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EFFECTS OF A LIGHT CRUDE (F3-) AND OF TWO SELECTED OIL COMBAT METHODS IN EXPERIMENTAL TIDAL FLAT ECOSYSTEMS FINAL REPORT OIL POLLUTION EXPERIMENTS (OPEX) 1986

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1. INTRODUCTION

1.1 MARINE OIL POLLUTION

In recent years there has been increasing concern about the ecological effects caused by oil pollution at sea. The high intensity of shipping and off-shore oil production in the North Sea results in many minor spills of oil every year, and there remains the risk of a major oil spill in the production and shipping areas. It is generally recognized that the impact of oil spilled at sea is the strongest in areas with a limited opportunity for dilution in combination with high sedimentation rates and anaerobic conditions in the sediment (Scholten and Kuiper, 1987). Thus intertidal soft-bottom ecosystems, such as the Wadden Sea, appear to be highly sensitive to oil pollution. The Dutch sector covers 240 km^2, i.e. 40% of the entire Waddensea. Some 50% of this area consists of intertidal flats.

The tidal flat sediments are inhabited by enormous numbers of worms, crustaceans and molluscs, and therefore provide a rich food source for birds and fish. The Wadden Sea area is considered to be of great ecological, economical, cultural and scenic value, and is designated as a protected area (Rijkswaterstaat, 1987). Indeed, large parts of it are already governed by nature conservancy regulations. In an area of such natural importance, the prevention of oil pollution is essential. Therefore, there is a need for comprehensive contingency plans concerning appropriate oil combat strategies specifically designed for the morfology of the Wadden Sea

1.2 THE OPEX PROJECT

In 1984 a research project called Oil Pollution EXperiments (OPEX) was commenced, in order to gain a scientifically based understanding of the effects of oil pollution on the tidal flat ecosystem of the Wadden Sea and to investigate the possibilities of reducing such effects using appropriate combat strategies. Long term experiments were carried out in large-scale artificial tidal-flat ecosystems called MOdel TIdal Flats (MOTIFs). Such model systems allow relevant information about a tidal flat to be obtained, because different ecological processes and relevant organisms can be studied in an interconnected manner.

Preliminary experiments with the MOTIFs were carried out in the period 1981-1983 (1) (Kuiper et al, 1983, 1984 and 1985). These experiments showed that it was indeed possible to use the MOTIFs as a reliable tool in large-scale ecotoxicological research, since identical MOTIF systems behaved similarly and their ecological functioning was comparable to that of natural tidal flats. Treatment of the systems with oil caused reproducable effects, that were consistent with the (scarce) observations from field situations following large oil spills.

The OPEX-project was split into two parts. In the first two years (1984-1985), the fate and effects of a common North Sea light-crude oil, produced in the Forties field, were studied in the MOTIFs in combination with a common chemical dispersing agent of the concentrate type (hydrocarbonic). The direct and indirect effects of the dispersant as a combat strategy were examined in these experiments. The results of these studies have already been published (2) (Kuiper et al, 1986; Dekker and van Moorsel, 1987) and their main observations are reviewed below (see 1.3). In the third year (1986), the research was directed to investigate another type of oil (from the F3-block) and some alternative combat methods. Publications on the MOTIF research are listed in appendix 1.

1.3 OPEX 1984-1985

It was observed that the acute, toxic effects on the phytoplankton, zooplankton and the benthic fauna depended on the concentration of oil that was dispersed or dissolved in the water.
Sedimentation of oil caused a prolonged exposure of the ecosystem because of the slow degradation of oil in the anoxic sea-bed. Bioturbation by lugworms enhanced the penetration of oil into the sediment, especially in the intertidal ecosystem where floating oil was left behind at low tide. The presence of high amounts of oil in the sediment caused a long-lasting perturbation of the benthic community. Furthermore, leakage of the oil from the sediment, resulted in a chronic elevation of oil concentrations in the shallow water in which limited possibility for dilution were presented.
Acute or chronic effects on the zooplankton or benthic fauna resulted in an increase of the microalgal biomass, as the reduction of grazing pressure upon algae exceeded the inhibition of the primary productivity. The higher algal concentration increased the amount of food available for the surviving organisms, resulting in faster growth. Furthermore, an explosive

development of rapidly growing and reproducing (opportunistic) species such as Hydrobia, nematodes, and copepods was observed when the oil concentration in the water had decreased. These species benefit from the extra algae as a result of reduced competition for food.Corophium and ostracods seemed to be the best indicators of oil pollution, as the former shows a clear dose-dependent population increase, while the latter is sensitive to extremely low oil concentrations. The increased mortality among cockles in oil polluted MOTIFs during a period of severe frost, even 9 months after the application of oil, indicated that long-term toxic effects appear to interact with natural stress factors. Dispersion of the oil resulted in an aggravation of the effects as a consequence of increased oil concentrations in the shallow watercolumn. Penetration of oil into the sediment cannot be prevented by chemical dispersion.

It was conduded that the environmental impact of an oil spillage on a marine ecosystem depends on:

1. The amount of oil floating on the water surface, which obviously affects birds, shores and coastal vegetation by directly smothering.
2. The concentration of oil that is dispersed or dissolved in the water, which determines the uptake by organisms and resulting toxic effects.
3. The amount of oil accumulated in the sediment which is of primary importance over the longer term, because this creates a source of oil pollution within the ecosystem resulting in a virtually indefinite perturbation.

Therefore, an oil combat technique should be designed that meet as much as possible of the following requirements:

1. A quick removal of floating oil, without enhancement of the oil concentration in the water or the sediment.
2. No perturbation of the system, resulting from the combat technique itself.
3. No chemical inhibition of the biodegradation of the oil. On the contrary a stimulation of the biodegradation would be welcome.
4. The application of the combat technique must be workable in the open field situation where access by boat or land may be difficult.

For the Wadden Sea area, chemical dispersion with hydrocarbonic "concentrate"-type dispersants clearly did not meet all these requirements.

1.4 OPEX 1986

The Nederlandse Aardolie Maatschappij B.V. intends to lay a pipeline through the Dutch Wadden Sea for the transport of an oil-gas mixture from the F3-block in the Northern part of the Dutch continental sector of the North Sea to the mainland (fig. 1). A malfunctioning of this pipeline, however unlikely, could result in an oil spillage in or near the Wadden Sea area. An appropriate contingency plan was considered essential and was subsequently prepared by the oil company (Jacobs, 1987).

In order to be able to optimize the contingency plan, information was necessary on the fate and effects of the F3 oil and potential oil combat methods. Amongst many combat strategies two seemed to meet the in section 1.3 mentioned requirements:

a) Removal by setting the oil on fire with the help of an ignition agent.
b) Mechanical removal of stranded oil by using amphibious or wide tracked vehicles.

The impact of an F3 oil spill on the tidal flat ecosystem was examined in the MOTIFs in 1986 in combination with mechanical removal and burning with the help of an ignition agent. Particular attention was paid to the feasibility of effect reduction using these two methods.

1.5 OVERALL PROJECT ORGANIZATION

The overall research programme was determined by a national steering group, while a project group helped in finding solutions for technical and scientific problems. The members of the steering and project groups, belonging to the research teams, governmental management bodies and oil industry, are listed in appendix 2.
The OPEX project is a co-operative investigation being carried out by the Laboratory of Applied Marine Research MT-TNO, the Netherlands Institute for Sea Research (NIOZ) and the Research Institute for Nature Management (RIN).

The experimental systems were built by the RIN, who also operated the technical installations. The project was coordinated by MT-TNO. Meiobenthos research was carried out by the RIN, macrozoöbenthic and granulometry research by the NIOZ and the plankton, phytobenthos, physico chemical parameters, caged mussel and oil research was carried out by MT-TNO.

Financial sponsoring of the project was obtained from several sources. Apart from the collaborating research institutes, funds for the 1986 experiments were obtained from the constituent companies of the society of the Netherlands oil industry: Verenigde Nederlandse Aardolie industrie (VNA), a Dutch petroleum company: Nederlandse Aardolie Maatschappij (NAM) B.V. and the Netherlands Ministry of Transport and Public Works (Rijkswaterstaat, contract no. R1-81). For the first years of the OPEX project partial funding came from the Commission of the European Communities, Directorate General XII under the third environment programme (contract no. ENV. 814. NL (N)).

2. MATERIAL AND METHODS

2.1 TECHNICAL SET-UP

The technical set-up of the MOTIFs is based on the design of indoor tidal-flat ecosystems developed by the NIOZ. Eight MOTIFs were built near the RIN station on the Wadden island Texel (Fig. 2).

A MOTIF pair consists of two interconnected concrete basins, each with an area of $6x3.5m^2$ and a depth of 1.2 m. The walls and the bottom were painted with two layers of biologically inert Colturit. After painting, the basins were allowed to dry for five days, filled with seawater, leached for a further seven days, then finally emptied.
A 45 cm high brick wall running the length of each basin separates a "tidal-flat" (6x3m), filled with a 45 cm sediment layer, from a "tidal channel" (6x0.5m) (Fig. 3).

Seawater was pumped back and forth between two connected MOTIFs, every six hours, to simulate tidal movements, so that low water in one basin coincided with high water in the other. Centrifugal pumps (0.55 kW Begemann Type "KZ 120-40") with a maximum capacity of $5m.^3\ hr^{-1}$ were used. Water-flow was directed by a system of electrically operated valves coupled to a time-switch. The combined suction and outlet pipe of a MOTIF is situated in the tidal channel. The tidal cycle lasted 12 hours as shown in Fig. 4. Once a week the tidal cycle in each pair of basins was shifted by 6 hours, so that low water was at 09.00 h. in the basin which had low water at 15.00 h. the week before, and *vice versa*. High water level was 50 cm above the tidal flat surface while low water level was fractionally below it.
The flow of North Sea water through the Wadden Sea was simulated by replacing 20% of the water in the MOTIFs each day (intake water).

All MOTIFs were connected to a permanent supply of sea water drawn from the NIOZ harbour at the western-most entrance to the Waddensea (Marsdiep) at Texel, using the same type of Begemann pump as described above. An overflow in each basin discharged the surplus water at high tide. All surplus water was pumped back to the Wadden Sea via an oil separator.
A wave board was installed at one end of all MOTIFs in order to provide water mixing and surface agitation.

The sediment was collected in 1985 from natural tidal flats in Mok Bay on the Island of Texel. A top layer of 10 cm, was scraped off and collected. The sand was spread out in the open air before use in the experiment in order to make it azooïc. Before transfer to the basins the sand was mixed thoroughly.

At the start of the experiment in March 1986, lugworms (<u>Arenicola marina</u>) and various species of bivalves (<u>Macoma balthica</u>, <u>Cerastoderma edule</u> and <u>Mytilus edulis</u>) were introduced into the systems at the densities listed in table 1. In addition, a quantity of smaller seabed organisms (<u>Nereis diversicolor</u>, <u>Corophium volutator</u>, <u>Hydrobia ulvae</u>) and a meiofaunal mixture (nematodes, ostacods, copepods etc.) were introduced into each MOTIF in order to promote ecosystem development. The infaunal organisms were randomnized and distributed evenly over the tidal flat. Mussels were placed in cages in the tidal channel in randomized lots of 25 specimens. Periwinkles (<u>Littorina littorea</u>) were put into the MOTIFs in an effort to keep the walls free of algae.

Bacteria, algae and zooplankton (copepods and larvae and juveniles of benthic fauna) were continually introduced with the intake water.

The interrelations between the different biotic compartments are shown schematically in Figure 5.

Table 1. Species introduced in the MOTIFs.

Species	Density	Sampling location	Sampling date	Introduction date
Cerastoderma edule	32 m^{-2}	Roggeplaat Eastern Scheldt	08/04/86	09/04/86
Macoma balthica	100 m^{-2}	Balgzand Waddensea	23/04/86-02/05/86	25/04/86-02/05/86
Arenicola marina	25 m^{-2}	Schorren, Texel Waddensea	01/04/86	02/04/86-03/04/86
Nereis diversicolor	40 $MOTIF^{-1}$	Mokbay, Texel Waddensea	14/04/86	15/04/86
Corphium volutator	700 $MOTIF^{-1}$	Mokbay, Texel Waddensea	14/04/86	15/04/86
Hydrobia ulvae	380 $MOTIF^{-1}$	Mokbay, Texel Waddensea	07/04/86	08/04/86
Littorina littorea	500 $MOTIF^{-1}$	Marsdiep, Dyke Texel Waddensea	07/04/86	07/04/86
Mytilus edulis	250 $MOTIF^{-1}$	Huisduinen, Northsea	09/04/86	10/04/86
Meiobenthos	$1.5 \times 10^{5} m^{-2}$	Mokbay, Texel Waddensea	07/04/86	08/04/86

2.2 EXPERIMENTAL SET-UP

On May 12, 1986 oil was applied to six MOTIFs. A relatively light, North Sea crude oil, a condensate, from the F3-block, kindly made available by the Nederlandse Aardolie Maatschappij (NAM) B.V., Assen, was used.
Some 80 liters of an oil-water mixture, acquired from an exploratory drilling by the NAM, was available for the experiments. It has been calculated by Nooijen en van Asselen (1984) that 45 volume-procenten would evaporate within 10 hours. Kuiper et al. (1985) showed that during the first 22 h. some 50% of the oil added to modelplanktonsystems evaporated, and another 30% during the remaining 4 weeks the experiment lasted.
For this reason it was tried to remove some 50% of the oil by evaporation, with the aid of a compressed air bubbler, in order to obtain a type of oil that was similar to oil floated on the sea surface for several hours before reaching a tidal flat area at low tide.
Due to ignorance of the water content of the raw oil mixture the forced evaporation lasted 100 hours, resulting in only 12% volume reduction. The remaining 70 liter formed two layers: a brownish oil-water mixture toplayer of 22 liter could be seperated from a colourless water layer. It was thought that the brownish layer would consist mainly of oil, and this layer was used for the oil application.

Special engineering designs on the pipeline quarantee that in case of a pipeline rupture the maximum amount of oil lost would be 700 m^3. Some 60% of this volume would immediately disappear due to flashing, so that only 280 m^3 would be found maximally on the water surface (Jacobs, 1987).
It was calculated by Nooyen en van Asselen (1984) that such a spill would cover a watersurface of 1-2 km^2 in about 10 hours, resulting in maximally 0.28 liter of oil m^{-2}.

In two of the MOTIFs, 5.4 liter of the oil layer was applied just before low tide (figure 6). The oil was distributed evenly over the tidal flat so that a mean oil spread of 0.3 liters per m^2 was obtained resembling the maximal oil spillage that may be expected after a rupture of the F3-oil pipeline. The magnitude of this oil treatment was intermediate to those in the first OPEX-experiments, where 0.5 l and 0.1 $l.m^{-2}$ of forties oil was applied in 1984 and 1985 respectively. The MOTIFs will be referred to as <u>'F3' MOTIFs</u>.

It was estimated by the OPEX steering and project group that ca. 10-15% of the oil would be mixed into the sediment if the oil would be removed with the help of amphibious or wide tracked crafts. Moreover, the structure of the sediment surface would be disturbed by vehicle-movement. These consequences of mechanical removal of the oil was simulated by an application of 0.7 liter of F3 oil (13% of that applied to the 'F3' MOTIFs) in each of two MOTIFs at low tide. This oil was then raked into the upper 5 cm of the sediment. These MOTIFs will be referred to as 'F3m' MOTIFs.

Removal by setting the oil on fire was realistically simulated in two other MOTIFs (figure 7, 8). These are further referred as 'F3b' MOTIFs. In these MOTIFs 3.2 liter of F3 oil was added, followed by an application of ca. 3kg of a proprietary, granular ingnition agent. The ignition agent reacted with water, producing high temperatures that set the oil on fire. Unfortunatly there was not enough F3-oil available to treat these MOTIFs with 5.4 liter of oil as in the normal oil treated 'F3' MOTIFs. The oil was applied just before low tide, the ignition agent at low tide. Immediatly after the burning tide was coming up.

The remaining two MOTIFs served as a control (referred to as 'C' MOTIFs). Table 2 summaries all MOTIF treatments. The general set-up of the 1986 MOTIFs experiment is given in figure 9.

Table 2: summary of MOTIF treatments in OPEX 1986, 1985, 1984.

Treatment	Reference	Year	MOTIFs number
Control	'C'	1986	3-4
'F'3 oil: o.3 l m^{-2}	'F3'	1986	7-8
Burning of 'F'3 oil	'F3b'	1986	1-2
Simulation of mechanical removal of 'F'3 oil=0.04 l m^{-2}	'F3m'	1986	5-6
Control	'C'	1985	5-6
Control	'C'	1984	7-8
Forties: 0.1 l m^{-2} 1 day	'Fo1.1'	1985	1-2
Forties: 0.1 l m^{-2} 3 days	'Fo1.3'	1985	7-8
Forties: 0.5 l m^{-2} 5 days	'Fo5.5'	1984	3-4
Forties: 0.1 l m^{-2} + dispersant	'Fo1.Fi'	1985	3-4
Forties: 0.5 l m^{-2} + dispersant	'Fo5.Fi'	1985	1-2
Dispersant	'Fi'	1984	5-6

2.3 MEASURING PROGRAM

The measuring program included the following parameters, (precise methodology and analytical techniques are presented in section 2.4):

Abiotic factors:
- temperature, pH and oxygen content of the water.
- dissolved nutrient concentration (nitrate, nitrite, ammonia, silicate and phosphate) of the water.
- oil concentration and composition in water and sediment.
- granulometry of the sediment.

Primary producers:
- Phytoplankton: Chlorophyll concentration, primary production, species composition
- Phytobenthos: Chlorophyll concentration, species composition, trials on measurements of benthic primary production, macroalgal biomass

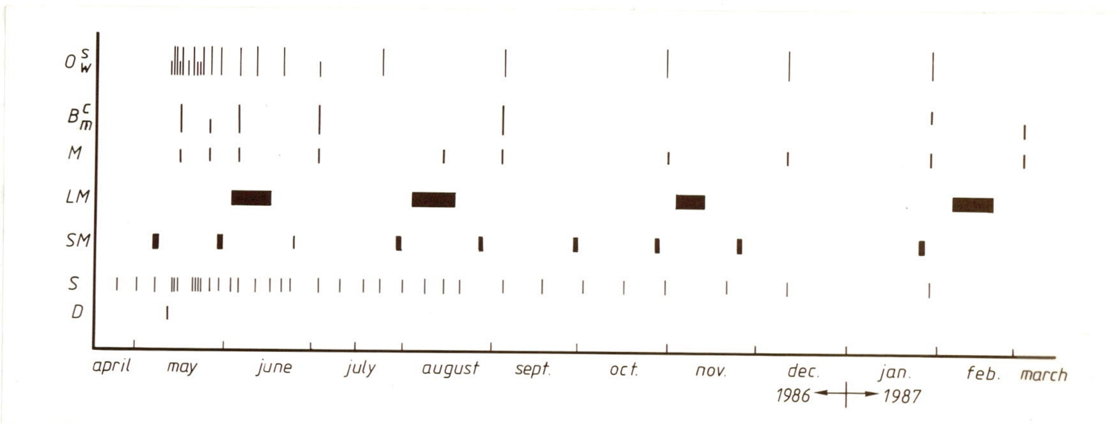
Secondary producers:
- Zooplankton: species composition, density
- Meiobenthos: species composition, density
- Macrozoobenthos: density, biomass and growth of *Mytilus*, *Cerastoderma*, *Macoma*, *Arenicola*, *Nereis*, *Corophium*, *Hydrobia*, *Littorina*, behavioural characteristics

Bacteria: total number in waterphase.

Bioaccumulation: Oil concentration in tissues of *Mytilus*, *Cerastoderma* and *Macoma*.

The frequency of measurement was dependent on the type of parameter, the season and the time of oil application (see table 3).

Tabel 3: Sampling frequency.



D = Dosage of oil and combatment of oil

S = Standard sampling of abiotics, plankton, fytobenthos and meiobenthos

SM = Small macrozoobenthos sampling

LM = Large macrozoobenthos sampling

M = Mussel sampling

B = Analysis of bioaccumulation of oil in m: mussel and c: cockle and balthic tellin

O = Analysis of oil in w: water and s: sediment

2.4 SAMPLING AND ANALYTICAL METHODS

2.4.1 Water: plankton, bacteria and physico-chemical parameters

Samples were taken from the water column in order to follow the development of the phytoplankton (chlorophyll content, and species composition), zooplankton (numbers of organisms and species composition), bacteria (total numbers), and various physico-chemical parameters (temperature, pH, concentration of oil, phosphate, silicate, ammonia, nitrite and nitrate, oxygen and suspended solids).

The sampling bottles were filled with a siphon to prevent contamination of the samples by the floating oil. This siphon was installed before the oil addition. Samples were taken at high water from a depth of approximately 25 cm, except those for zooplankton (see furtheron). The samples were transported to the laboratory as soon as possible and processed 0.5-2 h after sampling.

One litre of water was used for chlorophyll analyses according to the method of Strickland and Parsons (1968). One ml of a 1% magnesium carbonate suspension was poured onto a Whatman GF/C filter, through which the sample was subsequently filtered. The filters were then transferred to 10 ml of 90% acetone in water. After 1 minute of sonification with a Branson ultrasonic generator, the remnant was extracted for 24 hours in a refrigerator. After separation by centrifugation, pigment concentrations were measured in the supernatant with a Vitatron MPS photometer system using 1 cm cells. Concentrations of chloro-phyll and phaeopigments were calculated according to equations given by Lorenzen (1976).

The concentration of suspended solids was determined by filtration of 1 litre water over dried filter paper (Schleicher and Schull, Schwarzband no. 589) and measuring the weight of the material on the filter after drying for 24 hours at 110°C .

Two replicate subsamples of 5.0 ml water were fixed and preserved with 0.3 ml filtered formaldehyde solution for determination of bacterial numbers. These were counted by the acridine orange technique of Daley and Hobbie (1975) using a Zeiss fluorescence microscope.
A subsample of at least 100 ml water was fixed with 1 ml of Lugol's iodine solution (Vollenweider, 1969) and used for the determination of phytoplankton species composition, using a Zeiss inverted microscope (Utermohl, 1958). The main species were indentified where possible using nomenclature given by Drebes (1974) and Hendey (1964).
A subsample of 100 ml water was frozen for nutrient analyses. The concentrations of phosphate, reactive silicate, ammonia, nitrate and nitrite were measured by spectrophotometry with the aid of a Technicon AutoAnalyzer, according to Strickland and Parsons (1968) and Technicon procedures.
The pH of the water was measured in the remaining water with a Beckmann model 3550 pH meter. Temperatures were measured *in situ*.

For the determination of oxygen concentrations in the water, as well as for the measurements of the primary production by the phytoplankton, 6 oxygen bottles of approximately 100 ml were filled with the same siphon. Two bottles were fixed immediately with 1 ml 3M $MnCl_2$ and 2ml 6M NaOH/2m kI (Scholten, 1983), while the other four were transported to an incubator in the laboratory to measure oxygen production and consumption in two light and two dark bottles. The oxygen concentrations were determined with a sensitive Winkler titration using photometric end point determination (Williams et al., 1979; Tijssen, 1980; Scholten 1983). This latter method was also used to measure the oxygen production and consumption by the plankton, in order to estimate their primary productivity and respiration. To this end, 2 light and 2 dark oxygen flasks were held for approximately 4 hours in an incubator in the laboratory. This incubator consisted of a container above which 4 fluorescent lights (40W/47) were installed. The temperature in the incubator was maintained at the ambient MOTIF value. The oxygen concentration after incubation in the light bottles minus the oxygen consumption in the dark bottles, is a measure for the gross primary productivity.

Zooplankton samples were collected by draining 100 litres of water from a depth of 30 cm in the MOTIFs through a 55 µm plankton net. The zooplankton samples were preserved with 4% formaldehyde solution in seawater and the organisms were counted and identified by procedures described by Fransz (1976). Subsamples of the 100 l samples were examined until at least 150 organisms had been counted. Calanoid copepods were classified as nauplii, copepodites and adults.

Samples for oil analysis were collected in 1 l glass flasks which had been carefully cleaned with hexane (nanograde) to remove possible contaminants. The oil components were extracted within 4 hours after sampling with 2 x 50 ml hexane (nanograde). The extracts were held at 4°C until analysis.
The concentration of oil components in the water was measured in the hexane extracts. Before analysis the extracts were cleaned over aluminium oxide pentahydrate. The clean extracts were then injected into a gaschromatograph (HP 5880) with flame ionisation detection. Quantification of the oil components was done by means of retention times and external standards.

2.4.2 Sediment: fytobenthos, meiobenthos and oil.

Samples were taken from the sediment at low tide to follow the development of the phytobenthos (chlorophyll and species compositions on selected days) meiobenthos and oil in the sediment.
Sediment samples were collected by a plastic syringe core sampler with a diameter of 2.5 cm and a length of 12.5 cm. Each core was divided into sections (0-1, 1-2, 2-10 cm depth) directly after sampling. Additional samples were collected with a plexiglass core sampler (diameter 2.5 cm) from a depth of 35-40 cm.

The tidal flat was divided by a grid, each field of the grid measuring 12.5 x 12.5 cm (see Fig.). A gantry was installed over the tidal flat in each basin, so that each field of the grid could be sampled without disturbing the rest of the sediment. Twelve fields of the grid, regularly distributed over the flat, were sampled on each date.

Samples were transported to the laboratory as soon as possible, the sediments were mixed per depth within 0.5-2h after sampling. For chlorophyll analysis, subsamples of 1 g wet sediment were placed in 15 ml centrifuge tubes, 10 ml of 90% acetone was added and the procedure for water samples given above was followed. All chlorophyll values in the sediment are given on a dry weight basis.
The percentage dry weights of the sediments were determined after oven drying subsamples for 24 hours at 80°C.

A sample of 25 g of the 0-1 cm sediment layer was covered with lens tissues (Whatman 105) and incubated for 24 hours under light, in order to yield living and mobile epibenthic diatoms (Eaton and Moss, 1966). These diatoms were removed from the lens tissues by rinsing them with 4 ml conc. H_2SO_4 and 4 ml 30% H_2O_2. They were then preserved by treating the solution with sat. $KMnO_4$, conc. HCl and 30% H_2O_2. Species composition of the benthic diatoms were determined, using a Zeiss inverted microscope. All numerically important species were identified if possible. A more detailed description of the method used is given by Lemmen (1987).
Benthic macroalgae (mainly Entermorpha spp.) were removed every two weeks, in so far as possible without disturbing the benthic system. Biomass dry weight was determined.

Primary production of the benthic algae was measured in situ with the aid of bell-jars that were placed on the sediment surface and filled with 4-8 l. of filtered seawater. O_2 consumption or production was measured after dark and light incubation of the bell jars. Dissolved O_2 concentration of the water was measured continuosly during the incubation period with the aid of a yellow springs O_2-electrode. Calibration was achieved by determination of oxygen concentrations at the start and at the end of the incubation periods. Subsamples of the water were measured using a sensitive Winkler titration with end point determination. More detailed descriptions of the method used are given by Lemmen (1987).

Meiobenthic samples were collected from the larger sediment samples from 0-1, 1-2 and 2-10 cm depth every two weeks. These subsamples were put in plastic jars and were preserved with a 4% formaldehyde solution in seawater. Preserved meiofaunal samples were extracted from sandy sediments by means of simple decantation (Mc Intyre and Warwick, 1984) using a sieve with a pore size of 30 µm. Retained samples were washed off in a petridish and were toxinomically indentified and counted under a standard light microscope. In fresh sediment samples, from 0-1 cm depth, behaviour and percentage mortality of the different meiofaunal groups was determined before and after the oil addition.

The oil components were extracted from soils within 4 hours after sampling 40 g of sediment was weighed in a 100 ml erlenmeyer flask. 5 ml acetone (to loosen up the sediment), and 20 ml hexane (nanograde) were added, after which the erlenmeyer flask was placed in an ultrasonic waterbath at 0°C for 20 minutes to extract the oil components. These extracts were quantitatively collected and kept at -20°C until analysis. The same procedure adopted for oil analysis of water extracts was followed to determine oil in hexane extracts of the sediments.

On April 16 and December 3, 1986, from each MOTIF six sediment samples were collected at three depths (0-10, 10-20 and 20-30) to determine mud content and median grain size in order to characterize the sediments used in the experiments. Oven dried samples were sieved over 500, 315, 200, 125 and 50 µm meshes on an mechanical sieve- shaker. The mud content was estimated by after presieving through a 50 µm mesh.

2.4.3 Macrozoobenthos

Visual surface censuses

During the first 2 months after oil addition, freshly produced casts of Arenicola marina were counted regularly to obtain an impression of the total number of active lugworms. Other visual signs such as crawl tracks and the appearance of individuals at the sediment surface were noted.
Newly empty shells ("doublets") of bivalves that were found at the sediment surface were removed and counted once a fortnight. Although not each case of bivalve mortality resulted in the appearance of a doublet at the surface, this method was adequate to signal sudden changes in mortality.
Counts were carried out more frequently directly after oil addition.

Benthic macrofauna samples.
To exclude possible edge effects, samples were not collected in a zone within 0.5m from the walls and 1 m from the wave boards. The sampling area that remained was further diminished by the area that was used to collect bivalves for oil analyses. Therefore, only 8 m^2 per MOTIF was available in which to collect samples for the determination of macrofaunal abundance and biomass. The sampling strategy (i.e., sample sizes and frequencies) chosen, constituted a compromise between adequate sample size and the risk that sampling would affect the system severely by depleting the macrofauna. Samples were taken regularly over the substratum in such a way that inference with other sampling programs is minimal. During each Small Macrofaunal Sampling (SMS) benthic macrofaunal organisms were extracted by sieving the contents of 32 cores (6.6 cm diameter x 15 cm length). The total area sampled was 0.109 m^2 per MOTIF per sampling date. Specimens of Macoma balthica and Cerastoderma edule were not present in sufficient densities in the SMS's to be of any value and were replaced in the system. SMS's were taken at monthly intervals (see tabel 3) to follow the development of smaller macrofaunal organisms (Nereis diversicolor, Corophium volutator and Hydrobia ulvae).
During Large Macrofauna Samplings (LMS's), four 0.25 m^2 box cores were sampled to the bottom of the MOTIFs. LMS's gave larger numbers of Macoma balthica, Cerastoderma edule and Arenicola marina, but were taken less frequently than SMSs, viz., in June, August, November and February.

All samples were sieved through a 1 mm square gauze. The material retained, including the macrozoobenthos, was collected in plastic bags and stored not longer than 1 day at 3°C until further treatment and analysis. After sieving the remaining sediment was returned to the open core holes in the MOTIFs. Samples were sorted in shallow white dishes to species level. Shell length of bivalves was measured with a calipers to the nearest 0.1 mm. In *Cerastoderma edule* the width of the "winter ring" was also measured. Because the cockles had laid down this ring at the start of the experiments, individual growth thereafter could be calculated. Bivalves (not those collected for oil analyses) were briefly boiled in water in order to remove the soft parts from the shells. Sex, reproductive stage and infection rate by parasites were determined with a dissection microscope.
The following reproductive stages were discerned:
1. No gonad development
2. Vague gonad development
3. Clear gonad development, no difference between ovaria and testes
4. Further gonad development, unripe ovaria or testes
5. Further gonad development, gonads fully ripened
6. Spawning has taken place, but few gametes are still visible

For the purpose of ash free dry weight measurements, *Arenicola marina* and *Cerastoderma edule* were treated individually, while *Macoma balthica* were divided into 0.5 mm size groups. In *Corophium volutator* and *Nereis diversicolor* three size classes were discriminated. In the other species examined, all specimens from each MOTIF were pooled. All samples were placed in porcelain cups and dried to constant weight in a ventilated oven for at least 3 days at 60°C. After determination of the dry weights, samples were combusted in a furnace for two hours at 560°C. This resulted in a weight loss, the ash-free dry weight (AFDW), which is used as a biomass value in this report. Individual AFDW and total biomass. m^{-2} were then calculated. In the gastropod *Hydrobia ulvae* whole animals including shells were dried and ashed. Soft tissue AFDW of these animals was calculated as 90% of the total AFDW (Dekker 1979). In bivalves, a tissue condition index (CI) was calculated by dividing individual AFDW (mg) by the third power of the length (cm) (Beukema and de Bruin, 1977).

For *Mytilus edulis*, portions of 25 initially randomized specimens, were collected, on each sampling date; adhering sediment and (pseudo)faeces were removed and the cleaned mussels were frozen at -20°C. The shells were first measured and the empty shells counted. The soft tissues were then removed from the shell with a knife and homogenized; the dry and ash weights were determined, according to De Kock (1982).

All adult *Littorina littorea* were collected three times, in July, October and March. After counting and length measurments they were returned to the MOTIFs on the same day.

Oil analyses in the bivalves species were carried out by destruction of ca. 5 g of soft tissue homogenate with a pepsine solution for 12 hours at 37°C, after which the samples were steam distilled for 12 hours with extraction of the oil component in 10 ml of hexane (nanograde). Thereafter, the same procedure was followed as with the extracts of the water samples.

3. RESULTS

3.1 FATE OF THE OIL

The chromatogram of the original F3-oil (figure 10) supplied by the NAM B.V., was comparable to that of the F3-oil used previously in experimental plankton system studies (Kuiper et al, 1985). The chromatogram characterizes the F3-oil as a very light, condensate type crude oil.
It was decided to use the alkanes as indicators for the F3 oil in the gaschromatographic analyses (table 4). The alkane distribution had a maximum at around octane (C-8). Alkanes with a chain length of more than 21 C-atoms did not exceed 4 $g.kg^{-1}$, while all chromatographically detected alkanes in the range C8-C32 comprized 152 $g.kg^{-1}$, of the oilmixture in total. All the other peaks, representing all the other oil compounds that can be detected with a GC followed by a FID (flame ionisation detection), including cycloalkanes, aromatics and polynuclear aromatic hydrocarbons, comprized 191 $g.kg^{-1}$ of the oil mixture. The total amount of oil detected with the GC was 343 $g.kg^{-1}$ of the F3 mixture, excluding the Unresolved Complex Mixture (UCM) fraction of 104 $g.kg^{-1}$ of the F3-mixture.
This indicates that roughly 45 % of the original F3-mixture by weight consisted of oil compounds, which equals roughly 50 volume %.
Unfortunately, no measurements were avialable of the oil that was applied to the MOTIFs, after the period of 100 hours of forced evaporation. The amount of oil applied in the MOTIFs is discussed in Section 4.2.
Most of the applied oil penetrated the sediment rapidly when it was deposited on the tidal flats at low tide (Fig. 11). Only a small portion was released from the sediment with the following rising tide. An oil-film (sheen) was seen at the water surface and some oil droplets appeared to form small patches of mousse. However, no real floating oil layer formed.

The ignition agent burst into flames when it contacted sea-water and the temperature of the flames was measured with a Leybold-Heraeus Thermotron optical thermometer to be 800-1200°C. White carbide smelling fumes were released from the burning ignition agent (Fig. 12). Moreover, a biting phosphor-oxide like vapour was formed. When the oil itself was set on fire, black fumes indicated incomplete combustion. No serious heating of the sediment was measured during the fire. At a depth of 1 cm, an increase of only 3-4°C was measured, while at a depth of 3 cm there was no change at all.

Tabel 4: Composition of F3 oil used in OPEX 1986.

component	OPEX amount $(mg.kg^{-1})$
C- 8	17,6
C- 9	15,4
C-10	15,0
C-11	12,2
C-12	13,9
C-13	10,5
C-14	9,3
C-15	8,1
C-16	6,5
C-17	6,4
Pristane	1,6
C-18	5,6
Phytane	1,1
C-19	4,1
C-20	3,8
C-21	4,7
C-22	3,4
C-23	2,2
C-24	2,8
C-26	1,3
C-27	2,5
C-28	1,1
C-29	1,3
C-30	1,0
C-32	0,67
Other peaks	191
Total	343
UCM	104

After the fire a white material was suspended in the water. After sedimentation a thick layer of this material covered the sediment surface (fig. 13). It has been suggested that the white material was the remainder of the ignition agent. The calcareous white layer remained for months, encrusted on the sediment surface.
Apart from this white material a black sooty substance covered the sediment (probably the remainder from incomplete combustion of both the oil and the ignition agent). The ignition agent used in the MOTIF no. 2 had an organic coating.

No significant elevation of the concentration of oil was measured in the water in any one of the oil treated MOTIFs. In figure 14 the chromatograms of the analysis of oil in the water, one day after the oil addition were given. Only a slight elevation of oil in the range of C26-C29 alkanes was observed in the 'F3' and 'F3b' MOTIFs.
Because differences in the oil measurements between days are low, and no trend in changes of the oil concentration of the water could be observed, the mean concentration of oil in the water is given for two periods: 13 May - 21 May and 22 may - 3 July, i.e. the mean of 7 observations (table 5).

Table 5: Oil concentrations in the water measured in µg/l.
Mean values of two periods (± standard deviation)

		13 May - 21 May (n=7)	22 May - 3 July (n=7)
alkanes			
'C'	MOTIFs	30 (±22)	14 (±10)
'F3'	MOTIFs	39 (±27)	11 (±11)
'F3m'	MOTIFs	31 (±19)	15 (± 8)
'F3b'	MOTIFs	50 (±23)	18 (± 7)
other compounds			
'C'	MOTIFs	148 (±23)	24 (±17)
'F3'	MOTIFs	145 (±22)	18 (±16)
'F3m'	MOTIFs	161 (±33)	25 (±13)
'F3b'	MOTIFs	172 (±24)	27 (±13)
total			
'C'	MOTIFs	177 (±35)	38 (±27)
'F3'	MOTIFs	184 (±32)	29 (±27)
'F3m'	MOTIFs	192 (±43)	40 (±20)
'F3b'	MOTIFs	222 (±42)	45 (±20)

The differences between both periods are hard to explain. They may be related to the use of an improved detection technique used in the GC-method during the second period, or to the fact that the extracts of the samples of the second period were not analyzed immediatly. No differences were found between chromatograms of the water analysis of the different MOTIFs in June or September (Fig. 15).

In figure 16 the chromatograms of the analysis of oil in the upper 1 cm soil layer are given. Compared to the GC of the original F3 oil, no dominance of short-chain alkanes is observed in the oil penetrating the soil,indicating an evaporation of these components.
Table 6 lists the concentrations of alkanes (table 6a), other oil compounds (table 6b), the UCM fraction (table 6c) and the sum of these fractions (table 6a) in the different soil layers in the MOTIFs at various sampling dates. The measurements of paired MOTIFs are averaged.
Just as in the analysis of oil in the waterphase, the measurements done before 23 May were somewaht higher than after that date, although the differences are less pronounced in the sediments.
In the 'C' MOTIFs the concentration of alkanes are less than 1 mg per kg dry sediment. Other compounds yield ca. 3 mg per kg dry sediment. The UCM fraction is completely absent in these sediments.
The highest concentrations of oil were found in the 'F3' MOTIFs. Initially, more than 400 mg alkanes and 300 mg other compounds were measured in the upper 1 centimeter. These concentration gradually declined until only a slightly elevated concentration (4 $mg.kg^{-1}$ alkanes, 5 $mg.kg^{-1}$ other compounds) remained in January 1987. The oil concentrations decreased with increasing soil depth. At a depth of 1-2 cm maximum concentrations (65 $mg.kg^{-1}$ alkanes, 115 $mg.kg^{-1}$ other compounds) were found 7 weeks after the oil application, while at a depth of 2-10 cm maxima (45 $mg.kg^{-1}$ alkanes, 65 mg/kg^{-1} other compounds) were found 4 months after the oil application. This indicates burrowing of oil into the sediment due to bioturbation. At depths beyond direct influence of bioturbation (25-30 cm) no significant elevation of the oil was observed.

In the 'F3m' MOTIFs the oil concentrations in the 0-1 cm layer were about 10% of that of the 'F3' MOTIFs during the first days. The concentration of oil in the sediment of these MOTIFs rapidly declined. Within 4 weeks background levels were reached.
In the 'F3b' MOTIFs the concentration of oil in the upper 1 cm layer was not much lower then that of the 'F3' MOTIFs. The penetration of oil to deeper layers and the decline of the concentration of oil is somewhat slower compared to the 'F3' MOTIFs.

In table 7 list the mean concentration of alkanes in the upper 10 cm of the sediment of the different MOTIFs. During the first two days after the oil application the concentrations observed in the 'F3m' and 'F3b' MOTIFs were 11-15% respectively 75-76% of those in the 'F3' MOTIFs.

Table 7: Total alkane concentrations (C10 - C32) in sediment (mg. oil/kg dry sediment): mean of 0-10 cm depth.

	'F3b'	'C'	'F3m'	'F3 '
13/5/86	-	0.5	5.9	55.4
14/5/86	25.5	1.2	4.9	33.6
16/5/86	19.5	0.6	6.2	26.1
20/5/86	9.3	1.3	7.5	9.0
23/5/86	7.8	0.8	4.9	7.0
26/5/86	5.8	0.4	3.9	10.1
30/5/86	6.0	0.1	2.5	25.6
5/6/86	3.7	0.4	1.3	7.4
11/6/86	2.9	0.1	2.0	9.6
20/6/86	3.4	0.5	2.1	22.4
24/7/86	9.7	0.1	6.1	24.7
4/6/86	8.3	0.1	0.4	43.4
29/1/86	1.4	0.1	0.4	0.7

Chromatograms of oil in the upper 10 cm of the 'F3' and 'F3b' MOTIFs, at 1, 2, 4 and 8 months after the oil addition, indicate that especially the longer chained alkanes dissapeared with time, resulting in a redominance of short-chained alkanes.

Table 8: Retention times of the alkanes in the GC-procedure used for analysis of oil in OPEX 1988 (see figures 10, 14, 15, 16, 17 and 18).

Alkane	Retention time (minutes)
C- 8	1.46
c- 9	2.14
C-10	2.93
C-11	3.74
C-12	4.53
C-13	5.27
C-14	5.95
C-15	6.61
C-16	7.22
C-17	7.81
pristane	7.89
C-18	8.36
phytane	8.45
C-19	8.90
C-20	9.40
C-21	9.94
C-22	10.60
C-23	11.46
C-24	12.58
C-26	16.07
C-27	18.74
C-28	19.00
C-29	20.00
C-30	21.00
C-32	22.00

3.2 MACROZOOBENTHOS

3.2.1 Cerastoderma edule

By far the highest initial mortality was observed in the 'F3b' MOTIFs (Fig. 21). A slightly increased mortality was found in MOTIFs treated with 'F3' only. The pattern of densities observed in large macrofaunal samples (LMS's) confirmed almost 100% cockle mortality in 'F3b' MOTIFs and about 25% mortality in the 'F3' MOTIFs (Fig. 22). From December onwards mortality in the 'C' MOTIFs increased, especially in one of them (Fig. 23).
The fastest initial shell growth was found in the 'C' MOTIFs (Fig. 24). However, from autumn onwards the highest growth values were found among the few survivors of the 'F3b' treatment, while 'F3' cockles had intermediate values. Changes in the tissue condition indices showed the same long term picture (Fig. 25). From autumn onwards cockles from the 'F3b' MOTIFs showed condition indices which were twice as high as those of control animals. Condition indices may be regarded as biomass values which are corrected for size. This means that the biomass of 'F3b' cockles increased more than expected on the basis of increased growth of shell length only (Fig. 26). In the 'F3' MOTIFs, high individual growth compensated for higher mortality in terms of total biomass (Fig. 27).
Reproductive development was similar in all treatments (Fig. 28). Good condition in 'F3' and 'F3b' animals at the end of the experiment was reflected by a high percentage of individuals in stage 3 and 4: 60% (n=38) in the 'F3' MOTIFs; 100% (n=2) in the 'F3b' MOTIFs and 28% (n=43) in the 'C' MOTIFs. The difference between 'F3' and 'C' MOTIFs was significant ($p< 0.01$, test of independence using G-statistic; Sokal and Rohlf, 1981).

3.2.2 Macoma balthica

In this species the mortality pattern was comparable to that of Cerastoderma. (Fig. 29 and 30). In the 'F3b' MOTIFs, however, a larger part of the population survived (20%). Cumulative mortality of Macoma, based on the number of empty shells appearing at the surface, was underestimated to a greater extent (a factor of 2) than in Cerastoderma (Fig. 31).
In late spring and summer the control animals had the highest mean shell length, but were later passed by the survivors of the "F3b' treatment (Fig. 32). A clear short-term effect on tissue condition indices was seen, with the largest decrease in 'F3b' values, smaller decreases in 'F3' and 'F3m' and an increase in the 'C' values (Fig. 33). In summer, condition indices were more comparable but in autumn and winter, survivors of the 'F3b' treatment showed the highest values, with the condition indices 1.5 times of the control animals. Differences in total biomass decreased during the year, but the effect of mortality in the 'F3b' and 'F3' MOTIFs was still obvious at the end of the experiment (Fig. 34 and 35).
Reproductive development of the F3 and 'F3m' individuals was identical to those in the 'C' MOTIFs (Fig. 36). In the survivors of the 'F3b' treatment, gonad development was accelerated. In autumn, the part of the population in stage 4 or 5 was 46% (n=165), 42% (n=173) and 45% (n=109) in the 'C', 'F3m' and 'F3' MOTIFs respectively, while in the 'F3b' MOTIFs this was already 76% (n=29). The difference between F3b and C was significant ($p < 0.01$, test of independence using G-statistics).

3.2.3 Mytilus edulis

An appreciable mortality of mussels was only found in the 'F3b' MOTIFs. The larger individuals, introduced in the MOTIFs for additional stress-parameter measurements, seemed to be more sensitive than the smaller mussels used for growth measurements (table 9).

Table 9: Percentage of dead mussels (Mytilus edulis) found in the cages:
A: Mussels (2-3 cm length); mean mortality April 1986 - March 1987.
B: Mussels (4-4.5 cm length); mean mortality April 1986 - June 1987.

		A	B
'C'	MOTIFs	6%	8%
'F3m'	MOTIFs	6%	11%
'F3'	MOTIFs	7%	10%
'F3b'	MOTIFs	21%	45%

The individual biomass of the mussels is shown in figure 37. An inhibition of growth was observed in specimens from the 'F3', 'F3b' and 'F3m' MOTIFs, immediatly after the oil application. At the end of May, the differences between the MOTIFs were maximal and from this date onwards the growth of the 'F3b' mussels was excessive. Somewhat later, the 'F3' mussels showed an acceleration in growth.
From September onwards, biomasses of the mussels were more or less constant, although the 'F3b' mussels continued to grow until November. Final biomass of the 'F3m' mussels was significantly heavier (170% of the 'C' mussels), while the 'F3b' mussels had even higher biomasses (250% of that of 'C' mussels).

A similar trend was seen for the length of the mussels as a growth parameter although again this was somewhat delayed following the oil addition (Fig.38).

All mussel spat juveniles that had settled in the fine sediment of the MOTIF tidal channels were collected and weighed at the end of the experiment. The lowest recruitment was seen in the 'F3b' MOTIFs, but here, individual AFDW and tissue condition indices were the highest. With respect to these parameters, the mussels from the 'F3' MOTIFs were intermediate between the 'F3b' mussels at one side and the 'F3m' and 'C' mussels at the other side (table 9).

Table 10: Recruitment rate (n), length/growth (mm), individual ash free dry weight (mg) and tissue condition index of mussels (Mytilus edulis) settled in the tidal channels.

Treatment	'F3b'		'C'		'F3m'		'F3'	
MOTIF	1	2	3	4	5	6	7	8
n	12	9	20	46	21	34	18	19
Length (mm)	38.4	45.4	31.5	35.0	30.1	37.6	38.9	38.4
St.err.	1.6	1.8	1.4	.6	1.7	1.0	2.1	1.7
ind AFDW(mg)	419.9	634.0	151.6	218.6	144.1	271.5	410.2	328.3
St.err.	47.6	67.7	16.4	11.3	26.8	21.1	59.3	40.7
CI	7.14	6.75	4.71	4.90	4.50	4.82	6.22	5.41
St.err.	.30	.47	.21	.10	.21	.19	.28	.30

3.2.4 Arenicola marina

A pretreatment mortality of about 5 m^{-2} was observed. One day after ignition of the oil in the 'F3b' MOTIFs, lugworms had stopped producing casts. Many worms appeared at the sediment surface and died subsequently. After a fortnight Arenicola resumed bioturbation at a constant rate (Fig. 39). Survivorship in the 'F3b' MOTIFs appeared to be only 20% of that observed in the Controls (Fig. 40). Bioturbation activity, as a result of 'F3' only, showed a dip of 35% but no mortality occurred. One day after the addition of F3, fresh casts were produced next to old casts, which indicated that the lugworms were digging new burrows. A general decrease in numbers during the year was seen. Apart from natural mortality, this was partly caused by migration into sampled quadrats and also by mortality due to disturbance of the bottom layers by sampling instruments.

Individual ash-free dry weight values showed large variations (Fig. 41). Initially, the highest weights were found in 'C' lugworms and the lowest in the 'F3b' treatments. 'F3' and 'F3m' MOTIFs showed intermediate values. A decrease at the end of the summer was apparent, probably as a result of spawning.

Settlement of juvenile Arenicola occurred especially in the 'F3' MOTIFs and in the 'F3m' MOTIFs number 5 (Fig. 42). This is confirmed by the number of juveniles found in LMS samples (Fig. 43) and by the number of casts which were present in the tidal channel. In this part of the MOTIF the water supply enters directly when the tide is out.
Some larvae entering with the intake water settled in the thin layer of sand at the bottom of the tidal channel, probably because movement towards the tidal flat was restricted at low water. By September 6, no casts of juveniles were present in the tidal channels of 'F3b' and 'C' MOTIFs (1,2 and 3,4). However, in the 'F3m' and 'F3' MOTIFs (5,6 and 7,8) the total cast numbers of juveniles was 21, 4, 16 and 40 respectively.
The total biomass of Arenicola was reduced in the 'F3b' MOTIFs (Fig. 44).

3.2.5 Nereis diversicolor and Nereis virens

Population density started to increase in July. The highest abundances, of up to 3000 m^{-2}, occurred in the 'F3m' MOTIFs. The 'F3b' MOTIFs showed the lowest densities throughout the experiment. 'F3'and 'C' MOTIFs had intermediate densities which never exceded 1000 individuals m^{-2} (Fig 45).
The abundance pattern showed a striking relation to MOTIF rank: LMS 2, 3 and 4 all resulted in highest numbers in MOTIF 6 and decreasing numbers in both directions towards MOTIFs 2 and 8. MOTIF 1 had low numbers but none the less 20 times as much as in MOTIF 2. It was remarkable that in this MOTIF 1, in LMS and 4, 89 and 88% of all Nereis was found in two of the four subsamples. These subsamples were located in the north-western part of the MOTIF.
The individual ash free dry weight showed a reciprocal pattern to that of density, i.e., those MOTIFs with the lowest numbers of individuals ('F3b') had the highest AFDW values, while the 'F3m' MOTIFs with the highest densities of Nereidae had the lowest individual ash free dry weights (Fig. 39).

In the latter MOTIFs individual ash-free weight was about seven times the control value.
Reciproke development of abundance and individual ash-free dry weight led to much less diferences in total biomass (Fig. 47). The low abundance in MOTIF 2 resulted in a low total biomass, however.
With the fourth large macrofauna samples an attempt was made to separate individuals of Nereis diversicolor and N. virens. All Nereis in the 'F3b' treatment and 50% in the F3 MOTIFs number 8 belonged to Nereis virens. In other MOTIFs the majority of specimens belonged to Nereis diversicolor.

3.2.6 Corophium volutator

Numbers started to increase in June and reached a maximum of about 10000 m^{-2} in September, but only in the Control and 'F3m' MOTIFs (Fig. 48). Individual ash-free dry weights were high at the start of the experiment (introduced animals). A June decrease in the 'C' and 'F3m' MOTIFs and in August in the 'F3' and 'F3b' MOTIFs indicated mortality of large individuals and the appearance of a new generation (Fig. 49). In the 'C' and 'F3m' MOTIFs the winter generation consisted of many small Corophium. In the 'F3' and 'F3b' MOTIFs, the experiment ended up with a few large individuals having a weight of circa 3 times that of the the control animals.
In the 'C' and 'F3m' MOTIFs, maximum total biomass was reached before maximum density (August) (Fig. 50). In these treatments a summer generation of large animals was probably present.

3.2.7 Hydrobia ulvae

A new generation of Hydrobia appeared at the end of July (Fig. 51). In the 'C', 'F3m' and in 'F3' MOTIFs, high densities were found. The 'F3b' MOTIFs showed lowest abundances, while the other 'F3' MOTIF showed intermediate densities. Individual ash free dry weights showed a reciprocal development in comparison to abundance (Fig. 52). Mudsnails from the 'F3b' MOTIFs were twice as heavy as 'C' and 'F3m' animals. At the end of the experiment total biomass was similar in all treatments (Fig. 53).

3.2.8 Littorina littorea

In the 'F3b' MOTIFs resulted in a high mortality of periwinkles: only 30% survived (Fig. 54). These survivors had a high growth of shell length during summer, especially animals in one of the 'F3b' MOTIFs (no. 2) (see Fig. 55).

3.2.9 Capitella capitata

At the end of the experiment low densities of about 80 m^{-2} were found in the 'F3m' MOTIFs. In all other MOTIFs, densities of 250 to 1100 m^{-2} were reached.

3.2.10 Total biomass (mussels excluded)

At the start of the experiment a large part of the total biomass consisted of Arenicola marina (Fig. 56). This species was also responsible for development of the total biomass directly after the experimental treatment. During summer Cerastoderma and Nereis are contributed to a greater extend to the total biomass measurements.
At the end of the experiment total biomass ranged from 20 g AFDW m^{-2} in the 'C' MOTIFs to 24 g m^{-2} in the 'F3m' and 'F3' MOTIFs. In the two 'F3b' MOTIFs, only 9.5 (no. 1) and 4.3 g (no. 2) was present. The main cause of the differences between these MOTIF pairs was the variation in Nereis.

3.3 BIOACCUMULATION

3.3.1 Mussels

The highest internal concentrations of oil were determined in mussels from the 'F3' MOTIFs, immediately after the application of "F3' oil. Towards September the internal oil concentration in the above 'F3' mussels decreased to levels similar to that in 'C' mussels (table 11).

In the 'F3b' and 'F3m' MOTIFs the mussels had only slightly elevated internal oil concentrations during the month following the dosing of oil. It was remarkable that the 'F3b' mussels did not started to accumulate oil before 3 days after the oil dosage and ignition.

In figure 57 some chromatograms of oil in mussels tissues were given. Especially oil compounds with a relative low retention time (i.e. alkanes < 24-C atoms) were accumulated by mussels.

A striking feature is the level of oil concentrations determined in mussels harvested after the winter period in March 1987. The internal oil concentrations of mussels from the 'F3b' and 'F3' MOTIFs were relatively abnormally high compared to those of mussels harvested in the summer of 1986 whereas 'C' mussels always had similar background concentrations. The oil concentrations in the 'F3m' mussels were only slightly elevated above background levels. Whether the high oil concentrations determined in mussels collected in March '87 were the result of winter stress, or variation in chemical analyses due to differences in storage time before analysis is hard to say. The mussels collected in March, 1987 were analyzed immediately after their harvest, while those collected in 1986 were stored in a freezer at -20°C for 3 - 7 months before they were analyzed in December 1986.

Table 11: Internal oil concentration in mussels (Mytilus edulis) (mg oil kg^{-1} ash free dry weight).

a) C8-C32 alkanes

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
15/ 5/86	50.97	52.38	88.15	327.20
26/ 5/86	87.86	-	-	128.21
5/ 6/86	66.35	-	-	150.50
3/ 7/86	70.13	-	-	58.41
4/ 9/86	53.07	92.65	-	74.72
2/ 3/86	539.13	26.20	91.25	647.49

b) other compounds

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
15/ 5/86	327.65	436.30	700.22	942.94
26/ 5/86	770.46	-	-	610.74
5/ 6/86	468.98	-	-	755.63
3/ 7/86	225.47	-	-	316.09
4/ 9/86	201.53	433.29	-	359.71
2/ 3/86	5006.90	208.75	334.88	5389.85

c) UCM fraction

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
15/ 5/86	190.40	128.50	415.64	764.64
26/ 5/86	297.69	-	-	635.79
5/ 6/86	406.31	-	-	781.56
3/ 7/86	205.77	-	-	558.40
4/ 9/86	321.49	162.63	-	391.80
2/ 3/86	377.65	98.88	47.18	425.60

note: -: not measured

3.4.2 Cerastoderma edule

The internal oil concentration of the 'F3' cockles were much larger than that of the 'C' cockles up to the end of the experiment. This holds especially for the fractions 'other compounds' and U.C.M.. The oil concentrations of 'F3m' cockles were only slightly elevated compared to the 'C' cockles. Cockles of the 'F3b' MOTIFs were only sampled and analyzed three days after the fire; on the other dates not enough cockles could be collected for chemical analyses. The concentration of oil in the 'F3b' cockles were intermediate between those of the 'F3' and 'F3m' cockles (table 12). In figure 58 some chromatograms of oil in cockle tissues were given.

3.4.3 Macoma balthica

For Macoma the highest internal oil concentrations were found in the 'F3m' MOTIFs wereas the concentrations in the 'F3' specimens were slightly lower. In September internal concentrations of the alkanes reached similar levels as found in the control Macoma's. The other peaks and U.C.M. remained high until January. The 'F3b' Macoma's did not show an increase of internal oil before June (table 13). In figure 59 some chromatograms of oil in Macoma tissues were given.

Table 12: Internal oil concentration in cockles (<u>Cerastoderma</u> <u>edule</u>) (mg oil kg^{-1} ash free dry weight)

a) C8-C32 alkanes

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
16/ 5/86	211.73	41.86	56.71	587.11
5/ 6/86	+	-	34.46	98.63
3/ 7/86	+	-	63.05	4.95
4/ 9/86	+	53.07	23.75	25.05
29/ 1/87	+	-	-	25.95

b) other compounds

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
16/ 5/86	314.65	78.53	113.61	1084.33
5/ 6/86	+	-	288.22	227.60
3/ 7/86	+	-	90.52	5328.42
4/ 9/86	+	125.94	279.41	236.32
29/ 1/87	+	-	-	651.30

c) UCM fraction

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
16/ 5/86	118.93	-	23.01	417.39
5/ 6/86	+	-	60.96	101.27
3/ 7/86	+	-	18.12	12.97
4/ 9/86	+	-	202.64	71.99
29/ 1/87	+	-	-	371.06

note: +: to less surviving organisms

-: not measured

Table 13: Internal oil concentration in balthic tellins (<u>Macoma balthica</u>) (mg. oil.kg^{-1} dry weight).

a) C8-C32 alkanes

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
16/ 5/86	28	47	153	136
5/ 6/86	111	-	236	170
3/ 7/86	+	-	121	107
4/ 9/86	88	52	41	53
29/ 1/87	+	-	-	33

b) other compounds

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
16/ 5/86	243	444	565	185
5/ 6/86	3500	-	1035	754
3/ 7/86	+	-	473	325
4/ 9/86	479	323	796	727
29/ 1/87	+	-	-	489

c) UCM fraction

Date	MOTIFs 'F3b'	MOTIFs 'C'	MOTIFs 'F3m'	MOTIFs 'F3 '
16/ 5/86	86	87	55	103
5/ 6/86	71	-	87	139
3/ 7/86	+	-	172	221
4/ 9/86	0	-	199	258
29/ 1/87	+	-	-	88

note: +: to less surviving organisms

-: not measured

3.4 BENTHIC MEIOFAUNA

Benthic meiofaunal organisms were concentrated in the upper 0-1 cm of the sediment. Densities in the 1-2 and 2-10 cm layers were always much lower and in these layers no changes in densities were found after the addition of oil.

Therefore only observations of the upper 1 cm sediment layer were reported here.

At the time of oil addition, meiofaunal densities were low, despite innoculation with Mok Bay sediment (controls; 10 ind. per cm^{-2}, Mok Bay: 30 ind. cm^{-2}). Oil was therefore added to a developing meiofaunal population.

Treatment of the oil by burning ('F3b' MOTIFs) appeared to kill all nematodes (table 4) and other meiofaunal organisms, and it was only after a three week lag period that a new population started to develop. Mortality was also high in the 'F3' and 'F3m' MOTIFs. Until at least 3 months after the oil dosage, the behaviour of the remaining nematodes was sluggish. Low densities of the other meiofaunal groups did not allow conclusions to be made about acute effects.

Table 14: Percentage dead nematodes in MOTIFs (0-1 cm; n=100).

Treatment	MOTIF	Sampling date					
		6/5	14/5	23/5	29/5	17/7	8/8/86
'C' MOTIFs	3	11	6	12	23	0	0
	4	8	10	48	18	-	0
'F3m' MOTIFs	5	-	27	70	48	29	0
	6	-	22	90	37	-	0
'F3' MOTIFs	7	5	67	50	78	42	0
	8	3	84	74	76	-	0
'F3b' MOTIFs	1	8	100	100	100	-	0
	2	8	100	100	100	-	0

Population development of nematodes (by numbers the most important meiofaunal group) showed a sharp increase in numbers initially, followed by a slow but continuous rise, in the control MOTIFs (Fig. 60); Mechanical treatment of the oil ('F3m' MOTIFs) caused only slightly inhibited population development. The 'F3' MOTIFs showed a higher summer peak followed by lower densities in the autumn. The same applied to the 'F3b' MOTIFs, however, which had summer densities of about 1000 nematodes cm^{-2}. This summer peak in nematode density was caused by the benthic diatom feeding species Chromadora nudicapitata (Fig. 61).

Harpacticoid copepods showed two peaks, one in July and one in September/ October (Fig. 62). In general however, the oil seemed to have little effect on density. Oligochaetes were found in higher densities in both the 'F3' and 'F3b' MOTIFs (Fig. 63). Turbellarian densities increased towards a summer peak in all MOTIFs (Fig. 64). However, in the 'F3' and 'F3b' MOTIFs this increase was delayed in comparison to the controls.

Ostracods were the only group of meiofaunal organisms that did not show any population development in the oil contaminated MOTIFs during the summer period. The controls showed an expected summer increase in densities (Fig. 65).

3.5 ZOOPLANKTON

3.5.1 Calanoid copepods

Figures 66-68 show the densities of nauplii, copepodite and adult calanoid copepods during the experiment. As would be expected in animals with short lifecycles, the major peaks for both the juvenile stages and the adults occurred in quick succession. Thus for all stages, peaks were recorded in June and early July in the 'C' MOTIFs, while smaller peaks were observed in the 'F3m' MOTIFs at the same time. Activity of all stages was severely reduced in the 'F3' and 'F3b' MOTIFs with no obvious maxima. The numbers of all three stages in the intake water were very low until August 1986; a net production of calanoid copepods in the MOTIFs is thus assumed. A very small peak of naupplii was noted in the 'F3b' MOTIFs in August.

The most important species was *Erytemora velox*, which was largely responsible for the June bloom. Figure 69 represents the densities of all stages of this species combined. The aforementioned small August bloom in the 'F3b' MOTIFs was due to the development of *Acartia clausi* (Fig. 70).
Other species, such as *Temora longicornis* (max. 2 per litre), *Centropages hamatus* (max. 1.5 per litre) and *Pseudocalanus elongatus* (max. 0.4 per litre) were less abundant in the MOTIFs.

3.5.2 Harpacticoid copepods

The highest numbers of Harpacticoid copepods occurred in the late June -early July period in the 'F3m' and 'F3' MOTIFs (ca. 500 copepods per l^{-1}. Simultaneously, a peak of ca. 200 l^{-1} occurred in the 'C' MOTIFs. In the 'F3b' MOTIFs however, peak activity was reduced to <100 per litre and was delayed by at least two weeks. A second but smaller increase in densities occurred in early September to mid October. Highest numbers of copepods (ca. 100 l^{-1} occurred in the 'C' MOTIFs, with sligtly lesser numbers in the 'F3m' MOTIFs and hardly any abundance in the 'F3' and 'F3b' MOTIFs (Fig. 71).

3.5.3 Bivalve larvae

The highest bivalve larval densities occurred in the 'C' MOTIFs (13 per litre), with slightly lesser numbers in the 'F3m' MOTIFs and severely reduced number's in the 'F3' and 'F3b' MOTIFs (Fig. 72). In general, peak densities occurred in May-June, with little further activity, except for some very small peaks in the 'C' MOTIFs in July and August. The continuous low densities of larvae in the uptake water suggests that the bivalve larvae presented in the water of the MOTIF system in May-June were indigenous to these MOTIF systems.
As the identification of bivalve mollusc larvae is difficult and very time consuming, no attempts were made to determinations on the species level.

3.5.4 Polychaete worm larvae

The highest densities of polychaete worm larvae occurred in the period July to September (Fig. 73). Highest numbers occurred in the control and 'F3m' MOTIFs. Activity in the 'F3b' and 'F' MOTIFs was inhibited; except for some abundancy of low numbers in the 'F3b' MOTIFs from August to November. In general, the major peaks of larval activity in the MOTIFs coincided with higher numbers entering via the intake water, but the relative high numbers compared to the intake water suggests that most of the larvae are indigenous to the MOTIF systems.

3.5.5 Gastropod larvae

Gastropod larvae were detected form April through to November, with highest densities occuring in two phases (Fig. 74). From May to July, the highest densities occurred in the 'C' and 'F3m' MOTIFs, while densities in the 'F3' and 'F3b' MOTIFs were clearly depressed. However, from August onwards, this trend was reversed with highest numbers occuring in the 'F3' and 'F3b' MOTIFs. Where the gastropod larvae are concerned, the numbers entering via the intake water were relatively high throughout the experiment.
The majority of larvae entered via the inflow of Waddensea water.

3.5.6 Nematode larvae

In May a relative high numbers of nematode larvae appeared in the control MOTIFs. In the 'F3m' MOTIFs their density was somewhat lower, while in the 'F3b' and 'F3' MOTIFs almost no larvae were observed until half May. A second bloom was observed in July in the 'F3b' MOTIFs, at the same time as a high import via the intake water took place. In the other MOTIFs the density of nematode larvae remained low during this period (Fig. 75).

3.5.7 Microzooplankton

A peak of rotifers was observed in May in the control MOTIFs (Fig. 76). In the 'F3m' MOTIFs this bloom was somewhat reduced and postponed for 2-3 weeks. In the 'F3b' and 'F3' MOTIFs, almost no rotifers were observed during this period. A second peak did however appear in the 'F3b' MOTIFs from July to September.
Tintinids occurred in very low densities (less than 3 per litre). Until September their density was inhibited in the 'F3' and 'F3b' MOTIFs compared to the controls, while after September they were only seen in the 'F3' and 'F3b' MOTIFs.

3.6 FYTOBENTHOS

The highest concentration of chlorophyll in the sediment was found in the upper 1 centimeter layer. the concentrations in the deeper layers did not exceed 2 chlorophyll per kg dry sediment, and no differences between MOTIFs were observed. Therefore only the observations of the upper 1 cm were reported here (Fig. 77). Immediately after the application of the oil a sharp fall of the chlorophyll concentration was seen in the 'F3b' and 'F3m' MOTIFs.
In the period June-July, the chlorophyll concentration in the 'C'-MOTIF decreased, like that in the 'F3m' MOTIFs. The chlorophyll concentration in the 'F3' MOTIFs was substantially higher during this period, while in the 'F3b' MOTIFs the highest concentrations were found.
From August onwards the chlorophyll concentrations in the 'F3' and 'F3m' MOTIFs fell below that of the 'C'-MOTIFs. The concentration in the 'F3b' MOTIFs remained relatively high, especially from October onwards.

No significant change in species diversity in benthic microalgae as a consequence of oil treatments could be detected. However, some changes in abundance of species were observed (Fig. 78: abundancy of genera, Fig. 79: abundance of dominant species).

Stauroneis species were amongst the most abundant species at the time of the oil application. It was observed that the abundancy of Stauroneis gradually declined in the 'C' MOTIFs, while in the other MOTIFs their density remained high, especially in the 'F3b' MOTIFs. This was mainly due to S. constricta.

Navicula digitoradiata and an Amphora species only appeared in a high abundance in the 'C' MOTIFs, whereas N. peregrina was only found in the 'C' and 'F3m' MOTIFs. Nitzschia closterium and Caloneis amphisbaena had the greatest abundant in the 'F3m' MOTIFs. Nitschia was also abundant in the 'F3' MOTIFs. Both species were virtually absent in the 'F3b' MOTIFs.

Fragilaria species were somewhat reduced in the 'F3' and 'F3b' MOTIFs.

Navicula arenaria showed some initial low abundance in the oil treated MOTIFs, compared to the 'C' MOTIfs. 56 days after the oil treatment its abundance in the 'F3' and 'F3b' MOTIFs was high compared to the 'C' MOTIFs.

Tropidoneis occurred in all of the oil treatments, especially in the 'F3' MOTIFs, but was absent in the controls.

Other species did not show a marked difference in appearance between the various MOTIFs.

The gross primary production (GPP) of the microphytobenthos was strongly dependent on solar radiation, temperature and chlorophyll concentrations. In the 'C' MOTIFs the GPP was calculated to vary between 140-180 mg. O_2 $m^{-2}.h^{-1}$ until mid September, between 80-100 mg. O_2 $m^{-2}.h^{-1}$ from mid September until October, and about 20 mg. O_2 $m^{-2}.h^{-1}$ in November (fig. 80). Until mid September the GPP in the 'F3b' MOTIFs was low compared to the 'C' MOTIFs and in mid August it was only 40% of that of the controls. From then onwards the GPP in the 'F3b' MOTIFs steadily increased, and from mid September it was high in the 'F3b' MOTIFs compared to the control situation. By mid November the GPP in the 'C' MOTIFs was only 10% of that of the 'F3b' MOTIFs. This increase was primarily due to an increase of chlorophyll concentration in the sediment, as the GPP per unit chlorophyll did not change in the 'F3b' MOTIFs and was constantly varying between 150 and 250 µg. O_2. g. chl^{-1} h^{-1}. The decrease of the 'GPP' in the 'C' MOTIFs was partly caused by a reduction of the 'GPP' per unit chlorophyll. Until mid September this varied between 300 and 500 µg. O_2. g. chl^{-1} h^{-1}, which was significantly higher than in the 'F3b' MOTIFs. However, from mid September onwards it suddenly dropped to levels between 50-200 µg. O_2. g. chl^{-1} h^{-1}, which was lower than that in the 'F3b' MOTIFs (Fig. 81).

A substantial bloom of macroalgae, i.e. Enteromorpha, was found in the 'F3b' MOTIFs (Table 15). In the other MOTIFs almost no Enteromorpha biomass (< 2 gram dry weight) was present on the sampling dates.

A difference between both replicate 'F3b' MOTIFs was observed, basin no. 2 always yielded more Enteromorpha than basin no. 1. Total Enteromorpha biomass harvested from the 'F3b' MOTIFs was 1200 g dry weight (no. 1) and 2800 g dry weight (no. 2). From the other MOTIFs less than 2 gram dry weight was harvested.

Table 15: Biomass of Enteromorpha (gram dry weight) harvested from the 'F3b' MOTIFs.

harvest date	MOTIF 'F3b' no. 1	MOTIF 'F3b' no. 2
8- 8-1986	387	482
14- 8-1986	164	265
20- 8-1986	50	154
4- 9-1986	18	42
18- 9-1986	450	1020
26-10-1986	136	860
total	1205	2823

3.7 PHYTOPLANKTON

In the period June-July there was a net production of algae in the MOTIFs (Fig. 82). A decrease in algal biomass was observed directly after burning of the oil in the 'F3b' MOTIFs. Until July there were no consistent differences between MOTIFs. Maximum chlorophyll density in the 'F3m'-MOTIFs was somewhat earlier and in the 'F3b'-MOTIF, somewhat later when compared to the controls. The density in the 'F3'-MOTIFs was generally lower.

Later on, the density in the 'F3b'-MOTIFs, (and from August onwards also in the 'F3'-MOTIFs) remained at a higher level that that in the control and 'F3m'-MOTIFs, until mid October.

The gross primary production of the phytoplankton in the 'C' MOTIFs was high compared to that of the intake water, especially during the main bloom in May to July (Fig. 83). This was only partly due to higher chlorophyll concentrations, as the production per unit chlorophyll was also somewhat higher (Fig. 84).
A sharp decrease of the primary production was observed in the 'F3b' MOTIFs following the fire. This could also be related to lower algal biomass present after the event. Later on the primary production of the phytoplankton in these 'F3b' MOTIFs was the highest of all MOTIFs.
Until mid June the primary production in the 'F3' MOTIF water was relatively low. There after, it was similar to that of the 'C' MOTIFs, except for blooms in July and October. Differences between the 'F3m' and 'C' MOTIFs were small, except for the July bloom, which was larger in 'F3m' MOTIFs.
In general no differences in the primary production per unit chlorophyll were observed between the various MOTIFs, indicating that algal biomass primarly determines the production. Exceptions might be the relative high very efficient photo-systhesis of the algae in the 'F3' and 'F3m' MOTIFs during the October bloom, and the relative low efficiency of photosynthesis in these MOTIFs in the summerperiod July-September.

Concerning species composition, it was observed that Skeletonema, Chaetoceros, Nitzschia and Phaedactylum were the main genera produced in the 'F3b'-MOTIF (Fig. 85). Skeletonema and Nitzschia was also produced in the 'F3m'-MOTIF, directly after the oil treatment.
The development of microflagellates was delayed in the control MOTIFs compared to the treated ones and the uptake water.
Other species did not reach high densities at any stage in the experiment.

3.8 BACTERIA

At the beginning of a MOTIF experiment a net production of bacteria, is usually observed (Fig. 86). Later on the density in the MOTIFs is generally similar to that of the intake water. Immediatly after the oil treatment higher densities were found in the 'F3m'-MOTIFs. Whether or not these were oil degrading bacteria is unknown. However, later in the experiment the densities in the 'F3' and 'F3b' MOTIFs exceeded those of the controls. The bacterial counts for the 'F3' and 'F3b' MOTIFs were made difficult by high densities of small algae.

3.9 PHYSICO-CHEMICAL FACTORS

Oxygen

A net production of dissolved oxygen was measured in the MOTIFs during the summer (Fig. 87). In the period June to October, highest morning oxygen concentrations were found in the 'F3b'-MOTIFs and the lowest in the controls, while the 'F3' and 'F3m'-MOTIFs had intermediate values. Only burning of the oil caused a sharp decrease of the oxygen concentration for a few days.

pH

pH showed a similar pattern to the dissolved oxygen, with highest levels in the 'F3b'-MOTIFs (Fig. 88). Immediately after the burning of oil a sharp increase in pH was observed in the 'F3b'-MOTIFs. On the other hand the pH in the 'F3'-MOTIFs was relatively low in the first month after the oil dosage. during the summer the pH in the 'C' 'F3m' and 'F3' MOTIFs were similar.

Nitrate

There was a net consumption of nitrate in all MOTIFs until August, except for the 'F3b' MOTIFs immediately after the burning of the oil (Fig. 89). Nitrate may have been a limiting factor during the period May-August. In Autumn nitrate concentration in 'F3b'-MOTIFs were relatively low compared to the controls.

Nitrite

Until August there was a net consumption of nitrite. Directly after the oil applicaton a slight increase was found in 'F3b' and 'F3' MOTIFs (Fig. 90).

Ammonia

Until August there was a net consumption of ammonia. Directly after the oil application a sharp increase was found in the 'F3b' MOTIF. Ammonia may have been limited in summer (Fig. 91).

Phosphate

There was a net production (mineralisation and release from sediments) of phosphate in the MOTIFs (Fig. 92). Until July this was highest in the control MOTIFs. Later on it was highest in the 'F3b'-MOTIFs.

Silicate

There was also a net production of silicate, except for some periods: in May-June there was a silicate depletion in the control and 'F3m'-MOTIFs. In June-July and October onwards there was a net consumption in 'F3b'-MOTIFs (Fig. 93).

Granulometry

There were no significant differences in soil texture as based on grain size and mud content between the various MOTIFs. Mean mud content was about 0.70%, while the median grain size was 230 µm. No differences between depth layers were observed and there was also no change between April and December samples.

4. DISCUSSION

4.1 FUNCTIONING OF THE MOTIFs

In general the ecological development in each pair of similarly treated MOTIFs was quite comparable, especially where the 'C' MOTIFs were concerned. Moreover, the ecological functioning of these control MOTIFs approximates that of natural tidal flats in the nearby Waddensea, especially when the production and biomass values of ecological functioning groups are concerned. There is a small deviation concerning the species composition of the microflora and fauna.

The results of the previous two OPEX years suggested that the production of the systems was food limited, i.e. that the secondary production by zoobenthic and zooplankton species was limited by the primary production available, and that the primary production was limited by the nitrogen available.

The primary production of the microphytobenthos in the 'C' MOTIFs was estimated to vary from 180 mg O_2. m^{-2},h^{-1} (55 mg C.m^{-2} h^{-1}) during the summer months down to 20 mg O_2. m^{-2}. h^{-1} (5 mg C.m^{-2} h^{-1}) in November. This is comparable to the range of 15-70 mg Cm^{-2}. h^{-1} that Cadee & Hegeman (1974) and Colijn (1974) found for the dutch Waddensea.
Nevertheless, just as in 1984 and 1985, the chlorophyll 'a' concentrations in the upper centimeter of the sediment of the 'C' MOTIFs ranged from 2-5 mg kg^{-1}, corresponding to ca. 35-85 mg chlorophyll 'a' m^{-2}. That is somewhat lower than the values of around 80-130 mg chla. m^{-2} that were reported by Cadee & Hegeman (1974) and Colijn (1974) for the dutch Waddensea.
This indicates high grazing rates in the MOTIFs, removing a considerable amount of the primary production. Deposit feeders like *Macoma balthica*, *Arenicola marina*, *Corophium volutator*, grazers such as *Hydrobia ulva*, *Littorina littorea* and various nematode species may be responsible for heavy grazing of the microphytobenthos.
The microphytobenthos community found in the 'C' MOTIFs (table 16)is typical for sandy-silt tidal flats (van den Hoek *et al*, 1983).

Table 16:

Relative abundancies of species in the microbenthic flora of the 'control' MOTIFs at 5 sampling dates (from Lemmen, 1987).

Species	6 may	13 may	20 may	3 july	10 july
Achnanthes hauckiana		1.9			
Achnanthes spec. 1 (5)	1.4				
Achnanthes spec. 2 (12)	2.2				
Amphora coffeaeformis					2.6
Amphora ocellata					2.2
Amphora spec. 1 (2)					2.2
Amphora spec. 2 (4)			5.8	14.8	14.3
Amphora spec. 3 (8)				3.0	5.4
Caloneis amphisbaena	1.4	1.9	3.8		
Cocconeis peltoides	1.2				
Cymatosira belgica	1.2				
Eunoria spec. (1)	3.6				
Fragilaria brevistriata	2.4	4.5	1.8	5.3	11.8
Fragilaria schulzii	2.4		1.3		1.3
Navicula arenaria	3.8	12.0	28.2	4.3	2.9
Navicula biskanteri	3.1	10.4	3.8	6.3	3.8
Navicula cryptocephala		1.6	2.9	2.6	1.3
Navicula digitoradiata				2.3	3.5
Navicula diserta	6.4	5.5	2.2	5.6	3.5
Navicula flanatica		3.6	4.0		
Navicula pelliculosa	9.1	6.2	2.7	4.3	6.4
Navicula peregrina				12.5	3.5
Navicula rostella			1.6		
Navicula salinarum	2.2	2.3			
Navicula spec. 1 (9)			2.0		
Navicula spec. 2 (10)	12.2	2.3	2.2		1.9

continue table 16

Species		6 may	13 may	20 may	3 july	10 july
Navicula	spec. 3 (11)		6.2		5.6	3.5
Navicula	spec. 4 (13)		2.6			1.6
Navicula	spec. 5 (18)					6.7
Navicula	spec. 6 (19)	1.4				
Navicula	spec. 7 (20)	1.2		1.6		1.9
Navicula	spec. 8 (22)	1.4				
Navicula	spec. 9 (23)	4.3		1.3	1.3	
Navicula	spec.10 (24)	2.4				
Navicula	spec.11 (26)	1.9		2.2	6.3	1.6
Nitzschia	closerium		5.2	4.0		
Nitzschia	linearis				6.3	1.3
Nitzschia	palea	2.2	4.6	5.1		1.9
Opephora	spec.	1.7	2.9	1.8	2.3	1.6
Stauroneis	constricta	2.6	4.2	2.0		
Stauroneis	decipiens		2.9	1.8		
Stauroneis	spec. 1 (4)	1.4	2.6	1.1		
Stauroneis	spec. 2 (5)	6.4	1.9	2.2		1.3
Stauroneis	spec. 3 (8)			1.1		

note spec. = species taxonomically not specified, in brackets species numbering by Lemmen (1987)

The primary production of the phytoplankton in the 'C' MOTIFs was estimated to be 500-1000 mg. $O_2.m^{-3}h^{-1}$, which is equal to ca. 150-300 mg. $C.m^{-3}.h^{-1}$. These figures are high compared to those of Cadee & Hegeman (1977) observed in natural Waddensea water. They found production rates of ca. 10-30 mg.$Cm^{-3}.h^{-1}$ during the summer, which is similar to what was found for the intake water of the MOTIFs (20 mg. $Cm.^{-3}$ h^{-1} during summer, but 80 mg. Cm^{-3} h^{-1} during the <u>phaeocystis</u> bloom in May).

The high overall production in the 'C' MOTIFs may well be due to the relatively high chlorophyll concentrations in the water. Chlorophyll concentrations in the water of the 'C' MOTIFs were higher (rising to 60 mg. m^{-3} during the summer bloom) than those measured by Cadee & Hegeman (1979) in the Waddensea (10-40 mg.$m.^{-3}$ during the summer). It also exceeds the

concentration found in the intake water, indicating a considerable net production of phytoplankton within the MOTIFs.
These mean chlorophyll concentration was also higher than in the 1984 and 1985 'C' MOTIFs, where in the latter case, no net production of phytoplankton was recorded (table 17). The differences between 1984/85 and 1986 may be due to a lower density of mussels introduced to the MOTIFs in 1986. The relative high P/B reatio in the MOTIFs indicates a high grazing rate upon fytoplankton in the MOTIFs, like observed for the fytobenthos.
Filterfeeders like mussels (Mytilus edulis) and cockles (Cerastoderma edule) may be responsible for this grazing. There are several indications that food is limited for these filterfeeding organisms in the MOTIFs, and strong intra- and interspecific competition occur.
The species diversity of the phytoplankton community in the MOTIFs (table 18) is rather low as compared to Waddensea water. This also applies to the relatively low quality of the intake water with respect to algal species.

Silicate depletion only occur during the first fytoplankton blooms until mid June, in the control MOTIFs. In the oil treated MOTIFs a net production of silicate was seen during this time. From mid June onwards the silicate concentration increases in the intake water. The intensive algae blooms in the 'F3b', and to a lesser extend in the 'F3', MOTIFs result in relative low silicate concentrations compared to the controls, but no depletion, as reported by van Bennekom et al (1974) for the Waddensea, is observed even in the intake water. It seemed that algal growth in the MOTIFs in nitrogen limited. Just as in 1984 and 1985 the various nitrogen sources were depleted during the summer (May-October). The concentration of nitrogen in the intake water is low too. All the nitrogen entering the MOTIFs is rapidly used by the algae, whereas also nitrogen mineralized in the MOTIFs and nitrogen delivered from the sediments were rapidly assimilated.
There is a net production of phosphate, indicating mineralization and delivery from sediments exceed phosphate assimilation in the MOTIFs. Bacterial numbers of $2\text{-}3 \times 10^6 . \ ml^{-1}$, as counted for the 'C' MOTIFs, were commonly observed in the intake of Waddensea water. The high numbers found in the MOTIFs during the first months of the experiments were probably related to the establishment of the new systems; thus high microbial activity occurred on freshly disturbed organic sources.

Table 17:

Mean chlorophyll concentration during summer period in control MOTIFs.

1983: 11 mg chlor. m^{-3}
1984: 14 mg chlor. m^{-3}
1985: 6 mg chlor. m^{-3}
1986: 19 mg chlor. m^{-3}

Table 18:
List of fytoplanktonic species determined in the water of the MOTIFs in 1986.

Cerataulina bergonii
Chaetoceros sp.
Ditylum brightwelli
Eucampia zoodiacus
Exuviaella balthica
Gymnodinium sp.
Gyrodinium sp.
Melosira nummuloides
Micracanthodinium sp.
Navicula sp.
Nitzschia closterium
Nitzschia delicatissima
Oxyrrhis marina
Phaeocystis pouchetii
Phaeodactylum tricornutum
Plagiogramma sp.
Pleurosigma sp.
Prorocentrum micans
Rhizosolenia delicatula
Rhizosolenia setigera
Rhizosolenia shrubsolei
Skeletonema costatum
Thalassionema nitzschioides
Thalassiosira nordenskioldii
Thalassiosira rotula

Just as in the Waddensea, the zooplankton community in the MOTIFs was dominated by copepods, while in some periods meroplankton (polychaete, gastropod and bivalve larvae) peaks occurred as a result of the spawning activities of the macrozoobenthos. Most of the meroplanktonic larvae originated from outside the MOTIFs, and entered with the intake water.
This is primarly due to the poor quality of the intake water with respect to larval material, as a consequence of the indirect capture of seawater for suppletion of the MOTIFs. For bivalve larvae alone, was there an indication that a large fraction was indigenous to the MOTIFs.
The density of zooplankton in the 'C' MOTIFs was high compared to that occurring in 1985 and may be due to the higher phytoplankton concentrations in 1986. This holds especially for Eurytemora velox, a typical brackish-water species that showed a strong development within the MOTIF systems. Eurytemora was by far the most dominant species in the MOTIFs, whereas the four dominant species of the copepod communities in the Waddensea (i.e. Temora longicornis, Pseudocalanus elongatus, Acartia clausi and Centropagus hamatus) occurred in lower densities in the MOTIFs, indicating that they were not indigineous to the MOTIF systems and entered only via the intake water.

The meiofaunal community was dominated by nematodes, just as on natural tidal flats in the Waddensea area. Although the MOTIFs were innoculated with meiofauna in relatively low numbers, resulting in a poor, unripe community at the start of the experiment, a community comparable to that found in the Waddensea eventually developed. There were only slight differences between the community development in the 'C' MOTIFs and that in the Mok Bay, from which site, the meiofaunal innoculation orginated. Monhystera spec., Spirina parasitifera and Daptonema spp. were more abundant, whereas Atrochromadora microlaima showed a somewhat delayed development in the 'C' MOTIfs compared to the Mok Bay (table 19). Numbers of meiobenthic organisms in the 'C' MOTIFs in 1984/1985 and 1986 are of the same order.
Total biomass of the benthic macrofauna in the 'C' MOTIFs was about 20-30 gram AFDW. m^{-2} (mussels excluded). Beukema (1976) reported that most tidal flats carry about 10-40 gram AFDW m^{-2} of macrozoobenthos (with an average of 30 gram m^{-2}). The initial biomass level at the start of the experiment (ca. 20 gram m^{-2}) exceeded that of the experiments in 1984 and 1985 (ca. 6-10 gram m^{-2}). However, the maximal biomass observed in the summer and the final biomass were similar for the three years. This indicates that the maximal

biomass in MOTIFs is not depending on intial biomass figures, and that the biomass of the macrobenthos is probably regulated by the algal production. Biomass allocation over the various species is quite similar to that described by Beukema (1983) for natural zoobenthic communities in the Waddensea.
As in earlier MOTIF experiments, it was found that the intensity of the larval settlement was less on than natural tidal flats. This is probably caused by the long and indirect way the intake water from the Waddensea. Effects of the water supply system were found in the settlement of Arenicola and Nereis juveniles, although relatively less than in 1984 or 1983 respectively. There was also a strong variation of settlement between paired MOTIFs. It is possible that such factors also affected the settlement of Hydrobia. Improvement of the intake system is therefore highly recommended.

Just as in the former MOTIF-experiments, the average renewal of the water was 20% d^{-1}, resulting in an average water residence time of about 5 days (Flusihing time = 5 days, $t_{\frac{1}{2}}$ = 3,5 days). This was based on an estimation by Postma ( ) for the water exchange in a tidal flat area in the western Dutch Waddensea.
By more recent calculations it has been assessed that this is probably an underestimation (pers. comm. Ridderinkhof, EON) and the residence time of the water in the western Waddensea may well be as high as 12 days. As a consequence, the impact of the benthic system (including the changes exerted by the oil treatments) on the physico-chemical and biological parameters in the water may have been underestimated in the MOTIF experiments so far.

Technically the MOTIFs functioned without major problems. Difficulties may have arisen from a crack in the wall between one of the 'F3b' MOTIFs and a 'C' MOTIF. Some water flowed through the crack from one into the other MOTIF and the size of the crack gradually increased with time. Serious flow-through of water was observed from October onwards. Untill that day no consequences of the flow-through were observed. The extra mortality of cokles in the 'C' MOTIFs no 3 at the end of the experiment might be caused by leakage of toxic components from the 'F3b' to the 'C' MOTIFs.

	MOTIF control 3	MOTIF control 4	Mok Bay a	Mok Bay b	MOTIF control 3	MOTIF control 4	Mok Bay a	Mok Bay b
Monhystera sp.	38	69	-	-	-	-	-	-
Spirina parasitifers	21	9	2	2	-	-	15	15
Onyx sp.	13	9	-	-	-	-	-	-
Daptonema spp.	8	3	-	5	30	37	-	-
Chromadorita guidoschneideri	8	5	-	8	-	-	5	5
Sabatieria pulchra	4	5	25	20	-	-	-	-
Atrochromadora microlaima	4	-	23	20	43	43	5	5
Calyptronema maxweberi	4	-	-	-	-	-	2	5
Microlaimus sp.	-	3	2	-	-	-	5	5
Rhabditis marina	-	3	-	-	-	-	-	-
Odontophora rectangula	-	-	18	25	-	-	-	2
Paracanthonchus caeces	-	-	10	2	-	-	12	2
Eleutherolaimus stenosoma	-	-	8	2	-	1	8	5
Sphaerolaimus spp.	-	-	8	-	-	-	3	-
Metachromadora vivipara	-	-	2	3	-	-	10	8
Paralinhomoeus sp.	-	-	2	-	-	-	-	-
Axonolaimus paraspinosus	-	-	-	10	5	5	2	13
Oncholaimus brachycercus	-	-	-	3	2	4	-	2
Chromadora nudicapitata	-	-	-	-	17	5	-	-
Tripyloides marinus	-	-	-	-	1	4	-	-
Ascolaimus elongatus	-	-	-	-	1	-	-	-
Oxystomatina sp.	-	-	-	-	-	-	-	-
Viscosia viscosa	-	-	-	-	-	-	18	15
Enoploïdes labiatus	-	-	-	-	-	-	15	18
	100%	100%	100%	100%	100%	100%	100%	100%
Number of species	8	7	10	11	7	8	12	13
Nematodes/cm²	9	11	33	30	169	122	39	23
Sampling date	24/4	24/4	7/4	7/4	18/9	18/9	18/9	18/9

Table 19: Species composition of nematode community in control MOTIFs compared to the Mok Bay. Percentage abundance is indicated per species.

During January and February 1986 temperatures were so low that ice formed in the MOTIFs. All MOTIFs were then filled with 25 cm of water, the tidal flow was stopped and pumps and pipes were drained to prevent damage to installations. Most organisms survived the periods of ice cover.

4.2 OIL DOSAGE

The original 'F3' oil-mixture had a much smaller amount of oil than expected. Some 80 litres of an oil-mixture, acquired from an exploratory drilling, was supplied by the

Chemical analysis of a subsample, taken from the upper (and thus oily) layer in the barrel, proved that ca. 50 volumepercentage of this oily layer consist of compounds. The remainder must be water. The subsample was characterized as moussy and unstable.

Due to ignorance of the existance of the water layer, the forced evaporation lasted 100 hours in an attempt to reduce the total volume 45%. This was calculated by Nooijen and Van Asselen (1984) to be the fraction of the volume of F3 oil that would evaporate within 10 hours. Unless the pretreatment of the F3 oil-mixture by 100 hours of forced evaporatin, only a reduction of the volume with 10 litre (ca. 12%) was realized.

When the oil-mixture was tapped from the barrel it was found out that the mixture consists of two layers: a colourless waterlayer of ca. 50 litre and a brownish oil-water mixture at 22 litre.

We can assume that primarily the oil compounds evaporated from the barrel. Therefore we estimate that the oily layer had a volume of 22 litre (remains) + 10 litre (evaporated) = 32 litre. The analysis of this layer proved an oil content of 50 volumepercents, i.e. 16 litre of oil.

It was calculated that in the remaining oily layer the oil content was: 16 litre (initial) - 10 litre (evaporated) = 6 litre. This may be an overestimation when water from the lower waterlayer accumulated in the upper oily layer or when some oil compounds were dissolved in the lower water layer, due to the pretreatment of the oil by forced evaporation. Unfortunately no measurements of oil concentration of the 22 litre oil mixture, that was used in the experiments, was available. Based upon an oil content of 6 litre in the pretreated oil-mixture and an oil content of 16 litre in the original oil-mixture, it can be calculated that 62% of the oil was evaporated during the 100 hours of forced evaporation.

The estimated amount of oil applicated to the MOTIFs will be:

'Control' MOTIFs : no addition of oil

'F3' MOTIFs : addition of 5.4 litre oil-water mixture per MOTIF gives an addition of 1.473 litre of oil per MOTIF, or 82 ml of oil per m^2 tidal flat

'F3m' MOTIFs : addition of 0.7 litre oil water mixture per MOTIF gives an addition of 0.919 litre of oil per MOTIF, or 11 ml of oil per m^2 tidal flat

'F3b' MOTIFs : addition of 3.2 litre of oil water mixture per MOTIF gives an addition of 0.873 litre of oil per MOTIF, or 48 ml of oil per m^2 tidal flat

This can be compared to the application of Forties oil in 1984 and 1985:
1984: addition of 9 litre of oil per MOTIF, or 500 ml per m^2 tidal flat
1985: addition of 1-8 litre Forties oil per MOTIF, or 100 ml per m^2 tidal flat

Therefore it can be seen that the dosage of F3 oil in the 'F'3 MOTIFs was about 80% of the dosage of Forties oil, in the smaller application of Forties in 1985, and only 16% of the dosage in the larger application of Forties in 1984.

4.3 FATE OF THE OIL

From oil analysis of the sediments in the 'F'3 MOTIFs, one day after the oil addition, it can be concluded that from the applied 1473 ml F3 oil per MOTIF, only 355 ml (i.e. 24 volumepercent) was in fact present in the sediment at that time. The remainer (1118 ml) must be evaporated, degraded, dissolved in the water or adsorbed to walls, tidal channels, pumps etc. of the MOTIFs. As there was no real floating oil layer formed, and no measurable concentration of oil in the water column was found, evaporation, degradation and absorption

will be the main loss terms of oil preventing it from accumulation in the sediment. However, visual observations indicate that most of the oil rapidly penetrated the sediment during the first lowtide.
The analysis of oil in the sediments were based upon 12 sampling points per MOTIF, so that an heterogeneous distribution of the oil do not give large variation in oil concentrations measured.

In the experiments with Forties oil some 40-50 % of the oil was measured back in and on the sediments one day after the oil addition (Table 20).

Table 20: Total amount of oil measured in the sediments of the MOTIFs one day after addition of Forties or F3 oil in the OPEX-experiments.

Year	Oil	Application	In sediment	% in sediment
1984	Forties (Fo.5)	9000 ml	4125 ml	46 %
1985	Forties, floating oil layer removed after 1 day (Fo.1,1)	1800 ml	690 ml	38 %
1985	Forties (Fo.1,3)	1800 ml	937 ml	52 %
1986	F3 (F3)	1474 ml	355 ml	24 %

Regardless, the final concentrations of F3 oil in the sediment of the MOTIFs were much lower than those found in the 'Forties' treated MOTIFs in the former oil experiments. In these experiments even high concentrations of oil in the water and floating oil on the water were observed indicating that a large part of the oil remained in the waterphase. Probably the evaporation, degradation and absorption to MOTIF structures was less for Forties oil compared to F3 oil, resulting in relative high accumulation of oil in the sediment (40-50%). The amount of oil initially present in the sediment of the 'F3' MOTIFs was only 51% of that in 'Fo.1,1' MOTIFs, 38% of that in 'Fo.1,3'

MOTIFs, 38% of that in 'Fo.1,3' MOTIFs and 9% of that in 'Fo.5' MOTIFs. In the 'F3m' MOTIFs 54 ml of F3 oil was found in the sediment one day after the oil addition. This is 28% of the 191 ml that was applicated, which is comparable to the percentage of the applied oil found in the sediment of the 'F3' MOTIFs, despite the fact that the applied 'F3' oil was directly raked into the sediment.

However, 4 days after the oil application, there is still 54 ml of oil found in the sediment of the 'F3m' MOTIFs, whereas only 204 ml was found in the sediment of the 'F3' MOTIFs (Table 21). This indicates no further weathering of oil in the sediment of the 'F3m' MOTIFs and some 43% loss of oil from the sediment of the 'F3' MOTIFs. This was probably the fraction of oil that was absorbed on the sediment surface, that could be degraded. Moreover, bioturbation by lugworms was not hampered in the 'F3' MOTIFs, resulting in some delivery of oil from the sediment to the waterphase. In the 'F3m' MOTIFs bioturbation was hampered by the mechanical disturbance of the benthic structure.

Although only 13% of the oil applied to 'F3' MOTIFs was applied to the 'F3m' MOTIFs (simulating a removal of 87% of the oil by the use of mechanical removal as combat technique), after one day the concentration of oil in the sediment of the 'F3m' MOTIFs was 15% of that of the 'F3m' MOTIFs and after 4 days this was even 27%. It is indicating that mechanical removal hamper the "natural" loss of oil that is accumulated in or on the sediment by degradation or release to the water, as a consequence of a deeper accumulation of the oil and disturbance of the bioturbation due to the mechanical removal.

The use of an ignition agent to remove oil from the tidal flat ecosystem was not as succesful as expected. The powerful reaction seen during the use of the ignition agent seemed to be primarily caused by the burning of the agent itself. The use of the ignition agent results in a presence of 16% of the oil that was applied in the sediment one day after the oil addition. From this it can be calculated that at most, only, 35% of the oil was removed from the systems by burning.

However, 4 days after the burning there was still 101 ml oil found in the sediment of the 'F3b' MOTIFs, indicating a loss of only 27% of oil from the sediment. This was less than observed in the 'F3' MOTIFs. As a consequence the use of the ignition agent results in only a reduction of oil present in the sediment of ca. 15% compared to uncombatted oil. The reduced loss of oil is probably caused by the inhibition of the bioturbation following by the burning- and the formation of a calceous layer encrusted on the sediment and impeding delivery of oil from the sediments to the water column.

Table 21: Calculated amount of oil present in the sediments of the MOTIFs 1 and 4 days after oil addition.

	Applied	1 day	4 days	1-4
'C'	0 ml	14 ml	15 ml	+ 7%
'F3'	1473 ml	355 ml	204 ml	-43%
'F3m'	191 ml	54 ml	54 ml	- 0%
'F3b'	873 ml	138 ml	101 ml	-27%

The highest concentrations in the upper 1 cm were found immediately after the oil application. Seven weeks later maximum concentrations were found at a depth of 1-2 cm, while at a depth of 2-10 cm maxima were found 4 months after the oil application. At depths beyond direct influence of bioturbation (25-30 cm) no significant elevation of the oil concentration was observed.

Disturbance of the sediment surface, as a consequence of mechanical removal, causes a relative deep penetration of oil into the sediment directly after the oil addition. A somewhat slower decline of the oil concentration during the first weeks have probably been caused by a lesser degradation or release of oil from the sediment, due to less superficial accumulation of the oil in the sediment of the 'F3m' MOTIFs compared to the 'F3' MOTIFs. The inhibition of the bioturbation activity during a short period following the raking of the sediment may contribute to a lesser penetration of the oil to deeper sediment layers. However, after 4 months background levels were reached in the 0-2 cm sediment layer. At the 2-10 cm depth layer this was reached 8 months after the oil application.

In the 'F3' MOTIFs also no enhancement of the oil concentration in the waterphase was measured.

4.4 BIOACCUMULATION

Bioaccumulation of oil by the mussels correlated well with the concentration of oil in the sediments. Although no high levels of oil were detected in the water of the MOTIFs, the resulting bioaccumulation by the filter feeding mussel indicated a direct relationship between the amount of oil in the sediment and that in the water, from which medium the mussel can take up the oil compounds. There is probably a high turn over of oil in the watercolumn, after release from the sediment.
In particular the high concentrations of oil observed in the mussels collected in March 1987 were remarkable. This indicates a long-lasting low level release of oil from the sediments up to 10 months after the oil application. The reason why the concentrations in these mussels were so high compared to thos found in 1986 is still obscure. It may be as a result of winter stress, or a variation in the chemical analyses, as the mussels collected in March 1987 were analysed immediately, while previous samples were stored for 54 months at 20^{o}C before being analysed in February 1987. The relatively low bioaccumulation of oil by mussels in the 'F3b' MOTIFs on the first sampling dates may be due to reduced activity of the mussels as a result of the burning itself. No decrease in internal oil concentration, observed for 'F3m' and 'F3' mussels was found in the 'F3b' mussels. At the end of the experiment, in March 1987 almost no difference between 'F3b' and 'F3' mussels remained.

The cockles showed a similar bioaccumulation pattern as the mussels, although their internal oil concentrations were relatively higher, especially in the 'F3' and MOTIFs. This may be caused by the benthic way of life of the cockle. In the 'P3m' MOTIFs the internal concentration in cockles was low compared to mussels, probably because of the lower accumulation of oil in the upper 2 cm of the sediment. where the cockle is living.

As a consequence of high cockle mortality due to the burning in the 'F3b' MOTIFs, it was unfortunately impossible to collect enough cockles for oil analysis in these MOTIFs, apart from the first day, at which relatively high internal concentrations were measured, compared to the mussels.

The bioaccumulation of oil by Macoma was also quite similar to that described above, apart from the relatively high accumulation in Macoma from the 'F3m' MOTIFs. It is suggested that this was due to the fact that in the 'F3m' MOTIFs and 'F3b' MOTIFs the activity of the clams was not inhibited, as was the case in the 'F3' and 'F3b' MOTIFs. Inhibited activity would naturally result in lower accumulation rates. In the 'F3' and 'F3b' MOTIFs the internal concentration of oil in Macoma is low compared to mussels and cockles. Just as for mussels, no real decrease of internal concentration of Macoma's occurred in 'F3b' MOTIFs.

4.5 THE SHORT TERM EFFECTS OF THE F3-OIL

The primary short term effect observed was mortality among the benthic macrofauna. The mortality in Cerastoderma and Macoma as a result of the 'F3' treatment was similar. This was somewhat higher (Cerastoderma) and comparable (Macoma) to mortalities found in 1985 after the addition of 0.1 l Forties oil m^{-2} (Fo.1), but lower when compared to the addition of 0.5 l of Forties m^{-2} in 1984 (Fo.5). Mortality of Arenicola did not increase as a result of the 'F3' oil, just as after the Forties additions. A decrease in bioturbation activity, as observed in the 'F3' treated MOTIFs was also found in the 'Fo.5 treatment, but not in the 'Fo.1' treatment. Treatment of the 'F3' with the ignition agent resulted in almost 100% mortality in Cerastoderma. Such mortality was only seen before in the Forties, premixed with the dispersant Finasol (1985). The number of Arenicola, Macoma and Littorina that died in the 'F3b' MOTIFs was also higher than in any other previous OPEX experiment. The mortalities in the 'F3m' MOTIFs were very low and differ hardly from the 'C' MOTIFs.

Just as with the Forties treatments, no mortality of mussels was observed in the 'F3' and 'F3m' MOTIFs. An increased mortality of mussels was however observed in the 'F3b' MOTIFs. This mortality was similar to that found in this chemically dispersed Forties treated ('Fo Fi') MOTIFs.

Short term length/growth inhibition was observed for all the bivalves in the 'F3' and 'F3b' MOTIFs (crustoclama) and comparable (Macoma). The greatest inhibition was found in the 'F3b' MOTIFs.

The condition index of Macoma was reduced in the 'F3' MOTIFs compared to the 'C' MOTIFs, and in the 'F3b' MOTIFs it was even further depressed. The individual AFDW of Arenicola also showed some reduction in the 'F3' and especially in the 'F3b' treatment, immediately following that application.

Such severe short-term effects on macrofauna as found in the 'F3b' MOTIFs were never found in any of the previous OPEX treatments. On the other hand the effects of the 'F3'treatment were intermediate between those found after the addition of Fo. 5 and 0.1 l. Forties m^{-2}. The 'F3m' treatment did not result in any significant short-term effects on the benthic macrofauna.

The densities in which other macrobenthic faunal, meiobenthicfaunal and zooplankton organisms were present at the time of the oil addition were too small for the detection of any short term effects. However, an increased mortality of nematodes was observed.

4.6 THE LONG TERM, DIRECT EFFECTS OF F3 OIL

An inhibition of the population development of several smaller benthic and planktonic species by 'F3' or 'F3b' treatments was detected.

The amphipod Corophium volutator, which was found to be a reliable oil indicator in theformer OPEX experiments, had also a reduced population development in the 'F3' MOTIFs. This reduction was stronger than in the 'Fo.1' MOTIFs, but not as severe as that observed in the 'Fo-5' MOTIFs. In the 'F3b' MOTIFs almost no Corophium appeared.

Just as in the Forties experiment, ostracods appeared to be the most sensitive group of meiofaunal organisms, showing no population development whatsoever on oil polluted sediments throughout the experiment. This agrees with the findings of Elmgren et al (1979).

One obvious long-term effect was the increased mortality of cockles in the 'C' MOTIFs in December 1986. The 'C' MOTIF adjacent to an 'F3b' MOTIF has the highest mortality. This mortality is probably the result of leakage of water through a crack in the partition wal, from the 'F3b' MOTIF into the 'C' MOTIF. Oil compounds which were specific for the water of the 'F3b' MOTIFs have indeed been found in the 'C' MOTIFs.
Adult Nereis seemed to be highly affected by the 'F3b' treatment, while high densities of both Nereis (later in the year) and Capitella were found in the 'F3m' MOTIFs. Very high recruitment of Nereis in the 'F3b' by comparison with the 'C' MOTIFs was remarkable. In 1983 no Nereis was introduced in 8 identical MOTIFs. MOTIF 1 and 2 produced highest numbers (±300 m^2) and no

settlement took place in MOTIFs 6, 7 and 8. In 1984 200 Nereis MOTIF^{-1} were introduced and not much differences in numbers were eventually found (500-1500 m^{2}). In 1985 the introduction of 50 Nereis perMOTIF again resulted in higher numbers (2000-1500 m^{2}) in the 'C' MOTIF 6 and 'Fo 5' MOTIF 7.

Introduction of only 40 Nereis in the curent experiment is therefore probably responsible for the relatively large sequence effect with maximum densities of 2200 and 3600 Nereis m^{2} in MOTIFs 5 and 6. Effects of the water supply systems were also found in settlement of Arenicola juveniles, although less than in OPEX 1984. Moreover it was remarkable that all the Nereis in the 'F3b' MOTIFs and 50% of the Nereis in the 'F3' MOTIFs belonged to the carnivorous predator Nereis virens, instead of Nereis diversicolor which normally dominated the MOTIFS.
The 'F3' treatments also resulted in lower numbers of nematodes immediately after the oil addition. This was also seen after the addition of 0.5 Forties m^{-2}, but not after the addition of 0.1 l. Forties m^{-2}. From August onwards, the numbers in the 'F3' MOTIFs were again reduced when compared to the 'C' MOTIFs, just as was seen in both former OPEX experiments. The reduction of nematode numbers may be caused by hampering of their reproduction, especially that of Atrochromadora microlaima.

4.7 INDIRECT EFFECTS OF F3 OIL

As a consequence of the mortality of macrobenthic species and the reduced population density of some smaller benthic species, we may expect an increase of algal biomass, and productionm in the 'F3' and 'F3b' MOTIFs.
This is indeed observed in the 'F3b' MOTIFS, having the highest algal biomasses from June onwards, especially for the benthos.
The 'F3' MOTIFs showed only temporary elevated biomasses. In the benthic system these are observed from June until August. An increase of meiobenthic species feeding on the benthic algae may have caused the rapid decline of benthic algae density from July until November, resulting in even the lowest algal biomasses in the 'F3' MOTIFs compared to the others from August until November. In this period an elevation of the fytoplankton biomass was observed in the 'F3' MOTIFs compared to the 'C' MOTIFs.
The elevation of algal biomass in the 'F3' MOTIFs was low compared to that observedin the 'Fo.5' MOTIFs in previous experiments. It was comparable to the algal development in the 'Fo.1' MOTIFs.
Just as in former OPEX experiments, there are almost no differences in photosynthesis efficiency (photosynthesis per unit chlorophyll) of the fytoplankton between the MOTIFs. During the summer a small decrease of the photosynthesis efficiency was found in the 'F3' and 'F3m' MOTIFs.
The photosynthesis efficiency of the microphytobenthos was during the summer reduced in the 'F3b' MOTIFs compared to the 'C' MOTIFs. In autumn this was reversed. Unfortunately no measurements on the photosynthesis of the microphytobenthos of the 'F3' MOTIFs are available.
The increase of algal concentration means that the amount of food available for the surviving organisms increases.
The 'F3b' treatment resulted in low survival in Cerastoderma, Macoma and Littorina, and low recruitments in Nereis, Corophium, Hydrobia and Mytilus. Individual AFDW of the few animals of these species in this treatment were at least two times control values. In Nereis there was a sevenfold difference. With the bivalves Littorina, shell growth also increaed. A higher growth of periwinkles in MOTIF 2 was possibly the result of increased algal development in that MOTIF. Bivalves had also higher tissue condition indices and better development of reproductive stages.

In general the 'F3' treatment resulted in mortality or recruitment at levels intermediate between the 'C' and 'F3b' MOTIFs. The individual ASFD weights

found in Mytilu, Cerastoderma, Macoma, Nereis and Hydrobia were intermediate between those from the 'C' and 'F3b' MOTIFs. Corophium from the 'F3' MOTIFs were as heavy as animals from the 'F3b' treatments. This generally favorable development is also reflected by high shell growth, gonads condition (Cerastoderma) and the tissue condition index (all bivalves). The high summer peaks of nematodes were caused by species which feed exclusively on benthic diatoms. One of these species, Chromadora nudicapitata, seemed to be correlated to the presence of the macroalga Enteromorpha; it may feed on diatoms which grow on the surface of the macroalgae. Chromadora nudicapitata can reproduce very quickly in suitable circumstances, for example with a plentyful supply of food.
Oligochaetes also had the highest densities in the oil treated MOTIFs, which agreed well with previous findings.

In the zooplankton copepod species that have maximal population development in July-September (Acartia) the highest densities were found in the oil treated MOTIFs, with maxima in the 'F3b' MOTIFs. A similar stimulation of copepods, following on an initial inhibition immediately after the oil spill, was seen in the "Forties" treated MOTIFs. In all cases the increase of algal biomass results in higher population development, when the concentration of oil in the water is no longer that high that it can inhibit copepod production. A similar reaction to oil is seen on a shorter term (few weeks) in the protozoan community (Bak, 1987). Decrease of protozoans in 'F3' MOTIFs for a few days following the oil spill and an increase of protozoans during some weeks thereafter). Rotifers showed the same reaction at an intermediate time scale (month).

On the contrary to the (planktonic) copepods, benthic crustaceans (Corophium Ostracodes and copepods had a longer lasting inhibition of their development due to chronical high concentrations of oil in the sediment. These crustaceans can not benefit from the extra algal biomass in oil treated MOTIFs.

Planktonic harpacticoid copepods had only some benefit in June-July in the 'F3' and 'F3m' MOTIFs.

In the autumn both harpacticoid copepods and nematodes suffer from oil again, as reproduction rates decrease and an opportunistic response to algal blooms are not longer possible.

In autumn the improved development of surviving gastropods and high densities of nematodes in the 'F3' and 'F3b' MOTIFs are reflected by elevated numbers of gastropod and nematod larvae in these systems.

An interesting phenomenon is the interaction between Hydrobia ulva and Corophium volutator

Hydrobia has a gregarious distribution, as is reflected by large standard errors, e.g. there was a large difference between both 'F3' MOTIFs. However, just as with Corophium, Hydrobia had the highest numbers in the 'C' and 'F3m' MOTIFs, and the largest numbers in the 'F3b' MOTIFs. Thus, an inversely related population development of both species, indicating a competitive relationship, as occurred in all previous OPEX experiments, was not observed in 1986/87. Hydrobia densities during OPEX 1986 were relatively large (up to 3000 m^{-2}) compared to 1984 (800 m^{-2}) and 1985 (400 m^{-2}). A sequence effect of the water supply system might have been important in the settlement of Hydrobia, and might have supressed competition effects. The use of the ignition agent did result in a slower decrease of the concentration of oil in the sediment, probably due to lack of biodegradation and delivery of oil from the sediment as a consequence of the calceous layer encrusted on the sediment. The existance of this layer form more than 4 months was probably related to low bioturbation activity due to loss of benthic fauna following the burning of the oil, as well as inhibited settlement of new fauna due to the calceous layer on the sediment surface. The penetration of oil to deeper sediment layer seemed to be slower in the 'F3b' MOTIFs compared to the 'F3' MOTIFs.
As stated, a great portion of the oil penetrated the sediment when it stranded on the tidal flats at low tide. In contrast to the Forties oil from 84/85, the F3 oil did not adhere to the sediment surface, but appeared to be directly absorbed into it. Therefore no oil was lifted off again at high tide, as was see in the Forties experiments. Due to bioturbation and percolation processes the oil was found to have sunk deeper into the sediment with time.
The concentration of oil in the sediment decreased steadily, but at the end of the experiment, 10 months after the oil additions, concentrations in hte 'F3' MOTIFs remained higher than those in the 'C' MOTIFs.
No significant elevation of the oil concentration in the water was observed during the 1986/87 'F3' oil experiments, whereas this was the case in the 84/85 'Forties' experiments.

4.8 RELATIVE TOXITY OF F3 OIL

The environmental impact of oil can be divided into 4 categories:
Direct effects:

1. Physical impact of the floating oil mass by smoothering birds or coastal (intertidal) communities such as salt marshes and rocky shores.
2. Short term toxic impact of the oil (i.e. the dissolved or dispersed fraction) resulting in mortality of organisms.
3. Long term toxic impact of the oil (i.e. the fraction accumulated in the sediment) resulting in an inhibition of the development of seasonally fluctuating populations and the settlement of benthic larvae. This determines the recovery rate of the system against the short term effects.

Indirect effects:

4. Changes in structure and functioning of the ecosystems, i.e. shifts in species composition towards unstable, dynamical development of opportunistic species, that can benefit from the reduced competition with organisms that had a mortality or inhibited population development due to direct effects.

The indirect effects were primarily determined by the direct effects. The physical impact of the oil was not measured in the MOTIF experiments. However, no floating oil layer was formed with the dosage of oil used in the MOTIF experiments (82 m/F3 oil m^{-2}). In the MOPs experiments in 1985 a mousse was formed at the top of the waterlayer, at a dosage of 400-2000 ml oil per m^{-2}.

Direct toxic effects, and consequented indirect effects, observed in the MOTIFs treated with 82 ml F3 oil per m^{-2} were in the same order as those observed in MOTIFs treated with 100 ml Forties per m^{-2}. From this it can be concluded that a less amount of F3 can result in similar effects as a greater amount of Forties.
This is in agreement with observations in MOPs experiments, in which F3 oil was proved to be somewhat more toxic than the Forties oil.

Although no elevation of oil compounds in the water column could be observed, bioaccumulation rates and effects to filterfeeding organisms suggest a chronic release of oil from the sediments into the water column. There might be a high turn over of these oil fraction due to biodegradation.

4.9 IMPACT OF COMBAT TECHNIQUES

No additional impacts could be observed from the disturbance of the benthic system by as a consequence of mechanical removal, other than some inhibited activity of the benthos during 2 weeks. Within this time a full recovery of the benthos was observed.
Furthermore the systems can be seen as systems treated with a very small amount of F3 oil (11 ml per m^{-2}). This small dosage has no measurable effect to the tidal flat ecosystem.

The use of the ignition agent to remove oil from the tidal flat system was not succesful. It is estimated that at most 25% of the oil was burned and removed from the system. However, the impact of the calcereous layer encrusted on the sediment surface is severe. This layer prevents exchanges between sediment- and waterphase and the settlement (and thus recovery) of benthic species. In those systems the mortality or reduced population development of species was not compensated by extra growth of surviving species or opportunists. The total biomass of secondary producers was drastically reduced. This phenomenon was only observed after premixing Forties oil with Finasol dispersant in previous oil experiments.
The low density of secondary producers results in eutrophication like effects, such as blooms of phytoplankton and enteromorpha, and consequented peaks of opportunistic faunal organisms.

5. CONCLUSIONS

5.1 The ecological functioning of control MOTIFs approximates that of natural tidal flats in the nearby Waddensea, especially when the production and biomass values of ecological functioning groups are concerned. There is a small deviation concerning the species composition of the microflora- and fauna.

5.2 Relative high P/B ratio's of the fytoplankton and fytobenthos in the MOTIFs indicate a high grazing rate upon the primary producers. The secondary production by zoobenthic and zooplankton species is limited by the primary production available. Intra- and interspecific competition between secondary producers occur.

5.3 Silicate depletion only occured during the first fytoplankton blooms until mid June. During the summer algal growth is nitrogen limited.

5.4 No floating oil layer was formed after addition of 82 ml F3 oil per m^2 in the MOTIFs. No measurable elevation of oil in the water column was found, although high bioaccumulation rates in mussels and other filter-feeding organisms, indicate a chronic release of oil from the sediments into the water column until the end of the experiment (10 months after oil addition).

5.5 Due to bioturbation and percolation processes the F3 oil sunk deeper into the sediment with time. Due to delivery of oil from the sediment to the waterphase the concentration of oil in the sediment decreases with time. However, 10 months after the oil addition concentrations of oil in the 'F3' treated MOTIFs remained higher.

5.6 Mechanical removal of oil cases a deep penetration of the oil into the sediment. The decrease of the oil concentrations was therefore initially slower. However, after 8 months after the oil background levels were reached.

5.7 Th use of an ignition agent results in a removal of at most 25% of the oil by burning. Remainders of the ignition agent formed a calcereous layer encrusted on the sediment. This layer exist for more than 4 months. The decrease of the oil concentration in the sediment was very slow. At the end of the experiment higher concentrations of oil in the sediment remained compared to the 'F3' oil treated MOTIFs.

5.8 Direct toxic effects (mortality in Cerastoderma and Macoma) were highest in the 'F3b' MOTIFs and intermediate in the 'F3' MOTIFs. No toxic effects were observed in the 'F3m' MOTIFs.
Mortality of Arenicola and Littorina occur only in the 'F3b' MOTIFs.
Short term growth inhibitions were found in bivalves in the 'F3' and to a greater extend in the 'F3b' MOTIFs.

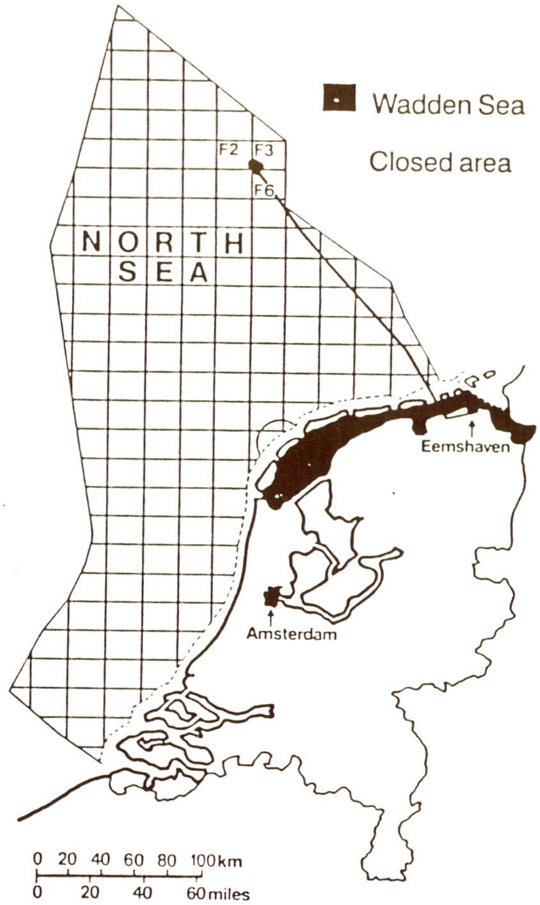
5.9 The long term toxic effects (inhibition of populations) development of Corophium, ostracodes, nematodes as Atrochromadora, benthic copepods and zooplankton, copepods as Eurytemora) were highest in the 'F3b' MOTIFs and intermediate in the 'F3' MOTIFs.
Almost no long term toxic effects were observed in the 'F3m' MOTIFs.

5.10 The development of Nereis and Hydrobria were primarily determined by variation of quality of intake water with respect to larvae numbers.

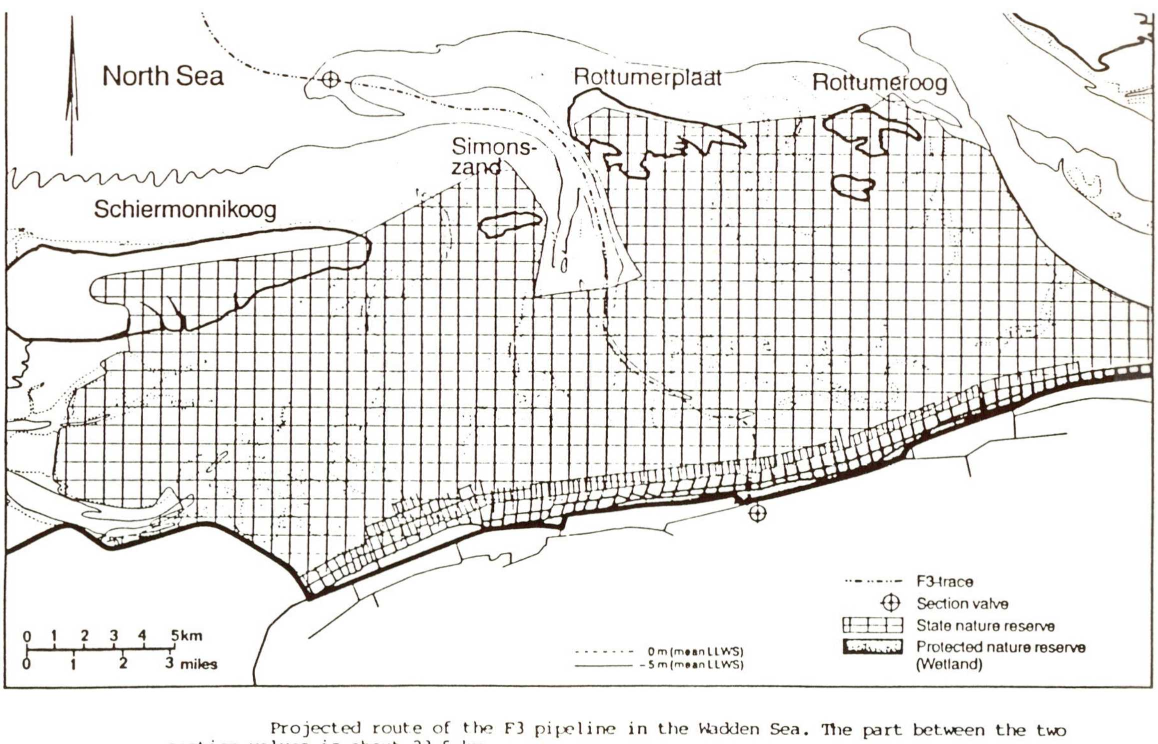
5.11 Indirect effects of F3 oil (extra algal production due to reduced grazing, followed by development of opportunistic species) were highest in the 'F3b' MOTIFs and intermediate in the 'F3' MOTIFs. Almost no indirect effects were observed in the 'F3m' MOTIFs.

5.12 The effects of application of 82 ml of F3 oil per m^2 were comparable even stronger than those of application of 100 ml of Forties oil per m^2. The actual accumulation of oil in the sediment one day after the oil application was 20 ml of F3 oil per m^2 respectively 40 ml of Forties oil per m^2.

5.13 The effects of the burning of oil with the help of the ignition agent were stronger than those observed after premixing Fories oil with the dispersant.



Situation of the F3 oil and gas field and the projected pipeline for transport of hydrocarbons to the shore.



Projected route of the F3 pipeline in the Wadden Sea. The part between the two section valves is about 23.5 km.

Figure 1. Projected route of F3 pipeline (source, Jacobs 1987).

Figure 2. MOTIF-systems.

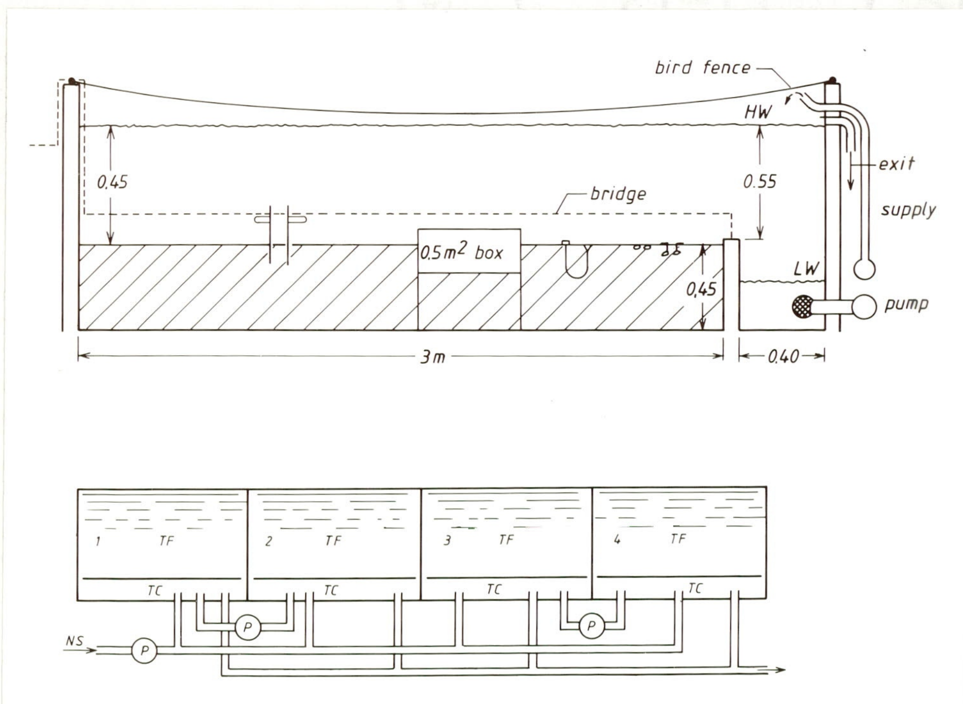


Figure 3. Diagram of the MOTIFs.

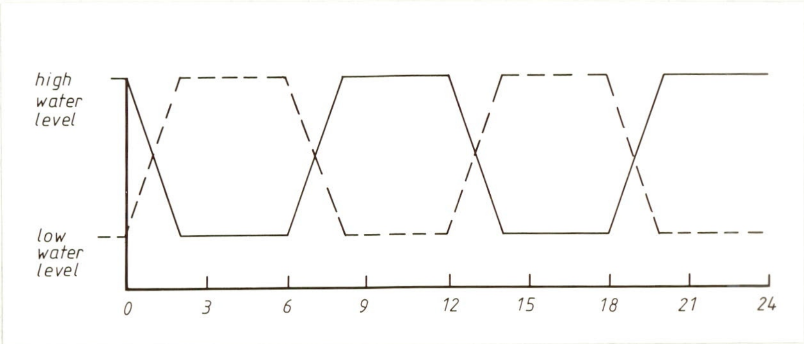


Figure 4. Tidal cycle of paired MOTIFs.

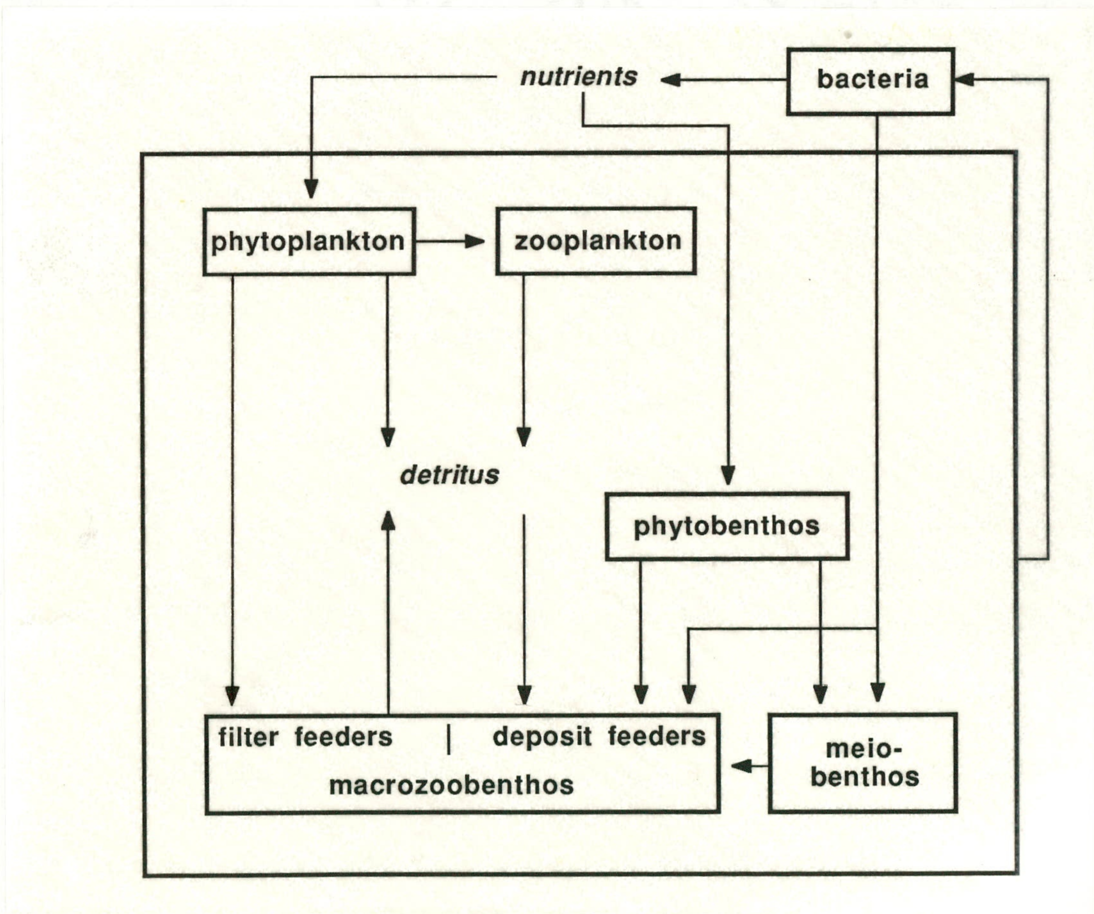


Figure 5. Interrelations between biotic components within MOTIFs.

Figure 6. Application of the F3 oil to the MOTIF systems.

Figure 7. Burning of oil with the help of an ignition agent.

Figure 8. Burning of oil.

Figure 9. General set-up of the MOTIFs experiment 1986.

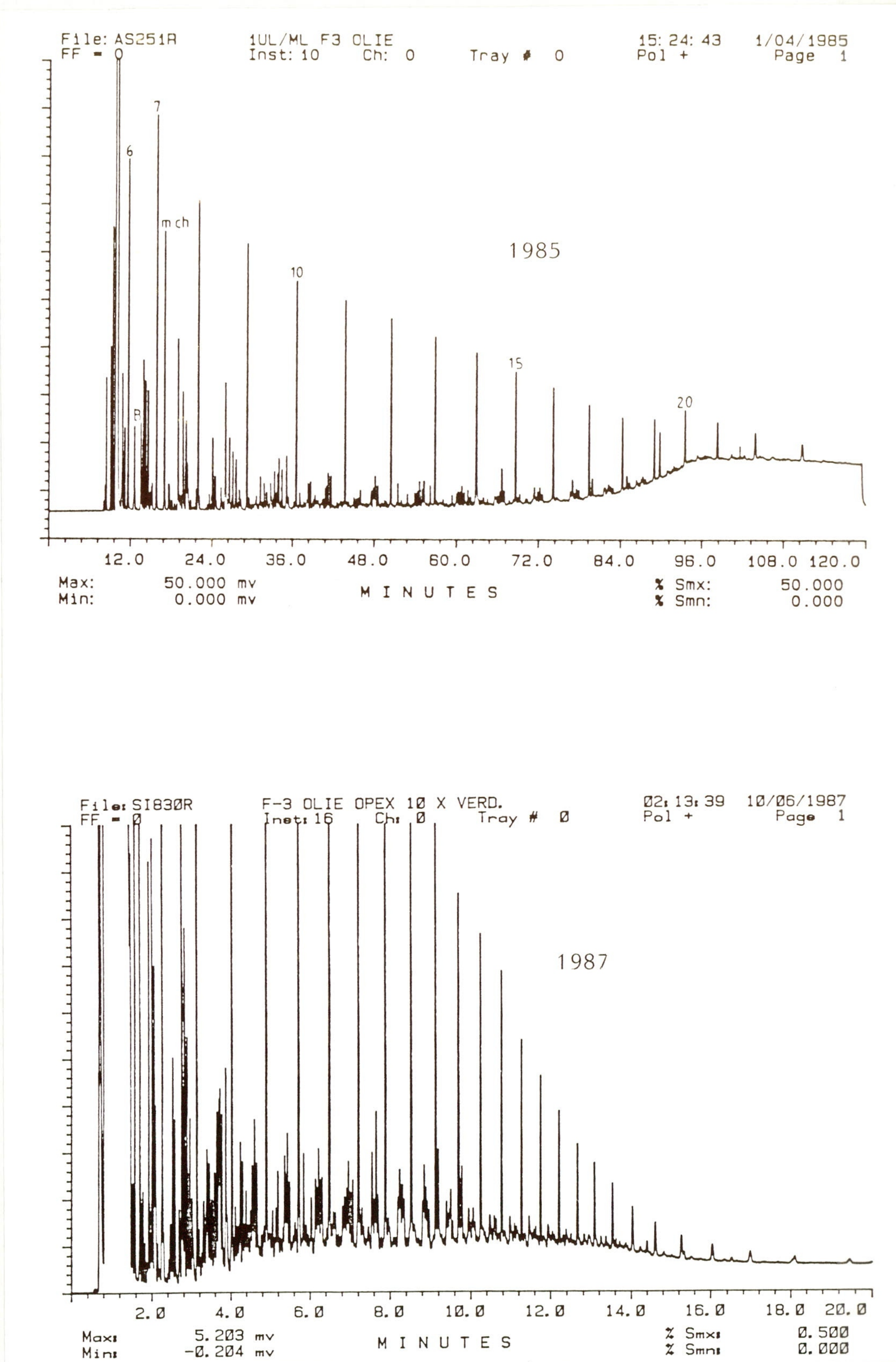


Figure 10. Chromatogram of original F3 oil used in the OPEX experiment (above), compared to that used in MOP experiments (below), from Kuiper et al., 1985.

Figure 11. F3 oil at the sediment surface measured at low tide in the MOTIFs, some 8 hours oil application.

Figure 12. White fumes released during the ignition of the F3 oil in the MOTIFs.

Figure 13. A white sedimentation layer, encrusted on the sediment surface of the MOTIFs after ignition of the F3 oil.

Figure 14. Chromatograms of oil in water from the 'C', 'F3', 'F3m' MOTIFs, on the day after the F3 oil addition (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

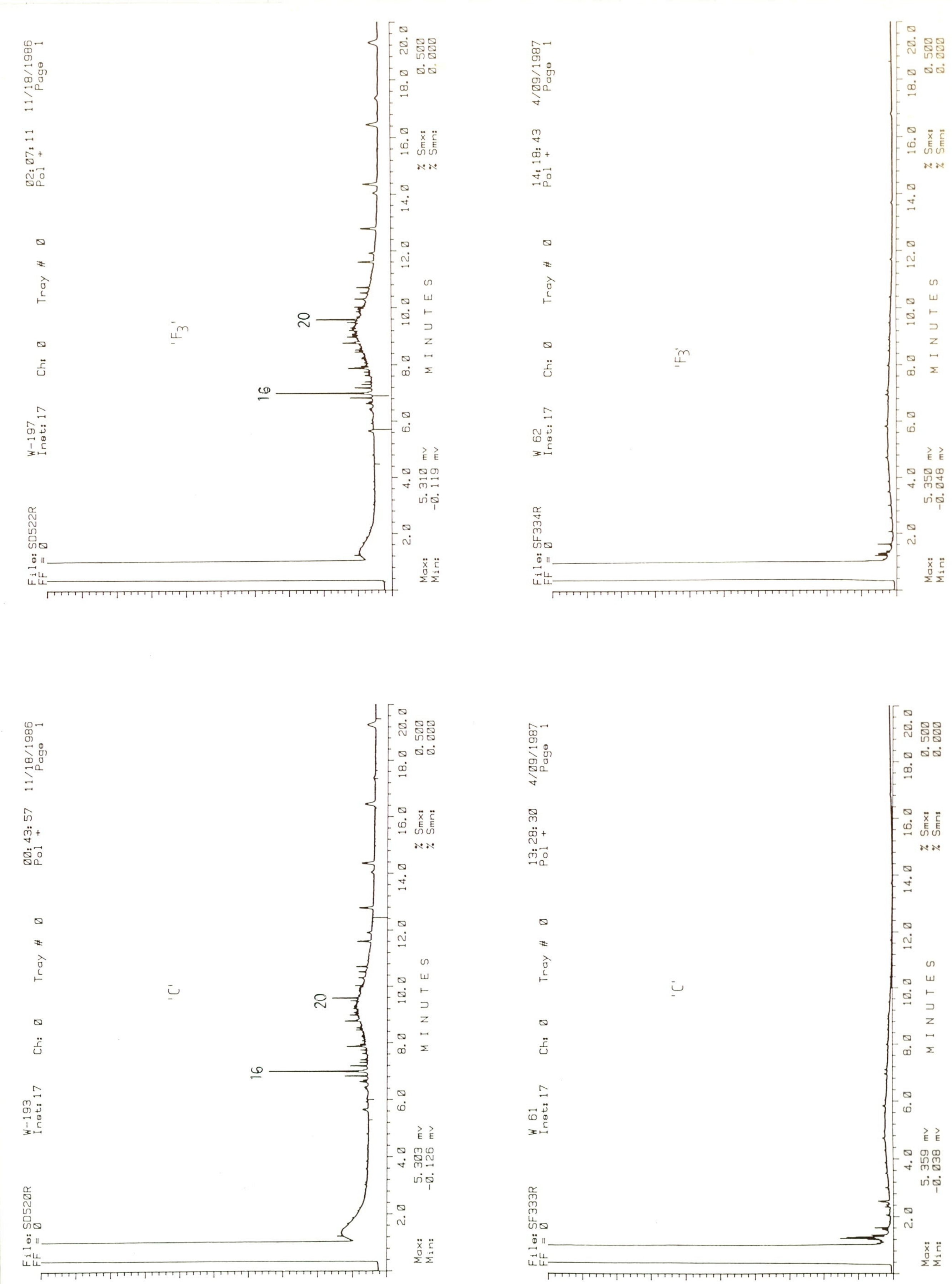


Figure 15. Chromatograms of oil in water from the 'C' and 'F3' MOTIFs in June 1986 (above) and September 1986 (below), resp. 4 months after the oil addition. Chromatograms of other MOTIFs are comparable (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

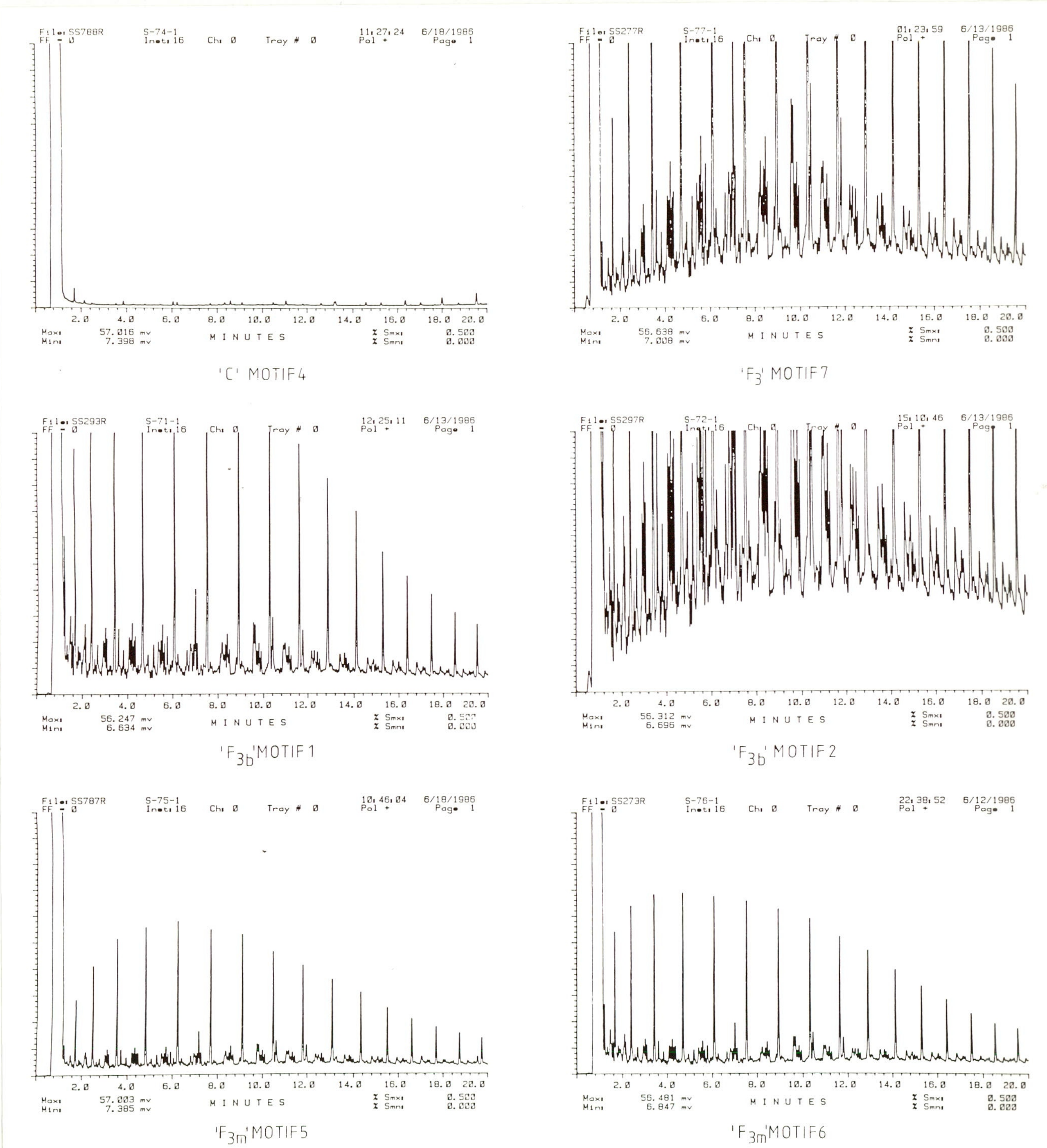


Figure 16. Chromatograms of oil in the upper 1 cm sediment layer of the 'C', 'F3', 'F3b' and 'F3m' MOTIFs, one day after the addition of F3 oil (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

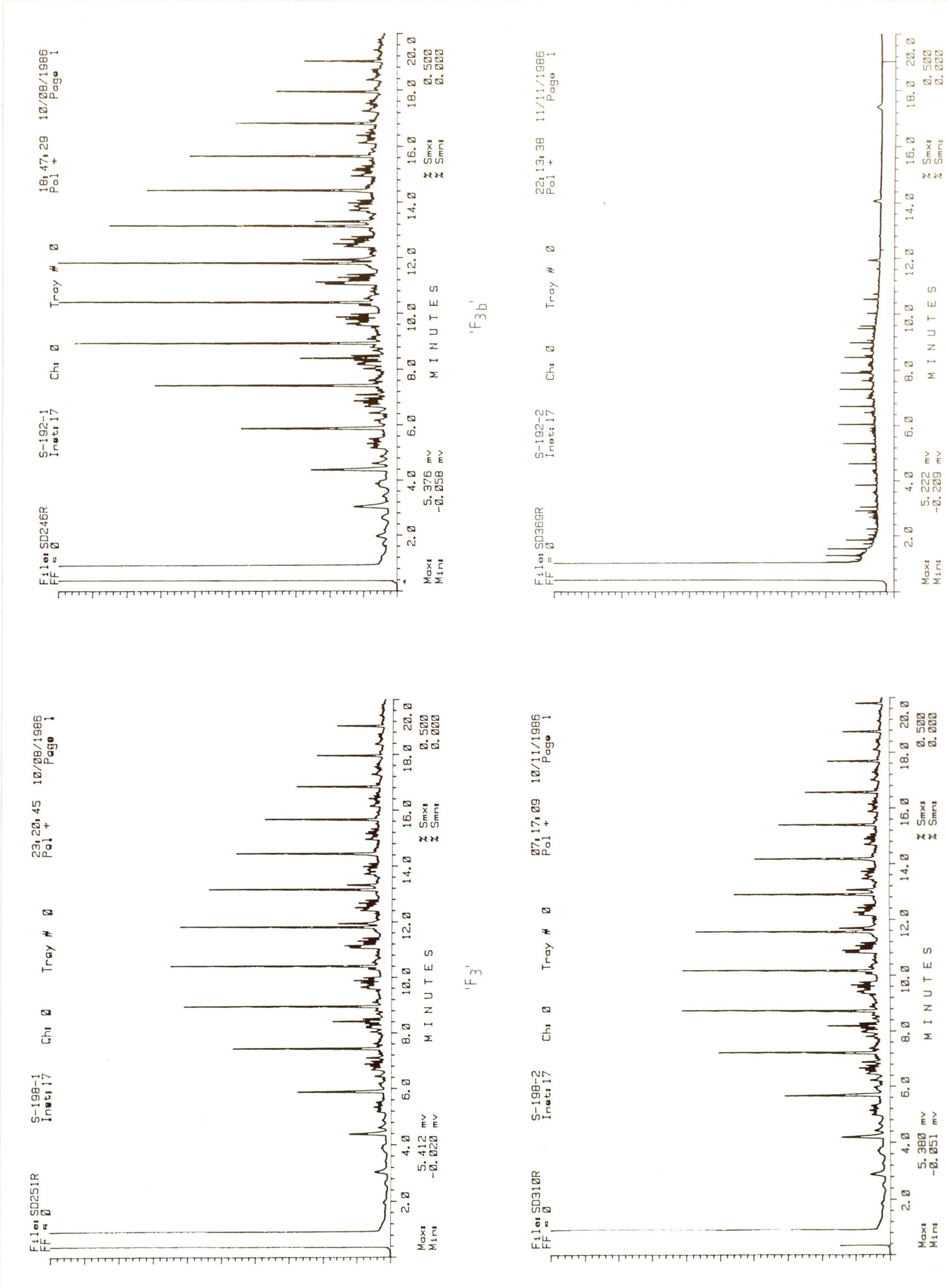


Figure 17. Chromatograms of oil in the sediment of the 'F3' and 'F3b' MOTIFs in June 1986, 1 month after the oil application, at 0-1 cm depth (above), 1-2 cm depth (below) (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

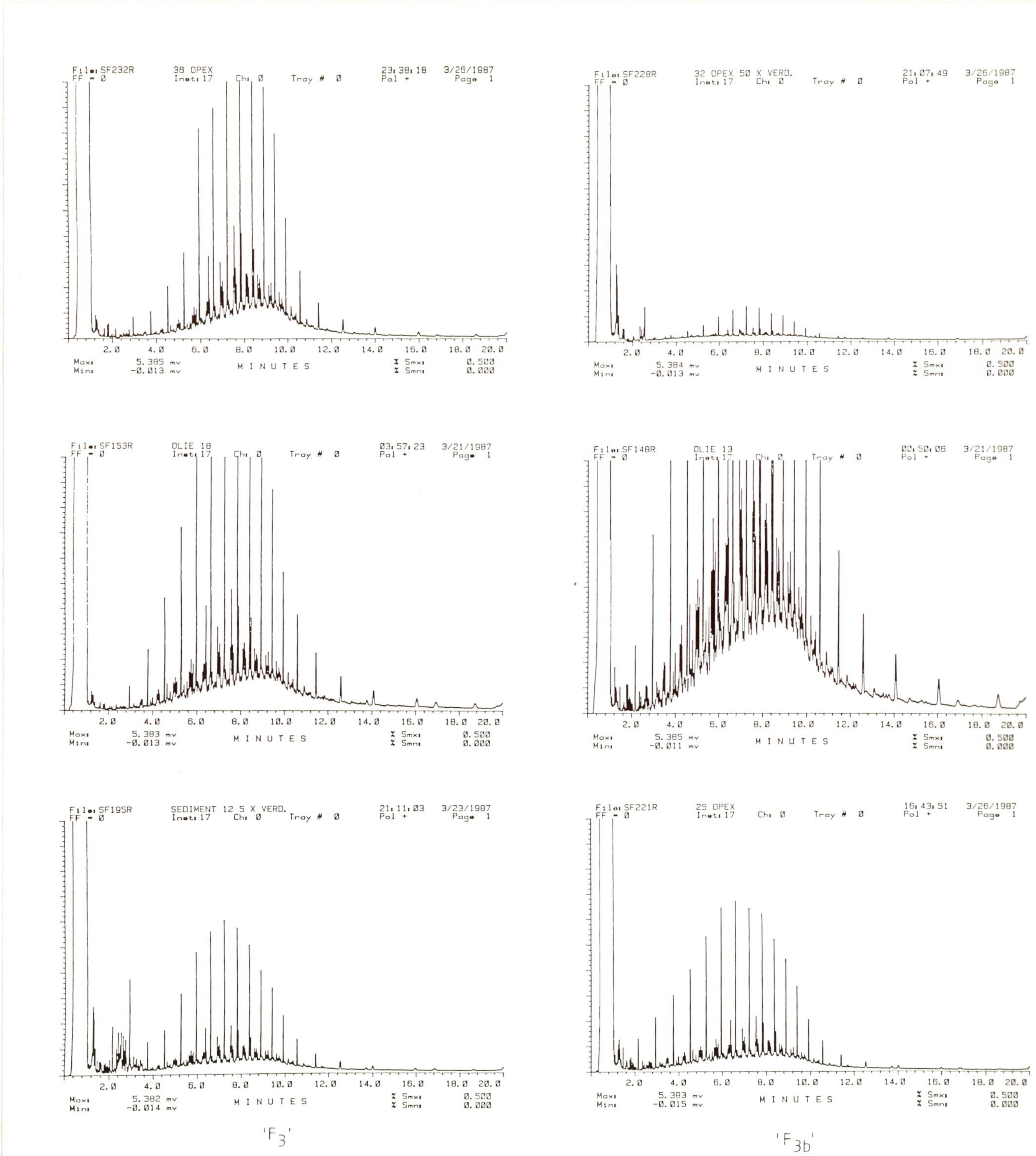


Figure 18. Chromatograms of oil in the sediment of the 'F3' and 'F3b' MOTIFs in July 1986, 2 months after the oil application, from 0-1 cm depth (above), 1-2 cm depth (middle) and 2-10 cm depth (below) (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

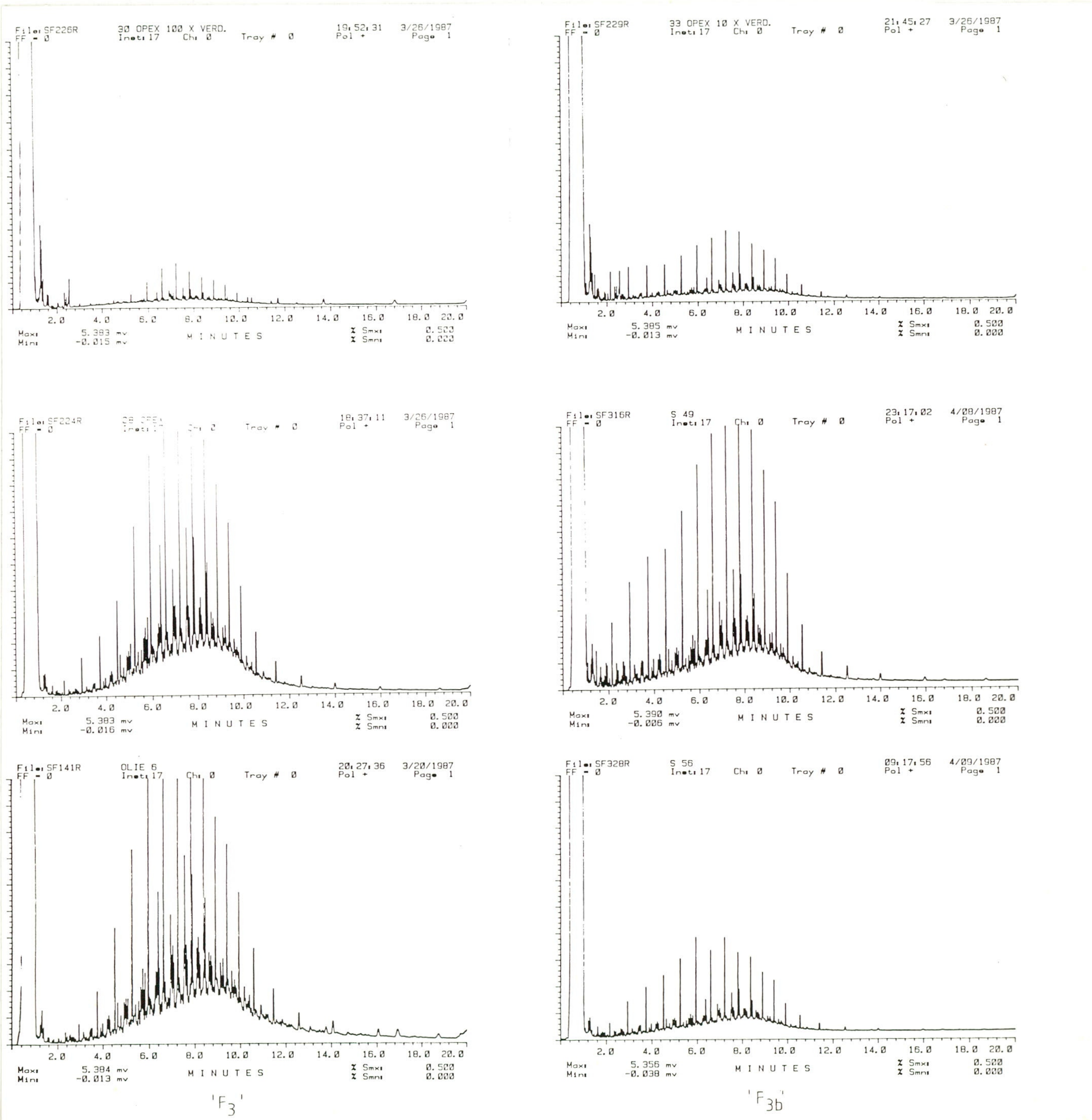


Figure 19. Chromatograms of oil in the sediment of the 'F3' and 'F3b' MOTIFs in September 1986, 4 months after the oil application, from 0-1 cm depth (above), 1-2 cm depth (middle) and 2-10 cm depth (below) (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

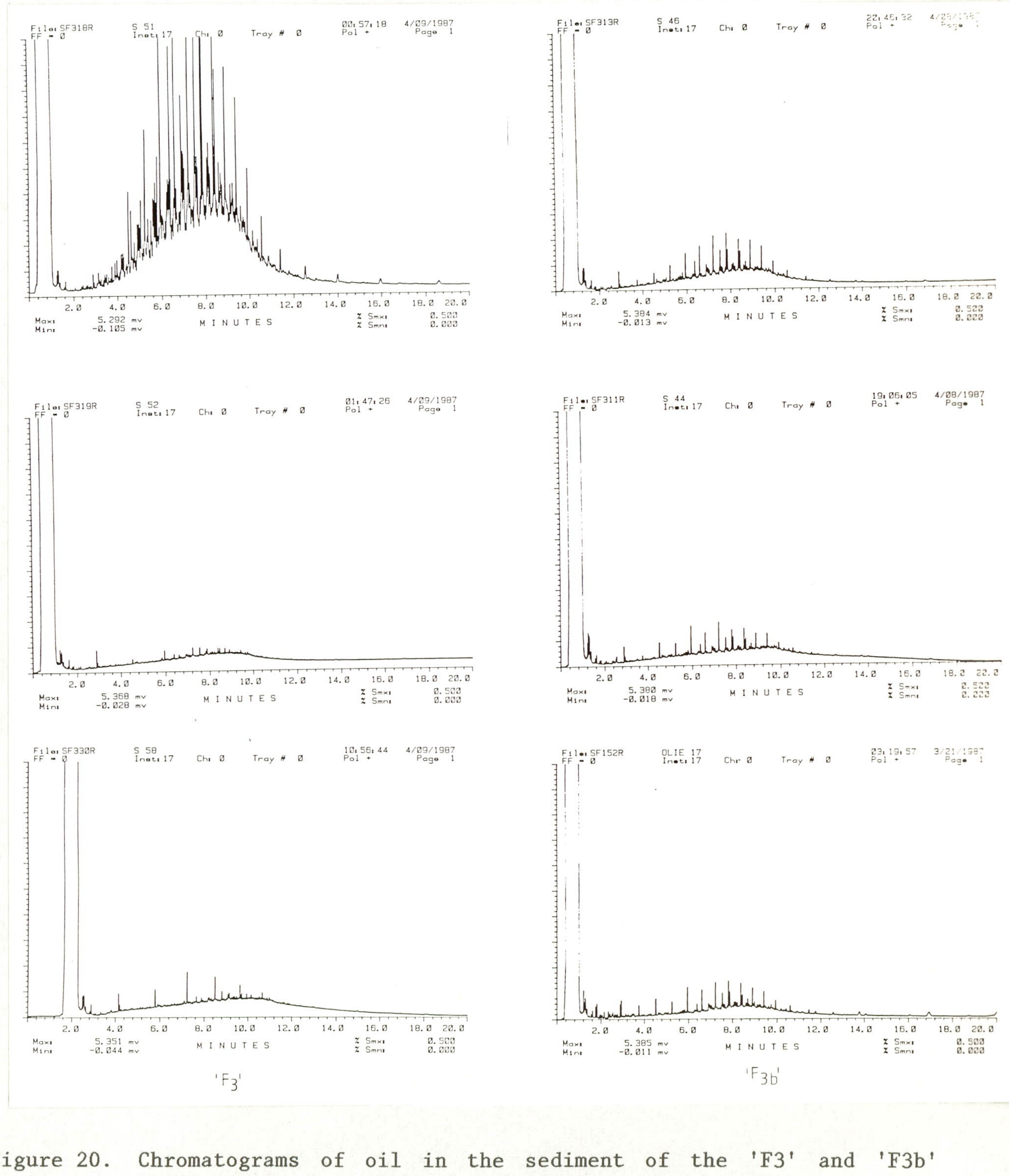


Figure 20. Chromatograms of oil in the sediment of the 'F3' and 'F3b' MOTIFs in January 1987, 8 months after the oil application, from 0-1 cm depth (above), 1-2 cm depth (middle) and 2-10 cm depth (below) (for the relation of alkanes to retention times see Table 8, at the end of paragraph 3.1).

Fig. 20

Figure 20. Chromatograms of oil in the sediment of the "F" and "[illegible]" marshes in January 1962, 8 months after the oil application, from 0-1 cm depth (above), 2-5 cm depth (middle) and 2-10 cm depth (below). (For the notation of [illegible] changes to [illegible] see Table 8, at the end of paragraph 3.1).

Figure 21.

Figure 22

Figure 23.

Figure 24.

Figure 21. Cerastoderma edule. Mortality expressed as the cumulative number of empty shells ('doublets') appearing at the sediment surface.

Figure 22. Cerastoderma edule. Numbers introduced and found at large macrofaunal samplings.

Figure 23. Cerastoderma edule. Survival per MOTIF expressed as cumulative number of shells ('doublets') which appeared alive or moribund sediment surface. Numbers are corrected for extraction of animals due to sampling.

Figure 24. Cerastoderma edule. Growth of shell length.

Figure 25.

Figure 26

Figure 27.

Figure 25. Cerastoderma edule. Changes in tissue Condition Index.

Figure 26. Cerastoderma edule. Changes in individual Ashfree Dry Weight.

Figure 27. Cerastoderma edule. Changes in Total Ashfree Dry Weight.

a.

b.

c.

d.

Figure 28. Cerastoderma edule. Reproductive development: relative proportion of specimens in different reproductive stages.

Figure 29. Figure 30.

Figure 31. Figure 32.

Figure 29. Macoma balthica. Mortality expressed as cumulative number of empty shells ('doublets') appearing at the sediment surface.

Figure 30. Macoma balthica. Numbers introduced and found at large macrofaunal samplings.

Figure 31. Macoma balthica. Survival per MOTIF expressed as cumulative number of shells ('doublets') which appeared alive or moribund at the sediment surface. Numbers are corrected for extraction of animals due to sampling.

Figure 32. Macoma balthica. Growth measured as change in shell length.

Figure 33.

Figure 34.

Figure 35.

Figure 33. Macoma balthica. Changes in tissue Condition Index.

Figure 34. Macoma balthica. Changes in individual Ashfree Dry Weight.

Figure 35. Macoma balthica. Changes in Total Ashfree Dry Weight.

a. b.

c. d.

Figure 36. *Macoma balthica*. Reproductive development: relative proportion of specimens in different reproductive stages.

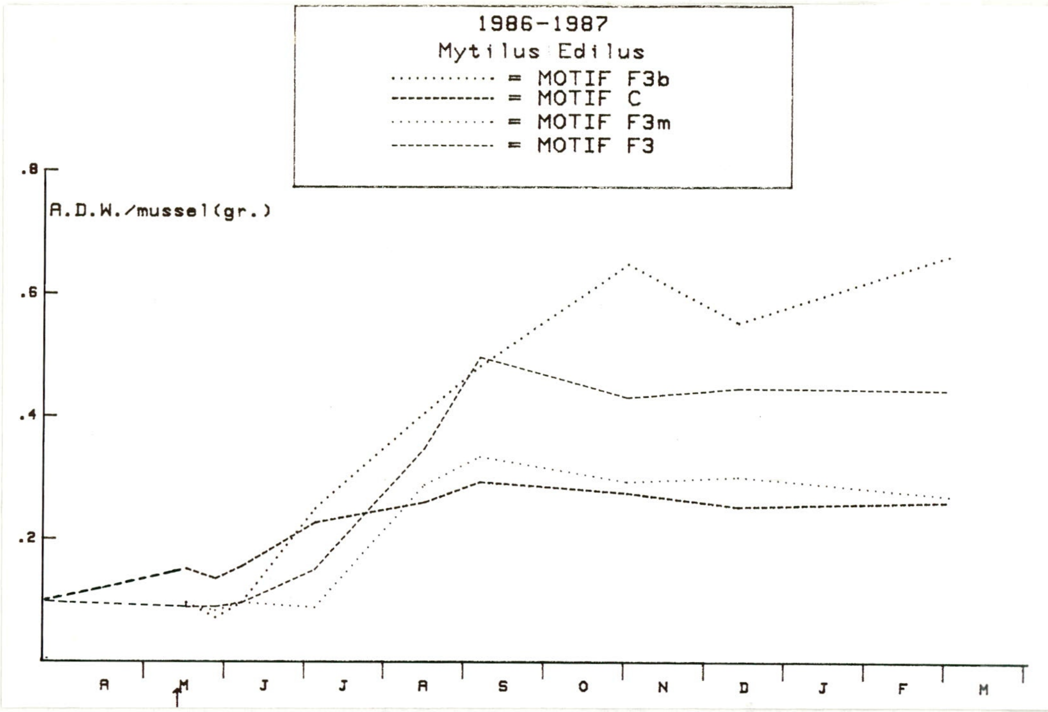


Figure 37. Mytilus edulis. Biomass in g ashfree dry weight.

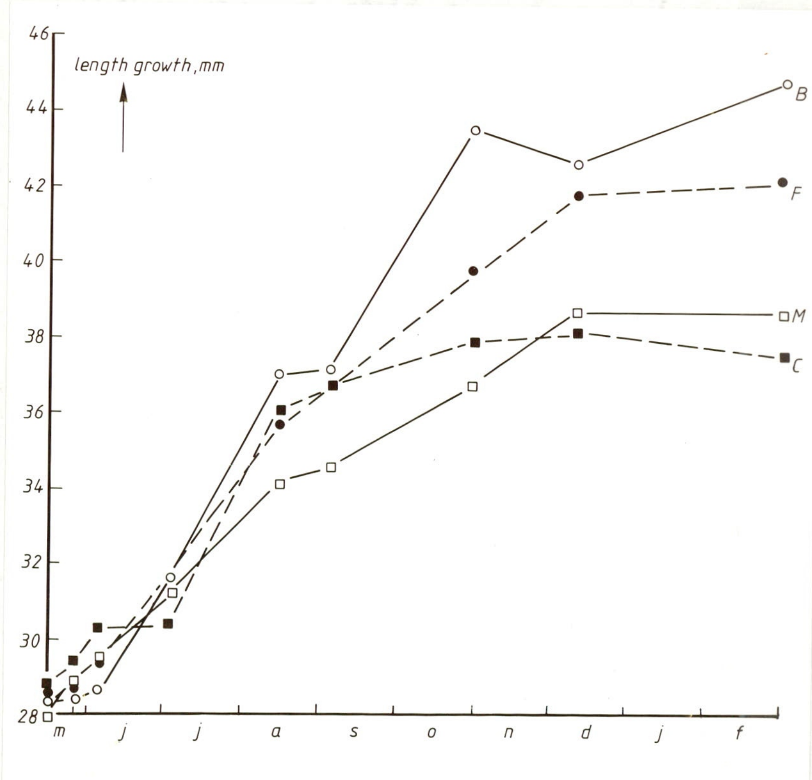


Figure 38. Mutilus edulis. Growth of measured as change in shell length.

Figure 39.

Figure 40.

Figure 41.

Figure 42.

Figure 43.

Figure 44.

Figure 39. Arenicola marina adults. Bioturbation activity expressed as number of freshly produced casts at the sediment surface.

Figure 40. Arenicola marina adults. Numbers introduced and retreived during large macrofaunal samplings.

Figure 41. Arenicola marina adults. Changes in individual Ashfree Dry Weight.

Figure 42. Arenicola marina juvenile. Bioturbation activity expressed as the number of freshly produced casts at the sediment surface.

Figure 43. Arenicola marina juveniles. Numbers per MOTIF, retrieved during large macrofaunal samplings.

Figure 44. Arenicola marina adults. Changes in Total Ashfree Dry Weight.

Figure 45.

Figure 46.

Figure 47.

Figure 48.

Figure 49.

Figure 50.

Figure 45. Nereis diversicolor. Numbers found at small macrofaunal samplings. Values per MOTIF.

Figure 46. Nereis diversicolor. Changes in individual Ashfree Dry Weight.

Figure 47. Nereis diversicolor. Changes in Total Ashfree Dry Weight.

Figure 48. Corophium volutrator. Numbers found at small macrofaunal samplings.

Figure 49. Corophium volutator. Changes in individual Ashfree Dry Weight.

Figure 50. Corophium volutator. Changes in Total Ashfree Dry Weight.

Figure 51.

Figure 52.

Figure 53.

Figure 51. Hydrobia ulvae. Numbers found at small macrofaunal samplings.

Figure 52. Hydrobia ulvae. Changes in individual Ashfree Dry Weight.

Figure 53. Hydrobia ulvae. Changes in Total Ashfree Dry Weight.

Figure 54.

Figure 55.

Figure 54. Littorina littorea. Changes in abundance.

Figure 55. Littorina littorea. Changes in shell length.

Figure 56. Changes in total biomass and contribution by different species.

Figure 57a. Chromatogram of the internal oil in tissues of mussel (Mytilus edulis) in the different MOTIFs one day after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

Figure 57b. Chromatogram of the internal oil in tissues of mussel (Mytilus edulis) in the different MOTIFs 4 months after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

Figure 57c. Chromatogram of the internal oil in tissues of mussel (<u>Mytilus edulis</u>) in the different MOTIFs 10 months after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

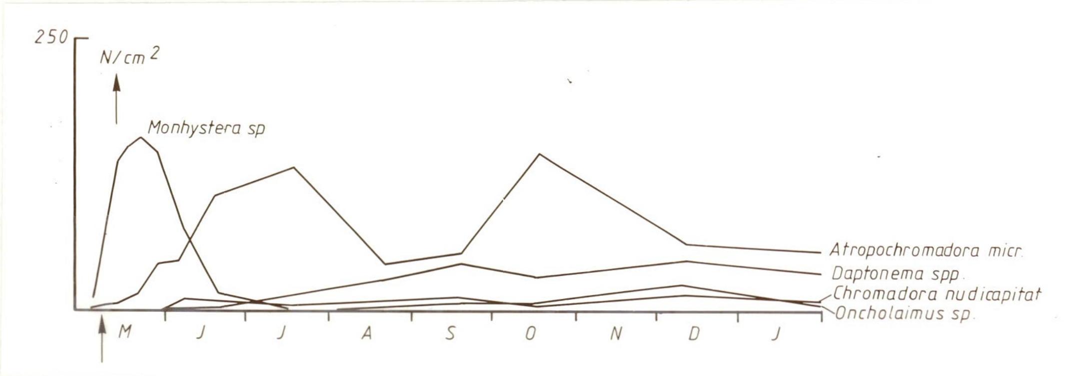
Figure 58a. Chromatogram of the internal oil in tissues of cockles (cerastoderma edule) in the different MOTIFs one day after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

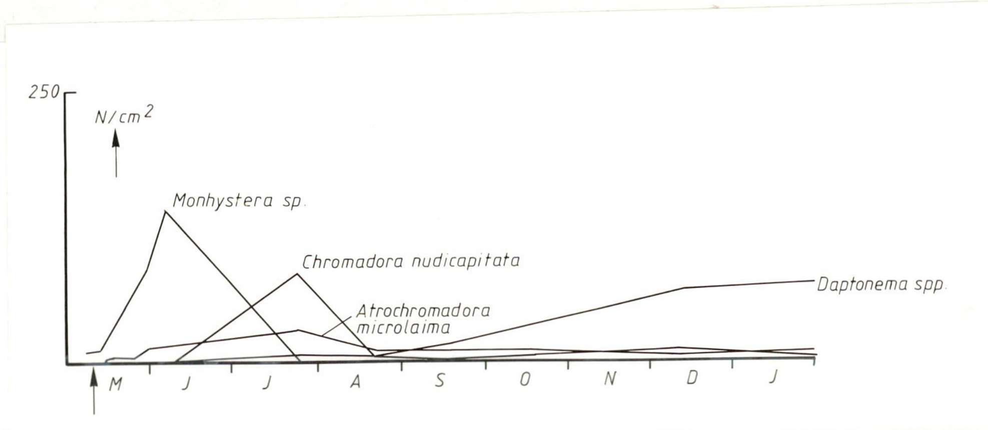
Figure 58b. Chromatogram of the internal oil in tissues of cockles (cerastoderma edule) in the different MOTIFs 4 months after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

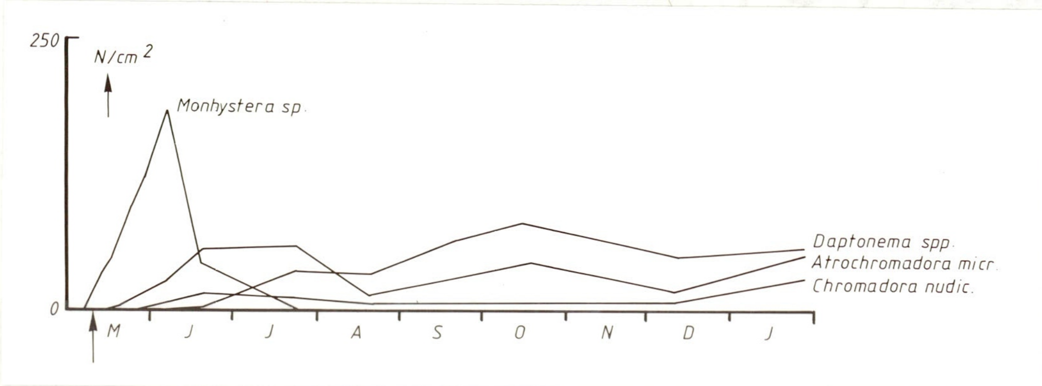
Figure 59a. Chromatogram of the internal oil in tissues of balthic tellings (Macoma balthica) in the different MOTIFs one day after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

Figure 59b. Chromatogram of the internal oil in tissues of balthic tellings (Macoma balthica) in the different MOTIFs 4 months after the F3 oil application (see for reference of retention time to alkane Table 8 at the end of §3.1).

Figure 60. Density of nematodes in the upper 1 cm layer of the MOTIF sediments. ● = 'F3b'; o = 'C'; □ = 'F3m'; ■ = 'F3'.







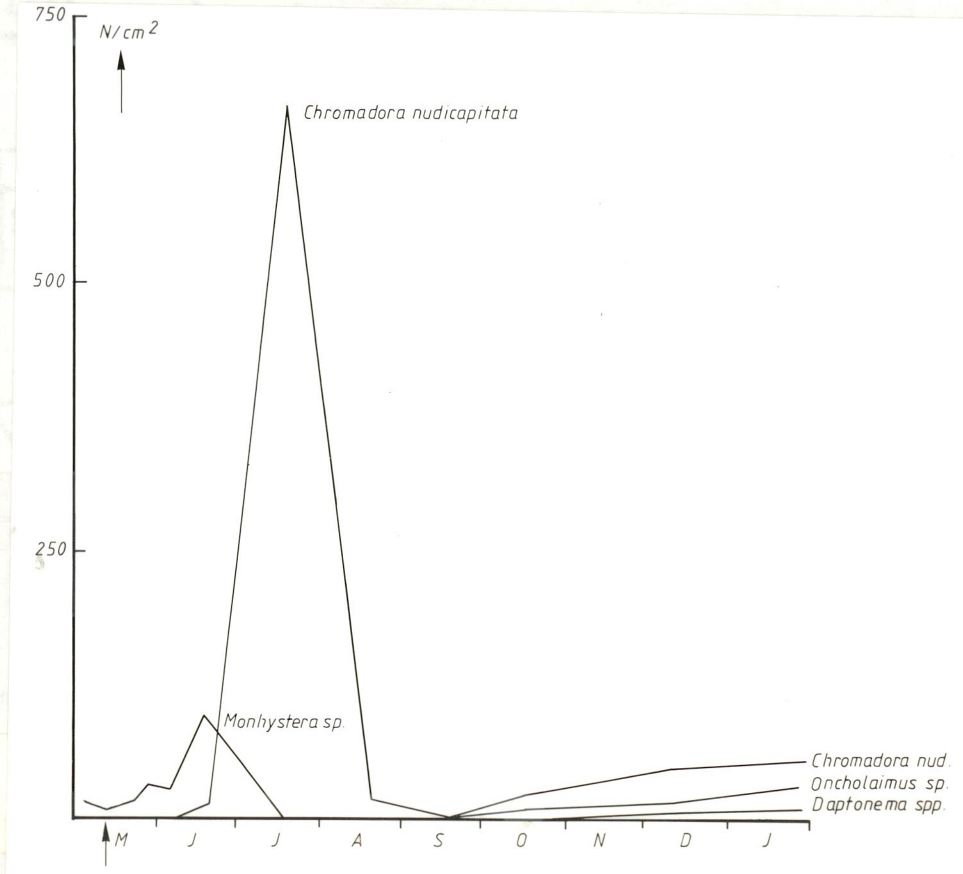


Figure 61. Density of dominant nematode species in the upper 1 cm layer of the MOTIF sediments.

Figure 62. Density of harpacticoöid copepods in the upper 1 cm layer of the MOTIF sediments. ● = 'F3b'; o = 'C'; □ = 'F3m'; ■ = 'F3'.

Figure 63. Density of oligochaete in the upper 1 cm layer of the MOTIF sediments. ● = 'F3b'; o = 'C'; □ = 'F3m'; ■ = 'F3'.

Figure 64. Density of turbellaria in the upper 1 cm layer of the MOTIF sediments. ● = 'F3b'; o = 'C'; □ = 'F3m'; ■ = 'F3'.

Figure 65. Density of ostracods in the upper 1 cm layer of the MOTIF sediments. ● = 'F3b'; o = 'C'; □ = 'F3m'; ■ = 'F3'.

Figure 66. Density of calanoïd nauplii in the MOTIFs.

Figure 67. Denisty of the calanoïd copepodites in the MOTIFs.

Figure 68. Density of the calanoïd adults in the MOTIFs.

Figure 69. Density of Eurytemora velox in the MOTIFs.

Figure 70. Density of Acartia clausi in the MOTIFs.

Figure 71. Density of harpacticoïd copepods in the MOTIFs.

Figure 72. Density of bivalve larvae in the MOTIFs.

Figure 73. Density of polychaete larvae in the MOTIFs.

Figure 74. Density of gastropod larvae in the MOTIFs.

Figure 75. Density of nematode larvae in the MOTIFs.

Figure 76. Density of rotifers in the MOTIFs.

Figure 77. Concentration of chlorophyll in the upper 1 cm sediment layer of the MOTIFs, adjusted mean values based on 3 data points.

Figure 78. Composition of the microbenthic flora in the MOTIFs.

Figure 79. Relative abundance of some dominant benthic diatom species in the MOTIFs.

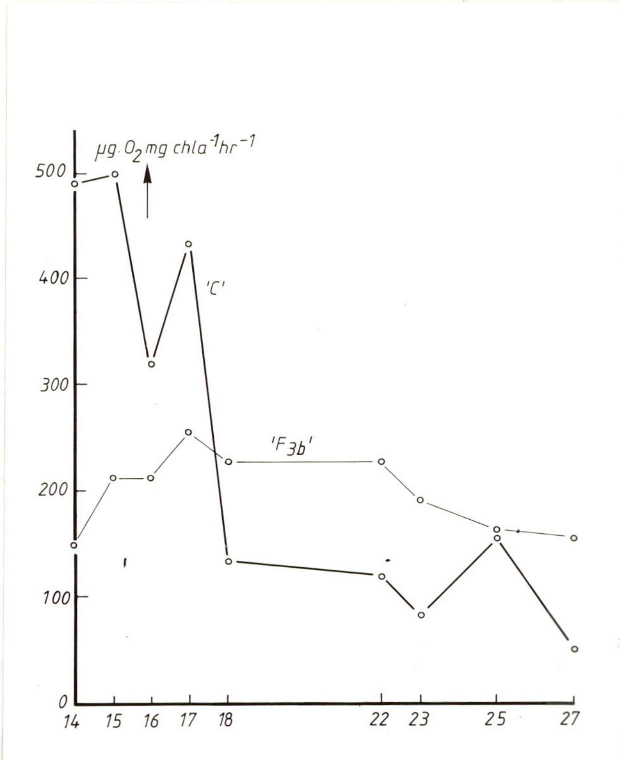


Figure 80. Gross Primary Production of the benthic microflora, and the community respiration of the benthos, in the 'C' and F3b' MOTIFs.

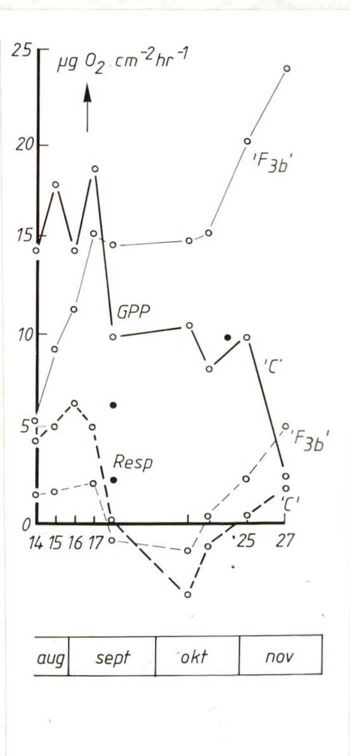
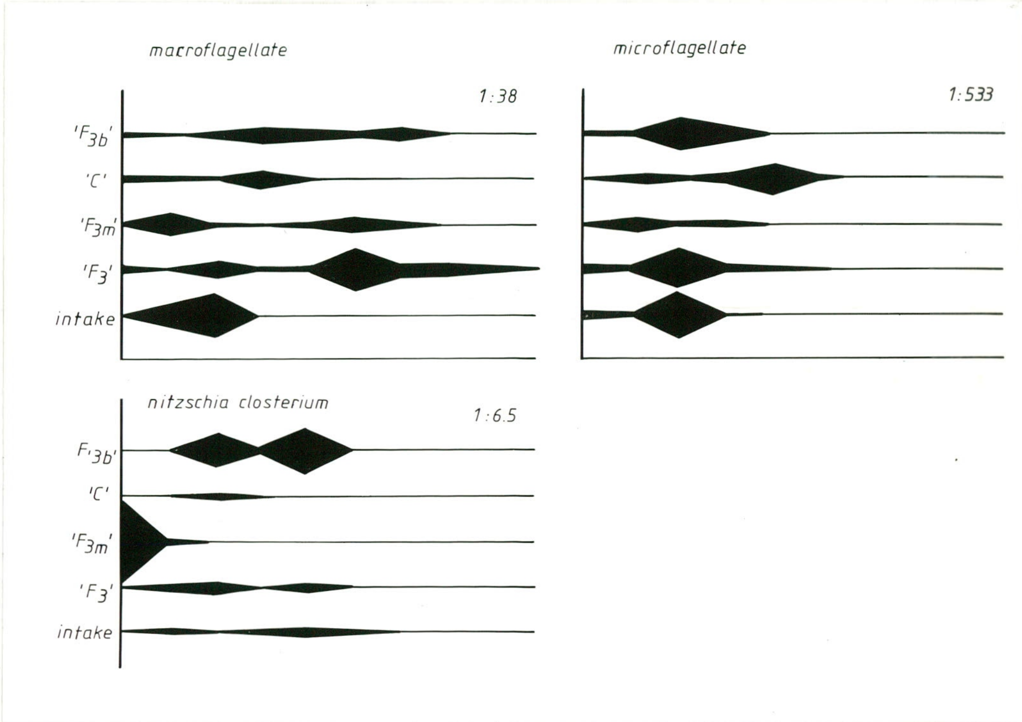


Figure 81. Gross Primary Production per unit chlorophyll a of the micro-benthic flora of the 'C' and 'F3b' MOTIFs.

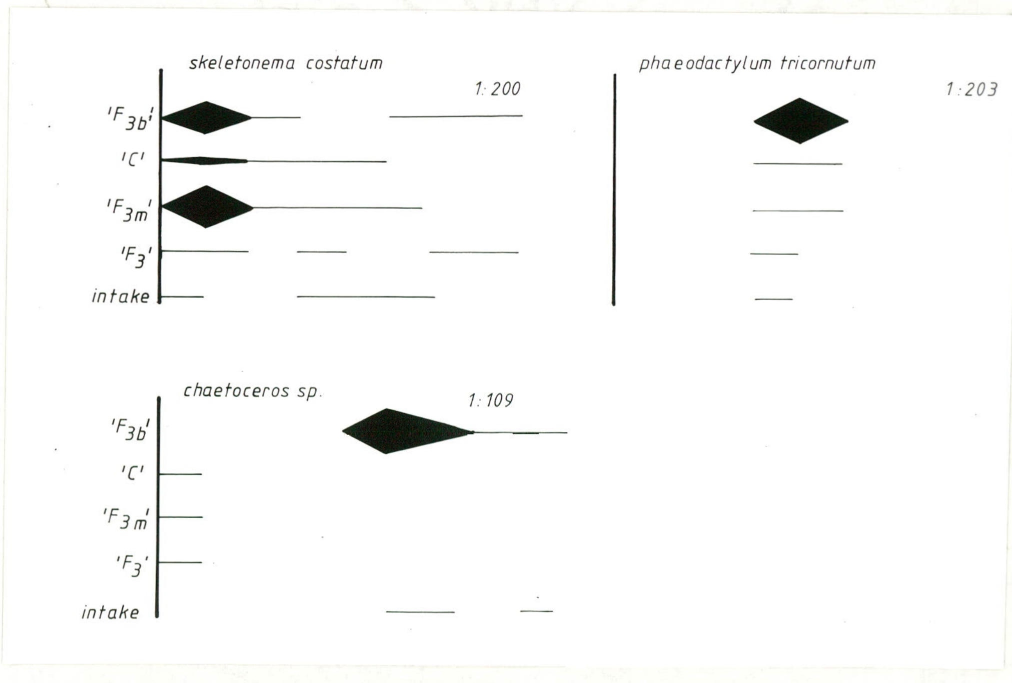
Figure 82.
Concentration of chlorophyll in the water of the MOTIFs. Adjusted mean values based on 3 data points.

Figure 83.
Gross primary production of the phytoplankton in the MOTIFs. Adjusted mean values based on 3 data points.

Figure 84. Gross primary production per unit chlorophyll a of the phytoplankton in the MOTIFs. Adjusted mean values based on 3 data points.



a.



b.

Figure 85. Relative abundance of some dominant phytoplankton taxons.

Figure 86. Density of bacteria in the water of the MOTIFs.

Figure 87. Concentration of dissolved oxygen in the water of the MOTIFs at 10.30 a.m. Adjusted mean values based on 3 data points.

Figure 88. pH of the water of the MOTIFs. Adjusted mean values based on 3 data points.

Figure 89. Concentration of nitrate in the water of the MOTIFs. Adjusted mean values based on 3 data points.

Figure 90. Concentration of nitrite in the water of the MOTIFs. Adjusted mean values based on 3 data points.

Figure 91. Concentration of ammonia in the water of the MOTIFs. Adjusted mean values based on 3 data points.

Figure 92. Concentration of phosphate in the water of the MOTIFs. Adjusted mean values based on 3 data points.

Figure 93. Concentration of silicate in the water of the MOTIFs. Adjusted mean values based on 3 data points.