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A trial in the Ems-Dollard estuary

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Contents

Summary

On March the 30th of 2017 the Ems-Dollard estuary was designated as a Special Protection Area under the Habitat Directive as part of the Wadden Sea Habitat Directive area (Amendment Decision Natura 2000 Area #1, 2017). The nature objectives formulated as a result of this included the improvement of the area and quality of various habitat types including intertidal mussel beds. Ways to initiate intertidal mussel beds were sought to aid this recovery. In the research described here, a method was developed and put to the test in the Ems-Dollard estuary. Because the Dutch-German national borders in the area are under debate, a joint Dutch – German research project was formed between Wageningen Marine Research (WMR) and Senckenberg am Meer (SaM). An important project objective was formed by the credo "learning by doing". This meant that the research should be focused on testing in practice the most promising method of constructing an intertidal mussel bed.

At the start of this project, it was clear that the environmental conditions in the study area, the Hond and Paap tidal flat in the Ems-Dollard, are not optimal for the survival of mussels and may even be inadequate to sustain vital intertidal mussel beds. Some pre-studies, limited in design, were conducted to get a better idea of the survival changes for mussel(s)(beds). To this end water quality data (originated form the Monitoring Waterstaatkundige Toestand des Lands, MWTL program) were analysed, the historical developments in intertidal mussel beds were investigated (data originated from the Wettelijke onderzoekstaken, WOT- fisheries) and a mussel survival test was carried out on site. For the survival test, mussels of various origins (intertidal- and subtidal beds as well as from a mussel seed collector), were set out at the Hond and Paap tidal flat to monitor survival and growth. Additional water quality measurements were also carried out as part of this test.

The extreme cold temperatures at the end of February 2018 has led to ice formation in the estuary damaging the installations of the survival experiment to such extent that it was terminated prematurely. Therefore, the results of the test were only of very limited use. The test did demonstrate that mussels could survive in the area for at least two months and that conditions at the northern side of the Hond and Paap tidal flat were a bit better compared to the southern side. Results of the MWTL water quality parameters that were analysed here (salinity, suspended solids and chlorophyll-*a* concentrations as well as the ratio between suspended solids and chlorophyll-*a*) showed that in the research area suspended solids concentrations at which negative effects on mussels can be expected have been frequently recorded. The suspended solids concentration seem to have increased in recent decades which is also evident from results of more elaborate studies on this subject. In the past decades, periods lasting for a year with above average suspended solids concentrations have been observed twice, that co-occurred with decreases in the acreage of intertidal mussel beds in the area. Further south of Hond and Paap tidal flat conditions deteriorate further by (further) reduced salinity levels. Analysis of the historical fluctuations in mussel bed acreage carried out here showed that a bed has been able to form recently (2016) that was able to maintain itself for at least four years. In the end it has been concluded that the environmental conditions for mussel beds at the Hond and Paap tidal flat are suboptimal but mussel bed survival is possible. However, the possibility for prolonged periods with unfavourable suspended solids and chlorophyll-*a* concentrations continue to pose a risk to mussel bed survival. It was concluded that successfully conducting a mussel bed trial on the Hond and Paap mud flat, difficult in itself, is therefore not impossible in advance but will be extra challenging in the study area.

To arrive at a trial design a brief literature study was conducted, recent attempts to construct a bed were evaluated and mussel bed researchers at WMR and SaM were consulted. The resulting experimental design consisted of collecting mussel seed using biodegradable collector nets after which the nets and mussels are placed and fixed on the Hond and Paap tidal flat. Because the mussels were not removed from the biodegradable nets after placement on the tidal flat, the structures made by the mussels by which they attach themselves to the ropes of the net remained intact. Over time the nets will biodegrade and disappear. In the meantime the nets offer the mussels an attachment substrate,

preventing erosion, and time to form and mature into a self-sustaining bed. The test sites on the tidal flat for the trials were selected using information of a habitat suitability map for intertidal mussel beds (Brinkman in preparation), maps of the emersion time on the tidal flat and the presence of (historical) natural mussel beds. In addition, the intended test sites were visited before the start of the trial.

As machinal construction of the nets with the desired fibres was not possible, nets were manufactured manually by C.I.V. in Den Oever. Nets, with a dimension of two by ten meters each, were made of cotton, hemp and sisal ropes. Nine nets were made in total, from each material three. Because on not all nine nets the capturing of the mussel spat was successful, the original experimental set-up, testing the three net materials at three different sites, all in one season and thus under similar environmental conditions, had to be abandoned. Not one but two trials were conducted spread out over two seasons under different environmental conditions. Furthermore, not one but two different starting situations were tested. Due to these alterations it was no longer possible to statistically investigate the effects of location and net material type.

Mussel spat collection was only successful at the nets that where hung out in the Inner harbour of Wilhelmshaven in 2018. Trial 1 started in spring of 2018 using those nets (three nets in total, one of each rope material). These nets were placed and fixed at one of designated sites on the Hond and Paap tidal flat the same year. Trial 2 started in spring 2019. In that year, none of the remaining six nets became colonized by mussel spat and mussel spat from a commercial mussel spat collector was used instead. In this trial two plots (plot 1 and plot 2) were constructed, each consisting of multiple tiles with mussels (dimension of two by two meter). On top of each of the tiles in plot 1 a (empty) net was strained. In plot 2 of this trial the effects of the presence of a net and the presence of a foundation of cockle shells for the erosion of mussels were tested. For this reason tiles with and without nets and with and without a foundation of cockle shells were constructed. In both trials the developments of the mussel population and the coverage of the mussel patches were monitored over time. To this end multiple fieldtrips were conducted. The mussel population was assessed by taking field samples to determine the mussel density, mussel shell length and biomass in the laboratory. The development in mussel patch coverage was examined by construction of an ortho map. In addition, changes in elevation were investigated by constructing a digital elevation model. The emersion time was determined with the use of a CTD-sensor. Rope samples were taken to assess the rate of biodegradation.

Trial 1 showed that it is possible to capture large quantities of mussels with each of the net materials tested (hemp, cotton and sisal). The efficiency of capturing spat on the nets is determined by several factors, and with the trials conducted here, only limited successes were obtained. The dominant reason(s) for the poor capture and development of spat on the nets hung out in the tugboat harbour (located in the Jade channel) in spring 2018 and in the inner harbour of Wilhemsharbour in spring 2019 are not known. In the tugboat harbour it is likely that the observed presence of juvenile crabs and the accumulation of silt on the nets may have prevented the development of the mussel population. It is not likely that mussel larvae present in the water column was insufficient. In the inner harbour it is less clear what might have caused the poor mussel spat development on the ropes. The observed colonization of barnacles and filamentous algae might have caused the poor settlement of mussel larvae on the ropes but it could also be that there were not enough mussel larvae in the water column or it might have been the results of yet another unknown reason(s).

The nets (containing mussel spat) that were used in trial 1, were placed in an area where a layer of mud of several centimetres deep had been build up. Only the presence of the nets made survival of the mussels for several months possible in this area. All mussels that were flushed off the nets at any point in time were not found back in subsequent monitor events and were likely buried in the mud. Instead, the presence of dead shell material in the more sandy (and dynamic) environment of test site used in trial 2 made survival of mussels possible. A large portion of the mussels placed on top of a bed of cockle shells survived their first year and also two years later patches with living mussels were found. It is believed that these mussels has been provided with a good chance to develop themselves. But as just one trial was conducted in this way, more trials are needed to test the robustness of this method and its performance under different environmental conditions. In addition, the positive contribution of the nets to mussel survival and washout could not be conclusively proven in the tests carried out here. The test location at trial 1 proved to be unsuitable while at the suitable location used in trial 2, the necessary attachment structures were not formed prior installation on the tidal flat. This showed that a firm attachment of mussel spat prior installation on the tidal flat is essential for its functioning in preventing mussel erosion.

On both the nets (trial 1) and the mussels that are placed on a bed of cockles (trial 2), losses of mussels occurred mainly in the inner areas. It is precisely in these areas, possibly as a result of food suppression and/or oxygen depletion, that individual mussels detach themselves from each other to move themselves upwards were conditions are better. By doing so they become susceptible to erosion that was observed in periods that contain wind speeds higher than average (5 to 6 Beaufort or higher). These observations could be used to improve the experimental design.

It is recommended to investigate the underlying reasons for the observed periods (up to a year) with elevated suspended solids concentration in the estuary first before conducting more mussel bed construction trials in the Ems-Dollard estuary. It is believed that during these periods survival changes of mussel beds decline. If these periods are the result of human activities (dredging for example) effort could be made to prevent in order to improve mussel bed survival chances. Measures that could decrease the suspended solids concentration will improve the survival changes of the intertidal mussel beds in the area as well. Examples of such projects include a project by which sludge is extracted from the estuary system and a project by which a salt march is constructed. The formation- and survival of the in 2016 established mussel bed proves that natural establishments of mussel beds is possible. As conditions allow for survival of beds, there are no reasons to think why beds could not form and develop naturally in the estuary. Human intervention is not needed. Due to the irregularity of years with good spat fall and variation in the locations where mussel spat falls, natural establishment of beds might require some patience. In situations where intertidal beds are unintended damaged or in situation where damaging them cannot be avoided, in construction of cables for instance, the availability of a technique to initiate a bed can be of use in order to compensate for the damage done.

There is room for and a need to further optimize and test the techniques that were tested here, as both the ‘cockle’ and ‘net’ technique are believed to have potential but have not be tested to its full extent nor are fully proofed to work. First of all the success rate of mussel spat collection on the nets need to be improved by finding more suitable and reliable sites. In the search for sites, the experience from commercial mussel seed collectors can be used. It is also advisable to investigate the environmental conditions at successful collector sites and use this information to find suitable locations. Secondly suitable test sites on the tidal flat need to be found. As the technique is still in a development phase, it is recommended to select sites with favourable conditions for mussel survival. They can be found at tidal flats located near sea inlets. On these flats, areas with a more sandy substrate and with presence of shell material, and preferably where historically mussel beds have been located, should be selected. Measures to decrease the chance of detachment and subsequent erosion of mussels should aim at decreasing the chance for food limitation and oxygen depletion. They could include testing variations in mussel densities, mussel patch coverage and net-mesh sizes. It is recommended to test variations in execution with enough replica’s to prevent outcomes to be the result of unforeseen differences in local autonomic developments. The 'learning by doing' principle is then abandoned or at least interpreted more broadly.

1 Introduction

The Ems-Dollard estuary was designated a Birds Directive area in 1991, along with the rest of the Wadden Sea. Because the Dutch-German land border in the Ems-Dollard estuary is under debate, the Ems-Dollard was kept out of the designation of the Wadden Sea as a Habitat Directive area in 2009. In 2010 consultation between Dutch and German authorities were started in order to achieve the designation of the entire Ems-Dollard Estuary as a Natura 2000 site. On 30 March 2017, the Ems-Dollard was designated as a Special Protection Area under the Habitats Directive as a result of this whereby it is included as part of the Wadden Sea Habitats Directive area (Amending Decision Natura 2000 Area #1, 2017).

The estuary of the Ems-Dollard consists largely of subtidal and intertidal areas (areas that fall dry during periods of low tide) that both are part of the estuary habitat type (H1130). The (Dutch) national condition of this habitat type is unfavourable (H1130 Estuaries, version 2016). The (Dutch) national objective for Estuaries is to expand the surface area (foreseen for the Western Scheldt) and to improve the quality. For the Ems-Dollard Estuary, the objective is to maintain the surface area and improve the quality. One of the improvement objectives is to restore the mussel beds as soon as possible, as these form a characteristic part of the good structure and function of H1130. The reference situation for H1130 is the year 2008 when the Ems-Dollard was placed on the list of Areas of Community Importance. In 2008 there were 118.3 ha of mussel beds (mainly on the Hond-Paap tidal flats), which had decreased to 12.8 ha in 2015 according to Baptist & Geelhoed (2016). The goal is to stimulate the formation of intertidal mussel beds in order to improve the quality of the estuary.

1.1 Construction of mussel beds

Various attempts that have been made in the past, and are currently being carried out, demonstrate the difficulty of initiating a new intertidal mussel bed, see Dankers & Fey-Hofstede (2015) and de Paoli (2017). Despite being mostly unsuccessful, these attempts have provided valuable insights into the factors that are important to consider in conducting an attempt. Mussel bed research, such as Mosselwad and Waddensleutels, among others, have also provided valuable insights into factors that are important in the successful formation of intertidal mussel beds. Important differences between the properties of intertidal- and subtidal mussel beds have become evident for example. However, all these studies have not led to a proven technique by which the formation of an intertidal mussel bed can be formed.

1.2 Research question

So far, there is no method available that has proven itself to form vital intertidal mussel beds. It is also unclear to what extent the environmental conditions in the estuary have contributed to the decline in tidal mussel beds in recent years.

In the research described in this report, the following two main questions are addressed:

(1) To what extent do the environmental conditions in the Ems-Dollard estuary support survival of intertidal mussel beds?
(2) Based on the most recent insights and with the highest chance of success, how could a vital intertidal mussel beds be formed?

For research question 1, the following sub-questions could be formulated:

- What are the environmental conditions (food quantity, suspension load and salinity) in the Ems-Dollard and how does it compare with other tidal basins located in the Dutch Wadden Sea?
- What is the trend over time of these parameters in the Ems-Dollard?
- Are there gradients present in the Ems-Dollard?
- How sensitive are mussels to those parameters?
- Can mussels from different origin, adapt, survive and grow under the current environmental conditions at the Hond and Paap tidal flat?

For research question 2, the following sub-questions were asked:

- What is the latest knowledge on variables that determine the stability of intertidal mussel beds?
- What can be learned from past attempts to establish an intertidal mussel bed?
- What methods are considered promising for constructing intertidal mussel beds, and where are suitable locations in the Ems-Dollard for conducting trials?

Since both Germany and the Netherlands have claims in the area, a close involvement of Germany in planning and restoring tidal mussel beds is essential for its success. An important project objective is therefore to set up a Dutch-German research collaboration. A second important project objective is to work according to the credo "learning by doing". The 'learning by doing' philosophy implies testing methods in practice (no laboratory experiments for instance) and to solely focus on methods that are believed to be the most promising ones. No effort should be made in investigating factors that determine mussel bed survival or to test less promising methods.

2 Pre-studies

Before developing and implementing the mussel bed initiation trial it was investigated to what extent the conditions in the Ems-Dollard area are suitable for mussel (bed) survival. This research consisted of a short literature review on the dose-effect relationships of suspended solids levels, an analysis of the environmental conditions (water quality), an analysis of the historical mussel bed occurrences in the area and a field experiment in which the growth- and survival of mussels in the area were tested.

2.1 Food, suspended solids and salinity

Mussels obtain their food by filtering suspended solids from the water. Suspended solids consists of inorganic- and living or dead organic material. Depending on the size and quality, the organic material constitutes food for the mussel (Dankers & Fey-Hofstede, 2015). Mussels filter particles from the water and transport suitable material to their mouth and remove unsuitable particles as pseudofaeces. In this process, mucus (slime) produced by the mussel is used as a transport carrier. The more indigestible particles are present in the suspended solids, the more energy the mussel has to spend on filtering, transporting, separating and excreting them. The lower the organic content of the suspended matter, the less energy (food) is gained in this process.

Excessive concentrations of (inorganic) suspended solids can have negative effects on mussels. Effects that can occur are a decrease in food uptake rate, increased production of pseudofaeces, decrease in pumping rate and thus decreased oxygen supply, increased energy requirements and respiration, modification of morphology, decreased growth and eventually mortality (Tamis, 2016; Essink et al., 1985 & 1999). The morphological response to elevated suspended solids concentrations is that mussels have smaller gills and larger palps (both organs are involved in filtering water and excreting undesirable particles) (Capelle et al., 2021; Essink, 1999). Mortality can occur at extreme high concentrations and has been observed at 1220 mg/l (Purchon, 1937).

The relation between suspended solids concentration and filtration rate show an optimum curve. At low suspended solids concentrations, filtration rates increases due to its role as food. At increasing suspended solids concentrations, energy cost and handling time to excrete the inorganic matter increases resulting in decreasing filtration rates. Starting from very low suspended solids concentrations, the filtration rate increases. Widdows et al., (1979) found that pseudofaeces production started already at 5 mg/l. Depending on the size of the mussel, the filtration rate reaches a maximum at 100 to 200 mg/l and then drops to zero at 250 to about 380 mg/l according to Widdows et al., (1979). Prins and Smaal (1989) found a decrease in the rate of food uptake already at lower concentrations, namely at concentrations of 50 mg/l. This difference in research results may be caused by the fact that the mussels used in Widdows' experiments originated from Lynher (an estuary in England) and may have been morphologically better adapted to high suspended solids concentrations compared to the mussels used by Prins and Smaal (1989) that originated from the Oosterschelde estuary. Like Widdows et al. (1979), Prins and Smaal (1989) also found a loss in mussel weight at suspended solids concentrations above 200 mg/l.

In the Wadden Sea, under the influence of the North Sea on the one hand and freshwater discharge on the other, gradients in salinity are present (Baptist et al., 2019; Bouma et al., 2005; de Kok, 2000). At the tidal inlets, which are under the influence of North Sea water, salinity levels are high and there is little fluctuation in salinity. Average salinities are lower in areas influenced by river mouths (estuaries) and by the discharge of fresh surface water into the Wadden Sea, for example at the Afsluitdijk in the Netherlands. Moreover, in these areas there are greater fluctuations in salinity as a result of variations in river discharge and discharge flow (de Kok, 2002). Mussels (*Mytilus edulis*) that occur in the Wadden Sea are adapted to survive at fluctuating salinity levels. Nagarajan et al., (2006) found that mussels can survive periods of reduced salinity by closing their valves and switching to anaerobic respiration. Low salinity levels may indirectly have a positive effect on mussels when, as a result of reduced salinities, the predation pressure exerted by starfish on subtidal mussels decreases according to Agüera Garcia (2015). Troost et al., (2015) found that in areas above the Afsluitdijk, where salinities are lower, subtidal mussel beds are most frequently found.

Although mussels have a high tolerance for variations in salinity, a reduction in salinity does affect mussel morphology and filtration and growth rates. Riisgard et al. (2013) investigated the adaptive capacity of mussels to reduced salinities using mussels originating from both the Baltic Sea and the Great Belt (Denmark). The filtration rate of mussels from the Great Belt, which had been acclimated to salinities of 17 PSU, was found to be significantly reduced when exposed to salinities lower than 10 PSU. The filtration rates of mussels from the Baltic Sea (acclimated to 20 SPU) showed only a transient decrease in filtration capacity when exposed to 6 PSU. Westerbom et al. (2001) and others investigated the influence of a salt gradient present in the Baltic Sea on the morphology and growth rate of mussels. They found that with decreasing salinity, both shell length and biomass decreased significantly and concluded that salinity was the most important factor determining the size structure and growth of the mussels in that area.

2.2 Water quality

The concentrations of, chlorophyll-*a* (as a measure for the availability of algae), total suspended solids and salinity were obtained from the MWTL (Monitoring Waterstaatkundige Toestand des Lands) program. By dividing the concentration of total suspended solids by the concentration of chlorophyll-*a*, ratios were calculated. High ratios indicate unfavourable conditions for mussels because there is then a relatively large amount of (inorganic) suspended solids present compared to the amount of algae. When the ratio between the suspended solids and chlorophyll-*a* concentration becomes very unfavourable (e.g. when there is a lot of suspended solids and little chlorophyll-*a* present), it will no longer be profitable to filter water. At that moment the mussel stops filtering the water and starts using its fat reserves.

Data from MWTL monitoring stations located in the Ems-Dollard estuary are compared with data from MWTL monitoring stations located in the other basin areas of the Wadden Sea for reference. For most stations data for the period 1988 to 2016 are used. By looking at data of different MWTL monitoring stations located within the Estuary, gradients in environmental conditions were investigated. For this, MWTL measurement data collected from 1988 to 1995 were used. In Table 1 an overview is given of the MWTL monitor stations and measurement periods used in the analysis. Figure 1 shows the locations of these MWTL monitoring stations in the Dutch Wadden Sea. In most cases, three measurements per month or more were available for each station. This study took place at the end of 2017.

Table 1. MWTL monitor stations in the different tidal basins and the period from which data of the stations were used in the analysis.

MWTL station	**Tidal basin**	**Measurement period**	
		from	*until*
Bocht van Watum	Ems-Dollard	1988	2016
Dantziggat	Borndiep	1988	2016
Doove Balg west	Marsdiep	1988	2016
Vliestroom	Vlie	1988	2016
Zoutkamperlaag	Zoutkamperlaag	1988	2009
Zuid Oost Lauwers oost	Lauwers	1988	2009
Bocht van Watum	Ems-Dollard (for gradient)	1988	1995
Groote Gat noord	Ems-Dollard (for gradient)	1988	1995
Huibertgat oost	Ems-Dollard (for gradient)	1988	1995
Oostfriese gaatje	Ems-Dollard (for gradient)	1988	1995
Zeehavenkanaaldijk monding	Ems-Dollard (for gradient)	1988	1995

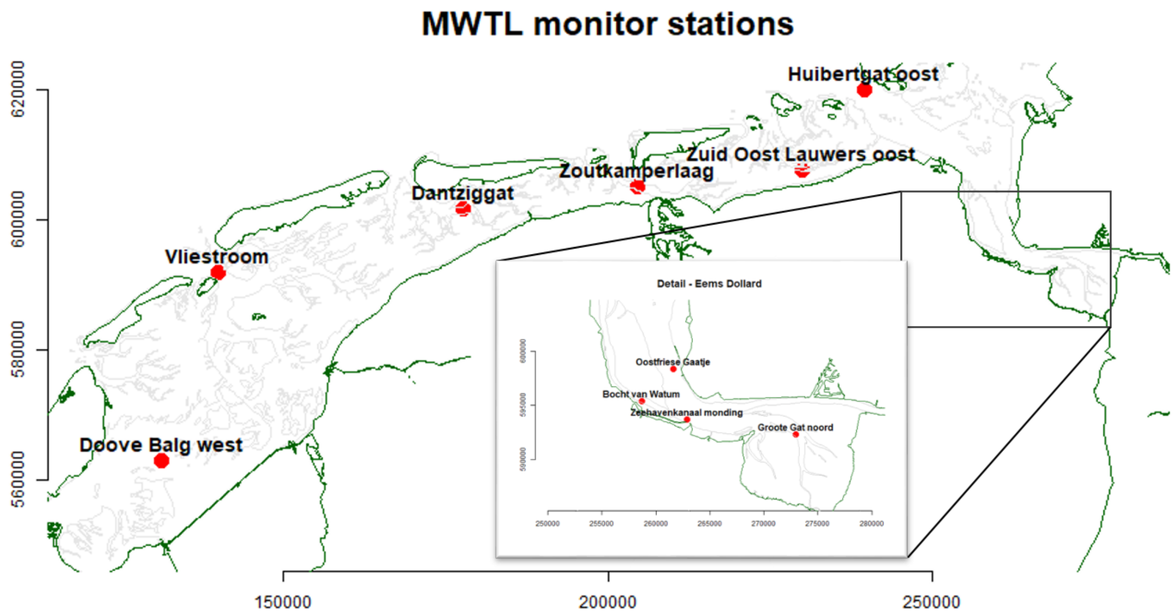

Figure 1. Map of the Wadden Sea showing the MWTL monitoring stations used in the analysis.

2.2.1 Comparison between tidal basins

Figure 2 shows the concentrations of suspended solids and chlorophyll-*a* in the water column as measured in the MWTL measuring program and for the stations located in the various tidal basins of the Wadden Sea, see also Table 1. Also the ratio between both parameters is calculated and shown in Figure 2. Low ratio values are an indication of more favourable food conditions for mussels than high values.

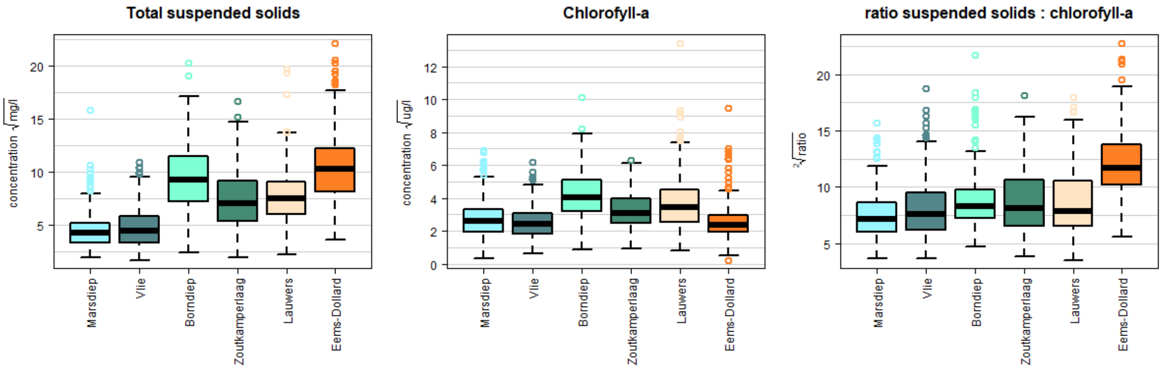

Figure 2. Boxplots of square root transformed concentration of suspended solids (left), square root transformed concentration of chlorophyll-*a* (middle) and double square root transformed ratio of suspended solids : chlorophyll-*a* (right) as measured in the MWTL monitoring program for stations located in the Ems-Dollard and for stations located in the other tidal basins in the Dutch part of the Wadden Sea in the period 1988-2016.

Figure 2 shows that compared to the other tidal basins the suspended solids concentrations are relatively high in the Ems-Dollard, where the highest maximum concentrations were measured. Concentrations are a bit lower in the tidal basins Borndiep, Lauwers and Zoutkamperlaag, followed by Marsdiep and Vlie. As described in the profile H1130, high turbidity is an abiotic characteristic of the habitat type estuary where sea and river currents meet (H1130 Estuaries - version 2016). The relatively high suspended solids concentrations found for the Ems-Dollard is therefore not unexpected from an ecological point of view.

The chlorophyll-*a* concentrations measured at the MWTL monitoring station in the Ems-Dollard are comparable to those in Marsdiep and Vlie, and lower than those in Borndiep and Zoutkamperlaag, see Figure 2.

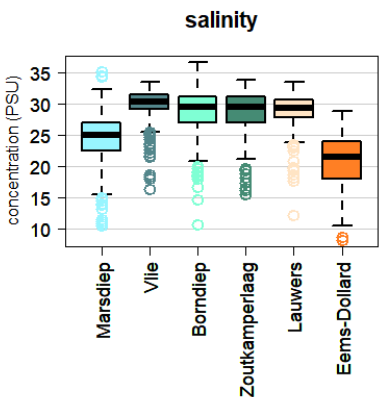

Figure 3. Boxplots of the variation in salinity as measured in the MWTL measurement program for stations located in the Ems-Dollard and at stations located in the other tidal basins in the Dutch part of the Wadden Sea in the period 1988-2016.

The salinity in the Ems-Dollard estuary is somewhat lower due to the influence of fresh water discharges from the Ems and other rivers and channels. This can be seen in Figure 3, where the salinity in the estuary at the monitor station Bocht van Watum, located about halfway between the mouth of the Ems and the tidal inlet, is compared with the salinity measured at the MWTL monitor stations located in the other tidal basins of the Wadden Sea. This is another natural phenomenon for estuaries that are influenced by rivers.

2.2.2 Trends over time

Figure 4 to Figure 6 show the monthly average concentrations (grey line) and the trend (black line) in the suspended solids, chlorophyll-*a* and the ratio between both parameters for the MWTL monitor station Bocht van Watum and for the period 1988-2016. The trend was calculated using a moving window of one year. On average a suspended solids concentration of 125 mg/l (± 69 mg/l) was measured during this period, see red- and red dashed lines in Figure 4. It can be seen that concentrations above 200 mg/l were measured regularly (grey line in Figure 4). There are also longer periods with elevated concentrations of suspended solids (see grey and black line). In the years 1990, 1998, 1999, 2008, 2010 and 2011 the suspended solids concentration was somewhat higher than the long-term average, see Figure 4.

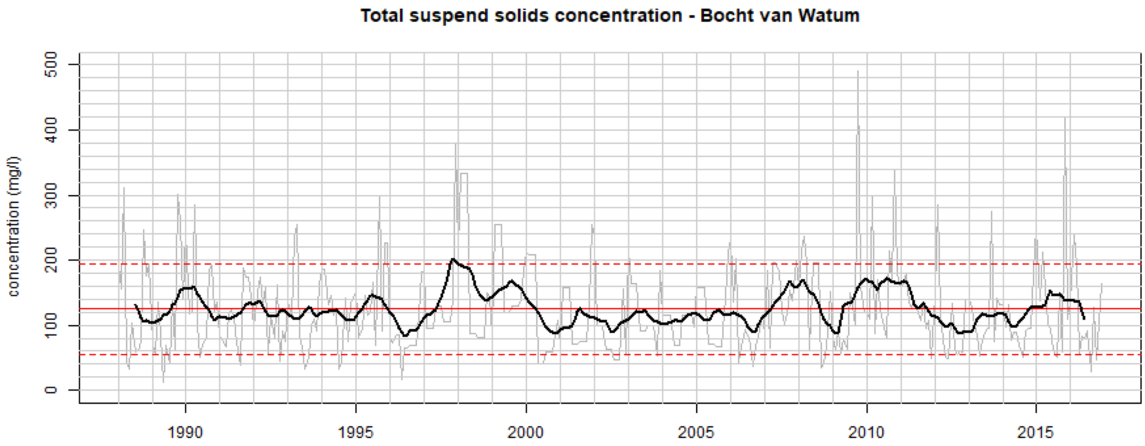

Figure 4. The suspended solid concentration as measured in MWTL monitoring station Bocht van Watum for the period 1988 to 2016. The grey line shows the monthly average value, the black line shows the moving average with a moving window of one year. The solid red line represents the average concentration over the measurement period and the red dotted lines the variation (± 1 standard deviation).

The chlorophyll-*a* concentration measured at the MWTL monitor station Bocht van Watum averages 7.8 µg/l (± 6.8 µg/l), see Figure 5. In the years 1991, 1994 and 2000 the concentrations were somewhat higher than the long-term average. The chlorophyll-*a* concentration in the periods 1988-1990 and 2010-2016 is lower than the long-term average, see Figure 5.

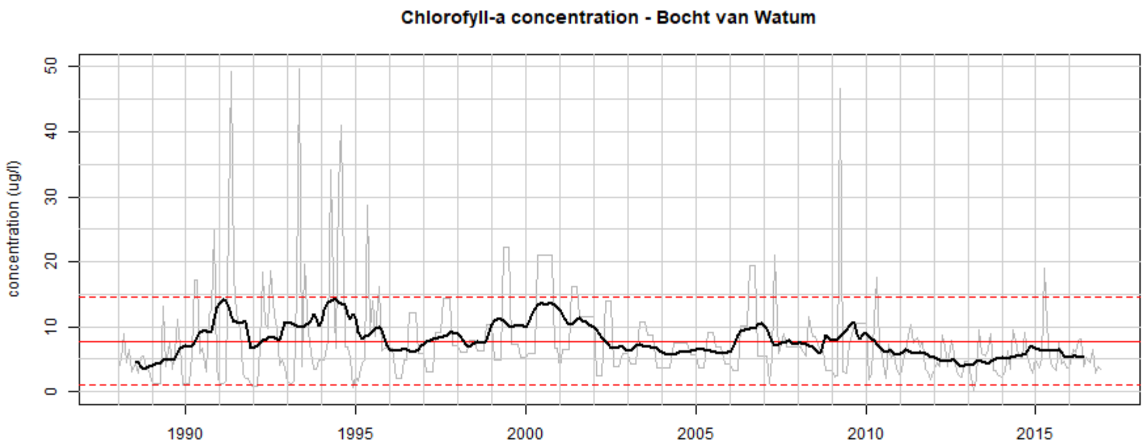

Figure 5. The chlorophyll-*a* concentration as measured at the MWTL monitor station Bocht van Watum for the period 1988 to 2016. The grey line shows the monthly average value, the black line shows the moving average with a moving window of one year. The solid red line represents the average concentration over the measurement period and the red dotted lines the variation (± 1 standard deviation).

The (double root transformed) ratio between the suspended solids and chlorophyll-*a* concentration for MWTL monitor station Bocht van Watum was on average 11.9 (± 3.0) over the measurement period, see Figure 6. In the periods 1988-1995 and 2010-2016, ratios were regularly above the long-term average. In the last decades, longer periods of low ratios have been observed twice. High ratio values are found from mid-2010 to late 2010 and from mid-2012 to mid-2013.

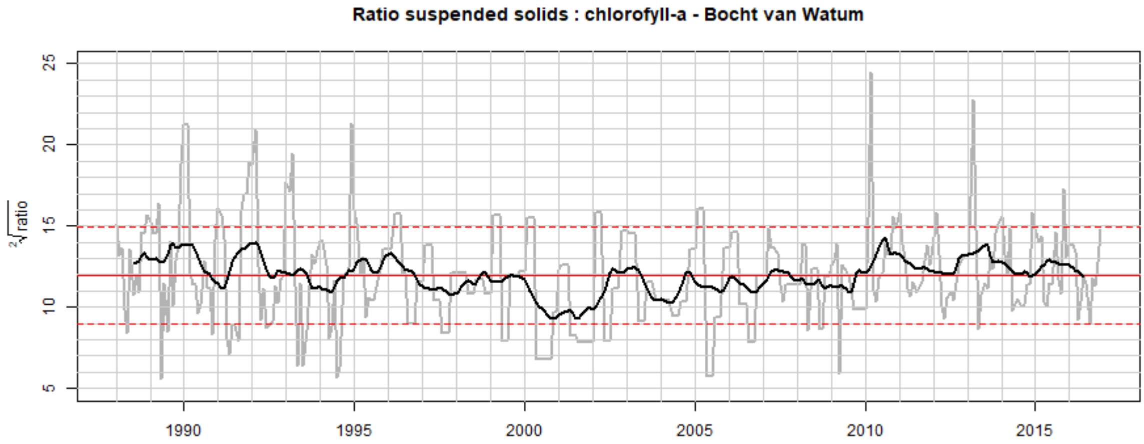

Figure 6. The double root transformed ratio between suspended solids and chlorophyll-*a* concentrations as measured in MWTL monitoring station Bocht van Watum for the period 1988 to 2016. The grey line shows the monthly average value, the black line shows the moving average with a moving window of one year. The solid red line represents the (double root transformed) average ratio over the measurement period and the red dotted lines the variation (± 1 standard deviation).

2.2.3 Gradients

In the early years of the MWTL measurement program, more monitoring stations were located in the Ems-Dollard than in more recent years. In order to see to what extent there are gradients in salinity, total suspended solids and chlorophyll-*a* concentrations in the Ems-Dollard, the data from the years 1988-1995 are selected. In this period 6 monitoring stations, on a transect from the tidal inlet to the mouth of the Ems river, were included in the program, see Figure 1 and Table 1.

Figure 7 shows boxplot graphs of the measured values for total suspended solid concentration, the chlorophyll-*a* concentration and the resulting ratios, as well as the measured salinity. The X-axis shows the MWTL monitor stations. The most north-eastern located sampling station, Huibertgat oost, is shown on the extreme left of the x-axis and the most south-eastern located sampling station, Groote Gat noord, on the extreme right.

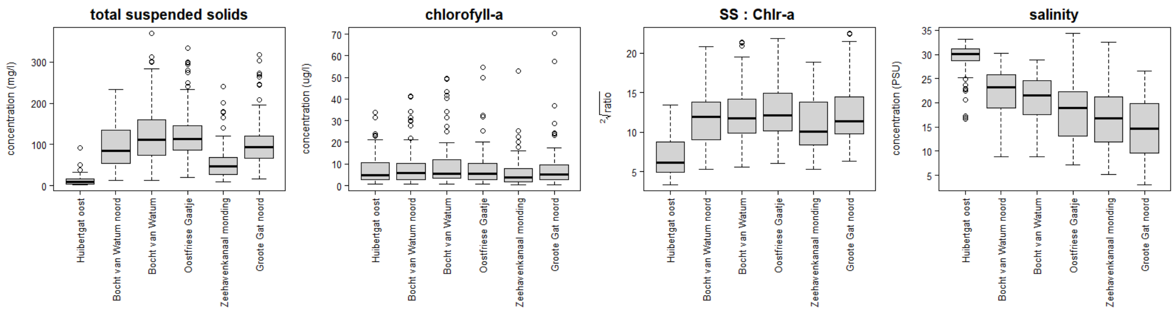

Figure 7. Boxplots showing the concentration of total suspended solids (far left), -chlorophyll-*a* (left from the middle) and double root transformed ratio of suspended solids and chlorophyll-*a* (right from the middle) and salinity (far right) as measured in the MWTL monitoring program for sampling points located in the Ems-Dollard in the period 1988-1995.

Figure 7 shows that in the north eastern part of the estuary (Huibertgat), near the tidal inlet, the concentration of suspended solids is low compared to more inland located stations. At this monitor station, an average suspended solids concentration of 12.4 mg/l was measured with a maximum of 92 mg/l. The relatively low concentrations measured here are the consequence of the inflow of clear North Sea water into the estuary. The concentration of suspended solids measured at locations further into the estuary, Bocht van Watum noord, Bocht van Watum, Oostfriese Gaatje and Groote Gat noord, are among the highest of the stations studied. The average concentrations are higher than the maximum concentration in Huibertgat oost. The difference between these locations is small. The average measured concentration varied from 97 mg/l for Bocht van Watum noord to 128 mg/l for

Oostfriese Gaatje. The measuring location Zeehavenkanaal monding shows an intermediate position with higher values than in Huibertgat oost and lower values than the other measuring locations. This deviation from nearby stations can be explained by the influence of outflow of fresh water originated from the Zeehavenkanaal (Delfzijl).

The average chlorophyll-*a* concentrations measured for the monitor stations during the period 1988 to 1995 do not show large differences, see Figure 7. However, the maxima are somewhat higher in the southern located stations. For the stations and period analysed here, an average chlorophyll-*a* concentration of 8 µg/l was measured.

Differences in (double root transformed) ratios of the suspended solids and chlorophyll-*a* concentrations between the monitor stations are therefore mainly determined by differences in suspended solids concentration rather than from chlorophyll-*a* concentrations. Based on these measurements, the conditions for the mussels in Huibertgat oost are the best, with an average (double root transformed) ratio of 6.9. This means that at this location there is relative small amount of suspended solids to be processed to obtain food. Near Bocht van Watum the ratios are higher (11.7) resulting in a deterioration of the environmental conditions for mussels as more suspended solids need to be processed by the mussels. Ratios do not change much at monitor stations located south of Bocht van Watum, that on average fluctuate around 11-12. Apparently, environmental conditions do not deteriorate further south of Bocht van Watum.

In Huibertgat oost the salinity is close to that of seawater (average 29 PSU). At the station Bocht van Watum, salinity has decreased by 7 PSU with an average of 22 PSU. It can clearly be seen that salinity decreases further moving towards the mouth of the Ems rivers, see Figure 7. Near Groote Gat noord an average salinity of 15 PSU has been found, while levels <10 PSU are regularly found.

2.2.4 Frequency of elevated suspended solids concentrations

In order to get an impression how often the suspended solids concentration in the estuary exceeds levels at which effects on mussels cannot be ruled out, measurements of the MWTL monitor stations Bocht van Watum (period 1988-2016) and Oostfriese Gaatje (period 1988-1995) were analysed. The share of the total measurements within each year and within six predefined concentration intervals is calculated, see Figure 8 and Figure 9.

Figure 8 shows that about half of the measurements (53%) at the monitor station Bocht van Watum are above a suspended solids concentration of 100 mg/l. A quarter of the measurements (25%) are above 150 mg/l. In 11% of the measurements the suspended solids concentration was even higher, namely above 200 mg/l and in about 6% of the measurements the concentration exceeds 250 mg/l.

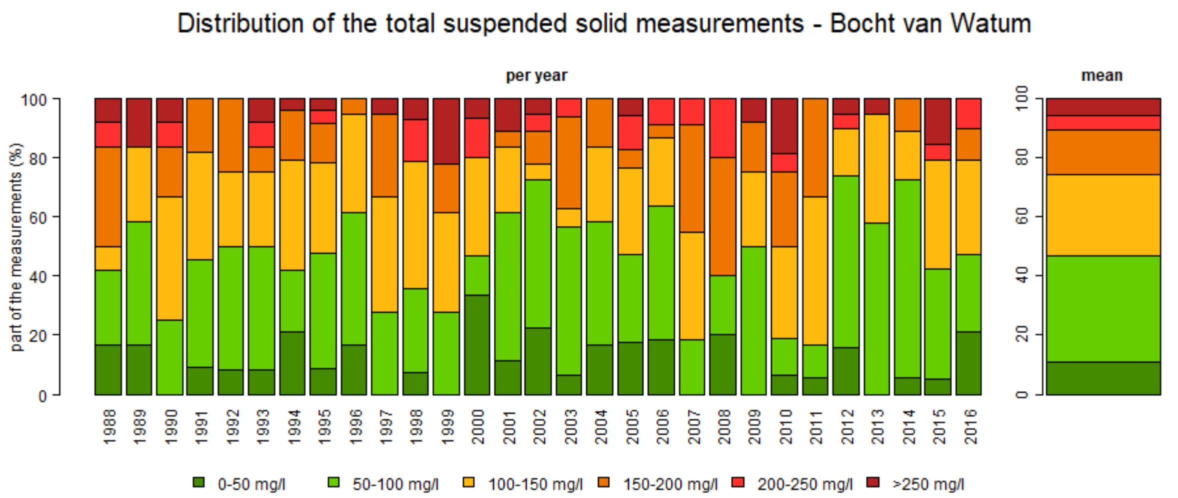

Figure 8. Bardiagram showing the distribution of the total suspended solids concentration measured at MWTL station Bocht van Watum over the period 1988 to 2016 divided over different concentration intervals. In the left panel the distribution of the measurements per year is shown and in the right panel the average distribution of the measurements over the period 1988-2016.

Figure 9 shows that on average in 68% of the measurements taken in Oostfriese Gaatje the suspended solids concentration was above a level of 100 mg/l and 22% of the measurements were above a level of 150 mg/l. In 11% of the measurements the suspended solids concentration was above 200 mg/l and in 6% above 250 mg/l. Similar to Bocht van Watum, the distribution of measurements exceeding certain levels varies considerably from year to year.

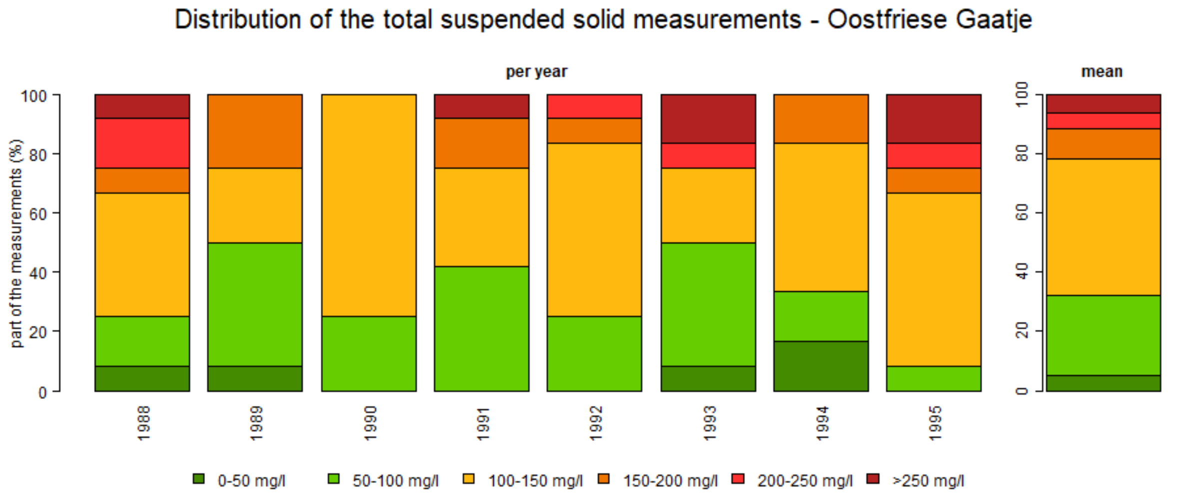

Figure 9. Bardiagram of the suspended solids concentration measured at the MWTL station Oostfriese Gaatje during the period 1988 through 1995, divided over different concentration intervals. In the left panel the distribution of the measurements per year is shown and in the right panel the average distribution of the measurements during the period 1988-1995.

For both the monitor stations Bocht van Watum and Oostfriese Gaatje, total suspended solids concentrations above 200 mg/l were found regularly. Negative effects on mussels have been demonstrated for such concentration levels, and during long-term exposure to such levels negative effect on mussels can be expected.

2.3 Occurrence of intertidal mussel beds

Since 1995 WMR has been estimating the total area of intertidal mussel beds, oyster reefs and mixed mussel and oyster beds in the Dutch Wadden Sea on behalf of the Ministry of Agriculture, Nature and Food Quality (LNV), (Troost et al., 2021). For definitions and methods we refer to Troost et al., (2021). In addition data from a study conducted by Dankers et al., (2005) were used to place the current acreage of mussel beds in a historical perspective. This data does not cover the tidal flats bordering the Germain mainland.

Since the beginning of the WOT survey (1995) intertidal mussel beds have been frequently found in the estuary (Troost et al., 2015). In 2004, Pacific oysters (further in the text as oysters) were found for the first time in the estuary, which probably fell into the area in 2003 as oyster seed (Dankers et al., 2005). In addition to pure mussel beds and pure oyster reefs, beds consisting of a mixture of both species have been found in recent decades. In Figure 10 and Figure 11 the development of the beds and reefs from 2006 to 2017 is shown. Most recent survey results can be found on the website https://shiny.wur.nl/Schelpdiermonitor_Banken/.

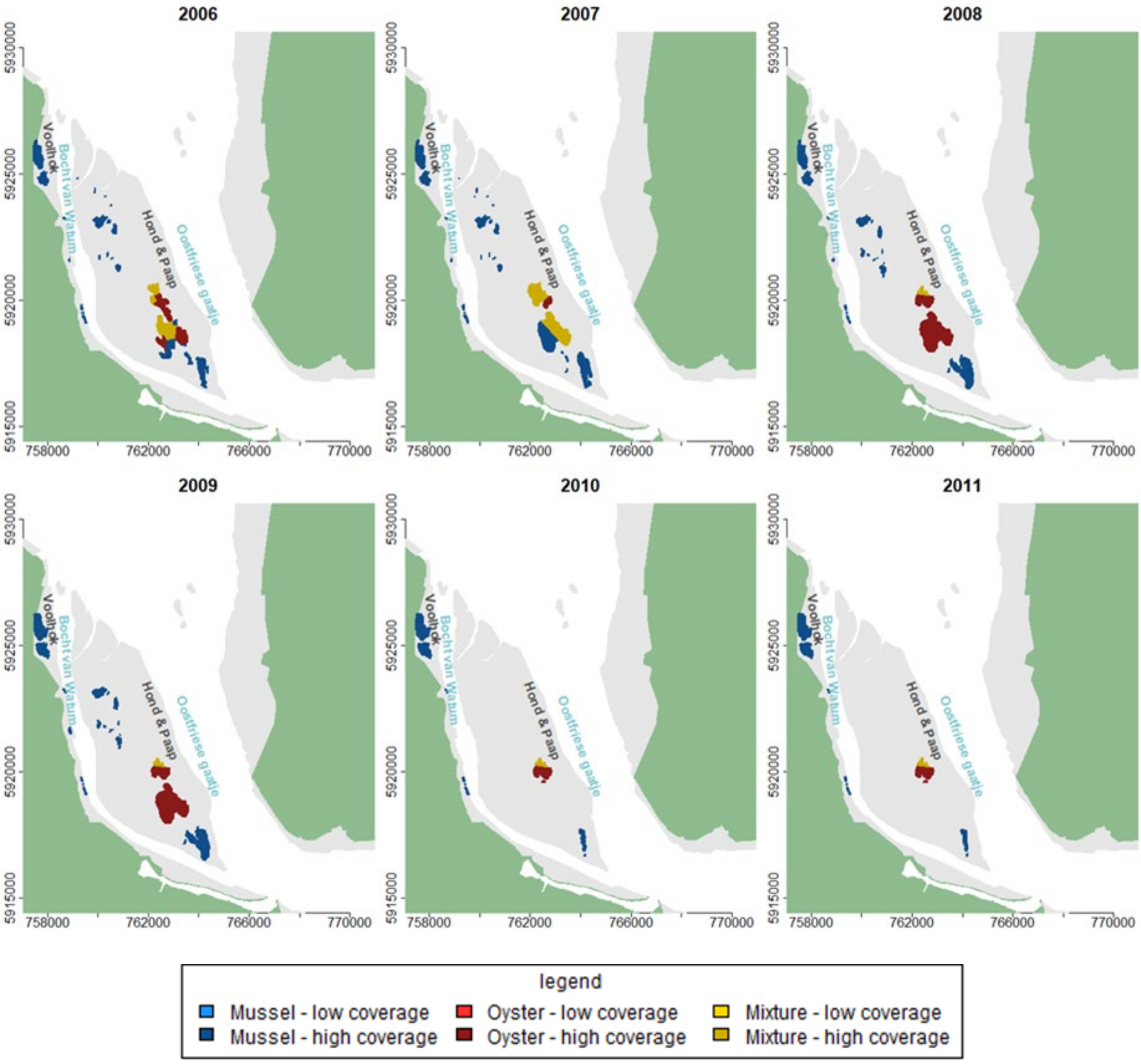

Figure 10. Presence of intertidal mussel beds, oyster reefs and beds consisting of mussels and oysters (mixture) in the Ems-Dollard Estuary for the period 2006 to 2011. Data from WOT fishery program carried out by WMR.

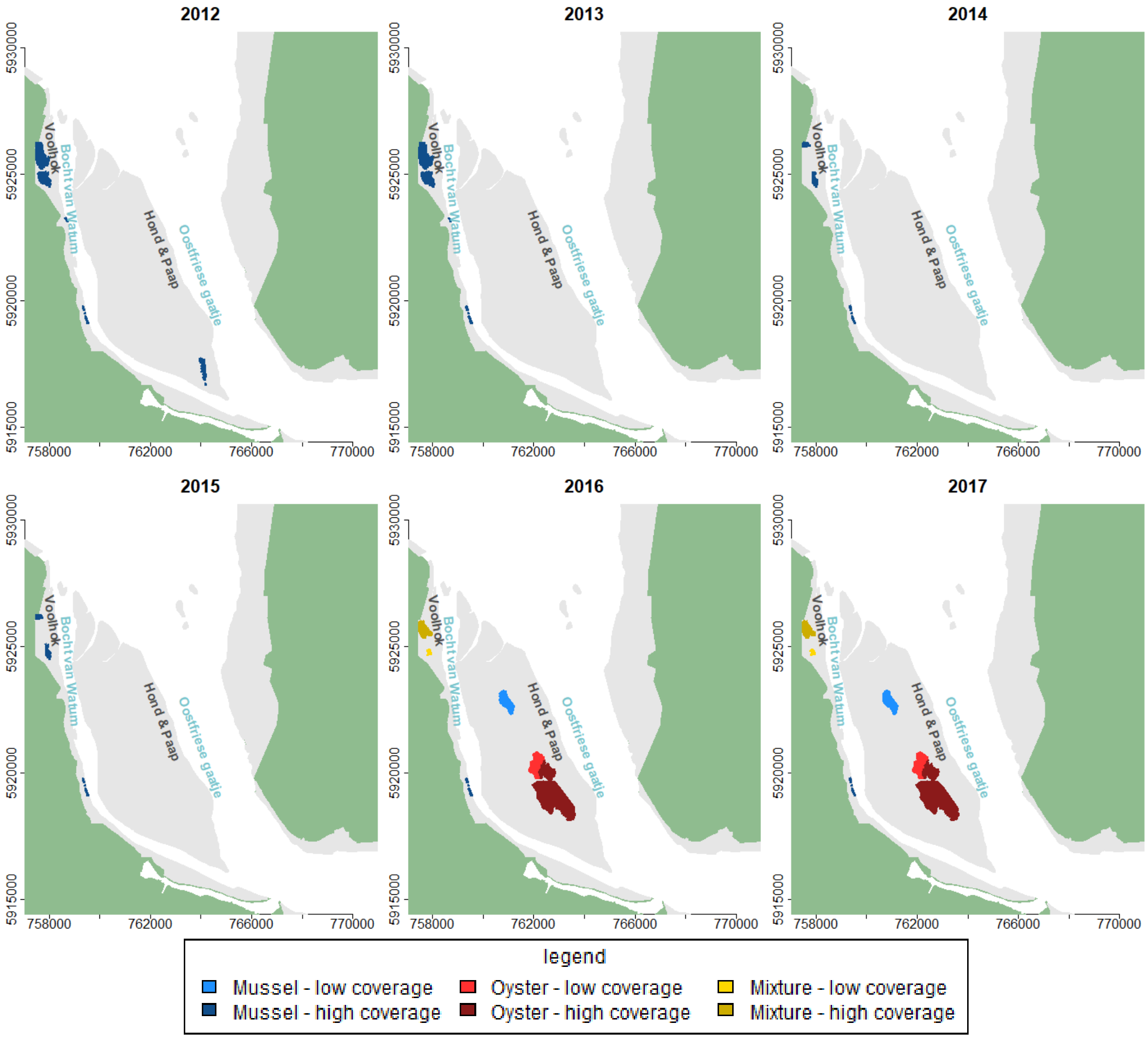

Figure 11. Presence of intertidal mussel beds, oyster reefs and beds consisting of mussels and oysters (mixture) in the Ems-Dollard Estuary for the period 2012 to 2017. Data from WOT fishery program carried out by WMR.

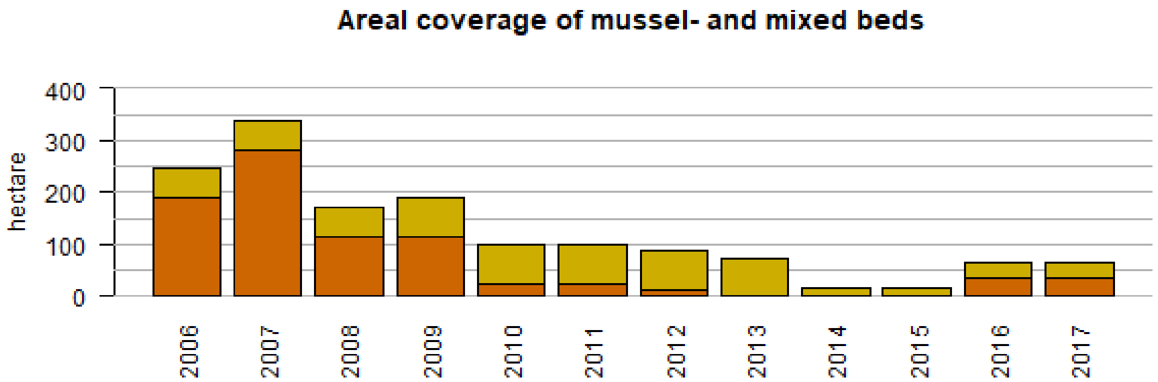

Figure 12. Bardiagram showing the acreage of mussel- and mixed mussel/oyster beds (in the Ems-Dollard estuary for the period 2006 to 2017. The areal coverage of these beds located on the Hond and Paap tidal flat is shown in orange and those found adjacent to the mainland of the Netherlands in yellow. The area was not visited in 2016 as part of this program. By estimating the age of beds encountered in 2017, the acreage in 2016 was reconstructed. Pure oyster reefs are not included in this diagram. Data from WOT fishery program carried out by WMR.

With the disappearance of mussel- and mixed mussel/oyster beds on the Hond and Paap tidal flat in 2010, the tidal flats adjacent to the Dutch shore became more important for the total bed area in the estuary, see Figure 12. In the following years, the remaining beds on the Hond and Paap tidal flat disappeared altogether (orange bars in Figure 12). In 2013, 2014, and 2015, no mussel- or mixed beds were found at all on the Hond and Paap tidal flat. The beds located on the tidal flats adjacent to the mainland of the Netherlands managed to persist but the area of these beds also decreased in 2013. In 2013 and 2014 a minimum was reached over the measurement period studied here. At that time, an area of 17 hectares was present in the estuary consisting entirely of beds located on tidal flats adjacent to the mainland.

In 2017, a new (pure) mussel bed was found on the Hond and Paap tidal flat, Figure 11. By looking at the mussel length, it was determined that it must have fallen as seed in 2016 (the area was not visited in 2016 as part of the WOT program). As a result, the bed area of mussel and mixed mussel-Japanese oyster beds increased somewhat again in 2016.

Figure 13. The mussel bank newly formed in 2016 on the Hond and Paap tidal flat and as encountered in 2017 in this research. Left photo the northern part of the bed, right photo a close up.

Pure oyster reefs were only found in the southern areas of the Hond and Paap tidal flat, see Figure 10 and Figure 11. Due to mortality these reefs disappeared for a large part in 2010 and in its entirety in 2012. Due to settlement of oyster larvae in the remaining structures of the (dead) oyster reef, pure oyster reefs were found again in the area in 2017 (personal communication Karin Troost). After reconstruction, it was determined that the oysters found in 2017 must have settled as larvae in 2016.

2.4 Survival test

It is not known whether mussels from elsewhere and from different origin are able to adapt to the conditions in the Ems-Dollard estuary. A field experiment was conducted to test this using mussels originating from a subtidal- and intertidal bed and from seed mussel collectors (SMC) were used in the experiment. Water quality measurements were conducted to relate survival and growth to environmental conditions. This research took place in late 2017 and continued through early 2018.

2.4.1 Methods

The survival and growth of mussels was tested at three different locations and at three different emersion times. Test were conducted in triplicate. The intertidal mussels were collected from the Roode Hoofd (below Schiermonnikoog), the subtidal mussels came from a bed located at Boontjes (south of Harlingen) and the SMC mussels were obtained from Neeltje Jans (Oosterschelde). The mussels were examined in the laboratory if they were alive and their shell lengths were measured. Mussels were placed in baskets and sampled at regular time intervals to estimate growth and survival.

The test sites were located roughly in the centre of the tidal flat and at three locations along a line going roughly from the north-west to the south-east across the tidal flat, see Figure 14. At each test site three poles with baskets containing mussels were placed at a distance of 100 meter apart from each other. By attaching the baskets at a height of -33 cm NAP, -9 cm NAP and +13 cm NAP to the poles, respectively 35, 40 and 45 percent emersion times were obtained. To make four sampling events possible four batches of 30 individuals of each type of origin were made and placed at each pole and height. This resulted in a total of nine poles with twelve baskets containing mussels on each pole (four baskets at each height) with in each basket 90 mussels (30 per type of origin).

Due to differences in the elevation of the tidal flat going from the north to the south, the distance between the baskets and the tidal flat differed between the three test location. At the most northern test location the (lowest) baskets were located a few centimetres above the sediment surface while in the most southern located test site the distance with the lowest basket containing mussels was several decimetres.

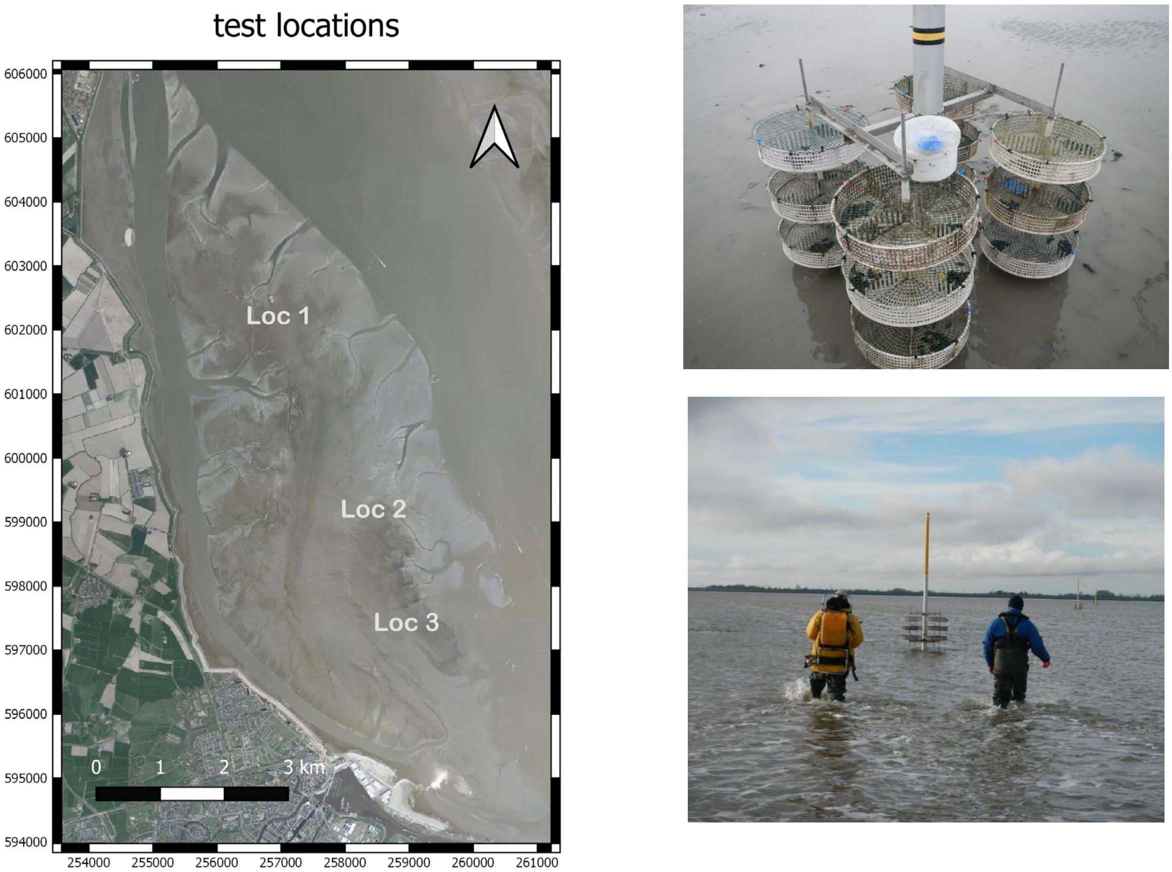

Figure 14. In the panel on the left a map showing the three test sites on the Hond Paap tidal flat. The photos shown in the right panels show the baskets containing mussels that are attached to the poles.

Due to differences in age and mussel population structure shell lengths differed between the mussels from different origin types at the start of the experiment. The intertidal mussel bed was formed in 2016 and was one year old when used for this experiment. Mussels with a shell length between 30 and 40 mm were selected for the experiment for this group (mean shell length was 36.0 ± 2.9 mm). The subtidal mussels came from a bed established in 2017. Mussels with a shell length ranging from 25 to 35 mm were selected (mean shell length of 28.7 ± 2.8 mm). The SMC mussels with a shell length of 25 to 35 mm were selected (mean shell length of 30.8 ± 2.7 mm). These measurements (T0) were conducted on 27 and 28 of November 2017 and on 29 and 30 of November in the same year the mussels were placed at the tidal flat.

Mussel sampling events and ice formation
On January 9 2018, the first monitoring took place (T1 measurement). At the end of the winter of 2017-2018 (late February early March 2018), a short but severe frost period occurred. February 28 was ranked as the coldest February day for 118 years. As a result of this frost period, large parts of the Wadden Sea froze and due to drift ice, the baskets and poles suffered damage to such extent that

sampling of mussels was no longer possible. An attempt was made to carry out a final sampling event before the frost period but this proved to be impossible. As a consequence the experiment was stopped prematurely and the T2, T3 and T4 samples were not taken. As a consequence, the experiment could not continue into the next growing season (spring).

Because the experiment was terminated prematurely and only took place during the winter months, only very limited growth could occur. The difference in average lengths of the 30 individuals between the measuring moments T0 and T1 were so small as a results of this that an analysis of the average shell lengths between groups was no longer meaningful. An attempt was made to determine individual growth instead. The shell lengths of individual mussels at T1 were linked to a length measured at T0 by putting the lengths in a sequence from small to large. In this way shell lengths of a large proportion of the mussels could be linked and used in the analysis. As an example of this procedure, see Figure 15 where mussel lengths in T1 (red dots) are linked to a length in T0 (blue circles). When the number of mussels recovered at T1 was lower than for T0, it was considered at which position in the mussel length sequence to skip in order to get the smallest possible deviation for the whole set of lengths.

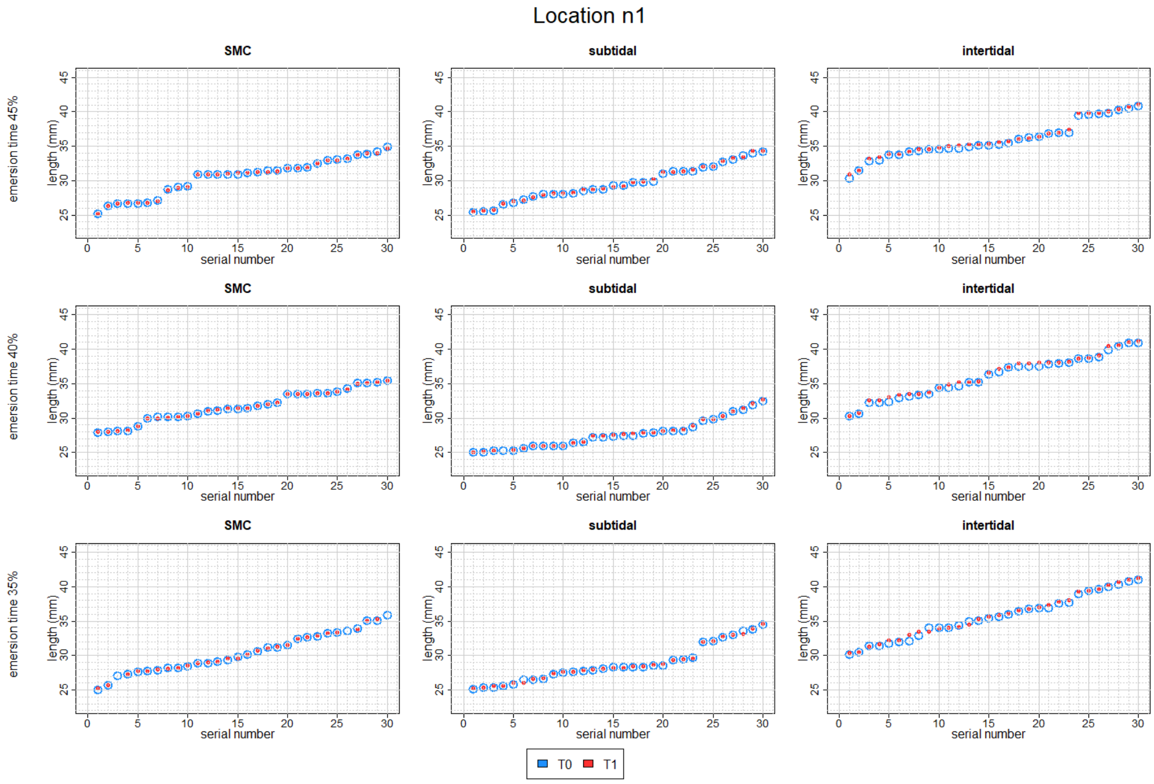

Figure 15. Deducing the shell lengths of mussels sampled at T1 to shell lengths at T0. This example shows mussels attached to pole 1 in site 1 (located in the northern area of the Hond and Pap tidal flat).

Water quality

In situ water measurements were conducted and water samples were taken for laboratory analysis to assess fluctuations of different water quality parameter during the survival experiment. In the field the transparency, the water temperature, the oxygen level and the salinity was determined. Water samples were collected to measure the concentration of total suspended solids, the fraction organic matter of the total suspended solids and the chlorophyll-*a* concentration in the laboratory. The water quality measurements were done at all three locations (Location numbers 1 through 3 in Figure 14) and two hours before- and after high tide. Water quality measurements were conducted on February 2nd (T1 measurement), March 3rd (T2 measurement) and May 3rd (T3 measurement) 2018.

The transparency has been determined using a Secchi disk. The distance in centimetres at which the distinction between the black and white surfaces could no longer be detected was noted. This measurement has been carried out in duplicate. The water temperature, salinity and oxygen content were measured with an HQ40D Hach meter in combination with probes for measuring the salinity

(PSU) and oxygen content (mg/l) in combination with the water temperature (°C). Readings were noted on a field form.

Water samples of 0.5 litres were taken to determine the chlorophyll-*a* and suspended solid concentrations. The water samples were taken from a depth of approximately 1 meter below sea-surface level using a stick, see Figure 16. Immediately after sampling the samples were stored in dark and cool bottles for transportation. The determination of the suspended solid concentrations was carried out at the SaM laboratory. Water samples were filtered and the filtrate was weighed on an analytical scale after drying (at 105 ^{0}C) and ashing (at 450 ^{0}C). Chlorophyll-*a* measurements were carried out the next day after the samples were taken in the laboratory of WMR. For this purpose, the BBE Moldaenke AlgeaLabAnalyser 1 Hz cuvette fluorometer was used and the procedure described in WMR protocol E_4_046 was followed. In short, the chlorophyll-*a* content in a water sample is determined by placing water in a cuvette after which the fluorescence is measured as a measure for the presence of chlorophyll-*a*.

Figure 16. Taking a water sample by Achim Wehrmann.

2.4.2 Survival rates

Because individual mussels appeared to be able to move between the compartments of the baskets, mussels of different origins within the same basket were mixed up. For determination of the survival rates all mussels present in the baskets were examined as one group, regardless of the mussels origin. Table 2 shows the percentage of mussels that were found alive at the T1 sampling event. It can be seen that most mussels survived the first two months (early December 2017 to early February 2018) well. The survival rate ranged from 94.4% to 100% and averaged 98.0%.

Differences in survival rates between location and emersion time were investigated using generalize linear models with binominal distribution and log link function. Models with location and emersion time as additive explanatory variables (m1) and with an interaction term between location and emersion time (m2) were constructed. The predictive ability of these models was compared using AIC criteria. The software program R (R Core Team, 2020) was used for the analysis and the basic packages that come with it. The analysis shows that model m1 predicts survival the best. By using this model it appears that the survival rates do not differ significantly between the various locations or the emersion time regimes.

Table 2 . Survival rates of mussels on February 2, 2018 for the different locations and emersion time regimes. In each case, the mean, minimum and maximum survival rates are shown.

Location	Emersion time 35%			Emersion time 40%			Emersion time 45%		
	gem	min	max	gem	min	max	gem	min	max
1 (north)	97.0	95.6	98.9	99.6	98.9	100.0	98.9	97.8	100.0
2 (centre)	99.1	98.3	100.0	97.4	95.6	98.9	95.6	94.4	96.7
3 (south)	99.6	98.9	100.0	97.8	94.4	100.0	97.4	95.6	98.9
average	98.6			98.3			97.3		

2.4.3 Growth

In Figure 17 the change in shell length of individual mussels between the T0 and T1 measurements is shown. Negative growth in shell length were found frequently (data point below the horizontal red line indicating no growth). This is a result of measurement error and/or incorrect coupling of individual mussels between T0 and T1 that was done retrospective and based on shell lengths, see paragraph 2.4.1.

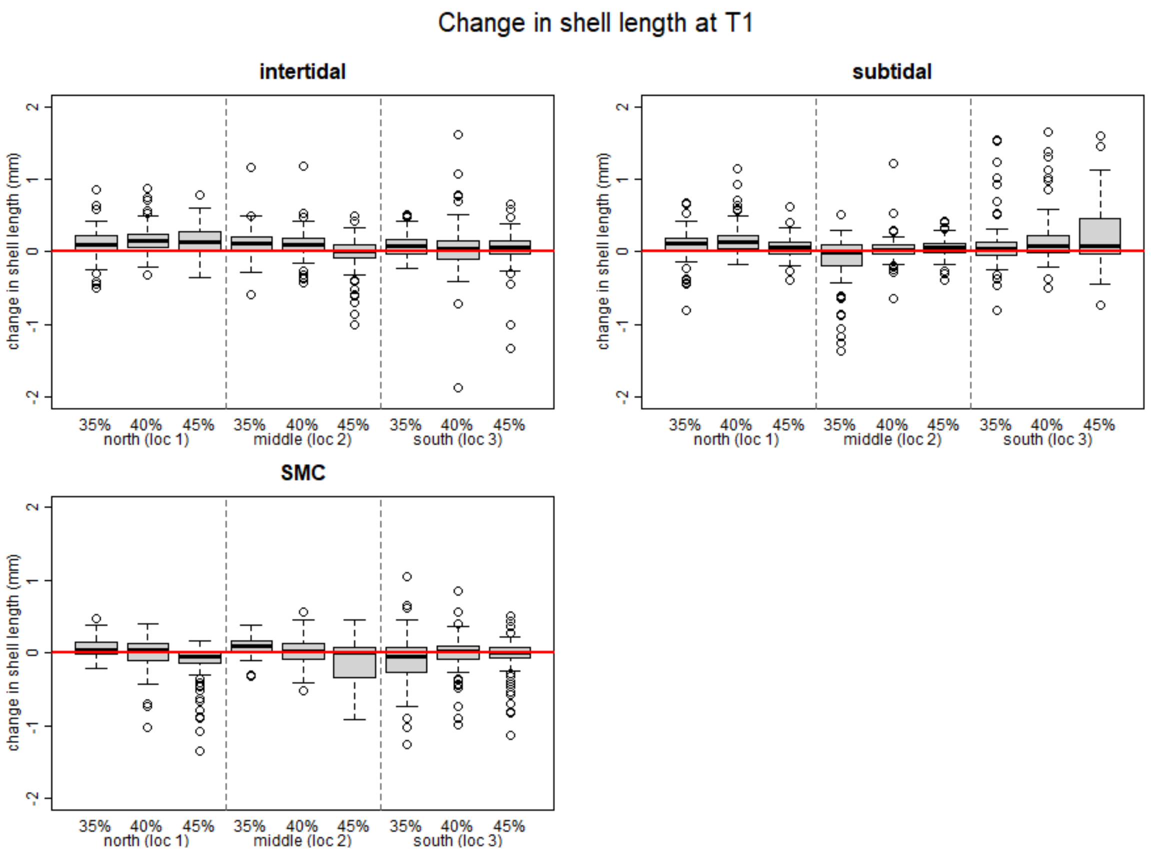

Figure 17. Boxplots showing the change in shell length at T1 compared to the T0 measurement for mussel originated from the intertidal- and subtidal beds and from a SMC installation. The horizontal red line indicates zero growth.

Differences in growth between the mussel types, location and emersion time between T0 and T1 were analysed using a linear model. Model combinations with less variables have also been tested but based on AIC criteria these model predictions appear to be less good. Analysis were performed using the R software package and model results are presented in Appendix 1 of this report.

The analysis show that there has been no increase in the length of the shells of mussels originated from the SMC installation and that there has been a small increase in shell length of mussels originated from intertidal- and subtidal beds. The increase in shell length for mussels subject to a 35% emersion time regime is a significant higher compared to a 45% emersion time regime. In the north (location 1, Figure 14) and south (location 3, Figure 14) of the tidal flat, the increase in shell length was also significant higher than in the middle (location 2, Figure 14). However, these differences are very small (<0.1 mm), see Appendix 1.

2.4.4 Water quality measurements

The influence of sampling moment (T1, T2 and T3), moment in the tide (before or after high water) and sampling location (location 1 t/m 3) on the water quality parameters has been investigated in R with linear models. In Appendix 1 of this report model results are presented. The chlorophyll-*a* concentration and oxygen content are log- and double root transformed, respectively, to meet the normality assumption.

Figure 18 shows the suspended solids concentration as measured in the water samples taken during the T1, T2 and T3 measurements. The suspended solids concentration is not dependent on the sample location, sampling time or the moment in the tide cycle, see the model results in Appendix 1. Overall, the average suspended solids concentration of 56.9 ±12.4 mg/l was measured.

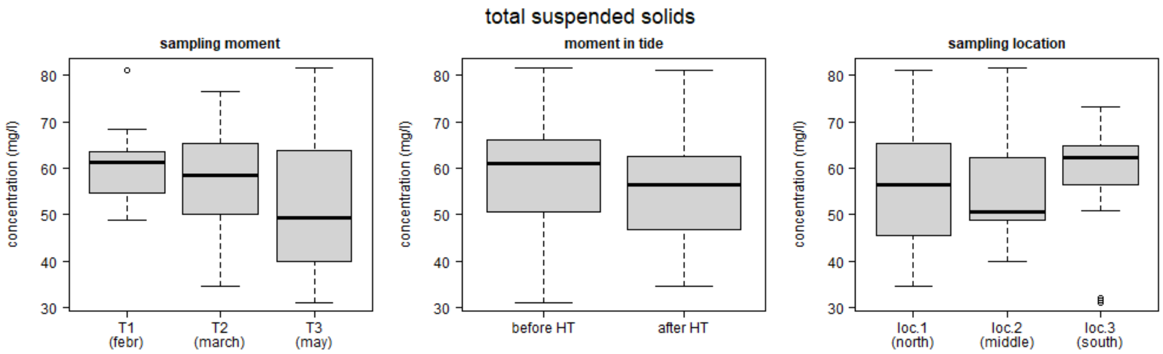

Figure 18. Boxplots with the concentration of suspended solids in the water samples per sampling moment (left figure), moment in the tide (middle) and sampling location on the Hond and Paap tidal flat (right). HT= high tide.

Figure 19 shows the fraction of organic carbon in the suspended solids as measured in the water samples. The organic matter fraction did not depend on the sampling location or the moment in the tide (before or after high tide), see Appendix 1. After the first sampling moment (T1) the proportion of organic matter in the suspended solids decreased significantly, see Figure 19 and Appendix 1. On T1 an average proportion of 68.0 ±10.8 % organic matter was measured, which decreased at the time the T2 samples were taken to an average of 46.5 ±9.9 %. The average proportion of organic matter in the suspended solids in the samples taken at the T3 was 50.6 ±13.4 % and was not significantly different compared to the T2 measurement (Tukey *post hoc* test).

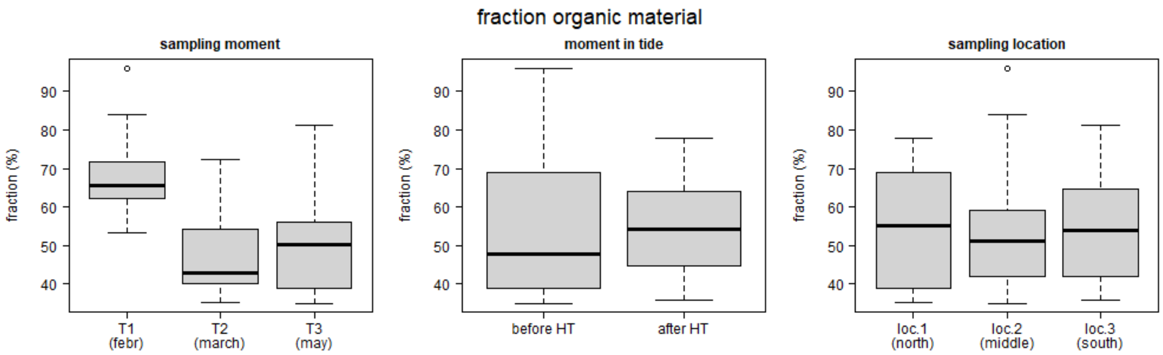

Figure 19 . Boxplots with the fraction of organic matter in the suspended solids broken down per sampling moment (left figure), moment in the tide (middle) and sampling location on the Hond and Paap tidal flat (right). HW= high tide.

The chlorophyll-*a* concentration is not depending on sampling location nor does it make a difference whether the water samples were taken before or after high tide, see Appendix 1. The chlorophyll-*a* concentration as measured in samples taken at the T2 was significantly higher compared to samples taken at the T1, see Appendix 1 and Figure 20. At T1 an average concentration of 5.3 ±0.5 µg/l was measured and at T2 14.1 ±2.7 µg/l. The chlorophyll-*a* concentration measured in the samples taken at T2 did not differ from that of samples taken at T3 (Tukey *post hoc* test) when 14.9 ±4.6 µg/l was present.

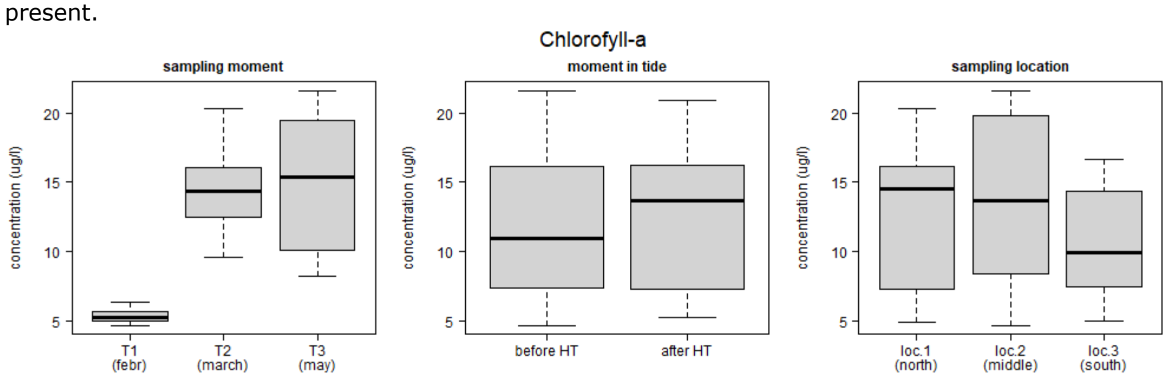

Figure 20. Boxplots with the concentration of chlorophyll-*a* in the water samples broken down per sampling moment (left figure), moment in the tide (middle) and sampling location on the Hond and Paap tidal flat (right). HT= high tide.

Figure 21 shows the ratio between the suspended solids and chlorophyll-*a* concentration. The sampling moment has the greatest influence on the ratio, which decreases strongly and significantly after T1 sampling, see Appendix 1 and Figure 21. The ratios are significant lower 2 hours after high tide and significant higher at sampling location 3. However, the influence of these variables on the ratio numbers is much smaller than the influence of the sampling moment, see Figure 21.

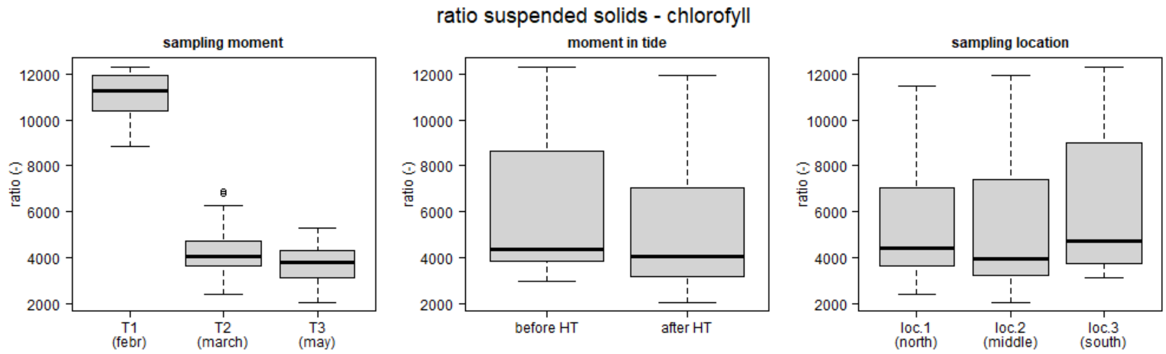

Figure 21. Boxplots with the ratio suspended solids : chlorophyll-*a* in the water samples broken down per measuring moment (left figure), moment in the tide (middle) and measuring location on the Hond and Paap tidal flat (right). HW= high tide.

Figure 22 shows the salinity values for the T1, T2 and T3 measurements. The measured salinity ranged from 14.5 to 24.2 PSU. Over time the salinity increased significantly, see Appendix 1 and Figure 22. At the T1 sampling event an average of 16.9 ±1.6 PSU was measured. The salinity increased at the T2 sampling event to an average of 19.0 ±1.8 PSU and increased further at the T3 sampling event when an average of 21.8 ±1.6 PSU was measured. The salinity in the most northern located location (location 1) was somewhat higher than in the more southern located locations, see Appendix 1 and Figure 22. The moment in the tide did not made a significant difference, see Appendix 1 and Figure 22.

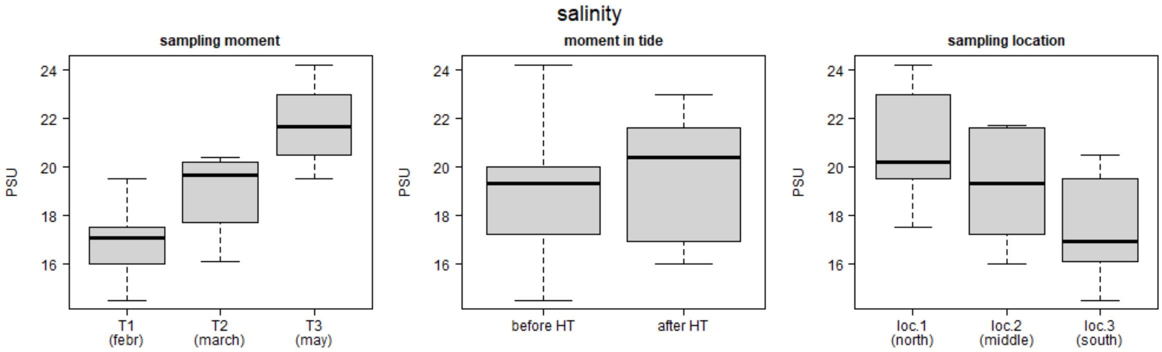

Figure 22. Boxplots with the saltinity of the water samples broken down per measuring moment (left figure), moment in the tide (middle) and measuring location on the Hond and Paap tidal flat (right). HW= high tide.

The water temperature increased significantly over the measuring period, see Appendix 1 and Figure 23. At T1 the average temperature was 2.5 ±0.2 degrees, which increased at T2 to an average of 7.3 ±0.5 degrees and increased further at T3 to 12.5 ±12.5 degrees. Furthermore, at the most northern located sampling location (location 1) and after high tide, water temperatures were significant higher, Appendix 1. The influence of those factors on the temperature was limited to a maximum of 0.4 degree.

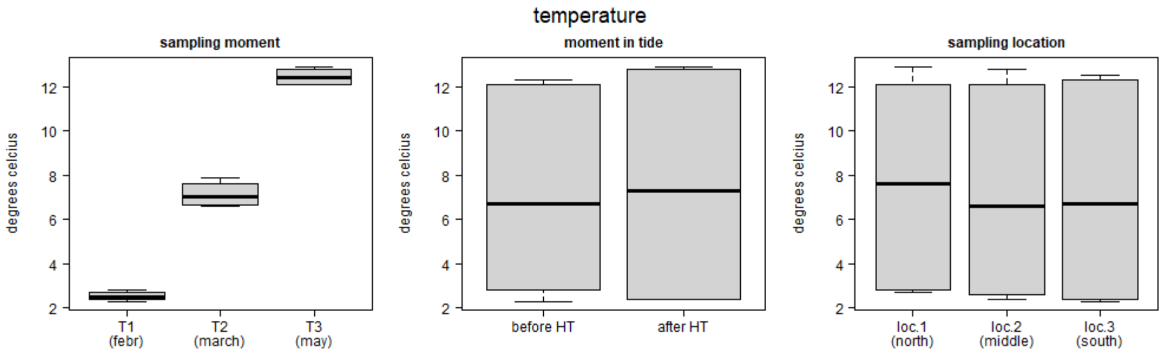

Figure 23. Boxplots with the water temperature in the water samples broken down per sampling moment (left figure), moment in the tide (middle) and sampling location on the Hond and Paap tidal flat (right). HT= high tide.

The oxygen content in the water was lower at T3 than at T1 and T2, see Appendix 1 and Figure 24. At the most northern located sampling location somewhat higher oxygen levels were measured. There was no difference in measurements taken before or after the high tide, see Appendix 1.

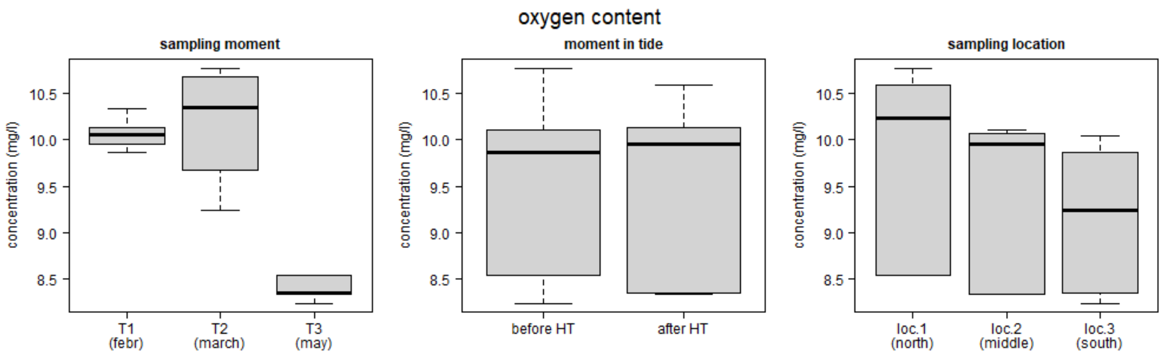

Figure 24 . Boxplots with the oxygen content in the water samples broken down per measuring moment (left figure), moment in the tide (middle) and measuring location on the Hond and Paap tidal flat (right). HW= high tide.

2.5 Discussion and conclusions pre-studies

The analyses carried out here are limited in scope but do show that, with the exception of the tidal inlet, suspended solids concentration and the ratio between suspended solid and chlorophyll-*a* are higher, and the salinity level is lower in the Ems-Dollard estuary compared to other tidal basins in the Dutch Wadden Sea, see Figure 2. This make the environmental conditions for mussel bed survival more challenging in the estuary. There are gradients in environmental conditions going from the outer estuary to the inner estuary. At the outer estuary, close to the tidal inlet, the here investigated parameters are more comparable to the rest of the (Dutch) Wadden sea but the tidal flats located there fall outside the research area for the trial. In 25% of the (MWTL) measurements taken near the Hond and Paap tidal flat (the research area) the suspended solids concentration exceeds 150 mg/l and in 11% of the samples concentrations exceeded 200 mg/l, see Figure 8. Effects on the water filtration rate are likely at those concentrations. In recent decades, two periods of one year with elevated suspended solids concentrations have been observed, which co-occurred with declines in mussel bed acreages. Although the salinity levels found at the Hond and Paap tidal flat are lower than that of sea water (around 22 PSU), they are within the tolerance limits of mussels. In areas located south of the Hond and Paap tidal flat the salinity levels decreased further. At MWTL sampling station located in the inner estuary, Groote Gat noord, salinity levels below 10 PSU were regularly observed. Negative effects such as a decrease in filtration rate, decrease in shell length and biomass at salinity levels below 10 PSU on mussels cannot be ruled out. Also own water quality measurements carried out during the survival experiment show a gradient in salinity levels. The formation of a mussel bed in 2016 on the (northern site) of the Hond and Paap tidal flat shows that natural establishment of mussel beds is possible and that they can survive for at least one year.

In the limited set up of the pre-study, chlorophyll-*a* concentration has been used as a proxi for food availability for mussels. The concentrations of chlorophyll-*a* can indeed be used to get an impression of the presence of algae (and thus the presence of food for mussels). But there are limitations. It does not tell which algae species are behind the chlorophyll-*a* concentration (determining the food quality) nor tells it how productive they are. With high turnover rates, low algae concentrations can lead to high primary productivity or vice versa. More comprehensive studies carried out on this subject support our findings and have shown that food conditions for shellfish in the estuary have deteriorated in recent years. Taal et al. (2015) show that in the period 1990 to 2011 suspended solids concentrations in the water of the Ems-Dollard area increased by 0.5-3% per year. The increased turbidity has reduced primary production in the form of phytoplankton. In a study conducted by Brinkman et al. (2015), measurements of primary production were also taken and analysed. Compared to the late 1970s, primary production in 2012-2013 was approximately halved in the estuary. In particular at locations south of the Hond-Paap tidal flat, suspended solids concentrations increased significantly compared to the late 1970s (from about 100 to 200 mg/l) and primary production decreased significantly in 2012-2013 (Brinkman et al., 2015).

The survival experiment was terminated prematurely due to a severe period of frost and before the growing season had started. As a result the value of this pre-study was limited. It was only possible to study the effects on mussel survival and growth during two winter months and analysis of the mean shell length of batches with mussels was no longer an option.

Although the greatest increase in shell length occurs in the spring, when food is plentiful and water temperatures are higher, it cannot be ruled out that shell growth occurs during the winter months as well. Seed (1968) found that between April and October 90% of the shell length growth occurred and 10% occurred in the remaining months. Although much effort has been made to correctly link shell lengths of individual mussels between the T0 and T1 measurements, and control plots show that this linkage appears to have been correct in the vast majority of cases, it is inevitable that errors have occurred. By using a digital calliper for the determination of the shell length it is possible to determine shell lengths to hundredths of a millimetre. Nevertheless, measurement errors in shell lengths may occur if the calliper is placed slightly askew over the shell. Because differences in shell length are often in the order of 1 mm to tenths of a mm measuring errors can influence the result. However, because mussels were randomly distributed, were about the same size when hung out, and were treated equally during the experiment, it is expected that measurement errors between groups will be equally large and that changes in shell lengths between groups are still of some value.

The limited results that were obtained show that most mussels survived the two winter months. No spatial differences or differences due to different emersion time regime on survival rates were found. It is however possible that the mussels have survived this period by using their fat reserves. The shell lengths of the mussels originated from intertidal- and subtidal beds increased a bit. The increase in shell length was higher in the most northerly located test sites and at a emersion time regime of 35%. For mussels originated from a SMC installation in the Oosterschelde no increase in shell length was observed at any of the study locations. It is expected that these mussels, of the mussels studied here, have had to adapt the most. The SMC in the Oosterschelde is under great influence from the North Sea with high salinity levels and low suspended solids concentrations as a result. These mussels had not only to adapt to the lower salinity levels at the Hond and Paap tidal flat but also to the tidal regime in which they fall dry twice a day. Also the mussels originated from the subtidal mussel bed had to adapt to the new tidal regime but they were at least already better adapted to a decrease in salinity levels due to freshwater discharge from Kornwerderzand (de Kok, 2002). It is unknown whether the absence of growth for the SMC mussels is the result of a somewhat longer and more intense adaptation period and whether they would have been able to grow in spring when the growth conditions have improved.

In summary, the data collected in the survival experiment are insufficient to conclude whether mussels placed from different origin and from areas outside the estuary can survive at the Hond and Paap tidal flat. However the differences in changes of the shell lengths and the collected water quality data show that the environmental conditions for mussel survival in the northern situated parts of the Hond and Paap tidal flat seem to be somewhat better. Moreover, mussel beds appear to be able to form and maintain themselves naturally in the area. However, the bed size fluctuates strongly due to the overall natural fluctuations but also due to the position within the estuary with its complex environmental conditions and water chemistry. It seems that especially the occasional occurrence of long periods with above average suspended solids concentrations and below average chlorophyll-*a* concentrations makes the preservation of mussel bed areas uncertain which can lead to sharply decreasing mussel bed acreages. In areas south of the Hond and Paap tidal flat, the environmental conditions for mussels are further decreased by a further reduction in salinity.

3 Trial design

To arrive at a trial design to initiate the formation of an intertidal mussel bed, a meeting was held in Groningen at the end of 2017. Dutch and German researchers, specialised in the field of mussel bed ecology and representatives of both governments attended this meeting. From WMR Norbert Dankers, Jacob Capelle and Sander Glorius were present and from SaM Achim Wehrmann was present.

The meeting discussed and evaluated attempts that had already been carried out. For this purpose, the overview as presented in chapter 5 of 'Een zee van mosselen' (Dankers and Fey-Hofstede, 2015) was used and supplemented with results from more recent mooring attempts. Research results obtained from, among others, the research projects Mosselwad (https://www.mosselwad.nl) and Waddensleutels were also used. The results were incorporated into ideas for an experimental design that was further elaborated by WMR and SaM.

To find suitable locations for the trial at the Hond and Paap tidal flat, results of the pre-studies (chapter 2 of this report) were used as well as other sources such as the habitat suitability map for intertidal mussel beds (Brinkman in preparation; Dankers & Fey-Hofstede, 2015) and a map of emersion times. In addition, a field visit took place on 26 June 2018 to assess the suitability of potential locations.

3.1 Experiences from attempts

This section gives a brief account of the attempts that have been made (in Europe) to establish mussel beds and mentions the most important findings that are relevant to the establishment of mussel beds. For more information we refer to the various references that are included in the text.

Table 3 reproduces the overview of past attempts to establish mussel beds as included in Chapter 5 of 'Een zee van mosselen' from Dankers & Fey-Hofstede (2015).

Table 3 . Summary of attempts to establish mussel beds. L = littoral (intertidal) and S= sublittoral (subtidal). Reprinted from 'Een zee van mosselen' Dankers & Fey-Hofstede (2015).

Project	Jaar	N	Aan-leg L/S	Opper-vlakte	Type	Oor-sprong L/S	Overleving	Oorzaak verdwijnen	Opmerkingen	Geslaagd
Jan Louw	2001	5	L	5 × 5 ha	zaad	L	1 maand – 14 jaar	? —	1 bank overleefde	Ja
Zeegras	2002	6	L	20 m²	oud	S	2 week	Predatie ?	Mossel op kokkelschelpen	Nee
Schier	1987	1	L	1 ha	1-6 cm	S	>1 jaar	Predatie/vis-serij	Nieuwe broedval belangrijk	
Jan Smit	1995	1	L	> 1 ha	3-5 cm	L	>10 jaar	—	Mosselen van elders stroomden in	Ja
Nedersaksen	?	?	L	> 10 ha	zaad	L	>1 jaar	visserij	Droogvallende percelen	Nee
Sleesw. Holstein	1998	2	L	1000 m²	4-5 cm	S	1-4 week	Predatie?	Ondergrond wadplaat en kiezels	Nee
K. Reise	2008	6	L	100 m²	Oester	L	> 6 jaar	—	Transplantatie-experiment	Ja
Zandkreek	2011	3 × 4	L	6-62 m²	zaad	MZI	maanden	?	70% sterfte	Nee
Bangor	veel	×	L/S	> 1 ha	zaad	L	> 1 jaar	Visserij —	Veel percelen, tijverschil 7 m	Ja
Wash	2000	?	L	> 1 ha	zaad	S	1 jaar	Visserij —	Niet goed gevolgd	Ja
Mosselwad	2013	4	L	4 × 2 ha	mix	S	2 maanden	Storm en predatie	Op wad, oester- en mosselbank	Nee
Mosselwad UU	2012	19	L	19 × 1 m²	oud	L	> 2 jaar	—	Patches als zode getransplanteerd	Ja
Waddensleutels	10-13		L	?		L/S	Dag – × week			Nee
Boyne (Ierland)	00-03	?	S	3300 ton	zaad	S	4 jaar	? Stroming?	Herstelproject na baggeren	Nee
LNV-RIVO	1952			variabel	sub-straat	L			Invang zaad (MZI) rap. Nico Laros	Nee
Cultuurperceel	veel	× ×	S	3000 ha	zaad	S	> 3 jaar	Visserij —	50% verlies	Ja

As Table 3 shows, many attempts to restore mussel beds have not been successful. Recent projects financed by the Wadden Sea Fund, Rijkswaterstaat and the provinces of Friesland and Noord-Holland, namely Mosselwad and Waddensleutels, have shown that especially storms hamper the experimental establishment of mussel beds. Additional experiments have also made clear that wave action is an important limiting factor for recovery by damaging the edges of a bed. Other important factors for successful construction of mussel beds are the suitability of the starting material (origin of the mussels), the possibility of forming aggregations of mussels and building up a spatial structure, and the role of predation by crabs at high tide and birds at low tide.

Recently, several PhD theses have been published on the stability of intertidal mussel beds in relation to hydrodynamic processes (Donker, 2015), on how constructed mussel beds can survive (de Paoli, 2017) and on the role of predation by crabs and birds on tidal mussel beds (Waser, 2017). New research projects have also been formulated using the recently acquired knowledge. For the Oosterschelde, a RAAK PRO project at HZ University of Applied Sciences has started "More value with Mussels" (2015-2019). In this project, mussel beds are constructed using mussel seed from SMCs. In this project, RWS, NGOs and the mussel sector are working closely with HZ University of Applied Sciences and various research institutes (including WMR, Deltares and NIOZ).

In addition, at the Feugelpôlle, southwest of Ameland, so-called BESE-elements (biodegradable crates) have been used to stimulate the natural recovery of mussel beds. The crates are made of potato remnants from a potato factory and should be degraded after about 10 years. The idea is that they serve as a temporary substrate to facilitate the establishment of mussels. The crates are held in place by wooden stakes installed on both sides of the crates. In 2014, the crates were set up in rows to capture mussel brood fall. Mussel spawn was soon found on the crates as well as growth of barnacles and anemones (pers. communication W. Lengkeek). As far as the authors of this report are aware, the presence of the crates and the mussels caught in them did not lead to the formation of mussel patches on the surrounding tidal flats.

De Paoli (2017) summarized some of her findings as recommendations for mussel bed restoration:

- Mussel density
    - The density should be sufficient to allow for aggregation (clumping) of mussels. A high density of mussels promotes aggregation and increases the persistence of mussel beds.
- Bulks
    - Mussel beds must be constructed on a flat substrate and (mussel) humps must form themselves over time. Bumps increase flow velocity and reduce survival. On stable mussel beds, bumps increase food availability (Donker, 2015).
- Origin of mussels
    - Sublittoral (subtidal) mussels are not suitable for restoration of intertidal beds, the thin shell makes them susceptible to predation and they attach too weakly to substrate to cope with hydrodynamic stress.
- Artificial substrate
    - The provision of alternative substrate in the form of coir matting is not suitable for the recovery of mussel beds, because intertidal mussels do not use it.

In addition, there are other factors that influence the chances of recovery projects:

- Shelter
    - The risk of washing away (newly) constructed beds is lower in sheltered areas. Natural or artificial shelter can improve conditions
- Predation
    - Mussels are eaten. Young mussels are eaten by shrimps and crabs at high tide. Larger mussels are eaten by birds, particularly gulls and oystercatchers, at low tide. Protection from predation can increase mussel survival.

This overview of the Paoli is a good summary of the experiences gained in the mussel bed experiments as well as the results of the research conducted into the stability of mussel beds in Waddensleutels and Mosselwad. These recommendations were also important in the realization of the trial design, which is described in the next section.

3.2 Trial design

The trial design consists of catching mussel larva present in the water column in spring on nets after which the nets with the collected mussel spat are placed on the Hond and Paap tidal flat to mature to an intertidal mussel bed. It is expected that the mussel spat will have firmly anchored themselves to the ropes of the net with byssus threats when the nets are placed in the water column to catch the mussel seed. Because the nets are not removed after placement on the tidal flat, the byssus thread structures made by the mussels remain intact. In this way a temporal structure is offered that may prevent erosion due to wind and wave actions. For this reasons the nets need to be made out of biodegradable materials.

Placing the nets with mussels on the tidal flat will create a surface of mussel seed with a more or less equal density of mussels. It is expected that the net structure will eventually disintegrate and disappear altogether. In the meantime, the mussels will have the opportunity to grow, develop a thick shell that can withstand tidal influences and predation pressure, and to organize themselves spatially to develop patches that are resistant to erosion caused by wind and waves. The hope is that the mussels will be able to survive for multiple years and developing into a bed with mussel patches, open (mussel free) areas, drainage channels in between them and form structures that are in the end suitable for collection of mussel spat for necessary rejuvenation.

This approach incorporates advice prepared by de Paoli (2017), see previous section. No use is made of mussels already fully adapted to the subtidal environment leading to, compared with the intertidal variant, thinner shells, and fewer byssus threads leading to looser attachment and increased susceptible to erosion and bird predation. Mussels from an intertidal bed are best equipped to survive on an intertidal flat, according to de Paoli, but logically these cannot be used because you would be damaging an ecosystem element elsewhere that you actually want to protect. For the trial it is important to place the nets soon after settlement to the tidal flat in order to prevent mussels to develop into subtidal mussels with their specific characteristics. Because the mussels are still young (a few weeks old) when they are first exposed to the wind and tidal influences of the tidal flat, it is expected that they are able to adapt to the intertidal conditions.

There are several possible materials that could be used to manufacture the nets. Since experience with the use of coir nets was poor (de Paoli, 2017), this material was not chosen. It was decided to construct nets from different materials in order to see if they perform differently. Nets made out of ropes of sisal-, hemp- and cotton fibres were constructed. Sisal is a fibre manufactured from plant species belonging to the genus *Agave*.

As also advised by de Paoli (2017), no height differences or open surfaces are created. It is intended that these will arise naturally over time if the mussels are able to survive. The trial design is in large parts in line with the natural development of beds. The creation of a natural intertidal mussel bed starts with a seed falling on a tidal flat. In that way a homogenous mat of mussel seed is formed with few differences in heights and few open areas without mussels. Over time, the mussels aggregate and climb upwards on each other, creating elevated (consolidated) mud bumps with mussels that alternate with open areas with little or no mussels and drainage channels. In 'Een zee van mosselen', this process is nicely depicted on page 48 (Dankers & Fey-Hofstede, 2015).

Both de Paoli (2017) and several other studies Liu et al., (2013) and Donker (2015) show the importance of mussel densities and the formation of spatial structures for increasing the bed stability. Individual mussels need each other to form strong patches building resilience against predation and erosion. In addition, as a result of competition for food, open spaces between mussel clusters are formed (de Paoli, 2017, Liu et al., 2013 and Donker, 2015). These competitive processes lead to the formation of mussel bands consisting of mussel patches in which mussels are attached to each other in more or less honeycombed patterns (Liu et al., 2013 and Dankers & Fey-Hofstede, 2015). On a larger spatial scale, elevated areas with mussels are alternated with areas with few mussels. Water currents over the mussel areas during high tide create vertical mixing of the water column leading to an increase in food availability (algae) for the mussels (Donker, 2015). By waiting to transplant the nets until a sufficient number of mussels are attached, it is ensured that there are enough individuals present to form clusters. In addition, the nets are constructed with a mesh size of approximately 5-8 cm to facilitate the formation of mussel aggregations on a small spatial scale.

The presence of the nets and several rows of bamboo sticks (for monitoring purposes) may scare birds to some extent. Placing structures on or around the nets to further prevent bird- and/or crab predations is believed to create undesirable water currents, that possibly causes erosion of mussels. Therefore, no further measures were taken to prevent predation.

3.3 Construction of nets

It turned out to be a quest to purchase nets of the desired materials or to have them manufactured by machines. Nets of the desired materials, rope thickness and mesh size were not for sale and machine-made nets were quickly discarded because the machines used to make nets could not cope with the fibrous structure of the ropes. Eventually the C.I.V. in Den Oever offered a solution by making the nets of the desired materials in the traditional manner, see Figure 25. From each material three nets of two meters wide and ten meters long were made. The net intersections were knotted and woven alternately, see Figure 25.

Figure 25 . Construction of a sisal net. Top picture showing the knotting and weaving of the net. Lower left picture shows the net intersections, lower right picture the rolls of sisal rope.

3.4 Spat collection

In order to allow for regular inspection of the nets, practical considerations led to the decision to hang out the nets in the vicinity of either the WMR office in Den Helder or the SaM office in Wilhelmshaven (Germany) to catch mussel spat. Due to well established contact between SaM and a local SMC entrepreneur active in the Jade it was decided to select Wilhelmshaven. Possibilities to attach the nets to the SMC installation of this entrepreneur were investigated, but disregarded because of the business risks involved and the logistically impracticality to conduct the desired monitoring.

Various other potential locations in the Jade were investigated, see symbols 'A' to 'D' in Figure 26. Locations 'B' and 'D' were discarded. Site 'D' was a jetty in the Hoekseiler harbour. It turned out that there were insufficient possibilities to hang the nets on this jetty without falling dry periodically.

Location 'B' concerned the jetties of the Eurogate container terminal in the Jade Weser Port. This proved to be difficult to access this site. Location 'C', a floating pontoon in a tugboat harbour, and location 'A' in the inner harbour of the Jade proved suitable for hanging out the nets.

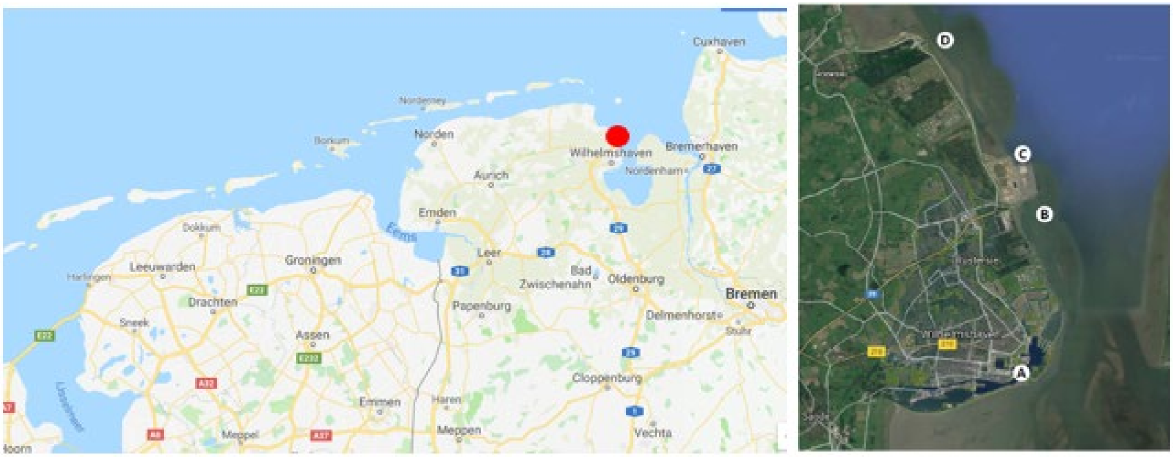

Figure 26 . Left a map showing the location of Wilhelmshaven and the Jade (red dot). On the right, a satellite map showing the location of the potential sites for the nets.

At both locations, salinity was about 30 PSU. At the tugboat harbour a tidal amplitude of approximately 3.5 meter is present. Due to the presence of a floating pontoon the nets would move along with the tide. The harbour is protected against wave influence by a structure of concrete, see Figure 27. In the inner harbour there was no tidal influence.


Figure 27. Left a picture of the floating pontoon (location 'A') and right the quay of the inner harbour (location 'B').

The nets were attached to a (floating) synthetic rope at the top and weighted down with bricks at the bottom, see Figure 28. Several buoys and fenders were attached to the top to keep the construction afloat.

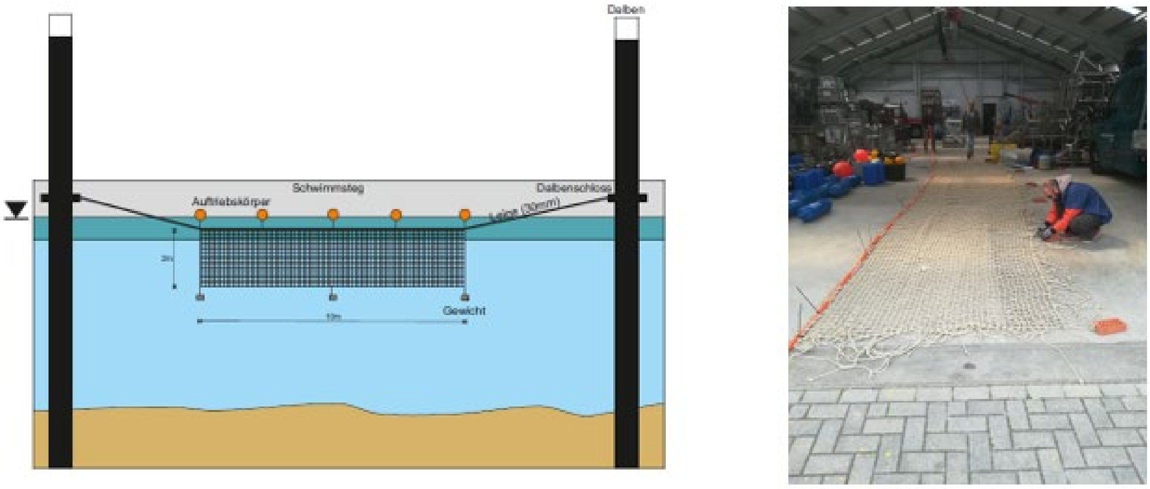

Figure 28. Left a schematic representation of the positions of a net hanging in the water column. Right: attaching the rope and stones to a net in the production hall of the SaM.

When the water temperature reaches 12 °C in spring, mussels release their sperm and egg cells and fertilization takes place in the water column (Dankers and Fey-Hofstede, 2015). After fertilization, mussel larvae stay in the water column for about a month feeding on plankton. Afterwards, the mussel larvae sink to the bottom or attach to suitable hard substrate. In the Dutch Wadden Sea, this means that, depending on the weather, the mussel seed reaches the establishment stage between May and June.

To the south of the harbour and in the Jade river are the mussel collection facilities of Musselzicht GmbH, where mussel seed has been harvested every year for decades (pers. communication with D. de Leeuw, owner of Musselzicht GmbH). It is therefore expected that mussel seed will be present at the tugboat port. From contact with Mr. de Leeuw it appeared that the moment that mussel seed settles on the ropes of the SMC in the Jade is somewhat later than in the Netherlands. In the inner harbour of Wilhelmshaven there is no experience with catching mussel seed.

3.5 Trial location

In order to find suitable locations for the trial on the Hond and Paap tidal flat, information from various sources was used in addition to the results obtained during the preliminary investigation; (1) a habitat suitability map, (2) data on historical mussel bed occurrences, (3) a map with emersion time and (4) observations made during an inspection of the tidal flat.

In order to draw up the habitat suitability map, Brinkman (in preparation) related the occurrence of intertidal mussel beds to abiotic characteristics. This showed that emersion time and average grain size are the most important variables that determine the presence or absence of beds, followed by the silt and salt content (Brinkman, in preparation). In addition, orbital velocity (wave action on the bottom) also contributes to the habitat suitability. This analysis also shows that the maximum flow velocity and the resulting shear stress on the bottom have only a minor influence.

Brinkman's analysis (in preparation) shows that the optimum emersion time values for mussel spat and half grown beds is 30-40%. For mature beds this is slightly shorter. The optimum average grain size (>16 µm) is 120-150 µm, but 80-100 µm half grown beds. The optimal mud content is at least 40%, but this varies widely. The optimum salinity appears to be 20-25 PSU. As stated earlier, flow velocities are of little consequence for the presence of intertidal mussel beds. The optimum maximum (orbital) velocity should not be too high and is 0.3-0.4 m/s for young beds and 0.2 m/s for mature beds in spring.

Variable	Optimal value	Note
Emersion time	30-40% shorter	Mussel spat and half-grown mussel beds Adult mussel beds
Average grain size	80-100 µm 120-150 µm	Half-grown mussel beds Adult mussel beds
mud content	> 40%	Variable
Salt content	20-25 PSU	
(orbital) flow rate	0,3-0,4 m/s 0.2 m/s	Mussel spat Adult mussel beds (spring)

It should be noted that the analysis of Brinkman was carried out using data of mussel beds in the Dutch Wadden Sea for the period 1992-2007. The habitat suitability of the Hond-Paap is high and optimal for the area where mussel beds are observed, on the western part of the Hond and southern part of the Paap (see figure 1, taken from Brinkman in preparation).

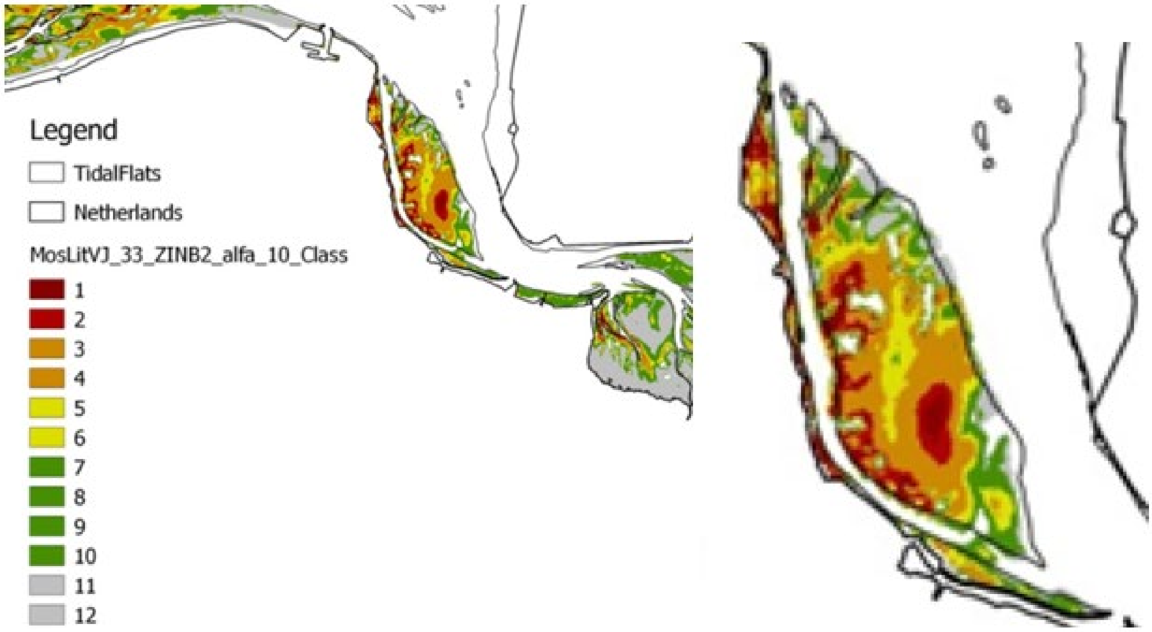

Figure 29. On the left the mussel bed habitat suitability map showing the classes for mature mussel beds in spring in the Ems-Dollard. On the right a detail of the map showing the Hond Paap tidal flat. Class 1 is most suitable, class 12 is the least suitable for mussel beds (taken from Brinkman in preparation).

Areas where mussel beds have historically been located are favourable for testing because (1) mussel beds have apparently been able to establish and maintain themselves to a certain extent in such areas (2) the substratum is probably more robust due to accumulated dead shell material in underlying soil layers. The frequency of presence of intertidal mussel beds was mapped by Troost et al. (2015). The map was made based on monitoring data over the period 1995-2011. The decline in mussel bed occurrence in the recent period (see section 2.3) is therefore not included in this map, which means that the map mainly says something about the historical occurrence of mussel beds in the area. The map shows that mussel beds were frequently found in the southern part of the Hond and Paap tidal flat, see Figure 30. This figure shows also that the beds are mainly found away from the edges of the tidal flat and at the gully outlets. The presence of mussel beds is in general in line with the habitat suitability map, but deviates in detail by a lower than predicted presence in general and a higher frequency of encounters in the southern part of the tidal flat than predicted.

Figure 30 . Frequency map of intertidal mussels on the Hond and Paap tidal flat for the period 1995-2011, taken from Troost et al., 2015).

The preliminary research carried out as part of this project (see chapter 2) shows the presence of a gradient in salinity on the Hond and Paap tidal flat which runs from the north, with a somewhat higher salinity, to the south, with a lower salinity. Based on own measurements the salinity in the northern areas was around 20 PSU. In the southern areas salinity is below the optimum range (<20 PSU) determined by Brinkman. The most southerly areas of the tidal flat are therefore estimated to be less suitable.

By combining the information obtained from the above sources, several locations were found to be suitable for the trial. These sites were visited to see if there were any gullies, eelgrass or shellfish beds present that would made them unsuitable as a test site and to get an estimate of the soil conditions. To spread the risk for unforeseen unfavourable circumstances and to test the approach at different locations it was decided to place three nets (of each material one) at three different sites instead of placing all nets at one site. Figure 31 shows the three selected trial locations (labelled as 'L1', 'L2' and 'L3') on an bathymetry map.

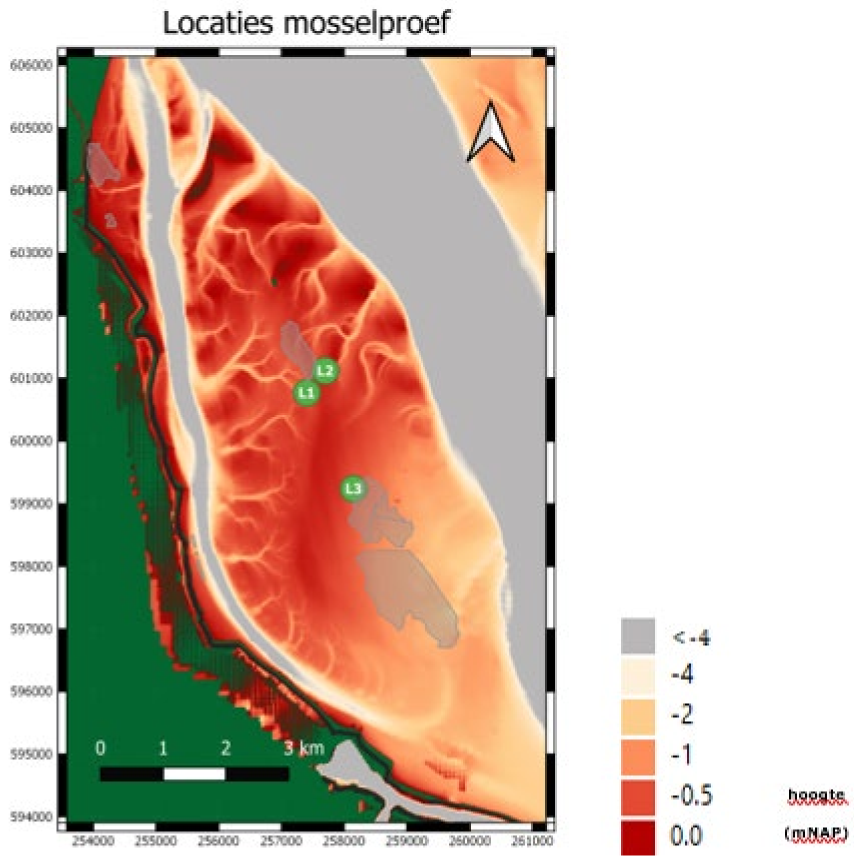

Figure 31 . The positions of the three trial sites on the Hond and Paap tidal flat, projected on an bathymetry map. Grey areas show the presence of a mussel- and mixed mussel/oyster bed (situation 2017).

Due to the fact that on not all nets (enough) mussel spat settled (see chapter 4), it became necessary to make adjustments to the above described plan. First of all the trial was split into two attempts. The first trial started in spring of 2018 using the nets on which spat was collected. Location 'L1' (Figure 31) was used for this trial. A second trial was carried out one year later (see chapter 5) using spat from a commercial spat collector installation. For this trial location 'L2' was used. Location 'L3' was not used at all in the end.

3.6 Monitoring

To monitor the development of the mussels the following parameters were determined: mussel density, -length, -weight and -cover, the emersion time, the rope degradation and changes in net elevation. This paragraph describes how the different parameters were measured. Information on sample frequency numbers differed per trial. In Appendix 2 this information is specified for each trial.

3.6.1 General impression nets and mussel coverage

A general impression of the development of the mussels on the nets was obtained by photographing all sides of each net. Photographs were taken from several meters away, making sure the entire side was photographed. In addition, detailed photographs were taken of the mussel patches. A stainless steel frame (50 x 50 cm²) was placed in the centre of the nets and photographed from above with a waterproof Nikon Coolpix W300 camera.

3.6.2 Mussel population

The mussel density, -length and -weight were determined by collecting mussel samples that were analysed in the laboratory. A PVC ring with a diameter of 10 cm and a height of 5 cm was used to take the samples. The ring was pushed into the sediment at mussel patches at random locations up to the edge of the ring and all the mussels within the ring were collected. The sample was then sieved in the field using local water (mesh size 1 mm) and placed in labelled plastic bags. All mussel samples were kept in a cool box for transport to the SaM laboratory for analysis. From each sample the total number of living individuals was determined as well as the total fresh weight (is weight including shell and adherent moisture). From 100 random individuals within each sample, individual shell lengths and fresh weights were determined as well. Ash free dry weight of the mussel flesh (so excluding shells) was determined per mussel group of similar size. For this purpose size classes per 5 mm were made.

In the execution of trial 2, three additional mussel samples were collected from a natural mussel bed that was established in 2016 and was located not far from the trial location. Samples were taken from the middle of the bed (in the longitudinal direction) at the northern side, the southern side and the central part of the bed. These samples were taken and processed in the same way the nets samples were taken and processed.

3.6.3 Degradation rope

Rope samples were taken in order to determine the integrity of the rope in the laboratory. Pieces of rope, several centimeters long, were cut off for this purpose. In the laboratory any mussels present were removed and the rope was rinsed to visually examined to what extent the structure was still intact.

3.6.4 Mussel cover and height

An innovative method was used to determine the mussel coverage and height. The method consists of creating an ortho-mosaic by merging a large number of overlapping photographs. These photographs were taken perpendicular to the tidal flat bottom, whereby care was taken to ensure large parts to overlap in successive photographs. Because the objects present on the picture were recorded from different angles, it is possible to construct a height model, or 'digital elevation model' (DEM), out of it. To calibrate the images and to geo-reference them, a number of reference points have been installed prior recording. These points were positioned throughout the nets and the geographical position and altitude of these reference points were accurately measured with an RTK-DGPS. The software program Agisoft Metashape Professional was used to merge the individual images into a mosaic and to construct the ortho- and DEM images.

In QGIS the ortho maps were used to identify and map areas containing mussels. The identification of the mussel patches was done manually. The dark coloured mussel shells were very distinctive from bare tidal flat and dead shell material. This information was stored in several shape files and used to calculate the area covered with mussels. It was also used to make maps of the areas covered with mussels.

There was no previous experience with mapping the presence of mussels in this way. As the project progressed, improvements were made in the implementation of it. In all cases a pole held by two persons and at which one to five cameras were attached to were used to take the photographs. At first one SLR camera was used that was operated with the use of a remote control. In a later stadium five go-pro cameras (with 2 sec. time laps) were used to take the pictures which made it possible to cover larger areas in the limited available time. At the end three remote controlled SLR cameras were used to increase the level of detail.

3.6.5 Emersion time

A CTD sensor (CTD-diver, Schlumberger Water Services) was attached to an aluminium pole and placed on the tidal flat. In order to calculate the emersion time of the nets the sensor was placed in the near vicinity of the trial location but at a lower elevation. The sensor was set to measure and log the temperature, salinity and pressure in 2-minute intervals. To correct for changes in air pressure, an air pressure sensor (Baro-diver, Schlumberger Water Services) was placed in the area as well (on the NAM island). The exact calculation to determine the water level over the sensor is described in the product manual. In short, the water pressure measured by the CTD is corrected for the density of the seawater and the air pressure determined by the Baro meter, after which a formula that is specified in the manual of the sensor can be used to calculate height of the water column above the sensor. By measuring the height of the sensor with a RKT DGPS all information is available to calculate the emersion time of the nets. The calculations were performed in the software package R (R core team, 2020).

3.6.6 Winddata

In order to gain an impression of the wind direction and speed during trials, data made public available by the KNMI (Royal Dutch Meteorological Institute) was used. From the KNMI website hourly data for the weather station Lauwersoog (number 277) were downloaded. This data was processed in R (R Core Team, 2020) and using the R package 'openair' (Carslaw & Ropkins, 2012) to produce wind roses showing the dominant wind force and directions for several time periods.

4 Trial 1

4.1 Collection of mussel spat

During late May and early June 2018, all nets were constructed by the C.I.V. Den Oever and could be placed in the water column at their destinated sites to collect mussel spat, see Figure 32. Six nets (two of each material) were hung out in the tugboat harbour, location 'A' in Figure 26. The three remaining nets (one of each material) were hung out in the inner harbour, location ‘C’ in Figure 26. The nets in the tugboat harbour were placed on 23th of May, 28th of May and 4th of June. In the inner harbour all nets were hung out on 28 May.

Figure 32. Nets hung out in the tugboat port (left) and in the inner port (right).

During the period June through mid-August 2018, the nets were inspected regularly for the presence of mussel spat (initially every other week and later more frequently). Rope samples were taken from a selection of the nets and examined with a microscope in the laboratory for the presence of mussel spat. During inspection of the nets on 12 June (tugboat harbour) and 13 June (inner harbour), mussel spat were found for the first time on the ropes, see Figure 33.

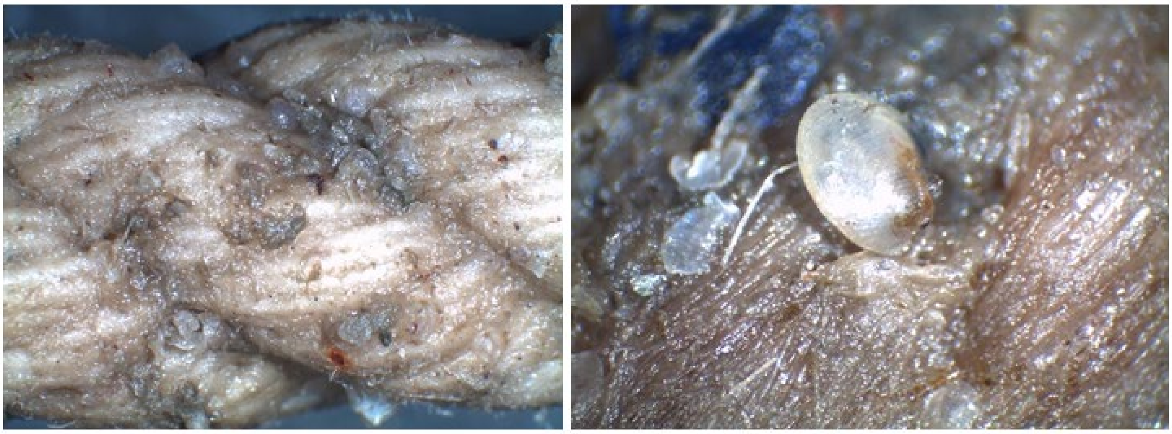

Figure 33. Mussel seed present on one of the cotton nets hung out in the tugboat harbour.

An inspection carried out on 6 July showed that the nets hung out in the tugboat harbour were for the most part covered with hydroid polyps, see Figure 34. Various types of macroalgae and barnacles were also found on the nets, as well as juvenile crabs. A layer of mud covered the nets hang out in this location fouling the nets and organisms on the nets.

Figure 34 . The fouling of species and mud on the nets hung out in the tugboat harbour. Left picture a net made of cotton, middle picture a net made of sisal and right picture a net made of hemp. Photos were taken on July 6, 2018.

The nets hung out in the inner harbour also became overgrown. Here, in addition to mussels, we found filamentous algae, sea squirts and barnacles. Crabs were not found.

Figure 35. The overgrowth on the nets hung out in the inner harbour. Left photo a net made of cotton, right photo a net made of hemp. Photos were taken on July 6, 2018.

To see how much mussel spat was present on the nets, pieces of it were removed for inspection at the laboratory. In the tugboat harbour three nets were sampled (one of each material). In the inner harbour, pieces of string were sampled from nets made from sisal and cotton. Slightly more mussels were found on the rope samples from nets hung out in the inner harbour compared to the net samples taken from nets hung out in the tugboat harbour, see Figure 36. The allocation of the nets to the Hond and Paap tidal flat was postponed from July 16th to August the 17th to give the nets in the tugboat harbour chance to become more densely covered with mussels.

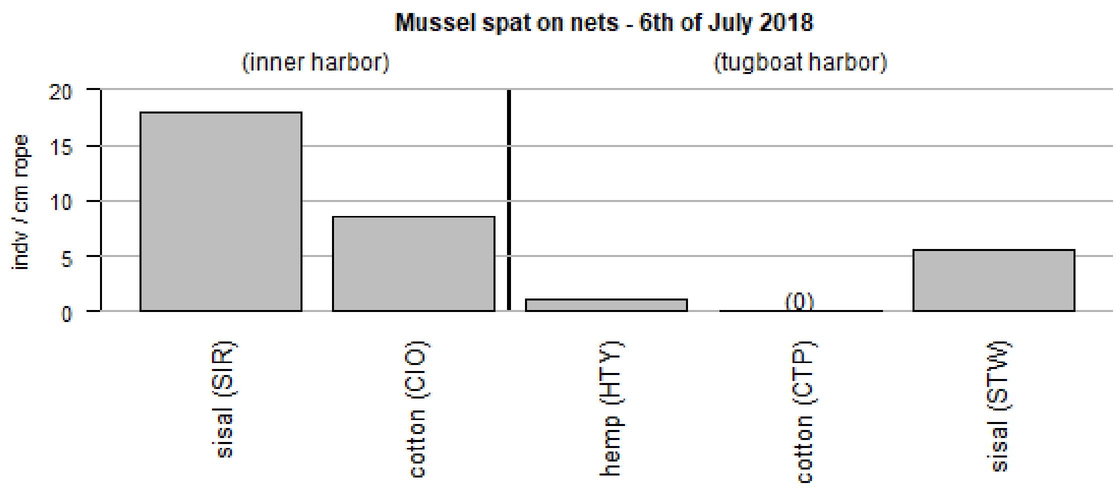

Figure 36. Bar charts showing the number of mussel spat expressed as number of individuals per cm of rope on samples taken on the 6th of July 2018 from nets hung out in the inner harbour and tugboat harbour. The rope sections did not contain knots or (fluffy) ends. The letter codes in brackets refer to the identification code. Where no mussels were found, this is indicated in the figure by "(0)".

In the weeks that followed, the nets in the inner harbour became increasingly thickly covered with mussels due to the growth of the mussels present and possibly also to additional spatfalls on the nets. On June 20 the nets in the inner harbour were fully covered with mussels, see Figure 37. Mussel shell lengths up to about 15 mm were present.

Figure 37. Net in the inner harbour almost completely covered with mussel seed. Photo taken on July 20, 2018.

The mussel population on the nets hung out in the tugboat harbour did not develop well. On 31st of July, pieces of rope were removed and examined in the laboratory for the presence of mussel spat. The number of mussels found per cm of rope was limited to <2 mussels per cm of rope, see Figure 38. On both nets made of cotton and on one of the nets made of sisal no mussels were found at all on the sampled pieces of rope. The mussel density on the nets made of hemp and sisal, where mussels were found, appeared to have decreased compared to the measurement conducted on 6 July 2018.

Due to the very limited presence of mussels on these nets, it made no sense to use these nets for the test and move them to the Hond Paap tidal flat. These nets were removed, roughly cleaned with fresh water, air-dried and stored in a shed at SaM in Wilhelmshaven for later use.

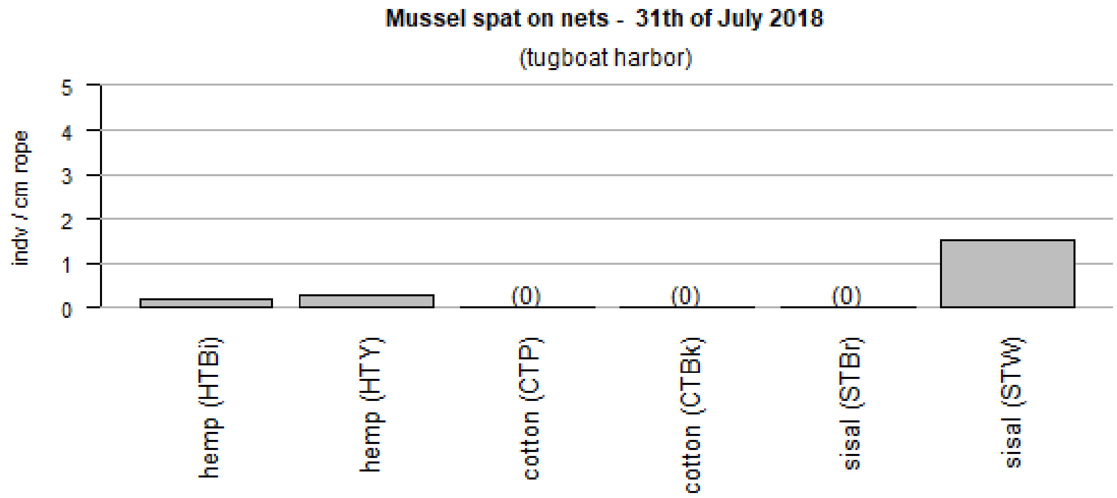

Figure 38 . Bar graphs showing the number of mussel spat expressed as the number of individuals per cm of rope for samples taken on 31th of July 2018 in the tugboat harbour. The rope sections did not contain knots or (fluffy) ends. The letter codes in parentheses refer to the identification code of the nets. Where no mussels were found, this is indicated in the figure by "(0)". Note the difference in scale for the Y axis compared to Figure 36.

4.2 Placement on the Hond en Paap tidal flat

Since only three of the nine nets were available for the trial, it was decided to use only trial site 'L1' on the Hond and Paap tidal flat (see Figure 31). To meet the requirements of the permit, the location was moved slightly further south so that the mats were located at a distance of > 200 meters from the natural mussel bed present there. During the period 13th to 17th of August 2018, the nets containing the mussels were moved from the Jade to the Hond and Paap tidal flat.

In the morning of Wednesday 15th of August, the nets were detached from their attachment points (other net or the quay) and towed with a RIB to a place where a crane truck stood. With the use of the crane the nets were lifted out of the water and placed into two plastic containers. The containers with nets were transported to the Eemshaven in an open truck and a trailer the same day. Here the nets were placed on deck of the boat *ZILVERVISJE* using the crane of the vessel *HARDER*. Assisted by the *HARDER*, the nets were transported with the *ZILVERVISJE* to the designated site on the Hond and Paap tidal flat during high tide on Wednesday. There the nets were manually put overboard, each secured by an anchor against drifting. The limited number of mussels that came loose from the nets during transportation were also put overboard. The low tide of Thursday morning was used to spread out the nets and secure them with iron staples. Additionally, the thick ropes used previously for installation in the harbour were removed as well. Figure 39 shows a pictorial report of these activities.

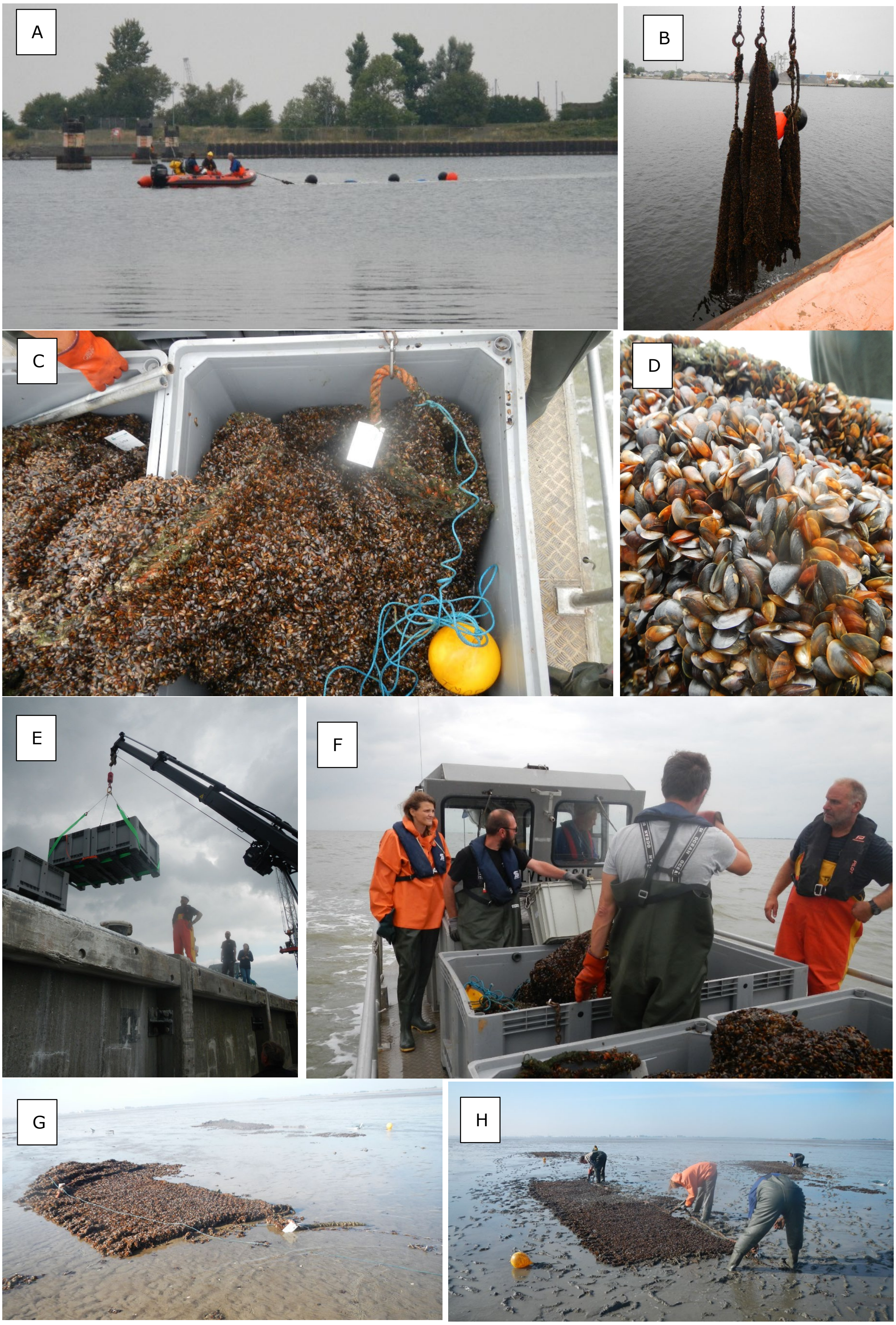

Figure 39. Visual report of translocation of the nets with mussels from the inner harbour of Wilhelmshaven to the Hond and Paap tidal flat in the Ems-Dollard estuary. 'A' transportation of nets to the quay, 'B' to 'D' transportation of nets in containers, 'E' placing the nets on the deck of the *ZILVERVISJE*, 'F' transportation of nets to the Hond and Paap tidal flat, 'G' position of nets at low tide the following day, 'H' positioning and anchoring of the nets.

The nets are located south of a natural mussel bed and east of a small gully that drains water to the Bocht van Watum channel, see Figure 40. Lengthwise, the nets are roughly orientated along a line running from north to south and are placed at a distance of around 10 meter apart from each other, see Figure 40.

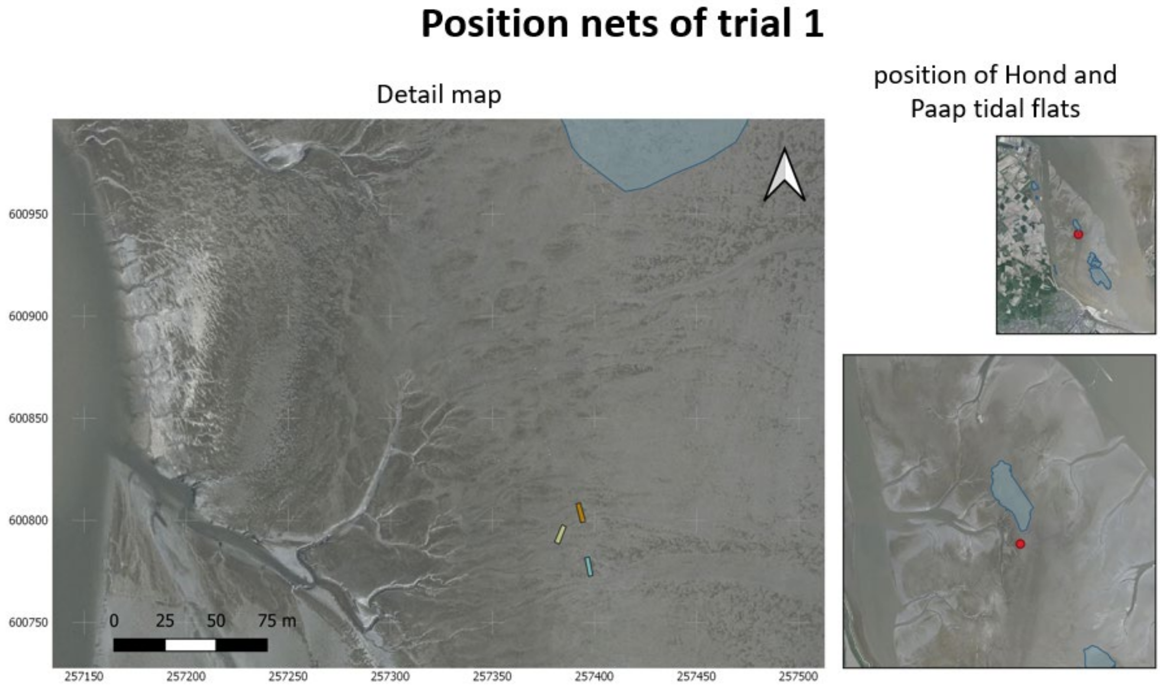

Figure 40. The position of the nets of trial 1 on the Hond and Paap tidal flat. On the right maps showing the position of trial one with a red dot on the Hond and Paap tidal flat. In the map on the left the locations of the three nets are shown. The net made from sisal ropes (orange rectangle), from cotton ropes (blue rectangle) and from hemp ropes (yellow rectangle). The blue contours show the naturally present mussel beds (situation 2017).

4.3 Development of the mussels of trial 1

This paragraph describes the development of the mussels of trial 1 using the information that was collected with the monitoring activities.

4.3.1 Emersion time

On average the nets of trial 1 have an emersion time of 26.9%. Due to differences in altitude between the nets and due to the influence of the phases of the moon and wind, the duration of emersion time varied between 24.6% and 34.3% over the monitoring period, see Figure 41. The difference between the nets were small (tenths of percentages).

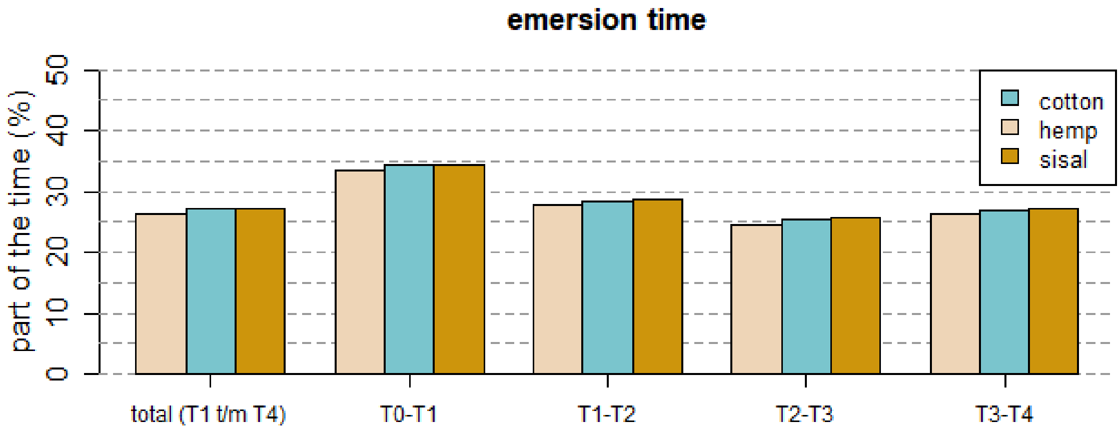

Figure 41. Bar chart showing the average emersion time during trial 1 for the different nets and between the different sampling events. The CTD logger was removed at T4 so no data is available for the period between the T4 and T5 sampling events.

4.3.2 Development in mussel coverage

During the course of trial 1 no mussels were found outside the nets except for the T1 sampling event (Monday 20 August 2018), see Figure 42. The mussels laying outside of the nets consisted most likely of mussels that were detached from the nets during the transportation from Wilhelmshaven to the Ems-Dollard estuary and were thrown overboard on Wednesday 16 August when the nets were placed on the tidal flat. In the following sampling session (T2, on the 3th of September) nearly all of the mussels laying outside the nets, have disappeared, see Figure 42. Except for the T1 sampling event, no mussel were found outside the nets in the other sampling events.

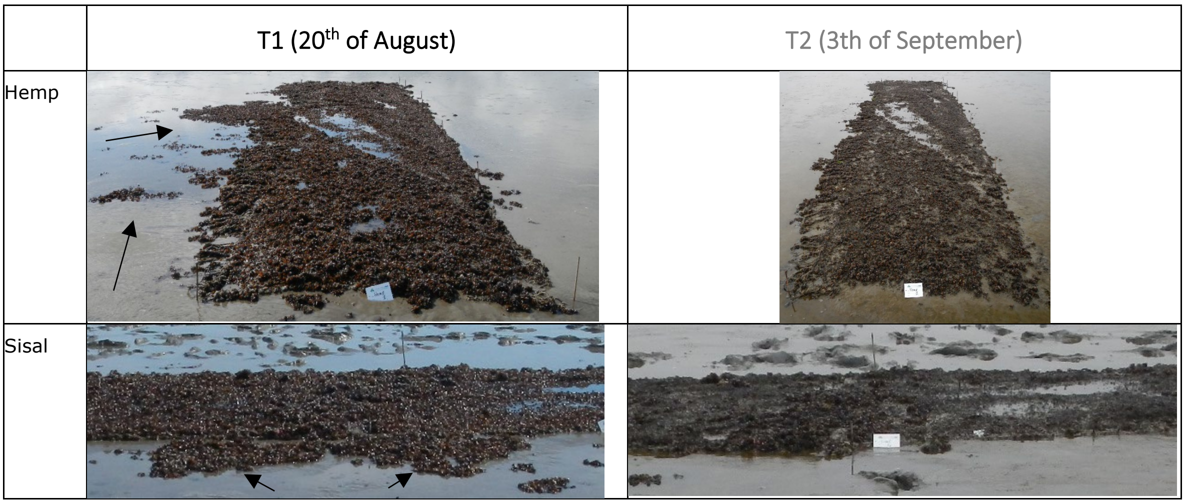

Figure 42. Photos showing mussel patches that layed outside the net made of hemp and cotton at T1 sampling event and their dissapearance at the T2 sampling event.

In the four months after the nets were placed on the tidal flat, the surface area covered by mussels decreased from almost 100% to <30%, see Figure 43 and Figure 44. Whereas the coverage of mussels in nets made of sisal was still >80% in October (T3), it had already decreased to <40% in nets made of cotton and hemp at T3, see Figure 43. At T4 the coverage of mussels on nets made of sisal had also decreased significantly to <35% of the net surface, see Figure 43. As part of a field visit for the second trial, the nets of trial 1 were visited again on 21 June 2019. At that time some very small and limited patches of mussels could be found present on the sisal net, while on both other nets the mussels had almost completely disappeared.

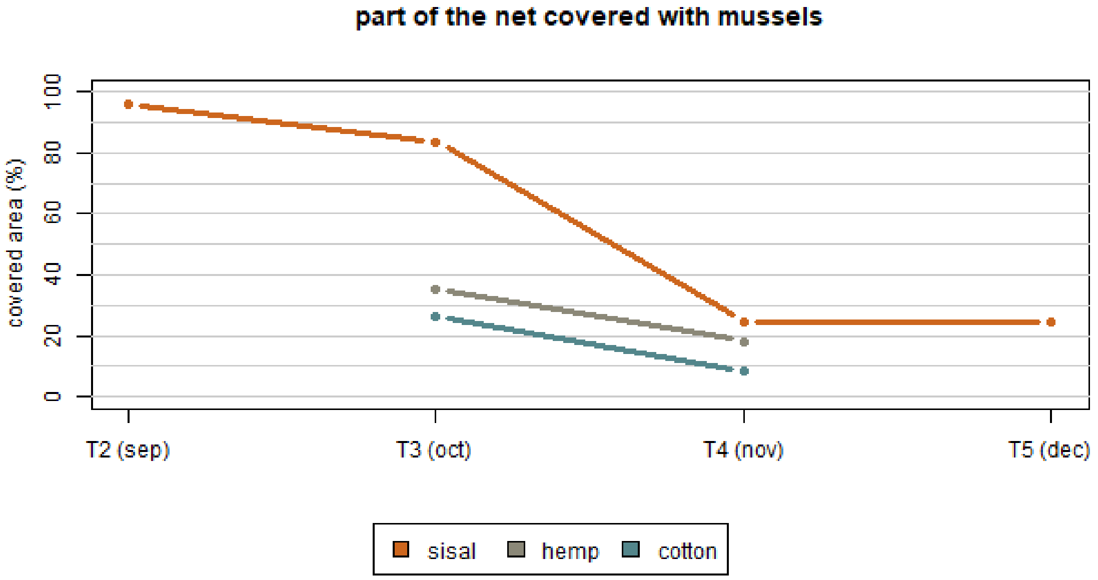

Figure 43. Development of mussel cover expressed as percentage of nets covered with mussels for the different nets and sampling times at which they were integrally mapped.

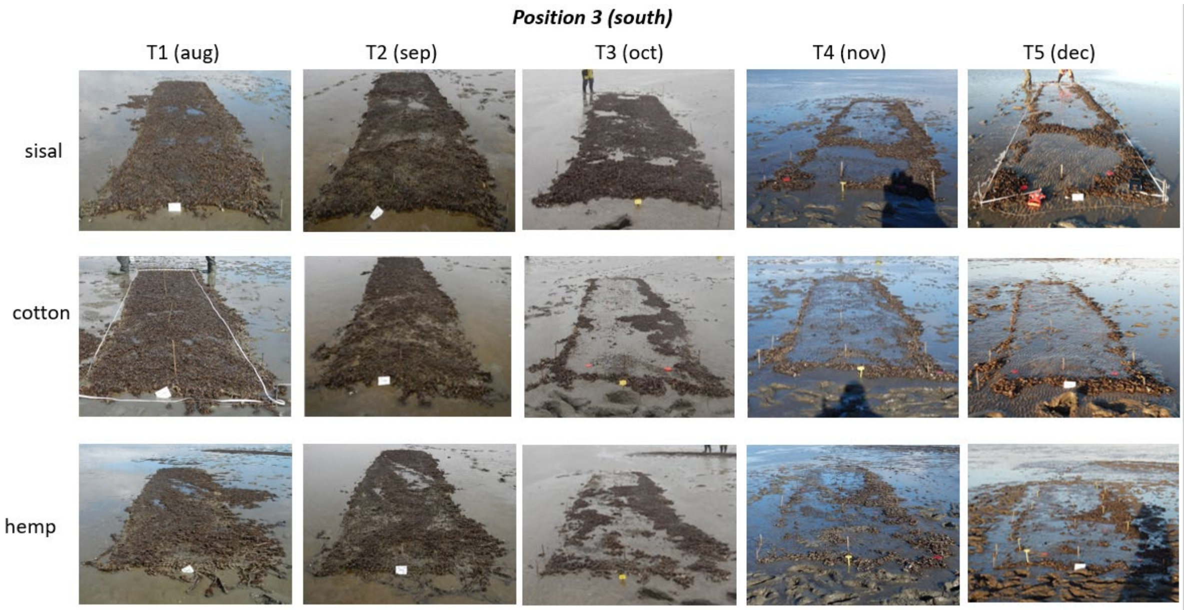

Figure 44 . Development of the mussel on the nets made from sisal, cotton and hemp of trial 1 photographed from the south at the sampling events T1 to T5.

Figure 45 show the disappearance of the mussels on the sisal net and the influence of wind direction and speed. Especially the somewhat stronger (>6 Bft) winds from the northwest between the T3 and T4 sampling events (third panel from the left in Figure 45) lead to the erosion of many mussels on the sisal net. Few mussels disappeared between the T4 and T5 measurements when wind speeds were lower. As has been observed in the field for all nets, mussels situated at the inner parts of the nets eroded the fastest while mussels located at the net edges remained for a longer period of time. At the northeastern edge of the sisal net, a thin band of mussels remained up to T5 sampling, see Figure 45. Also on the nets made from cotton and hemp, the mussels mainly remained on the (north-eastern) edge of the net, see Figure 44. However, in both nets more mussels disappeared between the T2 and T3 measuring periods compared to the sisal net, see Figure 43 and Figure 44.

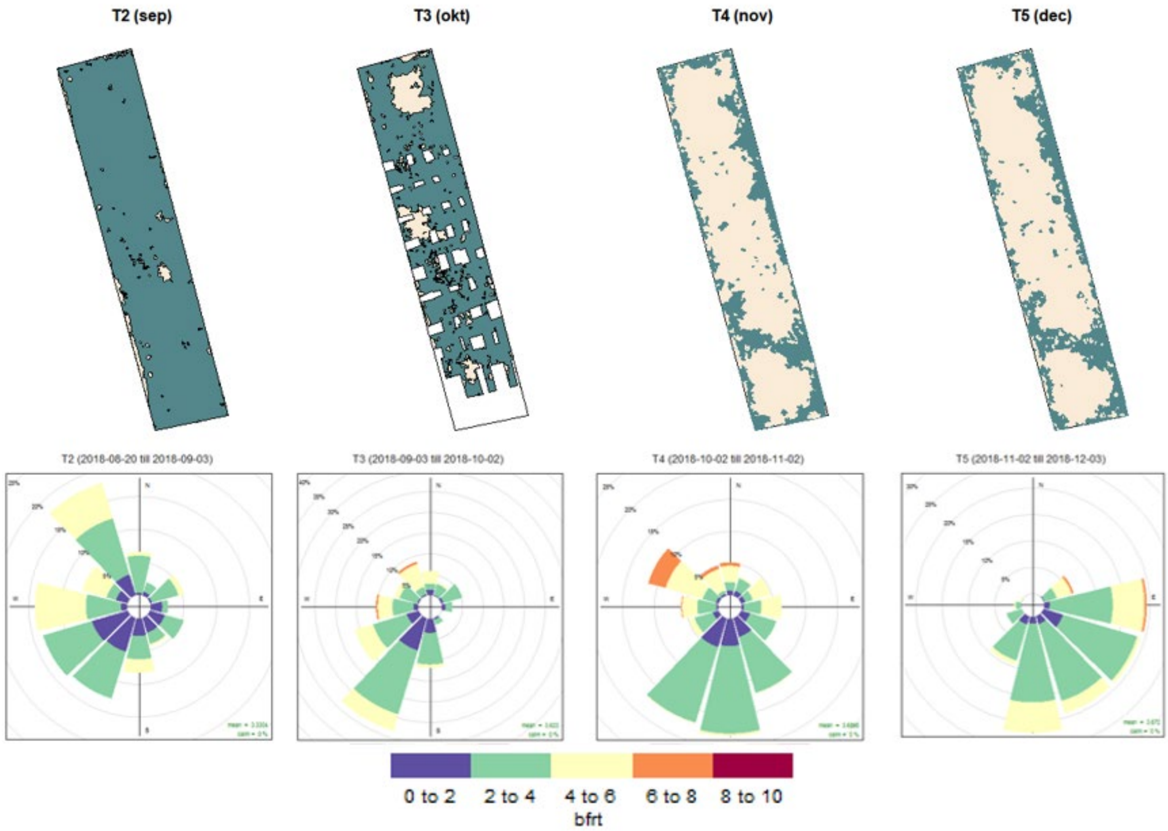

Figure 45. Top panels show the coverage of the sisal net with mussels (blue collord shapes) for the sampling events T2, T3, T4 and T5. On T3 the overlap of individual pictures was in some cases insufficient to construct a mosaic covering the entire surface. Not mapped areas are shown in white, mapped areas without mussel in yellow. The figures in the lower panel show the wind direction and wind speed between sampling events T1-T2 (far left), between T2 and T3 (left-middle), between T3 and T4 (right-middle) and between T4 and T5 (far right).

4.3.3 Mussel population

Figure 46, Figure 47 and Figure 48 show, respectively, the mussel abundance, mussel shell length and the ash-free dry weight of the mussel flesh from mussels in the mussel patches. Differences in these parameters between the nets and between the sampling events were investigated using linear models with net and sampling event as explanatory variables. The software package R (R Core Team, 2020) was used for the analysis. Model results are included in Appendix 3.

During sampling, it was observed that the sediment under the mussels was often black coloured, indicating low oxygen levels directly under the first mussel layer.

The abundance of mussels found in the mussel patches depended on the sampling time but not on the net material (see model results in Appendix 3). Figure 46 shows that on all nets the abundance of mussels in the samples gradually decreased from about 30,500 mussels per m^2 at T1 to 7,400 mussels per m^2 at T5. Note that here the abundance is given of the mussel patches, to obtain the abundance of mussels for each net, abundances need to be multiplied with the mussel coverage of the nets.

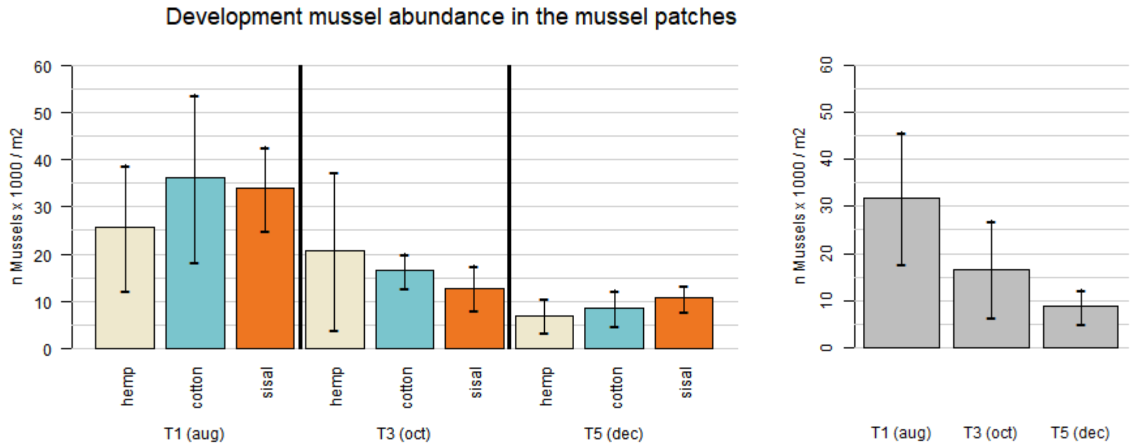

Figure 46. The left panel shows bardiagrams of the mussel density from the nine mussel patches sampled in each net and for the sampling events T1, T3 and T5. The right panel shows bardiagram of the mussel density for the sampling events T1, T3 and T5 for all three nets together. The vertical lines show ± 1 times the standard deviation.

Figure 47 and Appendix 3 show that the shell lengths of the mussels present in the samples increased slightly over time. At T1 an average length of 15.5 mm was measured and at T5 it averaged at 16.8 mm. The average shell length of mussels sampled on the net made of cotton was slightly smaller (-0.7 mm) than the shell length of mussels collected on hemp while the shell length of mussels collected on the net made of sisal was slightly larger (+0.7mm) than that of mussels collected on the hemp net. Note that the average shell length of mussels can increase due to mussel growth but also due to the disappearance of smaller mussels.

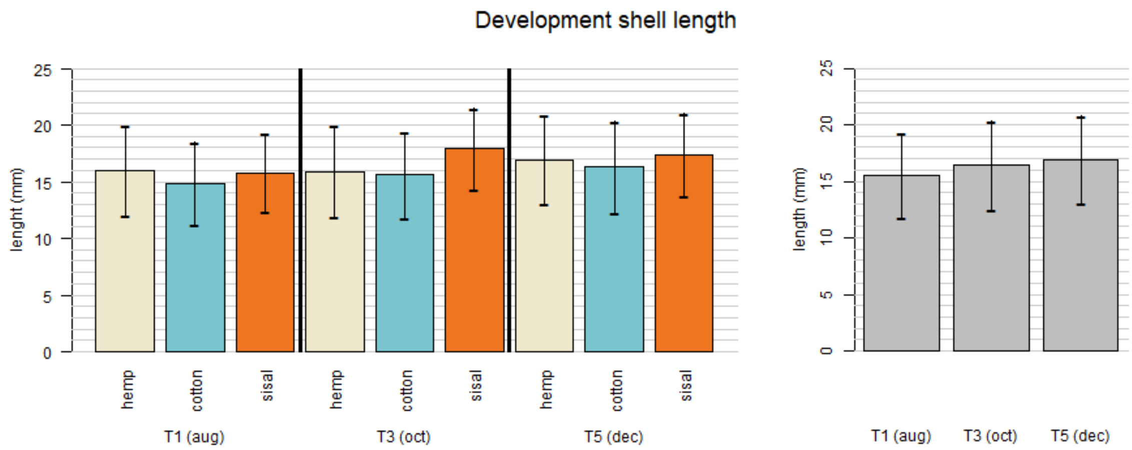

Figure 47. The left panel shows bardiagrams of the shell lengths of the mussels in the nine mussel patches sampled in each net and for the sampling events T1, T3 and T5. The right panel shows bardiagrams of the mussel shell lengths for the sampling events T1, T3 and T5 for all three nets together. The vertical lines show ± 1 times the standard deviation.

Figure 48 and Appendix 3 shows that the ash-free dry weight of the mussel flesh decreased between each sampling event. The ash-free dry weight decreased from 187 g/m^2 at T1 to 78 g/m^2 at T3 and to 43.8 g/m^2 at T5. The difference for the net made of sisal (see Appendix 3) that was found is caused by a higher weight of the mussels at the start of the trial for the net made out of sisal. No net differences in mussel biomass were found between nets after T1 as was shown by a model that included only T3 and T5. Also the fresh weight of the mussels (no diagram included) decreased. This is the weight of the mussels including shell and adherent fluid. On T1 an average fresh weight of 11.3 (±4.2) kg/m^2 was measured, on T3 this had decreased to 7.8 (±3.8) kg/m^2 and on T5 this decreased further to 4.9 (±2.2) kg/m^2.

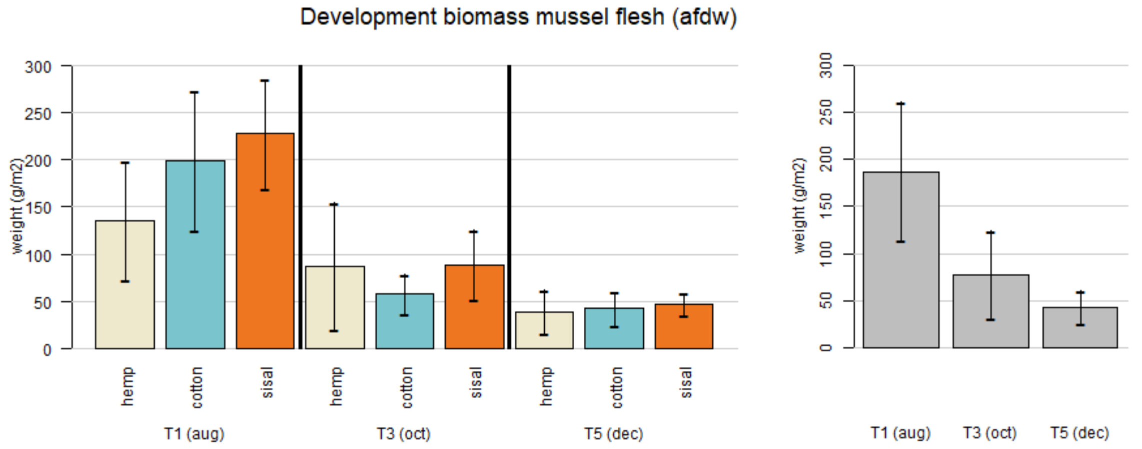

Figure 48. The left panel shows bardiagrams of the ash-free dry weight (afdw) of the mussel flesh from the nine mussel patches sampled in each net and for the sampling events T1, T3 and T5. The right panel shows bardiagram of the ash-free dry weight (afdw) of the mussel flesh for the sampling events T1, T3 and T5 for all three nets together. The vertical lines show ± 1 times the standard deviation.

Figure 49 shows the ash-free dry weight for mussels of various length classes and for the different sampling events. In order to adjust for differences in abundance, weights are expressed per individual. The figure shows that, especially for the larger individuals, the ash-free dry weight decreased over time. The ash-free dry weight of mussels up to a shell length of 20 mm decreased by about 30% between the T1 and T5 sampling events and for mussels with a shell length >20 mm by about 40% in the same period.

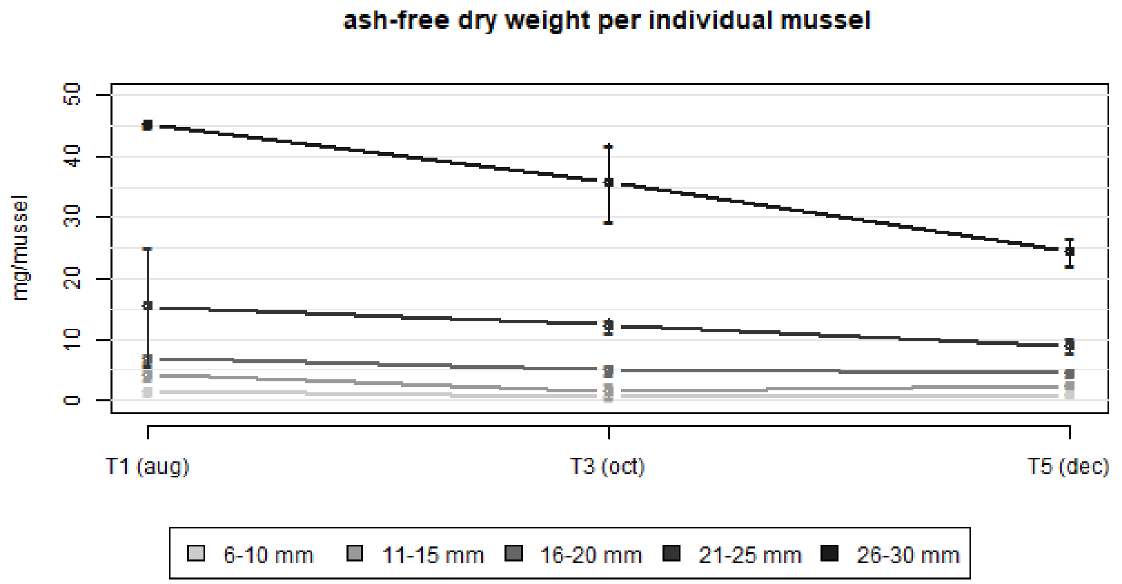

Figure 49. The mean ash-free dry weight of individual mussels per length class and for the sampling events T1, T3 and T5. The vertical lines show ± 1 times the standard deviation.

The mussel samples were taken in late summer, autumn and early winter. During this period, approximately one third of the mussel population in the mussel patches disappeared and the biomass of the mussels decreased by approximately 30-40%, which combined resulted in an 80% decrease in the ash-free dry weight of the mussels in the patches. The mussels present on the mussel patches have therefore been deprived of their fat reserves. This is in itself what you would expect when mussels from subtidal areas are transported to tidal areas and during winter months. The mussel population has also been reduced by mortality and erosion of mussels. It is unlikely that predation by birds or crabs played an important role in the disappearance of the mussels. Birds were never observed when entering the area for fieldwork nor footprints or other signs for the presence of birds were found. Crabs were also not observed during field trips.

4.3.4 Degradation of the nets

It was observed that the mussels completely encapsulated the ropes with their byssus threads, see Figure 50. These structures were removed first in order to inspect the rope itself.

Of the different rope types studied here, nets made from cotton degraded the fastest. From the first sampling event up to and including the T3 sampling event in October, it was still necessary to cut or slice cotton rope pieces in order to collect them. In later sampling events this was not required anymore and rope pieces could be collected by lightly pulling on them. Inspection at the laboratory showed that the cotton rope was already disintegrating strongly at the T3 measurement, see Figure 50. For the ropes made of hemp and sisal this was much less the case. The sisal rope appeared to remain intact the longest, although this rope also showed traces of degradation over time. In the last sampling event (T5) the sisal rope also loosened easily when pulled, but the structure did not disintegrate under the influence of wind and current alone. This was the case for the net made of cotton ropes, where net sections came loose as a result of wind and water currents and were spread across the tidal flat. The rope made of hemp took a middle position in terms of degradation speed.

Figure 50. Far left byssus threads of mussels completely encapsulating the ropes of the nets. Ropes made of cotton from the T1 sampling event (top middle) and from the T3 sampling event (bottom middle). Ropes made out of sisal of the T1 sampling event (upper right) and the T3 sampling event (lower right).

4.4 (interim) conclusions trial 1

The following conclusions can be drawn from the observations that have been made by execution of trial 1.

The following conclusions can be drawn regarding the performance of the nets in terms of capture of mussel seed and degradation of the ropes.

- Of the two locations used, only the nets placed in the inner harbour were successful in capturing mussel seed.
- It is not likely that there was no mussel spat present in the water of the tugboat harbour. Some individuals of mussel spat were found on the nets and at the installations of the mussel farmer nearby as well as on the nets in the inner harbour spat was successfully collected showing the presence of mussel spat in the area. The presence of silt in the inner harbour and the

accumulation of it on the nets as well as the predation pressure of juvenile crabs are more likely reasons for the unsuccessful spat collection on the nets.

- The successful collection of mussel spat on the nets hung out in the inner harbour showed that on each of the net materials studied here (hemp, cotton and sisal) mussel spat can be collected.
- Because only one net of each net material was available, differences in spat collection rates between the net types could not be investigated statistically. No standout differences were observed in the amount of spat collected on the nets however.
- The mussels first attach themselves to the net with byssus threads that at the end completely encase the ropes of the nets.
- Due to the firm attachment of the mussels to the nets and each other, just a few mussels came loose during transportation to and placement on the tidal flat.
- The net made of cotton degrade the fastest, the net made of sisal the slowest. Hemp took a middle position.

The following conclusions can be drawn regarding the development of the mussels once placed on the tidal flat.

- The test site of trial 1 proved to be unsuitable for the survival of mussels; all mussels that ended up outside the nets at any point in time were not found during subsequent visits. The presence of a layer of mud with a thickness of several centimetres probably contributed to this.
- Only the presence of the nets allowed the mussels to survive at the test site for several months.
- Over a five-month period, most of the mussels eroded from the nets regardless of the construction material used.
- It was observed that the mussels eroded less rapidly on the net made of sisal and most rapidly on the net made of cotton, but differences could not be investigated statistically. It might be related to a difference in degradation rates between the nets, which was slowest for sisal and fastest for cotton. Due to the absence of replicas this could be a tentative hypothesis that should be investigated further.
- The mussels move upwards, especially in the middle of the nets, probably due to competition for food. These mussels are the first to disappear.
- The mussels appeared to survive best on the edges of the nets, possibly because there is more water exchange and less competition for food. Apparently the mussels at the edges were not inclined to detach themselves and move upwards as the mussel in the central parts of the net did.
- The influence of elevated wind speeds might have led directly, and/or indirectly via wave action, to erosion of mussels. In periods with above average wind speeds (more than 5-6 Beaufort), most mussels eroded from the nets.
- The mussels have been metabolized into their fat reserves and the population has been depleted in five months. Certain reductions in mussel fat reserves and mussel densities during autumn and winter are a natural phenomenon explained by the lower availability of food and the lower quality of food during those months. The mussels were probably also well-fed at the time they were placed on the tidal flat. During collection on the nets the mussels were submerged all the time and could feed continuously while once placed on the tidal flat they were emersed part of the day during which they could not feed.

It should be noted that the summer of 2018 was extremely hot and dry. With an average of 18.6 °C, the summer of 2018 was 1.9 °C warmer than normal and the warmest summer since at least 1706 (KNMI). The high temperatures were associated with the observed extremely high cockle mortality on tidal flat in the Dutch Wadden Sea (Troost and Asch, 2018). It is expected that at high temperatures mussels spend a relatively large amount of energy on their metabolism and have more difficulties in maintaining their oxygen balance. It is possible that the weather extremes also influenced the development of the mussels on the nets.

The following recommendations were made for follow-up trials:

- Do not use the tugboat harbour but the inner harbour for collection of mussel seed on the nets.
- Select a site on the tidal flat with a slightly more dynamic current regime to reduce the chances for food limitation, oxygen depletion and mud accumulation.

- Enlarge the surface area of the net edges where chances for food limitations and oxygen depletion are lower.
- Transplant the mussel nets to the tidal flat as early in the season as possible so that the mussels can still make use of the algae present during the growing season. Obtaining a high density of mussels at the start is of less importance because the population will likely to be depleted anyway.

5 Trial 2

5.1 Collection of mussel spat

Six nets (two of each material) were still available after completion of trial 1. Despite the fact that these nets were rinsed, sun dried and stored in a dry and dark place, all of them, especially the nets made of cotton, showed traces of wear. The nets were therefore reinforced by the C.I.V. in den Oever and repaired. In order to strengthen the nets, five ropes running across the width of the nets (one at each end, one in the middle and one between the middle and the end on either side of the middle) were attached. In addition, vulnerable sections were repaired with the original rope material. After this, the nets were transported to Germany to collect mussel spat.

Because mussel spat capture was unsuccessful at the tugboat harbour at trial 1 all nets were hung out in the inner harbour on the 8th of May 2019.

On the 16th of May the first sampling took place. Two ropes were removed from each net to determine the presence of mussel at the SaM laboratory in Wilhelmshaven. No mussels were found in any of the samples.

The next net sampling took place on 20th of June. Again no mussels were found in the samples. At that time the SMC installations of David de Leeuw in the Jade had been colonized by mussel spat (personal communication). Again on June the 27th net samples were taken but no mussel seed was found. The ropes were heavily colonized with filamentous algae and barnacles. On 4th of July a final sampling took place where on three of the 18 rope samples some individuals were found. The mussel seed density was totally inadequate and it was decided not to wait any longer for colonization of the nets with mussel spat.

Due to the unsuccessful spat collection on the nets, it was decided to use mussel seed from an SMC installation of a commercial party instead. The nets (without mussel seed) are then stretched over a bed of previously applied mussel seed. The disadvantage of this approach is that the mussels are not anchored to the nets when they become subject to the tidal forces at the tidal flat. The mussels can only start making the necessary attachment structures after both the net and mussels are placed at the tidal flat. In addition the placement of the mussels on the tidal flat was rescheduled from early July to late July and less of the growing season could be utilized by the mussels. The mussel company Prins en Dingemanse was found willing to supply the required mussels for trial 2. These mussels came from SMC installations located in the western Dutch Wadden Sea.

5.2 Changes in trial design

The experiences gained from trial 1 lead to several changes in the design for trial 2. First of all the designated test site was reassessed. On 21 June, the Hond and Paap tidal flat was visited in order to find a location with a more sandy bottom as it indicated a more dynamic regime of water flows which is believed to be conducive for mussel survival, see paragraph 4.4. A suitable location was found east of a natural mussel bed located on the northern part of the Hond and Paap tidal flat, see location indicated with 'L2' in Figure 31. Next to the more dynamic character of the test site the trial site is located in an area where a mussel bed was present in 2016 to 2017 but which has since been diminished in the area. The historical presence of a mussel bed is also believed to be conducive for the survival of mussels, see paragraph 3.5.

A second adjustment was made to enlarge the net edges. This was realized by cutting the nets into pieces of approximately 2 by 2 meters by which the edge area was increased by 66%. A final adjustment in the trial consisted of testing the effect of a foundation of (dead) cockle shells in the survival of the mussels. This addition was not a result of experience gained at trial 1 but from insights of recent conducted experiments. Capelle et al., (2019) showed in flume- and field experiments that the erosion of mussel could be reduced when attached to cockleshells. In addition to this it is known that natural mussel beds form regular on a bed of (living) cockles preventing erosion (Dankers and Fey-Hofstede, 2015).

These changes led to a trial design consisting of two plots in which areas with mussels (a square area of 2 by 2 meter, further referred to as 'tiles') are alternated with open areas without mussels. In plot 1, all tiles consist of a layer of mussel spat covered by nets. The tiles of plot 2 had a different set up to test the effect of the nets and a foundation of dead cockle shell in the preservation of mussels. For this reason the tiles in plot 2 included tiles with mussels and nets (similar to plot 1) but also tiles with only mussels and no nets and tiles with a fundament of dead cockle shells, both with- and without nets.

The tiles of plot 1 were placed on a grid of 6 rows of 3 tiles with a spacing of about 2 meters between the tiles, see Figure 51. The three tiles in each row contained one net of each material type. The nets were distributed in such a way that the different net construction materials are located at different positions in the row. Plot 2 consists of 4 rows of 3 tiles. The distance between the tiles within each row was approximately 2 meter and a distance of about 5 meters was kept between the rows. The tiles of the first row (row number 7 in Figure 51) consisted of mussels and a net (from each material one). The second row from the top (row 8) consisted of only mussels and no nets or cockle shells. In the tiles of rows 9 and 10 a bed of cockleshells is placed under the mussels. At the tiles of row 9 no nets were applied while at the tiles of row 10 nets (one of each material) were spread over the tiles. See Figure 51 for the layout of both plots.

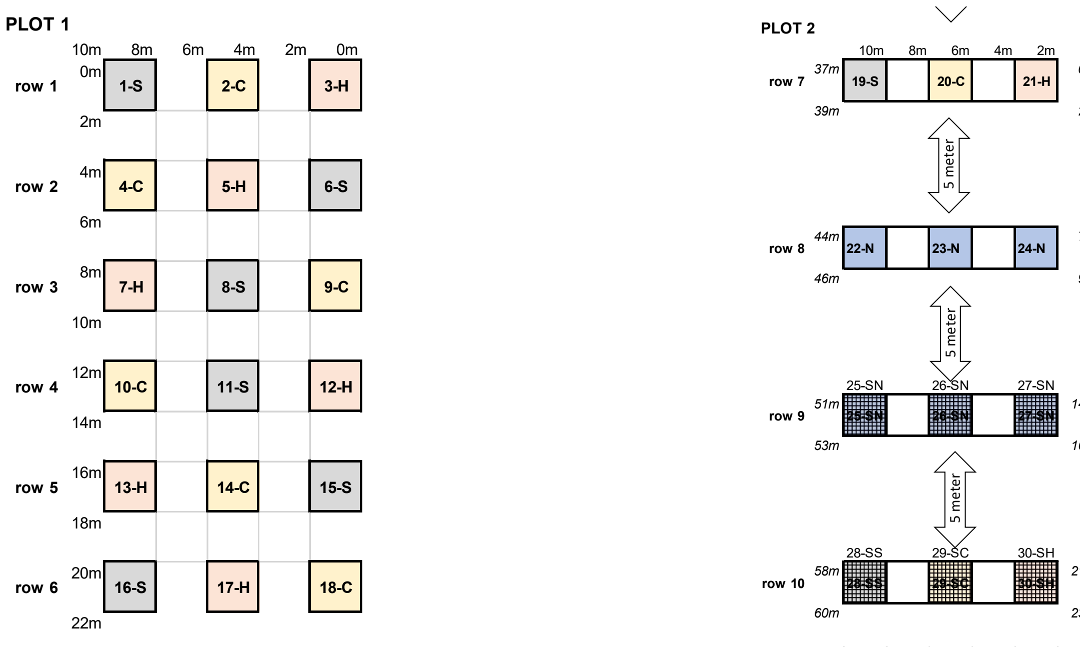

Figure 51. Layout of plot 1 (left) and plot 2 (right) of trial 2. Tiles covered with nets made of sisal are depicted with grey blocks, of cotton with yellow blocks and of hemp with orange blocks. Locations without nets are depicted with blue blocks. Under the tiles of the last two rows of plot 2 (row 9 and 10), a bed of dead cockleshells has been placed. This is depicted with a black grid.

Plot 1 and 2 are positioned approximately parallel in longitudinally direction to the eastern outer edge of the natural mussel bed, which lies at an angle of about 140 degrees. Plot 2 is located south of plot 1 at a distance of about 20 meters.

5.3 Construction of trial 2

At the visit of the tidal flat on 21th of June, bamboo sticks were placed in the mudflat to mark the test sites of plot 1 and 2 of trial 2 for orientation purpose for the construction of the plots at a later moment in time, a bed of dead cockle shells was spread out at the designated tile locations, see Section 5.2. The high tide of Friday 26th of July 2019 was used to jettison the cockleshells and at the following low tide a foundation of dead cockleshells of approximately 10 cm high was constructed at the intended locations using a rake.

On Monday 29th of July approximately 3000 kg of mussel seed in several big bags were delivered by Prins en Dingemanse to the NIOZ harbour on Texel. On Texel the mussels were placed in jute bags weighing approximately 25 kg. The jute bags were placed in plastic cubic containers and kept cool with water for transportation. The same day the mussels were transported to the Eemshaven. On the same day the nets were cut up into 2 by 2 meter pieces, labelled and transported from Wilhelmshaven to the Eemshaven. In the Eemshaven, cubic containers were filled with the jute bags and placed on the *ZILVERVISJE* with the help of the crane of the *HARDER*. The high tide of Tuesday morning 30 July was used to transport the nets and mussels to the tidal flat with the *ZILVERVISJE* and under supervision of the *HARDER*. Four jute bags with mussels were placed overboard on each tile. The nets were also put overboard. Tuesday's low tide was used to further construct the tiles of both plots. First the tiles were marked with bamboo sticks. The mussels were removed from the bags and spread out with a rake on the tiles. After that the nets were installed and secured. Figure 52 contains a pictorial report of the work described here.

Figure 52. Image report of the construction of the tiles of trial 2. 'A', jettisoning of the nets and SMC mussels. 'B', view of test site at the start of the low tide of Tuesday afternoon. ‘C', distribution of the mussels on one of the tiles. 'D', stretching nets on the tiles. ‘E', completion of the installation.

5.4 Development of the nets and mussels

On average the nets of trial 2 fell dry for 26.8% of the time, see Figure 53. Due to changing moon phases and wind conditions, the duration of the emersion time varied during the course of the trial. Between the sampling moments the emersion time varied between 30.4% (in the period between the T11 and T12 sampling and averaged over both plots) and 25.9% (in the period between the T13 and T14 sampling and averaged over both plots). Due to a gradient in elevation, with lower values in the northeast and higher values in the southwest, the emersion times of the tiles of plot 1 and plot 2 differed. With and longer emersion time of 1.2% for plot 2 compared to plot 1 this difference was small.

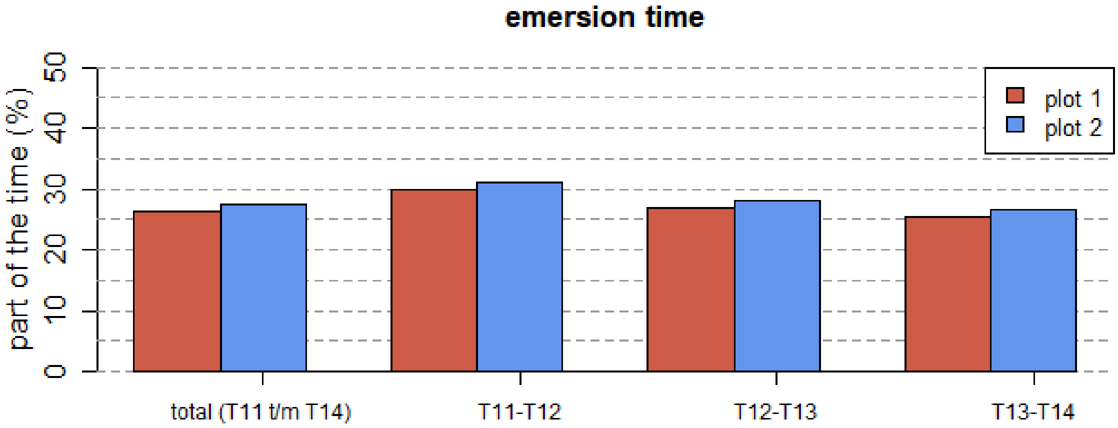

Figure 53 . Bardiagrams showing the average emersion time during trial 2 for plots 1 and 2 in the period between the sampling events of T11 and T14. Both the average emersion time between sampling events T11 and T14 is shown 'total' as for the different time intervals between the different sampling events. The CTD logger was removed at the T14 sampling event so no data was available in the period T14 to T15.

5.4.1 Surface covered with mussels

Immediately after installation, the mussels on the tiles positioned themselves above the nets. This was clearly visible at the first measuring moment (T11), one day after installation, see Figure 54.

Figure 54. The photo on the left shows the situation after installation of mussel tile '29SC' on Tuesday 30th of July. The mussels have been placed on a bed of (dead) cockle shells over which a net made of cotton has been stretched. The photo on the right shows the situation of the same tile on Wednesday 31th of July.

Figure 55 shows the area covered with mussels for both plot 1 and plot 2. Immediately after construction (T11), the total area covered with mussels in plot 1 was 76 m^2 and in plot 2 51 m^2. Over time the area covered with mussel decreased but in plot 2 the decrease was slower compared to plot 1. In plot 1 11 m^2 (14% from the start situation) was still covered with mussels one year after construction while at plot 2 there was still 21 m^2 (41% from the start situation) present.

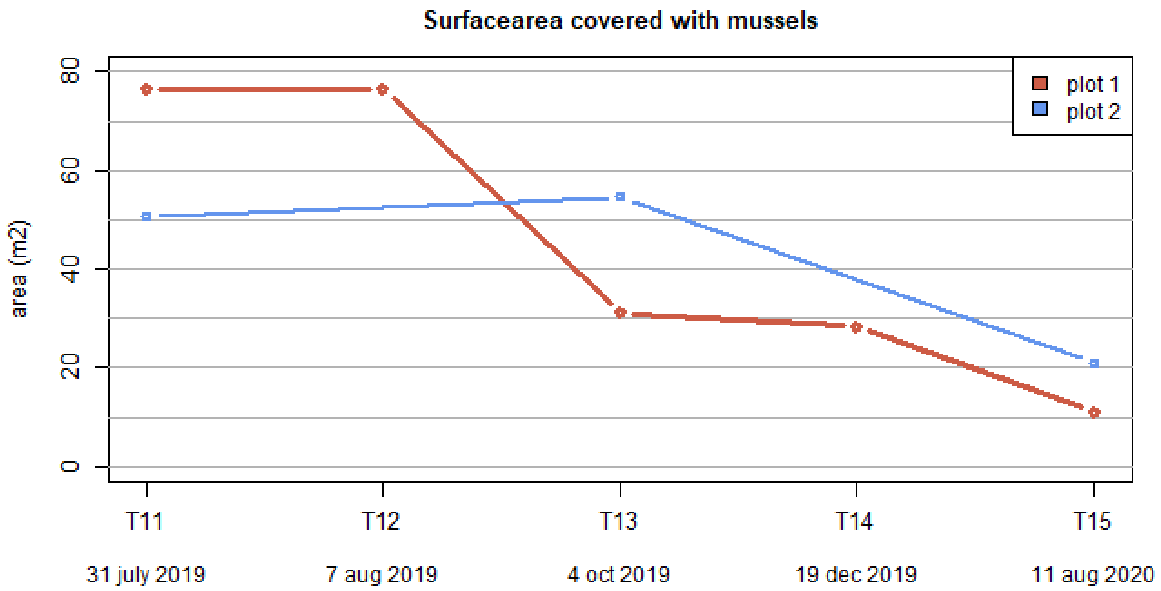

Figure 55. Surface area covered with mussels in square meters for the different sampling events and for plot 1 and plot 2 separately. Note that for plot 2 the surface area could not be determined at sampling events T12 and T14 due to time limitation during field work.

Figure 56 shows the number of mussel patches and the average patch size at the sampling events. In the first week after construction (sampling events T11 and T12) the average patch size was about 0.5 m^2 for both plots. At both sampling events there were large differences in size between individual patches, see the large standard deviations for T11 and T12 in Figure 56. Some patches covered almost the entire tile and were about 2 m^2 in size while others were a few tenths of a square meter in size. In the following (two) months both the average patch size and the differences in size of individual patches decreased while the number of patches increased, see the T13 sampling event in Figure 56. At that time a patch was on average about 0.03 m^2 in size and the number of patches increased with about a factor 10. Afterwards, in T14 and T15 sampling events (six months to one year after construction) the patch size did not changed much. After an initial increase in the number of patches in the first half year (T13 and T14), the number of patches decreased sharply one year after installation (T15), see Figure 58. This shows the disappearance of mussels patches as can be seen in Figure 55 as well.

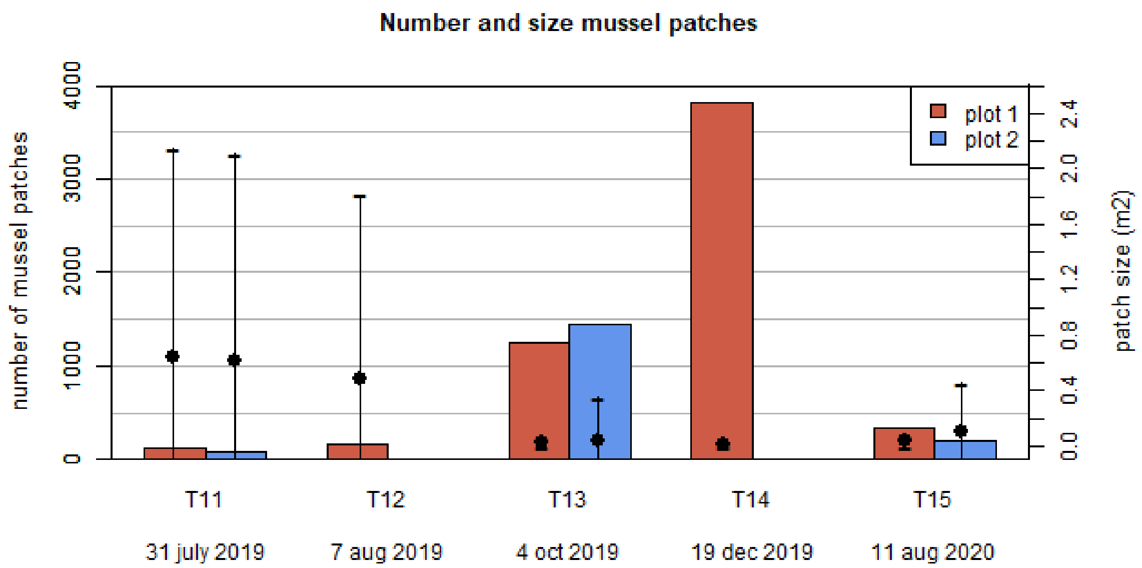

Figure 56. Number of mussel patches (bars) and mean (± 1 standard deviation) patch size (m^2) as mapped at the different sampling events for plot 1 and plot 2. Note that plot 2 was not mapped at T12 and T14.

Because in trial 1 the influence of the wind condition on the erosion and distribution patterns of the mussel patches became clear, the wind conditions during trial 2 were analysed as well. In Figure 57 the distribution of the windspeeds during the different sampling events in steps of 1 Beaufort are shown. Between the construction of the plots and second sampling event (T12), a period of 10 days, windspeeds stayed limited to 5 Beaufort at the maximum ('T0-T11' and 'T11 - T12' in the figure). Between the T12 and T13 sampling events (a period of 59 days) 11 separate periods with windspeeds over 6 Beaufort were recorded or 0.19 times per day on average. Between T13 and T14 sampling events (a period of 77 days) windspeeds exceeded 6 Beaufort more often when 27 separate events were recorded in total or 0.35 events per day on average. In the here investigated time consecutive periods with windspeeds over 6 Beaufort were most frequent encountered between the sampling events of T14 and T15 (a period of 235 days). Then 108 events were recorded or 0.46 events per day on average. Windspeeds of 8 Beaufort (stormy) were only found once between the sampling events of T12 and T13 and 7 times between the T14 – T15 sampling, see also Figure 57.

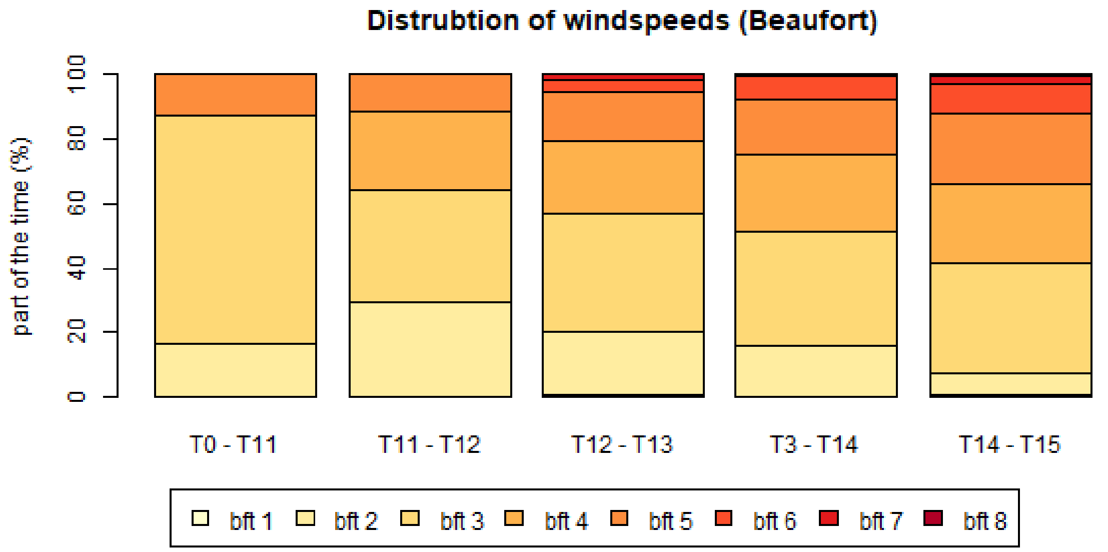

Figure 57. Distribution of the hourly average windspeeds in Beaufort (Bft) between the different sampling events and as measured at KNMI weather station Lauwersoog.

Figure 58 to Figure 64 show the spatial distribution of the mussel patches as recorded at the different sampling events. The distribution of the wind direction and -speed as measured by the KNMI weather station in Lauwersoog and for the period in between the sampling events are shown as well in the figures.

In the first week after installation, almost all the mussels are on the 'tiles', which are also still completely covered with mussels, see Figure 58 (T11) and Figure 59 (T12). At the T12 sampling 11 days have passed since the plots were constructed. In this period wind speeds were limited to 5 Beaufort.

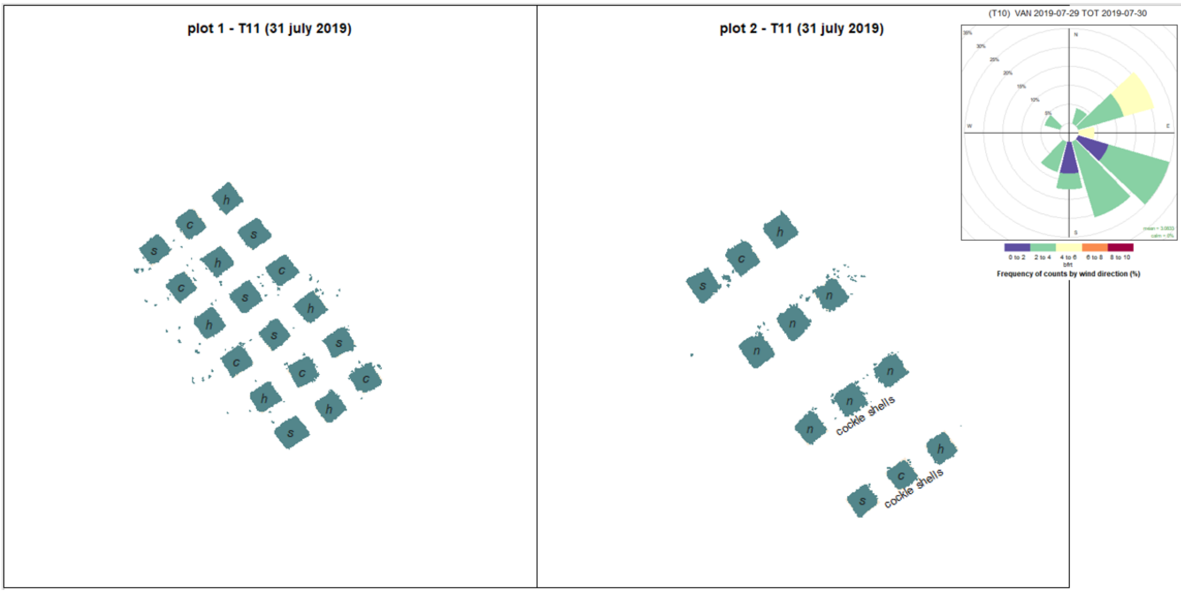

Figure 58. Spatial coverage of musselpatches as found at the T11 sampling event for plot 1 (left panel) and plot 2 (right panel). Areas covered with mussel patches are depicted in azure color. The tiles are shown in beige tint (only visible when not covered by mussels). Nets made of sisal are marked with 's', those made of cotton with 'c', those made of hemp with 'h', and places without nets with 'n'. The top right panel shows winddirection and -speed in the time period between the T0- and T11 sampling events.

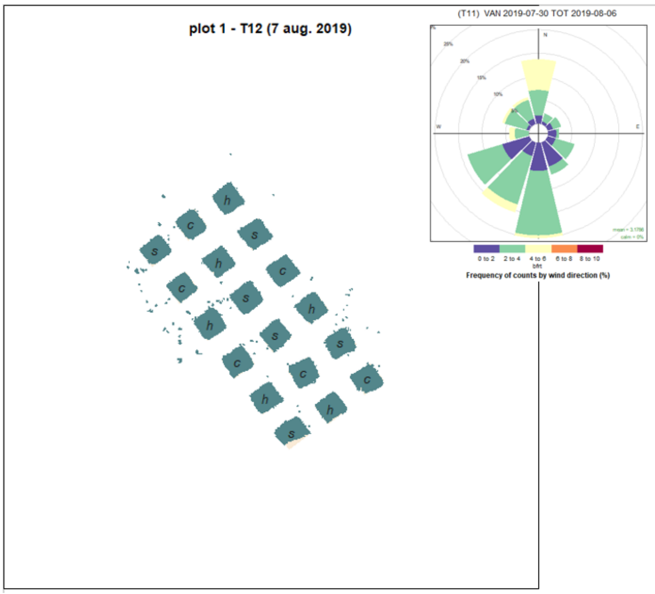

Figure 59. Spatial coverage of musselpatches as found at the T12 sampling event for plot 1. Areas covered with mussel patches are depicted in azure color. The tiles are shown in beige tint (only visible when not covered by mussels). Nets made of sisal are marked with 's', those made of cotton with 'c', those made of hemp with 'h', and places without nets with 'n'. The top right panel shows winddirection and -speed in the time period between the T11- and T12 sampling events. The part of the tile at the lower left side that is not covered with mussels is due to the incomplete recording of that area, and not because mussel are absent.

The Wadden Unit took photographs of plot 1 and 2 from a plane, one day after the T12 sampling events, see Figure 60 and Figure 61. These photographs show that also the tiles of plot 2 (not recorded in the field at T12) are completely covered with mussels. Furthermore it can be seen on the photographs that by the construction of the tiles with a foundation of cockle shells some of the shells ended up in areas northwest of the tiles, see the black ovals in Figure 61.

Figure 60. Aerial photographs of plot 1 and plot 2 of trial 2 taken by the Wadden Unit from a plane on August 8th 2019. In the photopgraph on the left all tiles of plot 2 are shown. In the photograph on the right some of the plot 2 tiles can be seen (left side of the image) and all tiles of plot 1 (right side of the image).

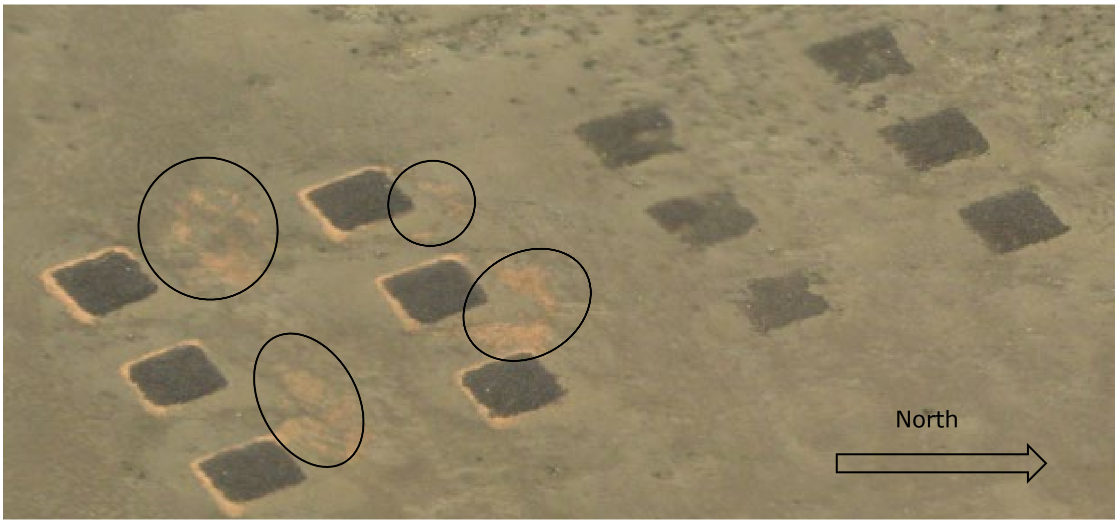

Figure 61 . Detail of aerial photograph of plot 2. With the balc ovals areas outside the mussel tiles are shown where some of the cockle shells ended up.

Two months after construction (T13 sampling event) the mussels were spread over a larger area and just a few were found at their original positions on the tiles, see Figure 62. Most were found in areas outside the plot in northeasterly direction of the plot. A smaller part of the mussel were found in areas in between the tiles and outside the plot in the other directions. In the time period between the T12 and T13 sampling event windspeeds up to 8 Beaufort were measured. The dominant wind direction was southwest. This has probably caused the (dominant) northeasterly movement of the mussels. Remarkably, mussels placed on a bed of dead cockleshells (bottom two rows of tiles of plot 2) were relocated to a much lesser extent, see Figure 62. On tiles where not only cockles but also a net has been stretched, the mussels moved the least.

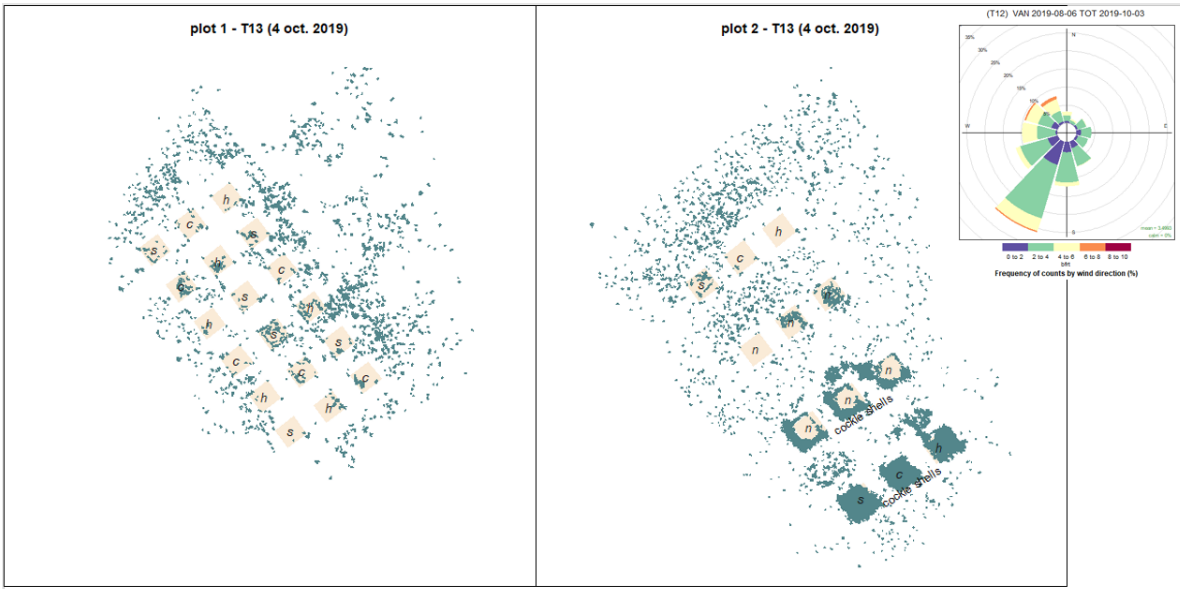

Figure 62. Spatial coverage of musselpatches as found at the T13 sampling event for plot 1 (left panel) and plot 2 (right panel). Areas covered with mussel patches are depicted in azure color. The tiles are shown in beige tint (only visible when not covered by mussels). Nets made of sisal are marked with 's', those made of cotton with 'c', those made of hemp with 'h', and places without nets with 'n'. The top right panel shows winddirection and -speed in the time period between the T12- and T13 sampling events.

At T14, six months after construction, the mussels of plot 1 have become more scattered around the area compared to the T13 situation, see Figure 63. Plot 2 was not successfully mapped at this sampling event. However, it was observed in the field that larger mussel patches were present on and near the tiles containing a bed of cockles shells. The dominant wind direction between the T13 and T14 sampling events was southwesterly and speeds up to 7 Beaufort were recorded.

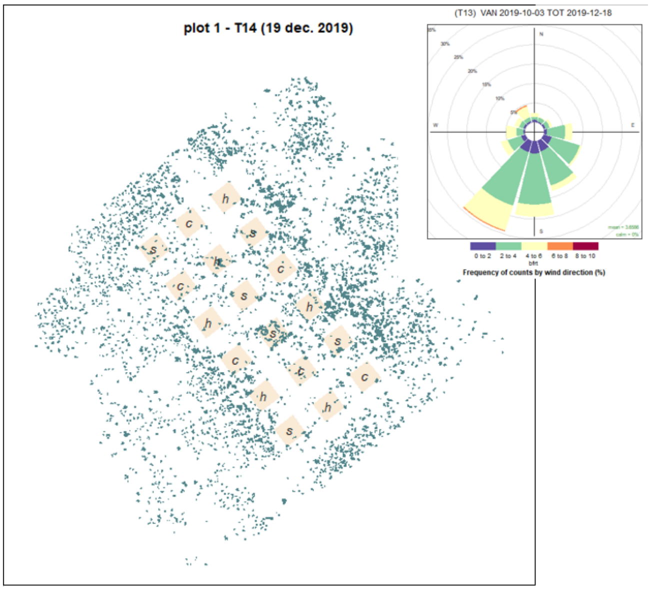

Figure 63. Spatial coverage of musselpatches as found at the T14 sampling event for plot 1. Areas covered with mussel patches are depicted in azure color. The tiles are shown in beige tint (only visible when not covered by mussels). Nets made of sisal are marked with 's', those made of cotton with 'c', those made of hemp with 'h', and places without nets with 'n'. The top right panel shows winddirection and -speed in the time period between the T13- and T14 sampling events.

One year after construction (T15) most of the mussels have disappeared from the area, see Figure 64. The largest areas covered with mussels were found at- and around the borders of the tiles containing a bed of cockleshells, see also Figure 64. No clear differences in mussel coverages could be found between the cockle shell containing tiles that included nets and tiles that did not. On the cockle containing tiles most mussel patches were found along the south-easterly and south-westerly sides of the tiles. Remarkably, in areas where cockleshells were left during construction, see text above and Figure 60, relatively large numbers of mussel patches were found as well.

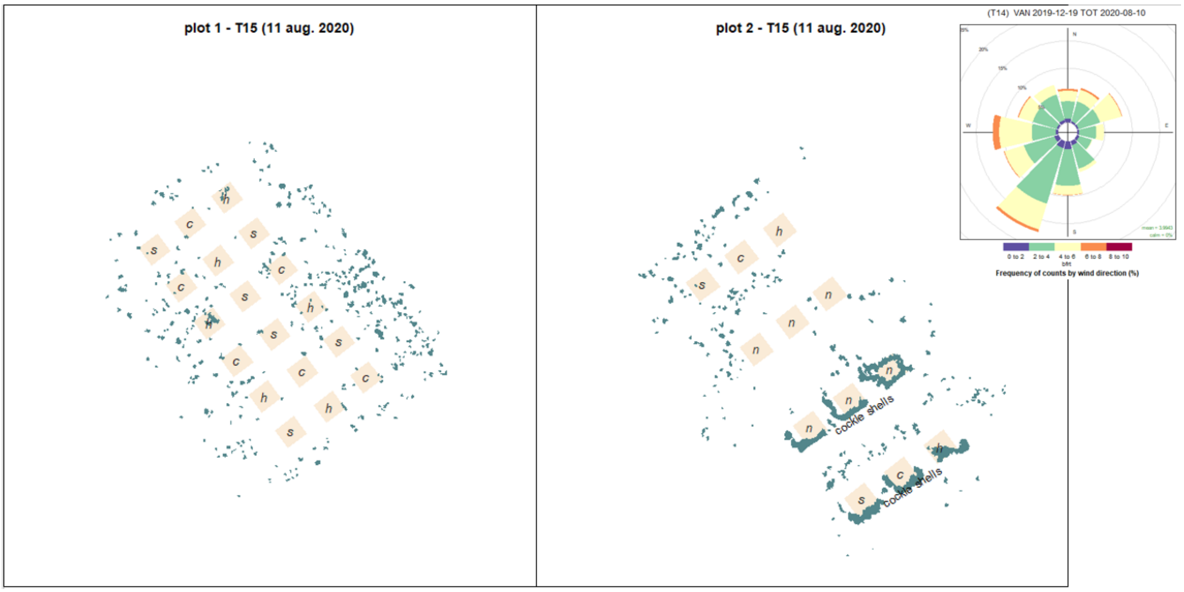

Figure 64. Spatial coverage of musselpatches as found at the T15 sampling event for plot 1 and plot 2. Areas covered with mussel patches are depicted in azure color. The tiles are shown in beige tint (only visible when not covered by mussels). Nets made of sisal are marked with 's', those made of cotton with 'c', those made of hemp with 'h', and places without nets with 'n'. The top right panel shows winddirection and -speed in the time period between the T14- and T15 sampling events.

Figure 65 show some of the photographs that were taken in the field during the T15 sampling event. The photos show the situation of plot 1 (pictures on the left side of the figure) and the mussel patches that were found on the tiles with cockleshells of plot 2 (pictures on the right side). Most of the mussels that were still present in plot 1 were attached to shell debris as can be seen in Figure 65. The detailed photos in the bottom (middle and right) show that the mussels at the cockle tiles have partially dug themselves in.

Figure 65. Situation one year after the construction of mussel plots 1 and 2 (measurement time T15, 11 August 2019). Photo top left shows the overview situation of plot 1 taken from the south. Photo top right, the tiles with a substrate of dead cockle shells in plot 2. Photo below left shows a detail of photo above left (the black framed area). Photo below centre, classification of two mussel patches from plot 1. Photo below right detail of mussels near a tile with a bed of dead clams.

Two years after contstruction (29th october of 2021) vital mussels could still be found at mussel patches located at and around the still intact tiles of plot 2 containing a foundations of dead cockle shells. Mussels were heavily colonized by barnacles.

5.4.2 Mussel population

Figure 66, Figure 67 and Figure 68 show respectively the mussel density, the shell length and the ash-free dry weight of the mussel flesh from mussels found in the mussel patches sampled during trial 2. Differences between net types, sampling event and presence of cockles were investigated by means of linear models with net (sisal, cotton, hemp or no net), presence of cockles (yes, no) and the sampling time (T12, T13, T15) as explanatory variables. The software package R (R Core Team, 2020) was used for the analysis. Model results are included in Appendix 4.

As has been observed during trial 1, also in trial 2 the sediment layer direct under the mussels was often black coloured, indicating anoxic conditions.

The mussel abundance in patches on plot 1 and 2 decreased significantly over time. Densities decreased from approximately 25,000 indiv/m^2 at the T12 sampling to approximately 6000 indiv/m^2 at the T13 sampling event, see Figure 66. After T13 the number of mussels decreased further to about 3500 indiv/m^2 at T15 but this decrease was not significant. The number of mussels did not depend on the presence of a net or the type of net. The mussel density was somewhat higher at tiles containing a foundation of cockle shells, but this was only the case for the T12 sampling event. At later moments in time the difference due to cockle shell disappeared. Figure 66 shows that only at the first sampling event (T12) the mussel abundance in the patches were considerably higher compared to those found at mussel patches in the natural bank. Due to the disappearance and a spread of mussels in the plots this was no longer the case for sampling events conducted later in time.

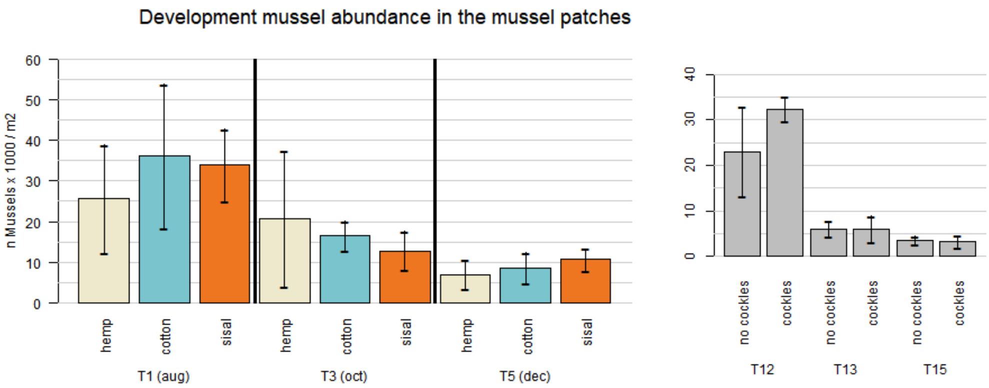

Figure 66. The development of the mussel density expressed as the number of individuals per square meter and for the samples taken at mussel patches in the tiles of plot 1 and 2 and at patches in the natural occuring mussel bed at the sampling events of T12, T13 and T15. The diagram on the left shows the situation broken down per sampling event, for tiles without nets and with the nets of different making and for the natural occuring mussel bed ('mussel bed'). Samples taken from plot 1 and plot 2 are combined in this diagram. In the diagram on the right the development of the mussel density is split up per sampling event and presence of a foundation of cockles. This diagram does not include data from the samples taken from the natural mussel bed.

There was no difference in the length of mussel shells whether or not a net was stretched, what material it was made of or if cockleshells were present, see Figure 67 and Appendix 4. Mussel shell lengths increased significantly only between the sampling times of T13 (16.7 mm on average) and T15 (21.5 mm on average). At the T12 sampling event the average shell length was 17.0 mm. Mussels collected at the natural occurring bed were, with a shell length of around 33 mm, larger compared to the mussels from plot 1 and 2. They did not seem to grow during the monitor period of one year.

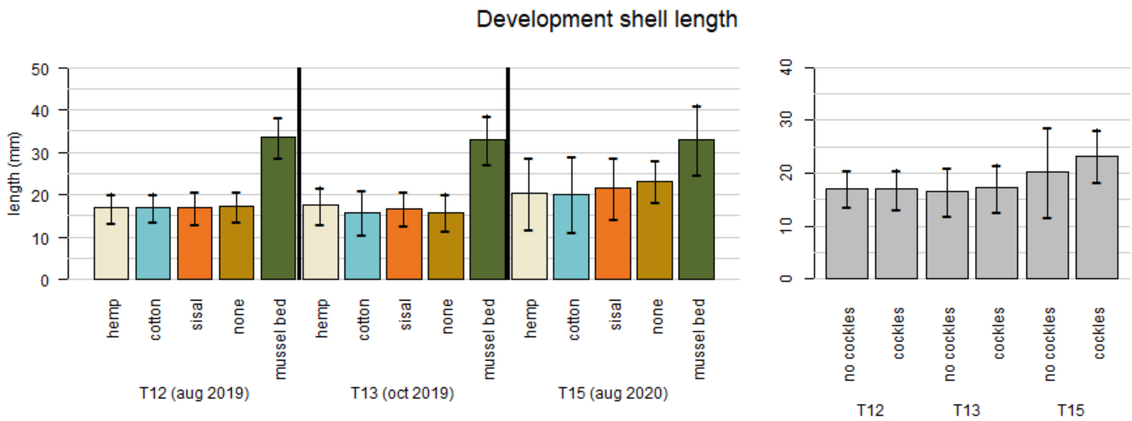

Figure 67. The development of the mussel shell lenghts in mm for the samples taken at mussel patches in the tiles of plot 1 and 2 and at patches in the natural occuring mussel bed at the sampling events of T12, T13 and T15. The diagram on the left shows the situation broken down per sampling event, for tiles without nets and with the nets of different making an for the natural occuring mussel bed ('mussel bed'). Samples taken from plot 1 and plot 2 are combined in this diagram. In diagram on the right the development of the mussel shell length is split up per sampling event and presence of a foundation of cockles. This diagram does not include data from the samples taken from the natural mussel bed.

The presence of a net nor the different net materials had an influence on the ash-free dry weight of the mussel flesh, see Figure 68 and Appendix 4. The ash-free dry weight was somewhat higher in areas with cockleshells, but this was caused by difference in weights at T12 only. At later times, weights were similar between sites with and without cockleshells. At the sampling event of T12 the ash free dry weight was about 300 mg/m^2 and at T13 it decreased (significantly) to 88 mg/m^2. At T15 the ash free dry weight was 95 mg/m^2 on average, but this increase compared with T13 values was not significant. The ash free dry weights of mussels found on the natural bed fluctuated between the sampling events, see Figure 68. At the T12 sampling events weights per square meter were comparable with those found at mussel patches of plot 1 and 2. Higher weights were found at the T13 and T15 sampling events, see Figure 68.

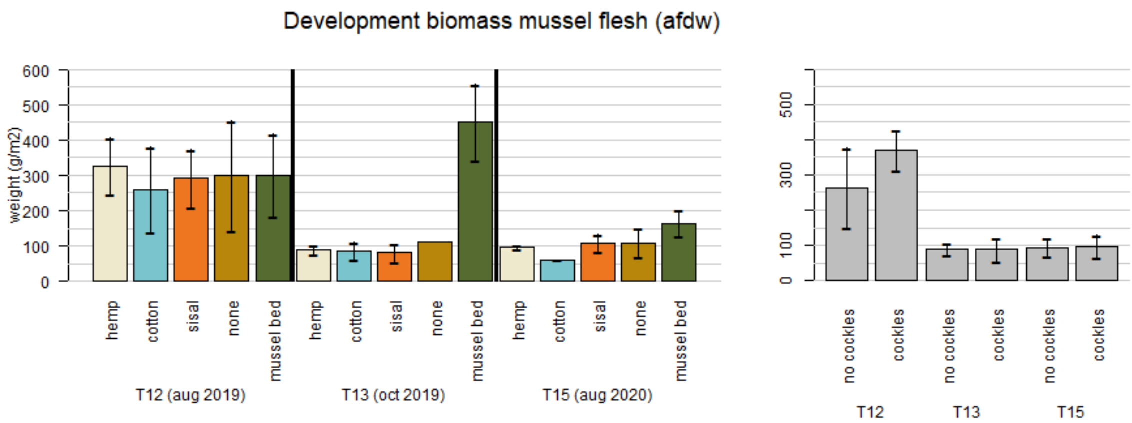

Figure 68. The development of the ash-free dry weight (afdw) of the mussel flesh expressed as grams/m^2 for the samples taken at mussel patches in the tiles of plot 1 and 2 and at patches in the natural occuring mussel bed at the sampling events of T12, T13 and T15. The diagram on the left shows the situation broken down per sampling event, for tiles without nets and with the nets of different making an for the natural occuring mussel bed ('mussel bed'). Samples taken from plot 1 and plot 2 are combined in this diagram. In diagram on the right the development of ash-free dry weight (afdw) of the mussel flesh is split up per sampling event and presence of a foundation of cockles. This diagram does not include data from the samples taken from the natural mussel bed.

Figure 69 shows the ash-free dry weight for various length classes of mussels and for the different sampling events. Weights are expressed per mussel and not per square meter in order to adjust for differences in mussel abundance between sampling events. This shows that only for the larger mussels (length class 26-30 mm) the ash-free dry weight decreased (25%) between the T12 and T13 sampling event. For the other length classes, the ash-free dry weight increased; between 20% and 48%, depending on the length class. Between the T13 and T15 sampling events, the ash-free dry weight increased only for the larger mussels (7%). For the other length classes, the ash-free dry weight decreased (between 7 and 50%). The largest relative decrease was found for the smallest mussels.

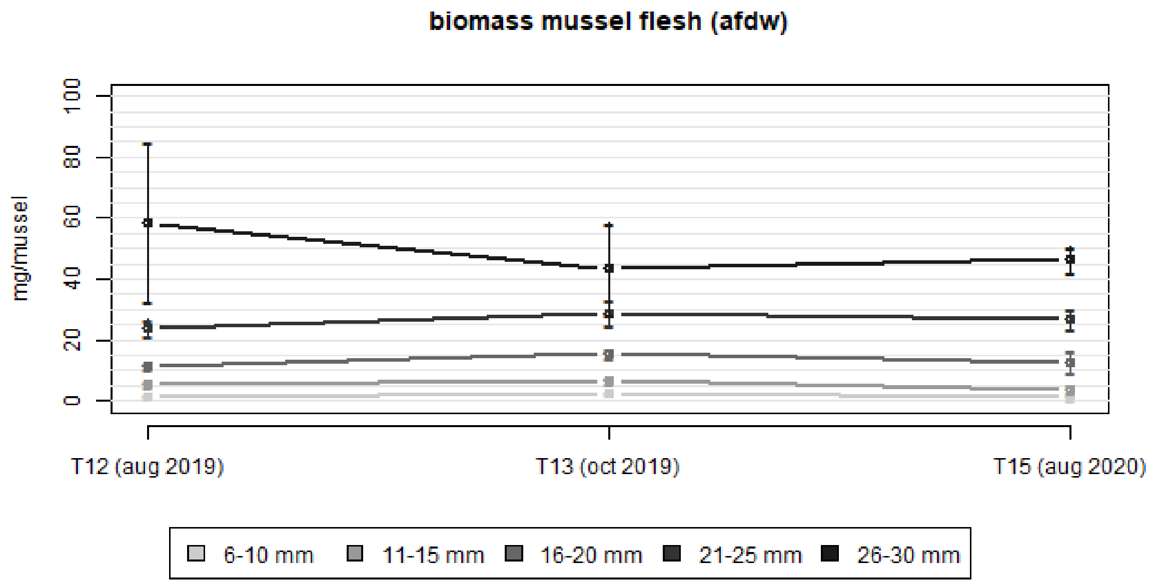

Figure 69 . The mean (± 1 standard deviation) of the ash-free dry weight (afdw) of individual mussels per length class and for the sampling events of T12, T13 and T15. Samples taken at the tiles of plot 1 and 2 are combined in the plot.

In summary, in the first winter after the trial was set up, the mussel abundance in the patches fell by about 70% and the mussel biomass by about 75%. Especially the larger mussels have reduced their fat content during this period. After the winter measurement in December, the mussel density did not decrease any further and a year after the mussels were released some small signs for growth could be observed.

5.5 (interim) conclusions trial 2

In summary, the following conclusions can be drawn from trial 2.

The following conclusions can be drawn concerning the functioning of the nets in collecting mussel spat and aiding the survival of mussels once placed on the tidal flat.

- The location used in the inner harbour is not every year reliable in the collection of mussel spat. The underlying reason is not known. Mud was not found on the nets and could not have hampered the collection of spat. It might have been that the amount of mussel larvae in the water was too low and or the waterflow over the nets was to limited preventing spat to come into contact with the nets or an earlier colonization of barnacles and filamentous algae on the ropes prevented mussel spat to settle on the nets.
- The value of nets in the preservation of the mussels on the tidal flat is absent or, at best, very limited when partly degraded and mussel spat is not captured and firmly attached to the net before placed on the tidal flat.

The following conclusions can be drawn regarding the development of the mussels on the tidal flat.

- The test site on the tidal flat used in trial 2 proofed to be suitable for the survival of the mussels. Most likely the absence of a layer of mud, the presence of dead shell material and better water exchange rates compared to the test site of trial 1 all contributed to this.
- It is likely that the mussel density on the tiles of trial 2 were not optimal but to high. This might have led to food limitation and oxygen depletions, motivating mussels to detach themselves and become more vulnerable for erosion.
- Dead shell material was frequently used by the mussels to attach themselves, helping in making the survival of a part of the mussels up to at least two years possible at trial site 2. Most likely the more open and more scattered shell-mussel patches that were formed in this way had a positive effect on the food- and oxygen supply to the mussels.
- Dead cockle shells proved to be an excellent substrate that can be supplemented to form the above described vital mussel patches consisting of dead shell materials and living mussels. Again the supplemented layer of dead cockle shells might have been sub-optimal (to high) leading to too condensed mussel patches that might have subsequently led to oxygen- and food depletion.
- Results show that especially in periods in which windspeeds exceeded 6 Beaufort erosion and dispersal of mussel patches took place.
- In winter months, the mussel population is depleted by about 70%. In the following spring limited growth is observed.

6 Discussion and conclusions

In this chapter the conclusions that can be drawn carrying out the pre-studies and both trials are discussed.

6.1 Environmental conditions at the Hond and Paap tidal flat

From the results of the (limited) pre-studies it was found that with the exception of areas around the tidal inlet between Borkum and Rottumeroog, the suspended solids concentration and the ratio between the suspended solids and chlorofyl-a concentrations are higher and the salinity levels are lower in the estuary compared to the other tidal basins in the Dutch Wadden Sea. In 25% of the (MWTL) measurements taken near the Hond and Paap tidal flat (the research area) the suspended solids concentrations exceeded 150 mg/l and in 11% of the samples concentrations exceeded 200 mg/l. Effects on the water filtration rate of mussels are likely at those concentrations. It is likely that mussels can endure short periods with high suspended solids conditions by closing their valves and cease food intake. But also long periods, up to a year, with above average suspended solids concentrations have occurred in the past and seem to form a risk for mussel bed survival.

Although the salinity levels found at the Hond and Paap tidal flat are lower than that of sea water (around 22 PSU), they are within the tolerance limits of mussels. In areas located south of the Hond and Paap tidal flat the salinity levels decreased further. At MWTL sampling station located in the inner estuary, Groote Gat noord, salinity levels below 10 PSU were regularly observed. Negative effects on mussels, such as a decrease in filtration rate, decrease in shell length and biomass at salinity levels below 10 PSU, cannot be ruled out. This leads to challenging environmental conditions for mussel(s)(beds).

Results of the survival experiment, carried out in this context, showed that conditions in the northern situated parts of the Hond and Paap tidal flat seem to be somewhat better compared to the southern parts of the tidal flat due to slightly higher salinity levels and slightly better suspended solids and chlorophyll-*a* ratios in the northern part. The formation of an intertidal mussel bed in 2016 on the Hond and Paap tidal flat and its survival up to at least 2020 show that natural establishment and survival for several years is possible. However, the sizes of the naturally occurring mussel beds fluctuates strongly in the area due to the overall natural fluctuations but also due to the position within the estuary with its complex environmental conditions and water chemistry. All results combined it seems that formation and survival of intertidal mussel beds is challenging but possible at the Hond and Paap tidal but also that especially prolonged suspended solids concentrations well above 150 mg/l seems to form a risk for bed survival.

When the growth rates of mussels that are sampled during trial 2 are compared to those found elsewhere in the Wadden Sea (sampled in the context of TMAP monitoring program), it can be seen that the growth of mussels on the Hond Paap tidal flat stays behind. The mussels from the trial 2 grew most in the months they spend on the SMC collectors when they were permanently submerged (reaching a length of up to 17 mm). In one year time on the tidal flat they grew by about 4 mm (up to 21 mm). Mussels from intertidal beds located west of Zuid Oost Lauwers grew by about 20 mm in their first year on the tidal flat (from about 5 to 25 mm the following year). Mussels from beds located on Horsbornzand (a tidal flat located east of Rottumeroog) grew by about 40 mm in their first year (Glorius et al., 2020). Mussels collected from the in 2016 naturally established bed on the Hond and Paap tidal flat show that they did not grow anymore after 2018. In 2020 (when the bank was 4 years old), the mussels had a shell length of approximately 34 mm. Same shell lengths were found by Erftemeijer et al., (2003) where the average shell lengths of a four year old bed sampled in 2003 on the Hond and Paap tidal flat was on average 35 mm. Compared to mussels from intertidal beds located outside the estuary in the eastern Dutch Wadden Sea, this is small, where four-year-old mussels grow to about 40-50 mm (Glorius et al., 2020). The observed limited growth illustrates the challenging environmental conditions for mussels in the estuary. It is likely that as a result of this mussels on the Hond and Paap tidal flat, rather than in areas elsewhere in the Wadden Sea, will tend to separate themselves from conspecifics sooner, after they become more vulnerable to erosion.

6.2 Functioning of the nets in capturing mussel spat

Trial 1 showed that it is possible to capture large quantities of mussels with each of the net materials tested here (hemp, cotton and sisal). The actual capturing of spat on the nets is determined by several factors, and with the trials conducted here, only limited successes were obtained. Several reasons could result to poor mussel development such as the absence of (sufficient) mussel larvae in the water column, predation by crabs and possibly starfish (Capelle et al., 2016; Dankers, 2015; Kamermans et al., 2009), limited or absence of water exchange carrying the spat to the nets, absence of attachment sites on the ropes of the net due to previous colonization by soft bodied species such as hydroid polyps and filamentous algae and accumulation of mud on the ropes. It is under debate if a previous settlement of hard bodied barnacles is preventing or helping mussel settlement and development on the ropes. SMC entrepreneurs state that barnacle settlement will lead to erosion of mussel spat while in the trails conducted here we did not observe erosion of mussels from rope sections at which barnacles have settled. The dominant reasons for the poor capture and development of spat on the nets hung out in the tugboat- (spring 2018) and inner harbour (spring 2019) are not known. In the tugboat harbour it is likely that the presence of juvenile crabs and the accumulation of mud on the nets may have played a role in it and not the absence of mussel larvae in the water column. The unsuccessful mussel spat collection on nets hung out in the inner harbour in spring 2019 might have been caused due to earlier colonization of soft bodied species such as filamentous algae on the ropes and/or due to too low densities of mussel larvae in the water column or yet other unknown reason(s). Mud and crabs were not found and did not cause the poor development of the mussel spat on those nets.

6.3 Development of the mussels on the tidal flat

Because the limited successes in the capturing of mussel spat on the nets, the original experimental set up, testing three net materials at three different sites, in one season and thus under similar environmental conditions, had to be abandoned. Not one but two trials were conducted spread out over two seasons under different environmental conditions. Furthermore, not one but two different starting situations were tested (nets on which spat was collected prior placement and nets without spat). Due to these alterations it was no longer possible to statistically investigate the effects of location and net material type.

Trial 1 showed that the nets are suitable for transportation of the mussels from the spat collection site to the tidal flat and do offer a temporal attachment structure for the mussels that prevent them to be buried or eroded right away. It also showed that all materials used here biodegraded over time, cotton the quickest and sisal the slowest. Trials 2 showed the need for appliance of undamaged nets and a firm attachment of mussel spat to the ropes of the net before placement on the tidal flat. In Trial 2, were mussel spat was not collected on the nets but were mussels and nets were first brought together on the tidal flat, lacking the necessary attachment structures, no positive net effect in the retention of mussels was found.

The mussels of trial 1 were placed in an area were a layer of mud of several centimetres thick had been build up. Only the presence of the nets prevented the immediate burial and death of mussels. Mussels and mussel patches flushed away from there net at any time died quickly afterwards. Instead the presence of dead shell material in the more sandy (and dynamic) environment of the test site of trial 2 proofed to be beneficial for mussel survival. A part of the mussels survived the complete monitoring period of one year and also two years after construction vital mussel patches were found. It could not be tested if the presence of a net in this environment would have had an additional positive effect in the retention of the mussels. For this undamaged nets at which mussel spat is firmly attached to prior placement is required, which was not present during execution of this trial.

Despite the fact that the nets of trial 1 did offer a temporal foundation for the mussels, the mussels disappeared over time and hardly any survived the first winter. Survival rates of mussels on the net made out of sisal were a bit higher compared to the nets made out of the other materials. It is likely that, compared to the other tested materials, the slower degradation rate of the sisal fibres has caused this. What has been observed was that in the inner sections of the nets, mussels detached themselves first and once climbed upwards they eroded in periods with higher than average windspeeds (>6 Beaufort). Similar observations were made during the execution of trial 2. Mussels on the edges of the tiles containing a layer of dead cockle shells survived, while those positioned at the centre of these tiles died. In trial 2, where mussels were not attached to the nets before placement, it was observed that mussels were able to organise themselves in polygon structures similar to natural beds within one day after placement. However, the adhesive power of mussels to each other and to the ropes formed in this process proved to be insufficient to prevent them being washed away.

On the tiles of trial 2 without a foundation of cockle shells, mussels disappeared altogether, so both from the inner parts as from the edges of the tiles and despite the nets and polygon structures formed. Only (a small part of) the mussels that were able to attach themselves to natural occurring shell debris were found back one year after construction. These patches were small in size and were laying scattered around the test site.

A too high mussel density in the inner parts of the nets of trail 1 and of the inner parts of the cockle tiles of trial 2 might have contributed to the poor survival rates in especially the inner located parts. High mussel densities might have led, in combination with the poor food conditions at the Hond and Paap tidal flat, to food limitations and oxygen depletions stimulating them to detach themselves and clime upwards becoming vulnerable to erosion. At the net edges of trial 1 and the edges of the cockle-tiles and the scattered mussel-shell patches of trial 2, survival rates were higher. Most likely the more open and more scattered shell-mussel patches that were formed in this way had a positive effect on the food- and oxygen supply to the mussels.

A large part of the mussels placed on the tiles containing a foundation of cockle shells survived the first year (estimated at 40% of the applied area and mainly found around the edges of the tiles). Also two years after construction vital mussel patches were found at those tiles which are still intact irrespective hydrodynamic impact by storm surges etc. Recently conducted flume tank and field experiments show the positive effect of cockle shells on the erosion rates of mussels up to certain flow velocities (Capelle et al., 2019). Patches consisting of mussels and cockleshells eroded less rapidly in the flume tank than when patches consisted of mussels only. Also in the field, fewer (factor of 3) mussels disappeared when shell material was present compared to a mussel-only situation (Capelle et al., 2019).

6.4 summary

Despite the sub-optimal environmental conditions it seems possible for mussel beds to survive at suitable areas on the Hond and Paap tidal. Suitable areas can be found at slightly more dynamic parts of the tidal flat consisting of a sandy sediment with the presence of shell material. Intertidal mussel beds can form naturally in the area. It is believed that the possibility for long periods of several months with elevated suspended solids concentrations forms a risk and can lead to the death of mussels and disappearance of beds, naturally formed or initiated.

A large proportion of the mussels placed on top of a bed of cockleshells survived their first year and also two years later vital mussels were found. It is believed that these mussels were given a good chance to develop. But as only one trial has been conducted more trials are needed to proof the robustness of this method and test its performance under different circumstances. In addition, the positive contribution of the nets for mussel survival and washout could not be conclusively proven in the tests carried out here. A firm attachment of mussel spat to the ropes of the net prior installation on the tidal flat is essential next to the suitability of the test location. The success rate of mussel spat collection on nets need to be improved.

On both the nets and the mussels that are placed on a bed of cockles, losses of mussels occur mainly in the inner areas. It is precisely in these areas, possibly as a result of food suppression, that individual mussels detach themselves from each other and move upwards, whereupon they become susceptible to erosion. They are especially washed away in periods when wind speeds are higher than average (5 to 6 Beaufort or higher). These observations could be used to improve the experimental design.

7 Recommendations

It is recommended to rely on the natural capacity in the formation of mussel beds on the Hond and Paap tidal flat first, before starting to initiate beds. As conditions allow for survival of beds, there are no reasons to think why beds could not form and develop naturally. It is free of cost and is the most natural way. Due to the irregularity of years with good spat fall and variation in the locations where mussel spat falls, natural establishment of beds might require some patience. In situations where intertidal beds are unintended damaged or where damaging them cannot be avoided, in construction of cables for instance, the availability of a technique to initiate a bed can be of hand in order to compensate for the damage done.

There is room for and a need to further optimize and test the techniques that were tested here, as both the 'cockle' and 'net' technique are believed to have potential but have not been tested to its full extent nor are fully proofed to work. A realistic success rate of any technique must be kept in mind. Research show that only about 40% of the natural established mussel beds survive the first winter and around 15% of them the first five years (van der Meer et al., 2019). Only after five years the necessary properties are formed that build up resilience of natural beds after which survival rates are improved substantially. So even by improvement of the survival changes in the first years by optimizing the techniques described here, survival up to five years are required to arrive at an mature and resilient mussel bed that is capable to thrive by itself. Improvements in the techniques described here would however not necessary lead to a technique or techniques that absolutely guarantee the survival of bed for the required five years. A more realistic goal would be to arrive at a technique that lead to a substantial improvement of the survival rate compared to the natural situation.

In the next paragraphs recommendations for this pursuit are made. In general it is recommended to test variations in execution with enough replica's to prevent outcomes to be the result of variations in local conditions. The 'learning by doing' principle is then abandoned or at least interpreted more broadly.

7.1 Collection of spat

- In order to increase a successful collection of mussel spat on nets, better suited locations need to be found. Some general rules in the selection process can be followed;
  - Open connection to the Wadden Sea to be certain of mussel larvae in the water column.
  - Low suspension load to prevent the building up of mud on the nets aiding the settlement and development of spat.
  - Prevent nets to be connected to the seafloor to limit access of predators such as crabs and starfish.
  - Allow for a water flow through and along the net to make sure large quantities of larvae come in contact with the ropes of the net.
- It is also advisable to make use of the experiences obtained by commercial spat collector parties and to invest the environmental conditions at those installations to find the most important factors determining a successful capturing of mussel spat and use this information in the selection of sites.
- When possible use locations near the vicinity of a commercial party. In that way you make sure that the location is suitable and you have a reference to map the developments of mussel spat on the nets.

7.2 Installation on the tidal flat

7.2.1 Sites

- As initiating an intertidal mussel bed is hard enough by itself, conduct new tests at locations with optimal environmental conditions. These can be found on tidal flats near sea inlets where salinity and suspension solids levels are more suitable.
- Select a place on the tidal flat with a sandy substrate and with presence of shell material.
- Select sites where historically mussel beds were present, as it is proved by nature as a favourable site.

7.2.2 Method

- Cockle shells and nets can be used to prevent erosion of mussels. Important in the usage of nets is that they should not be damaged and mussels have to be collected and attached to them before placing them on the tidal flat.
- Although not statistically tested, the net made out of sisal performed a bit better, likely due to the slower degradation rate and/or more rough fibres, compared to the net made of cotton and of hemp. So as different material types are to be tested, sisal should be preferred.

In order to prevent food limitation and oxygen depletion the following variations could be tested.

When using cockle shells:
- Test a range in mussel densities. Somewhere between 5.000 and 10.000 juvenile mussels per meter square seems to be sufficient.
- Test a range in different cockle shell densities, from an single layer up to several centimetres.
- Test different layouts were bands with cockle and mussels are alternated with areas without them. The distance between the bands should not exceed several meters.

When using nets:
- Test different net mesh sizes.
- Test variations in layout in which nets and open areas are alternated.

7.2.3 Monitoring

- If possible, monitor an existing natural bed in the vicinity of the experimental site as a reference for autonomic developments.
- Monitor both the spatial distribution of the mussel patches as well as the properties of the mussel population.
- Try to monitor the effect of isolated storm events, or events with above average windspeeds, on the erosion and distribution patterns of the mussel patches. This can be realized by for example the placement of a camera system that makes continuous monitoring possible. Another possibility could be to plan monitoring efforts before and after a period at which elevated wind speeds are forecasted.

7.3 Ems Dollard estuary

- Invest the underlying reasons for the prolonged periods (several months) with elevated suspended solids concentration first before attending new trials or experiments in the area. If they are the result of human actions (dredging for example) effort could be made to prevent it helping the survival of the intertidal mussel beds.
- Activities that are currently executed and that could decrease the suspended solids concentrations should be stimulated as it would improve the survival chances of intertidal mussel beds. Examples of already carried out projects include the construction of salt marsh near the harbour of Delfzijl and a project that will capture and remove sludge from the estuary.

8 Quality assurance

Wageningen Marine Research has an ISO 9001:2015 certified quality management system. This certificate is valid until 15 December 2021. The organisation has been certified since 27 February 2001. The certification was carried out by DNV GL.

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Accountability

Report C090/21A
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This report has been compiled with great care. The scientific quality of this report has been assessed internally by a fellow researcher and the responsible member of the Wageningen Marine Research management team.

Agreed:	Jeroen Wijsman Expert shellfish ecology
Signature:	
Date:	17th of November 2021
Agreement:	Jakob Asjes MT member Integration
Signature:	
Date:	17th of November 2021

Appendix 1 Model results survival test

Here the model results are given for the growth of mussels and for the different water quality parameters that were measured during the survival test. The column 'significance level' shows whether a variable is of influence. For p-values <0.001 the significance level is '***', for p-values between 0.001 and 0.01 the significance level is '**', between 0.01 and 0.05 it is '*', and between 0.05 and 0.1 it is '. Column 'p-value' shows the actual p-value.

Table 1. Model results for the change in shell length between the T0 and T1 measurement.

Variable	estimate	Std. Error	t value	P value	Significance level
(Intercept)	0.1201	0.0143	8.3870	< 2e-16	***
Origin – SMC	-0.1114	0.0133	-8.3670	< 2e-16	***
Origin – subtidal	0.0007	0.0134	0.0540	0.9571	
40% emersion time	0.0208	0.0134	1.5480	0.1217	
45% emersion time	-0.0361	0.0135	-2.6800	0.0074	**
Location 2	-0.0579	0.0134	-4.3090	0.0000	***
Location 3	-0.0232	0.0133	-1.7380	0.0824	.

Table 2. Model results for the suspended solids concentrations in the water samples.

Variable	estimate	Std. Error	t value	P value	Significance level
(Intercept)	61.8759	4.167	14.849	<2e-16	***
Sampling event - T2	-2.5167	4.167	-0.604	0.5487	
Sampling event – T3	-7.1278	4.167	-1.711	0.0936	.
After high tide	-3.3667	3.4024	-0.99	0.3274	
Location 2	-0.6722	4.167	-0.161	0.8725	
Location 3	0.4778	4.167	0.115	0.9092	

Table 3. Model results for the proportion of organic matter in the suspended solids concentrations in the water samples.

Variable	**estimate**	**Std. Error**	**t value**	**P value**	**Significance level**
(Intercept)	68.3419	4.0115	17.036	< 2e-16	***
Sampling event - T2	-21.5588	4.072	-5.294	3.25E-06	***
Sampling event – T3	-17.4699	4.072	-4.29	9.08E-05	***
After high tide	0.8577	3.2782	0.262	0.795	
Location 2	-0.4412	4.072	-0.108	0.914	
Location 3	-1.7111	3.9399	-0.434	0.666	

Table 4. Model results for the log transformed chlorophyll-*a* concentrations in the water samples.

Variable	**estimate**	**Std. Error**	**t value**	**P value**	**Significance level**
(Intercept)	0.72034	0.03747	19.224	< 2e-16	***
Sampling event - T2	0.41382	0.03657	11.316	2.46E-14	***
Sampling event – T3	0.42588	0.03657	11.646	9.80E-15	***
After high tide	0.02999	0.02833	1.059	0.2957	
Location 2	0.03228	0.03469	0.931	0.3574	
Location 3	-0.05974	0.03469	-1.722	0.0924	.

Table 5. Model results for the chlorophyll-*a* suspended solids ratios in the water samples.

Variable	**estimate**	**Std. Error**	**t value**	**P value**	**Significance level**
(Intercept)	11074.2	379.55	29.177	<2e-16	***
Sampling event - T2	-6756.22	370.41	-18.24	<2e-16	***
Sampling event – T3	-7343.44	370.41	-19.825	<2e-16	***
After high tide	-678.49	286.92	-2.365	0.0227	*
Location 2	-59.13	351.4	-0.168	0.8672	
Location 3	920.81	351.4	2.62	0.0122	*

Table 6. Model results for the salinity in the water samples.

Variable	**estimate**	**Std. Error**	**t value**	**P value**	**Significance level**
(Intercept)	18.3691	0.2712	67.728	< 2e-16	***
Sampling event - T2	1.713	0.2923	5.861	6.30E-07	***
Sampling event – T3	4.8167	0.2531	19.029	< 2e-16	***
After high tide	0.442	0.2285	1.934	0.0598	.
Location 2	-1.6552	0.2695	-6.143	2.48E-07	***
Location 3	-3.3152	0.2695	-12.303	1.63E-15	***

Table 7. Model results for the water temperature in the water samples.

Variable	**estimate**	**Std. Error**	**t value**	**P value**	**Significance level**
(Intercept)	2.68704	0.10124	26.54	< 2e-16	***
Sampling event - T2	4.57778	0.1091	41.958	< 2e-16	***
Sampling event – T3	9.91667	0.09449	104.953	< 2e-16	***
After high tide	0.1963	0.08529	2.302	0.026392	*
Location 2	-0.34778	0.10059	-3.457	0.001262	**
Location 3	-0.40778	0.10059	-4.054	0.000213	***

Table 8. Model results for the (double root transformed) oxygen levels in water samples

Variable	**estimate**	**Std. Error**	**t value**	**P value**	**Significance level**
(Intercept)	1.793308	0.003664	489.416	< 2e-16	***
Sampling event - T2	0.001685	0.003949	0.427	0.671675	
Sampling event – T3	-0.07916	0.00342	-23.148	< 2e-16	***
After high tide	0.002123	0.003087	0.688	0.495349	
Location 2	-0.01506	0.00364	-4.138	0.000165	***
Location 3	-0.02489	0.00364	-6.837	2.49E-08	***

Appendix 2 Measurement frequency of trials

In this appendix the measurement frequency are shown for trial 1 and 2.

Table 1. Measurement frequency of trial 1.

Paragraph this report	Measurement	T1	T2	T3	T4	T5
		20 Aug 2018	Sep 3 2018	Oct 2 2018	Nov 2 2018	3 Dec 2018
6.3.1	Overview and detail photos	x	x	x	x	X
6.3.2	Mussel samples	x		x		x
6.3.3	Rope degradation	x		x	x	x
6.3.4	Ortho- and DEM cards		x	x	x	x
6.3.5	Emersion time (CTD)	continuous				

At each sampling event, nine mussel samples were taken from each net. Only areas with mussels were sampled and samples were taken at random locations.

Only for the net made of sisal the ortho- and DEM maps were recorded at the sampling events in Table 1. For the nets made of hemp and cotton, the ortho and DEM maps were determined only at sampling events T3 and T4.

Table 2. Measurement frequency of trial 2.

Paragraph this report	Measurement	T11	T12	T13	T14	T15
		July 31 2019	Aug 7 2019	Oct 4 2019	Dec 19 2019	Aug 11 2020
6.3.1	Overview and detail photos	x	x	x	x	X
6.3.2	Mussel samples		x	x		x
6.3.4	Ortho- and DEM cards	X	x	x	x	x
6.3.5	Emersion time (CTD)	continuous				

In trial 2, no rope samples were taken but only field observations were made. The number of mussel samples taken varied between the monitoring events. On T13 21 samples were taken (9 on plot 1 and 12 on plot 2), on T13 12 samples were taken (9 on plot 1 and 3 on plot 2), on T15 9 samples were taken (5 on plot 1 and 4 on plot 2). The samples were taken at different locations but close to the original position of the tiles. In addition, on the sampling events of T12, T13 and T15, three samples were taken from the natural mussel bed.

Appendix 3 Model results trial 1

Significant changes between sampling event and/or net type are indicated in the tables in column 'significance level'. For p-values <0.001 the significance level is '***', for p-values between 0.001 and 0.01 the significance level is '**', between 0.01 and 0.05 it is '*', and between 0.05 and 0.1 it is '. Column 'p-value' shows the actual p-value.

Table 1. Model results for mussel densities in samples taken from trial 1.

Variable	estimate	Std. Error	t value	p-value	Significance level
(Intercept)	30509	2558	11.929	< 2e-16	***
Sampling event T3	-15166	2802	-5.413	6.96E-07	***
Sampling event T3	-23112	2802	-8.249	3.67E-12	***
cotton	2660	2802	0.949	0.345	
sisal	1363	2802	0.486	0.628	

Table 2. Model results for mussel shell lengths in samples taken from trial 1.

Variable	estimate	Std. Error	t value	p-value	Significance level
(Intercept)	15.5125	0.1009	153.729	< 2e-16	***
Sampling event T3	0.9815	0.108	9.089	< 2e-16	***
Sampling event T3	1.3477	0.1176	11.464	< 2e-16	***
cotton	-0.7003	0.1149	-6.097	1.14E-09	***
sisal	0.7365	0.1149	6.408	1.57E-10	***

Table 3. Model results for the ash-free dry weight of mussel flesh from trial 1.

Variable	estimate	Std. Error	t value	p-value	Significance level
(Intercept)	171637	12388	13.855	< 2e-16	***
Sampling event T3	-109160	13570	-8.044	9.07E-12	***
Sampling event T3	-144062	13570	-10.616	< 2e-16	***
cotton	12165	13570	0.896	0.3728	
Sisal[1]	33601	13570	2.476	0.0155	*

[1] *for a model without T1, there is no influence on the ash-free dry weight of the mussel flesh for the different nets.*

Table 4. Model results for fresh weight of mussels from trial 1.

Variable	estimate	Std. Error	t value	p-value	Significance level
(Intercept)	10638.8	839.5	12.673	< 2e-16	***
Sampling event T3	-3505.3	919.6	-3.812	0.000279	***
Sampling event T3	-6394.7	919.6	-6.954	1.07E-09	***
cotton	-104.3	919.6	-0.113	0.909987	
Sisal[2]	2157.8	919.6	2.346	0.021565	*

[2] *for a model without T1 there is no influence on the fresh weight of the mussel for the different nets.*

Appendix 4 Model results trial 2

Significant changes between sampling event and/or net type are indicated in the tables in column 'significance level'. For p-values <0.001 the significance level is '***', for p-values between 0.001 and 0.01 the significance level is '**', between 0.01 and 0.05 it is '*', and between 0.05 and 0.1 it is '. Column 'p-value' shows the actual p-value.

Table 1. Model results for mussel densities in samples taken from trial 2.

Variable	**estimate**	**Std. Error**	**t value**	**p-value**	**Significance level**
(Intercept)	22320	2790	7.999	2.06E-09	***
Sampling event T13	-20006	2470	-8.098	1.55E-09	***
Sampling event T15	-23190	2700	-8.588	3.89E-10	***
Net-hemp	4181	3282	1.274	0.2111	
Net-cotton	1495	3304	0.452	0.6537	
Net-sisal	1862	3177	0.586	0.5616	
Cockleshells (present)[1]	5426	2467	2.199	0.0346	*

[1] for a model without T12 there is no influence on the mussel density for sites with cockles.

Table 2. Model results for mussel shell lengths in samples taken from trial 2.

Variable	**estimate**	**Std. Error**	**t value**	**p-value**	**Significance level**
(Intercept)	17.04009	0.21182	80.445	<2e-16	***
Sampling event T13	-0.30148	0.20025	-1.506	0.1323	
Sampling event T15	4.36304	0.28732	15.185	<2e-16	***
Net-hemp	-0.06357	0.24522	-0.259	0.7955	
Net-cotton	-0.4176	0.25407	-1.644	0.1004	
Net-sisal	-0.03341	0.24355	-0.137	0.8909	
Cockleshells (present)	0.34152	0.19267	1.773	0.0764	.

Table 3. Model results for the ash-free dry weight of mussel flesh from trial 2.

Variable	estimate	Std. Error	t value	p-value	Significance level
(Intercept)	267146	32992	8.097	1.56E-09	***
Sampling event T13	-208890	29210	-7.151	2.44E-08	***
Sampling event T15	-209106	31928	-6.549	1.47E-07	***
Net-hemp	34746	38806	0.895	0.3767	
Net-cotton	-6115	39059	-0.157	0.8765	
Net-sisal	15212	37560	0.405	0.6879	
Cockleshells (present)[2]	58061	29171	1.99	0.0544	.

[2] for a model without T12 there is no influence on the mussel ash-free dry weight of mussels for sites with cockles.

Table 4. Model results for fresh weight of mussels from trial 2.

Variable	estimate	Std. Error	t value	p-value	Significance level
(Intercept)	8809.3	985	8.944	1.45E-10	***
Sampling event T13	-6431.8	872.1	-7.375	1.26E-08	***
Sampling event T15	-6781.9	953.2	-7.115	2.72E-08	***
Net-hemp	912.4	1158.6	0.788	0.4363	
Net-cotton	107.5	1166.1	0.092	0.927	
Net-sisal	835.5	1121.4	0.745	0.4612	
Cockleshells (present)[3]	1848.8	870.9	2.123	0.0409	*

[3] for a model without T12 there is no influence on fresh weight of mussels for sites with cockles.