Macrobenthos monitoring on the Belgian Part of the North Sea in the framework of the OSPAR eutrophication assessment (EUTROF)

- FINAL REPORT -

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Acknowledgments

We wish to thank everybody who has contributed to this project and especially the following persons:

- The crew of the research vessel Belgica and Zeeleeuw for their assistance during the sampling campaign.
- Bart Beuselinck, Niels Viaene, Annick Van Kenhove, Dirk Van Gansbeke and Ulrike Braeckman for sampling, sample processing and measurements of oxygen profiles.
- Bart Goes, Jan Wittoeck, Jozefien Derweduwen and Sofie Vandendriessche for sample processing and report input.

Prepared for

Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu
Directoraat-generaal Leefmilieu
Dienst Marien Milieu

Bestek nr. MM/WB/OSPAR-EMS-07

16/12/2009

Contents

1   Summary

The OSPAR convention for the protection of the marine environment states that the eutrophication status of marine waters should be evaluated regularly. The status of eutrophication also has to be documented in the European Marine Strategy Framework Directive (2008/56/EC) (EMS) by the description of loss in biodiversity, ecosystem degradation, harmful algae blooms and oxygen depletion in bottom waters. Both the OSPAR convention and the EMS aim to minimize human induced eutrophication by forcing the member states to take adequate measures. For the evaluation of the eutrophication problems regarding the OSPAR and EMS objectives, a monitoring programme for measuring nutrients, phytoplankton variables and benthos was introduced covering the entire Belgian Part of the North Sea (BPNS). Within the previous eutrophication assessment (2002, 2006), the parameter changes/death in benthos/fish was not taken into account.

The EUTROF project tackles the question regarding possible eutrophication impacts on soft-bottom benthos. The hypothesis tested in this study is whether eutrophication influences the natural seasonal pattern of the benthos (number of species, density and biomass) in certain areas, resulting from changes in sediment conditions (oxygen concentration, depth of the redox potential discontinuity layer [RPD], chlorophyll a concentration). To that purpose the benthos and related environmental parameters (chlorophyll a concentrations in water and sediment, depth of the RPD layer, and sedimentological parameters) were sampled or measured seasonally or monthly at 10 stations on the BPNS in 2009. These 10 stations cover the different benthic habitats (muddy sediments [*Macoma balthica* habitat], muddy sands [*Abra alba* habitat], medium sand [*Nephtys cirrosa*] and coarse sands [*Ophelia limacina*] and the natural spatial gradient on the BPNS. Therefore, this sampling design was not only appropriate for evaluating the eutrophication on the BPNS scale, is also useful in the context of the evaluation of the environmental status of the benthos (cf diversity) under the EMS.

The monitoring results showed that the different habitat types can differentially be affected by eutrophication. Off shore, no effect of eutrophication on the benthos was encountered in sandy sediments characterized as permeable sediments and located in areas with the lowest chlorophyll a concentrations in the water and sediment over the year (lowest potential). In the coastal cohesive muddy and muddy sandy sediments, the eutrophication potential was higher. In muddy sands, the eutrophication effect on benthos was low, except when the sediments lacked habitat structuring and bioturbating species. The eutrophication potential was high for muddy sediments, in which the RPD layer clearly decreased in depth from winter to summer. Despite these changes, the benthic seasonal pattern was not disturbed at the two monitored stations in this habitat; the typical species for this kind of environment were still encountered. The patterns observed in 2009 were conform the earlier observations on certain locations on the BPNS.

Based on the delineation of the problem/ problem free areas concerning eutrophication in the water column, the spatial area in which eutrophication can locally affect benthos is

mainly situated in the coastal zone, especially the eastern part, corresponding with the occurrence of the muddy habitat type.

The eutrophication status of the benthos is evaluated with different index types, i.e. the Benthic Quality Index (BQI), AZTI Marine Biotic Index (AMBI) and the Benthic Ecosystem Quality Index (BEQI) level 3, to test their applicability in the different habitat types of the BPNS. Additionally, the potential of using visual images of the sediment to assess the eutrophication status was investigated and was evaluated with the BHQ (Benthic Habitat Quality).

The BQI scores of muddy and muddy sandy sediments corresponded with the expectations for reduced sediments, but not for well-oxidized sandy sediments. The use of sediment profile images and the related index (BHQ), however, has proved to be a suitable tool for quick eutrophication assessment. The AMBI index did not result in consistent patterns related to dominance changes of certain species in time and place. The test with the BEQI index was preliminary, due to the fact that not all assumptions for optimal analyses were met. The evaluation of the eutrophication status with the BEQI is difficult, due to the lack of benthic reference data of the Belgian coast, not impacted by the eutrophication problems of the last decades. Nevertheless, the BEQI seems to be suitable for evaluating the overall ecological status of the benthic habitats on the BPNS, which is important in relation to the monitoring in the framework of the EMS.

2   Introduction

The OSPAR convention for the protection of the marine environment states that the eutrophication status of marine waters should be evaluated on a regular basis. The standard evaluation procedure encompasses three categories of eutrophication effects:

1. Nutrient enrichment effects: DIN/DIP concentrations, winter N/P ratios
2. Direct effects of enrichment: max. and average Chlorophyll *a* concentration, the presence and abundance of phytoplankton indicator species, and the presence and abundance of macro-algae
3. Indirect effects of enrichment: oxygen deficiency, **changes / mortality in benthos / fish**, the presence and abundance of organic substances

In the preceding two OSPAR evaluations (2002, 2006), the parameter changes / mortality in benthos / fish was not taken into account.

The eutrophication status evaluation is also a requirement of the European Water Framework Directive, and should be based on the documentation of biodiversity loss, ecosystem degradation, harmful algal blooms and oxygen depletion in bottom water. Within this framework, measures are proposed to minimize anthropogenically induced eutrophication.

Within this context of the eutrophication status evaluation, different ongoing projects / contracts keep close watch over the nutrient and phytoplankton concentrations at 10 monitoring stations in the Belgian Part of the North Sea (BPNS), based on measurements at a predefined frequency. Conform the WFD requirements, the status of the benthos is being evaluated in the 0-1 mile zone from the coastline. To be able to comply with the OSPAR and European Marine Strategy (EMS) requirements concerning the eutrophication issue, the monitoring needs to be extended.

Based on frequent sampling in 2009, the current project aims to evaluate the spatial distribution of the possible presence of eutrophication effects on the level of the benthos. These results, supplemented by existing scientific information, allow an assessment of the current eutrophication status in the BPNS. Additionally, the results enable an optimal spatial and temporal planning for future monitoring campaigns concerning eutrophication effects on benthic fauna, in the framework of the European Marine Strategy.

For the OSPAR evaluation, the BPNS is spatially divided in a coastal zone and an offshore zone based on the 34.5 salinity boundary. Based on this boundary, only the northwestern part of the BPNS resorts under the offshore zone. The rest, *i.e.* the coastal zone up to 10km from the coastline in the western BPNS and up to 30km offshore of Oostende, was marked as a problem area in the last OSPAR report (Figure 1). In the area, values of DIN, DIP, and maximum and average chlorophyll *a* concentrations are above limits. Additionally, the area is characterized by problematic *Phaeocystis* blooms. The offshore zone was labeled as non problem area (Figure 1). In this evaluation, however, the parameter changes / mortality of benthos was not taken into account. The current project therefore assessed whether there

actually is an effect of eutrophication on the benthos level, and if so, at what spatial scale the observed effect is measurable.
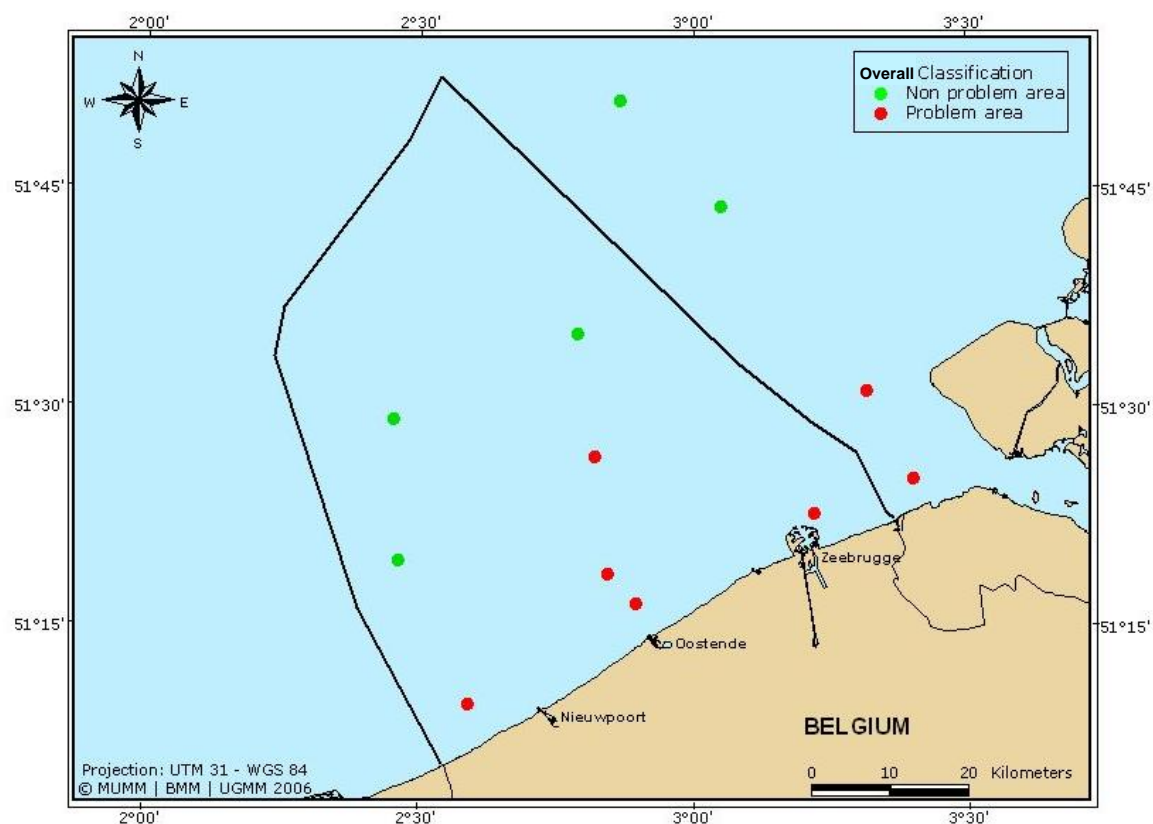

Figure 1. Overall area classification based on integration of the OSPAR assessment parameters (copy out of OSPAR 2nd assessment report).

3   Objectives

The current project aims to monitor the quality element macrobenthos in the BPNS in the framework of the OSPAR EUC and the EMS. The main goal is to evaluate the status of the benthos in relation to eutrophication based on:

1. The frequent macrobenthos monitoring in the BPNS at the MUMM monitoring stations (adapted to UGent or ILVO monitoring stations if necessary) and at station 115bis, 702. The monitoring data include information on density, diversity, species composition and biomass.
2. The monitoring of relevant environmental parameters (sediment characteristics, Chl *a* concentrations in the sediment, Chl *a* concentrations in the water, position of the redox potential discontinuity layer (RPD)) at the already mentioned stations, in order to be able to link benthos characteristics with eutrophication in the water column. At stations WO1 and 702 (eastern coastal zone), the environmental parameters are measured with a higher frequency, and additional data concerning the oxygen concentration in the sediment are collected.
3. A comparison of patterns observed in the macrobenthos and in the sedimentation and decomposition of phytoplankton during blooms (benthic-pelagic coupling), both during the described monitoring activities and previously described events (existing data).
4. The evaluation of the status of the macrobenthos in relation to eutrophication based on the results of tasks 1-3, and the use of relevant benthic indicators.
5. The evaluation of the 2009 monitoring strategy and its suitability for future monitoring in the framework of the EMS.

The hypothesis tested in this study is whether eutrophication influences the natural seasonal pattern of the benthos (number of species, density and biomass) in certain areas, resulting from changes in sediment conditions (oxygen concentration, depth of the redox potential discontinuity layer [RPD], chlorophyll a concentration). The normal seasonal recruitment pattern of benthos is characterized by an increase in density and number of species from spring towards autumn, followed by a decrease towards spring of the following year (Van Hoey et al., 2007). The sediment conditions will change due to eutrophication during the sedimentation of phytoplankton by a decrease in oxygen, changes in RPD layer and increase in Chlorophyll a concentration.

4   Material & Methods

4.1   Study area

The monitoring stations were distributed over the Belgian Part of the North Sea (BPNS) as visualised in Figure 2. These stations were regularly visited to sample the phytoplankton (chlorophyll and *Phaeocystis*) and chemical substances. In 2009 also the benthic fauna was collected. The overall monitoring stations of the MUMM were mostly located in the neighbourhood (within ± 1 mile) of a long-term monitoring station for benthos of ILVO or UGent. To fit the benthic data sampled in 2009 with the long-term monitoring, the benthic samples were taken at the long-term benthic monitoring stations, instead of the MUMM locations. In this report, the location name of the MUMM stations is used and in table 1 the link with the long-term benthic stations is made.

Table 1. The coordinates of the sample locations of the EUTROF project and the linking between the ILVO/UGent long-term monitoring locations and the MUMM locations.

| Station	N.B.	O.L.	Sample periods worked out
W01	51° 22,51	3° 11,469	Winter, spring, summer
W02	51° 13,5170	2° 51,5039	Winter, spring, summer
120 (W03)	51° 11,10	2° 42,07	Winter, spring, summer
330 (W05)	51° 26,00	2° 48,50	Winter, spring
315 (W06)	51° 19,37	2° 27,84	Winter, spring, summer
WTC13 (W07)	51° 34,329	2° 57,947	Winter, spring
415 (W08)	51° 24,00	2° 20,25	Winter, spring
840 (W09)	51° 44,22	2° 39,29	Winter, spring
830 (W10)	51° 42,54	2° 27,03	Winter, spring
702	51° 22,66	3° 18,578	Winter, spring, summer
115bis	51° 9,0760	2° 37,0717	Winter, spring, summer
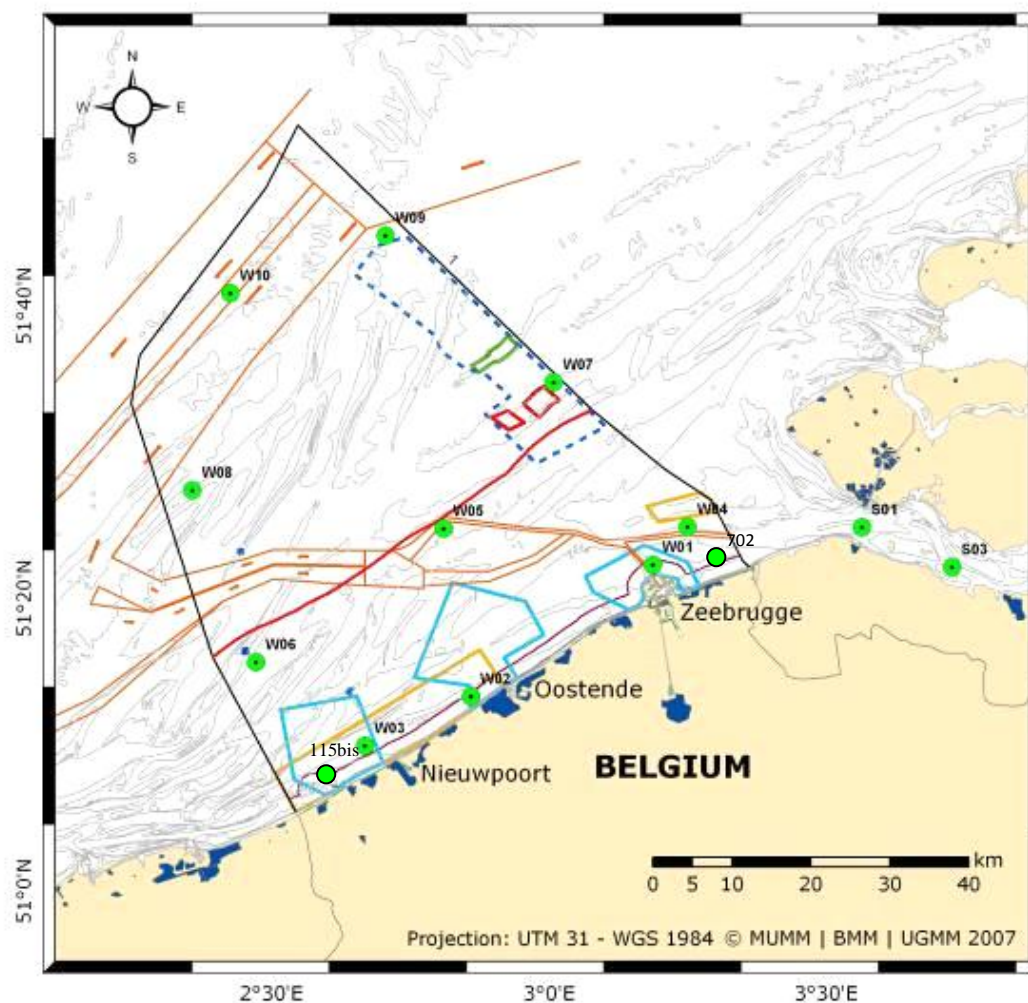
Figure 2. Map with the overall monitoringstations and 115bis and 702 (Map of the MUMM).

4.2 Sampling

The benthic samples were taken with a Van Veen grab (0.1 m²) and fixed with an 8 % formaldehyde solution. Afterwards the samples were sieved on a 1 mm sieve. The species in the samples were identified to species level when possible and the number of individuals was recorded. The macrobenthos sampling and analysis protocol is based on the ISO 16665:2005 standard ("Water quality – Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna"). From each Van Veen sample, a sediment sample was taken with a core. These samples were dried at 60 °C and analysed with a Malvern Mastersizer 2000 following a standardized protocol (see annex 6.7). Depth and position of each sample were also registered during the campaigns.

The water column was sampled 3 m below the air-sea interface using 10l Niskin bottles. To measure pigments, a fixed volume of water was filtered onto GF/F glass microfibre filters (i.d. 4.7 cm) using a vacuum pump. This procedure was repeated three times. The samples were kept in the dark, preserved at –20 °C on board and stored at –80 °C at the laboratory. Sediment was sampled using a Reineck boxcorer (surface area 180 cm^2). At all sampling moments, the Reineck box corer was deployed three times at each station. From each Reineck corer 2 perspex cores (i.d. 3.6 cm) were taken, 1 for pigment analysis and 1 for grain size analysis. At stations 702 and W01, an additional core (i.d. 3.6 cm) was taken for the assessment of oxygen profiles in the sediment. The upper 2 cm of the pigment core were subsamples using a 10 ml syringe from which the tip was removed. These subsamples were preserved at -20°C until analysis. Sediment cores collected for oxygen profiles were kept in the dark at *in situ* temperature and were aerated until measurement of the oxygen profiles. Time between sampling and measurements of oxygen profiles never exceeded 24 hours. Vertical oxygen profiles for Station 702 and W01 were measured using Unisense oxygen microsensors (type ox25) in vertical increments of 250 µm. Grain size analysis was performed using a Malvern Mastersizer 2000. Sediment fractions are expressed according to the Wenthworth scale (Buchanan, 1984). All pigment analyses (water and sediment) were done using HPLC (Gilson) following Wright & Jeffrey (1997). The depth of the Redox Potential Discontinuity (RPD) layers was visually assessed during sampling on one of the cores take out of the Reineck boxcorer.

For the benthic data, all the samples of the winter (campaign March) and spring (May) campaign were worked out. For the summer data, only the 3 replicates of stations W01, W02, W03 and W06 were worked out and 1 replicate of station 702 and 115bis (Table 1). For the environmental variables, all the samples of the winter, spring and summer campaign were worked out. The results of the autumn sampling campaign (benthos, environment) were not included in this report. All data will be delivered to the BMDC database of IDOD.

4.3 Data analysis

Different biological and environmental parameters were calculated using different tools, which were shortly outlined in this section.

First of all, the species data set was standardized by lumping some species (Cirratulidae spp, *Spio* spp, Anthozoa spp) and removing other species from the dataset because they did not belong to the macrobenthos *sensu strictu* (eg. Mysidacea). Nematoda were excluded because of inadequate sampling techniques for quantifying meiofauna.

The calculated univariate parameters were: (1) density (ind./m²); (2) biomass (g AFDW); (3) number of species (N_0). Cluster analysis by group averaging sorting based on a Bray-Curtis similarity dataset (on the fourth root transformed dataset) was used as multivariate analysis. This cluster analysis was complemented with a SIMPROF test (Similarity profile test) to define the significant different groups within the cluster analysis. The clustering was visualised using a non-metric Multi Dimensional Scaling analysis (MDS). A two-way ANOSIM (Analysis of similarities) was used to test the factors season and station as variance variables in the data. A one-way ANOSIM was used to test the significance of the SIMPROF defined cluster groups. A SIMPER analysis (Similarity/distance percentages, species/variable contributions) was performed to examine the contribution of each species to the average similarity within a cluster group. To test the linkage between the benthic patterns and the environmental variables, a BIOENV analysis (selects environmental variables) was executed. All multivariate analyses were performed with the PRIMER statistical program (version 6).

Differences in sedimentological variables between months were tested using ANOVA. When necessary, data were log-transformed in order to meet the assumptions for parametric analyses. When transformed data did not meet the assumptions for ANOVA, a non-parametric Kruskal-Wallis analysis by ranks was applied. All granulometric variables (median grain size and relative abundance of all fractions) and sediment pigment data for all stations for the months February, May and August were analysed using 2 way crossed ANOSIM, based on a Euclidean distance after normalization of data. This allows for a general interpretation of spatial differences between stations (taking into account the effect of sampling in different months) on the one hand, and for an interpretation of seasonal differences (taking into account differences between stations due to location) on the other hand. Pairwise comparisons were made when the general analysis revealed significant differences.

5   Results

5.1   Monitoring results of the environmental variables
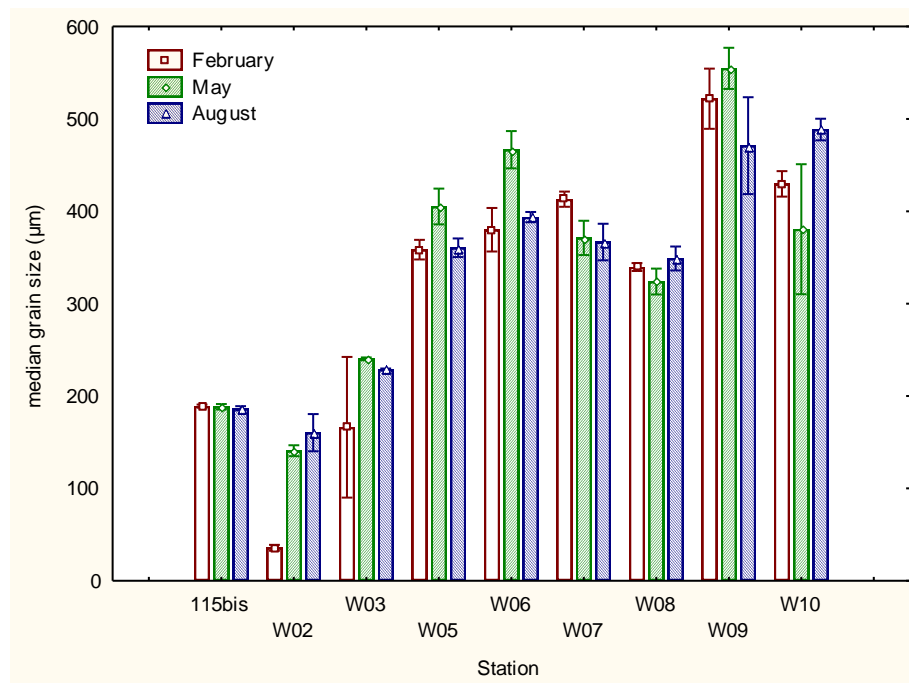
5.1.1   Granulometric variables

Figure 3. Mean (± SE) median grain size for 9 stations at the Belgian Part of the North Sea. Sampling was conducted in February, May and August 2009.

Median grain size (Figure 3) for stations sampled in February, May and August ranged between average values of 34 µm (W02 – February) and 554 µm (W09 – May) (Reneick samples). Excluding the extremely aberrant value for Station W02 in February, 3 stations (115bis, W02 and W03) can be classified as fine sandy (median grain size between 125-250 µm). Five stations (W05, W06, W07 and W08) have medium sand (median grain size varying around 300-400 µm) while W09 and W10 was classified as coarse sand (median grain size varying around 400-500 µm) in February and May. Apart from station W02 and W09, classifications did not change over the course of the seasons.

The clay-silt fraction (fraction < 63µm) was absent in stations W05, W06, W08, W09 and W10 (Figure 4). Relatively important fractions were observed at Station W02 (all sampling events > 20%) and W03 (10-20%), except in march (30%, high standard error). At station 115bis and W07, values were always below 10%.
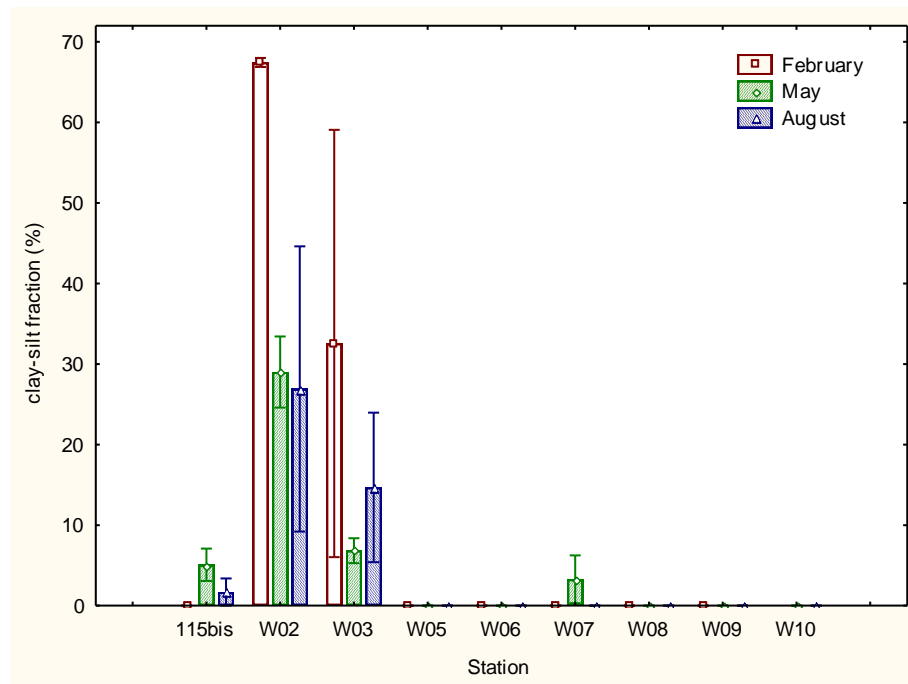
Figure 4. Mean (± SE) clay-silt fraction for 9 stations at the Belgian Contintal Shelf. Sampling was conducted in February, May and August 2009.

Sediments from the monthly sampled station 702 was classified as fine sand (Figure 5). ANOVA on log-transformed median grain size values revealed significant differences between months ($F_{6,14}$=4.9, p<0.01). Tukey HSD showed that median grain sizes from March were different from values obtained in April and May. Sediments at station W01 classified as silt (median grain size between 4-63μm) in February, April, August and September and as very fine sand (median grain size between 63-125 μm) in March and May. According to a Kruskal-Wallis analysis by ranks, median grain sizes were not significantly different between sampling dates at this station.
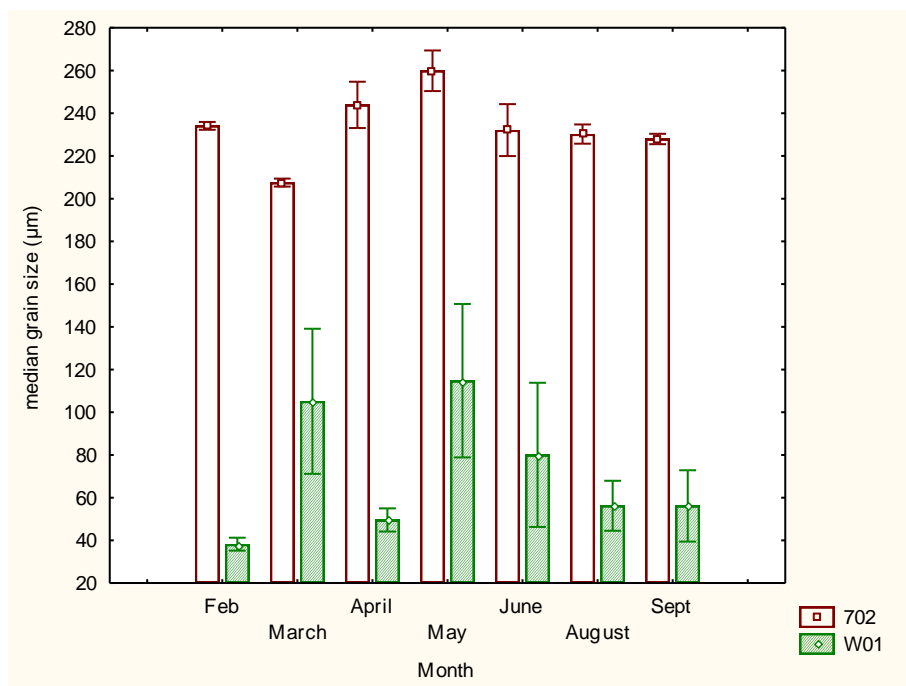
Figure 5. Mean (± SE) median grain sizes at the monthly sampled Station 702 and W01.

The clay-silt fraction was only present in March in very small quantities in station 702, while this fraction was present for more than 35% at all sampling events at station W01 (Figure 6). Although there was variability in clay-silt presence at station W01, there were no significant differences between sampling events ($F_{6,14}=1.33$; $p>0.05$).
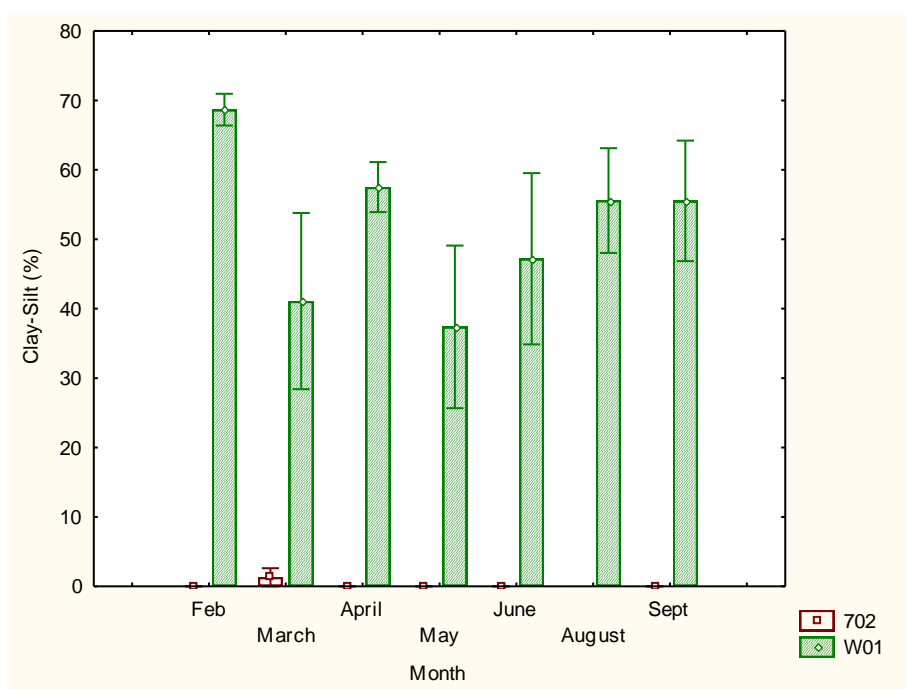
Figure 6. Mean (± SE) clay-silt fractions at the monthly sampled Station 702 and W01

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5.1.2 Pigment data
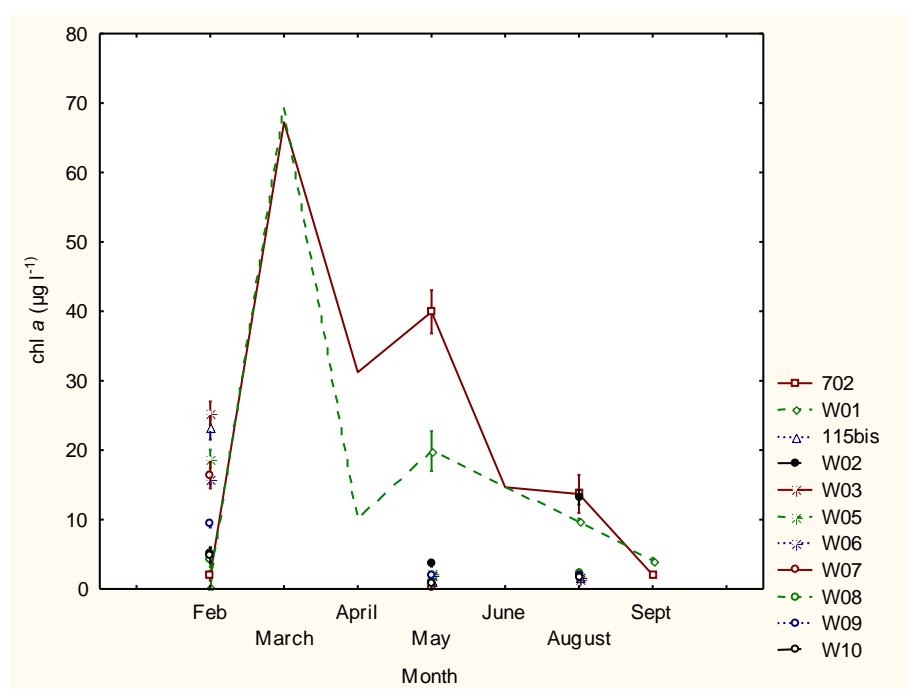
Figure 7. Mean (± SE) Chl a values in the water column. Full line: Monthly samples Station 702; dashed line: monthly samples Station W01

At both coastal stations near the Westerschelde, the phytoplankton bloom reached peak values at the end of March-beginning of April (Figure 7, March samples obtained at 27 March 2009) and a second peak in May (sampling 6 May). Values at other stations were lower during this period, and at the August sampling event and did not reveal clear trends.

Chl a values in the upper 2 cm of sediment (Figure 8) were highest in station W02 in February and May 2009. The other near-coastal stations 115bis and W03 showed much lower chl a values but at the more offshore stations even lower values were observed at most of the sampling periods.

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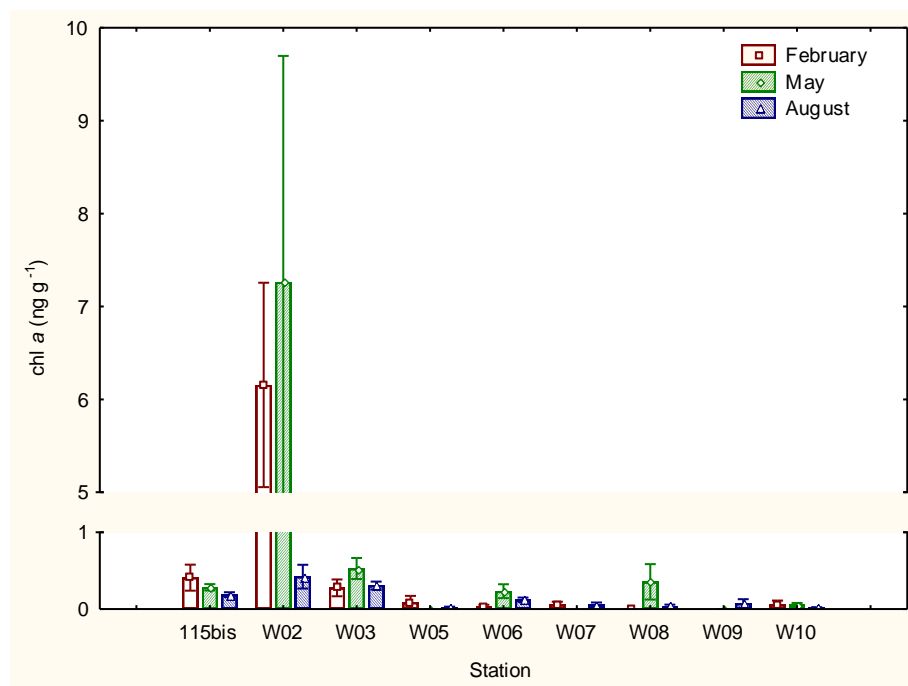
Figure 8. Mean (± SE) Chl *a* values in the upper 2 cm of sediment for 9 stations at the Belgian Contintal Shelf. Sampling was conducted in February, May and August 2009. Note the scale break in the y-axis.

At the east coast, sediment chl *a* values in the upper 2 cm were always higher at Station W01 compared to Station 702 (Figure 9). There were no significant differences in chl *a* values for the different months (Kruskal-Wallis analysis by ranks, p>0.05 for both stations).

Figure 9. Mean (± SE) Chl *a* value in the upper 2 cm of sediment at the monthly sampled Station 702 and W01

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5.1.3   Redox Potential Discontinuity Layer (RPD)

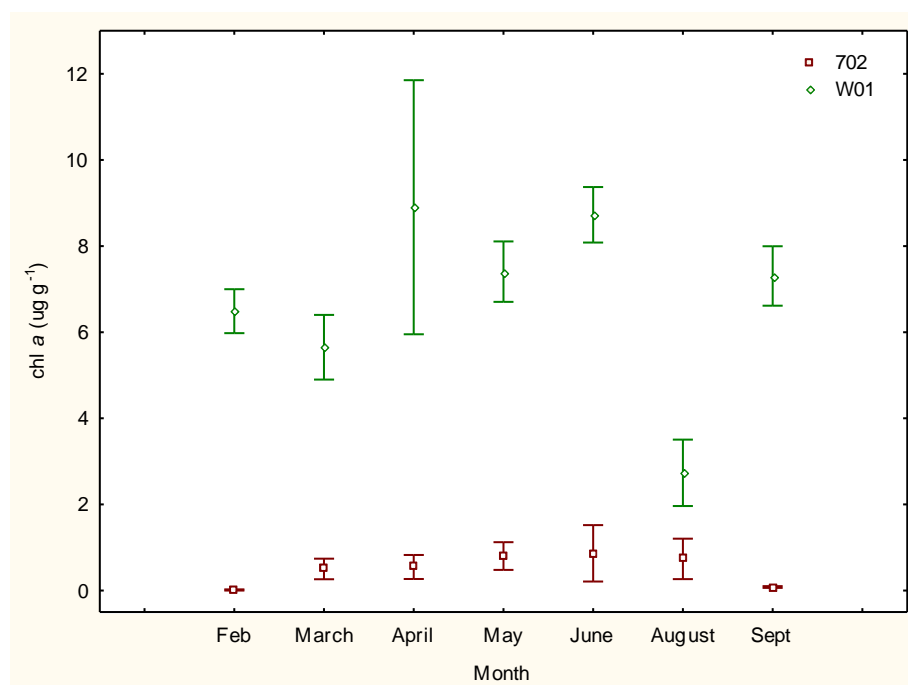Minimum and maximum depth of the RPD layers varied between replicates when this layer was present (Table 2) (depth of core varying between 10-15 cm). An RPD layer was always visible in the near-coast stations W01, W02, WO3 and 115bis, meaning anoxic parts present. At the more offshore locations W05, W06, W09 and W10 and the coastal station 702, the RPD layer was always absent (meaning completely oxic sediments). With the exception of Station 115bis, RPD layers were generally located at more shallow sediment depths in May/August compared to March. At Station W08 and W07 RPD layers were present in May and August respectively while they were absent in March.

Table 2. Minimum and maximum depth (cm) of RPD layer in 3 replicates (Depth of core varying between 10-15cm).

Station	February	May	August
W01	5-6	0-6	0-5
W02	4.5-5	2-7	1-5
W03	1-7	1-5	absent
W05	absent	absent	absent
W06	absent	absent	absent
W07	absent	absent	4- absent
W08	absent	1- absent	absent
W09	absent	absent	absent
W10	absent	absent	absent
115bis	0-3	2-3	0.5-2.5
702	absent	absent	absent

5.1.4   Oxygen profiles
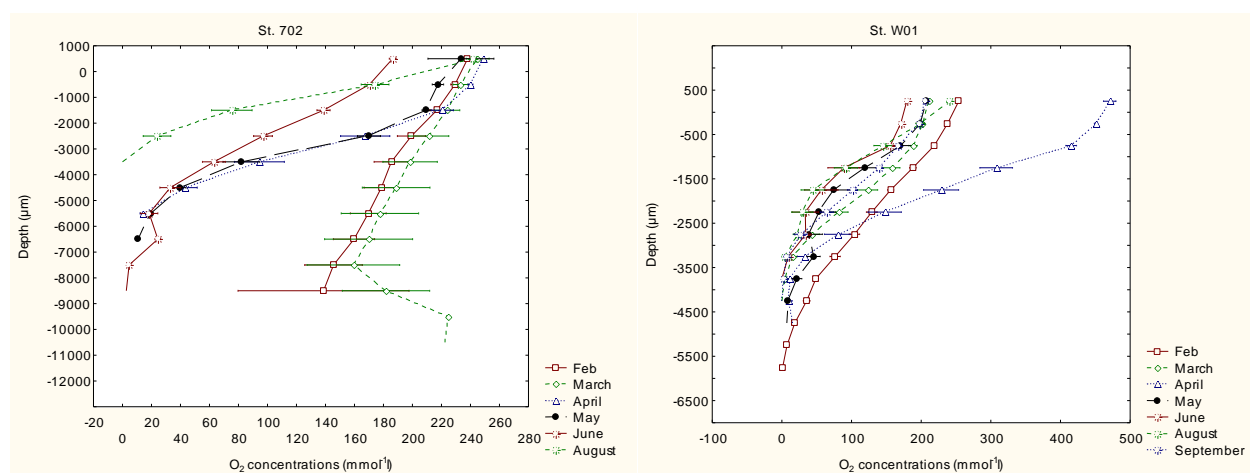
Figure 10. Mean (± SE) monthly oxygen concentrations at different sediment depths at Station 702 and W01.

Oxygen concentration profiles at the east coast stations 702 and W01 are depicted in Figure 10. At Station 702, oxygen penetration is deep in February and March. Later, the profiles are steeper and oxygen is depleted at 3.5 mm in August This trend is not obvious at Station W01

where oxygen depletion occurs at 5.25 mm in February. Maximum values decrease later in the year and oxygen is depleted between 3.5 and 4.5 mm in spring and summer.

5.1.5 Multivariate analysis

2 Way Crossed ANOSIM revealed differences between stations (R=0.705, [p=0.001]) and sampling months (R= 0.2, [p=0.002]). Pairwise comparisons revealed that only Station W01 and W02, and W09 and W10 were not significantly different. Pairwise comparisons between sampling months showed small (R<0.27 for all comparisons) but significant (p<0.002 in all cases) differences between sampling months.

5.2 Monitoring results of the macrobenthos

5.2.1 Overall
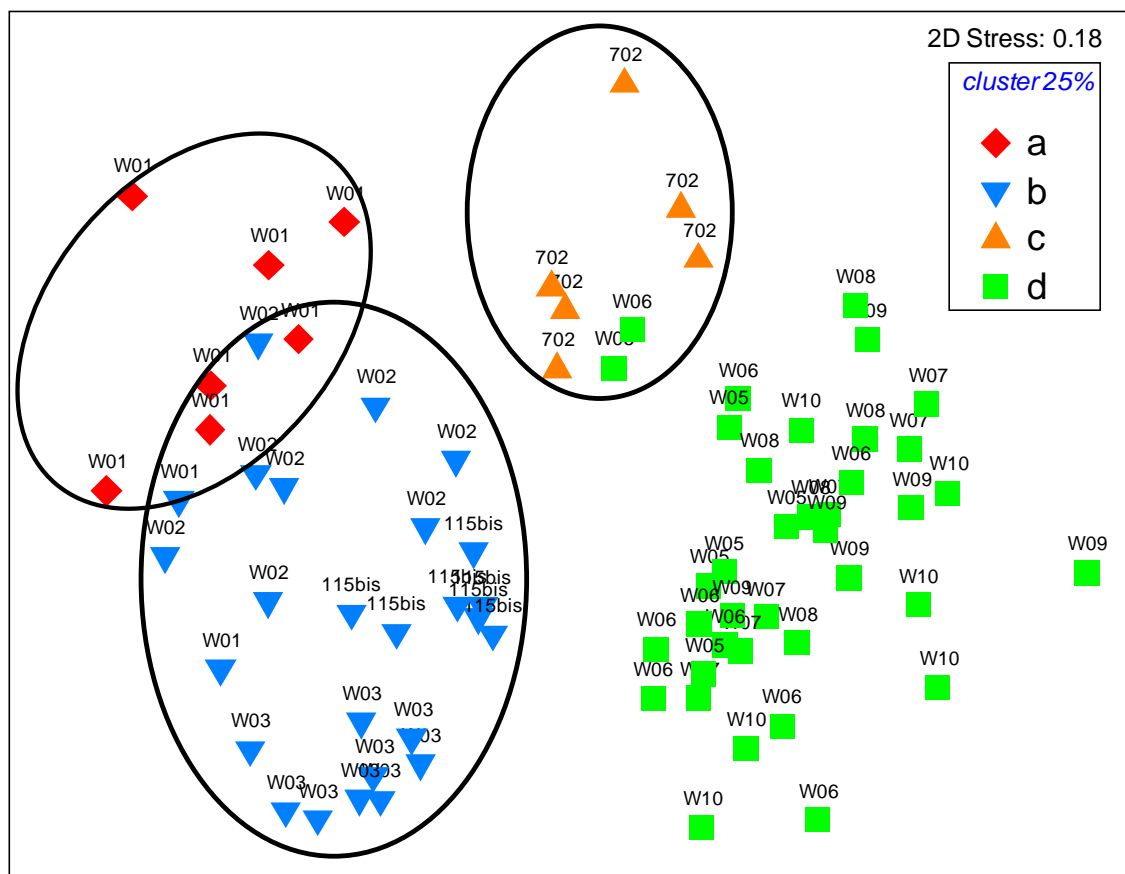
Figure 11. Multidimensional Scaling (MDS) with SIMPROF factor 25%, with indication of the stations and visualisation of the different cluster groups.

The benthic data was primary explored in order to find some structural patterns within the data. First of all, the structural factors 'season' and 'station' were analysed with a 2 Way Crossed analysis. Afterwards a cluster analysis (Bray Curtis similarity group averaging) with SIMPROF module is ran to find objectively biological patterns. Both season (ANOSIM: R: 0.686 [p: 0.001]) and station (ANOSIM: R: 0.859 [p: 0.001]) were significant, but the factor

'station' has a greater influence on the variation than the factor season (68.6% of the variation can be explained by the factor season while 85.9 % is due to differences between stations).

The factor SIMPROF 25% (ANOSIM: R: 0.794 [p: 0.001]) is also significant and four different clusters can be identified (Figure 11; Figure 12). These clusters can be linked to one of the known benthic communities on the Belgian Part of the North Sea (Van Hoey et al., 2004).

The typical species of each cluster and related community characteristics are determined based on a SIMPER analysis (Table 3).

Cluster a: The samples of this cluster were mainly originating from station W01. Both, the average density (507 ind/m² ± 141) and the average number of species (9 species/0.1 m²) were relatively low. The characteristic species of this cluster were (*Cirratulidae*, Oligochaeta, *Petricola pholadiformis* and *Macoma balthica*). These characteristics were typical for the *Macoma balthica* habitat. This was confirmed by the sedimentological characteristics, high mud content (46%) and low median grain size (44 µm).

Cluster b: The samples belonging to this cluster show the characteristics of the *Abra alba* habitat, due to the characteristic species (*Oligochaeta, Cirratulidae, Abra alba*) in combination with a very high average density (4752 ind/m² ± 1074) and a relative high species richness (21 species / 0.1 m²). The sediment was characterised by fine sand (median grain size of 151 µm) and relative high mud content (20%).

Table 3. Characteristic species and community parameters for the defined clusters.

	a	b	c	d
Habitat	*Macoma balthica* habitat	*Abra alba* habitat	Sandy/poor	*Nephtys cirrosa/ Ophelia limacina* habitat
Avg density (ind/m²)	507 ± 141	4752 ±1074	108 ± 17	1119 ± 267
Avg number of species	9	21	5	14
Total number of species	25	81	9	101
SIMPER species	*Petricola pholadiformis Cirratulidae Oligochaeta Capitella capitata Eteone longa Macoma balthica*	*Oligochaeta Cirratulidae Abra alba Nephtys juv*	*Nephtys cirrosa Cirratulidae*	*Nephtys juv Nephtys cirrosa Spiophanes bombyx Oligochaeta*
Median grain size (µm)	44.42 ± 3.00	151.51 ± 14.70	239.61 ± 2.50	407.79 ± 8.67
Mud content (%)	46.61 ± 3.15	21.61 ± 4.41	0	0.06 ± 0.06

Figure 12. Cluster diagram of the benthic data with SIMPROF factor 40%, with the indication of the stations and the habitat groups.

Cluster c: This cluster contains samples of station 702 and is defined by a very low average density (108 ind/m² ± 17) and poor number of species (5 species / 0.1 m²). The characteristic species were *Nephtys cirrosa* and *Cirratulidae.* No mud content was found and the median grain size show medium sand characteristic (239 μm). This cluster show similarity with the *Nephtys cirossa* habitat, but is clearly affected.

Cluster d: This cluster contains the offshore samples and is marked with a high average density (1119 ind/ m² ± 267) and a moderate diversity (14 species / 0.1 m²). The SIMPER species (*Nephtys juv, Nephtys cirrosa* and *Spiophanes bombyx*) characteristic for this cluster were typical for the *Nephtys cirrosa/Ophelia limacina* habitat and confirmed by the sedimentology. The median grain size was high (408 μm) and mud was nearly absent (0.06%).
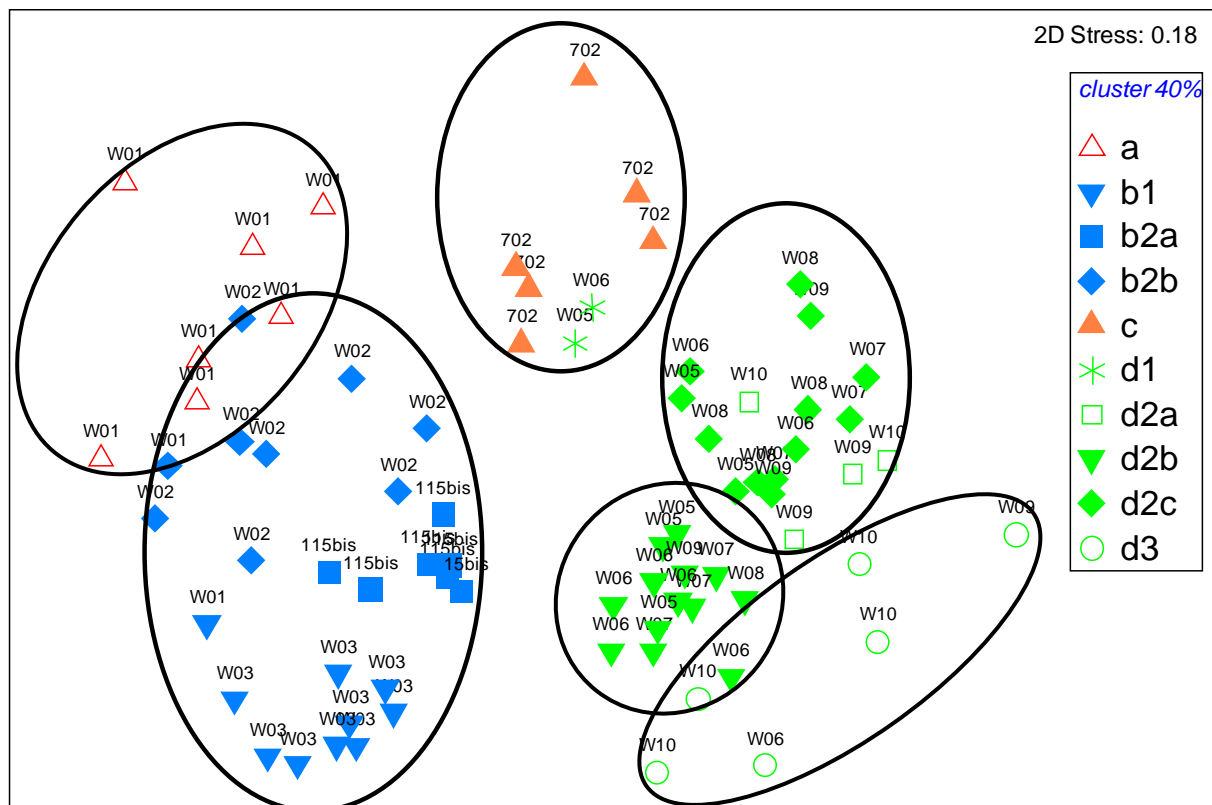
Figure 13. Multidimensional Scaling (MDS) plot with SIMPROF factor 40%, with indication of the stations & visualisation of the different cluster groups.

Within each cluster (Figure 13) (SIMPROF 40%: ANOSIM: R: 0.907 [p: 0.001]) some subgroups can be distinguished in cluster b and d. In cluster b, which contains the coastal samples, it can be observed that the samples of station W02 have more similarities with station W01 than with the other samples of cluster b (ANOSIM W01, W02: R: 0.263 [p: 0.015] vs. W02, W03: R: 0.842 [p: 0.001] and W02, 115bis: R: 0.656 [p: 0.001]).

Therefore and in combination with the sedimentological results, station W01 will be discussed together with station W02 (= habitat group 3: coastal muddy).

Cluster d, the offshore samples, can be divided in 3 main groups. One distinction (between d2b & d2c) can principally be explained by the seasonal variation (d2c = winter; d2b = spring/summer). The third group, including station W10 & W09 were situated in the most offshore regions.

Patterns in diversity, density and biomass of the benthos will be explained per habitat group (offshore sandy, 702, coastal muddy and coastal fine muddy).

5.2.2 Habitat group 1: Offshore sandy (*Nephtys cirrosa / Ophelia limacina*)

In each station, a raise in diversity can be seen (Figure 14) in more (W06, W07, W08 and W09) or lesser (W05 and W10) extend from winter to spring.
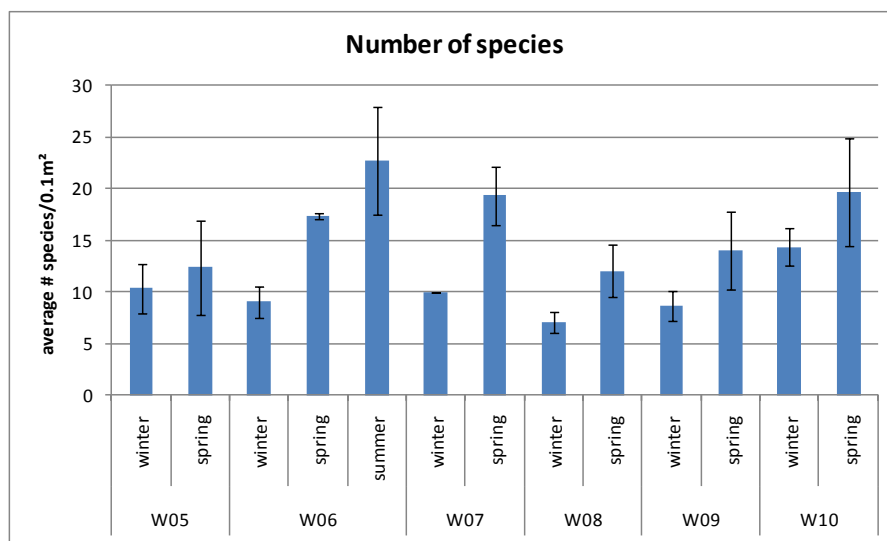
Figure 14. Average number of species/0.1 m² per season with standard error for the offshore stations

In stations W08, W09 and W10 there was a raise in density (Figure 15) but these stations were globally less dens than the other offshore stations. Therefore the differences in season were less obvious. In contrast, stations W05 → W07 showed a big jump in density between winter and spring. The density in spring was ± 5x the density in winter. In all these stations *Spiophanes bombyx* and Oligochaeta were the responsible species. The high standard error in W05 (spring) was due to the high numbers of *Spiophanes bombyx* (7680 ind/m²) in one of the replicates.
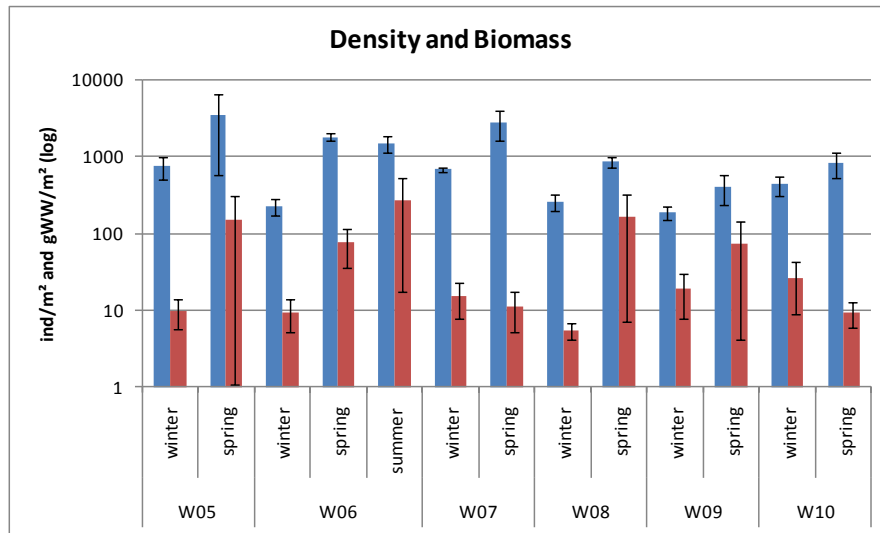
Figure 15. Average density (Ind/m²) and biomass (WW g/m²) with standard error per season for the offshore stations.

The biomass (Figure 15) in station W07 and station W10 was quit low and it's even a little descending from winter to spring. In station W05, W06 and W08 a big difference can be detected between the two seasons. The samples in winter were in both cases characterised by low values. In spring, the biomass was much higher because of the recruitment of *Echinocardium chordatum*.

Only for station W06, the summer samples have been analysed yet. The summer samples were more diverse and contained a higher biomass than in the other seasons. The latter is still due to the recruitment of *Echinocardium chordatum*. A little decrease in density can be noticed in comparison with the samples in spring.

Diversity and density of the most offshore stations (W09-W10) with the other offshore stations (Table 4) were compared. This division can also be seen in figure 3 (d3+d2a <-> d1+d2b+d2c). In both seasons; cluster W09-W10 was characterised by a poorer diversity and a poorer density than the other cluster. The dominant SIMPER species of this cluster were *Nephtys* juv but also *Polygordius appendiculatus* and *Glycera juv.* This species were typical species for coarser sediment.

Table 4. Density and diversity with standard error of cluster W09-W10 and cluster W05 to W08

		Cluster W09-W10	Cluster: W05-W06-W07-W08
Diversity (species/0.1 m²)	Winter	18 ± 1	24 ± 0.3
	Spring	22 ± 3	26 ±1
Density (Ind/m²)	Winter	451.67 ± 70.37	1298.33 ± 39.75
	Spring	780 ± 81.65	2931.11 ± 794.07

In cluster W05-W08 on the other hand the SIMPER-species like *Urothoe brevicornis, Bathyporeia guilliamsoniana* and *Gastrosaccus spinifer* were more characteristic for medium sandy sediments.

5.2.3 Habitat group 2: 702

This group only contains Station 702. This station is very poor and is characterised by low diversity (Figure 16) (avg ± 4.7 species/0.1 m²), low density Figure 17(avg ±108 ind/m²) and a low biomass (avg ± 2.13 g) (Figure 17). Between the different seasons, no big differences can be noted, indicating that the normal seasonal patterns was disturbed.

Figure 16. Average number of species/0.1 m² per season with standard error for station 702.

Figure 17. Average density (Ind/m²) and biomass (WW, g/m²) with standard error per season for station 702.

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5.2.4 Habitat group 3: coastal muddy (W01-W02) (*Macoma balthica*)

An increase in biomass, number of species and density over the seasons was observed for both stations.

For W01, the increase in number of species (Figure 18) occurred between winter and spring (from 6 species/ 0.1 m² in winter to 12 species /0.1 m² in spring) while there was only a slight raise between spring and summer.

In Station W02, the same phenomenon was detected but with a little delay. There were no big differences between diversity (Figure 18) in winter and spring (from 8 species/0.1m² to 9 species/0.1m²) but instead there was a great increase between spring and summer (16 species/0.1m²).
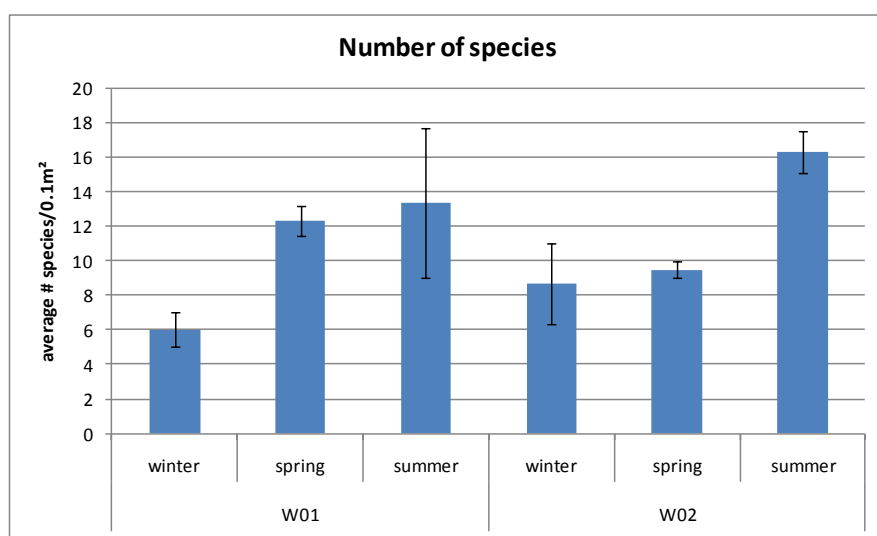
Figure 18. Average number of species/0.1 m² per season with standard error for stations W01 & W02.

As for density (Figure 19), the average ind/m² raised as expected in station W01. In W02 an enormous population increase was established. The density elevated to an average of 3806 ind/m² in the summer. The high number of *Macoma balthica* (7550 ind/m²) in one of the replicates caused the high standard error value. Station W02 is a heterogeneous station as there was a lot of variation between the replicates and the seasons. This heterogeneity was also reflected in the sedimentology.

The biomass (Figure 19) followed the same pattern as diversity in W01. In W02, there was also a slight increase in biomass but a lot less obvious than in W01.

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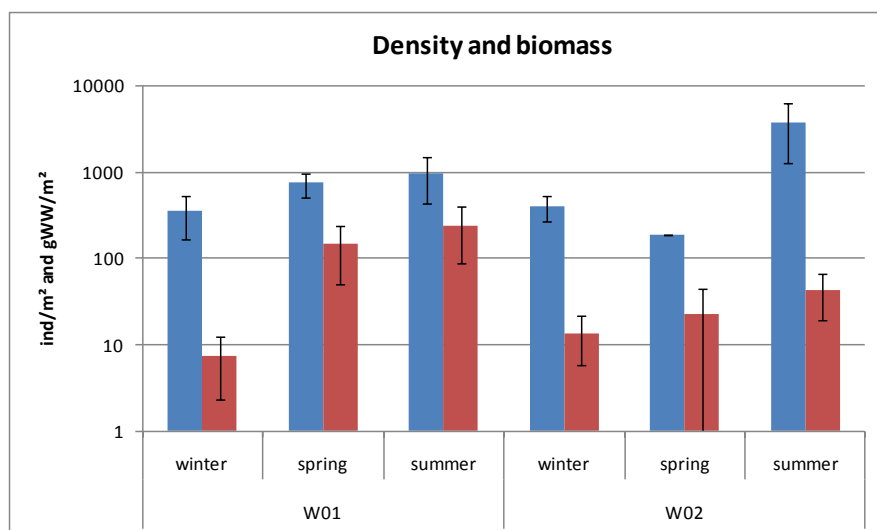
Figure 19. Average density (Ind/m²) and biomass (WW, g/m²) with standard error per season for stations W01 & W02.

5.2.5 Habitat group 4: coastal fine muddy (115bis, W03) (*Abra alba*)

Generally, the values in biomass, diversity & density were much higher for Station W03 than for Station 115bis. For both stations, the diversity (Figure 20) stayed more or less the same between winter and spring and increased slightly in the summer. For the density (Figure 21), the same trend was detected but a summer increase can be noted. This was due to the recruitment of *Ensis* spat. In 115bis for example, the number of individuals raised from ± 1337 ind./m² to ±9870 ind./m² (63% of the density from *Ensis* juv). This was due to the recruitment of the bivalve *Ensis directus*. This tendency is also reflected in the biomass (Figure 21): 89% of the total biomass came from this bivalve.
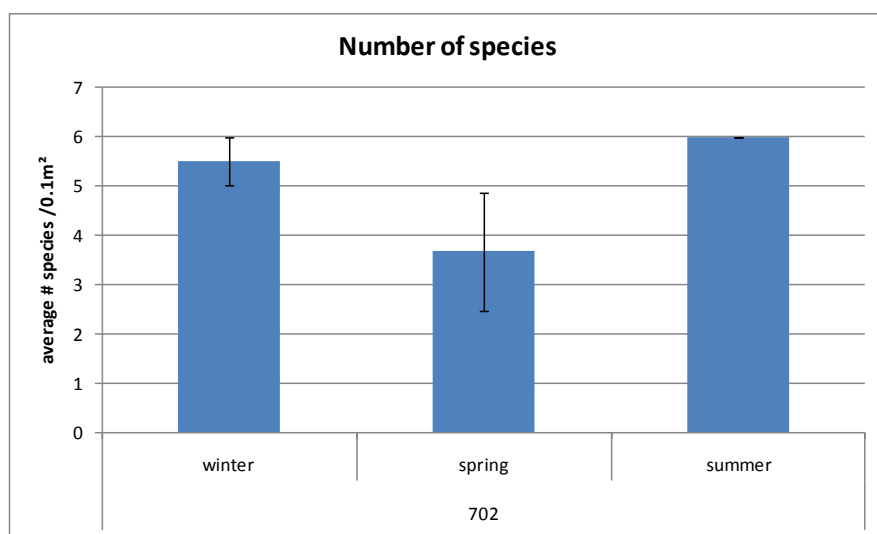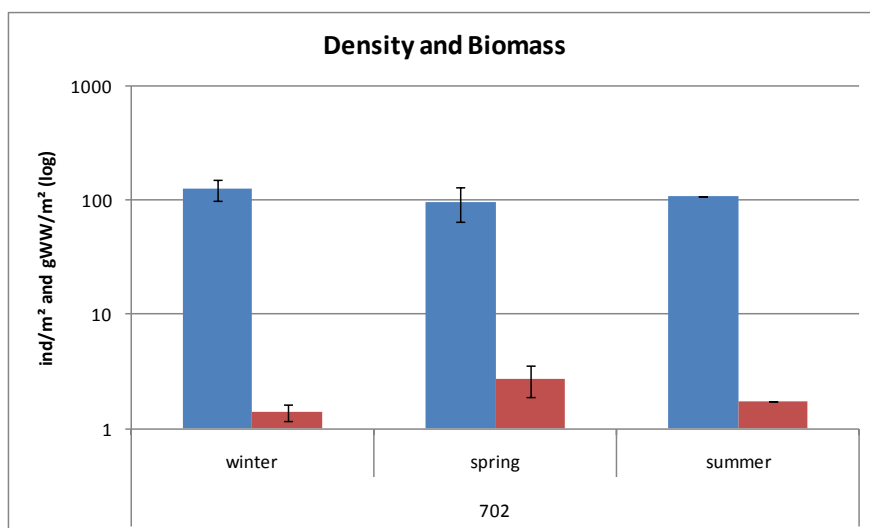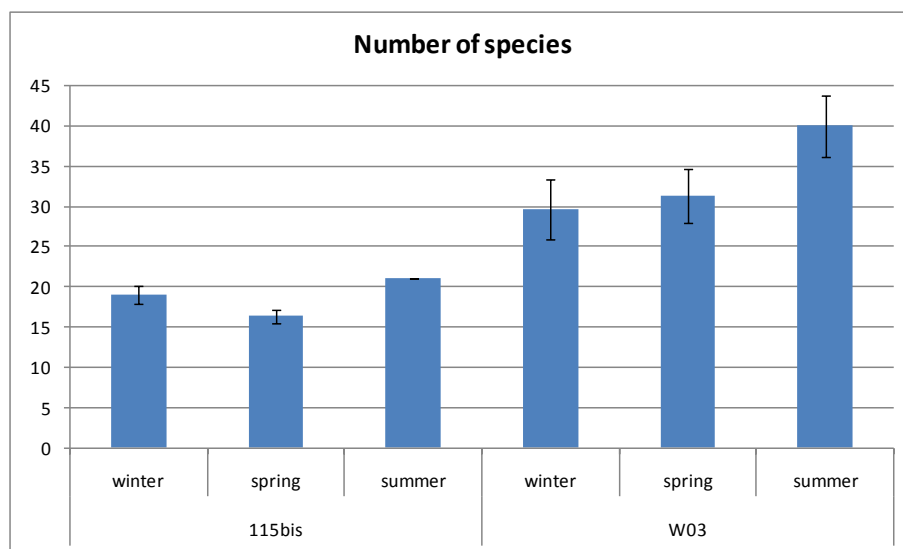

Figure 20. Average number of species/0.1 m² per season with standard error for station 115bis & W03.

28
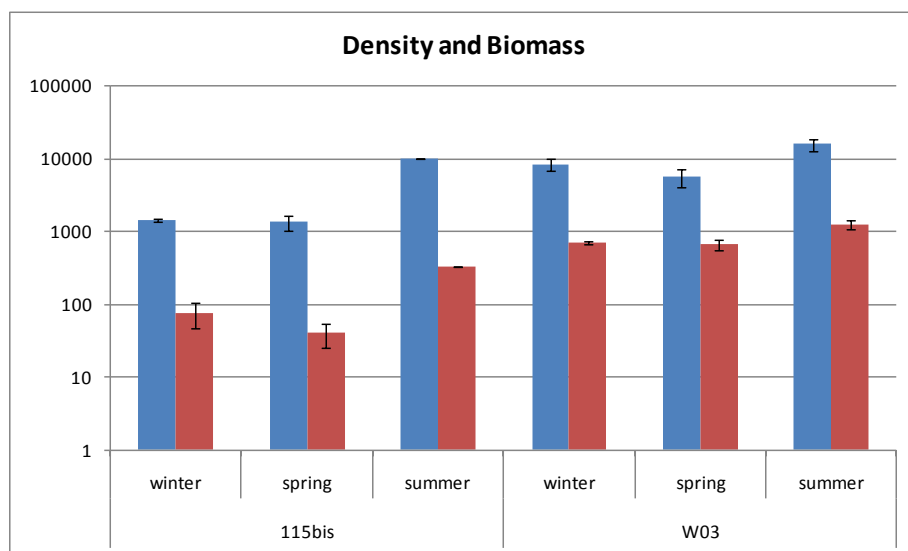
Figure 21. Average density (Ind/m²) and biomass (WW, g/m²) with standard error per season for stations 115bis & W03.

5.3 Benthic fauna – environmental variables
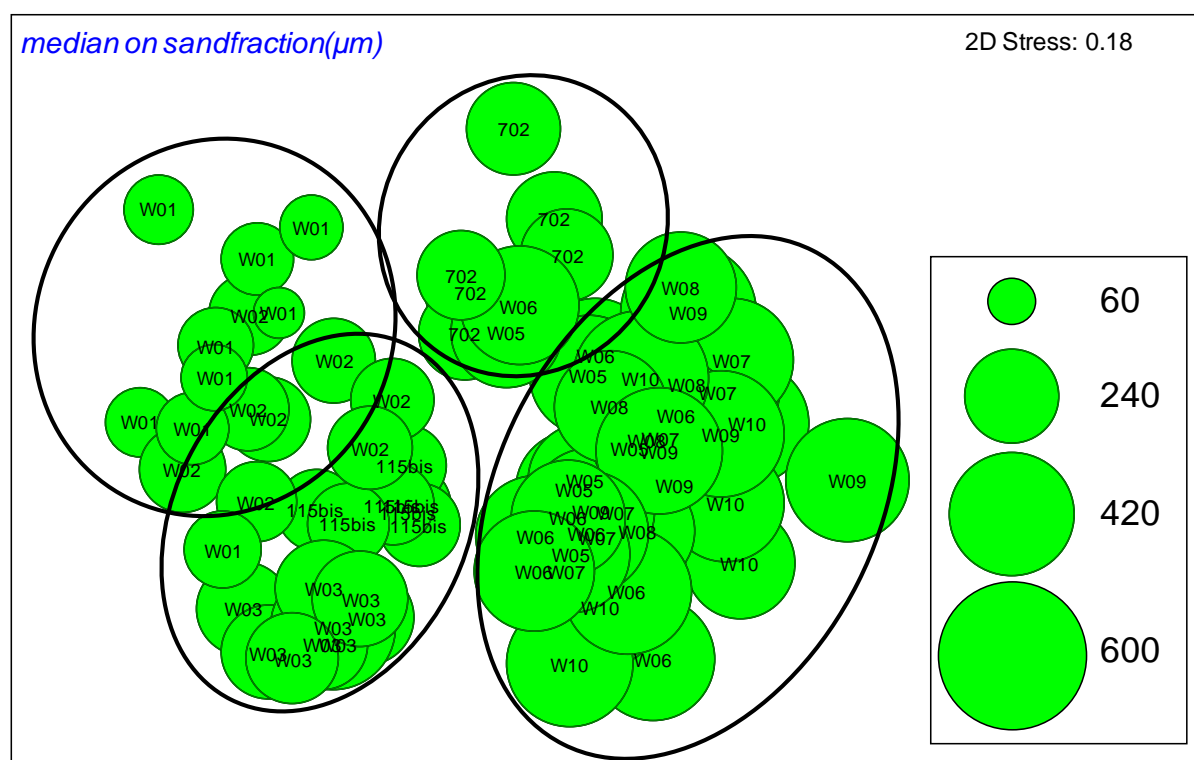
Figure 22. Bubble plot of the median grain size on the sand fraction on the biological data MDS.

The biological patterns observed in the benthic data, were linked to the monitored environmental variables. The not correlated environmental variables were Chl *a* sediment and water, sediment fractions <63μm, 125-250μm and > 1000μm and the median on the sand fraction (sedimentological characteristics from the Van Veen samples). The BIOENV STEPWISE analyses shows that the mud content (<63μm) and the median on the sand

fraction explain the biological patterns best (p = 0.589). All selected variables explain the variability in the benthic data with a p score of 0.609 (BIOENV analysis). The difference in environmental factors between the biological samples is visualised in the bubble plots on the MDS (Figure 22; Figure 23)

Figure 23. Bubble plot of the mud content (<63µm fraction) on the sand fraction on the biological data MDS.

The results of the benthic fauna and the environmental variables monitored at the 11 locations on the BPNS revealed that 3 habitat types can be distinguished, each with a different sensitivity to eutrophication. This is summarized in this section by giving the characteristics of these 3 habitats based on the biological and environmental variables. One sampling station, namely 702, is analyzed separately due to its different characteristics.

Figure 24. Box-Whisker plot of the parameters number of species and density of the different habitat types over the study period.

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The 3 habitat types could be distinguished in their biological characteristics, as in detail outlined above and summarized in Figure 24. The number of species is naturally highest in muddy sands, whereas in muddy sediments the species richness is low (median: 10 spp/0.1m²). In sandy sediments, the number of species is rather low, but can be diverse sometimes. Station 702, which is a sandy station, is characterized by a very low number of species. The median density is highest in the muddy sand habitat, where the densities can be very high, especially during the recruitment period (see higher). The median densities for the muddy and sandy habitats were similar and the variability lower. The density at station 702 is extremely low.The environmental characteristics of these 3 habitat groups were outlined based on mud content, median grain size, Chlorophyll *a* concentration in the water column and sediment.

The sedimentological characteristics of the 3 habitats were summarized in Figure 25. The median grain size is high in the sandy habitat and no mud content. In the muddy sand habitat the median grain size is around 200µm and the mud content below 20%. In the muddy habitat, the mud content is higher than 20% and the median grain size, mostly below 100µm. Station 702 shows a median grain size, which can be classified as fine to medium sand, but contains no mud.
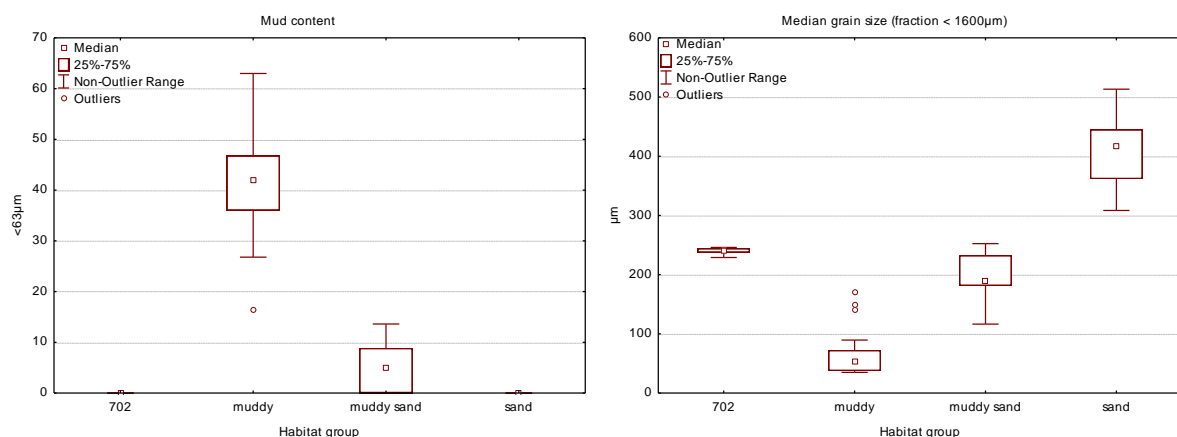
Figure 25. Box-Whisker plot of the parameters mud content and median grain size of the different habitat types over the study period.

The areas where the habitat groups were situated differ in the chlorophyll *a* characteristics in the water column and in the sediments (Figure 26). The chlorophyll *a* is dominantly high at station 702 during the study period in the water column, but a low amount is found back in the sediments. In the area, where the muddy sediments were situated on the BPNS, the chlorophyll *a* concentration is high, compared to the muddy sands and sand areas. The chlorophyll *a* concentration in muddy sediments is dominantly high. The chlorophyll *a* concentration in the water column in the areas of muddy sand is variable and depends on the seasonal cycle. The chlorophyll *a* concentration in the muddy sand sediments is higher than in sand habitats. The sand habitats, situated in the offshore area of the BPNS is characterised by low chlorophyll *a* concentrations in the water column and in the sediments.

The redox potential discontinuity layer (RPD) is absent in sandy sediments, whereas in the other sediment types, the RPD fluctuated seasonal, with a decrease in depth from winter to summer-autumn (Table 2).
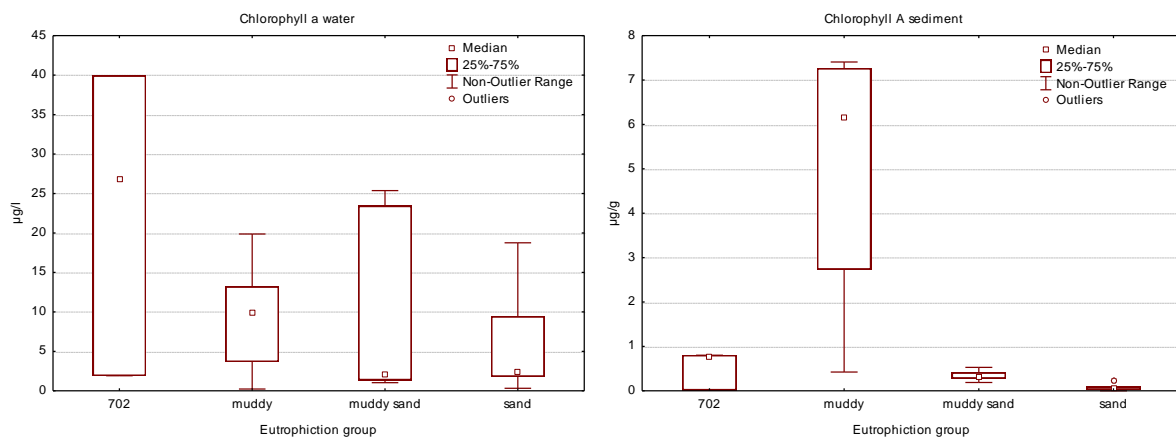
Figure 26. Box-Whisker plot of the parameters chlorophyll a water and sediment of the different habitat types over the study period.

5.4 Eutrophication assessment by indices
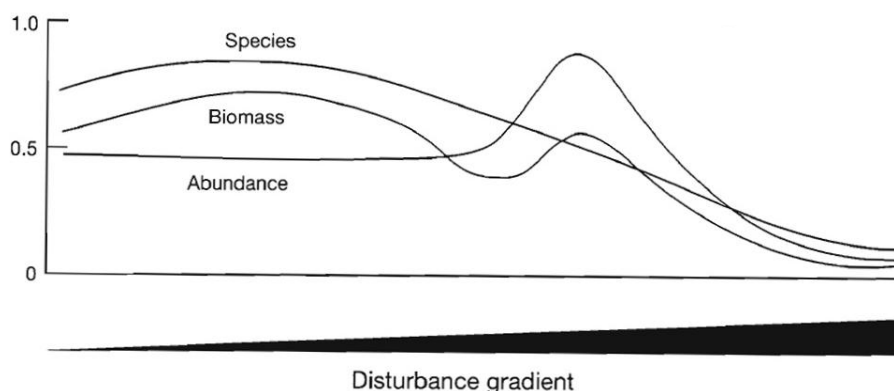
Figure 27. Pearson & Rosenberg model (Rosenberg et al., 2001).

Pearson and Rosenberg (1978) described a general model regarding the effects of organic enrichment on benthic organisms. According to this model, the first detectable change is a slow increase in number of species, followed by an increase in biomass and subsequently an increase in abundance at relatively high organic carbon content (Figure 27). At higher values of organic load, species diversity, biomass and abundance decrease dramatically since anaerobiosis installs. A change in community structure will follow favouring suspension feeders and burrowing detritus feeders (Aertebjerg et al., 2003), while in areas with high organic load opportunistic species will be favoured. While organic matter in sediments is an important source of food for benthic fauna, an overabundance can cause reductions in species richness, abundance, and biomass due to oxygen depletion and build up of toxic by-products (ammonia and sulphide) associated with the breakdown of these materials (Hyland et al., 2005).

A lot of benthic indices were developed based on the Pearson and Rosenberg principle, with the two most important ones being, the AZTI Marine Biotic Index (AMBI) (Borja et al., 2000) (Figure 28) and the Benthic Quality Index (BQI) / Benthic Habitat Quality (BHQ) (Rosenberg et al., 2004; Nilsson and Rosenberg, 1997) (Figure 29). An eutrophication status class for the different monitoring points on the BPNS were determined based on these indices to test their applicability in Belgian marine waters.

$$\text{Biotic Coefficient} = \{(0 \times \% \,GI) + (1.5 \times \% \,GII) + (3 \times \% \,GIII) + (4.5 \times \% \,GIV) + (6 \times \% \,GV)\}/100.$$
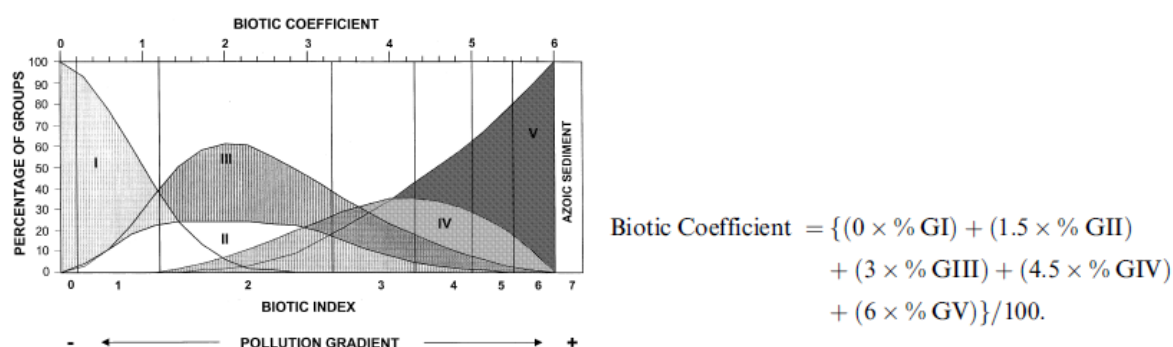
Fig. 2 Theoretical model, modified from Hily (1984), Hily et al. (1986) and Majeed (1987), which provides the ordination of soft-bottom macrofauna species into five ecological groups (Group I: species very sensitive; Group II: species indifferent; Group III: species tolerant; Group IV: second-order opportunistic species; Group V: first-order opportunistic species), according to their sensitivity to an increasing pollution gradient. The relative proportion of abundance of each group in a sample provides a discreet BI with eight levels (0–7) and an equivalent continuous BC (values between 0 and 6).

Table 1. Summary of the AMBI values and their equivalences (after Borja et al., 2000). The last column shows the proposed equivalent Ecological Status (WFD), as used in this study.

AMBI value	Dominating Ecological Group	Benthic Community Health	Site Pollution Classification	Ecological Status
$0.0 < BC \leq 0.2$	I	Normal	Unpolluted	High Status
$0.2 < BC \leq 1.2$		Impoverished		
$1.2 < BC \leq 3.3$	III	Unbalanced	Slightly Polluted	Good Status
$3.3 < BC \leq 4.3$		Transitional to pollution	Meanly Polluted	Moderate Status
$4.3 < BC \leq 5.0$	IV-V	Polluted		Poor Status
$5.0 < BC \leq 5.5$		Transitional to heavy pollution	Heavily Polluted	
$5.5 < BC \leq 6.0$	V	Heavily polluted		Bad Status
Azoic (7.0)	Azoic	Azoic	Extremely Polluted	

Figure 28. AMBI (AZTI Marine Biotic Index) – formula and concept (copy out of Borja et al., 2000)

The equation shown in the figure:

$$BQI = \left(\sum_{i=1}^{n} \left(\frac{A_i}{A_{tot}} \times ES_{50_{0.05i}} \right) \right) \times \log(S + 1) \qquad (1)$$

A_i	Abundance of individuals of species i at the considered station;
A_{tot}	Sum (at the considered station) of the abundances of individuals of all species for which it is possible to compute an $ES_{50_{0.05}}$;
$ES_{50_{0.05i}}$	$ES_{50_{0.05}}$ of species i;
S	Species richness at the considered station.
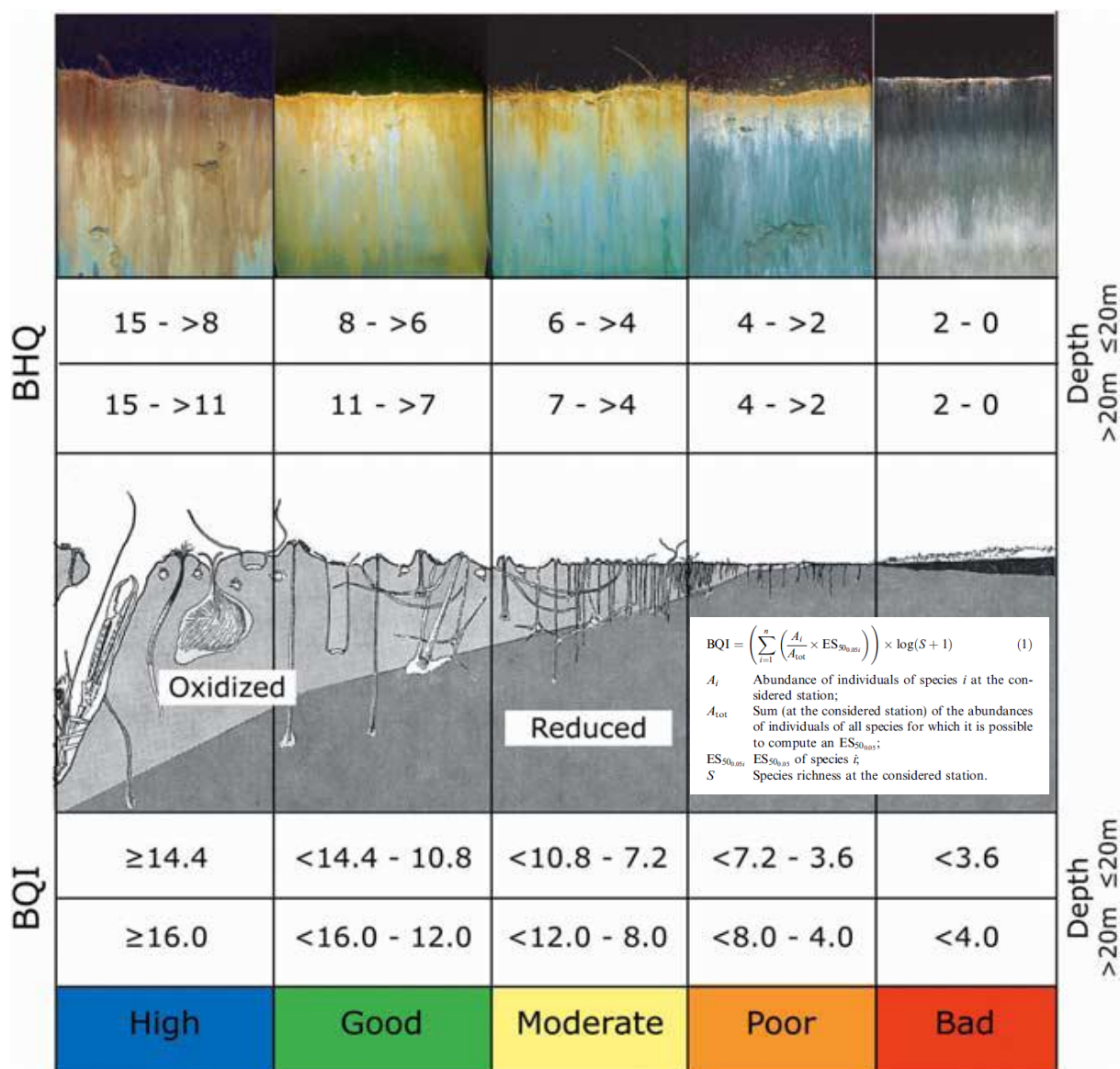
Figure 29. Pearson & Rosenberg model and the associated benthic-habitat quality (BHQ) index. Model of the faunal succession stages along a gradient of increasing disturbance from left to right (after Pearson and Rosenberg,1978). Sediment profile images (colours enhanced) are shown on the top where brownish colour indicate oxidised conditions and black reduced conditions, and the benthic habitat quality (BHQ) indices (Nilsson and Rosenberg, 1997) are presented for depths >20 m and 620 m. The benthic quality indices (BQIs) for the different environmental status accordingto the Water Framework Directive are presented for depths >20 m and 620 m at the bottom of the figure.

5.4.1 Benthic Habitat Quality (BHQ) / Benthic Quality Index (BQI)

The **BHQ** (Benthic Habitat Quality) is developed based on sediment profile images (SPI) to classify the physical (Redox potential layer, sediment type) and biological characteristics (presence of tubes/burrows of organisms) of the sediment. This could not be used for the evaluation of the Belgian monitoring stations, due to the lack of the ability to take SPI's. However, photographs of the Van Veen samples were available, which made it possible to visually compare the BHQ types defined by Nilsson and Rosenberg (1997) with these images.

Based on this information and expert judgment, an attempt was made to classify the different habitat types in the BHQ categories. Completely anoxic sediments were not observed during the current survey.

The offshore sandy sediments were classified as oxidised and not suffering from eutrophication. The Van Veen images show well oxidised sandy sediments (Figure 30).

Figure 30. Van Veen images of some offshore sandy stations in March 2009.

The coastal muddy/sandy sediments were slightly reduced, showing an oxidised top layer of varying thickness (Table 2, Figure 31). The depth of the RPD layer in these sediments is influenced by the phytoplankton bloom deposition and the activity of tube building benthic animals, which affect the biochemical status of the sediment (Braeckman et al., 2009). These sediments can be classified as reduced (BHQ < 6) (Figure 29).
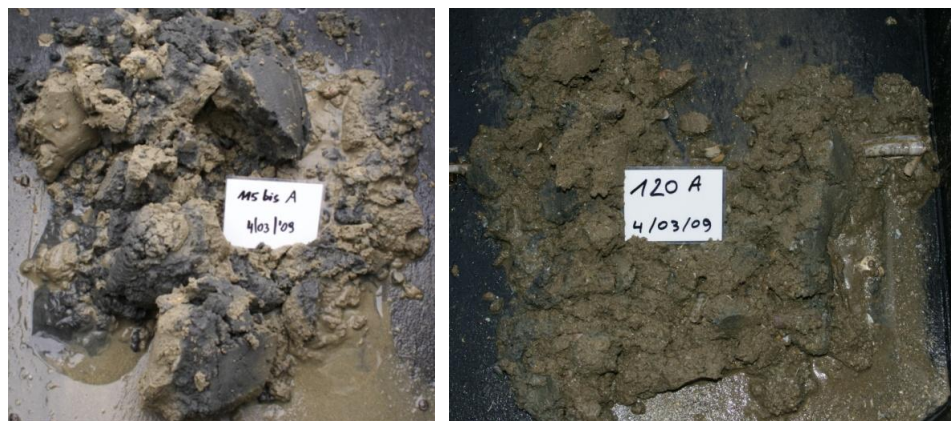

Figure 31. Van Veen images of the coastal muddy sand stations 115bis and 120 in March 2009.

The muddy sediments in our coastal area can be classified as strongly reduced, with a thin RPD layer (strong reduction in the oxidised layer) (Table 2). This is confirmed by the Van Veen images (Figure 32), that showed a very thin oxidised top-layer.

Figure 32. Van Veen images of the muddy sediments at station W01 and W02 in February 2009.

When the **BQI** (Benthic Quality Index) (Rosenberg et al., 2004) is calculated, based on the average per sample period for each station (Table 5), all stations were classified as poor to moderate. The $ES50_{0.05}$ value for each species is based on the list of the Marine North Sea dataset of MARBEF (Grémare et al., 2009). When lacking a value for an abundant species in Belgian marine waters, an expert value was assigned based on a similar species type in the list (e.g. interstitial polychaetes, Oligochaeta, Anthozoa, *Polydora, Petricola pholadiformis*). The BQI scores were seasonally affected, due to the increase in diversity towards the summer, leading to higher BQI scores.

The BQI scores for fine sandy sediments (*Abra alba*) and muddy sediments (*Macoma balthica*) correspond with the expectations for slightly reduced sediments (Figure 29) and reduced sediments. The BQI scores of the well-oxidised sandy sediments, however, do not correspond with the expectations. This sediment type was not affected by eutrophication, as shown in the results. Nevertheless, the BQI scores show that these sediments were less diverse and consisted mainly of tolerant species. The occurrence of tolerant species can be linked to the fact that the sediments are situated in a physically disturbed (both natural and anthropogenic) environment.

Table 5. BQI (Benthic Quality Index) values for the different station per sample period, with indication of the quality status.

BQI	Habitat	winter		spring		summer	
702	?	5.25	poor	3.14	poor	5.10	poor
W01	*Macoma balthica*	4.67	poor	5.88	poor	8.00	moderate
W02		4.82	poor	6.30	poor	8.83	moderate
115 bis	*Abra alba*	8.13	moderate	6.54	poor	8.53	moderate
W03		8.95	moderate	10.40	moderate	10.33	moderate
W05	*Nephtys cirrosa-*	5.90	poor	5.64	poor	9.51	moderate
W06		6.24	poor	6.67	poor		
W07		7.99	moderate	7.30	moderate		
W08	*Ophelia limacina*	5.55	poor	6.40	poor		
W09		6.63	poor	7.22	moderate		
W10		8.88	moderate	10.86	moderate		

The species classification (sensitivity/tolerance) of the BQI method is based on proportional abundance in relation to the diversity of samples, and classifies species tolerant to

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eutrophication (cf species in muddy sediments) or physical disturbance (cf species in sandy sediments) in the same way. Consequently, this index is not suitable for the evaluation of eutrophication in the Belgian Marine Waters. The use of sediment profile images and the related index (BHQ), however, seems to be alternative suitable tool for quick eutrophication assessment.

5.4.2 AZTI Marine Biotic Index (AMBI)

The AMBI (AZTI Marine Biotic Index), classifies the sensitivity and tolerance level of the species to organic enrichment, based on expert judgement. When the **AMBI** was calculated, based on the average per sample period for each station (Table 6), a scattered pattern (difference within the habitats and seasons) in status was observed. Differences in the dominance of the different species sensitivity/tolerance classes were visualised in Figure 33. The AMBI index declined from winter to summer for the coastal stations, except for W01. For the sandy habitats, the AMBI index increased in spring, mainly due to the recruitment of *Spiophanes bombyx* (Type III species). The sandy habitat stations seemed to have a good status, in relation to eutrophication assessment based on AMBI. For the *Abra alba* habitat, a ecological status difference was observed between 115bis (good) and W03 (poor-moderate), despite the fact that station 115bis was less diverse and dense than station W03. The high status in summer for 115bis is related to the recruitment of *Ensis spp* (Type I species), which is in fact an invasive species (Figure 33). The poor-moderate status at station W03 is related to the dominance of Oligochaeta and *Cirratulidae spp.* in otherwise diverse samples. For the *Macoma balthica* habitats, a good status was observed for station W01, despite the increase in AMBI towards summer, indicating a higher dominance of tolerant species towards summer. This is related to the disappearance of *Petricola pholadiformis* (Type I species) in the summer samples. At station W02, the poor status in winter was related to a very strong dominance of Oligochaeta and *Cirratulidae spp.*, whereas the recruitment of *Ensis spp.* was responsible for the good status in summer. Despite the poor conditions at station 702, the AMBI classified it as moderate to good.

Table 6. AMBI (AZTI Marine Biotic Index) values for the different station per sample period, with indication of the quality status.

AMBI	Habitat	winter		spring		summer	
702	?	3.55	moderate	2.05	good	1.36	good
W01	*Macoma balthica*	1.99	good	1.68	good	3.12	good
W02		5.16	poor	4.15	moderate	2.90	good
115 bis	*Abra alba*	2.11	good	1.79	good	0.18	high
W03		4.88	poor	3.73	moderate	3.41	moderate
W05	*Nephtys cirrosa-*	2.15	good	3.36	moderate		
W06		1.93	good	3.41	moderate	1.96	good
W07		1.43	good	3.19	good		
W08	*Ophelia limacina*	1.10	high	1.63	good		
W09		1.28	good	1.83	good		
W10		1.08	high	1.56	good		

The use of AMBI for eutrophication assessment did not result in consistent patterns related to dominance changes of certain species in time and place. The natural recruitment of certain type of species (e.g. *Ensis, Cirratulidae, ...*), can change the AMBI index value considerably, depending on their species type classification.
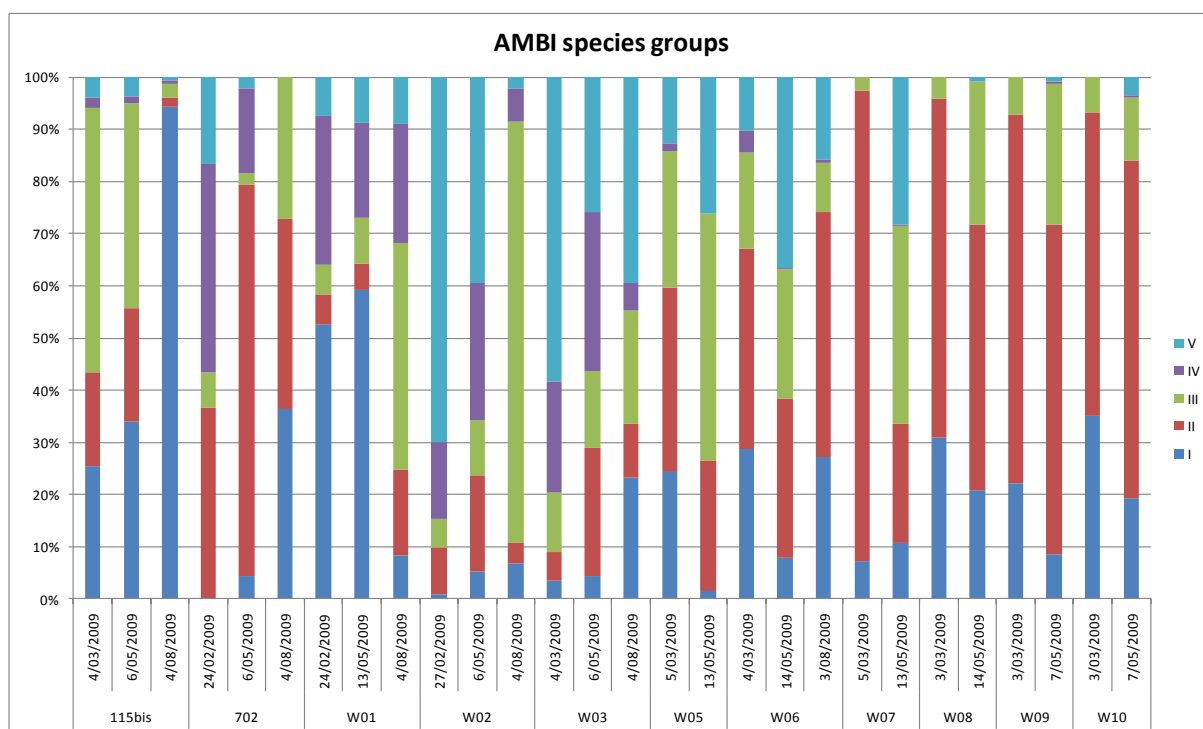
Figure 33. The proportional distribution of the different species sensitivity/tolerance classes at each station in each period.

5.4.3   Benthic Ecosystem Quality Index (BEQI), level 3

The two most important indices related to evaluating the effect of organic enrichment on benthos, show some shortcomings when used in the benthic habitats on the BPNS. Consequently, another index was considered.

The Benthic Ecosystem Quality Index (BEQI) can objectively evaluated changes in ecological status between current observations and a pre-defined reference status (Van Hoey et al., 2007). The current reference status of the benthic habitats (used in WFD context) is defined based on benthic autumn data within the 6 nautical mile zone of the BPNS in the period 1994-2004 (Van Hoey et al., 2008). When the eutrophication intensity changed between 2009 compared to the period 1994-2004, then the BEQI level 3 will score this. The hypothesis is that the changes in benthic habitat characteristics between now and 1994-2004 was not drastic, especially in relation to euthrophication, resulting in moderate or lower status for the BPNS.

A test was done by using the BEQI level 3 for the comparison of the winter data with the WFD reference for the different habitats (Table 7). The results indicated a good status for the three habitats, taking into account following observations:

- Seasonal difference between assessment (winter) and reference (autumn)
- The density values of the assessment seemed to correspond within what was expected, but the power of the parameter density was moderate to low. This indicates that more samples should to be used in the evaluation.
- The score for the number of species was high to good. Only for the *Nephtys cirrosa* habitat, a lot of new species were found in the assessment data but not in the reference, due to lack of typical offshore sand bank species in the reference (e.g. *Polygordius appendiculatus, Exogone spp., Syllidae species, Aonides paucibranchiata...*). Therefore, the parameter similarity for this habitat was low.

Table 7. BEQI (Benthic Ecosystem Quality Index) level 3 scores for the parameter density, number of species and similarity. * moderate power score; ** low porwer score.

Community	SS	Density (D)		Number of species (N0)		Similarity (S)	EQR - scores			
		Ass.	Ref.	Ass.	Ref.	Ass.	D	N0	S	Av.
Abra alba	0.60	4938	5477	50	147	0.55	0.93*	0.64	0.56	0.71
Macoma balthica	0.60	373	541	22	89	0.38	0.85**	0.69	0.57	0.70
Nephtys cirrosa	1.70	432	324	53	92	0.47	0.76*	0.83	0.45	0.68

The BEQI index shows that the benthic characteristics at the different habitats was not drastically changed in comparison to 1994-2004.

6 Discussion

Eutrophication process affecting primary production can have a drastic effect on coastal sediments of the North Sea as 24% of the ungrazed primary production is deposited on the sea floor of the Belgian Continental Shelf (Lancelot et al., 2005). The fate of this organic matter depends on the receiving sediment type (Franco et al., 2007; Franco et al., 2008). In physically driven permeable sediments, characterised by relatively coarse sediments, advective flows through the sediment hamper sedimentation of phytoplankton, provides the sediment with oxygen and increase the mineralisation of organic matter (Ehrenhauss et al., 2004; Huettel & Rush, 2000; Janssen et al., 2005; Bühring et al., 2006). Finer, so-called cohesive sediments, can be found in areas with lower hydrodynamic stress where phytodetritus settles easier at the sediment surface. In these sediments, sharp profiles of labile organic matter occur (Franco et al. ,2007), mineralisation of this organic matter results in oxygen stress (Graf, 1992) resulting in retarded breakdown of organic matter (Boon & Duineveld, 1998). Both sediment types are encountered at the Belgian Continental Shelf and very different biogeochemical, bacterial, meio-and macrobenthic responses to phytodetritus sedimentation were observed (Franco et al. 2007, 2008; Vanaverbeke et al. 2004a,b; Van Oevelen et al. 2009). Based on this, negative effects of eutrophication are expected to be most pronounced in fine grained sediments acting as cohesive sediments. Eutrophication affecting benthic fauna can lead to death (in complete anoxic areas) or changes in the benthic faunal composition. The hypothesis tested in this study is if the eutrophication in the Belgian waters causes problems in the seasonal pattern of the benthos (number of species, density, biomass) in certain areas, related to changes in sediment conditions (oxygen, RPD, Chlorophyll a). The normal season pattern of benthos in coastal waters is described in detail in several studies (Van Hoey et al., 2007) and result in an increase in density, biomass and number of species from the beginning of spring (coincidently with the spring phytoplankton bloom) towards autumn, due to recruitment of the benthic fauna. During autumn and winter, the benthic densities and number of species decline due to mortality.

Although the multivariate analysis in benthic fauna and environmental variables revealed differences between most of the sampling stations, our results confirm that we sampled both cohesive and permeable sediment types. Multivariate differences in environmental characteristics between locations within sediment type guarantee that all kinds of temporal responses (also significant in multivariate analysis) in environmental characteristics are incorporated in the sampling design. As such, potential different benthic responses to eutrophication at the east and west coast (resulting from possible differing external structuring factors for the water column processes) will become clear from our sampling design. It has to be mentioned that all cohesive sediments and some of the permeable sediments are located within areas that are listed among Problem Areas or Potential Problem Areas for eutrophication by Lancelot et al. (2008), and hence eventual negative effects of water eutrophication on the benthic environment will become clear in a routine monitoring programme.

Based on several studies (Van Hoey et al., 2004; Degraer et al., 2008), the benthic communities/habitats on the BCS are known and consist of the following main types: (1) the *Abra alba – Mysella bidentata* community characterised by high densities and diversity and

mostly found in **fine muddy sands** (*Abra alba* habitat); (2) the *Nephtys cirrosa* community characterised by very low densities and diversity and found in well-sorted **sandy sediments** (*Nephtys cirrosa* habitat); (3) the *Ophelia limacina – Glycera lapidum* community characterised by very low densities and diversity and found in **coarse sandy sediments** (*Ophelia limacina* habitat); and (4) the *Macoma balthica* community characterised by low densities and diversity and found in **muddy sediments** (*Macoma balthica* habitat). Those 4 habitat types were also reflected in the EUTROF monitoring programme, as outlined by the analysis on the benthic data. A distinction between the *Nephtys cirrosa* and *Ophelia limacina* habitat in the sandy offshore area was not made in this study, due to their higher similarity in comparison to the other habitat types. Only the most offshore stations (W09 and W10), were characterised by more coarser sediments, compared to the other offshore stations, which is partly reflected in their difference in benthic fauna (less divers and lower densities). The more offshore stations W05, W06, W07, W08, W09 and W10 were characterised as *Nephtys cirrosa* or *Ophelia limacina* habitat based on their biological characteristics. These stations and habitat types belong to the permeable sediments, as deduced from their coarser median grain size, lack of clay-silt fraction and very low values of chl *a* (proxy for labile organic matter). The coastal stations (from West to East: St. 115bis, W03, W02, W01 and 702) were characterised as *Abra alba* habitat (115bis and W03) or *Macoma balthica* habitat (W02, W01), except station 702, which seems to be characterised by a sandy habitat in an unstable environment. The biological characteristics at this site were poor and not directly comparable with what is expected in such sediment type. The *Abra alba* and *Macoma balthica* habitat can be characterised as belonging to the cohesive sediment type, due to lower median grain size and higher clay-silt fraction and higher chl a values. The linking between the position of the monitoring point and the distribution of the corresponding habitat area is visualised in Figure 34. The extent of the habitat area is based on modelling of the physical characteristics determining the habitat type (Degraer et al., 2008). The biological characteristics of the monitoring points corresponds with the habitat suitability expected in that area, except for station 702.

6.1 Eutrophication assessment

The occurrence of eutrophication effects on the benthic fauna on the BPNS is described for the different habitats separately, because they have a different potential to eutrophication (Franco et al., 2007; Franco et al., 2008). This is due to the difference in physical characteristics between the habitats (mainly sedimentology), which determine on their turn the benthic characteristics. The spatial distribution of these habitats in relation to the eutrophication area in the water column plays an important role in the potential negative eutrophication effect on the benthos (Figure 34).

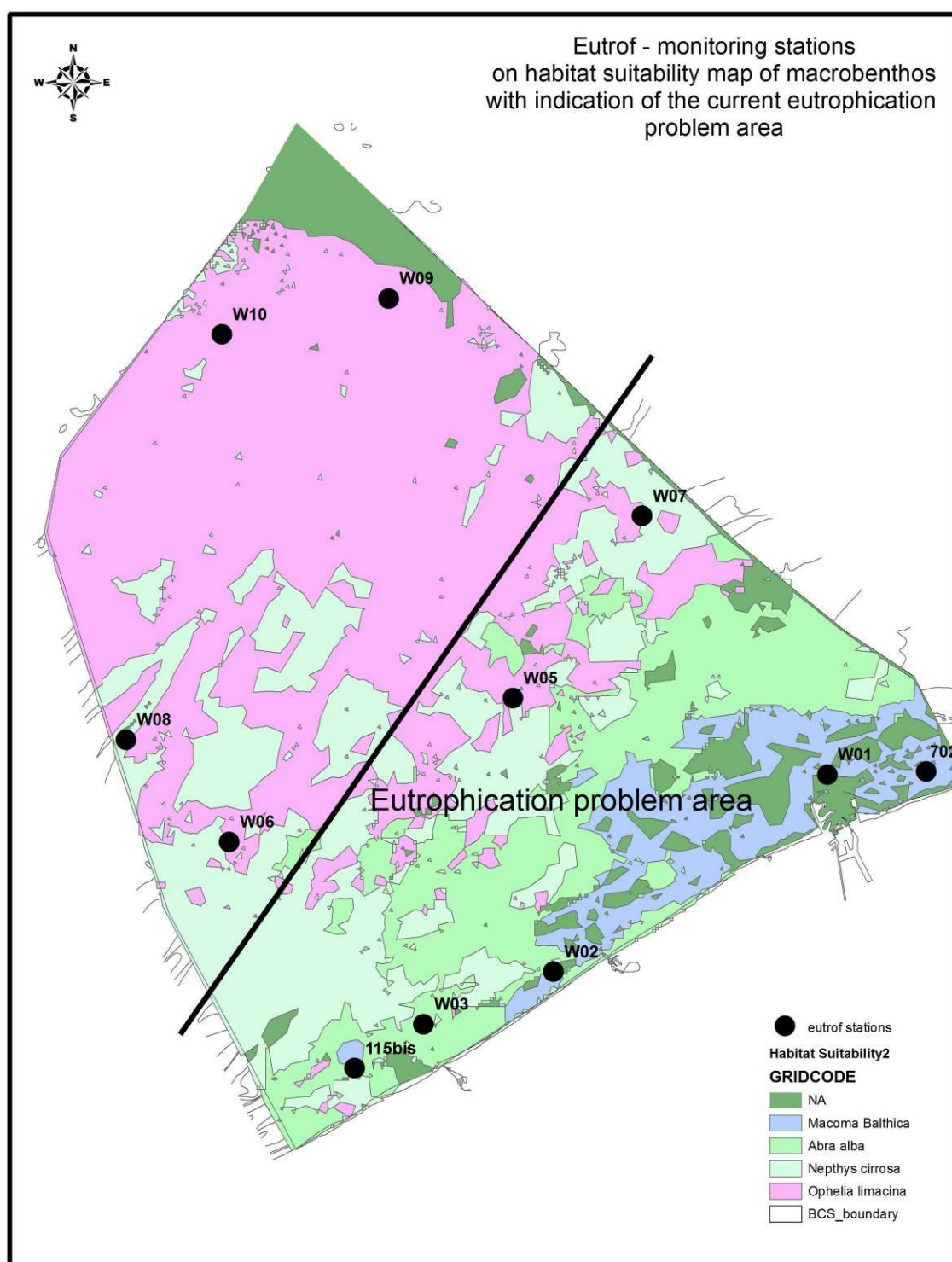**Figure 34. Map of the Belgian Part of the North Sea, with indication of the different benthic habitat types (habitat suitability), the EUTROF monitoring stations and the eutrophication problem area in the water column.**

At the more **offshore stations** W05, W06, W07, W08, W09 and W10, characterised as permeable sediments and located in areas with the lowest chl a concentrations in the water

column over the year, no effect of eutrophication on the benthos was encountered. The RPD layer was not present during all the sampling campaigns, except for 2 replicates and probably reflect a very local increased source of organic matter (e.g. a decaying larger organism), that is unrelated to sedimentation of organic matter as this would have resulted in the observation of an RPD layer in all replicates. The benthic characteristics in this area shows a normal seasonal pattern with higher densities, biomass and number of species in spring compared to winter, due to the recruitment of the benthic fauna. In 2009, the strongest recruitment in the offshore area was found for *Spiophanes bombyx* and *Echinocardium cordatum*. There can be concluded that the **offshore sandy sediments** has the **lowest potential to be impacted by eutrophication**.

Stations with lowest median grain sizes and highest clay-silt fractions are located at the **coast** (from West to East: Station 115bis, W03, W02, W01 and 702). Although a clear peak in phytoplankton sedimentation could not be observed in present study, the RPD layers was present in all stations in most of the sampling months and moved closer to the sediment-water interface after the winter months. This **increase in oxygen stress** during spring and summer was observed before at Station 115bis (Franco & Vanaverbeke, unpublished results) and resulted from increased sediment community oxygen consumption provoked by higher temperatures and increased availability of labile organic matter (Franco et al. 2009). In this context, a **deviation can be made between the muddy sands** (st 115bis, W03) **and muddy sediments** (W02, W01) due to some difference in biological and environmental characteristics.

In the **muddy sands**, the amount of organic matter is relatively high and the position of the RPD layer is variable. These sediments were also colonized by the **most diverse benthic community**, characterised by high densities and number of species. The seasonal pattern at station W03 (most diverse) was normal with an increase from winter towards the summer in density and number of species. At station 115bis, the seasonal pattern was less pronounced, except the very high recruitment of the bivalve *Ensis directus* in summer, and suffer also more from anoxic parts in the sediment. The benthic fauna at this station lacks the occurrence of structuring tube-building species and high densities of bioturbating animals, which can positively affecting the biochemical processes in the sediments (Braeckman et al., 2009). The reason for the benthic state at this station is not clear and can be all sorts (fishing, recruitment failure of certain species, eutrophication).

In the **muddy sediments**, the highest amount of organic matter in the water and sediment was found. There are also changes in the **RPD layer**, which decrease clearly in depth from winter to summer and is **permanently present**. Despite, these changes, the benthic seasonal patterns were not disturbed at station W02 and W01, because the species recruit in those areas and survive. The dominant species in this area were opportunistic polychaetes (*Capitella capitata, Cirratulidae spp*) and oligochaetes and those were adapted to partly anoxic sediments.

The observations at the **east coast** merit some special attention. Two sampling stations were located in this area as it was expected that the presence of the **Westerschelde mouth would increase the risk for eutrophication** (Brion et al. 2008; Lancelot et al. 2008), which was

reflected in the elevated chl *a* values in the water column observed in present study in this area. Data obtained at Station W01 indeed reflected the expectations of finding fine sediments with high amounts of clay-silt, chl *a* where oxygen profiles would reflect largely anoxic sediments as it was the case in earlier large-scale research (Vincx, 1990). Despite, the **anoxicity of the sediment**, some benthic animals, characteristic for this type of environment and adapted to it, where found and can scope the current eutrophication rate. However, data from Station 702 are not in accordance with this general expectation. Compared to the 1990'ies (Steyaert et al. 1999) **sediments became coarser**, with a lower amount of clay-silt and the presumed high oxygen stress was only present in August. This station is also characterised by the occurrence of few benthic species and no normal seasonal pattern, which indicate disturbance. This suggests that the area east of the harbour is **highly dynamic**, possible as a consequence of the enlargement of the harbour. This means that there were other **anthropogenic effects**, who plays a role on top of the eutrophication problem in this area (cf water column measures), and affecting the benthic fauna strongly. This should be backed up by more integrated research (e.g. combination of biology, biogeochemistry and geology) but at the moment this points to the fact that generalisations about the east coast cannot be made.

Based on the delineation of the problem/non-problem area concerning eutrophication in the water column (Figure 34), the **spatial area wherein eutrophication can affect benthos is mainly situated in the coastal zone**. The main occurring habitat types in the Belgian Coastal zone is the muddy sand and muddy habitat, which show potentials to be eutrophication, **especially the muddy habitat**, as described above. Therefore, the coastal area, with focus on the east coast, has to be monitored in the future regarding the eutrophication parameter changes/mortality in the benthos.

6.2   Eutrophication assessment by indices

The test of different index types for evaluating the euthrophication status of the benthos on the BPNS is important in selecting quick assessment methods appropriate for this monitoring goal. Most benthic indices were developed in function of certain objectives, in certain regions, to detect the effect of certain anthropogenic pressure, etc. Therefore, the eutrophication status of the habitat types in the BPNS were tested with 3 indices, the BQI (Benthic Quality Index) (Rosenberg et al., 2004), AMBI (AZTI Marine Biotic Index) (Borja et al., 2000) and the BEQI level 3 (Benthic Ecosystem Quality Index) (Van Hoey et al., 2007). Beside it, the potential of using visual images of the sediment, to assess the eutrophication status was investigated and can be evaluated with the BHQ (Benthic Habitat Quality) (Nilsson & Rosenberg, 1997).

The BQI and AMBI index were designed in function of assessing the eutrophication status of benthos by classifying species in sensitivity and tolerance species classes. The species class by AMBI were defined based on expert judgement, where the species class by the BQI is defined on their proportional abundance in relation to the diversity of samples. The BEQI index, level 3 does not classify species in certain sensitivity/tolerance classes, but rather

evaluated changes in species composition, which can be the results of any anthropogenic cause. It differs also in comparison to BQI and AMBI in the fact that this index scale the deviation between the assessment and the predefined reference data for benthic parameters (density, number of species and species composition). The BEQI is used for evaluating the environmental status of the benthos in the Belgian Coastal waters in the framework of the WFD. The BHQ evaluate sedimentological and biological characteristics visible on sediment profile images and is a complete different approach in comparison to the classical benthic indices.

The **BQI** index score correspond for fine sandy sediments (*Abra alba*) and muddy sediments (*Macoma balthica*) with the expectations for slightly reduced sediments and reduced sediments, but not for well-oxidized sandy sediments. This can be attributed to the fact that the species classification method, classifies species tolerant to eutrophication (cf species in muddy sediments) or physical disturbance (cf species in sandy sediments) in the same way. Consequently, this index is not direct suitable for the evaluation of eutrophication in the Belgian Marine Waters. The use of **sediment profile images** and the related index (BHQ), however, can be a **suitable tool** for quick eutrophication assessment. The **AMBI** index did not result in consistent patterns related to dominance changes of certain species in time and place.

And the test of the **BEQI** was **preliminary**, due to the fact that some aspects should be met first for an appropriate test.

- The assessment should to be done on autumn data (samples were taken, but not processed for this report), due to the fact that reference and assessment should be defined based on the same season to exclude seasonal effects on the assessment result.
- The reference status (cf WFD) of the offshore sandy habitats (*Nephtys cirrosa* habitat) is not adequately defined, based on the current 6 mile data. For the other habitats, the dataset should be expanded as well, but the current definition is more or less representative. A lot of benthic data of the BPNS is available to fill in this aspect.
- More samples (> 6), temporally or spatially spread, should be taken to assess the coastal muddy and muddy sand habitat with sufficient confidence, especially for the parameter density (cf WFD monitoring). The monitoring points selected for this study were representative for the different habitats on the BPNS, but for a yearly evaluation more sample locations within each habitat should be selected. When an evaluation can be done including several years, a higher temporal distribution of samples at the current locations would suffice.

When these assumptions were met, the BEQI seems to be suitable for evaluating the overall ecological status of the benthic habitats on the BPNS. The evaluation of the eutrophication status with the BEQI is difficult, due to the lack of benthic reference data of the Belgian Coast, which was not influenced by the eutrophication problems of the last decades.

6.3 Bentho-pelagic coupling: summary of previous projects

The patterns detected in macrobenthic densities largely **reflect earlier observations** on the BPNS (Franco et al. 2009; Van Hoey, 2000). Both biomass and densities increase in spring, rapidly after the establishment and subsequent sedimentation of the peak phytoplankton bloom in coarse sediments, reflecting the fast mineralisation processes described above. In finer sediments however, the increase in macrobenthic densities and biomass is delayed until late summer. This pattern was described before in a long-term analysis (1995-2003) of the diversity and densities of the macrobenthos from the same study area (Van Hoey et al. 2007).

This again shows that data collected following our sampling scheme reflect the general patterns observed in different sediment types at the BPNS.

6.4 Evaluation of the monitoring strategy

The selection of the overall monitoring stations on the BPNS, which were monitored for several purposes, seems to be fitting for covering the spatial distribution of the different benthic habitats. Those stations were not only valuable for evaluating the eutrophication on the BPNS scale, but can also be used to evaluate the benthic diversity in the different habitats on a longer term. Despite, the **good fitting of the monitoring stations**, some suggestions can be made for improvement of the programme:

- A better distinction between the station located in the sandy habitat (*Nephtys cirrosa)* and the coarse sandy habitat (*Ophelia limacina*) can be made in the future, but is not necessary for eutrophication assessment.
- It could be valuable to include a monitoring station at the east coast (e.g. North of the Vlakte van de Raan) to cover the *Abra alba* habitat at this site of the coast.
- For a correct assessment of eutrophication effects on the sediment at the east coast, it requires to incorporate more sampling stations at the area east of the Zeebrugge harbour.
- The use of other sampling techniques (cf SPI-camera) (see paragraph below), to allow a quicker eutrophication assessment in more detail and on a better spatial scale.

In this study, it was tested, based on limited number of benthic samples, whether eutrophication processes disturb the benthic seasonal pattern. This investigation of a limited number of spatially distributed samples in the different habitats shows quite good what the eutrophication potentials were for the different habitat types. Another interesting tool for evaluating eutrophication effects on benthos is the use of a SPI- camera (Sediment Profile Imaging). With this tool a rapid assessment of the bottom can be made on a larger spatial scale. Images made by this camera can be evaluated regarding sedimentological characteristics, RPD layer, tube density of benthic fauna, benthic fauna colonization stadia (Rhoads and Germano, 1982; Solan and Kennedy, 2002; Rosenberg et al., 2004). No such

instrument is currently tested in our waters and is also not available in the sampling equipment of Belgian research institutes.

6.5 Conclusion

Based on the above described patterns in benthic characteristics and environmental patterns, it can be concluded that the eutrophication potential of the different habitats on the Belgian Part of the North Sea is as follows:

- The offshore, sandy habitats (*Nephtys cirrosa* and *Ophelia limancina* habitat) are not affected by eutrophication, due to the physically driven permeable sediment characteristics. The RPD layer is not influenced and the benthic seasonal patterns were normal.
- The coastal, muddy-fine sand habitat (W03-115bis), a cohesive sediment type, has a slight potential to be affected by eutrophication, due to relative high chlorophyll a concentrations in the sediment. The RPD layer is also variable with anoxic sediment parts, which slightly affected the benthos, especially when habitat structuring species were absent (cf 115bis).
- The coastal muddy habitats (WO1-W02), a cohesive sediment type, has the highest potential to be affected by eutrophication, due to the high chlorophyll *a* concentrations in the sediment and the presence of a permanent RPD layer. Some benthic animals (opportunistic species), adapted to this environments, settle and survive in these areas and can scope the current eutrophication pressure.
- At the east side of the harbour of Zeebrugge strong changes were observed in sedimentology and benthic fauna over the last decades (cf station 702), which were not only related to eutrophication. Station 702 is characterised by sandy sediment, a poor benthic community, relatively low chlorophyll *a* concentration in the sediment and no RPD layer. The chlorophyll *a* concentration in the water column is very high compared to other areas on the BPNS.

With the knowledge of the spatial distribution of the different habitats on the BPNS (Figure 34), it can be concluded that the benthos at the eastern coastal side of the BPNS can potentially suffer from eutrophication. In the other part of the Belgian coast, the benthos can locally be affected by accumulated organic matter (enlarged by eutrophication), but on larger scale seems to be non problematic. The benthos in the offshore area of the BPNS is not affected by the current eutrophication problem.

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