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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 are 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.

1. 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 and Capelle, 2019). However, in some countries, mussel seeds are harvested from benthic mussel beds and relayed on designated bottom plots for grow-out (Dolmer and 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 and 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 et al., 2001; Dolmer and Frandsen, 2002; Dolmer, 2002; Eleftheriou and 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

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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 and 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 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 environmental 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., 2012a; 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 is 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 5 m). 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.

2. Materials and methods

2.1. 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 (Fig. 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 (Fig. 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 5 m), 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

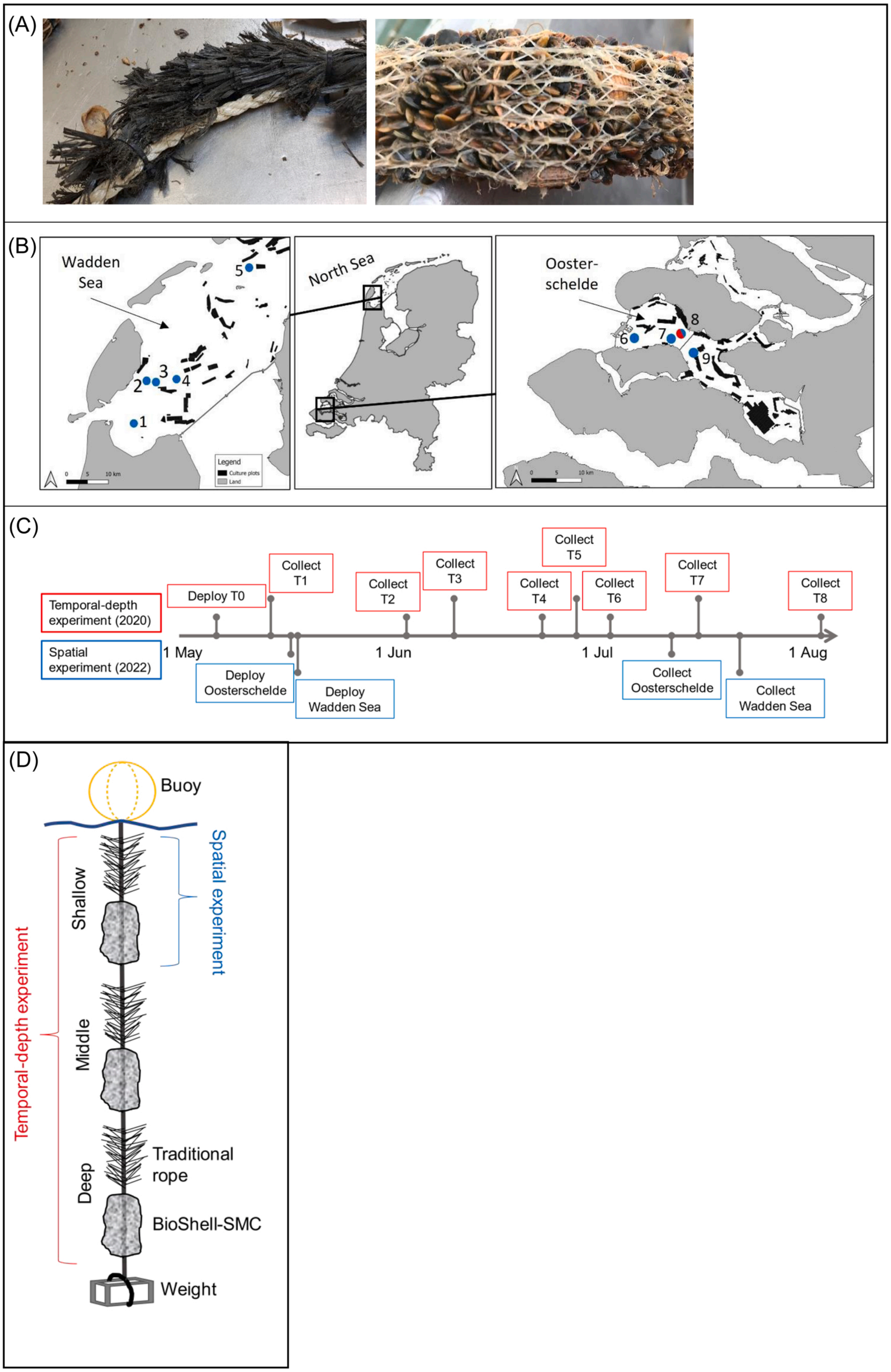

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Fig. 1. (A) The two types of mussel collector material. Left: traditional Xmas tree rope, and right: biodegradable BioShell-SMC. The BioShell-SMC consists of a coconut core surrounded by empty cockle shells that are held in place by a biodegradable 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 gray and water in white, mussel culture plots are shown in dark gray. 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 (± −1 m), middle (± −3 m) and deep (± −5 m). For the spatial experiment, only the upper part of the setup was used. The system consisted of two types of substrate: traditional rope (dark gray) vs. BioShell-SMC (light gray).

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.

2.2. Experimental setup

2.2.1. 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 Fig. 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. 1 m below the water surface), middle (approx. 3 m below surface), and deep (approx. 5 m below surface) (Fig. 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. 5 m. 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 (Fig. 1C). This means that sampling was conducted until commercial harvest time. At each depth and for each collector type, three samples of 2 – 10 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 h. The condition index (CI) was calculated (by dividing the AFDW by the cubed length) for every individual mussel in mg cm^{-3} (Beukema and De Bruin, 1977).

2.2.2. 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 (Fig. 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 and 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.

2.3. 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):

$$\text{Mussel weight} = 0{\cdot}0002 \times \text{shell length}^{2{\cdot}8} \quad (1)$$

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 Eq. (1). Because sample lengths differed, we expressed mussel biomass as a function of sample length (i.e., kg/m rope or BioShell-SMC).

2.4. 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).

2.4.1. Temporal-depth experiment

We wished to determine the effect of collector type (traditional rope vs. BioShell-SMC) and depth (1 m vs. 3 m vs. 5 m) 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 × two collector type × 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 ~ collector type × 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 × depth.

2.4.2. 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 × 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 × 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 × location). For the Oosterschelde, the best fit was a reduced model for biomass: Mussel biomass ~ location. The biomass data were not transformed.

3. Results

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

3.1.1. 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 (Fig. 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 1, Fig. 2). 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.

3.1.2. Number of mussels

In contrast to the increasing biomass, the number of mussels decreased over time (Fig. 2B). Major spat settlement occurred between the 3rd of June (T2) and the 23rd of June (T4), resulting in maximum numbers of almost 28,000 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 1, Fig. 2). 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.

3.1.3. 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 (Fig. 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 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 1, Fig. 2).

3.1.4. Mussel condition index

The condition index of mussels was significantly affected by deployment depth, collector type and the interaction between these factors (Table 1). At deep deployment depth, we found a significant higher condition index for mussels attached to the BioShell-SMC (Fig. 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, $p < 0.001$).

3.2. 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). 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

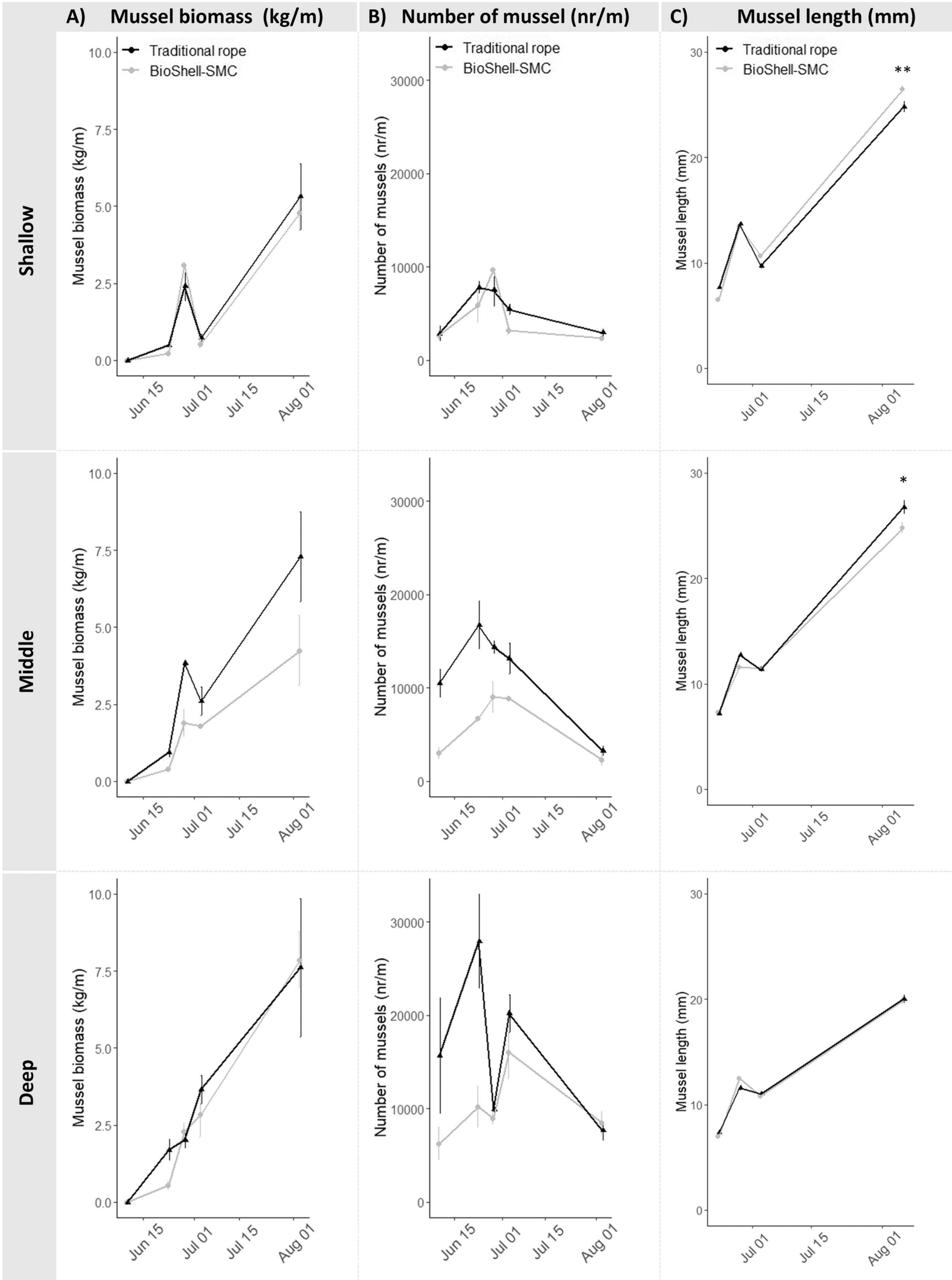

Fig. 2. Overview of development of mussel biomass (A, in kg/m), density (B, in nr/m) and length (C, in mm) over time at different deployment depths (shallow, middle and deep) at location Vuilbaard in the Oosterschelde. Dark gray: traditional rope (Xmas Tree), light gray: BioShell-SMC. Data are means ± 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.

Table 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***

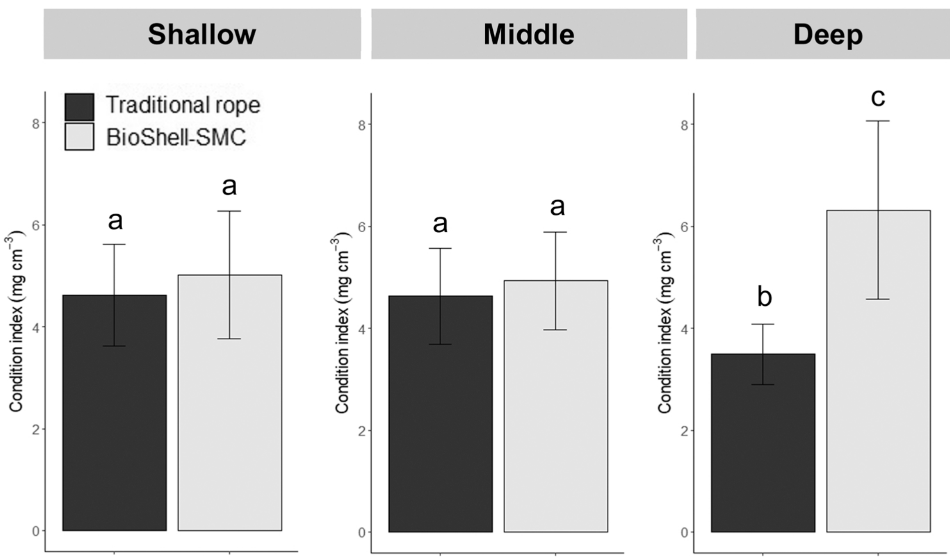

Fig. 3. Mean mussel condition index (mg cm^{-3}) at the end of the experiment at shallow, middle and deep deployment depth. Dark gray: traditional rope (Xmas Tree), light gray: BioShell-SMC. Data are mean ± SE (n = 60). Letters denote significance.

Table 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

significant interaction between location and collector rope (Table 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, $p = 0.049$) (Fig. 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). 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 (Fig. 5).

4. 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.

4.1. 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 and Buck, 2010; Filgueira et al., 2007; Walter and Liebezeit, 2003), including fine-branching algae and hydroids (Alfaro and Jeffs, 2002; Buchanan and Babcock, 1997). Biodegradable materials

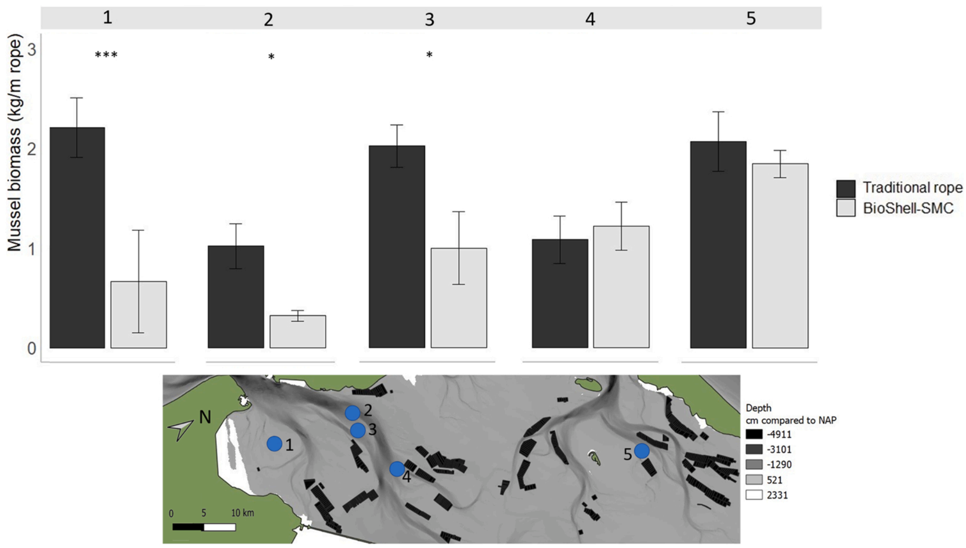

Fig. 4. Mussel biomass on traditional rope (Xmas Tree, dark gray) and BioShell-SMC (light gray) at five different locations in the Wadden Sea. 1: Zuidwal, 2: Burgzand, 3: Vogelzand, 4: Gat van Stompe, 5: Zuidmeep. Land is shown in green and water in greyscale. Mussel culture plots are shown in dark gray.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

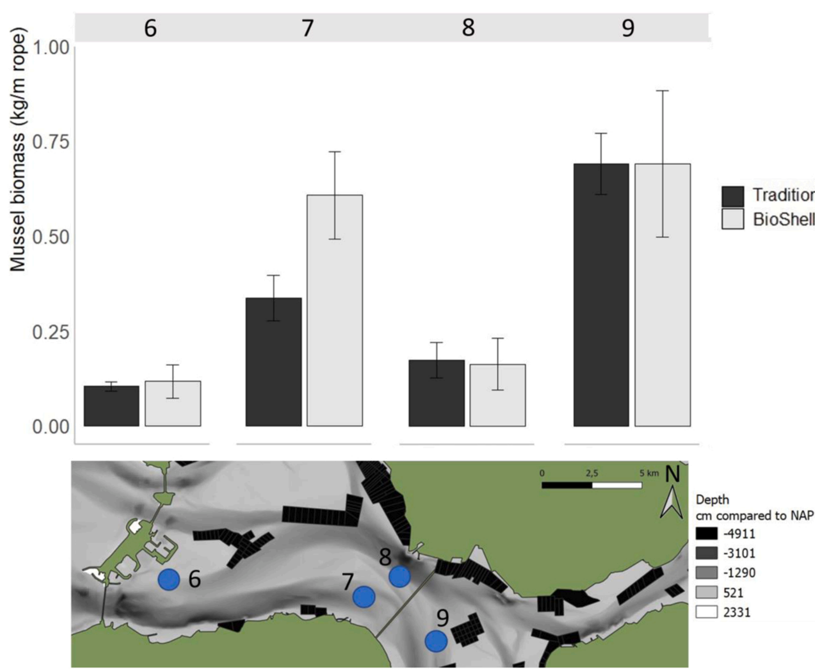

Fig. 5. Mussel biomass on traditional rope (Xmas Tree, dark gray) and BioShell-SMC (light gray) at four different locations in the Oosterschelde. 6: Neeltje Jans, 7: Schaar van Colijnsplaat, 8: Vuilbaard, 9: Vondelinge. Land is shown in green and water in greyscale. Mussel culture plots are shown in dark gray. Data are means ± SE.

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 and 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 and 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)feces 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.

4.2. 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 and 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 and 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 and 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.

4.3. 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 (Fig. 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, 2022; Fuentes and Molares, 1994; Kamermans et al., 2002; Karayücel and Karayücel, 2001), even on small spatial scales (Fuentes and Molares, 1994; Snodden and 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 and Molares, 1994; Marguš and 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 and Figueras, 1998), algal and microbial coverage associated with the substrate (Hunt and Scheibling, 1996), nutrient availability (Pechenik et al., 1990) and temperature and salinity (Brenko and Calabrese, 1969; Manoj Nair and 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.

4.4. 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 a 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 et al., 2020b). 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.

CRediT authorship contribution statement

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

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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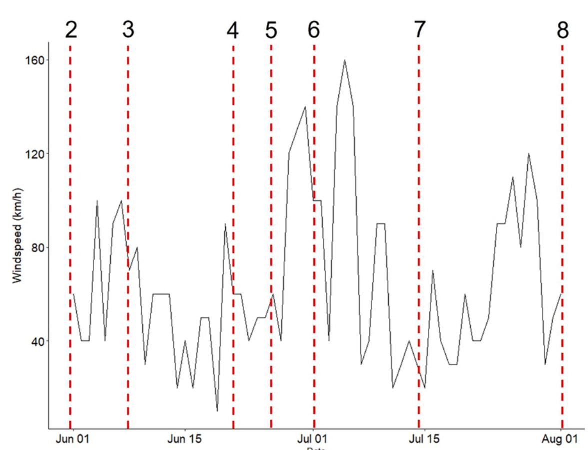
Appendix A



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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.

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