FINAL REPORT

Impact of OWEZ Wind farm on bivalve recruitment

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Benthos Recruitment T_1

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CONTENTS

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 IJmuiden. The OWEZ Wind farm with a surface area of approximately 8*4 km encloses 36 wind turbines with distances of 650-1000 m in between. Part of the OWEZ project was an extensive monitoring program, the Monitoring and Evaluation Program (NSW-MEP 2006-2012). According to this program, the environmental impact of the Wind farm on the marine ecosystem was monitored. The possible impact of the construction of the Wind farm on the benthic ecosystem was examined in two separate studies: 1) effects on the macrobenthos community (in- and epifauna > 1mm), covered in a report by Daan et al. (2009), and 2) effects on the recruitment of bivalves. The second study will be described in this report. In this NSW-MEP study we focused on the impact of the Wind farm on the settlement of juvenile benthos, being the start for the benthos populations in the coastal ecosystem. Potential impacts of the construction and operation of OWEZ during the 1-1.5 years preceding our field studies could be expected to show up in recently settled bottom fauna. We focused especially on the recruitment of juvenile bivalves, as their adult stages are a major food supply for fish and diving birds in the shallow coastal zone. Next to this, bivalve populations are a dominant factor in filtering particles from the water column enabling deposition and burial of (organic) material into the sediments.

OWEZ and its 500 m safety exclusion perimeter zone were closed to all shipping during the construction phase in 2006 and the operational phase in 2007. This resulted in an area of approximately 45 km^2 closed for fishery in the coastal zone in which trawling occurred regularly during the last decades (Rijnsdorp et al., 1998; Bergman and Santbrink, 2000; Kaiser, 2000). Among the possible effects of OWEZ on the settlement of bivalves this closure to fisheries could be one of the most important factors. Field experiments in the North Sea (Bergman and Santbrink, 2000) indicated that beam trawling caused direct mortality in various benthos species and led to instant mortalities up to 20% - 65% of the initial bivalve densities in the trawl track. 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). Such changes in community were observed even in areas with high chronic fishing disturbance (Reiss et al., 2009). Hinz et al. (2009) 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 derived by sampling 33 stations in 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 an unfished situation. Long-term effects of trawling on the composition of the benthic community were also demonstrated by comparing the 500 m fishery-exclusion zone around a gas production platform, established more than 20 years ago, with nearby regularly fished areas (Duineveld et al., 2007). The study showed greater species richness, evenness, and abundance of several burrowing mud shrimp and fragile bivalve species in the exclusion area.

Duineveld et al. (2007) found no evidence of greater recruitment in the relative small exclusion zone, despite its positive effect on adult survival. In the larger, 45 km^2 exclusion area formed by the OWEZ Wind farm positive effects on recruitment might occur more likely. In the absence of trawling and subsequent trawling mortality, higher numbers of bivalve species - especially those vulnerable to trawling- might settle, survive and grow up. If so, the species composition of benthic settlers in the non-fished Wind farm will be different from the surrounding, coastal zone. The fishery-stop might also lead to indirect effects caused by for example a lower frequency in resuspension of particles from the seabed (de Madron et al., 2005; Dellapenna et al., 2006). As stated by Witbaard et al. (2001), lower suspended matter concentrations lead to faster growth and higher survival of filter feeding benthos, since their filter efficiency is reduced by high loads. Accordingly better growth and survival of bivalve settlers could be expected in OWEZ. A related indirect effect might be a change in sediment composition due to the lower rate of resuspension by trawling activities. Hily et al. (2008) linked the changes in granulometry, i.e. a strong decrease in the mud fraction and increase in the fine sand fraction, over the last 35 years in the Bay of Biscay to the increase in bottom trawling effort inducing resuspension of fine mud particles and the homogenization of sediments. When the finer sediment fractions in OWEZ are no longer resuspended into the water column the sediment might become finer-grained. Such changes might have impact on species composition of benthic settlers. If so, the species composition of benthic settlers in the non-fished Wind farm will be different from the surrounding, coastal zone. At a small (some tens of meters) scale the construction of the turbines might have effect on sediment composition due to scouring around and predominantly downstream of the foundations. As a consequence sediment might become coarser with temporarily, during periods of calm weather, deposits of finer materials. This might have consequences for the settling response of benthic settlers, if they are selective with respect to the sediment composition.

In this NSW-MEP study we focussed on three questions: 1) do the autumn-densities of bivalve settlers show differences between OWEZ and the reference areas, 2) can possible differences in density of settlers be explained by environmental conditions and 3) do pelagic bivalve larvae respond differently to various sediment types offered in mesocosms.

To determine differences in settlement of young bivalve species we compared autumn-2007 densities in the non-fished OWEZ with densities in the 5 surrounding reference areas which were, just as before the construction of OWEZ, subject to regularly trawling. Median grain size and mud content of the sediment cores collected during this survey were also determined. From this dataset we used densities of *Spisula subtruncata* as an additional test to reveal the impact of OWEZ on settlement by comparing the actual numbers of juveniles in OWEZ Wind farm with the expected density of recruits required to maintain the present-day coastal density. This expected density was estimated from the 2007-density of adults found in OWEZ and surrounding reference areas (Daan et al., 2009) and the mortality estimate based on age converted length distributions of pre-OWEZ populations of *S. subtruncata*. If the fishery-stop in OWEZ led to substantial higher settlement of *S. subtruncata* their juvenile densities in 2007 should be at least some orders of magnitude higher than the expected density.

Differences found in the densities of the 2007-settlers between OWEZ Wind farm and the reference areas and differences in species distribution over the areas are discussed in view of gradients in abiotic variables like median grain size, mud content and water depth. Differences in juvenile densities between the non–fished Wind farm and the trawled reference areas are also discussed in the context of other environmental variables *in situ* measured by a submerged lander frame in the OWEZ Wind farm and another in one of the southern reference areas from February till October 2007. Autonomous instruments mounted at these landers measured current speed and direction, fluorescence, turbidity, salinity and temperature in 5 to 10 minutes intervals. Their recordings are discussed in view of existing knowledge of e.g. particle transport and mud (particles <63 μm) dynamics along the coast (Kleinhans et al., 2005).

The fishery-stop in OWEZ might induce changes in sediment composition by reducing the median grain size due to the lower rate of resuspension. At a smaller scale in the scours around and downstream the turbines foundations coarser sediment might occur. The settling response of bivalves to various sediment characteristics may lead to differences in species composition in the non-fished OWEZ and the reference areas. To examine effects of possible shifts in sediment characteristics on attractiveness for bivalve species to settle down, we mounted experimental settling trays (mesocosms) at the landers. In these mesocosms we offered three different fractions of coastal sediment in adjacent boxes: fine (200-500 μm), medium (500-1000 μm) and coarse (>1000 μm), based on the grain size distribution in OWEZ as stated by Jarvis et al. (2004). Two 3-weeks *in situ* mesocosms experiments were performed during the settling period in summer 2007 to examine preferences of settling bivalves for the range of sediment types.

This Final Report presents the results and conclusions of the project Benthos-Recruitment. The objectives of the study are described in the Introduction. The section Material and Methods includes a description of the survey areas, the set-up of the field survey, an outline of the landers and their instruments, the mesocosm experiments and describes the statistical methods used to analyse the data. In the chapter Results the various data sets are described, illustrated and 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.

Acknowledgement

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2. MATERIAL & METHODS

2.1. Area of investigation

The OWEZ Wind farm is situated in the coastal zone 10-18 km offshore Egmond aan Zee in water depths between 17 and 20 m. 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). In 2003 prior to the designation of the OWEZ area as a Wind farm and the construction of the turbines the median grain size was measured in several sites covering an area from approx. 20 km south to 15 km north of the present OWEZ (Jarvis et al., 2004). The median grain size was on average 504 µm (s.d. 122.8 µm) which is classified as medium sand. The median grain size within the present contours of OWEZ Wind farm was on average 466 µm (s.d.128.9) ranging from 207 to 655 µm. Mean mud (< 63 µm) content was 0.5 % (s.d. 2.25) ranging from 0 to 15% with the higher values found in a small patch in the centre of the Wind farm area. Mean gravel (> 2.0 mm) content was here 0.1 % (s.d. 0.8), and mean organic matter content was 0.49 % (s.d. 0.478).

Studies on mud dynamics in the shoreface off Noordwijk by Kleinhans et al. (2005) indicated that infiltration of mud into the sandy bed by pressure differences over bedforms is negligible. Mud inmixing into the bed is mostly coupled to macrobenthic activity, while re-entrainment is coupled to the sand mobilization during storms.

Total suspended matter (TSM, *i.e.* organic remains of algae and fauna, and silt) is the main source of turbidity in the water column under quiet wind conditions, whereas sand is mainly transported as bed load. By mixing with edible food particles TSM determines the efficiency filter feeders sieve their food from the water phase (Witbaard et al., 2001). Suspended matter concentrations in the water column are highly variable in time and space. In the shallow coastal zone TSM concentrations appear to be mainly determined by the wave heights (Suijlen and Duin, 2002), and TSM is distributed in bands which are roughly parallel to the coastline. In summer (May – November) the OWEZ Wind farm and its surrounding area are located in the zone with mean near-surface TSM values of 5-10 mg/l.

The macrobenthic fauna in the Dutch coastal zone is relative rich with a strong gradient of higher values towards the coast (Holtman et al, 1996). Biomass shows a relative stable spatial pattern over the last 20 years with 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). Abundances of macrobenthic fauna show the same relative stable spatial gradient with densities up to 3000 individuals per m^2 in this near shore zone (Daan and Mulder, 2006). The OWEZ Wind farm is situated between the relative rich near shore and relative poor offshore area. Despite the stable spatial gradients, large annual variations in biomass and density of 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

To measure possible differences in density of juvenile bivalve species that settled in and outside the fishery-free OWEZ Wind farm in 2007, a field survey was executed in OWEZ and in a number of surrounding regularly trawled reference areas. To measure the environmental parameters that might act as steering factors in the settlement of pelagic larvae of benthos species, we deployed a submerged lander with autonomous instruments in the non-fished OWEZ Wind farm and in one of the reference areas. Initially we decided to choose the same reference areas as in the NZW-survey on macrobenthos (>1 mm) executed in spring 2007 (see Daan et al., 2009). In that survey 6 surrounding reference areas were selected: three to the north and three to the south of the Wind farm.

In the OWEZ Wind farm the lander was planned in position 52º 36'N / 004º 27.14'E, circa 10.6 km from the coast and the reference lander was initially planned approximately 7.5 km to the south at a similar distance to the coast in Ref 5. Unfortunately this position was not allowed by the nautical authority. For logistic reasons and to avoid possible impacts of enhanced resuspension due to cable trenching and rock dumping in OWEZ during summer 2007 on the lander measurements we selected an alternative upstream site as close as possible east of Ref 5. The final position (52º32'N / 004º29'E) of the reference lander was 7.6

km off the coast, about 3 km more coast-nearby than the OWEZ lander (Fig. 1). Along the north-south axis the landers are still approx. 7.5 km apart. We subsequently skipped the most southerly and the most northerly reference areas used in the NZW study of Daan et al. (2009) since they were positioned at large distances (circa 20 km) from OWEZ. The final survey design comprised 5 reference areas in total (Fig.1). Two reference areas (Ref 2 and Ref 3) were situated to the north of OWEZ and three reference areas (Ref 4, Ref 5, and Ref L) were positioned to the south, all at a distance of approx. 9 km from the centre point of OWEZ, and 6.5 km off its outer border. Distances between the reference areas and the coast were approx. 16 and 12 km (in the north), and 20, 12, and 7 km (in the south) respectively.

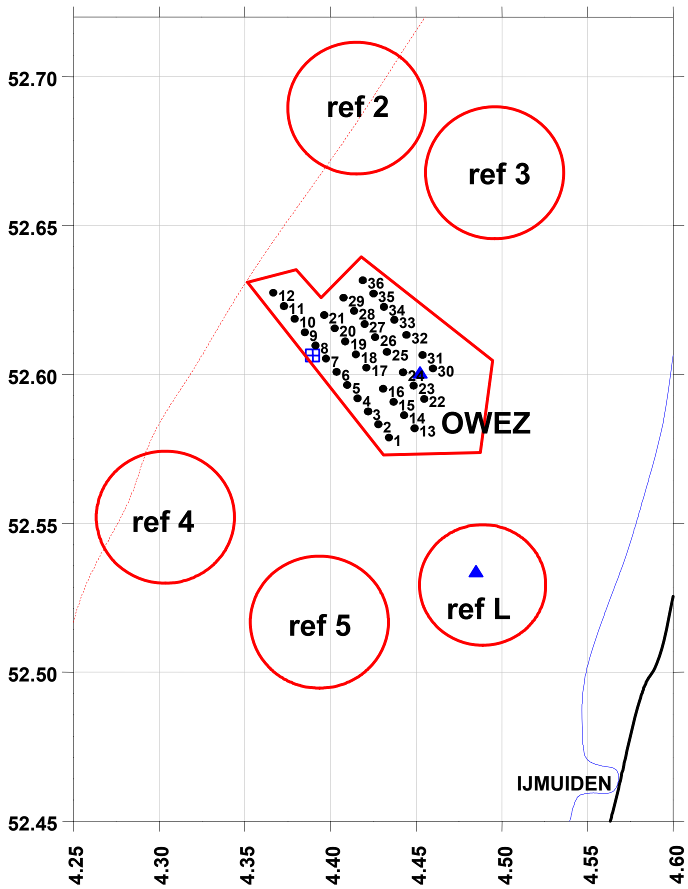


Fig. 1. Map of OWEZ Wind farm area enclosing the 36 turbines, and the 5 reference areas (Ref 2, Ref 3, Ref 4, Ref 5, Ref L). The Wind farm area is closed to fisheries in contrast to the reference areas. Lander positions in OWEZ and in Ref L (▲), the Meteomast (╬),the 10 m (blue) and 20m isobath (red) are indicated.

2.3. Field survey on juvenile benthos

In October 2007 a field survey was executed to compare the settlement of juvenile bivalve species in OWEZ Wind farm with the numbers in the 5 reference areas (Fig. 2). A total of 20 sample stations in OWEZ and 10 stations in each of the 5 reference areas were sampled with the NIOZ-boxcorer on board RV POSEIDON (IFM Geomar, Germany). This sampling equipment (Fig. 3) collects a 20 cm deep sample (diameter 30 cm) from the seabed. After carefully removing the layer of water, 3 cores (diameter 10 cm; height 10 cm) were pushed into the sediment surface of the boxcore sample. Sediment to a depth of 5 cm from each of these cores were collected with a spoon (Fig. 4) and stored separately, with 4% buffered formalin as preservative. In addition a 10 cm deep core (diameter 3 cm), conform the methods in the T_0-report (Jarvis et al., 2004) and in BIOMON (Daan and Mulder, 2006), was taken from each boxcore and stored in the cooling for sediment analysis (sediment grain size and mud content).

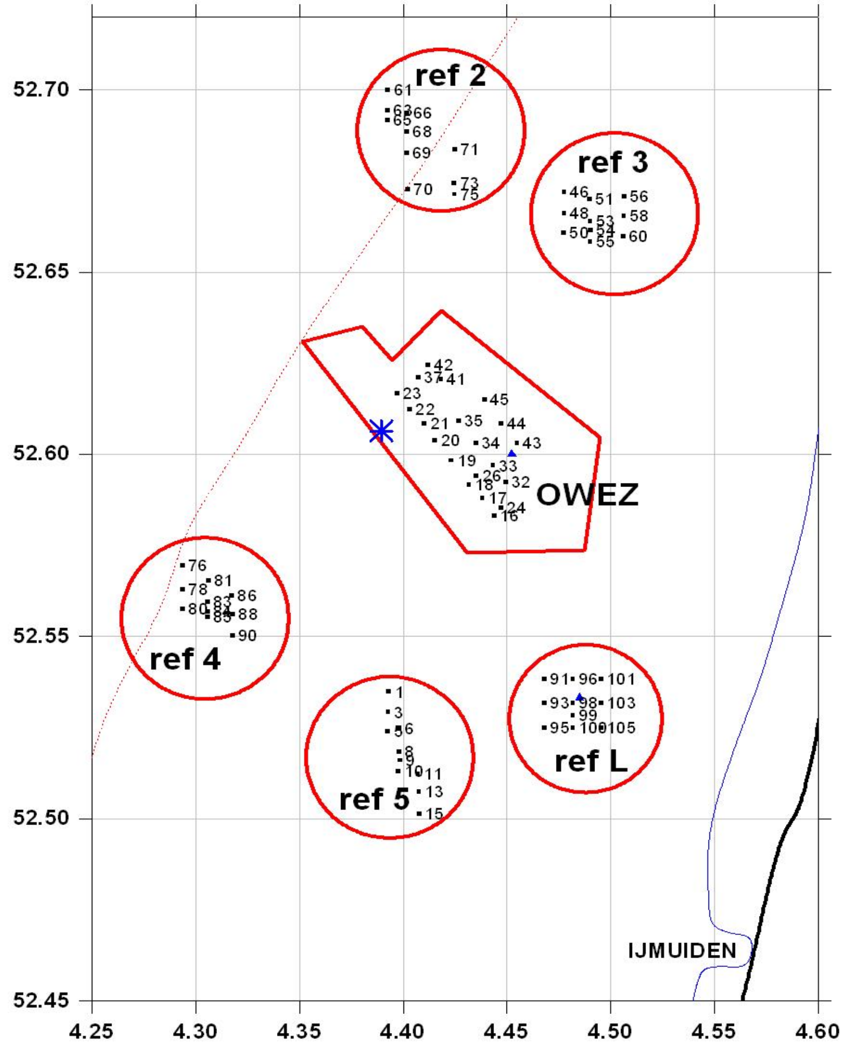


Fig. 2. Boxcorer stations for sampling juvenile bivalve species and sediment in OWEZ and the 5 reference areas. Lander positions in OWEZ and in Ref L (▲), the Meteomast (╬),the 10 m (blue) and 20m isobath (red) are indicated.

The sorting of the samples started in the laboratory in January 2008. Prior to the check for the presence of juvenile bivalves, the samples (n=210) were stained with Bengal rose and left for 24 hours before further processing. A decanting procedure was carried out to separate the small sized bivalves from the equally sized sand grains. During this procedure each sample was brought into a narrow cylinder and turned over carefully for ten times before the supernatant was poured into the stacked sieves of 1.0, 0.5 and 0.2 mm, respectively. This procedure was repeated 10 times to collect all bivalves from the sample. Juvenile bivalves from each sieve fraction were counted using a binocular and identified to lowest possible taxonomical level using morphological characteristics. Finally, the residue in the cylinder was poured over the stacked sieves again and material retained on the 1.0 and 0.5 mm sieves was inspected for the presence of larger sized bivalves that could not be decanted. The number of individuals found in these residues appeared to be negligible.

Fig. 3.
Reineck boxcorer
(sample size 0.07 m^2)
for sampling in- and epifauna.

Fig.4.
Boxcore sample (0.07 m^2; ~20 cm deep) with 3 sub-cores (diam. 10 cm; 0.024 m^2 total surface) inserted 5 cm deep for sampling juvenile bivalves. The smaller fourth core is for collection of sediment.

2.4. Landers and associated instruments

Submerged landers with instruments were deployed to record high resolution environmental data in OWEZ and in Ref L for a period of 8 months in 2007. The landers were also instrumental to execute *in situ* experiments using mesocosms (see section 2.5) in which a range of sediment types is exposed to bivalve settlers. The landers were constructed of aluminium tubes in the shape of an open tripod frame (3 m *3 m, and 2.5 m high) with a weight of circa 1200 kg (Fig. 5). The frame offered space to mount various instruments (current meter, fluorescence sensor, turbidity sensor, and sensors for salinity and temperature). All instruments were fitted at a distance of 1.5 m above seabed to avoid disturbance due to various structures (mesocosms, other instruments and frame constructions) and due to scouring around the lander feet. In order to offload environmental data, to collect benthic samples, to recharge batteries, and to remove fouling the landers were serviced every 3 to 4 weeks. Landers were retrieved on board using acoustically triggered releases that liberated a float pop-up buoy. Table 1 gives an overview of the time intervals the landers have been deployed in OWEZ and Ref L.

Fig. 5. Lander with autonomous sensors and mesocosms ready for deploy.

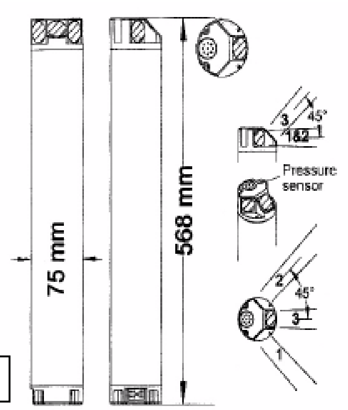
deployment #	start date	retrieval date	mesocosm	deployment (days)
1	27-02-2007	22-03-2007	-	23
2	25-03-2007	16-04-2007	-	22
3	18-04-2007	01-05-2007	-	13
4	03-05-2007	22-05-2007	-	19
5	25-05-2007	05-06-2007	-	12
6	14-06-2007	02-07-2007	-	19
7	10-07-2007	01-08-2007	yes	22
8	03-08-2007	21-08-2007	yes	18
9	24-08-2007	17-09-2007	-	24
10	01-10-2007	09-10-2007	-	8

Table 1. Overview of intervals in which landers have been deployed in OWEZ and Ref L.

The following instruments for recording environmental variables were fitted to the landers in OWEZ and Ref L:

Current meter
The NORTEK Aquadopp current meter (Fig. 6a) transmits a short sound pulse (2 MHz), and measures after recieving its echo the change in pitch or frequency of the echo. The instrument has 3 sensor heads with 2 beams in the horizontal plane and one slanted 45 degrees with respect to the vertical (Fig. 6b). The measurement cell (size 0.75 m) is located at a distance of 0.5 m from the sensors. The device also measures temperature and tilt. The instrument measures the current at a height of 1.5 m above seabed.

a



b

Fig. 6. (a) NORTEK Aquadopp current meter; (b) three sensor heads with 2 beams in the horizontal plane and one slanted 45 degrees.

Fluorescence and turbidity sensors
In the Compact-CLW data logger (ALEC Electronics) (Fig. 7a) a circular array of LED's emitting fluorescence and infrared light, respectively, provides the excitation light for the chlorophyll-a fluorescence and the turbidity backscatter sensors. Optical filters in front of the receivers separate the fluorescence signals from the backscattered light (turbidity). A wiper sweeps the optical surface before each sample to remove dirt and fouling (Fig. 7b). The instrument measures the parameters at a height of 1.5 m above seabed.

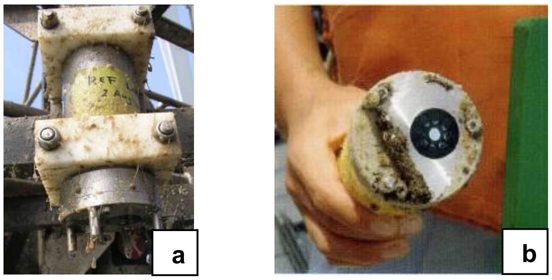


Fig. 7. (a) Compact-CLW data logger (ALEC Electronics); (b) CLW data logger after a one-month deployment. A wiper has regularly swept the optical surface to prevent fouling.

CTD for salinity and temperature measurements

The Seabird 37-SPM Microcat is a high-accuracy conductivity (salinity) and temperature Recorder (Fig. 8). The instrument measures the parameters at a height of 1.5 m above seabed.

Fig. 8. Seabird 37-SPM Microcat for recording conductivity and temperature.

2.5. Mesocosms

To study the settling response of larval bivalves facing a range of sediment types that could be expected to occur in the OWEZ wind farm, submerged settlement trays (mesocosms) were constructed. The mesocosms containing 3 different sediment types were mounted at the landers before their 3-weeks deployments in the OWEZ Wind farm and in Ref L. After retrieval the different sediments were sampled with small cores for density estimates of settlers in the different sediment types.

Each lander carried 3 mesocosm trays, each consisting of 6 small boxes (23*15 and 20 cm deep; Fig. 9 a, b). In each tray 3 boxes were filled each with a different fraction of defaunated sandy sediment. The sand fractions were sieved by hand from sand originating from river locations (grain size range 100 μm to >2 mm, mean grain size 760 μm (s.d. 470 μm), median grain size 590 μm, 5 weight % >2mm). Based on the grain size analyses of Jarvis et al. (2004) we used stacked sieves (200, 500 and 1000 μm) to get sand fractions just smaller and larger than the median grain size (*i.e.* 466, 504 μm) found in the coastal zone in and around OWEZ respectively in 2003. The fraction < 500 μm was chosen to simulate a finer grained sediment generated by lower resuspension as a possible consequence of the fishery-stop. The fraction > 1000 μm was introduced to mimic the coarser sediments that could be expected in the scour sites around the 20 m broad stone bed and downstream the turbines. The grain size ranges and the median grain size of the different fractions are listed in Table 2.

	range grainsizes (µm)	**med. grain size size (µm)**	**mud %<63 µm**
fine	110-780; 0% > 2 mm	370.5	0
fine	100-860; 0% > 2 mm	377.5	0
fine	110-780; 0% > 2 mm	380	0
mean value	**107-773; 0% > 2 mm**	**376**	**0**
medium	210-1320; 0% >2 mm	672	0.33
medium	190-1280; 0% >2 mm	680.5	0.33
mean value	**200-1300; 0% >2 mm**	**676.25**	**0.33**
coarse	>400; 25% > 2 mm	1641	0.061
coarse	>400; 25% > 2 mm	1648.5	0
mean value	**>400; 25% > 2 mm**	**1644.75**	**0.03**

Table 2. Sediment characteristics: range of grainsizes; median grain size (µm) and mud (% <63 µm) in the mesocosms prior to the deployment of the lander.

The design of the mesocosm trays is indicated in Fig. 10. In each tray the 3 middle boxes were filled with the 3 different fractions of defaunated sandy sediment. Both outer boxes of each tray were filled with two out of these three sand fractions and were used for technical tests only. A sixth box in each tray was filled with extreme muddy sediment also for test purposes. The sediment surface in the mesocosms was 70 cm above sea bed. At the top of each box just above the sediment surface a 1 cm thick plastic lattice (holes 1.5*1.5 cm) was attached to prevent washing out of sediment by the current. The two lids covering each tray were closed before deploy. During deployment the lids were programmed to open twice per day during the 2-hours intervals around the turn of the tide from ebb to flood. In that interval current speed is at the lowest and passive particles as well as larvae competent to settle (Hannan, 1984) are expected to sink to the near-bottom layers Although the lattice above the sediment in the trays might have affected the microscale hydrodynamics and the limited opening time of the trays might have caused a reduced larval settlement and resuspension (for details see discussion 4.4), we presume that these factors still permit a comparison of larval settlement between the different sand fractions mounted at a lander.

Upon retrieval after a 3 weeks exposure period the lids were pre-programmed to close while the lander was still at the seabed. On deck the lids were opened again and the surface of the sediment was drained carefully via holes in the bottom of the trays. To determine the amount of sediment washed out by the current the level of the sediment surface below the lower edge of the lattice was measured. Samples were taken from each box by impelling 4 cores (diameter 2.5 cm) into the surface to a depth of 5 cm (Fig. 9c). The content of the cores, sediment and faunal elements, was collected in separate containers and preserved in a buffered solution of 40% RCL2 + 60% ethanol. From each box a fifth core was taken to measure median grain size and mud content. To get detailed information on resuspension and deposition of sediment latter core was splitted in an upper layer (0-1 cm) and a deeper layer (1-10 cm). The mesocosm experiments were carried out during the deployments # 7(9/7/07 to 1/8/07) and # 8 (2/8/07 to 21/8/07; see Table 1).

In the laboratory the samples were stained using Bengal rose and left for a minimum of 24 hours before further processing. A decanting procedure was carried out to separate the small sized bivalves from the equally sized sand grains and other particles. During this procedure each sample was brought into a narrow 1000 ml cylinder that was filled up with filtered sea water to approximately the 600 ml mark. This cylinder was turned over carefully ten times before the supernatant was poured into a sieve of 0.05 mm. This procedure was repeated 10 times to collect all specimens from the sample. Juvenile benthos from the sieve was sorted out using a binocular, and specimens counted. The length of bivalves was measured using the 50x magnification. Finally, the sample residues from the cylinder were inspected for the presence of juvenile benthos. The number of individuals found in these residues appeared to be less than 0.1 % of the number of recovered specimens.

a

b

c

Fig. 9. Mesocosms with different sand fractions: (a) each lander contained 3 mesocosm trays containing 6 small boxes and closing lids; (b) each mesocosm tray contained 6 settlement boxes filled with different types of sediment; (c) sub samples were taken with small cores for juvenile benthos and with syringes to collect sediment.

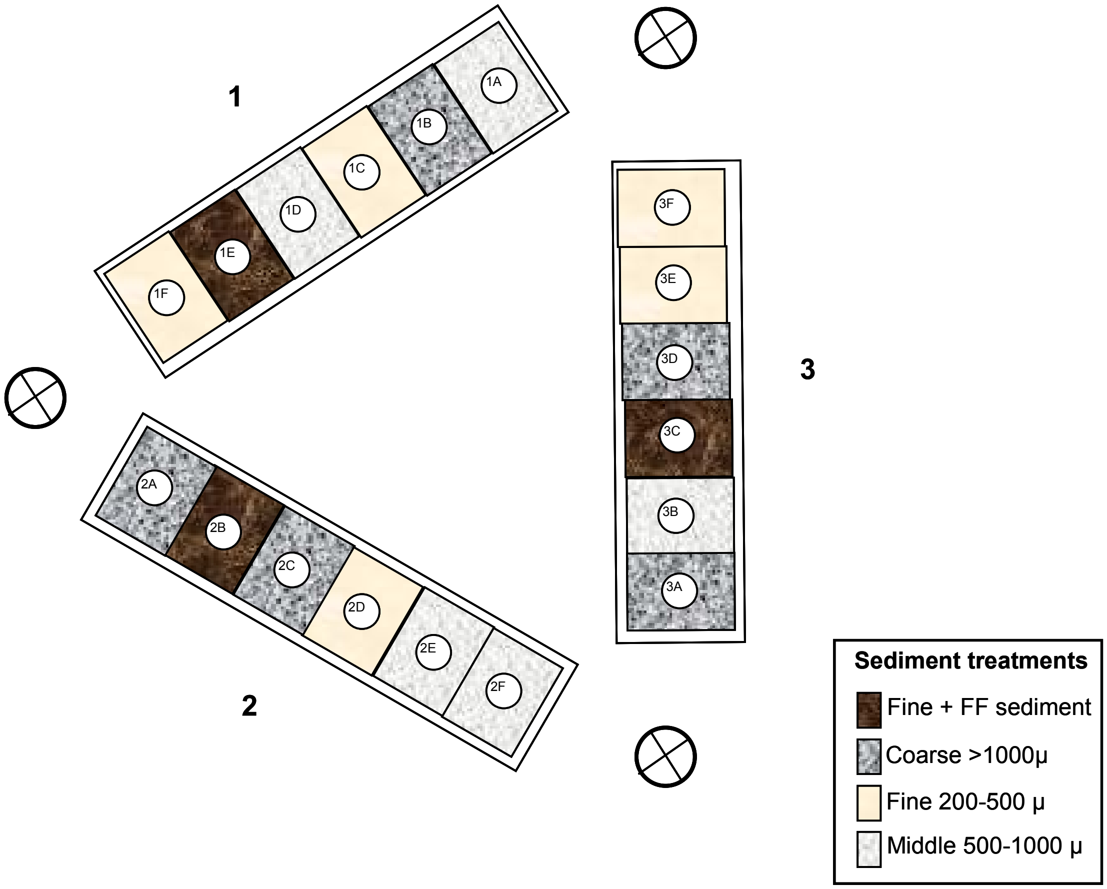


Fig. 10. Experimental design of *in situ* mesocosm experiments showing the three mesocosm trays fitted to a lander. The middle three boxes of each tray contained the 3 different sand fractions tested in this project. The outer boxes in each tray are filled with 2 out of the 3 fractions for technical tests. A sixth box was filled with an extreme muddy fraction for test purposes.

2.6. Sediment analysis

The grain size of the sediment samples collected from the boxcores in the October survey and from the mesocosms experiments was determined by dry sieving to weigh the larger fraction (>2mm) and measuring the particle distribution of 0.04-2000 mu with a Coulter LS230 Laser Diffraction Particle Size Analyser. No acidification or peroxide was practised. On basis of these data the median grain size and the percentage of mud (particles <63 µm) were calculated.

2.7. Statistical analyses

Data obtained during the survey of bivalve recruits with RV POSEIDON were statistically evaluated with respect to differences between OWEZ and reference areas using univariate and multivariate techniques. The results from the mesocosm experiment were evaluated by notched box and whisker plots.

POSEIDON survey

Univariate statistical tests were performed to detect statistical significant differences in central position (median) of abiotic (grain size, mud, water depth) and biotic (abundances, length of bivalves) variables between the six survey areas (*i.e*. OWEZ and the five reference areas).

We used the non-parametric Kruskal-Wallis analysis of variance in the SYSTATv12 and in the EXCEL/Analyse-it software packages. Significant differences were indicated by p- values less than 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. Differences were illustrated by notched box and whisker plots (SYSTAT v12) representing a multiple comparison of median values and their 95% confidence intervals.

Multivariate analyses of species patterns and relationships with environmental variables were executed in the statistical software package PRIMERv6 (Clarke and Gorley, 2006). Hierarchical clustering of the stations from all areas was performed with the CLUSTER analysis on the basis of a Bray-Curtis similarity matrix. Prior to analysis, abundance data were square root transformed. A SIMPROF test was conducted in the cluster analysis in order to discriminate between significant and insignificant sub-structures in the cluster dendrogram by calculating the significance of each node in the design at a 5% level. An ANOSIM test was done to determine significance of similarities in species composition between areas. With the SIMPER routine we examined the contribution of individual species in the separation between two groups of samples, or the “closeness” of samples within a group. In order to examine the best match between the distribution of bivalve species among samples and the environmental variables (mud content, median grain size, water depth) associated with these samples we used the BEST analysis in PRIMERv6. This analysis calculates Spearman’s rank correlations between the sample similarity matrix based on species abundances and different combinations of measured environmental variables. Highest values for ρ (rank correlation coefficient) mark the environmental variables that best explain the pattern among the samples. The statistical significance of the ρ is calculated in relation to the permutations (n=999) simulating the null hypothesis (Clarke and Gorley, 2006).

Mesocosm experiments

Notched box and whisker plots illustrate possible differences in abundances and length of bivalve setters in the sand fractions used in the mesocosm experiments.

3. RESULTS

3.1. Juvenile bivalve species and abiotic variables in OWEZ and reference areas

Table 3 summarises the data collected in OWEZ Wind farm and the 5 reference areas in October 2007: abiotic variables, the numbers of juvenile bivalves found per boxcore (0.024 m^2), and the numbers of identified species.

station #	abiotic variables			number of bivalve individuals found per boxcore		number of identified bivalve species > 0.5 mm found per boxcore								
	median grain size (µm)	mud content (%< 63 µm)	water depth (m)	n>0.2 mm	n>0.5 mm	*Abra alba*	*Donax vittatus*	*Mysella bidentata*	*Ensis* spp.	*Tellina* spp.	*Montacuta ferrigunosa*	*Chamelea gallina*	*Mytilus edulis*	*Spisula* spp.
OWEZ-16	233	3.4	20.4	92	7	0	1	0	2	0	0	0	0	0
OWEZ-17	217	8.7	21.5	570	103	1	0	27	6	1	0	0	6	1
OWEZ-18	299	0.0	19.2	25	1	0	0	0	0	0	0	0	0	0
OWEZ-19	279	0.0	17.3	11	0	0	0	0	0	0	0	0	0	0
OWEZ-20	271	0.0	18.2	15	1	0	0	0	0	0	0	1	0	0
OWEZ-21	256	0.0	19	19	3	0	0	0	2	0	0	0	0	0
OWEZ-22	256	0.0	19.6	13	1	0	0	0	0	0	0	0	0	0
OWEZ-23	259	0.0	19.2	22	1	0	0	0	0	0	0	0	1	0
OWEZ-24	244	0.0	19.3	45	3	0	0	0	1	0	0	0	0	0
OWEZ-26	303	0.0	19.2	115	5	0	0	0	1	0	0	0	0	0
OWEZ-32	231	3.4	20.7	373	27	0	1	4	7	11	0	0	0	1
OWEZ-33	266	1.8	21.1	241	15	1	0	0	11	0	1	0	0	0
OWEZ-34	289	0.0	17.5	51	0	0	0	0	0	0	0	0	0	0
OWEZ-35	273	0.0	18.2	2	0	0	0	0	0	0	0	0	0	0
OWEZ-37	259	0.0	19.1	19	2	0	0	0	1	0	0	0	0	0
OWEZ-41	248	2.8	19.9	60	9	0	2	0	1	0	1	0	3	0
OWEZ-42	257	0.0	19.9	26	3	0	0	0	0	0	0	0	0	0
OWEZ-43	254	2.3	20.9	309	22	0	1	0	9	1	10	0	1	0
OWEZ-44	278	0.0	19.6	25	2	0	0	0	0	0	0	0	1	0
OWEZ-45	280	0.0	17.6	36	0	0	0	0	0	0	0	0	0	0
mean	**263**	**1.1**	**19.3**	**104**	**10.3**	**0.1**	**0.3**	**1.6**	**2.1**	**0.7**	**0.6**	**0.1**	**0.6**	**0.1**
R2-61	288	0.0	21.8	31	3	0	2	0	1	0	0	0	0	0
R2-63	319	0.0	21.7	15	1	0	0	0	0	0	0	0	0	0
R2-65	329	1.3	22.5	42	0	0	0	0	0	0	0	0	0	0
R2-66	298	1.7	23.2	75	1	0	0	0	0	0	0	0	0	0
R2-68	276	2.1	23.2	52	4	0	0	0	2	0	0	0	0	0
R2-69	370	0.0	22.5	80	8	0	0	0	1	0	5	0	0	0
R2-70	233	0.0	22.6	208	44	10	2	0	1	16	11	0	0	0
R2-71	240	0.0	20.7	69	7	0	0	0	0	0	0	0	0	0
R2-73	242	0.0	19.6	90	4	0	0	0	2	0	1	0	0	0
R2-75	248	0.0	18.9	109	5	0	0	0	0	0	0	0	0	0

mean	**284**	**0.5**	**21.7**	**77**	**7.7**	**1**	**0.4**	**0**	**0.7**	**1.6**	**1.7**	**0**	**0**
R3-46	260	0.0	17.6	18	1	0	0	0	0	0	0	0	0
R3-48	260	0.0	17.9	55	2	0	0	0	0	0	0	0	0
R3-50	262	0.0	17.5	56	3	0	0	0	0	0	0	0	0
R3-51	266	2.6	20.7	235	24	4	0	1	7	5	3	0	0
R3-53	280	0.0	20.7	251	25	0	0	0	11	2	2	0	2
R3-54	274	0.0	20.2	106	12	0	0	0	2	1	4	0	0
R3-55	274	2.6	20.6	62	13	2	2	0	9	0	0	0	0
R3-56	270	0.0	20.9	13	0	0	0	0	0	0	0	0	0
R3-58	268	1.8	21.6	22	2	0	0	0	2	0	0	0	0
R3-60	276	3.0	22	81	9	1	0	0	6	1	0	0	0
mean	**269**	**1.0**	**20.0**	**90**	**9.1**	**0.7**	**0.2**	**0.1**	**3.7**	**0.9**	**0.9**	**0**	**0.2**
R4-76	246	0.0	20.3	22	3	0	0	1	0	0	0	0	0
R4-78	257	0.0	18.7	15	1	0	0	0	0	0	0	0	0
R4-80	263	0.0	17.9	13	0	0	0	0	0	0	0	0	0
R4-81	267	0.0	17.3	30	0	0	0	0	0	0	0	0	0
R4-83	266	0.0	18.2	26	0	0	0	0	0	0	0	0	0
R4-84	256	0.0	18.6	29	2	1	0	0	0	0	0	1	0
R4-85	256	0.0	18.9	15	0	0	0	0	0	0	0	0	0
R4-86	281	0.0	21.1	41	0	0	0	0	0	0	0	0	0
R4-88	277	0.0	22.7	67	1	0	0	0	0	0	0	0	0
R4-90	263	2.1	22.4	53	8	0	0	0	0	2	2	0	0
mean	**263**	**0.2**	**19.6**	**31**	**1.5**	**0.1**	**0**	**0.1**	**0**	**0.2**	**0.2**	**0.1**	**0**
R5-1	276	0.0	20.2	60	0	0	0	0	0	0	0	0	0
R5-3	251	2.9	20.3	73	0	0	0	0	0	0	0	0	0
R5-5	257	2.0	20.3	5	0	0	0	0	0	0	0	0	0
R5-6	248	1.9	20	157	4	0	0	0	0	1	3	0	0
R5-8	257	1.9	19.5	147	0	0	0	0	0	0	0	0	0
R5-9	275	0.0	18.8	18	0	0	0	0	0	0	0	0	0
R5-10	268	0.0	19.2	11	0	0	0	0	0	0	0	0	0
R5-11	254	1.8	19	202	1	0	0	1	0	0	0	0	0
R5-13	266	1.7	18.5	35	0	0	0	0	0	0	0	0	0
R5-15	203	3.1	21.4	168	22	0	0	1	3	9	0	0	0
mean	**256**	**1.5**	**19.7**	**88**	**2.7**	**0**	**0**	**0.2**	**0.3**	**1**	**0.3**	**0**	**0**
RL-91	277	0.0	16.2	3	0	0	0	0	0	0	0	0	0
RL-93	270	1.7	16.5	21	0	0	0	0	0	0	0	0	0
RL-95	283	2.3	17.4	31	1	0	0	0	0	0	0	0	0
RL-96	280	0.0	16.2	0	0	0	0	0	0	0	0	0	0
RL-98	276	1.9	16.4	24	2	0	0	0	1	0	0	0	0
RL-99	242	2.9	16	48	2	0	0	0	0	1	0	0	0
RL-100	251	2.4	16	43	1	0	0	0	1	0	0	0	0
RL-101	279	1.7	15.3	26	1	0	1	0	0	0	0	0	0
RL-103	266	4.8	15.9	29	2	0	0	0	0	0	1	0	0
RL-105	248	7.8	18.1	149	20	0	0	0	1	1	7	0	0
mean	**267**	**2.6**	**16.4**	**37**	**2.9**	**0**	**0.1**	**0**	**0.3**	**0.2**	**0.8**	**0**	**0**

Table 3. Summary of the data collected in OWEZ Wind farm and the 5 reference areas in October 2007. Station code, median grain size (μm), mud (% < 63 μm) and water depth (m) are indicated. Numbers of juvenile bivalves (>0.2 mm and >0.5 mm) found per boxcore (0.024 m^2), and numbers of identified species are given.

Abiotic variables

Sediment cores taken from the boxcores were analysed and median grain size (µm) and percentage of particles < 63 µm were calculated (see Table 3). Statistical analysis indicated no significant differences in median grain size among the six survey areas (Kruskal-Wallis test, p= 0.51). Notched box and whisker plots of the median grain size data visualise that the 95% confidence intervals of the grain size distribution in the various areas do overlap between all areas (Fig. 11). Median grain size of the samples in all areas was on average 266 µm (s.d. 24.1; ranging from 203-377µm).

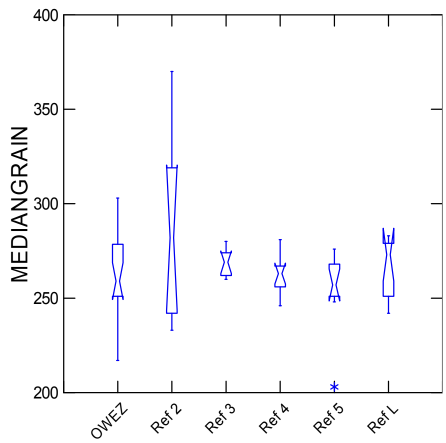


Fig. 11. Notched box and whisker plots of the median grain size (µm) in OWEZ Wind farm and the 5 reference areas showing the 95% confidence intervals. The overlap between the intervals of all areas points to absence of statistical significant differences. * means values between the lower or upper hinge (Q1 or Q3) and 1.5 a 3 times the Hspread (InterQuartileRange).

The mud data, *i.e*. the percentage of particles < 63 µm (see Table 3), showed a significant difference in mud content between the two or more of the six survey areas (Kruskal-Wallis test, p=0.0187). An additional pairwise test indicated no significant difference between OWEZ and any particular reference area, but exposed a significant difference between the reference areas Ref 4 (on average 0.2% mud) and Ref L (on average 2.5% mud; Kruskal-Wallis test, bonferroni adjusted, p=0.0214). The notched box and whisker plots in Fig. 12 illustrate the high mud content in Ref L, the station nearest to the coast. To illustrate possible gradients in mud content in the total survey area the spatial distribution of the percentage of mud is given in Fig. 13. The coastward trend in increasing mud content perceived between the Refs 4 and L, was also observed inside OWEZ Wind farm where the mud content in the 10 easternmost stations (on average 2.0%) was higher than in the 10 westernmost stations (on average 0.3%; Kruskal-Wallis test, p=0.056).

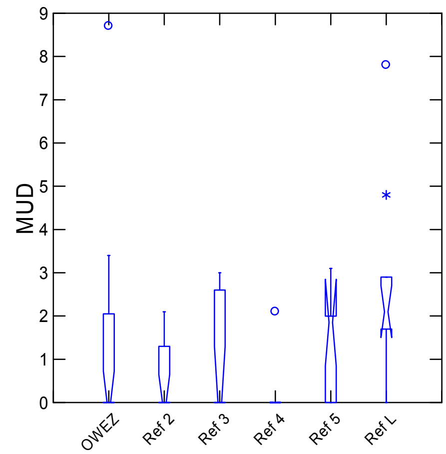


Fig. 12. Notched box and whisker plots of the mud content (%< 63 µm) in OWEZ Wind farm and the 5 reference areas showing the 95% confidence intervals. The non-overlapping confidence intervals between Ref 4 and Ref L point to a statistical significant difference. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

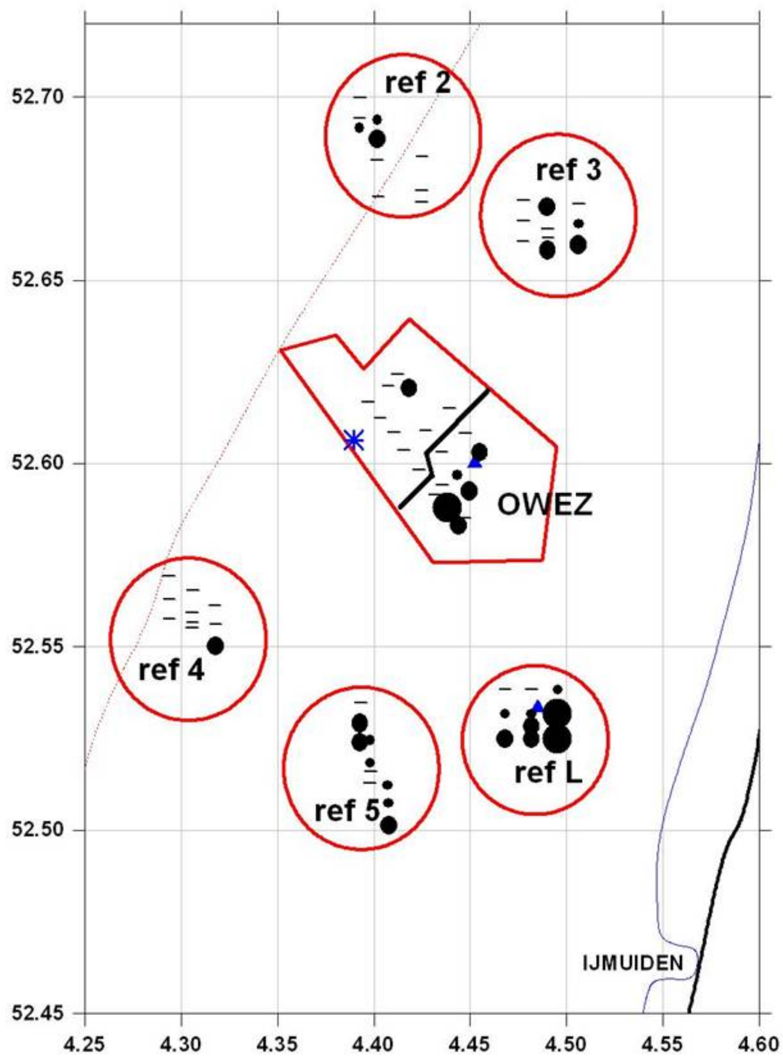


Fig. 13. Spatial distribution of mud (% <63 μm) in the survey areas (– 0%, • 0-2%, ● 2-4.5%, ⬤ 4.5-9%). The black line in OWEZ depicts the boundary between the stations positioned in the western and eastern part of the Wind farm.

Water depth was significantly different between two or more of the six survey areas (Kruskal-Wallis test, $p<0.0001$; see Table 3). A pairwise test showed that water depth in Ref L (on average 16.4 m) was significantly less than in all other areas including the Wind farm (Kruskal-Wallis test, bonferroni adjusted, $p<0.0011$). Ref 2 (mean depth 21.7 m) was significantly deeper than the Wind farm (mean depth 19.3 m) and Ref 4 (mean depth 19.6 m; Kruskal-Wallis test, bonferroni adjusted, $p=0.004$ and 0.027 respectively). The notched box and whisker plots illustrate the heterogeneity in water depth in the reference areas and the difference in water depth between the Wind farm and the reference areas L and 2 (Fig. 14).

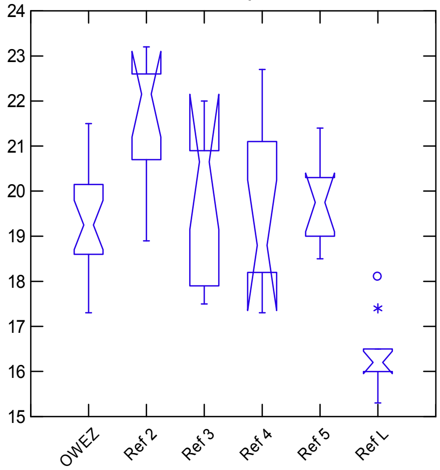
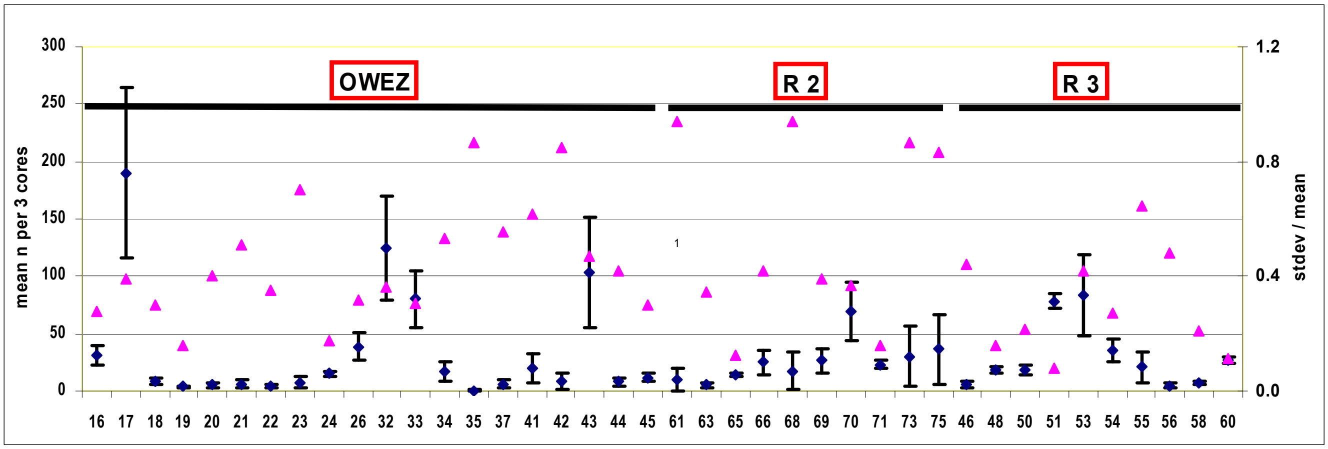


Fig. 14. Notched box and whisker plots of the water depth (m) in OWEZ and the 5 reference areas showing the 95% confidence intervals. The non-overlapping confidence intervals of Ref L with all other areas show its significant shallower water depth. On the other hand Ref 2 is significant deeper than OWEZ and Ref 4. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

Abundances of bivalve settlers

In the 210 samples a total of 5281 settled juvenile bivalves were counted, 4838 individuals of them were retained on the 0.2 mm sieve, 287 individuals on the 0.5 mm and 156 individuals on the 1.0 mm. Apart from these recruits, 18 *Abra alba* and 1 *Donax vittatus*, all settled in 2006 or earlier, were found on the 1.0 mm sieve. These specimens were not included in the dataset. A plot of the mean number of bivalves > 0.2 mm found in the three sub-cores per station indicated that the variation per station was relative small (Fig. 15) leading to a coefficient of variation C_v (s.d./mean) of on average 0.4 (ranging from 0.1 to 0.9). Apparently patchiness at the scale of the size of the boxcore (0.07 m^2) was relatively small. Because of the low variation among the three sub-cores, individuals originating from these triplets (total surface 0.024 m^2) taken from one boxcore were added together (see Table 3) in the statistical analyses.



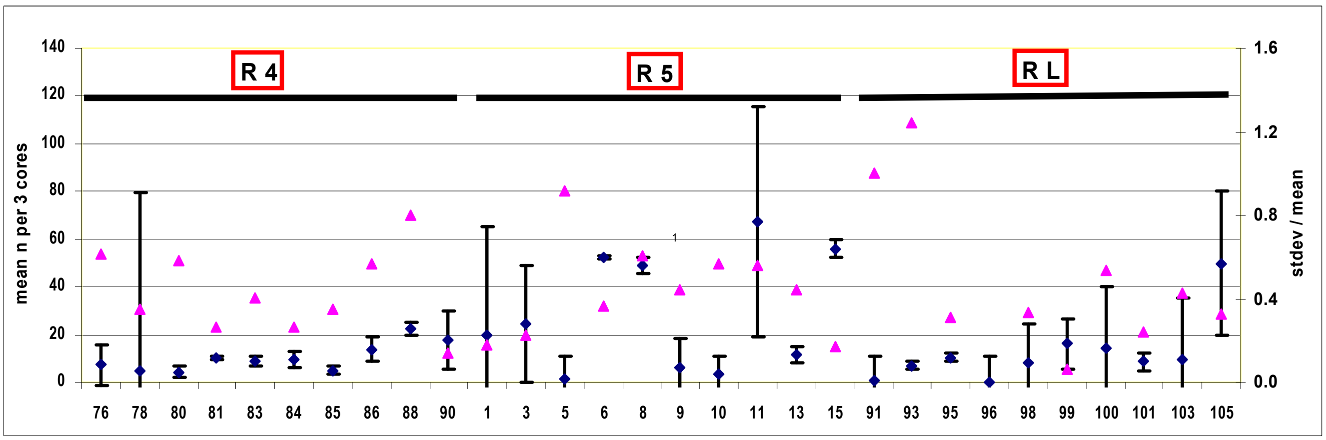


Fig.15. Plot of the mean numbers of bivalves (>0.2 mm) found in the three separate cores in the boxcore derived from one station. Standard deviation of the mean density found in the three cores and the coefficient of variation (s.d./mean: violet triangular dots) are indicated per station. Stations are grouped per survey area. NB x-axes scales are different.

Although the 3 highest peaks in mean density of juveniles > 0.2 mm were observed in OWEZ (Fig.15), statistical analysis revealed that no differences existed between the numbers of bivalve recruits found in the six survey areas (Kruskal-Wallis test, p=0.185). Average abundances ranged from 1296 to 4310 individuals per m^2 in the various areas (*i.e.* 4310, 3213, 3746, 1296, 3650, 1558 individuals per m^2 in OWEZ, Ref 2, Ref 3, Ref 4, Ref 5, Ref L, respectively). The box and whisker plots (on square rooted transformed data) in Fig. 16 illustrate the overlapping 95% confidence intervals of the bivalve abundances found in the six survey areas.

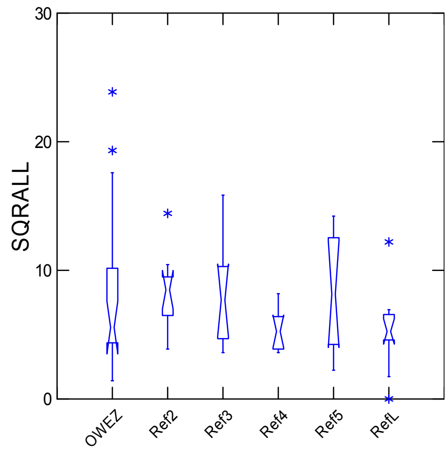


Fig. 16. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves >0.2 mm found in the three sub-cores per boxcore in OWEZ and the 5 reference areas showing the 95% confidence intervals. The overlap between the confidence intervals of all areas points to absence of statistical significant differences in bivalve abundances. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange).

If we focus on the larger sized settlers (> 0.5 mm; Table 3), supposedly older individuals that could have been affected by local environmental factors for a longer period, a significant difference existed between two or more of the survey areas (Kruskal-Wallis test, p=0.01). A pairwise Kruskal-Wallis test showed that abundances in OWEZ did not differ from those in any of the reference areas. But abundances in Ref 3 were significantly higher (383 per m^2) than in Ref 5 (113 per m^2); Kruskal-Wallis test, bonferroni adjusted, p=0.044). The box and whisker plots (Fig. 17) of the abundances of these larger recruits show that OWEZ, Ref 2 and Ref 3 scored higher values (mean densities 463, 321, 383 individuals per m^2, respectively) than Ref 4, Ref 5 and Ref L (mean densities 63, 113, 121 individuals per m^2, respectively). This result suggests an alongshore northwards increasing trend in densities in larger-sized individuals that, on the contrary, was not observed in the densities of the individuals > 0.2 mm (Fig. 16).

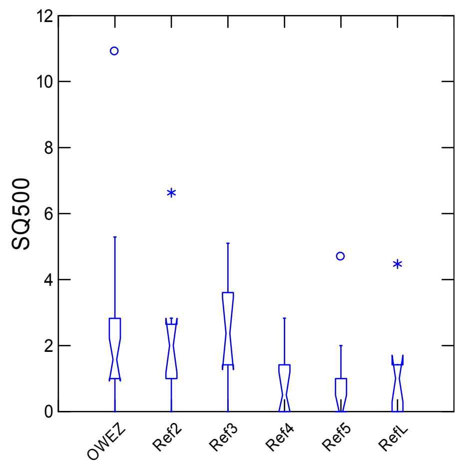


Fig. 17. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves >0.5 mm found in the three sub-cores per boxcore in OWEZ and the 5 reference areas showing the 95% confidence intervals. The non-overlapping confidence intervals of Ref 3 and Ref 5 points to a statistical significant difference in abundances of bivalves. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

The scatterplot in Fig. 18 illustrates the correlation between the numbers of small-sized settlers (0.2-0.5 mm) and larger-sized ones (>0.5 mm), as derived from Table 3. This correlation is statistically significant (Spearman rank correlation test; p<0.0001).

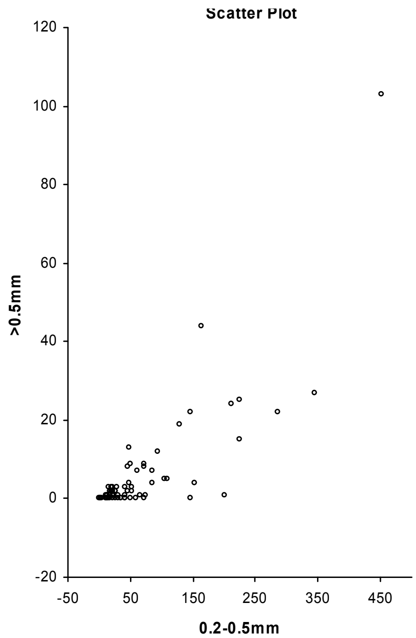


Fig. 18. Scatterplot of numbers of settlers of 0.2-0.5 mm versus numbers > 0.5 mm (data from Table 3).

Length of bivalve settlers

The length frequencies of the juvenile bivalves settled in 2007 (individuals >0.5 mm of all nine species; Table 4) were compared between the survey areas. Lengths of *Tellina* spp. were different between two or more of the six survey areas (Kruskal-Wallis test, p=0.0026). A pairwise test showed a significant lower length *of Tellina* in OWEZ (on average 2.3 mm) than in Ref 2 (on average 3.1 mm; Kruskal-Wallis test, bonferroni adjusted, p=0.0274). Their lengths in Ref 2 (mean 3.1 mm) and Ref 3 (mean 3.0 mm) were significantly larger than in Ref 5 (mean 2.0 mm; Kruskal-Wallis test, bonferroni adjusted, p=0.0056 and 0.0507 respectively). This suggests a higher growth rate or an earlier settlement in the northern reference areas. All other species (*Abra alba*, *Donax vittatus*, *Mysella bidentata*, *Ensis* spp, *Montacuta ferrigunosa*, *Chamelea gallina, Mytilus edulis* and *Spisula* spp*.)* showed no differences in length between the 6 survey areas.

species	OWEZ	n	Ref 2	n	Ref 3	n	Ref 4	n	Ref 5	n	RefL	n
Abra alba	2.7± 2.6	2	1.1 ±0.4	10	1.3 ±0.8	7	1.3	1	-	-	-	-
Donax vittatus	1.9 ±1.5	5	1.0 ±0.1	3	0.9 ±0.1	2	-	-	-	-	3.5	1
Mysella bidentata	2.0 ±0.6	30	-	-	0.7	1	1.2	1	0.9 ±0.0	2	-	-
***Ensis* spp**	1.7 ±1.1	44	2.1 ±0.8	7	1.6 ±0.5	41		-	1.8 ±0.3	3	1.1 ±0.3	5
***Tellina* spp.**	2.2 ±0.7	12	3.1 ±0.7	16	3.0 ±0.4	7	3.0 ± 0.7	2	2.0 ±0.9	10	1.9 ±0.2	2
Montacuta	2.0 ±1.4	12	1.5	17	2.1	7	3.2 ±	2	1.2	3	2.8	8

ferrigunosa			±0.8		±1.6		2.9		±0.1		±0.6	
Chamelea gallina	0.9	1	-	-	-	-	-	-	-	-	-	-
Mytilus edulis	0.7 ±0.1	15	-	-	0.8 ± 0	2	0.6 ± 0.0	2	-	-	0.6	1
***Spisula* spp.**	1.4	1	-	-	2.9	1	9.2 ± 0.8	2	-	-	-	-

Table 4. Mean length in mm (± s.d.) of juvenile bivalve species in OWEZ Wind farm and the reference areas.

Species composition of bivalve settlers

Of all bivalve specimens retained on the smallest of the stacked sieves (0.2 mm) only 6% was identifiable to genus or species level. Of the larger sized individuals retained by the 0.5 mm sieve 63% could be identified. We decided to focus on the latter size class as 1) the percentage of identified individuals (n= 281 out of a total number of n= 443) can be considered as being representative for their size class, and 2) the composition of the larger size class would reflect more clearly the environmental stress during the first months after settlement. In total 6 species (*Abra alba, Donax vittatus, Mysella bidentata*, *Mytilus edulis, Montacuta ferrigunosa, Chamelea gallina*) and 3 genera (*Ensis* spp., *Tellina* spp., *Spisula* spp.) could be distinguished on their morphological characteristics (see Table 3). In the respective genera the species *Ensis directus, Tellina fabula* and *Spisula subtruncata* are the dominant species in the coastal zone (Jarvis et al., 2004; Daan and Mulder, 2006).In order to illustrate the species composition and the number of species (species richness) in the six survey areas correctly, only 10 aselect but evenly distributed stations in OWEZ were included in Fig.19. Since the percentage of identified recruits differed among areas (ranging from 52 to 82 %, see Table 5), exclusively in Fig. 19 the species abundance per area has been corrected for the actual percentage of identified recruits in that area. Numbers were standardized to the total percentage (i.e. 71%) of individuals from these 60 stations that could be identified to species or genus level. Fig. 19 illustrates the species composition in 10 stations in OWEZ and in the reference areas. This Figure suggests that the bivalve fauna in OWEZ, Ref 2 and Ref 3 consists of a relative large variety of species occurring in relative high numbers per species.

	OWEZ	**Ref 2**	**Ref 3**	**Ref 4**	**Ref 5**	**Ref L**	**all areas**
ind. identified (%)	82	70	75	53	67	52	71

Table 5. Percentages of individuals in the size class >0.5 mm that could be identified in the different survey areas, and total percentage of these individuals that could be identified in the 60 stations. NB: Individuals of only 10 selected stations in OWEZ were included in this Table.

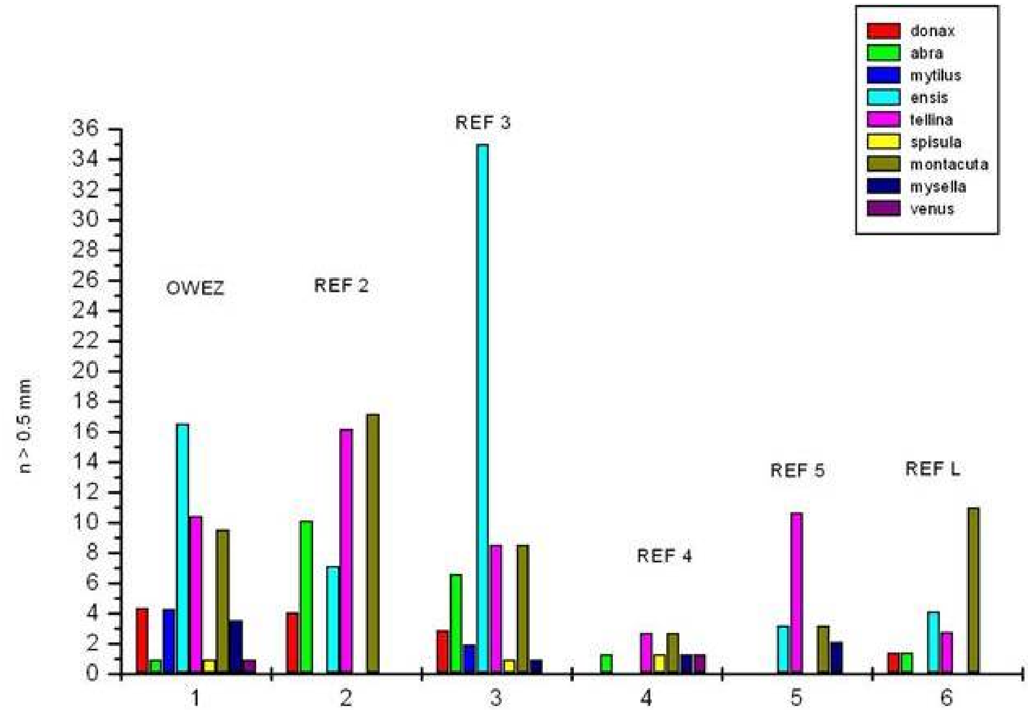


Fig.19. Species distribution of bivalves >0.5 mm in OWEZ and the reference areas based on the sum of individuals found in the three sub-cores per boxcore in 10 stations in OWEZ and in 10 stations in each of the reference areas. Numbers were standardized to the total percentage of individuals in these 6 areas that could be identified to species or genus level. NB. Species composition in OWEZ is based on 10 selected stations to avoid any bias due to an unequal total sample size. The 10 stations selected (*i.e*. stations 16, 18, 20, 23, 32, 34, 41, 42, 43 and 45) were evenly distributed across OWEZ. (*cf* Fig. 2).

In the following univariate and multivariate tests only the absolute, non-standardized abundances of larger-sized specimens (>0.5 mm) found in the 20 stations of OWEZ and 10 stations in each of the reference areas were tested for differences. For an univariate analysis of differences in species abundances between areas, we decided to focus on the 5 commonest species and genera: *Abra alba, Donax vittatus,, Mysella bidentata*, *Ensis* spp*.,* and *Tellina* spp. Abundances of *Ensis* spp. were different between one or more of the six survey areas (Kruskal-Wallis test, p=0.01), the other species showed no significant differences. A pairwise Kruskal-Wallis test revealed a significant difference in density of *Ensis* spp. between Ref 3 ($154.m^{-2}$) and Ref 4 ($0.m^{-2}$; bonferroni adjusted, p=0.016). The box and whisker plots in Fig. 20 illustrate the lower abundance of *Ensis* spp. in Ref 4 and, although not significant, in Ref 5.

A multivariate SIMPER analysis performed with the 9 species/genera showing the similarity indices belonging to the various areas indicated that *Ensis* spp. contributed the most to the average Bray Curtis similarity within the various survey areas: ranging from 70 to 78% in OWEZ, Ref 2, Ref 3, and Ref L. Other species contributed less than 20%. In Ref 5, however, contribution of *Ensis* spp. was 0%, whereas *Mysella* and *Tellina* spp. contributed here 56 respectively 44%. The contribution of *Ensis* spp. to the average Bray Curtis dissimilarity between groups (ranging from 84-98%) was also dominant (25-41%).

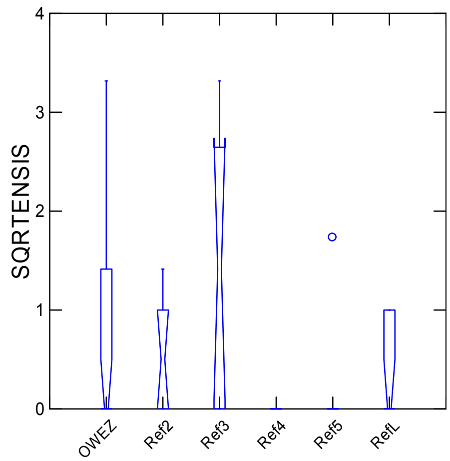


Fig. 20. Plot of (square rooted transformed) abundances of *Ensis* spp. found in OWEZ and the reference areas. The non-overlapping confidence intervals between Ref 4 and Ref 3 point to a statistical significant difference. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

Cluster analysis of the individual stations based on square root abundances of the nine species and genera (individuals > 0.5 mm) combined with a SIMPROF-test (p=0.05) revealed that 2 significant clusters could be identified: a top and a bottom cluster as depicted in Fig. 21. Most of the 16 stations in the top cluster were positioned in the easternmost parts of OWEZ and Ref 3 (Fig. 22) suggesting an offshore–coast, possibly combined with a slight south-north, gradient in species composition rather than an area-based trend in configuration. Their spatial distribution seemed to be correlated with relatively high mud contents. A statistical test revealed that the mud percentages of the stations in the top cluster were indeed significantly higher (on average 2.6%) than in the bottom cluster (on average 0.7%; Mann Whitney test, p=0.0002). Water depth of the stations in the top cluster was significantly higher than in the bottom cluster, on average 21.0 m and 19.0 m respectively (Mann Whitney test, p=0.0002), although not linearly correlated with the mud content (Fig. 23). The median grain size of the stations in the 2 clusters showed no significant difference (Mann Whitney test, p=0.079).

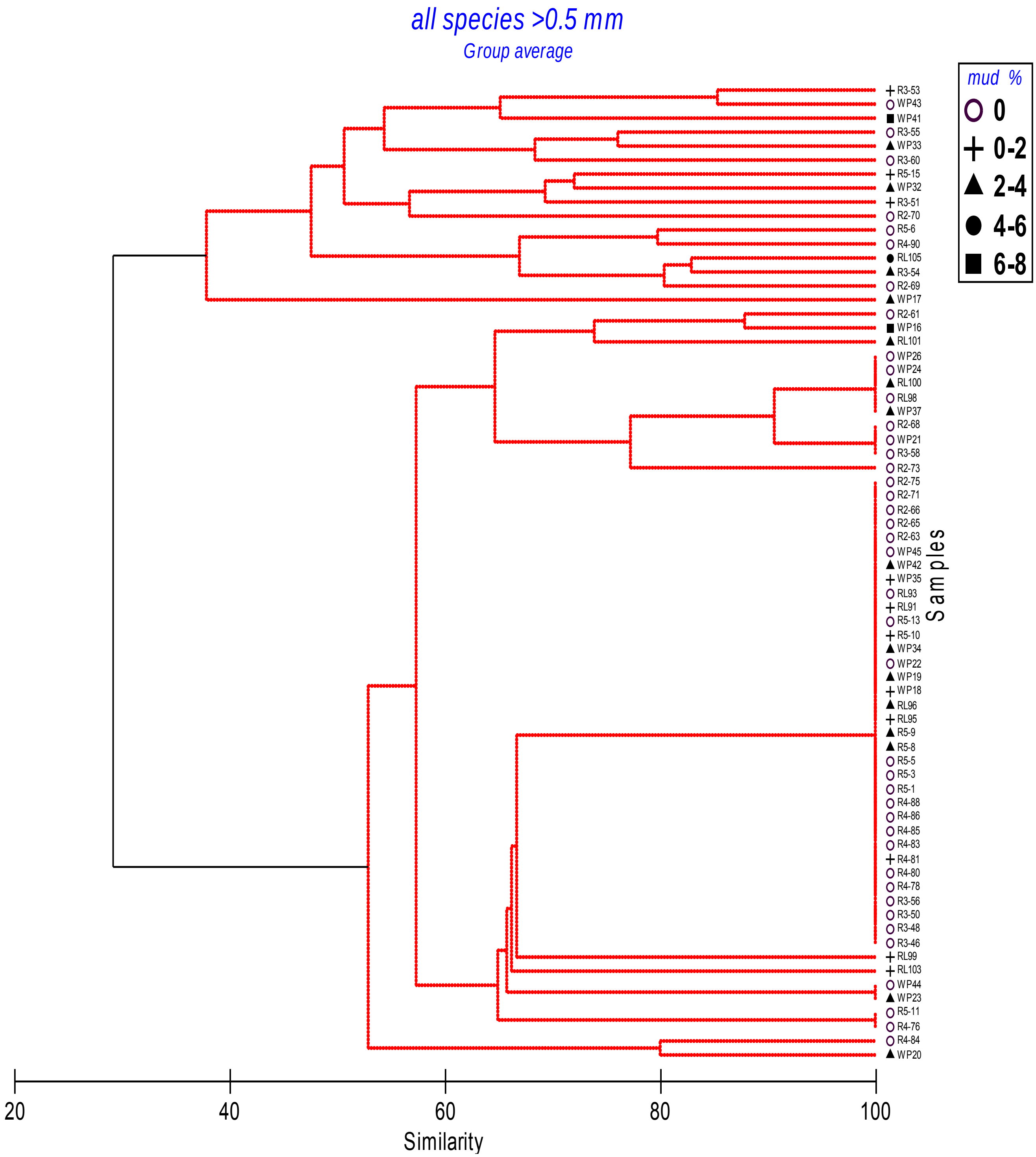


Fig. 21. Group average cluster analysis (PRIMERv6) of all species/genera (n=9) found in the stations (n=70) in OWEZ Wind farm and the reference areas. A top and a bottom cluster of stations indicated by black lines can be distinguished with a statistical significant difference (π =-11.46; p=0.001). Other clusters (red lines) are not statistically different. Mud content (% < 63 µm) of the stations is indicated.

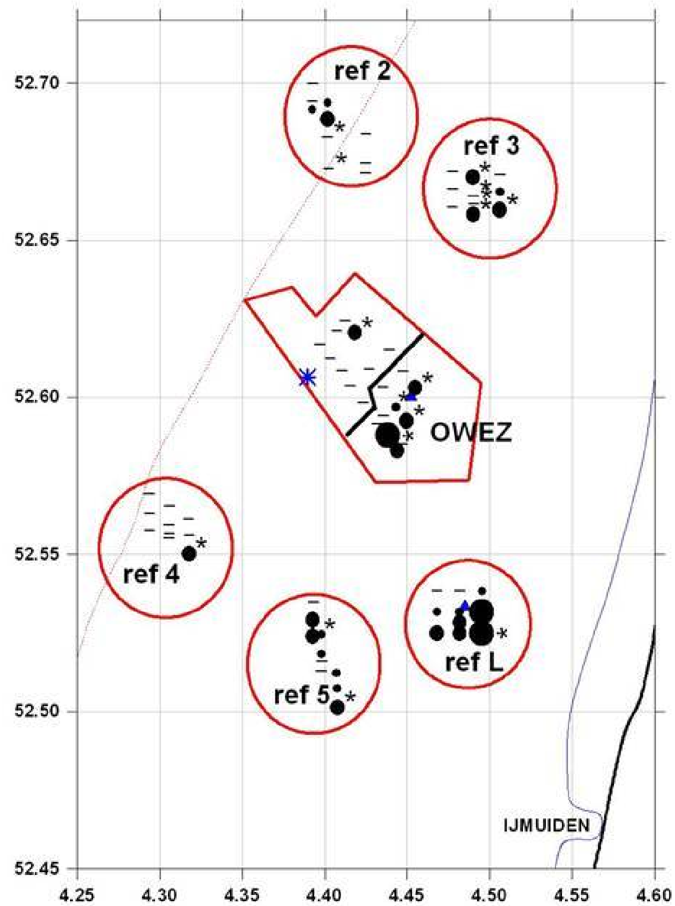


Fig. 22. Spatial distribution of the stations belonging to the top cluster (depicted with an asterisk) and the distribution of mud (% <63 µm) in the survey areas (– 0%, • 0-2%, ● 2-4.5%, ⬤ 4.5-9%).

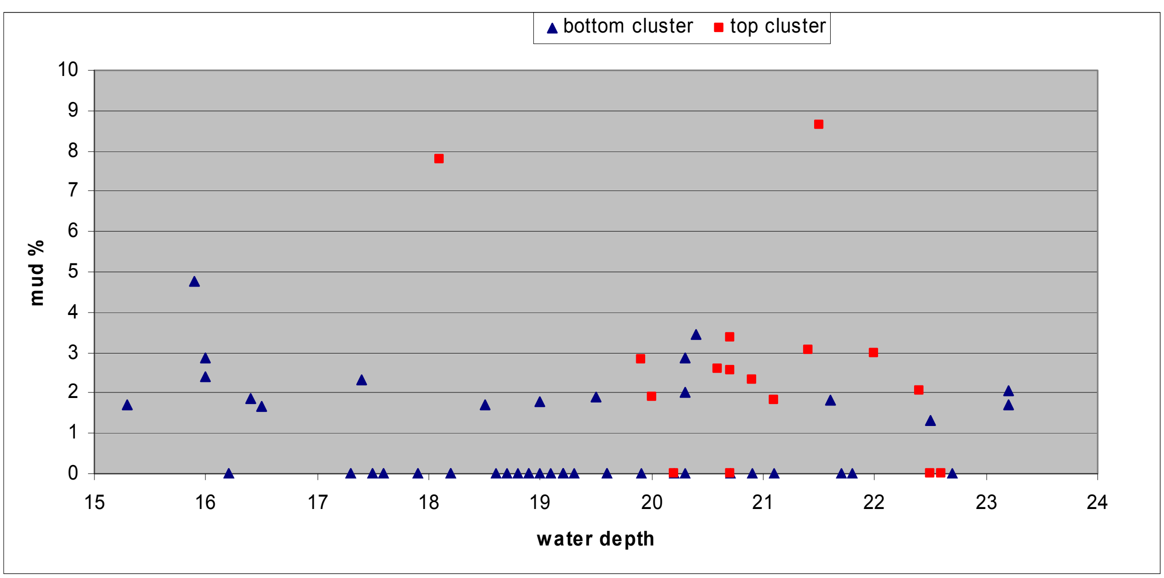


Fig. 23. Mud (< 63 µm) percentage and water depth (m) of the stations belonging to the top cluster (■) and the bottom cluster (▲) presented in Fig. 21.

The absence of area-based differences in species composition is confirmed by an ANOSIM analysis of the species composition (*i.e.* abundances) in the six survey areas showing no significant differences between areas (global R= -0.013; p=0.57). The strongest indications for differences in species distributions were found between the northerly Ref 3 and southerly Ref 4 (R=0.17 and p= 0.036; Table 6).

	OWEZ	**Ref 2**	**Ref 3**	**Ref 4**	**Ref 5**
OWEZ					
Ref 2	-0.05006				
Ref 3	0.01434	0.003444			
Ref 4	-0.0366	0.068444	0.170889		
Ref 5	-0.03972	0.029778	0.126444	-0.03556	
Ref L	-0.07585	-0.04733	0.044222	-0.01	-0.031

Table 6. Results of the pairwise test statistic R of the ANOSIM test (PRIMERv6) on square rooted data of 9 species/genera found in 20 stations in OWEZ and in 10 stations in each of the reference areas indicating no significant differences between the areas. The strongest indications for differences in species composition between areas were found between Ref 3 and Ref 4 (R=0.17, p=0.036).

A SIMPER analyse of the similarities within the clusters (Table 7) indicated that the average similarity in the top cluster was 44% with *Ensis* spp. as the highest contributor (38%), followed by *Montacuta ferruginosa* and *Tellina* spp*.* with 26 and 21% respectively. In the bottom cluster average similarity was much lower (5%) and could be mainly attributed to *Ensis* spp. (90%), with a minor role for *Donax vittatus* (3%). The average dissimilarity between the two clusters was attributed to *Ensis* spp, *Montacuta ferruginosa*, *Tellina* spp. and *Abra alba* with 26, 24, 19 and 10% respectively. From Table 6 it is also clear that the top cluster harboured a much denser bivalve population than the lower cluster of stations.

A	**Top Cluster: average similarity 44. 05**				
species	average abundance	average similarity	aim/SD	contribution %	cumulative %
***Ensis* spp.**	1.87	16.88	1.32	38.01	38.01
Montacuta ferrigunosa	1.35	11.64	0.79	26.21	64.22
***Tellina* spp.**	1.34	9.19	0.95	20.7	84.91
Abra alba	0.75	3.13	0.53	7.04	91.96
	Bottom Cluster: average similarity 5. 34				
***Ensis* spp.**	0.24	4.78	0.25	89.59	89.59
Donax vittatus	0.06	0.18	0.06	3.41	93.00

B	**Bottom & Top Cluster: average dissimilarity: 92. 93**					
species	average abundance bottom cluster	average abundanc e top cluster	average dissimilarity	dissim./SD	contribution %	cumulative %
***Ensis* spp.**	0.24	1.87	23.9	1.49	25.73	25.73
Montacuta	0.04	1.35	22.43	1.10	24.15	49.89

ferrigunosa						
***Tellina* spp.**	0.02	1.34	17.61	1.23	18.96	68.84
Abra alba	0.02	0.75	8.55	0.92	9.2	78.05
Donax vittatus	0.06	0.45	5.91	0.70	6.36	84.41
Mysella bidentata	0.04	0.57	5.42	0.53	5.83	90.24

Table 7. Results of SIMPER analyses (PRIMERv6) on square rooted data of the species and genera found in the stations belonging to the two clusters (see Fig. 21) indicating the contribution (as %) of a species to the similarities within the clusters (A) and dissimilarities between the clusters (B).

Correlations between bivalve species distribution and abiotic variables

Scatter plots based on the abundances (see Table 3) of small-sized (0.2-0.5 mm) and larger-sized settlers (>0.5 mm) and mud content (% <63 μm) of the stations illustrate the significant correlations between both size-classes and mud content (Spearman rank correlation test, p=<0.0001 and p=0.0018 respectively;Fig. 24).

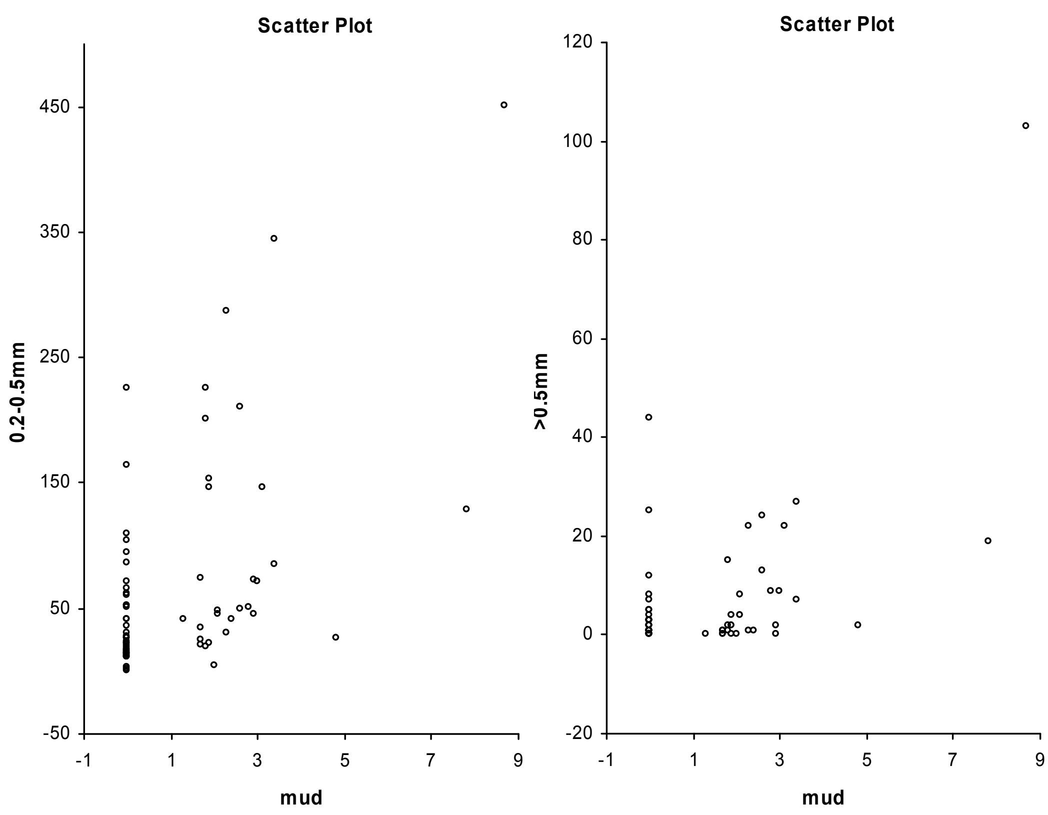


Fig.24. Scatter plots of the stations showing abundances of small-sized (0.2-0.5 mm; left-hand) and larger-sized settlers (>0.5 mm; right-hand) versus mud content (% <63 μm). Correlations between both size-classes and mud content were statistically significant (Spearman rank correlation test, p=<0.0001 and p=0.0018 respectively).

In order to identify the environmental variable(s) (mud content, median grain size, water depth) that best explain the patterns in the numbers of the nine bivalve species and genera that were found in the samples, we used the BEST analysis in PRIMERv6. This analysis was first performed using the complete datasets of the environmental variables and the bivalve recruits from all samples in OWEZ and the reference areas. To get insight whether the link between environmental variables and bivalve species might be affected by for instance ongoing trawling in the reference areas, separate BEST analyses were performed on the data from the trawled reference areas and the non-fished OWEZ. BEST analyses were performed with i) sample matrices based on abundances of smaller (0.2-0.5 mm) bivalves (Table 8a), ii) matrices based on total abundances and on species composition of larger-sized individuals >0.5 mm (Table 8a), and iii) matrices based on the abundances of individual species (Table 8b).

Results of the BEST-analyses in Table 8 show Spearman's rank correlation coefficients ρ (rho) and the p values for the various datasets. In case of a significant correlation, the three (combinations of) abiotic variables with the highest rank correlation have been listed. Table 8a shows a marked difference between results of OWEZ on one hand and those from the pooled reference areas and the combined dataset from OWEZ and the REFs on the other hand. While in OWEZ significant and relatively strong correlations between patterns in environmental variables and bivalve samples (small-sized and larger-sized abundances, all species > 0.5mm) existed, none were found in the analysis of the data from the pooled reference areas. In the significant correlations found in the data from OWEZ, the factor mud scored the highest correlation coefficient, although in combination with median grain size in the case of abundance of larger sized individuals.

A	**OWEZ and Refs**	**Reference areas**	**OWEZ**
all ind. 0.2-0.5 mm	ρ =0.131, p=0.06	ρ =0.106, p=0.08	**ρ =0.532, p=0.03** mud 0.532 mud, depth 0.518 all 0.454
all ind. >0.5 mm	ρ=0.200, p<0.001 all 0.2 mud, grain 0.192 depth, mud 0.191	ρ =0.106, p=0.08	**ρ =0.814, p<0.001** mud, grain 0.814 depth 0.761 all 0.712
all species > 0.5 mm	ρ =0.366, p<0.001 mud 0.366 grain, mud 0.318 all 0.305	ρ =0.237, p=0.02	**ρ =0.781, p<0.001** mud 0.781 mud, depth 0.649 all 0.571

B	**OWEZ and Refs**	**Reference areas**	**OWEZ**
***Ensis* spp.**	ρ=0.11, p=0.11	ρ =0.164, p=0.09	**ρ =0.473, p<0.001** mud 0.473 grain, mud 0.473 all 0.444
Abra alba	ρ =0.323, p=0.02 mud 0.323 mud, grain 0.257 mud, depth 0.251	ρ=0.208, p=0.084	ρ =0.636, p=0.06
***Tellina* spp.**	ρ =0.322, p=0.003 mud 0.322 grain, mud 0.279 grain 0.154	ρ=0.221, p=0.058	ρ =0.614, p=0.06
Donax vittatus	ρ =0.125, p=0.18	ρ =0.089, p=0.42	**ρ =0.634, p=0.002** mud 0.634 grain, mud 0.417 mud, depth 0.352
Mysella bidentata	ρ =0.28, p=0.004 Mud 0.28 Mud, grain 0.267 Grain 0.213	ρ =0.101, p=0.35	**ρ =0.646, p=0.006** Mud 0.646 Grain, mud 0.565 All 0.493
Montacuta ferrigunosa	ρ =0.194, p=0.055	ρ=0.268, p=0.017	ρ =0.31, p=0.11
Chamelea gallina	ρ = -0.034	ρ = -0.038	ρ =0.412, p=0.032
Mytilus edulis	ρ = 0.135, p=0.17	ρ = -0.013	ρ = 0.412, p=0.032

Table 8. Results of BEST analyses (PRIMERv6) performed on the datasets of environmental variables and juvenile bivalves in the six survey areas. A: matrices based on abundances of smaller sized (0.2-0.5 mm) bivalves, and on abundance and species composition of larger sized (>0.5 mm) bivalves. B: matrices based on dataset of environmental variables and abundances of single species. Separate tests were done using 1) the complete dataset from all samples in OWEZ and the reference areas, and 2) the datasets from the non-fished OWEZ and 3) the trawled reference areas. Rank correlation coefficient ρ and p value are indicated (bold indicates a statistical significance and relatively high correlation).

Table 8b shows that in the combined dataset of OWEZ and reference areas patterns in three species (*Abra alba, Tellina* spp., *Mysella bidentata.*) showed a significant but weak correlation with environmental variables of which mud ranked highest as explanatory variable. Data on single species derived from the trawled reference areas did also not yield any significant correlation with a strong correlation coefficient. In contrast, in the fishery-closed OWEZ Wind farm spatial patterns of *Ensis* spp*., Donax vittatus* and *Mysella bidentata* were significantly correlated with environmental variables with mud as the best explanatory one.

3.2. Environmental variables

Annual cycles of environmental parameters were measured in OWEZ Wind farm and Ref L, since they may influence settlement and survival of bivalve recruits. Figs. 25 to 32 show the annual variations in the parameters measured by the instruments mounted at the landers deployed in both areas (see Fig. 1). The basic recordings are also presented as plots of the consecutive deployments and presented in the Appendix I to VI. In Appendix VII the annual variation in wind speed (m/s) is indicated. Appendix VIII shows the variations in wind speed and direction in the consecutive deployments (source www.noordzeewind.nl). The wind data will be used in the sections 3.3 and 4.5.

Temperature

Temperature influences the development of the larvae, moment of settlement, growth of the juveniles and adults, and the reproduction. Fig. 25 shows that seawater temperature in both lander locations follow the same seasonal cycle. Started with temperatures of 8 ºC in March, temperature increased continuously until June. Maximum temperatures were reached in late August (19.5 ºC). Appendix I shows the variation in the basic recordings of temperature within each consecutive deployment. The two lander locations had an identical temporal pattern of the temperature variation and absolute differences in temperature between the locations were minimal i.e. on average 0.12 degrees with a median of 0.9 degrees.

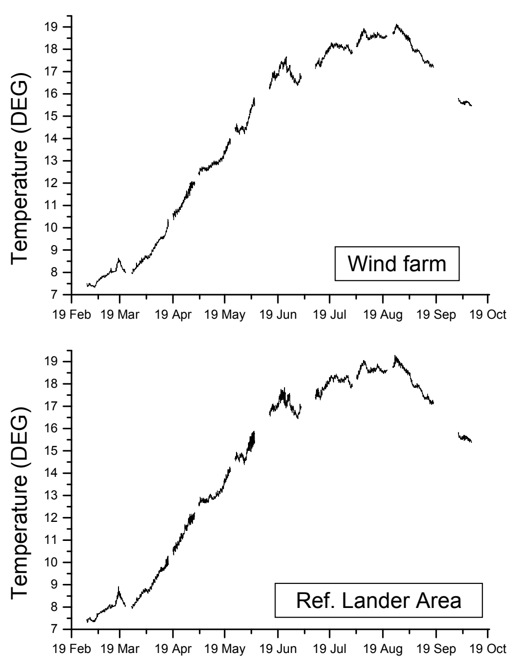


Fig. 25. Annual variations of ambient seawater temperatures (ºC) in OWEZ Wind farm (upper graph) and Ref L (lower graph) from February until October 2007.

Salinity

Salinity is a good indicator for the origin of water masses. It represents the dissolved salt contents of water. Salinity will be reduced in water masses containing fresh water run off from river mouths. On the other hand, more saline water originating from offshore areas enhances salinity. Salinity may affect the presence and abundance of marine species, which have their specific optimal salinity conditions, although they can live for longer periods under suboptimal conditions.

Fig. 26 indicates that salinity in the Wind farm and Ref L showed variations throughout the year. The observed declines in mid March, mid July and mid-August occurred simultaneously in both locations. A part of the fluctuations in salinity can be explained by fresh water discharge by the river Rhine (Fig. 27). This holds most prominently for the low levels in early March. The two lander locations which were separated in a coast-seaward direction by a distance of approx. 3 km showed a similar temporal pattern of variation of salinity. Appendix II shows the variation in the basic recordings of salinity within each consecutive deployment.

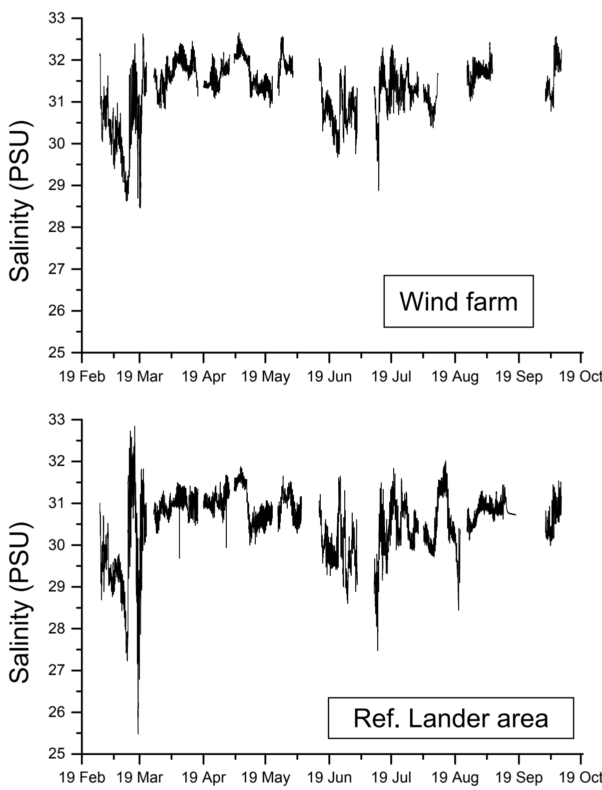


Fig. 26. Annual variations in salinity levels (PSU) in OWEZ Wind farm (upper graph) and ref L (lower graph) from February until October 2007.

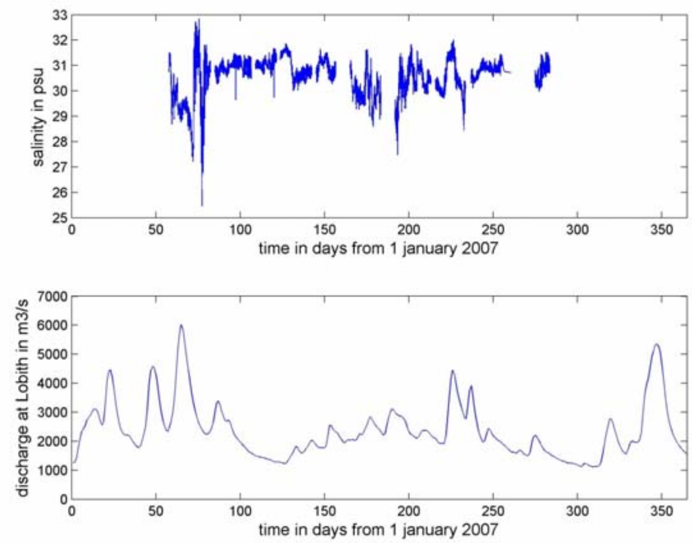


Fig. 27. Discharges at Lobith (m^3/s) and salinity (psu) in Ref L in 2007.

Fluorescence

Fluorescence is a measure for the presence of chlorophyll-a in the water column. Chlorophyll-a, a pigment in algae, is a useful proxy for phytoplankton biomass. Phytoplankton is a major food source for benthic species. Fluorescence has been measured to get insight in the annual and spatial differences in food particles available for filter feeding bivalves.

In both lander locations, chlorophyll-a concentrations were relatively low at the start of the first lander deployment (mid February; Fig. 28). At the start of spring, in the last two weeks of March, a rapid increase of the phytoplankton biomass occurred. Maximum chlorophyll-a concentrations were measured in mid April. The length of the phytoplankton bloom was similar for both locations (mid March – mid May). After this spring bloom, chlorophyll-a levels remained relatively low during the rest of 2007. To compare the amount of chlorophyll-a measured in the consecutive deployments in the Wind farm with Ref. L, the data per deploy were averaged and the integrated sum was calculated (Table 9). Although the two first deployments in the Ref L showed enhanced concentrations, this trend does not seem to continue during the later deployments. The Appendix III shows the variations in the basic data of fluorescence within each consecutive deployment.

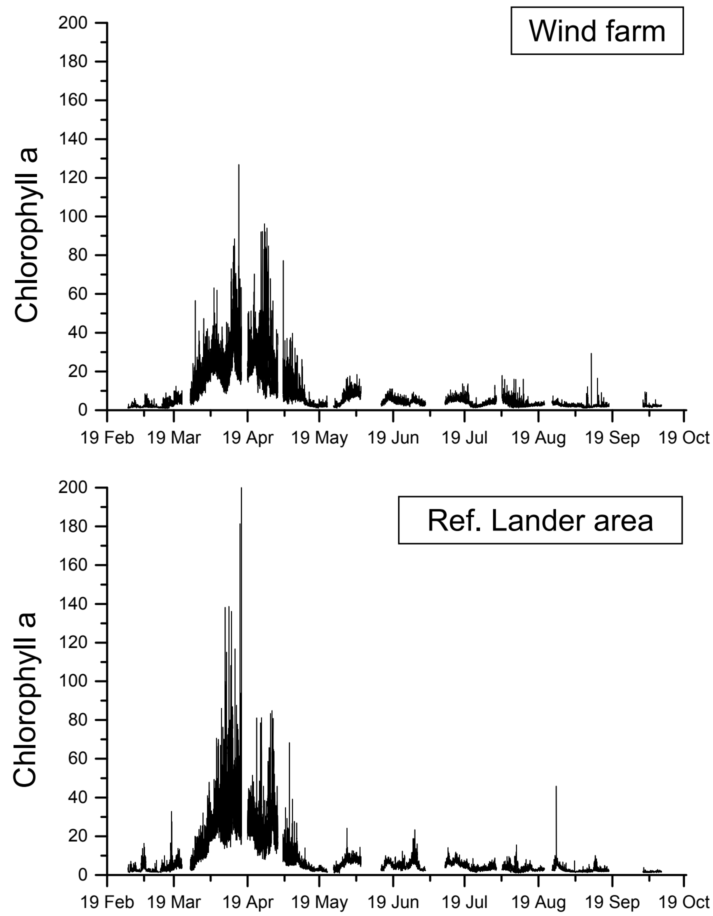


Fig. 28. Annual variations in fluorescence (in Uranine units ppb) at the Wind farm (upper graph) and Ref. L (lower graph) from February until October 2007.

Deploy #	Wind farm			Ref L		
	average	st dev	integr. sum	average	st dev	integr. sum
Deploy 1	2.60	1.57	58.82	3.35	2.16	75.85
Deploy 2	22.55	12.02	484.05	26.24	17.59	563.2
Deploy 3	26.65	13.61	337.83	25.19	10.98	319.33
Deploy 4	6.31	5.37	117.25	5.86	4.43	108.89
Deploy 5	6.44	3.18	73.92	6.26	2.55	71.86
Deploy 6	4.66	1.45	86.5	5.21	2.35	69.68
Deploy 7	4.21	1.73	90.67	4.53	1.82	97.48
Deploy 8	3.31	1.70	58.66	3.06	1.40	54.28
Deploy 9	2.41	1.05	57.18	2.65	1.51	63
Deploy 10	2.14	0.74	16.62	1.60	0.24	12.39

Table 9. Summary of fluorescence data (in Uranine units ppb) at the two locations (Windfarm and Ref. L) during the 10 deployments (average, standard deviation and integrated sum of the graph)

Turbidity

Turbidity has been measured in the Wind farm and Ref L as proxy for the concentration of suspended matter in the water column. Suspended matter (SPM) is a complex mixture of living and dead particles *e.g.* phytoplankton, benthos larvae, mud particles, and settled particles which are lifted from the seabed (*i.e.* resuspension) when the critical shear stress is exceeded. Sinking (sedimentation) and resuspension of particles is governed by the near-bottom current regime which is a combination of tidal (ebb and flood) currents and currents generated by (wind) waves.

Fig. 29 shows a difference in turbidity level at the two lander stations in the period February and October 2007. Peaks in turbidity in Ref L were more frequent and higher than in OWEZ. Table 10 shows the integrated sums of the separate deployments. The surfaces under the turbidity graphs in most of the subsequent deployments were twice to four times higher in the Ref L than in the Wind farm. Only two deployments showed lower turbidity values in Ref L. Appendix IV shows the basic data of turbidity for the consecutive deployments.

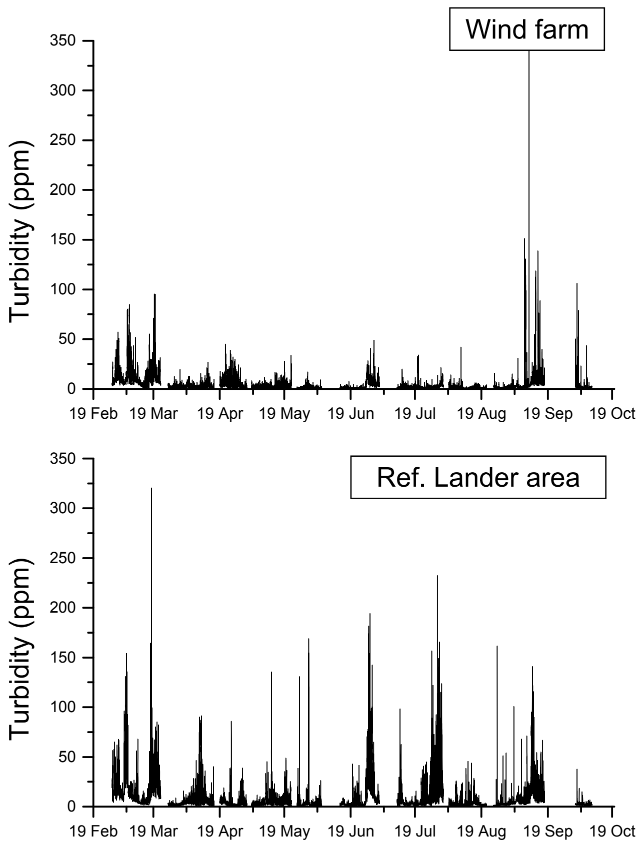


Fig. 29. Annual variations in turbidity concentrations (ppm) at OWEZ Wind farm (upper graph) and Ref L (lower graph) from February until October 2007.

deploy #	OWEZ Wind farm			Ref L		
	average	st.dev.	integr. sum	average	st.dev.	integr.sum
1	11.82	10.38	267.82	18.76	20.21	424.9
2	2.56	2.16	54.98	5.60	7.83	120.31
3	5.62	5.26	71.33	4.17	5.35	52.99
4	2.78	2.43	51.7	5.25	6.05	97.79
5	1.63	1.42	18.74	3.70	8.99	42.52
6	3.24	4.50	60.23	11.75	21.14	218.26
7	2.57	2.66	55.4	10.43	16.18	224.75
8	1.68	1.41	29.91	3.70	4.44	65.62
9	5.56	13.56	132.24	9.13	13.53	217.05
10	3.08	8.26	23.9	2.17	1.62	16.85

Table 10. Summary of turbidity data (ppm) in the two lander locations (OWEZ Wind farm and Ref L) during the 10 deployments (average, standard deviation and integrated sum of the graph).

Current speed and direction

As indicated above the current regime plays a dominant role in the resuspension of deposited materials. When the shear velocity of the current along the seabed remains below a critical value, there is no sediment resuspension. Increasing current speed enhances the shear stress and when it exceeds a critical value (critical shear velocity u_{crit}), the smallest particles are taken into suspension. Heavier or coarser material begins to roll or starts to bounce along the seabed as bedload, until shear velocity is high enough to bring them in suspension. Reduction in the current speed results initially in settling of the larger and heavier particles. Smaller particles will settle only very slowly or not at all. Data on current speed and direction form an essential part of the explanation for the differences in turbidity data from the Wind farm and the Ref L. The near bed tidal current speed can be augmented by (orbital) currents generated by surface waves (wave stirring) depending on the wave height, direction and depth. As our instruments were programmed to average over 10 min, effects of short term periodic waves are not properly represented in the data. In the Discussion (section 4.5), effects of waves on resuspension have been separately assessed based on wave height data near IJmuiden (www.waterbase.nl).

Fig. 30 shows the annual variations in current speed 1.5 m above the seabed at the two lander stations between February and October 2007. Due to a technical problem, current speed data of the first Wind farm deployment are not reliable. These data have been omitted from the dataset (appendices V and VI) and Fig. 30. The graphs clearly depict the alternation in spring and neap tides in the tidal cycle with intervals in between of approximately one week in both lander stations. The semi-diurnal tidal regime in the Dutch coastal zone encompasses two tidal cycles per 25 hours, covering two flood and 2 ebb periods (Fig. 31).Velocity during the flood (north-northeast going tide) is usually higher than during the ebb (south-southwest going tide), although strong northerly and easterly winds may change this pattern. Table 11 provides a summary of the current speed data in the two lander locations during the 10 consecutive deployments (average, standard deviation, median and integrated sum of the graph) and illustrates that the current speed did not show differences in both lander stations. Appendix V shows the occurrence (%) of current speed classes (m/s) in the basic data of the consecutive deployments.

Fig. 32 shows the annual sum patterns in the current direction per compass direction of 10° classes at the two lander stations between February and October 2007. North-north-east (023º) during the flood and south-south-west (203º) during ebb tide were the dominant current directions in both lander locations. Differences in the dominant current direction between the lander stations are not evident. Appendix VI summarizes the current patterns per compass direction of 10° for the consecutive deployments.

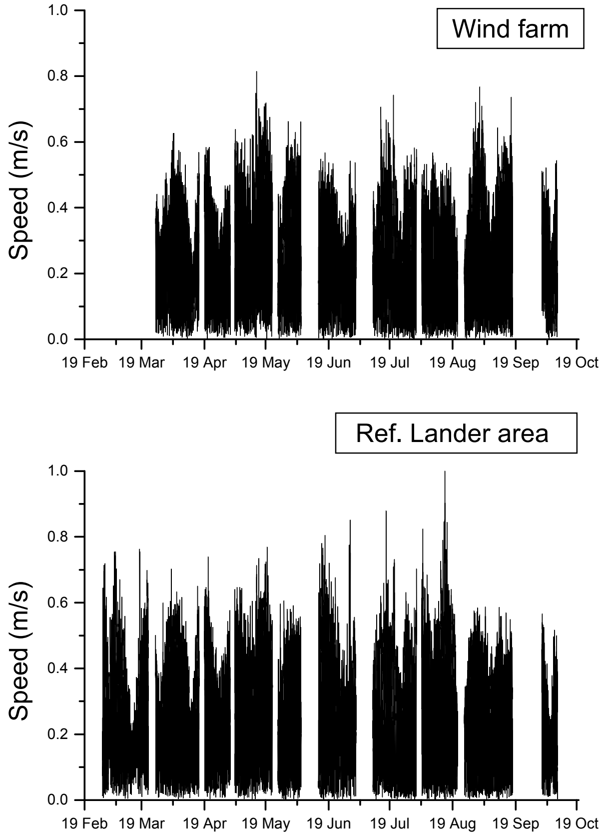


Fig. 30. Annual variations in current speed (m/s) in OWEZ Wind farm (upper graph) and Ref L (lower graph) from February until October 2007. Due to a technical problem, current speed data of the first Wind farm deployment are not reliable and not shown in this graph.

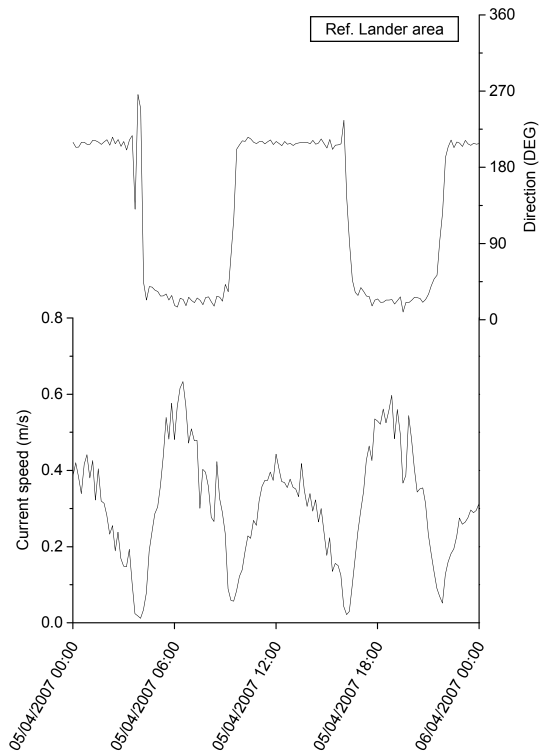
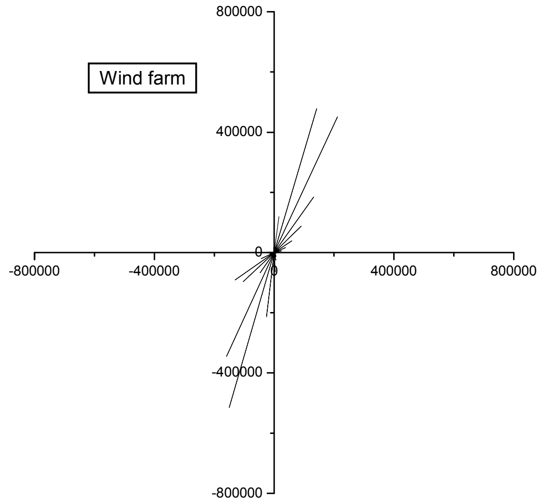


Fig. 31. Semi-diurnal tidal regime recorded in Ref L at 5 April 2007. Current speed (m/s) and direction towards the current flows (º) during the two flood and ebb periods are indicated.

Deploy nr	Wind farm				Ref L			
	Average	StDev	Median	Integr	Average	StDev	Median	Integr
Deploy 1	-	-	-	-	0.25	0.14	0.217	5.56
Deploy 2	0.25	0.12	0.240	5.25	0.25	0.13	0.245	5.43
Deploy 3	0.26	0.12	0.272	3.32	0.28	0.13	0.280	3.51
Deploy 4	0.32	0.15	0.328	5.93	0.31	0.15	0.314	5.73
Deploy 5	0.26	0.14	0.261	3.03	0.28	0.13	0.278	3.16
Deploy 6	0.24	0.11	0.240	4.51	0.27	0.15	0.254	5.02
Deploy 7	0.22	0.13	0.208	4.81	0.19	0.15	0.140	4.15
Deploy 8	0.24	0.12	0.239	4.21	0.30	0.16	0.299	5.35
Deploy 9	0.26	0.14	0.258	6.22	0.26	0.12	0.262	6.15
Deploy 10	0.25	0.11	0.252	1.91	0.22	0.11	0.214	1.68

Table 11. Summary of current speed data (m/s) in the two lander locations (Wind farm and Ref L) during the consecutive deployments (average, standard deviation, median and integrated sum of the graph).



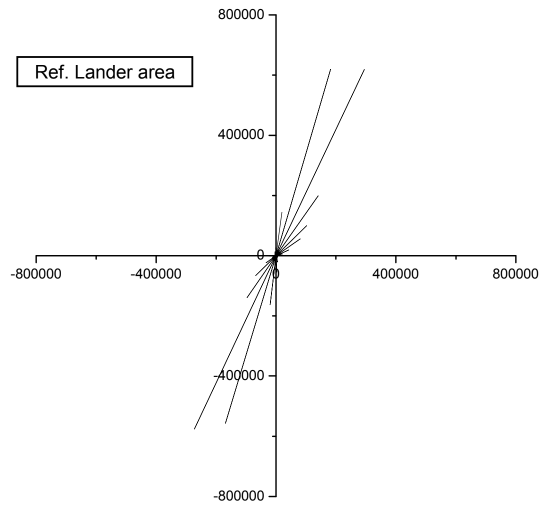


Fig. 32. Annual current patterns per compass direction (February-October 2007) for OWEZ Wind farm and Ref L. Lines present 10° classes and point in the direction the current is flowing to. Lengths of the lines reflect sum distances per direction (m) in 2007. The Wind farm graph doesn't include data of the first deployment since they are not reliable due to technical failure.

3.3. Settlement of bivalves in the mesocosms

Technical performance of mesocosms

The relevant technical data of the mesocosm experiments during the two deployments of the landers in OWEZ and Ref L are given in Table 2 (sediment data before deployment) and Table 12 (sediment data after deployment, number of 2-hours exposures realised). Checking the sediment characteristics after retrieval revealed that during Deploy 1 in Ref L the upper layer (0-1 cm) of the sediment in the mesocosm boxes had radically changed after the 3 weeks deployment (Table 12a). Mud percentages had increased from <0.3 to 9-39% and median grain sizes of the medium and coarse fraction had decreased accordingly to values below 300 µm, almost equalling the values of the original fine fraction. The reason for this mud deposition most likely is related to the wind conditions during the last week of Deploy 1, when only the lander in Ref L was operational (for details see Discussion section 4.4). This dramatic change in the upper layer of the sediment in the last week made a comparison impossible between the different sediment types in Ref L during Deploy 1. The extreme mud deposition also complicated a comparison with other deployments in Ref L or OWEZ. We therefore decided to leave out these results. In Deploy 1 in OWEZ we omitted for the same reason the results of box 3D, one of the boxes initially filled with the coarse sediment fraction.

The sediment values of most other boxes had remained well within the ranges of their original sand fractions, although mud content had slightly increased in most boxes up to 1 a 2%, and most coarse sand fractions consequently had slightly decreased in median grain size.

In Deploy 2 in Ref L (Table 12b) we identified also 3 boxes with extreme mud deposition (1C and 3E both filled initially with the finer fraction, and the box 3 B filled with medium fraction), but here we took the opportunity to compare these muddy boxes (9.8, 7.7 and 18.5% <63 µm, respectively) with the standard fine, medium and coarse fractions.

DEPLOY 1	**box**	**type of sand**	**before deploy**		**after deploy 0-1 cm deep**		**after deploy 1-10 cm deep**		**mean level below raster (cm)**	**start date**	**stop date**	**exp. (n)**
			med. grain size µm	mud %	med. grain size µm	mud %	med. grain size µm	mud %				
OWEZ	1C	fine	376	0.0	372	1.3	364	0.0	3.4	10/7/07	19/7/07	18
	2D	fine	376	0.0	375	2.7	365	1.0	2.9	10/7/07	19/7/07	18
	3E	fine	376	0.0	378	1.5	370	0.0	3.0	10/7/07	19/7/07	18
	1D	medium	676	0.3	718	1.4	695	0.1	2.0	10/7/07	19/7/07	18
	2E	medium	676	0.3	581	2.5	663	0.6	2.3	10/7/07	19/7/07	18
	3B	medium	676	0.3	599	2.0	644	0.4	1.9	10/7/07	19/7/07	18
	1B	coarse	1645	0.0	1541	0.4	1443	0.1	3.7	10/7/07	19/7/07	18
	2C	coarse	1645	0.0	1321	3.8	1515	0.1	2.6	10/7/07	19/7/07	18
	3D	coarse	1645	0.0	**540**	**9.8**	1479	0.1	2.3	10/7/07	19/7/07	18
Ref L	1C	fine	376	0.0	242	**20.9**	384	1.2	1.4	10/7/07	27/7/07	33
	2D	fine	376	0.0	215	**18.3**	358	2.6	1.9	10/7/07	27/7/07	33
	3E	fine	376	0.0	310	**26.4**	368	0.0	2.5	10/7/07	27/7/07	33
	1D	medium	676	0.3	**271**	**9.1**	659	6.8	1.4	10/7/07	27/7/07	33
	2E	medium	676	0.3	**176**	**29.1**	596	1.4	1.9	10/7/07	27/7/07	33
	3B	medium	676	0.3	541	**19.2**	653	0.4	1.4	10/7/07	27/7/07	33
	1B	coarse	1645	0.0	**285**	**18.6**	1607	0.0	1.1	10/7/07	27/7/07	33
	2C	coarse	1645	0.0	**154**	**38.6**	1533	0.4	1.6	10/7/07	27/7/07	33
	3D	coarse	1645	0.0	**898**	**26.4**	1667	0.3	2.4	10/7/07	27/7/07	33

Table 12a. Relevant data of the mesocosms in deployment 1 in OWEZ and Ref L: Average sediment data prior to deployment, sediment data after deployment in upper 0-1 cm and in deeper 1-10 cm, layer of sediment washed out by the current, dates of start and stop of experiment, number of 2-hours exposures of the trays. **Bold** values indicate large changes in sediment values in the upper layer compared to the original sand fractions prior to deployment.

DEPLOY 2	box	type of sand	before deploy		after deploy 0-1 cm deep		after deploy 1-10 cm deep		mean level below raster (cm)	start date	stop date	exp. (n)
			med. grain size µm	mud %	med. grain size µm	mud %	med. grain size µm	mud %				
OWEZ	1C	fine	376	0.0	388	1.1	360	0.8	3.3	03/8/07	20/8/07	33
	2D	fine	376	0.0	420	0.7	362	0.8	3.3	03/8/07	20/8/07	33
	3E	fine	376	0.0	484	0.6	370	0.8	2.0	03/8/07	20/8/07	33
	1D	medium	676	0.3	581	0.7	578	0.4	2.1	03/8/07	20/8/07	33
	2E	medium	676	0.3	664	0.6	637	0.4	0.6	03/8/07	20/8/07	33
	3B	medium	676	0.3	675	0.7	645	0.4	1.5	03/8/07	20/8/07	33
	1B	coarse	1645	0.0	1460	0.1	1574	0.1	3.0	03/8/07	20/8/07	33
	2C	coarse	1645	0.0	953	0.7	1092	0.1	3.9	03/8/07	20/8/07	33
	3D	coarse	1645	0.0	1094	0.5	1505	0.1	4.5	03/8/07	20/8/07	33
Ref L	1C	fine	376	0.0	328	**9.8**	382	0.7	2.9	03/8/07	20/8/07	33
	2D	fine	376	0.0	390	1.1	385	0.7	2.3	03/8/07	20/8/07	33
	3E	fine	376	0.0	336	**7.7**	375	0.8	2.9	03/8/07	20/8/07	33
	1D	medium	676	0.3	571	1.1	662	0.4	-	03/8/07	20/8/07	33
	2E	medium	676	0.3	650	1.0	707	0.4	2.5	03/8/07	20/8/07	33
	3B	medium	676	0.3	587	**18.5**	603	0.5	1.5	03/8/07	20/8/07	33
	1B	coarse	1645	0.0	1342	1.2	1508	0.2	1.6	03/8/07	20/8/07	33
	2C	coarse	1645	0.0	1063	1.1	1561	0.1	1.9	03/8/07	20/8/07	33
	3D	coarse	1645	0.0	1386	1.8	1490	0.1	2.6	03/8/07	20/8/07	33

Table 12b. Relevant data of the mesocosms in deployment 2 in OWEZ and Ref L: Average sediment data prior to deployment, sediment data after deployment in upper 0-1 cm and in deeper 1-10 cm, level of sediment washed out by the current, dates of start and stop experiment, number of 2-hours exposures of the trays. **Bold** values indicate large changes in sediment values in the upper layer compared to the original sand fractions prior to deployment.

Numbers of settlers

Juvenile bivalves were sorted from cores that were accepted with respect to their sediment values after deployment, and additionally from the 3 extreme muddy boxes in Ref L during Deploy 2. A total number of 481 (including the muddy fraction 556) juvenile bivalves was collected. A total of 222 specimens were found in the cores from the mesocosms that had been deployed in OWEZ during Deploy 1, while in total 96 and 163 (including the muddy fraction 238) individuals were detected in the cores from the mesocosms in OWEZ and Ref L, respectively, during Deploy 2. Average number of bivalves per core (4.9 cm^2) and standard deviation derived from the same original sediment fractions in the mesocosms trays, excluding the muddy trays, are given for each deployment (Table 13).

Deploy 1 OWEZ			Deploy 2 OWEZ			Deploy 2 Ref L		
box	**fraction**	**bivalves**	**box**	**fraction**	**bivalves**	**box**	**fraction**	**bivalves**
1Ca	fine	3	1Ca	fine	**0**	1Ca	fine	0 (muddy)
1Cb	fine	7	1Cb	fine	2	1Cb	fine	5 (muddy)
1Cc	fine	8	1Cc	fine	2	1Cc	fine	8 (muddy)
1Cd	fine	3	1Cd	fine	0	1Cd	fine	1 (muddy)
2Da	fine	4	2Da	fine	3	2Da	fine	0
2Db	fine	9	2Db	fine	1	2Db	fine	6
2Dc	fine	0	2Dg	fine	7	2Dc	fine	5
2Dd	fine	23	2Dc	fine	2	2Dd	fine	1
2De	fine	3	3Ea	fine	0	3Ea	fine	1 (muddy)
3Ea	fine	1	3Eb	fine	2	3Eb	fine	3 (muddy)
3Eb	fine	2	3Ec	fine	9	3Ec	fine	2 (muddy)
3Ec	fine	22	3Ed	fine	0	3Ed	fine	1 (muddy)
3Ed	fine	16						
mean; s.d.		**7.8; 7.8**			**2.3; 2.9**			**3.0; 2.9**
1Da	medium	7	1Da	medium	1	1Da	medium	4
1Db	medium	6	1Db	medium	2	1Db	medium	6
1Dc	medium	5	1Dc	medium	1	1Dc	medium	2
1Dd	medium	2	1Dd	medium	3	1Dd	medium	0
2Ea	medium	11	2Ea	medium	1	2Ea	medium	4
2Eb	medium	7	2Eb	medium	4	2Eb	medium	0
2Ec	medium	5	2Ec	medium	7	2Ec	medium	1
3Ba	medium	0	2Ed	medium	2	2Ed	medium	1
3Bb	medium	9	3Ba	medium	0	3Ba	medium	2 (muddy)
3Bc	medium	3	3Bb	medium	22	3Bb	medium	46 (muddy)
3Bd	medium	6	3Bc	medium	2	3Bc	medium	5 (muddy)
			3Bd	medium	0	3Bd	medium	1 (muddy)
mean; s.d.		**5.6; 3.1**			**3.8; 6.1**			**2.3; 2.2**
1Ba	coarse	7	1Ba	coarse	4	1Ba	coarse	34
1Bb	coarse	18	1Bb	coarse	1	1Bb	coarse	31
1Bc	coarse	11	1Bc	coarse	2	1Bc	coarse	11
1Bd	coarse	2	1Bd	coarse	1	1Bd	coarse	3
2Ca	coarse	4	2Ca	coarse	1	2Ca	coarse	0
2Cb	coarse	8	2Cb	coarse	4	2Cb	coarse	4
2Cc	coarse	9	2Cc	coarse	2	2Cc	coarse	7
2Cd	coarse	1	2Cd	coarse	1	2Cd	coarse	1
3Da	coarse	-	3Da	coarse	0	3Da	coarse	18
3Db	coarse	-	3Db	coarse	3	3Db	coarse	5
3Dc	coarse	-	3Dc	coarse	0	3Dc	coarse	13
3Dd	coarse	-	3Dd	coarse	4	3Dd	coarse	6
mean; s.d		**7.5; 5.5**			**1.9; 1.5**			**11.1; 11.3**

Table 13. Bivalve numbers found per core (4.9 cm^2) taken from the mesocosm boxes filled with different original sand fractions in Deploy 1 (OWEZ) and Deploy 2 (OWEZ and REF L). Average numbers and st.dev. per sand fraction are indicated for the boxes per deployment. Boxes with extreme muddy contents after retrieval are indicated as "muddy"and numbers were not included in the average numbers.

The main purpose of the mesocosm experiments was to test settlement preferences of bivalves with respect to different sand grain sizes irrespective of the location**.** Because of the dependency of the 4 cores per box and of the boxes within a tray a mixed model would be the best for statistical analyses. The limited numbers of observations, however, hamper the proper use of this test. In stead we present the results as box and whisker plots to illustrate the results. The differences between the numbers of bivalve settlers in the three types of sediment during each lander deployment in OWEZ are illustrated in Figs. 32 and 33. Fig. 34 illustrates the numbers found in the 4 different sediment fractions in Ref L during Deploy 2. In all deployments the differences in bivalve numbers between the sediment fractions were not significant.

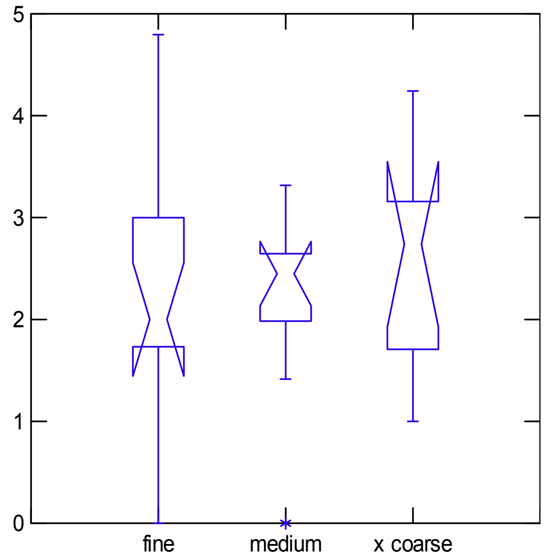


Fig 32. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves found in the three sediment types in Deploy 1 in OWEZ showing the 95% confidence intervals. The overlap between the confidence intervals of all sediment types points to absence of statistical significant differences in bivalve abundances. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange).

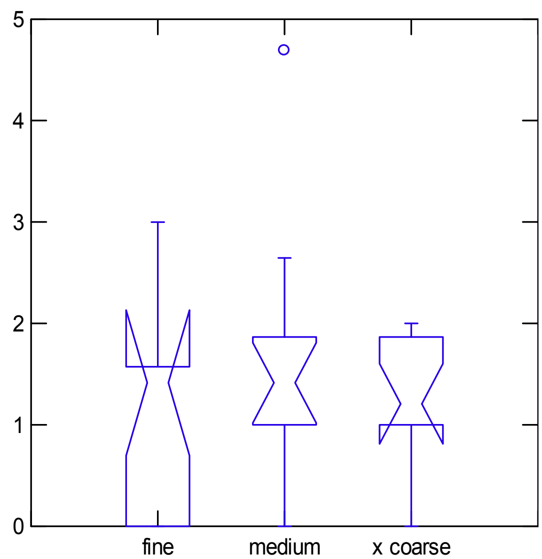


Fig 33. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves found in the three sediment types in Deploy 2 in OWEZ showing the 95% confidence intervals. The overlap between the confidence intervals of all sediment types points to absence of statistical significant differences in bivalve abundances O means values beyond 3 times Hspread (InterQuartileRange) from hinges

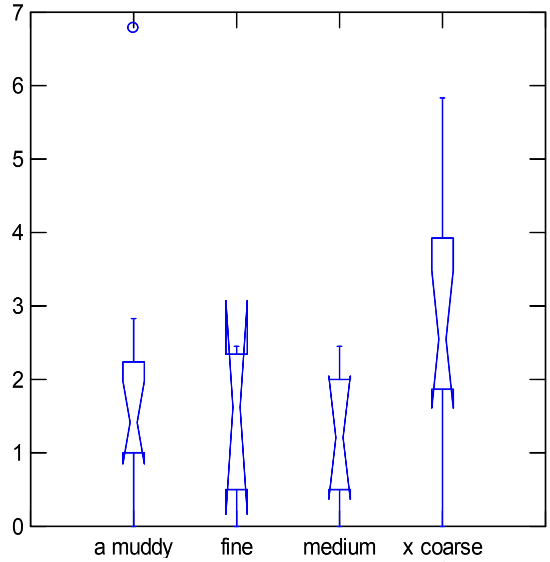


Fig 34. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves found in the three standard and the fourth muddy sediment types in Deploy 2 in Ref L showing the 95% confidence intervals. The overlap between the confidence intervals of all sediment types points to absence of statistical significant differences in bivalve abundances. O means values beyond 3 times Hspread (InterQuartileRange) from hinges

A comparison of the numbers of settlers in the 2 subsequent deployments in OWEZ revealed a remarkable temporal effect (Fig. 35). In fine sediment on average 7.7 settlers were found per core (4.9 cm^2) in Deploy 1 versus 2.3 settlers in Deploy 2, in medium sediment 5.5 versus 3.8 and in coarse sediment 6.3 versus 1.9. In medium and coarse sediments these temporal differences were significant. The higher numbers of settlers in Deploy 1 are remarkable since the number of 2-hours exposures was here only 18 while the number was 33 in Deploy 2.

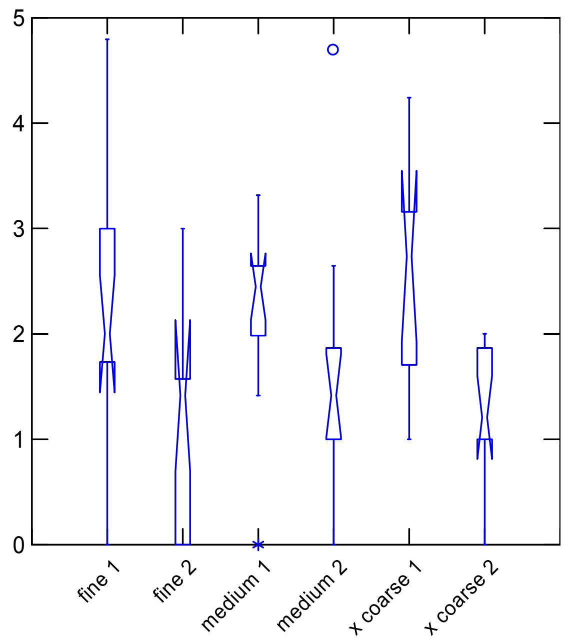


Fig. 35. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves found in the 3 sediment types in OWEZ in Deploy 1 and Deploy 2 showing the 95% confidence intervals. The non-overlap between the confidence intervals derived from Deploys 1 and 2 in medium and coarse sediment types points to statistical significant differences in bivalve abundances. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

As stated above, the experiment was not meant to test differences in settlement between OWEZ and Ref L. In fact, site specific differences appeared to be limited based on a comparison of the numbers of settlers per sediment type in OWEZ and Ref L during the second deployment (Fig. 36). The average numbers settled in Ref L and OWEZ in fine (2.8 and 2.3, respectively) and in medium sediment (6.0 and 3.8, respectively) were not significantly different. Numbers in the coarse fraction were significantly higher in Ref L than in OWEZ Wind farm (with average numbers of 11.1 and 1.9 per core, respectively).

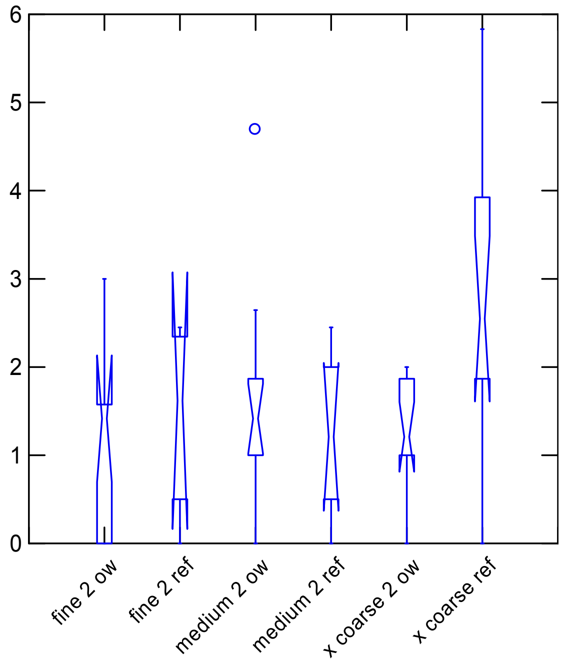


Fig. 36. Notched box and whisker plots of the total numbers (square rooted transformed) of bivalves found in the 3 sediment types in OWEZ and Ref L in Deploy 2 showing the 95% confidence intervals. The non-overlap between the confidence intervals derived from coarse sediment boxes points to statistical significant differences in bivalve abundances. O means values beyond 3 times Hspread (InterQuartileRange) from hinges

Length of settlers

The length of the bivalves found in the mesocosm cores was measured and ranged from 0.06 to 1.11 mm. Average length and standard deviation per type of sediment per lander deployment are given in Table 14. Differences in average length may be caused by differences in species of settlers, and/or differences in length and growth rates of subsequent groups of settlers. Notched box and whisker plots illustrate the in median lengths of settlers in the different types of sediment in each lander deployment. In all deployments in OWEZ and Ref L no significant differences in lengths were found (Figs. 37, 38, 39).

	DEPLOY 1 OWEZ			**DEPLOY 2 OWEZ**			**DEPLOY 2 Ref L**		
fraction	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n
muddy							0.166	0.205	71
fine	0.117	0.103	100	0.133	0.151	28	0.203	0.184	10
medium	0.107	0.031	61	0.129	0.073	42	0.147	0.094	40
coarse	0.123	0.070	60	0.198	0.164	22	0.137	0.124	129

Table 14. Length of bivalves (mm) found in the cores (average, s.d., number of ind.) from the three standard types and the fourth muddy type of sediment per deployment.

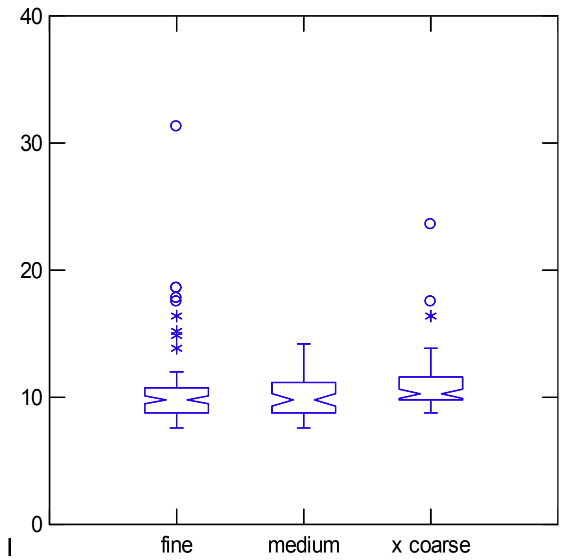


Fig. 37. Notched box and whisker plots of the lengths (square rooted transformed) of bivalves found in the three sediment types in Deploy 1 in OWEZ showing the 95% confidence intervals. The overlap between the confidence intervals of sediment types points to an absence of statistical significant differences in bivalve length. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

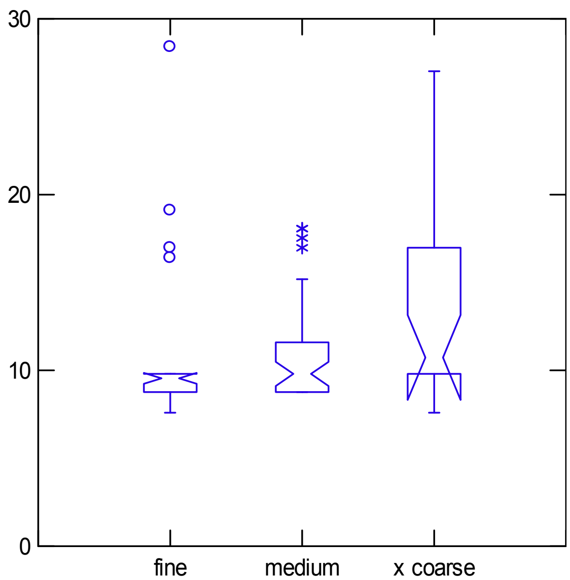


Fig. 38. Notched box and whisker plots of the lengths (square rooted transformed) of bivalves found in the three sediment types in Deploy 2 in OWEZ showing the 95% confidence intervals. The overlap between the confidence intervals of sediment types points to an absence of statistical significant differences in bivalve length. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread.

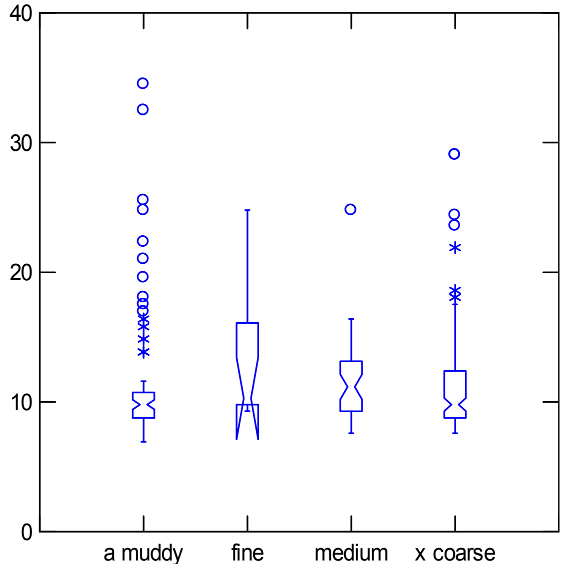


Fig. 39. Notched box and whisker plots of the lengths (square rooted transformed) of bivalves found in the three sediment standad types and the fourth muddy type in Deploy 2 in Ref L showing the 95% confidence intervals. The overlap between the confidence intervals of sediment types points to an absence of statistical significant differences in bivalve length. * means values between lower or upper hinge (Q1 or Q3) and 1.5 a 3 times Hspread (InterQuartileRange), O means values beyond 3 times Hspread

4. DISCUSSION

4.1. Abundance and species composition of juvenile bivalves in Wind farm and reference areas

Statistically significant differences in the total abundance of juvenile bivalves could not be proven between OWEZ and any of the separate reference areas (pair-wise comparisons). This holds for all individuals >0.2 mm (Fig. 16) as well as for the larger size class >0.5 mm (Fig. 17). In the pair-wise comparisons between reference areas a significant lower density of individuals of the latter size class was found in Ref 5 compared to Ref 3.

Comparison of the density distribution of the two size classes of bivalves over the six survey areas (Figs. 16 and 17) suggests a relative decline in numbers of larger sized (> 0.5 mm) recruits in the three southern Reference areas. Assuming that the larger size class consisted of older settlers, this could imply that environmental conditions in the three southern reference areas have been less suitable for the survival of settlers. On the other hand, it can not be excluded that the trend was caused by patchy settlement of recruits with different length.

In most larger-sized juveniles belonging to the 6 single species (*Abra alba, Donax vittatus, Mysella bidentata*, *Mytilus edulis, Montacuta ferrigunosa, Chamelea gallina*) and the 3 genera (*Ensis* spp*., Tellina* spp., *Spisula* spp.) significant differences in abundances between OWEZ and any of the separate reference areas (pair-wise comparisons) could also not be proven. Only *Ensis* spp. had significant lower densities in Ref 4 and Ref 5 (0 and 13 ind./m^2, respectively) than in Ref 3 (154 ind./m^2; Fig. 20). *Ensis* spp. were marked as the species contributing most to the similarity within the various areas (70 to 78%), although their role was nihil in Ref 5 where the contribution of *Mysella* and *Tellina* spp. was prominent (SIMPER-analyse). *Ensis* spp was also the dominant contributor (25-41%) to the average Bray Curtis dissimilarity between groups ranging from 84-98.

Focusing on the larger sized (>0.5 mm), identifiable recruits, numbers of species and individuals seemed to be higher in OWEZ, Ref 2, and Ref 3 (Fig. 19). However, differences in species composition among the various areas were not significant as indicated by an ANOSIM-test albeit that the largest difference in species composition was found between Ref 3 and Ref 4.

The question arises whether the 5 reference areas in this study (see section 2.2) are representative for the OWEZ Wind farm especially because the sampling has been done only once. Although the sediment composition in the survey areas did not differ in median grain size, the mud content (% of particles < 63 μm) showed a trend with higher values towards the coast (Fig. 13). This was supported by the higher mud content in Ref L (mean value 2.6%) than in, for instance, Ref 4 (0.2%). Within OWEZ wind farm this gradient in mud content was also present albeit less pronounced: higher values in the 10 eastern stations than in the western ones (2.0% versus 0.3%). Apart from this spatial gradient in mud content of the sediment, a gradient in water depth existed extending from the deepest area in the west (Ref 2; 21.7 m) to the shallowest area in the east (REF L; 16.4 m) with OWEZ (19.3 m) and Ref 4 (19.6 m) in between. So distinct gradients in mud content and water depth were present encompassed the survey areas in the coastal zone.

Although most variables in the water column measured by the instruments at the landers (temperature, fluorescence, salinity, current regime) did not show area-specific differences in seasonal or temporal variation in OWEZ and the more coast-nearby Ref L, the level of the turbidity in Ref L exceeded the values in OWEZ by a factor 2-4 (Fig. 29). In Ref L relatively high turbidity peaks in the water column were observed, caused by (re)suspended matter like detritus, silt and mud. So in addition to gradients in mud and water depth, gradients in SPM (suspended matter) existed in the zone enclosing the survey areas.

With OWEZ Wind farm holding an intermediate position within the gradients in deposited mud and water depth, and being less turbid than Ref L, statistically significant differences in abiotic variables between OWEZ and the surrounding reference areas during one single survey can only be proven when the values in OWEZ are deviant relative to the strong spatial gradients. This will likely also hold for faunal variables like abundances and species composition, given the number of studies showing a correlation between biotic and abiotic variables in the North Sea (Basford et al., 1990; Duineveld et al., 1990; Kunitzer et al., 1992).

At the start of our study we decided for the same reference areas as selected for the NZW-study of larger-sized (>1 mm) benthos densities (Daan et al., 2009), although we had to adapt the design of the reference areas slightly for logistical reasons (see section 2.2). For future impact studies in zones with strong abiotic gradients, it might be considered to select reference areas as close as possible to the central area of interest, especially if biotic variables are expected to be affected by environmental gradients. In such a design ecological effects of e.g. the construction of a wind farm or the implementation of a fishery-closed area can more clearly been proven in a single survey.

4.2. Recruit density of *Spisula subtruncata* in OWEZ (2007) compared to the estimated number to maintain the present-day population

To use an alternative test for the impact of OWEZ on bivalve settlement we compared the actual numbers of juvenile *S. subtruncata* found in the survey in OWEZ Wind farm in October in 2007 with the estimated number of recruits that are required to maintain the present-day (2007) density. If the wind farm promoted their settlement and early survival we expected to find abundances of settlers in OWEZ in an order of magnitude higher than the required number. For this estimate we calculate parameters for mortality and growth based on the population dynamics of *S. subtruncata* in pre-OWEZ conditions. We made use of NIOZ owned data on mortality and growth collected with the boxcore in coastal zone during the period 1991-1994. We used these data instead of the T0-boxcore data collected in 2003 (Jarvis et al., 2004), as latter do not provide population data over consecutive years and, hence, do not allow calculation of mortality rates. To estimate the number of recruits required to maintain the present-day coastal population we used the following parameters:

- instantaneous mortality
- growth rate
- density 2007
- age distribution 2007

Instantaneous mortality (Z) in this case is defined as (Brey, 2001):

$$dN/dt = -Z * N_t$$

or

$$N_t = N_o * e^{-Z * t}$$

Which is equivalent to:

$$\ln(N_t) = -Z * t + c \qquad [1]$$

This model assumes that mortality in each age (year)-class is a constant proportion of the stock. Ideally mortality estimates should be based on a population with easily distinguishable year classes with sufficient numbers of individuals. This is usually not the case as growth in older individuals slows down and older year-classes tend to overlap. In populations with overlapping year-classes but where growth is known (*e.g.* from shells bands, otoliths), mortality can be estimated using size-converted catch curves (Pauly, 1990).

For the *S. subtruncata* stock near Egmond, we have used an estimate of instantaneous mortality based on annual spring surveys made in the period April 1991- April 1994 at a nearby station (52^o 45.0'N, 04^o 30.0'E) circa 13 km northeast of OWEZ Wind farm and 3 km northeast of the Ref. 2. The *Spisula* stock at this station consisted basically of one year-class (cohort) which grew successive years and disappeared in 1995 (Fig. 40). In 1993 and 1994 small numbers of younger age classes were found – marked with asterix in Fig. 40 - but these were excluded from the calculation. Plotting ln(N_t) against time with 1 yr intervals (see formula 1) yields an estimate for Z of 0.51 (Fig. 41).

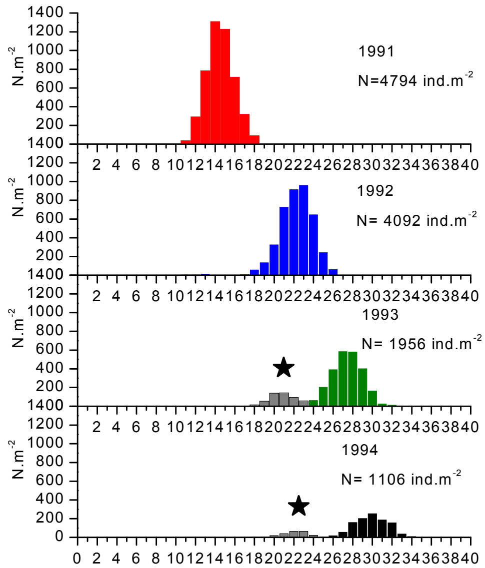


Fig. 40. Growth of *S. subtruncata* population 13 km north east of OWEZ Wind farm in 1991-1994. Younger age classes are marked with asterix.

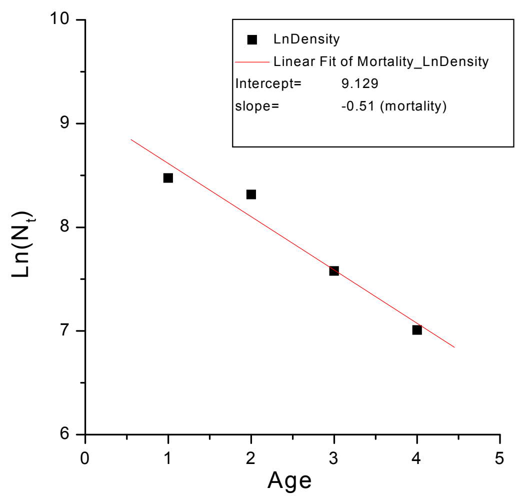


Fig. 41. Plot of $\ln(N_t)$ against time with 1 yr intervals.

Growth of the 1991-1994 cohort was modelled with a Von Bertalanffy Growth equation (VBG) being the most commonly observed type of growth among benthic invertebrates:

$$L_t=L_\infty*[1-e^{-k*(t-to)}]$$

Parameter values of the VBG were iteratively solved: k=0.51, L_∞=34.4 and t0=-0.05. The VBG growth curve based on these parameters (Fig. 42) is very similar to the one recently published by Cardoso et al. (2007) for a *S. subtruncata* population off Petten (circa 45 km north of OWEZ Wind farm) in 2001-2003. Because Cardoso et al. checked their growth curve against numbers of internal growth rings in the shell and both curves are similar, we are confident that length-at-age estimates in Fig. 42 are correct. A comparable growth rate

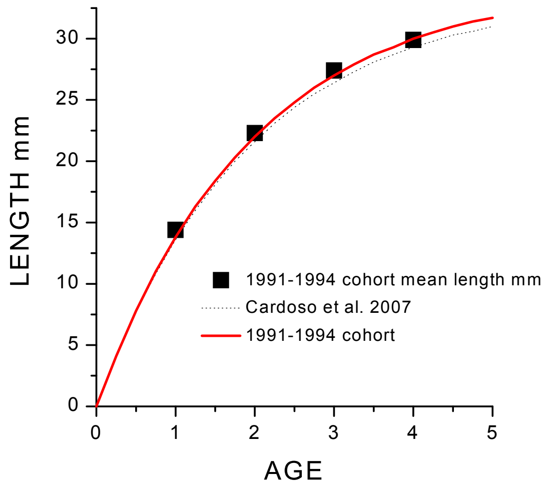


Fig. 42. Growth curves derived from Von Bertalanffy Growth equation (VBG) based on NIOZ-owned *S. subtruncata* data 1991-1994 and based on the population off Petten (Cardoso et al, 2007). Mean length of the cohorts in 1991 – 1994 are indicated.

of *S. subtruncata* as shown in Fig. 42 has been found by Craeymeersch and Perdon (2004) during spring surveys of the coastal zone with the majority of 1-yr old animals having a length of ~15 mm, 2-yr old animals of ~24 mm and 3 yr old animals of ~27 mm.

In 2006 the stock of *S. subtruncata* in the Dutch coastal zone was at its lowest since 1995 when monitoring of the stock begun (Perdon and Goudswaard, 2006). The average number of 1 yr old *S. subtruncata* in 2006 was 0.1 ind. per m^2. The authors further show a steady decline of 1 yr and older *S. subtruncata* along the coast from 2001 onwards and link this due to failing recruitment. In an earlier report, Craeymeersch and Perdon (2003) point at climate change and particularly the wind regime as probable cause for failing recruitment.

In the Benthos-density subproject of the NZW-project (Daan et al., 2009), the density of adult *S. subtruncata* was assessed in 2007 with two different methods: boxcores and Triple-D dredge. Earlier Bergman and van Santbrink (1994) demonstrated that the efficiencies of the Triple-D dredge and boxcorer with respect to bivalves larger than 1 cm were similar. The Triple-D dredge catches in OWEZ Wind farm and the reference areas (n=26; total sample surface 416 m^2) yielded a total number of 42 specimens of *S. subtruncata*. Average number within the Wind farm was 1.4 per Triple-D haul and in the reference areas 1.9 per haul. More than 85% of this number (n=38) were 2-yr old animals and the remaining ones younger. Back calculating the 2-yr old specimens to recruits taking an annual mortality of 0.51 (N_2=N_0*e^{2*Z}) gives a predicted number of 105 in 416 m^2 , *i.e* the sample surface of 26 Triple-D haules, thus ~0.25 individual per m^2.

This expected density of ~0.25 individual per m^2 was compared with the actual numbers of juvenile *S. subtruncata* found in OWEZ Wind farm and the reference areas in the October-2007 survey. Among the juvenile bivalves > 0.5 mm two individuals of *Spisula* spp. (most probably *S. subtruncata*) were found in OWEZ and one individual in Ref 3 and 2 individuals in Ref 4 (see Table 3). If we correct these densities for the species identification efficiency (Table 5) per area assuming a similar species composition for the unidentified percentages of bivalves > 0.5 mm, mean densities become 5.1.m^{-2} in OWEZ, 5.6.m^{-2} in Ref 3 and 15.7.m^{-2} in Ref 4. Since no *S. subtruncata* were found in the samples from other reference areas (Ref 2, Ref 5, Ref L), the average density becomes 4.3.m^{-2} in the reference areas. Comparing these actual densities of *S. subtruncata* recruits with the calculated juvenile densities needed to maintain the present-day adult population, *i.e.* ~0.25 individual per m^2, it can be concluded that numbers of recruits that we found in OWEZ in 2007 were circa 20 times higher than expected. The fact that densities were also ~20 times higher in the reference areas suggest that the present low density of the *S. subtruncata* population in OWEZ Wind farm and the trawled reference areas were due to other factors than fishing mortality in juvenile stages. These 2007-densities, however, are still too low to give rise to the dense populations (1100 to 4800 individuals per m^2; see Fig. 40) present in the 1980's and

1990's in the Dutch coastal area. Apparently recruitment is insufficient, even in the during 2 two consecutive years fishery-free Wind farm, to sustain the abundant populations before 2000. These results indicate that the similar low density of the *S. subtruncata* population in the reference areas most probably is not due to fishing mortality in juvenile stages but most likely due to low initial settlement. The reduced numbers of adult *S.subtruncata* stock in the southern North Sea might have contributed to low numbers of competent larvae. It can not be excluded that a future increasing adult stock would lead to enhanced numbers of settlers, possibly stimulated by the existence of a fishery-free area.

4.3. Correlation between species distribution of bivalves and abiotic variables

A marked difference was found between the environmental variables that best explained the spatial patterns in the numbers of juvenile bivalves in data from OWEZ on one hand and those from the trawled reference areas on the other hand (BEST–analyses; Table 8a). While in OWEZ significant and relatively strong correlations between patterns in environmental variables and bivalves abundances (both small-sized and larger-sized individuals) existed, none were found in the data from the pooled reference areas. In the significant correlations found in the data from OWEZ, the factor mud scored the highest correlation coefficient, whereas in combination with median grain size in the case of abundance of larger sized ones. BEST-analysis of patterns of environmental variables and single species showed that in the fishery-closed OWEZ Wind farm spatial patterns of *Ensis* spp*.*, *Donax vittatus* and *Mysella bidentata* were significantly correlated with mud as the best explanatory variable (Table 8b). In contrast, data on single species derived from the trawled reference areas did not yield any strong correlation.

The correlation between distribution patterns of bivalve settlers and mud in OWEZ can be explained by assuming that settlers respond similarly to the same hydrodynamics processes as mud particles. Planktonic bivalve larvae have swimming capabilities and can, for instance, actively position themselves in the water column (Knights and Crowe, 2006). However, they become passive particles in high flow and turbulent conditions (Hannan, 1984; Jonnson et al., 1991; Harvey et al., 1995). Similarly as mud particles, settled larvae can be resuspended from the sediment by high shear (St-Onge and Miron, 2007). Passive settlement of larvae in conjunction with fine sediment can be beneficial for the growth of the larvae since such conditions also favor deposition of nutritious light organic fluff (algae, detritus).

The correlation between distribution patterns of bivalve settlers and mud was not found in the reference areas. An explanation for this contrast might be that here the initial coupling between mud and bivalve recruits has been lost possibly as a result of disturbance such as ongoing trawling. Trawling with bottom gears disturbs the upper centimeters of the seabed and causes resuspension of the finest sediment and associated food particles (de Madron et al., 2005; Dellapenna et al., 2006). Bivalve recruits may be resuspended and redistributed or may face an abrupt change in sediment and associated food conditions. A similar disruption can be caused by strong wind and high waves. Our study, however, does not support the conclusion that the assumed disturbance of the initial sediment-recruit relationship had consequences for a lower survival and growth of recruits in the reference areas. At the end of the settling period the correlation between the abundances of small-sized settlers (0.2-0.5 mm) and larger-sized ones (>0.5 mm) was still significant (Fig. 18). Differences in abundance between OWEZ and the trawled reference areas could be proven neither for recruits >0.2 mm nor for > 0.5 mm. Nor did we find larger recruits in the non-trawled OWEZ Wind farm (Table 4). In stead, lengths of *Tellina* spp. were significant lower in OWEZ (and Ref L) than in Ref 2 and Ref 3 suggesting a higher growth rate or an earlier settlement in the northern reference areas. All other species showed no differences in length among the 6 survey areas.

The linkage between species composition and mud is endorsed by a Cluster-analysis. The two clusters distinguished by similarity in species composition of larger sized (>0.5 mm) recruits, comprising 16 and 54 stations (Fig. 21), were associated with significant differences in mud content (2.6% and 0.7%, respectively). Water depth was also different between the two clusters (21.0 m and 19.0 m, respectively; Fig. 23). Apparently, although the species composition of the bivalves is strongly mud-related, *cf* BEST-analysis, water depth is another important factor in the zonation of bivalve recruits. From an SIMPER analysis it is clear that the mud-associated cluster harboured a much higher bivalve population than the other cluster of stations. The similarity within the first cluster was mainly due to *Ensis* spp., *Montecuta ferrigunosa* and *Tellina* spp., whereas the similarity in the second cluster was entirely attributed to *Ensis* spp. The spatial distribution of the clusters (Fig. 22) suggests an offshore-coast, possibly combined with a slight south-north, trend rather than a survey area-based pattern. The latter was confirmed by an ANOSIM-test showing there were no differences in species composition between the 6 survey areas.

4.4. Abundances and lengths of settled bivalves in the different sand fractions in the mesocosms

The sediment characteristics of 26 out of 27 mesocosm boxes had remained well within the ranges of their original sand fractions during Deploy1 (OWEZ) and Deploy 2 (OWEZ and Ref L) (Table 12). In these 26 boxes mud content had only slightly increased probably by deposition of suspended matter. Consequently, most coarse sand fractions had slightly decreased in median grain size by deposition of finer grains and mud. Apparently moderate wind conditions didn't interfere with the *in situ* mesocosm experiments.

In contrast to this, the sediment characteristics in the upper layer (0-1 cm) of all mesocosm boxes in Ref L had changed radically during the 3 weeks deployment in Deploy 1. Mud contents up to 39% and median grain sizes below 300 µm were measured even in the medium and coarse fractions (Table 12a).The reason for this mud deposition most likely is related to the wind conditions during the last week of Deploy 1. In this week the 2 hours-exposures went on in Ref L, but were stopped in the mesocosm system in OWEZ because of technical problems. In this week (19/7-27/7) a wind force 6 Bft (*cf*. Appendices VII-VIII) led to waves (~3 m; *cf*. section 4.5, Fig. 44) high enough to resuspend mud and sand from the seabed. This resuspension most likely caused the massive deposition of mud in the mesocosm trays in the shallower Ref L. This dramatic change in the upper layer of the sediment in the last week made a comparison impossible between the different sediment types in Ref L during Deploy 1. The extreme mud deposition also complicated a comparison with other deployments in Ref L or OWEZ. We therefore decided to leave out these results. In Deploy 1 in OWEZ we omitted for the same reason the results of box 3D, one of the boxes initially filled with the coarse sediment fraction.

The mesocosm experiment had been set up to test the impact of possible changes in sediment characteristics in the OWEZ Wind farm due to the fishery-stop on the settlement of bivalves. It could be expected that a fishery-stop would reduce resuspension and consequently the median grain size (Hily et al. 2008), while in the scours around and downstream of the turbines coarser sediment would build up. So we decided to offer selected sand fractions just below and above the median grain size in OWEZ based on the sediment values reported by Jarvis et al. (2004). They mentioned an average median grain size of 504 µm in their overall survey area covering an area 20 km south to 15 km north of OWEZ in 2003. Within the contours of the future OWEZ Wind farm they reported an average median grain size of 466 µm (st dev 128 µm) ranging from 207 to 655 µm. Their findings, however, did not fit into the sediment analyses of the 5 stations covering a larger, 18 km wide coastal area ranging from 25 km south to 25 km north of OWEZ obtained in the long-term monitoring program BIOMON in 2003 and 2006 (Daan and Mulder, 2004; unpubl. 2006-data). Average median grain size was 250.8 µm (s.d. 39.9) and 254.2 µm (s.d. 48.8), in 2003 and 2006 respectively. Since the data were contradictory to each other we hypothesised that in and around OWEZ Wind farm a patch with coarser sediment would exist as indicated by a surface sediment map (RGD, 1986) where in and around OWEZ north-reaching slopes of southerly (i.e. south of Haarlem) patches of coarser sediments (250-500 mu) were depicted. Only 10 miles north of OWEZ even coarser sediments (500-2000 mu) reached the Dutch coast. On geomorfological maps ridges with heights 1-10 m are depicted in and near the OWEZ position (RWS, 1988). We decided for three sand fractions covering both finer and coarser size classes: 200-500 µm, 500-1000 µm and > 1000 µm. We did not include muddy sediments in the experiments because accumulation of mud in the OWEZ area seemed unlikely due to frequent resuspension caused by high wind force and associated wind waves (see also section 4.5).

Surprisingly, we found much smaller median grain sizes than reported by Jarvis et al. (2004) in OWEZ and the reference areas in the October 2007-survey: on average 266 µm (s.d. 24.1) ranging from 203 to 370 μm. The reason for the inconsistency between the analyses of Jarvis and our study is unknown. But having known the proper sediment composition of our survey areas already in 2007 before the start of the experiments, we had decided on a wider range of sand fractions covering finer fractions. Accidentally, we identified 3 boxes with extreme mud deposition (box 1C and 3E both filled initially with the finer fraction, and box 3 B filled with medium fraction) in Ref L Deploy 2 and we took the opportunity to compare these muddy boxes (9.8, 7.7 and 18.5% <63 μm, respectively) with the regular fine, medium and coarse fractions

Above the sediment surface a lattice was mounted in the trays to prevent washing out of sediment by the current. Although we assumed that this lattice might create hydrodynamic conditions that are not representative for the normal situation, we expected that these micro-scale variation would affect the larval settlement irrespective the type of sediment. Two lids covering each tray were closed before deploy and opened twice per day during the 2-hours intervals around the turn of the tide from ebb to flood during deployment. In that interval local current speed is at the lowest (see Fig. 31) and passive particles as well as larvae competent to settle (Hannan, 1984) are expected to sink to the near-bottom layers. Because of this schedule the numbers of larvae entering the mesocosm trays were restricted to the period when the lids are open. Theoretically the larvae were able to actively migrate to boxes containing attractive sediment types via the lattice and the few mm water layers just below the lids during the periods the lids are closed. We further presume that the degree of resuspension of larvae was similar for the different fractions although it might in all cases be lower than in normal conditions because of the limited time the lids were opened. Although the technical limitations might have affected the microscale hydrodynamics and caused a reduced larval settlement and resuspension we presume that these factors still permit a comparison of larval settlement between the different sand fractions mounted at a lander.

The mesocosm experiments revealed that settling bivalves did not show a preference for the three standard sediment types that we offered in the two deployments in OWEZ (Figs. 32, 33) and the same fractions including a fourth muddy type in the single deployment in Ref L (Fig. 34). No differences in average length were found in the settlers between the boxes with different sediment types at the lander in each of the deployments in OWEZ (Figs 37, 38) and Ref L (Fig. 39).

A comparison between the lander sites during Deploy 2 showed that in coarse sediments the abundance of settlers in Ref L was higher (average densities of 11.1 individuals per core of 4.9 cm^2) than in OWEZ with 1.9 per core (Fig. 35). These results suggest that competent larvae occur in patches leading to spatial differences in settlement. Since the average numbers of settlers in the fine and in medium fractions were not significantly different, the result may also point at coarse sediments being more attractive to certain bivalve species in coast-nearby zones like Ref L.

Another noteworthy result was the twofold higher numbers of settlers found in Deploy 1 (10-19 July) than in Deploy 2 (3-20 August) in all sediment types in OWEZ (Fig. 36). This is more remarkable as the number of 2-hours exposures were almost twice as high in Deploy 2. It suggests that competent bivalve larvae also arrive in patches in the coastal zone leading to a temporal pattern in settlement.

From the numbers of recruits found in the cores derived from the 3 sediment types after an exposure of 9 to 17 days (see Table 13) the average density of bivalve recruits per 0.024 m^2 was calculated for the various deployments (Table 15). Assuming an evenly distributed number of settlers over the days of exposure the average net settlement of bivalves in the mesocosms in OWEZ varied from 37.6 individuals per 0.024 m^2 per day in July to 7.8 individuals per 0.024 m^2 per day in August, whereas in August 19.6 individuals per 0.024 m^2 per day successfully settled in the boxes in Ref L. Table 15 further indicates that the average density of settlers in the mesocosm experiment was significantly higher than the density of recruits found in the field survey in OWEZ and Ref L in October 2007 (see Table 3). The numbers of recruits found in this field survey were the result of settlement and loss of recruits due to mortality and resuspension (see section 4.5) over a period of several months. Even if the resuspension of larvae from the mesocosms was reduced by the 2 hours-interval of opening of the lids around the turn of the tide, the data in Table 15 suggest a considerable loss of bivalve recruits during the first months of settlement in the field situation.

	mesocosm experiment		field survey October 2007	
	n >0.05 mm		n >0.2 mm	n >0.5 mm
	deploy 1 (9 days July)	deploy 2 (17 days August)		
OWEZ	338.0	132.2	103.5	10.3
REF L	-	333.1	37.4	2.9
Ref 2			77	7.7
Ref 3			90	9.1
Ref 4			31	1.5
Ref 5			88	2.7

Table 15. Mean numbers of settled bivalves (>0.05 mm) found in the two consecutive mesocosm experiments (averaged over the 3 sediment types) in July and August 2007 in OWEZ and one deployment in August 2007 in Ref L. Mean numbers of settled larvae (> 0.2 and >0.5 mm) found in the field survey in October 2007 in OWEZ and the reference areas. All densities in n per 0.024 m^2.

4.5. Gradients in environmental variables: causes of turbidity events

The annual recordings made by the instruments mounted at the landers showed that temperature, salinity, chlorophyll-a, and current regime did not differ markedly between OWEZ Wind farm and the reference areas during the period February -October 2007 (see section 3.2). Turbidity levels, however, showed a clear difference at the two lander stations in this period (Fig. 29). The concentration of suspended matter during the turbidity peaks in the Ref L was higher than in the Wind farm location. The difference was also indicated in the integrated sums of the separate deployments, showing twice to four times higher values in Ref L than in the Wind farm (Table 10). Some peaks which did occur in the Ref L were absent in the Wind park. The difference in distance to the coast might have effects for the TSM regime both landers were subject to. Long term recordings by Suijlen and Duin (2002) showed typically in winter (December-April) during and just after heavy storms higher TSM concentrations (i.e. 10-30 mg/l around the reference lander site than around the OWEZ lander site (~10 mg/l). The turbidity levels measured with the instruments at the lander showed clear differences also during the other months of the year (February-October). In this section of the discussion we try to identify the origin of these turbidity events and to understand the difference in turbidity between the two locations

Firstly, possible differences in local resuspension between the two sites are examined by investigating the different boundary conditions. Sediment becomes resuspended due to bottom shear stresses from (tidal) currents and waves. This can be expressed according to Van Rijn (1984):

$$\tau_{b,cw} = \tau_{b,c} + \tau_{b,w} \quad (1)$$

$$\tau_{b,c} = \left(\frac{1}{8}\right) \times \rho \times f_c \times \overline{v}_R^{2} \quad (2)$$

$$\tau_{b,w} = \frac{1}{4} \times \rho \times f_w \times \left(\hat{U}_\delta\right)^2 \quad (3)$$

In which:

ρ = the density of the sea water in kg/m^3

$\overline{v}_R^{2}$ = the depth average value of current velocity vector in m/s

$f_c = 8g / C^2$ = current related friction factor (4)

$C = 18\log(12h / k_S)$ = overall Chèzy-coefficient in $m^{0.5}/s$ (5)

$f_w = \exp\left[-6 + 5.2\left(\hat{A}_\delta / k_S\right)^{-0.19}\right]$ (6)

$\hat{U}_\delta$ = peak orbital velocity near the bed in m/s

$\hat{A}_\delta$ = peak orbital excursion near the bed in m

k_S = the effective bed roughness of Nikuradse in m

The bottom shear stress induced by the current is a factor of the density of the water, the depth averaged velocity and a friction parameter (formula 2). The bottom shear stress induced by the waves is a factor of the density of the water, the peak orbital velocity near the bed and a friction parameter (formula 3). The friction parameter in both formulas cannot be calculated as there were no measurements on bed-forms. Therefore the depth averaged current velocity and the peak orbital velocity near the bed will be used as parameters to be correlated with the suspended sediment concentration. Current parameters, needed to calculate depth averaged current velocity, were measured with the Aquadopp at 1.5 meter above the bed and averaged per 10 min intervals. The depth averaged velocities are assumed to have the same fluctuations as the velocities measured at 1.5 meter above the bed, although the absolute values will differ. Wave parameters, needed to calculate peak orbital velocity near the seabed, were measured at the 'Munitiestortplaats', by Rijkswaterstaat. This measurement location was positioned a few kilometres south of the position of Ref L. As the wave field will be relative constant over a few kilometres it is assumed to be representative for both Ref L and the Wind farm.

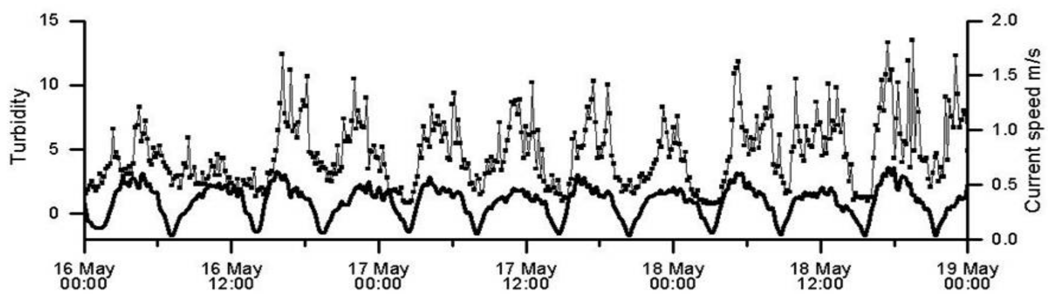


Fig. 43. Patterns of current speed (lower thick line) and turbidity (upper thin line) during a period of calm weather in Ref L (Lander data).

On a short time scale, the suspended sediment concentrations showed the same semi-diurnal fluctuations as was visible in the velocity data. This is illustrated in Fig. 43 which covers a few days with low wind speed (*cf* Appendices VII and VIII), minimal wave height (*cf.* Fig. 44) and low turbidity (*cf* Appendix IV). During maximum ebb and flood current speed, the suspended sediment concentration was also relatively high. During slack tides, the suspended sediment concentration was low because of mud settling on the seabed. Samples taken during slack tide showed a thin veneer of mud on the sediment surface (see Kleinhans et al., 2005). However, the maxima in current speed and turbidity did not occur simultaneously. The flood velocities were two hours in advance of the suspended sediment concentration. This means that there was e.g. a time lag in between acceleration of tidal velocities and the amount of suspended sediment concentration. This can be attributed to the scour lag and the settling lag. The scour lag means that particles need a certain threshold of tidal velocities before they will become resuspended (Postma, 1961). When tidal velocities drop, particles will not settle immediately, which is called the settling lag (Postma, 1961).

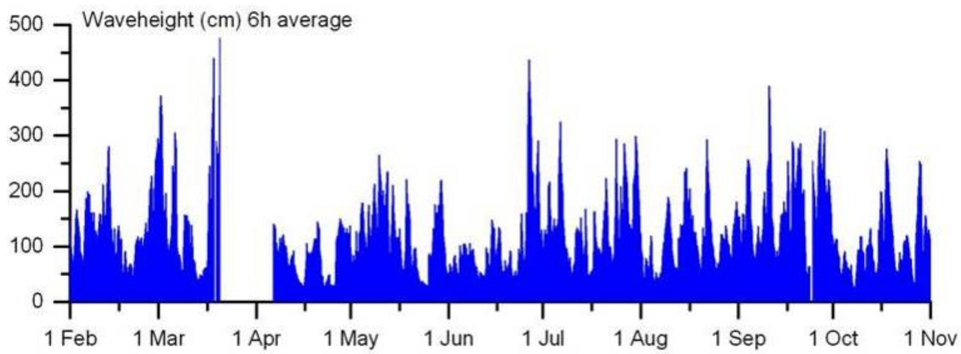


Fig. 44. Wave heights (cm) averaged over 6 hours intervals measured at Munittestortplaats in 2007

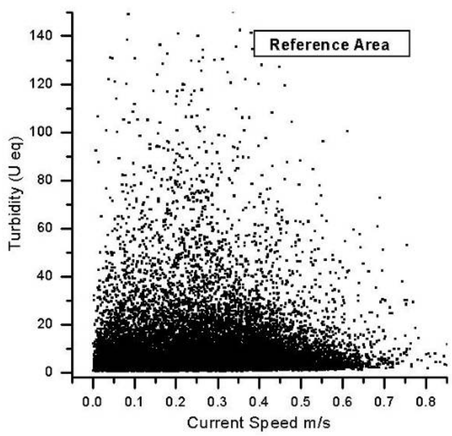


Fig. 45. Scatterplot showing relation between current speed and turbidity (Lander data) in Ref L

However, over a longer time scale there was no clear relation between (10 min average) current speed and turbidity (Fig. 45). Hence the suspended sediment concentration on time scales larger than the semi-diurnal tide is not determined by current velocities. What could have caused these peaks? The other factor determining the total bed shear stress is wave stirring. Because of the 10 min averaged intervals we adopted to enable long deployments, wave stirring has not been adequately measured by the Aquadopp. In stead, wave stirring was estimated by calculating the peak orbital velocities near the seabed (van Rijn, 1990) based on the wave parameters measured at the "Munitie stortplaats". As an example, peak orbital velocity in Ref L has been plotted for the period 9 July- 3 August (*cf*. Fig. 44) in Fig. 46b together with turbidity Fig 46c and tidal current speed Fig 46a. There is clear coincidence between peaks in wave stirring (orbital velocity) and those in turbidity (Fig. 46b, c) while such relation is absent with tidal current speed (Fig. 46a, c). Hence wave activity also induces local resuspension of sediment and explains much better the peaks in the turbidity on time scales larger than the semi-diurnal tide. This is further illustrated by the scatter plot in Fig. 47 showing the significant relation ($p<0.001$) between wave height and turbidity in Ref L over the whole period between February and October 2007. Corresponding data from OWEZ Wind farm also yield a significant relation but with a lower R (0.31 versus 0.58). So it seems that the small semi-diurnal variations in suspended sediment measured by the instrumented landers can be explained by high tidal velocities and the turbidity peaks by additional high wave activity.

During periods of strong wave stirring (example 17-18 March, wave height >3 m, Fig. 44), the calculated shear velocity due to currents and waves (0.04 m s^{-1} by water depth of 20 m, see Madsen, 1994) exceeds largely the critical shear velocity (0.017 m s^{-1}) of the local bottom sediment. Latter was calculated on basis of the critical Shields parameter θc using the formulation by Dorst et al. (2006) and the median grain size (466 µm) from Jarvis et al. (2004). This implies that part of the sand fraction may have been resuspended and added to the observed turbidity peaks. If we use the median grain size of 266 µm as measured in our analyses in 2007 (Table 3; Fig. 11), the critical shear velocity (0.013 m s^{-1}) shows that resuspension of sand grains may occur even at lower, and more frequently occurring, wave heights. Movement of the sandy seabed means that any fine sediment which under quiescent circumstances is stored below the surface, for instance by biological activity of the heart urchin *Echinocardium cordatum* (see Kleinhans et al., 2005; Borsje et al., 2009), is also released.

The question remains why the peaks in OWEZ Wind farm are considerably lower than in Ref L. The tidal velocities are about the same (see Fig. 46a) and their values cannot explain the difference in turbidity between the two sites. One of the differences between the two sites is the water depth. The water depth at OWEZ Wind farm is approximately 20 meter and the water depth at Ref L is approximately 16 meter. Orbital velocities decrease from the surface towards the bottom. A larger water depth will result in less stirring by waves at the bottom. The peak orbital velocity near the bottom is calculated for both the OWEZ Wind farm and Ref L (Fig. 46b). The peak orbital velocities near the bottom are about the same and the small difference in peak orbital velocity cannot be the reason for such large differences between turbidity peaks at the two sites.

It can be concluded that tidal velocities and water depth cannot explain the higher turbidity peaks in Ref L. Continued trawling activity in the Reference areas may also add to the higher turbidity in the water column. Hily et al. (2008) mentioned lower mud contents in sediment due to long-term trawl activities generating an increase in the resuspension of fine mud particles. Dellapennna et al. (2006) demonstrated that considerable suspended sediment concentrations were produced immediately behind the trawl net; an order of magnitude higher than pre-trawl levels. De Madron et al. (2005) reported that bottom trawls produce significant resuspension. The sediment clouds at several hundreds meters astern of the bottomtrawls are 3–6 m high and 70–200 m wide. Plume settling in the lower 1.5 m of the water column may take some time and particles re-settle primarily as flocs before they can be widely dispersed by local currents (Dellapenna et al., 2006).It's in our view unlikely that trawling activities in Ref L could have caused such a consistent increase in turbidity which 2-4 times higher levels than in the untrawled OWEZ Wind farm.

Another important factor determining the concentration of (re)suspended sediment in the water column is the availability of fine sediment in or on top of the seabed. If there is enough fine sediment available, the suspended sediment concentration can increase during periods of high dynamic energy. If fine sediment in the seabed is limited, the suspended sediment concentration will not increase any further when wave stirring increases. This is the most likely the factor explaining the difference in peak occurrence in turbidity between the Wind farm and Ref L. Analysis of the grainsize in bottom samples from both locations, show a difference in the number of samples comprising mud (particles <63 µm) and the mud contents. In Ref L 80 % of the samples contained mud, while of OWEZ Wind farm samples only 30 % contained mud (see Table 3). This difference is in accordance with the coast-offshore gradient in mud concentrations in the seabed as previously found in the MILZON project (van Scheppingen and Groenewold, 1990) and also mentioned by Kleinhans et al. (2005). This gradient reflects the elevated suspended mud concentrations carried by the coastal river in a narrow band along the Holland coast (Noordzee -atlas RIKZ, 2002). Burial of this suspended mud takes place during calm conditions under the influence of macrofauna (Kleinhans et al., 2005).

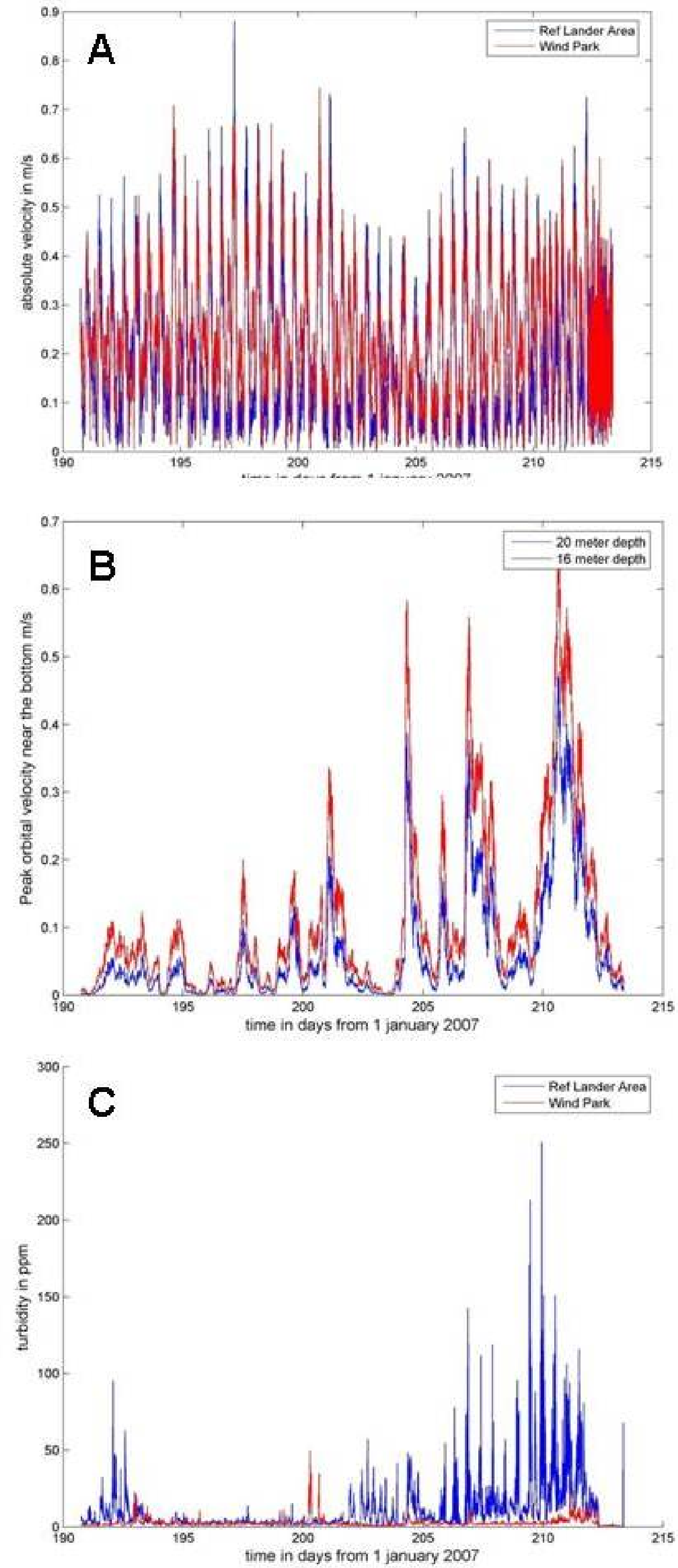


Fig. 46. Tidal currents (A), peak orbital velocity (B), and turbidity (C) in OWEZ Wind farm (red) and Ref L (blue) during the period 9 July- 3 August . Days are presented as numbers between 190 and 215.

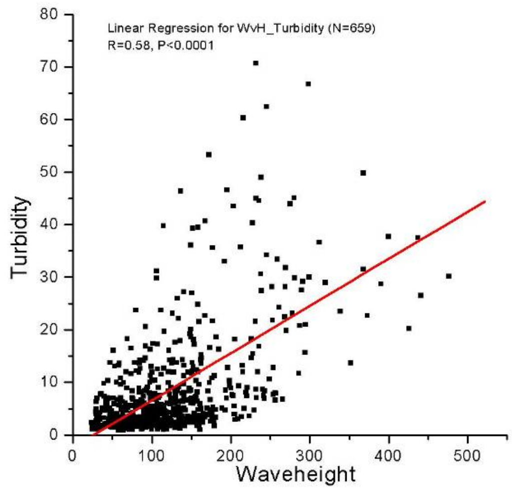


Fig. 47. Scatterplot showing the relation between wave height and turbidity over the whole measurement period in Ref L. The linear regression (red) is significant.

In summary, we conclude that tidal currents in combination with wave stirring most likely are the major causes for turbidity peaks observed at both lander sites, and that the difference between the sites in terms of turbidity peaks is probably predominantly due to a difference in availability of fine particles in the seabed. There is less fine sediment in OWEZ Wind farm to resuspend than in Ref L. The above observations imply that the termination of disturbance by trawling will unlikely lead directly to siltation of the Wind farm over time, because under the present conditions mud will be resuspended by tidal currents and occasional wave stirring and hence does not accumulate in the seabed. That is, when weather (wave) conditions do not change considerably compared to 2007 and the fauna does not change either.

4.6. Impact of the fishery-stop in OWEZ Wind farm on bivalve recruitment

Although small-sized bivalves are known to be less sensitive to trawling mortality than larger sized specimens of the same species (Bergman and van Santbrink, 2000), we yet hypothesized that the closure of OWEZ for fisheries could lead to a higher survival of bivalve settlers. Comparison of the 2007-densities of *S. subtruncata* recruits in OWEZ with the calculated juvenile densities needed to maintain the present-day adult population revealed that recruit numbers in OWEZ were ~20 times higher than calculated. The fact that densities were also ~20 times higher in the reference areas suggest that the present low density of the *S. subtruncata* population in OWEZ Wind farm and the trawled reference areas were due to other factors than fishing mortality in juvenile stages. These 2007-densities of recruits were far too low to give rise to the dense populations present in the 1980's and 90's in the Dutch coastal area (1100 to 4800 adults per m^2; see Fig. 40). The most likely reason for the low settlement is the reduced adult stock.

The results of the October 2007 field survey also showed no differences in abundances of the other species of bivalve recruits between OWEZ, closed for fishery in 2006 and 2007, and the regularly trawled reference areas (see section 4.1). Apparently not only the settlement of *S. subtruncata* but also of other bivalve species was regulated by other factors than fishery mortality in their first months. Hence, the presence of OWEZ wind farm did not initiate an enhanced bivalve settlement in 2007. Restoration of coastal bivalve populations like the former large *S. subtruncata* population may take years in which specimens due to reduced fishing mortality gradually accumulate within the closed OWEZ. Possibly the impact of the fishery-stop in OWEZ Wind farm becomes visible in 2011 when the final sampling survey will be executed.

Not only abundances of bivalve recruits but also species composition of recruits > 0.5 mm in the survey areas appeared to be not area-based (ANOSIM test; Table 6). Cluster analysis of the individual stations combined with a SIMPROF-test (p=0.05) identified 2 significant clusters as depicted in Fig. 21. The near-coast cluster of stations harboured a much denser bivalve population than the other cluster (Table 6). The locations of stations in the two clusters (Fig. 22) suggest an offshore–coast, possibly combined with a slight south-north, gradient in species composition rather than an area-based trend.. The mud percentages of the stations in the near-coast cluster were significantly higher (on average 2.6%) than in the other cluster (on average 0.7% (Mann Whitney test, p=0.0002. Water depth in the near-coast cluster was also significantly higher than in the other cluster, on average 21.0 m and 19.0 m respectively (Mann Whitney test, p=0.0002), although not linearly correlated with the mud content (Fig. 23). Scatter plots based on the abundances (see Table 3) of small-sized (0.2-0.5 mm) and larger-sized settlers (>0.5 mm) and mud content (% <63 µm) of the stations illustrate the significant correlations between both size-classes and mud content (Spearman rank correlation test, p=<0.0001 and p=0.0018 respectively; Fig. 24).

The presence of an area closed to fisheries (i.e. OWEZ) apparently does not affect abundances and species distribution of bivalve recruits. In contrast species composition appeared to be correlated to abiotic variables, such as mud content and water depth (BEST-analysis; Table 8). The initial correlation between mud and small recruit at the time of settlement might have advantages for growth and survival, and subsequent consequences if broken down as was observed in the reference areas. The reason for this break-down is unknown, although continuing trawling disturbance, possibly in combination with dispersion by wind waves, could form an explanation. The assumption that growth is reduced under such conditions could however not be proven since differences in bivalve lengths among the survey areas were not observed, except in *Tellina* where the largest specimens were found in the 2 northern reference areas. Our study also does not support the concept that the assumed disturbance of the initial sediment-recruit relationship had consequences for a lower survival and growth of recruits in the reference areas (see section 4.1). At the end of the settling period the correlation between the abundances of small-sized settlers (0.2-0.5 mm) and larger-sized ones (>0.5 mm) was still significant (Fig. 18).

A reduced turbidity was hypothesized for the fishery-closed OWEZ area. However, turbidity peaks in the survey areas, being most prominent in Ref L, appeared to be caused by tidally-induced resuspension of deposited mud particles on a semi-diurnal scale during calm periods and-by wave-induced resuspension in response to (incidental) strong wind conditions (section 4.5). Differences in turbidity between OWEZ and Ref L, are most likely caused by differences in available mud at and in the sea bed.These results imply that the cessation of bottom disturbance by trawling in OWEZ will unlikely lead to siltation of the area over time, because under the present conditions mud will be regularly resuspended by high tidal currents and occasionally during high wind speeds by additional wave stirring, and hence does not accumulate in the seabed. That is, when weather (wave) conditions do not change considerably compared to 2007 and the fauna does not change either. Even if the median sand fraction would be affected by the fishery-stop or for instance by maintenance activities like covering cables, the mesocosm results indicate that bivalve settlers will not be influenced in their habitat selection. Settlers showed no signs of being attracted to specific sand fractions (Figs. 32, 33, 34), although spatial and temporal differences in abundance of settlers were found such as the higher numbers of settlers in the coarse fraction in Ref L than in that in OWEZ Wind farm (Fig. 36) and the higher settling numbers in Deploy 1 than Deploy 2 (Fig. 35).

Based on our results we cannot prove some anticipated indirect impacts of the fishery-stop in OWEZ such as less turbidity in the water column leading to higher growth rate in filter-feeders (Witbaard et al., 2001), and the development of finer grained sediments leading to the settlement of other bivalve species. The main reason that these impacts will not become true is that turbidity and median grain size in OWEZ are largely governed by tidal and wind regimes and local mud deposits.

CONCLUSIONS

During the field survey in October 2007 no differences were found between the densities of small-sized (> 0.2 mm) bivalve recruits in OWEZ Wind farm and the 5 reference areas. For the larger (older) recruits > 0.5 mm differences in densities were found only between reference areas: 383 per m^2 in Ref 3 and 113 per m^2 in Ref 5.

Of recruits > 0.5 mm that could be identified as *Abra alba, Donax vittatus, Mysella bidentata*, *Ensis* spp*.,* and *Tellina* spp., only *Ensis* spp. showed a significant difference in density between some of the survey areas i.e. 154/m^2 in Ref 3 and none in Ref 4.

Cluster analysis of the individual stations on basis of recruits > 0.5 mm (*Abra alba, Donax vittatus, Mysella bidentata*, *Mytilus edulis, Montacuta ferrigunosa, Chamelea gallina, Ensis* spp*., Tellina* spp., *Spisula* spp) revealed 2 significant clusters. The average dissimilarity between the two clusters was attributed to *Ensis* spp, *Montacuta ferruginosa*, *Tellina* spp. and *Abra alba.* The position of the clusters did not coincide with survey areas but suggested an offshore–coast gradient in species composition. The absence of area-based differences was confirmed by an ANOSIM analysis of the species abundances in the six survey areas. The coast-nearby cluster of stations harboured a much denser bivalve population than the more offshore cluster. The two clusters differed in average mud contents and water depth and species composition in the coastal cluster was correlated with higher mud content and water depths exceeding 18-20 m.

Within the OWEZ Wind farm, which was closed for fishery in 2006 and 2007, significant correlations were found between environmental variables on the one hand, and total abundances and abundances of single species of recruits > 0.5 mm on the other hand. In case of total abundances the factor mud scored the highest correlation coefficient. Spatial distributions of recruits of *Ensis* spp., *Donax vittatus* and *Mysella bidentata* were also correlated with mud as the best explanatory variable. In contrast, no significant correlations were found between the environmental data and abundances of (single) recruits in the pooled reference areas. The coupling between sediment and bivalve recruits apparently has been broken down here likely by resuspension caused by for instance trawling and/or strong wind events.

The mesocosm experiments revealed that settling bivalves did not show a preference for any of the sand fractions that we used in the deployments in OWEZ and Ref L. In the second deployment, in coarse sediment only, settlement was remarkably higher in Ref L than in OWEZ, suggesting that competent larvae occur in patches leading to spatial differences in settlement. Since the average numbers of settlers in the fine and in medium fractions were not significantly different, the result may also point at coarse sediments being more attractive to certain bivalve species in coast-nearby zones like Ref L. There were indications that competent bivalve larvae arrived in patches in the coastal zone causing a temporal pattern in settlement. Differences in length of the settlers were not found in the deployments in OWEZ and Ref L.

Average net settlement of bivalves in the mesocosms in OWEZ varied from 1565 per m^2 per day in July to 324 per m^2 per day in August, whereas 816 per m^2 per day successfully settled in the submerged trays in Ref L in August. In October 2007, several months after initial settlement, densities of bivalve recruits (> 0.2 mm) varied from 4312 to 1558 per m^2 in OWEZ and Ref L, respectively, while densities of recruits > 0.5 mm varied from 429 to 121per m^2. Even if the resuspension of larvae from the mesocosms was reduced by the 2 hours-interval of opening of the lids around the turn of the tide, these data suggest a considerable loss of bivalve recruits during the first months of settlement in the field situation

Indirect effects of the fishery-stop in OWEZ such as a less turbid water column and finer sediments allowing better growth of bivalves and higher species richness, respectively, can not be expected. Turbidity and median grain size in OWEZ are primarily governed by tidal and wind (wave) regimes, and local mud deposits.

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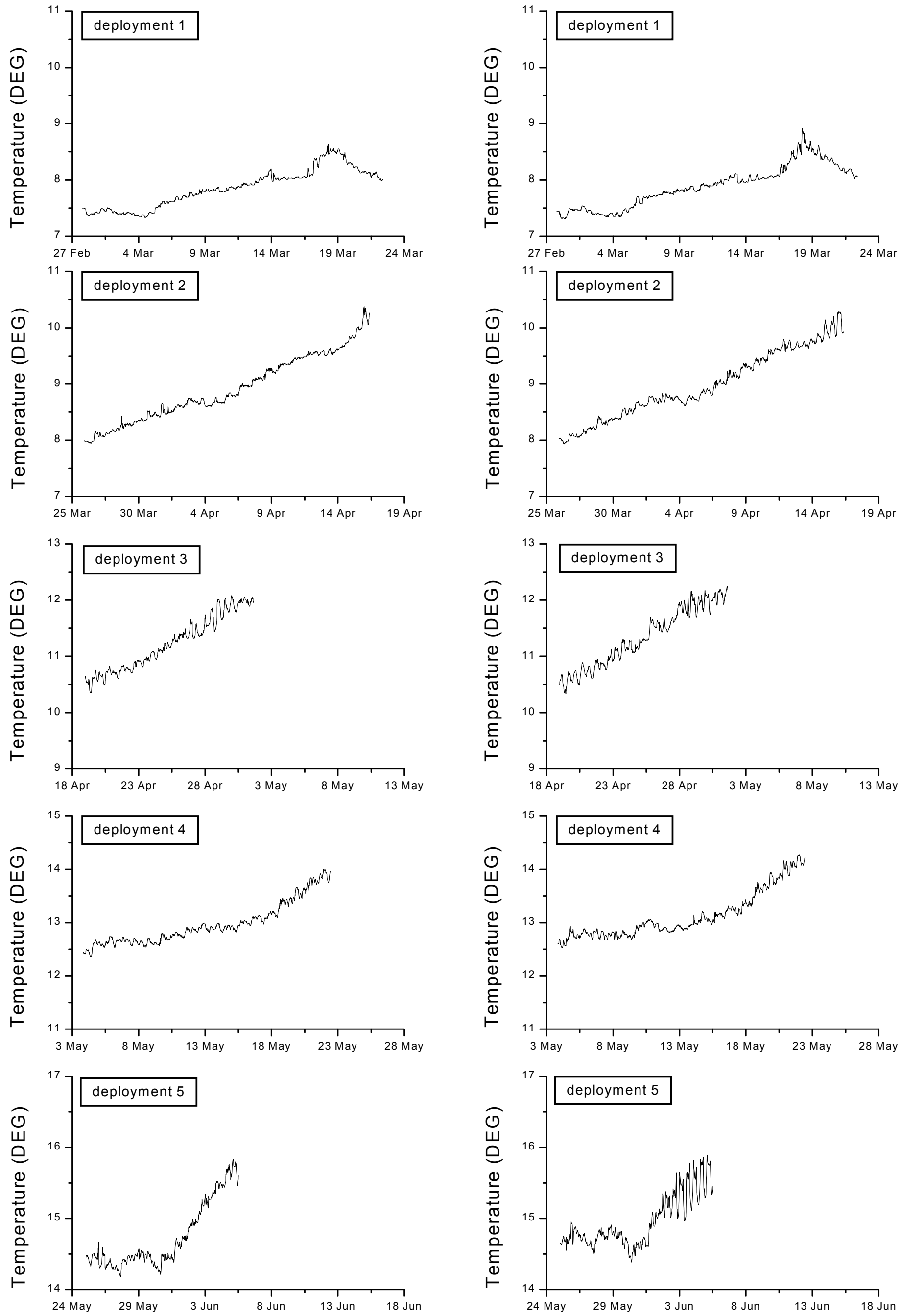
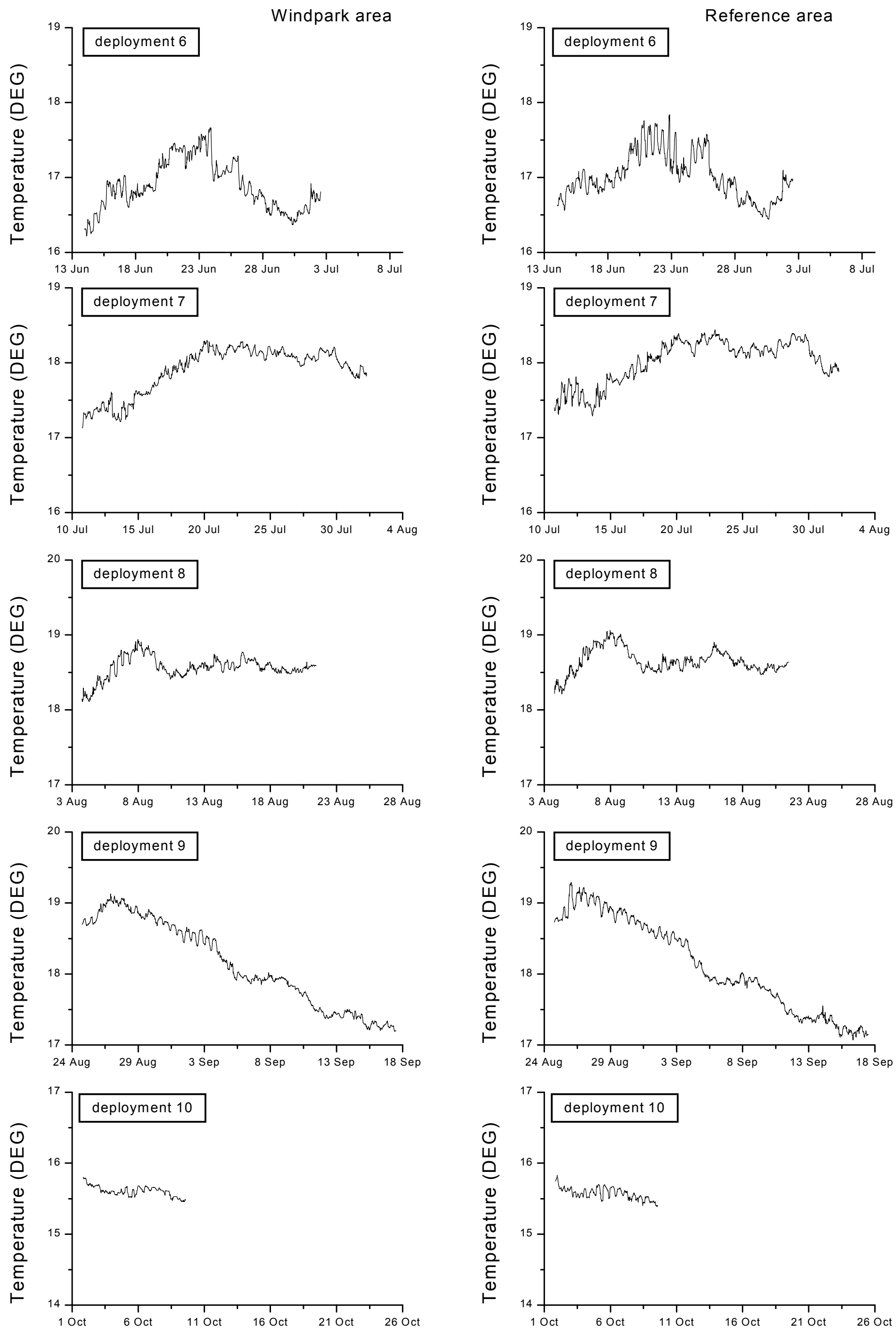
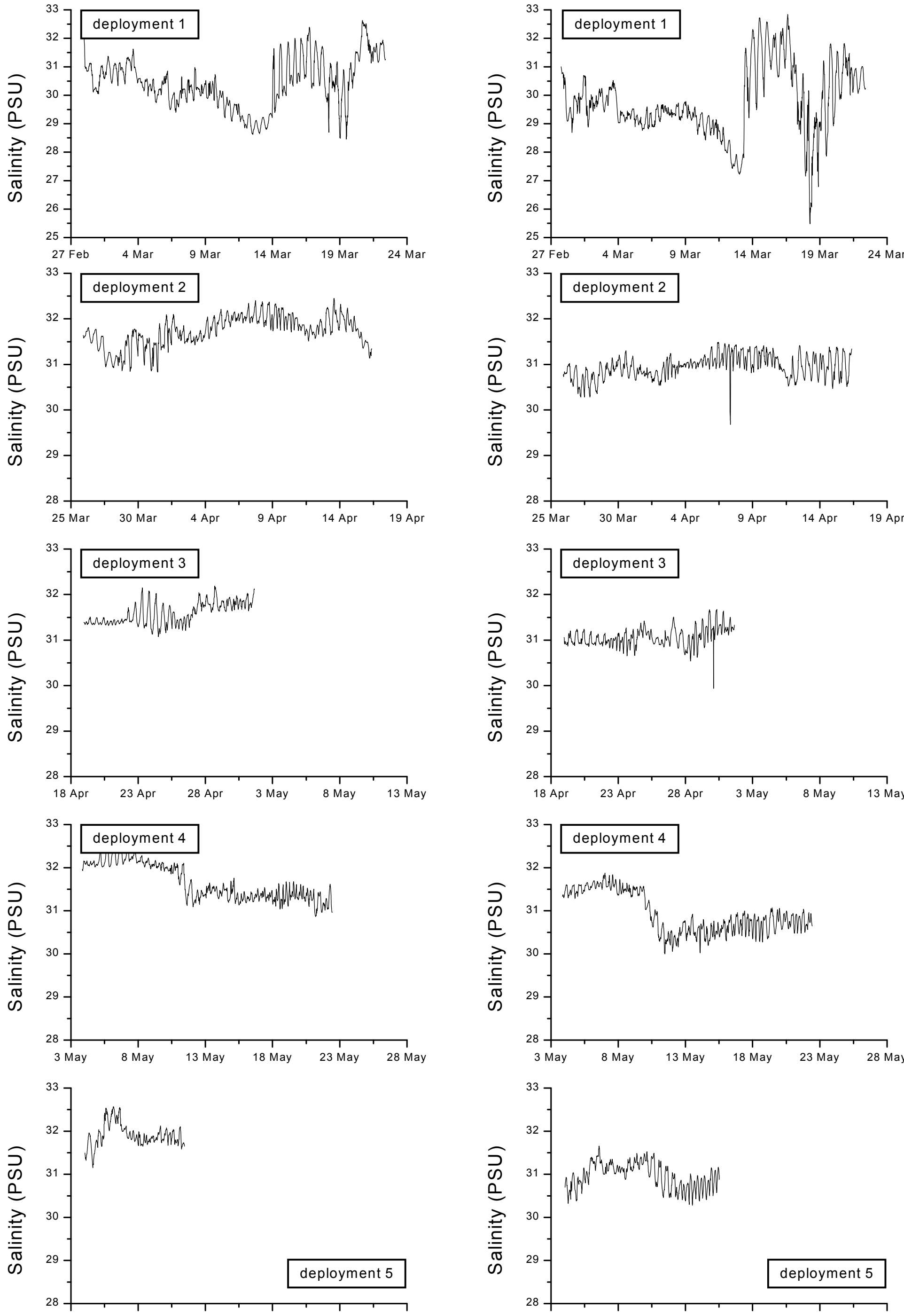
Appendix I. Variations of ambient seawater temperatures (ºC) for the first five deployments (February-June 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



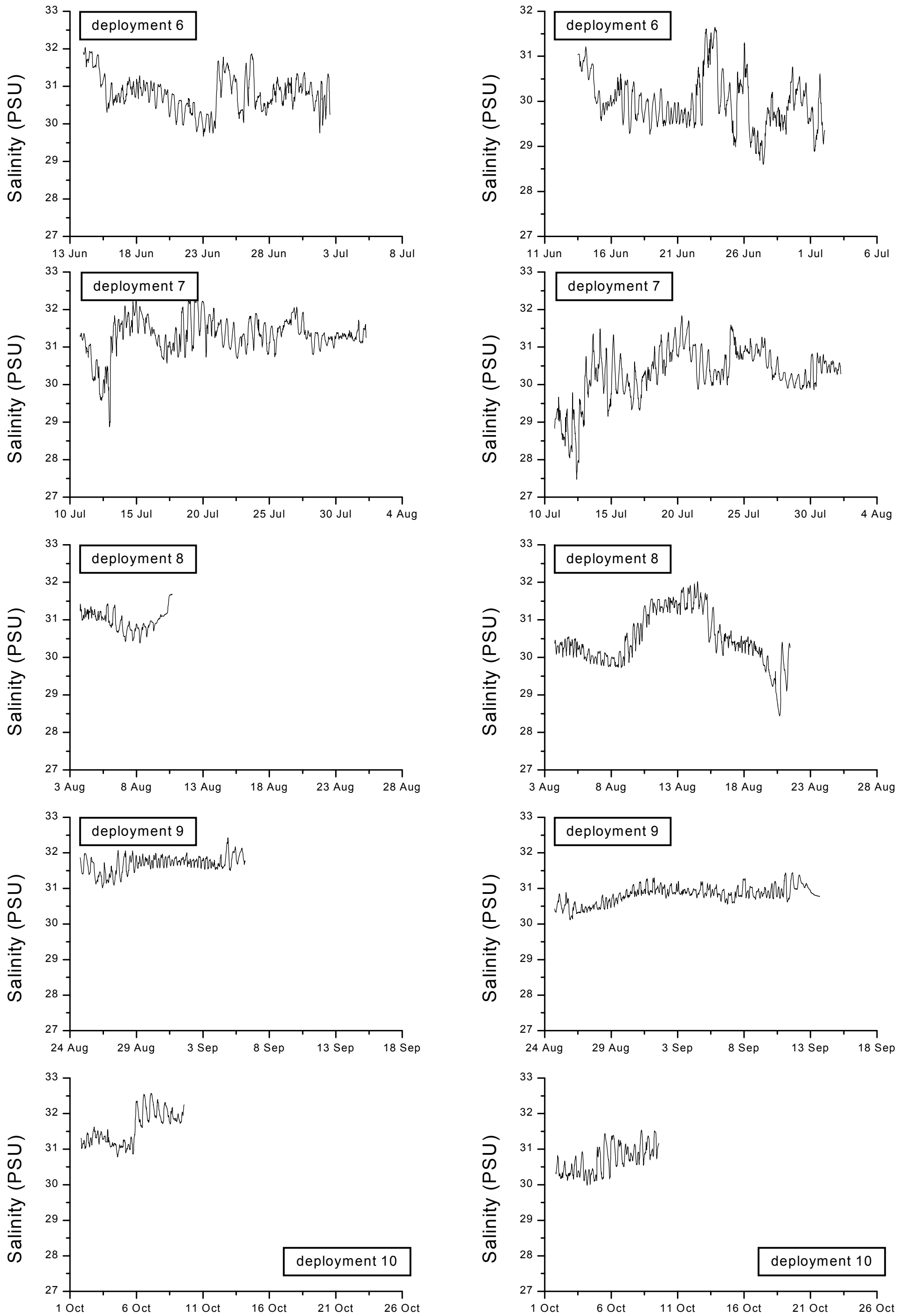
Appendix I continued. Variations of ambient seawater temperatures (ºC) for the last five deployments (June-October 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



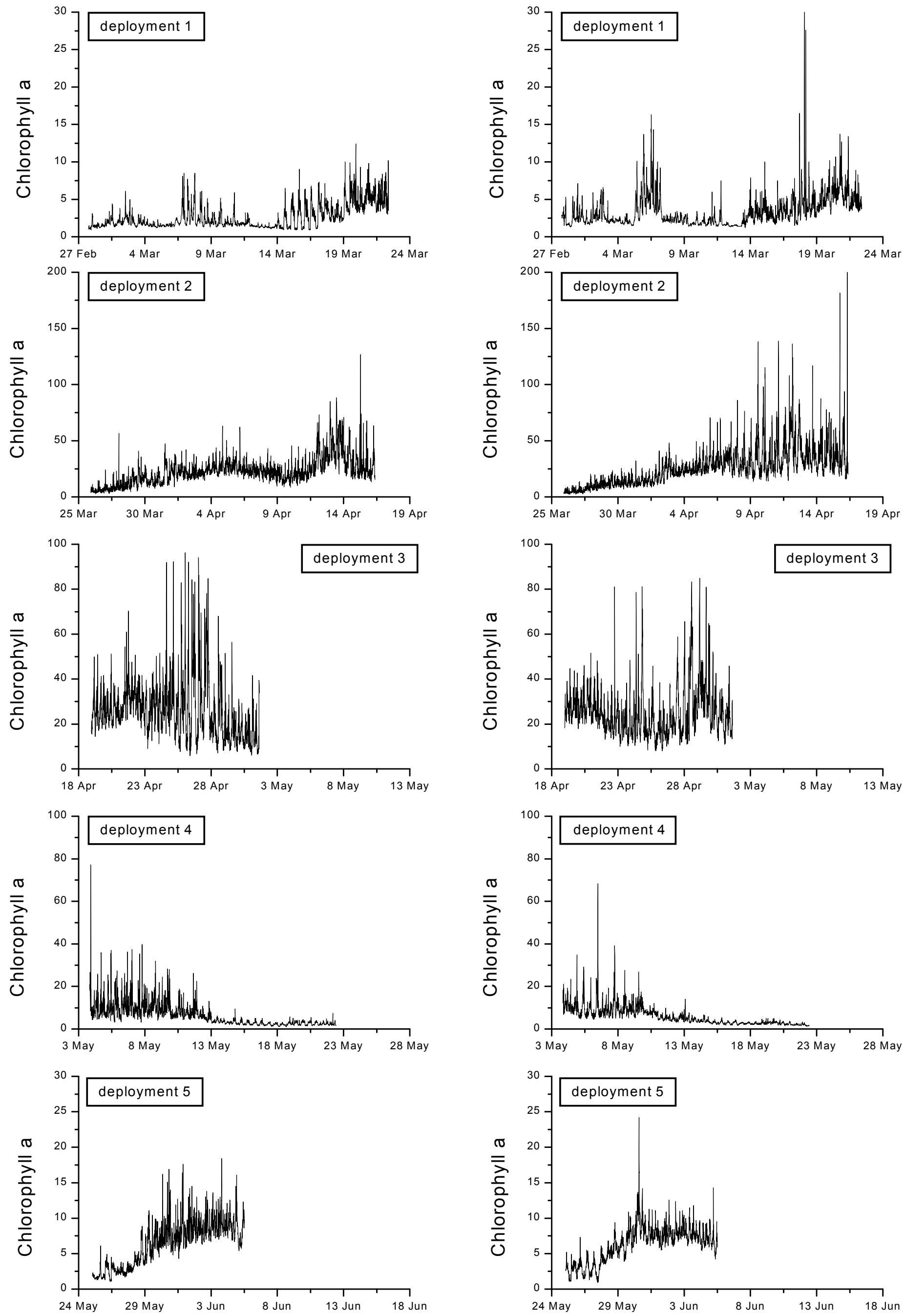
Appendix II. Variations of ambient salinity levels (PSU) for the first five deployments (February- June 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



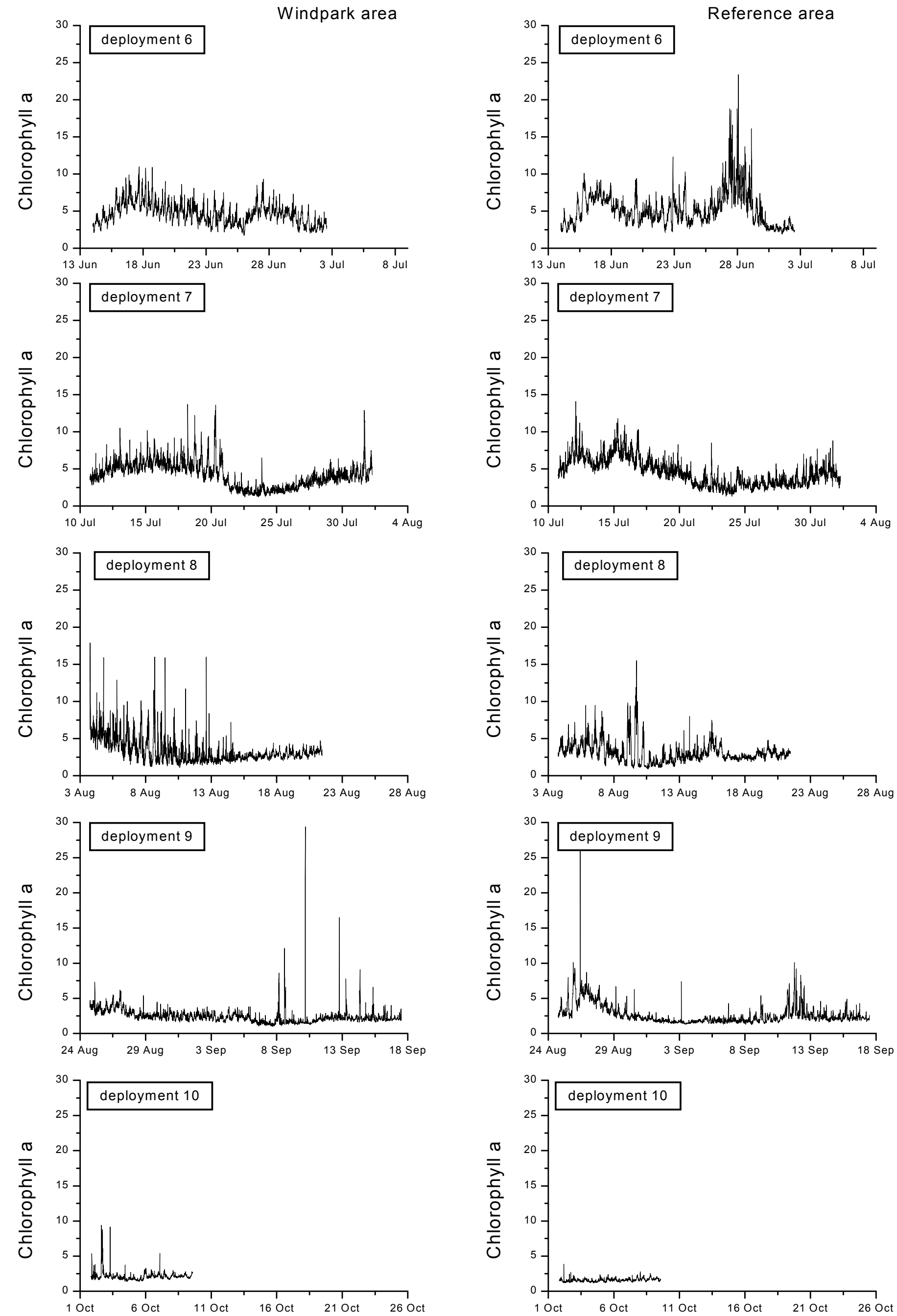
Appendix II continued. Variations of ambient salinity levels (PSU) for the last five deployments (June-October 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



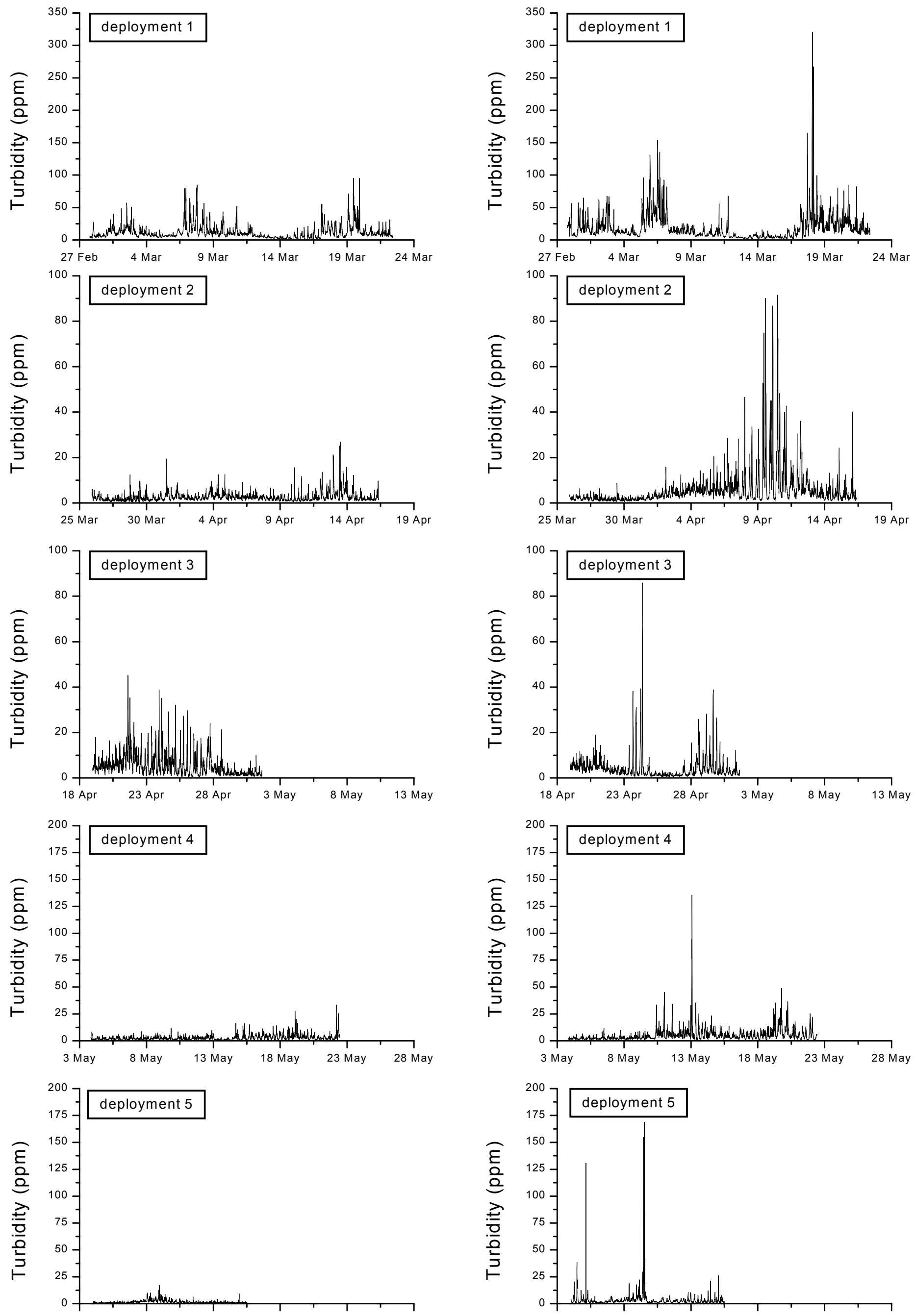
Appendix III. Variations in ambient fluorescence (in Uranine units ppb) for the first five deployments (February- June 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



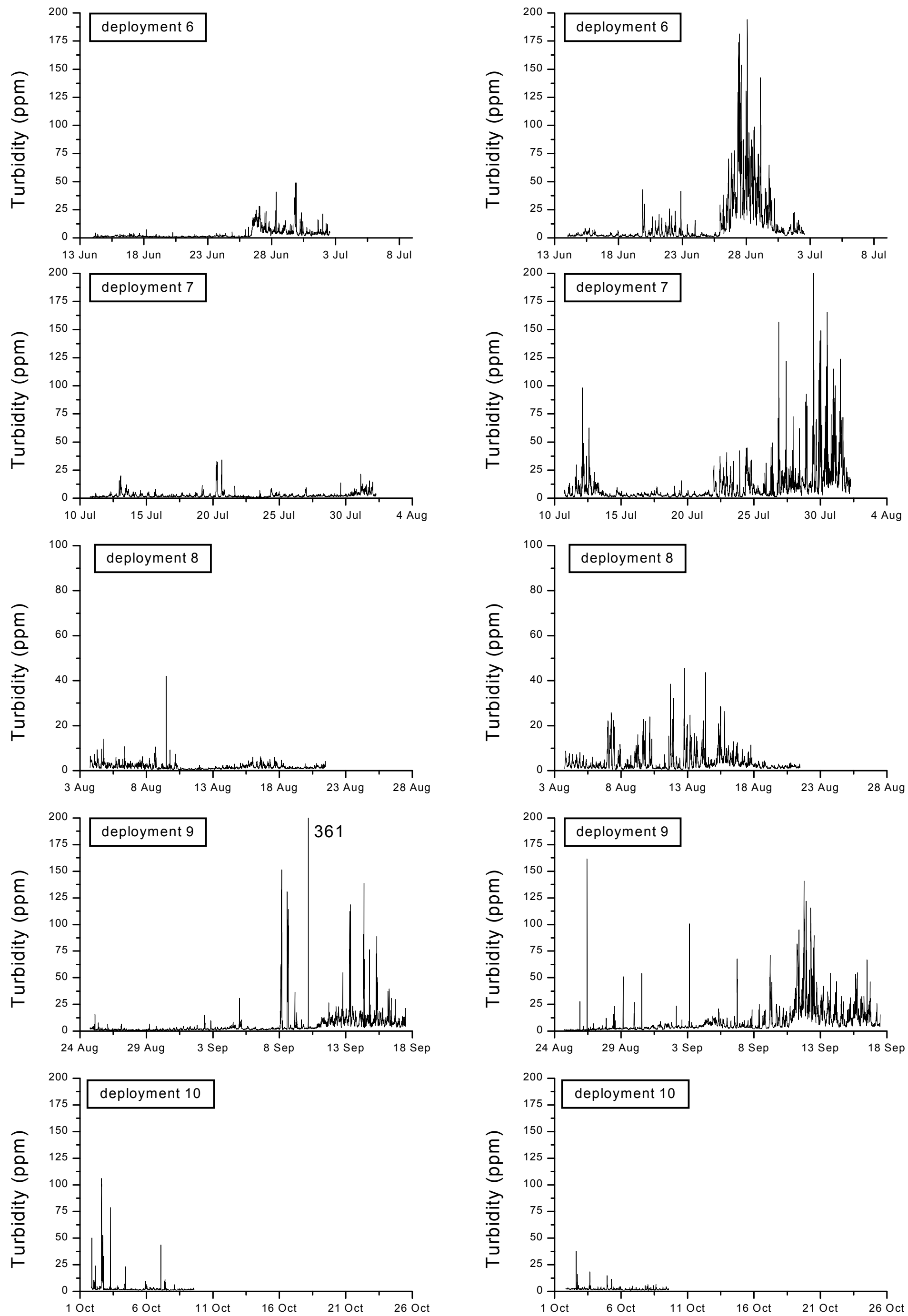
Appendix III continued. Variations in ambient fluorescence (in Uranine units ppb) for the last five deployments (June-October 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



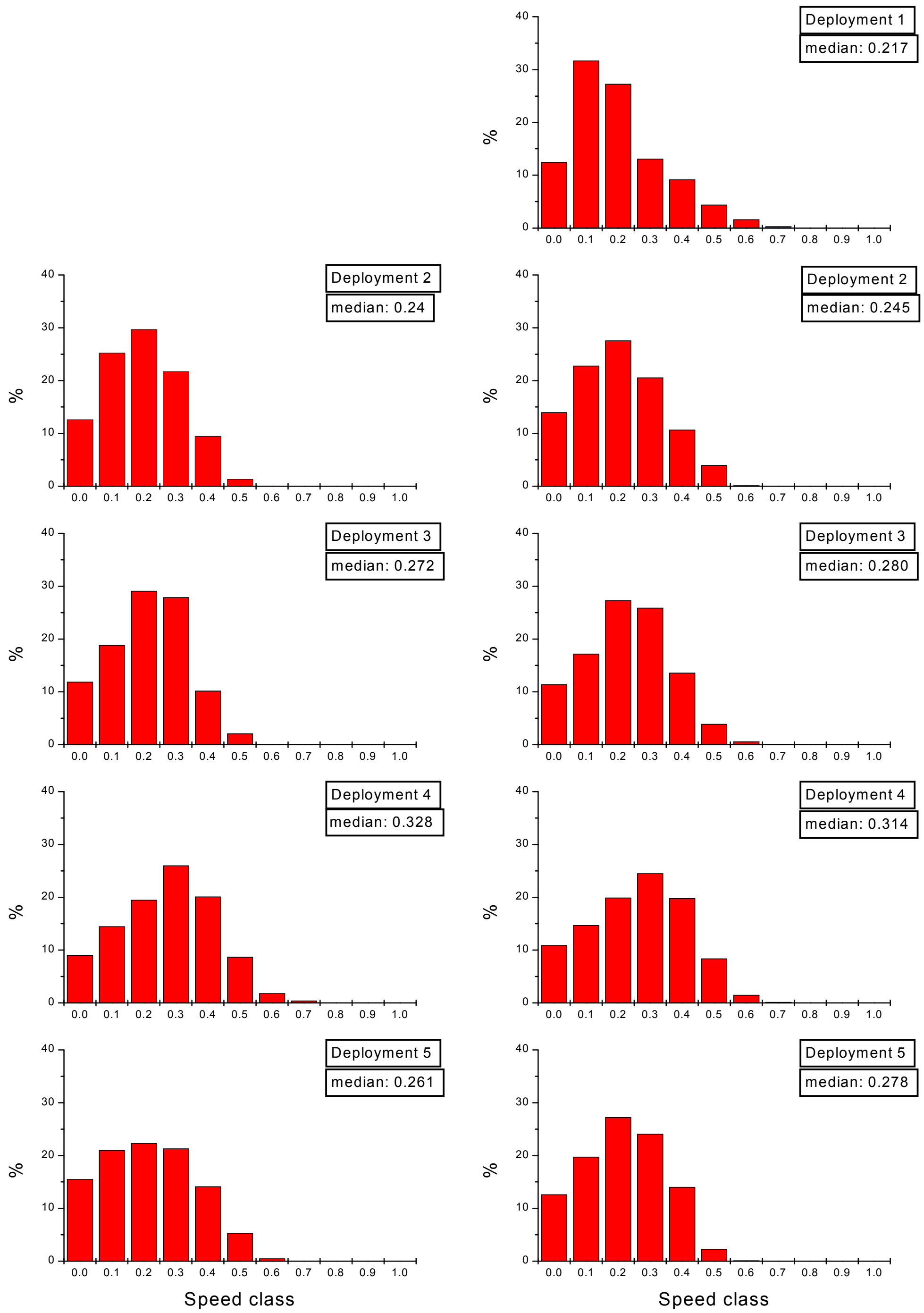
Appendix IV. Variations of ambient turbidity levels (ppm) for the first five deployments (February- June 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



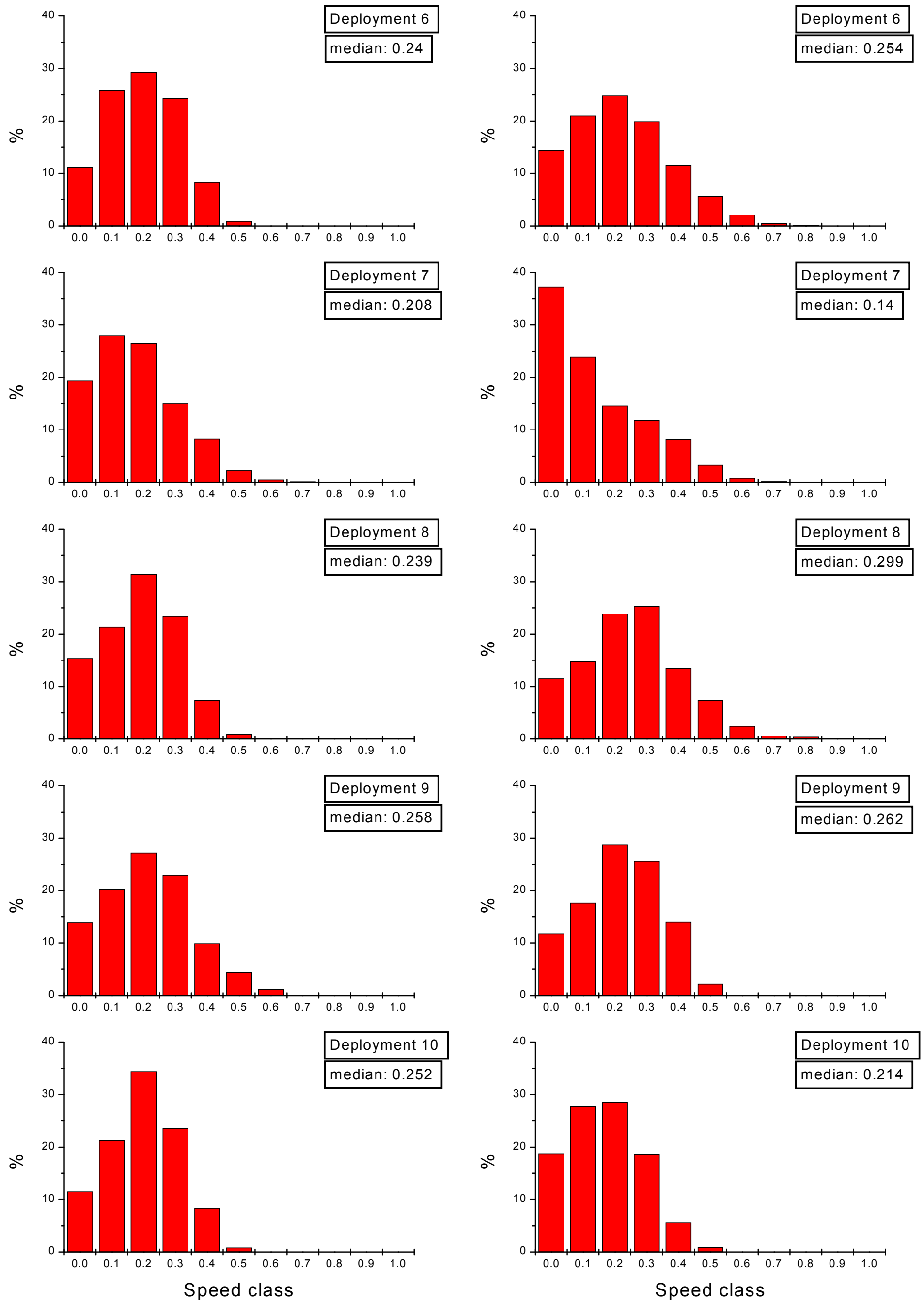
Appendix IV continued. Variations of ambient turbidity levels (ppm) for the last five deployments (June-October 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



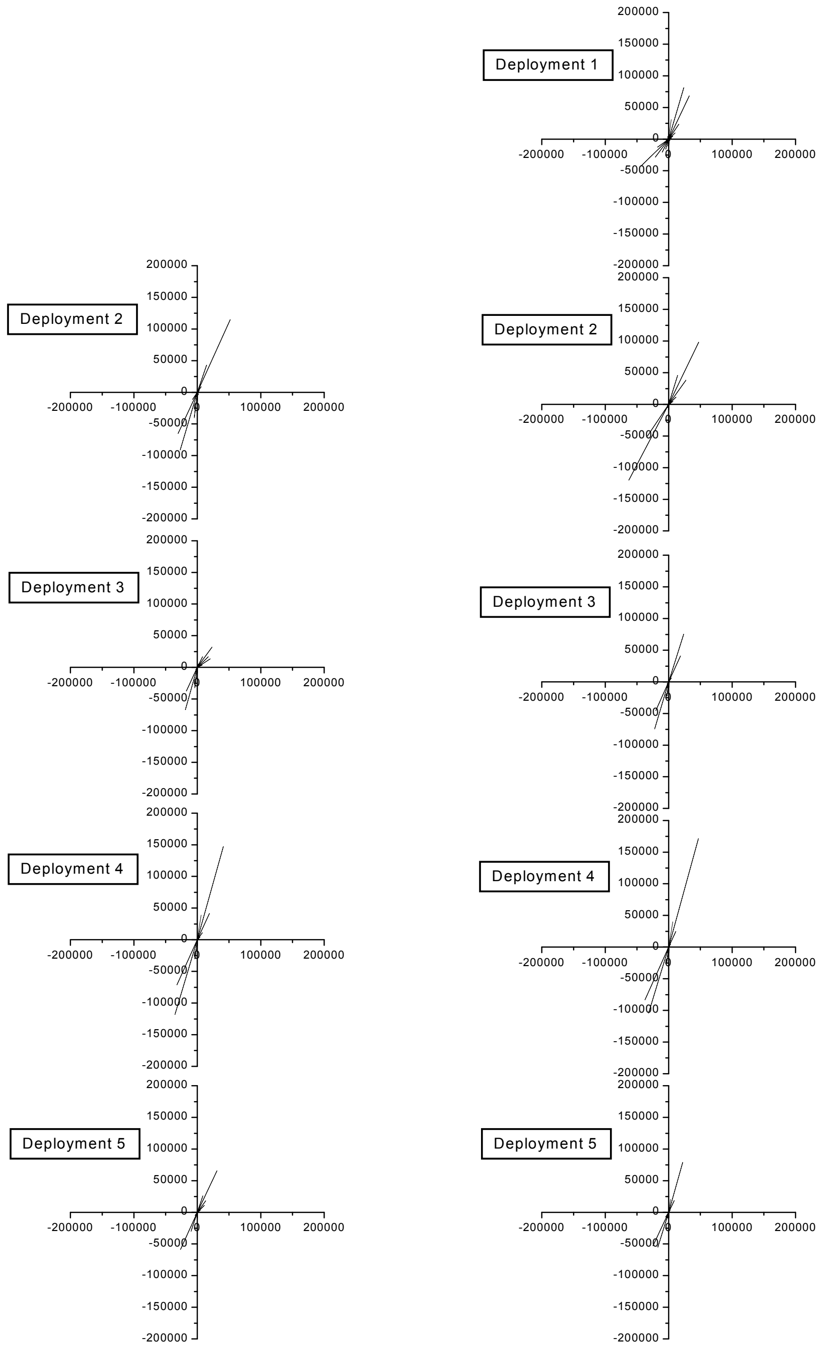
Appendix V. Occurrence (%) of current speed classes (m/s) for the first five deployments (February-June 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



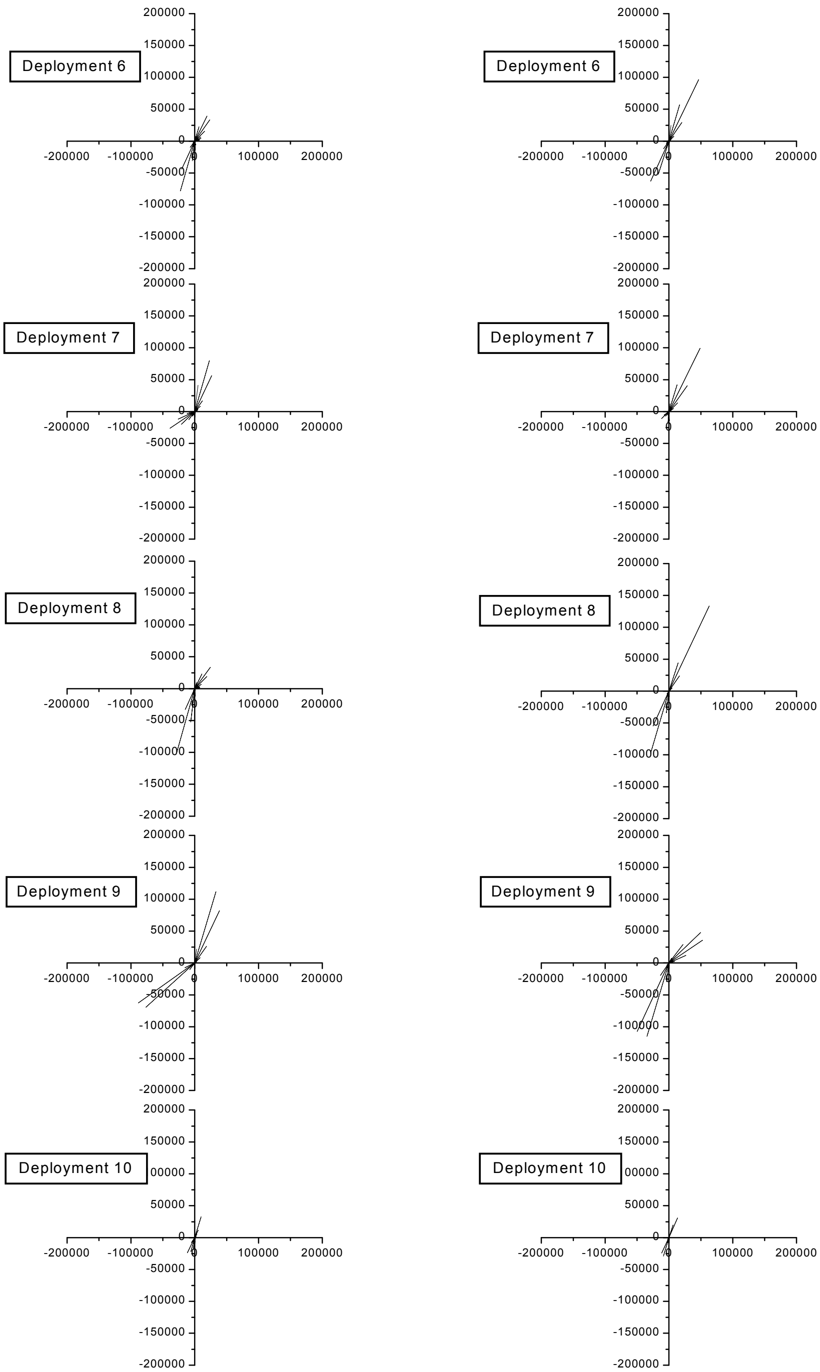
Appendix V continued. Occurrence (%) of current speed classes (m/s) for the last five deployments (June-October 2007). Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



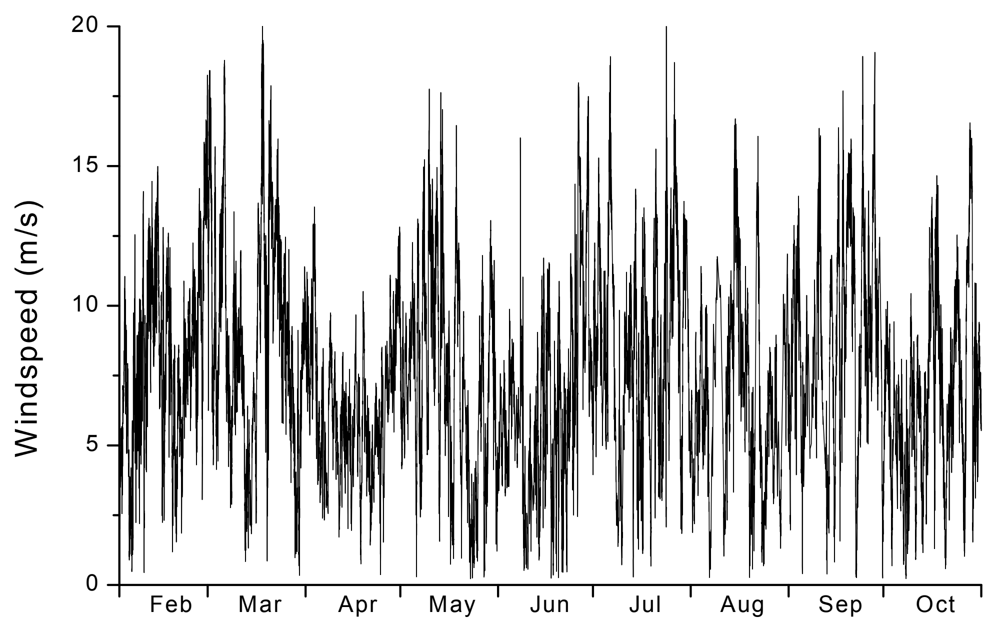
Appendix VI. Current patterns per compass direction for the first five deployments (February-June 2007). Lines are in 10° increments and point in the direction the current is flowing to. Lengths of the lines reflect total distances per direction (m) during deploy. Left column graphs represent the Wind farm location, right column graph represent the Ref. Lander area.



Appendix VI continued. Current patterns per compass direction for the last five deployments (June-October 2007). Lines are in 10° increments and point in the direction the current is flowing to. Lengths of the lines reflect total distances per direction (m) during deploy. Left column graphs represent the Wind farm location, right column graphs represent the Ref. Lander area.



Appendix VII. Annual variation in wind speed (m/s) during the period of the lander deployments in 2007. Data are measured by means of an acoustic recorder (instrument code 3D WM4/NW/21) mounted at the meteorological mast of the Wind farm area 21 m above sea level. Source: www.noordzeewind.nl.



Appendix VIII. Variations in wind speed (m/s) and direction (º) during the ten deployments (February - October 2007). Wind data are measured by means of an acoustic recorder (instrument code 3D WM4/NW/21) mounted at the meteorological mast of the Wind farm area 21 m above sea level. Wind speed data are symbolized by a black line, wind direction is shown with red dots. Source: www.noordzeewind.nl.