Changes in associated fauna on littoral commercial mussel plots in the Eastern Scheldt

Differences in community composition of different plot densities in mussel bottom culture

HZ University of Applied Sciences
5 June 2017

Research report

B. Schreur (66117)
Final thesis (CU11020)
Supervisor – A.C. Nijssen

Changes in associated fauna on littoral commercial mussel plots in the Eastern Scheldt

Differences in community composition of different plot densities in mussel bottom culture

Bo Schreur (66117)
HZ University of Applied Sciences
Delta Academy
Water management – Aquatic Ecotechnology

Hand in date – June 5, 2017
Vlissingen

Final thesis (CU11020)
Research report
Version 1.0

Supervisor *HZ University of Applied Sciences*:	A.C. Nijssen
Supervisor *Building with Nature research group*:	A. van den Brink
	J. Schotanus

Photo on the cover: (Telegraaf, 2015)

Preface

This research report is on my final thesis on the changes in associated fauna on littoral commercial mussel plots in the Eastern Scheldt. This final thesis was carried out at the Building with Nature research group, part of the Delta Academy at the HZ University of Applied Sciences. This research group is working on projects to find out how to make the maximum use of nature in coastal defence, while also creating opportunities for recreation. This final thesis and research report could not have been done without the help of several people.

I would like to thank Anneke van den Brink and Jildou Schotanus from the research group Building with Nature for their help, feedback and guidance during this final thesis. Their trust in me and my work stimulated me to get the best out of myself. In addition, I would like to thank Alco Nijssen for being my supervisor from the HZ University of Applied Sciences and for his help, support and trust.

Besides the supervisors in the company and from school I want to thank the other interns from the research group for their help during field monitoring days. The field collections were done efficiently and precise with their help. Furthermore, I want to thank the fellow students in the internship-office for helping out each other, for supporting each other, for learning each other variety of things, but most of all, thanks for the great time together and for all the fun we had. I also want to thank my closest friends for being there for me in times of a setback and for supporting me during the whole final thesis process.

And, last but not least, I want to thank my family for all the support during this final thesis. Gabriëlle Verbeeke, you are like family to me, thanks for reminding me daily to go home at the end of the workday at a decent time and for forcing me to get some rest and relaxation besides the work that needed to be done. I want to thank my parents and boyfriend for listening to me at rough times as well as at over-enthusiastic times, I also want to thank them for their support and the trust they had in me. And thanks to my parents and brothers for reading through my report and providing me with feedback.

Summary

From previous research in the western Wadden Sea it is clear that mussel plots are important hotspots for biodiversity. When the density of a mussel plot is higher, the biodiversity appears to be higher as well.

This final thesis project was a part of the bigger project, 'Added value with Mussels', executed by the Building with Nature research group at the HZ University of Applied Sciences, and focussed on getting more insight on the community composition in littoral commercial mussel bottom culture plots in the Eastern Scheldt with different densities. This water body is experiencing 'sand hunger' as an effect of the building of the storm surge barrier in 1986. Sand hunger is having a negative effect on the natural values of the water body as the existence of sandbanks is under threat and habitat loss is occurring. Previous research shows that mussel plots might be a solution to increase the natural values of an area. Within this final thesis the natural values in terms of the number of phyla and the community composition in the Eastern Scheldt were investigated in mussel plots.

The field research was performed between November 2016 and March 2017 and the date did not show to be a variable to cause a significant difference in the number of phyla found. However, the density of the mussel plots showed to have a significant effect on the number of phyla found. The denser a mussel plot the bigger the certainty that the density is a factor of influence for the number of phyla found within the mussel plot. The reference plots in this research showed to be having a significantly lower number of phyla found than the density plots with mussels. Therefore, community composition is affected by density of a mussel plot and mussel beds increase the natural values in the Eastern Scheldt. After having performed this research it is recommended to extend the research by using more sample points per density, by performing the monitoring over a longer period of time and using more sample locations, all in order to get a better insight, with a more stable result, in the community composition throughout the whole waterbody.

Samenvatting

Eerder onderzoek in de westelijke Waddenzee heeft aangetoond dat mosselpercelen belangrijke hotspots zijn voor biodiversiteit. Wanneer de dichtheid van een mosselperceel hoger is, blijkt de biodiversiteit ook hoger te zijn.

Dit afstudeerproject was onderdeel van het 'Meerwaarde met Mosselen' project, uitgevoerd door de Building with Nature onderzoeksgroep van de HZ University of Applied Sciences, en had ten doel meer inzicht te krijgen in de samenstelling van de leefgemeenschap in commerciële mossel bodemcultuur in de Oosterschelde en het effect van dichtheid van een mosselperceel. Dit waterlichaam is onderworpen aan zandhonger door de bouw van de Oosterschelde kering sinds 1986. Zandhonger heeft een negatief effect op de natuurwaarden van het waterlichaam, het bestaan van de zandbanken is bedreigd en er gaat habitat verloren. Eerder onderzoek heeft aangetoond dat mosselpercelen een optie kunnen zijn om de natuurwaarden in een gebied te verhogen. Binnen dit afstudeeronderzoek zijn de natuurwaarden in de vorm van samenstelling van de leefgemeenschap onderzocht in de Oosterschelde in mosselpercelen.

Het veldonderzoek is tussen november 2016 en maart 2017 uitgevoerd en de datum toonde geen factor te zijn welke een significant verschil geeft in samenstelling van de leefgemeenschap, gebaseerd op het aantal phyla dat is gevonden. De dichtheid van een mosselplot heeft echter wel een significant effect te hebben op het aantal phyla dat is gevonden. Hoe groter de dichtheid van een mosselperceel, hoe groter de kans dat de dichtheid een factor van invloed is op het aantal phyla dat is gevonden. Het referentie perceel blijkt in dit onderzoek een significant lager aantal gevonden phyla te hebben dan de percelen met mosselen. Hierdoor kan worden geconcludeerd dat de samenstelling van de leefgemeenschap wordt beinvloedt door de dichtheid van een mosselperceel en dat mosselpercelen de natuurwaarden in de Oosterschelde verhogen. Na dit onderzoek wordt er aanbevolen om het onderzoek uit te breiden met meer meetpunten per dichtheid, door de monitoring gedurende langere periode uit te voeren en om het onderzoek op meer locaties uit te voeren. Dit om meer inzicht, met een stabieler resultaat te krijgen in de samenstelling van de leefgemeenschap over het gehele waterlichaam.

Table of contents

Table of figures

Table of tables

Table of graphs

Use of definitions in this report

To understand the full aspect of this research it is important to understand several terms and what the difference and link between them is.

Biodiversity is the variety of different kinds of organisms that make up an ecosystem, it has two components: species richness, which is the number of different species in a community, and relative abundance, which is the proportion each species represents of all individuals in the community. Biodiversity is thus based on the taxonomic level of species.

Within this research community composition is the assembly of phyla that are found within the community of a littoral, commercial mussel plot.

As in this research the community composition will be based on the taxonomic level of phylum biodiversity is not used as a term in the research question and the results of this research. The terms biodiversity and community composition will however be used inter-changeable in this report, as most scientific literature is on biodiversity which can be used as an indicator for community composition. This is done by moving up the hierarch of taxonomic levels from species till phylum.

1. Introduction

1.1 Background

The research group Building with Nature of HZ University of Applied Sciences is performing an extensive research, named 'Added value with Mussels', together with commercial mussel farmers, nature organisations, Rijkswaterstaat and researchers from the NIOZ, Wageningen Marine Research and Deltares. The research explores the opportunities of creating multifunctional, littoral mussel plots in the Eastern Scheldt, as well as finding a way to maintain these by active management.

With experimental and comparative research new knowledge is generated about the factors that play a role in creating littoral mussel plots and about the value of a commercial mussel plot for its different functions. A commercial mussel plot creates economic value by the cultivation of mussels, natural values by providing a new habitat for species and it contributes to sediment stability by the reduction of wave energy. The natural values of an area are the values assigned to the area from the perspective of nature conservation, this can be based on several criteria, within this project the criterion will be ecology. The goal of the 'Added value with Mussels' project is to develop multiple methods to realise three goals:

1) Create new profitable production locations for the shellfish sector
2) Create nature friendly methods to protect intertidal zones from erosion
3) Increase the natural values in the intertidal zones with respect to the set Natura 2000 goals

In the project of 'Added value with Mussels' the (im)possibilities to connect these goals are investigated by looking at different forms of long term active management. The most important results in the end should be a method for the creation of commercial mussel plots and having a model with economic and ecological values included for insight in active management and for balancing of the values.

This final thesis report focusses on the goal of increasing the natural values in the intertidal zones. The community composition of benthos at littoral, commercial mussel plots in the Eastern Scheldt will be investigated over several months, where community composition will be recorded at the taxonomic level of phylum. The commercial mussel plot that will be used for this research is seeded in different densities to investigate whether this is a factor of influence on the number of phyla present and to what extent.

The project 'Added value with Mussels' is financed by *regieorgaan SIA*, department of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO). Partners in the project are *Roem van Yerseke B.V., de Ronde Beheer B.V., Rijkswaterstaat Dienst Zee en Delta, Natuurmonumenten, ARK Natuurontwikkeling, wereldnatuurfonds, NIOZ, IMARES* and *Deltares.* (HZ University of Applied Sciences, 2017)

1.2 Problem description

The composition of species in the Eastern Scheldt differs a lot from other comparable water bodies in Europe. The water body is in an unfavourable state when looking at conservation based on quality of tidal area and future prospects as the Eastern Scheldt is experiencing the process of 'sand hunger'. Sand hunger has a destabilizing influence on the sediment and is affecting the ecology by shrinkage of tidal sandbank area. To preserve and to recover the right balance in the water system the erosion of the sand banks needs to be mitigated and to improve the biodiversity, restoration of shellfish banks is required. The restoration of sublittoral and littoral shellfish banks is one of the important tasks set by the Natura 2000 for this area. (Noordzeeloket, 2006)

1.2.1 Sand hunger

After the flood of 1953 it became clear that Zeeland needed a better protection to flooding. Several dams have been built since 1960, which caused the Eastern Scheldt to lose its estuarine character. A storm surge barrier (finished in 1986) was built between the North Sea and the Eastern Scheldt, this barrier provides defence against future flooding events. The amount of the water flowing in and out of the Eastern Scheldt was reduced by the storm surge barrier which reduced the tidal differences. (Delta Expertise, 2017). To maintain the tidal differences as they were, the volume of the Eastern Scheldt water basin was reduced by creating compartments by building dams between the water body and its tributaries. With these interventions, the former estuary developed into a shallow bay with gullies and sandbanks and the balance of the water system was disturbed. The water velocity decreased and erosive processes became stronger than building processes, this causes a threat to sandbanks as they started to disappear below the water surface. Another effect of the storm surge barrier was that the inflow of sediment to the Eastern Scheldt decreased. The gullies become filled with the sediment that was eroded from the sandbanks. This process is called sand hunger (Figure 1.1). The expectation is that the intertidal area will decrease by 35% by 2060 compared with the surface area of the sandbanks in 2010. (Witteveen & Bos, Bureau Waardenburg bv, & Rijkswaterstaat Zee en Delta, 2013) Due to sand hunger the dykes, and therefore the water safety, are threatened as there are less sandbanks to attenuate the waves, so the dykes have to withstand more wave energy themselves (Rijkswaterstaat, 2011).

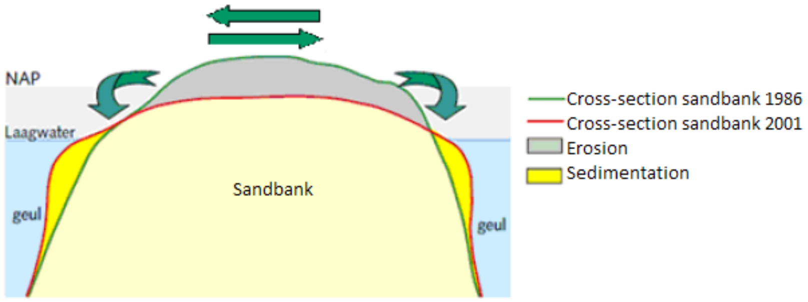


Figure 1.1: The process of sand hunger. Adjusted from (Witteveen & Bos, Bureau Waardenburg bv, & Rijkswaterstaat Zee en Delta, 2013).

Steps that can be taken to mitigate the effects of sand hunger include the use of supplementation or through stabilizing the sediment on the sandbanks by shellfish. Coastal protection systems can profit from structures coming from and activities by ecosystem engineering species, mussel beds can have an ecosystem engineering function. Mussels have the ability to climb on top of sediment that is deposited between them, they secure themselves in place with byssus threads and thereby the mussels trap and stabilize the sediment (Leeuwen, 2008). This, in turn, leads to an increased soil elevation and to attenuation of waves. These abilities make mussels suitable to function in coastal engineering systems. (Borsje, et al., 2011)

1.2.1.1 Effects of sand hunger on biodiversity

All organisms serve an important function that together create a well-functioning ecosystem that is in equilibrium. Within a biologically diverse ecosystem, organisms are dependent on each other for survival. Biodiversity can be threatened by habitat loss and a decrease in biodiversity weakens the ecosystem by weakening the community structure, which are the connections that exist among species. (Conserve Energy Future, 2017) (Perritano, 2017) Habitat loss can be the shrinkage of intertidal sandbanks as a result of sand hunger. The habitat modifications that reef building shellfish execute can contribute to stabilizing the bed of intertidal flats and the shellfish beds may enhance the biodiversity in an area (Borsje, et al., 2011).

The Eastern Scheldt is a complex system of creeks, gullies and sandbanks. The water body is often subject to changes: the water has an unstable turbidity, new species familiar in deeper water systems are perceived, like the Atlantic cod, and the salinity of the water body has increased since the building of dams for coastal protection. These changes negatively affect the natural value of the Eastern Scheldt which is quite diverse, see 2.1 Natural values of the Eastern Scheldt. The system of the Eastern Scheldt is not yet at equilibrium since the building of the storm surge barrier. (Ministerie van Economische Zaken, n.d.)

1.2.2 Biodiversity and mussel density

Due to sand hunger there is loss of habitat which has a negative effect on the biodiversity of the Eastern Scheldt. Restoration of shellfish banks may enhance the biodiversity because these provide substrata for the attachment of other species, like barnacles, and these provide in refuge and nesting area for e.g. fish and crustacean species. (Gutiérrez, Jones, Strayer, & Iribarne, 2003) (Borsje, et al., 2011)

Research performed in the Wadden Sea showed that the mussel density in mussel plots might be of influence on the biodiversity, where density is the coverage in percentage of mussels in a mussel plot. At plots where there were no or few mussels the biodiversity appeared lower than plots with a higher mussel density, therefore mussel plots are seen as hotspots for biodiversity. (Smaal, 2013) (Smaal, Craeymeersch, Glorius, van Stralen, & Drenth, 2017) The explanation might be that the more shells are aggregated the more organisms can settle on the shells as well as that there is more sheltered area when more shells are aggregated, however, the latter shows no linear function (Gutiérrez, Jones, Strayer, & Iribarne, 2003). However, to what extent density is of influence on the community composition in the Eastern Scheldt is still unclear.

1.3 Actual assignment

To maintain the high natural values and to reach the goals set by Natura 2000 for the Eastern Scheldt it was important to further investigate the community composition on different densities of mussel beds.

As part of the project ‘Added value with Mussels’ research was conducted to get more insight on the community composition of littoral commercial mussel plots. To perform research on the community composition of these bottom culture mussel plots, the benthos of three different densities in a mussel plot was monitored, as well as a control base area (the reference plot). The mussel plot used for this research was located at the Zandkreekdam, at the Eastern Scheldt side. Within the plot the mussels were found in three different densities, high, medium and low, see Figure 1.2. In Table 1.1 can be seen how dense the plots actually had to be (these ranges were set based on photos by use of ImageJ, a computer program, see Appendix A for underlying data).

Figure 1.2: Three different densities that were found in the mussel plot. Left: high. Middle: medium. Right: low. (Schotanus, 2016)

Table 1.1: Mussel plot densities

Mussel density	Coverage (#mussels)
High	± 70 % - 100%
Medium	± 35% - ±69%
Low	± 1% - ±34%
Reference	No mussels

The mussel plot was seeded with these densities by the mussel farmers. The dates for monitoring were between November 2016 and March 2017 and monitoring was performed every other month. During the field monitoring samples had to be gathered and taken back to the laboratory for further analysis of the community composition, this was done till the phylum level because of practical reasons, but also because there was be looked at trophic levels rather than individual species. Samples were taken in quadruple at the three different density plots and at the nearby reference plot. After the laboratory analysis the data was analysed and the research conclusions were made about the influence of density on the community composition.

1.4 Objective

The objective of this assignment was to gain insight on the community composition in and around littoral commercial mussel plots that have different mussel densities. This insight should help determine the natural values of a mussel plot as one of the main goals of 'Added value with Mussels' is to increase the natural values in the intertidal zone with respect to the set Natura 2000 goals. This research on community composition in the benthos of a littoral commercial mussel plot might contribute to this goal.

1.5 Research questions

To achieve the set objective, a research question was formulated with accompanying sub questions.

Research question:

How does the density of a littoral commercial mussel plot affect the community composition in the first four months after seeding the mussels?

Sub questions:

1) What organisms are present on the mussel plots?
2) Do the community composition and the relative abundance differ between different mussel densities?
3) Do the community composition and the relative abundance change over the first four months since seeding the mussels?

1.6 Preview

This research report will show the results from this research assignment and therefore gives insight in the community composition and the number of phyla in and around a littoral commercial mussel plot, for both infauna and epifauna based on the taxonomic level of phylum. Furthermore, it will give insight in the effect density has on the number of phyla and whether there is development in the community composition over several winter months.

2. Theoretical framework

Mussels are an important product for the aquaculture industry of the Netherlands, each year about 50 million kilograms of mussels are produced. The Dutch mussel fisheries have been active for over 150 years in the Wadden Sea and in the Eastern Scheldt. (Nederlands Mosselbureau, 2017) About 50% of the production of the blue mussel *(Mytilus edulis)* in Europe is produced in mussel bottom cultures (Figure 2.1) (Smaal, 2002). These bottom cultures can be natural, where the mussel plots exist naturally, or commercial, where the mussel plots are placed. The main product needed for this bottom culture is mussel spat. Juvenile mussels, or mussel spat, are collected from natural mussel beds or by mussel spat collector installations (Kamermans, Brummelhuis, & Smaal, Use of spat collectors to enhance supply of seed for bottom culture of blue mussels (Mytilus edulis) in the Netherlands, 2002).

Figure 2.1: Mussel bottom culture in the Wadden Sea (Telegraaf, 2015)

Mussel spat collectors (SMC) are a reliable source of mussel spat. However, SMC spat have thinner shells that make them more vulnerable to predation (Kamermans, Blankendaal, & Perdon, 2009). After collection of the juvenile mussels, they are seeded on subtidal or intertidal culture plots, where they grow for 1-3 years until they are large enough to be sold commercially (Gossling, 2003). Both the western Wadden Sea and the Eastern Scheldt estuary are important natural areas in the Netherlands and therefore specific regulations such as the Natura 2000 are put in place to protect the natural values. The natural values of an area are the values that are assigned to the area from the perspective of nature conservation, this can be based on several criteria, like for example biodiversity, ecosystem stability or species rarity. Most of the mussel bottom culture plots are situated in the Wadden Sea because this location is most suitable for mussel culture; there is a strong influence of the tides and therefore there is a higher availability of food for the mussels when compared to the Eastern Scheldt; However, the yield of the Wadden Sea is less stable than the yield of the Eastern Scheldt. Since the latter has a protected position the risk of losses by storms is smaller than the risk of losses in the Wadden Sea. (Capelle, Wijsman, van Stralen, Herman, & Smaal, 2016) (Nederlands Mosselbureau, 2017)

2.1 Natural values of the Eastern Scheldt

The Eastern Scheldt has a sheltered position between the islands of Zeeland and behind the storm surge barrier (Figure 2.2), which is important to its function as a nursery habitat. Furthermore, the sandbanks in the Eastern Scheldt are one of the biggest growth places for seagrass in the Netherlands. After the building of the Delta works the seagrass species have decreased as a consequence of the decrease of fresh water influence. The reduced tides have strongly altered the vegetation, as the sandbanks are flooded less often which causes establishment of vegetation. In the Eastern Scheldt, the last growth place of small cordgrass of the Netherlands occurs. The natural system of the Eastern Scheldt is not yet in equilibrium after the building of the storm surge barrier as the one-way erosion and the sediment transport continues. (Ministerie van Economische Zaken, n.d.)

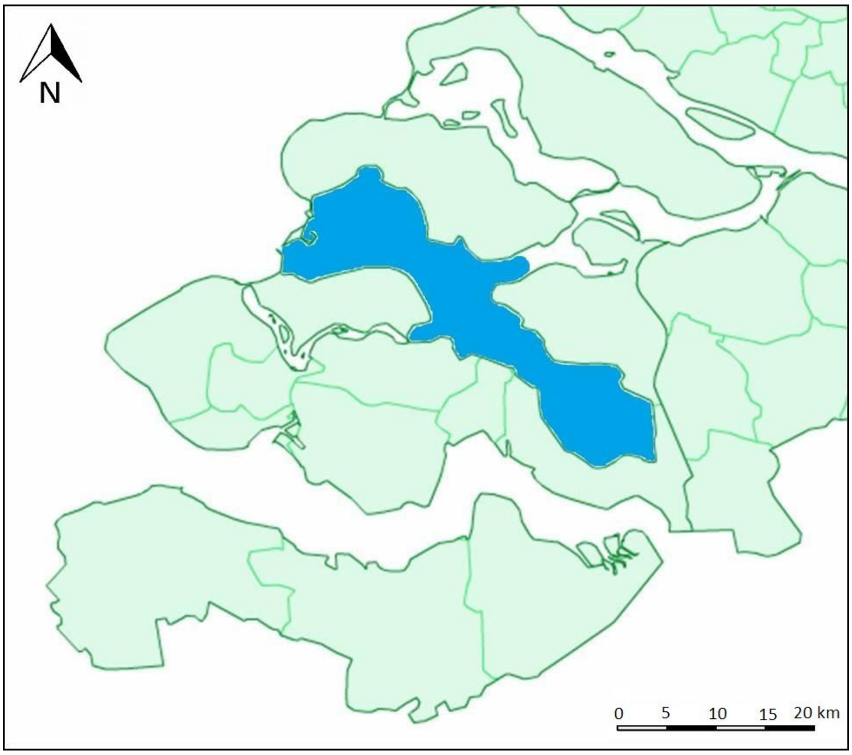


Figure 2.2: A map with the location of the Eastern Scheldt, which is shown in blue. Adjusted from (Altorf & van Gils, 2014).

The Eastern Scheldt is a Natura 2000 area, which means it is protected by the European Union in order to preserve the nature and its biodiversity. The Eastern Scheldt is a protected area by the habitat and bird directive and is thereby contributing to the preservation of European biodiversity. (Ministerie van Infrastructuur en Milieu; Rijkswaterstaat, 2016)

The Eastern Scheldt exists of a complex system of creeks, gullies and sandbanks, with many different biotopes (fresh and saline water, deep and shallow waters, strong and weak currents, sandbanks and rock shores) that accommodates many different ecosystems in the water body. In each ecosystem different species can be present, this causes high natural values in the Eastern Scheldt. (Nationaal Park Oosterschelde, n.d.) However, the Eastern Scheldt is subject to changes: the water has an unstable turbidity, new species, like the Atlantic cod or the European seabass, familiar in deeper water systems are perceived since the ‘70s and the salinity of the water body has increased since the building of dams for coastal protection. (Ministerie van Economische Zaken, n.d.) There are 33 typical species in the Eastern Scheldt, a typical species is a species that

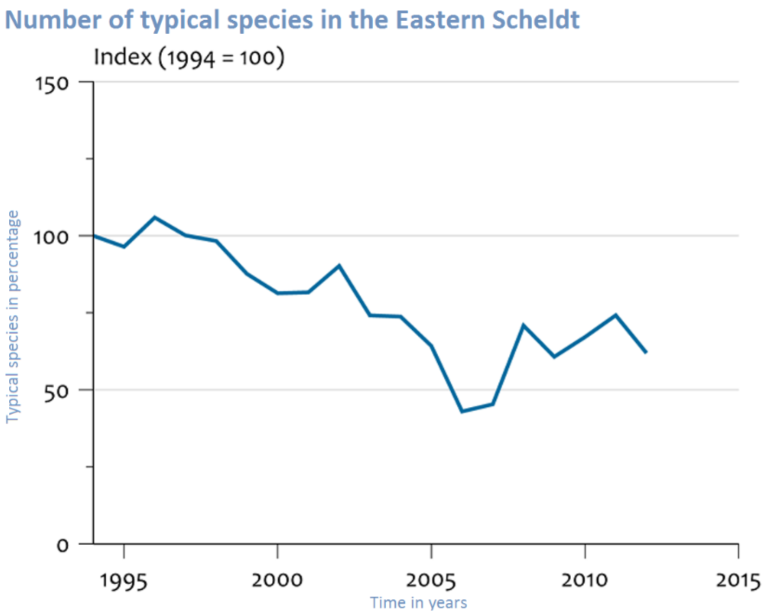


Figure 2.3: Typical species in the Eastern Scheldt. Adjusted from (CBS, PBL, Wageningen UR, 2014).

can be used as an indicator for good abiotic conditions or biotic structure. Fifteen of these typical species show a decrease which might be an effect of the changes in the water system (see Figure 2.3). (CBS, PBL, Wageningen UR, 2014). Several exotic species are present in the water body due to human introduction or due to increased natural distribution. Atlantic and southern species cause, in general, no problems in the water system. By natural enemies the populations are kept in equilibrium and most Atlantic species do not settle permanently. Non-European species, also called exotic species, may present a threat to the original flora and fauna. An example is the Pacific oyster (*Magallana gigas*), this species presents a threat for other shellfish. The *M. gigas* was introduced in the Eastern Scheldt to subsidise the farmers in the area in a time when the European flat oyster (*Ostrea edulis*) was suffering from parasites (Drinkwaard, 1998). However, the *M. gigas* inhabited the area and now, the *O. edulis* has almost disappeared due to the parasite and the succession of the *M. gigas.* The origin of new marine species in the Eastern Scheldt can be seen in Figure 2.4. (CBS, PBL, Wageningen UR, 2015) A different ecosystem is found on dikes along the Eastern Scheldt, species that are common at rocky coasts in Bretagne and South-England occur in these areas, e.g., sea-anemones, sponges, lichen and crayfish. At locations with high currents the flow of plankton allows for a rich diversity of organisms below the water surface. At places where currents are less high sand is deposited and e.g. flatfish and tubeworms occur. Porpoise, the common seal and the grey seal are noticed in the Eastern Scheldt occasionally. The sandbanks that reach

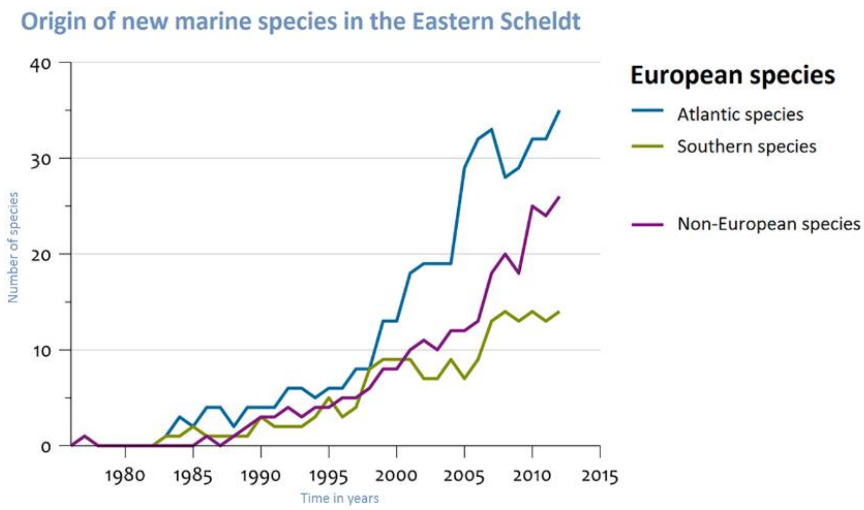


Figure 2.4: New species in the Eastern Scheldt, 1978-2012. Adjusted from (CBS, PBL, Wageningen UR, 2015)

above the surface at low tide are of great importance to them as well as to foraging birds. (Ministerie van Economische Zaken, n.d.)

2.2 Ecosystem engineering

An ecosystem engineer is an organism that has a big influence on a habitat, it can create, alter, maintain, modify or destroy it (Borthagaray & Carranza, 2007). The definition of an ecosystem engineer is only to be used for keystone species who strongly affect their surroundings. When conditions in a habitat change, the ecosystem engineers usually increase the diversity of niches that are present and affect the distribution and the abundance of other species in the region. To achieve a higher species richness the engineering species must fulfil two conditions; conditions that are not present elsewhere in the landscape must be created (1) and there must be species that are able to live only in the engineered patches which thus do not occur in unmodified areas (2) (Wright, Jones, & Flecker, 2002). All ecosystems contain several ecosystem engineering species. In 2004 Reise and Volkenborn performed a study on the influence of three engineers, at intertidal flats, on the composition of macrobenthic communities. The engineers in this research were the blue mussel (*Mytilus edulis*, Figure 2.7), the lugworm (*Arenicola marina,* Figure 2.5), and the cockle *(Cerastoderma edule,* Figure 2.6)*.* The *A. marina* is a species with a sediment destabilizing effect, the erosion of fine material is promoted by bioturbation. The *A. marina* hereby creates habitat for other species that inhabit sediment, which might have been absent without the *A. marina*. This species thus increases the biodiversity on the tidal flat where it is abundant. (Reise & Volkenborn, 2004)


Figure 2.7: Mytilus edulis (Wikipedia, 2016)


Figure 2.5: Arenicola marina (European lugworm, n.d.)


Figure 2.6: Cerastoderma edule (Ansomar, 2012)

The *C. edule* is a bioturbator because it decreases sediment stability, which however might create a habitat for other species at the same time. High densities of *C. edule* can locally increase sediment stability through biodeposition. (Donaldi, et al., 2015)

The *M. edulis* is known to control and affect the marine benthic environment by three traits (Gutiérrez, Jones, Strayer, & Iribarne, 2003):

1) Providing substrata for the attachment of sessile organisms. The more shells aggregated together, the more sessile organisms can settle on the shells.
2) Providing of refuge from tidal currents and predators by creating formations through enhancing the sediment organic matter and silt. According to a study performed in 2003 by Gutiérrez *et al.* there is no direct linear function between the number of shells and the number of species that seek refuge (Gutiérrez, Jones, Strayer, & Iribarne, 2003).
3) Controlling the transport of particles in the benthic environment. The degree of shell aggregation has an effect on the characteristics of the hydraulic flow over the shellfish bed.

By looking at the effects of these three species on their habitat they can be considered ecosystem engineers on mudflats. (Donaldi, et al., 2015)

2.3 Biodiversity

The biodiversity of a community is the variety of different kinds of organisms that make up a community. It has two components: species richness, which is the number of different species in a community, and relative abundance, which is the proportion each species represents of all individuals in the community. (Jackson, 2011a) Biodiversity is of importance for environmental monitoring and conservation because it is widely used to estimate the complexity, the stability, and the general health of an ecosystem. (Morris, et al., 2014) Communities that have a higher diversity are generally more productive and are better able to withstand and recover from environmental stresses than communities with a lower diversity. (University of Gothenburg, 2011)

Biodiversity is subject to seasonal variation, this shows in the study by Shimadzu *et al.* (2013) on seasonal variation in species abundance in estuarine fish assemblages in the Bristol Channel (United Kingdom). The data in this study is based on 30 years of records, sampled monthly. The fish species are divided into four seasonal groups, and the study concludes that these groups appear to be 'taking turns' at being abundant. The total abundance of this community exhibits some seasonal variation, which follows the classic predator-prey model, where the predator abundance lags behind the prey abundance. Shimadzu *et al.* (2013) concludes therefore that seasonal variation is linked to shifts in resource availability; When the competition for food is most intense the species are less homogeneous distributed that at times when the resource availability is sufficient. The winter and spring groups are more diverse in terms of spatial guild occupancy, because these are times of the year when competition for resources is likely to be greatest according to the study of Shimadzu *et al*. (2013). An interesting observance is that the abundance of species varied most in the winter time amongst the years. (Shimadzu, Dornelas, Henderzon, & Magurran, 2013)

Biodiversity is difficult to quantify partly because of the multitude of indices that can be used, but also as biodiversity cannot be captured completely by a single number (Purvis & Hector,

2000) (Morris, et al., 2014). A biodiversity index is a scale of the diversity of species at a study site and by this scale the study site can be compared with other sites. Comparing the diversity of multiple datasets can give an idea of the comparative stability and health of communities. Indices aim to describe general properties of communities, with these properties different regions, taxa, and trophic levels can be compared. (Morris, et al., 2014) Furthermore, diversity indices can give insight on the rarity and/or commonness of species in a community (Beals, Gross, & Harrell, 2000). Two diversity indices that are frequently used are the Shannon index and the Simpson index which both have their strengths and weaknesses. The Shannon index is a sample-size dependent index (Valdas, 2016) and is relatively easy in use as it incorporates both the species richness and the evenness, which can be seen as a strength and a limitation at the same time; This index provides a summary of the biodiversity in a single number, however this makes it difficult to compare communities that differ greatly in richness (Kerkhoff, 2010). As the Shannon index is sample-size dependent is it sensitive to site differences. The Simpson index is a dominance index as it weighs common or dominant species more heavily, therefore, rare species with few representatives will not affect the diversity much (protect u.s., n.d.). This makes the index less sensitive to species richness and is weighted towards the more abundant species, which can be seen as a strength and weakness at the same time.

Worldwide most of the marine species are benthic (Gray, 1997) and the sedimentary habitat is the most widespread one of the seabed, therefore it is of importance to study soft-bottom macrofauna. As determination of individuals to the level of species is labour-intensive and time consuming, research was performed on the use of higher taxonomic levels for biodiversity research. Olsgard & Sommerfield (2000) suggested that the process of sampling assemblages based on species level may be streamlined by reduction of the taxonomic resolution. Their study in the Norwegian sector of the North Sea shows that data based on taxonomic levels higher than species may be of use for research on community composition as little information about inter-sample relationships gets lost. In addition, this research showed that the correlation of the data from species level with higher taxonomic levels were all relatively high, the family taxon came out best, but also the phylum taxon showed a significant correlation with the species taxon. (Olsgard & Somerfield, 2000) (Anderson, Diebel, Blom, & Landers, 2004)

The study by Olsgard and Somerfield furthermore showed that Annelida, or more specifically Polychaeta, are the dominant phyla in soft-bottomed habitats, about 60%-70% of the individuals belong to this phylum. Mollusca comprises about 20% of the individuals and less than 10% of the individuals are Arthropoda, more specifically Crustacea. The Echinodermata were of minor importance in this survey, numerically seen. In the research by Olsgard (2000) Annelida is the most abundant phyla at the monitoring sites of this research, the initiation is that this phylum is more tolerant to changes and environmental stress than Mollusca, Arthropoda and Echinodermata (Olsgard & Somerfield, 2000). A study performed in 2007 in an area off the east coast of the United Kingdom supports this research in terms of proportional abundances of the phyla. The Annelida were the dominant group with 53%, followed by the Mollusca which comprised 17%. The smaller groups were the Arthropoda with 15%, 'others' with 9% and the Echinodermata comprised only 6%. (Barrio Froján, Boyd, Cooper, Eggleton, & Ware, 2008)

In 2006 IMARES performed a large-scale oyster removal experiment in the Eastern Scheldt, commissioned by the Province of Zealand (Wijsman, et al., 2008). One of the experiment locations was the Zandkreekdam. At this location monitoring of the biodiversity has been done over three years, each year the monitoring was performed in February. Five different phyla were identified in this study at this location: Annelida, Arthropoda, Mollusca, Nemertea, and Cnidaria. (Wijsman, et al., 2008)

In 2015 four locations in the Eastern Scheldt (Lokkersnol, Zeelandbrug, Westbout and Zuidbout) were sampled to investigate the infauna community of the Eastern Scheldt. Even though the research report is still in preparation there is already known that during this research nine different phyla were found, namely Annelida, Arthropoda, Bryozoa, Cnidaria, Echinodermata, Mollusca, Nemertea, Phoronida and Plathyhelminthes. (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016)

2.4 Trophic levels

A study performed by Wijsman *et al.* (2008) mentions that benthic species play an important role in the functioning of an ecosystem as they decompose organic material and as they are a resource for higher trophic levels, like fish and birds. A trophic level is the number of steps an organism is within the food chain (Encyclopaedia Brittannica, 2017). The biomass and the diversity of benthos is dependent of the surrounding environmental conditions and is subject to fluctuations, by e.g. disturbances or seasonal variation. As the benthos is a resource for the higher trophic levels a change in the benthic community might have effects on the higher trophic levels. (Wijsman, et al., 2008). Therefore, it is important to get insight in the trophic levels of organisms when investigating community composition. Previous research in the Eastern Scheldt by Wijsman *et al.* (2008) and Tangelder *et al.* (2016) indicates the three most abundant phyla are Annelida, Arthropoda and Mollusca. Furthermore, Cnidaria, Echinodermata, Nemertea, Phoronida and Chordata were found, however there were few individuals found for these phyla (Wijsman, et al., 2008) (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016).

The Annelida phylum exists of ringworms which are mainly found in wet ecosystems. The Polychaeta (bristle worms) is the most successful class within the phylum and abundant in the intertidal zone (The Columbia Encyclopedia, 6th ed., 2016). Previous research in the Eastern Scheldt shows this is indeed the most abundant class (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016). Polychaeta live in protected habitats and burrow or build tubes within the sediment and they can be active predators, deposition feeders or filter feeders (Ward, 2015).

The Crustacean is a sub-phylum of Arthropoda and forms a diverse group that is abundant in the Eastern Scheldt (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016). Orders that are likely to be found within this research are Amphipoda and Decapoda, as these are common in intertidal zones. Lowry *et al.* (2001) mentions Amphipoda are widespread, abundant and diverse Crustaceans which can be found in almost all aquatic habitats. Amphipoda are particularly important in marine environments as carnivores, herbivores, detrivores, micro predators and scavengers. (Lowry & Springthorpe, 2001) Most Decapoda are marine species and it is likely to find crabs at the monitoring site of this study. Crabs are scavengers and omnivores, feeding primarily on algae and taking other food like bivalve Mollusca, Annelida, other Crustacean species and detritus, depending on the availability (Washington Department of Fish and Wildlife, 2008).

Another phylum that was found in high abundance in a previous study in the Eastern Scheldt is Mollusca (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016). The Bivalves are a well-known class, to which also the *M. edulis* belongs. Mollusca are generally filter feeders and can filter a variety of algae or small Crustaceans, however, bigger sized Mollusca can eat other organisms as well, like crustaceans and other invertebrates (Yukozimo, 2017). Most Bivalves are filter feeders as they adopt a sedentary or sessile lifestyle. The *M. edulis* is however able to 'walk', as this species relies on random movements to balance the feeding and clumping behavior of individuals with the needs of the group. This allows the *M. edulis* to organize themselves into patchy mussel beds that are the optimal tradeoff between finding shelter and decreasing competition for food (de Jager, 2011).

2.5 Relationship between shellfish beds and biodiversity

Biodiversity can be threatened by habitat loss. Within a biologically diverse ecosystem, organisms are dependent on each other for survival. Loss of biodiversity weakens the connections that exist among species, which makes the ecosystem vulnerable. (Perritano, 2017) Shellfish beds may enhance the biodiversity in an area as they provide substrata for attachment, refuge and nesting area for bird species and they control the transport of particles and solutes in the benthic environment. (Gutiérrez, Jones, Strayer, & Iribarne, 2003) (Borsje, et al., 2011)

In a study performed in 2012 the comparison on the benthic community composition was made between mussel culture plots of hanging cultures and wild mussel beds in the Dutch western Wadden Sea (Smaal, Craeymeersch, Glorius, van Stralen, & Drenth, 2017). The study showed that the occurrence of mussels accommodates relatively high species numbers, both on wild beds and culture plots. Samples where no or few mussels were present showed lower species numbers, and therefore mussel plots are seen as hotspots for biodiversity. The comparison between wild beds and culture plots showed that the biodiversity of the cultured plots was slightly higher than the biodiversity on the wild beds, therefore it can be said that culture plots are important sites for benthic communities. It should be noted that the mussel culture plots in this research were located in areas with slightly higher salinity, close to the North Sea, and therefore could have a higher species richness than the natural mussel plots. (Smaal, Craeymeersch, Glorius, van Stralen, & Drenth, 2017)

A study performed in 2003 by IMARES on the restoration of shellfish reefs in the Dutch Wadden Sea, also considered whether a mussel reef is a hotspot for biodiversity (Smaal, 2013). The final conclusion on biodiversity in this research was that mussel culture plots are not only important for the benthic biodiversity, but also for the bird biodiversity in the area. Furthermore, more mussels in the mussel plot indicated a higher biodiversity in the area than with less mussels per mussel plot. (Smaal, 2013) Another study led to the insight that mussel banks contain approximately 40% more species than other nearby locations do, because the banks have more structure for species to grow on and to seek for shelter (Telegraaf, 2015).

2.6 Colonisation and ecological succession

After an area is cleared of organisms by a disturbance event, it may be colonised by a variety of species, which are gradually replaced by other species. This change within an ecological community is called ecological succession. Within an ecological community the species composition changes over time as some species become more prominent while others may go locally extinct. There are few types of succession; Primary succession occurs when an area that has never before supported life is colonized. Secondary succession occurs when an area that has supported an ecological community is disturbed or changed so that the original community is destroyed, and a new community inhabits the area. Primary succession is less common than secondary succession, the latter can be the result of natural disasters such as avalanches and floods, as well as human interference. (Friedl, n.d.) The harvesting of mussels by mussel farmers is seen as a human interference on an area. Research performed on the long-term benthic responses of aggregate dredging, off the east coast of the United Kingdom, showed that dredging has no significant effect on the proportion of each phylum (Barrio Froján, Boyd, Cooper, Eggleton, & Ware, 2008). Even though the harvesting of mussels is a human interference, it is less disturbing than dredging as it is done to shallower depths into the sediment. Harvesting of mussels from bottom culture is done by dragging trawl cages through the mussel bed, and thereby disturbing the seabed, and bringing in the trawl cages when filled with mussels. The kind of disturbances that have the highest influence on biodiversity are the moderate disturbances (Jackson, Community Ecology, 2011b), as the ecosystem is not fully destroyed, yet there is enough disturbance for the communities to be harmed.

When primary succession begins, pioneer species are the first species that colonize a new area. In general pioneering species are fast-growing, opportunistic, and able to disperse easily. (Friedl, n.d.) After the pioneer species have colonized an area, larger species start to colonize the area until the ecosystem reaches stability. When a community is stabilizing it is called a climax community, however real stability never happens. With secondary succession pioneering species do not prepare an area for other species, therefore succession can occur faster. (Robb, 2017)

2.7 Hypothesis

With the information from the previous paragraphs in the theoretical framework hypotheses on the sub questions are formulated.

What organisms are present on the mussel plots?

Mussel plots are seen as a hotspot for community composition and the expectation is to find at least four different phyla within this research. These will be Annelida, Arthropoda, Cnidaria and Mollusca as these are the most common phyla found in soft sediment.

Do the community composition and the relative abundance differ between different mussel densities?

Previous research showed that the biodiversity is higher when more mussels are present in a plot. Therefore, differences in the community composition and the relative abundance are expected. The largest community composition will be found in the mussel plot with the highest density and the smallest community composition will be found in the reference plot. Annelida will cause the highest relative abundance in all different plot densities as research indicates this is the dominant group in soft sediment habitats.

Do the community composition and the relative abundance change over the first four months since seeding the mussels?

The expectation is that the community composition will stay equal over the first four months as all monitoring will be performed in winter months. However, the relative abundance will differ, Annelida will become more abundant as it is a very tolerant species to changes in the environment.

3. Method

3.1 Method

The monitoring of the community composition was performed in both the field and the laboratory. For each plot density four locations were monitored during low tide, so sixteen monitoring locations in total. Figure 3.1 and Figure 3.2 show the location of the monitoring site in the Eastern Scheldt near the Zandkreekdam and the distribution of the mussels and the sample points at the monitoring site (the reference plot is east of the monitoring site). When in the field locations within the mussel plot with the right density for the monitoring were identified. Since mussels tend to 'walk' the monitoring locations were not the same during each month of monitoring.

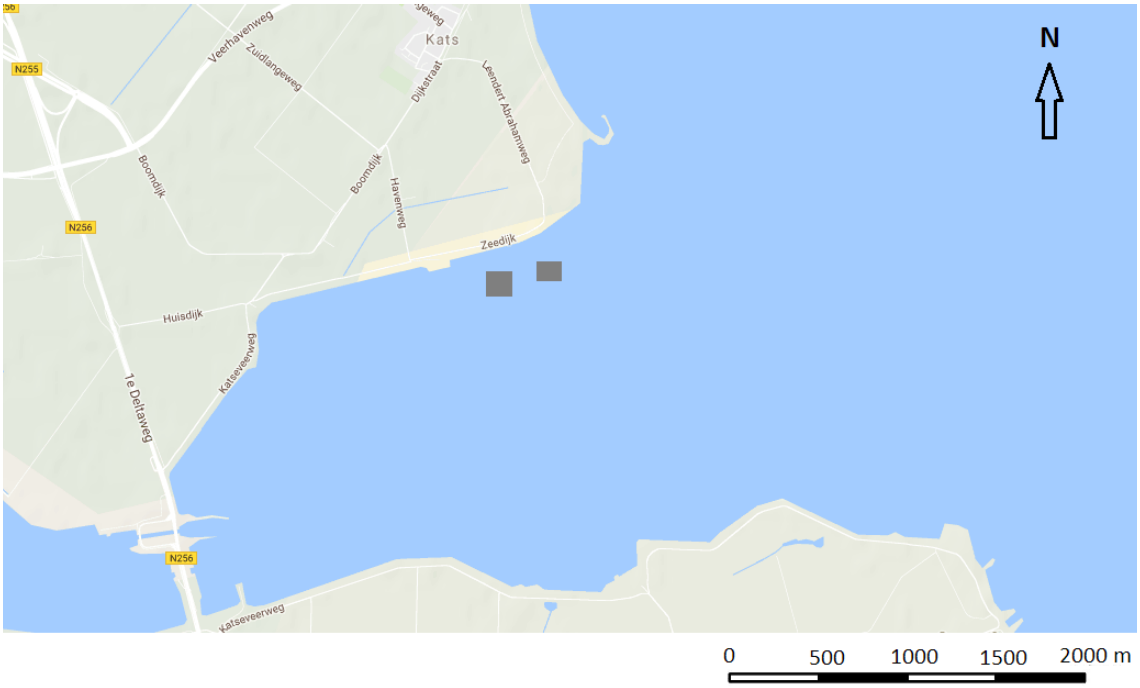


Figure 3.1: Location of the monitoring site at the Zandkreekdam. The left grey box is the location of the mussel plots and the right grey box is the location of the reference plot. Adjusted from (Google MyMaps, 2017).

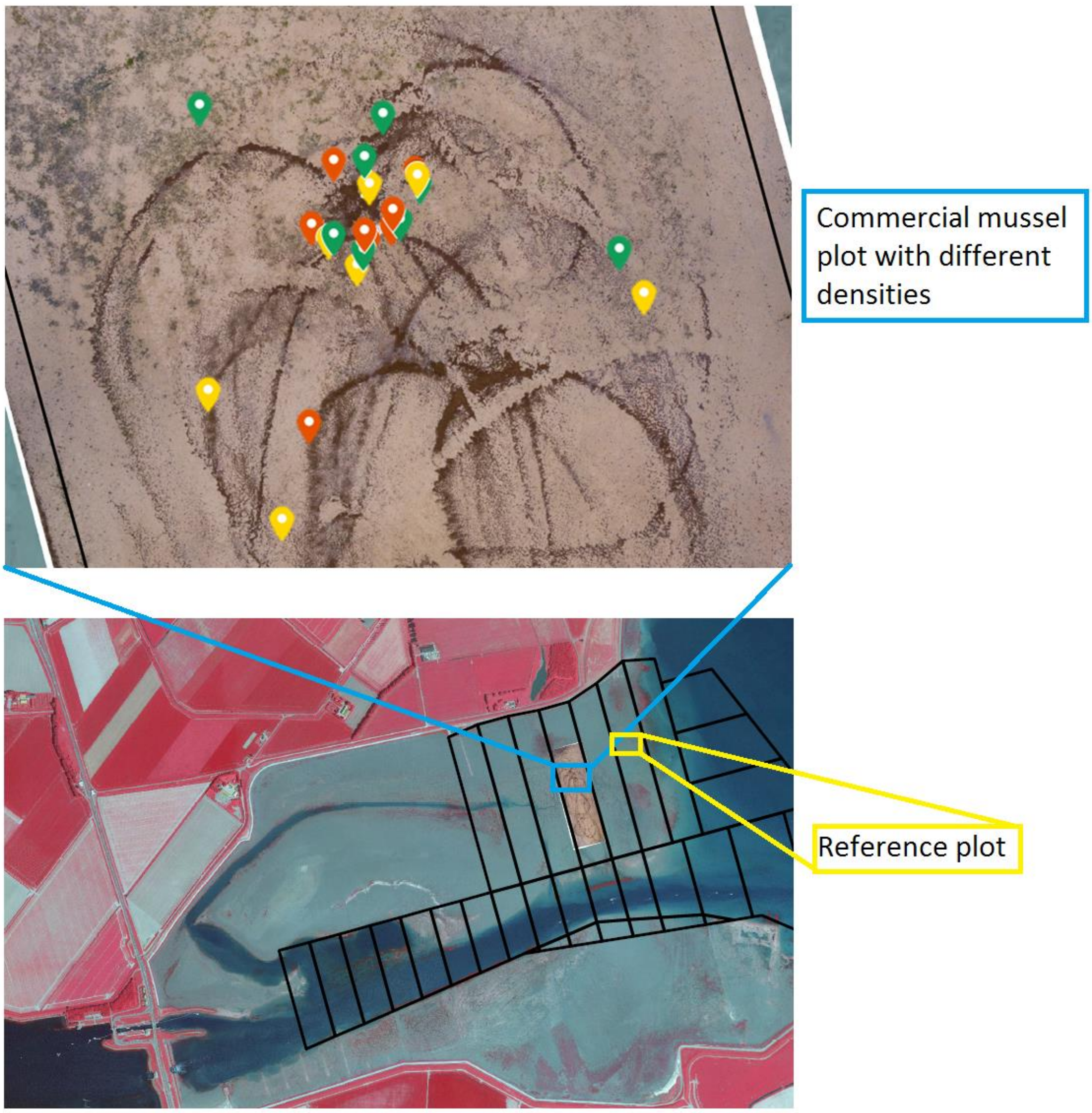


Figure 3.2: Distribution of the monitoring locations at the Zandkreekdam. Red = high density. Yellow = medium density. Green = low density. Adjusted from (Schotanus, 2016).

3.1.1 Field monitoring

All field monitoring, both epifauna and infauna, were performed during low tide. Before the fieldwork, all sample bottles were prepared by writing the ID of the sample location and the date of monitoring on both the bottle and on the lid.

In the field a monitoring location was sought by visible inspection, based on the set density percentages for each density. When at a monitoring location the quadrant was thrown once at random. Within the quadrant the epifauna species were determined, counted and recorded directly in the field.

Furthermore, three cores of sediment were taken at a monitoring location with the benthic corer to a depth of 30 cm, the benthic corer was dug out with a shovel. The cores of sediment were sieved in the field with a 1 mm sieve and the remaining material was put into the sample bottle of that location.

This field monitoring procedure was repeated for all sixteen monitoring locations.

NOTE: For the control base area and for each mussel density the number of cores that should be taken within the mussels and next to the mussels differs (see Table 3.1).

Table 3.1: Location of the sediment cores

Mussel density	Cores within the mussels	Cores next to the mussels
High	3	0
Medium	2	1
Low	1	2
Control base	0	3

3.1.2 Laboratory analysis

The preparation for the laboratory analysis was done by fixating the benthos samples with 96% ethanol and colouring the samples with rose bengal stain to be able to make a distinction between live and dead species at the time of collection.

The sample was sieved in the lab once again by making use of a 2 mm and a 1 mm sieve. The sieves were placed on top of each other, the biggest mesh was the upper sieve. Next the sample was rinsed with water: the remaining material in the 2 mm sieve was placed into a photo tray and the material in the 1 mm sieve was placed into petri dishes.

The material in the photo tray was examined and all organisms that were alive at the time of collection were removed from the tray. These organisms were put into a petri dish in a drop of water and were determined using a binocular microscope and determination books, the organisms were determined to phylum. For the phylum of Arthropoda a further distinction was made on the taxonomic level of order. All organisms were placed into small labelled sample jars with water and ethanol, these samples were preserved if further determination is required.

NOTE: In this research the *M. edulis* is not preserved. This is a familiar species and no further determination is therefore needed. This species is counted and afterwards thrown away.

The organisms from the 1 mm sieve, which were collected in a petri dish were sorted and preserved as stated above.

Since the determination of individuals is labour intensive and time consuming, the focus was on analysing three samples per density at first (12 samples in total per monitoring month), and if there would be enough time the fourth benthos sample would be analysed as well.

3.1.3 Data analysis

A Microsoft Office Excel file functioned as a database for all project data. The epifauna data from the field monitoring and the infauna data from the laboratory analysis were all put into this file. This file was therefore a source to see which organisms were found during this study.

To statistically analyze the data there was made use of RStudio, therefore the extensive data from Microsoft Office Excel was minimalized to only showing the date, the plot density and the number of phyla found.

In RStudio a bar graph with error bars, showing the standard error of the mean, was made to see the differences between the density plots based on the date.

Analysis of variance (ANOVA) tests were performed to find out whether or not there were significant differences between the plots based on date and/or density for the number of phyla found. When using ANOVA the goal is to use the simplest model which provides the most information, therefore the variables that did not show significant differences were taken out of the analysis. The significance levels were based on the p-values, in Table 3.2 can be seen which p-values correspond with which significance level.

Table 3.2: p-values and the corresponding significance levels.

p-value	Significance level
0 – 0.001	99.9%
0.001 – 0.01	99%
0.01 – 0.05	95%
0.05 – 0.1	90%
0.1 - 1	No significance

The first ANOVA performed was based on the interaction of density and date. When the interaction did not show to be causing a significant difference the interaction was taken out and another ANOVA is performed with the date and density as individual variables. When a variable shows not to be causing a significant difference it was taken out of the analysis as mentioned above. After performing multiple ANOVA's a model with only variables that caused significant differences remained.

Based on this simplest model a post-hoc Tukey-test was performed in order to find out between which density plots or dates the significant difference in the number of phyla occurred. These significant differences are indicated in the bar graph by adding letters above the significantly different bars.

For the epifauna data the regression was checked as well in RStudio to find out whether there was a relation between the number of phyla found and the number of mussels in the density plot. RStudio shows this by creating a graph and by calculating the p-value to check whether there is a significant relation.

The last analysis performed with RStudio was checking whether the data was normally distributed, RStudio creates a graph as output.

With Microsoft Office Excel a stacked bar plot was created to get insight in the share a certain phylum has in a density plot. This gave insight in the community composition within the different density plots as well as the change over the monitoring months.

3.2 Schedule of requirements

The research was bound to several limiting conditions that needed to be taken into account. Through an individual brainstorm-session a list of preconditions and limitations was formulated, this list was specified further to get to a schedule of requirements for this research.

- For this research mussel culture plots are needed where we have access to and where we are allowed to perform research;
- Within the mussel plot the needed densities have to be present;
- The field monitoring could only be done during low tide and with the right weather conditions;
- The monitoring locations within the density plots had to be ‘chosen’ at random;
- For the theoretical research scientific databases have to be used;
- For each density at least three sample points need to be analyzed to use a statistical analysis;
- For the analysis of the results there should be access to RStudio;
- The research report needs to give insight into the influence of density in mussel plots on the community composition;
- This research needs to be performed within four months.

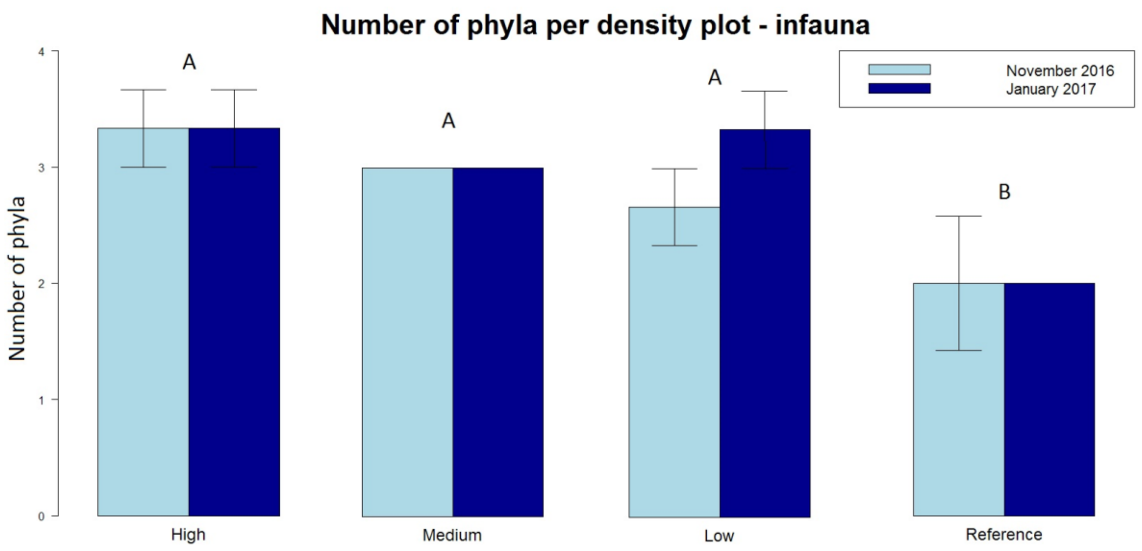
4. Results

The results of this research are divided into infauna results and epifauna results. Within this research six phyla were found, namely: Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata and Mollusca. Additional information on these six phyla can be found in **Fout! Verwijzingsbron niet gevonden.** in Appendix C.

4.1 Infauna

The infauna results of this community composition research are based on two months, November 2016 and January 2017, instead of all three monitoring months, as the benthos samples from the March monitoring could not be used for the analysis due to circumstances.

Before showing the results, it should be noted that due to the little amount of samples the data appears to be not normally distributed, the Q-Q plot can be found in Graph B.1 in Appendix C. Since the data is based on the number of phyla the steps in the Q-Q plot are discrete and forms clusters. Due to these clusters the line that indicates normal distribution remains almost horizontal, which is an indicator for no normal distribution. The number of phyla per plot density over the two months of monitoring of infauna is visualised in Graph 4.1. The results in this bar graph are based on data coming from three samples per month of each plot density, the standard error of the mean is included for each bar.



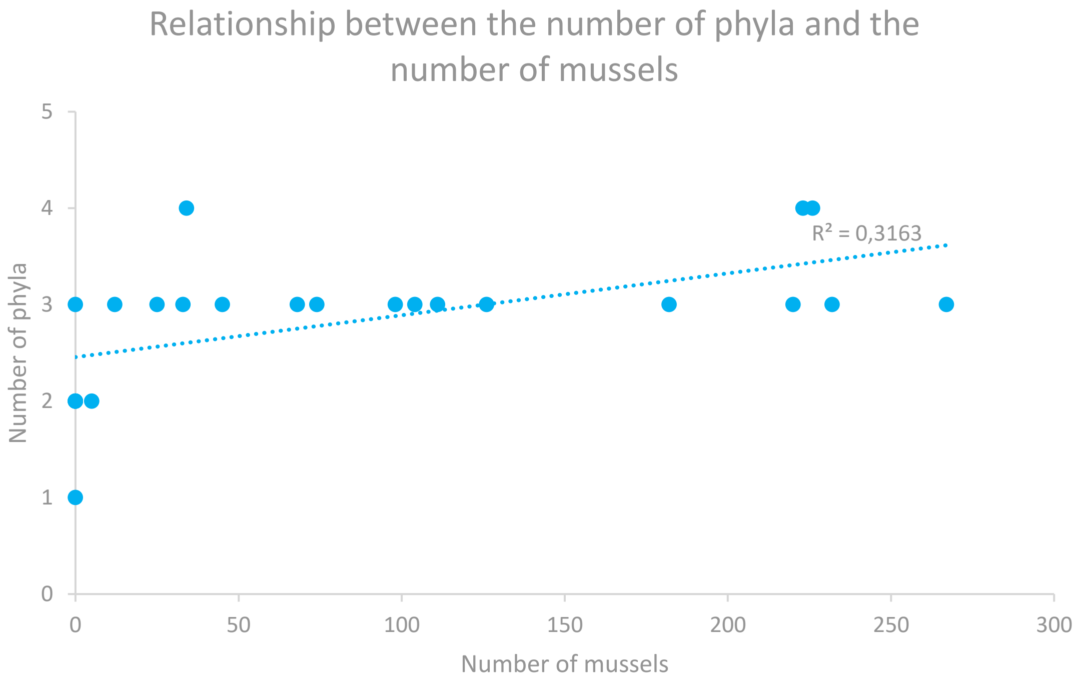
Graph 4.1: Number of phyla present per mussel plot density between November 2016 and January 2017. The A's and the B indicated that these plot densities are significantly different from each other. Adjusted from (RStudio, 2017).

The reference plot shows the lowest number of phyla on average (2 phyla) when compared to the other three densities (high=3.33 phyla, medium=3 phyla, low=3 phyla). Furthermore, for three out of four plot densities there was no increase in the number of phyla present, only in the low plot density there is a slight increase visible. Another remarkable feature is that the medium density has a standard error of 0 for both months, as during every monitoring the same number of phyla were found, and therefore has the most stable monitoring results.

To check whether there are significant differences between the data of the four plot densities and the months several ANOVA's have been executed. The differences between the number of phyla based on the interaction of density and date were tested with a two-way ANOVA. This statistical test showed there is no significant difference coming from the interaction of the density and the date (p=0.6419; df=3; F=0.571). Furthermore, this ANOVA (Table B.2 in Appendix C) indicated that the date might not be a factor of influence to cause a significant difference in the number of phyla found (p=0.4607; df=1; F=0.571), associated with density, which showed there might be a significant difference due to density (p=0.0035; df=3; F=6.857). With another two-way ANOVA (Table B.3 in Appendix C), without the interaction of density and date, the date indeed showed not to be causing a significant difference (p=0.4434; df=1; F=0.613). The date was taken out as a factor of influence and a one-way ANOVA with only the density remained. This ANOVA indicated that there is a 99% certainty that the density is causing a difference in the number of phyla between the density plots (p=0.0015; df=3; F=7.5) (Table B.4 in Appendix C).

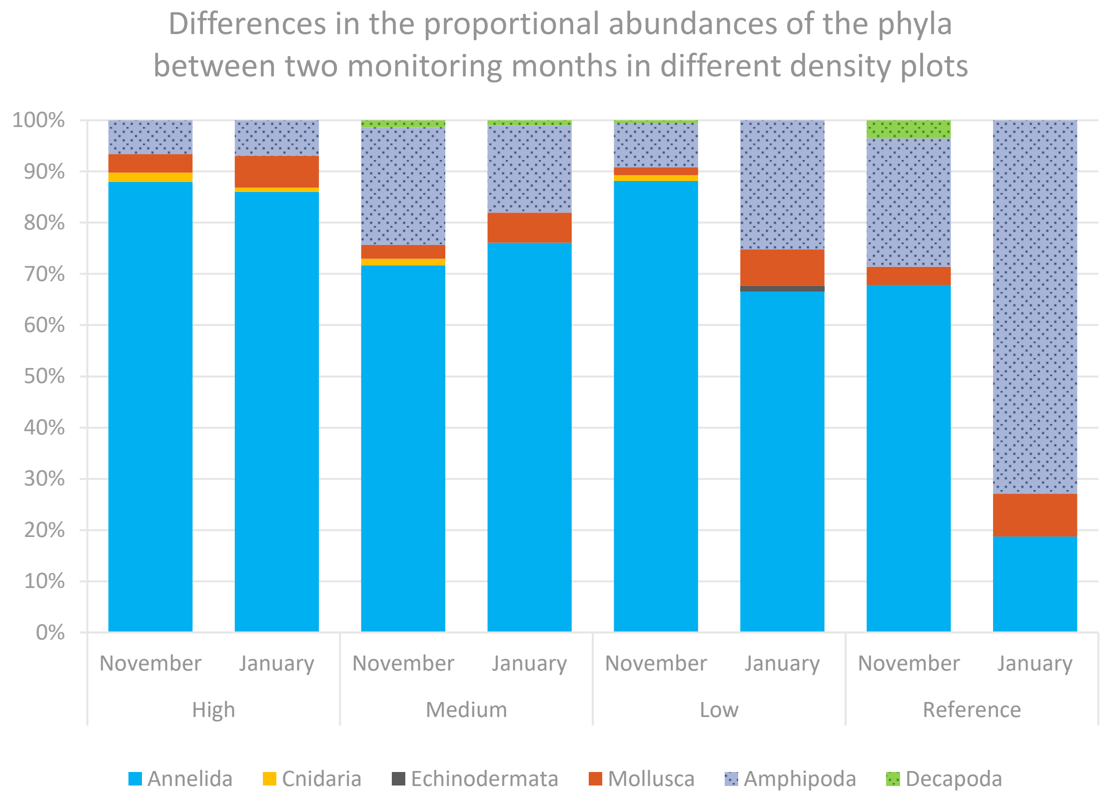
To find out between which density plots the significant difference occurred a post-hoc Tukey-test was executed. This test showed that the reference plot is showing significant differences with all other density plots. For the high density plot there is a 99% certainty that there is a significant difference with the reference plot (p=0.0012), for the medium and the low density plot this is a 95% certainty, both with a p-value of 0.0153 (Table B.5 in Appendix C). In Graph 4.1 these significant differences are indicated with 'A's' above the high, medium and low density plot and a 'B' above the reference plot.

Within this research the relationship between the number of mussels and the number of phyla found in the density plots was tested. As can be seen in the linear regression graph (Graph 4.2) the trend line is only slightly increasing with a R^2 value of 0,316. The data used for this linear regression is statistically analyzed and there is a 99% certainty that the number of mussels has a significant effect on the number of phyla in the density plots (p=0.0042) (Table B.6 in Appendix C).



Graph 4.2: Relationship between the number of phyla and the number of mussels

Five phyla were found in the monitoring of the infauna, namely Annelida, Arthropoda, Cnidaria, Echinodermata and Mollusca. The proportional abundances of these phyla differed per monitoring, which can be seen in Graph 4.3. Annelida dominated the benthic community with about 70% to 85% presence, only the reference plot from the January monitoring differs. The highest percentages of Annelida can be found in the high density plots. The phylum that dominated secondly was Arthropoda, which is for this graph the combination of Amphipoda and Decapoda; The Arthropoda are the dominant phylum in the reference plot from January. There should be noted that the Decapoda were not present in every monitoring, on the contrary to Amphipoda. The percentage of Arthropoda is different per density. The high density plot has the smallest percentage of Arthropoda out of all density plots (7%). The Mollusca shows in each density plot a slight increase in percentage over a period of two months, but remains one of the smaller phyla (2%-8%). The Cnidaria and the Echinodermata appear to be rare in the infauna samples (< 2%).



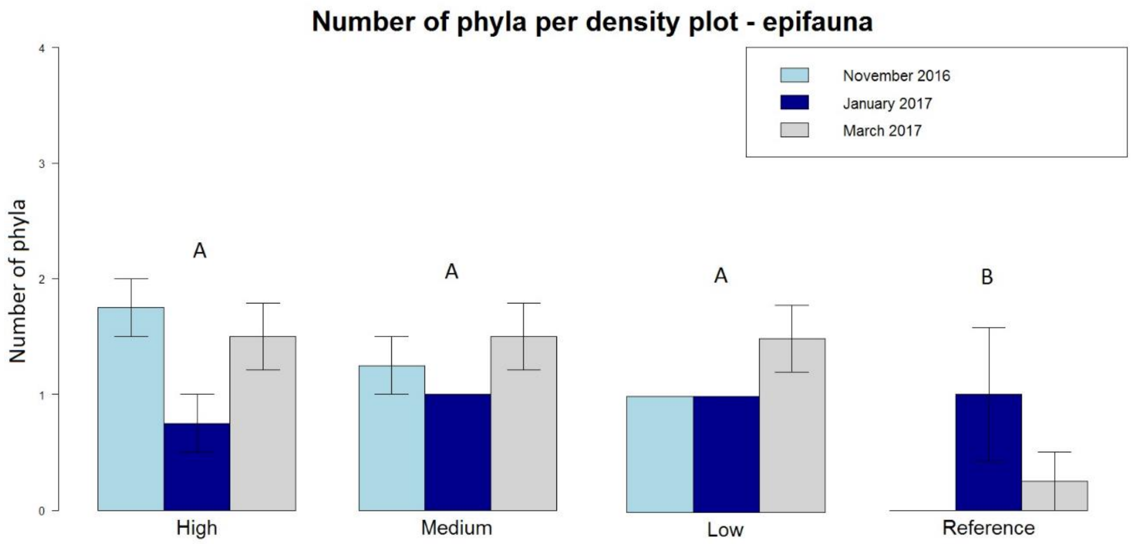
Graph 4.3: Distribution of the phyla in the different density plots in November 2016 and January 2017

4.2 Epifauna

The epifauna results of this community composition research are based on three months, November 2016, January 2017 and March 2017.

Before showing the results, it is relevant to indicate that the data appears to be normally distributed, the Q-Q plot can be found in Graph B.2 in Appendix C. Since the data is based on the number of phyla the steps in the Q-Q plot are discrete and forms clusters. Nevertheless, the line that indicates normal distribution is shows the epifauna data is normally distributed. Before showing the results of the epifauna, it should furthermore be noted that one of the samples from the January 2017 reference plot is taken out of the dataset as it does not show a true record since oysters were found within the sample point.

The change in number of phyla per plot density can be seen over the three months of monitoring of the epifauna in Graph 4.4. The results in this bar graph are based on the data coming from four samples per month of each plot density, the standard error of the mean is included for each bar.



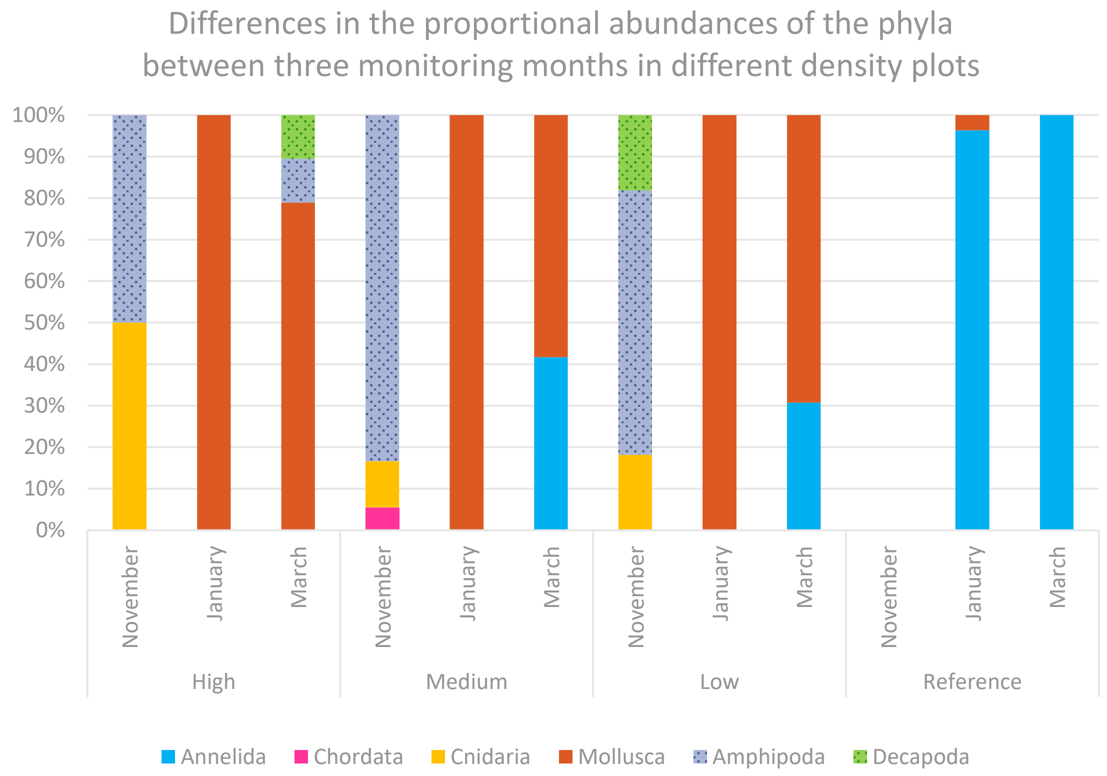
Graph 4.4: Number of phyla present per plot density between November 2016, January 2017 and March 2017. The A's and the B indicated that these plot densities are significantly different from each other. Adjusted from (RStudio, 2017).

The reference plot shows the lowest number of phyla on average (0.36 phylum) when compared to the other three densities (high=1.33 phylum, medium=1.25 phylum, low=1.17 phylum). Furthermore, the graph shows that in all three mussel plot densities the January monitoring is the lowest bar, whereas the January bar is the highest for the reference plot. For the three mussel density plots there is thus an increase in the number of phyla between January and March. The high density plot is the only density plot where the November monitoring is highest for its density. Furthermore, there are no phyla found during the November monitoring in the reference plot.

To check whether there are significant differences between the results of the four plot densities and the months several ANOVA were executed. With a two-way ANOVA the differences between the number of phyla as a function of the interaction of density and date was tested. This statistical test showed there is no significant difference coming from the interaction of the density and the date (p=0.6001; df=3; F=0.6299). Furthermore, this ANOVA indicated that the date might not be a factor of influence to cause a significant difference in the number of phyla found (p=0.3499; df=1; F=0.8951), on the contrary to the density, which showed there might be a significant difference due to density (p=0.0006; df=3; F=7.1994) (Table B.7 in Appendix C). With another two-way ANOVA test, without the interaction of density and date, the date indeed showed not to be causing a significant difference (p=0.3431; df=1; F=0.9194) (Table B.8 in Appendix C). The date was taken out as a factor of influence and a one-way ANOVA with only the density remained. This ANOVA indicates that there is a 99,9% certainty that the density is causing a difference in number of phyla between the density plots (p=0.0004; df=3; F=7.4087) (Table B.9 in Appendix C).

To find out between which density plots the significant difference occurs a post-hoc Tukey-test was executed. This test shows that the reference plot is showing significant differences with the all other density plots. For the high density plot it can be said with a 99.9% certainty that there is a significant difference with the reference plot ($p=<0.001$), for the medium and the low density plot this is a 99% certainty (respectively $p=0.0021$ and $p=0.0061$) (Table B.10 in Appendix C). In Graph 4.4 these significant differences are indicated with 'A's' above the high, medium and low density plot and a 'B' above the reference plot.

During the epifauna monitoring five phyla were found, namely Annelida, Arthropoda, Chordata, Cnidaria and Mollusca. The proportional abundances of these phyla differed per monitoring (Graph 4.5). The most striking feature in Graph 4.5 is that there are only two bars for the reference plot, as with the November monitoring no individuals were found. Other noticeable characteristics are that the January monitoring resulted in only Mollusca and Annelida. Arthropoda is a dominant group in the November results (this group is divided in Amphipoda and Decapoda in this graph). Furthermore, this graph shows that Decapoda and Chordata are groups that are found rarely and, when found, result in relatively low percentages (maximum 18%). The Cnidaria is a group that was only found in November.



Graph 4.5: Distribution of the phyla in the different density plots between November 2016, January 2017 and March 2017

5. Discussion

5.1 Results

The diversity of fauna inhabiting the intertidal flat at the Zandkreekdam was unexpected. With six phyla found in total it was slightly more diverse than the study of Wijsman et al. (2008), where only five phyla were found. These studies are comparable as for both the monitoring is performed in winter months. It should be noted however, that the study from Wijsman (2008) was not performed within a mussel plot. Tangelder *et al.* (2016) studied the infauna in the Eastern Scheldt in 2015, during this study 9 phyla were found. There should however, be noted that his research was not performed in shellfish beds and as the research report is still in preparation it is unclear during which season the infauna samples were taken. (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016)

The composition of the phyla for the infauna was more stable than the composition of the phyla for the epifauna. Therefore, the results from the infauna can be compared with previous research with more accuracy. The phylum Annelida is the most abundant phyla and constitutes about 70% to 85%, previous research in the Norwegian North Sea supports this result as Annelida was the most dominant phylum with 60% to 70% (Olsgard & Somerfield, 2000). Another previous study east off the United Kingdom showed Annelida has a proportional abundance of 53% (Barrio Froján, Boyd, Cooper, Eggleton, & Ware, 2008), which is much lower than this research at the Zandkreekdam. This difference might be explained by the seasonal variability and the number of samples in this study. For the study of Barrio Froján it is unclear in which season(s) the samples were taken, however, the data is from a period of 8 years so it may be a more true result as it shows the mean over a period of time. Furthermore, the research at the Zandkreekdam is in contrast with these previous studies as the Arthropoda show to be the second largest group, followed by the Mollusca. It may be possible that this order is different per type of waterbody, as Olsgard (2000) and Barrio Froján (2008) performed their research in the North Sea. Whereas Tangelder (2016) performed his research in the Eastern Scheldt and his study supports this order of dominance in phyla (Tangelder, van den Heuvel-Greve, & de Kluiver, 2016). The Echinodermata, Chordata and the Cnidaria are the smallest groups with a minor contribution to the total number of individuals found, this is supported by the study from Olsgard and the study of Barrio Froján. (Olsgard & Somerfield, 2000) (Barrio Froján, Boyd, Cooper, Eggleton, & Ware, 2008)

The results of this study show a significant regression between the number of phyla and the number of mussels. These results are supported by the results presented by Smaal (2013). Smaal (2013) furthermore indicated that more mussels in a plot led to a higher level of biodiversity than with fewer mussels per plot (Smaal, 2013).

A research from Smaal *et al.* (2017) supports the result that mussel density appears to be causing a significant difference in the number of phyla present. For the high density plot the certainty that the number of mussels was causing a significant difference was greater than at the other density plots. The reference plot showed the lowest number of phyla found and there was no significant difference found for this plot. This result is supported by a research performed in 2012 in the Wadden Sea, wherein was concluded that the occurrence of mussels accommodates higher species numbers, and therefore mussel plots are seen as hotspots for biodiversity. (Smaal, Craeymeersch, Glorius, van Stralen, & Drenth, 2017) The explanation for the relation between the number of mussels and the number of phyla can be found in the research of Gutiérrez (2003) which indicates that the more shells are aggregated, the more organisms can settle on this substratum. Furthermore, there is more space for species seeking refuge when there are more shells aggregates, however, Gutiérrez mentions there is no linear function between these numbers. (Gutiérrez, Jones, Strayer, & Iribarne, 2003)

5.2 Limitations

One of the limitations in this research was that all monitoring was performed in winter months. As there is seasonal variation in community composition and abundance according to Shimadzu *et al.* (2013). (Shimadzu, Dornelas, Henderzon, & Magurran, 2013) Within this research there was no significant difference in the number of phyla coming from the monitoring dates, maybe because there was so little data.

Another limitation in this research was that the reference plot was not everything it optimally should have been. The reference plot was in between the mussel plot and a nearby oyster plot. Therefore, the reference plot might have been under influence of these plots, as this study and previous studies show that shellfish beds have a higher biodiversity when compared to reference plots. This is especially a limitation as some oysters were present in the reference plot and thereby disturbed the 'reference' plot. A perfect example for this limitation is that one of the January samples from the reference plot of the epifauna was monitored with oysters in the quadrant. This sample point was taken out for the data analysis as it was an outlier by comprising more than thrice the number of phyla when compared to the other reference samples.

The number of sample points and the time were limitations as well. As this final thesis research had to be conducted within four months by one person the number of sample locations was just enough to have a research that could be statistically analysed. With this little sample points per plot density the data for infauna appeared not to be normally distributed as the number of phyla can be only in discrete steps and therefore forms clusters in the Q-Q plot.

Furthermore, the infauna results are coming from a dataset of two months instead of three. Due to circumstances the March samples could not be analysed in the laboratory and therefore there is no infauna data of the monitoring in this month.

The research for this final thesis was executed locally at one location in the Eastern Scheldt, therefore the results of this research might be different throughout the water body. The insight gotten in the natural values of the Eastern Scheldt based on community composition are local instead of regional, which eventually is the goal.

Research indicates that the level of phylum can be used to calculate significant differences, but, unfortunately, the level of phylum is too coarse to use a biodiversity index to calculate the species richness, the evenness and the abundance in absolute numbers. Furthermore, research indicates that the phylum taxon can be used without losing much inter-sample relations, however the family taxon is preferred above phyla.

6. Conclusion

From this study it can be concluded that mussel density is a factor of influence to cause a higher number of phyla. Statistics show that this can be said with a 99% certainty. Density plots with a higher number of mussels cause a higher number of phyla to be present in the plot, as has become clear with the regression analysis (R^2=0.316). The reference plot showed the lowest number of phyla in both the infauna and the epifauna monitoring. The reference plot is also the only density plot that was significantly different from the other density plots for the infauna monitoring (99% certainty for the high density and 95% certainty for the medium and the low density) when based on the number of phyla found. For the epifauna data the reference plot was significantly different from all other density plots as well, 99,9% certainty for the high density and 99% certainty for the medium and low density when based on the number of phyla found.

At least four different phyla were expected to be found within this study. These would be Annelida, Arthropoda, Cnidaria and Mollusca. In this study six different phyla were found, and therefore this hypothesis is accepted. Besides the four expected phyla Chordata and Echinodermata were also found.

Furthermore, differences in the community composition and the relative abundance within the density plots were expected. This hypothesis was found to be true as the biggest community composition was found in the high density plot and the smallest community composition was found in the reference plot. The most dominant phylum was Annelida in the infauna monitoring for all different densities, followed by Arthropoda.

The date, on the other hand, showed not to be a factor of influence on the number of phyla with the performed statistical analysis. As the date did not show significant difference in the number of phyla found the third hypothesis is rejected. The relative abundance of Annelida was expected to increase over a period of four months and the community composition would stay equal over this period, both were found not to be true.

Concludingly there can be said that density is definitely a factor of influence on the number of phyla found in the mussel plots, whereas the date showed not to be causing a significant difference. The density plots with mussels showed significantly higher numbers of phyla found when compared to the density plot, therefore the community composition is affected by the density of a mussel plot.

7. Recommendations and implementation

For community composition research in the Eastern Scheldt, there are several recommendations to be made.

First, it would be good to monitor more sample points per density. When more sample points are used the data might show to be normally distributed in the Q-Q plot as there might form more and/or bigger clusters. With more sample points the data will give a better view on the community composition in the density plots, especially the proportional abundance graphs might show more stable data.

Second, it is recommended to perform a community composition research over a longer period of time, throughout all four seasons. This research was performed over only three monitoring months, all in winter time. To get more insight in the community composition in mussel plots in the Eastern Scheldt it is needed to also monitor in the other seasons. This is also of use to see how seasonal variability is of influence on the community composition in the waterbody, as well as to see whether the individuals in the community follow the classic predator-prey model. As previous research showed the abundance of species varies most in winter months over the year it is recommended to perform community composition or biodiversity research over multiple years.

Third, the suggestion is to perform the community composition research at more than one location to have regional results instead of local results, as in this research. To get insight in the natural values in the Eastern Scheldt based on community composition, the research should be performed at more locations around the waterbody.

Fourth, it is recommended to determine individuals to a lower taxonomic level, family as the highest taxon, preferably to the species taxon. When family is used even less information about the inter-sample relations will be lost, when compared to the phyla taxon, but when the species level will be used biodiversity indices can lead to absolute numbers about the species richness and the relative abundance.

Fifth, it is recommended to create a food web of the water body based on community composition research in littoral, commercial mussel plots in order to get more insight in the community structure and the trophic levels. However, in order to make a food web the information on the individuals is needed to a less coarse level than the phylum.

Sixth, it is recommended to study the boundaries of the density ranges. For this study there was made use of only three photos per density to perform the density range analysis. During this analysis the photo's provided no information on the boundaries of the ranges, only on the average coverage of mussels in percentage.

Besides recommendations there is also thought of the relevance of this study for several stakeholders. The importance of this research for mussel farmers is that there now proof that their littoral, commercial mussel plots are of added value for the natural values in the Eastern Scheldt. Especially as this study shows that the density plots with mussels provide a significantly higher number of phyla when compared to the reference plot without mussels. Furthermore, this research has been important for the research group of Building with Nature as this is part of the research project 'Added value with Mussels' and community composition study is time consuming and labour intensive. This study contributes to one of the three main goals of the 'Added value with Mussels' project as it gives an indication for the influence of littoral, commercial mussel plots in the Eastern Scheldt on the community composition. Furthermore, this study is of relevance for nature organisations by providing insight in the community composition within the Eastern Scheldt. It shows that the natural values of the Eastern Scheldt can be increased by mussel plots within the strict regulations of the Natura 2000. In addition, this shows that building with nature, by mussel beds, is of added value for a sustainable and diverse environment.

8. References

Altorf, J., & van Gils, T. (2014). Retrieved May 29, 2017, from https://nieuwskroniek.files.wordpress.com/2014/03/kaart2.jpg

Anderson, M. J., Diebel, C. E., Blom, W. M., & Landers, T. J. (2004, December 17). Consistency and variation in kelp holdfast assemblages: Spatial patterns of biodiversity for the major phyla at different taxonomic resolutions. *Journal of Experimental Marine Biology and Ecology, 2005*(320), 35-56. doi:10.1016/j.jembe.2004.12.023

Ansomar. (2012). *Cockle*. Retrieved January 9, 2017, from Ansomar: http://www.ansomar.com/eng/cockle.html

Barrio Froján, C. R., Boyd, S. E., Cooper, K. M., Eggleton, J. D., & Ware, S. (2008). Long-term benthic responses to sustained disturbance by aggregate extraction in an area off the east coast of the United Kingdom. *Estuarine, Coastal and Shelf Science, 2008*(79), 204-212. doi:10.1016/j.ecss.2008.03.023

Beals, M., Gross, L., & Harrell, S. (2000). *Diversity indices: Shannon's H and E*. Retrieved February 28, 2017, from TIEM: http://www.tiem.utk.edu/~gross/bioed/bealsmodules/shannonDI.html

Borsje, B. W., van Wesenbeeck, B. K., Dekker, F., Paalvast, P., Bouma, T. J., van Katwijk, M. M., & de Vries, M. B. (2011). How ecological engineering can serve in coastal protection. *Ecological Engineering*, pp. 113-122. doi:10.1016/j.ecoleng.2010.11.027

Borthagaray, A. I., & Carranza, A. (2007). Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. *Acta Oecologica*(31), 243-250. doi:10.1016/j.actao.2006.10.008

Capelle, J., Wijsman, J., van Stralen, M., Herman, P., & Smaal, A. (2016, February). Effect of seeding density on biomass production in mussel bottom culture. *Journal of Sea Research*(110), 8-15. doi:10.1016/j.seares.2016.02.001

CBS, PBL, Wageningen UR. (2014, April 11). *Typische soorten van de Oosterschelde, 1994-2012*. Retrieved February 28, 2017, from Compendium voor de Leefomgeving: http://www.clo.nl/indicatoren/nl1563-typische-soorten-oosterschelde

CBS, PBL, Wageningen UR. (2015, November 24). *Aantal nieuwe soorten in de Oosterschelde, 1978-2012*. Retrieved February 28, 2017, from Compendium voor de Leefomgeving: http://www.clo.nl/indicatoren/nl1065-exoten-in-de-delta

Conserve Energy Future. (2017). *What is habitat loss and destruction?* Retrieved May 30, 2017, from http://www.conserve-energy-future.com/causes-effects-solutions-for-habitat-loss-and-destruction.php de Jager, M. (2011). Retrieved May 31, 2017, from https://www.youtube.com/watch?v=MaMBiRoZYvs

Delta Expertise. (2017). *Uitvoeren onderzoek naar zandhonger Oosterschelde i.h.k.v. MIRT*. Retrieved May 30, 2017, from https://www.deltaexpertise.nl/wiki/index.php/Uitvoeren_onderzoek_naar_zandhonger_Oosterschelde_i.h.k.v._MIRT#Het_proces_van_Zandhonger

Donaldi, S., van der Heide, T., Piersma, T., van der Zee, E., Weerman, E., van de Koppel, J., . . . Erikson, B. K. (2015, January 6). Multi-scale habitat modification by coexisting ecosystem engineers drives spatial separation of macrobenthic functional groups. (M. Bracken, Ed.) *Oikos Journal*, 1-9. doi:10.1111/oik.02100

Drinkwaard, A. (1998). Introductions and developments of oysters in the North Sea area: a review. *Helgoländer Meeresunters*(52), 301-308. doi:10.1007/BF02908904

Encyclopaedia Brittannica. (2017). Trophic level. Retrieved June 4, 2017, from https://www.britannica.com/science/trophic-level

European lugworm. (n.d.). Retrieved January 9, 2017, from Britannica: https://www.britannica.com/animal/European-lugworm

Friedl, S. (n.d.). What is ecological succession? - Definition,Types & Stages. Retrieved February 26, 2017, from http://study.com/academy/lesson/what-is-ecological-succession-definition-types-stages.html#transcriptHeader

Google MyMaps. (2017). *MyMaps*. Retrieved from MyMaps: https://www.google.com/maps/d/

Gossling, E. (2003). Bivalve Molluscs. In E. Gossling, *Bivalve Molluscs* (Vol. 9, pp. 296-303). Fishing New Books. Retrieved May 30, 2017, from https://malacolog.com/files/Gosling_2004.pdf

Gray, J. S. (1997). Marine biodiversity: patterns, threats and conservation needs. *Biodiversity and conservation*(6), 153-175. Retrieved May 30, 2017

Gutiérrez, J. L., Jones, C. G., Strayer, D., & Iribarne, O. (2003, April). Mollusks as ecosystem engineers: The role of shell production in aquatic habitats. *Oikos*(101), 79-90. doi:10.1034/j.1600-0706.2003.12322.x

HZ University of Applied Sciences. (2017). *Meerwaarde met Mosselen*. Retrieved January 2, 2017, from HZ University of Applied Sciences: https://hz.nl/projecten/meer-waarde-met-mosselen

Jackson, R. B. (2011a). Community ecology. In N. A. Campbell, P. V. Minorsky, J. B. Reece, L. A. Urry, M. L. Cain, S. A. Wasserman, & R. B. Jackson, *Campbell Biology* (9 ed., Vol. Unit 8, pp. 1246-1247). San Fransisco. Retrieved February 22, 2017

Jackson, R. B. (2011b). Community Ecology. In N. A. Campbell, P. V. Minorsky, J. B. Reece, L. A. Urry, M. L. Cain, S. A. Wasserman, & R. B. Jackson, *Campbell biology* (9 ed., Vol. VIII, pp. 1254-1255). San Fransisco. Retrieved February 26, 2017

Kamermans, P., Blankendaal, M., & Perdon, J. (2009). Predation of shore crabs (Carcinus maenas (L.)) and starfish (Asterias rubens L.) on blue mussel (mytilus edulis L.) seed from wild sources and spat collectors. *Aquaculture*(290), 256-262. doi:10.1016/j.aquaculture.2009.02.031

Kamermans, P., Brummelhuis, E., & Smaal, A. (2002). *Use of spat collectors to enhance supply of seed for bottom culture of blue mussels (Mytilus edulis) in the Netherlands.* Retrieved May 30, 2017, from http://edepot.wur.nl/344709

Kerkhoff. (2010, January 20). Measuring biodiversity of ecological communities. Retrieved May 31, 2017, from http://biology.kenyon.edu/courses/biol229/diversity.pdf

Leeuwen, B. v. (2008). *Modeling mussel bed influence on fine sediment dynamics on a Wadden Sea intertidal flat.* Master thesis, University of Twente; Deltares. Retrieved March 8, 2017, from http://www.waddenacademie.nl/fileadmin/inhoud/pdf/06-wadweten/Scripties/Bas_van_Leeuwen_scriptie.pdf

Lowry, J., & Springthorpe, R. (2001, September 2). Amphipoda: Families. Retrieved May 31, 2017, from crustavea.net: http://www.crustacea.net/

Ministerie van Economische Zaken. (n.d.). *Oosterschelde*. Retrieved February 7, 2017, from https://www.synbiosys.alterra.nl/natura2000/gebiedendatabase.aspx?subj=n2k&groep=2&id=n2k118

Ministerie van Economische Zaken. (n.d.). *Oosterschelde*. Retrieved February 13, 2017, from Beschermde natuur in Nederland: soorten en gebieden in wetgeving en beleid: https://www.synbiosys.alterra.nl/natura2000/gebiedendatabase.aspx?subj=n2k&groep=10&id=n2k118

Ministerie van Infrastructuur en Milieu; Rijkswaterstaat. (2016). *Oosterschelde - Natura 2000 Deltawateren.* Beheerplan. Retrieved February 13, 2017, from http://www.platformparticipatie.nl/Images/Oosterschelde%20Natura%202000%20Deltawateren%20Beheerplan%202016-2022_tcm318-379538.pdf

Morris, K. E., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., . . . Rillig, M. C. (2014). Choosing and using diversity indices: insights for ecological applications from the

German Biodiversity exploratories. *Ecology and Evolution*(4), 3514-3524. doi:10.1002/ece3.1155

Nationaal Park Oosterschelde. (n.d.). *Natuur*. Retrieved February 28, 2017, from Nationaal Park Oosterschelde: http://www.np-oosterschelde.nl/over-het-park/natuur.htm

Nederlands Mosselbureau. (2017). *Achtergrondinformatie*. Retrieved January 6, 2017, from Het Nederlands Mosselbureau: http://www.mosselbureau.nl/pers/persdossier.htm

Noordzeeloket. (2006). *Natura 2000 doelendocument.* Den Haag: Ministerie van Landbouw, Natuur en Voedselkwaliteit. Retrieved January 2, 2017, from https://www.noordzeeloket.nl/images/Natura2000%20doelendocument_861.pdf

Olsgard, F., & Somerfield, P. J. (2000, March). Surrogates in marine benthic investigations – which. *Journal of Aquatic Ecosystem Stress and Recovery, 2000*. doi:10.1023/A:1009967313147

Perritano, J. (2017). *How does biodiversity help an ecosystem?* Retrieved February 7, 2017, from How stuff works - science: http://science.howstuffworks.com/environmental/conservation/issues/how-does-biodiversity-help-an-ecosystem-1.htm

protect u.s. (n.d.). Student handout 1A: How to calculate biodiversity. Retrieved May 31, 2017, from http://entnemdept.ifas.ufl.edu/hodges/ProtectUs/lp_webfolder/9_12_grade/Student_Handout_1A.pdf

Purvis, A., & Hector, A. (2000). Getting the measure of biodiversity. *Nature*(405), 212-219. doi:10.1038/35012221

Reise, K., & Volkenborn, N. (2004). *Large worms as ecosystem engineers in intertidal sediments.* Retrieved January 8, 2017, from http://www.marmic.mpg.de/marmic/uploads_edit/Nils_Volkenborn.pdf

Rijkswaterstaat. (2011, September 20). Verkenning zandhonger Oosterschelde. Retrieved May 30, 2017

Robb, A. (2017). What is Succession in Biology? - Definition & Examples. Retrieved February 28, 2017, from http://study.com/academy/lesson/what-is-succession-in-biology-definition-examples.html

RStudio. (2017). Retrieved May 12, 2017

Schotanus, J. (2016). Retrieved June 1, 2017

Shimadzu, H., Dornelas, M., Henderzon, P. A., & Magurran, A. E. (2013). Diversity is maintained by seasonal variation in species abundance. *BMC Biology*. Retrieved May 30, 2017, from 10.1186/1741-7007-11-98

Smaal, A. (2002). European mussel cultivation along the Atlantic coast: production status, problems and perspectives. *Hydrobiologica*(404), 89-98. doi:10.1023/A:1021352904712

Smaal, A. (2013, March). *Sublittoral mussel reefs in the Dutch Wadden Sea: ephemeral hotspots for biodiversity*, (pp. 10-12). Yerseke. Retrieved January 8, 2017, from http://naturstyrelsen.dk/media/nst/Attachments/h_smaalkopenhagenmarch2013.pdf

Smaal, A., Craeymeersch, J., Glorius, S., van Stralen, M., & Drenth, J. (2017). *How to couple mussle culture and nature conservation?* Retrieved January 6, 2017, from aquaculture Europe: https://www.was.org/easonline/Mobile/Paper.aspx?i=1981

Tangelder, M., van den Heuvel-Greve, M., & de Kluiver, M. (2016). *Monitoring vooroeververdediging Oosterschelde 2015.* Retrieved May 31, 2017, from http://library.wur.nl/WebQuery/wurpubs/fulltext/393797

Telegraaf. (2015, April 16). Mosselbanken van levensbelang voor Waddenzee. Leeuwarden, Friesland, Netherlands. Retrieved January 8, 2017, from http://www.telegraaf.nl/binnenland/23931594/__Mosselbank_van_levensbelang__.html

The Columbia Encyclopedia, 6th ed. (2016). *Annelida*. Retrieved June 1, 2017, from http://www.encyclopedia.com/reference/encyclopedias-almanacs-transcripts-and-maps/annelida

University of Gothenburg. (2011, March 22). *Biodiversity leads to higher productivity.* Retrieved February 26, 2017, from Science daily: https://www.sciencedaily.com/releases/2011/03/110321183015.htm

Valdas, R. L. (2016). Retrieved May 31, 2017, from https://www.researchgate.net/post/what_is_the_deference_between_Shannon_Wiener_diversity_Index_and_Simpson_diversity_Index

Ward, P. (2015). *Annelidia - Annelids - Segmented worms*. Retrieved June 2, 2017, from Cool Galapagos: http://www.coolgalapagos.com/animals/annelida_segmented_worms.php

Washington Department of Fish and Wildlife. (2008). *Invasive species fact sheets*. Retrieved May 31, 2017, from https://web.archive.org/web/20080613141700/http://wdfw.wa.gov/fish/ans/identify/html/index.php?species=carcinus_maenas#feeding

Wijsman, J., Dubbeldam, M., de Kluijver, M., van Zanten, E., van Stralen, M., & Smaal, A. (2008). *Wegvisproef Japanse oesters in de Oosterschelde.* Eindrapport, IMARES Yerseke, Yerseke. Retrieved February 13, 2017

Wikipedia. (2016). *Blue mussel*. Retrieved January 9, 2017, from Wikipedia: https://en.wikipedia.org/wiki/Blue_mussel

Witteveen & Bos, Bureau Waardenburg bv, & Rijkswaterstaat Zee en Delta. (2013). *MIR-Verkenning Zandhonger Oosterschelde ontwerp structuurvisie.* Deventer. Retrieved February 7, 2017, from http://api.commissiemer.nl/docs/mer/p25/p2595/2595-045ontwerpstructuurvisie.pdf

Wright, J. P., Jones, C. G., & Flecker, A. S. (2002, February 25). An ecosystem engineer, the beaver, increases species richness at the landscape scale. *Oecologia*(132), 96-101. doi:10.1007/s00442-002-0929-1

Yukozimo. (2017). *What do Mollusks eat?* Retrieved June 1, 2017, from http://www.yukozimo.com/category/mollusca/

Appendices

A. Density ranges

The density ranges for the mussel density plots were set by using ImageJ. In this program each photo was uploaded separately and the scale was set based on the 1m*1m quadrant. With a polygon line the coverage in the quadrant by the mussels was indicated and the program provided the coverage in m^2. Based on this output the density ranges for the mussel density plots were set.

Figure A.1: These nine photos are used for the analysis in ImageJ to set the ranges for the three different densities. (Schotanus, 2016)

Table A.1: The output from ImageJ of the coverage by the mussels.

Set #	Area (m^2)	Coverage of the number of mussels
HIGH1	0,976	98%
HIGH2	1,014	101%
HIGH3	1,039	104%
MED1	0,561	56%
MED2	0,653	65%
MED3	0,608	61%
LOW1	0,276	28%
LOW2	0,219	22%
LOW3	0,226	23%

B. Material

For executing the method several materials are needed, these materials are divided into several sections.

Field monitoring – epifauna and infauna samples

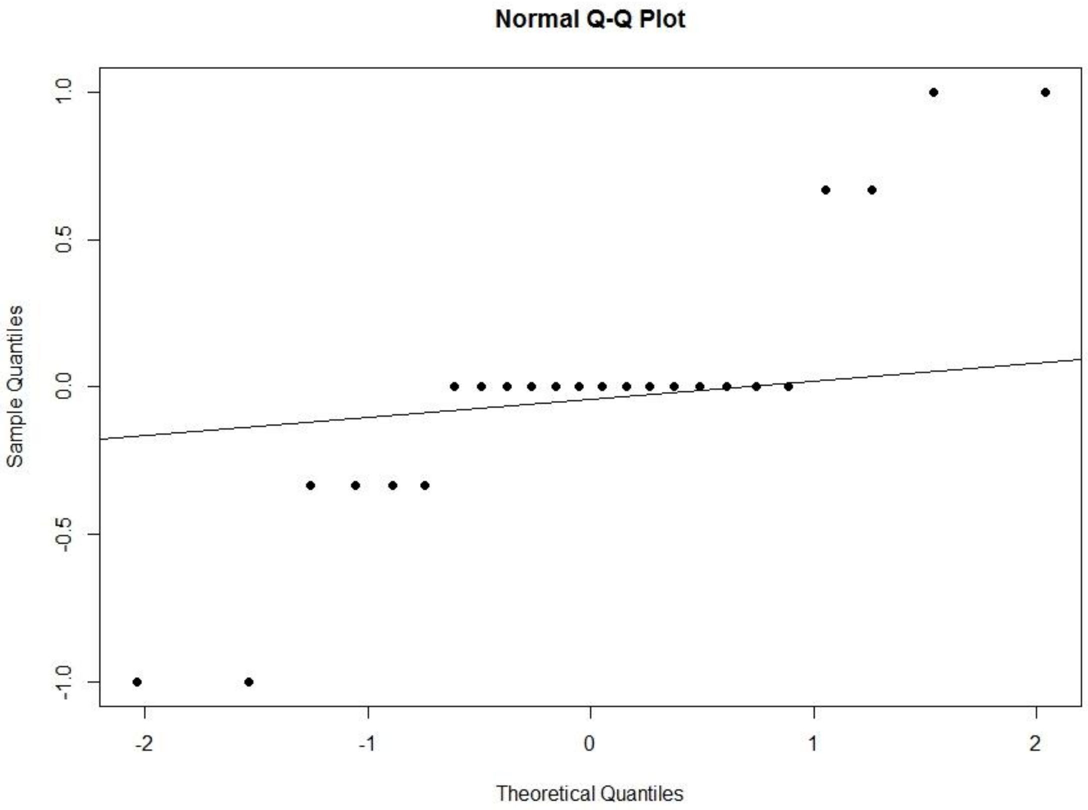
- Sample bottles + marker
- Notebook + pencil
- Waders
- Quadrant (0.5m*0.5m)
- Shovel
- Benthic corer
- Sieve (1 mm)
- Wash bottle
- Scoop

Lab analysis

- Infauna samples
- Fixation chemical – ethanol 96%
- Rose bengal stain
- Sieves (2 mm and 1 mm)
- Wash bottle
- Photo tray
- Petri dishes
- Binocular microscope
- Lights
- Tweezers + dissecting needle
- Books for the determination of benthos
- Notebook + pencil or a laptop with Microsoft Office Excel
- Sample bottles + etiquettes/marker

C. Results

Infauna



Graph B.1: Normal distribution of the infauna samples (RStudio, 2017)

Table B.2: Two-way Analysis of Variance based on the interaction of date and density (RStudio, 2017)

Two-way Analysis of Variance				
	Degrees of freedom	**F-value**	**p-value**	**Significance**
Density	3	6.8571	0.0035	**
Date	1	0.5714	0.4607	
Density * Date	3	0.5714	0.6419	
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Table B.3: Two-way Analysis of Variance based on the date and density as individual variables (RStudio, 2017)

Two-way Analysis of Variance				
	Degrees of freedom	**F-value**	**p-value**	**Significance**
Density	3	7.3548	0.0018	**
Date	1	0.6129	0.4434	
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Table B.4: One-way Analysis of Variance based on the density (RStudio, 2017)

One-way Analysis of Variance				
	Degrees of freedom	**F-value**	**p-value**	**Significance**
Density	3	7.5000	0.0015	**
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

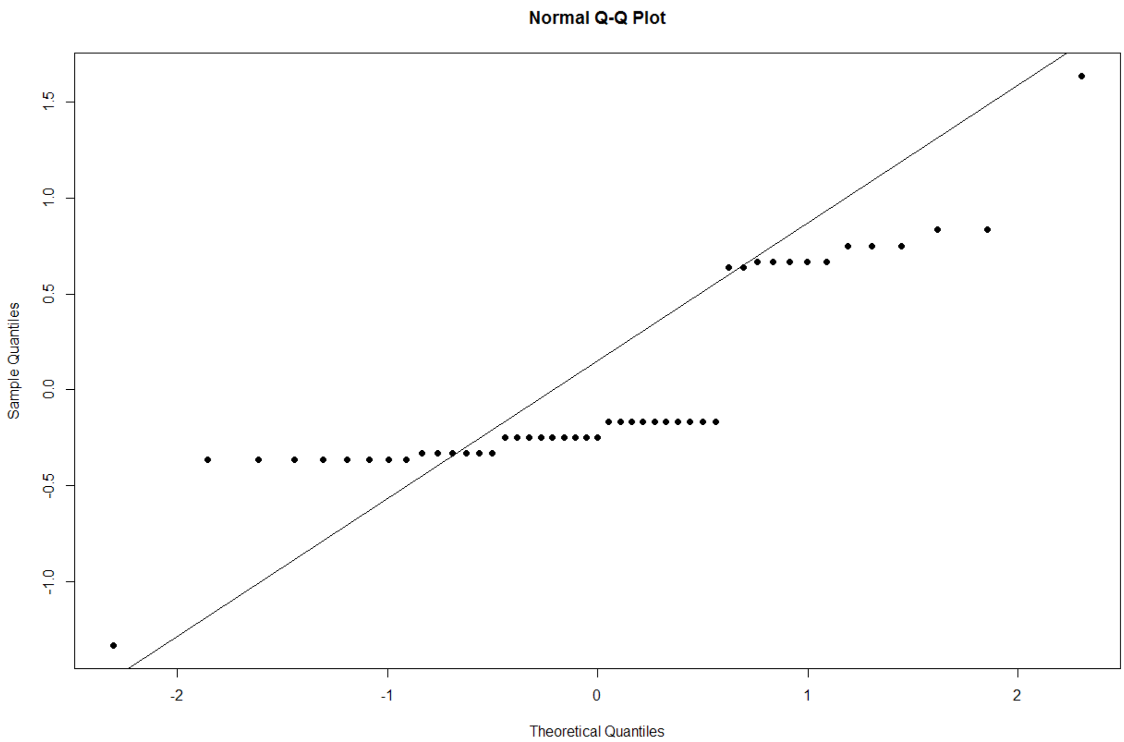
Table B.5: Post-hoc test to give insight between which density plots a significant difference occurs (RStudio, 2017)

Post-hoc test		
	p-value	**Significance**
High – Low	0.6829	
High – Medium	0.6830	
High – Reference	0.0012	**
Medium – Low	1.0000	
Medium – Reference	0.0153	*
Low – Reference	0.0153	*
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Table B.6: Analysis on the regression between the number of phyla and the number of mussels

Regression		
	p-value	**Significance**
Intercept	$9.59*10^{-13}$	***
Mussels	0.0042	**
R^2	0.3163	
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Epifauna



Graph B.2: Normal distribution of the epifauna samples (RStudio, 2017)

Table B.7: Two-way Analysis of Variance based on the interaction of date and density (RStudio, 2017)

Two-way Analysis of Variance				
	Degrees of freedom	**F-value**	**p-value**	**Significance**
Density	3	7.1994	0.0006	***
Date	1	0.8951	0.3499	
Density * Date	3	0.6299	0.6001	
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Table B.8: Two-way Analysis of Variance based on the date and density as individual variables (RStudio, 2017)

Two-way Analysis of Variance				
	Degrees of freedom	**F-value**	**p-value**	**Significance**
Density	3	7.3948	0.0004	***
Date	1	0.9194	0.3431	
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Table B.9: One-way Analysis of Variance based on the density (RStudio, 2017)

One-way Analysis of Variance				
	Degrees of freedom	**F-value**	**p-value**	**Significance**
Density	3	7.4087	0.0004	***
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Table B.10: Post-hoc test to give insight between which density plots a significant difference occurs (RStudio, 2017)

Post-hoc test		
	p-value	**Significance**
High – Low	0.8808	
High – Medium	0.9826	
High – Reference	<0.001	***
Medium – Low	0.9826	
Medium – Reference	0.0021	**
Low – Reference	0.0061	**
Significance codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Trophic levels

Diagram C.1: Characteristics on the found phyla

UNIVERSITY
OF APPLIED SCIENCES