PROGRESS REPORT FOR THE 2012 SAMPLING OF THE SYNOPTIC INTERTIDAL BENTHIC SURVEYS ACROSS THE DUTCH WADDEN SEA

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SUMMARY

To investigate potential effects of gas production, via surface sediment subsidence, on the macrobenthos in the East Frisian production area the synoptic intertidal benthic surveys of the Wadden Sea (SIBES) began in 2008. Gas production began in the East Frisian area in 2007. In the East Frisian area the current predicted subsidence effects on the surface are expected to be minimal as underground subsidence is less than 2 cm.

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, because they form the base of the food chain, changes in macrobenthos could affect the upper levels of the food chain.

This progress report will provide an assessment of macrobenthos data collected in the 2012 sampling campaign to examine if the abundance of macrobenthos in the area of the East Frisian production area differs from the remainder of the Dutch Wadden Sea. In addition, we will describe the physical environment of this area and the species that occur in this area.

We show that the East Frisian area is characterised by relatively fine sediment types (~107 µm) and long exposure times (0.4 fraction of time). Mean total biomass was lower in the East Frisian area compared to the mean total biomass observed across the remainder of the Wadden Sea. By contrast, the mean total abundance of all species was higher in the East Frisian area relative to the mean total abundance observed across the remainder of the Wadden Sea. On average 9 species were observed in a single core in the East Frisian area, compared to an average of 7 species in a core across the remainder of the Wadden Sea. Although these differences were observed, they were not significant indicating that total

biomass, total abundance and species richness, as defined by the number of species in a core, were no different in the East Frisian area relative to the natural variation observed across the entire Dutch Wadden Sea.

In total twenty species were observed in the East Frisian area. Species like *Pygiospio elegans* and *Macoma balthica* were most common. The Monte Carlo analysis showed that species abundances in this area did not differ relative to what one would expect if this area was not affected by gas production.

Conclusions

In agreement with the predicted minimal subsidence in the East Frisian area, this report and previous reports from the SIBES programme all show no or minimal change in the abundance of macrobenthos in the East Frisian area. The SIBES surveys thus provide a reference of the East Frisian area prior to larger underground subsidence, due to gas production. In addition, the current, and future collection, of macrobenthic and environmental characteristics in this area will ensure that short-term natural variability is not mistakenly interpreted as human driven change in this area.

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. 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 investigate potential changes in macrobenthic populations and sediments.

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 an early stage, 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 Dutch Wadden Sea is acknowledged for the wide range of ecological and economic services it provides The Netherlands. One such ecological service includes essential habitat to migratory shorebirds, which use this area to fuel-up prior to flying to the Arctic (Beukema, 1976, Wolff, 1983, van de Kam et al., 2004). Economic services include fisheries, gas and salt production and lugworm digging. It is estimated that a total of 20 billion cubic metres of natural gas lies beneath the Dutch Wadden Sea area (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. Since 2007 gas production began in the East Frisian area. With the exception of Zuidwal, most production areas are extracted by the Nederlandse Aardolie Maatschapij (NAM).

Gas production in the Dutch Wadden Sea can lead to land subsidence. Modelling studies estimate that sediment infilling should compensate for any land subsidence associated with gas production. However, habitat suitability for a range of organisms could be affected when infilling is slower than subsidence and when infilling of the subsidence area occurs with sediments differing from the original sediments. Land subsidence could therefore affect macrobenthos, defined as organisms larger than 1 mm that live in or on the mud. As base of the food chain, macrobenthos are an essential food source for bird and fish species across the Dutch Wadden Sea. Due to a combination of (1) a sedentary life style, (2) strong environmental associations and (3) short life-spans (<5 years) macrobenthos are known to show relatively fast responses to changing conditions in their environment. All over the world macrobenthos are therefore used as signalling species for anthropogenic driven changes in tidal flat environments (Beukema et al., 1999, Hewitt et al., 2001, Hewitt et al., 2007, Hewitt et al., 2008).

To examine whether a change in macrobenthos is occurring in the East Frisian gas production area, a field assessment study called the Synoptic Intertidal Benthic Surveys (SIBES) of the Wadden Sea 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. Another point is that 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 long-term data, the inherent short-term variability of tidal flat systems can mask the chronic and cumulative impacts of human activities, often until they reach critical levels. Alternatively, changes might be identified as human induced when they are actually part of a natural cycle (Hewitt & Thrush 2007)

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 are taken in the East Frisian area every year.

In the East Frisian area the current predicted subsidence effects are minimal (NAM contours < 2cm). In agreement with this minimal subsidence, previous reports from the SIBES programme all show no or minimal change in macrobenthos abundance in this area (Aarts et al. 2011; Compton et al. 2012; Compton et al. 2013). Furthermore, a comprehensive comparison of all gas production areas highlighted that macrobenthos in the East Frisian area showed no differences compared to the remainder of the Wadden Sea, whereas sites with longer production showed an increased chance of change compared to the remainder of the Wadden Sea (Compton et al. 2013).

THIS REPORT

This progress report will provide an assessment of macrobenthos data collected during the 2012 sampling campaign. First, we will characterise the physical environment that characterises this area and the species that occur in this area. We will then examine if there are differences in the macrobenthos from the East Frisian production area, relative to the remainder of the Wadden Sea, using an “in-house” developed testing approach (Monte-Carlo testing procedure).

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) (Bijleveld et al. 2012).

Sampling was completed over the summer of 2012 (6 June until 2 September 2012). 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 (n=186). In areas where it was too deep or muddy, small inflatable boats were used (n=3810). Sampling locations (total of n=3996) were found with a handheld GPS (WGS84 as map datum). At each site sampled by foot, a single core of 0.018 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).

In the areas of Moddergat and Ameland, additional gridded sampling points (250 x 250 m grid and an additional 10% random points) were taken to increase the power to detect differences due to potential land subsidence effects (see Figure 1, Aarts et al., 2011).

All macrobenthos samples were sieved on a 1 mm round mesh in the field. Large bivalves were separated and then frozen (shell samples), whereas the remaining macrobenthic species were preserved using a 4% formaldehyde solution (worm samples). Sediment samples were taken to a depth of 4 cm at all sample points.

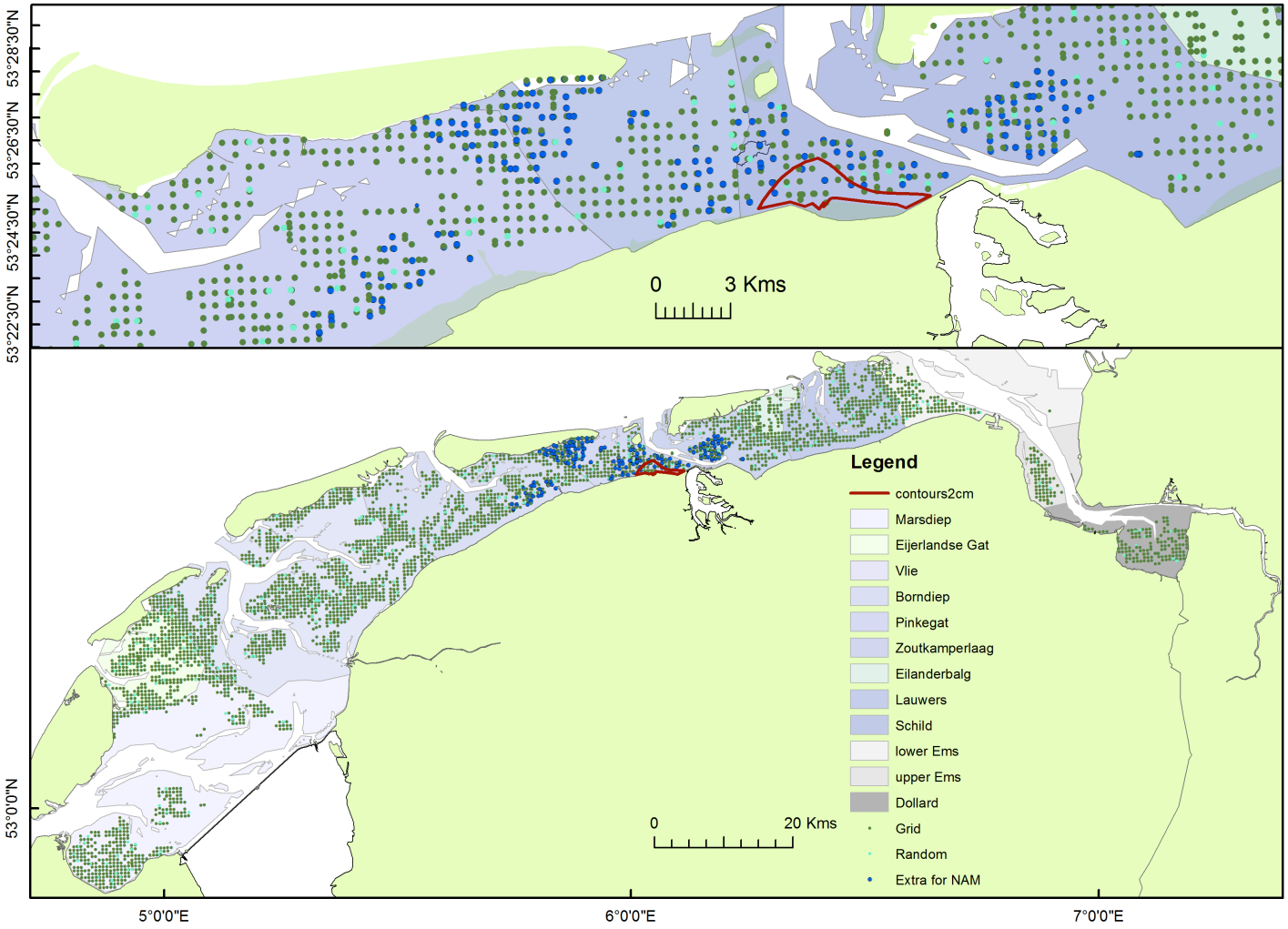
Laboratory analysis

The samples preserved in formalin (worm samples) were stained using rose Bengal dye (C.A.S. no. 632-68-8) for 24 hours in the laboratory, and 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 were 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 (Operational Taxonomic Units, OTUs). All molluscs from both the frozen and formalin samples were identified to a species level.

Once samples were counted and identified, the biomass or ash free dry mass (AFDM) of the OTUs were determined, with the exceptions of *Pygospio elegans*, *Spio martinensis* and Oligochaetes. In the cases of the bivalve molluscs (worm and shell samples), the flesh of all individuals were weighed separately when *Cerastoderma edule* and *Macoma balthica* > 6 mm, *Ensis directus* > 20 mm and when the other shell fish species were >8 mm, e.g. *Mya arenaria*, *Scrobicularia plana* and *Tellina tenuis*. In the cases of the polychaete worms, adults and juveniles were separated and weighed according to these rules: *Nereis* sp. and *Nephtys* sp. < 15 mm are juveniles, *Arenicola marina* < 50 mm are juveniles, *Marenzellaria* sp. and *Scoloplos* sp. with <20 mm are juveniles, and anything larger grouped and weighed as adults. 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.

Figure 1. SIBES sampling campaign from 2012. The red contour line indicates the deep subsidence in the East Frisian area. Additional points taken during the SIBES sampling campaign, in the Ameland-Moddergat region, are indicated in blue.



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 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. Median grain size (µm) is used in this report.

Environmental variables used to characterise the "reference" area

The four variables selected for this analysis: median grain size, fraction of exposure time, salinity and maximum current speeds, have linkages to benthic organisms in marine environments. Information on the physical variables is given below and also described in Compton et al. (2013). Median grain size, as measured during this sampling campaign, provides a measure of habitat association, e.g. some tube-building species need relatively coarse sediments to build their tubes (Dankers and Beukema, 1981).

Exposure time is correlated with the period of feeding 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). The modelled estimates of the average fraction of exposure time in 2011 were derived from measured water levels for these years. Water level data was interpolated from eight tidal poles positioned 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).

Current speed is correlated with substrate stability (Fegley, 1987) and the replenishment of phytoplankton food for suspension feeders or sediment erosion under very high velocities. 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 north west 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.

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). 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.

Characterisation and testing for differences in the East Frisian area

The contour line defining the East Frisian area (see Figure 1) was provided by the NAM at the end of 2011 and shows where there is an ~2 cm underground subsidence due to gas production. Thus this area is used to define the East Frisian gas production area or "IN" for our testing procedure.

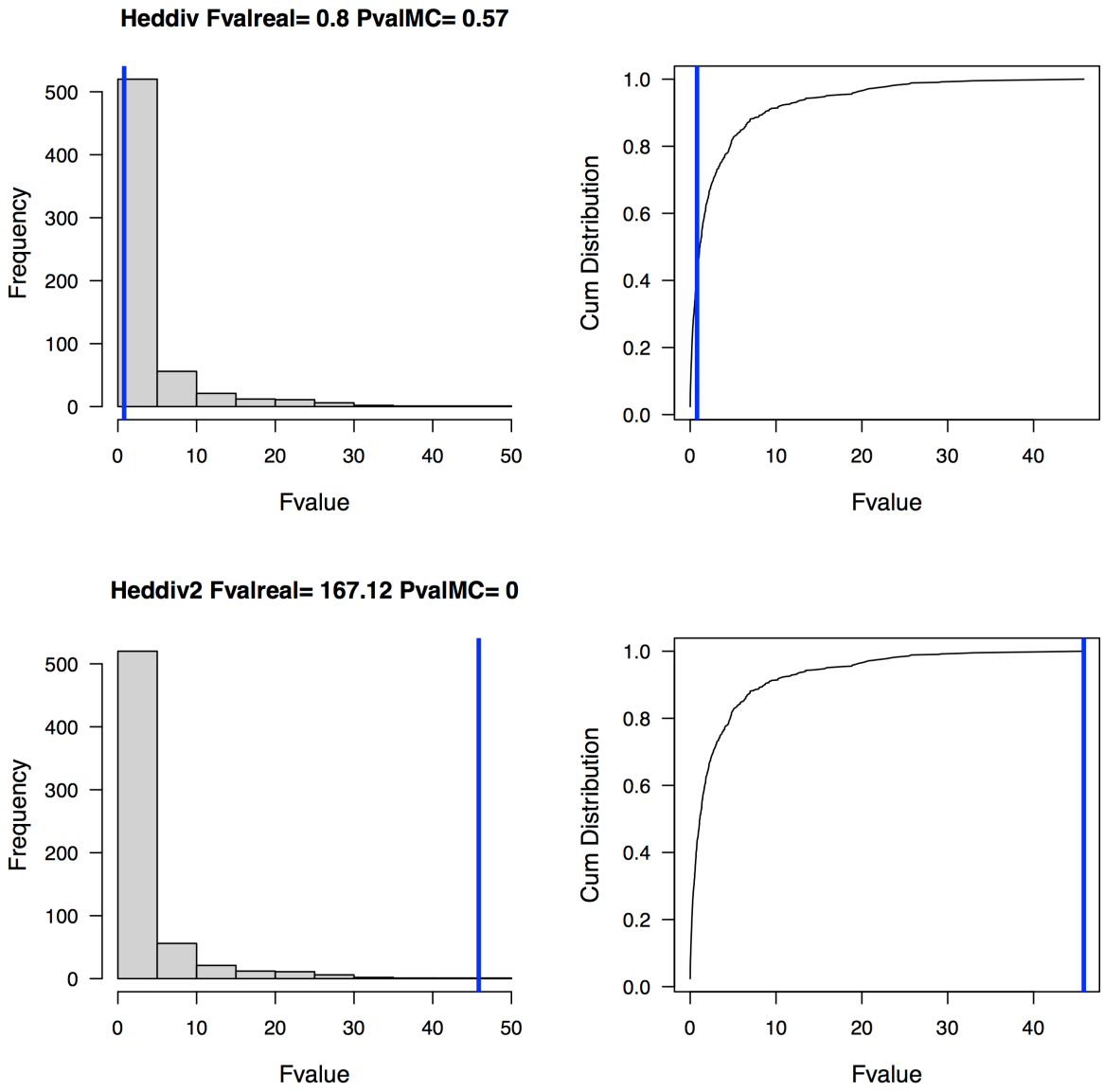
In this report, we characterise the macrofaunal and environmental characteristics of this area. Following this we test whether there are differences in the macrofaunal attributes of this area using a Monte Carlo testing procedure (as explained here and in Aarts et al. 2011; Compton et al. 2012; Compton et al. 2013).

The Monte Carlo testing procedure was developed to test for differences in an "IN" and "OUT" area, while alleviating problems associated with different sample sizes in the two testing regions and spatial autocorrelation. This "in-house" developed approach deals with these statistical issues by employing (1) its own test distribution and (2) by using the entire system as a reference for testing differences between the affected versus non-affected areas. Standard p-values would be biased by both of these issues.

The Monte Carlo testing approach involves two steps. First, to provide a reference of the variation in a specific macrobenthic attribute as found across the entire system, as sampled by SIBES, a statistical distribution is defined using a test statistic, e.g. the F-value. The test statistic is derived from a standard statistical test, e.g. a generalized linear model or anova. The standard statistical test examines whether a macrobenthic attribute from a random sample of adjacently located points (pseudo IN) differs from the same attribute in the remaining sample points outside of this sample (pseudo OUT). In this report, the number of points in the random sample (Pseudo IN) were equal in number to those found in the East Frisian area. To describe the variation in a specific macrobenthic attribute across the entire system, more than 1000 pseudo IN areas were randomly sampled and compared with the remaining pseudo OUT areas. These combined tests, i.e. Monte Carlo simulations, thus describe the variation in a specific macrobenthic attribute across the entire system. The histogram and cumulative distribution of values in Figure 2 provide an example of the statistical distribution that is described during this first step.

Second, to identify whether the same macrobenthic attribute in the area of gas production differs relative to the variation in the same macrobenthic attribute as found across the entire system, we compared the test statistic as derived from the "real" IN and OUT areas with the previously defined statistical distribution from the Monte Carlo simulations. When the test statistic describing the difference between the real IN and OUT falls far outside the range of what was identified by the test distribution then we conclude that the area of production differs to the remaining area. Specifically, the value of the test statistic value is examined for where it lies on the cumulative distribution of test values, as derived from the Monte Carlo simulation (above). When the test statistic from this "real" test, i.e. the comparison between the East Frisian area and the matching environment outside OUT (Appendix S1), falls upon a cumulative distribution value of greater than 0.95, i.e. a probability of 0.05, then the macrobenthic attribute is considered to be significantly different in the production area, relative to the variation observed in the remainder of the Wadden Sea (see the blue line in the bottom panel of Figure 2). By contrast, when the test statistic from this "real" test falls below the cumulative value of 0.95, then the test is not significant, and the variation observed in the testing area is no different to the variation found naturally across the system (Figure 2, top figure). Only those species that occurred in the East Frisian area were tested in this report. Although *Scrobicularia plana* was recorded in this area, there were not enough individuals to test this species in the analysis.

Figure 2. An illustrated example of how the testing procedure works. For example, a species that does not differ relative to the background environment (*Hediste diversicolor,* no change to the abundance) is shown versus a species with a significantly different response in an affected area (*H. diversicolor,* with a ten-fold increase in abundance in the East Frisian area). The blue line indicates the F-value from the "real" test. The histogram (left) and the cumulative distribution (right) of the test statistics (F-values) derived from the Monte Carlo simulations are shown. Only when the F-value from the "real" test (blue line) intersects with a value on the cumulative distribution >0.95 (i.e. a P-value of 0.05) is the test significant; indicating a difference in the affected area versus the remainder of the system.



In this report, statistical tests were run using a generalised linear modelling approach, with quasi-poisson distributed errors:

$$y \sim X \quad (1)$$

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 three positive occurrences were needed in each of the testing regions. In this report, outliers in the macrobenthic attributes were removed based on visual inspection, and outliers in the testing regions, as defined based on environmental values, were removed using Mahalanobis distances (Mahalanobis, 1936). Mahalanobis distances provide a relative measure of the distance between points, such that outlying points can be detected. This measure differs to Euclidean distance in that it takes into account the correlations of the data set and is scale-invariant.

All areas defining the reference for comparison with the simulated IN or real IN areas, i.e the pseudo OUT and OUT areas, were selected to have a similar environment as the IN areas based on the maximum and minimum values of all four environmental variables used in this analysis. Note that there are always more sites in the reference areas compared to the testing areas.

RESULTS AND DISCUSSION

Characteristics of the East Frisian area: environmental attributes

The environment of the East Frisian area is characterised by relatively intermediate fractions of exposure time (~0.4), relatively average maximum current speeds (0.42-0.46 cm/s), a tendency towards high salinities (~26 ppt), and finer grain sizes (<180 µm) compared to those observed across the remainder of the Dutch Wadden Sea (ws, Figure 3). The maps in Figures 4 and 5 show that the East Frisian area has fine grain sizes near the coastline (top panel and lower panel, Figure 4) and relatively long exposure times (Figure 5).

To perform our statistical analysis we selected sites with a similar environmental range as found in the East Frisian area (Figure 3, wss). These selected areas were mainly located in the eastern part of the Wadden Sea (see Appendix 1). There were 21 SIBES sampling sites in the East Frisian area and 148 SIBES sampling sites with a matching environment.

Figure 3.The environment as characterized by the entire SIBES sampling in 2012 (ws, excluding the East Frisian area), the selection of SIBES sampling points that match the East Frisian area in terms of environment (wss) and the East Frisian area (EastFr). All areas are characterised by the fraction of exposure time (ET), maximum current speed (Maxcurr), salinity in a dry period (Sal) and median grain size (MGS). The environmental ranges are characterized by a violin plot, this is a combination of a box plot and a kernel density plot.

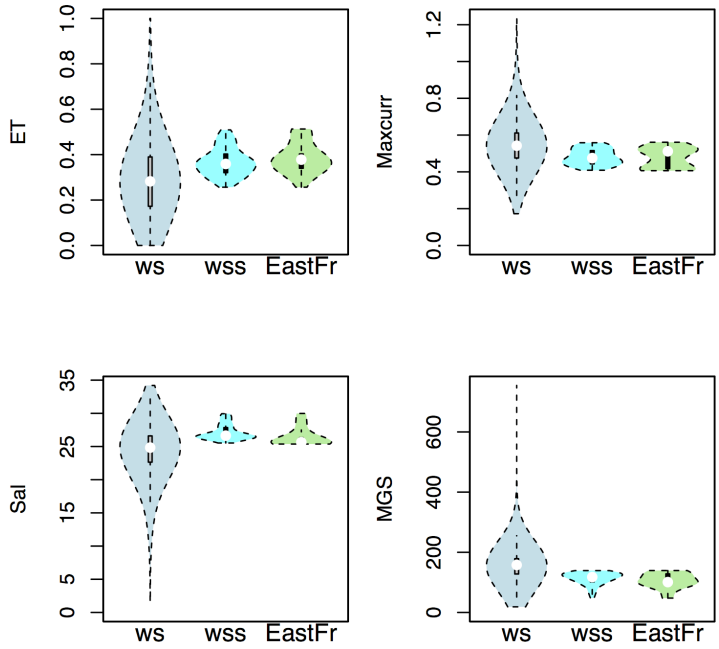


Figure 4. The sediment particle size across the Wadden Sea (bottom panel), with a close up on the East Frisian area in the top panel.

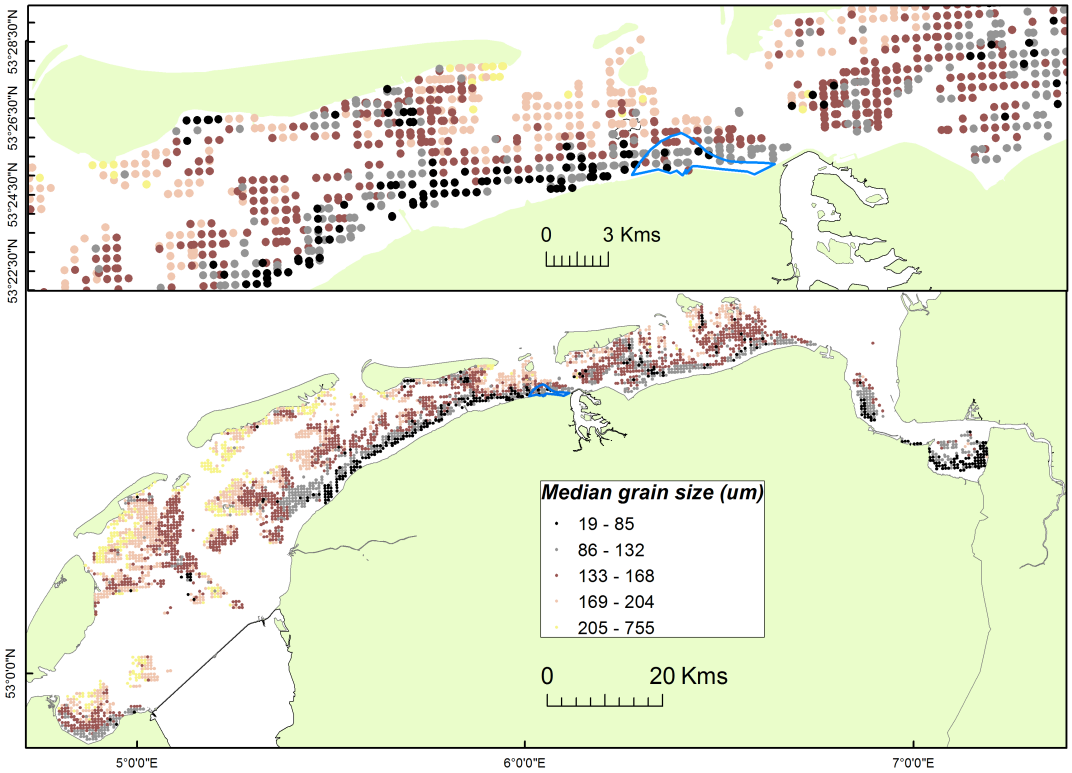
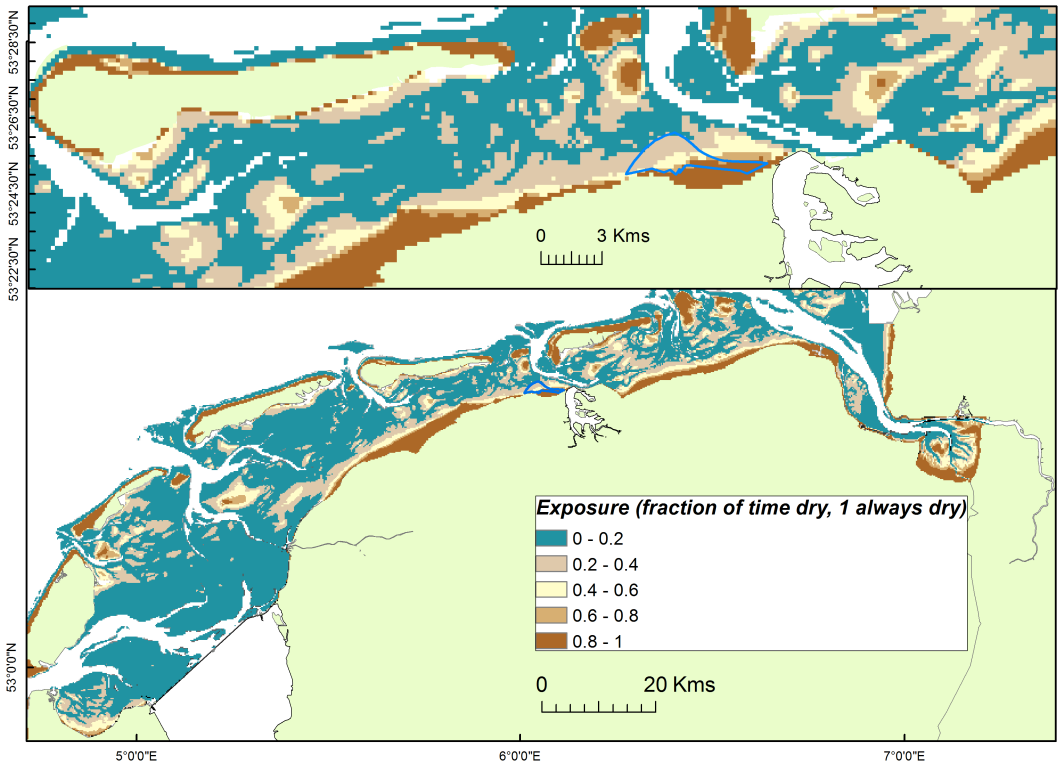


Figure 5.The fraction of exposure time across the Wadden Sea (bottom panel), with a close up on the East Frisian area in the top panel.



Characteristics of the East Frisian area: macrobenthic community attributes

The mean biomass in the East Frisian area was approximately 26 g/m^2 (sd 24, median 15 g/m^2, Figure 7), compared to 45 g/m^2 in the reference area that shared a similar environment (sd 50, median of 33 g/m^2). Across the entire SIBES sampling grid of the Wadden Sea, excluding the East Frisian area, the mean biomass was 82 g/m^2 (sd 578, median of 7 g/m^2).

The mean abundance of macrobenthic species in the East Frisian area was approximately 20638 nrs/m^2 (sd 26048, median 15671 g/m^2, Figure 8), compared to 13150 nrs/m^2 in the reference area that shared a similar environment (sd 22638, median of 5585 nrs/m^2). Across the entire Wadden Sea, excluding the East Frisian area, the abundance was 7014 nrs/m^2 (sd 14579, median of 2882 nrs/m^2).

Table 4.The Monte Carlo model results for the 19 species tested in this area. Although on first appearance there are differences between the IN and OUT areas (Appendix S1), these differences are no different to the variation in abundances observed across the remainder of the Wadden Sea (PvalMC).

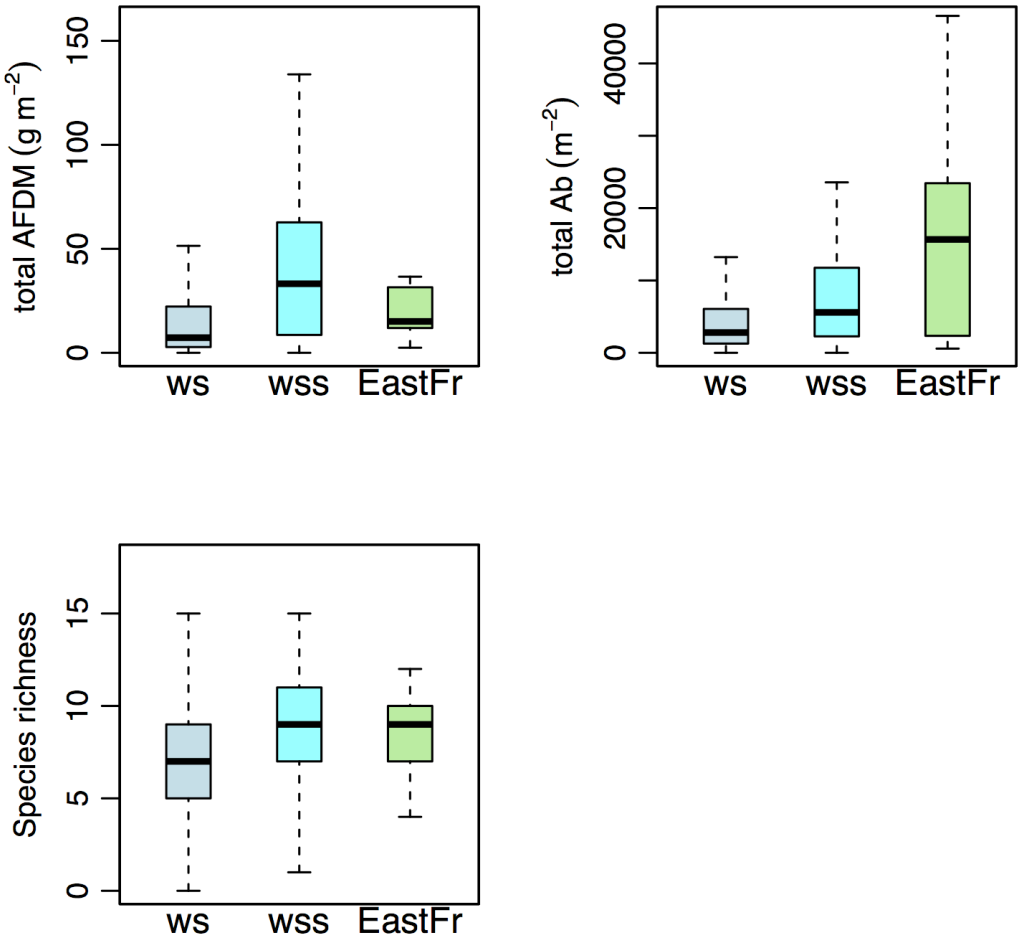


Figure 7. The total biomass across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel).

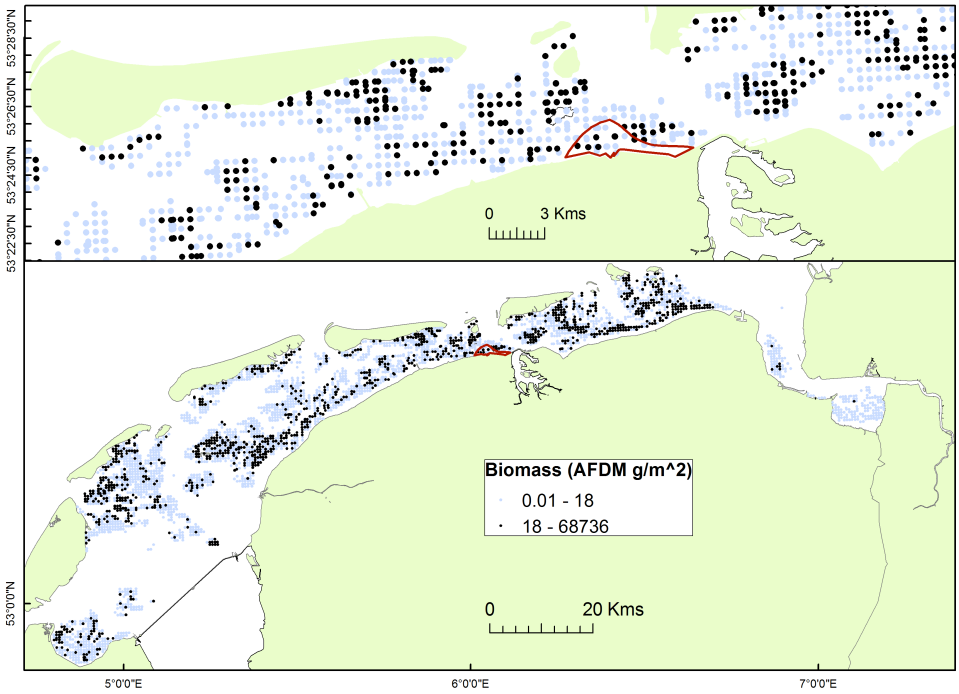
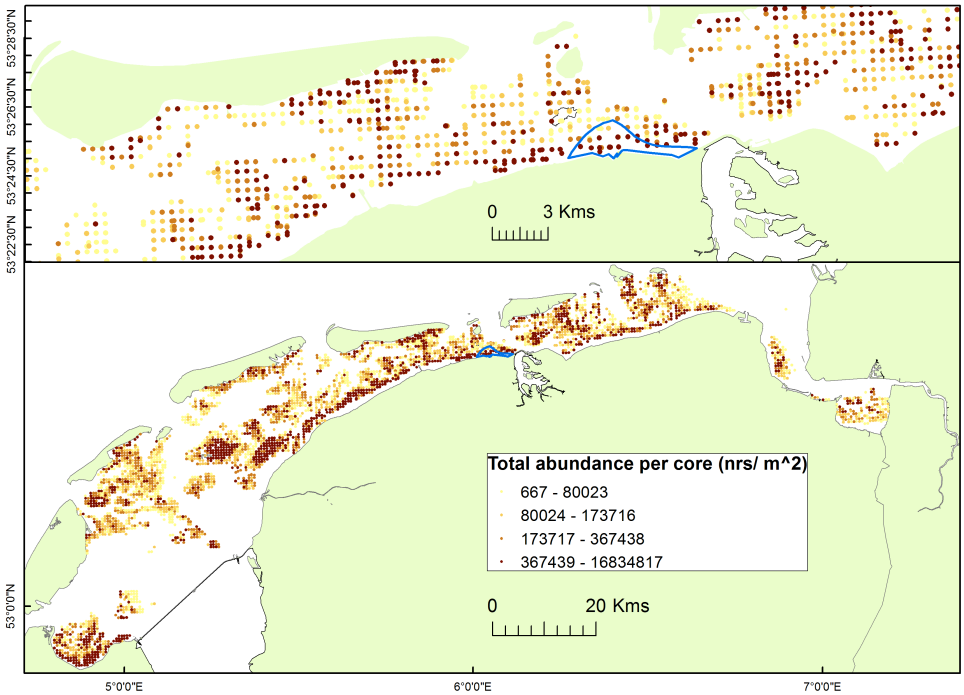


Figure 8. The total abundance (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel).



In the East Frisian area, the mean number of species in a core was approximately 9 (sd 3, median 9, Figure 9), compared to 9 in the reference area that shared a similar environment (sd 3, median of 9 g/m2). Across the entire Wadden Sea, excluding the East Frisian area, the mean number of species in a core was 7 (sd 3, median of 7).

The model using the "real data", i.e. macrobenthos from the East Frisian area (IN) versus OUT sites with a matching environment, showed that the largest difference between these areas was observed in total ash free dry mass, where biomass tended to be lower in the East Frisian area (Table 1). By contrast, total abundance was higher in the East Frisian area. However, the Monte Carlo simulation identified that total biomass, abundance and species richness were not different in the East Frisian area relative to what was observed across the remainder of the Wadden Sea (PvalMC >0.05, Table 1).

Figure 9.The species richness as defined the number of species in a core across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel).

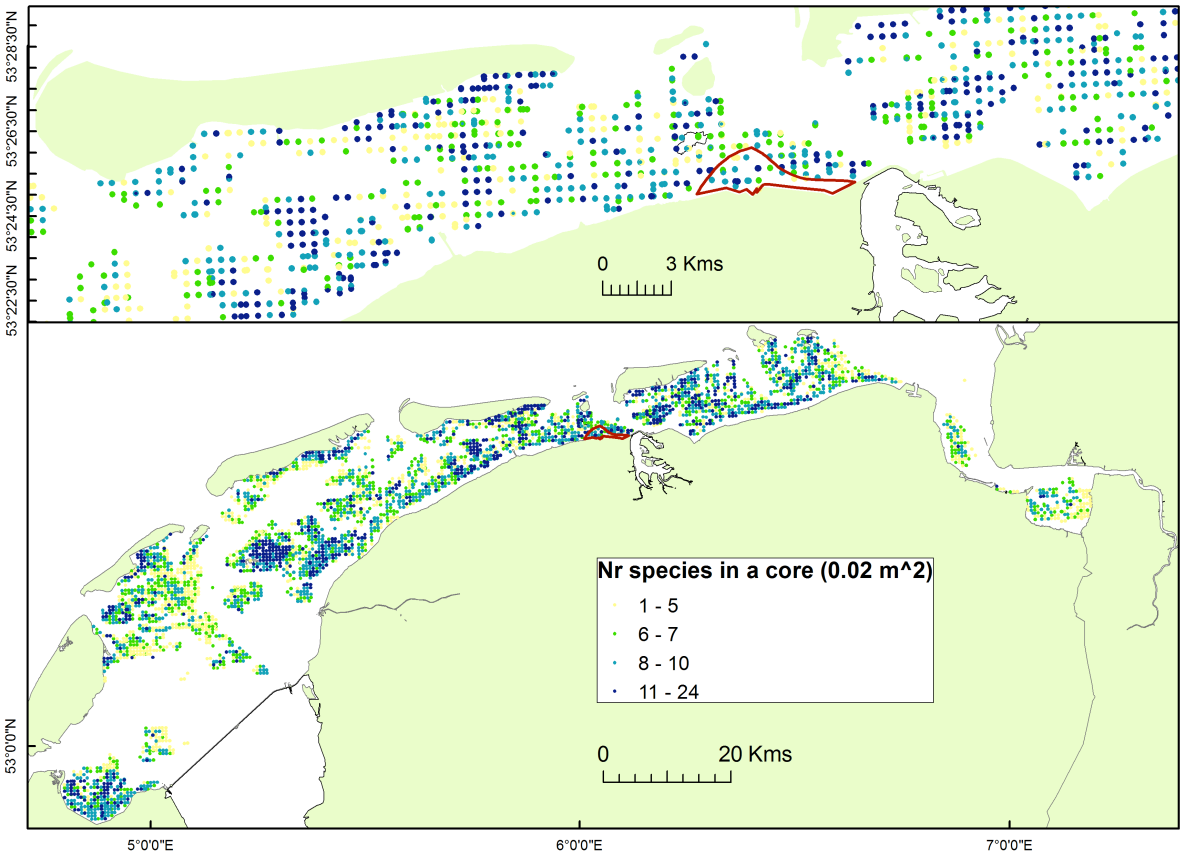


Table 1.The Monte Carlo model results for species richness (sr), total abundance (totab) and total afdm (totafdm). The Fvalue from the test using the real data (realFV) and the P value from the Monte Carlo simulation is shown (PvalMC). The model coefficients for the IN and OUT areas are given in the units of measurement (sr, nr of individuals per core, totab nr of individual per m^2 and afdm is the grams per m^2).

sp	in	out	realFV	PvalMc
sr	8.9	8.78	0.03	0.91
totab	20278	12922	1.71	0.41
totafdm	17	43	6.75	0.06

Characteristics of the East Frisian area: macrobenthic species attributes

In the East Frisian area, twenty species were observed, of which three were bivalves, one was a gastropod, four were arthropods, one was an oligochaete and the remainder were polychaetes. These species all showed a variety of traits, as listed in Table 2.

Table 2. The twenty species found in the East Frisian area, and their taxonomic classification and functional traits: longevity (Long), habitat, reproductive mode (Reprodn), position in the sediment (Posn), feeding mode (Feeding) and their approximate size (Size). The code for each species is also given.

Species	Class	family	Long	Habitat	Reprodn	Posn	Feeding	Size	Code
Arenicola marina	Polychaeta	Arenicolidae	>5	Burrow	Brooder	>10	Grazers	>5	Aremar
Capitella capitata	Polychaeta	Capitellidae	<2	Burrow	Brooder	2 to 10	Grazers	>5	Capcap
Heteromastus filiformis	Polychaeta	Capitellidae	<2	Burrow	Broadcast	>10	Deposit	>5	Hetfil
Aphelochaeta marioni	Polychaeta	Cirratulidae	>5	Burrow	Broadcast	<2	Deposit	>5	Aphmar
Nephtys sp.	Polychaeta	Nephtyidae	>5	Free	Broadcast	2 to 10	Scavengers	>5	Nephsp
Hediste diversicolor	Polychaeta	Nereididae	2 to 5	Burrow	Broadcast	2 to 10	Scavengers	>5	Heddiv
Scoloplos armiger	Polychaeta	Orbiniidae	2 to 5	Burrow	Brooder	>10	Grazers	>5	Scoarm
Eteone longa	Polychaeta	Phyllodocidae	<2	Free	Broadcast	2 to 10	Scavengers	>5	Etelon
Phyllodoce mucosa	Polychaeta	Phyllodocidae	<2	Free	Brooder	2 to 10	Scavengers	>5	Phymuc
Bylgides sarsi	Polychaeta	Polynoidae	<2	Burrow	Broadcast	<2	Scavengers	>5	Bylsar
Pygospio elegans	Polychaeta	Spionidae	2 to 5	Tube	Brooder	2 to 10	Deposit	1 to 5	Pygele
Oligochaeta sp.			<2	Burrow	Broadcast	2 to 10	Grazers	>5	Oligoc
Urothoe sp.	Crustacea	Urothoidae	<2	Burrow	Brooder	2 to 10	Deposit	<1	Urospe
Corophium sp.	Malacostraca	Corophiidae	<2	Burrow	Broadcast	<2	Deposit	<1	Corosp
Crangon crangon	Malacostraca	Crangonidae	2 to 5	Burrow	Brooder	<2	Scavengers	1 to 5	Cracra
Carcinus maenas	Malacostraca	Portunidae	>5	Free	Brooder	<2	Scavengers	>5	Carmae
Cerastoderma edule	Bivalvia	Cardiidae	2 to 5	Burrow	Broadcast	2 to 10	Suspension	1 to 5	Ceredu
Scrobicularia plana	Bivalvia	Scrobiculariidae	>5	Burrow	Brooder	>10	Deposit	>5	Scrpla
Macoma balthica	Bivalvia	Tellinidae	>5	Burrow	Broadcast	2 to 10	Deposit	1 to 5	Macbal
Hydrobia ulvae	Gastropoda	Hydrobiidae	<2	Free	Brooder	<2	Grazers	<1	Hydulv

The most common species were *Pygiospio elegans*, the ragworm *Hediste diversicolor*, the laver spire shell *Hydrobia ulvae* and the Baltic Tellin *Macoma balthica* based on the percentage of presences (>70% of sites, Table 3). Whereas, the two OTUs with the lowest percentage occurrence were *Urothoe* sp and *Corophium* sp (<14%). In terms of mean abundance *Hydrobia ulvae*, the cirratulids *Aphelochaeta marioni*, the common cockle

Cerastoderma edule and the *Oligochaeta* sp. were the most abundant, whereas the lugworm *Arenicola marina* and *Corophium* sp were the least abundant (Table 3).

Distribution maps of the species common to the East Frisian area are provided below (Figures 10 to 15). *P. elegans* was widespread across the Wadden Sea including the East Frisian area, and abundances of this species were highest in the vicinity of Griend and the Balgzand (Figure 10, lower panel). A close up of the East Frisian area shows that this species is not only common in the defined area, but also in the surrounding (Figure 10, top panel).

Table 3. The total abundance (Total, numbers per m^2), the percentage of presence records (%pres), and the mean, median and standard deviation of abundance (numbers per m^2) of the twenty species found in the East Frisian area. These descriptive measures are provided for the East Frisian area, the matching environmental area (environmental select OUT) and the remainder of the Wadden Sea. Species highlighted in blue are most common in each area.

	East Frisian area					Environmental Select from the Wadden Sea					Wadden Sea				
sp	Total	%pres	Mean	Median	Stdev	Total	%pres	Mean	Median	Stdev	Total	%pres	Mean	Median	Stdev
Aphmar	8026	57	382	115	528	140143	78	953	404	1321	1596259	44	381	0	960
Aremar	121	19	6	0	13	2719	29	18	0	45	65460	27	16	0	39
Bylsar	208	24	10	0	21	1363	14	9	0	27	23859	8	6	0	21
Capcap	624	24	30	0	72	11427	41	77	0	185	514097	47	123	0	266
Carmae	462	24	22	0	46	1559	11	11	0	38	42530	10	10	0	43
Ceredu	6121	67	291	115	454	74053	78	500	231	694	748783	35	179	0	522
Corosp	173	14	8	0	21	40620	15	274	0	1507	1781819	21	425	0	1927
Cracra	393	29	19	0	33	1213	8	8	0	31	23616	7	6	0	26
Etelon	1028	48	49	0	74	6996	41	47	0	83	227199	41	54	0	124
Heddiv	1548	71	74	58	79	8362	53	57	23	84	180566	30	43	0	114
Hetfil	953	33	45	0	96	16326	61	111	58	165	142616	26	34	0	96
Hydulv	387865	71	18470	13344	25500	1265308	44	8549	0	21504	13284084	25	3171	0	12883
Macbal	3592	71	171	115	162	35058	81	237	115	388	469871	51	112	57	273
Nephsp	248	24	12	0	22	393	5	3	0	12	11062	5	3	0	14
Oligoc	4100	52	195	58	392	146503	69	990	173	1789	1957635	36	467	0	1413
Phymuc	422	24	20	0	46	3215	20	22	0	67	100379	20	24	0	76
Pygele	3811	76	181	115	269	40850	65	276	115	582	2049685	67	490	115	1172
Scoarm	2731	33	130	0	250	29174	49	197	0	394	2439753	71	582	289	831
Scrpla	185	19	9	0	21	895	9	6	0	20	5068	2	1	0	9
Urospe	462	14	22	0	62	10913	10	74	0	341	989446	28	236	0	758

Figure 10. The abundance of *Pygiospio elegans* (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel). Purple dots indicate sites where no individuals were observed, although samples were taken.

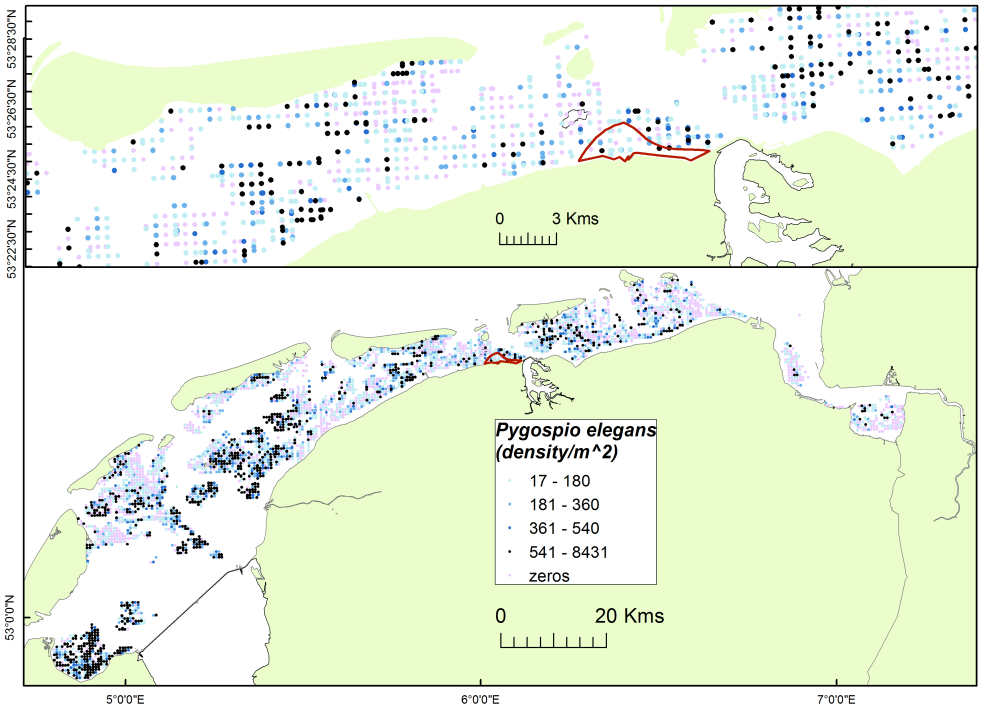


Figure 11..The abundance of *Macoma balthica* (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel). Purple dots indicate sites where no individuals were observed.

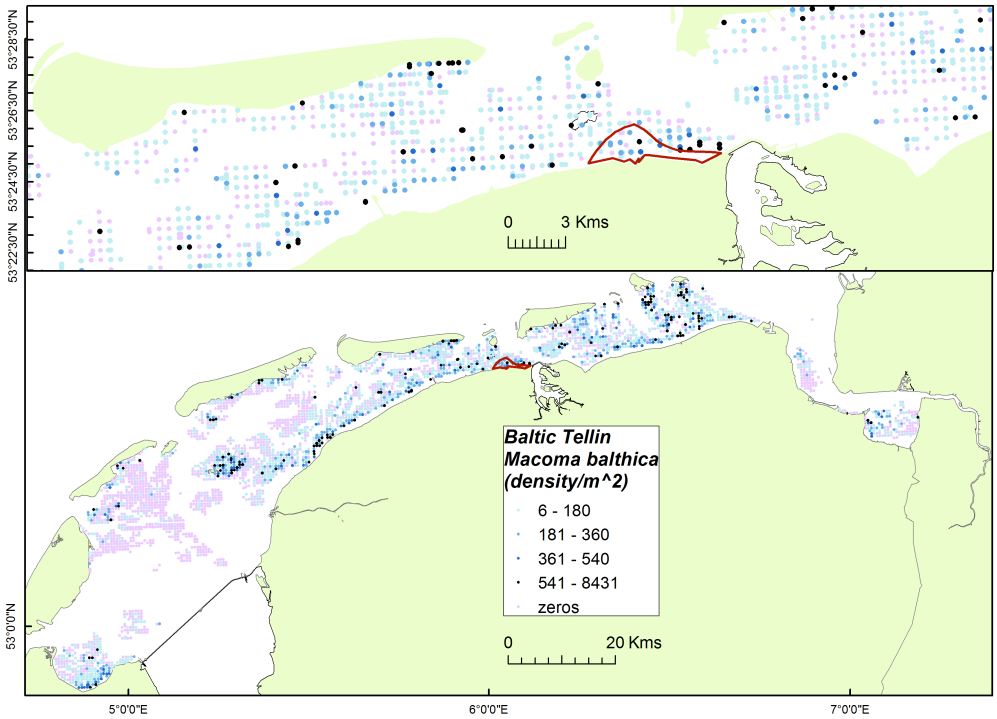


Figure 12. The abundance of *Cerastoderma edule* (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel). Purple dots indicate sites where no individuals were observed.

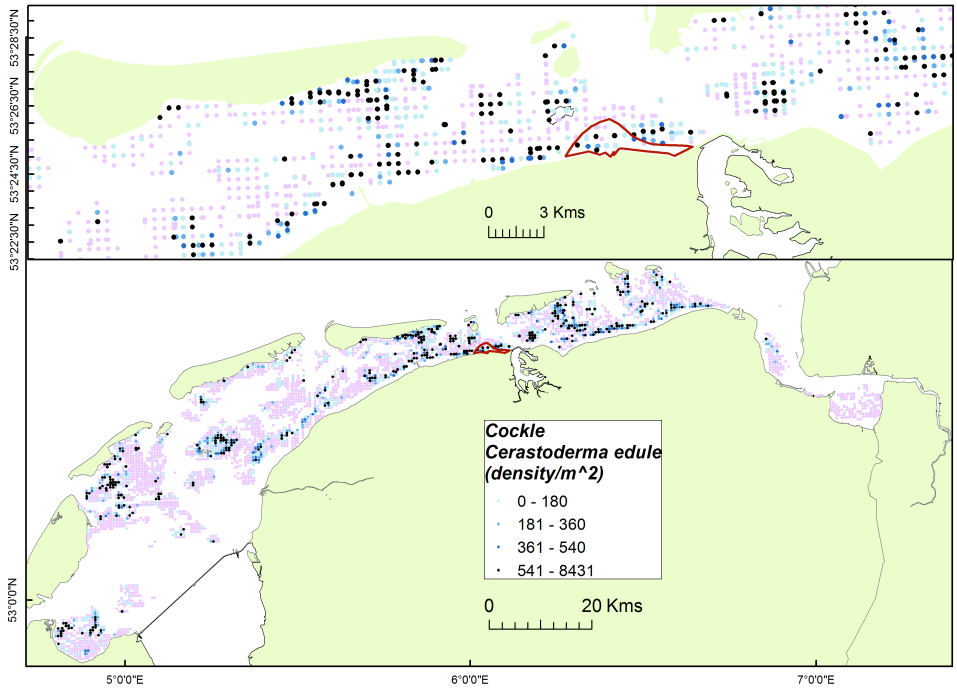


Figure 13.The abundance of *Scoloplos armiger* (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel). Purple dots indicate sites where no individuals were observed.

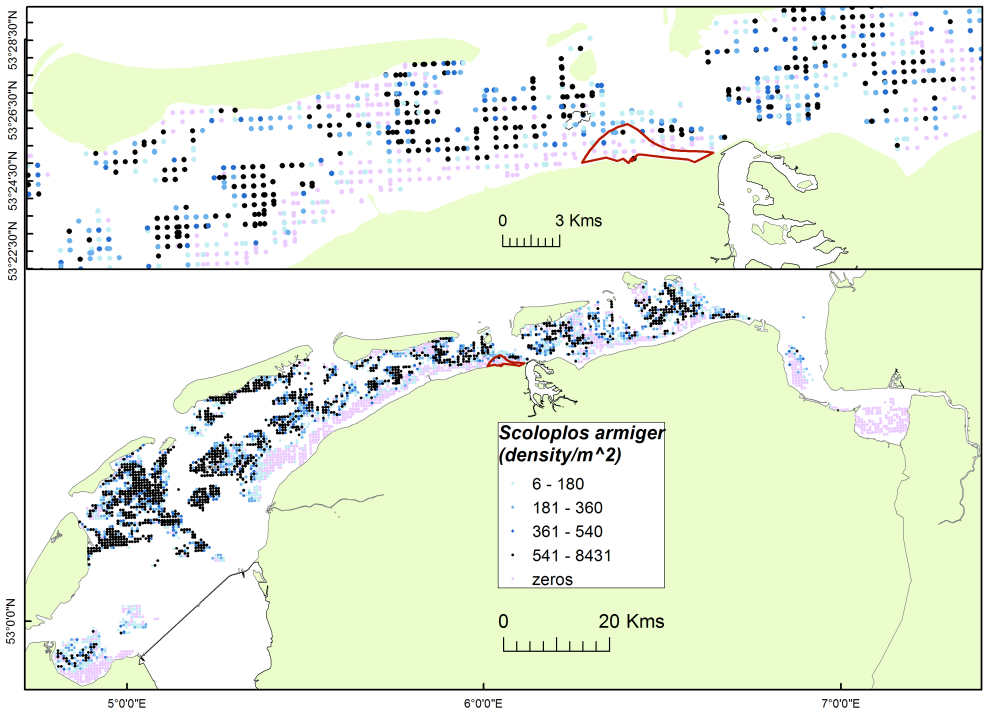


Figure 14. The abundance of *Hediste diversicolor* (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel). Purple dots indicate sites where no individuals were observed.

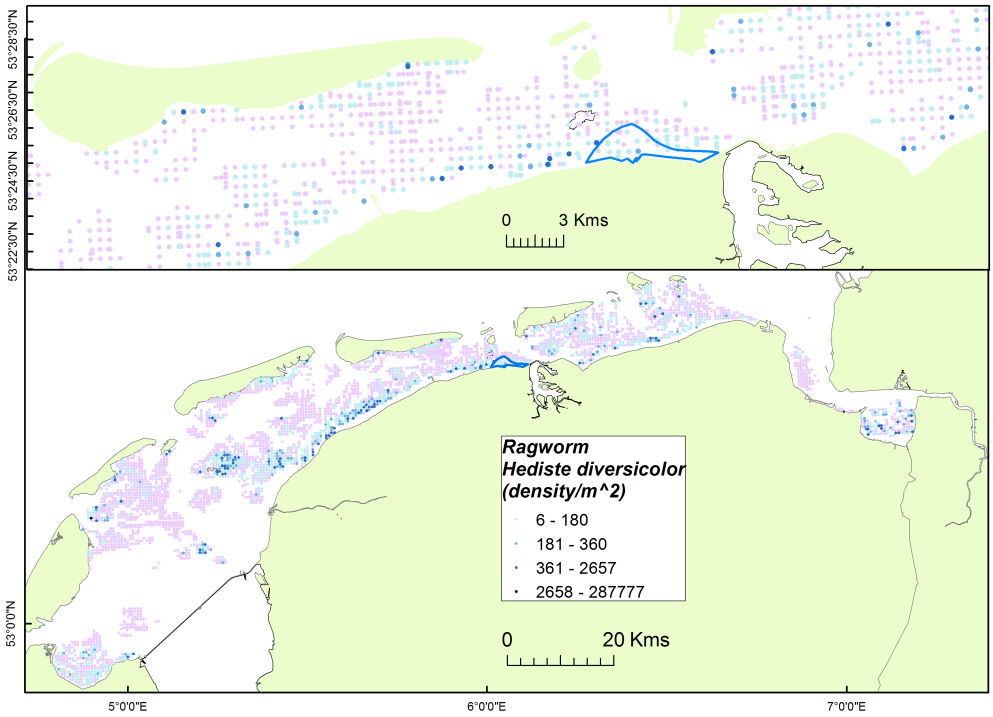
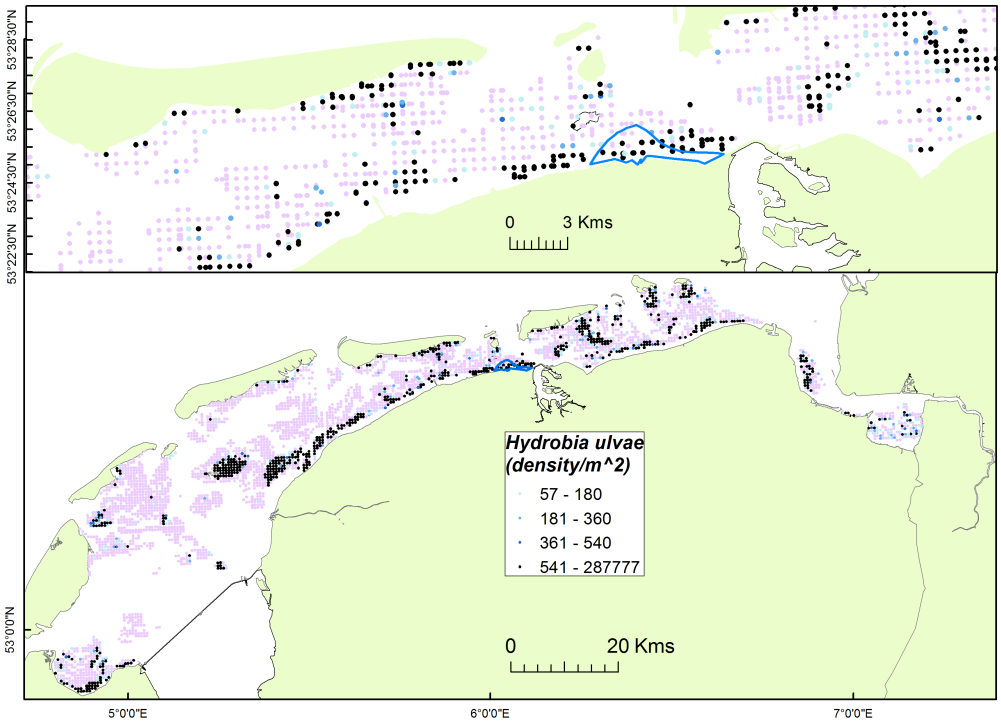


Figure 15. The abundance of *Hydrobia ulvae* (nrs/m^2) across the Dutch Wadden Sea in 2012 (below panel) and in the East Frisian area (defined by the red contour line in the top panel). Purple dots indicate sites where no individuals were observed.



The Baltic Tellin *M. balthica* is more commonly observed in the eastern as compared to the western part of the Wadden Sea, and especially along the coastlines in these areas (Figure 11, bottom panel). Although common in the East Frisian area, this species is also common along the broader coastline of the eastern Wadden Sea area (Figure 11, top panel).

The cockle *C. edule* has a patchy distribution with a preference for certain habitat types across the Dutch Wadden Sea (Figure 12, bottom panel). A closer inspection of the East Frisian area shows that the abundance and occurrence of this species does not appear to be strikingly different in the production area relative to the surrounding environment (Figure 12, top panel).

The bristleworm *S. armiger* occurs commonly and abundantly across the Dutch Wadden Sea, with the exception of coastal areas where it is not found widely (Figure 13, bottom panel). In the East Frisian area, *S. armiger* is not found along the coast but more towards the subtidal, in agreement with the observed distribution of this species in this area (Figure 13, top panel).

The ragworm *H. diversicolor* is found commonly along the coastline areas of the Wadden Sea (Figure 14, bottom panel). Consequently, this species is also commonly found along the coastline of the East Frisian area (Figure 14, top panel).

The laver spire shell *H. ulvae* has specific habitat preferences for areas that occur high on the intertidal, i.e. long exposure times, across the Wadden Sea (Figure 15, bottom panel). In accordance with this broader distribution, a close up of the East Frisian area shows that *H. ulvae* is very common and abundant near the coastline of the East Frisian production area (Figure 15, top panel).

In agreement with these mapped distribution patterns, the Monte Carlo testing analysis did not identify that the abundances of the species in the area of gas production differed markedly to the variation in the abundances of the same species as found across the remainder of the Dutch Wadden Sea (Table 4). These results are thus in agreement with the current expectation that there is no effect of land subsidence in the East Frisian area. By contrast, a comparison of older gas production areas identified differences between species in a report written for NAM earlier this year (Compton et al. 2013).

The strength of the SIBES programme is emphasized by this report, in that macrobenthos abundances and biomass in the East Frisian area are compared with what was

found across the entire system in this year. Given the comparison in space, via the maps, and also via the modelling approach we are confident that there are no changes in the East Frisian area associated with gas production. The ability to compare the distribution of the macrobenthos across the entire system shows that this programme will have the power to detect if changes occur in the long-term.

Table 4.The Monte Carlo model results for the 19 species tested in this area. Although on first appearance there are differences between the IN and OUT areas as compared in the real test, these differences are no different to the variation in abundances observed across the remainder of the Wadden Sea (PvalMC). The full names of the species from the species abbreviations given in this table (sp) can be found in Table 2.

sp	in	out	realFV	PvalMc
Aphmar	383	955	4.95	0.13
Aremar	3	17	3.52	0.17
Bylsar	8	9	0.02	0.91
Capcap	30	77	1.68	0.43
Carmae	22	10	1.27	0.54
Ceredu	292	500	2.04	0.42
Corosp	8	277	1.25	0.5
Cracra	19	8	1.73	0.7
Etelon	50	47	0.02	0.93
Heddiv	75	56	0.8	0.57
Hetfil	44	110	3.98	0.17
Hydulv	18489	8592	2.88	0.33
Macbal	171	237	0.66	0.63
Nephsp	11	2	5.06	0.46
Oligoc	195	993	6.23	0.17
Phymuc	19	21	0.02	0.82
Pygele	182	276	0.61	0.68
Scoarm	129	196	0.63	0.73
Urospe	22	74	0.68	0.61

CONCLUSIONS

In agreement with the predicted minimal subsidence in the East Frisian area, this report and previous reports from the SIBES programme all show no or minimal change in the abundance of macrobenthos in the East Frisian area. In total the abundances of the twenty species observed in this did not differ relative to what one would expect if this area was not affected by gas production.

The SIBES surveys currently provide a reference of the East Frisian area prior to larger underground subsidence, due to gas production. In addition, the current, and future, collection of data will ensure that short-term natural variability is not mistakenly interpreted as human driven change in this area.

Acknowledgements

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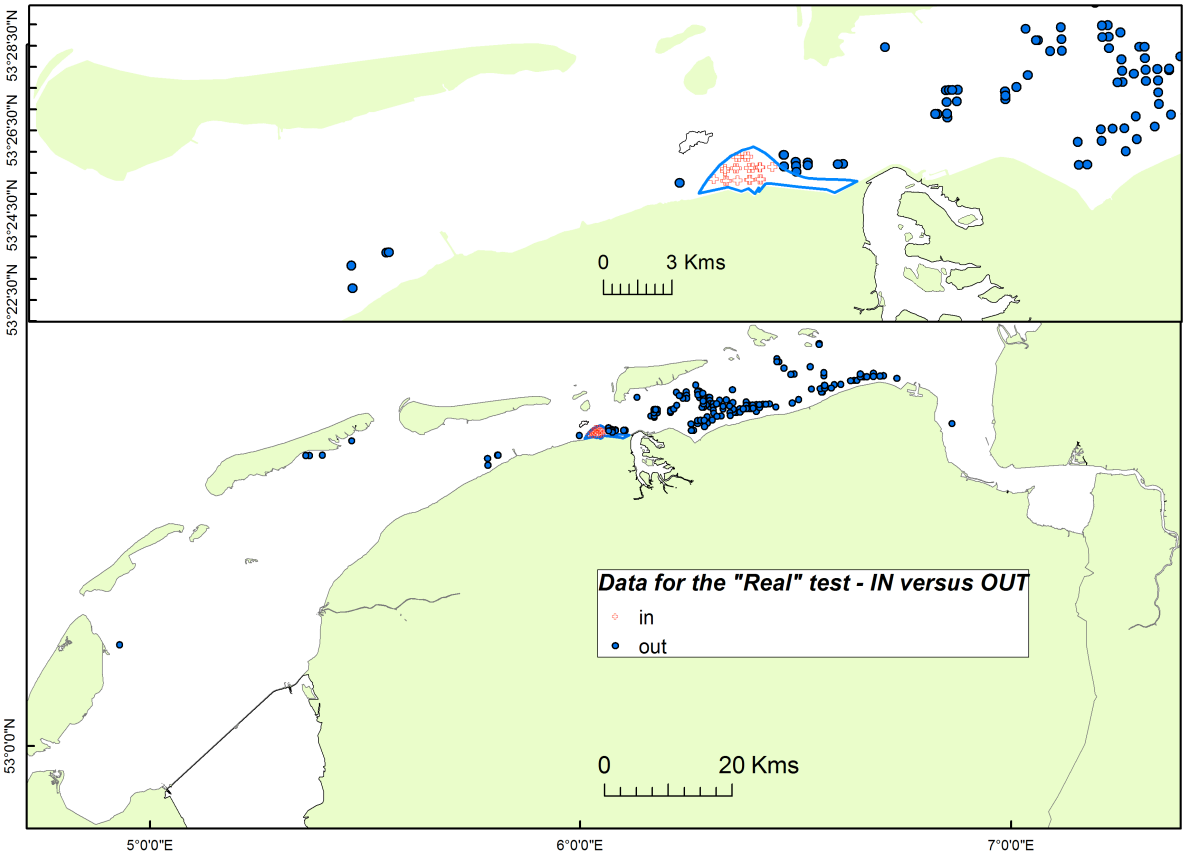
Appendix

S 1. SAMPLE POINTS USED IN THE "REAL" TEST.

Appendix S.1.The SIBES sampling points located in the East Frisian production area (n=21). The sampling points with a matching environmental space, used for the "real test" in the Monte Carlo analysis, thus the points with a matching environment outside of this area are also shown (OUT, n=148).



S 2. PROTOCOLS FOR SORTING BENTHIC SAMPLES

Field of Application

This procedure applies to all activities relating to the sorting of macrozoobenthic samples.

Objective

The objective of this procedure is to set down the activities relating to the sorting of macrozoobenthic samples in the field.

Definitions

Sorting: The process of separating dead macrozoobenthic material from material that is alive or has been preserved alive.

Qualified staff: Staff that is capable of independently doing preparatory work on and sorting benthic samples.

Sub-sample: A sample drawn from a sample that represents a known fraction of the total sample.

Responsibility

The co-ordinator in charge of the sorting work is responsible for the correct sorting of macrozoobenthic samples in the lab following the methods described in this procedure. The co-ordinator is also responsible for the correct documentation and for correctly handing over data and materials to the co-ordinator in charge of sample analysis.

Realization

Input

Macrozoobenthic samples (live samples in plastic bags)

Macrozoobenthic samples (dead, fixed in jars)

Lab facilities

Sorting equipment

Pretreatment of Samples

Live samples, cooled when taken in the field, are stored cool (4-5°C) until they are analysed.

Preserved samples are stained in the lab using a rose bengal solution. The stock solution is 1 gram of rose bengal dissolved in 100 ml water. Several millimetres of this solution are added to every sampling jar. Rose bengal stains proteins so that organisms contrast clearly with the dead material in the sample (shell grit in particular) and can be sorted out more efficiently. When the stain has been added, the sampling jars must be stored for at

least 24 hours before further treatment. Samples can be stained before they are rinsed and sorted. In this case, the entire sample is stained. Samples can also be stained after the first round of sorting. In that case, it is only the remaining material that is stained, which is subsequently rinsed and sorted again.

All samples must be rinsed in a fume cupboard before they are sorted. The sampling jar is first opened and decanted and the excess formalin is received into a collection vessel. The sample itself is then passed through a sieve with meshes of known size and rinsed with ample tap water. The sample may also be passed through a series of connected sieves with decreasing mesh sizes and rinsed with ample tap water. Any remaining formalin is thus removed and the sample is then divided into several size fractions, which facilitates sorting the material. The successive sample fractions are subsequently transferred to a photo developing tray filled with a layer of clean tap water and they are sorted using a table loupe with a light source.

Sorting

Before the sorting begins, it is important to consult the field form that comes with the sample for any information that might be relevant to the sorting process. Live samples are sorted in white photo developing trays on a working table in a room with ample illumination. The sample is divided and transferred to one or more sorting trays filled with sea water, depending on the sample size. Live organisms are put into a collection jar filled with sea water. This jar is stored cool after sorting.

Preserved samples are sorted under an extractor hood. All stained animals, or parts of animals, are taken from the sorting tray with a pair of tweezers and put into a collection jar, labelled with a waterproof label. Organisms may at this stage of the sorting process already be divided into 5 main groups during: Echinodermata, Mollusca, Crustacea, Polychaeta and 'rest'. These are then stored in separate tubes or jars. Mollusca are preserved in 70% ethanol, the other groups in 6% formalin. Tubes are marked with the initial letter of the taxonomic group (E, M, C, P or R) in felt-tipped pen and all tubes belonging to the same sample are stored in a single jar.

When sorting Wadden Sea samples, species that occur in large numbers and can easily be identified with the naked eye, such as the Laver spire shell, Hydrobia ulvae, can be subsampled. When using photo developing trays with ridges that run lenghtways, it is possible to sort one or half a length between the ridges after careful, equal distribution of the

species that is to be subsampled. The person doing the sorting records which fraction of the sample has been sorted for which specific species. Anyone sorting Wadden Sea samples must complete a form, indicating which species have been subsampled.

Qualifications of Staff Members

Qualified staff members can independently do preparatory work on and sort samples; non-qualified staff can only do this while being supervised by qualified staff. Supervision means that after having been sorted, some samples are randomly selected by a qualified staff member and checked.

Output

Jar with live material

Jar with preserved, stained material

Sorting Form Wadden Sea Grit

Forms

S 3. PROTOCOLS FOR ANALYSES OF BENTHIC SAMPLES

Field of Application

This procedure applies to all activities related to the analysis of macrozoobenthos samples.

Objective

The objective of this procedure is to set down all activities relating to the analysis of macrozoobenthos samples collected in the field.

Definitions

Qualified staff Staff capable of independently doing preparatory work on, sorting and identifying benthic samples

Biomass The amount of living material representing an organism or a group of organisms

Ash-free dry weight A commonly used unit of measurement for biomass: the weight of one or more organisms dried in the oven, minus the weight of the ash left behind after incineration in an oven.

Responsibility

The co-ordinator in charge of the analysis of macrozoobenthos samples is responsible for the correct analysis of the macrozoobenthos samples in the lab following the methods described in this procedure. The co-ordinator is also responsible for the correct documentation and the correct handing over of the acquired data and materials to the co-ordinator in charge of further processing and data entry.

Realization

Input

Macrozoobenthos samples (alive in trays)

Macrozoobenthos samples (dead, fixed in jars)

Laboratory facilities

Analysis equipment

Preparation

In case of living material, the entire sample is transferred to a white photo developing tray.

In case of preserved material, each sample is passed through a sieve with known mesh size with ample tap water as a preparatory treatment for analysis. Next, the sample is transferred to a Petri dish. The label of the sample is entered on the analysis/identification form, along with the date the sample was taken.

Analysis

Identification of species: analysis is the act of identifying and counting the individual macrozoobenthos species. Species are identified on the basis of external features, which are defined for the various species in the standard books. Polychaeta, Mollusca, Crustacea and Echinodermata are typically identified to species level, except for taxa that lack a sufficient number of recognisable features due to sample preservation (e.g. Nemertini and Oligochaeta), some juvenile organisms and organisms that have sustained too much damage.

Organisms > 1 cm are identified with the naked eye, unless their distinctive features are only clearly visible under a microscope. In that case, a stereo microscope is used for identification. Organisms < 1 cm are always identified under a stereo microscope. After identification, the total number of individuals of each species is entered on the counting form. As a guideline, it is only the heads that are counted. If a species cannot be identified at all or not with certainty, an external expert is consulted.

Molluscs and worms may also be identified and counted in the field as far as possible.

Biomass determination: next, the ash-free dry weight is determined for each species or taxon that is identified. There are two methods of determining this: (1) by actually drying and incinerating organisms; and (2) by measuring the wet weight or length of the organisms and converting this to the ash-free dry weight using the usual conversion factors.

Drying and incinerating: living bivalves are briefly immersed in boiling water before the flesh is separated from the shell. The flesh of the large, preserved bivalves (larger than 5 mm) is removed from the shell.

Biomass is determined by drying the entire animal or just the flesh in a porcelain crucible. Every crucible bears a number that is entered on the counting form after the name of the species. The crucibles are placed in a well-ventilated oven for 2 to 3 days at a temperature of 60°C. After this, the crucibles are put in a desiccator; when they have cooled to ambient temperature, they are weighed on digital scales that are connected to a computer, and the number of the crucible and the total weight of the crucible with its contents are registered.

After this first weighing session, the crucibles and their contents are placed in an oven for 3 to 5 hours at a temperature of 560°C for incineration. When they have cooled down, they are once more placed in a desiccator and when they have cooled to ambient temperature, they are weighed again, and the number of the crucible and the total weight of the crucible and its contents are again registered. The difference between the weights in the two weighing sessions is the ash-free dry weight.

In addition to determining biomass by drying and incinerating, biomass may also be determined by using conversion factors. Biomass of Mollusca and Echinoidea can be determined by measuring the length of each individual in the sample. Organisms > 5 cm are measured with an office ruler to an accuracy of 5 mm; organisms measuring between 1 and 5 cm are measured with a vernier calliper to an accuracy of 1 mm; and organisms measuring < 1 cm are measured with a measuring eyepiece to an accuracy of 0.5 mm. The lengths of the individual organisms are entered on the counting form.

The ash-free dry weight can subsequently be calculated on the basis of the relation between the shape W= a*Lb where W = ash-free dry weight in g, L =length in mm and a and b are factors with values that have been empirically determined previously.

Biomass of Polychaeta, large Crustacea, Ophiuroidea and the remaining groups is determined by establishing the wet weight of the collective organisms of each species using digital scales. The organisms are put on filter paper prior to weighing to make sure practically all excess water is absorbed. The wet weights measured are recorded on the counting form for each species.

Small Crustacea (Amphipoda and Cumacea) are attributed an ash-free dry weight of 0.3 mg for each individual organism.

Qualifications of Staff Members

Qualified staff may independently make analyses of/identify samples; non-qualified staff can only make analyses of/identify samples when supervised by qualified staff. Supervision means that after having been identified, some samples are selected randomly by a qualified staff member and checked.

Output

Counting forms

Forms

- Lab List Wadden Sea Worms Grit F.7.9
- Lab List Wadden Sea Molluscs Grit F.7.10
- Lab List Box Corer Wadden Sea Transect F.7.11

Lab List Balgzand Transect F.7.12

Lab List Sublittoral Open Sea F.7.13

- Lab List Identification Literature F.7.16

S 4. PROTOCOLS FOR SEDIMENT ANALYSES.

Field of Application

This procedure applies to all activities relating to the analysis of sediment samples.

Objective

The objective of this procedure is to set down all activities relating to the analysis of sediment samples.

Definitions

Coulter counter: Electronic device for counting particles

Responsibility

The co-ordinator in charge of the analysis of sediment samples is responsible for the correct analysis of sediment samples in the lab following the methods described in this procedure. The co-ordinator is also responsible for the correct documentation of the acquired data and materials and for correctly handing these over to the co-ordinator in charge of further data processing and data entry.

Realization

Input

Sediment samples

Laboratory facilities

Analysis equipment

Sediment analyses can be made with two different Coulter particle counters:
Coulter LS 230 and Coulter Beckman LS 13 320 with Autoprep.
Each Coulter counter has its own treatment and measuring methods:

Coulter LS 230

Preparation

Every sample is freeze-dried in a glass jar. This process may take from several hours up to 3 days, depending on the amount of water in the sample. Next, the sample is passed through a 2-mm-mesh sieve. Any material that is left behind on the sieve is weighed, as is the < 2-mm fraction. Both weights are recorded. Several grams of the < 2-mm faction are weighed out and kept for analysis by the Coulter counter.

Analysis

There are 3 methods of analysis:

a. Without chemicals: demineralised water is added to material that has been weighed out, and the sample is directly measured by the Coulter counter.

b. Adding H2O2: demineralised water and H2O2 are added to the weighed-out material. Next, the sample is placed in a sand bath or desiccation stove for 7 hours to remove all organic material. Next, demineralised water is added to the sample again, and the material is left to deposit from the liquid for 3 nights. Then the sample is drained by suction. The sample is subsequently measured by the Coulter counter in demineralised water to which sodium pyrophosphate has been added.

c. Adding H2O2 and HCl: demineralised water + H2O2 + HCl are added to the weighed-out material. Next, the sample is placed in a sand bath or desiccation stove for 7 hours to remove all organic material and calcium. Next, demineralised water is added to the sample again and the material is left to deposit from the liquid for 3 nights. Then the sample is drained by suction. The sample is subsequently measured by the Coulter counter in demineralised water to which sodium pyrophosphate has been added.

Coulter Beckman LS 13 320 with Autoprep

Preparation

Every sample is transferred to a plastic jar if required and subsequently freeze-dried. This process takes from several hours up to 3 days, depending on the amount of water in the sample. Next, there are two possible methods for further preparation: with and without preliminary treatment

Without Preliminary Treatment

The sample is passed through a 2-mm-mesh sieve into a 13-ml test PP tube (particles larger than 2 mm may damage the measuring cell and are therefore not measured). Next, RO (Reversed Osmosis) water is added to suspend the sediment particles. The filled tubes can subsequently be placed in the Autoprep module of the Coulter counter LS 13 320 and they are ready to be measured.

With Preliminary Treatment

The sample is passed through a 2-mm-mesh sieve into a 50-ml PP centrifuge tube (particles larger than 2 mm may damage the measuring cell and are therefore not measured). Next, 15 ml of RO water is added to each centrifuge tube. Then, 15 ml of 35% H¬¬2O2 solution (hydrogen peroxide) and 12 ml of 0.5N HCl solution (hydrochloric acid) are added. Next, the centrifuge tube is filled with RO water to the 45-ml marking. The tubes (there is room for 30 tubes per session) are then put into a drying oven at 80 °C for a night (approx 16 hours).

The next morning, the samples are taken out and left to cool down and extra RO water is added to compensate for evaporated water. Then the tubes are placed in a centrifuge at 3000 revolutions per minute for 5 minutes. Next, the chemicals on top of the sediment are drained by suction using a water vacuum pump. Then, 5 ml of RO water and 2.2 ml of a sodium pyrophosphate solution are added to the tubes, and they are subsequently homogenized in a vortex test tube shaker. The centrifuge tubes are filled with RO water to the 40-ml marking and centrifuged at 3000 revolutions per minute for 12 minutes. The liquid on top of the sediment is drained by suction and the sample is transferred to a 13-ml PP test tube. Next, the full tubes can be placed in the Autoprep module of the Coulter Beckman LS 13 320 and are ready to be measured.

Analysis

When the sample data have been entered into a computer that is connected to the particle counter, the samples can be measured. The Beckman Coulter LS 13 320 is a particle-size analyser designed according to the principles of laser diffraction and light scattering measurement (PIDS). The method roughly works as follows:

A laser beam is focused on the particles in the measuring cell. When the beam hits the particles, the light is scattered in various directions. Next, the 132 detectors fitted around the measuring cell absorb part of the light. The size of the particle can subsequently be calculated

with a complex algorithm on the basis of the intensity and angle of the light when it falls on the detectors.

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

Qualification of Staff Members

Qualified staff may independently analyse and identify samples; non-qualified staff may only analyse and identify samples when supervised by qualified staff.

Output

File with Sediment Data

6 Forms

Lab Registration Sediment Analysis F.7.14

MIDE RO Log F.7.15