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NIOZ Royal Netherlands Institute for Sea Research

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Abstract

The Wadden Sea is of paramount importance to wildlife. It is a breeding, wintering and refueling station for millions of birds, and an important nursery area for several species of fish. For their survival, growth and reproduction they 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 program, covering the entire intertidal zone of the Wadden Sea, a long-term effort named SIBES (Synoptic Intertidal Benthic Sampling). During the sampling campaign 3963 stations were sampled, in which more than 160 thousand individual organisms were observed, belonging to 76 species. In biomass (expressed as Ash Free Dry Mass (AFDM)), the most important species were 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 (*Nereis 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 higher regions in the Wadden Sea (e.g. the regions along the mainland and Island coast) contained most species.

Finally the data can be used to characterize the regions where, due to natural gas extraction, current and future land subsidence is to be expected. The 'Ameland-oost' and 'Moddergat-Lauwersoog-Vierhuizen' region were characterized by more individuals of *Hydrobia ulvae*, *Urothoe poseidonis*, *Lanice conchilega*, *Nephtys cirrosa*, *Malmgreniella lunulata*, *Phyllodoce mucosa*, *Magelona johnstoni* and *Nephtys longosetosa*. The *Scoloplos armiger* was less abundant. However, compared to the rest of the Wadden Sea, such deviations were only out of proportion for *Nephtys cirrosa*. The east Groningen region (with the exception of the Dollard which was not sampled in 2008), was characterized by more *Cerastoderma edule*, *Urothoe poseidonis*, *Lanice conchilega*, and less *Scoloplos armiger*, *Pygospio elegans*, *Oligochaeta spec.*, *Marenzelleria viridis*, *Spio martinensis* and *Nereis virens*. Compared to the rest of the Wadden Sea only the differences observed in *Pygospio elegans*, *Marenzelleria viridis* and *Spio martinensis* abundance were significant.

A comparison with the 2004 and 2006 data (non-synoptic sampling grid) illustrates large variability between years and regions. North of the east-Frisian coast (north of Moddergat), in 2004 and 2006, samples were taken both within and outside the area of expected land subsidence. These results suggest for most species (except *Ensis americanus*) a larger increase or less severe decline in the region of natural gas exploitation. It is unknown whether this can be attributed to land subsidence.

The 2008 sampling scheme and results presented in this report provide a solid reference to assess and test future changes in macrozoobenthos abundance. The way forward is to use the synoptic intertidal benthic sampling scheme to improve our understanding of how land subsidence and other natural and anthropogenic processes influence the spatial and temporal demography of the species of interest.

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 Sampling) Gedurende deze campagne zijn 3963 punten bemonsterd, waarin in totaal meer dan 160 duizend individuen zijn waargenomen en geteld. Deze individuen behoorden tot 76 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 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 de gebieden te karakteriseren waar nu of in de toekomst gas exploitatie (zal) plaatsvinden. Het gebied ten oosten van Ameland en het gebied nabij Moddergat-Lauwersoog-Vierhuizen worden gekenmerkt door meer individuen van het wadslakje, buldozerkreeftje, schelpkokerworm, het zagertje, *Malmgreniella lunulata*, *Phyllodoce mucosa, Nephtys longosetosa* en *Magelona johnstoni*. De wapenworm was minder aanwezig. Echter, als we deze afwijkingen vergelijken met de rest van de Waddenzee, kwam alleen het zagertje (*Nephtys cirrosa)* meer voor in bodemdalinggebieden dan verwacht. Het gebied ten noordoosten van Groningen (met uitzondering van de Dollard die alleen vanaf 2009 bemonsterd is) wordt gekenmerkt door meer individuen van de kokkel, buldozerkreeftje, schelpkokerworm, en minder wapenworm, zandkokerworm, *Oligochaeta*, *Marenzelleria viridis*, *Spio martinensis* en groene zeeduizendpoot. Op basis van simulaties, is deze afwijking alleen significant voor de soorten zandkokerworm, *Marenzelleria viridis* en *Spio martinensis*.

Gebruikmakend van bestaande data verzameld in 2004 en 2006 (geen complete Waddenzee brede dekking) zien we dat er grote veranderingen zijn tussen jaren en gebieden. In het gebied ten noorden van oost-Friesland, zijn er in alle jaren monsters genomen in het deel dat gekenmerkt wordt door zowel de aan- en afwezigheid van bodemdaling als gevolg van gasexploitatie. Deze resultaten laten zien dat voor de meeste soorten (met uitzondering van de Amerikaanse Zwaardschede) de toename groter of the afname geringer was in de gebieden waar gasexploitatie plaatsvindt. De achterliggende reden voor dit verschil is nog niet duidelijk.

De 2008 bemonstering en de resultaten die hier zijn gepresenteerd zullen in de toekomst gebruikt worden om verandering in macrozoobenthos te testen en kwantificeren. 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. 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.

In 2004 and 2006, a comparable but limited program covering only part of the Wadden Sea was conducted. This report will first describe the 2008 data which will be collected to carry out the long-term assessment that will take place in the upcoming years. Using this data, the areas of expected land subsidence will be characterized in terms of macrozoobenthos abundance and we will investigate how it has changed relative to 2004 and 2006 (see Kraan et al. 2007). 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 an area of paramount importance to wildlife. Millions of birds visit this area annually to overwinter, refuel or breed (van de Kam et al. 1999). The ecological importance of the Wadden Sea has lead 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 attributably to the large amount 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 productivity in the water and on the sea bottom (Dekker 1989, Herman et al. 1999). To conserve and restore the richness of the Wadden Sea food web, it is first 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. 2007)), 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 Western 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 3963 intertidal sites were sampled in June – October 2008. Of these stations, 3627 were placed on a regular 500 m grid and an additional 336 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. submitted). 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 (Piersma 1993, van Gils et al. 2005, van Gils et al. 2006, Kraan et al. 2007, Kraan et al. 2010). 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). 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).

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 8mm 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 Land 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 land 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 2 provides an overview of the predicted land subsidence for 2009 compared to 2005. Figure 3 shows the predicted land subsidence for 2025.

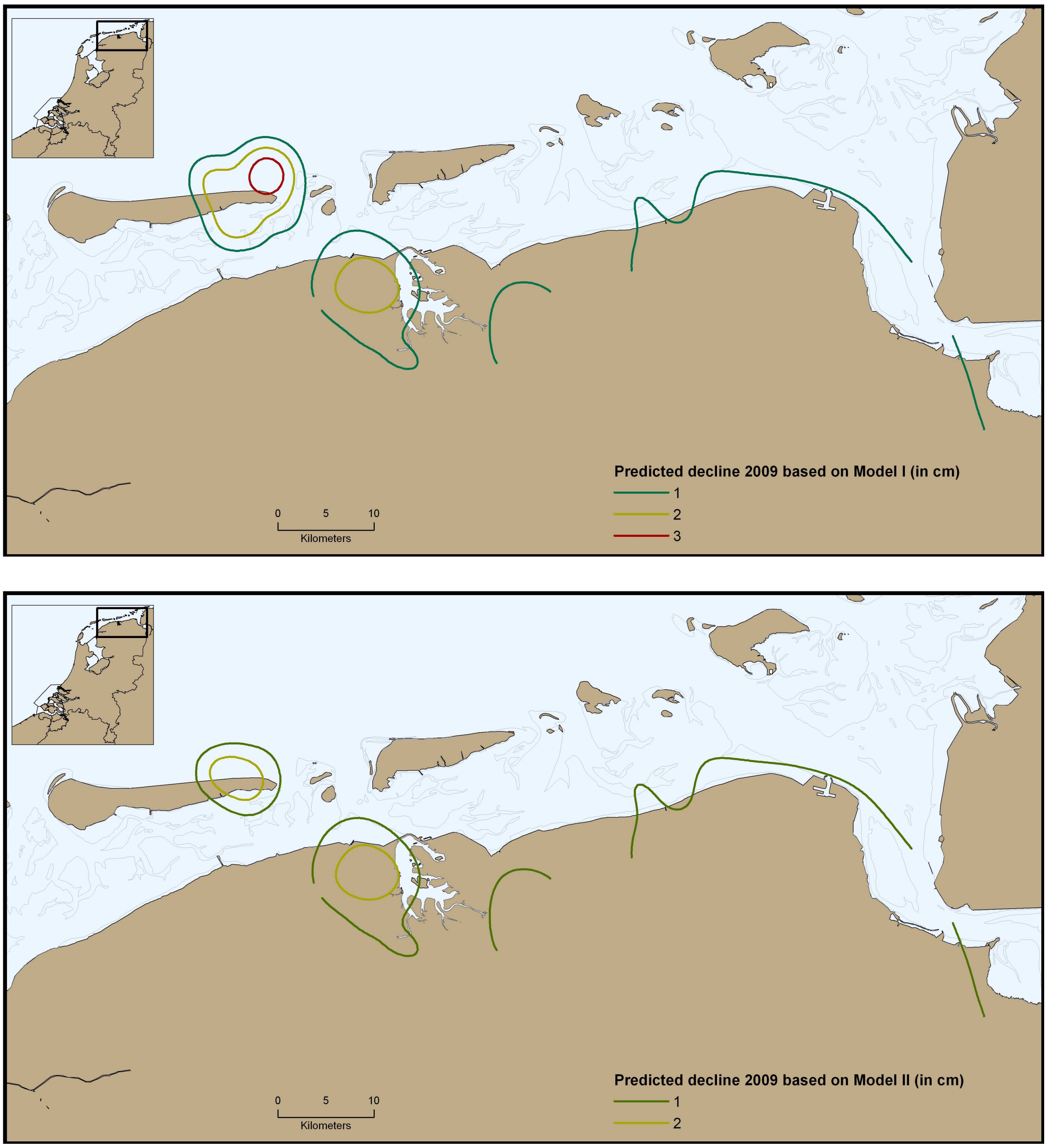
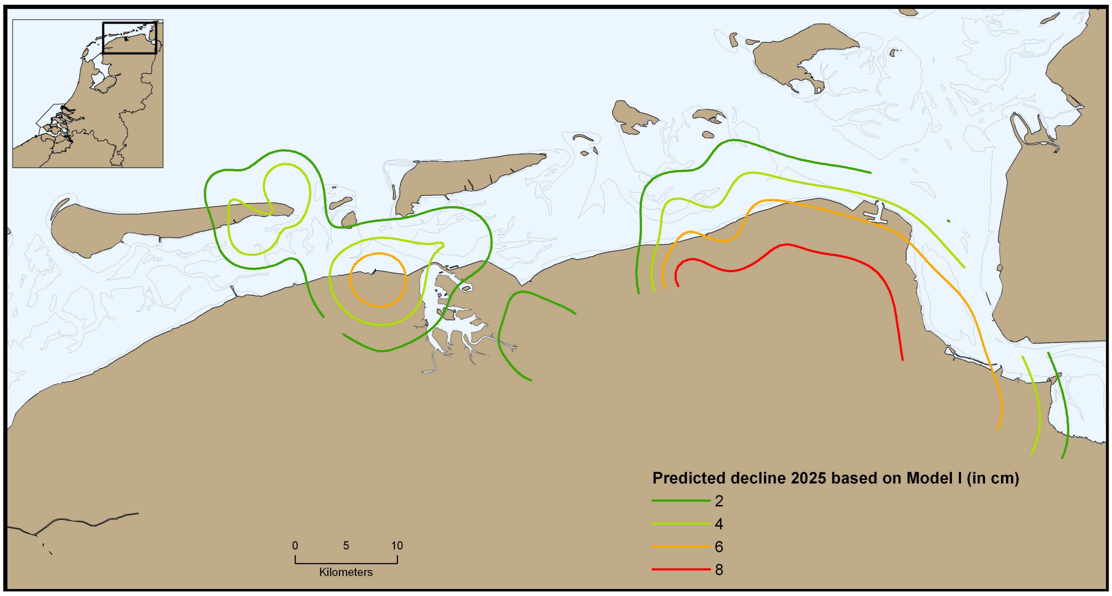


Figure 2 Predicted decline in 2009 relative to 2005 based on the land subsidence Model I and II.



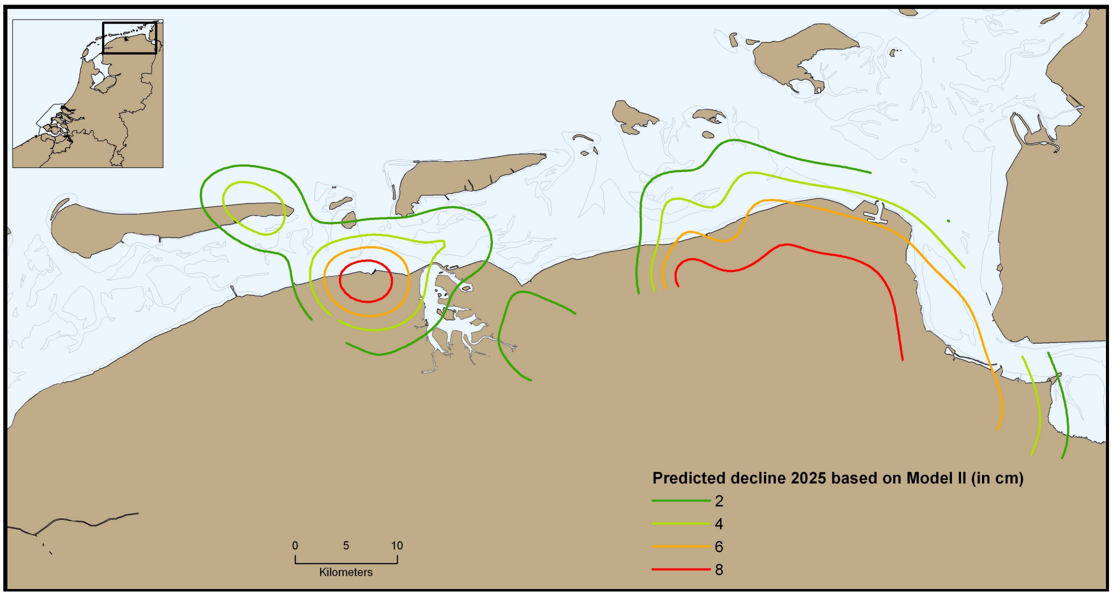


Figure 3 Predicted decline in 2025 relative to 2005 based on the land subsidence Model I and II.

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 (Kraan et al. in press, (Yates et al. 1993, Reise 2002). The inundation time can be calculated on the basis of depth measurements, 10 minute water-level measurements at stations distributed throughout the Wadden Sea (mostly in the vicinity of harbours) and tidal charts. Sediment data (Buchanan 1984) is collected at 1000 m intervals at the location of a macrozoobenthos sample (see Figure 3). In 2009 and the following years, sediment type will be collected at each macrozoobenthos sampling station (i.e. every 500m and the random plus-points). Depth data (Figure 5) is collected by the RIKZ, based on a dense grid of sampling points ('vaklodingen') and converted into a elevation map by NAM (NAM 2008; EP200905260877). Also human related covariates, such as manual 'fishing' of edible Cockles may be taken into account, however it is currently unclear whether this information is collected at a sufficient spatial and temporal resolution.

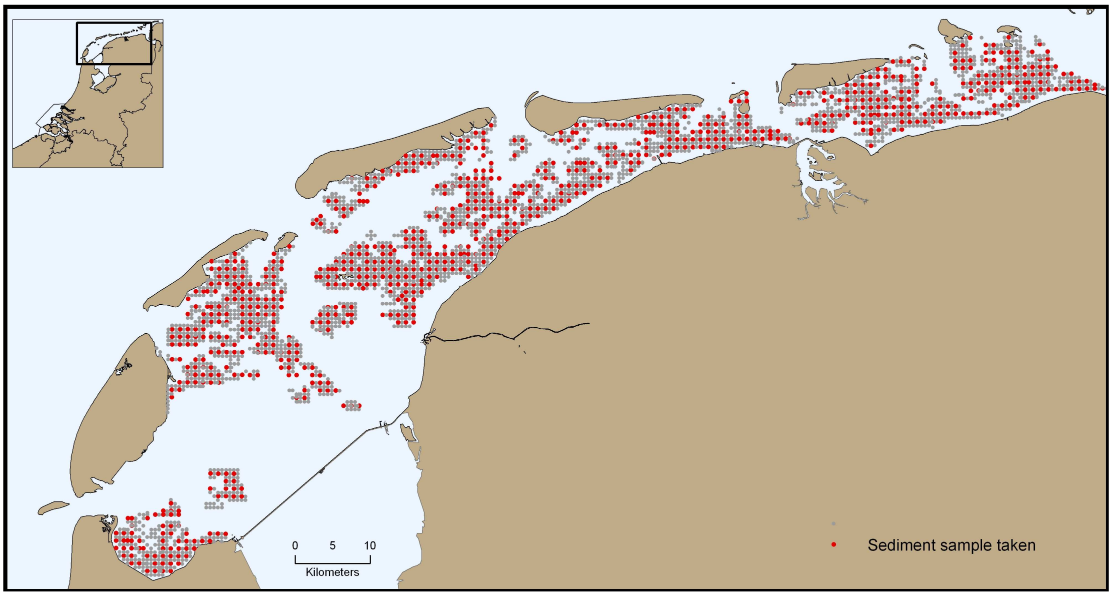


Figure 4 Spatial distribution of sampling stations at which sediment samples are taken in 2008. In 2009 sediment samples are taken at all stations.

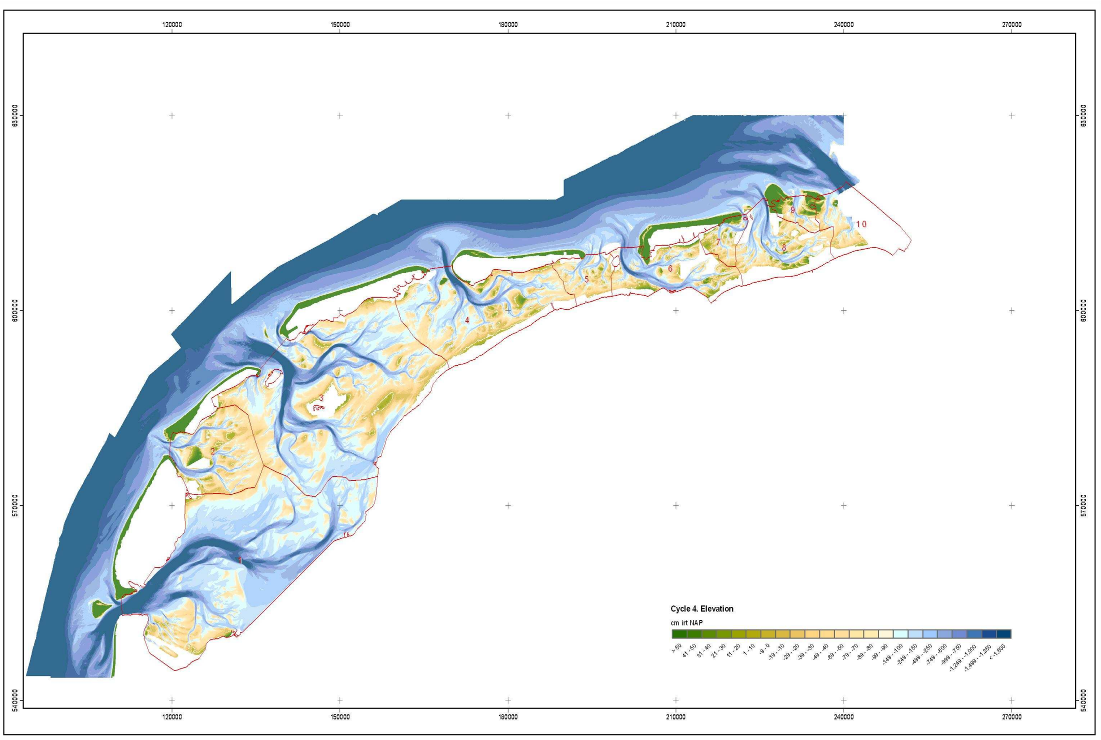


Figure 5 Depth based on RIKZ 'lodingen' 2005-2008 and NAM report EP200905260877

2.6 Characterizing species composition within and outside the area of future land subsidence

Future risk assessment studies will investigate whether the changes in macrozoobenthos communities occurring in the areas of land subsidence (due to gas exploitation), are out of proportion compared to regions elsewhere in the Wadden Sea. This report describes the results from the first extensive macrozoobenthos survey, and hence cannot look at such changes. Instead, this study will attempt to characterize the area were land subsidence is expected in the near future. 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 land subsidence, may be different due to other (e.g. natural) processes. E.g., if one organism is highly abundant in that region, this may lead to increased predation, and hence a larger absolute decline.

To assess whether the regions where natural gas exploitation occurs, are already different to start with, we classify each macrozoobenthos sampling point as either in- or outside the region of gas exploitation. This region is defined as the area where the predicted land subsidence is at least 2cm in 2025 (data provided by NAM, predictions based on Model II, see (NAM 2005)). This resulted in two regions; the Ameland and 'Moddergat-Lauwersoog-Vierhuizen' region, and the north-east Groningen region (see Figure 3). Next, for each species we investigate whether the abundance in- and outside the region of land subsidence is different. This is done by fitting a Generalized Linear Model to these count data. The count data is assumed to be quasi-Poisson distributed, which allows for possible under- or over dispersion. The variable 'in- or outside' is treated as factor.

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, 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 land subsidence is significantly different. To account for this, two approaches exist.

The first 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 et al. 1998, Diggle et al. 2003, Diggle and Ribeiro Jr. 2007). Furthermore, it requires a correct specification of all spatial dependences, e.g. by including all relevant environmental drivers, such 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.

Fitting the GLM as described above, results in a t-value and level of significance (p-value). Now we can randomly select a different region in the Wadden Sea consisting of a cluster of a similar number of sampling stations, fit a GLM, and extract the t-value. So in other words, we construct random regions in the Wadden Sea as if land subsidence would occur and investigate if it is different from regions elsewhere (see Figure 6 for some examples). We repeat this procedure 1000 times, resulting in an estimate of the t-distribution obtained through simulations. This is repeated for each species. Now it is possible to compare the t-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 factor 'in- or outside' *and* if such a large absolute t-value rarely occurs in the simulations (α=0.01), there is strong evidence that the abundance of the species of interest is indeed different in that region. Figure 11 provides the histogram of the simulated t-distribution and true t-value for a random selection of species and Table 1 provides the summaries for all species.

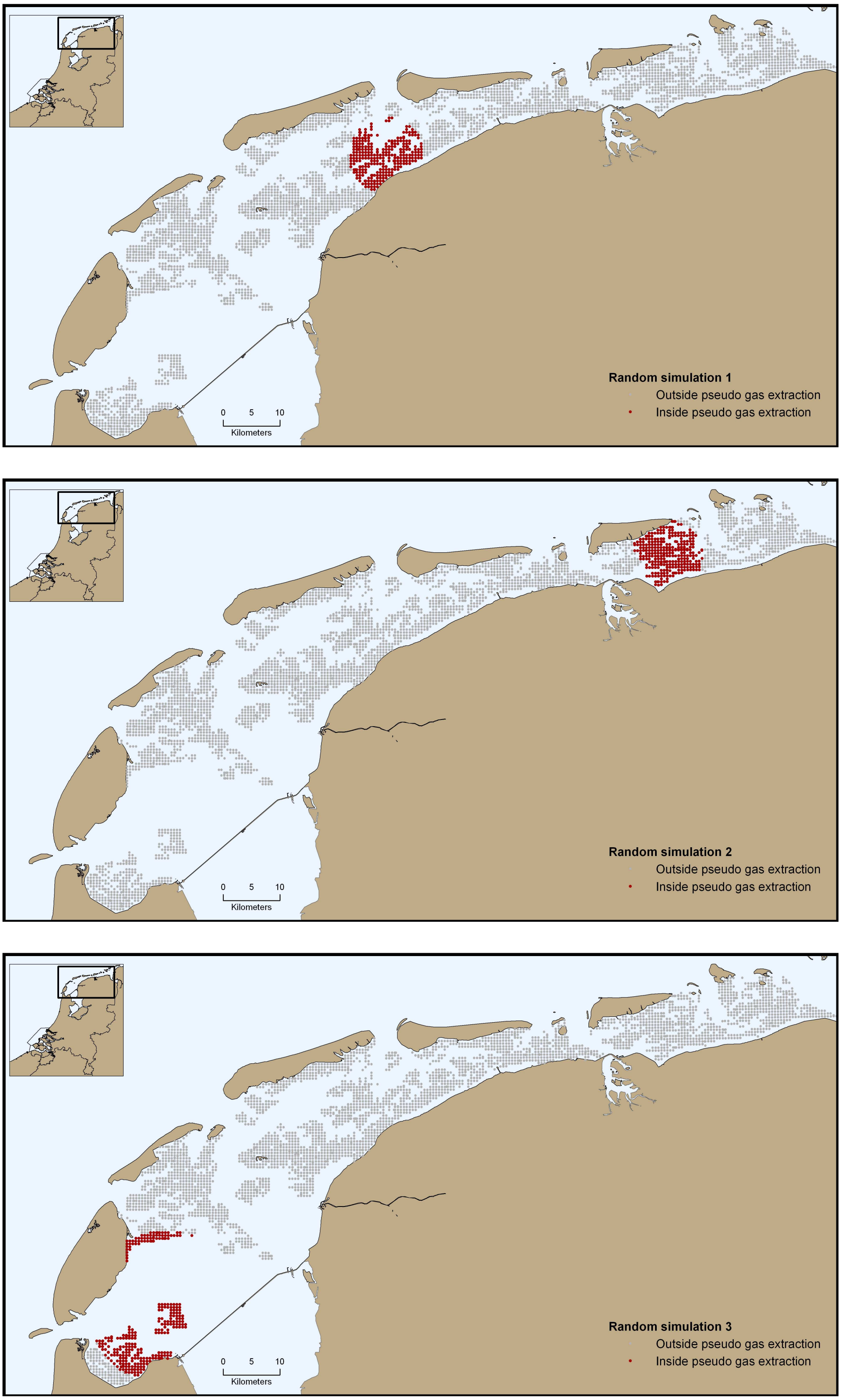


Figure 6 Three randomly generated pseudo gas extraction regions. The pseudo regions are constructed by randomly selecting a sampling point in the Wadden Sea and selecting the 311 nearest sampling station.

2.7 Comparison with 2004 and 2006

In 2004 and 2006 the NIOZ has collected macrozoobenthos data in both the Western and eastern Wadden Sea (see Kraan et al. 2007). These surveys differ from the current sampling design in that only a few regions were sampled, but at a higher spatial resolution, i.e. 250m (see Figure 7). Of these regions, the sampling stations south of Ameland-East and the eastern part of the "oost-Friesland" region are characterized by some expected land-subsidence in recent years (see Figure 2). In this analysis, we will look at the changes in species abundance using data from 2004 and 2006 (old sampling grid) and 2008 (SIBES grid). The avoid any spatial bias, for 2008 we will only use those sampling points which are also part of the 2004 and 2006 grid. In the analysis, 7 regions are defined; 1. the western Wadden Sea, 2. east-Ameland, 3. East-Friesland out and 4. East-Friesland in (the area out- and inside the expected land-subsidence, respectively), 5. Brakzand (the area just north-east of Lauwersoog) and 6. Zoutkamperlaag (the area just north of Lauwersoog) and 7. all remaining eastern sampling points ('Wad east'). First, for each region and year, the average number of individuals per sample and the corresponding 95% confidence intervals are estimated using an intercept-only Generalized Linear Model (see Figure 14). Secondly, for each region, species abundance is modeled using year as a covariate. This will illustrate whether there was a significant overall decline or increase.

Such results should be considered with care. First of all, the 2004 and 2006 sampling scheme did not cover the entire Wadden Sea and hence it is not possible to define whether statistically significant changes in the area of land-subsidence are out of proportion compared to changes elsewhere. Secondly, only when we have improved our understanding of the natural processes that influence macrozoobenthos distribution, will we be able to quantify the exact effect of land subsidence.

2.8 Future framework for assessing changes in macrozoobenthos

The sampling campaign of 2008 presented here is the first synoptic sampling program, and hence will only consider the current status of the gas exploitation region and how it relates to other regions in the Wadden Sea. In the following years it is possible to assess any changes occurring. 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 2009 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 land subsidence, and one can test if such changes do not occur elsewhere.

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 around the island Griend 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 land subsidence and no other relevant variables, there is strong evidence that it has an effect.

3. Results

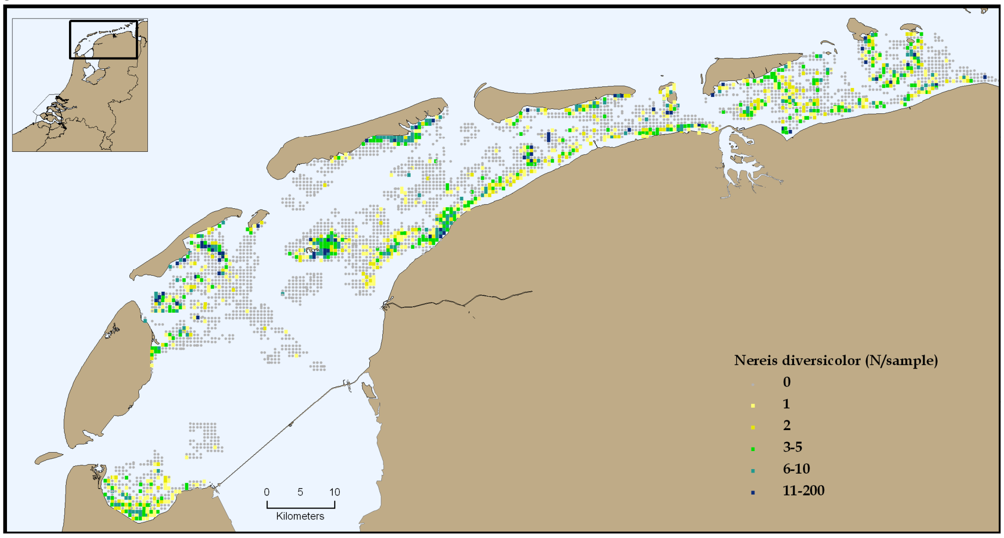
3.1 Sampling effort

In total 4376 stations were visited in the intertidal zone of the Wadden Sea. Of these, 413 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. 41 sampling stations with macrozoobenthos present according to observations in the field, did not appear in the laboratory analysis. These samples were probably lost during the field campaign or the PosKey may have been incorrectly recorded on the field lists. These sampling points were removed from the analysis. 6 samples analyzed in the laboratory could not be matched with PosKeys recorded on the field lists, and were also removed. These samples must belong to the 41 samples mentioned above
This resulted in a total of 3922 samples that could be used for the analysis, 332 of which were positioned on the random plus points. Based on these samples, a total of 162553 individuals were individually counted and measured.

3.2 Species specific Abundances and biodiversity measurements

In total 76 different species or genera have been identified. See Kraan et al. (2007) for a description of the most species. Table 1 provides for each species the number of individuals observed in the combined set of samples.
Figure 7 shows the spatial distribution 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 (*Nereis diversicolor*), Pacific giant oyster (*Crassostrea gigas*), Baltic tellin (*Macoma balthica*), bristleworm (*Scoloplos armiger*), Laver spire shell (*Hydrobia ulvea*) and a few rare species; bean-like tellin (*Tellina fabula)* and thin tellin (*Tellina tenuis)*

a.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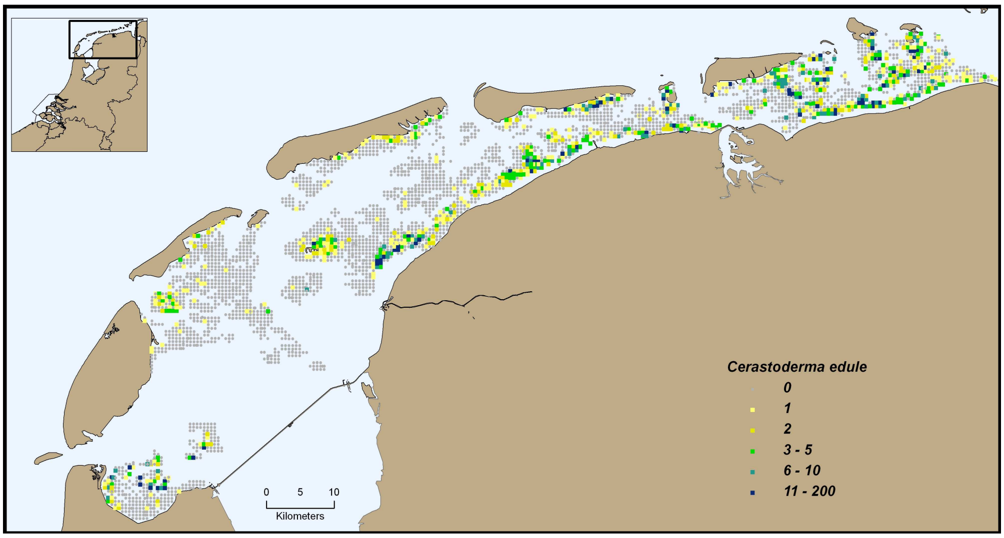
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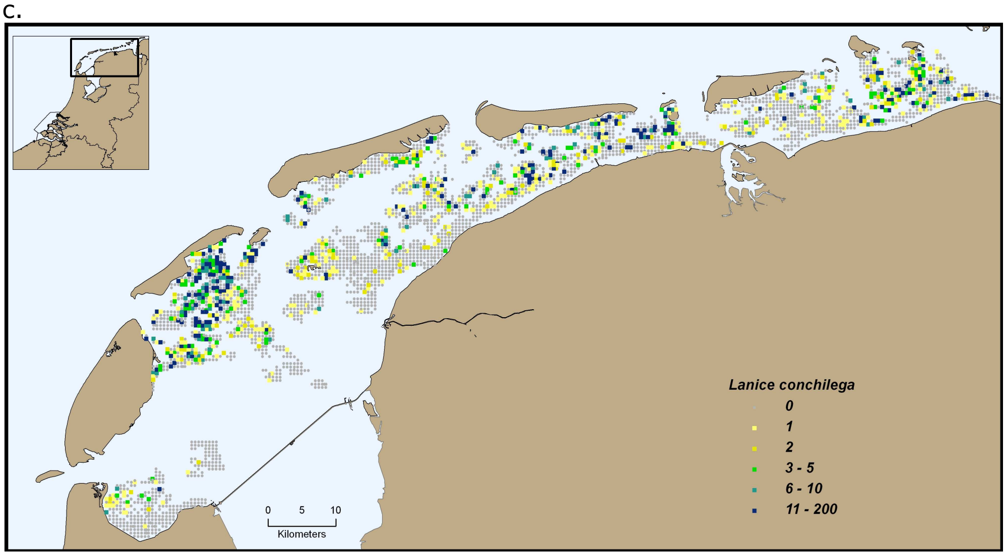
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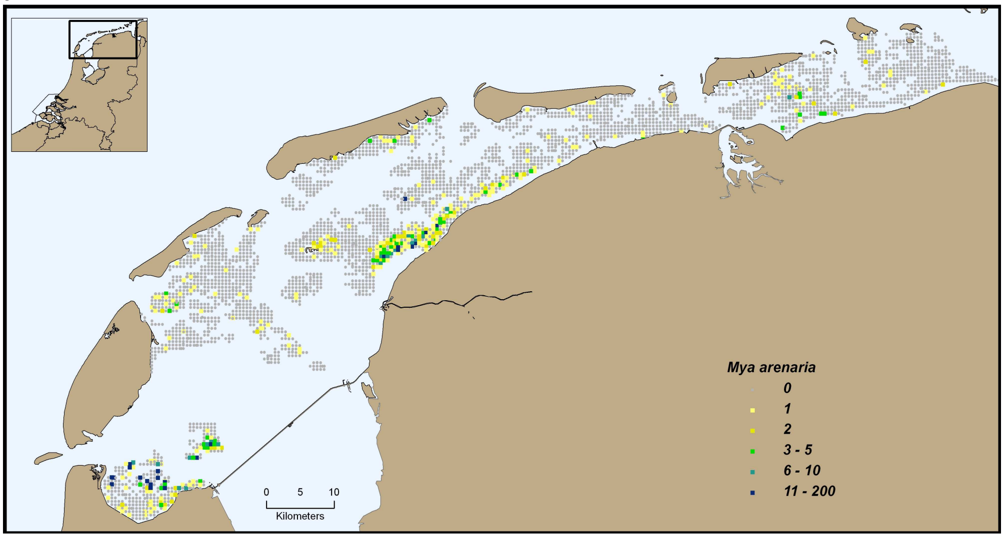
Mya arenaria
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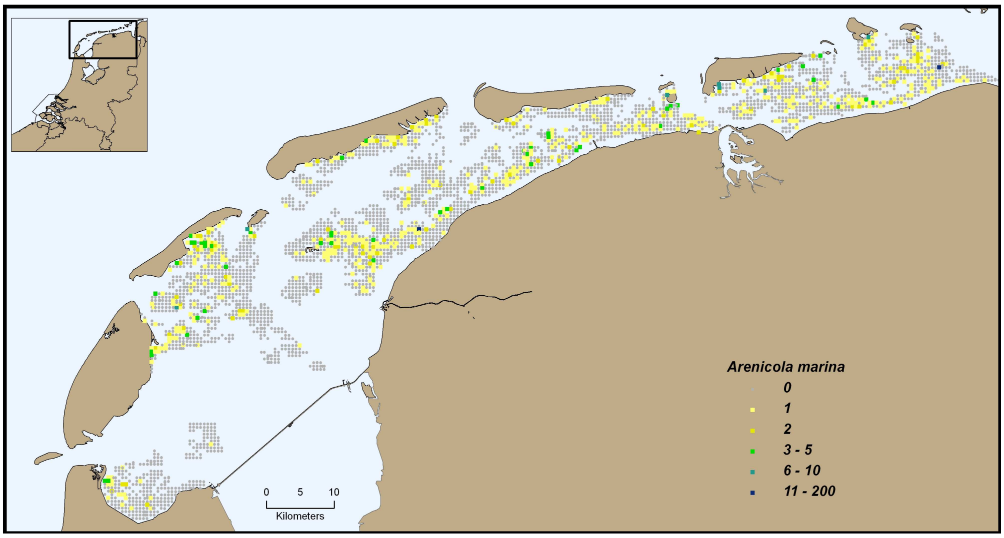
Kilometers

e.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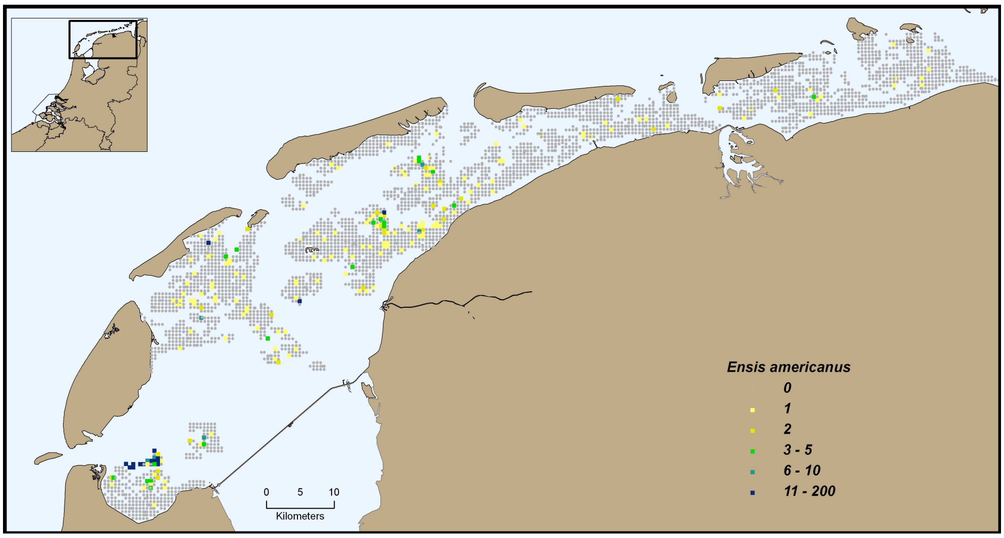
1
2
3 - 5
6 - 10
11 - 200
0 5 10
Kilometers

f.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2
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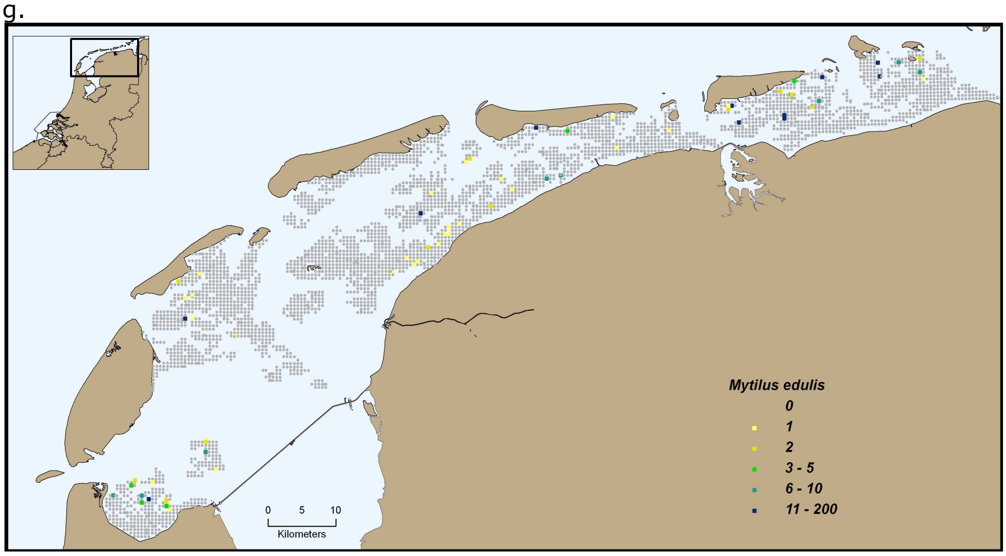
11 - 200
0 5 10
Kilometers

g.


Mytilus edulis
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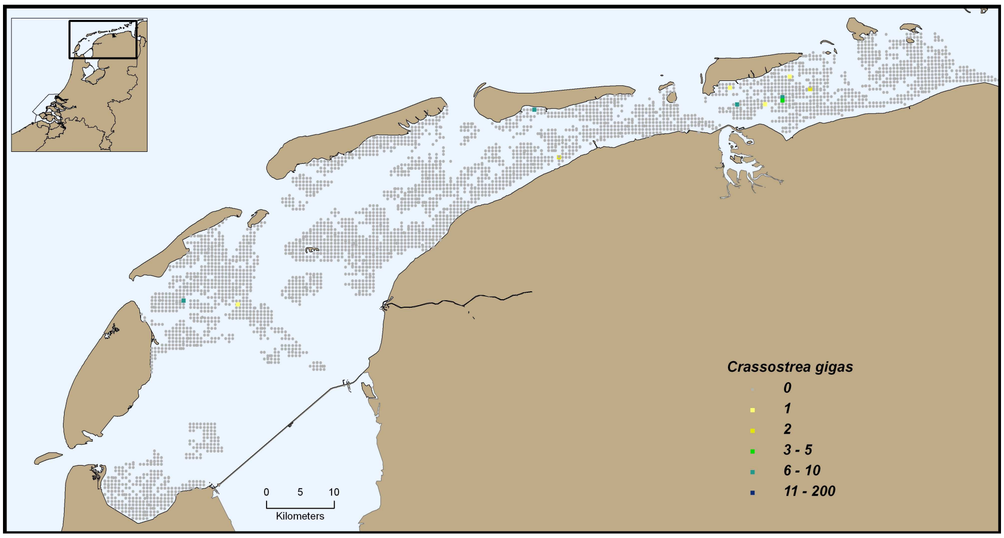
1
2
3 - 5
6 - 10
11 - 200
0 5 10
Kilometers

h.


Crassostrea gigas
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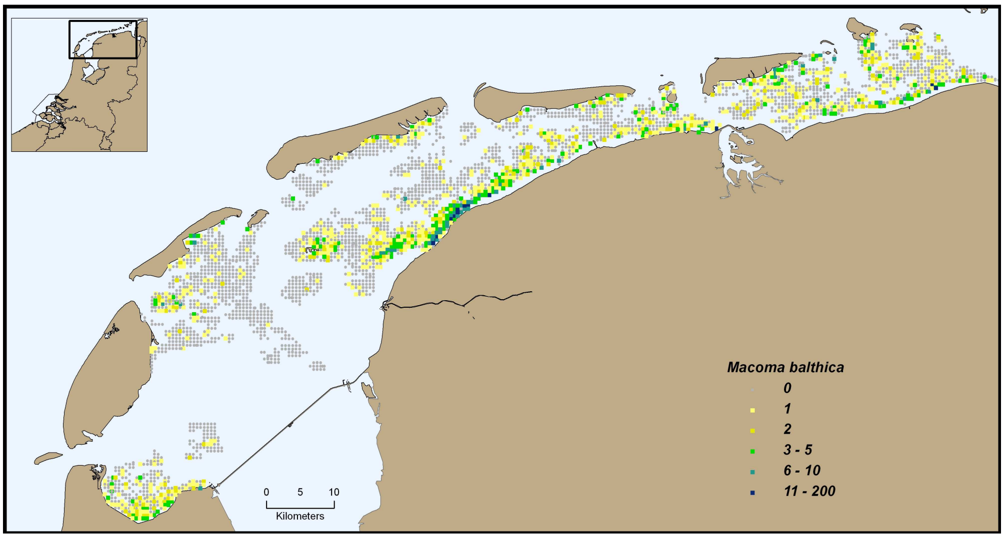
1
2
3 - 5
6 - 10
11 - 200
0 5 10
Kilometers

i.


Macoma balthica
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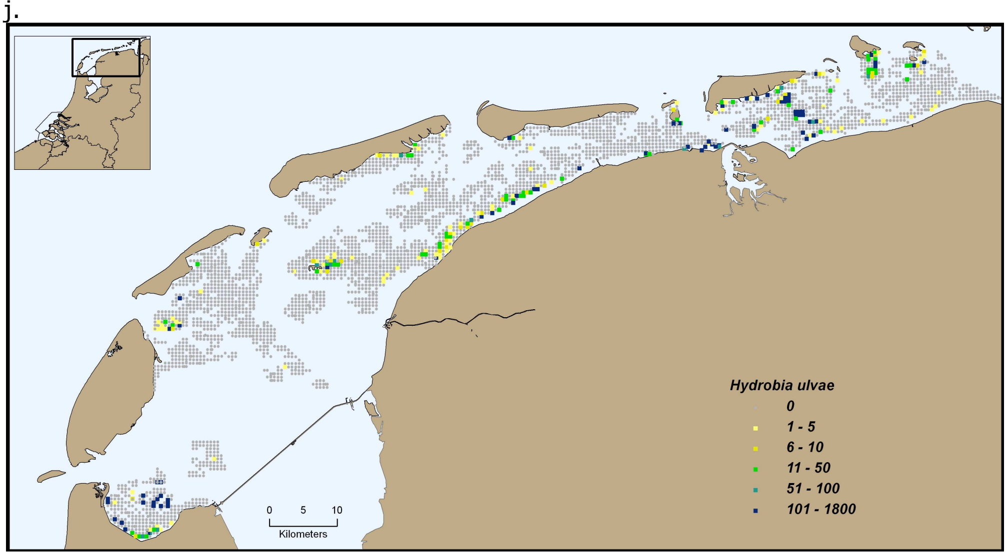
1
2
3 - 5
6 - 10
11 - 200
0 5 10
Kilometers

j.


Hydrobia ulvae
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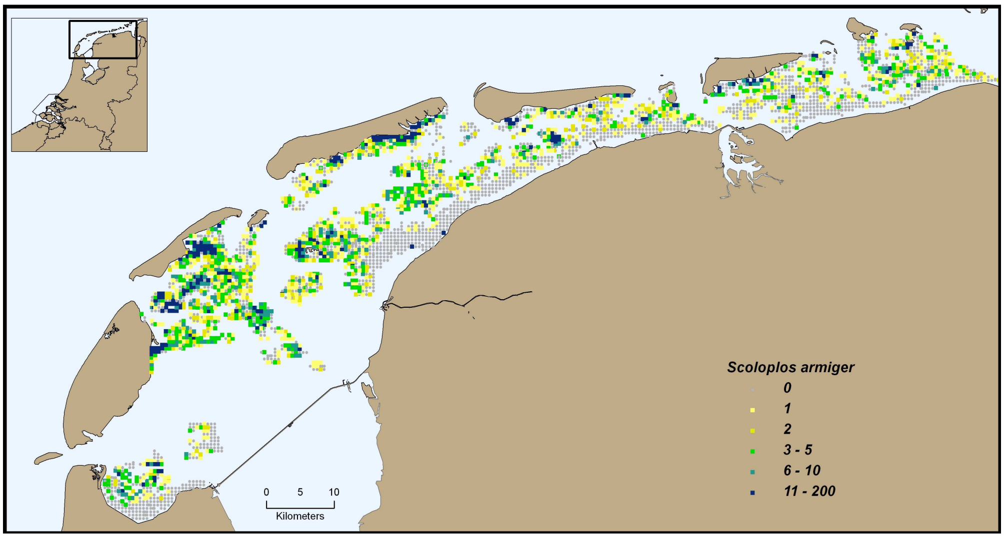
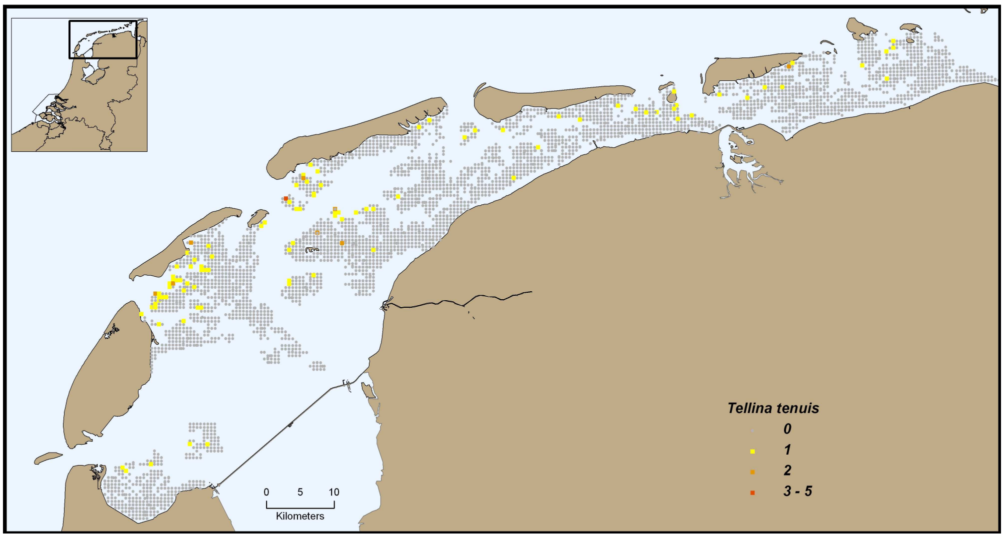
11 - 50
51 - 100
101 - 1800
0 5 10
Kilometers

k.


Scoloplos armiger
0
1
2
3 - 5
6 - 10
11 - 200
0 5 10
Kilometers

l.


Tellina tenuis
0
1
2
3 - 5
0 5 10
Kilometers

m.

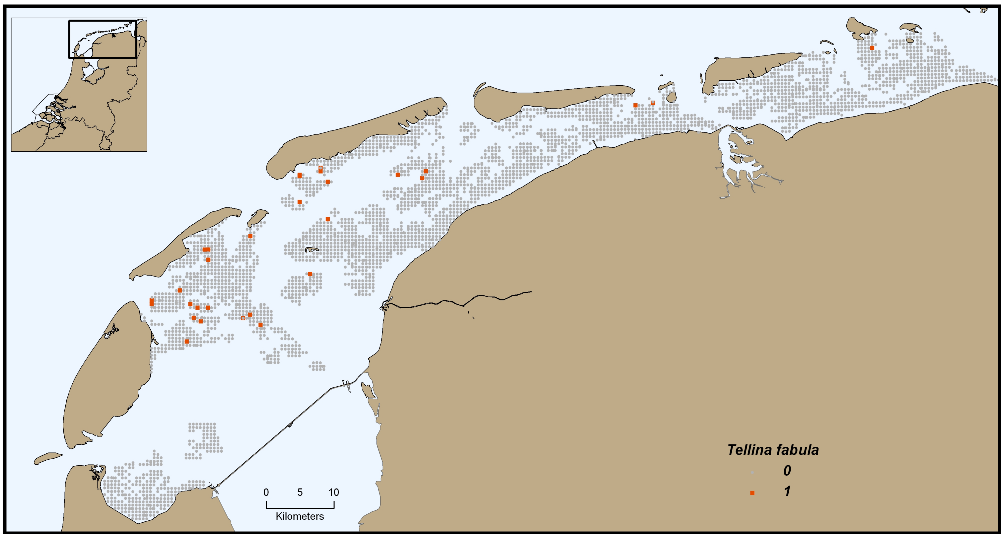


Figure 7 Spatial distribution of macrozoobenthos species a. Ragworm (*Nereis diversicolor*), b. Cockle (*Cerastoderma edule*), c. Sand mason (*Lanice conchilega*), d. soft-shell clam (*Mya arenaria*), e. Lugworm (*Arenicola marina*), f. American jack knife clam (*Ensis americanus*), g. Blue mussel (*Mytilus edulis*), h. Pacific giant oyster (*crassostrea gigas*), i. Baltic tellin (*Macoma balthica*), j. Laver spire shell (*Hydrobia ulvea*), k. bristleworm (*Scoloplos armiger*), l. thin tellin (*tellina tenuis)* and m. bean-like tellin (*tellina fabula)*.

Figure 7 illustrates most species occur throughout the Dutch Wadden Sea, but they differ considerably in there local preference. Some species, such as Ragworm (*Nereis diversicolor*), Cockle (*Cerastoderma edule*), soft-shell clam (*Mya arenaria*), Baltic tellin (*Macoma balthica*) and Laver spire shell (*Hydrobia ulvea*) prefer the muddy, higher regions. Also most slack-water areas (wantij), 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 plates.

Based on the number of individuals per sample it is possible to estimate the species richness for different areas in the Wadden Sea (Figure 8).

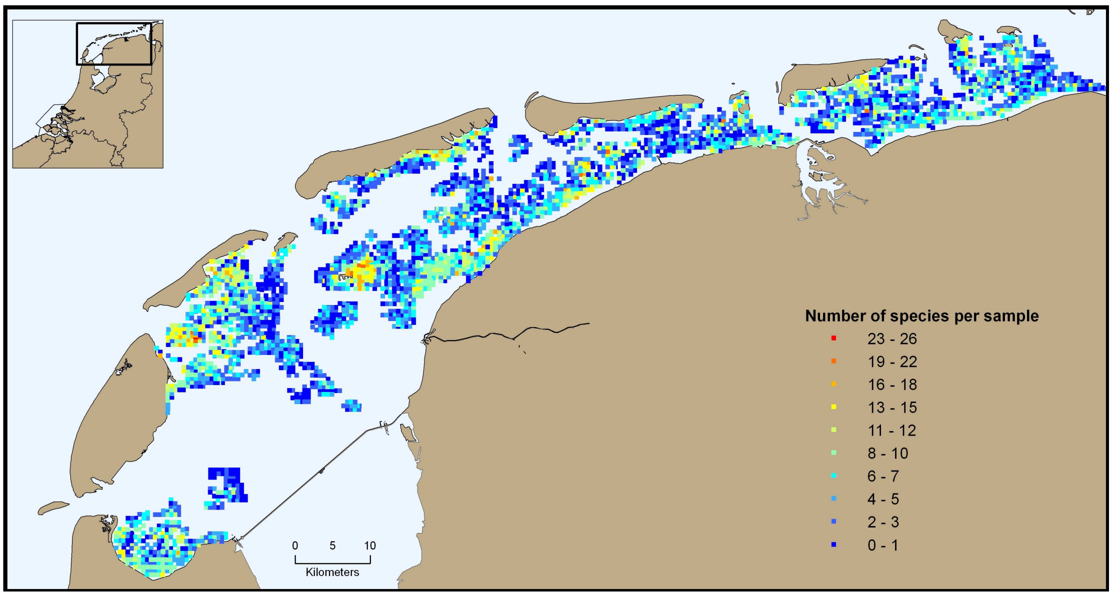


Figure 8. Distribution of species richness in the Wadden Sea.

In general it appears that the higher regions of 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 seperate growth, recruitment and mortality estimates. For example, Figure 9 shows the length distribution by age for Cerastoderma edule (cockle). Figure 10 shows the AFDM distribution for a selection of species; *Nereis diversicolor, Macoma balthica, Lanice conchilega, Hydrobia ulvae, Arenicola marina* and *Aphelochaeta marioni*. For *Hydrobia* two peaks are visible. The first peak show the distribution of recruits, while the second peak is probably a mixture of the older individuals.

Finally, the weight measurements could be used to estimate the total biomass of that species in the Wadden Sea. Table 1 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. Because the grid covers the entire intertidal region of the Wadden Sea, the ordering reflects the importance (in terms of biomass) of the different species. 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 1) shows that the Cockle, in terms of biomass, is the most important species. In terms of numbers of individuals, *Hydrobia* is most abundant.

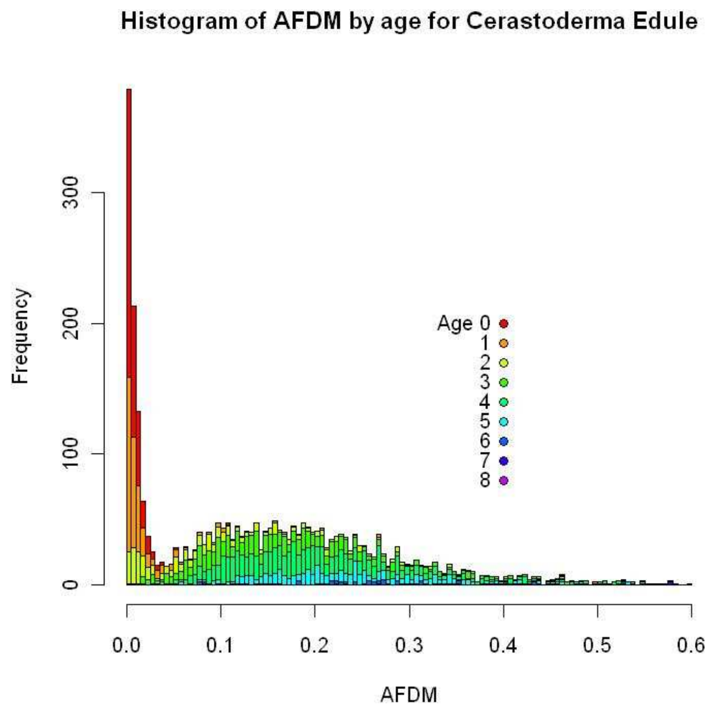


Figure 9 Cumulative histogram of AFDM by age for *Cerastoderma edule*. Ages are based on ring counts.

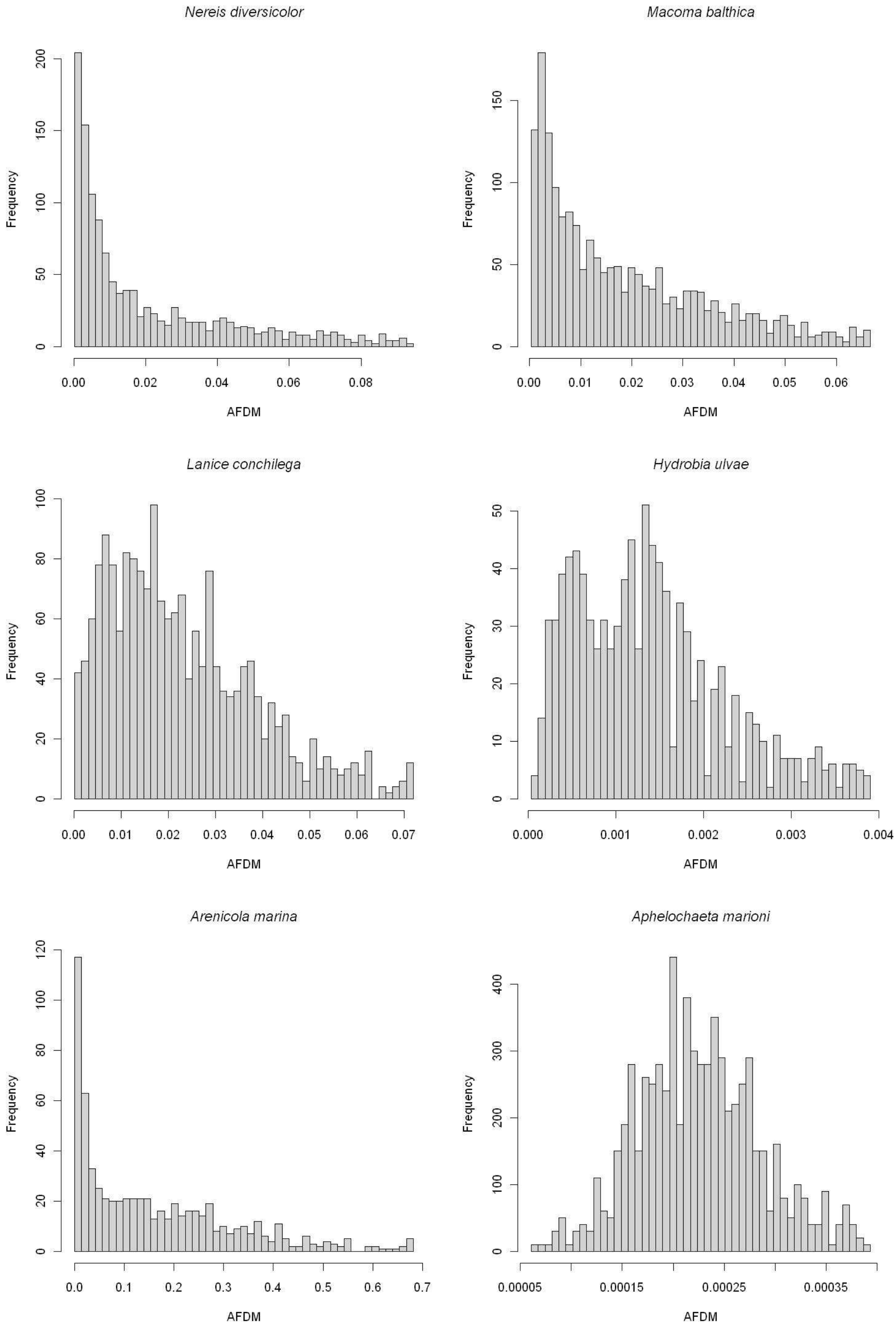


Figure 10 Distribution of AFDM per individual for some of the more abundant species.

Table 1. Number and AFDM of each species in the sample. N weighed is the number of individuals weighed, N the total number of individuals counted, AFDM the total Ash Free Dry Mass in gram and AFDM/individual is the average AFDM per individual.

Species name	N weighted	N	AFDM	AFDM/individual
Cerastoderma edule	3292	3293	454	0.1380
Lanice conchilega	9363	9406	183	0.0195
Mya arenaria	1323	1325	175	0.1320
Arenicola marina	726	841	102	0.1411
Ensis americanus	1825	1954	108	0.0590
Mytilus edulis	367	367	72	0.1952
Nereis diversicolor	3913	4014	68	0.0174
Crassostrea gigas	40	40	69	1.7280
Macoma balthica	1977	1979	43	0.0217
Carcinus maenas	450	451	34	0.0764
Hydrobia ulvae	12866	33471	10	0.0008
Scoloplos armiger	9775	9882	23	0.0024
Nereis virens	246	259	19	0.0773
Nephtys hombergii	819	896	18	0.0215
Scrobicularia plana	198	198	18	0.0932
Marenzelleria viridis	8328	8360	13	0.0016
Nereis succinea	449	458	9.0	0.0200
Nereis longissima	300	309	4.9	0.0164
Aphelochaeta marioni	18617	18696	4.1	0.0002
Corophium sp	8048	8052	3.3	0.0004
Urothoe poseidonis	8230	8230	3.2	0.0004
Oligochaeta sp	15433	15837	3.0	0.0002
Pygospio elegans	19049	19179	2.9	0.0002
Littorina littorea	43	43	2.7	0.0632
Crangon crangon	172	175	2.7	0.0154
Tellina tenuis	120	120	2.7	0.0224
Heteromastus filiformis	773	794	2.2	0.0028
Abra tenuis	1424	1424	2.1	0.0015
Capitella capitata	5271	5336	2.0	0.0004
Crepidula fornicata	13	13	1.7	0.1276
Nephtys cirrosa	215	223	0.99	0.0046
Echinocardium cordatum	5	5	0.97	0.1947
Petricola pholadiformis	12	12	0.91	0.0756
Polydora cornuta	2534	2539	0.85	0.0003
Nephtys caeca	124	131	0.74	0.0059
Eteone longa	645	648	0.71	0.0011
Tellina fabula	30	30	0.54	0.0180
Malmgreniella lunulata	178	185	0.46	0.0026
Hemigrapsus takanoi	4	4	0.45	0.1126
Nemertini sp	30	30	0.36	0.0120
Sagartia troglodytes	15	15	0.33	0.0221
Magelona johnstoni	39	39	0.31	0.0079
Nereis sp	74	77	0.30	0.0040
Phyllodoce maculata	175	176	0.24	0.0014
Gammarus sp	150	149	0.24	0.0016
Harmothoe sarsi	38	39	0.21	0.0055
Glycera alba	21	21	0.20	0.0095
Spio martinensis	974	974	0.19	0.0002
Bathyporeia sarsi	420	420	0.15	0.0004
Phyllodoce mucosa	111	111	0.14	0.0013
Spiophanes bombyx	206	206	0.14	0.0007
Pagurus bernhardus	3	3	0.14	0.0461
Metridium senile	14	14	0.13	0.0091
Scolelepis foliosa	8	8	0.11	0.0143
Streblospio shrubsolii	177	177	0.079	0.0004
Pectinaria koreni	3	3	0.075	0.0251
Autolytus prolifer	1	1	0.072	0.0716
Nephtys longosetosa	8	8	0.065	0.0082
Eumida sanguinea	74	74	0.061	0.0008
Lepidochitona cinerea	4	4	0.059	0.0148
Malacoceros fuliginosus	358	358	0.059	0.0002
Scolelepis bonnieri	19	19	0.056	0.0029
Mysella bidentata	15	15	0.032	0.0021
Mysta picta	16	16	0.030	0.0019
Pomatoschistus microps	1	1	0.028	0.0276
Abra alba	5	5	0.027	0.0055
Magelona mirabilis	11	11	0.022	0.0020
Manayunkia aestuaria	376	376	0.012	0.0000
Asterias rubens	2	2	0.012	0.0058
Travisia forbesii	2	2	0.010	0.0051
Harmothoe imbricata	6	6	0.007	0.0012
Nephtys spec	1	1	0.006	0.0059
Microphthalmus similis	9	9	0.002	0.0003
Harmothoe spec	1	1	0.002	0.0020

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

Based on the data points in- and outside of the area of future predicted land subsidence, it is possible to assess whether there is a difference between the two areas. The results are presented in Table 2. The 'Ameland-oost' and 'Moddergat-Lauwersoog-Vierhuizen' region were characterized by more individuals of *Hydrobia ulvae*, *Urothoe poseidonis*, *Lanice conchilega*, *Nephtys cirrosa*, *Malmgreniella lunulata*, *Phyllodoce mucosa*, *Magelona johnstoni* and *Nephtys longosetosa*. The *Scoloplos armiger* was less abundant. However, compared to the rest of the Wadden Sea, such deviations were only out of proportion for *Nephtys cirrosa*. The east Groningen region (except the Dollard region which is covered from 2009 onwards), was characterized by more *Cerastoderma edule*, *Urothoe poseidonis*, *Lanice conchilega*, and less *Scoloplos armiger*, *Pygospio elegans*, *Oligochaeta spec.*, *Marenzelleria viridis*, *Spio martinensis* and *Nereis virens*. Compared to the rest of the Wadden Sea only the differences observed in *Pygospio elegans*, *Marenzelleria viridis* and *Spio martinensis* was significantly different. These species are relatively small in size (some are <1mm) and not all individuals may be retained during the sieving process. So perhaps the differences in the sieving process (e.g. caused by sediment type) or differences in average size of these species in the north-east Groningen may also cause the observed difference in abundance.

Table 2. Assessment of the difference in species abundance between the region inside and outside the area of predicted land 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. Region 1 represents the Ameland and Moddergat-Lauwersoog-Vierhuizen region and region 2, the area north east of Groningen. Based on Monte-Carlo simulations comparing the deviations with regions elsewhere, only *Marenzelleria viridis*, *Pygospio elegans* and *Spio martinensis* are significantly less abundant in the north east Groningen region.

species	par. est. region 1	p-value region 1	par. est. region 2	p-value region 2
Abra alba	0.22	0.87	-15.39	0.99
Abra tenuis	-2.80	0.46	-4.34	0.59
Aphelochaeta marioni	0.15	0.47	-0.54	0.05
Arenicola marina	0.02	0.91	0.25	0.13
Asterias rubens	-14.89	0.99	-14.89	0.99
Autolytus prolifer	-15.18	0.99	-0.60	0.64
Bathyporeia sarsi	1.12	0.03	-2.54	0.28
Capitella capitata	-0.93	0.09	-1.37	0.04
Carcinus maenas	-1.08	0.06	-0.84	0.10
Cerastoderma edule	0.31	0.16	**0.67**	0.00
Corophium sp	-2.69	0.05	-5.94	0.38
Crangon crangon	-0.14	0.68	-0.80	0.08
Crassostrea gigas	1.18	0.17	-15.63	0.99
Crepidula fornicata	0.27	0.84	-15.03	0.99
Echinocardium cordatu	-15.32	0.99	-15.32	0.99
Ensis americanus	-2.66	0.43	-3.39	0.48
Eteone longa	-0.08	0.71	-0.30	0.19
Eumida sanguinea	0.66	0.15	-0.80	0.36
Gammarus sp	-0.55	0.45	-15.55	0.98
Glycera alba	-15.45	0.99	-15.45	0.99
Harmothoe imbricata	-14.90	1.00	-14.90	1.00
Harmothoe ljungmani	0.00	1.00	20.58	0.99
Harmothoe sarsi	0.65	0.23	0.21	0.72
Harmothoe spec	-14.89	0.99	-14.89	0.99
Hemigrapsus takanoi	1.32	0.37	-15.29	1.00
Heteromastus filiformis	-0.11	0.64	0.10	0.65
Hydrobia ulvae	**1.03**	0.00	-5.14	0.41
Lanice conchilega	**0.55**	0.01	**0.73**	0.00
Lepidochitona cinerea	-14.80	0.99	-14.80	0.99
Littorina littorea	0.49	0.55	0.28	0.75
Macoma balthica	0.23	0.11	0.19	0.19
Magelona johnstoni	**0.92**	0.01	0.12	0.80
Magelona mirabilis	-14.68	0.99	0.59	0.74
Malacoceros fuliginosus	-15.21	0.99	-15.21	0.99
Malmgreniella lunulat	**0.84**	0.00	-0.64	0.21
Manayunkia aestuaria	-15.17	0.99	-15.17	0.99
Marenzelleria viridis	-2.90	0.01	**-2.19**	**0.01**
Melita palmata	-14.89	1.00	-14.89	1.00
Metridium senile	-15.33	0.99	-15.33	0.99
Microphthalmus similis	-14.59	0.99	-15.59	1.00
Mya arenaria	-2.55	0.18	-3.19	0.21
Mysella bidentata	0.14	0.83	0.11	0.87
Mysta picta	0.11	0.89	-0.32	0.74
Mytilus edulis	-0.17	0.84	-1.39	0.33
Nemertini sp	-1.61	0.18	-1.48	0.18
Nephtys caeca	-0.21	0.60	-0.65	0.19
Nephtys cirrosa	**1.03**	0.00	-0.48	0.22
Nephtys hombergii	-0.05	0.74	0.00	0.99
Nephtys longosetosa	**2.30**	0.00	0.88	0.41
Nephtys spec	-15.07	0.99	-0.18	0.90
Nereis diversicolor	0.10	0.55	0.07	0.69
Nereis longissima	0.25	0.41	0.13	0.69
Nereis sp	-0.14	0.80	-1.21	0.17
Nereis succinea	0.44	0.21	0.36	0.31
Nereis virens	-0.09	0.87	**1.02**	0.00
Oligochaeta sp	-1.01	0.03	**-1.47**	0.01
Pagurus bernhardus	-15.58	0.99	-15.58	0.99
Pectinaria koreni	1.72	0.16	-14.89	0.99
Petricola pholadiformis	0.69	0.43	-15.62	0.99
Phyllodoce maculata	0.11	0.78	0.25	0.50
Phyllodoce mucosa	**1.25**	0.00	-0.08	0.90
Polydora cornuta	-0.23	0.47	-0.29	0.35
Pomatoschistus microp	-15.20	1.00	-15.20	1.00
Pygospio elegans	-1.23	0.02	**-2.16**	**0.01**
Sagartia troglodytes	-0.23	0.92	-14.83	0.99
Scolelepis bonnieri	-15.14	1.00	-15.14	1.00
Scolelepis foliosa	0.62	0.58	-15.68	0.99
Scoloplos armiger	**-0.95**	0.00	**-0.87**	0.00
Scrobicularia plana	0.47	0.09	0.43	0.13
Spio martinensis	-0.72	0.04	**-1.62**	**0.00**
Spiophanes bombyx	-1.38	0.44	-15.86	0.99
Streblospio shrubsolii	-0.48	0.53	-0.69	0.40
Tellina fabula	-0.24	0.74	-15.54	0.99

To assess whether the difference is significant, the Monte-Carlo simulations as described in the methods are used. Figure 11 and Figure 12 shows the distribution of the t-values. It is evident that the simulated t-distribution does not resemble the true t-distribution. In general, extreme values for t 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 t-distribution, we use the simulated t-distribution to derive the significance. Figure 11 shows a random selection of four species for which there is no significant difference between the area in- or outside the predicted land subsidence. Figure 12 shows the three species for which both the standard GLM and the comparison with simulations suggests that the abundance of *Spio martinensis*, *Pygospio elegans* and *Marenzelleria viridis* in the North east Groningen region differs significantly from regions elsewhere in the Wadden Sea.

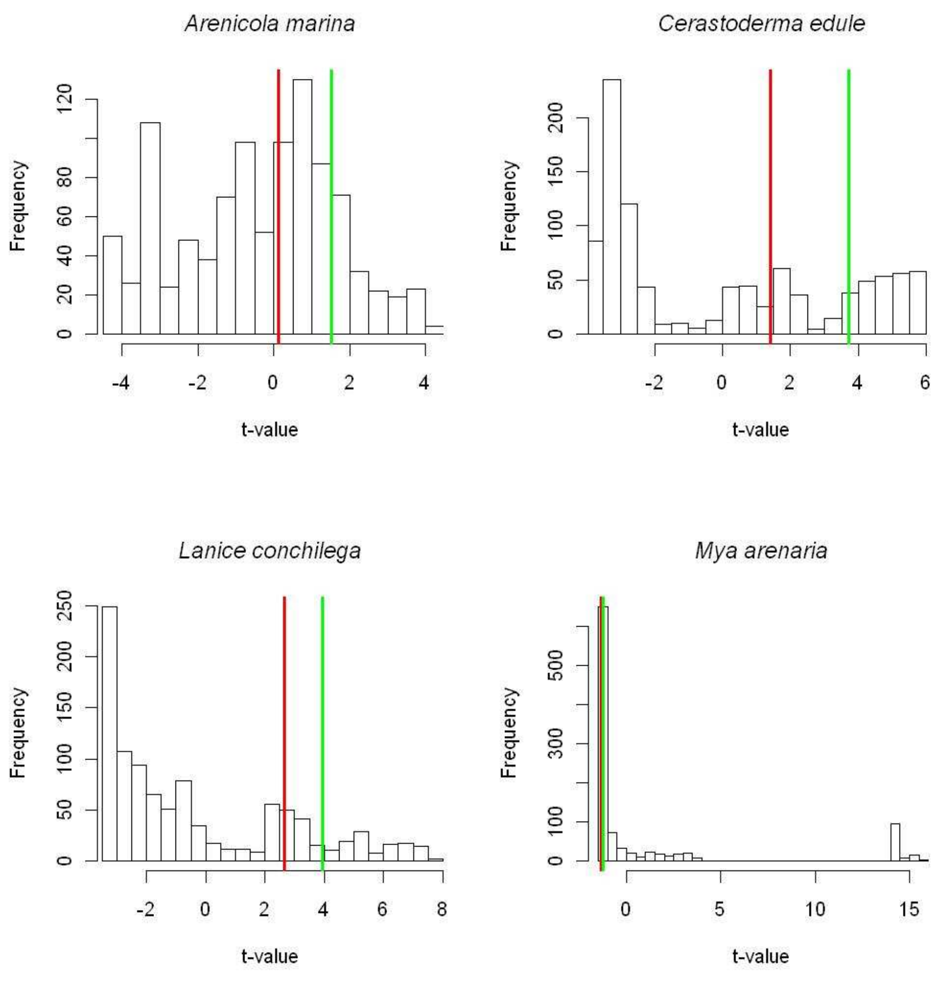


Figure 11 Example of the t-distribution obtained from 4 important species. None of these species significantly differ from other regions in the Wadden Sea. The red line represents Ameland-oost and Moddergat-Lauwersoog-Vierhuizen region and the green line North-east Groningen.

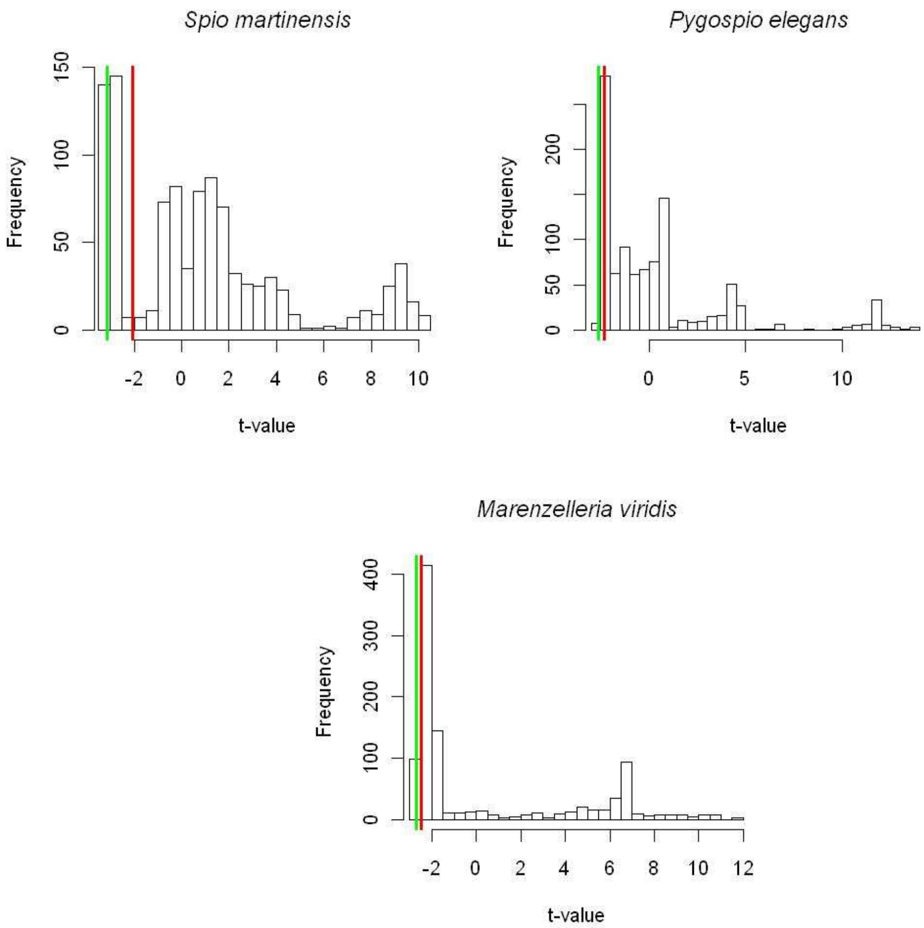


Figure 12. The three species which are significantly less abundant in the north-east Groningen region (green line).

3.5 Comparison with macrozoobenthos abundance in 2004 and 2006

Details on the distribution and abundance of macrozoobenthos observed in the 2004 and 2006 surveys can be found in Kraan et al. (2007). Figure 13 shows the distribution of sampling points in the 2004 and 2006 sampling program. Figure 14 shows the macrozoobenthos abundance for the years 2004, 2006 and 2008 for four abundant species. These results show that there is large variability between years and regions. For example, in 2008 *Macoma balthica* seems to have increased in the western Wadden Sea, while in the eastern Wadden Sea it declined. For another species, *Cerastoderma edule*, the patterns seems fairly consistent between the different regions.

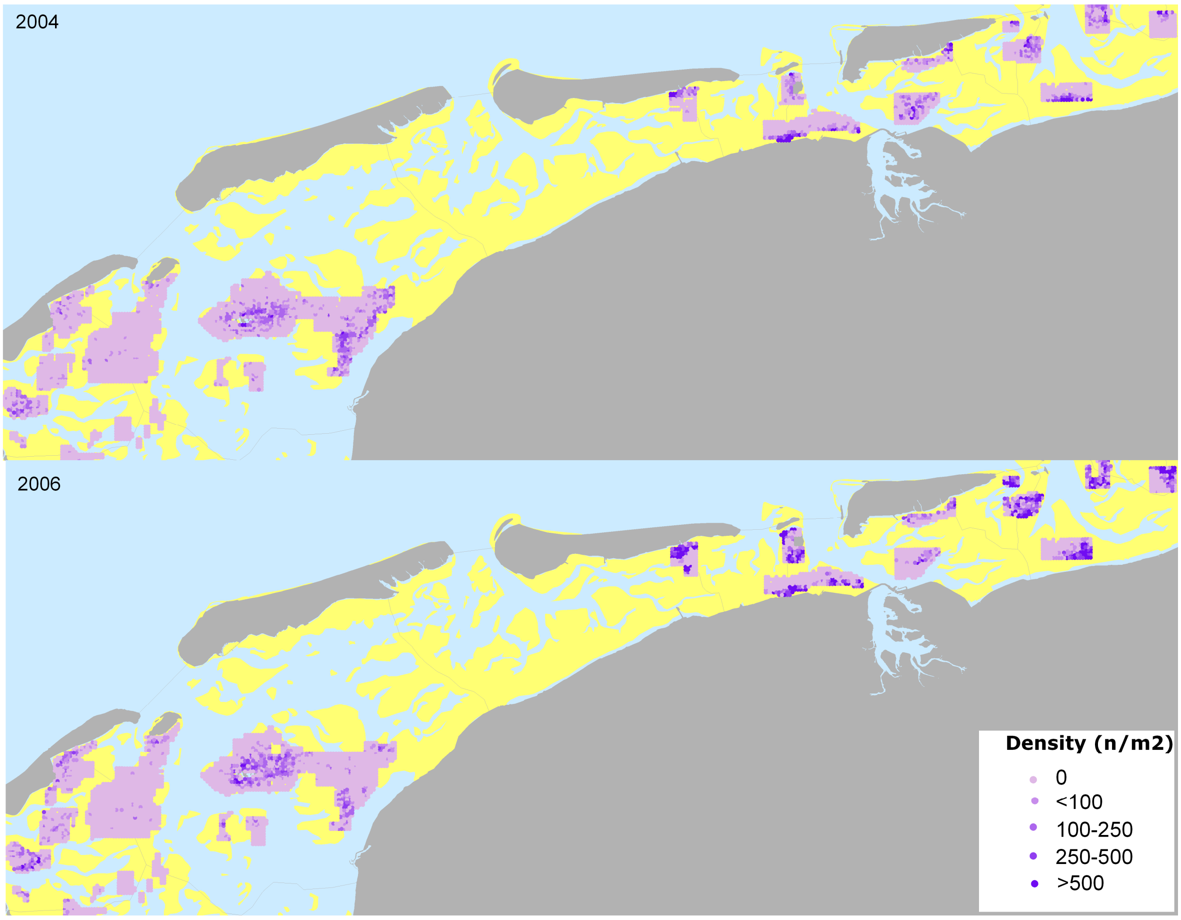


Figure 13 Example of the distribution of macrozoobenthos data (*Cerastoderma edule* in this case) collected in 2004 and 2006 (Kraan et al. 2007).

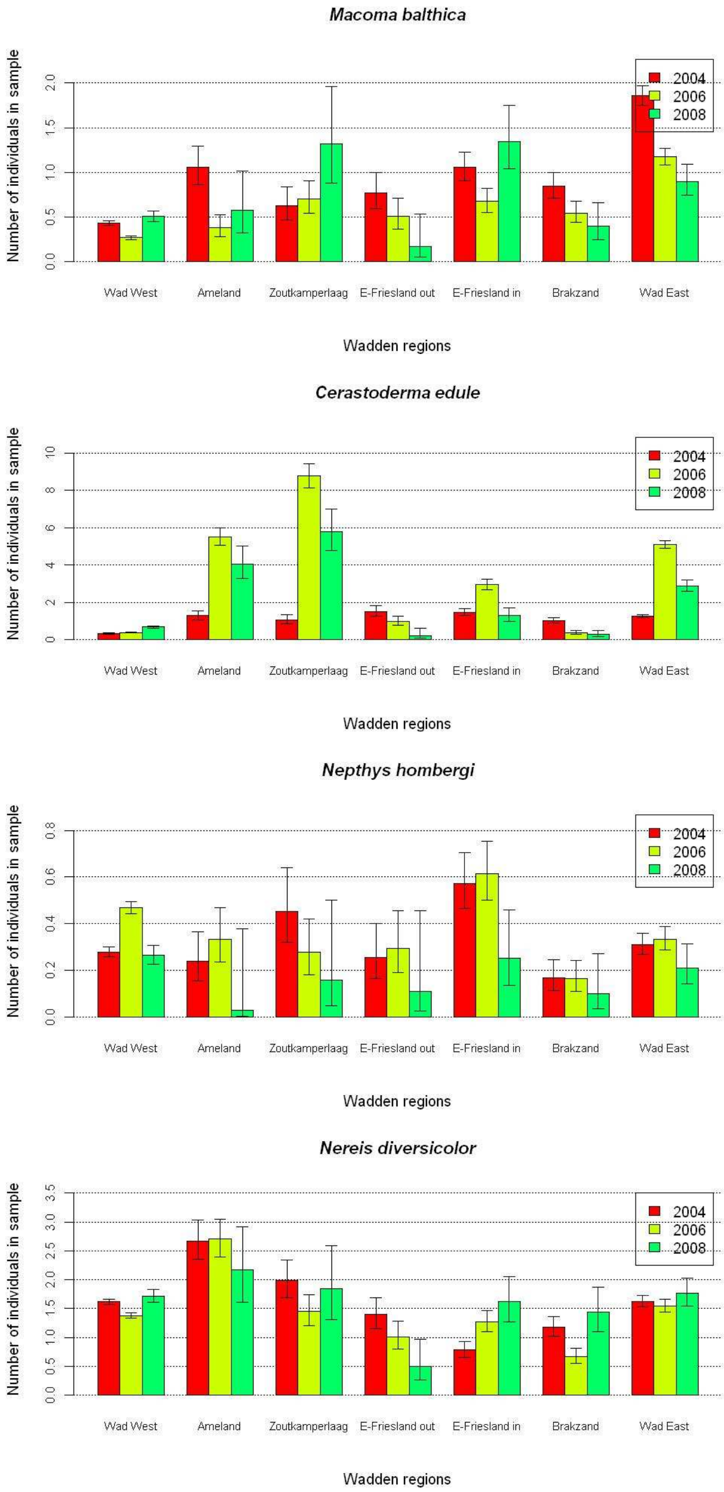


Figure 14. The number of species per sample for 2004, 2006 and 2008 for four abundant species; a. *Macoma balthica*, b. *Cerastoderma edule*, c. *Nepthys homergi* and d. *Nereis diversicolor*.

To provide information on all species for the regions of interest; Ameland and East-Frisian coast, Table 3 shows the overall decline or increase for each species. Table 4 shows the results which test for significant differences in the trend between the East Frisian mudflats in- and outside the area of expected land subsidence (>1cm from 2005 to 2009). These results suggest a larger increase or less severe decline in the region characterized by expected land subsidence for most species (*Carcinus maenas, Cerastoderma edule, Heteromastus filiformis, Hydrobia ulvae, Lanice conchilega, Macoma balthica, Marenzelleria wireni and Nereis diversicolor*). Only for *Ensis americanus*, the area of expected land subsidence is characterized by a decrease in abundance, while the area west of this shows a slight increase (see Table 3 and 4).

These results should be interpreted with care. First of all, due to the fact that the character of the sampling programs were quite different (500 versus 250m and synoptic versus local regions), only a subset of the data could be used for the comparison. In the upcoming years, the statistical power will improve considerably and a comparison will be based on a synoptic Wadden Sea wide sampling program complemented with additional data points in the region of expected land subsidence.

Table 3. The overall increase or decline (column 'estimate') for the areas characterized by land-subsidence; Ameland and the East Frisian mudflats. For the East-Friesland, a region within ('in') and outside ('out') predicted land subsidence can be defined.

species	region	estimate	p-value
Arenicola marina	Ameland	-0.03	0.853
	E-Friesland out	0.33	0.012
	E-Friesland in	0.1	0.28
Carcinus maenas	Ameland	0.09	0.804
	E-Friesland out	-0.96	0.061
	E-Friesland in	0.01	0.943
Cerastoderma edule	Ameland	0.32	<0.001
	E-Friesland out	-0.3	<0.001
	E-Friesland in	0.09	<0.001
Corophium volutator	Ameland	-9.17	0.997
	E-Friesland out	0	1
	E-Friesland in	9.75	0.994
Crangon crangon	Ameland	-0.94	0.012
	E-Friesland out	-9.06	0.996
	E-Friesland in	-0.34	0.218
Ensis americanus	Ameland	-9.02	0.996
	E-Friesland out	0.92	0.023
	E-Friesland in	-9.01	0.994
Eteona longa	Ameland	0.52	0.061
	E-Friesland out	0.37	0.457
	E-Friesland in	0.33	0.018
Harmothoe Sp.	Ameland	0.09	0.804
	E-Friesland out	0.37	0.457
	E-Friesland in	0.33	0.635
Heteromastus filiformis	Ameland	-0.11	0.01
	E-Friesland out	-0.2	<0.001
	E-Friesland in	-0.09	0.011
Hydrobia ulvae	Ameland	-9.02	0.996
	E-Friesland out	-8.71	0.996
	E-Friesland in	3.06	<0.001
Lanice conchilega	Ameland	0.27	<0.001
	E-Friesland out	-1.3	<0.001
	E-Friesland in	-0.36	<0.001
Macoma balthica	Ameland	-0.33	<0.001
	E-Friesland out	-0.27	0.001
	E-Friesland in	-0.01	0.837
Marenzelleria wireni	Ameland	-0.09	0.617
	E-Friesland out	-1.37	0.007
	E-Friesland in	0	0.98
Mya arenaria	Ameland	-0.26	0.214
	E-Friesland out	-0.51	0.179
	E-Friesland in	-0.13	0.614
Mytilus edulis	Ameland	0.37	0.07
	E-Friesland out	0.37	0.457
	E-Friesland in	0.18	0.029
Nepthys hombergi	Ameland	-0.06	0.577
	E-Friesland out	-0.07	0.56
	E-Friesland in	-0.09	0.084
Nereis diversicolor	Ameland	-0.03	0.394
	E-Friesland out	-0.2	0.001
	E-Friesland in	0.19	<0.001
Phyllodoce maculata	Ameland	0.74	0.097
	E-Friesland out	0	1
	E-Friesland in	-8.82	0.997
Pygospio elegans	Ameland	0.79	<0.001
	E-Friesland out	10.07	0.992
	E-Friesland in	1.83	<0.001
Scoloplos armiger	Ameland	-0.2	<0.001
	E-Friesland out	-0.12	0.281
	E-Friesland in	-0.02	0.772
Scrobicularia plana	Ameland	0.04	0.824
	E-Friesland out	0.15	0.54
	E-Friesland in	-0.03	0.888
Urothoe Sp.	Ameland	-0.37	0.36
	E-Friesland out	0.95	<0.001
	E-Friesland in	0.91	<0.001

Table 4. Testing the difference in the change in abundance between the area inside or outside the region of expected land subsidence. AIC without interaction (int.) represents the AIC of a model only including the covariate year and a factor indicating whether the data comes from within or outside the region of expected land subsidence. The AIC with interaction represents the AIC of a model which also includes the interaction between year and region. The column 'interaction' shows the model estimates. Positive values suggest that the increase was larger or decline was less severe within the area of expected land subsidence. The column significant, indicates whether the interaction term was significant (ΔAIC > 2).

Species	AIC without int.	AIC with int.	AIC diff	interaction	Significant
Arenicola marina	528.18	528.80	-0.62	-0.19	FALSE
Carcinus maenas	272.20	268.96	3.24	0.97	TRUE
Cerastoderma edule	4133.51	4098.97	34.54	0.39	TRUE
Corophium volutator	36.77	38.77	-2.00	9.75	FALSE
Crangon crangon	123.27	123.75	-0.48	7.72	FALSE
Crepidula fornicata	15.57	17.57	-2.00	9.57	FALSE
Ensis americanus	90.56	80.28	10.28	-9.41	TRUE
Eteona longa	234.50	236.50	-1.99	-0.04	FALSE
Gammarus locusta	92.77	94.77	-2.00	0.33	FALSE
Harmothoe Sp.	40.74	42.73	-2.00	-0.04	FALSE
Heteromastus filiformis	3068.18	3065.54	2.64	0.12	TRUE
Hydrobia ulvae	5216.23	5197.25	18.98	8.78	TRUE
Lanice conchilega	1778.34	1753.35	24.99	0.93	TRUE
Littorina littorea	19.57	21.57	-2.00	0.33	FALSE
Macoma balthica	1516.69	1510.42	6.28	0.26	TRUE
Marenzelleria wireni	304.81	288.65	16.16	8.97	TRUE
Mya arenaria	148.57	149.84	-1.27	0.38	FALSE
Mysella bidentata	46.73	48.73	-2.00	-0.05	FALSE
Mytilus edulis	633.01	634.87	-1.86	-0.19	FALSE
Nepthys hombergi	949.53	951.49	-1.96	-0.02	FALSE
Nereis diversicolor	2386.81	2354.17	32.63	0.39	TRUE
Phyllodoce maculata	18.20	20.20	-2.00	-9.32	FALSE
Pygospio elegans	495.28	494.66	0.62	-7.24	FALSE
Scoloplos armiger	1155.68	1156.73	-1.04	0.12	FALSE
Scrobicularia plana	218.85	220.52	-1.67	-0.17	FALSE
Tellina tenuis	87.52	89.13	-1.61	-0.48	FALSE
Urothoe Sp.	3719.43	3721.37	-1.94	-0.04	FALSE
Nereis virens	15.57	17.57	-2.00	9.57	FALSE

4. Discussion & Conclusions

This report endeavored to provide an overview of the data collected in 2008 and show how it can be used to understand the spatial distribution and demography of a large number of species. In total 3963 stations have been sampled, in which a total of 76 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 derivates (such as annual growth and mortality) can be used in assessing the possible effect of land subsidence.

In terms of biomass, the most important species were Cockle (*Cerastoderma edule*), Sand mason (*Lanice conchilega*), softshell clamm (*Mya arenaria*), Lugworm (*Arenicola marina*), American jack knife clam (*Ensis americanus*), Blue mussel (*Mytilus edulis*), Ragworm (*Nereis diversicolor*), Pacific giant oyster (*Crassostrea gigas*) and Baltic tellin (*Macoma balthica*).

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 7), 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 7). Combining the observations allows for the estimation of species richness (see Figure 8). 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.

The comparison in species abundances based on the 2004, 2006 and 2008 data, shows very large variability between regions and years (Figure 14). For one region, the East Frisian coast north of Moddergat, sample points from the 2004, 2006 and 2008 survey are positioned both in- and outside the region of expected land subsidence. For eight species, the area just north of Moddergat is characterized by a larger increase or less severe decline compared to the more western region with less or no expected land subsidence. For one species, *Ensis americanus*, the reverse is true. These results should be treated with care, because the mismatch in spatial resolution and extend between the current SIBES sampling program and the programs in previous years, did not allow us to use all data points.

This dataset will be used to carry out future assessments investigating the possible effect of land 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. (2009) 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 land subsidence.

The Wadden Sea is a highly heterogeneous environment in both space and time. It can be difficult to tell apart the effect of land 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 land subsidence.

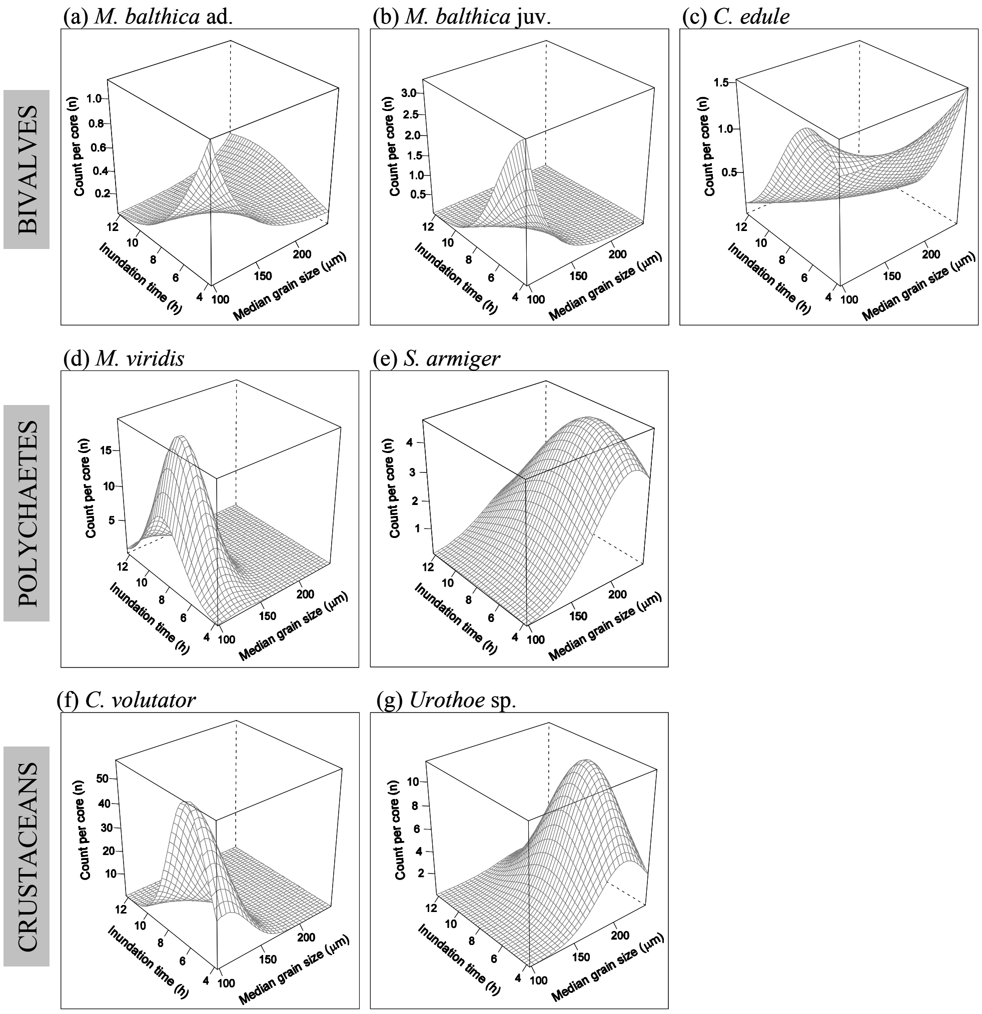


Figure 15 The preference of six individual species for two major environmental variables; inundation time and median grain size. These results show large species-specific variability. For example *Marenzelleria viridis* shows a strong preference for fine substrate, while *Scoloplos armiger* mostly prefers course sediment.

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Appendix A: Response to the 2008 Audit Commission

1. *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 extend 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 below shows the correlograms (based on the Moran's I) of most species. It can be seen that for the majority of species, the abundances are still correlated at distances > 500 meter.

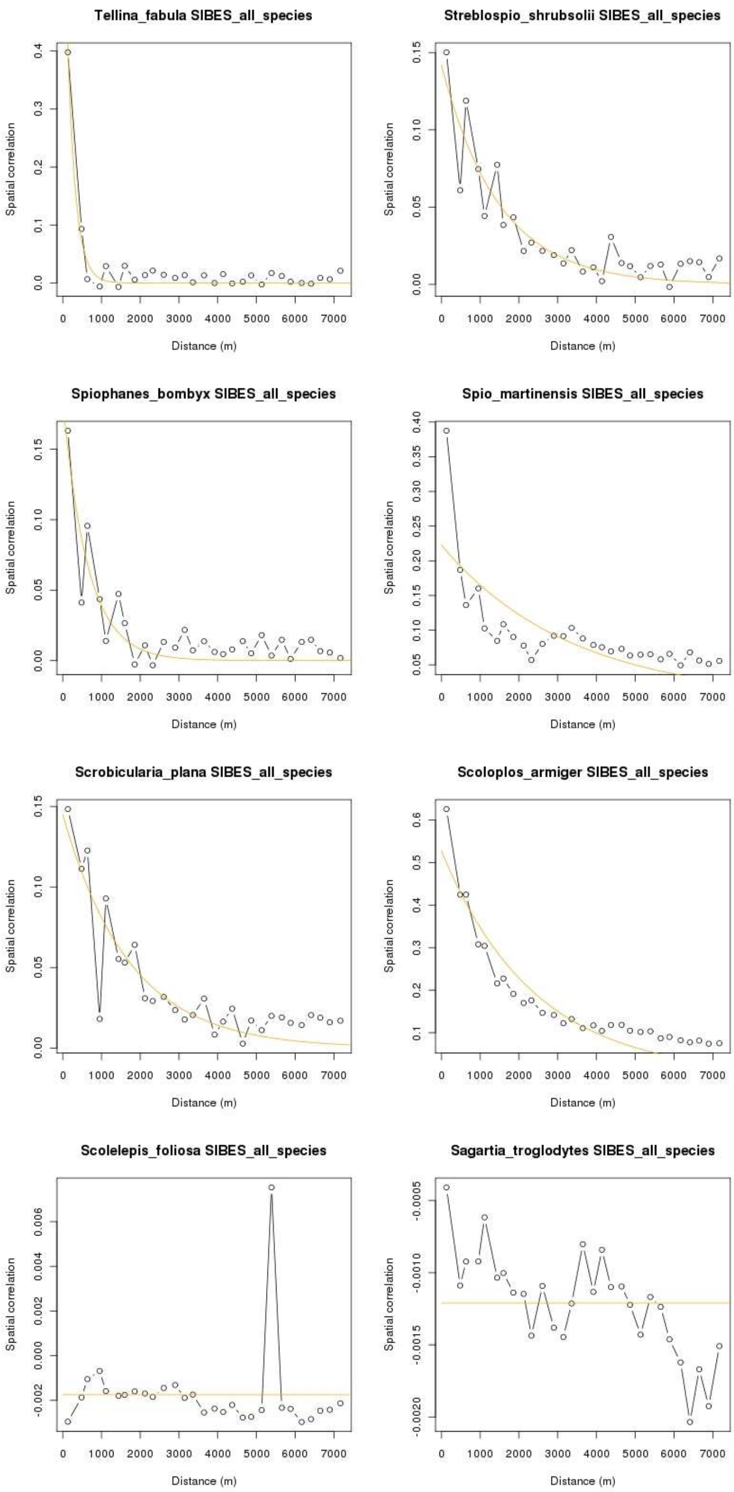
The results were not available last year, and therefore, to address the concern of the Audit commission expressed last year (2009), the 2009 sampling scheme has been extended. Currently each sampling station within 5 km of gas exploitation station, has been supplemented with another sample positioned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

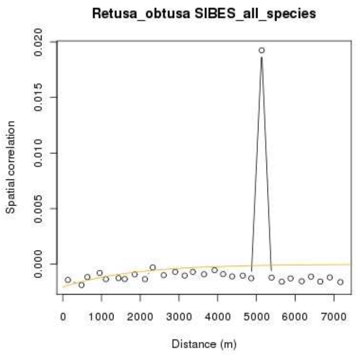
Alternatively, to define the most appropriate sample size, one could carry out some sort of power analysis. However, we argue that this is cannot be done at this stage. Any environmental variable (physical (e.g. current velocity or sediment type) , chemical (e.g. nitrogen content) or biological (e.g. macrozoobenthos abundance or bird breeding success)) that could potentially be influenced by natural gas exploitation, will also be influenced by other natural or human related processes. So to determine an effect it is first essential to know what proportion of the effect of these natural processes can be accounted for using some sort of model framework. For many biological processes, ecologist are just on the verge of developing such models (see e.g. Kraan et al. 2010). The challenge ahead is to further improve these models for macrozoobenthos using the synoptic sampling program. Only after this, a proper evaluation of the sampling scheme can be carried out.

2. *The audit commission advices to include the change in height of the tidal 'plates' (based on vaklodingen Rijkswaterstaat en GPS-metingen) in the macrofauna analysis.*

Data on the previous cycle (nr. 3) and the most recent cycle (nr 4.) is available. See Figure 5. However the uncertainty in the data (± 10 cm), is considerably larger compared to expected land subsidence due to natural gas extraction. So these maps could probably not be subtracted from one another to obtain an estimate of the amount of raise and declination due to land subsidence. However the data may still be used as a proxy to investigate the effect of any height in- or decrease on macrozoobenthos distribution. The feasibility of this approach is currently unknown to the authors of this report.

Figure 16. Below spatial correlogram of most species. It can be seen that many species are correlated at distance > 500 m.

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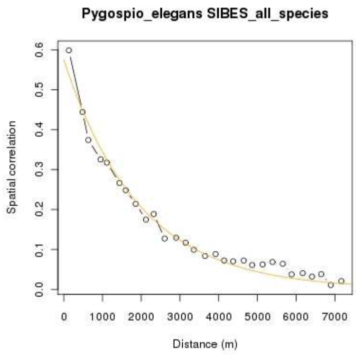
Spatial correlation
Distance (m)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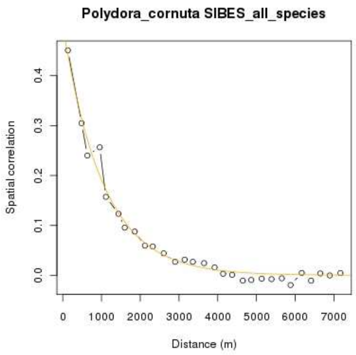
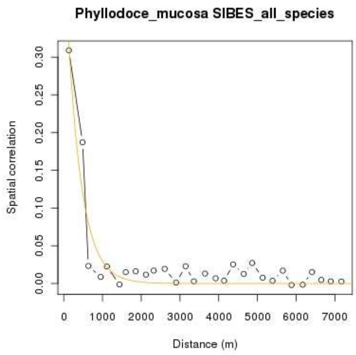
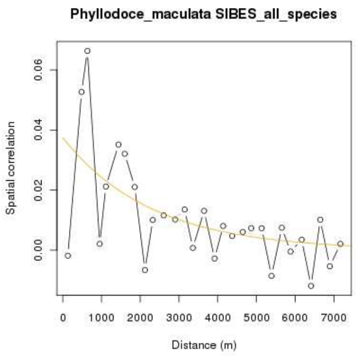
Spatial correlation
Distance (m)


Polydora_cornuta SIBES_all_species
Spatial correlation
Distance (m)


Phyllodoce_mucosa SIBES_all_species
Spatial correlation
Distance (m)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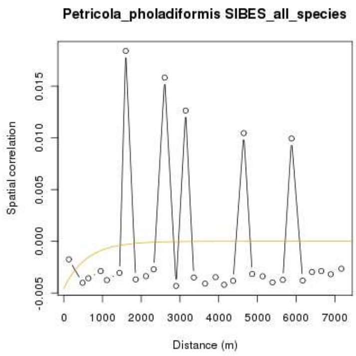
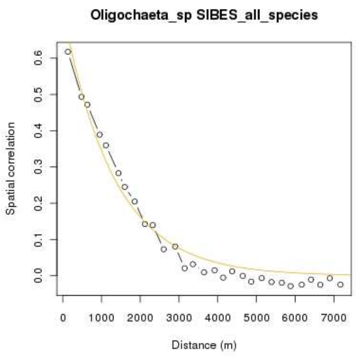
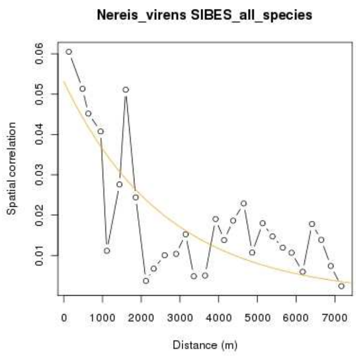
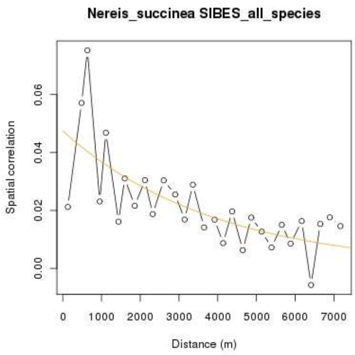
Spatial correlation
Distance (m)


Petricola_pholadiformis SIBES_all_species
Spatial correlation
Distance (m)


Oligochaeta_sp SIBES_all_species
Spatial correlation
Distance (m)


Nereis_virens SIBES_all_species
Spatial correlation
Distance (m)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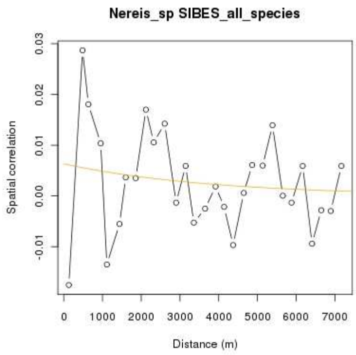
Spatial correlation
Distance (m)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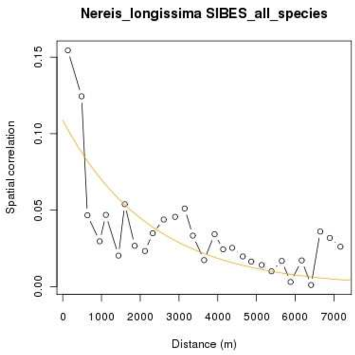
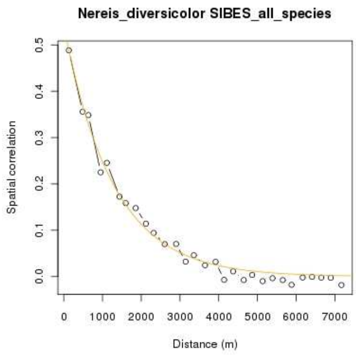
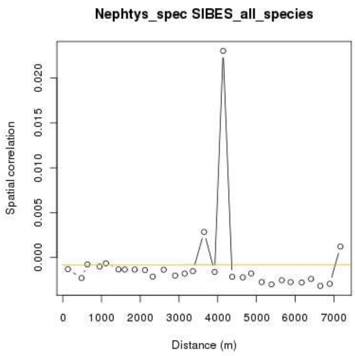
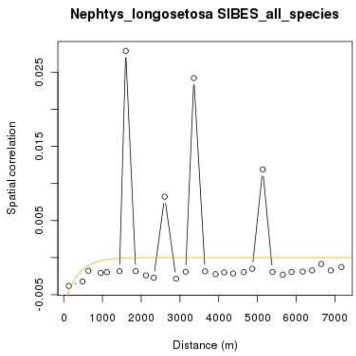
Spatial correlation
Distance (m)


Nereis_longissima SIBES_all_species
Spatial correlation
Distance (m)


Nereis_diversicolor SIBES_all_species
Spatial correlation
Distance (m)


Nephtys_spec SIBES_all_species
Spatial correlation
Distance (m)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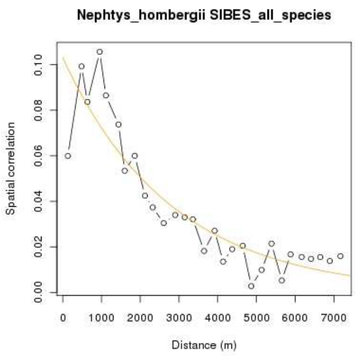
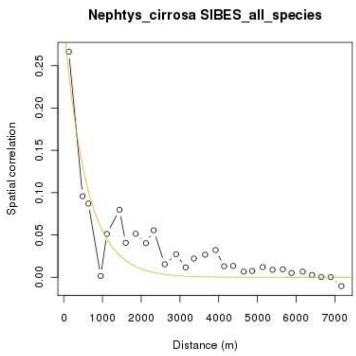
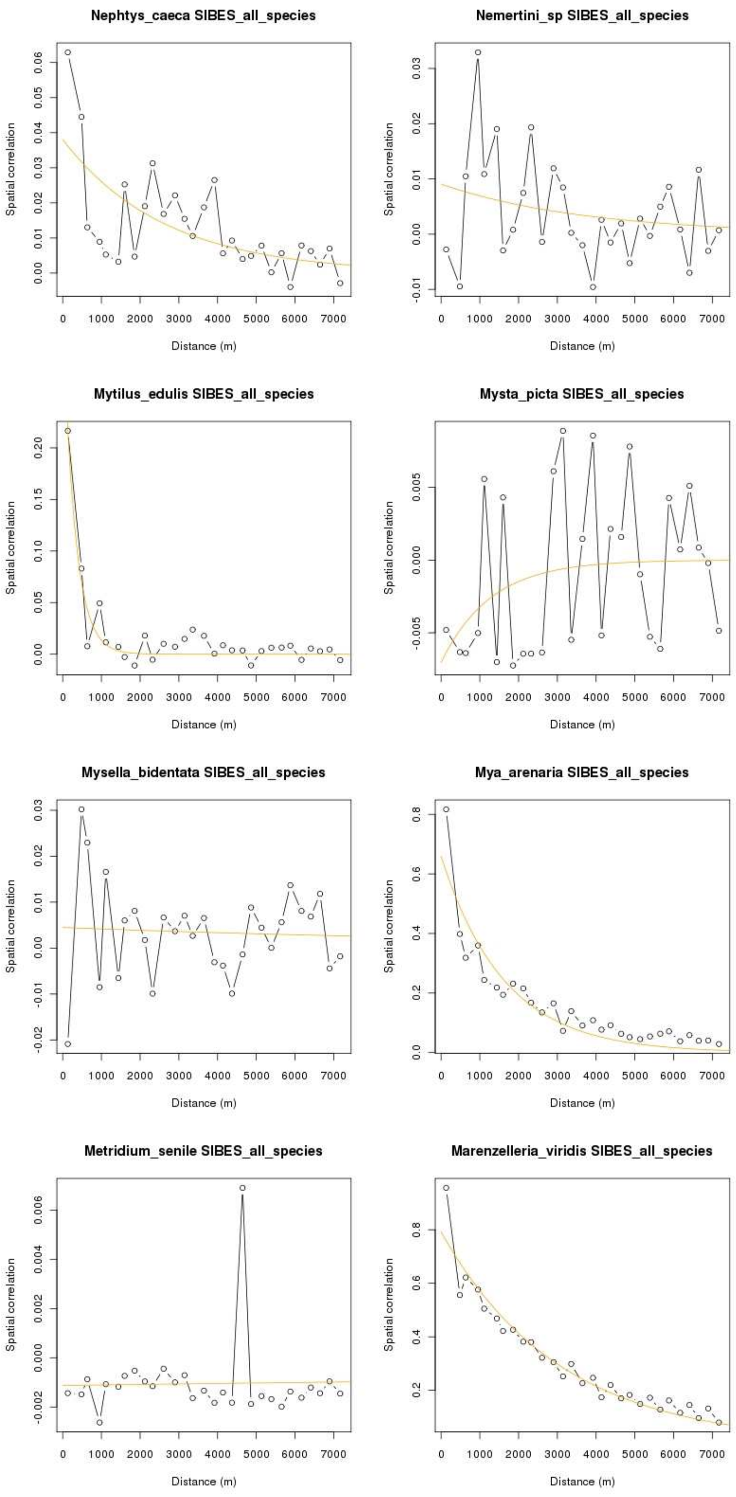
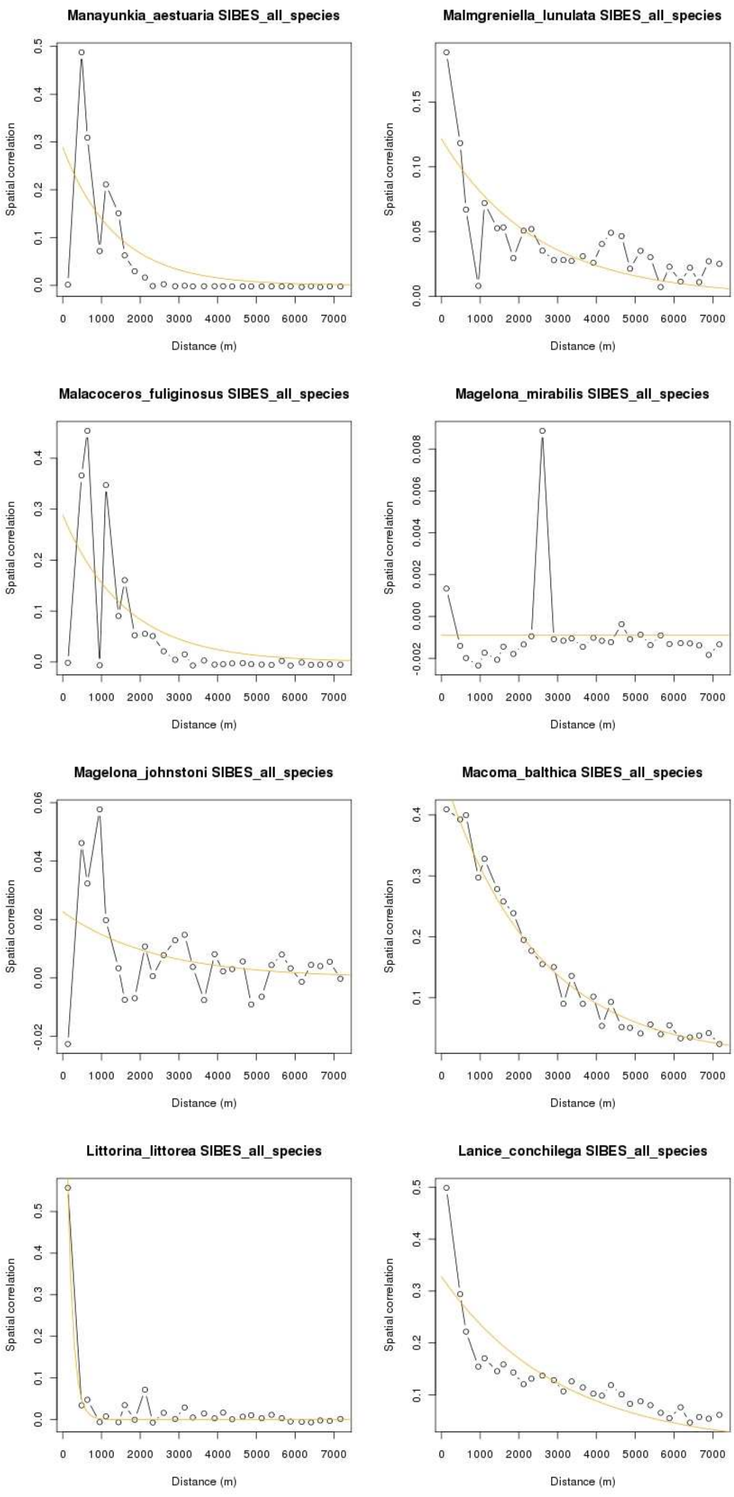
Spatial correlation
Distance (m)


Nephtys_hombergii SIBES_all_species
Spatial correlation
Distance (m)


Nephtys_cirrosa SIBES_all_species
Spatial correlation
Distance (m)


Nephtys_caeca SIBES_all_species
Nemertini_sp SIBES_all_species
Mytilus_edulis SIBES_all_species
Mysta_picta SIBES_all_species
Mysella_bidentata SIBES_all_species
Mya_arenaria SIBES_all_species
Metridium_senile SIBES_all_species
Marenzelleria_viridis SIBES_all_species
Spatial correlation
Distance (m)


Manayunkia_aestuaria SIBES_all_species
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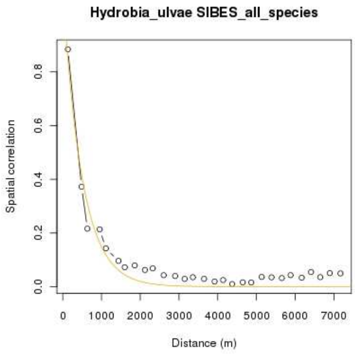
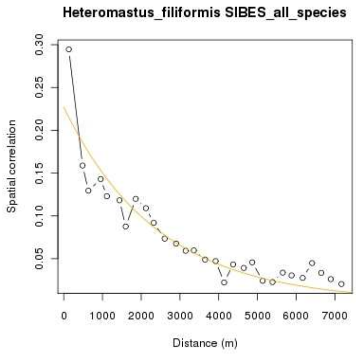
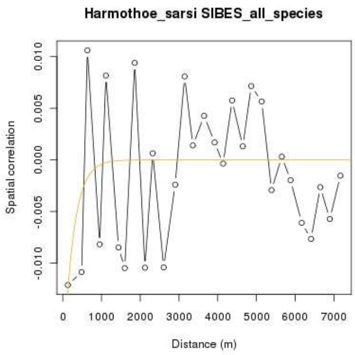
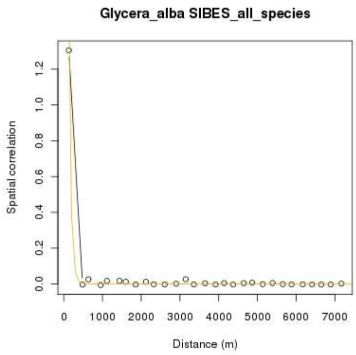
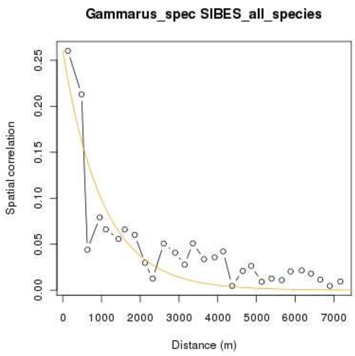
Malacoceros_fuliginosus SIBES_all_species
Magelona_mirabilis SIBES_all_species
Magelona_johnstoni SIBES_all_species
Macoma_balthica SIBES_all_species
Littorina_littorea SIBES_all_species
Lanice_conchilega SIBES_all_species
Spatial correlation
Distance (m)

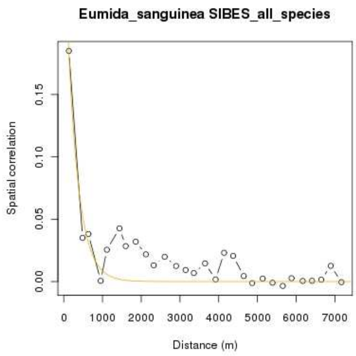

Hydrobia_ulvae SIBES_all_species
Spatial correlation
Distance (m)

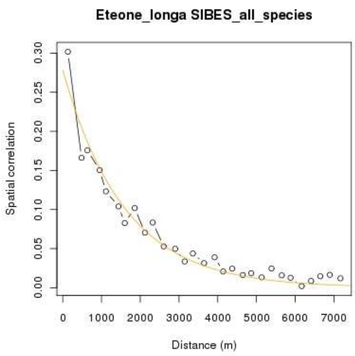

Heteromastus_filiformis SIBES_all_species
Spatial correlation
Distance (m)

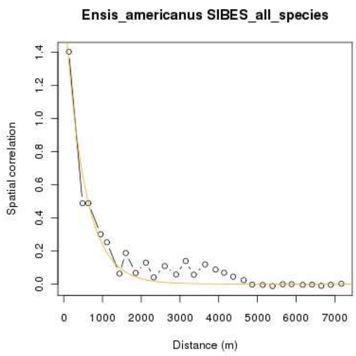

Harmothoe_sarsi SIBES_all_species
Spatial correlation
Distance (m)

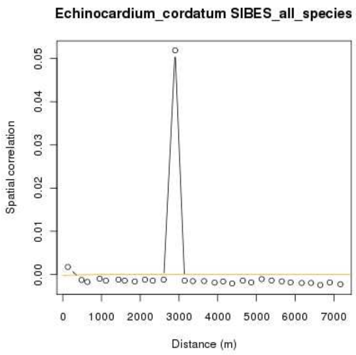

Glycera_alba SIBES_all_species
Spatial correlation
Distance (m)

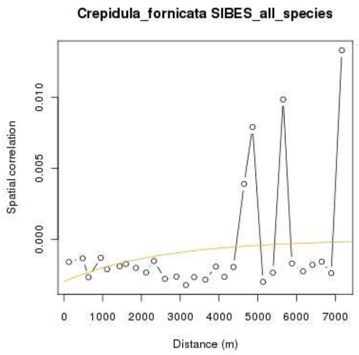

Gammarus_spec SIBES_all_species
Spatial correlation
Distance (m)

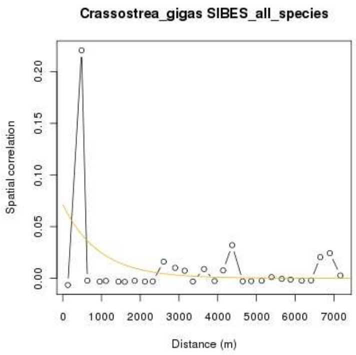

Eumida_sanguinea SIBES_all_species
Spatial correlation
Distance (m)

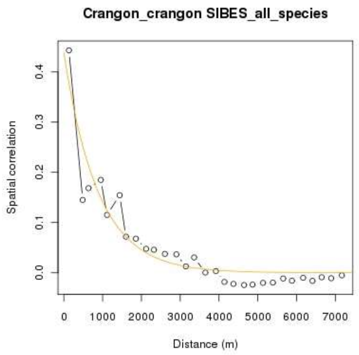

Eteone_longa SIBES_all_species
Spatial correlation
Distance (m)

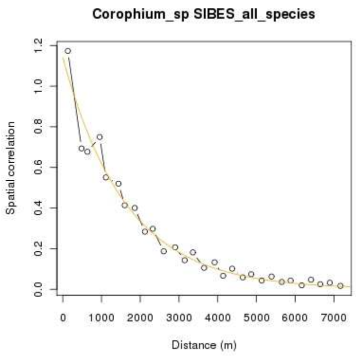

Ensis_americanus SIBES_all_species
Spatial correlation
Distance (m)

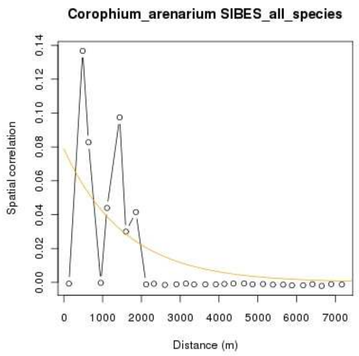

Echinocardium_cordatum SIBES_all_species
Spatial correlation
Distance (m)

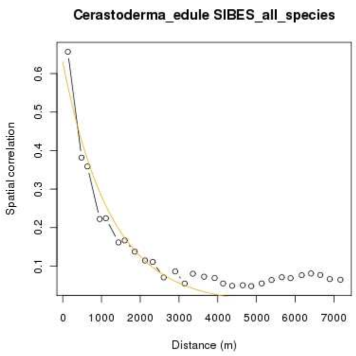

Crepidula_fornicata SIBES_all_species
Spatial correlation
Distance (m)

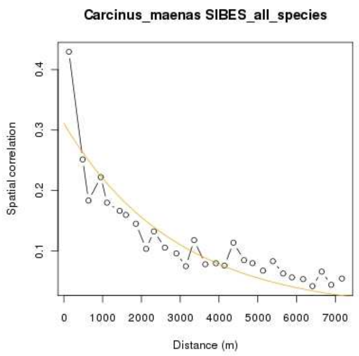

Crassostrea_gigas SIBES_all_species
Spatial correlation
Distance (m)

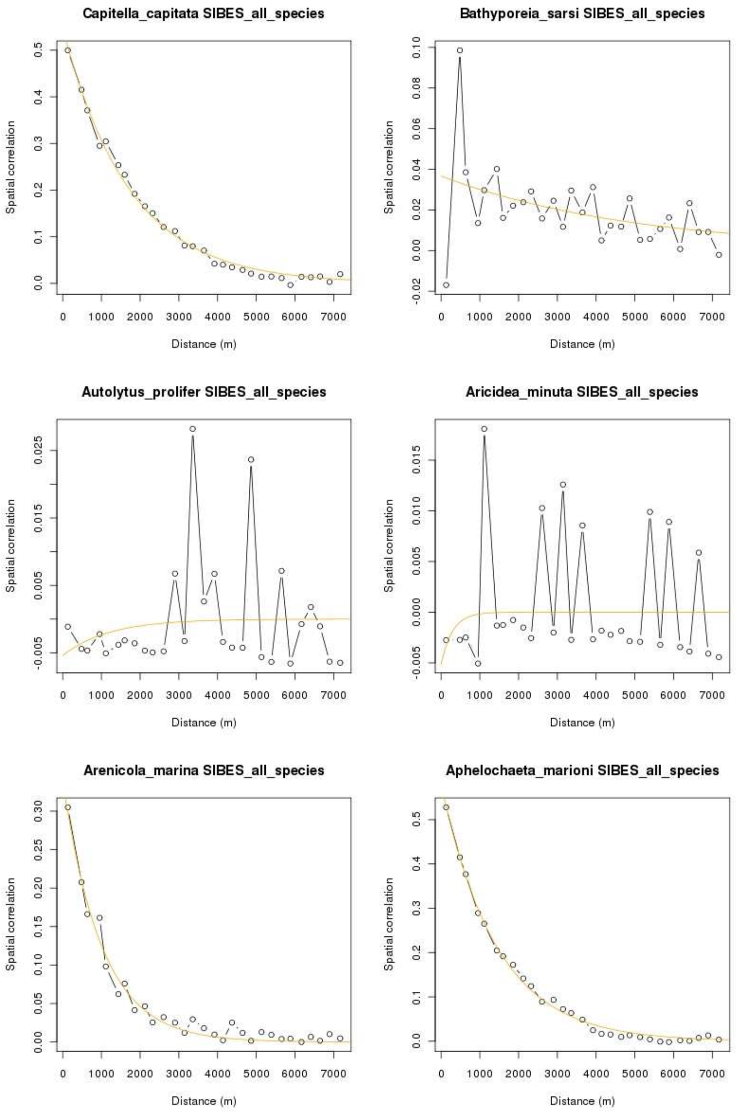

Crangon_crangon SIBES_all_species
Spatial correlation
Distance (m)

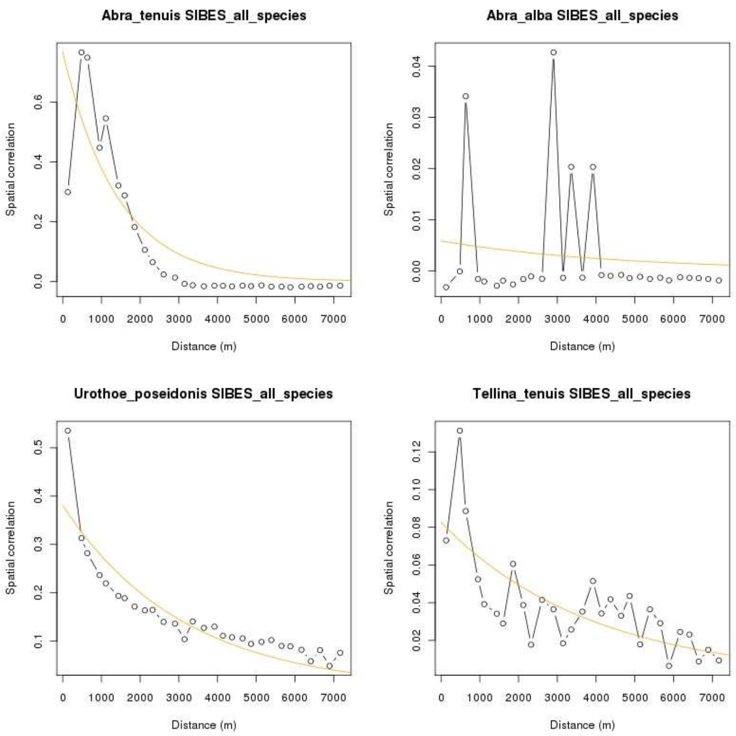

Corophium_sp SIBES_all_species
Spatial correlation
Distance (m)


Corophium_arenarium SIBES_all_species
Spatial correlation
Distance (m)


Cerastoderma_edule SIBES_all_species
Spatial correlation
Distance (m)


Carcinus_maenas SIBES_all_species
Spatial correlation
Distance (m)


Capitella_capitata SIBES_all_species
Bathyporeia_sarsi SIBES_all_species
Autolytus_prolifer SIBES_all_species
Aricidea_minuta SIBES_all_species
Arenicola_marina SIBES_all_species
Aphelochaeta_marioni SIBES_all_species
Spatial correlation
Distance (m)
0 1000 2000 3000 4000 5000 6000 7000


Abra_tenuis SIBES_all_species
Abra_alba SIBES_all_species
Urothoe_poseidonis SIBES_all_species
Tellina_tenuis SIBES_all_species
Spatial correlation
Distance (m)
0 1000 2000 3000 4000 5000 6000 7000