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Effects of a five year trawling ban on the local benthic community in a wind farm in the Dutch coastal zone

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SUMMARY

As part of a large impact study in a wind farm (OWEZ) in the Dutch coastal zone, the effects of exclusion of bottom trawling on the benthic community were studied by comparison with nearby reference areas which were regularly fished. In addition to a standard boxcorer for common macrofauna, a Triple-D dredge was used to collect longer-lived, more sparsely distributed infauna and epifauna. Multivariate analysis did not reveal any difference between the assemblages in and outside OWEZ with respect to abundance, biomass and production after a five year closure. The Shannon-Wiener diversity index pointed to a significantly higher diversity in OWEZ compared to some of the reference areas. A minority of the bivalve species assumed to be sensitive to trawling showed higher abundances (*Spisula solida)* or larger sizes (*Tellina fabula, Ensis directus)* in OWEZ than in some of the reference areas. In general, samples collected with the Triple-D dredge showed more differences between areas than boxcore samples. No evidence was also found that the species composition in OWEZ relative to the reference areas had changed in the period between one (2007) and five (2011) years after closure. The change observed in all areas between 2007 and 2011 was mainly due to relatively small variations in species abundances. In conclusion: five years after the closure of OWEZ to fisheries only subtle changes were measured in the local benthic community, *i.e.* a higher species

diversity and an increased abundance and lengths of some bivalves. Depleted adult stocks, faunal patchiness, and a limited time for recovery (5 years) might explain that a significant recovery could not be found. The current study shows that designation of large-scale MPA's as planned for the North Sea will not automatically imply that restoration of benthic assemblages can be expected within a relatively short period of years.

Keywords: benthic invertebrate community, impact of wind farm, North Sea, areas closed to fisheries, recovery, marine protected areas

INTRODUCTION

Many studies have reported on direct and long-term impacts of bottom trawling on benthic communities in the North Sea. Experimental beam trawling caused instant mortality in various benthic species mounting up to 65% of the initial bivalve densities in the trawl track (Bergman and van Santbrink, 2000). Demersal fishing was found to alter seabed habitats and to affect the structure and functioning of benthic invertebrate communities (Reiss *et al.*, 2009). Hinz *et al.* (2009) reported that changes in faunal composition and in benthic communities might impact the integrity of marine food webs. A size-based model showed that trawling reduced biomass, production and species richness (Hiddink *et al.*, 2006). Long-term effects of trawling on the composition of the benthic community were clearly demonstrated by comparing a 500 m exclusion zone around a gas production platform which had been closed to fishing for a period of 23 years with surrounding regularly fished areas (Duineveld *et al.*, 2007). Despite the small scale, the study showed greater species richness, evenness, and abundances of burrowing mud shrimps and fragile bivalves in the exclusion area. Areas with no fishing which allow studies on trawling effects and recovery at larger spatial scales were until recently lacking in the North Sea. With the construction of wind farms closed for fishery, new opportunities have arisen to explore impacts of larger-sized no-fishing zones on the development of benthic communities. In the meantime, European agreements like NATURA 2000 and the Marine Framework Directive require countries around the North Sea to safeguard and improve marine diversity, and protect valuable habitats. With (beam) trawling having one of the largest impacts, establishment of MPA's closed to fishing is underway (Anon, 2012). The wind farm studies provide insight into the potential of MPA's and their expected rates of recovery. The first results of such studies in the North Sea pointed to none or minor effects on the soft bottom faunal assemblages followinga 1 to 3 year closure to fishery (Bergman *et al*., 2010, Coates *et al.*, 2012, Daan *et al.*, 2009, Dannheim, 2007, Degraer *et al*., 2012, Spanggaard, 2005).

In 2006 the **O**ffshore **W**ind farm **E**gmond aan **Z**ee (OWEZ) was constructed at circa 14 km distance from the shore and approximate 18 km NW of IJmuiden. Consequently OWEZ became closed to all shipping, thereby creating a no-fishing area of approximately 25 km^2 in a coastal zone being frequently fished by beam- and shrimp trawlers for almost a century (Rijnsdorp *et al.*, 1998; Bergman and van Santbrink, 2000). The construction and exploitation of OWEZ was accompanied by an extensive Monitoring and Evaluation Program (NSW-MEP 2003-2012). In 2011 we examined the 5 year effect of the closure on the macrobenthic community in OWEZ (T_2-survey). Prior to this study, two other benthic surveys were conducted as part of the NSW-MEP, *i.e.* a T_0-survey (2003) 3 years before OWEZ construction covering the wind farm building site and 2 distant reference areas (Jarvis *et al*., 2004), and a T_1-survey (2007) just after OWEZ construction and following 1 year of fishery ban (Daan *et al*., 2009). The T_1 and the T_2-survey included 4 additional reference areas closer to OWEZ.

In the present paper which primarily deals with the T_2-data collected in 2011 we focus on possible changes in the macrobenthos in OWEZ relative to its regularly trawled surroundings after and over a period of 5 years. In the T_2-survey we employed a Triple-D dredge (Bergman and van Santbrink, 1994) next to a boxcorer in order to obtain also reliable estimates for longer-lived usually larger-sized and sparsely distributed species. Our hypothesis is that benthos species, especially longer lived ones, that are sensitive to trawling survive in higher numbers in the wind farm. As a consequence, the benthic community in OWEZ will become different from the surrounding trawled areas, similarly as we found around the gas platform further offshore (Duineveld *et al.*, 2007). We firstly compare OWEZ with 6 reference areas regarding species densities, biomass, production, and diversity in the 2011 situation. Additionally, we explore shifts in the species composition in OWEZ relative to the reference areas over the period 2007 – 2011 using the boxcore data from the T_1-survey by Daan *et al.* (2009).

MATERIAL & METHODS

Study area

OWEZ wind farm is situated in the Dutch coastal zone 11-17 km offshore of Egmond aan Zee in water depths between 12 and 20 m (Figure 1). The 36 turbines stand 650-1000 m apart. OWEZ and its 500 m safety zone were closed to all shipping since early 2006. In October 2007 OWEZ and surrounding reference areas had an average median grain size of 266 μm (range 203-370), and an average mud content of 0.92 % with a peak value of 8.7% in OWEZ (Bergman *et al*., 2010). The macrobenthic biomass in this coastal zone is relatively high compared to the offshore Southern Bight with a stable positive gradient towards the coast mainly due to high densities of bivalves (Duineveld *et al*., 1990; Holtman *et al*., 1996, Daan and Mulder, 2006). Until 2003, the once dense coastal beds of the bivalve *Spisula subtruncata* were commercially exploited (Craeymeersch and Perdon, 2004). After 2003, the invasive American jack knife *Ensis directus* and other species became dominant (Perdon and Goudzwaard, 2006). Overall, the macrobenthic biomass in the coastal zone has remained stable over the last 20 years. Despite the stable spatial biomass pattern in the coastal zone, large annual variations in density of small-sized single species have been recorded (Daan and Mulder, 2006).

The coastal zone around OWEZ is trawled regularly by shrimp and EURO-trawlers, although the exact trawling intensity between 2006 and 2011 cannot be calculated from the Vessel Monitoring System (VMS). Frequencies are probably underestimated by exclusion of EURO-cutters smaller than 15 m before 2011, and by trawlers that do not berth in adjacent ports. Conversely trawling intensity is most likely also overestimated, since algorithms do not discriminate between trawling and port approach procedures in coast-nearby regions. The best possible estimate of the distribution of trawling activities by EURO-trawlers (<300 HP, width of beam trawls <4.5 m) is given in Figure 1 which depicts "presence of trawlers" in and around OWEZ wind farm (N. Hintzen, IMARES, pers. comm.). Apparently trawlers stayed outside the area where the turbines were positioned including the 500 m restriction zone, and trawling frequencies in the reference areas fitted well in the range of frequencies in the coastal area.

Field survey's

The T_2-study was executed in February 2011, five years after the closure of OWEZ to fishery. Samples were collected in OWEZ (in approx. 9 km^2) and in six regularly trawled reference areas (R1 to R6; 2.2-4.4 km^2 each) positioned close to the wind farm (Figure 1). This design was similar to the

T_1-survey in March 2007, when benthos samples were also collected from RV "Pelagia" (NIOZ). For safety reasons all samples were taken more than 300 meters away from the turbines, and consequently hard substrate species related to the monopiles and the gravel scour protection layer (Bouma and Lengkeek, 2012) were largely excluded from the sampling. It was anticipated that in this T_2-study changes in species composition might be detectable among long-lived species that survived the 5 years without fishing in OWEZ. Therefore, an extensive survey was executed with the Triple-D dredge (Bergman and van Santbrink, 1994) directed to quantitatively sample the long-lived, larger-sized, more sparsely distributed in- and epifauna. The boxcore programme targeting the more common, small-sized (short-lived) species was consequently reduced compared to the T_1-study.

The T_2-survey with the Triple-D consisted of 14 single hauls along 3 transects running parallel to the wind turbines in OWEZ and 6 single hauls in each of the reference areas. The Triple-D effectively cuts a 20 cm deep strip of sediment with a width of 20 cm from the seabed. An odometer triggering a pneumatic opening/closing mechanism controls the exact length of the 100 m haul. The sediment is washed through the 7x7 mm meshes of a 6 m long net astern the dredge. Both in- and epifauna are retained in the net, representing the fauna in 20 m^2. Catches of the Triple-D dredge were sorted and identified on board (for details of Triple-D, see Witbaard *et al*., 2013).

The boxcorer used during the T_1- and T_2-studies had a diameter of 30.5 cm (0.078 m^2) and sampled to a depth >15 cm. A trip valve prevented flushing of water during ascent and loss of light animals (*e.g.* amphipods, cumaceans). The boxcoring programme in T_2 was reduced compared to T_1 (Daan *et al*., 2009) from 30 to 16 samples in OWEZ and from 15 to 8 stations in each of the 6 reference areas, but sampling stations were kept at the same positions. The OWEZ stations were arranged along 5 transects parallel to the turbines. The reference stations were arranged along three parallel transects per area. Per station a single boxcore was taken and immediately washed over a 1 mm sieve. The residue was preserved in a neutralized 6% formaldehyde solution and brought to the laboratory for identification and further analyses. In the T_2-study, a sediment core (diameter 2.5 cm) of the upper 10 cm was taken from every boxcore and frozen at -20°C. In the laboratory the median grain size and the percentage of mud were determined. In the T_1-study (Daan *et al*, 2009) no samples for sediment analysis were collected. Water depth at the stations was taken from the ships echo sounder, geographical positions from the ships logbook.

Sample treatment

Grainsize analysis

The sediment samples were freeze-dried up to 96 hours until dry. The samples were not treated with acid nor oxidized with peroxide. Median particle size and the percentage mud (fraction <63 mµ) of sediments were determined by means of a Coulter LS 13 320 particle size analyzer and an Autosampler using both laser diffraction (780 nm) and Polarization Intensity Differential Scattering (PIDS) (450 nm, 600 nm and 900 nm) technology.

Boxcore fauna

Samples were stained with Bengal Rose 24 hours prior to sorting and then sieved over a set nested sieves of 11.2, 6.7, 2.0 and 1.0 mm. Individuals from each fraction were sorted in categories (polychaetes, crustaceans, molluscs and echinoderms) and identified to species level. Juveniles and damaged animals were identified at higher taxonomic level (usually the genus). Representatives ofanthozoans, phoronids, oligochaetes, nemerteans and turbellaria were also identified at their taxon level. All individuals were counted. Individual lengths (mm) of molluscs and echinoids were measured and converted to ash-free dry weight (AFDW) using length-weight relations. Blotted wet weights of polychaetes, larger crustaceans, and ophiuroids were measured to the nearest mg. Remaining taxa were weighted per species or group. Blotted wet weights were converted to AFDW. Length-weight relations and conversion factors were derived from various sources viz. Daan *et al*. (2009), Ricciardi and Bourget (1998), Rumohr *et al*. (1987) and from unpublished NIOZ-data. Small crustaceans (amphipods and cumaceans) were only counted and assigned an average individual AFDW of 0.2-0.5 mg per individual. Total AFDW of a sample was obtained by summing individual weights.

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). To obtain the annual P/B ratio per species, the average energy content (kJ) was derived by multiplying the AFDW (g) with a factor 22 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

Specimens collected with the Triple-D were sorted, identified and counted on board. If needed, individual species were subsampled depending on their abundance in the catch. Lengths of all specimens in the (sub)samples were measured. For some species length of a particular part of the body was measured and later converted to total length. Of crabs the carapax width, of hermit crabs the length of the propodus, of the bivalve *Ensis* spp. the shell width, and of the bivalve *Lutraria lutraria* the siphon width was measured to the nearest mm. Of all other species total body length was measured to the nearest mm. Blotted wet weight per species was measured for each (sub)sample.

AFDW per species per dredge haul was calculated by conversion of the wet weight (WW). Conversion factors were taken from the same sources as used for boxcore fauna. Annual production was estimated using Brey's multi-parameter P/B-model (Brey 1999, 2001). To obtain annual P/B ratios per species the average energy content (kJ; see above) was derived from the AFDW (g) for all individuals of a species found in a sample. 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.

Statistical analyses

Abiotic data

To explore differences in abiotic variables among OWEZ and the six reference areas notched box and whisker plots, representing a multiple comparison of median values and their 95% confidence intervals, were made of the water depth, median grain size and mud content in 2011 (SYSTAT13™). Non-parametric Kruskal-Wallis analyses of variance tests on non-transformed data were applied to test the differences for statistical significance using SYSTAT13™ and the Analyse-it™ software packages for MS-Excel. Non-parametric analyses were used since the data were not normally distributed and in some cases (*i.e.* mud content) zero inflated. In case of a significant difference ($p<0.05$) a pairwise Kruskal-Wallis test with a Bonferroni adjustment was performed to assess which of the areas were different.

Boxcore fauna

data 2011 - multivariate tests

Differences between OWEZ and reference areas in 2011 with respect to abundance, biomass and annual production were evaluated with multivariate analyses in PRIMER6™ (Clark and Gorley, 2006) and PERMANOVA A+ for PRIMER (Anderson *et al*., 2008). The PRIMER software is flexible due to 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, homogeneity of variances, linearity). After 4^{th} root-transformation to reduce the effect of dominant species, Bray-Curtis similarity matrices were generated. Non-metric multi-dimensional scaling plots (MDS) were drawn to visualize the Bray Curtis similarity between the different samples. To test if OWEZ wind farm significantly differed from the reference areas, a PERMANOVA test with 2 factors was performed. The first factor divided the samples in two categories, *i.e*. OWEZ versus the reference areas. A second factor divided the samples in 7 different areas: OWEZ, and R1 to R6. This design enabled examining both the significant differences between OWEZ and the reference areas, and the significance of variability in benthic structure among all areas. To examine the relation between various co-variables (*e.g.* water depth, median grain size, mud content, distance to shore, fishery frequency) and faunal assemblages, and to correct for their potential effects on the differences between areas, the impact of these co-variables on the test results were tested sequentially in further PERMANOVA analyses. The (geometrical) components of variation of the relevant co-variables were calculated in a pseudo-F test. Their rooted values indicate the percentages of dissimilarity in each variable in terms of the similarity index (*i.e.* Bray-Curtis).

To examine the best match between the variance in species composition among the stations and abiotic variables (water depth, median grain size, mud content) a BEST analysis (PRIMER6™) was applied. In this analysis the BIOENV correlation was chosen which calculates Spearman’s rank correlations between the Bray-Curtis similarity matrix based on species abundances and the different combinations of environmental variables. The environmental variables that best explained the species composition among the samples achieved the highest ρ (rank correlation coefficient). The statistical significance of the ρ was calculated in relation to permutations (n=999) simulating the null hypothesis.

BEST analyses were performed on the total data set, and on the data sets from OWEZ and the reference areas separately.

data 2011 - univariate tests

Notched box and whisker plots (SYSTAT13™) were drawn for biotic variables (average abundances, biomass, production, and diversity indices) to explore possible differences between OWEZ and reference areas (SYSTAT13™). ANOVA-analyses were performed to test the univariate biotic variables for statistically significant differences (SYSTAT13™). Based on a graphical inspection of the normality of residuals and homogeneity of variance, it was decided to log-transform data on abundances, biomass and production before testing. Three types of diversity indices were tested, one being species richness, *i.e.* the number of species per sample, the second being the Shannon-Wiener index (^{2}log base) and the third one the Simpson index. Shannon-Wiener takes into account both the number of species in a community and the degree of evenness (Morin, 1999; Peet, 1974; Shannon and Weaver, 1949). The Simpson index (λ) represents the possibility that two randomly chosen individuals are the same species, and high values for λ indicate high dominance (Hill, 1973). In our tests the complement of Simpson index (1-λ) was used. Data of species richness and the Simpson index (1-λ) were log-transformed before testing.

comparison data 2007-2011 - multivariate tests

To explore differences between the boxcore samples from the T_1-survey in 2007 (Daan *et al.*, 2009) and the T_2-survey in 2011, one and five years after the closure, respectively, data were 4^{th} root-transformed and a Bray-Curtis similarity matrix was generated (PRIMER6™). Sediment characteristics could not be included in the tests as these data were not collected in 2007. To visualize changes in species composition between the two years, the centroids of the areas (*i.e*. the “gravity” centres representing all stations belonging to one particular area) were plotted in a MDS-plot. A PERMANOVA test (two way crossed design) was subsequently applied to test if the years differed from each other and if there were differences between the areas. The first factor divided the samples into year 2007 or 2011, the second factor divided the sample set in 7 different areas *i.e.* OWEZ and 6 reference areas. The resulting interaction term “area*year” revealed whether one of the areas had diverged over the 5 year period over a different distance or in a different direction than the other areas. To explore if the benthic assemblages in OWEZ were different from the reference areas, additionally a mixed design was tested in PERMANOVA. The test was done with the most conservative type 3 sums of squares.

Triple-D fauna

data 2011 - multivariate tests

Multivariate analyses to analyse differences in abundance, biomass and annual production of Triple-D samples between OWEZ and the reference areas were similar to those described for the 2011 boxcore samples. The same analyses were used to explore differences in abundance of relevant groups of species *i.e.* higher taxa, common species (contributing at least in one sample more than 10% to the abundances), rare species, epifauna, infauna, and scavengers. To explore if a possible clustering among the stations was associated with the presence of the no-fished OWEZ wind farm a hierarchical multivariate CLUSTER analysis was done (PRIMER6™). Abundance data were square root transformed. Based on a Bray-Curtis similarity matrix the samples were grouped in clusters showing a 67% similarity in species composition, and the clusters were visualized in a MDS plot. With the SIMPER routine (PRIMER6™) the contribution of individual species to the separation between the newly formed clusters was examined.

data 2011 - univariate tests

To tests for differences in average abundances, biomass, production, and diversity between Triple-D samples from OWEZ and reference areas, similar univariate ANOVA tests and notched box and whisker plots were used as for the analysis of boxcore data. Univariate tests were further performed on the lengths of 5 mollusc species (*Chamelea striatula, Tellina fabula, Donax vittatus, Ensis directus*, *Nassarius reticulatus*), and on the log(n+1) transformed abundances of species that either suffer from direct trawling mortality being *Corystes cassivelaunus*, *Spisula subtruncata*, *S. solida*, and *Echinocardium cordatum* (Bergman and van Santbrink, 2000), or were selected on their assumed vulnerability being fragile and in reach of the trawl (*Lanice conchilega*, *Donax vittatus*, *Lutraria lutraria*, *Spisula elliptica*, *Tellina fabula*, and *T. tenuis*).

RESULTS

Environmental variables

Water depths in the study areas ranged from 12 to 20 m, with R1 being significantly shallower than R2 and R3 (Figure 2A; Bonferroni adjusted, p=0.023 and 0.015, respectively). Median grain sizes ranged from 185 to 318 µm, with a mean value of 264.5 µm (st.dev. 17.6; Figure 2B), and differences between areas were statistically not significant (Kruskal-Wallis; p=0.086). Out of 64 samples only 21 contained mud with percentages up to 8.2%. OWEZ fitted well in the range of values found in the reference areas, with only a significant difference between R1 (0%) and R6 showing relatively high mud contents (Figure 2C; Kruskal-Wallis, Bonferroni adjusted, p=0.013).

Boxcore fauna

Data 2011

A total of 88 macrobenthic species were identified in the samples, of which 18 accounted for 90% of the total abundance. Polychaetes comprised 41 species, crustaceans 23, molluscs 15, echinoderms 3, while 6 species belonged to “other” phyla. A MDS-plot (Figure 3) based on the Bray-Curtis similarity matrix illustrates that stations were not grouped according to their original areas, indicating that species abundances in *e.g.* OWEZ did not differ from those in the six reference areas. PERMANOVA-tests indicated no significant statistical differences in species abundances between the areas (p=0.098), and OWEZ did not stand out relative to the variation among the reference areas (p=0.699). MDS-plots and PERMANOVA-tests of the biomass and production data gave similar result as the abundance data, *i.e.* no significant statistical difference between any of the areas (p=0.297 and 0.266, respectively), and OWEZ did not stand out in any way above the between reference areas variation (p=0.718 and 0.724, respectively). Further PERMANOVA tests pointed to water depth (p=0.0001) and median grain size (p=0.0004) as co-variables explaining most of the variation in the abundance data. After correction for their effects, however, OWEZ still did not differ from the reference areas (p= 0.844). The (geometrical) components of variation calculated in a pseudo-F test pointed to the largest variation at the boxcore level which a dissimilarity of circa 37%. At the level of areas an additional 11%

dissimilarity was calculated. Of the total variation in the faunal dataset approx. 83% was generated by individual boxcores, 8% is explained by areas, 9.6% by water depth and 3.3% by median grain size. Apparently most of the faunal variation cannot be explained by the two most relevant co-variables. The BEST analysis also identified mud content, median grain size and water depth as the co-variables best explaining the variation in species composition among OWEZ stations, though the maximum correlation appeared rather low (R= 0.469; significance level 0.01). When only samples from reference areas or from all areas were considered this correlation was even more trivial (R=0.259 and 0.294, respectively).

Abundances varied between 115 and 5670 individuals per m^2 with averages ranging from 1096 per m^2 in OWEZ to 1778 in R6. A notched box-whisker plot revealed no significant differences in average abundance between areas, which was confirmed by the ANOVA result (p=0.647). Biomass per station varied from 0.28 to 258 g AFDW per m^2. The average biomass was lowest in R1 (17.2 g AFDW per m^2) and highest in OWEZ (32.4). A notched box-whisker plot showed no significant differences between areas, and ANOVA (p=0.626) supported this conclusion. Production estimates 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 an average annual production ranging from 335.9 kJ/m^2/y in R4 to 524.4 in R3. A box-whisker plot showed no significant differences between areas, and ANOVA (p=0.749) confirmed this conclusion.

The number of species per boxcore (0.078 m^2) varied between 2 and 37. The average number of species per sample was lowest in R3 (13) and highest in R6 (20) with OWEZ (16) in between. A box-whisker plot and the ANOVA result (p=0.084) showed no significant differences between the areas. The Shannon-Wiener diversity values per sample ranged from 0.39 up to 4.11. Average value varied between 2.46 in R3 to 3.22 in R6, and OWEZ (average 2.86) which fitted well within this range. A box-whisker plot and the ANOVA result (p=0.158) showed no significant differences between the areas. The Simpson (1-λ) diversity of the samples varied from 0.15 to 0.93. The minimum average value (0.71) was found in R3 and the highest (0.84) in R5. A box plot and ANOVA (p=0.425) did not point to any difference between areas.

Comparison data 2007-2011

The distribution of the centroids representing the “centres of gravity” of the species abundances in each of the areas demonstrate a clear distinction between 2007 and 2011 (Figure 4), indicating that the benthic assemblages were different between these years. Relative to the reference areas, however, OWEZ did not change in or over a different direction or distance. Indeed a two-way crossed PERMANOVA proved a statistically significant difference between years (p=0.001) and between the areas (p=0.001), but not in the interaction term “area*year” (p=0.223) indicating that none of the areas had diverged differently over time. A further test (3-way mixed PERMANOVA) to examine the difference between OWEZ and the reference areas confirmed 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), implying that OWEZ did not differ from the reference areas in both years. A SIMPER analysis demonstrated that the distinction in species composition between 2007 and 2011 was mainly due to relatively small variations in species abundances (Table 1) and not caused by the introduction of new species or species loss.

Triple-D fauna

Data 2011

A total of 50 invertebrate species were identified: 18 crustaceans, 16 molluscs, 5 echinoderms, 6 polychaetes and 5 “other” species. Just 15 of them contributed 90% to the total abundance. A MDS plot based on species abundances shows that the OWEZ samples were dispersed among the reference samples (Figure 5), which seemed to be loosely arranged in clusters suggesting slight differences between reference areas. MDS ordination of biomass and production yielded similar configurations. PERMANOVA tests revealed differences in terms of abundance, biomass, and production between the areas (p=0.001), but OWEZ did not differ from the reference areas (Table 2). MDS plots depicting the differences between OWEZ and the six reference areas in terms of specific selections of species showed also no grouping of samples per area. For all faunal categories PERMANOVA analysis indicated significant differences between areas, but OWEZ did not differ from the between area variability among the reference areas (Table 2). Although the MDS plot of the abundances of 19 scavenger species showed the largest distinction between the reference areas, still OWEZ did not differ from the reference areas (Table 2).

The number of individuals per sample ranged from 3.6 to 65.9 per m^2. The average abundance was lowest (11.4/m^2) in R1 and highest (38.7) in R6. Median abundance was significantly higher in R6 than in OWEZ, ANOVA results supported this conclusion (p=0.04). The significant higher abundances of *e.g.* the shrimp *Crangon crangon,* the bivalve *Lutraria lutratria,* the brittle star *Ophiura albida,* and the polychaete *Ophelia limacina* in R6 (ANOVA on log (n+1) transformed data; p<0.0004) were possibly related to its relatively high mud content (Figure 2C). Biomass values varied between 19 and 320 g AFDW/m^2 per sample. Average biomass ranged from 61 g AFDW/m^2 in R4 to 134 in R6. Box-whisker plots suggested significantly higher median biomass in R6 than in OWEZ, although ANOVA did not support this conclusion (p=0.35). Production estimates per sample 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 production (43 kJ/ m^2/y), while R6 had the highest (119). Although box-whisker plots show significant higher median production in R6 than in OWEZ, ANOVA results could not confirm this difference (p=0.06).

The number of species per haul (20 m^2) varied between 13 and 28. Average numbers ranged from 15 in R3 to 21 in R5, with OWEZ (20) in between. Although Figure 6A suggests significantly lower median values in R3 than in R1, R2, R5, and R6, ANOVA did not support this (p=0.125). The average Shannon-Wiener diversity values per area varied between 2.5 in R6 and 3.3 in R1. Figure 6B shows lower median values in R6 than in R2 and OWEZ, which was confirmed by ANOVA results showing significant (p=<0.0001) lower values in R6 relative to R1, R2, R5 and OWEZ, and significant higher values in OWEZ than in R4. The average Simpson (1-λ) diversity values per area ranged between 0.64 in R6 and 0.85 in R1 and R2. Figure 6C shows significant lower median values in R6 compared to all other areas including OWEZ, ANOVA confirmed this (p=<0.0001).

Univariate tests on differences between areas in the abundances of 10 species sensitive to trawling yielded insignificant results for the bivalves *Spisula subtruncata, Tellina fabula, T. tenuis,* the echinoderm *Echinocardium cordatum,* and the crustacean *Corystes cassivelaunus*. The bivalves *Donax vittatus, Spisula elliptica*, *Lutraria lutraria*, *and the* polychaete *Lanice conchilega* showed only differences in abundance between reference areas. Only the bivalve *Spisula solida* was significantly more abundant in OWEZ than in R2 and R5 (n=0 in R2 and R5; ANOVA, Bonferonni adjusted, p=0.001), but differences in average shell length between OWEZ and the other reference areas (R1, R3, R4, R6) were not statistically significant (Kruskal Wallis test, p=0.085). *T. fabula* was significantly larger inside OWEZ (18.5 mm; ANOVA; p=<0.001) than in R1 (16.6 mm), R2 (18 mm) and R3 (17.4 mm), and widths of *E. directus* inside OWEZ were significantly larger (ANOVA; p=<0.001) than in R4, R5 and R6, but significantly smaller than in R3. Of the other abundant molluscs (*Chamelea striatula, D. vittatus, Nassarius reticulatus*) shell length in OWEZ fell within the range found in the surrounding reference areas.

A CLUSTER analysis of the abundance data identified 4 main clusters showing >67% resemblance in species composition (Figure 7). Although cluster C included the highest percentage of OWEZ samples (6 samples) combined with only 3 other samples (from R1 and R3), OWEZ samples occurred, although less frequently, in all other clusters. Apparently other factors than the fishery-free status of OWEZ contributed also to the similarity between stations. A SIMPER analysis revealed that *T. fabula, L. lutraria, S. solida, D. vittatus*, *L. conchilega,* and *O. albida* were the species contributing most to the dissimilarities between the 4 clusters.

DISCUSSION

Field and model studies have demonstrated the impact of trawling on abundance and biomass of benthic species, and on the structure and functioning of benthic communities in the North Sea (Bergman and van Santbrink, 2000, Hiddink *et al*., 2006, Hinz *et al*., 2009, Reiss *et al*., 2009). We hypothesized that the closure of OWEZ for fisheries could lead to higher local abundances and biomass of vulnerable species, and changes in its community structure. Faunal differences between OWEZ and reference areas can be expected to become more prominent, since license-buy-back program to counteract redistribution of trawling effort from inside OWEZ towards outside (Jennings, 2009) were lacking. Still the impact of the 5 year closure to fisheries on the benthic community in OWEZ was not demonstrable.

Univariate tests on average abundance per area based on boxcore samples did not expose any difference between OWEZ and reference areas after the 5 year closure. Triple-D samples showed a higher average abundance in R6 than in OWEZ, possibly related to its relatively high mud content (Figure 2C). In latter sampling the bivalve *Spisula.solida* had a significantly higher abundance in OWEZ than in R2 and R5, where no specimens were found, while shell length was not different between OWEZ and the other reference areas. Multivariate tests showed that irrespective of the sampling method, species abundances in OWEZ did not differ from the reference areas (Figures 3, 5).

In the boxcore sampling also no difference was found after correction for the effect of the significantly related co-variables water depth and median grain size. Again, Triple-D sampling exposed a significant higher abundance of some bivalves and polychaetes in R6 than in R1, R3, and R4, each contributing >5% to the dissimilarity between the areas (SIMPER–analysis). Apparently Triple-D sampling is more suited than boxcore sampling to detect spatial differences, most likely because *e.g*. the sparser older bivalves indicative for area-specific conditions are not adequately sampled with boxcores, and the Triple-D sampling integrates variation in abundances over a larger surface. Nonetheless, among the groups of selected Triple-D species only the scavenger species showed some distinction between reference areas, but still OWEZ was not different from the reference areas (Table 2). In conclusion, we found no convincing evidence for distinctiveness of OWEZ from the reference areas in terms of species abundances in 2011, not even among Triple-D samples targeting longer-lived species which presumably more accurately reflect the 5 year impact of the fishery ban in OWEZ. A multivariate comparison between the boxcore data collected in 2007 and 2011 revealed a distinct shift in species composition in all areas over time, but none of the areas including OWEZ had changed in a different direction or over a different distance when compared to the other areas (Figure 4). The main difference between the two years was caused by subtle changes in community composition in all areas, and not by the introduction of new species or species loss (Table 1). Apparently, the 5-year fishery ban in OWEZ has not led to a distinctive change in the boxcore fauna of OWEZ compared to surrounding areas evaluating the years 2007 and 2011.

Alike the absence of density differences, differences between the areas in average biomass and annual production were not found in 2011 (univariate tests). Multivariate tests gave similar results based on boxcore sampling, while Triple-D sampling (Table 2) indicated only significant differences between some reference areas. Based on the boxcore data, no differences between the areas were found in numbers of species, Shannon-Wiener and Simpson index. However, in the Triple-D dataset, both diversity indices pointed to R6 as an area with a relatively low diversity, possibly related to its high mud content, and to a significantly higher diversity in OWEZ than in R6 and R4 (Figure 6B, C). The higher diversity in OWEZ might be a first sign of recovery of the wind farm after the cessation of trawling 5 years earlier. The larger sizes of two fragile bivalve species (*Tellina fabula* and *Ensis directus*) in OWEZ than in some reference areas might also point to increased survival due to the fishery ban. Several factors (see below) might have contributed to the fact that apart from these minimal signs of recovery no evidence was found for a distinctive change in the species abundances in OWEZ compared to the reference areas after its 5 year closure period.

Sampling design

Because the Dutch coastal zone is characterized by a marked faunal zonation parallel to the coast (Duineveld *et al*., 1990; Holtman *et al*., 1996) the choice of reference areas may affect the comparison with OWEZ. In 2011 no distinction was found between OWEZ and the six reference areas with respect to water depth, median grain size and percentage mud and, moreover, correlations between these variables and the benthic community appeared to be weak as indicated by a multivariate BEST analysis. This suggests that it is unlikely that the choice of these particular reference areas would mask the recovery of the benthic community after the 5 year trawling ban in OWEZ. Neither was recovery obscured by a too faint contrast between trawling intensity in OWEZ and references areas. Trawling activity (in fact: presence of trawlers) in the closed area around the wind turbines in OWEZ appeared almost nil in the period 2006-2011, while all six reference areas were regularly trawled (Figure 1).

Faunal patchiness

The comparison between areas may have been affected by the patchy faunal distribution in and around OWEZ. In 2011 four fauna clusters with a >67% resemblance in species composition could be distinguished, each comprising Triple-D stations from various areas (Figure 7). Apparently species assemblages were patchy distributed over the study area. Patchiness in species composition was also evident at the relatively small scale (~9 km^2) of the OWEZ, as its Triple-D samples, although mostly related to cluster C, ended up in all clusters (Figure 7). The stations in cluster A represented a typical high abundant, species-rich community inhabiting the muddier sediments scattered over all study areas. The bivalve *Tellina fabula* was found almost exclusively in this cluster, together with the polychaete *Lanice conchilega* and the bivalve *Lutraria lutraria*. *L. conchilega* probably acts as ecosystem engineer in this faunal assemblage since its tubes reduce bottom shear stress, promote retention of fine particles and create refuge for young bivalves (Rabaut *et al*., 2007, van Hoey *et al.*, 2012). In the boxcore samples in 2011 the abundance of *L. conchilega* is indeed positively correlated with percentage mud in the samples (Spearman, rs 95%; p=0.013). It's further noteworthy that several wind farm studies have reported an increase of the opportunistic *L. conchilega* after the closure to trawling (Dannheim, 2007; Defew *et al*., 2012). In our study such an increase is not apparent in the boxcore nor in the Triple-D sampling in 2011 (ANOVA on log transformed data, p=0.66 and p=0.01, respectively), wherein the significance in the Triple-D sampling was related to differences between two reference areas.

Water depth and median grain size were the two most important explanatory co-variables of the variation in the 2011 faunal abundances in boxcore sampling, *i.e.* 10 and 3.3% respectively. The bathymetry of the study area with north-easterly, coastward directed gullies spanning depth gradients of more than 5 m (Figure 8) may have played a initiating and crucial role in creating faunal patchiness. Variation in hydrodynamic conditions across the bathymetry and hence of sedimentology may have promoted a patchy distribution of species and assemblages over the study area, especially when key engineering species with a distinct sediment preference are involved. This patchiness may have affected the responses of OWEZ and the reference areas to the contrasting fishery pressure. In this way the spatial distribution of key species such as *L. conchilega* may have overshadowed to some extent the potential benthic differences between OWEZ and the reference areas during first 5 years of the trawling ban.

Adult stocks

Chronic bottom trawling over large parts of the North Sea has led to a reduction in long-lived epifauna, and particularly of numbers and biomass of bivalves (Rumohr and Kujawski, 2000; Jennings *et al*., 2001; Callaway *et al*., 2007). In such regions the recovery of no-fishing zones may be retarded by the reduced larval supply from depleted adult stocks. Long-term trends in bivalve stocks in the Dutch coastal zone are documented for two commercially exploited species, *i.e. Spisula subtruncata* and *Ensis directus*. The population of *S. subtruncata* has drastically declined over the last decades, *i.e.* from 4000 per m^2 in the 1980's to 0.1 per m^2 in 2006 (Perdon and Goudswaard, 2006). It is evident that numbers of recruits that Bergman et al. (2010) counted in fall 2007 in the 2 year closed OWEZ and the reference areas (5.1 and 4.3 ind./m^2, respectively) were far below levels necessary to restore the previously dense *S. subtruncata* stock. The loss of *S. subtruncata* biomass has been compensated by the massive increase of the invasive jackknife *E. directus* in the shallow near shore zone. However, densities of *Ensis directus* were not significantly enhanced in 2011 in OWEZ even after a 5 year closure (ANOVA on log(n+1) transformed data, p=0.35).

During experiments with submerged colonisation trays in OWEZ in 2007, Bergman *et al*. (2010) observed substantial settlement of bivalve larvae of up to 1565 larvae m^{-2} d^{-1} in July. Unfortunately, the size of post-larvae did not permit species identification. Comparison between the settlement rate in July and abundance of juveniles (> 0.5 mm) 2 months later, *i.e.* ~100's per m^2 in October 2007, points to a significant mortality of bivalve recruits. Loss of habitat complexity due to long term trawling has been mentioned as a cause for low survival rates of settlers in the North Sea (Collie *et al*., 2000; Gray *et al*., 2006; Thrush and Dayton, 2002). Equally or possibly more important in this case is in our view the increased abundance of small predatory fish (solenette, juvenile plaice, dab, dragonet) since the mid 1990's as a result of trawling (Heessen, 1996; Tien *et al.,* 2004) and of invertebrate predators like shrimps (Campos *et al.*, 2010). Summarizing, whether the unsuccessful recovery of OWEZ is due to failing planktonic supply as result of diminished parent stocks, enhanced predation or a combination cannot be answered at this point.

Recovery time

Most reports on positive effects of a bottom trawling ban refer to observations made over periods longer than 5 years. Goñi *et al.* (2010) found positive spill-over effects of a MPA on an exploited lobster population within a decade. In a 23 year closed restriction zone in the southern North Sea larger species richness, evenness, and abundances of burrowing mud shrimps and fragile bivalve species were measured (Duineveld *et al*., 2007). After 20 years, a closed area in the Mediterranean had higher abundances of surface suspension feeders, epifauna and predatory fish, while burrowing scavengers and motile infauna decreased relative to the fished surroundings (Juan *et al*., 2007). Faunal recovery after dredging off the UK coast took at least 7 years (Cooper *et al.*, 2007). After a 5 year period without trawling, the production by scallops, green sea urchins and tube-building polychaetes on Georges Bank increased 5- to 10-fold (Hermsen *et al.,* 2003).

Reports on effects over periods shorter than 5 years are less conclusive. Two years after banning hydraulic clam dredging, a community near Canada was still in colonizing phase with increasing abundances of opportunistic polychaetes and amphipods, while recruitment of bivalves remained low (Gilkinson *et al.*, 2005). The higher abundances of infauna in Horns Rev wind farm 2 years after closure were probably a result of environmental conditions and lower predation by birds (Spanggaard, 2005). In a wind farm on Thornton bank no trends in diversity, species densities, biomass, and community composition were found over a 3 year period (Degraer *et al*., 2012). Settlement and survival of bivalve recruits were not enhanced in OWEZ after 2 years of closure (Bergman *et al*., 2010). Subtle faunal changes were reported 1 year after closure of a sandy area around a platform in the North Sea (Dannheim, 2007).

In view of above observations made over relatively short periods, it is not surprising that we could not demonstrate differences between the benthic fauna in the closed OWEZ and regularly trawled reference areas over the 5 year observation period. Perhaps the higher species diversity and higher abundances and lengths of some bivalve species in OWEZ could be interpreted as first signs of a recovery. After a longer recovery period the distinction may become more explicit although faunal patchiness remains a factor to account for in the design of a study. A systematic 5 yearly valuation, as proposed in NATURA 2000 areas in the near future, might be worthwhile. Besides, the depleted adult stocks in the wider region and the faunal patchiness are reasons for detailed studies on larval ecology and recruitment patterns. Such studies will enable predictions that can greatly enhance an effective management of future closed areas. The current study indicates that designation of large-scale MPA's as planned for the North Sea (Anon, 2012) will not imply that restoration of benthic assemblages can be expected within a relatively short period of years.

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Tables.

species	**2007 mean abundance**	**2011 mean abundance**	**contribution %**
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 1

	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

Table 2

Figures

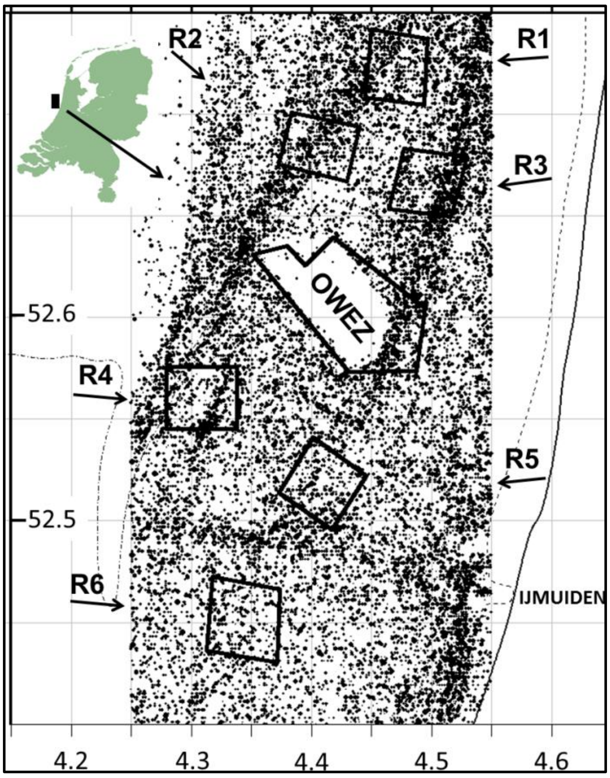

Figure 1

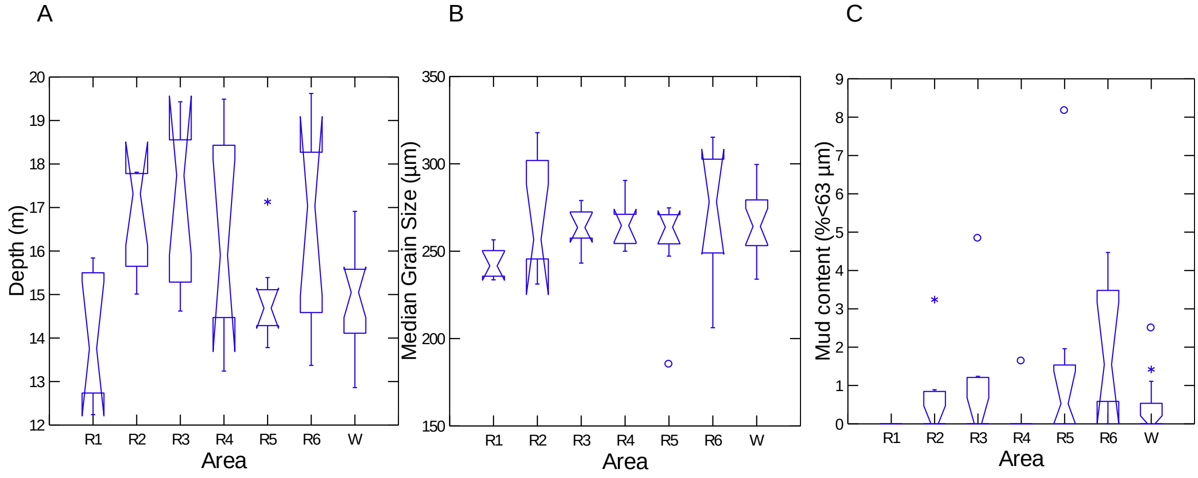
A
B
C
Depth (m)
Median Grain Size (µm)
Mud content (%<63 µm)
R1 R2 R3 R4 R5 R6 W
Area
Area
Area

Figure 2

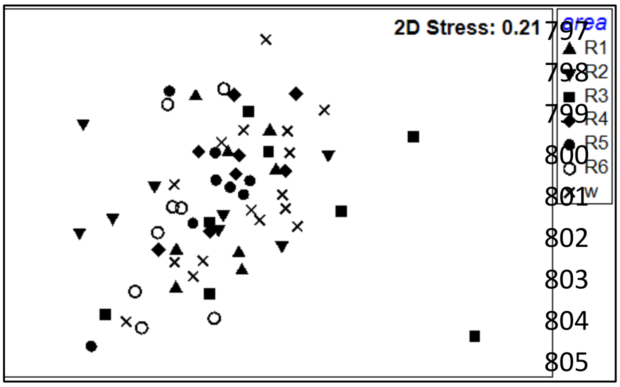

Figure 3

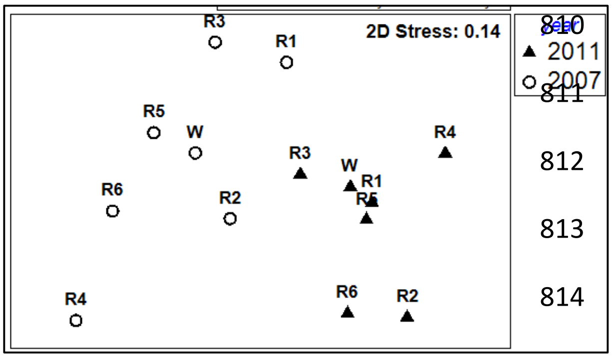

Figure 4

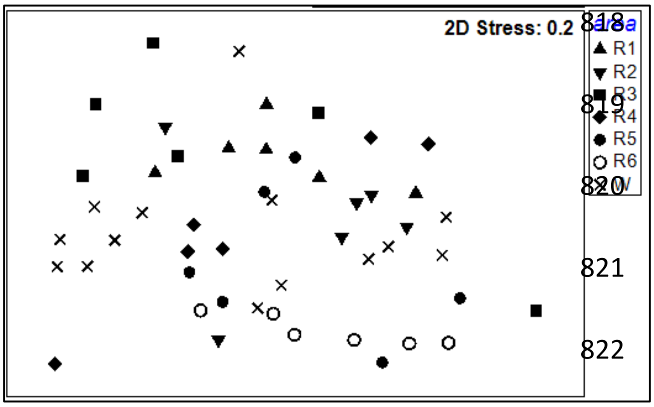

Figure 5

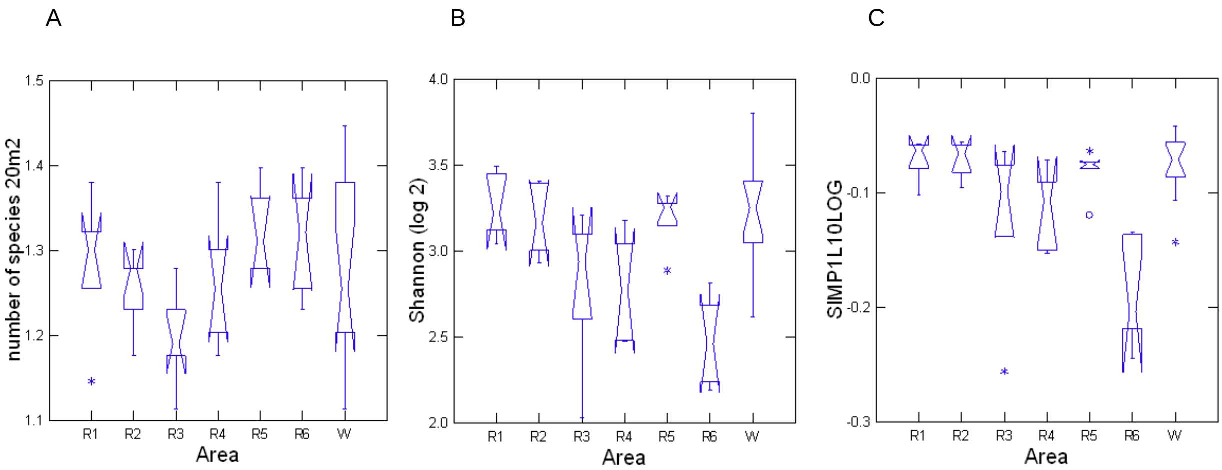
A
B
C
number of species 20m2
Shannon (log 2)
SIMP1L10LOG
R1 R2 R3 R4 R5 R6 W
Area

Figure 6

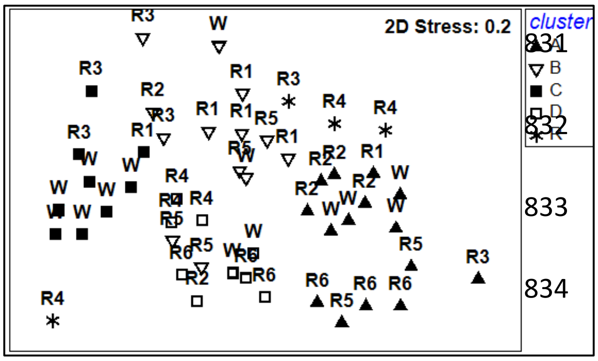

Figure 7

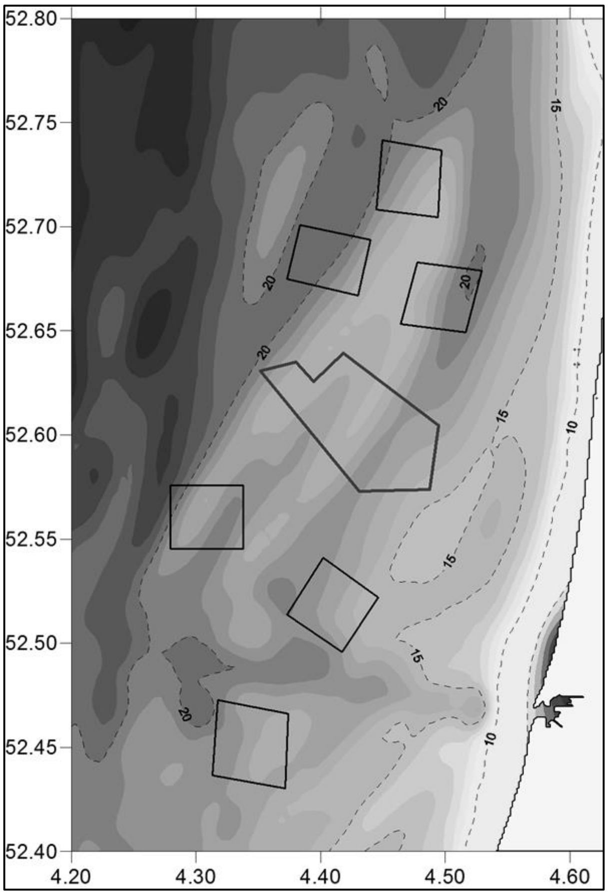
52.80
52.75
52.70
52.65
52.60
52.55
52.50
52.45
52.40
4.20
4.30
4.40
4.50
4.60
20
15
10

Figure 8

CAPTIONS for TABLES and FIGURES

Table 1. SIMPER-analysis showing the five species contributing most (%) to the average dissimilarities in species composition in all areas in 2007 and 2011. Average abundances are given based on fourth root transformed boxcore data (n/0.078 m^2).

Table 2. PERMANOVA results for the different variables and groups of species based on the Triple-D survey 2011. The first column shows the p values for difference between all areas. The second column shows the p values for the difference between OWEZ and the reference areas.

Figure 1. Map of the study areas showing delineated the concession area for OWEZ wind farm approx. 18 km NW of IJmuiden and the 6 reference areas sampled in 2007 and 2011. OWEZ wind farm comprising the 36 turbines plus a 500 m restriction zone around fitted well within the concession area, and has been closed to fisheries from 2006 onwards in contrast to the reference areas. The map presents an estimate of trawling activity of EURO trawlers in 2006-2011 (dots; based on VMS data provided by IMARES). Classification in total number of minutes in the period 2006-2011: ● 0-100, ●100-200, ●200-300, and ●>300. The 10 m and 20 m isobaths are indicated.

Figure 2. Notched box and whisker plots, with medians and notches that mark 95% median confidence intervals based on non-transformed 2011-data of the boxcore stations. A) water depth (m), B) median grain size (µm) and C) mud content (% particles <63 µm) in the OWEZ wind farm (W) and the six reference areas. Number of observations: n=16 in OWEZ, n=8 in references areas. *near outlier between 1.5 a 3 times the IQRs (Inter Quartile Range from Q1/Q3), ° far outliers exceeding 3 times the IQRs from Q1/Q3.

Figure 3. MDS-plot of species abundance (per m^2) data (Bray-Curtis index, 4^{th} root-transformed) of boxcore samples in OWEZ wind farm (W) and the six reference areas in 2011.

Figure 4. MDS-plot depicting the centroids (“centres of gravity”) of OWEZ (W) and the six reference areas, calculated on basis of the position of the single stations in each area, and based on abundance data from the boxcore samplings T_1 (2007) and T_2 (2011; Bray-Curtis index, 4th root transformed).

Figure 5. MDS plot of abundance per haul (n per 20 m^2; Bray-Curtis index, 4^{th} root-transformed) of Triple-D samples in OWEZ (W) and the six reference areas in 2011.

Figure 6. Notched box and whisker plots, with medians and notches that mark 95% median confidence intervals of three diversity indices for Triple-D samples in 2011 showing A) number of species per 20 m^2 (log-transformed data), B) Shannon-wiener, and C) Simpson index (1-λ; log-transformed data) in OWEZ (W) and the six reference areas. Number of observations: n=14 in OWEZ, n=6 in references 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.

Figure 7. MDS plot of the Triple-D 2011-samples of the stations in OWEZ (W) and the six reference areas showing 4 newly formed clusters (A, B, C, D) with each 67% resemblance. Cluster R contains 4 stations that do not fit in the other clusters.

Figure 8. Map showing north-easterly directed gullies across the study areas generating water depth variations of more than 5 m.