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Report on the 2008 and 2009 sampling program

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Abstract

The Wadden Sea is of paramount importance to wildlife. It is a breeding, wintering and refueling area for millions of birds, and an important nursery area for several species of fish. For their survival, growth and reproduction these consumers almost entirely depend on macrozoobenthos (all benthic organisms > 1mm) as their major source of food. To understand the functioning of the Wadden Sea ecosystem, monitoring and understanding the distribution and population dynamics of macrozoobenthos is essential.

In 2008, the NIOZ (thanks to NAM and NWO Sea and Coastal Research (ZKO) funding), initiated a synoptic intertidal benthic sampling programme, covering the entire intertidal zone of the Wadden Sea, a long-term effort named SIBES (Synoptic Intertidal Benthic Sampling). The objective of this study is to use the SIBES data to quantify the spatial and temporal variability of macrozoobenthos and to investigate if land subsidence caused by natural gas exploitation impacts the macro fauna community. This study describes the results of the 2009 sampling campaign and will address differences relative to 2008. In 2009, the survey for the first time also included the Eems-Dollard region. In total 4410 stations were sampled, containing more than 385 thousand individual organisms, belonging to 93 species. In 2009, substantially more individuals were observed due to a relatively large recruitment of several species. In biomass terms (expressed as Ash Free Dry Mass (AFDM)), the most important species were edible cockle (*Cerastoderma edule*), sand mason (*Lanice conchilega*), soft-shell clam (*Mya arenaria*), lugworm (*Arenicola marina*), American jack knife clam (*Ensis americanus*), blue mussel (*Mytilus edulis*), ragworm (*Hediste diversicolor*), Pacific giant oyster (*Crassostrea gigas*) and Baltic tellin (*Macoma balthica*). For each species the spatial distribution is estimated and presented. Also the species richness is estimated for the entire intertidal zone of the Wadden Sea, illustrating that the samples taken at higher elevations within the Wadden Sea (e.g. the regions along the mainland and Island coast) in general contained most species.

In this study we used the data to investigate changes in abundance in the regions where, due to natural gas extraction, current and future subsidence is to be expected. The Ameland-oost region characterized by 2-3 cm subsidence revealed a relative larger decrease of the green ragworm *Alitta virens* (p-value=0.002). The ‘Moddergat-Lauwersoog-Vierhuizen’ region was characterized by a relative larger decrease of the small crustacean *Bathyporeia sarsi*. When we compare all areas characterized by land-subsidence with the rest of the Wadden Sea, the subsidence areas showed a larger decrease of the small crustacean *Urothoe poseidonis*, but a larger increase of the polychaete worm *Magelona johnstoni*. The Ameland-oost region characterized by 1-3 cm subsidence revealed a relative larger significant increase of the polychaete worm *Heteromastus filiformis*. Compared to the rest of the Wadden Sea (using Monte Carlo simulations), such deviations were only out of proportion for *Alitta virens*, *Heteromastus filiformis*, *Magelona johnstoni* and *Urothoe poseidonis.* Because these species-specific deviations are not observed across all land-subsidence regions, it seems unlikely that they relate to gas exploration activities.

The results presented in this report provide a solid reference to assess and test future changes in macrozoobenthos abundance. As the data for more years become available, the power of this synoptic intertidal benthic sampling scheme to distinguish the possible effects of subsidence and other local natural and anthropogenic processes on the spatial and temporal demography of macrozoobenthic species and the consumers that depend on them, becomes ever larger. SIBES will become a powerful way to assess the ecological state of Wadden Sea.

Nederlandse Samenvatting

The Waddenzee is voor zowel Nederland als de rest van de wereld een belangrijk natuurgebied. Miljoenen vogels gebruiken het gebied om hun jongen voor te brengen, te overwinteren of gebruiken het als tussenstop gedurende hun trektocht van vaak duizenden kilometers. Ook voor veel vissoorten, is het gedurende de eerste fases van hun leven een belangrijk gebied. Het voedsel voor zowel vogels als vissen bestaat voornamelijk uit macrozoobenthos. Dit zijn alle organismen groter dan 1 mm, zoals schelpdieren, wormen en slakken. Om het belang van dit macrozoobenthos te begrijpen, is het noodzakelijk deze in eerste instantie in kaart te brengen.

Dankzij financiële steun van de Nederlandse Aardolie Maatschappij (NAM) en het NWO zee- en kustonderzoek (ZKO), is het NIOZ in 2008 begonnen met een voorgenomen langjarige synoptische macrozoobenthos bemonstering van alle litorale gebieden in de Waddenzee, genaamd SIBES (Synoptic Intertidal Benthic Survey). Het doel van deze studie is om de variatie van macrozoobenthos in ruimte en tijd te kwantificeren en te onderzoeken of bodemdaling als gevolg van gasexploitatie een invloed op de macrofaun gemeenschap heeft. Deze studie beschrijft de resultaten van gegevens verzameld in 2009, en zal waar nodig, vergelijkingen maken met 2008. In 2009 zijn 4410 punten bemonsterd, waarin in totaal meer dan 385 duizend individuen zijn waargenomen en geteld. Deze individuen behoorden tot 93 soorten. Uitgedrukt in biomassa (berekend door middel van asvrij drooggewicht (AFDM)), waren de belangrijkste soorten de kokkel, zandkokerworm, strandgaper, gewone zeepier, Amerikaanse zwaardschede, mossel, veelkleurige zeeduizendpoot, Japanse oester en nonnetje. Voor elke soort is de ruimtelijke verspreiding in kaart gebracht. Ook kan op grond van deze gegevens de soortenrijkdom per monster voor het hele litorale gebied van de Waddenzee berekend worden. Dit laat zien dat met name de hooggelegen gebieden (zoals de zone langs te Nederlandse kust en de Waddeneilanden), maar ook het gebied ten oosten van Griend, het rijkst zijn.

Uiteindelijk zijn deze data ook gebruikt om veranderingen in de gebieden te registreren waar nu of in de toekomst gas exploitatie (zal) plaatsvinden. Het gebied ten oosten van Ameland met een bodemdaling tussen de 2-3cm laat een grotere afname zien van *Alitta virens*. Het gebied nabij 'Moddergat-Lauwersoog-Vierhuizen' wordt gekenmerkt door een relatief grotere afname van *Bathyporeia sarsi*. Als we een vergelijking maken tussen alle gebieden gekenmerkt door bodemdaling met de rest van de Waddenzee, laten de bodemdalingsgebieden een relatief grotere afname van *Urothoe poseidonis* zien, maar een relatieve toename van *Magelona johnstoni*. Het gebied ten oosten van Ameland met 1-3cm bodemdaling liet een grotere toename van *Heteromastus filiformis* zien. Omdat er een zeer groot aantal tests zijn uitgevoerd en vanwege het feit dat dergelijke af- of toenames niet worden waargenomen in alle bodemdalingsgebieden, lijken deze resultaten er niet op te wijzen dat dergelijke veranderingen het gevolg van gasexploratie zijn.

De uitdaging is nu om de data van dit synoptische litorale bemonsteringsprogramma te gebruiken om een beter beeld te krijgen van het effect van alle natuurlijke en menselijke processen in de Waddenzee (zoals droogvalduur, sediment type, en eventueel menselijke activiteiten) en hoe dit de verspreiding en demografie van deze soorten beïnvloed.

0. Preface

In 2007, the Nederlandse Aardolie Maatschappij (NAM) requested NIOZ to monitor the macrozoobenthos in the Dutch Wadden Sea to detect any spatial and temporal changes which may result from natural gas exploitation. The first synoptic sampling program took place in 2008, which was preceded by a relative small scale monitoring program in 2006 (Kraan et al., 2007a). Sampling in the western Wadden Sea was partly funded through the NWO Sea and Coastal Research (ZKO) program, while all remaining samples (collected in the eastern Wadden Sea and part of the western Wadden Sea) were funded by the NAM and NIOZ.

This report will describe both the 2008 (see Aarts et al. 2010) and 2009 data which was collected to carry out the long-term assessment that will take place in the upcoming years with a 5 year evaluation in 2012. Using this data, the areas of expected subsidence will be characterized in terms of macrozoobenthos abundance and we will investigate how it has changed between these two years. Finally, we will introduce the methodology that will be used in future years to conduct the impact assessment. This assessment will be repeated annually. Future reports will use an identical structure, but will incorporate any improvements such as those suggested by the audit commission in the preceding year.

1. Introduction

The Wadden Sea is of paramount importance to wildlife. Millions of migrating birds visit this area annually to overwinter, refuel or breed (Kam et al., 1999). The ecological importance of the Wadden Sea has led to its protection under the conventions of Ramsar, Bonn and Bern and European guidelines, such as the 'Habitat- en vogel-richtlijn'. Recently it has been designated as an UNESCO world heritage site.

The richness of the Wadden Sea is directly attributable to the high production and standing stocks of macrozoobenthos. Macrozoobenthos are all large (>1mm) animals such as worms, crabs, snails and bivalves that live in marine soft sediments. They not only play a prominent role as food source for many bird species, but they are also the major consumer of primary production in the water and on the sea bottom (Dekker, 1989; Herman et al., 1999). To help conserve and restore the richness of the Wadden Sea food web, it remains essential to accurately assess the status and changes of this community.

The macrozoobenthos community consists of many species, each of which occupies a narrow environmental niche, defined by variables such as sediment type and inundation time. Several studies have already indicated that changes in the environmental conditions (e.g. due to human activities (Kraan et al., 2007b)), can lead to changes in abundance, growth and reproduction of at least some species of the macrozoobenthos community (van der Meer, 1991; Zajac et al., 2000). This makes macrozoobenthos a suitable bio-indicator to assess changes in the Wadden Sea.

The major objective of this study is to measure the abundance, composition and development of macrozoobenthos in the intertidal Dutch Wadden Sea and to investigate if natural gas exploitation influences those characteristics.

2. Methods

2.1 Macrozoobenthos field sampling

Within the Dutch Wadden Sea study area of 1500 km^2 (intertidal area) a total of 4771 intertidal sites were sampled in June – October 2009, however some were too deep. Details on the 2008 sampling program can be found in Aarts et al. 2010. Of these stations, 4227 were placed on a regular 500 m grid and an additional 544 were randomly placed along the gridlines connecting the sampling stations. This survey design also allows for the estimation of spatial processes at distances < 500 m, but still maintains the regular sampling design with which species distributions maps can be generated with high precision (Bijleveld et al., 2011). At each site, 0.0175m^2 and 0.018m^2 was sampled on foot or by boat (Figure 1), respectively, up to a depth of 20-25cm. For molluscs, a distinction was made between the upper (less than 4 cm deep) and lower (4cm or deeper) part of the sample. The sampling cores were sieved over a 1 mm mesh and all species that could be identified in the field, were recorded. Mollusks were collected and stored at -20 °C for later analyses in the laboratory (Kraan et al., 2010; Kraan et al., 2007b; Piersma, 1993; van Gils et al., 2005; van Gils et al., 2006). The remaining sample was stored in plastic containers containing a 4% formalin solution.

Figure 1 Sampling by boat (left) and on foot (right).

2.2 Worms and amphipod Lab-work

In the lab, the rose Bengal dye (C.A.S. no. 632-68-8) was added to the sample, which will only stain the protein containing worms, amphipods, bivalves and snails. After 24 hours, the samples were flushed with fresh water (for 10-20 minutes) over a 0.5 mm sieve to remove any remaining formalin. Next, using tweezers, all stained organisms were removed from the grit and sediment, placed in a container and topped up with a 6% formalin solution. At a later stage, all species in each sample were identified using a binocular (8-40 times magnification) and classified according the taxonomic rules outlined in Hartmann-Schröder (1996) and Hayward and Ryland (1995). In 2009, some taxonomic changes have taken place leading to some changes in species names (see Table 1). All individuals were counted and individuals from the same species were placed together in aluminum oxide or ceramic cups. Next these cups were dried at 60° C for 48 hours, cooled in a desiccator (i.e. moist free), incinerated for 5 hours at 560° C and again cooled in the desiccator. Prior to incineration and after the final cooling stage, the cups were weighed with a precision of 0.0001 g. The difference between these two results in the Ash Free Dry Mass (AFDM).

Table 1. Taxanomic changes between 2008 and 2009.

Species code	Old species name (2008)	New species name (2009)
5	*Harmothoe sarsi*	*Bylgides sarsi*
11	*Nereis diversicolor*	*Hediste diversicolor*
12	*Nereis succinea*	*Alitta succinea*
13	*Nereis virens*	*Alitta virens*
14	*Nereis longissima*	*Eunereis longissima*
15	*Nereis sp.*	*Nereide sp.*
43	*Stenelais boa*	*Sthenelais boa*
46	*Hydrobia ventrosa*	*Ventrosia ventrosa*
62	*Ensis americanus*	*Ensis directus*
70	*Gammarus salinus*	*Gammarus locusta*
106	*Molgula tubifera*	*Molgula socialis*
112	*Bathyporeia tenuipes*	*Bathyporeia elegans*
126	*Ophiura texturata*	*Ophiura ophiura*
127	*Ophiura albida*	*Psammechinus miliaris*
148	*Chaetogammarus marinus*	*Gammarus marinus*
164	*Harmothoe ljungmani*	*Malmgreniella ljungmani*

2.3 Bivalve and snails processing

The day prior to the processing of the bivalves, plastic bags were removed from the freezer. The following day, the bivalves species were identified (see Hayward and Ryland (1995)) and a record was made from which part (top (T), bottom (B) or hydrobia (H)) the individual is from. Next the length of each bivalve was measured at a precision of 0.01 mm. For *Macoma balthica* also the shell height was measured (at that same precision) and the inner and outer shell color was recorded. For *Hydrobia ulvae,* only the length was measured (0.5 mm precision). For bivalves larger than 8mm, the flesh was removed from the shell and placed in aluminum oxide or ceramic cups. Bivalves smaller than 8 mm were placed in the cups whole. Individuals of the same size and smaller than 8mm were placed in the same cup. Finally, all cups were dried, incinerated and weighed similar to the worms. However, at the end of the process the cups were also weighed empty, which allows for the estimation of flesh weight.

2.4 Subsidence

Several natural gas extraction regions under or in the proximity of the Wadden are currently in use. The 'Ameland-oost' region is in production since 1986. The 'Moddergat-Lauwersoog-Vierhuizen' region consists of a range of reservoirs, such as Lauwersoog oost, central en west, Moddergat, Vierhuizen-oost, but the subsidence is also influenced by regions further inland, such as Nes, Anjum, Ezumazijl and Vierhuizen-west. More details can be found in (NAM, 2005). Figure **Error! Reference source not found.** provides an overview of the total subsidence until 2009 and figure 3 shows the predicted subsidence between 2006 and 2009.

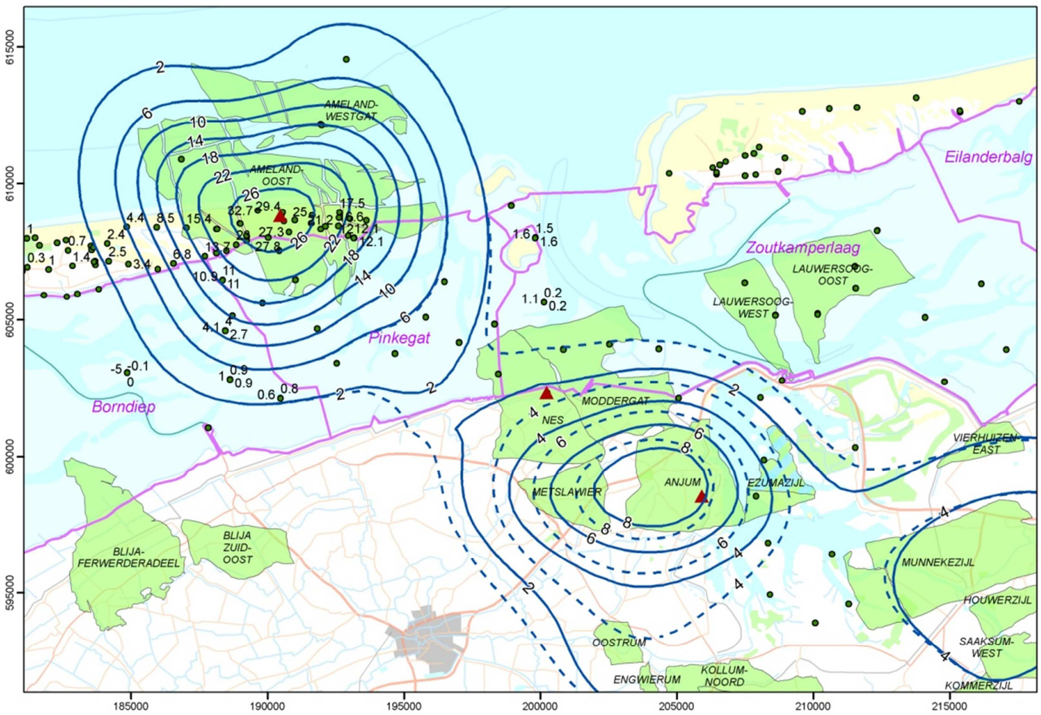


Figure 2 Predicted total subsidence (in cm) due to natural gas extraction from start of production until 2009. Blue lines illustrate the contours of the modeled subsidence. Dashed lines indicated modeled subsidence based on the 'old model parameters'. The green dots illustrate the height measurements taken from the start of the production until 2009. At three locations on top of the exploration areas Ameland-Oost, Nes/Moddergat and Anjum, continuous GPS measurements have been taken (red triangles).

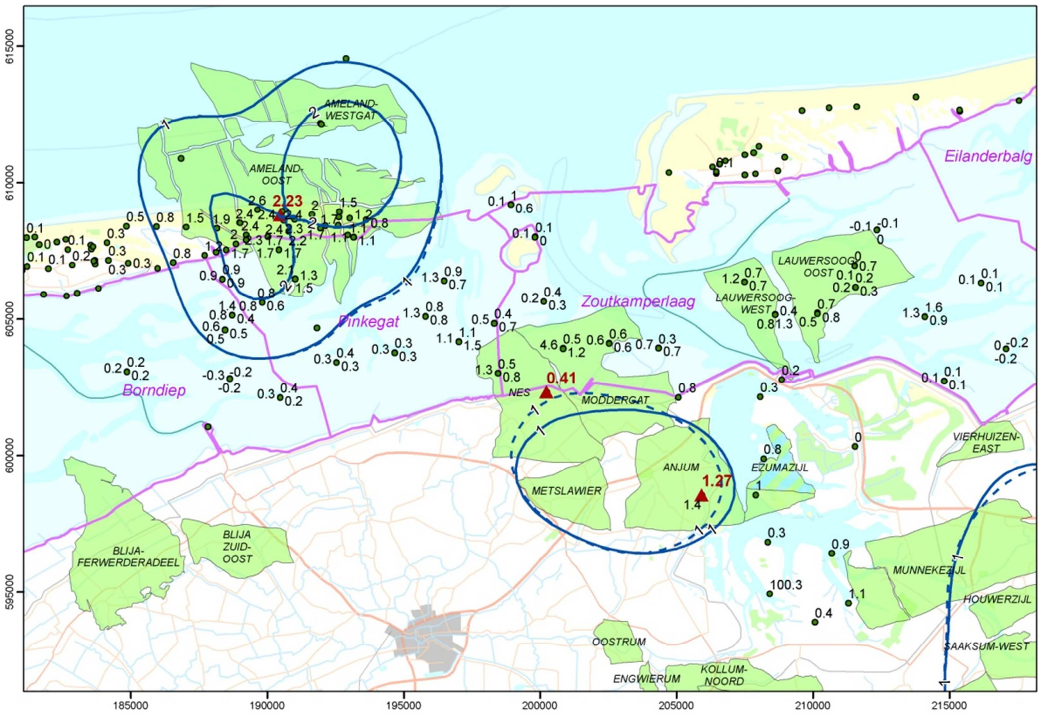


Figure 3. Predicted total subsidence (in cm) due to natural gas extraction from 2006 until 2009. Blue lines illustrate the contours of the subsidence based on the adapted and calibrated geo-mechanistic models. Dashed lines indicated modeled subsidence based on the 'old model parameters'. See for more details Fig. 2. These land-subsidence estimates are used for the analysis.

2.5 Physical, biological and human-related environmental variables

To understand the spatial preference of macrozoobenthos species, which will allow for the disentanglement between natural and human-related factors (such as land-subsidence), several environmental variables should be taken into account. The two most important drivers for benthos distribution are sediment type and inundation time (Compton et al., 2009; Kraan et al., 2010; Reise, 2002; Yates et al., 1993).

Inundation time is a function of the local elevation and water level, both of which vary in space and time. Ecocurves has developed a model which estimates inundation time by interpolating between a fixed set of tidal stations measuring water level every 10 minutes. ARCADIS has developed a hydrographic model. One major advantage of this model is that it does not require a linear extrapolation, but explicitly takes into account the geomorphology of the Wadden Sea. In addition to current speed and direction, the model estimates inundation time. Permission is requested to use this data.

Sediment data (Buchanan, 1984) was collected at 1000 m intervals at the location of a macrozoobenthos sample (see Figure 4). In 2009 and the following years, sediment data was and will be collected at each macrozoobenthos sampling station (i.e. every 500m and the random plus-points). At the sampling locations, 2-3 cores of the top 4 cm were taken using a

50ml plastic tube and stored in the freezer at –20 C. In the laboratory, prior to grain-size analysis, the sediment samples were freeze-dried for up to 96 hours till dry. Depending on the estimated grain size, between 0.5 and 5 grams of homogenized sample was weighed over a 2 mm sieve, in 13 ml PP Auto-sampler tubes. RO water was added and the sample was shaken vigorously on a vortex mixer for 30 seconds. Median particle size and the percentage silt (fraction < 63 µm) of sediments were determined using a Coulter LS 13 320 particle size analyzer and Auto-sampler. This apparatus measured particle sizes in the range of 0.04–2,000 µm in 126 size classes, using laser diffraction (780 nm) and PIDS (450 nm, 600 nm and 900 nm) technology. The optical module 'Gray' was used for the calculations.

Depth data (Figure) is collected by the RWS, based on a dense grid of sampling points ('vaklodingen') and converted into an elevation map by NAM (NAM 2008; EP200905260877). In 2010 a new elevation map was generated (NAM 2010; EP201005301455) and in 2011 this map will be updated by including improved RWS Lidar data. This map will be included in next year's assessment

Also human related covariates, such as manual 'fishing' of edible Cockles could be taken into account, however this information is till recently collected at an insufficient spatial and temporal resolution to be of any use in the analysis .

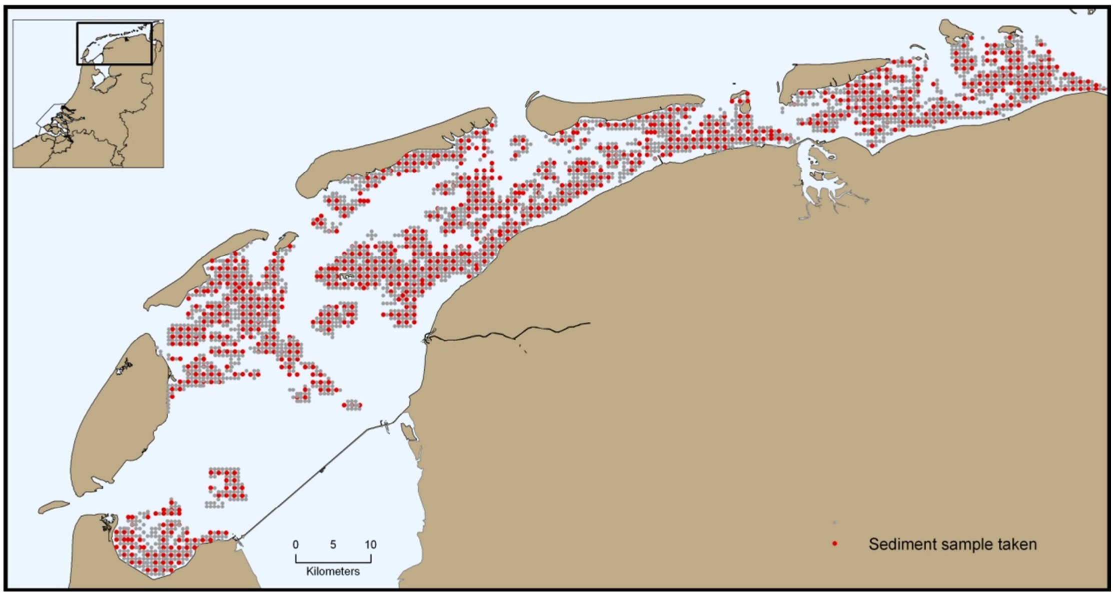


Figure 3 Spatial distribution of sampling stations at which sediment samples are taken in 2008. In 2009 sediment samples are taken at all stations.

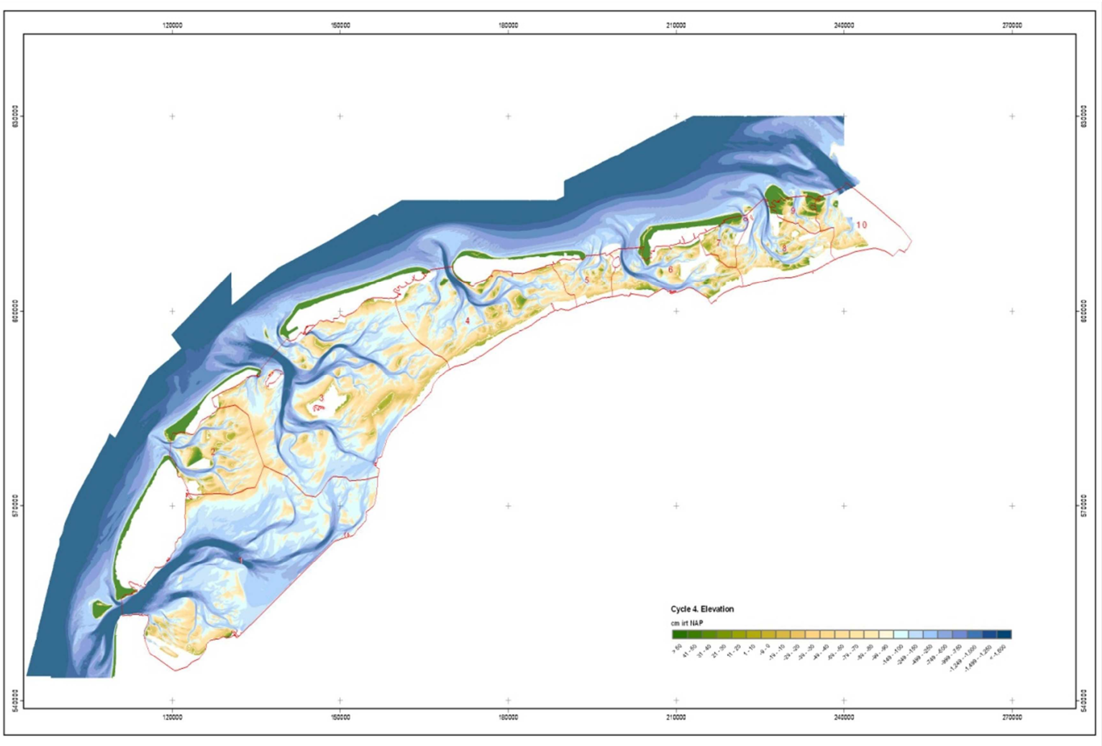


Figure 5 Depth based on RIKZ ‘lodingen’ 2005-2008 and NAM report EP200905260877

2.6 Assessment of the changes in species composition within and outside the area of future subsidence

Last year's assessment (Aarts et al., 2010) investigated whether the abundance of each species occurring in the areas of subsidence (due to gas exploitation), was out of proportion compared to regions elsewhere in the Wadden Sea. Such characterization is important, because the changes we may observe in the future for those species that are currently more or less abundant in the area of subsidence, may be different due to other (e.g. natural) processes. This report describes both 2008 and 2009 data, and therefore it is possible to investigate whether the *changes* in the macrozoobenthos communities inside the subsidence regions are significantly different from changes occurring elsewhere.

To carry out this analysis, we first need to classify each macrozoobenthos sampling point as either in- or outside the region of gas exploitation. In total there are three gas-exploitation sites; Ameland, 'Moddergat-Lauwersoog-Vierhuizen', and the north-east Groningen region. In all regions, the expected land-subsidence between 2006 and 2009 is less than 2cm, except for Ameland. The intertidal areas near the Ameland gas exploration site, may exhibit land-subsidence of up to 3cm. Therefore, in the analysis five regions have been identified

A. **A2-3cm**: Ameland-oost region with 2-3cm subsidence
B. **A1-3cm**: Ameland-oost region with 1-3cm subsidence (i.e. so it includes region A).
C. **MLV**: 'Moddergat-Lauwersoog-Vierhuizen' (1-2cm subsidence)
D. **G**: 'Groningen' region (1-2cm subsidence)
E. **All**: All sites characterized by at least 1cm subsidence

Next, for each species we investigate whether the change in abundance in- and outside the region of subsidence is different. This is done by fitting a Generalized Linear Model (GLM) to the count data. The response data is defined as the number of individuals for each species and is assumed to be quasi-Poisson distributed, which allows for possible under- or over dispersion.

The covariates included are year, 'in- or outside' (the area of subsidence) and the interaction between these two. All these covariates are treated as factors. If year is significant, it means there is a significant difference between 2008 and 2009. If the factor 'in- or outside' is significant, it means the species in question is more or less abundant. If the interaction is significant, this means that the *change* in abundance between years is different for the region characterized by land-subsidence.

Now this approach would be sufficient if the data from all sampling stations are independent from one another. Due to large scale spatially correlated natural processes, such as current velocity, inundation time, sedimentation, but also bird predation, the distribution of macrozoobenthos will also be spatially autocorrelated (Kraan et al., 2009), and hence the sampling points cannot be treated as independent. The consequence is that we will most often (perhaps incorrectly) conclude that the area of subsidence is significantly different. To account for this, two approaches exist.

One approach entails the incorporation of the (residual) spatial correlation into the model by assuming that the variance between sampling points increases with distance (e.g. by incorporating an exponential correlation function). Currently this approach is still computationally intensive (Diggle and Ribeiro Jr., 2007; Diggle et al., 2003; Diggle et al., 1998). Furthermore, it requires a correct specification of all spatial dependences, e.g. by including all relevant environmental drivers, such as sediment type and inundation time. This approach may be feasible in future assessments, but such methods are presently still in development. An alternative approach is to draw conclusions based on so-called Monte-Carlo simulations. This approach is applied in this study and works as follows.

First we randomly select a different region in the Wadden Sea consisting of a cluster of a similar number of sampling stations and fit a GLM as specified above. This model contains four components; an intercept, a year effect, a region effect (i.e. in or out-side the region of subsidence) and the interaction between the latter two. Using ANalysis Of VAriance (ANOVA) we can assess whether adding the interaction leads to a significant reduction in the explained deviance. For a quasi-Poisson GLM, the F-statistics is most appropriate (Hastie and Pregibon, 1992) and is extracted for each simulation.

So in other words, we construct random regions in the Wadden Sea as if subsidence would occur and investigate if the changes in species abundance between 2008 and 2009 are different from regions elsewhere (see Figure for some examples). We repeat this procedure 1000 times, resulting in an estimate of the F-distribution obtained through simulations. This is repeated for each species. Now it is possible to compare the F-value based on the correct assessment, with those attained through the simulations. If both the p-value from the correct GLM suggests a significant effect (α=0.01) of the interaction between 'in- or outside' and 'year', *and* if such a large absolute F-value rarely occurs in the simulations (α=0.01), there is strong evidence that the change in abundance of the species of interest is indeed different in that region. Figure 12 and 13 provide the histogram of the simulated F-distribution and true F-value for a random selection of species and Table 4 provides the summaries for all species.

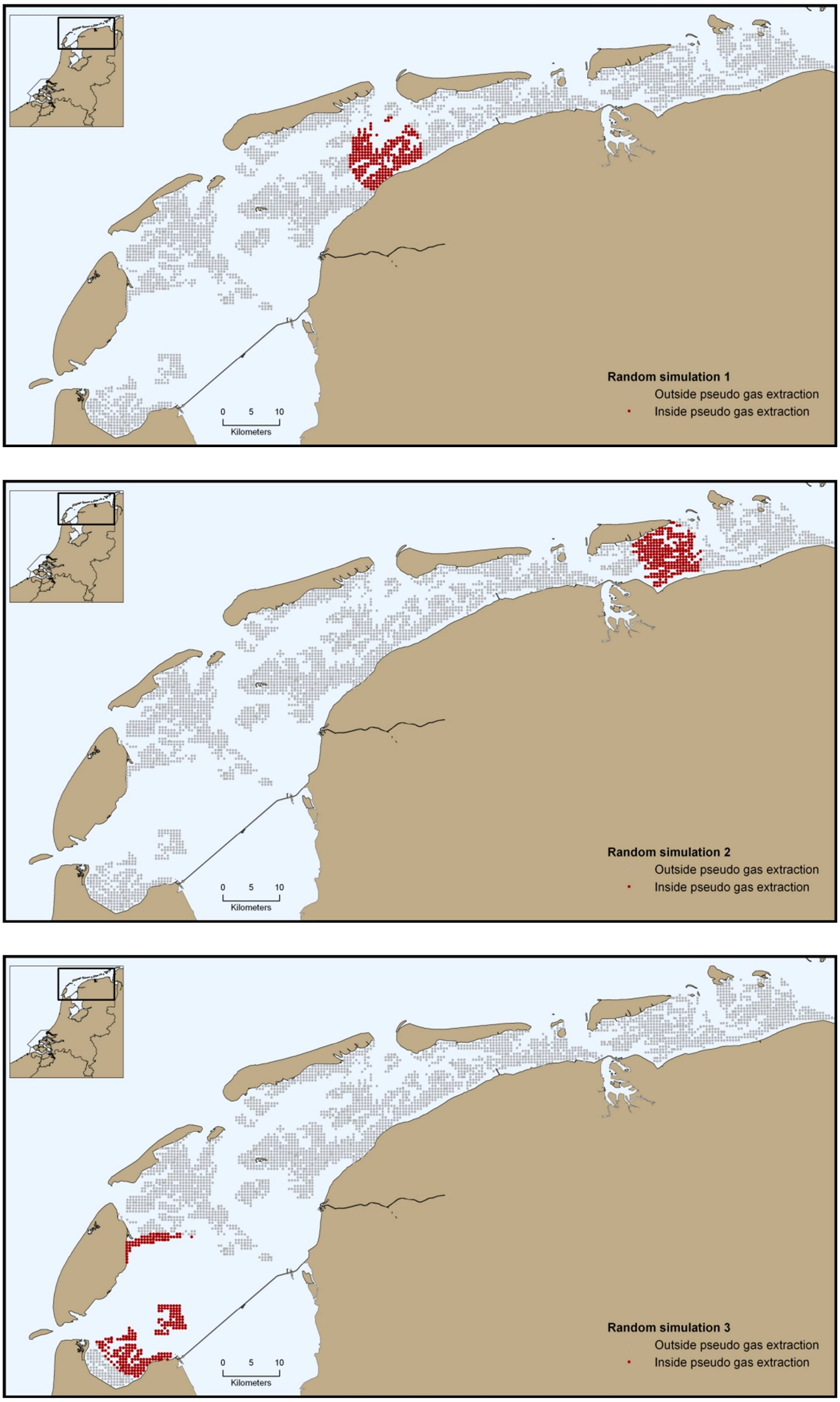


Figure 6 Three randomly generated pseudo gas extraction regions. The pseudo regions are constructed by randomly selecting a sampling point in the Wadden Sea and selecting the 311 nearest sampling station.

2.8 Future framework for assessing changes in macrozoobenthos

The sampling campaign of 2008 presented in Aarts et al. (2010), only considered the 2008 status of the gas exploitation region and how it relates to other regions in the Wadden Sea. The assessment presented here investigates whether changes in the gas exploitation are out-of-proportion compared to changes elsewhere. If strong changes occur, an investigation into how these changes occur need to take place. Therefore, the upcoming assessment will consist of two phases.

1. *Are changes in gas exploitation out-of-proportion compared to changes elsewhere?*
2. *If yes, are the observed changes most likely caused by natural gas exploitation or could they be due to other natural or human-related processes?*

1. Are changes in gas exploitation out-of-proportion compared to changes elsewhere?

In the assessment described above the parameters of interest are species-specific abundances. Because the sampling stations in 2008 and upcoming years will be positioned at the same geographic location, one can calculate for each species the change in abundance. Similar to the framework described above, a Generalized Linear Model can be used to investigate if the change in abundance is different in- or outside the region of subsidence, and one can test if such changes do not occur elsewhere. See details above.

2. What are the causes of these changes?

When the changes within the area of gas exploitation are out of proportion compared to regions elsewhere in the Wadden Sea, it may still be possible that these are the result of natural or human-related events that 'accidentally' happened within that region. To tackle this question, the first challenge is to quantify which physical, biological and human related variables influence the distribution of macrozoobenthos. Substantial progress has already been made using the macrozoobenthos data collected in the Western Wadden Sea in previous years (Kraan et al., 2010), and considerable improvements are expected using the synoptic sampling grid presented here. Using such habitat models, it is possible to predict the density of animals in space (and maybe time) and to compare these with the actual observations. If the deviations between model predictions and observation resemble the intensity of subsidence and no other relevant variables, there is strong evidence that it has an effect.

3. Results

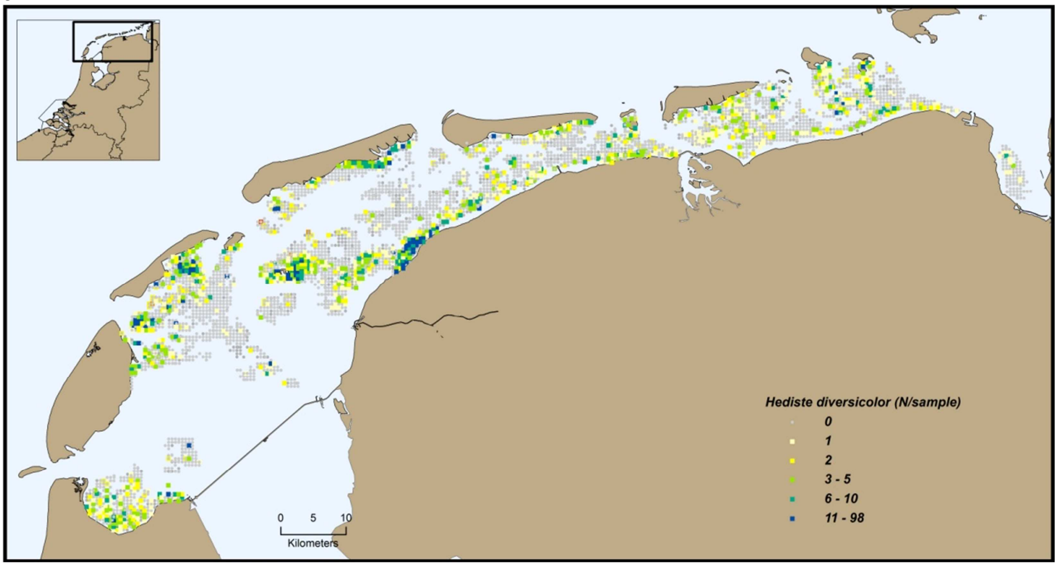
3.1 Sampling effort

In total 4376 stations were visited in 2008 and 4771 in 2009. In 2009 the Wadden Sea sampling program was extended with the Eems-Dollard intertidal zone. Of all samples in 2009, 349 where too deep (> 220m) and could not be sampled. If macrozoobenthos was present, a sample was stored (together with a plastic identification code, i.e. PosKey), for future laboratory analysis. This resulted in a total of 4410 samples that could be used for the analysis, 544 of which were positioned on the random plus points. Based on these samples, more than 385,000 individuals were individually counted and measured.

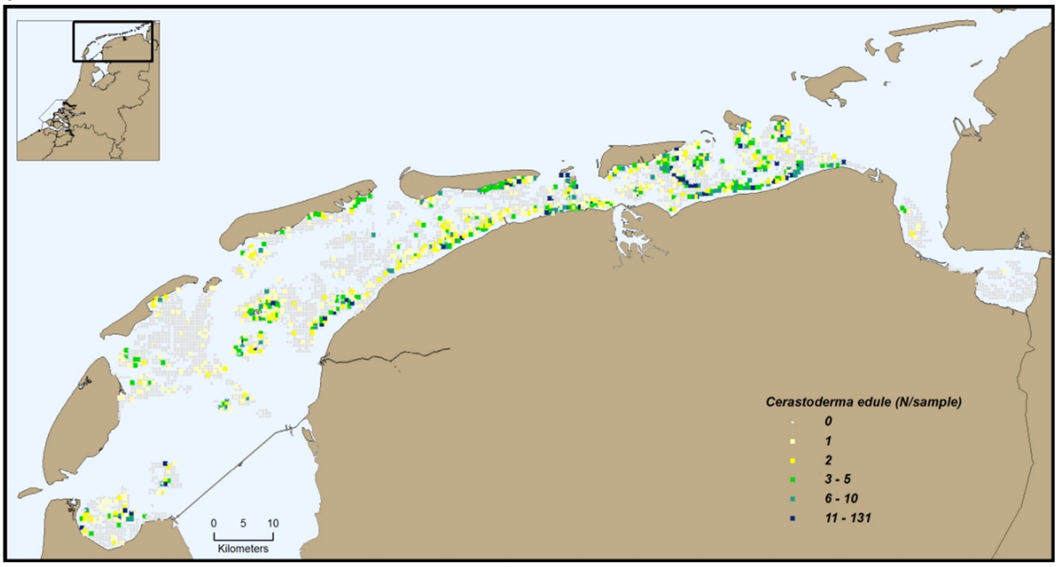
3.2 Species specific Abundances and biodiversity measurements

In both years combined, a total of 93 different species or genera have been identified. See Kraan et al. (2007) for a description of the most species. Table 2 provides for each species the number of individuals observed in the combined set of samples and Table 3 shows the estimated number of individuals and biomass in the entire Wadden Sea.

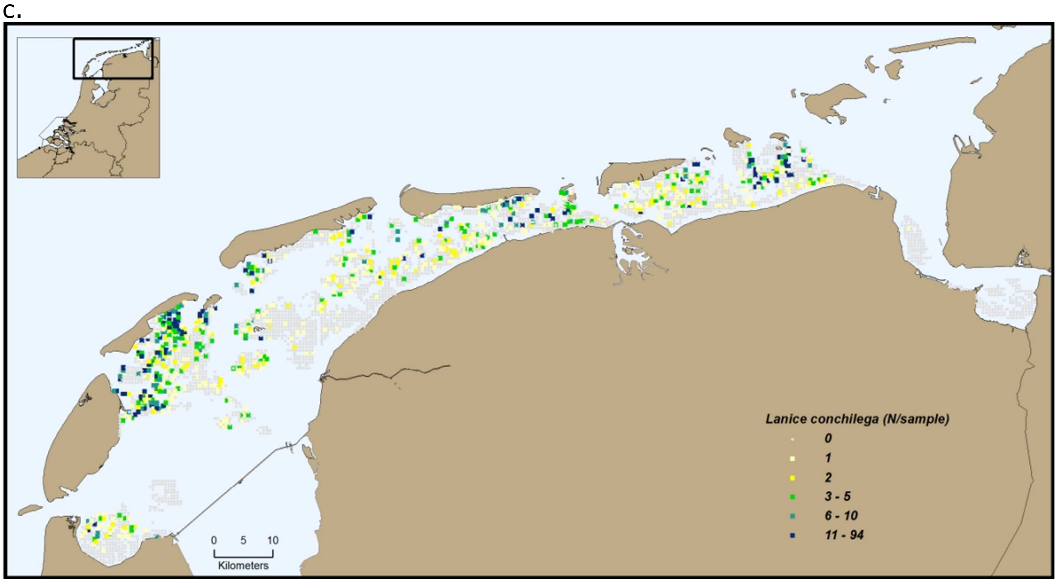
Figure 4 shows the spatial distribution in 2009 of some important species Cockle (*Cerastoderma edule*), Sand mason (*lanice conchilega*), soft-shell clam (*Mya arenaria*), Lugworm (*Arenicola marina*), American jack knife clam (*Ensis americanus*), Blue mussel (*Mytilus edulis*), ragworm (*Hediste diversicolor*), Pacific giant oyster (*Crassostrea gigas*), Baltic tellin (*Macoma balthica*), bristleworm (*Scoloplos armiger*), Laver spire shell (*Hydrobia ulvae*) and a few rare species; bean-like tellin (*Tellina fabula)* and thin tellin (*Tellina tenuis)* a.


Hediste diversicolor (N/sample)
0
1
2
3 - 5
6 - 10
11 - 98
0 5 10
Kilometers

b.


Cerastoderma edule (N/sample)
0
1
2
3 - 5
6 - 10
11 - 131
0 5 10
Kilometers

c.


Lanice conchilega (N/sample)
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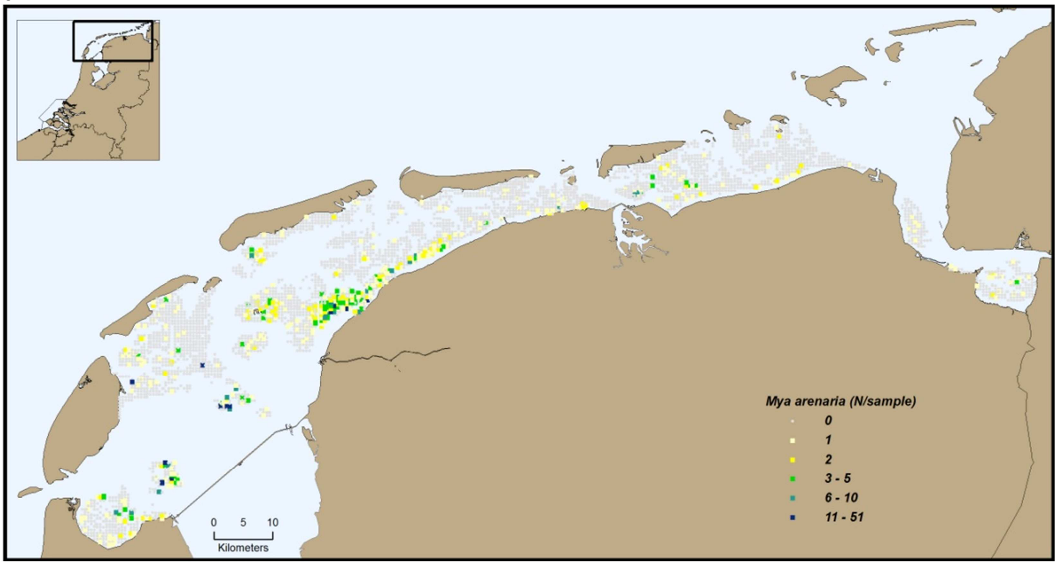
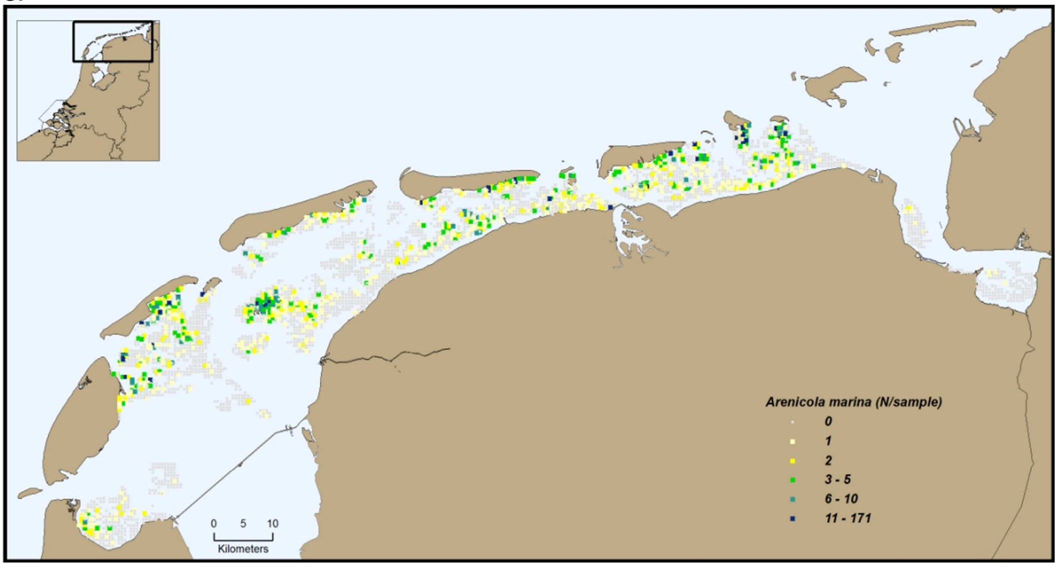
1
2
3 - 5
6 - 10
11 - 94
0 5 10
Kilometers

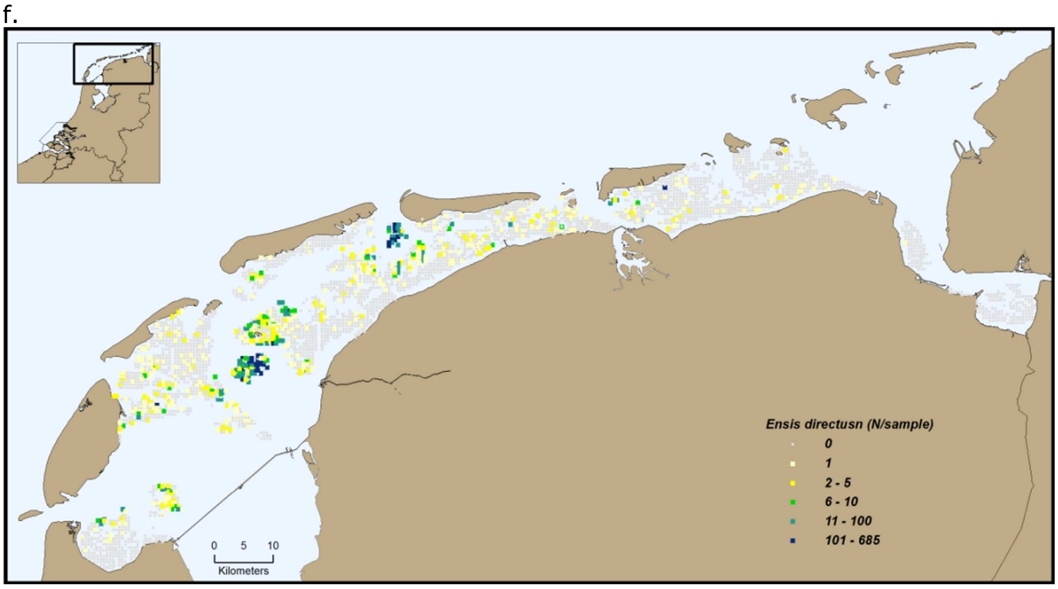
d.


Mya arenaria (N/sample)
0
1
2
3 - 5
6 - 10
11 - 51
0 5 10
Kilometers

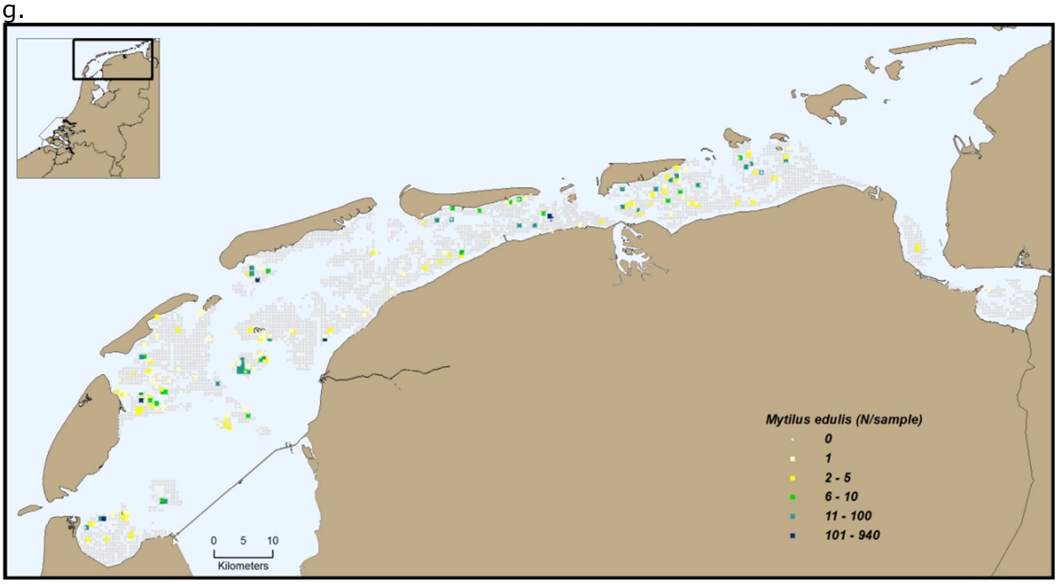
e.


Arenicola marina (N/sample)
0
1
2
3 - 5
6 - 10
11 - 171
0 5 10
Kilometers

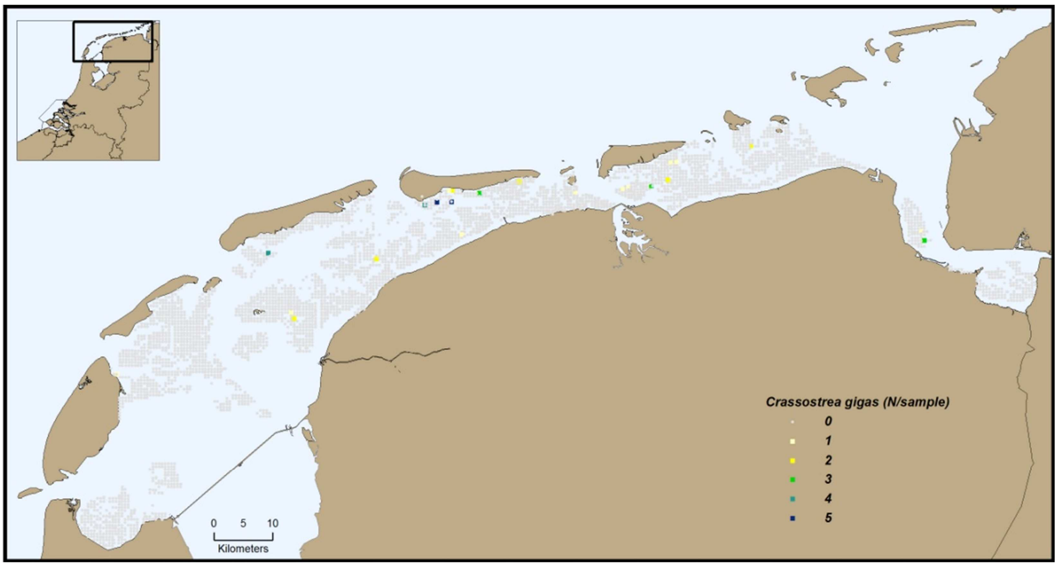
f.


Ensis directusn (N/sample)
0
1
2 - 5
6 - 10
11 - 100
101 - 685
0 5 10
Kilometers

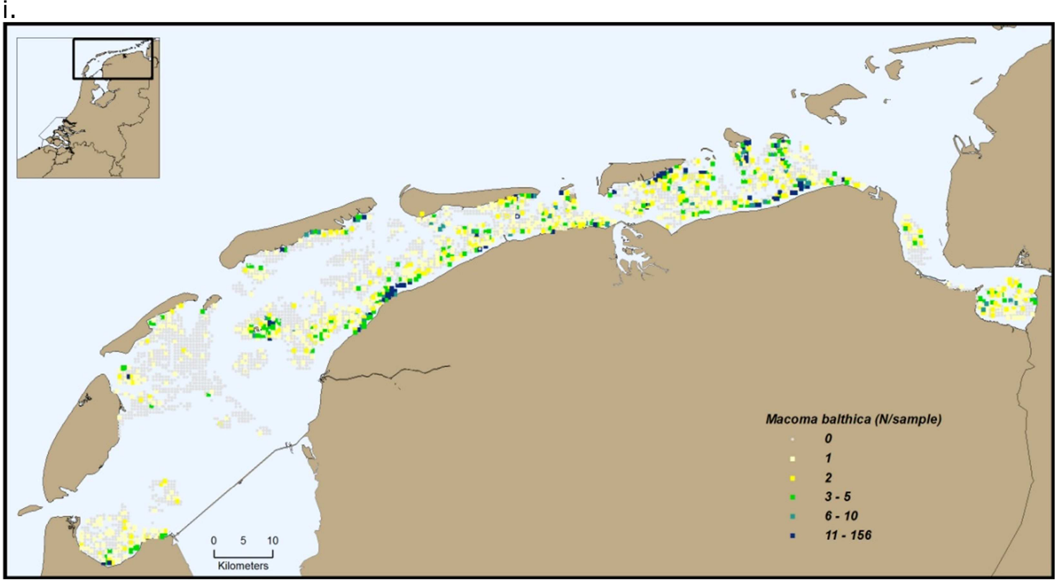
g.


Mytilus edulis (N/sample)
0
1
2 - 5
6 - 10
11 - 100
101 - 940
0 5 10
Kilometers

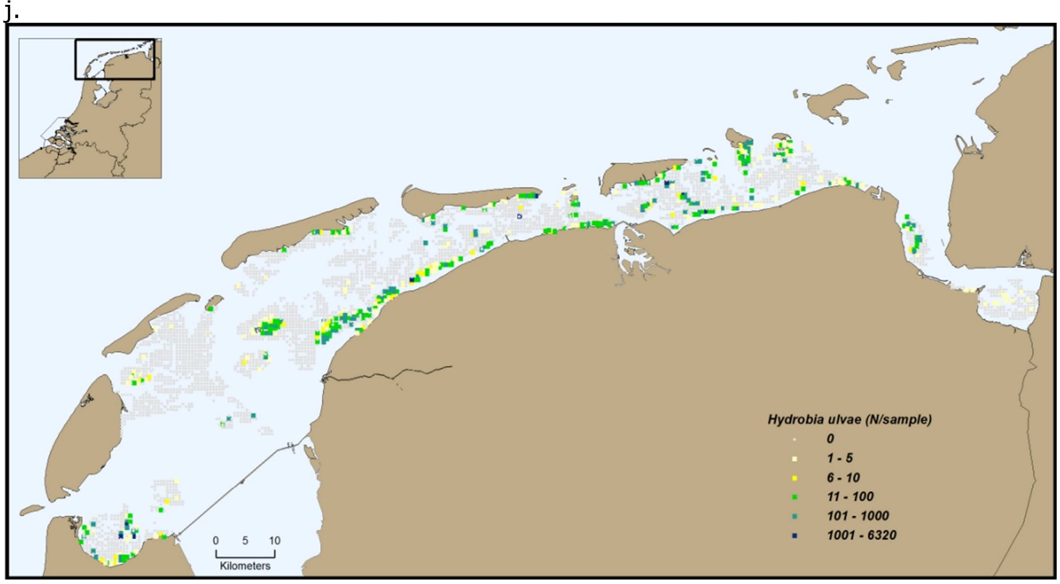
h.


Crassostrea gigas (N/sample)
0
1
2
3
4
5
0 5 10
Kilometers

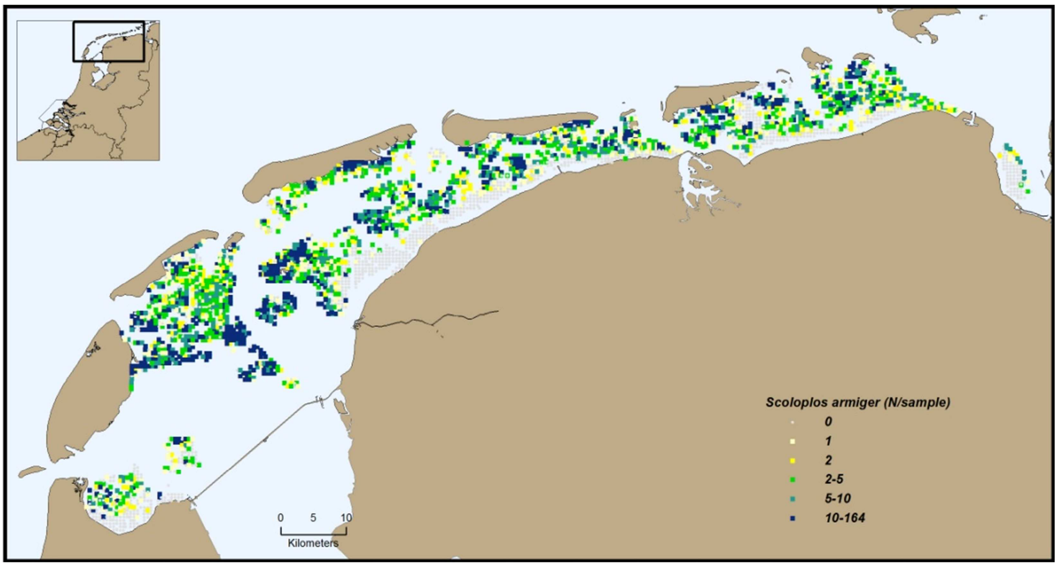
i.


Macoma balthica (N/sample)
0
1
2
3 - 5
6 - 10
11 - 156
0 5 10
Kilometers

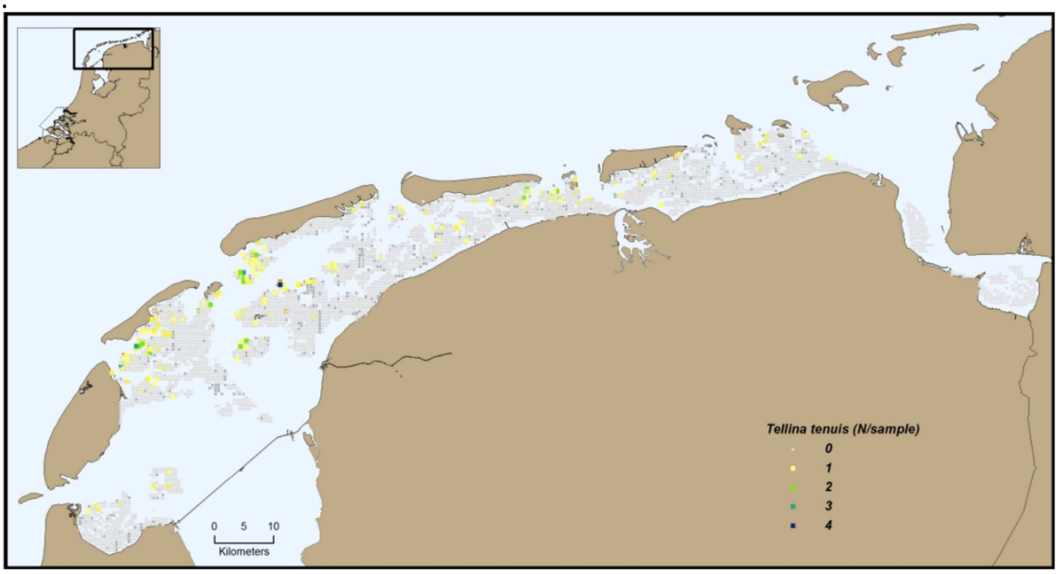
j.


Hydrobia ulvae (N/sample)
0
1 - 5
6 - 10
11 - 100
101 - 1000
1001 - 6320
0 5 10
Kilometers

k.


Scoloplos armiger (N/sample)
0
1
2
2-5
5-10
10-164
0 5 10
Kilometers

l.


Tellina tenuis (N/sample)
0
1
2
3
4
0 5 10
Kilometers

m.

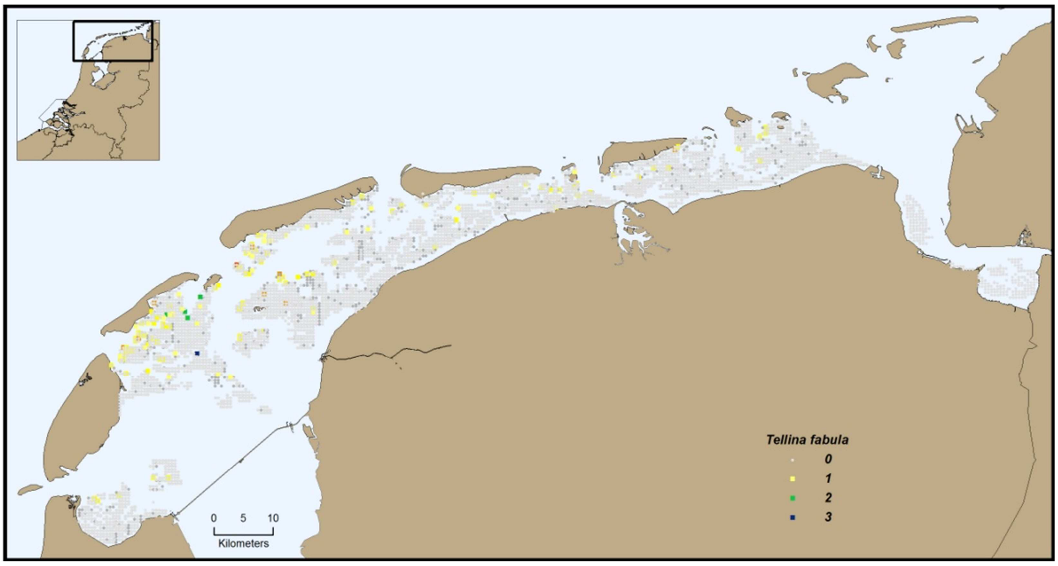
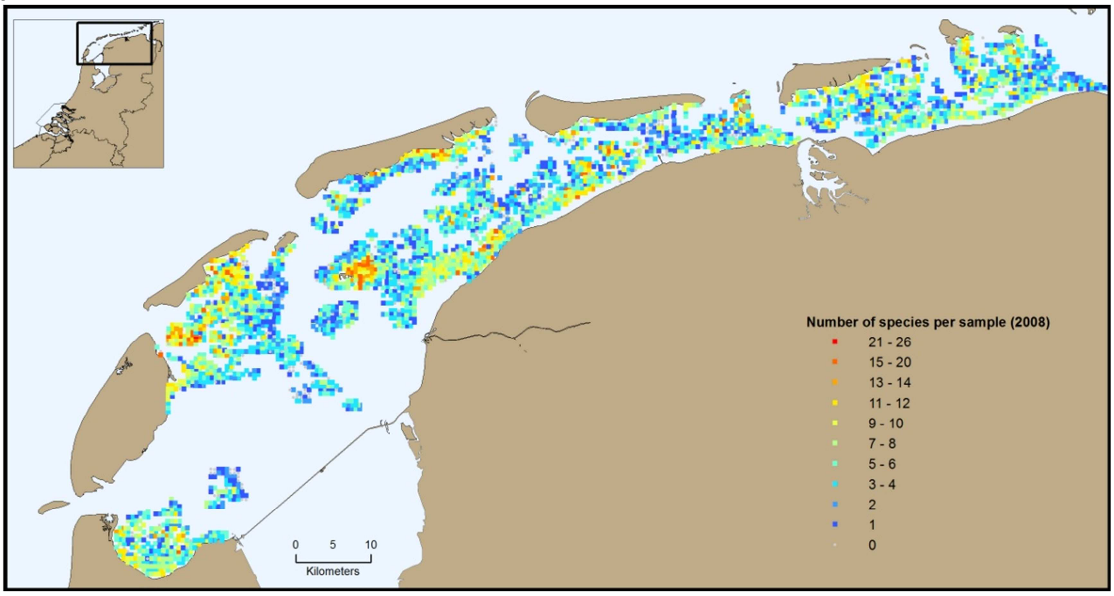


Figure 4 Spatial distribution in 2009 of macrozoobenthos species a. ragworm (*Hediste diversicolor*), b. edible cockle (*Cerastoderma edule*), c. sand mason (*Lanice conchilega*), d. soft-shell clam (*Mya arenaria*), e. lugworm (*Arenicola marina*), f. American jack knife clam (*Ensis americanus*), g. blue mussel (*Mytilus edulis*), h. Pacific giant oyster (*crassostrea gigas*), i. Baltic tellin (*Macoma balthica*), j. laver spire shell (*Hydrobia ulvae*), k. bristleworm (*Scoloplos armiger*), l. thin tellin (*Tellina tenuis)* and m. bean-like tellin (*Tellina fabula)*.

Figure 4 illustrates that in 2009 most species occur throughout the Dutch Wadden Sea, but they differ considerably in their local preference. Some species, such as Ragworm (*Hediste diversicolor*), Cockle (*Cerastoderma edule*), soft-shell clam (*Mya arenaria*), Baltic tellin (*Macoma balthica*) and Laver spire shell (*Hydrobia ulvae*) prefer the muddy, higher regions. Also most tidal divides, e.g. those running South from Schiermonnikoog, are clearly visible in the distribution of these species. Other species, e.g. thin tellin (*Tellina tenuis)* and bean-like tellin (*Tellina fabula)*, are distributed mostly on the edges of the tidal flats.

Based on the number of individuals per sample it is possible to estimate the species richness for different areas in the Wadden Sea (Figure 5).

a.



b.

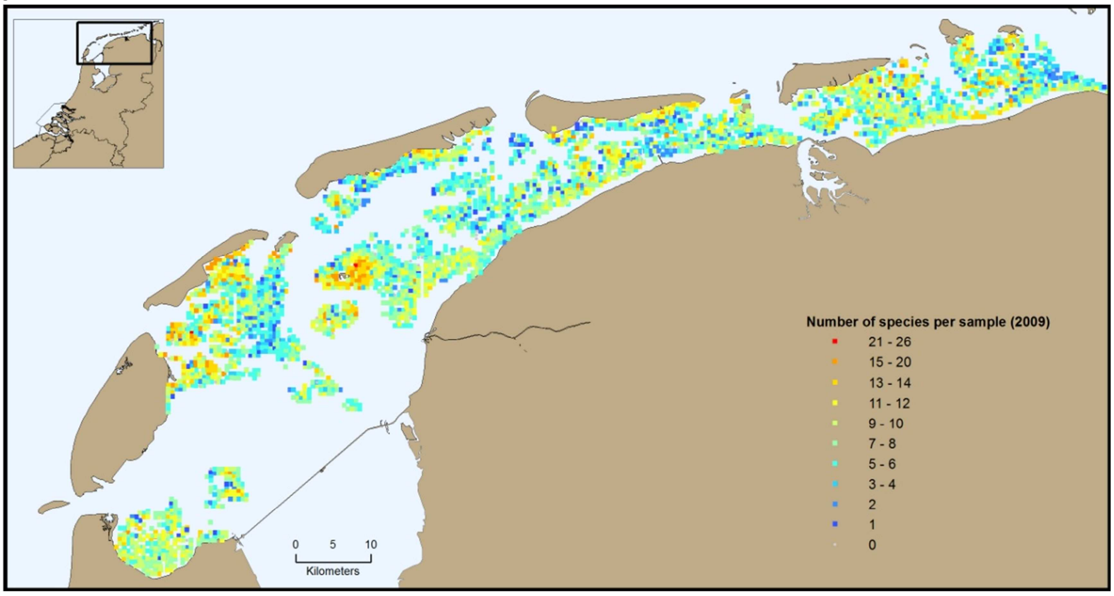


Figure 5. Distribution of number of species per sample in the Wadden Sea in 2008 (a) and 2009 (b).

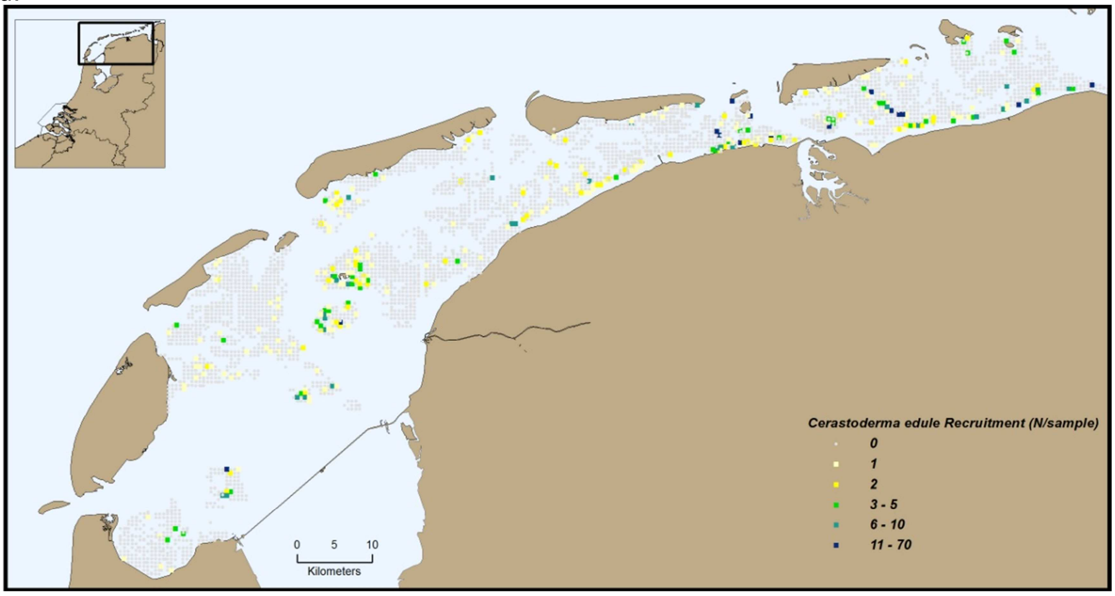
In general it appears that the samples taken at the higher tidal elevations within the Wadden Sea contain most species. This includes all areas close to the mainland and islands, but also the area north-east of the island Griend, around 'de Hengst' (between Texel and Vlieland) and 'Balgzand' (western Wadden Sea).

3.3 Length, weight and age measurements

For all mollusks, length, weight and age (by counting growth rings) measurements are made. For worms, only weight measurements are made. Such measurements become particularly useful when successive surveys are carried out, because it will allow for separate growth, recruitment and mortality estimates. For example, Figure 6 shows the recruitment of *Cerastoderma edule* (cockle) and *Macoma balthica*.

Finally, the weight measurements could be used to estimate the total biomass of that species in the Wadden Sea. Table 2 shows the total biomass in the sample (expressed in AFDM, which is approximately 10% of the total flesh weight). The species are sorted by their biomass in the total sample. Table 3 shows the estimated numbers and biomass in the intertidal area of the Dutch Wadden sea. It should be noted that some of the patchy distributed species of commercial interest, such as blue mussel (*Mytilus edulis*) and Japanese Oysters (*Crassostrea gigas*) may be better estimated using other existing species-specific stratified sampling schemes carried out by IMARES. The table (Table 2) shows that the Cockle, in terms of biomass, is the most important species. In terms of numbers of individuals, *Hydrobia* is most abundant.

a.



b.

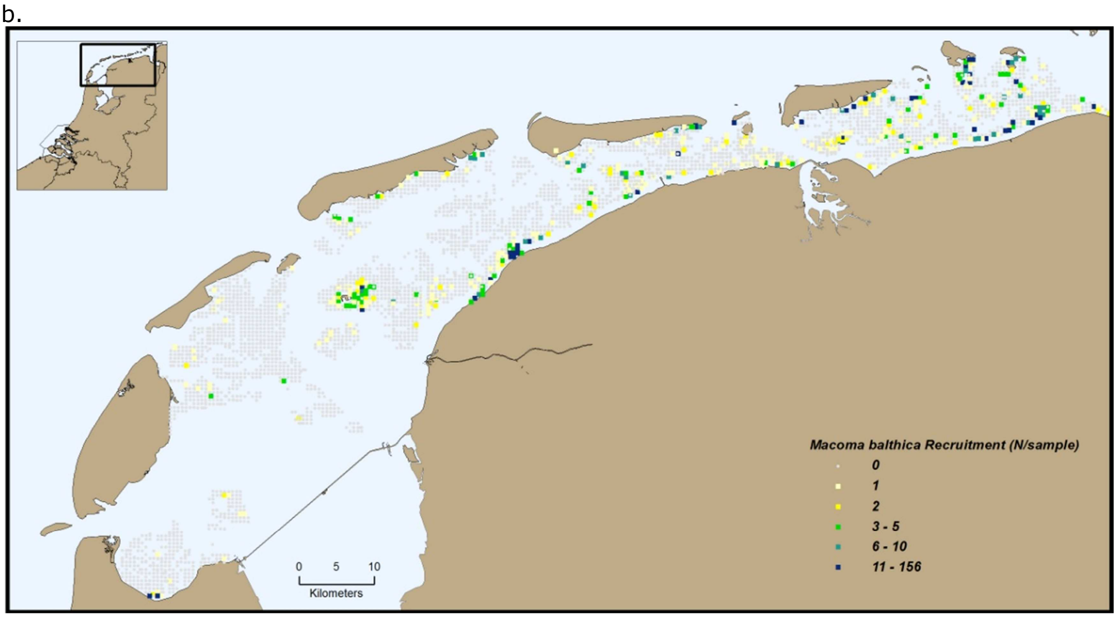


Figure 6 Spatial distribution of recruitment of *Cerastoderma edule* (a) and *Macoma balthica* (b) in 2009.

Table 2. Number and AFDM of each species in the sample. N tot. is the number of individuals counted, N weight the number of individuals weighed, Sum AFDM the total Ash Free Dry Mass in gram and AFDM/ind. is the average AFDM per individual. The number in brackets behind each year, represents the number of sampling stations on which these estimates are based.

	2008 (n=3914)				**2009** (n=4410)			
Species	***N tot.***	***N weight***	***Sum AFDM***	***AFDM/ind.***	***N tot.***	***N weight***	***Sum AFDM***	***AFDM/ind.***
Cerastoderma edule	3541	3515	449	0.1278	3930	3914	576	0.1471
Lanice conchilega	9394	9258	181	0.0195	5420	5384	109	0.0202
Mya arenaria	1581	1562	175	0.1118	1237	1201	228	0.1898
Arenicola marina	888	741	102	0.1374	3009	2760	184	0.0667
Ensis directus	1773	1770	81.1	0.0458	17112	8034	154	0.0192
Mytilus edulis	382	382	70.4	0.1844	3722	2427	134	0.0552
Crassostrea gigas	40	40	69.1	1.7280	49	49	64.4	1.3153
Hediste diversicolor	4186	4082	67.6	0.0166	5200	4955	69	0.0139
Macoma balthica	2299	2279	42.5	0.0187	4594	4236	55.4	0.0131
Scoloplos armiger	10755	10521	23.3	0.0022	25406	23439	36.5	0.0016
Alitta virens	257	244	19	0.0777	109	99	12.6	0.1277
Scrobicularia plana	204	201	18.2	0.0907	334	333	21.4	0.0643
Nephtys hombergii	937	821	17.4	0.0211	726	645	14.3	0.0222
Marenzelleria viridis	8705	8520	13.6	0.0016	11927	10538	16.7	0.0016
Carcinus maenas	478	471	10.8	0.0228	849	706	13.4	0.0190
Hydrobia ulvae	30428	13438	10.4	0.0008	76196	16823	11.5	0.0007
Alitta succinea	469	460	8.99	0.0195	2003	1920	12.1	0.0063
Eunereis longissima	357	344	4.64	0.0135	152	145	0.557	0.0038
Aphelochaeta marioni	20194	20099	4.33	0.0002	36173	33496	1.8	0.0001
Corophium sp.	8264	8254	3.35	0.0004	30742	29235	10.5	0.0004
Urothoe poseidonis	8801	8651	3.35	0.0004	13951	13230	4.69	0.0004
Oligochaeta sp.	16935	16549	3.2	0.0002	21198	18558	0.714	0.0000
Pygospio elegans	21792	21614	3.2	0.0001	84453	52429	2.72	0.0001
Crangon crangon	246	239	2.68	0.0112	266	264	2.36	0.0089
Tellina tenuis	123	121	2.66	0.0219	119	119	2.88	0.0242
Littorina littorea	52	48	2.37	0.0493	104	72	4.33	0.0601
Heteromastus filiformis	1035	914	2.25	0.0025	1533	1462	4.13	0.0028
Capitella capitata	6519	6450	2.18	0.0003	9743	8669	2.84	0.0003
Abra tenuis	1666	1666	2.16	0.0013	2028	1534	2.01	0.0013
Crepidula fornicata	19	19	1.66	0.0874	15	15	0.47	0.0313
Nephtys cirrosa	243	233	1	0.0043	200	192	1.13	0.0059
Polydora cornuta	3134	3116	0.99	0.0003	5158	2982	0.688	0.0002
Echinocardium cordatum	4	4	0.973	0.2433	3	3	1.07	0.3556
Petricola pholadiformis	13	13	0.907	0.0698	27	27	1.8	0.0668
Eteone longa	1017	1003	0.866	0.0009	6051	5608	3.07	0.0005
Nephtys caeca	137	129	0.741	0.0057	84	77	0.475	0.0062
Tellina fabula	30	30	0.54	0.0180	42	42	0.953	0.0227
Malmgreniella lunulata	203	195	0.469	0.0024	80	78	0.149	0.0019

Hemigrapsus takanoi	4	4	0.45	0.1126	7	7	0.221	0.0315
Nemertini sp.	57	56	0.365	0.0065	69	62	0.989	0.0160
Spio martinensis	2139	2130	0.344	0.0002	2464	1892	0.0939	0.0000
Sagartia troglodytes	15	15	0.332	0.0221	7	7	0.244	0.0349
Phyllodoce maculata	255	248	0.28	0.0011	270	243	0.154	0.0006
Gammarus spec.	228	224	0.263	0.0012	737	737	0.751	0.0010
Bylgides sarsi	45	42	0.212	0.0050	142	137	0.593	0.0043
Glycera alba	26	25	0.202	0.0081	6	4	0.0387	0.0097
Bathyporeia sarsi	525	524	0.19	0.0004	1187	1141	0.486	0.0004
Nereide sp.	215	135	0.175	0.0013	359	347	0.228	0.0007
Spiophanes bombyx	287	281	0.164	0.0006	236	211	0.261	0.0012
Phyllodoce mucosa	148	145	0.153	0.0011	1492	1398	0.9	0.0006
Pagurus bernhardus	4	4	0.138	0.0345				
Metridium senile	46	46	0.128	0.0028	70	70	0.145	0.0021
Streblospio shrubsolii	476	475	0.122	0.0003	232	224	0.042	0.0002
Scolelepis foliosa	14	13	0.117	0.0090	9	7	0.105	0.0150
Magelona johnstoni	83	82	0.0951	0.0012	30	29	0.0202	0.0007
Eumida sanguinea	146	144	0.0803	0.0006	217	215	0.103	0.0005
Pectinaria koreni	2	2	0.0675	0.0337	11	10	0.0234	0.0023
Nephtys longosetosa	10	10	0.0663	0.0066	12	11	0.0262	0.0024
Malacoceros fuliginosus	409	409	0.0639	0.0002	620	400	0.0666	0.0002
Lepidochitona cinerea	5	5	0.0598	0.0120	27	27	0.112	0.0042
Scolelepis bonnieri	19	19	0.056	0.0029	18	18	0.124	0.0069
Mysella bidentata	35	35	0.0429	0.0012	77	77	0.0708	0.0009
Mysta picta	34	34	0.0379	0.0011	5	5	0.0064	0.0013
Abra alba	10	10	0.0299	0.0030	34	34	0.134	0.0039
Pomatoschistus microps	1	1	0.0276	0.0276				
Magelona mirabilis	14	14	0.0239	0.0017	162	160	0.205	0.0013
Manayunkia aestuaria	392	392	0.013	0.0000	1502	604	0.0189	0.0000
Asterias rubens	2	2	0.0116	0.0058	45	45	0.122	0.0027
Travisia forbesii	3	2	0.011	0.0055	4	4	0.0019	0.0005
Nephtys spec.	6	4	0.0084	0.0021	34	28	0.0542	0.0019
Harmothoe imbricata	6	6	0.0071	0.0012	1	1	0.0073	0.0073
Autolytus prolifer	21	19	0.00438	0.0002	60	59	0.0047	0.0001
Aricidea minuta	13	13	0.0035	0.0003	16	16	0.0038	0.0002
Microphthalmus similis	11	11	0.003	0.0003	4	4	3.00E-04	0.0001
Harmothoe spec.	2	2	0.0024	0.0012	10	10	0.0513	0.0051
Retusa obtusa	6	6	0.0024	0.0004	63	63	0.0427	0.0007
Malmgreniella ljungmani	1	1	0.0016	0.0016				
Streptosyllis websteri	2	2	0.0011	0.0006				
Melita palmata	2	2	0.0011	0.0006	36	36	0.0161	0.0004
Phoxichelidium femoratum	2	2	0.001	0.0005				
Bodotria scorpioides	2	2	8.00E-04	0.0004	6	6	7.00E-04	0.0001
Jaera albifrons	4	4	8.00E-04	0.0002				

Eteone sp.	7	1	4.00E-04	0.0004	25	25	0.0157	0.0006
Polydora ciliata	2	2	4.00E-04	0.0002	2	2	3.00E-04	0.0001
Neomysis integer	1	1	2.00E-04	0.0002				
Tellimya ferruginosa	4	4	0	0.0000	1	1	6.00E-04	0.0006
Eulalia viridis	1	1	0	0.0000	2	2	4.00E-04	0.0002
Fish sp.	1	0	0					
Cerastoderma edule	3541	3515	449	0.1278	3930	3914	576	0.1471
Lanice conchilega	9394	9258	181	0.0195	5420	5384	109	0.0202

Table 3. Estimated total number of individuals and AFDM of each species in the intertidal Dutch Wadden Sea (excluding Eems-Dollard) for 2008 and 2009.

Species	N (billions)		Weight (thousand tons)	
	2008	2009	2008	2009
Cerastoderma edule	49.574	54.81	6.286	8.064
Lanice conchilega	131.516	75.866	2.534	1.54
Mya arenaria	22.134	16.912	2.366	3.15
Arenicola marina	12.432	41.944	2.016	3.556
Ensis directus	24.822	239.554	1.1284	2.478
Hediste diversicolor	58.604	68.936	1.0066	1.0108
Mytilus edulis	5.348	51.996	0.9856	1.974
Crassostrea gigas	0.56	0.63	0.9674	0.8722
Macoma balthica	32.186	61.292	0.595	0.756
Scoloplos armiger	150.57	354.564	0.3346	0.5334
Alitta virens	3.598	1.512	0.329	0.2212
Nephtys hombergii	13.118	9.982	0.2842	0.2422
Hydrobia ulvae	425.992	1036.63	0.2744	0.5866
Scrobicularia plana	2.856	4.676	0.2548	0.2996
Marenzelleria viridis	121.87	161.728	0.1918	0.2352
Carcinus maenas	6.692	11.844	0.1484	0.189
Alitta succinea	6.566	19.236	0.133	0.1414
Eunereis longissima	4.998	2.128	0.06706	0.008344
Aphelochaeta marioni	282.716	499.632	0.0609	0.02786
Oligochaeta sp.	237.09	287.154	0.04816	0.011998

2.5 Sediment type

In 2008, sediment samples were taken every 1000m. In contrast, in 2009 the sediment samples were taken at each sampling point. Laboratory analysis of the 2009 (and 2010) is not completed yet, and will be presented in the upcoming assessment. Figure 10, shows the distribution of the mean and median grain size in the Wadden Sea. The arrows indicate the grain sizes observed within the different gas exploration sites. The MLV region, which is closest to the Frisian coast, is characterized by a relative small grain size. The region south of Ameland shows large variability (Figure 11). The sediment near the eastern tip of Ameland is relative coarse. Going westwards, the sediment becomes finer and the area west of the Holwerd-Ameland ferry terminal is one of the muddiest places in the Wadden Sea, with mean and median grain sizes below 40 μm.

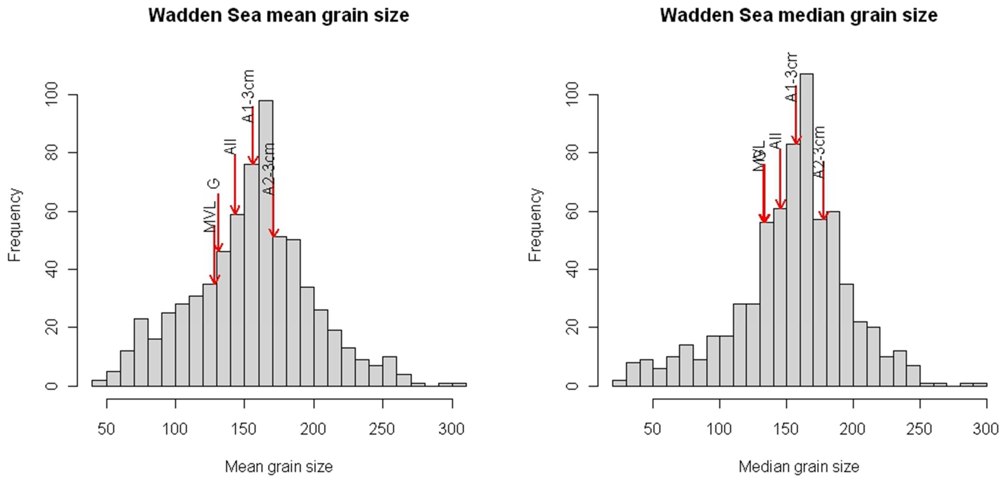


Figure 10 Distribution of mean and median grain size in the Wadden Sea and average grain sizes observed in the different subsidence regions.

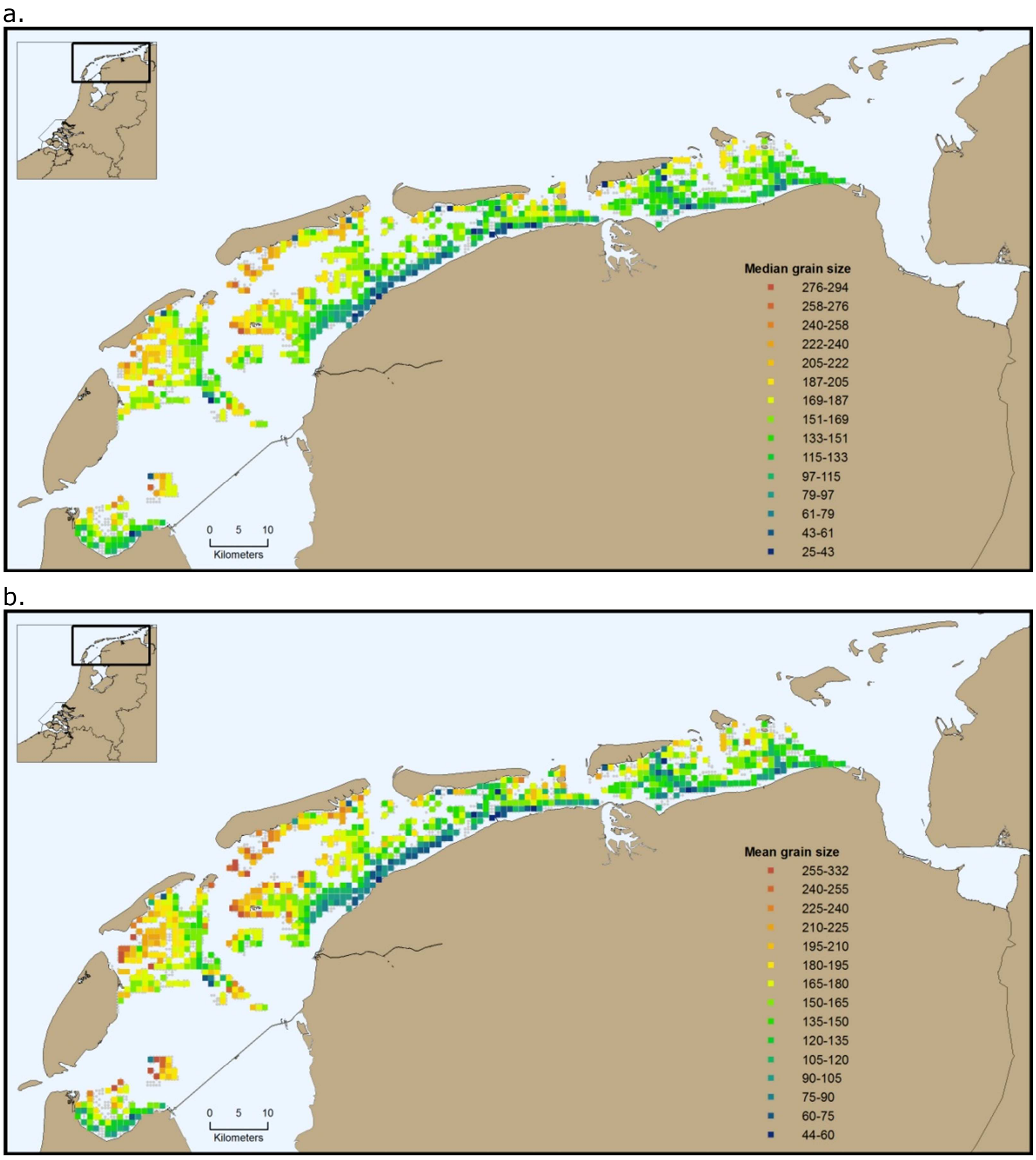


Figure 11 Spatial distribution of median (a) and mean (b) grain size in the Wadden Sea.

3.4 Changes in species composition in and outside the area of gas extraction

Based on the data points in- and outside of the area of present (predicted) subsidence, it is possible to assess whether there is a difference in the change in the abundance of species between the two areas. The results are presented in 4. The 'Ameland-oost' characterized by 2-3cm subsidence revealed a relative larger decrease of the green ragworm *Alitta virens* (p-value=0.002). The 'Moddergat-Lauwersoog-Vierhuizen' region was characterized by a relative larger decrease of the small crustacean *Bathyporeia sarsi* (p=0.01). When we compare all areas characterized by land-subsidence with the rest of the Wadden Sea, the areas characterized by subsidence showed a larger decrease of another small crustacean *Urothoe poseidonis* (p=0.004), but a larger increase of the polychaete worm *Magelona johnstoni* (p=0.01). The Ameland-oost region characterized by 1-3cm subsidence revealed a relative larger increase of the polychaete worm *Heteromastus filiformis* (p=0.008). These results, however, should be treated with care. Since a large numbers of tests have been carried out (see Table 4), purely by chance, it is very likely a few will appear to be significant. Furthermore, if these species are indeed effected by subsidence (or increased sedimentation), we expect the outcomes of these tests to be consistent across the different gas exploration sites. This however, is not the case.

Table 4. Assessment of the difference in a change in species abundance between the region inside and outside the area of predicted subsidence. Bold numbers of the parameter estimates and the corresponding p-values < 0.01, indicate whether the species is significantly more (green) or less (red) abundant. The region codes A1-3cm, A2-3cm, MLV, G and all, stand for Ameland Oost (characterized by 1-3cm and 2-3cm subsidence), Moddergat-Lauwersoog-Vierhuizen, north east of Groningen and all subsidence areas, respectively.

	A1-3cm		A2-3cm		MLV		G		All	
Species	**par.est.**	**pvalue**	**par.est.**	**pvalue**	**par.est.**	**pvalue**	**par.est.**	**pvalue**	**par.est.**	**pvalue**
Abra alba	-2.69	0.189	-1.181	1	12.845	0.646	-1.181	1	-1.553	0.384
Abra tenuis	-0.681	0.908	-1.248	0.874	8.899	0.93	-0.127	1	-0.163	0.976
Alitta succinea	0.983	0.175	0.525	0.592	1.296	0.576	1.686	0.427	1.373	0.03
Alitta virens	-1.608	0.113	**-4.129**	**0.002**	0.438	0.88	12.649	0.518	-0.897	0.337
Ampharete acutifrons	2.242	1	2.717	1	-17.374	1	-17.374	1	1.838	1
Aonides oxycephala	-17.543	1	-17.543	1	-17.543	1	-17.543	1	-17.543	1
Aphelochaeta marioni	0.661	0.387	0.176	0.893	0.242	0.533	2.236	0.123	0.157	0.644
Arenicola marina	0.578	0.617	-0.304	0.864	-1.048	0.345	-2.392	0.295	-0.225	0.74
Aricidea minuta	12.851	0.567	12.326	0.709	-0.071	1	-0.071	1	13.447	0.484
Asterias rubens	-3.044	1	-3.044	1	-3.044	1	-3.044	1	-3.044	1
Autolytus prolifer	10.952	0.827	-0.971	1	-0.971	1	-0.971	1	11.548	0.786
Balanus crenatus	-0.379	1	-0.598	1	-17.995	1	-17.995	1	-0.783	1
Bathyporeia elegans	-17.393	1	-17.393	1	-17.393	1	-17.393	1	-17.393	1
Bathyporeia guilliamsoniana	-16.988	1	-16.988	1	-16.988	1	-16.988	1	-16.988	1
Bathyporeia sarsi	0.758	0.75	1.257	0.788	**-4**	**0.01**	-0.728	0.921	-1.652	0.076
Bodotria scorpioides	-1.027	1	-1.027	1	-1.027	1	-1.027	1	-1.027	1
Buccinum undatum	-17.681	1	-17.681	1	-17.681	1	-17.681	1	-17.681	1
Bylgides sarsi	-0.247	0.901	-1.041	1	11.679	0.493	-0.53	0.737	-0.209	0.859
Capitella capitata	-0.444	0.703	-1.09	0.638	-0.868	0.601	10.012	0.785	-0.381	0.671
Carcinus maenas	0.496	0.865	-0.885	0.806	-11.998	0.707	-0.48	1	0.534	0.825
Cerastoderma edule	-0.945	0.064	-1.732	0.024	0.319	0.546	0.251	0.874	-0.38	0.295
Corophium sp.	3.303	0.158	11.815	0.324	1.692	0.766	12.152	0.378	3.563	0.054
Crangon crangon	-0.284	0.769	9.418	0.763	-1.165	0.476	11.739	0.494	-0.17	0.828
Crassostrea gigas	13.714	0.452	12.189	0.623	-0.001	1	-0.001	1	14.31	0.359
Crepidula fornicata	0.254	1	0.254	1	-14.264	0.425	0.254	1	-15.33	0.331
Echinocardium cordatum	0.226	1	0.226	1	0.226	1	0.226	1	0.226	1
Elminius modestus	-17.597	1	-17.597	1	-17.597	1	-17.597	1	-17.597	1
Ensis directus	-1.128	0.943	5.098	0.987	-0.484	0.974	5.321	0.981	-0.817	0.938
Eteone longa	0.018	0.986	10.131	0.465	-0.72	0.424	10.996	0.387	-0.185	0.772
Eteone sp.	-1.201	1	-1.201	1	-1.201	1	-1.201	1	-1.201	1
Eulalia viridis	-0.621	1	-0.621	1	-0.621	1	-0.621	1	-0.621	1
Eumida sanguinea	0.145	0.945	10.491	0.718	-0.292	1	11.427	0.675	0.722	0.705
Eunereis longissima	-13.621	0.137	0.909	1	-12.996	0.234	0.909	1	-13.621	0.048
Euridice pulchra	-17.681	1	-17.681	1	-17.681	1	-17.681	1	-17.681	1
Fish sp.	17.059	1	17.059	1	17.059	1	17.059	1	17.059	1
Gammarus locusta	-17.724	1	-17.724	1	-17.724	1	-17.724	1	-17.724	1
Gammarus obtusatus	-17.086	1	-17.086	1	-17.086	1	-17.086	1	-17.086	1
Gammarus spec.	11.126	0.743	8.684	0.895	-13.311	0.466	-1.099	0.999	-0.555	0.852
Glycera alba	1.534	1	1.534	1	1.534	1	1.534	1	1.534	1

Species	A1-3cm		A2-3cm		MLV		G		All	
	par.est.	pvalue	par.est.	pvalue	par.est.	pvalue	par.est.	pvalue	par.est.	pvalue
Glycera rouxi	0	1	0	1	0	1	0	1	0	1
Harmothoe imbricata	1.777	1	1.777	1	1.777	1	1.777	1	1.777	1
Harmothoe impar	-17.514	1	-17.514	1	-17.514	1	-17.514	1	-17.514	1
Harmothoe spec.	-1.586	1	-1.586	1	-1.586	1	-1.586	1	-1.586	1
Haustorius arenarius	-16.988	1	-16.988	1	-16.988	1	-16.988	1	-16.988	1
Hediste diversicolor	-0.841	0.107	-0.74	0.533	-0.764	0.135	0.318	0.821	-0.793	0.022
Hemigrapsus sanguineus	-17.597	1	-17.597	1	-17.597	1	-17.597	1	-17.597	1
Hemigrapsus takanoi	-0.459	1	-0.459	1	-0.459	1	-0.459	1	-0.459	1
Heteromastus filiformis	**2.689**	**0.008**	12.858	0.063	-0.998	0.122	0.232	0.865	0.382	0.356
Hydrobia ulvae	14.032	0.242	13.733	0.606	-1.746	0.34	13.599	0.555	0.532	0.634
Idotea balthica	-16.988	1	-16.988	1	-16.988	1	-16.988	1	-16.988	1
Idotea chelipes	-17.185	1	-17.185	1	-17.185	1	-17.185	1	-17.185	1
Jaera albifrons	17.446	1	17.446	1	17.446	1	17.446	1	17.446	1
Lanice conchilega	-0.653	0.178	-1.883	0.021	0.076	0.948	-1.661	0.581	-0.293	0.504
Lepidochitona cinerea	-1.615	1	-1.615	1	-1.615	1	-1.615	1	-1.615	1
Littorina littorea	10.311	0.898	9.786	0.935	-0.612	1	-0.612	1	10.907	0.874
Littorina saxatilis	-17.206	1	-17.206	1	-17.206	1	-17.206	1	-17.206	1
Macoma balthica	0.488	0.519	-0.246	0.837	-0.889	0.199	-0.541	0.692	-0.184	0.681
Magelona johnstoni	1.865	0.016	0.954	0.4	-11.936	0.491	-12.032	0.612	**1.749**	**0.01**
Magelona mirabilis	12.336	0.648	9.505	0.88	9.525	0.861	-2.278	1	11.985	0.558
Magelona spec.	0.227	1	-18.002	1	-18.002	1	2.717	1	0.922	1
Malacoceros fuliginosus	10.381	0.791	8.064	0.945	-0.334	1	-0.334	1	10.977	0.744
Malmgreniella ljungmani	17.059	1	17.059	1	17.059	1	17.059	1	17.059	1
Malmgreniella lunulata	0.5	0.576	13.046	0.324	13.507	0.25	12.863	0.346	1.169	0.161
Manayunkia aestuaria	-1.271	1	-1.271	1	-1.271	1	-1.271	1	-1.271	1
Marenzelleria viridis	0.738	0.814	0.721	0.891	0.768	0.828	-0.235	1	0.815	0.718
Melita palmata	11.863	0.799	10.338	0.871	-2.669	1	-2.669	1	12.459	0.751
Metridium senile	-0.348	1	-0.348	1	-0.348	1	-0.348	1	-0.348	1
Microphthalmus similis	1.083	1	1.083	1	1.083	1	1.083	1	1.083	1
Microprotopus maculatus	-17.76	1	-17.76	1	-17.76	1	-17.76	1	-17.76	1
Mya arenaria	1.192	0.788	10.492	0.817	2.519	0.491	11.121	0.718	1.912	0.488
Mysella bidentata	11.16	0.784	9.635	0.861	-14.974	0.134	-0.762	1	-2.521	0.198
Mysidacea sp.	-17.597	1	-17.597	1	-17.597	1	-17.597	1	-17.597	1
Mysta picta	1.93	1	1.93	1	1.93	1	1.93	1	1.93	1
Mytilus edulis	8.816	0.94	8.244	0.963	6.828	0.979	-2.198	1	9.456	0.923
Nemertini sp.	0.083	0.954	10.38	0.757	14.107	0.206	-0.017	1	0.997	0.428
Neomysis integer	17.059	1	17.059	1	17.059	1	17.059	1	17.059	1
Nephtys caeca	0.078	0.93	11.722	0.503	-0.554	0.695	12.35	0.309	0.202	0.772
Nephtys cirrosa	0.076	0.913	-0.423	0.776	0.261	0.756	11.374	0.556	0.234	0.659
Nephtys hombergii	-0.176	0.771	0.127	0.914	-1.29	0.215	0.242	0.728	-0.244	0.544
Nephtys longosetosa	14.578	0.168	12.666	0.636	0.269	1	0.269	1	15.174	0.099
Nephtys spec.	12.11	0.66	9.15	0.891	12.877	0.547	-1.554	1	13.246	0.476
Nereide sp.	-0.531	0.626	11.554	0.55	-0.901	0.758	-0.408	1	-0.221	0.821
Oligochaeta sp.	1.157	0.436	0.991	0.682	-1.115	0.437	2.499	0.704	0.23	0.791
Ophiura ophiura	-16.988	1	-16.988	1	-16.988	1	-16.988	1	-16.988	1
Pagurus bernhardus	17.446	1	17.446	1	17.446	1	17.446	1	17.446	1
Pectinaria koreni	-1.633	1	-1.633	1	-1.633	1	-1.633	1	-1.633	1

Species	A1-3cm par.est.	A1-3cm pvalue	A2-3cm par.est.	A2-3cm pvalue	MLV par.est.	MLV pvalue	G par.est.	G pvalue	All par.est.	All pvalue
Petricola pholadiformis	12.32	0.694	-0.602	1	-0.602	1	-0.602	1	11.916	0.627
Phoxichelidium femoratum	17.752	1	17.752	1	17.752	1	17.752	1	17.752	1
Phyllodoce maculata	-0.594	0.516	11.551	0.584	0.075	1	12.892	0.448	-0.023	0.979
Phyllodoce mucosa	11.705	0.565	9.791	0.764	-3.08	0.031	9.479	0.842	-1.509	0.17
Polydora ciliata	-0.216	1	-0.216	1	-0.216	1	-0.216	1	-0.216	1
Polydora cornuta	0.476	0.89	1.018	0.885	-1.272	0.638	-12.803	0.697	-0.623	0.73
Pomatoschistus microps	17.059	1	17.059	1	17.059	1	17.059	1	17.059	1
Praunus inermis	-16.89	1	-16.89	1	-16.89	1	-16.89	1	-17.89	1
Pseudopolydora pulchra	-17.086	1	-17.086	1	-17.086	1	-17.086	1	-17.086	1
Pygospio elegans	0.385	0.87	1.15	0.831	-0.868	0.703	0.573	0.909	-0.045	0.975
Retusa obtusa	12.709	0.484	12.05	0.672	-1.987	1	-1.987	1	13.305	0.392
Sagartia troglodytes	0.834	1	0.834	1	0.834	1	0.834	1	0.834	1
Scolelepis bonnieri	0.154	1	0.154	1	0.154	1	0.154	1	0.154	1
Scolelepis foliosa	0.514	1	0.514	1	0.514	1	0.514	1	0.514	1
Scolelepis squamata	-17.933	1	-17.933	1	-17.933	1	-17.933	1	-17.933	1
Scoloplos armiger	-0.567	0.292	-0.842	0.424	0.158	0.884	1.114	0.594	-0.132	0.771
Scrobicularia plana	0.582	0.56	11.248	0.468	0.563	0.527	12.065	0.424	0.592	0.367
Spio martinensis	-2.312	0.386	9.513	0.9	-0.68	0.826	-0.082	1	-1.603	0.422
Spiophanes bombyx	0.183	0.945	9.703	0.89	12.025	0.652	0.306	1	0.85	0.726
Spisula subtruncata	-17.681	1	-17.681	1	-17.681	1	-17.681	1	-17.681	1
Streblospio shrubsolii	12.692	0.39	11.83	0.627	0.331	0.906	0.824	1	1.262	0.513
Streptosyllis websteri	17.752	1	17.752	1	17.752	1	17.752	1	17.752	1
Tellimya ferruginosa	1.458	1	1.458	1	1.458	1	1.458	1	1.458	1
Tellina fabula	-15.704	0.067	-0.273	1	-0.273	1	-0.273	1	-14.857	0.096
Tellina tenuis	0.158	0.885	11.254	0.654	-13.355	0.346	0.163	1	0.196	0.833
Travisia forbesii	-0.216	1	-0.216	1	-0.216	1	-0.216	1	-0.216	1
Urothoe poseidonis	0.453	0.779	-0.724	0.822	-1.025	0.05	-1.892	0.204	**-1.314**	**0.004**
Ventrosia ventrosa	6.389	1	6.836	1	-17.067	1	-17.067	1	5.985	1

To assess whether the differences were significant, Monte-Carlo simulations are carried out. Figure 12 and 13 show the distributions of F-values. It is evident that the simulated F-distribution does not resemble the true F-distribution. In general, extreme values for F are very common, which would lead to an increase in type I error; i.e. it is more likely to reject the null-hypotheses (no difference), while in fact it is true. This is the result of non-independence in the data points due to spatial autocorrelation. Instead of using the true F-distribution, we use the simulated F-distribution to derive the significance. Figure 12 shows a random selection of four species for which there is no significant difference between the area in- or outside the predicted subsidence. Figure 13 shows the four species for which both the standard GLM and the simulations suggests that the change in abundance of *Alitta virens* (Ameland 2-3cm region), *Heteromastus filiformis* (Ameland 1-3cm region), *Magelona johnstoni* and *Urothoe poseidonis* (all subsidence regions) differs significantly from regions elsewhere in the Wadden Sea.

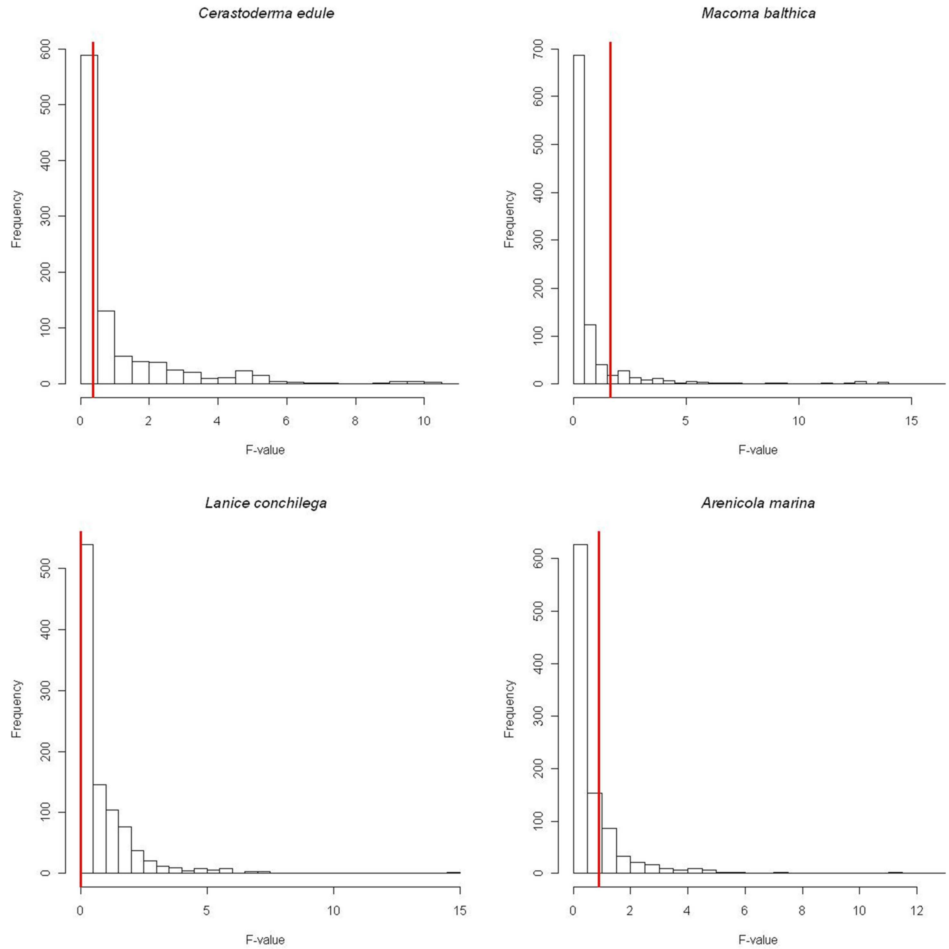


Figure 12 Example of the F-distribution obtained from 4 important species. None of these species significantly differ from other regions in the Wadden Sea. The red line represents the Moddergat-Lauwersoog-Vierhuizen region.

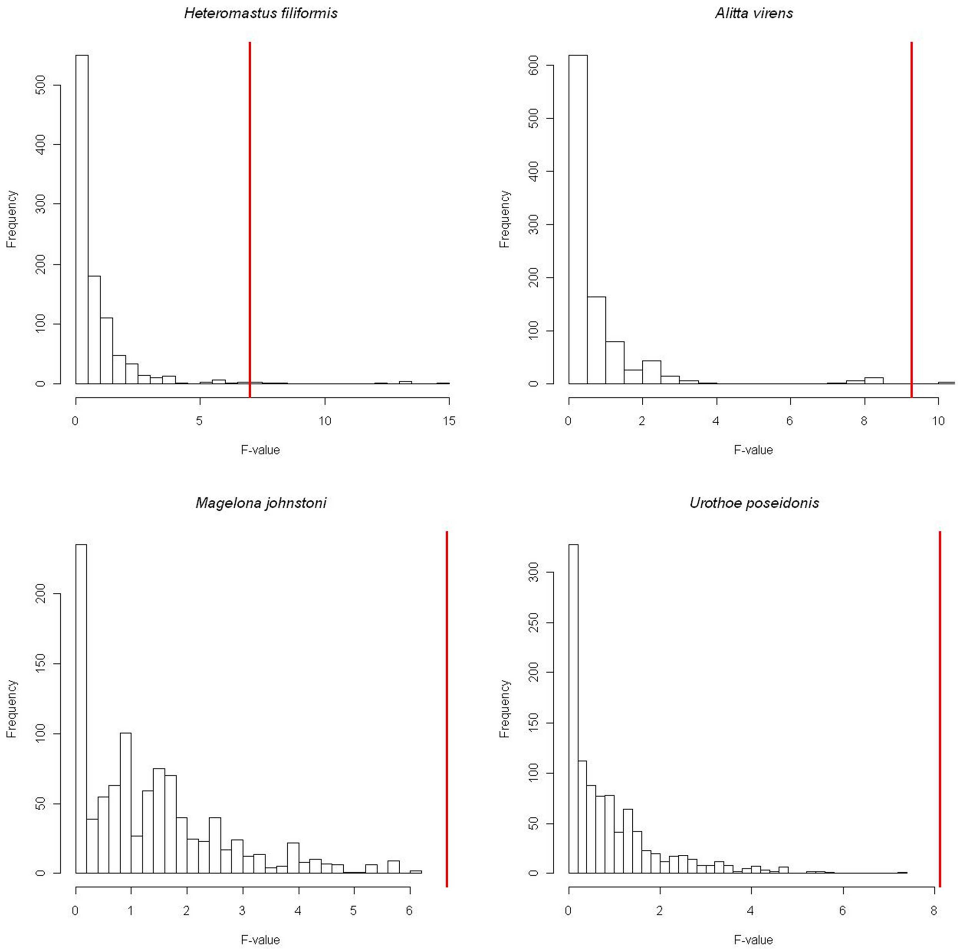


Figure 13. The four species for which the change in abundance in the area of land-subsidence (indicated by the red line) differed significantly from other regions in the Wadden Sea.

4. Discussion & Conclusions

This report endeavored to provide an overview of the data collected in 2008 and 2009 and show how it can be used to understand the spatial distribution and demography of a large number of macrozoobenthos species. In total 4410 stations have been sampled, in which a total of 95 species were found. The samples were post-processed to obtain estimates of AFDM (all organisms), age and size (bivalves and crustacean) and shell colour (*for Macoma balthica*). In future assessment all parameters and there derivatives (such as annual growth and mortality) can be used in assessing the possible effect of subsidence.

In terms of biomass, the most important species for both 2008 and 2009 were edible cockle (*Cerastoderma edule*), sand mason (*Lanice conchilega*), softshell clamm (*Mya arenaria*), lugworm (*Arenicola marina*), American jack knife clam (*Ensis directus,* formerly known as *Ensis americanus*), blue mussel (*Mytilus edulis*), ragworm (*Hediste diversicolor*, formerly known as *Nereis diversicolor*), Pacific giant oyster (*Crassostrea gigas*) and Baltic tellin (*Macoma balthica*). Their relative importance has changed however. The total biomass of *Lanice conchilega* in 2009 is only 60% of that in 2008. Also *Crassostrea gigas* has decreased slightly, but due to the low sampling size, this estimate is not very reliable. All other eight species have increased considerably in biomass, particularly *Mytilus edulis* and *Ensis directus,* which have more than doubled, which is due to a relative large recruitment. A large recruitment in 2009 was evident in several macrozoobenthos species, an interesting phenomenon that we will follow up on when the data for more years become available.

Maps of the spatial distribution show that most species occur throughout the Wadden Sea, but that the distribution heavily depends on local environmental conditions. E.g. Baltic tellin and cockle mostly occur in the muddy regions (Figure 4), while relative rare thin tellin (*Tellina tenuis)* and bean-like tellin (*Tellina fabula)* mostly occurs in more sandy and deeper regions close to the gully (Figure 4). Combining the observations allows for the estimation of species richness per sample (see Figure 5). Also here interesting patterns were observed. In general species richness seems to be highest in the regions with the shortest inundation time. One of the regions that springs out is the area east of the island Griend.

This synoptic data is used to investigate changes in abundance in the regions where, due to natural gas extraction, current and future subsidence is to be expected. The Ameland-oost region characterized by 2-3 cm subsidence revealed a relative larger decrease of the green ragworm *Alitta virens*. The 'Moddergat-Lauwersoog-Vierhuizen' region was characterized by a relative larger decrease of the small crustacean *Bathyporeia sarsi*. When we compare all areas characterized by land-subsidence with the rest of the Wadden Sea, the subsidence areas showed a larger decrease of the small crustacean *Urothoe poseidonis*, but a larger increase of the polychaete worm *Magelona johnstoni*. The Ameland-oost region characterized by 1-3 cm subsidence revealed a relative larger significant increase of the polychaete worm *Heteromastus filiformis*. Compared to the rest of the Wadden Sea (using Monte Carlo simulations), such deviations were only out of proportion for *Alitta virens*, *Heteromastus filiformis*, *Magelona johnstoni* and *Urothoe poseidonis.* Because these species-specific deviations are not observed across all land-subsidence regions, it seems unlikely that they relate to gas exploration activities.

This dataset will be used to carry out future assessments investigating the possible effect of subsidence. Such an assessment could be based on changes in abundance, recruitment, growth and community structure using the data from all species, hence leading to not one but more than hundred bio-indicators. The design chosen is based on (Bijleveld et al., 2011) and encompasses a regular grid (500 by 500 meter) distributed in the entire Wadden Sea, complemented with a set (approximately 10%) of random points which enable the estimation of small scale spatial processes. One could have chosen an alternative sampling scheme, concentrating most efforts in regions in the proximity of natural gas extraction. Although it may lead to an increased power to detect differences, at the end it often sheds little light on the actual causes of the differences. The reason for this is that at a small spatial scale, environmental variables are often correlated. Consequently one cannot find out to which of these variables the species responds. In statistical terms this is known as the problem of colinearity. By including regions elsewhere with different (more extreme) environmental conditions, it is more likely to disentangle the influence of the different environmental variables. In other words, when using a small scale sampling program, the question whether the observed temporal and spatial changes in species abundance, growth or mortality are mere local phenomena, or whether such changes also take place elsewhere in the Wadden Sea, would remain unanswered. Furthermore, since most species show a correlation beyond 500 meters, a sampling scheme at a higher resolution would lead to some level of pseudo-replication. However, prior to this investigation, on a precautionary basis, the 2009 sampling campaign has been extended by doubling the sample size within the regions of predicted subsidence.

The Wadden Sea is a highly heterogeneous environment in both space and time. It can be difficult to tell apart the effect of subsidence from other factors. Therefore, our challenge ahead is to improve our understanding of the effect of all physical, biological and anthropogenic variables on the distribution and demographic characteristics of species (Ellis et al., 2000). This can be done by developing habitat models (Kraan et al., 2010 and Figure 15), using such models to predict in space and relating model residuals (i.e. the difference between data observations and model predictions) with subsidence.

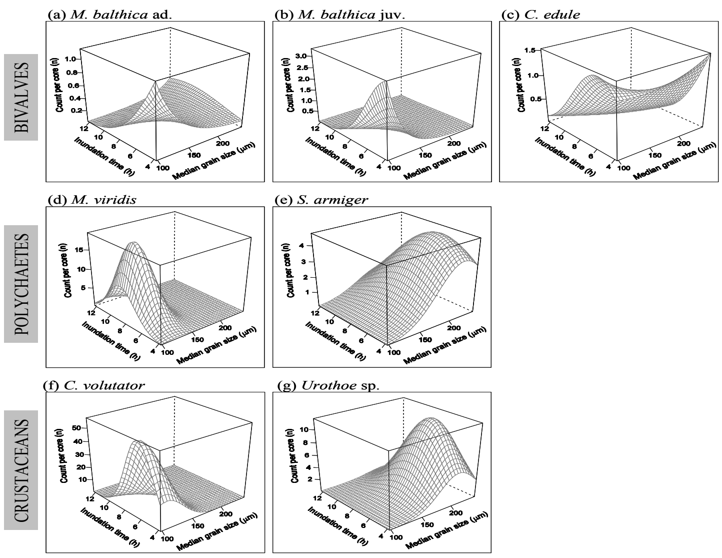


Figure 7 The preference of six individual species for two major environmental variables; inundation time and median grain size. These results show large species-specific variability. For example *Marenzelleria viridis* shows a strong preference for fine substrate, while *Scoloplos armiger* mostly prefers course sediment (Kraan et al., 2010).

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Appendix A: Response to the 2009 Audit Commission

1. *Sampling took place from June to October, during which considerable temporal changes in density and biomass may take place. The Audit commission advices to highlight in when and where sampling took place and what the consequences are for the interpretation of the results.*

We agree time of sampling will influence biomass and density estimates (Beukema, 1976). This will be particularly evident in 2008 when sampling first occurred in the Western Wadden Sea, and continued in the Eastern Wadden Sea later in the season. In 2010, attempts have been made to sample more evenly throughout the Wadden Sea. Currently, accounting for these effects (particularly in terms of changes in numbers) in the analysis is difficult, because one cannot differentiate between a space and time effect. This can only be resolved by sampling multiple times at the same locations. In 2010 some sites have been sampled both early and late in the season. This important point will be taken into account in future sampling programs.

2. *The report of 2013 will probably not take into account the data collected in 2012. The commission advices to discuss the consequences of this.*

Efforts will be made to complete at least some (e.g. bivalves only) of the laboratory analysis of 2012, such that it can be included in 2013 report. But it is correct to assume that probably not all data will be taken into account. This will indeed shorten the evaluation period and therefore will reduce some strength of the monitoring program.

3. *The Audit commission advices to discuss if the current spatial resolution of the sampling-scheme (i.e. 500 meter) will be sufficient to monitor the effect of the natural gas exploitation in the Moddergat-Lauwersoog-Vierhuizen region.*

It is true that the Wadden Sea is a very dynamic environment both in space and time. The question therefore is whether one sample is in any way representative for a 500 x 500 m region. For this to be the case, sampling stations should be correlated to some extent at distances beyond 500m. One of our research projects currently in progress is to look at the spatial autocorrelation and to compare it between species. Figures in last year's report shows the correlograms (based on the Moran's I) of most species. It could be seen that for the majority of species, the abundances are still correlated at distances > 500 meter.

The results were not available last year, and therefore, to address the concern of the Audit commission expressed last year (2009), the 2009 sampling scheme has been extended. Currently each sampling station within 5 km of gas exploitation station, has been supplemented with another sample positioned at a distance between 0-250m from that regular point sample.

In addition, this year we carried out a simple power analysis. The procedure is as follows: The sampling points can be classified into four groups depending on the year in which the samples are taken (e.g. 2008 or 2009) and whether they are positioned within or outside the MLV subsidence region. If subsidence negatively impacts the abundance, we expect a decrease in abundance in 2009 (or any year to come) relative to 2008 within the subsidence region. How much (or even if) it will be reduced, is unknown, but we can run several scenarios. First, we simulate for each species, the number of individuals we may find in a normal sampling core taken in 2008 or outside the area of subsidence. This is done by simulating from a Poisson distribution with expectation equal to the mean number of individuals found in each core throughout the Wadden Sea. If however, the sample is taken in 2009 within the area of subsidence, the mean expectation used to simulate the number of individuals found in a core, is reduced by x percent. In our simulation we imposed a reduction of 0% up to 100%, at 5% intervals. This simulation is repeated 100 times. Table 5 shows for each species and each reduction scenario, the percentage of simulations during which we correctly detect the imposed decline. A larger reduction obviously leads to a higher probability of rejecting the null-hypothesis (i.e. concluding there is an effect of gas exploration). Also detecting an effect is more likely to happen for those species that are most abundant (Table 5). For some species, such *Aphelochaeta marioni*, *Corophium sp.*, *Hydrobia ulvae*, *Oligochaeta sp.*, *Pygospio elegans* and

Scoloplos armiger an abundance reduction of only 25% allows us to reject the null-hypothesis. This simulation is only based on the Moddergat-Lauwersoog-Vierhuizen region and data from two years. Also including the Ameland-oost and Groningen region into the analysis, using data from more years (i.e. more samples) and doing a multi-species assessment, will improve the chances of detecting any effects of subsidence even further.

Table 5. Percentage of the simulations resulting in an observed significant effect of subsidence in the Moddergat-Lauwersoog-Vierhuizen region. If the reducing factor is 0, the expected number of individuals in a sample inside the area of subsidence is 0. If the reducing factor is 1, there is no effect of subsidence and the expected number of individuals in each sample taken in the subsidence area is equal those taken elsewhere. Only simulations from those species which occur in at least 100 samples, are presented.

	All individuals have disappeared due to subsidence								***⟵Reducing factor⟶***												*No effect of subsidence on species abundance*
Species	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95	1
Abra tenuis	100	100	100	95	96	84	68	63	63	41	28	20	17	12	11	8	11	12	7	9	10
Alitta succinea	99	92	85	76	64	60	47	34	33	27	22	13	17	14	8	9	12	7	7	11	4
Alitta virens	29	31	20	22	17	13	14	9	7	10	11	14	15	11	13	10	7	8	7	8	7
Aphelochaeta marioni	100	100	100	100	100	100	100	100	100	100	100	100	100	100	96	86	62	35	14	9	15
Arenicola marina	100	100	99	97	90	81	78	72	55	38	40	26	27	17	16	10	14	11	10	10	7
Bathyporeia sarsi	98	90	75	73	60	46	31	37	19	15	18	14	13	9	9	11	15	6	12	16	13
Bylgides sarsi	38	32	30	33	36	25	21	24	30	28	20	22	13	22	13	17	16	12	11	12	10
Capitella capitata	100	100	100	100	100	100	100	100	100	97	96	91	76	63	43	37	20	16	8	15	12
Carcinus maenas	96	85	58	48	38	34	28	28	22	18	14	14	10	11	8	11	8	10	12	9	11
Cerastoderma edule	100	100	100	100	98	96	96	94	81	72	56	44	37	36	26	8	14	14	8	7	6
Corophium sp.	100	100	100	100	100	100	100	100	100	100	100	100	99	100	90	72	46	30	12	6	6
Crangon crangon	38	28	29	22	20	14	15	12	13	8	12	7	9	9	8	6	15	12	8	10	11
Ensis directus	100	100	100	100	100	100	100	100	100	98	97	91	75	52	40	26	25	10	11	11	11
Eteone longa	100	100	100	100	100	100	98	92	85	72	69	40	33	35	19	14	13	8	9	15	11
Eumida sanguinea	22	16	24	17	19	17	10	11	4	12	13	10	4	4	8	8	11	5	9	11	14
Eunereis longissima	37	26	23	21	15	18	14	6	11	11	8	11	10	7	9	6	10	5	3	6	8
Gammarus spec.	76	61	48	43	36	24	28	15	17	13	11	9	5	3	8	10	11	12	12	7	10
Hediste diversicolor	100	100	100	100	100	100	100	98	97	84	72	66	41	45	27	17	16	10	7	11	9
Heteromastus filiformis	100	97	90	79	70	59	49	43	31	26	22	17	15	10	12	7	6	10	5	2	7
Hydrobia ulvae	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	88	67	23	5	7
Lanice conchilega	100	100	100	100	100	100	100	100	100	99	89	88	66	60	43	26	20	10	6	6	10
Macoma balthica	100	100	100	100	100	100	95	91	84	73	58	42	40	21	24	14	7	10	11	8	3
Magelona mirabilis	37	38	45	36	34	35	31	24	24	25	20	28	18	21	19	9	21	15	19	12	10
Malacoceros fuliginosus	87	66	60	39	37	27	23	26	8	15	17	11	6	8	14	12	16	8	11	7	5
Malmgreniella lunulata	23	27	23	18	22	13	9	12	12	6	9	8	9	8	8	10	15	3	10	11	13
Marenzelleria viridis	100	100	100	100	100	100	100	100	100	98	99	97	88	68	56	31	15	14	8	6	4
Mya arenaria	100	97	91	83	77	73	59	61	40	31	20	19	10	18	8	11	11	8	10	9	5
Mytilus edulis	100	100	100	97	93	82	82	71	61	49	34	34	18	18	8	13	15	8	4	8	11
Nephtys caeca	31	30	31	35	32	22	15	21	15	12	14	14	12	9	15	12	10	13	12	9	6
Nephtys cirrosa	25	22	17	11	14	14	13	13	16	7	12	13	5	8	8	10	8	8	10	10	9

Nephtys hombergii	98	95	72	70	60	48	38	32	32	29	18	13	13	10	11	5	10	8	5	10	8
Nereide sp.	31	28	27	27	14	9	16	14	10	10	12	9	9	9	7	8	3	5	4	11	12
Oligochaeta sp.	100	100	100	100	100	100	100	100	100	100	100	100	99	97	81	78	49	28	11	11	7
Phyllodoce maculata	34	28	29	22	19	12	13	16	6	13	5	15	9	6	11	11	7	9	7	7	7
Phyllodoce mucosa	99	86	80	66	54	29	31	18	18	17	10	9	10	8	8	5	5	6	5	5	10
Polydora cornuta	100	100	100	100	100	100	99	96	93	81	64	55	46	30	30	12	14	10	9	7	8
Pygospio elegans	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	95	56	26	13	5
Scoloplos armiger	100	100	100	100	100	100	100	100	100	100	100	100	99	96	83	73	41	17	14	13	11
Scrobicularia plana	35	38	24	24	15	18	13	14	13	12	9	11	8	9	10	5	6	5	10	8	10
Spio martinensis	100	100	100	100	94	90	80	73	64	44	37	36	15	18	10	12	10	10	7	8	13
Spiophanes bombyx	39	30	24	31	21	21	9	13	9	13	6	8	11	9	8	11	8	7	9	12	15
Streblospio shrubsolii	59	46	36	28	32	25	17	18	16	7	12	16	11	11	8	12	7	4	9	11	10
Tellina tenuis	34	25	28	25	22	17	18	15	14	13	16	16	15	10	10	9	11	8	8	10	7
Urothoe poseidonis	100	100	100	100	100	100	100	100	100	100	100	98	88	79	68	39	28	17	11	11	15

4. The Audit commission advices to carry out the analysis for Ameland-Oost and Moddergat-Lauwersoog-Vierhuizen' separately.

This year's analysis has performed the assessment for the regions separately.

5. *The Audit commission advices to include sediment composition and height (relative to NAP) into the statistical analysis.*

The NAM has agreed to include these variables into the macrozoobenthos analysis. The intention is to do this in the final 2012 evaluation. In the annual report these environmental covariates will be presented. Sediment and depth data are presented here. The best quality data on inundation time is not yet available, but will be presented in next year's assessment.