

# The effects of subtidal mussel seed fisheries in the Dutch Wadden Sea on sediment composition

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## 1. Introduction

The project PRODUS (a mnemonic for the Dutch name of the project 'Research Project Sustainable Shellfish fisheries') started in 2006. Main target was to investigate a number of processes linked to shellfish fisheries, among others the effects of seed mussel fisheries on the occurrence of natural sublittoral mussels and on the sublittoral nature values. In PRODUS, natural values have been translated into different aspects of the benthic ecosystem, such as the macro fauna community and sediment characteristics. For a more comprehensive description of the PRODUS background we refer to Smaal et al. (2013).

Mussel beds are dense aggregations of filter-feeding organisms. The roughness of these beds will cause water velocity and slow down suspended materials to settle on the sediment surface (ten Brinke et al. 1995). Moreover, mussels filter vast amounts of turbid water and excrete the fine silt as (pseudo)faeces. Hereby, mussel beds accumulate fine sediments and organic material over time (Ysebaert et al. 2009; Dankers et al. 2001).

In this report, the effect of seed mussel fishery activities on sediment composition is analysed. The hypothesis is that dredging for mussels brings fine silt in suspension. Tidal currents move silt away from the fishing site and a more coarse sediment is left behind (Piersma et al. 2001).

For PRODUS experimental plots of each 400 x 200 m have been marked out on sites where natural mussel seed occurred; on one half of the plots fishing was prohibited and on the other half fishing was allowed. Per plot 12 boxcorer samples were taken from which sediment samples were collected before and after fishing. Most plots have also been sampled later during the research, to test for increasing similarity of fished and control sites over time.

## **2. Sediment sampling and pre-treatment methods**

### **2.1 Experimental plots**

To study the effect of seed mussel fisheries on sediment characteristics, we followed a split-plot design (Ens et al. 2007). Sets of experimental plots, all with a 4 ha control (closed) and 4 ha impact (open) plot, have been marked out in the period 2006-2010 on sites where in autumn seed mussels appeared in the sublittoral parts of the Wadden Sea (Fig. 1). The precise position of plots was chosen shortly before fisheries, aiming to have both closed and open plots as equal as possible (same mussel density). A detailed description of the sampling strategy is provided in van Stralen et al. (2013). First sampling was conducted before fisheries took place to test for initial differences between open and closed plots. Sampling was repeated several weeks after fisheries, and one year after fisheries (Tab. 1).

### **2.2 Sediment sampling**

Sampling took place with a 20\*20 cm boxcorer from research vessel TX63. Sub-samples of the top 5 cm were taken with a 2 cm (diameter) test-tube, stored in poly-ethylene bottles in a -20 degrees C freezer. Shortly before analysis, the sub-samples were defrosted.

### **2.3 Sediment sample treatment**

All sediment samples have been pre-treated to remove organic matter and shell debris, a standard method to arrive at well-defined conditions. Large shell particles usually disturb the analysis and organic matter partly acts as a substance keeping clay particles together; mainly as a result of physical-chemical bonds between organic matter with a negative particle surface charge and clay particles with a positive particle surface charge. Next to such a 'glue'-effect, organic matter has completely different characteristics that are not well measured by the grain-size detection methods, and as such, these particles may largely obstruct a statistical analysis later on.

Pre-treatment consisted of several steps: First, the sample is sieved over a 2 mm<sup>2</sup> sieve to remove the really large particles. Next de-mineralised water (> 10 MΩ standardised resistance), and appropriate volume of 35% H<sub>2</sub>O<sub>2</sub> and 0.5N HCl is added. The samples are left for about 12 hours, and then heated on a 80 degrees C sand bath until no bubbles are produced anymore. After cooling, again de-mineralised water is added, leaving the sample for 48 hours as a minimum.

### **2.4 Grain size analysis**

After the pre-treatment, grain size analysis has been carried out with a Beckman LS 13 320, laser diffraction particle size analyser, equipped with a multi-wavelength Polarization Intensity Differential Scattering system for sub-micron particles (from 0.017 μm) and a single-wavelength module for particles >2 μm.

Volumetric contribution to the sample of classes from 0.04 to 2000 μm are produced. First the relatively large particles are classified as the fractions smaller than subsequently 2, 4, 8, 10, 16, 25, 32, 63, 125, 250, 500, 1000 and 2000 μm. The relatively small particles (smaller than 2 μm) are classified as the fractions smaller than 0.040, 0.044, 0.048, etc, in 48 steadily a bit increasing steps up to 4.24 μm. This series from 0.040 (etc) μm is continued in 31 steps to 63.4 μm, then in 21 steps to 500 μm and finally in 18 steps up to 2000 μm.

This amount of data leaves the statistical analysis with the choice what exactly can best be analysed, after examination of the data set itself. From the data, a median grain size can be computed, which is an

appropriate reflection of the data set under the condition of a perfect normal size distribution. Another possible choice is the use silt fraction (the volumetric part of all particles smaller than  $63 \mu\text{m}^1$ ) as basic characteristic. Actually, one wishes to test multi-possibilities to see what in fact is the best characteristic to apply for the analysis of the fishing experiment, but this is too time consuming to perform.

## 2.5 Sampling sites and dates

Sample sites are shown in Figure 1. Samples were taken in spring and autumn, in 2007 and 2009. In samples sizes per season and year are given.

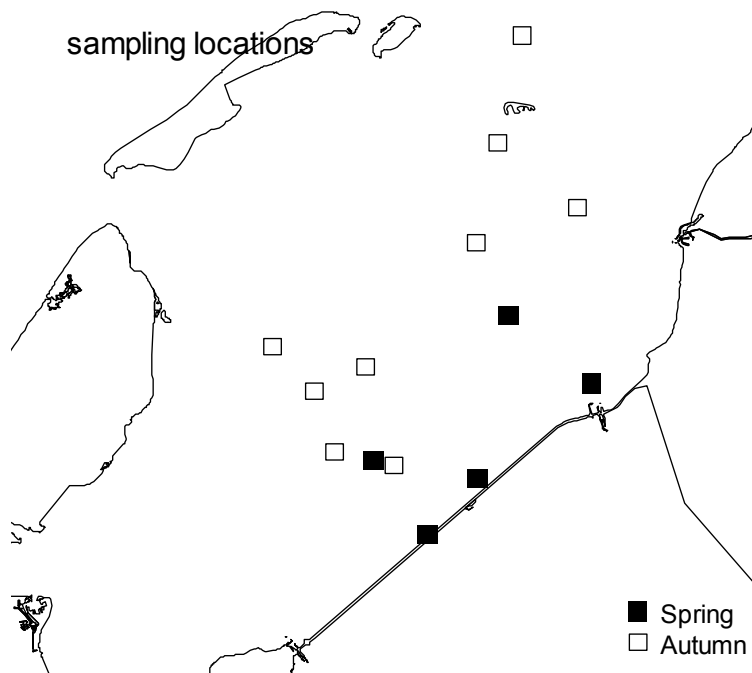


Figure 1. Sampling locations.

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<sup>1</sup> Note that  $63 \mu\text{m}$  has been used here as upper boundary of the silt class; internationally a common value. In standard soil classification in The Netherlands  $16 \mu\text{m}$  is taken as upper silt class boundary.

Table 1. Sample sizes per location per season and year.

<b>Location</b>	<b>Autumn</b>		<b>Spring</b>	
	<b>2007</b>	<b>2009</b>	<b>2007</b>	<b>2009</b>
Afsluitdijk - AD10				48
Breesem W		35		
Breesem Z		31		
Breezanddijk				47
Gat van Stompe	77			
Griend		43		
Inschot		47		
Kornwerd (Boontjes				47
Pollendam		47		
Stompe			80	
Stompe Zuid	78			
Westkom		36		
WestMeep		40		
Zuidoostrak				46
<b>n samples</b>	<b>155</b>	<b>279</b>	<b>80</b>	<b>188</b>
<b>n locations</b>	<b>2</b>	<b>7</b>	<b>1</b>	<b>4</b>

## 2.6 Statistical analyses

A statistical analysis was applied to test the above mentioned hypothesis. Grain size can be expressed in many ways. We choose median grain size as a measure for the overall sediment composition and volume percentage of grains smaller than 2  $\mu\text{m}$  and smaller than 63  $\mu\text{m}$  to reflect clay/silt fraction. Using a BACI (Before After Control Impact) approach, we tested for an interaction term between timing of sampling (before or after a fishing event) and the plot type (control or impact). Tests were carried out using different statistical approaches. In a first approach, we used linear mixed effect models (Pinheiro 2000) to explain the variation amongst individual sediment samples. In the second approach we integrated the 12 randomly collected sediment samples from each research plot per sampling occasion by calculating mean values. Mean values allowed for parametric tests without further randomization or modelling of variance structures.

## 2.7 Mixed modelling approach

Since we were not so much interested in each specific location, but in the overall effect of fishing, sampling location was modelled as a random factor. To conform to the statistical assumptions data were square root transformed. Different variance structures, allowing for heterogeneity within groups were tested and applied if significantly improving the model fit (based on AIC).

The data are modelled as follows:

$$M_{tps} = I + a_l + S \times T \times P + \varepsilon_{tps}$$

and

$$V_{tps} = I + a_l + S \times T \times P + \varepsilon_{tps}$$

Where  $M_{tps}$  and  $V_{tps}$  are respectively median grain size and volume fraction of grains smaller than 2  $\mu\text{m}$  in season  $S$  (spring or autumn) at time  $T$  (before or after fishing) for each plot type  $P$  (control or impact). The full model included main effects, a three way interaction, and three two way interactions.  $I$  is the intercept. The effect of location is modelled as random component  $a_l$  with constant variance, normally distributed and zero mean:  $a_l \sim \text{N}(0, \sigma_a^2)$ . The variance structure of the errors was modelled as  $\varepsilon_{tps} \sim \text{N}(0, \sigma_s^2)$  for the median grain size and  $\varepsilon_{tps} \sim \text{B}(n, p)$  for the volume fraction of grains smaller than 2  $\mu\text{m}$ . The variance has subscript  $s$  to denote that variance differed between seasons, plot type and time which allowed for heterogeneity between the two seasons (Pinheiro 2000).

Nested models of median grain size were compared by Akaike's Information Criterion (AIC) and tested using an F-test on the likelihood ratio which was calculated using restricted log likelihood (REML) for models with different variance structures and maximum log likelihood (ML) for models with different fixed effects. Nested models of the volume fraction of grains smaller than 2  $\mu\text{m}$  were compared using Akaike's Information Criterion (AIC) and tested using a chi-square test.

All calculations were performed using R (R Development Core Team) and the packages nlme (Pinheiro 2008) and lme4 (Bates et al 2011).



## **2.8 Linear modelling of plot means**

Basic linear modelling of plot means was based on a rather limited dataset of 15 "open" and 14 "closed" plots before and after fishing (n=58). To cope with plot-specific variation, the sediment composition (median grain size, silt fraction and clay fraction) at T1 was modelled as a function of the sediment composition at T0 plus the effect of fisheries ("open"/ "closed"). Considering the idea that mussels cause fine sediments to accumulate, the impact of fisheries was expected to be more obvious at sites characterized by coarse sediment than at sites where background sediment conditions are rich in fine silt. To deal with this effect, an interaction term between the factor "open"/"closed" and the sediment composition at T0 was included in the model.

## 2.9 Results

Data from 14 areas was analysed (9 in autumn, 5 in spring), summing up to 702 sediment samples. For most locations, the aimed 48 samples (12 in each block at each time) were collected. Median grain size and volume fraction of grains smaller than 63 or 2  $\mu\text{m}$  showed considerable variation between plots. This variation within and between plots is illustrated for the clay fraction in figure 2.

At the level of individual sediment samples, the silt ( $V\% < 63 \mu\text{m}$ ) and clay ( $V\% < 2 \mu\text{m}$ ) were highly collinear. Median grain size showed a non-linear relationship with both silt and clay fractions. Model output for silt and clay fractions was therefore comparable, while median grain size revealed rather different relation with the explanatory variables (Table 5).

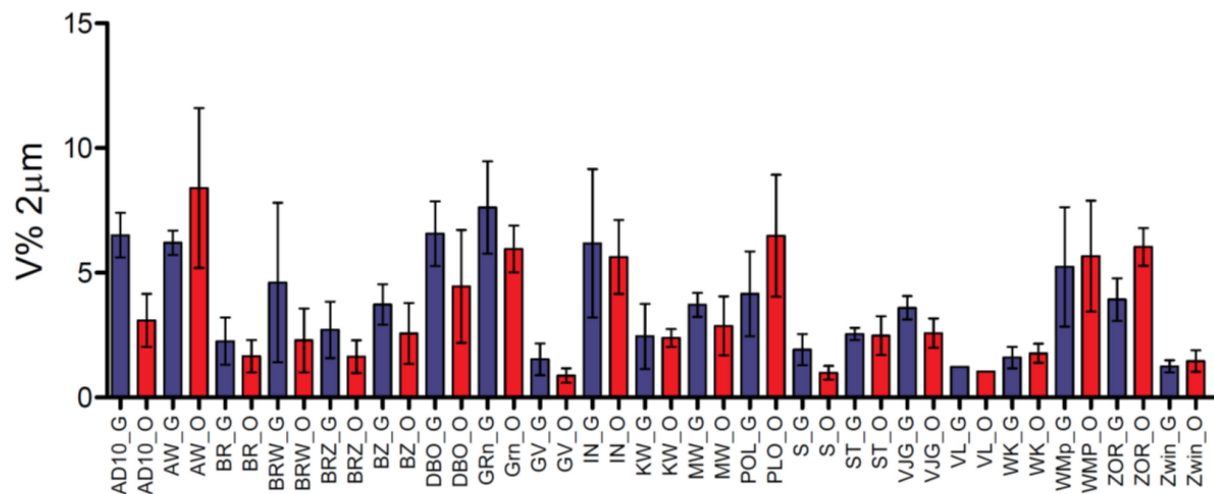


Figure 2: Mean clay fraction of the sediment ( $\% < 2 \mu\text{m}$ ) per plot. Blue bars represent plots that were closed for fisheries and red plots were open for fisheries. Error bars represent standard deviations of mean values over time.

## 2.10 Median grain size

The mean median grain size per plot ranged 73  $\mu\text{m}$  to 370  $\mu\text{m}$ . These values did not change between  $T_0$  and  $T_1$ . Approximately one year after fisheries ( $T_2$ ) the mean median grain size of the plots had increased with about 15%.

The residuals of the full model, including season, time and plot type and all interactions, plus location as a random variable, showed some patterns with its fixed effects. Including a variance structure for one or more fixed factors significantly improved the model. From models with various variance structures, the model with the lowest AIC was selected, which allowed different heterogeneity for season and time. Simplification of this model led to the exclusion of the three-way interaction, which was not significant (Likelihood ratio test  $p = 0.3691$ ,  $Df = 1$ ,  $LR = 0.8069$ ). From the two-way interactions, only season versus plot type was significant (Likelihood ratio test  $p = 0.0052$ ,  $Df = 1$ ,  $LR = 7.7931$ ). The interaction of time by plot type are presented in figure 3. If significant, these interactions could indicate an effect of fishery activities, but they were not (Likelihood ratio test  $p = 0.1267$ ,  $Df = 1$ ,  $LR = 2.3329$ ).

Table 2. Results of likelihood ratio testing of the different model components for median grain size. Probabilities of main effects are not given as a result of significant interactions.

Interactions	Log likelihood ratio	df	p-value
Season : Time	1.455064	1	0.2277
Season : Plot type	7.793106	1	0.0052
Time : Plot type	2.33288	1	0.1267
Season : Time : Plot type	0.8068525	1	0.3691

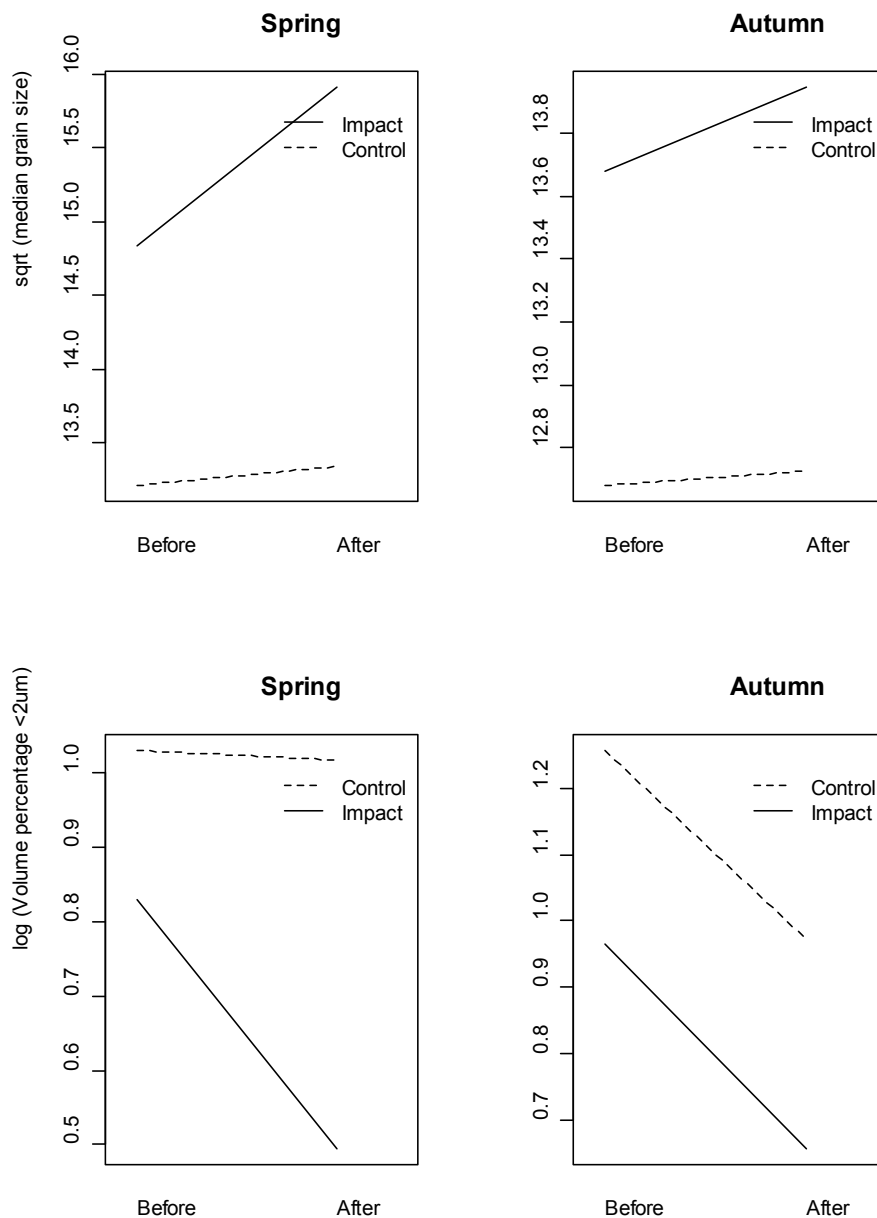


Figure 2. Interaction plot for median grain size (upper figures) and volume percentage of grains smaller than 2  $\mu\text{m}$  (lower panels), for spring and autumn between the  $T_0$  (before) and  $T_1$  (after).

### 2.11 Volume percentage of grains smaller than 2 µm

The mean clay fraction per plot ranged from 0.5 % to 10% of volume. The volume of these small grains was quite variable (Figure 2) and gradually decreased over time, with relatively large clay fractions at the start of the monitoring and a gradual decrease over months and years (Figure 4). In general, slightly higher clay fraction was found in control plots. This difference already existed before impact ( $T_0$ ) and decreased over time (Figure 3).

The three-way interaction of season by time by plot type was not significant ( $\chi^2=0.0338$ ,  $p=0.8541$ ). All two-way interactions were not significant either: season by time ( $\chi^2=0.0457$ ,  $p=0.8308$ ), season by plot type ( $\chi^2=0.0044$ ,  $p=0.9469$ ) and time by plot type ( $\chi^2=0.0053$ ,  $p=0.9417$ ). None of the main effects was significant; season ( $\chi^2=0.4182$ ,  $p=0.5178$ ), time ( $\chi^2=0.4668$ ,  $p=0.4944$ ) and plot type ( $\chi^2=0.2503$ ,  $p=0.6169$ ). Only the intercept was significant. See also Table 4.

Table 3. Results of likelihood ratio testing of the different model components for volume percentage of grains smaller than 2 µm. Probabilities of main effects are not given as a result of significant interactions.

Interactions	$\chi^2$	df	p-value
Season : Time	0.0457	1	0.8308
Season : Plot type	0.0044	1	0.9469
Time : Plot type	0.0053	1	0.9417
Season : Time : Plot type	0.0338	1	0.8541

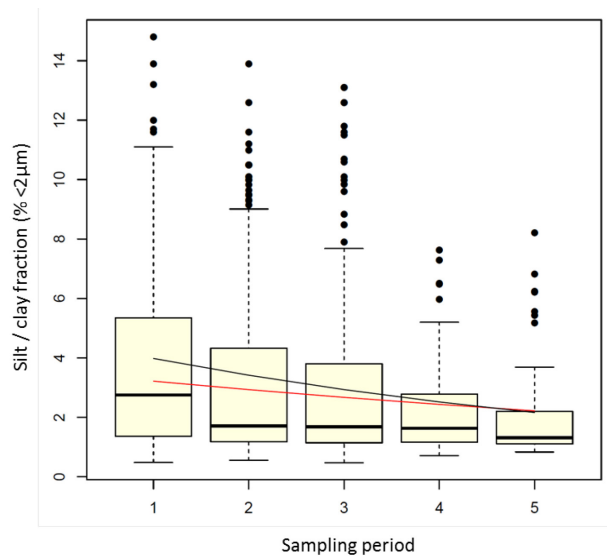


Figure 4. Clay fraction of the sediment ( $V\% <2\mu\text{m}$ ) per sampling period in time. 1 = before fishing; 2 = 2-4 weeks after fishing; 3, 4 and 5 are 1, 2 and 3 years after fishing, respectively.

### 2.12 Volume percentage of grains smaller than 63 µm

The mean silt fraction per plot ranged from 1.7 % to 68% of volume. Mean silt fraction shows a reduction of 20% between  $T_0$  and  $T_1$ , and a subsequent reduction of 15% from  $T_1$  to  $T_2$ .

Approximately one year after fisheries took place, the silt fraction decreased for most plots. Exceptions were locations Zuidostrak and Stompe, where silt accumulated in the fished and control plots.

### 2.13 Analysis of mean values per plot

Analysis of the median grain size based on the mean values per plot and sampling time is presented in figure 5. Figure 5 shows a tendency towards a decrease in median grain size in control plots in the period T<sub>0</sub>-T<sub>1</sub>. Exceptions are found and this result is not significant (Tab. 5). In the fished plots median grain size did not vary between T<sub>0</sub> and T<sub>1</sub>. The silt fraction (V% <63 µm) at T<sub>1</sub> was predominantly reduced compared to the situation at T<sub>0</sub>. An exception is formed by a series of control plots with relatively low silt fractions at T<sub>0</sub>. While the silt fraction for these locations decreased in fished plots, it clearly increased in the control plots. For the locations with relatively high silt fractions at T<sub>0</sub> this difference was not observed. Regression analysis revealed that this increase in silt fraction in control plots was significantly different from the decrease observed in fished plots (Table. 5). Very similar results were found for the clay fraction (V% <2µm), but these results were not significant (Table. 5).

*Table 5. Results for linear modelling of the impact of mussel fisheries (Estimate) on sediment characteristics. All three models included the interaction between the factor "open"/"closed" and the sediment composition at T<sub>0</sub>. Only the estimated effect of fishing is shown.*

<b>Response variable</b>	<b>Estimate</b>	<b>t-value</b>	<b>p-value</b>
Median grain size	-26.2	-0.906	0.3735 .
Silt fraction (<63 µm)	-11.3	-2.355	<b>0.0263 *</b>
Clay fraction (<2 µm)	-1.72	-1.838	0.0775 .

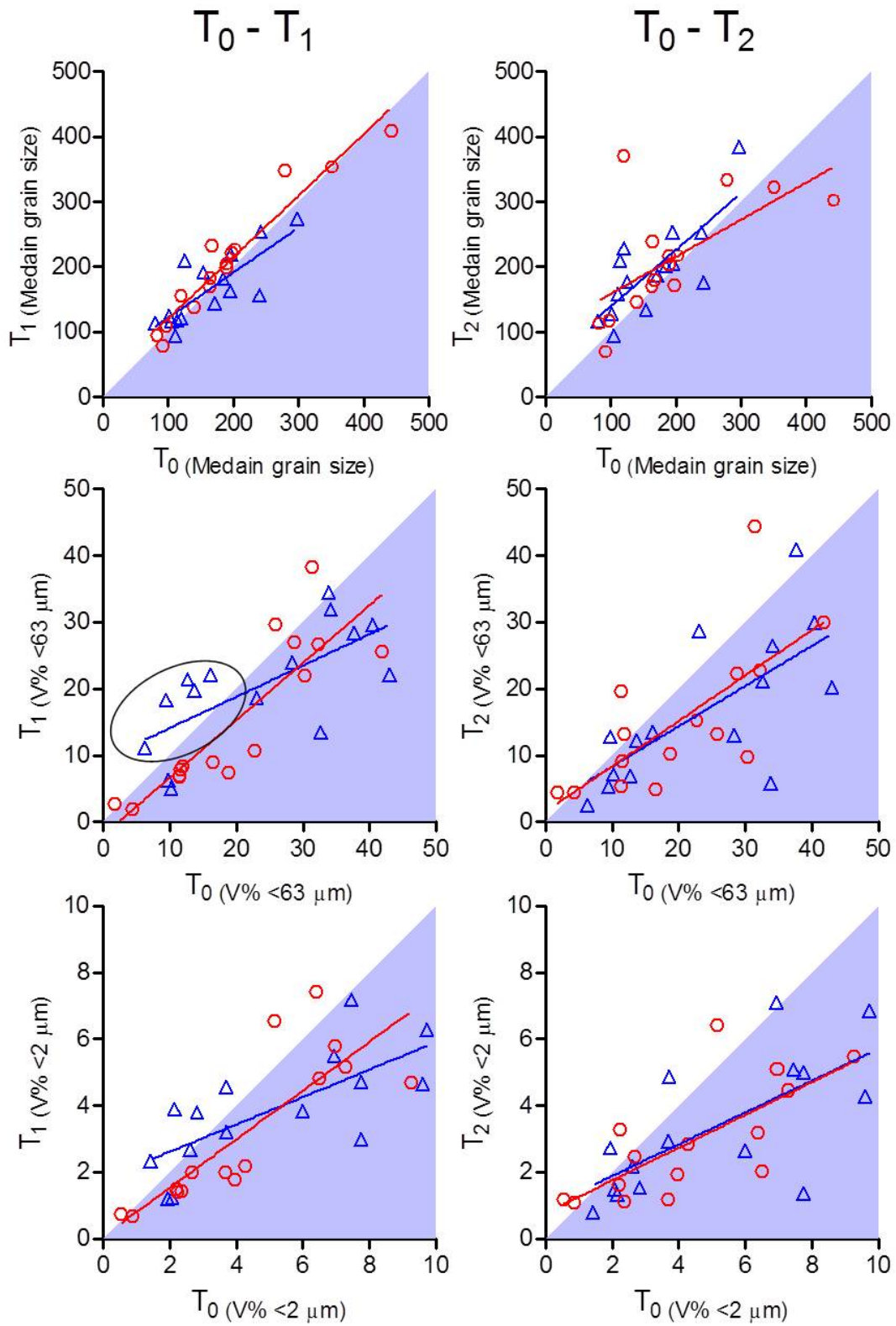


Figure 5. Change in sediment composition between  $T_0$  and  $T_1$  (left) and  $T_0$  and  $T_2$  (right) for fished (red) and control (blue) plots. Data points represent mean values per PRODUS plot. In the upper graphs results are presented for median grain size. The graph in the middle presents the change in silt fraction, and the lower graph presents the change in the clay fraction. Background of the plots is subdivided in a white (upper) and purple (lower) area. Data points in the purple area present a decrease over time, while data points in the white area present an increase over time. Regression lines are plotted to show whether there is interaction between the factor "fishing" and the sediment composition at  $T_0$ . Interaction is indicated by the difference in angle between the red and the blue line in each plot. The black circle in the  $T_0$ - $T_1$   $<63 \mu\text{m}$  silt fraction – plot demonstrates the data-point that show an increase in silt fraction in control plots, responsible for a significant effect of fisheries on the sediment composition.

## 2.14 Importance of mussels for sediment composition

In figure 6 we compare the mean change in the number of mussels from 12 boxcores per plot, with the change in mean sediment composition from the same boxcores. This comparison was made for the median grain size and for the clay fraction. Results showed that a decrease in mussel abundance between two subsequent sampling campaigns correlates with a decrease in clay fraction. For median grain size no correlation with the change in mussel abundance was observed.

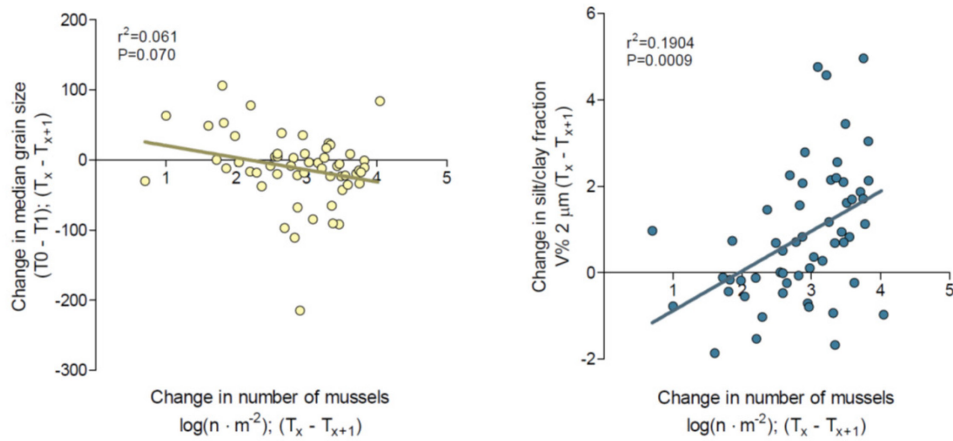


Figure 6. Change in mean sediment composition as a function of the change in mussel abundance at PRODUS plots.



### **3. Discussion and conclusion**

An important observation in this study was that sublittoral mussel beds show a high degree of variation in sediment composition at small spatial scale. Within the 100x100 m sample plots sediment compositions varied from fine to coarse sand with high and low silt content at some of the sites. Different statistical modelling approaches were taken to cope with this variation. The robust random intercept models with an additional variance structure diminished violation of spatial independence and homogeneity in the residual variance. These models did not detect an effect of mussel fisheries on sediment characteristics.

The simple linear models dealt with small-scale spatial variation by calculating plot averages on forehand (thereby ignoring any heterogeneity within locations) and modelling the sediment composition at  $T_1$  or  $T_2$  as a function of the situation at  $T_0$ . Some level of spatial dependence in the residual variance at the level of the PRODUS plots was solved by the interaction between the factor fishing and the background sediment characteristics. These simple linear models detected a negative effect on the silt fraction due to fisheries. What seems to happen is that at sites characterized by relatively coarse sediments the silt content increases between  $T_0$  and  $T_1$ . At fished sites, this effect was not found. In fact, a slight decrease in the silt content was observed. At sites with high silt content the presence of mussel beds nor the impact of fisheries on the silt content was observed. Only over time ( $T_0$ - $T_2$ ) a gradual decrease in the silt content was found for most sites, most probably due to the (almost) complete loss of mussels.

The accumulation of silt at coarse sand 'control' sites can be attributed to the presence and activity of mussels (Ragnarsson and Raffaelli, 1999). Callier et al. (2006) write that seed mussels contribute most to silt accumulation.

Overall, the silt content at locations decreased over time. Analysis of the relation between the silt content of the sediment and the presence of mussels in a sample demonstrated that mussels are indeed associated with a relatively high silt and clay content. We were only able to demonstrate this relation at the level of a boxcore (individual sample). Thereby, removal of mussels will affect the silt and clay content of the sediment at that very small spatial scale.

We conclude that mussel fisheries may decrease the silt and clay content of the sediment by removing mussels from the sea floor. This has an effect at the scale of a boxcore and at the scale of a location where background sediment characteristics are coarse.

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## **5. Quality Assurance**

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 57846-2009-AQ-NLD-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

## 6. Justification

Report number : C163/12  
Project number : 4308501015

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: Dr. F.E. Fey-Hofstede  
Researcher

Signature:

Date: 27 December 2012

Approved: Dr. B.D. Dauwe  
Head of department Delta

Signature:



Date: 27 December 2012

## Appendix A. Rapport van de Audit commissie en reactie van de Probus auteurs

Audit van het Project Onderzoek DUurzame Schelpdiercultuur (PRODUS)

### 4. Specifieke commentaren

#### 4.4. PR4: The effects of subtidal mussel seed fisheries in the Dutch Wadden Sea on sediment composition

Jammer genoeg is dit rapport weer in het Engels. De verwachting was dat, door accumulatie van faeces en pseudofaeces, de directe omgeving van de mosselen slibrijker zou worden naarmate de tijd zou vorderen. Visserij daarentegen, zou kunnen leiden tot verzanding als gevolg van bodemverstoring en het verwijderen van mosselen. Doordat er op de meeste locaties echter maar kort mosselen lagen, bleek het moeilijk om aan te tonen dat mosselen de bodem inderdaad slibrijker maken. Modellen die beviste en onbeviste proefvlakken één op één met elkaar vergeleken vonden geen aantoonbare verschillen. Echter, lineaire multiple regressie modellen, waarin het samenspel met de omgeving werd meegenomen, toonden wél korte termijn visserijeffecten aan. Deze effecten waren echter alleen zichtbaar op relatief zandige locaties. Hier zorgden mosselen voor verslibbing, terwijl visserij dit verhindert. Deze effecten namen af op de middellange termijn, vermoedelijk door het verdwijnen van mosselen op veel locaties.

Dit rapport kan nauwelijks op zichzelf staan, wat betreft inleiding en beschrijving van het monsterprogramma. De commissie stelt voor het samen te voegen met de rapportage over de andere variabelen in de boxcores, omdat het daarmee alle bemonsteringsdetails deelt, en de meeste hypothesen. Als het rapport op zichzelf blijft bestaan, moet gezorgd worden voor een correcte en volledige beschrijving van de bemonstering.

De auteurs hebben er voor gekozen om de rapporten niet samen te voegen. De volgende informatie is aan de methodensectie toegevoegd:

- Er is een referentie toegevoegd naar een kaart met bemonsteringslocaties
- Er wordt verwezen naar het onderzoekschip dat voor de bemonsteringen is gebruikt
- De methodensectie is opnieuw gestructureerd
- Voor verdere details over de bemonsteringsstrategie en de uitwerking daarvan wordt respectievelijk verwezen naar Ens et al 2007 en Van Stralen et al 2013.
- Voor meer achtergrond over het PRODUS-onderzoek wordt in de inleiding verwezen naar Smaal et al. 2013.

Er is een probleem met de cijfers in tabel 5. De (absolute waarde van de) t-waarde van klei is groter dan die van slib. Het aantal monsters is gelijk. Waarom is dan de p-waarde voor klei veel groter (en niet significant) dan voor slib? Ook in figuur 5 lijkt het effect minstens zo sterk voor klei als voor slib.

Dat klopt. Dit was een fout in de tabel. De betreffende t-waarde moet zijn -1.838. De p-waarde is wel correct. Het effect op de klei-fractie blijft dus niet significant. De tabel is gecorrigeerd.