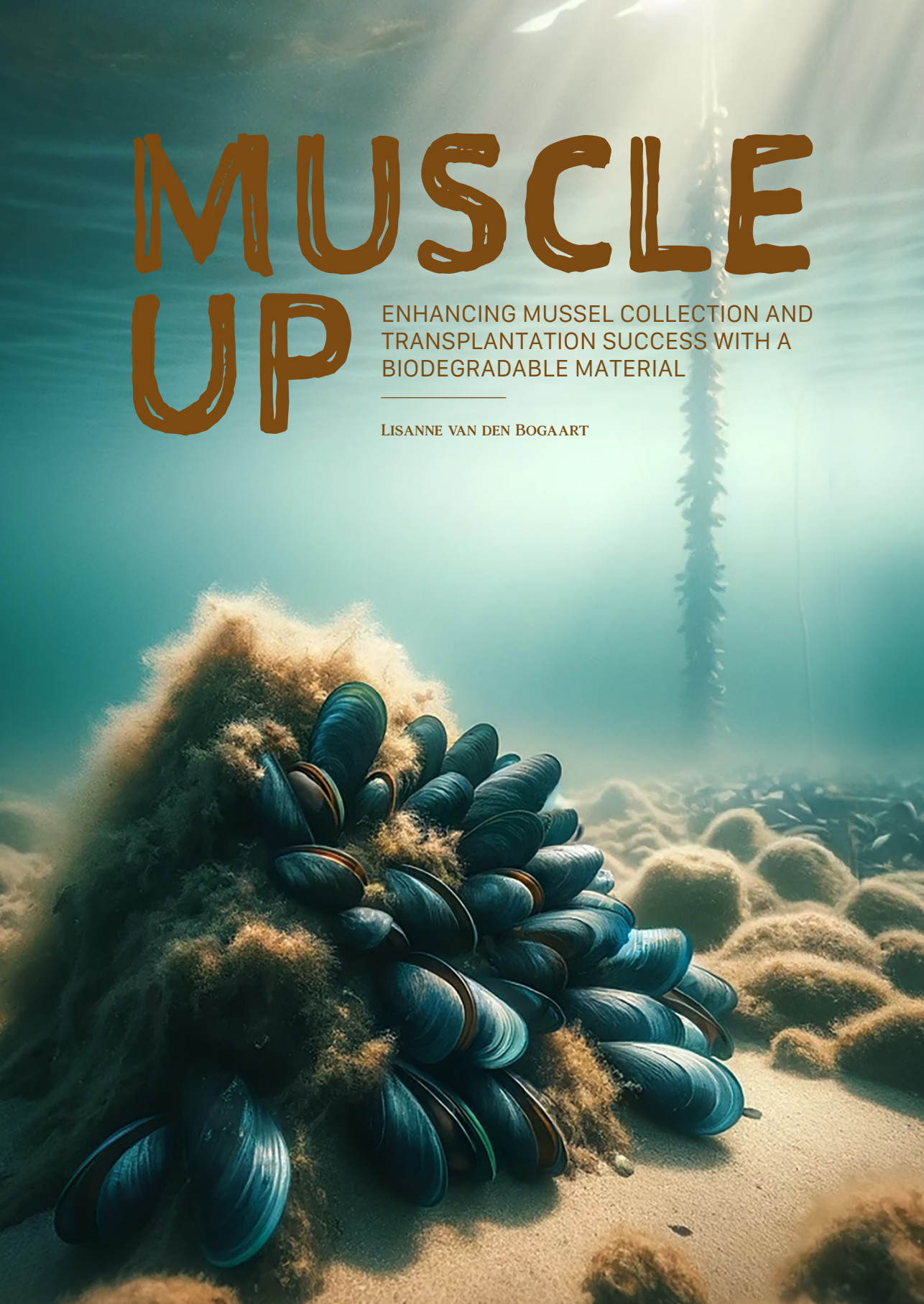

MUSCLE UP

ENHANCING MUSSEL COLLECTION AND TRANSPLANTATION SUCCESS WITH A BIODEGRADABLE MATERIAL

Lisanne van den Bogaart

Muscle-up: enhancing mussel collection and transplantation success with a biodegradable material

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Muscle-up: enhancing mussel collection and transplantation success with a biodegradable material

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(met een samenvatting in het Nederlands)

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Summary

In this thesis, I explore strategies to enhance the success of mussel transplantations. On one hand, transplantations can serve as a valuable tool to restore natural mussel beds. These efforts contribute to the restoration of important ecosystem services provided by mussel beds, such as habitat provisioning, water filtration and carbon sequestration. On the other hand, mussel transplantations occur on large scale in commercial practices, especially in light of a growing human population and the demand for more sustainable food sources. In **Chapter 1** the fundamental principles for transplantation of ecosystem engineers, with implications for both mussel bed restoration and aquaculture, are introduced. Mussel cultivation typically starts with collecting mussel spat or seed, either from natural spatfall using seed mussel collectors (SMCs) or by harvesting from benthic mussel beds. SMC-collected seed is more expensive (5 to 6 times) than traditional bottom fisheries due to increased effort and materials required. Achieving economic viability and successful mussel transplantation relies on improving the survival of SMC-collected seed. The vulnerability of young mussels to predation and dislodgment post-transplantation is a significant concern, leading to substantial losses during the critical settlement phase. Additionally, there are environmental concerns about SMC materials, often involving synthetic fibers and lead cores. What is imperative is the need for innovation to improve mussel survival rates after transplantation, cost-effectiveness, and environmental sustainability. The possible solution studied in this thesis centers around the use of biodegradable structures. These structures are expected to play an important role in kickstarting local-scale, self-facilitating feedback mechanisms, thereby mitigating the environmental stressors experienced during the initial post-transplantation phase. The overarching objective of this approach is to enhance the survival and growth of transplanted mussel seed, by using biodegradable structures that initiate self-facilitating feedback mechanisms. The biodegradable structure used in this thesis is the “BioShell-SMC”, which is an innovation of the traditional seed mussel collectors (SMCs) used in mussel cultivation. It does not contain any plastics, but consists of a biodegradable net based on a compound of aliphatic polyesters, filled with empty cockle shells around a coconut fiber rope.

Mussel larvae prefer settling on complex surfaces, that provides refuge from hydrodynamic forces and predation. Empty shells are complex surfaces that have shown to be a suitable attachment substrate for mussel larvae. Therefore, our BioShell-SMC filled with empty cockle shells could provide a promising innovation for mussel seed collection. In **Chapter 2** the effectiveness of mussel seed collection of the BioShell-SMC was compared with traditional nylon collectors for collecting mussel seeds. We monitored mussel biomass and density development and mussel length over time at different water depths and at different locations. The results showed that both collector types yielded a comparable mussel seed biomass in six out of nine locations. It turned out that the selection of deployment location held particular significance, as the more exposed areas resulted in higher biomass for the traditional ropes. This difference may be attributed to potential damage of mussel seed caused by crushing under harsh environmental

conditions due to the presence of shell fragments inside the BioShell-SMC. Besides, the study showed that mussel seed biomass was unaffected by deployment depth, although mussels were more abundant but smaller in deeper water. Over time, mussel density decreased while the overall biomass increased. Consequently, finding the right timing is crucial for optimizing mussel biomass yields.

Once mussel seeds are collected, they can be either relocated to a culture plot for cultivation or designated areas for restoration efforts. The size of the mussels is influenced by the timing of harvest. Furthermore, finding the optimal timing and method for seeding mussel seeds is crucial to maximize their survival. Therefore, in **Chapter 3**, we explored whether the BioShell-SMC could stimulate self-facilitating feedback mechanisms that enhance the survival of transplanted mussels. We conducted a field experiment to compare mussel survival when they were seeded attached to the BioShell-SMC versus loose mussels. Besides, we examined predation by crabs and sea stars in a mesocosm experiment to understand how predation affects mussels of different sizes. The results showed that larger mussel seeds had significantly higher survival rates when attached to BioShell-SMCs compared to loose transplanted mussels. Predation and dislodgment caused by hydrodynamic forces were key factors contributing to the higher losses among loose mussels. In the case of small mussel seeds, both loose mussels and those attached to BioShell-SMCs experienced a substantial decrease in mussel biomass during the initial three days of the experiment. This decline could be attributed to the small size of the mussels combined with relatively low mussel densities due to the small scale of the experiment. Overall, this study highlights the potential of using biodegradable structures to enhance self-facilitating feedback mechanisms for establishing ecosystem engineers in dynamic environments.

The results from Chapter 3 showed that the BioShell-SMC provides effective protection against wave impacts and predation, ultimately leading to improved mussel survival rates. However, the major disadvantage of the BioShell-SMC may be that mussel densities are initially high, leading to competition for food and space, impeding the growth and condition of the mussels. It is hypothesized that the cockle shells within the BioShell-SMC can disperse onto the surrounding substrate as the biodegradable net dissolves, facilitating the migration of mussels as a strategy to avoid such high competition. Therefore, in **Chapter 4**, we looked into the behavior of mussels attached the BioShell-SMC, particularly in high-density clusters, shedding light on their capacity to migrate onto the surrounding substrate. In a mesocosm experiment, we compared aggregation and performance between loose seeded mussels and mussels attached to the BioShell-SMC at different densities and sediment types (hard versus soft sediment). As expected, the results showed that the mussels attached to the BioShell-SMC showed more pronounced clustering compared to loose mussels, particularly in low density. Mussels in high density attached to the BioShell-SMC dispersed from the SMC on both hard and soft sediments, although we found a tendency for more patches on hard substrate. Loose transplanted mussels aggregated into higher biomass patches on soft sediment than in situations with attachment substrate, suggesting that mussels on soft sediment climbed on top of each other to access favorable positions, while those on hard sediment were able to occupy more space, attaching to the substrate. Furthermore, transplanted mussels attached

to the BioShell-SMC showed higher survival rates and had a better condition than loose mussels. This chapter contributes to understanding how facilitation and competition affect mussel dynamics in post-transplantation scenarios.

Encouraged by the promising results from the previous chapters, we aimed to conduct further research to improve and scale up transplantation efforts. This involved implementing another potential advantage of the BioShell-SMC, namely creating large-scale spatial patterns resembling the spatial organization of natural mussel beds to increases their resilience. In **Chapter 5**, we applied various large-scale configurations of BioShell-SMCs designed to mimic patterns observed in natural mussel beds. Natural mussel beds on soft sediment exhibit distinctive large-scale spatial patterns, characterized by high-density mussel bands (5 – 10 m apart) perpendicular to the tidal direction, alternating with bare sediment patches. These patterns result from a synergy of local positive feedback and larger-scale negative feedback mechanisms. In a large-scale field experiment, we tested whether different spatial configurations (low vs. high density labyrinth pattern and banded pattern) could increase transplantation success of mussel seed. The results showed high overall mussel losses (~75% after almost 300 days) across all configurations, with no significant variation. The results suggest that lack of mussels migrating horizontally from the BioShell-SMC structures and the high initial density hindered the initiation of optimal small-scale natural aggregations, that were conditional for the establishment of the large-scale patterns. Without horizontal migration, competition among mussels increased and survival decreased. The initial high mussel densities on the BioShell-SMC led to intense resource competition, resulting in mussels climbing on top of each other to reach favorable feeding positions, resulting in high mortality rates, mainly due to smothering. Besides, factors such as hydrodynamic dislodgement and burial, likely contributed to the observed losses.

In the general discussion in **Chapter 6**, the results of this thesis were summarized and contextualized within a broader framework. In conclusion, the BioShell-SMC has shown to be able to improve transplantation success substantially, when transplanted at the right time, in sufficient mussel densities and on small scale. Future research should further investigate the optimal density for mussels attached to the BioShell-SMC and scale-up restoration efforts. Additionally, I provided implications for both restoration efforts and aquacultural practices.

Samenvatting

In dit proefschrift onderzoek ik methoden om het succes van mosseltransplantaties te vergroten. Enerzijds kunnen transplantaties een waardevol instrument zijn om natuurlijke mosselbanken te herstellen. Deze inspanningen dragen bij aan het herstel van belangrijke ecosysteemdiensten die door mosselbanken worden geleverd, zoals het creëren van een habitat voor andere soorten, waterzuivering en koolstofvastlegging. Anderzijds gebeuren mosseltransplantaties op grote schaal in commerciële praktijken, vooral gezien de groeiende wereldbevolking en de vraag naar duurzamere voedselbronnen. In **Hoofdstuk 1** worden de fundamentele principes voor de transplantatie van biobouwers geïntroduceerd, met implicaties voor zowel het herstel van mosselbanken als aquacultuur. Mosselkweek begint doorgaans met het verzamelen van mosselzaad, afkomstig van natuurlijke zaadval met behulp van mosselzaad-invanginstallaties (MZI's) of door te vissen van mosselbanken op de zeebodem. Zaad dat verzameld wordt met MZI's is duurder (5 tot 6 keer) dan de traditionele bodemvisserij vanwege de extra inspanning en materialen die nodig zijn. Het bereiken van economische haalbaarheid en succesvolle mosseltransplantatie is afhankelijk van het verbeteren van de overleving van met MZI's verzameld zaad. De kwetsbaarheid van jonge mosselen voor predatie en wegspoeling na transplantatie is een grote zorg en resulteert in aanzienlijke verliezen tijdens de kritieke vestigingsfase. Daarnaast zijn er milieuzorgen over de materialen die in MZI's worden gebruikt, aangezien deze materialen doorgaans synthetisch zijn en loden delen bevatten. Wat van essentieel belang is, is de noodzaak om de overlevingskansen van mosselen te verhogen na transplantatie, kosteneffectiviteit te vergroten en milieuvriendelijkheid te bevorderen. De mogelijke oplossing die in dit proefschrift wordt bestudeerd, draait om het gebruik van biologisch afbreekbare structuren. Deze structuren spelen mogelijk een belangrijke rol bij het op gang brengen van zelf-faciliterende feedbackmechanismen op lokale schaal, waardoor de omgevingsstressoren die tijdens de eerste fase na de transplantatie worden ervaren, worden verminderd. Het doel van deze aanpak is het vergroten van de overleving en verbeteren van de groei van getransplanteerd mosselzaad, door het gebruik van bioafbreekbare structuren die zelf-faciliterende feedback mechanismen initiëren. De biologisch afbreekbare structuur die in dit proefschrift wordt gebruikt is de "BioShell-SMC", een innovatie van de traditionele MZI's die worden gebruikt in de mosselkweek. Het bevat geen plastics maar bestaat uit een biologisch afbreekbaar net op basis van een verbinding van alifatische polyesters, gevuld met lege kokkelschelpen rond een touw van kokosvezels.

Mossellarven vestigen zich het liefst op complexe oppervlakken, die bescherming bieden tegen stroming en predatie. Lege schelpen zijn complexe oppervlakken waarvan is gebleken dat ze een geschikt aanhechtingssubstraat vormen voor mossellarven. Daarom zou onze BioShell-SMC gevuld met lege kokkelschelpen een veelbelovende innovatie kunnen zijn voor de invang van mosselzaad. In **Hoofdstuk 2** is de effectiviteit van de BioShell-SMC voor het invangen van mosselzaad vergeleken met traditionele nylon MZI's. We hebben de ontwikkeling in

mosselbiomassa en -dichtheid en mossellengte doorheen de tijd op verschillende waterdieptes en locaties gemonitord. Uit de resultaten bleek dat beide collectortypen op zes van de negen locaties een vergelijkbare mosselzaadbiomassa opleverden. De keuze van de invanglocatie was hiervoor wel van belang, omdat de meer blootgestelde gebieden resulteerden in een hogere biomassa voor de traditionele touwen. Dit verschil kan worden toegeschreven aan mogelijke schade aan mosselzaad veroorzaakt door verbrijzeling onder zware omgevingsomstandigheden als gevolg van de aanwezigheid van schelpfragmenten in de BioShell-SMC. Bovendien toonde het onderzoek aan dat de biomassa van mosselzaad niet werd beïnvloed door de diepte, hoewel mosselen talrijker maar kleiner waren in dieper water. In de loop van de tijd nam het aantal mosselen af, terwijl de totale biomassa toenam. Daarom is het vinden van de juiste timing cruciaal voor het optimaliseren van de biomassa-opbrengsten van mosselen.

Zodra het mosselzaad is verzameld, kunnen ze worden verplaatst naar een kweekperceel of naar aangewezen gebieden voor restoratie. Het formaat van de mosselen wordt beïnvloed door het moment van oogsten. Het vinden van de optimale timing en methode voor het zaaien van mosselzaden is cruciaal om hun overlevingskansen te vergroten. Daarom hebben we in **Hoofdstuk 3** onderzocht of de BioShell-SMC zelf-faciliterende feedbackmechanismen kan stimuleren die de overlevingskansen van getransplanteerde mosselen vergroten. We hebben een veldexperiment uitgevoerd om de overleving van mosselen te vergelijken wanneer aan de BioShell-SMC waren gehecht in vergelijking met losse mosselen. De resultaten toonden aan dat grotere mosselzaadjes betere overleving hadden wanneer ze aan BioShell-SMC's waren gehecht in vergelijking met losse getransplanteerde mosselen. Predatie en stroming waren belangrijke factoren die bijdroegen aan de hogere verliezen onder losse mosselen. Voor kleinere mosselen was er gedurende de eerste drie dagen van het experiment een aanzienlijke afname in de biomassa, zowel bij de losse mosselen als bij de mosselzaadjes die aan de BioShell-SMC's waren gehecht. Deze afname kon worden toegeschreven aan het kleine formaat van de mosselen in combinatie met relatief lage dichtheden door de kleine schaal van het experiment, wat hen kwetsbaar maakten voor predatie door krabben. Dit werd bevestigd in een mesocosm-experiment waarin predatie door krabben en zeesterren werd onderzocht. Krabben richtten zich uitsluitend op de kleinere mosselen en lieten de grotere met rust. Al met al benadrukt deze studie het potentieel van het gebruik van biologisch afbreekbare structuren om zelf-faciliterende feedbackmechanismen te versterken voor de vestiging van biobouwers in dynamische omgevingen.

De bevindingen uit hoofdstuk 3 toonden aan dat de BioShell-SMC effectieve bescherming biedt tegen stroming en predatie, wat uiteindelijk leidt tot verbeterde overlevingskansen van mosselen. Het grote nadeel van de BioShell-SMC kan echter zijn dat de mosseldichtheden aanvankelijk hoog zijn, wat leidt tot competitie om voedsel en ruimte, waardoor de groei en conditie van de mosselen wordt belemmerd. Er wordt verondersteld dat de kokkelschelpen in de BioShell-SMC kunnen verspreiden over het omliggende substraat wanneer het biologisch afbreekbare net oplost, wat de migratie van mosselen vergemakkelijkt als een strategie om de intense concurrentie te vermijden. Daarom hebben we in **Hoofdstuk 4** het gedrag van mosselen onderzocht die aan de BioShell-SMC zijn bevestigd om meer inzicht te krijgen in hun vermogen om

zich naar het omringende substraat te verplaatsen. In een mesocosm-experiment hebben we aggregatie, overleving en groei vergeleken tussen los gezaaide mosselen en mosselen die vastgehecht waren aan de BioShell-SMC bij verschillende dichtheden en sedimenten (hard versus zacht). Zoals verwacht bleek uit de resultaten dat de aan de BioShell-SMC gehechte mosselen meer clustering vertoonden in vergelijking met losse mosselen, vooral bij lage dichtheid. Mosselen in hoge dichtheid gehecht aan de BioShell-SMC verspreidden zich vanuit de SMC op zowel hard als zacht substraat, hoewel we een neiging vonden naar meer clusters op een harde ondergrond. Mosselen die los werden getransplanteerd, vormden op zacht sediment clusters met een hogere biomassa dan in situaties met een harde ondergrond. Dit suggereert dat mosselen op zacht sediment boven op elkaar klommen om gunstige posities te bereiken, terwijl die op hard sediment meer ruimte konden innemen door zich aan het substraat te hechten. Bovendien vertoonden mosselen aan de BioShell-SMC hogere overlevingskansen en hadden ze een betere conditie dan losse mosselen. Dit hoofdstuk draagt bij aan het begrijpen hoe facilitatie en competitie de dynamiek van mosselen beïnvloeden na transplantatie.

Geïnspireerd door de veelbelovende resultaten uit de vorige hoofdstukken, wilden we verder onderzoek doen om transplantaties te verbeteren en op te schalen. Dit hield in dat we een ander potentieel positieve eigenschap van de BioShell-SMC toepasten, namelijk het creëren van grootschalige ruimtelijke patronen die lijken op de ruimtelijke organisatie van natuurlijke mosselbanken om hun veerkracht te vergroten. In **Hoofdstuk 5** hebben we ons verdiept in verschillende grootschalige configuraties van BioShell-SMCs, ontworpen om patronen na te bootsen die worden waargenomen in natuurlijke mosselbanken. Natuurlijke mosselbanken op zacht sediment vertonen opmerkelijke grootschalige ruimtelijke patronen, gekenmerkt door dichte mosselbanden (5 – 10 m uit elkaar) loodrecht op de getijdenrichting, afgewisseld met leeg sediment. Deze patronen zijn het resultaat van een synergie van lokale positieve feedback en grootschalige negatieve feedbackmechanismen. In een grootschalig veldexperiment hebben we getest of verschillende ruimtelijke configuraties (labyrintpatroon met lage versus hoge dichtheid en bandenpatroon) het transplantatiesucces van mosselzaad zouden kunnen vergroten. De resultaten lieten hoge totale mosselverliezen zien (~75% na bijna 300 dagen) in alle configuraties, zonder significante variatie. De resultaten geven aan dat het ontbreken van horizontale migratie van mosselen vanuit de BioShell-SMC structuren en de hoge initiële dichtheid de start van optimale kleinschalige natuurlijke aggregaties bemoeilijkten, wat noodzakelijk was voor de totstandkoming van de grootschalige patronen. Zonder horizontale migratie, wat leidde tot verhoogde concurrentie tussen mosselen, nam de overleving af. De aanvankelijk hoge dichtheid van mosselen op de BioShell-SMC resulteerde in grote concurrentie om voedsel en ruimte, waardoor mosselen boven op elkaar klommen om gunstige posities te bereiken, wat resulteerde in hoge sterftecijfers, voornamelijk als gevolg van verstikking. Bovendien hebben factoren zoals hydrodynamische wegspoeling en begraving waarschijnlijk bijgedragen aan de hoge verliezen.

In de algemene discussie in **Hoofdstuk 6** zijn de resultaten van dit proefschrift samengevat en geplaatst binnen een breder kader. In conclusie blijkt dat de BioShell-SMC aanzienlijk kan bijdragen aan het succes van transplantaties, mits deze op het juiste moment, met optimale mossel

dichtheden en op kleine schaal worden toegepast. Toekomstig onderzoek dient de optimale dichtheid van mosselen die aan de BioShell-SMC zijn bevestigd verder te onderzoeken en inspanningen voor herstel op grotere schaal te bevorderen. Daarnaast heb ik implicaties gegeven voor zowel restoratie als aquacultuur.

Chapter 1

General introduction

In the balance between a thriving environment and economic development, a resilient and innovative approach can possibly connect these two domains. With the expansion of the human population and the imperative for sustainable developments, an intriguing solution emerges—one that harnesses the potential of habitat restoration and aquaculture. Innovative strategies involving mussels have the potential to significantly enhance both environmental conservation and economic sustainability. Mussels have taken an important role in providing ecosystem services and meeting commercial demands. This duality of purpose, lays the foundation for the concept of the "BioShell-SMC". The BioShell-SMC is an innovation of the traditional seed mussel collectors (SMCs) used in mussel cultivation. It does not contain any plastics, but consists of a biodegradable net based on a compound of aliphatic polyesters, filled with empty cockle shells around a coconut fiber rope. The first section of this introduction chapter will address restoration, followed by a section dedicated to mussel aquaculture. The third part will bring together these seemingly distinct domains, revealing that their principles are more similar than one might initially assume.

Restoration

Ecosystems and their services

Over 625 million people inhabit the coastal zone, with a substantial part of them depending on the ecosystem services provided by coastal habitats (Neumann et al., 2015). Ecosystem services are characterized as the advantages that individuals derive, either directly or indirectly, from the ecosystem (Beaumont et al., 2007). They encompass a wide array of processes, resources, and functions that sustain life, enhance well-being, and drive economic activities (Millennium Ecosystem Assessment, 2005). The range of coastal ecosystem services is broad, including provisioning services like the production of food (i.e., fish and shellfish), materials (i.e., shells and pearls), and pharmaceutical products (Barbier et al., 2011; Millennium Ecosystem Assessment, 2005). It also includes regulating services, such as carbon sequestration (Mcleod et al., 2011) and coastal protection (i.e., wave attenuation during extreme weather events) (Barbier, 2016; Ferrario et al., 2014; Möller et al., 2014). Furthermore, habitats created by ecosystem engineers, such as mangroves, shellfish, corals, seagrass and saltmarshes, provide supporting services for other species. Moreover, these habitats function as nurseries for fish species and facilitate fish stock renewal (Beck et al., 2001; Zu Ermgassen et al., 2016). Lastly, these habitats offer cultural services, promoting activities like tourism, recreational opportunities, and generating scientific value (Millennium Ecosystem Assessment, 2005).

Decline of ecosystems

Human activities have exerted substantial pressures on coastal ecosystems globally, resulting in significant losses of ecosystems (Millennium Ecosystem Assessment, 2005). The functioning of global ecosystems changed more rapid in the latter half of the twentieth century than ever before in human history. The extent of these changes is accelerating due to the

simultaneous growth in population size and the intensity of economic activity. For example, the intensification of food production techniques, coastal development, deforestation, and the extensive exploitation of fisheries have collectively resulted in the depletion of natural resources and alterations to the functioning of ecosystems. This change in ecosystem functions may, for instance, lead to decline in water quality, air pollution, and more extreme weather events (Millennium Ecosystem Assessment, 2005).

Restoration of ecosystems

Acknowledging the vital role coastal ecosystems play in sustaining both natural processes and human livelihoods, ecological restoration is gaining growing recognition as a significant strategy for increasing the provision of ecosystem services (Bullock et al., 2011). By limiting the number of anthropogenic activities, ecosystems can, in some instances, naturally recover. Some studies demonstrate a natural recovery following the establishment of protected areas or the removal of a pollution source (Babcock et al., 1999; Bryars & Neverauskas, 2004; McClanahan, 2000). However, other studies have shown that protective measures alone are not always sufficient to restore a degraded ecosystem (Agardy et al., 2011; Christianen et al., 2014). For instance, in a Marine protected area (MPA) with seagrass habitat, green turtles were consuming seagrass faster than it can regrow. The ecosystem was approaching a tipping point, which could potentially lead to complete collapse of the seagrass habitat (Christianen et al., 2014). In such cases, more proactive approaches might be needed to aid restoration of ecosystems. Moreover, ecosystem restoration is an intentional activity that initiates or accelerates the recovery of a degraded, damaged or destroyed ecosystem (SER, 2004). In coastal ecosystems, a prevalent form of active restoration involves reintroducing, or transplanting, species that have been lost or diminished back into ecosystems that have undergone degradation (Byers et al., 2006). Transplantation of species often involves ecosystem engineers, which are species that have the ability to directly or indirectly influence the availability of resources for other species by inducing physical changes in biotic or abiotic materials (Jones et al., 1994). Examples of coastal ecosystem engineers are seagrasses, mangroves, corals and shellfish. These engineers play a crucial role in modifying, maintaining, and creating habitats within their ecosystem (Bruno et al., 2003; Ellison et al., 2005; Jones et al., 1994), thereby providing important ecosystem services to other species. What makes the restoration of ecosystem engineers particularly essential is the presence of an establishment threshold. These species often require specific conditions to be in place before they can successfully establish and thrive in an ecosystem. Besides, ecosystem engineers often form habitats that can be restored by focusing on the recovery of a single key species. This is because these engineers have such a significant impact on shaping the habitat that restoration can lead to a cascade of positive effects throughout the ecosystem.

Active restoration of our study system: blue mussel (*Mytilus edulis*)

Mussels are ecosystem engineers capable of forming reef-like structures through attachment to conspecifics or a substrate (Gosling, 1992). These mussel beds offer numerous ecosystem services. For example, they serve to stabilize sediment and offer a habitat and refuge to

numerous other species (Bouma, Olenin, et al., 2009). Besides, mussels have been cultivated for centuries for human consumption, forming a sustainable food source at the base of the food web (Smaal, 2002). They also serve as a significant food supply for birds and other animals, such as starfish, crabs, and bottom-dwelling fish (Mainwaring et al., 2014). In addition, mussels play a role in water quality regulation (Dame & Kenneth, 2011; Ferreira & Bricker, 2016; Lindahl et al., 2005) and carbon sequestration (Filgueira et al., 2019). Finally, mussels find utility in coastal defense and erosion control due to their wave dampening effect (Ysebaert et al., 2019). Hence, restoring or creating mussel beds holds significance in ensuring the provision of their essential ecosystem services.

Mussel bed restoration mimics the natural process of reef formation by harvesting mussels from their original location (such as aquaculture seed mussel collectors) and transplanting them to a designated restoration site on the seafloor. In recent years, the number of studies focusing on mussel bed restorations to strengthen ecosystem services has increased. For example, a study in Northern Ireland demonstrated that transplantation of the northern horsemussel can help restore marine benthic habitats through the development of a diverse community in a relatively short time (Fariñas-Franco & Roberts, 2014). However, many attempts to restore mussel beds have faced challenges, for example caused by insufficient seeding density (Capelle, Wijsman, et al., 2016), predation (Capelle, Scheiberlich, et al., 2016; Kamermans et al., 2009) or hydrodynamic dislodgement (Temmink et al., 2020).

Aquaculture

Cultivation cycle of blue mussel

Mussel spat or seed, obtained from the wild, serves as the starting material for mussel cultivation. The collection method of this seed varies based on local conditions and cultivation methods. In suspended longline culture, wild mussel seeds are often collected using seed mussel collectors (SMCs) and then grown to market size on the same systems, ensuring cost-effectiveness (Kamermans & Capelle, 2019). Alternatively, in some regions, mussel seeds are harvested from benthic mussel beds and relocated to designated bottom plots for further growth (Dolmer & Frandsen, 2002; Smaal, 2002). Referred to as benthic or mussel bottom culture, this approach demands minimal investment but is only suited for relatively shallow and sheltered tidal and sub-tidal flats. As a result, it is primarily practiced in Northern European countries, including The Netherlands, Germany, Denmark, and Ireland (Avdelas et al., 2021; Kamermans & Capelle, 2019).

The Netherlands is one of the largest producers of blue mussels in Europe (Smaal, 2002), particularly through the practice of bottom culture. The Dutch bottom cultivation cycle begins with collecting spat either from natural beds or by using seed mussel collectors (SMCs) (Figure 1.1). Once the mussel seed has been collected, the majority of it is relayed onto cultivation plots (Jacobs et al., 2016). These cultivation plots are primarily located in the Wadden Sea and to a lesser extent in the Oosterschelde. The shallow, nutrient-rich waters of the Wadden Sea are ideal for rapid mussel growth. When the mussels are around 10 to 15 months old and reach a size of about 4 to 5 cm,

they are moved to more sheltered plots. There they can grow into commercial sized mussels, reaching a size of 5 cm or larger. They typically reach this size after about 2 years (Smaal & Lucas, 2000).

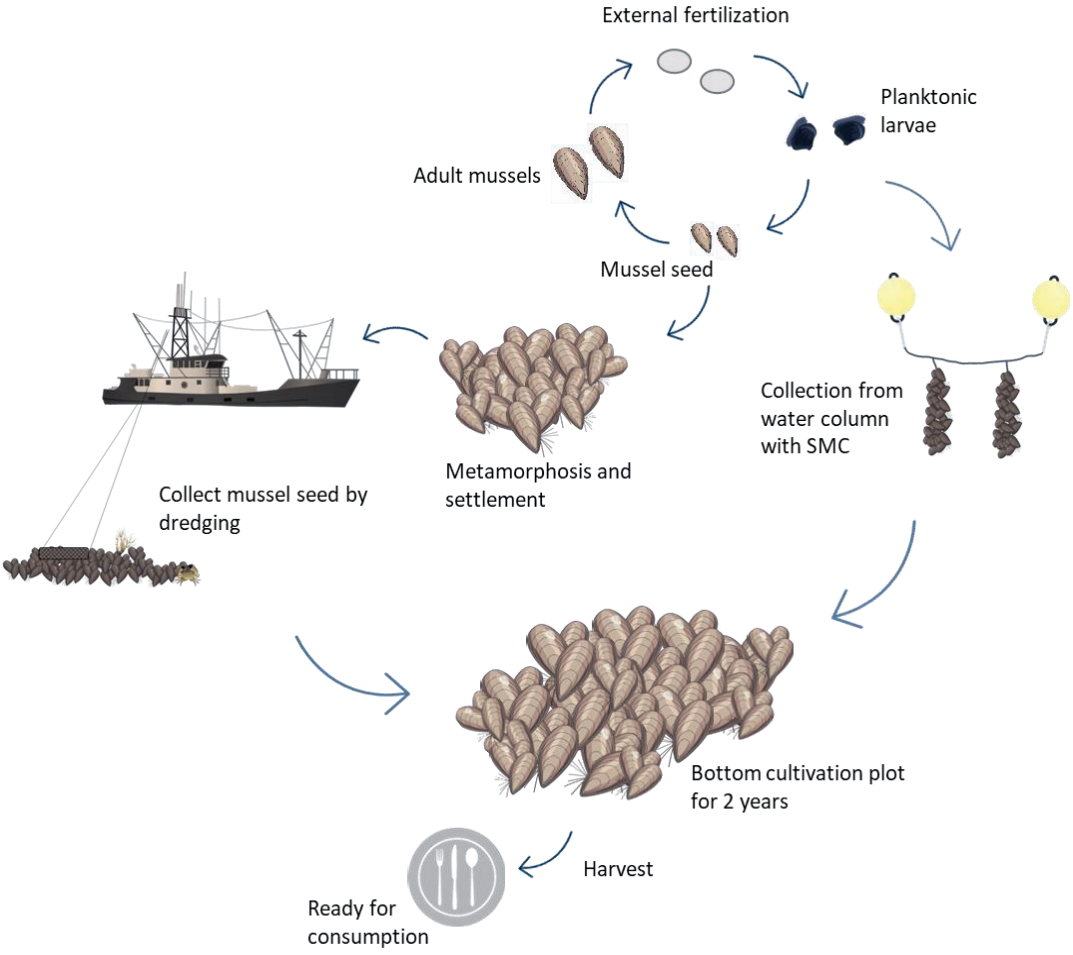


Figure 1.1. Dutch mussel cultivation cycle. The process initiates with the collection of spat from either natural beds or seed mussel collectors. The mussels are transplanted to culture plots for maturation. Symbols adjusted from Integration and Application Network (ian.umces.edu/media-library).

Mussel seed collection

The practice of dredging for mussel seed (Figure 1.2A) faced opposition from environmental organizations due to its impact on the development of natural mussel beds and the marine life on the seabed (Dolmer, 2002; Dolmer & Frandsen, 2002; Dolmer et al., 2001; Eleftheriou & Robertson, 1992; Van Hoof, 2012). To address this concern, a covenant was established in the Netherlands in 2009 involving mussel growers, nature organizations, and the government. This agreement outlined a gradual reduction in the utilization of bottom dredging for gathering mussel spat (Van Hoof, 2012). It initiated a new development to collect mussel seed in a more sustainable way, the seed mussel collector (SMC, Figure 1.2B). Mussel seed collected with SMCs in the water column is now gradually replacing the practice of bottom fishery for this purpose. This transition is expected to also ensure a more steady supply of mussel seed (Kamermans & Capelle, 2019), as the natural settlement of mussel spat on the seafloor exhibits greater variability compared to SMCs and experiences significant annual fluctuations (Capelle, 2017).

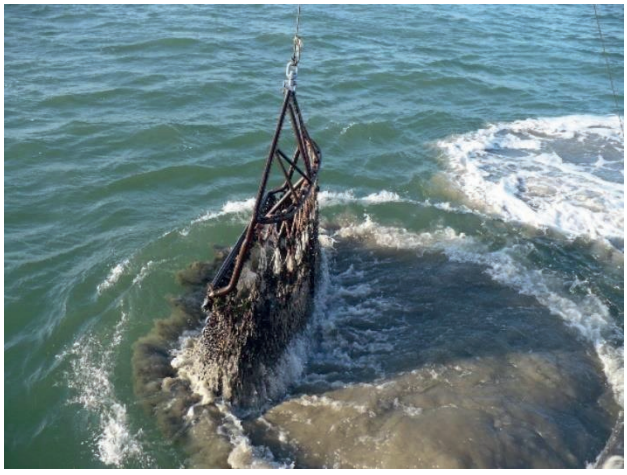
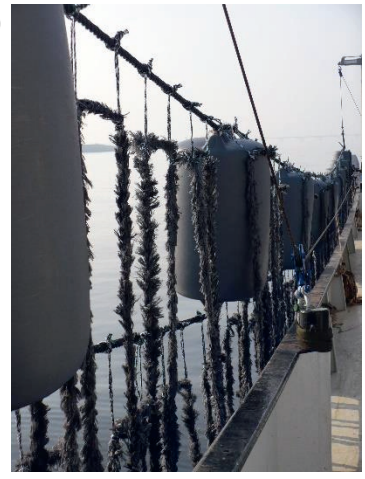
A)  **B)** 

Figure 1.2. A. Mussel dredge to harvest mussel seed from mussel beds. **B.** Seed mussel collector (SMC) system to collect mussel seed from the water column. Images from Nederlands Mosselbureau.

Bridging restoration and aquaculture

Transplantations of mussels can be applied for multiple purposes. On one hand, they can serve as a valuable tool to restore or create natural mussel beds (e.g., Alder et al., 2021; Schotanus et al., 2020). These efforts contribute to the re-establishment of important habitats. On the other hand, it also holds relevance in commercial contexts (Kamermans et al., 2002). This two-sided perspective emphasizes how mussel transplantations are not only a way to restore the environment, but also as a practical strategy that aligns with aquacultural practices.

Facilitation and competition

Understanding the interplay between facilitation and competition holds important implications for both restoration and cultivation of mussels. Mussels are reef-forming ecosystem engineers that aggregate into large complex beds by anchoring themselves to conspecific-substrate complexes (Christensen et al., 2015; Snover & Commito, 1998). Aggregation into high-density patches relates to the interplay of small-scale positive feedback (facilitation) and larger-scale negative feedback mechanisms (competition) (van de Koppel et al., 2008; van de Koppel et al., 2005). Localized aggregation at a small-scale (5 – 10 cm) leads to heightened local mussel densities, offering protection against displacement and predation (Figure 1.3A). However, this increased density also intensifies competition among mussels. Under some circumstances mussel beds on soft sediment display a distinct spatial pattern, characterized by dense bands of mussels (separated by 5 - 10 meters) perpendicular to the tidal flow, alternating with bare sediment patches (van de Koppel et al., 2005) (Figure 1.3B). These large-scale banded patterns decrease overall density, which, in turn, reduce competition for food. However, they maintain high local densities within the bands for safety and protection (van de Koppel et al., 2005). These large-scale patterns increase the resilience of mussel beds and diminish the likelihood of collapse (de Paoli et al., 2017; Liu et al., 2020). This interplay of facilitation and competition is also shown in other marine organisms, such as seagrasses and other reef-forming bivalves, where positive feedback mechanisms are needed to mitigate environmental stressors (He et al., 2013; Liu et al., 2014; van de Koppel et al., 2001; van der Heide et al., 2007). Seagrasses attenuate currents and trap sediment through heightened shoot density, which slows down the water flow and allows the seagrass to trap more sediment (Maxwell et al., 2017). Likewise, oyster reefs emerge when larvae settle on existing oyster shells, attracted by chemical cues, thus reinforcing the growth and stability of their habitat (Turner et al., 1994).

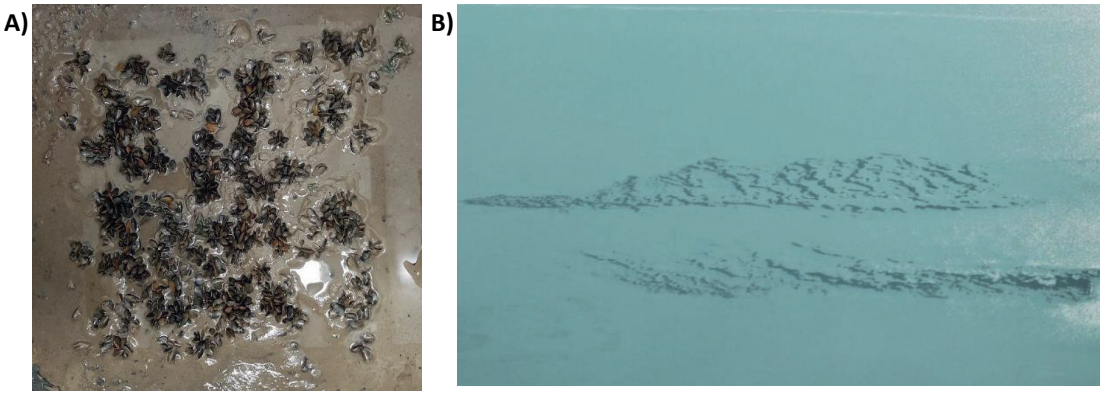


Figure 1.3. A. Small-scale aggregations among mussels on soft sediment. Photograph taken at the end of a mesocosm experiment presented in Chapter 4 of this thesis. **B.** Aerial photograph of a mussel bed displaying a large-scale banded pattern in the Wadden Sea, situated right below the island Ameland in The Netherlands. The mussel bed covers an area of about 1.2 ha. Photograph taken by Karin Troost on February 19th, 2019.

Role of facilitation and competition in restoration and aquaculture

In the context of restoration, knowing how facilitation and competition shape mussel beds helps conservationists design effective strategies to restore and enhance mussel populations. By creating conditions that encourage facilitation during the establishment phase, restoration efforts can enhance the survival and growth of mussel populations. Self-facilitating feedback mechanisms can be promoted by including an attachment substrate and sufficient densities. For mussel cultivation, this understanding provides insights into optimizing aquaculture practices. For instance, cultivating mussels in ways that encourage beneficial interactions between individuals could lead to higher yields and reduced vulnerability to environmental stressors. This could be achieved by providing an attachment substrate for the mussels to reduce the risk on dislodgement and predation. Furthermore, this knowledge can guide the configuration of mussels in bottom culture that mimic the natural spatial patterns observed in wild mussel beds, which could lead to increased resilience and sustainability in cultivated populations. By emulating the banded patterns that reduce competition and enhance survival in natural settings, aquaculture operations can potentially maximize yield while minimizing resource competition among mussels.

Crucial role of Window of opportunity and feedback mechanisms

The initiation of positive feedback mechanisms (facilitation) is often crucial in the establishment phase after transplantations. In a lot of cases, these positive feedback mechanisms can only arise when a certain density or size threshold is reached (Bouma, Friedrichs, et al., 2009; van der Heide et al., 2007). However, the occurrence of these mechanisms can be hindered, contributing to transplantation failures, due to the absence of a disturbance-free period right after transplantation. This critical period, referred to as a "window of opportunity," is essential for the establishment of these positive feedback mechanisms (Balke et al., 2014). Alternatively, a window of opportunity can also be described as the critical duration during which a suitable settlement substratum is available in the presence of recruits (Capelle et al., 2019).

Integrating the concept of a window of opportunity and positive feedback mechanisms into transplantation processes offers potential solutions to enhance transplantation success (Renzi et al., 2019; Valdez et al., 2020). This has been shown in for example salt marsh restoration, where incorporating positive intraspecific interactions through the use of clumped rather than dispersed transplant configurations improved the survival of the transplanted organisms (Silliman et al., 2015). But also for mussel bed restoration, stimulating the formation of an aggregated spatial configuration using fences between which the mussels were placed, led to lower loss rates of the transplanted mussels (Schotanus, Walles, et al., 2020). Similarly, these principles also hold relevance in mussel aquaculture practices, where manipulating the spatial arrangement of mussels can influence their growth and survival. By harnessing the benefits of positive intraspecific interactions, mussel farmers can potentially optimize production outcomes, while mitigating risks.

Challenges in restoration and aquaculture

Mussel restoration efforts and aquaculture practices are not without challenges. One significant challenge lies in the vulnerability of juvenile mussels, particularly during the initial phase

following transplantation to a soft bottom habitat. That is, during the transplantation procedure, mussel seeds are detached from the SMCs and subsequently placed on designated restoration sites or culture plots. The lack of alternative settlement substrates impedes their capacity to reattach to their environment rather than to conspecifics, making them more vulnerable to environmental stressors. The influence of environmental stressors, including hydrodynamic forces and predation, significantly impacts young mussels, leading to considerable losses during the initial month post-transplantation (Capelle, Scheiberlich, et al., 2016; Schotanus, Capelle, et al., 2020). Moreover, part of the mussel seeds obtained from SMCs are often harvested at a smaller size than bottom-fished seed to prevent them falling form the ropes, making them even more vulnerable to environmental stressors. Establishment of self-facilitating interactions among transplanted mussels is crucial. However, the creation of these interactions takes time and without specific environmental conditions, including calm conditions and the presence of suitable attachment substrates (window of opportunity), the resilience of mussel beds can be compromised (Liu et al., 2014). To optimize resilience and growth, ideal arrangements for mussel seeds should be included (Bertolini et al., 2019). Unfortunately, current seeding techniques in benthic aquaculture typically result in suboptimal configurations, with mussels adopting an eight-like pattern that mirrors the seeding tracks of mussel vessels. These tracks lead to local high-density areas and bare patches, resulting in high losses (on average 69%, Capelle et al., 2016). Another challenge with mussel seed derived from SMCs is the cost-effectiveness. This type of mussel seed is notably more expensive (5 to 6 times) compared to seed derived from bottom fisheries. Achieving economic viability of the mussel sector, relies on increasing the survival rate of SMC-collected mussel seed. This also extends to mussel restoration efforts, which are similarly characterized by considerable expenses. Furthermore, concerns associated with the composition of SMCs is that they are commonly made of multi-filament synthetic fibers around a core of coated lead, although the lead is increasingly replaced by more environmentally friendly materials such as rocks. Potential losses of SMC components due to natural forces such as storms or currents pose a littering risk on the seafloor or coastal areas (Kamermans et al., 2014; Sandra et al., 2019). For these reasons, there is a strong need for innovation in techniques to increase post-transplant survival of mussel seed, cost-effectiveness, and minimize the potential environmental impacts associated with mussel culture based on SMCs.

The BioShell-SMC: a dual-purpose innovation

In the context of ecosystem restoration and aquaculture, our proposed solution to the above problems revolves around the utilization of biodegradable structures. These structures serve to kickstart local-scale self-facilitating feedback mechanisms, thereby counteracting the environmental stressors experienced during the initial post-transplantation phase. The overarching goal of this approach is to enhance the survival and growth of transplanted mussel seed.

The so-called “BioShell-SMC” emerges as an innovation in marine ecosystem restoration and aquaculture, and may provide an excellent solution to the above-mentioned problems. The BioShell-SMC does not contain any plastics, but consists of a biodegradable net based on a compound of aliphatic polyesters, filled with empty cockle shells around a coconut fiber rope

(Figure 1.4). Empty shells have shown to provide an excellent attachment substrate for mussel larvae (Commito et al., 2014; wa Kangeri et al., 2014). One potential benefit of using BioShell-SMCs is that mussel seed can remain attached to the SMCs during transplantation to a cultivation or restoration site, eliminating the need for seed removal as required with traditional nylon SMCs. This means that mussels can be transplanted to the sea floor while staying attached to the BioShell-SMCs in high density clusters. Diverging from the relatively uncontrolled dispersal of loose mussel seeds on a subtidal culture plot or restoration site from traditional SMCs, the BioShell-SMC enables a more deliberate transplanting process. This involves the strategical positioning of intact BioShell-SMCs, which includes mussel seeds, empty cockle shells, biodegradable socking, and a biodegradable inner rope. Through the arrangement of these biodegradable structures that mimic the banded patterns found in natural mussel beds, we can possibly avoid the unfavorable large-scale consequences commonly associated with competitive interactions among mussels. That is, the water layer above a mussel bed becomes depleted of seston, but when it encounters a bare patch, the shortage of food is replenished through vertical mixing before reaching the next mussel patch (Saurel et al., 2013). In addition, the mussels in the high density clusters on the BioShell-SMC can reorganize themselves from this secure initial position. The clustering potentially provides safety against predators like crabs and sea stars, and enhancing resistance to hydrodynamic dislodgement. Moreover, as the starch nets gradually dissolve (intended to occur within a year), the enclosed cockle shells will slowly disperse. This dispersal could serve as a substrate for mussels to spread farther away from the BioShell-SMCs (where the availability of substrate offers a window of opportunity), allowing them to avoid competition for resources and space (Capelle et al., 2019).

This dissertation delves into the various facets of the BioShell-SMC, exploring its mechanisms, effectiveness, and implications for both restoration efforts and the mussel cultivation industry.

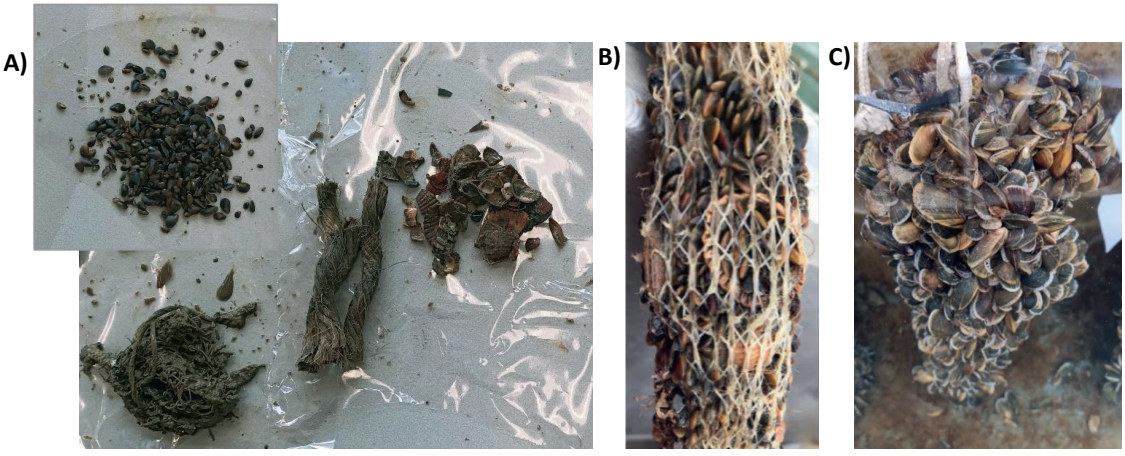


Figure 1.4. A. Components of the BioShell-SMC, including biodegradable netting, inner coconut rope, and fragments of empty cockle shells. Inset: Mussel seed collected using the BioShell-SMC. **B.** BioShell-SMC with small attached mussel seed. **C.** BioShell-SMC with large attached mussel seed.

Outline of this thesis

In the upcoming chapters, we explore strategies to enhance the success of mussel transplantation, which holds relevance for both mussel bed restoration and aquaculture applications. In all experiments, we used our BioShell-SMC to investigate if and how transplantation success can be increased.

The following research questions are addressed:

1. How does the collection performance of the BioShell-SMC compare to conventional nylon rope collectors in terms of mussel seed density and growth, considering varying water depths and collector locations?

Mussel larvae prefer settling on complex surfaces, that provides refuge from hydrodynamic forces and predation (Carl et al., 2012; Filgueira et al., 2007). Empty shells are complex surfaces that have shown to be a suitable attachment substrate for mussel larvae (Commito et al., 2014; wa Kangeri et al., 2014). Therefore, our BioShell-SMC filled with empty cockle shells could provide a promising innovation for mussel seed collection. While biodegradable socks made of cotton have been employed in submerged longline culture around the world, they have not been filled with shells for the purpose of mussel seed collection. Hence, there is a knowledge gap for applying socked-shells for mussel seed collection in the water column. **Chapter 2** aims to compare the effectiveness of the BioShell-SMC filled with cockle shells versus traditional nylon collectors for collecting mussel seeds. We monitored mussel biomass and growth at different water depths. In addition, this chapter provides further understanding on the performance of the SMCs across different locations in the Dutch Wadden Sea and the Oosterschelde.

2. Can a biodegradable structure promote self-facilitating feedback mechanisms, that increase survival of transplanted mussels?

To mitigate competition on collector ropes and minimize the risk of accidental dislodgment, mussel seed is traditionally harvested from the collector ropes and transplanted to subtidal cultivation plots or restoration sites when they are 2-3 cm long (Capelle, Wijsman, et al., 2016). After transplantation to the sea floor, mussels form aggregations by attaching to conspecifics, which is the result from self-facilitating feedback mechanisms. The adaptive benefit of aggregation is associated with the reduction of dislodgement by hydrodynamic forces and the protection against predators through stronger attachment and by a safety in numbers effect (Hunt & Scheibling, 2001). However, aggregation in high density patches also imposes disadvantages, particularly competition for space and food (Capelle et al., 2014; Newell, 1990). It has been shown that enriching mussel seeds with coarse shell material reduces mussel losses on culture plots (Capelle et al., 2019), and we hypothesize that this effect can be achieved by the presence of cockle

shells within the BioShell-SMCs as well. Seeded as entire system, the mussels attached to the BioShell-SMC are possibly better protected against dislodgment and predators than loose mussels, which enables earlier seeding. This early seeding has the potential to mitigate competition because the mussels are still smaller. However, it's important to note that early transplantation might increase the risks of predation and burial. **Chapter 3** aims to determine the optimal seeding time and method for mussel seed using BioShell-SMCs.

3. How do interactions between seeding method, mussel density and substrate type influence self-facilitation of mussels, and can these mechanisms be induced post-transplantation?

Aggregation into high-density patches relates to the interplay of facilitation (protection against predation and dislodgement) and competition for food and space. The major disadvantage of the BioShell-SMC may be that mussel densities are initially high, leading to competition for food and space, impeding the growth and condition of the mussels (Commito et al., 2014; van de Koppel et al., 2005). **Chapter 4** explores if mussels on BioShell-SMCs can disperse from high-density clusters, thereby actively reducing competition. We tested of the presence of attachment opportunities (shell debris) affects dispersion. This investigation contributes to understanding how facilitation and competition affect mussel dynamics in post-transplantation scenarios.

4. How can the implementation of BioShell-SMCs with diverse spatial configurations mimic the spatial patterns observed in natural mussel beds, and how does this affect the interplay between facilitation and competition in mussel populations after transplantation?

Natural mussel beds on soft sediment exhibit a distinctive spatial pattern, characterized by high-density mussel bands (5 – 10 m apart) perpendicular to the tidal direction, alternating with bare sediment patches. These patterns result from a synergy of local positive feedback and larger-scale negative feedback mechanisms (van de Koppel et al., 2008; van de Koppel et al., 2005). They enhance the resilience of mussel beds and reduce the likelihood of collapses (de Paoli et al., 2017; Liu et al., 2020). Inside the broader bands of the large-scale mussel patterns, individual mussels initiate small-scale movements and aggregations, enhancing protection. However, this behavior increases competition for resources (Frechette et al., 1992; van de Koppel et al., 2008). **Chapter 5** looks into various configurations of BioShell-SMCs that mimic patterns in natural mussel beds. We aim to encourage self-organization by using the BioShell-SMC, implementing diverse spatial arrangements that influence the interplay of facilitation and competition after transplantation.

Chapter 2

Comparing traditional vs. biodegradable seed mussel collectors (SMCs) for seed settlement, seed density, and seed growth: effect of deployment depth and location

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Abstract

Mussel bottom culture is historically based on transplanting wild mussel seed to designated culture plots. Seed mussel collectors (SMCs) that are deployed in the water column are gradually replacing benthic mussel beds for mussel seed resource provisioning. Traditional SMCs consist of weighted filamentous nylon ropes. The performance of SMCs is promising, but the major disadvantages are the increased cost, effort, and the use of non-sustainable materials. In this study, we developed an innovative SMC: the BioShell-SMC. It consists of a coconut core rope surrounded by empty cockle shells that are held in place by biodegradable socking. The advantage of this system compared to traditional SMCs is that it provides biodegradable and sustainable resource material suitable for on-bottom placement. We compared its relative performance to that of a traditional SMC at different deployment depths and locations used for SMC deployment in the Dutch Wadden Sea and Oosterschelde. The results from this experiment indicated that in six out of nine locations mussel seed biomass was comparable between the two collector types. On both collector types, mussel seed biomass was higher in the Wadden Sea than in the Oosterschelde. We also found that mussel seed biomass development was not affected by deployment depth, though mussels were more numerous and shorter in deep water. The results of the current study provide a promising start toward a more sustainable mussel seed collection for bottom cultivation.

Introduction

Wild-harvested seed mussels, also known as spat or juveniles, are used as starting material in mussel farming operations. Collection of this seed can be done in different ways, depending on the local circumstances and grow-out methods. In suspended longline culture, mussel seeds are collected from the wild, often by using seed mussel collectors (SMCs) and usually grown to market size on the same or similar systems to make it cost-efficient (Kamermans & Capelle, 2019). However, in some countries, mussel seeds are harvested from benthic mussel beds and relayed on designated bottom plots for grow-out (Dolmer & Frandsen, 2002; Smaal, 2002). This so-called benthic, or mussel bottom culture, requires a low investment but is restricted to tidal and sub-tidal flats that are relatively shallow and sheltered. For that reason, this method is mainly used in Northern European countries, such as The Netherlands, Germany, Denmark and Ireland (Avdelas et al., 2021; Kamermans & Capelle, 2019).

During the last three decades, dredging for mussel seed from wild mussel beds has received increased resistance from non-governmental organizations (NGOs) because of its possible negative impact on the sea bed and its associated benthic flora and fauna (Dolmer, 2002; Dolmer & Frandsen, 2002; Dolmer et al., 2001; Eleftheriou & Robertson, 1992). To reduce fishing pressure on wild mussel beds, management plans were realized in different countries. For example, in 2013 a new Mussel Fishery Management plan was implemented in Denmark to regulate the fishery. The mussel fishery was banned in vulnerable habitats, such as *Zostera* beds and rocky reefs, and restricted in other Natura 2000 areas (Frandsen et al., 2015). In the Netherlands, a covenant was signed in 2009 between mussel growers, NGOs, and the government that issued a stepwise decrease in using bottom dredging to collect mussel spat (Van Hoof, 2012). This agreement initiated new developments to collect mussel seed in more sustainable ways, such as mussel dredges with lower environmental impact (Frandsen et al., 2015) and seed mussel collectors (SMCs). In the Netherlands, mussel seed collected from SMCs in the water column is now gradually replacing mussel seed from the bottom fishery. This practice is expected to also ensure a more steady supply of mussel seed (Kamermans & Capelle, 2019), since natural spat settlement on the seafloor is much more variable than on SMCs and undergoes large yearly fluctuations (Capelle, 2017), probably due to the activity of benthic predators (van der Heide et al., 2014). The Dutch mussel growers are facing two major challenges in the application of SMCs for seed provisioning: (1) increased cost, since SMCs need to be purchased and maintained and are more labor intensive, and (2) environmental impact reduction.

A particular concern associated with using SMCs is that the increased cost of the mussel seed (€0.45-0.60 per kg for SMC-seed vs €0.10 per kg for seed from fishery (van Oostenbrugge et al., 2018)) is currently not yet compensated by increased productivity of the cultivation cycle. SMC seed is ideally harvested when densities are high and the mussels are still small enough to prevent them from falling off the SMC due to space regulated self-thinning (Cubillo et al., 2012; Lauzon-Guay, Hamilton, et al., 2005). Hence, the mussel seed is removed from the SMCs and relayed on

subtidal or intertidal (culture) plots before extensive seed loss occurs. However, on the culture plots the small size of the mussels and lack of hard substrate on soft-sediment (culture) plots makes the mussels highly vulnerable to loss factors such as hydrodynamic dislodgement and predation by crabs and sea stars (Kamermans et al., 2010; Murray et al., 2007). Applying the current best practice for seeding, which typically focuses on dredged juveniles, is not suitable for the small and clean SMC-seed. Moreover, the huge heterogeneity in mussel density and local biomasses that originates from dredged mussel-seeding techniques also causes major losses within the first month after seeding (approx. 69% in Capelle et al., 2016), due to competition-losses in the dense parts and hydrodynamic dislodgement in the sparse areas. Offsetting the increased cost of SMCs requires finding ways to increase the productivity of the cultivation cycle by enhancing the survival of the mussel seeds. Another concern associated with using SMCs is that they are typically made of multi-filament synthetic fibers around a core of coated lead, although the lead is increasingly replaced by more environmentally friendly materials such as stones. Potential loss of parts of the SMCs due to storms or currents, such as ropes or buoys, can lead to littering of the seafloor or washing up on shore (Kamermans et al., 2014; Sandra et al., 2019; Skirtun et al., 2022). Besides, the degradation of the synthetic filament fibers can lead to the release of microplastics into the marine environment. The concern of contaminating the environment could be resolved by using biodegradable SMCs.

To potentially improve SMCs by increasing post-harvest yields and overcoming pollution effects, we developed a new type biodegradable shell-filled seed mussel collector. We named it the BioShell-SMC and it consists of a biodegradable sock based on a compound of aliphatic polyesters, placed around a coconut-fiber carrying rope and filled with empty cockle shells. Empty shells increase the available attachment area and have shown to be an excellent attachment substrate for mussel larvae (Commito et al., 2014; wa Kangeri et al., 2014). Mussel larvae prefer settlement on complex substrates since this provides refuge from hydrodynamic forces and predation (Carl et al., 2012; Filgueira et al., 2007). By using shells inside the socks, the BioShell-SMC also provides resource material specifically suitable for on-bottom placement, because attachment substrate to the mussel seed is included. This method offers a more controlled seeding process compared to traditional mussel collectors. Instead of relying on the relatively uncontrolled process of relaying loose mussel seeds on subtidal culture plots, the BioShell-SMC method involves placing the intact collector system (consisting of mussel seeds, cockle shells, biodegradable socking, and coconut rope) on the sea floor to facilitate seeding. In previous research, addition of empty shells increased post-relay mussel survival due to reduced dislodgement risk and decreased competition (Capelle et al., 2019). If the BioShell-SMC indeed increases post-relay seed survival and seed growth, less SMCs will be needed per culture plot. If the annual costs of the BioShell-SMC are comparable with the traditional used seed collector systems, the overall costs per growing plot will thus decrease.

In the present study, we compare mussel seed (*Mytilus edulis*) density and growth between *i*) conventional mussel seed collectors consisting of nylon ropes and *ii*) the BioShell mussel seed collecting technique consisting of biodegradable socks filled with empty cockle shells. We tested if the relative performance of both systems was affected by deployment depth, by applying

the SMCs at contrasting water depths (1, 3 and 5m). In addition, we tested if the results were consistent across collector locations, by applying the mussel seed collectors across two marine systems where SMCs are deployed: the Dutch Wadden Sea and the Oosterschelde. We tested the hypotheses that (1) the biodegradable sock filled with empty cockle shells obtains a similar biomass of mussel seed compared to the conventional mussel seed collector; (2) the relative performance of both systems is consistent across locations. Overall, the results of our experiment will provide the mussel industry with more knowledge on a new potential sustainable and cost-efficient alternative for the conventional nylon mussel collectors.

Material and methods

Design of the seed mussel collectors

In this study we tested a prototype of an innovative seed mussel collector, the BioShell-SMC, and compared its performance to that of a traditional seed collector (Weighted Xmas Tree rope). The BioShell-SMC was composed of a central coconut core rope with a diameter of 15 mm, surrounded by empty cockle shells that were collected from North Sea shell deposits and ranged in size from shell fragments to intact shells of approx. 4 cm in length (Figure 2.1A). A quantity of 0.5 kg of cockle shells was used per meter of coconut rope, serving as an attachment substrate for the mussel seed. To hold the cockle shells in place, a biodegradable sock based on a compound of aliphatic polyesters was utilized. This sock is expected to decompose in the marine environment within a year. The BioShell-SMC was filled using a socking machine normally used to sock rope cultured mussels, but instead of mussels, cockle shells were socked around the coconut fiber rope. For the experiment, the BioShell-SMC was divided into small sections. On these small sections the sock was secured at the bottom and top to the coconut rope using a tie wrap. The traditional seed collector (Xmas Tree rope) was made of a frayed polypropylene rope with straight bristles and three strands of lead running through the center of the rope to help it hang vertically (Figure 2.1A).

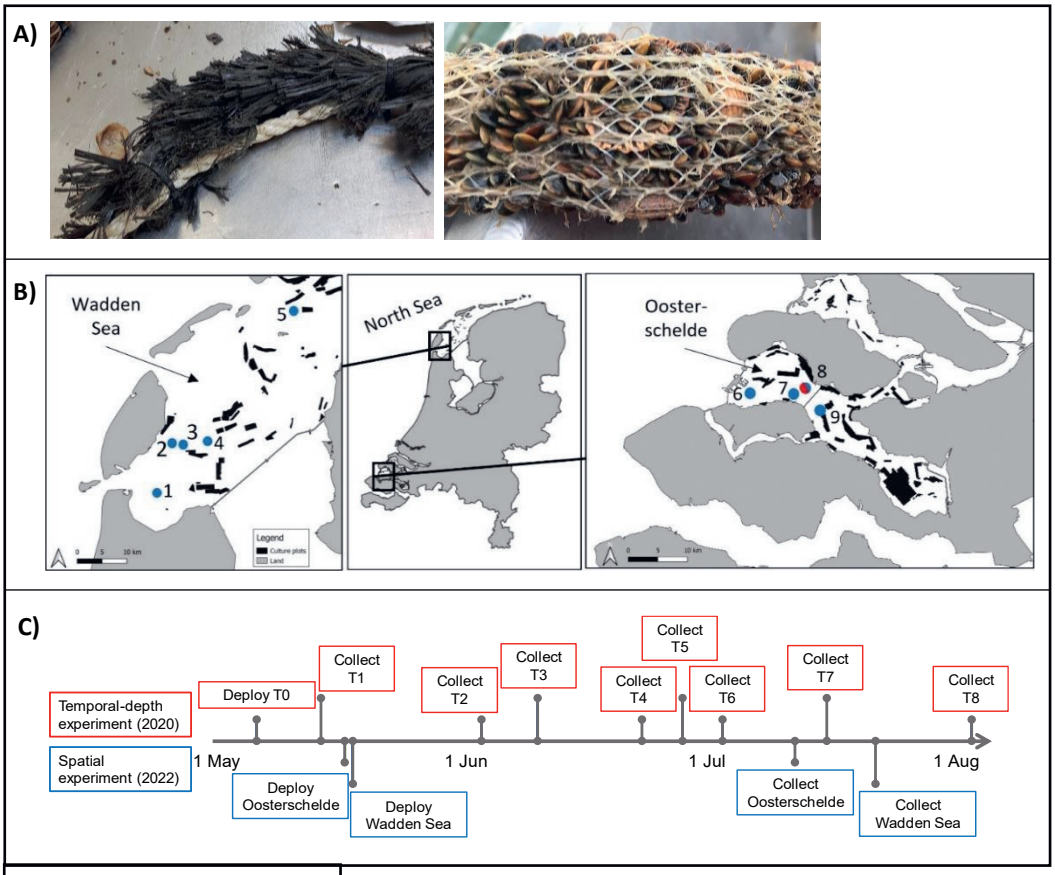
We tested the BioShell-SMC in two field experiments. The first experiment – the temporal-depth experiment – assessed the effects of seed collector type (BioShell-SMC vs. traditional rope), depth (1, 3 and 5m), and time (approx. every two weeks from May to August) on mussel spat (*Mytilus edulis*) density and growth. The second experiment – the spatial experiment – tested whether the effects of collector type (BioShell-SMC vs. traditional rope) varied among spat-catching locations (five locations in the Wadden Sea and four in the Oosterschelde). It is important to note that these experiments were primarily intended as pilot studies to assess the viability of the new methodology in the field. As such, we recognize that the low replication may limit the generalizability of our findings. Ideally, different locations within the SMC-locations would have been selected to place the experimental units and treat each unit as one replicate. However, logistical and material constraints made this unfeasible. Despite collecting the samples from the same experimental unit, we considered that the impact of the experimental unit itself on mussel seed settlement would be minimal. In commercial practice, mussel seed collectors are tightly

lashed together, forming a cohesive unit that functions as a single entity. This physical arrangement ensures that the collectors are in constant contact with each other, allowing for a homogeneous distribution of environmental factors such as water flow, sedimentation, and light exposure. Besides, mussel larvae possess limited mobility and tend to settle close to their origin, although we acknowledge the possibility of some larval movement within the unit. For the statistical analysis, we treated the samples from one experimental unit as independent replicates, since their impact on each other is expected to be minimal.

Experimental setup

Temporal-depth experiment

This experiment quantified the difference in mussel seed density and growth over time and at different water depths between the traditional rope and the BioShell-SMC. Eight experimental seed collector units were deployed at SMC location Vuilbaard in the Oosterschelde, The Netherlands (51.622558, 3.868734) in May 2020 (location 8 in Figure 2.1B). Each experimental unit was made up of a five-meter-long nylon carrying rope (with a diameter of approx. 10 mm), which was divided in three sections based on the deployment depth: shallow (approx. 1m below the water surface), middle (approx. 3m below surface), and deep (approx. 5m below surface) (Figure 2.1D). Each section consisted of a ~30 cm traditional rope (Weighted Xmas tree) and a ~30 cm BioShell-SMC, both attached to the carrying rope with tie-wraps to secure their position. For this experiment, a small area of the commercial SMC location Vuilbaard was utilized. The eight experimental units were tied to the nylon line “backbone” of the commercial Seed Mussel Collector system, which was connected to buoys. Suspended below them were lashed commercial Weighted Xmas tree ropes to depths of approx. 5m. Stone bricks were tied to the bottom of the experimental units to align them vertically in the water column and prevent entanglement. Roughly every two weeks (depending on the weather), one of the eight experimental units was taken out of the water between May and August 2020, and brought to the lab, where they were frozen for processing at a later stage (Figure 2.1C). This means that sampling was conducted until commercial harvest time. At each depth and for each collector type, three samples of 2 – 10



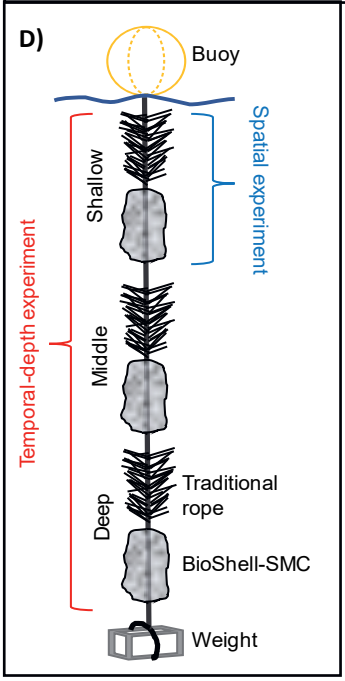


Figure 2.1. A. The two types of mussel collector material. Left: traditional Xmas tree rope, and right: biodegradable BioShell-SMC. The BioShell-SMC consists of a coconut core surrounded by empty cockle shells that are held in place by a biodegradable sock. Mussel seed can settle on the cockle shells. The biodegradable sock is based on a compound of aliphatic polyesters and dissolves after approx. one year. The traditional rope consists of nylon filaments around a core of coated lead. **B.** Maps of the study areas, land is shown in light grey and water in white, mussel culture plots are shown in dark grey. Left: map of locations in the Wadden Sea; blue dots represent site locations for the spatial experiment (2022); 1: Zuidwal, 2: Burgzand, 3: Vogelzand, 4: Gat van Stompe, 5: Zuidmeep. Right: map of locations in the Oosterschelde; blue dots represent site locations for the spatial experiment (2022) and red dot the single site for the temporal-depth experiment (2020); 6: Neeltje Jans, 7: Schaar van Colijnsplaat, 8: Vuilbaard, 9: Vondelinge. **C.** Timeline of temporal-depth experiment (top, blue) and spatial experiment (bottom, red). **D.** Schematic experimental setup. For the temporal-depth experiment, collector material (± 30 cm per piece) was used at three different deployment depths: shallow (± -1m), middle (± -3m) and deep (± -5m). For the spatial experiment, only the upper part of the setup was used. The system consisted of two types of substrate: traditional rope (dark grey) vs. BioShell-SMC (light grey).

cm were taken from the experimental unit for subsequent analysis, resulting in a total of 18 samples per sampling date, all obtained from the same experimental unit. We treated the samples from one experimental unit as independent replicates, since their impact on each other is expected to be minimal.

All mussels were removed from the samples, counted and weighted. Additionally, the length of the collector rope (traditional rope vs. BioShell-SMC) was measured to determine the average weight and number of mussels per meter. Mussel length and condition index were measured for a subset of mussels. During the initial two sampling periods (T1 and T2), no mussel seeds were discovered. The first mussel seed was observed on T3. However, these mussels were only used to obtain number of mussels and not for mussel biomass. For T4 and T8, 30 mussels per sample (or as many as present when less than 30 mussels were available) were measured for shell length. For T5 and T6, 60 mussels per sample were measured. T7 was lost during the experiment and, therefore, could not be taken into account. The condition index (mg cm^{-3}) of the mussels was only obtained at the final sampling day (T8). Therefore, 90 mussels for every depth and collector type were measured for length and weight. Ash-free dry-weight (AFDW) for every mussel was obtained by drying the flesh at 70°C for 2 – 4 days and ashing it at 560°C for 2 hours. The condition index (CI) was calculated (by dividing the AFDW by the cubed length) for every individual mussel in mg cm^{-3} (Beukema & De Bruin, 1977).

Spatial experiment

The difference in mussel seed biomass between the traditional rope and the BioShell-SMC was tested at different locations in the Wadden Sea and Oosterschelde. These locations were chosen since the Dutch government selected these areas for SMC deployment, making comparison with previous studies possible. A total of nine experimental units were deployed, five at locations in the Wadden Sea on May 18th 2022, and four at different locations in the Oosterschelde on May 17th 2022 (Figure 2.1B). Each experimental unit consisted of both traditional rope and BioShell-SMC. The locations in the Wadden Sea were separated by a minimum of 4 km and a maximum of 50 km. In the Oosterschelde, the locations were between 2 to 15 km apart. We retrieved the experimental units on the 12th of July in the Oosterschelde and the 22nd of July in the Wadden Sea. They were therefore collected well before commercial harvest time to prevent systems getting damaged or lost during commercial harvest activities. Due to rough weather, it was impossible to collect the experimental units in both marine systems at the same time. In the lab, we took four subsamples (~10 cm) of both traditional rope and BioShell-SMC from every experimental unit to estimate mussel biomass. This resulted in a total of eight samples per location, all originating from the same experimental unit.

Mussel biomass

Since the samples were frozen, we were not able to measure fresh weight of the mussels. Instead, we estimated the weight from shell length. Mussel biomass was based on length:biomass relationships established from culture plots in the Oosterschelde between 2014 and 2022 (based

on average weight and length values of 752 samples, with a minimum of 30 mussels per sample, resulting in approx. 30,000 mussels. Data from Wageningen Marine Research):

$$\text{Mussel weight} = 0.0002 \times \text{shell length}^{2.8} \quad (1)$$

For each sample, we estimated the mean mussel biomass by multiplying the number of mussels by the average mussel weight, which was converted from shell length using equation (1). Because sample lengths differed, we expressed mussel biomass as a function of sample length (i.e. kg/m rope or BioShell-SMC).

Statistical analysis

All statistical testing was carried out in R studio (R Studio Team 2022), with the critical alpha value for significance being set to $p = 0.05$. Prior to model fitting, we checked assumptions of normality and homogeneity of residuals visually, following the procedure described in Zuur et al. (2010). If necessary, data were transformed to meet assumptions. The Kenward-Roger method was used for obtaining degrees of freedom. Where relevant, pairwise comparisons were obtained by Tukey posthoc tests with the *contrast* and *lsmeans* functions from the lsmeans package (Lenth, 2016).

Temporal-depth experiment

We wished to determine the effect of collector type (traditional rope vs. BioShell-SMC) and depth (1m vs. 3m vs. 5m) on the response variables (mussel biomass, number of mussels, mussel length and condition index) at the final sampling date, since this harvest time is most relevant for aquaculture practice. We used an ANOVA to evaluate the mussel responses to collector type and depth. Each analysis evaluated 18 samples total (three replicates $\times$ two collector type $\times$ three depth strata = 18 samples, all originating from the same experimental unit). Because we wanted to know whether the response to collector type would be different depending on water depth, we included an interaction between collector type and depth (Response $\sim$ collector type x depth). Data of mussel biomass and number of mussels were not transformed. Model simplification for these variables was achieved by removing collector type and the interaction between collector type and depth, which resulted in these models: Mussel biomass ~ depth and Number of mussels ~ depth. Normality of residuals improved when the data of the response variables length and condition index were log-transformed. The best models for length and condition index based on AIC were: Length ~ collector type x depth and Condition index ~ collector type x depth.

Spatial experiment

We tested the effect of collector type (traditional rope vs. BioShell-SMC) and location (five locations in the Wadden Sea and four locations in the Oosterschelde) on the response variable mussel biomass. Since the mussel collectors of the spatial experiment were collected in two different ecosystems (Wadden Sea vs. Oosterschelde) and ten days apart, we first tested for

differences between means of mussel seed biomass of all locations within each system with a two-way ANOVA of the best model (Mussel biomass ~ collector type x ecosystem). Since this was significant, we separated both ecosystems to simplify further analyses. Because we wanted to know whether the response to collector type would be different depending on location, we evaluated the model that included an interaction between collector type and location (Response ~ collector type x location). The analysis of the Wadden Sea evaluated 40 samples total (four replicates × two collector type × five locations = 40 samples, all originating from the same experimental unit) and 32 in the Oosterschelde (four replicates × two collector type × four locations = 32 samples, all originating from the same experimental unit). Simplification of the Wadden Sea model did not result in a better fit and we therefore used the model with interaction (Mussel biomass ~ collector type x location). For the Oosterschelde, the best fit was a reduced model for biomass: Mussel biomass ~ location. The biomass data were not transformed.

Results

Temporal-depth experiment: effect of collector type and deployment depth on mussel biomass, number of mussels, mussel length and condition index

Mussel biomass - The average mussel biomass increased over time at all depths on both of the collector types, with a slight decrease observed at the end of June (Figure 2.2A). Both collector types showed a similar trend, with biomass levels increasing from almost 0 kg/m in May to over 2 kg/m in August. We found no significant effects of deployment depth or collector type on mussel biomass at the final sampling time (T8) (Table 2.1, Figure 2.2A). Additionally, we did not observe any significant interaction between deployment depth and collector type. These findings suggest that both collector types were equally effective in collecting mussel seed. The average biomass collected at the final date was 6.34 ± 2.42 kg/m.

Number of mussels - In contrast to the increasing biomass, the number of mussels decreased over time (Figure 2.2B). Major spat settlement occurred between the 3rd of June (T2) and the 23rd of June (T4), resulting in maximum numbers of almost 28000 mussels per meter found at the end of June. Subsequently, the number of mussels decreased until the final sampling date (T8). In the shallow depth, both collector types showed comparable numbers of mussels over time. However, in middle and deep water, greater numbers of mussels were observed per unit length on the traditional rope than on the BioShell-SMC up until the final sampling date. At the final sampling date, the number of mussels increased with deployment depth on both collector types (Table 2.1, Figure 2.2B). The greatest quantity of mussel seed was collected at deep deployment depths (8067 ± 1759 per meter), followed by middle (2887 ± 890 per meter) and shallow depth (2632 ± 464 per meter) (Tukey, $p < 0.001$). There was no significant difference in number of mussels between the traditional rope and BioShell-SMC, indicating again that collector type did not affect the number of mussel seed that settled on the collectors.

Mussel length - The total number of mussels measured in this experiment was 3216. Mussel length increased from approx. 7 mm in mid-June to almost 26 mm at the beginning of

August. A slight decrease in average length was observed at the end of June across all deployment depths (Figure 2.2C). This reduction in length, coupled with a decrease in mussel numbers, suggests disproportionate losses of larger specimens, particularly in shallower water. At the final sampling date, we observed a significant interaction between deployment depth and collector type (Table 2.1). Mussels near the surface were significantly longer on the BioShell-SMC (26.52 ± 2.64 mm) compared to the traditional rope (24.81 ± 4.79 mm) (Tukey, $p = 0.006$). In contrast, at middle deployment depth, mussels on the traditional rope were longer than those on the BioShell-SMC (26.74 ± 5.87 mm and 24.81 ± 4.92 mm, respectively) (Tukey, $p = 0.026$). For both the traditional rope and the BioShell-SMC, the shortest mussels were found in deep water (19.96 ± 3.71 mm), and there was no significant difference between the two collector types (Tukey, $p < 0.001$). We found no main effect of collector type on mussel length at the final sampling date (Table 2.1, Figure 2.2C).

Mussel condition index - The condition index of mussels was significantly affected by deployment depth, collector type and the interaction between these factors (Table 2.1). At deep deployment depth, we found a significant higher condition index for mussels attached to the BioShell-SMC (Figure 2.3). However, we observed no significant difference between collector types at shallow and middle deployment depths. Upon examining mussels on the traditional rope only, we found the lowest condition index in deep water, compared to both middle and shallow water (Tukey, $p < 0.001$). Conversely, on the BioShell-SMC, we observed opposite results, with a higher condition index for mussels at deep deployment depth compared to both middle and shallow depths (Tukey, $p < 0.001$).

Table 2.1. ANOVA results of the temporal-depth experiment, using mussel biomass (kg/m), number (nr) of mussels, mussel length (in mm) and mussel condition index (CI, in mg cm^{-3}) as dependent variables and collector type (traditional rope vs. BioShell-SMC) and deployment depth (shallow vs. middle vs. deep) as explanatory variables.

	Mussel biomass (n=3)				Nr of mussels (n=3)				Mussel length (n=90/180)				Mussel CI (n=60)			
Predictor	Sum of squares	*Df*	*F*	*p*	Sum of squares	*Df*	*F*	*p*	Sum of squares	*Df*	*F*	*p*	Sum of squares	*Df*	*F*	*p*
Collector	0.04	1	0.56	0.405	0.02	1	0.284	0.808	0.00	1	0.00	0.945	9.61	1	184.31	<0.001***
Depth	0.70	2	4.52	0.166	5.00	2	37.26	<0.001***	4.29	2	50.93	<0.001***	1.97	2	18.85	<0.001***
Collector x depth	0.41	2	3.67	0.523	0.15	2	1.09	0.520	0.53	2	6.27	0.002**	4.81	2	46.12	<0.001***

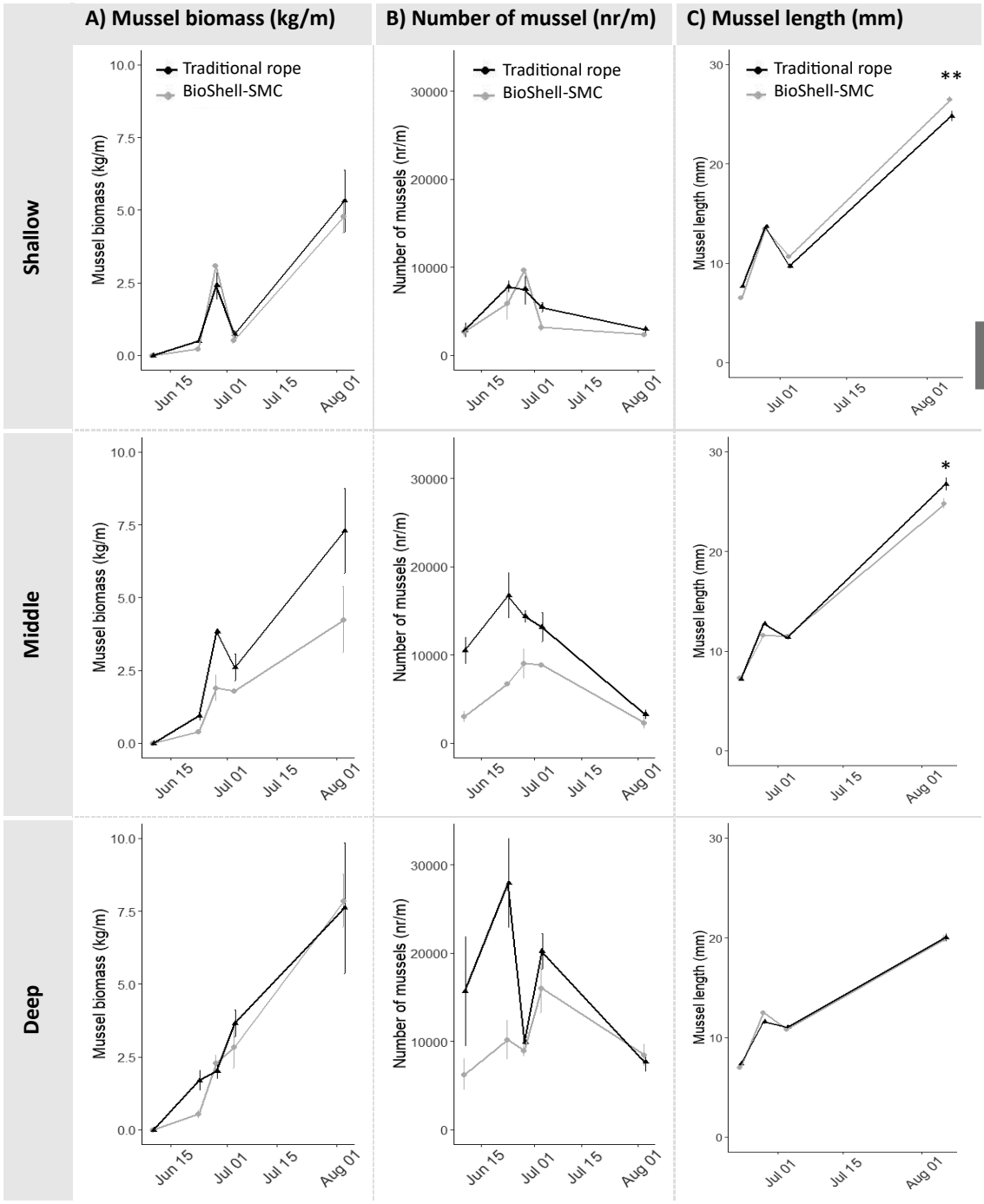


Figure 2.2. Overview of development of mussel biomass (**A**, in kg/m), density (**B**, in nr/m) and length (**C**, in mm) over time at different deployment depths (shallow, middle and deep) at location Vuilbaard in the Oosterschelde. Dark grey: traditional rope (Xmas Tree), light grey: BioShell-SMC. Data are means ± SE (n = 3 for biomass and number of mussels, n = 90 for length of T4 and T8 and n = 180 for length T5 and T6). Asterisk on top at final sampling date denote significance with * <0.05 and ** < 0.01.

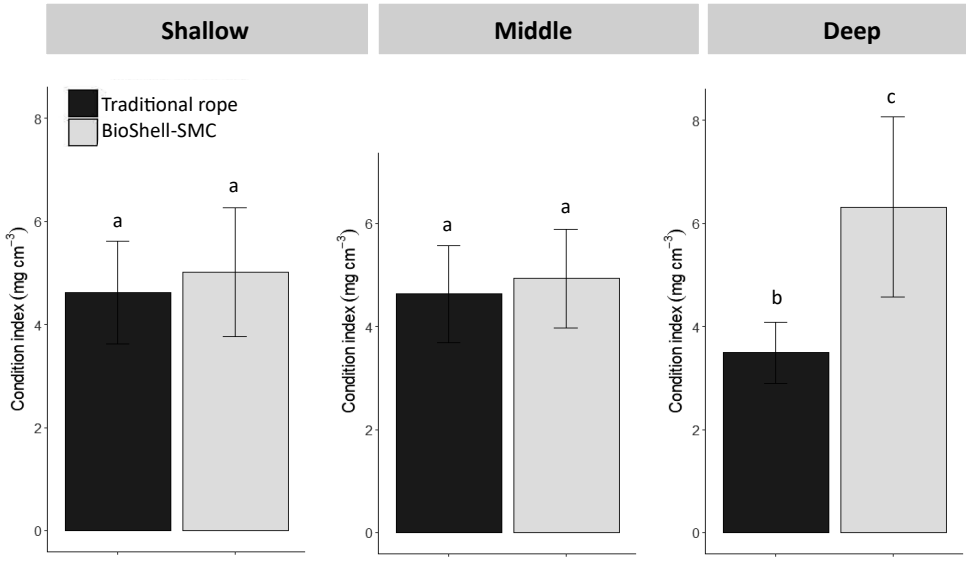


Figure 2.3. Mean mussel condition index (mg cm-3) at the end of the experiment at shallow, middle and deep deployment depth. Dark grey: traditional rope (Xmas Tree), light grey: BioShell-SMC. Data are mean ± SE (n = 60). Letters denote significance.

Spatial experiment: effect of collector type and location on mussel biomass

In the second experiment, we aimed to test whether location affected the relative performance of both collector systems. Since the Wadden Sea and the Oosterschelde are different ecosystems and data were collected ten days apart, we initially examined the effect of marine system (Wadden Sea vs. Oosterschelde) on final seed biomass. We measured 100 mussels per subsample (or less when not 100 mussels were present, with a minimum of 34) to obtain the total mussel biomass, resulting in a total of 6722 mussels. We found an interaction effect between the marine system and collector type (Table 2.2).

Table 2.2. ANOVA results of the spatial experiment using mussel biomass (kg/m) as dependent variables and collector type (traditional rope vs. BioShell-SMC) and origin (Wadden Sea vs. Oosterschelde) or location (five locations in the Wadden Sea and four locations in the Oosterschelde) as explanatory variables.

	Mussel biomass (n=4)					Mussel biomass Wadden Sea (n=4)				Mussel biomass Oosterschelde (n=4)			
Predictor	Sum of squares	*Df*	*F*	*p*	Predictor	Sum of squares	*Df*	*F*	*p*	Sum of squares	*Df*	*F*	*p*
Collector	0.02	1	0.02	0.891	Collector	2.38	1	4.96	0.034*	0.02	1	0.05	0.825
Origin	8.00	1	9.50	0.003**	Location	11.74	4	6.11	0.001**	19.77	3	21.59	<0.001***
Collector x origin	3.49	1	4.14	0.046*	Collector x location	6.80	4	3.54	0.018*	0.77	3	0.79	0.509

Additionally, a significant main effect of the marine system was observed, while no significant main effect of collector type was found. The average biomass on the experimental units in the Oosterschelde was lower than in the Wadden Sea. In the Wadden Sea, the units collected an average of 1.34 kg mussel seed per meter, and in the Oosterschelde, 0.36 kg/m was collected. When we only looked at the Wadden Sea, we found a significant interaction between location and collector rope (Table 2.2), which was explained by three locations: Zuidwal (Location 1, Tukey, $p < 0.001$), Burgzand (Location 2, Tukey, $p = 0.034$) and Vogelzand (Location 3, Tukey, $p = 0.049$) (Figure 2.4). In addition, we should note that the difference in mussel biomass in the Wadden Sea was much greater between these three locations than between collector types. At the other locations, we did not find a difference between mussel density on the two collector types. In the Oosterschelde, we found no effect of collector type on mussel biomass or an interaction between location and collector type (Table 2.2). We only found a main effect of location. The lowest biomass was found on the experimental units located at Neeltje Jans and Vuilbaard, and the highest biomass at Vondelinge and Schaar van Colijnsplaat (Figure 2.5).

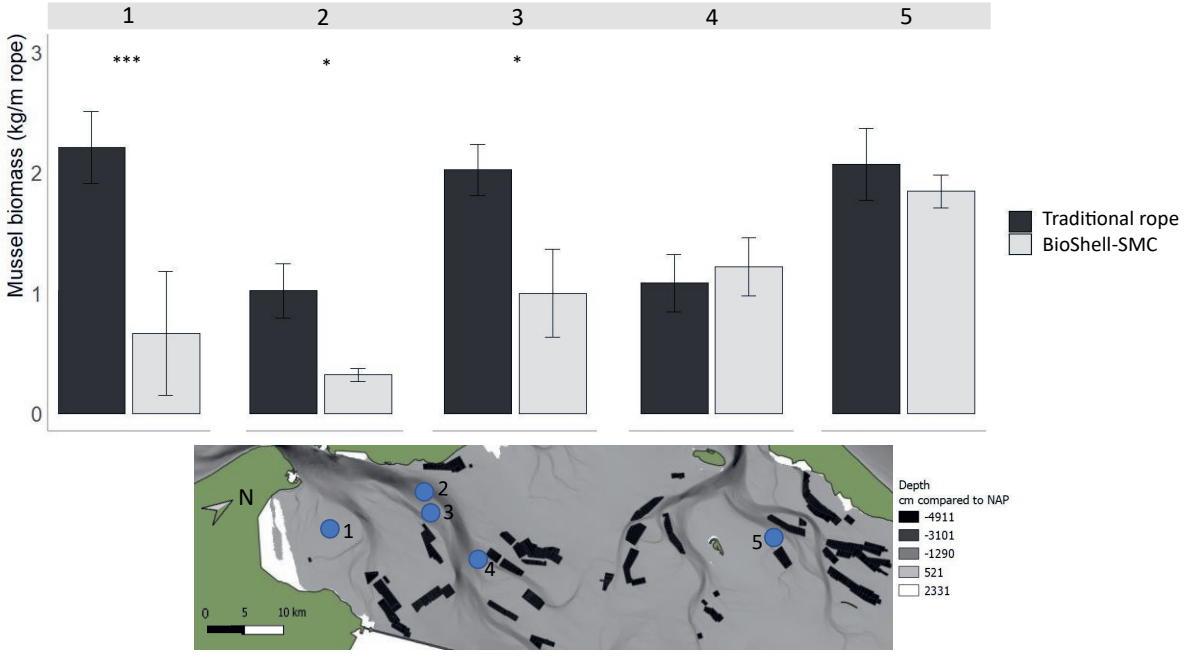


Figure 2.4. Mussel biomass on traditional rope (Xmas Tree, dark grey) and BioShell-SMC (light grey) at five different locations in the Wadden Sea. 1: Zuidwal, 2: Burgzand, 3: Vogelzand, 4: Gat van Stompe, 5: Zuidmeep. Land is shown in green and water in greyscale. Mussel culture plots are shown in dark grey. Data are means ± SE. Asterisk on top denote significance with * <0.05, ** < 0.01.

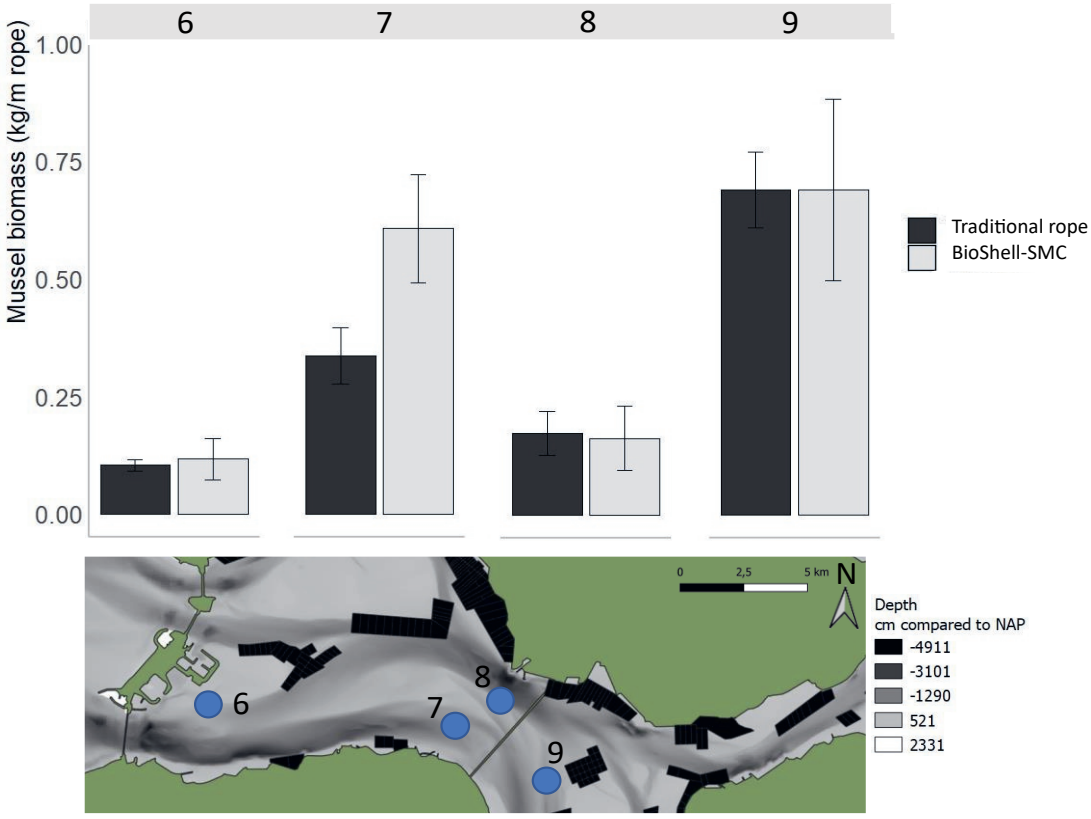


Figure 2.5 Mussel biomass on traditional rope (Xmas Tree, dark grey) and BioShell-SMC (light grey) at four different locations in the Oosterschelde. 6: Neeltje Jans, 7: Schaar van Colijnsplaat, 8: Vuilbaard, 9: Vondelinge. Land is shown in green and water in greyscale. Mussel culture plots are shown in dark grey. Data are means ± SE.

Discussion

We developed the BioShell-SMC and compared it with traditional Xmas Tree rope across deployment depth and location, using mussel cultivation in The Netherlands as a case study. The results of our field experiments showed that mussel density was comparable between the two collector types, except for three locations in the Wadden Sea. Overall, mussel density was spatially heterogeneous, both between and within marine systems. We also found that mussel seed biomass was not affected by deployment depth, while the mussel quantity increased with deployment depth. In addition, mussels in deep water were shorter than in shallower water.

Role of substrate on mussel seed settlement

Mussel larvae in the water column are capable of distinguishing between different settlement substrata (Gosling, 2003). Moreover, settlement of mussel seed is higher on rough compared to smooth surfaces (Carl et al., 2012b; Gribben et al., 2011), and filamentous collecting

substrata (Brenner & Buck, 2010; Filgueira et al., 2007; Walter & Liebezeit, 2003), including fine-branching algae and hydroids (Alfaro & Jeffs, 2002; Buchanan & Babcock, 1997). Biodegradable materials are increasingly being used in mussel cultivation around the world. This is seen in for example China with paper-like material used for a sock-type bag (Mao et al., 2019), a natural fiber mesh around a central SMC in Chile (Gonzalez-Poblete et al., 2018) and a cotton stocking in New Zealand (Skelton & Jeffs, 2021). In addition, there are various pilot studies looking at the durability of biodegradable SMCs and the possible applications in aquaculture (e.g. DSOLVE (uit.no/research/dsolve) and BIOGEARS (biogears.eu)). However, these developments all aim to grow out of seed to commercial sized mussels in longline culture, which is globally the most used culture method for mussels (Kamermans & Capelle, 2019). As far as we know, there has never been developed a sustainable SMC that could be applied to bottom culture to increase mussel yield.

In the present study, we expected to find comparable mussel seed biomasses on the BioShell-SMC compared to the traditional rope, since shell fragments are shown to create a suitable attachment substrate for mussel seed (Commito et al., 2014; wa Kangeri et al., 2014). Throughout our temporal-depth experiment, we observed a generally higher number of mussels on the traditional rope, except for the final sampling date in August (which coincides with commercial harvest time), where we found similar results on both the traditional rope and the BioShell-SMC. The reduction in the number of mussels on the traditional rope compared to the BioShell-SMC at the end of the experiment could be attributed to the cockle shells offering better protection for mussel seed from predators or hydrodynamic forces than the traditional frayed ropes in this sheltered location. Alternatively, as the biomass increases, there is less substrate available for attachment, resulting in a space forming between mussels and the traditional rope. This space gets filled up with (pseudo)faeces or fouling (personal observation), leading to the dislodgement of the mussels. In our spatial experiment, we observed a comparable mussel biomass on both types of collectors, except for three locations in the Wadden Sea, where the traditional rope had higher biomass than the BioShell-SMC. The mussel seed may have been better protected from the exposed locations on traditional ropes than on the BioShell-SMC, which is further discussed in paragraph 4.3. Another possible explanation for the higher biomass on the traditional rope might be the limited time that the experimental units were in the water. Indeed, in our temporal-depth experiment, we noted higher biomasses on the traditional rope at the beginning, but the biomasses became comparable to the BioShell-SMC by the end of the experiment. However, since we collected the experimental units in July for the spatial experiment, we cannot determine whether the biomass on the traditional ropes would have decreased more than the BioShell-SMC over summer. Our inability to prolong the experiment was due to logistical constraints and the start of the busy season for the mussel growers, which increased the risk of losing systems.

Role of depth on mussel seed settlement

In the present study, we found more and smaller mussels on the deeper parts of the mussel collectors compared to parts near the surface. The cause of the smaller size of the mussels in deeper parts remains unclear, although a size effect has been observed in other studies as well.

In a study with *Perna canaliculus*, they found smaller mussels at shallower depths and higher mussel abundances were seen at greater depths (Alfaro & Jeffs, 2003). According to the authors this happened because of the greater buoyancy and migratory capability of smaller mussels compared to generally heavier and larger mussels. The depths used in that study varied from 2 m (shallow) to 18 m (deep). In our study, the deepest part of the collector system was situated at 5 m, which might be too shallow to be explained by differences in buoyancy and migratory capability. Besides, the majority of seed losses in a study on *P. canaliculus* occurred while small-scale migrations took place, which is a process that enables juveniles to actively resettle on substrata (Skelton & Jeffs, 2020; South et al., 2017). These results suggest that mussel seed is highly vulnerable to loss factors during these migrations.

Variation in the vertical distribution of mussel seed biomass on collectors was found in more studies, with higher settlement in the upper and intermediate parts (1 and 5 m) than in the lower parts (9 m) (Fuentes & Molares, 1994), which is comparable with the results we found. The presence of a thermocline between 5 and 10 m during summer is one of the explanations given in the paper for the vertical differences. It is unlikely that such thermocline played a role in our experiment, since the water is well mixed in both Wadden Sea and Oosterschelde. However, due to the high average windspeeds at the end of June and the beginning of July (Appendix A), the heavier and larger mussels on the outside of the experimental unit might have fallen of which has led to a smaller average mussel size after the storm. Winds and waves have a bigger impact on the shallower part compared to the deeper part, which led to lower biomasses in the shallower parts. We expect that higher number of mussels in deeper water subsequently lead to increased competition for food and space between individuals (Newell, 1990; Okamura, 1986), leading to significantly smaller mussels, with a lower condition index at deeper water during the final harvest.

Role of location and time on mussel seed settlement

In 2022, the mussel biomass was on average twice as great in the Wadden Sea than in the Oosterschelde. The higher mussel biomass in the Wadden Sea may be attributed to the longer duration the mussels spent in the water (10 days). Although this time frame might seem insignificant, our results (Figure 2.2) demonstrated that it can result in a significantly increase in biomass, up to a doubling. Another explanation for the variation in biomass between the two ecosystems is that the Wadden Sea is characterized by a more frequently abundant spat fall (Capelle, 2017) and higher growth rates of the spat compared to the Oosterschelde (Van Stralen, 2016). This spatial variation within each ecosystem was also shown by Capelle (2022), who reported the mussel biomass at harvest per meter seed mussel collector at different locations in The Netherlands since 2010. He found biomasses varying from less than 1 kg/m SMC to over 5 kg/m, depending on the location and year. Natural spat fall shows large yearly fluctuations (Capelle, 2017), which can partly explain the differences in biomass. However, we should consider that the experimental units of our spatial experiment were in the water for a shorter period of time compared to the units in our temporal-depth experiment and the collectors from Capelle (2017). This might explain the much higher biomasses found by Capelle (1 kg/m SMC to over 5 kg/m) and in our temporal-depth experiment (6.34 ± 2.42 kg/m) compared to our spatial experiment (0.17 ±

0.11 kg/m) at location Vuilbaard. We saw a steep increase in biomass in the last weeks of our temporal-depth experiment, indicating that higher biomasses would have been obtained in the spatial experiment as well when the experimental units were kept in the water for a longer period of time.

Spatial differences in mussel larval settlement between locations have been extensively documented (Capelle, 2023; Fuentes & Molares, 1994; Kamermans et al., 2002; Karayücel & Karayücel, 2001), even on small spatial scales (Fuentes & Molares, 1994; Snodden & Roberts, 1997), as was the case in our study. Some locations were less than 1 kilometer away from each other, but still resulted in large differences in biomass (e.g. Schaar van Colijnsplaat and Vuilbaard). This indicates that factors that affect settlement, growth and survival vary on small scale. In studies on *Mytilus galloprovincialis*, higher settlement densities were found at locations more seaward compared to locations more upstream, while the locations were only 5 – 10 km removed from each other (Fuentes & Molares, 1994; Marguš & Teskeredžić, 1986).

In our study, we observed higher biomass on the traditional rope at the three most Western locations in the Wadden Sea. These locations are more exposed to the dominant South-western wind than the other locations in the Wadden Sea and Oosterschelde. The fourth location (Gat van Stompe) is relatively close to the third (Vogelzand), but it is situated on the other side of the gully, resulting in a more sheltered surrounding. The difference in biomass between traditional rope and BioShell-SMC might be due to the different effects of currents and hydrodynamics on both substrate types. Although initial settlement might be comparable, survival on the traditional rope is higher in exposed areas. A possible explanation is that the cockle shells in the BioShell-SMC rub against each other when water conditions are rough, resulting in decreased survival since small mussel seeds might get crushed. In areas with lower turbulence and predation, the final biomasses on both collector types were found to be comparable.

Our temporal-depth experiment showed better initial settlement on the traditional rope, but in the end, we did not observe any significant difference, suggesting that survival is higher on the BioShell-SMC. However, this observation is only valid for more sheltered areas, where the complexity of the substrate of the BioShell-SMC might seem to enhance survival and compensate for the lower initial settlement. Studies on the interaction between location and collector type are relatively scarce, but two studies in the Wadden Sea and in Norway found differences in the performance of collector type by substrate. (Kamermans et al., 2002; Lekang et al., 2003). These findings suggest that many factors are involved in mussel seed settlement (Peteiro et al., 2007), including timing and magnitude of mussel reproduction (Cáceres-Martínez & Figueras, 1998), algal and microbial coverage associated with the substrate (Hunt & Scheibling, 1996), nutrient availability (Pechenik et al., 1990) and temperature and salinity (Brenko & Calabrese, 1969; Manoj Nair & Appukuttan, 2003). Although we did not measure additional factors at the different sites in our experiment, the spatial variability in mussel settlement suggests that many factors likely contributed to our results. This can be due to differences in mussel seed settlement, but also due to variation in loss (e.g. current or storms) after settlement.

Implications for aquaculture practice

High mussel mortality shortly after seeding plays an important role in the overall production efficiency of mussel cultivation (Capelle et al., 2014; South et al., 2020). The small size of the mussels collected with SMCs and the lack of attachment substratum makes them highly vulnerable to loss factors when seeded on bottom culture plots, such as competition in high density areas and hydrodynamic dislodgement in sparse areas (Bertolini et al., 2019). The sustainable biodegradable BioShell-SMCs provide a new approach for mussel bottom cultivation. Mussel growers can gain more control on seeding, since these SMCs are harvested as an entire system rather than only the mussel seed, which offers opportunities for a larger control on the spatial deployment methods. That is, instead of a relative uncontrolled relaying of loose mussel seeds on subtidal culture plots form traditional mussel collectors, the BioShell-SMC allows seeding by placing the intact socked shells on the sea floor (i.e., mussel seeds, cockle shells, biodegradable socking and biodegradable inner-SMC). The mussels are already attached to a substrate that could potentially be suitable for long-term attachment, allowing them to avoid secondary migrations. This method is therefore specifically relevant to bottom culture.

The expected increase in seed survival and growth means that less mussel seed is needed per culture plot, which remains to be tested. Besides implications for mussel cultivation, our BioShell-SMC could also provide a promising solution to restoration of mussel beds in highly dynamic ecosystems, as attachment substratum has shown to increase retention of transplanted mussel seed (Schotanus, Capelle, et al., 2020). Since the BioShell-SMCs in our experiment showed a comparable collection success at most locations, the possible higher survival rates of mussel seed attached to the BioShell-SMC when seeded might result in higher yields. The results of the current study provide a promising start toward a more sustainable mussel seed collection for bottom cultivation, with prospects to improve overall yield.

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Chapter 3

Increasing mussel transplantation success by initiating self-facilitating feedback mechanisms

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Abstract

Transplantation success of ecosystem-engineering species can be low in dynamic environments, as such ecosystem-engineers often require density-dependent positive feedback mechanisms to overcome environmental stressors. These self-facilitating feedback mechanisms play an important role in self-organization, whereby complex systems tend to organize and create patterns in order to ameliorating physical and/or biological stressors. In this study we used biodegradable structures to ameliorate self-facilitating feedback mechanisms to overcome environmental stressors in the initial post-transplantation phase. The biodegradable structures tested are an innovation of the traditional Seed Mussel Collectors (SMCs) used in mussel cultivation. The so-called "BioShell-SMC" does not contain any plastic, but is made up of a coconut fiber rope surrounded by empty cockle shells and held together by a biodegradable net based on a compound of aliphatic polyesters. We tested if the survival of two size classes of blue mussel (*Mytilus edulis*) transplants, on a tidal flat in the Oosterschelde estuary in the Netherlands, increased when mussel seed was transplanted attached to the BioShell-SMCs instead of single mussels in combination with empty cockle shells. The results of this study revealed that the survival of larger mussel seed significantly improved when attached to the BioShell-SMC compared to those transplanted loosely. Factors contributing to the difference in mussel loss between BioShell-SMC mussels and loosely transplanted mussels include predation, competition and dislodgement due to hydrodynamic forces. For small mussel seed, mussel biomass decreased strongly in the first three days of the experiment, irrespective of transplantation method. This is due the small size of the mussels in combination with low mussel densities. Overall, this study highlights the potential of using biodegradable structures to initiate self-facilitating feedback mechanisms in establishment of ecosystem engineers in dynamic environments.

Introduction

Restoration of coastal ecosystems by transplantation of habitat-forming ecosystem-engineering species has become a key conservation tool to counteract coastal degradation (Byers et al., 2006). In recent years, incorporating ecological processes into restoration efforts such as harnessing of self-facilitation between transplants is increasingly recognized as a fundamental component of successful restoration (Ladd et al., 2018; Renzi et al., 2019; Silliman et al., 2015). Several studies have demonstrated that restoration success can be enhanced by using clumped individuals rather than spacing individuals out, or by stimulating the formation of natural aggregations (Fivash et al., 2022; Schotanus, Walles, et al., 2020; Shaver & Silliman, 2017; Silliman et al., 2015; Suykerbuyk et al., 2016). For example, salt-marsh grasses planted in clumps benefited each other by alleviating physical stressors such as anoxia and erosion that improved transplantation success (Silliman et al., 2015). Furthermore, by stimulating the formation of large-scale aggregation in transplanted mussels, the survival of a restored mussel bed increased (Schotanus, Walles, et al., 2020). All these restoration efforts were however still at an experimental scale. Integration of positive interactions on a large restoration scale as needed to have landscape-scale impact is still a challenge. Innovations are thus needed to improve the likelihood of truly large-scale restoration success, to decrease the numbers of transplants required for long-term restoration and to reduce the application costs (Temmink et al., 2020).

To learn about how to upscale restoration, we may look at how patterns form in natural landscapes. Self-organized spatial patterns as observed in a wide range of ecosystems (Rietkerk & Van de Koppel, 2008), including arid systems, peatlands, forests, mussel beds and diatom mats (Liu et al., 2020; van de Koppel et al., 2005; Weerman et al., 2010), typically result from positive intraspecific interactions at the local scale combined with negative intraspecific interactions at a larger scale. These local-scale self-facilitating interactions typically occur when a certain patch density and/or size threshold is surpassed (Bouma, Friedrichs, et al., 2009) following a window of opportunity, i.e. a sufficiently long period of calm conditions in which individual organisms can settle without being in a patch (Balke et al., 2011, 2014). Thus, establishment success of transplanted organisms in the absence of a natural window of opportunity may be increased, if a critical density threshold can immediately be surpassed in order to induce short-range facilitation. The latter may involve transplanting organisms in a way that mimics regular patterns, thereby induce local-scale self-facilitating while minimizing large-scale negative interactions (Rietkerk & Van de Koppel, 2008). Large-scale transplantations of individuals in regular patterns that mimic natural-patterned ecosystems can however be extremely time-consuming and costly. Hence, we propose to use innovative engineering measures that facilitate self-organization to increase transplantation success, using mussels as model system.

Mussel beds on soft sediment are an example of an ecosystem with a distinctive spatial patterning (van de Koppel et al., 2005). The patterning consists of high-density mussel clusters alternating with bare sediment patches and is thought to be the result of an interplay of facilitation and competition between mussels at different spatial scales (Liu et al., 2014; van de Koppel et al.,

2005). Aggregation in clusters facilitates protection against predators and increases resistance to erosion by waves and currents. However, if clusters become too large, it also increases competition for food and space, impeding the growth and condition of the mussel (Commito et al., 2014; van de Koppel et al., 2005). For example, mussels in the middle of a mussel patch tend to be smaller than mussels on the outside of a mussel patch (Svane & Ompi, 1993). Transplantations of juvenile blue mussels (*Mytilus edulis*) on bare sediment have been carried out as an attempt to restore natural mussel beds (de Paoli et al., 2015; Schotanus et al., 2020a), but is much more common to cultivate mussels for consumption (Capelle et al., 2014). In both cases, the mussel bed restorer and the mussel farmer, face the same problem: juvenile mussels (mussel seed) are vulnerable from environmental stressors, such as hydrodynamic forces and/or predation, and losses in the first month after transplantation are high (Capelle et al., 2016a; Schotanus et al., 2020b). The newly transplanted mussels need sufficient time to establish self-facilitating interactions in order to increase the resilience of the mussel bed (Liu et al., 2014). Here we propose to use biodegradable structures, to initiate local-scale self-facilitating feedback mechanisms to overcome these environmental stressors in the initial post-transplantation phase, which will ultimately enhance the survival and growth of transplanted mussel seed. By spacing-out these biodegradable structures, we can avoid the long-distance negative effects resulting from competition.

The biodegradable structures tested are an innovation of the traditional Seed Mussel Collectors (SMCs) used in mussel cultivation (Van den Bogaart et al., 2023a). Normally, these SMCs consist of frayed nylon ropes suspended in the water column on which mussel larvae settle. When the mussel seed is large enough (2-3 cm), they are harvested from the ropes and transplanted to culture plots to grow to commercial size (Kamermans et al., 2002). The innovative SMCs, or so-called “BioShell-SMCs”, do not contain any plastics, but comprise of a coconut fiber rope surrounded by empty cockle shells held together by a biodegradable net based on a compound of aliphatic polyesters (Figure 3.2). Empty shells have shown to be an excellent attachment substrate for mussel larvae (wa Kangeri et al., 2014). Results of a first comparative field study showed that the mussel seed yield of the BioShell-SMCs was comparable to that of the traditional nylon SMCs at most locations (Van den Bogaart et al., 2023a). One potential advantage of using BioShell-SMCs is that the mussel seed does not have to be removed from the SMCs before transplantation to a cultivation or restoration site, as is the case for the traditional nylon SMCs. That is, mussels can be transplanted attached to the BioShell-SMC, in high-density clusters, which may provide protection from predators, such as crabs and sea stars, and may increase resistance to dislodgement by hydrodynamics. In addition, when the starch nets gradually dissolve (aimed to happen within a year), the cockle shells within will slowly disperse, which may provide an attachment substrate for mussels to spread further away from the BioShell-SMC, escaping competition for food and space and increase resilience to environmental disturbances (Capelle et al., 2019).

To test if innovative engineering measures that facilitate self-organization can increase transplantation success, we carried out three experiments using mussels as model system: i) a *field transplantation experiment* in the Dutch Oosterschelde estuary, ii) an *anti-predation cage field experiment* also in the Dutch Oosterschelde estuary, and iii) a *mesocosm experiment* in the lab. In

the *field transplantation experiment*, we tested the hypothesis that the survival of mussel transplants increases when mussel seed is transplanted attached to the BioShell-SMCs, by providing a self-facilitating feedback mechanisms that help overcome environmental stress during the initial transplantation phase. In contrast, we expect that single mussels in combination with empty cockle shells do not have such self-facilitating feedback mechanisms, as we expect the empty shells do not provide a stable substrate for mussel-seed attachment but are prone to dislodgement. In addition to monitoring the biomass development of mussels attached to the BioShell-SMCs and mussels loose-seeded with shells, we also tested the intermediate treatment: the effect of cutting open the starch net on the development of the mussel biomass. We expect that the spilled-out cockle shells may provide a window of opportunity for mussels to escape competition after the initial transplantation phase, when a more stable environment is established. A complementary *anti-predation cage field experiment* was carried out within the field experiment to test the hypothesis that the growth of mussels in the cages will be lower when transplanted attached to a substrate in high-density clusters than loose transplanted mussels or mussels attached to cut-open nets due to greater competition for space and food, when the cages prevent thinning by predators and washing away by waves. Finally, a *mesocosm experiment* was carried out to test whether crabs or sea stars have a preference for foraging on loose mussels or mussels attached to substrate, in this case the biodegradable SMC. Since constrains such as the chance of predation or dislodgement are greater for smaller mussels while competition becomes more restrictive as the mussel grows larger, all experiments were carried out twice: once with small mussel seed in July and once with larger mussel seed in August.

Materials and Method

Field experiments: transplantation and anti-predation cages

Study site

The field experiment was conducted on an intertidal commercial mussel plot (51°33'26.6"N 3°53'55.0"E), located at the Oosterschelde, the Netherlands (Figure 3.1). The sheltered study area is characterized by sandy sediments and the dominant water flow direction is from the southwest. The experimental plots only emerged from the water during extreme low tides, which occurred approximately 3 times throughout the experiment. For this experiment, 150 m of the BioShell-SMC was deployed at a widely used SMC location in the Nearshore North Sea, (51°46'22.0"N 3°48'10.4"E). The BioShell-SMCs were placed in the water column in April 2020 and the first mussel larvae settled at the end of May 2020.

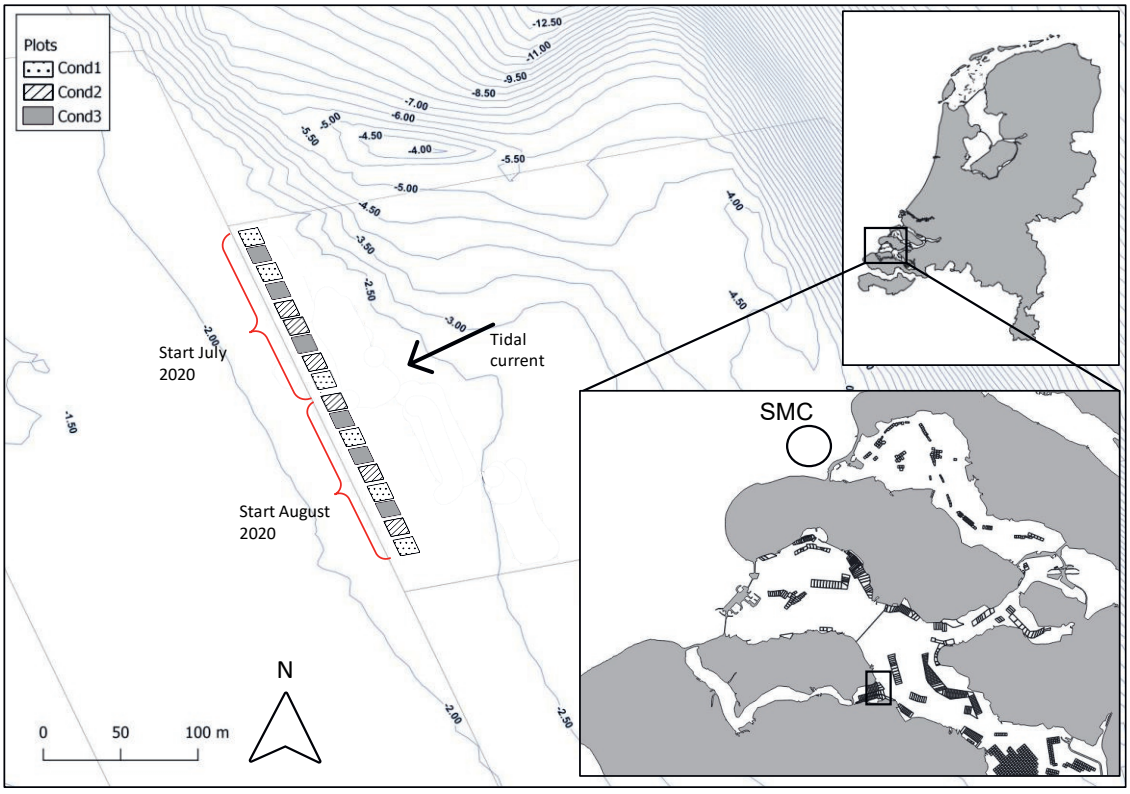

Figure 3.1. Map of the study area. Land is shown in grey and water in white. Mussel culture plots are shown in grey on the overview maps and grey bordered in the close-up image. SMC: origin of the seed (Spat Mussel Collector, SMC). The three transplantation methods are each represented with a different patterned plot. Cond1 (dots): loose mussels and shells; Cond2 (dashed): cut-open net; Cond3 (grey): intact net. The plots measure each 6x6 m and are spaced 1 meter apart. There were three replicates of each treatment. The experiment was conducted twice. The first round trial started in July 2020 and the second round trial in August 2020.

Setup of the field transplantation experiment

Mussels were transplanted in three configurations; (1) *Loose mussels and shells;* the biodegradable net was cut open, the coconut fiber rope and biodegradable net were completely removed and only the mussels and cockle shells were placed on the plots, (2) *Cut-open net;* the biodegradable net was cut open to make sure that the shells and mussels were able to disperse, (3) *Intact net*; the BioShell-SMC was kept intact completely (Figure 3.2). Each treatment comprised three replicates. Mussels were transplanted in plots (6x6 m), with randomly assigned treatments. Metal fences covered with chicken wire were placed around each plot to enclose them and prevent mussels from being washed out. The experimental plots were placed in a row to ensure that they were located at approximately the same depth and faced the prevailing current direction (southwest). To ensure an empty buffer zone between two plots, the mussels were placed in squares of 3x3 m within each plot. Each plot contained mussel seed from approx. 9 m of SMC. In treatment 1 the loose mussels and cockle shells were spread homogenously over the plot. In

treatments 2 (cut-open net) and 3 (intact net) the SMC-rope was transplanted to the experimental plots in one long line (9 m) and placed down as homogeneously as possible over the plot.

Mussel farmers normally harvest the mussel seed from the SMCs and transport them to commercial mussel plots when they are around 2-3 cm (Capelle, 2017). To examine how mussel

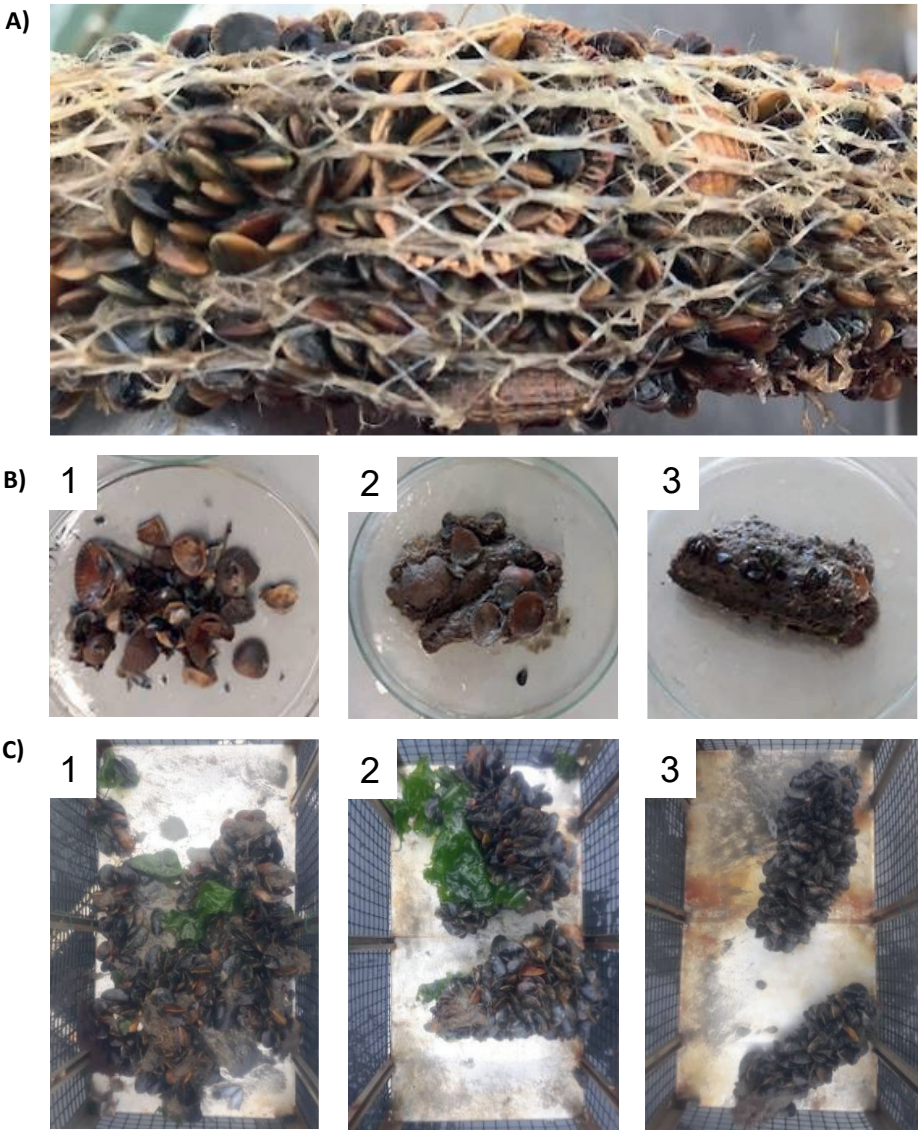

Figure 3.2. A. Biodegradable SMC ("BioShell-SMC"), which consists of a coconut core surrounded by empty cockle shells that are held in place by a biodegradable sock. Mussel seed has settled on the cockle shells. **B/C.** Three configurations of mussel transplantation methods: (1) loose mussels and shells; the biodegradable net was cut open, the coconut fiber rope and biodegradable net were removed and only the mussels and cockle shells were transplanted, (2) Cut-open net; the biodegradable net was cut open to make sure that the shells and mussels were able to disperse, (3) Intact net; the SMC was kept intact completely. **C.** The three configurations in the anti-predation cages field experiment.

size influences mussel survival and growth, the experiment was conducted twice: the first trial (9 plots) started at the 14th of July 2020 and the second trial (9 plots) at the 31th of August 2020. Thus, after August 31, the experiment consisted of 18 experimental plots. The initial average mussel length and condition are summarized in Table 3.1. The condition index (CI) was calculated by dividing the ash-free dry weight (AFDW) by the cubed length of each mussel, resulting in units of mg cm^{-3} (Beukema & De Bruin, 1977). Additionally, the average biomass and density of transplanted mussels in July and August are also provided.

Table 3.1. The initial average mussel length and condition and the average transplanted biomass and density of mussels harvested in July and August.

Start experiment	Shell length (cm ± SE)	Condition Index (mg cm^{-3} ± SE)	Biomass (kg m^{-2} ± SE)	Density (nr m^{-2} ± SE)
July 14, 2020	1.24 ± 0.04 (n=107)	6.4 ± 0.14 (n=107)	2.3 ± 0.1 (n=5)	12481 ± 698 (n=5)
August 31, 2020	2.46 ± 0.06 (n=89)	9.5 ± 0.22 (n=89)	2.6 ± 0.3 (n=9)	2867 ± 379 (n=9)

Setup of the anti-predation cages experiment

To gain a better understanding of the mussel losses caused by predation and hydrodynamic dislodgement and the interplay with intraspecific competition, a cage that excluded predators (anti-predation cage) was placed in each plot. The cages measured 40 x 20x 25 cm and were covered with chicken mesh with a mesh size of 1 cm. Moreover, they were equipped with a removable lid that was also covered with chicken mesh, allowing for easy picture capture during the experiment. The lid was secured to the cage using tie wraps. The cages contained mussels of two pieces of SMC of approximately 12 cm. The mussels were placed in the cages in the same configuration as the plot in which they were placed, thus (1) only mussels and shells, (2) cut open SMC, or (3) intact BioShell-SMC.

Monitoring of the transplantation experiment

Initial measurements - The mussels harvested for the first trial of experiments in July were by nature homogeneously distributed across the SMC-rope. Therefore, the initial mussel density and biomass were comparable for each plot. To estimate the initial mussel density and biomass in a plot, 4 subsamples of 10 cm SMC-rope were taken to the lab. The number of mussels were counted and weighed and the length of rope and amount of cockle shells were determined to estimate the average weight and number of mussels per 1 m SMC. The mussels harvested in August were already letting loose from the SMC as a result of the unexpected premature dissolution of the biodegradable net. The mussel seed was heterogeneously distributed along the rope, with alternately bare patches and very high-density mussel clumps. In order to estimate the initial mussel density and biomass for the plots started in August, top-view pictures were taken of all 9 m rope and the total rope was divided in 5 rope cross-section thickness classes, which included both the SMC and the mussels. The smallest rope thickness cross-section was 0 to 3 cm, which meant that the SMC was not baring any mussels. The thickest cross-section class contained all the ropes

that were thicker than 12 cm, meaning that there was a very thick layer of mussels attached to the SMC. To determine the average mussel density per cross-section thickness class, three subsamples of 10 cm for every rope thickness cross-section class (0-3, 3-6, 6-9, 9-12, >12 cm) were taken to the lab and the mussels were counted and weighted. The mussel number and weight for every thickness class was summed and the total initial density per plot was calculated.

Long-term measurements - Mussel density and biomass development inside the plots were monitored for a period of almost 10 months for the larger mussels; from August 16, 2020 until May 5, 2021. The monitoring of the plots with the small mussels (starting in July) was carried out for 1 month because mussel survival was nearly 0 in all experimental plots by August 10th. For the larger mussels (starting August), the experimental plots were monitored weekly during 4 weeks after transplantation, whereafter the monitoring frequency decreased to monthly. To estimate the biomass of mussels for every monitoring time, top-view pictures and samples (approx. 10 cm in length) were taken while snorkeling over the 18 mussel plots and the 1-m buffer-zone around the plots. Monitoring could only take place when visibility was good enough to distinguish the mussels. The analysis of the pictures and samples followed the same methodology as the initial measurements. However, in addition to that, the samples were categorized into three groups, namely: mussels attached to the rope, mussels aggregated into patches and mussels washed against the fences. Of every category, three random samples per plot were taken. In case of the mussels attached to the rope, the mussel density and biomass were estimated using the same method as with the determination of the initial mussel density per m rope in August. Thus, the average mussel biomass per rope thickness cross-section class (0-3, 3-6, 6-9, 9-12, >12 cm) was determined by analyzing three samples of approx. 10 cm in length. For the mussels aggregated into patches or washed against the fences, similar top-view pictures were taken while holding a ruler next to the patch. The patch area was then determined using the software program ImageJ. After taking pictures, three samples of whole mussel patches and patches washed against the fence were taken to the lab and the mussels were counted and weighed. These values were then correlated to the total mussel patch area to estimate the overall mussel biomass detached from the ropes. By adding up the estimated mussel biomass of the detached and the attached mussels, the overall mussel biomass inside a plot was determined.

Monitoring of the anti-predation cages experiment

Mussel growth and biomass development inside anti-predation cages were monitored from July 17, 2020 until May 5, 2021. At the end of the experiment, on May 5, 2021, all mussels were taken out of the anti-predation cages. Per cage the mussels were counted and weighted. One of the cages placed in July was excluded from the experiment because a hole in the chicken mesh made it possible for crabs to enter. From every cage a subsample of 30 mussels was taken and the shell length was determined. Some of the mussels that were initially attached to the SMC had dispersed from the rope to another location inside the cage. To ensure that there were no differences in growth between the mussels that had dispersed from the rope and the mussels that stayed inside the mussel clump, we subsampled 15 mussels of each condition, resulting in 30 mussels per cage total.

Mesocosm experiment

Setup of the food preference experiment

To investigate whether there is a difference in predation rates by crabs and starfish on different mussel seed sizes and various transplantation configurations, two food preference experiments were carried out in mesocosms; one for the mussel seed harvested in July (~1.24 cm, Table 1) and one for the mussel seed harvested in August (~2.46 cm, Table 1). These mussel seeds originated from the same location as the ones used in the field experiment. The experiment in July took place between July 27, 2020 and August 19, 2020 and the experiment with mussels harvested from August took place between August 23, 2020 and October 5, 2020. In the middle of a 1 × 1 × 1 m tank, filled with 900 L unfiltered Oosterschelde seawater, four configurations of mussels were placed on a 10 cm thick layer of sand collected in the Oosterschelde. For each mussel configuration a 10 cm piece of SMC, that was 100 percent covered with mussels, was cut-off. Configurations corresponded largely with the mussel transplantation methods used in the field experiment, namely: (1a) the biodegradable net the coconut fiber rope and cockle shells were removed, only the mussels were placed in the tank, (1b) like 1a, but now the mussels plus cockle shells were placed in the tank, (2) the biodegradable net was cut open to make sure that the shells and mussels were able to spill out, (3) the BioShell-SMC was kept intact completely. The configurations of mussels were then placed in a square 30 cm apart in the middle of the tank. In each tank two crabs with an average carapax width of 4.8 cm ± 0.37 sd (n=8) or four sea stars with an average wet weight of 65 gr ± 14.1 sd (n=9) were kept. When a crab or sea star died during the experiment, it was replaced with a new one. Each of these treatments was carried out in triplicate, resulting in 6 experimental units. Each tank was aerated. In addition, the water in all tanks circulated with an inflow and outflow port connected to an additional water tank, providing a steady circulation flow rate of 6 L h^{-1} $tank^{-1}$. Mussels were fed weekly with instant algae with a concentration of 2 billion cells per ml (shellfish diet 1800/Reed Mariculture Inc.). The tanks were cleaned and refilled with unfiltered Oosterschelde seawater between the experiment taking place in July with small mussels and the experiment in August, with larger mussels.

Monitoring of *the food preference experiment*

To measure the effect of the predators on the survival of the mussels, the number of mussels in each food source (loose mussels, loose mussels and shells, open net, intact net) was determined at the beginning and at the end of the experiment and the percentage difference was recorded as the mortality rate. A trendline was used to determine the correlation between the total weight of a 10 cm piece of SMC and the number of mussels on this piece of SMC. This correlation was used to estimate the initial number of mussels on the cut open and intact net food sources. Mortality rates have been corrected for the duration of the experiments, as the trial for the smaller mussels, harvested in July, lasted only 3 weeks, while the duration in August was 6 weeks.

Statistical analysis

All statistical analyses were carried out in R, 3.5.1 (R Core Team 2022). Prior to model fitting, all data were visually checked for normality (Q–Q plot) and homogeneity of residuals,

following the procedure described in Zuur et al. (2010). If necessary, data were transformed to meet assumptions. Models were simplified according to Akaike's information criterion (AIC) scores and non-significant factors were removed.

Field experiment: transplantation experiment

In order to compare the biomass loss rates between the three mussel transplantation methods (loose mussels and shells, cut open net, intact net) a survival analysis was carried out based on maximum likelihood (Miller, 1981) and comparable to the survival analysis carried out in Schotanus et al. (2020). In short, the mean loss rate (ε) per transplantation method was estimated as the inverse of the mean life time of a mussel bed (τ) for the mussels transplanted in July and the mussels transplanted in August:

$$\varepsilon = 1/\tau$$

The mean life time of a mussel bed (τ) was estimated by determining the difference in proportion of mussel biomass (ρ_i) for every monitoring time (t_i). Since most mussel beds did not disappear completely during the course of the experiment started in August, a correction for these right-censored observations was included to prevent underestimation of the mean life time:

$$\tau = 1/(1-\rho_{t\_end})\Sigma((1-\rho_{i+1})-(1-\rho_i))t_{i+1}$$

Finally, a one-way ANOVA was carried out with loss rates (ε) as the response variable and the transplantation method as the explanatory variable (Loss rate ~ Transplantation method).

For both the mussels seeded in July and in August the difference between transplantation methods in average final mussel biomass inside the experimental plots (3x3m) and outside the experimental plots, in the 1-m buffer zone, were analyzed with a one-way ANOVA, which resulted in the following models: Mussel biomass inside plot ~ Transplantation method and Mussel biomass outside plot ~ Transplantation method. The biomass data was square-root transformed to meet the assumptions for homogeneity of variance. Post-hoc Tukey tests were used to test for significant differences between treatments at specific timepoints (R-package emmeans, Lenth 2016).

Field experiment: anti-predation cages experiment

The effect of the starting month (July vs. August) and the transplantation method (loose mussels and shells vs. cut open net vs. intact net) on the average mussel biomass increase rate inside the anti-predation cages was analyzed with a two-way ANOVA (Mussel biomass increase rate ~ Transplantation method × starting month). In order to meet the assumptions, the biomass data was log-transformed prior to analysis. The effect of the transplantation method, starting month and location of the mussel (i.e. whether the mussels were located inside or outside the original mussel patch) on the average mussel length was analyzed with a linear mixed effect model, with the cage from which the mussels were sampled entered as random effect (Length ~ Transplantation method × starting month × Location + (1 | Cage). Pairwise comparisons were obtained by Tukey posthoc tests with the *contrast* and *lsmeans* functions from the lsmeans package (Lenth, 2016).

Mesocosm experiment: food preference experiment

The mussel mortality per week (proportion of dead mussels per week over start number) in the mesocosm experiment was analyzed with a quasi-binomial generalized linear model (GLM), implemented with the glm function (family set to quasi-binomial). The size of the mussels (small mussels harvested in July vs. larger mussels harvested August), transplantation method (loose mussels and shells vs. cut open net vs. intact net), predator type (crabs vs. sea stars) and the interactions between these three factors were entered as explanatory variables. The best model based on AIC resulted in: Mussel mortality ~ Predator × Mussel size. The analysis evaluated 12 samples total (three replicates × two predator types × two mussel sizes = 12 samples). We used a post hoc comparison on the least-squares mean (lsmeans) with no adjustment.

Results

Biomass loss rate field transplantation experiment

Small mussels transplanted in July

For the small mussels seeded in July, there was no significant difference in mussel loss rate between treatments ($F_{2,6}$=2.47, p=.17, Figure 3.3A). Regardless of the transplantation method, mussel biomass decreased strongly (98% ± 0.8 SE) in all plots in the first three days after transplantation on the 14th of July 2020 (Figure 3.3B). In addition, no mussels were found outside of the 3x3m plots, in the 1m buffer zone, or against the fences surrounding the plots, indicating there was no hydrodynamic dislodgement. After 4 weeks, on August 10th, mussel survival was 0% in plots with the configuration *loose seeded mussels and shells (treatment #1)* and nearly 0% for mussels transplanted attached to *cut open biodegradable nets (treatment #2)* or to *BioShell-intact nets (treatment #3)*. Monitoring of these plots was therefore concluded.

Larger mussels transplanted in August

The overall loss rate of the larger mussels that were transplanted on the experimental plots in August was much lower in comparison with the mussels seeded in July. Besides, there were significant differences between transplantation methods ($F_{2,6}$= 31.52, p<.001, Figure 3.3C). The biomass loss rate was significantly higher for the *loose seeded mussels and shells (treatment #1)* than for the mussels attached to *cut open nets (treatment #2)* (p=.001) or *BioShell-intact nets (treatment #3)* (p<.001).

After the first 4 weeks, in which the biomass decreased in all treatments, the mussel biomass stayed relatively stable over the remaining course of the experiment in the plots with mussels attached to *cut open* or *intact net* (Figure 3.3D). At the end of the experiment, mussel biomass was significantly higher in plots with mussels attached to *cut-open net* (p<.001) and *intact net* (p<.001) compared to plots seeded with *loose mussels and shells (treatment #1)*. The final biomass between plots with mussels attached to *cut-open net (treatment #2)* and *BioShell-intact net (treatment #3)* did not significantly differ (p=.092).

Larger mussels seeded in August washed against the fences, which indicates hydrodynamic losses (Figure 3.3E). This was especially true for the *loose seeded mussels with shells (treatment #1)*. On December 6th, 3 months after the start of the experiment, 100 % of the initially seeded mussels from the *loose mussels and shells* treatment were washed out of the experimental squares against the fences surrounding the plots, while almost none of the mussels washed out of the *cut-open net (treatment #2)* and *intact net (treatment #3)* plots. At the final sampling date, we found that there was a significant difference in mussels washed against the fences between the treatments *loose mussels and shells*, and *cut-open net* (p<.001) or *intact net* (p<.001) but no significant difference between *cut-open* and *BioShell-intact net*.

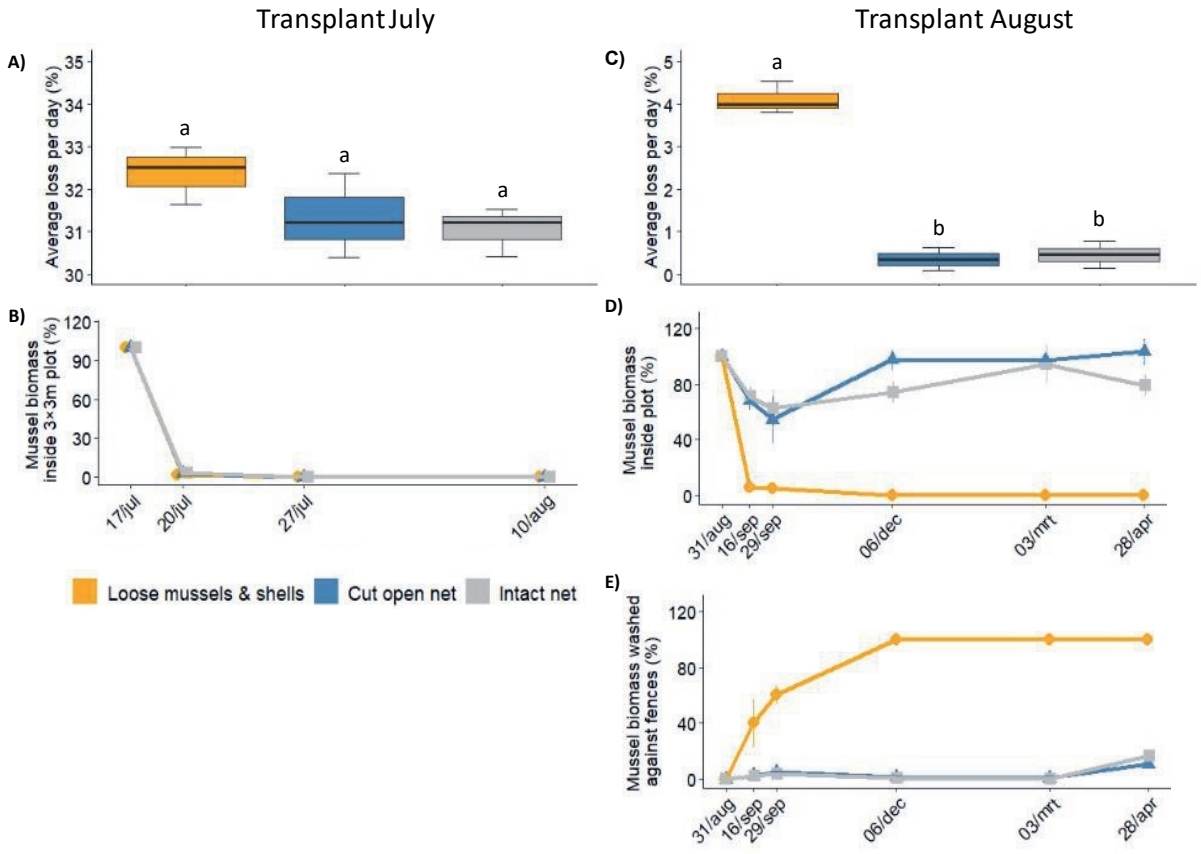

Figure 3.3. A. Average loss rate of mussels per day (%), between start and final measurement (23 days), for mussels transplanted in July. **B.** Mussel biomass development over time (%) inside experimental plot (3x3m) for mussels transplanted in July in the configurations: loose mussels & cockle shells, cut open BioShell-SMCs net, or intact BioShell-SMCs net. **C.** Average loss rate of mussels per day (%) between start and final measurement (240 days) for mussels transplanted in August. **D.** Mussel biomass development over time (%) inside experimental plot (3x3m) for mussels transplanted in August. **E.** Mussel biomass development over time (%) outside experimental plot, within 1m buffer zone, relative to initial mussel biomass inside experimental plot (3x3m) in August in the configurations: loose mussels & cockle shells, cut-open BioShell-SMCs net, or intact BioShell-SMCs net. Data are means ± SE (n=3).

Mussel survival and growth inside anti-predation cages field experiment

Mussel survival was high in all cages, both for the small mussels transplanted in July and the larger mussels transplanted in August. There was a significant interaction between initial mussel size and treatment ($F_{2,11}$=6.01, p=.017). Besides, overall biomass increase inside the cages was significantly affected by treatment ($F_{2,11}$=5.59, p=.021). The difference in biomass increase was explained by the lower mussel biomass increase of small mussels transplanted in July on *BioShell-intact nets (treatment #3)* (Figure 3.4), which was significantly lower in comparison with the biomass increase in July for *loose mussels & shells (treatment #1)* (p=.028), *cut open net (treatment #2)* (p=.034).

At the end of the experiment, the average mussel length was significantly lower in the cages with small mussels harvested in July than in the cages with larger mussels harvested in August ($F_{1,2}$=25.79, p<.001). Mussel length did not significantly differ between mussels located inside a mussel clump or mussels that moved outside the mussel clump. Therefore, these data have been merged. There was no significant interaction between initial mussel size and transplantation method and the transplantation method had no significant effect on the final length of the mussels.

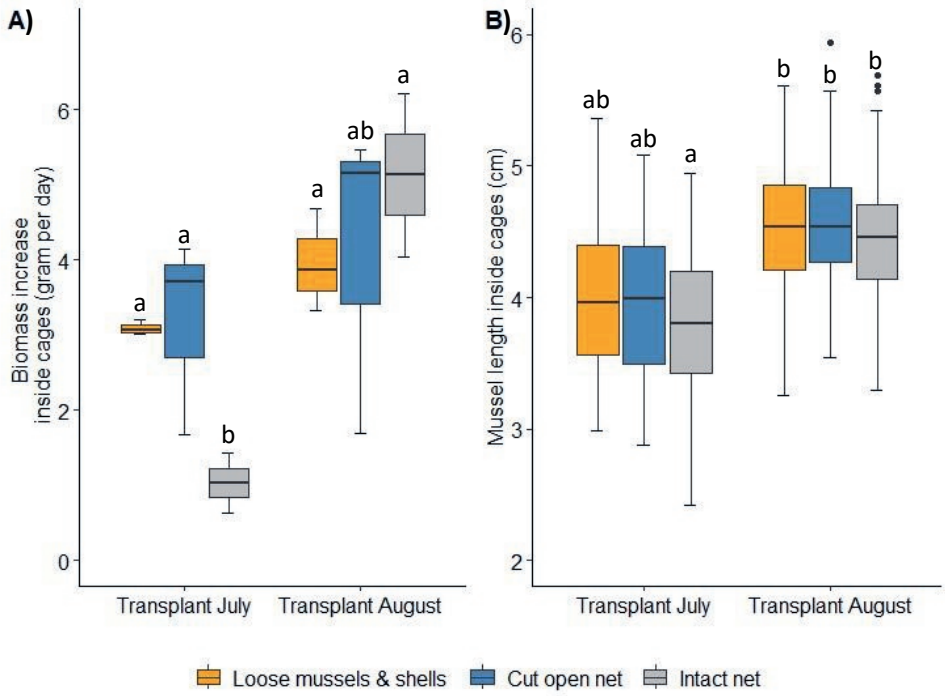

Figure 3.4. A. Mussel biomass development (gram per day) **B.** Mussel length inside the anti-predation cages at the end of the experiment for mussels transplanted in July and August in the configurations: loose mussels & cockle shells, cut-open BioShell-SMCs net, or intact BioShell-SMCs net.

Mussel predation in mesocosm experiment

In the mesocosm experiment, transplantation method (loose mussels – treatment #1a; mussels with shells – treatment #1b; cut-open SMC – treatment #2; and intact SMC – treatment #3) had no significant effect on the survival rate of the mussels when exposed to crabs or sea stars ($F_{3,41}$=1.57, p>.05). We found a significant interaction between predator type and initial mussel size ($F_{1,44}$=7.57, p=.006). Survival of smaller mussels was significantly lower when exposed to crabs than to sea stars (Figure 3.5).

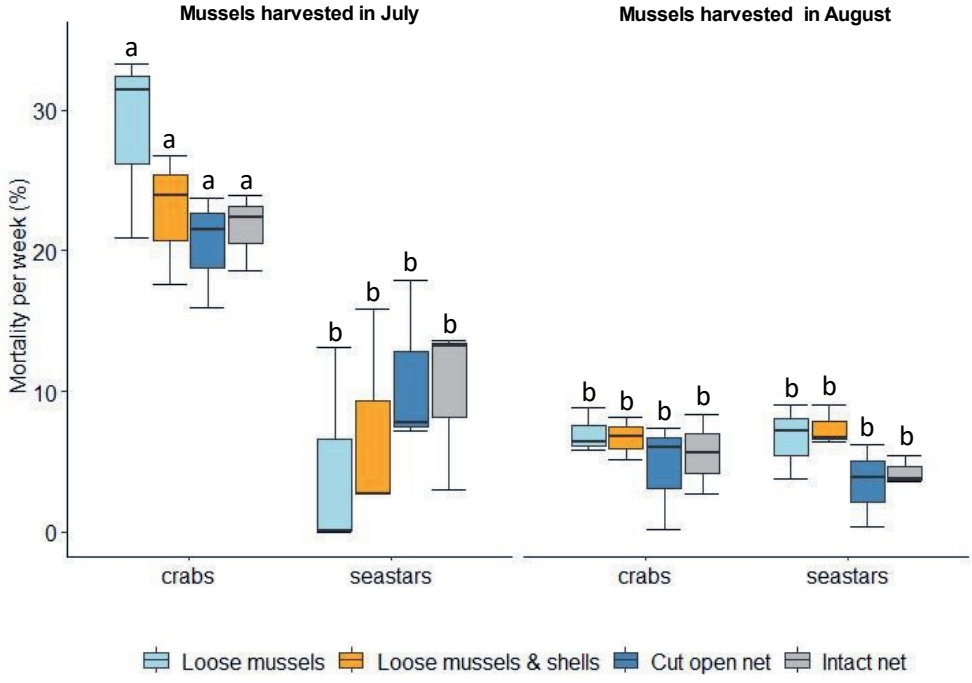

Figure 3.5. Average mussel survival (%) for mussels harvested in July and August for four configurations: Only loose mussels, loose mussels and cockle shells, mussels attached to cut open BioShell-SMC and mussels

Discussion

Transplantations of juvenile blue mussels (*Mytilus edulis*) are carried out as an attempt to restore natural mussel beds (de Paoli et al., 2017; Schotanus, Capelle, et al., 2020) and to cultivate mussels for consumption (Capelle et al., 2014). However, the small size of the mussels and the lack of attachment substratum after transplant makes them highly vulnerable to predation and hydrodynamic dislodgement, leading to huge initial losses (Capelle, Wijsman, et al., 2016). Hence, there is need to improve transplantation success, to decrease the number of individuals needed and to decrease costs of large-scale transplantations. Therefore, we tested transplantation of mussels attached to the innovative biodegradable BioShell-SMC and evaluated the transplant success compared to loose seeded mussels. Our findings demonstrated that mussel seed survival

in the field experiment was significantly higher when mussels were attached to the biodegradable BioShell-SMC than when they were transplanted without attachment. The cage experiment showed high mussel seed survival when predation and hydrodynamic dislodgement were excluded, and the mesocosm experiment revealed higher loss of small mussels compared to larger mussels due to predation by crabs. The higher loss of loosely transplanted mussels compared to BioShell-SMC-mussels are caused by a mix of factors such as dislodgement due to predation, hydrodynamic forces or competition.

Role of predation on losses

During the initial three days of the July trial, we observed a substantial decrease in mussel biomass across all transplantation methods, with no mussels found against the surrounding fences. However, high mussel survival rates were observed in the anti-predation cages, indicating that predation was the primary cause of losses in the plots. This is in line with a study by Alder et al. (2021), who showed that biodegradable substrates (coir matting and rope) were ineffective at preventing loss of cultured juvenile (10 – 30 mm) and subadult (30 – 70 mm) mussels against predation by snappers and rays within the first 24 hours following experimental set-up. In contrast, in our August experiment with larger mussels, the survival of mussels attached to the (cut-open) SMC was much higher compared to the loose mussels and compared to all treatments in July. The higher predation pressure observed in July may be due to the smaller size of the mussels in combination with low mussel densities. During the first week of the July-experiment, we observed many crushed mussel shells. According to Davidson (1986), crabs prey on mussels by either crushing the shell or chipping it, leaving behind only shell fragments. In contrast, sea stars utilize their tubefeet to force open the mussels' valves, resulting in two intact shells, rather than fragments (Ruppert & Barnes, 1994). Based on this observation, it appears that crabs were the primary predators in our July field experiment.

In the mesocosm experiment, a similar outcome was observed where crabs showed a higher predation rate on smaller mussels harvested in July, compared to larger mussels harvested in August. This suggests that crabs preferred smaller mussels, over larger mussels, irrespective of whether the mussels were attached to a substrate or not. Earlier work showed that crabs have a preference for certain size classes of mussels, and that the preferred prey size increases with crab size (Enderlein et al., 2003; Murray et al., 2007). Kamermans et al. (2009) demonstrated that small mussel seed (< 11mm) was consumed faster by crabs (carapace width of 44-63mm) than larger mussels with a size of 22mm. In our mesocosm experiment, the crabs had an average carapace width of 48mm, indicating that the small mussels in July (12mm) were easier prey for the crabs than the larger mussels in August (25mm), resulting in lower survival rates. Predation mortality could be expected to decrease with increasing mussel densities (Frandsen and Dolmer, 2002). Because of the small scale, our field-transplantation experiment used relatively low mussel densities, suggesting that the small mussels harvested in July may have been transplanted below the density threshold necessary to provide protection against predators, even with attachment substratum. Due to their larger size, the mussels harvested in August were not as vulnerable to predators. The mussels attached to the (cut-open) SMC showed an improved survival compared to

loose mussels, which is in line with previous research, indicating that increased substrate complexity (Frandsen & Dolmer, 2002; Reimer & Tedengren, 1997) and aggregation into dense clumps (Côté & Jelnikar, 1999) reduced predation risk.

Role of hydrodynamics on losses

The field experiment revealed that mussels attached to the cut-open and intact BioShell-SMC (i.e., treatment #2 & #3) experienced lower losses due to hydrodynamic force compared to those that were transplanted loosely, without attachment substrate (treatment #1). That is, during the experimental trial in August, a significant reduction in the number of mussels washed up against the fences surrounding the plots was observed when the mussels were attached to the BioShell-SMCs. These findings align with the results of a previous study by Bertolini et al. (2019), in which the hydrodynamically induced dislodgement thresholds of four different spatial patterns of mussels were tested. Here, mussel stripes created sufficiently dense patches that maximized resistance to dislodgement. The mussels attached to the biodegradable BioShell-SMCs in our study formed a comparable striped spatial pattern. Furthermore, the cockle shells in the biodegradable socks likely added extra weight to the mussel patches, which may have further increased the dislodgement threshold. This induced dislodgement for mussels attached to the BioShell-SMCs was not observed in July, which can be attributed to the fact that most mussels did not survive high crab predation during the initial three days of the experiment.

The addition of empty cockle shells during the transplantation of loose mussel seed did not appear to enhance the dislodgement threshold of the mussels, as evidenced by the majority of the loosely transplanted mussels washing up against the fences in the second trial, despite the use of empty shells (treatment #1). Previous studies have shown that the presence of a complex substratum can increase the chances of mussel establishment by enhancing the critical hydrodynamic dislodgement threshold (Capelle et al., 2019; Christensen et al., 2015). However, in these papers, the complex substratum was already naturally present in the form of coarse shell material or artificially added in the form of shells embedded in cement, which created more stable substrates than the loose shells used in our experiment. The loose mussels in our study may not have had sufficient time without experiencing hydrodynamic forces (i.e., window of opportunity) to establish positive feedback mechanisms before being washed away or preyed upon.

For the mussels in the cut-open net condition, we found no higher survival rates compared to those attached to the intact BioShell-SMCs. We originally expected that by cutting open the BioShell-SMCs, cockle shells could fall out of the SMC and thereby provide additional attachment substrate away from the SMC. This might increase mussel survival, as mussels could utilize these shells to escape high densities and subsequently reduce competition. However, this was apparently not the case, possibly because harsh environmental conditions did not offer benefits for mussels dispersing away from the SMC.

Role of competition on losses

Mussel losses on culture plots after seeding typically dependent on seeding density (Bertolini et al., 2020; Capelle et al., 2014; Capelle, Wijsman, et al., 2016; Gascoigne et al., 2005).

When seeding in bottom culture, the distribution of mussels is highly heterogeneous, with high densities in the spaces occupied by mussels (Capelle et al., 2014), leading to competition for food and space (Commito et al., 2014; Fréchette & Bourget, 1985; van de Koppel et al., 2005). Capelle et al. (2016) found that seeding in high density resulted in increased mortality due to intraspecific competition. In our experiment, we found that mussels attached to the BioShell-SMCs had significantly lower biomass increase compared to (surviving) loose mussels and mussels attached to a cut-open net in July. We did not find a similar difference in transplantation methods in August. The reason for this discrepancy might be that initial mussel density was more than four times higher in July compared to August. The difference in biomass increase in July suggests that mussel densities on the SMC may have been too high, leading to competition for food and space, hindering growth and condition. These mussels may not have had the opportunity to disperse, whereas loose seeded mussels and those attached to the cut-open net may have been able to escape competition by dispersal onto the empty cockle shells. Our findings are supported by (Christensen et al., 2015), who found lower growth rates on a complex substrate, and Eschweiler and Christensen (2011), who documented reduced growth rates for mussels in protective interspaces of a Pacific oyster reef. Frandsen and Dolmer (2002) demonstrated that complexity can negatively impact growth rate due to limited food supply caused by decreased water flow in cavities of complex substrates. Further research is needed to determine the optimal transplantation density for mussel seeds using our BioShell-SMCs.

Management implications and outlook

Creating density-dependent positive feedback mechanisms to mitigate environmental stressors during large-scale transplantation of ecosystem-engineers is a key challenge to overcome both for ecosystem restoration and aquaculture applications. Using mussels as a model system, we demonstrated that using biodegradable structures (i.e., BioShell-SMC) can initiate self-facilitating feedback mechanisms by keeping the mussels grouped together and enable outplacement in distinct patterns. More specifically, our results demonstrated that attaching larger mussel seed to the BioShell-SMC significantly improved their survival rate compared to those transplanted without any attachment substrate and compared to small mussel seed. This transplantation method holds great promise for restoring sub- and intertidal mussel beds for nature conservation, as well for efficiency gains in aquaculture. Since the yield of the BioShell-SMC was comparable to traditionally used SMCs , but with significantly increased survival rates, it could reduce the costs of long-term restoration by requiring fewer transplants and by larger control on the spatial deployment. Additionally, our BioShell-SMC approach could provide a promising solution to the significant losses in mussel bottom cultivation (Capelle, Wijsman, et al., 2016). Follow-up research should focus on optimizing degradation times for different applications and environmental settings. The biodegradable substrate should maintain long enough for mussels to settle, stabilize and grow. Nevertheless, our study's results provide a promising step towards developing a more successful approach to restore mussel beds. In line with studies on other species (Temmink et al., 2020), present approach of using biodegradable structures to restoring ecosystem engineering species is expected to have broad applicability beyond our case study on mussels. Since our BioShell-SMC

facilitates self-organization between individuals and provides greater control on transplanting in regular patterns that mimic patterns in natural ecosystems, restoration success can be enhanced by using our BioShell-SMC.

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Chapter 4

Using a biodegradable substrate to increase transplantation success: Effect of density and sediment on aggregation behavior of mussels

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Abstract

Habitat restoration through transplantation of ecosystem engineering species has become an increasingly popular conservation strategy. However, the success of these restoration efforts depends largely on the ability of transplanted organisms to establish and persist in their new environment. Ecosystem engineers typically occur in large numbers and rely on self-facilitating feedback mechanisms to overcome physical and/or biological stressors for successful establishment. These feedback mechanisms can only arise when a certain density or size threshold is reached and are driven by the interplay of facilitation and competition. To initiate the establishment of self-facilitating feedback mechanism, we used biodegradable structures known as "BioShell-SMCs". These structures are an innovation of the nylon seed mussel collectors (SMCs) commonly used in mussel cultivation. They consist of a biodegradable net based on a compound of aliphatic polyesters, filled with empty cockle shells around a coconut fiber rope. In a mesocosm experiment, we investigated competition and facilitation processes by comparing aggregation and performance between loose seeded blue mussels (*Mytilus edulis*) and mussels already attached to the BioShell-SMC at two different densities (high vs. low) and two sediment compositions (mud vs shell). Our results revealed that mussels attached to the BioShell-SMC showed more pronounced clustering compared to loose mussels, particularly in low density. Mussels in high density attached to the BioShell-SMC dispersed from the SMC on both sediment compositions. Furthermore, transplanted mussels attached to the BioShell-SMC showed higher survival rates and had a better condition than loose mussels. Overall, our study emphasizes the importance of considering ecological processes such as competition and facilitation when designing and implementing restoration projects. It provides a case for optimizing transplantation success of ecosystem engineers by including temporary substrate that provide positive feedback mechanisms at establishment, effectively creating a window of opportunity.

Introduction

Transplantations are intentional movements of populations or individual organisms across landscapes (Weeks et al., 2011). These can be applied to restore degraded ecosystems, for example, by reforestation (Horoszowski-Fridman & Rinkevich, 2016), for the provision of ecosystem services, such as mangroves to provide protection against sea-level rise and coastal storms (Barbier, 2016), or for commercial purposes, such as aquaculture (Kamermans et al., 2002). Unfortunately, transplantations, often involving foundation species, tend to have low success rates (Dodd Jr & Seigel, 1991; Godefroid et al., 2011; Griffith et al., 1989). Foundation species play a significant role in shaping community structure (Dayton, 1972) due to their abundance and capacity to create the physical and environmental conditions essential for the coexistence of other species (Bruno et al., 2003; Ellison et al., 2005; Stachowicz, 2001). These foundation species often rely on self-facilitating feedback mechanisms in the establishment phase to overcome physical (e.g., wave exposure, salinity) and/or biological (e.g., nutrients, predation) stressors in dynamic environments (He et al., 2013; Jones et al., 1997; Liu et al., 2014; van de Koppel et al., 2001; van der Heide et al., 2007). Seagrass and reef-forming bivalves are two examples of marine organisms that have evolved positive feedback strategies to mitigate environmental stresses (Hunt & Scheibling, 2001; Maxwell et al., 2017). Seagrasses have been found to attenuate currents and trap sediment more effectively with higher shoot density (Maxwell et al., 2017). This is because the increased surface area of the seagrass leaves and roots creates more drag, which slows down the water flow and allows the seagrass to trap more sediment. Similarly, reef-forming bivalves like mussels mitigate individual losses by attaching themselves to conspecifics and aggregating in large groups (Hunt & Scheibling, 2001). This creates a more stable environment for the bivalves, as they are better able to withstand currents and waves. Additionally, the bivalves can share resources and defend themselves against predators more effectively when they are aggregated. But in a lot of cases, these positive feedback mechanisms can only arise very early in the establishment phase and when a certain density or size threshold is reached (Bouma, Friedrichs, et al., 2009; van der Heide et al., 2007).

Transplantation failure may be partly explained by the lack of a disturbance-free period immediately after transplantation to establish positive feedback mechanisms, also referred to as a window of opportunity (Balke et al., 2014). Alternatively, the concept of a window of opportunity can also be understood as the critical minimal duration during which a suitable settlement substratum is available in the presence of recruits (Capelle et al., 2019). For instance, oyster reefs situated in soft sediment locations need the presence of hard substrate to facilitate their establishment (Walles et al., 2016). These periods of sufficient length might be necessary to initiate self-facilitation. Transplantation success can be enhanced by integrating positive feedback mechanisms in the transplantation process (Renzi et al., 2019; Valdez et al., 2020). For instance, incorporating positive intraspecific interactions through the use of clumped rather than dispersed transplant configurations improves the success of salt marsh restoration (Silliman et al., 2015). Likewise, loss rates of transplanted reef-forming bivalves in highly dynamic areas were lower when

the development of self-facilitating processes was promoted. For instance, Schotanus et al. (2020) accomplished this by stimulating the formation of an aggregated spatial configuration using fences between which the mussels were placed. The wave-dislodged mussels were trapped over time, resulting in banded mussel patterns with local high mussel densities, facilitating their attachment to one another . Apart from these few examples, positive inter- or intraspecific interactions are rarely intentionally included in restoration transplantations (Derksen-Hooijberg et al., 2018; Silliman et al., 2015). Therefore, to increase establishment success after transplantation, it is important to gain more insight into how interactions between biological and physical factors affect self-facilitating feedback mechanisms in restoration efforts. We address this issue by analyzing how self-organization in blue mussels (*Mytilus edulis*) is affected by the interaction between mussel transplantation method, sediment composition and mussel density.

Mussels are reef-forming ecosystem engineers that aggregate into large complex beds by anchoring themselves to conspecific-substrate complexes (Christensen et al., 2015; Snover & Commito, 1998). Aggregation behavior increases when a substratum large enough (> 0.85 mm: Young, 1983) to attach to is scarce. (Commito et al., 2014; Hunt & Scheibling, 1995; van de Koppel et al., 2005). Aggregation into high-density patches relates to the interplay of facilitation and competition. That is, the adaptive value of aggregation is associated with the reduction of dislodgement by hydrodynamic forces and protection against predators by a stronger attachment and by a safety in numbers effect (Hunt & Scheibling, 2001). However, aggregation in high density patches also imposes disadvantages, particularly competition for space and food (Capelle et al., 2014; Newell, 1990). The trade-off between intraspecific competition and protection against dislodgement and predation leads to self-organized aggregations of dense patches alternating with bare sediment (Saurel et al., 2013; van de Koppel et al., 2008).

Transplantations of juvenile blue mussels (*Mytilus edulis*) have been carried out as an attempt to restore natural mussel beds in soft sediment environments (de Paoli et al., 2017; Schotanus et al., 2020a), but is even more common to cultivate mussels for consumption (Capelle et al., 2014). In both situations, the small size of transplanted mussels and a lack of attachment substrate make them highly vulnerable to loss factors such as predation and hydrodynamic dislodgement (Kamermans et al., 2010; Murray et al., 2007). In addition, the newly transplanted mussels may not get the time to establish positive feedback mechanisms, such as intra-specific interactions before they are washed away or preyed upon, which leads to very high losses within the first month after transplantation (Capelle, Scheiberlich, et al., 2016).

To facilitate the establishment of positive feedback mechanisms after transplanting mussels, we propose to use biodegradable structures, the so-called “BioShell-SMC” (Van den Bogaart et al., 2023a). The biodegradable structures we tested are an innovation of the nylon seed mussel collectors (SMCs). The traditional SMCs are becoming more prevalent in mussel farming. Here, SMCs collect juvenile mussels (i.e. mussel seed) from the water column which, when they are large enough (2-3 cm), are transplanted to soft sediment grow-out plots. The BioShell-SMC consists of a biodegradable net based on a compound of aliphatic polyesters, filled with empty cockle shells around a coconut fiber rope. An advantage of using the BioShell-SMC is that the mussel seed can

be transplanted while still attached to the BioShell-SMC, instead of first harvesting and then transplanting loose individuals, which is commonly done in mussel farming and restoration. The advantages of transplanting the mussels in stable, high-density clusters are that the juvenile mussels are less susceptible to predation and dislodgement by hydrodynamics (Van den Bogaart & Schotanus et al. 2023b). The major disadvantage is that mussel densities on the BioShell-SMC might get very high, leading to competition for food and space, impeding the growth and condition of the mussels (Commito et al., 2014; van de Koppel et al., 2005). However, when the biodegradable nets dissolve, the cockle shells within will disperse, which may provide an attachment substrate for mussels to spread further away from the SMC, escaping competition for food and space (Capelle et al., 2019).

In this study, we examined how the interactions between biological and physical factors affect self-facilitation and if these mechanisms can be initiated after transplant. Since these interactions are rarely included in restoration attempts, we investigated this by comparing mussel aggregation and mussel performance between *i)* mussels attached to the BioShell-SMC and *ii)* loose mussels (Figure 4.1). In addition, we tested our expectation that mussels will disperse from high density clusters in order to escape competition when an attachment opportunity in the form of added shell debris, is available. On the other hand, we expected that mussels will not show dispersal behavior when densities are low (below density threshold, which is the minimum mussel density required to induce aggregation) or when no suitable attachment substrate is available (lack of window of opportunity). For loose mussels, intraspecific competition is low, but predation and dislodgement risk is high in the initial transplantation phase. In contrast with mussels attached to biodegradable substrate, they are expected to aggregate into patches for safety rather than disperse.

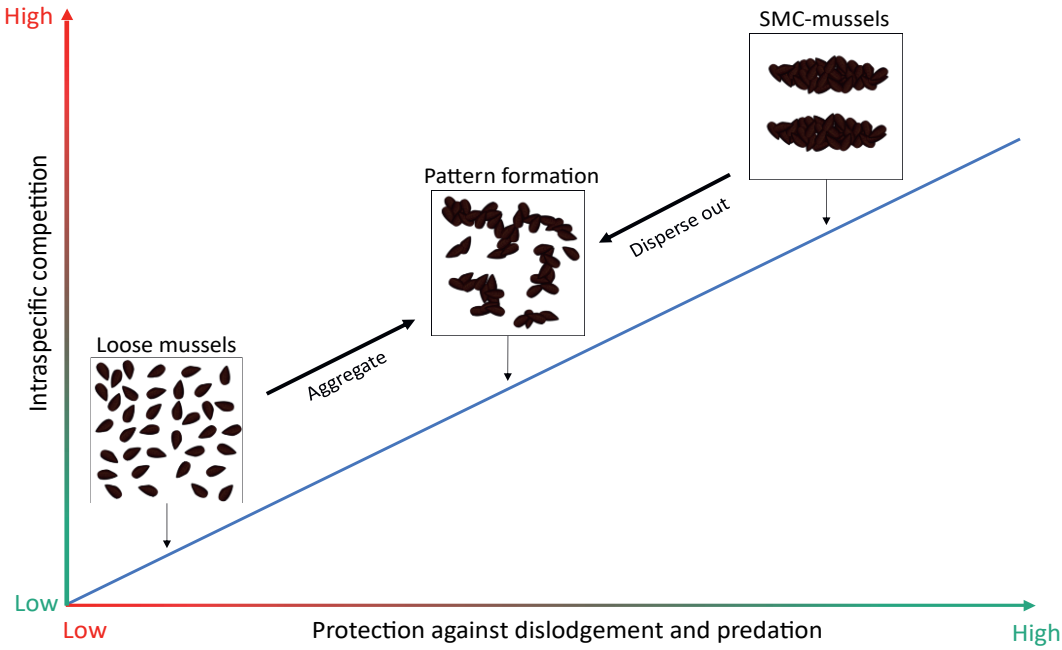

Figure 4.1. Schematical overview of expected pattern formation after transplant by loose mussels and mussels attached to the BioShell-SMC. Loose mussels will organize in patches to find protection against dislodgement and predation (aggregate), while SMC-mussels will disperse from the substrate in order to escape high competition (disperse out). Optimal mussel densities are a trade-off between intraspecific competition for food and space and protection against dislodgement and predation. Loose mussels experience low competition and low protection, while attached to substrate experience both high competition and protection.

Material and Methods

We tested how an innovative transplantation method, that consists of mussels settled on a biodegradable mussel seed collector substrate (the "BioShell-SMC"), influenced the aggregation behavior, survival and condition of mussels after transplant. A more detailed description of the BioShell-SMC can be found in Van den Bogaart et al. (2023a). In short, the BioShell-SMC consists of a biodegradable net based on a compound of aliphatic polyesters, wrapped around a coconut fiber rope, and filled with empty cockle shells. After a period in the water column to collect mussel spat, the entire SMC can be relayed on the seafloor. The biodegradable net will dissolve and the mussels and cockle shells will disperse. Empty shells create an excellent attachment substrate for mussel larvae floating in the water column (Commito et al., 2014) and after relay for juvenile mussels to increase resilience (Capelle et al., 2019; Frandsen & Dolmer, 2002; wa Kangeri et al., 2014). In the current study, we tested the effect of BioShell-SMC substrate and empty cockle shells on aggregation behavior, survival and condition of mussels, after relay. We mimicked this in the experiment by including the empty cockle shells in the substrate. We compared mussels attached

to the BioShell-SMC with loose mussels. In addition, we tested the extent to which aggregation behavior is affected by the interaction of sediment composition and mussel density.

Experimental design

In a mesocosm experiment, we tested the aggregation behavior of mussels as a response to transplantation method, sediment composition and mussel density. We tested (1) two transplantation methods: loose homogeneously spread mussels versus mussels attached to the BioShell-SMC (further referred to as "SMC-mussels"), (2) two types of sediment: mud versus coarse sand mixed with shells (further referred to as "shell") and (3) two mussel densities per covered area: low = 2.1 kg/m^2 (0.75 kg per tank) versus high = 8.3 kg/m^2 (3 kg per tank) for loose mussels and low = 7.8 kg/m^2 (0.75 kg per tank) versus high = 18.5 kg/m^2 (3 kg per tank) for mussels attached to the SMC. The SMC-mussels were more concentrated than the loose mussels because, although the amount of mussels per tank remained constant between the two densities, the SMC-mussels occupied a smaller area. All treatments were carried out in triplicate, which resulted in (2 x 2 x 2 treatments x 3 replicates =) 24 experimental units (Figure 4.2). Due to the limited number of experimental mesocosms available to carry out all treatments simultaneously, the experiment was conducted in two rounds; the first round tested the loose mussels, while the second round focused on SMC-mussels. During the first round, sediment and density were randomly allocated to the tanks, while in the second round, only density was assigned randomly as changing the sediment was logistically unfeasible. After the first round, we used a water vacuum cleaner to remove all the water along with the suspended sediment. Subsequently, we refilled the tanks with seawater and allowed the sediment to settle again until the water regained its clarity. Consequently, by ensuring the substrate was clean again at the beginning of the second round, we anticipated minimal impact from its repeated use.

Mussel source and acclimatization prior to the experiment

The mussels used in this study were obtained from a mussel culture plot in the Eastern Scheldt on the 21st of October 2020. To ensure similar treatment of the starting material, we provided attachment of all mussels to the BioShell-SMC. Therefore, we enveloped all mussels in 50 cm biodegradable fine-meshed socks based on a compound of aliphatic polyesters around a coconut fiber rope before the experiment started. Half of the mussels were kept in low density (0.375 kg mussels per 50 cm) and half of the mussels in high density (1.5 kg mussels per 50 cm). The ropes with mussels were kept in a tank with a flow-through system until the experiment started to ensure all mussels were attached to the coconut rope and/or the biodegradable sock.

Experimental treatments

The experiments were carried out in 1 x 1.2 x 1 m tanks with 900 L of seawater. All tanks were provided with a flow-through of seawater. We additionally fed the mussels every two days with a batch of living algae or 50 mL of instant algae (shellfish diet 1800; Reed Mariculture) at a concentration of 2 billion cells ml^{-1} $tank^{-1}$. The tanks were located in a climate chamber with a constant temperature of 18 ℃ and a continuous light source. Tanks were filled with a 10 cm layer of

either mud or coarse sand mixed with shells (further referred to as "shell"). Mud was collected from a mussel culture plot in the Eastern Scheldt and it consisted of particles that were too small for mussels to attach to. The shell substrate consisted of sand originating from the Eastern Scheldt combined with empty shells and shell fragments (e.g., cockle, oyster) collected at a beach at location Schelphoek. In contrast to the muddy substrate, the sandy substrate provided the mussels with the opportunity to establish attachment. The empty shells were added to mimic the shells that are normally within the BioShell-SMC, since the biodegradable net would not dissolve during the 30-day duration of the experiment. To observe changes in mussel patch location or shape, we installed GoPros above the tanks to observe the development of spatial patterns.

At the start of the experiment, we cut the netting of two BioShell-SMCs per tank and placed both of them on the sediment 60 cm apart (Figure 4.2 E – H), which resulted in a mussel density of 7.8 kg/m^2 for the low density treatment and 18.5 kg/m^2 for the high density treatment. The transplantation method with loose mussels was obtained by removing the biodegradable sock and detaching the mussels from the coconut rope. Mussels were homogeneously dispersed in the middle of the tanks using a 60 x 60 cm frame in a low density of 2.1 kg/m^2 and high density of 8.3 kg/m^2 for the respective treatments. In comparison, the typical average seeding density in Dutch mussel cultivation is 1.0 – 2.5 kg/m^2 on plot scale (Capelle et al., 2014). The experiment with the loose mussels started on the 9th of November 2020 and lasted 30 days (round 1). The experiment with the mussels attached to the BioShell-SMC started on the 14th of December 2020 and lasted 29 days (round 2).

Mussel measurements

Every tank was photographed from above at the start of the experiment and at the end (after 30 days) to determine the spatial organization of the mussels. The pictures of the final day were edited, whereby mussel patches and individual mussels were visualized in black and non-mussels in white. We defined a "patch" in this experiment as a spatially isolated aggregation of mussels, following Hunt & Scheibling (2001). This was done with the fuzzy select tool and fine-tuned manually in the GIMP 2.10.32 software (revision 1). For every tank, the variance-to-mean ratio (VMR), number of patches (NP), perimeter-to area ratio (P:A) and total mussel cover (A) were determined to compare aggregation behaviour.

Dispersion

To measure the extent of dispersion of mussels within the experimental tanks, we used the variance-to-mean ratio (VMR, also known as an index of dispersion; Hoel, 1943):

$$VMR = \sigma^2/\mu$$

where σ^2 is the variance of mussel cover (the degree of variability in the number of black pixels per treatment) and μ is the mean number of mussel pixels found within the pattern. If the distribution of the mussels is completely random, the VMR would be ± 1.0. High VMR values (> 1.0) correspond to clustered patterns, with the presence of clumps of mussels and subsequently less dispersion.

Small values (< 1.0) correspond to a regularized gridded pattern and more dispersion. By quantifying pattern formation in terms of the variance-to-mean ratio, we can estimate the ability of loose mussels to find each other and create patches, and the ability of SMC-mussels to disperse away from the BioShell-SMC to escape high densities.

Window of opportunity and density threshold

In order to examine the feasibility of creating a window of opportunity (i.e., the presence of a suitable settlement substratum) for the dispersal of mussels away from the SMC at high and low densities, we added empty shells and shell fragments to the sandy sediment rather than to the BioShell-SMC. We did not envelop these empty shells within the biodegradable net, since the net would not be dissolved within the time span of the experiment. Consequently, the empty cockle shells would not have been dispersed as intended. We quantified this window of opportunity by counting the number of patches and calculating the total mussel cover area (in %).

Patch characteristics

We quantified the perimeter-to-area ratio (P:A ratio) to get information regarding the shape of the patches; a higher perimeter-to-area ratio corresponds with increased boundary length, which indicates multiple smaller patches or a large patch with an irregular edge. Subsequently, few and larger patches with a uniform edge are indicated by a low P:A ratio. This ratio was obtained by dividing the total perimeter of all patches by the total patch area. For loose mussels, this parameter tells us if the mussels were able to find each other, with a high ratio indicating that the mussels were too far away from each other to aggregate into big patches, resulting in multiple small patches. For SMC-mussels, a higher P:A ratio means that mussels were able to disperse in smaller groups onto the sediment away from the SMC.

Mussel condition and survival

We quantified the condition of the mussels to test if the transplantation method had an effect on the condition of the mussels. At the start of each round, 100 mussels were randomly selected to obtain the condition. The mean initial condition index (CI) was 2.90 mg cm^{-3} (*SD* = 1.01, n = 100) at the start of the first round of experiments (loose mussels). At the start of the second round of experiments (SMC-mussels), the CI was 2.76 mg cm^{-3} (*SD* = 1.53, n = 100). There was no significant difference in initial condition index between these two rounds ($t(200) = 0.759$, $p = 0.449$), demonstrating that the additional month of acclimatization had no impact on the condition of the mussels. At the end of the experiment, the water was removed from the tanks and a 6x6 grid comprising 36 squares, each measuring 10x10cm, was placed on the sediment. Ten squares were randomly selected for each tank for sampling purposes. All mussels within a selected square were collected and pooled into two subsamples, one from top of the patch and one from the bottom of the patch, to test the effects of within patch position. In the event that a square was empty, the adjacent square was chosen instead. The collected mussels were subsequently measured for length (cm), weight (g) and condition index (mg cm^{-3}), resulting in an average of 92 mussels per tank. Ash-free dry-weight (AFDW) for every mussel was obtained by drying the flesh at 70°C and ashing it at

540°C until the difference in weight was less than 1% between two measurements. The condition index (CI, mg cm^{-3}) was calculated (by dividing the AFDW by the cubed length) for every individual mussel. To test if there was a difference in survival between treatments, we counted the number of living and dead mussels for the collected mussels for every tank. Survival was obtained by dividing the number of living mussels by the total number of mussels in the sample.

Statistical analysis

All statistical testing was conducted in R studio (R Studio Team 2020), with the critical alpha value for significance being set to $p = 0.05$. Prior to model fitting, we checked assumptions of normality and homogeneity of residuals visually, following the procedure described in Zuur et al. (2010). If necessary, we transformed data to meet the assumptions. Model simplification was achieved by a stepwise reduction in predictive factors, starting with the highest-order interactions. Parameters were retained when removal resulted in a significant reduction in model fit. The Kenward-Roger method was used for obtaining degrees of freedom. Where relevant, pairwise comparisons were obtained by Tukey posthoc tests (R-package emmeans, Lenth, 2016).

We wished to determine the effect of transplantation method (loose mussels vs. SMC-mussels), initial density (high vs. low) and sediment composition (mud vs. shell) on the response variables (variance-to-mean ratio, mussel cover, number of patches, perimeter-to-area ratio, mussel survival and condition index). Data of variance-to-mean ratio (VMR), perimeter-to-area ratio and mussel cover met the assumptions without transformation. These dependent variables were analyzed with a two-way ANOVA with transplantation method, initial density, sediment composition and the interactions between these parameters as predictive factors, resulting in the following models: Response variable ~ transplantation method × perimeter-to-area ratio × mussel cover. Differences in the number of patches was analyzed with a quasi-poisson generalized linear model (GLM), implemented with the glm function (family set to quasi-poisson). The transplantation method, initial density, sediment composition and the interactions between these parameters were entered as explanatory variables. Mussel survival was analyzed with a logistic regression because the response variable was a count (number of living mussels) that can be expressed as a proportion (living mussels/total mussels), using the ln-function of the SciViews package (Grosjean et al., 2019): ln(survival/(100-survival)). The condition index of the individual mussels followed the normality and homogeneity assumptions. The initial condition index of round 1 and 2 was compared with a two-sample t-test. The difference in condition index at the end of the experiment was analyzed using linear mixed-effects models with transplantation method, sediment composition, initial density, position of the mussels and the interaction between these parameters as predictive factors.

Results

Mussel measurements

The pictures in Figure 4.2 show how the mussels redistributed after 30 days from the initial situation. The left eight pictures (A – D) show the loose seeded mussels, which were equally distributed in the tanks at the start of the experiment. The mussels in high density were redistributed into patches and created a “labyrinth” like pattern (A and B). The mussels in low density aggregated in small patches of a few mussels (C and D). The mussels attached to the SMC (E – H) showed dispersion onto the sediment at high density (E and F). At low density, the mussels showed less dispersion onto the sediment (G and H).

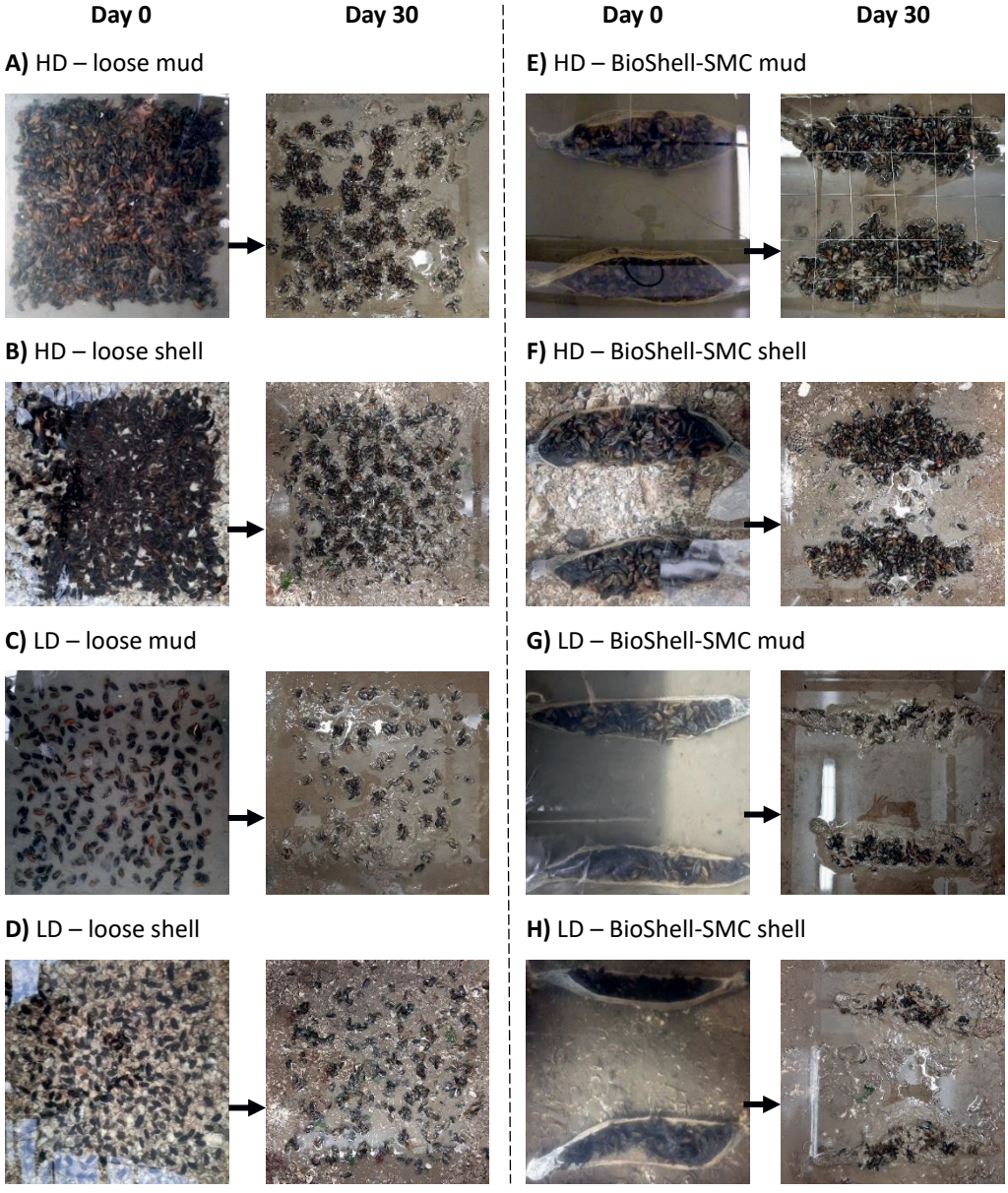

Figure 4.2. Overview of the mussels in the experimental units at the start (left) end of the experiment (right, after ±30 days). Experiments in round 1 included loose mussels of different densities (**A-D**) while the mussels in experimental round 2 were attached to the BioShell-SMC which was cut open on top (**E-H**). High density (HD): 8.3 kg/m^2 for loose mussel (**A**, **B**) and 18.5 kg/m^2 for mussels attached to the SMC (**E**, **F**); low density (LD): 2.1 kg/m^2 for loose mussels (**C**, **D**) and 7.8 kg/m^2 for mussels attached to the SMC (**G**, **H**). All treatments were carried out in triplicate.

Spatial clustering

At the end of the experiment (after 30 days), mussels attached to the SMC had a higher variance-to-mean ratio (VMR) than loose mussels ($F_{1,1}$ = 71.81, p < .001) (Figure 4.3), which indicates more intense clustering for SMC-mussels. Less mussels to start with (low density) intensifies clustering (i.e. reduced dispersal) as well, which is shown by a significant effect of density on the VMR ($F_{1,1}$ = 9.13, p = 0.008). Sediment composition also significantly influenced the VMR ($F_{1,1}$ = 47.08, p < .001), as well as the interaction between sediment composition and method ($F_{1,1}$ = 12.74, p = 0.003) and sediment composition and density ($F_{1,1}$ = 21.66, p < .001). This was explained by a higher VMR for loose mussels in high density on mud than on shell, indicating that mussels dispersed more on shell than on mud (Tukey, p < .001). We also found a significant interaction between transplantation method and density ($F_{1,1}$ = 28.29, p < .001), explained by a higher VMR for SMC-mussels in low density compared to high density (Tukey, p < .001).

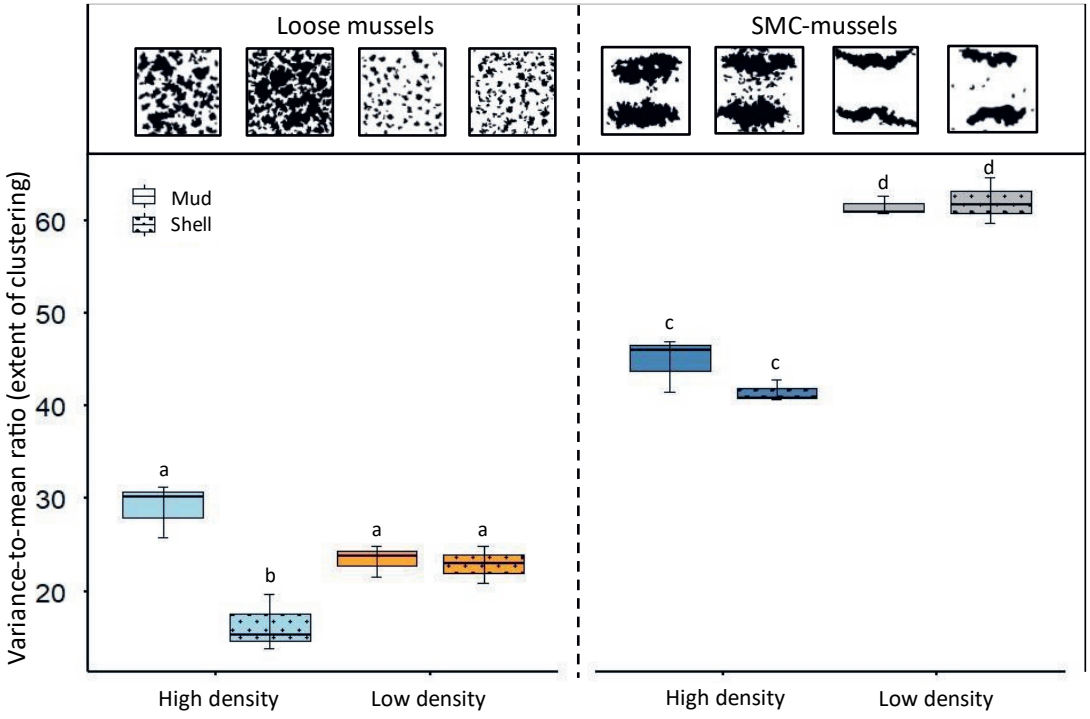

Figure 4.3. Variance-to-mean ratio for loose mussels (left) and SMC-mussels (right) in high and low density. Mud is represented with solid fill and shell with dotted pattern. Letters denote significance.

Window of opportunity and density threshold

The number of patches decreased over time for loose mussels in low density from approx. 300 to 100. This indicates that the mussels that were individually transplanted created patches of approx. 3 mussels when survival was 100%. In high density, we found an average number of patches of 28. For SMC-mussels, the number of patches increased, indicating that the mussels moved away from the rope. After 30 days, transplantation method ($F_{1,1}$ = 4.40, p = 0.036) and density ($F_{1,1}$ =

63.03, $p < .001$) significantly affected the number of patches (Figure 4.4A). Besides, the number of patches was affected by the interaction between these variables ($F_{1,1} = 57.70$, $p < .001$). We found more patches for loose mussels transplanted in low density compared to loose mussels in high density (Tukey, $p < .001$). Sediment composition showed an interaction effect with method ($F_{1,1} = 4.12$, $p = 0.04$), but this was only explained by differences between transplantation methods, not within loose nor SMC-mussels. However, comparing the number of patches on shell and mud for SMC-mussels in high density approached the level of significance (Tukey, $p = 0.079$).

Total mussel cover was significantly influenced by initial transplantation density ($F_{1,1} = 154.12$, $p < .001$) and sediment composition ($F_{1,1} = 16.13$, $p < .001$) (Figure 4.4B). Method did not significantly influence mussel cover, however, we found significant interactions between method and density ($F_{1,1} = 9.24$, $p = 0.007$) and method and sediment composition ($F_{1,1} = 11.46$, $p = 0.003$). Mussel cover was higher for loose mussels seeded in high density (53.3 ± 4.3%) compared to low density (18.3 ± 4.4%) ($p < .001$). This was also the case for SMC-mussels, with a coverage of 40.3 (± 4.6) % for high density and 17.4 (± 4.0) % for low density (Tukey, $p < .001$). Sediment composition

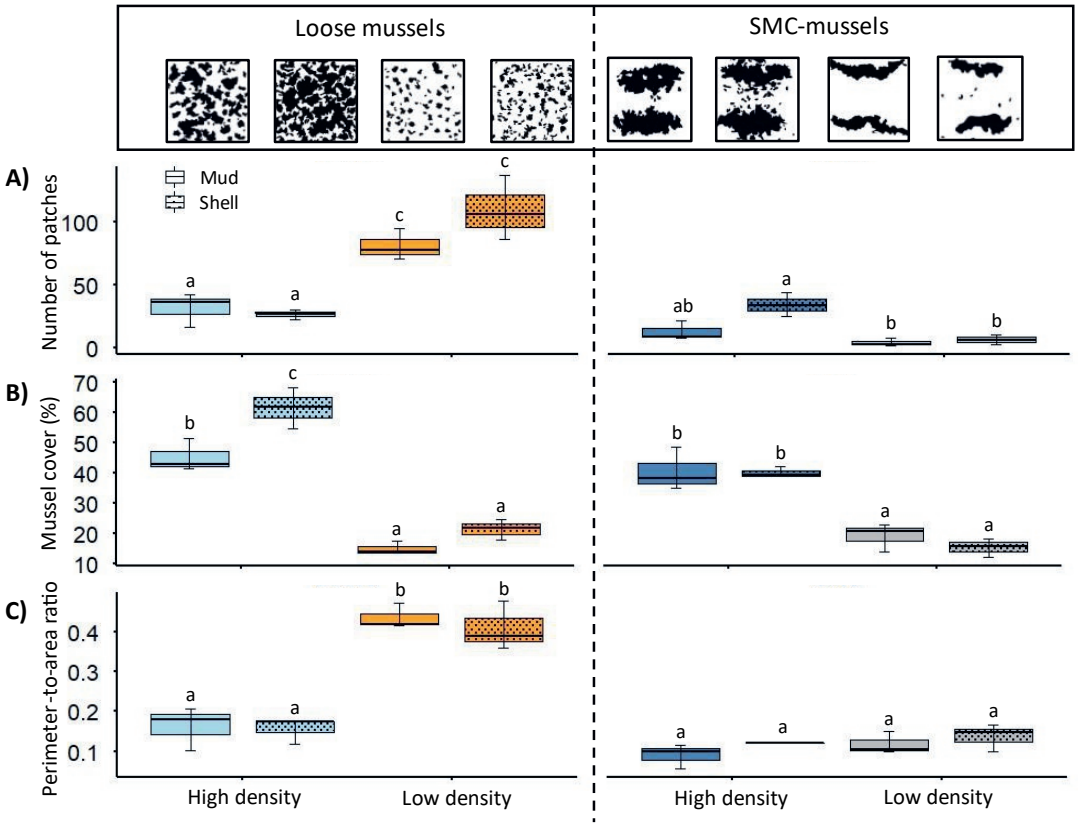

Figure 4.4. Patch characteristics at the end of the experiment for loose mussels (left) and SMC-mussels (right) in high and low density. Mud is represented with solid fill and shell with dotted pattern. Letters denote significance. **A.** Number of patches, **B.** Mussel cover (in %) and **C.** perimeter-to-area ratio, calculated as the total perimeter of all patches divided by the total mussel cover.

had an effect on loose mussels, with a larger cover area on shell compared to mud (p = 0.004). We did not find this for SMC-mussels.

Patch characteristics

We also looked at how the perimeter-to-area ratio (P:A ratio) of the mussel patches changed over time (Figure 4.4C). The ratio was affected by the transplantation method ($F_{1,1}$ = 6.71, p = 0.017), density ($F_{1,1}$ = 158.22, p < .001) and the interaction between these two variables ($F_{1,1}$ = 66.33, p < .001). Loose mussels transplanted in low density showed patches with a significantly higher perimeter-to-area ratio than loose mussels in high density (Tukey, p < .001), indicating a more fragmented pattern for low density. Treatments did not differ between SMC-mussels and between SMC-mussels and loose mussels in a high density. Sediment composition did not have an effect on the perimeter-to-area ratio for loose or SMC-mussels.

Mussel survival and condition

Survival of mussels after 30 days was influenced by transplantation method, mussels transplanted with substrate better survived than loose mussels ($F_{1,1}$ = 63.51, p < .001) (Figure 4.). A significant interaction between density and method ($F_{1,1}$ = 8.00, p = 0.013), is explained by differences between, but not within transplantation methods. The same applies for a significant three-way interaction between method, density and sediment composition ($F_{1,1}$ = 6.65, p = 0.021), it reveals differences between all loose mussel treatments vs. SMC-mussel treatments, but not within transplantation method.

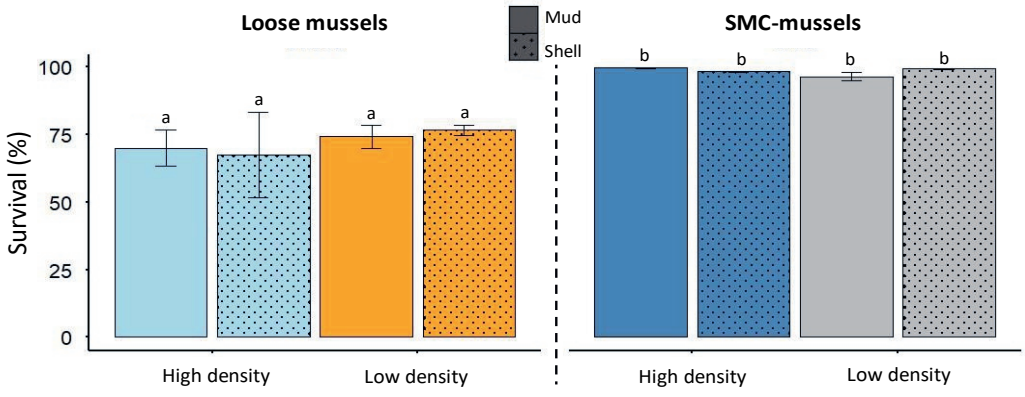

Figure 4.5. Survival of mussels at the end of the experiment for loose mussels (left) and SMC-mussels (right) in high and low density. Shell substrate is shown with a lined pattern and mud without pattern. Letters denote significance. Data are means ± SE.

At the end of the experiment, our analysis revealed significant main effects of density ($F_{1,1}$ = 4.75, p = 0.029), sediment type ($F_{1,1}$ = 11.20, p < .001) and transplantation method ($F_{1,1}$ = 63.45, p < .001) on the condition index (Figure 4.6). Besides, significant interaction effects between these factors were present. By loose mussels, we found no difference in condition between high and low density. For mussels attached to the SMC we found a higher condition for mussels seeded in low density than mussels seeded in high density (p < .001). Substrate type only affected the condition index for loose mussels in high density (p < .001), with a higher CI on shells. The condition index for loose mussels was not influenced by the position of the mussels (top or bottom). SMC-mussels, however, showed a higher condition index for mussels on the bottom (2.57 ± 0.04) than on top (2.41 ± 0.03) (p < .001).

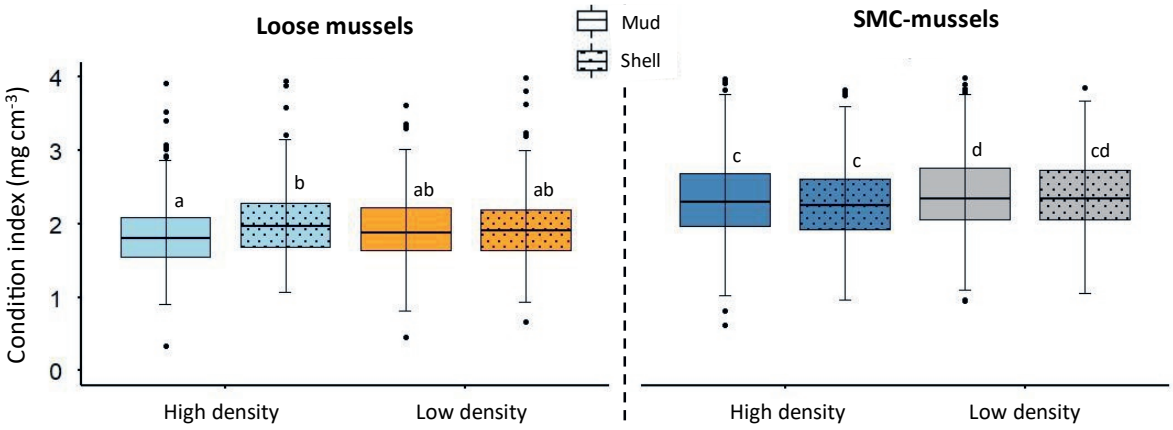

Figure 4.6. Condition Index (CI, in mg cm^{-3}) for loose mussels (left) and mussels attached to the SMC (right). Mud is represented with solid fill and shell with dotted pattern. Data are means ± SE. Letters on tope denote significance.

Discussion

In recent years, habitat restoration through transplantation of ecosystem engineers or foundation species has become an increasingly popular conservation strategy. However, the success of such restoration efforts depends largely on the ability of transplanted organisms to establish and persist in their new environment. Ecosystem engineers and foundation species typically occur in large numbers and depend on positive feedback, between individuals and the environment for establishment or extension. In this context, a better understanding of the underlying ecological processes, such as environmental context dependent competition and facilitation can provide crucial insights to improve restoration success. In this study, we investigated competition (measured through growth and condition) and facilitation processes (evaluated by measuring aggregation) among transplanted mussels using two different transplantation methods: loose seed and seed already attached to each other and to a substrate (BioShell-SMC). We conducted a mesocosm experiment to compare mussel aggregation and performance between the two transplantation methods at different densities and sediment compositions. Our findings showed that mussel aggregation patterns were influenced by attachment substrate and density, with mussels attached to the BioShell-SMC showing more intensified clustering compared to loose mussels, especially in low density. As expected, mussels in high density attached to the BioShell-SMC dispersed from the SMC. This, however, happened on both sediment types and not only when given the opportunity to escape the high competition by adding shell substrate mimicking cockle shells dispersed from the BioShell-SMC. Furthermore, transplanted mussels attached to the BioShell-SMC had higher survival rates and had a better condition than loose mussel seed. There are several biotic and abiotic environmental factors that affect spatial clustering and mussel survival after transplant. These factors include seeding method, density and sediment type.

Effect of density on spatial clustering and patch characteristics

Species density plays a key role in self-facilitation, which is a feedback mechanism to ameliorate environmental stressors. Initiating facilitation after transplantation by increasing densities of a mussel bed is only profitable until a certain density threshold is reached, whereafter the higher densities will increase competition between individuals, which may result in food shortage and even mortality (Bertness & Grosholz, 1985; Capelle, Scheiberlich, et al., 2016; Svane & Ompi, 1993). In contrast, if the density of mussels does not meet a specific threshold, they will not be able to form a consistent matrix, and instead, they will disperse into smaller clusters (Capelle et al., 2014), leading to reduced stability of the mussel bed (Bertolini et al., 2019).

Aggregation patterns for individually distributed mussels in low density in our study were compliant with observations in previous studies. After transplanting each mussel separately, aggregation resulted in clusters of about three mussels, which implies that the mussels were transplanted below the critical density threshold and could not form a uniform matrix. Capelle et al. (2014) found this threshold to fall between 2.5 kg m^{-2} and 5 kg m^{-2}, while we used a transplantation density of 2.1 kg m^{-2}. For these small clusters, the perimeter-to-area ratio was high,

indicating a large fragmentation. A study by Bertolini et al. (2020) also found mussel patches with a greater perimeter-to-area ratio in low density, even in patches of similar percentage cover. Seeding with such low density is not recommended, since increased fragmentation (i.e. more edge) can lead to greater losses from predation (Bertness & Grosholz, 1985; Capelle et al., 2019; Dolmer, 1998). Besides, increased edge size may lead to increased susceptibility to hydrodynamic forces on a mussel patch, i.e. gradual erosion of individual mussels on the bed edges (de Paoli et al., 2015). The patches of the SMC-mussels in our study had a lower P:A ratio compared to loose mussels and were less fragmented. This suggests that seeding mussels attached to the SMC would be beneficial because it reduces the risk of predation and vulnerability to dislodgement by hydrodynamic forces. Besides, the transplanted SMC-mussel patches had a greater ratio of variance-to-mean compared to the loose mussels, implying a more intense clustering. Here, SMC-mussels placed in high density exhibited a lower ratio than SMC-mussels placed in low density, indicating that they were less clustered. In conclusion, our results showed that transplanted loose mussels below the critical density threshold formed small, fragmented clusters, which may increase the risk of predation and erosion. Seeding mussels attached to SMCs was found to be beneficial, as it reduced fragmentation and clustering was more intense, particularly in low density conditions.

Surpassing thresholds is important for restoration success in other ecosystems as well. For example, the establishment of mangrove propagules requires an inundation-free period to develop roots of sufficient length to resist disturbances (Balke et al., 2011) and the establishment of seagrass can only happen above a certain density threshold (van der Heide et al., 2007). Besides, a study by Yuan et al. (2020) showed that a salt marsh can be successfully restored when physical (suitable tidal flat elevation) and biological (availability of propagules) thresholds are passed to open windows of opportunity for the establishment of the propagules.

Effect of sediment composition on spatial clustering and patch characteristics

The inclusion of an attachment substratum, such as empty shells, in mussel seeding has been found to impact self-organization, resulting in decreased clustering due to the provision of additional attachment points in a soft substrate environment (Capelle et al., 2019; Christensen et al., 2015; Frandsen & Dolmer, 2002; wa Kangeri et al., 2014). Our study revealed that loose mussels in high density exhibited greater mussel coverage on hard sediment (shells) than on soft sediment (mud), despite no significant difference in the number of patches. Additionally, they showed higher clustering intensity (i.e. higher VMR) on soft sediment than on hard sediment. This suggests that mussels on soft sediment climbed on top of each other to access favorable positions, while those on hard sediment were able to occupy more space, attaching to the substrate. When there is a lack of suitable attachment substratum such as on mud (Young, 1983), mussels will hold on to each other. Hence, mussels aggregate into higher biomass patches on soft sediment than in situations with attachment substratum (Capelle et al., 2019), which is confirmed by our results.

In adult zebra mussels, a greater density of mussels stimulated attachment to the substratum (Kobak, 2001). In our study, creating hard substrate in a soft sediment environment by adding shell debris created an opportunity for loose mussels in high density to aggregate to more favorable positions to optimize feeding and growth. In low density, this pattern did not occur. This

indicates that there was no density threshold to stimulate the formation of a different pattern. In contrast to these findings and to our hypothesis, adding shell debris to high density SMC-mussels did not increase dispersal; the number of patches on hard and soft sediment was comparable, as well as mussel cover. However, we found a tendency for more patches on hard than on soft sediment for SMC-mussels in high density, although this finding was not significant. An explanation for observing similar dispersion patterns on soft and hard sediment could be the presence of low levels of competition among individuals. This is supported by the high survival rates (nearly 100%) and favorable condition indices of SMC-mussels compared to loose mussels. Moreover, as competition levels were not too high, active resettlement was unnecessary since SMC-mussels were already attached to a settlement substratum. This stands in contrast to loose mussels, which needed to locate each other and aggregate for protection, a process that was found to be more effective on hard sediment than on soft sediment.

The interaction effects of substratum and density on positive feedback mechanisms for the establishment of biogenic reefs have, as far as we know, only been scarcely studied. Our study showed that adding shell debris to loose mussels in high density created an opportunity to aggregate, while this was not shown for loose mussels in low density. For SMC-mussels in high density, we observed a slight tendency for more dispersion on hard sediment compared to soft sediment, although the effect was not significant. Additional research is necessary to investigate the potential of using shell debris as an attachment substrate for SMC-mussels. Specifically, it remains unclear whether this method can effectively facilitate escape from high densities or whether it offers no significant advantage in situations where competition is moderate and suitable attachment substrates are already available, thereby reducing the need for resettlement.

Factors in mussel survival and condition

Mussels transplanted already attached to a substrate remained to score higher on cluster indices than loose mussels. This is in accordance with our expectations since the mussels attached to the SMCs were already highly concentrated at the start of the experiment. After 30 days, the SMC-mussels were still more intensely clustered than loose mussels, although they showed dispersal away from the SMC. This did not negatively affect the survival or the condition. Although it is known that high mussel densities increase *per capita* competition, our results revealed a substantial higher survival rate (98.2 ± 0.5 % vs. 72.0 ± 3.9 %) and condition index (2.47 ± 0.82 vs. 1.96 ± 0.53) for SMC-mussels than for loose mussels. Considering that there was no difference in the condition index at the start of the two rounds, we anticipated that the additional month of acclimatization did not impact this initial condition. The higher condition for SMC-mussels compared to loose mussels is in contrast with previous studies, where often condition decreased with increasing mussel clump size. Besides, mussel condition is often higher at the edge of a mussel bed or patch than in the center (e.g., in Knights, 2012; Newell, 1990; Svane & Ompi, 1993). In our study, we found no effect of position in the patch for loose mussels. For SMC-mussels, mussel condition was higher on the bottom of the patch than on the top of the rope. An explanation for the higher survival and condition of SMC-mussels compared to loose mussels might be handling

stress during removal and relaying of the loose mussels. Numerous studies have indicated that declumping of mussels can result in a range of negative outcomes, including shell damage, detachment of byssal threads from internal tissues, and loss of liquor, which can lead decreased health of mussels and even death (Calderwood et al., 2014; Capelle, Scheiberlich, et al., 2016; Dare, 1974; Slabyj & Hinkle, 1976). Additionally, bivalves can expend their energy reserves when exposed to disruptions in order to maintain their internal balance (Malham et al., 2003), leading to reduced growth (Garthwaite, 1985). What can be concluded from these results is that the mussels attached to the SMC in high density did not experience too much competition at the experimental scale, since the survival and condition were higher than for loose mussels. However, when scaling up, competition may become more intense. Nevertheless, this study indicates that the mussels were able to disperse on both soft and hard sediment, suggesting potential for successful mussel growth and survival on larger scales.

Implications for transplantation practice

Ecological restoration can greatly benefit from the transplantation of individuals or populations. However, these transplantations, particularly those involving foundational species, are commonly faced with challenges that result in significantly low success rates. Our study provides important insights into the competition and facilitation processes among transplanted mussels, which are key considerations for successful habitat restoration efforts. Our findings demonstrate that including a substrate (BioShell-SMC) as a transplantation method to enhance self-facilitation feedback mechanisms improves mussel survival and condition, indicating its potential as an effective technique for restoration projects. Our study also highlights the importance of understanding the interplay between attachment substrate and density in mussel aggregation. Specifically, pre-clustered mussels on the BioShell-SMC substrate remained a more intense clustering, which may have important implications for future transplantation efforts. Additionally, our results support the hypothesis that mussels will disperse from the BioShell-SMC in high density conditions, surprisingly on both soft and hard sediment conditions. Overall, our study emphasizes the need to consider ecological processes such as competition and facilitation when designing and implementing restoration projects, and provides a case for optimizing transplantation success of ecosystem engineers by including positive feedback mechanisms at establishment, effectively creating a window of opportunity.

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Chapter 5

Exploring the impact of spatial patterns on restoration efforts: promoting self-facilitating feedback mechanisms with an innovative biodegradable seed mussel collector

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Abstract

Transplantations of organisms in aquatic ecosystems play an important role in ecological restoration and commercial practices. However, success rates of these transplantations, especially when ecosystem engineers are involved, are often low. To enhance transplantation success, the promotion of self-facilitation between transplants that mitigate environmental stressors is crucial. Besides, spatial patterns resulting from self-facilitation can enhance ecosystem resilience. In this study we used biodegradable structures ("BioShell-SMCs") to (1) ameliorate self-facilitating feedback mechanisms to overcome environmental stressors in the initial post-transplantation phase, and (2) to increase (mussel) transplantation success by implementing large-scale spatial configurations, mimicking natural mussel bed patterns. These structures are an innovation of traditional seed mussel collectors (SMCs) used in mussel cultivation and do not contain any plastic. Instead, they consist of a biodegradable net based on a compound of aliphatic polyesters, filled with empty cockle shells around a coconut fiber rope. We tested whether different spatial configurations could increase transplantation success of mussel seed: low vs. high density labyrinth pattern and banded pattern. The results of this large field experiment showed high overall losses (~75%), with no significant variation between configurations. The lack of mussels migrating from the BioShell-SMC structures hindered the initiation of natural aggregations, resulting in increased competition among mussels. Besides, factors such as hydrodynamic dislodgement, burial and interannual variation, likely contributed to the observed losses. Overall, this research contributes to understanding the mechanisms that underlie successful transplantation strategies in aquatic ecosystems, with potential applications in ecological restoration and aquaculture practices.

Introduction

Transplantations of individuals or populations in aquatic ecosystems can be an important component in the success of ecological restoration (Horoszowski-Fridman & Rinkevich, 2016), the provision of ecosystem services (e.g., mangroves providing coastal protection (Barbier, 2016)) and commercial practices (e.g., aquaculture (Kamermans et al., 2002)). However, these transplantations tend to have low success rates, particularly when ecosystem engineers are involved (Godefroid et al., 2011; Griffith et al., 1989). Ecosystem engineers play a crucial role in modifying, maintaining, and creating habitats within their ecosystem (Bruno et al., 2003; Jones et al., 1994). This is due to their ability to directly or indirectly influence the availability of resources for conspecifics or other species by inducing physical changes in biotic or abiotic materials (Jones et al., 1994). In dynamic coastal environments, ecosystem engineers rely on self-facilitating feedback mechanisms that help to mitigate physical (e.g., wave exposure, salinity) and biological (e.g., nutrient availability, predation) stressors (Liu et al., 2014; van de Koppel et al., 2001; van der Heide et al., 2007). For example, macrophytes are ecosystem engineers that have the capability to decrease hydrodynamic energy and drag in their surroundings, resulting in local accretion or wave attenuation, which ultimately promotes the expansion of the species (Bouma et al., 2005; Maxwell et al., 2017). Likewise, oyster reefs emerge when larvae settle on existing oyster shells, attracted by chemical cues, thus reinforcing the growth and stability of their habitat (Turner et al., 1994). The lack of a disturbance-free period immediately following transplantation (also referred to as a window of opportunity), along with the absence of a suitable settlement substratum for new recruits to establish positive feedback mechanisms, could contribute to the failure of transplantations (Balke et al., 2014; Capelle et al., 2019). The promotion of self-facilitation between transplants is, therefore, increasingly recognized as an important component of transplantation success (Silliman et al. 2015; Ladd et al. 2018).

The emergence of spatial patterns in ecosystems can significantly increase the overall resilience of an ecosystem (Liu et al., 2014). Patterns arise from the interplay between facilitation and competition, that drives pattern formation at a small scale, while the influence of negative interactions like physical forcing leads to the development of spatial patterns on a larger scale (Rietkerk & Van de Koppel, 2008). Examples of ecological systems with large scale patterns are arid ecosystems (HilleRisLambers et al., 2001), wetlands (Foster et al., 1983), coral reefs (Mistr & Bercovici, 2003), and mussel beds (van de Koppel et al., 2005). Recent studies have shown that mimicking these natural spatial pattern formations in restoration efforts can increase transplantation success (de Paoli et al., 2017; Schotanus, Walles, et al., 2020; Shaw et al., 2020; Temmink et al., 2022). For example, when mussels were transplanted in high density bands (four bands of 16 x 5 m and spaced 5 m apart) positioned between fences, resulted in a loss that was over twice as small in comparison to the loss observed when mussels were homogenously transplanted at low density across a 16x40 m area (Schotanus, Walles, et al., 2020). Using blue mussels (*Mytilus edulis*) as a model organism, we explored the possibility of increasing large-scale

transplantation success in a subtidal ecosystem by (1) using biodegradable materials to promote self-organization into high-density clusters upon transplantation, thereby potentially offering protection against predators and increasing resistance to hydrodynamic dislodgement. We also tested (2) the efficiency of implementing different spatial configurations designed to mimic patterns found in natural mussel beds. To the best of our knowledge, no large-scale field study has previously attempted to create a subtidal ecosystem while incorporating spatial patterns.

Natural mussel beds on soft sediment often exhibit a distinctive spatial pattern, that is characterized by high-density mussel bands (5 – 10 m apart) perpendicular to the tidal direction, alternating with bare sediment patches (van de Koppel et al., 2005) (Figure 5.1). These patterns are thought to result from a combination of small-scale positive feedback, and larger-scale negative feedback (van de Koppel et al., 2008; van de Koppel et al., 2005). Small-scale aggregation leads to local high densities, which offers mussels safety from dislodgement and predation (Hunt & Scheibling, 2001). However, high density also increases competition among the mussels. To mitigate this competition, large-scale formation of banded patterns reduces the overall density, thereby decreasing competition for food, while maintaining a local high density within the bands to ensure safety (van de Koppel et al., 2005). These large-scale patterns enhance the resilience of mussel beds and reduce the likelihood of collapses (de Paoli et al., 2017; Liu et al., 2020). Therefore, implementation of large-scale patterns in mussel transplantations may increase transplantation success.

The biodegradable structures ("BioShell-SMCs") used in our experiment were used to mimic the large-scale spatial patterns in mussel beds and simultaneously provide substrate and

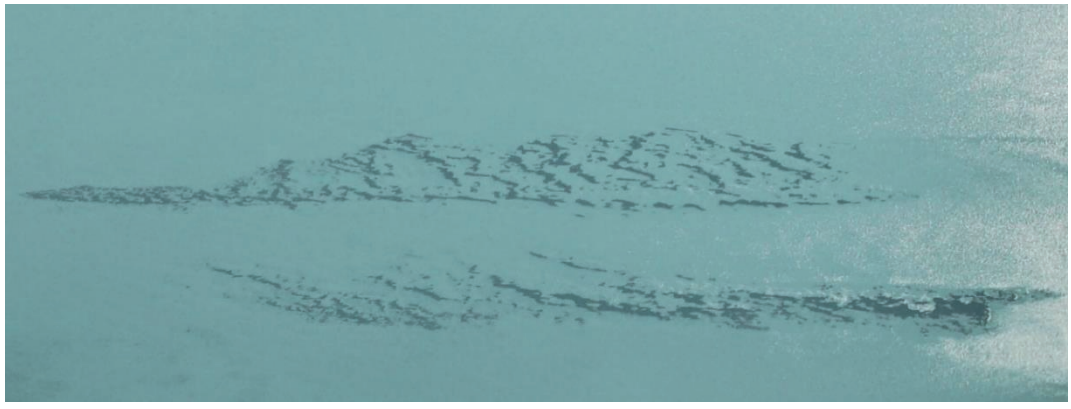

Figure 5.1. Aerial photograph of a mussel bed displaying a large-scale banded pattern in the Wadden Sea, situated right below the island Ameland in The Netherlands. The mussel bed covers an area of about 1.2 ha. Photograph taken by Karin Troost on February 19th, 2019.

protection for the establishment of self-facilitating interactions. BioShell-SMC is an innovation of traditionally used nylon seed mussel collectors (SMCs) (Van den Bogaart et al., 2023a). The BioShell-SMC consists of a biodegradable sock based on a compound of aliphatic polyesters, filled with empty cockle shells and placed around a coconut-fiber carrying rope. SMCs are used to collect mussel seed (juvenile mussels) from the water column (Kamermans et al., 2002), which can be used for aquaculture practices or for restoration efforts. Collection of mussel seed using BioShell-SMCs

was comparable with the biomass obtained with traditionally used SMCs (Van den Bogaart et al., 2023a). Normally, in benthic mussel culture mussel seed is removed from the SMCs and individual seeds are subsequently dispersed over the bottom. However, the small size of the mussels and the lack of hard substrate on soft-sediment (culture) plots makes the newly transplanted mussels highly vulnerable to loss factors, such as hydrodynamic dislodgement and predation (Kamermans et al., 2010; Murray et al., 2007). The use of the BioShell-SMC makes it possible to directly transplant the mussels onto the bottom in high-density clusters, while still attached to the biodegradable structure. The presence of pre-clustered mussels on the BioShell-SMCs has shown to increase survival of the mussels by offering protection against crab predation, as well as enhancing resistance to hydrodynamic dislodgement (Van den Bogaart & Schotanus et al. 2023b). Consequently, the BioShell-SMCs hold the potential to enhance restoration or cultivation success by reducing the number of transplants needed. To improve long-term transplantation success, establishment of spatial patterns is crucial (Schotanus, Walles, et al., 2020; Shaw et al., 2020; Temmink et al., 2022; van de Koppel et al., 2008). Although mussels typically self-aggregate into patterns, this process leads to substantial losses (Capelle, Scheiberlich, et al., 2016), and in dynamic environments, it can even hinder restoration success (de Paoli et al., 2015). Since mussels attached to the BioShell-SMC are more stable than loose mussels (Van den Bogaart & Schotanus et al. 2023b), we provide the mussels with an attachment substrate, and by mimicking spatial patterns of mature mussel beds, we offer them a kickstart that potentially increases transplant success.

In a large field experiment, mussels attached to the biodegradable substrate were relayed into three different patterns: *i*) *Low density labyrinth pattern*: low local density mussels attached to short pieces of BioShell-SMC homogeneously distributed, hypothesized to result in low competition among mussels but in an increased risk of hydrodynamic loss, *ii*) *High density labyrinth pattern*: high local density mussels attached to short pieces of BioShell-SMC homogeneously distributed, hypothesized to result in high competition but in a reduced risk of hydrodynamic loss, and *iii*) *Banding pattern*: high local density mussels attached to the BioShell-SMC placed in regularly spaced banded patterns, hypothesized to result in low competition and a reduced risk of hydrodynamic loss. Overall, the results of our experiment will help to understand the importance of combining (1) self-organization by using biodegradable substrates; and (2) optimal spatial configurations to increase (mussel) transplantation success.

Material and Methods

Study site

We conducted a field experiment on a subtidal mussel culture plot from the 7th of September 2021 until the 28th of June 2022. The study site was situated in a sheltered area of the Oosterschelde in the Netherlands, the Zandkreek (51°33'26.6"N 3°53'55.0"E) (Figure 5.2A). The location is characterized by sandy sediments and the dominant water flow direction is from the southwest (Figure 5.2B). The experimental plots had a depth varying from approx. 1 to 4 m,

depending on the tide. The mussels used in this experiment were collected on 5 km of biodegradable BioShell-SMC (Van den Bogaart et al., 2023a), which was deployed at a location in the nearshore North Sea (SMC in Figure 5.2A: 51°46'22.0"N 3°48'10.4"E) in May 2021. The BioShell-SMCs were harvested on the 6th of September 2021 and relayed on the study site the next day.

Setup of the field experiment

The BioShell-SCMs (Figure 5.3A) were relayed in three configurations: (1) short (4 m) fragments of single rope BioShell-SMC (low density) homogeneously distributed over the experimental plot (*Low density labyrinth pattern*); (2) short fragments (4 m) of three BioShell-SMCs tied together (high density) homogeneously distributed over the experimental plot (*High density labyrinth pattern*); and (3) eight long (8 m) ropes of three BioShell-SMCs tied together (high density) placed plot-wide in perpendicular lines (*Banding pattern*) (Figure 5.3B and C). Each configuration was replicated four times. The SMCs were manually dropped from a boat and placed in randomly assigned experimental plots measuring 20 x 24 meters. A buffer zone of approximately 5 meters was maintained between adjacent plots. The plots were arranged in a row to minimize variation in depth and current between the plots. In total, each plot contained approx. 850 kg of BioShell-SMC (structures and mussels). The initial mussel biomass attached to the BioShell-SMC was 6.0 kg/m. In comparison, the typical average obtained biomass with traditional seed collectors in the Dutch Voordelta is 2.6 kg/m, with fluctuations ranging from 0.3 to 5.0 kg/m (Capelle, 2023).

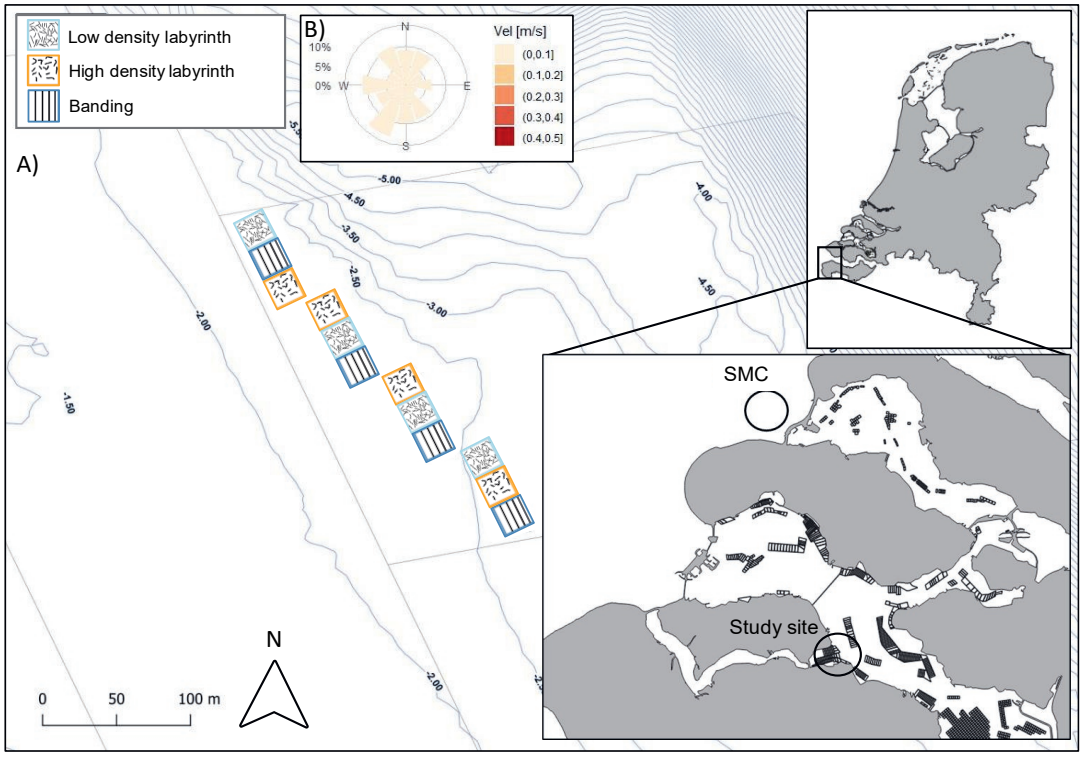

Figure 5.2. A. Map of the study area. Land is shown in grey and water in white. Mussel culture plots are shown in dark grey on the overview maps. SMC: origin of the seed (seed mussel collector). The three transplant configurations are: low density BioShell-SMC homogeneously distributed (Low density labyrinth pattern, light blue); high density SMCs homogeneously distributed (High density labyrinth pattern, orange); and high density perpendicular lines (Banding pattern, dark blue). The plots measure 20x24 m each and are five meter apart, with four replicates per configuration. **B.** Near-bed orbital velocity (m/s) and direction.

Mussel cover based on sonar data

We used side scan sonar (Kongsberg GeoAcoustics PulSAR Sidescan) to map change in mussel cover of the different configurations. Approx. every month (on days with little wind and current) from September 2021 until June 2022 we collected sonar data during high tide (water depth of 4 – 5 m) with a 7 m long vessel. The side scan towfish was connected with a tow cable to a reel and launched from starboard. A deck cable ensured data transmission from the towfish to the interface deck unit, which was connected to a laptop with PulSAR software (version 0100 B6-r7235) for real-time visualization. The vessel was equipped with a GPS (Trimble R8s with RTK positioning) for accurate positioning. For consistent sonar imagery, the vessel moved in a constant speed of approx. 8 km/h to avoid distortion and stretching of the scans. The transects were sailed as straight as possible and areas were scanned multiple times to ensure a good coverage. We used

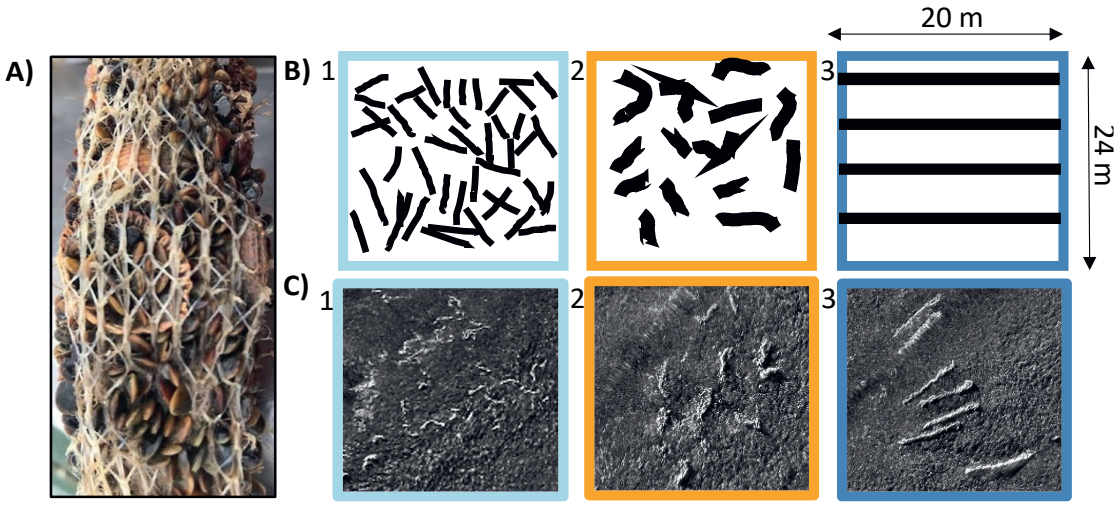

Figure 5.3. A. Biodegradable BioShell-SMC, consisting of a biodegradable sock based on a compound of aliphatic polyesters, filled with empty cockle shells and placed around a coconut-fiber carrying rope. Mussel seed can settle on the cockle shells. **B.** Schematic representation of the transplant configurations of the BioShell-SMC: 1. Low density SMCs homogeneously distributed (Low density labyrinth pattern); 2. High density SMCs homogeneously distributed (High density labyrinth pattern); 3. High density perpendicular lines (Banding pattern). The data were collected with four replicates per configuration. The SMCs were relayed on plots measuring 20 x 24 m, with an empty buffer zone of approx. 5 meter between two plots. **C.** Images obtained with sonar scanning, corresponding with configurations in **B**.

high-frequency scans (600 kHz) with a range of 20m. Captured side-scan images were saved as xtf files and the corresponding GPS coordinates were recorded.

The xtf files were imported into SonarWiz 7 software (V7.09.05) and processed into a complete geo-referenced image mosaic with a 2 cm resolution. Errors in the heading of the towfish were removed by visually selecting a threshold to remove outlying pings. The water column was removed with bottom tracking, since we were only interested in the sea floor. Then, empirical gain normalization was used to sum up and average out all amplitudes for every ping, the results is a smoother surface where artefacts were easier to spot. Removal of noise in the sonar images, such as ripple marks or stones, was done by time-varying-gain to equalize the backscatter. De-stripe filter was applied to slightly repair errors caused by waves, turns of the vessel and speed differences that occurred as stripes in the scan. Finally, map corrections were applied to sheave sonar offsets and the files were exported in greyscale.

The geo-referenced files were imported in Python (Version 3.9.7), where smaller tiffs were separately created for each experimental plot. To remove noise, median convolution was applied with a kernel of 8 by 8 pixels. This recalculates every pixel by taking the median from an 8 by 8 square around the pixel. The threshold value for mussels was defined on band 140. Band 0 resulted in a black pixel, while 255 resulted in a white pixel. To determine the percentage coverage, the number of white pixels (representing mussels) was divided by the total number of pixels within each plot. These numbers were then converted to square meters (m^2) coverage. We used 6 out of the 10 measurements, since they obtained good quality data.

Mussel biomass based on sample data

We conducted five moments where biomass of mussels attached to the BioShell-SMC was monitored, spanning from September 2021 to June 2022, including initial sampling. The third sampling campaign (January/February 2022) was interrupted due to unfavorable weather and tide conditions that intercepted the fieldwork. The fieldwork campaign could only be resumed 28 days after the start on the 19th of January. Since there was no significant difference in mussel biomass between these sampling moments in January and February ($t_{33.8} = 0.87$, $p = 0.389$), we used the average sampling date between those two dates to account for this division. During each sampling moment, three samples were taken per plot from different BioShell-SMCs, resulting in 12 samples per treatment and a total of 36 samples per sampling moment. Due to very limited visibility and because the experiment was always submerged, we relied on locating the SMCs by touch. The samples were obtained by snorkeling and selecting a piece of BioShell-SMC where we would come across. Using a knife, samples of approx. 10 cm in length were taken and transported to the laboratory. In the case of the high density treatments, only one of the three ropes tied together was sampled. For each sample, total weight and weight of the mussels were noted. To determine the mussel biomass, we calculated the proportion of mussel biomass attached to the BioShell-SMC. This was achieved by dividing the weight of the mussels by the total weight of the sample. We applied this approach since some samples did not include the inner carrying-rope from the BioShell-SMC, making it impossible to calculate mussel biomass per meter of collector material.

Mussel length and condition

We collected three random samples from the BioShell-SMCs at the start, analysing a total of 210 mussels for shell length. Over a period of nearly 10 months, we monitored mussel length development within the plots. During each sampling moment, we measured shell length using the same three samples from each plot that were used for density determination. In total, we measured 3,707 mussels by randomly subsampling 70 mussels (or fewer if there were less than 70 present) from each sample. The condition index (mg cm^{-3}) was obtained at the beginning (8th of September) and at the end (14th of June 2022) of the experiment. All 70 mussels from each initial sample (n = 3) were pooled together, as they originated from the same location and had no initial differences in seeding configuration. However, at the end of the experiment, mussels were processed individually to obtain more precise data. Ash-free dry-weight (AFDW) was obtained by drying the flesh at 65°C for 2 – 4 days and subsequently ashing it at 510°C for 4 hours. The condition index was calculated by dividing the AFDW (mg) by the cubed length (cm^3) for all mussels collectively in September, or for each individual mussel in June (Beukema & De Bruin, 1977).

Effect of hydrodynamics on SMCs

From the 12th of November 2021 until the 19th of April 2022, a wave gauge sensor (OSSI-10-003) was placed on a metal frame to measure hydrodynamic forces within the experimental area. In order to measure the impact of storms on the BioShell-SMCs, we deployed the sensor in November rather than September, as storms typically occur during the winter season in the Netherlands. This decision was also influenced by the battery capacity, as it would not have lasted

with the same measuring interval from September until June. The gauge was placed approx. 50 cm above the sediment. Pressure samples were recorded at a rate of 10 Hz (datapoints per second) with a burst length of 7 minutes and a burst interval of 15 (resulting in 4 recordings of 7 minutes with 10 Hz per hour). Spectral analysis was performed on the pressure measurements to obtain significant wave height, while accounting for depth-dependent pressure. Subsequently, near-bed orbital velocity (m/s) was calculated based on the linear wave theory.

Statistical analysis

All statistical testing was conducted in R studio (2023.03.1). Prior to model fitting, all data were visually checked for normality (Q-Q plot) and homogeneity of residuals, following the procedure described in Zuur et al. (2010). If necessary, data were transformed to meet the assumptions. Post-hoc comparisons were used to test for significant differences between configurations (r-package emmeans, Lenth, 2019).

Mussel cover based on sonar data

In order to compare the rate of cover loss between the three configurations, a survival analysis was carried out based on maximum likelihood (Miller, 1981). In summary, the average daily mussel cover loss rate (ε) per configuration was estimated as the inverse of the mean lifetime of the mussel structures (τ). To estimate the mean lifetime of the mussel structures, the difference in proportion of mussel cover (ρ_i) was determined for each monitoring time (t_i). Since the BioShell-SMCs did not completely disappear throughout the duration of the experiment, a correction for these right-censored observations was incorporated to avoid underestimation of the mean lifetime:

$$\tau = 1/(1-\rho_{t\_end})\Sigma((1-\rho_{i+1})-(1-\rho_i))t_{i+1} \quad (1)$$

Differences in cover loss rate (ε) were analyzed with a linear mixed-effects model from the R package *lme4* (Bates et al., 2014), with the log transformed cover loss rate as the response variable, the configuration as the explanatory variable and plot as random factor (Mussel cover loss rate ~ Configuration + (1|Plot)).

Differences in mussel cover over time were analyzed with a repeated measures ANOVA, with time as the within-subject factor (repeated), nested within the "Plot" factor, and configuration as the between-subjects factor (Mussel cover ~ Configuration + (1|Plot/Time)).

Differences in mussel cover at the final date were also analyzed with a linear mixed-effects model (Mussel cover on final day ~ Configuration + (1|Plot)).

Mussel biomass based on sample data

A similar survival analysis (eq. 1) as for cover loss was used to assess changes in mussel biomass. Here, instead of calculating the difference in the proportion of mussel cover at each monitoring time, the difference in the proportion of mussel biomass was determined. Mussel density loss rate (ε) could not be transformed to meet the assumptions; we therefore used Friedman's tests (based on ranks). Plot was included as blocking factor, similarly to the random factor in a linear mixed-effects models (Mussel density loss rate ~ Configuration | Plot).

Differences in mussel density over time were analyzed with a repeated measures ANOVA, with time as the within-subject factor (repeated), nested within the "Plot" factor, and configuration as the between-subjects factor (Mussel density ~ Configuration + (1|Plot/Time)).

Differences in mussel density at the final date were analyzed with a linear mixed-effects model, with mussel density as the response variable, the configuration as the explanatory variable and plot as random factor (Mussel density on final day ~ Configuration + (1|Plot)).

Mussel length and condition

Differences in mussel length between treatments was analyzed with a linear mixed-effects model, with mussel length as the response variable, the configuration as the explanatory variable and plot as random factor (Mussel length ~ Configuration + (1|Plot)). The condition index was analyzed in a similar way, with Condition index as the response variable.

Results

Mussel cover based on sonar data

Based on the sonar data, we found a significant difference in cover loss between treatments ($F_{2,6}$ = 12.64, p = 0.007, Figure 5.4A, Appendix B). Contrary to what was expected, rate of cover loss was higher for the high density Banding pattern than for the Low density labyrinth pattern (Tukey, p = 0.006) and nearly significantly higher for High density labyrinth pattern than for the Low density labyrinth pattern (Tukey, p = 0.050). Cover loss rate was similar between both high density treatments (Banding pattern and High density labyrinth pattern).

From September to mid-February, mussel cover was more or less constant across all experimental configurations (Figure 5.4B). A nearly 80% strong decline in mussel cover occurred from mid-February to mid-April. Coinciding with this timeframe, there were instances of elevated near-bed orbital velocities (reaching up to 50 cm/s), which might signify losses attributed to hydrodynamic impact. Following this decline, the cover remained relatively stable again until the end of May, after which a subsequent decrease was observed. The pattern in mussel cover was consistent and not different ($F_{2,9}$ = 0.04, p = 0.959) between treatments, which suggests the factors influencing mussel survival and distribution were not significantly influenced by the different spatial configurations.

At the start of the experiment, mussel cover was comparable for all treatments ($F_{2,9}$ = 1.43, p = 0.288). In June, at the end of the experiment, mussel cover was affected by treatment ($F_{2,6}$ = 6.08, p = 0.036). The high density banding pattern treatment exhibited significantly higher mussel cover compared to High density labyrinth pattern (Tukey, p = 0.033). Contrary to what we expected, mussel cover in the Low density labyrinth pattern was not distinct from the high density treatments and configuration.

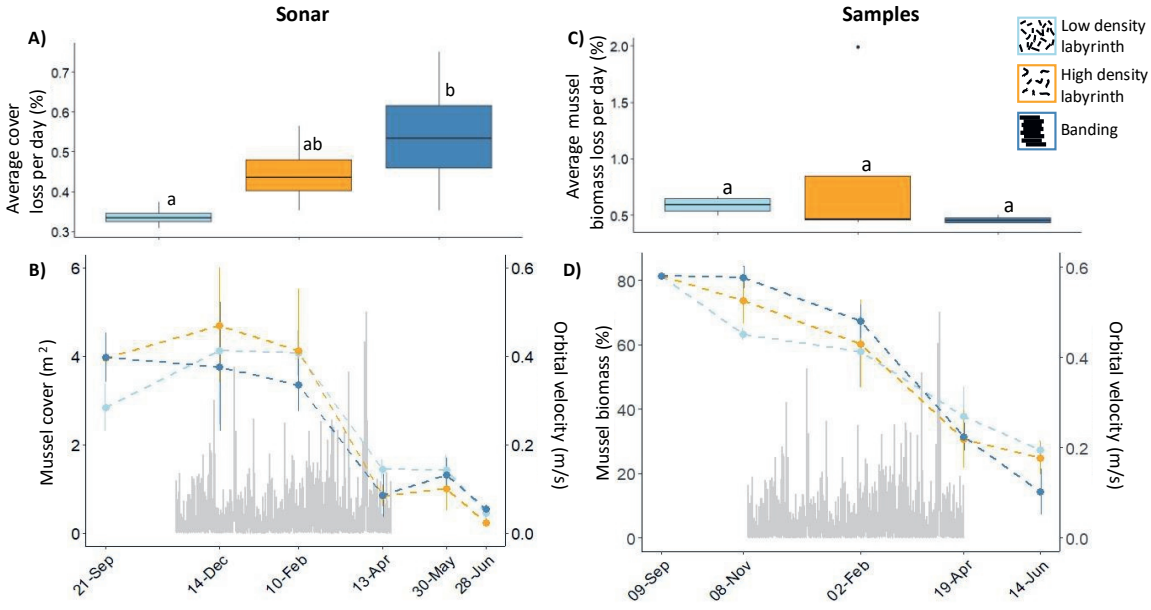

Figure 5.4. A. Average loss rate of mussel coverage per day (%), between start and final measurement (293 days) based on sonar data. **B**. Mussel coverage development over time (m^2) inside experimental plot (20x24m) for mussels transplanted in three configurations: low density homogenously spread SMC's (), high density homogenously spread SMC's (High density labyrinth pattern), and high density perpendicular SMC's (Banding pattern). Grey graph on background is the orbital velocity (m/s) **C.** Average mussel biomass loss per day (%) between start and final measurement (278 days). **D.** Mussel biomass (%) over time inside experimental plot (20x24m) for mussels transplanted in the same three configurations. Data are means ± SE (n = 4).

Mussel biomass based on sample data

We did not find a difference in average biomass loss per day between treatments ($\chi^2(2)$ = 2, p = 0.368, Figure 5.4D). This indicates that over the experimental period each treatment lost a comparable biomass of mussels from the BioShell-SMC.

We found a strong decline in mussel biomass (%) on the ropes from the start until the end of the experiment (more than 70%, Figure 5.4D). It started with more than 80% of the total weight being mussels and it ended with slightly more than 20%. We found no difference in proportion loss rates between configurations ($F_{2,9}$ = 0.08, p = 0.923).

At the end of the experiment, mussel biomass within the structures was affected by treatment ($F_{2,6}$ = 7.69, p = 0.021). However, we found no significant difference between groups with post-hoc analyses.

Mussel length and condition

Mean (± SE) mussel length at the start of the experiment was 22.34 ± 0.57 mm. At the end of the experiment after 278 days, mussels increased almost 75% to an average length of 38.95 ± 0.20 mm. The configuration in which the mussels were seeded, had no effect on mussel length at the final sampling date in June ($F_{2,9.6}$ = 0.3164, p = 0.736) (Figure 5.5A). A lower growth rate was observed from November until April.

The initial condition index of the mussels was 10.12 ± 0.40 mg cm^{-3}. This CI decreased over the experimental period for mussels in all configurations by 54% to 5.51 ± 0.06 mg cm^{-3} (Figure 5.5B). The condition of the mussels was not affected by the seeding configuration ($F_{2, 9.7} = 1.50$, $p = 0.271$).

Comparison previous study

Mussels transplanted while attached to the BioShell-SMC exhibited significantly higher survival rates compared to loose transplanted mussels (Van den Bogaart & Schotanus et al. 2023b). When we compared the results of the current study with those of the previous study, we found that the BioShell-SMC again outperformed loose mussels from the previous experiment (Figure 5.6). In particular, during the initial stages, the BioShell-SMC exhibited considerably lower mussel biomass loss compared to loose mussels. Nevertheless, when compared to the BioShell-SMC in the first experiment, our current study showed a more gradual decline in mussel biomass and by the end of April, the remaining biomass was on average only 41%, whereas it was 79% in the first experiment.

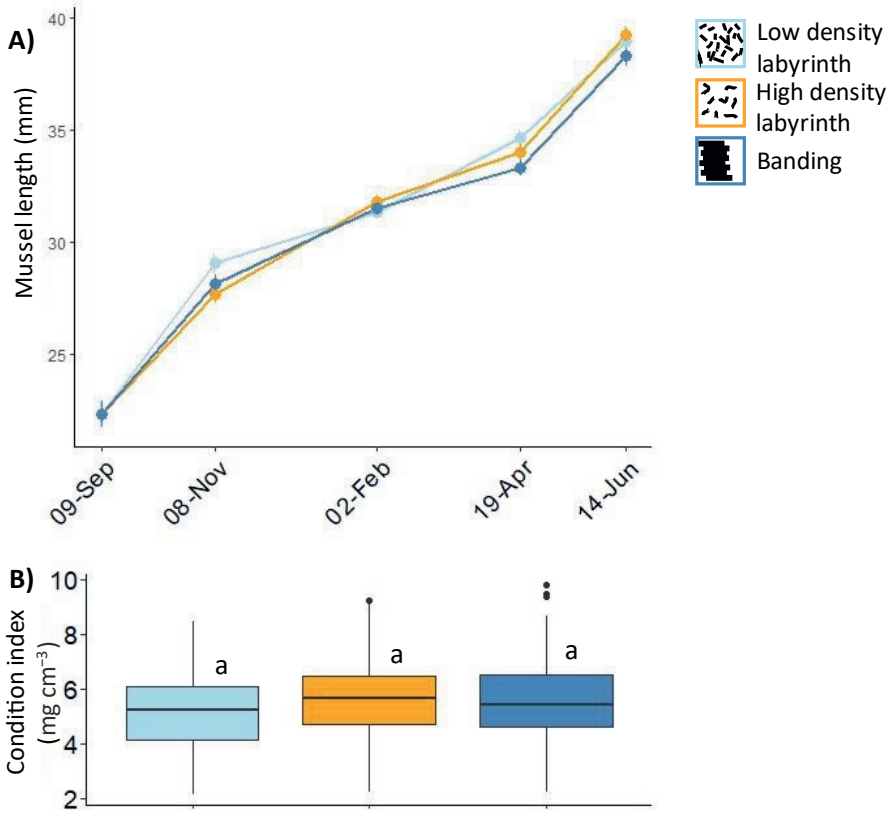

Figure 5.5. A. Length (mm) over time of mussels transplanted in three configurations: low density homogenously spread SMCs (), high density homogenously spread SMCs (High density labyrinth pattern), and high density perpendicular SMCs (Banding pattern). **B.** Condition index (mg cm^{-3}) of the mussels at the end of the experiment (June 2022) for each configuration.

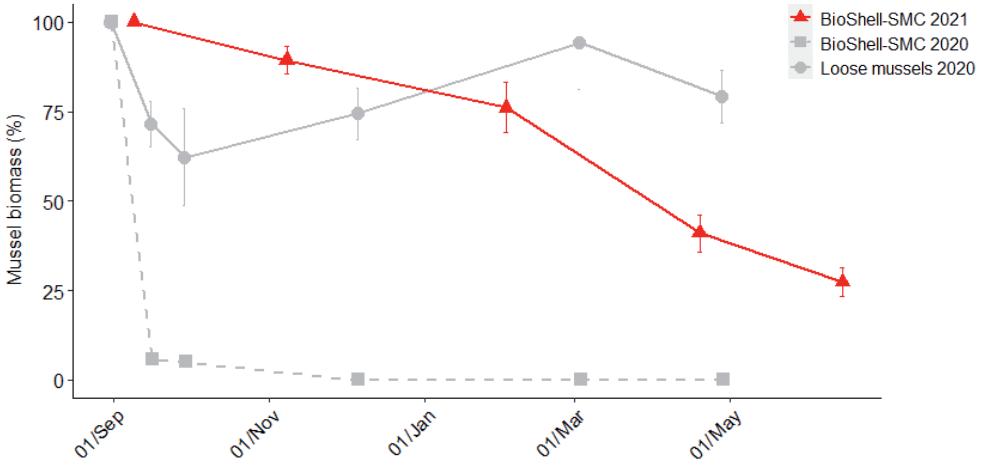

Figure 5.6. Comparison of mussel biomass (%) between BioShell-SMC (solid line) and loose mussels (dashed line) in the current study (2021, shown in red) and the previous study (2020, shown in grey). For the previous study, BioShell-SMC mussel biomass is calculated using data from both cut open and intact nets, as there was no significant difference between them (see Van den Bogaart & Schotanus et al. 2023b for detailed methodology). For the current study, mussel biomass is determined as the proportion of mussel weight to the total sample weight, with the initial mussel proportion set at 100%. Over time, mussel biomass is expressed as a percentage of the initial mussel biomass (%). All spatial configurations are combined since no significant differences were observed.

Discussion

In a previous field experiment at the same location, mussels that were transplanted while attached to the BioShell-SMC demonstrated significantly higher survival rates compared to loose mussels (Van den Bogaart & Schotanus et al. 2023b). Encouraged by these results, we aimed to conduct further research to improve and scale up restoration and aquaculture efforts, incorporating knowledge about the functioning of natural mussel beds. Specifically, during transplantation we (1) included a suitable attachment substrate for the mussels, to provide protection against predation and hydrodynamic dislodgement. And, we (2) tested the efficiency of implementing large-scale spatial patterns resembling the spatial organization of natural mussel beds to increases their resilience. As far as we are aware of, no large-scale field study has ever attempted to restore a subtidal ecosystem incorporating spatial patterns.

In contrast to our expectations, we observed overall high losses (approx. 75%) with no meaningful differences between spatial configurations. When comparing these findings with those from the earlier study (Van den Bogaart & Schotanus et al. 2023b), it became evident that the losses observed in the current study were more pronounced. Several factors may account for this difference in loss up to April, with one crucial consideration being the substantial impact of year-to-year variations on mussel survival. While the precise causes of this variability remain unclear, it is a well-recognized phenomenon that some years are more favorable for mussel survival than

others. Nevertheless, this comparison underscores that, although losses in our study were generally high, the BioShell-SMC still holds promise when compared to loose mussels from the previous study.

The losses found in our study were not excessive when compared to other experimental restoration efforts with mussels. For instance, Schotanus et al. (2020) reported an average decline of 65% in mussel coverage within the first month in their best-performing treatment. Similarly, Temmink et al. (2022) used biodegradable settlement substrates and observed a decline of intact structures during the first year, with a 42% decline due to burial, and an additional 28% of the structures being lost. However, when compared to mussel cultivation in subtidal areas, which is more similar in terms of environmental conditions with our experiment than the above intertidal restoration efforts, we observed a lower final mussel biomass. In mussel aquaculture, initial seeding losses are also substantial, reaching up to 69% (Capelle et al. in 2016), often influenced by seeding density. Nevertheless, this trend is counterbalanced by a subsequent rise in relative biomass production, resulting in an average harvest of 1.5–2.5 kg per kg mussel seed after approximately 1-1.5 years (Capelle, 2017). The low final cover and biomass observed in our study and previous restoration efforts highlight the difficulty of restoring mussel beds in dynamic environments. They also raise questions what factors might have contributed to the high losses observed in our study, even though our experiment was conducted within a designated mussel cultivation area. We delve into this problem from diverse viewpoints, including the way we implemented the spatial patterns, hydrodynamic dislodgement and burial.

Implementation of spatial patterns

Several studies have shown the importance of small-scale positive feedback-mechanisms in transplantation efforts by using clumped individuals rather than spacing individuals out, or by stimulating the formation of natural aggregations (Ladd et al., 2018; Silliman et al., 2015; Temmink et al., 2020). However, there has been comparatively less research on implementing large-scale pattern formations in restoration efforts, even though there are numerous studies underlying the importance of natural patterns in ecosystems (Lejeune et al., 2002; Pringle et al., 2010; Rietkerk et al., 2004). Promoting small-scale facilitation in transplantation efforts involves transplanting organisms in a density that meets specific threshold levels. Additionally, arranging these organisms on a large-scale aims to optimize the utilization of limited resources (Rietkerk & Van de Koppel, 2008).

Our experiment involved the attachment of mussels to biodegradable structures as a means to create stable small-scale aggregations. These structures provide shelter and attachment points during transplantation. They also enable mussels the potential to form patches by migrating away from these structures, while retaining the option to use them as stable structures for shelter or anchor points for forming clusters. Based on previous research (Van den Bogaart & Schotanus et al. 2023b; Van den Bogaart et al. 2023c), where migration behavior was observed, we had anticipated a greater degree of mussel dispersion from the structures in this study, due to the substantially higher initial densities. However, we did not observe this horizontal migration, either because it did not occur or because limited visibility at the study site prevented us from capturing

it. High initial densities (6 kg/m) triggered possibly intense competition for space and food, leading to mussels climbing on top of each other. This vertical migration might have resulted in smothering mussels positioned below, leading to elevated mortality rates (Capelle et al., 2014; Newell, 1990). This is consistent with findings reported by Capelle et al. (2014), who found that as mussel density increased, redistribution decreased.

Throughout the entire study period, the BioShell-SMC's mesh remained intact, preventing the dispersion of cockle shells onto the seafloor. Ideally, the net should have degraded within six months after its deployment in spring. This degradation would have allowed the cockle shells to naturally disperse, starting from mid-winter onward as the net gradually began to break down. Consequently, the limited availability of attachment points on the adjacent seafloor potentially impeded mussels from migrating away from the BioShell-SMC structures. Mussels favor hard substrate above soft substrate (Van den Bogaart et al., 2023c), a preference that may be facilitated by the earlier dissolution of the biodegradable net used in the BioShell-SMC. The initial high densities and the minimal dispersal away from the structures onto the surrounding substrate raises questions about the effectiveness of this method in initiating natural aggregations. The fact that we observed high biomass losses but limited migration from the SMCs onto the surrounding substrate, implies that either the mussels that undertook migration were subsequently lost, or migration itself was a rare occurrence. The suboptimal use of the available substrate area may have resulted in increased competition rather than facilitation in all treatments.

In addition to potential constraints at a small scale, there are also potential challenges arising from the large-scale spatial arrangement of the BioShell-SMCs. For instance, in terms of the optimal configuration, structures positioned too far apart from one another could potentially give rise to fragmented patterns rather than cohesive ones. This, in turn, could potentially limit mussel bed resilience to wave disturbance (de Paoli et al., 2017). Conversely, too closely spaced structures could lead to resource depletion, as indicated by Saurel et al. (2013). That is, the water flow over a mussel bed leads to seston depletion in the boundary layer. When bare patches without mussels are encountered, vertical mixing replenishes nutrients upon reaching the subsequent mussel patch. Re-suspension, as identified by Saurel et al. (2013), happens during high current velocities. Therefore, optimal configuration and mussel band size depends on prevailing environmental conditions. At our experimental location, we measured relatively low near-bed orbital velocities (mostly 0 to 10 cm/s). It is plausible that the patch dimensions in which the mussels were placed were unsuited for an environment where strong currents, causing re-suspension, are minimal. Another possible explanation for low survival rates might be the low initial cover. We placed the mussels in concentrated stripes and anticipated redistribution into wider striped patterns that are consistent with natural beds. With an initial cover of 0.75% in our plots, and a comparable amount of attachment substrate (i.e., BioShell-SMC) in every treatment, the significance of the initial spatial arrangement of the BioShell-SMCs might have been attenuated, resulting in comparable loss rates across all configurations. As demonstrated by Sleeman et al. (2005), slow-growing corals at low initial transplant densities (0.45% cover) had less dependency on specific spatial arrangements than evenly spaced gridded transplanting arrangements. To address this, reducing plot size and a better

dispersion of initial cover, rather than highly clustered aggregations, could enhance small-scale positive feedback and long-range negative feedback, ensuring regular patterns in the structures.

Hydrodynamic dislodgement and burial

A second factor to consider in relation to the overall high losses is hydrodynamic dislodgement. Despite relatively low average near-bed orbital velocities measured in our study, we found a decline in cover across all configurations, indicating a loss of structures or mussel clumps, notably between February and April. In this period a series of storms with wind originating from the northeast, had a notable impact on the near-bed orbital velocity. This was attributed to the bay's sheltered location by the surrounding landmass, resulting in diminished effects from winds originating from alternative directions. These strong winds from northeast direction possibly played a role in dislodging part of the structures or clusters of mussels, resulting in a significant reduction in coverage. When comparing the outcomes of this field experiment to those of a previous study conducted at the same location, we observed lower survival rates of mussels attached to the BioShell-SMC in the current study compared to the earlier one (41% vs. 79% by the end of April). When comparing the highest wind velocities originating from the northeast during the first experiment with those in our study, there were no indications of greater wind speeds in the current study. In fact, the earlier study documented higher maximum wind speeds from the northeast direction (79.2 km/h vs. 72 km/h). Other studies have shown that mussel in clumps or stripes persisted at higher orbital velocities than measured in our experiment, between 40 and 60 cm/s (Bertolini et al., 2019) and patches with shells even persisted at 70 cm/s (Capelle et al., 2019). Moreover, on soft substratum, similar to the conditions found at the Zandkreek, it was found that erosion around a mussel patch and sedimentation behind it contributed to enhancing patch stabilization by reducing the height above the sediment (Capelle et al., 2019). This scouring was linked to patch weight, indicating that heavier patches were less prone to hydrodynamic dislodgement. Therefore, we expected the BioShell-SMCs, especially the heavier high-density treatments, to be stable structures in the field due to reduced mussel dislodgement with increasing biomass. Hence, we anticipate that hydrodynamic dislodgement is unlikely to be the primary cause of the substantial losses.

Another factor worth considering as contributor to high losses is burial. It is possible that our structures were partly buried under the sediment and thus became invisible to the sonar, leading to a decrease in cover. This phenomenon was observed in a large-scale (20x10 m plots) mussel restoration experiment by Temmink et al. (2022), where biodegradable structures were placed in bands mimicking natural patterns. Suspended sediment deposition on top and around their structures caused significant burial (25% - 70%). Notably, bands closest to the gully were buried the least (25%), while bands behind the first row were buried most deeply (60-70%). Also on mussel plots, mussels are known to trap sediment and accumulate pseudofaeces up to 10 cm during the summer (ten Brinke et al., 1995). In our experiment, which was situated on a mussel culture plot in a sheltered area away from a gully, the structures might have experienced significant burial as well. This burial process could have contributed to the decrease in cover visibility observed with sonar. These findings raise concerns about the suitability of placing structures for future

mussel bed restoration efforts. Nonetheless, a more rapid degradation of the BioShell-SMCs would require the mussels to reorganize autonomously, which could potentially lead to increased survival.

Challenges and opportunities for restoration

Ecological restoration can greatly benefit from the transplantation of individuals or populations. However, such transplantations, when involving ecosystem engineers, are commonly faced with challenges that result in significantly low success rates. We hypothesized that lack of scale dependent feedback in the establishment phase is a major contributor to this low success rate. To our knowledge, this was the first large-scale field experiment attempting to increase mussel restoration success in subtidal areas by not only implementing self-facilitation feedback mechanisms at a small-scale, but also spatial patterns resembling natural mussel beds at a larger-scale. Furthermore, we used sonar analyses to monitor mussel cover, a technique that demonstrated its reliability, evident from the comparable results obtained in relation to mussel biomass. Transplanting BioShell-SMCs in different configurations was initially expected to enhance mussel survival by increasing resistance to hydrodynamic dislodgement, and minimizing competition and predation. However, our findings revealed significant cover and biomass loss rates across all configurations, indicating the challenges faced by mussel transplantations. The major bottleneck was the lack of migration from the BioShell-SMCs, resulting in suboptimal space utilization and fostering high competition rather than facilitation. Nevertheless, when compared to other studies involving mussel transplantations in intertidal zones, our losses were not considered excessive. But in comparison to mussel cultivation, which aligns more closely with our study in terms of environmental conditions, an increase of at least 1.5 to 2.5 times the original seeding amount would be expected within a year. The typical average seeding density in Dutch mussel cultivation is 1.0 – 2.5 kg/m^2 on plot scale, although there are locally higher densities up to 10 kg/m^2 (Capelle et al., 2014). In our study, with an initial mussel biomass of 6 kg/m on the BioShell-SMCs, the local densities on the plots became excessively high due to the intensely clustered configuration. Enhancing the dispersion of BioShell-SMCs and encouraging mussels to migrate from the structure—perhaps achieved through the earlier dissolution of the biodegradable mesh—holds the potential to reduce transplantation losses. It is important to note that mussel survival is significantly influenced by year-to-year variability, a phenomenon widely acknowledged among Dutch mussel farmers. The underlying causes of this interannual variation remain uncertain. To address and minimize this inherent variability, a promising approach would be to conduct the current experiment consistently over several consecutive years. In summary, our study highlights the complex interplay of factors influencing facilitation and competition of ecosystem engineers in dynamic environments. It also demonstrates that the lack of small-scale facilitation can hamper large-scale facilitation. Future research in this direction should aim to comprehend the potential benefits of implementing self-facilitation and spatial patterns simultaneously and to expand their applicability to a wider range of species and restoration contexts.

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Chapter 6

General discussion

As human population expansion continues and the need for sustainable development intensifies, the role of coastal ecosystems in providing essential services becomes increasingly important. Coastal ecosystems offer a wide range of benefits, from food production and carbon sequestration to coastal protection and cultural value (Millennium Ecosystem Assessment, 2005). However, human activities have exerted immense pressure on many ecosystems, leading to their degradation and the depletion of vital resources. Consequently, ecological restoration is gaining recognition as a critical component in restoring ecosystems and their services (Bullock et al., 2011). This involves efforts to restore degraded, damaged, or destroyed ecosystems, often by reintroducing ecosystem engineers. Nonetheless, it should be considered a component of a broader restoration strategy, rather than a standalone approach. An essential step is restoring the conditions that allow ecosystem engineers to reestablish themselves, as this is often a prerequisite for successful restoration. Blue mussels (*Mytilus edulis*) serve as ecosystem engineers, offering services like sediment stabilization, habitat provision, food supply, and water quality regulation (Bouma, Olenin, et al., 2009; Dame & Kenneth, 2011; Mainwaring et al., 2014; Smaal, 2002). Active restoration of mussel beds can be applied for preserving these services and often involves transplanting mussels to designated areas. Transplantation of mussels can also be applied to cultivate mussels. The cultivation cycle starts with obtaining mussel spat, collected either from the water column using seed mussel collectors or harvested from benthic mussel seed beds.

The main objective of this thesis was to explore strategies to enhance the success of mussel bed establishment after transplantations, either for restoration purposes or mussel cultivation. We used biodegradable structures ("BioShell-SMC") to (1) collect mussel spat in the water column, and to (2) increase mussel bed resilience by implementing large-scale spatial configurations, mimicking natural mussel bed patterns. This final chapter integrates the most important findings of my research. First, I will focus on the performance of the BioShell-SMC in mussel seed collection. Then I will discuss whether transplantation success can be enhanced by initiating self-facilitation and explore the factors that may constrain transplantation success. Finally, I will discuss implications for both restoration efforts and aquaculture.

Mussel seed collection

The collection of mussel seed (Figure 6.1) is a crucial step in restoration efforts, since it can be transplanted to subtidal or intertidal areas for mussel bed restoration. But also for mussel bottom culture, mussel seed serves as the start for cultivation practices. In the context of mussel seed collection, two critical factors come into play: the choice of deployment location and the timing of harvesting collectors to optimize efficiency.

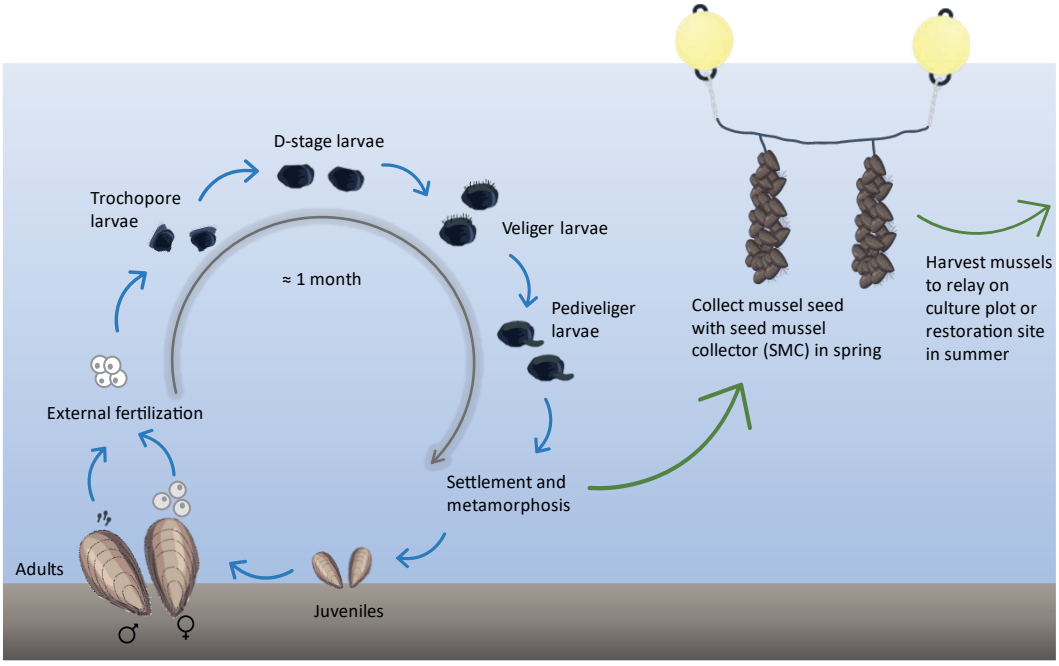

Figure 6.1. Life cycle of blue mussel (*Mytilus edulis*) and collection process. Larvae seek a suitable attachment substrate for settlement, which may include a seed mussel collector (SMC). The strategic deployment of SMCs in early spring aligns with the larvae's search for attachment sites. Symbols adjusted from Integration and Application Network (ian.umces.edu/media-library).

SMC deployment location

In Chapter 2 of this dissertation, it became evident that while the traditional Xmas Tree rope and the BioShell-SMC collected comparable mussel seed biomass in six out of nine locations, the selection of deployment location held particular significance. The three most Western locations in the Wadden Sea exhibited higher biomass on the traditional rope when compared to the BioShell-SMC. These locations, more exposed to dominant South-Western winds, experienced harsher environmental conditions. Although initial settlement may have been similar on the traditional rope and the BioShell-SMC, the higher survival rates on the traditional ropes could potentially be attributed to the packing density of cockle shells within the BioShell-SMC. Looser packing may have led to abrasion and subsequent loss of mussel spat due to crushing. This phenomenon has been observed in coral reefs where rubble movement damaged recruits and hindered settlement success (Cameron et al., 2016). On the other hand, looser packing allows more space for mussels to attach, aligning with their preference for complex substrates that offer protection from hydrodynamic forces and predators (Carl et al., 2012). The comparable final seed biomass between the two rope types in the less exposed locations suggests that either the initial settlement, survival rates, or a combination of both were similar. These results underscore the importance of strategically selecting deployment locations to maximize the efficiency of mussel seed collection and suggest that the BioShell-SMC is more suitable for locations with calmer conditions.

In our experiments described in Chapters 3 and 5, we obtained substantial quantities of mussel seed as starting material on the BioShell-SMC in the Voordelta. Although we did not directly compare the obtained biomass with traditional seed collectors, we can draw comparisons with previous studies. In the Voordelta, traditional SMCs collected an average of 2.6 kg/m over nine years, spanning from 2012 to 2022, with fluctuations ranging from 0.3 to 5.0 kg/m (Capelle, 2023). In our first transplant experiment presented in Chapter 3, we achieved an average collection of 2.9 kg/m with the BioShell-SMC, closely resembling the numbers from the previous study. In our field experiment discussed in Chapter 5, we observed an initial biomass of 6 kg/m at harvest, indicating the extreme high mussel density collected by the BioShell-SMC. These results show that the BioShell-SMC is a viable alternative for mussel seed collection, likely performing comparably to traditional ropes.

Timing of SMC harvest

In addition to the deployment location, the timing of seed harvest plays an important role in mussel seed collection. Typically, mussels originating from SMCs are harvested at a relatively young age, around 4 months old. At that stage mussels are still small, which prevents them from collectively falling from the ropes due to overcrowding. Mussel farmers have developed extensive experience in determining the optimal harvesting time. We did observe detachment of the mussel seed from the substrate during both years of deployment in the Voordelta. Whole clumps of mussels were observed to either fall off, or to easily fall off when collected into the boat or handled afterwards. On the other hand, collecting mussel seed when they are too small increases their vulnerability to predation, particularly by crabs. The mesocosm experiment presented in Chapter 3 revealed that crabs exhibited a stronger predation preference for smaller mussels harvested in July compared to larger mussels harvested in August. This observation was confirmed in our first transplant experiment (Chapter 3), during which we observed substantial losses in July, but not in August, when mussels were attached to the BioShell-SMC. Additionally, we observed high mussel survival rates within the anti-predation cages in both July and August. This suggests that, despite the additional protection provided by their attachment substrate, the mussels harvested in July were too small to effectively withstand the very high predation pressure. We had anticipated that the BioShell-SMC would offer sufficient protection for these smaller mussels, allowing for earlier harvesting and yielding a higher mussel number, since space-regulated self-thinning did not yet occur. However, it became evident that predation was the constraining factor for early harvesting in this particular location. This situation may differ in other areas with lower predation pressures by crabs. Earlier research has shown that crabs display preferences for specific mussel size classes, and as crab size increases, so does the size of preferred mussels (Enderlein et al., 2003; Murray et al., 2007). Based on these results, mussels of a size similar to those harvested in August (25 mm) during the first transplant experiment were used in the last field experiment (Chapter 5) to enhance their chances of survival. Nonetheless, the significant losses in that last experiment indicate that other factors besides size dependent predation played a role in these high losses.

Survival after transplantation

Survival after transplantation is a critical factor in determining the success of mussel restoration and aquaculture practices (relevant terminology explained in Box 1). It depends on the balance between facilitation and competition among mussels, while also being influenced by environmental stressors and other ecological factors. These processes are conceptualized in Figure 6.2. Here, we illustrate the strategies employed by mussels to optimize their survival. Loose mussels tend to aggregate, forming patches that provide protection against dislodgement and predation. In contrast, mussels attached to substrates disperse away from each other to mitigate intense competition. Loose mussels experience lower competition but reduced protection, while attached mussels face both heightened competition and increased protection. Understanding these dynamics can help to optimize the success of restoration and aquaculture efforts. In this section, I will explore the efficacy of the BioShell-SMC as an innovative solution to address these challenges.

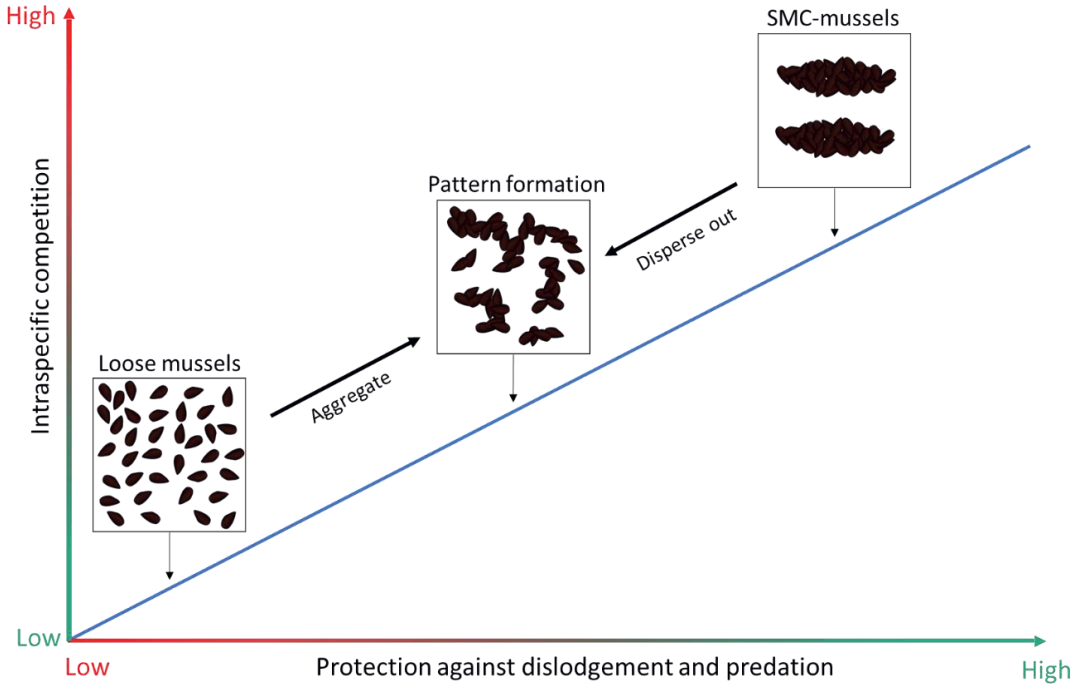

Figure 6.2. Schematic representation of expected pattern formation after mussel transplantation. Loose mussels tend to cluster into patches to seek refuge from dislodgement and predation (aggregate). SMC-mussels disperse away from the substrate as a strategy to evade intense competition, characterized as dispersion. Optimal mussel densities involve a trade-off between competition for resources and protection against dislodgement and predation. Loose mussels experience low competition and protection, while attached mussels face high competition and enhanced protection.

Facilitation and competition

Spatial patterns are often the result of facilitative and competitive shaping processes and can be found as such in many different ecosystems, including arid ecosystems (HilleRisLambers et al., 2001), wetlands (Foster et al., 1983), coral reefs (Mistr & Bercovici, 2003), and intertidal mudflats (Blanchard et al., 2000). The same applies to mussel beds. Facilitative processes drive the formation of extensive beds, as mussels anchor themselves to substrate complexes composed of conspecifics (Christensen et al., 2015; Snover & Commito, 1998). At a smaller scale (5-10 cm), mussels tend to aggregate in clumps and strings, offering advantages such as increased resistance to dislodgement from hydrodynamic forces and enhanced protection against predators (Hunt & Scheibling, 2001). However, the aggregation of mussels into high-density patches also carries disadvantages, notably in the form of competition for limited space and resources (Capelle et al., 2014; Newell, 1990). To mitigate this competition, larger-scale patterns emerge, forming distinctive bands (5 – 10 m apart) that reduce overall mussel density across their habitat (van de Koppel et al., 2005). Importantly, they maintain a high local density within the bands, ensuring a safety in numbers effect.

The BioShell-SMC was introduced as a solution to enhance

Box 1. Understanding mussel dynamics. Clarifying key terminology used throughout this thesis.

Self-facilitation refers to a phenomenon where organisms, often ecosystem engineers, facilitate their own growth and survival by modifying their environment in a way that benefits them. **Positive interactions** can take place when one organism enhances the local environment's favorability for another, either directly (e.g., reducing hydrodynamic, or nutrient stress through wave mitigation or nutritional symbioses) or indirectly (e.g., by eliminating competitors or hindering predators) (Bruno et al. 2003). In the case of mussels, they are classic examples of self-facilitators. Mussels create a complex three-dimensional matrix by attaching themselves to one another and the substrate. This structure provides protection from wave action and predators. As more mussels settle in the bed, they strengthen the overall structure, reducing the risk of dislodgment. They also bind sediments together with their byssal threads and shells. This stabilizes the substrate, reduces erosion, and improves water clarity by preventing sediment resuspension. A minimum density of mussels may be necessary to form the structural framework, which is referred to as a **density threshold**. Below this density, the facilitative effects may not be as pronounced. The self-facilitation by mussels in this manner can be seen as a **positive feedback mechanism**. As more mussels settle and grow, they enhance their own environment, making it even more attractive for mussel growth and the establishment of associated species. **Negative interactions**, or **competition**, can be observed when mussels compete with one another for resources in their environment. Mussels are filter feeders, meaning they obtain their nutrients by filtering small particles from the water. Competition for food may lead to reduced growth and reproduction for some individuals or beds. Negative interactions through competition can potentially constrain the realized niche of mussels, causing their distribution and abundance to be limited to areas where they are less impacted by competitive pressures.

mussel survival by promoting local-scale self-facilitating feedback mechanisms. It was hypothesized that transplanting mussels still attached to the BioShell-SMC, in high density clusters would provide protection from predators and increase resistance to hydrodynamic dislodgement (Chapter 3). We further hypothesized that by transplanting the BioShell-SMC onto the sediment would prevent self-thinning by offering the mussels the opportunity to migrate away from the high density clusters on the ropes. The starch net was supposed to gradually dissolve (intended to occur within half a year), whereafter the cockle shells within the BioShell-SMC could gradually disperse. This would create an additional sediment surface for mussels to attach to on the surrounding substrate. During the experiments, I observed that the net did not dissolve, within a period of approximately one year. I believe it would have been advantageous for the mussels if the net dissolved more quickly, allowing the cockles to disperse. This could potentially have provided an attachment substrate for mussels to migrate beyond the high-density clusters on the BioShell-SMC, reducing competition for food and space and enhancing their resilience to environmental disturbances. During fieldwork described in Chapter 3, mussels attached to the outside of the BioShell-SMC showed movement away from the ropes (Figure 6.3). This behavior enabled them to escape high competition on the BioShell-SMC, resulting in reduced local density and, consequently, improved survival rates compared to isolated mussels, all while still benefiting from the protective advantages of clustering. Encouraged by these findings, we conducted a mesocosm experiment to delve deeper into the interactions between biological and physical factors that influence self-facilitation (Chapter 4). We assessed different mussel densities to determine the presence of a density threshold and to assess whether mussels exhibited greater migration when transplanted in high density conditions. Additionally, we examined different substrates, hypothesizing that incorporating shell fragments (hard substrate) would promote mussel dispersion by offering attachment points, especially when surpassing the density threshold. The results showed that mussels attached to the BioShell-SMC in low density conditions (0.75 kg/m) displayed significantly more pronounced clustering by the end of the experiment compared to the high-density treatments (3 kg/m). This observation suggested that mussels in high-density conditions tended to disperse more, which was consistent with our findings from the field experiment. Adding shell debris created an opportunity for loose mussels in high density to migrate to more favorable positions to optimize feeding and growth. In low density, this pattern did not occur. This indicates that there was no density threshold to stimulate the formation of a different pattern. Surprisingly, substrate type (soft or hard) did not significantly impact mussel behavior when attached to the BioShell-SMC, although there was a notable trend towards more patches on shell substrates than on mud for SMC-mussels in high-density conditions. This trend indicated that mussels in high-density conditions had a preference for dispersing on shell substrates. This is what we expected based on previous literature, since mussels were found to cluster less in the presence of additional attachment points (Capelle et al., 2019; Christensen et al., 2015; Frandsen & Dolmer, 2002). However, the effectiveness of this concept, which had appeared promising in our field experiment (Chapter 3), as well as the mesocosm study (Chapter 4), came into question based on the results of the last field experiment (Chapter 5). In this large-scale experiment, we tested, building upon the findings of our previous smaller-scale studies, the

hypothesis that the success of transplantation could be improved by adopting spatial configurations that mimic patterns observed in natural mussel beds. We therefore tested three different spatial patterns: low vs. high density labyrinth pattern and banded pattern. Contrary to what we expected, we observed overall high losses, with no significant variation among the different patterns.

The notably low survival rate of mussels has inspired me to delve deeper into the underlying causes. Unlike the findings from our previous experiments, we did not observe the level of dispersion we had anticipated, although the mussels were transplanted with high initial mussel densities on the BioShell substrate. This is in accordance with a previous study, where redistribution decreased with increasing mussel density, and mussels did not spread out over a larger area when density was high (Capelle et al., 2014). In our study, it is still possible that horizontal dispersion did occur, and may have escaped my detection due to the exceptionally high density and poor visibility. However, if such dispersion did occur, it likely happened on a small scale, as there is no evidence in the sonar data, and I would have observed it otherwise. The high initial densities (6 kg/m) of mussels attached to the BioShell-SMC must have triggered intense resource competition, leading to mussels climbing on top of each other to reach favorable feeding positions. This implies the potential for migration, although it appears to have been predominantly in the vertical direction rather than horizontally. This vertical migration possible resulted in smothering mussels positioned below, leading to elevated mortality rates (Capelle et al., 2014; Newell, 1990). As competition gradually diminished over time, so did the need for dispersion. This is in accordance with the results

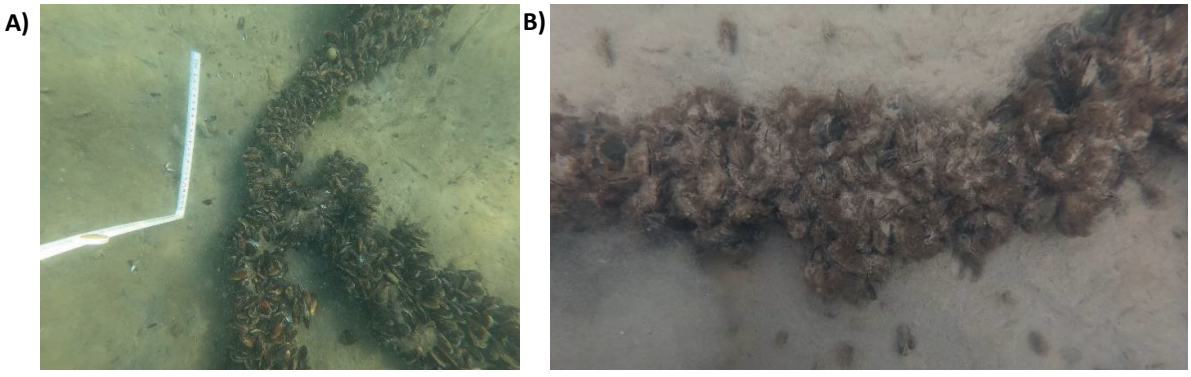

Figure 6.3. Mussels attached to the BioShell-SMC in transplant experiment 1 (Chapter 3). **A.** photo taken on 16th of September 2021; **B**. photo taken on 3rd of March. On the right photo, mussels have slightly dispersed onto the surrounding substrate.

presented in Chapter 4, which did not reveal a substantial migration preference for mussels in low density (0.75 kg/m) on shell substrate compared to mud, suggesting the absence of a clear density threshold leading to dispersion away from the BioShell-SMC. In conclusion, our study has demonstrated both in a small-scale field experiment and in a mesocosm experiment the efficacy of self-facilitation in mussel transplantation when attached to the BioShell-SMC. However, when implemented on a larger scale, its effectiveness appeared to diminish. The major bottleneck was the lack of horizontal migration and the suboptimal use of space, resulting in competition rather than facilitation. This finding opens the door to further research opportunities. To enhance the prospects of success, I recommend conducting future studies with a lower initial mussel density,

specifically in the range of 2 to 3 kg/m, as indicated by the promising results in Chapter 3 (where a density of 2.9 kg/m proved successful). This could be accomplished by reducing the amount of cockle shells used inside the socks, as this would result in less attachment material for the mussels. Additionally, the netting should be designed to dissolve within a 6-month period, starting from the moment the mussels are transplanted onto the site. Therefore, it is crucial to test the materials' degradability under various conditions. Furthermore, since the specific configurations did not seem to have a significant impact (Chapter 5), I would recommend avoiding attempts to seed in bands and instead distribute the mussels as evenly as possible. This approach would help disperse mussel density while still benefiting from the safety-in-numbers effect provided by clusters at the BioShell-SMC.

Hydrodynamic effect

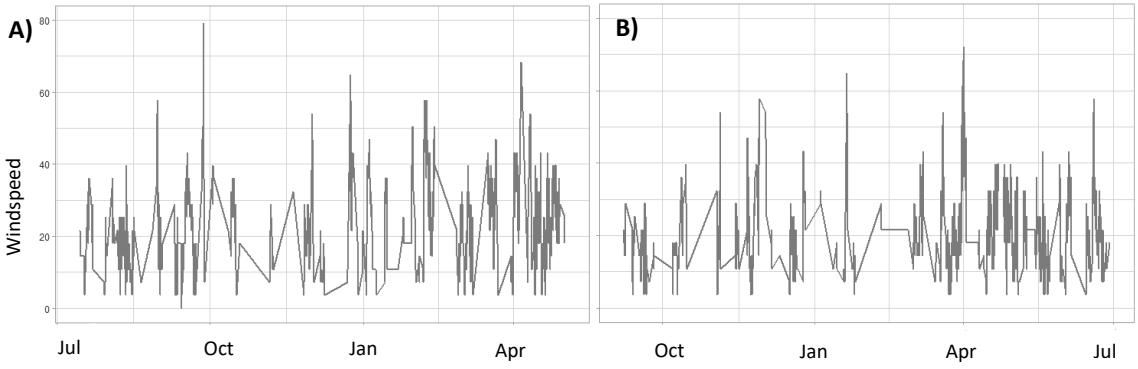

Figure 6.4. Windspeed during transplant experiment 1 (**A**) and 2 (**B**). Winds are measured as maximum wind gust (3 second mean wind speed) in the preceding hour. Only winds coming from the North, East, and all directions in between are considered because these wind directions had the most significant impact on the location. Other wind directions were shielded by land, and thus, their influence was negligible.

Mussel seed collection with the BioShell-SMC is probably affected by hydrodynamics, as explained earlier. But also after transplantation hydrodynamic conditions can significantly influence mussel survival. Moreover, various studies have attempted to restore mussel beds in highly dynamic areas, such as the intertidal flats of the Dutch Wadden Sea (de Paoli et al., 2015; Temmink et al., 2020) and the Oosterschelde (Schotanus, Capelle, et al., 2020). These studies were conducted in areas characterized by substantial wave impacts, leading to these high losses. In contrast, our experiments were situated on a subtidal mussel plot designed to culture high mussel biomass, where the conditions are comparatively less harsh. The recorded orbital velocity, as measured by a wave monitoring device during our last experiment, averaged between 0 and 10 m/s. When considering hydrodynamics alone, we anticipated a higher survival rate for mussels attached to the BioShell-SMC in our experiments, since heavier patches have shown to be less prone to hydrodynamic dislodgement (Capelle et al., 2019). This hypothesis was confirmed with the results from Chapter 3, the BioShell-SMC mussels displayed remarkable resilience to hydrodynamic forces. In contrast, a significant number of loose mussels were washed against the fences by the current. This validation supports our hypothesis that the BioShell-SMC provides effective protection against

waves and currents. Even with the low orbital velocities measured at our location, loose mussels were susceptible to dislodgement, while the BioShell-SMC remained steady. In the last field experiment, we observed a gradual decline in mussel cover over time. Given the stability provided by BioShell-SMC structures in the first transplant study, we initially expected minimal impact from wind and currents in this experiment. However, the unexpected decline in cover seen in the second experiment urged me to investigate whether there were more intense and frequent wind conditions during the last experiment in comparison to the first, which could potentially explain the greater loss in the final experiment. To study this, I compared the maximum wind speeds originating from the northeast between the first and second transplant experiments, as other wind directions were shielded by land (Figure 6.4). Surprisingly, I found no remarkable differences in maximum wind speeds between the two years. Since the structures remained stable under these wind conditions in the first experiment, it is unlikely that wind was the primary factor contributing to the substantial losses documented in Chapter 5. However, it is worth noting that orbital velocity showed sporadic peaks between February and April 2022, which coincided with the period of the most significant decrease in mussel cover. This suggests that orbital velocity may have played a role, although it may not align perfectly with wind speeds. Future research could incorporate the use of accelerometers to monitor the movement of the BioShell-SMCs, providing insights into their resilience to wind and potential links to high losses. Although we initially included accelerometers in our second transplant study, they were all damaged or lost. Nevertheless, such data could offer valuable information regarding the movement of ropes and whether hydrodynamics played a role in the disappearance of mussel clumps. It is important to note that this approach would primarily assess the overall structure's behavior and not specifically the loss of mussel clumps.

Macroalgae coverage

During our field trips for the last transplant experiment, I observed a substantial presence of macroalgae attached to the BioShell-SMCs in all configurations (Figure 6.5). Luckily, the side scan sonars' ability to distinguish between hard substrate (shells), vegetation and sediment has been shown to be largely unaffected by high algal cover (Greene et al., 2018). Several studies have examined the impact of algal epibionts on mussel populations, for example by reducing mussel growth by affecting mussel energetics (Dittman & Robles, 1991). This phenomenon is explained by the authors as epiphytes' ability to modify the internal temperature conditions of intertidal mussels through changes in reflectance and enhanced evaporative cooling. These temperature variations can have a significant influence on the reproduction and growth of *Mytilus* spp. (Hines, 1979). Furthermore, macroalgae have been suggested to potentially impede mussel feeding (Paine & Suchanek, 1983), although this effect was not confirmed in another study by O'Connor et al. (2006). In our experiment, accurately assessing the impact of overgrowth on mussel growth requires measurements of mussel growth in the absence of algae coverage. Unfortunately, we are unable to make such a direct comparison, since all mussels were extensively covered with algae. Comparing results across different years may not yield reliable insights, as mussel condition can be greatly influenced by the specific year and seasonal timing.

Figure 6.5. BioShell-SMC during the last field experiment covered with algae. This photo was taken on the 19th of April 2022.

Macroalgae coverage may also affect mussel survival by increasing flow-induced forces leading to dislodgement and increasing mussel loss rate by an increase in drag-induced lift (Denny, 1987; Dittman & Robles, 1991; O'Connor, 2010; O'Connor et al., 2006; Witman & Suchanek, 1984). As previously mentioned, wind and currents were not expected to be the primary factors causing mussel loss, given the relatively low orbital velocities we measured. However, when we consider that the dislodgement threshold significantly decreases when algae are attached to the mussels, it

raises the possibility that the structures may have been set in motion due to drag-induced lift, particularly between February and April. In contrast, we observed almost no algae during the experiment described in Chapter 3, which could potentially account for the greater resilience of the structures during that period. As discussed in O'Connor et al. (2006), even the removal of epibionts increased the likelihood of mussel survival. Nonetheless, it is essential to emphasize that without a direct comparison with uncovered mussels during the same time period, the overall mussel loss attributable to macroalgae remains speculative.

Upscaling

The small-scale experiment that was conducted in 3x3 m plots yielded positive outcomes, demonstrating an increase in mussel survival when they were attached to the BioShell-SMC (Chapter 3). However, the transition to a larger scale (20 x 24 m plots, Chapter 5), did not yield the same level of success. Ecological restoration is most effective when executed on an even larger scale in order to efficiently restore biodiversity and ecological functionality while remaining cost-effective (Schulte et al., 2009). Besides, mussel culture typically takes place on much larger plots, approximately around 5 hectares in size, often arranged in clusters. This clustering can lead to a substantial mussel population in a broader area. The observed losses in the large-scale experiment were greater than expected, which underscores the difficulty of scaling up restoration efforts. This does not necessarily imply that the large-scale approach has been the bottleneck; it is important to consider the impact of year-to-year variation on mussel survival. The exact causes of this variability remain unclear, but it is well-recognized that certain years are more favorable for mussel survival than others. When comparing the performance of the BioShell-SMC in the large-field study with loose mussels in the small-scale study, BioShell-SMCs outperformed loose mussels, although their performance in the large-scale field experiment might not have matched their performance in the previous study. This variation could possibly be attributed to year-to-year differences. In summary, spatial patterns did not significantly impact mussel survival, but BioShell-SMCs outperformed loose mussels from the first experiment. We did not include loose mussels again as an additional configuration in the last experiment because we had already tested them in Chapter 3. Due to resource and space limitations, we chose not to repeat this experiment, as we anticipated significant losses due to hydrodynamics once again.

In the process of upscaling experiments to a larger scale, the use of an efficient monitoring device becomes crucial. We carried out monitoring moments with the side scan sonar, and the outcomes were encouraging. The results were consistent with the sample data, indicating the sonar's effective functionality. This method proves to be less time-consuming in comparison to the conventional sampling technique of taking mussel samples for biomass and cover. In Ireland they used side-scan sonar to search for seed mussel during the annual seed mussel survey and to compare pro- and post-fishing data (Chopin & McCoy, 2021). This technology has the potential to replace intermediate sampling campaigns, although it remains valuable to take regular mussel samples, especially for growth and condition during the initial and final stages of the experiment.

Implications for mussel bed restoration

In coastal ecosystems, a common method of active restoration includes the reintroduction or transplantation of species that have disappeared or declined (Byers et al., 2006). The findings presented in this thesis demonstrate that the inclusion of an attachment substrate can promote facilitation. That is, mussels benefitted from a biodegradable structure that decreased the risk of predation and prevented them from being dislodged by water currents, ultimately enhancing their chances of survival. Aggregation of mussels in clumps and strings offers protection against hydrodynamic forces and predators (Chapter 3; Hunt & Scheibling, 2001; van de Koppel et al., 2008). Therefore, it is crucial to transplant mussels in a sufficiently high (but not too high) density to facilitate their aggregation behavior. If the density is below a certain threshold, mussels will cluster in small groups of just a few individuals. Conversely, when densities are excessively high, they will create a uniform matrix (Chapter 4). Neither of these scenarios is desirable, as low density increases the risk of dislodgement and predation, while high densities result in competition. The most favorable spatial configurations are found in between these extremes, resulting in string-like formations or a net-like structure. The concentrated string-like clusters mimicked with the BioShell-SMC provided increased survival rates for mussels in high-density clusters compared to homogenously spread mussels (Chapter 3). However, this increased density also leads to heightened competition for both food and space (Capelle et al., 2014; Newell, 1990), potentially leading to suffocation and high mortality, as documented in Chapter 5 of this thesis. In the context of mussel bed restoration, the primary objective is to establish and maintain mussel beds that can sustain themselves multiple years through the recruitment of mussel larvae. Consequently, it is crucial to ensure that a substantial number of mussels persist and are not washed away during storm events or are preyed upon in the first weeks. Initiating self-facilitating processes is crucial to strengthen the mussels' resilience against environmental stressors, enabling them to grow and reproduce before these stressors have a destructive effect. With the BioShell-SMC, one possible approach could involve reducing the density right before transplantation to ensure an initial density of around 2.5 to 3 kg/m, increasing their resilience. It is worth noting that retrieving the SMCs earlier than August is not recommended, as the small size of the mussels makes them highly vulnerable to predation, as discussed in Chapter 3. I would also suggest further refining the biodegradable netting to have a dissolution period of six months. This would enable the cockle shells and mussels to start migrating immediately upon transplantation into a restoration area. By incorporating the cockle shells, there would be sufficient attachment substrate to significantly enhance their chances of survival.

Natural mussel beds exhibit distinct spatial patterns, often featuring a banded configuration on a large scale due to a combination of facilitation and competition dynamics. In Chapter 5 of this thesis, we hypothesized that positioning the BioShell-SMCs in a high-density banded pattern would lead to increased mussel survival. However, despite previous research suggesting this as an optimal setup (van de Koppel et al., 2008), we observed low survival rates for the mussels in this configuration. Additionally, we did not find any significant differences between

this banded treatment and the two other treatments involving labyrinth patterns in low and high densities. Therefore, for future restoration efforts with the BioShell-SMC, as previously discussed, I would prioritize obtaining the optimal initial mussel density. Then, I would avoid investing excessive time and effort in arranging the SMCs in a banded pattern. This method proved to be the most time-consuming, as it was challenging to deploy the SMCs in straight lines perpendicular to each other. Moreover, tying three SMCs together was also a time-intensive task. Since there was no noticeable improvement in survival compared to the low-density SMCs, I would recommend eliminate this step. An alternative to the existing BioShell-SMC design could involve excluding the inner rope. In this modified version, only the cockle shells would be encased within the biodegradable netting, providing a surface for mussel seed attachment. These structures could be cut open when harvested, facilitating the dispersion of both mussels and cockle shells. Building on the findings by Capelle et al. (2019), it was observed that the inclusion of coarse shell material reduced mussel losses by a substantial factor three when compared to mussels seeded without shell debris. It could be interesting to explore whether seeding the BioShell-SMC without inner rope and the biodegradable netting cut open in spatially banded patterns might further enhance mussel survival rates.

Implications for mussel aquaculture

Mussel culture is a traditional sector of aquaculture. Since 2009, there has been a shift in mussel farming practices away from relying on wild beds and towards the use of SMCs for collecting mussel seeds. While SMCs produce a reliable seed source (Capelle, 2023), they come with increased effort and cost compared to seed from fisheries, leading to higher prices for mussel seed. This rise in resource costs has created financial challenges for mussel farmers, as it has not been matched by increased productivity in the culture cycle. The current seeding techniques are identified as a bottleneck, with significant losses occurring immediately after seeding due to density-dependent seeding losses (Capelle, Wijsman, et al., 2016).

Based on the findings presented in this thesis, the BioShell-SMC concept may still hold promise for aquaculture when employed with optimal initial densities to promote aggregation while avoiding excessive competition among the mussels. The reduction in numbers due to limitations in food or space is a natural occurrence often referred to as "self-thinning" (Frechette et al., 1992; Kautsky, 1982; Lauzon-Guay, Dionne, et al., 2005; Westoby, 1984). Over time, the number of individuals decreases due to this competition, but the surviving individuals tend to grow larger, resulting in increased biomass. This phenomenon was observed in Chapter 2 of our study; the number of mussels declined over time, while biomass increased. This is a desirable outcome in mussel cultivation, as it achieves a substantial final biomass. To achieve lower initial densities, it might be worth investigating the use of fewer cockle shells within the BioShell-SMC. Since cockle shells serve as attachment material for mussel seed, reducing their quantity could lead to fewer mussels settling, allowing for the optimization of the amount of mussel seed. This approach avoids

the need to remove mussels from the substrate, which can induce handling stress and significantly decrease survival (Calderwood et al., 2015).

Minimizing mussel loss after seeding through effective relaying has the potential to augment production efficiency per unit of mussel seed in on-bottom mussel farming. Research has shown that a more even distribution of mussel seeds at relatively low densities can enhance mussel survival (Capelle et al., 2014). Mussels themselves can create small patches that contribute to increased production efficiency over the long term. However, it has also been demonstrated that using clustered individuals rather than spacing them out can enhance restoration success in marine ecosystems, including mussels (Fivash et al., 2022; Schotanus, Walles, et al., 2020; Shaver & Silliman, 2017). This underscores the importance of considering site-specific environmental conditions. In challenging environments characterized by strong currents, waves, and limited attachment substrate, mussels often struggle to self-organize effectively due to a limited window of opportunity. This is where the BioShell-SMC can potentially offer a solution. However, in more favorable conditions like mussel culture plots, where environmental conditions are less harsh, mussels have greater chances to self-organize. Consequently, it could be worthwhile to build upon the findings by Capelle et al. (2014), and to consider seeding mussel seed along with cockle shells in large-scale spatial patterns without the inner rope in such areas. Although Chapter 3 revealed a rapid decline in mussel biomass for loose mussels, this decline could potentially be counterbalanced by the substantially greater number of mussels present within a large-scale mussel plot. By providing attachment substrate for the mussels and strategically seeding them in a banded pattern to mitigate resource competition, it enables mussels to organically self-organize into small-scale spatial patterns.

This thesis underscores the complexity of extensive aquaculture and the need for continued research and innovation to address the issues faced by mussel farmers. While the current study may not have yielded the desired results, it highlights the importance of ongoing efforts to enhance mussel aquaculture practices and ensure the sustainability of this sector in the Netherlands. The use of the BioShell-SMC in its current form does not appear to be a viable solution for mussel farmers. While the seed collection aspect shows promise (Chapter 2), additional research is required to determine where it can be competitive with traditional rope-based methods and where it may fall short. Transplanting the BioShell-SMC on a small scale yielded promising results (Chapter 3 and 4), but on a larger scale (Chapter 5), the outcomes did not meet our expectations. Therefore, further research is needed to enhance survival rates on a larger scale. Additionally, it would be interesting to investigate the effect on mussel survival when seeding loose mussels with cockle shells in a banded pattern to create large-scale patterns. This approach would facilitate the self-organization of mussels into small-scale spatial patterns. An important note is that collaborations between mussel farming companies, research institutes, and industry suppliers are essential for the success and large-scale adoption of these innovations. Regular communication and sharing of progress ensure that the entire industry benefits from these innovations.

Main conclusions

The primary objective of this research was to explore a strategy to enhance the success of mussel collection and transplantations for restoration and aquaculture purposes, particularly using biodegradable structures known as BioShell-SMCs.
Answers on the research questions defined in Chapter 1 (graphical summary of the main findings can be found in Figure 6.6):

1. *How does the collection performance of the BioShell-SMC compare to conventional nylon rope collectors in terms of mussel seed density and growth, considering varying water depths and collector locations?*

Deployment location significantly impacted mussel seed collection efficiency, with traditional ropes performing better than BioShell-SMCs in harsher, more exposed areas (**Chapter 2**). In calmer conditions, the final obtained biomass was similar. Timing of harvest was crucial; smaller mussels were more vulnerable to predation (**Chapter 3**). Water depth did not have an effect on the obtained mussel seed biomass (**Chapter 2**). Careful location selection and timing are thus essential for optimal results in mussel seed collection.

2. *Can a biodegradable structure promote self-facilitating feedback mechanisms, that increase survival of transplanted mussels?*

The use of the BioShell-SMC proved to be a promising approach to enhance the survival and establishment of larger mussel seed, enhancing self-facilitating feedback mechanisms in a coastal ecosystem (**Chapter 3**). While the BioShell-SMC provided protection against both hydrodynamic forces and predators, the extent of its effectiveness depended on mussel size, with smaller mussels being vulnerable to predation during the early days to weeks.

3. *How do interactions between seeding method, mussel density and substrate type influence self-facilitation of mussels, and can these mechanisms be induced post-transplantation?*

The BioShell-SMC can promote self-facilitating feedback mechanisms necessary for mussel establishment. Specifically, the BioShell-SMCs facilitated mussel clustering, particularly in low density (**Chapter 4**). Mussels in high density attached to the BioShell-SMC dispersed from the SMC on both muddy and sandy sediments, creating an opportunity to escape competition. The BioShell-SMC also increased survival of the mussel seed, and improved their condition.

4. *How can the implementation of BioShell-SMCs with diverse spatial configurations mimic the spatial patterns observed in natural mussel beds, and how does this affect the interplay between facilitation and competition in mussel populations after transplantation?*

Despite the different spatial configurations tested, there were high overall mussel losses (~75%) (**Chapter 5**). The lack of mussels migrating from the BioShell-SMC structures hindered the formation of natural aggregations, leading to increased competition among the mussels. Additionally, factors such as hydrodynamic dislodgement and interannual variation also contributed to these observed losses. Nevertheless, when compared to loose mussels, the BioShell-SMC exhibited promising potential for mussel survival (**Chapter 3**). Importantly, the survival of mussels was found to be largely unaffected by the seeding patterns, simplifying the implementation of this novel method without the need for specific banding patterns (**Chapter 5**).

In conclusion, the BioShell-SMC has shown to be able to improve transplantation success substantially, when transplanted at the right time, in right mussel densities and on small scale. Future research should further investigate the optimal density for mussels attached to the BioShell-SMC and scale-up restoration efforts.

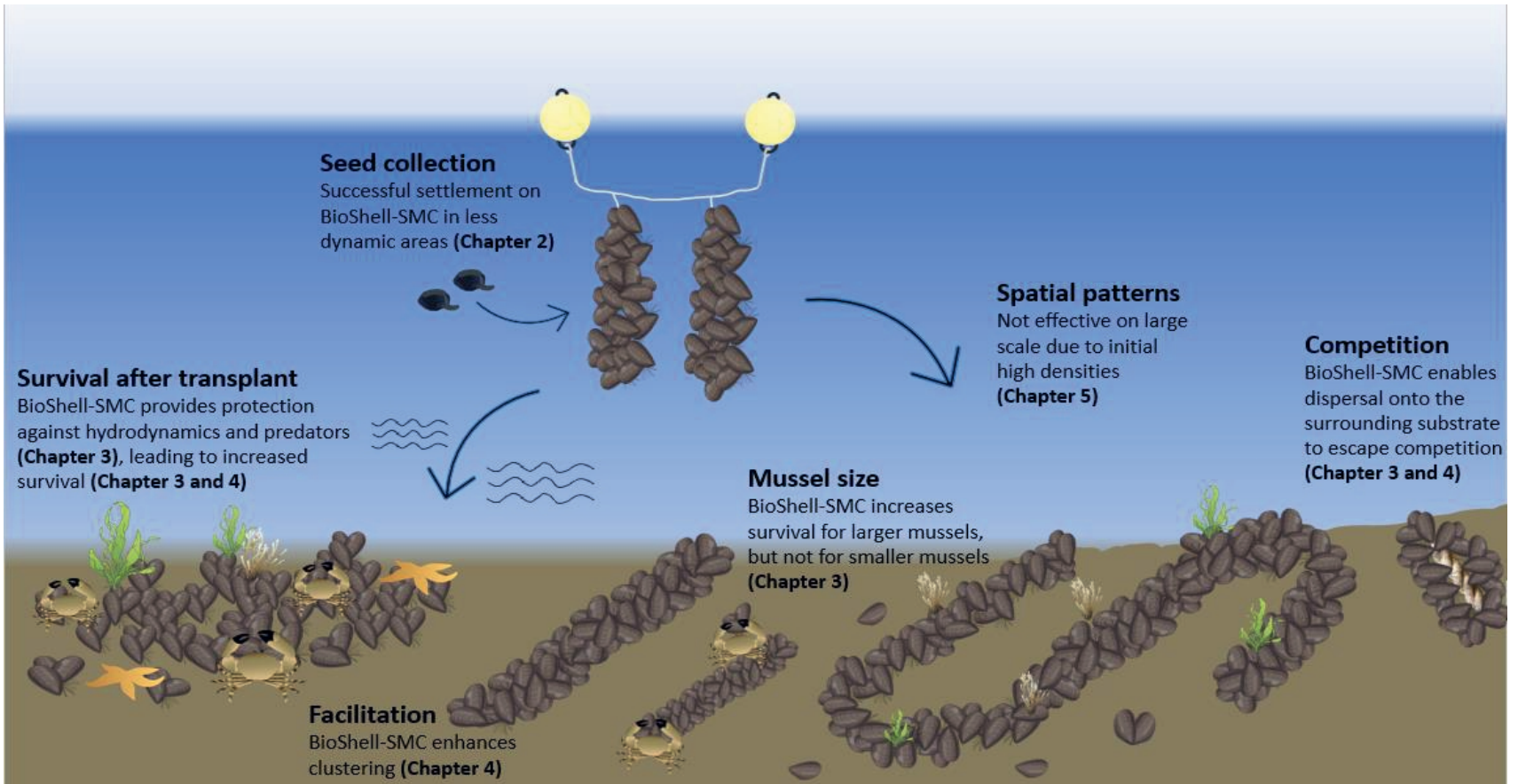

Figure 6.6. Summary of the main findings of this thesis. Symbols adjusted from Integration and Application Network (ian.umces.edu/media-library).

Aanbevelingen voor mosselkwekers

Op basis van de bevindingen in dit onderzoek wil ik enkele aanbevelingen delen voor mosselkwekers. In het kort heeft de BioShell-SMC getoond dat het potentieel heeft om transplantatiesucces te verbeteren, vooral wanneer deze op kleine schaal en op het juiste tijdstip en met de juiste mosseldichtheden wordt toegepast. Voor grootschaligere implementaties is echter aanvullend onderzoek nodig om de optimale dichtheid van mosselen bij het gebruik van BioShell-SMC's te bepalen. Bovendien is het van belang om rekening te houden met de jaarlijkse variatie in mosseloverleving, die kan leiden tot fluctuaties in sterfte zonder duidelijke oorzaak. Daarom wordt aanbevolen om de BioShell-SMC gedurende meerdere jaren te testen voordat grootschalige implementatie wordt overwogen. Mosselkwekers kunnen de volgende aanbevelingen in overweging nemen om hun strategieën voor mosselkweek te verbeteren.

1. **Invang van mosselzaad**: bij het invangen van mosselzaad is het belangrijk om de juiste locatie te selecteren. Traditionele Xmas Tree MZI's presteren beter in meer blootgestelde gebieden in vergelijking met de BioShell-SMC. In kalmere omstandigheden is er geen verschil in de opbrengst van biomassa. Bovendien blijkt de timing van de oogst van groot belang te zijn. Klein mosselzaad is vatbaar voor predatie, maar als je te lang wacht, kunnen hele mosselklompen van de MZI's loslaten. Het wordt daarom aanbevolen om regelmatig te controleren of de mosselen nog stevig vastzitten.
2. **Overleving na uitzaaien**: bij het gebruik van BioShell-SMC's voor mosselkweek is het belangrijk om aandacht te besteden aan de initiële mosseldichtheid. Deze mag niet te hoog zijn om competitie tussen de mosselen te voorkomen. Prioriteit moet worden gegeven aan het vinden van de optimale initiële dichtheid van mosselen om een succesvolle opschaling te garanderen zonder de overlevingskansen in gevaar te brengen. Daarnaast wordt aanbevolen om voldoende tijd te besteden aan het uitzaaien van de mosselen. Bij een te hoge lokale dichtheid, is de competitie te hoog en zal de overleving lager zijn.
3. **Ruimtelijke configuraties**: vermijd complexe ruimtelijke configuraties, eenvoud kan effectiever zijn dan complexe ontwerpen. Het creëren van een natuurlijk bandenpatroon met de BioShell-SMC is mogelijk niet het beste model voor hogere overleving en neemt veel tijd in beslag. Echter, verder onderzoek zou uit kunnen wijzen of deze configuratie wel effectief blijkt wanneer losse mosselen en kokkelschelpen in een bandenpatroon worden uitgezaaid.

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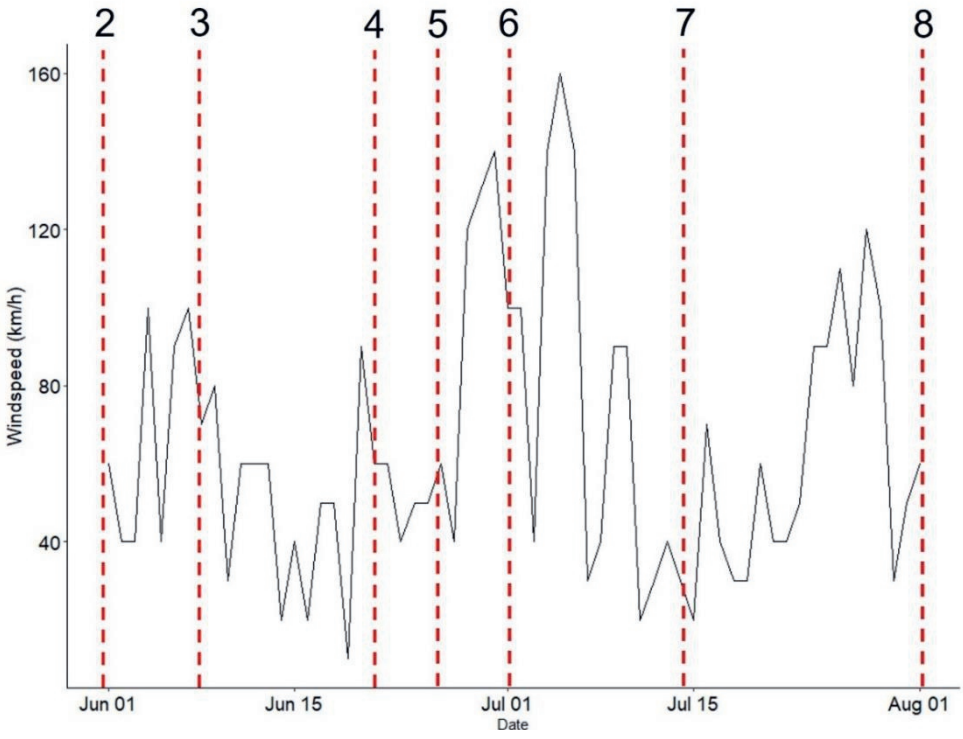
Appendix

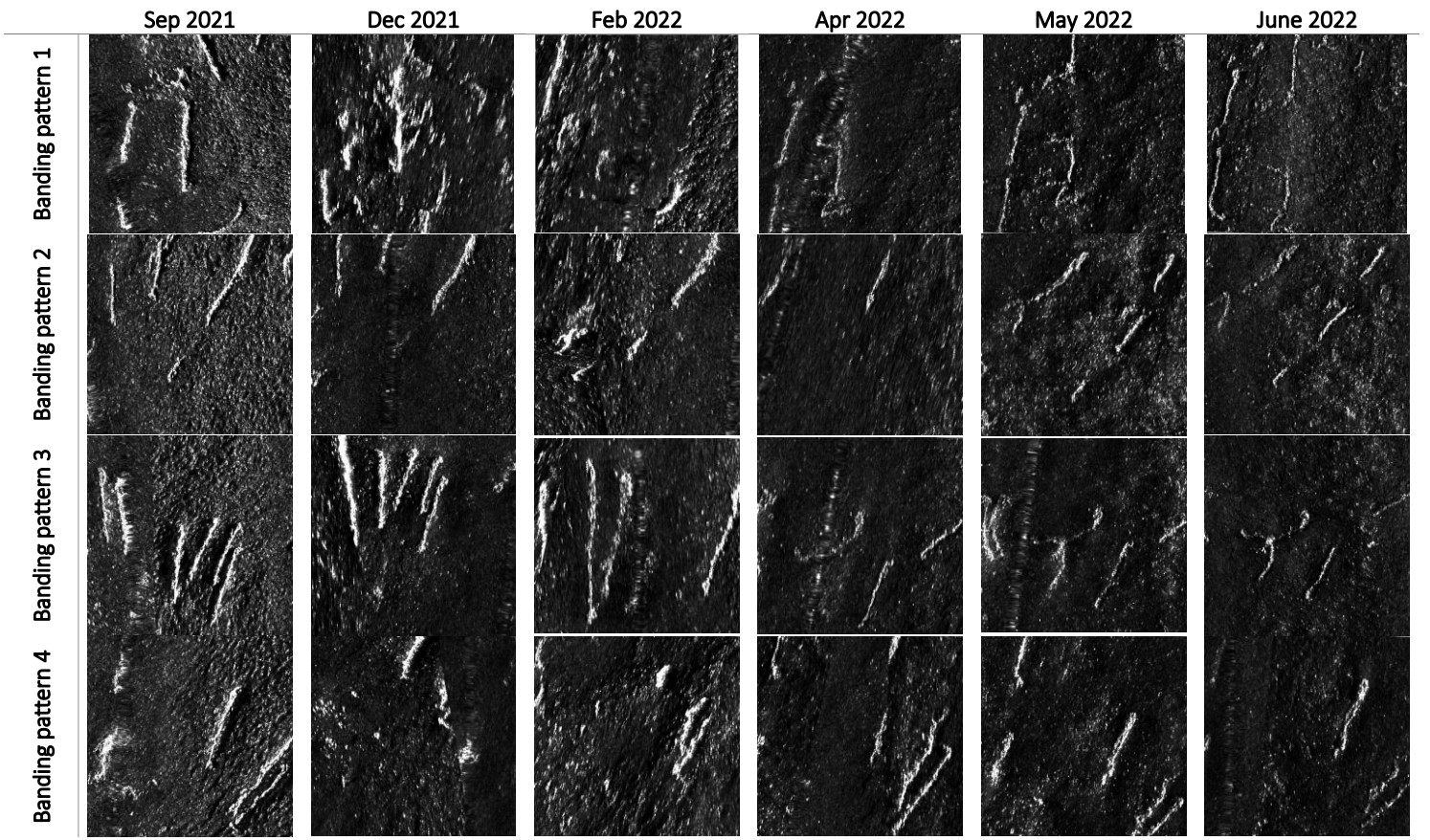
Appendix A

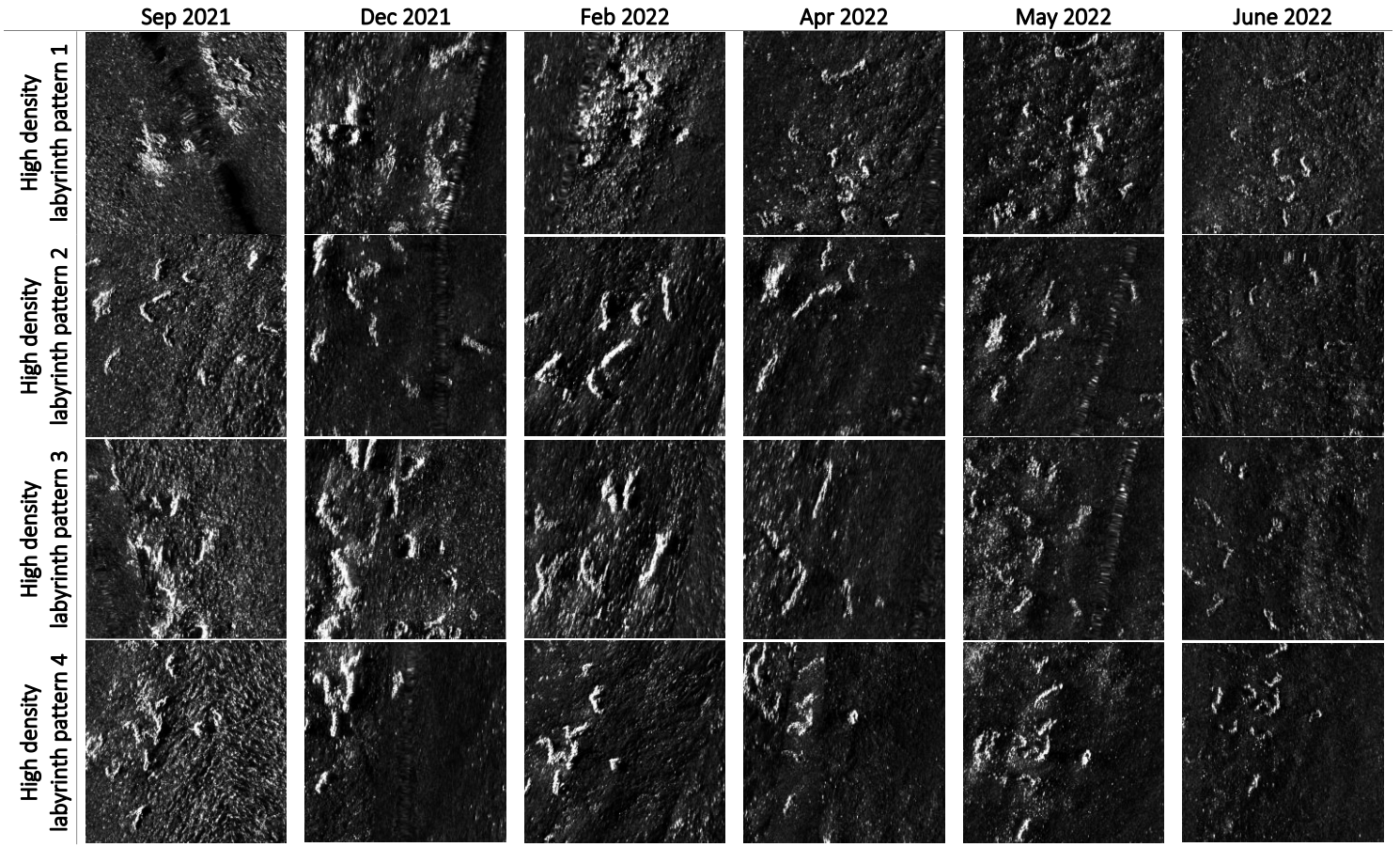


Hourly mean wind speed (in km/h) at station 312 in the Oosterschelde in 2020 (3.622 LON(east), 51.768 (LAT(north)) (Source: Royal Netherlands Meteorological). Red dashed lines represent collection of experimental units in the temporal-depth experiment with numbers on top corresponding to the sampling times.

Appendix B

Low density labyrinth pattern
Low density labyrinth pattern
Low density labyrinth pattern
Low density labyrinth pattern
Sep 2021
Dec 2021
Feb 2022
Apr 2022
May 2022
June 2022


Sep 2021
Dec 2021
Feb 2022
Apr 2022
May 2022
June 2022
Banding pattern 1
Banding pattern 2
Banding pattern 3
Banding pattern 4


Sep 2021
Dec 2021
Feb 2022
Apr 2022
May 2022
June 2022
High density labyrinth pattern 1
High density labyrinth pattern 2
High density labyrinth pattern 3
High density labyrinth pattern 4

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born in 1992 in the Netherlands, is a marine biologist. She completed her master's degree in Marine Biodiversity and Conservation at EMBC+, an international master program, and subsequently worked as a project manager and shellfish researcher at Wageningen Marine Research. In 2021, she embarked on a PhD journey at the Royal Netherlands Institute for Sea Research (NIOZ). Lisanne's interest lies in practical research, innovation, and future-oriented solutions. Her contributions to real-world challenges in marine ecosystems extend to this thesis book, an exploration of enhancing mussel transplantations.