Marine Ecosystem Restoration
in Changing European Seas MERCES
Grant agreement 689518
Coordinator: UNIVPM

Manual of restoration measures in soft bottoms based on surveys and experiments

MERCES Project Work Package 2
Deliverable 2.1
November 2019

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Cover photo: C. Boström

The MERCES project

The aims of MERCES (Marine Ecosystem Restoration in Changing European Seas http://www.merces-project.eu) are to restore degraded marine habitats within Europe, including coastal hard-bottom habitats, coastal soft-bottom habitats, and deep-sea habitats. MERCES seeks to assess the potential for various approaches and technologies to increase restoration success, develop new approaches, quantify the recovery of ecosystem services following restoration, and define the legal and political framework that can optimize restoration efforts.

MERCES Work Package 2 (WP2) focuses on shallow soft-bottom habitats, especially seagrass meadows and bivalve reefs. Using a combination of field surveys, aquarium and field experiments, and case studies, WP2 aims to

(a) determine the factors affecting seagrass restoration success,

(b) test whether integrating feedbacks and interactions in restoration increases success rates, and

(c) provide recommendations for managers and policy-makers.

MERCES WP2 includes 9 research groups in 7 countries (Croatia, Estonia, Finland, Italy, Netherlands, Norway, Turkey). Northern European (Baltic Sea, North Sea, Wadden Sea). Test species include eelgrass (*Zostera marina*), dwarf eelgrass (*Z. noltii*), blue mussels (*Mytilus edulis*) and Baltic clams (*Macoma balthica*). In Southern Europe (Adriatic Sea, Eastern Mediterranean), researchers are restoring the seagrasses *Cymodocea nodosa* and *Posidonia oceanica* and the endangered noble pen shell *Pinna nobilis*.

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SUBMISSION DATE: 30/11/2019

The importance of ecosystem engineers in coastal soft-bottom habitats

Coastal soft-bottom habitats are found around the world and include mangrove forests, seagrass meadows, salt marshes, and bivalve reefs. The species that dominate these habitats are known as engineers, as they build habitats that support high biodiversity by providing food and habitat for many other species. They also provide many other ecosystem services for human societies, including reducing coastal erosion and protection against storm surges, increasing water clarity, and mitigating climate change by storing carbon.

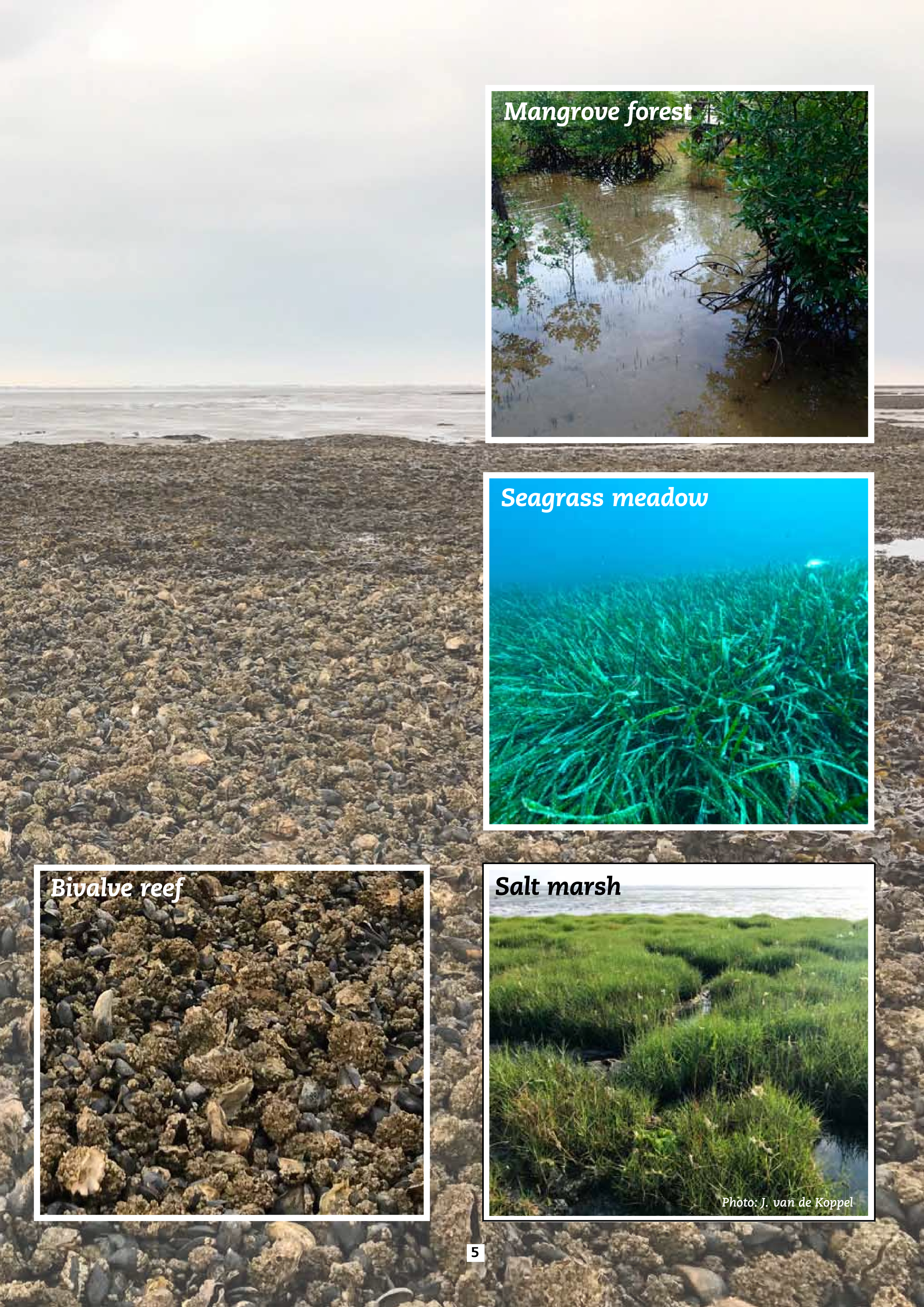

Mangrove forest
Seagrass meadow
Bivalve reef
Salt marsh
Photo: J. van de Koppel

Photo: C. Boström

Marine ecosystem engineers are being lost at alarming rates

Despite their importance, coastal ecosystems are being degraded and lost at alarming rates. Around the world, 30% of seagrass, salt marshes, and mangrove forests are estimated to have been lost, while up to 85% of oyster reefs are gone. In the EU, many of these habitats have been placed on the red list of habitats. For example, seagrass meadows are listed as near-threatened in the Baltic Sea, vulnerable in the Mediterranean, and critically endangered in the North East Atlantic. Oyster and mussel beds are also listed as near-threatened in the North East Atlantic and endangered in the Mediterranean Sea.

There are many reasons for habitat loss, but the majority are linked to human activities. Coastal development and dredging physically damage organisms, as well as increasing sediment load in the water column which chokes bivalves and reduces light available for seagrass. In many areas, salt marshes have been filled in and turned into grazing areas for livestock. Climate change also threatens coastal habitats: increased seawater and changing weather patterns cause higher wave intensity and prolonged heat waves, leading to reduced reproduction and higher mortality.

Simultaneously, it is becoming widely recognized that Green Infrastructure such as submerged aquatic vegetation (SAV) hold a great, but largely understudied, potential to mitigate the negative effects of human activities and wave disturbance and therefor increase coastal resilience. The high value submerged aquatic vegetation (SAV) including both seagrasses and freshwater plants, is related to several processes and ecosystem services (see below).

Seagrass ecosystem services

(a) trap organic and inorganic particles and reduce resuspension,

(b) increase sediment deposition, accretion and elevation rates,

(c) stabilize sediments by extensive root systems,

(d) regulate nutrient fluxes and trap nutrients in the sediment,

(e) produce oxygen,

(f) trap CO2 and function as blue carbon sinks, and

(g) support high biodiversity.

Marine ecosystem restoration – what are the challenges?

Restoration ecology began as a terrestrial field of study, for example, replanting forests after logging. In recent years, ecologists and managers have tried to apply the same techniques and principles in marine ecosystems, but success rates in the marine realm have been relatively low (Bayraktarov et al. 2016, van Katwijk et al. 2016). In addition, working underwater entails much higher costs and longer time scales than terrestrial work. Marine trophic networks also tend to be much more complex than terrestrial ones, so planting a single species does not seem to be enough to revive a whole ecosystem. This raises the question:

Can restoration help us regain lost biodiversity, functioning, and ecosystem services?

Habitat	Success rate (%)	Cost ha^{-1} (EUR)
Seagrass meadows	38	342 000
Mangrove forests	51	2 300
Salt marshes	65	135 000
Oyster reefs	56	170 000

Table source: Bayraktarov et al. 2016

Marine ecosystem restoration – what has been tried previously?

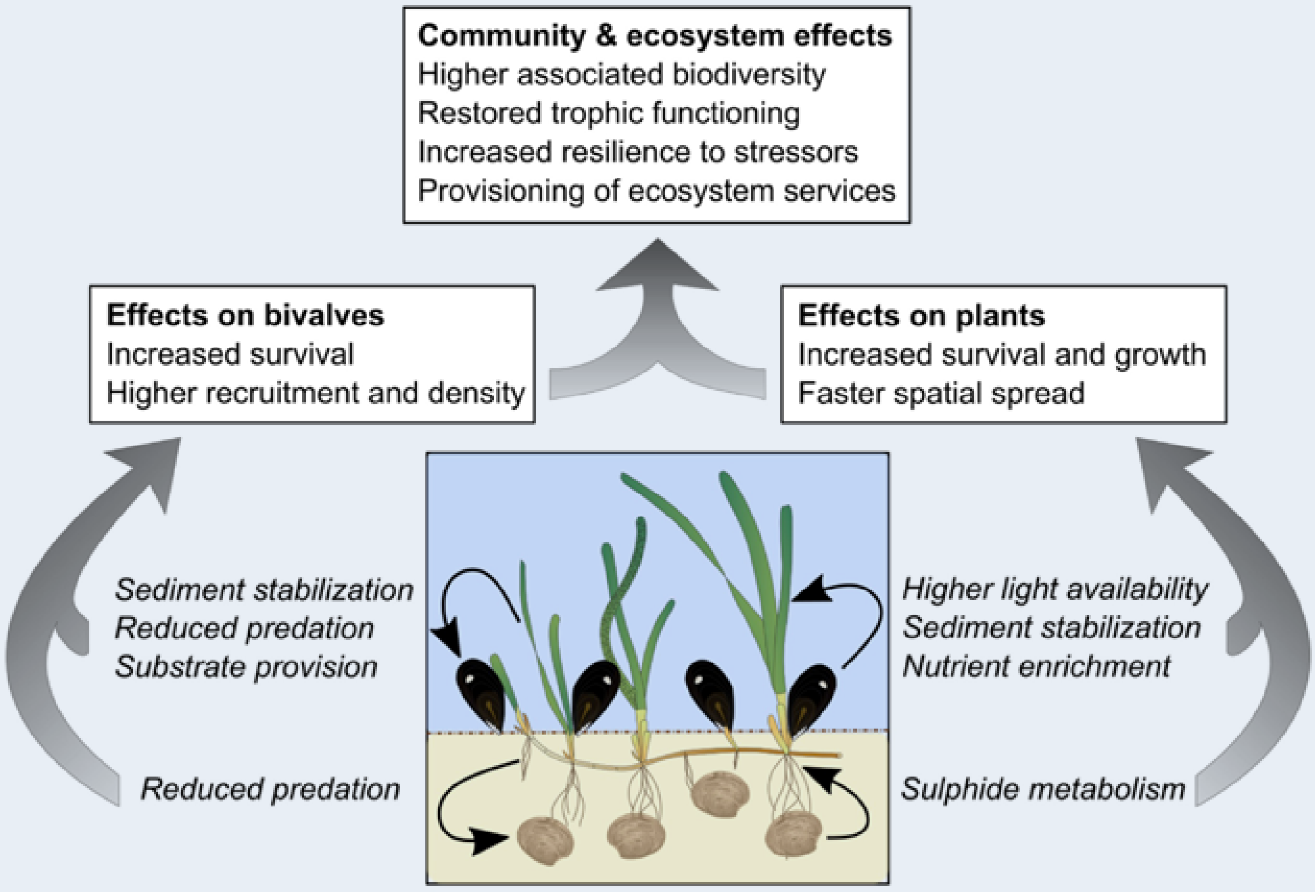
Despite the low success rates, there have been success stories in e.g. the Chesapeake Bay US, Australia, and New Zealand. Several studies have published guidelines for increasing restoration success (van Katwijk et al. 2016, Infantes et al. 2016, Paolo et al. 2019).

These include:

1. Large-scale planting
2. Careful site selection
3. Considering interactions and feedbacks
4. Single shoot planting instead of seeds
5. Reducing anthropogenic impacts prior to restoration

Integrating interactions and feedbacks into restoration

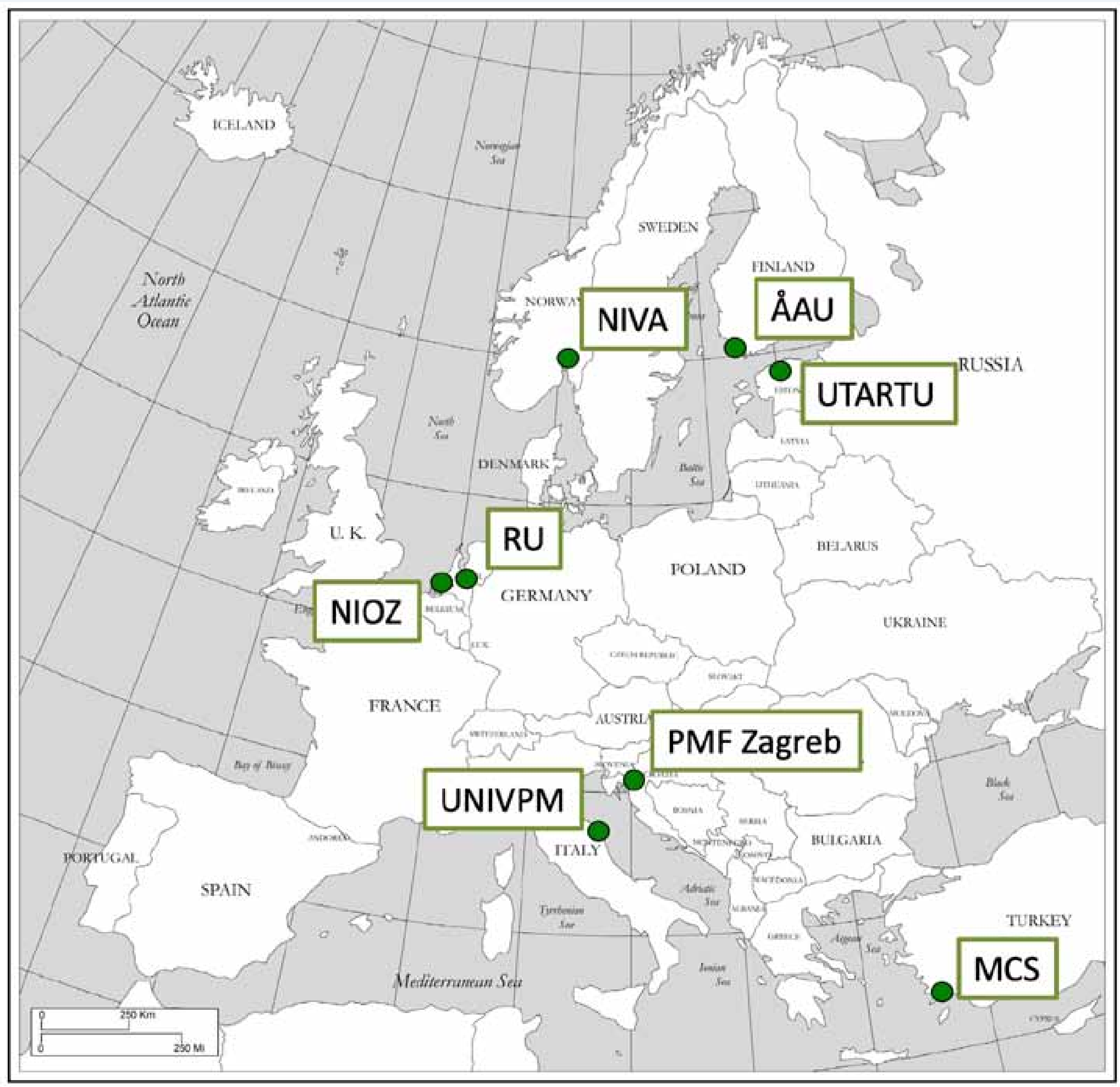
Habitats created by ecosystem engineers, such as seagrass meadows, are regulated by a complex network of interactions and feedbacks with other species and the surrounding environments (Maxwell et al. 2017). This means that when the habitat disappears, simply reintroducing the species alone may not work, as the environmental conditions (sediment type, water clarity) are no longer suitable (Moksnes et al. 2018). A global review of the literature on marine restoration (MERCES WP2) revealed that most interactions between plants and bivalves, especially plants and epifaunal bivalves, are positive (Gagnon et al., submitted). Plants provide shelter, protection and food for bivalves, while bivalves provide nutrients for plants and increase water clarity. Overall, these positive interactions lead to higher bivalve survival and abundance, and higher plant growth and density. Harnessing these interactions could increase both seagrass and bivalve restoration success and lower costs.



Source: Gagnon et al. submitted.

Seagrass and bivalve restoration in Northern Europe

MERCES WP2 study regions and species



Northern Europe

Mytilus edulis

Photo: C. Boström

Macoma balthica

Photo: C. Boström

Zostera marina

Photo: C. Boström

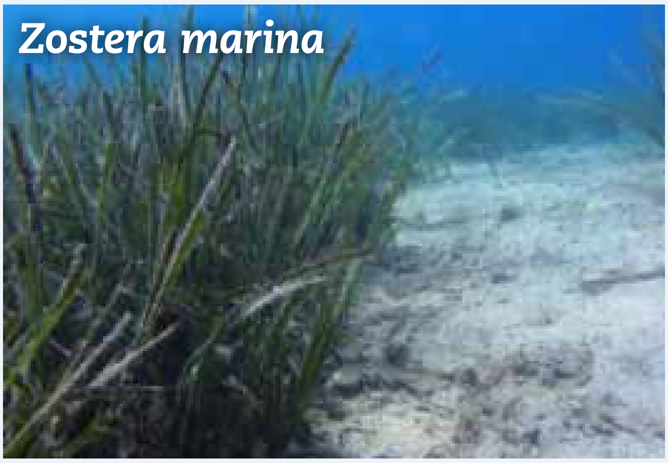
Zostera noltii

Photo: J. van de Koppel

Southern Europe

Pinna nobilis

Photo: D. Petricioli



Zostera marina

Cymodocea nodosa

Photo: S. Kipson

Posidonia oceanica

Photo: C. Boström

■ Seagrass restoration using Biodegradable EcoSystem Engineering elements

Biodegradable EcoSystem Engineering elements (BESE, producer: Bureau Waardenburg, The Netherlands) consist of 91x45.5x2 cm sheets that can be combined to form three-dimensional establishment structures. The modular units are designed to temporarily mitigate harsh environmental conditions to allow establishment of transplants, seeds or larvae of ecosystem engineering species. Once matured, these organisms should form biogenic structures that sufficiently improve the organism's own environment to allow it to thrive, after which the structures will naturally biodegrade.

Locations: Finland, Bonaire (Caribbean Netherlands), Sweden, USA, Croatia.

Species: *Zostera marina, Thalassia testudinum, Posidonia oceanica*

Question: Can BESE enhance establishment and restoration yields of seagrass transplants in exposed environments where mature meadows facilitate and maintain themselves by attenuating hydrodynamic energy and stabilizing sediments?

Approach/Protocol: Seagrass ramets were transplanted in 91x91x6 cm BESE-modules that were either placed above- or belowground, and compared with transplants in unmanipulated controls. Each of the three treatments was block-wise replicated four times per site.

Results: Results show that belowground BESE, by mimicking root mats of mature meadows, significantly enhanced establishment and growth in exposed environments, while having a non-significant negative effect in sheltered areas. Aboveground BESE positively affected *Thalassia* yields in Bonaire, but had neutral (Finland, USA) to negative effects on *Zostera* yields. In Croatia, almost half of BESE modules with seagrass ramets were lost during strong winter storms, with belowground structures being more resistant. Observed losses of BESE resulted from the breakage of the structures and not due to their inadequate anchoring.

Photo: M. Belosevic
Producer: Bureau Waardenburg

Conclusions: BESE can be used to enhance seagrass transplant establishment and restoration success at sites where key population-level traits generating self-facilitation (in our case anchoring and sediment stabilization by mature root mats) can be mimicked. There is a threshold in hydrodynamics beyond which the application of BESE modules may be severely compromised.

Photo: M. Belosevic
Producer: Bureau Waardenburg

■ Seagrass-bivalve co-restoration using *Zostera* and *Mytilus*

Locations: Estonia, Finland, Norway.

Species: Eelgrass *Zostera marina*, blue mussel *Mytilus edulis/trossulus*

Question: Can planting eelgrass and blue mussels together increase the restoration success (survival and growth) of either or both species? Does site exposure moderate this interaction?

Approach/Protocol: Six subtidal (2–4 m depth) sandy sites. Each country included an exposed and a sheltered site. In each site, we planted 30 plots including eelgrass alone, mussels alone, and eelgrass and mussels together, along with control plots. The eelgrass was collected from a donor site, then 16 shoots and rhizomes were attached to a plastic grid with cable ties. The grid was then buried several centimeters under the sediment and kept in place with 2–3 metal pins. The mussels (1 liter) were then placed on top of the plot. We checked eelgrass shoot density, growth, and mussel percent cover after 3 months (one growing season) and after 12 months (one winter season).

Results: In all exposed sites and one of the sheltered sites, the mussels disappeared from the plots within the first three months. The eelgrass survived in most plots after one growing season, but had disappeared from most plots after the winter season.

Conclusions: Though in aquaria mussels fertilized eelgrass and facilitated their growth, we could not detect any evidence in the field. Eelgrass had difficulty surviving in some sites, due to several site-specific factors: drift algal mats in Finland, sediment burial in Estonia and erosion in Norway. It was impossible to determine whether mussels could facilitate eelgrass growth and survival, as most of them were washed away from the plots. Attachment appear to be crucial (see below).

Mytilus-Zostera co-restoration plot Finland. Photo: K. Gagnon.

Mytilus Zostera co-restoration plot Estonia. Photo: K. Kaljurand

Mytilus-Zostera co-restoration plot Norway. Photo: C. With Fagerli

■ Seagrass-bivalve co-restoration using *Zostera*, *Mytilus* and BESE

Locations: Denmark, Estonia, Finland, Norway

Species: Eelgrass *Zostera marina*, blue mussel *Mytilus edulis/trossulus*

Question: Can biodegradable structures provide substrate for blue mussels in co-restoration efforts with eelgrass? Do blue mussels and/or BESEs facilitate eelgrass survival?

Approach/Protocol: Subbtidal sandy sites, one site per country except for two sites in Norway. At each site we set up 32 plots: 16 with BESEs and 16 on sand. Within these we had four plot treatments: control with organisms, eelgrass alone, mussels alone, eelgrass and mussels together. Like the previous experiment, we measured eelgrass shoot density and mussel cover after 2 months and 12 months.

Results: The mussels survived much better on the BESEs than on bare sand, and in some site, the BESEs also attracted new mussel recruits. Due to a heat wave across northern Europe in 2018, there was some eelgrass mortality across all treatments. After three months, eelgrass shoot density was highest in BESE plots with mussels suggesting that they both facilitated eelgrass growth. However, after 12 months, only BESE plots without mussels had any surviving eelgrass.

Conclusions: The BESEs are effective at retaining mussels and also at attracting new mussel recruits. The BESEs also seemed to increase eelgrass overwinter survival. Mussels facilitated eelgrass during the growing season, but not over winter, probably due to wave action destroying plants.

Photo: E. Rinde. (BESE producer Bureau Waardenburg)

Photo: K. Kaljurand. (BESE producer Bureau Waardenburg)

■ Seagrass-bivalve co-restoration using *Macoma* and *Zostera*

Location: Finland, Fårö, Archipelago Sea.

Species: Eelgrass *Zostera marina,* baltic clam *Macoma balthica*

Question: Can planting eelgrass and clams *(M. balthica)* together increase the restoration success (survival and growth) of either or both species?

Approach/Protocol: We planted 60 plots consisting of 16 eelgrass shoots each, attached to a 25x25cm plastic grid. The grid was buried several centimeters under the sediment and kept in place with 2–3 metal pins. We then added 10 densities (0–2800 ind. m^{-2}) of adult clams (>8mm) to the plots. Three replicates of each treatment were recollected after 75 days (n=30) and again after 14 months (n=30).

Results: All plots, independent of clam density, survived the 14 months and increased in biomass and size over time. Infauna samples indicated that most clams stayed in place during the first 2 months. While there was no detectable direct effect of clams density on plant traits, there was a significant interaction effect between clams and the underlying porewater nutrient gradient at the site. Shoot, root and rhizome biomass were highest at high clam densities in combination with low ammonium concentrations and *vice versa*. High clam densities combined with high ammonium concentrations however, resulted in an inhibition of biomass production. Since porewater nutrients were not sampled after 14 months, we could not test whether this effect was still apparent over time. The condition index of clams was significantly lower in plots and in the adjacent eelgrass meadow, compared to bare sand.

Conclusions: The effect of infaunal clams on eelgrass seems to be context-dependent, potentially due to increased nutrient release from the sediment. While the addition of *M. balthica* might facilitate eelgrass growth at sites with moderate nutrient concentrations, our results indicate that clams can have negative effects when nutrient levels are elevated. Our results further indicate that bivalve condition was worse in vegetated areas, potentially through reduced food availability. We thus recommend careful consideration when using of infaunal bivalves in seagrass restoration efforts.

Macoma-Zostera co-restoration. Photo: Alf Norkko

■ Seagrass-bivalve co-restoring using bivalves to trap eelgrass seeds

Location: Sven Lovén Centre for Marine Science, Kristineberg Station, Gothenburg University, Sweden

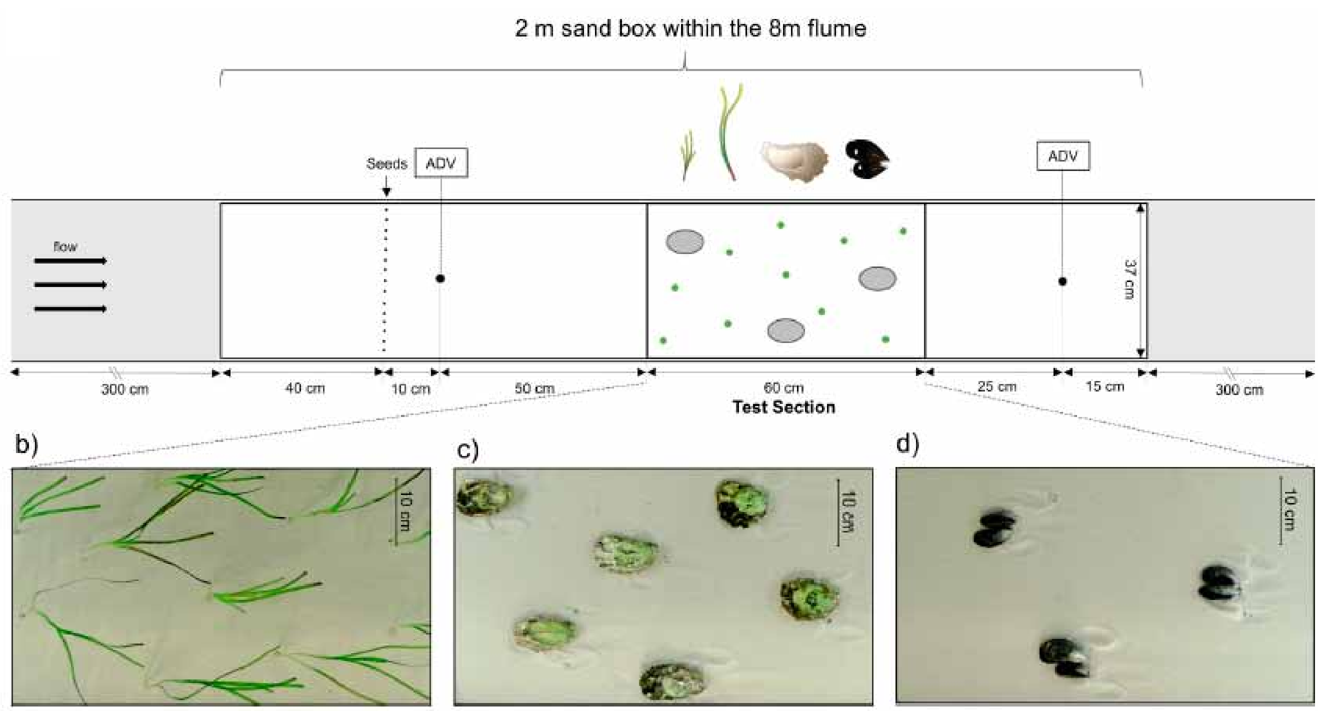
Species: Eelgrass *Zostera marina,* blue mussels *Mytilus edulis,* Pacific oyster *Magellana gigas*

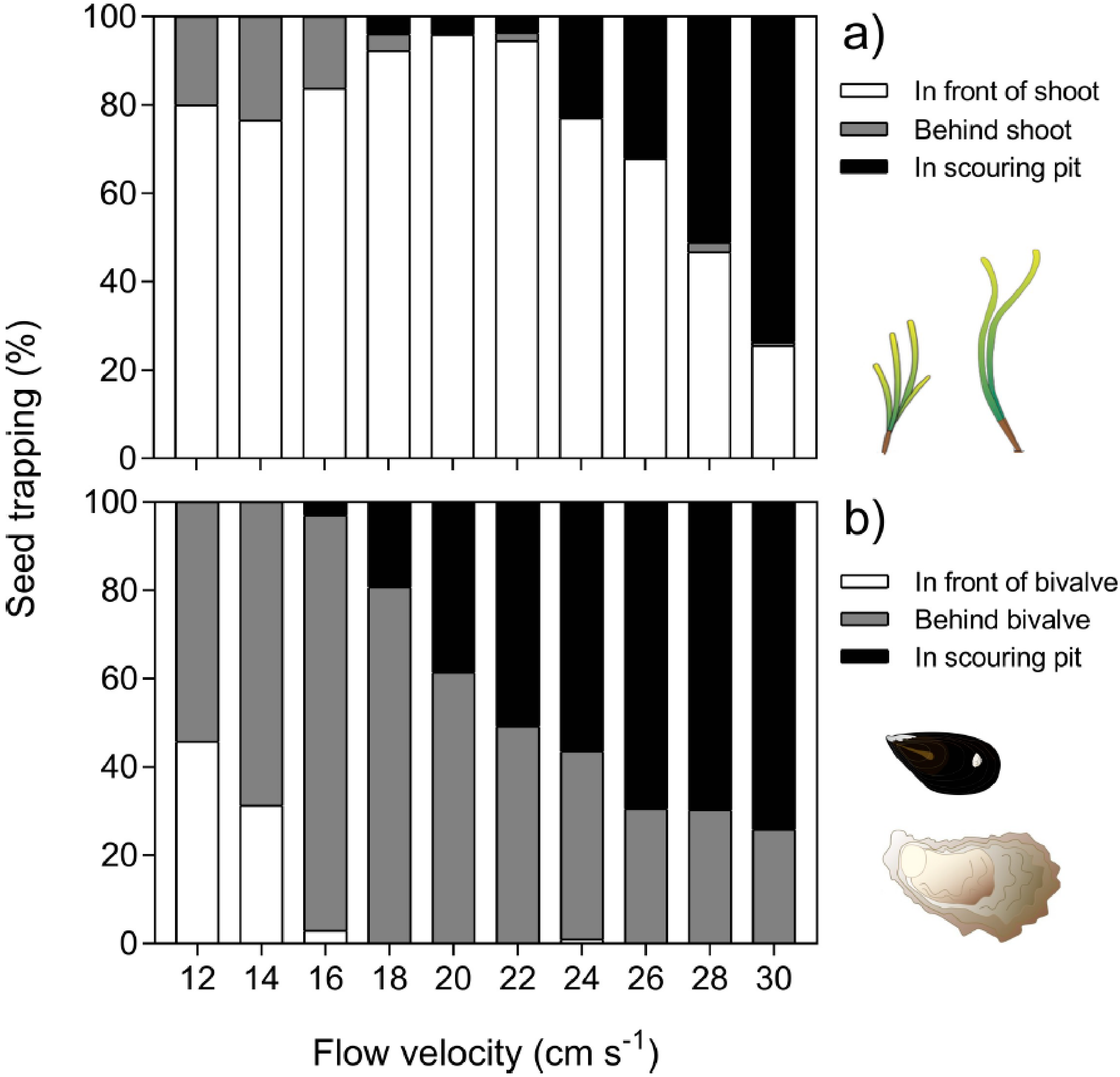
Question: Can ecosystem engineers such as oysters, mussels or eelgrass facilitate trapping and burial of eelgrass seeds?

Approach/Protocol: Main aims were to assess eelgrass seed dispersal and trapping by individual and combined synergistic effects of eelgrass shoots, pacific oysters *(Magellana gigas)* and blue mussels *(Mytilus edulis)* and to quantify which flow velocities affect sediment dynamics and seed retention by those ecosystem engineers. After placing different densities of oysters, mussels and eelgrass shoots in an embedded sand box within a hydraulic current flume (see below), we released 30 eelgrass seeds upstream and tracked dispersal at 10 flow velocities (12–30 cm s^{-1}). We counted the number of seeds that passed or retained this test section for each combination of engineering species and flow velocity. Since plants and bivalves affected sediment topography which in turn affected seed trapping, we also applied photogrammetry analysis to measure the size of the resulting scouring patterns.

Results: Both eelgrass shoots and bivalves effectively trapped seeds. Our results indicate that overall trapping of seeds increased with increasing habitat complexity (Meysick et al. 2019). Yet, plants and bivalves showed different trapping positions (see figure to the right) and flow velocity optimums for seed trapping. At low shoot densities, trapping in eelgrass was highest at low flow and decreased with flow velocity. Bivalves on the other hand were comparably ineffective at low flow velocities. Here, trapping success increased with flow due to strong reverse flow and sediment scouring patterns behind each specimen. Simultaneously, scouring also resulted in seed burial once the seeds were trapped. Combinations of bivalves and eelgrass reached constantly high trapping rates throughout the flow velocity gradient.

Conclusions: Seed dispersal is a critical part of the life cycle of seagrasses. Our results indicate that besides eelgrass shoots, also epifaunal bivalves such as oysters and blue mussels can facilitate trapping and burial of seeds, particularly under strong currents. This suggest for restoration efforts with seeds, site selection should carefully consider both the presence of co-occurring engineering species and the hydrodynamic regime.





Sources; Meysick L, Infantes E, Boström C (2019) The influence of hydrodynamics and ecosystem engineers on eelgrass seed trapping. PLoS ONE 14(9): e0222020. https://doi.org/10.1371/journal.pone.0222020

■ Intertidal seagrass restoration using eelgrass seed transplantation (I)

Location: The Netherlands, Uithuizen, North of the Dutch Groningen coast.

Species: Eelgrass *Zostera marina*

Question: Previous attempts at intertidal seagrass restoration in the Dutch Wadden Sea (e.g. the BuDS-method; Pickerell et al. 2005) had overall poor results. Here, we focused on the new DIS-method *(Dispenser Injection Seeding)* to determine how effective it is in different scenarios and how it can be optimized.

Approach/Protocol: Intertidal mudflat. *Zostera marina* seeds were collected in late summer from a substantial intertidal seagrass meadow in Schleswig-Holstein, Germany. In the Netherlands the seeds were separated from other plant material and organic debris. Once separated the seeds were treated with a low concentration of copper-sulfate (0.2 ppm) to combat a prevalent mold infection. Afterwards the seeds were stored in a cold and dark climate chamber over winter. In March, before seeding the seeds were soaked in freshwater for 24 h, with the goal to kickstart a stress reaction that initiates the germination process. After soaking the seeds were mixed with mudflat-sediment and the mixture was pushed into 300 ml dispenser tubes. In the field the seed-mud mixture was injected directly into the sediment with sealant-/caulking guns. We first investigated the viability of actual method, as well as how plot size (20 vs. 200 m^2) and seed density (2 vs. 20 seeds/injection) affect restoration success of *Zostera marina* in the intertidal zone.

Results: The experiment resulted in 100x higher plant densities (optimal treatment: ~1,8 plants m^2) than any of our experiments performed in previous years. As expected, plots injected with higher seed densities produced higher plant densities, but also significantly higher seed loss rates. Further optimization of the method was required. We found no effect of plot size on plant densities.

Conclusions: Overall, this first DIS-experiment was a success and provided us valuable insight on the potential of this method. However, only a few plants emerged the next summer, so our ultimate goal of establishing a self-sustaining seagrass population was still distant.

Photo: Laura Govers


Photo: Max Gräfnings


Photo: Laura Govers

■ Intertidal seagrass restoration using eelgrass seed transplantation (II)

Locations: (1) Intertidal sandflat Northeast of the Dutch Wadden Sea island Griend, (2) intertidal mudflat at Uithuizen, North of the Dutch Groningen coast.

Species: Eelgrass *Zostera marina* with an annual growing strategy

Question: After the first promising results in 2017 we aimed to further optimize the DIS (Dispenser Injection Seeding)-method. Additionally, we investigated if the method could be used successfully at both muddy and sandy sites.

Approach/Protocol: In a 1st experiment, we investigated how three seeding variables affected restoration success, crossing injection density (100 vs. 25 injects/m^2), seeding depth (4 vs. 2 cm) and seed density (20 vs. 2 seeds/inject). We seeded six 4-m^2 replicates of the eight treatments at a sandy and a muddy site (48 plots/site). In a 2nd experiment, we tested what seed density yields the highest plant numbers and the lowest seed loss. We tested five seed densities in the mesocosm (2, 4, 6, 8 & 10 seeds/inject) and seven densities in the field (2, 4, 6, 8, 10, 16 & 20 seeds/inject).

Results: The first experiment was successful at the sandy site, near the island of Griend. The best treatment (100 injections/m^2, 2 seeds/injection, 4 cm depth) resulted in high plant densities (>10 plants/m^2), with low seed loss (~94%) compared to previous experiments (99%), yielding up to 10-fold higher plant densities. However, low-tide drainage caused

by sediment trapping combined with mid-summer heat waves decimated these high-density plots. Nevertheless, we estimate that overall, over 10,000 adult plants emerged from our seeds at this site; approximately 1/3 of size of the largest current eelgrass population in the Dutch Wadden Sea. At the muddy tidal flat near Uithuizen, high seedling densities emerged in May. However, the majority of the plants washed away during June, which was most likely caused by the PVC-poles marking the experimental plots that caused heavy scouring here. In the second experiment, we found that the lowest seed densities (2 and 4 seeds/inject) performed best in the mesocosm as these densities produced similar plant densities compared to high-density treatments. We did not find any clear results in the field, as experimental plots were overrun by large aggregates of cockles, which dislocated/burrowed the majority of the seagrass seedlings.

Conclusions: We conclude that the DIS-method is viable for large-scale restoration in stable sediments. However, in contrast to earlier findings, we found that sediment trapping by high-density intertidal eelgrass beds enhances low-tide water drainage, increasing the populations' vulnerability to desiccation. Mesocosm experiments highlight that high-density seeding yields lower net germination, presumably due to intraspecific competition. We conclude that when focusing on single-species restoration, seeding should be done at relatively low densities at sites that remain moist during low tide.

Photo: Laura Govers

■ Seagrass and bivalve restoration in Northern Europe – challenges, solutions and recommendations

Challenges and barriers to restoration

- Mussel loss in high hydrodynamic conditions
- Mussel loss due to predation
- Seagrass loss due to filamentous algal blooms
- Seagrass over-winter survival is low
- Seagrass loss due to summer heat wave
- Low germination of seeds
- Seed loss in high hydrodynamics

Intertidal. Our results suggest that the DIS-method is very suitable for intertidal seagrass restoration. We have already been able to introduce high adult plant densities in the intertidal and by upscaling the restoration effort beyond an experimental setting, the plant numbers could be substantially increased. Our ability to introduce a new self-sustaining population is potentially not in the too distant future. By treating and storing the seeds overwinter we have been able to reduce winter mortality significantly, which in turn has made our seed-based restoration more economically viable and ecologically sustainable. Injecting the seeds directly into the sediment, also reduces seed losses. Methods that disperse the seeds on the sediment surface or in the water column suffer from very high seed loss rates, as the seeds easily wash away in the turbulent intertidal. We have also discovered that the injection depth plays an important role. Seeds injected at 4 cm depth produced significantly more plants than seeds injected closer to the surface at 2 cm depth. Closer to the surface the seeds wash away more easily, even though they are injected into the sediment. The DIS-method has shown potential on both muddy and sandy intertidal areas. The method doesn't perform well in exposed sites, as the seeds seem to wash away too easily even if they have been injected directly into the sediment.

Subtidal. In the subtidal zone, BESEs seem to be an effective tool to stabilise sediments and increase seagrass survival, especially in exposed sites with unstable sediments. BESEs are also very effective for bivalve restoration, as they provide a substrate to attach adult bivalves prior to transplantation, as well as for bivalve larvae to settle on. BESEs are designed to naturally biodegrade over time, so the bivalves should form natural reefs after the BESE structure has disappeared. Adding epifaunal bivalves (mussels) to seagrass restoration plots seems to have positive short-term effects on growth, possibly due to nutrient enrichment, but may be negative over the winter season. If nutrient enrichment is positive, one solution may be to add nutrients to seagrass restoration plots in the early stages after transplantation to aid in establishment and early growth. Adding other ecosystem engineers such as bivalves may increase seed trapping and thus restoration success.

Photo: C. Boström

Seagrass and bivalve restoration in Southern Europe

■ Seagrass restoration using biodegradable materials

Location: Gabicce Mare, North – Western Adriatic Sea, Parco del San Bartolo, Site of Community Importance)

Species: Slender seagrass *Cymodocea nodosa,* eelgrass *Zostera marina*

Question: Can biodegradable bags and jars facilitate the seagrass transplanting in coastal areas subject to high hydrodynamic conditions?

Approach/Protocol: Seagrass transplanting is based on the use of biodegradable bags inserted in biodegradable jars anchored with U-shaped stainless-steel rods. A corer was used to dig a clod from the donor seagrass meadow. This clod was immediately inserted in a biodegradable bag. The biodegradable bag was inserted in a biodegradable jar to maintain the consistency. Replicated experimental plots (n=3; 1×1m) have been prepared in bare sediments adjacent the existing seagrass meadows at similar environmental conditions of the seagrass meadow donor.

Results: Transplanted seagrasses are still present in the experimental plots after one year from the beginning of the experiments. The experimental plots show a strong seasonal variation in term of shoot density and biomass as reported also for existing seagrass meadows. Highest values of shoots density and biomass in transplanted seagrass plots are observed at the end of the summer.

Conclusions: This approach is efficient and successful. The transplanted seagrass survived during the severe hydrodynamic conditions occurred during the winter period. The effects of seagrass transplanting are still evident after one year from the beginning of the experiment. Good environmental conditions immediately after the transplanting favor the settlement and maintenance of the transplanting seagrass.


Photo: F. Torsani

Seagrass-bivalve co-restoration using *Pinna*, *Cymodocea* and *Zostera*

Location: Gabicce Mare North – Western Adriatic Sea, Italy. Parco del San Bartolo.

Species: Noble pen shell *Pinna nobilis*, slender seagrass *Cymodocea nodosa*, eelgrass *Zostera marina*

Question: Can planting seagrass and *P. nobilis* together increase the survival and growth of either or both species? Can transplantation of *P. nobilis* in existing meadows increase the growth/survival of the seagrasses?

Approach/Protocol: *P. nobilis* transplanting was performed using U-shaped stainless-steel rods. First of all a housing for the transplanting bivalve was prepared in the seabed using a corer. After that, the hole was partially filled with pebbles and the bivalve was anchored with the steel rod. We transplanted nine *P. nobilis* specimens in three experimental plots (1x1m): three specimens in bare sediments, three specimens in natural seagrass meadows and three specimens in transplanted seagrasses. *P. nobilis* abundance: 1 ind m^{-2} per each experimental plot. Seagrass transplantation using biodegradable bags. The experimental treatments included transplanting seagrass, transplanting seagrass and *P. nobilis* and existing seagrass as a control. Each experimental plot (1x1 m, n=3).

Results: The presence of seagrass favoured the survival of *P. nobilis* specimens while the severe hydrodynamic conditions occurred immediately after the beginning of the experiment have limited the success of the seagrass transplanting. The proposed method of anchorage for *P. nobilis* specimens resulted to be efficient. Plots with *P. nobilis* into existing seagrass meadows showed higher organic matter concentrations immediately after the translocation of bivalves. No differences among experimental plots in terms of meiofaunal abundance and diversity were observed immediately after the beginning of the experiment.

Conclusions: Environmental conditions immediately after translocation play a key role in the survival of *P. nobilis* and transplanted seagrasses. The presence of natural seagrass acts as a barrier for *P. nobilis* reducing the severe hydrodynamic conditions and avoiding possible burial effects. The presence of *P. nobilis* may increase the availability of food for benthic fauna associated with seagrasses meadows.

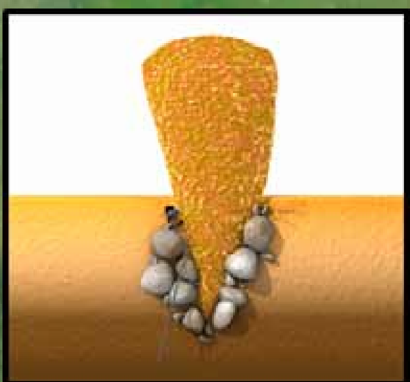


Photo: Z. Da Ros

■ Seagrass-bivalve co-restoration using *Pinna* and *Cymodocea*

Location(s): Javorike Bay, Brijuni MPA, North Adriatic Sea, Croatia

Species: Noble pen shell *Pinna nobilis*, slender seagrass *Cymodocea nodosa*

Question: Can transplantation of *P. nobilis* in existing seagrass meadows increase the growth/survival of either or both species?

Approach/Protocol: Noble pen shells were collected from a donor site and transplanted into the 1m^2 plots at 12 m depth with and without seagrass (on unvegetated sandy bottom). During transplantation, pen shells were carefully dug out and planted at host sites without provision of any additional anchoring substrate (burying approx. 1/3 of the shell-the anterior part, as ocurring naturally for this semi-infaunal bivalve). Plots with pen shells were assigned either to low (1 ind m^2) or high density (5 ind m^2) treatment whereas controls contained no pen shells. There were 5 replicates per each treatment (25 plots in total). We checked bivalve survival and growth, as well as seagrass growth after 1 and 2 years post-transplantation.

Photo: D. Petricioli

Results: Pen shell survival on bare sediment was high 5 months post-transplantation but was severely compromised by a late-autumn storm. In such an exposed site, transplanting pen shells within seagrass meadow substantially increased their survival. Moreover, growth of seagrass *C. nodosa* was enhanced by high-density pen shell treatment (5 ind m^2) and in general, nitrogen levels were higher (although not significantly) in plots with pen shells.

Conclusions: This is the first study to show mutual facilitation of the noble pen shell *P. nobilis* and a seagrass. Transplanting *P. nobilis* within seagrass meadow enhances its survival in exposed areas, given that transplantation is (ideally) carried out during early summer, thus providing enough time for pen shells to regenerate byssus and anchor well, prior to winter storms. Furthermore, transplanting pen shells in high density (e.g. 5 ind m^2) may enhance *C. nodosa* growth through a putative fertilization effect.

Photo: D. Petricioli

Photo: D. Petricioli

■ *Pinna* translocation using cages

Location: Gökova Bay, Turkey, Eastern Mediterranean.

Species: Noble pen shell *Pinna nobilis*

Question: Can covering with cage help *Pinna* establish after translocation?

Approach/Protocol: *P. nobilis* translocation was done by collecting small individuals from the vicinity and digging out with 50 cm radius and 50-60 cm deep sediment to protect the byssus as much as possible. All individuals were then transferred by covering attached sediment with plastic bag and carried underwater. They were placed and covered with their original sediment, and no support was used. After 1x1x0.5 m cages were used to cover the individuals.

Results: Transplanted *P. nobilis* individuals were alive and healthy after the winter and spring periods. Some new individuals were observed in spring on both cage covered and uncovered plots and few on the frame of the cages. However, in July 2018, due to parasite infection all individuals were either looking unhealthy (slowly closing their shell) or even dead.

Conclusions: It was observed that cages help pen shells to anchor after translocations and promote recruitment of new individuals, but a solid conclusion cannot be made due to disease outbreak that wiped out a large portion of the Mediterranean *P. nobilis* population.

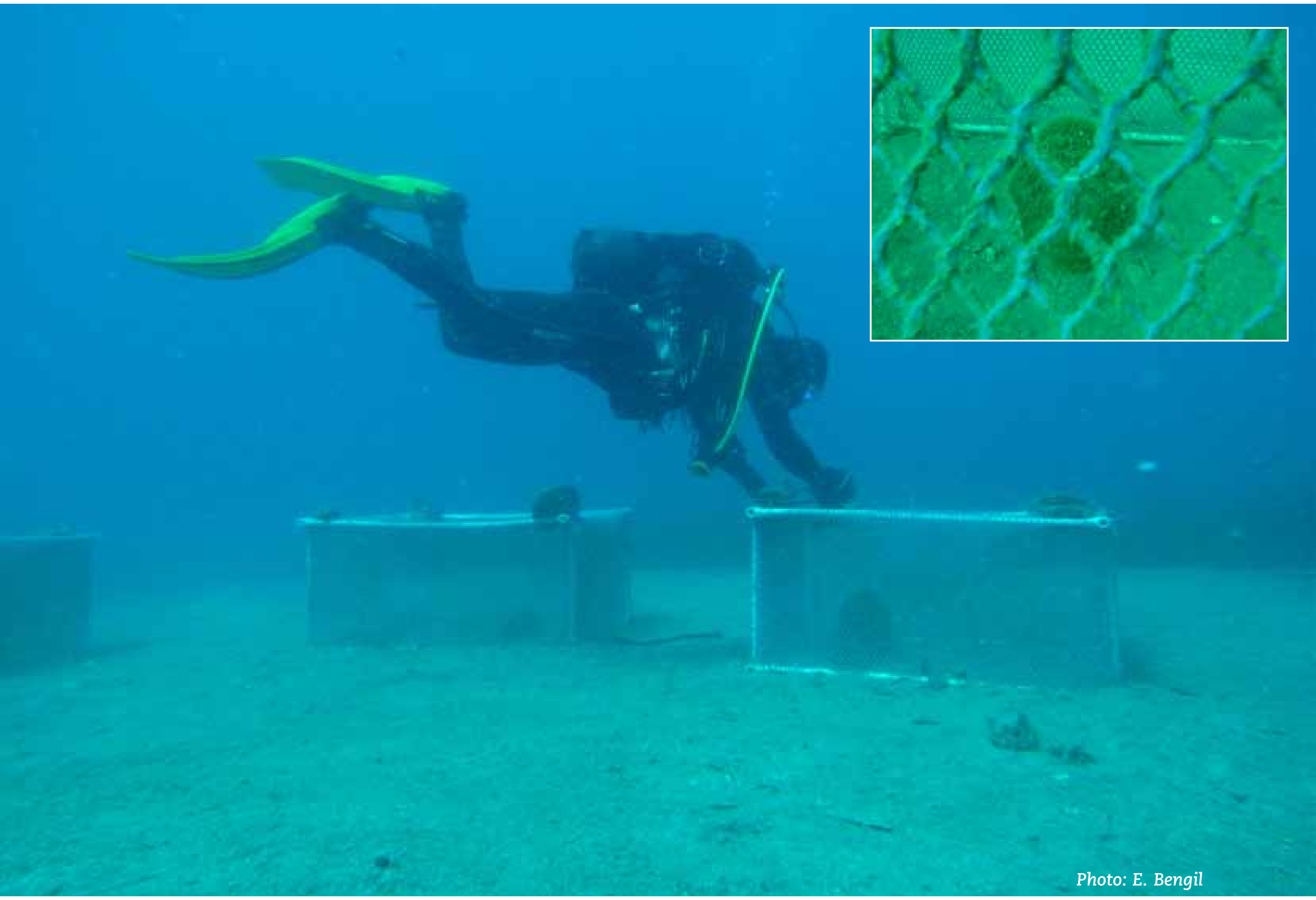


Photo: E. Bengil

■ *Posidonia* restoration using cages to prevent herbivory

Location: Gökova Bay, Turkey, eastern Mediterranean.

Species: Neptune grass *Posidonia oceanica*

Question: Can cages help seagrass transplantation success against grazing?

Approach/Protocol: Two controls (bare sediment and seagrass) and four experimental treatments were considered, with three replicates (1x1x0.5 m cages) each placed between 8-11 m depth. Treatments were bare sediment, bare sediment + transplanted *P. oceanica*, and already existing *P. oceanica*. *P. oceanica* transplantation was done by removing plants with their rhizomes using a shovel. Transplants were chosen from the same depth as the experimental plot and were placed to the plots by digging a hole and covering the rhizomes with the removed sand. To secure transplants, 70 cm long steel rods were pushed into the sediment and shoots were attached using cable ties.

Results: Shoot density in the natural *P. oceanica* meadows increased 45% and 11%, respectively in plots with and without cages. Conversely, transplanted shoots decreased 29% in both cases. Though cages provided protection against grazing on natural meadows, an increased grazing on transplants was observed. Additionally, cages protected transplants against anchoring damage.

Conclusions: Cages can be an effective tool to protect the transplants against anchoring damage as well as protect the natural meadows against grazing. However, cages need regular maintenance. In case of protecting transplants, it is not an effective method since it protects juvenile grazers from predation enabling juveniles to graze more efficiently.

Photo: E. Bengil

Seagrass and bivalve restoration in Southern Europe – challenges, solutions and recommendations

Challenges and barriers to restoration

- **Herbivory by invasive fish in eastern Mediterranean**
- **Large scale Pinna die-offs from disease outbreak**
- **Severe hydrodynamic conditions**

Despite failures, our results show that restoration could be an important tool in conserving populations of endangered endemic species in the Mediterranean Sea. However, other environmental factors must be considered in conjunction with translocating/ transplanting activities. *P. nobilis* survival is enhanced when translocating individuals to existing seagrass beds, as the seagrass reduces hydrodynamic stress and allows the bivalves to re-establish their byssus threads. An important aspect of increasing establishment success is to translocate *P. nobilis* during calm seasons to reduce stress. However, in recent years, *P. nobilis* mortality due to disease outbreaks has been an expanding problem, and destroyed experiments carried out (see above). More research is needed on the environmental factors involved in these outbreaks to properly plan conservation and restoration work. *P. oceanica* restoration is also possible in the Mediterranean. The transplantation of other seagrass species using sediment cores and biodegradable bags seems to be effective in promoting early establishment and resistance to the hydrodynamic stress. As with *P. nobilis*, transplantation seagrass during calm weather conditions and seasons reduces stress and increases survival. In the eastern Mediterranean, where herbivory by invasive fish is a limiting factor influencing seagrass growth, cages can increase early survival and allow seagrass to grow to a minimum size, at which they can withstand grazing. However, cages require maintenance, and are subject to breaking during strong storms, so restoration areas must be chosen with this in mind.

Facilitating seagrass restoration by means of bivalves

In northern-European habitats, our experiments failed to realize seagrass restoration on any meaningful scale. In aquarium systems, mussels were found to fertilize eelgrass and facilitate growth, but we could not detect any evidence of this occurring in the field. Eelgrass co-restored with *M. edulis* did not survive in Finland, Norway, Netherlands and Estonia due to physical disturbance. Field experiments showed that effects of infaunal clams on eelgrass is context-dependent, potentially due to increased nutrient release from the sediment. Flume experiments showed that epifaunal bivalves can facilitate trapping and burial of seeds, suggesting that both the presence of engineering species and the hydrodynamic regime are important factors to consider in restoration using seeds.

In southern European habitats, mutual facilitation of *P. nobilis* and a seagrass was observed and transplanting *P. nobilis* within seagrass meadow enhances seagrass survival, especially in exposed areas. Furthermore, transplanting *P. nobilis* at a density of 5 ind m^2 may enhance *C. nodosa* growth through fertilization. The presence of natural seagrass acts as a barrier reducing the severe hydrodynamic stress for *P. nobilis* and avoiding possible burial effects. Conversely, the presence of *P. nobilis* may increase the availability of food for benthic fauna associated with seagrasses meadows. In other words, bivalve facilitation may not only enhance seagrass restoration, but the interactions between bivalves and seagrass proved positive for both species.

BESE as supporting substrate for seagrass restoration

In northern European habitats, BESE-elements was found to enhance seagrass transplant establishment and restoration success at sites where self-facilitation is important. The BESE method proved particularly effective at retaining mussels as well as at attracting new mussel recruits. In northern European habitats, BESE-elements enhanced establishment of seagrass transplants and restoration success at sites where hydrodynamic energy is high and sediments are mobile. Here, BESE can be applied to mimic root mats of established seagrass meadows, thereby temporarily stabilizing the sediment bed. This in turn allows vulnerable transplants to establish, after which the BESE degraded and matured seagrass patches take over its sediment-stabilizing role. BESE also proved particularly effective in stimulating settlement of mussel recruits within the structure in the Dutch Wadden Sea. Similar to BESE mimicking seagrass root mats, the BESE was used to mimic the natural complexity of established mussel beds, providing suitable attachment substrate for settling larvae and reducing predation by shrimp and crabs. In the southern European sites, seagrass transplanted using biodegradable bags survived harsh hydrodynamic conditions and effects were still evident after one year. Hence, our study highlights the use of artificial, biodegradable substrates that can support establishing seagrass as well as other sessile species in dynamic intertidal areas.

General recommendations for managers and policy makers: Is prevention better than curing?

Restoration is a conservation tool, aiming to recover an ecosystem or habitat and its services on a specific location by helping keystone organisms to establish, facilitating positive interaction within and between species, so that in the end, a population or even an entire ecosystem can develop. The alternative to restoration is to conserve and protect what is there, or to allow and wait for natural recovery to occur. This often requires, however, that a far larger area is protected in a precautionary way, preventing further degradation, and stimulating for natural recovery wherever keystone organisms will emerge naturally. This, in the end, requires long time scales and a much larger area to be protected from human activities, and hence puts much more extensive limitations on economic activities. Thus, from an economic viewpoint, it would be preferable if damage to natural systems could easily be "repaired" by "on-the-spot-restoration", after the losses imposed by human activities become apparent. Restoration, if successful, allows for less control on human enterprises. Furthermore, other context-dependent factors can influence the decision to restore or protect, which may depend on operational, legal, social and often political (governance) constraints (Possingham et al. 2015). Other factors to be taken into account include relative costs of restoration *vs* conservation, habitat loss rates, and the expected time frame between habitat restoration and the subsequent recovery of ecosystem services.

Seagrasses and corals are the most expensive and difficult ecosystems to restore, and costs for restoration of one hectare marine coastal habitat are on average US$1600000, which is 10-400 times higher than the maximum cost for restoration of freshwater and terrestrial habitats (Bayraktarov et al. 2016). Moreover, succes rates in seagrass restoration has been particularly low (38%, Bayraktarov et al. 2016), and are basically absent on scales that are relevant for ecosystem management. This points out that conservation, and restoration by protecting ecosystems and improving their functioning on very large spatial scales - stimulating purely natural recovery - are the only viable option. This will require the reduction of economic activities on extensive spatial scales. Especially in systems where establishment thresholds reduce quick recovery of ecosystems, generating hysteretic ecosystem dynamics, environmental conditions may even have to be improved to near-pristine conditions before recovery will take place. This has been experienced in the Wadden Sea, were mussel and cockle fisheries on the tidal flats was forbidden entirely, and yet it took between 10-20 years before recovery of mussel beds was effective.

We have in this project tested a number of approaches to directly restore seagrass beds, involving several seagrass species and associated bivalves as well as different anchoring materials, and testing their effectiveness in different ecosystems along the European coast line. Our results show that bivalves can have a stimulating effect on seagrass growth, aiding conservation and restoration, but success is context and habitat dependent. We also tested the effectiveness of a three-dimensional biodegradable artificial substrate, which can provide a foothold for seagrass and bivalves and reducs wave-driven losses.

Photo: C. Boström

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Acknowledgementsnts

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 689518.

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