WP 2 Deliverable 2.2

D2.2: Restoration results in the case study sites

Marine Ecosystem Restoration in Changing European Seas MERCES

Grant agreement n. 689518

COORDINATOR: UNIVPM

LEAD BENEFICIARY: 11 – University of Tartu (UTARTU)

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SUBMISSION DATE: 30/11/2019

DISSEMINATION LEVEL
(Public)

PU	Public	**X**
CO	CO Confidential, only for members of the consortium (including the Commission Services)	

Seagrass *Zostera marina* in the Baltic Sea (photo by K. Kaljurand, UTARTU).

Citation

Pajusalu L, Bakran-Petricioli T, Boström C, Carugati L, Christie H, Da Ros Z, Danovaro R, Gagnon K, Gambi C, Govers L, Gräfnings M, Kaljurand K, Kipson S, Kotta J, Martire ML, Rinde E, van der Heide T, van de Koppel J, Martin G (2019) Restoration results in the case study sites. Deliverable 2.2. MERCES project, 53 pages.

Acknowledgments

We acknowledge the cooperation and generous contribution to MERCES activities in Viksfjord (Norway) from the local Viksfjord-project and project lead. PMF Zagreb team would like to thank Donat Petricioli, Mirko Radolović, Josip Tomišin and volunteer divers from diving club "Loligo" (Vodnjan, Croatia) for invaluable assistance in the field. We also acknowledge the financial contribution provided by Kermas Istra Ltd. for pen shell translocation. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 689518.

Summary

Human population increase has intensified the use of marine resources and as a consequence significantly increased the pressure placed upon the marine environment, as a result cause widespread habitat loss and change. The overall aim of MERCES Deliverable 2.2 was to carry out experimental restoration trials in shallow soft bottom habitats in five different MERCES case study areas and to investigate how restoration success varies across European coastal systems. This covers five MERCES case study sites, involving the Baltic Sea, Skagerrak, Dutch Wadden Sea, and Mediterranean Sea. Case studies have been carried out with the seagrasses *Zostera marina*, *Zostera noltii*, *Cymodocea nodosa* and the bivalve *Pinna nobilis*. Within the case study sites, different restoration methods were utilised from transplantation and translocation to seeding as a result of differing aims, species-specific requirements and site-specific conditions.

There were different starting motivations of the restorative actions in the five case study sites. For example, in addition to seasonal touristic pressures, a breakwater relocation had a deleterious effect on seagrass meadows located at Gabicce Mare in the north-western Adriatic Sea. During extreme eutrophication events in the 1980s *Z. marina* had disappeared from extensive areas in the Gulf of Riga, northern Baltic Sea. In the Dutch Wadden Sea, *Z. marina* subtidal meadows vanished due to the construction of the Closure Dam (“Afsluitdijk”) and a concurrent outbreak of wasting disease occurring in the 1930s. At the same time, the intertidal populations in the same area initially survived longer but had also almost completely disappeared by the 1970s to 1980s due mainly to eutrophication. The motivation for the translocation of the strictly protected *P. nobilis* was to avoid the smothering of this species during the construction of a new nautical centre located in Pula Harbour (North Adriatic Sea). In southern Norway (Viksfjord), annual blooms of green algae reduce the light conditions and the decomposition of the algae leads to oxygen hypoxia which threatens marine life and *Z. marina* meadows, calling for actions to improve the ecological conditions.

So far, the success rate of seagrass meadow restorations has been relatively low worldwide (under 50%). Despite this, within focused MERCES case study sites we found that in most cases restoration attempts were quite successful. The seagrass transplantation using biodegradable bags was found to be successful in Gabicce Mare. Transplanted seagrass survived over the winter, enduring harsh hydrodynamic conditions. In the Dutch Wadden Sea, the novel seeding-method ‘DIS-method’ provides a good example of this as it is a method that can be used to restore intertidal seagrass meadows. The DIS method was found to greatly increase seagrass shoot densities as well

as reducing seagrass seed loss. Additionally, the DIS-method has, in part, acted to the further proposed aim of enhancing self-sustaining seagrass meadows of the future. In the northern Baltic Sea, there was some success observed with the transplantation of *Z. marina* using the rope method after the first growing season. However, the transplanted seagrass did not survive over the winter. In the second attempt to transplant seagrass, the overall shoot density decreased but at the same time on some ropes the number of shoots increased significantly. In the Skagerrak region, the seagrass transplantation experiments had only a short-term success. Similarly like in the Baltic Sea, transplanted seagrass did not survive over the winter. Translocation of *P. nobilis* within a conservation action to protect this species was also successful in the Pula Harbour. After two years post-translocation, their survival was very high – 86.4%.

The success of restoration actions varied across European coastal systems, and success rate mainly depended on geographic location as well as the restoration method used. Knowledge developed in this study regarding the use of restoration methods identifies local environmental conditions as being of key importance to increasing the success rate of soft bottom marine habitat restoration attempts. In addition, an important aspect is to incorporate different stakeholders such as local communities, funding organisations, governmental bodies, scientists, citizens and volunteers into restoration projects.

Contents

Acronyms Used

BESE-elements: Biodegradable Elements for Starting Ecosystems
DIS: Dispenser Injection Seeding
EEC: European Economic Community
EU: European Union
H_2S: Hydrogen Sulfide
IVIV: County Governor from Indre Viksfjord Association
LC: Least Concern
MERCES: Marine Ecosystem Restoration in Changing European Seas
MPA: Marine Protected Area
SCUBA: Self-contained underwater breathing apparatus
SLIKKEN: Self-developed craft
UNEP: United Nations Environment Programme
UNESCO: United Nations Educational, Scientific and Cultural Organization
WP: Workpackage

1. INTRODUCTION

1.1. Scope of the Deliverable

The overall scope of MERCES WP2 Deliverable 2.2 is to give an overview of the restoration results from the case study sites. This covers five MERCES case study sites, involving the Baltic Sea, Skagerrak region, Dutch Wadden Sea, and Mediterranean Sea. Case studies have been carried out with the seagrasses *Zostera marina*, *Zostera noltii*, *Cymodocea nodosa* and the bivalve *Pinna nobilis*. The methods used in restoration actions included both transplantation and translocation of adults, as well as seeding. The individual case study methods include:

- Seagrass *Z. marina* transplantation with the 'rope method' in the northern Baltic Sea;
- Translocation of *P. nobilis* from disturbed sites in the north-eastern Adriatic Sea;
- Seagrass transplantation and monitoring in the north-western Adriatic Sea;
- Intertidal seeding-method in the Dutch Wadden Sea;
- Seagrass *Z. marina* transplantation and aeration of sediments in Viksfjord (Skagerrak)

1.2. Motivation of restorative actions

There were different starting motivations of the restorative actions in the five MERCES case study sites. For example, a deleterious effect on seagrass meadows at Gabicce Mare in the north-western Adriatic Sea because of seasonal touristic pressures and a breakwater relocation. During extreme eutrophication events in the 1980s *Z. marina* had disappeared from extensive areas in the Gulf of Riga, northern Baltic Sea. In the Dutch Wadden Sea, *Z. marina* subtidal meadows vanished following to the construction of the Closure Dam ("Afsluitdijk") and a concurrent outbreak of wasting disease occurring in the 1930s. At the same time, the intertidal populations in the same area initially survived longer but had also almost completely disappeared by the 1970s to 1980s due mainly to eutrophication. The motivation for the translocation of the strictly protected *P. nobilis* was to avoid the smothering of this species during the construction of a new nautical centre located in Pula Harbour (North Adriatic Sea). In southern Norway (Viksfjord), annual blooms of green algae reduce the light conditions and the decomposition of the algae leads to oxygen hypoxia which threatens marine life and *Z. marina* meadows, calling for actions to improve the ecological conditions.

1.3. Ecosystem engineers in the coastal soft-bottom habitats

Ecosystem engineers such as seagrass meadows and bivalve reefs are important components in shallow soft-bottom coastal habitats worldwide. They play a key role in these ecosystems by providing habitat for countless other species and act to support high levels of biodiversity. Moreover, seagrass meadows also provide numerous other ecosystem services such as fisheries production, coastline protection, increased water clarity, pollution buffering, and nutrient and carbon sequestration (Hemminga & Duarte, 2000). Such systems may have an important role in mitigating the effects of climate change and ocean acidification through the uptake and storage of carbon. Due to their large global coverage, the contribution of seagrass meadows to total blue carbon storage is second only to that of mangrove habitats (Pendleton et al., 2012). As a result, "blue carbon" is stored in seagrass meadows worldwide, in environments ranging from cold polar waters to that of warm regions located in the tropics (Röhr et al., 2018).

Human population increase has intensified the use of marine resources and as a consequence significantly increased the pressure placed upon the marine environment. Different marine activities such as fishing, shipping, and aquaculture combined with the land-use of coastal infrastructure through the practices associated with agriculture, forestry and industry act to place multiple pressures on the marine environment (Smith et al., 2016). Furthermore, global changes in ocean temperature and acidification have a strong influence on marine system function (IPCC, 2014). These factors have a strong negative effect on marine coastal ecosystems and as a result cause widespread habitat loss and change. Thus, the restoration of valuable marine habitats is seen as an essential component in the field of environmental conservation.

1.4. Restoration of marine soft-bottom habitats

In recent years, there has been an increasing interest in the restoration of marine ecosystems worldwide. The restoration of terrestrial ecosystems has been widely implemented, however, less is known about the requirements needed for the restoration of marine ecosystems. So far, the success rate of marine ecosystem restorations has been relatively low. For example, based on seagrass restoration projects the expected outcome for success is as low as 38% (Bayraktarov et al., 2016). In terms of the many ecosystems that are not able to recover without human intervention, there is a need to develop new techniques and approaches that increase the success rate for their restoration (Jones et al., 2018). For example, one new approach is to investigate plant and animal engineers that can be used in co-restoration (e.g. seagrass and mussels) to increase the chance of restoration success of marine habitats (Gagnon et al., in review).

While most studies have shown the relatively low success rates of seagrass restoration, there have been success stories as well. For example, transplantation of the seagrass *Posidonia australis* was carried out in the south coast of Western Australia. After 5 years of transplantation, shoot density of *P. australis* was similar to the shoot densities recorded within natural meadows (Bastyan & Cambridge, 2008). Furthermore, large restoration scales may benefit seagrass restoration successes as the initial number of shoots or seeds planted were directly related to survival in population (van Katwijk et al., 2016).

1.5. Case study sites and studied species

D2.2 of MERCES WP2 focuses on five different case study sites, involving the Baltic Sea, Skagerrak region, Dutch Wadden Sea and the Mediterranean Sea, located in northern and southern Europe respectively (Figure 1). The species used in restoration actions are the seagrasses *Zostera marina*, *Zostera noltii*, *Cymodocea nodosa*, and the bivalve *Pinna nobilis*. Restoration activities related to *Z. marina* meadows were carried out in four different locations within the northern Baltic Sea, Dutch Wadden Sea, Skagerrak and north-western Adriatic Sea. In addition, restoration attempts of *Z. noltii* were conducted in the Dutch Wadden Sea with attempts also made for *C. nodosa* in the north-western Adriatic Sea. Translocation of *P. nobilis* was carried out in the north-eastern Adriatic Sea. For each MERCES case study different restoration techniques were used due to species-specific requirements and site-specific conditions. The restoration actions ranged from transplantation and translocation to seeding. More detailed information on each case study site is presented under a standardized headline in section 2.

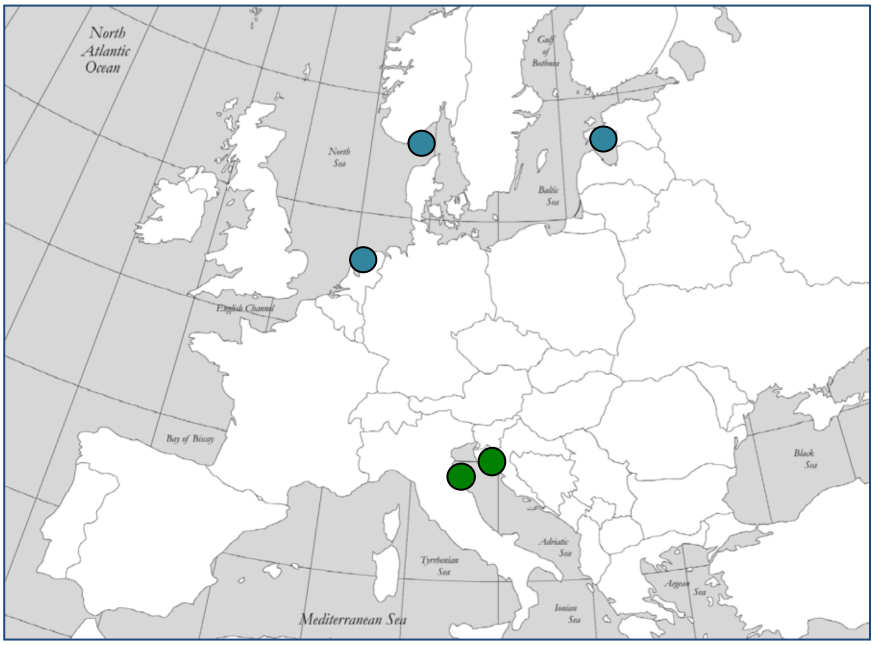

Figure 1. Locations of case study sites.

The most studied seagrass, *Z. marina*, is a widely distributed seagrass species found to inhabit temperate coastal waters in the Northern Hemisphere (Figure 2). *Zostera marina* grows across a wide range of environmental conditions (e.g. salinity and turbidity) in both the intertidal and subtidal zones. Similar to other seagrass species, *Z. marina* plays a critical role in the structure and function of coastal ecosystems (Boström et al., 2014). Eelgrass meadows create essential habitats for numerous commercially important fish and shellfish species. However, the distribution of *Z. marina* meadows are in serious decline, involving also the Baltic Sea (Boström et al., 2014), the Dutch Wadden Sea (Den Hartog, 1987; de Jonge et al., 2000) and the Mediterranean Sea (Boudouresque et al., 2009) mainly due to multiple human activities such as eutrophication, habitat destruction and climate change.

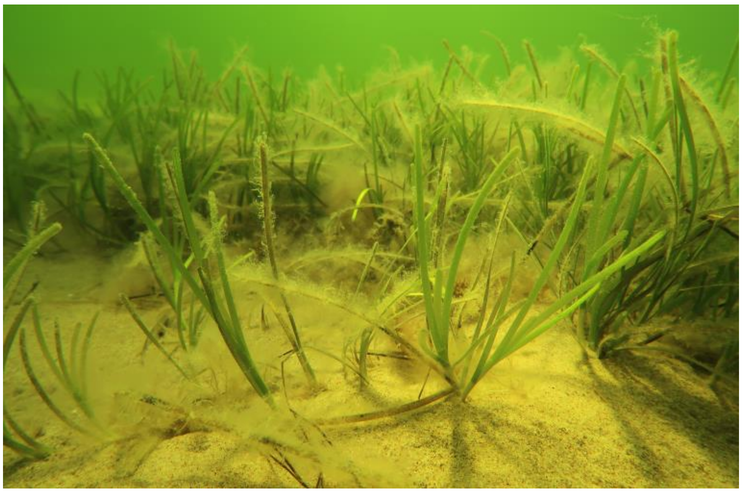

Figure 2. Seagrass *Zostera marina* communities in the Baltic Sea (photo by K. Kaljurand).

Cymodocea nodosa is one of the most common seagrass species in the Mediterranean Sea. It is a fast-growing species that spread mainly throughout shallow areas of the Mediterranean Sea (Figure 3). *Cymodocea nodosa* leaves reach a maximum length of 40 cm. It forms meadows on the seabed but at times may also grow interspersed with other seagrass species such as *Z. noltii* and *Posidonia oceanica*. The different future scenarios predict that the Mediterranean meadows of *C. nodosa* will suffer an important range contraction, estimated to the loss of habitat ranging from 20.8% to 46.5% by 2100 (Chefaoui et al., 2018).

Figure 3. Seagrass *Cymodocea nodosa* in the Mediterranean Sea (photo by T. Bakran-Petriciol).

Dwarf eelgrass, *Z. noltii*, is a perennial seagrass species that grows mainly in intertidal and shallow subtidal ecosystems (Figure 4). Dwarf eelgrass has a wide distribution margin throughout NE Atlantic and European Seas, and forms extensive meadows, providing food and structure for a wide variety of marine species and birds.

Figure 4. Intertidal *Zostera noltii* in the Dutch Wadden Sea (photo by M. Gräfnings).

The noble pen shell *P. nobilis* is an endemic Mediterranean species and it is considered one of the biggest bivalves in the world, reaching up to 120 cm in shell length (Zavodnik et al., 1991; Figure 5). It grows at a depth between 0.5 and 60 m, commonly found to reside within soft-sediment seagrass meadows such as those associated with *P. oceanica*, *C. nodosa*, *Z. marina* and *Z. noltii*. The population of *P. nobilis* has been greatly reduced due to several environmental and anthropogenic factors such as habitat destruction, anchoring, trawling, illegal extraction and a rapidly spreading disease in the Mediterranean (Basso et al., 2015; Deudero et al., 2015; Vázquez-Luis et al., 2017).

Figure 5. The noble pen shell *Pinna nobilis* (photo by M. Belosevic)

The main aim of MERCES Deliverable 2.2 was to carry out experimental restoration trials in shallow soft bottom habitats in five different MERCES case study areas and to investigate how restoration success varies across European coastal systems. It provides an overview on restoration results from the case study sites found in the northern Baltic Sea, Skagerrak, Dutch Wadden Sea, and Central Mediterranean Sea. Based on the results from the studied sites we have identified general factors and guidelines that can be utilized to improve the success rate of future projects.

2. CASE STUDY SITES

2.1. Seagrass *Zostera marina* transplantation with rope method - Estonia (UTARTU)

2.1.1. Motivation for initiating the restoration measures

Seagrass *Z. marina* has been historically reported in the NE region of the Gulf of Riga, north-eastern Baltic Sea but due to eutrophication and extreme storm events in the 1980s these communities have decreased significantly throughout the region (Figure 6).

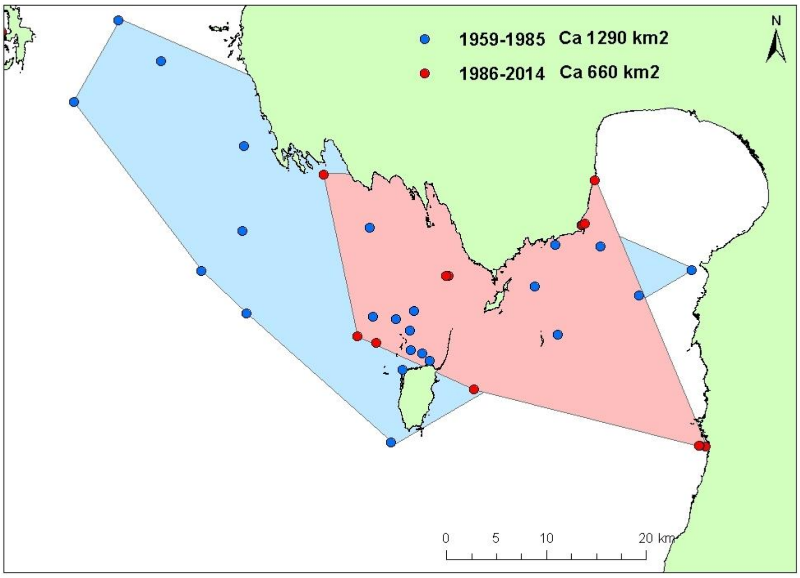

Figure 6. The decline in distribution area of *Zostera marina* communities compared to previous periods: from 1200 km^2 to 660 km^2 in the case study area.

2.1.2. Description of case study area

The Gulf of Riga is a wide, shallow basin situated between the Estonian and Latvian mainlands. In most parts of the Gulf, salinity is low such that it represents the lower survival limit for many species of marine origin. A suitable transplantation site was identified in the north-eastern region of the Gulf of Riga (area north of Kihnu Island), a location where *Z. marina* was known to have previously inhabited. The study area is moderately eutrophic with an average salinity of around 6.0 psu and consists primarily of sandy, soft bottom habitat. A suitable *Z. marina* donor site was identified in the western basin of the West Estonian Archipelago Sea (Figure 7). The donor site was selected because its environmental conditions (e.g. salinity, nutrients level, light conditions) are similar to that of those found in the case study site. Furthermore, *Z. marina* communities were found to be in good health within this area, providing a good stock for transplanting.

The first attempt of transplanting experiment failed, and we then select a new site with better hydrodynamic conditions. The new site was chosen to in the Kihelkonna Bay, in the West Estonian Archipelago Sea (Figure 7).

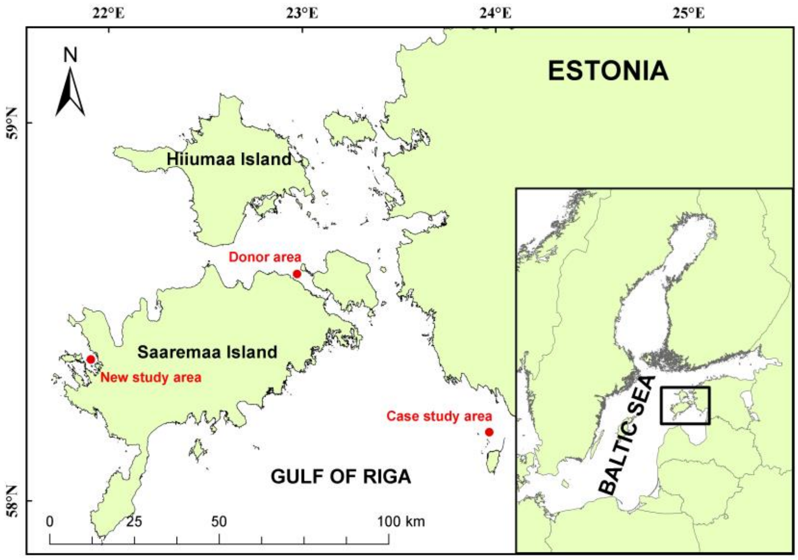

Figure 7. Location of *Zostera marina* habitat restoration activity in the Gulf of Riga, north-eastern Baltic Sea.

2.1.3. Description of the studied species

Zostera marina represents a subtidal marine species and is found to grow mainly on shallow sandy sediments within moderately exposed bays in the Baltic Sea. *Zostera marina* is considered a key species on the species-poor sandy bottoms of habitat (Kontula et al., 2012; Boström et al., 2014). In the north-eastern regions of the Baltic Sea, *Z. marina* is found to grow in its lowest observed salinity levels (salinity 5-7). Such levels may act to cause physiological stress for *Z. marina* communities and causes *Z. marina* to reproduce vegetatively, with flowering being a rare event. It has been documented that the decline in the distribution of *Z. marina* within the area is mainly a result of a reduction in underwater light as a consequence of eutrophication in the Baltic Sea (overview in Boström et al., 2014).

2.1.4. Methods used in restoration actions

As *Z. marina* only reproduces vegetatively in the case study area (northern Baltic Sea), restoration actions can be done via means of transplantation only. The restoration strategy involved transplanting *Z. marina* shoots from the donor area to the case study site. A first small-scale transplanting trial was carried out in July 2017 within an area 5 m^2 in size in the Gulf of Riga. A second trial was then conducted to test the success of the rope method in a 3-m^2 area in June 2018 in the Kihelkonna Bay. The rope method was utilized for the transplant experiment. In the lab, 10 shoots containing long rhizomes (rhizome length: 5-10 cm) were attached to a 1 m rope using cable ties (Figure 8a). Divers using SCUBA buried the ropes with containing the attached seagrass shoots under the sediment. The ropes were held in place by driving attached metal pins into the sediment at a depth of 3.0 m (Figure 8b). A density of 50 shoots per 1m^2 was achieved using this method. The transplant experiments trials were performed in five replicates for the Gulf of Riga and three replicates for the Kihelkonna Bay.

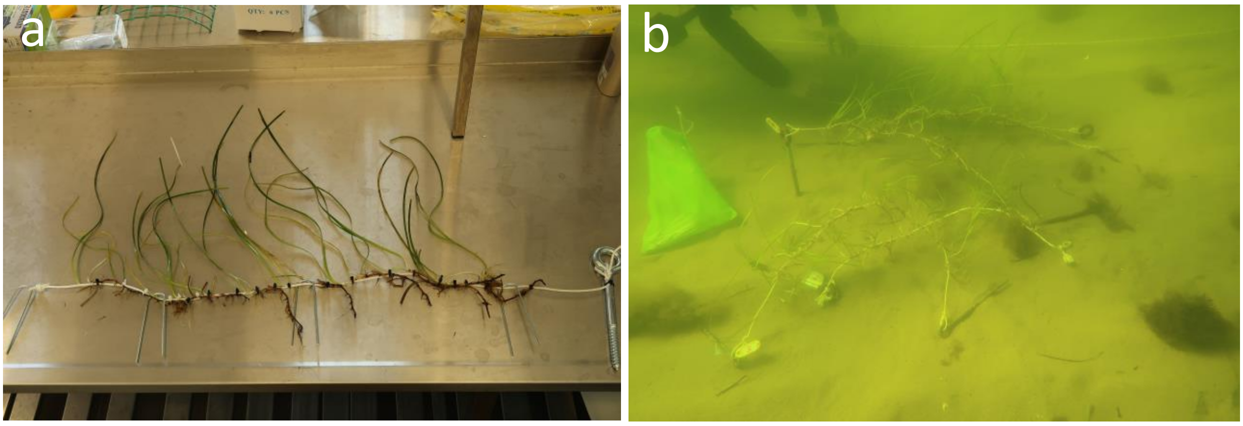

Figure 8. Seagrass *Zostera marina* transplantation with rope method. a) prepared in the lab, shoots of *Z. marina* with a long rhizome were attached to the ropes (photo by L. Pajusalu); b) the ropes with attached seagrass were buried under the sediment by divers using SCUBA at a depth of 3.0 m (photo by K. Kaljurand).

2.1.5. Restoration results from the case study site

Field trial I

First survey of the success of case study area took place at the beginning of November 2017. A 45% decrease in shoot density after the first growing season for the NE region of the Gulf of Riga was observed (Figure 9). This was deemed relatively successful for an exposed site. The experimental setup was left in place over the winter and visited again in June 2018. It was found that all ropes with seagrass had been buried under matts of dense drifting macroalgae (thickness ca 20-30 cm). No live *Z. marina* shoots were detected.

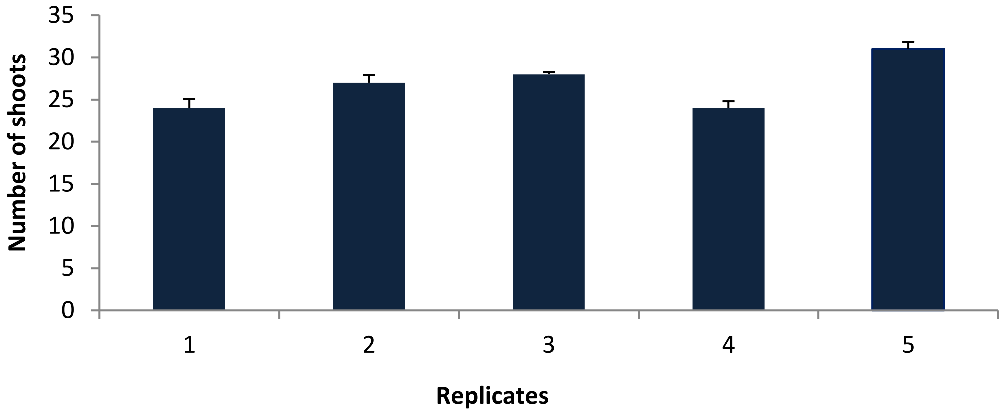

Figure 9. The number of *Z. marina* shoots observed after the first growing season in September 2017 in the Gulf of Riga case study site (± standard error, S.E. n = 50 in the beginning).

Field trial II

To test the success of the rope method of the new transplanting trial was carried out in June 2018 in the Kihelkonna Bay (less exposed site). Similarly, there was about a 40% decrease in shoot density after the first growing season in September 2018 (Figure 10). The experiment was left in place over the winter and visited again in spring. In May 2019, the shoot density of *Z. marina* had decreased a further 65% compared to autumn 2018. In September 2019, we found that most of the ropes were empty after the second growing season. However, there were two ropes where seagrass had expanded when compared to spring with the number of shoots increasing from 33 to 86.

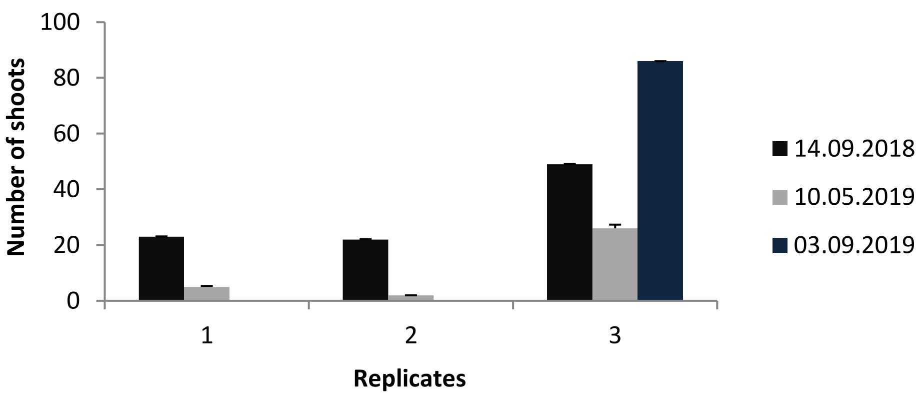

Figure 10. The number of *Z. marina* shoots for September 2018, May 2019 and September 2019 in the Kihelkonna Bay site (± standard error, S.E. n = 50 in the beginning).

2.1.6. Major issues/problems from the case study

The main reason for failure at the first case study site was likely due to drifting macroalgal mats (20-30 cm thickness) smothering the seagrass shoots. In summer 2018 large drifting algal mats were observed more frequently in the Estonian coastal waters than that of previous years, most probably due to an extraordinarily long and warm summer. In the Baltic Sea there is a large degree of seasonal variation resulting in variation in environmental factors between the years (e.g. harsh winter environmental conditions). Therefore, there are many uncontrollable factors for conducting experiments in this marine environment.

2.1.7. Relevant time scales for restoration

To reach high enough seagrass densities and aerial spread to support a self-sustaining population, it would be good practice to transplant *Z. marina* shoots every summer for about 4-6 years.

2.1.8. Conclusions

There was some success observed with the transplantation of *Z. marina* using the rope method in our case study site after the first growing season. However, no shoots were found to have survived the first winter, most probably due to the drifting mats of macroalgae. To test the success of the rope method, a second transplantation trial was carried out within a new site. Despite seagrass being lost from most ropes over the first winter, some ropes were found to have seagrass expansion after the second growing season, with the number of shoots increasing significantly since the initial establishment. To conclude, even if the majority of transplanted shoots do not survive, only a small number are required to survive in order to establish new seagrass meadow. Nevertheless, there is a need to test the transplantation of seagrass utilizing the rope method for longer periods of time before scaling up similar restoration activities.

2.2. Translocation of pen shell *Pinna nobilis* from disturbed site - Croatia (PMF ZAGREB)

2.2.1. Motivation for initiating the restoration measures

Coastal development, resulting in habitat loss or degradation, threatens many sessile marine species. To avoid smothering of the strictly protected noble pen shells during construction of a new nautical centre in the Pula Harbour (North Adriatic Sea, Croatia), the environmental impact assessment prescribed translocation of this bivalve as a conservation measure. Recognizing a valuable restoration case study for the EU Horizon 2020 project MERCES, http://www.merces-project.eu/), we approached the investor of the nautical centre, and as a win-win solution, organized pen shell translocation action assisted by local volunteer divers.

2.2.2. Description of case study area

Pula Harbour has been well known human port since ancient times due to its protected position (Figure 11). During the 20th century it was an important naval and military base. The area from which donor population originates was an active military base until the 1980s but was abandoned in the 1990s and has since then been neglected and used only for mooring by fishing boats. No cleaning or other maintenance has been performed in the area since then.

The donor site (Figure 11, orange circle) is shallow, mostly ~6 m deep (up to 11 m in some areas), covered with unconsolidated stones and litter interspersed with patches of muddy sand. Based on predominant size category of the individuals (most of them in the 40-45 cm length class), the pen shell population there was probably the result of only one or a few pulses of settlement. As previously noted, due to future building of a nautical port and danger of smothering, these pen shells had to be translocated to appropriate location.

Hence, we translocated them to the nearby Brijuni MPA (Figure 11) where their protection from adverse impacts of anchoring and illegal extraction could be ensured and monitoring of their survival could be performed. The selected host site already harbours a sparse *P. nobilis* population within seagrass bed, and it is located in the more sheltered part of MPA, not too exposed to hydrodynamism. The bottom covered by sand with small portion of mud is gently sloping towards south-south west.

Figure 11. A graphical presentation of a donor site where the noble pen shells lived (in the inner part of Pula Harbour) and host site to which they were translocated (Javorike Bay in Brijuni National Park), (photo by S. Kipson).

2.2.3. Description of the studied species

The strictly protected species *Pinna nobilis* (Linnaeus, 1758) is an endemic, long-lived Mediterranean species and one of the largest bivalves in the world, reaching up to 120 cm in shell length (Zavodnik et al., 1991). As a suspension-feeding habitat-former it provides important biogeochemical functions of water clarification and biodeposition, and enhances local biodiversity (e.g. Addis et al., 2009; Trigos et al., 2014; Rabaoui et al., 2015). Anthropogenic and environmental threats such as habitat loss or degradation due to intense coastal development, anchoring, trawling, illegal extraction and most recently, a rapidly spreading disease (see review in Basso et al., 2015; Vázquez-Luis et al., 2017) have contributed to the decline of its populations across the Mediterranean. This bivalve is listed as endangered under the 1992 European Council Directive on the conservation of natural habitats and wild fauna and flora (92/43/EEC, Annex IV). It has been protected by the Protocol for Specially Protected Areas Biological Diversity in the Mediterranean (Barcelona Convention: UNEP) since 1996. It is also strictly protected by national laws.

2.2.4. Methods used in restoration actions

We tested the translocation of *P. nobilis* as a previously suggested conservation action to protect the species (Katsanevakis, 2016; Bottari et al., 2017). Since its shells provide substrate for diverse epibiontic community, at the same time such an action can be also considered as a restorative measure for biogenic hard bottom habitat. Small trowels were used by SCUBA divers to carefully dig out pen shells from the sediment (Figure 12a), in order to avoid (aggressive) pulling that could damage byssus gland responsible for the production of byssus threads used for pen shell attachment to the substrate. Pen shells were collected in sub-divided plastic boxes, used for their transportation by divers while in the sea (Figure 12b). Until the last moment, pen shells were kept in the sea and were transferred to a larger boat at the time of departure (Figure 12c). Pen shells can survive very short time periods out of the water, as sometimes happens in shallow areas during low tides. On the boat, pen shells were placed in large tanks with a constant supply of fresh seawater (Figure 12d). We collected data on shell morphometry (total height and maximum shell width) for all rescued individuals. In the host habitat, we used a trowel or a metal rod to create holes in the sediment (Figure 12e) where we translocated pen shells by inserting anterior part of the shell, covering approximately 1/3 of the total shell height with sediment, as occurs naturally for this semi-infaunal bivalve (Figure 12f).

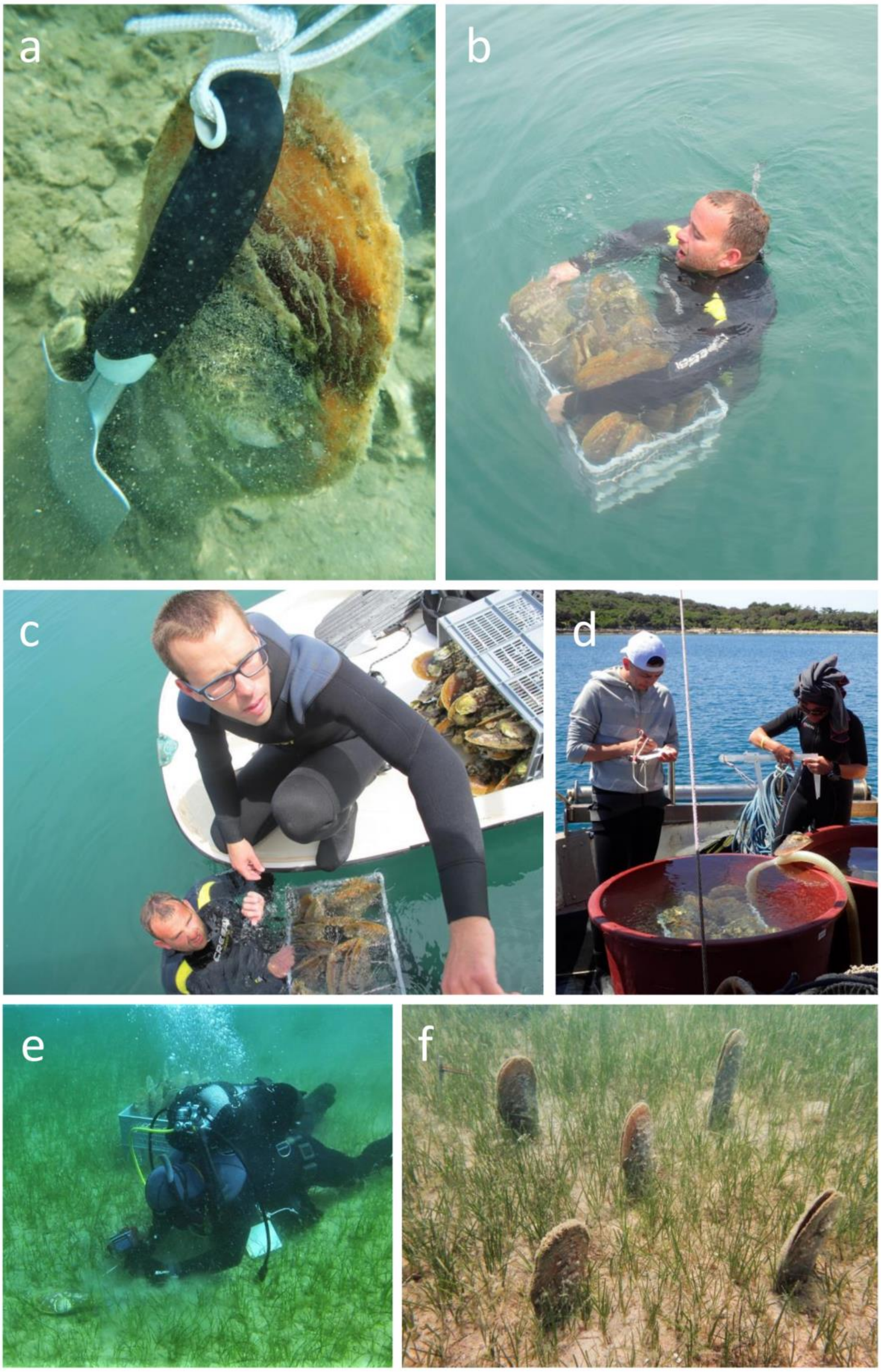

Figure 12. Translocation of the noble pen shell *Pinna nobilis* in the Northern Adriatic (Croatian coast): a) digging out pen shells using trowels in Pula harbour; b) volunteer diver transporting pen shells; c) transferring pen shells on board a bigger vessel; d) transporting pen shells in tanks supplied by fresh seawater and measuring them on board; e) translocating pen shells in a host *Cymodocea nodosa* meadow in Javorike Bay (Brijuni MPA); f) translocated pen shells in a host location. Photos a- c and e by S. Kipson, d and f by D. Petricioli.

2.2.5. Restoration results from the case study site

In June 2017, a total of 154 noble pen shells were translocated within the *C. nodosa* meadow at 3 sites in the nearby Brijuni MPA, at approximately 6 and 12 m depth. Two years post-translocation, their survival was very high – 86.4%, a result that is in line with estimated natural mortality of 7% per year (Katsanevakis, 2016). Of all dead individuals, half of them died immediately after translocation, probably due to associated stress and/or inappropriate handling. The shells of translocated individuals (both alive and dead) are being used as a substrate, shelter or foraging ground by numerous species typical of hard bottoms and not previously present within a host seagrass meadow. Hence, pen shell translocation increased local biodiversity.

2.2.6. Major issues/problems from the case study

The overall success of this case study stems from cooperation and understanding of all involved parties (the investor, governmental bodies, national park authority, scientists and citizen scientists-volunteer divers). However, several major points need to be addressed before, during and after translocation in order to secure its effectiveness.

Prior to translocation:

- Need for securing the funds necessary for the action.
- Selection of appropriate donor population (especially important during ongoing disease alerts, see conclusions below), giving priority to larger, less vulnerable individuals (> 8 cm shell width, Katsanevakis, 2016) and/or > 37 cm in total length, more likely to be already reproductive, Trigos et al., 2018).
- Selection of appropriate host location where pen shells' post-translocation survival would be enhanced. The best would be to select a location in which pen shells already live and preferably where there is a seagrass meadow e.g. *Cymodocea*; bare sand locations should be avoided as they could point to very strong hydrodynamism which could damage or dislodge translocated individuals. Additionally, selected sites should be devoid of adverse anthropogenic impacts such as anchoring, illegal harvesting, coastal construction.
- Selection of appropriate time of the year (sea and air temperatures should be similar during translocation process, and translocation individuals should have enough time prior to winter storms to re-grow byssus and firmly attach themselves to the bottom).
- Obtaining all the necessary permits, which can take considerable time.

During translocation and transfer of organisms:

- Careful manipulation/digging out of pen shells not to damage byssus gland and to preserve byssus.
- Continuous water aeration during transport of pen shells to new location.
- Similar sea water and air temperature during translocation process – to reduce stress on the pen shells
- Organised and well-trained diving team

After the translocation:

- Organisation of adequate monitoring (including securing necessary funds)

2.2.7. Relevant time scales for restoration

The immediate restoration goal was to ensure survival and growth of translocated specimens, both to protect the species and to provide biogenic hard substrate. The noble pen shell is a long living species and growth of adult specimens is slow. Therefore, we suggest a minimum of 5 years of post-translocation monitoring to confirm the steady survival rate (in the absence of extreme events) and to register the growth of the shell (concentric growth along the outer edge of the shell). In addition, to further ensure viability of the translocated population, the recruitment of juvenile pen shells in the area should be monitored.

2.2.8. Conclusions

Pen shell translocation was confirmed as an effective conservation method, resulting in high survival and increased function and services of the host habitat. Careful digging out of bivalves, not to damage byssus gland (as described above), proved to be critically important as failure to do so can result in subsequent pen shell mortality. In addition, an important aspect of increasing establishment success is to translocate pen shells during calm seasons (e.g. early summer) to allow for regeneration of byssus and appropriate pen shell attachment prior to winter storms.

Besides being the first official case in Croatia to implement translocation of a sessile marine species as a measure prescribed by the environmental impact assessment, this action additionally offered a compelling case for citizen science. Volunteer divers need to be educated about the major points in the process, as this can influence the success of translocation. In the light of recently reported pen shell mass mortalities due to a rapidly-spreading disease across the Mediterranean, every effort should be made to minimize more manageable impact in situ (e.g. of coastal construction, boat anchoring, trawling, illegal extraction) in order to support maintenance of its populations relying on survival of adults. However, given the current disease alert (September 2019), and as a means to mitigate the further spread of disease, we strongly advise against any noble pen shell translocation until concerns around the nature of the infected populations has been established. At the moment, greater knowledge on the factors involved in disease outbreaks is urgently needed in order to properly plan future conservation and restoration actions involving the noble pen shell.

2.3. Seagrass transplantation and monitoring - Italy (UNIVPM)

2.3.1. Motivation for initiating the restoration measures

The case of study selected by UNIVPM is located at Gabicce Mare (Central Mediterranean), a coastal site characterized by the co-occurrence of highly valuable natural resources (bordering a regional Park), seasonal touristic pressures (in summer) and anthropogenic structures (breakwaters aimed at preserving the beach). This site is characterized by the presence of three different seagrass species: *Z. marina*, *Z. noltii* and *C. nodosa*. UNIVPM has conducted pilot experiments of seagrass transplantation in Gabicce Mare since 2002. A breakwater relocation, whose management has at times involved temporary damage to seagrass beds, occurred in 2015. UNIVPM has set up new trials of seagrass restoration to test new techniques in the framework of the MERCES projects.

2.3.2. Description of case study area

Gabicce Mare is located in the north-western Adriatic Sea, near the “Parco del San Bartolo” and inside a Site of Community Importance of the Marche region (Italy) (Figure 13). The northern part of Gabicce Mare is limited by a touristic harbour and the Tavollo River, and the southern portion is confined by the rocky cliff, Monte San Bartolo, which gives the name to the Regional Natural Park created in 1997, to protect an area of great naturalistic importance (Colantoni et al., 2004). Gabicce Mare is characterized by important hot spots of benthic biodiversity and the presence of seagrass meadows of *Z. marina*, *Z. noltii* and *C. nodosa* (Coccioni, 2003; Balsamo et al., 2011). Gabicce Mare presents eroded Messinian deposits, mainly composed of sand and pelites (Tramontana et al., 2005 and references therein; Principi et al., 2011). Although this coastal area is characterized by lower primary productivity than the North Adriatic Sea (Zavatarelli et al., 2000), the contribution of local Apennine rivers and the nutrient enriched coastal current coming from the northern basin enhance its nutrient availability (Artegiani et al., 1997).

Figure 13. Location of the study area in the north-western Adriatic Sea (Central Mediterranean).

Along the coast of Marche region, important extractions of sediments were carried out in the 1960s and 1970s, and several harbours were built. As a result, a reduction of sediment flow was reported and several breakwaters and seawalls were placed to protect the coast from further erosional processes, typically occurring between the mouth of the Foglia River and that of the Metauro River, adjacent to the Natural Regional Park of Monte San Bartolo (Colantoni et al., 2004; Semprucci et al., 2010). In Gabicce Mare, the breakwaters are located ca. 100 m from the shore and parallel to the coast. The seawater circulation is mainly related to wave motion and its interaction with breakwaters, inducing local longshore and rip currents that control the sediment transport (Semprucci et al., 2010). The breakwaters were not highly effective in protecting the coastline of Gabicce Mare, which continue to erode. In addition, the water stagnation caused by the breakwaters caused hypoxic conditions in summer, reduced water quality and consequent low level of satisfaction of end users (i.e. tourists). Thus, to solve these critical issues, the breakwaters were relocated in 2015.

UNIVPM investigated the effects of breakwater relocation on benthic features and meiofaunal diversity (Carugati et al., 2018). To do this, a before–after comparison (2011–2017) was carried out since the relocation occurred in 2015. Sediment features and meiofaunal variables were altered by the relocation, especially in the areas colonized by seagrass meadows. Results of this study pointed out to an enrichment in organic matter, the loss of two sensitive taxa (*Cumacea* and *Ostracoda*) and a shift in the assemblage structure with the increase of the relevance of *Copepoda* and *Polychaeta*. These results indicate that the careful management of breakwater is crucial for planning adequate conservation practices and protecting seagrass habitats and their biodiversity (Carugati et al., 2018). During summer 2017 UNIVPM completed the mapping of the spatial distribution of the seagrass meadows in Gabicce Mare (Figure 14).

Figure 14. Spatial distribution of seagrass meadows in Gabicce Mare. Seagrass meadows mapped during summer 2017.

Seagrass meadows were composed of three species: *Z. marina*, *Z. noltii* and *C. nodosa*, as found in previous surveys carried out in 2011, before the breakwater relocation in 2015. Shoot density was measured in situ using 40 × 40 cm quadrats. Shoot density was 925-1925 shoots m^{-2} for *C. nodosa*, 269-1246 shoots m^{-2} for *Z. noltii* and 216-1093 shoots m^{-2} for *Z. marina*, respectively.

These values were comparable to densities reported in previous investigations carried out along the Italian coasts (Guidetti et al., 2001, 2002; Buia et al., 2003).

2.3.3. Description of the studied species

The species selected for this restoration project are *C. nodosa* and *Z. marina*. *Cymodocea nodosa* is one of the most common seagrass species of shallow-soft bottoms of the Mediterranean Sea. The leaves reach a maximum length of 40 cm during summer. This species is fast growing (up to 70 mm× day^{-1}) (Cancemi et al., 2002) and can spread vegetatively through the production of new shoots from the horizontal rhizome. This species flowers in spring (Buia & Mazzella, 1991). Also *Z. marina* is considered a fast-growing species with elongation rates that can reach about 10 mm×day^{-1} (Olesen & Sand-Jensen, 1993) during the summer season. This species can reproduce both sexually and asexually with rhizome propagation in the study area. In the intertidal zone, the production of seeds occurs annually (Figure 15a, b).

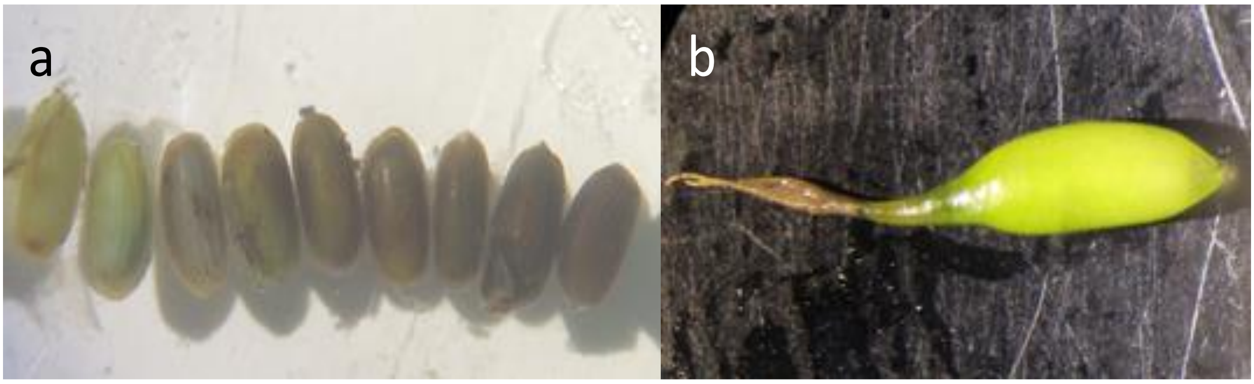

Figure 15. *Zostera marina* in different steps of reproduction. a) seeds of *Z. marina* in various maturation stages; b) a germinated seed (photo by Z. Da Ros).

2.3.4. Methods used in restoration actions

In May 2018 a new approach was tested for the restoration project. The seagrass transplantation was carried out using biodegradable bags inserted in biodegradable jars anchored with U-shaped stainless-steel rods (Figure 16a). A manual stainless-steel corer was used to dig a clod from the donor seagrass meadow. This clod was immediately inserted into a biodegradable bag to maintain the clod integrity. The biodegradable bag was included in a biodegradable jar to maintain the shape and consistency of the clod. The jar was then easily anchored with a U-shaped stainless-steel rod in the sediments. Several jars were planted in each 1m × 1m plot in order to cover the entire experimental plots.

Each jar contained 11-13 shoots of seagrass. Replicated experimental plots have been prepared as control plots in the bare sediments and in the original seagrass meadows (Figure 16b).

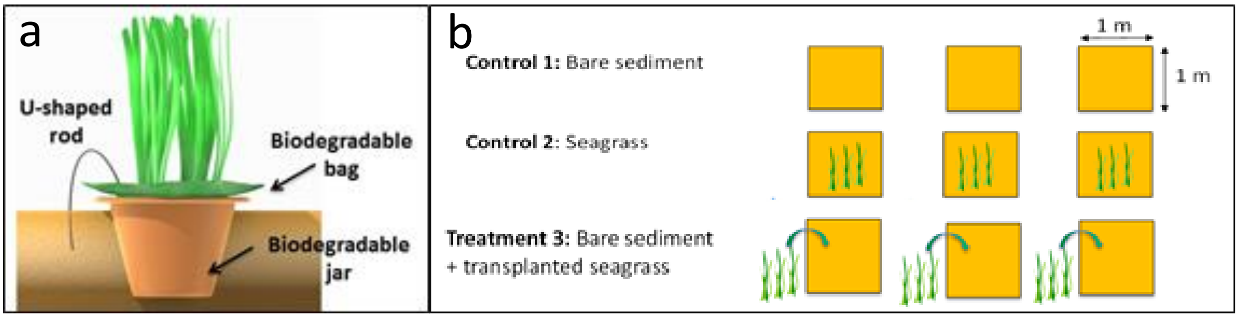

Figure 16. Seagrass transplanting in Gabicce Mare. a) seagrass transplantation based on biodegradable bags inserted in biodegradable jars anchored with U-shaped stainless-steel rods. b) experimental design with the replicated plots.

2.3.5. Restoration results from the case study site

Transplanted seagrasses are still present in the plots after more than one year after the beginning of the experiments (Figure 17a, b, c).

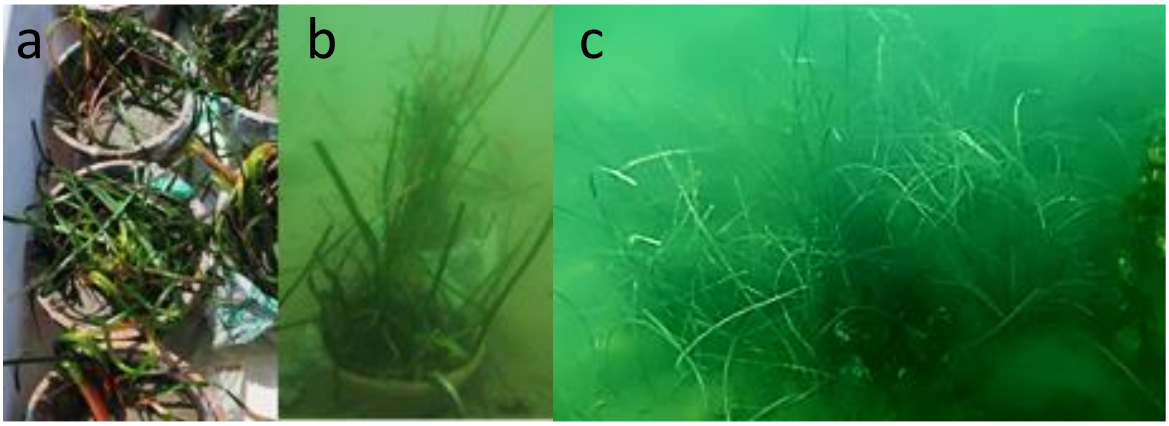

Figure 17. Seagrasses transplantation in Gabicce Mare. Seagrasses were taken from the donor meadow using a corer to dig a clod. a) the clods were inserted in biodegradable bags and jars; b) the jars were planted in the plots anchoring them with a U-shaped stainless-steel rod; c) one of the plots with transplanted seagrasses and transplanted bivalves (September 2018).

During the experiment (May 2018-July 2019), samples were collected for the analysis of the shoot density of the seagrasses in the transplanted plots, biomass of the seagrass (both from the existing meadows and the transplanted plots). Moreover, sediment samples were collected to quantify total phytopigments and biopolymeric carbon, to assess the potential effects of seagrass transplanting on the quantity of organic matter. Shoots density was lower in the experimental plots compared to that of the existing seagrasses, except in September 2018. Plots with transplanted seagrasses showed the highest density at the end of summer 2018, when the density of transplanted seagrass (619.7±271.4 shoots/m^2) was comparable to the existing meadows (874.1±134.4 shoots/m^2).

The temporal variability of shoot biomass followed the patterns observed for their density. Shoot biomass was lower in the transplanted seagrasses than in the other experimental plots with existing seagrasses. In September 2018, also biomass increased in the transplanted seagrasses (100.5±47.7 µgC /m^2), reaching values similar to those of the control plots (282.9±131.5 µgC /m^2).

We also investigated the availability of organic matter associated to existing and transplanted seagrass to assess the potential effects on the food sources for benthic fauna from May 2018 to February 2019. Total, phytopigments show a typical seasonal variability with higher concentrations in transplanted seagrass meadows in June and September 2018, at the beginning and the end of the summer period (Figure 18).

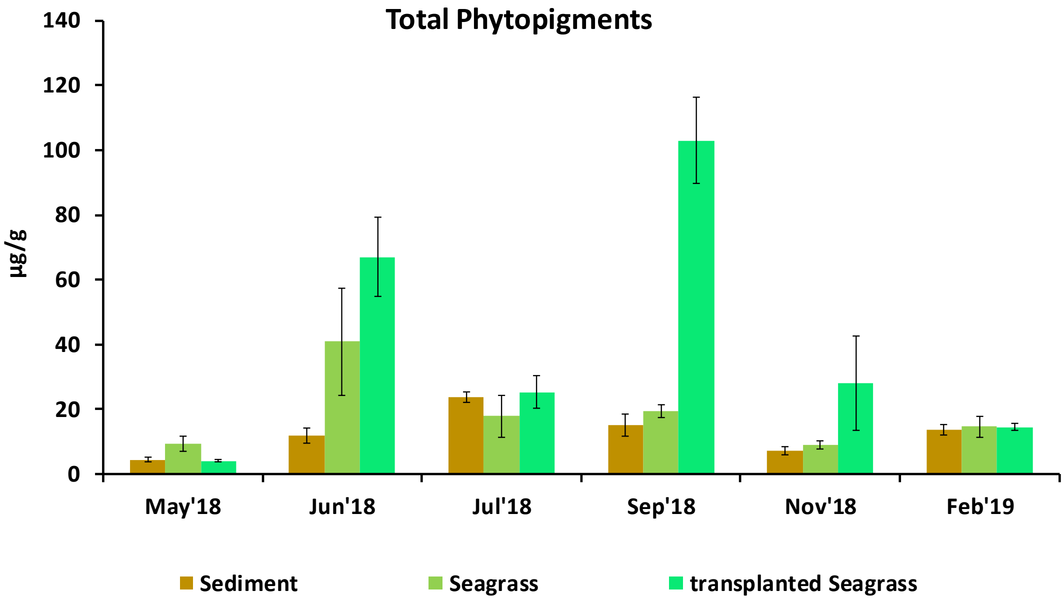

Figure 18. Temporal variability of total phytopigments in different experimental plots from May 2018 to February 2019.

The biopolymeric carbon showed high values in all experimental plots also in bare sediments suggesting a general enrichment of the sediments inside and close to seagrass meadows (both existing and transplanting) (Figure 19). Values reported here are generally higher compared to other coastal sediments (Mirto et al., 2010; Bianchelli et al., 2016) suggesting a high primary production, and thus high contribution of this production to the pool of organic matter available in the sediments and seagrass meadows. The organic matter availability can have a positive effect on benthic fauna associated to the experimental plots.

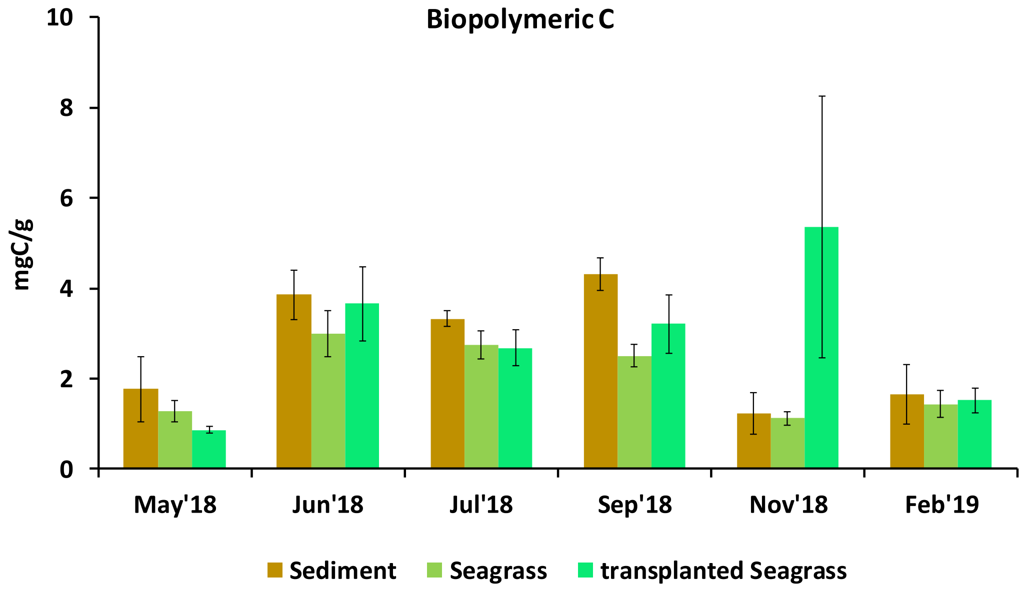

Figure 19. Temporal variability of the biopolymeric carbon in different experimental plots from May 2018 to February 2019.

Our results suggest also that the organic matter available in the transplanted seagrass is comparable to those observed in the existing seagrass immediately after the beginning of the experiments.

2.3.6. Major issues/problems from the case study

Despite the presence of breakwaters, the study of area of Gabicce Mare is subject to the occurrence of severe hydrodynamic conditions, especially during the winter. These events can compromise the survival and growth of the transplanted seagrasses. Good environmental conditions immediately after the transplanting can favor the settlement, maintenance and the vegetative growth of the underground rhizome of the transplanted seagrass. Thus, it is important to consider the timing of the restoration action. The study area is located close to a coastal area characterized by an important touristic activity during the summer and this can be an issue for the conservation of the experimental plots. However, a dedicated campaign to raise social awareness of the ecological importance of the seagrass habitats for coastal activities can support their preservation.

2.3.7. Relevant time scales for restoration

Transplanted *C. nodosa* and *Z. marina* require a certain time scale to anchor their roots to the new substrate, suggesting that the transplanted seagrasses can require a certain time scale to reach density and biomass comparable to those reported in existing seagrass meadows. Our previous experiences of seagrass transplanting in Gabicce Mare suggest that after a decade there are no differences between natural and transplanted seagrass meadows.

2.3.8. Conclusions

The method proposed here for seagrass transplanting appears to be successful. The use of biodegradable bags favors the preservation of the roots and their survival during the severe hydrodynamic conditions occurred in the winter period. The transplanted seagrasses show the same seasonal variability of shoots abundance and biomass as documented in the adjacent natural seagrass meadows. In addition, our experience suggests that the strict collaboration of civil society is a priority for the recovery and conservation of this important habitat.

2.4 Intertidal seeding method - the Netherlands (RU/NIOZ)

2.4.1. Motivation for initiating the restoration measures

Zostera marina meadows used to cover over 15 000 ha in the Dutch Wadden Sea, where they functioned as important habitats and nurseries for a vast array of marine species. The meadows mainly grew subtidally (~90%), but also covered vast intertidal mudflats. In the 1930s, most of the subtidal meadows vanished from the Dutch Wadden Sea due to the construction of the Closure Dam ("Afsluitdijk") and a concurrent outbreak of seagrass wasting disease. The intertidal populations survived longer, but eventually almost completely disappeared in the 1970-1980s, most likely due to eutrophication. Today only few small intertidal *Z. marina* populations remain, while subtidal seagrass has gone extinct in the Dutch Wadden Sea.

By restoring seagrass meadows in the Dutch Wadden Sea, we hope to kickstart the recovery of the whole damaged food web.

2.4.2. Description of case study area

The Wadden Sea is a shallow coastal sea, that extends from northwest Netherlands to the southwest coasts of Denmark. The Wadden Sea harbours the world's most extensive intertidal mudflat-system and is a hotspot for migratory birds and marine biodiversity. For its globally unique geological and ecological values the Wadden Sea is listed by UNESCO as “World Heritage”-site.

2.4.3. Description of the studied species

Common eelgrass, *Z. marina*, is a relatively large seagrass species that can be found in temperate waters in the Northern Hemisphere. Common eelgrass is very tolerant and can be found growing across a wide range of environmental conditions (e.g. salinity and turbidity). Despite its flexibility, common eelgrass has shown declining trends worldwide, mostly as a result of anthropogenic influences (e.g. eutrophication and overfishing). The species is mainly found subtidally but can also grow in the intertidal. In the subtidal, the species is perennial and mainly reproduces asexually. In the intertidal, common eelgrass is mostly annual. The survival of a population therefore heavily depends on seed production of the previous year – also because the seeds remain viable for only a single year.

Dwarf eelgrass, *Z. noltii*, is a perennial seagrass-species specialized to survive and thrive in the intertidal. The species forms extensive meadows, providing food and structure for a wide array of marine species and birds. Dwarf eelgrass has a wide distribution margin, spanning from Scandinavia to Mauritania. In the Dutch part of the Wadden Sea, *Z. noltii* can still be found at a few selected sites, where the plants form patchy landscapes with relatively low densities.

2.4.4. Methods used in restoration actions

The Dispenser Injection Seeding (DIS-method) is a novel seeding-method, specifically developed for seagrass restoration. With caulking guns, a seed-mud mixture is injected directly into the mudflat sediment, which greatly decreases seed displacement within the often-unstable sediments in the intertidal areas. Over the course of the MERCES project, the Dutch seagrass restoration group has continuously developed the method, yielding some very promising results (presented in section 2.4.5).

We collected the seeds late summer from Schleswig-Holstein, Germany, where seagrass is plentiful and has shown natural recovery during the last decades. Prior to seeding, the seeds are treated with a low concentration of copper-sulfate and stored in a cold and controlled environment overwinter. The copper-sulfate treatment kills a prevalent mold infection (*Phytophthora* spp.) that has been proven to reduce the fitness and germination rates of the seagrass seeds, while storing the seeds overwinter greatly reduces seed loss. Several days before seeding the seeds are soaked in freshwater, with the aim to cause a stress reaction that kick starts the germination process.

2.4.5. Restoration results from the case study site

The seagrass restoration experiments (2017-2019) in the Dutch Wadden Sea have been conducted at two sites (Griend and Uithuizen) (Figure 20, Table 1). Griend (53.263500, 5.289550) is a small uninhabited island in the middle of the Dutch Wadden Sea, surrounded by a large intertidal mudflat. Our restoration experiments have been conducted on the mudflat northwest of the island, that is relatively elevated (+30 NAP) and were the sediment is sandy. The second site, Uithuizen (53.465409, 6.681931), is located along the coast of Groningen. The experimental site is located ~300m from the dike and is characterized by muddy sediment. As the restoration results vary greatly between the sites, we will discuss the results separately per site.

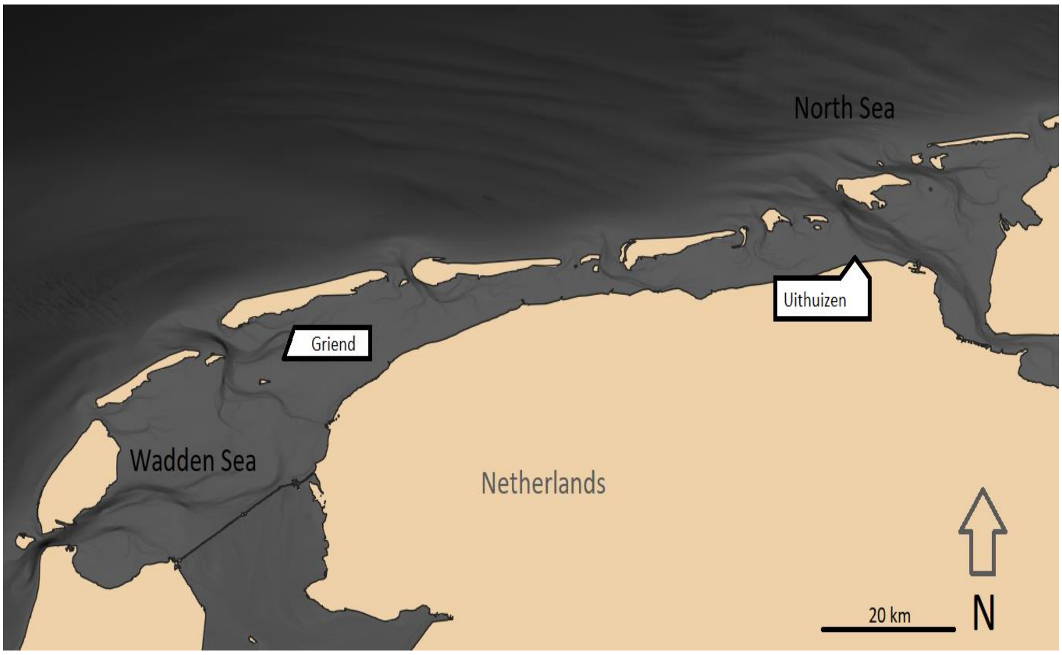

Figure 20. The Dutch Wadden Sea, with the two seagrass restoration sites highlighted.

Table 1. Overview of the seagrass restoration experiments conducted in the Dutch Wadden Sea 2017-2019.

	Griend	Uithuizen
2017	Seagrass seeding in the proximity of artificial and natural mussel beds. **Failed.**	First trial of the DIS-method. Effect of plot size and seed density. **Success.**
2018	Optimizing the DIS-method: Effect of Injection density, Seed density & Injection depth. **Success.**	Optimizing the DIS-method: Effect of Injection density, Seed density & Injection depth. **Failed.**
2019	*Zostera noltii* facilitation experiment & Elevation-gradient experiment. **Success** & **Success.**	*Zostera noltii* facilitation experiment. **Failed.**

Griend

The seagrass restoration efforts with the DIS-method started at Griend in 2017, when seeds were injected in the wake of artificial- and natural mussel beds. We hypothesized that the mussel structures would facilitate the introduced seagrass by decreasing sediment mobility and physical disturbances from waves. Although habitat suitability maps constructed a priori predicted that the selected site would be calm enough for seagrass settlement, this was unfortunately not the case. Even despite the presence of stabilizing structures, most seeds and plants washed away before they could germinate or produce seeds.

In 2018, the seeding experiments were conducted at a more sheltered site on the Griend mudflat, which resulted in immediate positive results. The 2018 experiment investigated how seeding depth (2 vs 4 cm), injection density (25 vs 100 injects/m^2) and seed density (2 vs 20 seeds/inject) affects restoration success (Figure 21, 22a, b). For the first time we managed to obtain plant densities >10/m^2, while significantly reducing seed loss (from >99% down to 94%) at the same time (optimal treatment: 100 injects/m^2, 2 seeds/inject & 4 cm injection depth).

Seed loss was significantly reduced by injecting the seeds deeper into the sediment and by using less seeds/inject. As expected, the higher injection density lead to higher plant densities in the experimental plots.

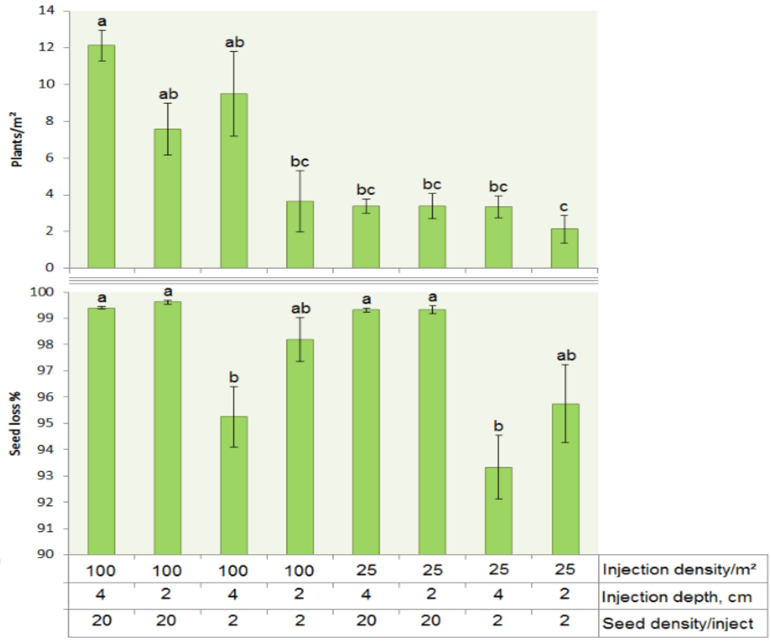

Figure 21. Differences in plants/m² and seed loss % between the different treatments of the 2018 seeding experiment at Griend. The small letters show significant differences between treatments.

After the success in 2018, two new seagrass restoration experiments were conducted at Griend in March 2019. With the first experiment, we aim to investigate potential facilitation mechanisms of *Z. noltii* on *Z. marina*. Observations in natural *Z. noltii* meadows in the Wadden Sea suggest that the smaller perennial seagrass may facilitate annual *Z. marina* in the intertidal by offering protection against desiccation and improve seed retention by stabilizing sediments. To investigate these potential facilitation mechanisms, we constructed *Z. noltii*-mimics by tying dried Raffia leaves to BESE-elements. With the mimics we aimed to create pool landscapes similar to natural *Z. noltii* meadows, that provide increased moisture for *Z. marina* during low tide and enhance seed retention of common eelgrass, thus increasing the survivability of *Z. marina* seeded in the vicinity of the mimics. The experiment is still ongoing, but early results suggest that the landscaping effects of *Z. noltii* can indeed offer common eelgrass protection against desiccation. In some of our experimental plots, very high plant densities (~60 plants/m²) emerged during the season, which further proves the DIS-methods suitability for intertidal seagrass restoration.

With the second experiment we aim to study how seagrass restoration success and plant survival differs along an elevation gradient. Twelve experimental plots (52m² plots) were constructed and seeded at 4 different elevations (+30, +10, -10 & -30 NAP) on the mudflat. Six BESE elements ($3m^2$) were placed in each plot, to investigate whether sediment stabilization by BESE would increase restoration success. The experiment is still ongoing, but early results suggest that restoration success was greatest at the highest elevation, since a majority of the seeds washed away or got buried at lower elevations. The BESE elements do not appear to affect restoration success here.

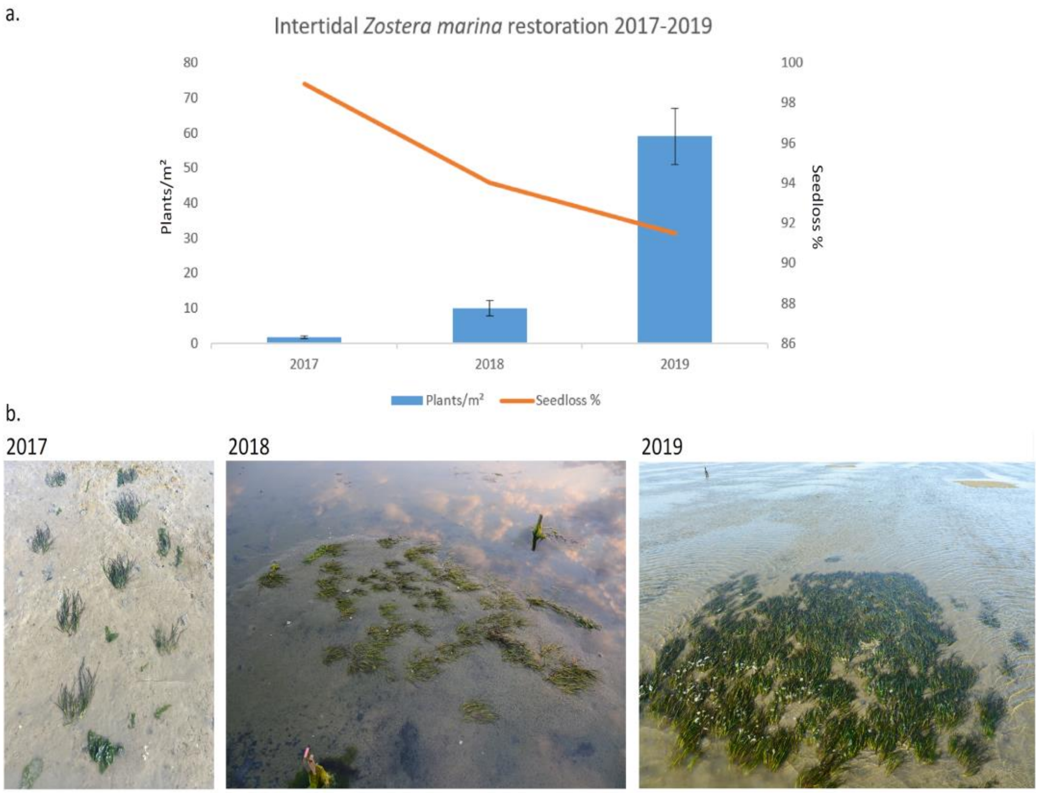

Figure 22. a. Shows the positive development of the restoration efforts in the Dutch Wadden Sea. Great increases in plant densities have been achieved while simultaneously reducing seedlosses. b. Pictures of the experimental plots with highest plant densities for each year, Uithuizen 2017, Griend 2018 & 2019 (photos by L. Govers).

In July 2018, we noticed that seeds had dispersed and germinated from the experimental plots over a 30 ha area on the Griend mudflat. After mapping the contour of the area, we estimated the number of plants inside the area with Batcheler-corrected point distance-method. We estimated ~10 000 adult plants inside the area, which corresponds to ⅓ of the total plants of the largest natural *Z. marina* meadow (Hond en Paapzand, 53.407780, 6.917032) in the Netherlands. In August 2019, the same procedure was repeated at the Griend mudflat. The area where plants were found had grown significantly (~100 ha) in a year and the plant numbers had also more than doubled on the mudflat, as we estimated over 25 000 plants in the area. This most likely means that the plants seeded in 2018 produced viable seeds that survived the winter and germinated in 2019. In fact, we presume that the majority of the population-growth on Griend originates from seeds produced in 2018, since in 2019 seed loss from the experimental plots was low.

Additionally, in 2019 the DIS-method was used for the first time for *Z. noltii* restoration. The dwarf eelgrass seeds were collected in August 2018 and seeded with the DIS-method in March 2019. Plants emerged early July and quickly established small patches, highlighting the potential of seed-based restoration also for the smaller eelgrass species. The survival and expansion of the introduced patches will be monitored in the coming years and additional larger scale experiments are being planned. The DIS-method might provide a valuable tool for large scale intertidal *Z. noltii* restoration, as seeding is potentially cheaper and logistically more feasible than previously used transplantation methods.

Uithuizen

The first DIS-experiment at Uithuizen was also conducted in 2017. We investigated, besides the viability of actual method, how plot size (20 vs. 200m²) and seed density (2 vs. 20 seeds/injection) affect restoration success of *Z. marina* in the intertidal. The experiment resulted in 100x higher plant densities (optimal treatment: ~1,8 plants m²) than any of our previous experiments performed before 2017 with other restoration methods. As expected, plots injected with higher seed densities produced higher plant densities, but also significantly higher seed loss rates. We found no effect of plot size on plant densities.

After the success in 2017, experiments were constructed again at Uithuizen both in 2018 and 2019, but with meager results to show. In 2018, a fair amount of seedlings (>5 plants/m² in the best treatment) emerged in our experimental plots, but most plants washed away in June and thus did not mature and produce seeds. In 2019, 99.99 % of the injected seeds either washed away or were buried. The problem seems to be that storms had hit the otherwise sheltered area rather hard, disturbing the mudflat too much for seagrass/seeds to survive. This also suggests that establishing a self-sustaining population is most likely not possible at Uithuizen, because seed retention is limited and inconsistent from year to year. Still, since the seeds germinated and the seedlings initially established in the muddy sediment, we believe that the DIS-method also has the potential to work in muddy environments, provided that the area is otherwise suitable.

2.4.6. Major issues/problems from the case study

Intertidal mudflats are harsh environments typified by strong variation in conditions driven by the tides and seasons. This is particularly the case for *Z. marina* that follow an annual growing strategy in these stressful conditions (i.e. sediment instability, low-tide desiccation). Consequently, it is vital to select areas most suitable for restoration in order to maximize chances of success. Selection of suitable sites, however, turned out to be rather difficult. Certain experiments during the MERCES project were placed in areas that predictive modelling had classified as suitable, while in reality these areas turned out to be highly unsuitable (high physical disturbance/sediment movement) for seagrass. Hence, the difficulties we experienced clearly highlight the importance of accurate suitability maps as a first step for successful seagrass restoration.

2.4.7. Relevant time scales for restoration

Although we have been able to reintroduce large plant numbers for two years in a row at Griend, we still do not yet know what the minimum viable population size will be at the longer term, and thus many successful seeding seasons will be required to establish a self-sustaining seagrass population. At present, we are therefore following the developments at Griend closely and aim to seed seagrass of both species at the location in the years to come.

2.4.8. Conclusions

Our results suggest that the DIS-method is very suitable for intertidal seagrass restoration. Through an iterative process of applying and adapting the seeding technique we have been able to greatly increase plant densities and reduce seed loss compared to previously used restoration methods and by upscaling the restoration effort beyond an experimental setting, the plant numbers could be substantially increased. Our method paves the way towards establishing self-sustaining meadows in the future. The DIS-method has shown potential on both muddy and sandy intertidal areas, but it does not perform well at exposed sites with unstable sediment. The method could potentially also be used successfully in the subtidal, but this needs further investigation.

2.5. Seagrass *Zostera marina* transplantation and aeration of sediments - Norway (NIVA)

2.5.1. Motivation for initiating the restoration measures

The seagrass beds in inner Viksfjord in southern Norway (Figure 23) are of national importance. Inner Viksfjord also includes valuable shallow un-vegetated soft-bottom habitats of importance for a rich bird life. The area has had status as a nature reserve since 1981 aiming to preserve an important wetland area in its natural state and to protect an especially rich and interesting bird life, vegetation, and other wildlife naturally associated with the area. Some part of the area, on land as well as in the sea, has since 2006 been protected due to landscape values to preserve a coastal area with a beautiful and unique natural and cultural landscape with the biological diversity that characterizes the landscape.

Figure 23. Location of inner Viksfjord (Varildsfjorden, marked by a red circle in the photo) in southern Norway. The location of Viksfjorden within Europe is shown by the red rectangle in the map of northern Europe (photo by G. Sider, Europe map - Gebco).

Annual blooms of green algae (mainly *Cladophora spp*.) threaten the important marine habitats and the rich birdlife, as well as greatly reduce the user value of the area for humans, particularly when floating algal mats occur (Figure 24). The green algal mat reduces the light conditions and the seagrasses ability to perform photosynthesis. Furthermore, the decomposition of the algae leads to oxygen hypoxia on the seabed and to reduced marine biodiversity and available food for birds and fish. Hence there is a need to test actions that can improve the ecological status of these marine habitats.

Figure 24. Floating green algae mats in inner Viksfjord (photo by G.E. Piene).

A mitigation project was funded in 2012 by the Norwegian Environmental Directorate through the County Governor of Vestfold, based on an application to the County Governor from Indre Viksfjord Association (IVIV), a voluntary organization of local interests. The aim of the project was to preserve the *Z. marina* seagrass beds in Viksfjord. The proposed measures were described in an action plan for the management of the Viksfjord area, including removal of floating green algae mats that bind a large quantity of nutrients, as well as actions to improve the water exchange rate. Since 2014, the seagrass beds have been monitored seasonally every year, to assess the effects of the various actions (Figure 25). The under-water registrations (photo and video filming) have been performed by IVIV in close collaboration with NIVA.

2.5.2. Description of case study area

Inner Viksfjord is located in southern Norway, in a very sheltered part of a larger fjord that extends through an archipelagic area out into Skagerrak. The total sea area of the fjord section is 494 000 m^2. It is surrounded by farmland, which also constitutes the main source of nutrient supply. Inner Viksfjord has been significantly influenced by particles from a nearby stone industry. However, imposed requirements for process water purification since 2014 have reduced the discharges of stone particles to the fjord. The seagrass bed in the area was mapped in 2008 and its total area was measured to be 67 500 m^2.

Figure 25. Hartvig Christie and Ivar Trondsen are preparing to monitor the ecological state of the *Zostera marina* bed in Viksfjorden, by diving. Jan Strand is driving “Slikken”, a vehicle developed by AGN Skjærgårdstjenester AS (photo by G.E. Piene).

2.5.3. Description of the studied species

The general biology and characteristics of *Z. marina* is described in sections 1.5; 2.1.3; 2.3.3 and 2.4.3. In Viksfjorden the maximum length of the leafs are 1 m, but leaf length varies within different parts of the fjord.

2.5.4. Methods used in restoration actions

Through the Viksfjord project, there has been a regular harvesting of green algae mats in the summer months, from 2012-2018 (Figure 26). In total, 2 700 tons of green algae have been harvested, corresponding to 2.4 tonnes nitrogen and 300 kg phosphorus. There has been a large annual variation in the amount of green algae harvested (Figure 27), probably related to annual variation in both temperature and wind conditions (high temperature stimulates algae growth and formation of oxygen bobbles that lift the algae upwards to the surface; wind direction and speed influence whether the algae mats accumulate or not along the deployed booms that are arranged to gather the mats to ease the uptake process. The booms are shown in Figure 26. Warm summers, little rain and weak south west wind, as well as a high proportion of moderate winds from the northeast, seemed to create appropriate conditions for successful green algae harvesting. The self-developed craft, "SLIKKEN", was built in 2014 by AGN Skjærgårdstjenester AS, to ease the transportation of the mats to the uptake location. This passive restoration effort has been accompanied by testing aeration of the sediment as a measure to improve the water quality within the bay. “SLIKKEN” has been used to deploy the boom, to perform the aeration of the sediments, and as a base for diving.

Figure 26. “SLIKKEN” in action, gathering the floating algae mats that have been trapped by booms. An excavator has been used to harvest the green algae to containers on land (photo by G.E. Piene).

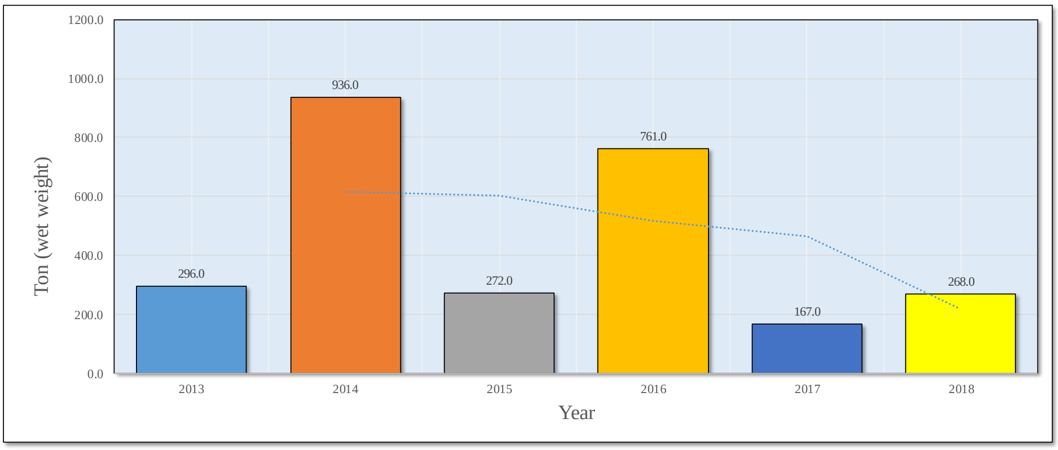

Figure 27. Annual uptake of the green algae mats (tons wet weight). A trendline based on moving average is shown. (Source; the annual report of the project; https://www.indreviksfjordvel.no/dokumentarkiv/arsrapporter/2017-2-2/)

The aeration has been done in an area without seagrass plants, to avoid destruction of the seagrass beds. A sledge was built for injecting oxygen into dead and non-vegetated areas close to the seagrass beds, to improve the ecological condition of the sediment and hopefully also for the nearby seagrass bed. The first tests of using the sledge was performed 3. Oct 2016. In total ca 300 m^2 was aerated within 3 hours. NIVA sampled sediment and water to assess the water quality and the sediment conditions at the site before (23 Sept 2016) and after the test (21 Oct 2016). Redox measures of sediment core samples were also taken.

On behalf of the project, Akvaplan NIVA performed hydrodynamic simulation studies of the water exchange rate within the fjord. The studies pointed out a significant degree of back and forth pumping of the water masses through the sound; 63% of released particles passing out at descending water level was returning at the next inflowing tide. The model was further used to explore different solutions to increase the water exchange rate, including removing bridge structures, dredging and developing a tidal port (https://www.indreviksfjordvel.no/dokumentarkiv/faglige-rapporter/akvaplan-niva/).

Within MERCES, NIVA has tested survival of transplanted seagrass plants with and without blue mussels and using plastic net to anchor the plants, as well as biodegradable structural engineering units (BESEs) (developed by BuWa/NIOZ). The first tests using plastic nets were done in 2017. Then 16 shoots were attached to 20 x 20 cm plastic nets. We transplanted 3 seagrass frames at the aerated site 25. June 2017. Later (7-8 July 2017) we transplanted 8 frames with seagrass shoots; 4 at the aerated site, and 4 at an area without aeration. The three previously deployed frames at the aerated site was then buried below a dense mat of filamentous algae, with a high abundance of tiny snails and mussels. The seagrass beneath the algae was still green.

2.5.5. Restoration results from the case study site

The redox measurements indicated at least a short time improvement of the oxygen conditions in the sediments. The sediments had a bad ecological state with respect to organic content (more than 100 mg/g of organic matter), and mainly redox potential of about -300 on 0-1. 5 and 10 cm down in the sediment. After aeration we got redox values around 0 and even positive values in the upper layer, but still at a level of -300 deeper in the sediments. However, the rapid growth of the green algae resulted in a short duration of the positive sediment conditions. Sampling of seagrass plants nearby, and studying the number of associated animals, indicated that the process of aerating did not have a negative impact on the nearby fauna. The latter could have been a result of released H_2S.

After four months, five out the seven mats deployed at the aerated part were gone, probably removed during the process of aeration (although the location of the sites had been reported to managers of IVIL). At the two remaining plastic nets, 30% of the shoots had survived, but the covered area had become reduced (60% of the original area). At the area without aeration, all nets were in place, and 40% of the shoots had survived, occupying a smaller area compared to when planted (i.e. 45% of the original area). After 16 months none of the seagrass shoots had survived.

On request of the local restoration project NIVA mapped the seagrass bed in inner Viksfjord in 2017. The mapping showed an enlarged seagrass area compared to the mapping in 2008, covering in total 26.1 ha, with a mixture of *Z. marina* and *Ruppia* sp plants. It is reasonable to think that the measures with uptake of turf algae has contributed to this substantial increase. However, due to the lack of a control area (we do not have seagrass beds in similar enclosed areas in this region) that can be used to conclude how the development would have been without the measure, we lack a scientifically sound evidence of this link.

The local restoration project lost its funding from the County Governor from 2019, since seagrass is no longer on the list of specially selected habitats in Norway, and because seagrass was not categorized as threatened on the updated version of the Norwegian red list (published in November 2018). NIVA plan to repeat mapping of the seagrass bed in October 2019, to check any deterioration / shrinkage of the meadows area as a possible response to the stop of the mitigation actions.

2.5.6. Major issues/problems from the case study

The only available area without seagrass plants within the area, providing the possibility to transplant seagrasses without clearing an area for seagrasses, was just outside the margin of the existing seagrass beds. Although, the lack of seagrass plants indicated that the conditions for the seagrass plants would not be optimal in this test area, we chose to this option to avoid destroying any part of the existing meadow. The sediment and depth of the test area was similar to other parts of the fjord where seagrass occur.

Because of the deep and very soft, muddy sediment, the visibility is reduced immediately when transplanting or doing measurements of the deployed plants within the area, thus working conditions can be difficult. Although we communicated well with the local restoration project, some of the nets were probably removed accidently by the team operating the aeration system in the first transplantation experiment.

2.5.7. Relevant time scales for restoration

The short-term survival of the sea grass shoots was relatively high for both experiments, however, none (using plastic) or only 2% (using BESE units) of the *Z. marina* plants survived over winter. We did not test if the timing of transplanting the seagrasses would influence the survival rate, e.g. whether transplanting in spring could have resulted in better anchoring or in other ways increasing the survival rate of the plants.

2.5.8. Conclusions

The local restoration project in inner Viksfjord was made possible due to; 1) a highly visible problem - the floating green algae mats, being a nuisance to recreation , 2) the green algae's threat to the seagrass bed, the saltmarshes and a rich bird life, 3) public funding of actions aiming to protect or improve the environmental conditions for seagrass beds. The restoration project has successfully removed large amounts of green algae biomass (2700 tons in total) and hence nutrients (including 2.4-ton nitrogen), improving the environmental conditions for the seagrass. Needed craft and solutions to gather the green algae and to pump air into the sediment have been developed. The aeration improved the oxygen condition in the sediment, although the effect was short-term. Through hydrodynamic simulation studies, several solutions to increase the water exchange rate and solutions to improve the ecological conditions have been explored. Evaluated solutions include removing bridge structures, dredging, and development of a tidal port. Because the public grant was stopped/reduced in 2018, the restoration project has been put on hold in 2019. The reduction in funding happened because seagrass beds in Norway was evaluated to be vital (i.e. red list category LC, least concern) in Nov 2018. The project has developed tools and approaches that can be used by others in similar conditions / circumstances.

The seagrass transplantation experiments had a short-term success (4 months), but almost none of the transplanted seagrass plants survived the winter season. It could be that the density and the area extension of the transplanted sea grasses were too small to be successful. A larger patch size or higher plant density might be needed for the sea grass plants to become sufficient anchored into the sediment, and to create the needed facilitation of the environment to survive the winter season. Timing of the transplantation should also be tested.

3. DISCUSSION – Key messages from the case study sites

3.1. Outcome from different case study sites

Valuable marine habitats are being lost at unprecedented rates due to multiple anthropogenic impacts. Thus, the restoration of vegetated marine habitats is seen as an essential part in the field of environmental conservation. MERCES WP2 of D2.2 gives an overview on restoration results from the case study sites found in the northern Baltic Sea, Skagerrak region, Dutch Wadden Sea, and Central Mediterranean Sea located in northern and southern Europe respectively. Within the case study sites, different restoration methods were utilised as a result of differing aims, motivations, species-specific requirements and site-specific conditions. Until now, the success rate of seagrass meadow restorations has been relatively low worldwide. Despite this, within focused MERCES case study sites we found that in most cases restoration attempts were quite successful. However, some methods used in seagrass restoration actions had only a short-term success and thus, there is a need for the further evaluation of these methods over longer periods of time.

The seagrass transplantation using biodegradable bags was found to be successful in Gabicce Mare (north-western Adriatic Sea). Transplanted seagrass survived over the winter, enduring harsh hydrodynamic conditions. Transplanted individuals are still present in the plots even after more than one year from the experiment being established. In the Dutch Wadden Sea, the dispenser Injection Seeding (DIS-method) was found highly suitable for intertidal seagrass restoration, as a result greatly increase plant densities and reduce seed loss compared to previously used restoration methods. In the northern Baltic Sea, there was some success observed with the transplantation of *Z. marina* using the rope method after the first growing season. However, the transplanted seagrass did not survive over the winter. In the second attempt to transplant seagrass, the overall shoot density decreased but at the same time on some ropes the number of shoots increased significantly. Consequently, there is a need for the further evaluation of seagrass transplantation using the rope method over longer periods of time before scaling up similar restoration activities. In the Skagerrak region, the seagrass transplantation experiments had only a short-term success. As in the Baltic Sea, transplanted seagrass did not survive over the winter. Translocation of *P. nobilis* within a conservation action to protect this species was also successful in the Pula Harbour (north-eastern Adriatic Sea). Results showed quite high transplant survival (86.4% of survival rate after two years) and increased function and services of the host habitat.

3.2. Best practices

One of the more significant findings was that the novel seeding-method (DIS-method) is very suitable for both muddy and sandy intertidal seagrass restoration that tested in the Dutch Wadden Sea. Through DIS-method a seed-mud mixture is injected directly into the sediment, which greatly decreases seed displacement in unstable sediments. This method was found to greatly increase shoot densities and reduce seed loss compared to previously used restoration methods. Further, study showed that the seeding depth is also an important as seeds injected at 4 cm depth produced significantly more plants compared to the surface at 2 cm depth. By scaling up similar restoration activities, the number of shoots could be substantially increased. Additionally, the DIS-method has, in part, acted to further our goal of enhancing self-sustaining seagrass meadows of the future. This method could potentially also be used successfully in the subtidal areas, but this needs further verification.

The new seagrass restoration method was found to be successful in the Mediterranean Sea. Transplantation of seagrass was carried out using biodegradable bags inserted in biodegradable jars anchored with U-shaped stainless-steel rods. Transplanted seagrass survived over the winter, enduring harsh hydrodynamic conditions and the individuals are still present in the plots even after more than one year from the experiment being established.

Translocation of pen shell *P. nobilis* was confirmed as an effective conservation method in the Mediterranean Sea. The study showed a high rate survival of *P. nobilis* in seagrass beds, and as a result of increased local biodiversity. One important aspect is carefully digging out of pen shells to avoid damaging to the byssus gland during the restoration activities. It is also important to pay attention to the time of translocation, i.e. to translocate pen shells during calm seasons to allow for regeneration of byssus and encourage their attachment prior to winter storms.

Transplantation activities of *Z. marina* in the Baltic Sea and Skagerrak region had a short-term success. There is a need for the further evaluation of these transplantation methods over longer periods of time, and at larger patch sizes, before scaling up these restoration activities.

3.3. Lessons learned

During the restoration studies we identified a range of factors causing failure of the restoration activities. Together these provide lessons learned that should be taken into account to improve the success rate of restoration of marine, shallow soft bottoms habitats in the future. The lessons learned from five MERCES case study sites depended on the geographic location, local environmental conditions and the methods used. An overview of causes of the failures for each case study site is presented in table 2.

Table 2. Lessons learned from the different case study sites and among the studied species within restoration actions.

Lessons learned	**Studied species and case study sites**						
	Zostera marina in northern Baltic Sea	*Z. marina* in central Mediterranean Sea	*Z. marina* in Skagerrak region	*Z. marina* in Dutch Wadden Sea	*Z. noltii* in Dutch Wadden Sea	*Cymodocea nodosa* in central Mediterranean Sea	*Pinna nobilis* in central Mediterranean Sea
Seagrass loss due to drifting algal mats	+		+				
Negative effect on seagrass due to harsh hydrodynamic conditions		+				+	
Negative effect on seagrass due to bad local environmental conditions			+				
Negative effect on seagrass due to seasonal touristic pressures		+				+	
Seagrass overwinter survival is low	+		+				
Seagrass loss due to summer heat wave	+						
Seagrass seed loss due to high hydrodynamics in intertidal mudflats				+	+		
Pen shell mortality due to inappropriate handling							+
Threat to translocated pen shells by a fast-spreading disease							+

3.4. General conclusions

The restoration of marine ecosystems is a developing field, particularly as global changes have increased the need to restore damaged and degraded habitats. Overall, our results showed that within focused five MERCES case study sites in most cases restoration attempts were quite successful. The success of *Z. marina* meadow restoration varied across European coastal systems, depending on geographic location as well as the restoration method used. Local environmental conditions are of key importance to increasing the success rate of soft bottom marine habitat restoration attempts. The novel seeding-method 'DIS-method' provides a good example of this as it is a method that can be used to restore intertidal seagrass meadows. The DIS method was found to greatly increase seagrass shoot densities as well as reducing seagrass seed loss. Thus, by upscaling restoration efforts beyond an experimental setting, plant numbers could be substantially increased. Additionally, the DIS-method has, in part, acted to the further proposed aim of enhancing self-sustaining seagrass meadows of the future.

Pen shell *P. nobilis* translocation was confirmed as an effective conservation method, resulting in high survival of individuals and as a result increased local biodiversity. Thus, restoration of marine habitats is an essential part in the field of marine species conservation. An important aspect is to incorporate different stakeholders such as local communities, funding organisations, governmental bodies, scientists, citizens and volunteers into restoration projects. One of the key challenges for conducting experiments within the marine environment is that there are many uncontrollable factors that may reduce the success of restoration actions and initiatives.

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