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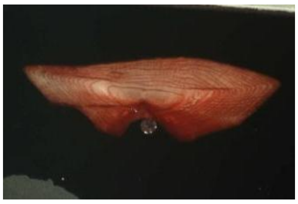

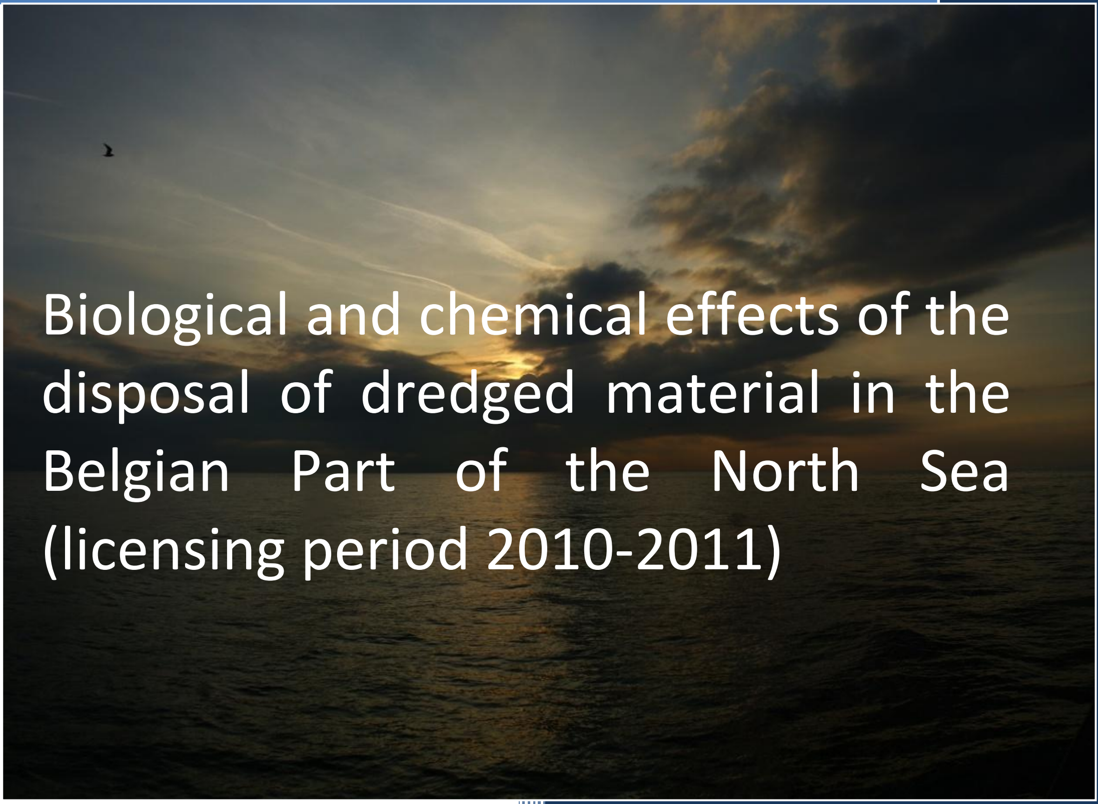

Biological and chemical effects of the disposal of dredged material in the Belgian Part of the North Sea (licensing period 2010-2011)

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1 Summary

It is important to investigate the effects of dumping of dredged material in the marine environment from ecosystem perspective, because it could lead to different responses of the ecosystem. Therefore, the regular dredging program from ILVO Fisheries is evaluating the impact of these dumping activities at different levels by looking at: i) differences in biological characteristics of the ecosystem components macrobenthos, epibenthos and demersal fish ii) the (bio)accumulation of contaminants in the marine ecosystem as investigated by chemical analysis of sediment and different biota species iii) biological effects of pollutants on marine organisms as indicated by the prevalence of fish diseases and by the measurement of enzymatic EROD activities in the liver of juvenile dab. We also conducted a study on the long term impact of dredged material dumping on the benthic habitats in the Belgian Coastal Zone. Secondly, we explored the benthic life in the dredging area's itself. Thirdly, we investigated the effect of some adaptations in the ecological sampling strategy of the routine monitoring program.

Long term impact of dredged material dumping on the benthic habitats

A long term analysis of changes in the benthic habitat characteristics in relation to the dumping intensity is executed at the five dumping sites. At ILVO, a benthic sampling program is running from 1978 onwards and changed strongly over this period, with a clear impact/control sampling strategy at each dumping site in the last years (2004 onwards). Previously, only a monitoring point inside the dumping zone Br&W S2 is followed up. Based on the multivariate analysis, we detected a transition in species assemblage. This changes in species composition can be attributed to the absence of mud loving species in the recent period. The average density and species richness were also significantly lower in the latest period ('01-'08) compared to the previous ones ('85-'90; '93-'00). These observed patterns coincide with a decrease in the dumping intensity in the recent period ('01-'08). We can conclude that dumping at Br&W S2 has a small positive effect, by supply of mud and organic matter to the more sandy environment. This species enrichment pattern at Br&W S2 is observed again in the monitoring of 2010 (see further).

The dumping activities at the five disposal sites have lead to benthic habitat changes at dumping site Br&W S1, while at the other sites, the benthic community seems to cope with the existing dumping regime. Especially in naturally more impoverished areas (dumping site Br&W Zeebrugge-Oost & Br&W Oostende), the impact is less pronounced than in more vulnerable benthic habitats (e.g. *Abra alba* habitat or sandy environments). We suggest that if an impact is detected, this is mainly related to the physical burial of the organisms (smothering, incorporation), or to the properties of the dredged-disposal (mud in more sandy areas), both causing habitat modifications.

Biological and chemical status of the disposal sites in the Belgian Part of the North Sea: 2009-2010

Biological status

The ecological status of the macrobenthos at the different disposal sites is evaluated with the benthic indicator BEQI. The observed patterns confirm those of the previous years. The

medium to high dumping intensity at Br&W Oostende and Br&W Zeebrugge Oost has no effect on the macrobenthic community. At dumping site Nieuwpoort however, the macrobenthic characteristics showed a high variability which could not be attributed to the low dumping activity. At Br&W S1, where the highest dumping activities occur, a steady loss of *Abra alba* habitat could still be observed in the dumping area. The tube building polychaete *Owenia fusiformis* however, has exponentially increased in the surrounding area. Despite the 'good status' of Br&W S2, some remarkable changes in the macrobenthic community appeared. The benthic characteristics of the samples indicate an enrichment of the Northern samples with mud 'loving' species, whereas the samples in the western part are more impoverished (lower diversity). If this could be attributed to the higher dumping intensities over the period 2009 (focus on Northern part) – 2010 (focus on Western part) than in previous years is not unambiguous. Still, this should be confirmed by further detailed analyses.

For the epibenthic and demersal fish fauna, no effects of dumping were clearly visible. This could be explained by the fact that most of those species have a high mobility and are able to flee from the dumping sites. Another possibility is the fact that the statistical power (due to a low number of samples caused by a switch in sampling strategy) was too low to detect any possible effects. Nevertheless, some significant differences in the epibentic and demersal fish characteristics occurred, probably due to a temporary dominance of certain species (e.g. starfish, brittlestar and goby) and/or by the natural variability of the habitat (e.g. dumping site Nieuwpoort). For future research, it is advisable to investigate the effect of dumping on certain functional or sensitive species.

Chemical status

Sediment Assessment

During the period 2009-2010, only small variations between impact and control assessment are observed for the measured parameters heavy metals, PCBs and pesticides. No major differences between the diverse dumping areas are noticed based on the assessment of sediment samples. Nevertheless, the levels of lead and PCBs must be followed in future since EAC values (OSPAR, MSFD, Belgisch Staatsblad) were approached or even exceeded at both, impact and control sites. Other measured heavy metals and persistent organic pollutants do not approach the formulated EAC values.

Accumulation of Pollutants in Marine Organisms

Chemical analysis was performed on diverse sentinel species to assess the (bio)accumulation level of persistent organic pollutants and heavy metals. Due to the omnipresence in the marine environment and relevance in the ecosystem, mainly starfish and brown shrimp were used to evaluate the chemical health status of the different dumping sites.

Based on the accumulated levels of POPs and heavy metals in marine biota species during 2009-2010, it can be concluded that differences in contamination between control and impact areas for dredge disposal sites are limited. The PAH levels were slightly elevated on the impact areas of dredge deposit sites Br&W Zeebrugge Oost, Br&W Oostende and Br&W S1 compared to the control areas. Based on the assessment of accumulation, dumping site Br&W Oostende could be distinguished from the other dumping sites based on higher levels of contamination in starfish.

Biological Effects of Pollutants

Externally visible fish diseases (e.g. ulcers, skeletal deformations, nodules, lymphocystis) and parasite infection were used as parameter for environmental stress and environmental health status. Most anomalies were due to parasitical infections and did show high variation in spatial and temporal distribution. The observed prevalence of *Glugea stephani* and *Acanthochondria cornuta* in the period 2009-2010 (the dumping site vs the coastal reference zone) was remarkably higher compared to the mean prevalence over the period 2000-2010. These diseases must be followed strictly in future .

Secondly, the biomarker EROD (7-ethoxyresorufin O-deethylase) activity is used as an indicator of xenobiotic substance accumulation in the flatfish dab (OSPAR indicator). The EROD induction in the liver of juvenile dab is clearly visible during winter and early spring, while during summer and autumn only a background level is recorded. During the period 2009-2010, no significant higher EROD activity on impact sites versus control sites was observed.

An exploration of the biological life in the dredging areas

One of the aspects that was not studied in the previous decade was the biological life in the dredging areas, especially in the gullies towards the harbor of Zeebrugge and the harbor itself. 48 benthic taxa were recorded in the dredging areas of Zeebrugge, whereof 27 (56%) taxa were only recorded once. Most taxa were found in the gullies towards Zeebrugge and only a few taxa (9; Cirratulidae spp, Oligochaeta spp, Nephtys juvenile, *Mytilus edulis, Streblospio benedicti, Abra alba, Macoma balthica, Anthzoa spp, Crangon crangon*) in the harbor itself. Not one of the observed species was a rare taxon within the benthic fauna on the BPNS. We can conclude that the dredging areas around Zeebrugge were characterised by a very poor benthic community, except for the 'Vaargeul 1' area. Input of benthic animals from the dredging areas towards the dumping areas is possible, but should not lead to species enrichments in the dumping zones, due to the low densities and species richness in the dredging areas.

Optimalization of the sampling strategy in the routine monitoring program

In the period 2009-2011, we invested in the optimalization of the ecological sampling strategy of the routine monitoring program at the dredge disposal sites. This was carried out to standardize the analysis according to European directives (e.g. MSFD) and to make the monitoring time and cost efficient. We adjusted the sampling protocol of the epibenthos and fish tracks (shortening of the duration) and the benthic sieving procedure (alive instead of fixated). Finally, we introduced quality assurance in the macrobenthos analysis (ISO 16665:2005) by achieving a BELAC accreditation certificate under ISO17025 norm.

Concerning the changes in the duration of the epibenthos and fish tracks, we observed that the rate of overestimation or underestimation varied between tracks and between species groups. This difference plays no part when tracks of similar kind (short) are taken and compared within the same time frame. A clear advantage of using short tracks in the dredge disposal research is the fact that the tracks fit within the borders of the dumping site. Like this, side effects are minimized and the short tracks seem to result in more reliable density and diversity estimates of the area.

Sieving alive has a clear negative influence on the density and species richness of the samples, compared to sieving fixed. Based on analyses, we can trust that data retrieved when sieving alive on a 0.5 + 1mm sieve is comparable with data retrieved for fixed sieving. Therefore, we can consider that this switch in sieving procedure will have a minor influence on the long term trend analysis at the benthic control stations. Since sieving with two sieves is only used at a certain subset of stations, we have to use conversion factors for analyzing a long term trend at the other stations.

Perspectives

In the ecological monitoring program, we will keep the current monitoring strategy, because it seems suitable to evaluate changes within the dumping area and its surroundings. By high dumping intensities, leading to habitat modifications, a clear impact is detected on the overall benthic characteristics (density, diversity). In the future, it is advisable to investigate the possible loss of the ecosystem functioning by this habitat modifications, by using functional traits analysis. Secondly, we have to consider the possibility to determine a critical boundary of dumping intensity leading to a certain impact.

In the chemical monitoring program, it will be a necessity to investigate the general toxicity of the environment. General toxicity tests will give information about the presence and bioavailability of toxic compounds in the environment. In addition to the assessment of fish diseases, it would be of main importance to monitor the general health of fish species, e.g. gonadosomatic index (GSI), quality index method (QIM), liver glycogen content (LGC), liver-somatic index (LSI), condition index (k), etc.

2 Introduction

Dredging and dumping of dredged material is one of the major human activities in the coastal area of the Belgian part of the North Sea (BPNS). This activity is necessary to maintain the accessibility for ships within and towards the Belgian harbors (Nieuwpoort, Oostende, Blankenberge and Zeebrugge). The main dredging works in Belgium are executed in the harbor of Zeebrugge and in its access channel. The dredged material is dumped at five dumping sites in the coastal area of the BPNS: 1) dumping site Br&W S1, 2) dumping site Br&W Zeebrugge Oost, 3) dumping site Br&W S2, 4) dumping site Br&W Oostende and 5) dumping site Nieuwpoort (Figure 11). The dumping intensity at the five sites differs considerably within the study period, and depends on their position with respect to the dredging zones. Br&W S1 and Br&W Zeebrugge Oost were the most intensively used sites, with nearby 5 million tons dry matter at average per year. There was a moderate use of the dumping site Br&W S2 and Br&W Oostende, varying between 500.000 and 1 million tons dry matter per year. The dumping intensities were lowest at Nieuwpoort, and varied around 100.000 tons dry matter per year.

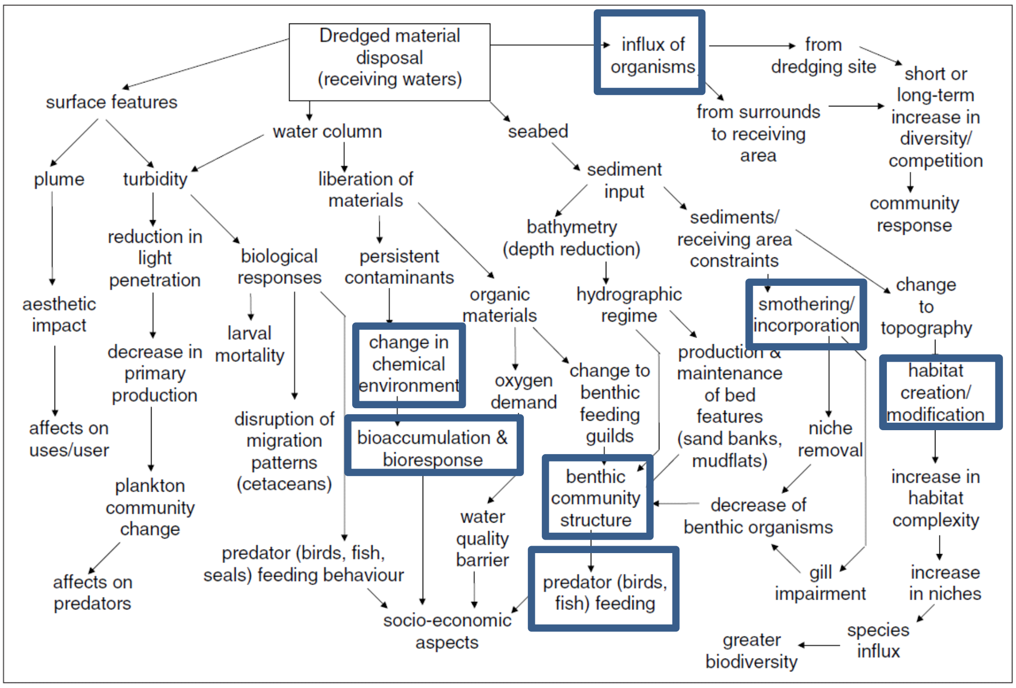


Figure 1. The potential environmental impacts of marine dredged material disposal – a conceptual model with indication of the research focus points in the ILVO monitoring program (adapted from Elliott & Hemingway, 2002)

It is important to investigate the effects of dumping of dredged material in the marine environment from ecosystem perspective, because it could lead to different responses of the ecosystem . The dumping itself has its consequences on the state of the water column and the seabed. The main effects within the water column lead to changes in the turbidity, which could be responsible for changes in the primary production and the release of materials (contaminants and organic material (Figure 1). The sediment input towards the seabed leads to habitat creation/modification, smothering of the fauna and changes in bathymetry. A side effect could be the influx of organisms, especially when the fauna in the dredging area is different from the dumping area.

The ILVO dredging monitoring program focused on evaluating the following impacts:

1. The effect of changes in the contaminants in the sediment and fauna at the disposal sites (bioaccumulation and bioresponse).
2. The effect of the dumping activity on the benthic organisms and their direct predators (epibenthos and demersal fish). Where they negatively affected, due to smothering or niche removal or even favored by changes in the habitat complexity.
3. The effect of the influx of organisms from the dredging areas on the native fauna at the disposal sites.

This report outlines the results of the research done at the dredged disposal sites by ILVO-fisheries in the context of the study 'biological, chemical and biochemical monitoring of sediment and bottom fauna at the dredged disposal sites of the Belgian Coast' (*cf.* protocol ILVO and MOW-aMT of 5 September 2003), over the period 2009-2010. This study focuses on the evaluation of the above mentioned impacts. In this period, the following tasks were set and the results of it described in this report:

- The monitoring program, investigating the effects of dumping of dredged material on the benthic fauna, is running for almost 30 years. The benthic sampling program strongly changed over this period, with a clear impact/control sampling strategy in the last years. A long term analysis of the changes in the benthic macrofauna in relation to the dumping intensity is executed and descripted in section 3.
- The results of the regular ILVO monitoring program over the period 2009-2010 were outlined in section 4. We described the differences observed in the biological community parameters of macrobenthos, epibenthos and demersal fish between the impacted area and the control area, if possible based on indicator assessments. The level and trends in heavy metals, PCB's, OCP's and PAK's in sediment and biota were measured within the chemical monitoring part. The histo-pathological part focused on the inventarisation of fish diseases in commercial fish species. Finally, the biochemical part investigated stress-indicators in juvenile dab to see whether the dredged material disposal has repercussion on the safety of the food chain (fish).
- We also investigated the biological life, focusing on the benthos, within the dredging areas of Zeebrugge (section 5). The aim was to analyse a possible difference in fauna of the dredging areas and the dumping areas and to examine whether an influx of organisms can occur.
- In section 6, we outlined the optimalisation of the sampling strategy and the sampling handling, with the intention to make the biological monitoring program more time- and cost efficient and to meet the international sampling and analyse criteria.

3 Long-term impact of dredged material dumping on the benthic habitats in the Belgian Coastal Zone

This chapter was made by Vyshal Delahaut in function of his master thesis in Marine and Lacustrine Science (academic year 2009-2010) and slightly adapted for this report.

3.1 Introduction

Approximately 11,000,000 tonnes dry matter (TDM) of sediments are disposed yearly at five designated dumping sites on the Belgian part of the North Sea (BPNS). The majority of these sediments derive from the so called maintenance dredging works. These are carried out on a regular basis and are necessary to keep the ports, harbours and channels functional for shipping traffic. To a lesser extent, sediments emerge from capital dredging works, as a result of deepening of channels and ports, or the construction of pipelines and cables. An alternative for dumping the dredged material at sea, is to use the sediments for beneficial purposes, such as shoreline stabilization, erosion control, construction material, ... (Paipai et al., 2003); which is not a common practice on the BPNS.

International agreements, such as the London convention (1972), the OSPAR convention, the Environmental Impact Assessment Directive (97/11/EEC), the Habitat and Species Directive, the Wild Birds Directive (79/409/EEC) and Strategic Environmental Assessment Directive (85/337/EEC) have to be taken into account before licenses for the disposal of dredged material at sea are granted. The legal basis for these activities on the BPNS can be found in the “MMM-act of 20 January 1999 on the protection of the marine environment in maritime areas under Belgian jurisdiction”. The procedure to obtain authorization to dump dredged-material is summarized in the Royal Decree of 12 March 2000.

Macrobenthic species are closely associated with the sediment and play an important role in marine ecosystems. They have been found to clearly reflect environmental changes in and near the bottom of the sea (Bilyard, 1987). Therefore, they have been shown to serve as valuable bio-indicators to monitor human activities, such as the disposal of dredged-material (Wildish and Thomas, 1985; Bilyard, 1978; Soule et al., 1988; Rees et al., 1992; Simonini et al., 2005; Rees et al., 2006). A possible impact may be merely of a physical nature, since organisms get buried immediately after a dumping event (cf. changes in foraging capacity, mobility,...; Morton, 1977). Additionally, the properties of the dumped sediments (e.g. mud content, median grain size), could contribute to a biological impact (Maurer et al., 1981; Maurer et al., 1982; Maurer et al., 1983). Chemical contamination of the sediments, organic enrichment or increased turbidity, has also been shown to affect the benthic organisms (Delvalls T. A. et al., 2004; Ware et al., 2009; Essink, 1999) (Figure 1). Since benthic species differ in susceptibility towards disturbance, the results of impact evaluation studies will be determined by the benthic community present at a given dumping site. More specifically it is indicated that species assemblages, adapted to a certain degree of stress, will recover faster after a disturbance event than assemblages occurring in an unstressed environment. The former ones are usually characterised by life-history traits, facilitating the recolonisation process (opportunistic species) (Bolam et al., 2003). The sampling design (number of samples and the degree of coverage of the study area) and the selected parameters contribute to the reliability of the obtained results. All these factors, together with the natural variability of the benthic communities, need to be taken into account, when a conclusion regarding the impact is drawn.

The ILVO (Institute for Agriculture and Fisheries Research) has a monitoring program to follow up disposal activities at the BPNS. Since 1979, two annual sampling campaigns are carried out, providing a large set of biological and sediment data, which were used for this study. We distinguish three sampling strategies in the history of the monitoring program. From 1979 until 2003, samples were taken at a limited number of stations on the BPNS. Each of these stations functioned as a reference station for nearby disposal sites, although not all disposal sites were operational. One exception is dumping site S2, since it already had a monitoring point inside the dumping zone since 1979. In 2004-2005, the former strategy was exchanged towards a control-impact strategy. From then onwards, each dumping site had one internal station (=disposal station) and one or two stations outside (=nearby-reference station) the dumping area. At the same time, additional sampling continued at the previously used reference stations. Since 2006, the sampling intensity was markedly increased, and each disposal site nowadays has 7 disposal and 4 to 6 nearby-reference stations.

The aim of this section is to address two main questions:

I. What is the relationship between the degree of dumping intensity on the one hand and the presence and diversity of the marine benthos on the other hand?

a. At long-term scale (e.g. the natural patterns or shifts over long-term)

b. On Control/impact design scale.

II. Do the different sampling designs have a different capacity in reflecting a possible impact of the dumping activity? Where the selected reference stations optimal for impact evaluation?

3.2 Material and Method

3.2.1 Description of the study area

The Belgian part of the North Sea (BPNS) has a surface area of 3454 km² and is situated in the southern part of the North Sea. Average depths are approx. 20 meters, with maximal depths of 35 meters. The main current has a SW-NE direction and turns into the open ocean. The area exhibits a high geomorphologic diversity (Degraer et al., 2008) due to the presence of a series of sandbanks orientated parallel to the coastline. This results in a wide variety of sediment types. Previous studies showed that this abiotic diversity create for the benthic ecosystem, a set of four macrobenthic habitats; the *Abra alba* habitat, the *Nephtys cirrosa* habitat, the *Ophelia limacina* habitat and the *Macoma balthica* habitat, which are mainly linked to certain sediment characteristics (cf. grain size and mud content,...; Van Hoey et al., 2004; Degraer et al., 2008).

This section focused on the dredged disposal sites situated in the coastal area of the BPNS. The samples used in this study were collected from (1) stations associated with current designated dumping sites (Nieuwpoort, Br&W Oostende, Br&W Zeebrugge Oost, Br&W S1 and Br&W S2) (2) stations associated with two former dumping sites (S3 and R4) and (3) 12 coastal reference stations on the BPNS (Table 1, Figure 2). The sampling of each station did not start at the same time. In addition, the sampling design did change over the years (Table 1), as described in detail per dumping area.

Table 1. Information about dumping sites and reference stations. Between brackets, the number of replicates per station.

		Function	1979-2003	Sampling period: 2004-2005	2006-2010
Dumping sites	Nieuwpoort	Disposal		2251(3*)	LNP.01-LNP.07
		Nearby Reference		2252(3)&2253(3)	LNP.08-LNP.11
	Br&W Oostende	Disposal		1401(3)	LOO.01-LOO.07
		Nearby Reference		1402(3)	LOO.08-LOO.13
	Br&W Zeebrugge Oost	Disposal		7001(3)	LZO.01-LZO.07
		Nearby Reference		7002(3)	LZO.08-LZO.13
	Br&W S1	Disposal		78001-78011(1)	LS1.01-LS1.11
		Nearby Reference		78012-78016(1)	LS1.17-LS1.22
	Br&W S2	Disposal	7101(3)	7101(3)	LS2.01-LS1.07
		Nearby Reference		7102(3)	LS2.08-LS2.11

	Station Code	Sampling period	Function (historic)
Far Reference Stations	115 (3)	2005-2009	Ref. Nieuwpoort
	120 (3)	1979-2009	Ref. Nieuwpoort
	140 (3)	1979-2008	Ref. Br&W Oostende
	150 (3)	2000-2008	Ref. Br&W Zeebrugge Oost
	230 (3)	2000-2009	Ref. Oostendebank
	250 (3)	1997-2008	Ref. Steendiep
	315 (3)	1997-1998, 2004-2009	Ref. Oost Dyck
	780 (3)	1983-2008	Ref. Br&W S1
	B031 (3)	2000-2008	<2004: Disposal S3, >2004: Ref Br&W S2
	B032 (3)	2004-2008	Ref Br&W S2
	B041 (3)	1997-2008	<2004: Disposal R4, >2004: Ref Br&W S2
	B042 (3)	2004-2008	>2004: Ref Br&W S2
	B08 (3)	1997-2008	Ref. Vlakte van de Raan
	ZEB (3)	2004-2005	Ref. Zeebrugge Eb
	ZVL (3)	1979-2008	Ref Br&W Zeebrugge Oost

Table 2. Dumped quantity of dredged material per year per disposal site (in wet tonnes and tonnes dry matter).

Period	Br&W S1	Br&W S2	Br&W Zeebrugge Oost	Br&W Oostende	Nieuwpoort
Wet tonnes (WT)					
April 1991-March 1992	14176222	7426064	10625173	4416386	
April 1992-March 1993	13590355	5681086	10901837	3346165	
April 1993-March 1994	12617457	5500173	10952205	3614626	
April 1994-March 1995	15705346	2724157	8592891	3286965	
April 1995-March 1996	14308502	2626731	8432349	4165995	
April 1996-March 1997	14496128	1653382	7609627	2763054	
Yearly average	14149002	4268599	9519014	3598865	
Tonnes dry matter (TDM)					
April 1997-March 1998	6045581	1563485	6593905	745147	
April 1998-March 1999	7455619	482108	2976919	467107	
April 1999-March 2000	9073402	131139	3189077	591605	
April 2000-March 2001	5557961	1399000	4971782	559332	
April 2001-March 2002	4558539	329798	2623069	565938	
April 2002-March 2003	5727875	1067492	3681589	491217	289949
April 2003-March 2004	6075792	741323	3573611	646276	142420
April 2004-March 2005	1826561	1826033	3003397	464307	71928
April 2005-March 2006	3017123	1234640	2973545	599905	
April 2006-March 2007	11722690	596317	2796772	819665	178269
2007	5592676	127704	2219780	460167	118100
2008	4589589	80014	4667225	864863	103541
Yearly average	5 936 951	798 254	3 605 889	606 294	150 701

Disposal site Nieuwpoort (Nieuwpoort, Figure 2) is situated between Nieuwpoort and Oostende, 8 kilometers offshore. It has a diameter of 1500 meters and is used since 2002. With a yearly average dumping amount of merely 113,798 (±46,046) tonnes of dry matter (TDM) over the period 2002-2008, it is also the least used disposal site of the BPNS (Table 2). The disposed sediments derive from the harbor of Nieuwpoort and its access channel. The history of the monitoring program at this site consists of no impact monitoring before 2004, followed by a control/impact design afterwards (1 impact/2 control samples in 2004-2005 and 7 impact/4 control samples in 2006-2008). The selected far reference stations for dumping site Nieuwpoort in the monitoring program were 120 and 115, situated in the "Westdiep area" (Table 1, Figure 2).

Disposal site Oostende (Br&W Ooostende, Figure 2) is situated just outside the harbour at 4 kilometers from the coast and has a diameter of 1500 meter. During the last eight years this dumping site has moved twice (in 2002 and 2004). Over the period 1997-2008, the disposal site Oostende received an average yearly amount of dredged disposal of 606,294 (±40,027) TDM (Table 2). Sediments derive from maintenance dredging works, such as the deepening of the access channel to Oostende or the harbor itself. Intensity maps of 2004-2008 indicate that the sediments are not disposed evenly, but that the majority is dumped at the south-western part (ANNEX 1). The history of the monitoring program at this site consist of no impact monitoring before 2004, followed by a control/impact design afterwards (1 impact/1 control sample in 2004-2005 and 7 impact/6 control samples in 2006-2008). The selected far reference stations for Br&W Oostende in the monitoring program was 140 (Table 1, Figure 2), which was relocated to the west in 2002.

Dumping site Zeebrugge Oost (Br&W Zeebrugge Oost, Figure 2) is situated nearby the harbor of Zeebrugge at the eastern site, 2 km offshore and has a diameter of 1500 meter. The dumped sediments mainly derive from the harbors of Zeebrugge or Blankenberge. With an average of 3,605,889 (±356,766) TDM (Table 2)) of sediments yearly (based on data from the years 1997-2008), dumping site Zeebrugge Oost is the second most used dumping site. The western part received more sediments than the eastern part over the period 2004-2008 (ANNEX 1). The history of the monitoring program at this site consists of no impact monitoring before 2004, followed by a control/impact design afterwards (1 impact/1 control in 2004-2005 and 7 impact/6 control in 2006-2008). The selected far reference stations for Br&W Zeebrugge Oost in the monitoring program were 150 and ZVL (Table 1, Figure 2).

Dumping site Br&W S2 (Figure 2) is situated on the western part of the "Vlakte van de Raan", nearly 11 km offshore. The legally permitted dumping area has the shape of a semi-circle, with a radius of 1500 meter. The sediments are derived from "Pas van Zand", the "Central part of the outer harbor of Zeebrugge", "Scheur Oost" and "Scheur West". With an average dumping amount of 798,254 (±174,254) TDM (Table 2)) yearly (based on data from 1997-2008), this site is used to a lesser extent as compared to the other disposal sites. According to intensity maps of 2004-2008 (ANNEX 1), the dumped sediments are more or less equally distributed over the disposal area. The history of the monitoring program at this site consist of long-term monitoring at station 7101 (LS2-01) (since 1980), which is located at the border of the dumping site, followed by a control/impact design from 2004 (1 impact/1 control sample in 2004-2005 and 7 impact/4 control samples in 2006-2008). Since 2004, the stations B031, B032, B041 and B042 are selected as the far reference stations for Br&W S2 (Table 1, Figure 2). The first two served as impact and control stations for the former back-up dumping site S3, while the latter two were impact and control stations for back-up dumping site R4, but both were never used.

Dumping site Br&W S1 is situated at a distance of 17 kilometers off the coast (Figure 2) and has a diameter of 3000 meters. The location of S1 is changed over the years towards the gully of the Akkaert bank. The site mainly receives sediments from “Pas van Zand”, the “Central part of the outer harbor of Zeebrugge”, “Scheur Oost” and “Scheur West”. This site is very frequently used (average yearly amount: 5,936,951 ±754,238 TDM, based on data from 1997–2008, Table 2). Dumping intensity maps show that the south-western part has been used more often during certain years, while the distribution of sediments occurred more equally during some other years (ANNEX 1). The dumping site has been sampled applying a detailed control-impact strategy since autumn 2004 (11 impact/5 control samples), whereas no monitoring was executed in the previous years. The 5 nearby-control stations were positioned more nearby the dumping area in 2006. The station 780 and 120 were selected as the far reference stations for Br&W S1.

3.2.2 Sampling method

Macrobenthos can be defined as organisms that spend most part of their life in the sediment, and that are retained on a 1 mm-meshed sieve. Table 1 shows the number of samples taken at each station (number between brackets). Macrobenthic samples were collected with a Van Veen grab (0.1 m²) on board of the marine vessel, the *Belgica*. They were immediately fixed with an 8% formaldehyde seawater solution. The samples were afterwards sieved on a 1mm sieve. The residue was stained with eosin in order to facilitate further sorting. Species were identified to the lowest, possible taxonomic level (species) and counted.

A separate Van Veen sample was taken at each station for granulometric analysis and chemical analysis of the sediment for the period 1979-2005. Since 2006 a single Perspex® core was taken from each Van Veen sample for further particle size analysis. These samples were dried in an oven at 60°C. From 1979 to 2006, a sieve tower was used for sediment analyses. Since 2007, sediment analyses were conducted using the Malvern Mastersizer 2000 analyzer following a standardized protocol.

4.2.3 Data analysis

The species dataset was standardized by lumping some species (Cirratulidae spp., *Spio* spp., Anthozoa spp.), and reduced by excluding species that did not belong to the macrobenthos sensu strictu (e.g. Mysida). Nematoda were excluded because of inadequate sampling techniques for quantifying meiofauna.

The multivariate analyses were performed by using version 6 of the PRIMER software package (Plymouth Routines In Multivariate Ecological Research, Clarke and Gorley, 2006). Prior to specific analyses, the few outlier samples (10) were identified and removed by conducting a non-metric Multi-Dimensional Scaling (nmMDS). Biological data was fourth-root transformed and the resulting dataset was used to create a Bray-Curtis similarity matrix. In order to create significantly different groups for further analyses, a group average cluster analysis with SIMPROF test (Similarity profile, test for structure in the data) was performed. Characteristic species for the different clusters were identified using the SIMPER function (Similarity/distance percentages, species/variable contributions). Analysis of similarity (ANOSIM) was used to detect possible differences in species composition within and between the sampled locations for four possible structuring factors (cluster name, year, season (winter, autumn) and station type (impact, nearby and far away control stations)). In addition, a 2-d ordination plot from the nmMDS's provided a visual representation.

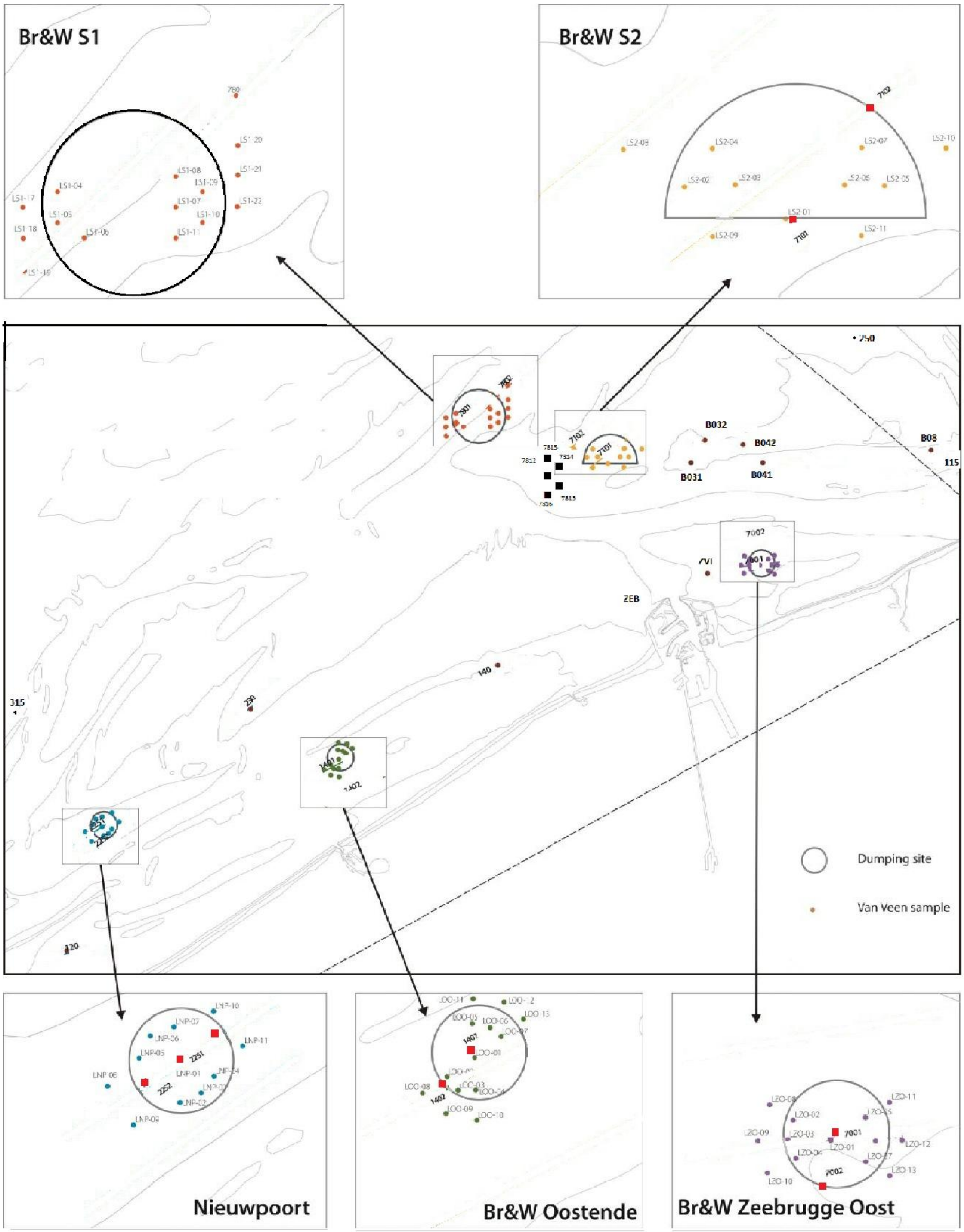


Figure 2. Map of the study area. Red squares represent the disposal and nearby-reference stations of the period 2004-2005 (in most cases identical to one of the disposal and nearby reference stations of the period 2006-2008). Black squares represent the first set of nearby –reference stations of dumping site S1.

Univariate analyses were executed with the software package R, version 2.10.1. The parameters used were; density, species number (S) and Hill's (1973) diversity and eveness indices (N1 and N2), computed from the standardized dataset. ANOVA analyses were carried out in order to detect significant differences in space and time. Post-hoc analysis was performed with the TukeyHSD-test. To meet the assumptions for parametric analysis, data was log-transformed where needed. If assumptions for parametric tests were not met, a Kruskal-Wallis test was performed, followed by the Wilcoxon-test when the former did identify significant p-values.

Finally, the Benthic Ecosystem Quality Index (BEQI, www.beqi.eu) was used to scale the relationship between dumping intensity and the degree of impact. The BEQI level 3 analyses were based on the parameters, total density (ind/ m²), number of species and similarity (Bray-Curtis similarity of fourth-root transformed density data). For this analysis, the disposal stations were compared with the two types of references stations (nearby-reference and far-reference). Together with the BEQI-analysis, a power assessment was performed. The power gives an indication of the chance to detect an impact, when there is one and is defined as 1-β (β is the probability of a type II-error). The result of this post-hoc power analysis will depend on the variance, the effect size and the choice of the level of significance (here set at 0.05%) (Van Hoey et al., 2007).

3.3 Results

3.3.1 Habitat characterization of the dumping sites and control stations

In this section, the habitat type of the dumping sites and the overall reference stations was determined based on their biological and sediment characteristics. The linking between the dumping sites and possible reference stations was previously based on a subjective assignment (Table 1). Therefore, this analysis is necessary to correctly compare dumping sites with similar (= with the same benthic habitat type) far-reference stations.

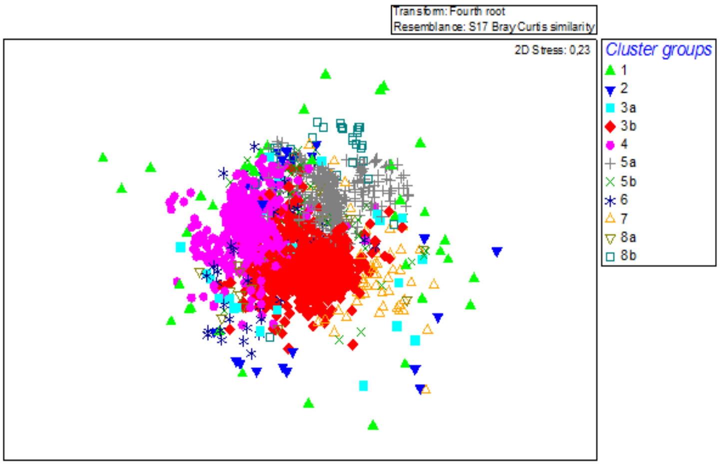


Figure 3. MDS-plot of cluster groups.

The cluster obtained from the hierarchical cluster analysis on the fourth root transformed density dataset was sliced at the 18.5%-similarity level, resulting in 11 cluster groups. Analysis of similarity confirmed that these groups were significantly different from each other regarding their species composition (ANOSIM R: 0.661 [p:0.1%]). Subsequently the known benthic habitat types (Van Hoey et al., 2004; Degraer et al.,2008) could be linked to the cluster groups based on their specific characteristics for the parameters; density, number of species, characteristic species (SIMPER) and the sediment characteristics (median grain size and mud content) (Table 3). There were three main sample groups (3b, 4 and 5a) central in the MDS plot, while these of the other eight groups were more diffused on the graph (Figure 3). A short description of the characteristics of each cluster group is given in Table 3 and described below:

Cluster 3b has samples characterised by high densities (2056 ± 40 ind/m^2) and a high average number of species (± 18 species/sample). Species playing an important role for this cluster are *Nephtys* species, *Spiophanes bombyx, Scoloplos armiger* and *Abra alba*. Mainly sediments with fine sands (grain size: 187 ± 24 µm) were found at the corresponding stations and had a relative low mud content (11 ± 3%). Based on these characteristics we could define cluster 3b as *Abra alba* habitat.

In cluster 4, the dominance of the polychaete *Nephtys cirrosa* and *Scoloplos armiger* as well as the moderate density (328 ± 225 ind/m^2) and low species number (± 7 species/sample) was the main reason for assigning this cluster to the *Nephtys cirrosa* habitat. This was confirmed by the relative high median grain size (223 ± 25 µm) and very low mud content (3±1%).

Cluster 5a, the moderate densities (615 ± 21 ind/m^2), low number of species (± 6 species/sample) and the dominant presence of *Cirratulidae* species and *Nephtys hombergii* are typical biological features for the third habitat type on the BPNS namely; the *Macoma balthica* habitat. Also the very fine sand fraction (median grain size: 123 ± 12 µm) and high mud contents (45 ± 8%) are characteristic for this type of habitat.

Cluster 1 was considered as an outlier group, because the biological parameters nor the sediment characteristics could clearly link these samples towards a certain habitat type.

Samples of cluster 2, scattered in the MDS plot, are characterised as *Macoma balthica* habitat, due to the low density (76 ± 9 ind/m^2), and low number of species (± 3 species/sample), the very fine sediments (median grain size: 86 ± 10 µm) and the high mud content (58 ± 10%). The characteristic species of this group were *Spiophanes bombyx, Nephtys spec., Diastylis rathkei, Barnea candida, Polydora spec.*

The few samples from cluster 3a were characterised by rather low densities (242 ± 10 ind/m^2) and a low number of species (± 5 species/sample). The main species found in this samples were three polychaetes species (*Nephtys hombergi, Nephtys cirrosa and Magelona johnstoni*), the bivalve *Spisula subtruncata* and *Bathyporeia* species. Sediments are characterised by fine sands (median grain size 155 ±18 µm) with mud contents (22 ± 4%). This cluster group shows correspondence with the *Abra alba* habitat.

The samples of cluster 5b are characterised by a rather low density (307 ± 16 ind/m2) and a low number of species (± 6 species/sample). Nevertheless, the presence of the bivalve *Abra alba*, the median grain size (154 ± 19 ind/m2) and the relatively high mud content (23 ± 4%) refer to an *Abra alba* habitat.

Cluster 6 contains samples which reflect a low average density (148 ± 14 ind/m^2) and a low averaged species number (± 6 species/sample). The characteristic species composition; Oligochaetes species, *Spiophanes bombyx*, *Nephtys cirrosa* and *Scoloplos armiger,* the median grain size (269 ± 31µm) and the low mud content (8 ± 2%) suggest to link this cluster to a *Abra alba- Nephtys cirrosa* transition habitat.

Samples from cluster 7 did represent stations with a high density (6710 ± 59 ind/m^2) and moderate species numbers (± 12 species/sample). The species found characteristic for this cluster are *Petricola pholadiformis* and *Capitella spec*., typical for a *Macoma balthica* habitat. Also the associated low median grain size (83 ± 11µm) and the high mud content (56 ± 11%) confirms this assumption.

Cluster 8a did contain samples with a rather low average density of organisms (164 ± 13 ind/m^2) and a low number of species (± 6/sample). Characteristic species for this cluster were *Capitella sp.* and *Scoloplos armiger*. These biological features, together with the average median grain size (183 ±18 µm) and relatively high mud content (19 ± 4%), indicated a *Macoma balthica-Abra alba* transition habitat.

8b is a cluster of samples with a very low density of organisms (71 ind/m^2) and a low number of species. The most dominant species group found here were Oligochaetes. Sediment characteristics showed a high median grain size (241±23 µm) and moderate mud contents (12±3%). The position of the samples on the MDS plot was the main reason for linking this cluster with the *Macoma baltica* habitat.

The dumping sites and its reference stations could be linked to a certain habitat type based on the number of samples of each disposal site associated with a certain cluster/habitat type. Consequently, disposal site Nieuwpoort was linked to the *Abra alba* habitat (96.84% of the samples were found within clusters with this habitat type). The *Macoma balthica*-cluster groups comprised 87.38% of the Br&W Oostende samples and 86.81% of the Br&W Zeebrugge Oost samples. Therefore the dumping sites Zeebrugge Oost and Oostende were recognized as *Macoma balthica* habitat. Disposal site Br&W S2 was identified as *Nephtys cirrosa* habitat because this cluster existed out of 50.22% of the samples from this site. At first this assumption may not seem correct, because 44% of the other Br&W S2-samples were found within and *Abra alba* cluster. A closer look revealed that 98% of these samples were taken before 2004. This may indicate a shift in community structure and will be explained in section 3.3.2. Regarding dumping site Br&W S1, the samples were not uniformly associated with one habitat type. Of the 161 samples collected at this site, 25.47% were found within *Abra alba* habitat, 44% in *Nephtys cirrosa* habitat, 14.29% in *Macoma balthica* habitat and 10.56% and 3.10% in resp. the *Abra alba-Nephtys cirrosa* and *Macoma balthica-Abra alba* transition habitats (Table 3). This habitat heterogeneity can be caused by the dumping activity (see further), but is partly naturally due to its position along the gully bank gradient (*Abra alba* habitat in gully, switching towards *Nephtys cirrosa* habitat at the bank).

Overall control stations ascribed to *Abra alba* habitat are the following: 115 (86.96%), 120 (98.68%), 230 (71.43%), 250 (66.67%), 780 (97.62%) and B08 (60.61%) (Table 3). In the *Macoma balthica*-clusters, the far reference station ZVL (89.58%) was the only one with a significant amount of samples belonging to this habitat type. Finally, the reference stations found for the *Nephtys cirrosa* habitat are the following: 315(97.50%), B031(89.80%), B032(96.30%), B041(67.16%) and B042(100%).

Table 3. Characterization of the cluster groups by the parameters: density (ind/m²), Average # species (/sample), SIMPER species, average median grain size (µm), average mud content (% < 63µm). The stations and amount of replicates (between brackets) per cluster group is listed.

	group 1	**group 2**	**group 3a**	**group 3b**	**group4**	**group 5a**	**group 5b**	**group 6**	**group 7**	**group 8a**	**group 8b**
Habitat	outlier group	Macoma balthica	Abra alba	**Abra alba**	**Nephtys cirrosa**	**Macoma balthica**	Abra alba	Nephtys cirrosa-Abra alba	Macoma balthica	Macoma balthica-Abra alba	Macoma balthica
Density (ind/m²)	102 ±8	76±9	242+10	2056±40	328±18	615±21	307±16	148±14	6710±59	164+13	71±7
# species (/sample)	3	3	5	18	8	6	6	6	12	6	4
SIMPER species	Microphthalmus	Spiophanes bombyx	Nephtys hombergii	Nephtys	Nephtys cirrosa	Cirratulidae	Abra alba	Scoloplos armiger	Petricola pholadiformis	Capitella	Oligochaeta
	Pectinaria koreni	Nephtys	Spisula subtruncata	Spiophanes bombyx	Nephtys	Nephtys	Nephtys	Microphthalmus	Polydora	Spio	Macoma balthica
	Gastrosaccus spinifer	Diastylis rathkei	Bathyporeia	Abra alba	Spio	Oligochaeta	Macoma balthica	Oligochaeta	Corophium	Oligochaeta	
	Nephtys	Barnea candida	Nephtys cirrosa	Scoloplos armiger	Magelona johnstoni	Macoma balthica	Spisula subtruncata	Spiophanes bombyx	Alitta succinea	Magelona johnstoni	
	Bathyporeia elegans	Polydora	Magelona johnstoni	Nephtys hombergii	Scoloplos armiger	Nephtys hombergii	Spio	Nephtys cirrosa	Capitella	Scoloplos armiger	
Median grain size (µm)	243±18	86±10	155±18	187±24	223±25	123±12	154±19	269±31	83±11	183±18	241±23
Mud content (<63µm)	20±4	58±10	22±4	11±3	3±1	45±8	23±4	8±2	56±11	19±4	12±3
Stations (# Samples)	120(2) B08(1) 140(2) LS1(3) 150(4) LS2(7) 250(1) LZO(9) B031(2) ZEB(2) B032(1) **ZVL(8)**	140(9) LS1(3) ZEB(2) **ZVL(7)**	140(1) 250(1) B041(2) LS1(2) LS2(9) ZVL(1)	**115(20)** **B08(36)** **120(145)** **LNP(90)** **140(21)** LOO(9) **230(5)** **LS1(30)** **250(11)** LS2(89) **780(122)** LZO(1) 780C(3) **ZVL(2)** B041(13)	115(2) **B032(26)** 140(1) **B041(45)** 150(16) **B042(27)** 230(2) B08(9) 250(5) LNP(1) **315(39)** LOO(4) 702(3) **LS1(34)** 710(3) **LS2(114)** 780(3) LZO(2) **B031(44)** ZEB(4)	115(1) LNP(2) 140(6) **LOO(87)** 150(8) LS1(11) 702(2) LS2(4) B041(1) **LZO(72)** B08(3) ZEB(1) **ZVL(69)**	120(5) 140(26) 780(1) B041(6) B08(1) LS1(2) LS2(2) ZVL(1)	140(3) 150(3) B08(12) LNP(2) LS1(17) ZVL(2)	140(7) B031(3) B08(3) **ZVL(51)** LNP(7)	150(1) LOO(2) LS1(10) LS2(2) LZO(2) ZVL(1) LNP(1)	150(11) 315(1) B08(1) LOO(1) LS1(5) LZO(5) ZVL(2) LNP(12)

3.3.2 Long-term pattern (1980-2008); focus on station 7101

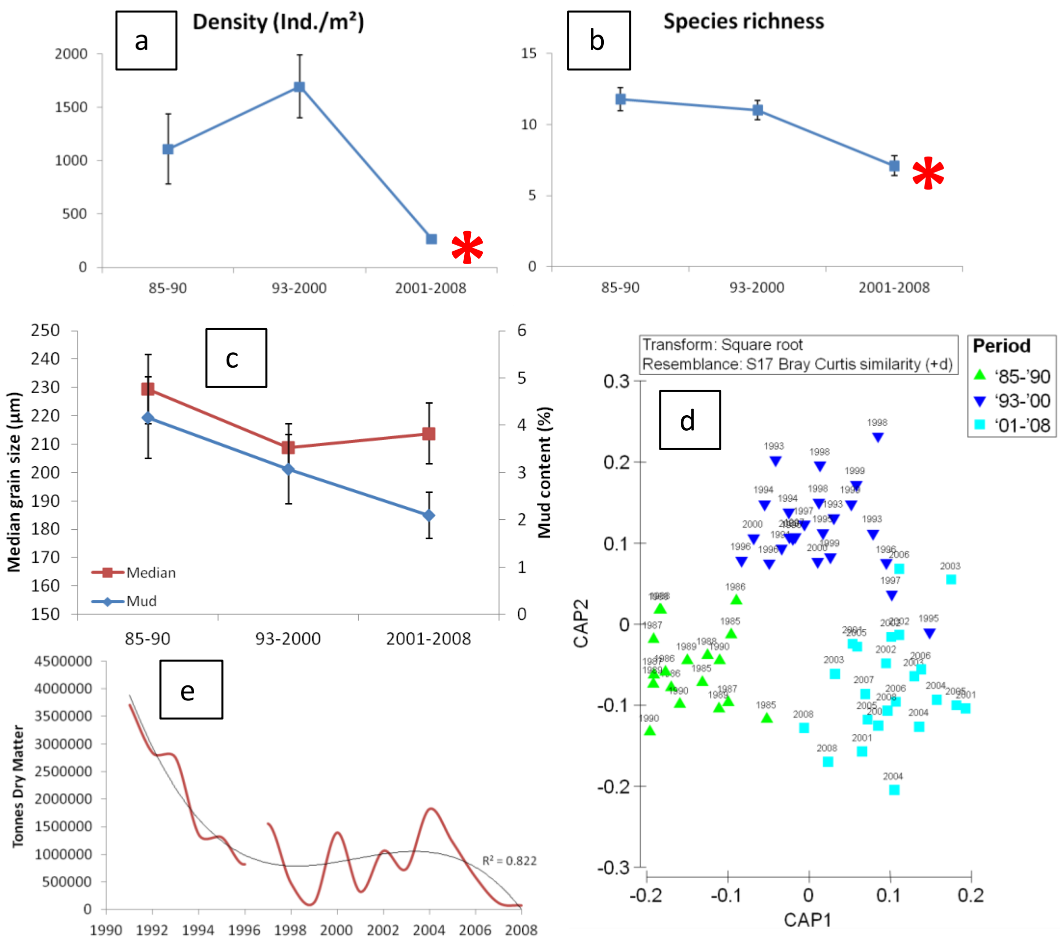


Figure 4. a) the average density with standard error at LS2-01 in the three periods; b) the average species richness; c) the average median grain size and mud content; d) CAP analysis of the species composition with delineation of the three periods; e) the dumping intensity at dumping site LS2 over the period 1990-2008 in tons dry matter. * significant difference

Station 7101 (LS2-01) is sampled already over a period of 30 years, which makes a long term analysis suitable. This station is situated at the border of the dumping site and could be a proxy for the natural and anthropogenic (dumping) changes in the benthic habitat characteristics in this part of the BPNS. The multivariate CAP analysis (Figure 4d) indicates a transition in species assemblage over the years. We can roughly distinguish 3 temporal assemblages (period '85-'90; '93-'00; '01-'08). The average density (Figure 4a) and species richness (Figure 4b) were significantly lower in the latest period compared to the previous ones. The changes in species composition can be attributed to the absence of mud loving species in the recent period, as *Abra alba, Nephtys hombergii, Spisula subtruncata, Capitella* and *Tellina fabula*. The dominant species in the recent period were *Nephtys cirrosa, Ensis directus*, *Scoloplos armiger* and *Bathyporeia guilliamsoniana*, which were species characteristic for more sandy sediments. When comparing the sediment over the different periods, we did not find any significant changes but nevertheless a decreasing trend in mud content is visible from 4 to 2% (Figure 4c). This little change in mud content leads already to a transition from the more diverse *Abra alba* habitat to the less diverse *Nepthys cirrosa* habitat (Van Hoey et al., 2004). Simultaneously, dumping intensity decreased under the 1 million tons (Figure 4e), which is an indication that the amount of dumped muddy sediments is decreased. This prevents recruitment of ‘mud loving’ species, causing a decrease in the benthic diversity coinciding with the decrease in dumping.

3.3.3 Impact at the disposal sites

Impact evaluation is done by using the impact and control samples belonging to the same habitat type for each dumping site. Only for Br&W S1, the control samples of the *Abra alba* and the *Nephtys cirrosa* habitat are used.

3.3.3.1 Dumping site Nieuwpoort

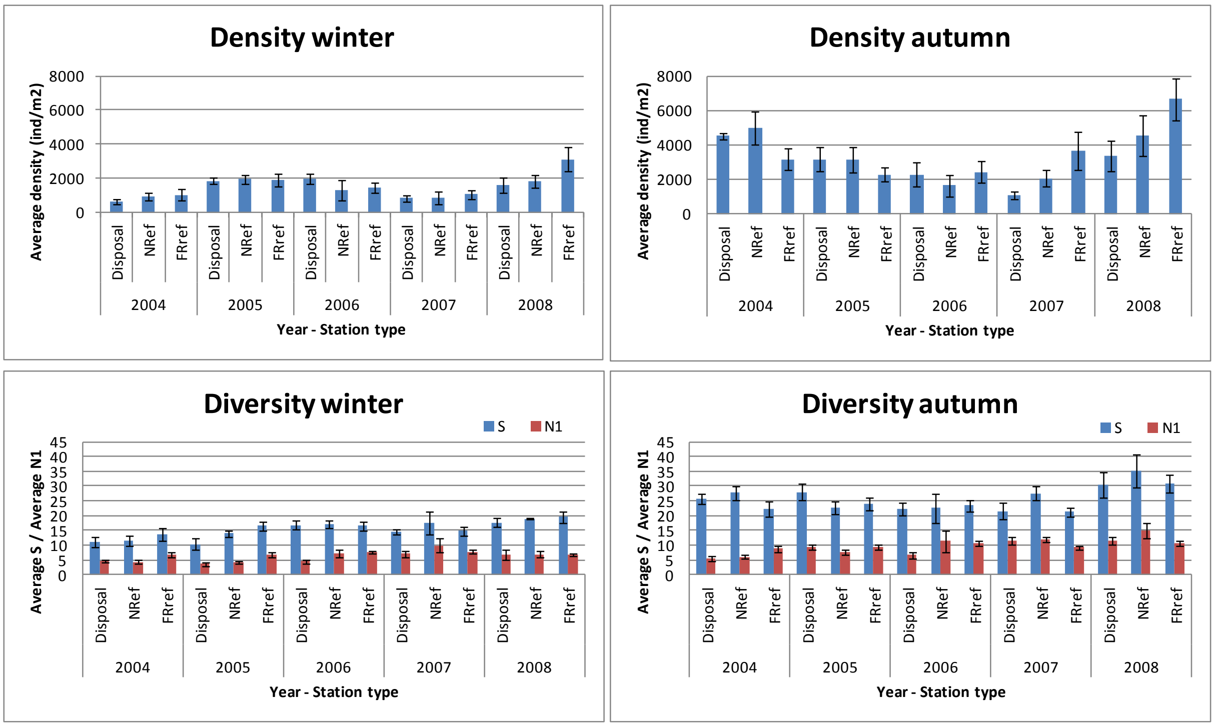


Figure 5. Density and diversity at dumping site Nieuwpoort in winter and autumn over the period 2004-2008.

The species composition did not differ significantly between the years 2004 and 2005, but there was found a small difference between the years over the period 2006-2008 (ANOSIM: R: 0.1 [p:0.1%]). Over both periods there was a seasonal difference in species composition (e.g. period 2006-2008; ANOSIM: R:0.114 [p:0.1%]). In general, the univariate parameters density and species number shown the same patterns (Figure 5). The values of the univariate parameters were higher in autumn compared to winter. No significant differences were seen between the disposal, nearby- and far reference stations over the investigated period with regard to the parameters density, species number and diversity for dumping site Nieuwpoort. Only a significant difference in species composition between the nearby-reference station and the far-reference station was found (e.g. ANOSIM: R: 0.234 [p:

0.1%]). Dumping intensity was low over the years 2004-2008 (maximum of 178,289 TDM) and absent in 2005 (Table 2).

3.3.3.2 Dumping site Br&W Oostende

The species composition did not differ between the years 2004-2005, nor a difference between the seasons within this period was detected. Over the period 2006-2008, both the factor year and season were found to be structuring factors based on the species composition (ANOSIM: R: 0.088 [p:0.1%]). Species composition differed between the disposal-and nearby-reference stations on the one hand and the far-reference stations on the other hand (e.g. Disposal-FRef; ANOSIM: R: 0.168 [p:0.1%]), but this was only the case for the period 2004-2005. Regarding the univariate parameters (density, number of species and diversity) there was not seen any significant difference between the three station types. An exception was autumn 2008, when far-reference station ZVL appeared to be characterized by a significant lower number of species and diversity than the stations at the dumping site (e.g. number of species at the disposal vs. far-reference stations, p: 0.0362). Generally, a lower density was seen at all the three station types in autumn 2008, with respect to the other years (Figure 6). The dumping intensity at this site was relatively low over the years 2004-2005, with a maximum of 864,863 TDM in 2008 (Table 2).

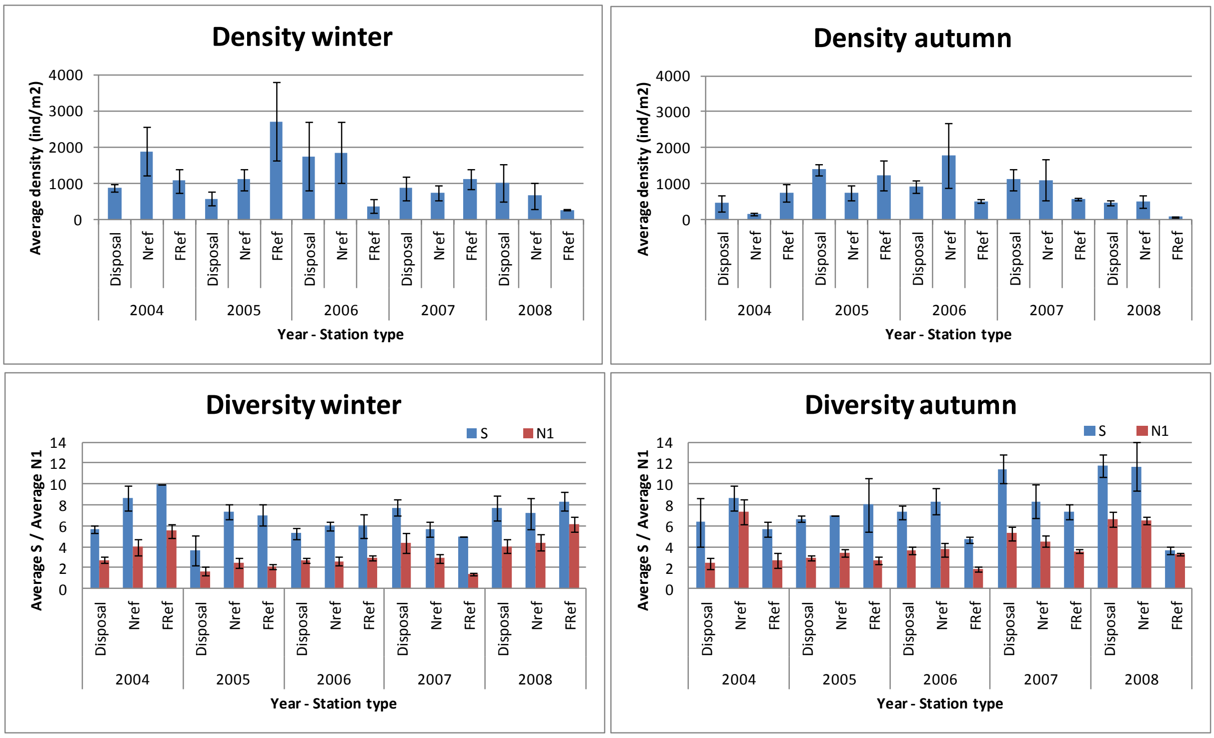


Figure 6. Density and diversity at dumping site Oostende in winter and autumn over the period 2004-2008.

3.3.3.3 Dumping site Br&W Zeebrugge-Oost

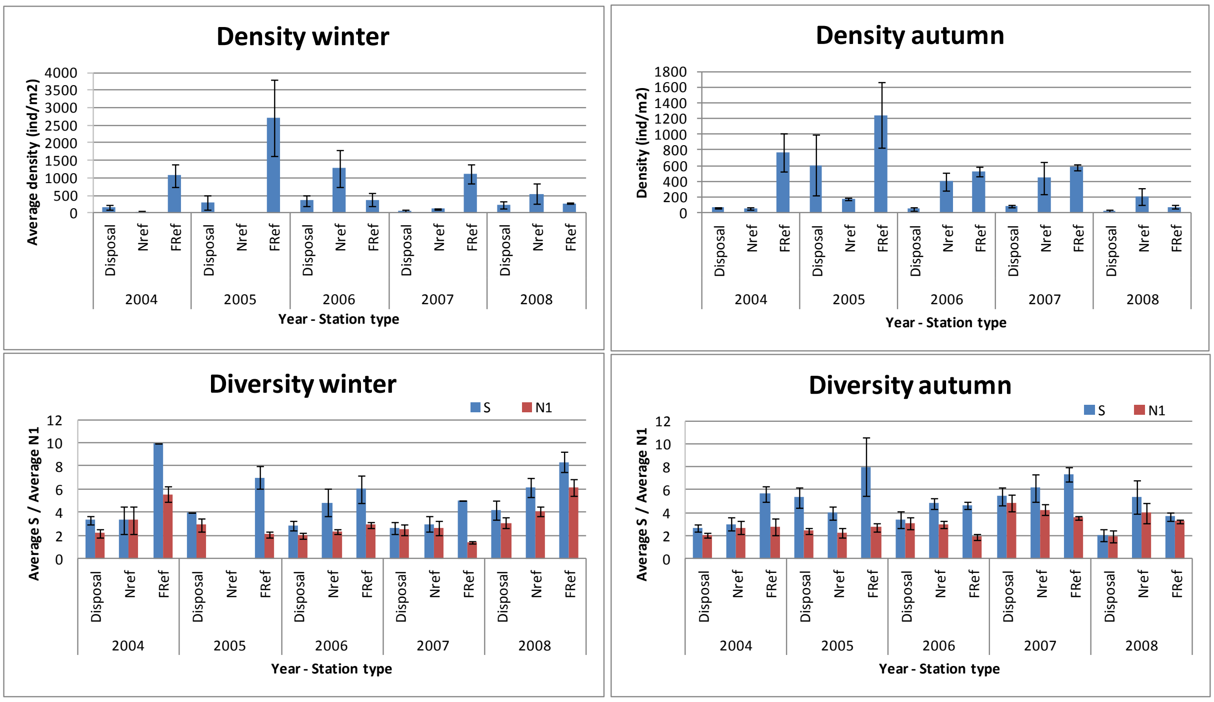


Figure 7. Density and diversity at dumping site Br&W Zeebrugge Oost in winter and autumn over the period 2004-2008 (No samples of NRef in winter 2005).

The year and season were not significant structuring factors over the period 2004-2005. A significant difference in species composition was seen between the years and between the seasons over the period 2006-2008, but the corresponding R-values were very low (e.g. Year; ANOSIM: R: 0.08 [p:0.1%]). The species composition did differ between the three station types over the period 2004-2005 (ANOSIM: R: 0.52 [p:0.1%]), but over the period 2006-2008 there was not seen any difference. For the parameter density we saw a significant difference between the disposal station and nearby-reference station in autumn 2006 (p: 0.018) and between the nearby-reference stations and far-reference stations in autumn 2006 (p: 0.011) and winter 2007 (p: 0.019). Finally a higher amount of species was seen between the far-reference station with respect to the disposal and nearby reference stations in the winter of 2004 (e.g. Disposal-FRef; p:0.0018). The diversity did never differ significantly between the three station types (Figure 7). Dumped quantities were relatively high over the years 2004-2008, with maximum of 4,667,225 TDM disposed sediments in 2008 (Table 2).

3.3.3.4 Dumping site Br&W S2

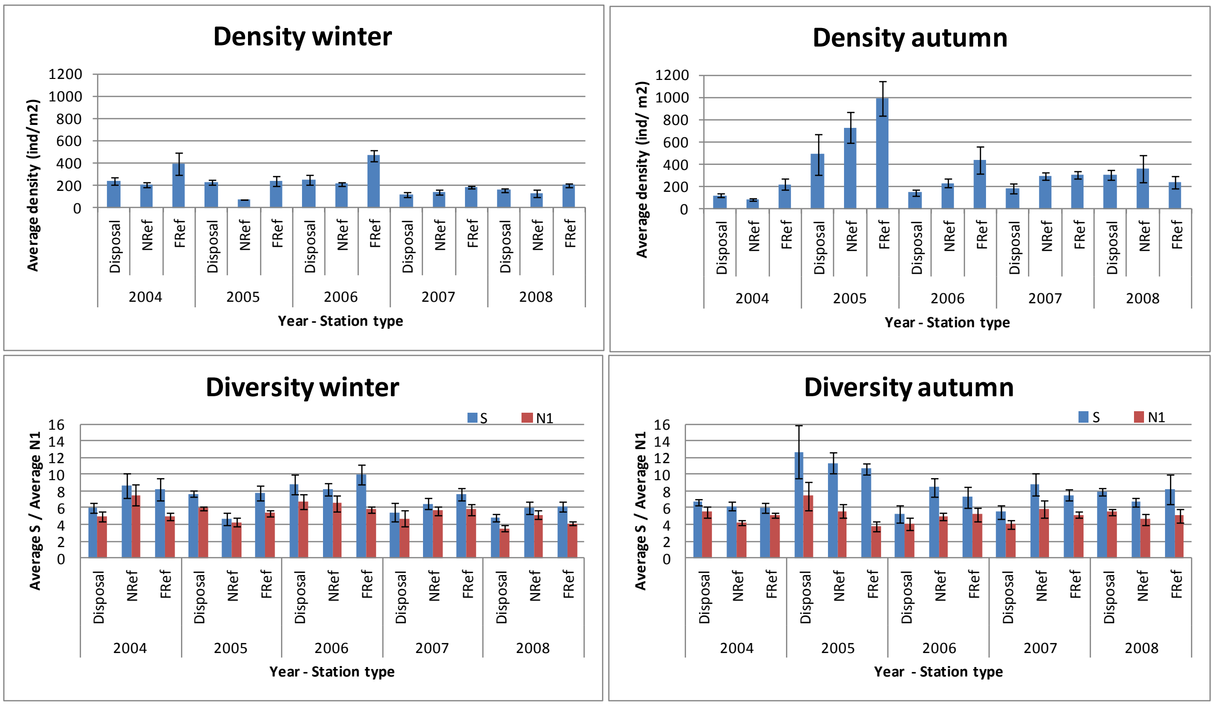


Figure 8. Density and diversity at dumping site Br&W S2 in winter and autumn over the period 2004-2008.

At dumping site Br&W S2, the factor year was considered as a significant structuring parameter over both periods; 2004-2005 (ANOSIM: R: 0.109 [p:0.1%]) and for 2006-2008 (ANOSIM: R: 0.093 [p:0.1%]). Also a significant difference between the two seasons was seen in both periods, but was more pronounced for the years 2004-2005 (ANOSIM: R: 0.109 [p:0.1%]). No significant differences were seen between the three station types regarding their species composition, density, species number or diversity (Figure 8). A notable observation was the high macrobenthic density and species numbers within all three station types in the autumn of 2005. Dumping intensities were high in 2004 (1,826,033 TDM) and in 2005 (1,234,640 TDM), but had a tremendous decrease in the years 2006-2008 (Table 2).

3.3.3.5 Dumping site Br&W S1

For the period 2006-2008 the species composition was significantly different between the corresponding years, but the R-value was very low (ANOSIM: R: 0.035 [p: 0.1%]). Season was a significant structuring factor regarding the species composition over both periods, but the R-values were again very low (2004-2005: ANOSIM: R: 0.047 [p:0.4%] and for 2006-2008: ANOSIM: R:0.037 [p: 0.2%]). Species composition over the period 2004-2005 was the same for the disposal and nearby-reference stations, but differed between disposal and far-reference stations (ANOSIM: R: 0.163 [p: 0.1%]). Over period 2006-2008 disposal and far-reference (ANOSIM: R: 0.282 [p: 0.1%]) stations also differed and additionally there was seen a significant difference between disposal and nearby-reference stations (ANOSIM: R: 0.282 [p: 0.1%]). In general, a significant lower value for the disposal stations in comparison with the nearby-reference stations and far-reference stations regarding the variable density was observed (e.g. winter 2006: disposal-NRef; p: 0.03). Only The only year where a significant indication for this pattern was not seen was in 2004. The number of species was also significantly lower in the disposal area with respect to the nearby- and far-reference stations in all years (e.g. winter 2007 disposal-FRef; p:0.004). A significant difference in diversity between the three station types was not found. Proportionately, the differences between the three station types did not change between winter and autumn samples regarding the three variables (Figure 9). Dumping intensities were always very high (>1,500,000 TDM) at this dumping site and took place throughout the year (Table 2).

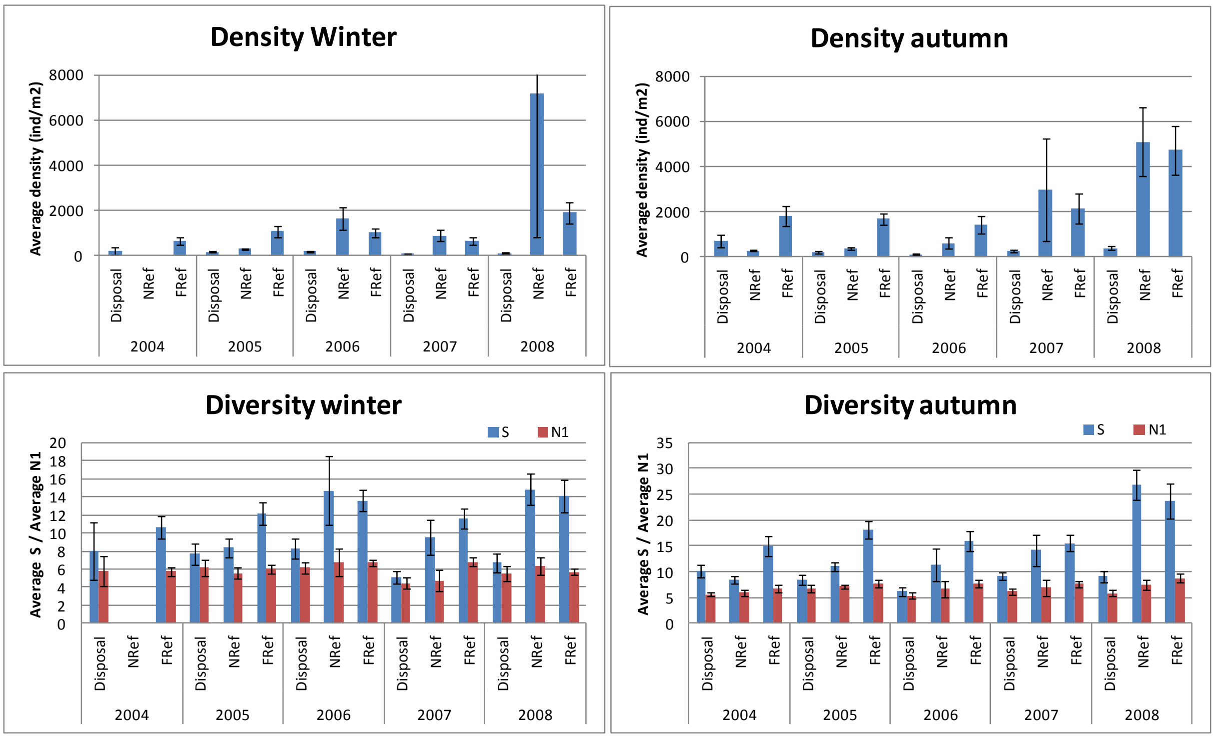


Figure 9. Density and diversity at dumping site Br&W S1 in winter and autumn over the period 2004-2008 (No samples of NRef in the winter of 2004).

3.4 Discussion

3.4.1 Impact of dumping of dredged material on the benthic fauna.

No significant impact of dredged-material disposal was seen at dumping site Nieuwpoort, which seems logical because the dumping intensities were very low. Nevertheless, we saw lower densities (but not significant) in the disposal stations than in the nearby-reference stations in the last two years. Because the dumping site is situated in a habitat with a high ecological value (*Abra alba habitat*), this site still needs to be monitored in the future, despite the low dumping intensity.

Although the moderate dumping intensities at Br&W Ooostende, we did not notice a negative effect of dumping activities at disposal site Oostende. This can be explained by the poor benthic community, occurring in this area (*Macoma balthica* habitat). Most species were adapted to a certain amount of stress and to the muddy environments, and are known to recover relatively fast (Bolam et al., 2003).

We did not indicate any significant effects of dumping over the period 2004-2008 at disposal site Br&W S2. The dumping area is characterised as a *Nephtys cirrosa* habitat, with sandy sediments and a low mud content. Therefore, we expect a negative effect of the dumping of muddy sediments, but although dumping intensities decreased since 2006, we did not see any reaction of the macrobenthic community. Nevertheless, we saw indications for a possible change in community structure at dumping site Br&W S2, reflected by the patterns observed at the long-term station 7101. The samples taken during the period 1980-2002, indicate that this area is more related with the *Abra alba* habitat, while the samples taken after 2003 suggest a *Nephtys cirrosa* habitat. A study by Van Hoey et al (2003), discovered that a transition from an Abra *alba* community towards a *Nephtys cirrosa* community is characterised by a decrease in the mud content (switch between 2-4%). Since this very small change in the mud content and the median grain size was seen, this can be the reason for the switch. This switch can be related to the decrease in the dumping intensity over the years (the pattern is partly biased due to the switch in units (WT vs TDM) and the related conversion over time). Nevertheless, it seems that dumping site Br&W S2 was more used in the past than in recent years, possibly reflected in less supply of mud and organic content towards the more sandy environment. It has been shown that dredged-disposal containing a certain amount of organic matter could have a positive effect on the diversity and density of the macrobenthic organisms (Stutterheim, 2002; Essink, 1999). If more muddy sediments with a high mud content were dumped before 2002, this could explain the observed pattern. Generally, increased fishing intensity could also have a destructive effect on the macrobenthos and consequently result in a poor community of only opportunistic species, but we do not have data to confirm this hypothesis. Since the long-term analysis at station ZVL, also indicated lower densities over the recent years, we also consider that large-scale processes (e.g. change in bottom currents or change in input by the Westerschelde) could have influenced the shift around the period 2000-2003.

At dumping site Br&W Zeebrugge Oost, there was no clear indication for an effect of dumping on our disposal stations with respect to our control stations (NRef and FRef). The only significant difference between the disposal and nearby-reference stations in the autumn of 2006, is probably related to the additional dumping of capital dredged-material (401,944 TDM). As for disposal site Oostende, the presence of a, by nature poor, *Macoma balthica* community, might be an explanation why we could not detect any effect on the disposal stations. Also the fact that our nearby-reference stations can be affected by some dumping events, and consequently minimizing/masking the impact, need to be considered.

Dumping site Br&W S1 is the most used dumping site in the years 2004-2008 and this was also observed in the results of our analyses. The impact of the high dumping amounts had a clear effect on the macrobenthos community at our disposal stations. 2004 missed significant values indicating an impact, but this can be explained by the lower amounts of dredged material disposed in that year (1,826,561 TDM), which could be a sign that quick recover of parts of the benthic community is possible. The dumping site Br&W S1 is located on a natural gradient of the gully-bank system of the Vlakte van de Raan. Therefore, this site is characterised in the deeper part as *Abra alba* habitat, whereas the top shows more affinity with the *Nephtys cirrosa* habitat, in the natural situation (Van Hoey et al, 2009). The former normally knows a rich diversity and high densities (Van Hoey et al., 2004). An event of disturbance such as dumping, will therefore be clearly indicated as a decrease in density and/or species richness. The dumping at Br&W S1 creates changes in the bathymetry and habitat characteristics, which is reflected by the scattered benthic community characteristics in this area (samples belonging to different cluster/habitat groups). This shows that the environment is not stable and that mainly opportunistic benthic species can occur here. As shown on the intensity maps (ANNEX 1), the dumped sediments are not always equally spread (higher intensity in the western part than in the eastern part). We saw a trend of lower densities and species numbers at points which received more dredged-material, but the differences were not significant. As suggested in Van Hoey et al. (2009), this is probably due to the fact that currents transport a certain amount of the sediments during the dumping procedure, before they settle down.

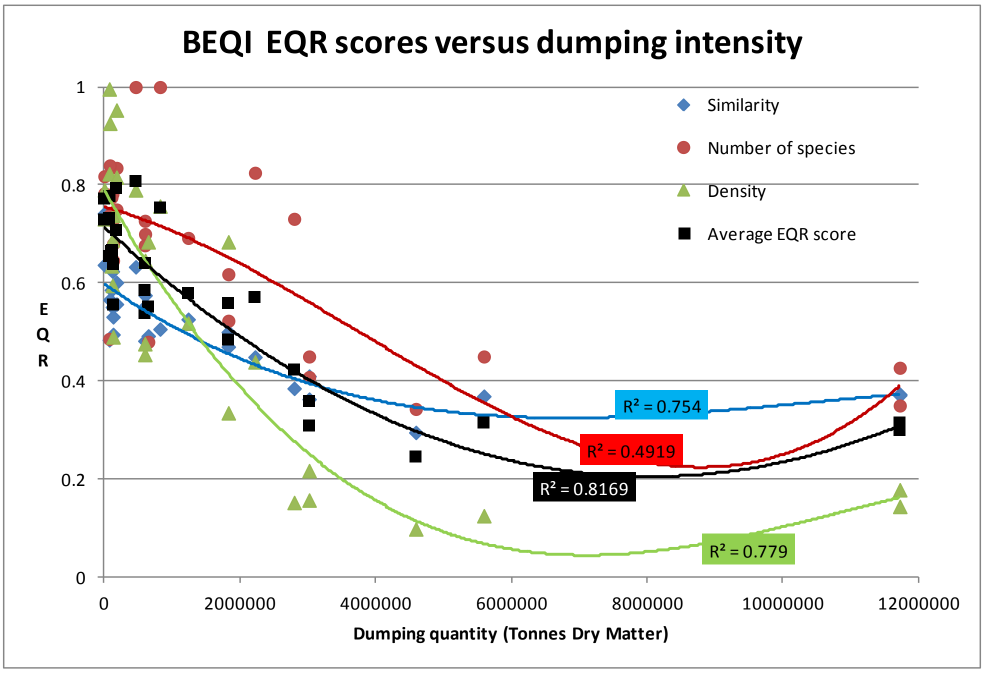


Figure 10. Relation between the dumping intensity and the BEQI scores for the parameters similarity (species composition), number of species and density. The BEQI overall score is the average of those three parameters. The trend line is a Polynomial order 3, with indication of the R^2 score.

The impact evaluation can be summarized by the use of a benthic indicator (BEQI, level 3). Therefore, we calculate an EQR score, which scales the difference in benthic parameters between the impact and control samples between 0 (bad status) and 1 (high status). The EQR score is calculated separately for each season and year with enough data, between 2004-2008. The difference between impact and control is calculated within each season and year to avoid temporal trends and to minimize cumulative effects. The indicator shows a clear relation between the yearly dumping intensity (maintenance and capital dredging) and its impact on the health of the benthic habitat (Figure 10). We can conclude that yearly dumping intensities, exceeding values of about 2,000,000 TDM, start to affect the benthic characteristics (corresponding with moderate to poor BEQI-values). The effect was smaller for the parameter number of species, than for density.

Literature provides a lot of explanations for the impact of dredged-disposal on the benthic communities (Windom, 1976; Morton, 1977; Elliot et al., 2002). In the particular case of the five Belgian disposal sites, we exclude large effects of chemicals, since there are specific regulations, which prohibit dumping of heavily polluted sediments and because ILVO-reports (Lauwaert et al., 2008; Lauwaert et al., 2009., Van Hoey et al., 2009) did not mention differences in contamination between disposal- and reference stations. Differences in organic matter have come up already as possible explanations (cf. Br&W S2 and Br&W Zeebrugge Oost), but knowledge about the Belgian coastal zone learns us, that the area is characterised by relatively high levels of organic matter, especially nearby the river-mouth of the river Schelde. Therefore, a positive contribution of the disposed sediments in increasing the organic matter is less likely, although we won't exclude a short-term effect. We suggest that if we see an impact, this is mainly related to the physical burial of the organisms, or to the properties of the dredged-disposal. When benthic organisms are buried under a certain amount of sediments, their feeding capacity will get altered or their mobility will decrease (Morton, 1977). Some species will be more resistant to direct burial than others, depending on their capacity to migrate upwards through the sediments. The sediment properties of the dredged material will mainly be responsible for the effect, when they are obviously different from the sediment characteristics of the dumping site. Dredged disposal usually has a high mud content, and will therefore have a higher chance to cause an effect in by nature, sandy environments.

3.4.2 Evaluation of the monitoring strategies.

Table 4. Power related to the overall BEQI-scores.

	Dumping site				
Sampling period	LNP	LOO	LZO	LS2	LS1
winter 2004	low	moderate	very poor	low	very poor
autumn 2004	moderate	low	very poor	low	moderate
winter 2005	moderate	low	low	low	moderate
autumn 2005	moderate	low	very poor	low	moderate
winter 2006	moderate	moderate	low	good	moderate
autumn 2006	moderate	low	moderate	moderate	moderate
winter 2007	moderate	moderate	low	good	moderate
autumn 2007	moderate	moderate	moderate	moderate	low
winter 2008	moderate	low	low	good	moderate
autumn 2008	moderate	low	low	good	moderate

The results from the BEQI-analysis did also provide us a good way to evaluate our two sampling designs (Table 4). Beside the scores for the parameters, we also obtained an indication for the power of our analyses, based on the number of samples taken at the disposal stations on the one hand and at the reference stations (nearby + far- reference) on the other hand. A high power means that the chance to make a type II-error (we do not detect an impact when there is one) is low. We consider a power classified as moderate as acceptable and giving a rather confident assessment. Very poor classification means that the results were not trustable.

We can conclude that the efficiency of the two designs differs between the dumping sites, caused by the difference in dumping intensity and the characteristic habitat type of the area. A dumping site in a diverse habitat, like Nieuwpoort, apparently does not require a real extensive sampling design (cf. 2006-2008) as long as the dumping intensity stays low. We already seemed to have enough control and impact samples over the years 2004-2005 to get a moderate power of our analyses. If the area is characterised by poor benthic characteristics, like at Br&W Ooostende and Br&W Zeebrugge Oost (*Macoma balthica* habitat), probably more samples or relocation of stations is needed to avoid type II errors (cf. low power of overall BEQI-scores,Table 4). Nevertheless, the detection of a possible impact will remains difficult due to resistance and the fast recovery of the opportunistic species. When the separation between nearby-and far-reference stations will proceed in the future, it is suggested to sample more far-reference stations for *Macoma balthica* habitats (instead of only ZVL). We see that when the dumping site is not uniformly chararcterized by one benthic community, a design comprising both benthic habitats will lead to reliable results (cf. Br&W S1). Regarding dumping site Br&W S2, the decision to enlarge the sampling intensity (1disposal/1 nearby reference stations to 7 disposal/4 nearby-reference stations) has positively affected the power of the overall BEQI-scores (Table 4).

The characterization of the stations and dumping sites (cf. section 3), revealed that four far-reference stations (780C, ZEB, 150 and 140) could not be linked to a certain habitat, based on their biological and sediment characteristics. The samples of 780C were found within the *Abra alba* cluster, but this station was sampled only once and therefore not taken up in our analyses. The station Zeebrugge eb (ZEB), did not cluster within one specific habitat and is probably not a good reference station. Also station 150 was found within the clusters of all the three habitat types and is not a good reference for Br&W Zeebrugge Oost, as originally considered (Table 1). Finally, the long-term station 140 was found within the *Abra alba* cluster (71.23% of the samples) based on the community analysis, but expert opinion did not recognise this station as a good reference station for an *Abra alba* habitat. Since the station was originally sampled as reference for Br&W Ooostende (*Macoma balthica* habitat), it is now recommended to reconsider whether to proceed sampling station 140 or not. The other far reference stations were considered as very useful for the dredge disposal assessment of the different dumping sites.

3.4.3 Conclusion

We can conclude that dumping activities have resulted in benthic habitat changes at dumping site Br&W S1. Whereas at the other dumping sites, the benthic community can scope the existing dumping regime. At Br&W S1, mainly the rich *Abra alba* community is affected, normally dominating the gully stations in the disposal area. At Br&W S2, it is possible that dumping has had a small positive effect, by supply of mud and organic matter to the more sandy environment (indicated by the switch between *Abra alba and Nephtys cirrosa* habitat). At dumping site LNP, with lowest dumping intensity, there was no impact. And finally, the dumping activity at site Br&W Zeebrugge Oost and Br&W Oostende had a smaller effect on the benthos, due to the more natural poor benthic environment.

Nevertheless, it needs to be kept in mind that this is a control/impact study, without knowledge about the natural situation, before the dumping activities started. Therefore, the characterization of the dumping sites might not be fully realistic as the dumping events could already have changed the original habitat and nearby environment. The relation between the benthos indicator BEQI and the dumping intensity shows that a benthic community seems to be affected from an average dumping regime of 2.000.000 tons dry matter a year. Based on this analyses, roughly 2.000.000 tons, regardless the surface area where it is dumped on, is proposed as critical boundary. It has to be mentioned that this critical boundary can differ for each habitat type. In other words, it is possible that a sandy or fine muddy sand habitat is more sensitive to it than a muddy habitat (*Macoma balthica* habitat), as some results show, but more detailed investigation is needed. This critical boundary needs to be seen as a boundary where the natural benthic recolonisation and recruitment cannot compensate for the loss of benthos due to smothering by the dumping of dredged material.

4 Biological and chemical status analyze of the disposal of dredged material in the Belgian Part of the North Sea: period 2009-2010.

4.1 Introduction

In this section, the results of the routine biological and chemical monitoring at the five dumping sites on the BPNS were described for the years 2009-2010. Firstly, we analyzed the sedimentology at the five sites, with indication of the sedimentological characteristics (mud content, median grain size) and the pollution (PCBs, PAHs, heavy metals, …) in the sediments. Secondly, we described and evaluated the difference or correspondence in biological characteristics of the ecosystem components macrobenthos, epibenthos and demersal fish between the dumping sites and the nearby control area. Thirdly, the (bio)accumulation of contaminants in the marine ecosystem is investigated by chemical analysis on different biota species. Finally, the biological effects of pollutants on marine organisms is investigated by the assessment of the prevalence of fish diseases and by the measurement of enzymatic EROD activities in the liver of juvenile dab. The observed patterns at the disposal sites were evaluated with indicators and threshold values as defined in Belgian or European legislation (e.g. Marine Strategy Framework Directive).

4.2 Material and method

4.2.1 Study area

All sampling locations were distributed in the shallow coastal zone of the Belgian Part of the North Sea (BPNS; limited to approximately 20 km offshore), since all dumping sites are situated in this zone (Figure 11). The impact samples are situated in the five dumping sites, while control samples are situated just outside the dumping sites and on some overall monitoring locations in the close surroundings (Figure 11). Table 5 give an overview of the stations used per dumping site, resp. as impact or control station for the assessment of macrobenthos and epibenthos and demersal fish.

There were changes introduced in the sampling strategy for epibenthos and demersal fish, as well as for the macrobenthos. The changes introduced were done to increase the confidence of the impact assessment. More details about the effect of the changes in the sampling program on the evaluation is outlined in chapter 6. For the epibenthos and demersal fish monitoring, the following adaptations were introduced:

- A shortening of the tracks to get a better spatial resolution in the analysis. The length of the fish tracks (3500m) is too long to fit within the delineation of the dumping sites, whereby fauna of within and aside the dumping site was collected.
- At dumping site S1, two tracks were taken in 2010 within the dumping site to cover the natural spatial variability within this area.

Table 5: Overview of stations used per dumping site as impact or control station for epibenthos- demersal fish and macrobenthos.

Dumping site	Impact/control	Epi- fish station	Macrobenthos station
Br&W Zeebrugge Oost	Impact	7001	LZO 01 – LZO 06
	Nearby Control	7002	LZO 08 – LZO 19
	Far reference	B10 / 140	
Br&W S2	Impact	7101	LS2 01 – LS2 07; LS2 12 – LS2 14
	Nearby Control	7102	LS2 08 – LS211; LS2 15 – LS2 16
	Far reference	B04 / B03	
Br&W S1	Impact	7801 / 7803 & 7804	LS1 01 – LS1 11
	Nearby Control	7802	LS1 17 – LS1 28
	Far reference	B04 / 230	
Br&W Oostende	Impact	1401	LOO 01 – LOO 07
	Nearby Control	1402	LOO 08 – LOO 17
	Far reference	140	
Nieuwpoort	Impact	2251	LNP 01 – LNP 07
	Nearby Control	2252	LNP 08 – LNP 11
	Far reference	120 / 230	

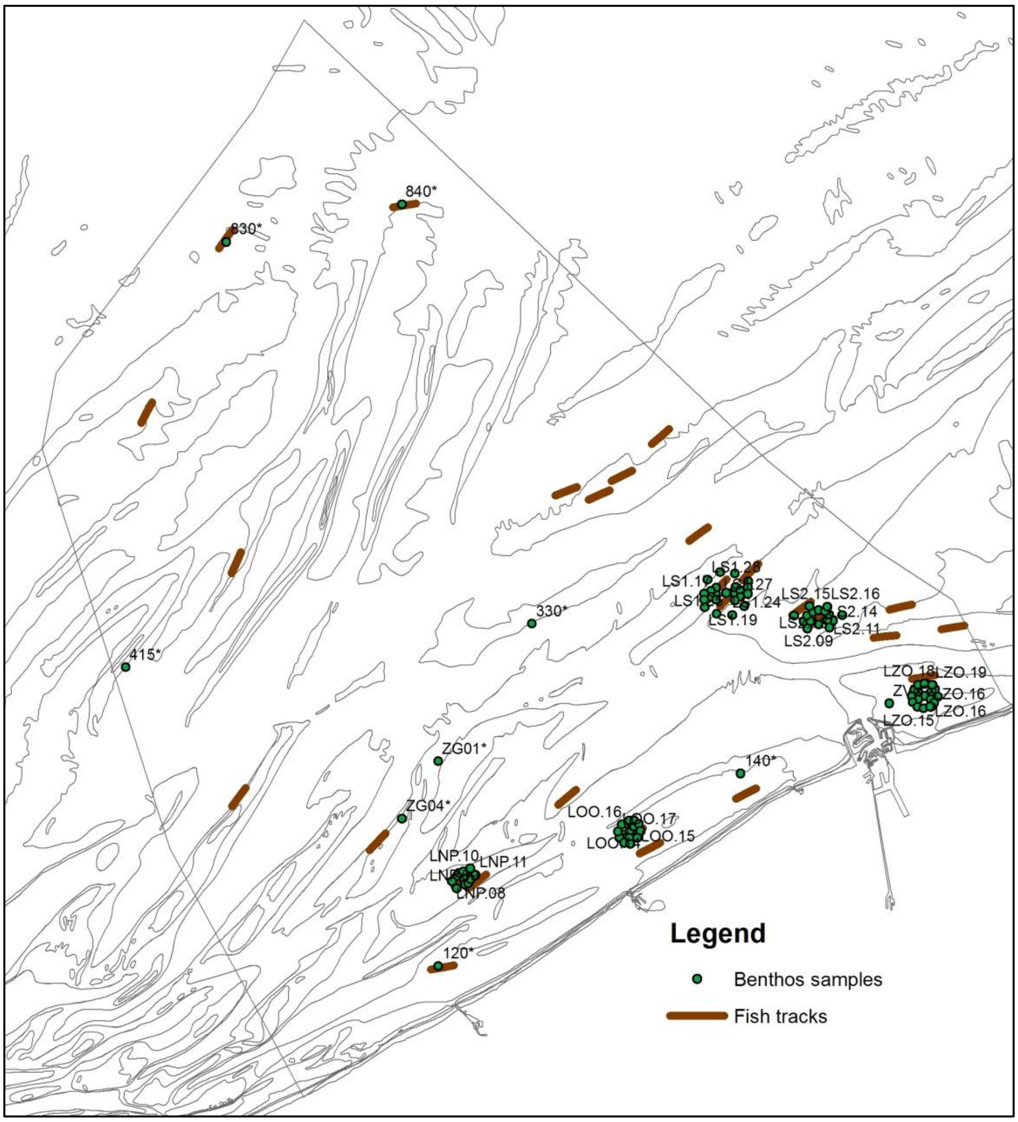


Figure 11. Overview map of the different Van Veen and fish track sampling locations included in chapter 4, 5 and 6. All locations were sampled in spring and autumn of both 2007 and 2008.

4.2.2 Sampling and data analysis

4.2.2.1 Macrobenthos

4.2.2.1.1 SAMPLING

Macrobenthos can be defined as the organisms that live for most part of their life in the sediment, and that are retained on a 1mm-meshed sieve. At the stations in the dumping sites, one sample was taken. The macrobenthos was sampled with a Van Veen grab (0.1 m²), and sieved on a 1mm sieve. Afterwards, the sample was fixed with an 8 % formaldehyde seawater solution and stained with eosin to facilitate sorting afterwards. Species were identified to species level when possible and counted. The macrobenthos monitoring (sampling, processing and analyzing) is executed following the ISO standard (ISO 16665:2005(E)) ("Water quality – Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna"). This procedure is under accreditation since 24/05/2011 under the BELAC ISO17025 norm (ILVO-DIER-ANIMALAB; CertificaatN°:BELAC T-315).

From each Van Veen sample, a Perspex core was taken for sediment analyses. These samples were dried at 60°C and analyzed with a Malvern Mastersizer 2000 following a standardized protocol. Depth and position of each sample were also registered during the campaigns.

Figure 12. Picture of Van Veen grab and macrobenthic sample

4.2.2.1.2 STATISTICAL ANALYSIS

The species dataset was standardized by lumping some species (Cirratulidae spp, *Spio* spp, Anthozoa spp), and reduced by excluding species that did not belong to the macrobenthos *sensu strictu* (e.g. Mysidacea). Nematoda were excluded because of inadequate sampling techniques for quantifying meiofauna.

The calculated univariate parameters were: density (ind./m²), biomass (Wet Weight/m²) and number of species (N_0). All analyses were performed within PRIMERv6 (Plymouth Routines in Multivariate Ecological Research; Clarke & Gorley, 2006). The univariate parameters per sample within and around each dumping side is mapped with GIS.

To scale the degree of impact, due to the dumping activities, a benthic index, the Benthic Ecosystem Quality Index (level 3), is used (Van Hoey *et al.*, 2007; Ysebaert *et al.*, in prep; www.beqi.eu). The BEQI was designed to evaluate the deviation in density, biomass, number of species and species composition between the impact and the control area.

4.2.2.2 Epibenthos and demersal fish

4.2.2.2.1 SAMPLING

Demersal fish fauna and epibenthos can be defined as organisms living on or in close association with the seafloor, and that are caught representatively and efficiently with a beam trawl. Both ecosystem components were sampled with an 8-meter beam trawl , equipped with a fine-meshed shrimp net (stretched mesh width 22mm in the codend) and a bolder-chain but no tickler chains (to minimize the environmental damage) (Figure 13). The net was dragged during 30 minutes at an average speed of 4 knots over the bottom. As such, an average distance of 3500 m was covered. This sampling method was used up to 2009. Since 2010, the tracks (short tracks) are half as long as the original tracks (long tracks) so the sampling distance and time is 1750m and 15 minutes (see chapter 7). Data were recorded on time, start and stop coordinates trajectory and sampling depth in order to enable a correct conversion towards sampled surface units. The fish tracks were positioned following depth contours that run parallel to the coastline, thereby minimizing the depth variation within a single track.

Figure 13. An 8-meter beam trawl with a fine-meshed shrimp net and a bolder chain.

The complete catch was sorted using a rinsing and sieving machine. As such, three fractions were obtained: a coarse fraction with mainly larger fish, adult starfishes and sea urchins; a shrimp fraction with mainly crustaceans, ophiuroids and smaller fishes and a fine fraction with mollusks and sea anemones. From these fractions, all fish, except gobies, were identified, measured and/or counted on board. After fish elimination, representative subsamples (2 to 10l) from each fraction were taken for epibenthos analyses. For a number of tows, the epibenthos (except gobies and small and/or rare species) subsamples were processed on board as well; for other tows, the subsamples were frozen for further laboratory analyses. Rare or peculiar species/individuals were stored for further reference or investigation.

4.2.2.2.2 STATISTICAL ANALYSIS

The net contents were divided into 'demersal fish' and 'epifauna'. These ecosystem components were dealt with separately concerning density, biomass (epibenthos only), diversity and community structure. Furthermore, from the epibenthos dataset, the polychaetes *Nereis* and *Nepthys* were deleted, ascidians and the barnacle *Balanus*, since these species were not representatively sampled. Gobiidae, belonging to the order of Perciformes, were treated separately due to their abundance and local importance.

The community structure of epifauna and demersal fish was analysed using the multivariate techniques non-metric available in Primer v6 (Plymouth Routines in Multivariate Ecological Research; Clarke & Gorley, 2006). These analyses were based on 4^{th} root transformed and reduced datasets of frequency of occurrence and density. Based on the multivariate results, structuring factors were determined. Since a different sampling method (short *versus* long tracks) was used in 2009 and 2010, analyses were conducted per year. The number of individuals per sample and per species was converted to number of individuals per $1000m^2$ (density). Biomass was expressed as grams of wet weight (WW) per $1000m^2$ and diversity was evaluated based on the expected number of species (ES) and N1 (Hill, 1973). Statistical differences between the parameters were calculated with Permutational MANOVA, an extend of Primer v6.

4.2.2.3 Chemical contaminants in sediment and biota

4.2.2.3.1 SAMPLING

Sediment samples (Table 6) were taken at different stations both inside and outside the dredge spoil disposal sites. Sampling stations more distant from the dumping sites are equally foreseen, as the silt is spread rapidly and distant from the original dumping point. Especially for chemicals which are known to strongly bind to silt, this spread is important. Analysis of sediment included grain size distribution, organic carbon content, carbonate content and analysis of CBs (chlorobiphenyls), chlorinated pesticides and metals on the fine fraction (< 63 µm). Results for sediment are always expressed on a dry weight (d.w.) basis.

Table 6. Overview of the stations used as impact and control for the chemical analysis on sediment and biota.

Dumping site	Impact/control	Biota	Sediment
Br&W Zeebrugge Oost	Impact	7001	LZO 01 – LZO 06
	Control	B10	LZO 08 – LZO 13
	Control		150/ZVL/ZEB
Br&W S2	Impact	7101	LS2 01 – LS2 07
	Control	B07	LS2 08 – LS211
	Control	B04	B031/B041
Br&W S1	Impact	7801	LS1 01 – LS1 11
	Control	7802	LS1 17 – LS1 22
	Control	350	780/B08/B031/B032
Br&W Oostende	Impact	1401	LOO 01 – LOO 07
	Control	140	LOO 08 – LOO 13
	Control		140/ZVL
Nieuwpoort	Impact	2251	LNP 01 – LNP 07
	Control	120	LNP 08 – LNP 11
	Control	230	120/230

The biota species used for chemical analysis are starfish, sea anemone, bivalves, crustaceans (brown shrimp, swimming crab and hermit crab) and fish (dragonet, goby and hooknose). Assessment of marine biota included the analysis of PCBs, chlorinated pesticides, metals and PAHs. All results for biota are expressed on a wet weight (w.w.) basis. Table 6 gives the sampled tracks at the dumping sites.

4.2.2.3.2 ANALYSIS

Metal analysis was carried out by CODA (Centre for Research in Veterinary and Agrochemistry, Tervuren). Metals were solubilised from sediment using a mixture of nitric acid, hydrofluoric acid and perchloric acid, as prescribed by QUASIMEME (Quality Assurance in Marine Environmental Monitoring in Europe), followed by a dilution step and quantification. Al and Fe were analysed as major components in sediments using ICP-AES (Inductively Coupled Plasma – Atomic Emission Spectrophotometry). As, Cr, Cu, Ni, Pb, Zn (in mg/kg) and Cd (in µg/kg) were quantified using ICP-MS (Mass Spectrometry) and Hg (in µg/kg) was analysed using gold amalgamation (AMA) followed by AFS (Atomic Fluorescence Spectrophotometry). All measurements were completed on the fine sediment fraction (<63 µm). Reference materials were included in analysis batches, and the lab successfully participated in international inter-calibration exercises. Metal assessment in biota was completed by the same lab, but Al and Ni were not quantified, and the applied pretreatment was different. Biota samples were extracted with nitric acid at high temperature by microwave digestion in pressure bombs. For final analysis, the same techniques as for sediments were applied.

PAHs were extracted from biota using hexane after a methanol-KOH digest, followed by clean-up with alumina oxide. After concentration of the sample at low temperature (30 °C) under a gentle nitrogen flow, samples were analysed with HPLC and fluorescence detection. The recovery of the procedure was checked with a 6-methylchrysene internal standard, and every batch included a blank and a reference sample. The participation in intercalibration exercises yields good results for some 95 % of the determinants. Following PAHs were quantified: acenaphtene, acenaphtylene, anthracene, benzo-a-anthracene, benzo-b-fluoranthene, benzo-k-fluoranthene, benzo-g,h,i-perylene, benzo-a-pyrene, chrysene, dibenz-a,h-anthracene, indeno-1,2,3(c,d)-pyrene, fluoranthene, fluorene, phenantrene and pyrene. All concentrations are expressed as µg/kg w.w.

For quantification of CBs and chlorinated pesticides, different extraction methods were used for sediment and biota. For sediments, typically 3 grams were extracted by hexane-acetone (v:v% 75:25) in a soxhlet for 6 hour, followed by desulphurisation using TBA (tetrabutyl ammonium sulphate), and solvent was largely evaporated at 30 °C under a gentle nitrogen stream. On biota samples, a fat extraction was generally performed by using a Bligh and Dyer extraction (chloroform-methanol). For bivalves except mussels, a Smedes extraction was applied. The fat content was determined by weighing after solvent evaporation.

The clean-up of biota and sediment extracts were achieved by bringing the extract onto an alumina oxide column, and eluted with hexane. Subsequently, the elution was concentrated to 0.7ml. Afterwards, a fractionation was achieved using a silica column.

The CBs were eluted by hexane, as well as HCB (hexachlorobenzene) and p,p'-DDE (dichlorodiphenylethylene). The remaining pesticides were eluted with hexane-diethylether (v:v% 90:10). After concentration, the samples were spiked with TCN (tetrachloronaphtalene) as analytical recovery standard, and diluted up to 1 ml for analysis.

Quantification was done with a GC equipped with ECD (Electron Capture Detector). The CB run included IUPAC numbers 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180; HCB and p,p'-DDE. The pesticide run included alpha-HCH (Hexachlorocyclohexane), gamma-HCH, aldrin, transnonachlor, dieldrin, p,p'-DDD (dichlorodiphenyldichloroethane) and p,p'-DDT (trichloro-diphenyldichloroethane). All results are expressed in µg/kg.

Reference and blanks samples were systematically included in all batches, and participation in international intercalibration exercises was done several times a year. For ease of interpretation of large data sets, concentrations of CBs might be summed (Sum of 10 CBs: IUPAC 28, 31, 52, 101, 105, 118, 153, 156, 180), the same is the case for sum DDT, including DDD, DDE and DDT values.

The normal distribution of the data was verified by the Kolmogorov-Smirnov Test and by Q-Q plots. Impact and control assessments on sediments for the different dumping sites are statistically evaluated using T-tests for independent samples.

4.2.2.4 Biological Effects of Contaminants: Fish diseases and parasites

4.2.2.4.1 SAMPLING

The Belgian Part of the North Sea was divided in different areas through assembling of the results of similar zones. The first sampled zone (Impact Zone) includes the dredge spoil dumping sites: Nieuwpoort, Br&W Oostende, Br&W Zeebrugge Oost, Br&W S1 and Br&W S2. The results for some additional sites, studied in the context of other monitoring programmes, were integrated in this study as reference zones. A first reference zone includes the coastal reference sites of the BPNS (Reference Zone 1/ coastal reference area): Westdiep, Oostende bank, Oostdyck, Buitenratel and Kwintebank. A second reference zone includes reference sites in open sea of the BPNS (Reference Zone 2/ offshore reference area): Scharrebank, Bligh bank, Fairybank, the Hinderbanken and other open sea points.

In some areas, the sampling size of certain fish species was still too small and not representative for the overall observed prevalence of the diseases. This is definitely the case for the coastal areas during spring 2010. Therefore, no overall observed prevalence was calculated and the results were not included in this report. Only sample sizes larger than 100 fishes are considered as representative. For each zone, all fish larger than 15 cm are collected.

4.2.2.4.2 ANALYSIS

The selected organisms should be abundant and exhibit diseases which are easily recognized. Dab (*Limanda limanda*) is an appropriate organism to use in monitoring programs for fish pathology because of its demersal location and low mobility (Bucke *et al.*, 1996). However, in the examined zones, dab was not always sufficiently present. Therefore, the monitoring was extended to most of the commercial flatfish species: dab (*Limanda limanda)*, plaice (*Pleuronectes platessa*), Dover sole (*Solea solea*) and flounder (*Platichthys flesus*). Roundfish species were chosen as additional organisms for disease monitoring: whiting (*Merlangius merlangus*), bib (*Trisopterus luscus*) and cod (*Gadus morhua*). The main focus was put on disease monitoring of the flatfish dab and the round fish whiting. The fishes were examined for the presence of the following diseases: skin ulcers, skeletal deformities (including scoliosis, lordosis, and vertebral compression), pigmentation, lymphocystis, papilloma, liver nodules and the following parasites: *Cryptocotyle lingua, Clavella sp., Acanthochondria cornuta, Lepeophtheirus pectoralis, Lernaeocera branchialis, Glugea stephani* and *Anisakis sp* (Figure 14).

Figure 14. Pictures of the diseases and parasites: Pigmentation, *Glugea sp.*, *Stephanostomum sp.*, *Lernaeocera sp.* Epidermal papilloma, *Anisakis sp.* and *Lepeoptheirus sp.*

4.2.2.5 Biological Effects of Contaminants: EROD activity as biochemical indicator of xenobiotic substance accumulation.

4.2.2.5.1 INTRODUCTION

The effects of contaminating substances (PCBs, PAHs, …) in living organisms can be studied through the upregulation of enzymatic activities involved in biotransformation of pollutants following their binding on the aryl hydrocarbon receptor (ArH receptor). Upregulated enzyme activities may serve as 'early warning' signals for pollution. Here, the biochemical biomarker EROD (7-ethoxyresorufin O-deethylase) activity is used as an indicator of xenobiotic substance accumulation in the flatfish dab. Assessment of the EROD activity is one of the required indicators of pollution by the Joint Assessment and Monitoring Programme (JAMP) under the coordination of OSPAR.

4.2.2.5.2 SAMPLING

The study area for monitoring the biological effects of contaminants comprises the main dredge spoil dumping sites: Nieuwpoort, Br&W Oostende, Br&W Zeebrugge Oost, Br&W S1 and Br&W S2 and three preference zones (Westdiep, Steendiep and Raan). If possible, the same control zones as for macro- and epibenthos monitoring were used. When insufficient sampling material was present, control zones could be expanded. The main goal of biochemical monitoring is a follow-up of the introduction of contaminants by dredge spoil disposal and the biological effects on biota of these contaminants.

Biochemical monitoring uses liver homogenates of small dab *Limanda limanda* (9-13 cm), caught in their natural environment. Dab is an appropriate organism because it is a demersal fish with a low mobility. However, the decreasing trend in the appearance of small dabs on the BPNS forms a major problem for the assessment of the biochemical EROD activity. Large sampling sizes are needed to use this biomarker in an integrated monitoring strategy.

4.2.2.5.3 ANALYSIS

Dab livers were excised and homogenised in a homogenisation buffer using a Potter-Elvjeghem homogenisation device. The homogenate was centrifuged to remove fat and debris, and the supernatant was used for EROD activity measurement. An ¼ dilution was made for the assessment of the protein concentration, needed to normalize the EROD activity results. EROD activity was measured using a fluorescence spectrophotometer to quantify kinetics of the conversion of ethoxy-resorufin (non fluorescent) to resorufin (fluorescent). A bicinchoninic acid (BCA) protein assay was used to determine the protein level of the supernatans (Smith et al. 1985). The BCA protein assay is based on the colorimetric detection of bicinchoninic acid using a spectrophotometer (UV-VIS). EROD activities are reported in (pmol)/(min.mg protein).

The EROD assay is based on the method of Burke & Mayer (1974). ICES guidelines are used for the assessment of the EROD activity in dab liver (Stagg & McIntosh, 1998). The protocol was adapted and participation in an inter-laboratory proficiency test organised by BEQUALM (Biological Effects Quality Assurance in Monitoring Programs) proved good analysis.

Normal distribution of the data was examined by the Kolmogorov-Smirnov Test and by Q-Q plots. The influence of sex on the EROD assay was evaluated using an non-parametric Mann-Whitney U test ($p<0.05$). Impact and control assessments are statistically evaluated by Kruskal-Wallis tests ($p<0.05$).

Besides the EROD assay, it is possible to assess other alkoxy resorufin deethylase (AROD) activities; e.g. methoxyresorufin-O-deethylase (MROD) activity and pentoxyresorufin-O-deethylase (PROD) activity. The MROD and EROD assays are CYP1A1 mediated deethylations, while the PROD assay deals with a CYP2B mediated deethylation. In all three AROD assays, the different substrates are converted to the fluorescent resorufin.

4.3 Results

4.3.1 Sedimentology

4.3.1.1 Sedimentological characteristics

The sedimentological characteristics were determined based on the median grain size (d(05)), median grain size of the sand fraction (D(sand)) and percentage mud (Figure 15). Dumping site Nieuwpoort is characterised by the highest average d(05) and d(sand) and a low mud content. The dumping sites Br&W Oostende and Br&W Zeebrugge Oost were characterised by the highest mud content (30-40% at average) and the lowest average median grain size (<200µm). Those dumping sites have similar sedimentological characteristics. Dumping site Br&W S2 is characterised by a very low mud content and an average median grain size of 230µm. The D(sand) and D(05) are similar, indicating that the area is characterised by clean, well sorted sands. Dumping site Br&W S1 is characterized by a relative high mud content and a median grain size of around 240-250µm.

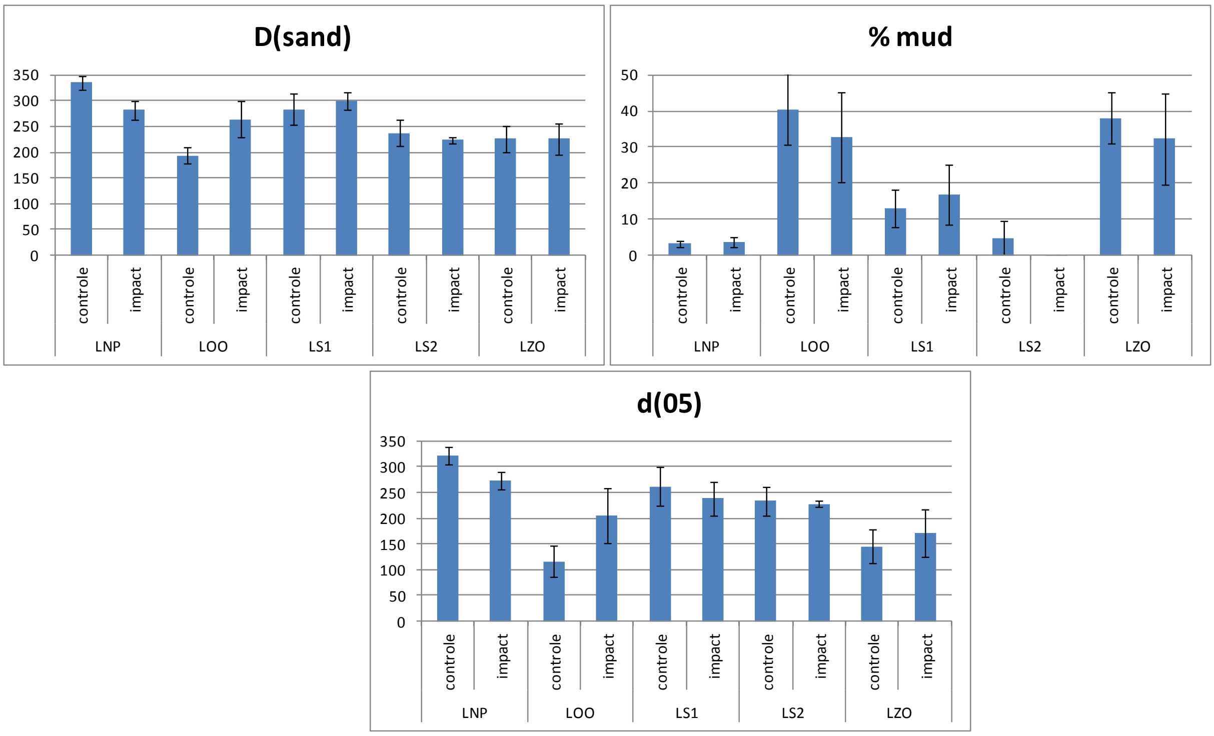


Figure 15. Overall sedimentological characteristics at the different dumping sites and nearby control area

4.3.1.1.1 DUMPING SITE NIEUWPOORT

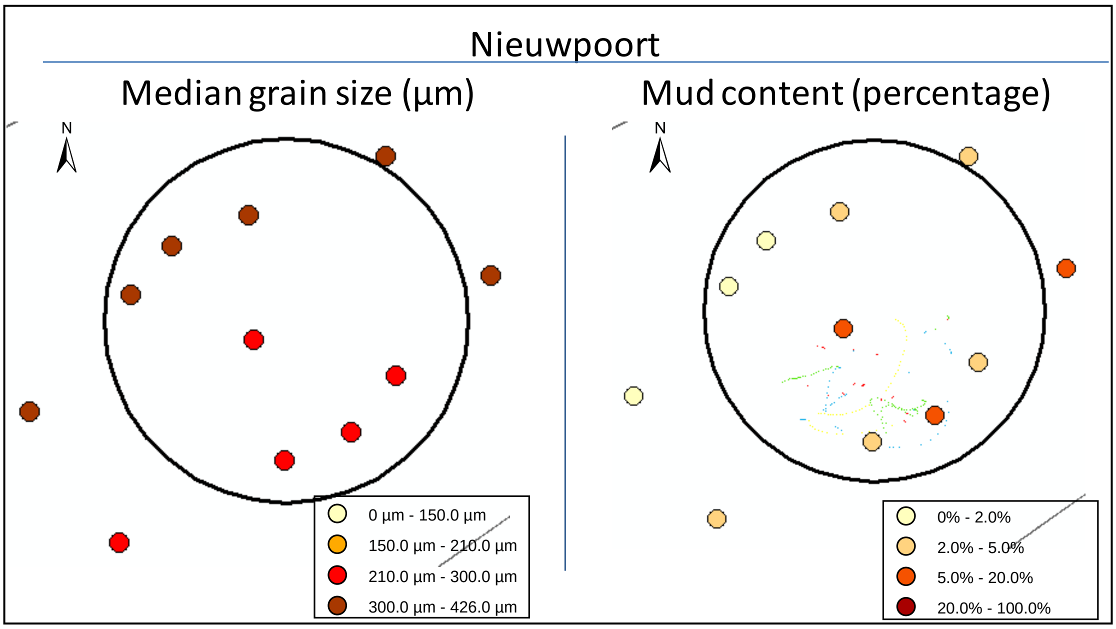


Figure 16. Map of dumping site Nieuwpoort with visualization of the median grain size and mud content of the samples. In the right figure, also the dumping intensity of 2009 and 2010 is visualized (increased intensity scale from yellow-green-blue to red).

The sedimentology at the dumping site Nieuwpoort is different in its Northern part compared to the Southern part (Figure 16). The median grain size is highest in the Northern part, whereas the mud content is lower there. This is maybe partly natural, but can also partly related to the dumping activity, which is most frequently situated in the Southern part.

4.3.1.1.2 BR&W OOSTENDE

The sedimentological characteristics at Br&W Oostende are very variable (Figure 17). The samples with the highest mud content and lowest median grain size were found at the southern border and in the Northern part of the dumping site. In the southern part of the dumping site, the median grain size of the samples are higher, with a relative high mud content. The sedimentology at this site can be defined as muddy.

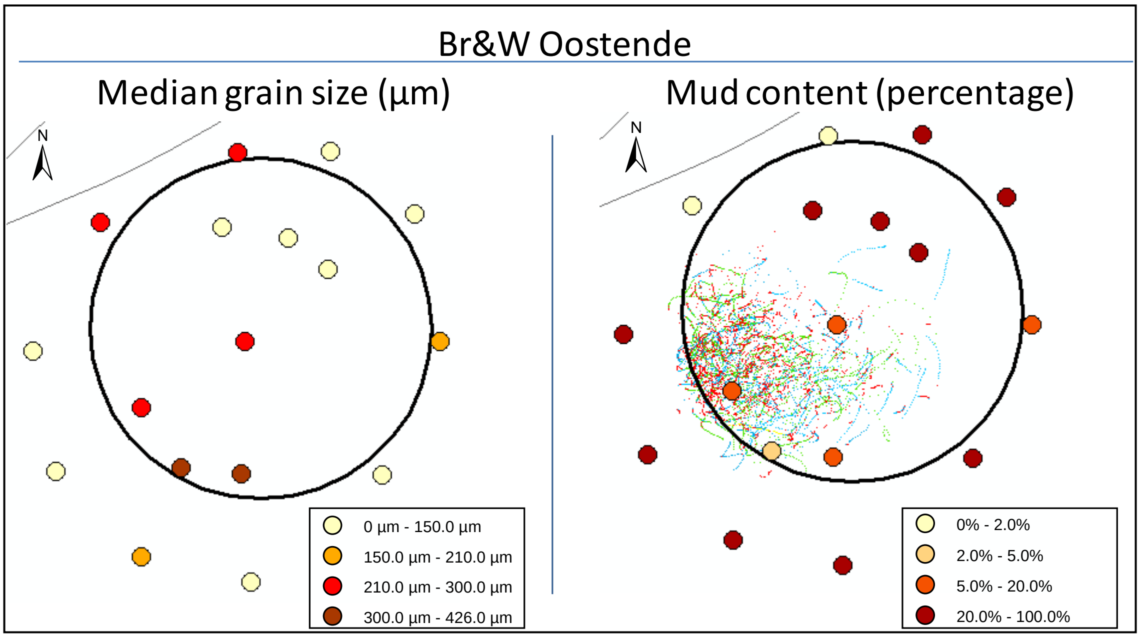


Figure 17. Map of dumping site Br&W Oostende with visualization of the median grain size and mud content of the samples. In the right figure, also the dumping intensity of 2009 and 2010 is visualized (increased intensity scale from yellow-green-blue to red).

4.3.1.1.3 BR&W S1

The samples in the southeastern part of the dumping site Br&W S1 are characterised by a low mud content, except a few just outside the site (Figure 18). In the Western part, the sedimentological characteristics are very variable, especially in mud content. This can be caused by the very frequently dumping activities in this part of the dumping site Br&W S1.

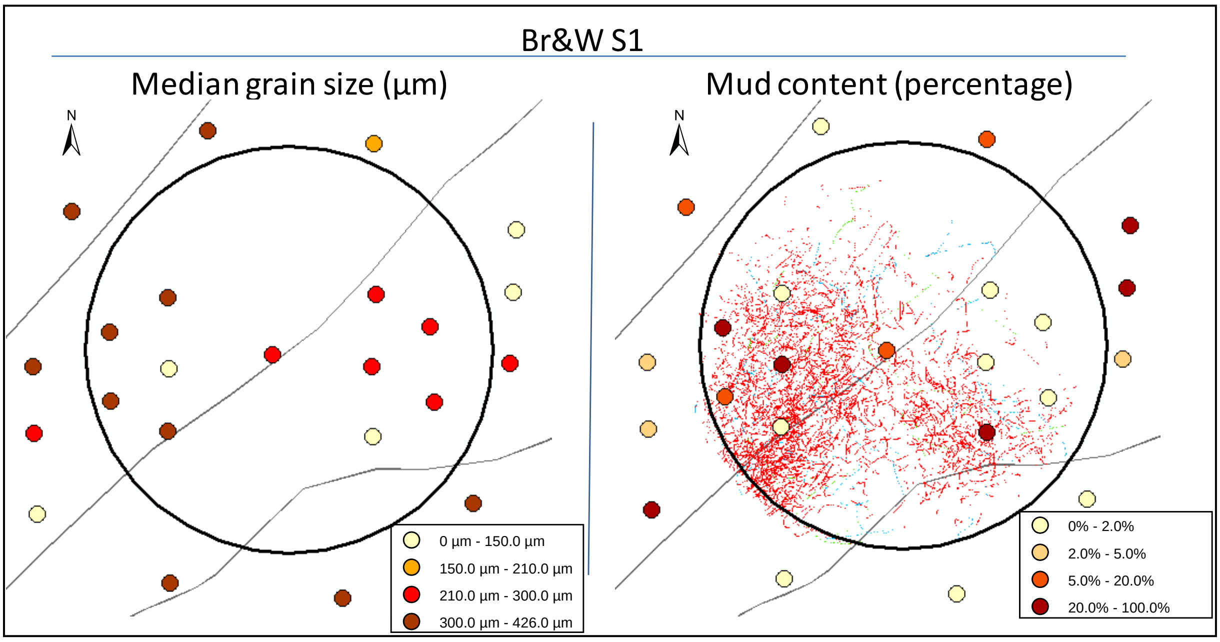


Figure 18. Map of dumping site Br&W S1 with visualization of the median grain size and mud content of the samples. In the right figure, also the dumping intensity of 2009 and 2010 is visualized (increased intensity scale from yellow-green-blue to red).

4.3.1.1.4 BR&W S2

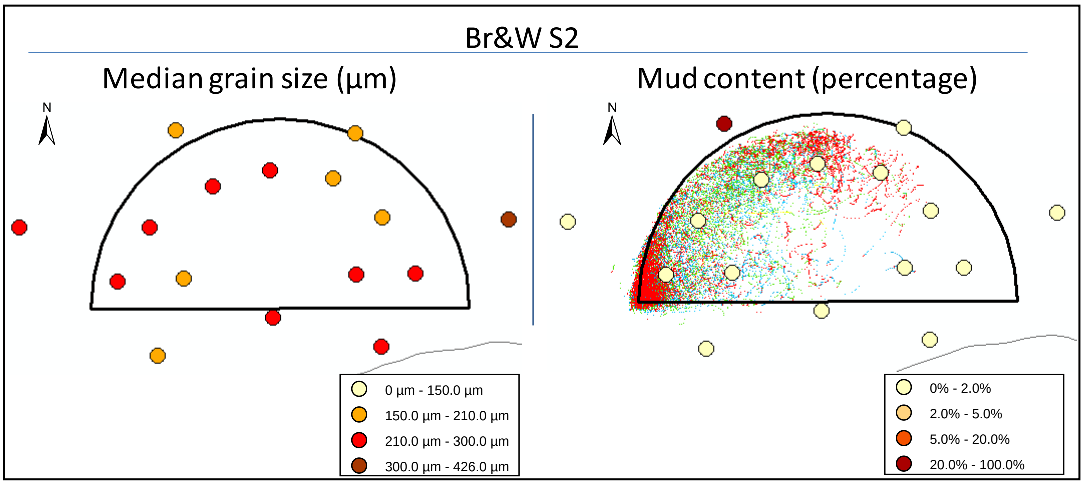


Figure 19. Map of dumping site Br&W S2 with visualization of the median grain size and mud content of the samples. In the right figure, also the dumping intensity of 2009 and 2010 is visualized (increased intensity scale from yellow-green-blue to red).

The mud content of the samples in and outside the dumping site Br&W S2 is very low, except for 1 sample (Figure 19). Therefore, the median grain size of the samples is very similar (around 210µm). This area can be characterised as a 'clean' sandy area. This sedimentological observations are contradictory with the higher dumping intensity in 2009-2010 at this side, especially in the western part.

4.3.1.1.5 BR&W ZEEBRUGGE OOST

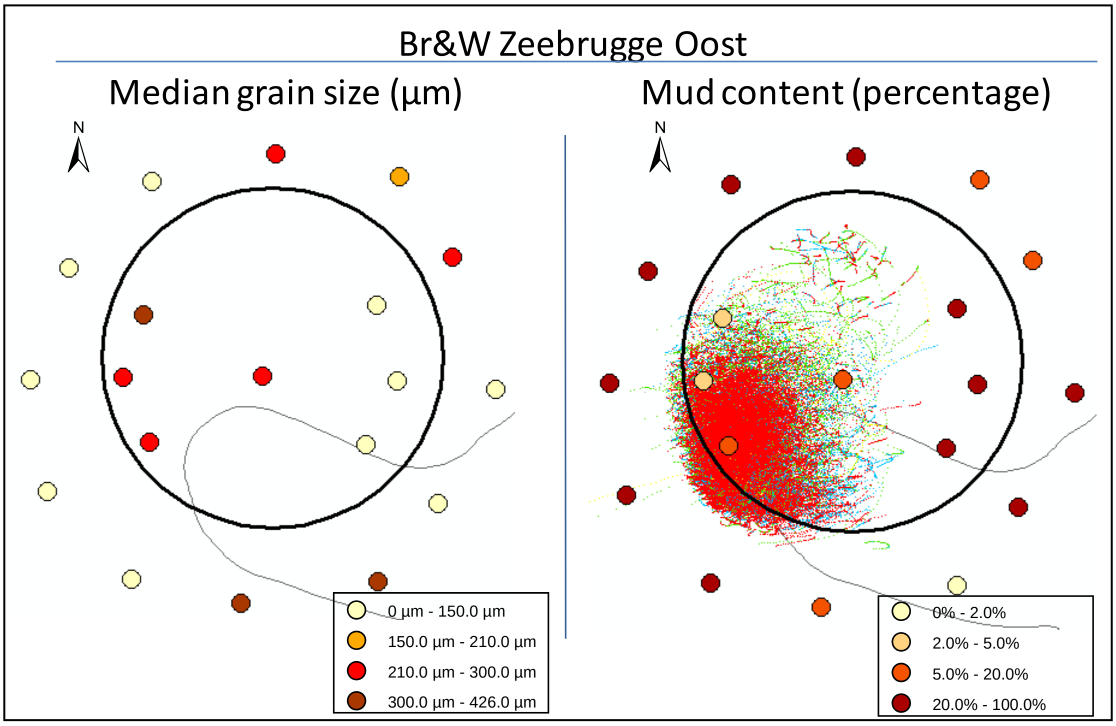


Figure 20. Map of dumping site Br&W Zeebrugge Oost with visualization of the median grain size and mud content of the samples. In the right figure, also the dumping intensity of 2009 and 2010 is visualized (increased intensity scale from yellow-green-blue to red).

The samples outside the dumping site Br&W Zeebrugge Oost are characterised by a high mud content and a low median grain size, except 2 samples in the south and 2 samples in the northeast (Figure 20). Within the dumping site, the samples in the eastern part are characterised by a higher mud content compared to those in the western part. The highest dumping frequency is observed in the western part, characterised by the highest sediment variability.

4.3.1.1.6 CONCLUSION

As we compare the sediment maps and the dumping intensity maps, we can conclude that the sediment characteristics of the samples within the area with the highest dumping intensity are more heterogeneous. The mud content, the main dredged material at most sites, is not obvious higher compared to the samples elsewhere within a dumping site. It is in some cases the opposite, that the samples further away, mostly in line with the mean current direction are characterised by the highest mud content. Therefore, it seems that the muddy material is deposited in a more wider area than the dumping spot itself.

4.3.1.2 Chemical contamination in sediment

4.3.1.2.1 INTRODUCTION

The chemical contaminants PAHs, CBs and chlorinated pesticides are known to be strongly associated with sediment, while being almost absent in the water phase. They mainly attach to the fine fraction while coarse particles present only few active spots. Metals, especially these known as toxic to marine life, can also be found mainly in sediment or suspension.

Metals analysed comprise Al and Fe, as major constituents (in %), As, Cr, Cu, Ni, Pb and Zn (in mg/kg) and finally the most toxic Cd and Hg (in µg/kg), all expressed on a dry weight (d.w.) basis of the fine fraction (<63 µm). Since results for Al and Fe varied very little, data are not shown.

CBs are always present in measurable quantities, and results are shown as sum of 10 CBs (IUPAC numbers 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180). Pesticide concentrations are quite low, except for DDT and breakdown products DDE and DDD (only pp' isomers), presented as sum of DDT. Values for alpha-HCH and gamma-HCH are close to or below LOD values, and rarely exceed 0.2 µg/kg d.w., the same holds for transnonachlor and HCB. It must be stated that HCB is present in the vast majority of the samples at a concentration of 0.1 µg/kg. Aldrin, dieldrin and endrin are never detected.

The normal distribution of the data was verified by the Kolmogorov-Smirnov Test and by Q-Q plots. Impact and control assessments on sediments for the different dumping sites are statistically evaluated using T-tests for independent samples.

4.3.1.2.2 DUMPING SITE BR&W ZEEBRUGGE OOST

For evaluation of chemical variation on this dumping site, the presence of the Scheldt as an important source has to be taken into consideration. This dumping area is characterized by very muddy sediments (average mud content comprises 20% of sediment).

Heavy Metals

Figure 21 present the heavy metal content (Cd, Hg, Cu and Pb) of sediment samples of deposit site Br&W Zeebrugge Oost. During 2009-2010, the observed concentrations are generally higher in the impact zone compared to the control area. In spring 2010, this difference between impact and control area was significantly for cadmium and mercury, followed by a reduction in concentrations in autumn.

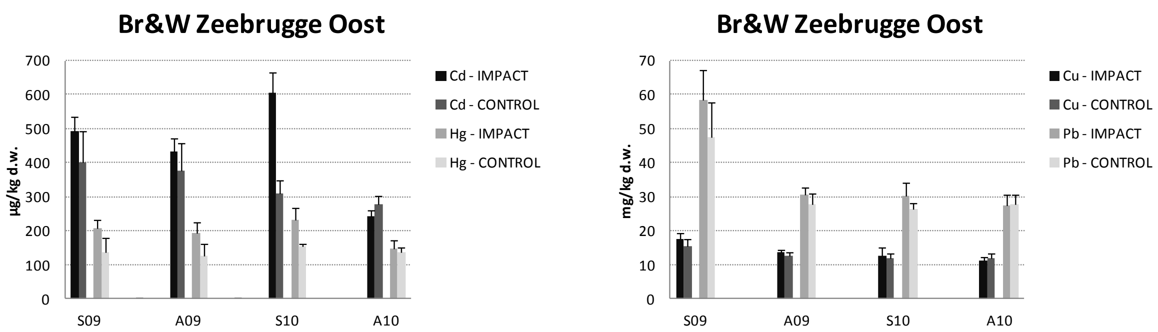


Figure 21. A) Average concentration and standard error for Cd and Hg in sediment samples of Br&W Zeebrugge Oost. Concentrations are expressed in µg/kg d.w.; B) Average concentration and standard error for Cu and Pb in sediment samples of Br&W Zeebrugge Oost. Concentrations are expressed in mg/kg d.w. Impact en Control are presented for each season. (S09: spring 2009, A09: autumn 2009, S10: spring 2010, A10: autumn 2010)

Chlorinated Biphenyls CBs

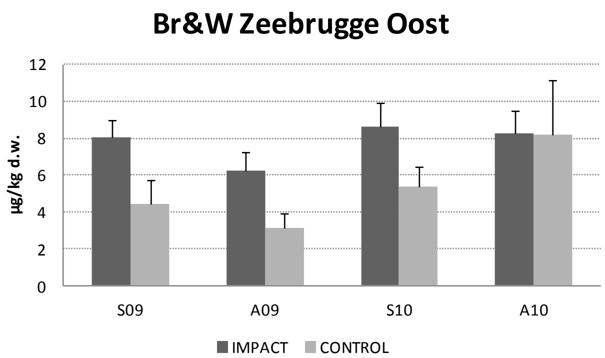


Figure 22. Average concentrations and standard errors for the sum of 10 CBs in sediment on dumping site Br&W Zeebrugge Oost. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season. (S09: spring 2009, A09: autumn 2009, S10: spring 2010, A10: autumn 2010)

With exception of autumn 2010, the detected CB concentrations in sediments of Br&W Zeebrugge Oost are higher for impact compared to control sites (Figure 22). Pesticide concentrations (DDT) found on Br&W Zeebrugge Oost support the same trend. The seasonal variation in CB content could be neglected.

4.3.1.2.3 DUMPING SITE BR&W SI

This dumping site is located at a larger distant from the Scheldt estuary, but close to an intensive shipping lane. It is important to mention the patchiness of the seafloor on deposit site Br&W S1. On this site, the mud concentration is occasionally lower on the impacted sites versus the control sites, which could make the interpretation of the results more difficult.

Heavy Metals

No major differences are noticed between the impact and control area of dumping site Br&W S1, and the seasonal variation on sediment pollution is negligible (Figure 23).

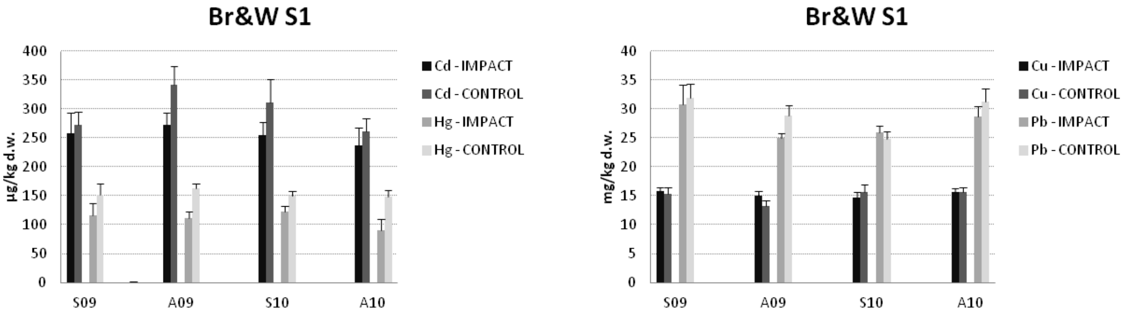


Figure 23. A) Average concentration and standard error for Cd and Hg in sediment samples of Br&W S1. Concentrations are expressed in μg/kg d.w.; B) Average concentration and standard error for Cu and Pb in sediment samples of Br&W S1. Concentrations are expressed in mg/kg d.w. Impact en Control are presented for each season.

Chlorinated Biphenyls CBs

With exception of autumn 2010, CB concentrations are higher at control zones compared to impact zones (Figure 24). In agreement, the DDT concentrations are slightly elevated on control sites (Figure 25). This trend could be supported by the very patchy seafloor and the possible higher mud concentrations on control sites compared to impact sites, originated by the sedimentation of dumped material. CB concentrations of sediments on dumping area Br&W S1 (and on Br&W S2) are lower compared to the other dumping sites, although this is not the case for DDT pesticides. Standard errors are remarkably higher for the control sites. By a Kruskal-Wallis statistical test, CB concentrations were found significantly higher during autumn 2010 for impact and control areas compared to the other seasons. Therefore, follow up in 2011-2012 is important.

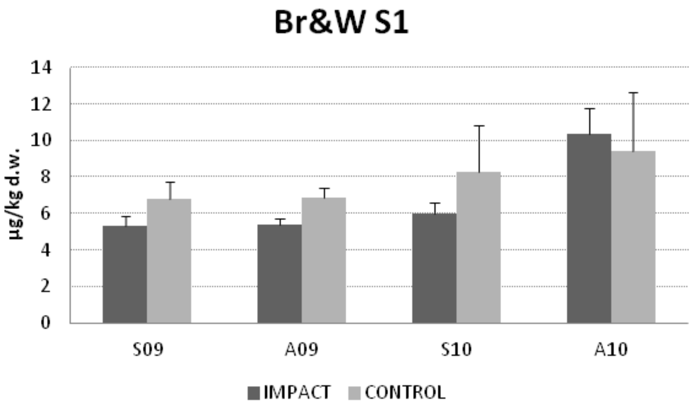


Figure 24. Average concentrations and standard errors for the sum of 10 CBs in sediment on dumping site Br&W S1. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season.

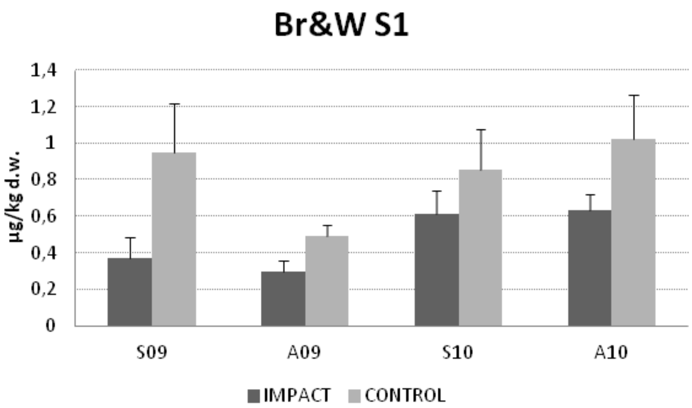


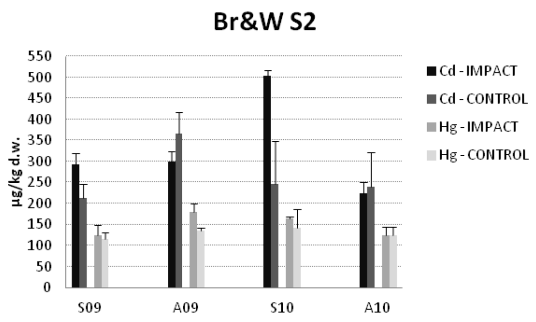
Figure 25. Average concentrations and standard errors for the sum of 10 DDT in sediment on dumping site Br&W S1. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season.

4.3.1.2.4 DUMPING SITE BR&W S2

The dumping site Br&W S2 is located quite near to the Scheldt estuary, so its influence has to be considered. Br&W S2 sediments are characterised as more sandy, with a very small mud content.

Heavy Metals

No trend between control and impact could be observed on dumping site Br&W S2 based on the metal content (Figure 26). During spring 2010, a significant higher cadmium concentration was observed in the impact area. This was also the case on dumping site Br&W Zeebrugge Oost, which is also located near to the Scheldt estuary.



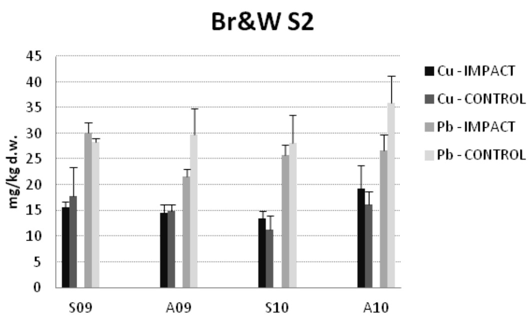


Figure 26. A) Average concentration and standard error for Cd and Hg in sediment samples of Br&W S2. Concentrations are expressed in µg/kg d.w.; B) Average concentration and standard error for Cu and Pb in sediment samples of Br&W S2. Concentrations are expressed in mg/kg d.w. Impact en Control are presented for each.

Chlorinated Biphenyls CBs

In agreement with the results on metals on dumping site Br&W S2, no significant trend between impact and control could be observed during 2009-2010 based on CB content (Figure 27). Standard errors were rather elevated, caused by the patchiness of the seafloor. Pesticide (DDT) fluctuations on dumping site Br&W S2 are consistent with the CB fluctuations. The seasonal variation in CB content could be neglected.

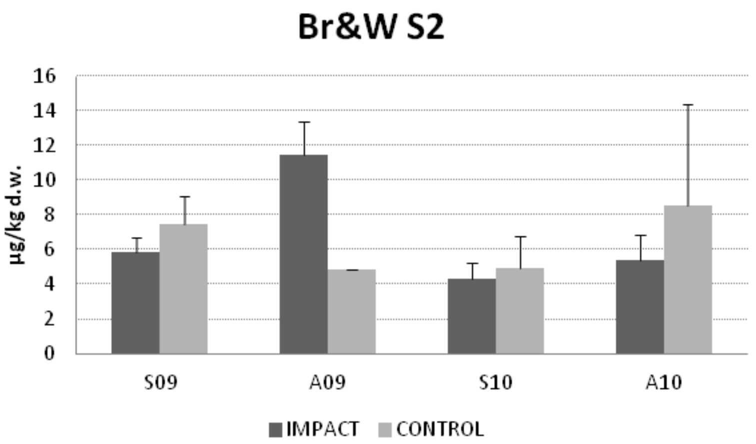


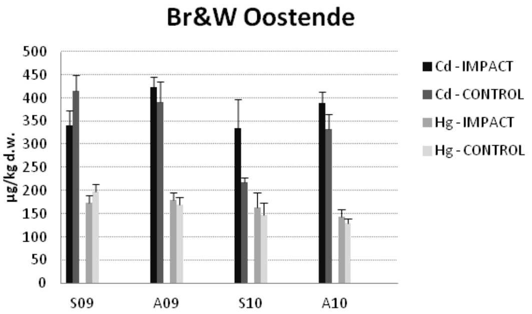
Figure 27. Average concentrations and standard errors for the sum of 10 CBs in sediment on dumping site Br&W S2. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season.

4.3.1.2.5 DUMPING SITE OOSTENDE

This dumping site is more distant from major polluting sources, and the intensity of dredged spoil disposal is rather low. This is reflected in very complete sampling and comparability of averaged data is good. Dumping site Br&W Oostende is characterised by muddy sediments.

Heavy Metals

With exception of spring 2009, a higher metal concentration (Cd, Hg, Cu & Pb) is measured on impact sites compared to control sites of dumping area Br&W Oostende (Figure 28). Consistent standard errors are obtained. No significant seasonal variation is observed.



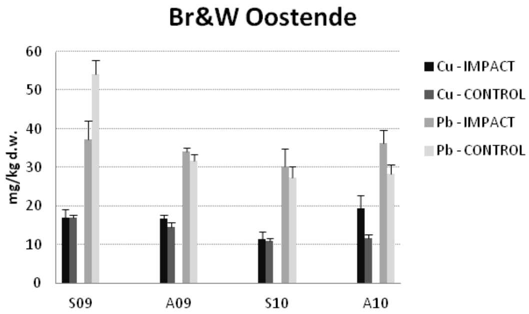


Figure 28. A) Average concentration and standard error for Cd and Hg in sediment samples of Br&W Oostende. Concentrations are expressed in µg/kg d.w.; B) Average concentration and standard error for Cu and Pb in sediment samples of Br&W Oostende. Concentrations are expressed in mg/kg d.w. Impact en Control are presented for each season.

Chlorinated Biphenyls CBs

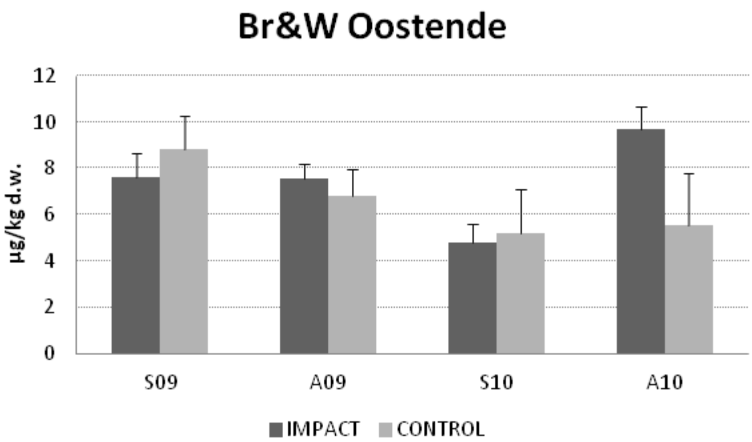


Figure 29. Average concentrations and standard errors for the sum of 10 CBs in sediment on dumping site Br&W Oostende. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season.

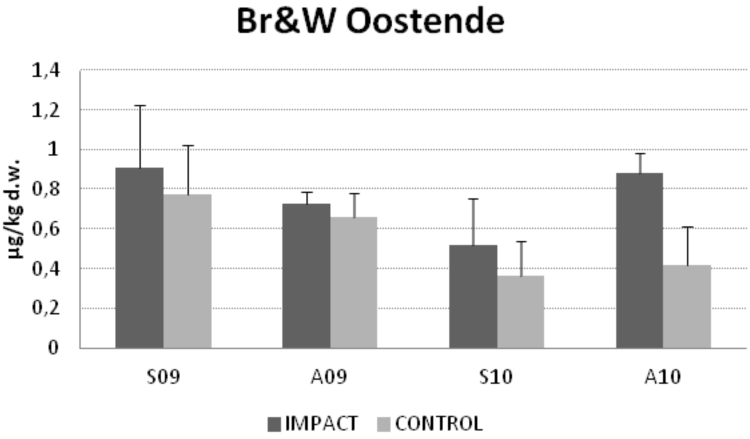


Figure 30. Average concentrations and standard errors for the sum of DDT in sediment on dumping site Br&W Oostende. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season.

No significant differences in CB concentrations are observed between impact on control sites, independent of the season (Figure 29). During 2009 and 2010, a higher DDT content is observed on the impact sites of dumping area Br&W Oostende compared to the control sites (Figure 30).

4.3.1.2.6 DUMPING SITE NIEUWPOORT

This dumping site is distant from major polluting sources and influenced by Atlantic water advection through the Channel. The intensity of dredged spoil disposal is rather low, which is reflected in very complete sampling. This dumping area is characterised by fine sandy sediments with a somewhat lower mud content.

Heavy Metals

Given that this dumping site is not intensively used, higher values might be caused by old dredged spoil or by older sediments that were replaced. Only a limited variation between impact and control was observed (Figure 31). Seasonal influence could be neglected. The smaller standard errors point at a less patchy seafloor.

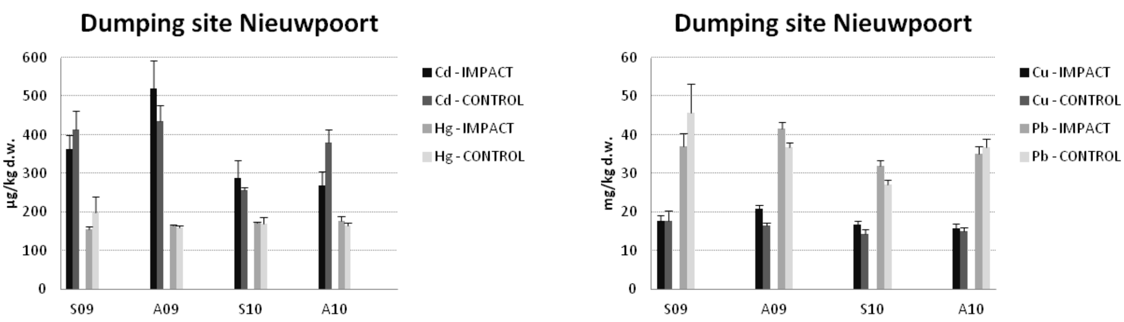


Figure 31. A) Average concentration and standard error for Cd and Hg in sediment samples of dumping site Nieuwpoort. Concentrations are expressed in µg/kg d.w.; B) Average concentration and standard error for Cu and Pb in sediment samples of dumping site Nieuwpoort. Concentrations are expressed in mg/kg d.w. Impact en Control are presented for each season.

Chlorinated Biphenyls CBs

Taking into account the low dumping activity, relatively high CB concentrations are obtained on dumping site Nieuwpoort (Figure 32). For autumn 2010, CB concentrations at the impact areas were higher than at the control areas More important, by far the highest DDT concentrations are observed at this site compared to other dumping sites. In 2010, an average concentration of >4 µg/kg d.w. was observed on the impact sites, while on the other dumping sites, concentrations of ≤1 µg/kg d.w. were recorded.

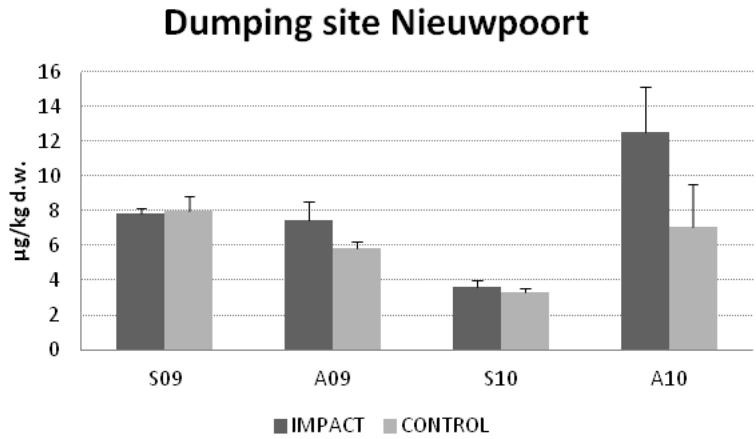


Figure 32. Average concentrations and standard errors for the sum of 10 CBs in sediment on dumping site Nieuwpoort. Concentrations are expressed in µg/kg d.w. Impact en Control are presented for each season.

4.3.1.2.7 OVERVIEW DUMPING SITES

To assess the chemical differences amongst the dumping sites, the sampling period 'autumn 2010' was selected and the levels of contamination were evaluated. This is illustrated by the levels of CBs and Pb in the sediment samples of the dumping areas during autumn 2010 (Figure 33). Based on the results of sediment analysis, only minor differences between the dumping sites could be observed.

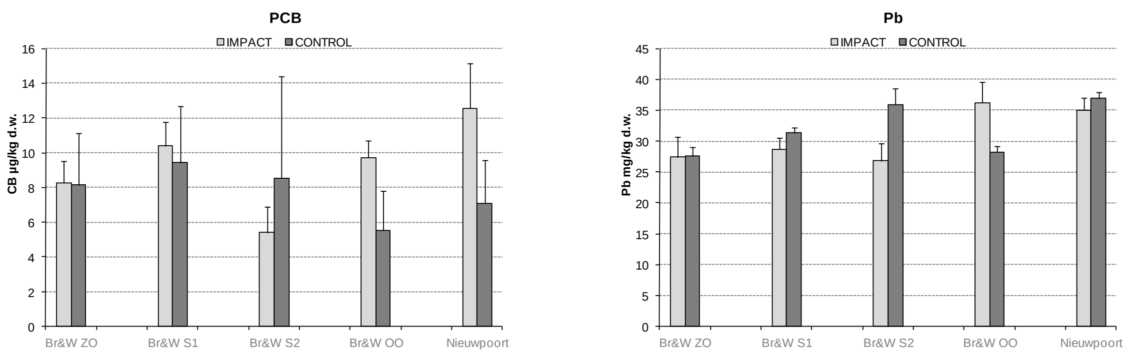


Figure 33. Levels of CBs and Pb in sediment samples during autumn 2010 for the Impact and Control sites of the different dumping sites. Concentrations are expressed in µg/kg w.w. for the sum of CBs. Concentrations are expressed in mg/kg w.w. for Pb.

4.3.1.2.8 CONCLUSION

Based on the results of 2009-2010, only small differences between impact and control assessments are observed. For dumping sites Br&W Zeebrugge Oost and Br&W Oostende, impact levels are slightly elevated compared to control levels. When there is a large difference in patchiness at a dumping site, larger standard deviations could be observed. However, no major differences between dumping sites were noticed. Influences by season are small, still it is important to take into account the sampling period before using data in monitoring.

Environmental assessment criteria (EAC) according to OSPAR, MSFD (Task group 8, Joint Report) and Belgisch Staatsblad can be used to interpret the observed levels of pollution. Consequently, monitoring data can be scored according to the EAC as presented in Table 7. If the assessed level of a specific pollutant reaches the EAC level it is indicated in red, if the level is lower, it is indicated as green. The EAC values for the heavy metals cadmium and lead introduced by the MSFD are clearly more strict compared to the EAC values of OSPAR and Belgisch Staatsblad. The observed levels of Cd and Pb on each site clearly exceed those EAC values of MFSD, while this is definitely not the case for the other EAC values. An similar observation could be made for the PCBs 118 and 153 by Belgisch Staatsblad and PCB 118 by MSFD. Based on the OSPAR monitoring criteria, only incidentally overrange levels of Pb and PCBs are observed (Table 7). Nevertheless, the levels of lead and CBs must be followed in future. Other measured heavy metals and persistent organic pollutants do not approach the formulated EAC values. It will be necessary to complete this table with monitoring results of the Belgian Part of the North Sea (BPNS) during the upcoming years. A major remark concerns the availability and relevance of environmental assessment criteria. Extra attention must be paid to the development of those EAC, which will be relevant for monitoring on the BPNS.

Table 7. Assessment criteria for sediment analysis according to OSPAR, MSFD and Belgisch Staatsblad (GREEN = OK according to EAC, RED = value above)

		Dumping site Nieuwpoort								Br&W Oostende								Br&W S1								Br&W S2								Br&W Zeebrugge Oost							
		2009				2010				2009				2010				2009				2010				2009				2010				2009				2010			
		S		A		S		A		S		A		S		A		S		A		S		A		S		A		S		A		S		A		S		A	
		I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C
SEDIMENT																																									
OSPAR	Cd	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Hg	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Pb	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Cu	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
MFSD	Cd	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED
	Hg	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Pb	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED
Belgisch Staatsblad	Cd	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Hg	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Pb	GREEN	RED	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	RED	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	Cu	GREEN	GREEN	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
OSPAR	∑7 CB	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	RED	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	pp-DDE	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
MFSD	CB118	RED	RED	RED	RED	RED	GREEN	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	GREEN	RED	GREEN	RED	RED	RED	RED
	CB153	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
Belgisch Staatsblad	CB118	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED
	CB153	RED	RED	RED	RED	RED	GREEN	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	RED	GREEN	RED	RED	RED	RED
	pp-DDE	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN
	pp-DDT	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN	GREEN

[1] OSPAR, Quality Status Report 2000, Region II Greater North Sea

[2] MSFD Marine Strategy Framework Directive, Task Group 8, Contaminants and pollution effects. *Law et al*. 2010.

[3] Belgisch Staatsblad N° 209, vrijdag 9 juli 2010

4.3.2 Macrobenthos

4.3.2.1 Patterns in Benthic characteristics

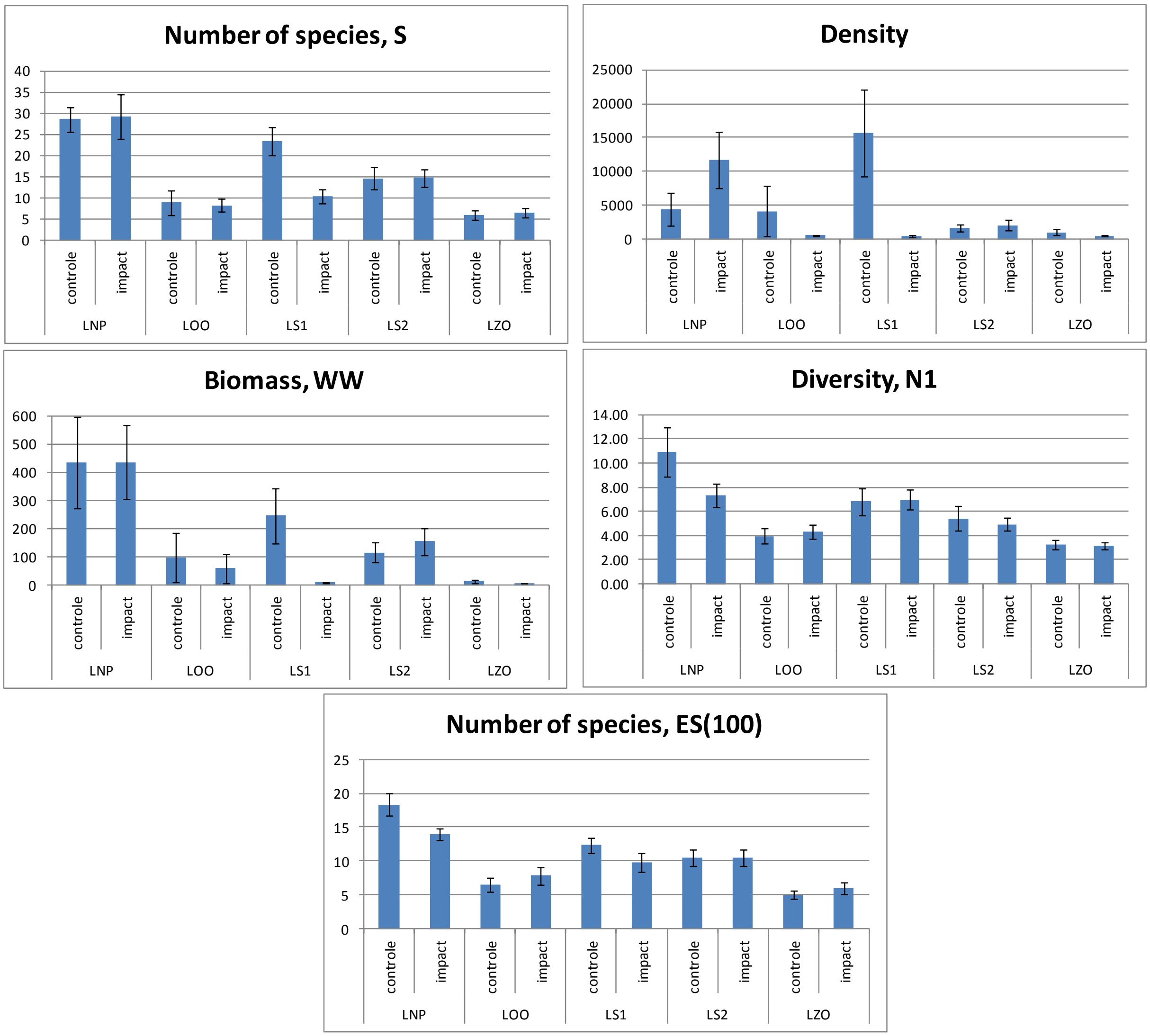


Figure 34. Benthic characteristics at the different dumping sites and near-by control area.

The differences in benthic characteristics (number of species, density, biomass, diversity) between the samples (year 2010) in the impacted and nearby control area for the different dumping areas were visualized in Figure 34. The highest number of species and diversity is recorded at the dumping site Nieuwpoort. Dumping site Br&W Zeebrugge Oost is characterised by the lowest diversity. The density and biomass is highest at dumping site Nieuwpoort, both in impact and control samples. At dumping site Br&W S1, the density and biomass is only high in the control samples. At the other dumping sites, the density and biomass is much lower and not significant different between impact and control samples. Only at site S1, significant difference between impact and control samples are observed for most benthic characteristics, with higher values in the control area.

4.3.2.1.1 NIEUWPOORT

Most samples at the dumping site Nieuwpoort are characterised by a species richness of more than 20 spp/0.1m² (Figure 35). The samples with the highest density are situated in the southern part of the dumping site and exceed 5000 ind/m². The dominant species in these samples is the tube building polychaete *Owenia fusiformis*. Other dominant species are the polychaetes *Heteromastus filiformis* and *Notomastus latericeus* and the bivalve *Abra alba*. The samples in the northwestern part of the dumping site are characterised by a different species composition, dominated by *Scoloplos armiger, Capitella spp., Spiophanes bombyx* and *Nephtys cirrosa*. These species, except *Capitella spp.*, are more characteristic for a more sandy substrate. This difference in species composition and density characteristics is confirmed by the sediment differences at this side (see Figure 16). This pattern is consistent over the samples of the previous monitoring years; which indicates that the observed patterns are probably a natural spatial difference at this site, especially because the influence of the yearly dumping activity is minimal (very low amounts per year) at this site.

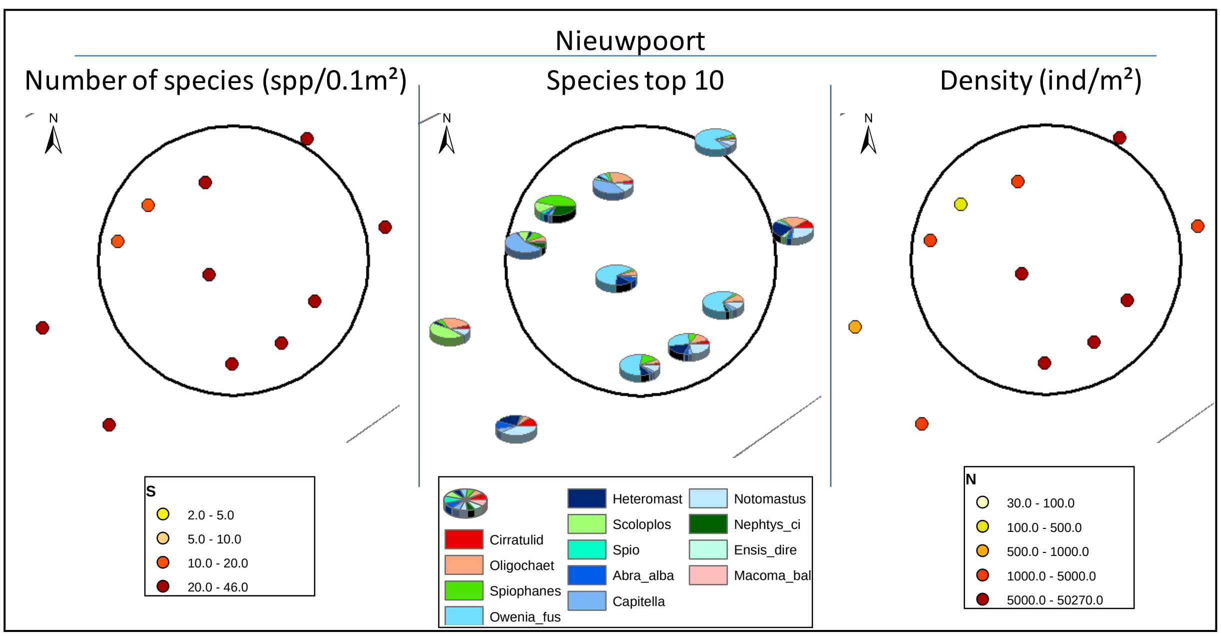


Figure 35. GIS maps of dumping site Nieuwpoort with visualisation of the benthic characteristics (Left: Number of species (spp/0.1m²); Middle: Relative presence of the 10 most abundant species; Right: Density (ind/m²)).

4.3.2.1.2 BR&W OOSTENDE

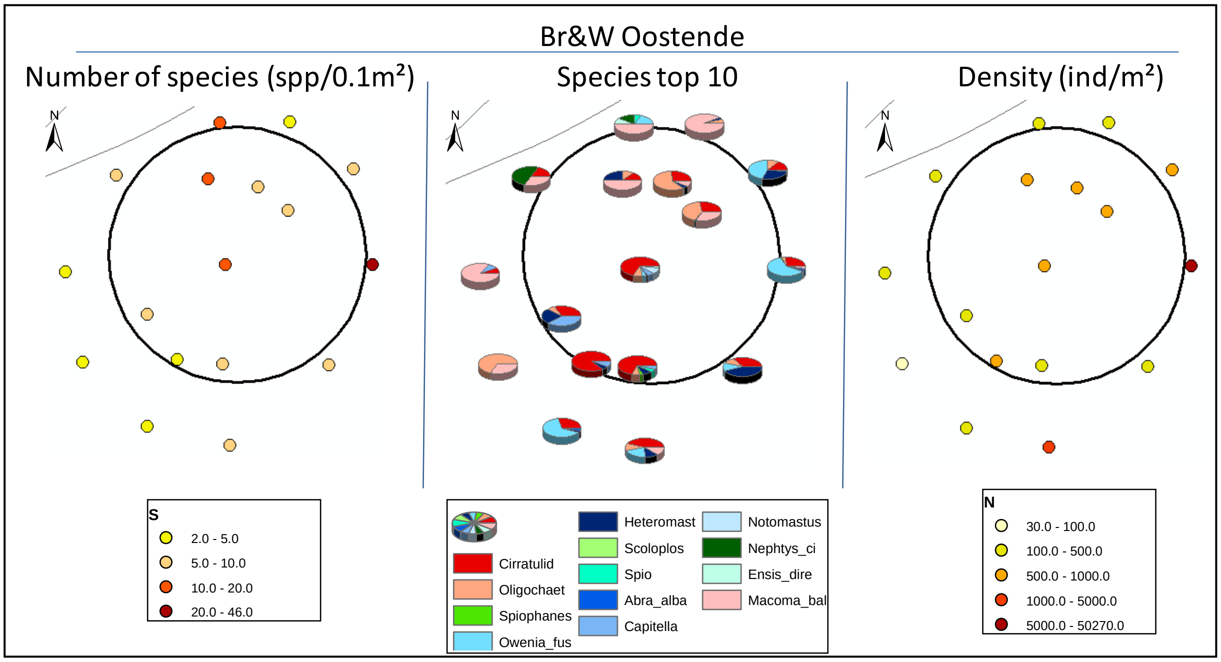


Figure 36. GIS maps of dumping site Br&W Oostende with visualisation of the benthic characteristics (Left: Number of species (spp/0.1m²); Middle: Relative presence of the 10 most abundant species; Right: Density (ind/m²)).

The benthic samples at dumping site Br&W Oostende are characterised by a very low number of species, especially in the southwestern part (Figure 36). The samples in the Northern part are containing a higher species richness. One sample (LOO.14), outside the dumping area in the east is characterised by a high species richness and density, due to the presence of the tube-building polychaete *O. fusiformis*. The most dominant species in and around the dumping site are *Macoma balthica*, *Cirratulidae spp* and *Oligochaeta spp.* Those opportunistic species are very characteristic for muddy sediments. The benthic density of those samples is mainly low, but slightly higher inside the dumping site compared to the samples around the site.

4.3.2.1.3 BR&W S1

The benthic characteristics of the samples in and around the dumping site of Br&W S1 are very different (Figure 37). First, some samples outside the dumping site in the Northeast and one in the Southwest are strongly dominated by young individuals of the tube building polychaete *O. fusiformis* (between the 35410- 46280 ind/m²). This is the highest measured density of this species for the moment on the BPNS. Those samples are also characterised by the highest species richness. Second, the samples within the dumping site are characterised by a variable species richness and species composition. Some samples are characterised by species of sandy substrates, as

Nephtys cirrosa and *Spiophanes bombyx*, whereas others by *Macoma balthica* and *Capitella spp*, which were mud loving species. The mixture of both species, accompanied with *Cirratulida spp, Oligochaeta spp* and *Spio spp.,* is also common. The density of the benthic species within the dumping site is at average very low for these type of sediment. These observations can indicate that this area is really disturbed by the very frequent dumping activity, preventing a sustainable development of a benthic community. Third, the samples outside the dumping area in the south, are characterised by low densities and a moderate species richness. The species composition is mainly determined by *Nephtys cirrosa*, *Spio spp., Spiophanes bombyx* and *Scoloplos armiger*. Finally, the two samples outside the dumping area in the Northwestern part are dominated by *Spiophanes bombyx*.

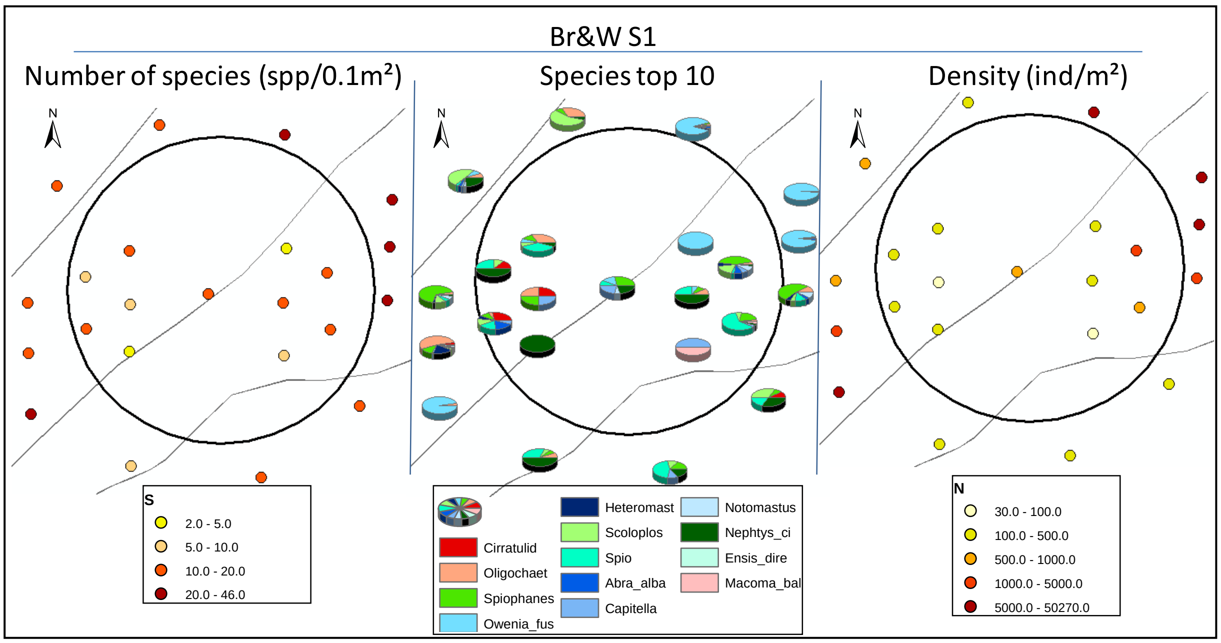


Figure 37. GIS maps of dumping site Br&W S1 with visualisation of the benthic characteristics (Left: Number of species (spp/0.1m²); Middle: Relative presence of the 10 most abundant species; Right: Density (ind/m²)).

There can be concluded that the dumping site Br&W S1 is impacted by the dumping activity, due to the variable and low benthic species numbers and density and the patchy species distribution inside the dumping area. If there is an effect on the surroundings of the dumping area, it is minimal are slightly positive (higher species richness). But the higher species richness in the neighborhood of the dumping site is mainly related to the structuring effect of *O. fusiformis* on the benthic habitat.

4.3.2.1.4 BR&W S2

The samples at dumping site Br&W S2 are characterised by a moderate species richness (between 10 to 20 spp/0.1m²), except at the western tip (Figure 38). This sample is characterised by a very low number of species and density and *Spio* spp. is the dominant species. The density of the samples at the dumping site and its surroundings is very variable. There is an indication that the samples in the western part are characterised by the lowest densities. This coincide with the fact that this part of the dumping side is frequently used over the period 2009-2010 for dumping dredged material. The samples are varying in species composition and follow a clear distribution pattern, with dominance of the bivalve *Ensis directus* in the Southern part and the dominance of the bivalve *Abra alba* in the Northern part. The northern part is also characterised by species characteristic for more muddy conditions, as *Spio spp., Abra alba* and *Cirratulidae spp.* Higher mud content in the sediment samples was not observed in this area, except for one (see Figure 19).

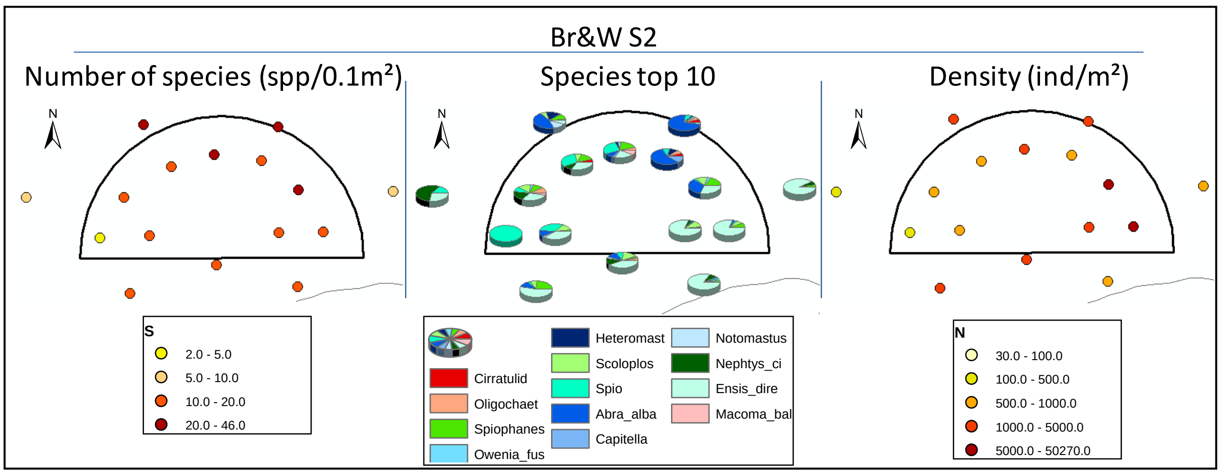


Figure 38. GIS maps of dumping site Br&W S2 with visualisation of the benthic characteristics (Left: Number of species (spp/0.1m²); Middle: Relative presence of the 10 most abundant species; Right: Density (ind/m²)).

4.3.2.1.5 BR&W ZEEBRUGGE OOST

The samples in and around the dumping area of Br&W Zeebrugge Oost are characterised by a low number of species and density (Figure 39). A few samples outside the dumping area has a higher benthic density (> 5000ind/m²). The area is clearly dominated by *Cirratulidae spp*., *Oligochaeta spp* and *Macoma balthica*. In a few samples also the presence of the tube building polychaete *Owenia fusiformis* is observed. At this side, it seems that the dumping area and its surroundings are characterised by the same benthic characteristics. The samples (control + impact) located in the western part of this area tend to be characterised by a lower number of species and density compared to the samples in the eastern part.

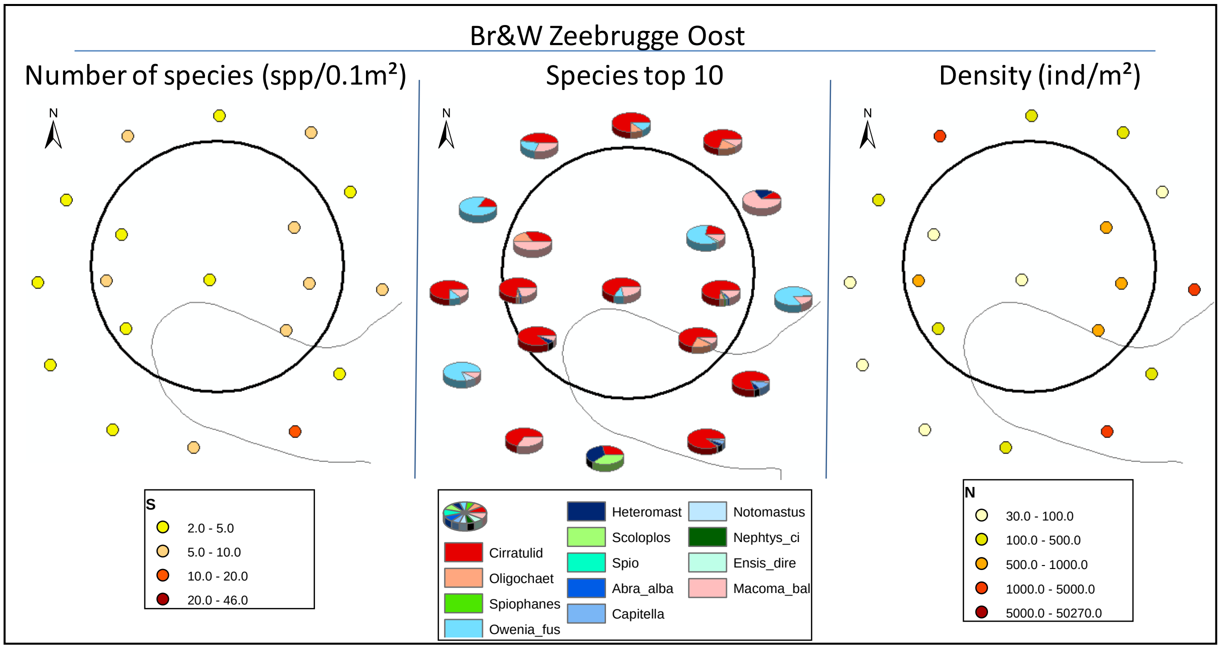


Figure 39. GIS maps of dumping site Br&W Zeebrugge Oost with visualisation of the benthic characteristics (Left: Number of species (spp/0.1m²); Middle: Relative presence of the 10 most abundant species; Right: Density (ind/m²)).

4.3.2.2 Benthic indicator (BEQI)

Table 8. Evaluation of the difference between control and impact samples in autumn 2010 at the five dumping sites for the ecosystem component macrobenthos. The values represent the BEQI-index, the colors the status(blue: high; green: good; yellow: moderate; orange: poor; red: bad).

BEQI	Dumping site Nieuwpoort	Br&W Oostende	Br&W S1	Br&W S2	Br&W Zeebrugge Oost
Density	**0.37**	0.81	**0.06**	**0.75**	0.77
Biomass	**0.97**	0.88	**0.08**	**0.68**	0.76
Similarity	**0.58**	**0.66**	**0.38**	**0.56**	**0.49**
Species	**1**	**0.73**	**0.45**	**1**	**0.74**
Average	**0.73**	0.77	**0.24**	**0.75**	0.68

The ecological status of the macrobenthos at the different disposal sites is evaluated with the benthic indicator BEQI, which weighed the difference in the parameters density, biomass, species composition (Bray-Curtis similarity) and number of species between the set of impact and control samples (Table 8).

Based on the BEQI evaluation, only dumping site Br&W S1, gives a score ‘poor’ for the status of the macrobenthos. This can mainly be attributed to the very low scores for density and biomass, which are much higher in the control samples compared to the impact samples. This difference is caused by the dominance of the tube building polychaete *Owenia fusiformis* in the control samples, with densities up to 46.000 ind/m². At this dumping site, the diversity (number of species) was also lower in the impacted area compared to the surroundings.

On average, the status of the macrobenthos is good at the dumping site Nieuwpoort. The lower score for density and similarity can be attributed to the dominance of *Owenia fusiformis* in the impacted area.

At dumping site Br&W Oostende and Br&W Zeebrugge Oost, the benthic parameters deviate not between the impacted area and the surroundings. The evaluation of the parameters density and biomass is less confident because the high variability between the individual samples. Both areas are characterised by a higher heterogeneity in their sedimentological and biological characteristics.

At dumping site Br&W S2, the macrobenthos status was good, which indicates that there is no significant difference observed between the impact samples and the control samples. Nevertheless, some remarkable differences are observed between the samples. The benthic characteristics of the samples indicate an enrichment of the Northern samples with mud 'loving' species, whereas the samples in the western part are more impoverished (lower diversity). At this side, the invasive alien species *Ensis directus* was rather dominant. If this could be attributed to the higher dumping intensities over the period 2009-2010 than in previous years is not unambiguous. From the dumping intensity maps could be derived that the dumping in 2009 was concentrated in the Northern part, whereas in 2010 it was in the western part.

4.3.2.3 Conclusion

The patterns observed at the five dumping sites in autumn 2010 confirm those of previous years. At the dumping sites Br&W Oostende and Br&W Zeebrugge Oost, naturally characterised by an instable, sandy mud and a poor benthic community, the medium to high dumping intensity does not really affect the benthic life. The variability in the benthic characteristics at dumping site Nieuwpoort is more likely natural, and is not related to the low dumping intensity. The changes in the benthic characteristics at site Br&W S1 are still the result of the loss of *Abra alba* habitat within the dumping area. This side is subjected to the highest dumping intensities over the period 2009-2010, especially in 2009 and contribute to those ecological changes. Despite, the good status at dumping site Br&W S2, some remarkable differences in benthic characteristics were observed between the western and northern part of the side, which can have a link with the dumping activity over the period 2009-2010.

4.3.3 Epibenthos and demersal fish

4.3.3.1 Habitat characterisation

For the sampling campaigns and analyses in the previous years, the position of the impact and control stations have been determined by expert judgement. Also, a number of overall monitoring stations have been assigned as control stations. To know whether those overall monitoring stations actually are appropriate as control stations for the accompanying impact stations, an objective habitat characterisation was conducted. A cluster- and MDS-analysis (see Figure 40) of the epibenthos data revealed five epibenthic clusters on a similarity level of 50%. Cluster 2, 4a and 4b show high affinity with the 'coastal 1'-group defined in Vandendriessche *et al.* (In prep.). The assemblage of this group is characterised by high densities and diversity. Further, cluster 5 corresponds well with the 'coastal 2'-group, which is typified by intermediate densities and diversity. Cluster 1 matches well with the 'coastal 3'-group, which is characterised by low densities and diversity. The density, number of species, most distinctive species and samples per cluster are visualised in Table 9.Table 9. Characteristic epibenthic species and community parameters for the defined clusters.

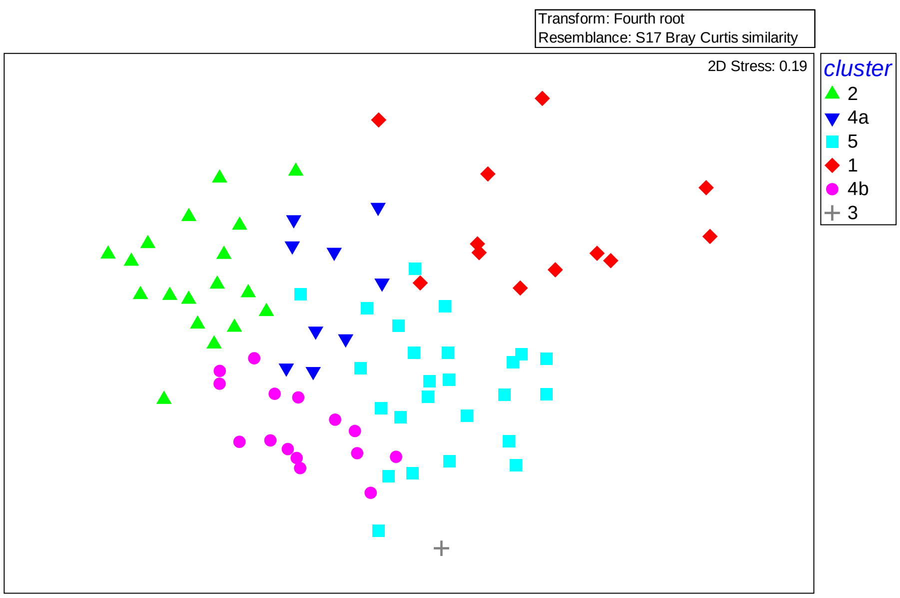


Figure 40. Multidimensional Scaling (MDS) plot of the epibenthic data of 2009-2010 with indication of the different biological clusters.

The habitat characterisation makes it possible to gain a clear insight into the relation between samples and habitat type. The overall monitoring stations and the impact stations which are clustering together are typified by the same habitat type. So, these overall monitoring stations can function as control stations for the corresponding impact stations. Table 10 gives an overview of the dumping sites, impact stations, control stations and overall monitoring stations which function as control stations.

Table 9. Characteristic epibenthic species and community parameters for the defined clusters.

cluster	1	2	3	4a	4b	5
habitat	coastal 3	coastal 1		coastal 1	coastal 1	coastal 2
Density (ind/m²)	89±127	2037±1251	216	1083±810	2357±1392	314±351
Number of species	10	17	7	9	9	9
SIMPER species	*Ophiura ophiura* *Macoma balthica* *Crangon crangon* *Asterias rubens*	*O. ophiura* *C. crangon* *A. rubens* *Ophiura albida*	*C. crangon* *A. rubens* *Liocarcinus holsatus* *Ensis*	*O. ophiura* *A. rubens* *C. crangon* *Pagurus bernhardus*	*C. crangon* *O. ophiura* *A. rubens* *L. holsatus*	*C. crangon* *O. ophiura* *L. holsatus* *A. rubens*
samples	ft140(3) C ft1401(2) I ft1402(2) C ft7001(3) I ft7002(2) C	ft120(3) C ft1401(1) I ft2251(3) I ft2252(3) C 230(1) C ft7801(2) I ft7802(4) C	ft1402(1) C	ft120(2) C ft230(4) C ft7802(1) C ftB03(1) C ft B07(1)C	ft140(1) C ft230(2) C ft7002(1) C ft7101(2) I ft7102(2) C ft7802(1) C ft7803(1) I ftB03(1) C ftB07(2) C ftB10(2) C	ft 140(2) C ft1402(1) C ft7001(2) I ft7002(2) C ft7101(2) I ft7102(2) C ft7803(2) I ft7804(3) I ftB04(5) C ftB07(2) C ftB10(2) C

Table 10. Overview of the impact stations, control stations and overall monitoring stations per dumping site, for epibenthos and demersal fish.

	Nieuwpoort	Br&W Oostende	Br&W Zeebrugge Oost	Br&W S1	Br&W S2
Impact stations	ft2251	ft1401	ft7001	ft7803 ft7804	ft7101
Control station	ft2252	ft1402	ft7002	ft7802	ft7102
Overall monitoring stations	ft120 ft230	ft140	ft140 ftB07 ftB10	ftB04 ft230	ftB04 ftB03

Similar analyses were conducted for the demersal fish data. The patterns in the fish dataset were not explicitly present as in the epibenthic dataset. However, we could distinguish a global consistency between both data sets. Therefore and for practical reasons, the same control stations were applied for both, epibenthos and demersal fish.

4.3.3.2 EPIBENTHOS

4.3.3.2.1 DUMPING SITE NIEUWPOORT

At dumping site Nieuwpoort, several significant differences between control and impact samples could be detected (Figure 41). The parameters density and biomass displayed significantly higher values in the control samples than in the impact samples of autumn 2009. This could be attributed to a much higher density (and biomass) of *Asterias rubens, Ophiura ophiura* and *Crangon crangon* in the control samples. The species *Diogenes pugilator* and *Mytilus edulis* were lacking in the impact samples. The estimated number of species (ES) was significantly higher in the impact area of winter 2009. Species which were present in the impact samples and not in the control samples were *Venerupis senegalensis*, *Euspira pulchella* and *Epitonium clathrus.* This global pattern was also visible in the other years and seasons but could not be statistically established. The diversity index N1 showed also higher values in the impact samples but those differences between control and impact samples were not significant.

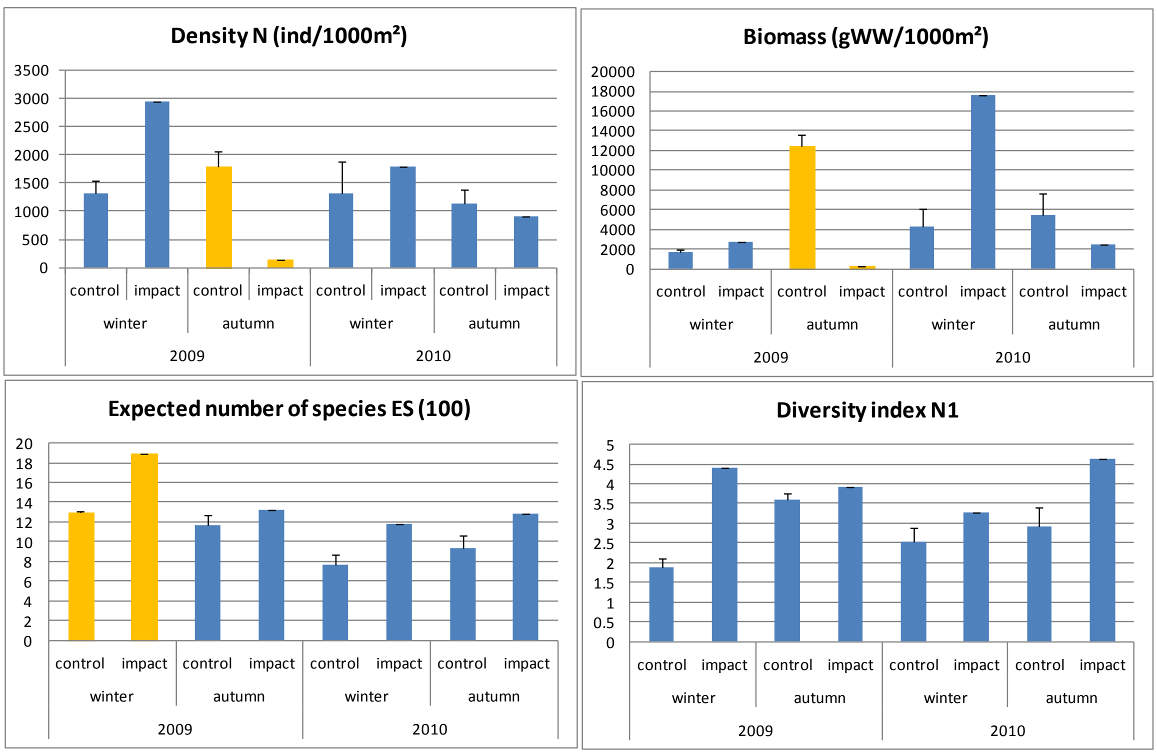


Figure 41. Univariate parameters (density, biomass, expected number of species ES(100) and diversity index N1) characterising the epibenthos in the impact and control area of dumping site Nieuwpoort for the period 2009-2010. Significant differences were highlighted in yellow (Permutational MANOVA).

4.3.3.2.2 BR&W OOSTENDE

At dumping site Br & W Oostende, no impact samples were available in autumn 2010. A significant density difference between control and impact samples was observed in winter 2009 (Figure 42). Particularly *O. ophiura* was responsible for this pattern because of its higher abundance in the control samples. *C. crangon* however, was more common in the impact area. For the parameters biomass, ES(100) and N1, no significant effect of dumping was detected. Nevertheless, there was a higher diversity (ES(100) and N1) in the impact samples of 2009 and a lower diversity in the impact samples of 2010.

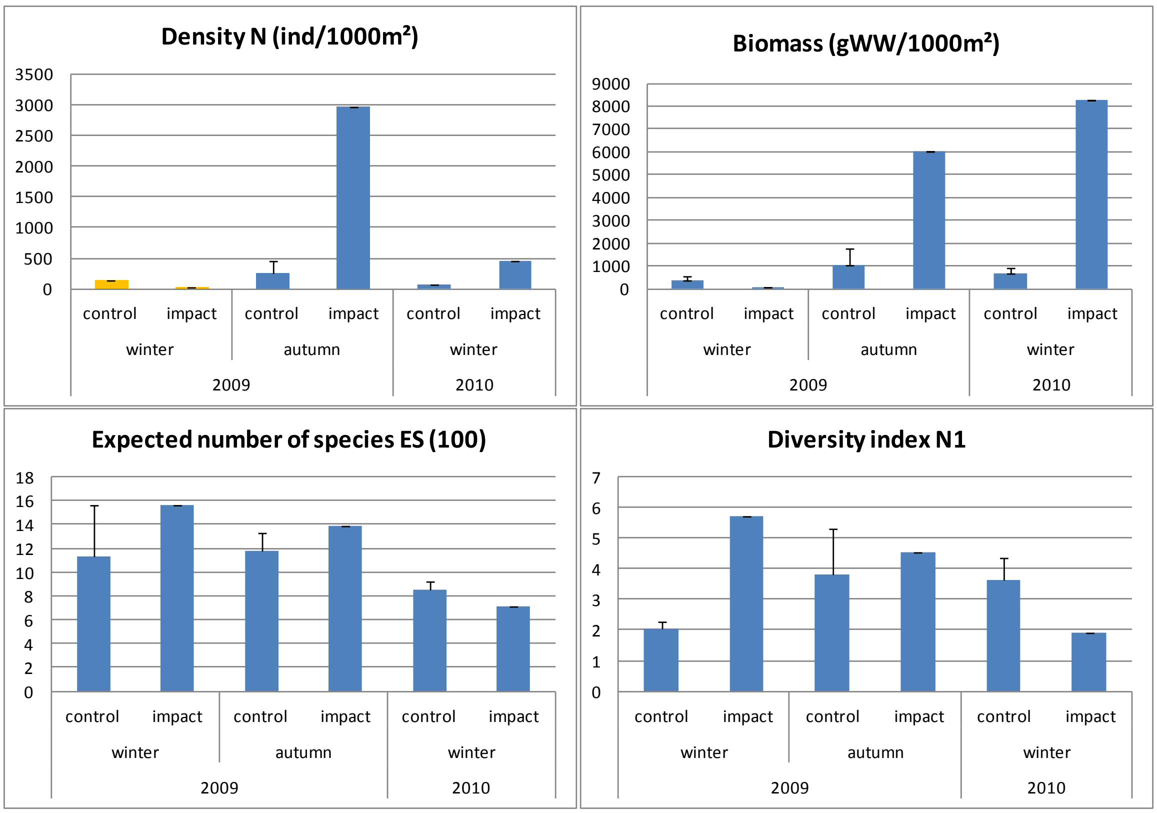


Figure 42. Univariate parameters (density, biomass, expected number of species ES(100) and diversity index N1) characterising the epibenthos in the impact and control area of Br & W Oostende for the period 2009-2010. Significant differences were highlighted in yellow (Permutational MANOVA).

4.3.3.2.3 BR&W S1

For dumping site Br&W S1, no significant effect of dumping on density, biomass, expected number of species and diversity index N1 could be established (Figure 43). However, lower densities and biomass values are visible in the impact areas for the last three seasons. This pattern is mainly caused by the species *O. ophiura* and *A. rubens* which occurred in higher densities in the control areas. In winter 2009, winter 2010 and autumn 2010, the graph of the expected number of species (ES(100)) shows lower values in the impact samples than in the control samples. Species like *Echinocardium cordatum* and *Liocarcinus depurator* were responsible for this pattern because of their absence in the impact samples.

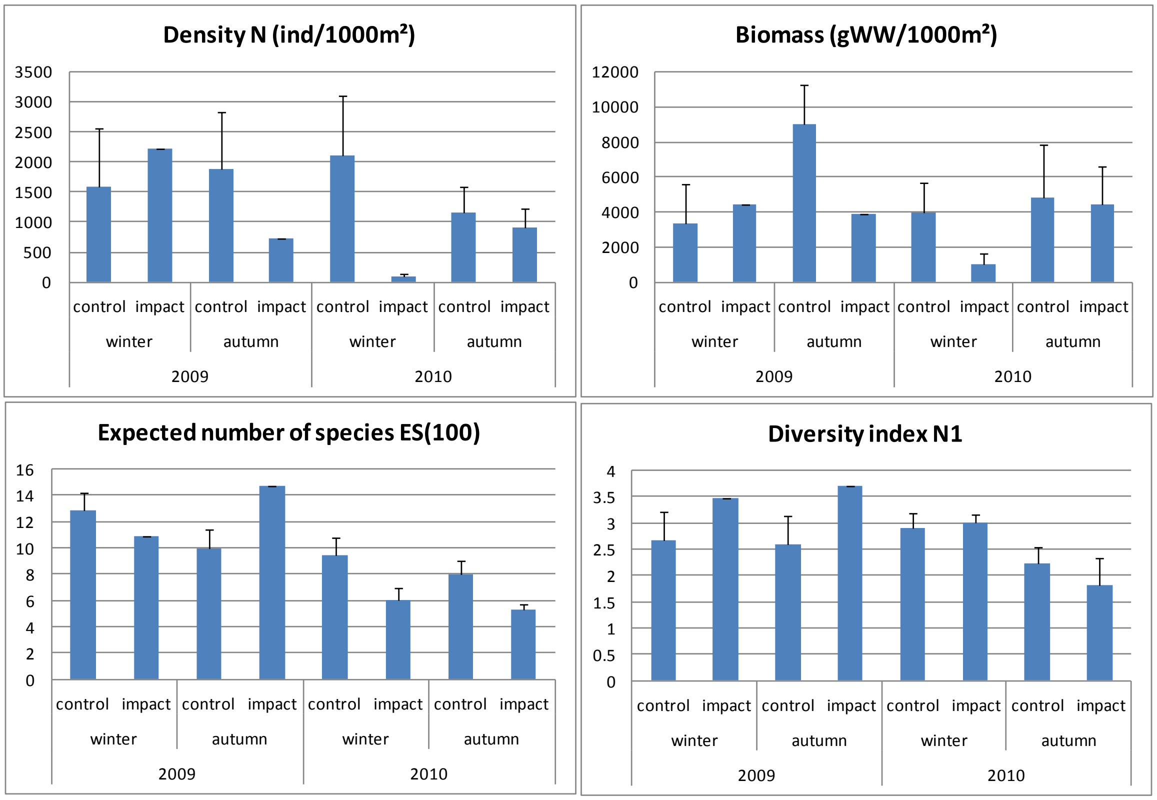


Figure 43. Univariate parameters (density, biomass, expected number of species ES(100) and diversity index N1) characterising the epibenthos in the impact and control area of Br&W S1 for the period 2009-2010.

4.3.3.2.4 BR&W S2

At dumping site Br&W S2, there were no significant effects of dumping on the parameters density, biomass, expected number of species ES(100) and diversity index N1 (Figure 44). But again, slightly lower density and biomass values were recorded in the impact samples. In winter 2009, mainly *C. crangon* and *Ensis sp.* were responsible for the higher control values. In autumn 2009, *L. holsatus* and *A. rubens,* in autumn 2010 *C. crangon* and *O. ophiura* and in winter 2010 *O. ophiura, A. rubens* and *Pagurus bernhardus* were more common species in the control samples than in the impact samples. The graph of the expected number of species and the diversity index N1 revealed no particular pattern.

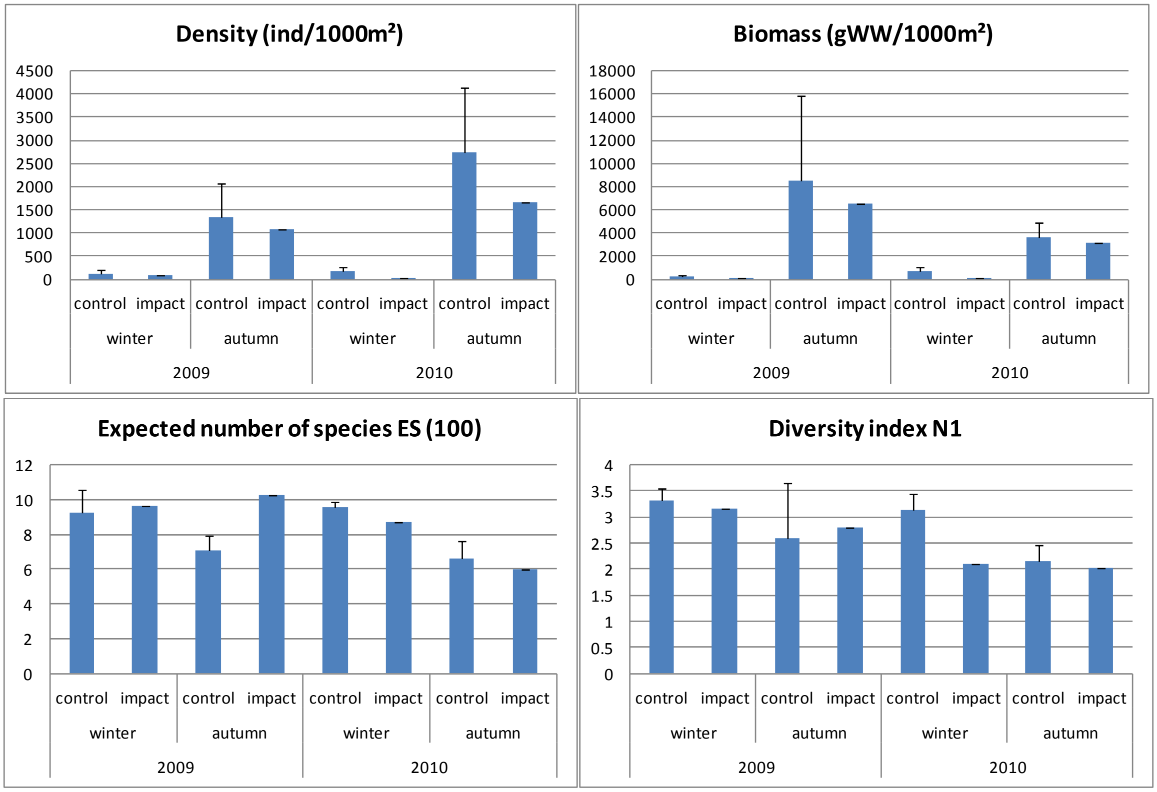


Figure 44. Univariate parameters (density, biomass, expected number of species ES(100) and diversity index N1) characterising the epibenthos in the impact and control area of Br&W S2 for the period 2009-2010.

4.3.3.2.5 BR&W ZEEBRUGGE OOST

In the dumping area Br&W Zeebrugge Oost, only one parameter was significantly affected by the dredging activities, i.e. biomass in winter 2009 (Figure 45). The control area displayed more biomass than the impact area, due to *O. ophiura, C. crangon* and *Macoma balthica* which were more abundant. Although the biomass and density differences between control and impact samples (mainly caused by *A. rubens*) were visibly more explicit in the other seasons (see Figure 45, these differences were not statistically significant. The other parameters ES(100) and N1 were again not influenced by the dredging.

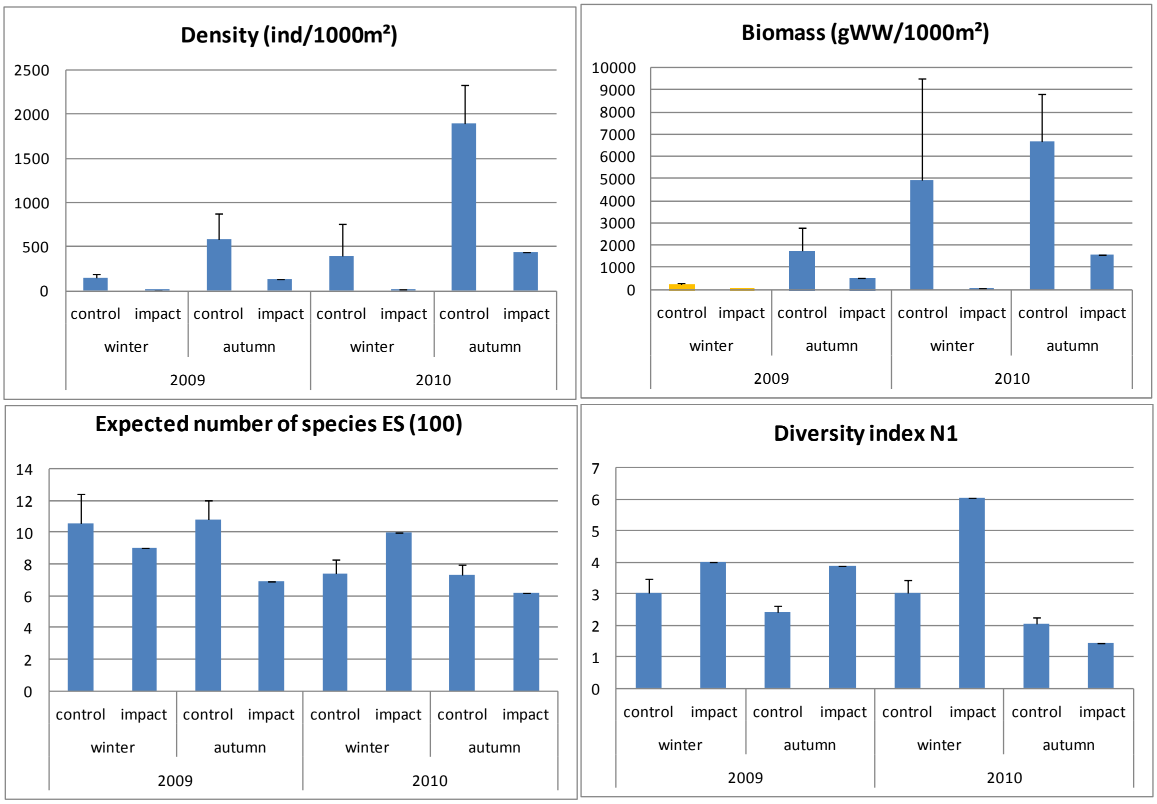


Figure 45. Univariate parameters (density, biomass, expected number of species ES(100) and diversity index N1) characterising the epibenthos in the impact and control area of Br & W Zeebrugge Oost for the period 2009-2010. Significant differences were highlighted in yellow (Permutational MANOVA).

4.3.3.3 DEMERSAL FISH

4.3.3.3.1 DUMPING SITE NIEUWPOORT

In autumn 2009, there was a significant difference between control and impact samples, both for the density and the diversity index N1 (Figure 46). The density of the control samples was higher than in the impact samples, which was mainly due to the fact that *Pomatoschistus sp.*, *Callionymus lyra* and *Merlangius merlangus* were more abundant in the control samples. The diversity index N1 showed significantly higher values in the impact samples of autumn 2009, whereas the expected number of species ES(100) was roughly equal.

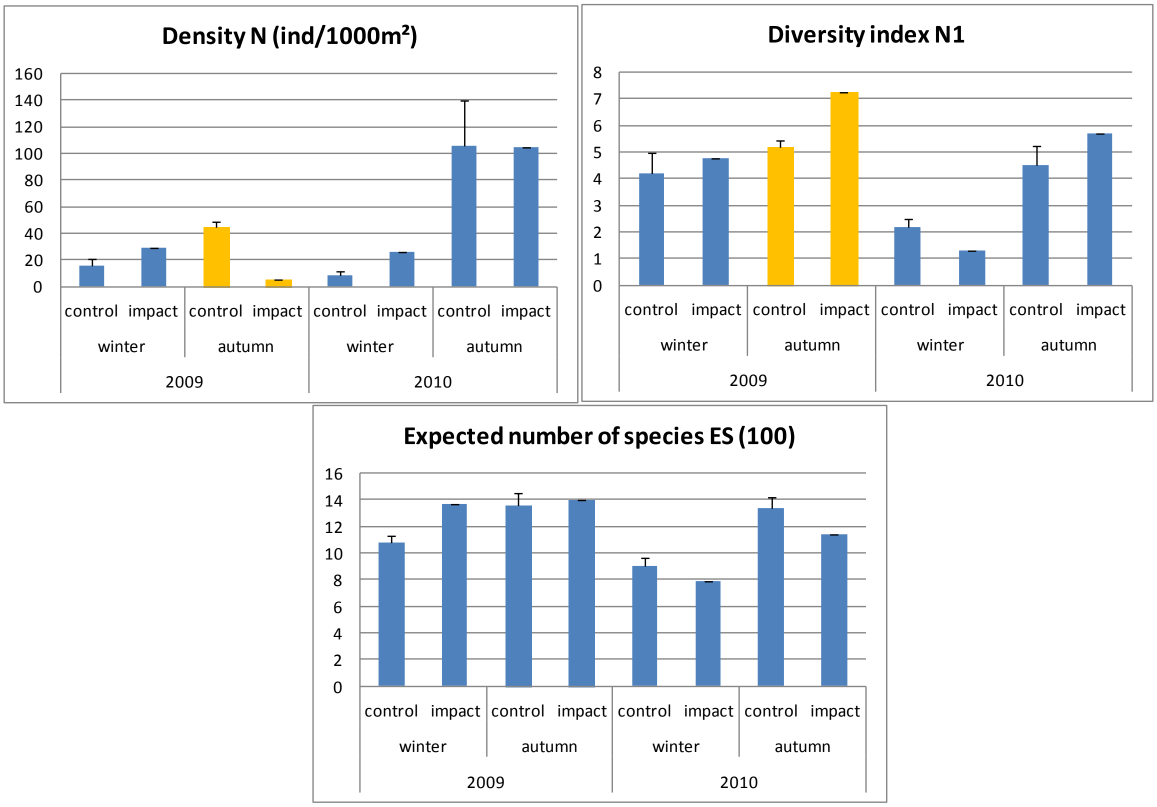


Figure 46. Univariate parameters (density, expected number of species ES(100) and diversity index N1) characterising the demersal fish in the impact and control area of dumping site Nieuwpoort for the period 2009-2010. Significant differences were highlighted in yellow (Permutational MANOVA).

4.3.3.3.2 BR&W OOSTENDE

For dumping site Br & W Oostende, again no impact samples were available in autumn 2010. For the other seasons, a general trend is visible for the three parameters. The higher values in the impact area (except N1 in winter 2010) could mainly be attributed to the higher abundance of *Pomatoschistus sp.* (Figure 47). However, this trend could not statistically be established and therefore is not significant.

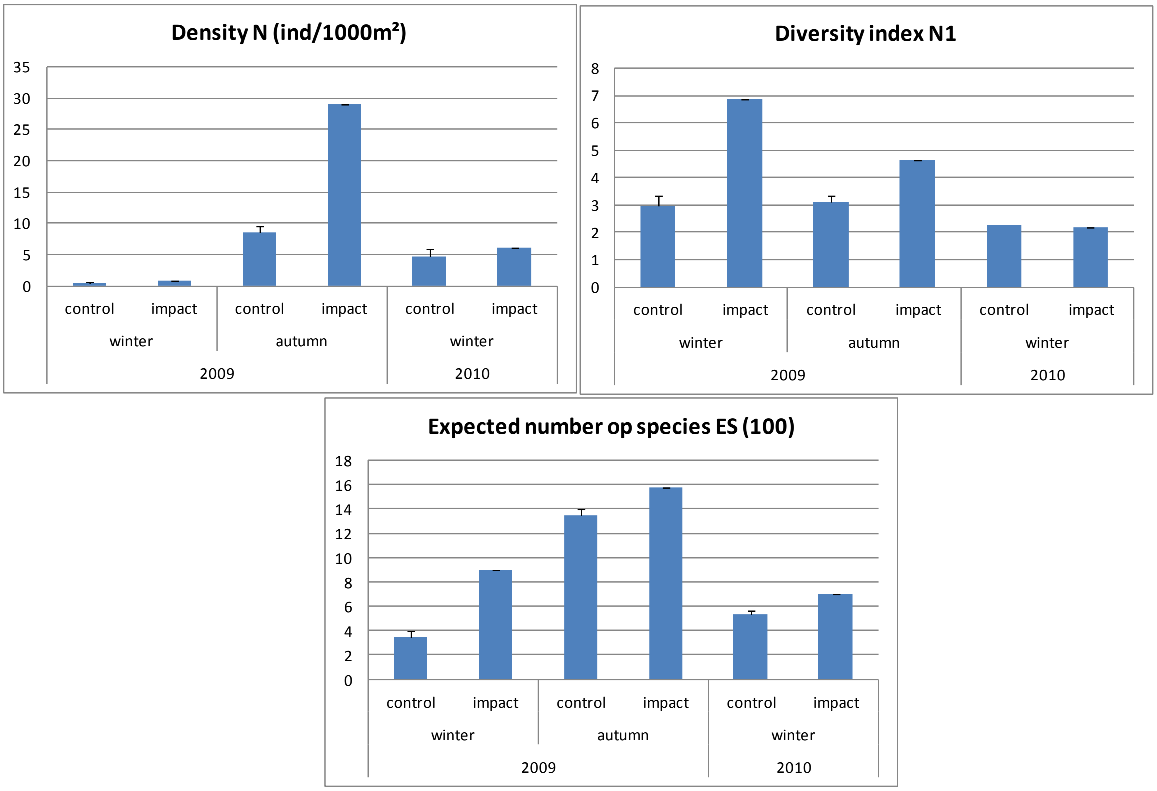


Figure 47. Univariate parameters (density, expected number of species ES(100) and diversity index N1) characterising the demersal fish in the impact and control area of Br & W Oostende for the period 2009-2010.

4.3.3.3.3 BR&W S1

Dumping site Br&W S1 revealed no significant patterns for density and ES(100) (Figure 48). Nevertheless, in autumn 2009, density values were higher in the impact samples due to a higher abundance of *Pleuronectes platessa* and *Limanda Limanda.* In autumn 2010, *Clupea harengus* was responsible for the higher values in the control samples*.* The diversity index N1 showed a significant difference between the control and impact samples of winter 2009, with higher diversity values in the impact area.

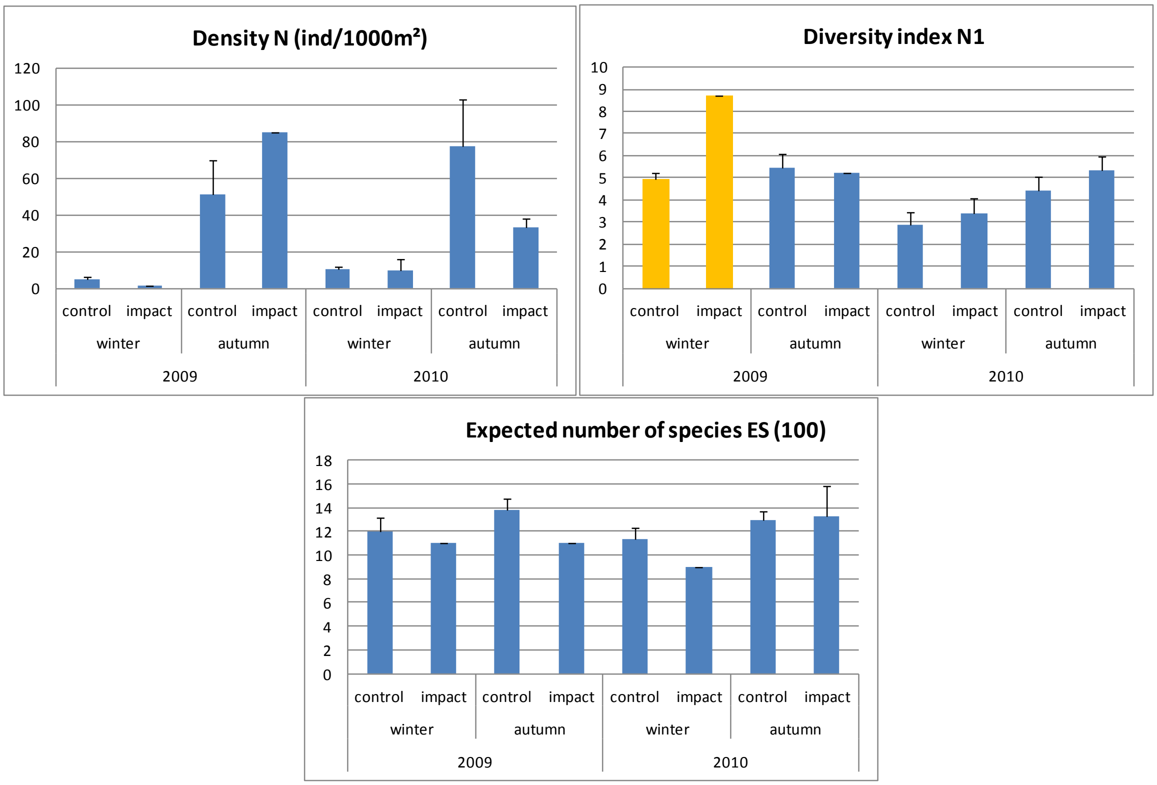


Figure 48. Univariate parameters (density, expected number of species ES(100) and diversity index N1) characterising the demersal fish in the impact and control area of Br&W S1 for the period 2009-2010. Significant differences were highlighted in yellow (Permutational MANOVA).

4.3.3.3.4 BR&W S2

Analogously with epibenthos, demersal fish was not significantly affected by the dredging activities at Br&W S2 (Figure 49). Nevertheless, the values for density and ES(100) were consequently lower in the impact area, except for the density in winter 2010. A species that was present in the control samples and not in the impact samples, in autumn was *Sprattus sprattus.* In winter, the absence of *C. harengus* and *M. merlangus* in the impact samples was notable.

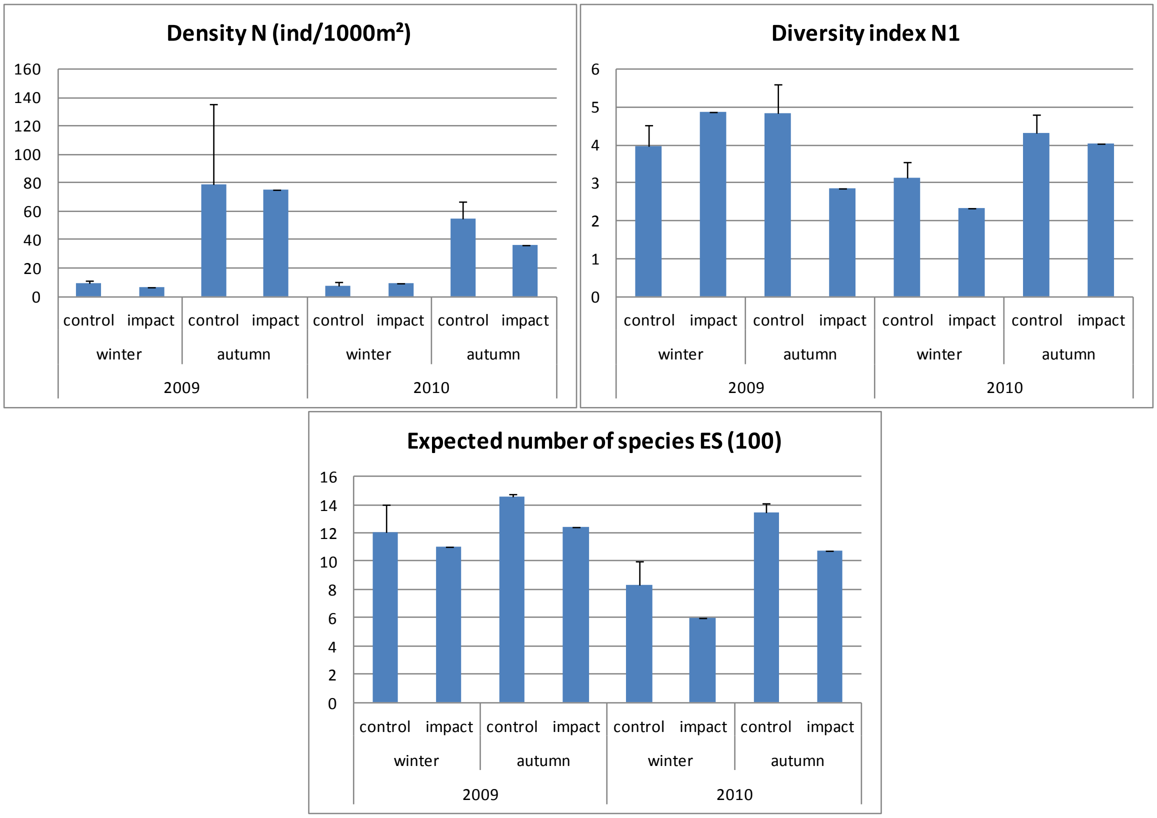


Figure 49. Univariate parameters (density, expected number of species ES(100) and diversity index N1) characterising the demersal fish in the impact and control area of Br&W S2 for the period 2009-2010.

4.3.3.3.5 BR&W ZEEBRUGGE OOST

The demersal density values differed significantly in autumn 2010, mainly due to the higher occurence of *Pomatoschistus sp.*, *Solea solea* and *P. platessa* in the control samples (Figure 50). The species *L. Limanda, Chelidonichthys lucernus* and *Callionymus lyra* were even lacking in the impact samples. In general, the diversity index N1 was higher in the impact area than in the control area but only in winter 2009 this difference was statistically significant.

Figure 50. Univariate parameters (density, expected number of species ES(100) and diversity index N1) characterising the demersal fish in the impact and control area of Br & W Zeebrugge Oost for the period 2009-2010. Significant differences were highlighted in yellow (Permutational MANOVA).

4.3.3.4 Conclusion Epibenthos and demersal fish

The ecological status of the epibenthos and demersal fish was also evaluated for the five dumping sites. To know whether dumping activities had an impact on density, biomass, estimated number of species (ES) and diversity index N1, a Permutational MANOVA-analysis was conducted.

At dumping site Nieuwpoort, several significant differences between control and impact samples could be detected. Concerning the epibenthos, the parameters density and biomass displayed significantly higher values in the control samples than in the impact samples of autumn 2009. This could be attributed to a much higher density (and biomass) of the starfish *Asterias rubens* in the control samples. The estimated number of species (ES) was significantly higher in the impact samples of winter 2009. This pattern was also visible in the other years and seasons but could not be statistically established. For the demersal fish, there was a significant difference between control and impact samples of autumn 2009, both for the density and the diversity index N1. The density of the control samples was higher than the impact samples, which was mainly due to the fact that gobies (*Pomatoschistus sp.*), dragonet (*Callionymus lyra*) and whiting (*Merlangius merlangus*) were more abundant in the control samples. The diversity index N1 showed higher values in the impact samples.

Table 11. Evaluation of the differences between control and impact samples in the period 2009-2010, for the five dumping sites and for the ecosystem components epibenthos and demersal fish. Red = significant difference; green = no significant difference.

	Dumping site Nieuwpoort				Br&W Oostende				Br&W S1				Br&W S2				Br&W Zeebrugge Oost			
	2009		2010		2009		2010		2009		2010		2009		2010		2009		2010	
	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A
EPIBENTHOS																				
Density	Green	Red	Green	Green	Red	Green	Green		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Biomass	Green	Red	Green	Green	Green	Green	Green		Green	Green	Green	Green	Green	Green	Green	Green	Red	Green	Green	Green
ES	Red	Green	Green	Green	Green	Green	Green		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
N1	Green	Green	Green	Green	Green	Green	Green		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DEMERSAL FISH																				
Density	Green	Red	Green	Green	Green	Green	Green		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red
ES	Green	Green	Green	Green	Green	Green	Green		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
N1	Green	Red	Green	Green	Green	Green	Green		Red	Green	Green	Green	Green	Green	Green	Green	Red	Green	Green	Green

In the dumping area of Br&W Oostende, a significant epibenthic density difference between control and impact samples was observed in the winter of 2009. Particularly the brittlestar *Ophiura ophiura* was responsible for this pattern, because of its higher abundance in the control samples. The demersal fish community showed no conspicuous changes as a result of the dumping activities.

Dumping sites Br&W S1 and Br&W S2 revealed no appreciable effects of dumping on epibenthos and demersal fish. The values of density, biomass and species richness were in most cases lower (but not significant) in the impact area than in the control area. Species like starfish *A. rubens* and brittlestar *O. ophiura* are present in lower densities in the impact area. The heart urchin *Echinocardium cordatum* and the blue-leg swimming-crab *Liocarcinus depurator* were even absent in the impact area. For demersal fish however, the diversity index N1 was significantly higher in the Br&W S1 impact samples in winter 2009.

For dumping site Br&W Zeebrugge Oost, the epibenthic biomass data showed a significant difference between the control and impact samples in winter 2009. Again, this difference could be explained by a higher abundance of the brittlestar *O. ophiura* in the control samples. The demersal density values differed significantly in autumn 2010, mainly due to the higher occurence of gobies (*Pomatoschistus sp.)* in the control samples. The diversity index N1 was significantly higher in the impact zone in winter 2009.

There is no clearly visible effect of dumping on the epibenthic and demersal fish fauna. This can mainly be attributed to the fact that most species have a high mobility and are able to avoid the dumping stress. The significant p-values (Table 11) can be explained by the temporary dominance of certain species (starfish, brittlestar, goby) and/or by the natural variability of the habitat (e.g. dumping site Nieuwpoort). Based on this analysis, a small effect of dumping on the higher trophic level of epibenthos and demersal fish cannot be excluded, because the number of samples per sampling event is too low to have sufficient statistical power. A way to reach a higher power is by combining data of several sampling events, which is not possible over the period 2009-2010 due to a switch in sampling strategy. Since dumping had no or a minor effect on the epibenthos and demersal fish community, it is advisable to investigate the effect of dumping on certain functional or sensitive species.

4.3.4 Chemical contamination in biota

4.3.4.1 Introduction

For chemical analysis, species of interest must be limited in mobility, sufficiently present in the catch, important in the ecosystem and potential accumulators of pollutants. Clams, starfish, sea anemone, several crustaceans (brown shrimp, swimming crab and hermit crab) and fish (dragonet, goby and hooknose) are selected. In general, bivalves show the highest concentrations of persistent organic pollutants (POPs), due to filter feeding, the lack of sophisticated detoxification pathways and their living inside the sediment. Due to the inconsistent occurrence of bivalve species on the different dredge spoil desposit areas, those results were not presented in graphs, only discussed within the text.

The abundance of certain species influenced the reporting approach on the dumping sites. Depending on the sample content on each dumping site, specific key species are selected. Different species were not pooled. In this case, the results are not influenced by the abundance or absence of certain species. Mainly data from autumn 2009 and autumn 2010 are reported. The most abundant key species are starfish and brown shrimp, which will be reported more frequently compared to other species.

Although results are not presented in detail, all datasets have been screened, in order to exclude unambiguous results. For chemical analysis, biota species must be alive on the specific locations. The absence of the species on certain spots is indicative for disturbance, although that does not mean the sludge poses a risk from the chemical point of view. As stated in the Materials and Methods section, results of analysis are expressed on a wet weight (w.w.) basis.

Concerning the contamination by PAHs, the results are shown as sum of 16 (US EPA) PAHs as proposed by the OSPAR guidelines (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, triphenylene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno (1,2,3-cd)pyrene).

Metals analyzed comprise Fe, Cr, Zn and Cu (in µg/g) and Cd, Pb and Hg (in µg/kg), all expressed on a wet weight (w.w.) basis.

CBs are always present in measurable quantities, and results are shown as sum of 10 CBs (IUPAC numbers 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180). Pesticide concentrations are quite low, except for DDT and breakdown products DDE and DDD (only pp' isomers), presented as sum of DDT. Values for alpha-HCH and gamma-HCH are close to or below LOD values, the same holds for transnonachlor and HCB. Aldrin, dieldrin and endrin were never detected.

4.3.4.2 Dumping site BR&W Zeebrugge Oost

4.3.4.2.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

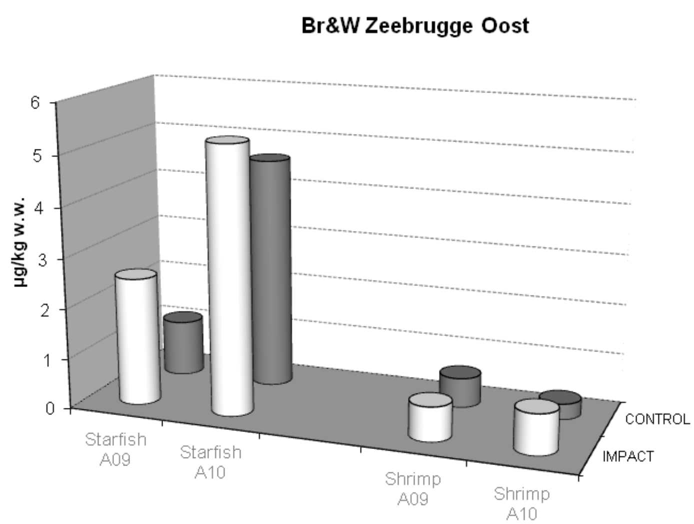


Figure 51. Concentration of PAHs (sum of 16 EPA PAHs) in starfish and brown shrimp during autumn 2009 and autumn 2010 on Impact and Control sites of dumping area Br&W Zeebrugge Oost. Concentrations are expressed in µg/kg w.w. Impact and Control are presented for each data set. (A09: autumn 2009, A10: autumn 2010)

Highest levels of PAHs are definitely found in bivalves, more specific in the bivalve species *Macoma balthica* (17-29 µg/kg w.w.). Results on bivalves are not presented due to small sampling sizes and the occurrence of diverse bivalve species. Figure 51 represents the PAH content in starfish and shrimp during autumn 2009 and 2010, for control and impact sites. Starfish accumulate more PAHs compared to the brown shrimp. For each data set, somewhat elevated levels are found in the key species of impact sites compared to those on the control sites.

4.3.4.2.2 CHLORINATED BIPHENYLS (CB)

Figure 52 shows an analogue graph for the PCB content of brown shrimp and starfish. In agreement with PAHs, starfish tend to accumulate more CBs compared to brown shrimp.

In disagreement with the levels of CBs on sediments of dumping site Br&W Zeebrugge Oost, the CB levels are not elevated in the impact areas compared to the control areas. The levels of pesticide content of both species on this dumping site describe a similar pattern. The highest concentrations CBs (>60 µg/kg w.w.) and DDTs (>2 µg/kg w.w.) were found in hermit crab during autumn 2009. For each dumping area, the highest CB and DDT levels are found in hermit crab.

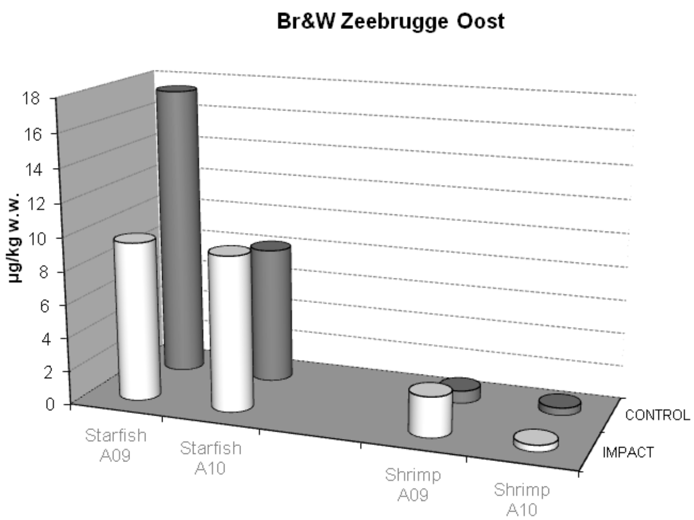
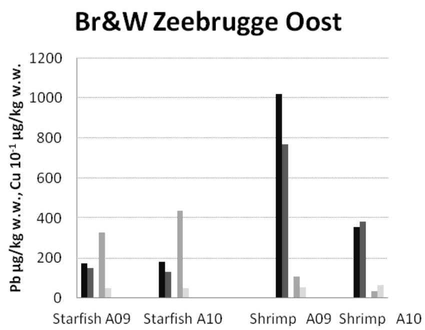


Figure 52. Concentration of CBs (sum of 10 CBs) in starfish and brown shrimp during autumn 2009 and autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control are presented for each data set on the dumping site Br&W Zeebrugge Oost.

4.3.4.2.3 HEAVY METALS

In the sampled sediments of dumping area Br&W Zeebrugge Oost, metal concentrations were elevated at impact sites compared to the control sites. This is reflected by the Cu and Pb concentrations in biota (Figure 53 A). Contrary, Cd and Hg levels in control areas equal or slightly exceed the levels in impact areas (Figure 53 B).



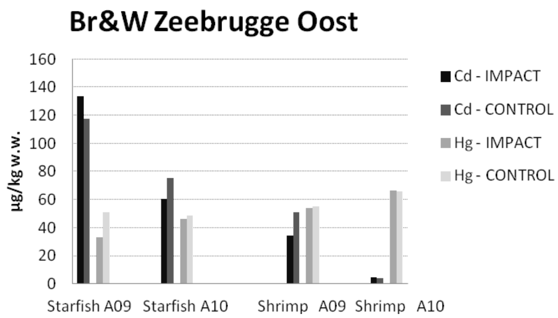


Figure 53. A) Cu and Pb concentration in starfish and brown shrimp during autumn 2009 and autumn 2010 on dumping site Br&W Zeebrugge Oost. Concentrations are expressed in µg/kg w.w. for Pb and in 10-1 µg/kg for Cu.; B) Cd and Hg concentration in starfish and brown shrimp during autumn 2009 and autumn 2010 on dumping site Br&W Zeebrugge Oost. Concentrations are expressed in µg/kg w.w. Impact and Control are presented for each data set.

4.3.4.3 Dumping site BR&W S1

4.3.4.3.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

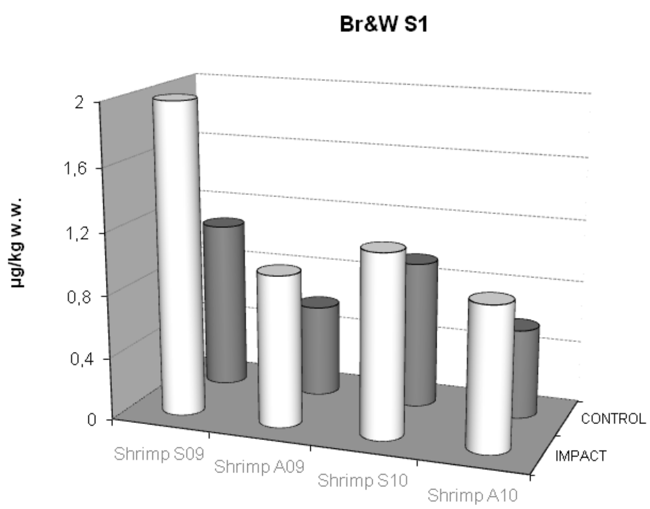


Figure 54. Concentration of PAHs (sum of 16 EPA PAHs) in brown shrimp during the period 2009-2010. Concentrations are expressed in µg/kg w.w. Impact and Control areas of dredge deposit area Br&W S1 are presented for each season. (S09: spring 2009, S10: spring 2010).

On dredge deposit site Br&W S1, the levels of PAH in diverse marine biota species are elevated on impact areas compared to control areas. This is reflected in Figure 54 via the level of PAHs in brown shrimp. This trend was also observed for other species, e.g. starfish. PAH concentrations in epibenthos species show a seasonal variability with higher values during spring. On dumping site Br&W S1, the bivalves *Spisula spp.* are sampled, which contain PAH concentrations up to 35 µg/kg w.w.

4.3.4.3.2 CHLORINATED BIPHENYLS (CB)

While de CB levels in sediments of Br&W S1 were elevated at control areas, no difference was observed in the CB levels of brown shrimp (Figure 55). A remarkably low level of CBs was observed in brown shrimp during autumn 2010. Due to small sampling sizes, no conclusion on seasonal variation could be formulated for the additional sampled species.

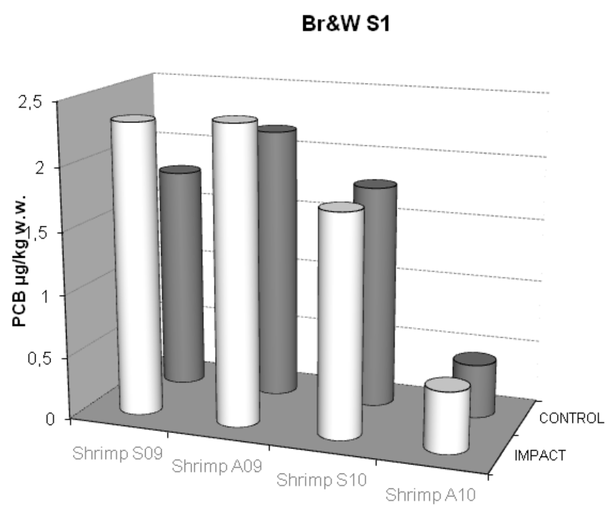


Figure 55. Concentration of CBs (sum of 10 CBs) in brown shrimp during the period 2009-2010. Concentrations are expressed in µg/kg w.w. Impact and Control areas of dredge deposit area Br&W S1 are presented for each season.

4.3.4.3.3 HEAVY METALS

Figure 56 demonstrates the uncommonly high copper content in proportion to the Pb level in biota samples of dredge deposit area Br&W S1, e.g. brown shrimp. This is definitely not the case in sediment samples, where the level of Pb exceeds the level of Cu. Generally, the recovered levels of heavy metals in biota samples are much lower compared to the levels of sediment samples. Brown shrimp tends to accumulate more Cu in proportion to Pb, and more Hg in proportion to Cd (Figure 56). It must be mentioned that the observed Cu-level in biota on Br&W S1 is higher compared to other dumping sites. No trend between impact and control sites is observed.

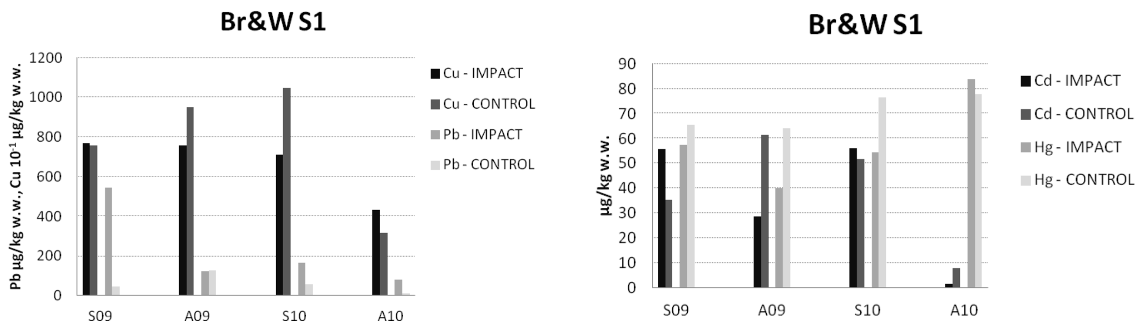


Figure 56. A) Cu and Pb concentration in brown shrimp during the period 2009-2010. Concentrations are expressed in µg/kg w.w. for Pb and in 10-1 µg/kg for Cu; B) Cd and Hg concentration in brown shrimp during the period 2009-2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping site Br&W S1 are presented for each season.

4.3.4.4 Dumping site BR&W S2

4.3.4.4.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

For all sampled species, no unambigous trend was observed between impact and control assessment of the levels of PAHs (Figure 57). For example on starfish during spring 2010, impact was elevated compared to control, while the opposite observation was noted during autumn 2010. A similar pattern was observed for brown shrimp. Figure 57 and other collected data suggest the higher accumulation of PAHs in the order: Bivalves >> Anemone > Hermit Crab > Starfish > Goby > Shrimp.

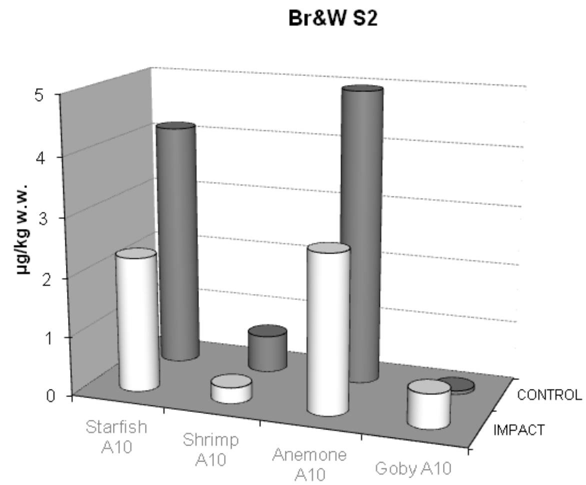


Figure 57. Concentration of PAHs (sum of 16 EPA PAHs) in 4 different species: starfish, brown shrimp, anemone and goby during autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping site Br&W S2 are presented for each data set.

4.3.4.4.2 CHLORINATED BIPHENYLS (CB)

The CB content at impact sites at Br&W S2 was not higher than at control areas, in agreement with the results on sediments of dredge deposit area Br&W S2. The same observations could be made for the pesticide levels in biota on Br&W S2. Figure 58 illustrates the CB levels in different biota species on dumping site Br&W S2 during autumn 2010.

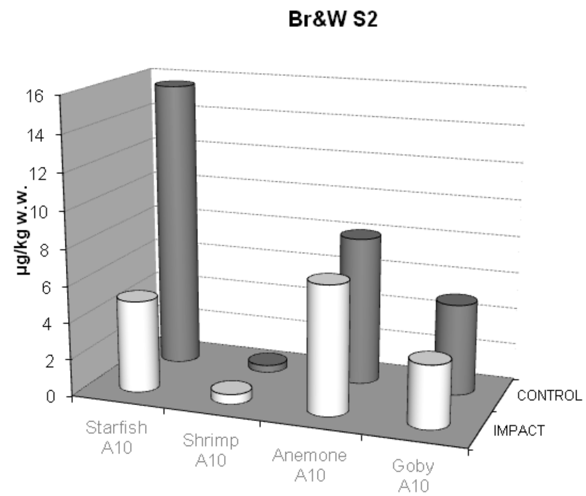


Figure 58. Concentration of CBs (sum of 10 CBs) in 4 different species: starfish, brown shrimp, anemone and goby during autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control are presented for each data set.

4.3.4.4.3 HEAVY METALS

In biota species of dumping site Br&W S2, no difference between the level of heavy metals on control and impact sites could be observed and no trend could be noticed (Figure 59).

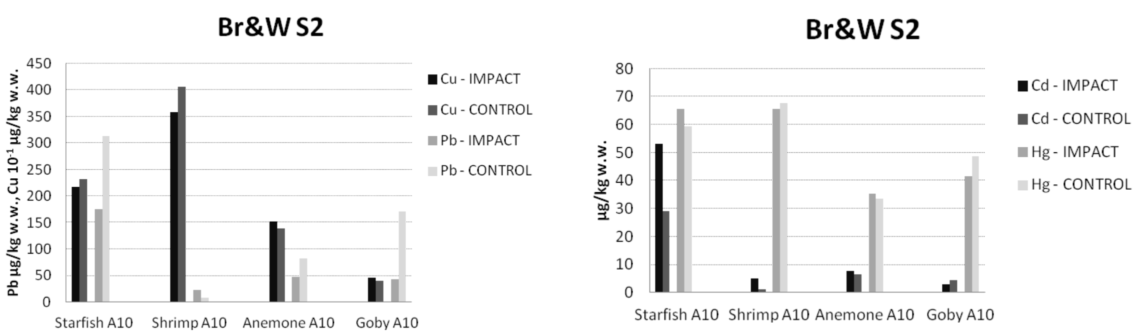


Figure 59. A) Cu and Pb concentration in starfish, brown shrimp, anemone and goby during autumn 2010. Concentrations are expressed in µg/kg w.w. for Pb and in 10-1 µg/kg for Cu; B) Cd and Hg concentration in starfish, brown shrimp, anemone, goby during autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of Br&W S2 are presented for each season.

4.3.4.5 Dumping site BR&W Oostende

4.3.4.5.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

Elevated levels of PAHs are observed on anemone, shrimp and goby on impact sites of dumping site Br&W Oostende (Figure 60). The highest PAH content noted in the period 2009-2010 was observed in *Macoma balthica* during spring 2009 on the impact area of dredge deposit area Br&W Oostende (48.5 µg/kg w.w.).

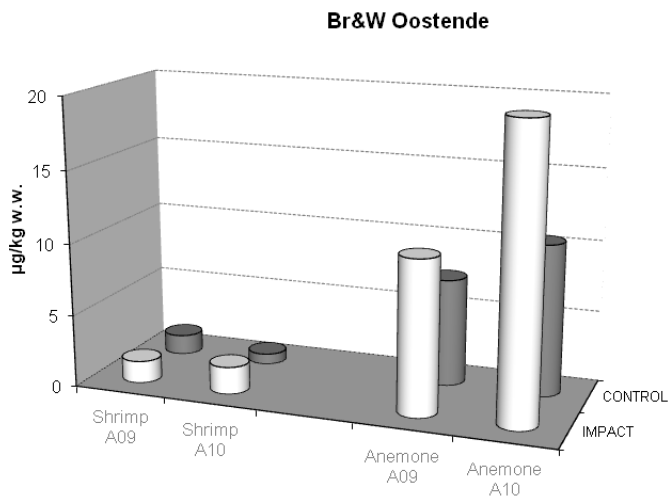


Figure 60. Concentration of PAHs (sum of 16 EPA PAHs) in brown shrimp and anemone during autumn 2009 and autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping site Br&W Oostende are presented for each data set.

4.3.4.5.2 CHLORINATED BIPHENYLS (CB)

In sediment, no differences between CB concentrations at impact and control areas of dumping site Br&W Oostende were noted. For CB levels in biota, starfish and goby reveal higher concentrations on impact sites of Br&W Oostende while for brown shrimp and anemone, impact levels equal control levels (Figure 61). Analogue observations are made for the DDT levels in the different biota species.

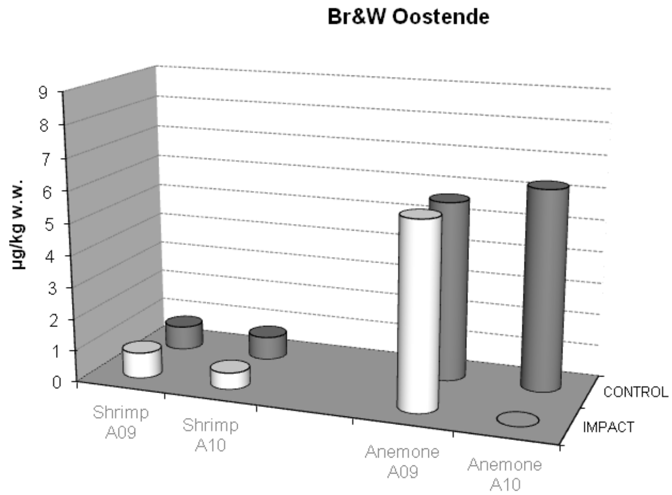
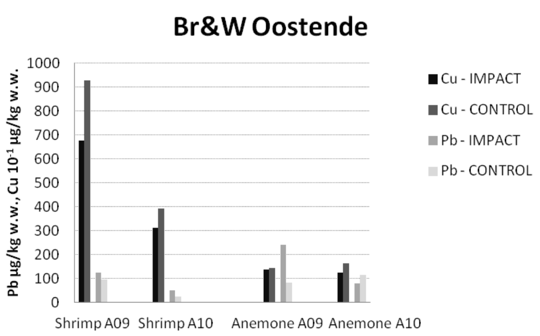


Figure 61. Concentration of CBs (sum of 10 CBs) in brown shrimp and anemone during autumn 2009 and autumn 2010. Due to extraction difficulties, no anemone sample was analysed for the impact site in autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dredge deposit area Br&W Oostende are presented.

4.3.4.5.3 HEAVY METALS

In contrast to the results on sediment, no elevated metal levels in biota are observed on the impact sites of Br&W Oostende compared to control sites (Figure 62).



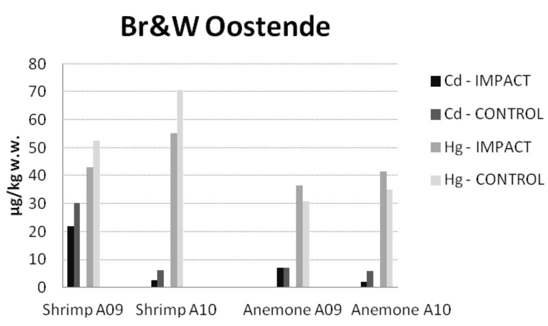


Figure 62. A) Cu and Pb concentration in brown shrimp and sea anemone during autumn 2009 and autumn 2010. Concentrations are expressed in µg/kg w.w. for Pb and in 10-1 µg/kg for Cu.; B) Cd and Hg concentration in brown shrimp and sea anemone during autumn 2009 and autumn 2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping area Br&W Oostende are presented for each data set.

4.3.4.6 Dumping site BR&W Nieuwpoort

4.3.4.6.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

No trend was observed in the PAH content of biota on impact and control sites of dumping area Nieuwpoort. Figure 63 represents the PAH level in starfish during the period 2009-2010. No clear seasonal variation was observed.

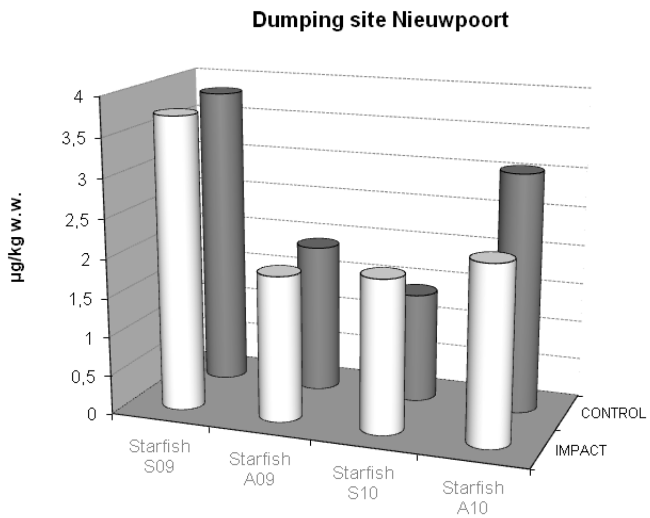


Figure 63. Concentration of PAHs (sum of 16 EPA PAHs) in starfish during the period 2009-2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping area Nieuwpoort are presented for each season.

4.3.4.6.2 CHLORINATED BIPHENYLS (CB)

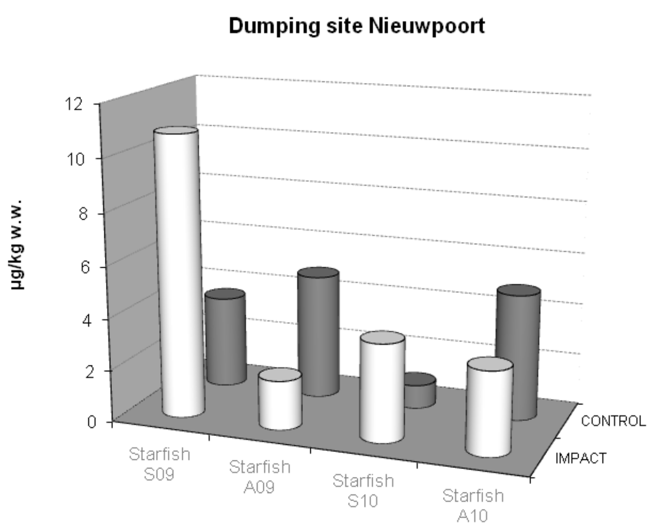


Figure 64. Concentration of CBs (sum of 10 CBs) in starfish during the period 2009-2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping area Nieuwpoort are presented for each season.

Though relatively high CB and DDT concentrations are found in the sediment of dumping area Nieuwpoort, CB and DDT levels in biota are not exceptionally high compared to the other dumping sites. A seasonal variation of CB and DDT levels is established (Figure 64). In contrast to the higher impact levels on sediment of dumping site Nieuwpoort during autumn 2009 and 2010, impact sites did not reveal higher CB levels in biota compared to control sites (Figure 64).

4.3.4.6.3 HEAVY METALS

Only the cadmium concentration in starfish was elevated in impact areas of dumping area Nieuwpoort compared to the control areas (Figure 65). Especially for Cd, a seasonal variation is observed in agreement with the results on sediments of dumping area Nieuwpoort. The Cd level was exceptionally high on impact sites during spring 2009. The Cu level in starfish caught on impact sites during spring 2010 was higher compared to the control sites and compared to the other sampling periods.

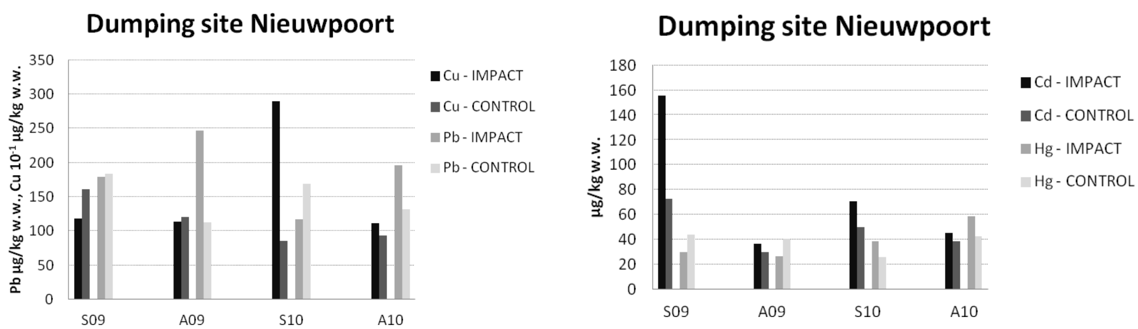


Figure 65. A) Cu and Pb concentration in starfish during the period 2009-2010. Concentrations are expressed in µg/kg w.w. for Pb and in 10-1 µg/kg for Cu.; B) Cd and Hg concentration in brown starfish during the period 2009-2010. Concentrations are expressed in µg/kg w.w. Impact and Control sites of dumping area Nieuwpoort are presented for each season.

4.3.4.7 Overview dumping sites

To assess the differences between the dumping sites based on the accumulation of contaminants in marine biota species, starfish was selected as key species and the sampling period ‘autumn 2010’ was considered. As discussed above, no difference between the dumping sites could be observed based on sediment chemistry. Based on the accumulation of contaminants, a clearly higher level of CBs and Pb was observed in starfish of dumping site Br&W Oostende compared to the other dumping sites (Figure 66). This trend could be observed in a lower extend for PAHs, Hg and Cu.

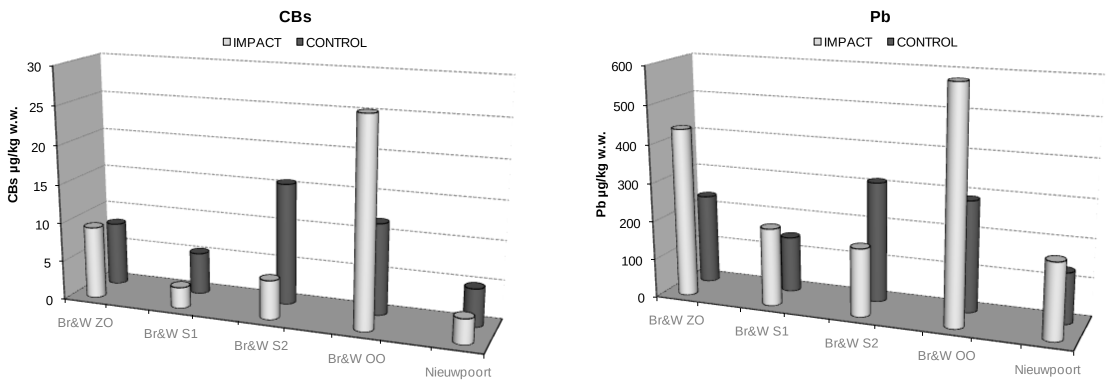


Figure 66. Levels of CBs and Pb in sediment samples during autumn 2010 for the Impact and Control sites of the different dumping sites. Concentrations are expressed in µg/kg w.w. for the sum of CBs. Concentrations are expressed in mg/kg w.w. for Pb.

4.3.4.8 Conclusion

Based on the accumulated levels of POPs and heavy metals in marine biota species during 2009-2010, it can be concluded that differences in contamination between control and impact areas for dredge disposal sites are limited. No difference was noted for CB concentrations while for PAH levels, slightly elevated concentrations were noted at the impact areas of the dumping sites Br&W Zeebrugge Oost, Br&W Oostende and Br&W S1. For heavy metals, elevated concentrations of Cu and Pb were detected at the impact sites of Br&W Zeebrugge Oost and Cd at the impact sites of Nieuwpoort. Based on the assessment of accumulation, dumping site Br&W Oostende could be distinguished from the other dumping sites based on higher levels of contamination in starfish.

The assessed levels of persistent organic pollutants or heavy metals in biota are obviously influenced by the investigated species. As mentioned before, very high levels of PAHs are found in bivalves, while for example high levels of PCBs are recovered in hermit crabs.

In agreement with the assessment on sediments, it would be of main interest to compare the results on biota with environmental assessment criteria (EAC) from OSPAR/MFSD (Joint Report, Task group 8)/Belgisch Staatsblad. To illustrate, Table 12presents the assessment criteria and the score (green or red) according to OSPAR/ Belgisch Staatsblad for blue mussel. It is not possible to compare the results on other biota species, e.g. starfish, goby, hooknose, other bivalves, hermit crab, etc., since those EAC values do not exist for those marine biota species. Table 12 indicates the over range levels of Pb and Hg in mussel tissue of dumping area Nieuwpoort according to OSPAR.

Table 12. assessment criteria for mussel according to OSPAR and Belgisch Staatsblad. (Colour colde: green = value OK according to EAC, red = value too high) for the control area of dumping site Nieuwpoort during autumn 2010.

		Dumping site Nieuwpoort	
			2010
			A
			C
Blue mussel			
OSPAR	Pyrene		
	Benzo(a)pyrene		
	Cd		
	Pb		
	Cu		
	Hg		
	pp-DDE		
	∑7 CB		
Belgisch Staatsblad	Hg		
	Hexachlorbenzen		

[1] OSPAR, Quality Status Report 2000, Region II Greater North Sea

[2] Belgisch Staatsblad N° 209, vrijdag 9 juli 2010

4.3.5 Biological Effects of Contaminants: Fish diseases and parasites

4.3.5.1 Introduction

Nowadays, it is generally accepted that the investigation of externally visible fish diseases may provide information on the occurrence of environmental stress. As a consequence, the study of the prevalence and distribution of diseases and parasites of wild marine fish became an essential part of integrated monitoring programmes to screen for the effects of environmental changes and marine pollution (Thain *et al*., 2008; Lang *et al*., 2002).

The aim of this epidemiological study is to monitor and compare the prevalence of contamination associated diseases and parasites of demersal fish on dredge spoil disposal sites and some reference zones on the Belgian Part of the North Sea (BPNS). Therefore, an important number of infectious and parasitical anomalies of the epidermis, the gills and the mouth of several fish species were recorded.

In this section the observed prevalence of contamination associated diseases and parasites during the spring and autumn of 2009 and 2010 are discussed. The diseases and parasites of fish were determined according to the ICES Training guide for identification (1996).

Dab (*Limanda limanda*) was chosen as primary fish species for monitoring, because it is a demersal fish with small mobility (Bucke *et al*., 1996). Dab is an abundant species and exhibits diseases which are easily recognised. The roundfish Whiting (*Merlangius merlangus*) was selected as additional species for monitoring.

In zones were dab has a limited abundance, other commercial flatfish species can be used: plaice (*Pleuronectes platessa*), flounder (*Platichtys flesus*) and Dover sole

(*Solea solea*). Disease monitoring on roundfish was extended to pouting (*Trisopterus luscus*) and cod (*Gadus morhua*). Due to the limited abundance and/or the lack of external diseases, species as sole, flounder and cod were not longer used for disease monitoring in 2010. If results on plaice, flounder, cod, pouting or sole are mentioned, it will only point to results from 2009.

As mentioned in the Materials and Methods, the Belgian Part of the North Sea was divided into different areas through assembling of the results of similar zones: impact area, coastal reference area and offshore reference area.

4.3.5.2 Results and Discussion

4.3.5.2.1 INFECTIONS, NODULES AND DEFORMATIONS

This part discusses the overall observed prevalence of skeletal deformations, ulceration, epidermal papilloma, liver nodules, lymphocystis and pigmentation. Especially ulcers, skeletal deformations, nodules and lymphocystis can provide valuable information on changes in the environmental health and may act as an 'alarm bell'.

Pathologically important diseases of the skin (fins and eyes included), the gills and liver were not or rarely observed on the BPNS.

Skeletal deformations were only registered on dab and whiting with a very small prevalence (max 1% prevalence). Higher prevalence is observed in coastal areas compared to offshore areas, no difference could be detected between the dredge deposit zones and the coastal reference zone.

Ulcerations are easy to locate and can be clearly seen. They are mostly associated with bacterial infections. Acute, healing and healed ulcerations were found on dab, whiting and plaice (2009) at very low frequencies. Geographic variations in occurrence are observed on the BPNS. On the dredge dumping sites a maximal observed prevalence of 0.82 % could be reported on dab in the autumn of 2009. No major difference could be observed between the dredge deposit zones and the reference areas.

Lymphocystis has a viral aetiology (Iridovirus). The nodules are the result of hypertrophy of connective tissue cells. During the last years, an overall decreasing trend in the occurrence of *Lymphocystis* on dab has established in the North Sea. On the BPNS, *Lymphocystis* is not detected since 2007.

Epidermal papilloma could only be detected on dab and were all stage 1 lesions (less than 4 lesions per individual). Important skin papillomas were never observed on the BPNS. The cause of papilloma on the skin of marine fishes is unknown, but a viral aetiology is assumed. On the dumping zone, the highest observed prevalence (0.2%)

was noticed in 2009. From 2004 to 2010, a declining trend in the overall prevalence on dab was observed on the BPNS (Figure 67). Remarkably, on the offshore reference zone the overall observed prevalence appeared to be increasing again (1.84 %) during 2010. This was not the case in the coastal zones or impact zones, where no increase was observed during 2010.

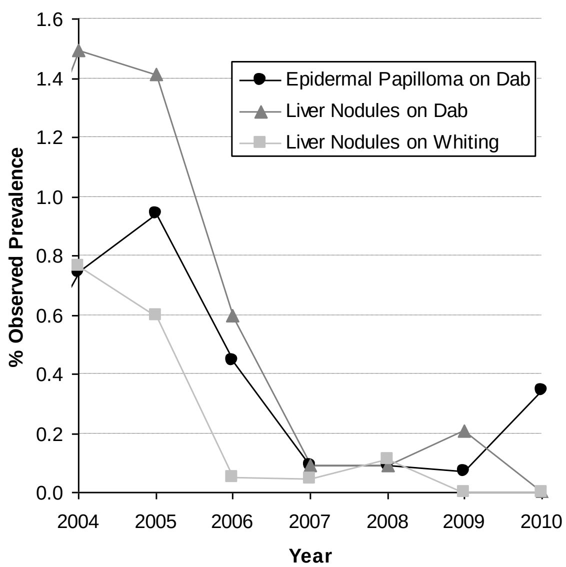


Figure 67. The overall observed prevalence per year of epidermal papilloma on dab and liver nodules on dab and whiting on the BPNS (2004-2010).

Liver nodules were observed in dab and cod (2009). The sampling size of cod was too small to calculate the observed prevalence on the separate sampling areas. At de dredge dumping zone an overall observed prevalence of 0.27% was obtained on dab and in de the second reference zone a similar prevalence (0.29%) was observed in 2009. No considerable difference could be observed between the different geographic areas. No liver nodules were observed in 2010. Generally, a continuing general downward trend in prevalence is apparent in the North Sea (Figure 67).

Hyper-melanisation was not observed on dab or other flatfish species during the years 2009-2010. Pigmentation was detected in dab, sole, flounder and plaice in all monitored zones. Often, the sampling sizes were too small to calculate the overall observed prevalence. The highest observed prevalence on plaice (8.16 %) was found during the spring of 2010 and on dab (5.56 %) during the autumn of 2009, both in the second reference zone (offshore). Long-term prevalence data (2000-2010) for dab revealed no important difference between the zones. In the dredge disposal zone, the trend appeared to be increasing during the last three years.

4.3.5.2.2 PARASITES

Most of the parasitical infections are relatively innocent when they are present at low numbers. The results show that the infection rates can vary considerably in space and time. The most common parasites on dab include *Acanthochondria cornuta, Glugea stephani* and *Stephanostomum baccatum*, while the most widespread parasites on whiting consist of *Cryptocotyle lingua, Lernaeocera branchialis* and *Clavella sp.*

It is problematic to define background levels or environmental assessment criteria for fish disease data due to the natural variation in disease prevalence on a temporal and regional scale. As a consequence, long-term prevalence data is used as a guideline. In this report, long-term prevalence data from 1996 until 2010 was used (whiting and dab). The overall observed long-term prevalence of fish disease on the BPNS was introduced by a poster presentation on the PRIMO 16 conference (Devriese *et al.*, 2011). Overall prevalence peaks were detected with intervals of a few years, depending on the investigated parasitical infection.

PARASITES ON FLATFISH

The overall observed prevalence of the trematode *Stephanostomum baccatum* was very low in the reference zones, and the individual infection rate was low (1 to 2 parasites per fish). This trematode was not observed on the dredge deposit zones during 2009 and 2010. And since 2003, a higher prevalence is observed in coastal reference sites compared to dredge disposal sites.

Lepeoptheirus pectoralis was found on dab, plaice and especially on flounder. Due to the small sampling size of flounder, no prevalence is calculated. A low overall observed prevalence is obtained on dab, depending on the season and sampling location, with the highest occurrence (3.27 %) during spring 2010 in the offshore reference zone. From 2009 to 2010, a small increase in prevalence is observed on all areas, mainly in the offshore reference zone (Figure 68).

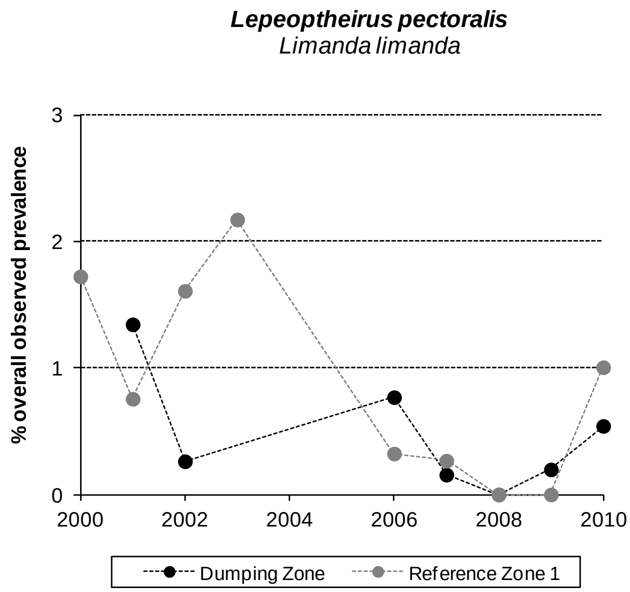


Figure 68. Overall observed prevalence of *Lepeoptheirus spp.* on dab during the period 2000-2010 in coastal sites. (Sampling sizes were too small during 2003 -only dumping zone-, 2004, 2005)

Externally attached copepods, such as *Acanthochondria spp*. on flatfish, are considered harmless. Especially flounder is host to these crustaceans, but the sampling size per zone was too small to consider the observed prevalence. On dab, a maximal prevalence of 9.56% was observed during autumn 2009 on the dredge dumping zone. The assembled data on *Acanthochondria spp*. show a higher prevalence during autumn compared to spring. The abundance of *Acanthochondria spp.* increased clearly during 2009 and 2010, particularly in coastal areas (Figure 69). Considering mean prevalence data of the years 2000-2010 (Figure 70) the occurrence was higher in coastal areas (2.89 %), especially in the dumping zones (3.37 %), compared to the more offshore zones (1.88 %). During 2010, no difference between the dumping sites and the coastal reference sites was observed.

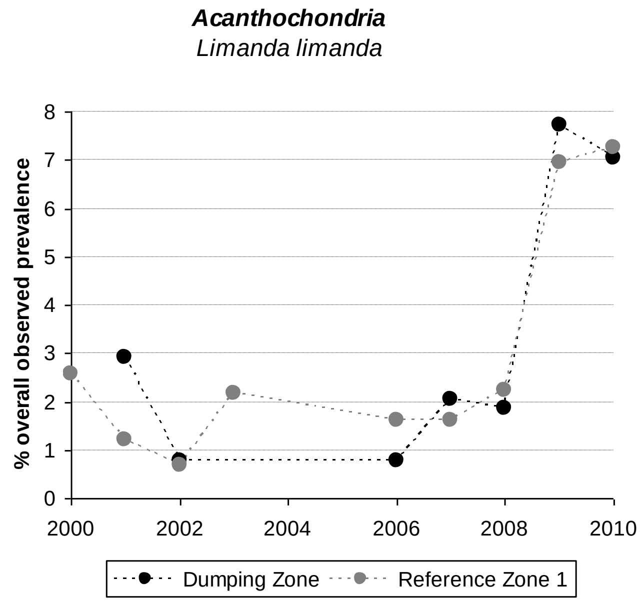


Figure 69. Overall observed prevalence of *Acanthochondria spp.* on dab during the period 2000-2010 in coastal sites. (Sampling sizes were too small during 2003 -only dumping zone-, 2004, 2005)

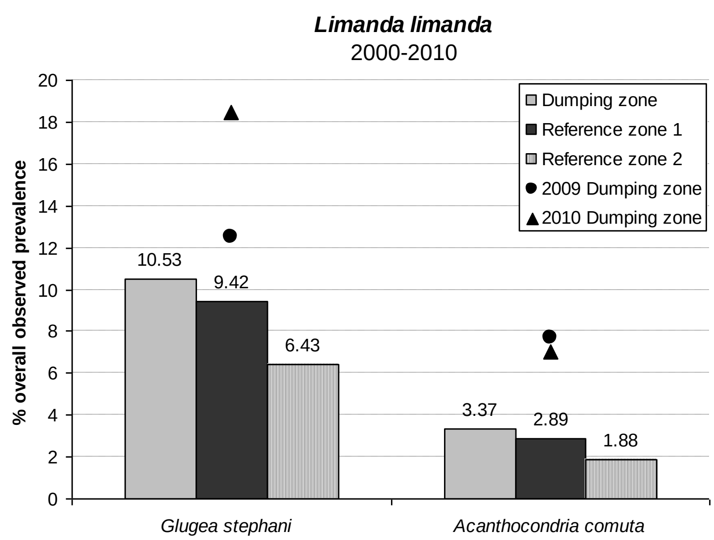


Figure 70. The mean observed prevalence of 2 different parasitical infections (Acanthochondria spp. and Glugea spp.) on dab over the years 2000-2010. The overall observed prevalence for the dredge disposal sites is separately given for 2009 and 2010.

The parasite of the intestines, *Glugea stephani*, was mainly present on dab. The highest prevalence (18.18 %) was observed on the dumping sites in autumn 2010. A higher occurrence of the parasite during autumn compared to spring was noticed. The assembled data of the years 2000-2010 on *Glugea stephani* reveal an overall increasing trend in coastal areas (Figure 71). Mean prevalence data of the period 2000-

2010 show that the observed prevalence at dredge dumping sites turned out to be slightly higher compared to the other coastal reference sites and clearly higher compared to the offshore reference zone. On the dumping zone, the observed prevalence during 2009 and 2010 was significantly higher compared to the mean observed prevalence using data from 2000-2010.

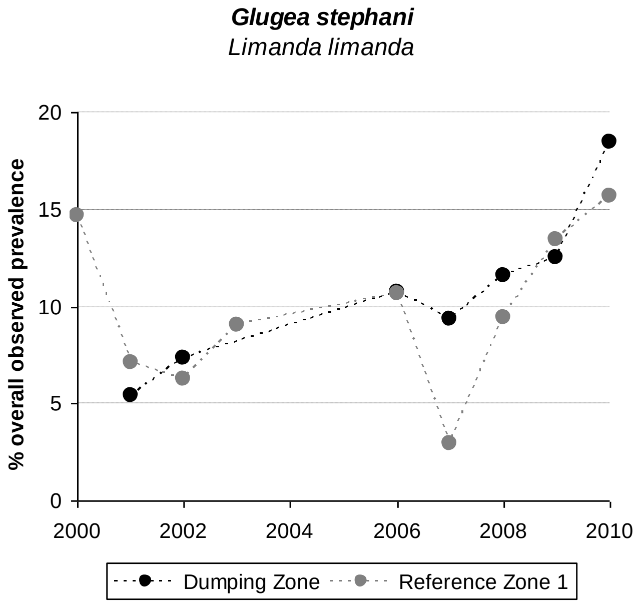


Figure 71. Overall observed prevalence of Glugea stephani on dab during 2000-2010 in coastal areas. (Sampling sizes were too small during 2003 (only dumping zone), 2004, 2005)

PARASITES ON ROUNDFISH

The 'black spot' disease caused by the skin parasite *Cryptocotyle lingua* (trematode) is expressed in highly variable concentrations on roundfish, especially on whiting and pouting. On whiting, data of the years 2009-2010 insinuate no clear difference between the dumping and reference zones. At the dredge dumping sites, the observed prevalence tend to decrease slightly since 2000, with exception of higher occurrence in the year 2009. Long-term prevalence data suggest higher occurrence at coastal areas (Figure 72). Data from 1996 to 2010 also suggest a higher observed occurrence of this trematode during spring.

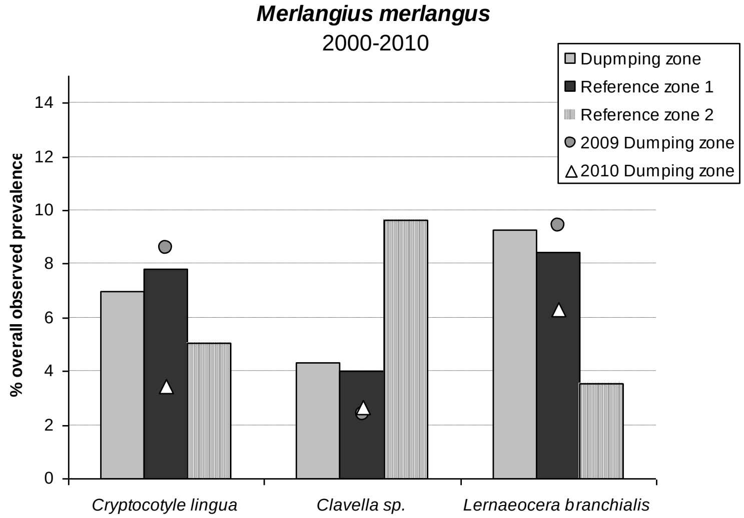


Figure 72. the mean observed prevalence of 3 different parasites on whiting over the years 2000-2010. The overall observed prevalence for the dredge disposal sites is separately given for 2009 and 2010.

The harmless externally attached copepod *Clavella sp.* Is generally more widespread at offshore areas. On whiting, the highest observed prevalence (14.74%) was obtained at the offshore reference area during spring 2009. Long-term prevalence data confirmed the higher occurrence of *Clavella sp.* at offshore areas, especially during spring (Figure 73). No considerable difference could be observed between the dredge deposit zone and the coastal reference zone. Data from 1996-2010 confirm an undulating prevalence pattern during the years (Figure 73), which is typical for most parasitic infections. As a consequence, a decrease of prevalence is observed during 2009 and 2010 compared to 2007 and 2008. The predicted prevalence could be used as assessment criteria for parasitic disease monitoring. Similar models are formulated for the other parasitic diseases.

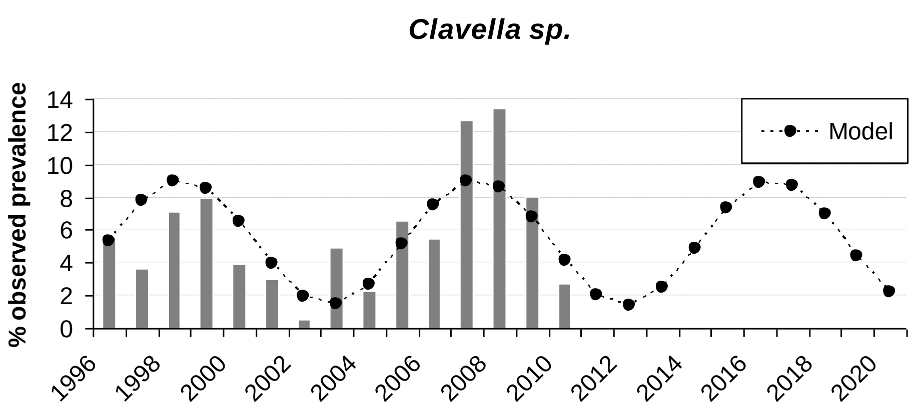


Figure 73. Observed prevalence of Clavella sp. on whiting during 1996-2010 and the predicted prevalence using Solver until 2020.

The parasitical copepod, *Lernaeocera branchialis* was noted on the three observed roundfish species: whiting, cod and pouting. The occurrence varied according to species, sampling zone and period. The elevated prevalence in coastal zones was confirmed by data from 2009-2010 as well as by the long-term prevalence data. A slightly higher prevalence in the dumping area compared to the coastal reference zone could be deduced from the mean prevalence 2000-2010 (Figure 72).

4.3.5.3 Conclusions

Severe diseases such as skin ulcers, nodules, skeletal malformations and lymphocystis, which might indicate effects of pollution, are rare on the investigated zones of the BPNS. The overall trend for these diseases appeared to be decreasing in the North Sea. No significant differences could be detected on the basis of the data between the dredge dumping sites and the reference zones. Because the overall prevalence of those diseases is very low, examination of histopathological liver lesions in flatfish may provide additional information about the condition of the dredge disposal sites in future.

Most of the observed anomalies were due to parasitical infections. The presence of these parasites showed considerable variation in spatial and temporal distribution.

Parasites such as *Cryptocotyle lingua*, *Clavella sp.* and *Lernaeocera branchialis* are more abundant during spring, in contrast to *Glugea stephani* and *Acanthochondria cornuta* which are more widespread during autumn. Long-term prevalence data from 1996 until 2010 showed undulating prevalence patterns during the years for all examined parasitical infections. Models can be formulated to predict the prevalence of those diseases in future. Those models can be used as assessment criteria to interpret monitoring data.

Parasites such as *Lernaeocera branchialis, Cryptocotyle lingua*, *Acanthochondria cornuta* and *Glugea stephani* have a larger prevalence in coastal areas compared to the more offshore sites. As a consequence of the spatial variation, the dredge disposal sites must be compared to the coastal reference sites. For the period 2000-2010, *Glugea stephani* and *Acanthochondria cornuta* on dab and *Lernaeocera branchialis* on whiting showed a slightly higher prevalence on the dumping sites compared to the coastal reference sites, while *Stephanostomum baccatum* and *Lepeoptheirus pectoralis* on dab and *Cryptocotyle lingua* on whiting showed the opposite trend.

In future, the obtained data should be compared with the long-term prevalence data. Significant differences in prevalence between coastal reference zones and dredge deposit areas and between long-term prevalence data and recent data must be examined thoroughly and followed in future.

Finally, it is important to remind the effect of migration on the observed species. Based on the migratory properties, flat fish species were chosen as monitoring organism. The results can, especially for roundfish, be influenced by migration. As a result, the data rather reflects the health condition of a big area instead of smaller zones like the dredge deposit zones. Nevertheless, some important regional differences in parasitical affection could be observed.

In upcoming surveys, only results on the main species whiting and dab will be gathered. Due to the small sampling size, flounder, Dover sole, cod, pouting and plaice will not be used for the assessment of contaminant associated fish diseases. If the sampling size allows, dab species will be divided in two subgroups (15-19 cm and 20-24 cm) according to the ICES guidelines.

Recently, histopathological liver lesions in flatfish have been recommended as one of the methods to be used for biological effect monitoring under the OSPAR Joint Assessment and Monitoring Programme (JAMP). This research strategy could provide additional or complementary information on contaminant associated fish diseases.

In addition to the assessment of fish diseases, the general health of fish species (e.g. gonadosomatic index GSI, quality index method QIM, liver glycogen content LGC, liver-somatic index LSI, condition index k,…) will be monitored during future sampling surveys.

4.3.6 Biological Effects of Contaminants: EROD activity as biochemical indicator of xenobiotic substance accumulation

4.3.6.1 Introduction

Studying the possible harmful effects of pollutants present in the marine environment on biota species is an essential research topic in environmental monitoring. In this part, the relationship between the dumping of dredge spoil with associated pollutants and the biological effect of those pollutants on local fish populations is investigated. It is of major interest to identify if pollutants effect the biological activity of marine organisms or if they are transformed/metabolized and excreted.

The effects of contaminating substances (PCBs, PAHs, …) in living organisms can be studied through the upregulation of enzymatic activities involved in biotransformation of pollutants following their binding on the aryl hydrocarbon receptor (ArH receptor). Upregulated enzyme activities may serve as ‘early warning’ signals for pollution. Here, the biochemical biomarker EROD (7-ethoxyresorufin O-deethylase) activity is used as an indicator of xenobiotic substance accumulation in the flatfish dab (*Limanda limanda*). Assessment of the EROD activity is one of the required indicators of pollution by the Joint Assessment and Monitoring Programme (JAMP) under the coordination of OSPAR.

ILVO participated to the OSPAR expert group 'Study Group for the Integrated Monitoring and Biological Effects' (SGIMC) to define the assessment criteria for the EROD assay for Atlantic cod, flounder, dab and plaice by using EROD data submitted in ICES database.

4.3.6.2 Results and discussion

4.3.6.2.1 EROD ACTIVITY IN DAB LIVER

To assess the induction in EROD activity, the livers of juvenile dab were excised and homogenized. The small livers of juvenile dab may assumed to be homogeneous in contrast to the livers of larger dab. This assumption was confirmed by analyzing different parts of large and small dab livers during 2009-2010. Besides, on juvenile dab no significant difference (α=0.05) was obtained between EROD results on male and female dab and therefore the EROD results for male and female dab were pooled into one group.

The seasonal model of the EROD induction shows a peak during late winter/early spring. This peak is inversely correlated with the concentrations of POP's in liver fat. The obtained EROD data clearly demonstrate the EROD induction during late winter/early spring, while only a background level is recorded during autumn. This is illustrated by the EROD induction in dab liver during the four sampling campaigns of 2009-2010 on a reference site (Figure 74).

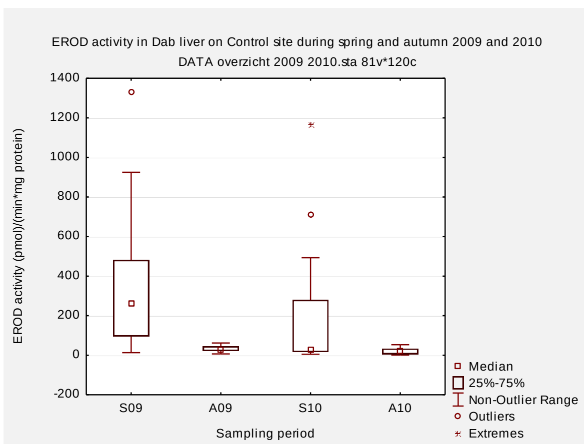


Figure 74. The EROD activity, expressed as pmol/(min.mg protein) measured on juvenile dab caught on a control site during spring 2009 – autumn 2009 – spring 2010 – autumn 2010.

Due to the small sampling sizes of juvenile dab during 2009-2010, no overview of EROD activities on dab of the different dumping sites could be proposed. The EROD results for dumping site Nieuwpoort and Br&W S1 are presented in Figure 75 for the impact and control sites. On Br&W S1, spring and autumn 2010 are presented, while for dumping site Nieuwpoort spring and autumn 2009 are shown. Only on dredge deposit site Nieuwpoort during spring 2009, a significant difference between impact and control sites was obtained. During the period 2009-2010, no significant higher EROD activity on impact sites versus control sites was observed on the different dumping areas.

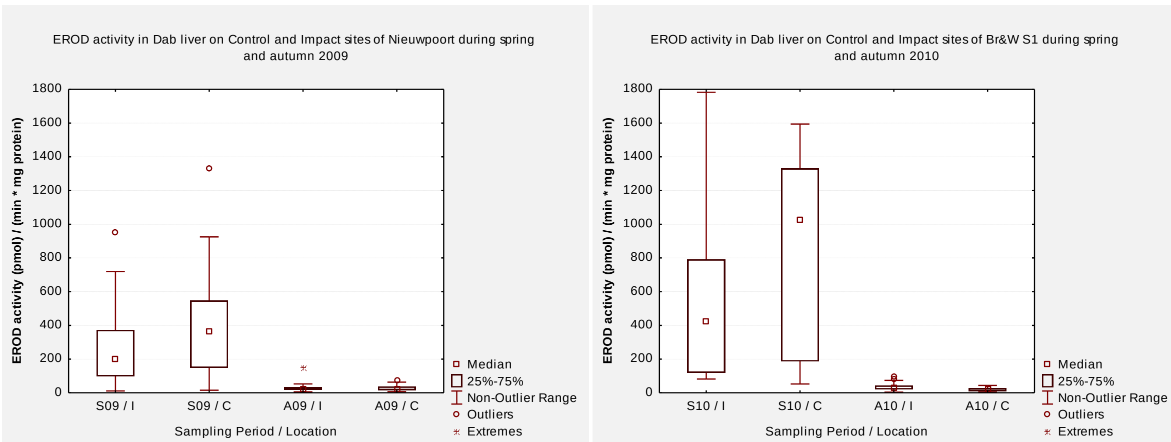


Figure 75. The EROD activity, expressed as pmol/(min.mg protein) measured on juvenile dab caught on dredge deposit site Br&W S1 and dumping site Nieuwpoort (Impact versus Control, Spring versus Autumn)

Former data indicated the influence of the freezing process (liquid nitrogen or -80°C) of the livers on the EROD activity. During the years 2009 and 2010, samples are taken to define this deviation on the EROD activity in order to compare EROD data on freshly prepared liver homogenates and livers stored in liquid nitrogen or at -80°C during 1 month. For this test, juvenile dab livers were divided and one piece was analyzed immediately, while the other piece was stored (liquid nitrogen/ -80°C) during one month before measurement. As expected, the EROD activities were significantly elevated in the freshly analyzed livers. A correlation coefficient of 0.655 could be formulated.

4.3.6.2.2 EROD, MROD AND PROD ACTIVITY IN DAB LIVER

Besides the EROD assay, preliminary tests for the MROD (7-methoxyresorufin O-deethylase) and PROD (7-pentoxyresorufin O-deethylase) assay are conducted on the livers of juvenile dab.

To assess the differences between the assays, the livers were divided in three pieces, one for each assay (EROD, MROD, PROD). The assays were implemented on liver samples from spring 2009 and autumn 2010. During autumn, the MROD and PROD activities did not exceed the detection limit. In general, the PROD assay wasn't suitable on dab livers, the induction of PROD activity was often too small for measurement. The MROD and EROD data showed a similar trend, but the EROD activity was about 14.7 times higher compared to the MROD activity (Figure 76). In conclusion, the EROD activity is definitely the most effective assessment on dab liver.

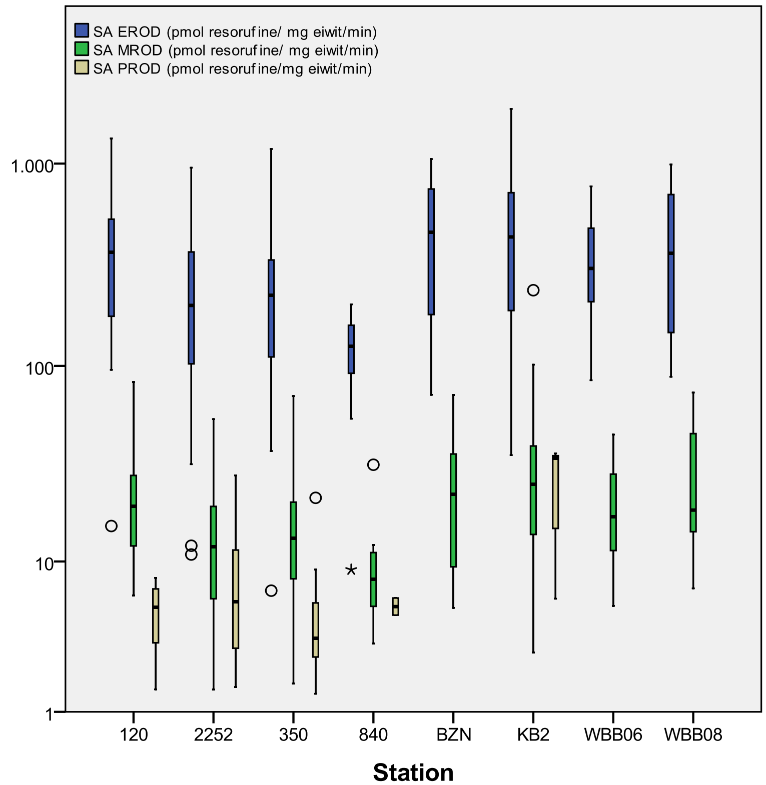


Figure 76. The EROD, MROD and PROD activity in dab liver on different sampling sites during spring 2009

4.3.6.3 Conclusion

On the dumping areas, no significant difference between impact and control sites could be observed based on the enzymatic EROD activity during 2009-2010.

Although the measurement of the enzymatic EROD activity is an important tool to assess the effects of anthropogenic activities, the sample sizes are often too small to allow statistical interpretation, especially during spring. The biochemical EROD assay depends on different parameters such as season, water temperature, size of the fish, maturity of the fish, sex of the fish, ... and could be difficult to interpret. It is of main interest to keep searching for other biomarkers (genomic or biochemical) on relevant abundant species. Recently, the first efforts were made to develop a genomic biomarker on starfish.

5 An exploration of the biological life in the dredging areas

5.1 Introduction

One of the aspects, that was not looked after in the previous decade was the biological life in the dredging areas, especially in the gullies towards the harbor of Zeebrugge and the harbor itself. This is necessary to make a comparison between the fauna in the dredged material and those occurring on the dumping sites. Species occurring in the dredging areas can be transported to the dumping areas and locally enrich the fauna if they survive (Figure 1). This should have a minor effect for the dumping areas on the Belgian Part of the North Sea, because the dredging areas are expected to be characterised by a poor faunal composition.

5.2 Material and Method

5.2.1 Sampling

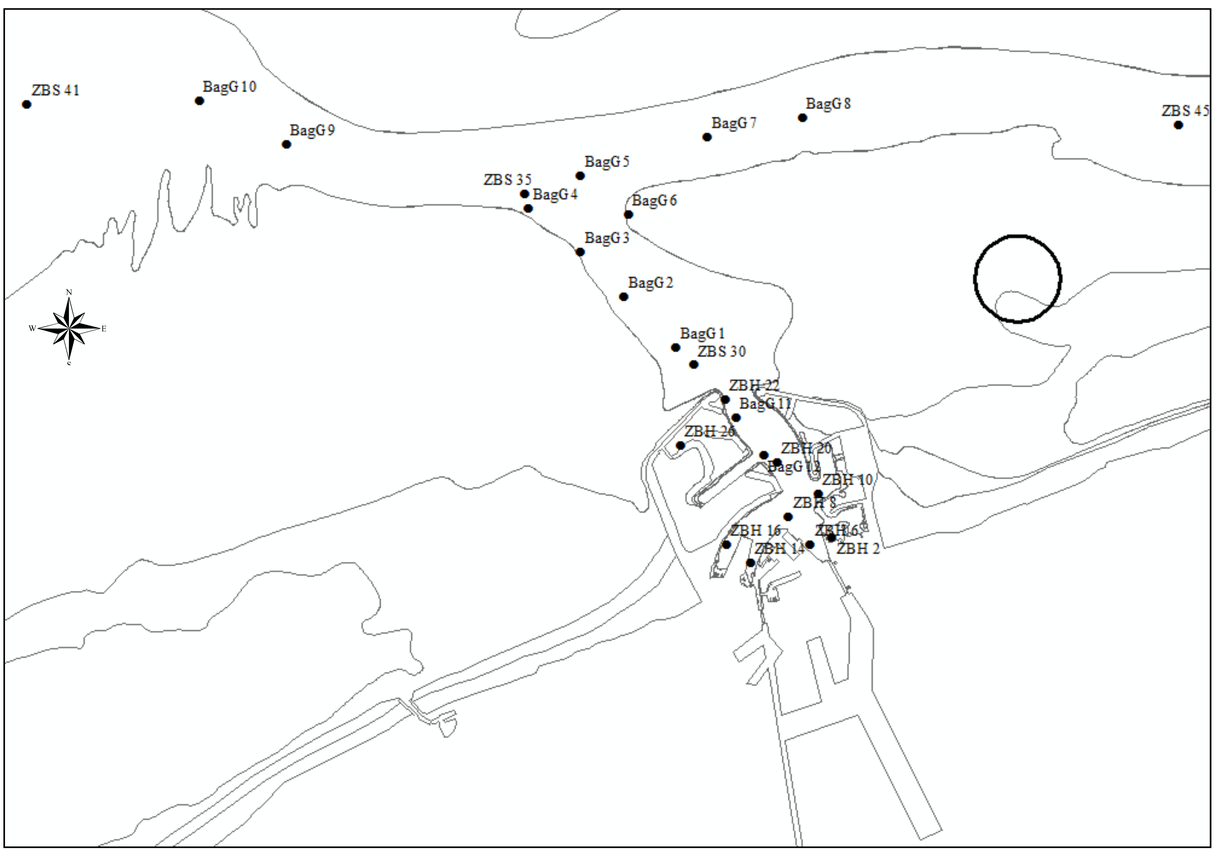


Figure 77. Map of the Zeebrugge area with indication of the benthic samples taken in 2007 and 2011.

Benthic samples were taken in two different years (2007 and 2011). In the first year (2007), mainly samples in the harbor of Zeebrugge were taken, whereas in 2011, most samples were originated from the gullies towards the harbor (Pas van 't Zand, Scheur, Vaargeul 1).

The samples in december 2007 were taken on board of the Brandaris in coincidence with the sediment monitoring program of the harbors and their entrances (Lauwaert et al., 2008). At 13 of the 42 sampling points an extra benthic sample with a Van Veen grab (12L) was taken (Figure 77).

In February 2011, a benthic sampling program was executed to monitor the gullies towards the harbor of Zeebrugge. Therefore, a Van Veen grab (22L) was taken at 12 sampling points, which were distributed over this area (Figure 77). No biota were found in the samples ZBH6 and BagG11.

All the samples were sieved over a 1mm sieve to extract the benthic fauna. The samples of 2007 were sieved after fixation, whereas those of 2011 were sieved alive.

5.2.2 Biological analysis

Species were identified to species level when possible and counted. 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 Perspex core was taken for sediment analyses. These samples were dried at 60°C and analyzed with a Malvern Mastersizer 2000 following a standardized protocol. Depth and position of each sample were also registered during the campaigns.

The species dataset was standardized by lumping some species (Cirratulidae spp, *Spio* spp, Anthozoa spp), and reduced by excluding species that did not belong to the macrobenthos *sensu strictu* (e.g. Mysidacea). Nematoda were excluded because of inadequate sampling techniques for quantifying meiofauna. By this standardization, two more stations were than characterised by the absence of benthic fauna (ZBH8, BagG12).

Cluster analysis by group average sorting based on a Bray-Curtis similarity dataset (fourth root transformed) was used as multivariate analysis. This cluster analysis was complemented with a SIMPROF test (Similarity profile, test for structure in the data) to define the significant different groups within the cluster analysis. The clustering was visualized using a non-metric Multi Dimensional Scaling analysis (MDS). 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. Factors structuring the observed multivariate pattern were tested with ANOSIM analysis (Analysis of similarities). All multivariate analyses were performed within PRIMERv6 (Plymouth Routines in Multivariate Ecological Research; Clarke & Gorley, 2006).

The calculated univariate parameters were: density (ind./m²), biomass (Wet Weight/m²) and number of species (N_0).

5.3 Results

5.3.1 Macrobenthos

48 benthic taxa were recorded in the dredging areas of Zeebrugge, whereof 27 (56%) taxa were only recorded once (Table 13). Most taxa were found in the gullies towards Zeebrugge and only a few taxa (9; Cirratulidae spp, Oligochaeta spp, Nephtys juvenile, *Mytilus edulis, Streblospio benedicti, Abra alba, Macoma balthica, Anthzoa spp, Crangon crangon*) in the harbor itself. 58% of the taxa belongs to the phylum Polychaeta, whereas 19% to the Mollusca. Taxa of the Crustacea and Echinodermata has a contribution of 10%.

Not one of the observed species was a rare taxon within the benthic fauna on the BPNS. Most species were common within the *Abra alba* and/or *Macoma balthica* habitat (Van Hoey et al., 2004). Only the bivalve *Mytilus edulis* is more characteristic for hard-substratum and the specimens found in our samples were mostly recruits. Those were temporally settled or washed-out from the hard substrate around the harbor.

Table 13. Species list of the samples taken in the dredging areas of Zeebrugge harbor. The species in grey were also found in the harbor itself.

Species name	Phylum	# samples	Tot. density (ind/m²)	Species name	Phylum	# samples	Tot. density (ind/m²)
Cirratulidae spp.	Polychaeta	16	4625	Ophiura albida	Echinodermata	1	130
Oligochaeta spp.	Polychaeta	12	2632	Tellina fabula	Mollusca	1	93
Nephtys juv.	Polychaeta	9	1020	Ophiura juv.	Echinodermata	1	93
Mytilus edulis	Mollusca	8	392	Spisula subtruncata	Mollusca	1	93
Owenia fusiformis	Polychaeta	7	3173	Abludomelita obtusata	Crustacea	1	74
Streblospio benedicti	Polychaeta	6	2630	Ensis directus	Mollusca	1	40
Abra alba	Mollusca	5	4668	Microphthalmus similis	Polychaeta	1	40
Nephtys hombergii	Polychaeta	5	195	Pholoe minuta	Polychaeta	1	37
Macoma balthica	Mollusca	4	129	Lanice conchilega	Polychaeta	1	20
Anthozoa spp.	Cnidaria	4	86	Sthenelais boa	Polychaeta	1	19
Heteromastus filiformis	Polychaeta	4	70	Eteone flava	Polychaeta	1	19
Ophiura ophiura	Echinodermata	3	94	Glycera juv.	Polychaeta	1	19
Mediomastus fragilis	Polychaeta	2	141	Echinocardium cordatum	Echinodermata	1	19
Notomastus latericeus	Polychaeta	2	140	Phyllodoce mucosa	Polychaeta	1	19
Scoloplos armiger	Polychaeta	2	84	Magelona spp.	Polychaeta	1	19
Petricola pholadiformis	Mollusca	2	80	Venerupis senegalensis	Mollusca	1	19
Capitella spp.	Polychaeta	2	30	Asterias rubens	Echinodermata	1	10
Crangon crangon	Crustacea	2	29	Gammarus salinus	Crustacea	1	10
Glycera spp.	Polychaeta	2	29	Gastrosaccus spinifer	Crustacea	1	10
Eunereis longissima	Polychaeta	2	29	Ophelia spp.	Polychaeta	1	10
Pectinaria koreni	Polychaeta	2	29	Nephtys longosetosa	Polychaeta	1	10
Kurtiella bidentata	Mollusca	1	593	Polydora cornuta	Polychaeta	1	10
Spiophanes bombyx	Polychaeta	1	204	Malmgreniella castanea	Polychaeta	1	10
Pariambus typicus	Crustacea	1	130	Spio spp.	Polychaeta	1	10

There is a clear pattern in the benthic community characteristics in the dredging areas of Zeebrugge (Figure 78). In the central gully within the harbor of Zeebrugge almost no benthic individuals (max 1) were found. Two samples (ZBH16 and ZBH2) within a corner of the harbor were characterised by a higher species richness, respectively 7 and 5 spp./0.054m²), whereas in the other samples the species richness was very low. The highest number of species, densities and biomass were found in 'Vaargeul 1, especially at station ZBS41 (32 spp./0.054m²; 13981 ind./m²; 1124 g/m² Wet weight). The samples in the 'Scheur' area showed a low species richness and a moderate density. The high density spot within the harbor of Zeebrugge (ZBH16) is caused by the presence of the spionid *Streblospio benedicti*.

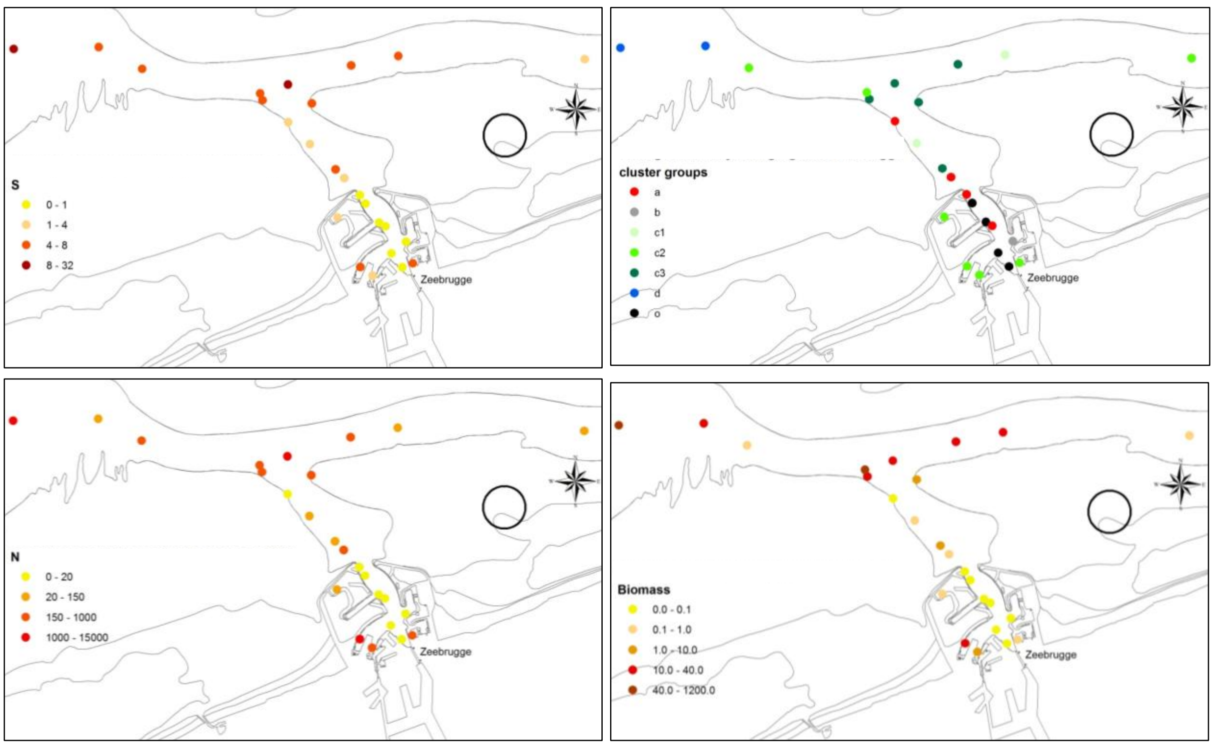


Figure 78. Map with indication of the number of species (S, top-left hand corner), density (N, bottom-left hand corner), biomass (bottom-right hand corner) and the cluster groups (top-right hand corner).

Most samples shows a high affinity with each other and were grouped in cluster C (C1, C2 and C3) (Figure 79). This cluster is characterised by the presence of Cirratulidae spp., Oligochaeta spp. and *Nephtys* juveniles. The samples of this cluster were localized in the 'Scheur' area and within the harbor of Zeebrugge. Within this cluster C, we recognized on a higher similarity level, three sub-clusters, which were not significantly different regarding the SIMPROF analysis. These sub-clusters shows a deviation between the samples located within the harbor and those of the 'Scheur' area.

Cluster A is characterised by the presence of *Mytilus edulis* and the samples were located in the central part of the harbor and in the entrance gully.

Cluster B is characterised by one sample (ZBH 10), containing one taxa (*Nephtys* juvenile). Cluster D, contains two samples which were characterised by the highest diversity (especially ZBS 41) and were located in 'Vaargeul 1' area. The three species mainly characterizing this cluster were *Abra alba*, Cirratulidae spp. and *Eunereis longissima.*

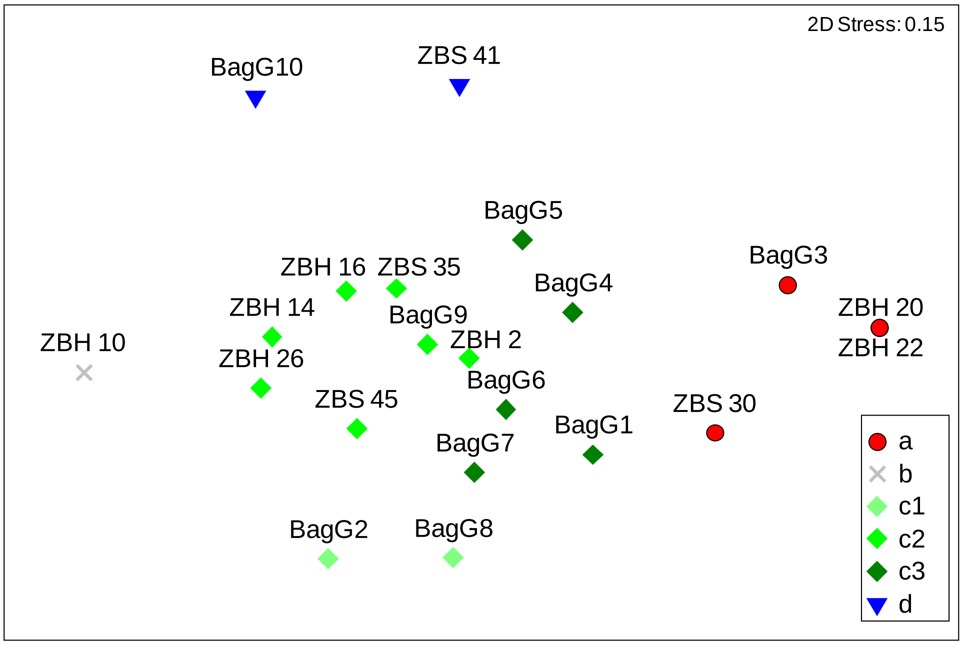


Figure 79. MDS plot of the benthic samples with indication of the four simprof cluster groups.

5.4 Conclusion

We can conclude that the dredging area's around Zeebrugge were characterised by very poor benthic community characteristics, except the 'Vaargeul 1' area. Input of benthic animals from the dredging areas towards the dumping areas is possible, but should not lead to species enrichments in the dumping zones, due to the low densities and species richness in the dredging areas.

6 Optimalization of the sampling strategy in the routine monitoring program

6.1 Introduction

In the period 2009-2011, we invested in the optimalisation of the sampling strategy of the routine monitoring program at the dredge disposal sites. This was carried out to standardize the analysis according to European directives and to make the monitoring time and cost efficient. The first is necessary because the ecological and environmental status of the marine waters will be evaluated following the guidelines of the Water Framework Directive and Marine Strategy Framework Directive. Therefore, the monitoring programs of every Member state has to be adapted in the near future towards these guidelines. ILVO started this process of monitoring standardisation in the previous years. Secondly, monitoring programs are costly and time consuming, especially biological ones. Therefore, some sampling protocols were adapted to collect similar data within less time.

In this section, the changes to the sampling protocols of the routine monitoring program of ILVO were outlined and discussed:

- The changes in the duration of the epibenthos and fish tracks from 30 minutes towards 15 minutes.
- Sieving of the macrobenthos samples alive instead of fixated.
- Introduction of quality assurance in the macrobenthos analysis (ISO 16665:2005) to achieve a BELAC accreditation certificate under ISO17025 norm.

6.2 Short versus long epibenthos-fish tracks

6.2.1 Introduction

When evaluating the previous campaigns, it became clear that an adaptation of the sampling method, and more precisely the length and the number of a fish track, was needed. The cause for this specific adaptation was twofold:

- The length of the fish tracks (3500m) was too long to fit within the delineation of the dumping sites, whereby fauna within and aside the dumping site was collected.
- Local effects within the dumping sites are hard to detect using long tracks, since all fauna over a length of 3500m are pooled in a single catch and information about the small-scale ‘patchiness’ of fauna is largely lost. A

shortening of the tracks and an increase in the number of tracks would result in a higher spatial resolution in the analysis, which would decrease the detection level of local changes.

However, when implementing shortened fish tracks in the monitoring program, the confidence of such tracks was first tested experimentally during the autumn campaign of 2009 (Vandendriessche et al., 2010). Secondly, both track types were taken at some monitoring stations on the BPNS during the monitoring activities of 2010. The analysis of both campaigns is shown in this section.

6.2.2 Material and methods

Table 14. List of epibenthic and demersal fish tracks used for testing the difference between short and long tracks, within different time periods (years and seasons).

Station	year	season	Station	year	season
ft120	2010	winter	ft7802	2010	winter
ft140	2010	winter	ft820	2009	autumn
		autumn	ft830	2010	winter
ft215	2009	autumn			autumn
	2010	winter	ft840	2010	winter
		autumn			autumn
ft230	2009	autumn	ftB04	2010	winter
	2010	winter			autumn
		autumn	ftB07	2010	autumn
ft315	2010	autumn	ftB10	2010	autumn
ft340	2010	winter	ftWT2	2009	autumn
		autumn	ftWT3	2009	autumn
ft415	2010	autumn	ftWT5	2009	autumn
ft421	2010	winter	ftWT9	2009	autumn

Epibenthos and demersal fish were sampled with an 8-meter beam trawl, equipped with a fine-meshed shrimp net (stretched mesh width 22mm in the codend) and a bolder-chain but no tickler chains (to minimize the environmental damage) (Figure 13). For the long tracks, the net was dragged during 30 minutes at an average speed of 4 knots over the bottom. As such, an average distance of 3500 m was covered. The short tracks are half as long as the original tracks (long tracks) so the sampling distance and time is 1750m and 15 minutes (see chapter 7). Both track types were taken aside each other. Data were recorded on time, start and stop coordinates trajectory and sampling depth in order to enable a correct conversion towards sampled surface units. The fish tracks were positioned following depth contours that run parallel to the coastline, thereby minimizing the depth variation within a single track.

The test was done for different locations, distributed on the Belgian Part of the North Sea and at different time periods (2009 & 2010 and for winter and autumn) (Table 14; Figure 11).

The complete catch was sorted using a rinsing and sieving machine. As such, three fractions were obtained: a coarse fraction with mainly larger fish, adult starfishes and sea urchins; a shrimp fraction with mainly crustaceans, ophiuroids and smaller fishes and a fine fraction with mollusks and sea anemones. From these fractions, all fish, except gobies, were identified, measured and/or counted on board. After fish elimination, representative subsamples (2 to 10l) from each fraction were taken for epibenthos analyses. For a number of tows, the epibenthos (except gobies and small and/or rare species) subsamples were processed on board as well; for other tows, the subsamples were frozen for further laboratory analyses.

The net contents were divided into 'demersal fish' and 'epifauna'. These ecosystem components were dealt with separately concerning density, biomass (epibenthos only), diversity and community structure. Furthermore, from the epibenthos dataset, the polychaetes were deleted, ascidians and the barnacle *Balanus*, since these species were not representatively sampled. Gobiidae, belonging to the order of Perciformes, were treated separately due to their abundance and local importance. The number of individuals per sample and per species was converted to number of individuals per 1000m² (density). Biomass was expressed as grams of wet weight (WW) per 1000m² and diversity was evaluated based on the number of species.

6.2.3 Results

6.2.3.1.1 EFFECT ON DENSITY

Comparison of fish density and epifaunal density showed that the standardisation of short tracks to number or weights per 1000m² resulted in an underestimation of fish densities (average 7%) and epifaunal densities (average 36%) compared to the long tracks (Figure 80). The rate of overestimation or underestimation varied between tracks and between species groups. Large differences in the estimates of epibenthic densities were found at stations situated in the coastal area (ft120, ft230, ft215). The difference is much lower for tracks on sandy bottoms situated more offshore. The most common epibenthic species groups (Crangonidae, Paguridae, Portunidae, Asteriidae and Ophiuridae) are characterised by an underestimation of the density by taking long tracks (Table 15). This underestimation is the highest for sea stars (Asteridae) with a value of 148%. The most common demersal fish species groups (Pleuronectidae, Soleidae, Callionymidae, Gobiidae) are underestimated by long fish tracks (Table 15). This underestimation is on average very low for Callionymidae.

Table 15. Average deviation percentages (based on density data) per species groups (epibenthos and demersal fish). Positive values represent overestimation, negative values represent underestimation.

Epibenthos	%	Demersal fish	%
Crangonidae	83	Pleuronectidae	37
Paguridae	66	Soleidae	49
Portunidae	89	Callionymidae	3
Asteriidae	148	Gobiidae	52
Ophiuridae	97		

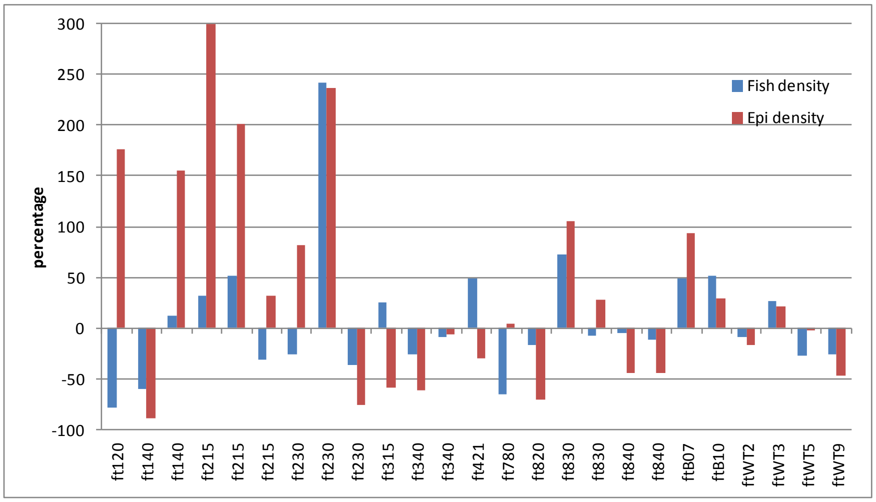


Figure 80. Percentage difference between short and long tracks in the density of fish and epibenthic species.

6.2.3.1.2 EFFECT ON SPECIES RICHNESS

Since the number of observed species S strongly depends on sample size (Soetaert & Heip, 1990), this parameter is logically underestimated during short tracks compared to long tracks (Figure 81). The number of species for epibenthos is at average 12% lower, compared to long fish tracks. For demersal fish, there is an underestimation of the number of species with 8%. Next to the actual number of species, the expected number of species per 1000 individuals (ES1000) was calculated (Primer 6). This diversity measure is less influenced by sample size (Soetaert & Heip, 1990), and therefore a better proxy for species richness. By the use of ES1000, we found an average underestimation of the number of species for epibenthos and demersal fish of respectively 8 and 4%.

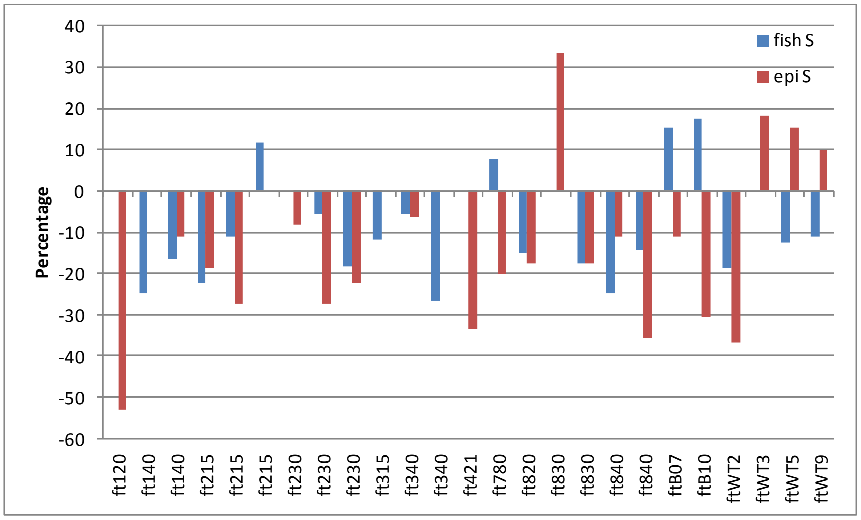


Figure 81. Percentage difference between short and long tracks in the number of species for fish and epibenthic species

Reduction of work load per track?

The average volume of the catch was reduced from 125 liters in the standard tracks to 75 liters in the short tracks, which corresponds with an average reduction of 41% (range 10% - 64%). In the case of reasonably small catches that are entirely processed, the workload on board is obviously reduced. In the case of large catches, such as de ones at station 230, the subsampling procedure (including the use of a rinsing and sieving machine) was applied to both track types, and the amount of organisms to be processed remained identical. In this case, only the tow duration and the covered distance were reduced.

6.2.4 Conclusion

The rate of overestimation or underestimation varied between tracks and between species groups. For the offshore tracks, a better correspondence between estimates was found compared to the coastal tracks. It seems that in areas with high epibenthic densities (coastal area), the net attained to be filled up, which influenced the net efficiency. Therefore, short tracks gives a better density estimate compared to long tracks. Estimations of diversity of short tracks are reliable, providing that the appropriate measure (expected number of species) is used. Detailed analyses of diversity (e.g. drafting species lists), however, should be based on longer tracks.

For subsequent analyses, the occurrence of overestimations or underestimations compared to standard tracks, should be taken into account. Additionally, indices which do not depend on sample size should be adopted. This difference plays no part when tracks of similar kind (short) are taken and compared at the same time. A clear advantage of using short tracks in the dredge disposal research is the fact that the tracks fit within the borders of the dumping site. Like this, side effects are minimalised and the short tracks seem to result in reliable density and diversity estimates of the area.

6.3 Fixed versus live sieving

6.3.1.1.1 INTRODUCTION

One of the elements that often varies among macrobenthic studies is the sieving procedure: sieving alive versus sieving after fixation. Before 2010, the ILVO monitoring programs used sieving after fixation as sieving procedure. This is not common in European macrobenthos monitoring and results in the use of high volumes of the carcinogenic formaldehyde. Therefore, from 2010 onwards, we switched to sieving alive. This difference in sieving procedure has its consequences on diversity, species densities and community structure (Degraer et al., 2007). Diversity indices and the density of some species, mainly small, interstitial polychaetes, were negatively impacted. No major impact on the community composition was found.

Due to the effect of the sieving procedure on the benthic characteristics, we tested if sieving alive on a 0.5mm + 1mm sieve compensates for the loss of species. It is expected that a smaller mesh size sieve could nullify the loss of specimens due to sieving alive (Degraer et al., 2007). This is important to know whether a change on long-term basis is caused by a change in sampling procedure.

In this section, we investigated the difference between sieving alive, sieving on 2 sieves (0.5mm+1mm) and sieving fixed, for some long-term monitoring stations.

6.3.1.1.2 MATERIAL AND METHOD

In winter and autumn 2010 6 replicate samples (whereof 3 were sieved fixed and the other three were sieved alive on a 1mm and 0.5mm sieve) were taken at some stations (120, 140, 330, 415, 830, 840, ZG01, ZG04, ZVL),. The species were identified to species level when possible and counted. The macrobenthos sampling and analysis protocol is based on the ISO 16665:2005 standard.

The dataset was standardised and the univariate parameters number of species, density and biomass (wet weight) were calculated. The statistical differences in those benthic parameters between the three sieving procedures was tested with a one-way PERMANOVA (PRIMER6).

6.3.1.1.3 RESULTS

A difference in the benthic parameters between the three sieving procedures was found. There was a decrease in average number of species, density and biomass from sieving fixed over sieving alive with 0.5+1mm sieve towards sieving alive on a 1mm sieve only. An average loss of 29% for diversity and 34% for density was detected when sieving alive compared to sieving fixed. There was also an average loss of 8% for diversity and 16% for density when there is sieving alive on a 0.5+1mm sieve compared to sieving fixed.

Table 16. Permanova results (p level) for the parameter density, number of species (N0), biomass and species composition (ssp com) between the three sieving procedures. The non-significant results (p level >0.05).

N0 / Density	Fixed	Alive 0.5+1mm	Alive 1mm		Biomass/ Spp com	Fixed	Alive 0.5+1mm	Alive 1mm
Fixed		**0.4833**	0.0014		Fixed		**0.8373**	0.0199
Alive 0.5+1mm	**0.6551**		0.0039		Alive 0.5+1mm	**0.6975**		**0.2423**
Alive 1mm	0.0012	0.0019			Alive 1mm	**0.7271**	**1**	

There was no statistical difference between sieving fixed and sieving alive on a 0.5+1mm sieve for the parameters number of species and density (Table 16). Sieving alive on 1mm sieve gives significant lower results for the benthic parameters compared to the other methods. For the parameter biomass (wet weight), there was no significant difference between the sieving procedures. For the species composition there was only a significant difference between the fixed sieving procedure and the sieving alive on a 1mm sieve procedure.

6.3.1.1.4 CONCLUSION

Sieving alive has a clear negative influence on the density and species richness of the samples, compared to sieving fixed. Therefore, caution is needed, in particular in habitats dominated by small, interstitial and/or larger, slender polychaetes. Larger polychaetes, polychaetes with obvious head capsules and appendages and more rigid species, such as amphipods and bivalves, are less prone to the impact (Degraer et al., 2007). Based on the analysis on the effect of the sieving procedure on the benthic characteristics in the main benthic habitats, we can trust that data retrieved when sieving alive on a 0.5 + 1mm sieve is comparable with data retrieved for fixed sieving. Therefore, we can consider that this switch in sieving procedure will have a minor influence on the long term trend analysis at the benthic control stations. Since sieving with two sieves is only used at a certain subset of stations, we have to use conversion factors at the other stations for analyzing a long term trend.

6.4 Quality control macrobenthos analysis (accreditation)

A standardization and harmonization of the analysis procedures between the European countries is necessary, due to the requirements of the European environmental legislation (KRW, MSFD) and its monitoring. Due to the fact that macrobenthos is considered as an important ecosystem element for environmental monitoring in different directives, it is necessary to follow the international standards. Therefore, we adopted the ISO 16665:2005 standard for macrobenthos analysis ("Water quality – Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna"). This guideline was already adapted in our benthic monitoring, except for the quality control and traceability of the samples procedure. A way to fill this in is to obtain an accreditation for certain analyses and lab working. In 2010-2011, our microscopy lab and macrobenthos analyses have been accreditated under BELAC ISO17025 norm (ILVO – DIER – ANIMALAB; CertificaatN°: BELAC T-315). This label is obtained on 24/05/2011. For the extern control of the counting and determination, we participate in the BEQUALM/NMBAQC scheme (UK).

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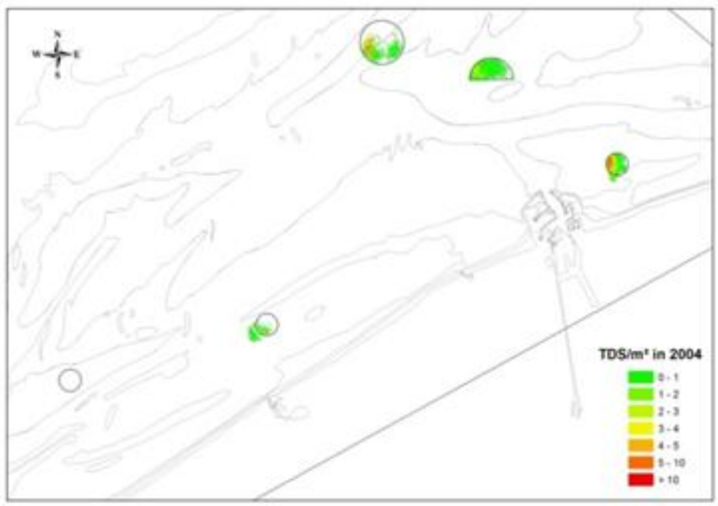
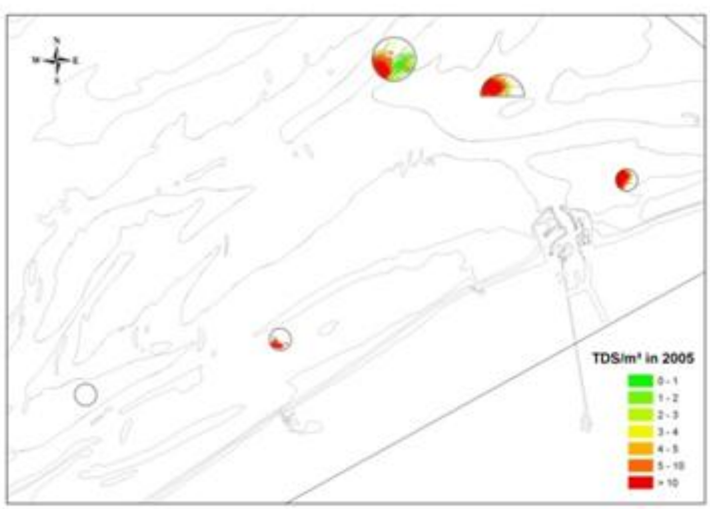
8 ANNEX 1

Annex 1.Dumping intensity maps of the disposal sites (2004-2008):

- Maps of “2004”, “2005” and “2006 ” are based on point observations.
- Maps of “2007” and “2008” are based on polygons
- For 2006; only S1-data was available



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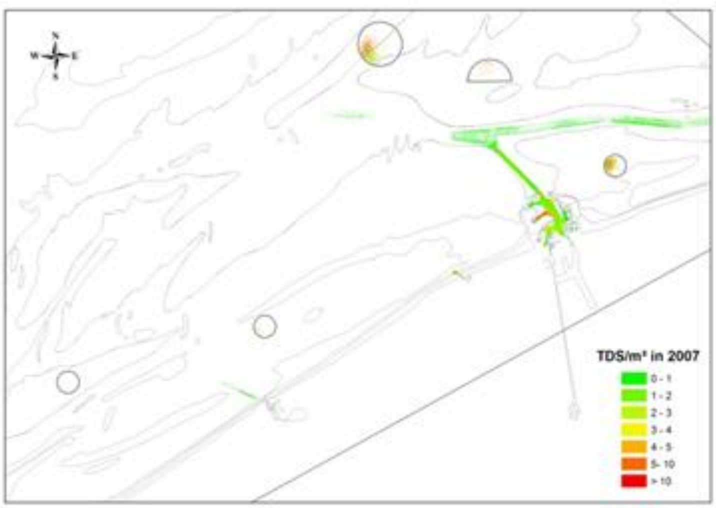
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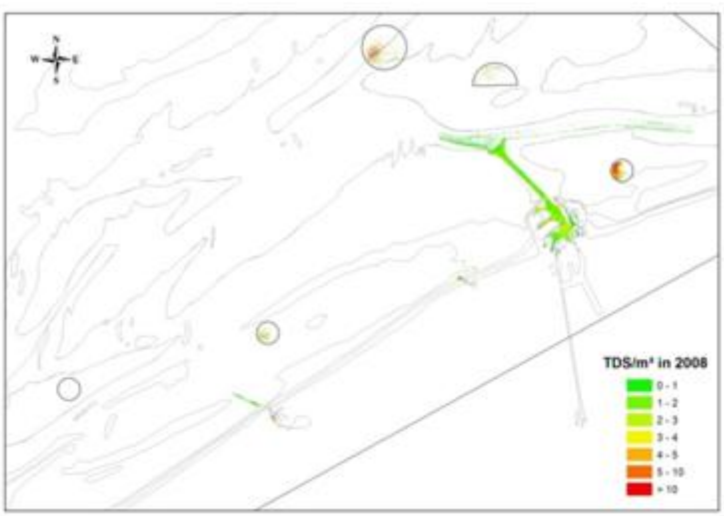
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