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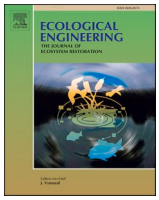

# Using a biodegradable substrate to increase transplantation success: Effect of density and sediment on aggregation behavior of mussels

Lisanne A. van den Bogaart [a,b,*], Jildou Schotanus [c], Jacob J. Capelle [c], Tjeerd J. Bouma [a,b]

[a] Netherlands Institute for Sea Research, PO Box 140, 4400AC Yerseke, the Netherlands
[b] Faculty of Geosciences, Department of Physical Geography, Utrecht University, 3508 TC Utrecht, the Netherlands
[c] Wageningen Marine Research, PO Box 77, 4400AB Yerseke, the Netherlands

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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.

## 1. 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 and 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 and 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

* Corresponding author at: Netherlands Institute for Sea Research, PO Box 140, 4400AC Yerseke, the Netherlands.
*E-mail addresses:* lisanne.van.den.bogaart@nioz.nl (L.A. van den Bogaart), jildou.schotanus@wur.nl (J. Schotanus), jacob.capelle@wur.nl (J.J. Capelle), tjeerd.bouma@nioz.nl (T.J. Bouma).

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mitigate environmental stresses (Hunt and 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 and 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 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 and 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 and 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 and 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 et al., 2016b).

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., 2023). 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 (Schotanus & van den Bogaart et al., submitted.). 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 (Fig. 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.

## 2. 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. (2023). 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.,

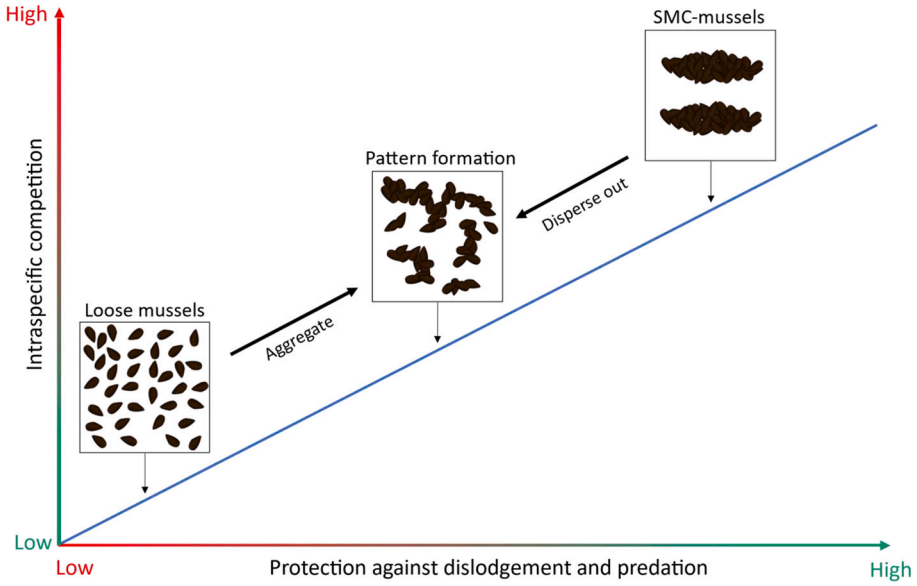


**Fig. 1.** Schematical overview of expected pattern formation after transplant by loose mussels and mussels attached to the BioShell-SMC. Loose mussels will organize in patches to find protection against dislodgement and predation (aggregate), while SMC-mussels will disperse from the substrate in order 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.

2019; Frandsen and 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.

### 2.1. 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 × 2 × 2 treatments × 3 replicates =) 24 experimental units (Fig. 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.

### 2.2. 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.

### 2.3. Experimental treatments

The experiments were carried out in 1 × 1.2 × 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 °C 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 (Fig. 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 × 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

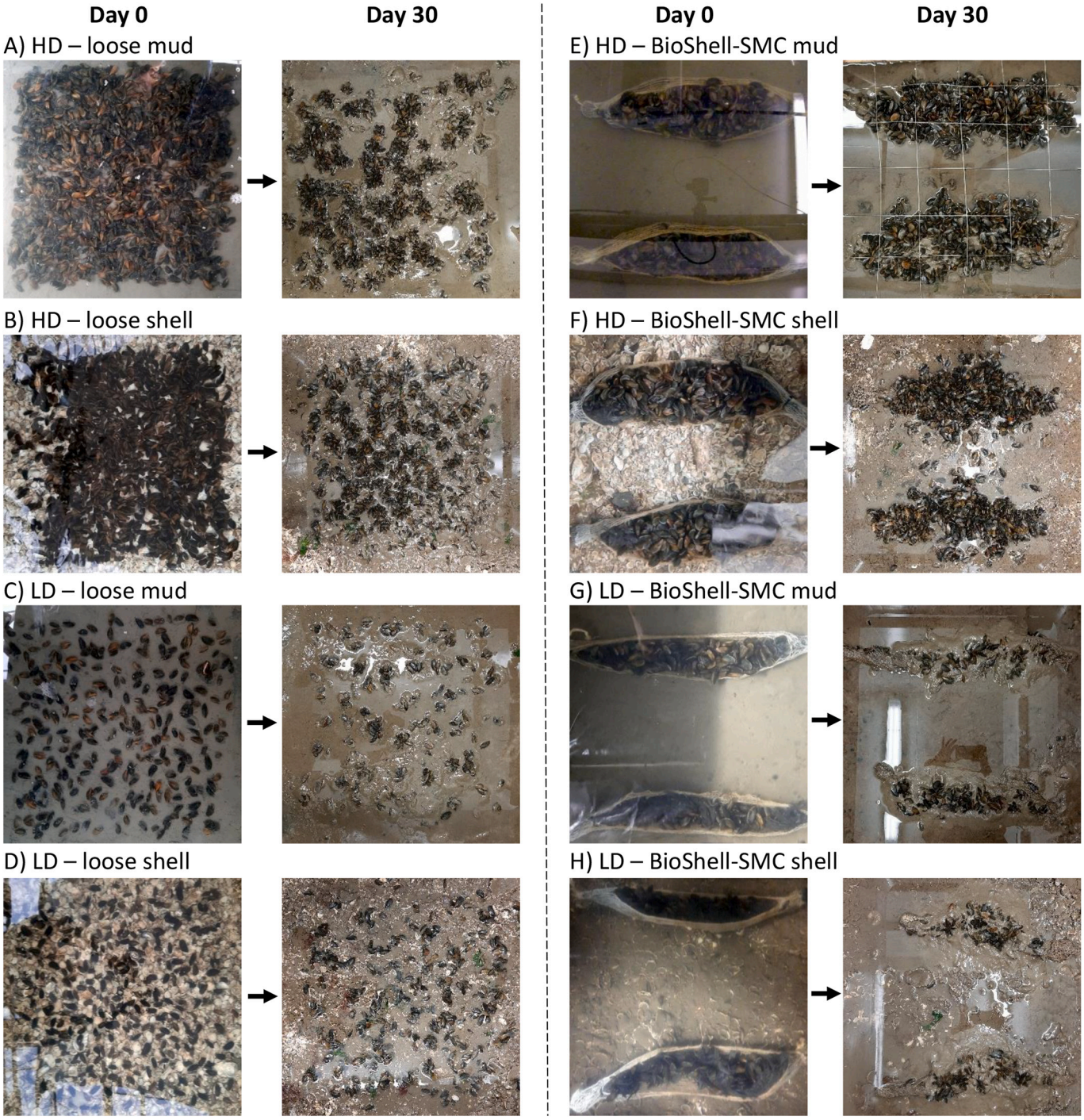


**Fig. 2.** Overview of the mussels in the experimental units at the start (left) end of the experiment (right, after ±30 days). Experiments in round 1 included loose mussels of different densities (A-D) while the mussels in experimental round 2 were attached to the BioShell-SMC which was cut open on 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.

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).

### 2.4. 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 behavior.

#### 2.4.1. 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 $\sigma^2$ is the variance of mussel cover (the degree of variability in the number of black pixels per treatment) and $\mu$ 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.

#### 2.4.2. 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 %).

#### 2.4.3. 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.

#### 2.4.4. 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 ($t(200) = 0.759$, $p = .449$), 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 6 × 6 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 <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.

### 2.5. Statistical analysis

All statistical testing was conducted in R studio (R Studio Team 2020), with the critical alpha value for significance being set to $p = .05$. 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.

## 3. Results

### 3.1. Mussel measurements

The pictures in Fig. 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).

#### 3.1.1. 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 ($F_{1,1} = 71.81$, $p < .001$) (Fig. 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 ($F_{1,1} = 9.13$, $p = .008$). Sediment composition also significantly influenced the VMR ($F_{1,1} = 47.08$, $p < .001$), as well as the interaction between sediment composition and method ($F_{1,1} = 12.74$, $p = .003$) 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, $p < .001$).

### 3.1.2. 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 ($F_{1,1} = 4.40$, $p = .036$) and density ($F_{1,1} = 63.03$, $p < .001$) significantly affected the number of patches (Fig. 4). 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 ($F_{1,1} = 4.12$, $p = .04$), 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, $p = .079$).

Total mussel cover was significantly influenced by initial transplantation density ($F_{1,1} = 154.12$, $p < .001$) and sediment composition ($F_{1,1} = 16.13$, $p < .001$) (Fig. 4). Method did not significantly influence mussel cover, however, we found significant interactions between method and density ($F_{1,1} = 9.24$, $p = .007$) and method and sediment composition ($F_{1,1} = 11.46$, $p = .003$). 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 had an effect on loose mussels, with a larger cover area on shell compared to mud ($p = .004$). We did not find this for SMC-mussels.

### 3.1.3. Patch characteristics

We also looked at how the perimeter-to-area ratio (P:A ratio) of the mussel patches changed over time (Fig. 4). The ratio was affected by the transplantation method ($F_{1,1} = 6.71$, $p = .017$), 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.

### 3.1.4. Mussel survival and condition

Survival of mussels after 30 days was influenced by transplantation method, mussels transplanted with substrate better survived than loose mussels ($F_{1,1} = 63.51$, $p < .001$) (Fig. 5). A significant interaction between density and method ($F_{1,1} = 8.00$, $p = .013$), 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 ($F_{1,1} = 6.65$, $p = .021$), it reveals differences between all loose mussel treatments vs. SMC-mussel treatments, but not within transplantation method.

At the end of the experiment, our analysis revealed significant main effects of density ($F_{1,1} = 4.75$, $p = .029$), sediment type ($F_{1,1} = 11.20$, $p < .001$) and transplantation method ($F_{1,1} = 63.45$, $p < .001$) on the condition index (Fig. 6). Besides, significant interaction effects between these factors were present. By loose mussels, we found no difference in

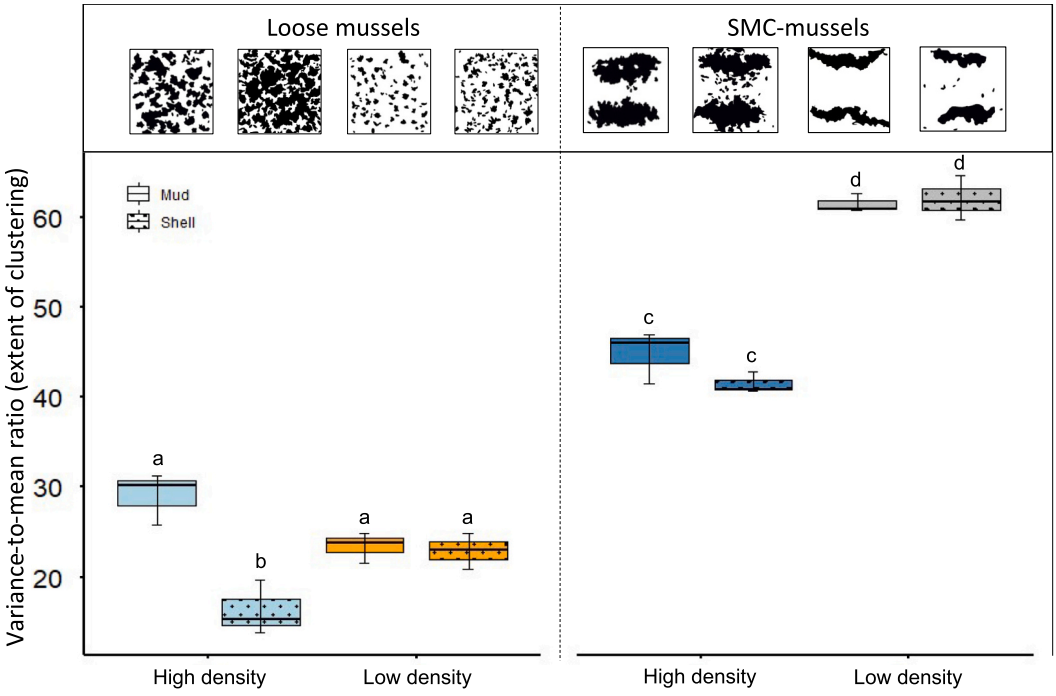


**Fig. 3.** Variance-to-mean ratio for loose mussels (left) and SMC-mussels (right) in high and low density. Mud is represented with solid fill and shell with dotted pattern. Letters denote significance.

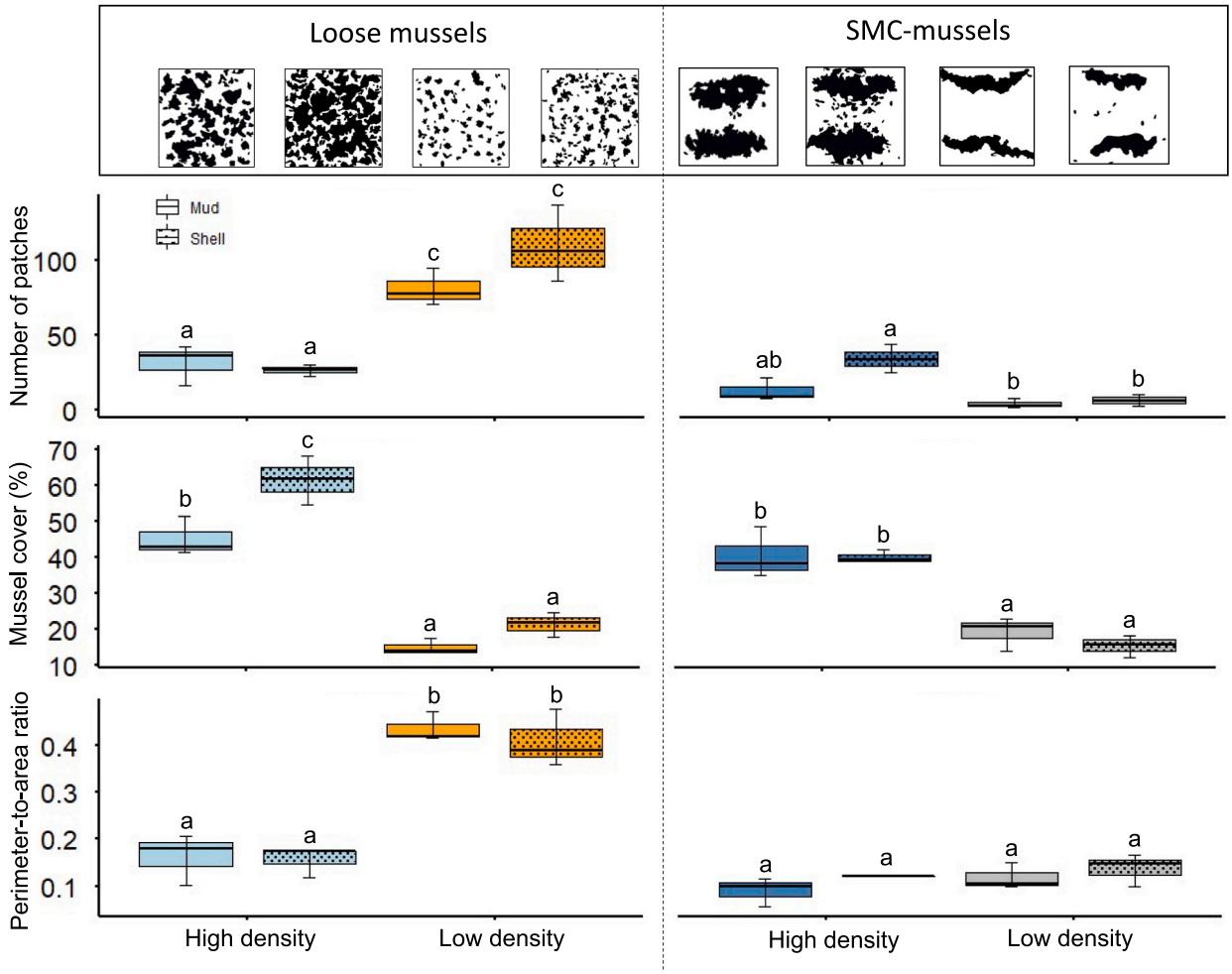


**Fig. 4.** Patch characteristics at the end of the experiment for loose mussels (left) and SMC-mussels (right) in high and low density. Mud is represented with solid fill and shell with dotted pattern. Letters denote significance. A) Number of patches, B) Mussel cover (in %) and C) perimeter-to-area ratio, calculated as the total perimeter of all patches divided by the total mussel cover.

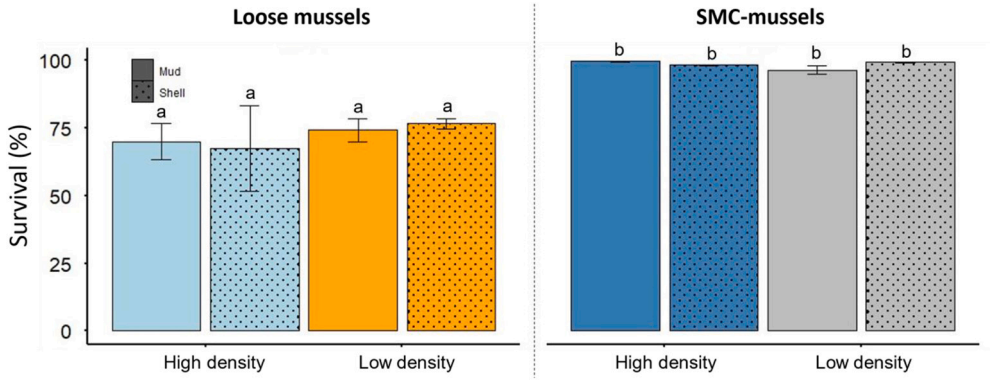


**Fig. 5.** Survival of mussels at the end of the experiment for loose mussels (left) and SMC-mussels (right) in high and low density. Shell substrate is shown with a lined pattern and mud without pattern. Letters denote significance. Data are means ± SE.

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) ($p < .001$).

## 4. 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

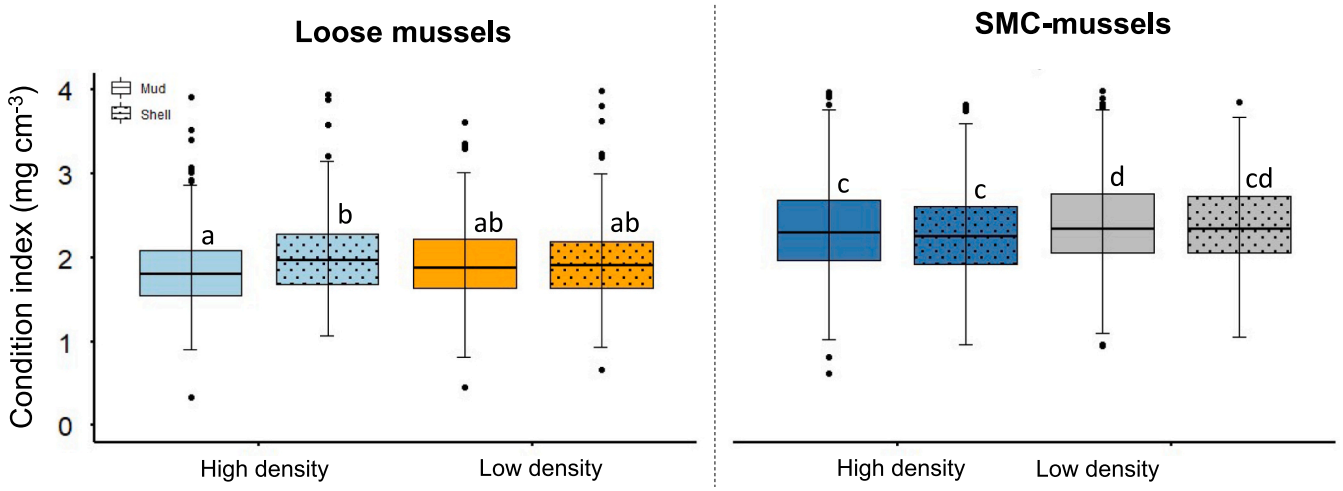


**Fig. 6.** Condition Index (CI, in mg $cm^{-3}$) for loose mussels (left) and mussels attached to the SMC (right). Mud is represented with solid fill and shell with dotted pattern. Data are means ± SE. Letters on tope denote significance.

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.

### 4.1. *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 and Grosholz, 1985; Capelle et al., 2016b; Svane and 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 and 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.

### 4.2. *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 and 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.

### 4.3. 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 $\pm$ 0.5% vs. 72.0 $\pm$ 3.9%) and condition index (2.47 $\pm$ 0.82 vs. 1.96 $\pm$ 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 and 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 et al., 2016a; Dare, 1974; Slabyj and 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.

### 4.4. 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.

## Statement

During the preparation of this work the authors used ChatGPT in order to enhance the readability of their own sentences. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

## CRediT authorship contribution statement

**Lisanne A. van den Bogaart:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Jildou Schotanus:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision. **Jacob J. Capelle:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. **Tjeerd J. Bouma:** Conceptualization, Writing – review & editing, Supervision,

Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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