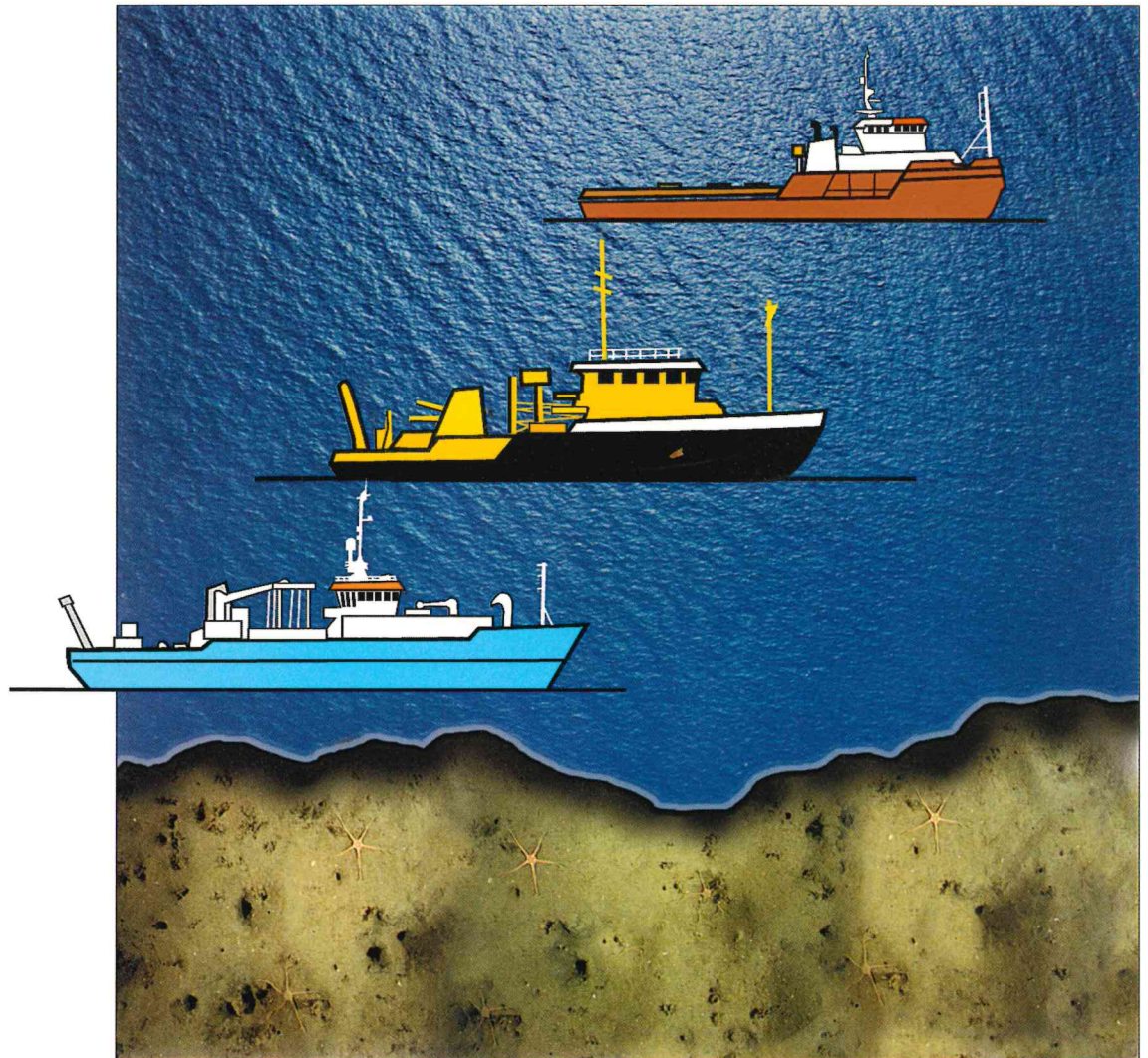


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Nederlands Instituut voor Onderzoek der Zee

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NETHERLANDS INSTITUTE FOR SEA RESEARCH

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SUMMARY AND CONCLUSIONS

In the period July 1993 - August 1994 a field monitoring programme has been carried out to assess the environmental effects associated with drill cutting discharges after drilling with ester based muds (EBM). The study was performed at a location in the Dutch sector of the North Sea, where an exploration well was drilled in August-September 1993, and included a baseline survey just before drilling started and 3 post-drilling surveys, which took place 1 month, 4 months and 11 months after termination of drilling respectively. During these surveys sediment samples were collected in the close vicinity of the location and at increasing distances, up to 3 km from the location. Ester concentrations in the sediment were determined for the 3 post-drilling surveys. Macrofauna analyses were made for the baseline survey and the 2nd and 3rd post-drilling surveys.

For the 1st and 2nd post-drilling surveys esters were consistently detected for all stations within 200 m from the location. Concentration levels in individual samples were extremely variable and ranged between 2 and 4700 mg·kg⁻¹ dry sediment. For the 2nd survey the concentrations measured were higher than during the 1st survey, which might be explained by redistribution of discharged material in the intervening period. At distances between 500 and 3000 m concentrations were at detection level. Esters were still detected during the 3rd post-drilling survey up to 200 m and concentrations ranged between 1 and 250 mg·kg⁻¹ dry sediment. Between 500 and 3000 m esters could no longer be detected. The results suggest a substantial decrease in the whole area. The mean of several estimates of degradation rates of the esters pointed at a half-life time value of 133 days. This value reflects the composite effects of biodegradation and sediment relocation.

During the 2nd post-drilling survey gross effects on the macrofauna could be detected up to 200 m from the location. Most species showed reduced abundance within this zone and there appeared to be an overall reduction in species richness, total fauna abundance and relative fauna abundance. Also the presence of *Capitella capitata* was an obvious sign of disturbed sediment conditions in this area. A few species showed abundance gradients over the whole sampling transect and occurred in reduced abundance up to 500 or 1000 m compared to the 3000 m reference station. The echinoderm *Echinocardium cordatum*, which was found in high numbers before drilling started, had completely disappeared at all stations within 500 m from the location. The 3rd post-

drilling survey still clearly revealed effects at 75 m from the location. Effects were detected at species level and at community level. At 125 - 200 m effects were less pronounced but species richness and relative fauna abundance were still significantly reduced compared to the 3000-m station. At distances ≥ 500 m persistent effects could no longer be demonstrated. The fact that adult *E. cordatum* were still absent at 500 m was considered just the 'long-term' consequence of a short-term effect and not as the consequence of actual stress.

The results of this study are compared with those of a study earlier performed in the Norwegian sector and contrasting outcomes are discussed. Although the results of the present study show that effects did not have completely disappeared after one year, there are signs of recovery of the macrobenthos, because a reasonable number of species had recolonised the area in the vicinity of the well site and particularly at 200 m in fairly high numbers. It is suggested that recovery of sediment conditions had set in particularly in the 3-5 cm top layer, which subsequently has provided a suitable substratum for recolonisation of several species.

In conclusion, the results may be summarized as follows:

1. Four months after termination of drilling, concentrations of esters in the sediment ranged between 2 and 4700 mg·kg⁻¹ dry sediment within 200 m from the location and traces were detectable up to 3000 m from the location.
2. Eleven months after termination of drilling, esters were still detected within 200 m (at one station up to 250 mg·kg⁻¹ dry sediment), but concentrations were at lower levels than after 4 months. At distances ≥ 500 m the concentrations were below detection level.
3. Estimates of the decay rate of the esters indicated a mean half-life time of 133 days and a lower confidence limit of 68 days.
4. Four months after termination of drilling, the macrofauna was severely impoverished within 200 m from the location. A few species showed reduced abundance up to 500 or 1000 m.
5. Eleven months after termination of drilling, significant effects on the macrobenthic community could still be detected within 200 m from the location. At distances ≥ 500 m persistent effects could no longer be demonstrated.
6. Within 200 m the sediment was recolonised by several species after 11 months, which indicated that recovery of the natural macrobenthic community had set in.

SAMENVATTING EN CONCLUSIES

In de periode juli 1993 - augustus 1994 is een veldonderzoek uitgevoerd, teneinde de effecten vast te stellen van lozing van boorgruis met restanten boorspoeling op basis van esters. De studie werd uitgevoerd rond een lokatie in de Nederlandse sector van de Noordzee, waar gedurende augustus-september 1993 een exploratieboring is verricht, en omvatte een baseline survey (vlak voor met boren werd gestart) en 3 surveys die respectievelijk 1 maand, 4 maanden en 11 maanden na beëindiging van de boring werden uitgevoerd. Tijdens deze surveys vond bodem-bemonstering plaats in de onmiddellijke omgeving van de lokatie en op enkele stations op toenemende afstand van de lokatie, tot op maximaal 3 km. Esterconcentraties in het sediment werden bepaald voor elk van de 3 surveys die na de boring plaats vonden. Makrofauna-analyses werden uitgevoerd aan de hand van monsters verzameld tijdens de baseline-survey en die verzameld tijdens de 2de en 3de survey na boring.

Tijdens de 1ste en 2de survey na boring werden esters in het sediment gedetecteerd op alle stations binnen een straal van 200 m van de lokatie. Het concentratieniveau in afzonderlijke monsters was uiterst wisselvallig en varieerde tussen 2 en 4700 mg·kg⁻¹ droog sediment. De concentraties gemeten tijdens de 2de survey waren over het algemeen hoger dan tijdens de eerste survey, kennelijk als gevolg van redistributie van het materiaal in de tussenliggende periode. Op afstanden tussen 500 en 3000 m lagen de concentraties rond detectieniveau. Tijdens de 3de survey na de boring werden esters zonder uitzondering nog steeds waargenomen op de stations binnen 200 m afstand van de lokatie, in concentraties die varieerden tussen 1 en 250 mg·kg⁻¹ droog sediment. Tussen 500 en 3000 m kon de aanwezigheid van esters niet meer worden aangetoond. De resultaten wijzen op afnemende concentraties in het hele gebied. Schattingen van de afbraak-snelheid van de esters komen uit op een gemiddelde halfwaarde-tijd van 133 dagen. Deze waarde is de resultante van twee processen, nl. biodegradatie en sedimentverplaatsing tussen opeenvolgende surveys.

Tijdens de 2de survey na de boring werden verscheidene effecten op de makrofauna vastgesteld tot op 200 m afstand van de lokatie. De meeste soorten kwamen binnen deze zone in verlaagde aantallen voor en een algehele reductie kon worden vastgesteld in soortenrijkdom, absolute fauna-abundantie en relatieve fauna-abundantie. Ook de aanwezigheid van de als opportunistisch bekend staande soort *Capitella capitata* vormde een duidelijke indicatie van sedimentverstoring. Enkele soorten vertoonden qua talrijkheid in voorkomen een gradient die zich uitstreckte over het gehele bemonsterde transect en kwamen tot op 500 of 1000 m voor in verlaagde aantallen vergeleken met het 3000-m referentiestation. Zeeëgels (*Echinocardium cordatum*), die nog in grote

aantallen werden aangetroffen voordat met boren was begonnen, bleken volledig verdwenen te zijn tot op 500 m van de lokatie. Tijdens de 3de survey na boring konden nog steeds duidelijk effecten worden vastgesteld op 75 m van de lokatie. Deze effecten manifesteerden zich zowel op soortniveau als op het niveau van de totale faunagemeenschap. Op afstanden van 125 tot 200 m waren de effecten minder uitgesproken, maar toch werd hier een significant lagere soortenrijkdom en relatieve fauna-abundantie aangetroffen dan op het 3000-m station. Vanaf 500 m konden geen effecten meer worden waargenomen. Het feit dat adulte *E. cordatum* nog steeds ontbraken op dit station dient te worden gezien als het 'lange-termijn' gevolg van een korte-termijn effect en niet als het gevolg van permanente stress.

De resultaten van deze studie zijn vergeleken met die van een studie die eerder werd uitgevoerd in de Noorse sector en voor zover de uitkomsten met elkaar in tegenspraak zijn is daar een discussie aan gewijd. Hoewel de voorliggende studie aantoont dat effecten een jaar na lozing nog steeds kunnen worden waargenomen, zijn er tekenen van intredend herstel van het macrobenthos, gezien het feit dat bij een aantal soorten rekolonisatie van het sediment kon worden vastgesteld, met name op het 200-m station in soms vrij hoge aantallen. De indruk bestaat dat herstel van sediment-condities met name is opgetreden in een 3 tot 5 cm dikke toplaag, hetgeen voor verscheidene soorten een geschikt substraat voor rekolonisatie heeft opgeleverd.

De resultaten en conclusies van dit onderzoek kunnen als volgt worden samengevat:

1. Vier maanden na beëindiging van de boring varieerden concentraties van esters in het sediment binnen 200 m van de lokatie tussen 2 en 4700 mg·kg⁻¹ droog sediment terwijl sporen van esters detecteerbaar waren tot op 3000 m.
2. Elf maanden na beëindiging van de boring waren esterconcentraties nog steeds meetbaar binnen 200 m (op één station werd 250 mg·kg⁻¹ droog sediment gemeten), maar aanzienlijk lager dan na 4 maanden. Vanaf 500 m waren de concentraties beneden detectieniveau.
3. Schattingen van de afbraaksnelheid van de esters kwamen gemiddeld uit op een halfwaarde-tijd van 133 dagen met als statistische betrouwbaarheidsongdergrens 68 dagen.
4. Vier maanden na beëindiging van de boring was de makrofauna aanzienlijk verarmd binnen 200 m van de lokatie. Van enkele soorten waren de aantallen verlaagd tot op 500 of 1000 m van de lokatie.
5. Elf maanden na beëindiging van de boring werden significante effecten nog steeds waargenomen binnen 200 m van de lokatie. Vanaf 500 m konden geen effecten meer worden aangetoond.
6. Binnen 200 m hadden na 11 maanden verscheidene soorten het sediment gerekoloniseerd, hetgeen er op wijst dat een herstel van de natuurlijk makrofauna-gemeenschap was ingetreden.

1 INTRODUCTION

1.1 GENERAL PART

During drilling activities in the North Sea large amounts of drill cuttings are dumped on the sea-bed. The material is always contaminated with residuals of 'drilling muds', necessarily employed to perform the drilling process. Initially these drilling muds were based on water as the basic carrier fluid (water based muds = WBM). However from the early 80's there was increasing use of oil based muds (OBM), which substantially improved the drilling efficiency, particularly in deep salt layers, and also enabled deviated drilling. Although most of the OBM were regained after the cuttings were brought to the surface, residuals of OBM on cuttings always reached the sea-bed. Concern about the environmental impact of these discharges led to a research programme in the Dutch North Sea sector that is running from 1985, to assess the effects of drill cutting discharges on the benthic system around well sites. The broad outcome of these and similar studies in other North Sea sectors was that dumping of OBM cuttings involves high disturbance of the benthic fauna composition up to some hundreds of meters from platforms and that subtle effects can be detected up to a few kilometres. Moreover, there are no convincing indications that oil in the sediment is biodegraded for the greater part at the long term. Particularly in the deep anaerobic sediment layers in the vicinity of a former drilling site oil concentrations have shown to be high even eight years after termination of OBM cutting discharges. These results have contributed to the Dutch governmental decision to put a complete ban on discharges of oil contaminated drill cuttings from 1993. Oil companies are still allowed to use OBM, but are bound to bring oil contaminated cuttings to land.

The new measure has enhanced the costs for the oil companies in those cases where drilling cannot completely be performed by using WBM. It is not surprising therefore that great pains have been taken to develop new muds based on alternative carrier fluids that have the same operational efficiency as OBM but not the adverse environmental effects. In 1990, a new mud was introduced that is based on esters (EBM) and brought out under the trade-mark 'Petrofree'. The specifications guaranteed that this drilling fluid allows for a technical performance equivalent to mineral oil, does not contain aromatic (petroleum) hydrocarbons and therefore does not raise the level of toxic hydrocarbons in the sediment when it is dumped on the sea-bed and, finally, is rapidly biodegradable in both aerobic and anaerobic environments. As yet, there is no general governmental permission in the Netherlands to dump drill cuttings contaminated with these muds on the sea floor. For the present there has been given only one licence to use and discharge EBM at a location in the Dutch sector (known as K14-13), on

the condition that the activities should be accompanied by a field monitoring programme to assess possible effects on the benthic system around the location and to estimate degradation rates of esters in the sediment after discharge. This report describes the research programme and presents the results obtained during a number of field surveys around K14-13 carried out before and during a period of 1 year after the discharges took place.

Location K14-13 is situated in the western part of the Dutch Continental Shelf (Fig. 1a) in 30 m water depth, on the edge of a longitudinal north-south depression in the sea-bed, known as 'Teakettle Hole'. The location may be considered as lying outside the sphere of influence resulting from former drilling activities in the area. The nearest well site is a WBM location at 3 km distance and since locally prevailing currents follow a direction between north and east (Ruijter *et al.*, 1987) this location may be considered as lying 'downstream' from K14-13 (Fig. 1b). Other drilling sites are all lying at ≥ 8 km from K14-13.

An exploration well was drilled at K14-13 in August - September 1993. The top hole was drilled with WBM but during drilling of the deeper transects EBM was used. Discharges of EBM drill cuttings took place from the middle of August to the beginning of September. The cuttings were dumped through a chute which extended to a depth of about 5 meters above the sea-bed. Amounts and composition of the material are listed in Table 1. Before drilling started a baseline survey was carried out in July 1993. Sediment samples were taken at 6 stations to assess the natural macrofauna composition around the location under undisturbed conditions. Post-drilling surveys were carried out 1 month, 4 months and 11 months after termination of drilling. During the 1st post-drilling survey samples were collected only for analyses of ester concentrations in the sediment. Samples collected during the 2nd and 3rd post-drilling surveys were used for both chemical and faunistic analyses. The occurrence of biological effects was detected by studying possible gradual changes in fauna composition and spatial abundance patterns of individual species that could be related to distance to the discharge site, and by comparing species specific abundances after drilling to the predrilling situation.

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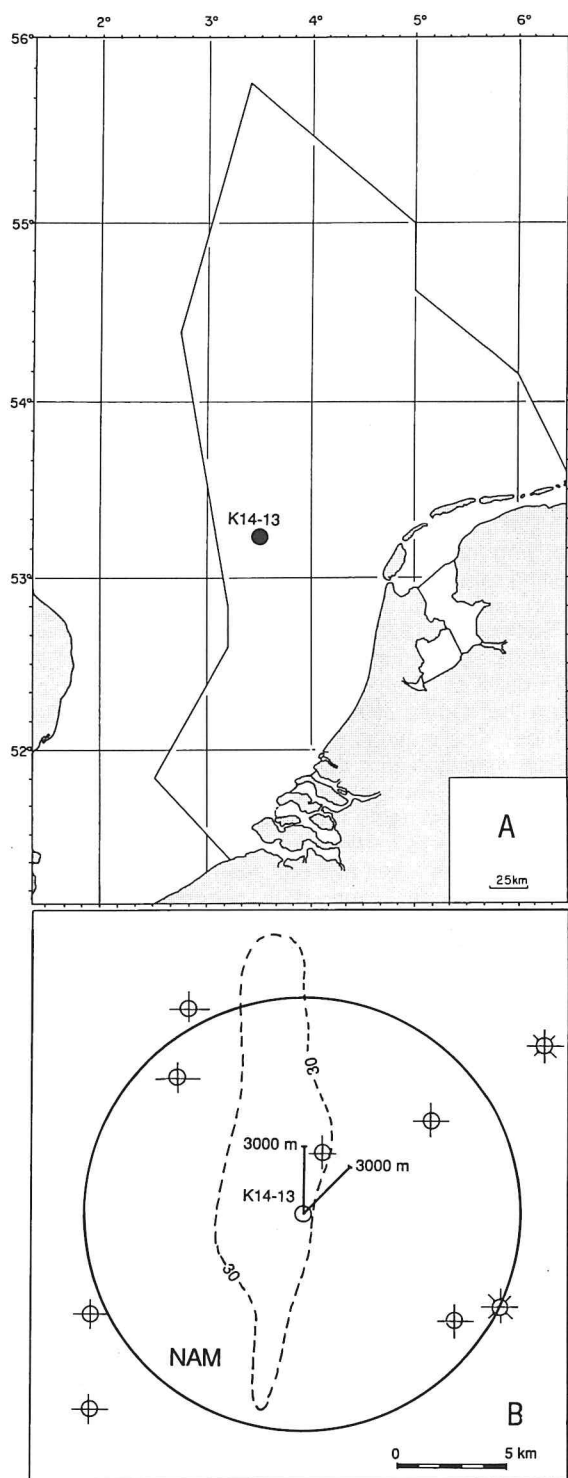


Fig. 1. Position of well site K14-13 in the Dutch sector of the North Sea (a) and its position in relation to neighbouring well sites (b). The dotted line (b) gives the contour (30-m isobath) of 'Teakettle hole'.

fieldwork. J. van der Hoek sorted the macrofauna samples. The assistance and advice of M. Dekker and W. Pool on the MS analysis is also gratefully acknowledged. Finally we would like to thank M. van Arkel for his organising helpfulness.

2 METHODS

2.1 SURVEY PROGRAMME AND SAMPLING METHODS

A baseline survey was carried out in July 1993 on board of the m.v Field Express, before the drilling platform had arrived at the location. Accurate positioning was performed by using DGPS (accuracy in position finding 5-10 m). Sampling stations were chosen at increasing distances from the location along a transect in the direction 45° (NE, see Fig. 2a), which was thought to be the local residual current direction according to Ruijter *et al.* (1987). In the opposite direction (225°) also 1 station was sampled at 75 m. Bottom samples (8 per station) were collected with a 0.16 m² Van Veen grab. The samples were washed through a sieve (mesh size 1 mm) and the residual macrofauna was preserved in a 6% neutralized formaldehyde solution for later analysis in the laboratory.

Because a steep gradient was observed in the sediment composition along the 45° transect sampled during the baseline survey, that was reflected in a

TABLE 1

Amounts and composition of drilling muds discharged at K14-13. WBM used in first transect (holesize 24") and second transect (holesize 16"). EBM used in third transect (holesize 12.25").

product	used in WBM (tonnes)	used in EBM (tonnes)
baryte	209.3	243
bentonite	35	-
caustic soda (NaOH)	0.6	-
carboxymethylcellulose	3.5	-
Xanthan gum	0.3	-
CaCl ₂	-	23.7
Amine-treated lignite	-	11.0
Polyamine	-	11.6
Organo-clay	-	2.2
Lime (Ca(OH) ₂)	-	2.6
sulphonated ester	-	0.1
Amidoamine	-	1.6
Polymeric fatty acid	-	0.9
Petrofree ester	-	180.5
Total WBM	248.7 (833 m ³)	-
Total EBM	-	477.2 (361 m ³)

gradual but unmistakable change in the natural fauna composition (see chapter 3) it was decided that this transect was unsuited for further use to assess possible biological effects in post-drilling surveys. The reason is that possible changes in the fauna composition after drilling, which are most likely to be expected in the close vicinity of the location, can not be related to an adequate reference situation in the 45° direction. The need was felt to search for an alternative sampling transect, that was more likely to meet the requirement of more or less homogeneous natural sediment characteristics. Therefore a main transect was chosen for the post-drilling surveys in the direction 0° (N). This choice was based on the fact that local depth contours are running northward, so that the assumption seemed to be justified that the sampling stations over this transect did not differ substantially in depth. Moreover, according to an oceanographic rule of thumb, prevailing bottom currents generally tend to run parallel to depth contours, which implies that stations along the 0° transect would be situated more or less downstream from the K14-13 discharge site.

A limited survey was carried out in October 1993, one month after termination of the EBM cutting discharges. During this survey four stations were sampled in the vicinity of the platform (Fig. 2b) to assess contamination levels in the sediment shortly after the material was dumped. At each station 8 Van Veen grab samples were collected and at the 125-m station also 8 boxcores were taken. The samples were merely used for chemical analyses, however not only to assess ester concentrations but also to check the absence of possible oil contamination in the sediment. This check was felt necessary because an extensive oil-slick had been observed by the crew of the platform 2 weeks before the survey took place. The oil-slick, which did not have any relation with the local drilling activities, was observed when it was floating exactly over the location and the possibility should be reckoned with that traces of oil could have reached the sea-bed around the location. The oil analyses were carried out by using FTIR (according to NEN 6675) and were performed by NAM (see Appendix 4).

The 2nd and 3rd post-drilling surveys were carried out in January 1994 and August 1994, *i.e.* 4 months and 11 months after termination of EBM drilling respectively. Since the drilling platform had been removed from the location, the well site had to be traced by an accurate positioning system. To that end DGPS was used. During both surveys 6 stations were sampled along the 0° transect and also 4 stations within 200 m from the location, that had been sampled during the baseline survey (Fig. 2c). At each station 8 grab samples were taken. Additionally, at the station 125 m (0°) 8 boxcores were collected.

Surface sediments for chemical analyses were collected by taking subsamples from Van Veen grabs

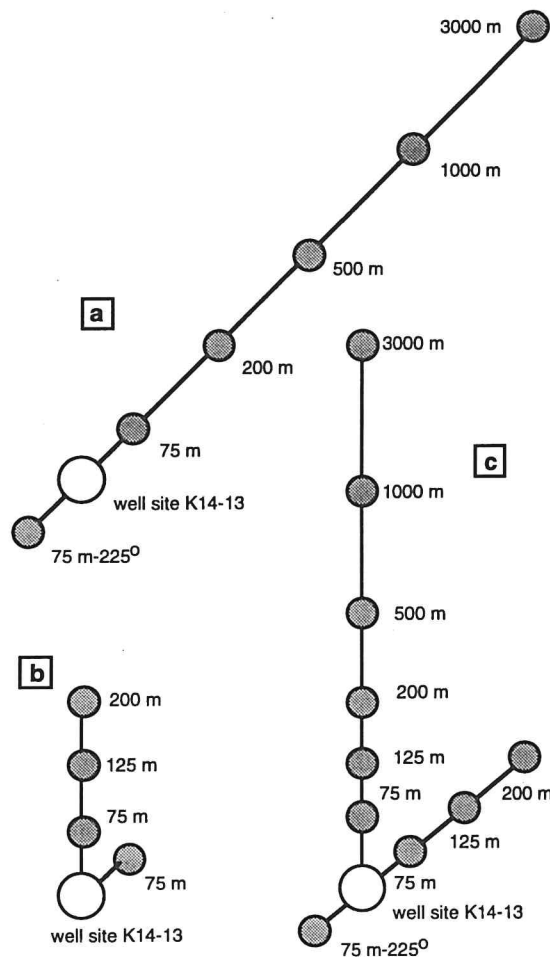


Fig. 2. Positions of the sampling stations during the baseline survey (a), the 1st post-drilling survey (b) and the 2nd and 3rd post-drilling surveys (c).

and boxcores. From each grab or boxcore, 2 subcores were taken (diameter 28 mm, depth 10 cm). Except for the 0° 125 m station the subcores of two grabs were pooled on board of the ship. At the 0° 125 m station, the 2 subcores were pooled for each grab or box separately. All samples were stored in 200 ml glass jars, that had been heated overnight at 300° C, and immediately frozen at -18° C. During the 1st post-drilling survey, a two kg sample was collected for the preparation of an Internal Reference Material (IRM). This sample was thoroughly mixed and stored in 100 ml glass jars. Upon arrival in the laboratory, sediment samples were stored at -80° C. When necessary, the samples were further pooled in the lab by mixing equal wet weights in a glass beaker, while preserving the original unpooled samples.

TABLE 2
Survey schedule and programme of analyses; VV = Van Veen grab; BC = Boxcorer.

<i>Baseline survey (July 1993, 1 month before EBM drilling)</i>						
<i>station</i>	<i>sampling gear</i>	<i>number of samples</i>	<i>ester analyses</i>	<i>oil (THC) analyses</i>	<i>counts of E. cordatum</i>	<i>fauna analyses</i>
45° - 75 m	VV	8			8	6
200 m	VV	8			8	6
500 m	VV	8			8	6
1000 m	VV	8			8	6
3000 m	VV	8			8	6
225° - 75 m	VV	8			8	6
<i>First post drilling survey (October 1993, 1 month after EBM drilling)</i>						
0° - 75m	VV	8	1	2		
125 m	VV	8	8	2		
125 m	BC	8	8			
200 m	VV	8	1	2		
45° - 75 m	VV	8	1	2		
<i>Second post-drilling survey (January 1994, 4 months after EBM drilling)</i>						
0° - 75m	VV	8	1		8	6
125 m	VV	8	8		8	6
125 m	BC	8	8		8	
200 m	VV	8	1		8	6
500 m	VV	8	1		8	6
1000 m	VV	8	1		8	6
3000 m	VV	8	1		8	6
45° - 75 m	VV	8	1		8	
125 m	VV	8	1		8	
200 m	VV	8	1		8	
225° - 75 m	VV	8	1		8	
<i>Third post-drilling survey (August 1994, 11 months after EBM drilling)</i>						
0° - 75m	VV	8	1		8	5
125 m	VV	8	8		8	5
125 m	BC	8	8		8	
200 m	VV	8	1		8	5
500 m	VV	8	1		8	5
1000 m	VV	8	1		8	5
3000 m	VV	8	1		8	5
45° - 75 m	VV	8	1		8	
125 m	VV	8	1		8	
200 m	VV	8	1		8	
225° - 75 m	VV	8	1		8	

TABLE 3

Ester composition (%) in the standard mixture as determined by the relative FID response area.

<i>standard</i>	<i>85 µg/mL</i>	<i>138 µg/mL</i>	<i>average</i>	<i>difference</i>
C8	5.82	5.96	5.9	0.1
C10	5.11	5.17	5.1	0.1
C12	66.69	66.55	66.6	0.1
C14	19.94	19.74	19.8	0.2
C16	2.43	2.58	2.5	0.1

During fieldwork, numbers of sea urchins (*Echinocardium cordatum*) were counted in all samples, except for the 1st post-drilling survey where the samples were used for chemical analyses only. Not all samples were analysed in the laboratory. Table 2 gives an overview of the complete programme of analyses applied to the samples collected during the 4 surveys.

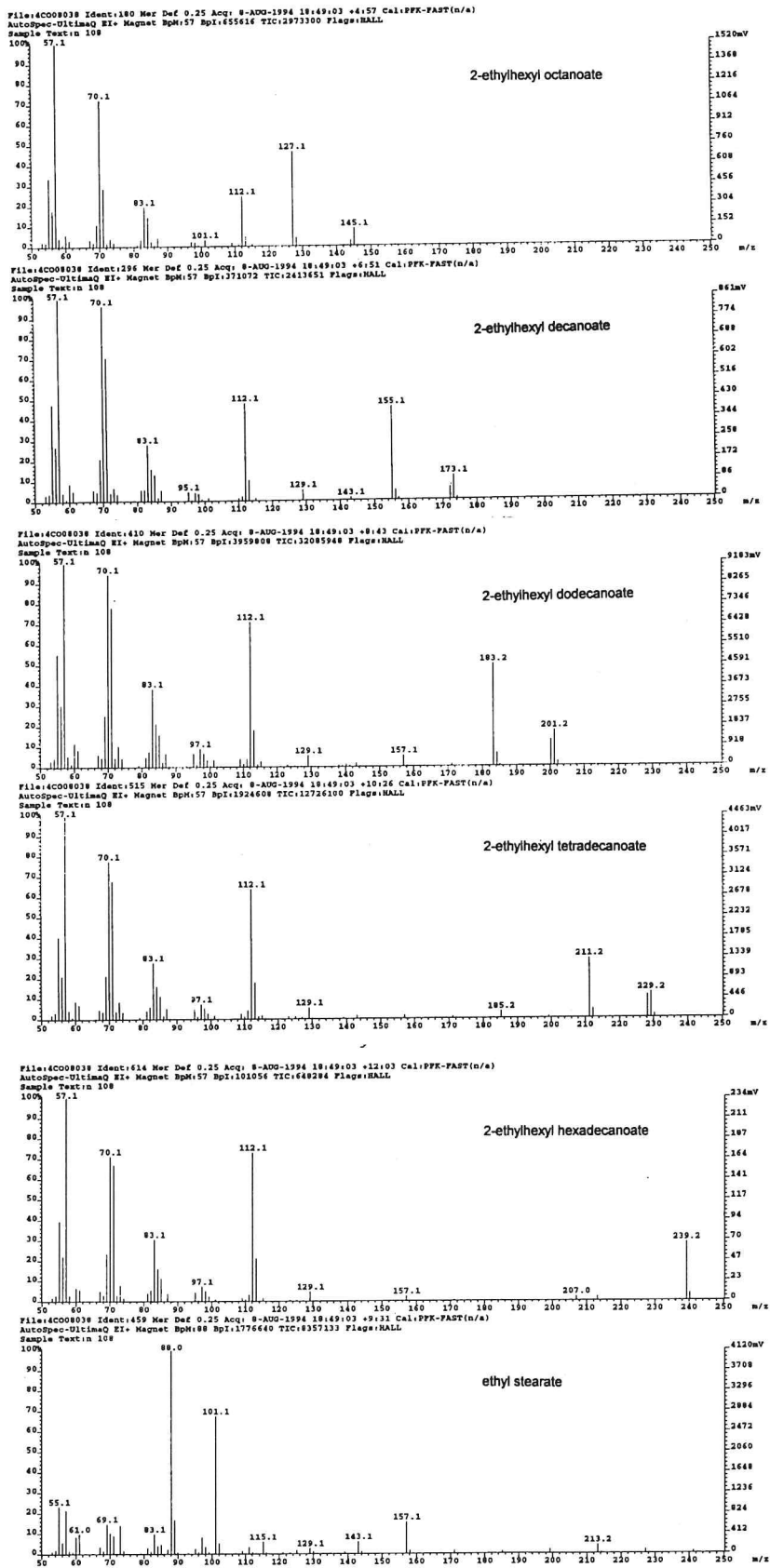


Fig. 3. Mass spectra of the components of the ester mixture and of ethyl stearate (f).

2.2 CHEMICAL ANALYSIS

2.2.1 MATERIALS AND METHODS

The compounds to be analysed are the 2-ethyl hexanol esters of unbranched aliphatic acids with 8, 10, 12, 14 and 16 carbon atoms. A sample of the mixture used during the drilling was supplied by Baroid. Three calibration standards were prepared in 2,2,4-trimethylpentane (TMPE) with concentrations of 5, 100 and 180 $\mu\text{g}/\text{mL}$. The relative composition of the components in the mixture was determined by evaluating the relative areas in the FID response (Table 3). Ethyl stearate and pyrene D₁₀ were used as internal standards. The former was added at the beginning of the extractions, the latter was added prior to injection. Calibration mixtures were prepared by adding 100 μl of both internal standard solutions to 800 μl of the ester standards. Response functions were calculated by weighed linear regression of the relative peak areas of the esters and pyrene D₁₀ versus the ester concentration. Calibration functions were calculated by taking the inverse of the response function. The amounts of the esters were corrected for the recovery of ethyl stearate. When necessary, samples were diluted 10-100 times in order to meet the restrictions posed by the linear range of the detector. The amount of sediment to be extracted was always in excess of 4 g wet weight (3 g dry weight) in order to avoid prob-

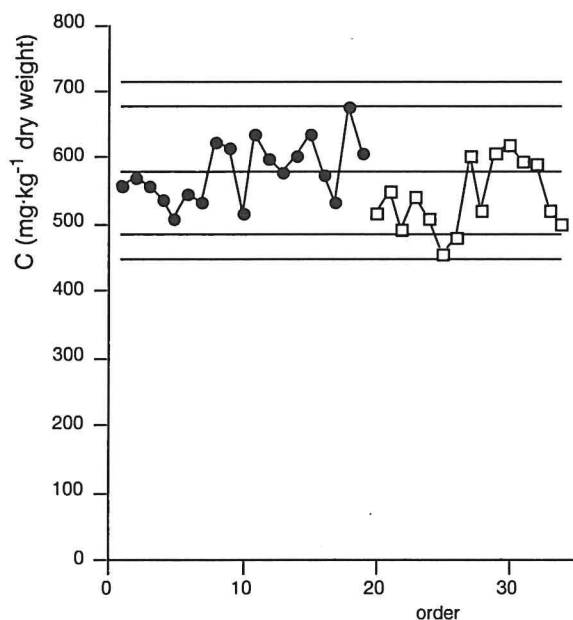


Fig. 4. Total ester concentration in the Internal Reference Material for the learning set (circles) and for the IRMs analysed together with the survey samples (squares). Drawn lines indicate average, and 95% and 99% confidence limits of the learning set.

TABLE 4

Total ester concentrations in the Internal Reference Material and ethyl stearate recovery as determined by three extraction methods. Values are listed as average \pm standard deviation.

method	C ester (mg/kg)	CV (%)	recovery ethyl stearate
soxhlet	597 \pm 37	6	0.90 \pm 0.17
acetone+acetone/hexane	554 \pm 46	8	0.86 \pm 0.14
DCM/MeOH/water	584 \pm 49	8	0.96 \pm 0.14
all	578 \pm 46	8	0.91 \pm 0.15

lems with sub-sample inhomogeneity.

Samples were injected on a HP 5890 series II gas chromatograph equipped with a HP 7673 autosampler, and a CP-Sil 5CB capillary column, (25 m, 0.32 mm id, film thickness 0.12 μm), carrier gas He. Injection mode: cold on column. The GC temperature program was: initial temperature 100° C (hold 0 min), increase 10 °C/min, final temperature 300 °C (hold 5 min). The GC was coupled to a Fisons Auto-Spec Ultima/Si84QU mass spectrometer. Ionisation mode electron impact (70 eV), scan range 400-50, scan rate 0.8 s/decade, scan delay 0.25 s.

2.2.2 QUALITY ASSURANCE & QUALITY CONTROL

2.2.2.1 DETECTION AND IDENTIFICATION

The mass spectra of the petrofree esters and of ethyl stearate are shown in Fig. 3. The petrofree esters have a common ion at $m/z=112$, which can be identified as an ethyl hexanol fragment (C_8H_{16}). Other prominent peaks are the acid fragments $\text{C}_8\text{H}_{15}\text{O}$, $\text{C}_{10}\text{H}_{19}\text{O}$, $\text{C}_{12}\text{H}_{23}\text{O}$, $\text{C}_{14}\text{H}_{27}\text{O}$ and $\text{C}_{16}\text{H}_{31}\text{O}$ ($m/z=127$, 155, 183, 211, and 239 respectively). At low ester to hydrocarbon ratios interference with alkane residues may be expected for the $m/z=112$ ion. The acid fragment trace was used to confirm the identity of the $m/z=112$ trace by calculating the peak height ratio of the $m/z=112$ trace and the acid fragment trace. The quantitation limit was thus estimated as 0.05 mg/kg for the individual components. Components were listed as not-detected whenever the signal to noise ratio of either the $m/z=112$ trace or the acid fragment trace was smaller than 3 (≈ 0.01 mg/kg).

2.2.2.2 COMPARISON OF EXTRACTION METHODS

Three methods for the extraction of the esters were compared. These methods are described in detail in Appendix 1-3, and briefly are as follows. The soxhlet method was adapted from Bakke & Laake (1991), and comprised an initial extraction of the wet sediment with methanol, followed by a second extraction with DCM. The combined extracts were shaken with bidistilled water, and extracted two times with DCM.

Extracts were concentrated and the DCM replaced with 2,2,4-trimethyl pentane (TMPe). Next to the Soxhlet method, two cold blend methods were used. In one method, the wet sediment was extracted with acetone on a table shaker (1h), followed by a 1.5 h extraction with acetone/hexane (30/70) (Jensen *et al.* 1977). The combined extracts were shaken with water. After addition of 1 mL TMPe, the organic phase was concentrated to about one mL. In the second cold blend method the wet sediment was extracted with a single phase mixture of DCM, MeOH and water (1/2/0.8 v/v) (Bligh & Dyer, 1957; Booij & Van den Berg, 1994). After extraction DCM was added to obtain a volume ratio DCM:MeOH:water=2:2:0.8. Then water was added and the DCM phase was isolated.

The results are summarized in Table 4. No significant differences were observed between the three methods for either the means or the standard deviations. The recovery of ethyl stearate was 86-96%. Differences between methods were not statistically significant. The DCM/MeOH/water method was selected for the remainder of the project, because of the lower solvent demand and the greater ease of operation.

2.2.2.3 STABILITY OF THE ANALYSIS

With each series of samples, one or two IRM samples were processed in order to check the stability of the analysis. A Shewhart chart of IRM results is shown in Fig. 4. Results for the extraction method comparison were used as a learning set (circles). Average and 95% and 99% confidence limits are shown as drawn lines. Results for all IRMs analysed during processing of the samples for the three surveys are indicated as squares. All measurements fall within the alarm limits (upper and lower 99% confidence limits). This indicates that the analysis has been under control for the entire project. The coefficient of variation for all IRMs analysed during the project was 9%.

The average recovery of ethyl stearate for all samples analysed during the project was 0.95 with a standard deviation of 0.15.

2.3. MACROFAUNA ANALYSES

In the laboratory a selection of the fauna samples was stained with Bengal rose and sorted using a magnifier. Then molluscs, crustaceans, polychaetes and echinoderms were identified under a stereomicroscope and counted on species level. Remaining taxa were not further identified and recorded only at higher taxonomic levels. When species were broken by handling, only heads were counted.

For each survey the presence of significant gradients in the frequency of occurrence of individual species over the sampled transect was tested by logit regression (see Jongman *et al.*, 1987). The regres-

sion was applied to all species of which at least 20 specimens were found, according to the improved procedure described by Daan *et al.* (1994). This implies that in first instance model (0)

$$\text{logit}(\pi) = \ln(\pi/(1-\pi)) = \exp(b_0) \quad (1)$$

(predicting that the probability (π) of a species being present in a sample does not depend on distance to platform) is tested against model (1)

$$\text{logit}(\pi) = \ln(\pi/(1-\pi)) = \exp(b_0 + b_1 \cdot \log(d)) \quad (2)$$

(predicting that there is a systematic increase or decrease of π with increasing distance from the platform). Here d is distance to platform and b_0 and b_1 are model parameters, which are estimated according to the maximum likelihood principle. Rejection of Model (0) in favour of Model (1) does not necessarily mean that Model (1) gives a perfect fit. There still may be a considerable deviation of the observed values and the fitted values, due to over-dispersion in the data. Therefore model (1) is further tested against the full model

$$\text{logit}(\pi) = \ln(\pi/(1-\pi)) = \exp(b_i) \quad (3)$$

where b_i is directly estimated from the relative frequency of occurrence of the species at the i^{th} station. When the difference in log-likelihood between the full model and model (1) is large this may be reason to decide that a possible significant gradient in frequency of occurrence as established by the first test is due to over-dispersion in the data.

Possible shifts in the macrofauna community along the sampled transects were tested by comparing the relative abundance of all identified species at each of the stations (ANOVA). This method is described in detail by Daan *et al.* (1990).

3 RESULTS

3.1 CONCENTRATIONS OF ESTERS IN THE SEDIMENT

Concentrations of the esters in the sediment are summarized in Table 5a-c.

3.1.1 VARIABILITY AT THE 0° 125M STATION

For the 0° 125 m station samples were analysed for the individual boxes and grabs. The results were tested for normality by applying the Lilliefors test to the untransformed and log-transformed data (Table 6). If the variable is normally distributed, then p gives the probability of finding a deviation from normality larger than or equal to the value listed in Table 6. For the grabs of the first survey and the grabs and the boxes of the second survey the untransformed data

TABLE 5
Ester concentrations (mg/kg⁻¹ dry sediment) in grabsamples (G) and boxcores (B).

<i>a. First post-drilling survey</i>								
<i>transect (°)</i>	<i>distance (m)</i>		<i>C8</i>	<i>C10</i>	<i>C12</i>	<i>C14</i>	<i>C16</i>	<i>sum</i>
0	75		8.4	12.3	138.7	42.2	4.3	205.8
0	125	B1	1.6	1.8	25.9	5.9	0.6	35.8
0	125	B2	4.1	5.4	72.1	15.6	1.5	98.7
0	125	B3	7.7	8.3	100.4	30.2	3.2	149.8
0	125	B4	23.3	26.7	271.0	98.4	12.6	432.0
0	125	B5	17.8	20.8	198.2	73.1	9.0	318.9
0	125	B6	31.8	34.2	313.7	116.4	14.7	510.8
0	125	B7	38.2	46.3	405.0	159.3	21.1	669.9
0	125	B8	7.0	7.8	111.6	28.4	2.7	157.4
0	125	box mean						296.7
0	125	G1	3.4	3.5	44.8	12.6	1.3	65.7
0	125	G2	0.6	0.6	7.2	2.4	nd	10.8
0	125	G3	3.6	3.7	49.2	13.0	1.2	70.6
0	125	G4	2.4	2.4	35.8	9.8	1.0	51.4
0	125	G5	37.3	45.4	426.1	170.9	21.3	701.1
0	125	G6	5.6	6.3	87.3	23.8	2.1	125.1
0	125	G7	t	0.1	1.4	0.2	t	1.8
0	125	G8	0.1	0.1	1.9	0.3	0.1	2.4
0	125	grab mean						128.6
0	200		2.2	2.7	38.4	9.9	1.1	54.2
45	75		0.4	1.0	14.2	2.7	0.3	18.6
<i>b. Second post-drilling survey</i>								
<i>transect (°)</i>	<i>distance (m)</i>		<i>C8</i>	<i>C10</i>	<i>C12</i>	<i>C14</i>	<i>C16</i>	<i>sum</i>
0	75		17.0	19.5	282.8	68.4	5.6	393.2
0	125	B1	4.8	4.8	54.9	12.5	0.9	78.0
0	125	B2	21.3	27.5	252.0	77.0	6.0	383.7
0	125	B3	38.0	53.2	468.2	151.1	13.0	723.5
0	125	B4	0.7	0.8	7.4	2.4	0.3	11.5
0	125	B5	39.2	63.5	403.2	176.0	16.1	698.0
0	125	B6	3.8	4.5	36.5	12.3	0.8	57.8
0	125	B7	205.2	268.5	3142.2	961.6	92.0	4669.5
0	125	B8	2.3	3.0	31.6	9.1	0.7	46.7
0	125	box mean						833.6
0	125	G1	0.1	0.1	1.2	0.3	0.1	1.7
0	125	G2	4.0	4.7	53.9	13.1	0.9	76.7
0	125	G3	73.5	97.0	866.1	308.2	20.4	1365.1
0	125	G4	0.8	0.8	7.1	2.6	nd	11.3
0	125	G5	13.8	17.6	177.2	51.9	3.9	264.5
0	125	G6	3.2	6.2	73.1	14.8	1.1	98.3
0	125	G7	3.8	4.1	46.7	12.6	0.8	67.9
0	125	G8	1.8	2.1	22.2	6.4	0.5	32.9
0	125	grab mean						239.8
0	200		5.2	7.7	118.9	27.2	2.0	160.9
0	500		t	t	0.4	0.1	t	0.5
0	1000		t	t	t	t	t	t
0	3000		t	t	t	t	t	t
45	75		0.9	1.5	14.0	4.5	0.5	21.4
45	125		1.6	2.0	27.8	7.2	0.6	39.2
45	200		1.0	1.3	15.0	4.0	0.4	21.7
225	75		19.1	19.8	296.3	69.6	5.6	410.4

c. Third post-drilling survey

transect (°)	distance (m)		C8	C10	C12	C14	C16	sum
0	75		1.6	3.1	61.5	16.2	1.7	84.2
0	125	B1	0.6	0.8	12.3	3.9	0.5	18.0
0	125	B2	0.5	0.6	7.8	3.0	0.4	12.4
0	125	B3	0.5	0.8	12.7	3.7	0.4	18.1
0	125	B4	nd	0.1	2.9	1.0	0.1	4.1
0	125	B5	nd	0.1	1.9	0.7	0.1	2.7
0	125	B6	nd	0.2	3.7	1.5	0.2	5.5
0	125	B7	0.1	0.2	3.8	1.3	0.1	5.5
0	125	B8	0.1	0.5	6.1	2.3	0.2	9.3
0	125	box mean						9.5
0	125	G1	0.8	0.9	12.1	4.2	0.6	18.5
0	125	G2	1.0	1.5	27.0	7.2	0.9	37.6
0	125	G3	0.9	0.9	12.0	4.1	0.5	18.4
0	125	G4	0.1	0.3	4.3	1.6	0.3	6.7
0	125	G5	0.6	0.7	10.1	3.5	0.5	15.4
0	125	G6	nd	0.2	3.1	1.1	0.1	4.4
0	125	G7	0.7	0.8	8.7	3.1	0.4	13.7
0	125	G8	nd	0.1	1.2	0.4	nd	1.6
0	125	grab mean						14.5
0	200		1.3	2.0	39.0	11.1	1.3	54.6
0	500		nd	nd	nd	nd	nd	nd
0	1000		nd	nd	nd	nd	nd	nd
0	3000		nd	nd	nd	nd	nd	nd
45	75		0.3	0.7	10.7	3.7	0.5	15.8
45	125		t	0.1	1.8	0.6	0.1	2.6
45	200		nd	t	0.3	0.1	t	0.5
225	75		8.5	15.9	164.4	57.3	5.3	251.4

deviate substantially from normality ($p=0.002$). The probability of the largest deviations of normality for the log-transformed concentrations is larger than 0.22 in all cases.

In the following, log-normality will be assumed for the concentration data. The average of the log-transformed data (*i.e.* the maximum of the log normal distribution) gives after back transformation the geometric mean (C_{geom})

$$\exp\left(\frac{\sum_{i=1}^N \ln(C_i)}{N}\right) = \sqrt[N]{\prod_{i=1}^N C_i} = C_{\text{geom}} \quad (4)$$

The distributions can be characterised by a mean and a standard deviation ($\ln(C_{\text{geom}}) \pm s$). After back transformation, the standard deviation transforms into an uncertainty factor:

$$\exp(\ln C_{\text{geom}} \pm s) = C_{\text{geom}}^{*/} \div \exp(s) \quad (5)$$

3.1.2 BOX-GRAB COMPARISON

Means and standard deviations of the log transformed data are shown in Table 7. The mean of the box core results for the first survey is significantly different from mean of the Van Veen grab results. For the other surveys no significant differences were observed for the means. For the standard deviations, no significant differences were observed between boxes and grabs. Standard deviations for the third survey were significantly smaller than those of the first and second survey at the 95% and 99% confidence level (two-tailed F-test). This result indicates that the spatial variability decreases with time.

3.1.3. POOLING AND CONFIDENCE INTERVALS

In order to reduce the number of samples to be analysed, the subsamples from the individual grabs were pooled prior to analysis. The consequence of not analysing the individual samples is that neither the geometric means nor their variances can be estimated. Instead, the arithmetic mean of the distribution is measured only. Estimates of the confidence intervals

TABLE 6
Results of the Lilliefors normality test for boxes and grabs at the 0° 125 m station.

survey	sampling device	untransformed data		log transformed data	
		deviation from normality	p	deviation from normality	p
1	box	0.233	0.254	0.163	1.000
1	grab	0.381	0.001	0.220	0.348
2	box	0.403	0.000	0.183	0.739
2	grab	0.370	0.002	0.159	1.000
3	box	0.240	0.211	0.184	0.730
3	grab	0.238	0.221	0.237	0.225

of the arithmetic mean were obtained by the bootstrapping technique for all stations for which the individual samples were analysed (boxes and grabs 125 m 0° stations). Sampling was simulated by selecting eight samples randomly from the available measurements and calculating the average. After 1000 simulations the upper (L^+) and lower (L^-) confidence limits were estimated by specifying the values that comprised 95% of the calculated means (2.5% larger than the upper limit, 2.5% smaller than the lower limit). The results are summarized in Table 8a-c. The ratios average/ L^- and L^+ /average show that the confidence intervals are non-symmetrical. The lower confidence limit is about a factor of 4 smaller than the mean, the upper confidence limit is about a factor of two larger than the mean. This implies that concentration differences between surveys should be larger than about a factor of 8 in order to be statistically significant.

3.1.4 SPATIAL DISTRIBUTION

The spatial distribution of the esters in the three post-drilling surveys is shown in Fig. 5. For the 0° transect (top panel) the concentrations are fairly constant at distances smaller than 200 m, and drop rapidly to trace levels at larger distance. Qualitatively, the spatial distribution is very similar for the three surveys. For the 45° transect (bottom panel) ester concentrations have a constant level of about 30 mg/kg in the second survey. In the third survey concentrations decrease rapidly to trace levels at 200 m from the well.

3.1.5 TEMPORAL TRENDS

Some qualitative notions on the time evolution of ester concentrations can be derived from Fig. 5. Without exception the concentrations for the second survey were higher than for the first survey by a factor of 1.2 to 3. These differences are not statistically significant when the stations are compared on an individual basis. The average concentration difference between first and second survey was a factor of 2.0. This value is significantly different from zero at the 95% confidence level (two tailed t-test, $N=5$). The reason for this difference could be that contaminated sediment

at short distance from the well (<75 m) has spread out into the sampling region in the time period between the first and the second survey. Except for the 45° 75 m station, concentrations during the third survey are substantially lower than during the second survey.

More quantitative estimates of the time evolution can be obtained by assuming a first order decay process that can be described by

$$C=C_0 \exp^{-kt} \quad (6)$$

or

$$\ln(C) = \ln(C_0) - kt \quad (7)$$

where C_0 is the concentration at $t=0$ and k is the first order decay constant. The reciprocal value of k has the dimension of time, and can be regarded as a characteristic time scale for decrease. Equation (7) was applied to the data of the first, second and third survey, as well as for the second and third survey only. The results are summarized in Table 9. Rate constant estimates followed a normal distribution and attained a mean value of 0.0052 day^{-1} . This value corresponds to a half-life time ($t_{1/2}$) of 133 days (95% decrease in 580 days). This value is significantly different from zero at the 95% confidence level (one-

TABLE 7

Comparison between box cores and Van Veen grabs for sampling at the 0° 125m station after log transformation of the data (mg/kg). A two-tailed t-test was applied to the means. A two-tailed F-test was applied to the standard deviations. Significant differences are indicated by *) (95% confidence level).

survey		mean	std	comparison	comparison
				of means	of std
				t-value	F-value
1	box	5.35	1.05	2.49)*	0.31
1	grab	3.45	1.89		
2	box	5.28	2.00	1.19	0.86
2	grab	4.05	2.15		
3	box	2.05	0.75	-0.79	1.13
3	grab	2.33	0.71		

TABLE 8a

Estimates of the arithmetic mean (avg.), upper and lower 95% confidence limits of the mean (L^+ and L^-) and ratios between these quantities. Arithmetic means were computed by bootstrapping of the concentration measurements (mg/kg) of the 125 m station on the 0° transect.

survey		L^-	avg.	L^+	avg./ L^-	L^+ /avg.
1	box	152	295	445	1.9	1.5
2	box	138	833	1946	6.0	2.3
3	box	6	9	14	1.7	1.4
1	grab	26	127	297	5.0	2.3
2	grab	41	239	594	5.8	2.5
3	grab	8	15	23	1.9	1.6

TABLE 8b

Estimates of the geometric mean (avg.), upper and lower 95% confidence limits of the mean (L^+ and L^-) and ratios between these quantities. Geometric means were computed by assuming a log normal distribution of the concentration measurements (mg/kg) of the 125 m station on the 0° transect.

survey		L^-	avg.	L^+	avg./ L^-	L^+ /avg.
1	box	88	211	507	2.4	2.4
2	box	37	196	1045	5.3	5.3
3	box	4	8	15	1.9	1.9
1	grab	6	32	153	4.9	4.9
2	grab	10	57	346	6.0	6.0
3	grab	6	10	19	1.8	1.8

TABLE 8c

Ratio between arithmetic and geometric means for concentrations at the 125 m station on the 0° transect.

survey		ratio
1	box	1.4
2	box	4.2
3	box	1.2
1	grab	4.0
2	grab	4.2
3	grab	1.4

tailed t-test). The 95% confidence limit of the mean k was 0.010 day^{-1} . This value corresponds to a half-life time of 68 days (95% decrease in 294 days). This would indicate that the degradation rates in the field situation were substantially lower than found under (anaerobic) experimental conditions, where 83% of the esters degraded in 35 days ($t_{1/2} = 14$ days, Steber *et al.*, 1994). In mesocosm experiments 99% decrease was observed over a period of 184 days ($t_{1/2} = 27$ days, Bakke & Laake, 1991). It should be noted, however, that both biodegradation and sediment redistribution determine the value of the measured decrease rates in the present study. Biodegradation rates may be higher or lower than the observed decrease rates. On one hand, if highly contaminated sediments continuously, or intermittently, form deposits on less contaminated sediments, the biodegradation rates could be higher than the

observed decrease rates. On the other hand, if new deposits are formed by less contaminated sediments, the biodegradation rates would be smaller than the observed decrease rates.

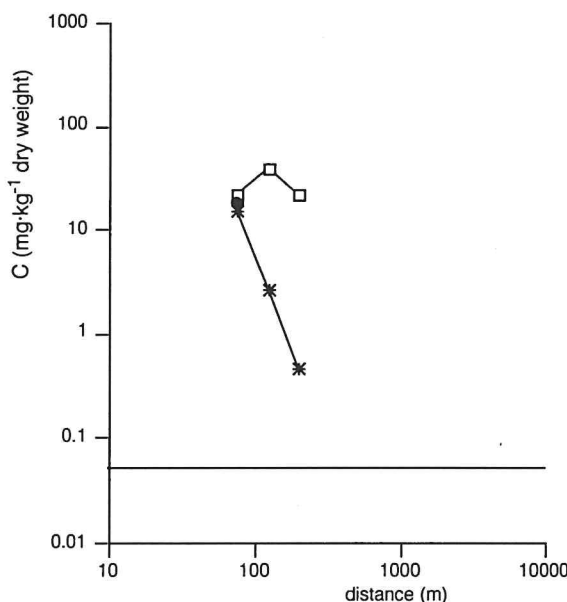
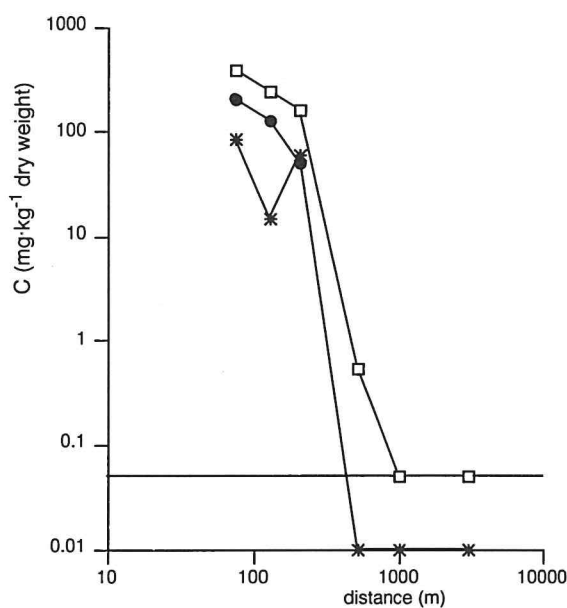


Fig. 5. Geographical distribution of esters in the sediment for the 0° transect (top panel) and the 45° transect (bottom panel) during the 1st (circles), 2nd (squares) and 3rd (asterisks) post-drilling survey. The lines $C = 0.05$ and $C = 0.01$ mg/kg represent trace amounts and non detected values respectively.

TABLE 9

First order decay rate constants (k) of esters in the sediment.

surveys			1+2+3	2+3
transect	distance	sampler	k (day ⁻¹)	k (day ⁻¹)
0	75	grab	0.0036	0.0073
0	125	grab	0.0081	0.0132
0	200	grab	0.0008	0.0051
45	75	grab	0.0007	0.0014
45	125	grab		0.0128
45	200	grab		0.0181
225	75	grab		0.0023
0	125	box	0.0128	0.0211
avg.			0.0052	0.0086
std			0.0052	0.0073
t			2.23	3.34
p (one-tailed)			p<0.05	p<0.01

3.1.6 RELATIVE COMPOSITION

The concentration ratios of the various esters in the sediments did not change with distance from the well, and were fairly constant for all three post-drilling surveys. Fig. 6 shows the ratio of the ester concentra-

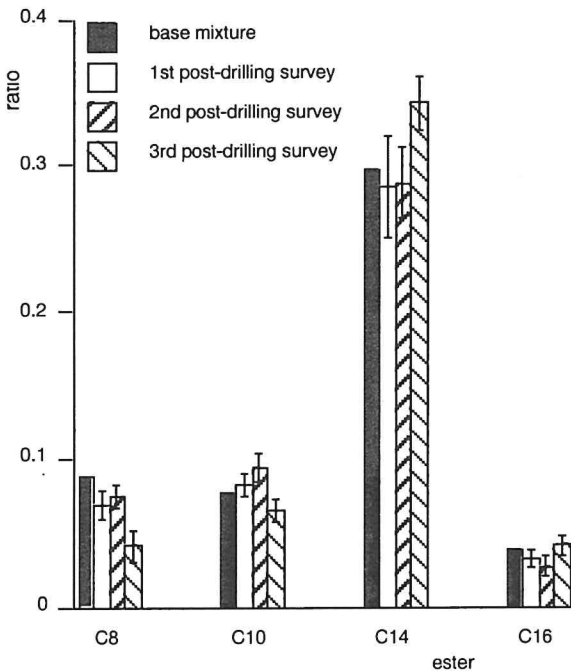


Fig. 6. Ester concentrations relative to the concentration of the C12 ester in the original ester mixture (black bar), and the 1st (white), 2nd (hatched upward) and 3rd (hatched downward) post-drilling surveys. The error bars represent the 95% confidence limits of the means.

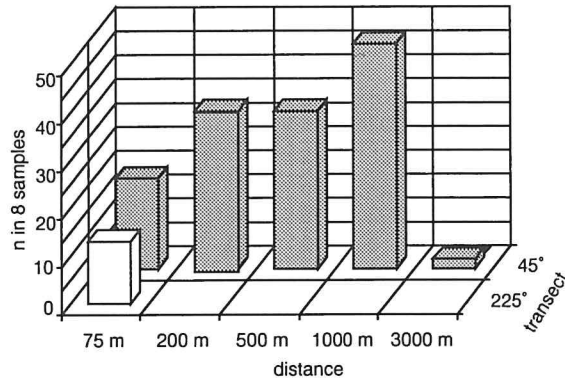


Fig. 7. Baseline survey, July 1993: Spatial abundance pattern of *Echinocardium cordatum* (number of specimens >15 mm in 8 samples).

tions relative to the concentration of the C12 ester for the original ester mixture oil (black bar) and for the sediments collected during the three surveys (white and hatched bars). No significant differences were found between the ratios for the first and second survey. C8/C12 and C10/C12 ratios for the third survey were significantly smaller than for the first and second survey at the 99% confidence level. C14/C12 and C16/C12 ratios were larger than for the first and second survey (99% and 95% confidence level respectively). These results indicate that for the third survey, the lower molecular weight esters are depleted relative to the higher molecular weight esters. This may be caused by differences in solubility as well as by differences in degradability of the ester components.

3.2 MACROBENTHOS

3.2.1 BASELINE SURVEY, JULY 1993

3.2.1.1 ON BOARD OBSERVATIONS

Based on visual inspections of the grab samples during fieldwork, the impression was that the sediment consisted of fine sand mixed with silt up to 200 m from the location and large specimens (>15 mm) of *Echinocardium cordatum* occurred frequently in the samples (see Fig. 7). Numbers ranged from 1 to 8 per sample, i.e. 5 - 50 m⁻². Between 200 and 500 m there was an abrupt change in the sediment composition. The silt fraction disappeared and there was an increase of more or less coarse sand. High numbers of *E. cordatum* were still present in the samples up to 1000 m. At 3000 m the sediment composition was different again and contained much very coarse material in the form of large numbers of empty shells and only 2 specimens of *E. cordatum* were found.

3.2.1.2 GENERAL FAUNA DESCRIPTION

The macrofauna analyses of 30 samples collected up to 1000 m from the location yielded 73 identified species. In Table 10 their percentual occurrence in the samples is summarized. The original data are listed in Appendix 5. The fauna in the area was dominated by juvenile *Echinocardium cordatum* (within 200 m) and by the polychaete *Magelona papillicornis* (500 - 1000 m). Within 200 m *E. cordatum* accounted for 86% of the total macrofauna and at 500 - 1000 m *M. papillicornis* accounted for 28%. The total fauna abundance was 4 - 8 times higher at the stations within 200 m than at the 500 m and 1000 m stations, due to the high numbers of juvenile *E. cordatum* within 200 m (Fig. 8a). However, when the dominant species are

excluded, the total fauna abundance appears to fluctuate within a narrow range of 500 - 700 ind·m⁻² at all stations (Fig. 8b).

There was no systematic spatial trend in the numbers of species at the different stations. The numbers ranged between 41 and 49 per station (Fig. 9). Also the mean number of species per sample for each station fluctuated within a narrow range (20.5 - 22.8 species per sample) and significant differences between stations were not detected (ANOVA).

3.2.1.3 ABUNDANCE PATTERNS OF INDIVIDUAL SPECIES

There were 25 species of which ≥20 specimens were

TABLE 10
Baseline survey, July 1993. Percentage of occurrence of each species in the total number of samples (30).

POLYCHAETA		<i>Lanice conchilega</i>	77	<i>Pseudione spec.</i>	3
		<i>Lagis koreni</i>	43	<i>Megaluropus agilis</i>	7
<i>Harmothoe lunulata</i>	10	<i>Sabellaria spinulosa</i>	3	<i>Melita obtusata</i>	7
<i>Harmothoe longisetis</i>	43			<i>Atylus swammerdami</i>	10
<i>Gattyana cirrosa</i>	3	MOLLUSCA		<i>Atylus falcatus</i>	3
<i>Sigalion mathildae</i>	47			<i>Hippomedon denticulatus</i>	3
<i>Pholoe minuta</i>	37	<i>Nucula turgida</i>	30	<i>Orchomenella nana</i>	10
<i>Sthenelais limicola</i>	37	<i>Chlamys spec. juv.</i>	50	<i>Leucothoe incisa</i>	33
<i>Eteone longa</i>	20	<i>Montacuta ferruginosa</i>	93	<i>Urothoe poseidonis</i>	33
<i>Mysta barbata</i>	7	<i>Mysella bidentata</i>	63	<i>Bathyporeia guilliamsoniana</i>	33
<i>Anaitides maculata</i>	23	<i>Venus striatula</i>	13	<i>Bathyporeia elegans</i>	80
<i>Anaitides spec. juv.</i>	23	<i>Venus verrucosa</i>	3	<i>Pericolodes longimanus</i>	10
<i>Eumida sanguinea</i>	27	<i>Mactra corallina</i>	7	<i>Pontocrates arenarius</i>	7
<i>Ophiodromus flexuosus</i>	10	<i>Tellina fabula</i>	80	<i>Synchelidium haplocheles</i>	47
<i>Gyptis capensis</i>	73	<i>Tellina tenuis</i>	3	<i>Caprella spec.</i>	13
<i>Nereis longissima</i>	17	<i>Abra alba</i>	33		
<i>Nereis spec. juv.</i>	23	<i>Abra nitida</i>	7	ECHINODERMATA	
<i>Nephtys hombergii</i>	93	<i>Ensis ensis</i>	3		
<i>Nephtys caeca</i>	10	<i>Cultellus pellucidus</i>	43	<i>Amphiura filiformis</i>	43
<i>Nephtys spec. juv.</i>	13	<i>Natica alderi</i>	97	<i>Acronida brachiata</i>	7
<i>Glycera alba</i>	3			<i>Ophiura albida</i>	27
<i>Glycera spec. juv.</i>	3	CRUSTACEA		<i>Ophiura spec. juv.</i>	67
<i>Goniada maculata</i>	43			<i>Echinocardium cordatum</i>	87
<i>Lumbrineris latreilli</i>	17	<i>Pagurus bernhardus</i>	17	<i>Echinocardium cordatum juv.</i>	100
<i>Scoloplos armiger</i>	17	<i>Macropipus spec. juv.</i>	17	<i>Echinocyamus pusillus</i>	3
<i>Poecilochaetus serpens</i>	27	<i>Ebalia cranchii</i>	7		
<i>Spio filicornis</i>	3	<i>Ebalia tuberosa</i>	3	OTHER TAXA	
<i>Spiophanes bombyx</i>	97	<i>Corystes cassivelaunus</i>	43		
<i>Aonides paucibranchiata</i>	7	<i>Callianassa subterranea</i>	43	Nemertinea	60
<i>Magelona papillicornis</i>	63	<i>Decapoda larven</i>	27	Nematoda	10
<i>Chaetozone setosa</i>	67	<i>Eudorella truncatula</i>	3	Phoroniden	43
<i>Scalibregma inflatum</i>	70	<i>Iphinoe trispinosa</i>	10	Harp. copepoda	57
<i>Ophelina acuminata</i>	7	<i>Pseudocuma longicornis</i>	3	Oligochaeta	10
<i>Mediomastus gracilis</i>	7	<i>Diastylis bradyi</i>	13	Anthozoa	23
<i>Notomastus latericeus</i>	60	<i>Ione thoracica</i>	7		

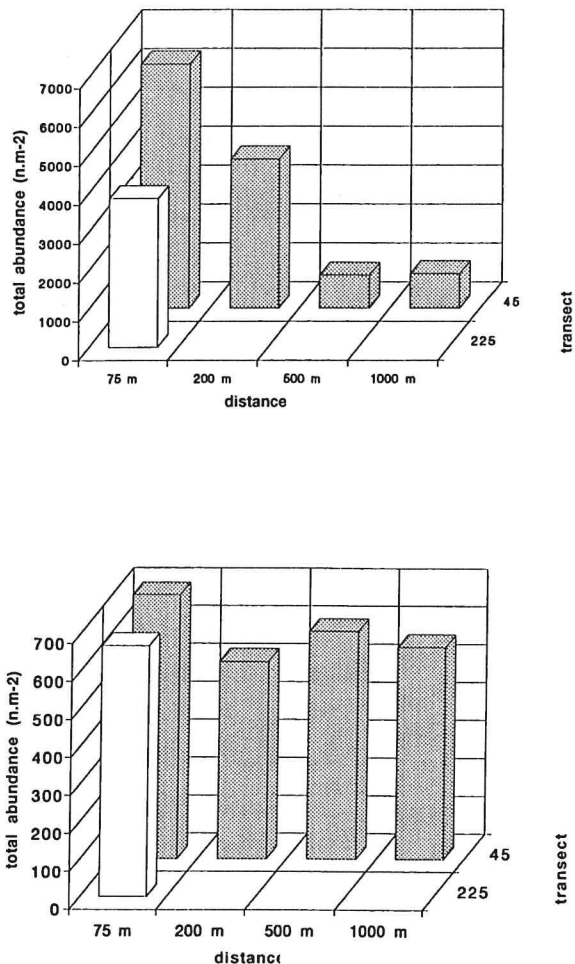


Fig. 8. Baseline survey, July 1993: Total macrofauna abundance along the 45° and 225° transects. A: all species included; B: dominant species excluded (*Echinocardium cordatum* < 15 mm and *Magellona papillicornis*).

found. These species were selected for further inspection of their abundance patterns. Of these species 18 showed a gradient in their abundance along the sampled transect (Fig. 10a-d). In 50% of these species highest densities were found at 500 or 1000 m and 50% were most abundant within 200 m from the location. Application of logit regression confirmed that there was a significant gradient in frequency of occurrence along the transect in 12 of these species, but also in the polychaete *Lagis koreni* (Table 11). Of these species 6 were found more frequently in the samples at remote stations and 7 occurred more frequently in the vicinity of the location. In other words,

this strongly indicates that there was a gradual change in species composition along the transect. In view of the gradient in sediment composition already observed during the fieldwork, it may be interesting to see if the species that occurred in abundance within 200 m were characteristic of fine sand with silt, and if the species that were more abundant at larger distance were indeed characteristic of a more coarse-sandy sediment type. Table 12 shows the sediment preferences of the 18 species according to data of Holtmann & Groenewold (1992) and according to our own observations in different parts of the Dutch sector, i.e. the P-block ($\leq 1\%$ silt), the K-block (9% silt), the L-block (12-26% silt) and the F-block (18% silt). Of the species that were abundant within 200 m most appear to prefer a muddy or intermediate sediment type. Only *Spiophanes bombyx* is generally more common in sandy areas. On the other hand, the majority of the species that were more abundant at distances ≥ 500 m are known to prefer sandy sediment. Hence, the gradient in the fauna composition along the 45° transect may be explained very well by the gradient in sediment composition that was visually observed.

3.2.1.4 RELATIVE MACROFAUNA ABUNDANCE

A plot of the mean relative abundance, calculated as the mean rank of each station for all identified species (Fig. 11), shows that the mean rank was close to 3 for all stations. Analysis of variance revealed no significant differences between stations. This means that there was no station where most species were less or more abundant than at any of the other stations. It suggests also that the total number of species that were more abundant at the inner stations was about the same as the number that were more abundant at the remote stations.

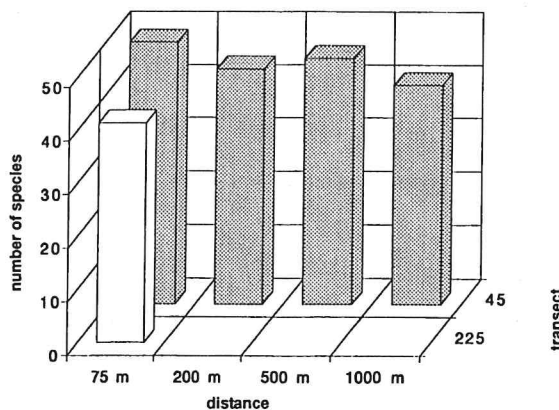


Fig. 9. Baseline survey, July 1993: Number of identified species (in 6 samples) per station.

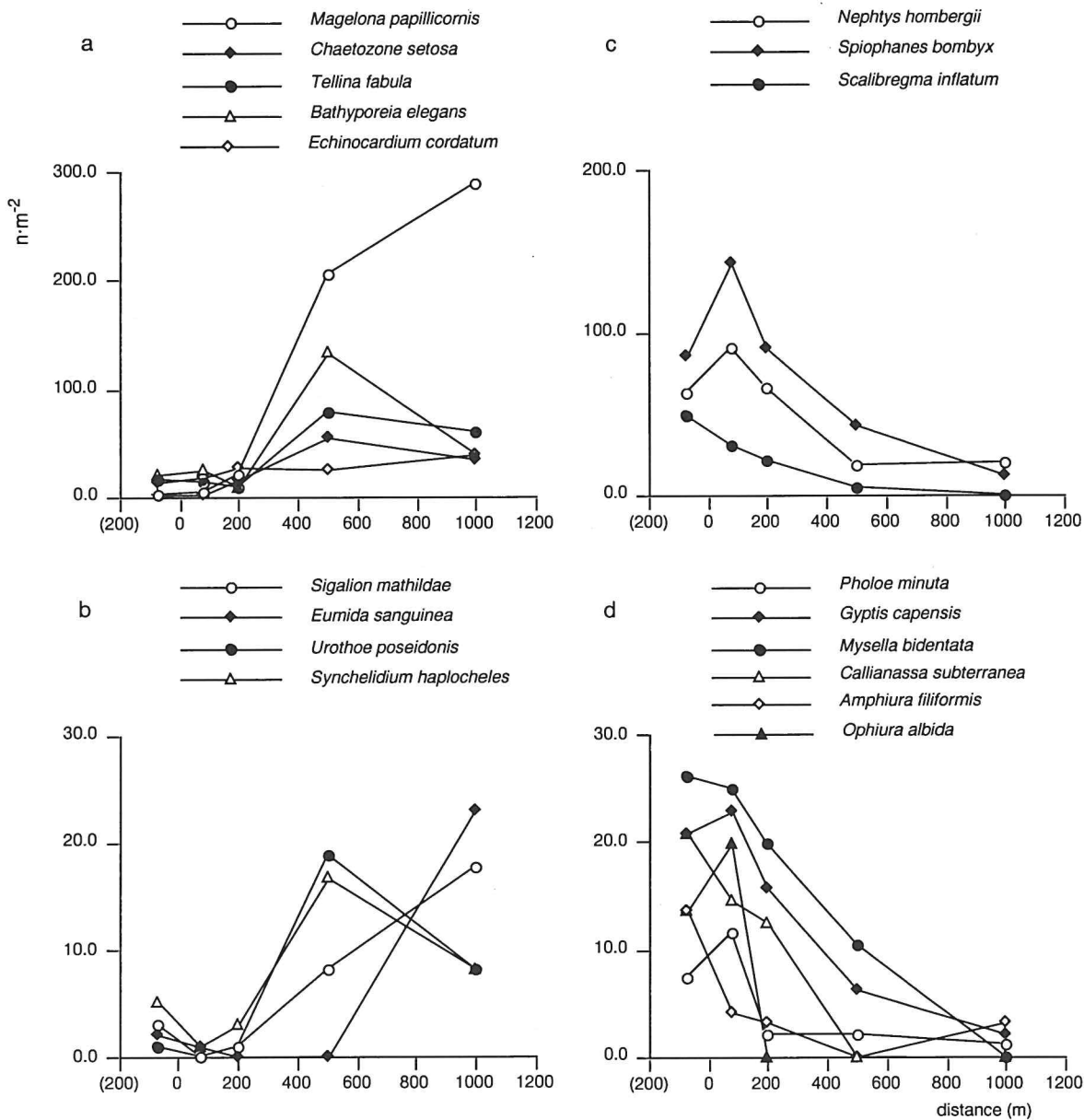


Fig. 10. Baseline survey, July 1993: Distribution patterns of 18 abundant species along the 225° and 45° transects.

3.2.1.5 REPERCUSSIONS

The observed heterogeneity of the sediment structure and the fauna composition along the 45° transect during the baseline survey was reason to decide that this transect was unsuited for further use to assess possible biological effects in post-drilling surveys. It was anticipated that possible changes in the fauna composition after drilling, which were most likely to be

expected in the close vicinity of the location, could not be related to an adequate reference situation in the 45° direction. Therefore an alternative main sampling transect was chosen for the post-drilling surveys, as explained in chapter 2.1. Nevertheless, the fauna composition within 200 m from the location as observed during the baseline survey may be considered to reflect the initial pre-drilling situation around the location to which possible future changes can be

TABLE 11

Baseline survey, July 1993: List of species for which gradients in frequency of occurrence were tested by logit regression. Sign of the gradient (+/-) and significance level are indicated: + = increasing frequency of occurrence away from the location; - = decreasing frequency of occurrence away from the location.

	sign	sign. level (%)
<i>Sigalion mathildae</i>	+	0.1
<i>Pholoe minuta</i>	-	n.s.
<i>Eumida sanguinea</i>	+	0.1
<i>Gyptis capensis</i>	-	0.5
<i>Nephtys hombergii</i>	-	n.s.
<i>Goniada maculata</i>	-	n.s.
<i>Spiophanes bombyx</i>	-	n.s.
<i>Magelona papillicornis</i>	+	0.1
<i>Chaetozone setosa</i>	+	0.1
<i>Scalibregma inflatum</i>	-	0.1
<i>Notomastus latericeus</i>	+	n.s.
<i>Lanice conchilega</i>	+	n.s.
<i>Lagis koreni</i>	-	0.5
<i>Montacuta ferruginosa</i>	+	n.s.
<i>Mysella bidentata</i>	-	0.1
<i>Tellina fabula</i>	+	n.s.
<i>Natica alderi</i>	+	n.s.
<i>Callianassa subterranea</i>	-	0.1
<i>Urothoe poseidonis</i>	+	5
<i>Bathyporeia guilliamsoniana</i>	+	n.s.
<i>Bathyporeia elegans</i>	+	n.s.
<i>Synchelidium haplocheles</i>	+	0.5
<i>Amphiura filiformis</i>	-	5
<i>Ophiura albida</i>	-	0.1
<i>Echinocardium cordatum</i>	-	0.1

compared. Particularly the fact that *Echinocardium cordatum*, which in former studies has shown to respond very sensitive to discharges of oil contaminated drill cuttings, occurred in abundance in this area before drilling, provides an excellent opportunity to observe its response after termination of the EBM-cutting discharges.

3.2.2 SECOND POST-DRILLING SURVEY, JANUARY 1994

3.2.2.1 ON BOARD OBSERVATIONS

A visual inspection of the samples at all 75-m stations (0°, 45° and 225°) gave the impression that the sediment was largely anaerobic at these stations. Large parts were black-coloured and the smell of H₂S was frequently observed. In a few samples an 'oily' film appeared on the overlaying water. To a lesser extent similar observations were made at the stations up to 200 m from the discharge site. At 500 m (0°) only 4 samples looked more or less anaerobic. At 1000 m and 3000 m such visual indications of disturbed sedi-

ment conditions were no longer observed. Apart from the presence of drill cuttings, observed in the samples up to 200 m, there was no indication of differences in the grain size composition between stations along the 0° transect. The sediment along this transect may be described as fine sand mixed with some silt. In contrast to the baseline survey, living adult *Echinocardium cordatum* were not observed up to 500 m from the location. However, parts of the exoskeleton of dead specimens were found in one or a few samples of each of the stations within 500 m, indicating that mortality in *E. cordatum* had recently occurred. The first living specimen (and some fragments of living specimens) was observed at the 1000-m station and 9 specimens (all in the size class 35-50 mm) were found at 3000 m from the location, indicating a spatial gradient in the abundance of *E. cordatum* related to distance to the location (Fig. 12b).

3.2.2.2 GENERAL FAUNA DESCRIPTION

A total number of 60 (identified) species were found during this survey. Their percentual occurrence in the samples is summarized in Table 13. The original data are listed in Appendix 6. Juvenile specimens of the bivalve *Abra alba* dominated the fauna by number. On average they accounted for 61% of the fauna. This generation was not yet found during the baseline survey in July of the preceding year, so spatfall of larval stages had apparently settled in the second half of 1993 in the area, including the contaminated zone in the vicinity of the location. Densities of living *A. alba* at 1000 m and 3000 m were substantially higher than within 500 m, but in the samples from stations within 500 m empty shells were frequently found, the delicate valves still connected to each other, which indicated that the animals had died a short time before. At 1000 m and 3000 m juvenile *Echinocardium cordatum*

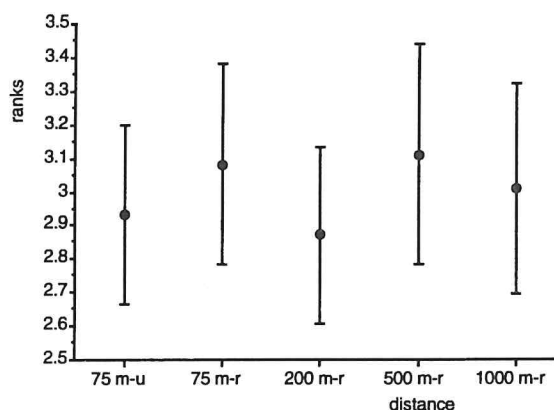


Fig. 11. Baseline survey, July 1993: Relative macrofauna abundance (mean ranks and 95% confidence limits).

TABLE 12

Preferential sediment types of 18 species that showed abundance gradients over the 45° transect at K14-13 (baseline survey, July 1993).

	Holtmann & Groenewold, 1992	Own observations
species abundant at $\leq 200\text{m}$		
<i>Mysella bidentata</i>	muddy sand	muddy sediment
<i>Callianassa subterranea</i>	muddy sand	muddy sediment
<i>Amphiura filiformis</i>	muddy sand	muddy sediment
<i>Pholoe minuta</i>	muddy sand	muddy sediment
<i>Scalibregma inflatum</i>	muddy sand	intermediate sediment type
<i>Gyptis capensis</i>	muddy sand	intermediate sediment type
<i>Spiophanes bombyx</i>	fine/ coarse sand	sandy sediment
<i>Nephtys hombergii</i>	no preference	intermediate sediment type
<i>Ophiura alba</i>	no preference	no preference
species abundant at $\geq 500\text{m}$		
<i>Magelona papillicornis</i>	fine/coarse sand	sandy sediment
<i>Tellina fabula</i>	fine/coarse sand	sandy sediment
<i>Bathyporeia elegans</i>	fine/coarse sand	sandy sediment
<i>Urothoe poseidonis</i>	fine/coarse sand	sandy sediment
<i>Sigalion mathildae</i>	no preference	sandy sediment
<i>Synchelidium haplocheles</i>	-	sandy sediment
<i>Eumida sanguinea</i>	no preference	no preference
<i>Chaetozone setosa</i>	no preference	no preference
<i>Echinocardium cordatum</i>	no preference	no preference

um were to a lesser extent also dominant, contributing 13% of the total macrofauna. Within 500 m from the location they were completely absent. In a qualitative sense the abundance pattern of juveniles was similar to that of the adults (Fig. 12).

The total fauna abundance was relatively low within 200 m and continuously increased beyond that distance (Fig. 13). In view of the fact that the total abundance is often disproportionally determined by the numbers of a few dominant species, in the present

situation by juveniles of *A. alba* and *E. cordatum*, a second plot is given in Fig. 13, where these species are excluded. This figure shows a continuous increase of fauna numbers with increasing distance up to 500 m, after which the numbers become stable at a level of 400-500 ind·m⁻². These numbers are slightly lower than during the baseline survey which has likely to be explained as a seasonal effect, since macrofauna abundance has been shown to follow a seasonal cycle with increasing numbers during sum-

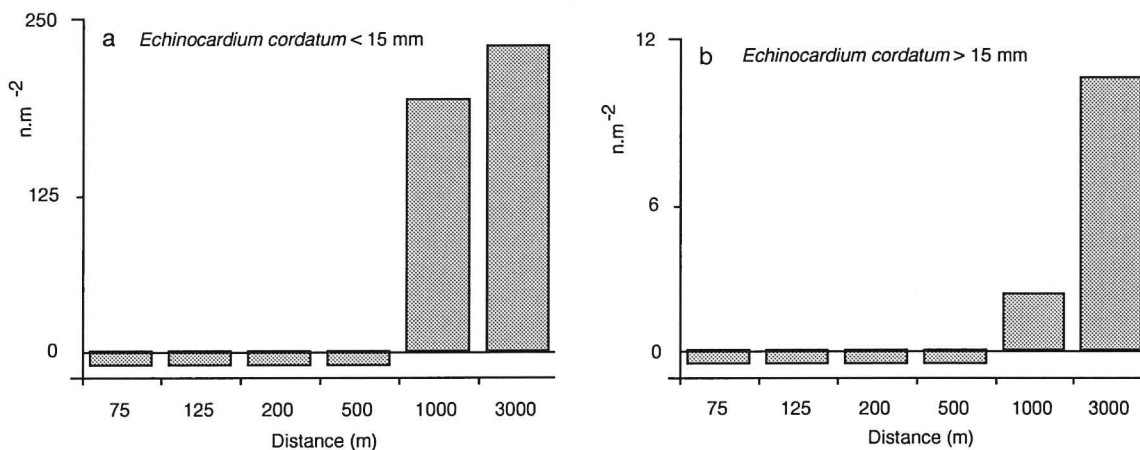


Fig. 12. 2nd post-drilling survey, January 1994. Spatial abundance pattern of *Echinocardium cordatum*. a: specimens < 15 mm; b: specimens > 15 mm. Zero values are given below X-axis.

TABLE 13

Second post-drilling survey, January 1994. Percentage of occurrence of each species in the total number of analysed samples (36).

POLYCHAETA		<i>Mediomastus gracilis</i>	6	<i>Iphinoe trispinosa</i>	14
		<i>Notomastus latericeus</i>	8	<i>Eurydice spinigera</i>	3
<i>Harmothoe lunulata</i>	14	<i>Lanice conchilega</i>	22	<i>Gammarus marinus</i>	3
<i>Harmothoe longisetis</i>	11	<i>Lagis koreni</i>	19	<i>Orchomenella nana</i>	11
<i>Pholoe minuta</i>	39	MOLLUSCA		<i>Leucothoe incisa</i>	25
<i>Sthenelais limicola</i>	17			<i>Urothoe poseidonis</i>	6
<i>Anaitides groenlandica</i>	17	<i>Nucula turgida</i>	25	<i>Bathyporeia guilliamsoniana</i>	6
<i>Anaitides maculata</i>	6	<i>Montacuta ferruginosa</i>	17	<i>Bathyporeia elegans</i>	17
<i>Anaitides spec. juv.</i>	3	<i>Mysella bidentata</i>	64	<i>Perioculodes longimanus</i>	3
<i>Eumida sanguinea</i>	8	<i>Mactra corallina</i>	3	<i>Caprella spec.</i>	8
<i>Ophiodromus flexuosus</i>	22	<i>Spisula spec. juv.</i>	6	<i>Corophium insidiosum</i>	3
<i>Gyptis capensis</i>	67	<i>Tellina fabula</i>	8	ECHINODERMATA	
<i>Nereis longissima</i>	6	<i>Abra alba</i>	17	<i>Amphiura filiformis</i>	44
<i>Nephtys hombergii</i>	67	<i>Abra alba juv.</i>	100	<i>Amphiura chiajei</i>	8
<i>Nephtys hombergii juv.</i>	89	<i>Onoba vitrea</i>	8	<i>Ophiura albida</i>	47
<i>Nephtys caeca</i>	3	<i>Natica alderi</i>	97	<i>Ophiura spec. juv.</i>	53
<i>Nephtys longosetosa</i>	3	<i>Cylichna cilindracea</i>	6	<i>Echinocardium cordatum</i>	14
<i>Glycera spec. juv.</i>	11	CRUSTACEA		<i>Echinocardium cordatum juv.</i>	31
<i>Glycinde nordmanni</i>	3	<i>Processa parva</i>	19	OTHER TAXA	
<i>Goniada maculata</i>	14	<i>Pontophilus trispinosus</i>	3	Nemertinea	36
<i>Lumbrineris latreilli</i>	3	<i>Macropipus marmoreus</i>	6	Turbellaria	6
<i>Poecilochaetus serpens</i>	11	<i>Macropipus spec. juv.</i>	3	Phoroniden	33
<i>Spio filicornis</i>	3	<i>Ebalia cranchii</i>	6	Harp. copepoda	6
<i>Polydora pulchra</i>	11	<i>Corystes cassivelaunus</i>	3	Oligochaeta	3
<i>Spiophanes bombyx</i>	69	<i>Callianassa subterranea</i>	53	Anthozoa	3
<i>Magelona papillicornis</i>	3	<i>Gastrosaccus spiniifer</i>	6		
<i>Chaetozone setosa</i>	6	<i>Eudorella truncatula</i>	3		
<i>Scalibregma inflatum</i>	6				
<i>Ophelina acuminata</i>	8				
<i>Capitella capitata</i>	39				

mer and decreasing numbers during winter (Rachor & Gerlach, 1978; Ziegelmeier, 1978).

Between 500 and 3000 m the numbers of species ranged between 33 and 39 per station and, on average, between 14 and 16 per sample (Fig. 14). These numbers are lower than during the baseline survey, which again might be explained as a seasonal effect. There were no significant differences in species richness within the stations at 500, 1000 and 3000 m. On approach of the discharge site the numbers of species strongly decreased to 13 species at 75 m (on average 5 per sample). The numbers of species per sample appeared to be significantly lower within 200 m than at ≥ 500 m (ANOVA).

3.2.2.3 ABUNDANCE PATTERNS OF INDIVIDUAL SPECIES

There were 12 species of which ≥ 20 (adult) specimens were found. All occurred in abundance also

during the baseline survey, except for the polychaete *Capitella capitata*. Their abundance patterns are shown in Fig. 15 and can be compared by their mean abundance in the close vicinity of the location (≤ 200 m) just before drilling started. All species, except for the echinoderm *Ophiura albida* and the crustacean *Callianassa subterranea*, showed a more or less clear gradient in their abundance along the sampled transect. Logit regression confirmed that gradients in frequency of occurrence were significant in *Pholoe minuta*, *Nephtys hombergii*, *Spiophanes bombyx*, *Capitella capitata*, *Natica alderi*, *Montacuta ferruginosa* and *Echinocardium cordatum* (Table 14). Densities of most species were lowest at 75 m, the station closest to the discharge site, but *C. capitata* showed peak numbers at the stations nearest to the discharge site. This opportunist species, generally rare by nature in the offshore region of the Dutch sector (Holtmann & Groenewold, 1992; Duineveld, 1992; Duineveld & Belgers, 1993), is known as a rapid coloniser of disturbed and organically enriched sediment

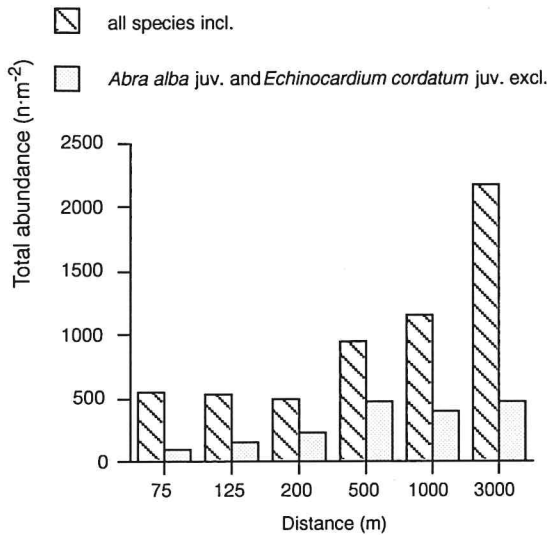


Fig. 13. 2nd post-drilling survey, January 1994. Total macrofauna abundance along the 0° transect. a: all species included; b: dominant species (*A. alba* juv. and *E. cordatum* juv.) excluded.

and to be highly tolerant to anoxic conditions. Increased abundances of this species have also often been observed at locations where OBM cuttings had been dumped (Addy *et al.*, 1984; Davies *et al.*, 1984; Hannam *et al.*, 1987; Kingston, 1987; Levell *et al.*, 1989; Gray *et al.*, 1990).

The polychaete species *Gyptis capensis* and *Nephtys hombergii* seemed to reach stable densities at 200 m and the bivalve *Mysella bidentata* and the echinoderm *Amphiura filiformis* at about 500 m. *G. capensis*, *N. hombergii* and *M. bidentata* showed an overall decrease compared to the baseline situation, whereas *A. filiformis* decreased within 200 m and increased at ≥ 500 m. The other species, the polychaetes *Pholoe minuta* and *Spiophanes bombyx*, the molluscs *Montacuta ferruginosa* and *Natica alderi*, and *Echinocardium cordatum* showed a strong decrease in abundance within 200 m compared to the baseline situation. At larger distance there was only a slight decrease (*S. bombyx*, *M. ferruginosa* and *E. cordatum*) or even an increase (*P. minuta* and *N. alderi*). The species of which the densities were severely depressed over the largest distance were *M. ferruginosa* and *E. cordatum*. Of all species they are those which in earlier OBM studies have been identified as most sensitive to discharges of contaminated drill cuttings (Daan & Mulder, 1994).

3.2.2.4 RELATIVE MACROFAUNA ABUNDANCE

A plot of the mean relative abundance of all macrofauna species (Fig. 16) shows that the densities of

TABLE 14

Second post-drilling survey, January 1994: List of species for which gradients in frequency of occurrence were tested by logit regression. Sign of the gradient (+/-) and significance level are indicated: + = increasing frequency of occurrence away from the location; - = decreasing frequency of occurrence away from the location.

	sign	sign. level (%)
<i>Pholoe minuta</i>	+	5
<i>Gyptis capensis</i>	+	n.s.
<i>Nephtys hombergii</i>	+	0.1
<i>Spiophanes bombyx</i>	+	0.1
<i>Capitella capitata</i>	-	0.1
<i>Montacuta ferruginosa</i>	+	0.1
<i>Mysella bidentata</i>	+	n.s.
<i>Natica alderi</i>	+	1
<i>Callianassa subterranea</i>	+	n.s.
<i>Amphiura filiformis</i>	+	n.s.
<i>Ophiura albida</i>	-	n.s.
<i>Echinocardium cordatum</i>	+	0.1

the species were on average lowest at 75 m and increased with increasing distance to the location. At 500 m and further away the relative macrofauna densities were generally stable. Analysis of variance confirmed that there were highly significant ($P < 0.001$) differences in mean relative abundance of the different stations. A LSD-test subsequently applied to all pairs of means, to detect whether they were significantly different, showed that the stations at ≤ 200 m were all different from those at ≥ 500 m (Table 15). Within both groups of stations the differences were generally not significant.

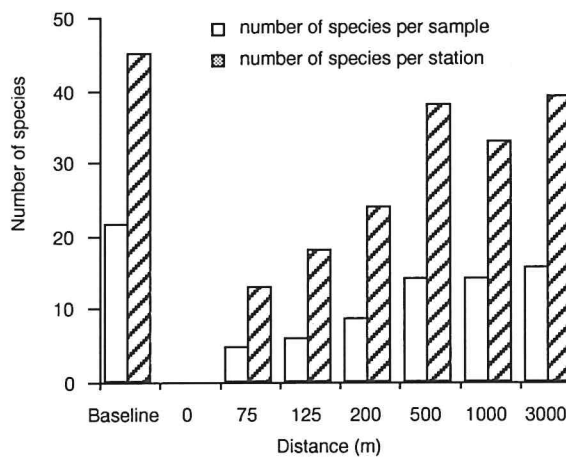


Fig. 14. 2nd post-drilling survey, January 1994. Species richness along the 0° transect. Dashed bars: numbers of species per station; open bars: numbers of species per sample.

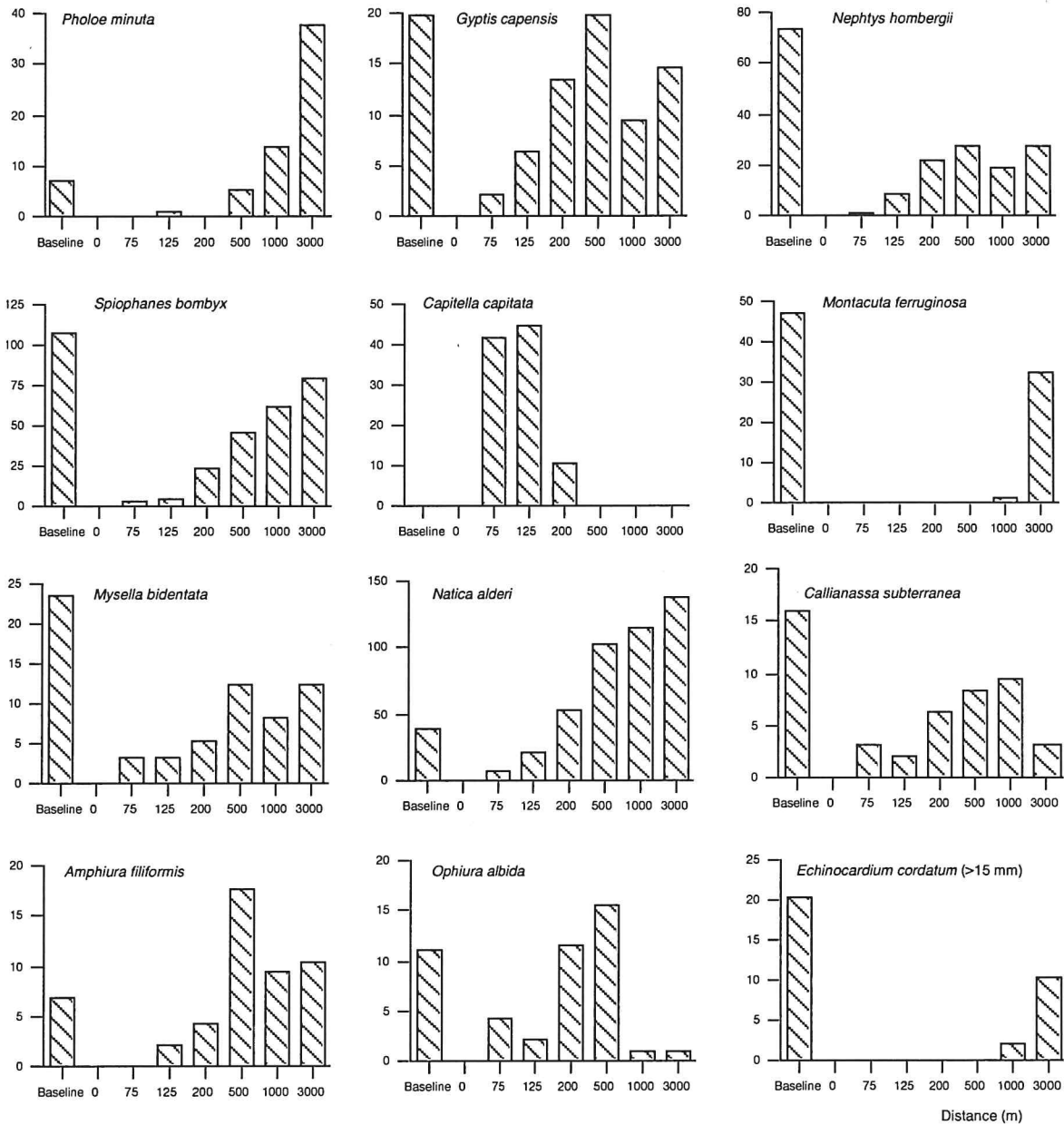


Fig. 15. 2nd post-drilling survey, January 1994. Abundance patterns ($n \cdot m^{-2}$) of 12 common macrofauna species along the 0° transect. Left bar: baseline values (mean density baseline survey (stations at ≤ 200 m)).

3.2.3 THIRD POST-DRILLING SURVEY, AUGUST 1994

3.2.3.1 ON BOARD OBSERVATIONS

Inspection of the samples during fieldwork provided a visual impression of the sediment condition around the location. At all stations lying at ≤ 200 m from the

discharge site, most samples looked in a greater or less degree anaerobic. The sediment was black-coloured and the smell of H_2S was frequently observed. An oily smell was also frequently observed and sometimes an oily film appeared at the surface of the overlying water, possibly originating from residuals of fish-oil. The most strongly polluted sediment seemed

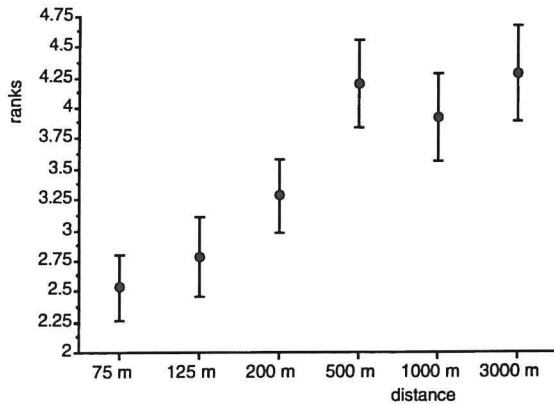


Fig. 16. 2nd post-drilling survey, January 1994. Relative macrofauna abundance along the 0° transect (mean ranks and 95% confidence limits).

TABLE 15

Second post-drilling survey, January 1994: Statistical significance (LSD test) of differences in relative macrofauna abundance between stations. Significance level (%) indicated.

	75 m	125 m	200 m	500 m	1000 m	3000 m
75 m	-					
125 m	n.s.	-				
200 m	1	n.s.	-			
500 m	0.1	0.1	0.1	-		
1000 m	0.1	0.1	5	n.s.	-	
3000 m	0.1	0.1	0.1	n.s.	n.s.	-

to occur at the 75-m station in the 225° direction. The sediment was for the major part black coloured, produced a strong H₂S smell and a persistent oily film appeared on the overlaying water of most samples. Inspection of vertical profiles in the boxcore samples collected at 125 m (0° direction) indicated that anaerobic conditions particularly occurred in the deeper sediment layers. There was always a top layer of about 3-5 cm, where the sediment showed its natural grey colour.

Fully grown *Echinocardium cordatum* of the size class 35-50 mm - a generation that occurred in abundance around the well site during the baseline survey and of which several specimens were found at the stations at ≥1000 m during the 2nd post-drilling survey - seemed to have almost completely disappeared from the area. Only at the 3000-m station one specimen was found. However a new generation of (sub) adult individuals appeared to have recolonised the area. On the basis of the size of these specimens (15-20 mm) and known growth rates of *E. cordatum* in the field (Duineveld & Jenness, 1984) it seems most likely that these animals represented the surviving part of the 1993 spatfall generation. Juveniles of this generation were found in abundance during the

baseline survey but were no longer found up to 500 m from the well in January 1994. It is not surprising therefore that only a few specimens of the 1993 generation were found within this zone during the August survey (2 specimens at 75 m 225° and 2 specimens at 125 m 0°, see Fig. 17b). However, outside this zone the 15-20 mm size class appeared frequently in nearly all samples. Mean densities amounted to 45 ind·m⁻² at 1000 m and 86 ind·m⁻² at 3000 m.

3.2.3.2 GENERAL FAUNA DESCRIPTION

A total number of 67 (identified) species were found during this survey. Their percentual occurrence in the samples is summarized in Table 16. The original data

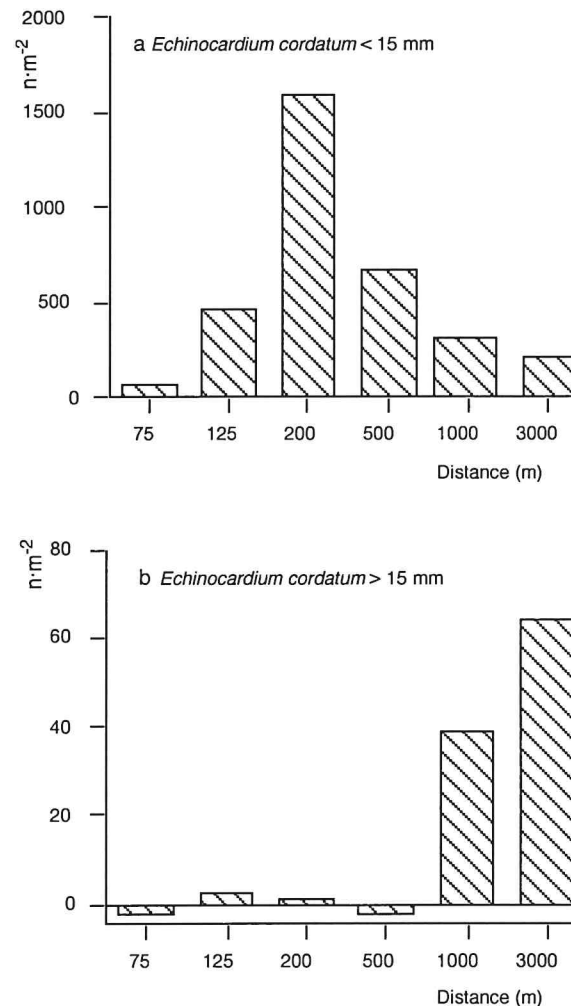


Fig. 17. 3rd post-drilling survey, August 1994. Spatial abundance pattern of *Echinocardium cordatum*. a: specimens < 15 mm; b: specimens > 15 mm. Zero values are given below X-axis.

TABLE 16

Third post-drilling survey, August 1994. Percentage of occurrence of each species in the total number of analysed samples (30).

POLYCHAETA		<i>Ophelina acuminata</i>	7	Decapoda larven	10
		<i>Capitella capitata</i>	7	<i>Eudorella truncatula</i>	7
<i>Aphrodita aculeata</i>	17	<i>Mediomastus gracilis</i>	7	<i>Iphinoe trispinosa</i>	10
<i>Harmothoe lunulata</i>	10	<i>Notomastus latericeus</i>	60	<i>Diastylis bradyi</i>	3
<i>Harmothoe longisetis</i>	43	<i>Owenia fusiformis</i>	90	<i>Ione thoracica</i>	17
<i>Harmothoe spec. juv.</i>	10	<i>Lanice conchilega</i>	90	<i>Megaluropus agilis</i>	3
<i>Sigalion mathildae</i>	13	<i>Lagis koreni</i>	100	<i>Orchomenella nana</i>	20
<i>Pholoe minuta</i>	90	<i>Pectinaria belgica</i>	3	<i>Leucothoe incisa</i>	27
<i>Sthenelais limicola</i>	30			<i>Urothoe poseidonis</i>	13
<i>Eteone longa</i>	100	MOLLUSCA		<i>Bathyporeia guilliamsoniana</i>	23
<i>Anaitides groenlandica</i>	97	<i>Nucula turgida</i>	47	<i>Bathyporeia elegans</i>	67
<i>Anaitides maculata</i>	87	<i>Kellia suborbicularis</i>	3	<i>Synchelidium haplocheles</i>	3
<i>Eumida sanguinea</i>	40	<i>Montacuta ferruginosa</i>	20	<i>Caprella spec.</i>	27
<i>Ophiodromus flexuosus</i>	40	<i>Mysella bidentata</i>	93	ECHINODERMATA	
<i>Gyptis capensis</i>	93	<i>Venus spec. juv.</i>	7	<i>Asterias rubens</i>	7
<i>Nereis longissima</i>	70	<i>Mactra corallina</i>	13	<i>Amphiura filiformis</i>	17
<i>Nereis spec. juv.</i>	3	<i>Tellina fabula</i>	83	<i>Ophiura texturata</i>	43
<i>Nephtys hombergii</i>	93	<i>Abra alba</i>	97	<i>Ophiura albida</i>	67
<i>Nephtys caeca</i>	13	<i>Abra alba juv.</i>	90	<i>Ophiura spec. juv.</i>	100
<i>Nephtys spec. juv.</i>	3	<i>Cultellus pellucidus</i>	37	<i>Echinocardium cordatum</i>	40
<i>Glycera alba</i>	20	<i>Thracia phaseolina</i>	7	<i>Echinocardium cordatum juv.</i>	100
<i>Glycera spec. juv.</i>	40	<i>Natica alderi</i>	100		
<i>Glycinde nordmanni</i>	17	<i>Cylichna cilindracea</i>	7	OTHER TAXA	
<i>Goniada maculata</i>	50			Nemertinea	67
<i>Lumbrineris latreilli</i>	7	CRUSTACEA		Turbellaria	30
<i>Scoloplos armiger</i>	23	<i>Processa parva</i>	30	Phoroniden	63
<i>Poecilochaetus serpens</i>	23	<i>Pagurus bernhardus</i>	10	Harp. copepoda	17
<i>Spio filicornis</i>	20	<i>Macropipus marmoreus</i>	3	Oligochaeta	10
<i>Spiophanes bombyx</i>	87	<i>Macropipus spec. juv.</i>	13	Holothuroidea	3
<i>Scoelepis bonnierii</i>	3	<i>Corystes cassivelaunus</i>	57	Anthozoa	7
<i>Magelona papillicornis</i>	53	<i>Callianassa subterranea</i>	80		
<i>Chaetozone setosa</i>	13				
<i>Scalibregma inflatum</i>	93				

are listed in Appendix 7. The tube-building polychaete *Lagis koreni* and juvenile *Echinocardium cordatum* dominated the fauna by number. These species accounted for 43% and 18% respectively. Both must have settled in the area in the current season and were so abundant that they were found in all samples along the 0° transect. Hence, logit regression (which is based on presence-absence data) was not appropriate to verify whether there was a gradient in their frequency of occurrence. Both species occurred in lowest abundance at 75 m and in highest abundance at 200 m.

The total fauna abundance was highest at 200 m, with densities over 5000 ind·m⁻² (Fig. 18). The lowest numbers were found at 75 m (1000 ind·m⁻²), whereas at the other stations the numbers fluctuated between 2700 and 3600 ind·m⁻². Because of the prevailing contribution of the dominant species to the total fauna abundance Fig. 18 also shows the fauna abundance after deducting the numbers of *L. koreni* and juvenile

E. cordatum. The fauna numbers again appear to be lowest at 75 m (455 ind·m⁻²), whereas at the other stations the numbers range between 1100 and 1500, which is about twice as much as found during the baseline survey (500-700 ind·m⁻²).

A continuous increase was observed in the number of species found per station with increasing distance from the location (Fig. 19). At 75 m a relatively low number (36) of species was found, but between 125 m and 3000 m the numbers per station (41-53) were in a similar range as during the baseline survey (41-49), although the number of analysed samples was one fewer than in 1993. The mean number per sample was also low in the vicinity of the location and increased with increasing distance. At 75 m the numbers per sample appeared to be significantly (5% level) lower than at ≥500 m and at 125 m and 200 m the numbers were significantly lower than at 500 m and 3000 m (but not significantly different from 1000 m, ANOVA).

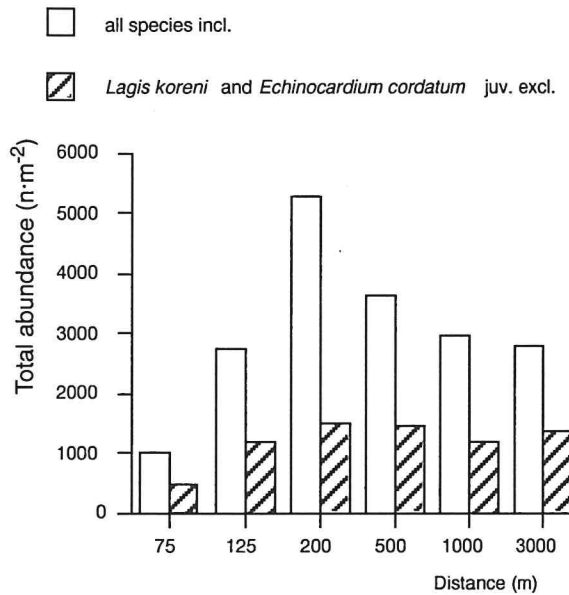


Fig. 18. 3rd post-drilling survey, August 1994. Total macrofauna abundance along the 0° transect. Open bars: all species included; dashed bars: dominant species excluded.

3.2.3.3 ABUNDANCE PATTERNS OF INDIVIDUAL SPECIES

There were 28 species of which ≥ 20 specimens were found. Most of them (20) were also more or less common during the baseline survey (≥ 10 specimens found at 3 stations within 200 m from the location). On the other hand, during the baseline survey there were 4 common species of which less than 20 specimens were found now. An inspection of the abundance data of these $28+4=32$ species (Table 17) reveals that the lowest mean abundance was observed at the 75-m station in August 1994 in 13 species. There was no species that occurred in the highest abundance at this station. The highest abundance was found at the 3000-m reference station in August 1994 in 11 species. A comparison between the mean abundance of species at 3000 m and at each of the other stations (Table 18) shows that most species appeared to be more abundant at 3000 m than at any of the other stations. At 3000 m the mean abundance of most species was also higher than during the baseline survey around the location. A sign-test revealed that there were significantly (5% level) more species that were found in higher abundance at 3000 m than at 75 m and at 125 m. The differences between 3000 m and the other stations and between 3000 m and baseline survey were not significant. Of the 32 selected common species, 12 were found at 75 m in higher abundance than during the baseline, 15 were found in lower abundance than during the

baseline, and 5 in the same abundance as during the baseline.

Fig. 20 illustrates the abundance patterns of 11 species. The species selected here are in principle the same as in Fig. 15 (2nd post-drilling survey), but the polychaete *Capitella capitata* and the echinoderm *Amphiura filiformis* were not included because of their low numbers, whereas the polychaete *Lagis koreni* was added because of its high abundance. Except for the polychaete *Gyptis capensis*, all species clearly show relatively low numbers at 75 m compared to the 3000-m reference station. To a lesser extent, and with the exception of the polychaetes *Nephtys hombergii* and *L. koreni*, the comparison between 125 m and 3000 m reveals similar differences in species specific abundances. Spatial gradients in abundance are most pronounced in the bivalves *Montacuta ferruginosa* and *Mysella bidentata*, the crustacean *Callinassa subterranea* and in *Echinocardium cordatum*. A few species (the polychaetes *Pholoe minuta* and *L. koreni*) occurred especially in high numbers at the 200-m station. A comparison with the situation before drilling reveals that *P. minuta* and *L. koreni* showed an overall increase in the area, but to a less degree at the stations in the vicinity of the location than at the stations at ≥ 200 m. An overall decrease in abundance was observed in *N. hombergii* and *M. ferruginosa*. For *M. bidentata*, *C. subterranea* and *E. cordatum* there was a decrease within 200-500 m and an increase at ≥ 1000 m.

Logit regression, applied to presence-absence data of the 28 common species, confirmed that there was a significant relation between frequency of occur-

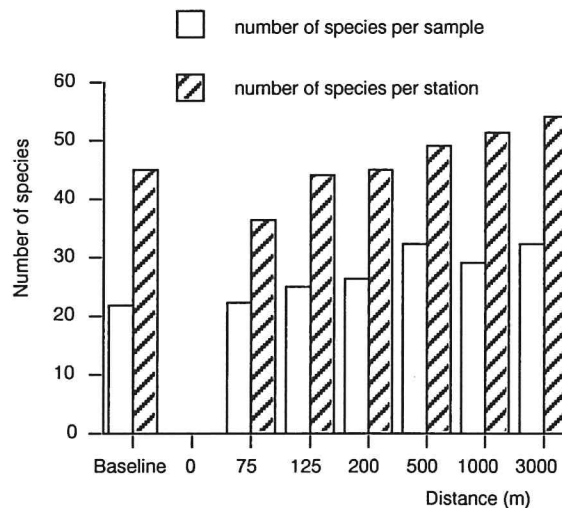


Fig. 19. 3rd post-drilling survey, August 1994. Species richness along the 0° transect. Open bars: numbers of species per station; dashed bars: numbers of species per sample.

TABLE 17

Mean densities ($n \cdot m^{-2}$) of 32 species common during the 3rd post-drilling survey and/or the baseline survey. Per species the stations are arranged in ascending order of abundance. The 75-m (white backgr.), 3000-m (black backgr.) and baseline (grey backgr.) stations are marked.

<i>Harmothoe longisetis</i>	200 m	75 m	500 m	3000 m	75u b	200r b	1000 m	125 m	75r b
	0	2,5	3,8	3,8	4,2	4,2	5	6,3	8,3
<i>Pholoe minuta</i>	200r b	75u b	75r b	125 m	75 m	1000 m	500 m	3000 m	200 m
	2,1	7,3	11,5	20	21,3	37,5	71,3	87,5	117,5
<i>Eteone longa</i>	200r b	75u b	75r b	75 m	3000 m	1000 m	125 m	500 m	200 m
	0	0	1	60	108,8	120	145	366,3	406,3
<i>Anatides groenlandica</i>	75r b	200r b	75u b	75 m	200 m	500 m	125 m	1000 m	3000 m
	0	0	0	17,5	33,8	60	62,5	123,8	158,8
<i>Anatides maculata</i>	200r b	75r b	75u b	3000 m	1000 m	75 m	500 m	125 m	200 m
	3,1	4,2	6,3	8,8	20	26,3	30	56,3	186,3
<i>Ophiodromus flexuosus</i>	75 m	75u b	125 m	200 m	75r b	200r b	500 m	1000 m	3000 m
	0	0	1,3	1,3	2,1	2,1	7,5	7,5	7,5
<i>Gyptis capensis</i>	500 m	1000 m	125 m	200r b	200 m	75u b	75r b	75 m	3000 m
	10	12,5	13,8	15,6	17,5	20,8	22,9	25	26,3
<i>Nereis longissima</i>	75u b	75r b	75 m	200r b	500 m	125 m	200 m	1000 m	3000 m
	0	1	1,3	2,1	10	15	35	41,3	43,8
<i>Nephtys hombergii</i>	75 m	1000 m	3000 m	125 m	200 m	500 m	75u b	200r b	75r b
	17,5	20	32,5	35	41,3	43,8	63,5	65,6	90,6
<i>Goniada maculata</i>	125 m	75r b	75 m	1000 m	200r b	200 m	75u b	500 m	3000 m
	1,3	2,1	2,5	2,5	3,1	3,8	7,3	7,5	10
<i>Spiohanes bombyx</i>	75 m	200 m	125 m	500 m	75u b	3000 m	1000 m	200r b	75r b
	2,5	42,5	51,3	86,3	86,5	87,5	90	90,6	143,8
<i>Magelona papillicornis</i>	1000 m	75u b	3000 m	75r b	200 m	500 m	75 m	125 m	200r b
	0	1	1,3	5,2	6,3	6,3	8,8	18,8	19,8
<i>Chaetozone setosa</i>	75 m	125 m	1000 m	75r b	200 m	3000 m	75u b	500 m	200r b
	0	0	0	1	1,3	1,3	2,1	5	15,6
<i>Scalibregma inflatum</i>	75 m	200 m	200r b	75r b	500 m	3000 m	75u b	1000 m	125 m
	12,5	20	21,9	30,2	31,3	32,5	49	56,3	106,3
<i>Notomastus latericeus</i>	500 m	200r b	75r b	75u b	125 m	200 m	75 m	1000 m	3000 m
	1,3	2,1	3,1	3,1	3,8	5	8,8	16,3	83,8
<i>Owenia fusiformis</i>	75r b	200r b	75u b	75 m	200 m	125 m	3000 m	1000 m	500 m
	0	0	0	6,3	26,3	27,5	33,8	37,5	40
<i>Lanice conchilega</i>	75 m	125 m	200r b	500 m	200 m	3000 m	1000 m	75r b	75u b
	11,3	13,8	15,6	18,8	20	21,3	33,8	65,6	103,1
<i>Lagis koreni</i>	75u b	75r b	200r b	75 m	125 m	3000 m	1000 m	500 m	200 m
	3,1	17,7	20,8	455	1116,3	1200	1448,8	1498,8	2200
<i>Nucula turgida</i>	200r b	75 m	125 m	200 m	75r b	1000 m	75u b	500 m	3000 m
	1	1,3	2,5	2,5	3,1	5	7,3	8,8	12,5
<i>Montacuta ferruginosa</i>	75 m	125 m	200 m	500 m	1000 m	3000 m	75u b	75r b	200r b
	0	0	0	0	5	17,5	35,4	50	55,2
<i>Mysella bidentata</i>	75 m	200 m	200r b	125 m	75r b	75u b	3000 m	1000 m	500 m
	5	15	19,8	21,3	25	26	47,5	56,3	63,8
<i>Tellina fabula</i>	1000 m	200r b	3000 m	75r b	75u b	75 m	500 m	200 m	125 m
	3,8	8,3	12,5	14,6	15,6	25	36,3	45	66,3
<i>Abra alba</i>	75r b	75u b	200r b	1000 m	75 m	500 m	3000 m	200 m	125 m
	4,2	4,2	5,2	58,8	73,8	100	131,3	153,8	207,5
<i>Natica alderi</i>	75 m	75r b	125 m	75u b	1000 m	200r b	200 m	500 m	3000 m
	25	31,3	33,8	39,6	45	46,9	47,5	55	68,8
<i>Corystes cassivelaunus</i>	75 m	75r b	125 m	1000 m	3000 m	200r b	75u b	200 m	500 m
	0	1	3,8	3,8	3,8	6,3	6,3	7,5	8,8
<i>Callianassa subterranea</i>	75 m	200 m	125 m	200r b	75r b	75u b	500 m	3000 m	1000 m
	5	7,5	11,3	12,5	14,6	20,8	27,5	30	57,5
<i>Bathyporeia guilliamsoniana</i>	1000 m	75r b	75 m	3000 m	200r b	200 m	500 m	125 m	75u b
	0	1	1,3	1,3	2,1	2,5	3,8	3,8	8,3
<i>Bathyporeia elegans</i>	125 m	1000 m	3000 m	75 m	200r b	200 m	75u b	500 m	75r b
	3,8	3,8	5	8,8	9,4	13,8	19,8	20	26
<i>Amphiura filiformis</i>	75 m	200 m	125 m	1000 m	3000 m	200r b	500 m	75r b	75u b
	0	0	0	2,5	2,5	3,1	3,8	4,2	13,5
<i>Ophiura texturata</i>	75r b	200r b	75u b	125 m	200 m	1000 m	500 m	75 m	3000 m
	0	0	0	1,3	2,5	3,8	5	8,8	10
<i>Ophiura albida</i>	200r b	200 m	1000 m	125 m	75 m	75u b	75r b	500 m	3000 m
	0	3,8	3,8	5	11,3	13,5	19,8	26,3	33,8
<i>Echinocardium cordatum</i>	75 m	500 m	200 m	125 m	75u b	75r b	200r b	1000 m	3000 m
	0	0	1,3	2,5	14,6	18,8	27,1	38,8	63,8

TABLE 18

Comparison of mean abundances of 32 common species at 5 stations and during baseline survey with mean abundance at the 3000-m reference station. Significant differences (*) and non-significant differences (n.s.) are indicated.

	nr. of species			nr of comparisons n=32-C	critical T-value (5%-level, one-sided test)	test value T=A-B
	Abundance higher at 3000 m (A)	Abundance lower at 3000 m (B)	Abundance at 3000 m equal (C)			
75 m	27	4	1	31	11	23 (*)
125 m	22	9	1	31	11	13 (*)
200 m	20	11	1	31	11	9 (n.s.)
500 m	17	13	2	30	10	4 (n.s.)
1000 m	19	10	3	29	11	9 (n.s.)
baseline	19	13	0	32	12	6 (n.s.)

rence and distance to the location in 11 species (Table 19). Most species occurred more frequently away from the location and in 9 of them a gradient was significant. The species for which such signifi-

TABLE 19

Third post-drilling survey, August 1994: List of species for which gradients in frequency of occurrence were tested by logit regression. Sign of the gradient (+/-) and significance level are mentioned: + = increasing frequency of occurrence away from the location; - = decreasing frequency of occurrence away from the location. 0 = no gradient.

	sign	sign. level (%)
<i>Pholoe minuta</i>	+	0.1
<i>Eteone longa</i>	0	-
<i>Anaitides groenlandica</i>	+	0.1
<i>Anaitides maculata</i>	-	0.1
<i>Ophiidromus flexuosus</i>	+	0.5
<i>Gyptis capensis</i>	-	n.s.
<i>Nereis longissima</i>	+	n.s.
<i>Nephtys hombergii</i>	+	n.s.
<i>Goniada maculata</i>	+	n.s.
<i>Spiophanes bombyx</i>	+	0.5
<i>Magelona papillicornis</i>	-	5
<i>Scalibregma inflatum</i>	+	n.s.
<i>Notomastus latericeus</i>	+	n.s.
<i>Owenia fusiformis</i>	+	n.s.
<i>Lanice conchilega</i>	+	0.1
<i>Lagis koreni</i>	0	-
<i>Nucula turgida</i>	+	0.5
<i>Montacuta ferruginosa</i>	+	0.1
<i>Mysella bidentata</i>	+	0.1
<i>Tellina fabula</i>	-	n.s.
<i>Abra alba</i>	-	n.s.
<i>Natica alderi</i>	0	-
<i>Corystes cassivelaunus</i>	+	n.s.
<i>Callianassa subterranea</i>	+	0.1
<i>Bathyporeia elegans</i>	-	n.s.
<i>Ophiura texturata</i>	+	n.s.
<i>Ophiura albida</i>	+	n.s.
<i>Echinocardium cordatum</i>	+	n.s.

cant gradients were detected were not always those of which a gradient was expected on the basis of their abundance pattern. For instance, *E. cordatum* did not show a significant gradient. However there appeared to be considerable over-dispersion in the data, which influenced the result of the test. Further, logit regression failed to detect any trends in *Eteone longa*, *Lagis koreni* and *Abra alba*. These species were so abundant that they appeared in all samples.

In 2 species, the polychaetes *Anaitides maculata* and *Magelona papillicornis*, a significant negative gradient (indicating decreasing frequency of occurrence away from the location) was observed, although for the latter species the significance level was relatively weak (5%). Moreover, since there was no increase in abundance of *M. papillicornis* in the vicinity of the location compared to the baseline situation, the decreasing frequency of occurrence at larger distance might possibly reflect the presence of an incidental natural gradient. The gradient in *A. maculata* should be considered in a different way. At 3000 m the abundance of *A. maculata* was about the same as found during the baseline survey around the location. However, within 1000 m the species seems to have increased abundance. Particularly at 125 m and 200 m the numbers per m² were much higher than a year before.

3.2.3.4 RELATIVE MACROFAUNA ABUNDANCE

The spatial pattern in relative fauna abundance (Fig. 21) looks similar as that found in the January survey. On average species were found in the lowest abundance at 75 m, whereas the relative abundance increased up to 500 m. Analysis of variance revealed highly significant ($P < 0.001$) differences between stations and an LSD-test showed that the relative abundance at 75 m was significantly lower than at all other stations (Table 20). At the 125-m and 200-m stations the relative abundance was also significantly lower than at 500 and 3000 m. Between the stations 500 m, 1000 m and 3000 m there were no mutual differences.

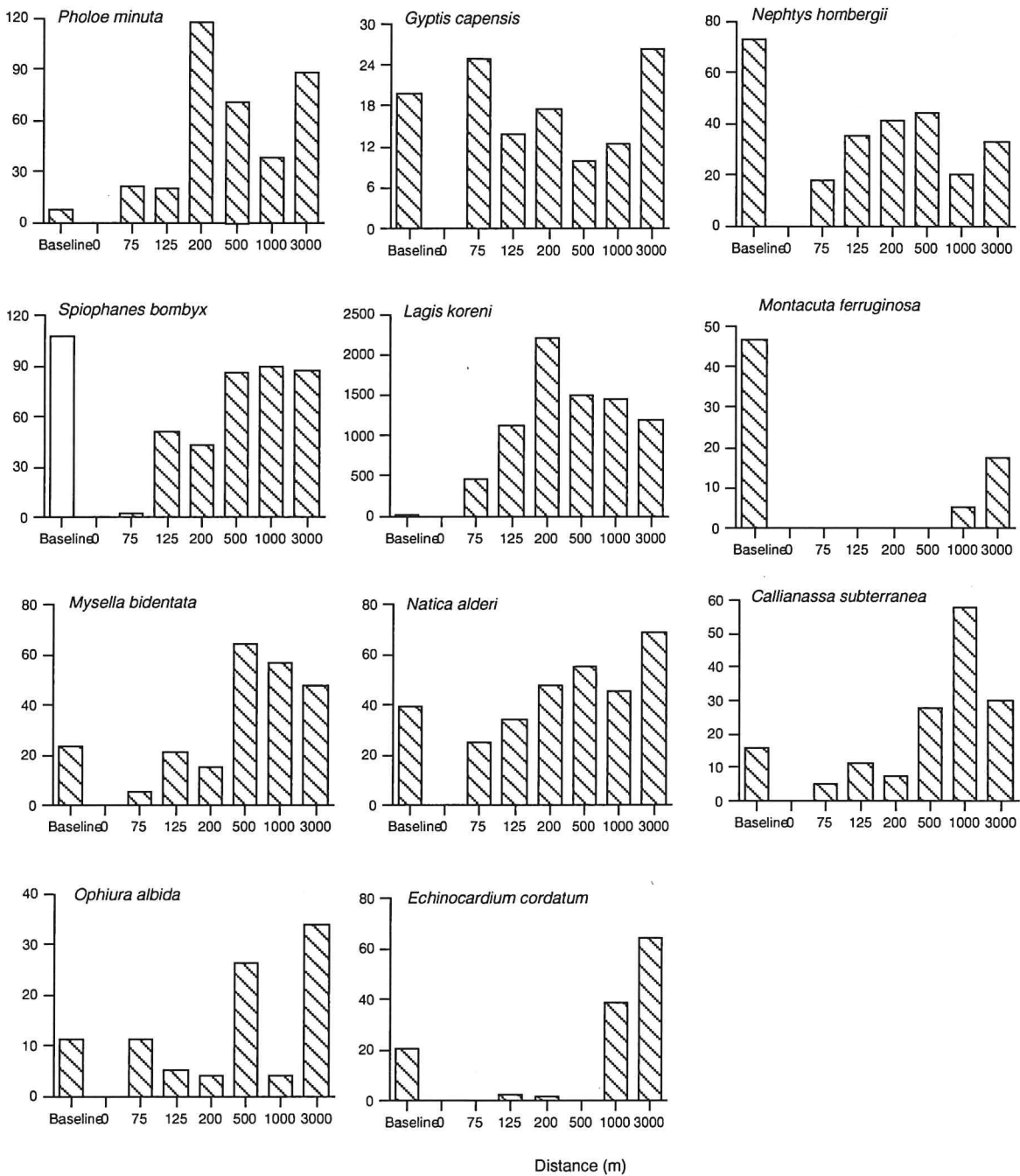


Fig. 20. 3rd post-drilling survey, August 1994. Abundance patterns ($n \cdot m^{-2}$) of 11 common macrofauna species along the 0° transect. Left bar: baseline values (mean density baseline survey (stations at ≤ 200 m)).

3.2.3.5 COMPARISON WITH BASELINE SURVEY

The question may arise to what extent the fauna composition at the different stations sampled during the

3rd post-drilling survey resembled the fauna composition found during the baseline survey. Since the baseline situation was described on the basis of the fauna composition at a few stations in the close vicinity of

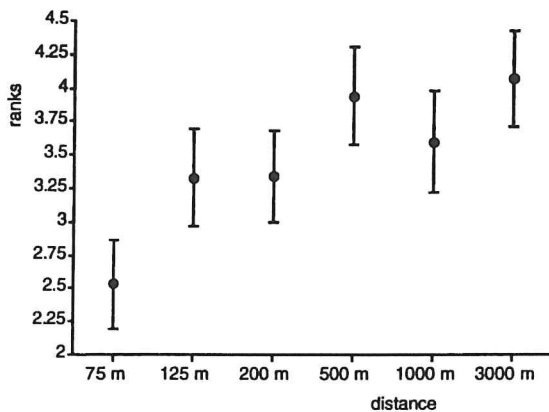


Fig. 21. 3rd post-drilling survey, August 1994. Relative macrofauna abundance along the 0° transect (mean ranks and 95% confidence limits).

the location, the relevance of this question would particularly appear if (a) the similarity between nearby and remote stations were lower than between nearby stations and baseline situation or (b) similarity in the fauna composition between stations in the vicinity of the well site and baseline situation were higher than between remote stations and the baseline. In both cases there would be reason to doubt whether the gradients in fauna composition observed during the 3rd post-drilling survey should be attributed to a gradient of stress. One might argue that these gradients largely could reflect natural spatial inhomogeneity over the sampled transect and that there actually was no appreciable difference with the baseline situation.

A comparison of between-station and between-survey similarities was made on the basis of the Bray-Curtis index for percentage similarity between species assemblages (Bray & Curtis, 1957). The index (range 0-100%) has been found to reflect accurately true similarity (Bloom, 1981). We calculated the index both from quantitative data and from presence absence data of species in samples. The use of quantitative data implies that relatively few (common) species may dominate the calculated similarities, whereas the use of presence-absence data weights 'rare' species as heavily as more common species

TABLE 20

Third post-drilling survey, August 1994: Statistical significance (LSD test) of differences in relative macrofauna abundance between stations. Significance level (%) indicated.

	75 m	125 m	200 m	500 m	1000 m	3000 m
75 m	-					
125 m	0.5	-				
200 m	0.5	n.s.	-			
500 m	0.1	5	5	-		
1000 m	0.1	n.s.	n.s.	n.s.	-	
3000 m	0.1	1	1	n.s.	n.s.	-

(Gray *et al.*, 1988). The results of both sets calculations (Table 21) show that similarities within the group of stations sampled during the 3rd post-drilling survey were consistently higher than between each of these stations and the baseline situation. In other words, the difference in fauna composition between the nearby stations (75-200 m) and the remote stations was smaller than the overall difference in fauna composition between the 2 surveys. Further, both sets of calculations show that the nearby stations differed in about the same degree from the baseline situation as the remote stations. In fact the 3000-m station appeared to have the highest similarity with the baseline situation according to both sets of calculations. This means that there is no reason to suppose that, before drilling started, the 3000-m station was more different from the baseline situation than the nearby stations.

The similarity data indicate that differences in the fauna composition in the area between the 2 surveys, due to natural processes like mortality and settlement of new generations in the intervening period, were larger than differences in fauna composition between nearby and remote stations, due to the presence of a gradient of decreasing stress between these stations. Particularly the poor inter-survey similarity appearing from the comparison based on quantitative data (even the similarity of the least differing 3000-m station with the baseline was poor) implies that the baseline data can not be assumed to provide a reliable reference, *i.e.* an approximate description of the fauna in the vicinity of the location during the 3rd post-drilling survey in the theoretical absence of the stressor.

TABLE 21

Values of the Bray-Curtis index for percentage similarity between stations sampled during the 3rd post-drilling survey and for percentage similarity of these stations with the baseline situation (stations 75m-45°, 200m-45° and 75m 225°). A: Calculation of the index based on quantitative data; B: calculation of the index based on presence-absence data.

A	75 m	125 m	200 m	500 m	1000 m	3000 m
75 m	x					
125 m	55	x				
200 m	37	66	x			
500 m	46	75	78	x		
1000 m	47	75	68	84	x	
3000 m	49	77	64	78	84	x
baseline	26	23	15	24	25	27
B	75 m	125 m	200 m	500 m	1000 m	3000 m
75 m	x					
125 m	78	x				
200 m	73	84	x			
500 m	72	78	78	x		
1000 m	63	72	73	74	x	
3000 m	66	73	76	80	84	x
baseline	61	67	64	64	65	69

4 DISCUSSION

In a study like the present one, where the environmental effects of a point source of disturbance are investigated along a gradient of decreasing stress, the basic idea is that effects on the fauna composition (or individual species) can be detected by comparing the data from stations in the vicinity of the source of stress to a reference situation. The reference situation, in our study the fauna composition at a station 3000 m north of the drilling location, is assumed to give an approximate description of the fauna in the vicinity of the location in the theoretical absence of the stressor. This should not necessarily mean that densities of individual species in the latter situation would be exactly the same as at the reference site, because variance is a natural component of the benthic community. However, the assumption implies that there are no systematic gradients in fauna composition due to incidental gradients in one or more natural environmental variables. The major significance of carrying out a baseline survey is to verify this assumption. The meaning of a baseline survey as a direct reference is of secondary importance, because the detection of effects by temporal changes in the benthic community after the introduction of a stressor may be hampered by relatively large seasonal and year to year fluctuations that can naturally occur in the composition of the benthic community (see Rachor & Gerlach, 1978; Ziegelmeier, 1978). Our data on inter-station and inter-survey similarities (section 3.2.3.5) support the occurrence of these large year to year fluctuations and illustrate that an evaluation of effects by comparing data obtained one year after drilling with baseline data would indeed be hampered by these unpredictable natural fluctuations. In this study, the baseline survey revealed an evident gradient in the fauna composition along the originally planned 45° transect that could be explained very well by the presence of the gradient in sediment composition that was visually observed. This convinced us that the 45° 3000 m station could not be considered to represent a useful reference situation and that we had to switch to another transect for the post-drilling surveys. The consequence of this decision is that there is no real verification now of the assumption that natural gradients in the benthic community along the 0° transect were absent. Nevertheless, in retrospect this assumption seems to be justified, not only by the observation that there was no appreciable gradient in sediment composition along this transect. Also the fact that the similarity indices, calculated to compare data from the 3rd drilling survey with the baseline data, showed that the 3000-m 0° station was not more different from the baseline than the nearby stations does not encourage the idea that natural gradients could have been present over the 0° transect already before drilling started. There are other good reasons to reject the hypothesis that

the gradients along the 0° transect observed during the post-drilling surveys can be considered as incidental natural gradients. Firstly, the majority of abundance patterns observed in individual species pointed in both post-drilling surveys at gradients of decreasing density on approach of the location, which resulted in similar gradients in species richness and relative fauna abundance. This is in contrast with a situation like found during the baseline survey, where natural gradients of decreasing density along the 45° transect occurred as frequently in one direction as in the other and were not accompanied by similar patterns in species richness and relative abundance. Indeed, in the natural situation a shift in sediment composition will involve a differentiation in habitats and therefore a shift in species composition, but a shift in fauna abundance or species richness should not necessarily be expected. Moreover the pattern of overall decreasing densities near the location is the characteristic pattern found near well sites in the Dutch sector where OBM cuttings have been discharged (e.g. Daan *et al.*, 1990). A second reason to attribute the observed gradients to stress from the discharges can be found in the abundance patterns of species which earlier have been identified as most sensitive to OBM cutting discharges. Daan *et al.* (1994) have presented a list of 15 such species and of those species that were more or less common in the area around K14-13 all displayed a tendency for decreasing frequency of occurrence on approach of the location during both post-drilling surveys (Table 22). Apparently these species show a similar response to EBM cutting discharges. Finally, the presence of parts of exoskeletons of recently died *Echinocardium cordatum* in the samples during the 2nd post-drilling survey provided indisputable evidence that mortality had occurred within 500 m from the location and strongly suggests that mortality in the vicinity of the location should explain the gradient observed.

In a qualitative sense, there seems to be a striking similarity between the type of initial effects related to OBM cutting discharges and those resulting from EBM cutting discharges as observed in this study. Particularly the fact that *Echinocardium cordatum* and its symbiont *Montacuta ferruginosa*, two species that have shown to respond most sensitive to OBM (Daan *et al.*, 1994), seemed to be affected in their abundance to the largest distance underlines this similarity. In this respect a parallel can also be recognized with the position of *E. cordatum* in the generalized gradient pattern suggested by Pearson & Rosenberg (1978) for macrobenthic communities along gradients of organic enrichment. In this generalized pattern *E. cordatum* was considered as a representative of 'normal' communities at the lower end of organic enrichment gradients. This parallel indicates that there is an initial effect of EBM and OBM cutting discharges that both have in common with stress resulting from

TABLE 22

Evaluation of the abundance patterns of 15 OBM sensitive species during the 2nd and 3rd post-drilling survey.

+ = tendency for higher abundance away from the location

? = less than 20 specimens found

Significant gradients (5% level) are indicated (*).

species	2nd p-d survey	3rd p-d survey
<i>Scalibregma inflatum</i>	?	+
<i>Pholoe minuta</i>	+ (*)	+ (*)
<i>Nephtys hombergi</i>	+ (*)	+
<i>Glycinde nordmanni</i>	?	?
<i>Cylichna cilindracea</i>	?	?
<i>Chaetozone setosa</i>	?	?
<i>Nucula turgida</i>	?	+ (*)
<i>Amphiura filiformis</i>	+	?
<i>Owenia fusiformis</i>	sp. not found	+
<i>Callianassa subterranea</i>	+	+ (*)
<i>Mysella bidentata</i>	+	+ (*)
<i>Echinocardium cordatum</i>	+ (*)	+
<i>Harpinia antennaria</i>	sp. not found	sp. not found
<i>Gattyana cirrosa</i>	sp. not found	sp. not found
<i>Montacuta ferruginosa</i>	+ (*)	+ (*)

organic enrichment. Most likely this effect should be associated with disturbance of the oxygen supply in the sediment due to a change in the physical or chemical sediment properties. This disturbance might be attributed to elevated bacterial activity, but also by blocking of interstitia (Pearson & Rosenberg, 1978). It is not likely that the latter is merely caused by solid materials (drill cuttings), since in earlier studies on WBM cutting discharges we did not observe the effect on *E. cordatum*. In the case of EBM cutting discharges the possibility of increased oxygen consumption as a result of degradation seems most plausible as a cause of oxygen depletion of the sediment and of the water layer immediately overlying the sediment, but additional physical blocking of interstitia can not be excluded. An explanation related to disturbed oxygen conditions is supported by our observations in boxcosm experiments (Daan *et al.*, 1990) where particularly echinoderms, including *E. cordatum*, responded very sensitively to oxygen depletion. In these experiments, echinoderms were the first to come out of the sediment when aeration of the boxcosms became suboptimal.

During the 2nd post-drilling survey gross effects could be detected up to 200 m from the location. Most species showed reduced abundance within this zone and there appeared to be a clear reduction in species richness, total fauna abundance and relative fauna abundance. Also the presence of *Capitella capitata* was an obvious sign of disturbed sediment conditions in this area. Between 500 m and 1000 m there only seemed to be a few species that occurred in reduced abundance (*Pholoe minuta*, *Echinocardium cordatum* and *Montacuta ferruginosa*). This pattern of decreasing effects with increasing distance from the location

corresponded very well with the gradual decrease of ester concentrations along the transect.

The 3rd post-drilling survey, carried out 11 months after termination of the discharges, still revealed clear effects at 75 m from the location. Effects can be recognized at species level (> 50% of the more or less abundant species occurred in reduced abundance at 75 m compared to the 3000-m reference station and in 9 species a significant gradient of increasing frequency of occurrence away from the location was detected), but also at the community level (relatively low numbers of species per sample, low overall fauna abundance and significantly reduced relative fauna abundance). At 125 m and 200 m effects were clearly less pronounced, but at these stations we still found significantly less species per sample and reduced relative fauna abundance compared to the 3000-m station. At 500 m persistent effects could no longer be demonstrated. In fact, the absence of (large) *Echinocardium cordatum* at this station was expected since this species, including the juvenile generation of 1994, did not survive the first post-drilling period. It would, therefore, take *a priori* more than 1 year before a new generation of adults could have build up. Hence, the absence of *E. cordatum* at 500 m should be considered the 'long-term' consequence of a short-term effect and not as the consequence of actual stress.

Although effects did not have completely disappeared after one year, there are signs of recovery of sediment conditions compared to the situation in January 1994. The onset of recovery is not only indicated by the observation that a substantial fraction of the esters had disappeared after one year, but also by the notion that a reasonable number of species has recolonised the area in the vicinity of the well site. Particularly at 200 m where detectable ester contamination had almost disappeared, several species reappeared in fairly high numbers. On the basis of visual inspection of the boxcores during the fieldwork, which showed a 3-5 cm top layer of oxidized sediment, it seems plausible that this top-layer has provided a suitable substratum for many species to recolonise. Particularly for a superficially burrowing species like the tube-building polychaete *Lagis koreni* this top-layer seems to have provided an opportunity to settle in large amounts. Nevertheless the relatively low numbers at 75 m suggest that there is still an adverse effect of the discharged material on the percentage survival of this species. Another sign of recovery of the natural fauna composition is the abundance decrease of the opportunist *Capitella capitata*, in January frequently found up to 200 m, but in August 1994 present in only one sample at 75 m and in one at 125 m. Additionally, a comparison between Fig. 10 and Fig. 15 shows that the difference in species richness between stations in the vicinity of the location (in particular the 125 m and 200 m stations) and remote stations was clearly smaller in August than in January. A

similar decrease could further be observed in the difference in relative fauna abundance between the 125 m and 200 m stations *versus* the remote stations (compare Fig. 12 and 17).

In the past, there has been one other field study on the effects of EBM cutting discharges. This study was performed at Ula well site 7/12-9 in the Norwegian sector between 1990 and 1992 (Smith & Moore, 1990; Smith & May, 1991; Smith & Hobbs, 1993). During 2 years following EBM drilling the authors monitored the extent of disturbance and subsequent recovery of the macrobenthic community. Immediately after drilling they detected a severely affected zone within 100 m from the well site, where the fauna was strongly impoverished. Between 100 m and 200 m there was an abrupt change in macrofauna and the authors concluded that there were no significant effects noticeable at 200 m and beyond that distance. The sharp demarcation between the affected and non-affected zone was attributed to the short time interval between drilling and subsequent sampling. One year later, they carried out a second survey and found that the zone of disturbance within 100 m from the location had disappeared, indicating recovery of the sediment in the area. During that survey there was only one slightly anomalous station at 500 m from the well, where a patchy but dominant population of *Capitella capitata* was found, but the authors did not consider the presence of this population as serious evidence for a local effect of stress. Our data from the 2nd and 3rd post-drilling surveys at K14-13 indicate a larger extent of the initially affected zone and a slower rate of recovery of sediment conditions. In this context, it should be noted that the amount of esters discharged at K14-13 (180 t) was about twice as much as at the Ula well site (96 t). A second difference is that the Ula well site is situated in 67 m water-depth, which is twice as much as at K14-13 (30 m). This may imply that there is a much larger effect of bottom currents on the redistribution of discharged material at K14-13. A further point to be mentioned is that the biological impact of the discharges at K14-13 was assessed for the first time during the 2nd post-drilling survey, *i.e.* 4 months after drilling. Hence, the benthic community had been exposed considerably longer than at the moment of sampling at the Ula location. Together these factors may have caused that the initial impact observed at K14-13 stretched over a wider area than at the Ula well site. However, the question remains why there seemed to be almost complete recovery of the benthic community at the Ula well site after 1 year, whereas at K14-13 there was still a zone of disturbance detectable. There seem to be only speculative explanations for these contrasting results. One point to be mentioned is that there might have been a difference in the accuracy of position finding. In our study we used DGPS, which allows for an accuracy of positioning within 5-10 m. At Ula well site radar was used and the authors noticed

that their conclusions should be tempered by the knowledge that positioning was difficult (Smith & Hobbs, 1993). Indeed, the use of radar may be subject to considerably more deviation in positioning, particularly when the reference object is at a distance of 4000 m or more. It is not inconceivable therefore that, if there still had been a small area of disturbance, this area was missed. An other possible explanation to be considered is that the day grab used at the Ula well site surveys samples the sediment to a depth of about 10 cm, whereas the larger Van Veen grab we used penetrated to a depth of 15-20 cm. This difference in sampling device may have consequences if there are vertical gradients in the distribution of contaminants in the sediment. Such gradients could possibly have developed as a result of faster degradation of esters in the top layer of the sediment or to sedimentation of fresh material during the period following termination of drilling. This could have allowed for recolonisation of the top layer, whereas deeper living species would still experience the effects of stress. This idea is supported by our visual inspection of the boxcore samples at the 125 m station which always showed a 3-5 cm layer of naturally (grey) coloured sediment on top of the deeper black coloured sediment layers.

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APPENDICES

Appendix 1. Extraction of aliphatic esters from sediments using soxhlet extraction MeOH/DCM

1. Goal

Quantitative extraction of aliphatic esters from marine sediments using flow through soxhlet extraction with methanol followed by dichloromethane

2. Introduction

This SOP is a modification of existing methods for the extraction of aliphatic esters from marine sediments (e.g. Bakke and Laake, 1991 NIVA report), which in turn are modifications of the extraction of total hydrocarbons from sediments.

3. Materials

Methanol (MeOH)

Dichloromethane (DCM)

Trimethyl pentane (TMPe)

Bidistilled water

Flow through soxhlet set-up

Separatory funnel

internal standards (ethyl stearate and pyrene D10)

4. Methods

Determination of water content

Mix the homogenate with a spoon for at least 20 s, especially taking care to scrape the walls and the bottom.

Add accurately about four gram of sediment to the petri dish. Dry at 105 °C until constant weight. Determine the fraction of dry sediment in the sample.

Extraction

For the required solvent amounts, refer to the table below.

Place a plug of quartz wool in the soxhlet tube. Add a weighed amount of wet, homogenised sediment. Place a second plug of glass wool on top of the sample. Add 100 L of the ethyl stearate solution.

Reflux for 3.5 h with methanol (→ Table)

Transfer the MeOH extract to a separatory funnel.

Place DCM (→ Table) in the soxhlet set-up. Reflux overnight.

Add bidistilled (→ Table) to the separatory funnel. Extract with DCM (→ Table).

Extract the MeOH/water phase a second time with DCM (→ Table).

Combine all DCM extracts.

Wash the DCM extract twice with bidistilled water (→ Table) in a separatory funnel.

Transfer the DCM layer to a round bottom flask. Dry the extract with anhydrous Na₂SO₄.

Evaporate to about 5 ml, using a Kuderna Danish column.

Add 1 ml trimethyl pentane.

Evaporate to about 0.5 ml.

Transfer the concentrated extract to a GC vial, add 100 L of the pyrene solution and bring the total volume to 1 ml using TMPe.

Table: Required solvent amounts in mL.

wet sample mass (g)	soxhlet extraction		extraction of MeOH phase		DCM extract wash
	MeOH	DCM	water	DCM	water
15-20	200	200	200	60	100
10-15	150	150	150	45	75
5-10	100	100	100	30	50
< 5	50	50	50	15	25

Appendix 2. Extraction of aliphatic esters from sediments using acetone + acetone/hexane

1. Goal

Quantitative extraction of aliphatic esters from marine sediments using acetone followed by acetone/hexane (30/70)

2. Introduction

Polar solvents are to be preferred over non-polar solvents, since the extraction fluid should be compatible with the polarity of the matrix. Loss of relatively volatile components is a problem that may arise when drying the sediment prior to extraction. Therefore, the wet sediment is extracted and the fraction of dry sediment is determined separately. This SOP is a modification of the procedure described in the SOP "Extraction of PCBs and penta- and hexachlorobenzene". This SOP was based in turn on Jensen et al. (Anal. Chem. 49, 316-318, 1977).

3. Materials

Acetone

Hexane

Bidistilled water

Centrifuge tube 50 mL

Shaking table

Petri dishes (weighed)

Oven (105 °C)

Balance (0.01 g accuracy)

measuring cylinders

internal standards (ethyl stearate and pyrene D10)

4. Methods

Determination of water content

Mix the homogenate with a spoon for at least 20 s, especially taking care to scrape the walls and the bottom.

Add accurately about four gram of sediment to the petri dish. Dry at 105 °C until constant weight. Determine the fraction of dry sediment in the sample.

Extraction procedure

Before taking a sub-sample from the jar, the sediment is mixed for at least 20 s with a spoon, or a spatula. The sub-sample is transferred to a centrifuge tube. The tube is covered with a cleaned sheet of aluminium foil. A volume of an internal standard solution is added to the mixture.

Extraction

For each gram of sediment 1.5 mL of acetone is added. The tube shaken for 1 h on a table shaker.

The tube is centrifuged for 10 min at 3000 min⁻¹. The solvent is decanted into a separatory funnel.

For each gram of wet sediment, 0.3 mL of acetone is added to the centrifuge tube. The residue is resuspended by shaking. Then, for each gram of wet sediment, 0.7 mL of hexane is added.

The tube is shaken for 1.5 hours on a table shaker.

If the hexane/acetone layer is turbid, the tube is centrifuged for 10 min at 3000 min⁻¹.

The fluid decanted into the separatory funnel. The residue is rinsed with a small volume of hexane.

For each gram of wet sediment, 1 mL of bidistilled water is added to the separatory funnel. The funnel is shaken for one minute. After phase separation, the lower (water/acetone) layer is discarded. The hexane layer (upper) is washed once with an equal volume of bidistilled water. The lower layer is discarded, the upper layer is transferred for concentration. Entrained water droplets are bound with anhydrous sodium sulphate (1 g for each mL of water).

Appendix 3. Extraction of aliphatic esters from sediments with MeOH/DCM/water

1 Goal

Quantitative extraction of aliphatic esters from marine sediments using a one-phase solvent (methanol/dichloromethane/water)

2. Introduction

Because of the relatively polar nature of the organic matrix, polar extraction solvents are more suitable than non-polar solvents. This SOP is a modification of the total lipids extraction described by Bligh and Dyer (Can. J. Biochem. Physiol., 37:911-917, 1959). First, water, methanol and dichloromethane are mixed in a proportion that produces a one phase system. The addition of dichloromethane causes phase separation. The addition of water causes a transfer of the esters to the dichloromethane layer. Because the relative proportion of the solvents is critical, the water content of the sample should be determined first.

3. Materials

shaking table

Methanol

Dichloromethane (DCM)

bidistilled water

erlenmeyer flask

round bottom flask

centrifuge tube

balance (0.01 g accuracy)

3 measuring cylinders (10 or 25 ml)

Internal standards: ethyl stearate and pyrene D10

4. Methods

Determination of water content

Mix the homogenate with a spoon for at least 20 s, especially taking care to scrape the walls and the bottom.

Add accurately about four gram of sediment to the petri dish. Dry at 105 °C until constant weight. Determine the fraction of water in the sample.

Selection of required solvent amounts and tube sizes

From the amount to be extracted, calculate the approximate mass of water in the subsamples. From the table below, the mass of water that is next higher than the amount present in the sub-sample. The row in this table determines the volume of all solvents and the volume of the centrifuge tube.

Extraction procedure

Mix the sub-sample with a spoon for at least 20 s, especially taking care to scrape the walls and the bottom.

Transfer a sub-sample to the erlenmeyer flask.

Calculate the amount of water in the sub-sample.

step 1

Add to the sub-sample:

- water (bring the total water mass to the required amount)
- methanol (table)
- dichloromethane (table)
- 100 ul of the ethyl stearate

Mix for 1.5 h on the shaking table at 90 min⁻¹

step 2

Add dichloromethane (table)

Mix for 1 h on the shaking table at 90 min⁻¹

step 3

Add bidistilled water

Mix .

Decant the solvent mixture into a centrifuge tube. Centrifuge for 10 min at 3000 min⁻¹. The tubes may be tarred with bidistilled water.

Remove the upper (methanol/water) layer with a syringe as far as possible

Transfer the lower layer quantitatively to a round bottom flask, with a syringe or a capillary pipette.

Add 1 ml TMPe.

Concentrate the extract in the round bottom flask on a water bath (75-80 °C) down to 2-5 ml.

Transfer the extract quantitatively to a test tube, taking care not to transfer particles.

Concentrate the extract to 1 ml, using a micro Snyder set-up.

Step 1			Step 2	Step 3	volume centrifuge tube (ml)
water (g) (in sample + added water)	methanol (ml)	CH ₂ Cl ₂ (ml)	CH ₂ Cl ₂ (ml)	water	
4	10	5	5	5	50
6	15	7.5	7.5	7.5	50
8	20	10	10	10	100
10	25	25	12.5	12.5	100

Appendix 4. Results oil analyses 1st post-drilling surveys.

Mineral oil concentrations were determined by NAM for the 0⁰ transect (75, 125, 200m) and the 45⁰ transect (75m). The analysis was made according to NEN 6675, which comprises soxhlet extraction with freon, followed by a clean-up step with florisil for removal of polar compounds, and quantitation by measuring the absorption at 3030 cm⁻¹, 2958 cm⁻¹ and 2925 cm⁻¹. Total hydrocarbon concentrations were in the range 10-23 mg/kg wet weight (\approx 13-30 mg/kg dry weight), with a negligible contribution of aromatic hydrocarbons (see Table).

Table

station	grabs	Hydrocarbons incl. aromatics (mg.kg ⁻¹ wet weight)	Hydrocarbons excl. aromatics (mg.kg ⁻¹ wet weight)
0 ⁰ -75m	1,3,5,7	13	13
0 ⁰ -75m	2,4,6,8	23	23
0 ⁰ -125m	1,3,5,7	16	16
0 ⁰ -125m	2,4,6,8	13	13
0 ⁰ -200m	1,3,5,7	12	12
0 ⁰ -200m	2,4,6,8	14	14
45 ⁰ -75m	1,3,5,7	10	10
45 ⁰ -75m	2,4,6,8	11	11

Appendix 5. Data macrofauna, baseline survey (July 1993).					
Mean densities (n.m ⁻²).					
Number of samples () in which species are present.					
Tot. number of ind. per m ² per station.					
Number of identified species.					
R= residual current transect, U= upstream transect.					
Distance to well site (m)	75 R	200 R	500 R	1000 R	75 U
Number of analyzed samples	6	6	6	6	6
POLYCHAETA					
<i>Harmothoe lunulata</i>	2.1 (1)			2.1 (2)	
<i>Harmothoe longisetis</i>	8.3 (4)	4.2 (4)	1.0 (1)		4.2 (4)
<i>Gattyana cirrosa</i>	1.0 (1)				
<i>Sigalion mathildae</i>		1.0 (1)	8.3 (5)	17.7 (6)	3.1 (2)
<i>Pholoe minuta</i>	11.5 (4)	2.1 (2)	2.1 (1)	1.0 (1)	7.3 (3)
<i>Sthenelais limicola</i>	1.0 (1)		4.2 (4)	4.2 (3)	3.1 (3)
<i>Eteone longa</i>	1.0 (1)		4.2 (4)	1.0 (1)	
<i>Mysta barbata</i>	1.0 (1)		1.0 (1)		
<i>Anaitides maculata</i>	4.2 (3)	3.1 (2)			6.3 (2)
<i>Anaitides spec. juv.</i>		3.1 (2)			8.3 (5)
<i>Eumida sanguinea</i>	1.0 (1)			22.9 (6)	2.1 (1)
<i>Ophiodromus flexuosus</i>	2.1 (2)	2.1 (1)			
<i>Gyptis capensis</i>	22.9 (6)	15.6 (6)	6.3 (3)	2.1 (2)	20.8 (5)
<i>Nereis longissima</i>	1.0 (1)	2.1 (1)	2.1 (2)	1.0 (1)	
<i>Nereis spec. juv.</i>	1.0 (1)	2.1 (2)		7.3 (3)	2.1 (1)
<i>Nephtys hombergii</i>	90.6 (6)	65.6 (6)	18.8 (5)	19.8 (5)	63.5 (6)
<i>Nephtys caeca</i>	1.0 (1)		3.1 (2)		
<i>Nephtys spec. juv.</i>	2.1 (1)	1.0 (1)	1.0 (1)		1.0 (1)
<i>Glycera alba</i>	1.0 (1)				
<i>Glycera spec. juv.</i>					1.0 (1)
<i>Goniada maculata</i>	2.1 (2)	3.1 (2)	4.2 (2)	8.3 (4)	7.3 (3)
<i>Lumbrineris latreilli</i>	1.0 (1)	3.1 (3)			1.0 (1)
<i>Scoloplos armiger</i>		1.0 (1)	2.1 (2)	1.0 (1)	1.0 (1)
<i>Poecilochaetus serpens</i>	1.0 (1)	5.2 (5)	1.0 (1)		1.0 (1)
<i>Spio filicornis</i>					1.0 (1)
<i>Spiophanes bombyx</i>	143.8 (6)	90.6 (6)	42.7 (6)	12.5 (5)	86.5 (6)
<i>Aonides paucibranchiata</i>			2.1 (2)		
<i>Magelona papillicornis</i>	5.2 (3)	19.8 (4)	205.2 (5)	287.5 (6)	1.0 (1)
<i>Chaetozone setosa</i>	1.0 (1)	15.6 (5)	55.2 (6)	35.4 (6)	2.1 (2)
<i>Scalibregma inflatum</i>	30.2 (6)	21.9 (5)	4.2 (4)		49.0 (6)
<i>Ophelina acuminata</i>	1.0 (1)	1.0 (1)			

Appendix 5. continued.					
Distance to well site (m)	75 R	200 R	500 R	1000 R	75 U
Number of analysed samples	6	6	6	6	6
<i>Mediomastus gracilis</i>	2.1 (2)				
<i>Notomastus latericeus</i>	3.1 (3)	2.1 (2)	14.6 (6)	4.2 (4)	3.1 (3)
<i>Lanice conchilega</i>	65.6 (6)	15.6 (3)	8.3 (3)	88.5 (6)	103.1 (5)
<i>Lagis koreni</i>	17.7 (5)	20.8 (5)	1.0 (1)		3.1 (2)
<i>Sabellaria spinulosa</i>				1.0 (1)	
MOLLUSCA					
<i>Nucula turgida</i>	3.1 (2)	1.0 (1)	1.0 (1)	1.0 (1)	7.3 (4)
<i>Chlamys spec. juv.</i>		1.0 (1)	8.3 (4)	14.6 (5)	10.4 (5)
<i>Montacuta ferruginosa</i>	50.0 (4)	55.2 (6)	29.2 (6)	55.2 (6)	35.4 (6)
<i>Mysella bidentata</i>	25.0 (4)	19.8 (4)	10.4 (5)		26.0 (6)
<i>Venus striatula</i>		1.0 (1)		2.1 (2)	1.0 (1)
<i>Venus verrucosa</i>			1.0 (1)		
<i>Mactra corallina</i>			3.1 (1)	1.0 (1)	
<i>Tellina fabula</i>	14.6 (4)	8.3 (3)	79.2 (6)	59.4 (6)	15.6 (5)
<i>Tellina tenuis</i>		1.0 (1)			
<i>Abra alba</i>	4.2 (4)	5.2 (3)	1.0 (1)		4.2 (2)
<i>Abra nitida</i>			1.0 (1)	1.0 (1)	
<i>Ensis ensis</i>			1.0 (1)		
<i>Cultellus pellucidus</i>	4.2 (4)	1.0 (1)	8.3 (5)	2.1 (2)	1.0 (1)
<i>Natica alderi</i>	31.3 (6)	46.9 (6)	27.1 (6)	34.4 (6)	39.6 (5)
CRUSTACEA					
<i>Pagurus bernhardus</i>	1.0 (1)	1.0 (1)	2.1 (2)	1.0 (1)	
<i>Macropipus spec. juv.</i>		1.0 (1)	2.1 (2)	3.1 (1)	2.1 (1)
<i>Ebalia cranchii</i>	1.0 (1)				1.0 (1)
<i>Ebalia tuberosa</i>		1.0 (1)			
<i>Corystes cassivelaunus</i>	1.0 (1)	6.3 (4)	2.1 (2)	2.1 (2)	6.3 (4)
<i>Callianassa subterranea</i>	14.6 (4)	12.5 (5)			20.8 (4)
Decapoda larven	3.1 (3)	1.0 (1)		2.1 (2)	4.2 (2)
<i>Eudorella truncatula</i>		1.0 (1)			
<i>Iphinoe trispinosa</i>	1.0 (1)	1.0 (1)	1.0 (1)		
<i>Pseudocuma longicornis</i>			1.0 (1)		
<i>Diastylis bradyi</i>		2.1 (1)		2.1 (2)	3.1 (1)
<i>Ione thoracica</i>	2.1 (1)	1.0 (1)			
<i>Pseudione spec.</i>		1.0 (1)			

Appendix 5. continued.					
Distance to well site (m)	75 R	200 R	500 R	1000 R	75 U
Number of analysed samples	6	6	6	6	6
<i>Megaluropus agilis</i>			2.1 (2)		
<i>Melita obtusata</i>				3.1 (2)	
<i>Atylus swammerdami</i>			1.0 (1)	2.1 (2)	
<i>Atylus falcatus</i>			1.0 (1)		
<i>Hippomedon denticulatus</i>				1.0 (1)	
<i>Orchomenella nana</i>			3.1 (2)	1.0 (1)	
<i>Leucothoe incisa</i>	3.1 (2)	2.1 (2)	4.2 (2)	4.2 (2)	2.1 (2)
<i>Urothoe poseidonis</i>		1.0 (1)	18.8 (5)	8.3 (3)	1.0 (1)
<i>Bathyporeia guilliamsoniana</i>	1.0 (1)	2.1 (2)	4.2 (2)	8.3 (3)	8.3 (2)
<i>Bathyporeia elegans</i>	26.0 (4)	9.4 (3)	133.3 (6)	39.6 (6)	19.8 (5)
<i>Perioculodes longimanus</i>	3.1 (3)				
<i>Pontocrates arenarius</i>				2.1 (2)	
<i>Synchelidium haplocheles</i>	1.0 (1)	3.1 (1)	16.7 (5)	8.3 (5)	5.2 (2)
<i>Caprella spec.</i>	8.3 (1)	3.1 (2)			3.1 (1)
ECHINODERMATA					
<i>Amphiura filiformis</i>	4.2 (4)	3.1 (2)		3.1 (2)	13.5 (5)
<i>Acronida brachiata</i>	1.0 (1)				1.0 (1)
<i>Ophiura albida</i>	19.8 (5)				13.5 (3)
<i>Ophiura spec. juv.</i>	21.9 (6)	13.5 (5)	2.1 (2)	2.1 (2)	12.5 (5)
<i>Echinocardium cordatum</i>	18.8 (4)	27.1 (6)	26.0 (5)	39.6 (6)	14.6 (5)
<i>Echinocardium cordatum juv.</i>	5585.4 (6)	3293.8 (6)	42.7 (6)	42.7 (6)	3166.7 (6)
<i>Echinocyamus pusillus</i>				1.0 (1)	
OTHER TAXA					
Nemertinea	P (4)	P (3)	P (6)	P (1)	P (4)
Nematoda	1.0 (1)				4.2 (2)
Phoroniden	P (5)	P (4)			P (4)
Harp. copepoda	5.2 (4)	3.1 (2)	8.3 (4)	7.3 (5)	3.1 (2)
Oligochaeta			9.4 (3)		
Anthozoa	2.1 (1)	1.0 (1)	3.1 (1)	16.7 (3)	1.0 (1)
Total nr. of individuals	6285	3834	848	890	3830
Nr. of identified species	49	44	46	41	41
P= present (not counted)					

Appendix 6. Data macrofauna, 2nd post-drilling survey (January 1994).						
Mean densities (n.m-2).						
Number of samples () in which species are present.						
Tot. number of ind. per m2 per station.						
Number of identified species.						
R= residual current transect.						
Distance to well site (m)	75 R	125 R	200 R	500 R	1000 R	3000 R
Number of analysed samples	6	6	6	6	6	6
POLYCHAETA						
<i>Harmothoe lunulata</i>			1.0 (1)	1.0 (1)		3.1 (3)
<i>Harmothoe longisetis</i>				1.0 (1)	2.1 (2)	1.0 (1)
<i>Pholoe minuta</i>		1.0 (1)		5.2 (3)	13.5 (5)	37.5 (5)
<i>Sthenelais limicola</i>				4.2 (2)	2.1 (2)	2.1 (2)
<i>Anaitides groenlandica</i>				1.0 (1)	3.1 (2)	6.3 (3)
<i>Anaitides maculata</i>						3.1 (2)
<i>Anaitides spec. juv.</i>					1.0 (1)	
<i>Eumida sanguinea</i>						3.1 (3)
<i>Ophiodromus flexuosus</i>	1.0 (1)		1.0 (1)	3.1 (3)	3.1 (2)	1.0 (1)
<i>Gyptis capensis</i>	2.1 (2)	6.3 (3)	13.5 (4)	19.8 (5)	9.4 (5)	14.6 (5)
<i>Nereis longissima</i>				1.0 (1)		1.0 (1)
<i>Nephtys hombergii</i>	1.0 (1)	8.3 (2)	21.9 (3)	27.1 (6)	18.8 (6)	27.1 (6)
<i>Nephtys hombergii juv.</i>	28.1 (6)	45.8 (4)	61.5 (5)	144.8 (6)	74.0 (6)	33.3 (5)
<i>Nephtys caeca</i>				1.0 (1)		
<i>Nephtys longosetosa</i>			1.0 (1)			
<i>Glycera spec. juv.</i>				1.0 (1)	5.2 (2)	1.0 (1)
<i>Glycinde nordmanni</i>		1.0 (1)				
<i>Goniada maculata</i>				2.1 (2)	1.0 (1)	3.1 (2)
<i>Lumbrineris latreilli</i>	1.0 (1)					
<i>Poecilochaetus serpens</i>			2.1 (1)	3.1 (1)	2.1 (1)	1.0 (1)
<i>Spio filicornis</i>		2.1 (1)				
<i>Polydora pulchra</i>			1.0 (1)	1.0 (1)	2.1 (2)	
<i>Spiophanes bombyx</i>	3.1 (2)	4.2 (2)	24.0 (3)	45.8 (6)	62.5 (6)	79.2 (6)
<i>Magelona papillicornis</i>			-	1.0 (1)		
<i>Chaetozone setosa</i>			2.1 (1)	1.0 (1)		
<i>Scalibregma inflatum</i>				1.0 (1)		1.0 (1)
<i>Ophelina acuminata</i>				2.1 (2)		2.1 (1)
<i>Capitella capitata</i>	41.7 (5)	44.8 (6)	10.4 (3)			
<i>Mediomastus gracilis</i>				2.1 (2)		
<i>Notomastus latericeus</i>				1.0 (1)	1.0 (1)	2.1 (1)
<i>Lanice conchilega</i>			2.1 (2)	3.1 (3)	1.0 (1)	5.2 (2)
<i>Lagis koreni</i>	1.0 (1)	1.0 (1)	2.1 (2)		3.1 (2)	1.0 (1)

Appendix 6. continued.						
Distance to well site (m)	75 R	125 R	200 R	500 R	1000 R	3000 R
Number of analysed samples	6	6	6	6	6	6
MOLLUSCA						
<i>Nucula turgida</i>		3.1 (2)		2.1 (1)	4.2 (4)	2.1 (2)
<i>Montacuta ferruginosa</i>					1.0 (1)	32.3 (5)
<i>Mysella bidentata</i>	3.1 (2)	3.1 (3)	5.2 (4)	12.5 (5)	8.3 (5)	12.5 (4)
<i>Mactra corallina</i>						1.0 (1)
<i>Spisula spec. juv.</i>		3.1 (2)				
<i>Tellina fabula</i>	1.0 (1)		1.0 (1)	1.0 (1)		
<i>Abra alba</i>				3.1 (3)	1.0 (1)	4.2 (2)
<i>Abra alba juv.</i>	457.3 (6)	379.2 (6)	254.2 (6)	463.5 (6)	572.9 (6)	1454.2 (6)
<i>Onoba vitrea</i>			2.1 (2)			1.0 (1)
<i>Natica alderi</i>	7.3 (5)	21.9 (6)	52.1 (6)	102.1 (6)	114.6 (6)	137.5 (6)
<i>Cylichna cilindracea</i>					1.0 (1)	1.0 (1)
CRUSTACEA						
<i>Processa parva</i>			2.1 (2)	1.0 (1)	4.2 (2)	2.1 (2)
<i>Pontophilus trispinosus</i>		1.0 (1)				
<i>Macropipus marmoreus</i>			1.0 (1)	1.0 (1)		
<i>Macropipus spec. juv.</i>	1.0 (1)					
<i>Ebalia cranchii</i>				1.0 (1)	1.0 (1)	
<i>Corystes cassivelaunus</i>			1.0 (1)			
<i>Callianassa subterranea</i>	3.1 (3)	2.1 (1)	6.3 (3)	8.3 (4)	9.4 (5)	3.1 (3)
<i>Gastrosaccus spinifer</i>		1.0 (1)			1.0 (1)	
<i>Eudorella truncatula</i>					2.1 (1)	
<i>Iphinoe trispinosa</i>		1.0 (1)	1.0 (1)		3.1 (2)	1.0 (1)
<i>Eurydice spinigera</i>	1.0 (1)					
<i>Gammarus marinus</i>				1.0 (1)		
<i>Orchomenella nana</i>					2.1 (2)	2.1 (2)
<i>Leucothoe incisa</i>			1.0 (1)	1.0 (1)	5.2 (5)	3.1 (2)
<i>Urothoe poseidonis</i>				1.0 (1)	1.0 (1)	
<i>Bathyporeia guilliamsoniana</i>				1.0 (1)		2.1 (1)
<i>Bathyporeia elegans</i>				12.5 (2)	3.1 (2)	3.1 (2)
<i>Perioculodes longimanus</i>						1.0 (1)
<i>Caprella spec.</i>			2.1 (2)		2.1 (1)	
<i>Corophium insidiosum</i>		1.0 (1)				

Appendix 6. continued.						
Distance to well site (m)	75 R	125 R	200 R	500 R	1000 R	3000 R
Number of analysed samples	6	6	6	6	6	6
ECHINODERMATA						
<i>Amphiura filiformis</i>		2.1 (1)	4.2 (2)	17.7 (4)	9.4 (5)	10.4 (4)
<i>Amphiura chiajei</i>				1.0 (1)		2.1 (2)
<i>Ophiura albida</i>	4.2 (3)	2.1 (2)	11.5 (4)	15.6 (6)	1.0 (1)	1.0 (1)
<i>Ophiura spec. juv.</i>	1.0 (1)	2.1 (1)	1.0 (1)	20.8 (4)	15.6 (6)	16.7 (6)
<i>Echinocardium cordatum</i>					2.1 (1)	10.4 (4)
<i>Echinocardium cordatum juv.</i>					188.5 (5)	230.2 (6)
OTHER TAXA						
<i>Nemertinea</i>		P (1)	P (2)	P (4)	P (3)	P (3)
<i>Turbellaria</i>					1.0 (1)	1.0 (1)
<i>Phoroniden</i>			P (1)	P (5)	P (3)	P (3)
<i>Harp. copepoda</i>	1.0 (1)		1.0 (1)			
<i>Oligochaeta</i>			1.0 (1)			
<i>Anthozoa</i>				1.0 (1)		
Total nr.of individuals	559	538	492	943	1160	2165
Nr. of identified species	13	18	24	38	33	39
P=present (not counted)						

Appendix 7. Data macrofauna, 3rd post-drilling survey (August 1994).						
Mean densities (n.m-2).						
Number of samples () in which species are present.						
Tot. number of ind. per m2 per station.						
Number of identified species.						
R= Residual current transect.						
Distance to well site (m)	75R	125R	200R	500R	1000R	3000R
Number of analysed samples	5	5	5	5	5	5
POLYCHAETA						
<i>Aphrodita aculeata</i>				3.8 (2)	2.5 (2)	1.3 (1)
<i>Harmothoe lunulata</i>			1.3 (1)		1.3 (1)	1.3 (1)
<i>Harmothoe longisetis</i>	2.5 (2)	6.3 (2)		3.8 (3)	5.0 (3)	3.8 (3)
<i>Harmothoe spec. juv.</i>				2.5 (2)	2.5 (1)	
<i>Sigalion mathildae</i>				2.5 (2)		2.5 (2)
<i>Pholoe minuta</i>	21.3 (3)	20.0 (4)	117.5 (5)	71.3 (5)	37.5 (5)	87.5 (5)
<i>Sthenelais limicola</i>	1.3 (1)	3.8 (2)	1.3 (1)	5.0 (2)		3.8 (3)
<i>Eteone longa</i>	60.0 (5)	145.0 (5)	406.3 (5)	366.3 (5)	120.0 (5)	108.8 (5)
<i>Anaitides groenlandica</i>	17.5 (4)	62.5 (5)	33.8 (5)	60.0 (5)	123.8 (5)	158.8 (5)
<i>Anaitides maculata</i>	26.3 (5)	56.3 (5)	186.3 (5)	30.0 (4)	20.0 (4)	8.8 (3)
<i>Eumida sanguinea</i>			7.5 (2)	10.0 (5)	2.5 (2)	3.8 (3)
<i>Ophiodromus flexuosus</i>		1.3 (1)	1.3 (1)	7.5 (3)	7.5 (4)	7.5 (3)
<i>Gyptis capensis</i>	25.0 (5)	13.8 (5)	17.5 (5)	10.0 (4)	12.5 (4)	26.3 (5)
<i>Nereis longissima</i>	1.3 (1)	15.0 (3)	35.0 (5)	10.0 (2)	41.3 (5)	43.8 (5)
<i>Nereis spec. juv.</i>			1.3 (1)			
<i>Nephtys hombergii</i>	17.5 (4)	35.0 (5)	41.3 (5)	43.8 (5)	20.0 (4)	32.5 (5)
<i>Nephtys caeca</i>		1.3 (1)		2.5 (2)	2.5 (1)	
<i>Nephtys spec. juv.</i>	1.3 (1)					
<i>Glycera alba</i>		5.0 (3)	1.3 (1)	2.5 (2)		
<i>Glycera spec. juv.</i>		2.5 (2)	8.8 (4)	10.0 (3)	1.3 (1)	5.0 (2)
<i>Glycinde nordmanni</i>			1.3 (1)		5.0 (3)	1.3 (1)
<i>Goniada maculata</i>	2.5 (2)	1.3 (1)	3.8 (2)	7.5 (4)	2.5 (2)	10.0 (4)
<i>Lumbrineris latreilli</i>					1.3 (1)	1.3 (1)
<i>Scoloplos armiger</i>		2.5 (2)	2.5 (1)	2.5 (2)	1.3 (1)	1.3 (1)
<i>Poecilochaetus serpens</i>				2.5 (1)	5.0 (3)	6.3 (3)
<i>Spio filicornis</i>	3.8 (2)	7.5 (2)	7.5 (1)	1.3 (1)		
<i>Spiophanes bombyx</i>	2.5 (2)	51.3 (5)	42.5 (4)	86.3 (5)	90.0 (5)	87.5 (5)
<i>Scolecopsis bonnierii</i>		1.3 (1)				
<i>Magelona papillicornis</i>	8.8 (4)	18.8 (5)	6.3 (3)	6.3 (3)		1.3 (1)
<i>Chaetozone setosa</i>			1.3 (1)	5.0 (2)		1.3 (1)

Appendix 7 (continued).						
Distance to well site (m)	75R	125R	200R	500R	1000R	3000R
Number of analysed samples	5	5	5	5	5	5
<i>Scalibregma inflatum</i>	12.5 (5)	106.3 (5)	20.0 (4)	31.3 (4)	56.3 (5)	32.5 (5)
<i>Ophelina acuminata</i>		1.3 (1)			1.3 (1)	
<i>Capitella capitata</i>	1.3 (1)	1.3 (1)				
<i>Mediomastus gracilis</i>						2.5 (2)
<i>Notomastus latericeus</i>	8.8 (2)	3.8 (3)	5.0 (2)	1.3 (1)	16.3 (5)	83.8 (5)
<i>Owenia fusiformis</i>	6.3 (3)	27.5 (5)	26.3 (5)	40.0 (5)	37.5 (5)	33.8 (4)
<i>Lanice conchilega</i>	11.3 (3)	13.8 (4)	20.0 (5)	18.8 (5)	33.8 (5)	21.3 (5)
<i>Lagis koreni</i>	455.0 (5)	1116.3 (5)	2200.0 (5)	1498.8 (5)	1448.8 (5)	1200.0 (5)
<i>Pectinaria belgica</i>					1.3 (1)	
MOLLUSCA						
<i>Nucula turgida</i>	1.3 (1)	2.5 (2)	2.5 (2)	8.8 (3)	5.0 (2)	12.5 (4)
<i>Kellia suborbicularis</i>						1.3 (1)
<i>Montacuta ferruginosa</i>					5.0 (2)	17.5 (4)
<i>Mysella bidentata</i>	5.0 (3)	21.3 (5)	15.0 (5)	63.8 (5)	56.3 (5)	47.5 (5)
<i>Venus spec. juv.</i>		1.3 (1)	1.3 (1)			
<i>Mactra corallina</i>	6.3 (1)	1.3 (1)			1.3 (1)	1.3 (1)
<i>Tellina fabula</i>	25.0 (4)	66.3 (5)	45.0 (5)	36.3 (5)	3.8 (2)	12.5 (4)
<i>Abra alba</i>	73.8 (5)	207.5 (5)	153.8 (5)	100.0 (5)	58.8 (4)	131.3 (5)
<i>Abra alba juv.</i>	23.8 (2)	126.3 (5)	63.8 (5)	76.3 (5)	63.8 (5)	38.8 (5)
<i>Cultellus pellucidus</i>		6.3 (3)	7.5 (4)	2.5 (2)	1.3 (1)	1.3 (1)
<i>Thracia phaseolina</i>		1.3 (1)	1.3 (1)			
<i>Natica alderi</i>	25.0 (5)	33.8 (5)	47.5 (5)	55.0 (5)	45.0 (5)	68.8 (5)
<i>Cylichna cylindracea</i>				1.3 (1)		1.3 (1)
CRUSTACEA						
<i>Processa parva</i>	3.8 (3)			5.0 (3)	2.5 (1)	3.8 (2)
<i>Pagurus bernhardus</i>				1.3 (1)	1.3 (1)	1.3 (1)
<i>Macropipus marmoreus</i>						1.3 (1)
<i>Macropipus spec. juv.</i>	1.3 (1)		2.5 (2)	1.3 (1)		
<i>Corystes cassivelaunus</i>		3.8 (2)	7.5 (5)	8.8 (4)	3.8 (3)	3.8 (3)
<i>Callianassa subterranea</i>	5.0 (2)	11.3 (4)	7.5 (3)	27.5 (5)	57.5 (5)	30.0 (5)
<i>Decapoda larven</i>	7.5 (3)					
<i>Eudorella truncatula</i>					2.5 (2)	
<i>Iphinoe trispinosa</i>		2.5 (1)	1.3 (1)			1.3 (1)

Appendix 7 (continued).						
Distance to well site (m)	75R	125R	200R	500R	1000R	3000R
Number of analysed samples	5	5	5	5	5	5
<i>Diastylis bradyi</i>				1.3 (1)		
<i>Ione thoracica</i>			2.5 (1)		10.0 (3)	2.5 (1)
<i>Megaluropus agilis</i>			1.3 (1)			
<i>Orchomenella nana</i>				1.3 (1)	7.5 (2)	5.0 (3)
<i>Leucothoe incisa</i>	2.5 (2)	2.5 (2)		7.5 (2)	1.3 (1)	2.5 (1)
<i>Urothoe poseidonis</i>	1.3 (1)			2.5 (2)	1.3 (1)	
<i>Bathyporeia guilliamsoniana</i>	1.3 (1)	3.8 (1)	2.5 (1)	3.8 (3)		1.3 (1)
<i>Bathyporeia elegans</i>	8.8 (4)	3.8 (3)	13.8 (4)	20.0 (5)	3.8 (1)	5.0 (3)
<i>Synchelidium haplocheles</i>	1.3 (1)					
<i>Caprella spec.</i>	1.3 (1)		5.0 (2)	11.3 (3)	3.8 (2)	
ECHINODERMATA						
<i>Asterias rubens</i>	1.3 (1)				1.3 (1)	
<i>Amphiura filiformis</i>				3.8 (1)	2.5 (2)	2.5 (2)
<i>Ophiura texturata</i>	8.8 (3)	1.3 (1)	2.5 (2)	5.0 (2)	3.8 (2)	10.0 (3)
<i>Ophiura albida</i>	11.3 (5)	5.0 (2)	3.8 (2)	26.3 (5)	3.8 (2)	33.8 (4)
<i>Ophiura spec. juv.</i>	33.8 (5)	51.3 (5)	88.8 (5)	141.3 (5)	150.0 (5)	75.0 (5)
<i>Echinocardium cordatum</i>		2.5 (2)	1.3 (1)		38.8 (5)	63.8 (4)
<i>Echinocardium cordatum juv.</i>	68.8 (5)	460.0 (5)	1585.0 (5)	667.5 (5)	287.5 (5)	198.8 (5)
OTHER TAXA						
<i>Nemertinea</i>		P (4)	P (4)	P (4)	P (4)	P (4)
<i>Turbellaria</i>	2.5 (2)	1.3 (1)	8.8 (3)	2.5 (2)		1.3 (1)
<i>Phoroniden</i>	P (2)	P (2)	P (2)	P (4)	P (5)	P (4)
<i>Harp. copepoda</i>			3.8 (2)	1.3 (1)	1.3 (1)	1.3 (1)
<i>Oligochaeta</i>			5.0 (2)	1.3 (1)		
<i>Holothuroidea</i>					1.3 (1)	
<i>Anthozoa</i>				2.5 (2)		
Tot. nr. of individuals	1006	2736	5276	3629	2935	2756
Nr. of identified species	36	44	45	49	51	54
P= present (not counted)						

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