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Biodegradation of **Anco Green** and **Novaplus** Drilling Muds on Cuttings Deposited in Benthic Chambers

REPORT

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Abstract

Discharge of drilling fluids attached to bore hole cuttings may affect the benthic environment on the deposition sites in the North Sea. The effects may vary according to dose and properties of the base fluid and other components of the mud recipe. In the present study, biodegradation and environmental effects of two new drilling muds, *Anco Green* (based on an ester produced from fish oil fatty acids) and *Novaplus* (based on a linear olefin), was investigated in a six months simulated seabed experiment performed in benthic chambers at Marine Research Station Solbergstrand. Exponential regression analyses yielded the following rank of half-lives: *Anco Green* < *Petrofree* (reference ester) < *Ultidrill* (reference olefin) < *Novaplus* < *Safemul* (reference mineral oil). Effects of the ester based cuttings on redox -potentials and macrobenthic communities were more severe than the effects of olefin based cuttings. The most severe effects were observed in the *Petrofree* treatment. All chambers treated with olefin cuttings or low-organic control sediment, maintained diverse benthic communities classified by the end of the six months enclosure, as *good* or *fair* according to general criteria for fjord and coastal environments. Concentrations of drilling fluids, barium and two biomarker enzymes were measured in the polychaete *Hediste diversicolor* after six months exposure in the chambers. Several indications were found on bioaccumulation of olefins and mineral oil. Different levels of enzyme activities between control and treated chambers, showed that the polychaete had been affected by components present in some of the cuttings.

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1. oljeboring	1. oil drilling
2. kjemikalier	2. chemicals
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PREFACE

This report describes the results of NIVA projects O-95144 and O-95145. Project O-95144 on biodegradation of *Anco Green* as compared to biodegradation of previously studied reference muds was performed for Norsk Hydro a.s. on request from SFT. Project O-95145 on the biodegradation of *Novaplus* was funded by M-I Norge a.s. The experimental work was done at NIVA Marine Research Station Solbergstrand (MFS) during the period June 1995 - January 1996. The chemical analyses of the drilling fluids was performed at NIVA´s laboratory in Oslo. SINTEF Industrial Chemistry was subcontracted to perform the chemical analyses of barium. I wish to thank the contractors, *Ingegerd Rustad* at SINTEF and all project participants at NIVA for their contributions to the present report. In particular, the report rests heavily on the skills and reliability of *Oddbjørn Pettersen* and coworkers at Solbergstrand, *Helle Juul Rasmussen* at the chemical laboratory and *Bodil Ekstrøm* and *Pirkko Rygg* at the biological laboratory. *Ketil Hylland* performed biological sampling and analyses of biomarkers in the polychaete *Hediste diversicolor*. *Rainer Lichtenthaler* was responsible for the GC-MS analyses and the macrofauna was interpreted by *Brage Rygg*. *Torgeir Bakke* was responsible for quality assurance of the project.

Oslo, May 9. 1996

Morten T. Schaanning

SUMMARY AND CONCLUSIONS

Introduction and objectives of the experimental study

Synthetic drilling fluids have replaced much of the mineral oil previously used in mud systems for offshore drilling operations. Thus, new chemicals associated with bore hole cuttings are being discharged from installations in the North Sea. Dependent on particle properties, water depth and the prevailing current regime, some lateral transport will occur, but most of the cuttings will be deposited on the sea bed within a few km from the discharge site. Subsequently, some of the deposits may become buried by bioturbation and sediment transported with bottom currents.

Three types of synthetic drilling fluids are currently in use. *Ester* and *ether* compounds are characterised by oxygen atoms inserted in the carbon chains. The ester bond is more easily hydrolysed than the corresponding ether bond, and previous studies have shown that biodegradation of ester base fluids on cuttings occurred more rapidly than the biodegradation of ether base fluids. The third type of synthetic drilling fluids are based on *olefin* compounds. Olefins contain no oxygen atoms and biodegradation is probably initiated by an initial enzymatic cleavage of carbon-carbon double bonds. Previous investigations have shown that biodegradation of *polyalphaolefin* base fluids, which during the polymerisation process had lost their double bond, were comparable to the biodegradation of ether and mineral oil base fluids. A more recent *linear olefin* (*Ultidrill*), was however, found to biodegrade at rates intermediate between the esters and the more slowly degrading mineral oil, ether and polyalphaolefins.

The objective of the present investigation was to assess the environmental fate and biological effects of two new mud systems: *Anco Green* delivered from *Anchor Drilling Fluids*, was based on a mixture of esters derived from naturally occurring (fish oil) fatty acids. The other mud system, *Novaplus* delivered from *MI*, was based on an *internal olefin*.

The present assessment was based on a six months comparative study performed in benthic chambers at Marine Research Station Solbergstrand (MRS). Previously studied mineral oil, ester and olefin based muds on cuttings were used for reference substances. The reference ester delivered from *Baroid* under the trade name *Petrofree* was a mixture of five homologous fatty acid esters, mostly 2-ethylhexyl dodecanoate (C_{12}:C_8) and 2-ethylhexyl tetradecanoate (C_{14}:C_8). The reference olefin delivered from *Schlumberger Dowell IDF* under the trade name *Ultidrill*, was composed of two olefin compounds with stoichiometries corresponding to $C_{14}H_{28}$ and $C_{16}H_{32}$. The third reference mud, marketed under the trade name *Safemul*, was based on a low aromatic mineral oil.

Test set-up and sampling strategy

The *Novaplus* synthetic cuttings were made up in the laboratory by allowing an aliquot of a sample of *Novaplus* mud, which was supplied from MI 23.06.95, to soak into a sample of non-polluted control sediment which had been ignited to remove any organic carbon. The *Ultidrill* cuttings sample was left over from a previous test. The sample had been collected at *Frøy Platform*, and had been stored under appropriate conditions at Solbergstrand since October 1994. The remaining cuttings samples (*Anco Green, Petrofree and Safemul*) were collected during the last weeks of May 1995 at various drilling sites in the North Sea.

In previous tests identical sediment communities have been obtained by filling the chambers from a concrete mixer. In the present experiment undisturbed sediment communities were transferred from 200 m depth in the Oslofjord. Thus, at the cost of larger initial variation between chambers, the

maintenance of animal tubes and burrows and the presence of a much larger number of species provided a more realistic test environment.

The experiment comprised twelve identical, transparent acrylic chambers. Each chamber measured 0.5 x 0.5 x 0.4 m and contained an almost undisturbed 25-35 cm section of layered seabed covered with a 5-15 cm layer of seawater. The chamber water was continuously renewed with aerated, filtered sea water supplied from 60 m depth in the nearby fjord. Thus, the water entering each chamber was always close to saturation with oxygen and the range of temperature and salinity was 7-11°C and 32-35 PSU, respectively.

In order to obtain similar concentration of drilling fluids, the *Novaplus* and *Petrofree* cuttings were diluted with various amounts of pre-ignited sediment. On day zero, each chamber was treated with a slurry made up from 150-180 g wet cuttings, 200-220 g wet, non-contaminated, marine clay sediment and a small volume of seawater. The slurries were carefully poured into the chamber water and allowed to settle during gentle stirring. This way, similar particle loads of 200-250 g dry sediment and similar contamination levels of 1.0-1.5 g of drilling fluids, were added in a 1-2 mm layer on top of the natural sediment in each chamber.

Each type of cuttings was added to two replicate chambers. Thus, ten chambers were treated with drilling fluids. The remaining two chambers were treated with a slurry made up from 100 g of pre-ignited sediment, 210 g wet, non-contaminated sediment and some sea water. Thus, the two control chambers were prepared in order to correct for possible physical effects of the particle load and possible biodegradation effects of the refractory organic carbon ($\leq$ 1 g) added with the wet clay sediment.

The addition was done on July 3rd. 1995. During the following six months, the chambers were sampled for various parameters. Chemical analyses of drilling fluids and barium was determined in sediment samples collected monthly. pH and redox potentials were also recorded monthly at 15 mm depth at three different locations in each chamber. Oxygen consumption was determined twice a week by measuring the flow of water through each chamber and the difference in oxygen concentration between the header tank and the outlet from each separate chamber. After termination of the experiment (day 187), the macrofauna was sampled by sieving the sediment through 5mm and 1mm sieves.

Before addition of cuttings, 40 individuals of *Hediste (Nereis) diversicolor* had been added to each chamber. All individuals of this species were removed from the sieves and treated separately for analyses of bioaccumulation and biomarker responses.

Chemical identity of the drilling fluids

The *Anco Green* ester was identified by GC/MS as a mixture of isopropyl esters of saturated and unsaturated fatty acids. The chain length of the fatty acids is varying from C_{14} over C_{16},, C_{18} and C_{20} up to C_{22}.

Novaplus consisted of an isomeric mixture of mono-olefins (hydrocarbons with one double bond). The number of carbon atoms varies from C_{16} over C_{18} up to C_{20}.

The base fluid of *Safemul* was confirmed to be a mineral oil with almost non-detectable amounts of aromatic components.

In previous studies, the *Petrofree* base fluid have been found to be a mixture of five homologous fatty acid esters, of which the main component is 2-ethylhexyl dodekanoate, and the *Ultidrill* olefins have been found to be a mixture of tetra- and hexa-decenes with stoichiometries corresponding to $C_{14}H_{28}$ and $C_{14}H_{32}$.

Qualitative changes of component composition in drilling fluids

After 66 days, the compositional patterns of *Anco Green* ester were significantly changed. Components identified as the unsaturated fatty acid esters in the mixture were more rapidly lost compared to the saturated fatty acid esters. This effect was probably due to preferential biodegradation and was more pronounced for the higher homologs (C_{18}, C_{20} and C_{22}) compared to the lower ones (C_{14} and C_{16}). After 158 days both saturated and unsaturated fatty acid esters had more or less completely disappeared. The components still present at the end of the experiment were either minor impurities in the original *Anco Green* ester product and/or more resistant organic components inherent in the cuttings or fjord sediment.

In the *Petrofree* sediment samples, all (saturated) fatty acid ester components (C_8, C_{10}, C_{12}, C_{14} and C_{16}) disappeared at approximately equal rates. Because of their much higher concentrations in the original product, the C_{12} and C_{14} esters were still present at day 66.

With regard to *Novaplus*, even if the total concentration was significantly reduced, the relative abundance of each component in the isomeric mixture of C_{16},, C_{18} and C_{20} mono-olefins remained virtually unchanged over the experimental period. Thus, it may be concluded that all components had the same rate of disappearance.

In the *Ultidrill* sediment samples, the two main component groups, tetradecenes (C_{14}) and hexadecenes (C_{16}), showed different rates of disappearance. The C_{14}-components were more rapidly removed compared to the C_{16}-homologs, a phenomenon presumably caused by preferential biodegradation of the lower boiling fraction.

Finally, in *Safemul*, the lack of preferential disappearance of n-alkanes compared to their iso-alkane isomers, confirmed that biodegradation of mineral oil is a slow process. Such preferential biodegradation has otherwise been well documented in many studies of the fate of petroleum hydrocarbons in marine sediments.

Recovery of added barium and drilling fluids

The test was designed to assess the fate of the pool of cuttings present on the sediment surface on day one. Any loss during test set-up of drilling fluids or barium in colloidal particles, droplets or dissolved fractions will not affect test results. The recoveries, i.e. the fraction of the added cuttings recovered on the sediments on day 1, were determined only for the purpose of quality assurance of sampling procedures, analytical methods and inherent assumptions.

The range of recoveries of barium of 67-90% (mean = 78.6%) was interpreted primarily to result from wash-out of cuttings particles which had not been deposited on the sediment surface before circulation and exchange of chamber water was initiated. A very similar mean loss of 78.0% of the drilling fluids indicated that both barium and drilling fluids were firmly associated with the same particulate fractions. Both chambers treated with *Safemul* did, however, yield consistently low recoveries. Some preferential loss of mineral oil (relative to barium) might be expected from low boiling points of some of the mineral oil fractions.

The much wider range of recovery of 35-117% for the drilling fluids, as compared to barium, was thought to result mainly from analytical errors which appeared to be larger for the drilling fluids than for barium. (Because the cited recoveries were ratios between single determinations, unfortunate combination of errors in numerator and denominator may add up to yield large random variation of the ratios).

Initial and final concentrations of carbon and nitrogen in the sediment samples

The initial treatment resulted in a range of concentrations of total carbon of 29.2-33.9 $mg \cdot g^{-1}$ (dry wght) in the sediments treated with cuttings. This was significantly higher than the concentration of 25.7-26.5 $mgC \cdot g^{-1}$ observed in the two control chambers. During the experimental period the loss of carbon of 5.1 and 6.0 $mgC \cdot cm^{-2}$ from the two *Anco Green* chambers was reasonably consistent with the loss of esters of 5.7 and 6.2 $mg \cdot cm^{-2}$. (Carbon accounts for about 80% of the molecular weight of the average *Anco Green* ester). Similarly, the absence of any significant loss of carbon from the two *Safemul* chambers confirmed the slow disappearance of mineral oil.

As compared to organic matter from natural sources, the content of nitrogen is low in the drilling fluids. Therefore, the quality of the organic matter (in terms of the C:N ratio) present in all chambers treated with drilling fluids, deviated significantly from the quality found in the two control chambers. This relationship did not change during the experimental period. The drilling fluids represent a source of carbon for growth and energy-consumption of the micro-organisms. However, the nitrogen required for protein synthesis must be supplied from other sources. Thus it cannot be ruled out, that nitrogen availability represent a limitation to the growth of the decomposer communities associated with cuttings deposits.

Disappearance (total loss) of drilling fluids

Assuming first order kinetics, the half-lives (τ) of the drilling fluids were determined from exponential regression analyses of the change of concentration with time. Calculated probabilities (p) showed the significance of the decrease with time, and correlation coefficients (r) showed the fit of the observations to the various exponential models. 95% confidence intervals were calculated to show the precision of the half-life estimates. The regression analyses was performed on concentration data for each chamber as well as the data pooled for the two replicate chambers.

Most of the disappearance of drilling fluids was assumed to result from biodegradation and loss of cuttings particles from the sampled 0-3 cm depth interval. Barium was assumed to disappear via cuttings particles only. Thus, the decrease with time of the ratio between the concentration of drilling fluids and the concentration of barium was considered a better estimate of biodegradation than the rate of disappearance calculated from the concentration of drilling fluids alone.

Anco Green esters disappeared from the sediments at half-lives of 15.7 and 17.7 days, respectively, in the two chambers. Pooling the data from both chambers yielded a half-life of 16.6 days with a confidence interval from 13.9 to 20.8 days. Clear downward trends of barium were observed in both chambers and the regression analyses of the pooled ester:Ba ratios yielded a half-life of 17.9 days with a confidence interval between 15.0 and 22.0 days. This was the shortest half-lives ever found for any drilling fluid tested at Solbergstrand.

Petrofree esters disappeared with half-lives of 20.8 and 23.6 days in the two replicate chambers and 22.2 days for the pooled data. The confidence interval for the pooled data was 18.5 to 27.5 days. This agreed well with previously found half-lives close to 20 days for the degradation of *Petrofree* esters in several tests with correspondingly dosed chambers. No significant change of barium was observed in the two *Petrofree* chambers and the regression analyses of the ester:barium ratios gave a half-life 22.0 days with a confidence interval between 18.5 and 27.0 days. The absence of any loss of barium was consistent with the poor macrofauna communities found in the chambers at the termination of the experiment. Probably bioturbation was inhibited as a result of low redox potentials and the appearance of bacterial mats shortly after the addition of cuttings.

The half-life of *Ultidrill* olefins was 44.8 and 60.4 days, respectively, in the two replicate chambers. For the pooled data the half-life was 51.2 days with a confidence interval between 40.3 and 71.9 day. In a previous test, four chambers treated with *Ultidrill* cuttings and base fluid yielded halflifes from 36.0 to 49.9 and a mean value of 42.6 days. A significant decrease of barium was only found in one of

the *Ultidrill* chambers. Pooled data for the *Ultidrill*:barium ratios gave a half-life of 55.9 days and a 95% confidence interval of 45.1 to 75.5 days.

In all chambers treated with esters and *Ultidrill* olefins, the decrease with time was highly significant ($p \leq 0.002$), and the data showed good fits to the various regression curves ($r \geq 0.91$).

The *Novaplus* data showed less good fits to the exponential models than did the ester and *Ultidrill* data. The two chambers gave half-lives of respectively, 59.2 days (n=7, p=0.019, r=0.84) and 97.4 days (n=7, p=0.050, r=0.75). Pooled for both chambers, the half-life was 73.7 days with a confidence interval between 47.9 and 167.8 days (n=14, p=0.002, r=0.75). If some of the scatter found in the *Novaplus* olefin data was the result of patchiness within the sampled surface layer, produced by animal activities, the olefin:barium ratios should yield better curve fits than the olefins proper. Thus, the half-lives for the *Novaplus*:barium ratios in the two chambers of, respectively, 86.3 days (n=7, p=0.008, r=0.88) and 131 days (n=7, p=0.045, r=0.77), seemed to confirm the importance of bioturbation in the *Novaplus* chambers. As shown by the ratio half-life of 104 days for the pooled data (n=14, p=0.008, r=0.79) and the confidence interval of 70.2 to 201 days, biodegradation of *Novaplus* olefins was clearly more slow than biodegradation of both ester products and the *Ultidrill* olefins.

After day 90, the evidence for any decrease of concentration of the *Novaplus* olefins was rather weak. and by the end of the experiment, 21.9% of the initial concentration was still present in the sediment. However, the experimental period was to short to conclude that biodegradation actually did slow down towards the end of the experimental period. Neither did the GC-analyses yield any evidence for the presence of refractory fractions of the *Novaplus* olefins.

From a number of experimental studies and offshore surveys, mineral oil is known to undergo slow degradation in marine sediments. In a recent review of tests performed at NIVA a model was applied which assumed a 60 days lag phase before exponential degradation. Pooled data from two tests resembling the present test gave a half-life of 142 days for the period after day 60.

In the present test, the data on *Safemul* mineral oil were to scattered to justify the application of a lag-phase model. Downward slopes were observed in both chambers, but the decrease was not significant at 95%. The chamber with the best fit data yielded a half-life of 108 days for the mineral oil proper (n=7, p=0.101, r=0.67) and 168 days for the ratio (n=7, p=0.054, r=0.75). Omitting the concentrations determined in one of the samples in each chamber, correlations improved to yield 105 days (n=12, $p<0.05$, r=0.81) for the mineral oil and 158 days for the ratio (n=12, $p<0.05$, r=0.75).

Even though the results on mineral oil from the present test indicated somewhat shorter half-lives than those found in previous tests, the poor fits of the mineral oil data in all tests and the close correspondence between present and previous results on the half-lives of *Ultidrill* olefins and *Petrofree* esters, it could not be concluded that the much richer benthic communities in the present test set-up had had any impact on the respective rates of biodegradation. However, in most of the chambers of the present test, the loss of barium was significantly larger than in previous tests. Most of the difference was concluded to result from a larger bioturbative loss to sediment depths below the sampling interval of 3 cm.

Sediment oxygen consumption

Every 3-4 days, the rate of sediment oxygen consumption (SOC) was determined by measuring flow rates and the decrease of the concentration of oxygen between in- and outlet from each chamber.

In the control chambers, SOC decreased from initial rates of 400-600 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$ towards final rates of 200-400 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$. This more or less steady trend was interrupted by a temporary increase during days 90-120 of the experiment. The increase occurred simultaneous to an anomalous influence of a more surficial water type which resulted in increased temperatures from 6.5°C to 11°C in source water and chambers. In previous experiments, characteristic rates of 100-300 $\mu mol \cdot m^{-2} \cdot h^{-1}$ have been observed in control chambers. The high initial rates of SOC in the present experiment was

thought to result from recent sedimentation of phytoplankton spring blooms at the fjord location and more enriched benthic communities than those applied in previous studies. Possibly also, recent deposits from the anomalous large river flooding which had occurred this year, had stimulated decomposer activities in the sampled sediments.

In the *Anco Green* chambers, two major peaks of SOC occurred successively at about day 20 and day 50. Maximum rates (smoothed) were approximately 1500 $\mu mol \cdot m^{-2} \cdot h^{-1}$. During days 50 to 140, the rates slowly decreased to a stable level of about 400 $\mu mol \cdot m^{-2} \cdot h^{-1}$ which was maintained throughout the remaining experimental period. The characteristic double peak during the first months has previously been observed in chambers treated with *Petrofree* esters, probably as the result of microbial succession and the development of sulphide oxidising bacteria mats.

In the *Petrofree* chambers, no clear peaks were observed. During the first two weeks, maximum rates of SOC of 1000-1200 $\mu mol \cdot m^{-2} \cdot h^{-1}$ were observed in both chambers. The rates decreased slowly to about 800 $\mu mol \cdot m^{-2} \cdot h^{-1}$ on day 60, after which SOC declined in both chambers two a minimum of about 200 $\mu mol \cdot m^{-2} \cdot h^{-1}$ (smoothed) 95 days after addition of the cuttings. Probably stimulated by the rise of the water temperature, SOC increased in both chambers during the 95-110 days period, but whereas one of the chambers maintained a stable level of SOC of 500-600 $\mu mol \cdot m^{-2} \cdot h^{-1}$, the SOC in the other *Petrofree* chamber continued to oscillate about a level corresponding to the level observed in the two control chambers.

Over the entire experimental period, the accumulated oxygen consumption was 3620 $mmol \cdot m^{-2}$ and 3710 $mmol \cdot m^{-2}$, respectively, in the two *Anco Green* chambers and 2520 $mmol \cdot m^{-2}$ and 2940 $mmol \cdot m^{-2}$ in the *Petrofree* chambers as compared to 1440 $mmol \cdot m^{-2}$ and 1860 $mmol \cdot m^{-2}$ in the two control chambers. As shown by the mass balance calculations, oxygen consumption could only account for mineralisation of 22-32% of the total disappearance of *Petrofree* esters. This was low as compared to 80% mineralisation observed in a previous test at similar initial concentrations. The low total oxygen consumption as well as the absence of the characteristic peaks, was assumed to result from underestimation of anaerobic degradation due to the presence of oxidised minerals in the ignited sediment added to the *Petrofree* chambers, but not to the *Anco Green* chambers. Thus, rather than consuming oxygen in the mat communities on the sediment surface, the hydrogen sulphide produced by the sulphate reducing bacteria was assumed to react with ferric and manganese oxides abundant in the pre-ignited sediment. Such mineral competition for hydrogen sulphide would also be consistent with the earlier recession of the mat communities (see below) in the *Petrofree* chambers as compared to the *Anco Green* chambers.

In the *Novaplus* chambers, oxygen consumption rates decreased slowly from an initial level of about 900 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$ towards a final level between 500 and 700 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$. Thus during the last month of the experiment, oxygen consumption rates were higher in the *Novaplus* treatment than in any of the other treatments. Certainly, this did not confirm any slow-down of the degradation process, as might be indicated by the concentration data. Over the entire experimental period, oxygen consumption was 3110 and 3400 $mmolO_2 \cdot m^{-2}$, respectively, in the two chambers.

In addition to the *Petrofree* chambers, pre-ignited sediment was added to *Novaplus* and control chambers. Because, however, sulphate reduction and production of hydrogen sulphide appeared not to be an important pathway of biodegradation of the olefins, experimental bias resulting from preignited sediment was not expected to affect any of the results on *Novaplus*. Neither was any indication found on the presence of such bias in the *Novaplus* chambers.

In the *Ultidrill* chambers, oxygen consumption rates were frequently up to 1200 $\mu mol \cdot m^{-2} \cdot h^{-1}$ during the first two months of the experimental period. 60 days after addition of cuttings, the rates decreased to slightly below 800 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$. The rates continued to decrease slowly, and the final rates observed on day 185 were very similar to the rates observed in the control chambers. Over the entire experimental period, the cumulative oxygen consumption was 3000 and 3030 $mmolO_2 \cdot m^{-2}$, respectively, in the two *Ultidrill* chambers, which was slightly less than the consumption observed in the *Novaplus* chambers.

Of all chambers treated with drilling fluids, the two mineral oil chambers had the lowest rates of oxygen consumption. From initial rates of 800 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$, the consumption decreased slowly to a final level of 300-600 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$, which was slightly higher than SOC in the control chambers. The total consumption during the experimental period was 2170 and 2580 $mmolO_2 \cdot m^{-2}$, which, disregarding the collapsed community in one of the *Petrofree* chambers, was lower than the cumulative oxygen consumption in any other treated chamber.

Thus, in addition to confirming the collapse in the mat communities in the *Petrofree* chambers, oxygen consumption measurements showed highest total respiration in the *Anco Green* treatment followed by fairly similar consumptions in the four chambers treated with olefins. In the mineral oil treatments, respiration rates were lower than the corresponding rates in the other treatments, but throughout the experimental period oxygen consumption in mineral oil treatments was clearly stimulated relative to control chambers.

Mass balance

A mass balance of the drilling fluids was calculated for each separate chamber for the period 0-186 days. In this mass balance it was assumed that the difference between the amount of drilling fluids present in the sediment on day 1 and day 186 had been lost by dissolution to the water flowing through the chambers (assumed small), biodegradation to CO_2 (oxygen consumption equivalents) and organic metabolites, or by loss of drilling fluids attached to particles (equivalent to loss of barium). Particles may have been lost by sampling, resuspension or bioturbative mixing downwards to sediment depth below the sampling depth of 3 cm.

The frequent loss of 35-50% of the barium, was large compared to previous tests. The zero loss of barium from the two *Petrofree* chambers seemed to hold the clue as to the most important factors causing particle loss from the chambers. Sampling activities were similar in all chambers and could not explain any difference between treatments. Resuspension may have been hampered by bacteria mats which were more developed in the *Petrofree* chambers than in any other treatment. However, the mats were present for less than 1/3 of the experimental period and pump-driven resuspension, if important, should have had time enough to remove measurable amounts of barium. Thus, the collapse of the benthic macrofauna appeared to be the only factor left to explain the difference between the *Petrofree* and the other treatments. Both downwards bioturbative mixing to depths below the sampling depth of 3 cm, as well as bioturbative-driven resuspension of particles may have contributed. This appeared to implicate that in offshore areas with rich benthic communities, bioturbation may be an important factor contributing to the dissipation of drilling fluids deposited on the sediment surface.

The mass balances showed that of the *Anco Green* esters, nothing was left in the sediment, 35% had been completely mineralised to CO_2, 40% had been lost by bioturbation and 25% could not be accounted for. Also for the *Petrofree* esters, nothing was left in the sediment, but only 27% could be accounted for by oxygen consumption and because nothing had been lost by bioturbation the mass balance deficit became as high as 73%. The present mass balance for the *Petrofree* esters was concluded to be strongly biased by the addition of pre-ignited sediment which had implicated that degradation of *Petrofree* esters via sulphate reduction had failed to show up as oxygen consumption.

Of the mineral oil present on day 1, 29% was left in the sediment six months later, 32% was accounted for by oxygen consumed during the period and 34% by the loss of barium. Thus, the mass balance deficit was a negligible 4%.

Of the *Novaplus* olefins, 14% was left in the sediment, 38% had been mineralised and 40% had been lost by bioturbation. Thus, only 8% of the initial presence of *Novaplus* olefins could not be accounted for.

Of the *Ultidrill* olefins, 10% was left in the sediment on day 186 and 33% had been mineralised. The two replicate chambers differed greatly with regard to loss of barium. Possibly, most of the bioturbation was produced by a few large individuals missing in one of the *Ultidrill* chambers which

showed zero loss of barium despite the presence of a diverse and numerous benthic fauna. In the other chamber bioturbation could account for a loss of 35% of the drilling fluids, leaving a mass balance deficit of 22%.

Disregarding the biased *Petrofree* chambers, the mass balances did not reveal any major differences between the drilling fluids with regard to mass balance deficits and the fractions accounted for by oxygen consumption and loss of barium. The three items showed larger variation within than between replicate treatments. The non-degraded fractions were, however, larger in the mineral oil chambers than in the olefin chambers and negligible in the ester chambers.

Visual changes on sediment surface

During monthly sampling and inspection, visual changes on the sediment surface were noted and documented by photography.

On day 1, the added material was observed present on top of the sediment as an approximately one millimetre thin layer. The presence of several fragile biological structures and numerous perturbations and tracks in the freshly deposited layer, gave no indications that the transfer from the seabed *in situ* and the addition of slurries, had caused severe disturbance of the sediment communities.

In the control chambers the sediment surfaces were reworked by animal activities so that the patchiness increased during the course of the study. Colours varied from light grey (slightly reddish in chamber 10) to dark brown. 65 days after the addition of cuttings, maximum numbers of 3-4 dead bivalves were counted on the sediment surface of each chamber. Neither dead polychaetes, bacteria mats nor spots of white (presumably elemental sulphur) or black (presumably FeS) precipitates were ever recorded in any of the two control chambers.

36 days after addition of cuttings, continuous mats of sulphide oxidising bacteria had grown to cover the entire sediment surface in both *Petrofree* chambers, and white precipitates were estimated to cover 50-75% of the surfaces. In each chamber, two dead polychaetes and 20-30 small bivalves had been captured beneath the mat. On day 65, the white precipitates had disappeared and the mats had receded slightly. No dead animals were recorded in the *Petrofree* chambers on day 65. On day 96, only fragments of the mats were seen curled up along the walls of the chambers. In the two chambers, respectively 5 and 11 large polychaetes and several smaller ones were observed, decaying on the sediment surface. Throughout the remaining experimental period the sediment surfaces were mostly pale grey, with black areas surrounding animal remnants. Large rust-coloured patches occurred in both chambers on day 156 and were also present during the final sampling on day 186. Tracks, holes and other indications on an active sediment community were scarce, and a microscopic heterotrophic organism which invaded the sediment surfaces and the walls and tubes of most of the chambers during the 96-126 days period, failed to settle in the *Petrofree* chambers.

Also in the *Anco Green* treatments, mats of sulphide oxidising bacteria developed during the first month after addition of cuttings. The mats appeared, however, different from those observed in the *Petrofree* chambers. They never covered the entire sediment surface, but they persisted for a longer period of time. Thus, on day 35, the mats were estimated to cover 10% of the sediment surface in chamber 8 and 25% in chamber 2. On day 65 the mats had increased to cover approximately 25% and 60% of the respective sediment surfaces. On day 96, the mats had receded slightly, but they still appeared as dominant features in both *Anco Green* chambers. On day 126, most of the mats and the white sulphur precipitates had disappeared leaving small black and white patches. During the last two months, the areas which had not been covered by mats maintained a very patchy appearance. Dead polychaetes were never recorded in the *Anco Green* chambers and the number of dead bivalves was less than those observed in the *Petrofree* chambers.

In the *Novaplus* chambers, the patchiness ranging from light grey to dark brown, tended to increase throughout the experimental period. Small black and white spots were observed on day 65 and all subsequent surveys. Apart from some small, yellow/orange patches which appeared on day 65 and

later sampling occasions, the *Ultidrill* chambers changed their appearance in a way much similar to the change in the *Novaplus* chambers. During the final sampling on day 186, some biofilm formation was observed in both *Ultidrill* chambers and one of the *Novaplus* chambers. Dead polychaetes were never recorded in the olefin chambers, and counts of dead bivalves never exceeded six individuals.

In the mineral oil treatment, one dead polychaete was observed in chamber 12 on day 36, and another one in chamber 1 on day 65. Large dark patches had developed on day 65 and remained present throughout the experimental period. Apart from those characteristic patches the appearance of the chambers treated with mineral oil were not much different from the appearance of the control chambers.

Effects on pH and redox potentials

The pH and redox potential (E_h) are integrating chemical parameters controlled by, respectively, acid-base and redox equilibria in the sediment/pore-water system. Addition of chemicals might trigger spontaneous reactions, but most deviations from control sediments occur slowly as a result of altered biological and microbiological processes or altered diffusion caused by capping with cuttings material.

pH and redox potentials were measured monthly at 15 mm depth in the sediments at three different locations in each chamber. Statistically significant differences from control chambers (ANOVA, Tukey HSD multiple comparison) were determined for each sampling occasion as well as for the entire experimental period.

pH

Throughout the experimental period the pH in the two control chambers varied between 7.5 and 7.8. On day 2, positive pH differences of 1.4-1.5 pH-units were observed in the two chambers treated with mineral oil. A similar effect has been observed previously, in chambers treated with mineral oil cuttings. The effect has been assumed to result from the presence of mineral buffers in the mud system. With time, the effect dissipated and since day 97 the difference from control chambers were $\leq$0.4 pH units, which was not significant ($p \geq 0.052$). For the entire experimental period, the mean differences of 0.46-0.57 pH units were highly significant ($p < 0.001$).

In the other treatments the pH never exceeded 8.4. However, 36-126 days after addition of the cuttings, pH-maxima which did not occur in the control chambers, were observed in all chambers treated with drilling fluids. Significant positive differences were observed on day 36 and 65 in all chambers treated with esters, and on day 97 in one of the *Novaplus* chambers. None of the chambers treated with *Ultidrill* olefins showed any significant difference from control chambers. For the entire experimental period, the pH was 0.10-0.18 pH-units higher in chambers treated with *Novaplus* and *Ultidrill* olefins than in control chambers, and 0.17-0.33 pH-units higher in the chambers treated with *Anco Green* and *Petrofree* esters.

Possibly apart from the pH values exceeding 9.0 in both chambers treated with *Safemul* mineral oil, the moderate differences of pH observed in chambers treated with esters and olefins, were not believed to have any effects on benthic organisms.

E_h

On day 2, the mean redox potential in the two control chambers was 334 mV (standard deviation = 36 mV, n = 6), which was not significantly different from the mean redox potentials between 300 and 377 mV observed in the treatments.

A consistent trend of the E_h, showing an initial decrease followed by normalisation towards the end of the experimental period, was observed in all chambers. Thus, E_h minima were observed 2-4 months

after addition of cuttings. In the control chambers, capping effects of the added sediments, was thought to control the decrease to the minimum value of 244 mV, which was observed on day 65. The lowest E_h of -115 mV was observed in the *Petrofree* chambers on day 97. In the *Anco Green* treatment, a minimum E_h of -45 mV was observed during the same survey. In the olefin and mineral oil treatments, negative potentials were never observed. On day 126, minima of 60 mV and 180 mV, respectively, were observed in the *Ultidrill* and *Novaplus* treatments. In the mineral oil treatment, the redox potential decreased to a level close to 200 mV which was maintained throughout the 36-126 days period.

For the entire experimental period, the mean differences between treated and control chambers were -255 mV for the *Petrofree* treatment, -210 mV for *Anco Green*, -94 mV for *Ultidrill*, -68 mV for *Safemul* and -63 mV for the *Novaplus* treatments. At each sampling occasion, the E_h in the two replicate treatments was compared with the E_h in each one of the two control chambers. Thus, after the initial measurements on day 2, 24 cases of comparisons between each treatment and control chambers were performed (two treated vs. two control at six sampling occasions). The *Petrofree* chambers yielded significantly lowered E_h in all 24 cases ($p \leq 0.05$). *Anco Green* yielded significant negative deviations in 22 of the 24 cases as compared to frequencies of six in *Ultidrill*, four in *Novaplus* and only one significant difference in the mineral oil treatments.

Thus, severe and persistent lowering of redox potentials were observed in all chambers treated with ester based drilling fluids. In the other treatments, the lowering of redox potentials were more moderate. In the *Petrofree* chambers the E_h was slightly lower than the E_h in the *Anco Green* chambers. Similarly, the E_h in the *Ultidrill* chambers were slightly lower than the E_h in mineral oil and *Novaplus* treatments.

No doubt, the redox differences in the ester treatments had resulted from hydrogen sulphide produced by anaerobic degradation of esters by sulphate reducing bacteria. Redox deviations of this magnitude are normally believed to implicate severe effects on the benthic fauna. Potential fauna effects of the more moderate redox deviations observed in the olefin and mineral oil treatments are less certain.

Macrofauna community structure

By the end of the experiment, the benthic fauna from each chamber was collected. The animals were mostly identified to species level and counted. Two diversity indexes were determined, as well as an index which shows the relative contribution in the fauna of species sensitive to pollution. Quality assessment relative to normal status in field situations in fjords was derived.

The polychaete *Heteromastus filiformis* was particularly abundant and frequently accounted for approximately 50% of the total number of individuals. In the two *Petrofree* chambers, however, the pollution tolerant polychaete *Paramphinome jeffreysii* was the most abundant species, and the only species which did not suffer a severe decline. Several groups which were common in the other chambers were not found. Thus, neither mussels (mostly *Thyasira* spp.) nor the polychaete *Chaetozone setosa* were observed in the *Petrofree* samples. The polychate *Prionospio* was reduced in numbers in all treatments compared to the controls.

Effects of the treatments were most obvious in the two *Petrofree* chambers. In the two replicate chambers, 4-6 species and 32-83 individuals showed a *bad* environment relative to normal field conditions.

The fauna in the chambers treated with *Anco Green* esters was less affected than the fauna in the *Petrofree* treatments, but more strongly affected than the rest of the treatments and the controls. 14-35 species and 283-809 individuals yielded *poor* and *fair* environmental conditions, respectively, in the two chambers.

The least severe effects on the benthic communities were observed in the *Novaplus* treatments. As compared to 22-26 species and 308-338 individuals in the two *Ultidrill* chambers, the *Novaplus* chambers had higher numbers of species (30-36) and individuals (588-647). Environmental quality

was classified as ***fair*** in both Ultidrill and one of the Novaplus chambers and ***good*** in the second Novaplus chamber.

The two chambers treated with low-aromatic mineral oil showed lowered species numbers (18-20), individual numbers (226-309) and diversity indexes as compared to control (36-39 species, 281-856 individuals) and olefin treatments. Environmental quality was classified as ***fair*** in both mineral oil treatments, and the effects on benthic communities appeared to be intermediate between the moderate effects of the linear olefins and the larger effects of the esters.

The effects on the benthic communities were probably caused by redox deviations and presence of hydrogen sulphide in the sediment, as well as feeding interference and lack of oxygen supply from the water caused by bacterial mats. Possibly, extreme initial pH-values in the mineral oil treatment caused some acute mortality which may have accounted for the effects observed on the benthic fauna in this treatment.

Biomarker responses

Any perturbation of the environment will initially affect single individuals in the ecosystem. In fact, changes will initially be apparent at the cellular level within the individual. To most marine organisms it will appear as though their environment is changing continuously, although most such changes will be easily handled by the organism. Some changes, specifically those foreign to the ecosystem, may however initiate deleterious processes leading to impairment or death of organisms. In general, there are three main motives for using biomarkers in identifying and/or quantifying the influence of such processes. Firstly, responses can be measured at an early stage in a possibly damaging process; secondly, responses will be apparent at low levels of a given contaminant. Thirdly, many biomarkers are sufficiently specific to enable causal links to be established between ambient contaminants and the observed response. The two biomarkers used herein, glutathione reductase and catalase, are both components of a cellular defence against free radicals, including oxyradicals. The reason for this choice was that drilling muds are not very toxic in themselves and that the major stressors in the systems were expected to be related to low oxygen-availability.

Forty individuals of the ragworm *Hediste (Nereis) diversicolor* were added to each chamber. At the end of the 6-months exposure 0-12 individuals were retrieved. In two of the chambers, both treated with *Petrofree*, there were no surviving *Hediste*. In the remaining 10 chambers, mortality was thought to be predominantly due to predation and there were no differences between control and treated chambers. The mean size or number of surviving individuals was not obviously related to chemical/physical parameters in the chambers.

The levels of one of the biomarkers measured in *Hediste diversicolor*, glutathione reductase, was significantly elevated in worms exposed to *Anco Green* compared to the control group. Enzyme activities were however also weakly related to the mean size of the worms.

Catalase-activities were significantly increased in *Hediste diversicolor* kept in chambers with *Anco Green*, *Ultidrill* and *Novaplus* added. The increase appeared to be related to oxygen-consumption in the sediment (i.e. oxygen availability to organisms), either directly or through associated processes.

The responses of the two biomarkers glutathione reductase and catalase indicated that *Hediste diversicolor* kept in chambers treated with *Anco Green*, *Ultidrill* and *Novaplus* were negatively affected by these treatments. The observed responses are normally found associated with oxidative stress or tissue damage. In the present study it is not possible to distinguish between direct responses to ambient conditions and responses related to tissue breakdown or changes in metabolism.

Bioaccumulation

Sub-samples of the polychaete homogenates used for biomarker analyses, were analysed for barium (x-ray fluorescence) and drilling fluids (GC/MS).

No sample material was available to study the possible bioaccumulation of *Petrofree* ester, and as all *Anco Green* esters had disappeared from the sediments prior to day 186, it was not surprising that *Anco Green* esters were below detection limits in the polychaete samples.

Novaplus was found in the polychaetes. Comparison of *Novaplus* composition in sediment and polychaete, revealed that the relative abundance of the single components in the groups of C_{16}-, C_{18}- and C_{20}-olefins had remained mainly unchanged. Two of the samples showed high concentrations of olefins of 34.1 and 49.5 $mg \cdot kg^{-1}$ wet wght. (0.00 $mg \cdot kg^{-1}$ in control). However, also barium was high in those two samples, yielding olefin:barium ratios of 0.10 and 0.24, respectively. The similarity between those two ratios in the polychaetes and the corresponding ratios of 0.14 and 0.39 observed in simultaneously sampled sediments was a strong indication that most of the *Novaplus* olefins observed in those polychaetes were associated with sediment particles which had passed through the cleaning procedures.

A much lower concentration of 2.37 $mg \cdot kg^{-1}$ *Novaplus* olefins was observed in the third polychaete sample, in which the concentration of barium was below the detection limit of 3 $mg \cdot kg^{-1}$. Thus, the olefin:barium ratio of >0.79 was larger than all ratios observed in the sediment more than 35 days after addition of the cuttings. This result suggested some bioaccumulation of *Novaplus* olefins in the polychaete *Hediste diversicolor*. Any bioaccumulation which may have occurred in the two other polychaete samples would have been masked by the large amounts of olefins associated with sediment contaminations.

The concentrations of *Ultidrill* olefins in the three polychaete samples were calculated to 7.77, 3.56 and 6.26 $mg \cdot kg^{-1}$ wet weight. (Control = 0.00 $mg \cdot kg^{-1}$). The corresponding olefin:barium ratios were 0.37, 0.11 and 0.08 as compared to the range of 0.08-0.19 in the four sediment samples collected towards the end of the experimental period. Thus, even if barium was assumed unavailable for uptake so that all barium and an equivalent amount of drilling fluids were present as sediment contamination in the gut or in between body appendages, indications on bioaccumulation of *Novaplus* and *Ultidrill* olefins in the polychaete *Hediste diversicolor* was only found in one out of three samples. If some of the barium observed in the polychaete samples really was bioaccumulated, all six samples might have been interpreted as bioaccumulation of olefins.

Comparison of the composition of *Ultidrill* in the base fluid, sediment and polychaete revealed some remarkable differences. In the base fluid, the C_{14}-olefins were present at higher concentration than the C_{16}-olefins. In the sediment at the end of the experiment (day 158), the C_{14}-olefins were considerably less abundant than the C_{16}-components. This was most likely due to preferential biodegradation of the lower molecular weight fraction in the sediment. Both in sediment and polychaetes the loss of main components (straight chain C_{14} and C_{16}-olefins) had been large compared to the loss of associated components, which were tentatively identified as branched C_{14}- and C_{16}-olefins. Surprisingly, in the polychaetes the distribution of the components was again reversed. The C_{14}-olefins were again the dominating group. One possible explanation might be the preferential bioaccumulation of the lower molecular fraction.

The polychaetes exposed to *Safemul* showed a significantly higher level of petroleum hydrocarbons compared to controls. The component distribution differed, however, considerably from the component distribution in sediment samples. The total petroleum hydrocarbon content in the three polychaete samples was calculated to 2.98, 5.11 and 4.16 $mg \cdot kg^{-1}$ wet weight. (Control = 0.00 $mg \cdot kg^{-1}$). The concentration of barium was less than 3 $mg \cdot kg^{-1}$, in all samples of polychaetes from *Safemul* and control chambers. The corresponding hydrocarbon:barium ratios of at least 0.99, 1.78 and 1.39 were significantly larger than the mean ratio of 0.65 (standard deviation = 0.27) for the ten sediment samples collected after day 35. This was consistent with previous reports on bioaccumulation of petroleum hydrocarbons.

Conclusions - all treatments

- The use of undisturbed box-core samples represented a major improvement of test set-up with regard to simulation of *in situ* seabed conditions.
- By the end of the six months experimental period, the macrofauna communities in both control and seven treated chambers were classified, according to environmental quality criteria for fjords and coastal environments, as *fair* or *good*.
- Three chambers treated with esters were classified as *poor* or *bad*.
- Significant loss of barium in all but the Petrofree treatment, showed that bioturbation was larger in the present as compared to previous tests at Solbergstrand.
- The responses of the two biomarkers glutathione reductase and catalase indicated that the polychaete *Hediste diversicolor* kept in chambers treated with *Anco Green*, *Ultidrill* and *Novaplus* were negatively affected by these treatments, whereas no significant effects were found in chambers treated with *Safemul* mineral oil. (In *Petrofree* chambers, the polychaete did not survive to yield sufficient amount of sample material).

Conclusions - ester treatments

- The *Anco Green* ester was identified as a mixture of isopropyl esters of saturated and unsaturated fatty acids. The chain length of the fatty acids varied from C_{14} over C_{16},, C_{18} and C_{20} up to C_{22}.
- The unsaturated fatty acid esters in the mixture were more rapidly lost than the saturated fatty acid esters.
- By the termination of the 6 months experiment *Anco Green* components could not be detected neither in sediment samples nor in polychaete extracts.
- The disappearance from sediments showed good fits to exponential models with half-lives between 13.9 and 20.8 days (95% confidence limits) as compared to between 18.5 and 27.5 days for *Petrofree* esters.
- Sediment oxygen consumption was higher than in any other treatment. For the experimental period, the consumed oxygen was equivalent to complete mineralisation to CO_2 of 32 to 39% of the initial load of esters.
- Throughout most of the experimental period, redox potentials were significantly lower than redox potentials in control chambers. The potentials were also lower than the potentials observed in mineral oil and olefin treatments, but not as low as in the *Petrofree* treatment.
- Low redox potentials and mats of, presumably, sulphide oxidising bacteria indicated high activities of sulphate reducing bacteria below the sediment surface in all chambers treated with *Anco Green* and *Petrofree* esters.
- At their maximum extensions the mats covered 25 and 65%, respectively, in the two *Anco Green* chambers, but 100% in both *Petrofree* chambers.
- In the ester treatments, the disturbance of the benthic fauna was most probably caused by the combined effects of hydrogen sulphide toxicity and mat extension. In the two *Petrofree* chambers the benthic communities collapsed 1-3 months after the addition of cuttings, and only 4-6 species and 32-83 individuals survived the experiment, as compared to 14-35 species and 283-809 individuals in the two *Anco Green* chambers.

Conclusions - olefin and mineral oil treatments

- *Novaplus* consisted of an isomeric mixture of mono-olefins (hydrocarbons with one double bond). The number of carbon atoms varies from C_{16} over C_{18} up to C_{20}.
- All components of *Novaplus* seemed to disappear at similar rates, whereas in Ultidrill samples the C_{14}-components were more rapidly removed compared to the C_{16}-homologs.
- By the end of the 6 month experiment, components present in the original base fluids were still present in sediments and polychaetes sampled from both olefin treatments.

- Enrichment of the ratio between drilling fluids and barium in the polychaete *Hediste (Nereis) diversicolor*, as compared with simultaneously sampled sediments, did indicate some bioaccumulation of both types of olefins and the mineral oil.
- In order to confirm or reject this hypothesis, an experiment designed specifically for the study of bioaccumulation of drilling fluids is recommended.
- The disappearance of *Ultidrill* olefins showed good fits to exponential models with half-lives between 40 and 72 days (95% confidence interval). This was consistent with previous test results on this product.
- The *Novaplus* and mineral oil data showed less good fits to the exponential regression models than did the *Ultidrill* olefins and the esters.
- The estimated half-lives of 74 days for *Novaplus* olefins and 105 days for Safemul mineral oil, indicated that biodegradation of *Novaplus* occurred more slowly than the biodegradation of *Ultidrill*, but more rapidly than mineral oil.
- For the entire experimental period, oxygen consumption were slightly higher in the *Novaplus* chambers than in the *Ultidrill* chambers, but mineralisation of 38% of the initial load of *Novaplus* olefins was not significantly different from the mineralisation of neither *Ultidrill* olefins nor *Anco Green* esters.
- The slow change of concentration of *Novaplus* olefins and *Novaplus*:barium ratios towards the end of the experimental period was not confirmed by the oxygen consumption rates which indicated rapid mineralisation in the *Novaplus* chambers at the end of the experimental period.
- Redox potentials were lowered relative to control chambers but the difference was rarely significant and hydrogen sulphide were never detected in any of the chambers treated with olefins or mineral oil.
- Effects on the benthic fauna were less severe in the olefin-treatments than in the ester treatments. Both number of species and number of individuals were higher in the *Novaplus* chambers as compared to mineral oil and *Ultidrill* treatments, and pollution sensitive species were abundant in both *Novaplus* chambers.
- Mineral buffers present in the *Safemul* mud was the most probable cause of high initial pH values in the mineral oil treatments. Possibly, the lower diversities in the mineral oil as compared to the olefin treatments was a result of this pH anomaly.

Recommendations

- The *Anco Green* esters will disappear rapidly and completely from offshore discharge sites. Effects on the benthic communities during and for a limited period of time after the discharge period, will be more severe than the effects of olefin based muds. Apparently, however, the effects will be less severe than the effects of Petrofree, possibly as a result of the natural (fish oil) source of the fatty acid components of the *Anco Green* esters.
- Redox effects of discharges of olefin based drilling muds will be less than the corresponding effects of ester based muds, and less for *Novaplus* as compared to *Ultidrill* olefins.
- None of our tests have, so far, showed complete disappearance of any olefin products and apparently low availabilities to sulphate reducing bacteria may slow down biodegradation of buried olefins. Offshore surveys or a differently dosed benthic chamber experiment might elucidate this problem.
- In order to confirm or reject indications found on bioaccumulation of olefins in the polychaete *Hediste diversicolor*, an experiment designed specifically for the study of bioaccumulation of such drilling fluids is recommended.

1. INTRODUCTION

Offshore drilling operations using oil-based mud (OBM) were prior to the late eighties a significant source of discharges of oil to the North Sea. OBMs were originally formulated on a diesel base, but environmental concern led to the development and increasing use of alternative base oils with a lower aromatic content and reduced toxicity (Dicks et al. 1986/87). Following the prohibition of the discharge of cuttings containing OBM, synthetic drilling fluids have been developed and formulated into synthetic based muds (SBM). During the most recent years, the discharge of cuttings containing SBMs has increased rapidly in the Norwegian sector of the North Sea.

Dependent on particle properties, water depth and the present current regime, some lateral transport will occur, but most of the cuttings will be deposited on the sea bed less than a few km from the discharge site. Elevated levels of hydrocarbons (THC) have been found out to 1-12 km from the discharge sites (Zevenboom et al. 1993). Biological effects in benthic communities may occur to 2-5 km for some installations (Reiersen et al 1989, Gray et al 1990) and perhaps beyond 10 km (Bakke et al. 1989a, Olsgaard, 1994). All of these results apply to deposition and effects of OBMs. Until now, field surveys have yielded little information about the spread and effects of SBMs. However, effects on the benthic fauna in the vicinity of discharges of *Petrofree* esters have been reported at Ula and Oseberg well sites (Smith and Hobbs, 1993, Kaarstad et.al., 1994) and at well site K14-13 in the Dutch sector (Daan et al, 1995). The effects appeared to result from the rapid biodegradation of the esters.

So far, three types of organic chemicals, esters, ethers and olefins have been introduced for mineral oil substitutes. Thus in the *Petrofree* mud system, the mineral oil has been replaced by a mixture of five homologous fatty acid esters, of which the main component is 2-ethylhexyl dodekanoate (Oreld, Døhl og Gjøs, 1991). Biodegradation of *Petrofree* esters were first investigated by Bakke and Laake, 1991, and has later been used for reference material in all tests performed by NIVA. *Petrofree* esters degrade rapidly with a half-life of approximately 20 days at initial concentrations of approximately 5 $mg \cdot cm^{-2}$. The dose-response relationship has never been properly investigated, but much longer half-lives were found for the *Petrofree* esters in a test using 3x higher initial concentrations.

Biodegradation of Aquamul ethers and Novasol poly-alpha-olefins (PAO) have been found to be relatively slow. Thus, half-lives exceeded 100 days and oxygen consumption rates were never significantly different from those produced by mineral oil (Laake et al, 1992, Schaanning and Laake, 1993, Schaanning, 1994, Schaanning, 1995a).

In the test preceding the present, the test object was a new type of olefin produced by *Schlumberger Dowell IDF* and marketed as *Ultidrill*. The base fluid was a mixture of tetra- and hexa-decenes with stoichiometries corresponding to $C_{14}H_{28}$ and $C_{14}H_{32}$. *Ultidrill* disappeared exponentially from the test sediments with a half-life of 40-50 days, which was clearly intermediate between the two reference fluids, *Petrofree* ester and Novasol PAO. The relatively short half-life was confirmed by oxygen consumption rates which were also intermediate between *Petrofree* ester and PAO. To the benefit of the pore water environment, redox effects in the *Ultidrill* treatment were less severe than those observed in *Petrofree* treatments.

The objective of the present investigation was to assess the environmental fate of the *Anco Green* esters and the *Novaplus* olefins. According to information given by Bjørn Egeland, Pronova, the base fluid in the *Anco Green* mud system produced by Anchor Drilling Fluids, was a natural fatty acid ester, produced from fish oil. The *Novaplus* olefin was claimed to be a mixture of two internal C_{16} and C_{18} olefins (HMS Datablad, 27.07.94, M-I Norge).

Cuttings contaminated with muds based on *Petrofree* esters, *Ultidrill* olefins and mineral oil were used for reference purposes. Thus, the present study made possible a direct comparison between the biodegradation and effects of five different drilling fluids.

The biological effects of the treatments were assessed by analyses of the benthic community in the chambers as well as growth and biomarker responses of added polychaetes (*Hediste diversicolor*).

Biomarkers were selected to quantify responses in the cellular antioxidant defence (glutathione reductase and catalase). Such effects may conceivably arise both from exposure of organisms to radical-generating contaminants (e.g. carcinogenic PAHs, some pesticides, some metals) and from changes in the intracellular availability of oxygen. The latter effect is directly related to the role of those enzymes in normal cells, which is the "detoxification" of reactive oxygen species (see Halliwell & Gutteridge 1989 for a review).

None of the muds contained significant amounts of substances regarded as micropollutants, so no such biomarkers were included.

2. MATERIAL AND METHODS

2.1 TEST SET-UP AND ENVIRONMENTAL CONDITIONS

The test principle has been developed through several similar projects (ref. NIVA reports). The idea is to establish a series of replicate experimental systems (Figure 2.1), which are maintained in easily accessible indoor basins (Figure 2.2). Each system is referred to as a benthic chamber and the biodegradation environment inside the chambers is made to resemble the conditions at the North Sea seabed as closely as suitable to the purpose.

The chambers had an area of 48 x 48 cm and a height of 35 cm. The four walls of the chamber was dismounted and brought on board FF Trygve Braarud, UiO, for sampling of test communities at 200 m depth in the Oslofjord nearby Solbergstrand. The acrylic chamber walls fit tightly into the steel box of a USNEL box corer. The box corer with the chamber walls were lowered to a penetration depth of 25-30 cm into the seabed. By reversing the winch the drag of the wire releases a spade which digs into the sediment below the sample before bringing the entire 48x48x30cm section of the seabed to the surface. Onboard the boat, a bottom plate was inserted between the spade and the sample before

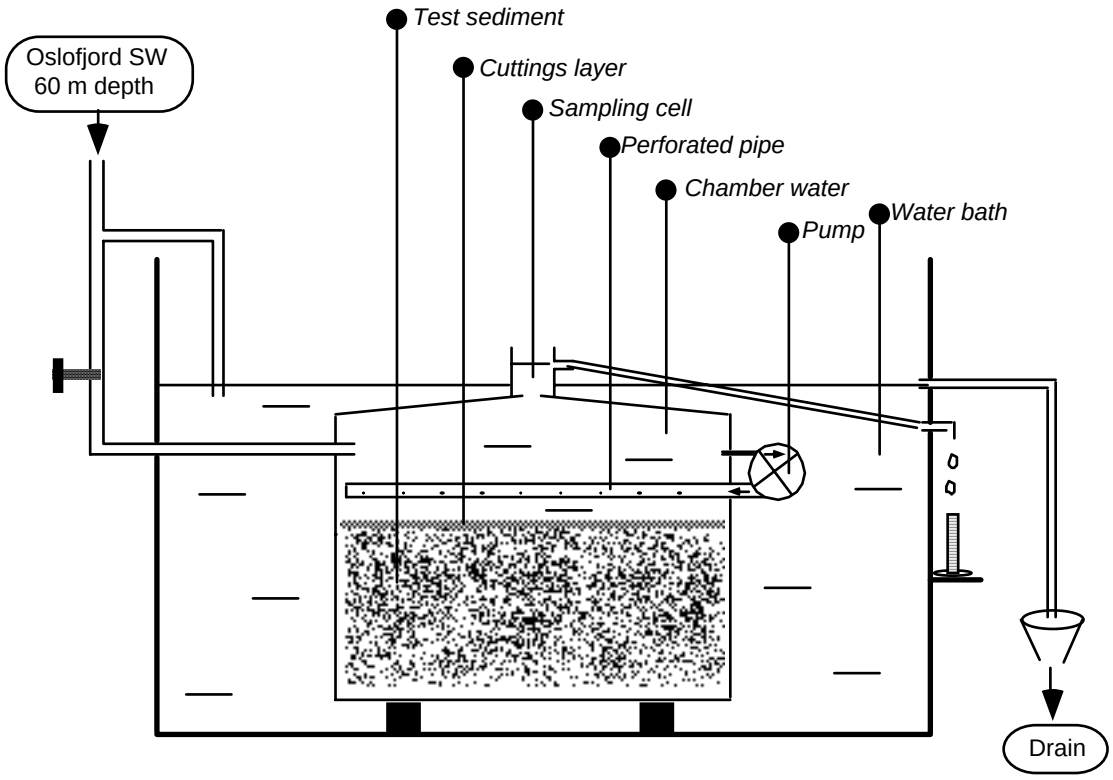

Figure 2.1. Schematic drawing of benthic chamber used for experimental study on biodegradation of pseudo oils on cuttings. Each chamber had a surface area of 50x50cm and a depth of 35 cm.

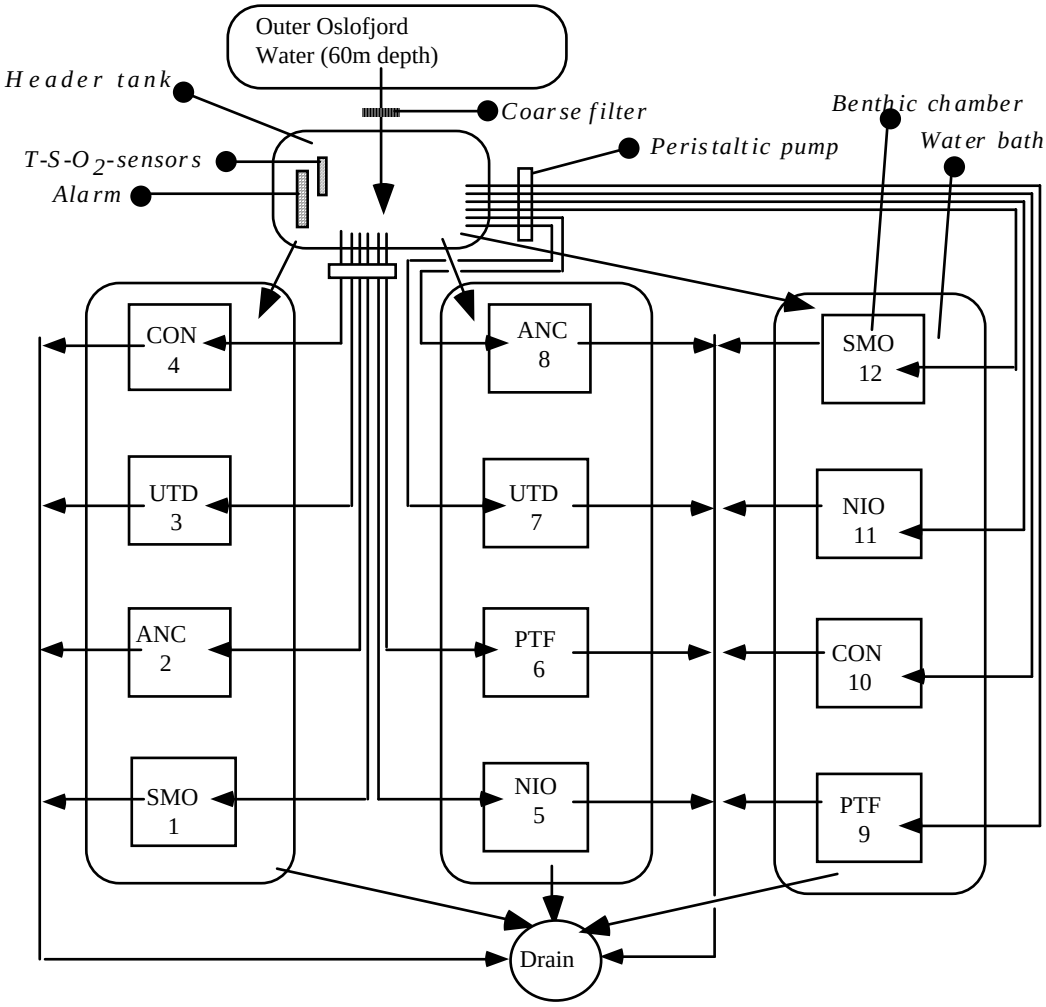

Figure 2.2. Schematic drawing of test set-up and water flow through the test basin. In the present test a second peristaltic pump and a third tray with another four chambers was added to the set-up shown above.

removal of the chamber from the steel box. A temporary lid was placed on top of the chamber and textile bands were strapped around each chamber to prevent the overlying water from spilling during handling and transportation.

At the Solbergstrand laboratory the boxes were placed at their respective locations in the trays The sediments appeared very similar with regard to colours (shades of grey and brown) and approximate number of animal structures. Typical depth between the rim of the box and the sediment surface was 15 cm, corresponding to a water volume of 34 l. Two boxes differed with regard to volume of overlying water. Those two boxes which had water volumes of 27l and 40l, respectively, were chosen for control purposes. The remaining ten boxes were distributed at random in the three trays shown in Figure 2.2. The transportation lids were then replaced by tightly fitted chamber tops with outflow through a sampling cell, as shown in Figure 2.1.

As shown in Figure 2.2, two six-channel peristaltic pumps maintained separate flows of seawater from the header tank through each chamber. Thus, throughout the experimental period, the chamber water was continuously renewed with a turnover ranging from 12 to 32 hours, depending on oxygen consumption rates.

A laminar type internal circulation system was maintained by submersed, aquarium pumps driving water through a perforated pipe positioned along one side of the chamber. By timer-control, the

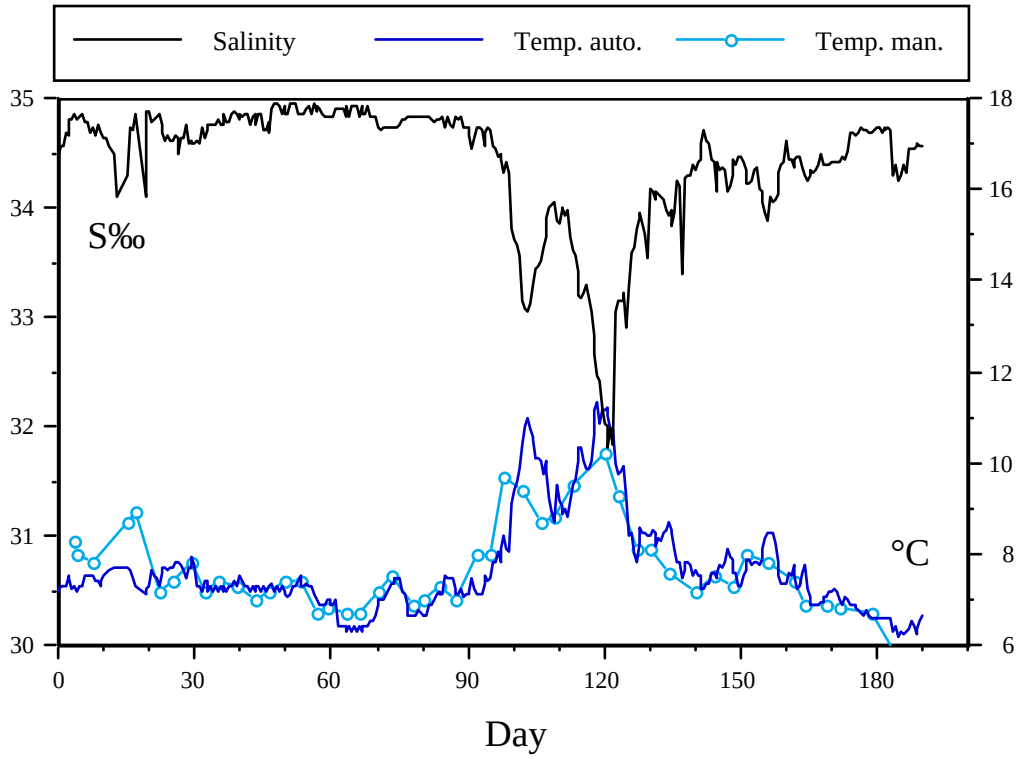

Figure 2.1. Temperature and salinity in header tank during experimental period.

Table 2.1. Test conditions in sediments and overlying water.

Parameter	Status
Sediment type	Marine, soft clay, transplanted from 200m depth
" size fraction<63µm	92-97%
Sediment redox state	Oxygen always available at sediment surface
Oxygen saturation	50-100% in overlying water
Mean temperature	7.69°C (n=371)
Mean salinity	34.39 PSU (n=371)
Current velocities	up to ca 10 $cm \cdot s^{-1}$
Current generation	15 minutes every two hours
Illumination	Dim to dark, bright light only during sampling and inspection

pumps were activated for 15 minutes every two hours. The pumps generated characteristic current velocities of 5-10 $cm \cdot sec^{-1}$. No visible resuspension of cuttings or sediments were ever observed to result from the internal circulation system.

Test conditions are summarised in Table 2.1. Temperature, salinity and oxygen concentration was recorded automatically, every 12 hours, on sensors located in the header tank. As shown in Figure 2.1 and Table 2.1, during the entire experimental period from 01.07.95 to 7.01.96, salinity and temperature in the source water ranged 31.8-35.0 PSU and 6.2-11.3°C, respectively. As shown in Figure 2.1 an influence of more surficial watermasses occurred during the 90-130 days time interval. The event was probably triggered by rather unusual weather conditions during the period. The increase of water temperature was accompanied by a temporary increase of oxygen consumption rates in control chambers and in most of the treated chambers (Figure 3.7).

The extreme values of temperatures and salinities observed in the chambers during the 90-120 days period are unlikely to occur at the offshore seabed location. However, the difference between the

mean salinity and the salinity at depths in the North Sea will be negligible with regard to biological impacts. The temperature at the Brage sea-bed may be a few degrees lower than the experimental mean temperature of 7.7°C. Because of temperature adaptation, it is not clear that such a temperature difference will have any effect on the actual degradation rates.

Temperatures in the benthic chambers were kept within ±0,5°C of the source water. By flow adjustments determined by oxygen consumption rates in each chamber, oxygen concentrations were maintained at concentrations 10-50% lower than the concentration in the source water.

Particle size fraction smaller than 63 µm was determined in samples of the top 3 cm layer of the two control chambers collected on day 1. Of the sample collected in CON 4, 97.1% of the particles were washed through the 63µm mesh size sieve, as compared to 92.1% of the sample from CON 10.

2.2 ADDITION OF CUTTINGS

The objectives of the initial treatment was to obtain an evenly distributed layer of cuttings contaminated with 4-6 $mgDF \cdot cm^{-2}$ of the respective drilling fluids and to obtain similar particle loads in all chambers. Such doses would correspond to the lower range of concentrations applied in previous tests, and similar concentrations of mineral oil (THC) have been found at distances up to 500 m away from offshore installations (Bakke et al, 1993).

Three of the five different types of cuttings were sampled from recent drilling operations in the North Sea. Thus, according to the information supplied from the operators, the *Anco Green* cuttings were sampled 22.05.95 at Brage, Well 31/4-A-28, the *Petrofree* ester cuttings were sampled 29.05.95 at Ula, 81/2 and the *Safemul* Mineral Oil based cuttings were sampled 29.05.95 at Oseberg B, 30/9-B-16. The cuttings arrived at NIVA during June, 2nd. - 8th., 1995 in carefully sealed polyethylen bottles.

The *Petrofree* cuttings had a high concentration of 17.3% of the drilling fluid. As shown in Table 2.2, dilution was obtained by addition of 192 g of powdered, marine, clay sediment which had been ignited at 550°C to remove all organic carbon.

The *Ultidrill* cuttings sample was left over from a previous test. The sample was collected at Frøy Platform, 25/5-A5(P4) and had been stored since October 1994 under appropriate conditions (tightly sealed containers, in the dark, 4°C) at the Solbergstrand Laboratory.

Synthetic cuttings were prepared from a sample of *Novaplus* mud delivered 23.06.95 from M-I Norge. According to Pål Helmichsen (M-I Norge Field Service Laboratory Report, 22.06.95) the mud sample had a content of *Novaplus*/Base Fluid/IO of 36.69%. By allowing 207 g of the mud sample to soak into 601 g of the ignited clay sediment, the laboratory prepared cuttings should contain 9.4 % of the drilling fluid (Table 2.2).

The cuttings were added to each chamber by preparation of slurries in a steel whirl mixer. As shown in Table 2.2, the slurries were made to contain similar amounts of particles, similar amounts of drilling fluids and similar amounts of a marine clay sediment which had a low content (10 $\mu g \cdot g^{-1}$) of mostly old, refractory organic carbon. The two control chambers were treated with similar loads of particles and similar amounts of the clay sediment, but no drilling fluids or any other mud components. Sea water was added to all slurries to obtain the appropriate viscosity for sprinkling of the slurries into the overlying water in each chamber.

Particles were allowed to settle for 20 hours, before initiation of the peristaltic pumps (Figure 2.2), which maintained continuous renewal of the chamber water.

The amount to be added of each cuttings sample was calculated from the respective content of base fluids as determined by retort analyses on the offshore sampling location. This information was

Table 2.2 **Initial treatment of experimental chambers. In the two right-hand columns, observed concentration of drilling fluids in the slurries are compared to concentrations calculated from the information (retort analyses) enclosed with the cuttings samples. DF=Drilling Fluid.**

			Composition of slurry (g)				Added	Est. layer	DF in slurry (wet wght)	
Treat-ment	Ch. no	DF in cuttings	wet cuttings	ignited sediment	wet con. sed.	sea water	slurry g	thickness mm	Cuttings info.	Observed on GC
ANC	2	8.70 %	320	0	412	696	562	1.66	1.95 %	2.63 %
ANC	8	"	"	"	"	"	478	1.41	"	2.68 %
SMO	1	8.70 %	358	0	409	758	604	1.78	2.04 %	1.80 %
SMO	12	"	"	"	"	"	637	1.88	"	1.90 %
PTF	6	17.3 %	170	192	433	585	613	1.81	2.13 %	1.95 %
PTF	9	"	"	"	"	"	601	1.78	"	1.38 %
UTD	3	9.70 %	306	0	424	693	562	1.66	2.09 %	1.87 %
UTD	7	"	"	"	"	"	478	1.41	"	1.82 %
NIO	5	9.41 %	334	0*	429	526	611	1.81	2.44 %	3.31 %
NIO	11	"	"	"	"	"	513	1.52	"	2.61 %
CON	4		0	204	422	427	545	1.61	-	-
CON	10		"	"	"	"	480	1.42	-	-

*Ignited sediment was used for preparation of this cuttings sample (see text).

supplied from the various operators. As shown in the two right-hand columns of Table 2.2, the calculated concentrations of 1.95-2.44 % in the slurries were in reasonably good agreement with the concentrations determined by GC-analyses in the NIVA-laboratory.

From the added 478-637 g slurry (Table 2.2) and the corresponding water contents of 54-68% (Table AI-2), the total load of particles added to each chamber ranged between 85 and 126 $mg \cdot cm^{-2}$. By assuming a water content in the added layer of 50%, the mean thickness of the layers was estimated to 1.4 -1.9 mm (Table 2.2).

As shown in Table 2.2, ignited clay sediment was added to *Petrofree*, *Novaplus* and control, but to none of the other chambers. The purpose of the ignition was to maintain similar loads of organic carbon (other than cuttings) in all chambers.

2.3 CHAMBER SAMPLING

2.3.1 Sediment samples

The slurries were added to the chambers 03.07.95. One sediment sample for analyses of base fluid and barium was taken from each chamber 1, 35, 66, 98, 127, 158 and 187 days after addition. Thus, the last series were sampled 05.01.96.

Each sediment sample was pooled from the top 0-3 cm section cut off from five separate cores (ID≈15 mm). Each core was drawn from the grid locations shown in Figure 2.2. The grid co-ordinates were chosen using the random number function of a spreadsheet program.

The total weight of the pooled sample was determined before the samples were put to storage at -20°C. The drilling fluids were extracted less than two weeks after sampling. Extracts were stored at -

20°C until GC-analyses on the complete time series could be performed in January 1996 (ref. Analytical methods, p.32).

Barium was analysed on pelletised samples of the dried sediment using x-ray fluorescence, by Ingegerd Rustad, SINTEF Chemical Industry (ref. Table AI-2).

2.3.2 Sample work-up and concentration units

The set-up of the experiment implicated that the cuttings and contaminants were present in a thin layer at the sediment-water interface. Thus concentrations measured against sediment mass (wet or dry), will be crucially dependent on the sediment depth at which the core-sample is cut off. This depth may

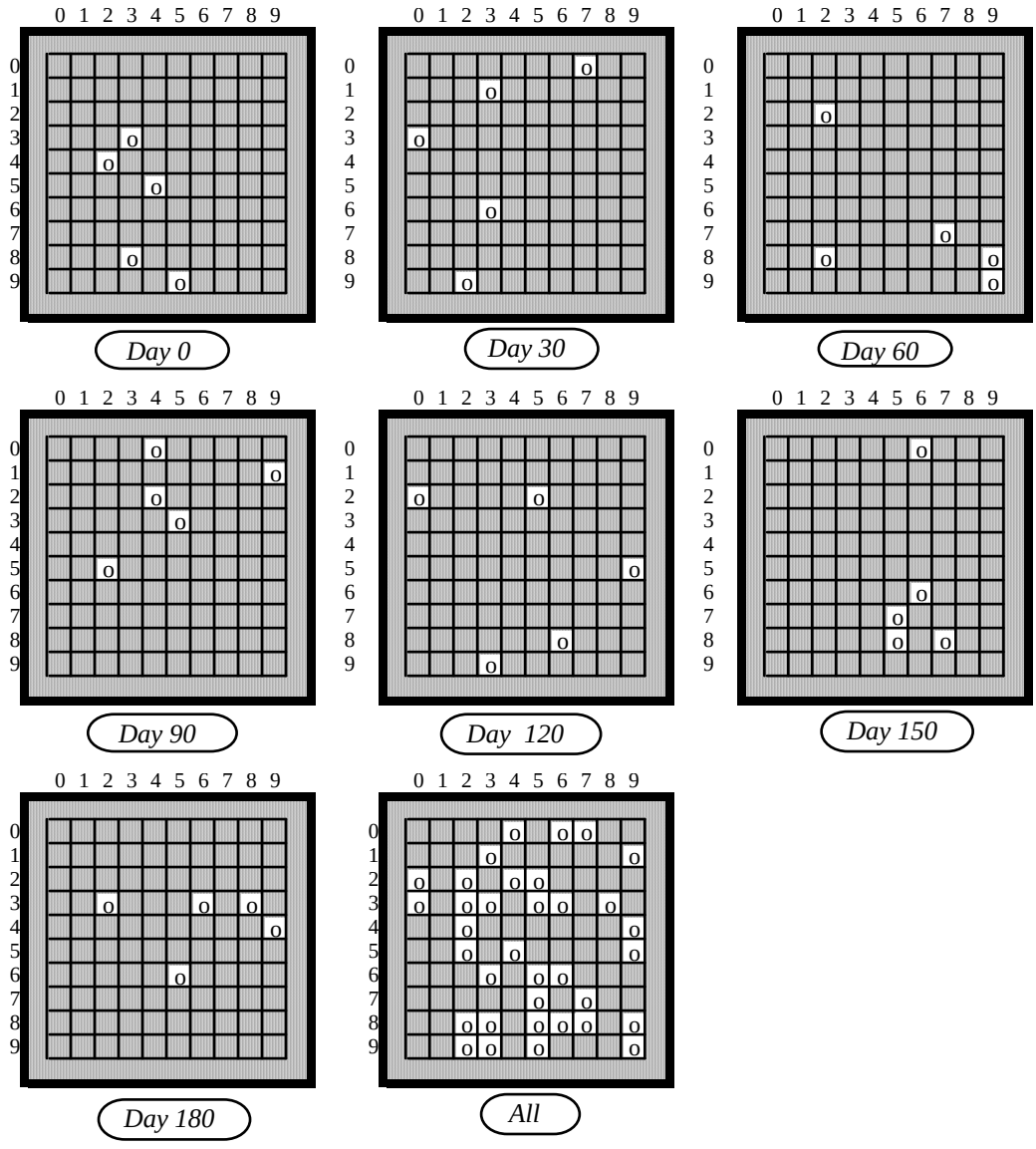

Figure 2.2. Grid system and randomly chosen locations for each one of the five core samples at each sampling occasion. The lower plate shows all sampled grids by the end of the experiment.

vary intentionally or unintentionally, and concentration units such as $mg \cdot kg^{-1}$ dry sediment will vary accordingly. In the present test the half-life and mass balance calculations depend on estimates of the total amount of drilling fluids present within each chamber or below a given sediment area, and the preferred units were such as $mg \cdot chamber^{-1}$ or $mg \cdot cm^{-2}$.

Therefore, the total wet weight of the five core samples were determined during sampling. In the laboratory, the drilling fluids were extracted from a sub-sample of the wet sediment. The concentration of drilling fluids was calculated from:

Equation 2.1 $$\mathbf{C_a = I_{GC} \cdot M_s / M_{GC} \cdot 5 \cdot A_{core}}$$

in which:

C_a = concentration of drilling fluid ($mgDF \cdot cm^{-2}$)
I_{GC} = integrated GC peak area, corrected for reagent blank (mgDF in extract)
M_{GC} = mass of sub-sample for extraction (g wet sediment)
M_s = total mass of sediment sample pooled from five cores (g wet sediment)
A_{core} = area of each core sample

Following this procedure errors resulting from inaccurate core sectioning and false assumptions of mass-volume ratios in the sediment were eliminated. The sampled area was calculated from accurate measurements of the core diameter using a sliding calliper.

2.3.3 Oxygen consumption

Oxygen consumption was determined every 3-4 days by successive measurements of concentration of oxygen in the inlet water in the header tank (HT, Figure 2.2) and in the outlet water in the sampling cell on top of each chamber (Figure 2.1), using WTW oximeter and electrodes. The flow of water through each separate chamber was measured gravimetrically after collection of outflow water for at least 4 minutes.

Thus, the sediment oxygen consumption SOC, was calculated from the equation:

Equation 2.2 $$\mathbf{SOC = (C_i - C_o) \cdot F \cdot 10^3 / A \cdot M_{O2}}$$

in which

SOC is the sediment oxygen consumption ($\mu mol \cdot m^{-2} \cdot h^{-1}$)
C_i is the concentration of oxygen in the water entering the chamber ($mg \cdot kg^{-1}$)
C_o is the concentration of oxygen in the water leaving the chamber ($mg \cdot kg^{-1}$)
F is the flow of water through the chamber ($kg \cdot h^{-1}$)
A is the area of the chamber (m^2)
M_{O2} is the molecular weight of oxygen = $32\ g \cdot mol^{-1}$

The contribution to SOC from oxygen consumed by micro-organisms present in the water or attached to tubes and chamber walls was assumed to be small compared to the amount of oxygen consumed in the sediment. Furthermore, if the total background respiration was similar in control and treated chambers, the difference between treated and control sediment (excess SOC or ΔSOC) should correspond to the respiratory activity produced by components in the contaminants added.

As shown in Table 2.3, over the entire experimental period, the mean concentration of oxygen was 7.8 $mg \cdot kg^{-1}$ at the inlet and 5.2-6.5 $mg \cdot kg^{-1}$ at the various outlets. If oxygen dropped to values below 4.0 the flow rate through the chamber was immediately increased. Thus, the lowest oxygen concentrations shown in Table 2.3 were never allowed to prevail for more than a day or two.

Table 2.3. Mean, maximum and minimum values and standard deviations during the experimental period of concentration of oxygen and flow rates through each chamber.

		Mean	Max.	Min.	Std.dev.	n
Oxygen ($mg \cdot kg^{-1}$)						
Header tank		7.8	9.2	6.5	.8	50
CON	4	6.1	7.5	3.1	.8	50
CON	10	6.5	8.1	5.3	.8	50
ANC	2	5.7	7.5	3.0	.9	50
ANC	8	6.1	8.8	4.2	.8	50
PTF	6	5.7	8.0	1.7	1.2	50
PTF	9	6.0	8.0	2.6	1.4	50
UTD	3	5.9	8.1	3.6	1.0	50
UTD	7	5.6	8.5	4.0	.9	50
SMO	1	5.7	7.8	3.1	.9	50
SMO	12	5.7	6.8	4.3	.6	50
NIO	5	5.2	8.3	1.5	1.3	50
NIO	11	5.6	7.8	3.4	1.0	50
Flow ($ml \cdot min^{-1}$)						
CON	4	29.8	44.4	12.6	5.4	50
CON	10	33.3	47.3	20.5	8.0	49
ANC	2	45.9	75.9	6.7	21.9	50
ANC	8	54.7	84.6	15.3	18.6	49
PTF	6	39.9	109.3	25.6	13.9	50
PTF	9	48.6	94.3	23.4	24.3	49
UTD	3	50.2	85.0	12.9	21.6	50
UTD	7	36.0	70.9	11.6	10.7	49
SMO	1	30.3	55.8	14.8	6.0	50
SMO	12	34.6	61.6	16.9	8.3	49
NIO	5	41.3	94.7	17.2	21.9	49
NIO	11	44.7	103.8	24.4	21.8	49

This test strategy implied that in order to maintain similar concentrations of oxygen, some variation in flow rates between the chambers had to be accepted. If the higher flow rates had implied higher current velocities at the sediment water interface, increased oxygen supply to sediment organisms and a higher sediment oxygen consumption might have occurred. However, the highest flow rates shown in Table 2.3, correspond to no more than rapid dripping from the outlet tubes. The water movements imposed by these flow rates were small compared to the movements generated by the circulation pumps. Thus, the dominating impact of the circulation pumps (section 2.1) ensured similar current regimes in all chambers.

2.3.4 Electrode measurements

pH and redox potentials (E_h) were determined at one month intervals after addition of cuttings. Electrodes were inserted directly into the submersed sediments at three different locations within each chamber. At each location, potentials were recorded in chamber water (OW) and 15 ± 5 mm below the sediment-water interface. The readings were taken as soon as the pH gave a stable value.

pH was measured using a Sentron ion-specific field effect transistor (ISFET) pH-meter and sensor. Redox potentials were determined on a standard Radiometer P101 platinum electrode against a Ag|AgCl reference electrode.

Before each series of measurements, the pH was calibrated in standard, low-ionic strength, buffers of pH 4.0 and 7.0. All calibrations and measurements were done at the experimental temperature of 6-10°C.

The redox circuit was checked in a ZoBell Fe(II)-Fe(III) redox-buffer solution with a redox potential of 430 mV at 20°C. At the experimental temperature the E_h recorded in the buffer solution before each of the seven series of measurements had a mean value of 432 mV and a standard deviation of 10 mV. At 10°C the half-cell potential of the Ag|AgCl reference electrode was 231 mV (Radiometer, technical information). As recommended by ZoBell, 1946, electrode performance was checked, but not calibrated with the redox buffer. Therefore, the E_h of the samples were obtained by adding 231 mV, to the potential recorded on the Pt-electrode.

2.3.5 Reproducibility of pH and E_h measurements

The reproducibility of electrode measurements has been determined by repeatedly inserting the electrodes to a given depth at different locations in similarly treated sediments kept in different chambers. Thus, the reproducibility of pH measurements was found to be ±.09 pH-units. The corresponding reproducibility of the E_h was ±33 mV.

2.3.6 Retrieval of benthic organisms

By the end of the experiment, the benthic fauna was collected by washing the top 20 cm of the sediment from each chamber through a 1 mm mesh size sieve.

Individuals of *Hediste diversicolor* were carefully removed from the sieve and transferred to clean seawater. Following three rinses in clean seawater each individual was blotted dry, weighed, pooled and frozen in liquid nitrogen. No surviving individuals of the polychaete was found in the *Petrofree* chambers. From the other treatments, three replicate pools of *Hediste diversicolor* were prepared for separate analyses of drilling fluids, barium and biomarker enzymes.

The remaining animals were preserved in 4% neutralised formalin, for later sorting and species identification.

2.3.7 Biomarker analyses

Pools of *Hediste diversicolor* were homogenised in ice-cold homogenising buffer (0.1 M potassium phosphate, pH 7.8, 0.15 M KCl, 2 mM glutathione, 10% glycerol, complete® protease inhibitor set) using a Potter-Elvehjem homogeniser. The homogenates were centrifuged at 10 000 *g* for 30 mins at 4°C, the S9-supernatant withdrawn, distributed into five eppendorf-vials and stored at -80°C until analysis.

Glutathione reductase was adapted to microplate analysis from the protocol described by Livingstone (1990). Briefly, 40-200 µL of S9 (corresponding to 1-5 µg of tissue) was added to a reaction mixture in each of 8 wells. Buffer only was added to 8 adjacent wells (blanks). Glutathione was then added as substrate to start the enzymatic consumption of NADPH, which was then measured photometrically at 340 nm. The amount of glutathione reductase present in the sample will be proportional to the rate of NADPH-consumption.

Catalase was measured according to Livingstone (1990) with some minor modifications. A reaction mixture was made from 0.1 M Na-phosphate buffer (pH 7.6) with the substrate, hydrogen peroxide, added (40 µL to 20 mL buffer). The enzymatic transformation of peroxide to oxygen and water was initiated by the addition of S9 to the sample cuvette. The rate of decrease was determined spectrophotometrically at 240 nm and used to calculate the catalase-activity present in the samples.

Both assays were optimised for pH and sample volume. All samples were analysed blind.

2.4 CHEMICAL CHARACTERISATION AND ANALYSES

2.4.1 Chemical characterisation

Anco Green ester

The base fluid has been characterised qualitatively using gas chromatography coupled with a mass selective detector, allowing to separate and tentatively to identify the different components in the mixture.

Figure 2.3, showing the total ion chromatogram, revealed major groups of components. Mass spectral identification, partly based on library search, revealed that the components consisted of isopropyl-esters of fatty acids of varying chain lengths and varying degree of unsaturation.

In Figure 2.3 chain length is indicated by C_n (where n = number of carbon atoms in the respective fatty acid group) and the degree of saturation is indicated by µ (unsaturated - 1 double bond) and s (saturated). The unsaturated fraction of each chain length group may consist of several components, which are likely isomers (varying location of double bond in the fatty acid chain).

In conclusion, it was confirmed that the product consisted of esters produced from naturally derived fatty acids (fish oil).

Novaplus

The base fluid has been characterised qualitatively using gas chromatography coupled with a mass selective detector, allowing to separate and tentatively to identify the different components in the mixture.

Figure 2.4, showing the total ion chromatogram, revealed three major groups of components. Mass spectral identification, partly based on library search, identified the components as unsaturated hydrocarbons (olefins, mostly with one double bond) with varying numbers of carbon atoms (C_{16}, C_{18} and C_{20}).

Ultidrill, Petrofree and Safemul

These products have been characterised in previous studies.

Ultidrill base fluid is an isomeric mixture of tetradecenes and hexadecenes.

Petrofree base fluid is a mixture of five homologous fatty acid esters with 2-ethylhexyl dodecanoate as main component.

Safemul is based on a low-aromatic mineral oil.

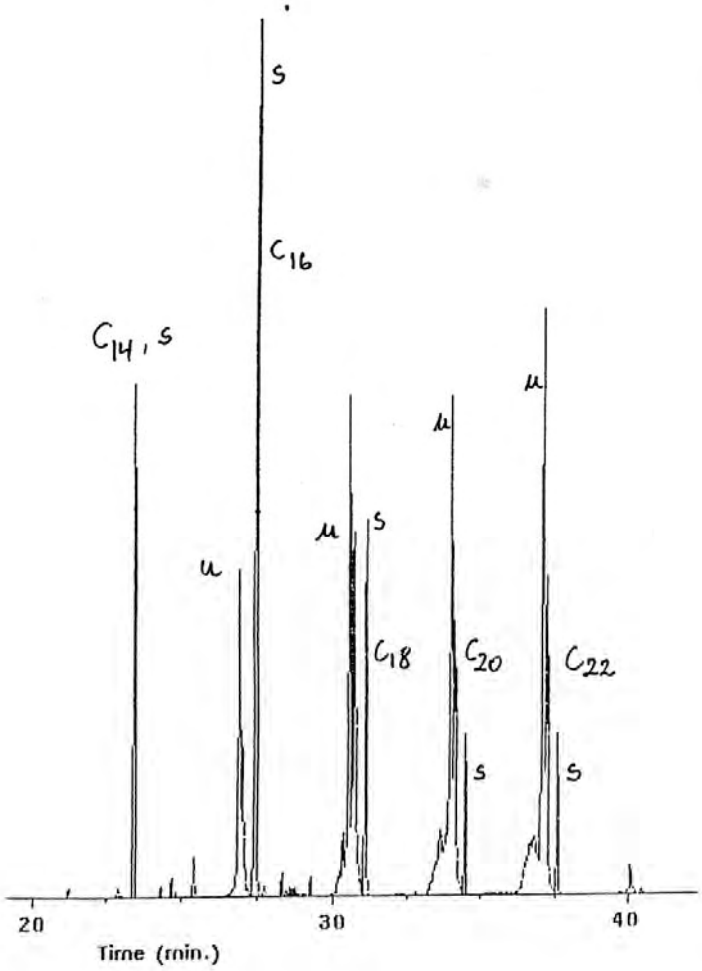

Figure 2.3 ***Anco Green*** **ester.**

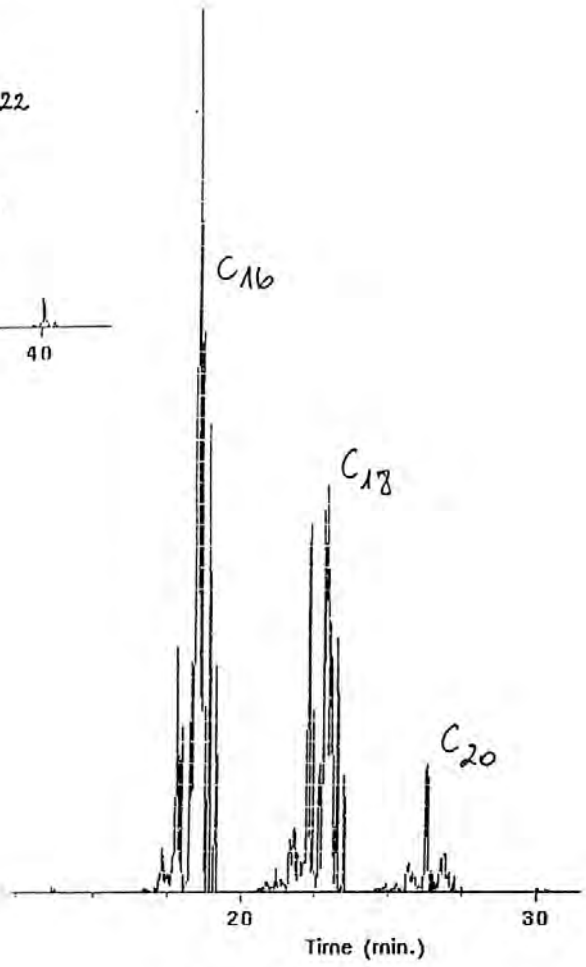

Figure 2.4 ***Novaplus*** **olefins.**

2.4.2 Chemical analyses

Analytical methods

Work-up procedure, sediments

Wet sediment samples weighing 1-5 g were homogenised and placed in a soxhlet tube. Internal standards, 1-dodecen (for *Ultidrill* and *Novaplus* samples) and ethyloctanoate (for *Anco Green*- and Petro free-ester samples) were added. Tubes were refluxed with 100 ml methanol for 2.5 hours to remove water. The methanol was decanted and the samples refluxed with 150 ml dichloromethane over night (min. 16 h). The methanol extract was diluted with 100 ml distilled water and extracted twice with 40 ml dichloromethane. The dichloromethane extracts were combined, washed twice with 50 ml water and dried over sodium sulphate for min.16 h. Finally, he extracts were evaporated to a suitable volume (5-25 ml) and analysed by gas liquid chromatography (GC).

Determination by GC-FID

Quantitation of olefin and ester based components was carried out by measuring the flame ionisation detector (FID) response of the area of the components of interest. This area was compared to the corresponding response of known amounts of the internal standards.

Quantitation of SMO was carried out by measuring the area of the flame ionisation detector response of the components. This area was compared to the corresponding response of known amounts of SMO as an external standard.

The gas chromatographic analyses were carried out under the following conditions, identical for all five components:

Gas chromatograph : HP 5890II with autosampler HP 7673
Column : 12.5 m, 0.20 mm i.d., fused silica, cross-linked with dimethyl silicone
Detector : Flame ionisation detector.
Total flow : 5.0 psi
Split : 9.0 ml/min 1:10
Column flow : 0.9 ml/min
Septum purge : 1.5 ml/min
Carrier gas : Hydrogen
Injection vol. : 1 µl
Temperatures:
Column : 50°C, 1 min-10°C/min-250°C, 10 min. total: 31 min.
Detector : 325°C
Injector : 258°C
Data system : HP-Chem station.

Quality assurance

Equipment and reagents

Trace analysis requires control of the background levels of chemicals and equipment.
The following chemicals were used:

Dichloromethane, Rathburn HPLC-grade.
Methanol, Merck p.a.
Deionized water
Sodium sulphate, Merck for org. trace analysis.

Most of the equipment was rinsed with acetone and heated at 600°C over night. Some equipment, such as soxhlet and graduated flasks were rinsed with dichloromethane three times before use.

The analytical procedure was controlled for possible contamination by analysing procedural blanks.

The instrument was regularly calibrated during the period of analysis using appropriate standards. The validity of the standard curve was verified by analysing control standards. These controls were required to be within +/- 10 % of the expected values for acceptance.

Accuracy

For all components, except for SMO, an internal standard was added to the sediment samples prior to the extraction, in order to compensate for possible losses during the preparation.

The accuracy of the methods for all five components was checked by analysing sediment samples with known amounts of the components. An average recovery of > 95% was obtained for all five components after work up and analysis of two replicates pr.component.

Reproducibility

The reproducibility of the analytical procedure was determined by repeated analyses of samples taken early in the experiment. A relative standard deviation of ±2% was obtained for all five components.

3. RESULTS AND DISCUSSION

3.1 SEABED REMEDIATION

3.1.1 Recovery of the cuttings added

With regard to test results, the recovery of drilling fluids and barium is not important. Biodegradation and effects will depend on the actual presence of contaminants after the sedimentation of cuttings on day zero. The calculated recoveries serve primarily as a quality assurance and to reveal errors in set up procedures, sampling and analytical methods.

Table 3.1 shows recoveries of barium and drilling fluids. The recovery was calculated as the ratio between the amount of chemical observed in the sediment on day one and the amount added with the slurry on day zero.

The recoveries of barium of 67-90% (mean = 78.6%) revealed a moderate loss of 10-33% (mean = 21.4%) of cuttings particles. Probably, most of this loss occurs by washing out of fine particles after initiation of water circulation and exchange. Both peristaltic pumps (Figure 2.2) and circulation pumps (Figure 2.1) were allowed to run for 8 hours before the first sampling. During this period some remaining turbidity was seen to disappear from the chamber water. No resuspension of settled sediments could be seen.

The recovery of the drilling fluids ranged from 35% in SMO 12 to 117% in ANC 8. If water soluble fractions had been a significant source of loss of one or more of the drilling fluids, the mean recovery of drilling fluids might have been expected to be less than the mean recovery of barium. However, as shown in Table 3.1, the mean recovery of 78.0% of the drilling fluids was almost identical to the mean recovery of barium of 78.6%. This supported the hypothesis that both barium and drilling fluids were firmly associated with the same particulate fractions.

Random errors originating in patchy distribution or collection and handling of the sediment samples, should have the same effect on concentrations of Ba and DF. Therefore, the difference with regard to the magnitudes of the random error was most likely to be found in the analytical procedures. Also, the concentration difference of more than 20% in several replicate samples of slurries (Table 2.2) could not possibly result from patchy distribution or core sampling procedures. In previous experiments, variations of 20% or more have frequently been found, between replicate determinations of drilling fluids in sediments from the same chamber. In ratios between single determinations errors may add up to approximately twice the magnitude of the maximum analytical error. Therefore, and consistent with experience from previous tests, large random variation was expected to occur in the recoveries of drilling fluids.

Thus, in NIO 5 the concentration of 3.31% determined in the slurry (Table 2.2) appeared high as compared to 2.61% determined in the replicate sample of the thoroughly mixed slurry. 3.31% was also high compared to the concentration of 2.44% predicted from the retort analyses enclosed with the cuttings samples. Assuming that the true concentration in the NIO slurry was 2.61% the calculated recovery in NIO 5 would be 51% rather than the 40% given in Table 3.1. However, also the concentration of drilling fluid in the sediment appeared spuriously low. As shown in Table 2.2, 611 g of the slurry was added to NIO 5 as compared to only 513 g added to NIO 11. This explained the 14%

Table 3.1. Recovery of drilling fluids (DF) and barium (Ba) on the sediment surface on day one. In the third column, the amount of drilling fluids added was calculated from the information (retort analyses) supplied with the cuttings samples

		Added DF (mg.cm-2)			DF	Added Ba	(mg.cm-2)	Ba
Treat-ment	Ch. no	Cuttings info.	Observed in slurry	sediment	Recovery sed./slurry	Obs. in slurry	Obs. in sediment	Recovery sed./slurry
ANC	2	4.86	6.55	5.72	87 %	6.12	4.16	68 %
ANC	8	4.13	5.68	6.64	117 %	6.70	5.03	75 %
SMO	1	5.47	4.82	2.21	46 %	1.74	1.36	78 %
SMO	12	5.77	5.35	1.87	35 %	1.87	1.45	77 %
PTF	6	5.79	5.28	4.30	81 %	3.41	2.29	67 %
PTF	9	5.67	3.69	4.19	114 %	2.77	2.17	79 %
UTD	3	5.20	4.65	3.97	86 %	3.71	2.89	78 %
UTD	7	4.42	3.85	3.76	98 %	2.60	2.34	90 %
NIO	5	6.60	8.96	3.59	40 %	4.01	3.51	87 %
NIO	11	5.54	5.92	4.51	76 %	3.54	3.07	87 %
Mean ± st.dev.					78.0±29.2			78.6±7.7

higher concentration of barium observed in the sediment in NIO 5 as compared to NIO 11. If the concentration of drilling fluids in NIO 5 was assumed to be 14% higher than the concentration determined in NIO 11, the calculated recovery of drilling fluid would have increased further to a value of 73%. Thus, the anomalous low recovery observed in NIO 5 may have resulted from an unfortunate combination of errors in both numerator and denominator.

Another example of spurious analytical results was the concentration of 1.38% esters determined in the slurry added to PTF 9 (Table 2.2). This was low as compared both to the concentration of 1.95% determined in the replicate sample of the slurry and 2.13% predicted from the retort analyses. Assuming that 1.95% was the true concentration in the slurry added to both chambers, the recalculated recovery of drilling fluids in PTF 9 would have been 80% rather than 114% as shown in Table 3.1.

The two examples showed that in single determinations of drilling fluids, random analytical errors of 20% may have been present to explain even larger errors in the recoveries calculated for a separate chamber.

The two SMO chambers showed, however, consistently low recoveries. Sediment samples collected later in the test (day 30) confirmed the level at about 2.0 $mg \cdot cm^{-2}$ in the sediments (Figure 3.7), and the retort analyses confirmed a level of about 5.0 $mg \cdot cm^{-2}$ added. This did indicate some preferential loss of mineral oil fractions during addition, but more data is needed to confirm the significance of this hypothesis.

3.1.2 Carbon and nitrogen

The concentrations of carbon (TOT-C) and nitrogen (TOT-N) was determined in sediment samples collected on day 1 and day 186. The results are shown in Table 3.2 and Figure 3.1.

In the control chambers, the initial concentration of carbon of 26.1 $mgC \cdot g^{-1}$ (dry wght.) was not much different from the final concentration. The addition of cuttings resulted in an initial increase of total carbon to a mean concentration of 30.5 $mgC \cdot g^{-1}$ (range = 29.2-33.9 $mgC \cdot g^{-1}$) for all treated chambers.

Table 3.2 Loss of carbon and nitrogen in each chamber during the experimental period.

Chamber	CARBON Day 1 $mgC \cdot g^{-1}$	Day 186	Loss	Loss $mg \cdot cm^{-2}$	NITROGEN Day 1 $mgN \cdot g^{-1}$	Day 186	Loss
UTD 3	29.8	30.1	-0.3	-0.3	2.9	3.1	-0.2
UTD 7	29.6	28.9	0.7	0.8	2.8	2.9	-0.1
PTF 6	29.8	28.8	1.0	1.1	2.4	2.6	-0.2
PTF 9	29.2	28.3	0.9	1.0	2.5	2.5	0.0
SMO 1	30.6	33.0	-2.4	-2.7	3.7	3.6	0.1
SMO 12	30.5	31.5	-1.0	-1.1	2.8	3.0	-0.2
ANC 2	32.0	27.5	4.5	5.1	3.3	2.6	0.7
ANC 8	33.9	28.6	5.3	6.0	3.0	2.9	0.1
NIO 5	30.0	27.1	2.9	3.3	2.7	2.5	0.2
NIO 11	29.8	29.4	0.4	0.5	2.3	2.5	-0.2
Treated mean	*30.52*	*29.32*	*1.20*	*1.35*	*2.84*	*2.82*	*0.02*
Std. deviation	*1.42*	*1.80*	*2.40*	*2.70*	*0.42*	*0.36*	*0.28*
CON 4	25.7	25.4	0.3	0.3	3.1	3.5	-0.4
CON 10	26.5	25.2	1.3	1.5	3.8	3.8	0.0
Control mean	*26.10*	*25.30*	*0.80*	*0.9*	*3.5*	*3.7*	*-0.2*

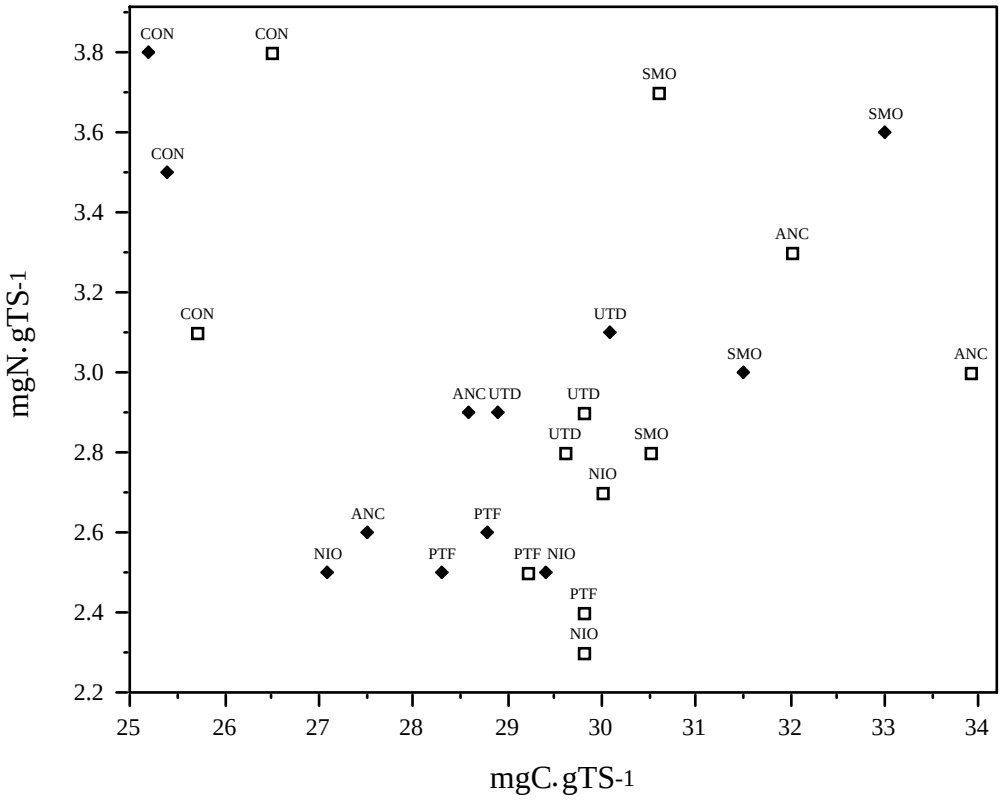

Figure 3.1 Initial (open squares) and final (crossed diamonds) concentrations of carbon and nitrogen in the top 0-3 cm of sediment in each chamber.

During the experimental period a loss of carbon was observed in most chambers. The loss of 5.1-6.0 $mgC \cdot cm^{-2}$ observed in the two *Anco Green* treatments was reasonably consistent with the complete loss of the added 5.7-6.2 $mgDF \cdot cm^{-2}$ (Table 3.1,Table 3.2). (Assuming an average stoichiometry of $C_{21}H_{42}O_2$ for the *Anco Green* esters, the carbon equivalent to the added esters was 4.4-4.8 mg ester-carbon $\cdot cm^{-2}$.) Also the absence of any decrease of carbon in the mineral oil treatments was consistent with the absence of any significant change of the concentrations of hydrocarbons (3.1.3). Thus, in spite of the moderate fraction accounted for by drilling fluids, the general trends of the concentrations of carbon was reasonably consistent with the observed loss of drilling fluids. However, the variations were masked by the large back-ground concentration of carbon, and significant trend analyses could only be performed on the analyses of the specific drilling fluids.

Because of the low content of nitrogen in the drilling fluids, the addition of cuttings resulted in a lowering (dilution) of the concentration of nitrogen from a mean value of 3.55 $mg \cdot kg^{-1}$ in control chambers to 2.83 $mg \cdot kg^{-1}$ in treated chambers. No significant differences were found between initial and final concentrations of nitrogen, neither in control nor in treated chambers.

The persistent difference between control and treated sediments with regard to the quality of the organic matter was clearly revealed in the plot of carbon versus nitrogen in Figure 3.1. The drilling fluids represent a source of carbon for growth and energy-consumption of the micro-organisms. However, the nitrogen required for protein synthesis must be supplied from other sources. Thus it cannot be ruled out, that nitrogen availability represent a limitation to the growth of the decomposer communities associated with cuttings deposits. Nutrient limitation in decomposition of drilling fluids have been indicated in several studies by NIVA and others.

3.1.3 Disappearance of drilling fluids and barium

In previous studies, first order kinetics have been found most appropriate for the description of the loss of drilling fluids with time. The general form of a first order reaction is:

Equation 3.1 $\mathbf{C = C_0 \cdot 10^{-kt}}$

in which:

C = concentration at time t
C_0 = initial concentration
t = time
k = rate constant

From Equation 3.1 it can be shown that if the half-life, τ, is the time at which $C = C_0/2$, then $\tau = 0.302/k$.

Barium may be considered a conservative property of the cuttings. After deposition, concentrations of barium should only change as a result of loss of cuttings particles by sampling, by resuspension to the water flowing through the chambers, or by burial to sediment layers below the sampling depth of three centimetres. Thus, if relocation of cuttings particles were significant, the change of the drilling fluid:barium ratio should be a better measure on biodegradation than the change of the concentration of drilling fluids alone. Also, the DF:Ba ratio will eliminate artefacts of non-representative sampling of the cuttings layer.

The change with time of the concentration of drilling fluids and barium are shown in Figure 3.2 to Figure 3.7. Results of exponential regression analyses of the time trends are shown in Table 3.3. Results are shown for each separate chamber (*A&B*) as well as pooled for the two replicate chambers (*Both*). Prior to the statistical analyses, the concentration data were normalised against initial concentration in each chamber. Such normalisation will have no effect on the calculated half-lives, but the correlation coefficients for pooled data will improve and confidence intervals shrink in cases of different dose levels. The correlation coefficients (r) show how well the data fits the model, whereas the probability (p) states the significance level for the change of the concentration (ratio).

Table 3.3 Results of exponential regression analyses of time trends of normalised concentrations of drilling fluids and DF:Ba ratios (see text). Italic numbers highlight cases of not significant decrease with time ($p \geq 0.05$).

Parameter analysed:		Drilling fluid			Drilling fluid : Barium ratio		
Chamber:		A	B	Both	A	B	Both
Number of analyses:	n	7	7	14	7	7	14
Anco Green ester							
Correlation coefficient	r	0.931	0.981	0.952	0.939	0.981	0.957
Probability	p	0.0023	0.0001	0.0001	0.0017	0.0001	0.0001
Intercept	C_0	1.85	1.84	1.84	1.67	1.98	1.82
Slope	k*1000	17.1	19.2	18.2	15.7	18.2	16.9
Halflife (days)	τ	17.7	15.7	16.6	19.2	16.6	17.9
95% Lower Confidence	τ	12.2	12.8	13.9	13.5	13.5	15.0
95% Upper Confidence	τ	32.1	20.4	20.8	33.2	21.4	22.0
Petrofree ester							
Correlation coefficient	r	0.981	0.946	0.955	0.984	0.952	0.959
Probability	p	0.0001	0.0013	0.0001	0.0001	0.0009	0.0001
Intercept	C_0	1.29	1.26	1.28	1.24	1.25	1.24
Slope	k*1000	12.8	14.5	13.6	12.8	14.6	13.7
Halflife (days)	τ	23.6	20.8	22.2	23.6	20.7	22.0
95% Lower Confidence	τ	19.1	15.0	18.5	19.5	15.1	18.5
95% Upper Confidence	τ	30.5	34.3	27.5	29.6	32.5	27.0
Ultidrill olefins							
Correlation coefficient	r	0.985	0.953	0.913	0.979	0.951	0.930
Probability	p	0.0001	0.0009	0.0001	0.0001	0.0010	0.0001
Intercept	C_0	1.12	1.02	1.05	1.17	1.11	1.03
Slope	k*1000	5.0	6.7	5.9	5.0	5.7	5.4
Halflife (days)	τ	60.4	44.8	51.2	60.4	53.0	55.9
95% Lower Confidence	τ	50.3	32.8	40.3	48.7	38.7	45.1
95% Upper Confidence	τ	75.5	70.4	71.9	79.5	83.9	75.5
Novaplus olefins							
Correlation coefficient	r	0.836	*0.754*	0.754	0.884	0.766	0.791
Probability	p	0.0192	*0.0504*	0.0018	0.0082	0.0445	0.0008
Intercept	C_0	1.32	*1.83*	1.18	1.14	1.54	1.16
Slope	k*1000	5.1	*3.1*	4.1	3.5	2.3	2.9
Halflife (days)	τ	59.2	*97.4*	73.7	86.3	131.3	104.1
95% Lower Confidence	τ	33.9	*49.5*	47.9	53.9	67.1	70.2
95% Upper Confidence	τ	251.7	-	167.8	215.7	3020.0	201.3
Mineral oil							
Correlation coefficient	r	*0.668*	*0.184*	*0.438*	*0.746*	*0.001*	*0.346*
Probability	p	*0.1011*	*0.6935*	*0.1176*	*0.0542*	*0.9981*	*0.2262*
Intercept	C_0	*1.01*	*1.12*	*1.05*	*1.13*	*1.15*	*1.14*
Slope	k*1000	*2.8*	*0.5*	*1.6*	*1.8*	*0.002*	*0.90*
Halflife (days)	τ	*107.9*	*604.0*	*188.8*	*167.8*	*131878*	*335.6*
95% Lower Confidence	τ	*46.5*	*91.5*	*79.5*	*81.6*	*127.4*	*120.8*
95% Upper Confidence	τ	-	-	-	-	-	-

Rest concentrations of drilling fluids in treated chambers compared to "back-ground" concentrations of corresponding chemicals in non-treated control chambers are shown in Table 3.4. (The observed levels in control chambers were measured against reagent blanks, and may result from cross-contamination and/or interference from the sediment matrix).

Anco Green esters

As shown in Figure 3.2 and Table 3.3, *Anco Green* esters disappeared rapidly from the sediments in both chambers (significance levels 99.99% and 99.77%) and the regression coefficients showed good fits to the exponential models (r-values of 0.93 and 0.98). The regression analyses in Table 3.3 yielded halflives of the ester of 15.7 and 17.7 days, respectively, in the two chambers. Pooling the data from both chambers yielded a halflife of 18.2 days with a 95% confidence interval of 13.9 to 20.8 days.

Some of the disappearance of the esters may result from loss of cuttings particles from the sampled 0-3cm layer by bioturbation, resuspension and/or sampling activity. This loss should apply to barium as well as to the ester. Clear downward trends of barium were observed in both chambers (Figure 3.2) and the regression analyses on the ester:Ba ratios yielded halflives of approximately one day more than the halflives of the esters proper (Table 3.3).

Petrofree esters

Also the disappearance of *Petrofree* esters were highly significant (p = .0001-.0013) and fitted well with the exponential model (r = .946-.981) yielding a halflife of 22.2 days for the pooled data, and a corresponding 95% confidence interval between 18.5 and 27.5 days. This agreed well with previous results on the degradation of *Petrofree* esters at similar initial concentrations.

As shown in Figure 3.3, no loss of barium was observed during the experimental period in the *Petrofree* chambers. Consequently the ratio between *Petrofree* esters and barium gave similar half-lives. Resuspension and sampling activities were similar in all chambers. The fauna analyses revealed, however, a dramatic lowering of the number of species and individuals in the *Petrofree* chambers as compared to all other chambers (Ch. 3.2.3). This suggested that bioturbation was a major factor causing a significant loss of barium from the sampled layer.

Novaplus olefins

The curve fits (r = .754-.836) of the *Novaplus* olefins were not as good as the fit of the esters, and the decrease with time in NIO 11 was not significant at 95% (p=.0504).

In NIO 5 the concentration tended to decrease steadily throughout the experimental period (Figure 3.4), whereas in NIO 11 most of the decrease occured during the first month after addition of cuttings. The reliability of the initial concentration in chamber NIO 11 was supported by the concentration expected from the amount of cuttings added (Table 3.1), and the concentrations observed towards the end of the experimental period were reasonably consistent between the two chambers. Also, the concentration of barium was observed to decrease during the first month and the samples drawn later in the experiment, confirmed that the initial decrease was a permanent change of the abundance of cuttings in the 0-3 cm layer.

Bioturbation may affect sediment concentrations in two ways. If bioturbation mixes cuttings particles to a depth beyond the sampling depth of 3 cm, the concentration measured within the 0-3 cm layer will decrease and stay low. The permanent decrease in concentration of both barium and olefins observed after the first month in NIO 11, as well as the general downward trends of the barium concentrations in most of the chambers, most probably resulted from this type of bioturbation. If, on the other hand, animal activity redistributes the cuttings into small mounds or patches, concentrations averaged over the entire surface should not change, but the increased patchiness will represent a

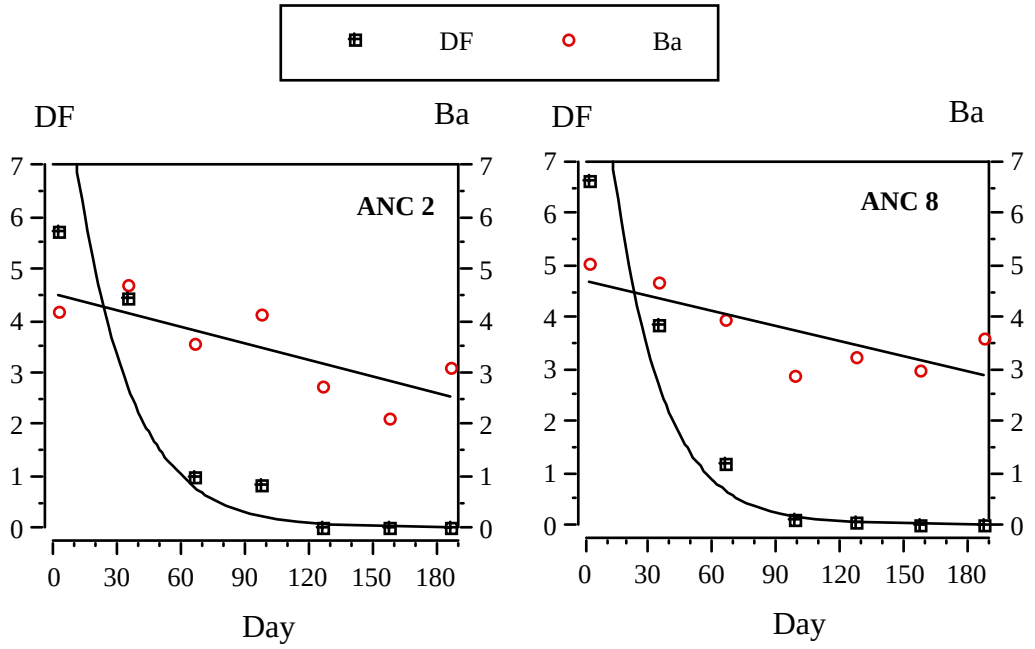

Figure 3.2. Variation with time of *Anco Green* esters and barium in the 0-3 cm depth interval of the sediment in chamber 2 and 8. Units = mg·cm^{-2}. Dotted line = linear regression curve for barium data. Full line = exponential regression curve for ester data.

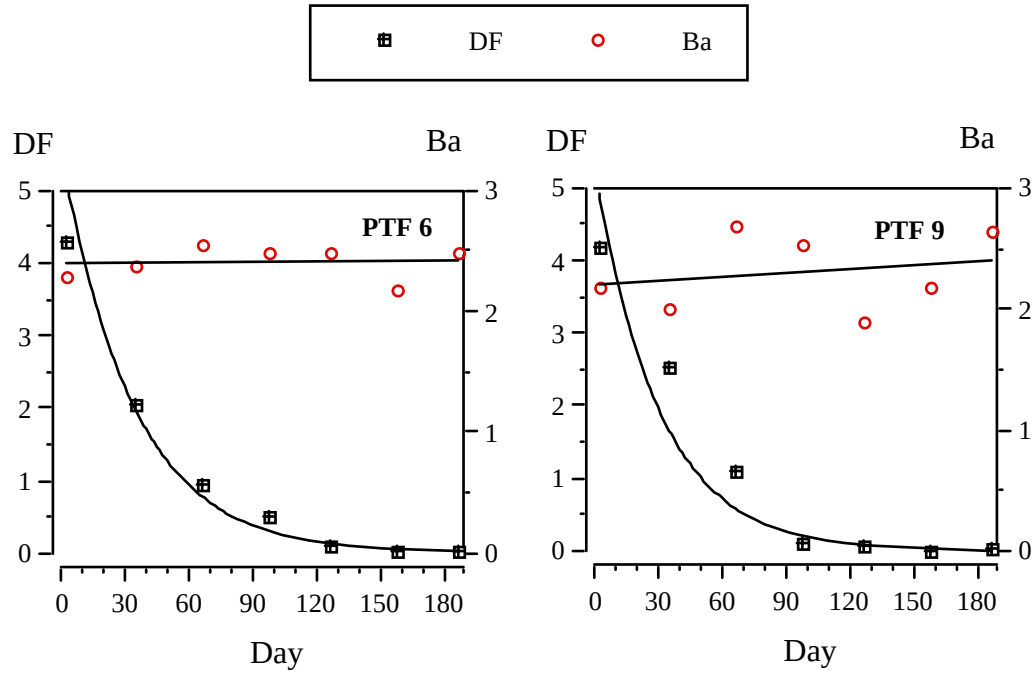

Figure 3.3 Variation with time of *Petrofree* esters and barium in the 0-3 cm depth interval of the sediment in chamber 6 and 9. Units = mg·cm^{-2}. Dotted line = linear regression curve for barium data. Full line = exponential regression curve for ester data.

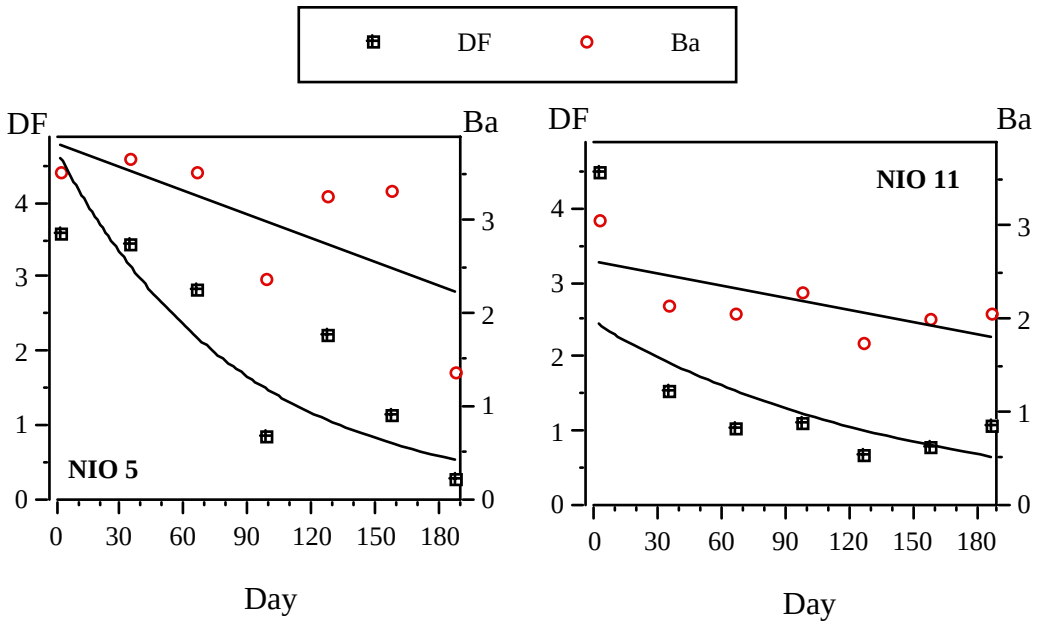

Figure 3.4 Variation with time of *Novaplus* olefins and barium in the 0-3 cm depth interval of the sediment in chamber 5 and 11. Units = mg·cm^{-2}. Dotted line = linear regression curve for barium data. Full line = exponential regression curve for ester data.

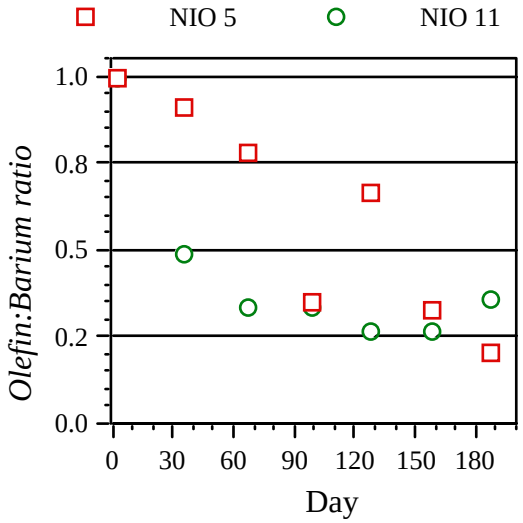

Figure 3.5 Normalised olefin:Barium ratios in NIO 5 and NIO 11.

sampling problem. As evident from Figure 3.4, barium and olefins frequently varied in similar patterns in both chambers. Thus, even though each sample was pooled from five randomly chosen core locations, the occasional increase in barium and drilling fluid from one sampling occasion to the next showed that errors resulting from patchy distribution was not completely eliminated.

If then, the rich macrobenthic communities in both *Novaplus* chambers (Ch.3.2.3) had been responsible for much of the disturbance relative to the ideal exponential degradation model, the curve fits for the olefin:barium ratios should be better than the curve fits for the olefins proper. As shown in Table 3.3, the decrease with time of the ratios was significant in both chambers (p = .0082-.0445) and correlation coefficients were larger than the corresponding coefficients for the olefins. The ratios are plotted in Figure 3.5.

Because barium is lost at a more or less constant rate, independent of biodegradation, the difference between the half-lives calculated from the DF:Ba ratios and the DF proper should increase with decreasing biodegradability. For the rapidly degrading *Anco Green* esters this difference was not more than one day corresponding to 7%. In NIO 5 and NIO 11, the half-lives increased from 59-97days for the olefins proper to 86-131 days for the ratios, or an average increase of 41%.

Ultidrill olefins

In both chambers, the change of the concentrations of *Ultidrill* olefins (Figure 3.6), showed good curve fits, yielding correlation coefficients (r) of .953 and .985 (Table 3.3). The half-life for the pooled concentration data was 51.2 days with a 95% confidence interval between 40.3 and 71.9 days.

Barium showed no significant decrease in UTD 3. Macrofauna communities were quite similar in the two chambers and unless, bioturbation in the other chambers have been driven primarily by a few highly active individuals of which the equivalents have been absent from UTD 3, the lack of a significant decrease in UTD 3 was most probably the result of random errors inherent in the barium determinations, in particular those originating in patchy distribution. In the other chamber, the half-life of the ratio was 8.2 days (18%) longer than the half-life of the olefin proper.

Thus the degradation rate of *Ultidrill* was clearly more slow than the degradation of both types of esters, but considerably faster than the degradation of *Novaplus*. The GC-analyses showed that the C_{14}-component, which represented the major fraction (60-70%) of the *Ultidrill* product, degraded faster than the C_{16}-component. This confirmed the results of a previous test which showed that the C_{14}:C_{16} ratio declined from an initial value of 1.8 to a final value of 0.6 (after a 176 days degradation period) (Oreld, 1995). The *Novaplus* olefins, being composed primarily of C_{16} and smaller fractions of C_{18}- and C_{20}-isomers and as it appears from **Error! Reference source not found.**, more branched structures, would be expected to degrade at a slower rate than the *Ultidrill* olefins.

In a previous test, using similar initial concentrations but an initially mixed sediment, half-lives of 43 and 19.6 days were found for *Ultidrill* and *Petrofree*, respectively. It appeared then, that if the present set-up have had any impact at all on rates of disappearance, the effects have been opposite of the increase one might have expected from the more diverse sediment community and undisturbed sediment layering.

Safemul mineral oil

From a number of experimental studies and offshore surveys, mineral oil is known to undergo slow degradation in marine sediments. In a recent review of tests performed at NIVA (Schaanning et al, in prep), a model was applied which assumed a 60 days lag phase before onset of exponential degradation. Pooled data from two tests ressembling the present test gave a halflife of 142 days for the period after day 60. (In the present test, the data were to scattered to justify any suggestion of a similar lag-phase.)

Downward slopes were observed in both chambers, but the decrease was not significant at the 95%

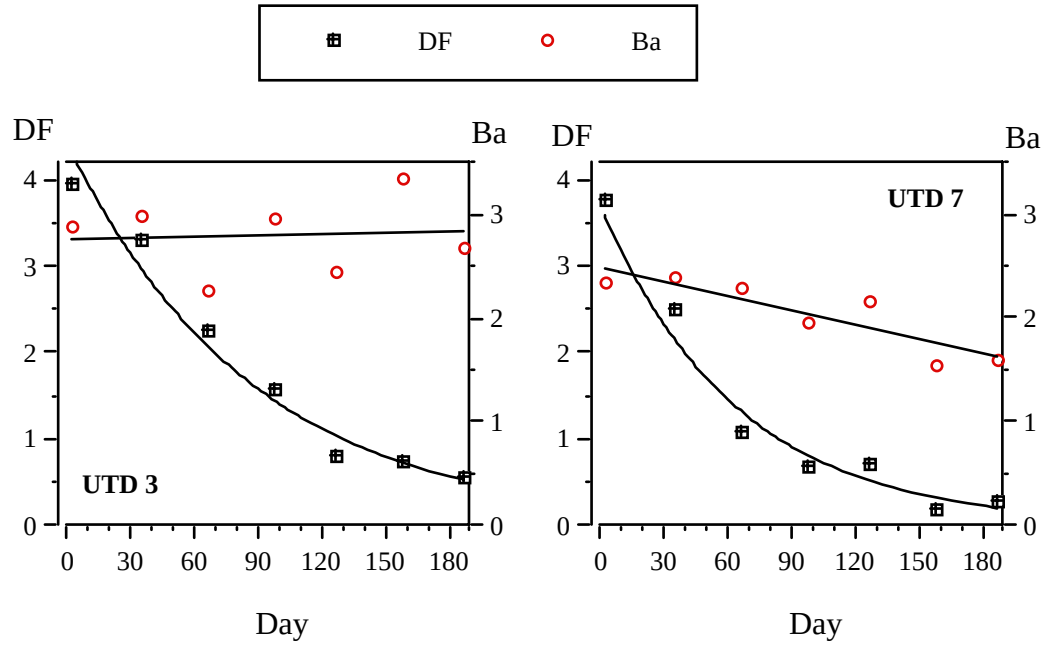

Figure 3.6. Variation with time of *Ultidrill* olefins and barium in the 0-3 cm depth interval of the sediment in chambers 3 and 7. Units = mg·cm^{-2}. Dotted line = linear regression curve for barium data. Full line = exponential regression curve for olefin data.

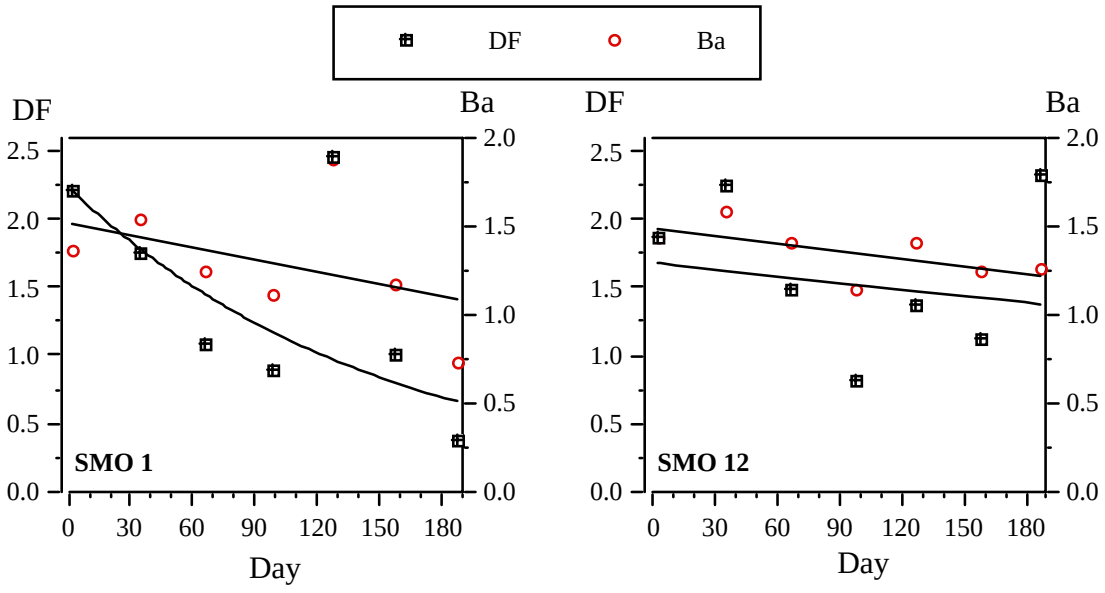

Figure 3.7. Variation with time of *Safemul* mineral oil and barium in the 0-3 cm depth interval of the sediment in chambers 1 and 12. Units = mg·cm^{-2}. Dotted line = linear regression curve for barium data. Full line = exponential regression curve for THC data.

Table 3.4 Final concentrations of drilling fluids in treated sediments (= mean day 158 and day 187)(n=4) compared to mean concentrations determined in non-treated control chambers.

	Rest concentration		Control sed
	Normalised	$mg \cdot cm^{-2}$	$mg \cdot cm^{-2}$
Anco Green esters	0.2%	0.009	0.001
Petrofree esters	0.6%	0.026	0.001
Novaplus olefins	21.9%	0.822	0.038
Ultidrill olefins	11.4%	0.445	0.017
Mineral Oil	40.7%	0.833	0.042

significance level. The best fit (SMO 1) yielded a half-life of 108 days for the mineral oil proper and 168 days for the ratio. Pooled for both chambers, halflives were 189 days for the oil and 336 days for the oil:Ba ratio.

The large scatter of the mineral oil data may result partly from patchy distribution of cuttings particles (Figure 3.7, SMO 1, four-month sample), but because of the multi-component nature of mineral oil analytical errors might be larger than for the pseudo-oils. Thus, a spuriously high concentration of mineral oil was observed in the final sample in SMO12 (Figure 3.7). If these two observations were omitted, the correlation coefficients for the pooled data (r) increased to .809 on mineral oil proper and .752 for the oil:Ba ratio. The corresponding half-lives were 105 and 158 days respectively, which in fact was quite similar to the half-lives calculated for SMO 1 using all data.

Thus, the concentration of mineral oil did decrease during the experimental period, but the concentrations remaining in the sediment towards the end of the experimental period (Table 3.4), corresponding to 40.7% of the addition, confirmed the slow disappearance of mineral oil as compared to the synthetic drilling fluids.

3.1.4 Qualitative changes of gas chromatographic patterns of drilling fluids

Anco Green esters

Figure 3.8 shows the gas chromatographic patterns of *Anco Green* esters in *Anco Green* base fluid (pure product) used in the production of drilling mud and *Anco Green* esters extracted from sediments sampled in chamber ANC 8 on day 2, 66 and 158.

The patterns in standard *Anco Green* esters and sediment extract on day 2 were to a large extent identical, indicating that no changes in chemical composition had occurred during the production and use as drilling fluid.

After 66 days, patterns revealed significant changes. Components identified as the unsaturated fatty acid esters (marked μ) in the mixture were more rapidly reduced compared to the saturated fatty acid esters (marked s). This effect was probably due to preferential biodegradation and was more pronounced for the higher homologs (C_{18}, C_{20} and C_{22}) compared to the lower ones (C_{14} and C_{16}).

After 158 days both saturated and unsaturated fatty acid esters had more or less completely disappeared. The components still present at the end of the experiment were either minor impurities in the original *Anco Green* ester product and/or more resistant organic components inherent in the cuttings or fjord sediment.

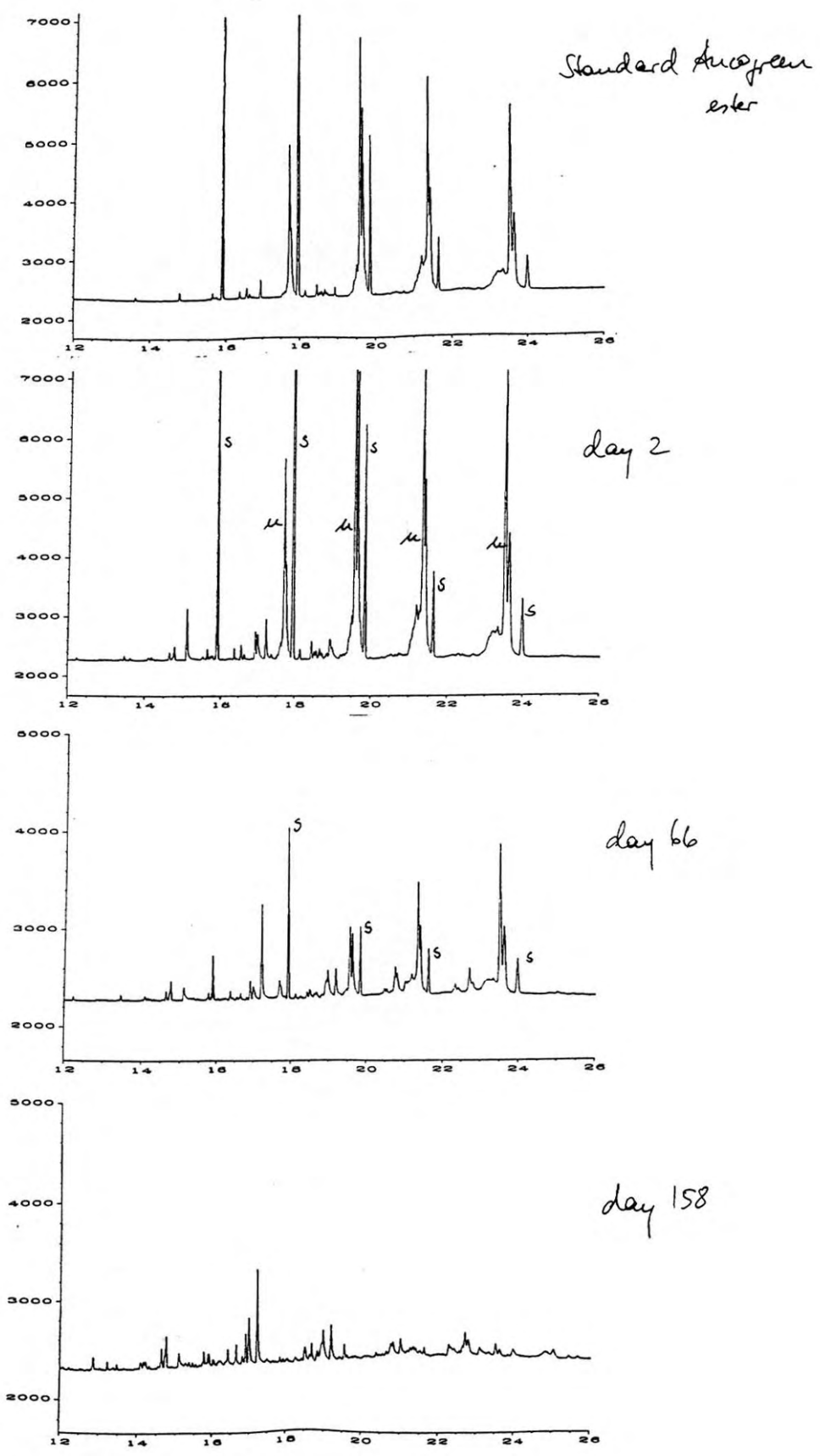

Figure 3.8 Gas chromatographic patterns of *Anco Green* base fluid (250 mg·kg^{-1}) (top) and extracts of sediments sampled in ANC 8 on day 2, day 66 and day 158 (bottom).

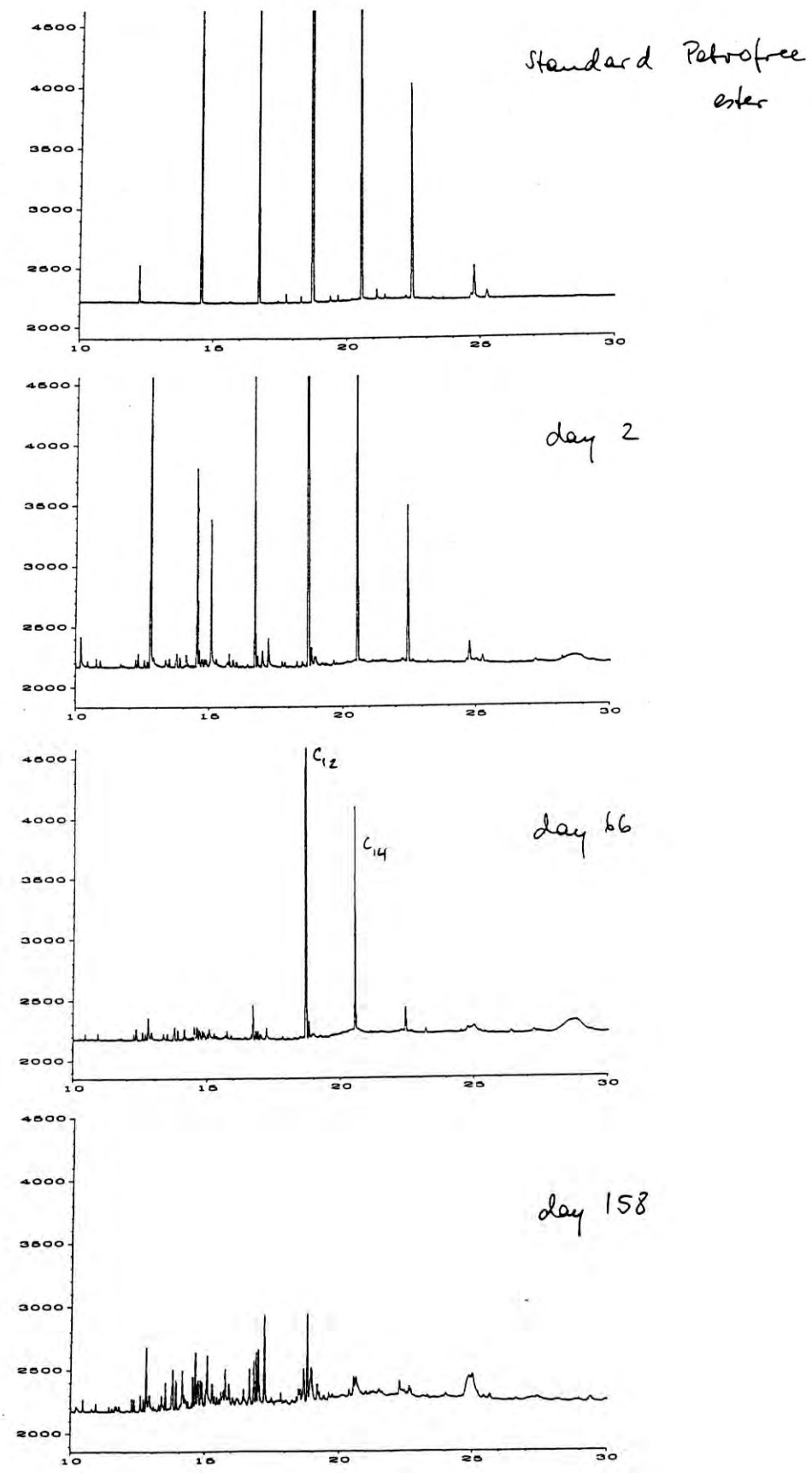

Figure 3.9 Gas chromatographic patterns of *Petrofree* base fluid (250 mg·kg^{-1})(top), and extracts of sediments sampled in PTF 9 on day 2, day 66 and day 158 (bottom).

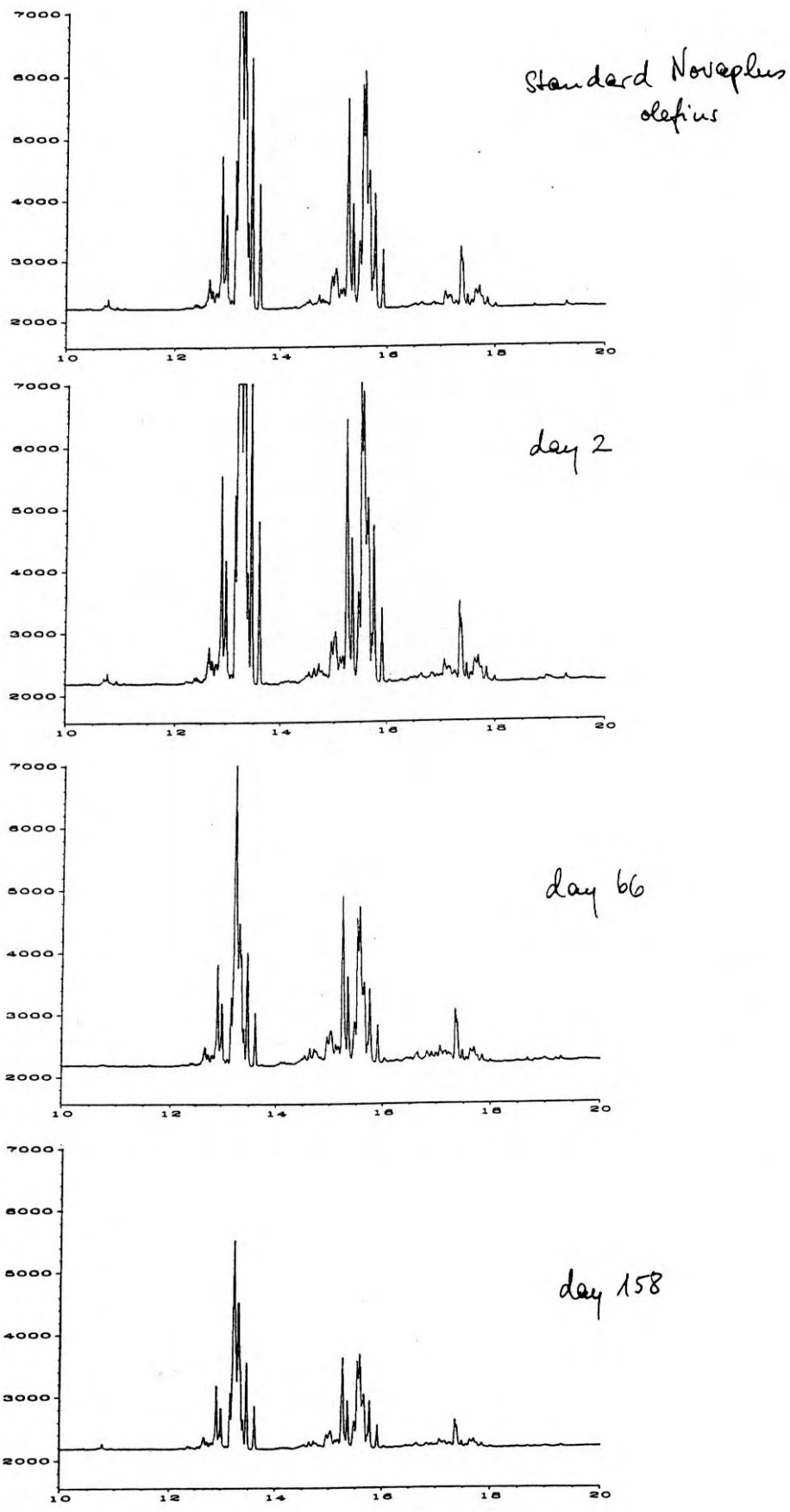

Figure 3.10 Gas chromatographic patterns of *Novaplus* base fluid (250 mg·kg^{-1})(top), and extracts of sediments sampled in NIO 11 on day 2, day 66 and day 158 (bottom).

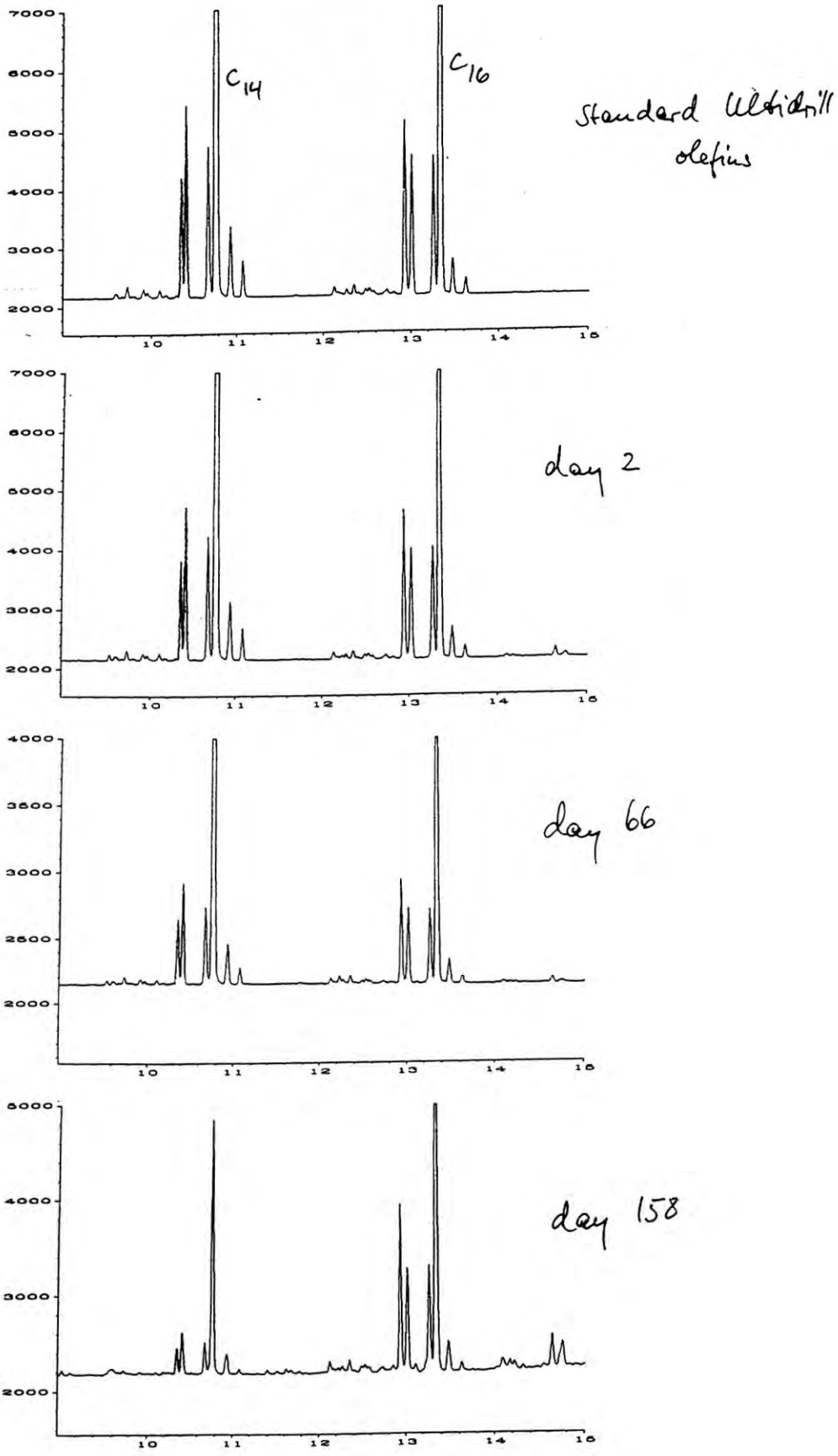

Figure 3.11 Gas chromatographic patterns of *Ultidrill* base fluid (250 mg·kg^{-1})(top), and extracts of sediments sampled in UTD 7 on day 2, day 66 and day 158 (bottom).

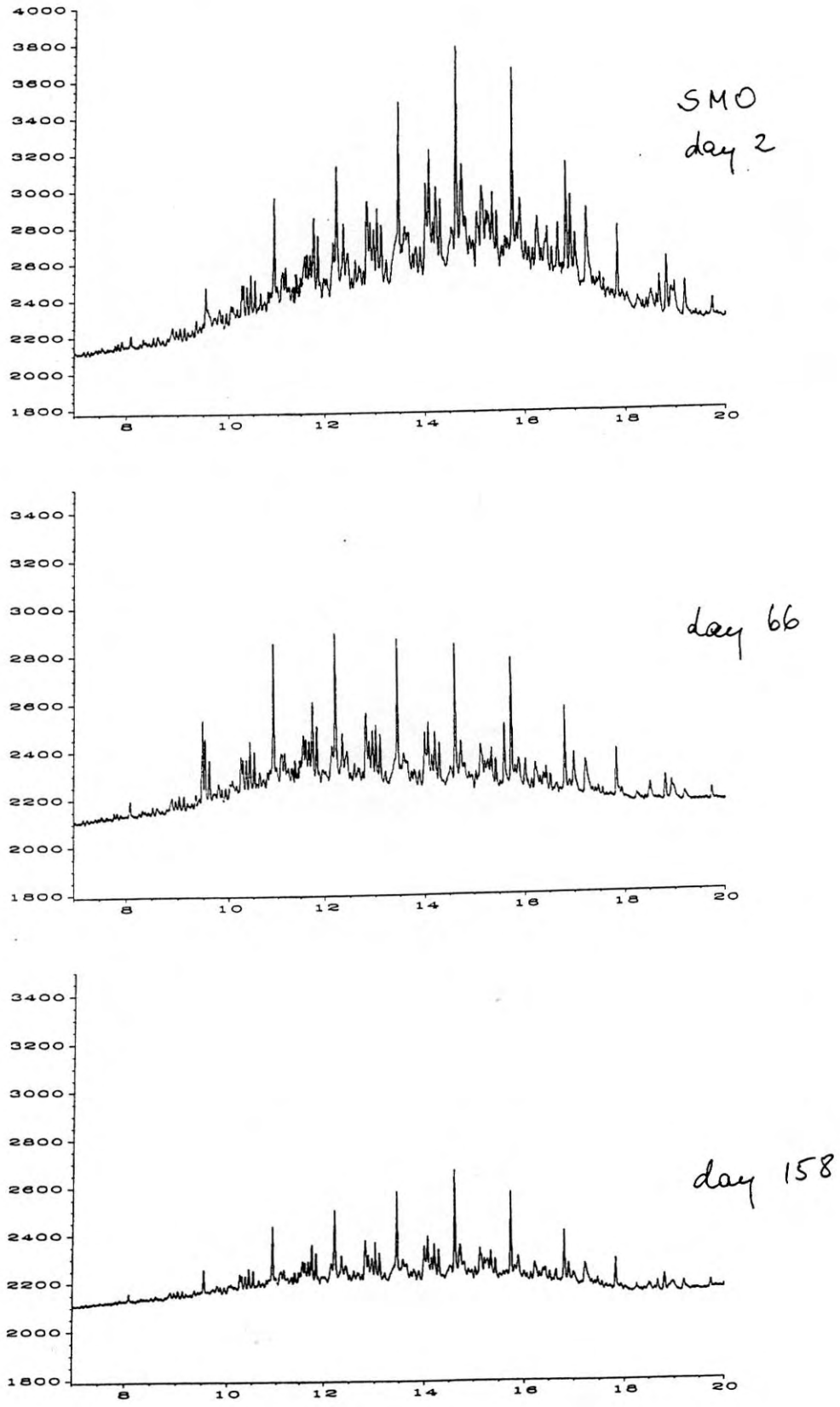

Figure 3.12 Gas chromatographic patterns of extracts of mineral oil in sediments sampled in SMO 1 on day 2 (top), day 66 (middle) and day 158 (bottom).

Petrofree esters

Figure 3.9 shows the gas chromatographic traces of *Petrofree* base fluid and sediment extracts from the PTF 9 chamber day 2, 66 and 158.

All (saturated) fatty acid ester components (C_8, C_{10}, C_{12}, C_{14} and C_{16}) showed approximately equal rates of disappearance. Because of their much higher concentrations in the original product, the C_{12} and C_{14} esters were still present at day 66.

Novaplus olefins

Figure 3.10 shows the gas chromatographic patterns of *Novaplus* base fluid and sediment extracts from day 2, day 66 and day 158. Even if the total concentration was significantly reduced, the relative abundance of each component in the isomeric mixture of C_{16},, C_{18} and C_{20} mono-olefins remained virtually unchanged over the experimental period. Thus, it may be concluded that all components had the same rate of disappearance.

Ultidrill olefins

Figure 3.11 shows the gas chromatographic patterns of *Ultidrill* base fluid and sediment extracts from day 2, day 66 and day 158. The two main component groups, tetradecenes (C_{14}) and hexadecenes (C_{16}), showed different rates of disappearance. The C_{14}-components were more rapidly removed compared to the C_{16}-homologs, a phenomenon likely caused by preferential biodegradation of the lower boiling fraction.

Safemul Mineral Oil

Figure 3.12 shows the gas chromatographic patterns of mineral oil extract from sediments collected in SMO 1 on day 2, day 66 and day 158. (*Safemul* base fluid was not available.)
Even if some qualitative differences between the three extracts did exist, the lack of preferential disappearance of n-alkanes compared to their iso-alkane isomers, confirmed that biodegradation of mineral oil is a slow process. Such preferential biodegradation has otherwise been well documented in many studies of the fate of petroleum hydrocarbons in marine sediments.

3.1.5 Biodegradation

Results of the sediment oxygen consumption (SOC) measurements are shown in Figure 3.13 and Figure 3.14. Figure 3.13 shows the variation of bi-weekly measured rates, whereas Figure 3.14 shows the development of the total accumulated oxygen consumption in all chambers. Table 3.5 shows a ranked list of the total oxygen consumption for the entire experimental period.

The highest rates and the largest cumulative oxygen consumption was observed in the two chambers treated with *Anco Green* esters (Table 3.5), and throughout most of the experimental period, the lowest rates were observed in the control chambers. Disregarding the anomalous low oxygen consumption in both *Petrofree* chambers, the mineral oil chambers consumed less oxygen than any other treatment. The four olefin treatments consumed intermediate amounts of oxygen, the *Novaplus* chambers slightly more than the *Ultidrill* chambers. As shown in Figure 3.13, whereas *Ultidrill*

Table 3.5 Total oxygen consumption (ranked) in each chamber during the 186 days experiment.

Chamber		$mmolO_2 \cdot m^{-2}$
CON	10	1 438
CON	4	1 863
SMO	1	2 166
PTF	9	2 518
SMO	12	2 579
PTF	6	2 935
UTD	7	2 999
UTD	3	3 032
NIO	11	3 111
NIO	5	3 399
ANC	8	3 623
ANC	2	3 708

showed higher rates during the first two months, the *Novaplus* chambers showed higher rates of oxygen consumption towards the end of the experimental period. Thus, observations of SOC gave no evidence to support the slow change of the olefin:barium ratio which was observed in NIO 11 towards the end of the experimental period (Figure 3.5).

In the control chambers, SOC decreased from initial rates of 400-600 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$ towards final rates of 200-400 $\mu molO_2 \cdot m^{-2} \cdot h^{-1}$. This more or less steady trend was interrupted by a temporary increase during the 90-120 days time interval. The increase occurred simultaneous to the anomalous influence of a more surficial water type which resulted in increased temperatures from 6.5°C to 11°C in source water and chambers (Figure 2.1). In previous experiments, characteristic rates of 100-300 $\mu mol \cdot m^{-2} \cdot h^{-1}$ has been observed in control chambers. The higher initial rates in the present experiment may have resulted partly from the fact that the samples were collected at a time of frequent sedimentation of phytoplankton spring blooms in the fjord, and partly as a result of the richer benthic communities. Furthermore, shortly before sediment transplantation, the extraordinary flooding of rivers in the South East Norway had carried large amounts of debris into the fjord environment. The sedimentation of this material may also have contributed to stimulated decomposer activities in the sampled sediments.

The relatively large difference between the two control chambers, was probably a result of the initial selection of deviating samples for control purposes. This was done to minimize experimental bias in treated chambers. Thus, CON 4 had a slightly tilting sediment surface and the largest volume of overlying water of all chambers. CON 10, on the other hand, had the smallest volume of overlying water. Furthermore, the analyses of the benthic fauna at the end of the experiment (Ch. 3.2.3), revealed “sub-normal” numbers of species and individuals in CON 10 which had the lowest SOC.

In the *Petrofree* chambers, oxygen consumption was high during the beginning of the experiment. However, the characteristic double peak, similar to those observed in the *Anco Green* chambers at about day 20 and day 50, and similar to those observed in several previous chambers treated with *Petrofree* esters, never occurred. On the contrary, 60-70 days after the addition of cuttings, the oxygen consumption declined to low rates in both *Petrofree* chambers. Thus, in Figure 3.14 the *Petrofree* curves can be seen crossing over the four olefin curves during the 60-120 days time interval. Whereas the PTF 6, retained more normal rates after day 100, the decline in PTF 9 appeared permanent, and towards the end of the experiment the cumulative SOC in PTF 9 was seen to cross over one of the mineral oil chambers as well.

The double peak of the oxygen consumption rates have frequently been observed simultaneous to the development of bacterial mats on the sediment surface. Probably, the first peak is dominated by the oxygen consumed by the bacterial community developing before the mats colonise the sediment surface. The mats will provide a physical barrier towards exchange of any compounds between the sediments and the overlying water. Thus a temporary decrease of oxygen consumption might be expected to occur shortly after mat formation. This first event may result in a clear peak, as was

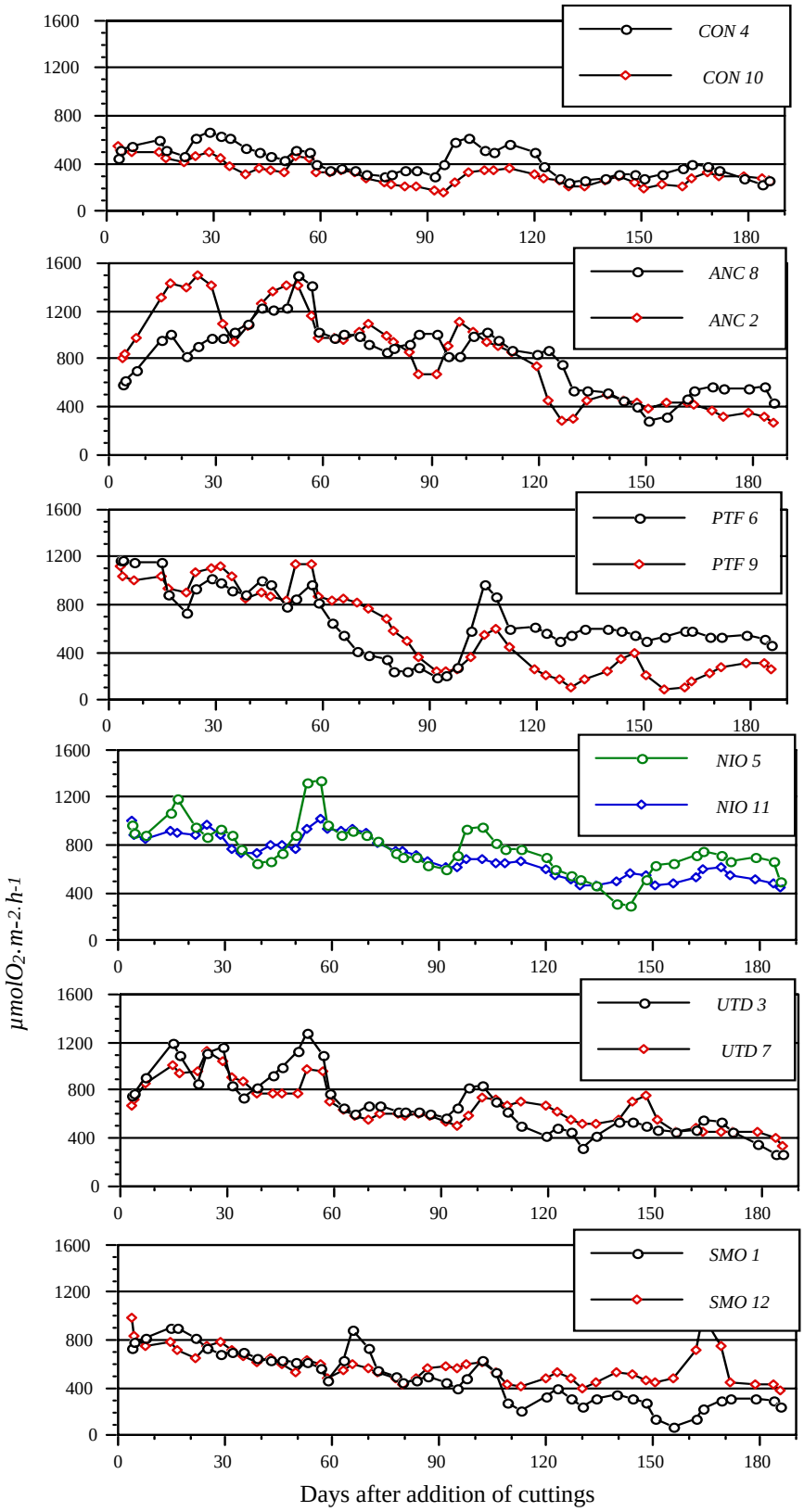

Figure 3.13. Variation during the test period of the rate of oxygen consumption in each chamber. Data were smoothed using a 3 point binomial function.

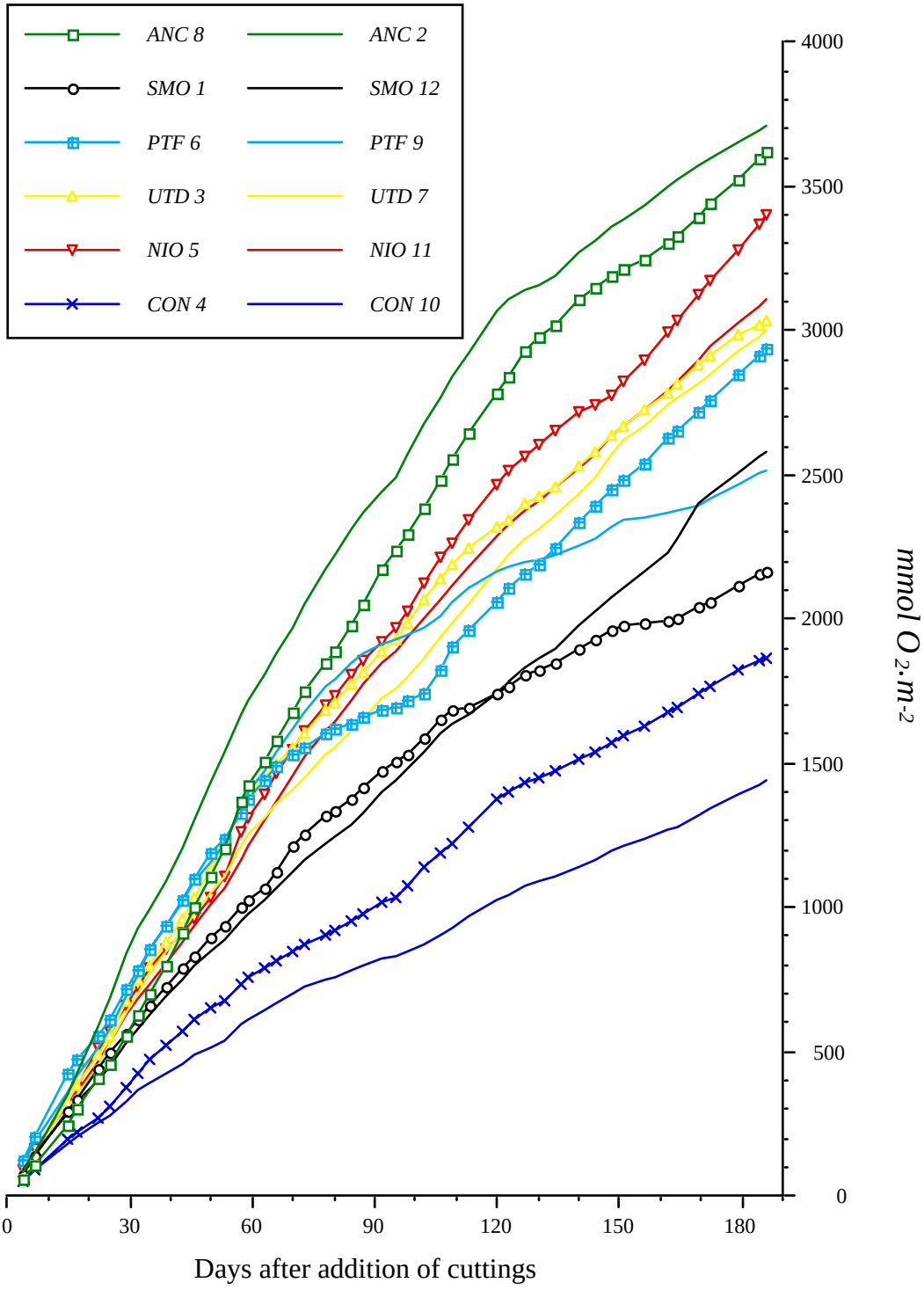

Figure 3.14. Cumulative sediment oxygen consumption in each chamber during the 186 days experimental period.

observed in ANC 8 (Figure 3.13) or a small kick-back as observed in ANC 2. The mats obtain their energy by mediation of the reaction between hydrogen sulphide produced by sulphate reducing bacteria (SRB) in the sediments, and oxygen supplied from the watermass. Thus, dependent on the availability of hydrogen sulphide, the mat community may consume large amounts of oxygen by mediation of the chemically spontaneous reaction between oxygen and hydrogen sulphide. During the decomposition event the highest SOC-rates have frequently been observed to occur during this second peak.

In order to dilute the *Petrofree* cuttings without altering the load of organic carbon, ignited sediment was added to the *Petrofree* chambers (Table 2.2). As indicated by the more reddish appearance of the sediment after ignition, the heating in the presence of atmospheric oxygen, had increased the content of ferric iron- and manganese(IV)-oxides in the ignited sediment. These minerals will react rapidly to consume hydrogen sulphide produced by the SRB´s, thus competing with the sulphide-demand of the mats. As described in ch. 3.2.1, in the *Petrofree* chambers the mats receded during the 36-65 days sampling interval. In the *Anco Green* chambers, however, the mats did not recede until after day 97. Thus the addition of ignited sediment to the *Petrofree* chambers may explain both the early disappearance of the mat communities and the disturbance of the characteristic pattern of oxygen consumption, in particular the absence of the previously observed large peaks occurring at about two months after the addition of cuttings.

3.1.6 Mass balance of the drilling fluids

In this section the mechanisms responsible for the observed loss of drilling fluids from the sediment between day 2 and day 186 shall be elucidated in a mass balance budget. Separate budgets for each chamber is shown in Table 3.6 in concentration units as well as normalised to the initial sediment concentration. Figure 3.15 shows budgets averaged on the two replicate chambers.

The primary input in the budget was the concentration of drilling fluids determined in samples of the 0-3 cm section of the sediments on day 2 and day 186. In Table 3.6 the difference between final and initial concentration was taken to represent the total loss over the period. In Figure 3.15 the total loss was calculated as the difference between mean initial concentration and final concentrations estimated from best fit regression curves (Table 3.3). For mineral oil the best fit was obtained by omitting two of the 14 concentration values (see text Ch.0 p.42).

The total loss was assumed to result partly from complete mineralisation of drilling fluid carbon to CO_2, which could be calculated from the accumulated excess oxygen consumption and the theoretical amount of oxygen required to oxidise an average drilling fluid carbon atom to CO_2, and partly from loss of drilling fluids via loss of cuttings particles, which was estimated from the relative decrease of the concentration of barium given by the slopes of the regression curves shown in Figure 3.2 to Figure 3.7. In the present mass balance, bioturbation was assumed to be the primary process responsible for loss of barium.

The deficit, which will represent an object of speculation, may result from errors and false assumptions or processes for which the data required for quantitation is absent or inadequate.

Table 3.6 revealed that whereas the esters were completely lost from the sediment, 6-21% of the olefins were still present during the final sampling. For mineral oil, the best estimate of the fraction remaining in the sediment was 29% (Figure 3.15). The GC-chromatograms (Figure 3.8 - Figure 3.12) confirmed, also qualitatively, the presence of olefins and mineral oil at the end of the experimental period. The remaining fraction of 6-14% of the *Ultidrill* olefins was reasonably consistent with the fraction of 3-10% found to remain present in the sediment in a previous test (Schaanning, 1995). Also the remaining mineral oil fraction of 29% (17-60%) confirmed the large remaining fractions of 45-84% of the added mineral oil reported by Schaanning and Laake, 1993 and Schaanning, 1994.

Complete mineralisation to CO_2 could account for 21-45 % of the added drilling fluids. Mineralised

Table 3.6. Mass balance of drilling fluids in each chamber (see text).

	ANC 2	ANC 8	PTF 6	PTF 9	NIO 5	NIO 11	UTD 3	UTD 7	SMO 1	SMO12
Units = mg DF·cm^{-2}										
In sediment day 186	.02	.01	.03	.03	.29	1.09	.56	.29	.37	1.12*
+ mineralised	2.22	2.12	1.38	.93	1.62	1.35	1.28	1.25	.47	.85
+ bioturbated	2.58	2.31	.00	-.18	.00	1.31	1.78	1.31	.77	.36
+ other loss	.91	2.20	2.89	3.41	-.10	.91	2.13	.97	.60	-.45
= in sediment day 2	5.72	6.64	4.30	4.19	3.59	4.51	3.97	3.76	2.21	1.87
Normalised										
In sediment day 186	0	0	1	1	8	21	14	6	17	60
+ mineralised	39	32	32	22	45	30	32	33	21	45
+ bioturbated	45	35	0	-4	50	29	0	35	35	19
+ other loss	16	33	67	82	-3	20	54	26	27	-24
= in sediment day 2	100	100	100	100	100	100	100	100	100	100

*concentration day 158

fraction of 32-45 % found in previous tests (cited above) with similar initial concentrations confirmed the present results on mineral oil, but 44-60% mineralisation of *Ultidrill* olefins (corresponding to 1.46-1.60 mgDF·cm^{-2} in 176 days) reported in Schaanning, 1995, was somewhat higher than the mineralisation rates (32-33%, 1.25-1.28 mgDF·cm^{-2}) observed in the present test.

With regard to bioturbation (barium loss) and "other loss" the previous test on *Ultidrill*l olefins gave quite similar results as the present test. Most of the previously tested chambers have, however, yielded much lower loss rates of barium (mean values 0% and 11% in two previous tests) than those observed in most of the present chambers (mean = 31% in chambers other than PTF). Probably, this was due to more numerous and larger individuals of benthic animals in the present test. No previous results exists on barium loss in chambers treated with mineral oil.

The mineralisation of no more than 0.93-1.38 mg·cm^{-2} of the *Petrofree* esters was low compared to previous results of 1.89 mg·cm^{-2} (or 45% of added esters) (Schaanning, 1994), and 2.44 mg·cm^{-2} (80% of added esters) (Schaanning, 1995). As indicated by visual appearance and redox potential measurements, the anoxic events have been similarly severe in all tests and it appeared not very likely that a collapse of an initially rich benthic community should result in a slow down of the activity of the sulphate reducing bacteria (SRB).

In previous tests the highest rates of oxygen consumption have been observed after the formation of sulphide oxidising bacteria mats on the sediment surface. In the present experiment, a moderately sized, but characteristically shaped, "post-mat" boom of oxygen consumption was observed in the *Anco Green* chambers during the 30-60 days period (Figure 3.13). These high rates have been maintained by microbial mediated reoxidation ($H_2S + O_2 = H_2SO_4$) of the hydrogen sulphide produced by the SRB´s below the biofilms. If, however, oxidised iron and manganese is abundant in the sediments a larger fraction of the hydrogen sulphide might become oxidised by mineral agents and trapped as ferrous sulphide (for example via $3/2H_2S + FeOOH = FeS + 1/2\ S_0 + 2H_2O$ and $H_2S + MnO_2 = S_0 + Mn^{2+} + 2OH^-$). Less hydrogen sulphide will be available and less oxygen can be consumed by the bacteria mats.

As shown in Table 2.2, approximately 100 g of ignited sediment was added to each of the *Petrofree* and control chambers, but to none of the others. The production of ferric and manganese oxides occurring at such temperatures (450°C) was indicated by a change from grey to a characteristic red colour, and rather than a post-mat boom, the oxygen consumption rates in the *Petrofree* chambers decreased after mat formation (after day 35, Figure 3.13). Thus, in the mass balance for the *Petrofree* chambers, the very large "other loss" of 67-82% was most probably a result of the addition of ignited sediment which had trapped much of the hydrogen sulphide produced by anaerobe mineralisation of the esters. It follows that if the ignited sediment had not been added the (true) mineralised fraction would have increased at the cost of "other loss".

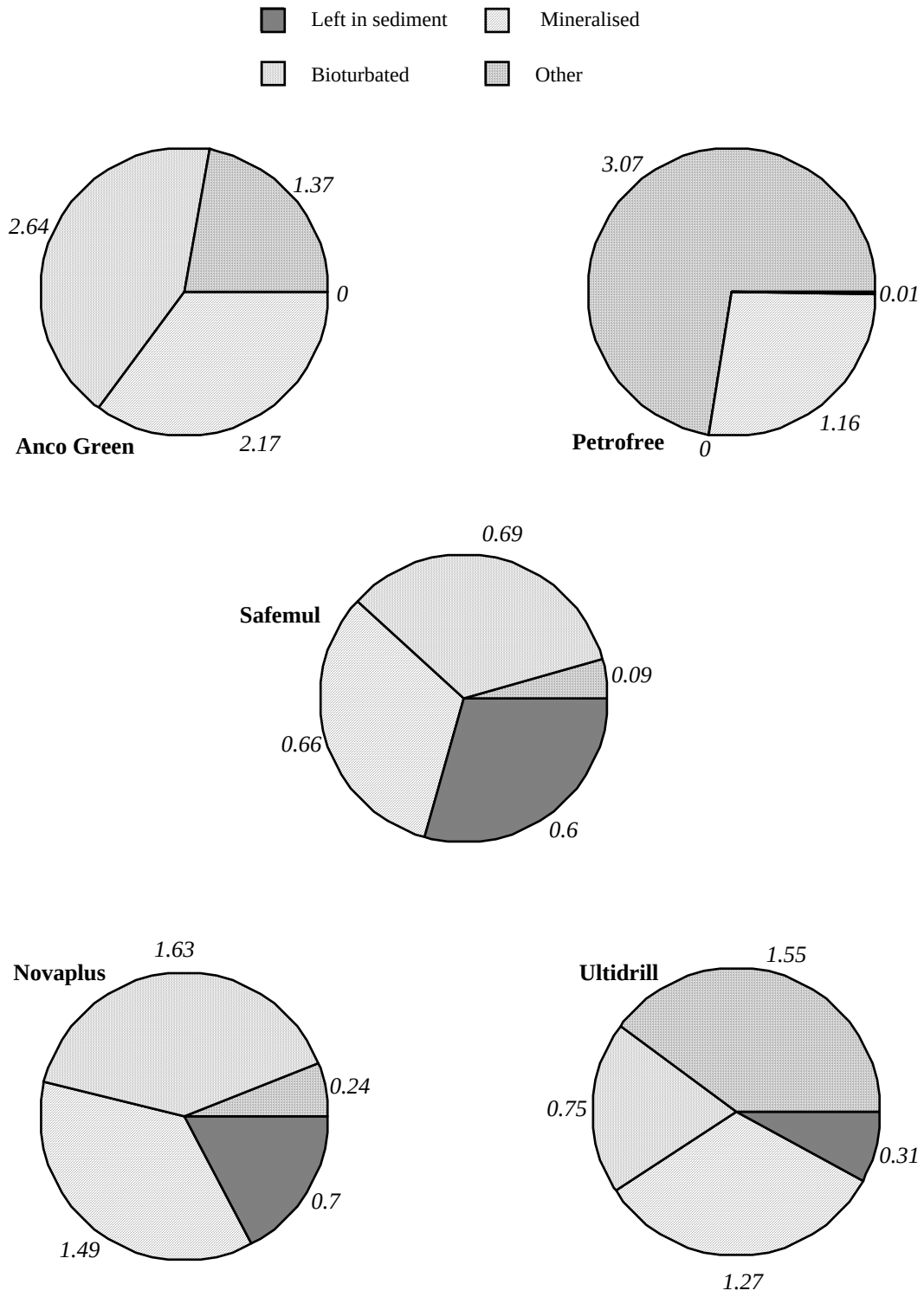

Figure 3.15 Mass balance of drilling fluids on cuttings added on day zero. Units = mg·cm^{-2}. (See text).

3.2 EFFECTS OF ADDITION OF CUTTINGS

3.2.1 Visual effects

Control chambers

The initially deposited sediment material was rapidly reworked by animal activities to produce a more patchy surface ranging from light grey to dark brown. The control chambers maintained this patchiness throughout the experimental period. Numerous tubes, wholes and mounds, were seen on the sediment surface. In both control chambers (and most of the treated chambers), stationary polychaetes survived in the same positions throughout the experiment. No dead polychaetes and no more than 4 dead bivalves were ever counted on the sediment surface in the control chambers. During the sampling interval between day 97 and 126 a dark coloured (almost black), microscopic organism settled on the sediment surface and walls of chambers and tubes in control and olefin treatments, but to a lesser extent in *Anco Green* and not at all in the *Petrofree* chambers.

Ester treatments

Inspection of the sediment surface revealed severe changes in the sediments treated with *Petrofree* esters. Thus, on day 35, continuous bacteria mats were observed to cover the sediment surface in both chambers and sulphur precipitates covered 50-75% of the surface areas. In each chamber two polychaetes and 20-30 small bivalves had perished beneath the mats. Mats and white sulphur precipitates were also observed in the two *Anco Green* chambers, but the mats were not continuous and the sulphur precipitates covered no more than 10% and 25% of the chamber areas in ANC 8 and ANC 2, respectively. Apart from five small bivalves in ANC 2, no dead animals were found in the two *Anco Green* chambers.

On day 65, sulphur precipitates had increased to cover 25% of the surface in ANC 8 and 60% of the surface in ANC 2, but the mats were not continuous and dead animals were scarce (4 dead bivalves in ANC 8). In the two *Petrofree* chambers, the white precipitates had disappeared and the mats had receded slightly. Sediments were described as evenly grey with several large black spots. No dead polychaetes were noted and the number of dead bivalves had decreased.

Then on day 97, respectively 5 and 11 large polychaetes and several smaller ones, were found on the sediment surface in the two *Petrofree* chambers, and several individuals were observed climbing in spider-like webs on the chamber walls. In the *Anco Green* chambers, no dead animals were noted and the visual appearance of the sediment surfaces had not changed much since the previous inspection.

On day 126, both *Petrofree* chambers were characterised by pale grey sediments and very few structures were observed to indicate presence of faunal activity. Several black sulphide images revealed polychaete remnants. On day 158 and 186, large rust coloured areas were observed in both *Petrofree* chambers.

In the *Anco Green* chambers, most of the precipitated sulphur had disappeared within day 126, and only small black and white patches were observed on the sediment surface. The surface appeared much more reworked by animal activity (wholes, mounds, tracks) than the *Petrofree* chambers. On day 158 a particular pattern of sulphur precipitates were observed at several locations in both *Anco Green* chambers. The structures were composed of one central colony surrounded by 6-8 satellite

colonies in a circle with a radius of 1-2 cm. The colonies were connected via tiny threadlike structures. Similar colonies have never been observed in any other treatments at Solbergstrand. The diverse appearance of the sediment surface was maintained in both *Anco Green* chambers until day 186.

Olefin treatments

The four chambers treated with olefins maintained a remarkably similar visual appearance throughout the experimental period. The general impression was that of powerful brown patches on a more greyish surface, high fauna activity and negligible mortalities of polychaetes and bivalves.

On day 35, the surfaces of the four olefin treatments were described as grey with numerous dark brown patches. The counts of 0-6 bivalve skeletons were not different from the numbers counted in the two control chambers. During the next month, some small yellow spots developed in both *Ultidrill* treatments, but none of the *Novaplus* treatments. On day 97, white precipitates were seen in all four chambers, but in much smaller amounts than in the ester treatments and in smaller amounts in the *Novaplus* than in the *Ultidrill* treatments. A few small black and white spots were observed in both treatments on day 126 and 158. By the time of the final sampling on day 186, thin grey biofilms were observed to cover approximately 30% of the sediment surface in UTD 3 and approximately 2% of the surface in UTD 7. A similar thin biofilm was observed over approximately 30% of the surface in NIO 5, but neither biofilms nor white sulphur precipitates could be seen in NIO 11.

Mineral oil

A few black areas occurred on the sediment surface 65-97 days after addition of cuttings and remained present throughout the experimental period. White, yellow or red spots were never observed. One dead polychaete and three small bivalves was observed in SMO 12 on day 35, and the same was observed in SMO 1 on day 65

3.2.2 pH and redox potentials

All measurements of pH and redox potentials are given in Table AI-I. Differences between the pH and redox potentials observed at each depth and time in the treated chambers and the corresponding observations in the control chambers are shown in Table 3.7 and Figure 3.16. Significant differences from control chambers at corresponding points of time were calculated by ANOVA analyses using the SYSTAT© statistical software (Tukey HSD multiple comparisons). Significance levels of 95% or better, are shown by bold characters in the two tables. The last rows show mean differences averaged over the entire experimental period.

pH

As shown in Figure 3.16, the pH variations in the control chambers were small and ranged between 7.5 and 7.8.

In the chambers treated with *Safemul* mineral oil, very high pH-values were observed two days after addition of cuttings. In several previous studies similar pH-deviations have been assigned to mineral buffer components added to the mud. The strong initial effect gradually dissipated and by the end of the experiment, the pH was not significantly different from the pH in the control sediment.

Table 3.7 Mean difference of pH between treated and control chambers (treated - control). Bold values show significant (p<0.05) difference from the respective control chamber (Systat© statistical software: one-factor ANOVA: Tukey HSD multiple comparison). (pH-units).

Day	Chamber	CON10	ANC2	ANC8	PTF6	PTF9	NIO5	NIO11	UTD3	UTD7	SMO1	SMO12
2	CON 10		0.18	0.18	0.07	0.28	0.05	0.04	0.17	0.07	**1.53**	**1.41**
"	CON 4	-0.05	0.13	0.13	0.02	0.23	0.00	-0.01	0.12	0.02	**1.48**	**1.36**
36	CON 10		**0.49**	0.42	**0.48**	**0.51**	0.29	0.16	0.27	0.13	**0.56**	**0.78**
"	CON 4	0.09	**0.58**	**0.50**	**0.57**	**0.60**	0.38	0.24	0.35	0.22	**0.65**	**0.87**
65	CON 10		**0.41**	**0.69**	**0.52**	**0.50**	0.10	0.35	-0.10	0.18	**0.47**	**0.73**
"	CON 4	0.01	**0.42**	**0.69**	**0.53**	**0.50**	0.11	0.36	-0.09	0.19	**0.47**	**0.74**
97	CON 10		0.16	0.36	0.24	0.33	**0.54**	0.04	0.21	0.35	0.27	0.38
"	CON 4	-0.08	0.08	0.28	0.17	0.25	**0.46**	-0.04	0.13	0.27	0.20	0.30
126	CON 10		0.11	0.16	0.16	0.30	0.13	0.12	0.30	0.07	0.17	0.26
"	CON 4	-0.01	0.10	0.15	0.15	0.29	0.12	0.11	0.29	0.07	0.16	0.25
158	CON 10		0.30	0.10	-0.02	**0.36**	0.11	0.10	0.07	0.08	0.24	0.31
"	CON 4	-0.05	0.25	0.06	-0.07	0.32	0.06	0.05	0.03	0.03	0.19	0.27
186	CON 10		0.03	-0.02	-0.25	0.06	0.08	-0.07	-0.15	-0.09	0.01	0.10
"	CON 4	0.04	0.07	0.02	-0.21	0.10	0.12	-0.03	-0.11	-0.05	0.05	0.14
all	CON 10		0.24	**0.27**	0.17	**0.33**	0.19	0.11	0.11	0.11	**0.46**	**0.57**
"	CON 4	-0.01	0.23	**0.26**	0.17	**0.33**	0.18	0.10	0.10	0.11	**0.46**	**0.56**

Table 3.8. Mean difference of redox potentials between treated and control chambers (treated - control). Bold values show significant (p<0.05) difference from the respective control chamber (Systat© statistical software: one-factor ANOVA: Tukey HSD multiple comparison). (Values in mV).

Day	Chamber	CON10	ANC2	ANC8	PTF6	PTF9	NIO5	NIO11	UTD3	UTD7	SMO1	SMO12
2	CON 10		38	11	71	-39	64	34	75	73	-14	10
"	CON 4	-63	-25	-52	8	**-103**	0	-29	12	10	-77	-54
36	CON 10		**-230**	**-230**	**-287**	**-262**	-12	-17	-101	-55	-97	-66
"	CON 4	-19	**-249**	**-249**	**-306**	**-281**	-32	-36	-120	-75	-116	-85
65	CON 10		**-228**	**-211**	**-228**	**-278**	16	17	3	-71	-28	11
"	CON 4	-44	**-272**	**-254**	**-272**	**-322**	-28	-27	-41	-115	-72	-32
97	CON 10		**-335**	**-311**	**-423**	**-399**	-86	-65	-158	-213	-47	-148
"	CON 4	-3	**-338**	**-314**	**-426**	**-402**	-89	-68	-161	-216	-50	-151
126	CON 10		**-301**	**-244**	**-255**	**-329**	-121	-107	**-247**	**-197**	-165	-2
"	CON 4	-22	**-322**	**-266**	**-277**	**-351**	-143	-129	**-269**	**-219**	-187	-23
158	CON 10		**-292**	-106	**-214**	**-276**	**-178**	-22	-105	-89	-89	-69
"	CON 4	-57	**-349**	**-163**	**-271**	**-333**	**-236**	-79	**-162**	**-146**	**-147**	-126
186	CON 10		**-167**	-112	**-151**	**-278**	**-163**	-15	-10	3	-13	-12
"	CON 4	-14	**-180**	**-126**	**-164**	**-292**	**-176**	-29	-24	-10	-27	-26
all	CON 10		**-216**	**-172**	**-212**	**-266**	-69	-25	-78	-78	-65	-39
"	CON 4	-32	**-248**	**-204**	**-244**	**-298**	-100	-57	**-109**	**-110**	-97	-71

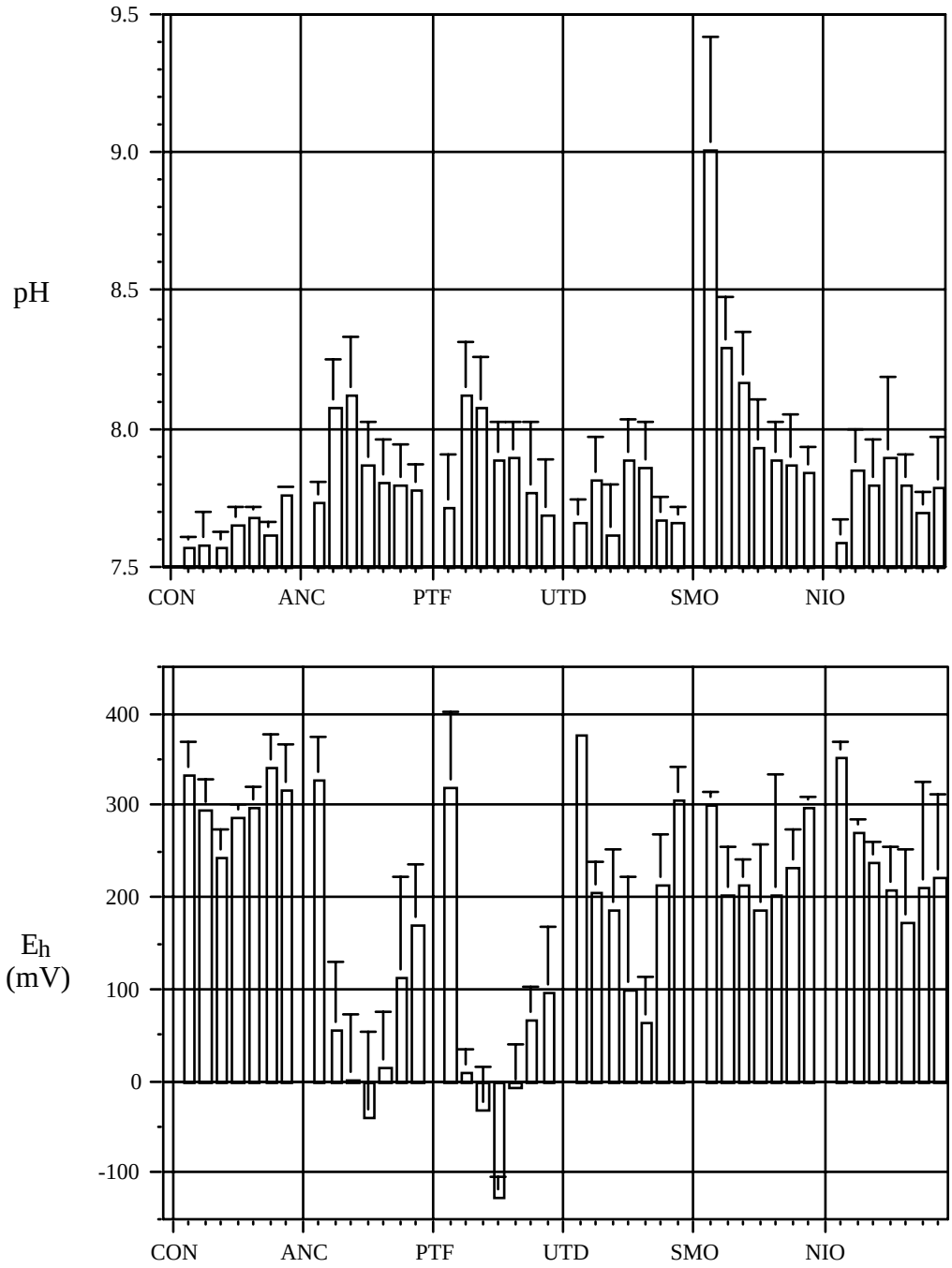

Figure 3.16. Variation with time of pH and Eh at 15 mm depth in the sediments in treated and control chambers. Each column represent mean of six recorded values in two replicate chambers. Vertical bar shows the magnitude of one standard deviation. First column after the grid line represent the observations taken two days after the treatment. The subsequent columns represent observations taken 1, 2, 3, 4, 5 and 6 months after the treatment.

In the chambers treated with esters, a small positive deviation was observed on day two. 1-2 months later, relatively high pH values were observed in all four chambers. The deviation decreased towards the end of the experiment.

Figure 3.16 revealed that also in the chambers treated with olefins, small positive deviations were frequently observed during the period 1-4 months after addition of the cuttings. However, in the olefin treatments the deviations were never beyond the normal range of pH found in most sublittoral, marine sediments.

The bottom lines in Table 3.7 showed that for the entire experimental period, all treated chambers had positive deviations from both control chambers, but the differences were significant only in ANC 8, PTF 9 and both mineral oil treatments.

E_h

On day two, similar redox potentials were observed in all chambers (Figure 3.16).

In control chambers, redox potentials decreased during the first two months of the experiment. Probably, this was a capping effect of the added particle layer which act to reduce the diffusive exchange of oxygen and metabolic products between the pore water and the continuously renewed chamber water. The maximum deviation was not larger than approximately 100 mV and after five months the pH in the control sediments were no longer significantly different from the initial values.

In all treated chambers, the decrease of the redox potential was larger than in the control chambers. The maximum decrease of E_h was observed in chambers treated with ester based drilling fluids. The deviations culminated three months after the addition of cuttings, and the lowest values were recorded in the chambers treated with the *Petrofree* ester.

In the chambers treated with olefin based fluids, a moderate lowering of the redox potentials were observed to culminate four months after addition of cuttings.

In the mineral oil treatment, the redox lowering event was less regular. However, throughout the 1-5 months time interval, the mean redox potentials were approximately 100 mV less than the potentials observed 0 and 6 months after addition of the cuttings.

For the entire experimental period, all treated chambers had a lower redox potential than any of the two control chambers. The mean redox deviation was, however, not significant at the 95% level in either of the four chambers treated with Mineral oil or *Novaplus* olefins. Compared to CON 4 but not to CON 10, the deviation of the mean redox potential was significant in both chambers treated with *Ultidrill* olefins. Finally, all chambers treated with esters obtained highly significant deviations from both control chambers.

3.2.3 Composition of benthic communities

By the end of the experiment, the benthic fauna from each chamber was collected by washing the sediments on a 1 mm mesh size sieve. The preserved samples were then sorted into main taxonomic groups and further identified to species level. In a few cases, identification was only done to a higher taxon. Below, those taxons will also be treated and referred to as species. Shannon-Wiener (H) (Shannon & Weaver 1963) and Hurlberts (ES_{100}) (Hurlbert 1971) diversity indexes were determined, as well as an index (AI) which shows the fraction of species sensitive to pollution (Rygg 1995a). Thus, a low value of AI shows that few sensitive species are present. Quality assessment relative to

Table 3.9 Number of species (S) and individuals (N), diversity (H og ES_{100}), index on indicator species (AI), and quality status. Because of the low number of individuals, ES_{100} could not be estimated in PTF 6 and PTF 9.

Chamber	S	N	H	ES_{100}	AI	Quality status	
ANC 2	14	283	2.20	11.12	5.92	**III**	Poor
ANC 8	35	809	2.31	13.66	6.87	**II**	Fair
PTF 6	6	32	1.90	-	4.24	**IV**	Bad
PTF 9	4	83	0.87	-	4.68	**IV**	Bad
NIO 5	30	647	2.70	13.89	7.22	**II**	Fair
NIO 11	36	588	3.33	19.71	7.68	**I**	Good
UTD 3	26	338	3.16	16.44	7.23	**II**	Fair
UTD 7	22	308	2.88	14.33	7.11	**II**	Fair
SMO 1	18	226	2.51	13.65	6.32	**II**	Fair
SMO 12	20	309	2.51	13.18	7.35	**II**	Fair
CON 4	39	856	2.97	17.74	7.20	**II**	Fair
CON 10	36	281	3.65	22.94	7.47	**I**	Good

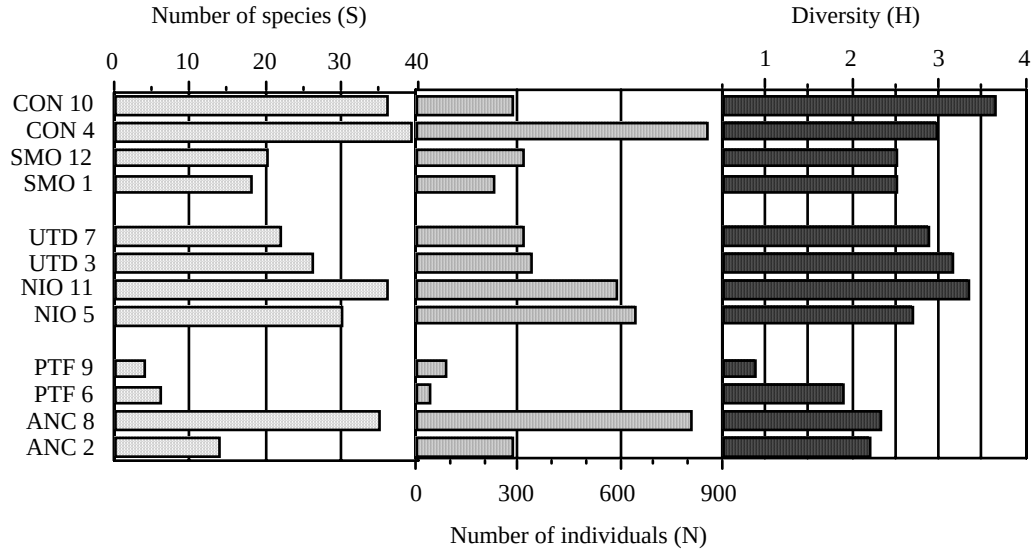

Figure 3.17 The macrofauna community in each chamber at the end of the experimental period.

normal status in field situations in fjords was derived from diversity classification (Rygg & Thélin 1993), species composition, number of species and number of individuals.

Complete taxonomic lists are given in Appendix (Table A1-6). A total number of 60 different species were identified. As shown in Table 3.9, each chamber contained up to 39 species and 856 individuals. The polychaete *Heteromastus filiformis* was particularly abundant and could frequently account for approximately 50% of the total number of individuals. In the two *Petrofree* chambers, however, the

Table 3.10 Abundance of the most common species in each chamber at the end of the experimental period.

	ANC	ANC	PTF	PTF	NIO	NIO	UTD	UTD	SMO	SMO	CON	CON
Species/Taxon												
Nemertinea indet	14	29	4	7	28	37	24	13	26	20	94	14
Paramphinome jeffreysii	41	44	14	69	51	56	48	37	27	29	81	27
Prionospio cirrifera		7			1	1		1		1	53	32
Chaetozone setosa	5	20			28	13	6	8	5	4	35	7
Heteromastus filiformis	165	513	11	6	332	248	126	128	119	166	419	99
Thyasira equalis	17	45			49	49	42	42	13	30	12	26
Thyasira obsoleta	4	6			11	5	14	4	4	3	10	5
Thyasira pygmaea	12	39			54	38	13	24	6	17	15	17
Thyasira sarsi	13	46	1		47	21	19	26	9	17	7	2

pollution tolerant polychaete *Paramphinome jeffreysii* was the most abundant species, and the only species which did not suffer a severe decline in the *Petrofree* chambers (Table 3.11). Several groups which were common in the other chambers were not found. Thus, neither bivalves (mostly *Thyasira* spp.) nor the polychaete *Chaetozone setosa* were observed in the *Petrofree* samples. The polychate *Prionospio* were reduced in numbers in all treatments compared to the controls (Table 3.11). Species of *Prionospio* (especially *P. cirrifera*) are known to disappear when oxygen is low (Rygg 1981; 1995b).

Both control chambers had high number of species (Figure 3.17), but the number of individuals was much lower in CON 10 than in CON 4. The reason for this difference is not clear. Analyses of carbon and nitrogen (Table 3.2) gave no evidence of a different food availability in the two chambers and the difference in particle size (Ch. 2.1, Table 2.1) was negligible. However, ever since the first week of the experiment, oxygen consumption rates in CON 4 were higher than in CON 10. Also, the different penetration of the box core sampler may have resulted from different types of sediments at the fjord location. Thus the difference between the two control chambers at the end of the experiment was probably an inherent effect of an initial difference.

Effects of the treatments were most obvious in the two *Petrofree* chambers. Species numbers were very low and the fact that the Shannon-Wiener diversity index was as high as 1.90 in PTF 6 should be considered an artefact of the extremely low number of individuals.

The fauna in the two chambers treated with *Anco Green* esters was less affected than the fauna in the *Petrofree* treatment. In ANC 8, number of species and number of indivduals were both high. In ANC 2, both numbers were low. Thus the diversities were similar in the two chambers and low as compared to the diversities found in the control chambers. It appeared that in ANC 8, the community had not suffered from extensive bacterial mats covering the sediment. Up to 25% of the sediment area was covered in ANC 8, while as much as 60% of the sediment was covered by bacterial mats in ANC 2.

The least severe effects on the benthic communities were observed in the *Novaplus* treatments. As compared to the *Ultidrill* chambers, the *Novaplus* chambers had higher numbers of species and individuals. The diversities in the four olefin chambers were fairly similar. Two of the chambers (one from each treatment) had Shannon-Wiener indexes within the range of the control chambers (Table 3.9). Only NIO 11 had a value of the ES_{100} index within the range of this index in control chambers. Finally, as indicated by the AI index, pollution sensitive species were abundant in both *Novaplus* treatments.

The two chambers treated with mineral oil were consistent with regard to low numbers of species and diversity indexes as compared to control and olefin treatments. However, the abundance of pollution sensitive species was clearly lowered in SMO 1, only. Thus, the effects on benthic communities of this low-aromatic mineral oil appeared to be intermediate between the moderate effects of the linear olefins and the larger effects of the esters.

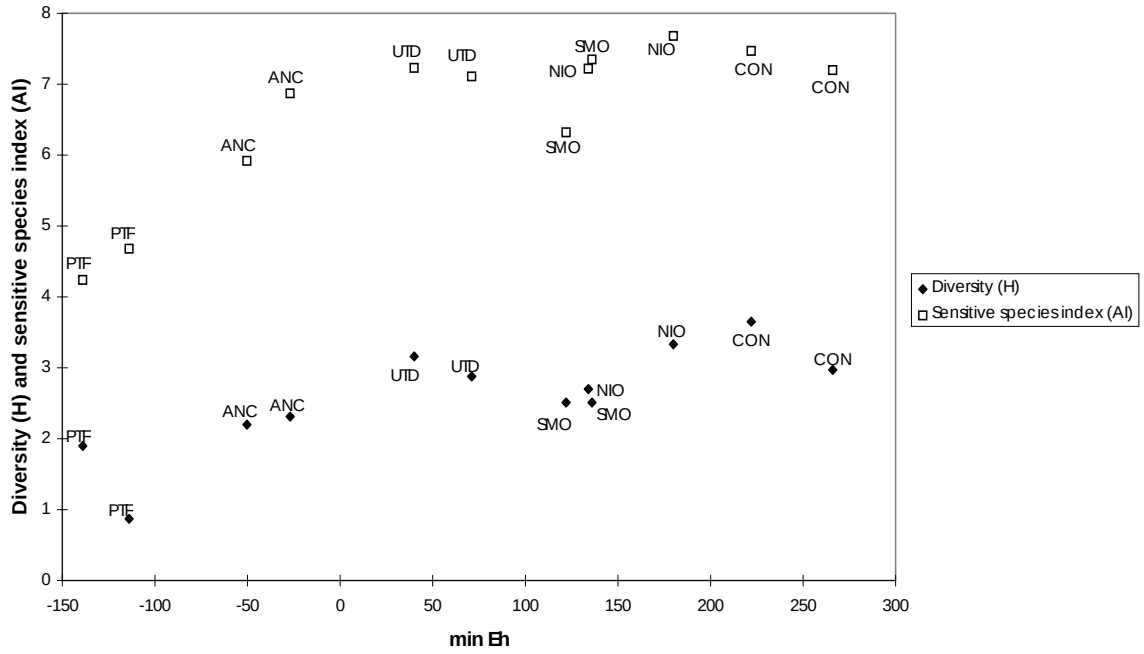

Figure 3.18 Plot of H and AI vs. lowest sediment E_h (min E_h) during the test period

The effects on the benthic communities were probably caused by redox deviations and presence of hydrogen sulphide in the sediment, and feeding interference and lack of oxygen supply from the water caused by bacterial mats. Probably, minimum redox values during the test period caused acute mortality. In Figure 3.18, the diversity index H and the sensitive species index AI are plotted vs. minimum redox values (E_h). The *Petrofree* treatments (PTF) had the lowest E_h values and showed also the lowest H and AI values. The *Anco Green* treatments (ANC) showed higher E_h, H and AI values than *Petrofree*, but lower values than the rest of the treatments and the controls (except the AI value of one of the mineral oil (SMO) treatments).

3.2.4 Bioaccumulation of drilling fluid in polychaete

Survival and growth of test organism

40 individuals of the polychaete *Hediste (Nereis) diversicolor* were added to each chamber at the start of the experiment. Of these, 0-12 individuals were recovered at the end of the experimental period (Table 3.11).

In the two chambers treated with *Petrofree* ester, there were no surviving *Hediste* (or other large polychaetes). Obviously, this was a result of the severe redox conditions in this treatment (Figure 3.16).

In the other treatments, the number and biomass of individuals surviving was rather variable and the variation within replicate chambers were no less than the variation between different treatments. There were numerous carnivores present in the sediments at the end of the study (*Nephthys ciliata*, *Eteone*, *Nereimyra* and others) and it is probable that the missing *Hediste* fell prey to one or more of these.

In a previous experiment (Schaanning, 1995), positive correlation was found between sediment oxygen consumption and body weight of juvenile *Hediste (Nereis)* inherent in the test communities. As shown from the data given in Table 3.11, no such correlation was found in the present test, probably because of the much larger individuals of the added test organisms.

Table 3.11 Number of individuals of the polychaete *Nereis diversicolor* found in each chamber by the end of the experiment.

Chamber	number of individuals	mean body size (mg wet wght.)
CON 4	10	198
CON 10	3	282
ANC 2	8	257
ANC 8	9	348
PTF 6	0	-
PTF 9	0	-
UTD 3	4	206
UTD 7	10	220
SMO 1	2	153
SMO 12	4	256
NIO 5	2	175
NIO 11	12	261

Anco Green esters

Figure 3.19 shows the gas chromatographic traces of the *Anco Green* ester base fluid, an extract of sediment collected at the end of the experiment (day 158) and the "ester fraction" extracted from polychaetes sampled in the *Anco Green* chambers (day 160). The results from both quantitative and qualitative analyses show that *Anco Green* ester had been completely removed from the sediment at the end of the experiment. Thus, it was not likely to find *Anco Green* bioaccumulated in polychaetes. However, a relatively large amount of components were found in the "ester fraction" extracted from the polychaete samples. These components were different from those found in the base fluid and, since they also occurred in the polychaetes sampled from control sediments, they were believed to be natural components of the biological tissue.

Petrofree esters

No sample material was found in the sediments after termination of the experiment.

Novaplus olefins

Figure 3.20 shows the corresponding gas chromatographic patterns of *Novaplus* olefins (base fluid, sediment extract day 158 and the “non-polar” fraction extracted from polychaetes sampled in the *Novaplus* chambers). Comparison of the three samples revealed that the relative abundance of the single components in the groups of C_{16}-, C_{18}- and C_{20}-olefins had remained mainly unchanged. The concentrations of *Novaplus* olefins in the three polychaete samples were calculated to 2.37, 34.1 and 49.5 $mg \cdot kg^{-1}$ wet wght. (0.00 $mg \cdot kg^{-1}$ in control) (Table 3.12).

Table 3.12. Concentrations of drilling fluids and barium in the polychaete *Hediste diversicolor* after six months exposure to sediments treated with cuttings contaminated with, respectively, *Ultidrill* (UTD), *Novaplus* (NIO) and *Safemul* (SMO) drilling muds.

Sample	DF $mg \cdot l^{-1}$	Ba	DF:Ba
UTD1-1	7.77	21	0.37
UTD2-1	3.56	32	0.11
UTD2-2	6.26	76	0.08
NIO1-1	2.37	<3	>0.79
NIO2-1	34.1	334	0.10
NIO2-2	49.5	203	0.24
SMO1-1	2.98	<3	>0.99
SMO2-1	5.11	<3	>1.70
SMO2-2	4.16	<3	>1.39
CON1-1	0.00	<3	0.00
CON1-2	0.00	<3	0.00
CON2-1	0.00	<3	0.00

The concentrations of barium of <3 (detection limit), 334 and 203 $mg \cdot kg^{-1}$, yielded olefin:barium ratios of >0.79, 0.10 and 0.24. The similarity between the two lowest ratios in the polychaetes and the corresponding ratios of 0.14 and 0.39 observed in simultaneously sampled sediments was a strong indication that most of the *Novaplus* olefins observed in the polychaetes were associated with sediment particles which had not been properly removed by the applied cleaning procedures. The ratio in the first polychaete sample was, however, larger than the ratios observed in all sedimentsamples collected more than 35 days after addition of the cuttings. This result suggested some preferential uptake of *Novaplus* olefins in the polychaetes.

Ultidrill olefins

The gas chromatographic patterns of *Ultidrill* base fluid, a sediment extract from day 158 and the "non-polar" fraction extracted from polychaetes collected in *Ultidrill* chambers, are shown in Figure 3.21. Comparison of the three samples revealed some remarkable differences. In the base fluid, the C_{14}-olefins were present at higher concentration than the C_{16}-olefins. In the sediment at the end of the experiment (day 158), the C_{14}-olefins were considerably less abundant than the C_{16}-components. This was most likely due to preferential biodegradation of the lower molecular weight fraction in the sediment.

Surprisingly, in the polychaetes the distribution of the components was again reversed. The C_{14}-olefins were again the dominating group. One possible explanation might be the preferential bioaccumulation of the lower molecular fraction. The component marked * might give an indication of such a process.

Both in sediment and polychaetes the loss of main components (straight chain C_{14} and C_{16}-olefins) had been large compared to the loss of associated components, which were tentatively identified as branched C_{14}- and C_{16}-olefins.

The concentrations of *Ultidrill* olefins in the three polychaete samples were calculated to 7.77, 3.56 and 6.26 $mg \cdot kg^{-1}$ wet weight. (Control = 0.00 $mg \cdot kg^{-1}$) (Table 3.12). The corresponding olefin:barium ratios were 0.37, 0.11 and 0.08 as compared to the range of 0.08-0.19 observed in the four sediment samples collected during the last two surveys (day 158 and 187).

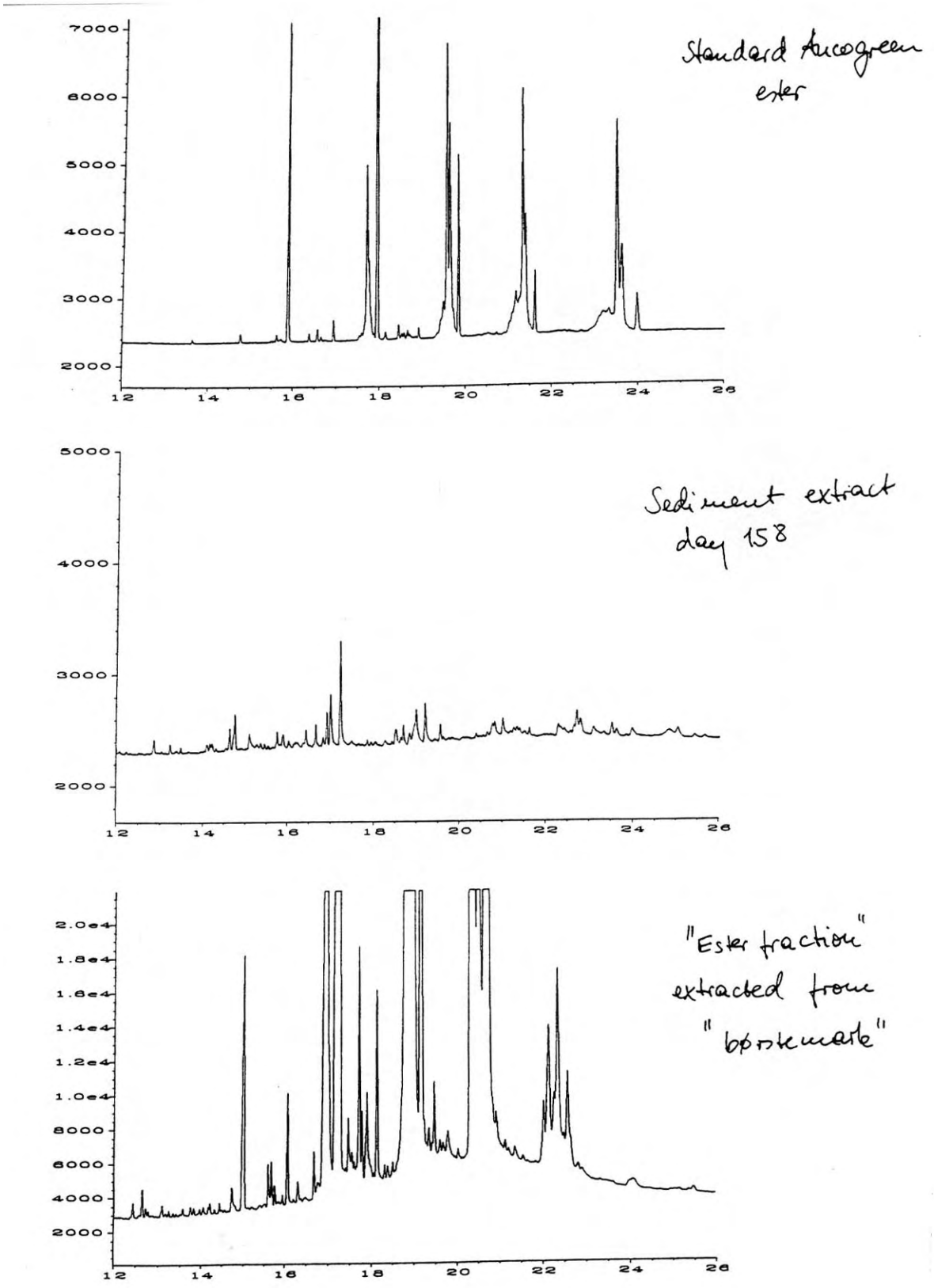

Figure 3.19 Gas chromatographic patterns of *Anco Green* base fluid (top), sediment extracts from ANC 8 day 158 (middle) and the "Ester fraction" extracted from polychaetes sampled in ANC chambers at the end of the experimental period (bottom).

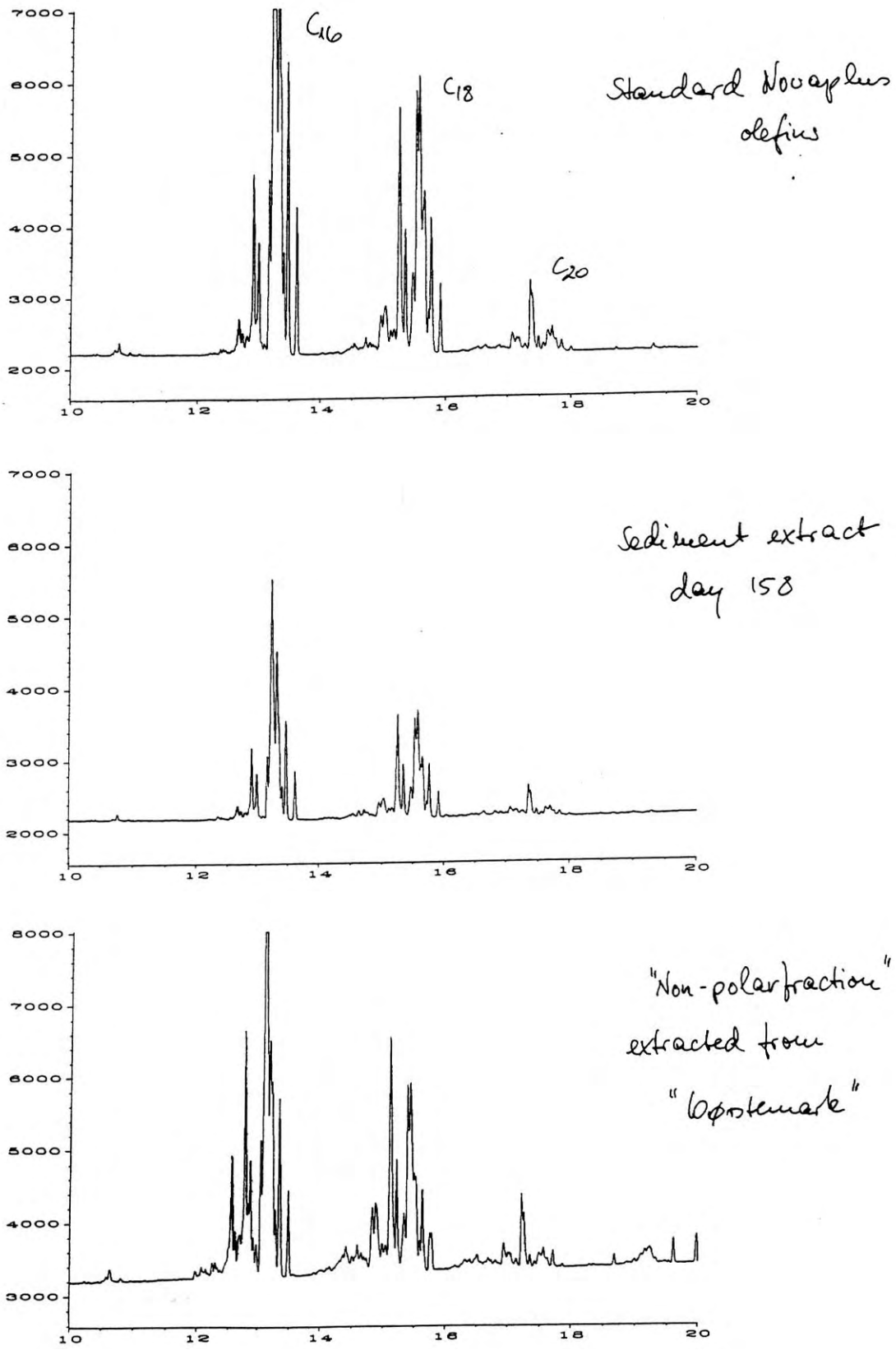

Figure 3.20 Gas chromatographic patterns of *Novaplus* base fluid (top), sediment extracts from NIO 11 day 158 (middle) and the "Non-polar fraction" extracted from polychaetes sampled in NIO chambers at the end of the experimental period (bottom).

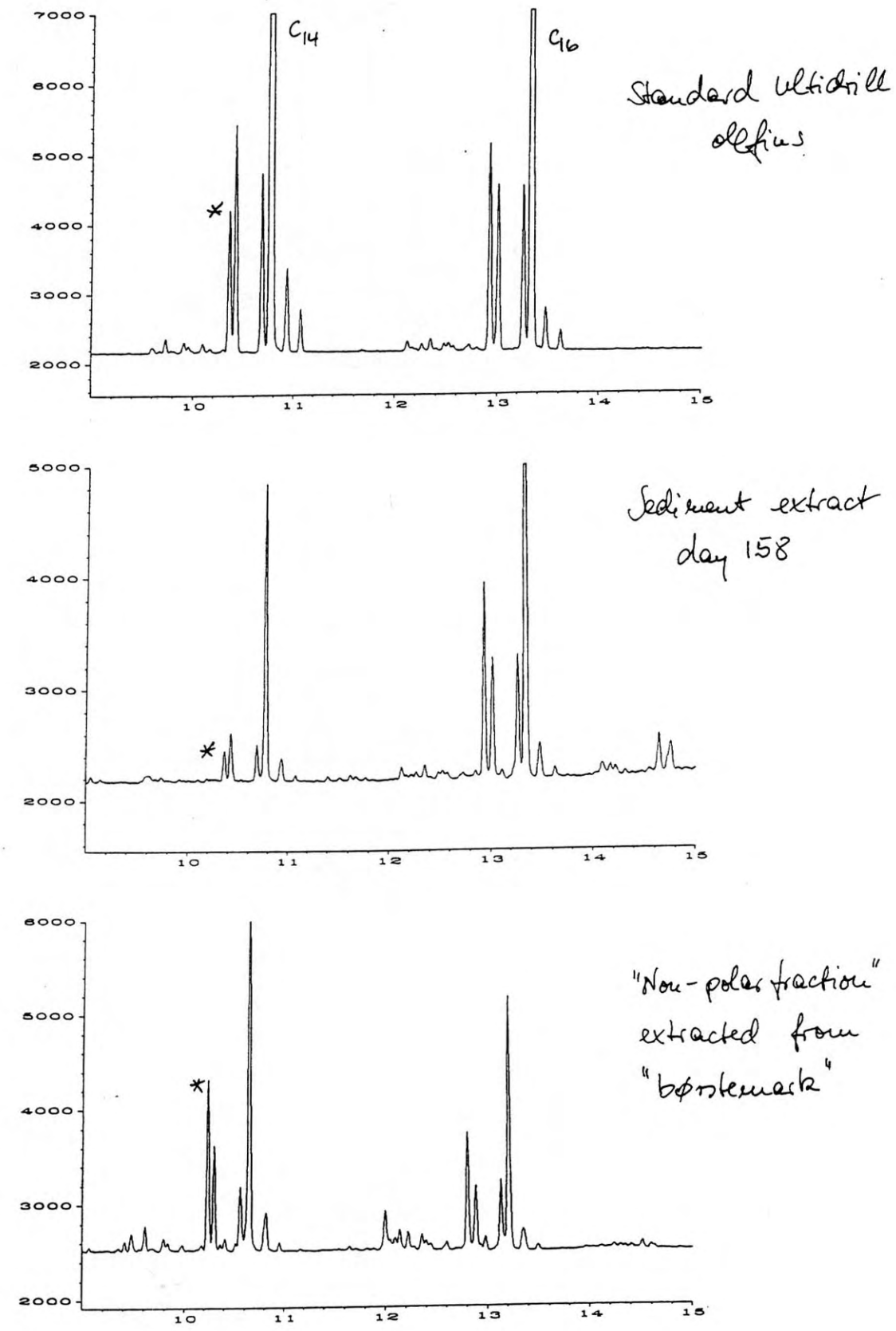

Figure 3.21 Gas chromatographic patterns of *Ultidrill* base fluid (top), sediment extracts from UTD 7 day 158 (middle) and the "Non-polar fraction" extracted from polychaetes sampled in UTD chambers at the end of the experimental period (bottom).

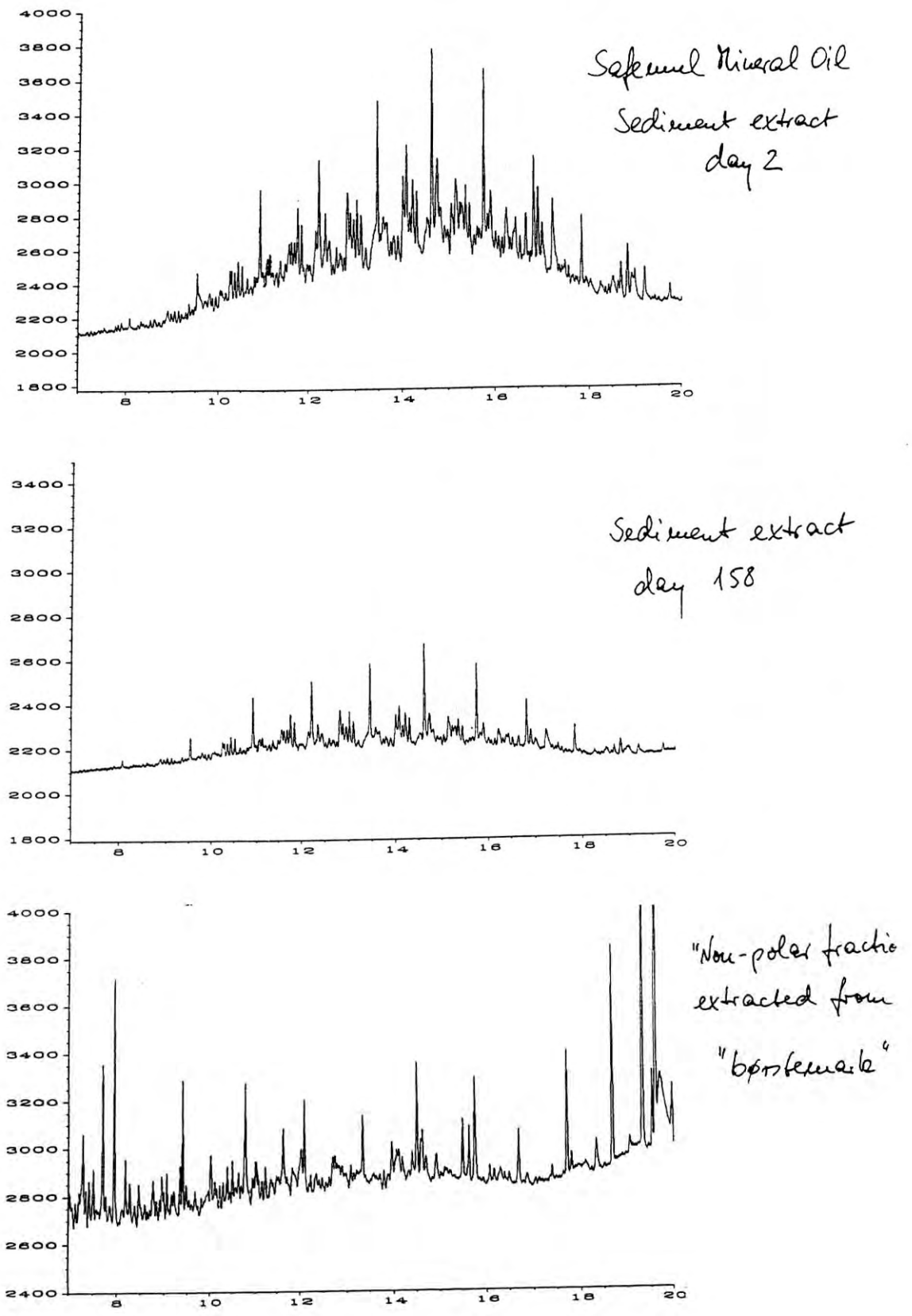

Figure 3.22 Gas chromatographic patterns of sediment extracts from SMO 1 day 2 (top) and day 158 (middle) and the "Non-polar fraction" extracted from polychaetes sampled in SMO chambers at the end of the experimental period (bottom).

Thus, even if barium is assumed unavailable for uptake, indications on bioaccumulation of *Novaplus* and *Ultidrill* olefins in the polychaete *Hediste diversicolor* was found in one of the three samples collected from each treatment. If some of the barium observed in the polychaete was bioaccumulated and not only present in sediment particles present in the gut or in between body appendages, all six samples might have been interpreted as bioaccumulation of olefins.

Safemul mineral oil

Figure 3.22 shows the gas chromatographic patterns of sediment extracts (day 2 and day 158) as well as the "non-polar" fraction extracted from a polychaete sample from the SMO chambers (day 160). Compared to animals from control sediments, a significantly higher level of petroleum hydrocarbons was found. The component distribution differed, however, considerably from the component distribution in sediment samples. The total petroleum hydrocarbon content in the three polychaete samples was calculated to 2.98, 5.11 and 4.16 $mg \cdot kg^{-1}$ wet weight. (Control = 0.00 $mg \cdot kg^{-1}$) (Table 3.12).

The concentration of barium was less than 3 $mg \cdot kg^{-1}$, in all samples of polychaetes from *Safemul* and control chambers. The corresponding hydrocarbon:barium ratios of at least 0.99, 1.78 and 1.39 were significantly larger than the mean ratio of 0.65 (standard deviation = 0.27) for the ten sediment samples collected after day 35. This was consistent with several reports on bioaccumulation of petroleum hydrocarbons in fish (Payne et al, 1989, Barron, 1990 and others).

3.2.5 Biomarker responses in *Hediste diversicolor*

Glutathione reductase is the enzyme that will catalyse the "regeneration" of oxidised glutathione. The enzyme is regulated by the intracellular levels of glutathione and may thus be viewed as an "integrator" of intracellular glutathione-availability. One of the most important roles of the tripeptide glutathione within the cell is radical scavenging (i.e. protection against cellular damage caused by radical generation). Levels of the enzyme were found to be significantly elevated in *Hediste* kept in chambers treated with *Anco Green* (Figure 3.23), which were the treatments with the highest cumulative oxygen consumption. However, polychaetes kept in chambers with *Anco Green* were also larger than *Hediste* found in the other chambers and there was also a weak relationship between size and glutathione reductase activity (Figure 3.24).

Catalase is an enzyme that will catalyse the breakdown of peroxide into molecular oxygen and water. The enzyme is inducible by the presence of oxyradicals and may thus be elevated under situations when there is oxidative stress. This enzyme was significantly elevated in all mud-exposed groups except the group kept in SMO (Figure 3.25) and there was a clear relationship between elevated activities of the enzyme and a general measure for low-oxygen stress such as total oxygen consumption summed over monthly means (Figure 3.26).

Biomarker-responses indicate that the polychaetes kept in ANC-, UTD- and NIO-treated sediments were experiencing increased oxidative stress. Conceivably, the opposite should be the case, since those treatments offered decreased ambient oxygen availability. A situation of decreased oxygen could however affect the cellular antioxidant defence systems in at least two ways that would explain these observations; (i) changed cellular metabolism (cf. Schöttler et al. 1984) causing increased generation of reactive metabolites, (ii) increased breakdown of tissue, either in response to low food-availability, low oxygen availability or a combination of the two. In mammalian systems, it has been found that elevations in antioxidant enzymes commonly accompany tissue damage or inflammatory processes. Corroborating the results found in this study, Abele-Oeschger et al. (1994) observed induction of catalase in *Hediste* kept under anoxic conditions for 6 h. Peroxide in itself (the substrate for Catalase) may also be generated in marine sediments, but predominantly in normoxic or hyperoxic intertidal sediments.

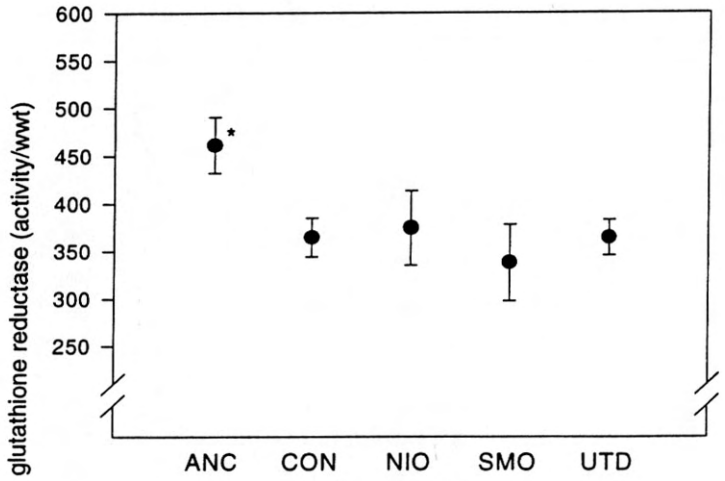

Figure 3.23 Glutathione reductase activity in *Hediste diversicolor* from different treatments (three pools analysed from each treatment, mean ± standard error); *significantly different from control (ANOVA, Dunnett`s test, p<0.05).

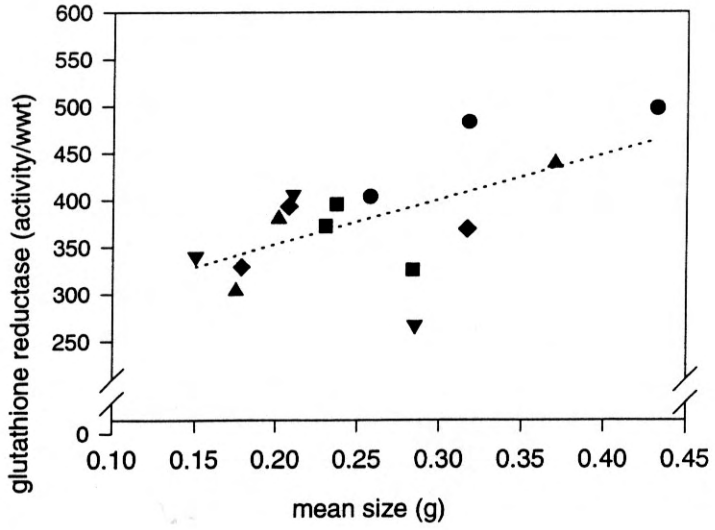

Figure 3.24 Relationship between the mean body size of *Hediste diversicolor* and the activity of glutathione reductase; simple linear regression with ANOVA: $r^2 = 0.35$, $p = 0.02$.

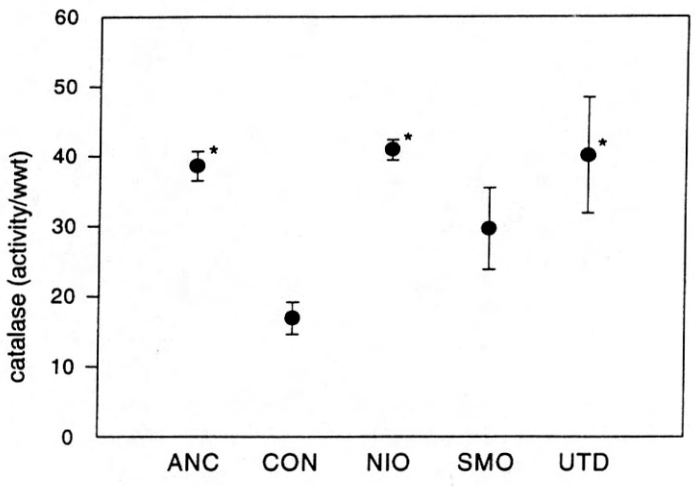

Figure 3.25 Catalase activity in *Hediste diversicolor* from different treatments (three pools analysed from each treatment, mean ± standard error); *significantly different from control (ANOVA, Dunnett´s test, p<0.05).

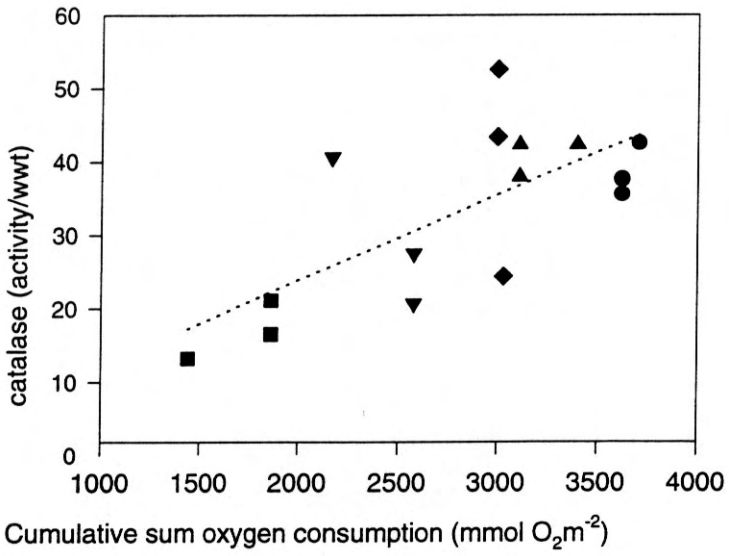

Figure 3.26 Relationship between the mean size of *Hediste diversicolor* in different pools and the activity of catalase; simple linear regression with ANOVA: $r^2 = 0.49$, $p = 0.004$.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 ALL TREATMENTS

- The use of undisturbed box-core samples represented a major improvement of test set-up with regard to simulation of *in situ* seabed conditions.
- By the end of the six months experimental period, the macrofauna communities in both control and seven treated chambers were classified, according to environmental quality criteria for fjords and coastal environments, as *fair* or *good*.
- Three chambers treated with esters were classified as *poor* or *bad*.
- Significant loss of barium in all but the Petrofree treatment, showed that bioturbation was larger in the present as compared to previous tests at Solbergstrand.
- The responses of the two biomarkers glutathione reductase and catalase indicated that a sediment dwelling polychaete exposed for six months in chambers treated with *Anco Green*, *Ultidrill* and *Novaplus* were negatively affected by these treatments, whereas no significant effects were found in chambers treated with *Safemul* mineral oil. (In *Petrofree* chambers, the polychaete did not survive to yield sufficient amount of sample material).

4.2 ESTER TREATMENTS

- The *Anco Green* ester was identified as a mixture of isopropyl esters of saturated and unsaturated fatty acids. The chain length of the fatty acids varied from C_{14} over C_{16},, C_{18} and C_{20} up to C_{22}.
- The unsaturated fatty acid esters in the mixture were more rapidly lost than the saturated fatty acid esters.
- By the termination of the 6 months experiment *Anco Green* components could not be detected neither in sediment samples nor in polychaete extracts.
- The disappearance from sediments showed good fits to exponential models with half-lives between 13.9 and 20.8 days (95% confidence limits) as compared to between 18.5 and 27.5 days for *Petrofree* esters.
- Sediment oxygen consumption was higher than in any other treatment. For the experimental period, the consumed oxygen was equivalent to complete mineralisation to CO_2 of 32 to 39% of the initial load of esters.
- Throughout most of the experimental period, redox potentials were significantly lower than redox potentials in control chambers. The potentials were also lower than the potentials observed in mineral oil and olefin treatments, but not as low as in the *Petrofree* treatment.
- Low redox potentials and mats of, presumably, sulphide oxidising bacteria indicated high activities of sulphate reducing bacteria below the sediment surface in all chambers treated with *Anco Green* and *Petrofree* esters.
- At their maximum extensions the mats covered 25 and 65%, respectively, in the two *Anco Green* chambers, but 100% in both *Petrofree* chambers.
- In the ester treatments, the disturbance of the benthic fauna was most probably caused by the combined effects of hydrogen sulphide toxicity and mat extension. In the two *Petrofree* chambers the benthic communities collapsed 1-3 months after the addition of cuttings, and only 4-6 species and 32-83 individuals survived the experiment, as compared to 14-35 species and 283-809 individuals in the two *Anco Green* chambers.

4.3 OLEFIN AND MINERAL OIL TREATMENTS

- *Novaplus* consisted of an isomeric mixture of mono-olefins (hydrocarbons with one double bond). The number of carbon atoms varies from C_{16} over C_{18} up to C_{20}.
- All components of *Novaplus* seemed to disappear at similar rates, whereas in Ultidrill samples the C_{14}-components were more rapidly removed compared to the C_{16}-homologs.
- By the end of the 6 month experiment, components present in the original base fluids were still present in sediments and polychaetes sampled from both olefin treatments.
- Enrichment of the ratio between drilling fluids and barium in the polychaete *Hediste (Nereis) diversicolor*, as compared with simultaneously sampled sediments, did indicate some bioaccumulation of both types of olefins and the mineral oil.
- In order to confirm or reject this hypothesis, an experiment designed specifically for the study of bioaccumulation of drilling fluids is recommended.
- The disappearance of *Ultidrill* olefins showed good fits to exponential models with half-lives between 40 and 72 days (95% confidence interval). This was consistent with previous test results on this product.
- The *Novaplus* and mineral oil data showed less good fits to the exponential regression models than did the *Ultidrill* olefins and the esters.
- The estimated half-lives of 74 days for *Novaplus* olefins and 105 days for Safemul mineral oil, indicated that biodegradation of *Novaplus* occurred more slowly than the biodegradation of *Ultidrill*, but more rapidly than mineral oil.
- For the entire experimental period, oxygen consumption were slightly higher in the *Novaplus* chambers than in the *Ultidrill* chambers, but mineralisation of 38% of the initial load of *Novaplus* olefins was not significantly different from the mineralisation of neither *Ultidrill* olefins nor *Anco Green* esters.
- The slow change of concentration of *Novaplus* olefins and *Novaplus*:barium ratios towards the end of the experimental period was not confirmed by the oxygen consumption rates which indicated rapid mineralisation in the *Novaplus* chambers at the end of the experimental period.
- Redox potentials were lowered relative to control chambers but the difference was rarely significant and hydrogen sulphide were never detected in any of the chambers treated with olefins or mineral oil.
- Effects on the benthic fauna were less severe in the olefin-treatments than in the ester treatments. Both number of species and number of individuals were higher in the *Novaplus* chambers as compared to mineral oil and *Ultidrill* treatments, and pollution sensitive species were abundant in both *Novaplus* chambers.
- Mineral buffers present in the *Safemul* mud was the most probable cause of high initial pH values in the mineral oil treatments. Possibly, the lower diversities in the mineral oil as compared to the olefin treatments was a result of this pH anomaly.

4.4 RECOMMENDATIONS

- The *Anco Green* esters will disappear rapidly and completely from offshore discharge sites. Effects on the benthic communities during and for a limited period of time after the discharge period, will be more severe than the effects of olefin based muds. Apparently, however, the effects will be less severe than the effects of Petrofree, possibly as a result of the natural (fish oil) source of the fatty acid components of the *Anco Green* esters.
- Redox effects of discharges of olefin based drilling muds will be less than the corresponding effects of ester based muds, and less for *Novaplus* as compared to *Ultidrill* olefins.
- None of our tests have, so far, showed complete disappearance of any olefin products and apparently low availability's to sulphate reducing bacteria may slow down biodegradation of

buried olefins. Offshore surveys or a differently dosed benthic chamber experiment might elucidate this problem.

- In order to confirm or reject indications found on bioaccumulation of olefins in the polychaete *Hediste diversicolor*, an experiment designed specifically for the study of bioaccumulation of such drilling fluids is recommended.

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APPENDIX I: Tables of results

Table AI.1. E_h and pH at 15 mm depth in the sediment.

		pH			E_h (mV)		
Day	Chamber	pH	Mean	stdev	E_h	mean	stdev
2	SMO1	9.29			282		
2	SMO1	9.36			291		
2	SMO1	8.57	9.073	0.437	293	289	6
2	ANC2	7.61			307		
2	ANC2	7.79			304		
2	ANC2	7.78	7.727	0.101	411	341	61
2	UTD3	7.73			390		
2	UTD3	7.64			369		
2	UTD3	7.78	7.717	0.071	374	378	11
2	CON4	7.60			367		
2	CON4	7.56			370		
2	CON4	7.62	7.593	0.031	361	366	5
2	NIO5	7.49			370		
2	NIO5	7.72			365		
2	NIO5	7.57	7.593	0.117	364	366	3
2	PTF6	7.68			372		
2	PTF6	7.56			373		
2	PTF6	7.61	7.617	0.060	376	374	2
2	UTD7	7.58			375		
2	UTD7	7.66			374		
2	UTD7	7.59	7.610	0.044	379	376	3
2	ANC8	7.68			348		
2	ANC8	7.80			314		
2	ANC8	7.69	7.723	0.067	279	314	35
2	PTF9	7.76			318		
2	PTF9	8.09			311		
2	PTF9	7.62	7.823	0.241	161	263	89
2	CON10	7.50			293		
2	CON10	7.54			298		
2	CON10	7.59	7.543	0.045	317	303	13
2	NIO11	7.60			330		
2	NIO11	7.56			340		
2	NIO11	7.60	7.587	0.023	341	337	6
2	SMO12	9.38			309		
2	SMO12	9.02			313		
2	SMO12	8.46	8.953	0.464	315	312	3
36	SMO1	8.05			203		
36	SMO1	8.31			250		
36	SMO1	8.20	8.187	0.131	112	188	70
36	ANC2	8.07			-27		
36	ANC2	8.07			147		
36	ANC2	8.21	8.117	0.081	47	56	87
36	UTD3	8.09			157		
36	UTD3	7.70			202		
36	UTD3	7.88	7.890	0.195	195	185	24
36	CON4	7.50			266		
36	CON4	7.51			324		
36	CON4	7.60	7.537	0.055	324	305	33
36	NIO5	7.95			272		
36	NIO5	7.83			275		
36	NIO5	7.96	7.913	0.072	272	273	2
36	PTF6	8.22			-5		
36	PTF6	8.12			25		
36	PTF6	7.98	8.107	0.121	-25	-2	25
36	UTD7	7.72			212		
36	UTD7	7.71			233		
36	UTD7	7.83	7.753	0.067	245	230	17
36	ANC8	7.77			72		
36	ANC8	8.25			-33		
36	ANC8	8.10	8.040	0.246	127	55	81

36	PTF9	8.23			12		
36	PTF9	8.35			22		
36	PTF9	7.82	8.133	0.278	37	24	13
36	CON10	7.52			247		
36	CON10	7.53			304		
36	CON10	7.82	7.623	0.170	305	285	33
36	NIO11	7.68			291		
36	NIO11	7.66			262		
36	NIO11	8.00	7.780	0.191	253	269	20
36	SMO12	8.50			258		
36	SMO12	8.23			194		
36	SMO12	8.49	8.407	0.153	207	220	34
65	SMO1	8.15			163		
65	SMO1	8.10			204		
65	SMO1	7.87	8.040	0.149	216	194	28
65	ANC2	7.81			-29		
65	ANC2	7.95			-63		
65	ANC2	8.20	7.987	0.198	75	-6	72
65	UTD3	7.48			229		
65	UTD3	7.48			247		
65	UTD3	7.47	7.477	0.006	199	225	24
65	CON4	7.51			254		
65	CON4	7.56			260		
65	CON4	7.63	7.567	0.060	284	266	16
65	NIO5	7.54			205		
65	NIO5	7.76			256		
65	NIO5	7.73	7.677	0.119	254	238	29
65	PTF6	7.85			53		
65	PTF6	8.26			-11		
65	PTF6	8.18	8.097	0.217	-60	-6	57
65	UTD7	7.90			198		
65	UTD7	7.75			66		
65	UTD7	7.61	7.753	0.145	190	151	74
65	ANC8	8.37			-72		
65	ANC8	8.29			89		
65	ANC8	8.12	8.260	0.128	18	12	81
65	PTF9	7.88			-75		
65	PTF9	8.08			-32		
65	PTF9	8.25	8.070	0.185	-61	-56	22
65	CON10	7.64			194		
65	CON10	7.59			227		
65	CON10	7.49	7.573	0.076	246	222	26
65	NIO11	7.93			243		
65	NIO11	7.97			227		
65	NIO11	7.88	7.927	0.045	248	239	11
65	SMO12	8.27			236		
65	SMO12	8.23			240		
65	SMO12	8.41	8.303	0.095	225	234	8
97	SMO1	8.12			219		
97	SMO1	7.83			241		
97	SMO1	7.69	7.880	0.219	252	237	17
97	ANC2	7.62			-96		
97	ANC2	7.90			-100		
97	ANC2	7.77	7.763	0.140	45	-50	83
97	UTD3	7.97			-28		
97	UTD3	7.63			187		
97	UTD3	7.85	7.817	0.172	219	126	134
97	CON4	7.65			275		
97	CON4	7.68			288		
97	CON4	7.72	7.683	0.035	299	287	12
97	NIO5	8.21			273		
97	NIO5	7.99			138		
97	NIO5	8.24	8.147	0.137	185	199	69
97	PTF6	7.78			-129		
97	PTF6	7.92			-146		
97	PTF6	7.85	7.850	0.070	-141	-139	9

97	UTD7	8.03			139		
97	UTD7	7.95			-83		
97	UTD7	7.88	7.953	0.075	157	71	134
97	ANC8	7.93			-58		
97	ANC8	8.02			105		
97	ANC8	7.95	7.967	0.047	-128	-27	120
97	PTF9	8.09			-101		
97	PTF9	7.75			-150		
97	PTF9	7.96	7.933	0.172	-92	-114	31
97	CON10	7.69			263		
97	CON10	7.58			297		
97	CON10	7.55	7.607	0.074	293	284	19
97	NIO11	7.56			216		
97	NIO11	7.75			223		
97	NIO11	7.63	7.647	0.096	220	220	4
97	SMO12	7.98			167		
97	SMO12	7.83			57		
97	SMO12	8.14	7.983	0.155	185	136	69
126	SMO1	7.70			176		
126	SMO1	7.92			-17		
126	SMO1	7.90	7.840	0.122	207	122	121
126	ANC2	7.56			43		
126	ANC2	7.78			-45		
126	ANC2	8.01	7.783	0.225	-38	-13	49
126	UTD3	8.12			93		
126	UTD3	7.94			45		
126	UTD3	7.85	7.970	0.137	-17	40	55
126	CON4	7.63			292		
126	CON4	7.69			307		
126	CON4	7.72	7.680	0.046	328	309	18
126	NIO5	7.99			239		
126	NIO5	7.68			87		
126	NIO5	7.73	7.800	0.166	173	166	76
126	PTF6	7.79			30		
126	PTF6	7.73			14		
126	PTF6	7.98	7.833	0.131	52	32	19
126	UTD7	7.71			119		
126	UTD7	7.82			55		
126	UTD7	7.71	7.747	0.064	97	90	33
126	ANC8	7.91			12		
126	ANC8	7.82			-1		
126	ANC8	7.76	7.830	0.075	119	43	66
126	PTF9	7.99			-18		
126	PTF9	7.89			-65		
126	PTF9	8.03	7.970	0.072	-43	-42	24
126	CON10	7.65			266		
126	CON10	7.66			291		
126	CON10	7.71	7.673	0.032	305	287	20
126	NIO11	7.77			235		
126	NIO11	7.85			240		
126	NIO11	7.76	7.793	0.049	65	180	100
126	SMO12	7.79			199		
126	SMO12	8.06			358		
126	SMO12	7.94	7.930	0.135	300	286	80
158	SMO1	7.53			254		
158	SMO1	8.01			205		
158	SMO1	7.97	7.837	0.266	209	223	27
158	ANC2	8.04			36		
158	ANC2	7.79			3		
158	ANC2	7.85	7.893	0.131	22	20	17
158	UTD3	7.81			202		
158	UTD3	7.60			260		
158	UTD3	7.60	7.670	0.121	160	207	50
158	CON4	7.66			362		
158	CON4	7.64			368		
158	CON4	7.63	7.643	0.015	378	369	8

158	NIO5	7.75			72		
158	NIO5	7.70			69		
158	NIO5	7.66	7.703	0.045	260	134	109
158	PTF6	7.58			83		
158	PTF6	7.43			96		
158	PTF6	7.72	7.577	0.145	116	98	17
158	UTD7	7.66			145		
158	UTD7	7.69			259		
158	UTD7	7.67	7.673	0.015	265	223	68
158	ANC8	7.79			139		
158	ANC8	7.65			238		
158	ANC8	7.66	7.700	0.078	242	206	58
158	PTF9	8.13			27		
158	PTF9	7.87			49		
158	PTF9	7.88	7.960	0.147	33	36	11
158	CON10	7.63			286		
158	CON10	7.60			320		
158	CON10	7.56	7.597	0.035	330	312	23
158	NIO11	7.81			315		
158	NIO11	7.63			317		
158	NIO11	7.64	7.693	0.101	238	290	45
158	SMO12	7.99			290		
158	SMO12	7.89			185		
158	SMO12	7.85	7.910	0.072	255	243	53
186	SMO1	7.75			285		
186	SMO1	7.82			306		
186	SMO1	7.80	7.790	0.036	302	298	11
186	ANC2	7.91			229		
186	ANC2	7.84			62		
186	ANC2	7.67	7.807	0.123	141	144	84
186	UTD3	7.69			240		
186	UTD3	7.64			325		
186	UTD3	7.57	7.633	0.060	336	300	53
186	CON4	7.74			356		
186	CON4	7.77			373		
186	CON4	7.71	7.740	0.030	244	324	70
186	NIO5	7.80			204		
186	NIO5	8.13			87		
186	NIO5	7.66	7.863	0.241	153	148	59
186	PTF6	7.48			156		
186	PTF6	7.70			152		
186	PTF6	7.41	7.530	0.151	172	160	11
186	UTD7	7.63			305		
186	UTD7	7.74			309		
186	UTD7	7.69	7.687	0.055	328	314	12
186	ANC8	7.72			227		
186	ANC8	7.74			159		
186	ANC8	7.82	7.760	0.053	209	198	35
186	PTF9	7.75			64		
186	PTF9	7.92			1		
186	PTF9	7.85	7.840	0.085	33	33	32
186	CON10	7.77			283		
186	CON10	7.81			323		
186	CON10	7.76	7.780	0.026	326	311	24
186	NIO11	7.71			311		
186	NIO11	7.80			300		
186	NIO11	7.63	7.713	0.085	275	295	18
186	SMO12	7.84			310		
186	SMO12	8.00			302		
186	SMO12	7.80	7.880	0.106	283	298	14

Table AI.2. Barium in slurries and sediment samples. (Analyses by I. Rustad, SINTEF Industrial Chemistry.)

Sample/ day	Treat-ment	Ch. no.	Tot wet wght. (g)	Dry wght. %	Barium $mg \cdot kg^{-1}$	Barium $mg \cdot cm^{-2}$
2	CON	4	41.52	25.05	780	.807
35	CON	4	39.44	26.22	770	.792
66	CON	4	40.45	26.54	780	.833
98	CON	4	39.57	26.93	768	.814
127	CON	4	41.71	24.05	776	.775
158	CON	4	38.24	25.74	733	.718
187	CON	4	39.62	25.37	785	.785
2	CON	10	36.51	25.21	780	.715
35	CON	10	37.76	25.42	790	.754
66	CON	10	40.85	24.20	760	.748
98	CON	10	39.95	24.81	773	.763
127	CON	10	40.41	24.83	782	.781
158	CON	10	39.23	25.21	787	.775
187	CON	10	38.77	25.26	766	.746
Slurry	ANC	2	547.00	45.11	56 000	6.124
2	ANC	2	35.48	21.92	6 100	4.722
35	ANC	2	36.26	22.83	6 400	5.272
66	ANC	2	39.82	25.06	4 330	4.300
98	ANC	2	38.98	24.44	5 100	4.835
127	ANC	2	39.61	23.78	3 670	3.441
158	ANC	2	39.05	22.72	3 130	2.763
187	ANC	2	38.79	23.23	4 160	3.731
Slurry	ANC	8	578.00	40.22	65 000	6.697
2	ANC	8	36.69	21.94	7 000	5.608
35	ANC	8	36.63	23.00	6 300	5.283
66	ANC	8	39.82	24.03	4 860	4.627
98	ANC	8	39.19	23.80	3 830	3.555
127	ANC	8	40.37	23.03	4 250	3.933
158	ANC	8	40.27	23.40	3 920	3.676
187	ANC	8	38.78	24.01	4 620	4.281
Slurry	PTF	6	613.00	46.44	27 000	3.407
2	PTF	6	34.56	21.66	3 800	2.830
35	PTF	6	37.40	22.94	3 500	2.988
66	PTF	6	41.33	24.17	3 300	3.280
98	PTF	6	41.08	24.55	3 190	3.202
127	PTF	6	38.65	22.58	3 580	3.109
158	PTF	6	40.06	23.97	3 000	2.867
187	PTF	6	40.11	24.20	3 300	3.188
Slurry	PTF	9	601.00	38.50	27 000	2.769
2	PTF	9	35.12	22.39	3 500	2.738
35	PTF	9	37.43	23.74	3 000	2.653
66	PTF	9	41.25	24.46	3 400	3.414
98	PTF	9	40.73	25.16	3 200	3.264

127	PTF	9	39.52	24.04	2 720	2.571
158	PTF	9	39.40	23.76	3 060	2.851
187	PTF	9	40.34	25.30	3 320	3.373
Slurry	UTD	3	562.00	39.15	38 000	3.706
2	UTD	3	35.43	22.88	4 300	3.468
35	UTD	3	36.11	23.84	4 200	3.598
66	UTD	3	39.92	23.77	3 130	2.955
98	UTD	3	40.10	25.44	3 640	3.696
127	UTD	3	39.00	24.18	3 340	3.134
158	UTD	3	40.47	23.62	4 240	4.034
187	UTD	3	39.54	25.39	3 410	3.407
Slurry	UTD	7	478.00	32.34	38 000	2.604
2	UTD	7	35.64	21.40	3 800	2.885
35	UTD	7	37.53	23.13	3 500	3.024
66	UTD	7	39.78	23.59	3 185	2.974
98	UTD	7	41.17	23.50	2 750	2.648
127	UTD	7	37.34	22.68	3 300	2.782
158	UTD	7	39.34	22.70	2 460	2.187
187	UTD	7	39.22	23.13	2 500	2.257
Slurry	NIO	5	611.00	39.01	38 000	4.014
2	NIO	5	36.33	22.69	5 000	4.102
35	NIO	5	37.71	23.37	4 900	4.297
66	NIO	5	40.05	24.87	4 260	4.223
98	NIO	5	38.12	23.62	3 370	3.020
127	NIO	5	38.55	23.67	4 310	3.914
158	NIO	5	39.02	24.00	4 290	3.998
187	NIO	5	38.87	22.62	2 280	1.995
Slurry	NIO	11	513.00	43.19	36 000	3.535
2	NIO	11	36.40	23.05	4 400	3.674
35	NIO	11	37.34	24.45	3 100	2.817
66	NIO	11	40.55	24.48	2 820	2.786
98	NIO	11	39.05	25.68	3 020	3.014
127	NIO	11	40.98	23.34	2 560	2.437
158	NIO	11	39.39	23.71	2 870	2.667
187	NIO	11	40.02	25.19	2 790	2.800
Slurry	SMO	1	604.00	38.86	16 700	1.737
2	SMO	1	34.85	20.84	2 600	1.879
35	SMO	1	36.27	24.03	2 500	2.168
66	SMO	1	40.05	25.27	1 960	1.974
98	SMO	1	39.48	24.55	1 880	1.813
127	SMO	1	40.38	23.84	2 690	2.578
158	SMO	1	40.12	23.68	1 970	1.863
187	SMO	1	37.95	23.40	1 545	1.365
Slurry	SMO	12	637.00	41.35	16 000	1.868
2	SMO	12	36.64	20.04	2 700	1.973
35	SMO	12	36.80	22.99	2 600	2.189
66	SMO	12	38.86	23.46	2 270	2.059

98	SMO	12	38.71	23.32	2 000	1.797
127	SMO	12	40.53	22.91	2 240	2.070
158	SMO	12	39.42	22.81	2 110	1.889
187	SMO	12	39.13	23.48	2 100	1.920

Table AI.3. Concentration of drilling fluids in slurries and sediment samples.

Sampling Date:	Sampling Day:	Chamb. No.:	Concentration $g \cdot kg^{-1}$ (dry wght)	Chamb. No.:	Concentration $g \cdot kg^{-1}$ (dry wght)
Novaplus					
Slurry	0	5	84.83	11	60.32
04.jul	2	5	4.33	11	5.35
07.aug	35	5	3.87	11	1.66
07.sep	66	5	2.81	11	0.99
09.okt	98	5	0.91	11	1.09
07.nov	127	5	2.41	11	0.69
07.des	158	5	1.17	11	0.81
07.jan	186	5	0.30	11	1.06
Ultidrill					
Slurry	0	3	47.64	7	56.22
04.jul	2	3	4.91	7	4.93
07.aug	35	3	3.83	7	2.88
07.sep	66	3	2.35	7	1.14
09.okt	98	3	1.53	7	0.71
07.nov	127	3	0.83	7	0.81
07.des	158	3	0.78	7	0.20
07.jan	186	3	0.56	7	0.32
Safemul					
Slurry	0	1	46.37	12	45.85
04.jul	2	1	3.02	12	2.53
07.aug	35	1	1.85	12	2.51
07.sep	66	1	1.08	12	1.65
09.okt	98	1	0.86	12	0.86
07.nov	127	1	2.56	12	1.48
07.des	158	1	1.03	12	1.21
07.jan	186	1	0.41	12	2.54
Petrofree					
Slurry	0	6	41.88	9	35.96
04.jul	2	6	5.78	9	5.35
07.aug	35	6	2.38	9	2.86
07.sep	66	6	0.95	9	1.08
09.okt	98	6	0.51	9	0.094
07.nov	127	6	0.12	9	0.065
07.des	158	6	0.026	9	0.0069
07.jan	186	6	0.034	9	0.031
Ancogreen					
Slurry	0	2	58.36	8	66.68

04.jul	2	2	7.38	8	8.29
07.aug	35	2	5.37	8	4.60
07.sep	66	2	0.99	8	1.24
09.okt	98	2	0.86	8	0.13
07.nov	127	2	0.015	8	0.062
07.des	158	2	0.011	8	0.0046
07.jan	186	2	0.014	8	0.0054

Table AI.4 Result of analyses of *Safemul* mineral oil, *Ultidrill* olefins, *Novaplus* olefins, *Petrofree* esters and *Anco Green* esters in control chambers.

Chamber:	CON 10	CON 10	CON 10	CON 4	CON 4
Analyses:	SMO	UTD	NIO	PTF	ANC
Day			mg/cm2		
2	.035	.016	.046	.000	.000
35	.172	.026	.042	.000	.000
66	.000	.037	.046	.000	.000
98	.064	.013	.033	.000	.000
127	.006	.021	.035	.000	.000
158	.042	.000	.040	.004	.000
186	.011	.008	.025	.000	.004
average	.047	.017	.038	.001	.001
stdev	.060	.012	.008	.001	.002

Table AI.5. Sediment oxygen consumption in each chamber ($\mu molO_2.m^{-2}.h^{-1}$).

	ANC	ANC	SMO	SMO	PTF	PTF	UTD	UTD	NIO	NIO	CON	CON
Day	8	2	1	12	6	9	3	7	5	11	4	10
3	573	895	847	1054	1371	1155	819	638	1005	1068	473	568
4	647	821	751	813	1120	1033	747	770	899	825	530	514
7	622	809	770	693	1068	947	762	724	826	823	527	468
15	912	1461	969	823	1352	1064	1351	1201	966	960	638	517
17	1376	1494	925	780	878	1080	1331	911	1532	953	580	502
22	351	1258	807	460	438	530	359	741	709	723	257	288
25	1198	1610	739	889	1195	1455	1414	1434	858	1141	751	542
29	847	1540	636	790	920	837	1251	909	1028	869	677	491
32	1005	961	734	694	1037	1268	726	900	816	700	589	453
35	1041	901	692	707	918	1089	697	961	854	788	706	419
39	1035	1031	653	537	775	722	843	699	535	665	437	238
43	1287	1328	628	680	1075	881	940	755	650	820	521	375
46	1320	1386	626	690	1073	1093	986	880	835	888	515	464
50	898	1346	623	320	651	389	1033	560	599	579	320	95
53	1767	1605	596	784	743	1459	1467	1109	1527	1021	573	675
57	1583	1126	668	635	1235	1241	1184	1121	1628	1121	567	437
59	693	814	346	352	661	631	544	516	580	809	298	256

63	1132	1127	475	590	683	939	806	693	1080	981	395	365
66	908	855	1250	635	584	823	441	622	826	935	303	325
70	1074	1001	548	528	345	840	764	433	926	910	417	369
73	925	1239	590	582	370	789	689	707	856	857	272	252
78	758	888	473	456	410	647	553	562	675	652	287	218
80	976	981	446	407	190	622	683	600	739	842	333	276
84	873	937	410	482	182	450	584	593	678	676	318	139
87	992	561	589	585	411	462	653	629	677	710	428	296
92	1144	645	392	610	64	74	523	507	504	567	184	104
95	720	829	431	537	232	350	618	523	697	611	401	168
98	733	1351	339	585	281	162	897	444	1006	689	594	222
102	1128	875	848	679	340	340	906	908	1036	738	732	366
106	953	1009	511	548	1351	581	637	688	728	595	402	330
109	1033	*900*	*270*	374	*850*	667	*620*	620	*760*	647	*500*	331
113	811	788	31	424	370	472	599	769	803	703	613	410
120	849	977	477	426	818	162	222	668	703	583	527	290
123	846	182	345	615	470	228	600	615	590	517	332	280
127	970	443	404	497	500	212	558	574	544	563	319	290
130	247	72	74	315	513	15	75	483	498	384	153	159
134	682	587	396	427	637	177	551	528	531	558	318	221
140	540	581	360	622	605	319	516	514	*350*	378	230	246
144	303	239	248	462	572	173	568	650	103	651	326	314
148	627	737	411	536	572	719	519	1008	671	610	345	314
151	59	16	14	327	464	-16	433	335	618	354	204	14
156	365	774	106	618	494	129	461	512	650	533	369	412
162	499	161	93	329	636	83	432	482	702	473	303	41
164	532	644	278	1587	577	153	585	443	816	662	443	374
169	574	237	290	499	501	239	585	451	659	613	376	299
172	595	346	321	435	545	283	381	449	721	574	320	309
179	451	362	324	422	531	302	451	466	600	467	338	287
184	723	328	305	470	545	356	134	419	852	524	146	291
186	346	238	220	340	431	226	315	303	390	414	288	251

Table AI.6. De enkelte artene og deres individtall i prøve S1-S12.

GRUPPE	FAMILIE	ART/TAKSON	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
ANTHOZOA		Anthozoa indet		2	1	5	2		1			1	2	
	Cerianthidae	Cerianthus lloydi Gosse	1							1		1	1	
NEMERTINEA		Nemertinea indet	26	14	24	94	28	4	13	29	7	14	37	20
POLYCHAETA	Amphinomidae	Paramphinome jeffreysii (McIntosh 1868)	27	41	48	81	51	14	37	44	69	27	56	29
	Polynoidae	Harmothoe sp				2								
	Sigalionidae	Pholoe anoculata Hartmann 1965				2						1		
	Sigalionidae	Pholoe minuta (Fabricius 1780)	1	3	12	12	7	1	5	13		2	13	1
	Phyllodocidae	Eteone sp	1		1	1	1			6		1		
	Phyllodocidae	Phyllodocidae indet								1				
	Hesionidae	Nereimyra punctata (O.F.Mueller 1788)			1	4				2			3	
	Syllidae	Exogone sp				1	1		1			1	2	
	Syllidae	Typosyllis cornuta (Rathke 1843)					1						1	
	Nereidae	Ceratocephale loveni Malmgren 1867	3	2	4	3	7		4	1		6	4	5
	Nereidae	Nereis virens Sars 1835		1			1	1		3				
	Nephtyidae	Nephtys ciliata (O.F.Mueller 1776)							1					
	Goniadidae	Goniada maculata Oersted 1843				1								
	Onuphidae	Onuphis fiordica Fauchald 1974			5	3	4		3	3	1	3	3	4
	Onuphidae	Onuphis quadricuspis M.Sars 1872	1		3	1	1		1	1		1	1	2
	Lumbrineridae	Lumbrineris sp	2			1	2		1	1		2	1	
	Orbiniidae	Orbinia sp			1									
	Paraonidae	Paraonis gracilis (Tauber 1879)	3	2	1	1	2		2	1		2	5	
	Paraonidae	Paraonis lyra (Southern 1914)	3		2	6	2					2	2	1
	Spionidae	Prionospio cf. cirrifera Wiren 1883				53	1		1	7		32	1	1
	Spionidae	Prionospio malmgreni Claparede 1868				1								
	Spionidae	Spiophanes kroeyeri Grube 1860	1		2	8				1		1	6	
	Cirratulidae	Caulleriella sp				34	1			1			5	
	Cirratulidae	Chaetozone setosa Malmgren 1867	5	5	6	35	28		8	20		7	13	4
	Cirratulidae	Tharyx sp							1					
	Flabelligeridae	Diplocirrus glaucus (Malmgren 1867)				2				1			1	
	Opheliidae	Ophelina sp				3						5	1	

GRUPPE	FAMILIE	ART/TAKSON	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
	Capitellidae	Heteromastus filiformis (Claparede 1864)	119	165	126	419	332	11	128	513	6	99	248	166
	Maldanidae	Rhodine loveni Malmgren 1865			1	1	1			1		1	1	1
	Oweniidae	Myriochele oculata Zaks 1922				2	1					1		
	Pectinariidae	Pectinaria koreni Malmgren 1865								1		2		
	Ampharetidae	Melinna cristata (M.Sars 1851)			1	2							1	
	Ampharetidae	Melythasides laubieri Desbruyeres 1978										3	1	
	Trichobranchidae	Terebellides stroemi M.Sars 1835				4				1				1
	Trichobranchidae	Trichobranchus roseus (Malm 1874)					1							
	Sabellidae	Fabriciinae indet							2					
	Sabellidae	Sabellidae indet				8						5		1
PROSOBRANCHIA	Nassariidae	Nassarius reticulatus (L.)	1											
CAUDOFOVEATA		Caudofoveata indet			6	2				1			6	2
BIVALVIA	Nuculidae	Nucula cf. turgida Leckenby & Marshall											19	
	Nuculidae	Nucula tumidula (Malm)			1		3			1				
	Nuculidae	Nuculoma tenuis (Montagu)		2	1	2	1			1		4	7	
	Thyasiridae	Thyasira croulinensis (Jeffreys)			1									1
	Thyasiridae	Thyasira equalis (Verrill & Bush)	13	17	42	12	49		42	45		26	49	30
	Thyasiridae	Thyasira eumyaria (M.Sars)					1							
	Thyasiridae	Thyasira ferruginea (Forbes)				1								
	Thyasiridae	Thyasira obsoleta (Verrill & Bush)	4	4	14	10	11		4	6		5	5	3
	Thyasiridae	Thyasira pygmaea (Verrill & Bush)	6	12	13	15	54		24	39		17	38	17
	Thyasiridae	Thyasira sarsi (Philippi 1845)	9	13	19	7	47	1	26	46		2	21	17
	Scrobiculariidae	Abra nitida (Mueller 1789)				6	3			13		2	6	
OSTRACODA	Cypridinidae	Philomedes lilljeborgi G.O.Sars				7			1	1		1	4	
AMPHIPODA	Melitidae	Eriopisa elongata Bruzelius										1		
SIPUNCULIDA		Onchnesoma steenstrupi Koren & Danielssen			2		3		2	1		1	18	3
		Phascolion strombi (Montagu 1804)								1			2	
		Sipunculida indet										1		
OPHIUROIDEA		Ophiuroidea indet				4				2			4	
VARIA		Ubestemt indet										1		