SYNOPTIC INTERTIDAL BENTHIC SURVEY

SIBES

ACROSS THE DUTCH WADDEN SEA

Report on data collected from 2008 to 2010

Royal Netherlands Institute for Sea Research
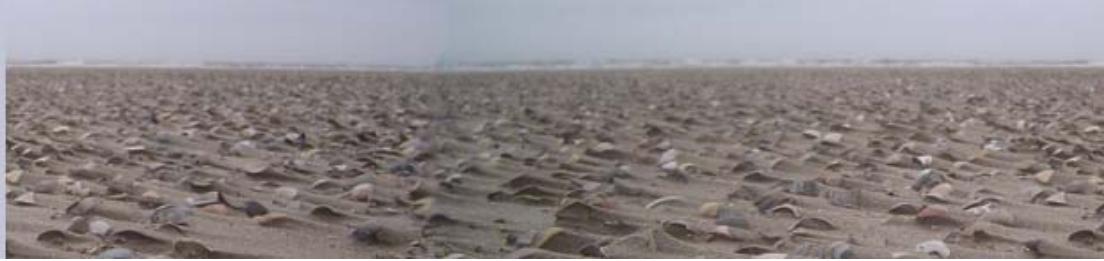
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Tanya J. Compton, Sander Holthuijsen, Anita Koolhaas, Anne Dekinga, Job ten Horn, Jeremy Smith, Ysbrand Galama, Maarten Brugge, Jaap van der Meer, Henk W. van der Veer and Theunis Piersma

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CONTENTS

 Appendices

1.   SUMMARY

The Wadden Sea, recognised as a wetland and heritage site of international importance (Ramsar status, UNESCO World Heritage Site), covers an area of almost eight thousand square kilometres and stretches from The Netherlands to Denmark, with a large part of this ecosystem falling within The Netherlands. Ecosystem services provided by the Wadden Sea to neighbouring coastal areas are numerous. Economic services include fisheries, and ecological services include essential habitat to migratory shorebirds that fuel-up in the Wadden Sea prior to flying to the Arctic or Africa.

Benthic macrofauna are an integral component of the Wadden Sea ecosystem. Defined as organisms greater than 1 mm in size, they provide food sources to humans and many animal species, especially migratory shorebirds. In addition, they are pivotal in keeping the ecosystem functioning, as they recycle nutrients, decompose organic matter and regulate nutrient cycles. Thus substantial changes to the macrofauna community could induce changes that cascade through the ecosystem.

To assess whether there are changes in the ecosystem due to land subsidence, a "Synoptic Intertidal Benthic Survey" (SIBES) has been completed annually across the entire Dutch Wadden Sea since 2008. The SIBES survey is comprehensive in the Dutch context and covers an area of 2483 km^2 or ~4500 sampling stations.

Results from the SIBES survey over the last three years show indications that some species had higher densities in the areas of predicted subsidence, however, these species differed among years. In 2010, *Macoma balthica*, *Alitta succinea* and *Ensis directus* had significantly higher densities in the areas of predicted subsidence. In 2009, it was observed that *Alitta virens*, *Urothoe poseidonis*, *Magelona johnstoni* and *Heteromastis filiformis* differed significantly in the predicted gas subsidence regions relative to the remainder of the Wadden Sea. In 2008, *Nephtys cirrosa*, *Pygospia elegans*, *Marenzelleria viridis* and *Spio martinensis* were different in the predicted subsidence areas. Land subsidence by gas extraction might be a direct cause for significant differences in species abundance within the predicted subsidence areas. However, a better understanding of other environmental drivers of species abundance will be necessary to confirm our findings. Elucidation of the drivers of species abundance, in combination with repeated surveys will increase the power of our analyses.

Despite the observed spatial and temporal variation in community and species patterns, interesting parallels in the distribution patterns, densities, biomass and species richness of the benthic macrofauna were noted between years. For example, high densities, high biomass and high species richness of the macrofauna were found along the Frisian coastline, close to Schiermonnikoog, near Griend and also in the vicinity of Vlieland. A comparison of the areas predicted to subside versus the remainder of the Wadden Sea showed that the

total biomass of all macrofauna at a single sampling point (g AFDM/m^2) was consistently lowest in the Wadden Sea near East Groningen for all three years. These results are similar to the alpha diversity estimates. Such recurring patterns between years suggests that given more SIBES surveys are completed we will be able to understand factors driving macrofaunal species distributions, and identify effects of gas subsidence.

In conclusion, the SIBES time series indicates that some species show differences in the predicted areas of subsidence. However, there is also a large amount of variation in the species responses between years, indicating the inherent natural variability of the system. We strongly suggest that the SIBES sampling continues so that insights into factors driving natural variation can be gained, and consequently the role of anthropogenic factors can be determined.

2.   PREFACE

The Wadden Sea is an important intertidal area (Ramsar status, UNESCO World Heritage Site recognition) that provides numerous economic and ecological services to the Dutch population. To assess whether there are changes in the ecosystem due to land subsidence, a "Synoptic Intertidal Benthic Survey" (SIBES) has been completed annually across the entire Dutch Wadden Sea since 2008. The SIBES survey covers an area of 2483 km^2 or ~4500 sampling stations.

The goal of SIBES is to monitor macrofauna abundance, biomass and community composition for changes associated with land subsidence. But to detect changes in macrofauna, e.g. due to land subsidence, it is aimed that the sampling be conducted over long temporal scales, as well as large spatial scales. Long-term sampling is essential to teasing apart inherent natural variation from anthropogenic effects.

SIBES was funded by the ZKO Wadden Sea programme for monitoring of the western Wadden Sea and by NAM for the ecological monitoring of the gas drilling areas in the eastern Wadden Sea.

3.   INTRODUCTION

The Wadden Sea recognised as a wetland of international importance (Ramsar status, UNESCO World Heritage Site recognition), covers an area of almost eight thousand square kilometres and stretches from The Netherlands to Denmark. The Wadden Sea not only provides important ecological services but also numerous economic services to populations along its coastline (Wolff 1983). Economic services include fisheries, and ecological services include essential habitat to migratory shorebirds who use this area to fuel-up prior to flying to the Arctic or Africa (Beukema 1976, Wolff 1983, Zwarts 1996, van de Kam et al. 2004).

The Dutch Wadden Sea forms a large part of this dynamic tidal system; ~2500 of the 8000 km^2 (Wolff 2000). The proximity of the Dutch Wadden Sea to numerous coastal populations has meant that this area has a long history of human exploitation and environmental change (Wolff 2000). For example, a large area of tidal flats were lost after the closing of the Afsluitdijk in 1932 and seagrass beds disappeared after the outbreak of a disease in 1932 (see reviews by Wolff 2000, Wolff 2005, Kraan et al. 2011). Although these changes are well known, it is difficult to pinpoint the consequences of such changes because at that time the Wadden Sea was not monitored.

In summary, it is difficult to detect change in a system without monitoring both before and after an impact (Underwood 1997). Thus to identify whether factors associated with human exploitation are having negative impacts we need to monitor the system. But teasing apart the drivers of change either due to natural variation or human induced factors is a major challenge. To tease apart these factors, data needs to be collected across large spatial and temporal scales.

4.   SIBES

In 2008, the NIOZ initiated a synoptic intertidal benthic survey (SIBES) across the entire Dutch Wadden Sea with the goal of monitoring mud-dwelling organisms or macrofauna for their abundance, biomass and composition. To tease apart effects of subsidence, due to gas drilling, from the inherent natural variation in the system it is aimed that this survey be conducted over long temporal and large spatial scales. Without long-term data, short-term natural variability can mask chronic and/or cumulative impacts, often until critical changes occur (Hewitt et al. 2001, Hewitt & Thrush 2007).

Macrofauna are defined as benthic organisms that are greater than 1 mm in size (Gray & Elliott 2009), and were chosen as the unit of examination for this work as they are relatively cost-effective to monitor and have proven to be highly responsive to change (Beukema et al. 1999, Hewitt et al. 2001, Hewitt & Thrush 2008, Hewitt & Thrush 2007, Warwick et al. 2010). Furthermore, as most macrofauna are sessile during most of their life cycle and relatively long-lived they can provide an integrated response to environmental variation at a particular place over a relatively long time. Apart from these aspects, macrofauna are also of considerable economic value to the Dutch population, not only as a food source to humans, e.g. blue mussels *Mytilus edulis*, but also for the ecosystem services they provide, e.g. decomposition of organic matter, nutrient cycling, and regulation of nutrients (Dame et al. 1991, Dame & Prins 1997, Levin et al. 2001, Gray & Elliott 2009). Also from an ecological perspective macrofaunal species provide an important food source for migratory and non-migratory shorebirds (Zwarts 1996, Kam et al. 2004), and other species living in the Dutch Wadden Sea (Wolff 1983). In summary, macrofauna are an integral component of the Wadden Sea ecosystem, and substantial changes to the macrofauna community could induce changes that cascade through the ecosystem.

Long-term data series are powerful for detecting factors driving change, as they provide useful information on the temporal dynamics of species and thus can be used for identifying the factors driving natural variation. For example, some of the year-to-year variability (Beukema et al. 2010), and seasonal variability of macrofaunal species abundances (Beukema 1974) were untangled using the long-term data series from the Balgzand. In the Baltic Tellin *Macoma balthica* adult density fluctuations were linked to recruitment variability, whereas growth and survival were associated with food limitation, competition and predation (van der Meer et al. 2001). Such recruitment variability appears to be associated with larval survival in the water column (Bos et al. 2006). In another study, variation in the population densities of two polychaete species, were described by predator prey dynamics, as the prey population density of *Scoloplos cf. armiger* was negatively related to the density of the predator *Nephtys hombergii* (van der Meer et al. 2000). Hewitt & Thrush 2008 could identify the influence of the El Niño oscillation on macrofaunal abundances, relative to local scale environmental variables, using 13 years of monitoring data from Manukau Harbour, in New Zealand.

Understanding the natural variation of macrofauna within a sedimentary system provides a basis for estimating whether anthropogenic drivers are having effects in coastal sedimentary systems (Hewitt et al. 2001). For example, using the long-term Balgzand data, it was shown that the macrofauna abundances doubled along with the doubling of planktonic algae stocks, as a consequence of mild eutrophication (Beukema 1997). Other studies have shown that structural and functional changes of macrofauna occur when there are natural and anthropogenic sedimentation events (Turk & Risk 1981, Peterson 1985, Johnson & Frid 1995, Smith & Kukert 1996 in Ellis et al. 2000).

Data obtained across large spatial and long-term data series, via the SIBES programme, will provide insights into whether subsidence due to gas drilling is affecting macrofaunal species distributions. We have currently analyzed three years of data (2008 to 2010, this report), and have started to process data from the fourth year (2011). In 2012 the monitoring program on subsidence by gas production will be evaluated by combining all ecological data collected over the period 2008-2011. The evaluation report will be published in May 2013.

5.   AIMS

In this report we will examine the most recently analysed SIBES data from 2010, as well as previous data (2008 and 2009), with respect to the areas of predicted tidal-flat subsidence.

First, we will compare community attributes of the macrofauna at the predicted subsidence areas versus the remainder of the Wadden Sea.

Second, we will compare the density responses of individual species responses in the areas of subsidence versus the remainder of the Wadden Sea.

6.   METHODS

Field sampling

Long-term monitoring programmes aim to measure the change in macrofaunal species abundance across an area over time. It is well known that the sampling design for such programmes is key to obtaining an accurate estimation of annual abundance estimates, and other associated changes (van der Meer 1997, Hewitt & Thrush 2007). Thus prior to implementing SIBES, the best sampling design for monitoring macrofauna at large temporal and spatial scales was identified given the goals of SIBES (Bijleveld et al. 2012). This sampling design allows for the estimation of spatial processes at distances of less than 500 m but still maintains the large-scale sampling overview, and thus should provide the ability to tease apart human from environmental drivers of species distributions.

The sampling design encompasses the entire intertidal Dutch Wadden Sea, with sampling at 500 m intervals and 10% random sample points (see Figure 1). From 2009 onwards, additional sampling points were added to the basic SIBES sampling design to (1) incorporate the Ems Dollard region and (2) to increase

the sampling resolution in the areas of Oost Ameland and Moddergat (see blue dots in Figure 2).

Sampling was completed on an annual basis in summer from June to about October, depending on weather. The NIOZ research vessel, Navicula, was used as a platform to access the sample areas across the Wadden Sea. During low tide we sampled by foot. In areas where it was too deep or muddy to access by foot, small inflatable boats were used to sample the SIBES sites. Sampling locations, ~4500 in total, were found with a handheld GPS (WGS84 as map datum). At each site sampled by foot, a single core of 0.0175 m^2 was taken to a depth of ~25 cm. By boat, two cores of 0.018 m^2 were taken to a depth of ~25 cm.

All macrobenthos samples were sieved on a 1 mm mesh in the field. After sieving, bivalves were separated from the other macrobenthic species for later analysis in the laboratory. The bivalve samples were frozen, whereas the remaining macrobenthic species were preserved using a 4% formalin solution.

Sediment samples were taken using a centrifuge tube to a depth of 4 cm and then frozen at -20°C. The sediment samples were taken at all sample points in 2009 and 2010, i.e. every 500 m and at the random sample points, and at 1 km intervals in 2008.

Laboratory analysis

All shellfish were identified to species level (Hayward & Ryland 1995) and measured for their maximum length using digital callipers (precision of 0.01 mm). An ash free dry mass (AFDM) was then determined for each individual greater than 8 mm in size or for multiple individuals together when their size was smaller than 8 mm in length. To determine the AFDM, the wet meat of an organism is placed into a crucible and then dried at 60°C for 48 hours. Once dried, the dry sample goes into a desiccator to cool prior to weighing to an accuracy of four decimal places (Mettler Toledo XS204). The dried organism is then put into an incinerator where the organic matter is burnt, so that only the ashed matter remains (560°C for 5 hours). Once incinerated, the ashed sample goes into a desiccator to cool prior to weighing the ashed mass to an accuracy of four decimal places.

Figure 1. SIBES sampling sites across the Dutch Wadden Sea (blue), with additional points (Extra) around the Oost Ameland and Moddergat region denoted in dark blue and random sample points denoted in purple. In 2008, 2009 and 2010 we sampled 4307, 4733, 4528 sample points, respectively.
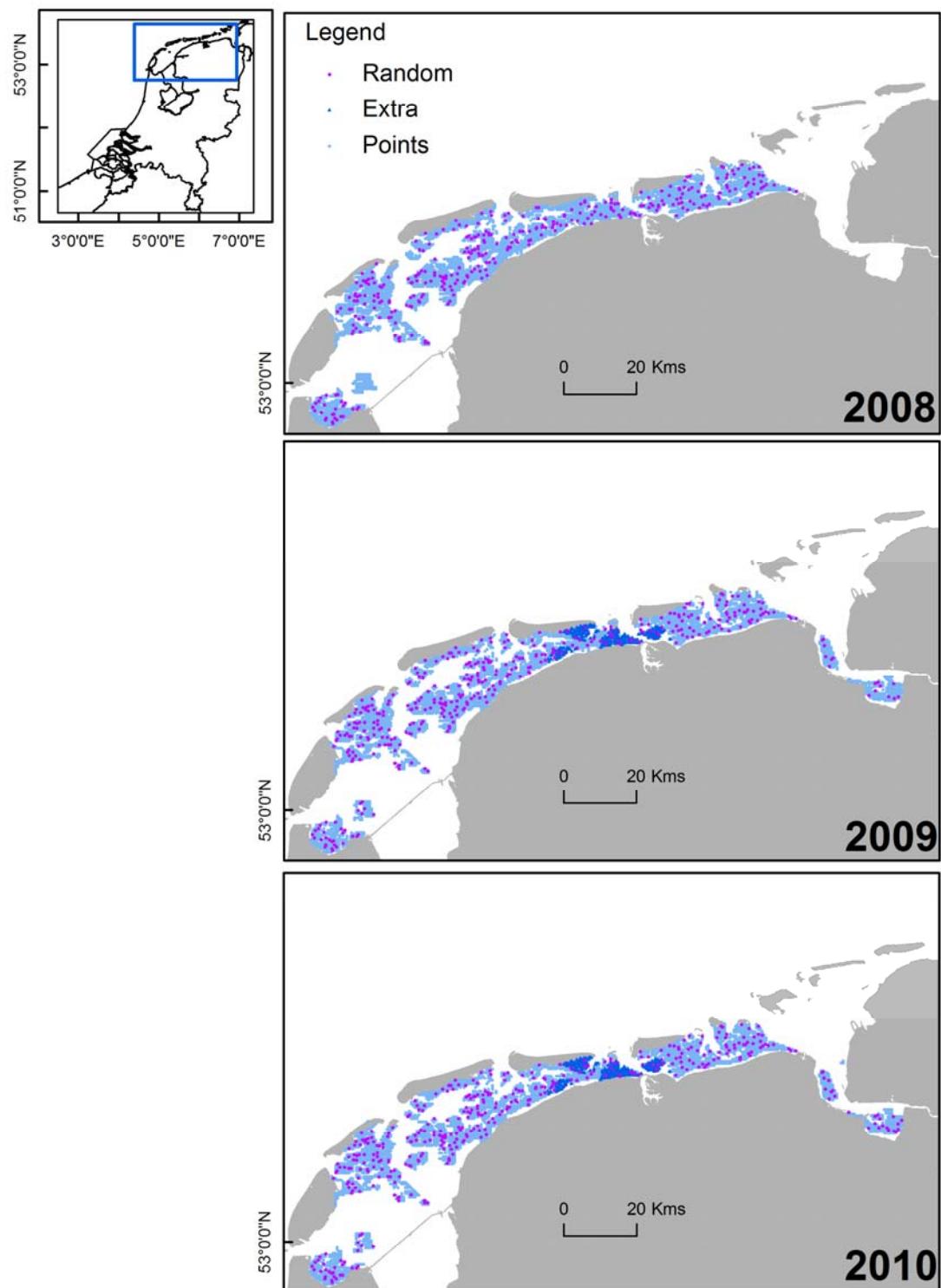
All other smaller organisms, predominantly crustaceans and worms, were identified to the highest taxonomic level possible; hereafter named operational taxonomic units (OTUs). In the first instance, these samples were stained using rose Bengal dye (C.A.S. no. 632-68-8) for 24 hours. These samples were then flushed with fresh water for 10-20 minutes over a 0.5 mm sieve to wash away the remaining formalin. The organisms were then carefully placed on a petridish for identification and counting under a binocular microscope (8 - 40 x magnification). Identification of the macrobenthic species was completed according to the ISO guidelines (ISO 9001:2008 nr. K57663/01); and classified according to Hartmann-Schröder (1996) and Hayward and Ryland (1995). For protocols, see Appendix 10. Specifically, worms and amphipods were identified to either a genus or species level, whereas oligochaetes were identified to a class level. We currently recognize that there are cryptic species in the Wadden Sea, however, without further taxonomic and genetic analyses we cannot discriminate between these species (Luttikhuizen et al. 2011). Once samples were counted and identified, the AFDM of either individuals or multiple individuals of the same species were determined.

Sediment analysis

Prior to analysis the samples were freeze-dried for up to 96 hours prior to analysis and then homogenized with a mortar and pestle. Homogenized samples were weighed to within 0.5 to 5 grams, depending on the observed estimated grain size, and placed into 13 ml Polypropylene Auto-sampler tubes with degassed Reversed Osmosis water. The sediment samples were analysed using a "biological" approach, meaning that after sieving over a mesh size of 2 mm, the calcium carbonate, including shell fragments and organic matter, were not removed from the samples. Samples were then shaken vigorously with a vortex mixer for 30 seconds before their grain size was determined using a coulter counter. The coulter counter then uses laser diffraction and Polarization Intensity Differential Scattering technology to estimate grain sizes (Coulter LS 13 320, optical module "gray", grain sizes from $0.04 - 2000$ μm in 126 size classes). For protocols, see Appendix 11.

Currently the sediment samples from 2008 and 2010 have been analysed, and the 2009 samples are in the process of being analysed.

Predicted subsidence and SIBES

There are several gas extraction regions located in close proximity to the Dutch Wadden Sea. These include "Ameland-Oost" (Figure 2), which has been in operation since 2006, the "Moddergat-Lauwersoog-Vierhuizen" region, which has been in operation since 2007 and spans two regions of the Wadden Sea, the northern Lauwersoog region and the East Groningen region of the Wadden Sea (Figure 2). In this report we will use predictions of land subsidence up to 2012 for our sampling sites (see Figure 2), as determined by a model from the NAM.

Figure 2. Predicted subsidence at the SIBES sampling points near the three areas of gas extraction in the eastern Dutch Wadden Sea: A – Oost Ameland, B – Moddergat and C- Oost Groningen. The shaded background indicates the Wadden Sea bathymetry (RIKZ, 2009). Depth data is collected by the Rijkswaterstaat (RIKZ), using a dense grid of sampling points ('vaklodingen' 2008 data, methods in Perluka et al. 2006) and converted into an elevation map by NAM (EP200905260877).
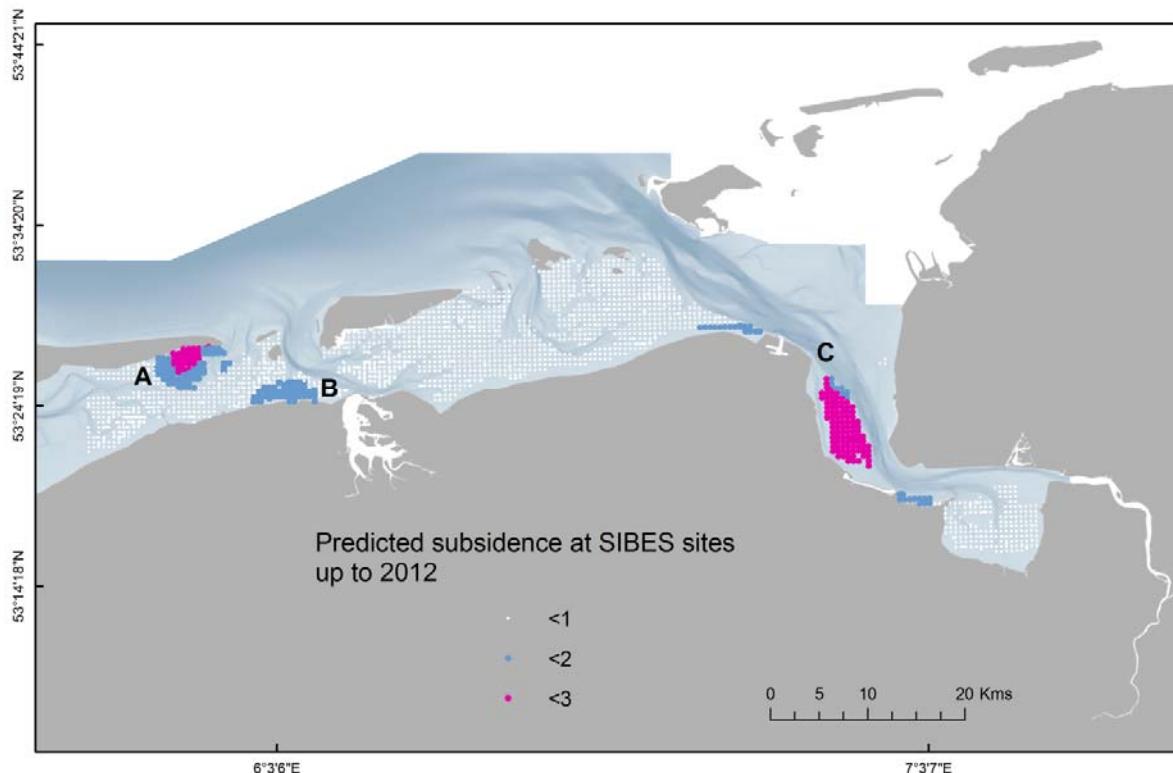
7.   ANALYSIS

Community patterns

To identify general trends in community patterns in the areas of land subsidence versus outside of these areas, for all years we calculated total macrofauna density per point (nrs/m^2), the number of macrofaunal species in a sample core (alpha diversity) and the total biomass of macrofauna in a sample (g AFDM/m^2). Using the predictions of subsidence as provided by the NAM, we classified our sample sites either as Oost-Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) or remainder of the Wadden Sea (Ws, see Figure 2).

Individual species

Generalized linear model analysis – raw data

Using the statistical approach, as described by Aarts (2010, 2011), we examined whether we could detect changes in the operational taxonomic unit (OTU) densities from the SIBES data of 2010 with respect to the predicted areas of subsidence; "in" indicating those sample sites inside the aforementioned gas regions and "out" indicating the sites outside of this region. The modelling outcomes are presented in two stages. In the first, we use the true data to test whether there are significant differences between densities of an operational taxonomic unit (OTU) "in" or "out" a region. But due to the inherent spatial variability of the Wadden Sea, and thus the chance that a significant difference between areas is more general across the Wadden Sea, a second approach (Monte Carlo simulations) is required to test whether a significant effect is indicative that the areas of predicted subsidence really differ compared to other areas in the Wadden Sea.

To examine whether changes in mean OTU densities differed significantly inside the gas drilling areas versus outside, we ran a generalized linear model for 2010 alone, with quasi-Poisson distributed errors:

y ~ G   equation 1

where y is the density of an OTU (nrs/m^2) and G is the factor "in" versus "out".

We tested whether changes in the density of an OTU were occurring: (a) for all areas, (b) for the Oost-Ameland region alone, (c) for the Moddergat region alone or (d) for the Oost Groningen region alone. For all models, we had a criteria that at least eight individuals should be found within the "in" and "out" regions for a model to run.

Generalized linear model analysis – simulations

To run the Monte Carlo simulations and thus to test whether the results in the predicted subsidence area differed relative to the rest of the Wadden Sea, 1000 randomly simulated "in" and "out" data were tested using the same models (equation 1, also see Appendix 5). Sampling regions were selected with replacement and were chosen by randomly selecting a single random point within

the SIBES data set and taking the closest points within the region of this single point. The number of randomly selected sample points representing the "in" region matched the number of points sampled in the areas of predicted subsidence, i.e. Oost Ameland, Moddergat and Oost Groningen. Similarly, to the previous models the criteria for a model to run was at least eight individuals recorded within the "in" and "out" regions.

By running the GLM model 1000 times on random subsets of the SIBES data outside of the gas drilling areas, we were able to estimate an F-distribution from the model runs for a particular OTU. This F-distribution could then be compared with the true GLM model F-value (above) for the same OTU to determine whether the previously determined significant value ($p < 0.01$) is rare in the simulations, and thus indicates a significant probability that the areas of subsidence differ relative to the other areas of the Wadden Sea.

To examine these results within the context of the data collected in 2008 and 2009, we examined the densities of the OTUs identified as significantly different by the models in more detail. Specifically, we compared the densities of these OTUs in the predicted subsidence regions versus the remaining Wadden Sea using boxplots and mapped distributions for all three sampling years. Maps were drawn in ArcGIS 9.3.

8.   RESULTS

Community patterns

Densities of total macrofauna in a sample (nrs/m^2) showed that on average differences in total macrofaunal densities were minimal between the predicted subsidence areas versus the remainder of the Wadden Sea in 2009 and 2010 (Figure 3), and tended to be lowest at Oost Groningen and Ameland in 2009. The mapped total densities of macrofauna highlighted temporal and spatial differences from 2008 to 2010 (Figure 4). Notably, in 2008 the highest macrofaunal densities were more spatially restricted compared to the highest recorded macrofaunal densities in 2009 and 2010. For all three years, an interesting spatial pattern reoccurs. Highest densities of macrofauna occur along the Frisian coastline, near Vlieland, Balgzand and in between Schiermonnikoog and the mainland (Figure 4).

The total number of OTUs counted in a sample point (alpha diversity, Figure 3), showed that Oost Groningen consistently had a lower number of species relative to the remaining areas of predicted subsidence and the Wadden Sea (~5 versus ~8 per core) for all years. Furthermore, a comparison of the mapped alpha diversity, based on all operational taxonomic units (OTUs), at the SIBES sample points showed interesting parallels between years. Alpha diversity appeared to be highest around northern Texel and Vlieland and near Griend and low in the Ems Dollard (Figure 5). Similarly to the density observations (boxplots and maps, Figures 3 and 4), mapped alpha diversity was lowest in 2008 compared to 2009 and 2010. Total OTU richness for the entire Wadden Sea (Gamma diversity) was also lowest for 2008 (Gamma diversity: 86 OTUs) compared to the species richness estimated in 2009 and 2010 (Gamma diversity 2009: 100 and Gamma diversity 2010: 93 OTUs).

A comparison of the areas predicted to subside versus the remainder of the Wadden Sea showed that the total biomass of all macrofauna within a point (g AFDM/m^2) was consistently lowest in the Wadden Sea area near Oost Groningen for all three years, similar to the alpha diversity estimates (Figure 3). There was also a slight tendency for biomass to be higher at Moddergat in 2008 and 2009 compared to the other sites (Figure 3). In 2010, the greatest variation in biomass was encountered at Oost Ameland (Figure 3). Similar to the mapped densities of all macrofauna, biomass tended to be highest along the Frisian coastline, near Vlieland, Balgzand and in between Schiermonnikoog and the mainland (Figure 6). The Ems Dollard region had the lowest biomass. Mapped biomass appeared to be highest in 2009 relative to the other two years.

Figure 3. A comparison of the data distributions of total density (nrs/m^2, square root transformed), richness and biomass (g AFDM/m^2) within a sample at the predicted subsidence areas versus the remaining Wadden Sea. Boxplots represent the median value (solid middle line) and the upper and lower quartile (upper and lower hinges of the box), and the whiskers represent the range of values around the box. The dots (blue) indicate the mean values. The location names are abbreviated Oost Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) and the remaining Wadden Sea (Ws).
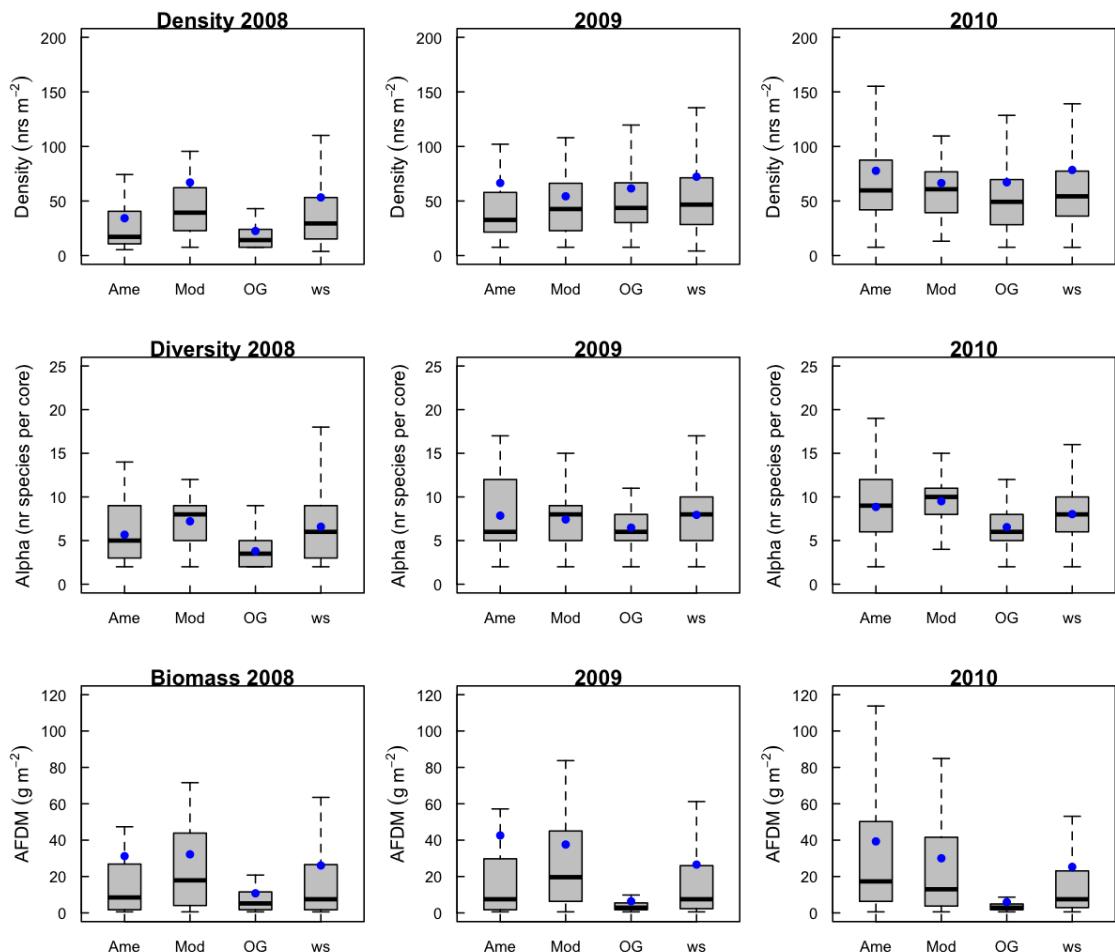
Figure 4. Densities of macrofauna (numbers per square metre, nrs/m^2) at the SIBES sample sites from 2008 to 2010. Darker colours indicate higher densities.
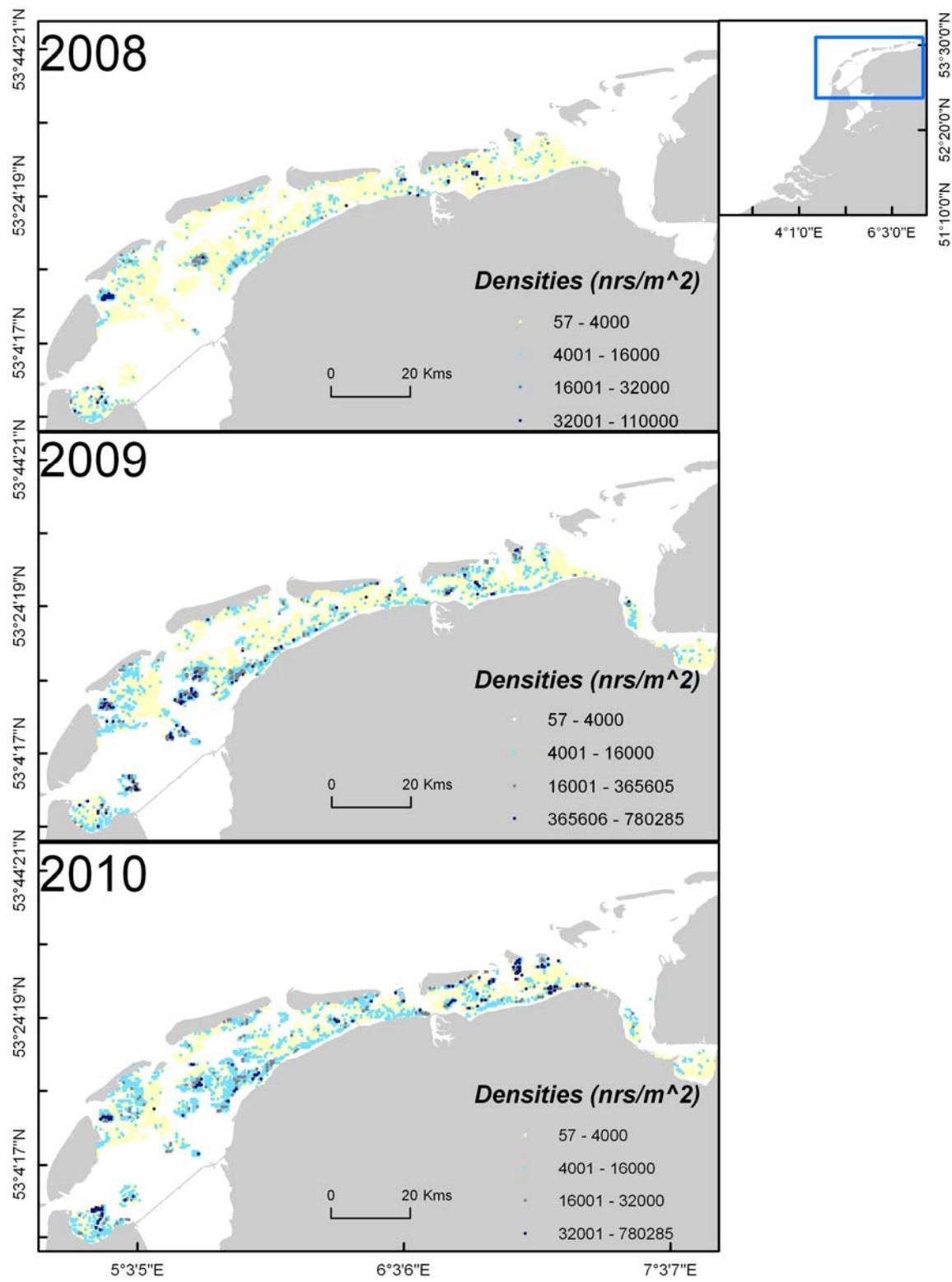
Figure 5. Number of operational taxonomic units (OTUs) or alpha diversity, at each sample point from the SIBES survey for all years. Darker colours highlight areas of higher alpha diversity.
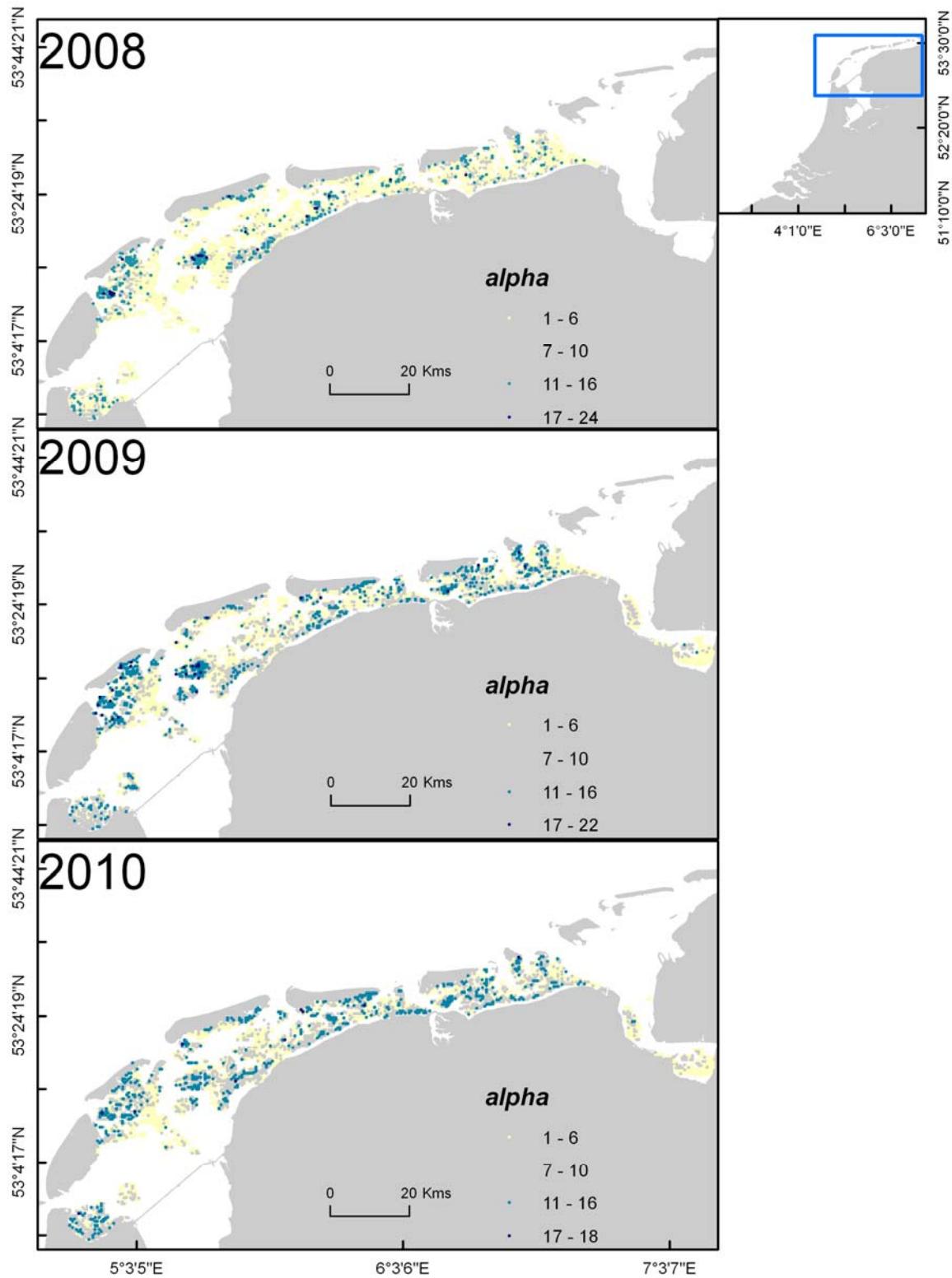
Figure 6. Total biomass (g AFDM/m^2) at the SIBES sample points for 2008 to 2010. Darker colours indicate areas of highest biomass.
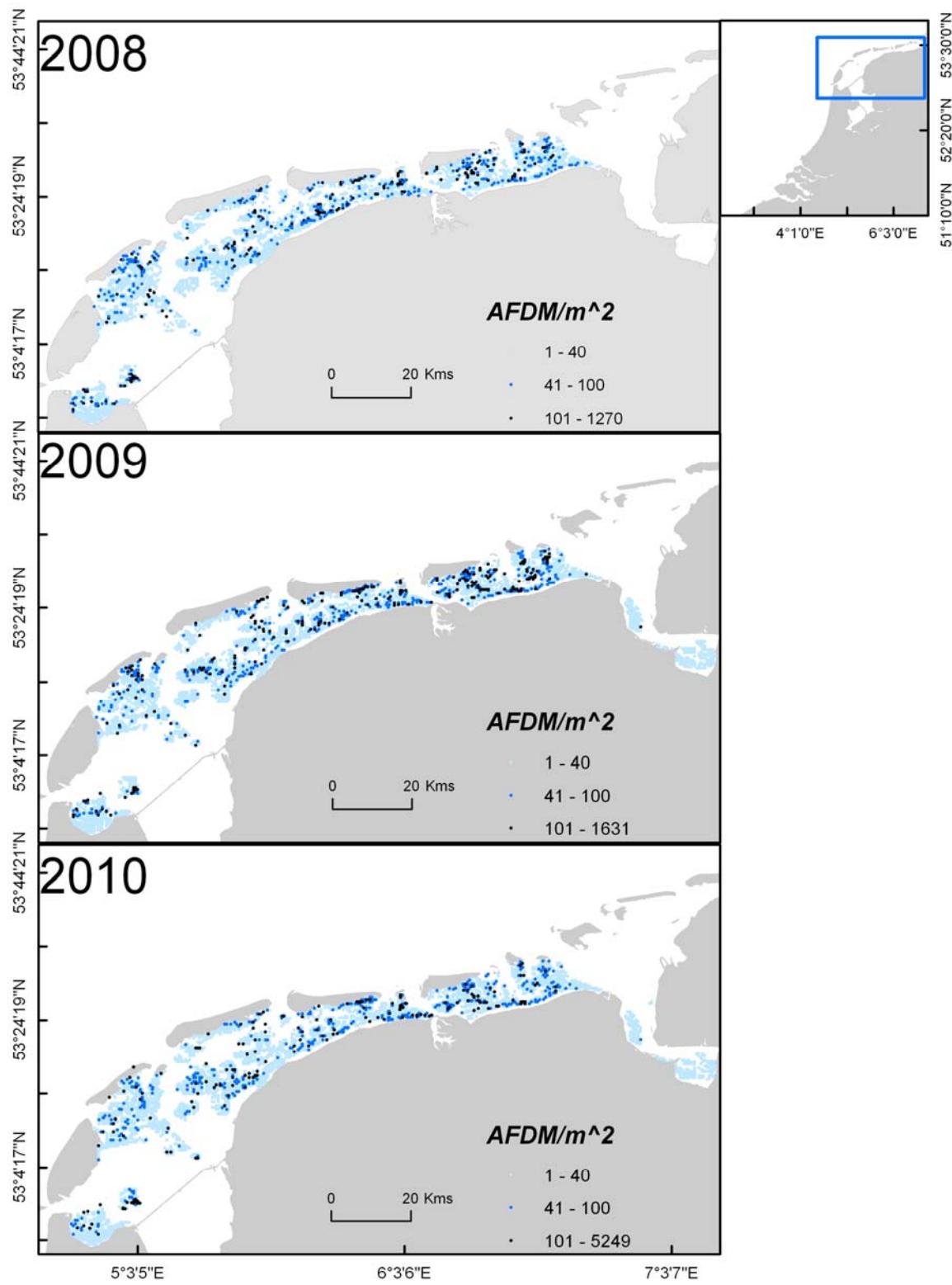
Individual species

Operational taxonomic unit (OTU) results from the Generalised Linear models (GLM raw data) can be found in Appendix 1. Here, significant OTU results from the GLM models are presented where all areas of predicted subsidence "in" (Oost Ameland, Moddergat and Oost Groningen) are compared with the remainder of the Wadden Sea "out".

Figure 7. The histogram (A) provides a comparison between the F-value from the raw data model (line) versus the F-values from the simulated GLMs (bars). In the case of *Macoma balthica*, the true F-value (42) occurs infrequently in the simulated models, indicating the densities in the predicted subsidence regions ("in") differ relative to the remainder of the Wadden Sea ("out"). Boxplots show the data distribution of *Macoma balthica* densities (nrs/m^2, square root transformed) in the predicted areas of subsidence versus the remainder of the Wadden Sea. In the first boxplot (B), the data used in the GLM model is represented: "in" versus "out". In the lower boxplots (C-E), densities of *M.balthica* at the three areas of predicted subsidence are compared with the remainder of the Wadden Sea for the years of 2008, 2009 and 2010. The location names are abbreviated Oost Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) and the remaining Wadden Sea (Ws). In all boxplots the dot (blue) gives the mean value. Photo by M.Mulder.
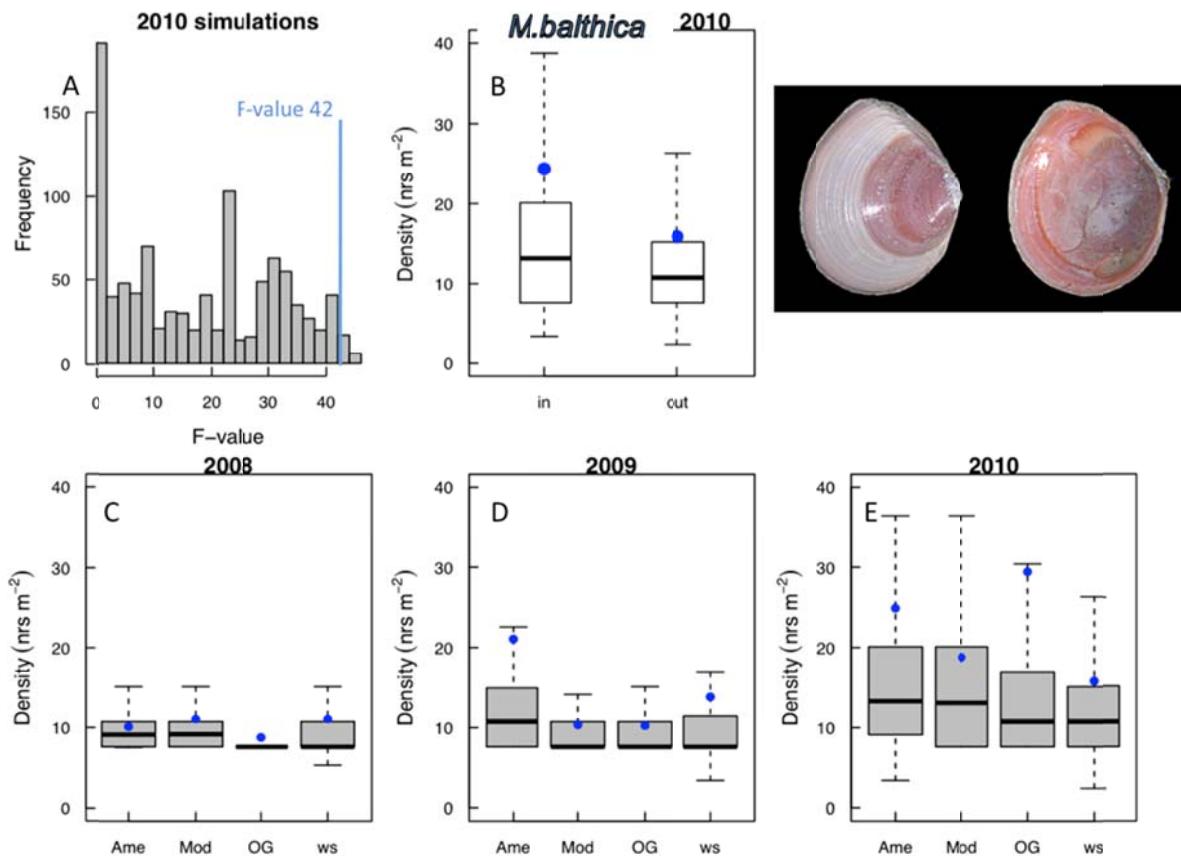
Figure 8. Densities (nrs/m^2) of The Baltic Tellin *Macoma balthica* across the Dutch Wadden Sea in the three survey years. Darker colours indicate higher densities. Note that the Ems Dollard was not sampled in 2008.
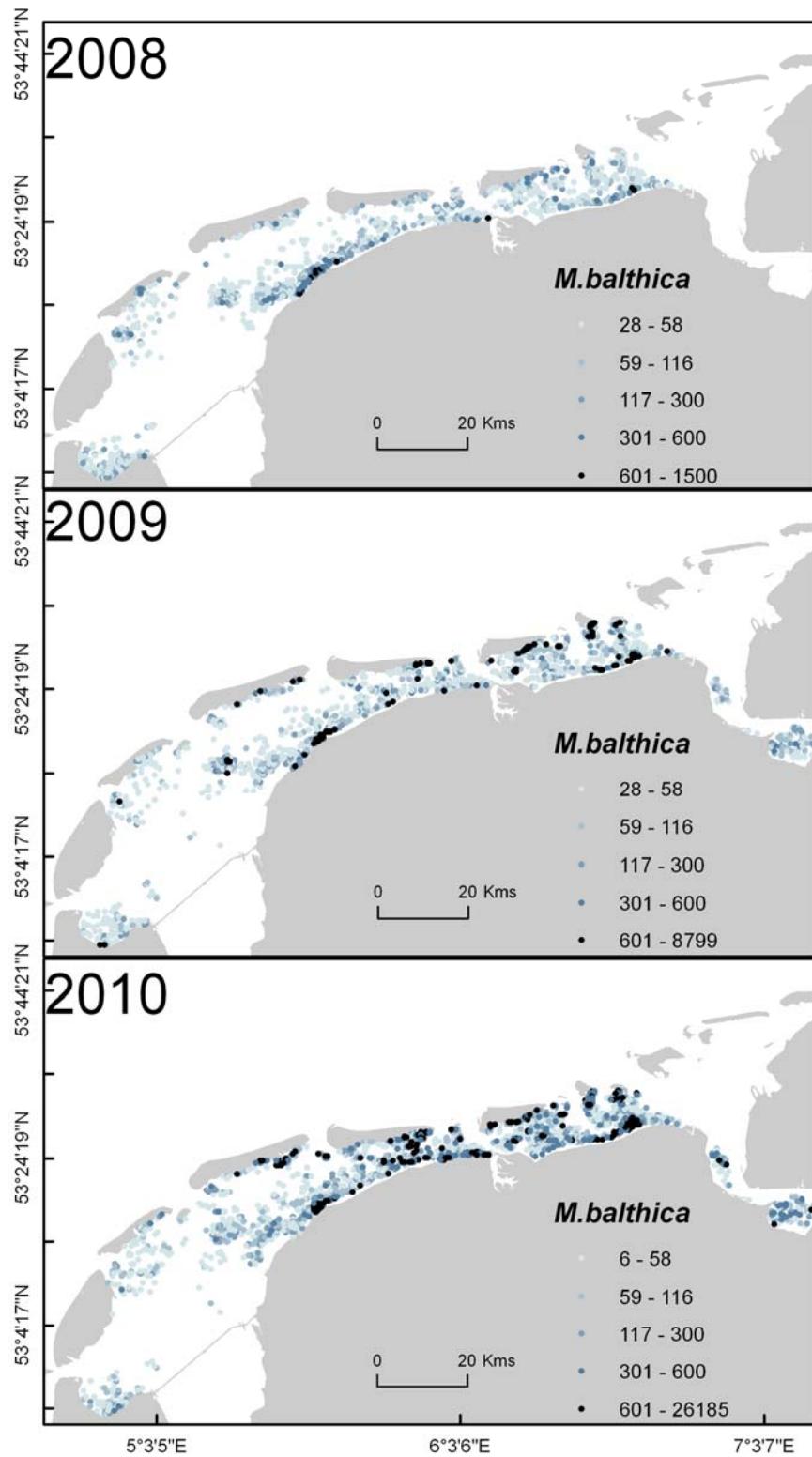
The models identified that three OTUs had significantly different densities either "in" or "out" the subsidence regions of the Wadden Sea in 2010. The OTUs that had a positive density increase in the predicted subsidence regions included the Baltic Tellin *Macoma balthica* (Appendix 1, est 1.13, F-value = 42, p < 0.001), the polychaete worm *Alitta succinea* (est 1.84, F-value = 137, p<0.001) and the razor shell *Ensis directus* (Appendix 1, est 1.25, F-value = 25, p<0.001). A comparison of these results with the simulated GLM model results indicated that the "in" regions differed relative to the remainder of the Wadden Sea (see histograms of simulated F-values in Figures 7, 9, 11).

OTUs that had a negative density response included the polychaete worm *Scoloplos cf. armiger* (Appendix 1, est -0.57, F-value = 25, p<0.001) and the capitellid worm *Capitella capitata* (Appendix 1, est -0.84, F-value = 12, p<0.001). However, the GLM simulations indicated that these results were not significant, i.e. that these density decreases were not something exceptional for the predicted subsidence regions but also occurred when simulated in other areas of the Wadden Sea (Figures 13 and 15, see histograms and lines representing the GLM-raw data F-values).

In the case of *M. balthica*, densities in the predicted subsidence areas "in" were significantly higher than densities in the remainder of the Wadden Sea "out" in 2010 (Appendix 1, Figure 7). An examination of the density data showed that mean densities in the subsidence regions were generally higher and showed a greater range of variation than the remaining Wadden Sea. A more detailed comparison of *M. balthica* densities for all three years showed that Ameland, Moddergat and Oost Groningen all had higher mean densities and higher maximum values than found in the remainder of the Wadden Sea (Figure 7). Notably, mean densities of *M. balthica* were lowest in 2008 but increased in 2009 and 2010. In 2009 Oost Ameland had the highest mean densities of *M. balthica* relative to the remainder of the Wadden Sea.

The mapped distributions of *M. balthica* showed that the areas with the highest densities occurred along the Frisian coastline and between Ameland and Schiermonnikoog in all three surveyed years. An observational comparison with the sediment data showed that these are some of the muddiest areas of the Wadden Sea (Figure 17). Lowest densities of *M. balthica* were found in the Western Dutch Wadden Sea. Although there is a trend for *M. balthica* to be found in highest densities at similar locations between years, it is notable that there is considerable variation in the densities between years, and in the patch size of such high-density areas. For example, a comparison between the years shows that densities were lowest in 2008 and highest in 2010 and that patch sizes were larger in 2010.

Figure 9 The histogram (A) provides a comparison between the F-value from the raw data model (line) versus the F-values from the simulated GLMs (bars). For *Alitta succinea*, the true F-value (135) does not occur in the simulated models, indicating the densities in the predicted subsidence regions ("in") differ relative to the remainder of the Wadden Sea ("out"). Boxplots show the data distribution of *Alitta succinea* densities (nrs/m^2, square root transformed) in the predicted areas of subsidence versus the remainder of the Wadden Sea. In the first boxplot (B) the data used in the GLM model is represented: "in" versus "out". Here, the F-value is rare in the simulations indicating the densities in the predicted subsidence regions differ relative to the remainder of the Wadden Sea. In the lower boxplots (C-E), densities of *A. succinea* at the three areas of predicted subsidence are compared with the remainder of the Wadden Sea for the years of 2008, 2009 and 2010. The location names are abbreviated Oost Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) and the remaining Wadden Sea (Ws). In all boxplots the dot (blue) gives the mean value. Photo by M.Mulder.
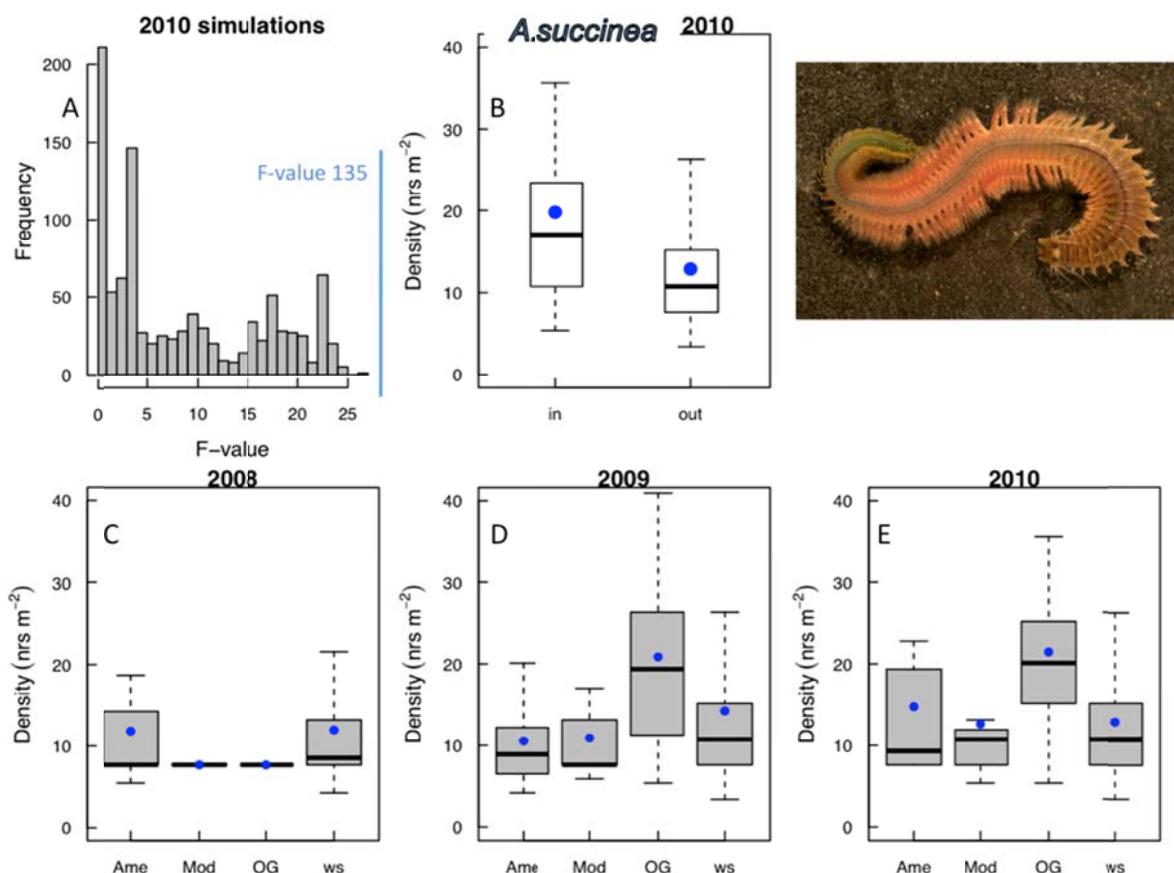
In the case of the polychaete species *A. succinea*, a significant response of the "in" versus "out" was observed using the GLM simulations for 2010 (Appendix 1, Figure 9). The very high F-value indicated that this species has much higher densities in the areas of predicted land subsidence versus the remainder of the Wadden Sea (F-value = 135). A comparison of the raw data "in" versus "out" used for this analysis in the boxplots, also shows that the median values and the range of values were higher in the predicted subsidence areas (Figure 9). A comparison of the densities of this species between the three predicted

subsidence regions in 2010 showed that highest densities were observed in the Oost Groningen region, whereas lowest densities were observed in Moddergat. Furthermore, a comparison between the three survey years showed that there was considerable variation in the densities of this species over time. Densities were lowest in 2008, but increased in 2009 and 2010. In both 2009 and 2010 densities were highest in Oost Groningen. The other areas are relatively similar to each other.

Figure 10 Densities (nrs/m^2) of the polychate *Alitta succinea* across the Dutch Wadden Sea in the three survey years. Darker colours indicate higher densities. Note that the Ems Dollard was not sampled in 2008.
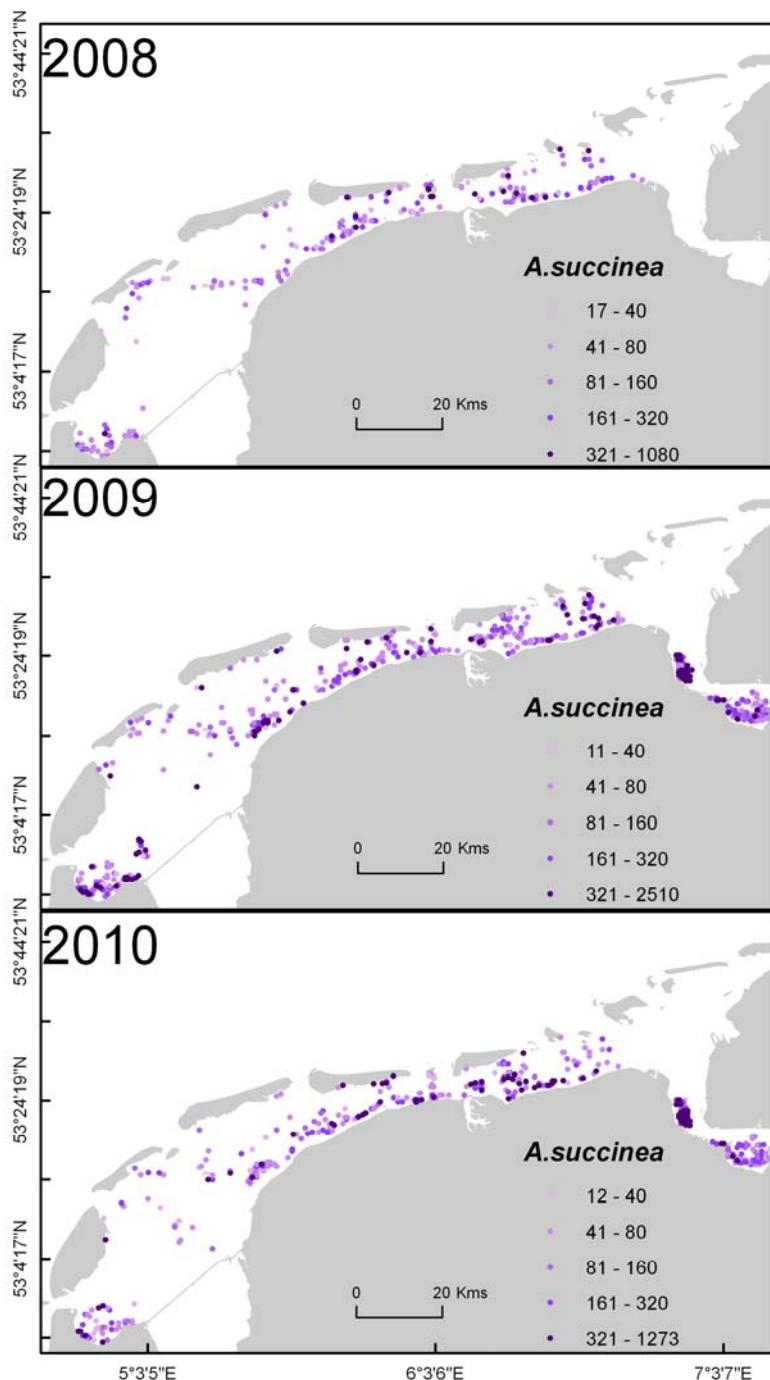
A comparison of the mapped distributions of *A. succinea* showed that densities of this polychaete species were highest in the Oost Groningen area, compared to the remainder of the Wadden Sea. Consequently, the significant result found in the GLM analysis should be interpreted with caution, as this result reflects the distribution of this species rather than a subsidence effect (Figure 10). This species is also relatively common in the Balgzand area and along the Frisian coastline, but is very uncommon across the remainder of the Western Wadden Sea. Areas where this species was most common were generally muddy (Figure 17). A comparison between the three survey years showed that this species had the highest density distributions in 2009 and 2010 and lowest densities in 2008.

Another species that showed significantly higher densities (Appendix 1, F-values 25 from 1000 simulations, Figure 11) in the predicted subsidence areas than in the remainder of the Wadden Sea was the razor shell *Ensis directus*. A comparison of the density distributions of this species both "in" and "out" the predicted subsidence regions showed that in 2010 this species had a higher mean and range of density values in the predicted subsidence regions relative to the remainder of the Wadden Sea. A closer examination of the density distributions of this species in 2010 showed that densities were particularly high in the Oost Ameland region (Figure 11). By contrast, densities were relatively similar between the other predicted subsidence regions and the remainder of the Wadden Sea in 2010. A comparison of *E. directus* densities at the predicted subsidence regions and the remainder of the Wadden Sea showed that the high densities observed at Oost Ameland in 2010 were an exception, as densities of *E. directus* were generally low at the predicted subsidence areas but higher in the remainder of the Wadden Sea in previous years (Figure 11). A comparison of densities between years showed that in 2009 mean densities were highest measured across all years for the remainder of the Wadden Sea (Ws).

The mapped distributions of *E. directus* in 2010 showed that densities were indeed highest in Oost Ameland and relatively low in the remaining areas of the Wadden Sea (Figure 12). The mapped comparisons of *E. directus* densities between years showed that densities were lowest in 2008 and 2010 and relatively highest in 2009. In 2008, high densities of *E. directus* were observed at the edge of Balgzand, near the Marsdiep channel. In 2009, high densities of *E. directus* were observed through the middle reaches of the Wadden Sea. These areas are relatively sandy with median grain sizes of $\sim$250 μm (Figure 17). Densities were generally low in the remaining areas of the Wadden Sea in all years.

By contrast with the aforementioned species, we now examine results from two species that showed a negative response in the predicted areas of subsidence (GLM raw data), but whose responses were not significantly different when compared with the remainder of the Wadden Sea (GLM simulations).

Figure 11 The histogram (A) provides a comparison between the F-value from the raw data model (line) versus the F-values from the simulated GLMs (bars). For *Ensis directus*, the true F-value (25) occurs rarely in the simulated models, indicating the densities in the predicted subsidence regions ("in") differ relative to the remainder of the Wadden Sea ("out"). Boxplots show the data distribution of *Ensis directus* densities (nrs/m^2, square root transformed) in the predicted areas of subsidence versus the remainder of the Wadden Sea. In the first boxplot (B), the data used in the GLM model is represented: "in" versus "out". In the lower boxplots (C-E), densities of *E. directus* at the three areas of predicted subsidence are compared with the remainder of the Wadden Sea for the years of 2008, 2009 and 2010. The location names are abbreviated Oost Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) and the remaining Wadden Sea (Ws). In all boxplots the dot (blue) gives the mean value. Photo by M.Mulder.
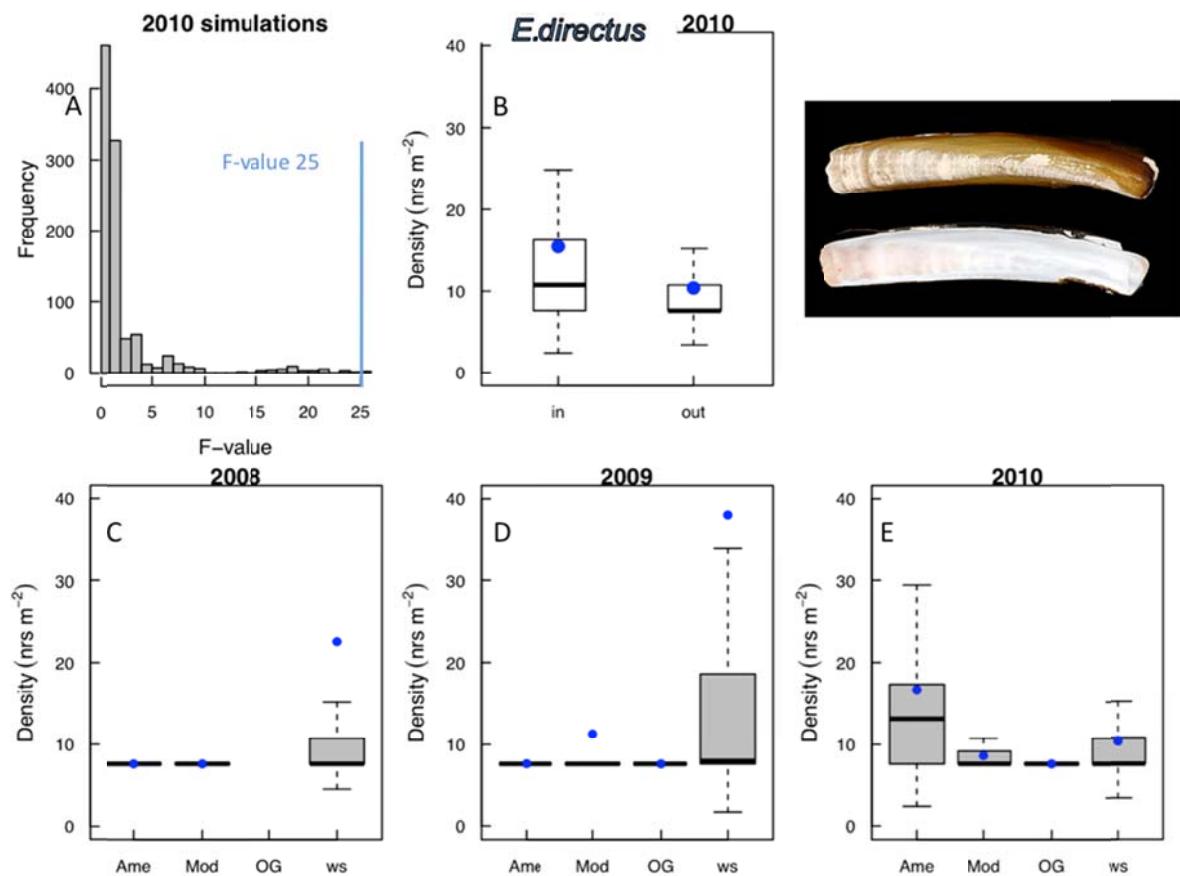
Figure 12 Densities (nrs/m^2) of the razor clam *Ensis directus* across the Dutch Wadden Sea in the three survey years. Darker colours indicate higher densities. Note that the Ems Dollard was not sampled in 2008.
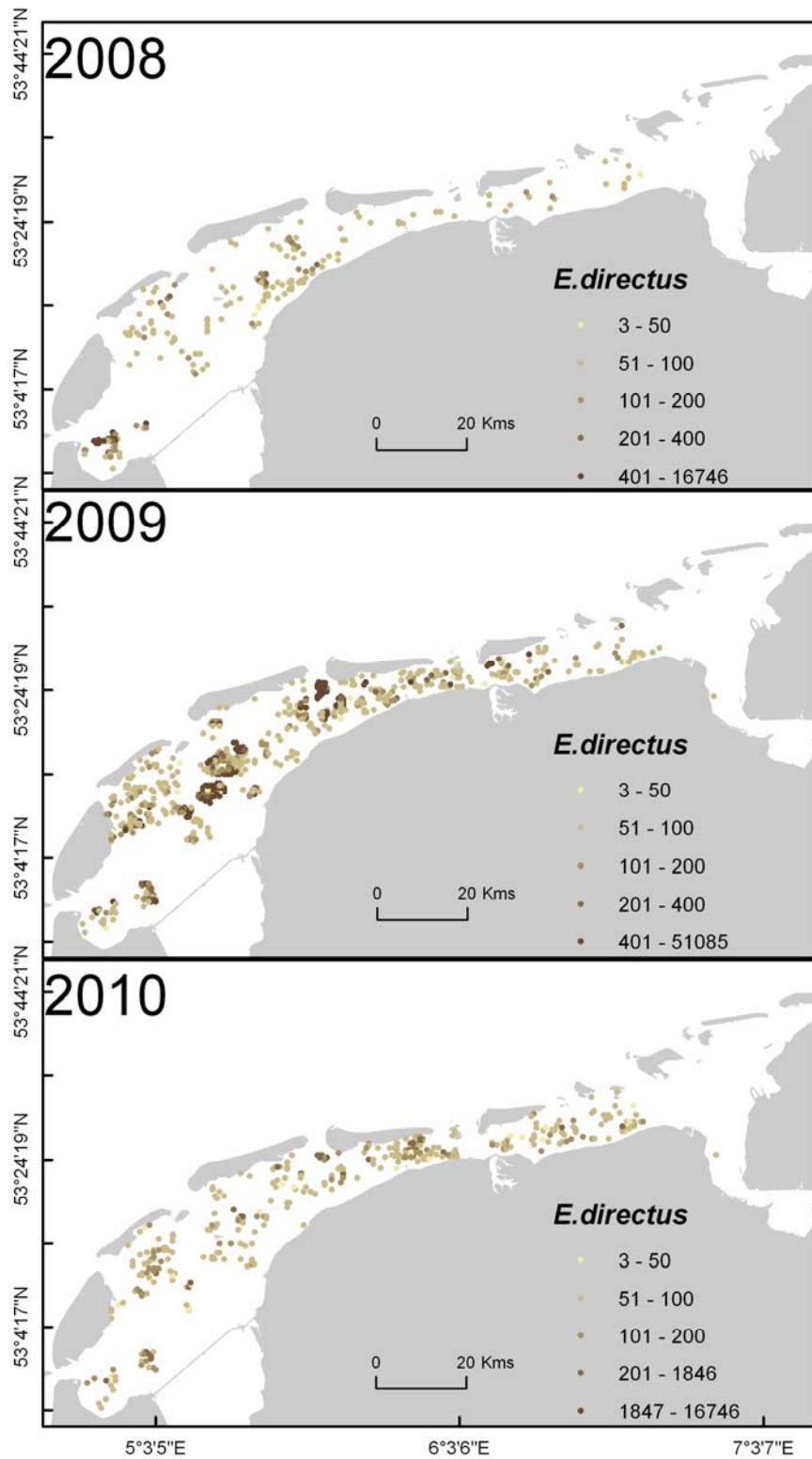
The polychaete bristle worm *Scoloplos cf. armiger* was a species with lower densities in the predicted subsidence regions relative to the remainder of the Wadden Sea (Appendix 1, Figure 13). Note, however, this negative response was not significantly different to other areas in the Wadden Sea (Figure 13, histogram of F-values from GLM simulations showed that this significant F-value was common by comparison with the F-values from the GLM raw data). The distribution of the densities in 2010 showed the mean was higher in the remainder of the Wadden Sea relative to the predicted subsidence regions. However, the range of densities was similar between the predicted subsidence areas and the remainder of the Wadden Sea.

Figure 13 The histogram (A) provides a comparison between the F-value from the raw data model (line) versus the F-values from the simulated GLMs (bars). For *Scoloplos cf. armiger*, the true F-value (25) occurs frequently in the simulated models, indicating the densities in the predicted subsidence regions ("in") do not differ relative to the remainder of the Wadden Sea ("out"). Boxplots show the data distribution of *Scoloplos cf. armiger* densities (nrs/m^2, square root transformed) in the predicted areas of subsidence versus the remainder of the Wadden Sea. In the first boxplot (B), the data used in the GLM model is represented: "in" versus "out". In the lower boxplots, densities of *S.armiger* at the three areas of predicted subsidence are compared with the remainder of the Wadden Sea for the years of 2008, 2009 and 2010 (C-E). The location names are abbreviated Oost Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) and the remaining Wadden Sea (Ws). In all boxplots the dot (blue) gives the mean value. Photo by M.Mulder.
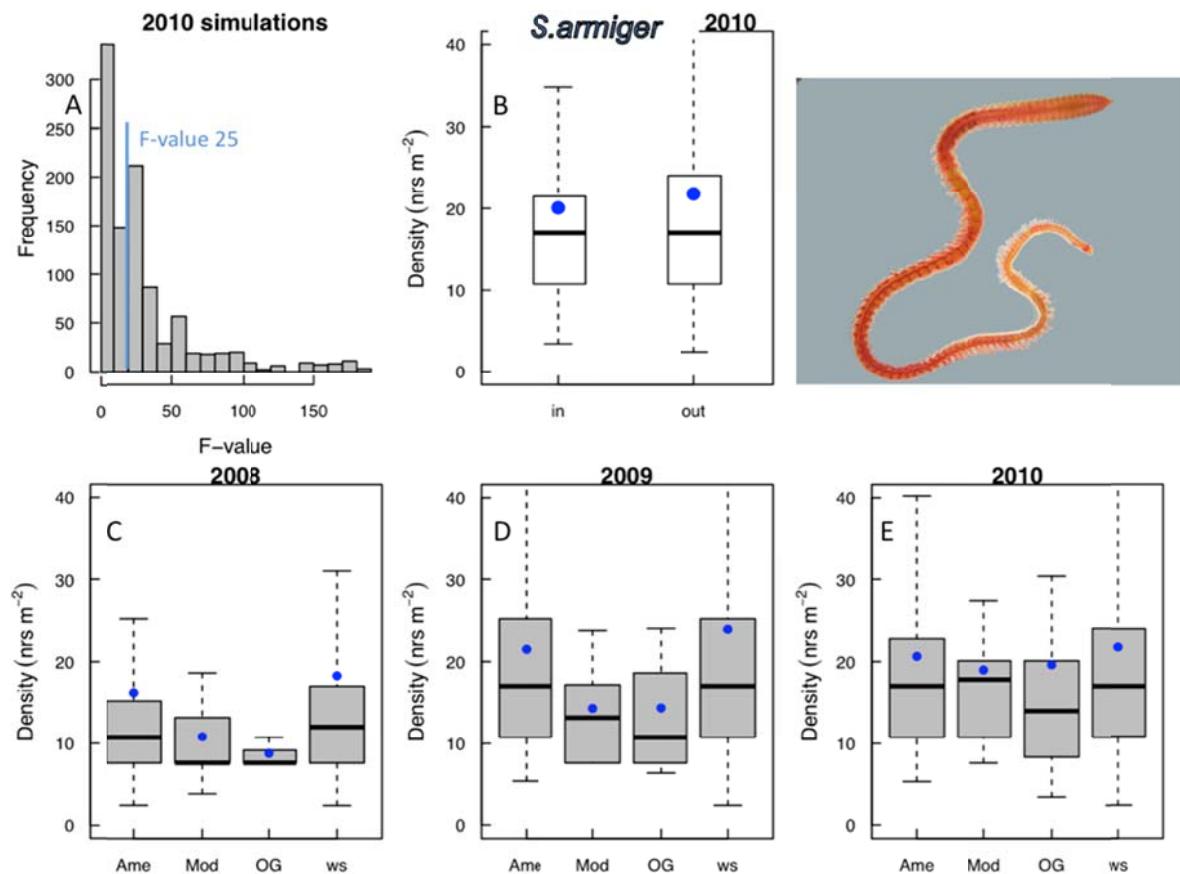
Figure 14 Densities (nrs/m^2) of the polychaete *Scoloplos cf. armiger* across the Dutch Wadden Sea in the three survey years. Darker colours indicate higher densities. Note that the Ems Dollard was not sampled in 2008.
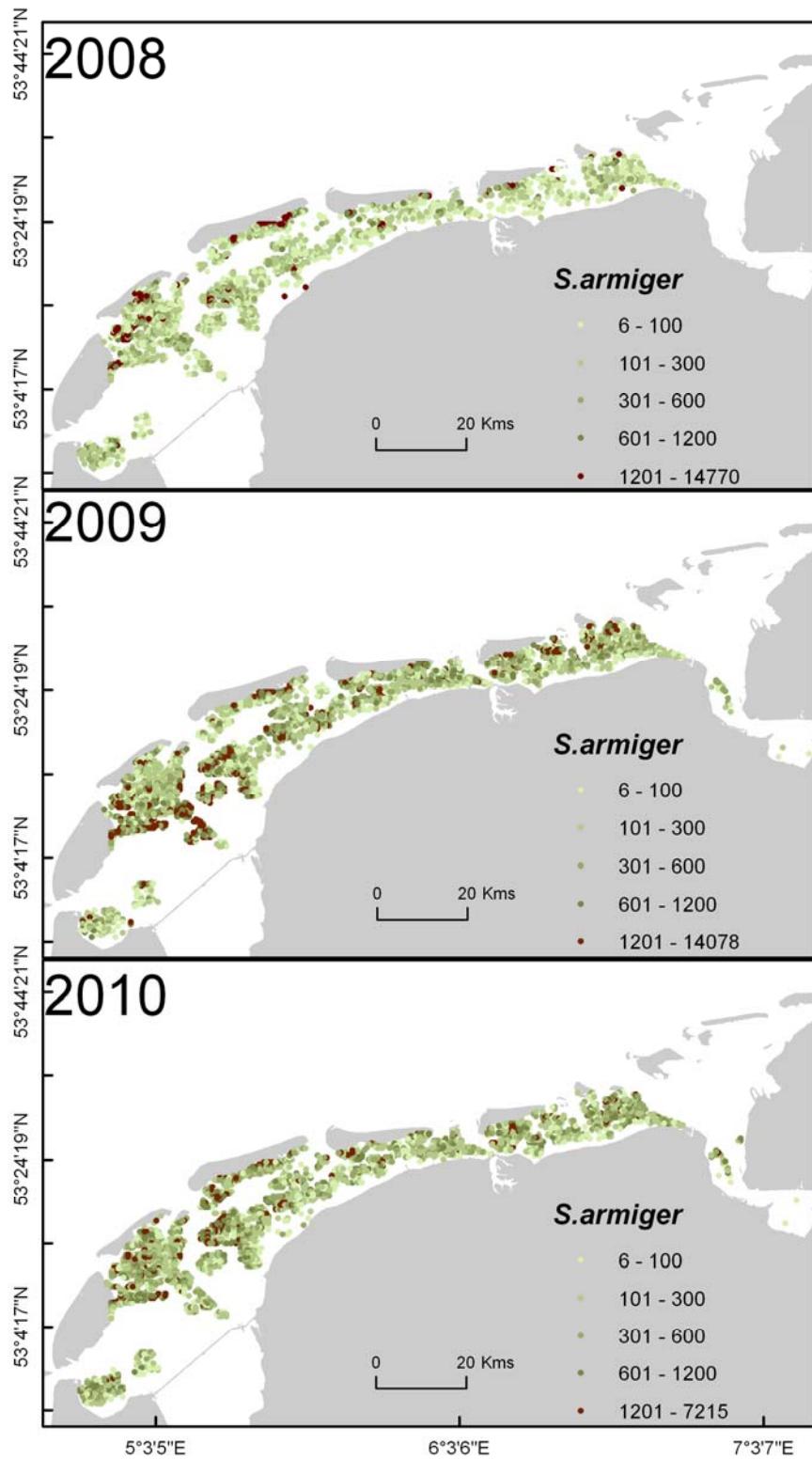
A comparison of the *S. armiger* densities for all three survey years showed that the range of densities were consistently higher in the remainder of the Wadden Sea relative to the areas of predicted subsidence (Figure 13). Furthermore, *S. armiger* densities were lowest in 2008, especially in the Oost Groningen area.

The mapped distribution of *S. armiger* highlights that this species is found widely across the entire Dutch Wadden Sea (Figure 14), mainly where sediments are relatively sandy (Figure 17). Similarly to the boxplots, the mapped distributions highlight that average densities were lowest in 2008 relative to the other two survey years. The mapped distributions tend to suggest that densities were highest in the Vlieland region (2009 and 2010).

The polychaete species *Capitella capitata* also showed a negative density response within the predicted areas of subsidence (Appendix 1, Figure 15). However, this response was not significantly different to other regions in the Wadden Sea (Figure 15, histogram). A comparison of the density distribution of *C. capitata* showed that the range of density values was similar between the "in" and "out" areas. A comparison of the density distributions of *C. capitata* over the three survey years (Figure 15) showed that the mean densities were generally higher in the remainder of the Wadden Sea relative to the areas of predicted subsidence, and that the range in density values was smallest in Moddergat and Oost Groningen in 2009 and 2010.

The mapped distributions of *C. capitata* showed that this species was broadly distributed across the entire Dutch Wadden Sea (Figure 16), but does not occur close to the Frisian coastline. This species thus shows an affiliation with sandier sediment types (Figure 17). Highest densities have generally been observed in the Western Wadden Sea by Vlieland, Balgzand and Griend, and also in between Schiermonnikoog and the mainland in 2009 and 2010. Densities were consistently lowest in 2008 by contrast with densities observed in 2009 and 2010 for this species.

The results from the five species shown here indicate that species densities vary both in space and time across the Dutch Wadden Sea, but also show some interesting parallels in their distribution patterns between years. Effects of land subsidence, however, cannot be discerned from the current analyses, as a better understanding of this spatial and temporal variation is needed prior to detecting effects due to other factors.

Figure 15 The histogram (A) provides a comparison between the F-value from the raw data model (line) versus the F-values from the simulated GLMs (bars). For *Capitella capitata*, the true F-value (12) occurs frequently in the simulated models, indicating the densities in the predicted subsidence regions ("in") do not differ relative to the remainder of the Wadden Sea ("out"). Boxplots show the data distribution of *Capitella capitata* densities (nrs/m^2, square root transformed) in the predicted areas of subsidence versus the remainder of the Wadden Sea. Boxplots show the data distribution of *Capitella capitata* densities (nrs/m^2, square root transformed) in the predicted areas of subsidence versus the remainder of the Wadden Sea. In the first boxplot (B), the data used in the GLM model is represented: "in" versus "out". In the lower boxplots (C-E), densities of *C. capitata* at the three areas of predicted subsidence are compared with the remainder of the Wadden Sea for the years of 2008, 2009 and 2010. The location names are abbreviated Oost Ameland (Ame), Moddergat (Mod), Oost Groningen (OG) and the remaining Wadden Sea (Ws). In all boxplots the dot (blue) gives the mean value. Photo by H. Hillewaert.
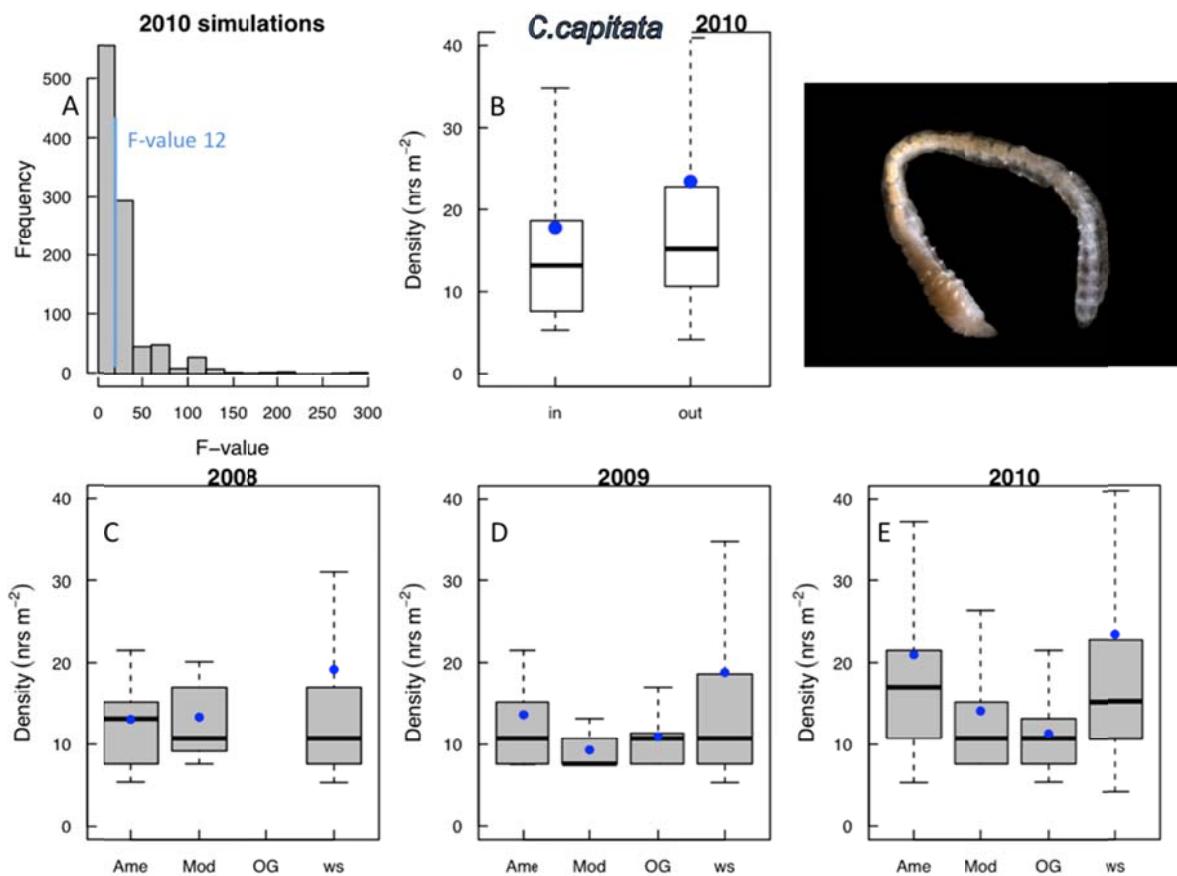
Figure 16 Densities (nrs/m^2) of the polychaete *Capitella capitata* across the Dutch Wadden Sea in the three survey years. Darker colours indicate higher densities. Note that the Ems Dollard was not sampled in 2008.
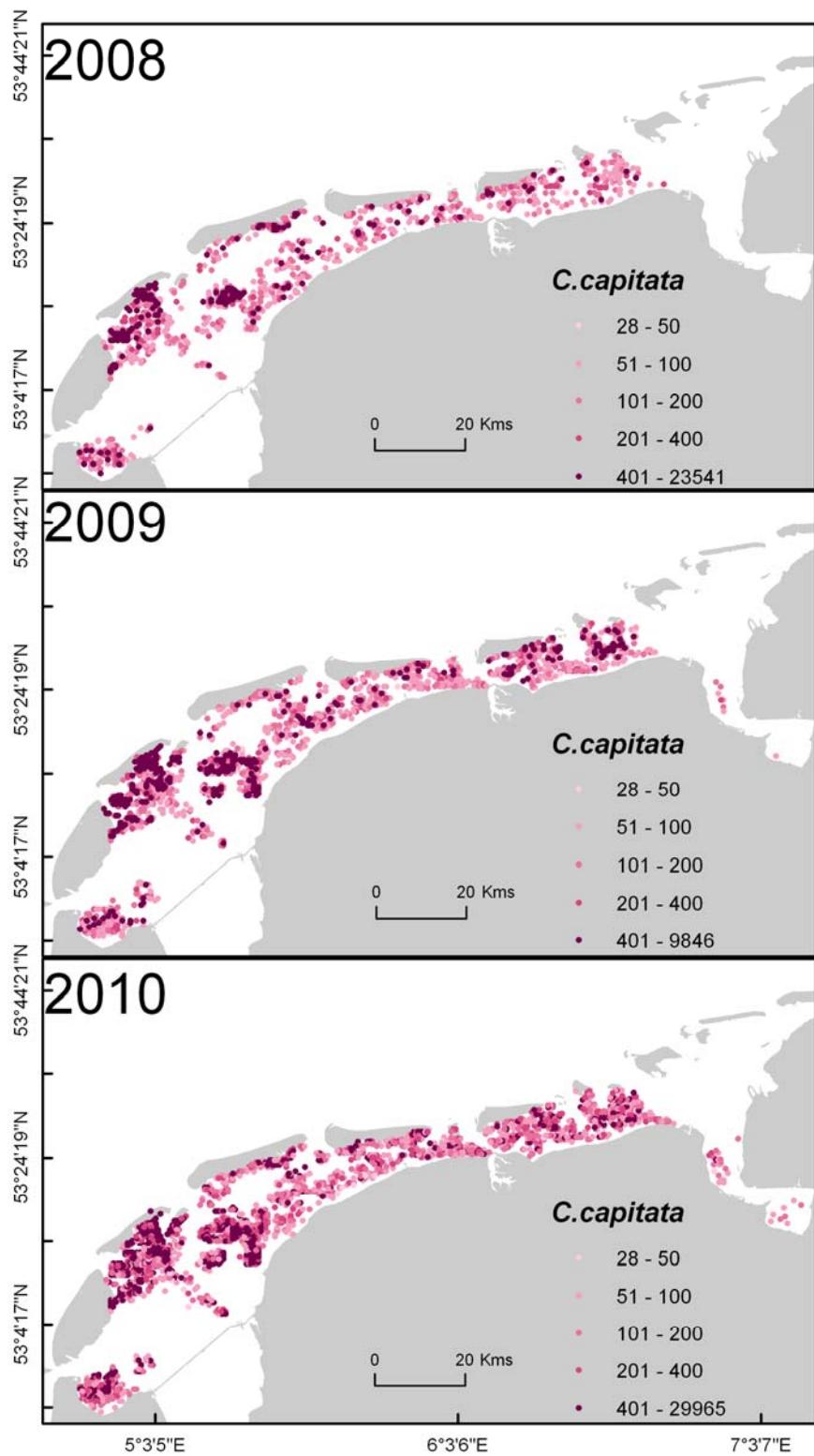
Figure 17 Sediment grain size composition across the Dutch Wadden Sea in 2008 and 2010 (Median grain size, μm). Note that the Ems Dollard was not sampled in 2008. An inverse distance weighting interpolation was used to provide an interpolated surface of grain sizes across the Dutch Wadden Sea (ArcGIS 9.3). Sediments for 2009 are currently being analysed.
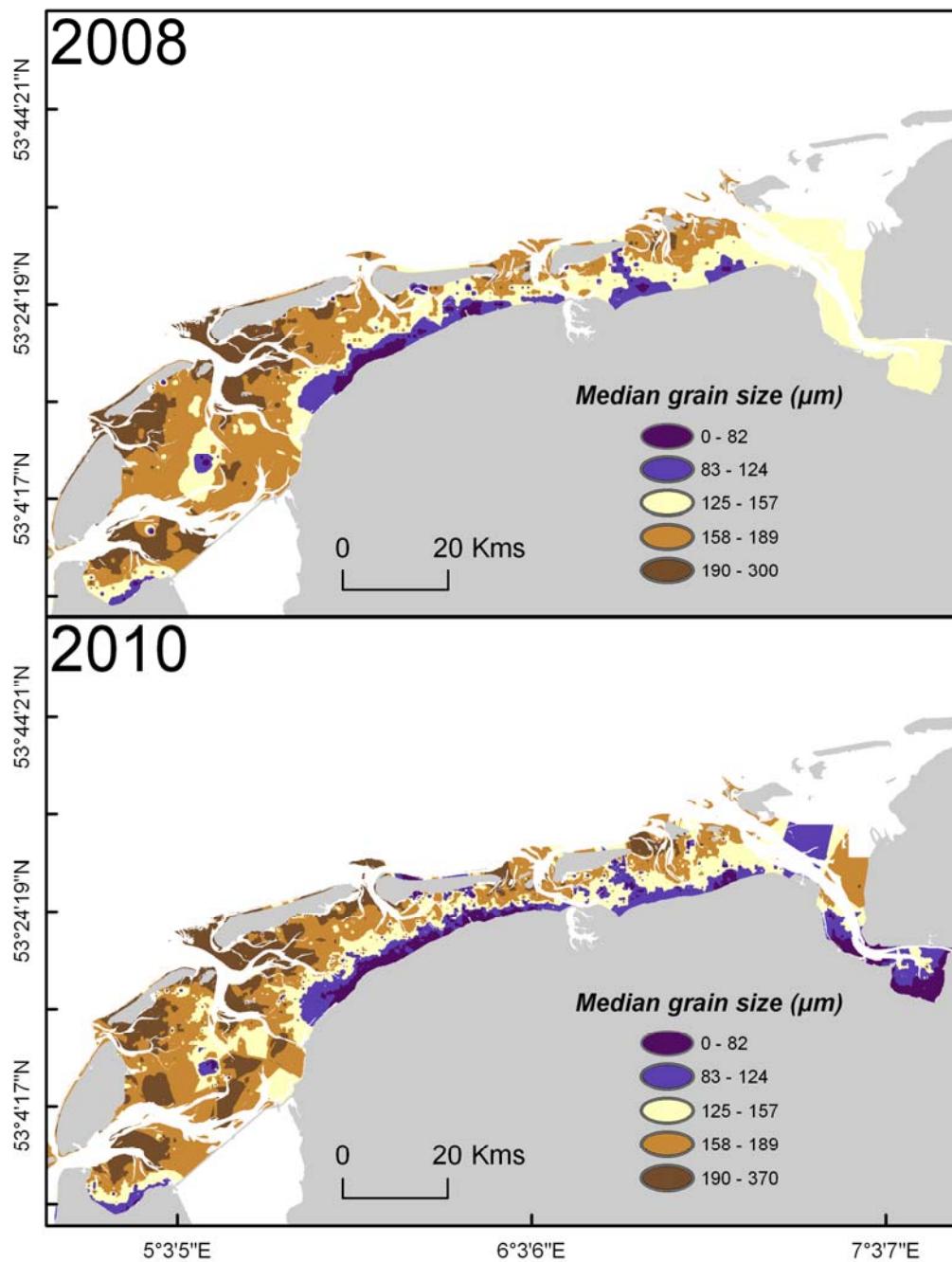
9.   DISCUSSION

Results from the three SIBES surveys (2008, 2009, 2010) currently show that some species had significantly higher densities in the areas of predicted subsidence. However, these species vary over the three years, so to identify whether land subsidence by gas extraction might be a direct cause for these significant differences in species abundance within the predicted subsidence areas, we need a better understanding of other environmental drivers of species abundance.

Three species were identified as having significantly higher densities in the areas predicted to subside for the 2010 survey data, i.e. *Macoma balthica*, *Alitta succinea* and *Ensis directus*. By comparison in 2009, it was observed that *Alitta virens*, *Urothoe poseidonis*, *Magelona johnstoni* and *Heteromastis filiformis* differed significantly in the predicted gas subsidence regions relative to the remainder of the Wadden Sea. Whereas in 2008, *Nephtys cirrosa*, *Pygospia elegans*, *Marenzelleria viridis* and *Spio martinensis* were different in the predicted subsidence regions. An examination of the distribution maps of these three species in 2010 showed that these species had much higher densities in the eastern Wadden Sea relative to the western Wadden Sea. In the case of *A. succinea* highest densities were observed in the Ems Dollard area, whereas highest densities of *M. balthica* were observed along the relatively muddy Frisian coastline. In addition, a comparison of these three species at the predicted subsidence regions through time showed that the annual change in density was greater than the location effect.

The two species identified as having lower densities in the predicted subsidence regions, *S. armiger* and *C. capitata*, did not have a significantly different response to other areas of the Wadden Sea (simulations). Instead, the mapped distributions of these two species showed that they were widespread throughout the Wadden Sea, with the highest densities often in the Vlieland area.

In the current data, species densities and richness were lowest in 2008. The relatively low numbers in 2008 could indicate harsh winter weather in previous years (Honkoop & Beukema 1997, Beukema et al. 1998, Beukema 1991). However, according to the Hellman classification the winters of 2006/7 and 2007/8 were mild (<20 days below freezing in both, http://www.knmi.nl/klimatologie/lijsten/hellmann.html). By contrast, the subsequent winters were harsher according to the Hellman classification (55 and 95 days below freezing for 2009 and 2010). Another possibility might be that the low numbers in 2008 are on an increasing trajectory from a severe winter event in previous years. But these possibilities need further investigation. Note that such temporal variability might be predictable given more data is collected over time, as the temporal variability in abundance of the large majority of common taxa is often not random (Hewitt & Thrush 2007).

Another trend that emerges when comparing the community responses between years is that density, biomass and richness of macrofauna were highest along the Frisian coastline, close to Schiermonnikoog, near Griend and also in the

vicinity of Vlieland, but lowest in the Ems Dollard region. Environmental variables explaining these patterns need further investigation, as sediment grain size and inundation time do not visually appear to be the sole variables that can explain these patterns (see Appendix 9). A possible reason for the higher richness, diversity and biomass in the Vlieland region might be tidal current velocities, which could continuously replenish the planktonic food sources in this area. By contrast, the low richness and biomass in the Ems Dollard might be related to the freshwater influence in this region.

In summary, our current analysis highlights that there may be an indication of that species are responding differently in the area of predicted subsidence, but also that the species identified as significant by the models differed between years and were observed to have very different spatial distributions, and thus habitat preferences, across the Wadden Sea. This observation is not new, as previous studies of Wadden Sea macrofauna have shown that macrofaunal species have different sediment grain size and inundation time associations (van der Meer 1991, Compton et al. 2008, Compton et al. 2009, Kraan et al. 2010). But what is new in the SIBES data is that we are examining macrofauna distributions at much larger spatial scales than previously measured across the Wadden Sea. Thus we need to get a better handle on the factors driving the spatial distributions of our species, before we can understand or predict what would happen with increased land subsidence. For example, other variables that we need to acquire include measures of microphytobenthos productivity (van der Wal et al. 2008, van der Wal et al. 2010), tidal current speeds, orbital velocities and salinity. These variables are currently not easily accessible, thus work is in progress to obtain this data for SIBES. In the future, we also need to take into account in our analyses that sampling is conducted over a period of about four months (see Appendix 6).

A previous study exploring possible effects of land subsidence and sea level change predicted that effects of land subsidence should be minimal across the Wadden Sea (Beukema 1998, Beukema 2002). This prediction was based on the association between macrofaunal biomass, richness and density with respect to inundation time and assuming that gas subsidence would be <10 cm over a few decades, with an expected rate of subsidence of <2 mm per year (see references in Beukema 2002). The observation that the macrofaunal densities did not show any striking differences in the predicted subsidence regions in this report might suggest that our results currently support this prediction.

But although the predictions provided by Beukema (2002) are a useful starting point they should be interpreted with considerable caution, as land subsidence may affect other environmental variables that could change the macrofaunal densities or species composition. Although Wang and Eysink (2005) concluded that land subsidence resulting from gas extraction would not result in changes in sediment transport and orbital velocities, longer inundation times could also have effects on other properties of the ecosystem, e.g. microphytobenthos densities. As we currently do not have a description of many other environmental variables that could affect community composition across the Wadden Sea we need to

obtain these variables so that we can describe macrofaunal composition with respect to these variables and then examine if we can identify changes in the areas of predicted subsidence.

Current evidence suggests that we need to get a better handle on the variables that explain the spatial distribution of species in the Wadden Sea. In addition, we need to continue sampling so that we can tease apart variation due to natural circumstances, versus variation due to human factors. It is well known from many other long-term studies of macrofauna that in attempting to detect impacts one needs to first identify the factors driving natural variability among sites (Hewitt et al. 2001). Furthermore, in ecology it is known that generality can be conferred by conducting studies at a number of sites, on a number of occasions, or on a number of different organisms (Hewitt et al. 2001).

10. CONCLUSIONS

In conclusion, there are some indications from the SIBES time series that some species are significantly different in the predicted areas of subsidence. However, responses appear to be variable in space and time. The power of our analysis will increase over time with more years of study, and thus will allow us to disentangle the anthropogenic effects from the natural variation in the future. Thus we strongly suggest that the SIBES sampling continues so that insights into factors driving natural variation can be gained, and consequently the role of anthropogenic factors can be determined.

11. ACKNOWLEDGEMENTS

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Appendix 1. **Results from the generalized linear model (GLM, quasi poisson distributed errors) to examine whether there are significant differences between the densities of individual OTUs across the different regions in 2010. Models were only run when there were eight individuals both inside and outside the regions of interest. The results are presented for all regions, Oost Ameland, East Groningen and Moddergat. The estimates (est) and their standard errors, the t-values and the F-values and the associated significance values (p-ttest and p-Ftest) for each GLM model are given below. Significant results are given in bold and positive estimate values indicate a positive significant difference, i.e. that the densities are highest "in" the subsidence region, whereas negative values indicate that densities were lower "in" the subsidence regions. The densities inside and outside the region are given (ab.in and ab.out), as well as the occurrences (presences, pr.in and pr.out).**

spec	est	se	tvalue	p_ttest	Fvalue	p_Ftest	ab.in	ab.out	pr.in	pr.out
2010 All regions										
Scoloplos armiger	-0.5687	0.1244	-4.5704	0.0000	24.9329	0.0000	55205	1167389	137	2455
Ensis directus	1.2509	0.2204	5.6745	0.0000	25.1238	0.0000	10053	34457	42	320
Macoma balthica	1.1290	0.1550	7.2841	0.0000	41.9530	0.0000	101454	392827	171	1557
Alitta succinea	1.8437	0.1378	13.3829	0.0000	136.6908	0.0000	28150	53337	72	322
Capitella capitata	-0.8442	0.2744	-3.0764	0.0021	12.4456	0.0004	38079	1060614	121	1927
Pygospio elegans	-1.2704	0.6341	-2.0035	0.0452	6.2080	0.0128	72139	3076975	163	2607
Heteromastus filiformis	0.5124	0.2055	2.4935	0.0127	5.4659	0.0194	11519	82623	93	723
Oligochaeta sp	-0.5534	0.2774	-1.9949	0.0461	4.7258	0.0298	73425	1529059	92	1343
Scrobicularia plana	0.5928	0.2996	1.9788	0.0479	3.3837	0.0659	1187	7854	19	112
Corophium sp	-0.4769	0.2813	-1.6957	0.0900	3.3284	0.0682	96610	1863767	93	945
Aphelochaeta	0.2393	0.1363	1.7557	0.0792	2.8910	0.0891	182138	1716861	173	1962

marioni										
Eteone longa	0.2242	0.1297	1.7288	0.0839	2.8133	0.0936	31442	300878	138	1715
Eumida sanguinea	0.9366	0.5351	1.7504	0.0801	2.4877	0.1148	2541	11924	12	57
Lanice conchilega	0.3781	0.2348	1.6103	0.1074	2.3501	0.1253	40809	334797	87	1004
Mya arenaria	-0.3856	0.2707	-1.4246	0.1544	2.2803	0.1311	1642	28915	27	354
Streblospio shrubsolii	0.4587	0.3198	1.4344	0.1515	1.8300	0.1762	1328	10052	12	118
Urothoe poseidonis	-0.2910	0.3049	-0.9545	0.3399	0.9934	0.3190	39647	635078	50	1048
Carcinus maenas	0.4666	0.4435	1.0520	0.2929	0.9829	0.3215	3753	28185	36	295
Nephtys hombergii	-0.6226	0.7744	-0.8040	0.4214	0.7860	0.3754	912	20360	17	288
Crangon crangon	-0.2458	0.3192	-0.7701	0.4413	0.6377	0.4246	1288	19724	19	232
Polydora cornuta	0.2258	0.2917	0.7741	0.4389	0.5639	0.4527	13772	131578	66	456
Marenzelleria viridis	-0.1114	0.1612	-0.6912	0.4895	0.4933	0.4825	288305	3859158	121	2158
Magelona mirabilis	0.2599	0.4138	0.6282	0.5299	0.3681	0.5440	693	6398	9	85
Nereide sp	-0.2760	0.5130	-0.5381	0.5906	0.3142	0.5751	930	14670	12	119
Hydrobia ulvae	-0.2574	0.5620	-0.4580	0.6470	0.2264	0.6342	417983	6474427	78	634
Spio martinensis	-1.0851	3.0095	-0.3606	0.7185	0.1870	0.6654	1155	40927	12	176
Bylgides sarsi	0.1534	0.4142	0.3704	0.7111	0.1316	0.7168	3261	33497	30	396
Phyllodoce mucosa	0.1590	0.4945	0.3216	0.7478	0.0990	0.7530	9037	92301	21	399
Hediste diversicolor	0.0441	0.1782	0.2474	0.8046	0.0604	0.8058	27803	318563	98	1337
Bathyporeia sarsi	-0.0850	0.6022	-0.1411	0.8878	0.0204	0.8864	3129	40785	12	121
Cerastoderma edule	0.0571	0.5567	0.1025	0.9183	0.0103	0.9190	15311	173167	83	725
Nephtys caeca	-0.0444	0.4699	-0.0944	0.9248	0.0090	0.9243	635	7950	10	105
2010 Oost Ameland										
Ensis directus	2.1052	0.2217	9.4951	0.0000	58.7002	0.0000	9476	34457	34	320
Eteone longa	0.8802	0.1462	6.0191	0.0000	28.4756	0.0000	24305	300878	73	1715
Lanice conchilega	1.1822	0.2473	4.7801	0.0000	16.8286	0.0000	36583	334797	59	1004
Macoma balthica	1.3142	0.1826	7.1961	0.0000	37.1971	0.0000	48977	392827	79	1557
Marenzelleria viridis	0.7475	0.1633	4.5766	0.0000	16.9920	0.0000	273003	3859158	76	2158
Corophium sp	-2.3406	1.1172	-2.0950	0.0362	11.1045	0.0009	6011	1863767	21	945

Eumida sanguinea	1.8500	0.5396	3.4287	0.0006	7.8164	0.0052	2541	11924	12	57
Carcinus maenas	1.1056	0.5100	2.1677	0.0302	3.5150	0.0609	2852	28185	24	295
Pygospio elegans	-1.6560	1.2305	-1.3457	0.1785	3.3579	0.0670	19679	3076975	54	2607
Phyllodoce mucosa	1.0064	0.5058	1.9896	0.0467	3.0242	0.0821	8459	92301	12	399
Urothoe poseidonis	-0.6384	0.5457	-1.1699	0.2421	1.6963	0.1928	11237	635078	33	1048
Scrobicularia plana	0.5752	0.4577	1.2568	0.2089	1.3387	0.2473	468	7854	9	112
Scoloplos armiger	-0.1771	0.1597	-1.1093	0.2674	1.3020	0.2539	32760	1167389	77	2455
Crangon crangon	0.4139	0.3631	1.1399	0.2544	1.1502	0.2836	1000	19724	13	232
Heteromastus filiformis	-0.3441	0.4769	-0.7215	0.4707	0.5821	0.4455	1962	82623	21	723
Capitella capitata	-0.2037	0.3189	-0.6387	0.5231	0.4354	0.5094	28984	1060614	66	1927
Hydrobia ulvae	-0.5666	1.0351	-0.5474	0.5841	0.3620	0.5474	123074	6474427	15	634
Hediste diversicolor	-0.1499	0.3021	-0.4963	0.6197	0.2583	0.6113	9186	318563	32	1337
Cerastoderma edule	0.2396	0.8027	0.2984	0.7654	0.0829	0.7735	7372	173167	40	725
Oligochaeta sp	0.0543	0.3204	0.1694	0.8655	0.0282	0.8667	54081	1529059	38	1343
Polydora cornuta	-0.0867	0.5261	-0.1647	0.8692	0.0279	0.8674	4042	131578	24	456
Nephtys hombergii	-0.1274	0.9677	-0.1316	0.8953	0.0180	0.8932	601	20360	10	288
Aphelochaeta marioni	0.0309	0.2315	0.1333	0.8940	0.0176	0.8945	59318	1716861	70	1962
Bylgides sarsi	0.0023	0.6871	0.0034	0.9973	0.0000	0.9973	1125	33497	18	396
2010 Oost Groningen										
Alitta succinea	2.6765	0.1407	19.0210	0.0000	232.8366	0.0000	24858	53337	54	322
Marenzelleria viridis	-3.3236	1.2102	-2.7464	0.0061	31.4609	0.0000	4458	3859158	20	2158
Scoloplos armiger	-1.0520	0.2530	-4.1584	0.0000	25.0399	0.0000	13073	1167389	34	2455
Capitella capitata	-2.3266	0.9284	-2.5061	0.0122	15.7951	0.0001	3320	1060614	26	1927
Heteromastus filiformis	1.0976	0.2461	4.4593	0.0000	14.8726	0.0001	7940	82623	57	723
Hediste diversicolor	-1.8014	0.6902	-2.6101	0.0091	13.4676	0.0002	1686	318563	19	1337
Eteone longa	-1.1039	0.3859	-2.8608	0.0042	12.0894	0.0005	3199	300878	34	1715
Macoma balthica	0.9858	0.2532	3.8938	0.0001	11.6177	0.0007	33757	392827	39	1557
Oligochaeta sp	-2.0620	0.9053	-2.2778	0.0228	11.5310	0.0007	6236	1529059	25	1343

Aphelochaeta marioni	-1.0311	0.3958	-2.6048	0.0092	9.7454	0.0018	19633	1716861	51	1962
Polydora cornuta	-1.1133	0.8887	-1.2528	0.2104	2.3279	0.1271	1386	131578	13	456
Pygospio elegans	-1.0275	0.9225	-1.1139	0.2654	1.7797	0.1823	35310	3076975	58	2607
Corophium sp	0.3324	0.3067	1.0837	0.2786	1.0632	0.3026	83324	1863767	62	945
Cerastoderma edule	-1.3471	1.7845	-0.7549	0.4503	0.9286	0.3353	1444	173167	10	725
Mya arenaria	-0.1020	0.3787	-0.2693	0.7877	0.0749	0.7843	837	28915	13	354
Hydrobia ulvae	0.1986	0.7288	0.2725	0.7853	0.0699	0.7915	253204	6474427	50	634
2010 Moddergat										
Aphelochaeta marioni	1.2086	0.1785	6.7693	0.0000	32.7824	0.0000	103187	1716861	52	1962
Hediste diversicolor	1.0856	0.2256	4.8123	0.0000	17.0515	0.0000	16930	318563	47	1337
Scrobicularia plana	1.6293	0.3771	4.3209	0.0000	12.2367	0.0005	719	7854	10	112
Marenzelleria viridis	-1.8542	0.7823	-2.3702	0.0178	11.4448	0.0007	10844	3859158	25	2158
Scoloplos armiger	-0.8045	0.2956	-2.7212	0.0065	9.8028	0.0018	9372	1167389	26	2455
Macoma balthica	0.9766	0.2795	3.4935	0.0005	9.2283	0.0024	18720	392827	53	1557
Polydora cornuta	1.2623	0.3749	3.3667	0.0008	8.0114	0.0047	8344	131578	29	456
Urothoe poseidonis	0.8654	0.3631	2.3833	0.0172	4.4143	0.0357	27082	635078	15	1048
Capitella capitata	-1.1928	0.7096	-1.6809	0.0928	4.3504	0.0371	5774	1060614	29	1927
Corophium sp	-1.5255	1.0242	-1.4895	0.1364	3.9179	0.0478	7276	1863767	10	945
Oligochaeta sp	-0.7389	0.6299	-1.1730	0.2409	1.7775	0.1825	13108	1529059	29	1343
Alitta succinea	0.6097	0.4409	1.3827	0.1668	1.5924	0.2071	1761	53337	11	322
Pygospio elegans	-1.1694	1.3274	-0.8809	0.3784	1.1837	0.2767	17150	3076975	51	2607
Eteone longa	-0.3157	0.3506	-0.9003	0.3680	0.9002	0.3428	3938	300878	31	1715
Cerastoderma edule	0.7373	0.8590	0.8583	0.3908	0.5926	0.4415	6496	173167	33	725
Hydrobia ulvae	-1.0247	1.7776	-0.5764	0.5644	0.4786	0.4891	41705	6474427	13	634
Carcinus maenas	0.5770	0.8809	0.6550	0.5125	0.3606	0.5482	901	28185	12	295
Lanice conchilega	-0.3683	0.6962	-0.5290	0.5968	0.3165	0.5738	4158	334797	26	1004
Heteromastus filiformis	0.0865	0.5281	0.1638	0.8699	0.0261	0.8717	1617	82623	15	723

Appendix 2.   Summed total biomass of OTUs in the Dutch Wadden Sea from 2008 to 2010. The total ash free dry mass of an OTU as sampled across the entire Dutch Wadden Sea in a single year is provided (sumAFDM), the mean ash free dry mass for an individual is given (meanAFDM), the number of individuals weighed (n wt) and their taxonomic affiliation are shown. Only the first 17 species with the highest biomass are shown for each year of sampling.

sp	sumAFDM	MeanAFDM	sdAFDM	n wt	phylum	class	genus
2008							
Cerastoderma edule	452.0772	0.5567	0.7473	809	Mollusca	Bivalvia	Cerastoderma
Lanice conchilega	193.4112	0.1928	0.4677	999	Annelida	Polychaeta	Lanice
Mya arenaria	174.5490	0.4680	1.0435	371	Mollusca	Bivalvia	Mya
Ensis directus	110.7823	0.5153	1.1649	215	Mollusca	Bivalvia	Ensis
Mytilus edulis	69.7502	1.0898	2.3970	64	Mollusca	Bivalvia	Mytilus
Crassostrea gigas	69.1198	6.2836	6.6673	11	Mollusca	Bivalvia	Crassostrea
Hediste diversicolor	68.2106	0.0638	0.0877	1064	Annelida	Polychaeta	Hediste
Macoma balthica	43.9068	0.0421	0.0541	1039	Mollusca	Bivalvia	Macoma
Carcinus maenas	37.3582	0.1448	0.7975	256	Arthropoda	Malacostraca	Cancer
Scoloplos armiger	23.9026	0.0129	0.0182	1837	Annelida	Polychaeta	Scoloplos
Alitta virens	19.5076	0.1355	0.2137	143	Annelida	Polychaeta	Alitta
Scrobicularia plana	18.2296	0.1098	0.0913	165	Mollusca	Bivalvia	Scrobicularia
Nephtys hombergii	17.7508	0.0230	0.0254	769	Annelida	Polychaeta	Nephtys
Marenzelleria viridis	13.6744	0.0185	0.0525	735	Annelida	Polychaeta	Marenzelleria
Hydrobia ulvae	10.4897	0.0379	0.0624	270	Mollusca	Gastropoda	Hydrobia
Alitta succinea	8.9766	0.0470	0.0697	191	Annelida	Polychaeta	Alitta
Eunereis longissima	4.9504	0.0250	0.0451	193	Annelida	Polychaeta	Eunereis
2009							
Cerastoderma edule	578.1390	0.5793	0.8842	996	Mollusca	Bivalvia	Cerastoderma
Mya arenaria	241.3391	0.4623	1.0898	520	Mollusca	Bivalvia	Mya
Ensis directus	215.3145	0.2811	0.7370	765	Mollusca	Bivalvia	Ensis
Mytilus edulis	133.8151	0.6827	4.0481	194	Mollusca	Bivalvia	Mytilus
Lanice conchilega	109.6402	0.1170	0.2491	932	Annelida	Polychaeta	Lanice

Macoma balthica	81.3870	0.0603	0.7039	1335	Mollusca	Bivalvia	Macoma
Hediste diversicolor	69.4085	0.0539	0.0794	1282	Annelida	Polychaeta	Hediste
Crassostrea gigas	64.4491	2.8021	4.7088	23	Mollusca	Bivalvia	Crassostrea
Scoloplos armiger	42.1299	0.0161	0.0955	2592	Annelida	Polychaeta	Scoloplos
Carcinus maenas	29.6997	0.0801	0.6577	366	Arthropoda	Malacostraca	Cancer
Scrobicularia plana	21.4208	0.0927	0.0994	231	Mollusca	Bivalvia	Scrobicularia
Marenzelleria viridis	16.6707	0.0160	0.0422	1031	Annelida	Polychaeta	Marenzelleria
Nephtys hombergii	14.4969	0.0231	0.0217	627	Annelida	Polychaeta	Nephtys
Alitta virens	12.6385	0.1389	0.2767	91	Annelida	Polychaeta	Alitta
Alitta succinea	12.1631	0.0238	0.0309	508	Annelida	Polychaeta	Alitta
Hydrobia ulvae	12.0396	0.0225	0.0395	532	Mollusca	Gastropoda	Hydrobia
Corophium sp.	10.5006	0.0119	0.0207	860	Arthropoda	Malacostraca	Corophium
2010							
Cerastoderma edule	380.6100	0.4746	0.7391	788	Mollusca	Bivalvia	Cerastoderma
Mya arenaria	264.8625	0.6589	1.2308	397	Mollusca	Bivalvia	Mya
Macoma balthica	185.3322	0.1096	2.2186	1665	Mollusca	Bivalvia	Macoma
Ensis directus	112.6106	0.2940	0.7529	380	Mollusca	Bivalvia	Ensis
Mytilus edulis	96.3238	0.9174	1.5492	103	Mollusca	Bivalvia	Mytilus
Hediste diversicolor	66.9758	0.0489	0.0775	1359	Annelida	Polychaeta	Hediste
Scoloplos armiger	65.9947	0.0266	0.0300	2465	Annelida	Polychaeta	Scoloplos
Crassostrea gigas	65.2539	2.9661	4.2870	22	Mollusca	Bivalvia	Crassostrea
Scrobicularia plana	38.1324	0.2685	0.2786	140	Mollusca	Bivalvia	Scrobicularia
Lanice conchilega	36.7015	0.0351	0.0685	1039	Annelida	Polychaeta	Lanice
Marenzelleria viridis	31.7816	0.0147	0.0340	2130	Annelida	Polychaeta	Marenzelleria
Littorina littorea	24.6275	1.0708	3.2405	23	Mollusca	Gastropoda	Littori
Alitta virens	22.6824	0.3240	0.7400	70	Annelida	Polychaeta	Alitta
Corophium sp.	17.6948	0.0180	0.0374	955	Arthropoda	Malacostraca	Corophium
Alitta succinea	13.7269	0.0366	0.0633	367	Annelida	Polychaeta	Alitta
Capitella capitata	11.4581	0.0064	0.0290	1743	Annelida	Polychaeta	Capitella
Carcinus maenas	11.3937	0.0365	0.1819	286	Arthropoda	Malacostraca	Cancer

Appendix 3.   Total density of the most densely occurring OTUs across the SIBES sampling sites (Sum Density). The taxonomic affiliation of each species is also provided. The first fifteen species with the most abundance are provided for each year of sampling.

sp	Sum Density	phylum	class	order	family	genus
2008						
Hydrobia ulvae	1761621	Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobia
Pygospio elegans	1240283	Annelida	Polychaeta	Spionida	Spionidae	Pygospio
Aphelochaeta marioni	1157301	Annelida	Polychaeta	Terebellida	Cirratulidae	Aphelochaeta
Oligochaeta sp.	972175	Annelida	Clitellata			
Scoloplos armiger	611973	Annelida	Polychaeta		Orbiniidae	Scoloplos
Lanice conchilega	539926	Annelida	Polychaeta	Terebellida	Terebellidae	Lanice
Urothoe poseidonis	499591	Arthropoda	Crustacea	Amphipoda	Urothoidae	Urothoe
Marenzelleria viridis	494070	Annelida	Polychaeta	Spionida	Spionidae	Marenzelleria
Corophium sp.	480122	Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium
Capitella capitata	367104	Annelida	Polychaeta		Capitellidae	Capitella
Hediste diversicolor	242281	Annelida	Polychaeta	Phyllodocida	Nereididae	Hediste
Cerastoderma edule	195386	Mollusca	Bivalvia	Veneroida	Cardiidae	Cerastoderma
Polydora cornuta	178112	Annelida	Polychaeta	Spionida	Spionidae	Polydora
Macoma balthica	127007	Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma
Spio martinensis	119962	Annelida	Polychaeta	Spionida	Spionidae	Spio
2009						
Hydrobia ulvae	4162560	Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobia
Corophium sp.	1720739	Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium
Scoloplos armiger	1462766	Annelida	Polychaeta		Orbiniidae	Scoloplos
Ensis directus	1007507	Mollusca	Bivalvia	Euheterodonta	Pharidae	Ensis
Pygospio elegans	853485	Annelida	Polychaeta	Spionida	Spionidae	Pygospio
Urothoe poseidonis	795117	Arthropoda	Crustacea	Amphipoda	Urothoidae	Urothoe
Marenzelleria viridis	673363	Annelida	Polychaeta	Spionida	Spionidae	Marenzelleria

|						
Capitella capitata	544942	Annelida	Polychaeta		Capitellidae	Capitella
Aphelochaeta marioni	533014	Annelida	Polychaeta	Terebellida	Cirratulidae	Aphelochaeta
Eteone longa	342134	Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Eteone
Lanice conchilega	307102	Annelida	Polychaeta	Terebellida	Terebellidae	Lanice
Hediste diversicolor	305545	Annelida	Polychaeta	Phyllodocida	Nereididae	Hediste
Polydora cornuta	278178	Annelida	Polychaeta	Spionida	Spionidae	Polydora
Macoma balthica	263236	Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma
Oligochaeta sp.	254268	Annelida	Clitellata			
2010						
Hydrobia ulvae	6760038	Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobia
Marenzelleria viridis	3796196	Annelida	Polychaeta	Spionida	Spionidae	Marenzelleria
Corophium sp.	1871170	Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium
Scoloplos armiger	1160482	Annelida	Polychaeta		Orbiniidae	Scoloplos
Capitella capitata	1018808	Annelida	Polychaeta		Capitellidae	Capitella
Urothoe poseidonis	641962	Arthropoda	Crustacea	Amphipoda	Urothoidae	Urothoe
Macoma balthica	476366	Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma
Lanice conchilega	354384	Annelida	Polychaeta	Terebellida	Terebellidae	Lanice
Hediste diversicolor	326533	Annelida	Polychaeta	Phyllodocida	Nereididae	Hediste
Eteone longa	309621	Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Eteone
Abra tenuis	190047	Mollusca	Bivalvia	Veneroida	Semelidae	Abra
Cerastoderma edule	185084	Mollusca	Bivalvia	Veneroida	Cardiidae	Cerastoderma
Polydora cornuta	136373	Annelida	Polychaeta	Spionida	Spionidae	Polydora
Phyllodoce mucosa	95546	Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Phyllodoce
Heteromastus filiformis	89284	Annelida	Polychaeta		Capitellidae	Heteromastus

Appendix 4.   The OTUs with the widest distributional range, in terms of occurrence, across the Dutch Wadden Sea from 2008 to 2010. The number of positive observations is provided (n) and the proportion of sites where a species was sampled is given (%) for each year. The first most frequently observed species for each year are shown.

2008			2009			2010		
sp	n	%	sp	n	%	sp	n	%
Scoloplos armiger	1853	43	*Scoloplos armiger*	2609	55	*Scoloplos armiger*	2483	55
Pygospio elegans	1364	32	*Eteone longa*	1803	38	*Marenzelleria viridis*	2162	48
Aphelochaeta marioni	1256	29	*Capitella capitata*	1519	32	*Capitella capitata*	1804	40
Hediste diversicolor	1069	25	*Macoma balthica*	1349	29	*Eteone longa*	1769	39
Macoma balthica	1042	24	*Hediste diversicolor*	1288	27	*Macoma balthica*	1691	37
Lanice conchilega	1003	23	*Urothoe poseidonis*	1155	24	*Hediste diversicolor*	1370	30
Capitella capitata	932	22	*Marenzelleria viridis*	1040	22	*Urothoe poseidonis*	1055	23
Cerastoderma edule	812	19	*Cerastoderma edule*	998	21	*Lanice conchilega*	1045	23
Urothoe poseidonis	801	19	*Lanice conchilega*	937	20	Corophium sp.	981	22
Nephtys hombergii	771	18	*Heteromastus filiformis*	932	20	*Cerastoderma edule*	802	18
Oligochaeta sp.	761	18	Corophium sp.	886	19	*Heteromastus filiformis*	783	17
Marenzelleria viridis	738	17	*Ensis directus*	766	16	*Hydrobia ulvae*	682	15

Appendix 5.   Examples of three of the 1000 randomly generated subsidence regions used in a single simulated GLM model (see methods section). These regions are constructed by randomly selecting a sampling point in the Wadden Sea and selecting the nearest points.
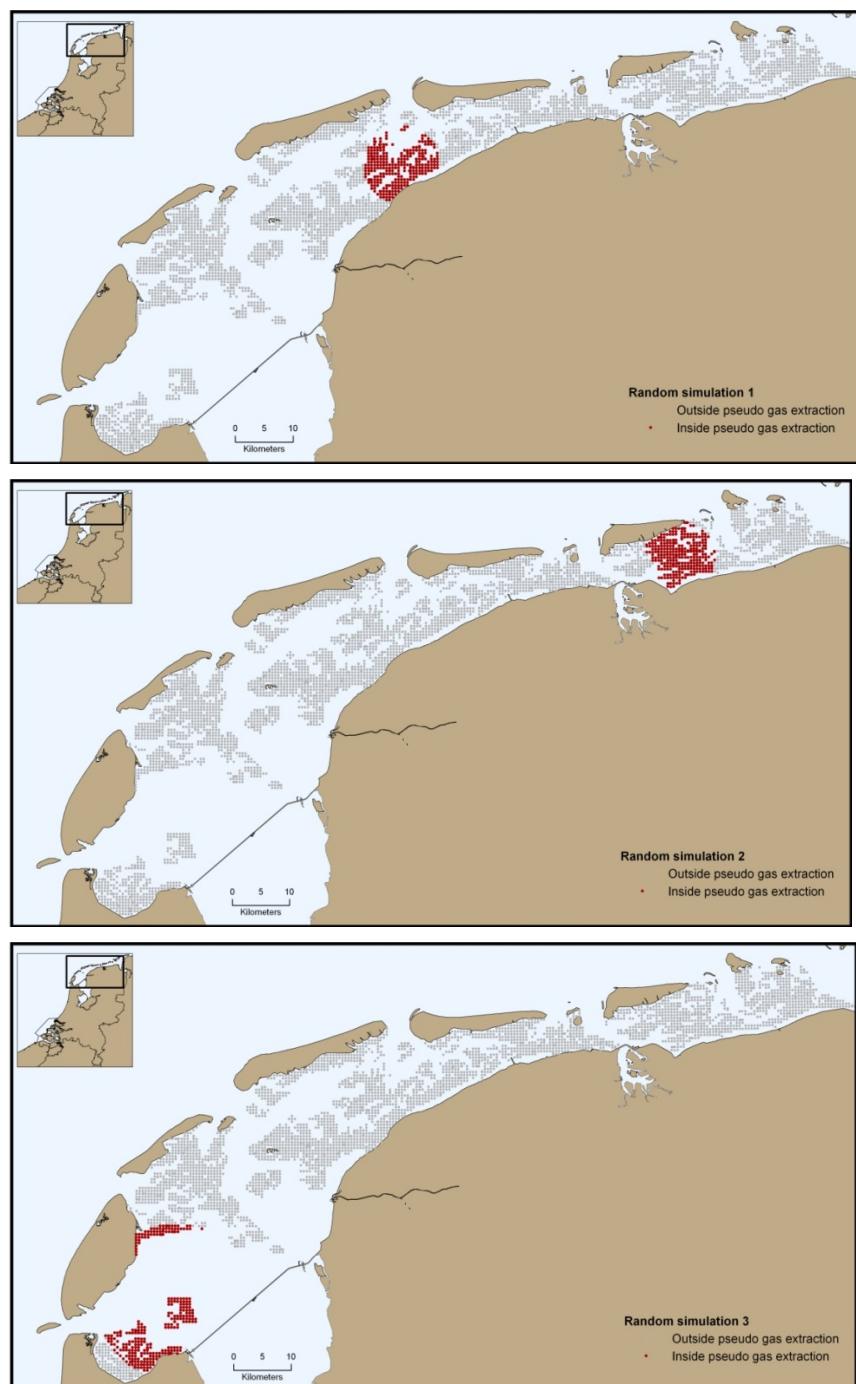
Appendix 6. Areas sampled in each month of the SIBES survey from 2008 to 2010.
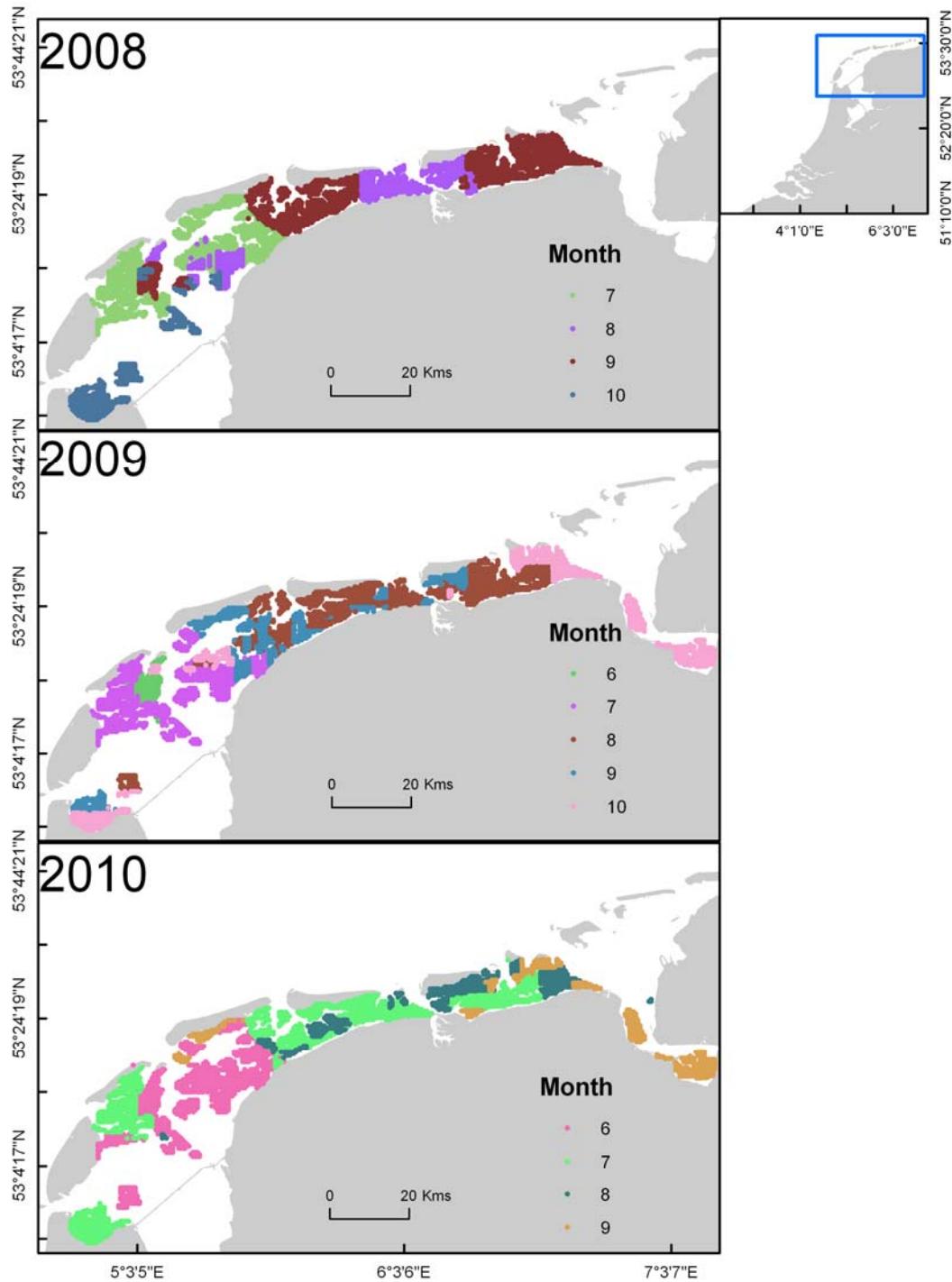
Appendix 7. **Distribution of *Cerastoderma edule* in 2008, 2009 and 2010. Darker colours indicate higher densities.**
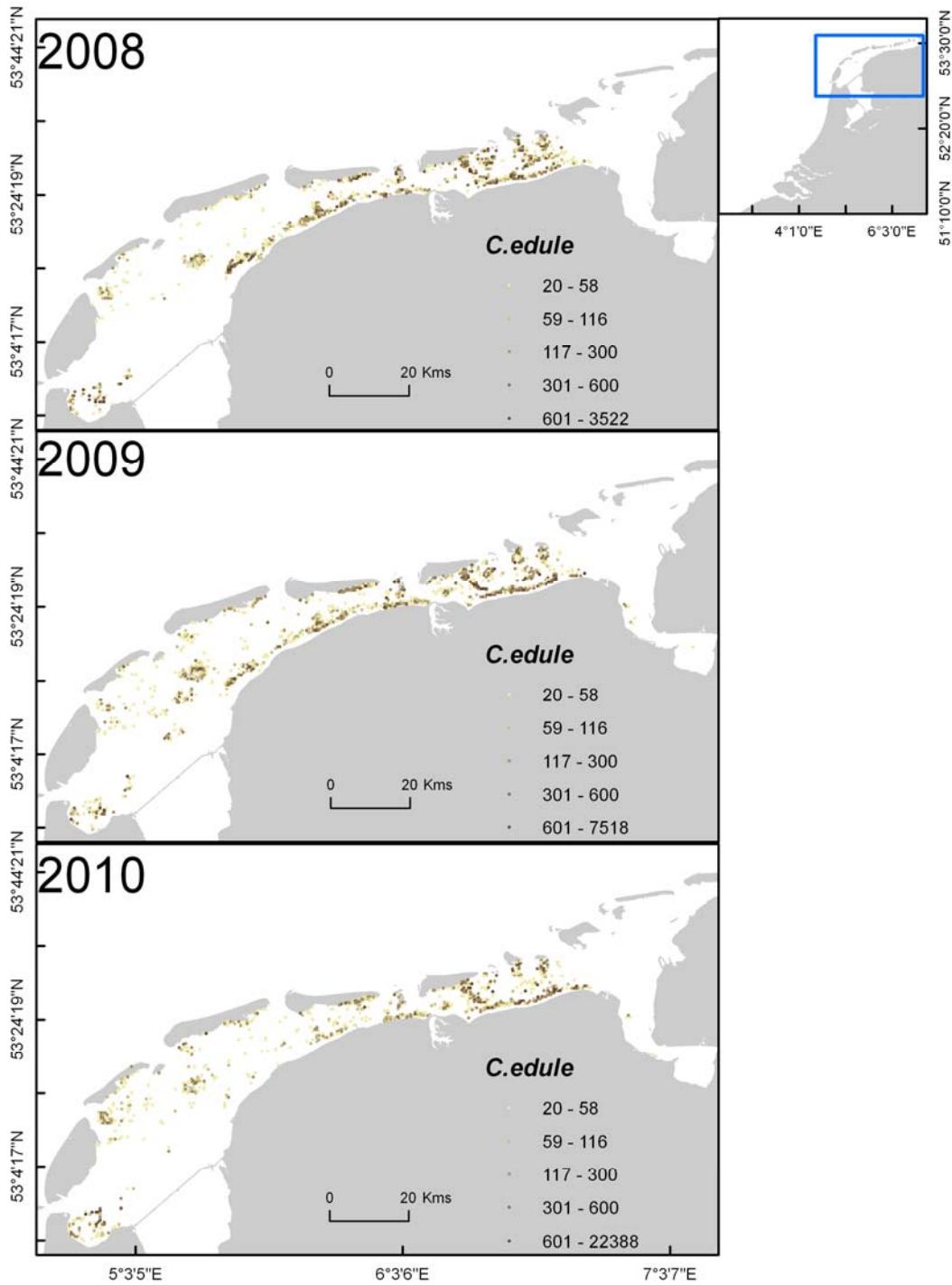
Appendix 8. **Distribution of *Lanice conchilega* in 2008, 2009 and 2010.**
Darker colours indicate higher densities.
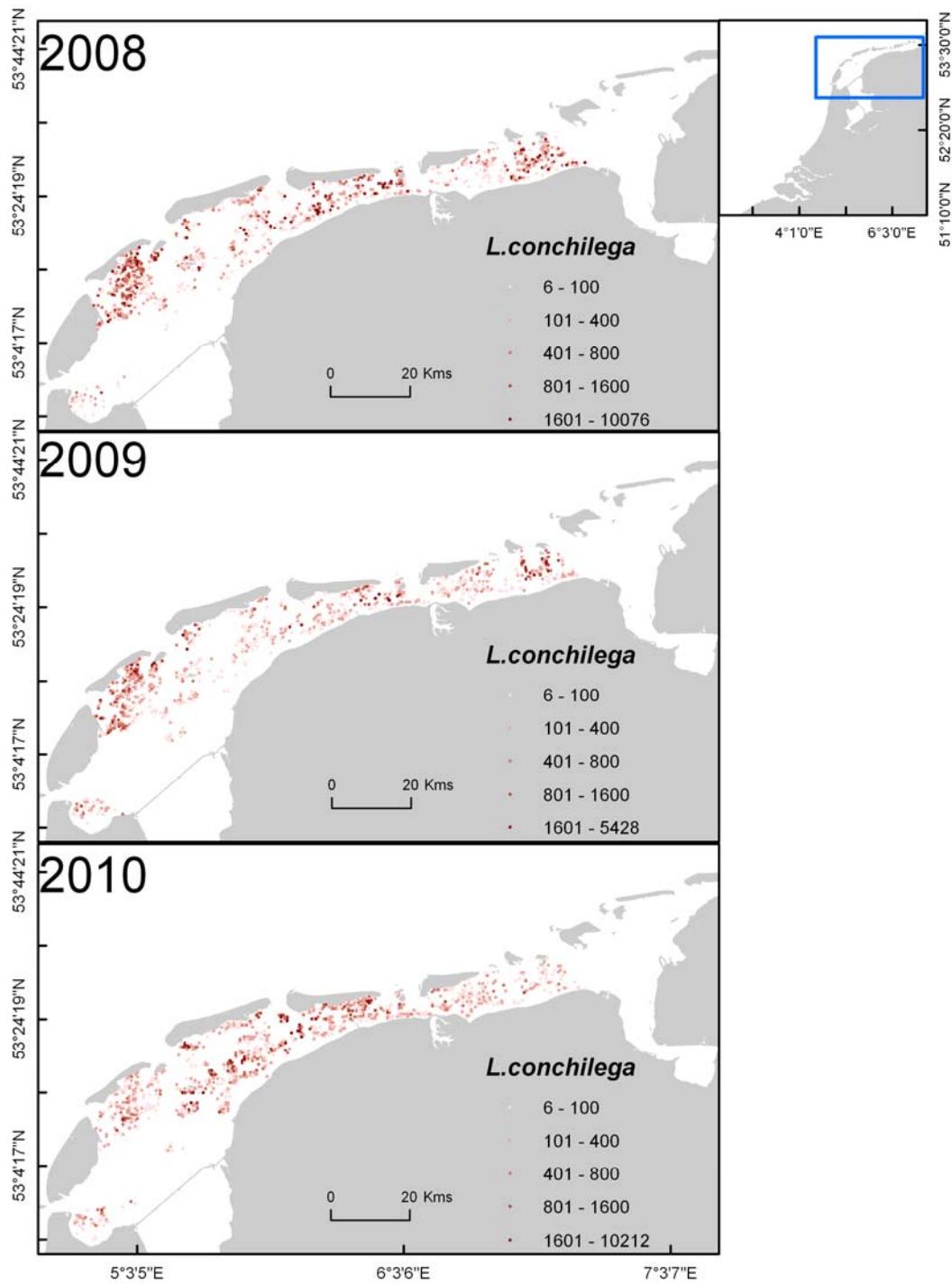
Appendix 9.   Tidal exposure time (%) as estimated by the Rijkswaterstaat (Droogvalkaarten Voordelta by Habets LCGJM and Stoorvogel AA).
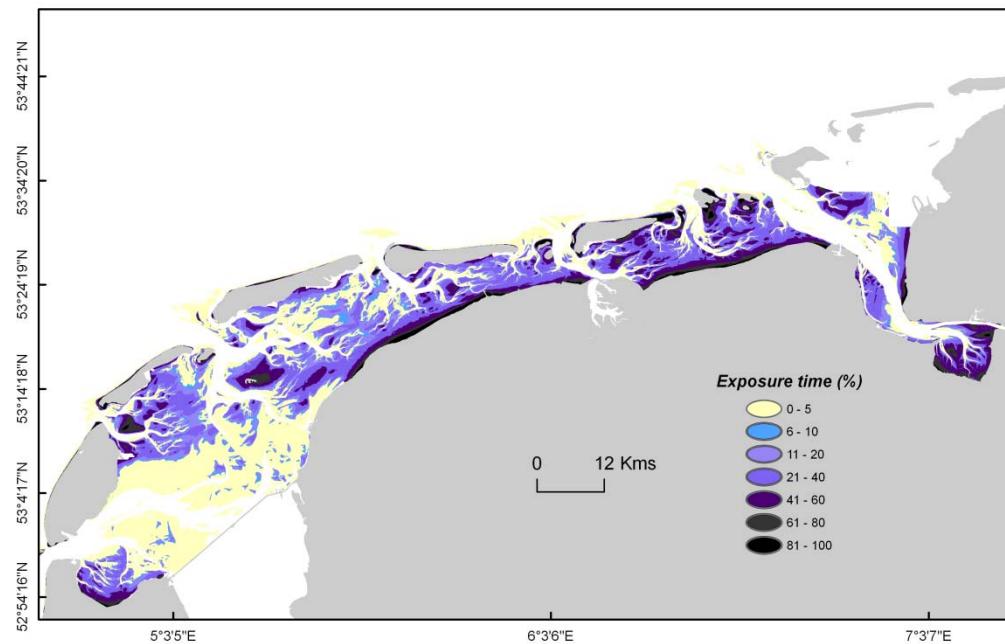
Appendix 10.   Protocols for macrofauna analyses.

Toepassingsgebied

Deze procedure is van toepassing op alle activiteiten behorende bij de analyse van macrozoöbenthos monsters

2   Doel

Het doel van deze procedure is het vastleggen van de activiteiten behorende bij de analyse van in het veld verzamelde macrozoobenthos monsters.

3   Definities

Gekwalificeerd personeel   Personeel met de vaardigheid om zelfstandig benthosmonsters voor te bewerken, uit te zoeken en te determineren

Biomassa   De hoeveelheid levend materiaal die een organisme of groep van organismen vertegenwoordigt

Asvrijdrooggewicht   Een gangbare maat voor biomassa: het gewicht van één of meer in een oven gedroogde organismen verminderd met het gewicht aan as die overblijft na verbranding van die organismen in een verassingsoven.

4   Verantwoordelijkheid

De aangewezen coördinator voor de analyse van macrozoobenthos monsters is verantwoordelijk voor de correcte analyse van de macrozoobenthos monsters in het lab volgens de in de procedure omschreven handelingen.

De aangewezen coördinator is tevens verantwoordelijk voor een goede documentatie en overdracht van de verkregen gegevens en materialen aan de coördinator die voor de verdere verwerking en data invoer verantwoordelijk is.

5   Uitvoering

Voorbereiding

Bij geconserveerd materiaal wordt ter voorbereiding van de analyse elk monster in een zeef van bekende maaswijdte onder stromend water gespoeld. Vervolgens wordt het monster in een petrischaal overgebracht. Het label van het betreffende monster wordt op het analyse/determinatieformulier ingevuld.

Analyse

Determinatie van soorten: De analyse bestaat uit het determineren en tellen van de afzonderlijke macrozoobenthos soorten. Determinatie vindt plaats aan de hand van uiterlijke kenmerken, die beschreven zijn voor de verschillende soorten in standaardwerken. Determinatie van Polychaeta, Mollusca, Crustacea en Echinodermata vindt in principe plaats tot op soortniveau, behalve voor taxa die als gevolg van de conservering onvoldoende herkenbare kenmerken vertonen (bijv. Nemertini en Oligochaeta), sommige juveniele organismen en organismen

die te zeer beschadigd zijn. Organismen > 1 cm worden met het blote oog gedetermineerd, tenzij onderscheidende kenmerken alleen microscopisch goed te zien zijn. In dat geval wordt een stereomicroscoop gebruikt. Organismen <1 cm worden altijd onder een stereomicroscoop gedetermineerd. Na determinatie worden per soort de aantallen geteld en op het telformulier genoteerd. Als leidraad geldt dat alleen koppen worden geteld. Indien een soort wordt gevonden die niet of niet met zekerheid gedetermineerd kan worden, wordt een externe expert geraadpleegd.

Eventueel worden reeds in het veld schelpdieren en wormen voor zo ver mogelijk gedetermineerd en geteld.

Biomassabepaling: Van iedere onderscheiden soort of taxon wordt vervolgens het asvrijdrooggewicht bepaald middels droging en verassing van organismen.

Droging en verassing: Van geconserveerde tweekleppigen wordt het vlees uit de schelp gehaald. Dit gebeurt alleen bij de grotere (vanaf 5 mm) exemplaren. Biomassabepaling geschiedt door dieren in hun geheel dan wel alleen het vlees van de dieren in een porseleinen kroes te drogen. Iedere gevulde kroes draagt een nummer dat op het telformulier achter de betreffende soort wordt genoteerd. De kroezen worden gedurende 2 tot 3 etmalen in een geventileerde stoof geplaatst bij een temperatuur van 60°C. Na droging worden de kroezen in een exsiccator geplaatst, en na afkoeling tot omgevingstemperatuur gewogen op een elektronische balans, gekoppeld aan een computer, waarbij nummer van de kroes en totaal gewicht van de kroes met inhoud genoteerd wordt. Na deze eerste weging worden de kroezen met inhoud geplaatst in een oven om bij een temperatuur van 560°C gedurende 5 uur te worden verast (verbrand). Na te zijn afgekoeld worden de kroezen weer in een exsiccator geplaatst, en na afkoeling tot omgevingstemperatuur voor een tweede maal gewogen, waarbij waarbij wederom nummer van de kroes en totaal gewicht van de kroes met inhoud genoteerd wordt. Het verschil tussen beide wegingen levert het asvrij drooggewicht op.

Kwalificatie van medewerkers

Uitvoeren analyse/determinatie van monsters kan door gekwalificeerd personeel op zelfstandige basis worden uitgevoerd, door niet gekwalificeerd personeel uitsluitend onder supervisie van gekwalificeerd personeel. Onder supervisie houdt in dit geval in dat determinaties steekproefsgewijs gecontroleerd worden door gekwalificeerd personeel.

Appendix 11.   Protocols for sediment analyses.

Toepassingsgebied
Deze procedure is van toepassing op alle activiteiten behorende bij de analyse van sediment monsters.

2   Doel
Het doel van deze procedure is het vastleggen van de activiteiten behorende bij de analyse van sediment monsters.
3   Definities
       Coulter counter: Elektronische deeltjesteller
4   Verantwoordelijkheid
De aangewezen coördinator voor de analyse van sediment monsters is verantwoordelijk voor de correcte analyse van de sediment monsters in het lab volgens de in de procedure omschreven handelingen.

De aangewezen coördinator is tevens verantwoordelijk voor een goede documentatie en overdracht van de verkregen gegevens en materialen aan de coördinator die voor de verdere verwerking en data invoer verantwoordelijk is.

5   Uitvoering
Binnen de sedimentanalyse wordt gebruik gemaakt van twee Coulter "deeltjes-tellers": de Coulter LS 230 en de Coulter Beckman LS 13 320 met Autoprep. Deze hebben ieder hun eigen behandel- en meetmethode.

Coulter LS 230

Voorbereiding
Ieder monster wordt in een glazen potje gevriesdroogd. Dit proces duurt afhankelijk van de hoeveelheid water in het monster enkele uren tot 3 dagen. Vervolgens wordt het monster gezeefd over een 2 mm zeef. Indien er materiaal op de zeef achterblijft wordt dit gewogen, alsook de fractie < 2mm. Beide gewichten worden genoteerd. Van de fractie < 2 mm worden nu enkele grammen afgewogen en bewaard voor analyse met de Coulter counter

Analyse
De analyse kan nu op 3 manieren plaatsvinden:
a.   Zonder chemicaliën: Aan het afgewogen materiaal wordt demiwater toegevoegd waarna het monster direct in de Coulter counter wordt gemeten.
b.   Na toevoeging van H_2O_2: Aan het afgewogen materiaal wordt demiwater + H_2O_2 toegevoegd. Vervolgens wordt het monster 7 uur op het zandbad of in de droogstof geplaatst. Op deze wijze wordt alle organisch materiaal uit het monster verwijderd. Aan het monster wordt nu weer demiwater toegevoegd waarna het materiaal minstens 3 nachten de tijd krijgt om te bezinken. Nu wordt het monster afgezogen. Vervolgens wordt het monster doorgemeten in de Coulter counter in demiwater waaraan natriumpyrofosfaat is toegevoegd.
c.   Na toevoeging van H_2O_2 en HCl: Aan het afgewogen materiaal wordt demiwater + H_2O_2 + HCl toegevoegd. Vervolgens wordt het monster 7 uur op het zandbad of in de droogstof geplaatst. Op deze wijze wordt alle organisch materiaal en kalk uit het monster verwijderd. Aan het monster wordt nu weer demiwater toegevoegd waarna het materiaal minstens 3 nachten de tijd krijgt om te bezinken. Nu wordt het monster afgezogen. Vervolgens wordt het monster doorgemeten in de Coulter counter in demiwater waaraan natriumpyrofosfaat is toegevoegd.

Coulter Beckman LS 13 320 met Autoprep

Voorbereiding
Ieder monster wordt waar nodig in een kunststof potje over gebracht en gevriesdroogd. Dit proces duurt afhankelijk van de hoeveelheid water in het monster enkele uren tot 3 dagen.
Hierna kan de voorbereiding op twee manieren worden voortgezet: Niet voorbehandeld en voorbehandeld.

Niet voorbehandeld
Het monster wordt over een 2 mm zeef ingewogen in een 13 ml PP reageerbuis (deeltjes groter dan 2 mm kunnen de meetcel beschadigen en worden dus niet gemeten). Vervolgens wordt RO (Reversed Osmosis) water toegevoegd om de sediment deeltjes in suspensie te brengen. Vervolgens kunnen de gevulde buizen in de Autoprep module van de Coulter Beckman LS 13 320 gezet worden en zijn ze gereed om gemeten te worden.

Voorbehandeld
Het monster wordt over een 2 mm zeef ingewogen in een 50 ml PP centrifugebuis (deeltjes groter dan 2 mm kunnen de meetcel beschadigen en worden dus niet gemeten). Vervolgens wordt aan elke centrifugebuis 15 ml RO water toegevoegd. Hierna volgt respectievelijk 15 ml 35% H_2O_2-oplossing (waterstofperoxide) en 12 ml 0.5N HCl-oplossing (zoutzuur) toegevoegd. Daarna wordt de centrifugebuis aangevuld tot de 45 ml markering met RO water. De buizen (er kunnen 30 buizen per serie gemeten worden) worden een nacht (± 16 uur) bij 80 °C in een stoof gezet.
De volgende ochtend worden de monsters, na afkoeling, aangevuld met RO water om het verdampte vocht te vervangen. Hierna gaan de buizen 5 minuten bij 3000 toeren per minuut in een centrifuge. Vervolgens worden de chemicaliën boven het sediment afgezogen met behulp van een waterstraalpomp. Aan de buizen wordt respectievelijk 5 ml RO water en 2,2 ml Natriumpyrofosfaat-oplossing toegevoegd, waarna de buizen met behulp van een vortex reageerbuisschudder gehomogeniseerd worden. De centrifugebuizen worden tot de 40 ml markering aangevuld met RO water en 12 minuten bij 3000 toeren per minuut gecentrifugeerd. De vloeistof boven het sediment word afgezogen en het monster wordt overgespoeld in een 13 ml PP reageerbuis. Vervolgens kunnen de gevulde

buizen in de Autoprep module van de Coulter Beckman LS 13 320 gezet worden en zijn ze gereed om gemeten te worden.

Analyse
Na het invullen van de monster gegevens in de aan de het apparaat gekoppelde computer kunnen de monsters gemeten worden. De Beckman Coulter LS 13 320 is een deeltjesgrootte analyser die werkt volgens het principe van laserdiffractie en lichtverstrooiingsmeeting (PIDS).
De methode werkt ruwweg als volgt;
Een laser vuurt een laserstraal af op de deeltjes in de meetcel. Het licht dat op de deeltjes komt wordt verstrooid in verschillende richtingen. Vervolgens pikken de 132 detectoren die rondom de meetcel geplaatst zijn (een deel) het licht weer op. Aan de hand van de intensiteit van het licht en de hoek waaronder deze op de detector valt kan via een complex algoritme de grootte van het deeltje berekend worden.

Standard Operating Procedure (SOM) Coulter LS 13 320 Autosampler
File name: SIBES-autoprep_alm_ap.som
SOM Description: SIBES-autoprep
Sample Description:
File ID:
Sample number:

Comment 1:
Comment 2:
Run number:
Control Sample: No
Sample Density: 0 g/mL

Fluid: Water
Include PIDS: Yes
Use Auto-Prep Station: Yes
File Name Template: <F20>_<S20>_<R4>_<U1>.<X>

Run folder: C:\LS13320\Runfiles
Run length: 90 seconds
Number of runs: 1
Pump speed: 76
Sonicate before first run: No
Sonicate during run: No
Compute sizes: Yes
Optical model: grijs.rf780d   PIDS included
Save file: Yes
Export size data: Yes
Print report: No

Repeat Cycle: Yes
Auto Rinse first: No
Measure Offsets: Yes
Align: Yes
Measure Background: Yes
Measure Loading: Load sample using Auto-Prep Station
Enter Sample Info: No
Start Run(s):   Yes
Auto Rinse Last: Yes

Auto-Prep Station Settings
Sonicate for   5 seconds
Sonicate Power: 5
Empty tube for   4 seconds
Pulsed Flush for   3 seconds
Wait after emptying for   2 seconds
Auto-Dilute: No

Kwalificatie van medewerkers
Uitvoeren analyse/determinatie van monsters kan door gekwalificeerd personeel
op zelfstandige basis worden uitgevoerd, door niet gekwalificeerd personeel
uitsluitend onder supervisie van gekwalificeerd personeel.