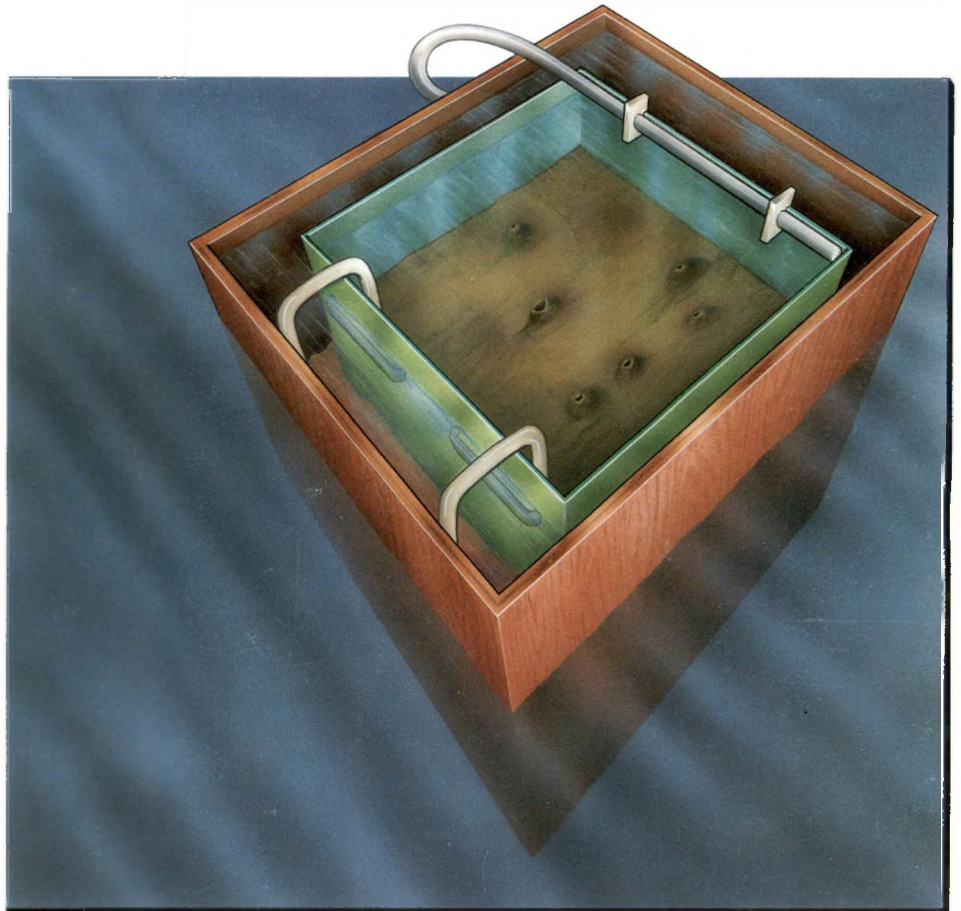


# THE ASSESSMENT OF A NO-EFFECT CONCENTRATION OF OIL IN THERMALLY TREATED OBM DRILL CUTTINGS FOR MARINE BENTHOS; ECOTOXICITY

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**THE ASSESSMENT OF A NO-EFFECT  
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This study was commissioned by the North Sea Directorate (RWS)  
and carried out in 1990

**NETHERLANDS INSTITUTE FOR SEA RESEARCH**

Beleidsgericht Wetenschappelijk Onderzoek NIOZ

## SUMMARY AND CONCLUSIONS

During drilling activities in the North Sea large amounts of drill cuttings are dumped on the seabed. The material is contaminated with adhering drilling muds, necessarily employed to perform the drilling process. In particular when oil based muds (OBM) are used, these discharges are a source of concern with respect to their impact on the marine environment.

In the 1980's several measures have been taken to reduce the load of toxicants in the discharged material and, thus, to minimize possible adverse effects for health and environmental reasons. *E.g.* in the Dutch sector of the North Sea the use of diesel based muds was forbidden in 1985 and according to the current legislation only the use of oil with a low aromatic content is allowed. Furthermore so called 'washing' techniques were developed to regain as much as possible of the OBM from the cuttings before they are dumped, thus reducing the emission of oil in a quantitative way. Nevertheless, these techniques failed to completely eliminate adverse effects on the benthic community. Since 1991 most operating companies in the Dutch Continental Sector voluntarily refrained from dumping oil-contaminated drill cuttings.

During these years, a new technique was developed by Solid Control Services (SCS). The method includes a thermal treatment of the cuttings, resulting in oil concentrations of  $\leq 1\%$  in the waste material. Till now, this method was only applied experimentally in land-based drillings, but it has the potential to be applied offshore. However, before implementation the effectiveness of this method should be evaluated. The central question is to what extent this treatment contributes to a reduction of adverse effects on the benthic community and to what levels oil concentrations should be reduced to eliminate such effects.

This report describes the results of research on the possible effects of discharges of thermally treated cuttings on a North Sea macrobenthic community. Experiments were performed in boxcosms with intact sediment sections collected in the 'Frisian Front' area, at the 5000-m reference station of drilling site L5-5. The boxcosms were stocked with a test species (*Echinocardium cordatum*) and discharges were simulated by artificially dosing various amounts of SCS cuttings. Since not the effect of the amount of material dosed was questioned, but the effect of the resulting oil concentrations in the sediment, a complementary amount of uncontaminated material was added to the lower doses to standardize the total amount of material dosed. To this end SCS cuttings were used that were cleaned from any organic material by combustion at 550°C. The standard amount was in correspondence with the maximum

amount to be expected at 100 m from an imaginary discharge point, as calculated by a simulation model. Two types of controls were used, *viz.* one without any addition and one with addition of the standard amount of combusted cuttings, to test possible effects due to the composition of the oil-free material dosed.

Biological effects are described in terms of observed lethal and sublethal effects in test animals and estimated mortality rates among natural infauna species. Three series of oxygen measurements were carried out to detect whether the dosings affected the penetration depth of oxygen in and the potential oxygen demand of the sediment. Additional chemical analyses were performed by the Netherlands Organisation for Applied Scientific Research (TNO, dept. den Helder, see GROENEWOUD & SCHOLTEN, 1992a).

The results and conclusions may be summarized as follows:

1. The sediment utilized appeared to be contaminated by unexpectedly high and variable background concentrations of oil. Unfortunately the presence of this contamination was detected after termination of the experiments. The origin of the oil in the sediment can not be explained. The composition was different from that assessed in the washed OBM cuttings discharged at drilling site L5-5.
2. Although the background concentrations for total oil in the boxcosms were 2-13 times higher than the minimum effect concentration of base oil for *Echinocardium* as previously assessed ( $\approx 20$  mg-oil-kg<sup>-1</sup> dry sediment), the species readily survived the experimental period. Overall mortality was less than 3% and sublethal effects were not observed. Apparently the oil already present in the sediment did not affect the functioning of the test species.
3. With respect to the doses, slight mortality occurred in *Echinocardium*, but was not related to the added doses of the thermally treated cuttings. Apparently there was no toxic effect of the oil in the cuttings on *Echinocardium*.
4. Mortality among the natural infauna was variable between species, but was generally not related to any kind of oil contamination. Only *Mysella bidentata* showed a significant response to the dosed SCS cuttings. At concentrations of 3.8 mg oil from cuttings per kg dry sediment (and higher) mortality seemed to be significantly elevated compared to the controls. However, there are several indications that this statistical significance was accidental and that interpretation of the outcome in terms of a causal relationship between mortality and dosed cuttings should be considered a statistical Type I error.

5. Dosing of combusted SCS cuttings did not result in adverse effects on the test species or the natural infauna.
6. In the high dose boxes the aerobic layer was significantly thinner than in the controls, probably due to the increased load of organic matter and oil originating from the cuttings.
7. The fact that the simulated discharges of thermally treated cuttings did not result in any substantial biological effect may indicate that the thermal treatment changed the composition of residual base oil in favor of non-toxic components or decreased the bioavailability of toxic components.
8. It is conceivable that, in general, adverse effects on the benthic community associated with discharges of oil contaminated drill cuttings are not merely due to toxicants but also to disturbance of the natural sediment structure. In this respect the SCS treatment also might include an improvement compared to traditional treatments.
9. Due to the powdery structure of the thermally treated cuttings, a floating dust film was observed, which existed until 2 weeks after dosing.
10. If discharge at sea is considered, further investigation is needed.

## SAMENVATTING EN CONCLUSIES

Tijdens boringen op de Noordzee worden aanzienlijke hoeveelheden boorgruis geloosd op de zeebodem. Het materiaal is verontreinigd met aanhangende boorspoelingen, vloeistoffen die bij het uitvoeren van de boring noodzakelijkerwijs worden toegepast. Met name wanneer het boorspoeling op oliebasis ('oil based muds'=OBM) betreft, vormen deze lozingen een bron van zorg met betrekking tot hun effect op het mariene milieu.

In de tachtiger jaren zijn verschillende maatregelen genomen, bedoeld om de concentraties aan toxische componenten in het geloosde materiaal te reduceren en daarmee eventuele negatieve effecten op het oecosysteem te minimaliseren. In de Nederlandse sector van de Noordzee werd bijvoorbeeld het gebruik van diesel based muds in 1985 mede om arbeidshygiënische redenen verboden en is volgens de huidige wetgeving alleen het gebruik van olie met een laag aromaatgehalte toegestaan. Verder werden zogenaamde 'was-technieken' ontwikkeld teneinde zoveel mogelijk olie uit het boorgruis terug te winnen alvorens het geloosd wordt. Op deze wijze tracht men de emissie van olie in kwantitatieve zin te reduceren. Toch slaagde men er niet in het toepassen van deze techniek niet in negatieve effecten van lozingen op de benthische levensgemeenschap geheel te elimineren. Sinds 1991 heeft een groot aantal maatschappijen het lozen van oliehoudend boorgruis op het Nederlands Continentaal Plat vrijwillig gestaakt.

Inmiddels werd door de firma Solid Control Services (SCS) een nieuwe techniek ontwikkeld, die in feite neerkomt op een thermische behandeling van het opgeboorde gruis. Men kan met deze methode het oliegehalte in het gruis terugbrengen tot  $\leq 1\%$ . De behandeling werd tot nog toe alleen uitgevoerd bij boringen op het vasteland, maar zou in de toekomst op OBM boorgruis afkomstig van offshore activiteiten toegepast kunnen worden. Voordat dat ook daadwerkelijk gebeurt, is het wenselijk de effectiviteit van de methode aan een nader onderzoek te onderwerpen. De kernvraag is, in welke mate de behandeling bijdraagt tot een reductie van negatieve effecten op de bodemfauna en tot hoever olie-concentraties dienen te worden teruggebracht teneinde dergelijke effecten geheel te voorkomen.

Dit rapport beschrijft de resultaten van onderzoek naar de mogelijke effecten van lozingen van thermisch behandeld boorgruis op de benthische fauna in de Noordzee. Experimenten werden uitgevoerd in boxcosms met intacte sedimentkernen afkomstig uit het Friese Front gebied, nl. het 5000-m referentiestation van de eerder onderzochte boorlocatie L5-5. In de boxcosms werden proefdieren uitgezet (*Echinocardium cordatum*) en vervolgens werden

lozingen gesimuleerd door kunstmatig verschillende doseringen SCS-gruis op het sediment aan te brengen. Aangezien hier niet de vraag aan de orde was welk effect een bepaalde hoeveelheid gedoseerd materiaal teweeg bracht, maar wel welk effect de resulterende olieconcentraties in het sediment opleverden, werden de lagere doseringen aangevuld met een bepaalde hoeveelheid niet verontreinigd materiaal, teneinde de totaal aangebrachte hoeveelheden in alle boxcosms op een standaard niveau te houden. Dit complementaire materiaal bestond uit SCS-gruis, waaruit door verassing al het organische materiaal was verdreven. De standaard hoeveelheid correspondeerde met de maximale hoeveelheid gruis die naar verwachting terecht komt op 100 m van een denkbeeldig lozingspunt. Deze laatste hoeveelheid is berekend aan de hand van een simulatiemodel. De experimenten werden uitgevoerd tegen een tweetal controles: één zonder enige toevoeging van boorgruis en één met een standaard hoeveelheid verast SCS-gruis teneinde mogelijke effecten van dosering van dit olie-vrije materiaal te controleren.

Biologische effecten worden beschreven aan de hand van waargenomen lethale en sublethale effecten op de proefdieren en de geschatte sterfetsnelheden onder natuurlijke infauna-soorten. In de loop van het experiment werden drie series zuurstofmetingen verricht om na te gaan of de doseringen invloed hadden op de penetratiediepte van zuurstof in, en het potentiële zuurstofgebruik door het sediment. Daarnaast werden chemische analyses uitgevoerd door TNO-den Helder (zie GROENEWOUDE & SCHOLTEN, 1992a), teneinde olieconcentraties in het sediment als gevolg van de doseringen vast te stellen.

De resultaten en conclusies kunnen als volgt worden samengevat:

1. Het in de proeven gebruikte sediment bleek te zijn verontreinigd met onverwacht hoge en bovendien variabele achtergrondconcentraties olie. Helaas werd de aanwezigheid van deze verontreiniging pas na afloop van het experiment ontdekt. De herkomst van deze olie kon niet worden achterhaald. De samenstelling ervan was anders dan die welke eerder werd vastgesteld in het gewassen OBM-boorgruis dat geloosd is op de lokatie L5-5.
2. Hoewel de achtergrondconcentraties olie in het sediment van de boxcosms 2 tot 13 keer hoger waren dan de tot nog toe aangenomen minimale effectconcentratie van OBM-olie voor *Echinocardium* ( $\approx 20$  mg olie-kg<sup>-1</sup> droog sediment), bleek de soort de incubatieperiode gemakkelijk te overleven. De totale mortaliteit bedroeg niet meer dan 3% en sublethale effecten werden niet waargenomen. Blijkbaar had de olie

- die in het sediment reeds aanwezig was geen invloed op het functioneren van de proefdier-soort.
3. Mortaliteit kwam bij *Echinocardium* op geringe schaal voor en was niet gerelateerd aan de hoogte van de doseringen thermisch behandeld gruis. Kennelijk ondervond *Echinocardium* geen effect van de olie in het boorgruis.
  4. Onder de natuurlijke infauna-soorten was de mortaliteit variabel, maar in het algemeen niet gerelateerd aan enige vorm van oliecontaminatie. Alleen *Mysella bidentata* vertoonde een significante respons op het gedoseerde SCS-gruis. Bij concentraties van 3,8 mg olie (afkomstig uit dit gruis) per kg droog sediment en hoger leek de mortaliteit significant verhoogd in vergelijking tot de controles. Er zijn echter verschillende aanwijzingen, dat deze statistische significantie van toevallige aard moet zijn geweest. Interpretatie van de statistische uitkomst in de zin van een causale relatie tussen gedoseerd SCS-gruis en mortaliteit zou vermoedelijk neerkomen op een (in statistische zin) fout van de eerste orde.
  5. Dosering van uitsluitend verast SCS-gruis bleek geen negatieve effecten te hebben op de proefdieren of de natuurlijke infauna.
  6. In de boxcosms met de hogere doseringen was de  $O_2$ -houdende laag significant dunner dan in de controles, waarschijnlijk als gevolg van de verhoogde organische belasting en de olie afkomstig uit het thermisch behandelde boorgruis.
  7. Het gegeven dat de gesimuleerde lozingen van thermisch behandeld gruis geen waarneembaar biologisch effect hadden, wijst er op dat de thermische behandeling mogelijk van invloed is op de samenstelling van de rest-olie, die achteraf nog in het gruis aanwezig is, en een verschuiving teweegbrengt ten gunste van non-toxische componenten.
  8. Het is in het algemeen denkbaar, dat negatieve effecten op de bodemfauna als gevolg van lozingen van oliehoudend boorgruis niet alleen toe te schrijven zijn aan toxische stoffen, maar deels ook aan een aantasting van de natuurlijke structuur van het sediment. Het lijkt er op, dat de SCS-behandeling ook in dit opzicht een verbetering betekent in vergelijking met tot nog toe gebruikelijke methoden.
  9. Als gevolg van de poederachtige structuur van het thermisch behandelde gruis, ontstond direct na dosering een laagje drijvend stof op het wateroppervlak, dat pas na twee weken verdwenen was.
  10. Indien lozing van thermisch behandeld boorgruis op zee zou worden overwogen, is nader onderzoek geboden.

## 1 INTRODUCTION

### 1.1 GENERAL PART

During drilling activities in the North Sea large amounts of drill cuttings are usually dumped on the seabed. The material is contaminated with adhering drilling muds, which may contain toxic components. In particular when oil based muds (OBM) are employed, the material disposed may be expected to be toxic. As a result, these discharges entail adverse effects on the benthic community. Such effects have been demonstrated to occur to over 1 km from drilling sites (e.g. MULDER *et al.*, 1987, 1988; REIERSEN *et al.*, 1989; GRAY *et al.*, 1990; DAAN *et al.*, 1990, 1991).

Since 1988 OBM cuttings are cleaned by sieving and washing procedures before discharge and, according to the current legislation, the material dumped should contain maximally 10% oil by weight. However, since the total amount of drill cuttings may amount to several hundreds of tonnes per drilling, still several tonnes of adhering oil will be discharged on the sea floor. Consequently, adverse effects on the benthic community still do occur and subtle biological changes were observed upto  $\approx 1$  km from such a drilling location where washed drill cuttings had been discharged (DAAN *et al.*, 1991).

Recently a new method to clean OBM cuttings was developed by Solid Control Services (SCS). The technique includes grinding of the cuttings and simultaneous heating, by which the oil evaporates for the greatest part. In the resulting powder the oil content is reduced to or below 1%.

This report deals with the results of experimental research on possible effects of discharges of such thermally treated drill cuttings on the benthic community. The central question underlying this study, carried out in the period September 1990 - January 1991, was to what extent the thermal treatment contributes to a reduction of adverse effects on the benthic fauna and to what levels oil concentrations in discharged cuttings should be reduced to eliminate such effects. The experiments were performed with intact sediment cores, collected at the 5000-m reference station of the earlier investigated drilling site L5-5 in the Frisian Front area in the Dutch part of the Continental Shelf (see DAAN *et al.*, 1991). The cores, including the natural infauna, were incubated in an indoor basin and discharges were simulated by dosing various amounts of SCS-cuttings to these 'box-cosms'. Possible responses to these dosings were studied by estimating the percentage survival of the natural infauna in the cores and by observing behaviour and mortality of introduced test animals (adult *Echinocardium cordatum*). In the course of the experiment O<sub>2</sub>-profiles were measured 3 times. Oil con-

centrations in the sediment were determined after termination of the experiment.

The biological research presented was carried out by the Netherlands Institute for Sea Research (NIOZ) in close cooperation with the Netherlands Organisation for Applied Scientific Research (TNO, dept. den Helder). TNO performed the chemical analyses to assess oil-contamination levels in the sediment and possible accumulation of oil in the tissue of test animals (GROENEWOUD & SCHOLTEN, 1992a).

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Ing. M. de Krieger (RWS, North Sea Directorate)  
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## 2. METHODS

## 2.1. EXPERIMENTAL SET-UP

The sediment employed in the boxcosms was collected in the first week of September 1990 in the Frisian Front area at 53°49'N and 4°25'E. This location was earlier sampled in 1989 as the 5000-m reference station of the 'washed' drilling site L5-5 (DAAN *et al.*, 1991). The sediment at the location was sampled first for macrofauna by taking 10 Reineck boxcores (round cores, diameter 30 cm, depth  $\approx$  40 cm). These box-core 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. Then the sediment sections to be used in the boxcosms were collected. These intact boxcores were taken with a (modified) Scripps corer. The stainless steel boxes (50x50x60 cm) were furnished with a mica-teflon coating. The Scripps corer tended to dig about 40 cm deep, thus collecting the major sediment layers inhabited by the benthic infauna. On board 14 such cores were placed in waterproof plywood cases with cooling water and fixed in sand. The water on top of the cores was regularly changed during the transport to the laboratory. After transport the complete cases were placed in an indoor basin and incubated at  $\approx$  16°C. During the period of incubation (4 months), the temperature was gradually lowered to  $\approx$  10°C, according to *in situ* temperatures. The water on the cores was continuously replaced with filtered and O<sub>2</sub>-saturated water from the Wadden Sea, with salinity varying between 29 and 31‰. Apart from inspections, incubation took place continuously in the dark. During the period of incubation the boxcosms were inspected daily for mortality and activity of test animals and natural infauna. Some small crabs (*Processa parva* and *Macropipus holsatus*) were removed to minimize mortality by predation.

## 2.2. TEST ANIMALS

The boxcosms were stocked with test animals (adult *Echinocardium cordatum*, 3.5 - 5 cm) in the third week of September. Per boxcosm 20 specimens were introduced. In former experiments, both in boxcosms (DAAN *et al.*, 1990, 1991) and bioassays (ADEMA, 1991), the species has appeared to be very susceptible to oil contamination of sediments.

## 2.3. DOSING OF THERMALLY TREATED CUTTINGS

SCS-treated drill cuttings were dosed to the boxcosms on October 10, *i.e.* 3 weeks after introduction of the test animals. A range of concentrations was tested, all in duplicate, against 2 untreated controls. The selection of concentrations was based on a worst case scenario; resulting from computer simulations performed by Delft Hydraulics. According to these unpublished calculations the total immission of SCS cuttings at 100 m from a theoretical discharge point, where one well has been drilled, would maximally amount to 700 gram·m<sup>-2</sup>. Assuming that this dose would turn out to be realistic in the natural field situation, the equivalent amount of 175 g SCS-cuttings per boxcosm was considered as a basic dose to be tested (Table 1). A factor 3.2 stepwise dilution was applied to test lower concentrations, the lowest being 5.6 g SCS cuttings per boxcosm. Since not the amount of contaminated cuttings was questioned, but the effect of the resulting oil concentration in the sediment, a complementary amount of uncontaminated material was added to the lower doses to standardize the total amount of material dosed. To this end SCS-cuttings were applied that were cleaned from organic contaminants by combustion at 550°C. It was expected that adding cuttings treated like this would not affect the grain-size distribution of the material dosed. Preliminary analyses had revealed that treatment (precombustion) of the SCS-cuttings

TABLE 1  
Schedule of experiments. s.d. = standard dose

Box nr.	treatment	SCS cuttings dosed (g)	uncontaminated material added (g)	expected oil concentration (mg·kg <sup>-1</sup> dry sed.)
1/2	untreated control	0	0	0
3/4	treated control	0	175	< 0.3
5/6	.032 x s.d.	5.6	169.4	1.2
7/8	0.10 x s.d.	17.5	157.5	3.8
9/10	0.32 x s.d.	56.0	119.0	12
11/12	st. dose	175	0	38
13/14	3.2 x s.d.	560	0	120

resulted in elimination of more than 99.9 % of the oil (GROENEWOUDE & SCHOLTEN, 1992a). To test possible effects of dosing oil-free cuttings, the precombusted material was dosed also in the standard amount to one pair of boxcosms as a check on experimental conditions ('treated control', Table 1). Finally 1 overdose of untreated SCS-cuttings ( $3.2 \times$  standard dose) was tested.

In the last column of Table 1 the oil concentrations are listed, that would be expected in the sediment of the boxcosms after dosing. The tabulated values are based on the results of analyses performed by GROENEWOUDE & SCHOLTEN (1992a), who found the oil concentration in the untreated SCS-cuttings to be 0.76% by weight, and on the assumption that the material dosed would stay in the upper 10 cm sediment layer during the experiment.

Since the untreated SCS-cuttings had appeared to be hydrophobic, the material was carefully brought into suspension before it was dosed. By adding small amounts of water and thoroughly stirring, some kind of sludge was obtained, that then was added to the boxcosms. Although most of the material then gradually descended on the sediment, still a film of dust

appeared on the water. In particular in the high-dosed boxes this floating dust could be observed up to two weeks later.

Since dosing of SCS-cuttings to the boxcosms took place on October 10 and the incubation was terminated at January 10, the total experimental period took 3 months. Actual contamination levels in the sediment were determined at termination of the experiment from small sediment cores, 9 of which were taken from each of the boxcosms. Oil concentrations were analysed according to the GCMS technique (gas chromatograph mass spectrometer). Concentrations of the fractions of alkanes ( $C_8$ - $C_{32}$ ), unidentified complex matter (UCM) and 'other components' were quantified. Methods and results are given in detail by GROENEWOUDE & SCHOLTEN (1992a).

#### 2.4. O<sub>2</sub>-PROFILES

Oxygen profiles were measured 6, 8 and 12 weeks after dosing of the SCS cuttings. The electrodes were constructed according to REVSBECH *et al.*, (1983) and sheathed in 10 cm stainless steel injection needles (outer diameter 1 mm). They had a sensing tip of 10

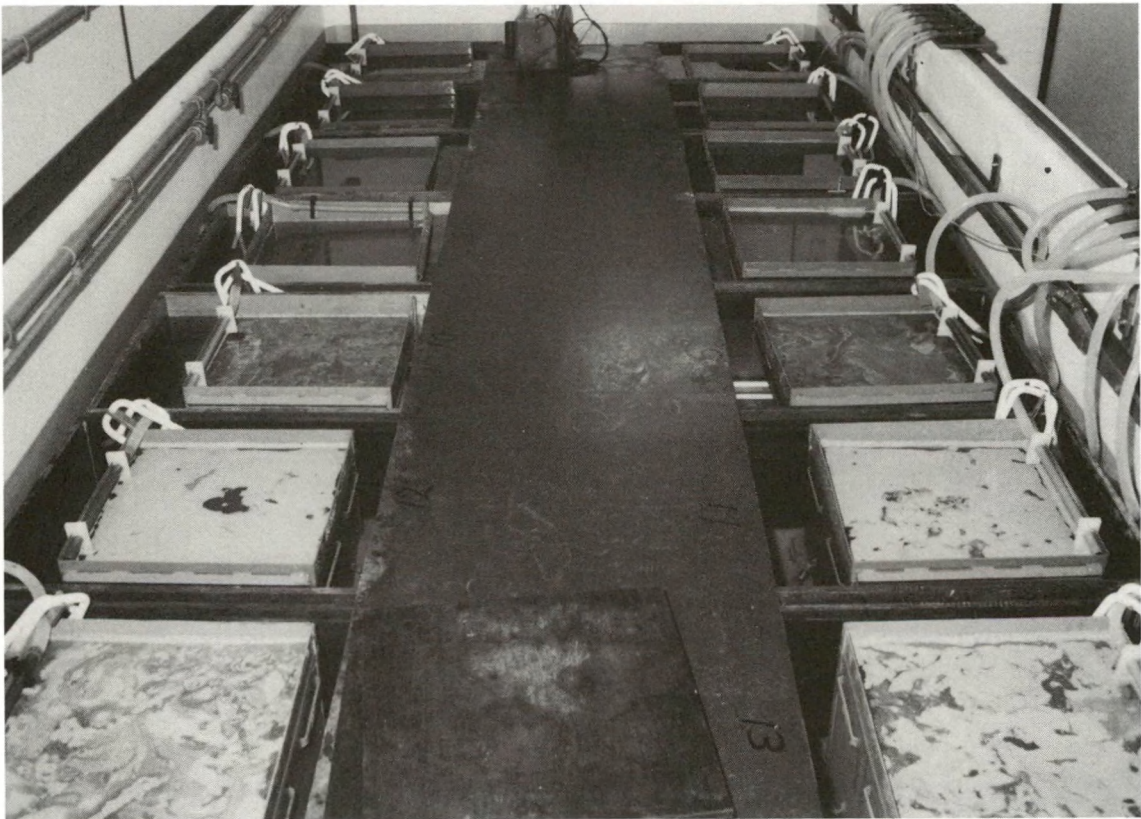


Fig. 1. Boxcosms in the indoor basin.

$\mu\text{m}$ , which was gold-plated and covered by a cellulose-acetate membrane. Microprofiles were measured with an automated micromanipulator on line with a solid state memory. Five oxygen electrodes and a Ag/AgCl reference electrode were connected to a platform. The platform was driven by a linear step-motor with discrete steps of  $100 \mu\text{m}$ . During the measurements the flow-through of seawater was stopped. In every boxcosm the platform was lowered two or three times, resulting in 2 or 3 sets of 5 simultaneously measured profiles. The probes were positioned approximately 1 cm above the sediment/water interface and allowed to stabilize and polarize for 15-20 minutes. Subsequently the measuring of profiles started. The sediment/water interface was reconstructed by comparing the change in slopes between the benthic boundary layer and the sediment itself. This invoked an error of  $\pm 100 \mu\text{m}$ . The electrodes give a linear response between the zero signal (the signal read from the lower part of the profiles) and the signal read in the overlying water. Triplicate seawater samples were taken and oxygen contents were determined after Winkler.

### 3. RESULTS

#### 3.1 GENERAL REMARKS

In this chapter the visual effects of dosing SCS-cuttings and the resulting concentrations of oil in the

sediment, as described by GROENEWOUD & SCHOLTEN (1992a), are summarized.

As already indicated in chapter 2.3, the method of dosing SCS-cuttings to the boxcosms did not prevent that a film of dust appeared at the water surface, due to the hydrophobic property of the untreated material. The extent of this dust film was proportional to the amount of material dosed (Fig. 1). In the treated control such a film was not observed. Possibly, this floating dust was more severely contaminated with oil than the material that settled down on the sediment. As remains of the dust film were visible up to two weeks after dosing (in the highest dosed boxcosms), part of the adhering oil may have evaporated during this period. If indeed such a loss occurred, the oil concentrations in the sediment would have been expected to be lower than the values listed in Table 1.

In contrast, the sediment analyses performed after termination of the experiment revealed much higher oil concentrations than were predicted from the amounts of material dosed. Moreover, there was no significant correlation between total oil concentrations measured and amounts of material dosed (Fig. 2). Indeed, part of the oil could be identified as (probably) originating from the SCS-cuttings, but the remaining part must have been present in the sediment in variable concentrations before the experiments started. The origin of this oil is unknown. As GROENEWOUD & SCHOLTEN (1992a) conclude from-

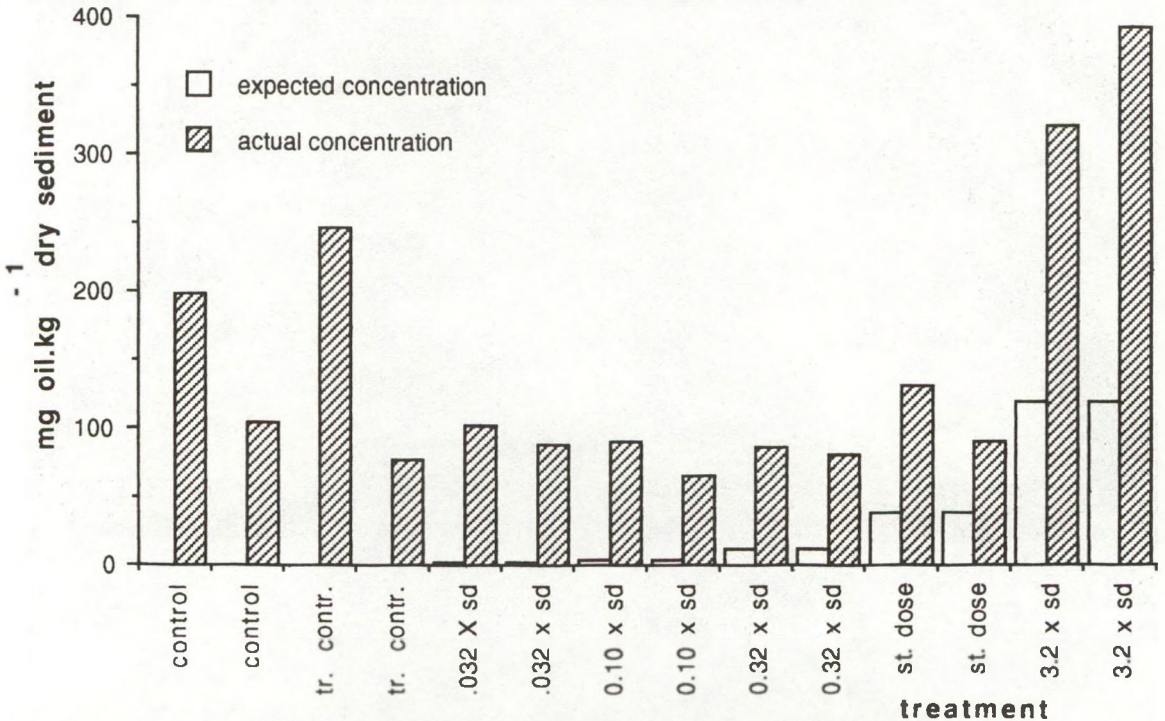


Fig. 2. Expected and actual oil concentrations in 14 boxcosms dosed with a range of thermally treated drill cuttings. tr. contr.=treated control (only precombusted SCS cuttings dosed) sd=standard dose (175 g SCS cuttings dosed).

TABLE 2

Natural infauna: mean initial densities ( $n \cdot m^{-2}$ ) estimated from 10 Reineck samples.

POLYCHAETA		<i>Mysia undata</i>	4.2
		<i>Abra alba</i>	2.8
<i>Aphrodita aculeata</i>	.0	<i>Cultellus pellucidus</i>	1.4
<i>Harmothoe longisetis</i>	4.2	<i>Mya truncata</i>	.0
<i>Gattyana cirrosa</i>	9.9	<i>Corbula gibba</i>	.0
<i>Pholoe minuta</i>	53.5	<i>Thracia convexa</i>	.0
<i>Sthenelais limicola</i>	9.9	<i>Cingula nitida</i>	14.1
<i>Ophiodromus flexuosus</i>	4.2	<i>Turritella communis</i>	1.4
<i>Gyptis capensis</i>	5.6	<i>Natica alderi</i>	5.6
<i>Synelmis klatti</i>	5.6	<i>Cylichna cylindracea</i>	5.6
<i>Exogone hebes</i>	4.2		
<i>Nereis longissima</i>	1.4	CRUSTACEA	
<i>Nereis spec. juv.</i>	1.4	<i>Pagurus bernhardus</i>	2.8
<i>Nephtys hombergii</i>	9.9	<i>Ebalia cranchii</i>	1.4
<i>Nephtys incisa</i>	1.4	<i>Corystes cassivelaunus</i>	.0
<i>Nephtys spec. juv.</i>	4.2	<i>Upogebia stellata</i>	.0
<i>Glycera rouxii</i>	5.6	<i>Upogebia deltaura</i>	.0
<i>Glycera alba</i>	.0	<i>Upogebia spec. juv.</i>	2.8
<i>Glycera spec. juv.</i>	2.8	<i>Callianassa subterranea</i>	100.0
<i>Glycinde nordmanni</i>	2.8	<i>Nebalia bipes</i>	1.4
<i>Goniada maculata</i>	5.6	<i>Eudorella truncatula</i>	2.8
<i>Lumbrineris latreilli</i>	288.7	<i>Diastylis bradyi</i>	2.8
<i>Lumbrineris fragilis</i>	25.4	<i>Ione thoracica</i>	.0
<i>Orbinia sertulata</i>	.0	<i>Melita spec.</i>	1.4
<i>Paraonis spec.</i>	2.8	<i>Orchomenella nana</i>	1.4
<i>Poecilochaetus serpens</i>	8.5	<i>Ampelisca brevicornis</i>	11.3
<i>Spio filicornis</i>	.0	<i>Ampelisca tenuicornis</i>	15.5
<i>Polydora pulchra</i>	.0	<i>Ampelisca spec. juv.</i>	4.2
<i>Polydora guillei</i>	.0	<i>Cheirocratus sundevalli</i>	1.4
<i>Spiophanes bombyx</i>	36.6	<i>Harpinia antennaria</i>	2.8
<i>Magelona papillicornis</i>	1.4		
<i>Magelona alleni</i>	4.2	ECHINODERMATA	
<i>Chaetopterus variopedatus</i>	16.9	<i>Amphiura filiiformis</i>	981.7
<i>Tharyx marioni</i>	2.8	<i>Amphiura chiajei</i>	7.0
<i>Scalibregma inflatum</i>	5.6	<i>Ophiura albida</i>	.0
<i>Notomastus latericeus</i>	15.5	<i>Ophiura spec. juv.</i>	2.8
<i>Heteromastus filiiformis</i>	4.2	<i>Echinocardium cordatum</i>	15.5
<i>Owenia fusiformis</i>	1.4		
<i>Myriochele heeri</i>	.0	OTHER TAXA	
<i>Lagis koreni</i>	7.0	Nemertinea	P
<i>Pectinaria auricoma</i>	19.7	Turbellaria	1.4
<i>Sosane gracilis</i>	7.0	Phoroniden	P
		Harp. copepoda	4.2
MOLLUSCA		Parasitaire copepoda	1.4
<i>Nucula turgida</i>	.0	Holothuroidea	2.8
<i>Thyasira flexuosa</i>	.0	Echiurida	4.2
<i>Lepton squamosum</i>	.0	Sipunculida	97.2
<i>Montacuta ferruginosa</i>	9.9	Ascidiacea	1.4
<i>Mysella bidentata</i>	181.7		
<i>Dosinia lupinus</i>	.0		
<i>Venus striatula</i>	1.4		

the chromatograms, it seems not likely that the oil originated from discharged washed OBM cuttings discharged at location L5-5.

### 3.2 SURVIVAL OF THE NATURAL INFAUNA

An estimate of the survival rate of the natural infauna in the boxcosms is obtained by comparing the abundance of living fauna present at the end of the experiment with estimates of the initial macrofauna abundance. Estimates of the initial abundance are based on the Reineck box samples that were taken simultaneously, when the sediment sections for the boxcosms were collected. The sediment depth sampled by the Reineck corer is similar to that in the boxcosms (35 - 50 cm).

Together the Reineck samples (10) yielded 54 identified species. Estimated initial densities of all species ( $n \cdot m^{-2}$ ) are listed in Table 2. A complete list of the original data (estimated densities in individual samples) is given in the appendix (Table 4). In these tables a number of species is included, of which no specimens were found in the Reineck samples. Some specimens of these species were later found, however, in one or more boxcosms. Table 2 shows that *Amphiura filiformis* predominated the macrofauna by number. Almost 50% of the individuals be-

longed to this species. Less numerous, but still abundant were *Pholoe minuta*, *Lumbrineris latreilli*, *Spiophanes bombyx*, *Mysella bidentata* and *Callianassa subterranea*. On average all these species were represented by more than 2 specimens per Reineck sample.

The fauna abundance was obviously higher in the initial situation than after incubation. Estimates of the total abundance ranged from 1500 to 3100 individuals per  $m^2$  (mean 2000 per  $m^2$ ) before incubation, whereas these numbers varied between 300 and 1300 (mean 640 per  $m^2$ ) after incubation (Table 4 and 5, appendix). This undeniably indicates that mortality occurred in all boxcosms. Mortality was not restricted to a few species, but seemed to occur among most of the species. Indeed, all abundant species suffered mortality, but not in the same degree: *E.g.*, in *Pholoe minuta* mortality was almost complete, whereas  $\approx 30\%$  of *Amphiura filiformis* survived and  $\approx 70\%$  of *Callianassa subterranea*.

A visual inspection of the data concerning the untreated controls (box 1 and 2) and the treated controls (box 3 and 4) shows that there is no indication that dosing of precombusted SCS cuttings did enhance mortality. In fact the total abundance was higher in the treated controls and several of the abundant species occurred in the highest numbers in box 3.

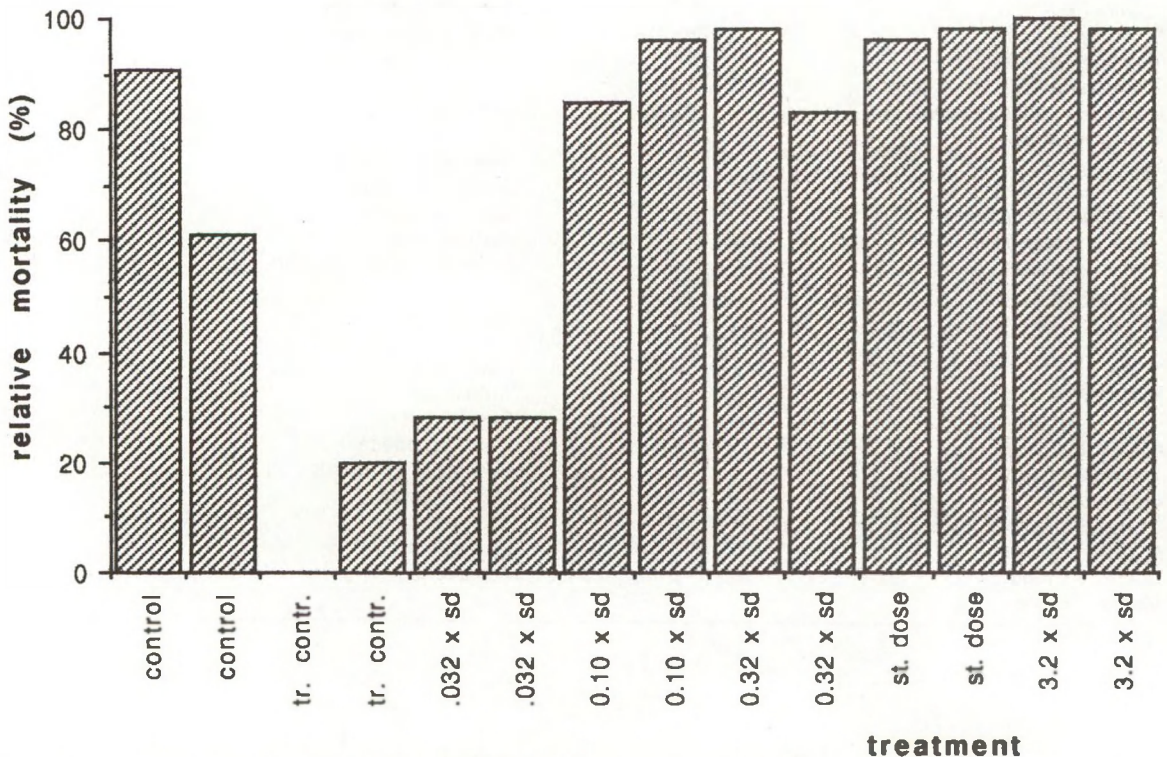


Fig. 3. Estimated relative mortality (%) in *Mysella bidentata* during incubation of the boxcosms. tr. contr.=treated control (only precombusted SCS cuttings dosed) sd=standard dose (175 g SCS cuttings dosed).

A statistical comparison of mortality rates in boxcosms with different doses of SCS cuttings is only relevant for species that were abundant at the start of the experiment, *i.e.* those species of which on average at least 2 specimens were found per Reineck sample. Other species are pooled in higher taxonomic groups, *i.e.* 'other Polychaetes' and 'other Molluscs'. Pooling of 'other Crustaceans' and 'other Echinoderms' failed to obtain abundance values of  $\geq 2$  individuals per Reineck sample.

In first instance analysis of variance was performed to test the equality of relative mortality rates in the different boxcosms. The relative mortality rate is defined as:

$$m_i = (\ln(n_o) - \ln(n_i)) / t$$

where:

$m_i$  = mortality rate in the  $i^{\text{th}}$  box  
 $n_o$  = mean initial number per box  
 $n_i$  = final number in the  $i^{\text{th}}$  box and  
 $t$  = time in weeks

The analysis of variance revealed no significant differences in nearly all species, except for *Mysella bidentata*. The latter species appeared to have suffered significantly ( $P = 0.016$ ) less mortality in the controls and the lowest dosed box ( $0.032 \times \text{sd}$ ) than in the higher dosed ( $\geq 0.10 \times \text{sd}$ ) boxes. As Fig. 3 shows mortality was always more than 80% in the boxcosms with 10% or more SCS cuttings, whereas mortality was in general much lower in the other boxcosms. Nevertheless it should be noted here that mortality was remarkably high in at least one of the untreated controls.

In the procedure performed in the analysis of variance described above, the assumption was made that adding oil-free material (precombusted SCS cuttings) to the boxcosms did not affect mortality rates. Moreover, the use of a mean initial density implicitly ignores variance on initial densities in the boxcosms. Finally, the presence of variable oil concentrations in the sediment before the experiments started may have interfered the results. Therefore, an alternative procedure (analysis of covariance) was performed, aimed to assess to what extent all individual factors explain a significant part of the total variance on numbers of individuals in the sediment before and after incubation. When the density of a species in a box sample is possibly affected by time (effect of incubation), presence of added oil-free material, presence of added oil in SCS cuttings and concentration of oil already present in the sediment, the following expression may represent a model which can be tested by analysis of covariance:

$$Y_i = \mu + c_i \cdot \alpha + d_i \cdot \beta + \gamma \cdot p_i + \delta \cdot r_i + \epsilon$$

where:

$Y_i$  = log (density ( $n \cdot m^{-2}$ ) in the  $i^{\text{th}}$  box),  
 $i = 1, \dots, 24$   
 $\mu$  = mean density of the population  
 $\alpha$  = effect of incubation  
 $\beta$  = effect of oil-free material (precombusted SCS cuttings)  
 $\gamma \cdot p_i$  = effect of oil in SCS cuttings added, with  
 $p_i$  = log (oil concentration)  
 $\delta \cdot r_i$  = effect of oil already present in the sediment, with  
 $r_i$  = log (oil concentration)  
 $\epsilon_i$  = random deviation  
 $c_i$  and  $d_i$  are dummy variables

The analysis of covariance applied to test this model resulted in a significant (5% level) effect of incubation on all abundant species and pooled taxonomic groups, *i.e.* numbers found after incubation were significantly lower than before, apparently due to mortality. In general however, there was no significant effect of oil-free material, oil in dosed SCS cuttings or oil in the sediment on this mortality, with one exception. Numbers of *Mysella bidentata* after incubation appeared to be significantly ( $P < 0.001$ ) lower in the boxcosms with higher doses of SCS cuttings, which suggests that the survival rate of *Mysella* was affected by oil in the SCS cuttings. There was, however, another significant effect, *viz.* that of adding reference material ( $P = 0.025$ ). The latter significance would imply that only adding reference material enhances the survival rate of *Mysella*. It should be noted here that this is merely a statistical outcome and not necessarily a conclusion (see Discussion). Finally, the analysis revealed no effect of the background concentrations of oil in the sediment on numbers of *Mysella* in the boxcosms.

### 3.3 BEHAVIOUR AND SURVIVAL OF TEST ANIMALS

Earlier experiments with *Echinocardium* have shown that the presence of sediment contamination is often clearly indicated by disturbed burrowing behaviour of the test species (DAAN *et al.*, 1990, 1991). In oil contaminated sediment the animals appear frequently at the sediment surface and may finally not burrow anymore.

Daily inspections revealed that most of the test animals remained under the sediment for the whole period. Over the total period of incubation, the mean fraction of animals that could be observed at or on the sediment surface was zero in box 2 (untreated control), box 6 ( $0.032 \times$  standard dose) and box 12 (standard dose). In the other boxes the percentual presence of animals at or on the sediment surface varied between 0.09% (once an animal observed) and 1.4 % (16 times an animal observed). Even the

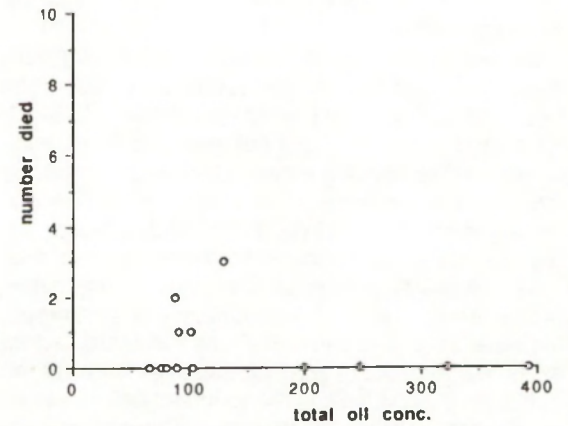
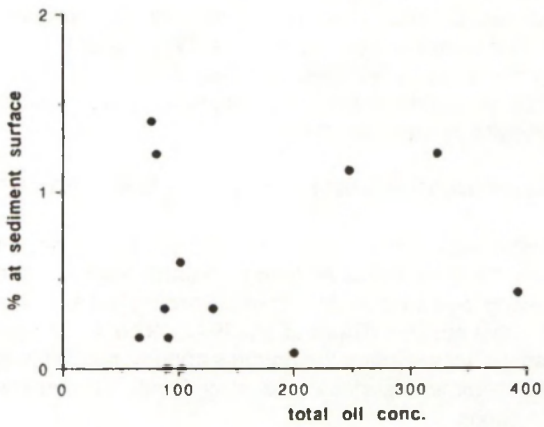
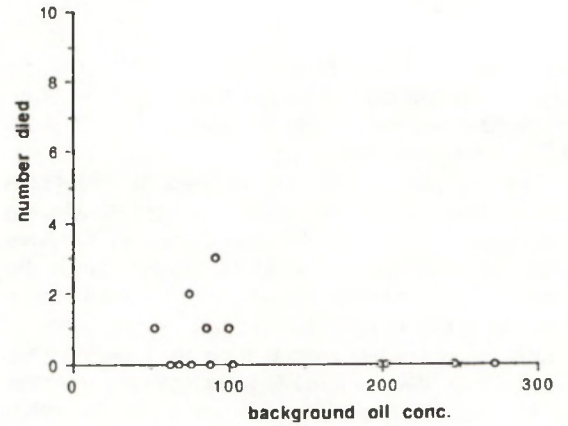
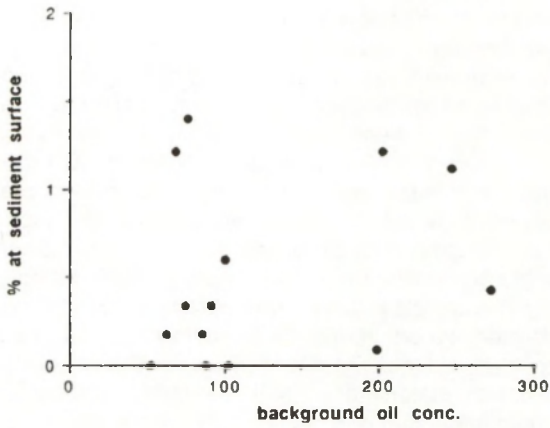
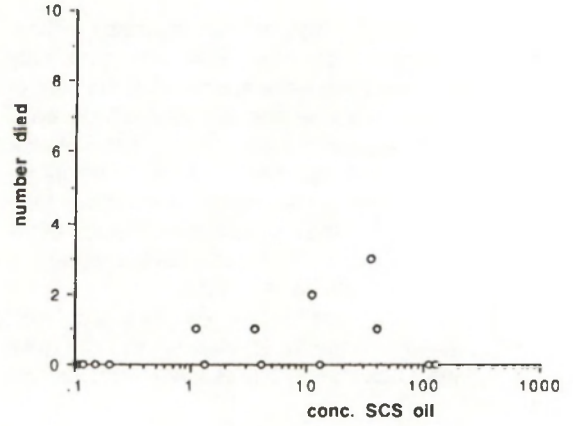
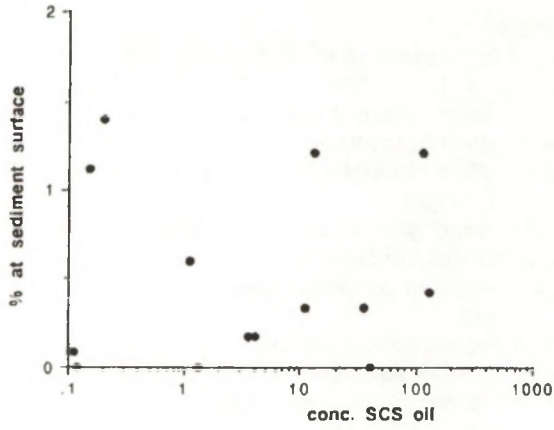


Fig. 4. *Echinocardium cordatum*: Test animals (%) daily present at the sediment surface plotted against: a. concentration of oil from SCS cuttings in the sediment b. background concentration of oil c. total oil concentration in the sediment (all concentrations in mg·kg<sup>-1</sup> dry sediment).

Fig. 5. *Echinocardium cordatum*: Number of test animals died during incubation plotted against: a. concentration of oil from SCS cuttings in the sediment b. background concentration of oil c. total oil concentration in the sediment (all concentrations in mg·kg<sup>-1</sup> dry sediment).

latter frequency is too low to be considered abnormal, c.q. indicative of sediment contamination. In most boxcosms, where animals were observed more than once, these observations concerned one or two animals that remained at the sediment surface for some days. Moreover there was no indication that the animals appeared more frequently at the sediment surface as the contamination level of the sediment increased. Fig. 4 shows that there was not any trend related to either the amount of SCS-cuttings dosed, to the total actual oil concentration, or to the concentration of the oil that must have been present in the sediment sections initially (=total oil minus oil in SCS-cuttings dosed).

During the experimental period 4 dead test animals were removed from the boxcosms. Only one of them showed slight loss of spines at the ventral side. At termination of the experiment another 4 animals appeared to have died. Loss of spines, a common sublethal effect in *Echinocardium* when it is introduced to oil contaminated sediment, did not occur in any of the surviving animals.

Table 3 shows that the highest mortality in *Echinocardium* was found in the boxcosms with the standard dose (700 g untreated SCS-cuttings per m<sup>2</sup> or 38 mg oil·kg<sup>-1</sup> dry sediment) and that no mortality occurred in the controls. However, there was no evidence for a significant increase of mortality with increasing amounts of SCS-cuttings dosed (see also Fig. 5). In the boxcosms with the highest dosing of SCS-cuttings all animals survived. Fig. 5 also shows that plots of mortality against total oil concentrations in the sediment or against 'background' concentrations do not suggest any relationship between mortality and oil in the sediment.

### 3.4 O<sub>2</sub>-PROFILES

A clear relation was observed between the thickness of the aerobic layer of the sediment and the amount of SCS cuttings dosed (Fig. 6). In the boxes with 0.32, 1.0 and 3.2×standard dose the averaged thickness ranged between 4.2 and 6.5 mm, whereas in both controls (with and without addition of precombusted cuttings) this zone ranged between 7.0 and 10.4 mm. The other boxes showed intermediate values.

The mean oxygen concentration at the sediment/water interface did not differ significantly between the boxes nor between the 3 measuring days and varied between 75 and 92% saturation. The oxygen decrease in the thin aerobic layer of the sediment is illustrated in Fig. 7. The slope is a measure of the respiratory activity and positively related to the oxygen consumption (REVSBECH *et al.*, 1983; HOFMAN *et al.*, 1991). Since no measurements were carried out of porosity and the apparent diffusion coefficient, fluxes could not be calculated from the slopes. The slopes can be compared since temperature changes are small: 12.5°C in week 6, 11.5°C in week 8 and 10.2°C in week 12. The control without any addition had the lowest slopes throughout the experiment, 39.6 - 45.4 μmol oxygen·l<sup>-1</sup> porewater·mm<sup>-1</sup>. In the 3 boxes with the highest doses clearly enhanced slopes were seen, 58.3 - 113.9 μmol·l<sup>-1</sup>·mm<sup>-1</sup>. The low dose boxes showed slightly enhanced slopes, ranging from 45.0 - 68.8 μmol oxygen·l<sup>-1</sup>·mm<sup>-1</sup>. There was no relation with the total oil content of the sediment. The relatively high ADW content of the SCS cuttings may explain the observed increase in respiratory activity.

TABLE 3

Mortality of *Echinocardium* in the boxcosms. tr. contr.=treated control; sd.=standard dose.

Box nr.	treatment	expected oil conc. (mg·kg <sup>-1</sup> dry sediment)	actual oil conc. (mg·kg <sup>-1</sup> dry sediment)	number died	mortality (%)
1	control	0	199	0	0
2	control	0	103	0	0
3	tr. contr.	0	247	0	0
4	tr. contr.	0	76	0	0
5	.032 × sd.	1.2	101	1	5
6	.032 × sd.	1.2	89	0	0
7	0.10 × sd.	3.8	90	1	5
8	0.10 × sd.	3.8	66	0	0
9	0.32 × sd.	12	87	2	10
10	0.32 × sd.	12	80	0	0
11	st. dose	38	130	3	15
12	st. dose	38	90	1	5
13	3.2 × sd.	120	322	0	0
14	3.2 × sd.	120	392	0	0



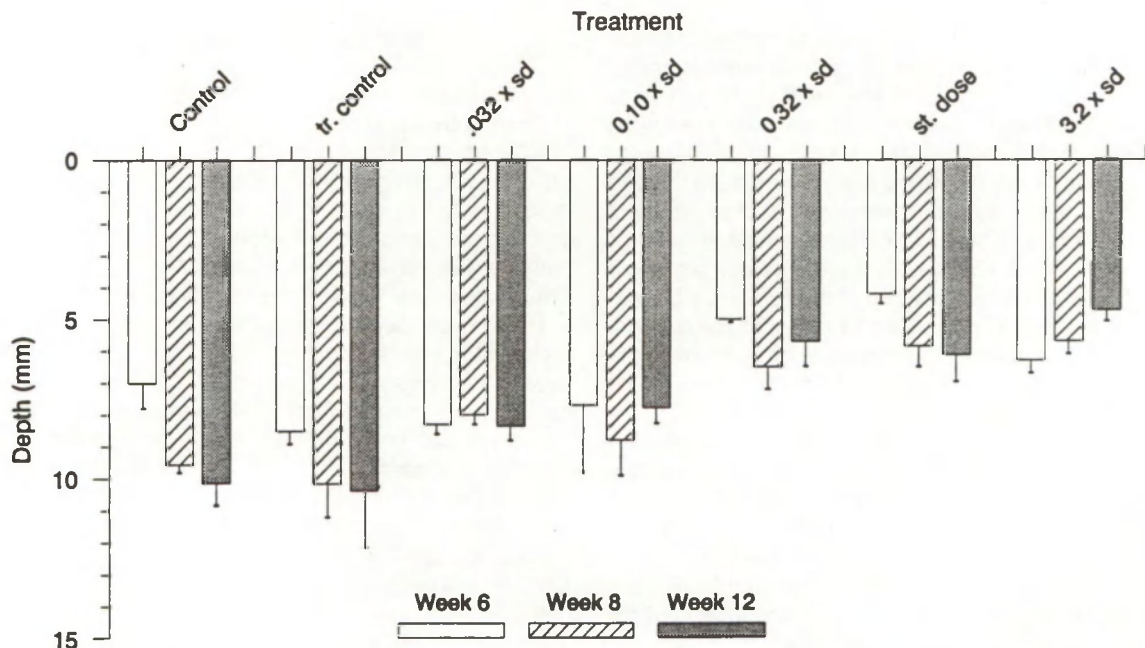


Fig. 6. Thickness of the aerobic zone in the boxcosms measured 6, 8 and 12 weeks after dosing. The bars show mean values of 10-15 profiles, vertical lines represent +1 s.e.. tr. contr.=treated control (only precombusted SCS cuttings dosed) sd=standard dose (175 g SCS cuttings dosed).

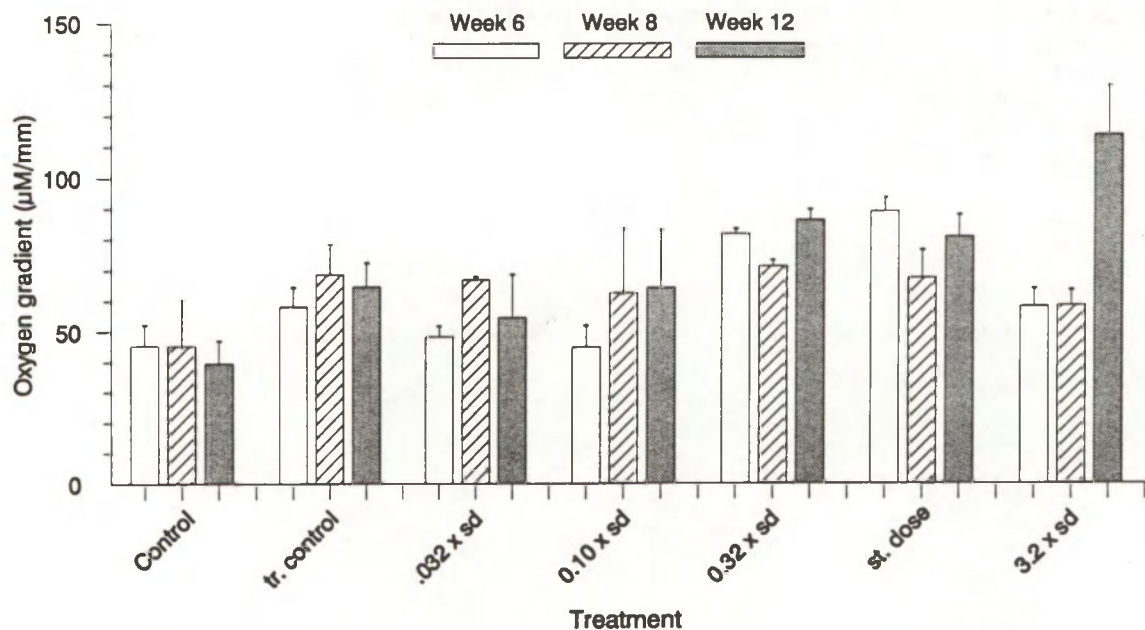


Fig. 7. Oxygen slopes in the boxcosms measured 6, 8 and 12 weeks after dosing. The bars show mean values of 10-15 profiles, vertical lines represent +1 s.e. tr. contr.=treated control (only precombusted SCS cuttings dosed) sd=standard dose (175 g SCS cuttings dosed).

#### 4 DISCUSSION

A prior condition required to an experimental set-up like used in the present boxcosm study is the use of undisturbed sediment. Unfortunately this condition was not met, due to the presence of unexpectedly high background concentrations of oil in the sediment. Moreover, the concentrations were variable between boxcosms, the highest and lowest levels differing by a factor 4. There is no explanation for the origin of the oil in the sediment, but according to GROENEWOUD & SCHOLTEN (1992a) it is not likely to be base oil like that which occurs in drilling wastes. Probably the sediment was contaminated during the last year before the sediment sections used in the boxcosms were collected, since the background levels were considerably higher than found at the same point during previous field studies in 1989 around drilling location L5-5. These studies included oil analyses of the sediment at this very point, viz. the 5000-m reference station (residual current direction) of L5-5. In 1989, the concentrations found were 19 mg oil·kg<sup>-1</sup> dry sediment in May (mean concentration in 10 VAN VEEN grabs), 8 mg·kg<sup>-1</sup> in September (idem) and 2, 2, 2 and 3 mg·kg<sup>-1</sup> respectively in 4 boxcosms incubated between September and December of that year (GROENEWOUD & SCHOLTEN, 1992b). Although these values are in some cases slightly elevated compared to usual background levels (1-2 mg·kg<sup>-1</sup> or, according to BAKKE *et al.*, 1991: 1-5 mg·kg<sup>-1</sup>), these values are consistently lower than in the boxcosms in 1990 (52-272 mg·kg<sup>-1</sup>).

Due to the high and variable background levels, the concentrations of oil found in the sediment after termination of the experiments did not show any relationship with the calculated amounts of oil dosed. The presence of background contamination could have completely disturbed the experimental results, in particular if the background oil would have been toxic. If the test species (*Echinocardium cordatum*), which has been shown to be susceptible to sediment contamination with base oil (ADEMA, 1991; DAAN *et al.*, 1990, 1991), would have suffered severe mortality in both the artificially treated boxcosms and the controls, then any possible effect of the dosed SCS cuttings would have been masked. At the observed background concentrations it seemed likely that this could occur, but, as a matter of fact, it did not. *Echinocardium* appeared to readily survive in all boxcosms and hardly showed any loss of spines, a common feature occurring as a sublethal effect of oil contamination. Overall mortality in *Echinocardium* was less than 3% and could not be related to any kind of oil contamination. Apparently there was no toxic effect of either the background oil or the oil in the SCS cuttings, even not in the highest doses. Since the highest doses must have induced concen-

trations considerably beyond the NOEC (no effect concentration) adopted till now, viz. 1-10 mg·kg<sup>-1</sup> dry sediment (see DAAN *et al.*, 1990; GRAY, 1991) the latter value seems to be contradicted by the present results. A plausible explanation may be that the composition of the residual base oil in the cuttings was changed by the SCS treatment, in favor of non-toxic components. An additional factor may be, that the powder resulting from the SCS treatment disturbs the structure of the sediment to a lesser extent than traditionally treated OBM cuttings do.

Also among the natural infauna, there was no convincing evidence for an adverse response (in terms of increased mortality among species or species groups) to oil concentrations in the sediment. The statistical calculations failed to detect any significant response to the background concentrations by the abundant species. In this respect, however, one should take into account the possibility that the high variability in initial fauna densities can mask a possible response. This problem was also met in boxcosm experiments with sediment from the same location (DAAN *et al.*, 1991). Dosing of SCS cuttings resulted in a significant response by only one species, *Mysella bidentata*. After incubation, this species was found in significantly lower numbers in the 0.1×sd and higher dosed boxes than in the 0.032×sd boxcosms and the controls. The statistical analysis revealed, however, another remarkable result, which suggested that merely adding oil-free material (precombusted SCS cuttings) significantly enhanced the survival rate of *Mysella*. Indeed, the numbers found in the treated controls and the 0.032×sd boxcosms were higher than in the untreated controls. Since it does not seem possible to find a reasonable plea to explain a causal relationship, acceptance of the statistical outcome as a realistic conclusion may be challenged and easily be explained as a statistical 'Type I' error. *E.g.* the initial abundance of *Mysella* may have been, merely by accident, high in box 3 up to 6, and, thus, have resulted in high numbers found after incubation. If indeed the significance of a positive effect of oil-free material on the survival rate of *Mysella* has to be rejected as a Type I error, the same holds for the significance of the negative effect of the oil in the SCS cuttings, since these statistical outcomes are interrelated. There is another reason to question the reproducibility of the significant response shown by *Mysella*. Although it was concluded from earlier field studies that *Mysella* is susceptible to oil contamination (DAAN *et al.*, 1990), the species can not be qualified as highly sensitive. It was never found to be affected in its natural densities at concentrations below 20 mg·kg<sup>-1</sup> (The present experiments suggested elevated mortality already at 3.8 and 12 mg·kg<sup>-1</sup>). In general, *Mysella* is absent only in the sediment in the very close vicinity of OBM

discharge points, where contamination levels are extremely high. The species usually occurs in reduced densities only in the area within 250 m from a discharge site. In several cases *Mysella* was found in natural (unaffected) densities at contamination levels of more than 30 mg·kg<sup>-1</sup> and even up to a level of 440 mg·kg<sup>-1</sup>. A relative of *Mysella*, viz. *Montacuta ferruginosa*, appeared in all cases to be much more sensitive. The area in which its natural abundance was affected was always much larger and at contamination levels beyond 20 mg·kg<sup>-1</sup> its densities were always reduced. Hence, if the higher mortality displayed by *Mysella* in the higher dosed boxcosms would have been caused by the oil in the cuttings, a clear response should certainly be expected in *Montacuta*. There was, however, no indication for such a response. Actually *Montacuta* was found in the boxcosms after termination of the experiment in even higher numbers than before incubation (see Table 4 and 5). This evidently supports the conclusion that the high mortality in *Mysella* in the higher dosed boxcosms should not be interpreted in terms of a causal relationship with oil concentrations.

The fact that the oxygen concentration at the sediment/water interface remained at a constant nearly saturated level throughout the experiment, suggests a sufficient supply of oxygen to the sediment. Oxygen depletion did not occur in spite of the enhanced respiratory activity and the thinner oxic layer in the boxcosms with the highest doses. Hence no harmful effects on the macrobenthic infauna should be expected. On the other hand, the somewhat higher overall macrofauna abundance in the low dosed boxes and the controls may have increased bioturbation and, thus, have contributed to a thicker oxic layer.

Summarizing this discussion it is concluded that the presence of high background concentrations did not interfere the experiment to an unacceptable degree, since the test animals readily survived the incubation period. The fact that the simulated discharges of thermally treated cuttings did not result in any substantial effect on either the test species or the natural infauna, strongly indicates that the treatment is effective in minimizing concentrations of toxic components in oil contaminated drill cuttings. However, in view of the hydrophobic properties of the resulting powder, it seems likely that discharge at sea would result in high concentrations of suspended matter and cause large patches of floating dust at the water surface.

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## APPENDIX

Table 4. Boxcosm experiment SCS, start values. ( 10 Reineck boxcores).  
 Estimated numbers of individuals per m<sup>2</sup> at the start of the incubation  
 period (6 September 1990).

	1	2	3	4	5	6	7	8	9	10
POLYCHAETA										
<i>Aphrodita aculeata</i>	--	--	--	--	--	--	--	--	--	--
<i>Harmothoe longisetis</i>	--	--	--	14.1	--	--	14.1	--	14.1	--
<i>Gattyana cirrosa</i>	28.2	--	14.1	14.1	--	--	--	--	--	42.3
<i>Pholoe minuta</i>	98.6	126.8	28.2	42.3	98.6	14.1	28.2	28.2	14.1	56.3
<i>Sthenelais limicola</i>	14.1	14.1	--	--	--	14.1	28.2	28.2	--	--
<i>Ophiodromus flexuosus</i>	--	14.1	--	--	--	14.1	14.1	--	--	--
<i>Gyptis capensis</i>	--	--	14.1	--	--	--	14.1	--	14.1	14.1
<i>Synelmis klatti</i>	14.1	--	--	14.1	14.1	14.1	--	--	--	--
<i>Exogone hebes</i>	--	14.1	--	--	--	--	14.1	--	14.1	--
<i>Nereis longissima</i>	--	--	--	14.1	--	--	--	--	--	--
<i>Nereis spec. juv.</i>	--	--	--	--	--	--	--	--	--	14.1
<i>Nephtys hombergii</i>	28.2	14.1	14.1	--	--	28.2	14.1	--	--	--
<i>Nephtys incisa</i>	--	--	--	--	--	--	--	--	--	14.1
<i>Nephtys spec. juv.</i>	--	--	--	--	--	--	14.1	28.2	--	--
<i>Glycera rouxii</i>	--	14.1	--	--	--	--	--	--	42.3	--
<i>Glycera alba</i>	--	--	--	--	--	--	--	--	--	--
<i>Glycera spec. juv.</i>	--	--	--	14.1	14.1	--	--	--	--	--
<i>Glycinde nordmanni</i>	--	--	--	--	--	14.1	--	14.1	--	--
<i>Goniada maculata</i>	14.1	--	--	--	--	--	14.1	--	14.1	14.1
<i>Lumbrineris latreilli</i>	112.7	281.7	323.9	352.1	239.4	464.8	366.2	140.8	338.0	267.6
<i>Lumbrineris fragilis</i>	197.2	14.1	--	14.1	--	14.1	--	14.1	--	--
<i>Orbinia sertulata</i>	--	--	--	--	--	--	--	--	--	--
<i>Paraonis spec.</i>	--	--	--	--	28.2	--	--	--	--	--
<i>Poecilochaetus serpens</i>	--	42.3	--	14.1	--	14.1	--	14.1	--	--
<i>Spio filicornis</i>	--	--	--	--	--	--	--	--	--	--
<i>Polydora pulchra</i>	--	--	--	--	--	--	--	--	--	--
<i>Polydora guillei</i>	--	--	--	--	--	--	--	--	--	--
<i>Spiophanes bombyx</i>	56.3	--	56.3	28.2	14.1	14.1	70.4	42.3	14.1	70.4
<i>Magelona papillicornis</i>	14.1	--	--	--	--	--	--	--	--	--
<i>Magelona alleni</i>	--	14.1	--	--	--	14.1	--	--	14.1	--
<i>Chaetopterus variopedatus</i>	28.2	14.1	--	42.3	--	--	14.1	--	42.3	28.2
<i>Tharyx marioni</i>	14.1	--	--	--	--	14.1	--	--	--	--
<i>Scalibregma inflatum</i>	14.1	14.1	14.1	14.1	--	--	--	--	--	--
<i>Notomastus latericeus</i>	84.5	14.1	--	14.1	--	--	--	--	42.3	--
<i>Heteromastus filiformis</i>	14.1	14.1	--	--	--	--	--	14.1	--	--
<i>Owenia fusiformis</i>	--	--	--	--	--	--	--	--	14.1	--
<i>Myriochele heeri</i>	--	--	--	--	--	--	--	--	--	--
<i>Lagis koreni</i>	14.1	14.1	--	14.1	14.1	--	14.1	--	--	--
<i>Pectinaria auricomma</i>	--	42.3	14.1	--	28.2	42.3	14.1	--	56.3	--
<i>Sosane gracilis</i>	--	--	14.1	28.2	--	--	--	--	28.2	--
MOLLUSCA										
<i>Nucula turgida</i>	--	--	--	--	--	--	--	--	--	--
<i>Thyasira flexuosa</i>	--	--	--	--	--	--	--	--	--	--
<i>Lepton squamosum</i>	--	--	--	--	--	--	--	--	--	--
<i>Montacuta ferruginosa</i>	--	14.1	28.2	--	14.1	14.1	14.1	--	--	14.1
<i>Mysella bidentata</i>	366.2	267.6	154.9	98.6	154.9	239.4	253.5	112.7	84.5	84.5
<i>Dosinia lupinus</i>	--	--	--	--	--	--	--	--	--	--
<i>Venus striatula</i>	--	--	--	--	--	--	14.1	--	--	--
<i>Mysia undata</i>	--	14.1	14.1	14.1	--	--	--	--	--	--
<i>Abra alba</i>	14.1	--	--	14.1	--	--	--	--	--	--
<i>Cultellus pellucidus</i>	--	--	--	--	--	--	--	--	--	14.1
<i>Mya truncata</i>	--	--	--	--	--	--	--	--	--	--
<i>Corbula gibba</i>	--	--	--	--	--	--	--	--	--	--
<i>Thracia convexa</i>	--	--	--	--	--	--	--	--	--	--
<i>Cingula nitida</i>	--	84.5	14.1	--	28.2	14.1	--	--	--	--

Table 4 (continued)

	1	2	3	4	5	6	7	8	9	10
<i>Turritella communis</i>	--	--	--	--	--	14.1	--	--	--	--
<i>Natica alderi</i>	42.3	--	--	--	14.1	--	--	--	--	--
<i>Cylichna cilindracea</i>	--	28.2	14.1	--	--	--	--	14.1	--	--
CRUSTACEA										
<i>Pagurus bernhardus</i>	28.2	--	--	--	--	--	--	--	--	--
<i>Ebalia cranchii</i>	--	--	14.1	--	--	--	--	--	--	--
<i>Corystes cassivelaunus</i>	--	--	--	--	--	--	--	--	--	--
<i>Upogebia stellata</i>	--	--	--	--	--	--	--	--	--	--
<i>Upogebia deltaura</i>	--	--	--	--	--	--	--	--	--	--
<i>Upogebia spec. juv.</i>	--	--	--	--	--	--	--	--	--	28.2
<i>Callianassa subterranea</i>	126.8	84.5	154.9	140.8	84.5	56.3	28.2	98.6	84.5	140.8
<i>Nebalia bipes</i>	--	14.1	--	--	--	--	--	--	--	--
<i>Eudorella truncatula</i>	--	14.1	14.1	--	--	--	--	--	--	--
<i>Diastylis bradyi</i>	--	--	14.1	--	--	14.1	--	--	--	--
<i>Ione thoracica</i>	--	--	--	--	--	--	--	--	--	--
<i>Melita spec.</i>	--	14.1	--	--	--	--	--	--	--	--
<i>Orchomenella nana</i>	14.1	--	--	--	--	--	--	--	--	--
<i>Ampelisca brevicornis</i>	--	--	14.1	--	--	--	14.1	42.3	--	42.3
<i>Ampelisca tenuicornis</i>	--	--	--	56.3	28.2	14.1	14.1	--	28.2	14.1
<i>Ampelisca spec. juv.</i>	--	--	28.2	--	--	--	14.1	--	--	--
<i>Cheirocratus sundevalli</i>	--	--	--	--	--	--	--	--	--	14.1
<i>Harpinia antennaria</i>	--	--	--	--	14.1	--	--	--	--	14.1
ECHINODERMATA										
<i>Amphiura filiformis</i>	1647.9	1197.2	971.8	1084.5	746.5	1112.7	619.7	1014.1	662.0	760.6
<i>Amphiura chiajei</i>	14.1	--	--	28.2	--	--	--	14.1	--	14.1
<i>Ophiura albida</i>	--	--	--	--	--	--	--	--	--	--
<i>Ophiura spec. juv.</i>	--	--	14.1	--	--	--	--	--	14.1	--
<i>Echinocardium cordatum</i>	42.3	28.2	14.1	--	28.2	28.2	14.1	--	--	--
OTHER TAXA										
Nemertinea	P	P	P	P	--	P	--	P	--	P
Turbellaria	--	--	--	--	--	--	14.1	--	--	--
Phoroniden	P	P	P	P	P	--	--	--	--	--
Harp. copepoda	--	14.1	--	14.1	--	14.1	--	--	--	--
Parasitaire copepoda	--	--	--	--	--	--	--	--	--	14.1
Holothuroidea	--	--	14.1	--	--	--	--	--	14.1	--
Echiurida	14.1	--	14.1	--	--	--	--	--	--	14.1
Sipunculida	126.8	112.7	56.3	56.3	28.2	28.2	225.4	56.3	126.8	154.9
Ascidiacea	14.1	--	--	--	--	--	--	--	--	--
Tot. number of ind.	3103.9	2463.8	1958.2	2087.5	1562.7	2228.4	1627.8	1628.7	1554.7	1667.6
Nr. of identified species	25	25	20	20	15	22	21	14	18	18
P=present, not counted.										

Table 5. Boxcosm experiment SCS, natural infauna.  
Mean numbers of individuals per m<sup>2</sup> at the end of the incubation  
period (10 January 1991).

BOXCOSM	1	2	3	4	5	6	7	8
POLYCHAETA								
<i>Aphrodita aculeata</i>	--	--	4.0	--	4.0	4.0	--	4.0
<i>Harmothoe longisetis</i>	--	--	4.0	--	--	--	--	--
<i>Gattyana cirrosa</i>	8.0	4.0	4.0	4.0	4.0	24.0	4.0	12.0
<i>Pholoe minuta</i>	--	8.0	8.0	4.0	--	--	4.0	--
<i>Sthenelais limicola</i>	4.0	4.0	--	4.0	--	--	--	--
<i>Ophiodromus flexuosus</i>	4.0	--	4.0	4.0	--	--	--	--
<i>Gyptis capensis</i>	--	16.0	4.0	4.0	16.0	4.0	--	--
<i>Synelmis klatti</i>	4.0	--	--	8.0	4.0	--	--	4.0
<i>Exogone hebes</i>	--	--	--	--	--	--	--	--
<i>Nereis longissima</i>	4.0	8.0	4.0	--	12.0	--	4.0	--
<i>Nereis spec. juv.</i>	--	--	--	--	--	--	--	--
<i>Nephtys hombergii</i>	12.0	--	8.0	16.0	4.0	12.0	--	8.0
<i>Nephtys incisa</i>	--	--	--	--	4.0	--	4.0	12.0
<i>Nephtys spec. juv.</i>	--	--	--	--	--	--	--	--
<i>Glycera rouxii</i>	--	20.0	8.0	--	--	8.0	4.0	--
<i>Glycera alba</i>	--	--	--	--	--	--	--	4.0
<i>Glycera spec. juv.</i>	4.0	--	--	--	--	--	--	--
<i>Glycinde nordmanni</i>	--	--	--	--	--	4.0	--	--
<i>Goniada maculata</i>	4.0	--	4.0	--	4.0	--	8.0	--
<i>Lumbrineris latreilli</i>	44.0	140.0	220.0	96.0	64.0	124.0	44.0	--
<i>Lumbrineris fragilis</i>	--	4.0	12.0	--	--	--	4.0	--
<i>Orbinia sertulata</i>	4.0	--	--	--	--	--	--	4.0
<i>Paraonis spec.</i>	--	--	--	--	--	--	--	--
<i>Poecilochaetus serpens</i>	--	--	--	--	--	--	--	--
<i>Spio filicornis</i>	--	--	--	--	4.0	--	--	--
<i>Polydora pulchra</i>	--	--	--	--	--	--	--	--
<i>Polydora guillei</i>	--	--	16.0	--	--	--	36.0	--
<i>Spiophanes bombyx</i>	--	--	--	--	--	--	--	--
<i>Magelona papillicornis</i>	--	--	--	--	--	--	--	--
<i>Magelona alleni</i>	--	--	--	--	--	--	--	--
<i>Chaetopterus variopedatus</i>	20.0	12.0	28.0	4.0	20.0	40.0	20.0	20.0
<i>Tharyx marioni</i>	--	--	--	--	--	--	--	--
<i>Scalibregma inflatum</i>	--	--	--	--	--	4.0	--	--
<i>Notomastus latericeus</i>	--	--	4.0	--	--	4.0	--	--
<i>Heteromastus filiformis</i>	--	--	--	--	--	4.0	--	--
<i>Owenia fusiformis</i>	4.0	--	--	4.0	--	--	--	8.0
<i>Myriochele heeri</i>	--	--	4.0	--	--	--	--	--
<i>Lagis koreni</i>	--	--	4.0	--	4.0	--	--	--
<i>Pectinaria auricomma</i>	16.0	--	8.0	--	--	12.0	--	--
<i>Sosane gracilis</i>	--	--	--	--	--	--	--	--
MOLLUSCA								
<i>Nucula turgida</i>	4.0	4.0	4.0	4.0	--	4.0	--	4.0
<i>Thyasira flexuosa</i>	--	--	--	--	--	--	--	--
<i>Lepton squamosum</i>	--	--	--	--	--	--	4.0	--
<i>Montacuta ferruginosa</i>	8.0	8.0	24.0	44.0	12.0	12.0	12.0	12.0
<i>Myrella bidentata</i>	16.0	72.0	188.0	148.0	132.0	132.0	28.0	8.0
<i>Dosinia lupinus</i>	--	--	--	--	--	--	4.0	--
<i>Venus striatula</i>	4.0	12.0	--	4.0	12.0	--	4.0	--
<i>Mysia undata</i>	4.0	--	--	--	--	--	--	--
<i>Abra alba</i>	--	--	--	--	--	--	--	--
<i>Cultellus pellucidus</i>	--	8.0	--	--	--	--	--	--
<i>Mya truncata</i>	--	--	4.0	4.0	--	--	4.0	--
<i>Corbula gibba</i>	--	12.0	4.0	4.0	--	--	--	--
<i>Thracia convexa</i>	--	--	4.0	--	--	--	4.0	--

Table 5 (continued) Boxcosm experiment SCS, natural infauna.  
 Mean number of individuals per m<sup>2</sup> at the end of the  
 incubation period (10 January 1991).

BOXCOSM	9	10	11	12	13	14
<b>POLYCHAETA</b>						
<i>Aphrodita aculeata</i>	--	--	4.0	--	4.0	--
<i>Harmothoe longisetis</i>	--	--	--	--	4.0	--
<i>Gattyana cirrosa</i>	16.0	12.0	--	8.0	4.0	24.0
<i>Pholoe minuta</i>	--	--	--	4.0	8.0	4.0
<i>Sthenelais limicola</i>	--	--	--	--	--	--
<i>Ophiodromus flexuosus</i>	--	--	--	--	--	--
<i>Gyptis capensis</i>	4.0	--	12.0	8.0	12.0	16.0
<i>Synelmis klatti</i>	8.0	4.0	--	--	--	4.0
<i>Exogone hebes</i>	--	--	--	--	--	--
<i>Nereis longissima</i>	4.0	--	--	--	8.0	--
<i>Nereis spec. juv.</i>	--	--	--	--	--	--
<i>Nephtys hombergii</i>	4.0	--	--	8.0	4.0	12.0
<i>Nephtys incisa</i>	--	4.0	4.0	4.0	--	--
<i>Nephtys spec. juv.</i>	--	--	--	--	--	--
<i>Glycera rouxii</i>	--	16.0	4.0	8.0	8.0	4.0
<i>Glycera alba</i>	12.0	--	--	--	--	8.0
<i>Glycera spec. juv.</i>	--	--	--	--	--	--
<i>Glycinde nordmanni</i>	--	--	--	--	--	--
<i>Goniada maculata</i>	--	4.0	--	--	--	--
<i>Lumbrineris latreilli</i>	168.0	116.0	72.0	64.0	44.0	40.0
<i>Lumbrineris fragilis</i>	8.0	--	4.0	--	--	4.0
<i>Orbinia sertulata</i>	--	--	--	--	--	--
<i>Paraonis spec.</i>	--	--	--	--	--	--
<i>Poecilochaetus serpens</i>	--	--	--	--	--	--
<i>Spio filicornis</i>	--	--	--	--	--	--
<i>Polydora pulchra</i>	4.0	--	--	--	--	--
<i>Polydora guillei</i>	--	4.0	20.0	12.0	--	--
<i>Spiophanes bombyx</i>	--	--	--	--	--	--
<i>Magelona papillicornis</i>	--	--	--	--	--	--
<i>Magelona alleni</i>	4.0	--	--	--	--	--
<i>Chaetopterus variopedatus</i>	12.0	16.0	4.0	36.0	28.0	40.0
<i>Tharyx marioni</i>	--	--	--	--	--	--
<i>Scalibregma inflatum</i>	--	--	--	--	--	--
<i>Notomastus latericeus</i>	--	--	--	16.0	--	--
<i>Heteromastus filiformis</i>	--	--	--	--	--	--
<i>Owenia fusiformis</i>	4.0	--	--	--	--	--
<i>Myriochele heeri</i>	--	--	--	--	--	--
<i>Lagis koreni</i>	--	--	--	--	--	--
<i>Pectinaria auricoma</i>	--	--	--	--	--	--
<i>Sosane gracilis</i>	--	--	--	--	--	--
<b>MOLLUSCA</b>						
<i>Nucula turgida</i>	8.0	4.0	--	--	--	--
<i>Thyasira flexuosa</i>	--	--	4.0	4.0	--	--
<i>Lepton squamosum</i>	--	--	8.0	8.0	--	--
<i>Montacuta ferruginosa</i>	12.0	20.0	12.0	12.0	28.0	24.0
<i>Mysella bidentata</i>	4.0	32.0	8.0	4.0	--	4.0
<i>Dosinia lupinus</i>	--	--	--	--	--	--
<i>Venus striatula</i>	--	4.0	--	--	--	8.0
<i>Mysia undata</i>	--	--	--	--	--	--
<i>Abra alba</i>	--	--	--	--	--	--
<i>Cultellus pellucidus</i>	--	--	--	--	--	--
<i>Mya truncata</i>	--	--	--	--	--	--
<i>Corbula gibba</i>	8.0	--	--	--	4.0	4.0
<i>Thracia convexa</i>	--	8.0	--	--	--	--



Table 5 (continued)  
BOXCOSM

	1	2	3	4	5	6	7	8
<i>Cingula nitida</i>	--	4.0	--	--	--	--	--	--
<i>Turritella communis</i>	4.0	4.0	16.0	16.0	16.0	--	20.0	28.0
<i>Natica alderi</i>	4.0	4.0	8.0	--	--	--	--	--
<i>Cyllichna cylindracea</i>	4.0	--	8.0	12.0	--	--	--	--
CRUSTACEA								
<i>Pagurus bernhardus</i>	--	--	--	--	--	--	--	--
<i>Ebalia cranchii</i>	4.0	--	--	--	--	--	4.0	--
<i>Corystes cassivelaunus</i>	--	--	--	4.0	--	--	--	--
<i>Upogebia stellata</i>	--	4.0	12.0	--	--	--	--	--
<i>Upogebia deltaura</i>	--	8.0	--	--	--	4.0	8.0	--
<i>Upogebia spec. juv.</i>	--	--	--	--	--	--	--	--
<i>Callianassa subterranea</i>	76.0	80.0	124.0	64.0	56.0	64.0	80.0	80.0
<i>Nebalia bipes</i>	--	--	--	--	--	--	--	--
<i>Eudorella truncatula</i>	--	--	--	--	--	--	--	--
<i>Diastylis bradyi</i>	--	--	--	--	--	--	--	--
<i>Ione thoracica</i>	--	--	--	--	--	--	--	4.0
<i>Melita spec.</i>	--	--	--	--	--	--	--	--
<i>Orchomenella nana</i>	--	--	--	--	--	--	--	--
<i>Ampelisca brevicornis</i>	--	--	--	--	--	--	--	--
<i>Ampelisca tenuicornis</i>	--	--	--	--	--	--	--	--
<i>Ampelisca spec. juv.</i>	--	--	--	--	--	--	--	--
<i>Cheirocratus sundevalli</i>	--	--	--	--	--	--	--	--
<i>Harpinia antennaria</i>	--	--	--	--	--	--	--	--
ECHINODERMATA								
<i>Amphiura filiformis</i>	504.0	124.0	480.0	328.0	348.0	704.0	308.0	84.0
<i>Amphiura chiajei</i>	4.0	--	--	4.0	--	--	--	--
<i>Ophiura albida</i>	4.0	--	--	--	--	--	--	--
<i>Ophiura spec. juv.</i>	--	--	--	--	--	--	4.0	--
<i>Echinocardium cordatum</i>	8.0	--	28.0	12.0	8.0	24.0	12.0	8.0
OTHER TAXA								
Nemertinea	P	P	P	P	P	P	P	--
Turbellaria	--	--	--	--	--	--	--	--
Phoroniden	P	--	P	--	P	P	P	P
Harp. copepoda	--	--	--	--	--	--	--	--
Parasitaire copepoda	--	--	--	--	--	--	4.0	--
Holothuroidea	--	--	--	--	--	--	--	--
Echiurida	4.0	4.0	--	--	--	--	--	4.0
Sipunculida	80.0	112.0	60.0	12.0	24.0	176.0	28.0	76.0
Ascidacea	--	--	4.0	--	--	--	--	4.0
Tot. number of ind.	780.0	560.0	1252.0	796.0	728.0	1188.0	616.0	300.0
Nr. of identified species	26	22	31	23	19	19	22	16

Table 5 (continued)

BOXCOSM	9	10	11	12	13	14
<i>Cingula nitida</i>	4.0	--	--	--	--	--
<i>Turritella communis</i>	4.0	8.0	12.0	4.0	4.0	4.0
<i>Natica alderi</i>	8.0	--	8.0	--	--	--
<i>Cylichna cylindracea</i>	--	4.0	4.0	--	--	--
CRUSTACEA						
<i>Pagurus bernhardus</i>	--	--	--	--	--	--
<i>Ebalia cranchii</i>	--	--	--	--	4.0	--
<i>Corystes cassivelaunus</i>	--	--	--	--	--	--
<i>Upogebia stellata</i>	--	--	4.0	--	--	--
<i>Upogebia deltaura</i>	--	4.0	4.0	8.0	--	--
<i>Upogebia spec. juv.</i>	--	--	--	--	--	--
<i>Callianassa subterranea</i>	68.0	56.0	36.0	76.0	56.0	52.0
<i>Nebalia bipes</i>	--	--	--	--	--	--
<i>Eudorella truncatula</i>	--	--	--	--	--	--
<i>Diastylis bradyi</i>	--	--	--	--	--	--
<i>Ione thoracica</i>	--	--	--	4.0	16.0	--
<i>Melita spec.</i>	--	--	--	--	--	--
<i>Orchomenella nana</i>	--	--	--	--	--	--
<i>Ampelisca brevicornis</i>	--	--	--	--	--	--
<i>Ampelisca tenuicornis</i>	--	--	--	--	--	--
<i>Ampelisca spec. juv.</i>	--	--	--	--	--	--
<i>Cheirocratus sundevalli</i>	--	--	--	--	--	--
<i>Harpinia antennaria</i>	--	--	--	--	--	--
ECHINODERMATA						
<i>Amphiura filiformis</i>	156.0	340.0	184.0	96.0	192.0	36.0
<i>Amphiura chiajei</i>	--	--	--	--	--	--
<i>Ophiura albida</i>	--	--	--	--	--	--
<i>Ophiura spec. juv.</i>	--	--	--	--	--	--
<i>Echinocardium cordatum</i>	20.0	28.0	12.0	--	12.0	4.0
OTHER TAXA						
Nemertinea	P	P	P	P	--	P
Turbellaria	--	--	--	--	--	--
Phoroniden	--	P	P	P	--	P
Harp. copepoda	--	--	--	--	--	--
Parasitaire copepoda	--	--	--	--	--	8.0
Holothuroidea	16.0	--	8.0	4.0	8.0	8.0
Echiurida	8.0	--	4.0	8.0	12.0	12.0
Sipinculida	28.0	60.0	12.0	44.0	92.0	32.0
Ascidacea	--	--	--	--	--	--
Tot. number of ind.	540.0	684.0	420.0	384.0	440.0	292.0
Nr. of identified species	22	19	19	18	17	18
P=present, not counted						

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