FINAL REPORT

Impact of OWEZ wind farm on the local macrobenthos community

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SUMMARY

Univariate comparison of the benthos community in the fishery-closed OWEZ Wind farm with that in six regularly trawled reference areas did not show any difference in total abundances, total biomass and total annual production in the 2011-survey, five years after the closure. Also multivariate species composition, biomass, and annual production in OWEZ did not differ from those in the reference areas. The same holds for various relevant groups of species like the most common, the most uncommon, all epifauna, all infauna, the separate taxa and all scavengers. The bivalve *Spisula solida* was the only species among nine other species specified as being sensitive to trawling that had higher abundances in OWEZ than in two of the reference areas. Shell length of the bivalve *Tellina fabula* and shell width in the bivalve *Ensis americanus* was larger in OWEZ than in three of the reference areas, but four other mollusc species reached their largest dimensions in one of the reference areas. Both diversity indices, Shannon-Wiener and Simpson index, pointed to OWEZ tending to have a higher diversity, higher number of species and higher eveness than two of the reference areas. In general, Triple-D sampling targeting larger-sized, longer-lived in- and epifauna resulted in more differences between OWEZ and the reference areas than boxcoring.

There is no evidence that the species composition in OWEZ when comparing 2007 (one year after the closure) and 2011 (five years after the closure) has changed relative to that in the reference areas. The distinction observed in all areas between the years 2007 and 2011 was mainly due to relatively small variations in species abundances and not caused by the introduction of new species or species loss. Total numbers of individuals, total biomass, and diversity in OWEZ were not different from the values in the combined reference areas in and between 2003, 2007, and 2011.

Five years after the closure to fisheries of OWEZ only subtle impact on the local benthos community can be measured. The faunal patchiness steered by local factors, the depleted adult stocks in the wider region, and a limited time for recovery (5 years) might have delayed the recovery of OWEZ. It cannot be excluded, however, that the higher species diversity, the higher abundances of *Spisula solida*, and the larger sizes of *Tellina fabula* and *Ensis americanus* found in OWEZ relative to those in (some of) the reference areas is a first step towards the recovery of the local benthos community.

SAMENVATTING

Univariate vergelijking van de benthosgemeenschap in het voor visserij gesloten OWEZ Windpark met die in zes regelmatig beviste referentiegebieden heeft geen verschillen in totale dichtheid, totale biomassa en totale jaarlijkse productie aan het licht gebracht in de 2011- bemonstering, vijf jaar na de sluiting. Ook multivariate soortsamenstelling, biomassa en jaarlijkse productie in OWEZ verschilde niet van die in de referentiegebieden. Hetzelfde geldt voor de verschillende relevante selecties van soorten zoals de meest algemene, de minst algemene, alle epifauna, alle infauna, de afzonderlijke taxa, en alle aaseters (scavengers). De tweekleppige *Spisula solida* was de enige soort van de tien voor visserijsterfte gevoelige soorten die in OWEZ hogere dichtheden vertoonde dan in twee van de overige referentiegebieden. Schelplengte van de tweekleppige *Tellina fabula* en schelpbreedte van de tweekleppige *Ensis americanus* was groter in OWEZ dan in drie van de referentiegebieden, maar vier andere mollusken waren het grootst in een van de referentiegebieden. Beide diversiteit indices, de Shannon-Wiener en de Simpson index, gaven aan dat OWEZ tendeert naar een groter aandeel zeldzame soorten, een groter aantal soorten en een hogere “eveness” dan twee van de referentiegebieden. In het algemeen resulteerde de Triple-D bemonstering in meer verschillen tussen OWEZ en de referentiegebieden dan het boxcoren.

Er zijn geen aanwijzingen dat de soortsamenstelling in OWEZ was veranderd ten opzichte van die in de referentiegebieden indien 2007 (een jaar na de sluiting) en 2011 (vijf jaar na de sluiting) vergeleken worden. De in alle gebieden zichtbare verandering tussen 2007 en 2011 werd voornamelijk veroorzaakt door kleine variaties in soortdichtheden, en niet door de introductie van nieuwe soorten of het verdwijnen van soorten. De totale aantallen individuen, de totale biomassa en de diversiteit in OWEZ verschilden niet van die in de gecombineerde referentiegebieden in en tussen 2003, 2007, en 2011.

Vijf jaar na de sluiting van OWEZ voor visserij kunnen slechts subtiele effecten op de lokale benthosgemeenschap worden gemeten. Enerzijds kan het herstel van OWEZ vertraagd zijn door de “patchiness” van de fauna gestuurd door locale factoren, de sterk gereduceerde volwassen populatie in de wijdere omgeving, en de beperkte herstelperiode (5 jaar). Anderzijds kan het niet worden uitgesloten dat de hogere diversiteit aan soorten, de hogere dichtheden van *Spisula solida*, en de grotere afmetingen van *Tellina fabula* and *Ensis americanus*, zoals gevonden in OWEZ in vergelijking met die in (enkele) van de referentiegebieden, een eerste stap in de richting van een herstel van de lokale benthosgemeenschap zijn.

1. INTRODUCTION

In 2006 the Offshore Windfarm Egmond aan Zee (OWEZ) was constructed in the Dutch coastal zone, 10 -18 km offshore Egmond aan Zee just north of the IJ-Geul leading to IJmuiden. The OWEZ Wind farm with a surface area of approximately 5.5*4 km encloses 36 wind turbines with distances of 650-1000 m in between. OWEZ and its 500 m safety exclusion zone were closed to all shipping during the construction phase in 2006 and the entire operational phase. This resulted in an area of approximately 25 km^2 closed for fishery positioned in the coastal zone where trawling with smaller trawlers (EURO-beam trawlers and *e.g.* shrimp trawlers) occurs regularly during the last century (Rijnsdorp et al., 1998; Bergman and van Santbrink, 2000; Kaiser, 2000). Exact estimates of trawling frequency in the surroundings of OWEZ are unknown, but the best estimate of trawling frequency is included in this report.

In an extensive monitoring program, the Monitoring and Evaluation Program (NSW-MEP 2003-2012), the environmental impact of OWEZ Wind farm on the marine ecosystem was monitored. Among the possible impact of OWEZ on the local macrobenthos community (invertebrate in- and epifauna > 1 mm), its closure to fisheries might be one of the key factors since many studies revealed the impacts of trawling on benthos. Field experiments in coastal and offshore areas in the North Sea indicated that beam trawling caused direct mortality in various benthos species and led to *e.g*. instant mortalities up to 20% - 65% of the initial bivalve densities in the trawl track (Bergman and van Santbrink, 2000). Demersal fishing altered seabed habitats and affected the structure and functioning of benthic invertebrate communities in the German Bight (Reiss et al., 2009). Chronic trawl disturbance led to clear changes in community composition of benthic infauna and epifauna in the Irish Sea (Hinz et al., 2009). They concluded that trawl impacts are cumulative and can lead to profound changes in benthic communities, which may have far-reaching implications for the integrity of marine food webs. Hiddink et al., (2006) demonstrated with a theoretical, size-based model and field data from the North Sea that trawling reduced biomass, production, and species richness. The model showed that the bottom trawl fleet reduced benthic biomass and production by 56% and 21%, respectively, compared with a pristine, non-fished situation. Long-term effects of trawling on the composition of the benthic community were clearly demonstrated by comparing the 500 m fishery-exclusion zone around a gas production platform, established more than 20 years ago, with surrounding regularly fished areas (Duineveld et al., 2007). The study showed greater species richness, evenness, and abundances of several burrowing mud shrimp and fragile bivalve species in the exclusion area.

It can be postulated that in the absence of trawling, and thus trawling mortality, higher numbers of specific species - especially those sensitive to trawling - might settle, survive and grow up in the 25 km^2 exclusion area formed by the OWEZ Wind farm. If so, the species composition of the benthos community in the Wind farm will be different from the surrounding, coastal zone. To explore possible faunal deviations in OWEZ both the small-sized, short-lived, often abundant ànd the larger-sized, long-lived, and less abundant benthos species were surveyed, each by means of their specific sampling instrument, the boxcore and the Triple-D dredge (Bergman and van Santbrink, 1994), respectively.

To examine the possible impact of the Wind farm on the macrobenthos community two studies were executed so far: 1) a T_0-field study prior to the construction in 2003 (Jarvis et al, 2004), and 2) a T_1-field study on the short-term (one year) impact in 2007 (Daan et al., 2009). The latter study concluded that OWEZ did not differ in any way from (the majority of) the reference areas. Short-term effects of the construction of the Wind farm on the local benthic fauna composition could not be demonstrated. In parallel study on the short-term (one year) impact of OWEZ, focusing on the recruitment of bivalve species, also no indications were found for any effects (Bergman et al, 2010).

It was anticipated that in OWEZ Wind farm, after being closed to fisheries during 5 years, a measurable change among the long living benthic species could be expected in the present T_2-study in 2011, as this fauna category is considered to be more indicative. Therefore, the present study focuses more than the T_1-study at the larger-sized, often lower abundant species that underwent the specific conditions in an area for longer periods. An extended survey was executed with the Triple-D benthos dredge (Bergman and van Santbrink, 1994), dedicated to the quantitative sampling of larger-sized, more sparsely distributed in- and epifauna. The boxcore programme was reduced compared to T_1, but remained substantial.

In the T_0-study in 2003 the fauna was sampled in OWEZ and only two reference areas at a relatively large distance (Jarvis et al., 2004). As described in Daan et al. (2009) prolongation of this original T_0-

design in the T_1-study in 2007 would have implied that only extremely large differences would be detectable since the difference in the fauna composition between the two reference areas was too large. It was decided therefore to spread the sampling effort in the T_1 over six control areas positioned closer to the Wind farm. In the T_2-field survey the fauna was sampled in the fishery-closed OWEZ Wind farm and in the same six surrounding, regularly trawled reference areas as surveyed in the T_1 study.

In the present NSW-MEP T_2-study we focus on the longer-term impact of OWEZ Wind farm on the benthos community, 5 years after its construction. To determine differences in species composition we compare spring-2011 densities in the non-fished OWEZ with densities in the six surrounding reference areas which are supposed to be subject to regularly trawling. Additionally, shifts between T_0, T_1 and T_2 in the species composition, biomass and diversity of OWEZ relative to the reference areas are explored. As a consequence of the changes in number of sampling areas and type of dredge, data collected with the dredge in the T_0 survey were not used in this comparison.

This Final Report presents the results and conclusions. The section Material and Methods includes a description of the survey areas, the set-up of the field survey, the sampling methods, the subsequent analyses in the laboratory, and describes the statistical methods used to analyse the data. In the chapter Results the various data sets are described, illustrated and statistically analysed. In the Discussion the results are discussed, and possible explanations for observed facts and trends are offered. In the section Conclusions the findings are summarized.

Offshore Wind farm Egmond aan Zee (OWEZ)

2. MATERIAL & METHODS

2.1. Description of coastal zone including OWEZ Wind farm

The OWEZ Wind farm is situated in the coastal zone 10-18 km offshore Egmond aan Zee in water depths slightly less than 20 m depth. The sediment in the Dutch coastal zone north of IJmuiden consists of fine to medium sands (125-500 µm; Duineveld et al., 1990) with some coarse sand patches (500-2000 µm; RGD, 1986). We found an average median grain size of 266 µm ranging from 203 to 370 µm in OWEZ and four surrounding reference areas in October 2007, with an average mud content of 0.92 % and the highest value (8.7%) in the Wind farm area (Bergman et al., 2010).

The macrobenthic fauna in the Dutch coastal zone is relatively rich with a strong gradient of higher values towards the coast (Holtman et al., 1996). Over the last 20 years biomass and abundances show a relatively stable spatial pattern with densities up to 3000 individuals per m^2 and ash free dry weights up to 80 g/m^2 in the near shore zone between the OWEZ Wind farm and the coast (Daan and Mulder, 2006). The OWEZ Wind farm is situated partly in the relatively rich nearshore and partly in the relatively poor offshore zone. Despite the stable spatial gradients over long time-spans, large annual variations in density and biomass of single species have been observed over the last 20 years in the BIOMON monitoring program (Daan and Mulder, 2006).

2.2. Design of the survey areas

In the T_0-study in 2003 the fauna in the area was sampled in only three subareas: OWEZ and two reference areas at a distance of circa 20 km: one area north of OWEZ, the other south of it (Jarvis et al., 2004). As described in the T_1-report (Daan et al., 2009) continuance of this original design in the T_1-study in 2007 would imply that only extremely large differences would be detectable with any statistical significance. A power analysis on the T_0 data showed that the difference in the fauna composition between the two reference areas was too large to statistically detect changes in the test area that could possibly result from the construction of the Wind farm. It was decided therefore to spread the sampling effort in the T_1 over more and smaller control areas positioned closer to the Wind farm. Between OWEZ and R1 two new control areas were designed (R2 and R3), the same took place between OWEZ and R6 (R4 and R5) (Fig. 1). The centres of the four new reference areas were situated at a distance of approx. 9 km from the centre point of OWEZ, with 3.5-5.5 km between the outer borders of OWEZ and reference areas. R1 and R6 are still positioned at the original T_0 areas at a distance of 14 and 18 km from OWEZ, respectively (Fig. 1). Distances between the centres of the reference areas and the coast were approx. 10.5, 15 and 9.5 km (in the north), and 20, 12.5, and 15.5 km (in the south), respectively. To examine the possible impact of OWEZ on in the benthos community after a period of 5 years, a T_2-field survey was executed in 2011. The fauna was sampled in a circa 9 km^2 fishery-free part of OWEZ Wind farm concession area (i.e. well within the area comprising the 36 turbines plus a 500 m restriction zone around) and in the same six surrounding, regularly trawled, circa 2.2-4.4 km^2, reference areas, all positioned between 52.4396° and 52.7336°N and 4.2834° and 4.5082°E (Fig. 1).

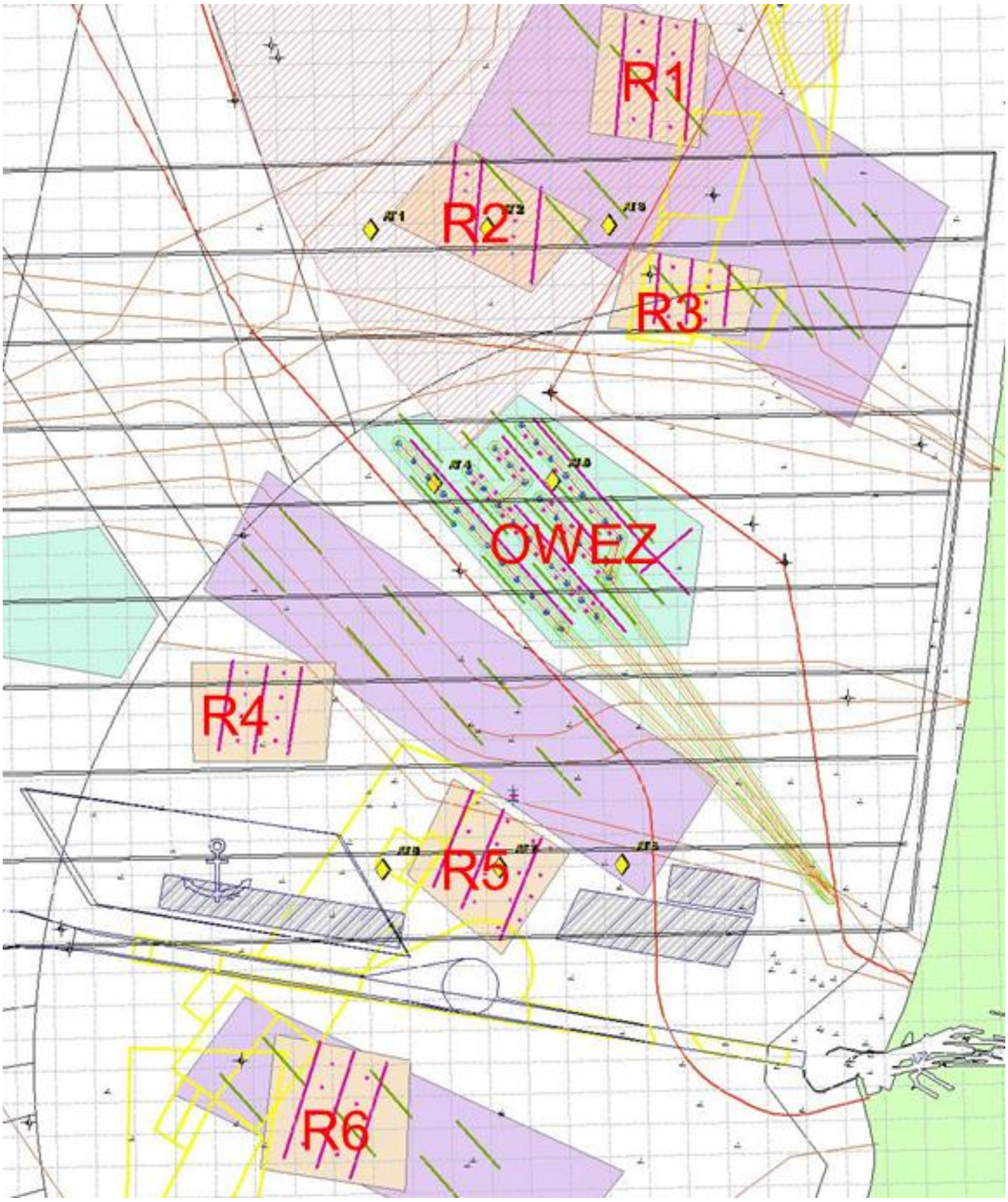


Fig. 1. Map of the study areas showing the OWEZ Wind farm concession area (green) enclosing the area comprising the 36 turbines (blue dots) plus a 500 m restriction zone around them all, forbidden for ships. Further showing the 6 reference areas sampled in 2007 and 2011 (only R1 and R6 were sampled in de T_0 in 2003). OWEZ Wind farm (*i.e.* the area strictly enclosing the turbines plus a 500 m restriction zone around) is closed to fisheries in contrast to the reference areas. N.B. Purple coloured lines and dots in OWEZ and the reference areas refer to small sub-areas characterised as “open” to survey activities (boxcores and Triple-D) by the OWEZ-authorities.

2.3 Trawling activities in and around OWEZ Wind farm

Data on trawling intensity in and around OWEZ Wind park are available from the Vessel Monitoring System (VMS). Trawling intensity near the Dutch coast between 2006 and 2011 cannot be calculated exactly and is probably underestimated, as only from 2011 onwards trawling frequencies of EURO-cutters smaller than 15 m (including shrimp trawlers) are present in the VMS. Prior to that year only trawling frequencies of trawlers >15m, including most of the EURO beam trawlers that are allowed in the coastal area, were registered in VMS. A second reason for a possible underestimate is the fact that only trawlers performing landings in adjacent ports were included. On the other hand the trawling intensity is most likely also overestimated since in coast-nearby regions algorithms do not discriminate between trawling and port approach procedures, so VMS data are in fact data on "presence of trawlers". A rough estimate of trawling activities of EURO cutters (<300 HP, width of beam trawls <4.5 m) in and around OWEZ Wind farm based on the VMS data is given in Fig. 2 (pers. comm. Niels Hintzen, IMARES). Comparing Fig. 2 with Fig. 1, showing the actual configuration of turbines within the OWEZ Wind farm consession area, clearly shows that trawlers indeed remain outside the area where the turbines are positioned inclusive the 500 m restriction zone around them. Fig. 2 indicates that the trawling frequencies in the reference areas fit well in the regular trawling frequencies in the adjacent coastal areas.

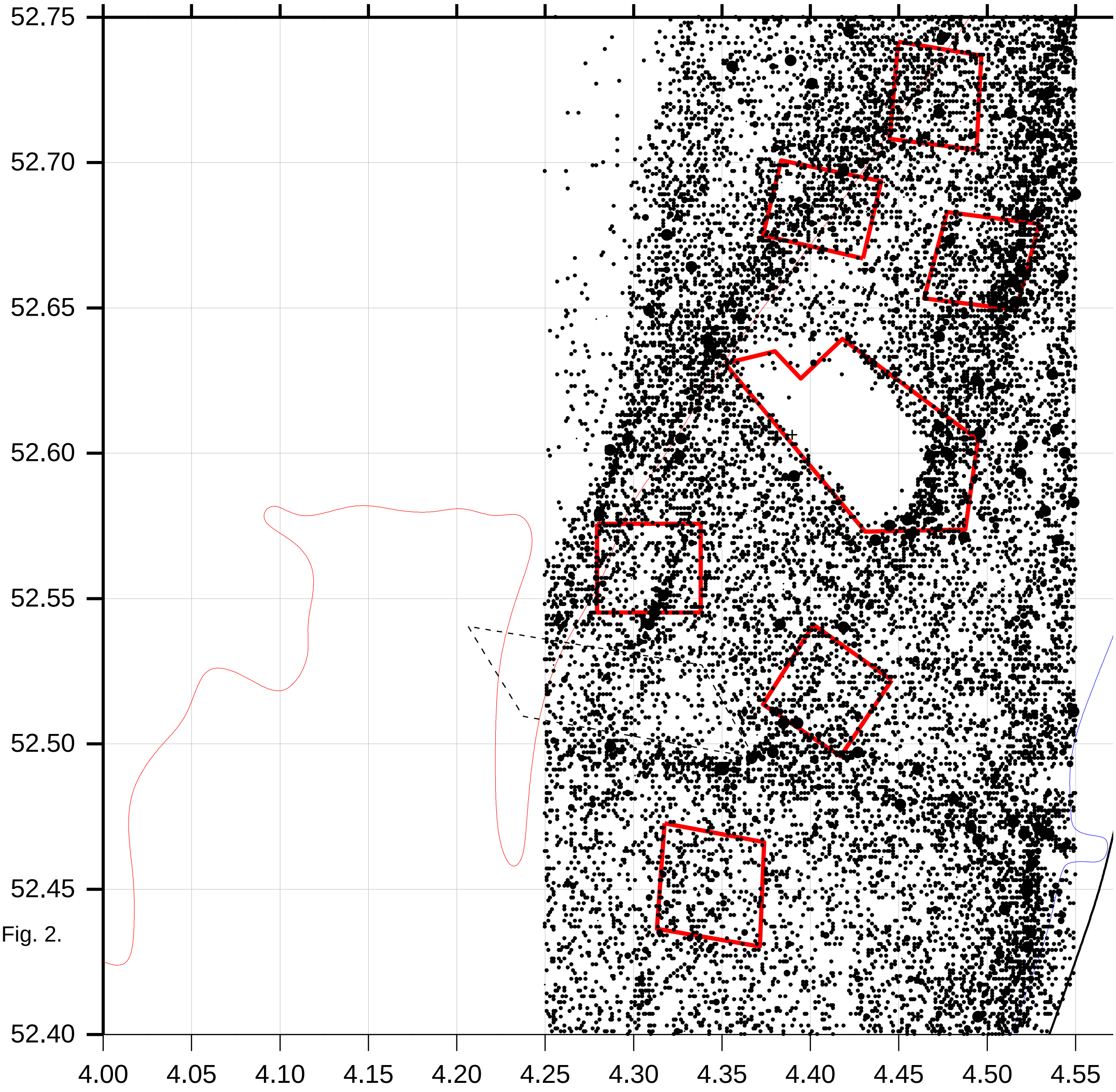


Fig. 2.

Estimate of spatial distribution of "presence of trawlers" cumulated over the years 2006-2011 when OWEZ was closed for trawling (based on VMS data provided by IMARES). The map presents a rough estimate of trawling activity of EURO trawlers in 2006-2011 in and around OWEZ and the 6 reference areas. Classification in total number of minutes in the period 2006-2011: ● 0-100, ●>100-200, ●>200-300, and ●>300. The 10 m isobath (blue) and 20 m isobath (red) are indicated.

2.4 Field survey

sampling methodology

It was anticipated that in the OWEZ Wind farm, after being closed to fisheries during the last 5 years, a measurable change could be expected among larger sized, long living benthic species that endured the fishery-free conditions in OWEZ for several years. The emphasis in the present T_2-study was therefore, in contrast to the T_1-study, on the Triple-D programme designed to sample quantitatively larger-sized, longer living macrobenthos ("megafauna"). During the surveys in T_1, and T_2 the numbers of Triple-D dredge samples in OWEZ were similar (14), in the reference areas the number of hauls increased from 2 to 6. Dredge samples collected in T_0 were not included in this report because of changes in sampling design and type of dredge between T_0 and T_1/T_2 (see 2.2). The boxcore programme in T_2 was reduced compared to T_1, but remained substantial. During the entire sampling series in T_0, T_1, and T_2 the numbers of boxcore samples declined: in OWEZ from 68, via 30, to 16, and in the reference areas from 25, via 15, to 8.

Fieldwork was carried out in the period from 17- 25 February 2011 on board of the research vessel PELAGIA (NIOZ). Aim of the cruise was to collect benthic samples inside OWEZ Wind farm and the six reference areas (Fig. 1). The benthic macrofauna was sampled with a boxcorer and a Triple-D benthos dredge (Bergman and van Santbrink, 1994). For safety reasons all samples were taken more than 300 meters away from the turbines and thus hard substrate species related to the gravel beds around and to the turbines itself (Bouma and Lengkeek, 2012) were missed in the sampling.

boxcore
The boxcorer (sample size 0.078 m^2, diameter 30.5 cm, sampling depth >15 cm) was used to sample the small (generally 1 to 10 mm) more or less abundant fauna species. The box-cores were sieved over a 1 mm sieve (Fig. 3). The residue was preserved on a neutralized 6% formaldehyde solution and taken to the laboratory for identification and further analyses.

Fig. 3. Processing the samples of the Reineck boxcorer (sample size 0.078 m^2).

Sampling stations for boxcoring in the present T_2 study were chosen out of the boxcore stations used in the T_1-study described in Daan et al. (2009). The 16 T_2 boxcores in OWEZ were selected from the 30 boxcore stations in the T_1-study, one boxcore per station (Fig. 4). The stations were arranged along

5 transects running parallel to the rows of wind turbines. The eight T_2 boxcores in each of the six reference areas were selected from the 15 boxcore stations in the T_1-study. The boxcore stations were arranged along three parallel transects in each reference area, one boxcore per station. Out of every boxcore a sediment core (2.5 cm diameter) of the upper 10 cm was taken. The samples were immediately frozen at -20°. In the laboratory the median grain size and the percentage of mud (particles <63 μm) were determined. Water depth at the boxcore stations was taken from the ships echo sounder. Geographical positions of the stations were taken from the ships logbook (Appendix 1).

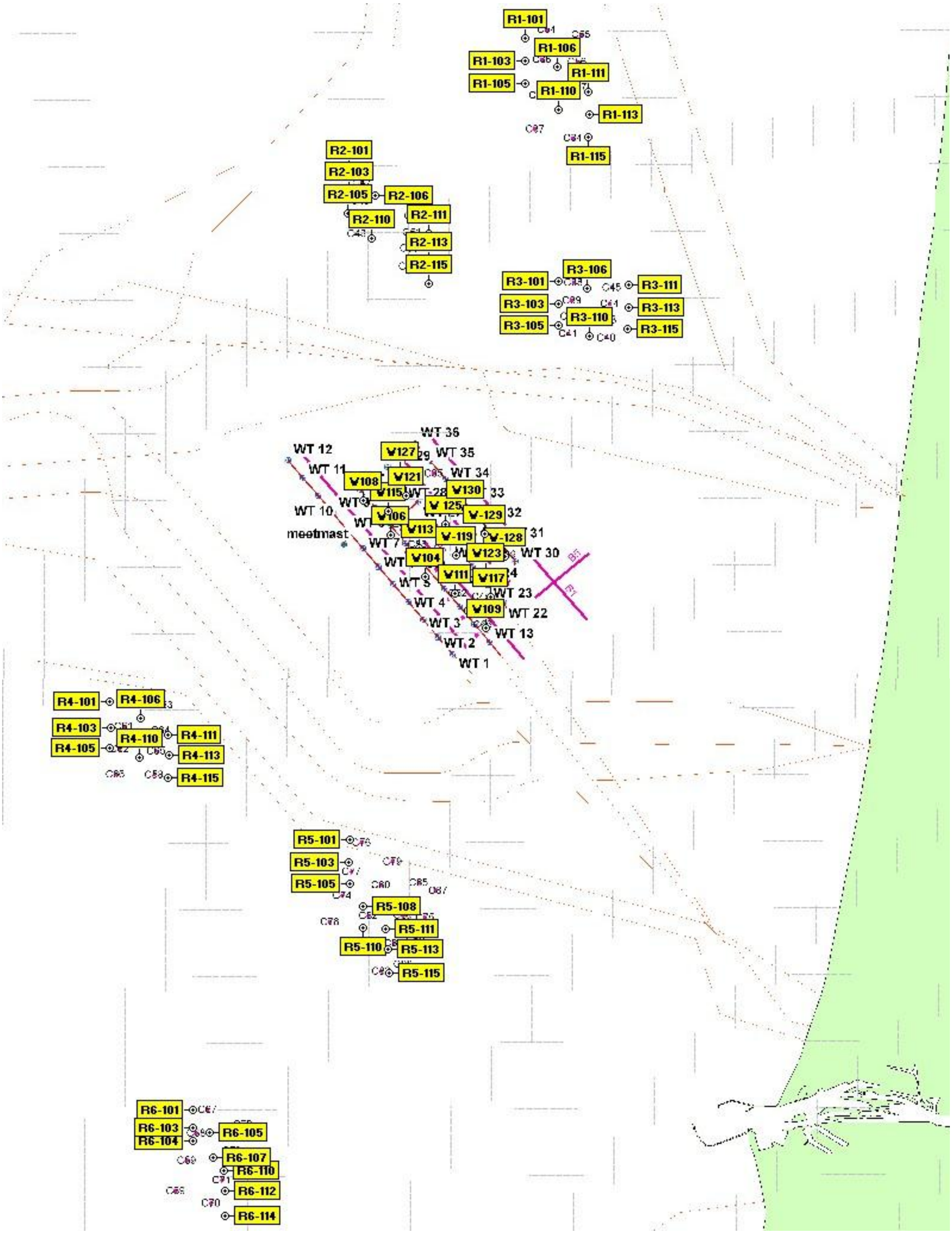


Fig. 4. Boxcore stations survey 2011.

Triple-D

The Triple-D was used to sample the less abundant and larger species, which cannot be sampled quantitatively by a boxcorer because of its small sample size. The Triple-D cuts effectively a 18-20 cm deep strip sediment from the seabed with a width of 20 cm. This sediment is washed through the meshes of the 6 m long net with a mesh size of 7x7 mm before taken onboard. Larger specimens (“megafauna”) both in- and epifauna are retained in the net. The dredge is equipped with a measuring wheel and a pneumatic opening/closing mechanism that ensures the exact length of a dredge haul. Haul length was 100 m, so each sample represented the fauna present in 20 m^2. The catches were sorted, identified and counted on board and individual lengths of specimens in the samples (or in subsamples) were measured. Blotted wet weight per species was measured for each sample on board. For crabs the carapax width, for hermit crabs the length of the propodus, for the bivalve *Ensis* spp. the shell width, and for the bivalve *Lutraria lutraria* the siphon width was measured to the nearest mm. For all other species total body length was measured to the nearest mm (Fig. 5). Some species were taken back to the laboratory for further taxonomic identification.

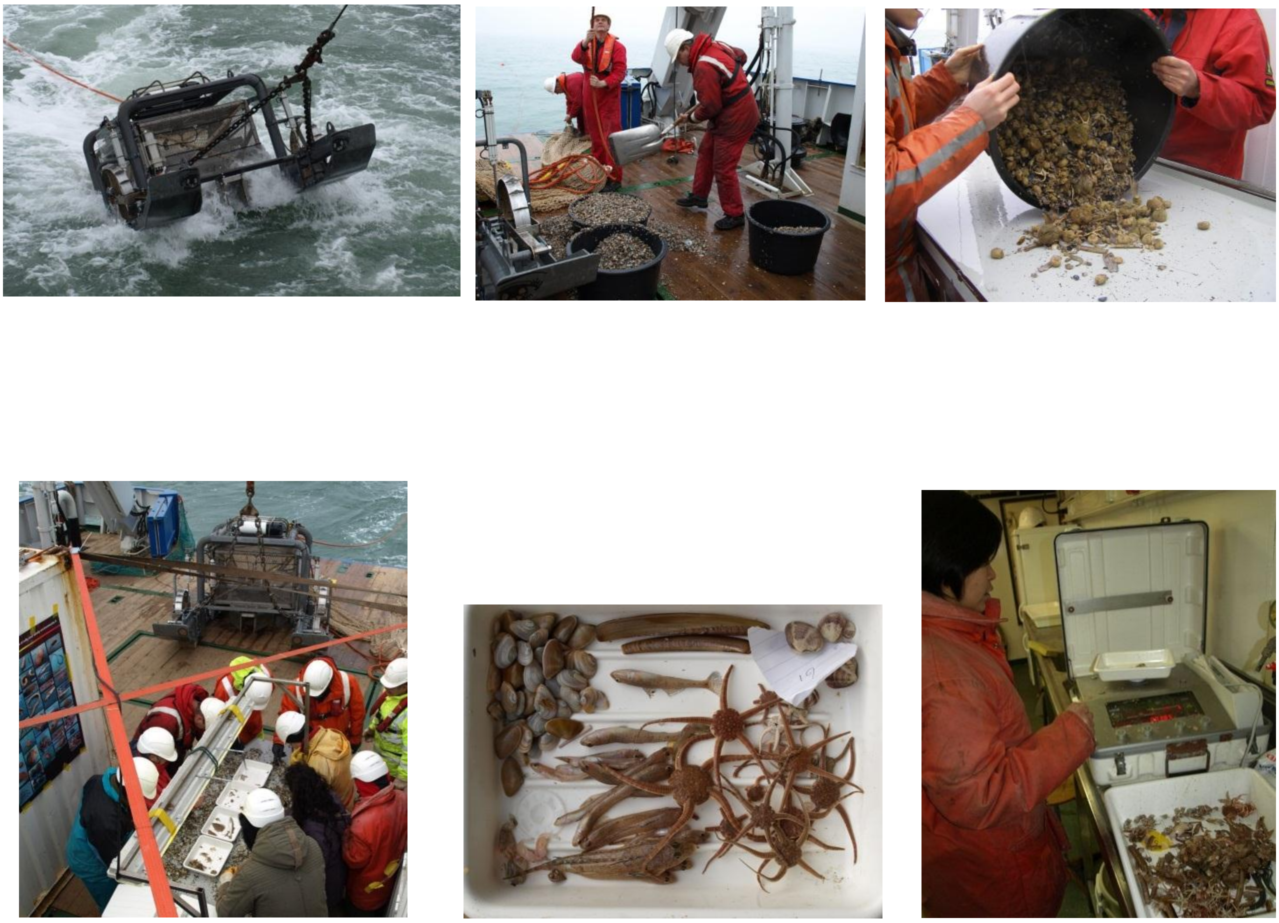


Fig. 5. Processing the Triple-D catches (sample size 20 m^2).

Triple-D hauls were made in the same 14 stations along 3 transects in OWEZ Wind farm as in the T_1-study (Fig. 6). In the six reference areas 2 of the 6 dredge stations were positioned on top of the only 2 stations sampled in the T_1-study. The other 4 stations were evenly distributed over each of the reference areas. One dredge haul was performed in each station.

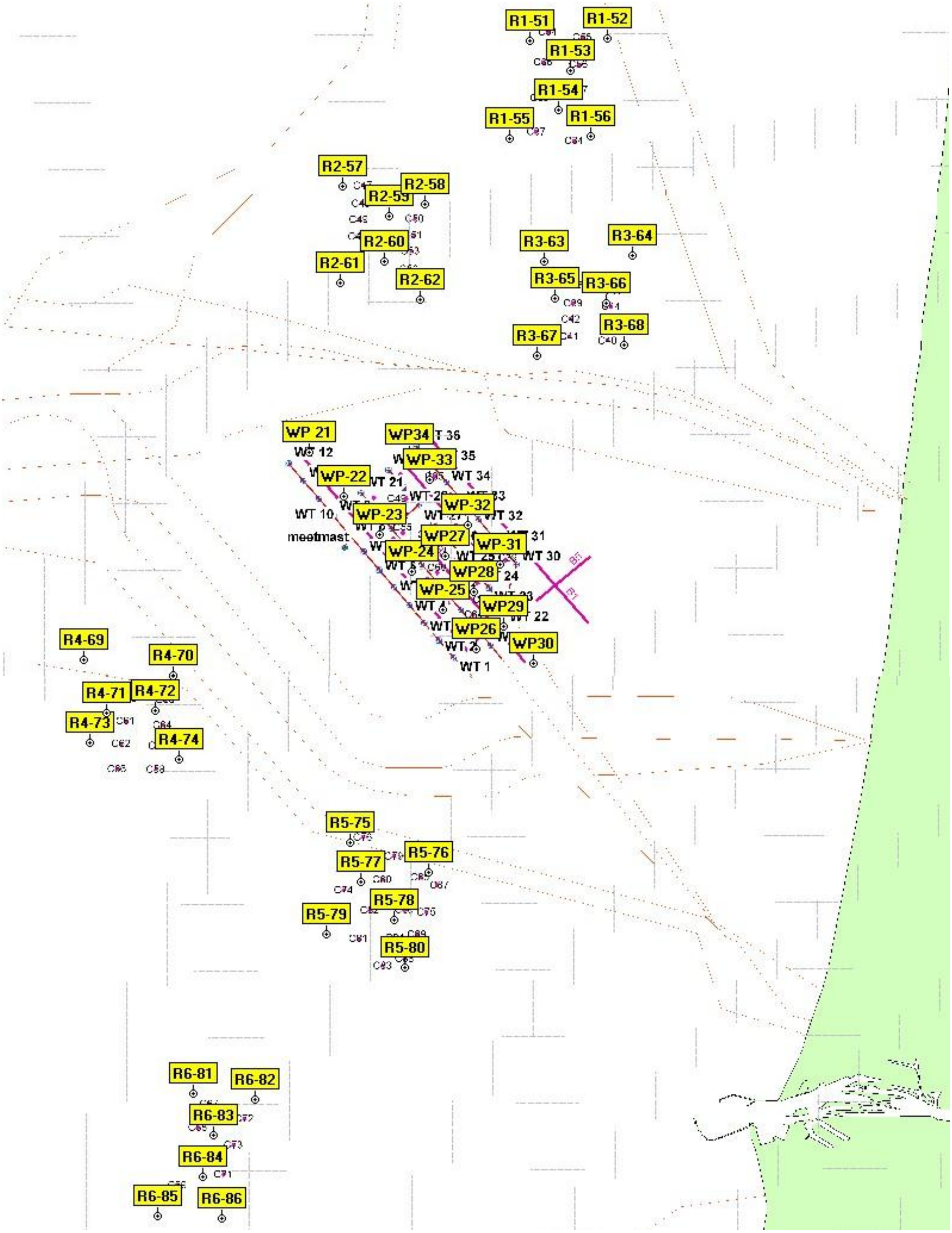


Fig. 6. Triple-D stations survey 2011.

sample treatment

sediment

The sediment samples were freeze-dried up to 96 hours till dry. Prior to grain-size analysis an amount of between 0.5 and 5 gram of homogenized sample, depending on the estimated grain size, was weighed over a 2 mm sieve. No acidification or peroxide was practiced. Reversed Osmosis (RO) water water was added and the sample was shaken vigorously on a vortex mixer for 30 seconds. Median particle size and the percentage mud (fraction < 63 mµ) of sediments were determined using a Coulter LS 13 320 particle size analyzer and Autosampler. This apparatus measures particle sizes in the range of 0.04–2.000 µm in 126 size classes, using laser diffraction (780 nm) and Polarization Intensity Differential Scattering (PIDS) (450 nm, 600 nm and 900 nm) technology.

boxcore fauna

The boxcore samples were treated in the laboratory in the same way as the samples from the 2007 survey (Daan et al., 2009). To facilitate the sorting process, samples were stained with Bengal Rose at least 24 hours prior to sorting. Samples containing benthos individuals, shell debris and dead material were sieved over a set of five nested sieves with different mesh size (11.2 mm, 6.7 mm, 2 mm, 1 mm and 0.7 mm). Under an illuminated magnifying lens individuals were collected from every fraction down to 1 mm sieve and sorted in five basic categories of species. The 0.7 mm sieve fraction was preserved for potential future analyses. Subsequently the macrofauna was identified to species level when possible under a stereomicroscope. The most common categories (polychaetes, crustaceans, molluscs and echinoderms) were identified to species level. Juveniles and damaged animals, which because of their small size could not be identified to species level, were identified at higher taxonomic level (usually the genus). Complicated taxa like anthozoans, phoronids, oligocheates, nemerteans and turbellaria where identified on their taxon level. All individuals were counted. Individual lengths (mm) of molluscs and echinoids were measured. Blotted wet weights of polychaetes, larger crustaceans, and ophiuroids were measured with a Mettler PJ300 balance to the nearest mg, and remaining taxa were determined per species/group. Small crustaceans (amphipods and cumaceans) were only counted.

Biomass values were expressed as ash free dry weight (AFDW). Total AFDW per boxcore was calculated by aggregation of the AFDW per species/taxon identified, each acquired by conversion of lengths for molluscs and echinoids, and of wet weight (WW) for other taxa. Conversion factors for species and taxa were derived from Daan et al. (2009), and if needed extended and updated with data from Ricciardi and Bourget (1998), Rumohr et al. (1987) and from unpubl. NIOZ-data (see Appendix 2a). For small crustaceans (*e.g.* amphipods, cumaceans) an average AFDW of 0.2-0.5 mg per individual was used (see also Daan et al., 2009).

Estimates of the annual production of the benthic community were obtained from empirical relations between production, total biomass and individual weight of species/taxa. Production per species was calculated by using annual Production/Biomass (P/B) ratios derived from Brey's multi-parameter P/B-model (Brey 1999, 2001; Appendix 3). To obtain the annual P/B ratio per species, the average energy content (kJ) was derived from the AFDW (g) for all individuals of a species found in the 2011 survey. Annual production ($kJ/m^2/y$) was then calculated by multiplying the annual P/B ratio with the energy content per unit area (kJ/m^2) per species per sample.

Triple-D fauna

Catches of the Triple-D dredge were sorted and identified on board. For each dredge haul individual length (mm) of all animals and blotted wet weight per species were measured on board. All data were included in a databank on board. Besides some checks on taxonomic accuracy no further laboratory handling of the samples was necessary.

AFDW per species per dredge haul was calculated by conversion of the wet weight (WW) measured on board. Conversion factors were derived from Daan et al. (2009), Ricciardi and Bourget (1998), Rumohr et al. (1987), and from unpubl. NIOZ-data (see Appendix 2b).

Annual production was estimated by using Brey's multi-parameter P/B-model (Brey 1999, 2001). To obtain annual P/B ratios per species the average energy content (kJ) was derived from the AFDW (g) for all individuals of a species found in a sample (haul). In contrast to the P/B ratios for the boxcore fauna, the P/B ratios for the Triple-D fauna were calculated based on the average body mass (kJ) per species <u>per haul</u>. This is considered as a more precise method because higher body masses tend to have relatively lower P/B ratios and vice versa. Annual production ($kJ/m^2/y$) was then calculated by multiplying the annual P/B ratio with the energy content per unit area (kJ/20m^2 or kJ/m^2) per species per sample (haul).

2.5 Statistical analyses

Benthos and environmental data obtained during the survey 2011 were statistically evaluated with respect to differences between OWEZ and the six reference areas using multivariate and univariate techniques. A summary of the main hypotheses, given as short research questions, and the statistical methods used for testing are given in Table 1. Several additional tests (BEST-analysis, CLUSTER-analysis and SIMPER-analysis) are not included in the Table and described in the next paragraphs.

Type of data	Research question: do differences exist between OWEZ and the reference areas?	Statistical method
Boxcore data		
environmental factors 2011	median grainsize, mud, depth and distance to coast	- box and whisker plots - Kruskal Wallis test
fauna 2011	abundance, biomass, and production	multivariate asymmetrical nested PERMANOVA test
fauna 2011	total density, total biomass, total production, number of species, Shannon-Wiener, and Simpson index	- box and whisker plots - one-way ANOVA test
comparison fauna 2003-2007-2011	total density, total biomass, number of species, Shannon-Wiener, eveness and Simpson index	- box and whisker plots*
comparison fauna 2007-2011	abundance	multivariate crossed and mixed PERMANOVA tests
Triple-D data		
fauna 2011	abundance, biomass, and production	multivariate asymmetrical nested PERMANOVA test
fauna 2011	abundances of various selections of species: separate taxa, (un)common species, epifauna, infauna , scavengers, species vulnerable for trawling,	multivariate asymmetrical nested PERMANOVA test
fauna 2011	total density, total biomass, total production, number of species, Shannon-Wiener, and Simpson index, lengths of bivalves, density of species vulnerable for trawling	- box and whisker plots - one-way ANOVA test

Table 1. Summary of the main hypotheses, described as short research questions, and the statistical methods used for testing.*reference areas pooled.

environmental factors

To explore univariate differences in abiotic variables among the areas (*i.e*. OWEZ and the six reference areas) data on median grain size, mud content, water depth, and distance to the shore are presented in notched box and whisker plots representing a multiple comparison of median values and their 95% confidence intervals. These analyses were executed with SYSTAT software for windows, version 13. In such plots outliers are denoted as follows: near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3) with *****, far outliers exceeding 3 times the IQRs from Q1/Q3 with **o**. To test the differences on their statistical significance we applied non-parametric tests Kruskal-Wallis analysis of variance tests on non-transformed data, using SYSTATv13 and the EXCEL/Analyse-it software packages. A non-parametric test was chosen as the data were not normally distributed and in some cases (*i.e.* mud content) zero inflated. Significant differences were indicated by p- values <0.05. In case of a significant difference a pairwise Kruskal-Wallis test with a Bonferroni adjustment was performed to assess which of the pairwise areas were different.

boxcore data

multivariate tests 2011 data
Multivariate analyses using Plymouth Routines In Multivariate Ecological Research (PRIMER™) software version 6 (Clark and Gorley, 2006), and PERMANOVA A+ for PRIMER software (Anderson et al., 2008) were executed to test differences between OWEZ and reference areas with respect to boxcore abundance (per m^2), biomass (g AFDW per m^2) and annual production (kJ per m^2 per year) data. For this analyses all data were rearranged into matrices (Appendix 4a). The PRIMER routine is free of assumptions on data distribution (like normality or homogeneity of variances).

To explore statistically significant differences in abundance, biomass and production of the benthos community between OWEZ Wind farm and the reference areas a Bray-Curtis similarity matrix was generated. Data was 4^{th} root-transformed to reduce the effect of dominant species. A non-metric multi-dimensional scaling plot (MDS) visualizes the Bray Curtis distance between the different samples. Different colour and symbols are given to the marks within the plot to distinguish between the different areas. To essentially proof if OWEZ significantly differs from the reference areas a PERMANOVA test was performed. Two factors were included in the design for this test. The first factor “IvC” divides the samples in two categories: Impact and Control. OWEZ is revered to as “Impact” and the reference areas are revered to as “Control”. The second factor is called “Area” and divides the samples in 7 different areas namely: OWEZ and Refs 1 to 6 .This test was designed to 1) test the significance of variability in benthos structure among all areas and 2) to detect significant differences between Impact area (OWEZ Wind farm) and Control areas (reference areas). The test design is shown in Table 2. The test is called asymmetrical because there is only one Impact area compared to several Control areas.

Factor	Nested in	Fixed/random	Contrast
IvC	-	Fixed	-
Area	IvC	random	-

Table 2. Asymmetrical design for PERMANOVA test

In order to examine the best match between the variance in species composition among the stations and the environmental variables associated with these stations (mud content, median grain size, water depth, distance to coast) we used the multivariate BEST analysis (PRIMER™ v6 Clark and Gorley, 2006). In this analysis the BIOENV correlation was chosen that calculates Spearman’s rank correlations between the sample Bray-Curtis similarity matrix based on species abundances and the different combinations of measured environmental variables. Highest values for ρ (rank correlation coefficient) mark the environmental variables that best explain the species composition among the samples. The statistical significance of the ρ is calculated in relation to permutations (n=999) simulating the null hypothesis. In the BEST analysis abundance data derived from boxcoring could be used since the environmental data were directly derived from boxcore samples (median grain size, mud content) or taken at boxcore stations (water depth, distance to coast) and therefore more accurate. BEST analysis was performed on the total data set and next to that on the data sets from OWEZ and the reference area separately.

univariate tests 2011 data
To test univariate measures for statistical significant differences between samples from different areas, i.e. OWEZ and the separate reference areas, one way analyses of variance (ANOVA) were performed (SYSTATv13). Tests could only be performed if data met the assumption of the normality and homogeneity of variance (Zuur et al., 2010). Assumptions on homogeneity and normality of residuals were assessed by graphs. Notched box and whisker plots visualize the possible differences between OWEZ and reference areas (SYSTATv13). Data of abundances, biomass and production were log-transformed before testing. Univariate tests were performed on:

- total density per m^2
- total biomass per m^2
- total production per m^2

One way analyses of variance (ANOVA) were also performed to test some univariate diversity indices. Three diversity indices were chosen with the simplest one being species richness, i.e. the number of species per sample. Next to that the Shannon-Wiener index, most commonly used to quantify diversity, is selected (Shannon and Weaver 1949; Morin 1999). This index takes into account both the number of species in a community and the degree of evenness, *i.e*. the way that individuals in a community are distributed among species. The third one, the Simpson index (λ) is particularly sensitive to the abundance of the commonest species and can therefore be regarded as a measure for dominance (Hill, 1973). We choose to use its complement 1-λ which represent the possibility that two randomly chosen individuals are of different species. A high index points to high diversity and evenness. Assumptions on homogeneity and normality of residuals were assessed by graphs. Notched box and whisker plots visualize the possible differences between OWEZ and reference areas (SYSTATv13). Data of total number of species and Simpson (1-λ) were log-transformed before testing, data of Shannon Wiener index were not transformed. Univariate tests (SYSTATv13) were performed on:

- total number of species per sample
- Shannon-Wiener (^{2}Log base) per sample
- Simpson index (1-λ) per sample

<u>*tests comparing data 2003-2007-2011*</u>

Sediment data were not included in the statistical tests since median grain sizes measured in T_0 (on average 504 μm; Jarvis et al., 2004) did not fit into the sediment analyses obtained in the long-term monitoring program BIOMON in 2003 and 2006 (on average 250.8 μm and 254.2 μm respectively; Daan and Mulder, 2004; unpubl. 2006-data) and also not in the dataset obtained in the October 2007 - survey in OWEZ and the reference areas (on average 266 μm; Bergman et al., 2010). The reason for the inconsistency between the analyses of Jarvis and these study's is unknown. In the T_1 in 2007 sediment analyses from boxcore samples were not taken (Daan et al, 2009)..

To explore differences between T_1 (2007) and T_2 (2011), based on surveys using similar designs, multivariate statistics were executed using PRIMER™ software version 6 (Clark and Gorley, 2006), and PERMANOVA A+ for PRIMER software (Anderson et al., 2008). The matrix used for this comparison was an abundance data matrix that contains all species per sample in both years. Data was 4th root-transformed before a Bray-Curtis similarity matrix was generated. To visualize the changes over the two years, the centroids (*i.e*. "gravity" centres representing all stations belonging to one particular area) of the areas were plotted in a MDS-plot. A PERMANOVA test was applied to test if the years differ from each other and if there were differences between the areas (including OWEZ) To do so, next to the factor 'Area' a new factor 'Year' was added, which divided the samples into those from year 2007 or 2011. The resulting interaction term Year*Area makes clear whether one of the areas diverged further or in a different direction over time than the other areas. The following PERMANOVA design was used (Table 3):

Factor	Nested in	Fixed/random	Contrast
Year	-	Fixed	-
Area	-	Fixed	-

Table 3. Two way crossed design for PERMANOVA test

To further explore if the benthos assemblages in particular OWEZ were different from the reference areas a mixed design was tested in PERMANOVA (Table 4). In such tests the partitioning can be done in different ways, the so called "Types" of sums of squares (SS). The test was done twice: with the most conservative version Type 3 SS, and the most sensible version Type 1 SS. The latter test was executed with Impact versus Control (IvC) as the first factor, meaning that the potential overlap in variability will contribute to that factor giving it the best chance to show a statistical significant difference.

Factor	Nested in	Fixed/random	Contrast
IvC		fixed	
area	IvC	random	
year		fixed	

Table 4. Three-way mixed design for PERMANOVA test

As an alternative for latter design we explored also if the benthos assemblages in OWEZ in 2011 were different from the fauna composition in OWEZ in 2007 plus reference areas in both 2007 and 2011 (suggested by Onno van Tongeren, WD, pers comm.). In this design we presume a “before impact” situation in 2007, although it was more than 1 year after the closure to fishery of OWEZ. Under the supposition that only OWEZ in 2011 will be affected its benthos composition is tested against all other areas in 2007 and 2011, including OWEZ in 2007 (“after*impact”). We also tested whether the pooled reference areas in 2007/2011 were different from OWEZ (“impact/pooled refs”; Table 5).

Factor	**Nested in**	**Fixed/random**	**Contrast**
before/after		fixed	
impact/pooled refs		fixed	
after*impact		fixed	

Table 5. Three-way design for PERMANOVA test

.
As explained in Daan et al. (2009) and briefly in section 2.2 the number of only two reference areas as used in the T_0-study in 2003 was too low, and the difference in the fauna composition between these two reference areas was too large, to statistically detect changes in the Wind farm that could possibly result from its construction. As a consequence, the authors stated that statistical comparisons of T_0 data with data from the T_1 (2007) and consequently also with T_2 (2011) surveys are disputable or even senseless. Yet, to explore possible differences between OWEZ and reference areas in the consecutive years of surveying 2003, 2007, and 2011 univariate tests (SYSTATv13 software) were used. Total number of individuals per m^2, total biomass per m^2, and a number of diversity indices are visualized in notched box end whisker plots showing 95% confidence intervals of the median value in order to compare OWEZ with the pooled reference areas.

Triple-D data

multivariate tests data 2011
Multivariate analyses (PRIMER™ v6 (Clark and Gorley, 2006), PERMANOVA A+ for PRIMER (Anderson et al., 2008)) were executed to test Triple-D data on abundance (number per 20 m^2) (Appendix 4b), biomass (AFDW per 20 m^2) and annual production (kJ per 20 m^2 per year) on differences between OWEZ and the reference areas. The procedure is identical with that described in the multivariate tests on the Boxcore 2011 data (Table 2).

Next to tests including all species, similar multivariate PERMANOVA tests on the abundance data of relevant selections of species were applied. The following groups of species were selected:

- four taxa separated (crustaceans, polychaetes, echinoderms, and molluscs)
- common species (i.e. the 15 species contributing more than 10% to the total abundances at least in one station)
- the 30 most uncommon species
- ten species most sensible to trawling (derived from Bergman and van Santbrink, 2000)
- epifauna species
- infauna species
- scavenger species

To explore if other factors than the presence/absence of OWEZ wind farm are involved in the clustering of the stations in terms of species composition a multivariate CLUSTER analysis was performed PRIMER™ v6 (Clark and Gorley, 2006). Hierarchical clustering of the stations from all areas was performed on the basis of a Bray-Curtis similarity matrix and samples were regrouped according to their 67% similarity. Prior to analysis, abundance data were square root transformed. With the SIMPER routine (Clark and Gorley, 2006) we examined the contribution of individual species to the separation between the newly formed clusters, and listed the species that contributed most to the dissimilarities. MDS plots of the newly formed groups were made to visualize the Cluster results. Abundances of the six species that contributed most to the divergence of the four groups were superimposed upon these MDS plots. Next to that total abundance per haul, total species richness per haul, median grain size and mud content were superimposed upon the MDS plots.

univariate tests data 2011

To test for differences in univariate measures between areas, and between OWEZ and the reference areas, one way analyses of variance (ANOVA) were performed, providing data met the assumption of the normality and homogeneity of variance. Assumptions on homogeneity and normality of residuals were assessed by graphs. Data on abundance, biomass, production, total number of species and the Simpson (1-λ) were log-transformed before testing, data of Shannon Wiener index were not transformed. Notched box plots demonstrate the possible differences between OWEZ and the reference areas. Univariate tests (SYSTAT v13) were performed on:

- total density per m^2
- total biomass per m^2
- total production per m^2
- diversity indices: total amount of species per haul, Shannon-Wiener (^{2}Log base) per haul, and Simpson index (1-λ) per haul
- lengths of five bivalve species (*Chamelea striatula, Tellina fabula, Donax vittatus, Ensis americanus*) and the gastropode *Nassarius reticulatus*
- abundances of ten species sensible to trawling derived from a study on direct mortality in the trawl path (Bergman and van Santbrink, 2000)

3. RESULTS

3.1 Environmental factors

In Fig. 7 the water depth and distance to the coast of the stations within the Wind farm and the six reference areas are depicted in notched box and whisker plots. Median values, notches that mark 95% median confidence intervals, and outliers are indicated.

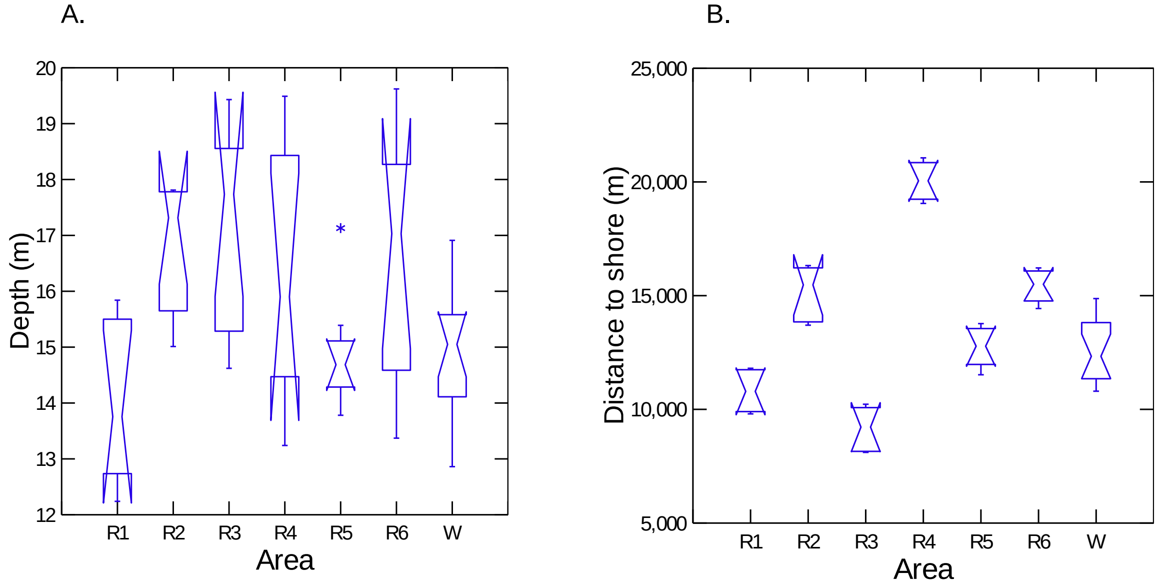


Fig. 7. Notched box and whisker plot, with median and notches that mark 95% median confidence intervals based on non-transformed data of A) water depth (m) and B) distance to shore (m) in the OWEZ wind farm and the six reference areas. *near outlier between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3).

Water depths (Fig. 7A) ranged from 12.2 to 19.6 m. A Kruskal-Wallis test on non-transformed data revealed significant statistical differences between some of the survey areas (p=0.003). A pairwise comparison (Bonferroni adjusted) indicated that R1 was significant different from R2 and R3 (p= 0.023 and 0.015, respectively). Distance to shore (Fig. 7B) varied between circa 9 and 20 km. A Kruskal-Wallis test showed significant differences between some of the areas (p<0.0001). Bonferroni test for pairwise comparison revealed that all survey areas differed significantly (p<0.026) from each other except the following pairs: OWEZ versus R5, R2 versus R6, and R1 versus R3.

The spatial distributions were made of median grain sizes (µm) and mud content (% < 63 µm) derived from sediment samples collected from each boxcore station in OWEZ and the reference areas. Median grain sizes ranged from 185 to 318 µm (Fig. 8), with mean of 264.5 µm (st.dev. 17.6). The spatial distribution of sizes demonstrate that OWEZ fits well in the range of medians found in the reference areas, with R1 tending to finer and R6 to coarser medians. The notched boxplot (Fig. 10A) suggests that R1 seems to differ from all other areas except R2 and R6. A Kruskal-Wallis test, however, revealed there was no significant difference (p=0.086) between the median grain sizes among the stations in the different survey areas. Mud content analyses revealed that 21 out of 64 samples contained mud with percentages up to 8.2 % (Fig. 9). The spatial distribution of mud content demonstrate that OWEZ fits well in the total range of values found in the reference areas, with R1 showing 0% mud and R6 showing relatively high mud contents. The notched boxplot (Fig. 10B) suggests that R1 seems to differ from R6. A Kruskal-Wallis test revealed significant differences between some of the areas (p=0.023). A pairwise comparison (Bonferroni adjusted) demonstrated that R1 and R6 are significantly different (p =0.013).

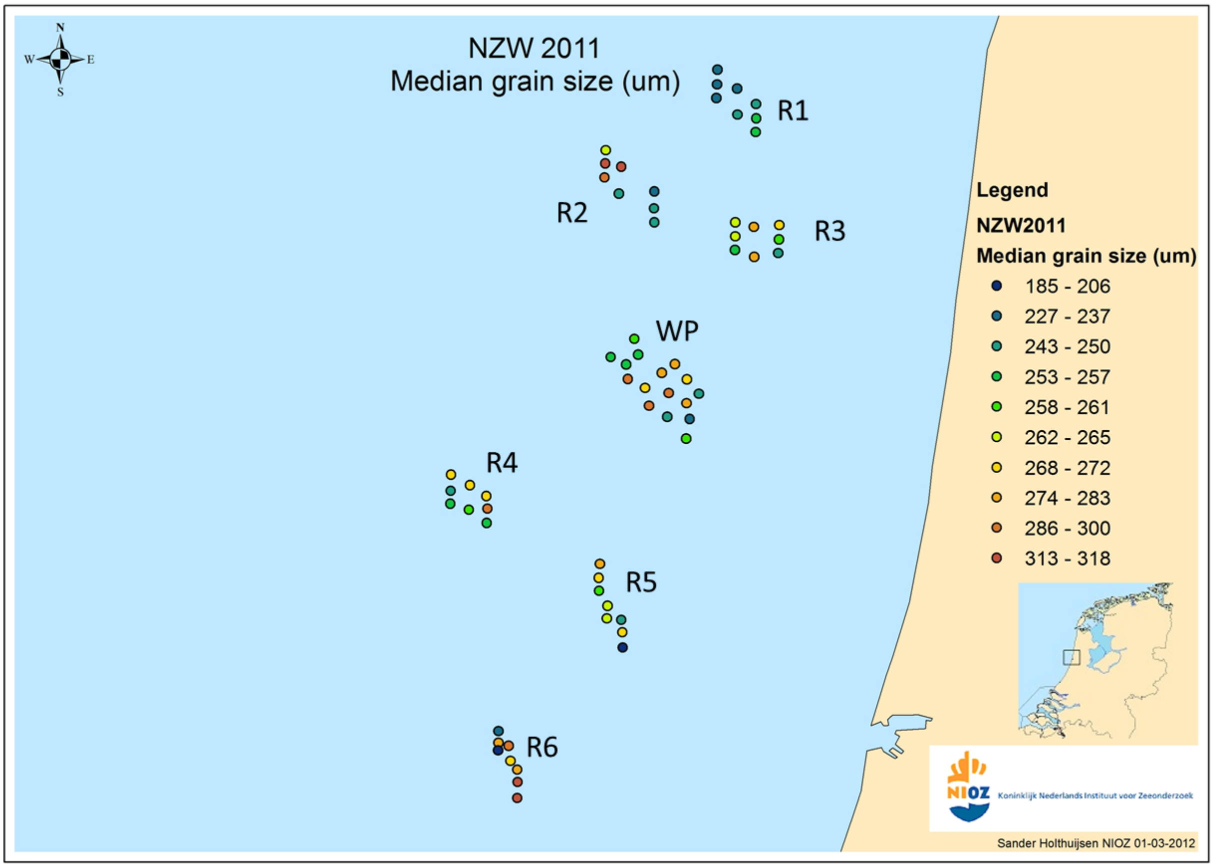


Fig. 8. Spatial distribution of median grain sizes (µm) in OWEZ and reference areas.

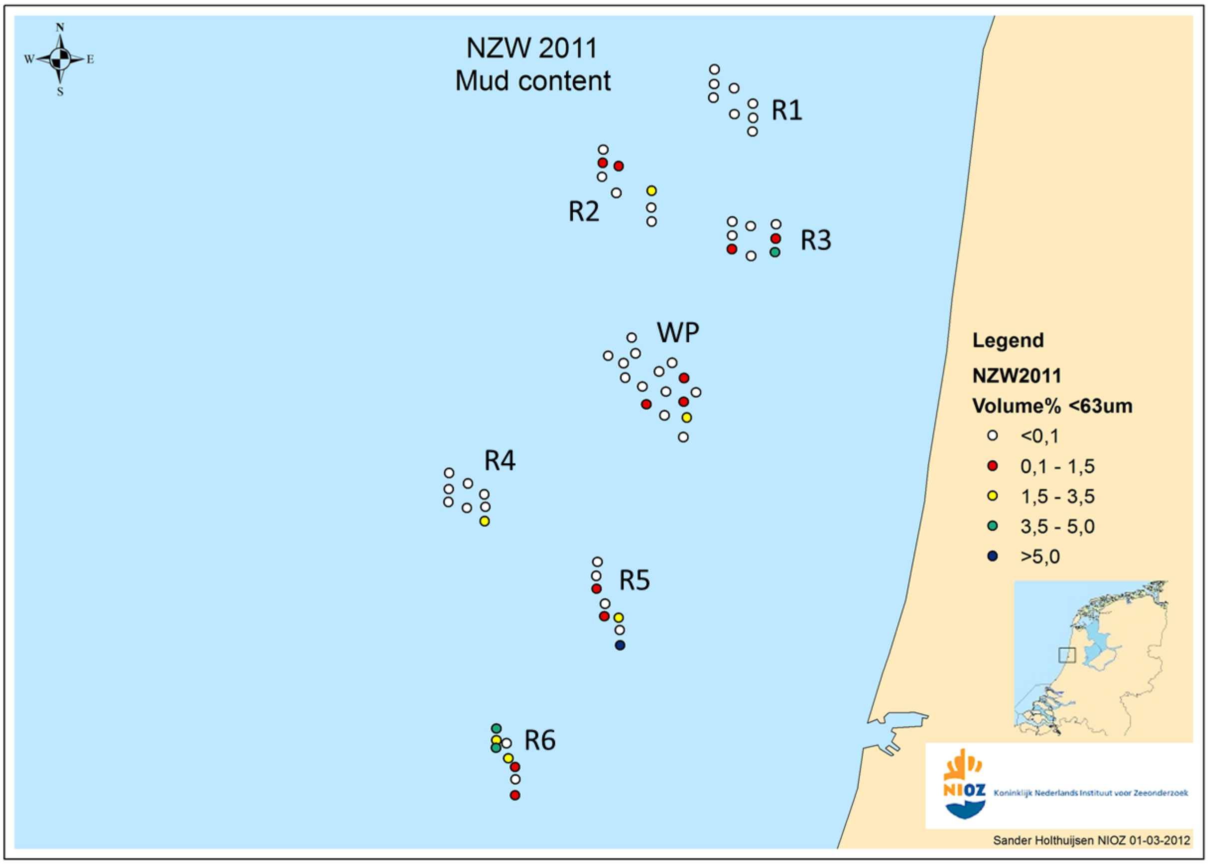


Fig. 9. Spatial distribution of mud content (% <63 µm) in OWEZ and reference areas.

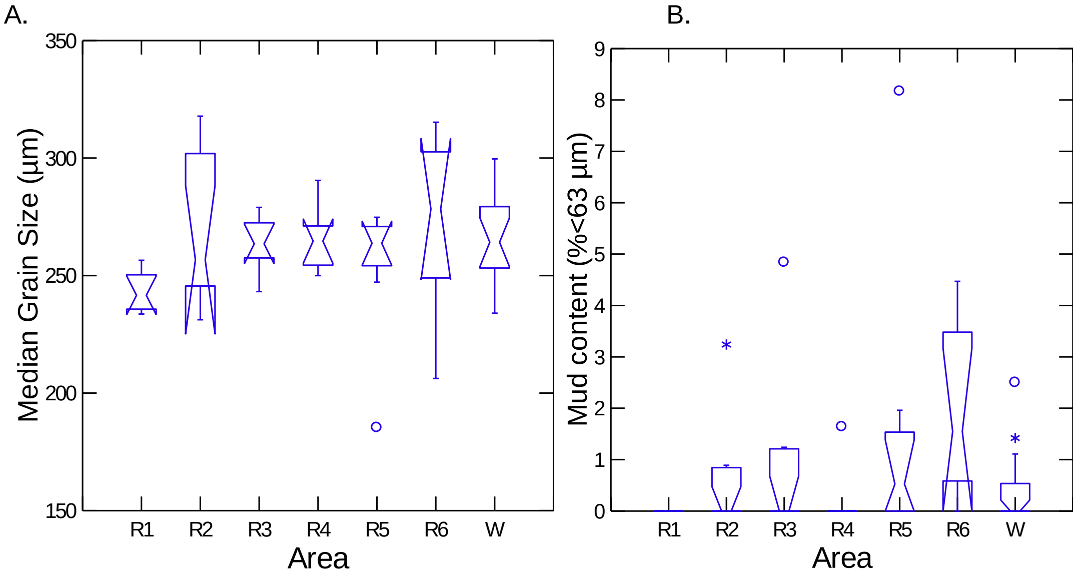


Fig. 10. Notched box and whisker plot, with median and notches that mark 95% median confidence intervals based on non-transformed data of A) median grains size (µm), and B) mud content (% particles <63 µm) in OWEZ and the six reference areas. *near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), ^{o} far outliers exceeding 3 times the IQRs from Q1/Q3.

3.2 Boxcore fauna

data survey 2011

Within the boxcore samples 88 species were identified, (Appendix 4A) of which 18 species contributed to 90% of the total abundances. Highest number of species (41) were found in the phylum of the polychaetes (Fig. 11). Crustaceans encompassed 23 different species, molluscs 15 and echinoderms 3. Six species were categorised as belonging to "other" phyla.

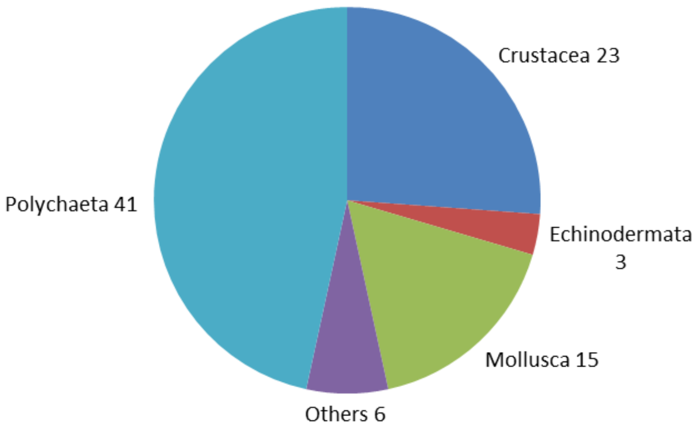


Fig. 11. Pie chart showing the different phyla and the number of species found within each phylum.

A MDS-plot (Fig. 12) based on the Bray-Curtis similarity matrix illustrates that macrobenthos abundances in OWEZ did not differ from the six reference areas. In fact, the grey crosses (representing OWEZ samples) are dispersed all over the plot. Grouping of stations does not occur within the reference areas as well. The p-value (p=0.098) generated in the PERMANOVA test (asymmetrical nested design) signified that the species composition showed no significant statistical difference between the areas. OWEZ did not differ more than the between area variation from the reference areas (p=0.699).

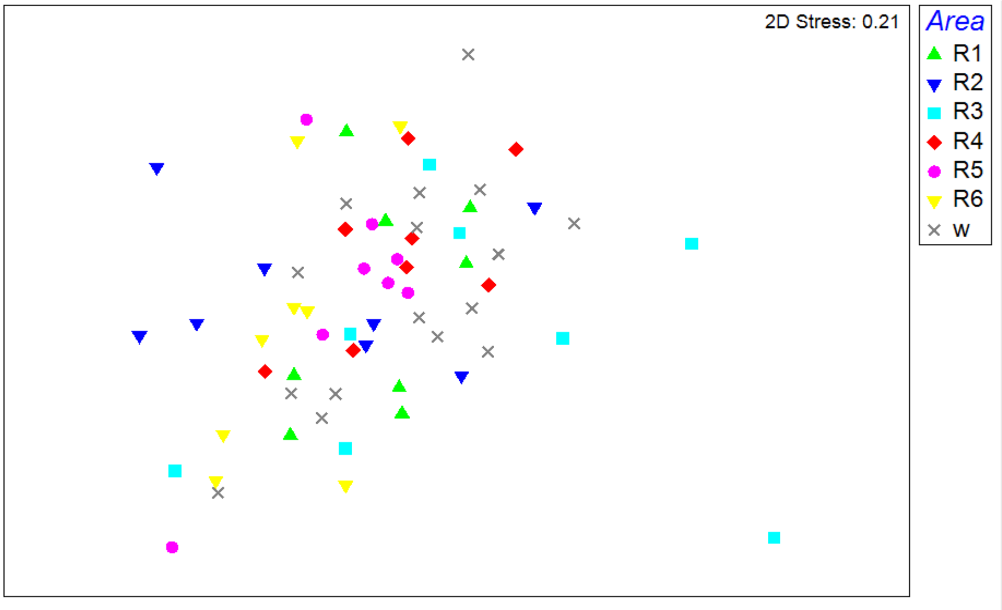


Fig. 12. MDS-plot on species abundance (per m^2) data (Bray-Curtis index, 4^{th} root-transformed) of all boxcore samples in OWEZ and the six reference areas.

A MDS-plot based on the biomass (Fig. 13A) and production data (Fig. 13B; Bray-Curtis similarity matrix) demonstrates the same result as the MDS-plot on abundance data. The wind farm did not differ from the reference areas. Besides that, no grouping in any of the reference areas is visible. PERMANOVA results on the biomass and production data revealed that there was no significant statistical difference between any of the areas (p=0.297 and 0.266 for biomass and production, respectively), and OWEZ did not stand out in any way above the between area variation (p=0.718 and 0.724 for biomass and production, respectively).

A. B.

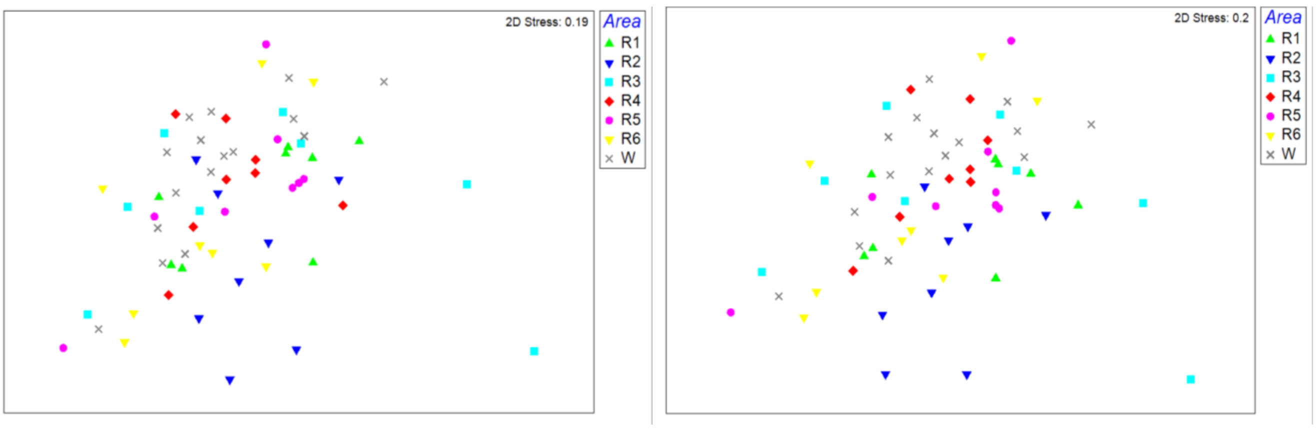


Fig. 13. MDS-plot on A) biomass (g AFDW/m^2) and B) production (kJ/m^2/y) data (Bray-Curtis index, 4^{th} root-transformed) of all boxcore samples in OWEZ and the six reference areas.

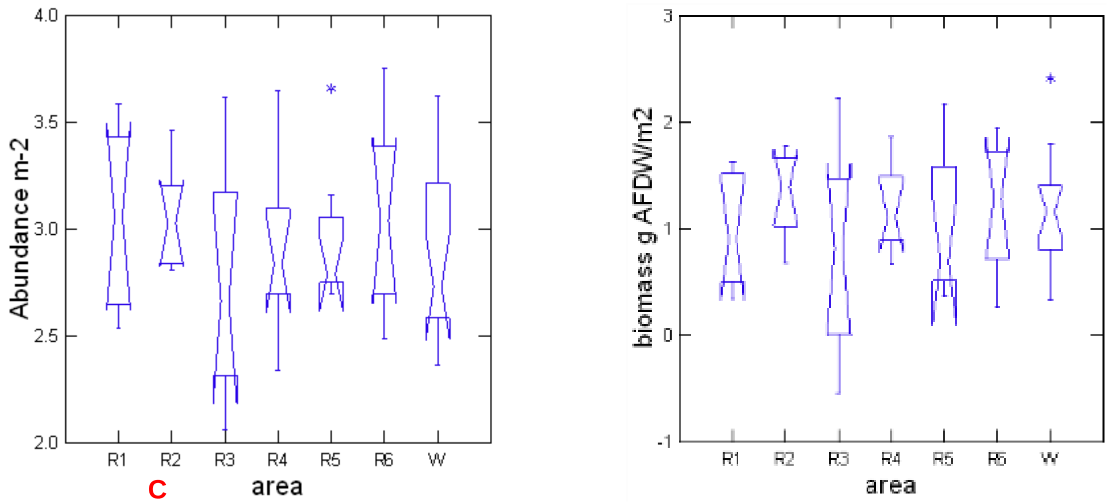
Results of the BEST analyses presents that the variation in species composition of all stations, is best explained by two environmental variables, *i.e.* mud content and water depth (Table 6). The variation in the OWEZ stations is best explained by the three environmental factors mud content, median grain size and depth. The same holds for the variation in the reference stations. In case of OWEZ this correlation was rather low (R=0.469), in the reference areas even more trivial (R=0.259).

	correlation coef. (R)	environmentals
All survey areas		
	0.294	1,4
	0.288	1,2,4
	0.252	1,2
OWEZ		
	0.469	1,2,4
	0.440	2,4
	0.430	All
Reference areas		
	0.259	1,2,4
	0.258	1,4
	0.247	All

Table 6. Results of BEST analyses. BIOENV correlation between abundance data and environmental factors: 1) mud content (% < 63 mμ), 2) median grain size, 3) distance to shore, 4) water depth. The three highest correlation coefficients (R) are given (significance level 0.01) in three different subdivisions of stations: 1) all areas, 2) OWEZ, and 3) reference areas.

Next to multivariate analyses, multiple tests on univariate measures were performed to explore possible differences between OWEZ and the reference areas. Median values with 95% confidence intervals of total abundance (m^2), biomass (g AFDW/m^2) and production (kJ/m^{2}/y) are presented per area in notched box and whisker plots (Fig. 14).

A. **B.**



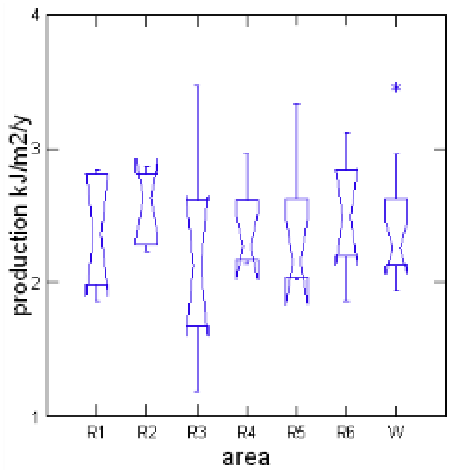


Fig. 14. Notched box and whisker plots, with median and notches that mark 95% median confidence intervals, on log-transformed boxcore data per area. A) abundance (per m^2), B) biomass (g AFDW/m^2), and C) production (kJ/m^2/y) in OWEZ and the six reference areas. ***near outliers between 1.5 a 3 times IQRs (Inter Quartile Range from Q1/Q3), o far outliers exceeding 3 times the IQRs from Q1/Q3.**

Abundances of macrobenthos varied between 115 and 5670 individuals per m^2. Average number of individuals per m^2 varied per area from 1096 in OWEZ to 1778 in R6. Relatively low numbers were also in R3, R4 and R5; relatively high numbers also in R1. The box plot (Fig. 14A) reveals no significant differences between areas, implying that OWEZ did not differ from any of the reference areas. The ANOVA result (Table 7) supported this conclusion.

Biomass per station had a minimum value of 0.28 and a maximum of 258 g AFDW per m^2. Lowest average was found in R1 with a value of 17.2 and highest in OWEZ with a value of 32.4 g AFDW per m^2. The box plot (Fig. 14B) presents no significant differences between areas, indicating that OWEZ did not differ from any of the reference areas. The ANOVA (Table 7) result supported this conclusion.

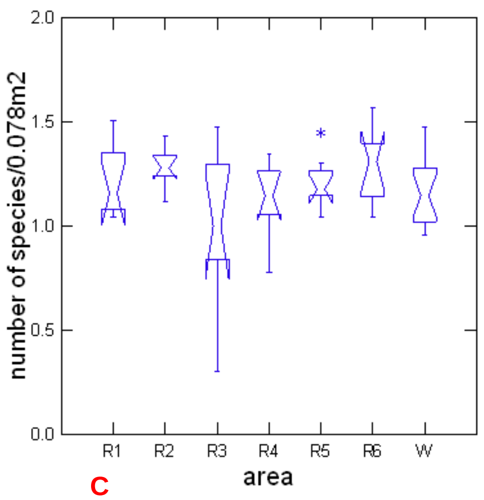
Production showed a large range varying between 15.6 and 2909.7 kJ/m^2/y per station (i.e. 0.7 and 132.3 g AFDW/m^2/y), with the on average highest annual production of 524.4 kJ/m^2/y (26.2 g AFDW/m^2/y) in R3 and the on average lowest annual production of 335.9 (16.8 g AFDW/m^2/y) in R4. The box plot (Fig. 14C) shows no significant differences between areas, revealing that OWEZ did not differ from any of the reference areas. The ANOVA (Table 7) result confirmed this conclusion.

	p
abundance (per m^2)	0.647
biomass (g AFDW/m^2)	0.626
productivity (kJ/m^2/y	0.749

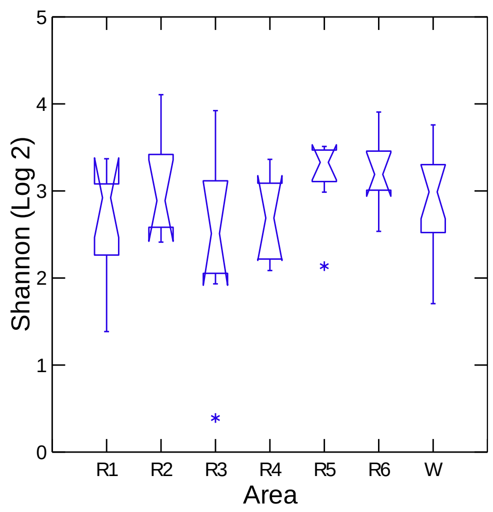
Table 7. Results of ANOVA tests to compare total abundance, total biomass, and total production between OWEZ and the reference areas. Tests on log-transformed data.

Differences between OWEZ and the reference areas were further explored by testing three diversity indices. The total number of species per boxcore (n/0.078m^2), the Shannon-wiener index (^{2}Log base), and the Simpson index (1-λ) are presented in box and whisker plots showing median values and 95% confidence intervals (Fig. 15).

A



B



C

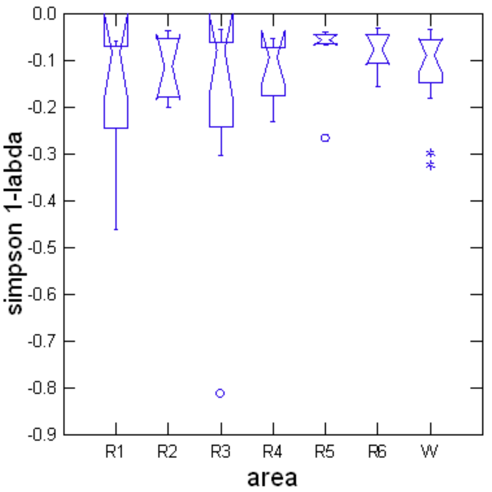


Fig. 15. Notched box and whisker plots with median and notches that mark 95% median confidence intervals, of three diversity indices describing OWEZ and the six reference areas. A) number of species per box (log-transformed), B) Shannon-Wiener index, and C) Simpson index (log-transformed). *near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), ° far outliers exceeding 3 times the IQRs from Q1/Q3.

A total of 88 different species were found in the boxcore samples. The number of species per boxcore varied between 2 and 37. The lowest average number of species (13) per box occurred in R3, the highest average number (20) in R6. The box plot (Fig. 15A) and the ANOVA result (Table 8) show no differences in number of species between the areas. OWEZ with an average number of 16 species did not seem to differ from the range of values in the reference areas.

The Shannon-Wiener diversity values per sample ranged from 0.39 up to 4.11. Average values per area varied between 2.46 in R3 to 3.22 in R6. OWEZ with an average of 2.86 fitted well within the range of the values observed in the reference areas. The boxplot of the Shannon-Wiener index (Fig. 15B) shows no differences between the areas. ANOVA results (Table 8) support this result. The Shannon-Wiener index did not seem to be different for OWEZ and the reference areas.

The Simpson (1-λ) diversity of the samples varied from 0.15 up to 0.93. The minimum average value (0.71) was found in R3 and the highest (0.84) in R5. The box plot (Fig. 15C) and ANOVA (Table 8) do not point to any difference between areas. The average value of OWEZ (0.78) did not diverge from the reference areas.

	p
number of species per box	0.084
Shannon (2log)	0.158
Simpson (1- λ)	0.425

Table 8. Results of ANOVA tests to compare number of species (log-transformed data) , Shannon-Wiener index, and Simpson index (log-transformed data) between OWEZ and the reference areas. P is indicated.

comparison data surveys 2003-2007-2011

To compare the macrobenthos abundances in OWEZ and the six reference areas between the years 2007 and 2011, both surveys exploiting similar spatial designs with 6 reference areas, the data were merged in a Bray-Curtis similarity matrix. A MDS-plot (Fig. 16) depicts the centroids, representing the “centres of gravity” of the single stations in each of the seven separate areas.

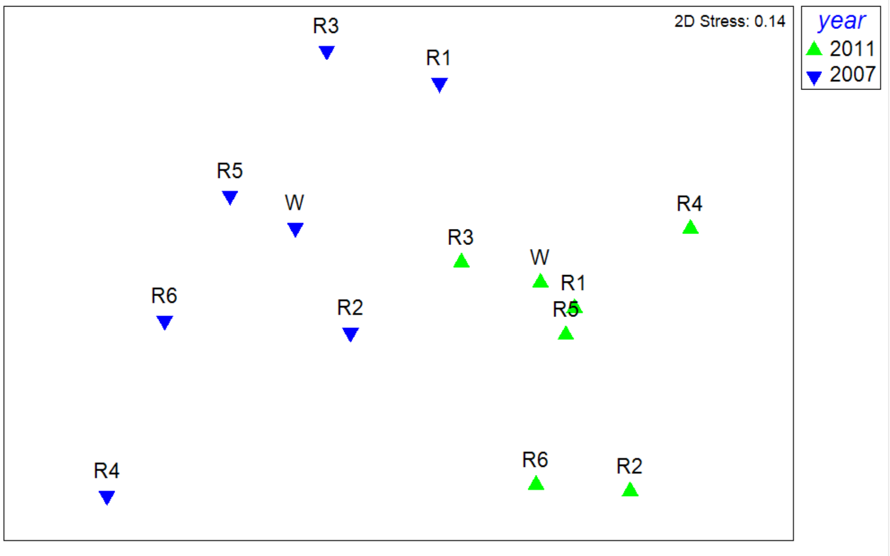


Fig. 16. MDS-plot depicting the centroids (“centres of gravity”) in OWEZ and in the six reference areas that were calculated on basis of the position of single stations in each of the areas (based on abundance data Bray-Curtis index, 4th root transformed) in T_1 (2007) and T_2 (2011).

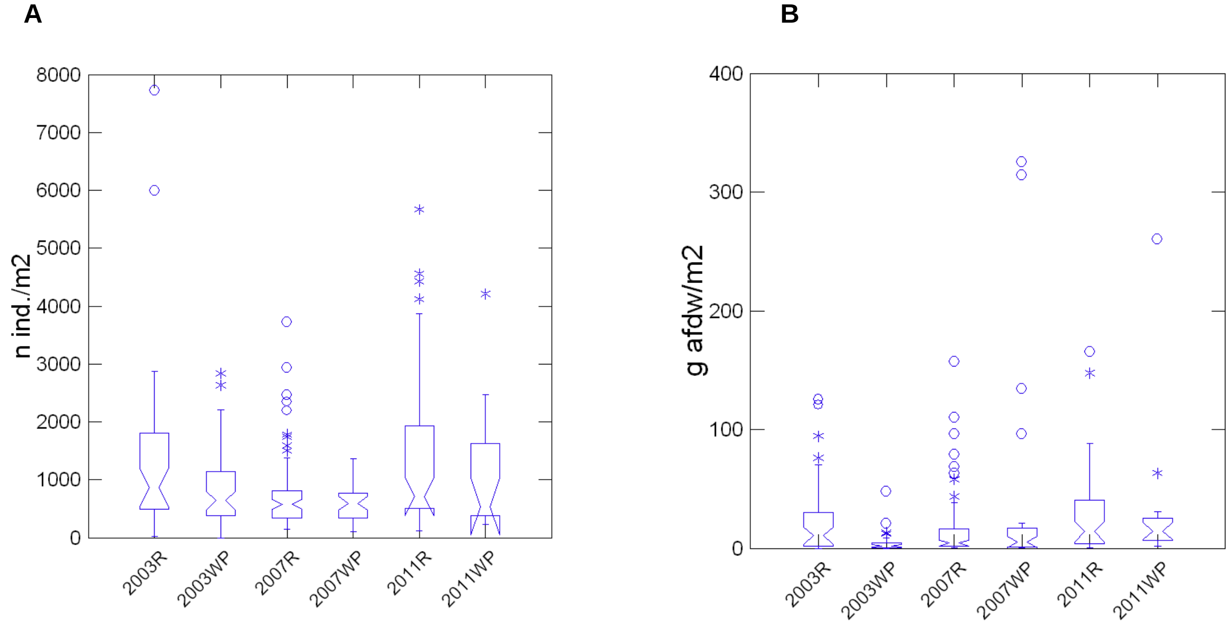
The MDS-plot demonstrates a clear distinction between the years. This suggests that the macro benthos community in the areas was different comparing the years 2007 and 2011. In comparison to the reference areas OWEZ did not seem to have changed in a different direction nor over a different distance in this interval. Indeed a two-way crossed PERMANOVA executed on the separate samples proved that there was a statistically significant difference between years (p=0.001) and between the areas (p=0.001). However, no significant difference was found in the interaction term “Area*Year” (p=0.223) indicating that none of the areas had diverged differently over time than one of the other areas. A 3-way mixed PERMANOVA test to examine the difference between in particular OWEZ and the reference areas proved that there was a statistically significant difference between years (p=0.001) and between areas (p=0.001). However, no significant difference was found between OWEZ and the reference areas (p=0.74 in the conservative Type 3 SS; p=0.71 in the sensible Type 1 SS). This implies that OWEZ did not differ from the reference areas in the years 2007-1011. The alternative 3-way crossed design also gave significant differences between years (p=0.001) and between all pooled reference areas and OWEZ (p=0.012), but could not prove a significant difference (p=0.77) between OWEZ in 2011 versus OWEZ in 2007 plus all reference areas.

A SIMPER analysis demonstrates which species are most important for the difference in among sample variation found in all areas (including OWEZ) between 2007 and 2011. The distinction between the years 2007 and 2011 was mainly due to relatively small variations in species abundances (Table 9) and not so much caused by the introduction of new species or species loss.

	2007	**2011**	**contribution %**
	mean abundance	mean abundance	
Urothoe poseidonis	1.47	1.54	5.78
Eteone longa	0.21	1.08	4.55
Bathyporeia elegans	1.19	1.26	3.66
Phoronida	0.38	0.79	3.66
Scolelepis bonnieri	0.78	0.99	3.53

Table 9. Results of SIMPER-analysis on species contribution (%) to the average dissimilarities in species composition in all areas between 2007 and 2011. Only the five species contributing most to the dissimilarities are shown. Average abundances per species in all areas in 2007 and 2011 are given based on fourth root transformed data per boxcore ($n/0.078m^2$).

Because of the strong methodological changes in sampling strategy between the year 2003 (two reference areas), and the years 2007 and 2011 (six reference areas), possible differences between the T_0, T_1, T_2 are most reliably explored with univariate methods comparing overall values in OWEZ with those in the pooled reference areas. Notched box and whisker plots present total number of individuals found per m^2, their total biomass, number of species per boxcore, and a number of diversity indices for the three surveys showing the 95% confidence limits of the median values (Fig. 17). The box plots indicate that values in OWEZ never deviate out of the range of values found in the surrounding reference areas.



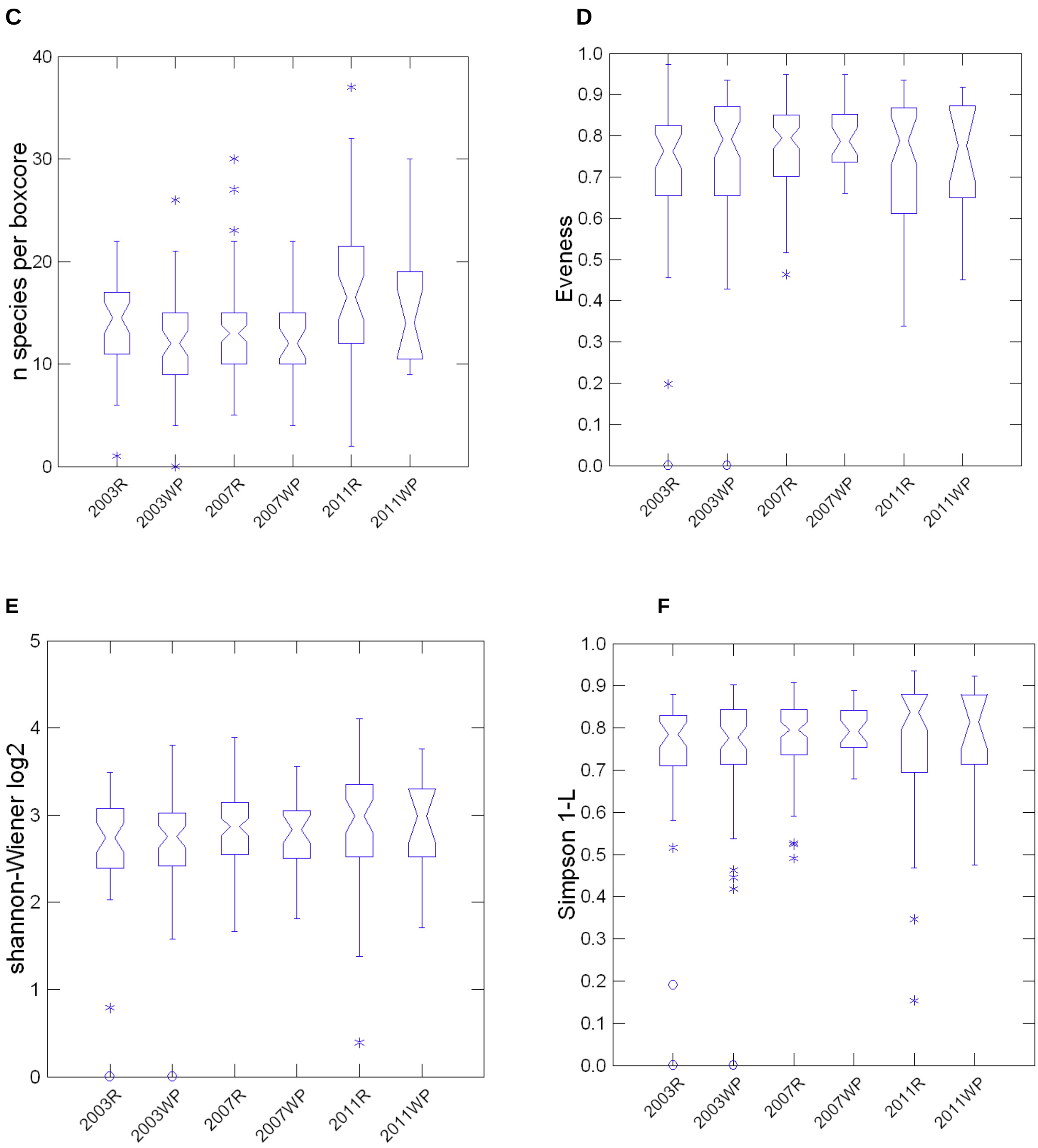


Fig. 17. Univariate comparisons of parameters in OWEZ and the combined reference areas over the years 2003, 2007, 2011. A) number of individuals per m^2, B) their biomass (g AFDW/m^2), C) number of species per boxcore, D) Pilou's eveness, E) Shannon-Wiener log2 index, and F) Simpson (1-λ) index. The box and whisker plots show the 95% confidence intervals of the median values. *near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), ^{o} far outliers exceeding 3 times the IQRs from Q1/Q3.

3.3 Triple-D fauna

In total 50 different species were collected with the Triple-D dredge (Appendix 4B) of which 15 species contributed to 90% of the total abundance. Highest number of species (18) were found in the (sub)phylum of the crustaceans. Lower number of species were recorded in the other phyla: 16 molluscs, 5 echinoderms, 6 polychaetes and 5 "other" species (Fig. 18).

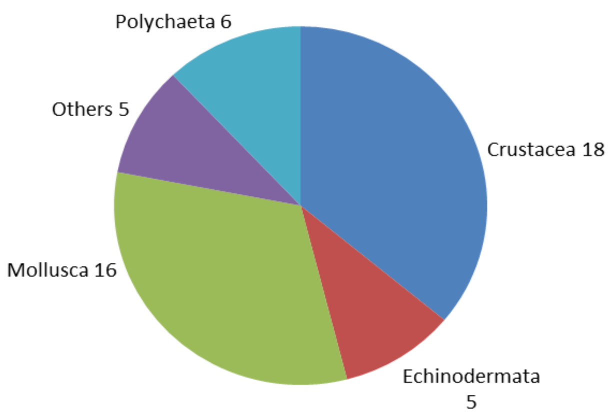


Fig. 18. Pie chart showing the different phyla and the number of species found within each phylum in Triple-D returns.

A MDS plot (Fig. 19) based on the Bray-Curtis similarity matrix illustrates that macrobenthos abundances in OWEZ did not differ from the six reference areas. In fact, the grey crosses (representing OWEZ samples) are dispersed all over the plot. Although grouping of samples also does not occur within the reference areas, the plotted samples per area (except R5) seem to be arranged in elongated parallel clusters suggesting differences between the areas.

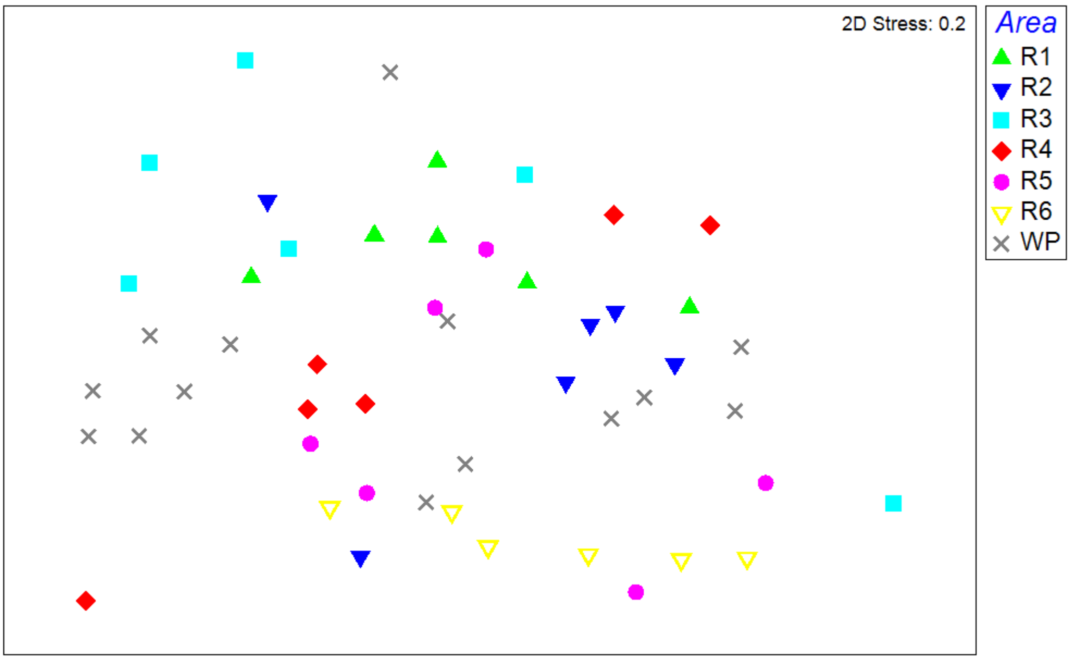


Fig. 19. MDS plot of abundance data per haul (n per 20 m^2) of Triple-D fauna (Bray-Curtis index, 4^{th} root-transformed) of all Triple-D samples in OWEZ and the six reference areas.

MDS plots depicting biomass (Fig. 20A) and production (Fig. 20B) per station show a similar configuration. The samples seem to be arranged in similar clusters.

A. B.

Fig. 20. MDS plot of A) biomass (g AFDW per 20 m^2) and B) production (kJ per 20m^2 per Y) data of Triple-D fauna (Bray-Curtis index, 4th root-transformed) in OWEZ and the six reference areas.

To proof differences in species composition of the benthos community between the areas in terms of abundance, biomass, and production, data were analysed with PERMANOVA (asymmetrical nested design). In all cases PERMANOVA revealed differences between the areas (p=0.001). But OWEZ did not differ from the among area variability in the reference areas (p=0.859, 0.721 and 0.723 for abundance, biomass and production, respectively; Table 10).

To get more detailed answers on the impact of OWEZ Wind farm on specific relevant categories of species, different selections of species were made. The first selection comprised the 15 most common species (*i.e.* species that contributed at least in one sample more than 10% to the abundances). The MDS plot is given in Fig. 21A, and shows no grouping of samples derived from any area. The PERMANOVA analysis on abundance data of common species revealed the same result as the analysis on abundance data of all species (Table 10): some areas clearly differed from each other (p=0.001), but OWEZ did not differ from the among area variability in the reference areas (p=0.61). The second selection comprised the 30 most uncommon species. A MDS plot or this category does not indicate any grouping of the samples belonging to the separate areas (Fig. 21B). The PERMANOVA analysis showed that OWEZ did not differ from the among area variation in the reference areas (p =0.7).

A **B**

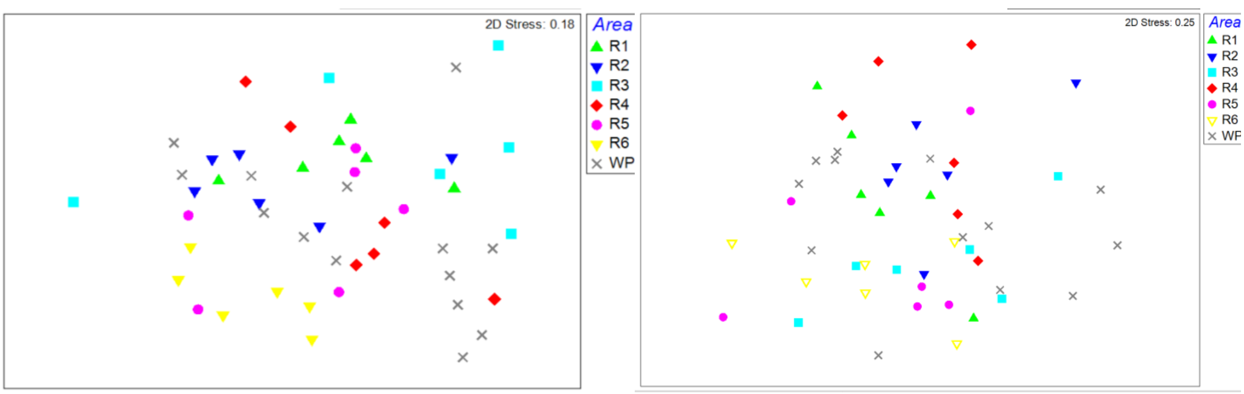


Fig. 21. MDS-plot of the Triple-D abundance data (Bray-Curtis index: 4th root-transformed) of A) the 15 most common species (contributing more than 10% to total abundance in at least one sample) and B) 30 most uncommon species in OWEZ and the six reference areas.

PERMANOVA analyses were performed also on Bray-Curtis 4th root-transformed abundance data of other selected groups of species. The PERMANOVA analyses of the four taxonomic groups (echinoderms, molluscs, polychaetes and crustaceans) indicated for all groups significant differences between areas (p=0.001), but revealed no differences between OWEZ and the reference areas (Table 10). PERMANOVA analyses on abundance data of epifauna, infauna and the ten species vulnerable to trawling (based on Bergman and van Santbrink, 2000) gave similar results: no distinction of OWEZ compared to the reference areas (Table 10). For all these selected groups of species MDS plots are not shown because none of them showed distinction of OWEZ or grouping of any of the areas.

Most distinction between the different areas was demonstrated in the MDS plot on the abundance data of 19 scavenger species, such as starfish, brittle stars, crabs, shrimps, and snails. Quite clearly the different areas are grouped together in the MDS-plot (Fig. 22). But the grey crosses representing OWEZ are dispersed all over the plot. The PERMANOVA results indeed indicated significant differences between areas (p=0.001), but OWEZ did not differ from the reference areas (p=0.566; Table 10).

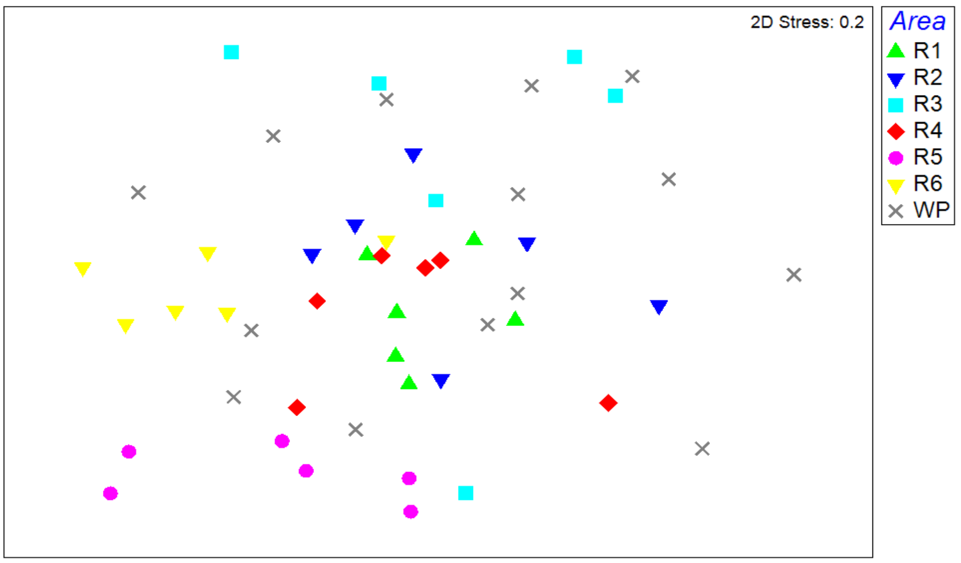


Fig. 22. MDS-plot of the Triple-D abundance data (Bray-Curtis index: 4th root-transformed) of 19 mobile scavenger species in OWEZ and the six reference areas.

	PERMANOVA p	
	areas	**OWEZ versus reference areas**
abundance	0.001	0.859
biomass	0.001	0.721
production	0.001	0.723
common species	0.001	0.61
uncommon species	0.005	0.7
echinoderms	0.001	0.308
molluscs	0.001	0.859
polychaetes	0.001	0.561
crustaceans	0.001	0.87
epifauna	0.001	0.563
infauna	0.001	0.867
scavenger species	0.001	0.566
vulnerable to trawling species	0.033	0.594

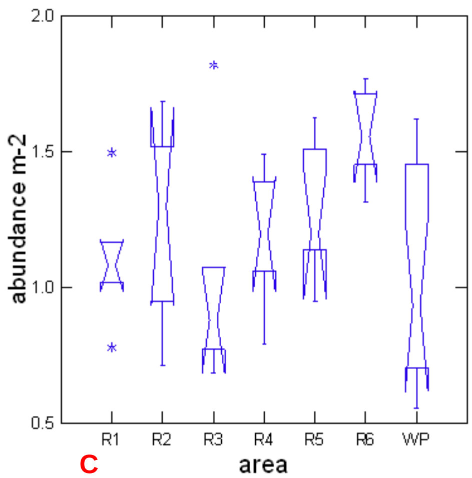
Table 10. PERMANOVA results for the different variables and groups of species. “Area” column shows the p values for differences between the all areas. “OWEZ versus reference areas” column shows the p values for the difference between OWEZ and the reference areas (whether OWEZ differs more than the between among area variation from the reference areas).

Next to multivariate analyses, multiple univariate tests were performed to compare total abundance, total biomass and production between areas. A total of 50 species were collected from the Triple-D samples. The number of individuals per sample ranged from 72 to 1318 (per 20 m^2; i.e. 3.6 to 65.9 per m^2). The average abundance was lowest in R1 with 288 individuals and highest in R6 with 774 individuals per 20 m^2 (i.e. 11.4 and 38.7 per m^2, respectively). Comparison of total abundance (per m^2) between the areas is visualized by notched box and whisker plots of log-transformed data showing median abundances in R6 and OWEZ were statistically significant different (Fig. 23). ANOVA results supported this conclusion (Table 11). According to the list of species abundances (Appendix 4b) this difference seemed primarily to be caused by higher overall abundances in contrast to by the dominance of a single species.

Biomass values varied between 19 and 320 (g AFDW/m^2) per sample. Minimum average biomass of 61 g AFDW/m^2 was found in R4. Maximum averaged 134 g AFDW/m^2 in R6. Although Fig. 23 shows significant higher median biomass in R6 than in OWEZ, the ANOVA results pointed not to differences in total biomass between areas (Table 11).

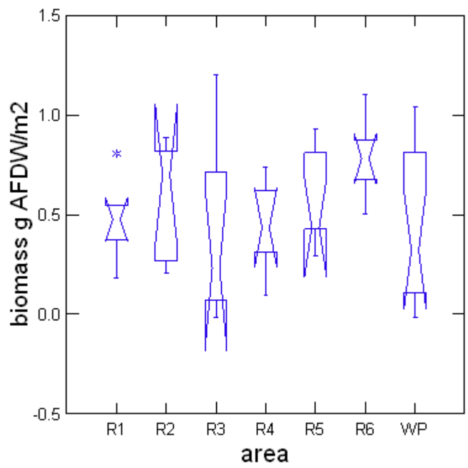
Production within samples varied between 13 and 228 (kJ/m^2/ Y; *i.e* 0.6 and 10.4 g AFDW/m^2/y). R1 had the lowest average value 43 (kJ/ m^2/y; i.e 1.9 g AFDW/m^2/y), R6 had the highest average value of 119 (i.e 5.4 g AFDW/m^2/y). Although Fig. 23 shows significant higher median production in R6 than in OWEZ, the ANOVA results pointed not to differences between areas (Table 11).

A



C

B



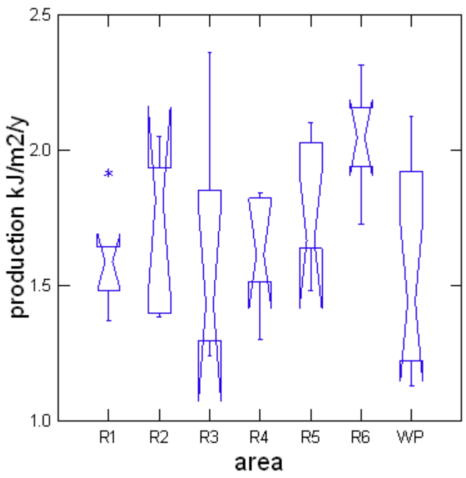


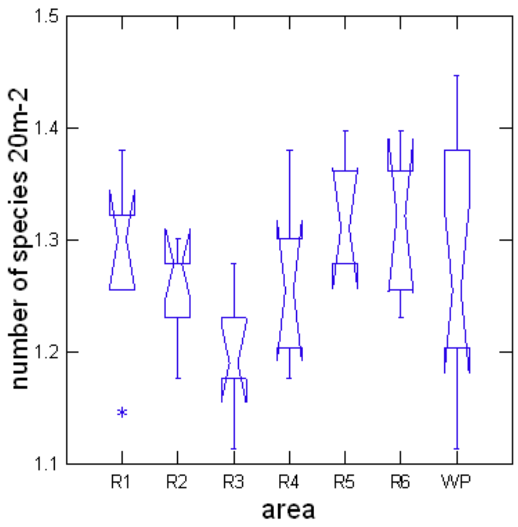
Fig. 23. Notched box and whisker plots with median and notches that mark 95% median confidence intervals on log-transformed Triple-D data per area: A) abundance per m^2, B) biomass g AFDW/m^2, and C) production kJ/m^2/y in OWEZ and the six reference areas. * near outlier between 1.5 a 3 times IQRs (Inter Quartile Range from Q1/Q3). *near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), ^{o} far outliers exceeding 3 times the IQRs from Q1/Q3.

	p	sign. difference between areas	highest value in:
Abundance (per m^2)	0.04	R6 vs. WP	R6
Biomass (g/m^2)	0.35		
Productivity (kJ/m^2/y)	0.06		

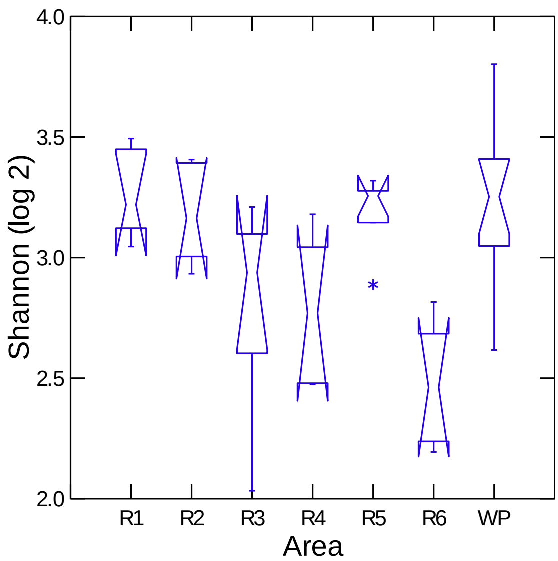
Table 11. Results of ANOVA analyses on differences between areas in total abundance, biomass, and production; data were log-transformed before testing. In case of a significant difference, Post-hoc Bonferroni-test was used to explore pairwise differences between areas. Areas with the highest values are indicated.

Diversity indices (number of species per Triple-D haul (per 20m^2), Shannon-Wiener, and the Simpson index) were explored in univariate tests to reveal differences between areas. All indices were not transformed before analyses and presented in notched box and whisker plots in Fig. 24.

A.



B.



C

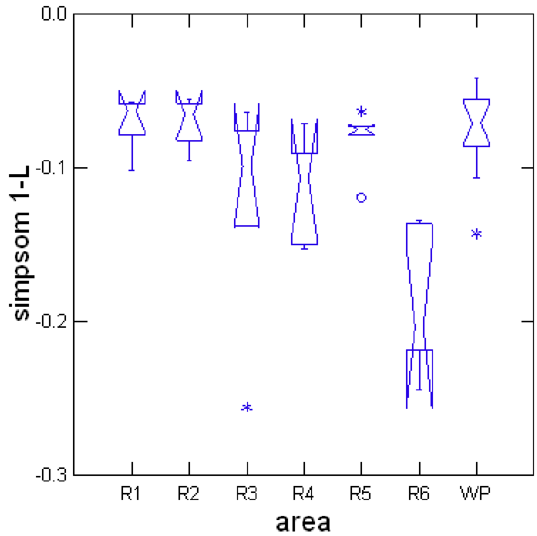


Fig. 24. Notched box and whisker plots, with median and notches that mark 95% median confidence intervals of three diversity indices for Triple-D samples. A) number of species per 20m2 (log-transformed data), B) Shannon-wiener, and C) Simpson index (log-transformed data) for OWEZ and the six reference areas. *near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), ^{o} far outliers exceeding 3 times the IQRs from Q1/Q3.

A total of 50 different species were found in the Triple-D hauls. The number of species per haul (20 m^2) varied between 13 and 28. The lowest average number of species (15) per haul was found in R3, the highest average number (21) in R5. The box plot (Fig. 24) shows that the median values in R3 were significantly lower than in R1, R2, R5, and R6. The ANOVA result (Table 12), however, shows no differences between the areas. OWEZ with an average number of species of 20 did not seem to differ from the range of values in the reference areas.

The Shannon-Wiener index, most commonly used to quantify diversity, is sensitive to the number of species in a community and the degree of evenness, i.e. the way that individuals in a community are distributed among species. The average Shannon-Wiener diversity values per area varied between 2.5 in R6 to 3.3 in R1. The boxplot of the Shannon-Wiener index (Fig. 24) shows lower median values in R6 than in R2 and OWEZ. ANOVA results (Table 12) pointed at significant lower values in R6 relative to R1, R2, R5 and OWEZ, whereas OWEZ had significant higher values than R4. This result indicates that the R6 samples were characterized by a relatively low diversity, whereas OWEZ tended to have a higher diversity than R4 and R6.

The Simpson index (λ) is particularly sensitive to the abundance of the commonest species and can therefore be regarded as a measure for dominance. We choose to use the expression 1-λ which represent the possibility that two randomly chosen individuals are of different species. The average Simpson (1-λ) diversity values per area ranged between 0.64 in R6 and 0.85 in R1 and R2. The box plot (Fig. 24) shows significant lower median values in R6 compared to all other areas including OWEZ. The ANOVA results (Table 12) showed lower values in R6 relative to R1, R2, R3, R4, R5, and OWEZ. This result indicates that R6 had a high dominance hence relatively low diversity and low evenness compared to all other areas including OWEZ.

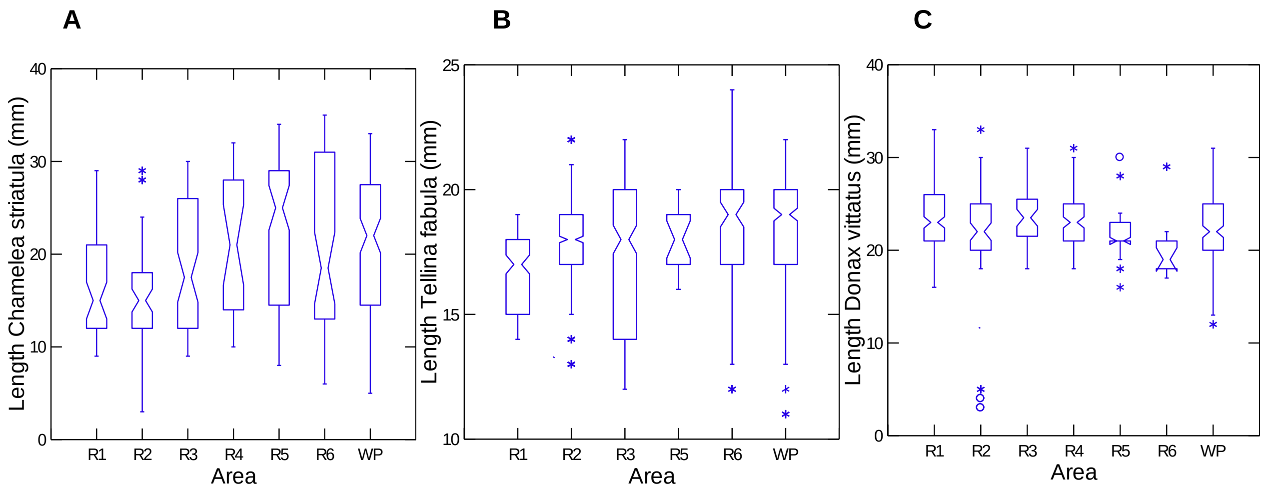
	p	sign. difference between areas	highest value in:
Number of species	0.125		
Shannon (log2)	<0.0001	R6 vs. R1, R2, R5, WP; WP vs. R4	R1, R2, R5, WP WP
Simpson (1-lambda’)	<0.0001	R6 vs R1, R2, R3, R4, R5, WP	R1, R2, R3, R4,R5,WP

Table 12. Results of ANOVA analyses on differences between areas with respect to diversity indices based on Triple-D returns (per 20m^2): number of species (log-transformed data), Shannon (log2), and Simpson (1-lambda’) (log-transformed data). In case of a significant difference, post-hoc Bnferonni test was used to explore pairwise differences between areas. Areas with the highest values are indicated.

Next to abundance, biomass, production and diversity indices, mollusc shell lengths were univariately tested. In Table 13 an overview is given of the average shell lengths and standard deviations of the 5 most abundant mollusc species, the bivalves *Chamelea striatula, Tellina fabula, Donax vittatus, and Ensis americanus* and the gastropod *Nassarius reticulates*. Red numbers indicate in what area the highest average shell length of a species were found. Black asterisks (*) mark the areas where shell lengths were significantly smaller than in the areas with the highest average value (ANOVA test and post-hoc Bonferroni tests). Other significant differences in length between areas are not indicated in Table 13. Shell lengths of *T. fabula* were significantly larger inside OWEZ than in R1, R2 and R3. Shell widths of *E. americanus* were inside OWEZ significantly smaller than in R3, but significant larger than in R4, R5 and R6. The other species all had their significant largest lengths in one of reference areas. Notched box and whisker plots of median values and 95% confidence limits of shell length in the different areas are depicted in Fig. 25. OWEZ did not seem to stand out with respect to shell lengths and fell within the range found in the surrounding reference areas.

		R1	R2	R3	R4	R5	R6	WP	ANOVA p
Chamelea striatula	Average	16.8*	15.3*	18.9*	20.5	**22.7**	20.8	20.7	<0.001
	Standard deviation	5.6	6.7	7.1	7.1	7.5	9.0	7.3	
Tellina fabula	Average	16.6*	18*	17.4*		18.2	18.4	**18.5**	<0.001
	Standard deviation	1.4	1.7	3.0		1.4	2.5	2.0	
Donax vittatus	Average	23.6	21.3*	**23.8**	23.2	21.6*	20.4*	22.9	<0.001
	Standard deviation	3.2	6.6	2.9	2.8	2.2	3.9	3.6	
Ensis americanus	Average	12.9*	12.6*	**15.4**	9.1*	11.7*	10.9*	13.6*	<0.001
	Standard deviation	5.4	5.5	6.0	3.7	5.4	4.7	6.1	
Nassarius reticulatus	Average					23.9	**24.3**	22.1	0.016
	Standard deviation					4.3	3.7	6.6	

Table 13. ANOVA results (non-transformed data), average shell lengths (mm; N.B. width for *Ensis americanus*) and standard deviation of five mollusc species (*Chamelea striatula, Tellina fabula, Donax vittatus, Ensis americanus* and *Nassarius reticulates)* per area. Red marked numbers are highest average values. Averages marked with an * represent significantly smaller shell lengths than the red marked average values.



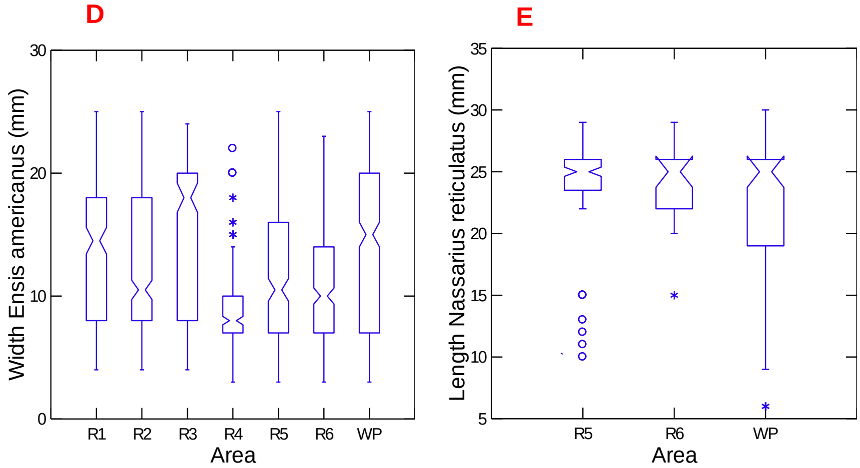


Fig. 25. Notched box and whisker plots, with median and notches that mark 95% median confidence intervals, of non-transformed shell lengths for A) Chamelea striatula, B) Tellina fabula, C) Donax vittatus, D. Ensis americanus (N.B. width mm) and E) Nassarius reticulates. *near outliers between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), o far outliers exceeding 3 times the IQRs from Q1/Q3.

The impact of a fishery-closed OWEZ wind park on the ten benthos species indicated by Bergman and van Santbrink (2000) as most vulnerable to trawling, was tested with ANOVA on log (n+1) transformed data. Table 14 revealed statistically significant differences between areas for *Chamelea striatula* and *Spisula solida*. Post-hoc Bonferonni test revealed that the abundance of *S. solida* was significantly higher in OWEZ than in R2 and R5. *C. striatula* abundance was higher in R5 than in R4.

species	p	sign. difference between areas	highest densities
Chamelea striatula	0.012	R4 vs R5	R5
Spisula solida	0.001	R2, R5 vs WP	WP
Spisula subtruncata	0.57		
Ensis americanus	0.35		
Tellina fabula	0.197		
Tellina tenuis	0.21		
Euspira catena	0.069		
Echinocardium cordatum	0.33		
Ophiura texturata	0.22		
Thia scutellata	0.36		

Table 14. ANOVA p-values on log(n+1)-transformed data for comparison of abundances of ten species vulnerable to trawling between OWEZ Wind farm (WP) and the six reference areas.

To further explore the spatial distribution of species in the coastal zone encompassing OWEZ and the reference areas, and to examine the possibility if other factors (or species) than the presence/absence of OWEZ wind farm may contribute to the similarity of the stations in terms of species composition a CLUSTER analysis was performed on the Triple-D data from all stations in OWEZ and the six reference areas. As a result 4 new groups (A, B, C and D) were formed. Within each cluster samples originating from stations in different areas had a > 67% resemblance in species composition. Only 4 samples did not fit in any of the formed clusters and were named rest group (R). All 4 newly formed groups contained stations from different areas. The 5 groups are presented in a MDS plot (Fig. 26).

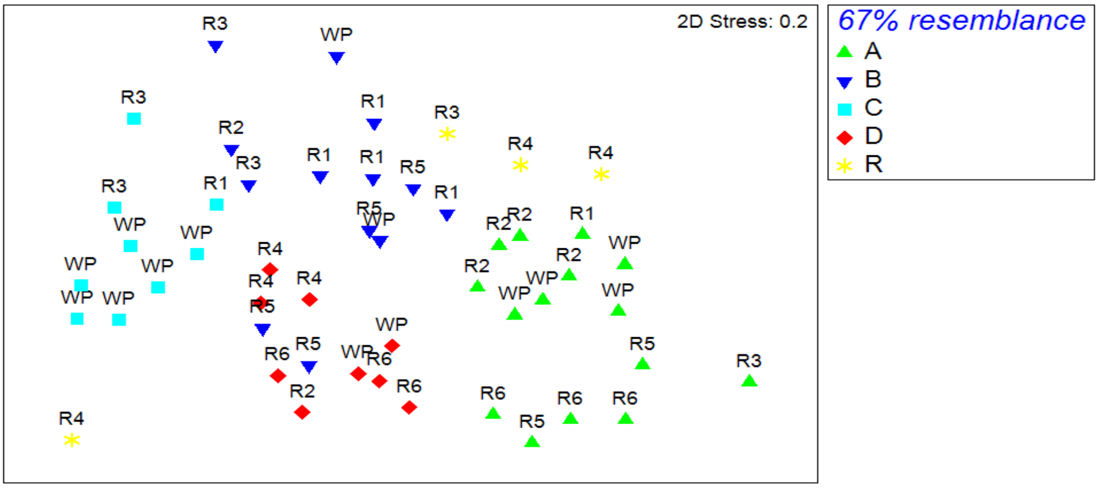


Fig. 26. MDS plot of the Triple-D samples of all stations divided into 4 clusters with each 67% resemblance. The colours represent the four newly formed clusters. The yellow (R) is a rest cluster comprised of 4 stations not fitting in the other groups.

Group A is the cluster comprising the highest number of different areas (missing only R4), group B was missing R4 and R6, and group D was missing R1, R3 and R5. Group C included the highest percentage of OWEZ samples (6 samples), combined with only 3 samples from R1 and R3. Although OWEZ samples were most dominant in group C they occurred, though less frequently, in all other groups.

To detect the species that are contributing most to the dissimilarities between the newly formed groups a SIMPER analysis was performed. The analysis (Table 15) revealed that the bivalves *Tellina fabula, Lutraria lutraria, Spisula solida, and Donax vittatus* the polychaete tube worm *Lanice conchilega,* and the brittle star *Ophiura albida* are the six most important species for the among sample variation between the four groups.

	Group A	Group B	
Species	av. abund.	av. abund.	contrib.%
Tellina fabula	2.62	0.29	9.20
Lutraria lutraria	2.13	0.45	6.47
Lanice conchilega	2.33	0.67	6.36
Diogenes pugilator	0.53	0.97	3.99
Nassarius reticulatus	1.07	0.36	3.91

	Group A	Group C	
Species	av. abund.	av. abund.	contrib.%
Tellina fabula	2.62	0.00	8.73
Lutraria lutraria	2.13	0.11	6.75
Lanice conchilega	2.33	0.89	4.68
Crangon crangon	3.70	2.33	4.49
Ensis americanus	3.02	1.76	4.18

	Group B	Group C	
Species	av. abund.	av. abund.	contrib.%
Spisula solida	0.08	1.32	7.07
Donax vittatus	1.98	1.05	5.50
Ophelia limacina	0.23	0.97	5.31
Ophiura albida	1.45	0.62	5.28
Diogenes pugilator	0.97	0.35	4.97

	Group A	Group D	
Species	av. abund.	av.abund.	contrib.%
Tellina fabula	2.62	0.30	9.05
Spisula elliptica	0.16	1.56	5.29
Ophelia limacina	0.52	1.52	4.72
Ophiura albida	1.54	2.20	4.61
Lanice conchilega	2.33	1.50	4.05

	Group B	Group D	
Species	av. abund.	av. abund	contrib.%
Ophelia limacina	0.23	1.52	6.61
Spisula elliptica	0.32	1.56	6.08
Ophiura albida	1.45	2.20	5.59
Lanice conchilega	0.67	1.50	5.53
Lutraria lutraria	0.45	1.35	4.62

	Group C	Group D	
Species	av. abund.	av. abund.	contrib.%
Ophiura albida	0.62	2.20	9.46
Lutraria lutraria	0.11	1.35	6.57
Lanice conchilega	0.89	1.50	5.59
Crangon crangon	2.33	3.30	5.26
Ophelia limacina	0.97	1.52	5.23

Table 15. Results of SIMPER-analysis on species contribution (%) to the average dissimilarities between the four newly formed groups A, B, C, D each with 67% resemblance. Only the five species contributing most to the dissimilarities between pairwise combinations of areas are shown and average abundances (based on fourth root transformed from n per 20 m^2) per species is given.

The SIMPER- results (Table 15) indicate that species contributing most to the differences between group C, dominated by OWEZ samples, and the other groups show mainly lower average abundances in group C. Only in the comparison between group C and B the average abundances of two species (the bivalve *S. solida* and the polychaete *Ophelia limacine*) appeared higher in group C.

To visualize the abundances of the six most distinctive species identified by the SIMPER analysis in each of the groups A, B, C, and D, their abundances were superimposed on the MDS plots of the clusters (Fig. 27). The size of the bubbles indicates the abundance of a species in that particular station. *S. solida* occurred most frequently and in relatively high densities in group C stations. This species was found less frequently and in lower abundances in group D stations. *D. vittatus* was present in all group B stations, but less frequently and less abundant in other groups. *Ophiura albida* is present in relatively high densities in all group D stations, but less frequently and less abundant in the other groups. *T. fabula* was found almost exclusively in group A stations, together with *L. conchilega* and *L. lutraria*, although the latter two were also found - although in lower densities - in the other groups. The stations in group A, all comprehending high numbers of *T. fabula*, *L. conchilega* and *L. lutraria*, have also higher total abundances and a relatively high number of species than the stations in the other groups (Fig. 28). Superimposing on the MDS plot the variation in median grain size in the stations did not show a visual correlation with the four different groups. Superimposing mud content revealed relatively frequent and high levels of mud in group A (Fig. 29).

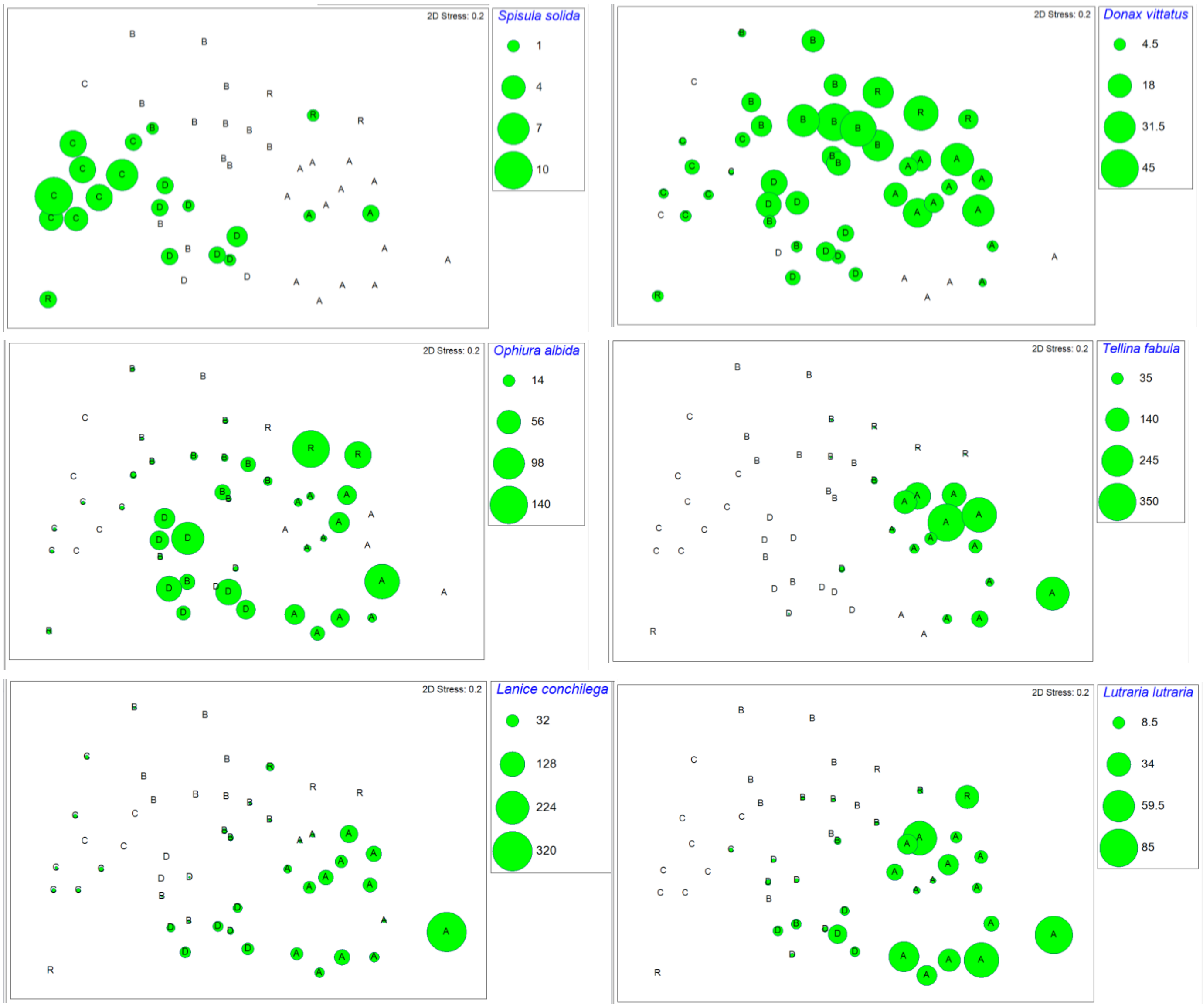


Fig. 27. MDS-plots showing the 4 clusters (based on the abundance data Bray-Curtis, 4th root) overlaid with bubble plots of 6 most distinctive species *Spisula solida, Donax vittatus*, *Ophiura albida*, *Tellina fabula, Lanice conchilega* and *Lutraria lutraria*. A, B, C and D indicate the newly formed 67% resemblance clusters. R represents the rest group.

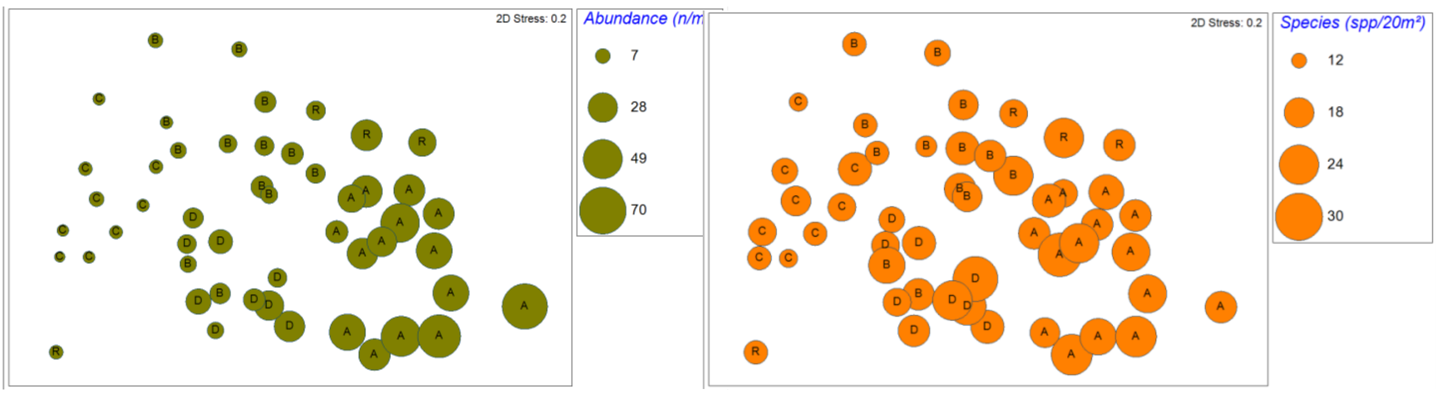


Fig. 28. MDS-plots showing the 4 clusters (based on the abundance data Bray-Curtis, 4th root) overlaid with bubble plots of the total abundance (per m^2; blue) and total number of species per sample (spp/20m^2; red). A, B, C and D indicate the newly formed 67% resemblance clusters. R represents the rest group.

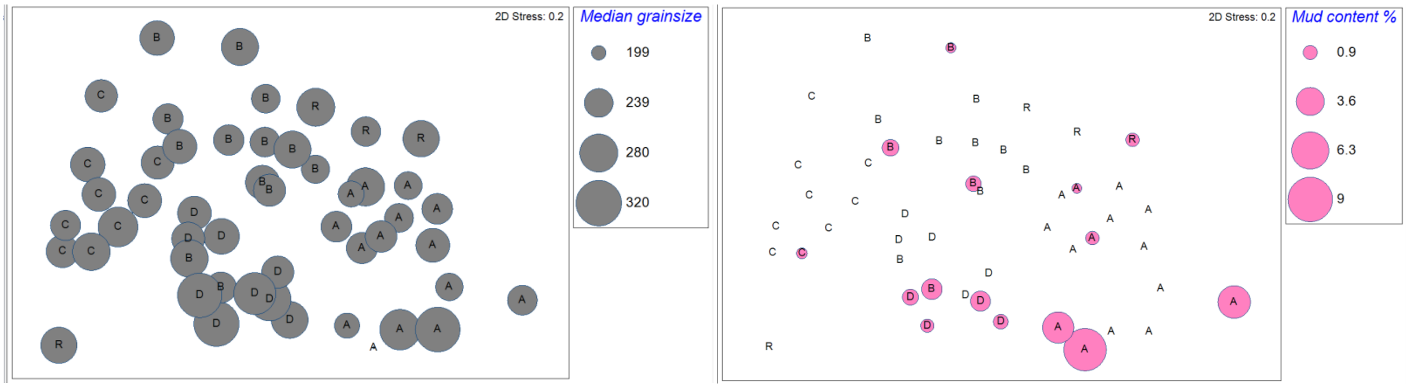


Fig. 29. MDS-plots showing the 4 clusters (based on the abundance data Bray-Curtis, 4throot) overlaid with bubble plots of the medium grainsize (µm; grey) and mud content (% <63 µm; red). A, B, C and D indicate the newly formed 67% resemblance clusters. R represents the rest group.

4. DISCUSSION

The focus of this present study was primarily on the possible differences between OWEZ and the reference areas in 2011, 5 years after the start of OWEZ Wind farm. These results will be discussed in section 4.1. Differences between the faunal situation in 2003, 2007 and 2011 will be discussed in section 4.2. In the final section (4.3) the outcomes of 4.1 and 4.2 will be discussed against the background of the design of the consecutive studies, the selected reference areas, the local patchiness, the reduction in fishery effort and the possibilities for recovery.

4.1 Impact of OWEZ on the local macrobenthos community - 2011 study

number of species and total abundance

In the boxcore sampling 88 species were identified, whereas 50 species were collected from the Triple-D samples (Figs. 11, 18). This difference was mainly caused by mesh size selection (boxcore: 1 mm; Triple-D: 7 mm knot to knot) leading to higher number of polychaete species in the boxcore. On the contrary, the Triple-D collected higher numbers of mollusc and echinoderm species primarily as a consequence of the larger surface area sampled (boxcore 0.078 m^2; Triple-D 20 m^2). Mesh size selection also created the difference in total macrobenthos abundances in a sample ranging from 115 and 5670 individuals per m^2 in boxcore sampling, and from 72 to 1318 per 20 m^2 (3.6 to 65.9 per m^2) in Triple-D sampling.

Average number of individuals in the samples varied per area. Median and average abundance of individuals showed in univariate tests no significant differences between areas if based on boxcores (Fig. 14A, Table 7), implying that OWEZ did not differ from any of the reference areas. Triple-D sampling, especially targeting on the larger sized and often older individuals that underwent the specific conditions in an area for longer periods, is considered to give a more overall impression of the impact of OWEZ. Triple-D sampling revealed that median and average total abundances in R6 were significant higher than in OWEZ (Fig. 23, Table 11). According to the list of species abundances (Appendix 4b) this difference seemed primarily to be caused by higher overall abundances in R6 in contrast to by the dominance of a single species. The higher total abundances in R6 might be related to the relatively high mud content (Fig. 10B).

species composition

Multivariate visualization of the data of both boxcore and Triple-D sampling illustrated that macrobenthos abundances (*i.e*. species composition) in OWEZ did not differ from the six reference areas. In fact, symbols representing OWEZ boxcore samples (Fig. 12) were dispersed all over the plot amidst the samples derived from the reference areas, that similarly did not show any grouping. Most of the plotted Triple-D samples (Fig. 19), mainly consisting of the larger sized and often older individuals, seemed to be arranged in elongated parallel clusters (except R5) suggesting some differences between the areas. This differences were likely more visible in Triple-D data as these individuals experienced the specific conditions in an area for longer periods. The observations were supported by results of the associated PERMANOVA tests revealing no differences between areas in the boxcore sampling and significant differences between the areas in the Triple-D sampling. But in both types of sampling OWEZ did not differ from the among area variability in the reference areas.

Since Triple-D sampling targeted on individuals that faced the specific conditions in an area for longer periods, we expected that several specific groups of species relevant to the research question might display differences between OWEZ and the reference areas. The 15 most common species showed almost no grouping of samples derived from separate areas, and OWEZ samples were dispersed amidst the reference samples (Fig. 21A). PERMANOVA tests advocated this result: some areas significantly differed from each other, but OWEZ did not differ more than the among area variability from the reference areas (Table 10). The selection of the 30 most uncommon species did also not show any grouping of areas (Fig. 21B). Similarly, multivariate tests of the four taxonomic groups (echinoderms, molluscs, polychaetes and crustaceans) indicated for all groups significant differences between areas, but revealed no differences between OWEZ and the reference areas (Table 10). Multivariate analyses on abundance data of epifauna, infauna and the ten species most vulnerable to trawling gave the same results: no distinction of OWEZ compared to the reference areas (Table 10). Univariate testing of these vulnerable species, however, revealed that the bivalve *Spisula solida* had significant higher abundances in OWEZ than in R2 and R5, while the bivalve *Chamelea striatula* was more abundant in R5 than in R4 (Table 14). Other vulnerable species displayed no differences between areas. Only in the MDS-plot of 19 scavenger species (starfish, brittle stars, crabs, shrimps, and snails) some distinction between the different areas was demonstrated. Quite clearly the different reference areas were grouped together (Fig. 22), although the symbols representing OWEZ are dispersed all over the plot. The PERMANOVA results indeed indicated significant differences between areas, but again OWEZ did not differ more than the between area variation from the reference areas (Table 10).

In conclusion: we could not prove that OWEZ differed more than the between area variability from the reference areas. OWEZ did not differ from the reference areas in terms of overall species composition or in composition of relevant faunal selections. This conclusion holds even if based on the Triple-D sampling, a method revealing clear impacts of fishery-free zones on benthos communities in previous studies in the North Sea (Duineveld et al., 2007). The fact that the bivalve *Spisula solida* had significant higher abundances in OWEZ than in R2 and R5, is hardly evidence for a distinctive difference between OWEZ and the reference areas.

biomass and production

In the boxcore sampling total biomass per station had a minimum value of 0.28 and a maximum of 258 g AFDW/m^2; in the Triple-D sampling these values ranged from 19 to 320. Total annual production per station had a minimum value of 15.6 and a maximum of 2909.7 kJ/m^2/y (132.3 g AFDW/m^2/y) in the boxcore sampling; in the Triple-D sampling these values ranged from 13 to 228 kJ/m^2/y (0.6 to 10.4 g AFDW/m^2/y). The discrepancies in the estimates of total biomass and annual production for stations and areas prompted by the sampling method is explained primarily by the different mesh size selection.

Univariate tests on total biomass and annual production per station measured in the boxcores revealed no significant differences in median and average values between areas, indicating that OWEZ did not differ from any of the reference areas (Figs. 14B, C; Table 7). In the Triple-D catches, targeting on larger-sized and older fauna, the significant higher median total biomass and annual production in R6 than in OWEZ (Fig. 23) was not supported by the ANOVA results (Table 11).

Multivariate visualization of the data of boxcore sampling illustrated that macrobenthos biomass and annual production data in OWEZ did not differ from the six reference areas (Figs.13A, B). Besides that, no grouping in any of the reference areas was visible. On the contrary, the biomass and annual production data of the Triple-D sampling seemed to be arranged, similar to the abundance data, in elongated parallel clusters per reference area suggesting differences between these areas (Figs. 20A, B). However, quite the opposite holds for the Triple-D samples representing OWEZ: they were dispersed all over the plot amidst the reference samples. PERMANOVA test on the biomass and annual production data based on the boxcores indeed revealed that there was no significant statistical difference between any of the areas, and OWEZ did not stand out in any way above the between area variation. The biomass and annual production data from the Triple-D sampling, however, did show significant differences between areas, but OWEZ did not differ more than the between area variation in the reference areas (Table 10). The results on biomass and annual production based on both sampling methods are consistent with the conclusion that species composition did not differ between OWEZ and the reference areas.

diversity

Almost half (41) the number of all species (88) found in the boxcore samples (Appendix 4a) belonged to the phylum of the polychaetes (Fig. 11). The dominant group found in the Triple-D hauls (18 out of 50 species) belonged to the phylum of the crustaceans (Fig. 18; see species list appendix 4B). The number of species varied per boxcore (0.078m^2) between 2 and 37, and per Triple-D haul (20 m^2) between 13 and 28 (i.e 0.65 to 1.4 per m^2). Variations in numbers of species found per sampling method are caused by mesh size selection and sample size (see also first paragraph 4.1).

The boxcore sampling revealed no differences in median and average number of species between the areas (Fig. 15A; Table 8). The Triple-D sampling, however, showed that the median number of species in R3 are significantly lower than in R1, R2, R5, and R6 (Fig. 24A), although ANOVA tests (Table 12) did not support this conclusion and pointed to absence of differences between the areas. Apparently the number of species in OWEZ did not seem to differ from the range of values in the reference areas.

Although the number of species in OWEZ did not differ from the reference areas, diversity indices pointed to subtle differences. The Shannon-Wiener index (^{2}Log base) used to quantify diversity and sensitive to the number of species in a community and the degree of evenness, varied based on boxcores between on average 2.46 in R3 to 3.22 in R6, and based on Triple-D samples between 2.5 in R6 to 3.3 in R1. Again, variations in index values per sampling method were caused by differences in mesh size selection and sample size (see first paragraph 4.1). The boxcore sampling revealed no differences in the median and average Shannon-Wiener index between the areas (Fig. 15B, Table 8). The Triple-D sampling, however, showed lower median values in R6 than in R2 and OWEZ (Fig. 24B). ANOVA results (Table 12) pointed to lower average values in R6 relative to R1, R2, R5 and OWEZ, whereas OWEZ had significant higher values than R4. This result indicates that the R6 Triple-D samples were characterized by a relatively low diversity, whereas total abundances were higher than in OWEZ (Fig. 23A, Table 11). These characteristics are most probably related to the relatively high mud content in R6 (Fig. 10B). In conclusion: Triple-D samples consisting of relatively longer sized and often older specimens that endured the local conditions for longer series of years showed that OWEZ tended to have a higher diversity, a higher number of species and a higher eveness than R4 and R6.

The Simpson index (1-λ) providing high index values in case of high diversity and eveness, ranged based on boxcore sampling from on average 0.71 in R3 to 0.84 in R5, and based on Triple-D samples from 0.64 in R6 to 0.85 in R1 and R2. The boxcore sampling revealed no differences in the median and average Simpson index between areas (Fig. 15C, Table 8). The Triple-D sampling, however, showed significant lower median and average values in R6 compared to all other areas including OWEZ (Fig. 24C, Table 12). Thus R6 had a relatively low diversity and low evenness compared to all other areas including OWEZ. In conclusion both diversity indices, if based on Triple-D sampling, pointed to R6 as an area with a relatively low diversity of larger sized species, a high dominance hence relatively low diversity, and low evenness, possibly related to the high mud content. On the contrary, OWEZ tended to have a higher diversity of larger sized species than R6 and R4. Since any difference in diversity indices was not found in the T_1 one year after the start of OWEZ Daan, et al, 2009) the higher indices found in 2011 might be a first sign of recovery of OWEZ Wind farm directing to a higher diversity.

mollusc shell length
Of the five most abundant mollusc species (the bivalves *Chamelea striatula, Tellina fabula, Donax vittatus, Ensis americanus* and the gastropod *Nassarius reticulates)* average shell lengths of *T. fabula* was significantly larger inside OWEZ than in R1, R2 and R3, whereas width of *E. americanus* was inside OWEZ significantly larger than in R4, R5 and R6, although smaller than in R3 (Table 13). Since the three other species all had their largest lengths in one of reference areas, and OWEZ fell within the range found in the surrounding reference areas (Fig. 25), the 5 fishery-free years apparently did not lead to the overall presence of larger-sized molluscs in OWEZ. The larger dimensions of *T. fabula and E. americanus* might however be interpreted as a first sign of increased survival.

4.2 Impact of OWEZ on the local macrobenthos community - comparison 2003-2007-2011

Fluctuations in environmental factors initiate dynamic processes in coastal benthos communities leading to large differences in abundances of species between years (see section 4). The variation in successful spawning and survival of juveniles contribute to this annual variation. Therefore, differences in the benthos community in OWEZ between years can not be correlated with the presence of OWEZ Wind farm. Comparison of OWEZ with the reference areas between three years, however, is in this project hampered by the inadequate sampling design in T_0 and the change in sampling design between T_0 and T_1. As described in section 2.2 the fauna in the T_0-study in 2003 was sampled in only three subareas: OWEZ and two reference areas at a distance of circa 20 km (Jarvis et al., 2004). A power analysis on the T_0 data showed that the difference in the fauna composition between the two reference areas was too large to detect significant changes in OWEZ Daan et al. (2009).. The difference in species composition was not unexpected as the macrofauna south of IJ-geul appeared to belong to another Twinspan-cluster than the fauna north of IJ-geul in the 1989 survey as reported by van Scheppingen and Groenewold (1990). Continuance of the initial T_0 design in the T_1-study in 2007 would have implied that only extremely large differences in OWEZ would be detectable with statistical significance. It was decided therefore to spread the sampling effort in the T_1 (and also in T_2) over a number of possibly more representative reference areas positioned closer to OWEZ. Daan et al (2009) stated that because of this change in study design statistical comparisons between T_0 and T_1 data were at least disputable and probably senseless.

An adequate comparison of the macrofauna in the Triple-D dredge samples between T_0 and T_1 is further hindered by methodological differences between the sampling methods as described in Daan et al. (2009). Between T_0 and T_1 the type of dredge changed: the first study being done with a dredge of 1 m wide cutting blade (15 cm below runners) and a haul length of approximately 100 m resulting in a sample surface of ≈100 m^2 per haul. At T_1 Triple-D dredge hauls of 0.2 m width (cutting blade 20 cm below runners) and of 80 m length, accurately measured by the revolutions of a wheel triggering a pneumatic closing system, resulted in a sampled surface of 16 m^2 per haul. At T_2 the haul length was 100 m resulting in a sampled surface of 20 m^2. Additional differences were a smaller mesh size used at T_0 (6 mm) than used at T_1 (7 mm), and the fact that some abundant hyperbenthic species like shrimps were apparently not included in the counting's at T_0.

Comparison between T_1 and T_2 is further impeded by the fact that at T_1 the Triple-D program was very restricted and limited to only two hauls in the reference areas, merely aimed to get an impression of possible short-term changes in the larger-sized fauna in OWEZ, one year after its construction. It was anticipated, however, that a measurable change among larger-sized and older benthic species could be expected only after at least several years without fisheries. Therefore an extended Triple-D sampling program was executed in the T_2 in 2011, with six hauls in each of the reference areas.

Considering the above we decided to test with multivariate methods the possible differences between the benthos community in OWEZ and reference areas only between 2007 and 2011 and based on abundances of species found in boxcore sampling. Additional univariate tests were performed to compare total abundance, total biomass, and a number of diversity indices between 2003-2007-2011, also based on boxcore sampling. In latter tests, the six reference areas were pooled in the notched box and whisker plots.

comparison 2003-2007-2011

The macrobenthos abundances based on boxcore sampling in OWEZ and the reference areas in the years 2007 and 2011 and depicted as centroids (representing the "centres of gravity" of the single stations in each of the seven separate areas) in a MDS plot (Fig. 16) demonstrated a clear distinction between the years. However, PERMANOVA tests revealed that over the years 2007-2011 none of the areas, including OWEZ, had changed in a different direction or over a different distance when compared with the other areas. OWEZ did not differ from the reference areas in the years 2007-1011, and a significant difference between OWEZ in 2011 versus OWEZ in 2007 plus all reference areas could not be proved. In conclusion: we cannot prove that the species composition in OWEZ 5 years after its construction and after 5 years of being closed to fisheries had been changed relative to the species composition in the reference areas. Changes in fauna assemblages observed in all of the areas including OWEZ between 2007 and 2011 may dominate potential subtle differences between OWEZ and reference areas. In other words: the effect of 5-years fishery-stop in OWEZ is not more explicit than the faunal variations in the offshore coast due to all types of environmental variation. According to the SIMPER analyses (Table 7) the main difference between the two years was caused by relatively small changes in community composition in all areas**,** and not by the introduction of new species or species loss.

Notched box plots visualizing the differences between the total numbers of individuals in OWEZ and the pooled reference areas in 2003, 2007, and 2011 show that OWEZ fitted well in the range of reference areas (Fig. 17A). Significant differences between T_0 and T_1 data were also not reported by Daan et al. (2009). In other words, there seemed no measurable effect of OWEZ Wind farm on the overall fauna abundance. At the species level there were marked differences between T_0 and T_1. Of the ten species that were overall the most abundant in OWEZ, R1 and R6 during T_1, only the two most abundant ones (the amphipod *Urothoe poseidonis* and the polychaete *Nephtys cirrosa*) were also among the ten most abundant species at T_0. At T_0 (2003) the fauna was dominated by two tube-building polychaetes, *Spiophanes bombyx* and *Lanice conchilega*, both relatively short-living species with strongly fluctuating annual densities (Daan and Mulder, 2006).

OWEZ fitted well in the ranges of total biomass in the pooled reference areas in 2003, 2007, and 2011 (Fig. 17B). Daan et al. (2009) pointed to much higher mean biomass values within OWEZ and R6 at T_1 than at T_0 due to the presence of banks of adult *Ensis americanus* in 3 stations in OWEZ and in 2 stations in R6. In R1, where no *E. americanus* banks were found at T_1, the average biomass was at a similar level as at T_0. In T_2 OWEZ and R6 had again the highest abundances of *E. americanus*, although densities were much lower than in T_1. Daan et al. (2009) pointed also to the relatively high contribution of the sea urchin *Echinocardium cordatum* to the community biomass at T_1, in fact belonging to the top two species ranking in biomass in all areas. Both abundance and biomass values were at a similar level as usually found in the coastal and southern offshore area during the BIOMON program (Daan and Mulder, 2006). However, during T_0 the species did not occur within the top ten species in any of the three areas investigated then. We don't have a plausible explanation for this dissimilarity between T_0 and T_1. Although these natural shifts and unexplained discrepancy in *E. cordatum* density affected overall biomass, no indication was found that overall production in OWEZ has changed between T_0 and T_1 as a result of a changing fauna composition (Daan et al., 2009).

OWEZ fell in the ranges of species richness, eveness, Shannon-Wiener index and Simpson index in the pooled reference areas in 2003, 2007, and 2011 (Fig. 17C-17F). Daan et al. (2009) concluded that species richness and diversity indices did not indicate differences between T_0 and T_1, considering the difference in sample size: 0.068 m^2 at T_0 and 0.078 m^2 at $T_{1.}$ In the 2011 survey, however, OWEZ tended to have a higher diversity of larger sized species than R6 and R4 (see 4.1).This might be a first sign of recovery of OWEZ Wind farm directing to a higher diversity of species.

4.3 Discussion on the impact of OWEZ on local macrobenthos community

In this section possible reasons are discussed why OWEZ Wind farm apparently has had no impact on the local benthos community 5 years after its construction.

study design

A BACI design (Before, After, Control, Impact) with several control areas is a commonly used outline for a study like the present one (Underwood, 1992). Relevant co-variables should be included, at least the co-variables grain size, mud content, water depth. The median grain sizes reported from the T_0 study seemed unrealistic (on average 504 µm; Jarvis et al., 2004) compared to long-term monitoring programs, to other analyses obtained in 2003 and 2006 (on average 250.8 µm and 254.2 µm respectively; Daan and Mulder, 2004; unpubl. 2006-data) and to the 2007-dataset in OWEZ and the reference areas (on average 266 µm; Bergman et al., 2010). The reason for the inconsistency between the analyses of Jarvis and these study's is unknown. In the T_1 in 2007 sediment analyses from boxcore samples were not taken (Daan et al, 2009). Therefore sediment data were not included in the statistical tests.

A number of multivariate statistical analyses can be used, e.g. multiple regression analysis, covariance analysis (ANCOVA), redundancy analysis (RDA), canonical correspondence analysis (CCA), principal component analysis (PCA), and PRIMER. Statistical assumptions determine the selection of a particular test to be applied. We selected the PERMANOVA test (PRIMER vs6 package; Clark and Gorley, 2006) and PERMANOVA (Anderson et al., 2008) because it is flexible due to its reliance on a resemblance measure and robust since it acts only on the ranks of dissimilarities and makes no explicit assumptions regarding the distributions of variables (e.g. normality and linearity).

During the progression of this project the study design has improved due to insight in the local faunal situation. This resulted in a change in number and position of the reference areas between the T_0 and the T_1/T_2 study. Due to the switch in the institute that organized the T_0 and the T_1/T_2 studies, the type of dredge has been changed as well. Both modifications in study design made statistical comparisons complicated between T_0 and the later surveys. It would be advisable for future studies to determine a proper and statistically sound set-up prior to the start of the project that should be followed during all stages of the study. Although the results of the present study fail to show significant changes in the benthos community in the fishery-free OWEZ Wind farm in the 5 years of restoration, it is quite possible that a longer period is needed. The faunal patchiness driven by strong local factors and the depleted adult stocks in the wider region are arguments to consider an additional survey 10 years after the closure to fishery.

reference areas

Environmental factors determine the benthos communities in the dynamic coastal zone. To answer the question if OWEZ Wind farm has affected the local macrobenthos community after 5 years, it is essential to compare this community with that in reference areas steered by a similar set of environmental variables. A number of these environmental factors in the reference areas in 2011 were checked for their degree of conformity with those in OWEZ. Water depths in the entire study area ranged from 12 to 20 m (Fig. 7A). R1 was shallower than R2 and R3, and was therefore not the best reference area to be selected, but could not be circumvented because of its initial selection in the 2003 study. OWEZ fitted well in the range of water depths. Distance to shore in the entire area varied between circa 9 and 20 km (Fig. 7B), and all survey areas differed from each other except the following pairs: OWEZ versus R5, R2 versus R6, and R1 versus R3. Median grain sizes in the whole study area ranged from 185 to 318 µm (Fig. 8) with a mean value of 264.5 µm, and although R1 tended to finer medians (Fig. 10A), there was no significant difference between the median grain sizes among the stations in the different survey areas. The average median grain size in 2011 almost equals that of 2007 (266 µm; Bergman et al., 2010. Mud content analyses revealed that 21 out of 64 samples contained mud with percentages up to 8.2 % in R5 (Fig. 9). The spatial distribution of mud content demonstrated that OWEZ fitted well in the total range of values found in the reference areas. A pairwise comparison demonstrated that R1 and R6 were significantly different, with R1 showing 0% mud and R6 showing relatively high mud contents up to 8.2 % (Fig. 10B). Maximum mud content in OWEZ was 2.5%. In 2007 the highest values of mud content (8.7%) were found in OWEZ (Bergman et al., 2010), whereas in 2003 values up to 15% were reported here (Jarvis et al., 2004). The above results on environmental variables indicate that the only distinctive factor for OWEZ compared to the reference areas was distance to shore, a factor not considered to directly affect benthos communities. From this point of view it cannot be stated that the reference areas were inadequately selected as areas for comparison.

Multivariate BEST analysis used to identify which environmental variable(s) best explain the variation in species composition of all stations, signified mud content and water depth as most prominent (Table 6). In case of OWEZ Wind farm this correlation was rather low (R=0.469), in the reference areas even more trivial (R=0.259), implying that a strong correlation between these factors and the benthos community in the particular areas is not likely. Therefore it cannot be proved that the reference areas including the muddy R6 were not suited to be used for comparison with OWEZ. It is remarkable, however, that in 2007 in the study on the impact of OWEZ on recruitment of juvenile bivalves (>0.5 mm) almost 2 years after the closure to fisheries also a higher correlation between total abundances and mud content was found inside OWEZ than in the reference areas (Bergman et al., 2010). The results from 2007 and 2011 suggest that species in areas undisturbed by fishery maintain their initial correlation with mud content for longer periods than fauna in trawled areas. A plausible reason would be the fishing intensity, that is nil in OWEZ and at a normal level in the reference areas since 2006 (Fig. 2). High resolution data on fishery effort, till now unavailable, when included in the BEST analyse may provide more insight in the correlation between fishery intensity and variation in species composition.

scale of faunal patchiness

Environmental variables including the above mentioned ones play a role in the spatial variation of the benthos communities. The 4 newly formed clusters with >67% resemblance in species composition, each including Triple-D stations from different areas, illustrate this spatial variation in the study area (Fig. 26). At a scale of 5.5*4 km (*i.e.* OWEZ) patchiness in species composition was evident: OWEZ samples were most dominant in group C, but they occurred, though less frequently, in the three other groups. Multivariate SIMPER analysis used to identify the species that were contributing most to the dissimilarities between the newly formed groups, signified the bivalves *Tellina fabula, Lutraria lutraria, Spisula solida, and Donax vittatus,* the polychaete tube worm *Lanice conchilega,* and the brittle star *Ophiura albida* (Table 15). Remarkable was that *T. fabula* was found almost exclusively in group A stations, together with *L. conchilega* and *L. lutraria*. The stations in group A, originating from all study areas, have also higher total abundances, a relatively higher number of species, and higher mud content than the stations in the other groups (Figs. 28, 29). Apparently, patchiness initiated by local factors and occurring at unknown scales, is more responsible for clustering of stations in this coastal benthos community than the assumed contrasting impact of the fishery-free OWEZ and the regularly trawled reference areas. This conclusion was also made based on the clustering of the bivalve recruits of *Abra alba, Donax vittatus, Mysella bidentata*, *Mytilus edulis, Montacuta ferrigunosa, Chamelea gallina, Ensis* spp*.*, *Tellina* spp., *Spisula* spp in 2007, almost two years after the closure to fishery of OWEZ (Bergman et al., 2010). The position of the two significant clusters in that study did not coincide with survey areas but pointed to an offshore–coast gradient in species composition.

Bio-engineering species like the tube-building, habitat structuring polychaete *L. conchilega*, dominant in group A, affects both sediment composition and community structure (Rabaut et al., 2007). The effect of the protruding tubes on hydrodynamics results in the retention of fine sediment particles. Other species are favored by the habitat modifications, which create and regulate refuge for species, alter the interactions between local species and change the physical factors of the environment. It's imaginable that *e.g*. the tube builder *L. conchilaga* by reducing bottom shear stress facilitates mud deposition creating favorable environmental conditions for settlement and survival of *T. fabula* and *L. lutraria.* If so, differences in species composition in the entire study area could be initiated more by unpredictable settlement and successful survival of *Lanice* larvae than by the closure to fishery of OWEZ during the last 5 years. The resulting patchiness apparently acts on a relatively small scale, even within the dimensions of an single study area (Fig. 26). Biogenically structured habitats are probably more affected by fishing than unconsolidated sediment habitats. It's thinkable that a random distribution of *e.g.* the cluster A community (dominated by *L. conchilega, T. fabula*, and *L. lutraria)* would contribute not only to the faunal differences between areas, but also to their responses to being regularly trawled or being free of fishery, and thus to their recovery. This spatial distribution of faunal bio-engineering communities could have had implications for the results of the present study, and could contribute to the fact that the study could hardly prove any impact of OWEZ on the local benthos community. Although nothing is known on spatial dimensions and spatial distribution of the patchiness it might be advisable to select future reference areas more closely around OWEZ to minimize the variability between OWEZ and reference areas.

trawling activity
For trustworthy conclusions based on the present study it is important to know the trawling frequencies in OWEZ and reference areas over the last 5 years. An estimate of trawling activity in and around OWEZ confirms the assumption that trawling activity (in fact: presence of trawlers) in OWEZ was strongly reduced and almost nil in the last 5 years, while the six reference areas were regularly and with an almost similar frequency trawled (Fig. 2). Apparently trawlers did not surpass the borders of the closed area around the wind turbines (*c.f.* Fig. 1). Superimposing the stations of boxcore and Triple-D sampling 2011 (Figs. 5, 6) on the map of trawling frequencies (Fig. 2) confirms that all boxcore stations and all Triple-D stations except WP30 were positioned inside the fishery-free OWEZ Wind farm. Only after receiving the VMS data on fishery frequencies and after constructing Fig. 2 we recently realized that Triple-D station WP 30 was positioned inside the borders of the concession area of OWEZ, but just outside the fishery-free OWEZ Wind farm where trawling frequencies remained unchanged the last 5 years. Inspection of the benthos abundances (Appendix 4b) suggest, however, that WP 30 did not differ from the other Wind farm stations, and most likely did not contribute to any bias in the results.

It can be concluded that the spatial distribution of the trawling frequencies over OWEZ and the reference areas was as expected at forehand, and thus most likely was not the reason for the fact that this T_2-study could hardly prove any impact of OWEZ on the local benthos community.

post-fishery faunal recovery
Previous field studies demonstrated the impact of trawling on benthos species and communities. Instant mortality (Bergman and van Santbrink, 2000), alterations in seabed habitat, structure and functioning of communities (Reiss et al., 2009), and long term changes in in- and epifaunal community composition (Hinz et al., 2009) were reported. Model studies Hiddink et al. (2006) demonstrated that trawling reduced biomass, production, and species richness. In the long run, trawling effects become manifest as shifts in community structure: from long-lived to short-lived, from large sized to small sized, and from slow growing to fast growing species. Almost one century of trawling has shifted the benthic community in the North Sea towards a low biomass/high productivity system (Dannheim, 2007). Since benthos species and communities are clearly impacted by trawling we hypothesized that the closure of OWEZ for fisheries could lead to higher abundances of vulnerable species and changes in the community structure. Considering the probability of redistribution of trawling effort from inside the fishery-closed OWEZ towards outside (Hiddink et al., 2006), faunal differences between OWEZ and surrounding reference areas can be expected be become even more pronounced. The question, however, remains whether already fishery-impacted communities are able to restore in structure and in what period of time. Restoration is documented over longer periods of recovery. In a fishery exclusion area around a gas production platform in the southern North Sea greater species richness, evenness, and abundances of several burrowing mud shrimp and fragile bivalve species was reported, 23 year after its designation (Duineveld et al., 2007). A muddy area in the NW Mediterranean that had not been fished for 20 year was characterized by higher abundances of surface suspension feeders than a similar fished area that on the contrary had a higher abundance of burrowing epifaunal scavengers and motile burrowing infauna (Juan et al., 2007).

Over shorter periods of post-impact recovery, however, less conclusive results are reported. In Horns Rev Wind farm (Spanggaard, 2005), the coastal infauna inhabiting medium-fine sand showed 2 years after the construction higher abundances of the common species . Changes in species composition were probably a result of the highly variable environmental conditions and of less predation by birds. In the T_1-study Daan et al. (2009) did not find any indication of recovery of the local macrobenthos (>1 mm) in OWEZ one year after the construction. The same holds for the settlement and survival of juvenile bivalves (>0.2 mm) in OWEZ measured almost 2 years after the closure to fisheries (Bergman et al., 2010). In another North Sea study on trophically relevant aspects, the fishery-closed area around a platform (fine sand, 28 m water depth) showed subtle faunal differences compared to two normally trawled reference areas (Dannheim, 2007). After 14 months predating/scavenging species were reduced in the fishery-free area, and interface feeders and deposit feeders, amongst them prominently the tube building polychaete *Lanice conchilega*, were increased. Apparently the absence of artificial and additional food sources (discards, bycatch, gear-induced mortality) caused emigration of predators and scavengers, and the absence of physical sediment disturbance facilitated settlement and survival of less mobile species. Remarkably and contrary to the expected recovery of long living species, opportunistic species such as *L. conchilaga* were the first to benefit from the closure. Nevertheless, this can be considered as a first step in the recovery towards a mature community that demands much longer time span and possibly larger scale of closed area (Dannheim, 2007). Collie et al. (2000) stated that much of the recolonization was through immigration into disturbed patches, and mainly by small-bodied taxa such as polychaetes. A comparison between a Bay closed to fisheries and a normally trawled one at the east coast of Scotland showed little difference in measures of diversity between the sites, but video analysis identified a greater number of *L. conchilega* beds in the fishery-closed St Andrews Bay (Defew et al., 2012). The authors interpret the presence of these habitat-structuring, tube dwelling polychaetes as a response to the absence of trawler disturbance. They stated that Lanice beds have the potential to stabilise the sediment to the extent where a more diverse community may develop. It is likely that recovery of the faunal structure including long-lived bivalve species demand longer recovery periods.

adult stocks

The low abundance of the juvenile bivalve *Spisula subtruncata* in the trawled reference areas and in OWEZ almost two year after its closure to fishery suggested that their low densities was most likely due to low initial settlement (Bergman et al., 2010). In 2006 the stock of *S. subtruncata* in the Dutch coastal zone was at its lowest since 1995 the start of the monitoring (Perdon and Goudswaard, 2006). Average numbers of one year old *S. subtruncata* were reduced to 0.1 ind. per m^2, compared to up to 4000 adults per m^2 present in the 1980’s and 90’s. The authors link the steady decline in population to failing recruitment, due to the extremely reduced adult stock producing low numbers of competent larvae. Collie et al. (2000) also stated that recovery seems slower if the spatial scale of impact was larger, as it would be on heavily trawled fishing grounds like most parts of the southern North Sea. It’s reasonable to expect that areas closed for fishery will recover hardly if wider surroundings are depleted of parent stocks. Significant reductions due to intensified fisheries since the beginning of the 20th century are reported for spatial distributions of long-lived, slow-growing epifauna (Callaway et al., 2007), and for biomass of bivalves and spatangoids (burrowing sea-urchins) (Jennings et al., 2001) in the southern and central North Sea. This was even a motive to argue that for an effective faunal recovery its desirable to combine the implementation of a fishery-closed areas with a national license-buy-back program to compensate fishers and to eliminate unwanted impacts elsewhere (Jennings, 2009).

Although the decline in adult abundances is evident in *S.subtruncata,* it is likely that most populations of bivalves and crustaceans sensible to trawling mortality have declined over the last century in the heavily trawled coastal zone of the southern North Sea. If so, successful settlement and survival of juvenile stages is limited by low numbers of competent larvae. This is supported by the 2007-survey in OWEZ almost two years after the its closure for fishery targeting the settlement of juvenile bivalves, that showed no indication for increased settlement (Bergman et al., 2010). In conditions with strongly reduced adult stocks restoration of dense benthos populations in the fishery-free OWEZ cannot be expected. This might be an explanation for the results of the present study that point to a minimal impact of OWEZ on the local benthos community even after a closure to fishery for 5 years. If in future adult stock would increase leading to enhanced numbers of competent larvae, successful settlement and survival most likely will be stimulated by the existence of this fishery-free area.

time scale of recovery

Almost certainly the most significant effect of trawling on benthic assemblages is that of habitat homogenization and the reduction in habitat complexity due to the removal of sessile epifauna (Thrush and Dayton, 2002; Collie et al., 2006). Natural sedimentary coastal environments contain a variety of three dimensional structure: stones and patches of different types of sediment. Biological alteration of the sediment is extremely important; shells, animal tubes of a variety of shapes, sizes and durability, fecal piles, holes and pits are all key elements of the structure and functioning of these habitats. Such structures are important cues for settlement processes of many organisms, and can act as refugia from predators (Gray et al., 2006). The patchy spatial distribution of the tube-building worm *Lanice conchilega,* an engineering species generating 3D structure and in the present study often found in assemblages with the bivalves *Lutraria lutraria* and *Tellina fabula,* might be a key factor in successful settlement. Recruitment and survival of *L. conchilega* and other species is quite unpredictable as it is affected by an extremely dynamic coastal environment that becomes manifest in high temperature variability including extremely cold winters, strong tidal currents, variable riverine input including nutrient and sediment load, as well as in wind induced swell and sediment resuspension. Recovery of other species can easily be delayed by a limited larval supply due to variation in those factors and additional variation in larval drifting, competition, predation and food supply. In the T_2-study Bergman et al. (2010) did not find any indication for a higher settlement and survival of juvenile bivalves (>0.2 mm) in OWEZ almost 2 years after the closure to fisheries. Comparisons between larval (>0.2 mm) settlement in the mesocosms, being up to 1565 per m^2 per day in OWEZ in July 2007, and larval (> 0.5 mm) abundances at the seabed, being some hundreds per m^2 in October 2007, suggest a considerable loss of bivalve recruits during the first months of settlement in the field situation. This mortality is most likely generated by variability in environmental and biotic factors. This process may lead to longer period of years before any recovery can be recorded. It is reasonable that recovery may take a longer period than the 5 years that were included in the present study. It cannot entirely be excluded that the higher species diversity in OWEZ than in R4 and R6, the higher abundances of the bivalve *Spisula solida* in OWEZ than in R2 and R5, and the largest length in *Tellina fabula* and width in *Ensis americanus* in OWEZ are a first step towards the recovery of the benthos community.

5. CONCLUSIONS

- Total abundances, total biomass and total annual production in 2011 were not different between areas based on boxcore sampling; Triple-D samples showed higher values in R6 than in OWEZ.
- Multivariate species composition (abundances), biomass, and annual production were not different between areas based on boxcore sampling; Triple-D samples signified differences between areas, but in both sampling methods OWEZ did not differ from the reference areas.
- Multivariate species composition (abundances) of selected groups of species from Triple-D sampling like the 15 most common, the 30 most uncommon, all epifauna, and all infauna species were not different between areas, and OWEZ did not differ from the reference areas.
- Multivariate species composition (abundances) of selected groups of species from Triple-D sampling like the 4 separate taxa and all 19 scavenging species differed between some of the areas, but OWEZ did not differ from the reference areas.
- The bivalve *Spisula solida* had higher abundances in OWEZ than in R2 and R5.
- The number of species was not different between areas based on boxcore sampling; Triple-D samples showed higher values in R3 than in R1, R2, R5, R6.
- The Shannon Wiener indices were not different between areas based on boxcore sampling; Triple-D samples showed higher values indicating a higher diversity, number of species and eveness in OWEZ than in R4 and R6, and lower values in R6 than in R1, R2, R5, and OWEZ.
- The Simpson indices were not different between areas based on boxcore sampling; Triple-D samples showed lower values indicating lower diversity and evenness in R6 than in all other areas.
- Both diversity indices, if based on Triple-D sampling, point to R6 as an area with a relatively low diversity, and a low evenness. OWEZ tended to have a higher diversity, number of species and eveness than R6 and R4.
- Shell length of the bivalve *Tellina fabula* was larger in OWEZ than in R1, R2, R3, and shell width of *Ensis americanus* was larger in OWEZ than R4, R5, R6; four othe mollusc species reached their largest dimensions in one of the reference areas.
- We cannot prove that the species composition in OWEZ comparing 2007 and 2011 has changed relative to the species composition in the reference areas. The distinction between the years 2007 and 2011 was mainly due to relatively small variations in species abundances in all areas and not caused by the introduction of new species or species loss.
- Total numbers of individuals, total biomass, and diversity (expressed in terms of species richness, eveness, Shannon-Wiener and Simpson indices) in OWEZ were not different from the values in the pooled reference areas in and between 2003, 2007, and 2011.
- It can be concluded that the spatial distribution of the trawling frequencies over OWEZ and the reference areas was as expected at forehand, and thus most likely was not the reason that this study could hardly prove any impact of OWEZ on the local benthos community.
- The faunal patchiness steered by local factors and the depleted adult stocks in the wider region may lead to a longer recovery time than 5 years.
- It cannot be excluded that the higher species diversity, the higher abundances of the bivalve *Spisula solida*, and the largest sizes of bivalves *Tellina fabula* and *Ensis americanus* found in OWEZ compared to (some of) the reference areas are first steps towards the recovery of the local benthos community.

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Appendix 1. Environmental data (derived from boxcore stations and samples): geographical position (degree, dec.degree), median grain size (µm), mud %, distance to shore (m0, water depth (m).

Stationnumber	Latitude	Longitude	Median	Volume%<63um	Distance to shore (m)	Depth (m)
R1-101	52.7326	4.4648	235.7	0	11803	15.84
R1-103	52.7267	4.4648	233.6	0	11753	15.64
R1-105	52.7213	4.4645	235.7	0	11730	15.36
R1-106	52.7252	4.478	237.1	0	10906	14.23
R1-110	52.7147	4.4785	246.1	0	10674	13.28
R1-111	52.719	4.4906	246.2	0	9959	12.95
R1-113	52.7134	4.4909	254.5	0	9845	12.24
R1-115	52.7078	4.4904	256.5	0	9802	12.52
R2-101	52.6998	4.3922	265.3	0	16322	17.81
R2-103	52.6946	4.3921	317.8	0.8	16248	16.99
R2-105	52.6889	4.3917	289.2	0	16190	17.64
R2-106	52.6934	4.4028	314.6	0.89	15502	17.79
R2-110	52.6826	4.4013	246.2	0	15439	17.77
R2-111	52.6837	4.4246	231.2	3.24	13898	16.11
R2-113	52.6769	4.4246	248.1	0	13795	15.19
R2-115	52.6714	4.4249	244.9	0	13700	15.01
R3-101	52.6718	4.4779	263	0	10223	15.54
R3-103	52.6661	4.478	264	0	10111	15.03
R3-105	52.6607	4.4778	254.4	1.18	10037	14.62
R3-106	52.67	4.49	279	0	9304	17.97
R3-110	52.658	4.4906	276.1	0	9127	17.5
R3-111	52.6708	4.5068	268.8	0	8157	18.11
R3-113	52.6651	4.5067	260.6	1.24	8143	19
R3-115	52.6597	4.5063	243.2	4.84	8105	19.43
R4-101	52.5695	4.2939	268.6	0	21056	16.32
R4-103	52.563	4.294	250	0	20896	14.67
R4-105	52.5579	4.2939	256.1	0	20799	14.27
R4-106	52.5654	4.3064	270.4	0	20135	13.24
R4-110	52.5555	4.306	260.7	0	19957	15.48
R4-111	52.5611	4.3174	271.8	0	19319	17.48
R4-113	52.5561	4.3181	290.5	0	19155	19.38
R4-115	52.5504	4.3178	252.7	1.64	19054	19.49
R5-101	52.5347	4.3923	274.8	0	13770	14.83
R5-103	52.5291	4.3917	271.1	0	13666	14.75
R5-105	52.5239	4.392	261.2	1.11	13434	15.39
R5-108	52.5181	4.3976	262.3	0	12869	14.62
R5-110	52.5129	4.3973	265.2	1.05	12682	14.47
R5-111	52.5124	4.4067	247.2	1.96	12103	14.1
R5-113	52.5075	4.4075	270.6	0	11842	13.78
R5-115	52.5014	4.4079	185.3	8.17	11520	17.13
R6-101	52.4674	4.3275	226.5	4.47	16218	16.9
R6-103	52.4628	4.3276	282.5	1.83	16121	18.92
R6-104	52.4597	4.3273	206.2	4.47	16043	19.62
R6-105	52.4617	4.3343	292	0	15662	17.16
R6-107	52.4556	4.3355	271.5	2.49	15338	17.62
R6-110	52.4521	4.3402	274.1	1.27	14860	15.36
R6-112	52.4472	4.3404	315.2	0	14670	13.81
R6-114	52.4408	4.3404	313.3	1.17	14432	13.37
W-104	52.5981	4.423	286.1	1.07	12916	13.29
W-106	52.6086	4.4088	288	0	14005	14.36
W-108	52.6173	4.3974	252.6	0	14870	14.45
W-109	52.5852	4.4475	260.3	0	11040	15.13
W-111	52.5938	4.435	248.4	0	12060	15.06
W-113	52.6052	4.4202	268.2	0	13180	12.86
W-115	52.6144	4.4076	257	0	14176	15.04
W-117	52.5931	4.4498	234	2.5	11035	15.8
W-119	52.6033	4.4356	299.6	0	12127	13.26
W-121	52.6185	4.4153	253.8	0	13692	15.96
W-123	52.5993	4.4475	278.4	1.11	11283	16.28
W-125	52.6113	4.431	275.9	0	12536	14.58
W-127	52.6246	4.4127	258	0	13939	15.36
W-128	52.6031	4.4554	244.3	0	10796	16.91
W-129	52.6089	4.4475	268	1.42	11408	15.35
W-130	52.6149	4.4395	280.3	0	12021	13.86

Appendix 2a. AFDW conversion factors (g wet weight to g AFDW) for boxcore species.

Boxcore					
Group	**Species name**	**Conversion ^**	*****	**Unit**	**Source**
Crustacea	Apherusa ovalipes		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Atylus falcatus		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Bathyporeia elegans		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Bathyporeia guilliamsoniana		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Bathyporeia nana		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Bathyporeia tenuipes		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Callianassa tyrrhena		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Cancer pagurus juv.		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Corystes cassivelaunus		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Crangon crangon		0.1732	ww	Rumohr 1987
Crustacea	Diastylis bradyi		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Diogenes pugilator		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Gammaropsis spec.		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Gastrosaccus spinifer		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Ione thoracica		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Leucothoe incisa		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Orchomene nana		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Perioculodes longimanus		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Pontocrates altamarinus		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Synchelidium maculatum		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Thia scutellata		0.1650	ww	Ricciardi en bourget 1998
Crustacea	Urothoe brevicornis		0.0003	aantal	Rapport Daan et al. 2009
Crustacea	Urothoe poseidonis		0.0003	aantal	Rapport Daan et al. 2009
Echinodermata	Echinocardium cordatum	3.2196	0.00000361	length	Pers com Marc Lavaleye NIOZ
Echinodermata	Ophiura albida	2.7598	0.000063	length	Pers com Marc Lavaleye NIOZ
Echinodermata	Ophiura texturata	2.7598	0.000063	length	Pers com Marc Lavaleye NIOZ
Mollusca	Abra alba	2.7477	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Chamelea striatula	3.0471	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Donax vittatus	3.1501	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Ensis americanus	3.8222	0.0000	length	Pers com Rob Witbaard
Mollusca	Ensis ensis	3.8222	0.0000	length	Pers com Rob Witbaard
Mollusca	Ensis spec.	3.8222	0.0000	length	Pers com Rob Witbaard
Mollusca	Euspira pulchella	2.9047	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Mactra corallina	2.5114	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Mysella bidentata	2.9631	0.00001308	length	Pers com Marc Lavaleye NIOZ
Mollusca	Nassarius reticulatus	2.8849	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Spisula elliptica	2.5114	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Spisula subtruncata	2.5114	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Tellimya ferruginosa	2.9631	0.00001308	length	Pers com Marc Lavaleye NIOZ
Mollusca	Tellina fabula	3.0000	0.0000	length	Pers com Marc Lavaleye NIOZ
Mollusca	Tellina tenuis	3.0000	0.0000	length	Pers com Marc Lavaleye NIOZ
Others	Anthozoa spec.		0.143	ww	Ricciardi en bourget 1998
Others	Hydrozoa		-	-	-
Others	Nemertini		0.22	ww	Rumohr 1987
Others	Oligochaeta		0.0539	ww	Ricciardi en bourget 1998
Others	Phoronida		0.16	ww	Ricciardi en bourget 1998
Others	Turbellaria		0.2520	ww	Ricciardi en bourget 1998
Polychaeta	Aricidea minuta		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Capitella capitata		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Chaetozone christiei		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Chaetozone setosa		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Chaetozone spec. juv.		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Eteone foliosa		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Eteone longa		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Eumida bahusiensis		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Eumida sanguinea		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Eumida spec.juv.		0.1600	ww	Ricciardi en bourget 1998

Appendix 2a continued.

Polychaeta	Harmothoe spec. juv.		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Lanice conchilega		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Magelona filiformis		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Magelona johnstoni		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Magelona mirabilis		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Malmgrenia marphysae		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Mediomastus fragilis		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Nephtys assimilis		0.175	ww	Rumohr 1987
Polychaeta	Nephtys caeca		0.175	ww	Rumohr 1987
Polychaeta	Nephtys cirrosa		0.175	ww	Rumohr 1987
Polychaeta	Nephtys hombergii		0.175	ww	Rumohr 1987
Polychaeta	Nephtys incisa		0.175	ww	Rumohr 1987
Polychaeta	Nephtys indet.		0.175	ww	Rumohr 1987
Polychaeta	Nephtys longosetosa		0.175	ww	Rumohr 1987
Polychaeta	Nephtys spec. juv.		0.175	ww	Rumohr 1987
Polychaeta	Nereis longissima		0.149	ww	Rumohr 1987
Polychaeta	Nereis virens		0.149	ww	Rumohr 1987
Polychaeta	Notomastus latericeus		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Ophelia limacina		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Pholoe minuta		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Phyllodoce mucosa		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Poecilochaetus serpens		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	pol. rest		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Scolelepis bonnieri		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Scoloplos armiger		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Sigalion mathildae		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Spio armata		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Spio decorata		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Spio filicornis		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Spiophanes bombyx		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Streptosyllis websteri		0.1600	ww	Ricciardi en bourget 1998
Polychaeta	Travisia forbesii		0.1600	ww	Ricciardi en bourget 1998

Appendix 2b. Conversion factors (g wet weight to g AFDW) for Triple-D species.

Triple-D				
Group	**Species name**	**Conversion**	**Unit**	**Source**
Anthozoa (actiniaria)	Metridium senile	0.1430	ww	Ricciardi en bourget 1998
Anthozoa (actiniaria)	Sagartia troglodytes	0.143	ww	Ricciardi en bourget 1998
Anthozoa (actiniaria)	Sagartiogeton undatus	0.143	ww	Ricciardi en bourget 1998
Crustacea	Callianassa subterranea	0.165	ww	Ricciardi en bourget 1998
Crustacea	Callianassa tyrrhena	0.165	ww	Ricciardi en bourget 1998
Crustacea	Corystes cassivelaunus	0.165	ww	Ricciardi en bourget 1998
Crustacea	Crangon allmanni	0.173237	ww	Rumohr 1987
Crustacea	Crangon crangon	0.173237	ww	Rumohr 1987
Crustacea	Diogenes pugilator	0.165	ww	Ricciardi en bourget 1998
Crustacea	Idotea linearis	0.165	ww	Ricciardi en bourget 1998
Crustacea	Liocarcinus (heel klein)	0.165	ww	Ricciardi en bourget 1998
Crustacea	Liocarcinus arcuatus	0.165	ww	Ricciardi en bourget 1998
Crustacea	Liocarcinus depurator	0.165	ww	Ricciardi en bourget 1998
Crustacea	Liocarcinus holsatus	0.165	ww	Ricciardi en bourget 1998
Crustacea	Liocarcinus klein	0.165	ww	Ricciardi en bourget 1998
Crustacea	Macropodia spec.	0.165	ww	Ricciardi en bourget 1998
Crustacea	Pagurus bernhardus	0.165	ww	Ricciardi en bourget 1998
Crustacea	Pontophilus trispinosus	0.165	ww	Ricciardi en bourget 1998
Crustacea	Portumnus latipes	0.165	ww	Ricciardi en bourget 1998
Crustacea	Processa spec.	0.165	ww	Ricciardi en bourget 1998
Crustacea	Thia scutellata	0.165	ww	Ricciardi en bourget 1998
Echinodermata	Acrocnida brachiata	0.074	ww	Ricciardi en bourget 1999
Echinodermata	Asterias rubens	0.112	ww	Ricciardi en bourget 2000
Echinodermata	Echinocardium cordatum	0.03	ww	Rumohr 1987
Echinodermata	Ophiura albida	0.085	ww	Ricciardi en bourget 2002
Echinodermata	Ophiura texturata	0.085	ww	Ricciardi en bourget 2003
Hydrozoa	Tubularia larynx	0.252	ww	Ricciardi en bourget 2004
Mollusca	Abra alba	0.05634	ww	Rumohr 1987
Mollusca	Chamelea striatula	0.036	ww	Pers com Marc Lavaleye NIOZ
Mollusca	Donax vittatus	0.058	ww	Ricciardi en bourget 2003
Mollusca	Ensis americanus	0.046	ww	Pers com Marc Lavaleye NIOZ
Mollusca	Ensis siliqua	0.046	ww	Pers com Marc Lavaleye NIOZ
Mollusca	Euspira catena	0.075	ww	Ricciardi en bourget 1998
Mollusca	Euspira pulchella	0.075	ww	Ricciardi en bourget 1998
Mollusca	Laevicardium crassum	0.058	ww	Ricciardi en bourget 1998
Mollusca	Lutraria lutraria	0.058	ww	Ricciardi en bourget 1998
Mollusca	Mactra corallina	0.058	ww	Ricciardi en bourget 1998
Mollusca	Nassarius reticulatus	0.063	ww	Pers com Marc Lavaleye NIOZ
Mollusca	Spisula elliptica	0.058	ww	Ricciardi en bourget 1998
Mollusca	Spisula solida	0.058	ww	Ricciardi en bourget 1998
Mollusca	Spisula subtruncata	0.058	ww	Ricciardi en bourget 1998
Mollusca	Tellina fabula	0.058	ww	Ricciardi en bourget 1998
Mollusca	Tellina tenuis	0.058	ww	Ricciardi en bourget 1998
Nemertea	Cerebratulus fuscus	0.2	ww	Ricciardi en bourget 1998
Polychaeta	Glycera	0.16	ww	Ricciardi en bourget 1998
Polychaeta	Lanice conchilega	0.16	ww	Ricciardi en bourget 1998
Polychaeta	Nephtys hombergi	0.16	ww	Ricciardi en bourget 1998
Polychaeta	Nereis longissima	0.16	ww	Ricciardi en bourget 1998
Polychaeta	Ophelia limacina	0.16	ww	Ricciardi en bourget 1998
Polychaeta	Pectinaria spec.	0.16	ww	Ricciardi en bourget 1998
Polychaeta	Phyllodoce	0.16	ww	Ricciardi en bourget 1998

Appendix 3. Boxcore P/B ratios per species.

Taxa	Species name	P/B ratio KJ
Mollusca	Abra alba	1.10701004
Others	Anthozoa spec.	0.265678651
Crustacea	Apherusa ovalipes	2.753494926
Polychaeta	Aricidea minuta	4.252563136
Crustacea	Atylus falcatus	2.753494926
Crustacea	Bathyporeia elegans	2.753494926
Crustacea	Bathyporeia guilliamsoniana	2.753494926
Crustacea	Bathyporeia nana	2.753494926
Crustacea	Bathyporeia tenuipes	2.753494926
Crustacea	Callianassa tyrrhena	0.695204112
Crustacea	Cancer pagurus juv.	2.411101113
Polychaeta	Capitella capitata	4.416379063
Polychaeta	Chaetozone christiei	3.312353688
Polychaeta	Chaetozone setosa	3.136722103
Polychaeta	Chaetozone spec. juv.	4.568188993
Mollusca	Chamelea striatula	0.627372077
Crustacea	Corystes cassivelaunus	0.610730103
Crustacea	Crangon crangon	1.05496313
Crustacea	Diastylis bradyi	2.753494926
Crustacea	Diogenes pugilator	1.996497042
Mollusca	Donax vittatus	0.639110382
Echinodermata	Echinocardium cordatum	0.273174579
Mollusca	Ensis americanus	0.269028066
Mollusca	Ensis ensis	0.4326871
Mollusca	Ensis spec.	0.35849206
Polychaeta	Eteone foliosa	3.551469387
Polychaeta	Eteone longa	3.766355566
Polychaeta	Eumida bahusiensis	3.475391223
Polychaeta	Eumida sanguinea	3.222809843
Polychaeta	Eumida spec.juv.	4.568188993
Mollusca	Euspira pulchella	1.192973948
Crustacea	Gammaropsis spec.	2.753494926
Crustacea	Gastrosaccus spinifer	3.179504693
Polychaeta	Harmothoe spec. juv.	4.568188993
Crustacea	Ione thoracica	5.667194218
Polychaeta	Lanice conchilega	0.941290212
Crustacea	Leucothoe incisa	2.753494926
Mollusca	Mactra corallina	1.369997426
Polychaeta	Magelona filiformis	3.481361298
Polychaeta	Magelona johnstoni	2.737947368
Polychaeta	Magelona mirabilis	2.493069144
Polychaeta	Malmgrenia marphysae	2.179699727
Polychaeta	Mediomastus fragilis	4.284275024
Mollusca	Mysella bidentata	2.684137596
Mollusca	Nassarius reticulatus	0.55385121
Others	Nemertini	1.158432677
Polychaeta	Nephtys assimilis	0.825155356
Polychaeta	Nephtys caeca	0.872010032
Polychaeta	Nephtys cirrosa	1.641077437
Polychaeta	Nephtys hombergii	1.200799727
Polychaeta	Nephtys incisa	1.348799587
Polychaeta	Nephtys indet.	3.39874222
Polychaeta	Nephtys longosetosa	1.438040374
Polychaeta	Nephtys spec. juv.	3.491517168
Polychaeta	Nereis longissima	0.942266137
Polychaeta	Nereis virens	0.512675028
Polychaeta	Notomastus latericeus	1.212411829
Others	Oligochaeta	3.475237784
Polychaeta	Ophelia limacina	1.666550546
Echinodermata	Ophiura albida	0.722183175
Echinodermata	Ophiura texturata	0.443933603
Crustacea	Orchomene nana	2.753494926
Crustacea	Perioculodes longimanus	2.753494926
Polychaeta	Pholoe minuta	2.955880047
Others	Phoronida	2.171279667
Polychaeta	Phyllodoce mucosa	2.515210975
Polychaeta	Poecilochaetus serpens	2.548536311
Crustacea	Pontocrates altamarinus	2.753494926
Polychaeta	Scolelepis bonnieri	1.683681704
Polychaeta	Scoloplos armiger	2.209784792
Polychaeta	Sigalion mathildae	1.363342317
Polychaeta	Spio armata	3.844388195
Polychaeta	Spio decorata	3.344589062
Polychaeta	Spio filicornis	3.844388195
Polychaeta	Spiophanes bombyx	3.043806556
Mollusca	Spisula elliptica	2.296737162
Mollusca	Spisula subtruncata	0.929760305
Polychaeta	Streptosyllis websteri	4.568188993
Crustacea	Synchelidium maculatum	2.753494926
Mollusca	Tellimya ferruginosa	2.161150494
Mollusca	Tellina fabula	0.910542172
Mollusca	Tellina tenuis	3.397024774
Crustacea	Thia scutellata	1.147248511
Polychaeta	Travisia forbesii	1.82407248
Others	Turbellaria	2.418758044
Crustacea	Urothoe brevicornis	2.753494926
Crustacea	Urothoe poseidonis	2.753494926

Appendix 4a. Boxcore species list (n/0.078m^2) in OWEZ and reference areas.

	R1-101	R1-103	R1-105	R1-106	R1-110	R1-111	R1-113	R1-115	R2-101	R2-103	R2-105	R2-106	R2-110	R2-111	R2-113	R2-115	R3-101	R3-103	R3-105	R3-106	R3-110	R3-111	R3-113	R3-115
Abra alba																								
Anthozoa spec.	1																							
Apherusa ovalipes										2														
Aricidea minuta										1		2												
Atylus falcatus										1					1									
Bathyporeia elegans	20	27	12	1	2	1	1	9	11	1	17	2	8	14	22	26	12		30	1			8	
Bathyporeia guilliamsoniana	10		2	2	3			2	2		2	2	7	1	1	3		1			2		1	
Bathyporeia nana	3		2			1			2		1				1	1							1	
Bathyporeia tenuipes																								
Callianassa tyrrhena		2									1													
Cancer pagurus juv.																								
Capitella capitata	1										16										1		1	17
Chaetozone christiei	2	3	2	1	1						2		3		1						2		1	1
Chaetozone setosa	1	1				2								2	1								3	
Chaetozone spec. juv.			1																					
Chamelea striatula	1														1	1								
Corystes cassivelaunus																								
Crangon crangon							1									1								1
Diastylis bradyi																								
Diogenes pugilator																1								
Donax vittatus	1		2	1									1											
Echinocardium cordatum	2		2	1					2			1		2	1			1			6		2	3
Ensis americanus				1									1											1
Ensis ensis									1		4	1												1
Ensis spec.																								
Eteone foliosa					1																			
Eteone longa	2	2			3	3	2	3	3	3	6	3	2	2		4			2	1		3	3	3
Eumida bahusiensis										1														
Eumida sanguinea	2											1												4
Eumida spec.juv.												1												
Euspira pulchella	1	1																						1
Gammaropsis spec.																								
Gastrosaccus spinifer							1								1									1
Harmothoe spec. juv.																								1
Hydrozoa	33	1				2		1	1	1	124	12	3	2	1						2		3	13
Ione thoracica											2													
Lanice conchilega	10		11						3	16	16	20											2	54
Leucothoe incisa		1	1								1	2			1									
Mactra corallina																								
Magelona filiformis	1	9	2	4				4	1		4	4	1	2	1				2		2		1	
Magelona johnstoni	3	3	3			1			1				1	2	1				1		11		5	7
Magelona mirabilis	1	2	1	1	1	3	4	1				2	2	3	1				2		5	2	1	1
Malmgrenia marphysae	5		2				1		1	8	10	5						1				1		27
Mediomastus fragilis																								
Mysella bidentata			1								1													7
Nassarius reticulatus																								
Nemertini	3	2	2	2	2		1	2		1	4	2	2	1					1		6	1	1	4
Nephtys assimilis																								
Nephtys caeca																								
Nephtys cirrosa	4	4	3	5	13	9	10	11	5	11	1	3	5	9	8	10		7	9	1	2	8	9	2
Nephtys hombergii		1	2			1					2	2												
Nephtys incisa																								
Nephtys indet.												1												
Nephtys longosetosa	1							1	1													1		1
Nephtys spec. juv.	1			1					3							1								
Nereis longissima	1	1	1																					1
Nereis virens												1												
Notomastus latericeus			2								1													15
Oligochaeta					1						1				1									
Ophelia limacina										6	1	3												
Ophiura albida										1														
Ophiura texturata						1				1														
Orchomene nana																					1			1
Perioculodes longimanus															1									1
Pholoe minuta																								
Phoronida	1	218	27	11	1				1			2	5	1			1				3			
Phyllodoce mucosa											7	7	1											
Poecilochaetus serpens	1									2	2	2												8
Pontocrates altamarinus																1			1				1	
Scolelepis bonnieri	1	5		3	2	2	2	4					1	3	4	4		4	5			5	7	
Scoloplos armiger	2		4			1	2	1	3	1	1	2	6	1		3						1		
Sigalion mathildae																								
Spio armata																								
Spio decorata																				2				
Spio filicornis																							1	
Spiophanes bombyx										1														3
Spisula elliptica																								
Spisula subtruncata																								
Streptosyllis websteri																								
Synchelidium maculatum		1										1								1	1			
Tellimya ferruginosa	2		8	4								1		3				2			8		5	23
Tellina fabula	4	1	1	1									1	1							1		1	9
Tellina tenuis													1	1										
Thia scutellata																							1	
Travisia forbesii																								
Turbellaria																								
Urothoe brevicornis	3		5	2				1	10	18	1	6		1				1			3	1	1	2
Urothoe poseidonis	77	17	112	171	7		8						74	75	1	1		3	1	3	134	1	13	109

Appendix 4a continued.

	R4-101	R4-103	R4-105	R4-106	R4-110	R4-111	R4-113	R4-115	R5-101	R5-103	R5-105	R5-108	R5-110	R5-111	R5-113	R5-115	R6-101	R6-103	R6-104	R6-105	R6-107	R6-110	R6-112	R6-114
Abra alba																			3					
Anthozoa spec.																1								
Apherusa ovalipes																								
Aricidea minuta																								
Atylus falcatus																			1					1
Bathyporeia elegans	8	12		1	6	3	9	3	8	2	9	11	2	10			2	1	2	7	1	14		2
Bathyporeia guilliamsoniana	2					1	1	6	4	4	10	5	2	4					4	2	3	9	2	
Bathyporeia nana	1						1				3								1					
Bathyporeia tenuipes					2																			
Callianassa tyrrhena						1													1					
Cancer pagurus juv.																								1
Capitella capitata										1				1					2	1	1			
Chaetozone christiei															2		2	1		1		2	1	
Chaetozone setosa									1								1	1	2					
Chaetozone spec. juv.																								
Chamelea striatula														1										
Corystes cassivelaunus																			1					
Crangon crangon								1	1															
Diastylis bradyi						1								1										
Diogenes pugilator											1					1								
Donax vittatus	2		1											1	1									
Echinocardium cordatum	1	1	1	1	1		2	4		1		1				26		6	4	1	1	1		
Ensis americanus							1			1									1		2			
Ensis ensis																						1		
Ensis spec.								1				1												
Eteone foliosa																	2							
Eteone longa	3	2	3	2	1	2	1	4	4	1	3	3	3	3	5	2			1	2	3	9	1	2
Eumida bahusiensis																								
Eumida sanguinea																4	2							
Eumida spec.juv.																								
Euspira pulchella																4			1	2		1		
Gammaropsis spec.																1								
Gastrosaccus spinifer						1					1									1				
Harmothoe spec. juv.																1								
Hydrozoa	1				2			2		9	1	3	1	15	6	21	8	1	3	4	7	9	4	1
Ione thoracica																								
Lanice conchilega							1	5		1		2				69	5		11	3	9	2		
Leucothoe incisa																1	2		3		3	2		
Mactra corallina												1		1										
Magelona filiformis	3		1				1	2	4		3	3	1	3			2	7	3	1	1	1		
Magelona johnstoni		2	1		1		5	2	4	1	4		2				4	1	2	1				
Magelona mirabilis	2	1			1	1	1		6	4	1	2		1	3	3	1	3	3			3	3	4
Malmgrenia marphysae								3								35	3		5	1	7	1		
Mediomastus fragilis																			17					
Mysella bidentata																92	1	9	41		3			
Nassarius reticulatus																1					1			
Nemertini	2	2	1	3	1	1	4	2					4		4	3			10		3			1
Nephtys assimilis								1																
Nephtys caeca																1								
Nephtys cirrosa	9	2	9	6	2	6	6	2	8	4	3	7	10	10	3		6	1		8	1	6	4	2
Nephtys hombergii																1			1		2			
Nephtys incisa									1															
Nephtys indet.																								
Nephtys longosetosa								1		1												1		
Nephtys spec. juv.		1										1							1			1		
Nereis longissima																			2					
Nereis virens																								
Notomastus latericeus																		1	5					
Oligochaeta	2												2						1					
Ophelia limacina		2	1				1	1		1												2	1	2
Ophiura albida						1												1						
Ophiura texturata																1								
Orchomene nana																								
Perioculodes longimanus																								
Pholoe minuta																4								
Phoronida					47	6	9	139		1	1		1		1	16	2		43		48			
Phyllodoce mucosa																1					1	1		
Poecilochaetus serpens							2	4											4		4			
Pontocrates altamarinus	1						2														1			
Scolelepis bonnieri	14	20	3	4	2	2	1		2	1	3	2	9	14	12		1	1				8	4	7
Scoloplos armiger		5			2		7	6	1	1	1			1			9	2	8		4		1	
Sigalion mathildae																2		2						
Spio armata																		1	1					
Spio decorata																								
Spio filicornis																		1				1		
Spiophanes bombyx									1	1			1		1			1	5		3			
Spisula elliptica																								
Spisula subtruncata										1					1	1					1		1	
Streptosyllis websteri																					1			
Synchelidium maculatum						1																		
Tellimya ferruginosa							2			1				1		22		43	60		6			
Tellina fabula								1								2		1	15		3			
Tellina tenuis																								
Thia scutellata																								
Travisia forbesii																							1	
Turbellaria																		1						
Urothoe brevicornis	1	2	11		6	10	2	2	1			1	3	1		1	2	3	2	6		1	12	1
Urothoe poseidonis	1	2				10	69	154	2	76	3	5		2		39	5	84	173	2	91	46		

Appendix 4a continued.

	W-104	W-106	W-108	W-109	W-111	W-113	W-115	W-117	W-119	W-121	W-123	W-125	W-127	W-128	W-129	W-130
Abra alba																
Anthozoa spec.																
Apherusa ovalipes																
Aricidea minuta																
Atylus falcatus		1							1						1	1
Bathyporeia elegans	5		2	22	1	24	6	1	1	1	20		1	3		8
Bathyporeia guilliamsoniana		7		3			3			3	2	1	1	4	1	
Bathyporeia nana				2							3					
Bathyporeia tenuipes			1	3						1						
Callianassa tyrrhena											1					
Cancer pagurus juv.																
Capitella capitata								9			3			1		
Chaetozone christiei			1	1	1			1			2			2		
Chaetozone setosa							1	1								
Chaetozone spec. juv.																
Chamelea striatula					1					1						
Corystes cassivelaunus																
Crangon crangon																
Diastylis bradyi																
Diogenes pugilator				2					1							
Donax vittatus	1						1				1					
Echinocardium cordatum	1	2	1	2		1	2	2	1	1	1			2	1	
Ensis americanus								4						1		
Ensis ensis										1						
Ensis spec.												1				
Eteone foliosa															1	
Eteone longa	2	5	4	2	3	1	3	7	1	8		2	7	2	6	
Eumida bahusiensis																
Eumida sanguinea								2						1		
Eumida spec.juv.																
Euspira pulchella								1			4					
Gammaropsis spec.																
Gastrosaccus spinifer	1		1						1				1			
Harmothoe spec. juv.								3								
Hydrozoa			1	7	5			12		21	14		3	6	1	1
Ione thoracica																
Lanice conchilega				4				36		1				10		
Leucothoe incisa								3						1		
Mactra corallina																
Magelona filiformis	1	1	1	5			2	1			1	1	2	6	2	
Magelona johnstoni				7	1		1	27			5			7	1	
Magelona mirabilis				1	5	1	2	1		3	2	1	1			
Malmgrenia marphysae				2				15		1				5		
Mediomastus fragilis																
Mysella bidentata								2			2			3		
Nassarius reticulatus																
Nemertini			1		2	1	1	6			2	2	1	2		
Nephtys assimilis																
Nephtys caeca						1		1								
Nephtys cirrosa	8	8	9	3	3	8	9		6	3	4	7	9	2	9	9
Nephtys hombergii								2								
Nephtys incisa																
Nephtys indet.																
Nephtys longosetosa							2								1	1
Nephtys spec. juv.					1		1									1
Nereis longissima																
Nereis virens																
Notomastus latericeus								8								
Oligochaeta															1	
Ophelia limacina			1													
Ophiura albida																
Ophiura texturata																
Orchomene nana																
Perioculodes longimanus																
Pholoe minuta																
Phoronida	1		92	2	2			3	2		3			5		
Phyllodoce mucosa								1								
Poecilochaetus serpens														1		
Pontocrates altamarinus				1			1				1					
Scolelepis bonnieri	2	1	4		5	1	2		3	5		2	6	3	3	1
Scoloplos armiger	2									1	1			1	2	
Sigalion mathildae								3								
Spio armata																
Spio decorata																
Spio filicornis																1
Spiophanes bombyx								1								
Spisula elliptica										1						
Spisula subtruncata										1	1					
Streptosyllis websteri																
Synchelidium maculatum	1					1			1							
Tellimya ferruginosa	1	2	1	1			1	15		6	10			1		
Tellina fabula							1	16			1			6		
Tellina tenuis																
Thia scutellata														2		
Travisia forbesii																
Turbellaria																
Urothoe brevicornis	1	1				1		3	5	1	1					1
Urothoe poseidonis	2	64	8	57	1	3	2	142		12	108	1		71	10	

Appendix 4b. Triple-D species list ($n/20m^2$) in OWEZ and reference areas.

	R1-51	R1-52	R1-53	R1-54	R1-55	R1-56	R2-57	R2-58	R2-59	R2-60	R2-61	R2-62	R3-63	R3-64	R3-65	R3-66	R3-67	R3-68
Abra alba																		8
Acrocnida brachiata											1							
Asterias rubens	2	4	2		3	1		4	3	4	2	1	1	13	2	13	1	27
Callianassa subterranea																		
Callianassa tyrrhena	10	2	6	4	8				2	2	3						1	
Cerebratulus fuscus	2				1													
Chamelea striatula	10	18	5	9	3	6	9	14	5	18	8	5	7	33	11	6	7	4
Corystes cassivelaunus		1	2		2	1		2				1						2
Crangon allmanni							1		2									
Crangon crangon	122	50	42	40	52	52	39	194	116	74	76	28	54	41	92	80	48	200
Diogenes pugilator		1				1					1				2			
Donax vittatus	34	44	31	33	16	7	7	8	14	18	11	12	2	14	2	30		
Echinocardium cordatum	4	2	1	3	1	1	3	6	4	2	3	1	2	2	1	8	2	4
Ensis americanus	52	38	43	31	42	8	32	114	80	54	116	12	8	10	3	28	4	200
Ensis siliqua	6																	
Euspira catena		4	3									1			1			
Euspira pulchella		1	1		3						1				1			
Idotea linearis			1															
Laevicardium crassum																		
Lanice conchilega	64		2				25	32	4	14	3		3		1	14	3	320
Liocarcinus (heel klein)																		
Liocarcinus arcuatus																		
Liocarcinus depurator																		8
Liocarcinus holsatus	4	2	2	4	2	3		28	9	11	20	4	1	2	2	3		5
Liocarcinus klein																		
Lutraria lutraria	8	1	1	1			1	25	68	16	24							84
Macropodia spec.										1						2		8
Mactra corallina													1					
Metridium senile																		
Nassarius reticulatus																		
Nephtys hombergi	38	13	15	20	37	12	8	30	72	32	45	9	7	19		2	13	40
Nereis longissima	14		1				1	4										
Ophelia limacina							2			2								
Ophiura albida	36	5	8	6	3	4	20	42	6		7	1		3	2			
Ophiura texturata	34	32	51	44	89	6	10	88	68	50	24	7	14	6	12	6	4	12
Pagurus bernhardus	16	3	4	3	4	4	2	4	6	4	2	2		9	2	34	5	100
Pectinaria spec.																		
Phyllodoce							2											
Pontophilus trispinosus		10	6	5	12	4						9	4	2	6	2	1	
Portumnus latipes						1							2				2	
Processa spec.			1					2		1								
Sagartia troglodytes	12															2		
Sargatiogeton undatus																		4
Spisula elliptica						1	6						1					
Spisula solida						2							5	1				
Spisula subtruncata	2		2			1	1			4	1					4		8
Tellina fabula	146	1	11		1		1	348	178	10	136					2		276
Tellina tenuis								4						1				
Thia scutellata	10	6	3	5	13	4	8	16	20	6	5	10	6	8		2	6	8

Appendix 4b continued.

	R4-69	R4-70	R4-71	R4-72	R4-73	R4-74	R5-75	R5-76	R5-77	R5-78	R5-79	R5-80	R6-81	R6-82	R6-83	R6-84	R6-85	R6-86
Abra alba						3					2					4		
Acrocnida brachiata												2						
Asterias rubens	3	1				1	2		3		11	19	2	8	10	24	12	1
Callianassa subterranea											2							
Callianassa tyrrhena	7		1			8	1					1						
Cerebratulus fuscus																		
Chamelea striatula	6	4	4		1	11	20	21	30	16	26	4	2	18	6	14	6	8
Corystes cassivelaunus							1		1		1							
Crangon allmanni																		
Crangon crangon	55	51	132	62	91	142	85	62	114	126	232	170	520	300	636	684	364	195
Diogenes pugilator						2	20	8	26	24	28	152						
Donax vittatus	38	17	22	4	20	12	41	5	14	4	4			6		2	6	
Echinocardium cordatum	6	5	3	2	1	4	4	1	2	3		4	14	2	18	16	2	6
Ensis americanus	54	21	11	11	15	80	41	19	22	8	82	64	72	16	60	106	21	12
Ensis siliqua																		
Euspira catena							1		2		2	5	2		2		2	
Euspira pulchella	1			1				1		2	4	2			2	12	1	
Idotea linearis				1														
Laevicardium crassum														2				
Lanice conchilega		1					2	1	6	2	4	20	32	30	50	20	9	15
Liocarcinus (heel klein)														2				
Liocarcinus arcuatus												3			2			
Liocarcinus depurator															2	2	2	
Liocarcinus holsatus	2	3	1		2			1	3	6	3	2	2	4	4	8	4	3
Liocarcinus klein																4		
Lutraria lutraria	2	1	1		2	32				6	14	24	54	6	30	72	21	6
Macropodia spec.						1						1	2		2	4		
Mactra corallina	1																	
Metridium senile																		
Nassarius reticulatus						1	7	3	8		72	22	10	6	2	6	1	
Nephtys hombergi	25	6	4	4	7		5	11	12	4	4	8	16	10	14	12	3	15
Nereis longissima	6	1	1	1														
Ophelia limacina		7		5	1			3		8		0	8	54	44	4	14	52
Ophiura albida	136	104	43	3	36	72	22	3	24	24	120	20	40	36	34	8	67	64
Ophiura texturata	213	131	24	23	33	36	36	13	26	32	168	28	52	40	58	32	8	11
Pagurus bernhardus	6	4	2	1	4	14	7	7	10	1	34	77	8	48	22	36	8	4
Pectinaria spec.	1																	
Phyllodoce																		
Pontophilus trispinosus		1			2			1				2					1	4
Portumnus latipes								1	2	2								
Processa spec.	6			1												4		
Sagartia troglodytes											2					8		
Sargatiogeton undatus																		
Spisula elliptica	14	5	7		8	8		5		2		2		12			4	8
Spisula solida	1	1	2	2	2												1	2
Spisula subtruncata	14	1			1	20	1	2		2	4	3	2	2	4	10	3	
Tellina fabula	1					1					18				22	68		
Tellina tenuis							1	1	2			1						
Thia scutellata	23	9	6	2	4	43	15	9	10	4	2	8	12	6	6	18	7	8

Appendix 4b continued.

	WP-21	WP-22	WP-23	WP-24	WP-25	WP-26	WP-27	WP-28	WP-29	WP-30	WP-31	WP-32	WP-33	WP-34
Abra alba								2	2	2	4			
Acrocnida brachiata														
Asterias rubens	3	1	3		2	9	3	6	8	10	10	4	1	1
Callianassa subterranea								1						
Callianassa tyrrhena						4			10	4	4		1	
Cerebratulus fuscus														
Chamelea striatula	6	7	9	7	6	13	12	8	6	12	2	6	16	13
Corystes cassivelaunus	1		1						1			1		1
Crangon allmanni														1
Crangon crangon	69	16	33	30	28	119	24	72	72	190	100	5	33	45
Diogenes pugilator	2		1			2	1						2	2
Donax vittatus	9		18	3	4	27	1	12	12	32	14	3	16	7
Echinocardium cordatum	1	1	2	6	1	3	1	6	12	4	4	2	6	2
Ensis americanus	18	10	31	11	18	55	9	30	58	170	62	11	18	12
Ensis siliqua														
Euspira catena						1			4					
Euspira pulchella	1					1				2				
Idotea linearis														
Laevicardium crassum														
Lanice conchilega	18	3	7	5	4	31		22	46	42	50	2		
Liocarcinus (heel klein)														
Liocarcinus arcuatus														
Liocarcinus depurator								1						
Liocarcinus holsatus	4		1	2		1	1	3	2	2	4		1	
Liocarcinus klein														
Lutraria lutraria	5		3			3	1	2	2	6	10			
Macropodia spec.	2					1				2	4			
Mactra corallina						1								
Metridium senile									2					
Nassarius reticulatus	5					28		3	12	28				
Nephtys hombergi	17	4	7	8	5	18	5	11	12	10	16	6		9
Nereis longissima	6					1				4	10			
Ophelia limacina	1	11		2	10	1	1	6				9		2
Ophiura albida	3	1	3			5	2		4			1		1
Ophiura texturata	6	2	48	10	2	240	17	91	240	236	4	17	40	25
Pagurus bernhardus	19	3	1	2	2	6	4	7	6	22	17		2	
Pectinaria spec.								1						
Phyllodoce														
Pontophilus trispinosus	8	1		7				1				1		7
Portumnus latipes	1						1	1				1		
Processa spec.	1		1											
Sagartia troglodytes													1	
Sargatiogeton undatus														
Spisula elliptica	2	5		2				5	2			1	4	7
Spisula solida	3	4		5	4	1	7	2		2		10		5
Spisula subtruncata			12			7		1	4	6	2		1	1
Tellina fabula	9					23			38	46	304			
Tellina tenuis						1			4	4			1	
Thia scutellata	4	3	11	11	7	12	11	14	9	2	4	6	10	2
Tubularia larynx	3													