How different are subtidal *Mytilus edulis* L. communities of natural mussel beds and mussel culture plots in the western Dutch Wadden Sea?

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Pictures on front and back cover are from Bert Brinkman.

Background, surface of a box core sample with mussel (*Mytilus edulis*) clump and associated fauna.

Pictures on back cover clockwise starting left. Box corer device. Core with *Asterias rubens* and empty shells of *Mytilus edulis. Enis directus* protruding from core sediment surface. Core surface with *Mytllus edulis* covered with *Balanus sp*., also empty shells of *Cerastoderma edule*, *Ensis directus* and *Mya arenaria* are visible*. Echinocardium cordatum* from a core washed over 1mm Ø mesh.

Contents

Abstract

In the subtidal western Dutch Wadden Sea an on-bottom mussel culture is practiced where seed mussels are dredged from natural settled mussel beds and transferred to mussel culture plots. This activity takes place in an area covered under European Habitat Conservation Regulations (Council Directive92/43/EEC Annex I). Of concern is the impact of the relocation of mussels on the diversity of the mussel bed community. Mussels and the macrozoobenthic community on mussel culture plots were compared with those of natural occurrences. Culture plots are located closer to the tidal inlets where the seawater salinity is markedly higher than at the naturally settled mussel beds. Mussels on mussel culture plots were larger and had higher flesh contents than naturally occurring mussels outside mussel culture plots. Numerical densities and total biomass of mussels were higher in samples from inside than from outside mussel culture plots. Number of species per box core sample was lower at mussel culture plots than at natural beds. The higher number of species per box outside mussel culture plots was due to a higher number of soft sediment species, hard substrate species numbers did not differ. At the ecosystem scale the species pool at mussel culture plots is larger than at the natural beds. Densities of macrozoobenthos are higher in natural mussel beds than in mussel culture plots. At natural beds densities of hard substrate species per unit mussel shell surface are higher than inside mussel culture plots.

Species composition of the macrozoobenthos community differs between mussel culture plots and natural mussel beds.

Introduction

In coastal soft sediment systems like the Wadden Sea *Mytilus edulis* L. can develop extensive beds on the sediment surface. Compared to their surroundings mussels beds in soft sediment systems are complex epibenthic structures consisting of a matrix of mussels attached to each other with byssal threads. These biogenic structures provide a settlement surface for other hard substrate species and within the matrix species may find shelter from predation. Generally the mussel bed community is more species rich and contains different species than the surrounding soft sediment habitat (Dittmann 1990, Buschbaum & Saier 2003, Norling & Kautsky 2008, Bush & Reise 1997, Commito et al. 2008). Mussel beds in the Wadden Sea area can be considered biodiversity hotspots (Buschbaum et al. 2009).

Mussels occur both in the intertidal and the subtidal. Traditionally in the Wadden Sea most research has been done on intertidal mussel beds and relatively little is known about subtidal mussel beds. There is however some information available that sites in the subtidal with mussels are relatively species rich. In the northern Wadden Sea epibenthos was dredged and found to be more species rich when mussels were present than when mussels were absent (Buhs & Reise 1997). More recent in 2008 a subtidal benthic survey was done in the western Dutch Wadden Sea. Box core samples with mussels had on average twice as many species as cores without mussels (Dekker & Drent 2012). In the northern Wadden Sea there was also a comparison made between associated epifauna from intertidal and subtidal mussel beds (Saier 2002). It turned out that the subtidal mussel bed community was more species rich and diverse than a nearby intertidal mussel bed community.

In the subtidal of the western Dutch Wadden Sea a mussel bottom culture is practised. This started in the beginning of the twentieth century (Van Leunen 1998). The culture takes place on culture plots that are leased from the government. Between 1949 and 1960 the surface area of culture plots was expanded from 10 km^2 to 70 km^2 (Dankers & Zuidema 1995). In general mussel plots are located in areas where it is expected that mussel growth is faster and mortality is less than on the natural beds. The present practise is that mussel culture plots are stocked with seed mussels (around 20 mm shell length) dredged from natural subtidal mussel beds in the western Wadden Sea or collected from spat collectors. During the culture period of approximately 1.5-2 years mussels may be dredged several times to control densities and move mussels between different culture plots. Also measures are taken to control predatory starfish (*Asteria rubens*) numbers (Dankers & Zuidema 1995, Dijkema 1997).

It is not unlikely that relocating handling and harvesting of mussels during the culture process will impact the ecological role of mussels. Due to fishery disturbance mussels may not be able to develop the complex physical habitat structure harbouring a diverse assemblage of associated fauna to the same degree as without fisheries (Buschbaum & Saier 2003). On the other hand relocating mussels to sites with better survival and growing conditions could also benefit the associated fauna (Murray et al 2007). Up to now several studies showed that infaunal soft sediment species are impacted by relaying mussels on the sediment surface (Ragnarsson & Raffaelli 1999, Beadman et al 2004, Smith & Shackley 2004). Species numbers decline and the community becomes dominated by opportunistic deposit feeders. At the local scale epibenthic species profit from mussel additions (Günther 1996, Ragnarsson & Raffaelli 1999, Ysebaert et al. 2009), however at the sites where the mussels are taken epibenthic species are lost (Ragnarsson & Raffaelli 1999, Dolmer 2002) and

biogenic structures and habitat complexity become reduced, limiting settling opportunities for epibenthic hard substrate species (Dolmer 2002, Dolmer & Frandsen 2002).

Considering the positive effect of subtidal mussels for biodiversity and other natural values of the ecosystem and that the culture and seed harvesting areas are specifically covered under European Habitat Conservation Regulations (Council Directive92/43/EEC Annex I) it is important to evaluate the possible impacts of mussel culture activities. Part of this task is to provide a description of the subtidal mussel communities and the differences between natural beds and mussel culture plots. This is information that should aid in developing the future policy of the shellfish fishery. In Ens et al. (2007) this is discussed in more detail.

In this descriptive study mussels and the macrozoobenthos community in box core samples from natural sublittoral mussel occurrences and from mussel culture plots in the western Dutch Wadden Sea were compared with each other. We compared total density, total biomass and species richness of the macrozoobenthos communities outside and inside mussel culture plots and also compared the mussel populations. We quantified the relationship between species richness and mussel biomass and tested for effects of environmental factors on species richness and differences between bed-types. Finally the macrozoobenthos community composition at sites with mussels outside and inside mussel culture plots was compared. In almost every aspect studied distinct differences between natural beds and mussel culture plots were found.

Materials and methods

Area description

The western Dutch Wadden Sea is a shallow coastal sea protected from the North Sea by a row of barrier islands (Fig. 1). The surface is about 1500 km^2, half of this area is always submerged (subtidal or sublittoral), the other half are tidal flats. There are three tidal basins, Marsdiep, Eirlandse Gat and Vlie. These tidal basins are connected with the North Sea by tidal inlets between the barrier islands. In 1932 a dam, the "Afsluitdijk" was completed that separated the formerly "Zuiderzee" into the western Dutch Wadden Sea to the north and a freshwater lake, The "IJsselmeer", to the south. Fresh water from the "IJsselmeer" enters the Wadden Sea through sluices in the south western and north eastern ends of the "Afsluitdijk". The inflow of fresh water causes a salinity gradient from the south east towards the tidal inlets on the north western side of the western Dutch Wadden Sea.

Mussels occur on natural mussel beds and mussel culture plots. Both bed-types are dredged for mussels. Natural beds are dredged for mussel seed collection or half grown mussels either in a restricted period in spring or autumn. Mussel culture plots are always open for dredging activities. Mussel culture plots covered 77 km^2 in 2008, this is about 10% of the subtidal area (Wijsman & Jol 2009). The majority of mussel culture plots are located in the shallow subtidal between the low water line (approximately -1m NAP) and -5 m NAP. Only 40 km^2 of these plots is really used for mussel culture.

Sampling

Sampling was done in 2008, 2009 and 2010. Each year in autumn mussels and the local macrozoobenthos community were sampled with a box core from natural subtidal mussel beds and mussel culture plots. Results from a yearly mussel inventory in the western Dutch Wadden Sea were used to choose the positions of natural mussel bed stations (Van Stralen 2008, 2009 & 2010). Sampling positions on mussel culture plots were selected using information about mussel presence on culture plots. This information was obtained from the local mussel farmers (Van Stralen, pers. comm.). At each selected location box cores were taken until a core contained at least one mussel and had penetrated at least 15 cm into the sediment. If after five boxes at one station still no mussels were encountered the station was abandoned. Only boxes containing mussels were analysed. In total 159 successful box core samples with mussels were collected, 79 from natural beds/mussel occurences and 80 from mussel culture plots (Table 1, Fig. 1).

Sampling was done with a 0.06 m^2 Reineck boxcorer operated from research vessel Navicula. After collection, samples were immediately washed over a 1mm mesh and preserved in a 4% formaldehyde solution. In the lab species were identified to the species level and counted. After that the flesh was dried at 60°C for three days and then weighed to the nearest 1E-4 g. The flesh was then ashed for four hours in an oven at 560° and weighed again. The difference in mass between the two weighings is the ash free dry mass (AFDM). Of a few species no AFDM was measured, these were mainly small and rare species or firmly attached hard substrate species. The species with no biomass measurements were: *Alcyonidioides mytili, Aricidea minuta, Campanularia, Cheirocratus sundevalli, Clytia hemisphaerica, Conopeum reticulum, Electra pilosa, Farrella repens, Hartlaubella gelatinosa, Hediste diversicolor, Hydractinia echinata, Mactra stultorum, Magelona johnstoni,*

Microphthalmus similis, Obelia dichotoma, Obelia longissima, Pedicellina cernua and *Procerastea halleziana*. In addition the longest length of the bivalves was measured to the nearest mm and the age estimated from growth marks on the shell.

Condition index of *M. edulis* was calculated as AFDM/l^3, where *AFDM* is ash free dry mass in mg and *l* the longest shell length in cm.

At each station a sediment sample (diameter 1.6 cm) was taken of the top 8 cm from the surface downwards. This sample was taken out of the same box as the macrozoobenthos before washing it over the mesh. Sediment grain size distribution was analysed on a coulter laser diffraction analyser (Coulter LS 13 320) after freeze drying and HCl and H_2O_2 treatments to remove calcareous material and organic matter respectively. Methods are described in more detail in Compton et al. (2012).

Depth in meters below NAP at each station was extracted from a GIS raster data set compiled from depth soundings made in the area in the period between 2003 and 2008 under responsibility of Rijkswaterstaat. Salinity information at the stations was approximated by averaging values extracted from two raster files describing salinity during a wet period in 1988 with high fresh water discharges and in a dry period in 1992 with very little fresh water inflow. These maps were made with a 2D water movement model for the coastal zone (Kuststrookmodel). For details and further references see Jager & Bartels (2002). Maximum current speed information was extracted from a raster data set accompanying Zwarts (2004).

Statistical analyses

Analyses were done with R version 2.15.0 (2012-03-30). Package beanplot was used to draw the beanpots (Kampstra 2008). A bean consists of a one-dimensional scatter plot (white horizontal lines, which becomes longer with overlapping values), its distribution as a density shape and an average line (solid black line) for the distribution. Next to that, an overall average for the whole plot is drawn (stippled line over the entire width of the graph). Package Vega version 2.0-3 (Oksanen et al 2012) was used for mutivariate statistics.

Results

Environmental variables

Stations outside mussel culture plots are spatially separated from stations on mussel culture plots (Fig. 1). Of the 79 stations outside the culture plots on the natural mussel beds 64 are located in the Marsdiep tidal basin. Culture plot stations are mainly concentrated in the Vlie tidal basin (58 out of 80). These mussel plot stations are on average closer to the tidal inlets that connect the Wadden Sea with the North Sea, than the stations outside mussel culture plots. The consequence is that on average the salinity is much lower at the stations outside mussel culture plots (17.3 PSU) than inside culture plots (22.1 PSU) (Fig. 2). Unlike salinity, there is no consistent difference in depth, median grain size and maximum current speeds between stations inside and outside mussel culture plots (Fig. 2).

Macrozoobenthos

As a first step in the analysis several exploratory test were run comparing mussel density, biomass flesh content, species richness subdivided in soft sediment species and hard substrate species, inside and outside mussel culture plots (Table 2). Mussels appear more abundant, have higher biomass and flesh content inside mussel culture plots. Abundance and biomass of soft sediment species is highest at sites outside mussel culture plots. There was no difference in densities and biomass of hard substrate species. Overall species richness is higher inside mussel culture plots. At the level of a box species richness is higher at mussel stations outside mussel culture plots. This is the effect of more soft sediment species, hard substrate species numbers do not differ inside and outside mussel culture plots.

total biomass and density

Overall the number of macrozoobenthos species in the data set is 108 (Appendix 1). Of these species 55 were classified as soft sediment species 29 as mobile hard substrate species and 20 sessile hard substrate species. Four species were unclassified. Out of the total 108 species 16 were invasive. These invasive species were all present both outside and inside mussel culture plots.

Geometric mean total biomass of the macrozoobenthos over all stations was 191 gm^{-2} (back transformed mean of log(biomass) (Fig. 3). Macrozoobenthos biomass was 145 gm^{-2} at stations outside mussel culture plots, significantly less than 251 gm^{-2} (lm: $F_{1,157} = 7.65$, $P = 0.006$) on the culture plots. There were no significant year and year:bed-type interaction effects. Biomass of the macrozoobenthos other than *M. edulis* differed more strongly (lm: $F_{1,157} = 32.21$, $P < 0.001$) inside and outside mussel culture plots and was higher outside mussel culture plots (Fig. 3). Geometric mean biomass excuding mussels outside mussel culture plots was 79 gm^{-2} and only 20 gm^{-2} at stations inside mussel culture plots.

Total number of individuals of macrozoobenthos species (m^{-2}) was significantly (lm: $F_{1,157} = 22.39$, $P < 0.001$) higher outside (9.8E+3 nm^{-2}) than inside the mussel culture plots (3.6E+3 nm^{-2}). This pattern was consistent between years (Fig. 4). Making the same comparison without incorporating mussel densities gives very similar results. Geometric mean marozoobenthos density excluding mussels is

also significantly (lm: $F_{1,157} = 34.97$, $P < 0.001$) higher outside (9.1E+3 nm^{-2}) than inside mussel culture plots (2.9E+3 nm^{-2}).

In Fig. 5 the total macrozoobenthos numerical densities are divided into hard substrate and soft sediment species. Hard substrate species are less abundant than soft sediment species, both outside and inside culture plots. There were no significant differences in densities of hard substrate species inside and outside mussel culture plots (Table 3). There was a significant year effect with lower abundances in 2009. Soft sediment species were much more abundant in the sites outside the mussel culture plots (lm: $F_{1,157} = 69.86$, $P < 0.001$). There were no significant year or interaction effects.

Mytilus edulis

Four metrics of *M. edulis*, numerical density, biomass, shell length and condition index inside and outside mussel culture plots were compared during three years (Fig. 6, Table 4). Numerical densities (nm^{-2}) of mussels were similar inside and outside culture plots in 2008. In 2009 and 2010 densities remained stable inside the mussel culture plots but declined at mussel occurrences outside mussel culture plots. Factors plot, year and the interaction between plot and year were all significantly contributing in a linear model describing numerical density (Table 4). Biomass (AFDM gm^{-2}) was highest inside culture plots in all three years . There was no significant effect of year or the interaction between plot and year (Table 4). Each year shell length (mm) was longest inside the mussel culture plots and did not show much change between years (Table 4). The condition index (mg mm^{-3}), differed with factors plot, year and the plot: year interaction (Table 4). Condition index was highest in 2008 inside the mussel culture plots. The following year the condition index inside the mussel culture plots was lower but always remained above the condition index outside the mussel culture plots (Fig.6).

Shell length distributions outside mussel culture plots differ from those inside mussel culture plots and also between years (Fig. 7). Outside mussel culture plots there were many small mussels <30 mm in 2008, but hardly any in the two following years. Median shell length outside the mussel culture plots increased from 19 mm in 2008 to 41 mm in 2009 and then to 55 mm in 2010. Inside the culture plots small mussels <35 mm were less abundant than outside the plots. Most small mussels were found inside mussel culture plots in 2009. In 2008 and 2010 mussels >40 mm dominated the mussel culture plots. The median sizes inside the mussel culture plots in 2008, 2009 and 2010 were 55, 32 and 50 mm respectively.

Ages of the mussels are compared in Fig. 8. Most mussels outside mussel culture plots are in their first year. Mussels on culture plots are much older the majority is in their third year or more. Between years age distribution are variable. In the three year period 2008-2010 average age increased with time outside mussel culture plots. On the culture plots older mussels dominate in 2008 and 2010, in 2009 there are a lot of mussels in their first or second year.

Species richness

Of the total 108 macrozoöbenthos 84 species were found in the box cores (79 of 0.06 m^2) outside mussel culture plots and 102 in the box cores (80) taken inside mussel culture plots. Five species were exclusively found outside mussel culture plots (Table 5). There were 23 species that were only

encountered inside the mussel culture plots (Table 6). These unique species were only found at low numerical densities in one or a few boxes.

Average number of species at a station of 0.06 m^2 was 20.4 outside mussel culture plots and 18.3 inside mussel culture plots. Although the average number of species in box cores was highest outside mussel culture plots total number of species found was highest inside mussel culture plots. Total species found outside mussel culture plots was 84 (in 79 cores of 0.06 m^2) and lower than 102 species (in 80 cores) found inside mussel culture plots.

These differences between stations outside and inside mussel culture plots were consistent during all three years (Fig. 9). The fractions of total species number belonging to hard substrate species was similar inside (0.43) and outside (0.46) mussel culture plots.

In Fig. 10 the species area curves are plotted for hard and soft substrate species and also total species. At the lowest sampling effort of one station hard substrate species numbers are similar inside and outside mussel culture plots. Soft sediment species numbers are higher outside than inside mussel culture plots at one at the lowest sampling effort. Obviously total species numbers per sample are then highest outside mussel culture plots. With increasing sample sizes however species numbers increase fastest in stations from inside mussel culture plots. Above 10 stations sampled outside mussel culture plots less species are found than inside mussel culture plots.

The distribution of species counts per sample inside and outside mussel culture plots during three years (Fig. 11, Table 7) shows consistently more species outside than inside the mussel culture plots. In 2010 more species were found per sample than in the two previous years, causing a marginally significant year effect (Table 7). When the species counts are subdivided by substrate (Fig. 12) it is clear that the larger species richness outside mussel culture plots is caused by higher soft sediment species numbers (11 versus 9). Hard substrate species numbers do not differ significantly between stations outside and inside mussel culture plots (Table 7). Besides a plot effect there is also a slight year effect influencing soft sediment species richness in the box cores (Table 7).

Effects of Mytilus edulis on species richness

To quantify the effect of *M. edulis* on species richness it is first necessary to quantify *M. edulis* presence itself. It can be argued that for hard substrate species that use the M. edulis shell to attach to, the shell surface area of *M. edulis* would be an adequate measure. An approximation of shell surface is the shell length squared. An additional refinement is to include the age of shell as well in the metric because chances that species will have settled on the shell surface increases with the time the shell was exposed to settlers. A practical metric then is the shell length squared times the age summed over total of individuals in the sample. Age was divided in three classes, 1 for spat< 1 year old, 2 for second year mussels and 3 for older mussels. This surface*age metric correlates strongly with biomass (AFDM, r=0.96). Using biomass as metric is more convenient because *Crassotrea gigas* can also be included in the statistical models using the same methodology. Length and age measurements of *C. gigas* are not as reliable as in *M. edulis* because the erratic shell formation in *C. gigas*.

Number of species in a box core was modelled with a Poisson generalized linear model (GLM). This was done for the count of all species, only hard substrate species and soft sediment species. The

most parsimonious model was selected with a backward forward stepwise procedure. A penalty of k=4 was set for addition of extra factors to the model. The explanatory variable mussel plot (factorial) was set to always be included in the model. The other explanatory variables were: Mytedu biomass (log_{10} transformed), Cragig biomass (log_{10} transformed), sediment silt content, year (factorial), height (NAP), average salinity, tidal basin (factorial) and presence of stones (factorial). In addition also second order interaction terms between mussel plot and the other explanatory variables as well as mussel biomass and the other variables were included in the model selection process.

The resulting model describing the total number of species including *M. edulis* includes six explanatory variables and two interaction terms. The null model had a residual deviance of 344 the final model 203. Model coefficients are listed in Table 8. Species richness is lower inside mussel culture plots than outside mussel culture plots. Silt and height have negative effects on species richness. Species richness increases with mussel biomass and oyster biomass. In 2010 species richness was significantly higher than in 2008 and 2009.

To judge the contribution of the explanatory variables used in the model each term was dropped separately from the final model and the deviance of the model excluding one term compared with the final model. This took two steps a first one with the final selected model including interaction terms and a second step using the final model excluding the interaction terms. In terms of deviance units the interaction terms contribute least to the explanatory power of the model (Table 9). In decreasing order of contribution to a model without the interaction terms (Table 9), the most important factor explaining species richness is mussel biomass. The next term is mussel plot so whether the observation is made inside or outside a mussel culture plot. Then silt content of the sediment is the most important environmental factor in the model. After that comes the year of observation. The contribution of oyster biomass and height contribute least in the model without interaction terms. In Fig. 13 the positive relationship between mussel biomass and species richness is plotted together with the location of the samples inside or outside the mussel culture plots.

The same stepwise model selection procedure was used on two subsets of the total species data set split into hard substrate species and soft substrate species. The final model describing the hard substrate species richness included; plot, mussel biomass, oyster biomass, height, year and the interaction terms mussel biomass : year and musselplot : oyster biomass. Null deviance was 307 and residual deviance was 133.This model is very similar to the one describing the overall species richness except that silt is not included and the interaction terms are different. The coefficients are summarized in Table 10. Number of hard substrate species is positively related with the mussel biomass and oyster biomass. Height had a negative effect on the number of hard substrate species. Number of hard substrate species per box is higher outside mussel plots than inside. Number of species was lower in 2009 than in the other two years 2008 and 2010.

The increase in residual deviance after dropping the explanatory variables separately from the model with and without interaction terms is listed in Table 11. Of the two interaction terms mussel biomass : year contributed most. Mussel biomass is also by far the most important explanatory variable in the model without interaction terms, followed by oyster biomass. Year and height only contribute little. Factor mussel plot decreases residual deviance least.

The strong relationship between hard substrate species number and mussel biomass including the effect of factor mussel plot is plotted in Fig. 14.

The stepwise model selection for soft sediment species number resulted in the simplest model with only silt content, height and factor mussel plot, without interaction terms. The null deviance was 252 the residual deviance 177. Number of soft sediment species was lower inside mussel culture plots than outside (Table 12). Height and silt content both had a negative effect on species richness. Dropping silt from the model had the largest effect on the residual deviance, followed by factor mussel plot (Table 13). Height had the smallest effect (Table 13). For comparison with Fig. 13 and 14 Fig. 15 shows the number of soft sediment species against mussel biomass.

Tidal basin effect

Because the stations are not equally distributed among the two tidal basins a basin effect might also cause les rich boxes from inside mussel culture plots than outside mussel culture plots. Therefore a separate analysis of species richness was done with only stations from the Marsdiep tidal basin.

The stepwise model selection procedure resulted in a model very similar to the model describing the entire data set. Terms in the model were; mussel plot, mussel biomass, silt content, oyster biomass, year, height and two interaction terms mussel plot : mussel biomass and musselplot : oyster biomass (Table 14). Null deviance was 189 and residual deviance was 78. Directions of effects are the same as in the model covering all stations. Interaction terms contribute little in explaining deviance. Dropping single terms from the model without interaction terms shows that mussel biomass is most important explaining species richness per station while factor mussel culture plots contributes least (Table 15). So when only taking the Marsdiep tidal basin stations into account the effect of mussel culture plot is less important than when the entire dataset is considered.

Salinity effect

The environment of the mussel culture plots is more saline than at the natural subtidal mussel beds (Fig. 2). It is well known that species richness in estuarine systems is strongly depending on salinity with lowest species numbers in low salinity areas. Thus differences in salinity could cause different species numbers at the sampling stations in and outside mussel culture plots. Salinity was added as factor in the model selection procedure but was never included in the resulting model. However distinction between effect of salinity and mussel culture plot is difficult because of the unevenness in the data set. In a separate analysis the relationship between species richness and salinity is explored. In Fig. 16 number of species in a box core sample is plotted against salinity for stations outside and inside mussel culture plots. There is a positive relationship while number of species is higher relative to salinity in the stations outside mussel culture plots than inside mussel culture plots.

In a subset of the data only including hard substrate species there again is a positive relationship between species number and salinity. Mussel plot and the interaction between mussel plot are much less important factors than in the case when all species are taken into account.

Soft sediment species numbers are mainly influenced by factor mussel plot and hardly by salinity. Salinity seems less important for soft sediment species number than for hard substrate species numbers.

Mussel shell surface area

The chance that a mussel shell will get colonized by other hard substrate species is likely to depend on the shell surface area and the amount of time this surface is available. To take surface area and time into account for comparisons of epifauna relative measure for mussel shell surface weighed for age was calculated by taking the shell length squared and multiplying this with variable depending on the age. Surface area of first year mussels was multiplied by 1, second year mussels by 2 and mussels older than 2 years by 3. Inside mussel culture plots this age weighed surface area is larger than outside mussel plots (Fig. 17). The numerical density of macrozoöbenthos species per unit of age weighed shell surface area is much higher outside than inside mussel culture plots. When densities are categorized in hard substrate species (excluding mussels) and soft sediment species the same pattern emerges of a higher numerical density of macrozoöbenthos per unit age corrected shell surface area outside than inside mussel culture plots (Fig. 17). The comparison of hard substrate species is most meaningful here and tested with a linear model including salinity as covariate. Both salinity and bed-type contributed significantly in explaining the variance (Table 16). Hard substrate species density per age corrected shell surface declined with salinity and is significantly higher on natural beds. Salinity and bed-type explain to a large degree the same part of the variance. Single term deletions (Table 16) show that bed type had the largest independent contribution to the model.

Species composition

Bray Curtis similarities between stations were calculated with numerical density data of all macrozoobenthos species encountered at the sampling stations. The similarity matrix is summarized in a nonlinear MDS plot (Fig. 18). (This ordination significantly correlates with salinity (R^2=0.40, p=0.001) and median grain size (R^2=0.13, p=0.001). The centroids of stations inside and outside mussel culture plots also differ significantly (p=0.001) and explain 14% of the between station variation in the MDS.). A PERMANOVA showed that mussel culture plot, salinity and median grain size have significant effects on the species composition (Table17). Plot and salinity are correlated and both variables can to some degree explain the same part of the variance. Together plot and salinity explain 9.5% of the variance, 2.1 % can be attributed to plot, 3.7% to salinity and 3.7% is explained by both terms. The 36 species contributing most to the difference between stations inside and outside mussel culture plots (results SIMPER analysis) are plotted in (Fig. 18). Together these species account for 70% of the difference between the bed types. Of the 36 contributing species *Carcinus maenas*, *Alitta virens*, *Capitella capitata*, *Heteromastus filiformis* and *Alitta succinea* were most important, together explaining 16% of the difference in similarity outside and inside mussel culture plots.

Differences in numerical densities of macrozoobenthos species inside and outside mussel culture plots were larger for soft sediment species than for hard substrate species (Fig. 19). In general soft sediment species are more abundant outside mussel culture plots than inside. Outside mussel culture plots *Peringia ulvae* is much more abundant (4 orders of maginitude) than on mussel culture plots, however this species is not common, it is only present at six stations outside plots and at two stations inside plots. The more common species *Mya arenaria*, *Pygospio elegans*, *Marenzelleria viridis*, *Petricolaria pholadiformis* and *Cerastoderma edule* are also more abundant outside mussel culture plots. Few soft substrate species reach higher densities inside than outside mussel culture

plots, noteable are two bivalve species *Ensis directus* and *Abra alba* and the pea crab *Pinnotheres pisum*.

Hard substrate species that are more abundant on mussel culture plots are Bryozoans *Alcyonidioides mytili* and *Conopeum reticulum* the polychaetes *Harmothoe impar* and *Harmothoe imbricate* and the Echinoderm *Asterias rubens* . Hard substrate Species with clearly higher densities outside mussel culture plots are the Polychaete *Polydora ciliate* the bivalve *Crasostrea gigas*, the sea squirt *Molgula socialis* and the Athropod *Gammarus locusta*.

A comparison of species occurrences outside and inside bed types is made in Fig. 20. There were 23 species with significantly different occurrences depending on factor mussel culture plot. In box cores from mussel culture plots *Alcyonidioides mytili, Carcinus maenas, Harmothoe imbricata, Harmothoe impar, Nephtys hombergii, Melita palmata, Eunereis longissima* and *Sthenelais boa* occur significantly more often than in boxes from outside culture plots. Summarized per Phylum these are one Bryozoan, 5 Annelids and 2 Atrhopods. Outside mussel culture plots *Aphelochaeta marioni, Oligochaeta, Balanus crenatus, Heteromastus filiformis, Polydora cornuta, Scoloplos armiger, Pygospio elegans , Mya arenaria, Marenzelleria viridis, Crassostrea gigas, Macoma balthica, Eteone longa, Petricolaria pholadiformis* and *Cerastoderma edule* are significantly more common than inside mussel culture plots. At the phylum level these are eight Annelids, one Arthropod, and 5 Mollusca.

Comparison of species typical for the habitat

Species typical for the subtidal habitat (Natura 2000 Habitat directive) are listed in Table 18 and compared between bed types. One species *Nephtys hombergii* occurred significantly more often inside culture plots than outside mussel culture plots. *Macoma balthica* and *Mya arenaria* were more common outside mussel culture plots. Note that the comparison of mussel (*M. edulis*) occurrence is not meaningful here because stations were only included when they contained mussels. Abundance of the species at the occupied sites was more often dissimilar outside and inside mussel culture plots than occurrence. The mussel (*Mytilus edulis*), the starfish (*Asterias rubens*) and the crab (*Carcinas maenas*) were more abundant inside the culture plots. The anemones *Metridium senile* and *Sagartia troglodytes*, the polychaete *Alitta virens* and the bivalve *Mya arenaria* were more abundant outside mussel culture plots.

Discussion

Almost every comparison made between stations outside and inside mussel culture plots showed that there were differences. A first important fact to recognize is that environmental conditions at stations inside and outside are not necessarily the same which is in particular the case for salinity. Mussel culture plots are situated in more saline conditions than where mussel settle naturally. Salinity gradients have well known effects on the macrozoobenthos diversity, within the salinity range relevant for this study (between 10 and 30 PSU) species richness increases with salinity (Remane 1934). In contrast macrozoobenthos abundance shows a reversed trend with salinity, in the subtidal western Dutch Wadden Sea lowest abundances are found in the areas with highest salinity (Dekker & Drent 2013). Differences in soft sediment species density outside and inside mussel culture plots can be largely explained by differences in salinity. However macrozoobenthos biomass differences are not caused by salinity. On mussel culture plots a large fraction of the biomass is mussel biomass, exactly where these beds are intended for. The biomass excluding mussels is much lower than on the natural beds. This effect is likely caused by high mussel densities impairing the soft sediment species like reported by Beadman et al. (2004) and in agreement with a lower endofaunal biomass on mussel culture plots in the Oosterschelde (Ysebaert et al. 2009). However results of an additional analysis with a larger dataset also including stations without mussels do not support this phenomenon in the subtidal western Dutch Wadden Sea (Appendix 3). Instead of an impaired soft sediment fauna inside mussel culture plots it turns out that the soft sediment species density and richness are significantly higher at mussel occurrences outside mussel culture plots. Soft sediment species densities inside mussel culture plots did not significantly differ from background levels without mussels.

The mussels on the mussel culture plots are often more abundant, larger, comprise more biomass and have a larger flesh content than on the natural beds (Fig. 6). Apparently the higher mussel densities of larger mussels on the culture plots do not result in stronger food competition than on natural beds, because if this would be the case a lower condition would be expected on the culture plots while the contrary is the case (Fig. 6). This suggests that on mussel culture plots mussel production is larger than at natural mussel beds, under the assumption that mortality due to the culture process is not larger than on the natural beds. Length and age distribution differ strongly outside and inside mussel culture plots. Outside the mussel culture plots the 2008 cohort is largely responsible for the dynamics of the length and age distribution (Fig. 6 & 7). On the culture plots cohort developments are disturbed and the population is dominated by relatively large and old mussels compared to the natural beds.

Compared to the surrounding soft sediment habitat mussel beds and mussel culture plots are species rich. There were 108 species found in 159 samples from both bed types while in a benthic survey in the same area in 2008 only retrieved 92 species out of 397 samples from habitat without mussels (Dekker & Drent 2013). Comparing between bed-types it turns out that culture plots harbour more species (102) than natural beds (84) in nearly 5 m^2 sampled in each bed-type. This higher species number on mussel culture plots agrees with the general trend of increasing species richness with salinity. This result does not mean that there are no negative effects of fisheries on species richness on mussel culture plots. In the northern Wadden Sea where salinity gradients are not as extreme as in the western Dutch Wadden Sea species richness was lower in mussel culture

plots than on natural beds and this observation could be explained as a fishery effect, however in this study silt accumulation in the culture plots may have been a confounding factor (Westphalen 2006).

Undisturbed beds under the same conditions may have harboured even more species, unfortunately this condition is not met in the western Dutch Wadden Sea so the comparison cannot be made. A comparison that can be made is one with an epibenthic species list from the northern Wadden Sea (Reise & Buhs 1999). This list contains 27 species that were present in 1988 and 1992 near Sylt, an area that is also impacted by dredging. On the culture plots in the western Wadden Sea 14 species matched with this list, on the natural mussel beds it were 9 species but these were not different from those found on the culture plots. Assuming that all the epibenthic species recorded from the northern Wadden Sea could potentially occur associated with mussels in the western Dutch Wadden Sea it would mean that a potential doubling of the epibenthic species number is possible, suggesting that subtidal mussel beds could be even richer than they are at present. Interesting was that one long time missing epibenthic species in the northern Wadden Sea, *Palaemon elegans*, was found in one box core from the culture plots.

Where mussel culture plots harbour more species at the ecosystem scale the number of species found in one box core is less than in mussel occurrences outside mussel culture plots. This difference is limited to the soft sediment species which are more diverse in the natural beds. That this is caused by higher mussel densities on the mussel culture plots that impair soft sediment species does not seem to be the case (see Fig. 15 and appendix 3). It however matches a pattern described by Beukema and Dekker (2012), who showed that along an environmental gradient encounter rates of species may change in an opposite direction as the size of the species pool. This can result in reversal of trends in species richness depending on sample size. Numerical densities on natural beds are higher than on mussel culture plots which makes chances higher to find more species in one box core (alpha diversity), however the number of new species found in each new boxcore is lower (beta diversity) resulting in a lower overall diversity (gamma diversity) on the natural mussel plots compared to the mussel culture plots (Figs. 9 & 10).

With several approaches we tried to separate the effects of environment and mussel culture plot on the number of species in a box. Stepwise selection of terms in a glm model always dropt salinity in favour of bed-type. Considering all model variants, using subsets of species and of subsets of stations a bed effect remained apparent. Conclusion must be that alpha diversity is lower on mussel culture plots than at sites with mussels outside mussel culture plots.

Interesting was that silt content had a negative effect on the soft sediment species richness, which could be an indirect bed effect on the benthos by deposition of fine material, filtered out of the water column by the mussels. However this would have been more likely if we had found a negative effect of mussel biomass on the number of soft sediment species. Hard substrate species richness did not respond to silt. This is a different result than found in the northern Wadden Sea where silt accumulation in the mussel bed resulted in low epifaunal diversity (Westphalen 2006). It is also clear that besides mussels also oysters increase hard substrate species richness in the mussel beds.

Shell surface of mussel serves as settlement substrate for other species. The number of hard substrate individuals per shell surface unit corrected for salinity effects is higher in natural beds than in mussel culture plots. A similar result was reported by Westphalen (2006), however in that study

silt accumulation played a role as well. The lower density of hard substrate species on the mussel shells from culture plots could have been cause by the higher mussel densities limiting effective settlement surface per mussel. Mussel handling by fishery might also cause a reduction of densities of epifauna, in the end heavily overgrown mussels are not marketable.

Species composition differs between mussel culture plots and natural mussel beds. Main factors explaining this difference are bed-type salinity and median grain size. Especially differences in soft sediment species between bed types are pronounced.

Limitations of the study

It is important to recognize that the distribution of mussel culture plots is without doubt human controlled. Therefore any differences in bed-types are human induced. Going one step beyond and trying to distinguish between environmental effects and fishery handling effects is more complicated with the present data. This study is purely descriptive and cannot be conclusive about causal relationships. Main concern is the unevenness in distribution of mussel culture plot and natural bed stations which at least causes a difference in seawater salinity between bed-types. There may even be more confounding factors which we did not recognize yet. This means that given the fact that the distribution differences between bed types are human induced, any difference in mussels and their associated communities from outside and inside mussel culture plots are human induced effects. With the present data set the effect are not unambiguously attributable to as direct handling effects (e.g. dredging, transporting and predator removal) or indirect effects because of differences in the environmental conditions between sites where mussel naturally settle and where mussel culture plots are located.

Conclusions

Mussel culture plots differ from natural mussel beds in several ways.

Important difference is the environment; salinity is higher in areas with mussel culture beds than in areas with naturally occurring mussel.

Number of species per box core is lower inside than outide mussel culture plots, the higher number of species per box outside mussel culture plots is due to a higher number of soft sediment species.

The species pool at mussel culture plots is larger than at the natural beds, which is most likely due to the higher salinities at mussel culture plots compared to the natural settlement areas of the mussels.

Densities of macrozoobenthos are higher outside than inside mussel culture plots.

Outside mussel culture plots densities of hard substrate species per unit mussel shell surface are higher than inside mussel culture plots.

Species composition differs between mussel culture plots and natural mussel beds.

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Table 1. Number of box core samples of 0.06 m^{-2} containing mussels (*Mytilus edulis*) collected outside and inside mussel culture plots during autumn in the years 2008, 2009 and 2010.

	year			
plot	2008	2009	2010	Totals
outside	25	29	25	79
inside	21	30	29	80
Totals	46	59	54	159

Table 2. First explorative tests of differences in mussels and macrozoobenthos metrics inside and outside mussel culture plots. Information on significance of test result and type of test and data transformation are included.

metric	unit	outside culture plot	inside culture plot	sig. diff.	test
total cores	n	79	80		no test
mussel density	n/m2	742	857	***	LM(log)
mussel biomass	g ADW/m2	92	489	***	LM(log)
mussel condition index	mg/cm3	3.3	4.6	***	LM
fraction mussels > 4 cm	%	22	68		no test
total biomass (excluding mussel)	g ADW/m2	151	50	***	LM(log)
hard substrate biomass (ex. mussel)	g ADW/m2	26	16	ns	LM (log+0.002)
biomassa soft sediment species	g ADW/m2	125	34	***	LM (log+0.002)
total density (ex mossel)	n/m2	27620	5045	***	LM(log)
density hard substrate (ex mossel)	n/m2	2810	3020	ns	LM(log+1)
density soft sediment species	n/m2	24810	2025	***	LM(log+1)
total species	n	84	102		no test
total species per box	n/0.06m2	19.4	17.3	*	GLM, quasi.
hard substrate species	n/0.06m2	6.7	7.7	ns	GLM, quasi.
soft sediment species	n/0.06m2	12.7	9.6	***	GLM, quasi.

Table 3. Anova table of a linear model comparison of total densities (nm^{-2}) of hard substrate species excluding mussels, between outside and inside mussel culture plots in three years (2008, 2009 & 2010).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
plot	1	0.031	0.031	0.047	0.828	
year	2	10.446	5.223	8.011	<0.001	**
plot:year	2	0.585	0.293	0.449	0.639	
Residuals	153	99.75	0.652			

Table 4. Anova tables of linear models with response variables density, biomass, shell length and condition index of mussels from outside and inside mussel culture plots. Explanatory factors are plot with two levels, outside and inside and year sampled.

Response:	Log10(Density)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
plot	1	7.047	7.0467	15.4899	0.0001249	***
Year	1	2.075	2.0748	4.5608	0.034282	*
plot:year	1	3.447	3.4475	7.5782	0.0066137	**
Residuals	155	70.513	0.4549			
Response:	Log10(Biomass)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
plot	1	24.818	24.8177	44.4021	4.41E-10	***
Year	1	1.303	1.3032	2.3316	0.1288	
plot:year	1	0.795	0.7947	1.4218	0.2349	
Residuals	155	86.634	0.5589			
Response:	Shell length					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
plot	1	1786.8	1786.79	14.2918	0.0002229	***
Year	1	737.2	737.16	5.8962	0.0163182	*
plot:year	1	722	721.96	5.7746	0.0174409	*
Residuals	155	19378.5	125.02			
Response:	Condition index					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
plot	1	67.57	67.573	32.3292	6.30E-08	***
Year	1	26.11	26.109	12.4912	0.0005392	***
plot:year	1	15.27	15.273	7.3073	0.0076342	**
Residuals	155	323.98	2.09			

Table 5. Species unique for 79 box cores (surface area 0.06 m^2) with mussels taken outside mussel culture plots. Occ. Is the number of boxes in which the species was found. Max density is in nm^{-2} (17 nm^{-2} is one individual per core) Substrate categories are Soft) soft sediment, Heterog.) heterogeneous sediments, Hard mob) mobile species on hard substrate, Hard ses) sessile species attached to hard substrate.

Species	**Phylum**	**Class**	**feeding**	**substrate**	**occ.**	**max density**
Eteone sp.	Annelida	Polychaeta	carnivore		1	17
Photis reinhardi	Arthropoda	Malacostraca	deposit	Hard mob	1	17
Phyllodoce maculata	Annelida	Polychaeta	carnivore	Soft	2	17
Procerastea halleziana	Annelida	Polychaeta	carnivore	Hard mob	1	17

Spisula subtruncata	Mollusca	Bivalvia	suspension	Soft	1	8

Table 6. Species unique for 80 box cores from mussel culture plots. Occ. Is the number of boxes in which the species was found. Max density is in nm^{-2}. See table 4 for an explanation of substrate types.

Species	**Phylum**	**Class**	**feeding**	**substrate**	**occ.**	**max density**
Abludomelita obtusata	Arthropoda	Malacostraca	deposit	Hard mob	3	33
Aeolidia papillosa	Mollusca	Gastropoda	carnivore	Hard mob	3	17
Amphipholis squamata	Echinodermata	Ophiuroidea	suspension	Hard mob	3	33
Angulus fabula	Mollusca	Bivalvia	deposit	Soft	2	33
Aonides oxycephala	Annelida	Polychaeta	deposit	Soft	1	17
Bathyporeia sarsi	Arthropoda	Malacostraca	deposit	Soft	1	17
Cancer pagurus	Arthropoda	Malacostraca	omnivore	Hard mob	2	17
Cheirocratus sundevalli	Arthropoda	Malacostraca	deposit	Hard mob	1	17
Eualus cranchii	Arthropoda	Malacostraca	omnivore	Hard mob	1	17
Hydractinia echinata	Cnidaria	Hydrozoa	suspension	Hard ses	1	17
Kurtiella bidentata	Mollusca	Bivalvia	suspension	Soft	4	83
Lepidochitona cinerea	Mollusca	Polyplacophora	deposit	Hard mob	1	17
Mactra stultorum	Mollusca	Bivalvia	suspension	Soft	1	17
Nemertea	Nemertea		carnivore		2	17
Ophiothrix fragilis	Echinodermata	Ophiuroidea	suspension	Hard mob	1	150
Pagurus bernhardus	Arthropoda	Malacostraca	carnivore	Soft	1	17
Palaemon elegans	Arthropoda	Malacostraca	omnivore	Hard mob	1	33
Pisidia longicornis	Arthropoda	Malacostraca	omnivore	Hard mob	4	50
Psammechinus miliaris	Echinodermata	Echinoidea	carnivore	Hard mob	1	17
Sthenelais boa	Annelida	Polychaeta	omnivore	Hard mob	9	33
Travisia forbesii	Annelida	Polychaeta	deposit	Soft	1	50
Urothoe poseidonis	Arthropoda	Malacostraca	deposit	Soft	2	33
Venerupis corrugata	Mollusca	Bivalvia	suspension	Heterog.	4	17

Table 7. Anova tables of generalized linear models, family quasipoisson describing species richness of box cores with mussels taken outside and inside mussel culture plots in 2008, 2009 and 2010. Tests are done for all species together and also separately for hard substrate and soft substrate species only.

Response:	All species					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
NULL			158	343.98		
plot	1	9.772	157	334.21	0.029	*
Year	2	12.072	155	322.14	0.053	.
plot:year	2	0.417	153	321.72	0.904	

Response:	Hard substrate species					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
NULL			158	307.18		
plot	1	3.956	157	303.22	0.143	
Year	2	4.898	155	298.32	0.265	
plot:year	2	2.664	153	295.66	0.485	
Response:	Soft sediment species					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
NULL			158	251.51		
plot	1	34.005	157	217.5	<0.001	***
Year	2	7.786	155	209.72	0.055	.
plot:year	2	0.74	153	208.98	0.759	

Table 8. Coefficients of a GLM model describing species number per box core outside and inside mussel culture plots.

Coefficient	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
(Intercept)	2.73E+00	7.72E-02	35.349	<0.001	***
$plot_{inside}$	-3.57E-01	1.19E-01	-3.001	0.003	**
mussel biomass	1.07E-01	3.52E-02	3.04	0.002	**
Silt	-1.79E-02	3.72E-03	-4.805	<0.001	***
oyster biomass	2.26E-01	5.07E-02	4.455	<0.001	***
$year_{2009}$	7.71E-02	4.63E-02	1.665	0.096	.
$year_{2010}$	1.67E-01	4.66E-02	3.597	<0.001	***
height	-2.92E-04	6.85E-05	-4.256	<0.001	***
$plot_{inside}$: mussel biomass	1.72E-01	5.30E-02	3.235	0.001	**
$plot_{inside}$: height	4.00E-04	1.47E-04	2.715	0.006	**

Null deviance: 343.98 on 158 degrees of freedom
Residual deviance: 202.70 on 149 degrees of freedom

Table 9 Effect of dropping a single term from the GLM model with and without interactions describing species number per box core outside and inside mussel culture plots.

Change in fit when dropping a single term from the model including interaction terms

Term	**Df**	**Deviance**	**AIC**	**LRT**	**Pr(>Chi)**	
<none>		202.7	977.9			
silt	1	226.7	999.9	23.9966	<0.001	***
oyster biomass	1	220.02	993.22	17.3203	<0.001	***
year	2	215.9	987.09	13.1942	0.001	**
plot : mussel biomass	1	213.25	986.45	10.5457	0.001	**
plot : height	1	210.12	983.32	7.4192	0.006	**

Change in fit when dropping a single term from the model excluding interaction terms

Term	Df	Deviance	AIC	LRT	Pr(>Chi)	
<none>		217.12	988.31			
plot	1	241.34	1010.54	24.228	<0.001	***
mussel biomass	1	264.77	1033.97	47.66	<0.001	***
silt	1	238.14	1007.34	21.026	<0.001	***
oyster biomass	1	232.3	1001.5	15.185	<0.001	***
year	2	234.56	1001.76	17.443	<0.001	***
height	1	228.1	997.3	10.985	<0.001	***

Table 10 Coefficients of a GLM model describing hard substrate species number per box core outside and inside mussel culture plots.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	1.43E+00	1.63E-01	8.802	<0.001	***
plot $_{inside}$	-2.08E-01	6.76E-02	-3.074	0.002	**
mussel biomass	2.64E-01	7.28E-02	3.626	<0.001	***
oyster biomass	3.37E-01	7.01E-02	4.803	<0.001	***
height	-2.29E-04	9.60E-05	-2.387	0.017	*
year $_{2009}$	-7.01E-01	2.21E-01	-3.17	0.002	**
year $_{2008}$	1.49E-01	1.95E-01	0.764	0.445	
mussel biomass : year $_{2009}$	4.04E-01	9.80E-02	4.125	<0.001	***
mussel biomass : year $_{2010}$	3.13E-02	8.77E-02	0.357	0.720	
plot$_{inside}$: oyster biomass	2.16E+00	9.78E-01	2.205	0.027	*

Null deviance: 307.18 on 158 degrees of freedom
Residual deviance: 132.80 on 149 degrees of freedom

Table 11 Effect of dropping a single term from the GLM model with and without interactions describing hard substrate species number per box core outside and inside mussel culture plots.

Change in fit when dropping a single term from the model including interaction terms

Term	Df	Deviance	AIC	LRT	Pr(>Chi)	
<none>		132.8	761.48			
height	1	138.35	765.03	5.5504	0.018	*
mussel biomass:year	2	156.37	781.04	23.5616	<0.001	***
plot:oyster biomass	1	137.22	763.9	4.4206	0.036	*

Change in fit when dropping a single term from the model excluding interaction terms

Term	Df	Deviance	AIC	LRT	Pr(>Chi)	
<none>		159.23	781.9			
plot	1	165.83	786.51	6.608	0.010	*
mussel biomass	1	252.08	872.75	92.856	<0.001	***
oyster biomass	1	181.76	802.43	22.533	<0.001	***
height	1	167.35	788.02	8.119	0.004	**
year	2	171.01	789.68	11.787	0.003	**

Table 12 Coefficients of a GLM model describing soft sediment species number per box core outside and inside mussel culture plots.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	2.6361712	0.0610096	43.209	<0.001	***
plot $_{inside}$	-0.2283182	0.0488249	-4.676	<0.001	***
silt	-0.0267341	0.005002	-5.345	<0.001	***
height	-0.0001677	0.0000773	-2.17	0.03	*

Null deviance: 251.51 on 158 degrees of freedom
Residual deviance: 177.21 on 155 degrees of freedom

Table 13 Effect of dropping a single term from the GLM model describing soft sediment species number per box core outside and inside mussel culture plots.

Term	Df	Deviance	AIC	LRT	Pr(>Chi)
<none>		177.21	851.13		
plot	1	199.25	871.17	22.04	<0.001
silt	1	207.46	879.38	30.25	<0.001
height	1	181.81	853.73	4.603	0.032

Table 14 Coefficients of a GLM model describing number of species in a box core with mussels form outside and inside mussel culture plots in the Marsdiep tidal basin

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	2.72E+00	9.19E-02	29.572	<0.001	***
plot $_{inside}$	-5.84E-01	1.61E-01	-3.624	<0.001	***
mussel biomass	1.16E-01	3.85E-02	3.011	0.003	**
silt	-1.74E-02	5.12E-03	-3.404	<0.001	***
oyster biomass	2.19E-01	5.20E-02	4.199	<0.001	***
year $_{2009}$	6.52E-02	6.23E-02	1.047	0.295	
year $_{2010}$	1.95E-01	6.31E-02	3.097	0.002	**
height	-2.70E-04	7.16E-05	-3.779	<0.001	***
plot $_{inside}$: mussel biomass	1.89E-01	8.27E-02	2.282	0.023	*
plot $_{inside}$: oyster biomass	5.31E+00	1.86E+00	2.862	0.004	**

Null deviance: 188.674 on 85 degrees of freedom
Residual deviance: 78.469 on 76 degrees of freedom

Table 15 Change in deviance by excluding single terms from a GLM describing species numbers in box cores from outside and inside mussel culture plots in the Marsdiep tidal basin.

Change in fit when dropping a single term from the model including interaction terms

Term	**Df**	**Deviance**	**AIC**	**LRT**	**Pr(>Chi)**	
<none>		78.469	508.18			
silt	1	90.434	518.14	11.9651	<0.001	***
year	2	88.551	514.26	10.0816	0.006	**
height	1	92.301	520.01	13.8317	<0.001	***
plot : mussel biomass	1	83.748	511.46	5.2784	0.022	*
plot : oyster biomass	1	86.012	513.72	7.5425	0.006	**

Change in fit when dropping a single term from the model after interaction term were removed

Term	**Df**	**Deviance**	**AIC**	**LRT**	**Pr(>Chi)**	
<none>		94.713	520.42			
plot	1	101.992	525.7	7.2788	0.007	**
mussel biomass	1	117.604	541.31	22.891	<0.001	***
silt	1	104.242	527.95	9.5294	0.002	**
oyster biomass	1	110.378	534.09	15.6657	<0.001	***
year	2	105.253	526.96	10.5407	0.005	**
height	1	108.573	532.28	13.8602	<0.001	***

Table 16 Anova table and table of single term deletions of a linear model describing hard substrate species density on the age corrected shell surface of mussels from outside and inside mussel culture plots.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
salinity	1	13.32	13.32	38.024	<0.001	***
plot	1	4.298	4.298	12.269	<0.001	***
salinity:plot	1	1.025	1.025	2.927	0.089	.
Residuals	155	54.296	0.35			

Single term deletions of model without interactions

	Df	Sum of Sq	RSS	AIC
<none>			55.321	-161.86
salinity	1	2.288	57.609	-157.42
plot	1	4.298	59.619	-151.97

Table 17. Anova table of a permutational multivariate analysis of variance of the distance matrix of stations outside and inside mussel culture plots. Terms were added sequentially from first to last.

Source	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
plot	1	2.479	2.47904	10.3136	0.05792	0.001	***
Salinity	1	1.598	1.59761	6.6466	0.03733	0.001	***
median grain size	1	0.652	0.65205	2.7127	0.01523	0.001	***
plot : salinity	1	0.458	0.45825	1.9065	0.01071	0.012	*
salinity: median grain size	1	0.688	0.68839	2.8639	0.01608	0.001	***
plot : median grain size	1	0.389	0.38933	1.6198	0.0091	0.028	*
Residuals	152	36.536	0.24037		0.85363		
Total	158	42.8			1		

Table 18. Differences in occurrences and densities of species typical for the habitat 1101, between natural beds and mussel culture plots. When the difference in occurrence is significant the location either outside or inside a mussel culture plot with the highest occurrence is mentioned. Differences in densities only refer to the subset of box core samples in which the species was encountered. Position relative to mussel culture plots where the density at the occupied sites is significantly highest is listed.

Dutch name	Scientific name	Occurrence	Density
Zeeanjelier	*Metridium senile*		outside
Slibanemoon	*Sagartia troglodytes*		outside
Zandzager	*Nephtys hombergii*	inside	
groene zeeduizendpoot	*Alitta virens*		outside
gladde zeepok	*Balanus crenatus*	no records	no records
Strandkrab	*Carcinus maenas*		inside
gewone zwemkrab	*Liocarcinus holsatus*	no records	no records
gewone zeester	*Asterias rubens*		inside
Nonnetje	*Macoma balthica*	outside	
Strandgaper	*Mya arenaria*	outside	outside
Mossel	*Mytilus edulis*		inside

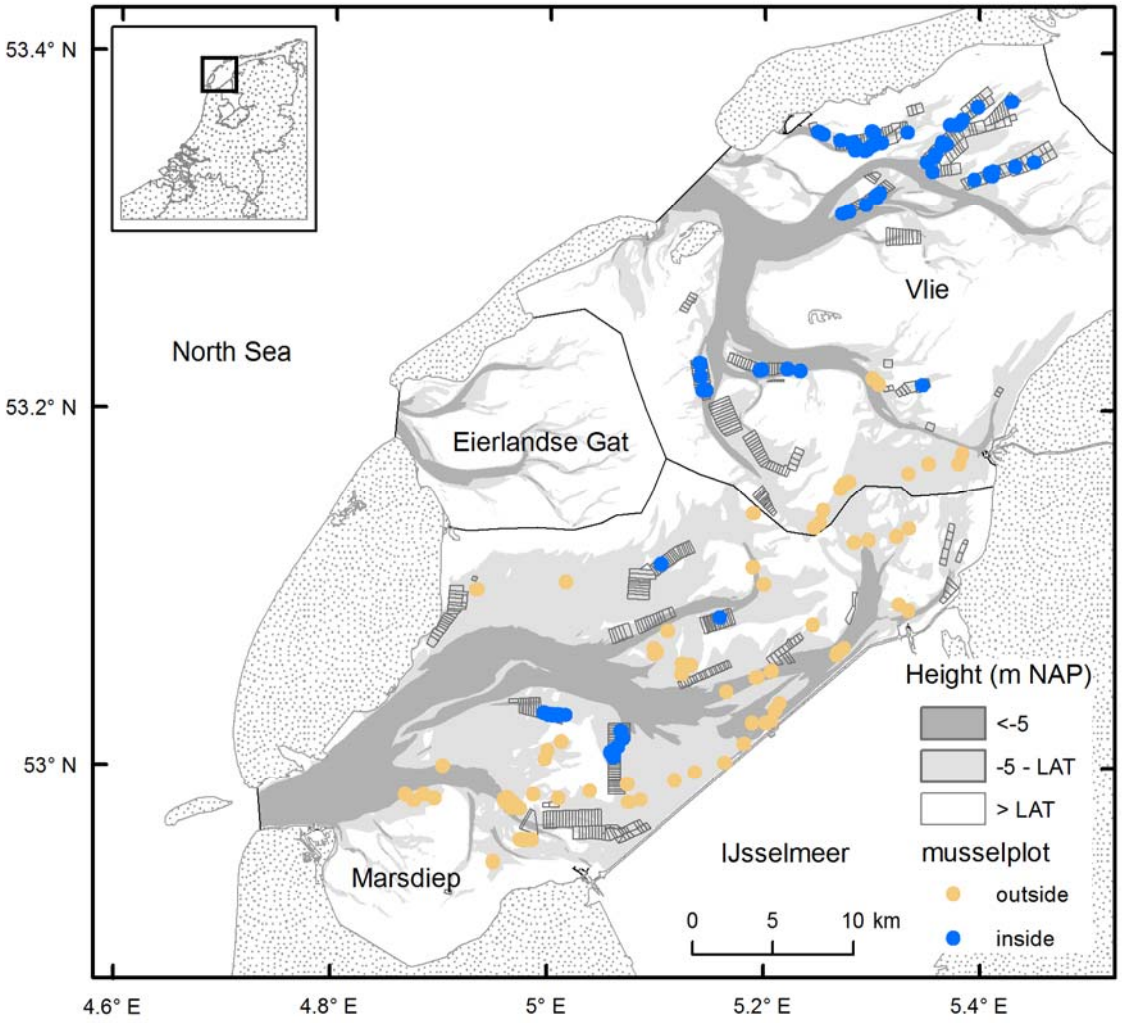

Figure 1. Map of the western Dutch Wadden Sea with sampling stations and outlines of the mussel culture plots. Borders and names of the tidal basins are also indicated.

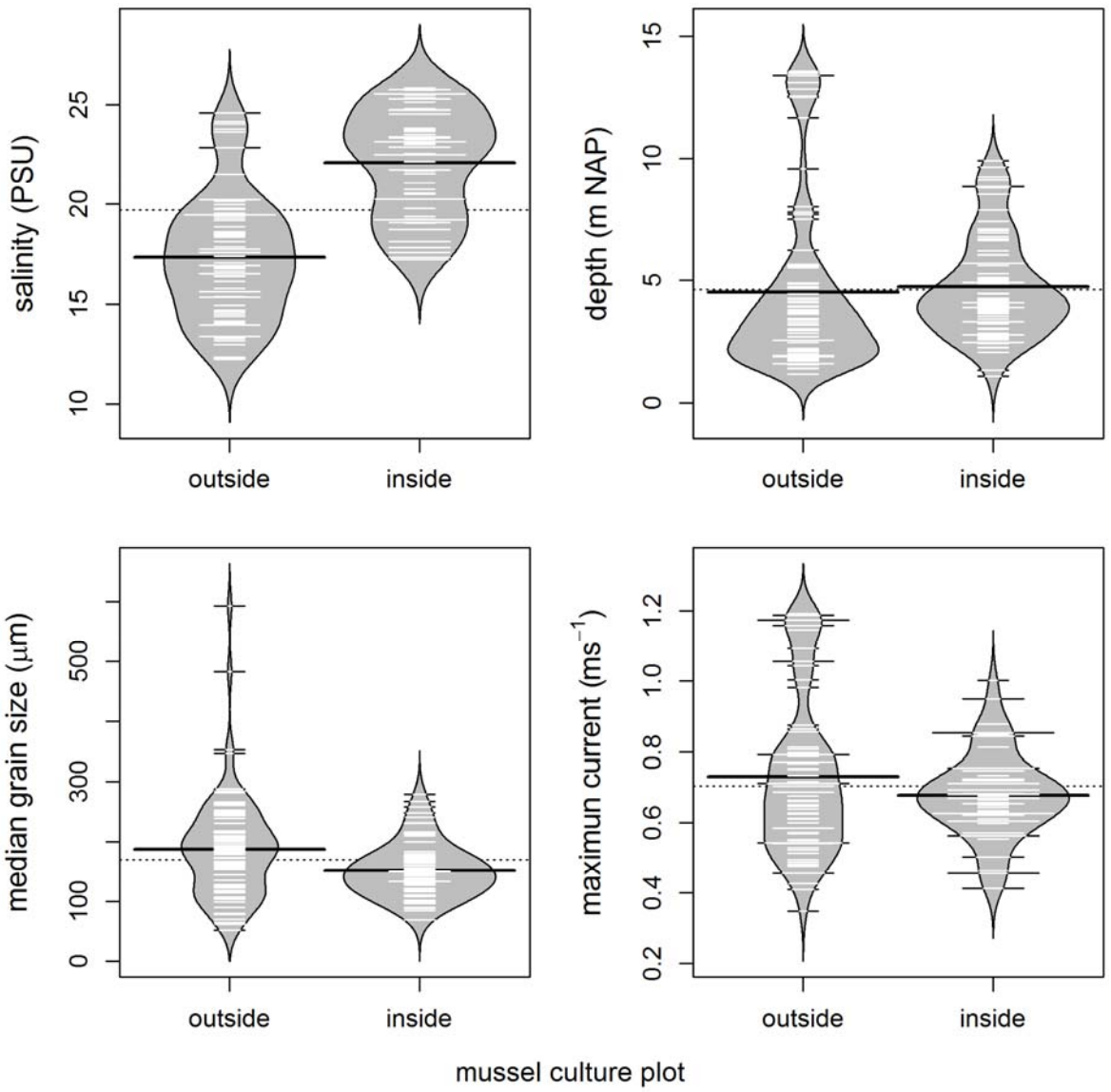

Figure 2. Seawater salinity, depth, sediment median grain size and maximum current speed at the stations outside and inside mussel culture plots in the subtidal western Dutch Wadden Sea. Median gran rise was measured from a sediment sample out of the box core, salinity and maximum current speeds are model predictions, depth is extracted from a sounding chart.

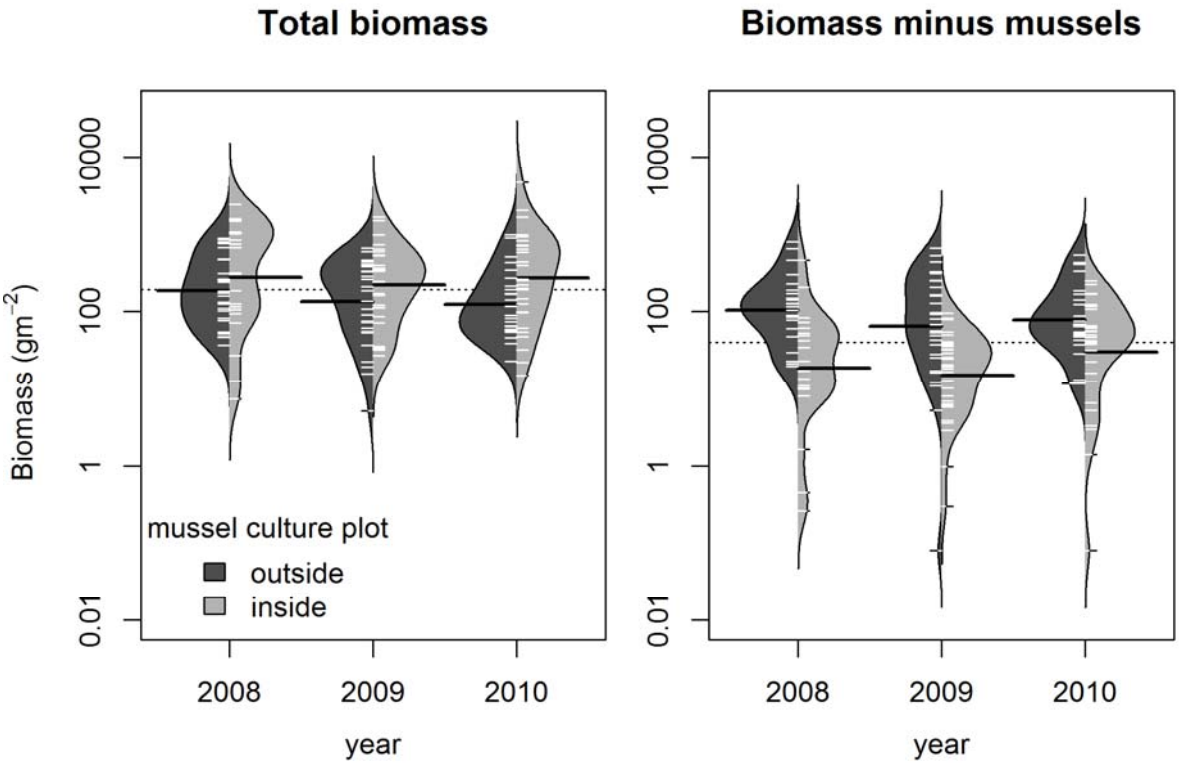

Figure 3. Total biomass of the macrozoobenthos (AFDM gm^{-2}) at stations inside and outside mussel culture plots in the three sampling years in the left panel. Right panel is total biomass excluding mussels biomass.

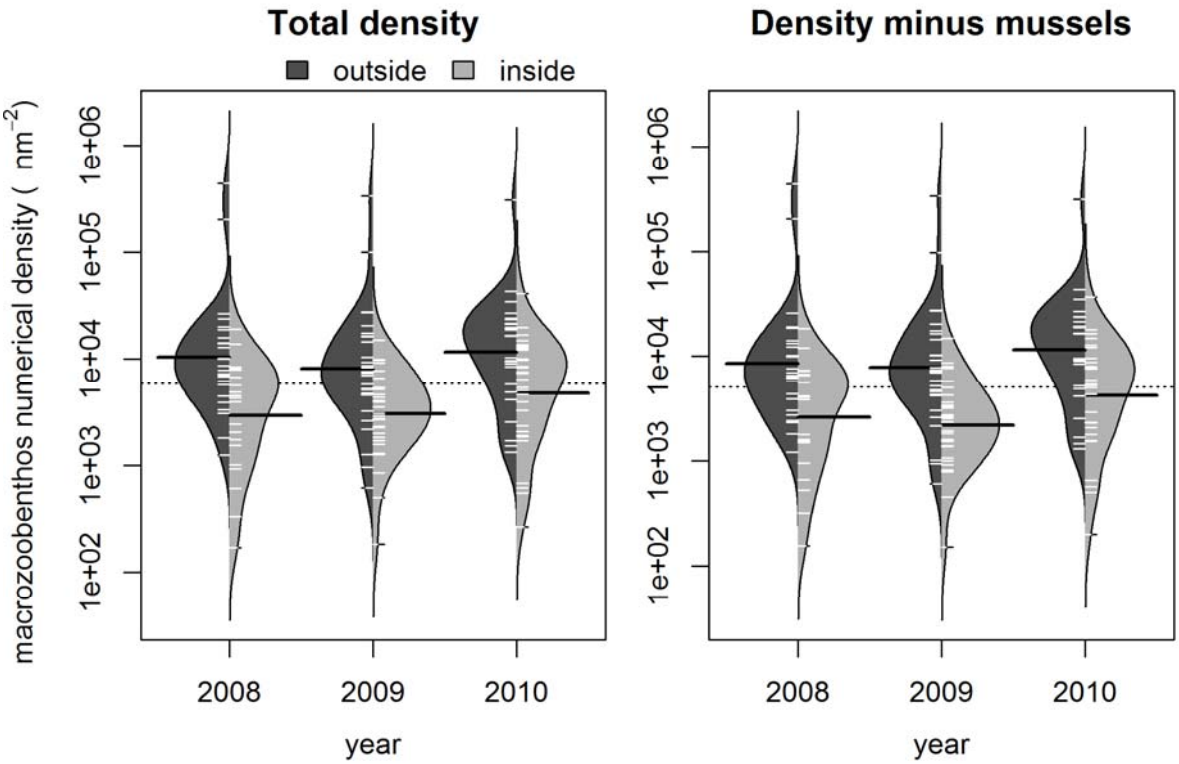

Figure 4. Total numerical density of the macrozoobenthos at the stations inside and outside the mussel culture plots during the three years in the subtidal of the western Dutch Wadden Sea. Left panel is total density including mussels, right panel is total density excluding mussels.

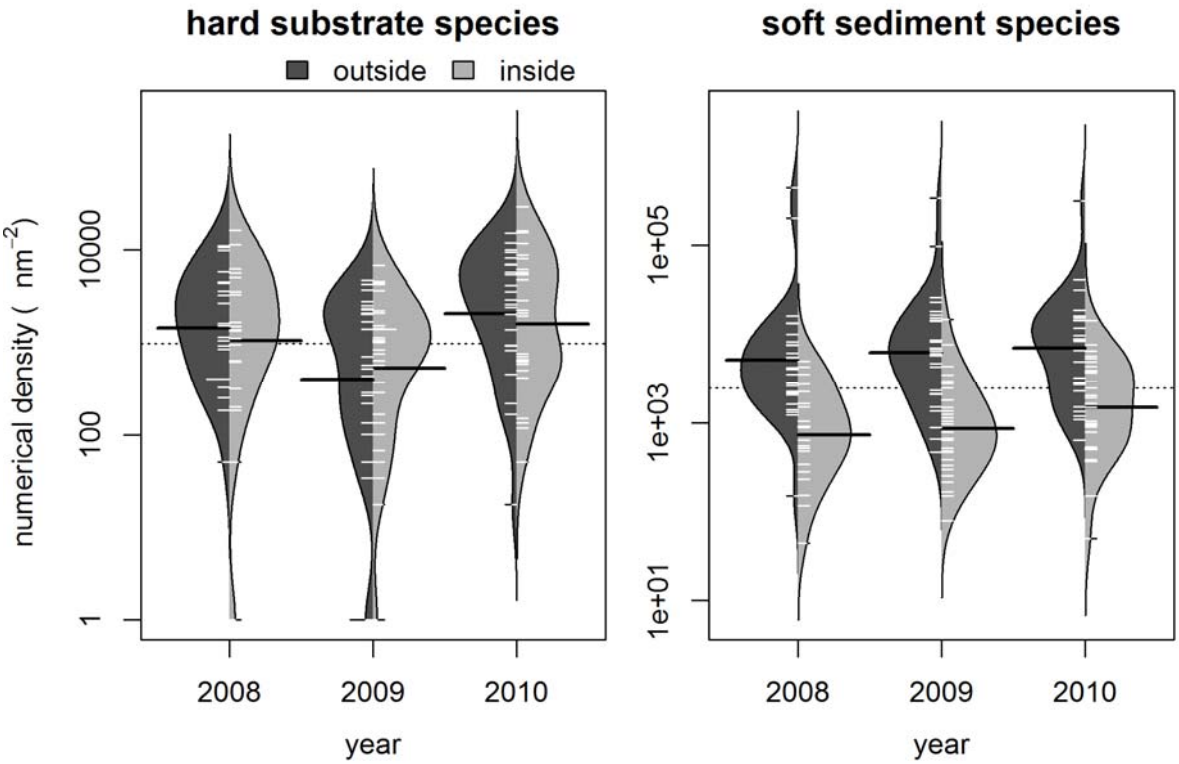

Figure 5. Total density of the macrozoobenthos at stations outside and inside mussel culture plots during three years in the western Dutch Wadden Sea. Macrozoobenthos is divided in hard substrate species excluding mussels (left panel) and soft sediment species (right panel). Note different y-scales.

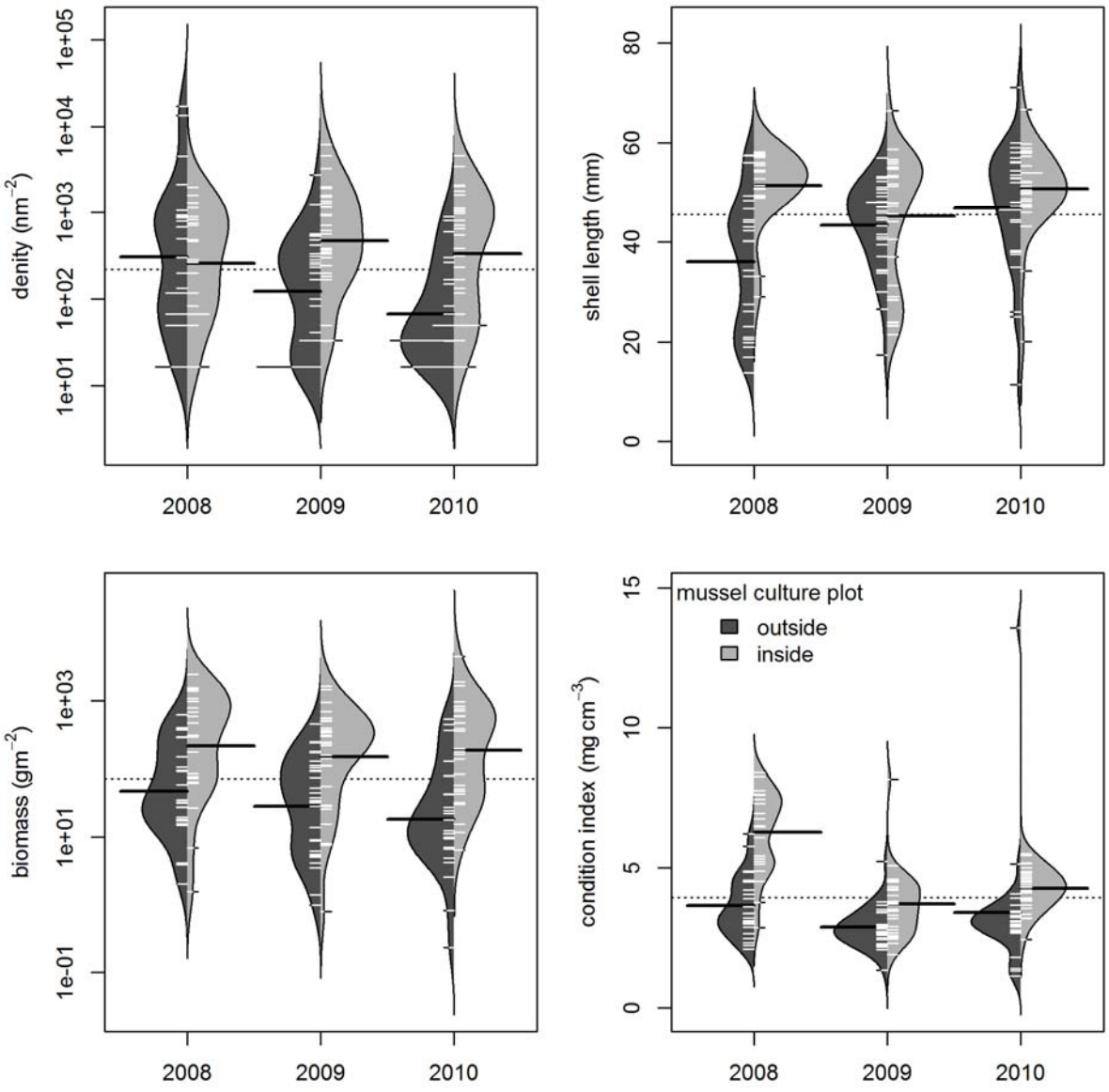

Figure 6. Comparison of numerical density (top left), biomass (bottom left), shell length (top right) and condition index (bottom right) of mussels (*Mytilus edulis*) from outside and inside mussel culture plots in the subtidal western Dutch Wadden Sea during three years, 2008, 2009 and 2010.

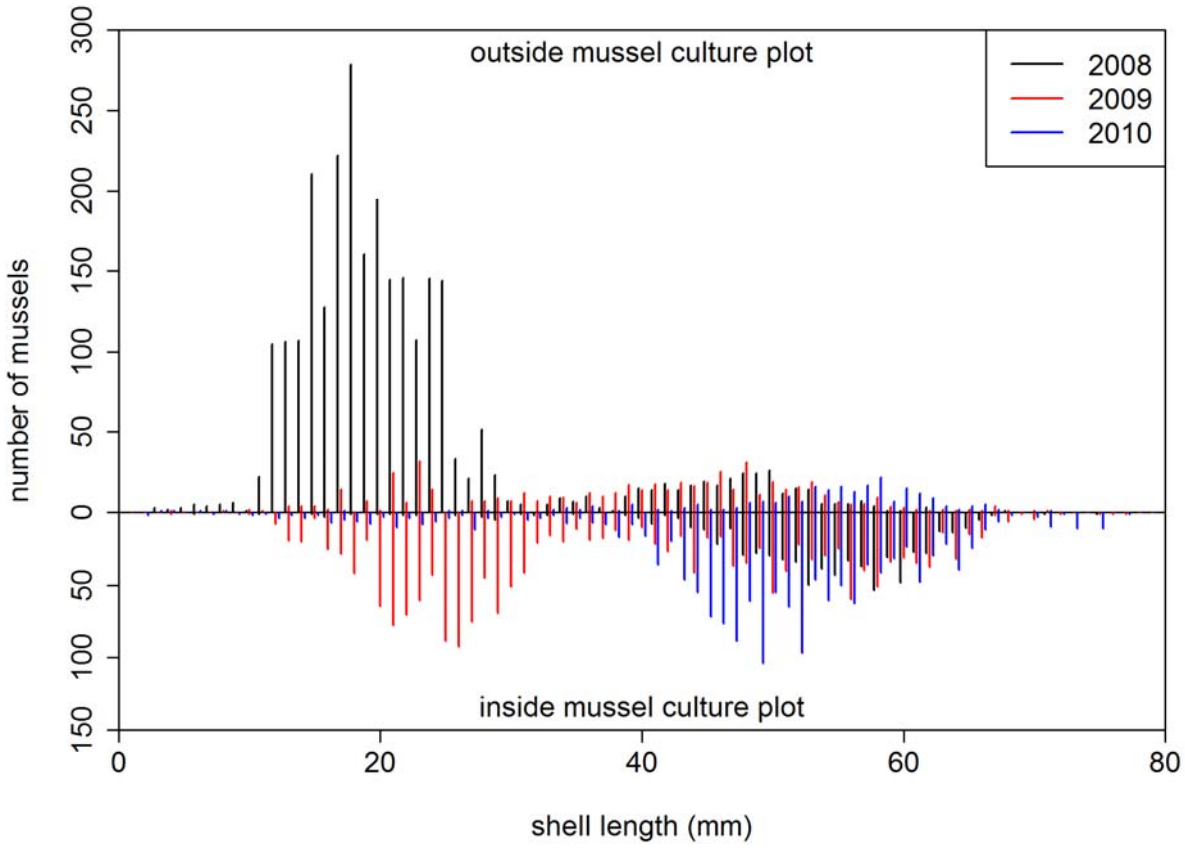

Figure 7. Shell length distribution of mussels (*Mytilus edulis*) at stations outside and inside mussel culture plots in the subtidal western Dutch Wadden Sea during 2008, 2009 and 2010. Shell length data are from all mussels collected from 159 box cores of 0.06 m^2.

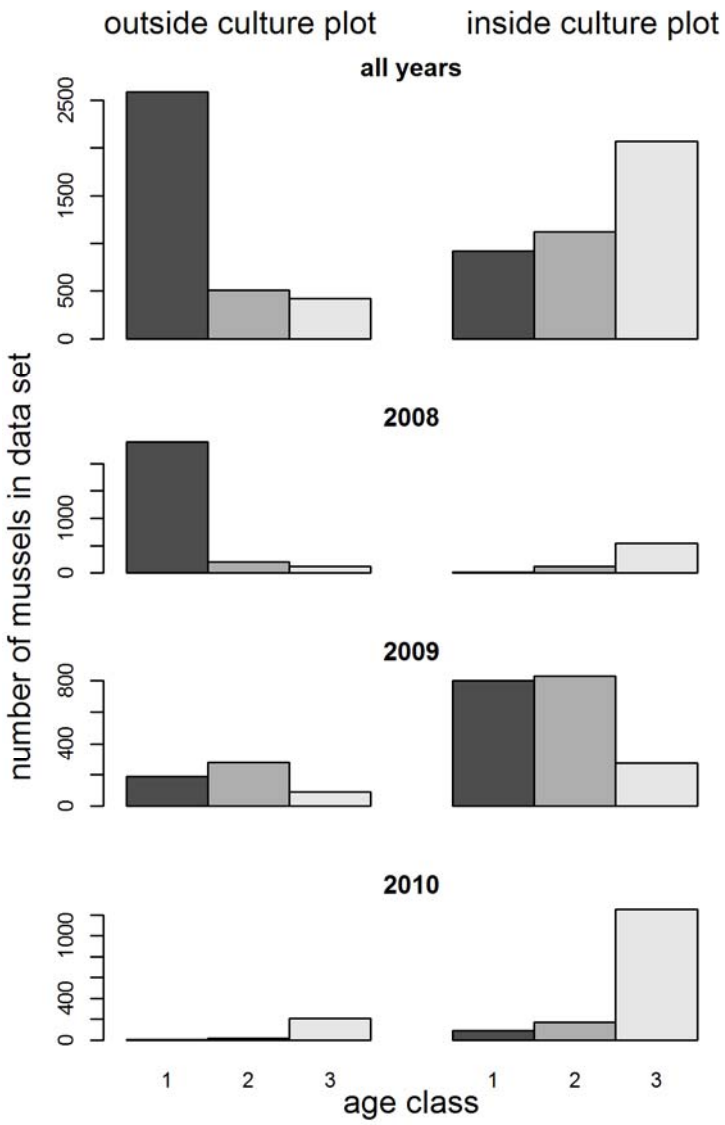

Figure 8. Mussel age distributions from stations inside and outside mussel culture plots in the subtidal of the western Dutch Wadden Sea. In the top panel all years are included. The lower three panels are separate distributions for each year. Age classes are: 1) first year after birth, 2) second year and 3) third year and older.

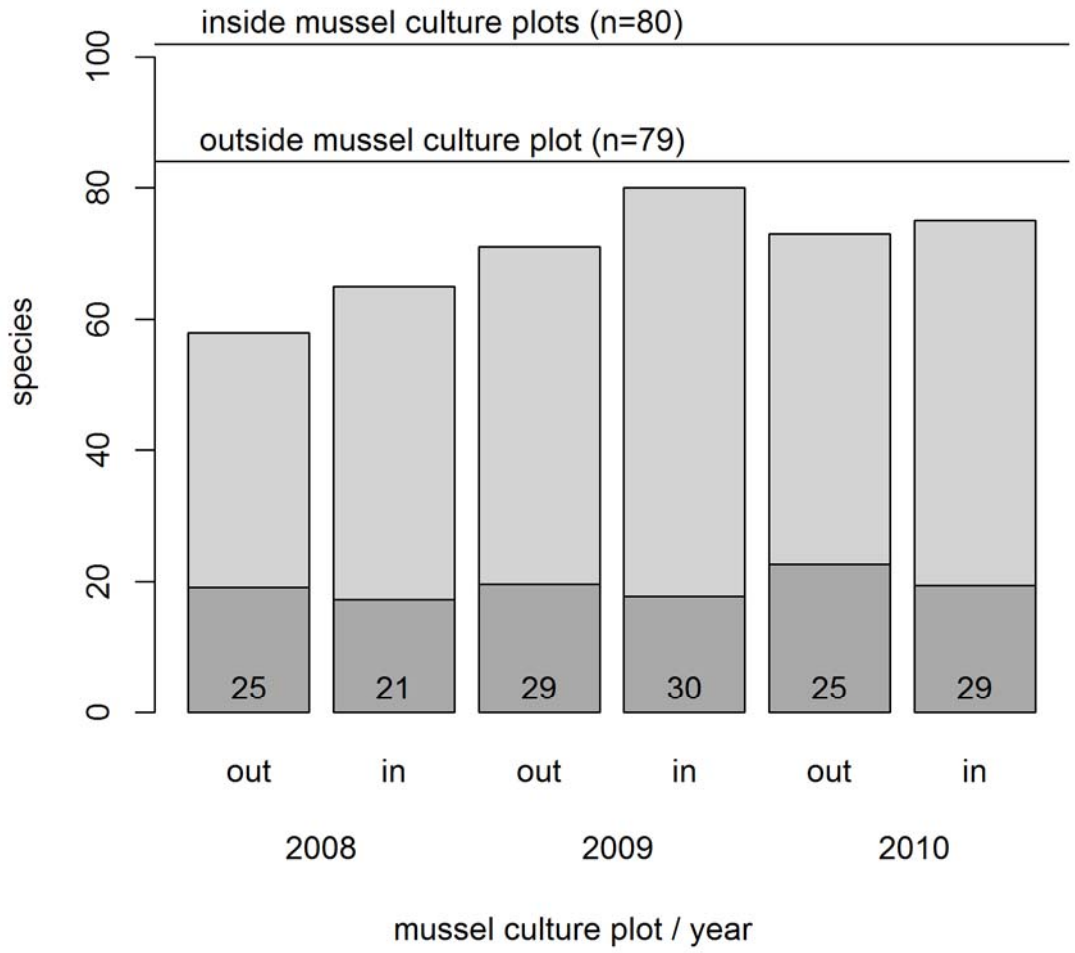

Figure 9. Number of species found at the stations outside and inside mussel culture plots categorized per year. The dark shaded lower parts of the bars indicate the average number of species in an area of 0.06 m^2. Horizontal lines indicate the total number of species found per bed type when taking all three year together. Numbers refer to number of box cores per category/bar.

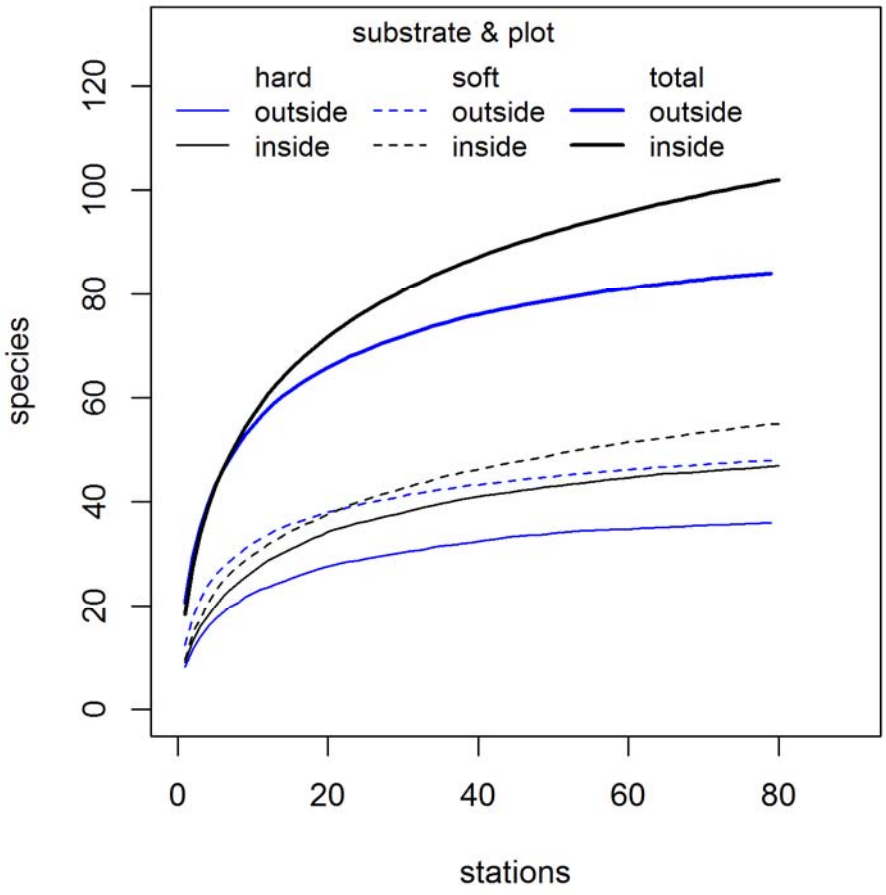

Figure 10. Species area curves showing the cumulative species number with increasing number of stations. There are curves for hard substrate and soft sediment species inside and outside mussel culture plots and the curves of the sum of hard substrate and soft sediment species. Stations were sampled in the subtidal western Dutch Wadden Sea with a box corer with surface area of 0.06 m^2.

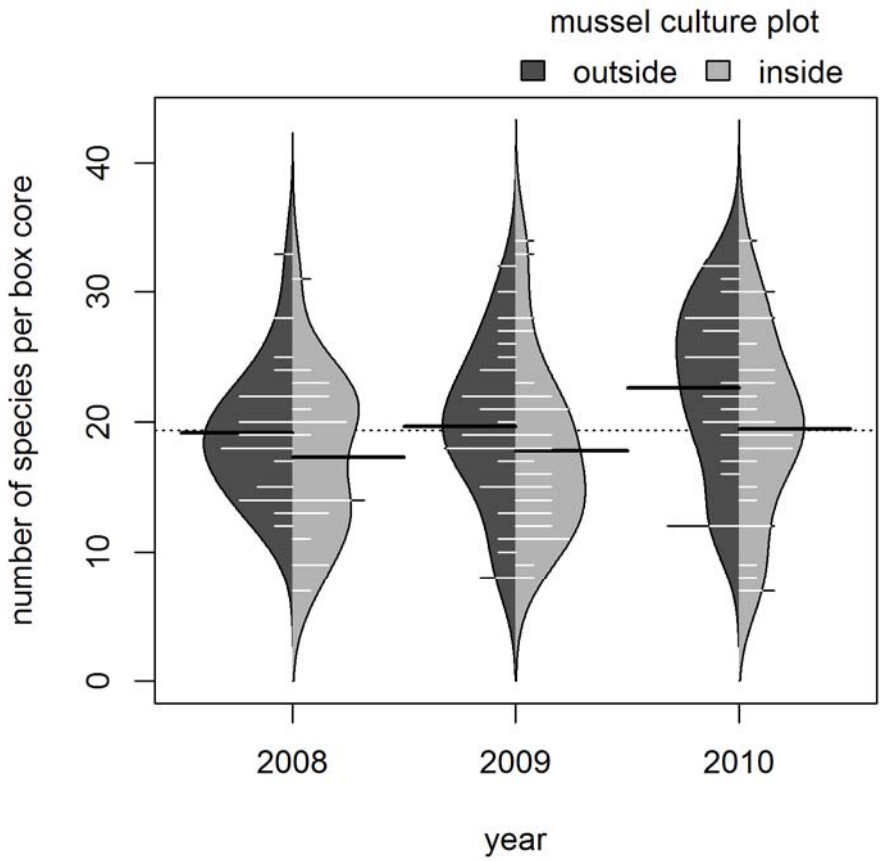

Figure 11. Beanplot of number of species in a box core of 0.06 m^2 inside and outside mussel culture plots in the subtidal of the western Dutch Wadden sea during the years 2008 2009 and 2010.

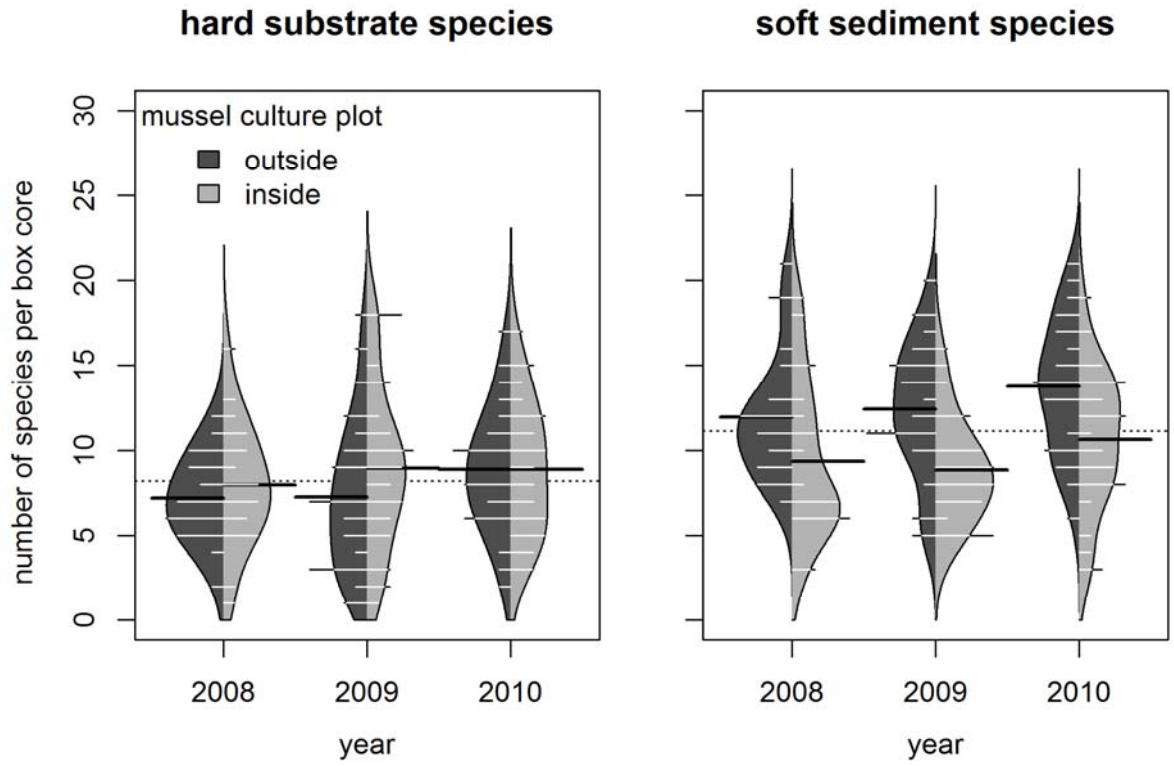

Figure 12. Number of species in 0.06 m^2 box core samples inside and outside mussel culture plots in the subtidal of the western Dutch Wadden Sea during three years divided in hard substrate (left panel) and soft sediment species (right panel).

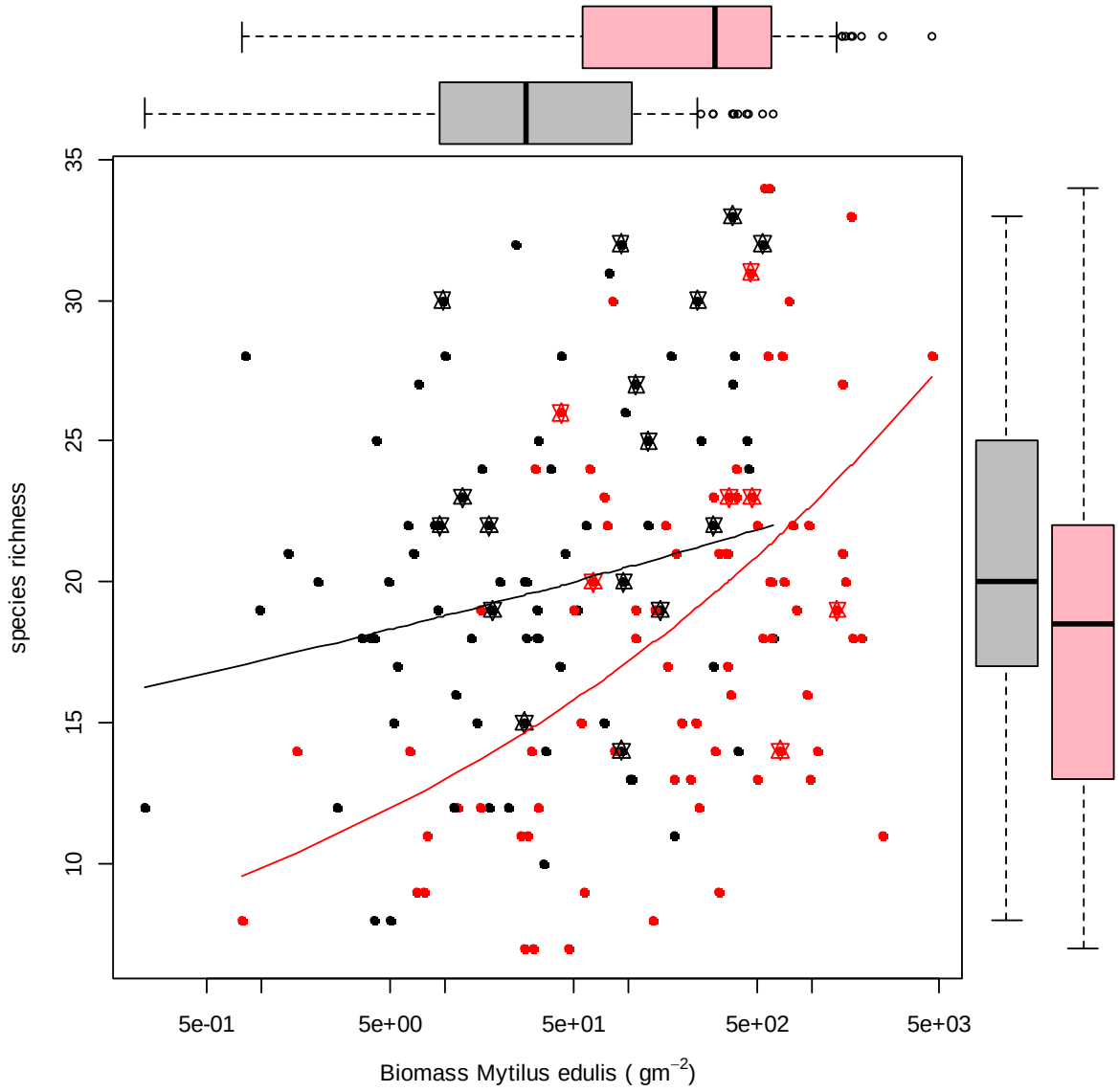

Figure 13. Relationship between species richness in a 0.06 core sample and biomass of Mytlius edulis in the same sample. Black dots are for cores outside mussel culture plots and red dots for cores inside mussel culture plots. Lines are fitted GLM model results for inside and outside mussel culture plot observations. Boxes in the margins show the distribution of the observations. Stations with *Crassostrea gigas* are indicated with stars.

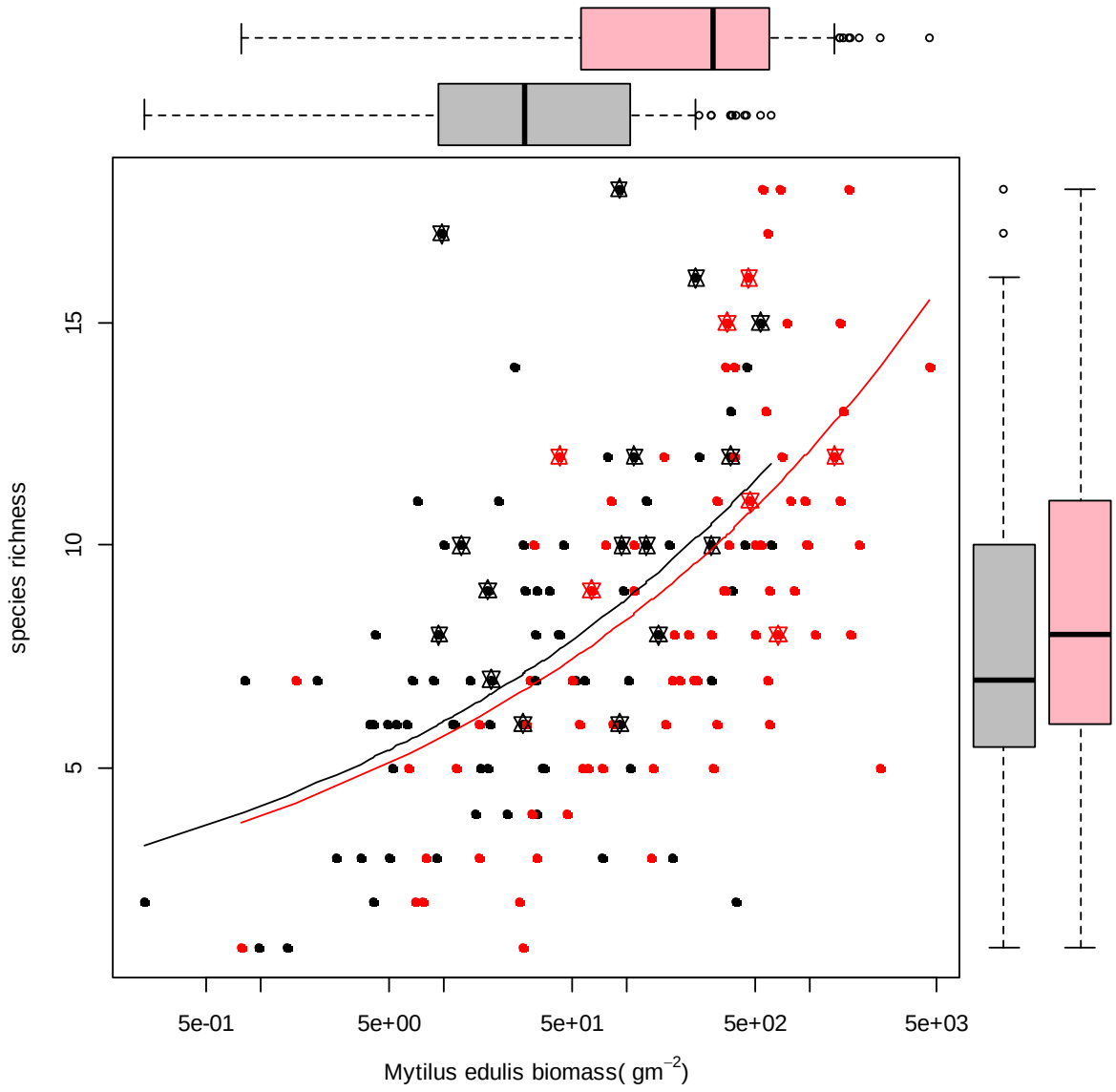

Figure 14. Same as Fig. 13 but for hard substrate species only.

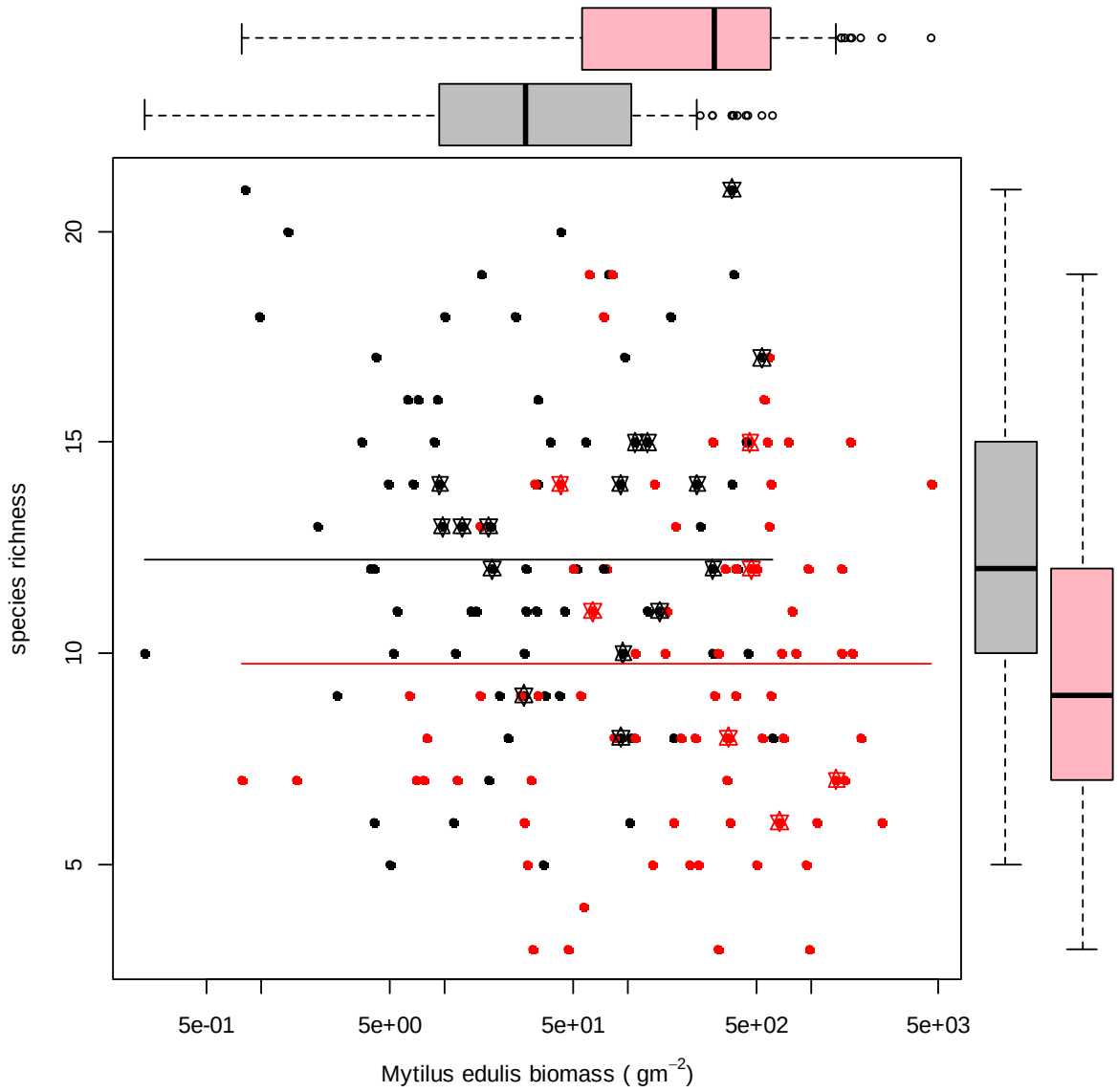

Figure 15. Same as Fig 13 but now only for soft sediment species.

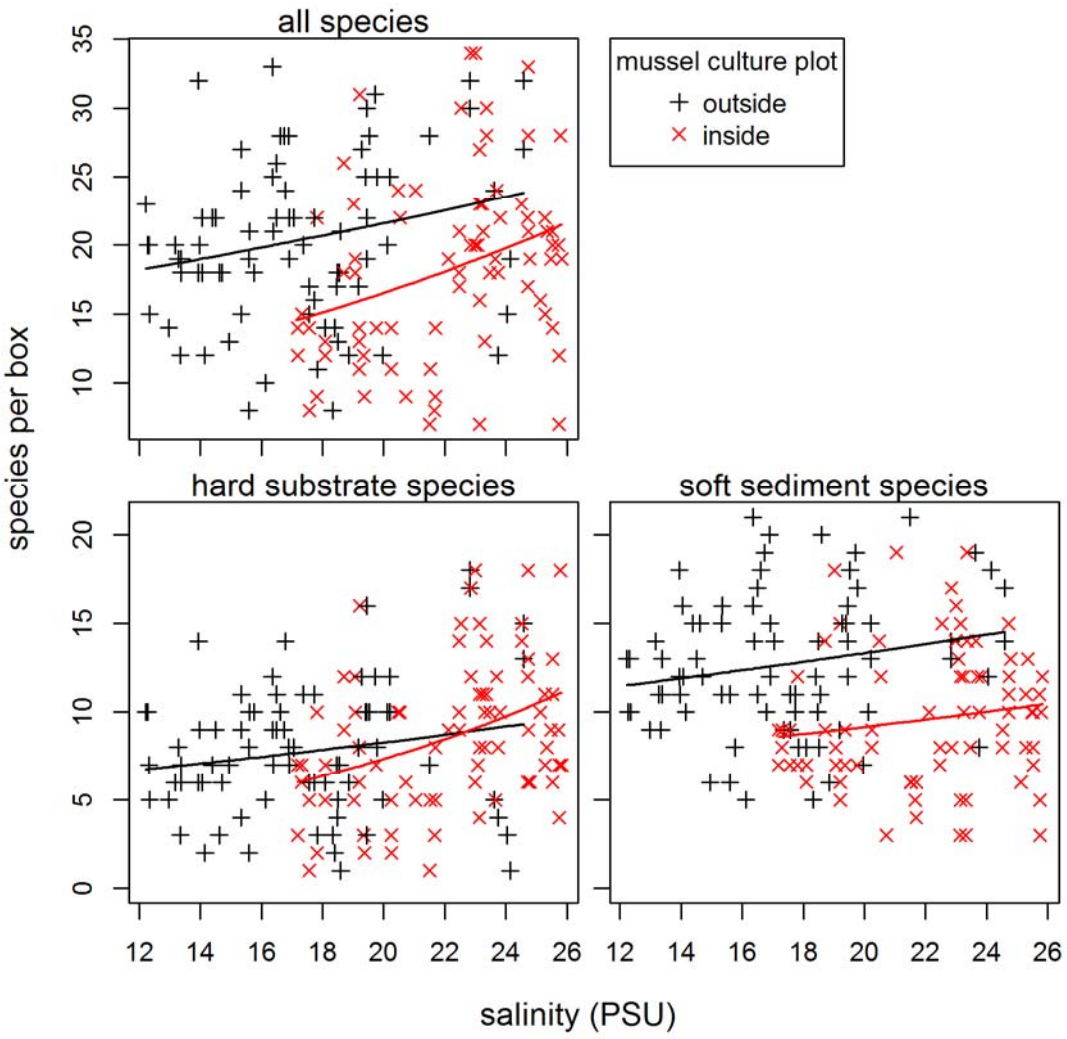

Figure 16 Species richness (species per sample outside and inside mussel culture plots, plotted against salinity. Lines are fitted values of a GLM with salinity, mussel culture plot and the interaction term salinity * mussel culture plot as explanatory variables. Top panel includes all species, in the two lower panels the species are divided in two groups. In the lower left panel hard substrate species are selected, in the lower right panel only soft sediment species are included.

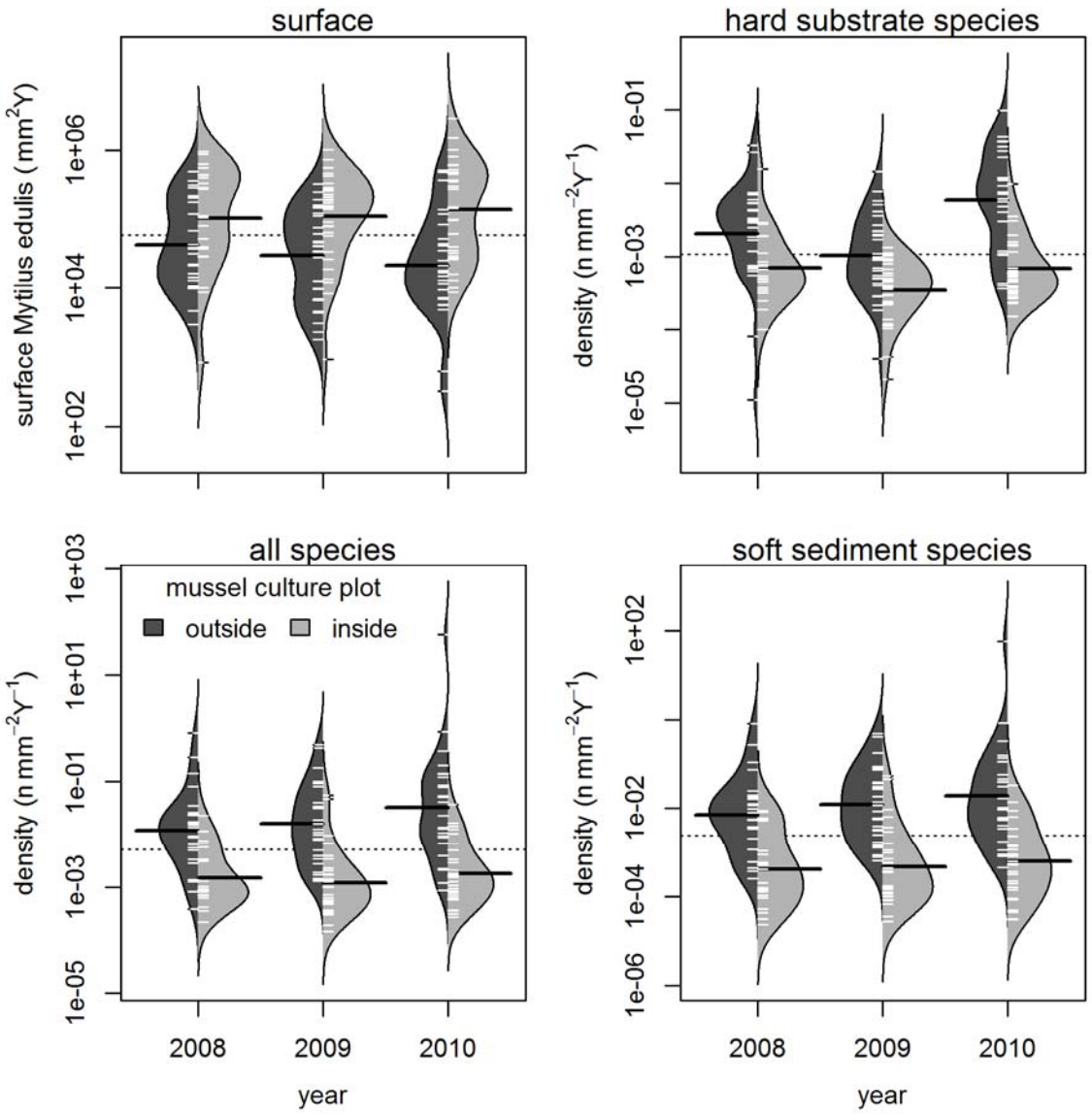

Figure 17 Surface area of the Mytlus edulis shells multiplied by age (top left), inside and outside mussel culture plots in the western Dutch Wadden Sea during three years. Macrozoöbenthos density (excluding mussels) per shell surface (lower left).Hard substrate macrozoöbenthos density (excluding mussels) per shell surface (top right). Soft sediment macrozoöbenthos density per shell surface (lower right).

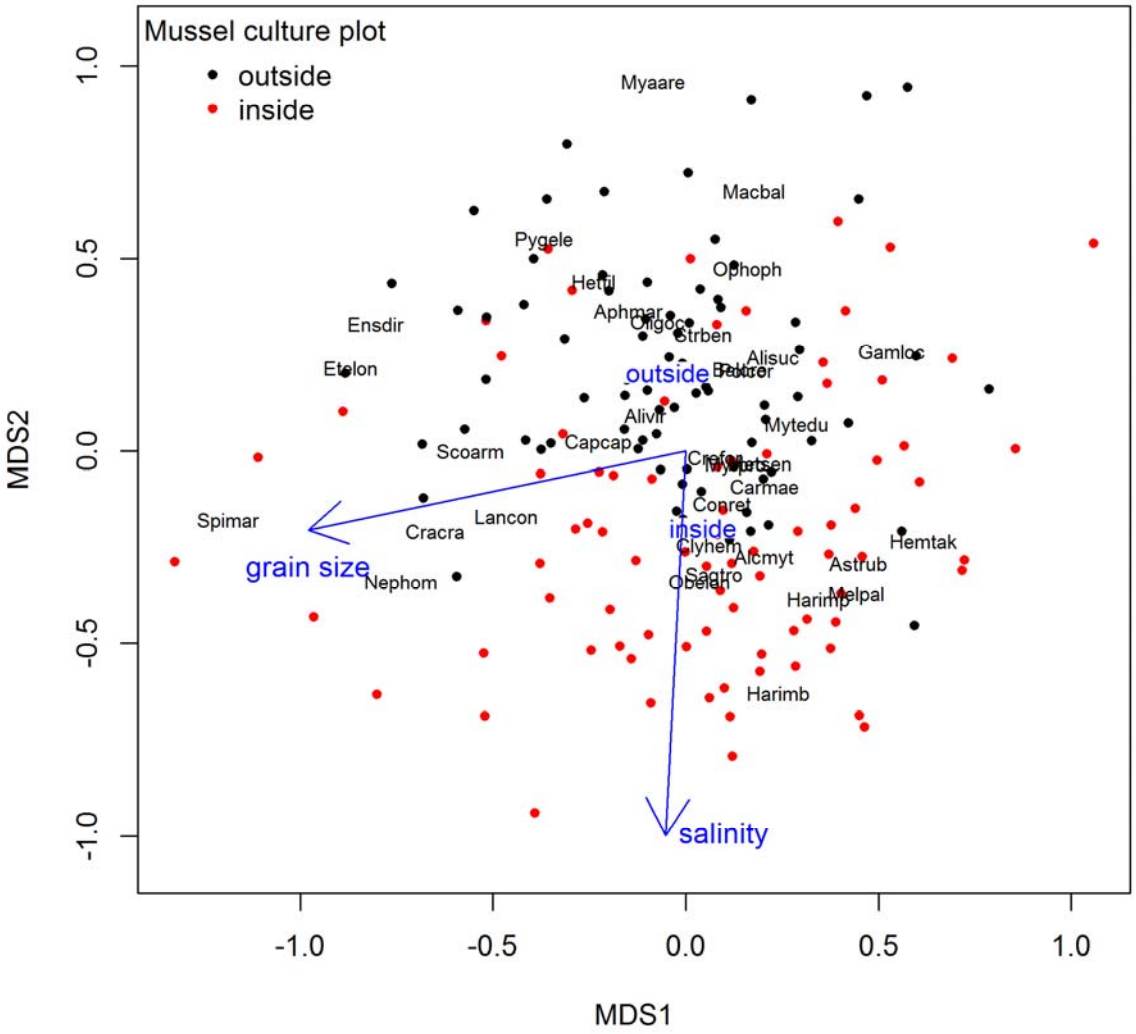

Figure 18. Nonlinear MDS plot of mussel bed community at stations outside and inside mussel culture plots in the subtidal western Dutch Wadden Sea. Species centroids of 36 species that contribute most to the difference between the bed types are indicated with abbreviated names. The centroids of the station values of natural beds and mussel culture plots are printed in blue. Salinity and median grain size gradients are shown by arrows. The nMDS is based on a Bray Curtis similarity analyses on Wisconsin double standardized square root transformed density data. Stress = 0.27.

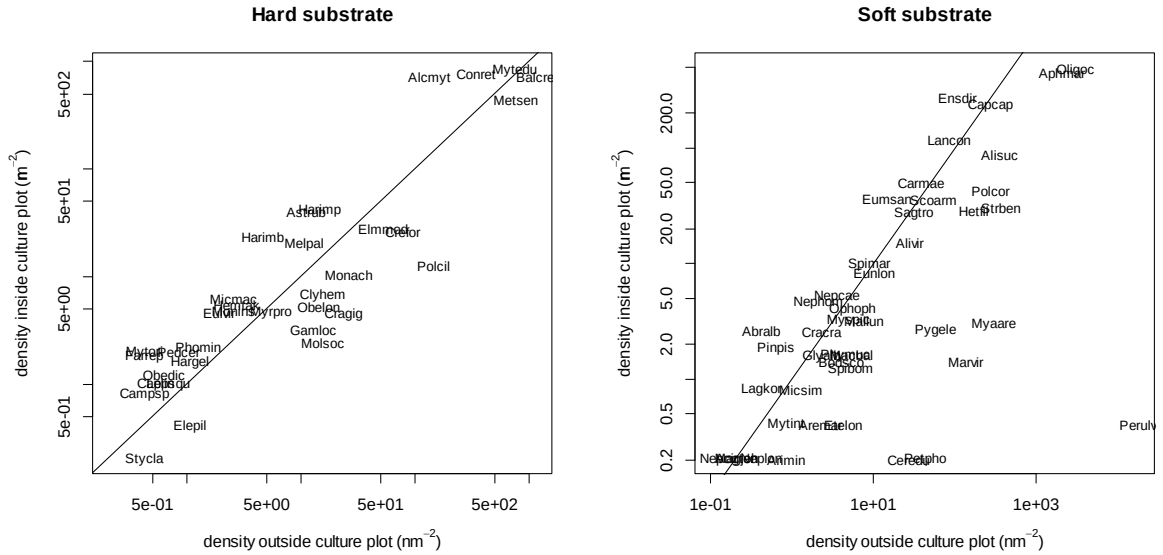

Figure 19 Plots of species densities inside mussel culture plots against species densities outside mussel culture plots. Left panel for hard substrate species, right panel soft substrate species. Lines are x=y.

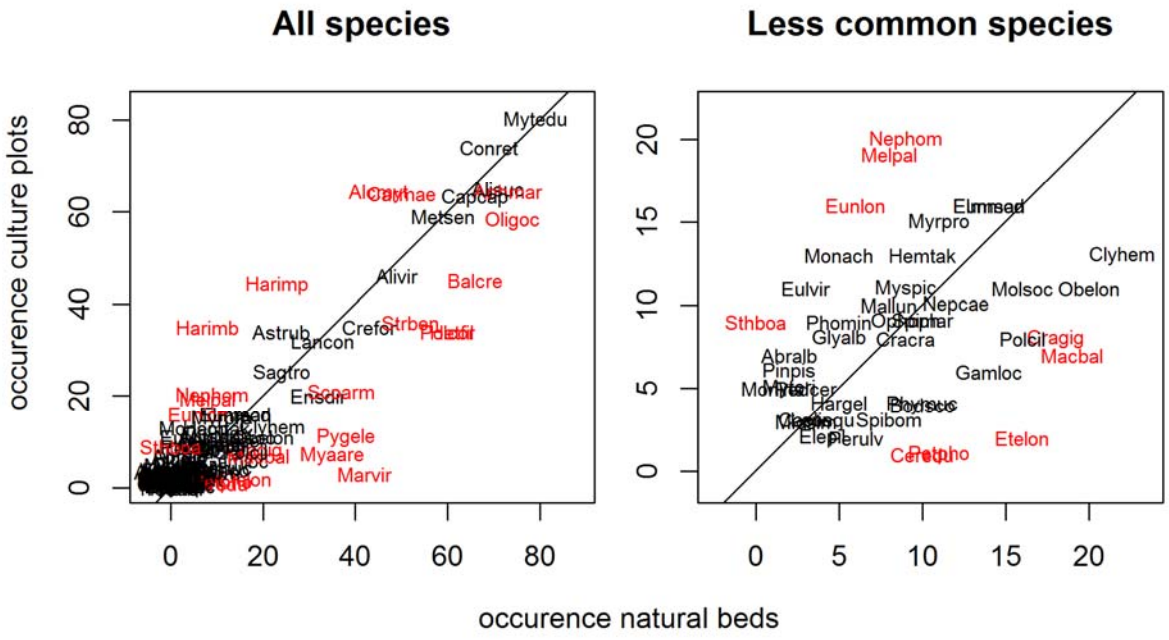

Figure 20. Number of occurrences of species inside culture plots, plotted against number of occurrences outside culture plots/on natural beds. Unit is number of stations. The right panel is a detail of the lower left part of the plot in the left panel. For clarity species with less than five occurrences combined over both bed types are left out of the right panel. Abbreviations of species with significantly differing occurrences in both bed types are in red. Lines are x=y.

Appendix 1

Macrozoobenthos species list

Macrozoobenthos species encountered in 179 box cores (0.06 m^2) from mussel occurrences inside and outside mussel culture plots in 2008, 2009 and 2010. Phylum and class are given in bold rows. Species is the scientific names using nomenclature updated with World Register of Marine Species http://www.marinespecies.org/. Feeding are feeding types. Substrate types are; soft sediment (soft), heterogeneous sediments (hetero.), hard substrate mobile species (hard m.), hard substrate sessile (hard s.) and undefined (undef.). Column benthos indicates whether the species is included as macrozoobenthos. Species are ascribed invasive based on Wolff (2005) and Buschbaum et al. (2012).

Code	Species	Feeding	Substrate	Benthos	Invasive
Annelida / Clitellata					
Oligoc	Oligochaeta	deposit	soft	yes	no
Annelida / Polychaeta					
Aremar	*Arenicola marina*	deposit	soft	yes	no
Arimin	*Aricidea minuta*	deposit	soft	yes	no
Capcap	*Capitella capitata*	deposit	soft	yes	no
Hetfil	*Heteromastus filiformis*	deposit	soft	yes	no
Scoarm	*Scoloplos armiger*	deposit	soft	yes	no
Trafor	*Travisia forbesii*	deposit	soft	yes	no
Alisuc	*Alitta succinea*	deposit	hetero.	yes	no
Alivir	*Alitta virens*	omnivore	soft	yes	yes
Etelon	*Eteone longa*	carnivore	soft	yes	no
Eteosp	*Eteone sp.*	carnivore	undef.	yes	no
Eulvir	*Eulalia viridis*	carnivore	hard m.	yes	no
Eumsan	*Eumida sanguinea*	carnivore	soft	yes	no
Eunlon	*Eunereis longissima*	omnivore	soft	yes	no
Glyalb	*Glycera alba*	carnivore	soft	yes	no
Harimb	*Harmothoe imbricata*	carnivore	hard m.	yes	no
Harimp	*Harmothoe impar*	carnivore	hard m.	yes	no
Heddiv	*Hediste diversicolor*	omnivore	soft	yes	no
Lepsqu	*Lepidonotus squamatus*	carnivore	hard m.	yes	no
Maldar	*Malmgreniella darbouxi*	carnivore	soft	yes	no
Micsim	*Microphthalmus similis*	deposit	soft	yes	yes
Myrpro	*Myrianida prolifera*	carnivore	hard m.	yes	no
Myspic	*Mysta picta*	carnivore	soft	yes	no
Nepcae	*Nephtys caeca*	carnivore	soft	yes	no
Nepcir	*Nephtys cirrosa*	carnivore	soft	yes	no
Nephom	*Nephtys hombergii*	carnivore	soft	yes	no
Neplon	*Nephtys longosetosa*	carnivore	soft	yes	no
Phomin	*Pholoe minuta*	carnivore	hard m.	yes	no
Phymac	*Phyllodoce maculata*	carinovore	soft	yes	no
Phymuc	*Phyllodoce mucosa*	carnivore	soft	yes	no
Prohal	*Procerastea halleziana*	carnivore	hard m.	yes	no

Code	Species	Feeding	Substrate	Benthos	Invasive
Sthboa	*Sthenelais boa*	omnivore	hard m.	yes	no
Aonoxy	*Aonides oxycephala*	deposit	soft	yes	no
Magjoh	*Magelona johnstoni*	deposit	soft	yes	no
Marvir	*Marenzelleria viridis*	deposit	soft	yes	yes
Polcil	*Polydora ciliata*	deposit	hard s.	yes	no
Polcor	*Polydora cornuta*	suspension	hetero.	yes	no
Pygele	*Pygospio elegans*	deposit	soft	yes	no
Spibom	*Spiophanes bombyx*	deposit	soft	yes	no
Spimar	*Spio martinensis*	deposit	soft	yes	no
Strben	*Streblospio benedicti*	deposit	soft	yes	yes
Aphmar	*Aphelochaeta marioni*	deposit	soft	yes	yes
Lagkor	*Lagis koreni*	deposit	soft	yes	no
Lancon	*Lanice conchilega*	suspension	soft	yes	no
Arthropoda / Malacostraca					
Ablobt	*Abludomelita obtusata*	deposit	hard m.	yes	no
Batsar	*Bathyporeia sarsi*	deposit	soft	yes	no
Caplin	*Caprella linearis*	omnivore	hard m.	yes	no
Chesun	*Cheirocratus sundevalli*	deposit	hard m.	yes	no
Gamloc	*Gammarus locusta*	deposit	hard m.	yes	no
Melpal	*Melita palmata*	deposit	hard m.	yes	no
Micmac	*Microprotopus maculatus*	omnivore	hard m.	yes	no
Monach	*Monocorophium acherusicum*	deposit	hard m.	yes	no
Monins	*Monocorophium insidiosum*	deposit	hard m.	yes	no
Phorei	*Photis reinhardi*	deposit	hard m.	yes	no
Uropos	*Urothoe poseidonis*	deposit	soft	yes	no
Bodsco	*Bodotria scorpioides*	deposit	soft	yes	no
Canpag	*Cancer pagurus*	omnivore	hard m.	yes	no
Carmae	*Carcinus maenas*	carnivore	soft	yes	no
Cracra	*Crangon crangon*	carnivore	soft	yes	no
Euacra	*Eualus cranchii*	omnivore	hard m.	yes	no
Hemtak	*Hemigrapsus takanoi*	carnivore	hard m.	yes	yes
Pagber	*Pagurus bernhardus*	carnivore	soft	yes	no
Palele	*Palaemon elegans*	omnivore	hard m.	yes	no
Pinpis	*Pinnotheres pisum*	parasite	undef.	yes	no
Pislon	*Pisidia longicornis*	omnivore	hard m.	yes	no
Messla	*Mesopodopsis slabberi*	omnivore	soft	no	no
Arthropoda / Maxillopoda					
Mytint	*Mytilicola intestinalis*	parasite	undef.	yes	yes
Mytori	*Mytilicola orientalis*	parasite	hard m.	yes	yes
Balsp	*Balanus sp.*	suspension	hard s.	yes	no
Elmmod	*Elminius modestus*	suspension	hard s.	yes	yes
Bryozoa / Gymnolaemata					
Conret	*Conopeum reticulum*	suspension	hard s.	yes	no
Elepil	*Electra pilosa*	suspension	hard s.	yes	no
Alcmyt	*Alcyonidioides mytili*	suspension	hard s.	yes	no

Code	Species	Feeding	Substrate	Benthos	Invasive
Farrep	*Farrella repens*	suspension	hard s.	yes	no
Chordata / Ascidiacea					
Molsoc	*Molgula socialis*	suspension	hard s.	yes	yes
Stycla	*Styela clava*	suspension	hard s.	yes	yes
Cnidaria / Anthozoa					
Metsen	*Metridium senile*	suspension	hard s.	yes	no
Sagtro	*Sagartia troglodytes*	carnivore	hetero.	yes	no
Cnidaria / Hydrozoa					
Hydech	*Hydractinia echinata*	suspension	hard s.	yes	no
Campsp	*Campanularia sp.*	suspension	hard s.	yes	no
Clyhem	*Clytia hemisphaerica*	suspension	hard s.	yes	no
Hargel	*Hartlaubella gelatinosa*	suspension	hard s.	yes	no
Obedic	*Obelia dichotoma*	suspension	hard s.	yes	no
Obelon	*Obelia longissima*	suspension	hard s.	yes	no
Echinodermata / Asteroidea					
Astrub	*Asterias rubens*	carnivore	hard m.	yes	no
Echinodermata / Echinoidea					
Psamil	*Psammechinus miliaris*	carnivore	hard m.	yes	no
Echinodermata / Ophiuroidea					
Ampsqu	*Amphipholis squamata*	suspension	hard m.	yes	no
Ophfra	*Ophiothrix fragilis*	suspension	hard m.	yes	no
Ophoph	*Ophiura ophiura*	carnivore	soft	yes	no
Entoprocta					
Pedcer	*Pedicellina cernua*	suspension	hard s.	yes	no
Mollusca / Bivalvia					
Ensdir	*Ensis directus*	suspension	soft	yes	yes
Myaare	*Mya arenaria*	suspension	soft	yes	yes
Mytedu	*Mytilus edulis*	suspension	hard s.	yes	no
Cragig	*Crassostrea gigas*	suspension	hard s.	yes	yes
Abralb	*Abra alba*	deposit	soft	yes	no
Angfab	*Angulus fabula*	deposit	soft	yes	no
Angten	*Angulus tenuis*	deposit	soft	yes	no
Ceredu	*Cerastoderma edule*	suspension	soft	yes	no
Kurbid	*Kurtiella bidentata*	suspension	soft	yes	no
Macbal	*Macoma balthica*	deposit	soft	yes	no
Macstu	*Mactra stultorum*	suspension	soft	yes	no
Petpho	*Petricolaria pholadiformis*	suspension	hetero.	yes	yes
Spisub	*Spisula subtruncata*	suspension	soft	yes	no
Vencor	*Venerupis corrugata*	suspension	hetero.	yes	no
Mollusca / Gastropoda					
Crefor	*Crepidula fornicata*	suspension	hard s.	yes	yes
Perulv	*Peringia ulvae*	deposit	soft	yes	no
Aeopap	*Aeolidia papillosa*	carnivore	hard m.	yes	no
Mollusca / Polyplacophora					
Lepcin	*Lepidochitona cinerea*	deposit	hard m.	yes	no

Code	Species	Feeding	Substrate	Benthos	Invasive
Nemertea					
Nemert	Nemertea	carnivore	undef.	yes	no

References

Buschbaum, C., D. Lackschewitz, and K. Reise. 2012. Nonnative macrobenthos in the Wadden Sea ecosystem. Ocean & Coastal Management 68:89-101.

Wolff, W. J. 2005. Non-indigenous marine and estuarine species in The Netherlands. Zoologische Mededelingen Leiden 79:1-116.

Appendix 2

Analysis of a subset of data with smaller differences in salinity

Salinity effects were not easily separated from the mussel culture plot effect. In this additional analysis a subset of the data is used where the salinity differences inside and outside mussel culture plots are less pronounced than in the entire data set.

In the Marsdiep tidal basin 22 stations were visited inside mussel culture plots. In the same area station coverage outside mussel culture plots was reasonable. There were 22 stations outside the mussel culture plots selected that were nearest to the stations inside the culture plots and at salinity higher than 15 PSU (Fig. 1). Environmental conditions were still not identical between stations inside and outside mussel culture plots (Fig. 2). The similarity in distribution of salinity values is much improved in the new selection of data compared to the entire data set. The range in height of the stations is strongly reduced in the new selection of stations. The difference in height between outside and inside mussel culture plots increased a little. Stations within culture plots are slightly deeper than stations outside mussel culture plots. Differences in sediment median grain size increased in the new selection. Sediment at stations inside mussel culture plots is considerably finer than outside mussel culture plots. The distribution of current velocity was quite equal in the entire data set. In the new selection current velocities are markedly higher outside than inside mussel culture plots.

At the new selected stations 79 species were found. Most species were found outside the mussel culture plots, 74 in total. Inside mussel culture plots 60 species were found. In table 1 the species are specified based on substrate. Hard substrate species numbers are very similar. The largest difference between outside and inside mussel culture plots is in species numbers of soft sediment species. There are 10 soft sediment species more in mussel occurrences outside mussel culture plots.

Outside mussel culture plots there were more species found per station than inside (GLM, $F_{1,42}$=6.33, p=0.016, Fig. 3). There were on average 24 species in the boxes from outside mussel culture plots and 20 species in boxes from within mussel culture plots.

Number of species in a box increases with the biomass of mussels in the box (Fig. 4, Table 2). At the same mussel biomass species number is significantly higher at sites outside mussel culture plots (Table 2). Slopes of the relationships inside and outside mussel culture plots do not differ significantly (Table 2).

This analysis on a selection of the dataset with similar salinities at stations inside and outside mussel culture plots shows that there are differences in species richness between mussel occurrences inside and outside that are consistent with the results of the analysis with the full data set. The consistent results are first that at the scale of a box there are more species in mussel occurrences outside than inside mussel culture plots. Second that the intercept of the relationship between species richness and mussel biomass is higher outside mussel culture plots than inside mussel culture plot.

Different from the previous analysis was that total number of species encountered at the mussel occurrences outside mussel culture plots was higher than the total number of species found on inside the mussel culture plots.

The reason for the species richness differences between mussel occurrences outside and inside mussel culture plots is that the number of soft sediment species is higher outside mussel culture plots. This lower soft sediment species richness may be an effect of the in general higher mussel densities on the mussel culture plots. These high mussel densities can increase deposition of fine sediment and organic material within the mussel bed. This may benefit a few small opportunistic deposit feeding species but decrease species richness (But see appendix 3 which shows that a more likely explanation is an enhanced soft sediment species richness at sites with natural mussel occurrences instead of a negative effect of mussels on soft sediment species inside mussel culture plots.)

Interesting is that the number of hard substrate species is similar inside and outside mussel culture plots. This suggests that there is not a strong effect of mussel handling by mussel culture on the hard substrate species living within the mussel matrix and attached to the mussels. However it must be stressed that this was not a controlled experiment. Also in this subset of data environmental conditions are different between sites outside and inside mussel culture plots. An experiment to test for mussel culture handling effects could be done by harvesting and relaying mussels at the same site with appropriate local controls.

Table A2.1. Number of macrozoobenthos species per substrate type at 22 stations outside and 22 stations inside mussel culture plots in the Marsdiep tidal basin in the western Dutch Wadden Sea.

	mussel culture plot	
substrate	**outside**	**inside**
not defined	3	1
soft sediment	37	27
heterogeneous sediment	4	3
hard substrate mobile	13	12
hard substrate sessile	17	17
totals	74	60

Table A2.2. Analysis of deviance table of a quasipoisson GLM testing the effect of mussel biomass (log_{10} transformed), factor mussel culture plot and the interaction between both terms on the number of species in a box.

Term	Df	Deviance	Res. Df	Res. Dev	F	Pr(>F)	
NULL	43	78.47					
Biom. Mytilus	1	14.85	42	63.62	10.65	0.002	**
Mussel culture plot (MCP)	1	8.29	41	55.34	5.94	0.019	*
Biom. Mytilus : MCP	1	0.43	40	54.91	0.31	0.583	

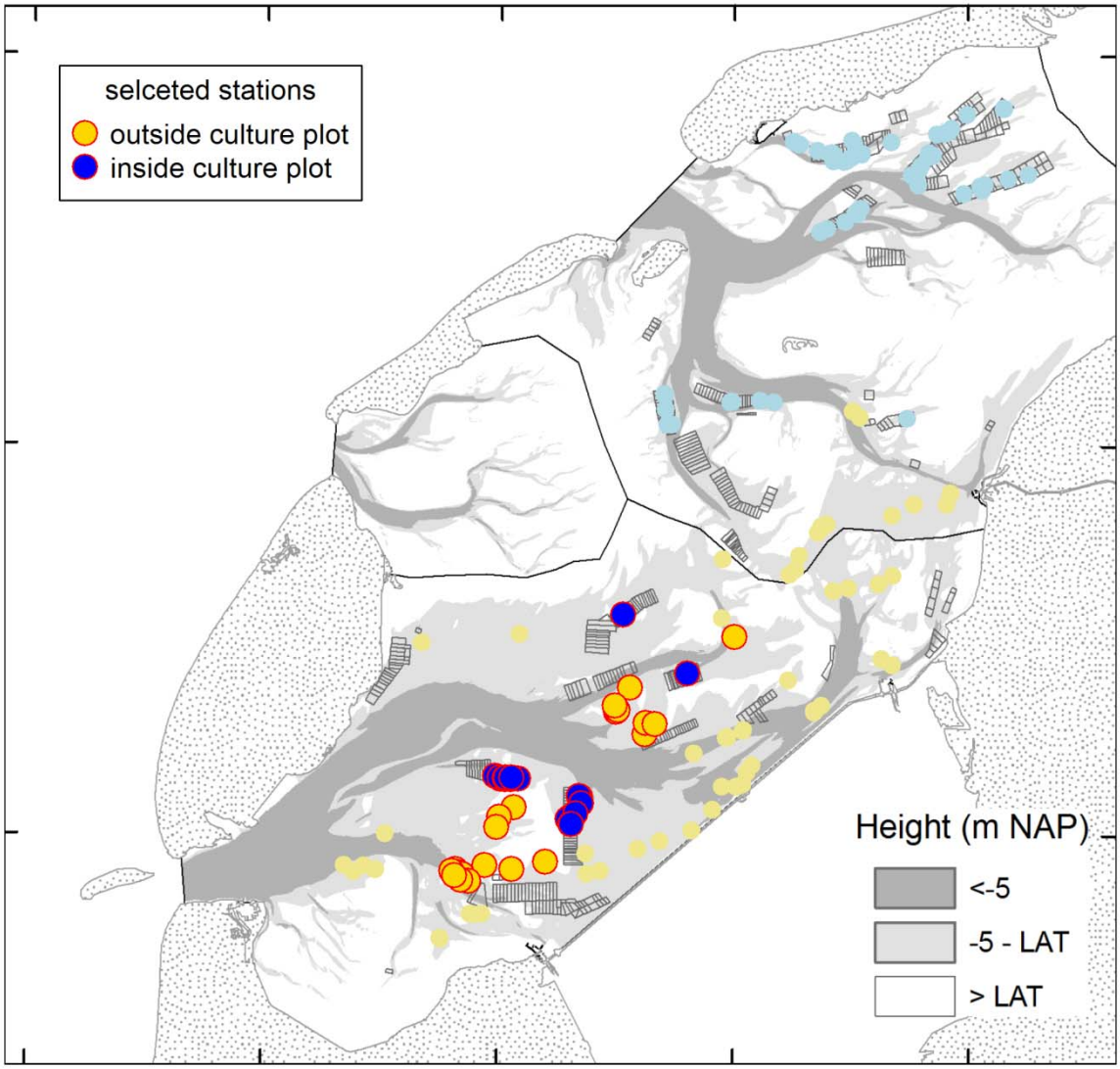

Figure A2.21 Map of the western Dutch Wadden Sea with mussel sampling stations inside (blue) and outside (beige) mussel culture plots. A subset of all available stations with equal number of stations inside and outside mussel culture plots were selected for analysis.

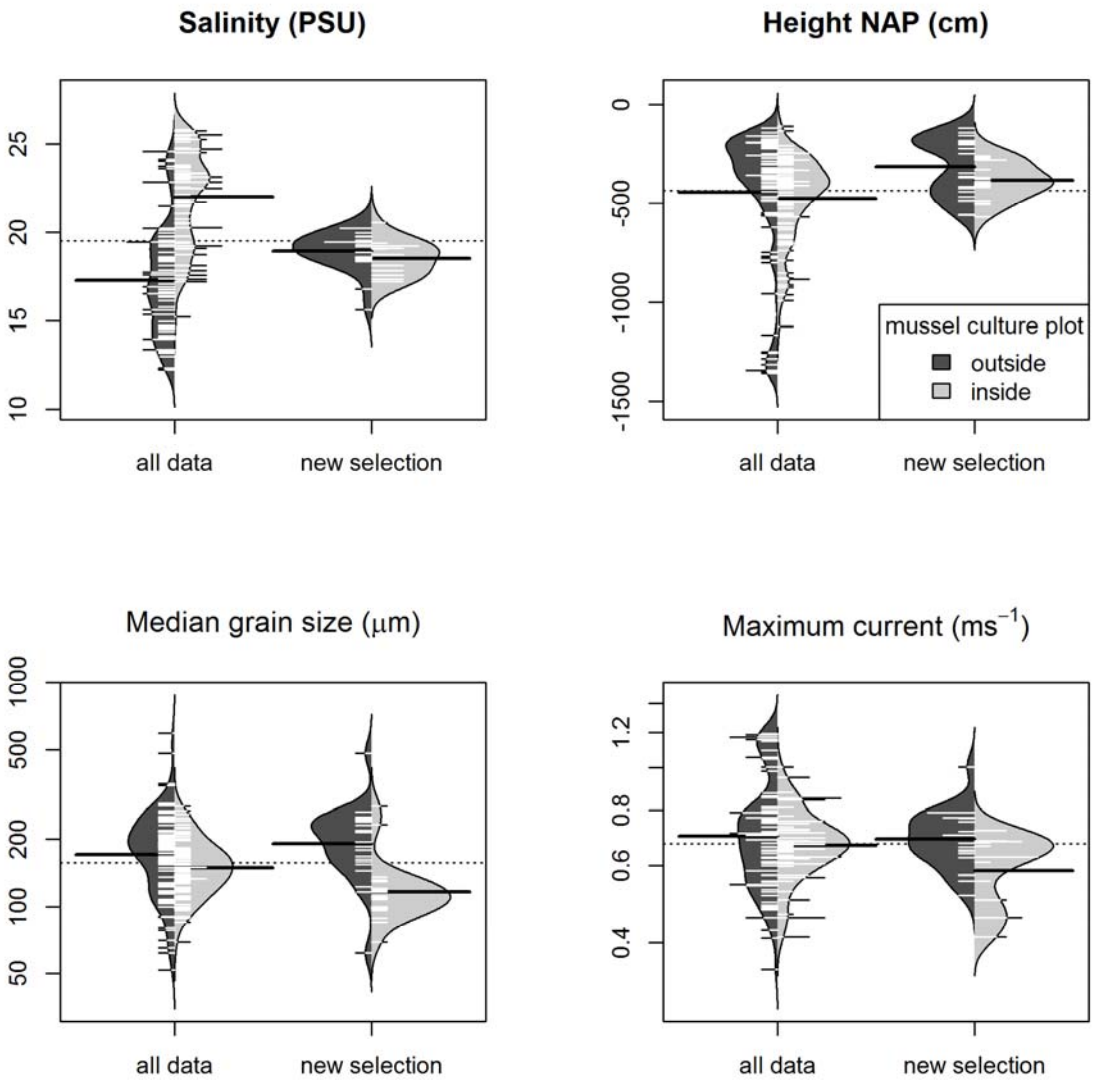

Figure A2.22 Comparison of environmental variables inside and outside mussel culture plots. All data refers to the entire data set of 179 stations in the western Dutch Wadden Sea, new selection to the 44 stations selected in the Marsdiep tidal basin.

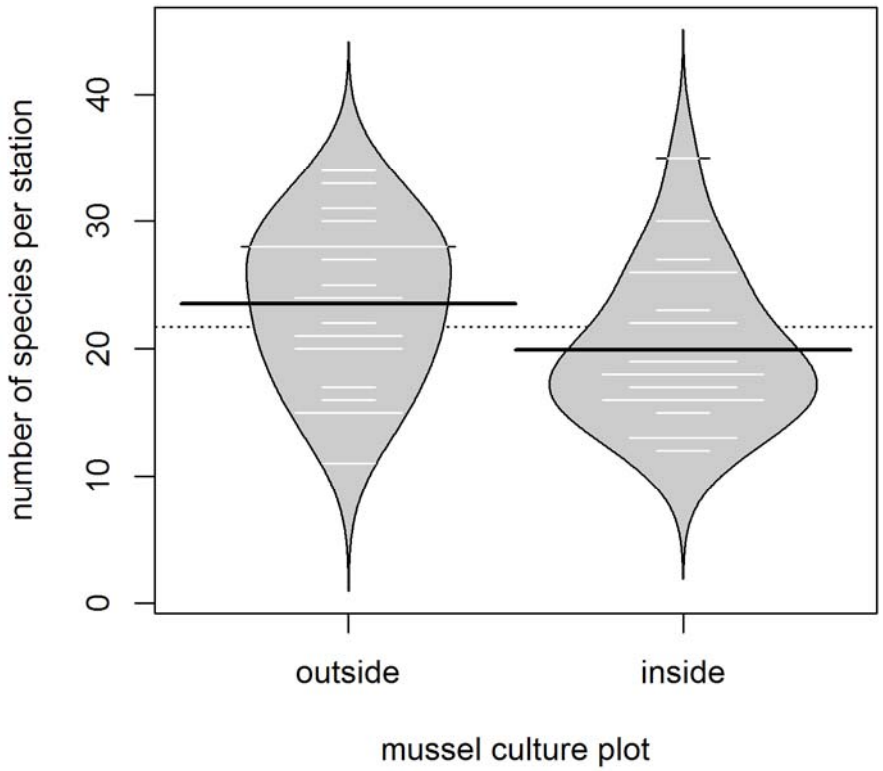

Figure A2.3 Number of species per box at stations outside and inside mussel culture plots. Stations were a selection of 22 outside and 22 inside mussel culture plots in the Marsdiep tidal basin.

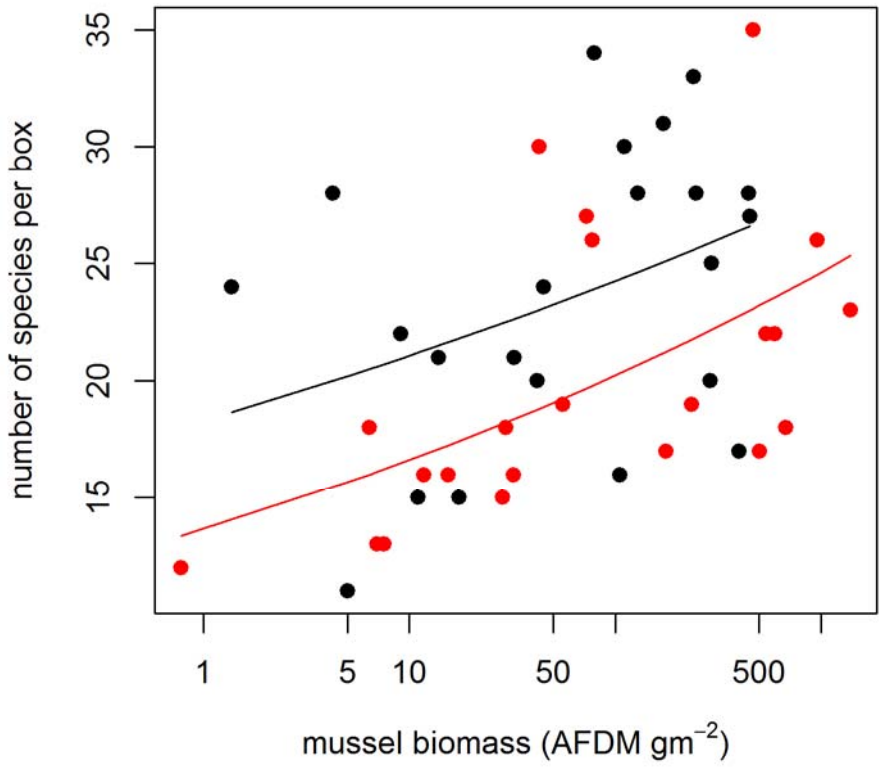

Figure A2.4. Number of macrozoobenthos species in a box core as function of the mussel biomass in the same core, for boxes outside mussel culture plots (black dots and line) and boxes inside mussel culture plots (red dots and line). Lines are result from a GLM model.

Appendix 3

Comparison of macrozoobenthos numerical densities and species richness at stations with and without mussels outside and inside mussel culture plots

Introduction

What are the differences in macrozoobenthos species richness in mussel communities outside and inside mussel culture plots? This was the main question treated in this report. Mussel communities are of major interest because they in general are more species rich and are inhabited by other species than in surrounding areas without mussels. The added species are predominantly sessile hard substrate species that use the mussel shell as attachment surface and mobile species that live within the mussel bed matrix. On the other hand infaunal soft sediment species richness may be reduced by the bed structure and organic enrichment by faeces and pseudofaeces produced by the mussels causing anoxic sediments.

Here we presented a comparison of numerical densities and number of macrozoobenthos species in box core samples from the subtidal western Dutch Wadden Sea. Boxes core samples were taken along transects (397 samples, see Dekker & Drent 2013) and at sites with mussels either outside culture plots or inside culture plots. Locations of the stations are mapped in Fig. A3.1. The distribution of boxes inside and outside mussel culture plots containing mussels or not is summarized in Table A3.1.

Results

Numerical densities of soft sediment species were similar outside and inside mussel culture plots when box cores did not contain mussels (Fig. A3.2). Mussels had a strong positive effect on the density of soft sediment species outside mussel culture plots. Inside mussel culture plots there was no significant effect of mussels on densities. Hard substrate species occurred in very low densities when no mussels were present in the boxes (Fig. A3.2). Especially box observations without mussels outside mussel culture plots are dominated by zeros. At station with mussels, hard substrate species were much more abundant. Hard substrate species abundance is highest inside mussel culture plots, both with and without mussels in the box core sample. Densities of all species combined are higher at stations with mussels than without mussels (Fig. A3.2). Densities of macrozoobenthos species are highest at stations with mussels outside mussel culture plots.

Species richness of soft sediment species did not differ inside and outside mussel culture plots in case the boxes did not contain mussels (Fig. A3.2). At box core stations with mussels soft sediment species richness was higher outside mussel culture plots than inside mussel culture plots. Soft sediment species richness inside mussel culture plots did not differ significantly between stations with and without mussels. Hard substrate species richness was higher when mussels were present (Fig. A3.2). In both cases with and without mussels hard substrate species richness was higher inside mussel culture plots. Total species richness without counting mussels was clearly elevated when

mussels were present in the box core (Fig. A3.2). The difference in total species number between stations with and without mussels was largest for box cores outside mussel culture plots.

Within stations where mussels occurred it was tested if macrozoobenthos densities and species richness were quantitatively related to mussel biomass. Soft sediment species densities were slightly positively related to mussel biomass and were clearly higher outside mussel culture plots (Fig. A3.3). Hard substrate species densities were positively related to mussel biomass. Increase of density with mussel biomass was stronger inside mussel culture plots (Fig. A3.3). Summed density of all macrozoobenthos species was overall not significantly related with mussel biomass (Fig. A3.3), however there was a significant interaction term indicating that the response of the macrozoobenthos density to mussel biomass differed inside and outside mussel culture plots. There was no response outside mussel culture plots and a positive response inside mussel culture plots.

Species richness of soft substrate macrozoobenthos was unrelated to mussel biomass (Fig. A3.3). Soft sediment species richness at stations with mussels was higher outside than inside mussel culture plots. Hard substrate species richness is strongly positively related to mussel biomass (Fig. A3.3). The relationships do not differ outside and inside mussel culture plots. Overall macrozoobenthos species richness at stations with mussels is highest outside mussel culture plots (Fig. A3.3). It is positively related with mussel biomass (Fig. A3.3). The slope of this relationship is highest inside mussel culture plots.

In figures A3.4, A3.5 and A3.6 densities of soft sediment species hard substrate species and densities of the combined macrozoobenthos species are plotted against salinity, depth, median grain size and maximum current speeds. Soft sediment species are consistently more abundant outside than inside mussel culture plots along all four environmental gradients (Fig. A3.4). Also inside mussel culture plots soft sediment species abundance is elevated relative to the stations without mussels, however judging from the statistical results in Fig. A3.2 this is not significantly higher. Hard substrate species abundance tends to be positively related to all four environmental variables except salinity (Fig. A3. 5). Hard substrate species densities are much higher at stations with mussels than without. Compared to that the differences inside and outside mussel culture plots are negligible. Total species density is highest at stations with mussels outside mussel culture plots along all four gradients (Fig. A3.6). Densities at stations with mussels inside mussel culture plots have intermediate densities. Lowest overall macrozoobenthos densities are found at stations without mussels. Environmental do not have strong effects on this pattern.

Patterns of species richness of soft sediment species along environmental gradients differed depending on the presence of mussels at the stations and whether the stations were inside or outside mussel culture plots (Fig. A3.7). Species richness at stations with mussels outside mussel culture plots increased along all four gradients considered. Inside mussel culture plots this was only convincingly so along salinity and grain size gradients. Interesting was that species numbers in boxes without mussels declined along depth and maximum current velocity gradients while the trends were opposite in boxes with mussels from outside mussel culture plots (Fig. A3.7). Hard substrate species richness at stations without mussels was virtually zero and unrelated to the environmental gradients (Fig. A3.8). Hard substrate species richness at stations with mussels as well inside as outside mussel culture plots was positively related to all environmental variables. Levels of hard substrate species richness were similar inside and outside mussel culture plots. Total

macrozoobenthos species richness at stations with mussels was positively related with all four environmental gradients considered (Fig. A3.9). Total macrozoobenthos species richness at stations without mussels was much lower and not strongly related with salinity and median grain size. It was negatively related with depth and maximum current speed. These trends with depth and current speed were positive at sites with mussels leading to strongly diverging differences in species richness between stations with and without mussels with increasing depth and current speed.

Discussion and conclusions

With this larger data set that also included stations without mussels it was shown that densities and species richness of macrozoobenthos at the level of a box core are elevated at stations that contain mussels relative to stations without mussels. Species richness was slightly higher at stations outside mussel culture plots than inside mussel culture plots. A more stinking difference between mussel stations outside and inside mussel culture plots was the contribution of soft sediment species to the total species richness per box core. Outside mussel culture plots at sites with naturally settled mussels the soft sediment species densities and richness are higher than at sites without mussels. This is not the case at sites with mussels inside mussel culture plots. Increased species richness inside mussel culture plots is an effect of increased hard substrate species richness. This hard substrate species richness is strongly and positively related to mussel biomass, independent of location relative to mussel culture plots. Because mussel biomass is higher in boxes inside than outside mussel culture plots hard substrate species richness is higher inside mussel culture plots. This higher hard substrate species richness inside mussel culture plots largely compensates for the differences in soft sediment species richness resulting in only small differences in total species richness inside and outside mussel culture plots.

Interesting is that at the scales studied and analysed there is no evidence for a reduced soft sediment species community within sites where mussels occur. The contrary seems to be true at sites where mussels occur naturally. This may be the result of a settlement preference of mussels at sites that are relatively species rich. Or in other words these preferred settlement sites are not species specific. An alternative explanation is that the presence of mussels creates a soft sediment habitat that is favourable for additional species compared to the surrounding soft sediment without mussels. This possible soft species promoting effect of mussels does not seem to be transferred along with the seed mussels to the culture plots because here soft sediment species densities and richness are not elevated compared to mussel culture plots without mussels. The reason for this could be that inhibiting effects of the higher mussel densities inside mussel culture plots are counterbalancing the possible richness enhancing effects observed at the natural occurring mussels outside the mussel culture plots. However within the range of mussel biomass on the mussel culture plots there was no evidence of declining densities or species richness with increasing mussel biomass (Fig. A3.3).

Habitat modification by mussels besides the contribution of hard substrate shell material clearly increasing hard substrate species richness inside and outside mussel culture plots, could be the amelioration of stressful environmental conditions. Example of this effect might be the increasing effect of mussels on soft sediment species richness along the current velocity gradient. Mussels may stabilize the seabed and improve conditions for soft sediment species. However proper experiments will be more definitive in providing evidence for these habitat ameliorating effects of mussels.

Major conclusions of the comparison between the densities and species richness of mussels communities outside and inside mussel culture plots, or in other words between naturally settled mussels and trans located mussels, first are that hard substrate species richness is similar when correcting for mussel biomass differences. Second that in contrast to hard substrate species soft sediment species density and richness differ outside and inside mussel culture plots. Soft sediment species were more abundant and species rich In box core samples from outside mussel culture plots, containing mussels, than at statins without mussels. This effect was not observed at sites with mussels inside mussel culture plots.

Reference

Dekker R. & Drent J. (2013) The macrozoobenthos in the subtidal of the western Dutch Wadden Sea in 2008 and a comparison with 1981-1982. NIOZ-Report 2013 2013-5

Table A3.3 Distribution of box cores depending on position relative to mussel culture plots either inside or outside and presence of mussels in the box.

	mussels in box	
plot	no	yes
outside	328	99
inside	57	84

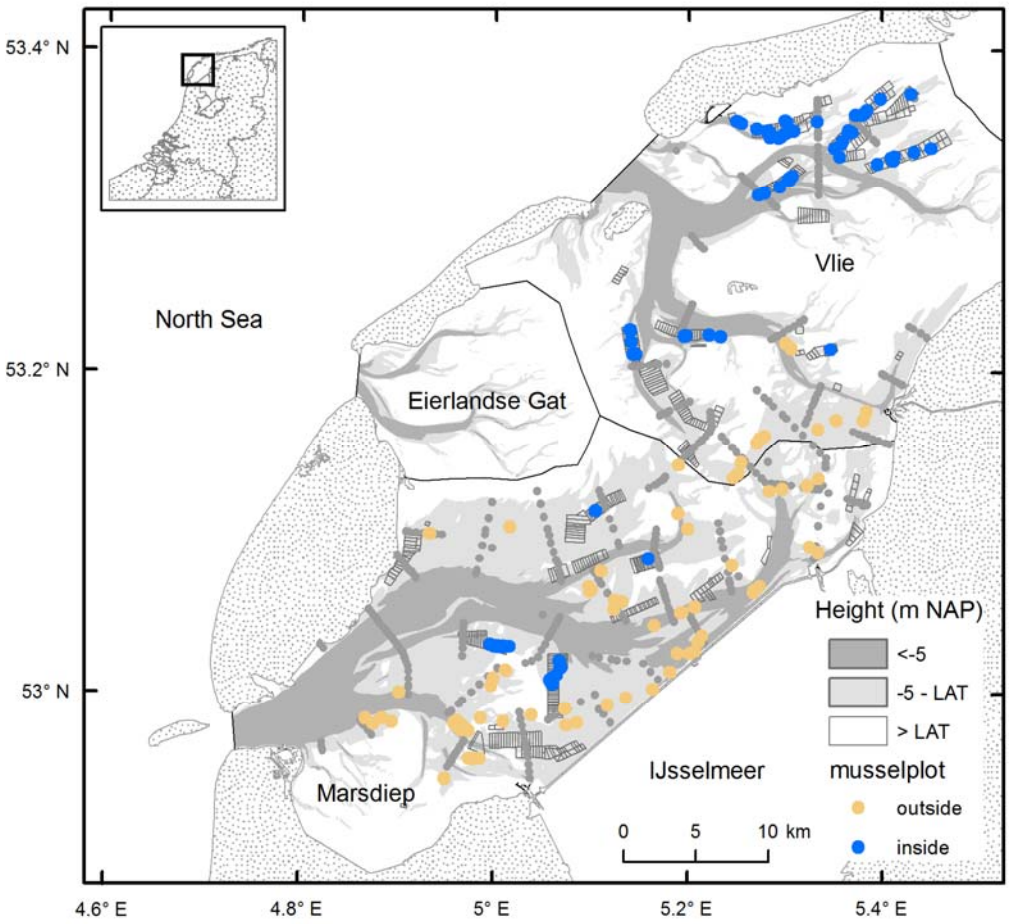

Figure A3.23. Map of the western Dutch Wadden Sea with contours of mussel culture plots, sampling stations specifically aiming for on mussels inside and outside mussel culture plots and sampling stations arranged along transects from a survey in 2008.

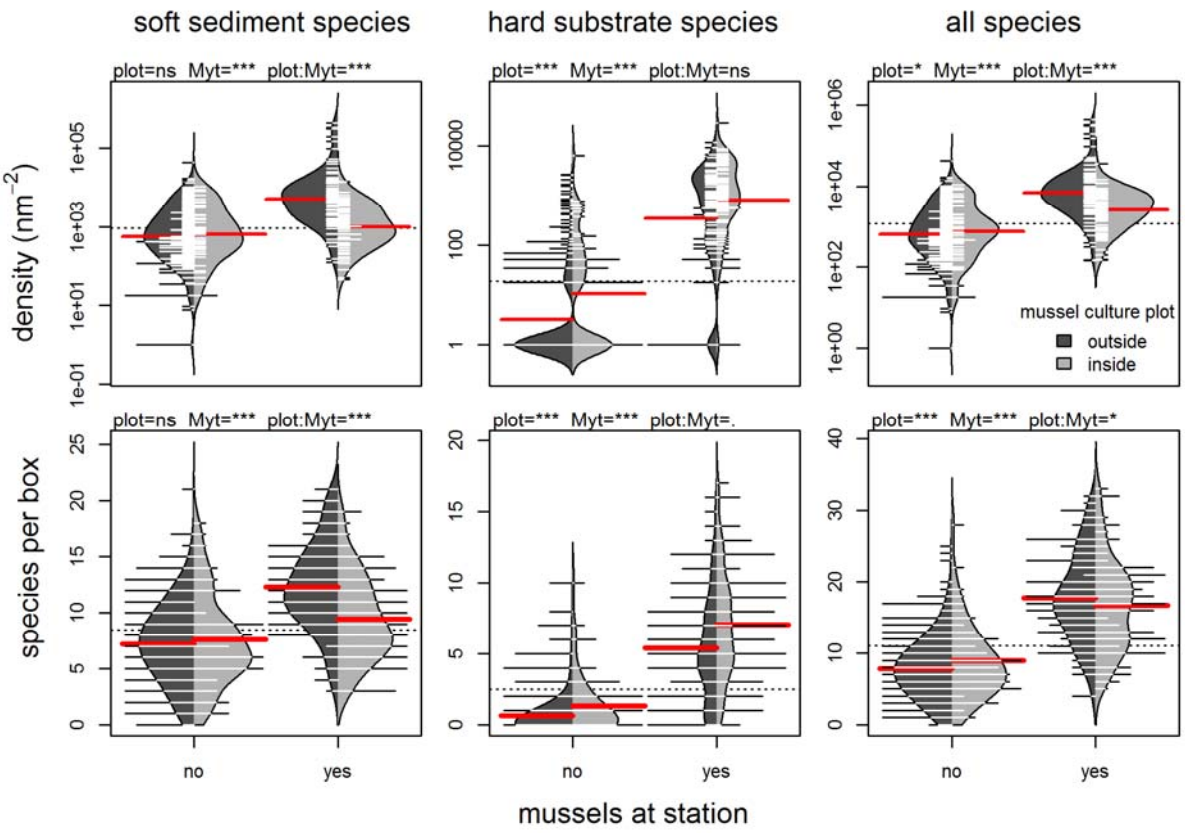

Figure A3.24. Comparison of the summed densities (top row) and species numbers (bottom row) of soft sediment species, hard substrate species and these two macrozoobenthos groups combined as all species, depending on mussels in the sample and location of the sample either outside or inside mussel culture plots. Statistical test results of plot, mussel (Myt) and interactive effects (plot:Myt) are summarized above the graphs. Significance codes are *, **, *, . and ns for p<0.001, <0.01, <0.05, < 0.1 and ≥0.1 respectively.**

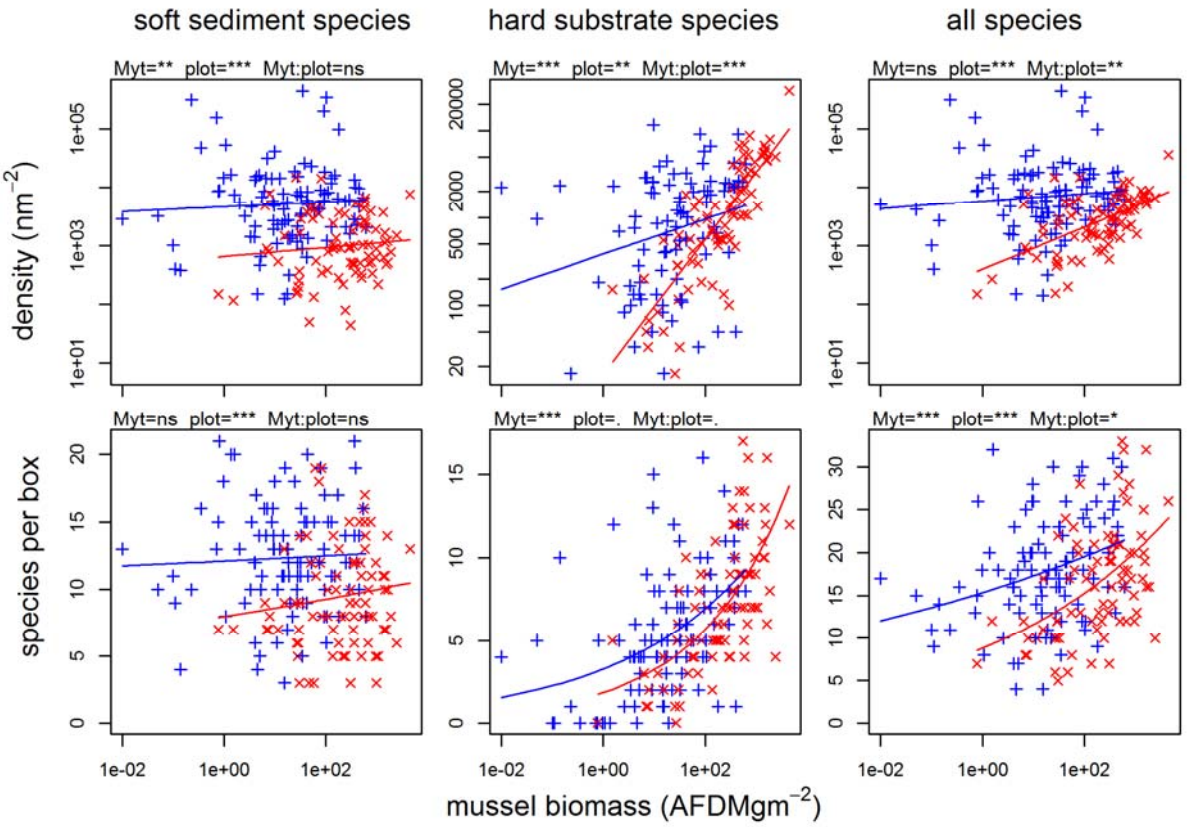

Figure A3.25. Relationships between numerical densities of macrozoobenthos and mussel biomass in box core samples with mussels (top row), and macrozoobenthos species richness per box and mussel biomass in the same box (bottom row). Macrozzobenthos is divided in soft sediment species (left column) and hard substrate species (middle column) and total macrozoobenthos (right column). Relationships are plotted for boxes outside and inside mussel culture plots. Mussels are not included in densities and species richness values. Significance of mussel biomass (Myt), position relative to mussel culture plot (plot) and the interaction between these two terms are indicated above each panel. Significance codes are explained in Figure A3.2.

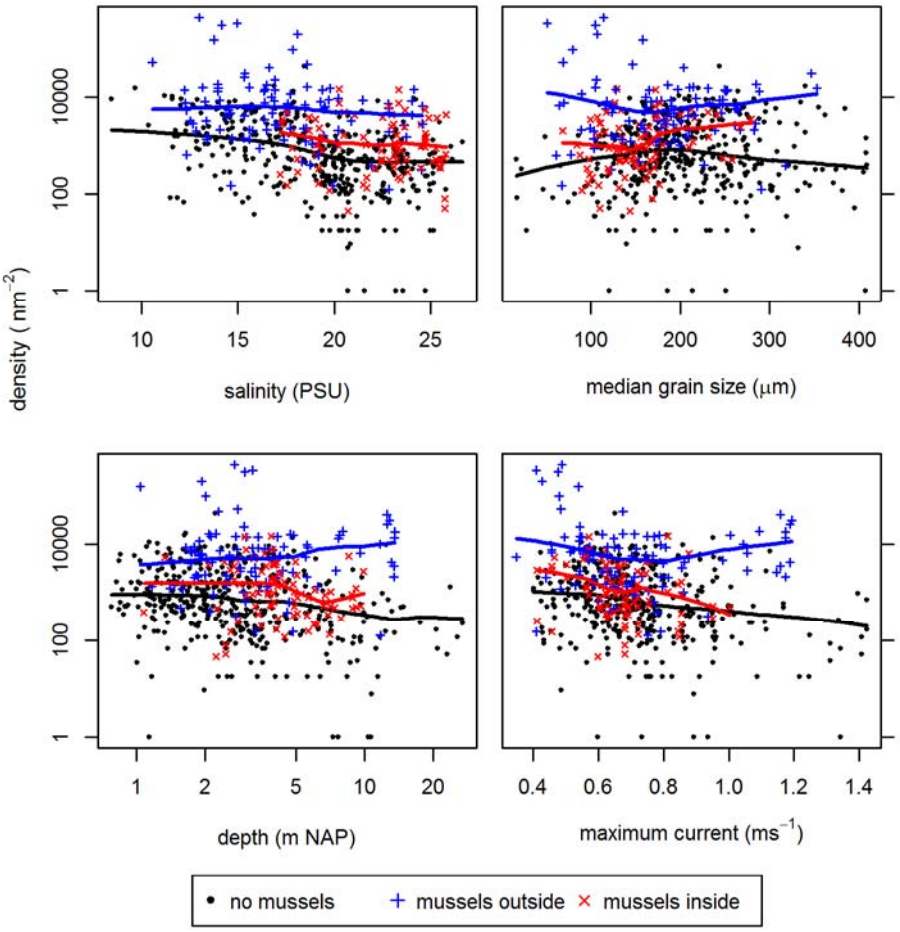

Figure A3.26. Summed numerical densities of soft sediment macrozoobenthos species at box core stations along four environmental gradients in the subtidal western Dutch Wadden Sea. Box core stations are categorized based on the presence of mussels. Box cores with mussels are further separated in boxes from outside and inside mussel culture plots. Lines are lowess smoothers.

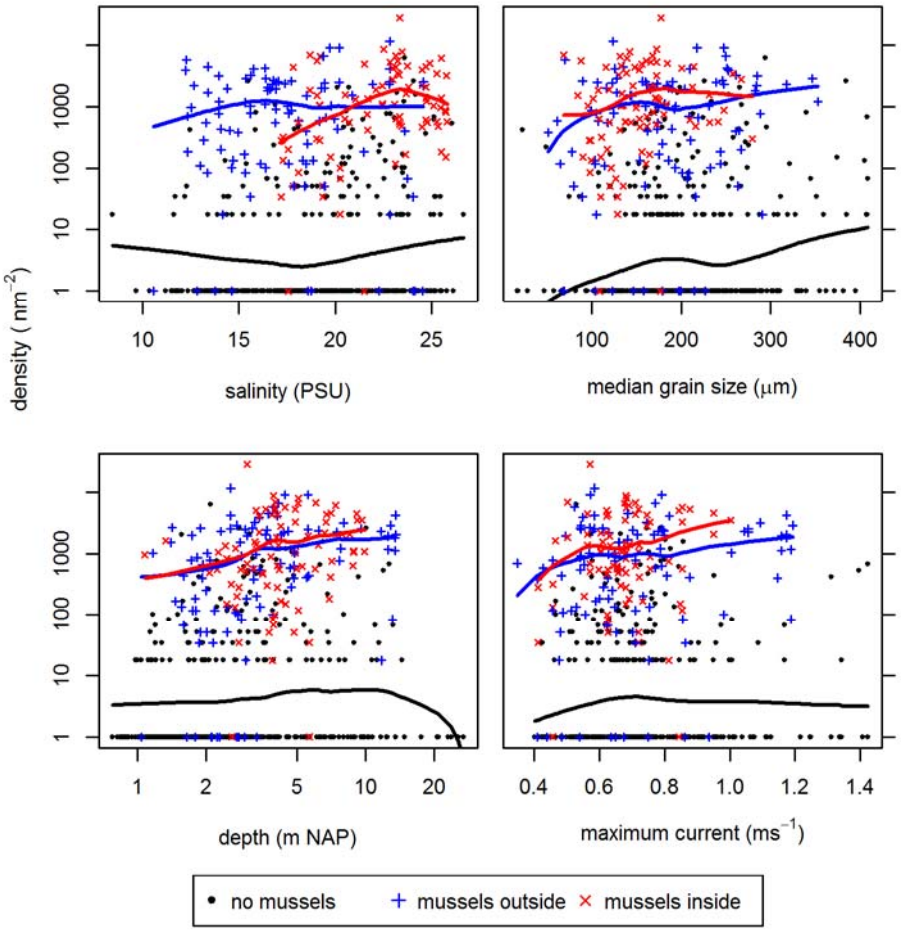

Figure A3.27. Summed numerical densities of all hard substrate macrozoobenthos species per box core station along four environmental gradients in the subtidal western Dutch Wadden Sea. Box core stations are categorized based on the presence of mussels. Box cores with mussels are further separated in boxes from outside and inside mussel culture plots. Lines are lowess smoothers.

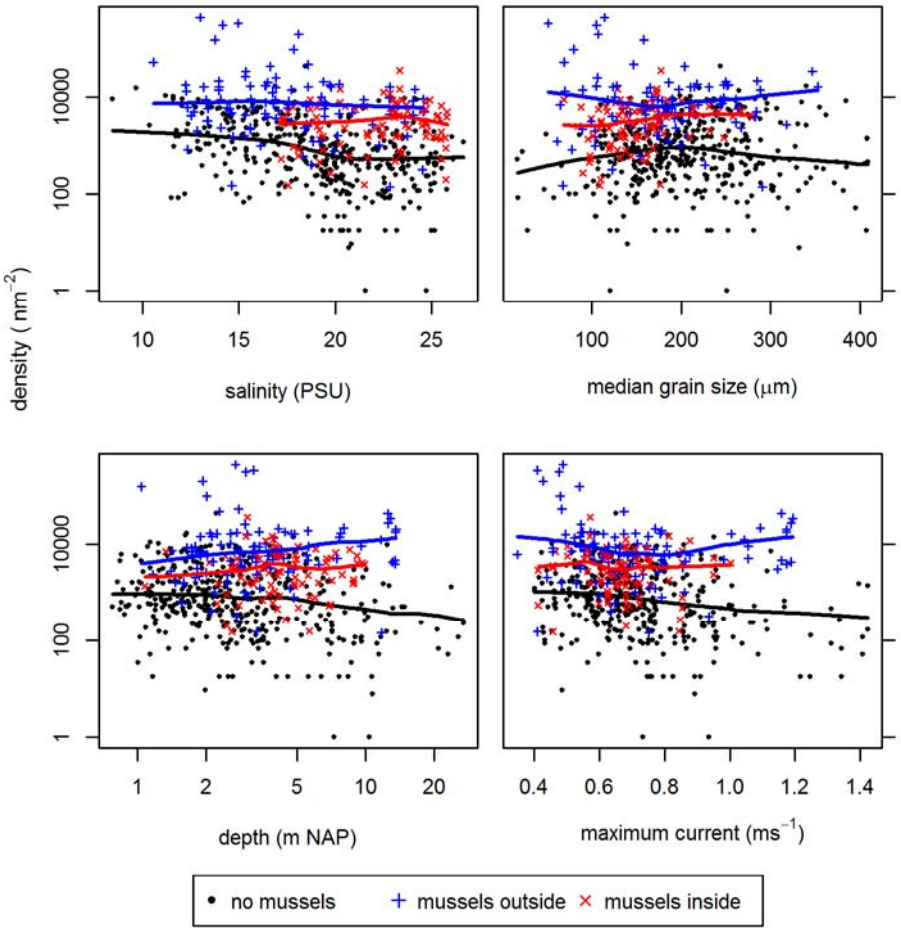

Figure A3.28. Numerical densities of the total macrozoobenthos but excluding mussels along four environmental gradients in the western Dutch Wadden Sea. Box core stations are categorized based on the presence of mussels. Box cores with mussels are further separated in boxes from outside and inside mussel culture plots. Lines are lowess smoothers.

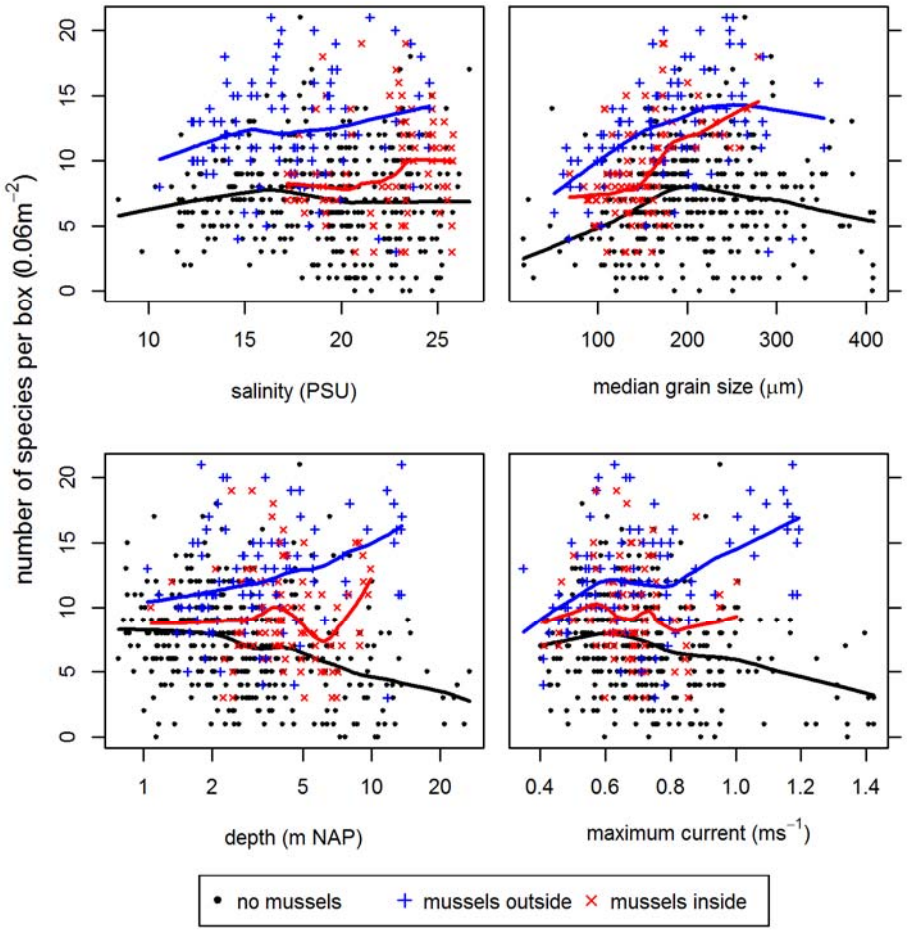

Figure A3.29. Soft sediment macrozoobenthos species numbers per box along four environmental gradients in the subtidal western Dutch Wadden Sea. Box core stations are categorized based on the presence of mussels. Box cores with mussels are further separated in boxes from outside and inside mussel culture plots. Lines are lowess smoothers.

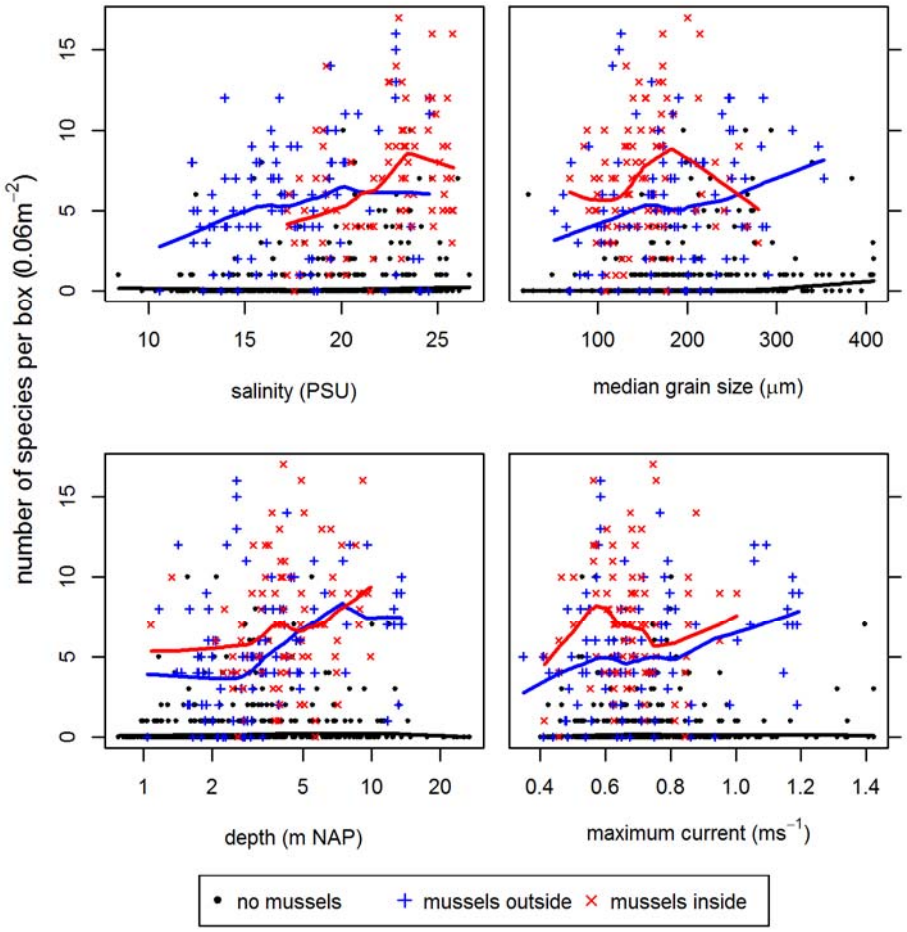

Figure A3.30. Hard substrate species richness at the level of a box core along four environmental gradients in subtidal of the western Dutch Wadden Sea. Box core stations are categorized based on the presence of mussels. Box cores with mussels are separated in boxes from outside and inside mussel culture plots. Lines are lowess smoothers.

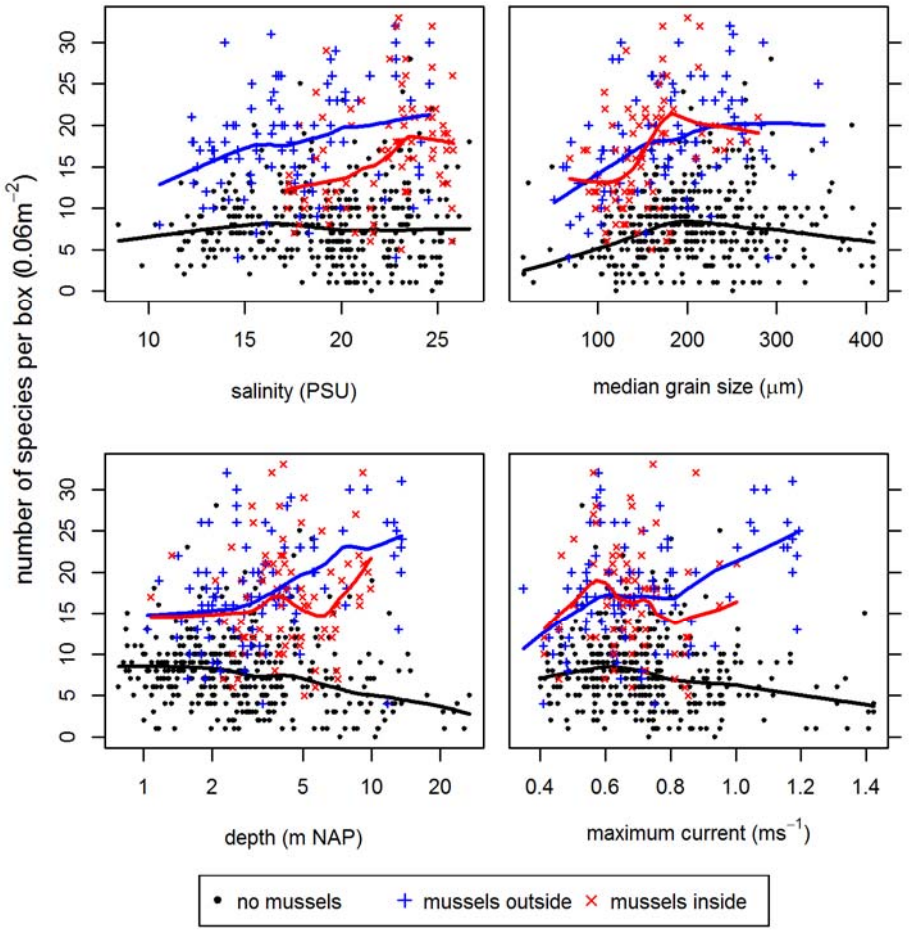

Figure A3.31. Macrozoobenthos species richness at the level of a box core along four environmental gradients in subtidal of the western Dutch Wadden Sea. Box core stations are categorized based on the presence of mussels. Box cores with mussels are separated in boxes from outside and inside mussel culture plots. Lines are lowess smoothers.

Appendix 4

Comparison between mussel culture plots in the Marsdiep and Vlie tidal basins

Mussel culture plots and sites where mussels settle naturally are at different places (Fig 2 main report). Mussel culture plots are closer to the tidal inlets than the natural mussel settling grounds. An important environmental difference between these areas is salinity. A comparison between mussels and the macrozoobenthos community inside and outside mussel culture plots is not only comparing between different mussel bed types but also comparing between different salinities. To get an idea of the effect of the salinity gradient between the tidal inlets and the sluices in the Afsluitdijk on the mussels and associated community two locations in the gradient are compared, namely the mussel culture plots from the Vlie tidal basin that are in the most saline area of the western Wadden Sea and the mussel culture plots in the Marsdiep tidal area that are in a more brakish area influenced by the fresh water discharge from the IJsselmeer. This comparison is done by redoing the graphs from the report but now not comparing between ourside and inside mussel culture plots but comparing between mussel culture plots in both basins, representing different parts in the salinity gradient.

Fig. A4.2 shows that indeed the average salinity is much higher in the Vlie tidal basin than in the marsdiep tidal basin. The other variables do not differ that much between basins and are comparable with the small differences in environmental differences between outside and inside culture plots.

Total biomass of the macrozoobenthos is higher in the Vlie basin than in the Marsdipe basin (Fig. A4.3). This is similar to the pattern when comparing between outside and inside mussel culture plots. However when leaving mussel biomass out of this comparison biomass values are comparable between Marsdiep and Vlie tidal basins. In the same comparison between inside and outside mussel culture plots remaining biomass was lower inside mussel culture plots than outside.

Differences in densities were not consistent between Marsdiep and Vlie mussel culture plots Fig. A4.4. Removal of mussels from the comparison did not change the general pattern. In the comparison between inside and outside mussel culture plots numerical densities were consistently higher outside culture plots. This remained like that when mussels were removed from the comparison.

There were no consistent differences in densities of hard and soft substrate species between the mussel culture plots in the Vlie and Marsdiep tidal basins (Fig. A4.5). In the original comparison between outside and inside mussel culture plots also no consistent differences were found in densities of hard substrate species. However densities of soft substrate species were markedly higher outside than inside mussel culture plots. This pattern did not emerge from the comparison between culture plots between basins in Fig. A4.5.

In Fig A4.6 a comparison is made between mussels in the Vlie and Marsdiep tidal basins. Numerical densities are rather similar. Biomass tends to be lower in the Marsdiep tidal basin especially in 2009 and 2010. Shells are larger on the culture plots in the Vlie tidal basin. Flesh content of the mussels in

the Vlie basin was markedly higher than in the Marsdiep tidal basin. Densities of mussels outside and inside mussel culture plots were less similar than between culture plots in the Vlie and Marsdiep tidal basins and diverged during the three years. Culture plots had relative stable mussel densities while the mussel number in boxes from natural mussel occurrences outside the mussel culture plots declined strongly during the years of measurement. Biomass was consistently lower outside mussel culture plots, more or less comparable with the difference in mussel biomass on culture plots between the basins. Mussel were much smaller outside than inside mussel culture plots in 2009. In the years after that mussels were large outside the culture plots and more similar to those found inside the plots. In the comparison between the tidal basins the mussels on the Marsdiep culture plots remained smaller over the entire period. Condition index was always higher on the mussel culture plots than outside mussel culture plots, this is very similar to the differences in mussel condition between Marsdiep and Vlie, where the conditions/flesh content is was always higher in the three year period.

Shell length distributions are rather similar between Marsdiep and Vlie culture plots Fig. A4.7. In the Marsdiep there is a higher proportion of small mussels while the mussels longer than 50 mm are more abundant in the Vlie basin. The mussel length distributions outside and inside mussel culture plots differed much more than those compared on culture plots between the basins. Where small mussel were dominant on sites outside mussel culture plots in 2008, most small mussels were found on the mussel culture plots in 2009. Also mussels longer than 40 mm were relatively to smaller mussels much more abundant on the culture plots than outside culture plots. Size distributions inside and outside mussel culture plots really differ far beyond a basin effect.

Age distributions of mussels differ strongly between years but are relatively similar between the tidal basins (Fig, 4A.8). Age distributions of mussels outside culture plots differ from those inside culture plots. Outside culture plots the youngest age class dominates while inside culture plots the oldest age classes dominate.

Inside mussel culture plots most species were found in the Vlie basin Fig A4.9. However the comparison between basins is in this figure is complicated by different sampling efforts. There were more than two times more box cores collected from mussel culture plots in the Vlie basin than from the Marsdiep basin. Species area relationships in Fig A4.10 show that number of species increases faster with increasing number stations in the Vlie tidal basin. This holds for all species as well as for soft sediment and hard substrate species. There seem to be more species in the Vlie tidal basin. This looks similar to the difference between inside and outside mussel culture plots. Inside mussel culture plots more species were found than outside. An interesting difference however is the faster accumulation of soft sediment species outside than inside the mussel culture plots resulting in crossing soft sediment species curves a little above 20 stations.

Average number of species per box was higher in the Marsdiep basin in 2008 but lower than Vlie in 2009 and 2010 (Fig A4.11). This differs from the inside outside comparison. Average species numbers per box were higher during all three years outside mussel culture plots than inside. Distinguishing between hard and soft sediment species did not lead to clear differences in species numbers per box between basins. (Fig. A4.12). Contrary to that specifying between species substrate type in the inside outside plot comparison showed that every year of measurement there were 2 to 3 more soft sediment species in boxes from outside than from inside mussel culture plots.

Available shell surface area may be an important determinant of the number of hard substrate species that can occupy a mussel bed. In two out of three years available shell surface area was larger in the Vlie tidal basin (Fig, A4.17). Density of hard substrate species per shell surface area was higher in the Marsdiep tidal basin during two years, in one year it was higher in the Vlie basin. In the comparison between outside and inside mussel culture plots, available shell surface was always larger on mussel culture plots. Largest densities of hard substrate species per unit shell surface area was consistently found outside mussel culture plots.

Conclusion from this comparison is that most of the differences outside and inside mussel culture plots are not similar to the differences on culture plots between basins. One clear exception is the flesh content of mussels this was consistently higher in the Vlie tidal basin than in the Marsdiep tidal basin and also consistently higher inside mussel culture plots than outside mussel culture plots. This is a likely basin or gradient effect, the mussels in the Vlie tidal basin near the tidal inlet probably experience better feeding conditions leading to higher flesh contents than the mussels in the Marsdiep basin that are further from the tidal inlet. The comparison showed that species numbers are higher in the Vlie tidal basin than in the Marsdiep basin. This suggests that the local species pool depends on local environmental conditions and is an important determinant for the species richness encountered on a mussel bed.

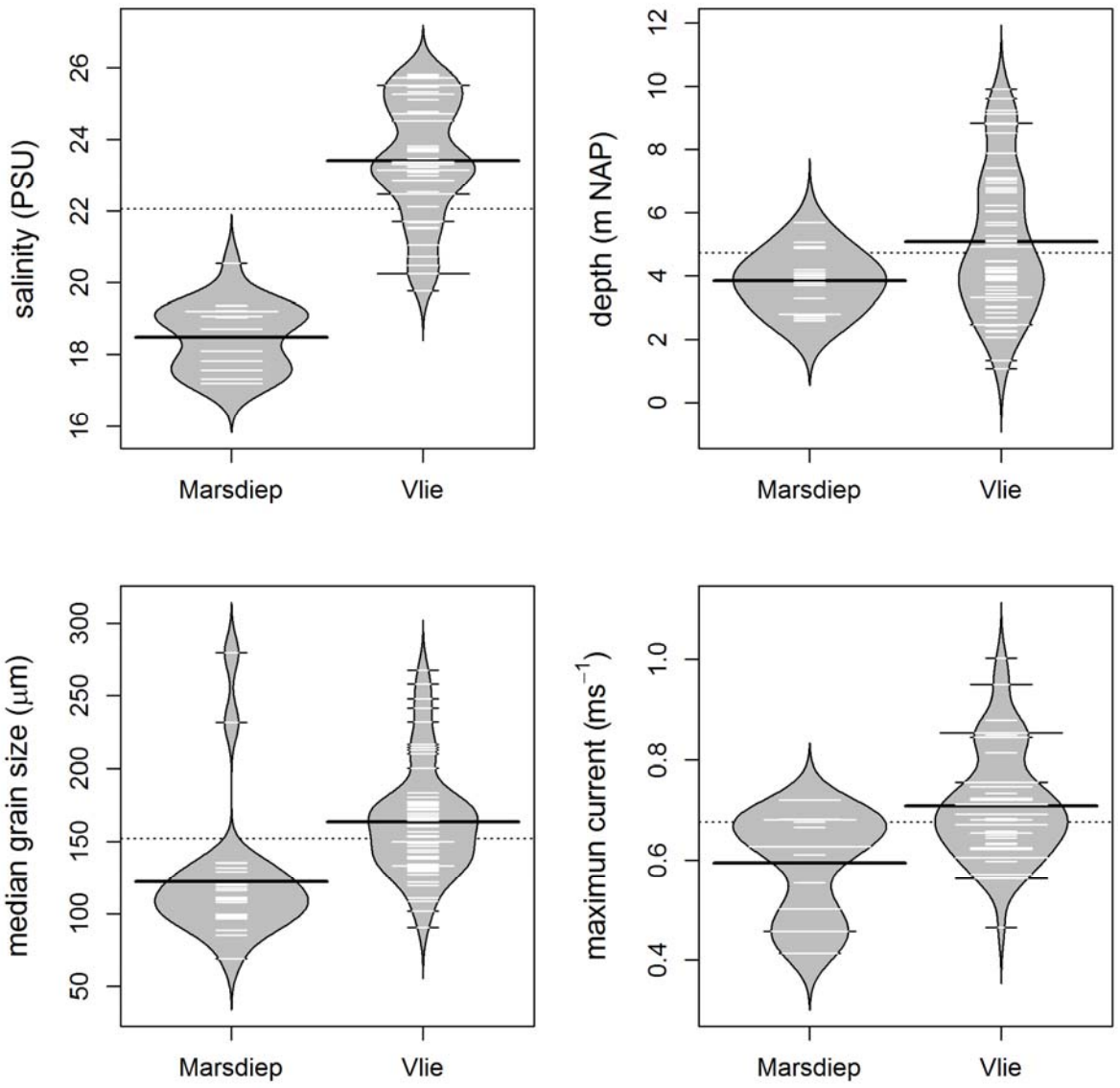

Fig. A4.2 Seawater salinity, depth, sediment median grain size and maximum current speed at subtidal stations in the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea. Median gran rise was measured from a sediment sample out of the box core, salinity and maximum current speeds are model predictions, depth is extracted from a sounding chart.

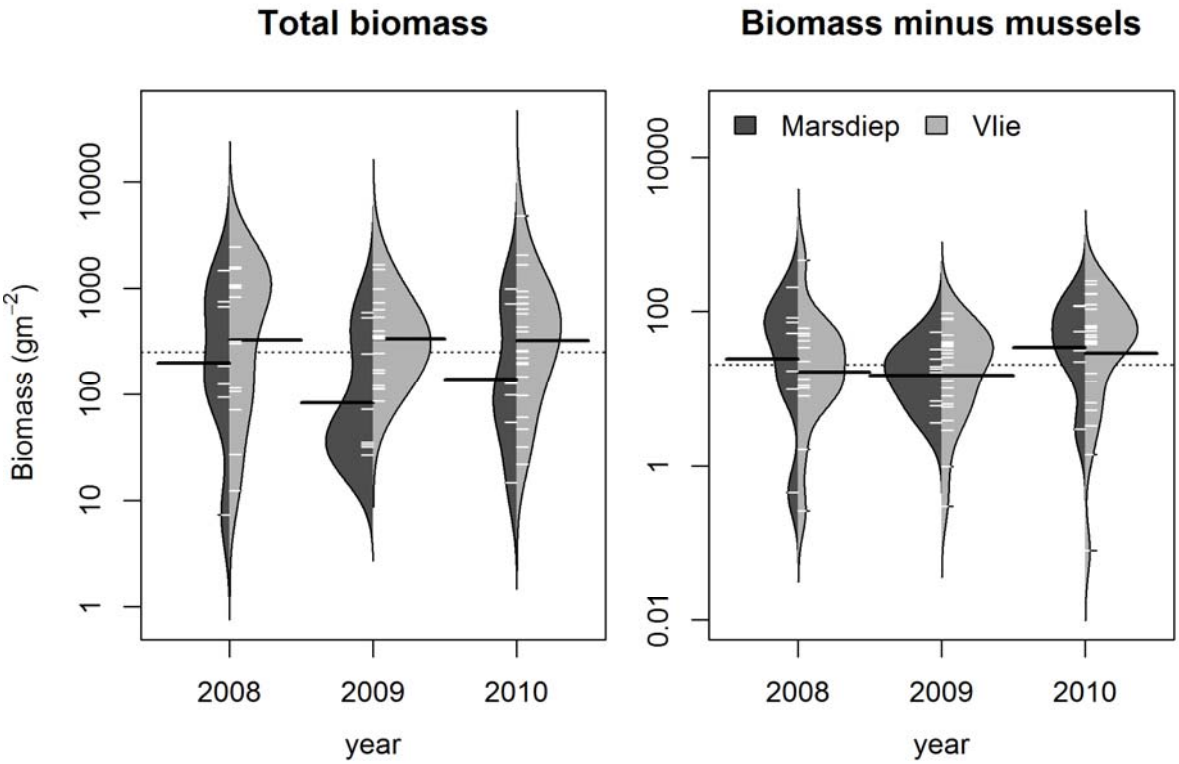

Fig. A4.3 Total biomass of the macrozoobenthos (AFDM gm^{-2}) at stations inside mussel culture plots in the Marsdiep and Vlie tidal basins during the three sampling years (left panel). Total biomass excluding mussels biomass is plotted in the right panel.

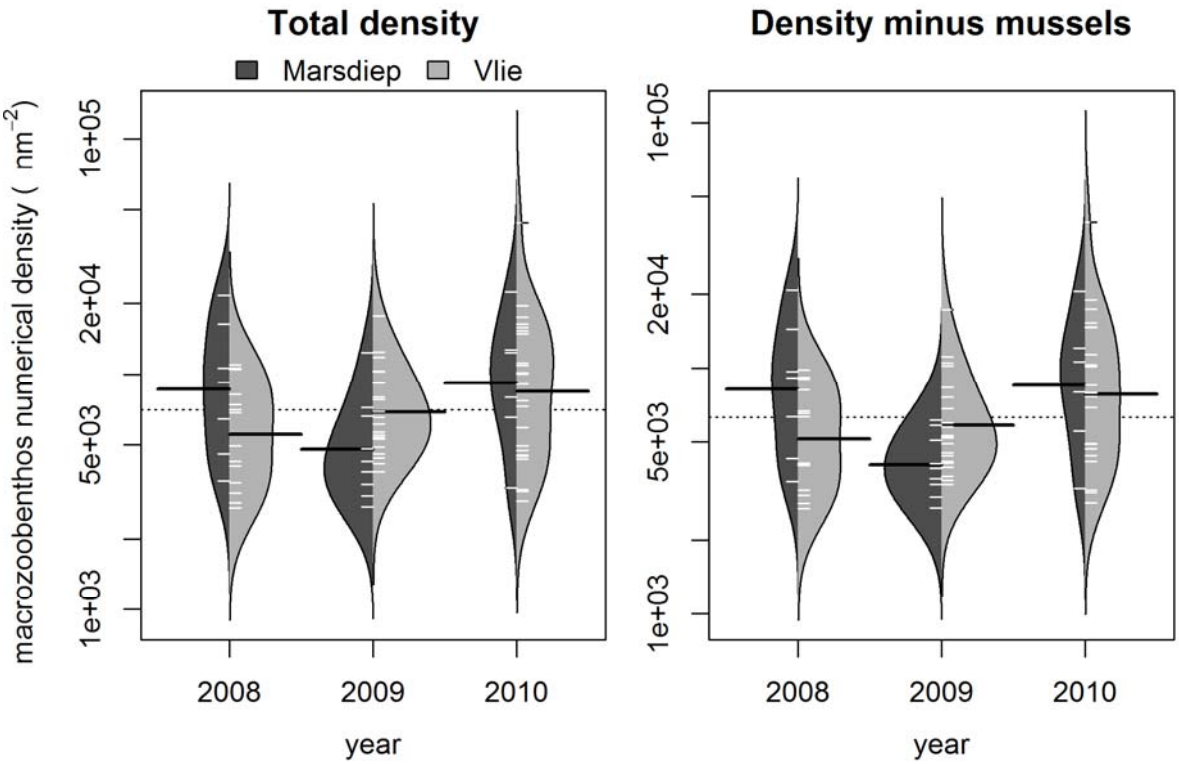

Fig. A4.4 Total numerical density of the macrozoobenthos at the stations inside mussel culture plots in the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea, during the three years. Left panel is total density including mussels, right panel is total density excluding mussels.

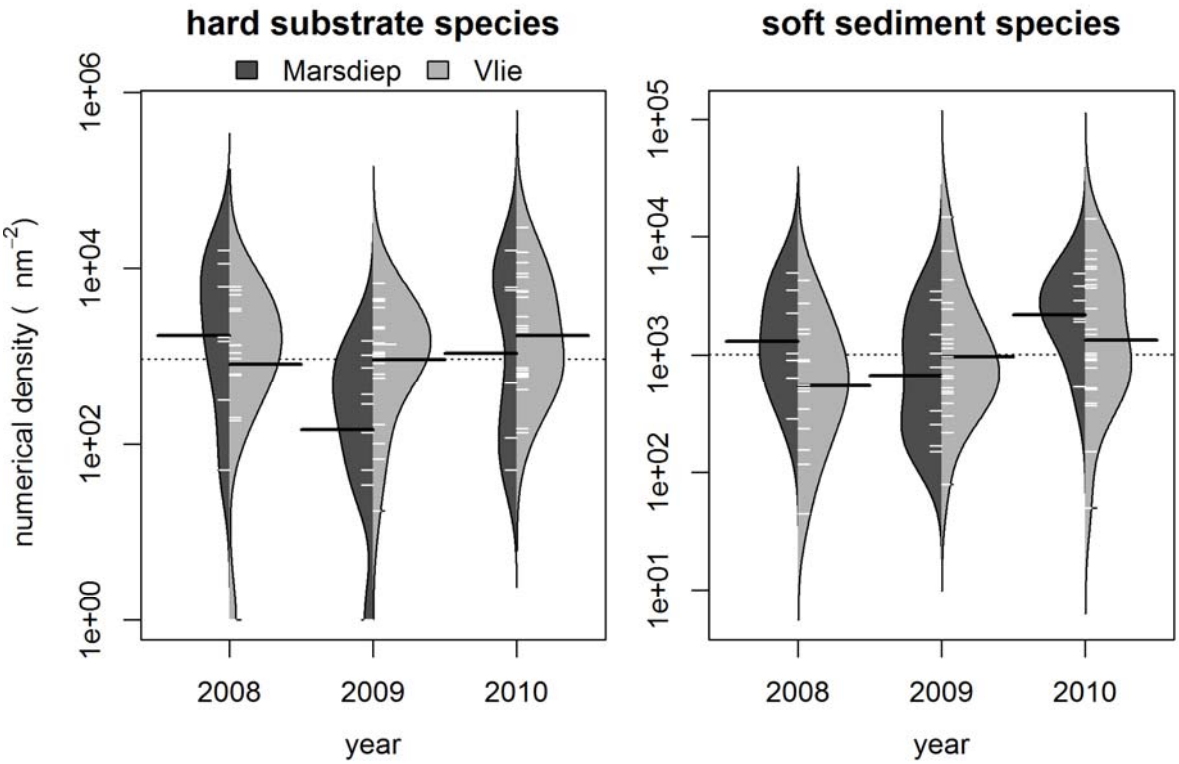

Fig. A4.5 Total density of the macrozoobenthos at stations inside mussel culture plots in the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea during three years. Macrozoobenthos is divided in hard substrate species excluding mussels (left panel) and soft sediment species (right panel). Note different y-scales.

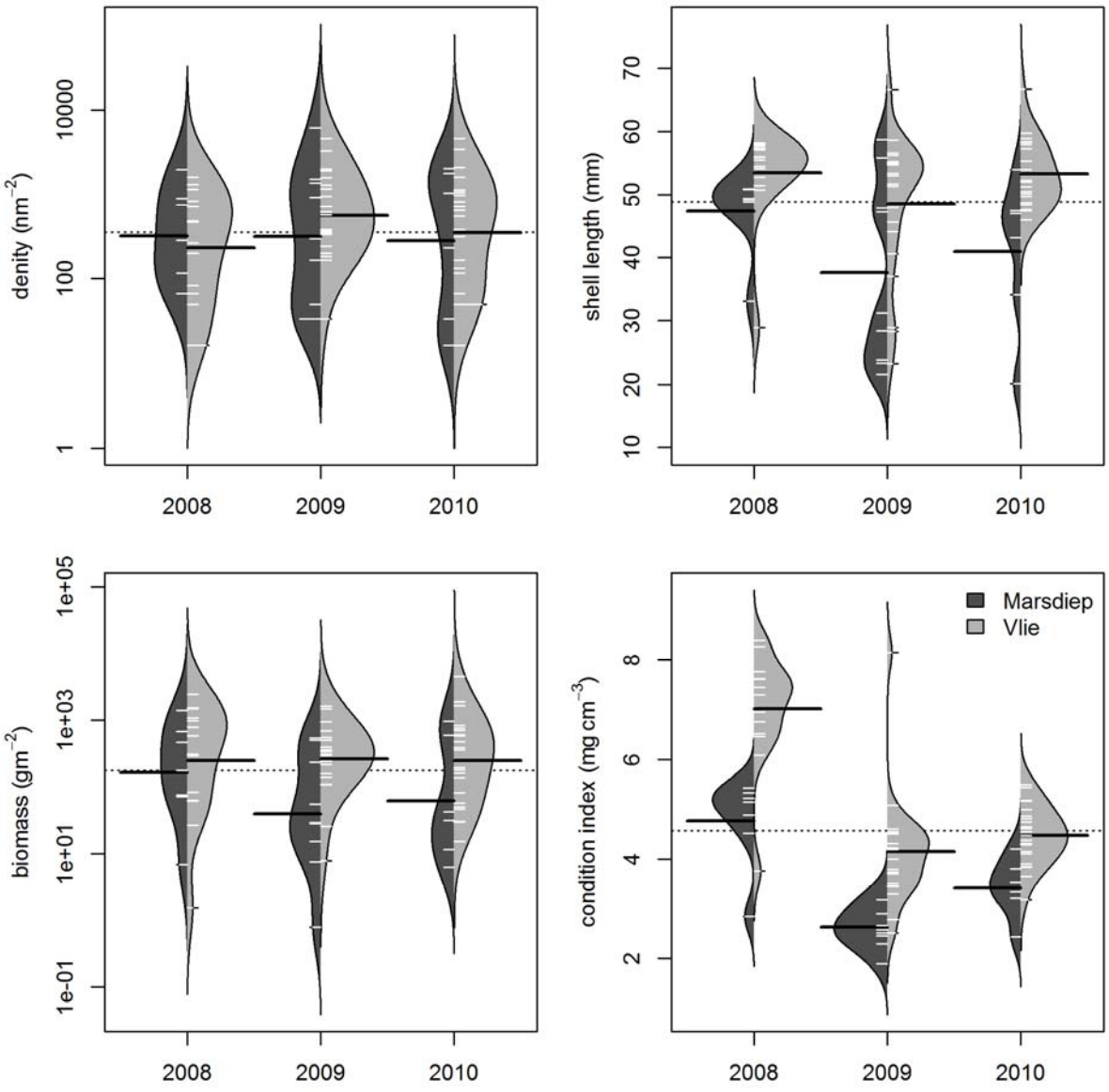

Fig. A4.6 Comparison of numerical density (top left), biomass (bottom left), shell length (top right) and condition index (bottom right) of mussels (*Mytilus edulis*) inside mussel culture plots in the Marsdiep and Vlie Tidal basins in the subtidal western Dutch Wadden Sea during three years, 2008, 2009 and 2010.

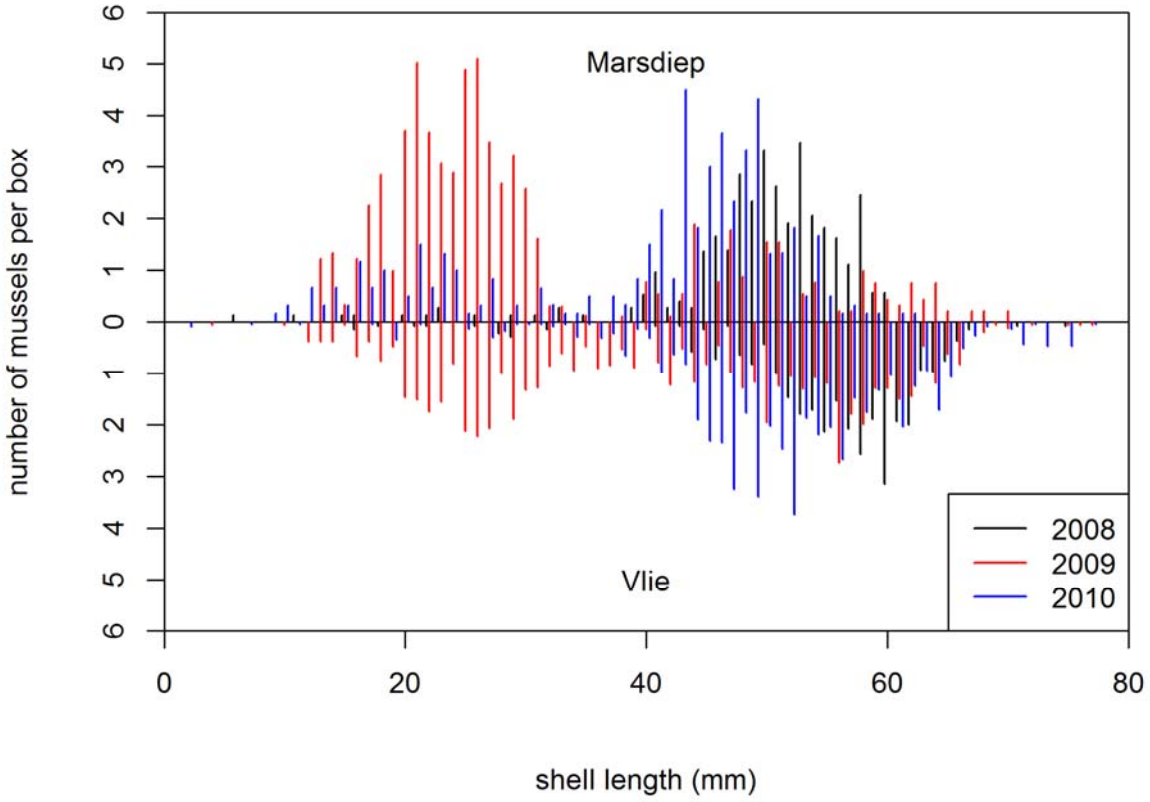

Fig. A4.7 Shell length distribution of mussels (*Mytilus edulis*) at stations inside mussel culture plots in Marsdiep and Vlie tidal basins in the subtidal western Dutch Wadden Sea during 2008, 2009 and 2010. Shell length data are from all mussels collected from box 80 cores of 0.06 m^2.

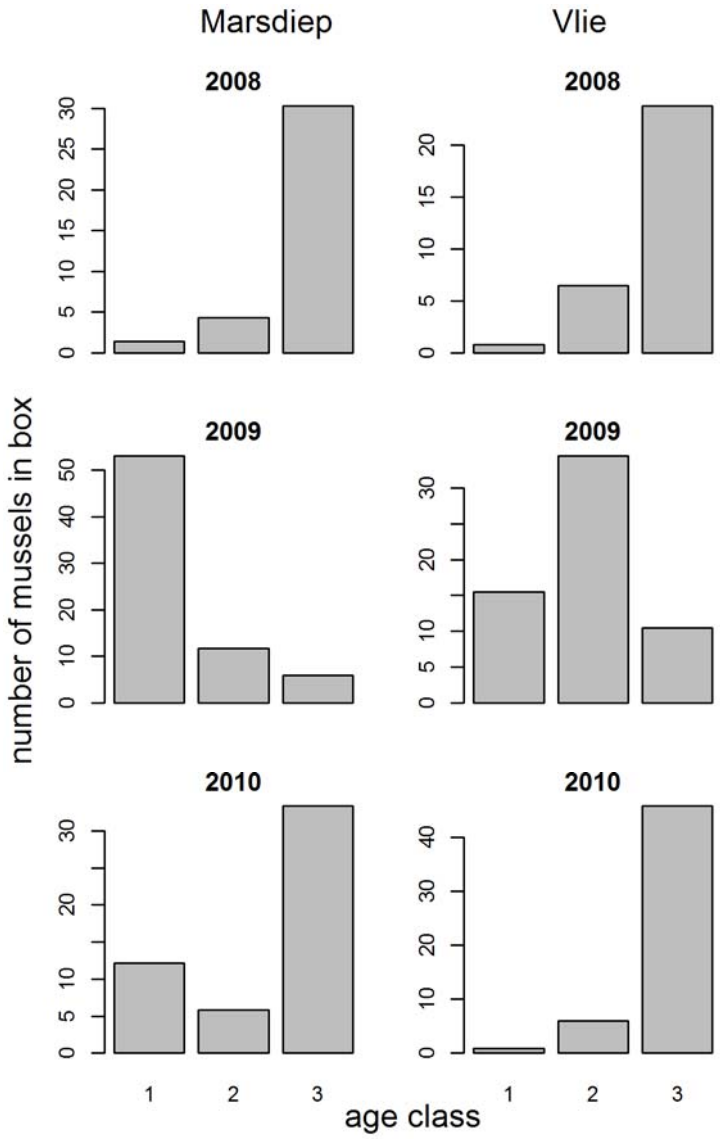

Fig. A4.8 Mussel age distributions of three years from stations inside mussel culture plots in the subtidal of the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea. Age classes are: 1) first year after birth, 2) second year and 3) third year and older.

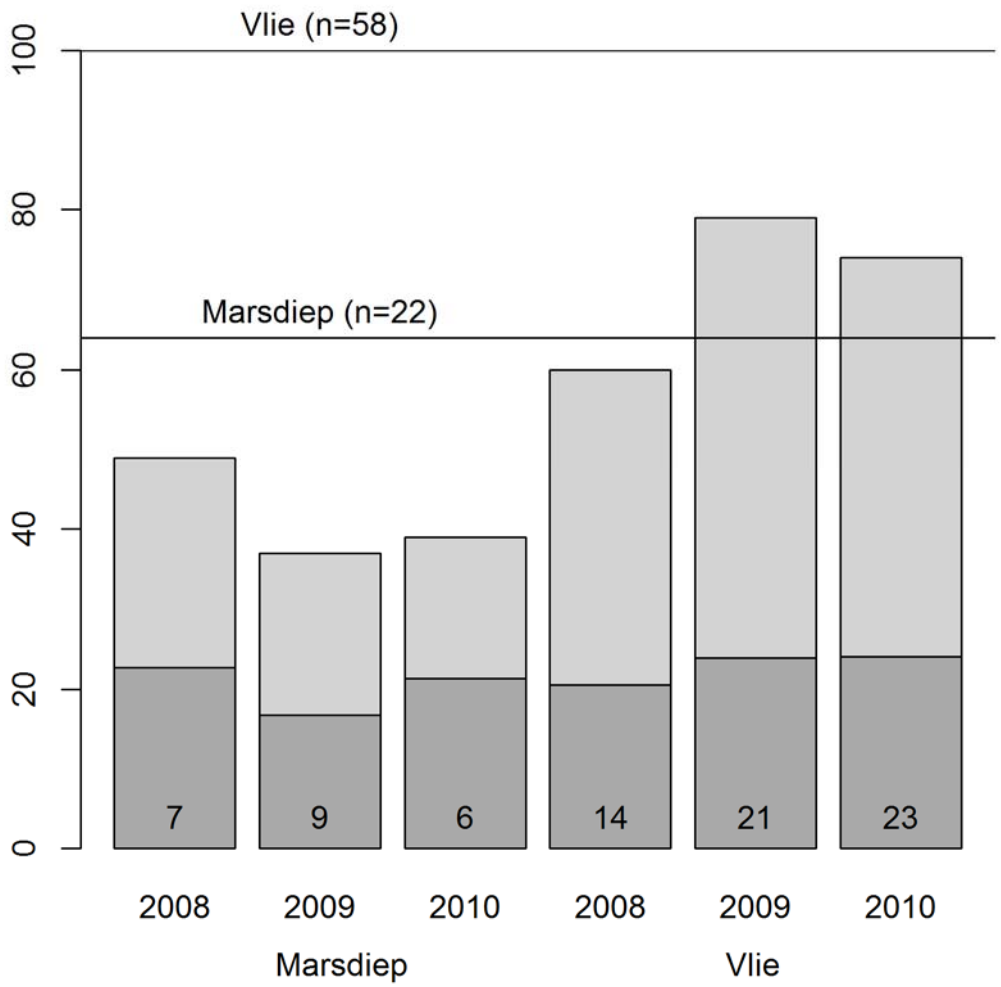

Fig. A4.9 Number of species found, categorized per year, at the stations inside mussel culture plots in Marsdiep and Vlie tidal basins. The dark shaded lower parts of the bars indicate the average number of species in an area of 0.06 m^2. Horizontal lines indicate the total number of species found per bed type when taking all three year together. Numbers refer to number of box cores per category/bar.

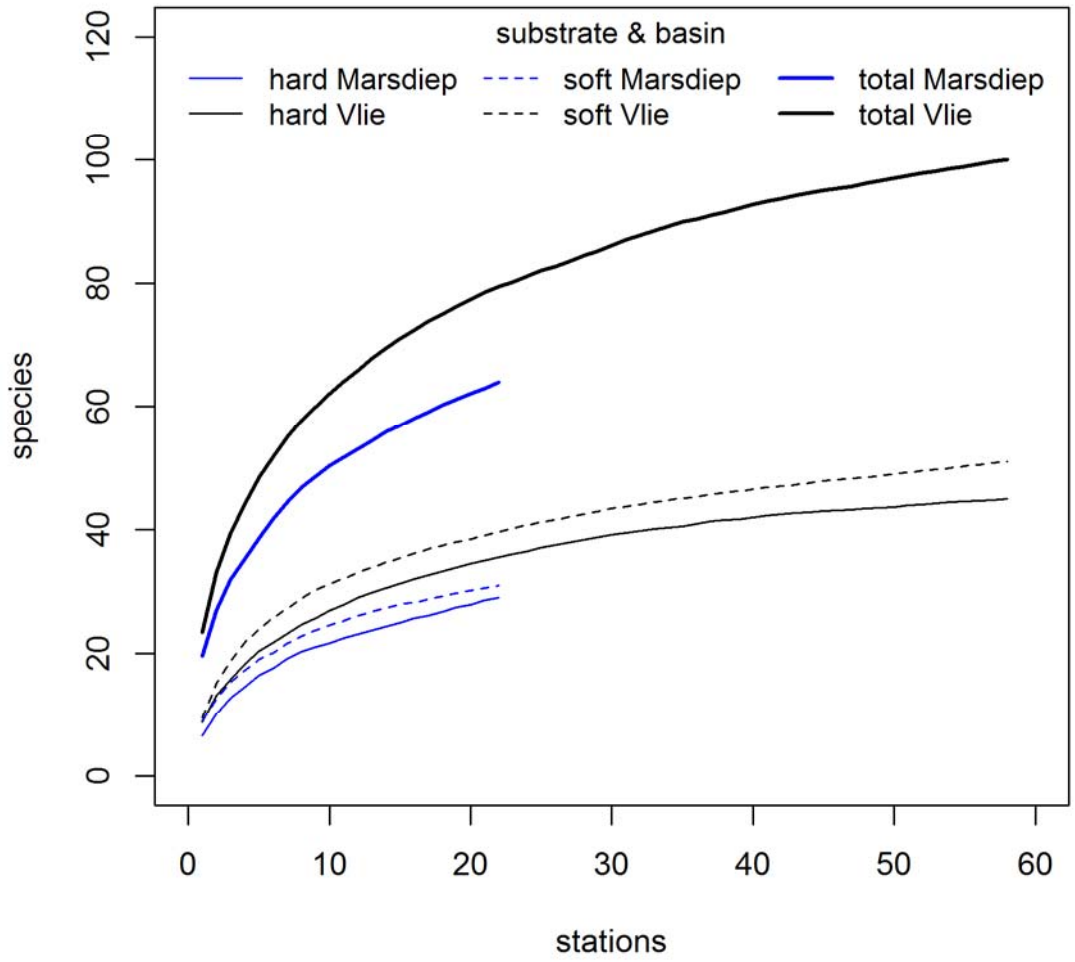

Fig. A4.10 Comparison of species area curves from mussel culture plots in the subtidal of the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea. There are separate curves for hard substrate and soft sediment species and curves of the sum of hard substrate and soft sediment species. The sampled surface per station was 0.06 m^2.

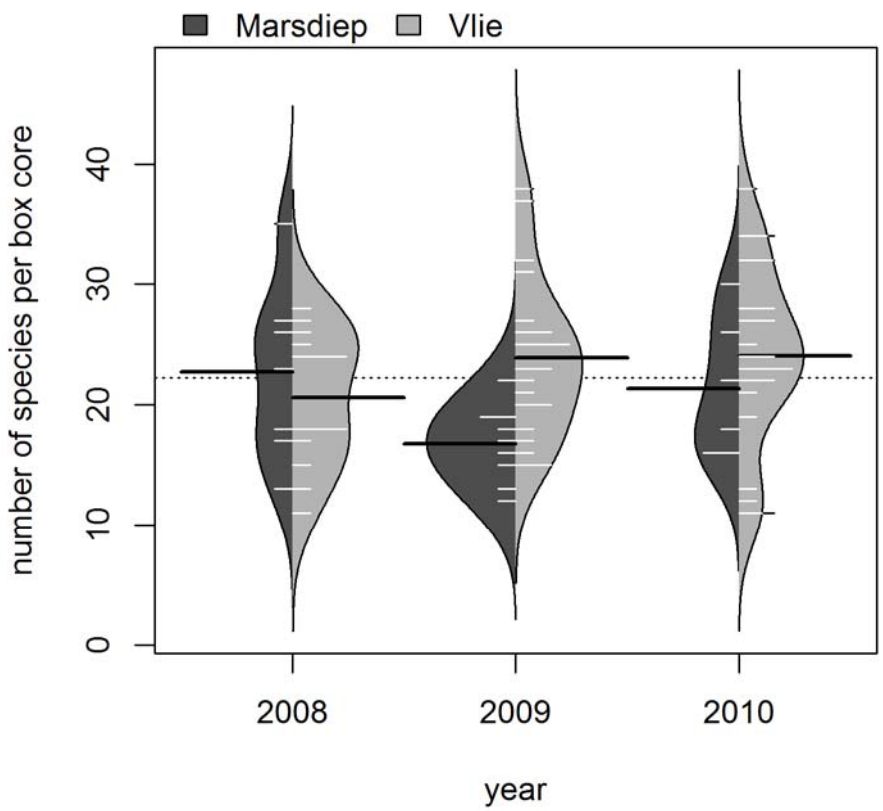

Fig. A4.11 Beanplots of number of species in a box core of 0.06 m^2 inside mussel culture plots in the subtidal of the Marsdiep and Vlie tidal basins in western Dutch Wadden sea during the years 2008 2009 and 2010.

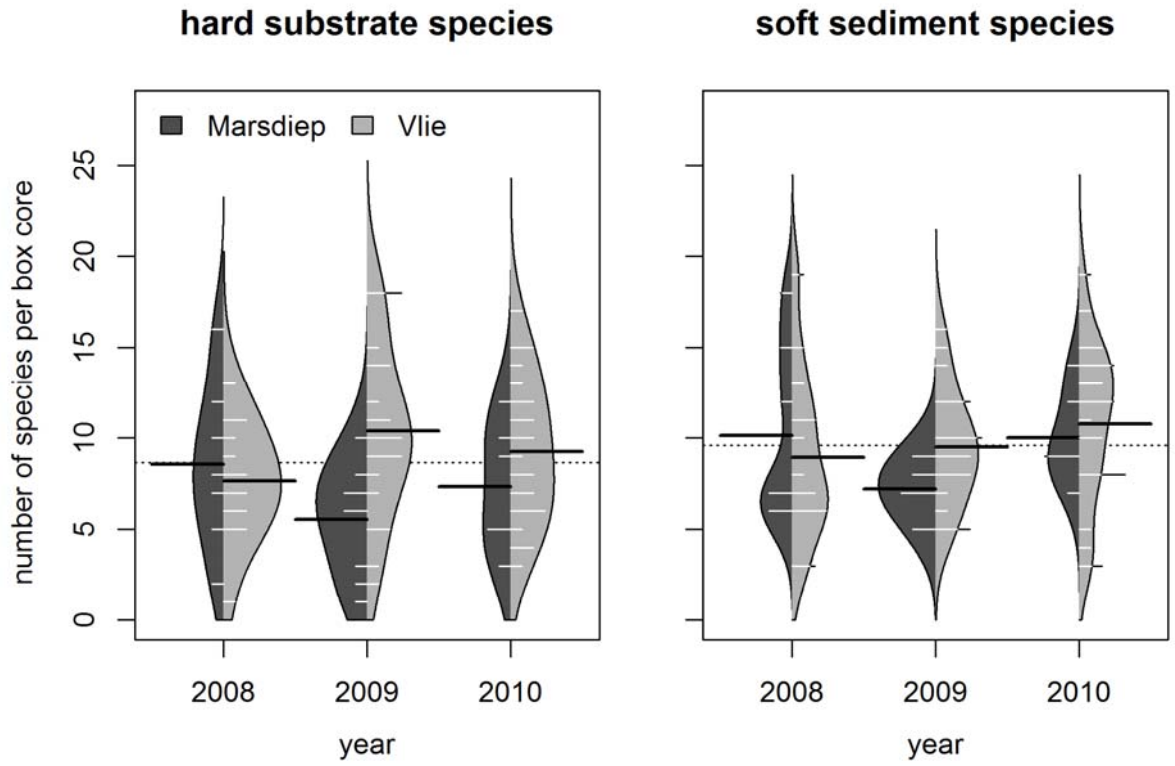

Fig. A4.12 Number of species in 0.06 m^2 box core samples inside mussel culture plots in the subtidal of the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea during three years divided in hard substrate (left panel) and soft sediment species (right panel).

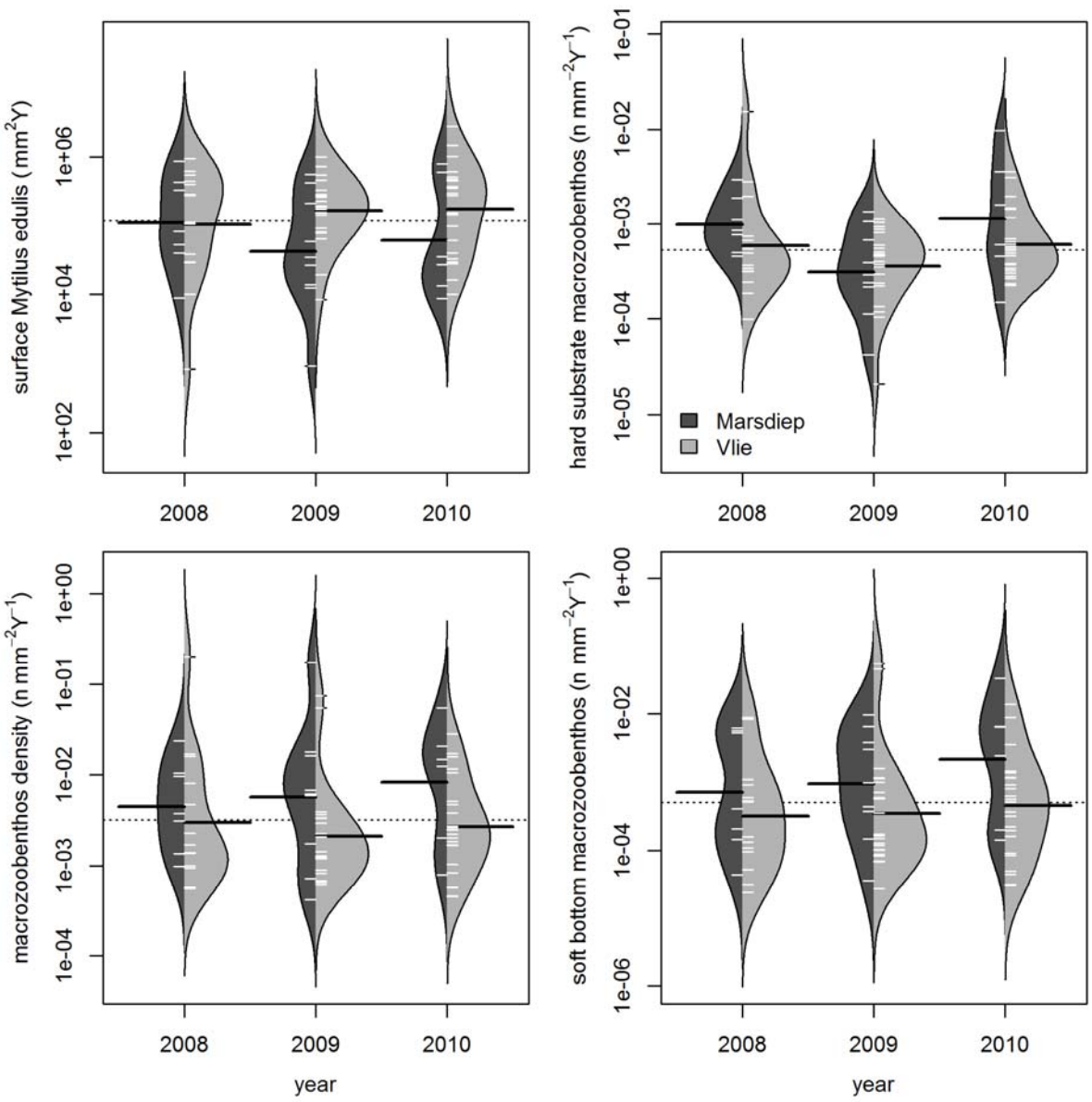

Fig. A4.17 Surface area of the Mytlus edulis shells multiplied by age (top left), inside mussel culture plots in subtidal of the Marsdiep and Vlie tidal basins in the western Dutch Wadden Sea during three years. Macrozoobenthos density (excluding mussels) per shell surface (lower left).Hard substrate macrozoobenthos density (excluding mussels) per shell surface (top right). Soft sediment macrozoobenthos density per shell surface (lower right).

Audit report with response of the authors.

4.6. PR2: How different are sublittoral Mytilus edulis L. communities of natural mussel beds and mussel culture plots in the western Dutch Wadden Sea?

Dit rapport is geschreven in het Engels. Dat is jammer omdat het ook voor een breder publiek interessante informatie bevat. De belangrijkste conclusies uit het rapport zijn duidelijk. Er zijn verschillen in soortensamenstelling tussen mosselpercelen en natuurlijke banken. Er worden meer soorten per monster aangetroffen in de boxcoremonsters van natuurlijke banken, maar het totale aantal soorten in alle boxcoremonsters samen is groter op percelen. Het rapport toont uitzonderlijk mooie en informatieve figuren, die ook in andere deelrapporten niet zouden misstaan. Dit deelrapport is als enige in staat geweest ook naar de externe literatuur te verwijzen, zij het met mate.
Mosselpercelen liggen veelal op plaatsen waar het zouter is dan op de plaatsen waar natuurlijke bedden worden gevonden. De auteurs zijn niet in staat om het effect van percelen en zoutgehalte goed uit elkaar te trekken. Er is voor dit probleem geen heel duidelijke oplossing, al zou stratificatie misschien uitkomst kunnen bieden.

In zekere zin is dit geprobeerd door ook apart alleen de stations in het Marsdiep te analyseren. In het Marsdiep zijn de verschillen tussen percelen en wildemossel voorkomensminder verschillend dan in het Vlie. In deze analyse bleef na modelselectie factor bedtype in het model en verviel saliniteit als verklarende variable van soortenaantallen.
De kernvraag is echter of en indien ja wat de verschillen zijn tussen mosselen en de geassocieerde fauna binnen en buiten percelen. Zoutgehlate kan een van de oorzaken zijn van de gevonden verschillen, primair zullen die verschillen altijd aan de kweekpraktijk kunnen worden toegeschreven omdat nu eenmaal de activiteit is die ervoor zorgt dat de mossels op de percelen terrecht komen. Natuurlijk is het interessant om te weten in hoeverre er mechanische schade van geassocieerde soorten wordt veroorzaakt door het opkorren van mossels en of bestrijding van zeesterren ook nadelige gevolgen heeft voor de andere geassocieerde fauna. Helaas lieten de praktijk van de mosselkweek en de opzet dit onderzoek niet toe om definitief uitsluitsel te geven over het precieze mechanisme waardoor mossels en hun geassocieerde fauna verschillen buiten en binnen mosselpercelen.

In de methoden wordt beschreven dat er is bemonsterd in 2008, 2009 en 2010. Het is echter onduidelijk of de natuurlijke bedden al waren bevist voordat ze zijn bemonsterd, of dat alle monsters zijn genomen voor de bevissing van dat jaar.

Alle mosselsbemonsterd buiten de percelen lagen in principe in gebieden waar gevist kon worden. De waarneming geven een beeld van de situatie bij de bestaande praktijk waar de natuurlijke zaadval bevist wordt en getransloceerd naar de percelen. Er moet van uitgegaan worden dat buiten en binnen percelen visactiviteit is geweest.

Ook wordt in deresultaten helemaal geen vergelijking gemaakt van deze monsters met de boxcoremonsters uit de experimentele PRODUS plots. Dit gemis toont duidelijk een gebrek aan samenhang tussen de deelrapporten aan.

Deze vergelijking werd bemoeilijkt door een verschil in het taxonomische detailvan determineren met name bij de hard substraat soorten. Deze zijn bij de experimentele PRODUS plots niet allemaal tot op soortsniveau gebracht. Hierdoor heeft een vergelijking een beperkte zeggingskracht en is achterwege gelaten.

Deze dataset heeft een belangrijke beperking. Er werden boxcores genomen, en alleen die cores die mosselen bevatten werden gehouden voor verdere analyse. Het aantal boxcores zonder mosselen werd niet genoteerd en blijft onbekend. Als een punt na vijf pogingen geen 'goed' monster opleverde werd het verlaten. Het gevolg van deze strategie is dat de resultaten niet kunnen worden gebruikt om gemiddelde dichtheid of biomassa van mosselen of andere fauna te berekenen. De resultaten die hierover toch worden gegeven zijn ongeldig, want het is volstrekt ongedefinieerd op welke vierkante meters deze 'dichtheid per vierkante meter' slaat. Het is informatief om het aantal mossels per boxcore te vermelden, maar men moet vermijden dit als een dichtheid uit te drukken. Zelfs binnen PRODUS is deze informatie niet goed doorgegeven: het eindrapport (PR1) vermeldt dichtheden zonder vermelding van hun echte betekenis. De resultaten van deze boxcores zijn vooral bruikbaar om andere soorten af te zetten tegen dichtheid of biomassa van mossels. Ook het aantal soorten per boxcore, als een soort absoluut gegeven, is van een twijfelachtige geldigheid in statistische vergelijkingen. Ook hier geldt dat dit gegeven slechts op een select deel van de mogelijke stukjes zeebodem slaat, en dat het veiliger is deze gegevens uitsluitend in relatie tot de lokale mosseldichtheid (aantal mossels per core) te tonen en te bespreken. Het is bij dit alles erg te betreuren dat een belangrijk eindlid van de gradiënt, namelijk monsters zonder mossels, ontbreekt in de dataset.

De vergelijking waar het bij dit onderzoek om ging was in hoeverre mosselvoorkomens binnen en buiten percelen van elkaar verschillen. De gebruikte aanduiding van natuurlijke bedden is wat dat betreft misschien verwarrend. Er zijn niet zoals voor het litoraal kaarten met contouren die aangeven waar en hoever banken zich uitstrekken. Er moest in het sublitoraal gewerkt worden met een globale aanwijzing van waar wel eens mossels zouden kunnen liggen. Alle resultaten hebben dan ook betrekking op de specifieke vergelijking tussen boxen met mossels van binnen en buiten mossel percelen. Natuurlijke banken suggereert dat het om banken gaat, het is echter beperkter dan dat en is alleen mosselvoorkomen. Met een scherpere definiering van wat de boxen representeren is er naar ons idee geen bezwaar tegen de huidige analyse en presentatie van de gegevens. De terminologie is nu wel zo veel mogelijk aangepast in binnen en buiten mosselperceel in plaats van banken te noemen. Het eindlid monsters zonder mossels worden gerapporteerd in Dekker & Drent (2013) en in toegevoegde appendix 3 wordt de situate zonder mossels wel meegnomen.

In het rapport worden diverse maten gepresenteerd voor het oppervlak beschikbare mosselschelp, als maat voor het voorkomen van soorten van hard substraat. Geen enkele van deze maten werkt echt goed, en er worden goede correlaties gevonden met de simpele maat van mosselbiomassa in de core. Wellicht is het eenvoudiger dit als de proxy voor mosselschelp te nemen. Overigens is opvallend dat ten opzichte hiervan, het voorkomen van hard-substraat soorten vrijwel gelijk is tussen banken en percelen.

Omdat de verschillende maten niet tot dezelfde conclusies leiden is het waarschijnlijk beter om deze verschillen te laten zien ipv een maat te kiezen. Want welke kies je dan, de maat doie het minste verschil tussen binnen en buiten percelen laat zien of juist het meeste verschil?

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