SYNOPTIC INTERTIDAL BENTHIC SURVEYS ACROSS THE DUTCH WADDEN SEA 2008 to 2011

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SUMMARY

The Synoptic Intertidal Benthic Survey Monitors macrobenthos across the Wadden Sea

This report will show that (1) the synoptic intertidal benthic surveys of the Wadden Sea (SIBES) has the power to detect change; a requirement for the continuous monitoring of ecological effects of gas exploitation and that (2) with time, in combination with the other monitoring programmes, SIBES will have the power to determine the influence of actual land subsidence to benthos in the East Frisian area, where subsidence is currently minimal.

The Dutch Wadden Sea is acknowledged for its ecological importance, but also for its natural resources like fisheries, gas and salt. In total it is estimated that more than 20 billion cubic metres of gas lie beneath the Dutch Wadden Sea. In the last decades, gas production has taken place under the Wadden Sea (Zuidwal and Ameland) and the province of Groningen (Slochteren). Since 2007, gas production also began in the East Frisian area.

Modelling studies estimate that sediment infilling should compensate for land subsidence that occurs with gas production. In the case that either sediment infilling or land subsidence are taking place, both factors could affect habitat suitability for a swath of organisms. Currently, along the coast of NE Friesland subsidence has been predicted to be less than 2 cm. By contrast, other areas that have been drilled over a longer period show greater subsidence. Thus, examining areas where production has taken place for a longer period might provide an indication of changes in the macrobenthos associated with gas production.

Macrobenthos, organisms larger than 1 mm that live in or on the mud, are commonly used as signalling species for anthropogenic driven changes in tidal flat environments. These species are suitable indicators because many species are sedentary and thus cannot escape adverse situations, and also have strong environmental associations, in combination with relatively short life-spans, such that they show relatively fast responses to adverse conditions. Furthermore, they form the base of the food chain. Thus if habitat changes, due to gas production, are occurring in the tidal flat area of the Wadden Sea it could be expected that changes in the composition, abundance or biomass of macrobenthic organisms might occur.

To examine whether macrobenthic organisms across the tidal flats of the Dutch Wadden Sea differ in the areas of gas production, we compared macrobenthos populations in the four areas of gas production: Zuidwal, Ameland, the East Frisian area and Groningen. Macrobenthos and sediment samples were collected across the tidal area of the Wadden Sea in the summer months of 2008 to 2011 during the SIBES sampling programme (see Preface). The 2012 data is currently being analysed. SIBES runs one year behind the remaining programmes. Contour intervals derived from Nederlandse Aardolie Maatschapij (NAM) models were used to identify areas of predicted subsidence.

To test whether the macrobenthos attributes in areas of predicted subsidence IN differed from macrobenthos in areas with no subsidence, that have a matching environment, OUT we used a quasi-poisson regression. The macrobenthos attributes included total abundance, total biomass and single species abundances. Monte Carlo simulations were run to determine whether a macrobenthic attribute, if identified as different in the gas production area IN, was more different than the natural variation for that macrobenthic attribute across the system.

To test the sensitivity of this Monte Carlo approach for detecting change in the Wadden Sea system, we ran a sensitivity analysis. In the sensitivity analysis, all production areas were excluded and 350 random IN areas were simulated across the system. An increase or decrease in abundance was then simulated in each of these random IN areas to test the effect size needed to observe a change in a macrobenthic attribute. Our analyses of two species, *Scoloplos armiger* and *Cerastoderma edule* showed that this Monte Carlo approach could detect an 8-fold increase in abundance or a 10-fold decrease; in the case of *S. armiger*.

The models identified that at Zuidwal and Groningen total biomass differed compared to the remainder of the system. At Zuidwal, total abundance was different relative to the remainder of the system. Of the 76 species that were tested at each of the four gas production areas (n>15), 10 species showed different abundances relative to the reference areas (OUT). The majority of these species (n = 8) were polychaetes, a group known to be highly responsive to change. Of the 10 species showing differences, 6 species had a higher abundance in the gas production areas. Zuidwal was the area where most species showed differences in abundance (n= 5 species).

A nearest neighbour distance analysis was used to identify the direction of change in a macrobenthic parameter, while accounting for the effect of environment. Silt and exposure times in the OUT area were matched to identical sites in the IN area. Macrobenthic abundance was then correlated for these environmentally identical points in the IN and OUT areas. The nearest neighbour distance analysis identified that 9 of the 10 species had higher abundances in the IN area relative to OUT, when accounting for environment.

Community composition, as examined using multidimensional scaling analysis, also showed that macrobenthic communities in all four areas overlapped in community space with the communities not affected by subsidence, but which share a similar physical environment. Only in Zuidwal was there a slight trend for communities to be associated with longer tidal coverages (short exposure times).

As current predicted subsidence effects in the East Frisian area are small (<2 cm), SIBES currently provides a reference of the system prior to larger subsidence effects. Thus given the obligation - exploitation with “hand on the tap” - we can only conclude that the SIBES sampling must continue. In the case of the East Frisian Area, the SIBES efforts will become more valuable in time, as the duration of production increases. With the increasing power of the macrobenthos data set, and with increasing and more precise knowledge about environmental changes (as also determined by the other monitoring programmes), insights into the factors driving change will be gained; with an appreciation of the role of anthropogenic factors.

Conclusions

The sensitivity analysis in this study showed that our statistical approach has the power to detect changes in macrobenthos abundance. The results from our approach highlighted that total biomass differed, and tended to be lower, in Zuidwal and Groningen, the two areas where production has taken place the longest. In total ten of 76 species showed differences in the IN areas, and most differences tended to be positive. Interestingly, most differences in species abundance were observed at Zuidwal; an area of long-term drilling.

Continuation of the SIBES monitoring is a cost-effective way to monitor possible effects of gas production, and subsidence, related changes on the core driver of the Wadden Sea food web, i.e. the macrobenthos of the Wadden Sea.

Photo 1. The NIOZ research vessel Navicula is used as a base for the SIBES sampling campaign in the summer months.

PREFACE

In 2008, building on the experience of previous large-scale grid sampling, the NIOZ initiated Synoptic Intertidal Benthic Surveys of the Wadden Sea (SIBES) across the entire tidal flat area of the Dutch Wadden Sea, i.e. from the Marsdiep to the Ems. The goal of the SIBES monitoring programme is to monitor macrobenthic tidal flat organisms. One important application is to monitor for effects of gas production and its associated effects like land subsidence. The SIBES survey covers an area of 2483 km^2 or ~4500 sampling stations.

Determining what provides a suitable reference area for detecting an anthropogenic change in communities of interest provides a challenge for any monitoring study (Osenberg and Schmitt, 1996), especially in a dynamic system such as the Wadden Sea. To determine whether change is occurring, it is imperative that multiple reference areas are available in space and time for comparison with areas perturbed by human impacts. A comparison of different sampling designs, identified that the most powerful and cost effective sampling design for detecting changes was gridded sampling interspersed with random points (Bijleveld et al., 2012). Thus, the SIBES design can draw on the entire system as a reference area to monitor macrobenthic populations and sediments for change.

To distinguish impacts of subsidence, due to gas production, from the inherent natural variation in the system, sampling should be conducted over long temporal and large spatial scales. Without long-term data, short-term natural variability can be mistakenly interpreted as human driven change (Hewitt et al., 2001, Hewitt et al., 2007). In the case of the East Frisian area where production is still in its early stages, long-term monitoring provides an opportunity to monitor if changes occur in the benthos or grain size parameters. Currently, such data is unavailable in the other long-term production areas.

The SIBES survey effort was funded by the Nederlands Aardolie Maatschapij (NAM), the Zee and Kust Onderzoeks programma (ZKO) of NWO and the Royal Netherlands Institute for Sea Research from 2008 to 2012.

INTRODUCTION

The Wadden Sea

The Wadden Sea is a long and narrow system that stretches from The Netherlands to Denmark. This tidally driven system shares multiple connections with the North Sea and is an exit area for several major European rivers. In 2009, the Wadden Sea received World Heritage status from UNESCO, additionally to its Ramsar status, in recognition of its unique landscape and wildlife (http://www.waddensea-worldheritage.org/). The Wadden Sea not only provides important ecological services, but also numerous economic services to human 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 (Beukema, 1976, Wolff, 1983, van de Kam et al., 2004).

Gas production in the Dutch Wadden Sea

The Dutch part of the Wadden Sea forms more than a quarter of the international Wadden Sea; comprising ~2500 of its total extent of 8000 km^2 (Wolff, 2000). In total it is estimated that 20 billion cubic metres of gas lie beneath the Dutch Wadden Sea (http://www.nam.nl/nl/projects/gas-production-waddensea/backgroundinformation.html). In the last decades, gas production has taken place in the areas of Zuidwal, the island of Ameland, and Slochteren in Groningen. In addition, since 2007 gas production began in the area of the East Frisian area. With the exception of Zuidwal, most production areas are extracted by the Nederlandse Aardolie Maatschapij (NAM).

Current hydrodynamic modelling studies estimate that effects of gas production should be minimal, as sediment infilling, should take place (Wang and Eysink, 2005). Nevertheless, tidal flats might subside, which would lead to longer exposure times. In the case that either sediment infilling and/or land subsidence is occurring, both factors could affect habitat suitability for benthic organisms.

Different types of monitoring studies have been conducted by the NAM since 2008 in the area of the East Frisian area. The integration of the results from these studies should provide a basis to determine whether changes are occurring in the East Frisian area over time.

Photo 1. Ensis directus, Alitta succinea and Macoma balthica are found in the Dutch Wadden Sea.

Macrobenthos

The macrobenthos community of the intertidal and subtidal areas constitute the interface between primary production and the higher trophic layers of fish, birds and marine mammals. Thus they are the core drivers of the Wadden Sea foodweb. Macrobenthos are defined as benthic organisms that are larger than 1 mm in size (Herman et al., 1999) and include crustaceans, polychaetes and molluscs. The ecosystem functioning of tidal flat systems depends on having healthy populations of macrobenthos, as these organisms recycle nutrients, decompose organic matter and regulate nutrient cycles (Levin et al., 2001). For example, suspension feeders transport sediments across the sediment water interface, bioturbators increase the turnover in nutrients and sediments, and biogenic builders generate structure and consolidate sediments (see review by Levin et al., 2001). Macrobenthos species also provide an important food source for migratory and non-migratory shorebirds (Zwarts and Wanink, 1993, Zwarts, 1996, van de Kam et al., 2004) and other species living in the Dutch Wadden Sea (Wolff, 1983).

Macrobenthos are commonly used to assess changes due to human perturbations, as they are highly responsive to change as they are relatively sedentary and short-lived (< 5years in most cases, Beukema et al., 1999, Hewitt et al., 2001, Hewitt et al., 2007, Hewitt et al., 2008). For example, short-lived opportunistic species are known to respond quickly to environmental perturbations (Grassle and Grassle, 1974). In the Dutch Wadden Sea (Beukema, 1976, Compton et al., 2008, Kraan et al., 2010), as in other systems (e.g. van der Meer, 1991, Ysebaert et al., 2002, Thrush et al., 2003), it has been shown that the abundance of macrobenthos species are associated sediment grain size and exposure time. For example, Kraan et al., (2010) identified that *Macoma balthica* was more abundant in areas of relatively finer sediments and longer exposure times, whereas *Cerastoderma edule* was more abundant in areas with relatively sandier sediments and shorter exposure times. In addition, recent analyses have shown that macrobenthos assemblage composition changes across the multiple environmental gradients of the Wadden Sea (Compton et al., 2013).

In summary, macrobenthos can act as a signalling species for human induced changes and are also an integral component of the Wadden Sea tidal flat ecosystem.

Detecting change

A previous study exploring possible effects of land subsidence and sea level rise on macrobenthos predicted that effects of land subsidence should be minimal (Beukema, 1998, Beukema, 2002). This prediction was based on a modelled association between macrobenthos biomass, richness and density and the period that these organisms are under water (the inverse of exposure time). This particular study assumed 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).

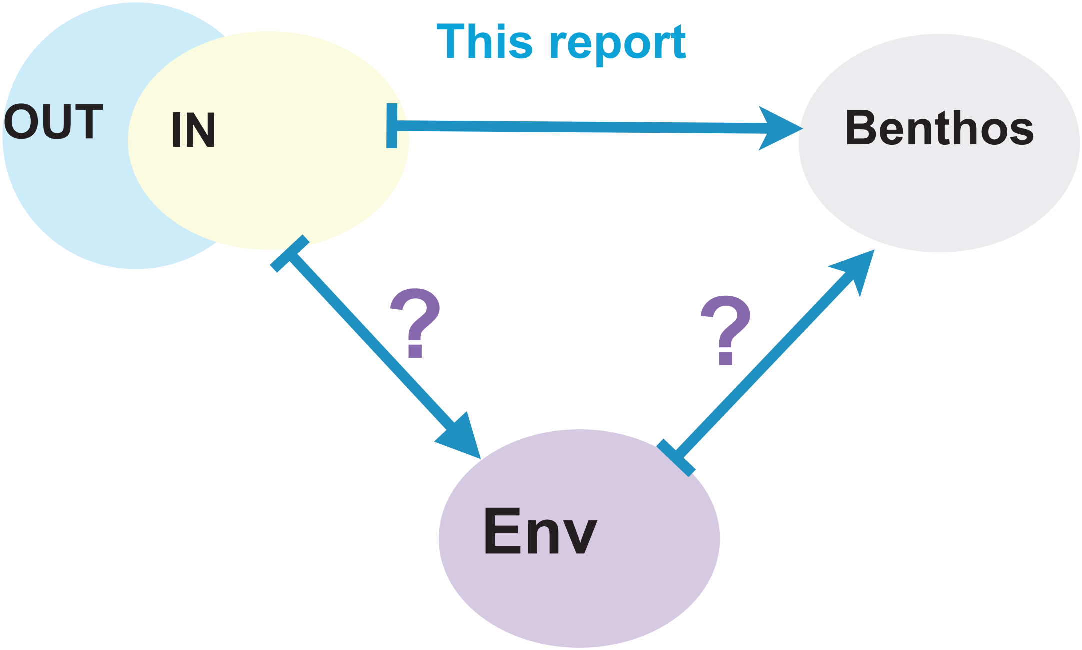
These studies (Beukema, 1998, Beukema, 2002) provide a useful starting hypothesis. However, as the expectations from these studies are based on modelled data and not on actual observations from tidal flat areas where gas production has occurred, they should be interpreted with caution. This is notable, because a change in environment due to gas production need not lead to a change in exposure time, but might induce a change in macrobenthos via a change in grain size or another unconsidered variable, e.g. change of tidal flat area (Wang and Eysink, 2005). Thus to assess whether macrobenthos differ in areas of subsidence relative to areas with no subsidence monitoring is completed.

As a way to examine whether a change is occurring in the area of gas production around the East Frisian area (start date 2007), a field assessment study called the Synoptic Intertidal Benthic Surveys of the Wadden Sea (SIBES) began in 2008. The goal of a field assessment study is to compare the state of the system in the presence of an activity with the state it would have assumed had that activity not occurred (Osenberg and Schmitt, 1996). However, determining what provides a suitable reference area for detecting a significant anthropogenic change in an area of activity provides a challenge to any monitoring study

(Osenberg and Schmitt, 1996), especially in such a dynamic system as the Wadden Sea. Thus to determine whether a change is occurring it is imperative that multiple reference areas are available in space and time for comparison with the area of activity.

Prior to starting the SIBES sampling programme in 2008, a comparison of different sampling designs was completed (Bijleveld et al., 2012). The result of this analysis identified that gridded sampling interspersed with random points across the entire tidal flat area of the Wadden Sea was the most powerful and cost effective for detecting changes in macrobenthos (Bijleveld et al., 2012). This is because this design can draw on the entire system as a reference area to monitor macrobenthic populations and sediments for change. Furthermore, to increase our power to detect an effect in the area of the East Frisian area region, additional sample points were taken.

Figure 1. Flow diagram indicating the goal of this report. We aim to estimate whether benthos in the IN areas differ to OUT areas; while accounting for environment. We cannot determine whether changes to environment have happened in the past, at long term production sites, and if so how they would have affected the benthos (?).



In the East Frisian area the current predicted subsidence effects are minimal (NAM contours < 2cm), suggesting that effects on benthos are likely to be small or non-existent. In this case, we expect that SIBES provides an estimate of what the system looks like prior to larger predicted subsidence effects in the East Frisian area.

By contrast to the East Frisian area, the other sites that have experienced subsidence by gas productions over a period of years or decades, and which are predicted to have subsided significantly, do not have benthic monitoring programmes associated with them (Zuidwal, Ameland and Groningen). The current SIBES monitoring design provides an opportunity to assess whether there are differences in macrobenthos populations in the areas of long-term subsidence IN versus areas outside OUT (see Figure 1). However, this report cannot assess how changes in environment, associated with gas production, could have affected macrobenthos populations (question marks given in Figure 1).

Many aspects of change due to human activity can only be detected and accurately assessed in the light of comparing long-term trends with short-term fluctuations (Thrush et al., 1996). Without a long-term view, macrobenthic fluctuations might mistakenly be taken to be a result of human perturbation; instead of natural variation (Thrush et al., 1996). To describe the macrobenthos of the Dutch Wadden Sea system in their current state, with respect to gas production areas, we removed the noise of the short-term natural fluctuations by averaging the four years of SIBES data, per sample point. We expect that if there are differences in macrobenthic populations at long-term production sites, and/or even the East Frisian area, these changes will be apparent when comparing average measures at each sample point for composition, abundance and biomass in the IN and OUT regions.

AIMS

Using the data collected during the Synoptic Intertidal Benthic Surveys of the Wadden Sea (SIBES) from 2008 to 2011, we examined whether macrobenthic attributes differ in the areas of gas production: Zuidwal, Ameland, the East Frisian area and Groningen. Macrobenthos and sediment samples were collected across the tidal area of the Wadden Sea in the summer months of 2008 to 2011 during the SIBES sampling programme (see Preface). The 2012 data is currently being analysed. SIBES runs one year behind the remaining programmes. Contour intervals derived from Nederlandse Aardolie Maatschapij (NAM) models were used to identify areas of predicted subsidence.

To test whether the macrobenthos attributes in areas of predicted subsidence IN differed from macrobenthos in areas with no subsidence, that have a matching environment, OUT we used a quasi-poisson regression. The macrobenthos attributes included total abundance, total biomass and single species abundances. Monte Carlo simulations were run to determine whether a macrobenthic attribute, if identified as different in the gas production area IN, was more different than the natural variation for that macrobenthic attribute across the system. To test the sensitivity of this Monte Carlo approach for detecting change in the Wadden Sea system, we ran a sensitivity analysis. We also examined whether trends in total abundance, biomass and sediment attributes were apparent in the proximate areas of gas production, as defined by predicted subsidence contour lines, and whether community composition differed in the predicted subsidence areas IN versus areas with no production OUT.

METHODS

Sampling

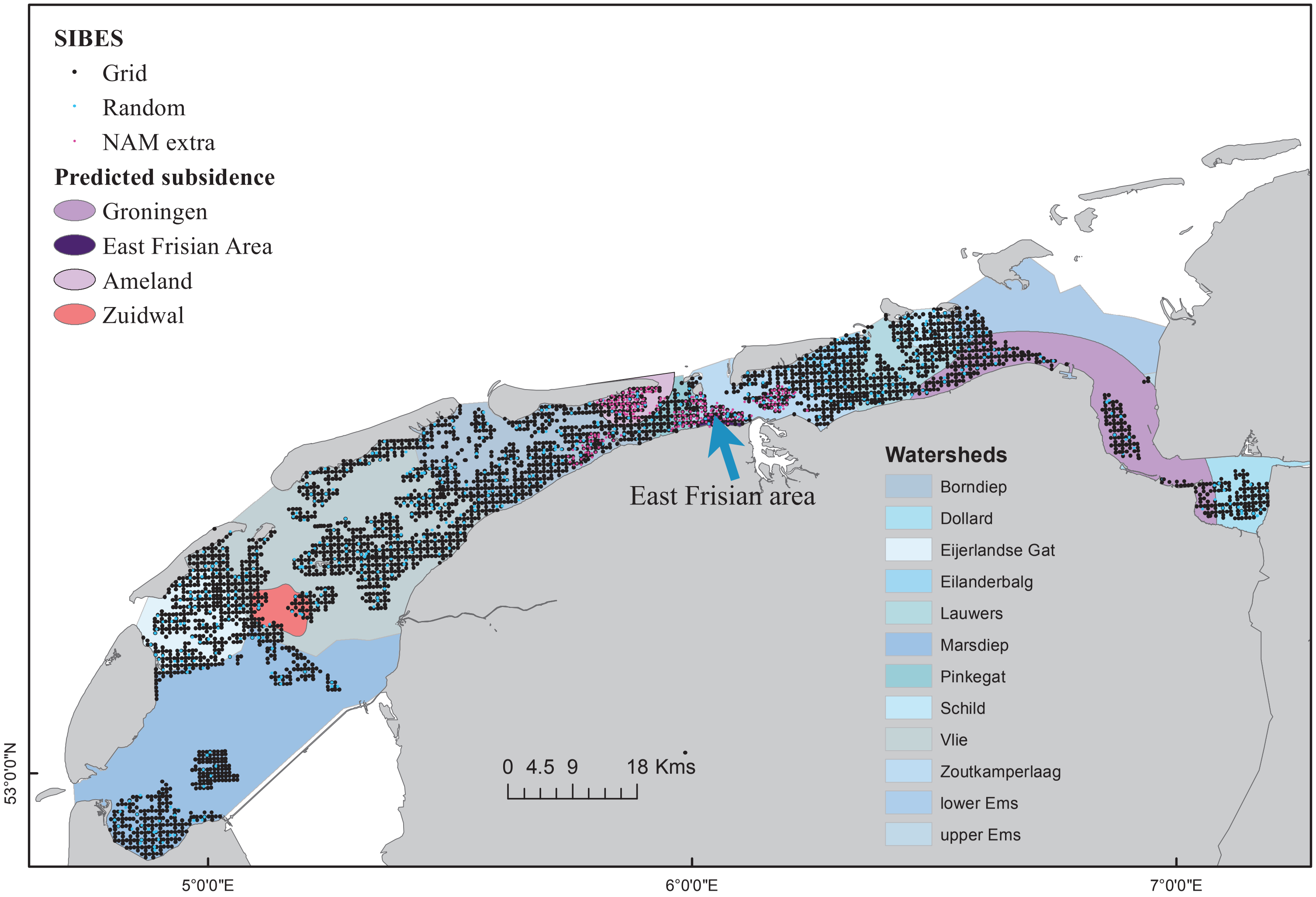
The Synoptic Intertidal Benthic Surveys of the Wadden Sea, SIBES, encompasses the entire intertidal Dutch Wadden Sea (Figure 1). Sampling combines both gridded sample points (500 x 500 m) and a percentage of random points (10% stratified by mudflat) (Osenberg and Schmitt, 1996). In the area of the East Frisian area, additional gridded points were taken to increase the power to detect change in these areas (Aarts et al., 2011). From 2009 onwards, the Ems region was incorporated into the SIBES monitoring (Figure 2). This effort is complementary to the long-term monitoring of the macrobenthos at the western end of our study area in the Balgzand (Beukema et al., 2002b).

Please note that to remove the signal of short-term "noise" the data used in the analysis of this report was based on the average abundance, biomass, richness and sediment grain size attributes per sample point for the four consecutive years (2008 to 2011). See the introduction for a description entailing why the averages of the four years at a point are used in this report.

Photo 2. Sample taken when walking on the tidal flats.

Photo 3. Samples are sieved on a 1 mm mesh.

Figure 2. The SIBES sampling programme, with gridded, random and extra points, and the areas of predicted subsidence (Groningen, East Frisian, Ameland (< 2cm) and Zuidwal). The underlying base layer indicates the different watersheds of the Dutch Wadden Sea.



Sampling was completed in the summers of 2008 to 2011 (June to about October). The NIOZ research vessel, the RV *Navicula*, was used as a platform to access the sample areas across the Dutch Wadden Sea. During low-tide sample sites were accessed by foot. In areas where it was too deep or muddy, small inflatable boats were used. Sampling locations, ~4500/year in total, were found with a handheld GPS (WGS84 as map datum). At each site sampled by foot, a single core of 0.0177 m^2 was taken to a depth of ~25 cm. By boat, two cores were taken to a depth of ~25 cm (combined area of 0.0173 m^2). Both methods yield similar results (Kraan et al., 2007). All macrobenthos samples were sieved on a 1 mm round mesh in the field. Large bivalves were separated and then frozen, whereas the remaining macrobenthic species were preserved using a 4% formaldehyde solution. Using a centrifuge tube, sediment samples were taken to a depth of 4 cm. The sediment samples were taken at 1 x 1 km intervals in 2008 and at 500 x 500 m grid intervals in the remaining years.

Laboratory analysis

Photo 4. All samples are analysed

Photo 5. Small organisms are stained with rose bengal.

All molluscs were identified to species level. All other smaller organisms, predominantly crustaceans and polychaetes, were identified to the finest taxonomic level possible; hereafter named operational taxonomic units (OTUs). Small organisms were stained using rose Bengal dye (C.A.S. no. 632-68-8) for 24 hours, then flushed with fresh water for 10-20 minutes over a 0.5 mm sieve prior to being 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 according to Hartmann-Schröder, (1996) and Hayward and Ryland, (1995). Polychaetes and crustaceans were identified to either a genus or species level, whereas oligochaetes were identified to a class level. Once samples were counted and identified, the biomass of either individuals or multiple individuals of the same species (shells < 8 mm) were determined. The AFDM was determined by first drying the sample for 2 to 3 days at 60°C in a ventilated stove, then taking a dry weight (dry mass). Following this, the sample was incinerated for 5 hours at 560°C and then weighed again to obtain the ash free dry mass (AFDM). Weighing was completed to an accuracy of four decimal places (Mettler Toledo XS204).

Sediment analysis

Sediment samples were freeze-dried for up to 96 hours 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. Samples were then shaken vigorously with a vortex mixer for 30 seconds prior to determining the grain size using a particle size analyser. The particle size analyzer 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). All sediments were analysed according to the 'biological approach', i.e. the organic matter and calcium carbonate were not removed from the samples. Measures of sediment grain size provided in this report include the median grain size in µm, the percentage of silt (< 63 µm), the range in grain sizes (D90 − D10) and the standard deviation in grain sizes. The latter two measures provide an indication of sediment sorting, i.e. how homogenous or heterogeneous a sample is.

Characteristics of areas possibly impacted by subsidence

In this report, we compare average macrobenthos attributes from 2008 to 2011, per sample point, in the four areas of predicted subsidence with samples taken outside of these areas (Figure 2). We use the predicted contour intervals, as provided by the NAM, to examine both macrobenthic responses and sediment grain size attributes in the proximate area of gas production. To link the macrobenthic and sediment data to the NAM contours we used ArcGIS 10.

To examine community composition inside the proximate area of gas production (contour < 2cm), we used barplots to describe which species contributed proportionally the most to the total abundance or biomass. Barplots display the relative frequency of observations from a categorical variable. Note that comparable barplots for the tidal part of the Dutch Wadden Sea are available in Compton et al. (2013). We also examined whether there were trends in macrobenthos abundance (number of individuals per core) and biomass (grams/core) at each of the defined contour intervals (NAM), in the proximate areas of the four gas production sites, were examined by plotting the average and standard errors of abundance, biomass or species richness at each contour interval. These graphs were made using the function lineplot.CI from the package Sciplot in R.

Background environment

To identify reference areas, i.e. OUT areas, that shared a similar environment, as found in the gas production areas we first obtained other physical variables, i.e. fraction of exposure time, maximum tidal current speed and salinity in dry spells, in addition to the SIBES sediment grain size information. Note that these variables were used to characterise the environment at large spatial scales, but cannot be used to infer anything about physical changes in the areas of production, as they are too coarse in resolution.

The five variables selected for this analysis have linkages to benthic organisms in marine environments. Specifically, median grain size and silt are a measure of habitat association, e.g. some tube-building species need relatively coarse sediments to build their tubes (Dankers and Beukema, 1981). These variables are also correlated with physical variables like current speed, which is correlated with substrate stability (Fegley, 1987). Exposure time is correlated with the period of feeding time and thus may be a limiting factor for some suspension feeders like *C. edule*, *M. arenaria* and *M. edulis* (Smidt 1951 in Dankers and Beukema, 1981, Kamermans, 1993). Salinity is important, as only few species are able to tolerate very low salinities e.g. *M. balthica* and *M. arenaria* (Beukema, 1979, Dankers and Beukema, 1981). Maximum tidal current speeds could be associated with the replenishment of phytoplankton food for suspension feeders or sediment erosion under very high velocities.

Information on the physical variables are given below and are also described in (Compton et al. 2013). The modelled estimates of the average fraction of exposure time from 2008 to 2011 were derived from measured water levels for these years. Water level data was interpolated from eight tidal poles found spread around the Wadden Sea area. The bathymetric grid used to estimate tidal exposures was Cycle 5, estimated by Deltares, but originally derived from data collected by the Rijkswaterstaat (RIKZ). The water levels and the bathymetric grid were implemented into a geometric triangular grid model, and used in an algorithm to interpolate exposure times across the Wadden Sea (Rappoldt and Ens, 2011). The water level data for this model was downloaded from the Rijkswaterstaat (www.waterbase.nl).

Maximum tidal current speeds (m s^{-1}) were estimated based on dynamic model computations using the WADPLUS model (Rijkswaterstaat). The maximum tidal current speeds were computed given tides on 13-15 February 1989 when there was a NW storm (500 × 500 m grid size, Brinkman, 2002). The main shortcoming of these gridded layers is that they are calculated for these specific dates in 1989 and thus one climatic condition, but they are currently the only estimates readily available for the entire Wadden Sea.

Freshwater discharge into the Dutch Wadden Sea was estimated from data collected at fourteen freshwater discharge points in March 1988, a wet month (Jager and Bartelds, 2002). A more recent synthesis is currently unavailable. Based on this data and a 2-D model (Kuijper 1993 in Jager and Bartelds, 2002) salinity concentrations were interpolated across the Dutch Wadden Sea (Jager and Bartelds, 2002).

Photo 6. The Ems Dollard is a brackish environment where the sediments are muddy, as seen in this photo.

Selection of IN and OUT regions for testing

To identify outliers in the environmental data, i.e. points that might have incorrect estimates or measurement errors, we used the Mahalanobis distance metric. The Mahalanobis distance is simply the distance of a test point from the centre of the majority of points, divided by the width of the ellipsoid in the direction of the test point. The Mahalanobis distance metric is a scale-invariant estimate that takes into account the correlations between variables.To identify outliers, a quantile plot was used to identify outlying distances. These points were excluded from the analysis.

To identify the areas where there is no gas production (OUT area), but that also share a similar environment to that found in each gas production area (IN areas), we selected the OUT area to have a similar environment as the IN area based on the maximum and minimum values of all five environmental variables (Table 1). Thus all OUT areas would have a similar environmental range, as the IN areas shown in Table 1.

Table 1. A summary of the maximum and minimum principal component 1 axis (PC1), principal component 2 (PC2) at each of the areas predicted to subside by 2 cm, i.e. Zuidwal (Zuid), Ameland (Ame), East Frisian (EastFr) and Groningen (Gron). In addition, the average, as well as the range, in environmental variables are given for each of these areas. The environmental variables used in the PCA analysis included silt (<63 %), median grain size (mgs), exposure time (ET), tidal current speeds (maxcurr) and salinity under dry conditions (saldry).

area	n	et	silt	mgs	maxcurr	saldry
Zuid	106	0 to 0.5	0 to 31.7	0 to 217.3	0.45 to 0.91	22.46 to 25.78
Ame	157	0 to 0.5	0 to 63.9	0 to 215.9	0.35 to 1.21	23.73 to 26.65
EastFr	25	0 to 0.9	0 to 49.2	0 to 147.3	0.40 to 0.56	25.37 to 32.51
Gron	370	0 to 0.7	0 to 70.2	0 to 196.0	0.25 to 1.04	14.24 to 34.13

Model and testing

Identifying whether changes in macrobenthic attributes are occurring in areas of older gas production and East Friesland, we needed a reference area for comparison, in this case the whole Wadden Sea, to test whether differences between IN and OUT exist. To further refine the selection of the OUT areas, so that they each had a similar environmental range as found in each gas production area, the OUT areas were selected based on the maximum and minimum values of all of the environmental variables in the IN areas (Table 1). This refinement answers a comment of the Audit commission that the referential OUT areas should be similar in environmental space as the IN areas. Although there is data selection, there are always more sites in the OUT area than in the IN area. The availability of four years of data, instead of a single year, has enabled us to remove the “noise” of short-term variation and provide an average estimate of difference between these areas. To ensure that no outliers remained in the macrobenthic species abundances, we used Mahalanobis distances to check for outliers and removed outlying values.

Analyses were completed in two stages at each site; according to Aarts et al. (2010, 2011). First, we used the actual data from the site of gas production to determine whether there were differences between the IN and OUT regions. Tests were done using a simple generalised linear modelling analysis, with quasi-poisson distributed errors:

$$y \sim X$$

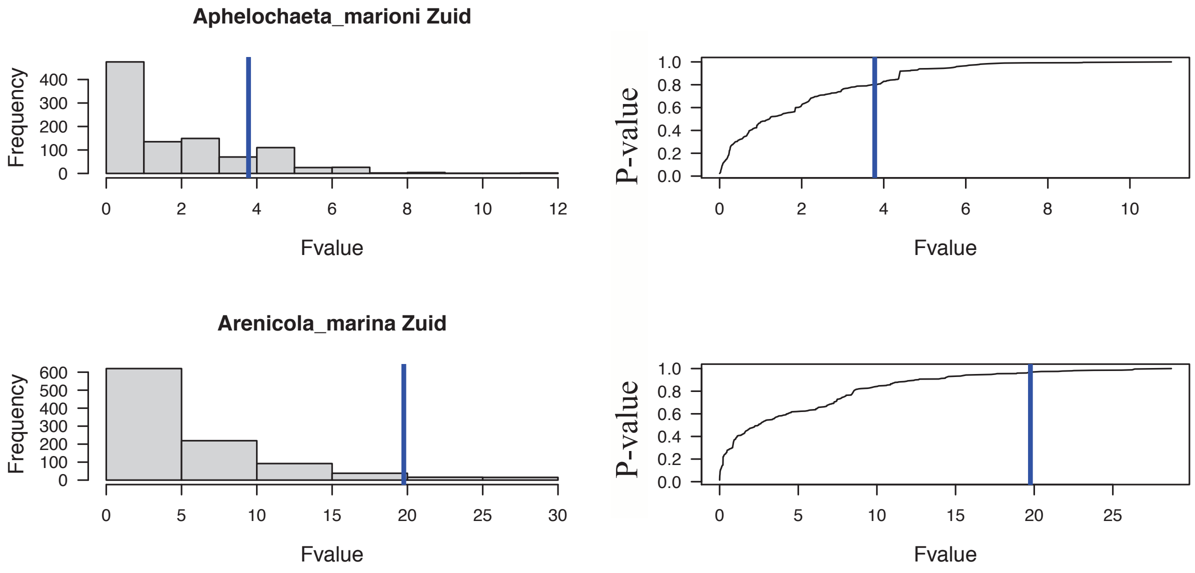
where y is the macrobenthic attribute and X is the factor IN versus OUT. The criteria for a model to run was that at least fifteen positive occurrences were needed in the IN and OUT areas. Macrobenthic attributes were considered to show significant effects when $p \leq 0.05$. Note that all gas production areas were examined separately.

However, due to chance and spatial autocorrelation, a macrobenthic attribute in the IN area might be identified as different to the OUT area. Thus to ensure that the macrobenthic attribute in the IN area was significantly different with respect to gas production, we used a Monte Carlo simulation to check whether a macrobenthic attribute from the IN area did not lie within the natural variation of the overall system.

To describe the natural variation within the system we used Monte Carlo simulations. Monte Carlo simulations entailed randomly sampling “in” and “out” areas across the Wadden

Sea 1000 times, while excluding all gas production areas from the SIBES sampling. The IN areas were identified by randomly sampling a point from the SIBES sample points using the `sample` function in R, and then identifying the 109 points in the closest vicinity of this point. The IN and OUT areas were selected to have the same environment, based on all five environmental variables (Table 1). Using the simulated "in" and "out" areas, we could then test for differences in macrobenthic attributes in these areas using the quasi-poisson regression; as above. A criteria for a simulation to run was that at least ten positive occurrences were needed in each area. F-values were extracted from the regressions, and were then used to draw a cumulative distribution of F-values (function `ecdf` in R) and thus provide a description of the natural variation in the system. An F-value is a ratio of the mean regression sum of squares divided by the mean error sum of squares. Its value will range from zero to an arbitrarily large number.

Figure 2. A significant species (*A. marina*) versus a non-significant species (*A. marioni*), as identified by the Monte Carlo testing approach. The blue line indicates the F-value from the "real" test. The histogram (left) and the cumulative distribution (right) of the F-values from the Monte Carlo simulations are shown for both tests. Only when the F-value from the "real" test (blue line) intersects with a P-value of greater than 0.95 is the test significant; indicating a difference in the area of gas production relative to the remainder of the system.



To test whether the data from the “real” test of the gas production area versus the OUT area was different to that encountered across the system, we matched the F-value from the “real” test with the closest F-value from the cumulative distribution of F-values obtained from the Monte Carlo simulations. At the point where the F-value from the “real” test and the Monte Carlo simulations were identical or closest, we obtained the probability value from the cumulative distribution function. If the probability value was >0.95 then the result from the Monte Carlo simulation was significant, but if less than this this showed that the macrobenthic attribute was not significantly different from what was found in the system.

Sensitivity analysis

A sensitivity analysis was run on the abundances of *Scoloplos armiger* and *Cerastoderma edule* to determine the effect size needed to find significant change in the Monte Carlo tests. To do this we first excluded all production areas, and the Ems, from the data. Then using the remaining SIBES data, we randomly simulated 350 IN areas and OUT areas, and ensured they shared a matching environment. In the case of each simulated IN area, we then increased and decreased the abundances and tested whether there were statistically observable differences between the IN and OUT areas. To increase the abundances we multiplied the observed abundance by either 1, 2, 4, 6, 8, 10, 14, and 20, or we divided the abundances by the same values.

For each simulated IN area (n=350) we then examined which changes in effect size were significantly different from what was observed in the remainder of the system. To do this we ran a Monte Carlo simulation (like described previously) to draw a cumulative distribution function from the modelled F-values to describe the natural variation in the system. At the point where the F-value from the change in effect size in the IN area and the Monte Carlo simulations were identical or closest, we obtained the probability value from the cumulative distribution function. If the probability value was >0.95 then the result from the Monte Carlo simulation was significant. All statistical analyses were done in R.

Nearest neighbour distance analysis

To observe whether abundances in the IN areas were higher or lower than expected, while accounting for the effect of environment, we further refined our selection of the OUT areas. We first defined an OUT area, as having the same environment as the gas production areas (as done previously, Table 1). We then took all points from the gas production area IN and found points with identical silt and exposure times, i.e. the nearest neighbour in environmental space. To find the nearest neighbour, we calculated the nearest neighbour distances between the sample points in the IN and OUT area. Silt was scaled to values between 0 and 1 to remove the undue influence of magnitude between silt and exposure time. The closest distances indicated points with identical environments (n=109 points in the IN area ~ 109 points in the OUT area). We then correlated the abundances of an individual OTU given identical silt and exposure times. In the case that abundances in the IN sites were higher or lower than the identical OUT sites, and were uncorrelated with abundances in the OUT sites, then they were considered different to the OUT sites; independent of environment.

Community responses

To explore whether community composition, i.e. beta diversity, differed in the IN versus OUT areas, we ran a non-metric multidimensional scaling analysis (nMDS, Clarke, 1993) (function `isoMDS` library `MASS`) on the Bray-Curtis dissimilarities of the log transformed abundance data from SIBES (log transform: log(x+min(x)), function `vegdist` library `Vegan`). Models only ran when the number of OTUs encountered at a site were greater than 3 and had more than 3 records. The goal of nMDS is to find a configuration in a given number of dimensions, which preserves rank-order dissimilarities. A measure of the goodness of fit is given by the stress value, the lower this value the better the fit. To explore how community composition was associated with the fraction of exposure time we used the `ordisurf` function in vegan. This function fits a smooth surface using thinplate splines in a generalised additive model to the nMDS fitted values.

RESULTS

Characteristics of areas possibly impacted by subsidence

At all four production areas, *Hydrobia ulvae* contributed the largest part of the total abundance on average; with highest contributions in Ameland and Groningen (Figure 3, top panel). The contribution of other species to the total abundance varied per location, e.g. at Zuidwal *Ensis directus* made up a large part of the abundance, *Marenzellaria viridis* was common only at Zuidwal and Ameland, and *Corophium* sp. was common at the East Frisian area and Groningen. At the four production areas, the species contributing most to the overall biomass were *Mya arenaria*, *Cerastoderma edule*, *Arenicola marina* and *Lanice conchilega* (Figure 3, bottom panel). *Alitta succinea* was found only in the East Frisian area and Groningen.

An exploration of the trends in community and sediment attributes with respect to the contour intervals in the Zuidwal, Ameland and Groningen areas can be seen in Figure 4. In Zuidwal, total abundance, total biomass and species richness appeared to be highest in the 8 cm contour interval; uncorrelated with sediment and exposure time. In the Ameland area, there was a trend for total biomass to increase from 21 cm predicted subsidence, in concordance with increasing exposure times in this range (Figure 4). No clear trends were seen in total abundance or species richness with greater predicted subsidence. In Groningen there was a clear trend for total biomass to decrease towards contour intervals of greater than 14 cm. This was concordant with the pattern for the fraction of exposure time to decrease in these areas. Species richness also showed a trend to decrease in areas predicted to subside (> ~14 cm, Figure 4) and then to increase again at about 19 cm predicted subsidence. There were no clear changes in silt (Silt %) and the range in grain sizes (Range) with contour intervals in this area.

Figure 3. Composition of OTUs in terms of those that contribute the most in terms of abundance (top panel) and biomass (bottom panel). All values are standardized as a percentage of the total abundance or biomass.

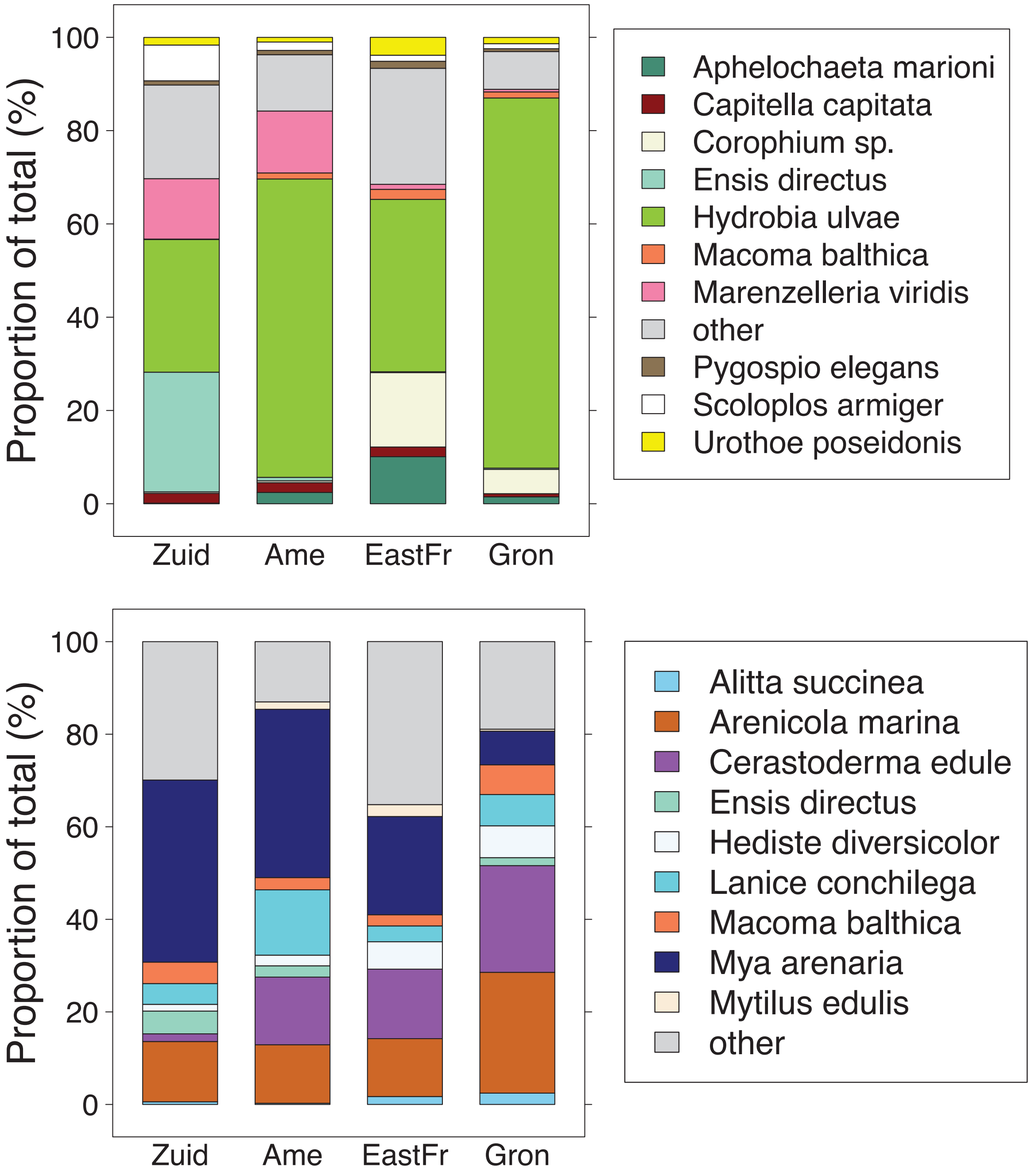
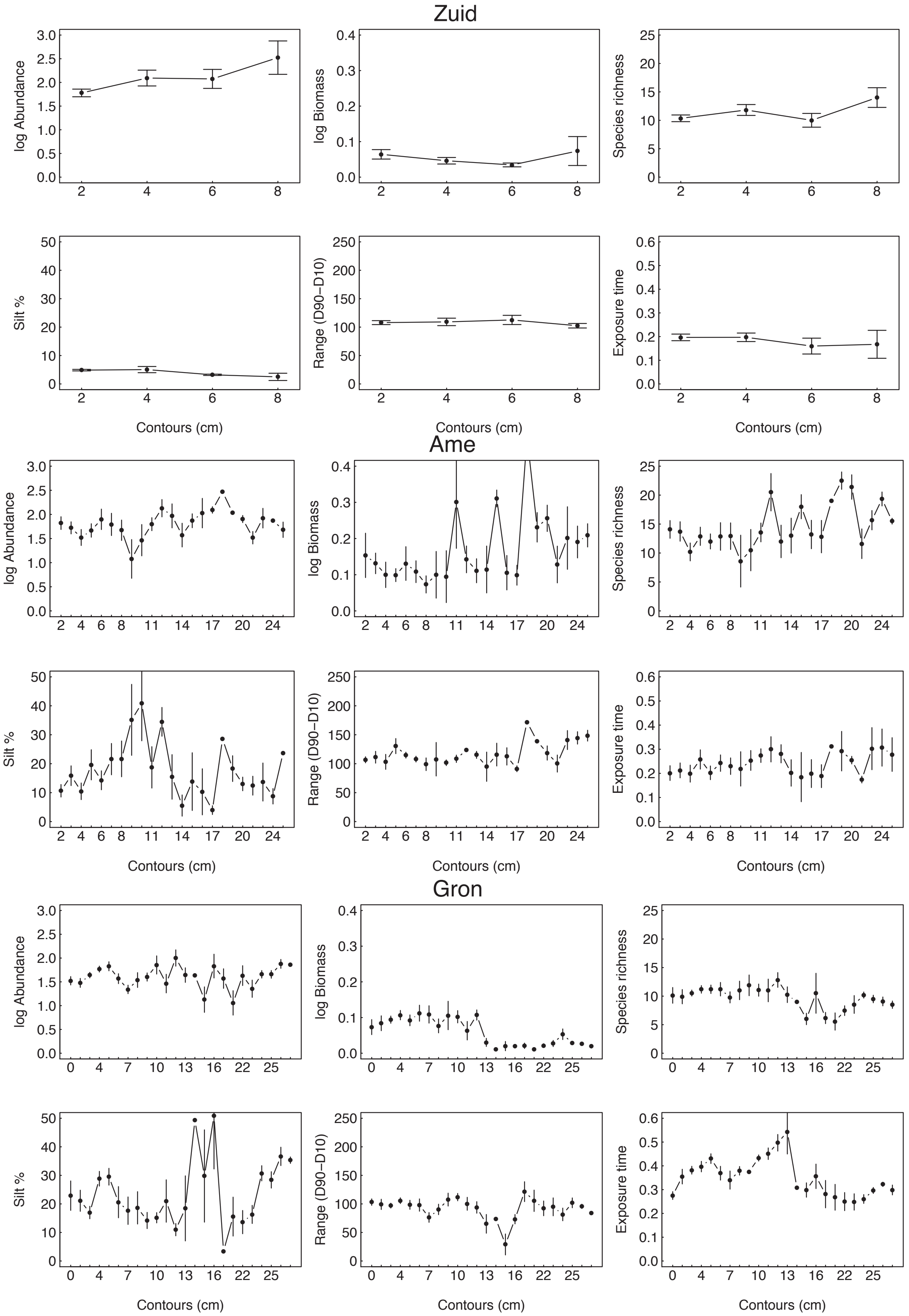


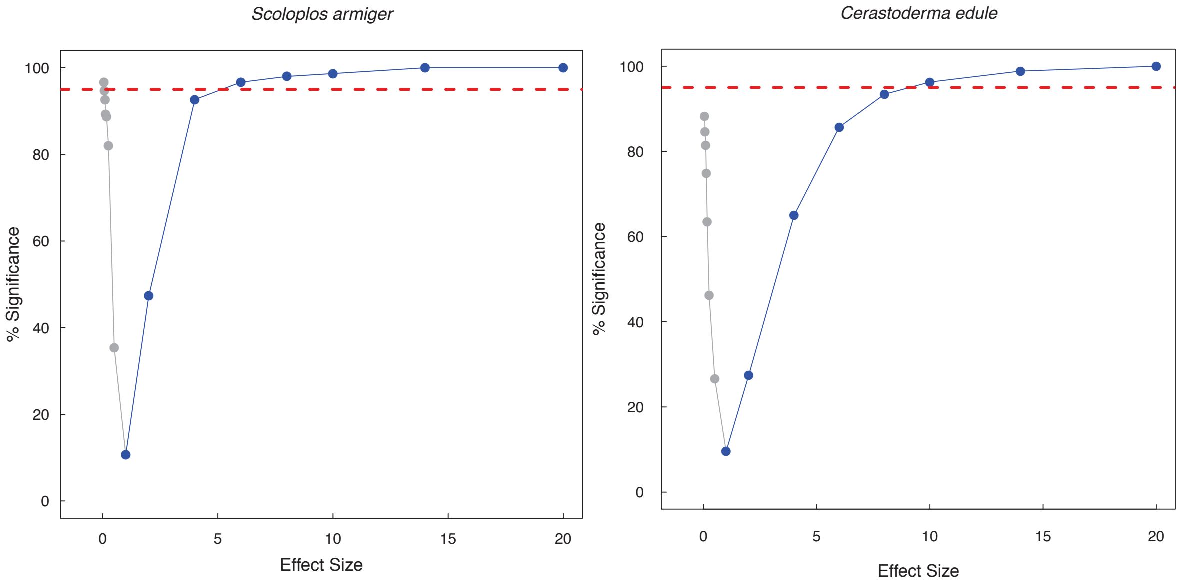
Figure 4. Trends in abundance (log10), biomass (log10), point richness, silt (<63%), range in sediment grain sizes (D90-D10) and the fraction of exposure time with respect to the predicted contour intervals of subsidence in the Ameland (Ame) and Gronginen (Gron) gas extraction areas.



Sensitivity analysis

The results from the sensitivity analysis of *Scoloplos armiger* and *Cerastoderma edule* showed that the Monte Carlo approach had the power to detect significant changes in macrobenthos abundance. Specifically, when abundances increased by ~8-fold in the case of *S. armiger* and ~10-fold in the case of *C. edule* then the chance of detecting an effect was >95% indicating a significant effect (Figure 5). By contrast, decreases in abundance were harder to detect in *S. armiger,* with significant effects when changes were >14-fold, and not detectable in the case of *C. edule* (Figure 5). These results showed that the Monte Carlo approach used here to detect changes in the areas of gas production is sensitive to detecting increases in abundance.

Figure 5. Sensitivity test of the Monte Carlo analysis using two example species: *Scoloplos armiger* and *Cerastoderma edule* in the OUT areas. All production regions were excluded from this test. Random IN areas were simulated in the OUT region for testing. In these IN areas the abundance of these species was increased or decreased by either 1, 2, 4, 6, 8, 10, 14, and 20 (Effect size). The significance of an effect was then tested with Monte Carlo simulations. Values greater than 95% (red line) indicates highly significant.



Model results: abundance and biomass

Comparisons of total abundance and biomass in the IN and OUT areas using generalised linear models, and a Monte-Carlo simulation, showed that total abundance was significantly different in the Zuidwal region relative to the remainder of the system (Figure 6), but not in the other production areas. Total biomass was significantly different in Zuidwal and Groningen areas relative to the other production areas (Figure 6), but not in the other production areas.

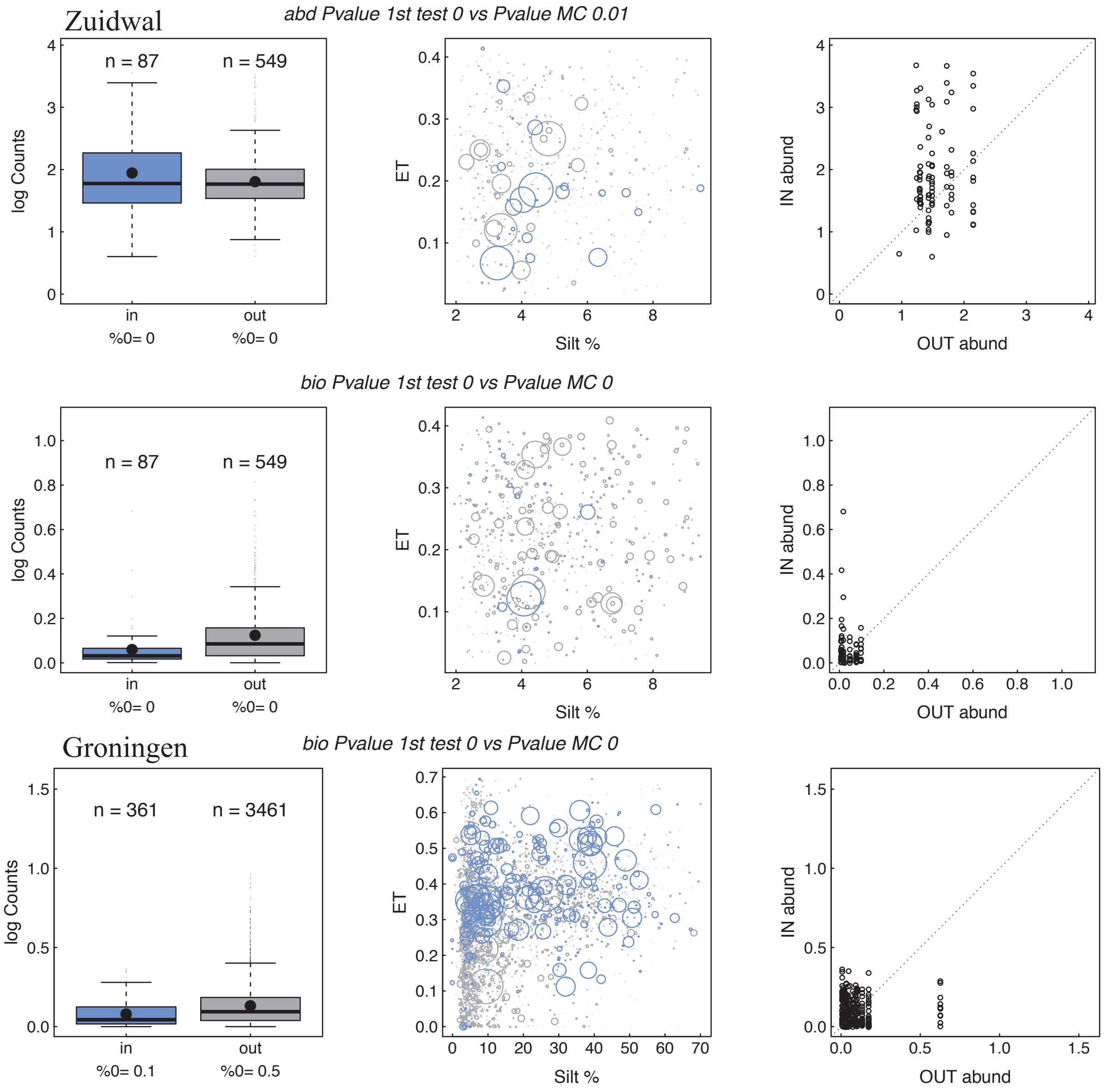
Abundance in Zuidwal appeared to be lower in the IN area according to the difference in the model coefficients , but results from the presence only data (boxplots) and the nearest neighbour distance analysis suggested abundances were higher in the IN area (Table 2, Figure 6). Biomass in Zuidwal was lower relative to the OUT areas, according to the boxplots of the positive data and the model coefficients, but higher given the nearest neighbour distance analysis. In Zuidwal, both abundance and biomass appear to be highest towards short exposure times and low silt values, relative to the OUT area (scatterplot, Figure 6).

In the case of Groningen, biomass was lower in the OUT areas according to the boxplots of the positive data and the nearest neighbour distance analysis but higher according to the model coefficients (Table 2, Figure 6). In Groningen, biomass appears to be higher at a range of silt values and towards long exposure times, relative to the OUT area (scatterplot, Figure 6).

Table 2. Direction of change in abundance and biomass at Zuidwal and Groningen. Only parameters identified as significant by the Monte Carlo simulations are shown. The directional changes are summarised for the boxplots (no zeros), the model coefficients and the nearest neighbour distance analysis. Higher abundances in the IN area are indicated by “plus”, and lower by “minus”.

area	**parameter**	**boxplot**	**model**	**nnd**
Zuidwal	abund	plus	min	plus
	biom	min	min	plus
Gron	biom	min	plus	min

Figure 6. Production areas where significant differences in total abundance and total biomass exist relative to the system (Pvalue 1st test versus the Pvalue of the Monte Carlo simulation (MC)). The relative abundance or biomass in the IN (blue) versus OUT (grey) areas with respect to silt and exposure time (ET, presence data only) are shown in the scatterplot. Abundance and biomass values were all scaled to values between 0 and 1. The last plot indicates the correlation between abundances IN and OUT, once corrected for environment using a nearest neighbours analysis.



Model results: species

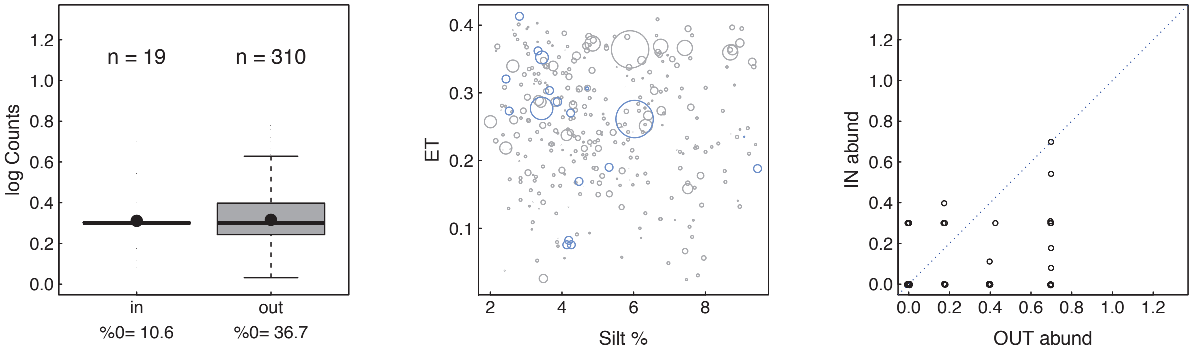
In total 76 species (n>15 occurrences) were tested for differences in the four gas production areas. In Zuidwal, four polychaetes and a bivalve species showed different abundances in the production area IN versus the area with a matching environment OUT, according to the Monte Carlo simulations (Figure 7). Of these five species, three were predicted to be lower in abundance in the IN area relative to OUT, according to the model coefficients (Table 3). But the boxplots of the positive observation, i.e. no zeros, and the nearest neighbour distances showed that the majority of species had higher abundances in the IN area relative to OUT (Table 3, Figure 7). The scatterplots showed that these species all occur in areas of low silt and low exposure times (Figure 7).

Table 3. Direction of change in abundance at the four production areas. Only OTUs identified as significant by the Monte Carlo simulations are shown. The directional changes are summarised for the presence only boxplots, the model coefficients and the nearest neighbour distance analysis. Higher abundances in the IN area are indicated by “plus”, and lower by “minus”.

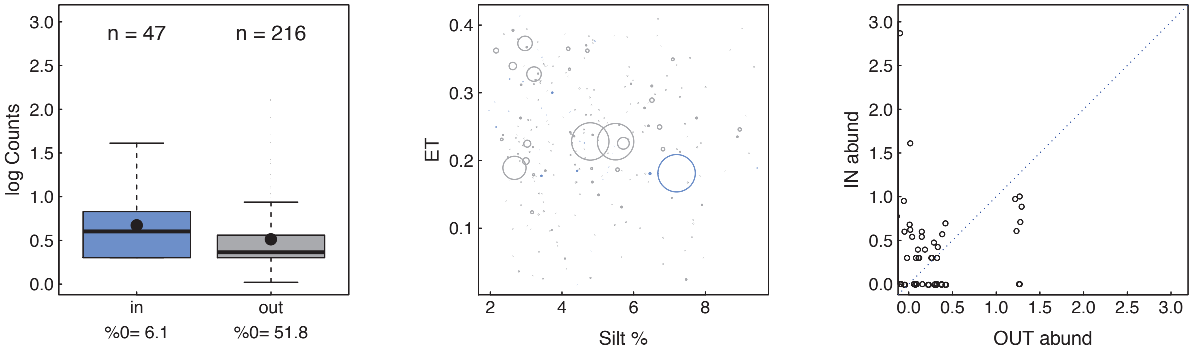
area	**parameter**	**boxplot**	**coef**	**nnd**
Zuidwal	*Arenicola marina*	-	min	min
	Cerastoderma edule	plus	plus	plus
	Pygospio elegans	plus	min	plus
	Scoloplos armiger	plus	min	plus
	Spiophanes bombyx	plus	plus	plus
Ame	*Eteone longa*	plus	min	plus
	Eumida sanguinea	plus	plus	plus
	Hydrobia ulvae	plus	plus	plus
EastFr	*Hediste diversicolor*	plus	plus	plus
Gron	*Alitta succinea*	plus	plus	plus

Figure 7A. OTUs with abundances identified as being different in the Zuidwal IN area, relative to the remainder of the system. The boxplots are shown on the log-scale but the analyses were run using untransformed data. The number of positive records are shown on the top of the boxplots. The relative abundance in the IN (blue) versus OUT (black) areas with respect to silt and exposure time (ET, presence data only) are shown in the scatterplot. Abundance values were all scaled to values between 0 and 1. The last plot indicates the correlation between abundances IN and OUT, once corrected for environment using a nearest neighbours analysis.

Arenicola_marina Pvalue 1st test 0 vs Pvalue MC 0.04



Cerastoderma_edule Pvalue 1st test 0 vs Pvalue MC 0



Pygospio_elegans Pvalue 1st test 0 vs Pvalue MC 0.04

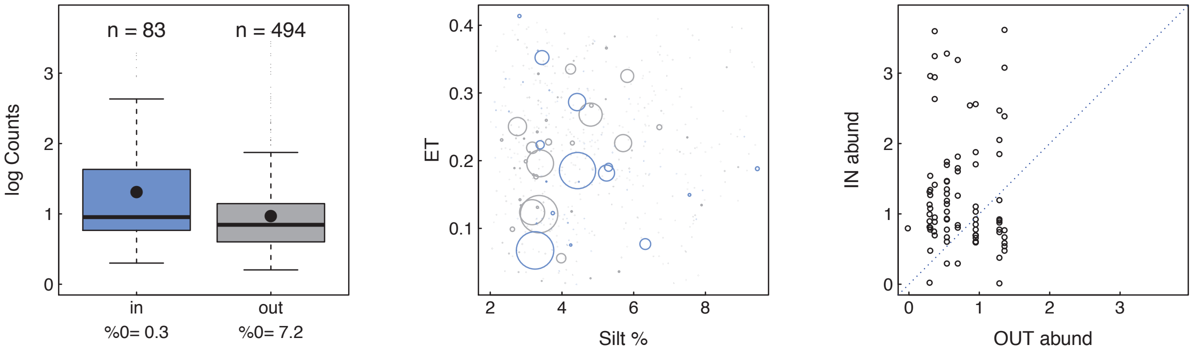
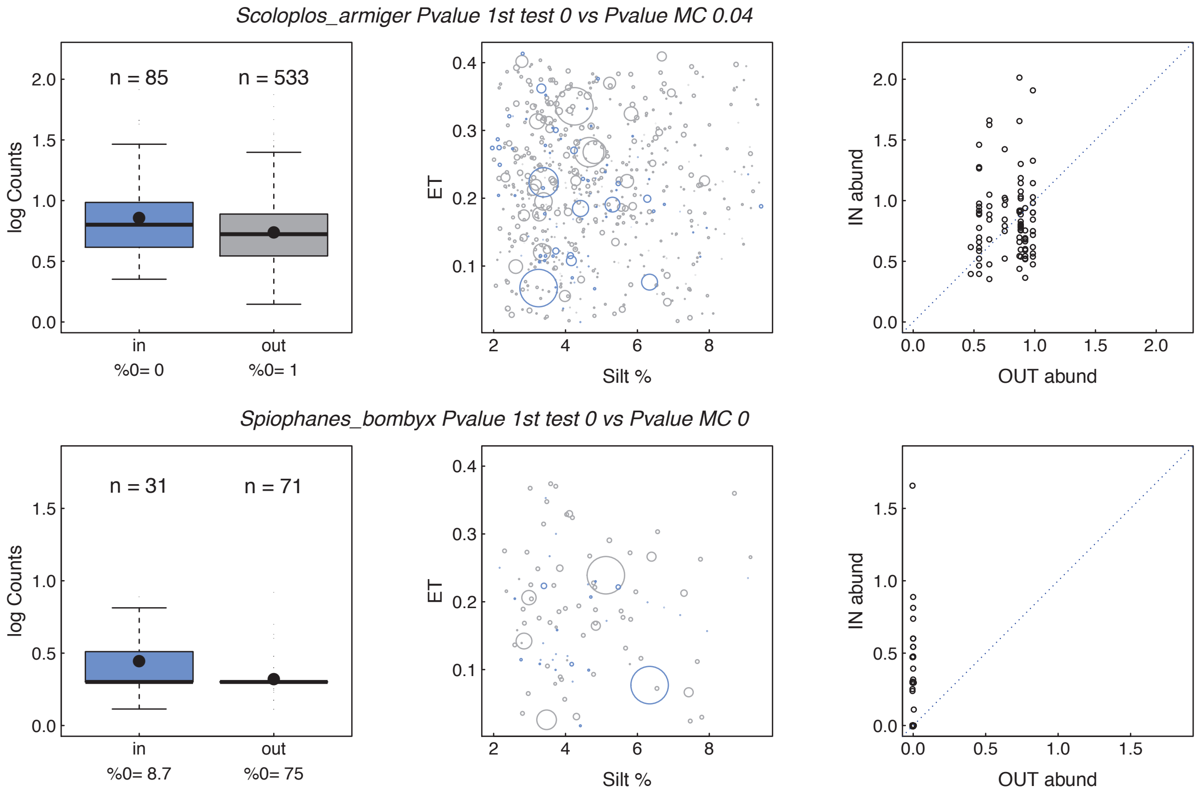


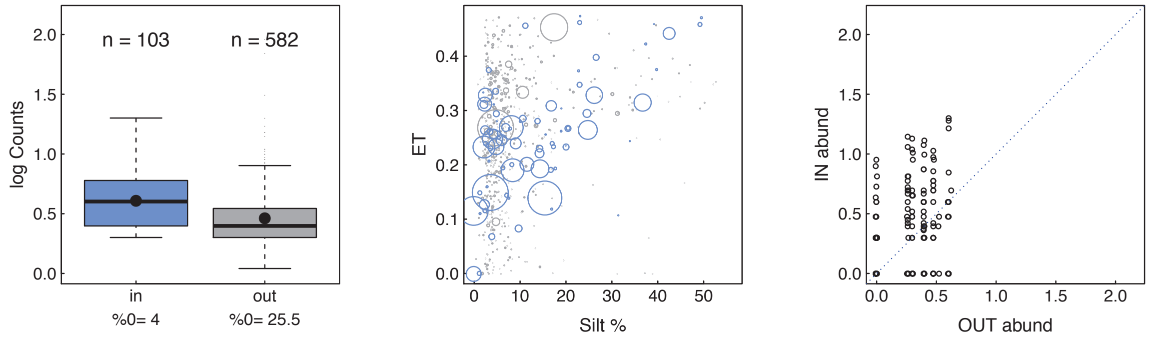
Figure 7B. OTUs with abundances identified as being different in the Zuidwal IN area, relative to the remainder of the system. The boxplots are shown on the log-scale but the analyses were run using untransformed data. The number of positive records are shown on the top of the boxplots. The relative abundance in the IN (blue) versus OUT (black) areas with respect to silt and exposure time (ET, presence data only) are shown in the scatterplot. Abundance values were all scaled to values between 0 and 1.The last plot indicates the correlation between abundances IN and OUT, once corrected for environment using a nearest neighbours analysis.



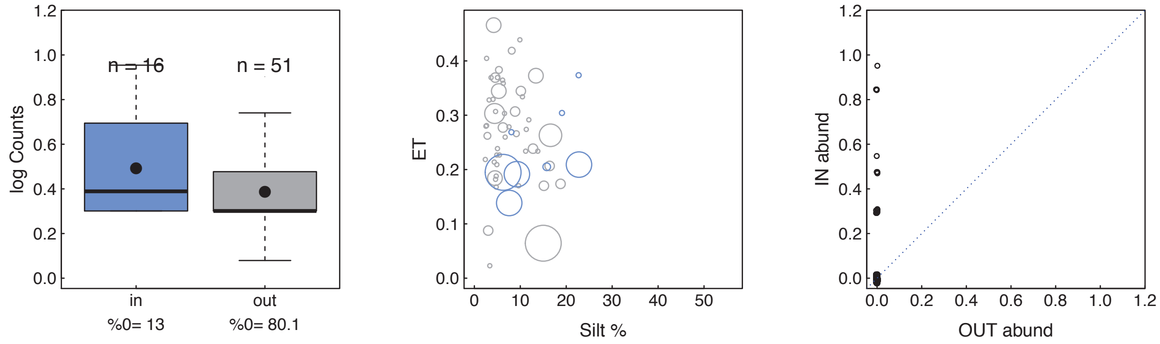
In Ameland, two polychaete species and a mollusc were identified as different in the production area IN versus the OUT area with matching environment (Table 3). A single species *E. longa* was predicted as having a lower abundance in the gas production areas, based on the model coefficients, the other two species were predicted to have higher abundances (Table 3). The boxplots and nearest neighbour distances showed that these species all had higher abundances in the gas production areas IN versus the reference area (Figure 8). The scatterplot showed that these species mainly occurred towards longer exposure times (>0.3) and across a range of silt (Figure 8).

Figure 8. OTUs with abundances identified as being different in the Ameland IN area, relative to the remainder of the system. The boxplots are shown on the log-scale but the analyses were run using untransformed data. The number of positive records are shown on the top of the boxplots. The relative abundance in the IN (blue) versus OUT (black) areas with respect to silt and exposure time (ET, presence data only) are shown in the scatterplot. Abundance values were all scaled to values between 0 and 1. The last plot indicates the correlation between abundances IN and OUT, once corrected for environment using a nearest neighbours analysis.

Eteone_longa Pvalue 1st test 0.01 vs Pvalue MC 0



Eumida_sanguinea Pvalue 1st test 0 vs Pvalue MC 0



Hydrobia_ulvae Pvalue 1st test 0 vs Pvalue MC 0

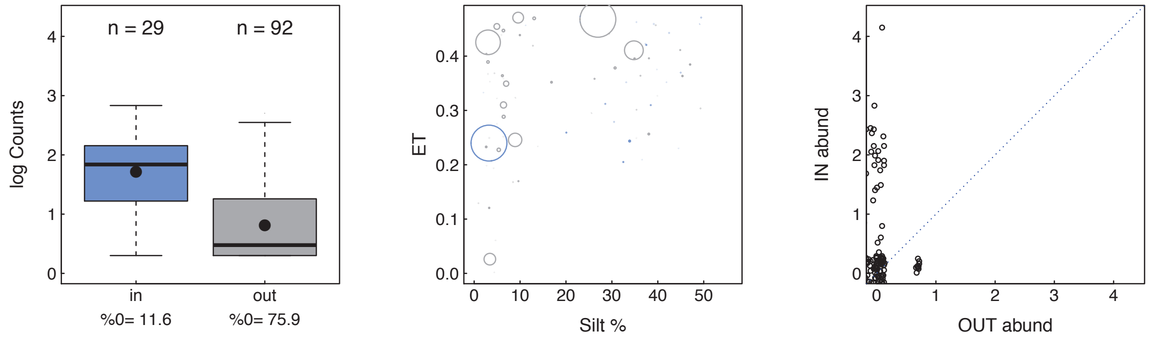
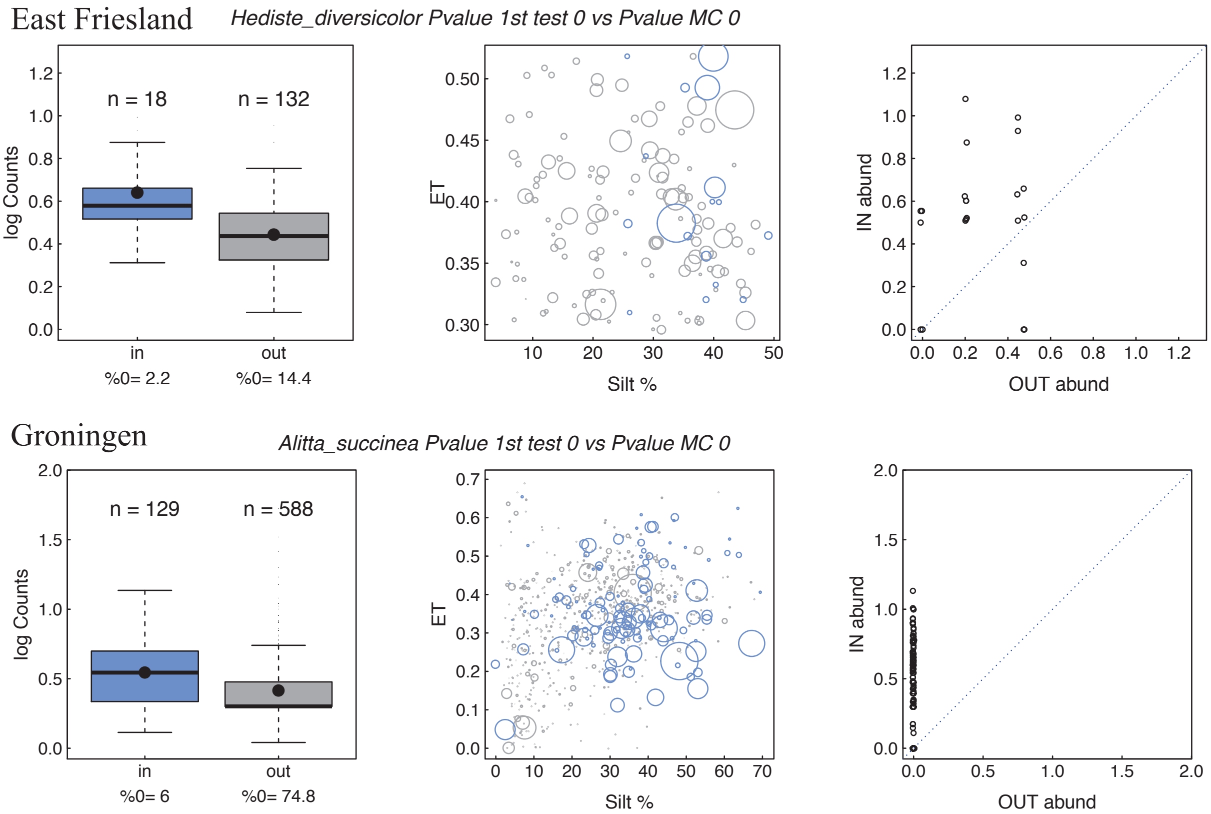


Figure 9. OTUs with abundances identified as being different in the East Friesland (*Hediste diversicolor*) and Groningen (*Alitta succinea*) IN area, relative to the remainder of the system. The boxplots are shown on the log-scale but the analyses were run using untransformed data. The number of positive records are shown on the top of the boxplots. The relative abundance in the IN (blue) versus OUT (black) areas with respect to silt and exposure time (ET, presence data only) are shown in the scatterplot. Abundance values were all scaled to values between 0 and 1.The last plot indicates the correlation between abundances IN and OUT, once corrected for environment using a nearest neighbours analysis.



In East Friesland, a single polychaete, *H. diversicolor*, was identified as different in the gas production area IN relative to the reference area OUT. This species was predicted to have higher abundances in the IN area relative to outside according to the model coefficients, the boxplot and the nearest neighbour distance analysis (Table 3, Figure 9). In East Friesland, this polychaete tended to occur in higher abundance where silt values were higher and towards relatively long exposure times (>0.3, Figure 9).

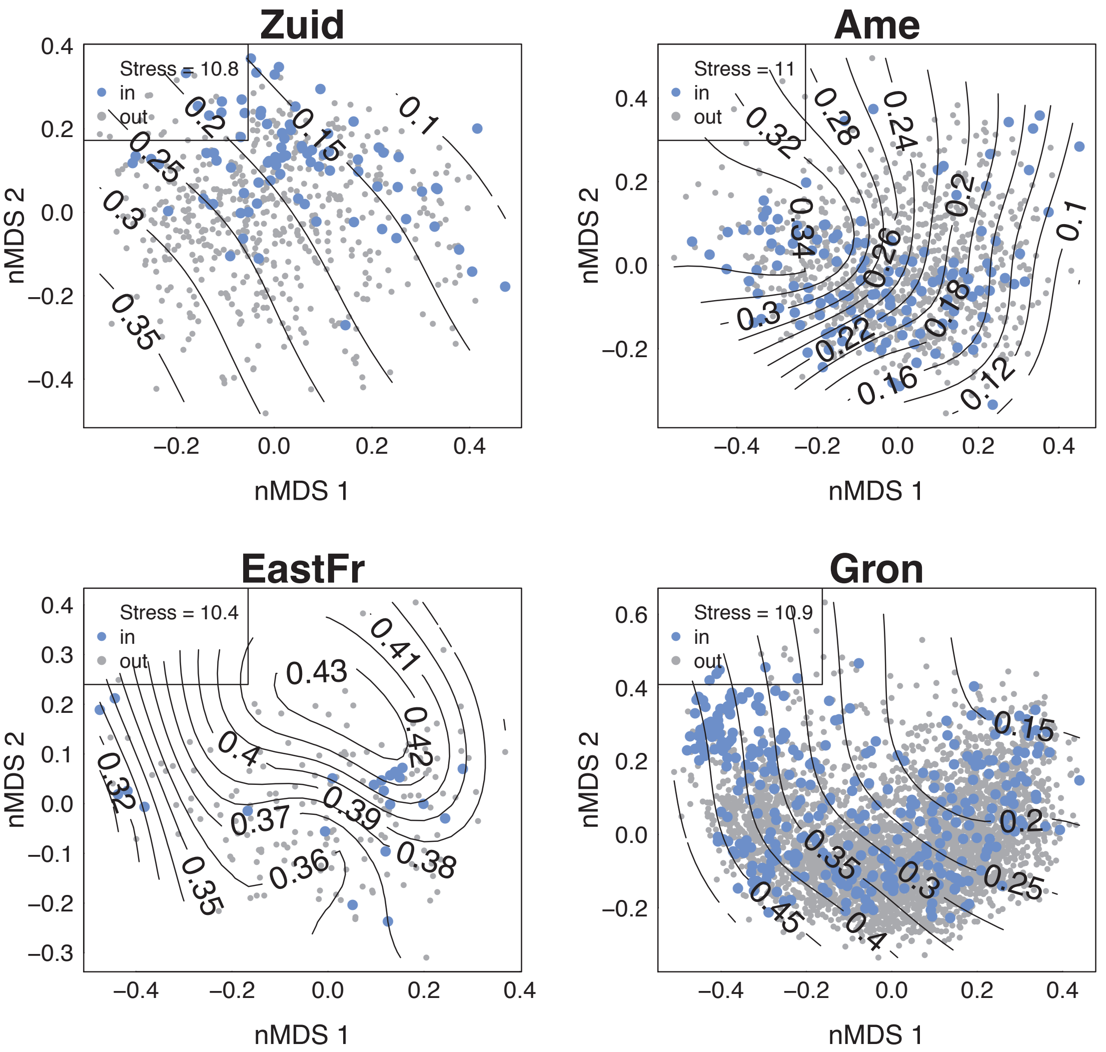
In Groningen, a single polychaete species, *A. succinea*, was identified as different in the gas production area IN, relative to the reference area OUT (Table 3, Figure 9). This species was identified as having a higher abundance in the IN area relative to OUT (Table 3, Figure 9). This species tended to have higher abundances in areas where there is higher silt and longer exposure times (Figure 9).

Community Responses

A non-metric multidimensional scaling analysis was used to visualise how community composition differed between the IN and environmentally matching OUT areas. The results showed that communities in the IN regions did not show marked differences with communities in the OUT regions (Figure 10). Communities in the IN regions shared a degree of compositional overlap with communities in the OUT region, with the most overlap between IN and OUT seen in Groningen and Ameland. The communities from Zuidwal and East Frisian area only shared a partial amount of overlap with the background community (Figure 10).

A simple smooth surface, fitted to the nMDS plot, showed that the community inside the predicted subsidence area of Zuidwal occurred towards deeper waters (ET < 0.2, Figure 10). Whereas, communities in Ameland, East Friesland and Groningen occurred across the entire intertidal range (0.1-0.4 ET).

Figure 10. Community composition in the IN and OUT regions of each of the four gas production regions: Zuidwal (Zuid), Ameland (Ame), East Frisian (EastFr) and Groningen (Gron). The scatterplot in the last panel shows the community attributes in the SIBES sites in the IN (blue) versus OUT (dark grey) areas.



DISCUSSION

A comparison of OTUs inside the areas of predicted subsidence versus outside (Figure 3 and 6), showed that *H. ulvae* contributed the largest share of the biomass in all four production areas, followed by *M. arenaria*, *C. edule* and *A. marina*. Each production area has a unique species composition that appears to concur to some degree with their geographic placement within the Wadden Sea (see Compton et al. 2013 for comparison).

A sensitivity analysis showed that the Monte Carlo testing approach is powerful for detecting significant changes, and thus that this approach has the power to detect changes in the macrobenthos. Specifically, our examination of two species, *Scoloplos armiger* and *Cerastoderma edule* showed that this Monte Carlo approach could detect an 8-fold increase in abundance or a 10-fold decrease; in the case of *S. armiger*. By contrast, decreases in abundance were harder to detect in the case of both species; and only became significant in the case of *S. armiger*.

In the production areas of Zuidwal and Groningen, where production has taken place the longest, total biomass was different in the production areas relative to outside(Figures 7A and B). There was a tendency for biomass to be decreased in the areas of production, however, this was not significant in the case of Groningen (positive model coefficient, where zeros are taken into account).In the case of Zuidwal, total abundance was slightly higher than in the reference area, i.e. the mean size of the organisms will be smaller, but this trend was not significant (negative model coefficient).

Of the 76 species tested in this study, 11 species showed significant responses in the gas production areas, according to the Monte Carlo analysis.The majority of species found to be different were in the Zuidwal production area. The OTUs that showed responses were mainly polychaetes; a group known to be sensitive to changes in environment (Jones and Kaly, 1996). Most of the 11 species tended to be higher in abundance in the gas production areas relative to the reference areas.

In Zuidwal, five species had different responses relative to the reference area based on the Monte Carlo analysis. The majority of these species tended to have higher abundances in the IN area relative to OUT, although only two of these five species were significantly higher in abundance (positive model coefficients). In Ameland, three species were found to be different in the gas production area. Most of these species showed an increase in abundance, relative to the reference area, in the gas production area. Only one of these was not a significant increase (negative model coefficient). In East Friesland, a single polychaete *H. diversicolor* showed a response in the gas production area. This species was higher in abundance in this area. In Groningen, a single polychaete *A. succinea*, a species known to occur in areas of high silt, was found in higher abundances relative to the environmentally matching reference area. This species is predominantly found in the eastern part of the Wadden Sea (Compton et al. 2013).

As patterns in community composition have and are often used to identify whether community composition differs in an area of a human affected perturbation versus outside (Clarke, 1993), e.g. with respect to oil production platforms (Clarke, 1999), we compared community composition in the production areas with those outside. In the cases of East Friesland, Ameland and Groningen, the IN and OUT communities shared a large degree of overlap in community space, whereas the Zuidwal community overlapped with other communities in the direction of longer exposure times.

Perspectives

Gas production is likely to have consequences that are unlike other studies on human perturbations where changes are more immediate in the zone of impact, e.g. with pollution (Azzurro et al., 2010) or oil production platforms (Clarke, 1999). Thus the differences observed here are difficult to qualify as production effects, because in this report we cannot answer the question whether these changes were induced by changes in the environment, as a consequence of gas production, or whether these are differences caused by another factor. At this moment we can identify that there are differences between areas where production has taken place and the “rest” of the Wadden Sea.

Future

It is well acknowledged that the power of our analysis will increase with more years of study, and thus will allow us to disentangle the anthropogenic effects from the natural variation in the future. Given the obligation that production can happen only with a “hand on the tap”, it is imperative that the SIBES sampling continues. Especially, as current predicted subsidence effects in the East Frisian area are small (NAM < 2cm), SIBES provides a reference of the system, prior to increasing subsidence effects. Thus, in the case of the East Frisian area, the SIBES efforts will become more valuable in time, as the duration of production increases. With the increasing power of the macrobenthos data to detect change, and also with the addition of more precise knowledge of the associated environmental changes associated with production, as determined by the other monitoring programmes, deeper insights into the factors driving change will be gained, with an unprecedented appreciation of the role of anthropogenic factors.

In summary, (1) SIBES has the power to detect change, a requirement for the continuous monitoring of ecological effects of gas production, and (2) with time and the concerted efforts of the other programmes, especially with the aid of improved environmental parameters, we will have a real chance to determine the contributions of actual subsidence of the land (an anthropogenic effect) and the accompanying biotic responses to the long-term changes of an internationally important ecosystem.

Acknowledgements

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Appendix

S 6. 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.

S7. 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¬¬2O2-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.