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A STUDY OF MARINE OIL POLLUTION IN
OUTDOOR MODEL ECOSYSTEMS REPRESENTING
A TIDAL FLAT (OPEX)
- FINAL REPORT -

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SUMMARY

For several reasons the fate and ecological effects of oil pollution are not easily studied in natural marine ecosystems. Likewise, the results of laboratory tests cannot easily be extrapolated to natural systems. A logical objective of ecotoxicological research is, therefore, to simulate natural ecosystems in model ecosystem experiments.

In this report results of a feasibility study with Model Tidal Flat ecosystems (MOTIFs) are given. The systems described should represent a tidal flat ecosystem typical of the Wadden Sea and other European coastal areas.

Research in the first year of this feasibility study was aimed at providing an answer to the following questions:

- Is a MOTIF in a large outdoor concrete basin sufficiently representative of a natural tidal flat ecosystem?
- What is the variation with time of biotic and abiotic parameters between similarly treated MOTIFs?
- Can crude mineral oil be added to the MOTIFs in such a way that its behaviour is similar to that under field conditions in a natural tidal flat ecosystem?
- What kind of effects are produced by an oil spill on these model tidal flat ecosystems?

The experiment started in October 1981 using 4 MOTIFs. Each MOTIF was housed in a basin of 3.5 x 6 m containing a sediment layer about 50 cm deep and a tidal channel. A tidal regime was provided by pumping water between pairs of basins. Part of the water was renewed continuously with water pumped from the Wadden Sea. The average residence time of the water was similar to that in the nearby Wadden Sea (1 week). A number of representative macro-invertebrates was introduced in the systems at densities similar to those found in the nearby Wadden Sea.

It was found that the structure (species composition, population densities) and function (productivity, bioturbation, mineralization, growth and decline of several organisms) of the community in the MOTIFs was indeed similar to that found on natural tidal flats in many aspects, demonstrating that it is possible to build a representative model ecosystem.

Only four MOTIFs were used, so that information on the variation between replicates is limited. The information obtained, however, indicates that the variation between identically treated systems is generally small. In some cases a large variation was found, but this may have been caused by sampling errors.

In May an oil mousse was added to two of the four MOTIFs. The mousse consisted of a mixture of 16 l fuel oil and 2 l gas-oil and contained 70% water. Remaining floating oil was removed from the MOTIFs after an exposure period of one week.

Clear effects on several components of the ecosystem could be recorded directly after addition of the oil. The oil in the systems did not show an artificial behaviour, only a small part sticking to the walls of the basins. An important finding from an ecological point of view was that part of the oil mousse was transported into the sediment as a result of bioturbation activity of worms (mainly *Arenicola marina*) in the sediment. After the removal of the floating oil, this sediment-bound fraction caused a prolonged exposure of the system to oil components. This prolonged exposure to low concentrations of oil resulted in turn in long-term effects; for example mass mortality of the edible cockle, *Cerastoderma edulis*, was found in August, two months after most of the mousse had been removed from the MOTIFs. Other species, such as *Macoma balthica* and nematods, also suffered higher mortality in the contaminated MOTIFs. Another long-term effect was a reduction in settlement success of larvae (e.g. of *Heteromastus filiformis* or *Littorina littorea*) produced in the system or entering with the incoming water.

These events resulted in large differences in community structure between the contaminated and the uncontaminated MOTIFs. These differences persisted until the end of the experiment (December 1982).

1. INTRODUCTION

Modern industrialized societies produce large amounts of chemicals and chemical waste. Accidents during production or transport and deliberate disposal of wastes in the sea may cause severe pollution problems.

Coastal waters are generally most exposed to pollution, and Dutch coastal waters, including the Wadden Sea, are no exception to this rule.

An important aspect of ecotoxicology is the development of test procedures to produce information about the fate and effects of pollutants in ecosystems. This information should help the authorities to set standards limiting damage to the ecosystem in question and should also serve as a basis for clean-up operations in case of accidents.

Ecotoxicology, however, is still in its infancy (Hueck-Van der Plas and Hueck, 1979) and the test procedures being considered for national or international use must be submitted to continuous review. Because pollution problems occur in the field, it seems logical to assess the influence of pollutants in field experiments. With only a limited number of exceptions (BIOS, 1982; Level, 1976; Little et al., 1980), it is generally impossible to experiment with the natural marine system, although oil pollution catastrophes and other dumping practices might be considered as large scale experiments (e.g. Boucher, 1980; O'Sullivan, 1978; Conan et al., 1981). In such cases, however, detailed information on pre-spill, conditions is often lacking.

Most studies in the field of aquatic toxicology, biodegradation and bioaccumulation are performed in the laboratory. Although these experiments yield useful information, extrapolation of the results to field conditions is difficult. The deficiencies of small scale laboratory experiments have been recognized by many authors (Gray, 1974; Menzel and Case, 1977; Perkins, 1979; De Kock and Kuiper, 1981; Anon, 1981).

It is clear that methods should be developed which enable the results of ecotoxicological experiments to be extrapolated to natural conditions.

In an attempt to evaluate the fate and effects of pollutants, many authors (Saward et al., 1975; Ringelberg and Kersting, 1978; Dortland, 1980; Oviatt et al., 1980, 1982; Giesy, 1981, Grice and Reeve, 1982) have recently used more complex systems which can be considered to approximate field conditions more closely than do laboratory experiments. Most of these experimental systems were derived from natural systems by enclosing part of the environment

or by transplanting elements of the natural system to an experimental situation. Model ecosystems have also been used to study the fate and effects of oil (crude or refined products) on marine ecosystems (Elmgren et al., 1981, 1982; Farrington, 1982; Davies et al., 1981, Hodson et al., 1977).

In 1978 the Laboratory for Applied Marine Research MT-TNO initiated discussions between several organizations (Netherlands Institute for Sea Research (NIOZ), Research Institute for Nature Management (RIN), Shell International Research, The Hague, the North Sea Directorate of "Rijkswaterstaat" (RWS, Dutch Ministry of Transport and Public Works) and MT-TNO) about the possibilities of building sediment-water model ecosystems typical for the Wadden Sea and other coastal and estuarine areas of temperate seas.

The various organizations were interested from different viewpoints. Large outdoor facilities for experiments with large amounts of natural seawater were available at the RIN (Drinkwaard, 1972), the NIOZ had a number of years experience with two indoor model tidal flat systems (De Wilde and Kuipers, 1976). MT-TNO had used model plankton ecosystems for several years to study fate and effects of chemicals and were interested in including the sediment compartment in their ecotoxicological studies (Kuiper, 1981, 1982a). The North Sea Directorate of RWS and the oil industry were interested because initial application of the MOTIFs was to be in relation to oil pollution problems.

A large amount of literature on the fate of oil in the environment and the effects of oil compounds on various organisms is available (Moulder and Varley, 1971, 1975, 1978, 1979; Samson et al., 1980).

The oil related activities in the North Sea (exploration and exploitation) have increased continuously since about 1970 and large amounts of crude oil and refined products are transported by tankers.

Large numbers of relatively small spills have been reported from the coastal waters of the Netherlands, Germany and Denmark (Anon., 1982a). In July 1982 for example 109 oil slicks were recorded by aerial survey in Dutch coastal waters (RWS, 1982). In June 1982 a large spill (1100 m³ of oil) also occurred here (Anon., 1982b). The chance of a large oil spill in the neighbourhood of the Wadden Sea is ever present (Smets, 1981; Bergman, 1982) and at the moment only scattered scientific information is available to combat such a oil spill.

A three year project to gather information on the fate and effects of an oil spill on the tidal flat ecosystems of the Wadden Sea and on the effects of possible clean-up operations (e.g. use of dispersants) has been proposed (Hueck et al., 1979). In this study Model Tidal Flat ecosystems (MOTIFs) in concrete basins with a sediment layer and overlying water with a tidal cycle containing organisms characteristic for the Wadden Sea area would be studied. Before this project could start it was necessary to carry out a one-year feasibility study, in which answers to the following questions could be obtained:

- Is an experimental ecosystem in a large concrete basin sufficiently representative of a natural tidal flat ecosystem?
- What is the variation with time of biotic and abiotic parameters between identically treated MOTIFs?
- Can crude mineral oil be added to the MOTIFs in such a way that its behaviour is similar to that under natural conditions?
- What kind of effects can be expected from an oil spill on these model tidal ecosystems?

Four organizations cooperated as follows in the feasibility study. The RIN built the experimental systems and operated the technical installations, the North Sea Directorate of RWS provided the oil mousse. The NIOZ and the Laboratory for Applied Marine Research MT-TNO carried out the research programme and the latter institute coordinated the project.

Preliminary results have been given in two interim reports (Kuiper, 1982b,c). In this final report a detailed account of the research in the first year is presented.

2. MATERIALS AND METHODS

2.1 TECHNICAL SET-UP OF MOTIFs

The technical set-up of the MOTIFs was based on the design of indoor tidal-flat ecosystems developed by the NIOZ (De Wilde and Kuipers, 1976).

The MOTIFs were built near the RIN station on the Wadden island of Texel, where basic facilities were already present (Fig. 1).

Basically, each MOTIF consisted of a concrete and brick basin with an area of $6 \times 3.5 \text{ m}^2$ and a depth of 1.2 m (Fig. 2). A brick wall of 45 cm height on the bottom separated the "tidal flat" from the "tidal channel". The tidal flat ($6 \times 3 \text{ m}$) was filled with natural tidal-flat sediment. Near the brick wall the sediment was level with the top of the wall; from there it sloped upwards very gently.

The floor and one of the sides of the basin consisted of concrete which had been in contact with seawater for 10 years. The remaining three sides and the brick wall were built with concrete bricks (MBI-betonsteen). The outer brick walls were plastered with cement and all the walls and the bottom were painted with two layers of Colturit. After painting the basins were allowed to dry for 5 days, filled with salt water, leached for 7 days and finally emptied.

The basins were coupled in pairs. Each pair was provided with one pump to perform the tidal water movements. Water was pumped back and forth so that low water in one basin coincided with high water in the other.

0.55 kW Begemann Type "kz 120-40" centrifugal pumps with a maximum capacity of $3 \text{ m}^3 \cdot \text{hr}^{-1}$ at 2 m watercolumn were used. Water-flow was directed by a system of magnetically operated valves coupled to a time-switch. The suction and outlet ends were both in the tidal channel.

Two pairs of basins were constructed. All basins were connected to a permanent supply of Wadden Sea water at $0.1 \text{ m}^3 \cdot \text{hr}^{-1} \cdot \text{basin}^{-1}$ using the same type of Begemann pump as described above. This resulted in an average renewal of 7.5% per tidal cycle (average water residence time ca 1 week). 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 (Fig. 3).

The tidal cycle lasted exactly 12 hours with slack tide at about 4.00, 10.00, 16.00 and 22.00 hours. Once a week the tidal cycle in each pair of

basins was shifted by 6 hours, so that low water was at 10.00 in the two basins which had had low water at 16.00, and vice versa. Fig. 4 gives the shape of the tidal curve. High water was 0.45 m above tidal flat level; at low water the tide fell to the level of the surface of the tidal flat.

The water was pumped through pipes with an internal diameter of 3.3 cm; consequently the flow rate was $0.9 \text{ m} \cdot \text{sec}^{-1}$. The pumps revolved at 0.38 r.p.s.

Sediment for the tidal flat models was collected from the natural tidal flats of the Mok Bay at the southern tip of Texel island. The top layer of about 0.1 m thickness was scraped off an area of about 500 m² in October 1981. After transport to the experimental area it was spread out on an asphalt platform in the open air, where it remained for 30 days and was subjected to all kinds of weather, including rain. The sediment was then mixed as thoroughly as possible and transferred to the basins, which had already been filled with seawater. This procedure ensured that the sediment did not contain air and was thoroughly mixed with seawater. Tabel 1 gives the grain size composition of the sediment.

Table 1 Mean percentages of grain size fractions (f = lower limits of fraction) of the original sediment in the basins.

f	%
0 μm	0.6
50	0.4
63	0.6
80	0.3
100	9.8
160	22.4
200	58.8
315	5.6
400	1.5
> 630	0.1

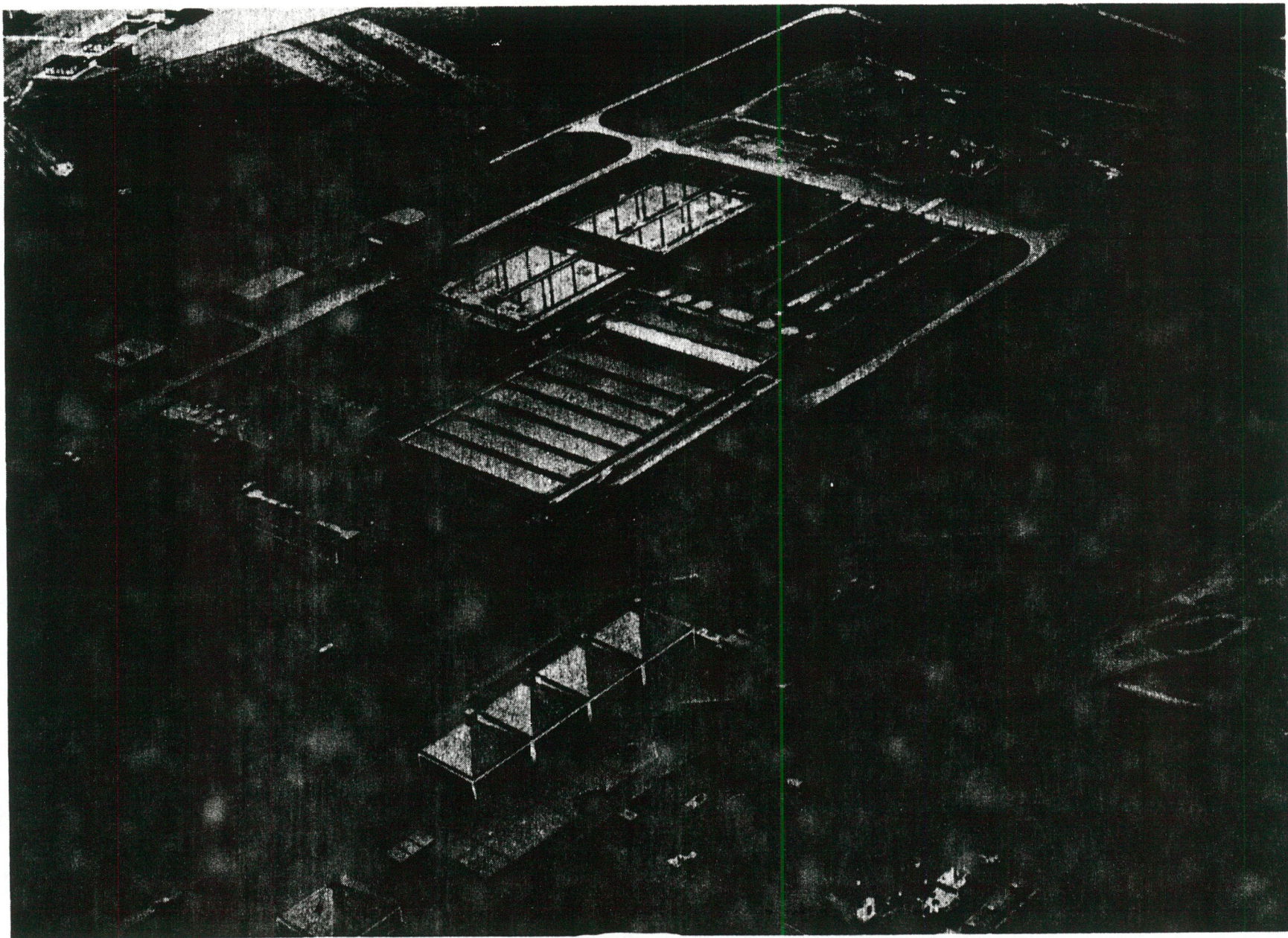


Fig. 1 Aerial photograph of experimental facilities of the Research Institute for Nature Management at 't Horntje, Texel.



Fig. 2 An overview of the four MOTIFs.

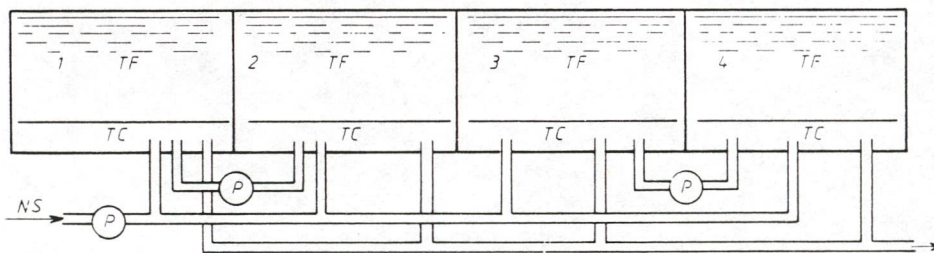


Fig. 3 Diagram of the MOTIFs.

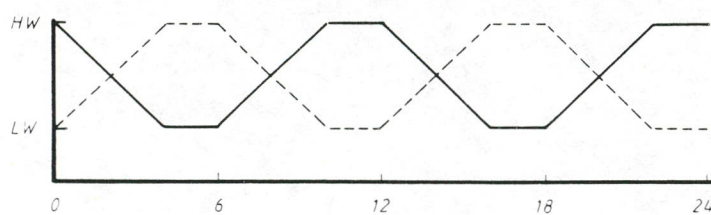


Fig. 4 Tidal cycle of the MOTIFs.

HW = high water;
LW = low water.

2.2 EXPERIMENTAL SET-UP

The experiment started in October 1981 with four MOTIFs (nos. 1-4) coupled in two pairs. The first samples were taken on 29 October. In order to accelerate the maturation of the ecosystem, representative larger macrofauna species were introduced into the system from the Wadden Sea in numbers similar to the average density in nearby areas of that Sea (Beukema, 1976). Table 2 shows the species which were introduced, together with some basic data concerning their population densities, origin, dimensions, etc.).

Table 2 Species of macro-invertebrates brought in the model ecosystems at the start of the experiment, together with some other data.

Species	Number in the model system	Average population density in Wadden Sea (N/m ²)	Dimensions	Origin	Date of introduction
<i>Mytilus edulis</i>	200 per basin	20	3.0-3.5 cm	dike Huisduinen	12.11.81
<i>Littorina littorea</i>	500 per basin		ca 1 cm	dike 't Horntje	19.02.82
<i>Cerastoderma edule</i>	28/m ²	34	ca 3 cm	Balgzand	07.01.82
<i>Macoma balthica</i>	100/m ²	113	ca 2 cm	Zoutkamperlaag	04.11.81
<i>Arenicola marina</i>	25/m ²	17	ca 10 cm	Balgzand	07.01.82

These species (*L.littorea* excluded) form about 80% of the macro-invertebrate biomass in the Wadden Sea. The periwinkle *L.littorea* was introduced into the systems to clean the walls of the basin of algae and thus to minimize wall effects.

The organisms which live in the sediment were distributed evenly over the model tidal flat. The mussels were placed in cages in the tidal channel in randomized portions of 25 specimens.

Fig. 5 shows a simplified diagram of the interrelations between the different biotic compartments and the abiotic factors influencing the development

of the organisms in the system. Table 3 lists the parameters measured during the programme which was carried out from October 1981 to December 1982.

No oil was added to the MOTIFs in the first six months. During this period information on the variation in time of biotic and abiotic parameters between identical MOTIFs was gathered. Table 3 also shows the sampling frequency during this period.

Sixty litres of an oil mousse was added to each of MOTIFs 3 and 4 on 24 May 1982. The mousse consisted of 16 l fuel oil, 2 l gas-oil and 42 l sea-water by vigorously mixing for at least 2 h using a submersible centrifugal pump (Koops pers. comm.). This mixture was chosen because these types of oil products are transported in bulk along the Dutch coast and through the Wadden Sea and because this mixture had a relatively high content of aromatic compounds (31% by IR analyses).

Aromatic compounds are held responsible for the bulk of biological effects by many authors (e.g. Neff and Anderson, 1981; Bergman, 1982).

The oil mousse was added to the systems 3 and 4 just before low tide by placing small amounts of mousse (ca 1 l portions) on top of the water. The mousse was initially spread over the whole water surface by gently moving the water. At the same time the overflow of all systems was changed so that the oil slick could not escape from the MOTIFs.

One week (the average residence time of the water in the western Wadden Sea) after the addition of the oil mousse, all the oil floating on the water was removed from the two systems and the original condition of the overflow was restored. An intensified sampling programme was carried out after the addition. Daily samples for many parameters were taken during the first week. Throughout the following month samples were taken three times a week, thereafter twice a week and from September the pre-spill sampling frequency was used again. In the first week after the addition, the fate of the oil was (in addition to the sampling programme) also monitored by time-lapse photography and by video-recording.

According to the contract with the EEC the project ended on 30 September, but additional samples were taken in the period October-December 1982 to detect possible long-term effects of the oil.

Table 3 Summary of the sampling programme conducted in the MOTIFs.

Compartment	parameter determined	determination frequency per month (pre-spill period)
Phytoplankton	Species composition (qualitative)	4
	Chlorophyll	4
	Phaeopigments	4
	Pigment index	4
Zooplankton	Species composition (semi-quantitative)	4
Bacteria (water)	Total numbers (fluor- escence microscopy)	4
Phytobenthos	Species composition (qualitative)	irregular
	Chlorophyll)	2
	Phaeopigments) in sediment	
	Pigment index) profiles	2
Bacteria (sediment)	Electron transport system (ETS) in (sediment profiles)	2
	Community respiration	irregular
Meiofauna	Biomass and speciesgroups (semi-quantitative)	1
Macrofauna	Total numbers per species	1
	Biomass (production)	1
	Growth	1
Physico-chemical	pH	4
Factors	Temperature	4
	Oxygen	4
	Nutrients (ammonia, nitrite, nitrate, silicate, phosphate)	4
	Salinity	4
	Suspended particles	4
	Light intensity	Continuously
	Added oil	irregular

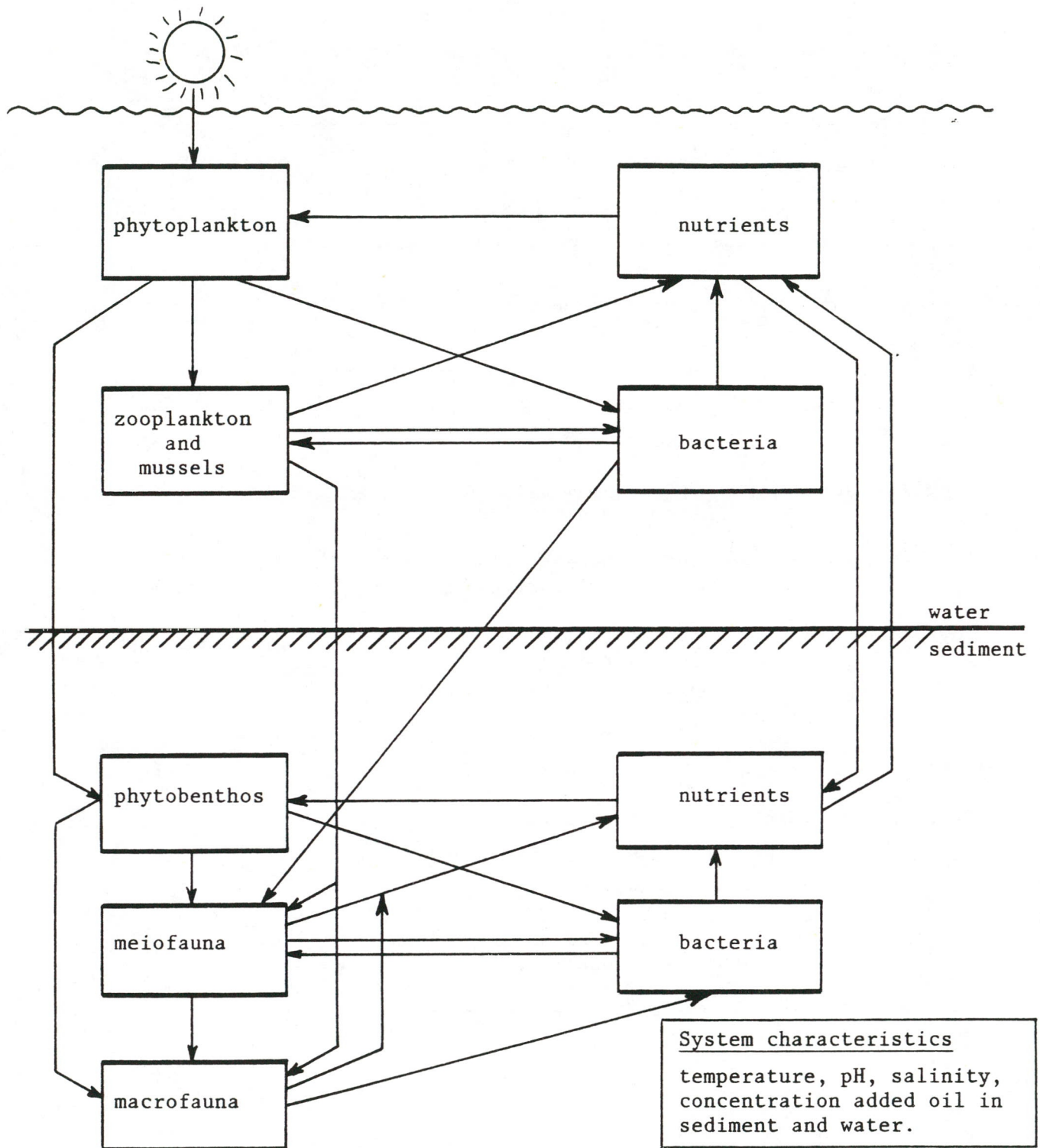


Fig. 5 Simplified diagram of the interrelations between the different biotic compartments and the abiotic factors influencing the development of the organisms in the compartments of the community in a MOTIF.

2.3 SAMPLING METHODS

2.3.1 Microflora, microfauna and physico-chemical parameters

2.3.1.1 In water

Samples were taken from the water layer covering the sediment to follow the development of the phytoplankton (by chlorophyll, phaeopigments, pigment index and species composition), the zooplankton (numbers of organisms and species composition), bacteria (numbers) and various physico-chemical parameters (temperature, salinity, pH, concentration of oil remaining, phosphate, silicate, ammonia, nitrite and nitrate, oxygen concentration and concentration of suspended solids).

Before the addition of the oil, water samples for phytoplankton, bacteria and the physico-chemical parameters were collected with a water sampler (Kuiper, 1981), in which three sampling bottles of 1 l could be placed. Samples were taken at high water from a depth of approximately 30 cm. The samples were transported to the laboratory as soon as possible and processed 0.5-2 h after sampling.

One litre was used for chlorophyll analyses and one litre was filtered to measure the concentration of suspended solids. The remaining litre was used for the other analyses. Replicate subsamples of 5.0 ml were fixed and preserved with 0.3 ml filtered formaldehyde solution for determination of bacterial numbers. A subsample of at least 100 ml was frozen at -20°C for later nutrient analyses, a subsample of 100 ml was fixed with 1 ml Lugol's iodine solution (Vollenweider, 1969) and used for phytoplankton species composition determination. pH was measured in the remaining water, temperatures were measured in situ, as was oxygen concentration.

After the oil addition, the sampling bottles (including those in the controls) were filled with a siphon to prevent contamination of the samples by the floating oil. This siphon was installed in the tidal channel before the oil addition.

Samples for oil analysis were collected in 1 l glass flasks which had been carefully cleaned and heated at 350°C for at least 4 h to remove possible contaminants. These samples were frozen at -20°C as soon as the samples arrived in the laboratory and kept frozen until analysis. Samples of the floating oil slick were taken with a glass beaker and also frozen at -20°C.

Before the oil addition, zooplankton samples were collected by towing a 55 μ m plankton net (aperture 20 cm) a distance of 10 m through the basin. Assuming a 100% efficiency, 60 l water was filtered in this way. After the addition of the oil, zooplankton samples were collected by filtering 30 l water through the net from the siphon in each basin. The zooplankton samples were preserved with 4% formaldehyde solution in seawater.

2.3.1.2 In sediment

Samples were taken from the sediment at low water to follow the development of the phytobenthos (chlorophyll, phaeopigments, pigment index, species composition on selected days) and the bacteria (electron transport system (ETS)). After the oil addition, samples were also taken to follow the oil in and on the sediment. Most sediment samples were collected with a core sampler with a diameter of 2.5 cm and a length of 12.5 cm. Each core was divided into different sections (0-1, 1-2, 2-10 cm depth) directly after sampling. The tidal flat was divided by a grid, each field of the grid measuring 12.5 x 12.5 cm.

A movable bridge 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. Twenty fields of the grid, regularly distributed over the flat, were sampled on each sampling date. In this way a particular field was not sampled more than twice during the year. The tidal flat was divided into a lower reach (close to the tidal channel), a middle reach and an upper reach. This division was based on the finding in the NIOZ indoor model tidal flats that the development of the system was different in the different reaches. Additional samples were collected from the middle reach with a plexiglass core sampler (diameter 2.5 cm) from a depth of 35-40 cm. On each sampling date 10 samples per basin were thus available for chlorophyll and ETS analysis. Samples were transported to the laboratory as soon as possible, sediment was mixed, and 0.5-2h after sampling subsamples were used for chlorophyll and ETS analyses, and for the determination of the wet and dry weight of the sediment.

After the oil addition, the same sampling procedure was used to collect sediment samples for oil analysis: subsamples of ca 150 g wet weight were frozen at -20°C in glass flasks. On selected dates samples to investigate the vertical distribution of the oil in the sediment were taken. To prevent

contamination of deeper layers by contaminated samplers, a clean core sampler (diameter 2.5 cm, length 45 cm) was used on selected spots in the systems. These cores were divided into sections (surface 2 mm, 0.2-1 cm, 1-2 cm, 2-5 cm, 5-10 cm, 10-20 and 35-40 cm) and frozen at -20°C until analysis.

2.3.2 Meiofauna and macro fauna

To follow the development of the populations of macrofauna organisms, sediment cores with a diameter of 154 mm $\emptyset$ and a depth of about 30 cm, were taken at low water in 20 grid fields as described above. Samples were taken at monthly intervals, although the sampling programme was intensified in the period shortly after the application of the oil-mousse. The sediment cores obtained were sieved over 1000 μ m $\emptyset$ gauze. The material retained, including the living organisms, was collected in plastic bags and stored at 3°C until further treatment and analysis. To minimize the effect of sampling on the MOTIFs themselves, the sieved sediment was replaced in the open bore holes in the mud flat.

For *M.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.

Meiofauna were also sampled at monthly intervals. Twenty samples per MOTIF were taken with a 15.5 mm corer up to a depth of 100 mm. Five cores were mixed in a plastic jar and preserved with 40% formaldehyde solution).

In addition in May, before the oil addition, and in June and September, 8 sediment cores were collected in glass bottles of 100 mm $\emptyset$ and a depth of 12-15 cm in the central part of MOTIF 2 (control) and MOTIF 4 (oil-polluted) for measuring benthic community respiration and for detailed investigation of the bottom fauna. Four cores were obtained from a smooth area, 2 from an *Arenicola* funnel, and 2 from an *Arenicola* cast; this roughly reflects the surface topography of the MOTIFs. The intact sediment cores were carefully transported to the laboratory and maintained at in situ temperature until incubation.

On 4 June and 15 July samples of *M.balthica* and *C.edule* were collected in the central part of MOTIFs 1 and 3 for oil analysis of the soft tissues of these species.

All MOTIFs were visually inspected weekly to detect possible particularities in the behaviour of the macrofauna organisms and to remove empty shells of dead bivalves if present. Dead periwinkles were not removed.

During the last week of December a final sampling programme was carried out. In addition to the usual microbenthos and water samples, a special macrofauna programme was carried out. Firstly, all newly produced casts of *Arenicola marina* were counted to obtain an impression of the total numbers of active lugworms in each MOTIF. Eighteen iron squares of 0.1 m² each were then placed randomly in each MOTIF, the sediment under each plate was carefully dug out to a depth of 10 cm and sieved over a 5 mm gauze. The bivalves retained were collected. In this way a more accurate estimate of the population densities was obtained than with the usual sampling method. Finally, large parts of the mudflat were dug over with a spade for non-quantitative collection of cockles and lugworms. Part of these samples was stored at -20°C prior to oil analysis.

2.4 ANALYTICAL METHODS

2.4.1 In water

The chlorophyll concentration of 1 litre water samples was measured according to 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 90% acetone and, after 1 minute of sonification with a Branson ultrasonic generator, the remnant was extracted for 24 h in a refrigerator. After clarification by centrifugation, pigment concentrations were measured with a Vitatron MPS photometer system using 1 cm cells. Concentrations of chlorophyll and phaeopigments were calculated according to equations given by Lorenzen (1976).

The pigment index described by Margalef (1965) was calculated from the corrected extinctions at 665 and 430 nm ($\text{index} = (D_{430} - D_{750}) / (D_{665} - D_{750})$).

The samples of phytoplankton were preserved with Lugols iodine and examined with a Zeiss inverted microscope (Utermohl, 1958). The main species were identified where possible using nomenclature given by Drebes (1974) and Hendey (1964).

Before the oil addition, the oxygen concentrations were measured with an Orbisphere Laboratories model 2603 oxygen meter. After the oil addition, most oxygen concentrations were determined with a sensitive Winkler titration with 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 the primary productivity and the respiration of the plankton. To this end light and dark bottles (contents ca 120 ml) were incubated in situ for ca 4 h. By comparing the oxygen production in the flasks, by the phytoplankton, with the oxygen productions in the basin, an indication on the productivity of the phytobenthos in the system was obtained.

The concentration of suspended solids was determined by filtering 1 l samples over a dried paper filter (Schleicher and Schull, Schwarzband no. 589) and measuring the dry weight of the material on the filter after drying for 24 h at 110°C.

The concentrations of phosphate, reactive silicate, ammonia, nitrate and nitrite were measured with a Technicon AutoAnalyzer according to Strickland and Parsons (1968) and Technicon procedures.

The pH was measured with a Beckmann model 3550 pH meter. Salinity was calculated from chlorinity titrations according to Strickland and Parsons (1968).

Numbers of bacteria were counted with a acridine orange technique of Daley and Hobbie (1975) using a Zeiss fluorescence microscope.

The zooplankton organisms were counted and identified by procedures described by Fransch (1976). Subsamples of the 60 and 30 l samples were examined until at least 150 organisms has been counted. Calanoid copepods were classified as nauplii, copepodites and adults.

2.4.2 In sediment

For chlorophyll analysis subsamples of 1 g wet sediment were placed in 15 ml centrifuge tubes, 10 ml 90% acetone was added and the further procedure for water samples was followed.

Electron Transport System (ETS) activity was measured in subsamples of ca 5 g wet sediment according to the method of Olanczuk-Neyman and Vosjan (1977) and Vosjan (1982). Extinctions were measured on a Vitatron MPS photometer using a 1 cm flow cell.

All chlorophyll and ETS values in the sediment are given on a dry weight basis. The dry weights of sediment subsamples were determined after drying for 24 h at 110°C.

For the measurement of community metabolism the following procedure was used.

The glass bottles with the sediment cores were topped up with sea water at in situ temperature from the MOTIFs, which had been previously filtered over 0.5 μm millipore filters to remove suspended particles. Each bottle was then connected in a closed circuit with a peristaltic pump which produced a constant flow of about 1.5-2.0 l per hour of recirculated water over the sediment.

The oxygen concentration of the circulating water was continuously monitored with a Yellow Springs Oxygen electrode Y.S. 5793. The incubation period generally lasted about 12 hours. Six hours the bottles were exposed to an artificial light source (4 cool white tubes) to allow estimation of the relatively primary production in the bottle by phytobenthos. For the remaining time the bottles were kept in the dark to measure the oxygen demand of the mud-flat sediment and the living organisms therein. The benthic community respiration was calculated from the measured decrease in oxygen concentration in such a way that consumption below the level of 70% O_2 saturation was ignored.

To relate the estimated oxygen consumption to the biomass of the fauna elements present in the bottle, the faunal species composition of each bottle was carefully determined. To this end the sediment cores were sieved over 400 μm nylon gauze and the collected animals sorted and determined as described in § 2.4.4.

2.4.3 For meiofauna

Preserved meiofauna samples were elutriated for 15 minutes by flushing with about 2 l of sea water per min., following the methods described by Uhlig et al. (1973). The material washed out was retained on 50 μm nylon screen and stained with Bengal rose. The stained material was poured into a measuring cylinder and diluted to a volume of 30 cm^3 . The particles were brought in suspension by gently shaking, after which three 1 cm^3 aliquots were collected with a pipette. Subsamples were placed in a petri-dish with a mm grid, and then examined and counted under a stereo-microscope for taxonomic iden-

tification. Organisms were only determined on the level of groups: nematodes, oligochaetes, juvenile and adult copepods and larval and juvenile stages of benthic macrofaunal organisms.

2.4.4. For macrofauna

Macrofauna samples were spread out in shallow photo dishes and carefully sorted on the species level under a stereo microscope at low magnification. Oligochaetes and large nematodes were only determined on the group level. The length of each specimen of the bivalve species *Cerastoderma* and *Macoma* was measured with an accuracy of 0.5 mm. The shells were opened by immersion of boiling water and the soft parts were removed.

Animals belonging to one species, group or length class were placed in porcelain cups and dried in a well ventilated stove at 70°C to constant weight. Finally the ash content of the cups was estimated after combustion for 2 hours at 540°C. The ashfree dry weight (ADW) of the animals was then calculated.

M. edulis samples were processed as follows. The shells were first measured and the empty shells counted. The soft tissues were then removed from the shell with a metal knife and homogenized; the dry and ash weights were then determined, according to De Kock (1982).

Growth in weight was derived from the weight increases in individuals. Occasionally the so-called condition index - the ADW of an individual divided by its length to the 3rd power ($W.l^{-3}$) was calculated for the larger mollusc species.

The *Litorina* populations in the MOTIFs were only completely collected once (in October) to measure the length frequency distribution. After measurements they were released into the MOTIFs.

2.4.5 Oil analyses

Oil analyses were carried out using infra-red spectrometry (IR), fluorescence spectrometry, high pressure liquid chromatography (HPLC) and gas-chromatography (GC). Table 4 shows which compartments were analysed together with the technique used.

HPLC and GC was only used on selected samples. Extracts of all samples were made in hexane (for IR, fluorescence and HPLC analysis) and CS₂ (for GC). Detailed information on the analytical procedures used is given by Compaan (1982).

Table 4 The various compartments in the model system analysed for oil together with the method used.

	fluorscence	IR	HPLC	GC
mousse	+	+	+	+
water	+	+	+	+
sediment	+	+	+	+
organisms	+	-	+	-

3. RESULTS

3.1 TECHNICAL FUNCTIONING OF THE INSTALLATIONS DURING THE EXPERIMENT

The technical functioning of the installations during the experiment caused few problems. The following points should, however, be mentioned.

The high-pressure magnetic valves used required much maintenance for good performance.

In future work with the basins, other valves, which require less maintenance, should be used.

In December 1981 and January 1982 temperatures were so low that ice was formed in the basins, the pipes and the pumps. All basins were therefore filled with 45 cm of water, and the pumps and pipes were drained. Tidal flow was thus stopped. The systems survived two separate periods of ice cover of 12 days in December and 14 days in January, although some of the paint, where the ice touched the walls, flaked off.

In September 1982 spat of mussels (*Mytilus edulis*) was discovered in the pipe supplying Wadden Sea water to all basins; these were removed.

In addition to ca 50 litres of oil mousse which was removed from the MOTIFs at the end of the exposure period in early June, about 13 dm³ of oil was skimmed off from the oil separator in the period between 1 June to 20 September.

3.2 DEVELOPMENT OF THE MOTIFs

Because a very large number of measurements was made during the experiment only the main results will be given in this section. Examples are chosen to provide answers to the main questions posed in this research (i.e. development of the MOTIFs, fate and effects of the added oil). All other results of measurements in the MOTIFs are given in Appendix I.

3.2.1 Physico-chemical parameters

Fig. 6 shows the air and water temperatures during the experiment. In December and January temperatures were so low that ice was formed, the tidal flow was stopped and all basins were filled with 45 cm of water to prevent freezing of the sediment and consequent damage to the organisms therein.

After the ice had melted, water movement (tidal flow, suppletion of water) was restored.

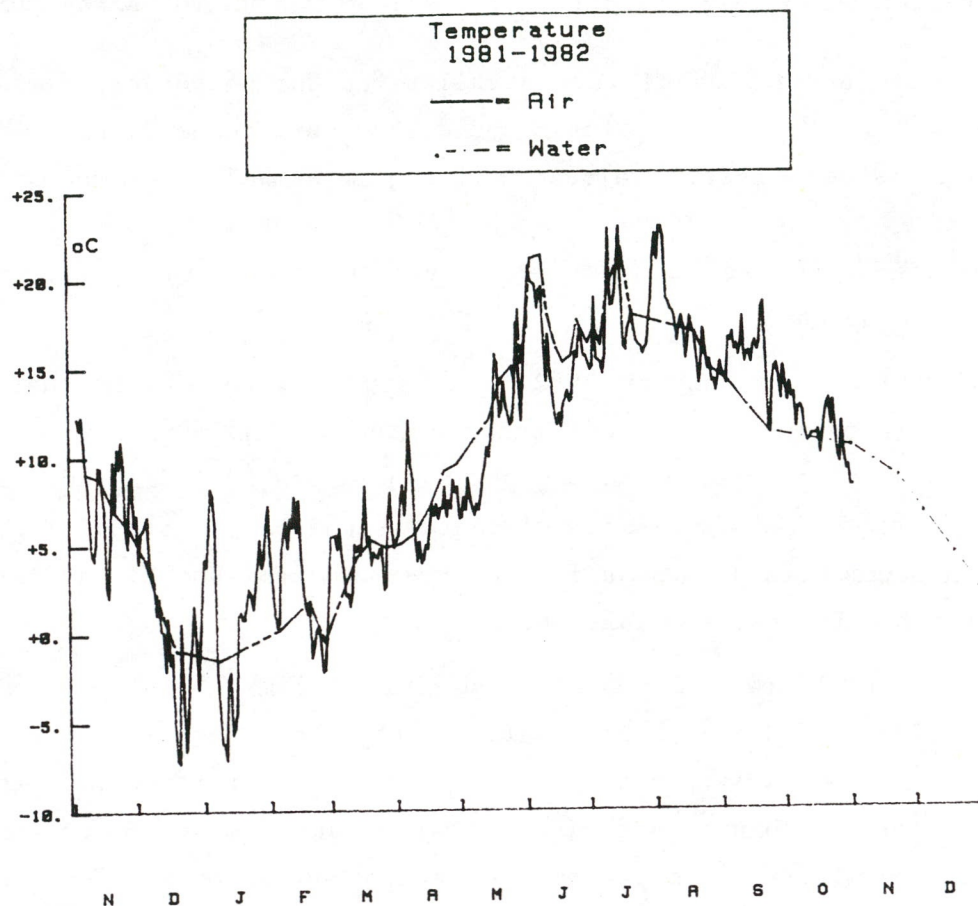


Fig. 6 Water and air temperatures in the MOTIFs during OPEX.

Fig. 7 shows the salinity in the different MOTIFs during the experiment. The salinities were generally within the normal range for the western Wadden Sea, although low values were measured sometimes, which cannot easily be explained. The salinity was generally similar in all basins, at any time, showing that the water had similar residence times, and that differences in other parameters such as nutrients and chlorophyll must be attributed to measurement errors or to differences in development between the different MOTIFs.

The concentrations of suspended particulate matter in the MOTIFs were around 10 mg/l throughout the year. This is low in comparison with concentrations found in the Wadden Sea, which is sediment laden (range 30-200 mg/l) because of the strong currents.

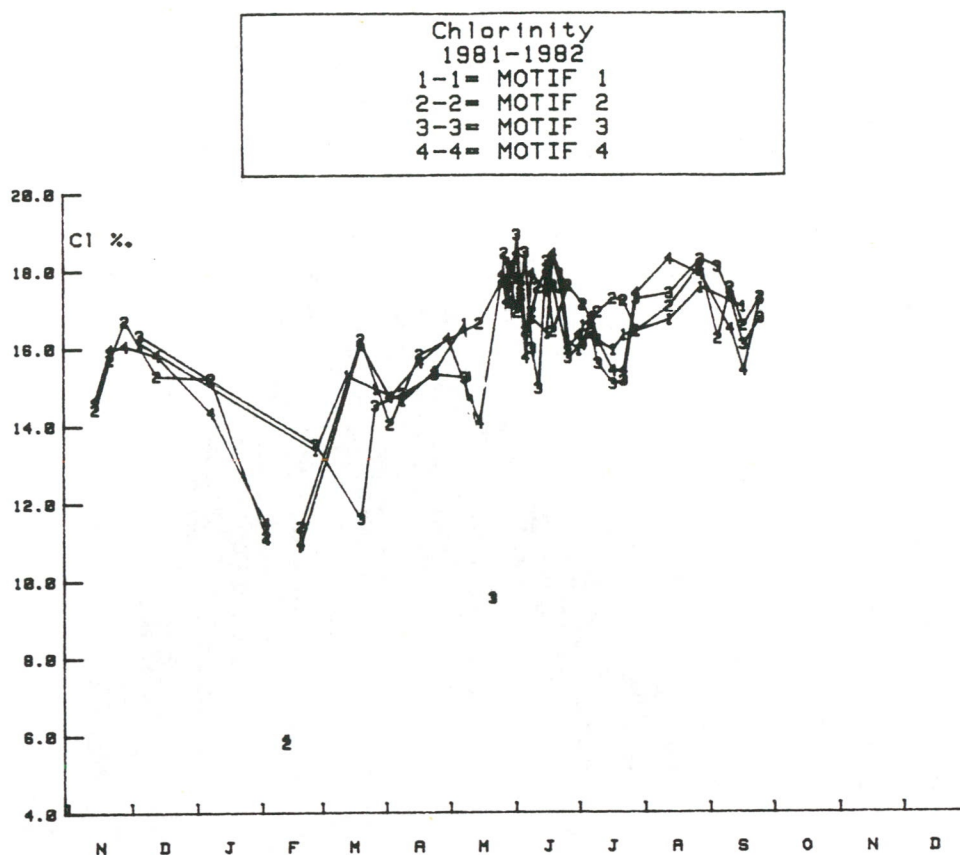


Fig. 7 Chlorinity in the MOTIFs during OPEX.
(salinity = $1.805 \times \text{chlorinity} + 0.03$).

Fig. 8 shows the global radiation as measured at the meteorological station at Den Helder. Measurements made in Den Helder do not differ by more than 10% from those on Texel (Cadee pers. comm.). In addition to the seasonal fluctuation, it can be seen that very much light was available in the period after the oil addition. This sunny period is also reflected in the high air and water temperatures (Fig. 6).

A few remarks on the texture of the sediment can be made here.

The compaction of the sediment increased after the start of the experiment. However, after the introduction of the lugworm *Arenicola marina*, bioturbation increased considerably and the water content of the top layers of the sediment increased again. In the controls this situation lasted for the rest of the experiment, although bioturbation activity decreased from September to the end of the year. After addition of the oil in May *A. marina* disappeared from the most contaminated locations in the MOTIFs (as a result of mortality or migration) and bioturbation was very low at these spots.

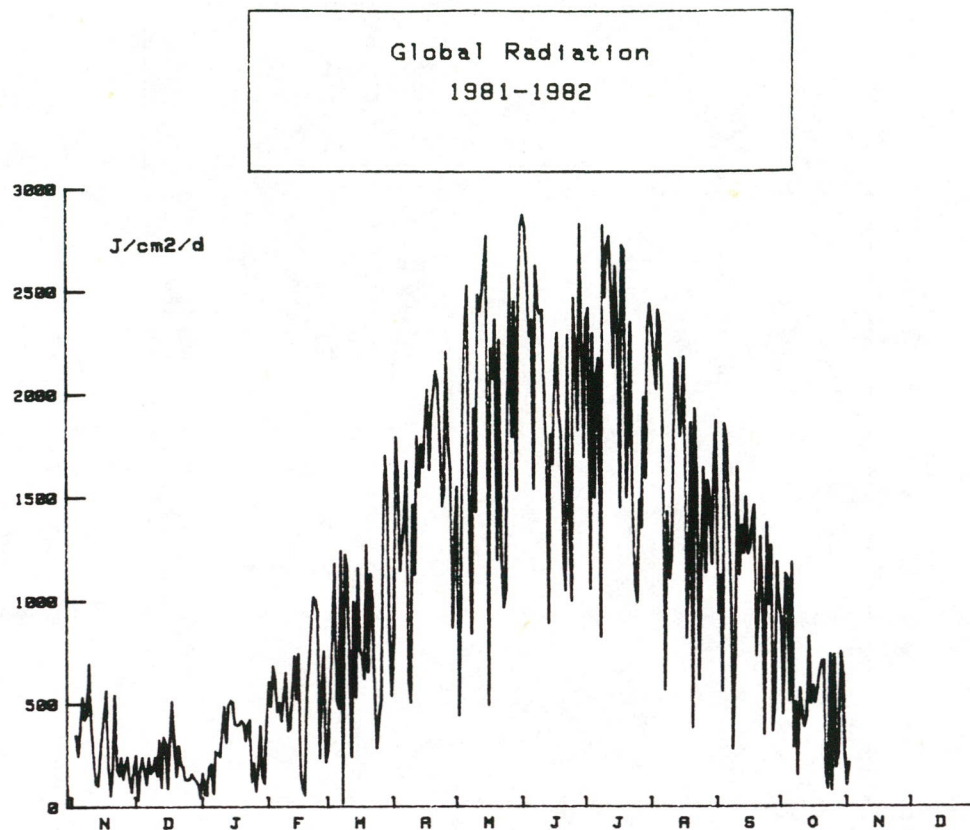


Fig. 8 Global radiation during OPEX at the meteorological station at Den Helder.

This decreased bioturbation activity resulted in low water content and a very compact sediment, into which it was difficult to push a core sampler. The water content of the sediment was less in the contaminated MOTIFs than in the controls for the rest of the experiment, indicating an overall decrease of bioturbation activity in the oil contaminated systems.

The results of the pH measurements and the concentrations of nutrients will be given in the section on the phytoplankton development (§ 3.2.7).

3.2.2 Oil in water, sediment and organisms

3.2.2.1 General picture and visual inspections

Fig. 9 shows how the oil mousse floated on the water 2 days after its addition to the systems. The oil mousse was evenly distributed over the water surface on only one day without wind. On other days the oil slick was driven to one of the ends of the basins. This wind effect resulted in a very uneven distribution of the oil on the sediment at low water.

When water rose, most of the oil floated on the water again, but part remained stuck to the sediment. It appeared that sediment deposited on top of the oil mousse by the bioturbation activities of worms (e.g. *Arenicola marina*) prevented the release of the oil from the sediment at high water.

This action of the worms trapped part of the oil in the sediment during the relatively short periods during each tidal cycle (2 of the 12 hours) that oil laid on the sediment. The oil was not evenly distributed over the tidal flat, because of the wind effects; most of it being concentrated in the northern corners of the basins. The floating oil was removed after one week of exposure; ca 50 l of oil mousse being removed from the two contaminated MOTIFs. In addition, 13 litres of oil was recovered in the oil separator at the end of the experiment. The remainder of the 36 litres oil added to the two MOTIFs had volatilized or was trapped by the sediment. During the remainder of the experiment a thin oil film was very often observed on the water surface in the oil contaminated MOTIFs, showing that oil components were released from the sediments until the end of the experiment (December 1982), 7 months after the one week exposure.

During the weeklong exposure period, part of the oil adsorbed to the walls of the basins when the tide fell. This layer was never more than ca 0.5 mm thick, indicating that a maximum of 4.5 litres of the 60 litres of mousse added, adhered to the walls. After the floating oil was removed, the walls of the basins were covered by a thin black layer of weathered oil of at most 0.1 mm thickness. In October-November a mat of small *Enteromorpha* spp. developed in the black layer, which then loosened from the walls (oil content of the layer was then 42 mg/g as measured by fluorescence).

During the week of exposure no visible amounts of oil mousse passed the pump generating the tide.

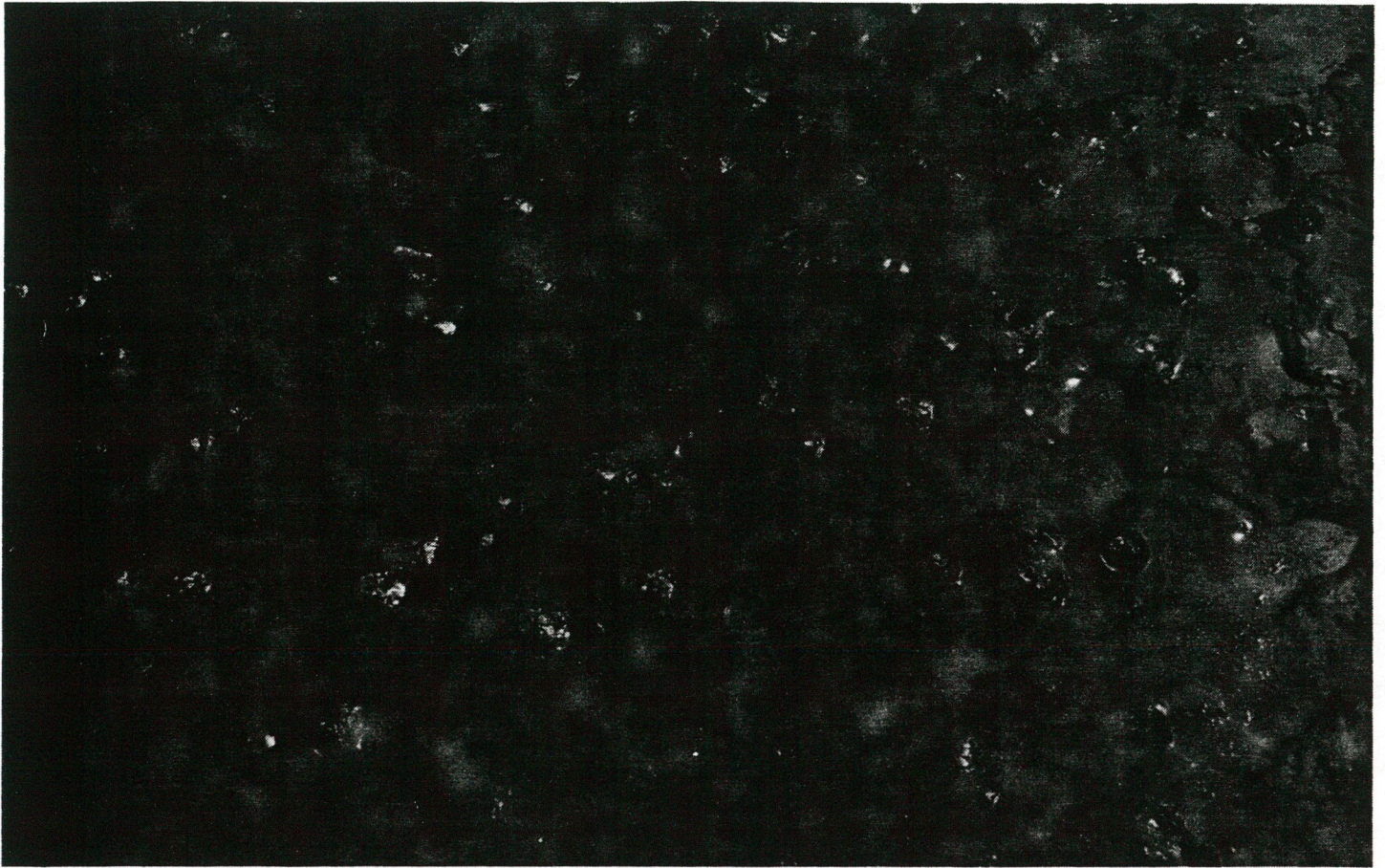


Fig. 9a The oil mousse covering the sediment at ebb; the pits and casts of *A. marina* can be clearly seen.

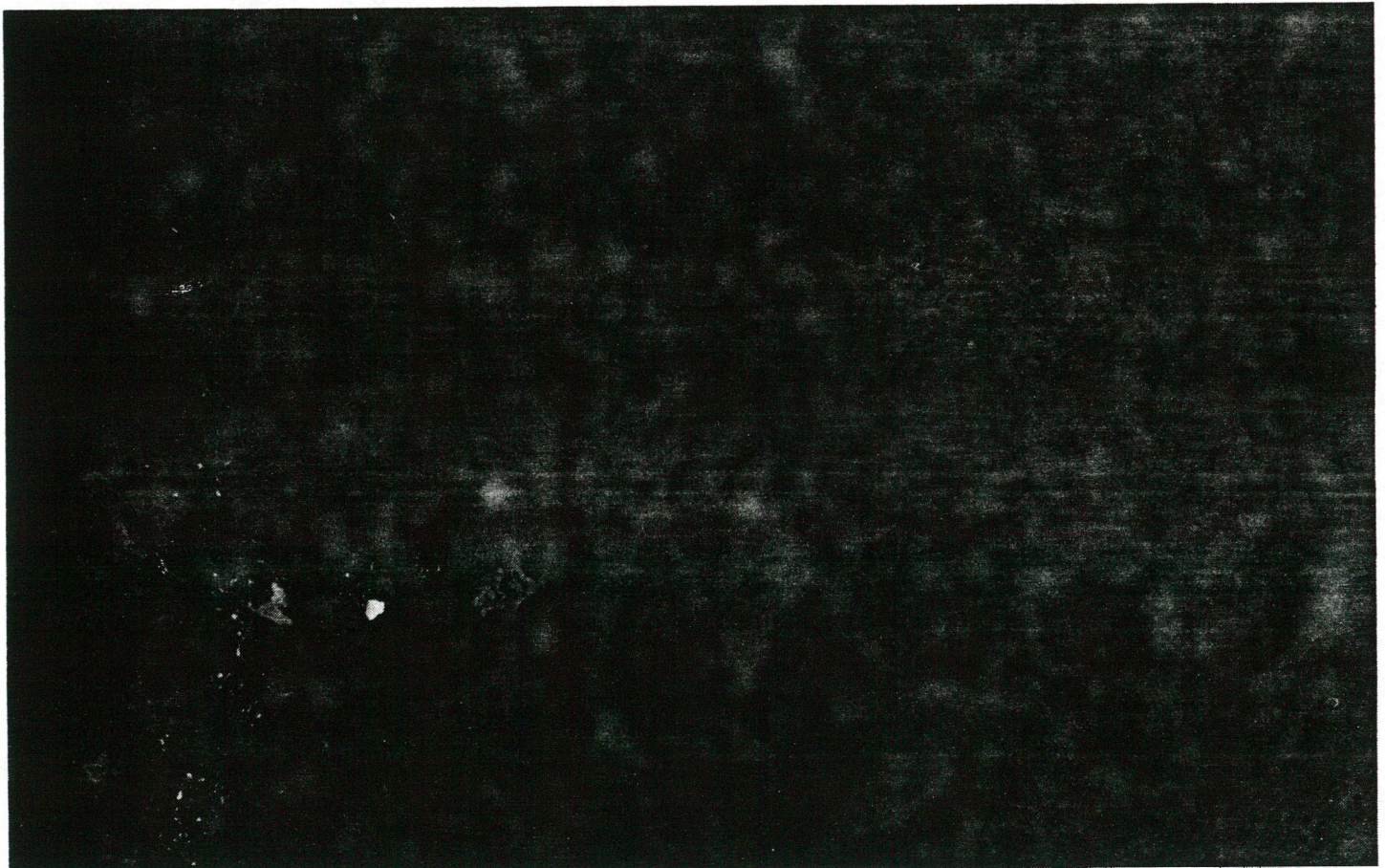


Fig. 9b Part of the mousse is lifted off the sediment by the rising tide.

3.2.2.2 Measurements on the mouse

IR analyses had indicated that the added oil contained ca 31% of aromatic compounds. The colour of the floating mousse changed during the week of exposure from dark brown to nearly black. The viscosity increased with time. The water content of the mousse was not monitored, but destabilization of the mousse probably occurred.

GC analyses of mousse samples taken from the floating slick during the first week showed that the lower alkanes (C8 - C12) disappeared in two days, and that also higher alkanes (C13 - C15) disappeared to a large extent during the week. This was probably a result of evaporation.

3.2.2.3 Oil in the watercolumn

Fig. 10 shows the concentration of oil in the water of the MOTIFs as measured with the fluorescence technique. Concentrations quickly rose after the addition of the oil mousse, and reached a maximum of around 400 $\mu\text{g/l}$ at the end of the week of exposure to the mousse. After this maximum concentrations remain relatively high throughout the rest of the experiment (around 100 $\mu\text{g/l}$), indicating that oil components were constantly released from the sediment to the overlying watercolumn. In the controls concentrations of around 10 $\mu\text{g/l}$ (having a different fluorescence spectrum) were recorded. The fluorescence spectrum in the contaminated systems did not change very much in time, indicating that the composition of the oil in the water also did not change very much with time. This was confirmed by compound analyses using HPLC and GC techniques. The IR analyses of the water samples did not give much information: oil concentrations being below the detection limit (0.2 mg/l) in all cases.

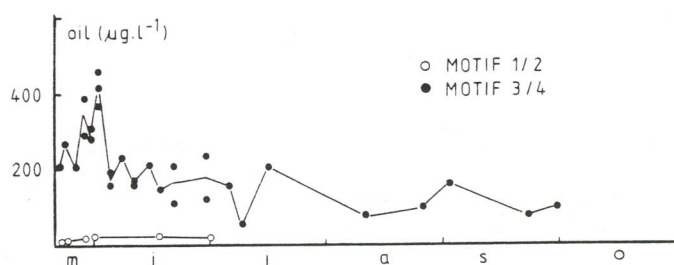


Fig. 10 Total oil concentrations in the water of the MOTIFs during OPEX (measured with fluorescence technique)

Fig. 11 and 12 show typical HPLC and GC results. Table 5 shows a number of compounds detected with these methods. By GC only trace amounts of aliphatic hydrocarbons were detected in the water and the method was operating near its detection limit.

Table 5 Various compounds detected with GC and HPLC techniques in the MOTIFs. The amounts ($\mu\text{g/l}^{-1}$) present in the original oil are given.

aliphatic hydrocarbons	traces
phenanthrene	2627
anthracene	83
fluoranthene	983
pyrene	4340
B(a)anthracene	960
chrysene	2340
B(j)fluoranthene	7870
B(e)pyrene	5821
perylene	205
B(b)fluoranthene	277
B(k)fluoranthene	86
B(a)pyrene	327
B(ghi)perylene	3614
indeno (1,2,3,-cd)pyrene	436
anthanthreen	300

3.2.2.4 Oil in sediments

Fig. 13 shows the concentrations of oil as measured with fluorescence and IR techniques in the upper cm of the oil contaminated sediments. It appears that oil remained in the sediments after the removal of the floating oil. More oil was caught in the sediment in MOTIF 4 than in that of MOTIF 3. Total oil amounts in the sediment were calculated using a trapezoid integration method. Results showed that a considerable part of the added oil (between 10 and 30%) remained in the sediment in the MOTIFs after removal of the floating oil.

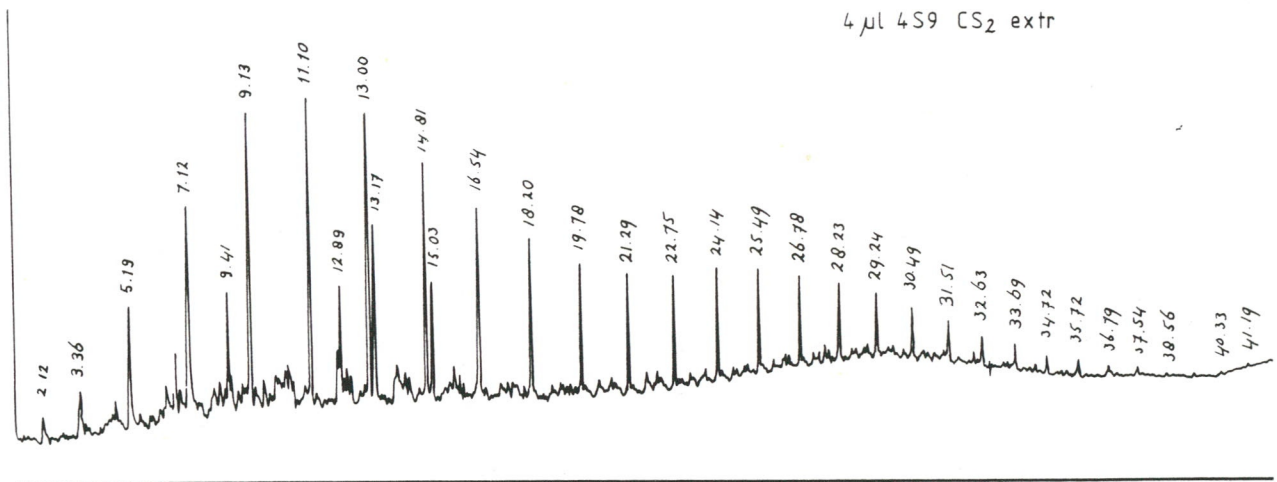


Fig. 11 Typical GC (gaschromatography) results of oil analysis during OPEX.

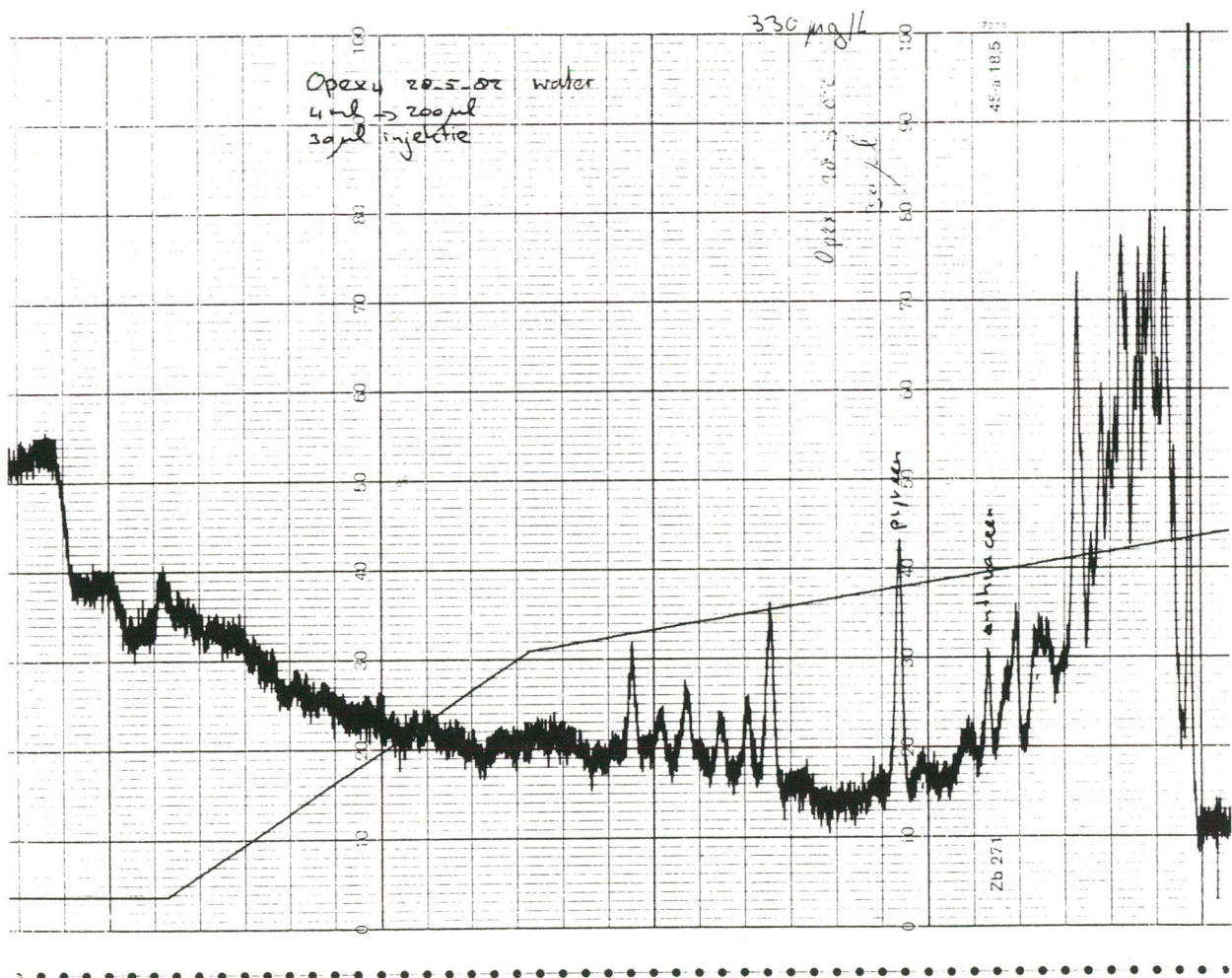


Fig. 12 Typical HPLC (High Performance Liquid Chromatography) results of oil analysis in water during OPEX.

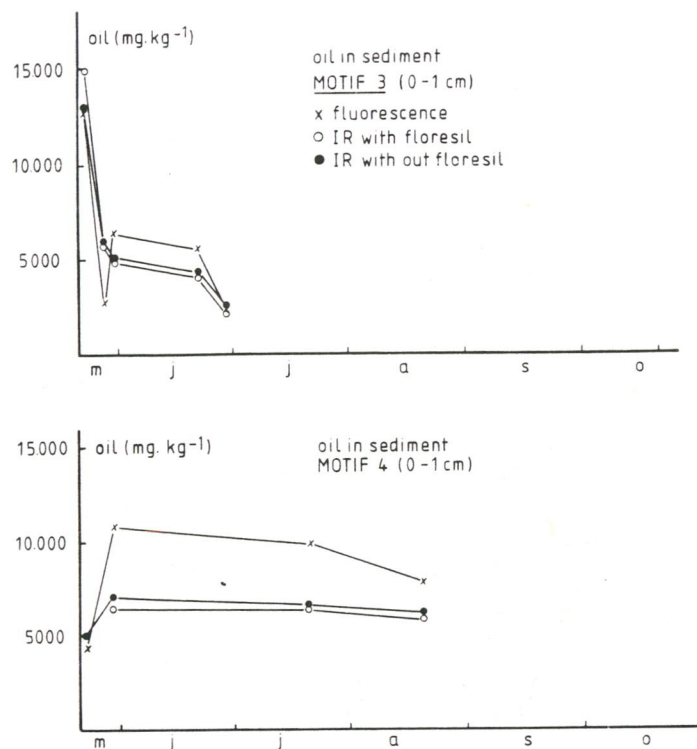


Fig. 13 Total oil concentrations (as measured with IR (Infra-red) and fluorescence techniques) in the upper cm of the sediment in the two contaminated MOTIFs (samples consisted of 20 subsamples, giving an average concentration for the whole tidal flat).

Fig. 14 shows the distributions of the oil with depth on a few selected days. Oil concentrations decreased strongly with depth. At the start of the experiment most of the oil was concentrated in the top mm, but after a few months the oil was distributed through the top few cm. On August 18, 1982 concentrations in the layer between 1 and 2 cm were even higher than those in the top cm, indicating that clean sediment from lower depths had been brought to the surface by bioturbation activity. Results shown in Fig. 14 refer to mixed samples from the whole basin. To study the vertical distribution of the oil in detail, samples were also taken on a few selected locations in the basins using a carefully cleaned sampler. Some of the results of these samples are shown in Fig. 15, in which the strong decrease with depth is also apparent. The samples from MOTIF 3 on 29 June, and from MOTIF 4 on 14 June, were taken at heavily oiled spots where bioturbation activity was much reduced, and therefore most oil remained in the top millimeters.

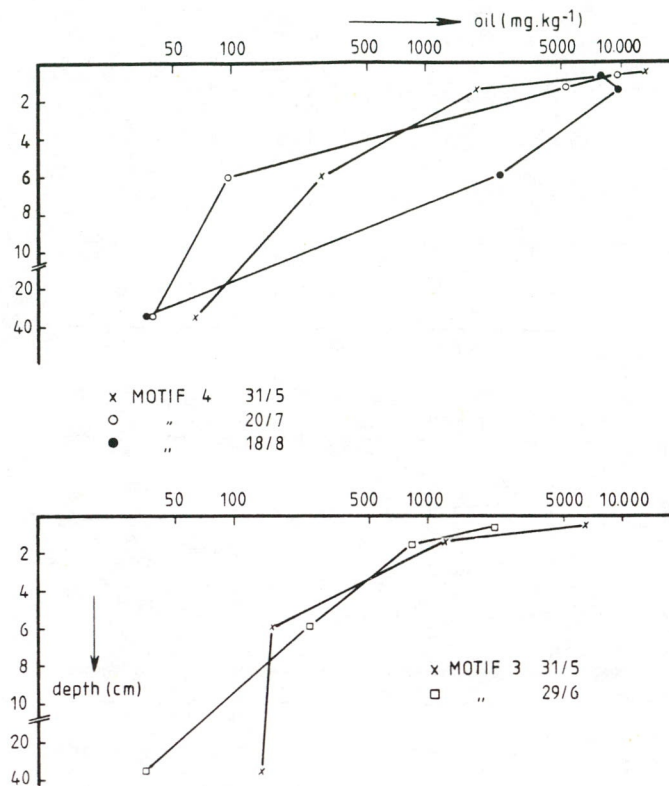


Fig. 14 Vertical distribution of total oil concentrations (measured by fluorescence) in the sediments of the oil contaminated MOTIFs on selected days. (samples consisted of 20 subsamples, giving an average concentration for the whole tidal flat).

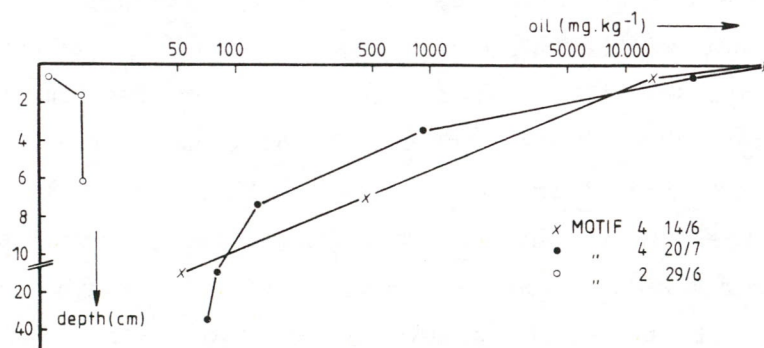


Fig. 15 Vertical distribution of total oil concentrations (measured by fluorescence) in some selected sediment samples.

3.2.2.5 Oil in organisms

Table 6 shows the oil content of mussels sampled from control MOTIFs 1 and 2 and oilcontaminated (MOTIFs 3 and 4) systems. Control concentrations were between 17 and 38 µg/g wet weight. After the oil addition, concentrations increased strongly and remained high until the end of the experiment. Unfortunately insufficient samples were taken to allow determination of the significance of the decrease in concentrations found between July and September.

Table 6 Oil content of mussels as measured by fluorescence.

origin	sampling date	oil content (mg/kg wet weight)
MOTIF 1	15.07.82	18
" 2	1.04.82	38
" 2	3.09.82	17
" 3	7.04.82	28
" 3	15.07.82	811
" 3	3.09.82	639
" 4	15.07.82	685
" 4	3.09.82	562

The following compounds were detected with HPLC in concentrations of 2-25 µg/kg wet weight in the mussel samples: fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno 6,2,3 (id)-perylene.

On 18 November 1982 one mussel sample was transplanted from the contaminated MOTIF 3 to control MOTIF 1, to investigate if oil concentrations in the mussels remained high because they did not eliminate the oil, or because they were in equilibrium with the oil concentrations in the water. Results were not available at the time of writing.

3.2.3 Development of the phytobenthos

Fig. 16 shows the development of the phytobenthos biomass as measured by chlorophyll concentrations in the different layers sampled in the sediment in one of the controls (no. 1). Since no important differences were found between the upper, middle and lower reach of the tidal flat, results were pooled, so that every value is the average of three analyses (with the exception of the 25-30 cm layer, which was only sampled in the middle reach).

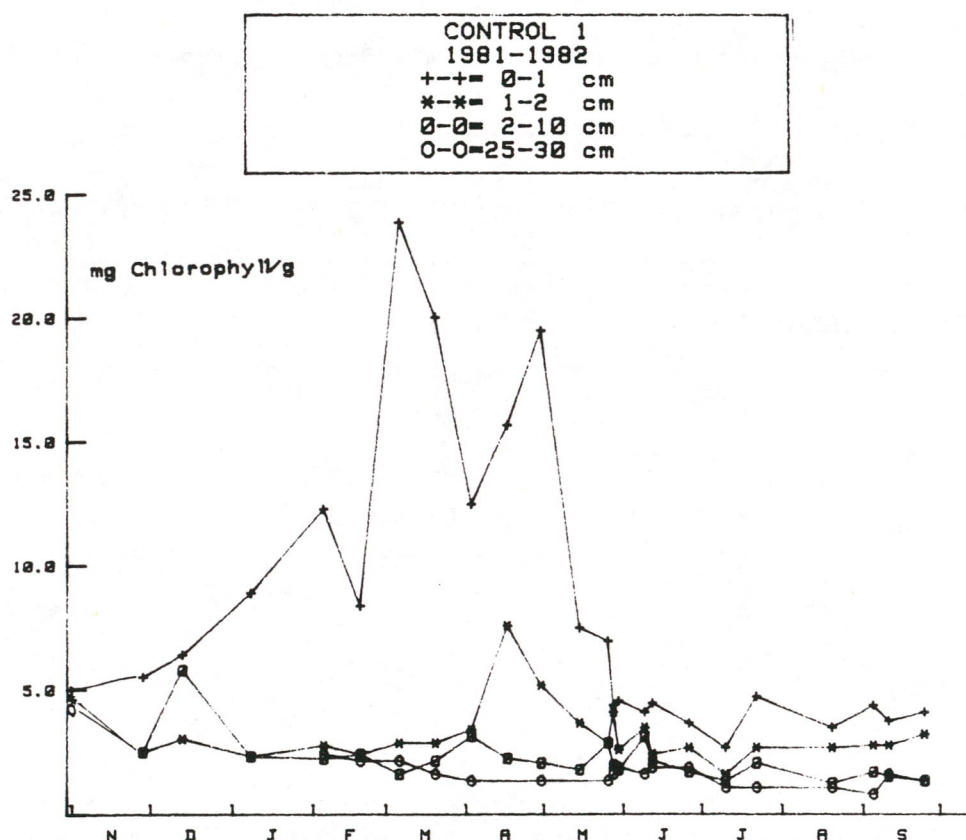


Fig. 16 Chlorophyll concentrations at different depths in the sediment of MOTIF 1.

A similar pattern was found in the other MOTIFs. In October 1981, at the start of the experiment, chlorophyll was homogenously distributed through the sediment at a concentration of 5 mg chlorophyll/g sediment. Directly after the start a stratification developed. At depths of 25-30 cm concentrations decreased from 5 to around 1.5 mg chlorophyll/g sediment.

As could be expected, most production of phytobenthos occurred at the surface, and chlorophyll concentrations increased there, even during the winter months, to around 10 mg/g in February. In March a large bloom of diatoms developed, forming thick brown algal mats on the sediment surface in all MOTIFs. These algae disappeared to a large extent at the end of May. From May to December (not shown in Fig. 16) concentrations of chlorophyll were nearly constant.

The species growing on the tidal flats were for the greater part pennate diatoms (e.g. *Nitzschia* spp., *Naviculaceae*, etc.).

Sometimes (in particular in the period April-July) macroscopic green algae (*Ulva* sp., *Cladophora* sp., *Enteromorpha* sp.) developed on the tidal flats; these are excluded from Fig. 16 because they were removed from the sampling grid before sampling. On several days the whole system was cleaned of these algae, because it was feared that anoxia would develop under these algal mats, killing many of the organisms. No new algal mats formed after July.

The average chlorophyll concentration of the upper 2 cm in the different MOTIFs is shown in Fig. 17. The development of the phytobenthos was similar in the different MOTIFs from the start of the experiment to the addition of the oil, six months later. Fig. 18 shows the same for the period May-July. Chlorophyll concentrations were not lower during the exposure to the oil mousse, however, higher chlorophyll concentrations were found in the oil contaminated systems thereafter (mid June). After June no differences, which could be attributed to the oil addition, occurred between the controls and the contaminated MOTIFs.

The species composition of the phytobenthos was not followed in detail. Macroscopically it was clear that large local differences occurred within the oil contaminated systems, depending on local oil concentrations in the sediment; hardly any algae could be seen in the northern corners of these basins.

In May, June and September oxygen production was measured in the laboratory using the intact sediment cores collected for the measurement of community respiration. Because these samples were exposed to artificial light, oxygen production values give information on relative primary production of the phytobenthos. Results of these measurements are given in Table 7.

Table 7 Potential primary productivity ($\mu\text{mol O}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) as measured in the community respiration experiments.

date	control	oil contaminated
middle May	1038	1114
end June	980	575
start September	781	595

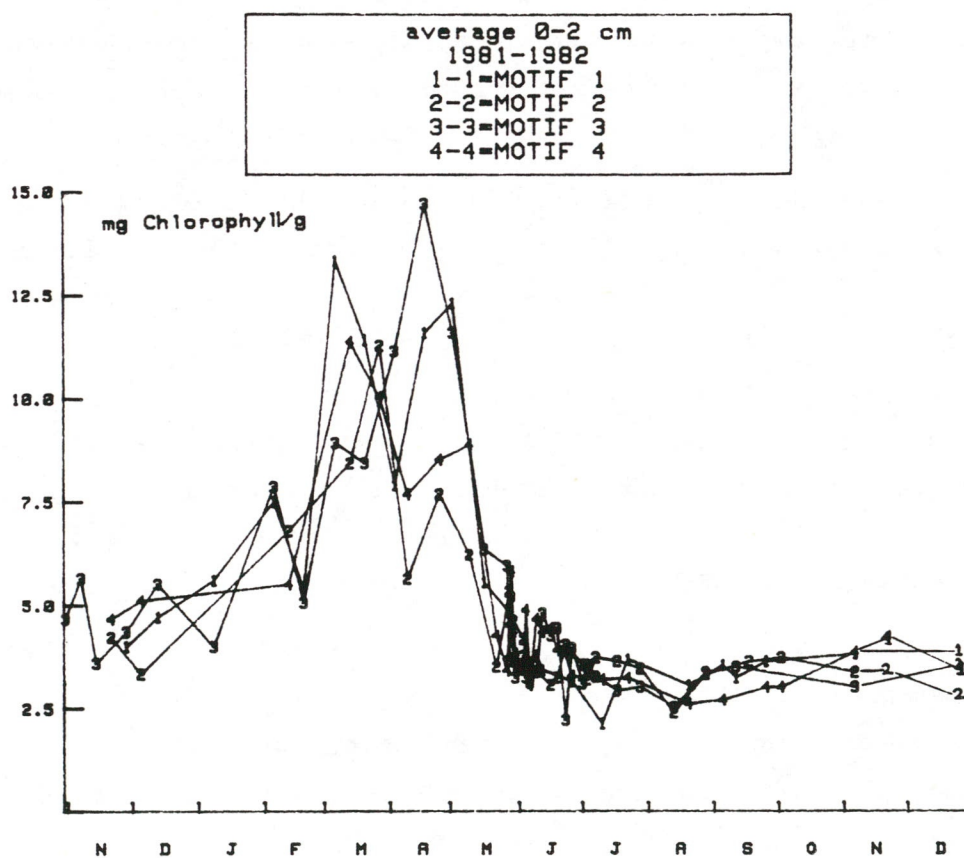


Fig. 17 Average chlorophyll concentrations in the upper 2 cm of the sediment in the different MOTIFS during OPEX.

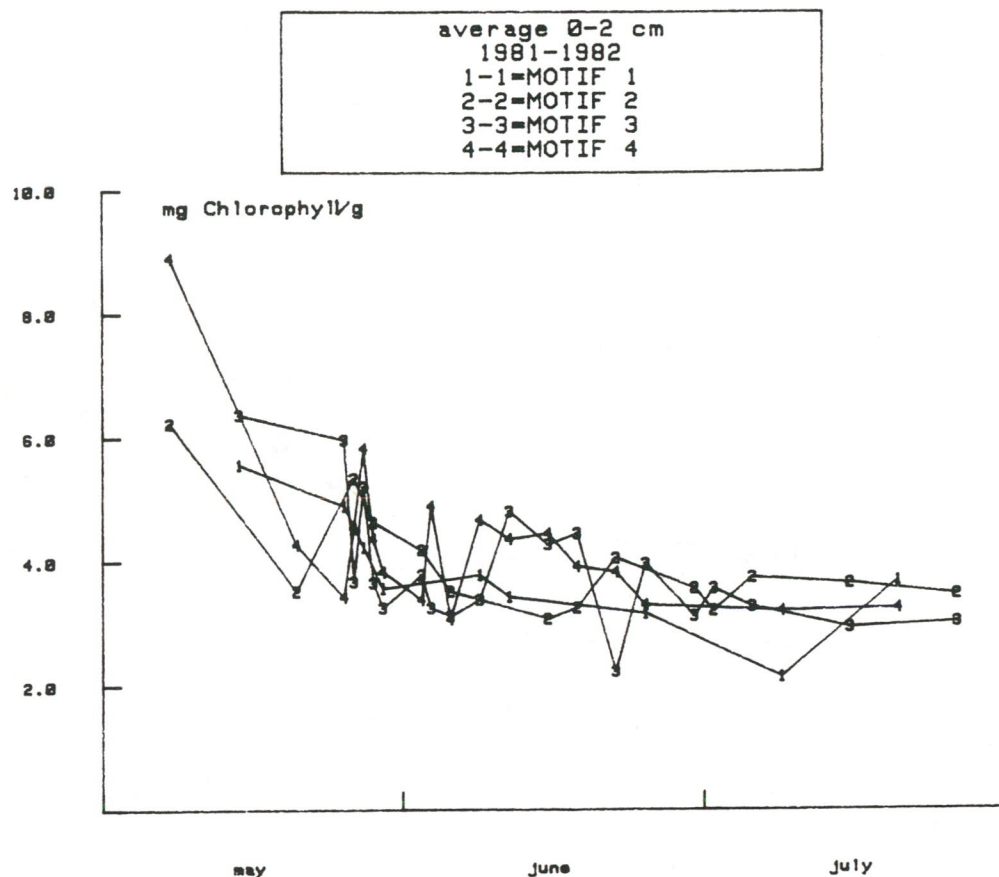


Fig. 18 Average chlorophyll concentrations in the upper 2 cm of the sediment in the different MOTIFs during the period May-July 1982.

In May very similar values were obtained in both MOTIFs (2 and 4). In June and September the values in the oil contaminated MOTIF no. 4 were lower than in the control. In June higher chlorophyll amounts were found in the oil contaminated system, in September chlorophyll values were similar to that in the control. The lower oxygen production of the phytobenthos per unit chlorophyll therefore indicates that the phytobenthos production was limited by the presence of the oil.

Fig. 19 shows the development of the pigment index of the phytobenthos. Very similar values were observed in the different MOTIFs before addition of the oil mousse. After the addition, values were constantly higher in the oil contaminated systems. This result is another indication that the phytobenthos activity was inhibited in the oil contaminated MOTIFs for the rest of the experiment.

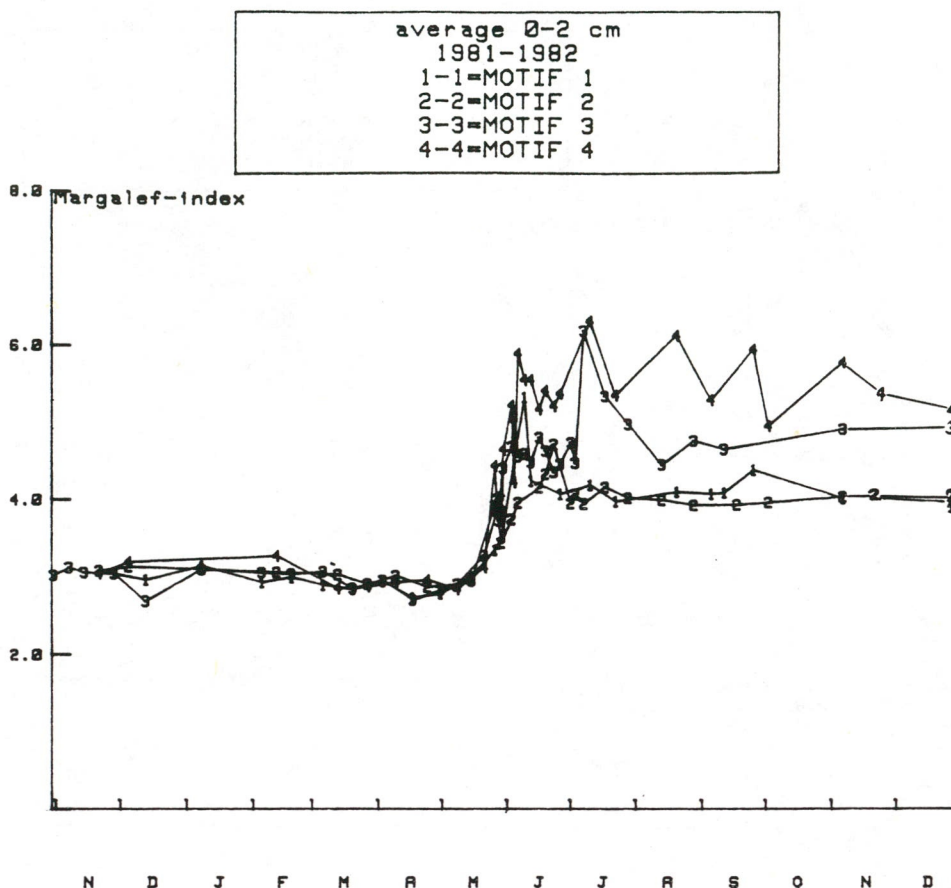


Fig. 19 Pigment index in the different MOTIFs during OPEX in the upper 2 cm of the sediment.

3.2.4 Development of the Electron Transport System (ETS) activity and community metabolism

Fig. 20 shows the development of the ETS activity in the different layers of the sediment sampled in one of the controls (no. 1). From the start of the experiment until March 19, 1982 the ETS activity ranged from 2-4 $\mu\text{mol O}_2/\text{g}$ dry sediment/h, being highest in the top 2 cm. ETS activity increased in this top layer simultaneously with the increasing phytobenthos populations. In May-June ETS activity increased in the deeper layers of the sediment in all MOTIFs, while there was a small decrease in the upper layer. From June until September ETS in the control remained high at depths of 1-30 cm, in the top cm somewhat lower values were recorded. From September to the end of the experiment decreasing ETS activities were found.

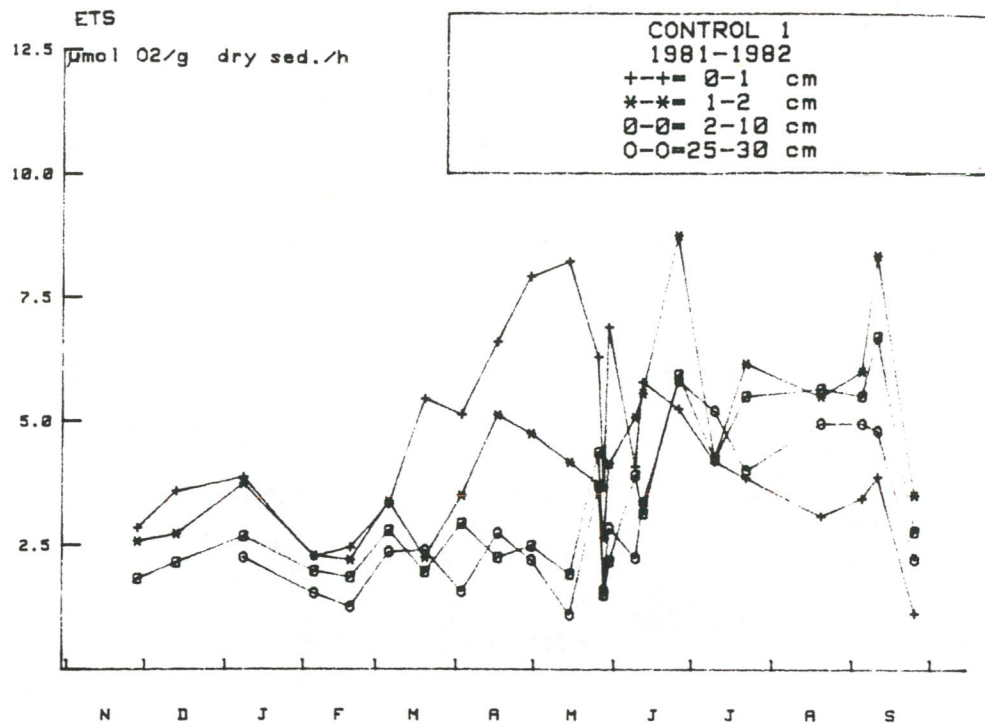


Fig. 20 Development of ETS (Electron Transport System) activity on different depths in the sediment of MOTIF 1.

Fig. 21 shows the average ETS activity in the 0-2 cm layer, in all MOTIFS. During the first six months of the experiment, the same trends were seen in all systems, although the timing of the first peak in April-May differed between systems, giving rise to large variations between the MOTIFS in May. After the oil addition, ETS activity showed very large variations in the oil contaminated systems. In the period July-September ETS activity was somewhat higher in these systems than in the control systems, but the variation between controls was of the same order of magnitude during this period.

The large variability directly after the addition of the oil may have been partly due to measurement errors caused by the presence of oil in the samples, but the high spatial variability of the oil concentrations in the sediment in the contaminated systems was probably also responsible.

Table 8 gives various results obtained from the incubated bottles in May, June and September; control MOTIF 2 is compared with contaminated MOTIF 4 for May (prior to exposure), June and September (after exposure to the oil mousse). As described above (§ 2.4.2) the contents of all of the incubation bottles were sieved over 400 μm nylon gauze and then meticulously inspected.

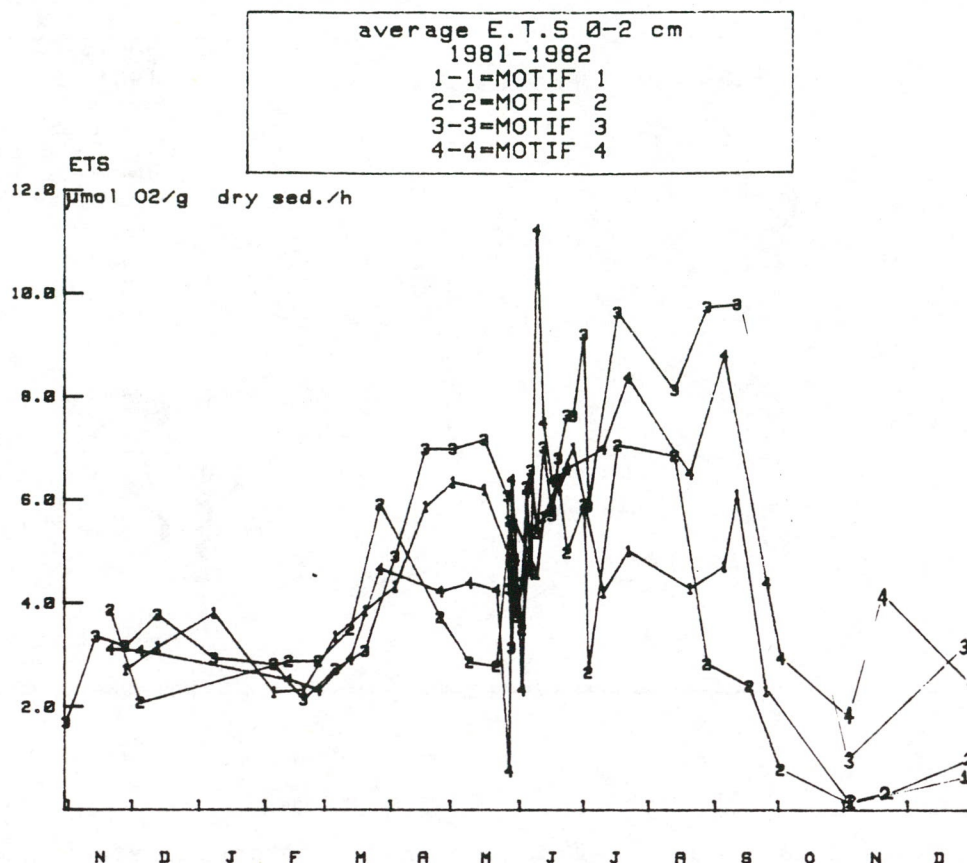


Fig. 21 Average ETS-activity in the upper 2 cm of the sediment in the different MOTIFs during OPEX.

This procedure provides much better information regarding average densities and weight for the majority of the small species than the normal sampling procedure. On the other hand, larger errors can be made in determining the population density of the larger organisms *Macoma*, *Cerastoderma* and *Arenicola*, than in the normal macrobenthos sampling procedure.

The average biomass per m² (= the weight of the total fauna assemblage expressed in grams ash-free dry weight (ADW) per m²) is a more useful parameter than the total number of organisms per m² (n.m⁻² in Table 8), because the organisms can range from macro- and mesofauna to extremely numerous meiofauna.

Table 8 shows the oxygen consumption of the complete community (= community metabolism, 3rd row from under, O₂-consumption per m² per gram (ADW) (2nd row from under), relative O₂-production by the incubated community under light conditions (bottom row). These relative O₂-production values give insight into the potential primary production of the benthic algae and can be related to the phytobiomass present (see 3.2.3).

Table 8 Results from the community respiration incubation experiment. The remarks in the right hand column indicate which parameter (n, ADW, etc.) in which months (J = June, S = September) is used to estimate the effect of the oil addition (for further explanation: see text).

Species	middle of MAY						end of JUNE						start of SEPTEMBER						Effect oil	Remarks
	MOTIF 2			MOTIF 4			MOTIF 2			MOTIF 4			MOTIF 2			MOTIF 4				
	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW		
Macoma ☆)	149	16.3	109.0	149	14.1	94.6	116	16.7	144.2	149	10.6	72.9	50	4.8	95.9	50	3.6	72.1	-	ADW in J
Cerastoderma ☆)	0	0	0	17	6.5	391.3	33	8.5	256.0	0	0	0	50	8.6	172.2	0	0	0	-	n in J, S
Hydrobia	182	0.2	0.8	82	<0.1	0.7	580	0.2	0.3	133	<0.1	0.4	1243	2.1	1.7	1027	2.0	2.0	?	
Corophium	17	0	0	0	0	0	133	<0.1	0.2	0	0	0	38857	2.1	<0.1	166	<0.1	0.1	-	n in J, S
Gammarus	0	0	0	0	0	0	713	0.4	0.6	0	0	0	0	0	0	0	0	0	-	n in J
Arenicola ☆)	17	4.4	265.5	22	4.8	224.4	12	1.7	145.0	5	0	0	0	0	0	0	0	0	?	
Nereis	0	0	0	17	0	0	133	2.1	0.4	928	0.4	0.4	1690	8.9	5.3	2320	18.9	8.1	+	n in J, S; ADW in S
Heteromastus	0	0	0	0	0	0	0	0	0	0	0	0	215	0.9	4.1	33	<0.1	1.4	-	n in S; ADW in S
Anatides/Eteone	0	0	0	0	0	0	14	0.1	7.8	50	0.2	3.2	50	0.1	2.7	66	0.2	3.6	?	
Pygospio	514	0.5	0.9	514	0.5	0.9	414	0.5	1.3	530	0.2	0.4	133	<0.1	0.3	66	<0.1	0.5	?	
Capitella	0	0	0	0	0	0	795	0.2	0.3	2651	0.4	0.2	0	0	0	0	0	0	+	n in J
Paranais	0	0	0	0	0	0	21375	0.7	<0.1	38541	1.1	<0.1	3927	0.4	0.1	12576	0.9	0.1	+	n in J, S
Oligochaet 1	2088	0.5	0.2	2601	0.6	0.2	2502	0.2	0.1	1740	0.2	0.1	2486	0.2	0.1	878	0.2	0.2	-	n in S
(large) Nematodes	5833	0.2	<0.1	5866	0.2	<0.1	5766	0.2	<0.1	6495	0.2	<0.1	3413	<0.1	<0.1	3960	0.2	<0.1	?	
total numbers	8800			9270			32590			51222			52114			21140			?	
total biomass		22.1			26.8			29.6			13.4			28.3			26.30		?	
total number excluding☆)	8634			9181			32425			51070			52014			21090				
total biomass excl.☆)		1.4			1.4			2.7			2.8			14.9			22.7		+	biomass in S
O ₂ -consumption in µgat.h ⁻¹ .m ⁻² (= community metabolism)		1588			1510			2340			2150			4316			4312		?	
O ₂ -consumption in µgat.h ⁻¹ .m ⁻² .g ⁻¹ (ash-free dry weight)		72.5			56.5			79.0			160.0			153.5			165.2		-	O ₂ -consumption per g in J
relative O ₂ -production in µgat O ₂ .h ⁻¹ .m ⁻²		1038			1114			980			575			781			595		-	O ₂ -production in J and S

In the 2 right hand column of Table 8 the effect of oil contamination on the various species and the complete community is estimated from the data on numbers, weight, biomass, O₂-consumption and production given in the table.

These effects are denoted negative (-) when a certain species, or parts of the complete community are thought to be adversely affected in one or the other way by the oil contamination in MOTIF 4. A positive (+) symbol indicated that the parameter measured had a higher value than in the controls; a question mark (?) indicated an unclear effect.

3.2.5 Development of the meiofauna

In this section the development of the meiofauna organisms will be presented per group.

Oligochaeta spp.

Population dynamics for an undetermined species ("oligochaet I") are given in Fig. 22. The lower lines of Fig. 22 (1000 μm sieve, collected with macrofauna samples) show only minor differences in numerical densities in the 4 MOTIFS. The species apparently reaches its maximum in May, with a decrease in June and July in all 4 MOTIFS. Comparison of the upper lines (400 μm sieve for MOTIFS 2 and 4) in which a much larger part of the populations, including juvenile and subadult specimens are sampled, indicates that high numbers of about 2500 per m^2 are maintained in the uncontaminated MOTIF 2 whereas numbers rapidly fall to a level of less than 1000 per m^2 in the contaminated MOTIF 4.

Oligochaetes generally have a short lifespan. The lower lines show a normal wax and wane of the adult populations. Oil contamination probably decreases the reproduction success of this species, as reflected in the upper lines.

Paranaís spec. (Oligochaeta)

Paranaís, a small oligochaete species, belongs to the group of opportunistic summer annuals which can produce dense populations under favourable environmental conditions. Data from sieving over 1000 μm represent large underestimates (lower lines of Fig. 23). Use of a 400 μm mesh revealed very high densities in June (upper lines). In the oil contaminated MOTIF 4, maximum values surpassed 40.000 m^2 , whilst in the uncontaminated MOTIF 2, maximum values amounted to about 20.000 per m^2 .

Paranaís is apparently a relatively insensitive species to oil, and perhaps the oil-contaminated sediment creates a favourable environment for reproduction and growth of this species.

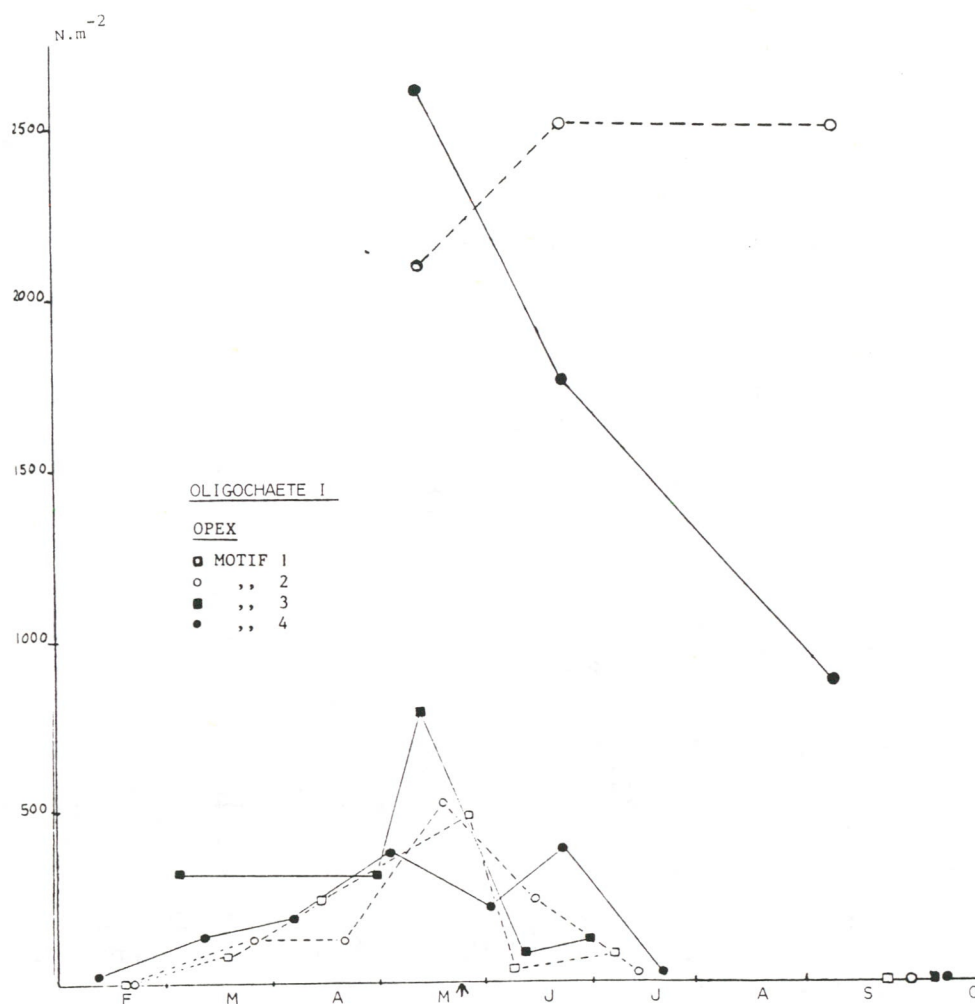


Fig. 22 Numbers of oligochaete I in the different MOTIFs. Lower lines represent numbers retained by a 1000 µm sieve; upper lines represent numbers retained by a 400 µm sieve.

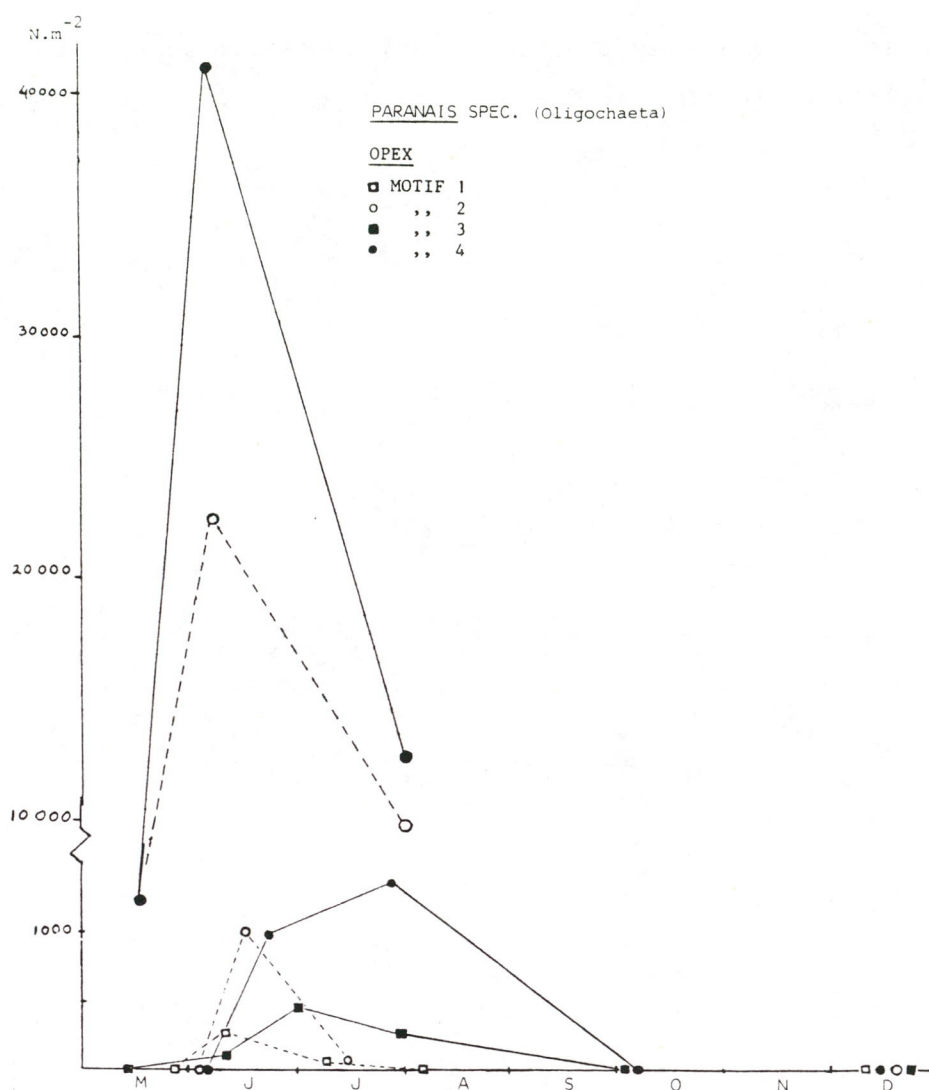


Fig. 23 Numbers of *Paranaïs* sp. in the different MOTIFs. Lower lines represent number retained by a 1000 µm sieve; upper lines represent numbers retained by a 400 µm sieve.

Nematodes

Some 200 species of nematodes are known to occur in the Wadden Sea, and they form by far the most important group of meiofauna organisms proper in the mud-flats. The largest species can reach a body length of about 10 mm and populations of this group in the MOTIFs 2 and 4, sampled with a 400 µm sieve are shown in the lower lines of Fig. 24. Although these numbers certainly do represent an underestimate, no significant differences were observed between MOTIF 2 and 4. The upper lines of Fig. 24 show the numbers of the complete nematode assemblage (small and large) obtained by elutriation.

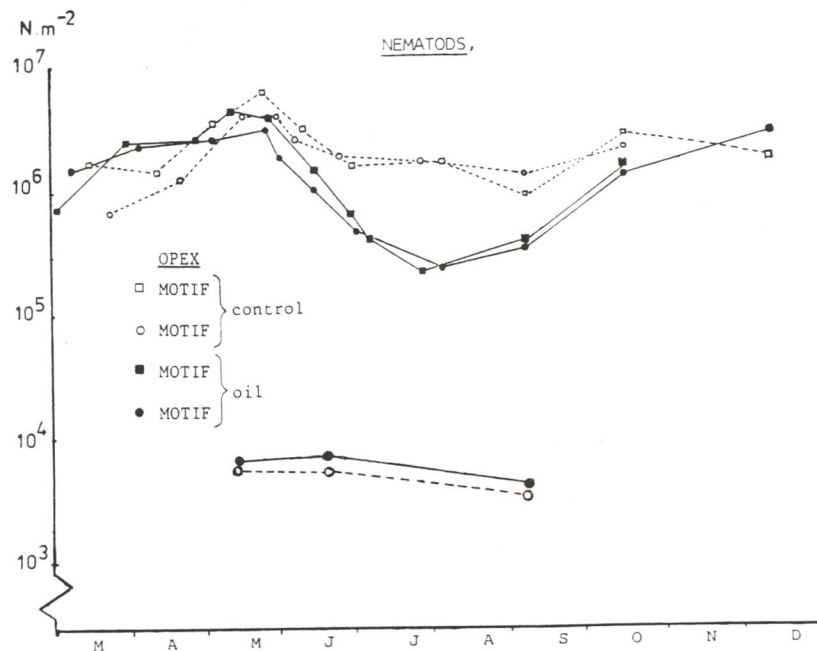


Fig. 24 Numbers of nematodes in the different MOTIFs. Lower lines represent numbers retained by 400 μ m sieve (larger nematodes), the upper lines represent numbers obtained by elutriation of the samples.

In spring the nematode populations increased and reached maximum values of about 6×10^6 nematodes per m^2 in May with a fall in June and July. This fall was, however, much more pronounced in the oil contaminated MOTIFs 3 and 4 with minimum values of about 0.5×10^6 per m^2 , than in the uncontaminated MOTIFs 1 and 2 with values of about 1.0×10^6 per m^2 . The populations in MOTIF 3 and 4 showed a recovery in September and October.

Harpacticoid copepods div. spec.

The lines in Fig. 25a, b show the population dynamics of harpacticides, adults and juveniles being plotted separately.

In spring, all the populations rapidly increased in numbers up to values of about 1.5×10^6 per m^2 . A sudden fall in numbers was observed early in June in the uncontaminated MOTIFs 1 and 2 whereas most remarkably numbers in the contaminated MOTIFs 3 and 4 continued to rise, with a decrease in July.

In autumn the picture became still more confusing. The numbers of adults recovered in MOTIF 1, 3 and 4, whilst the number in MOTIF 2 here in September was very low. The behaviour of the number of juveniles in autumn was also perplexing: MOTIFs 1 and 3 showing increases and MOTIFs 2 and 4 showing decreases.

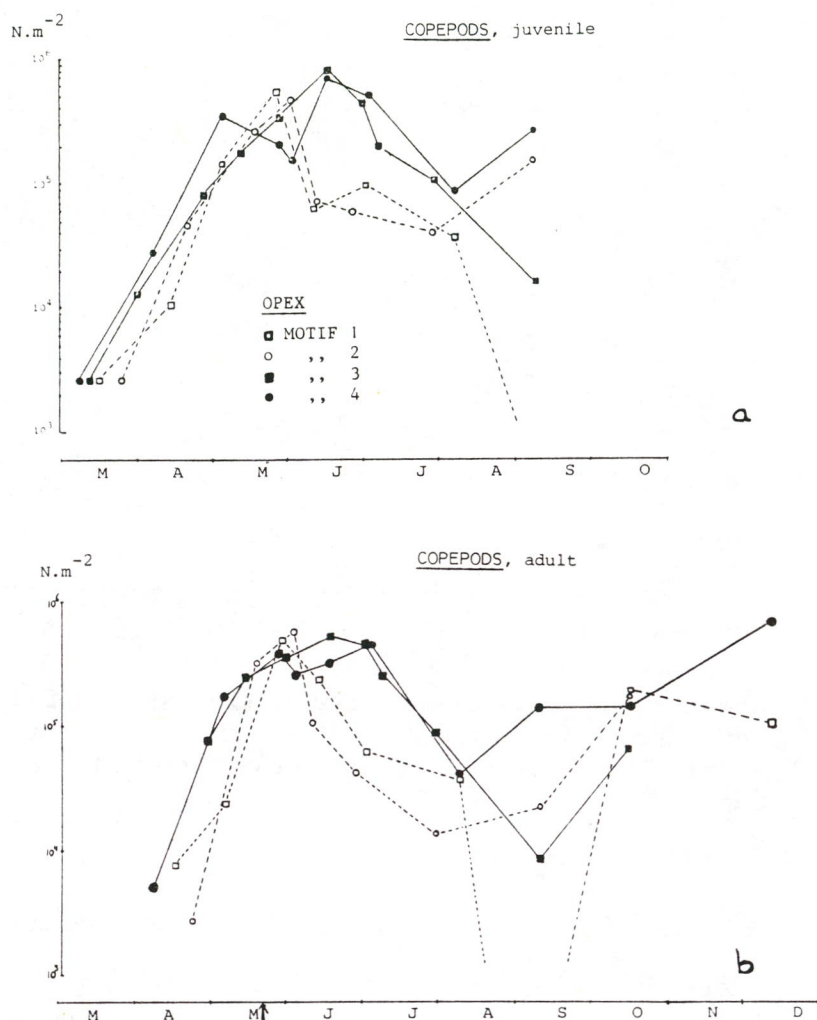


Fig. 25 Numbers of juvenile (a) and adult (b) harpacticoid copepods in the different MOTIFs.

3.2.6 Development of the macrofauna

In this section the development of the macroinvertebrates present in the MOTIFs will be presented per species. In addition to data on the species deliberately introduced at the start of the experiment (*Mytilis edulis*, *Cerastoderma edule*, *Macoma balthica*, *Littorina littorea* and *Arenicola marina*, data will also be given on other species which developed in the systems. The populations of these species may have developed from small stocks of individuals introduced with the sediment at the start of the experiment, from larvae introduced with the suppletion water.

Mytilus edulis

23-25 of the 25 specimens originally placed in each sample were recovered. Mortality was negligible in both the uncontaminated and the contaminated systems. Fig. 26 shows the increase in shell length in the different MOTIFs. The mussels increased in length by approximately 1 cm in all MOTIFs, no significant differences being found between uncontaminated and contaminated systems.

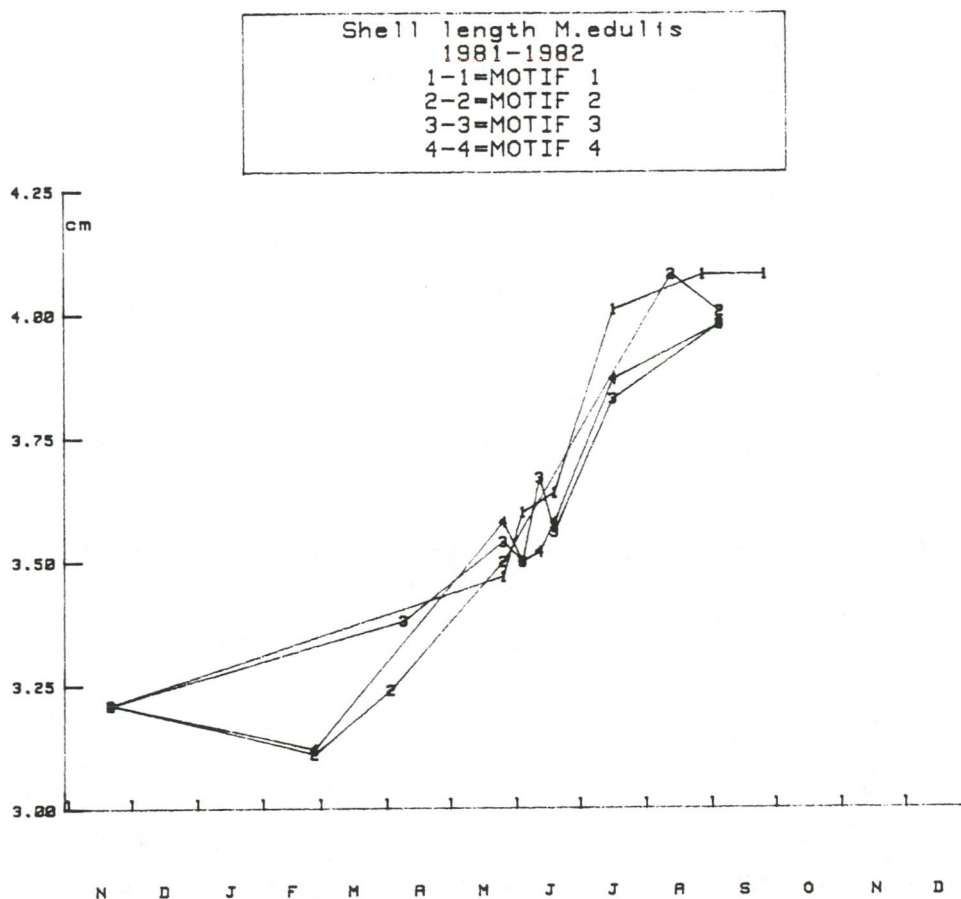


Fig. 26 Development of the length of shells of *M. edulis* in the different MOTIFs.

Fig. 27 shows the average ash-free dry weight (ADW) of the soft parts per mussel throughout the experiment. The increase in ADW after the winter was similar to that found under field conditions, with a maximum in September. ADW decreased in the oil contaminated MOTIFs following exposure, but this seemed to recover later.

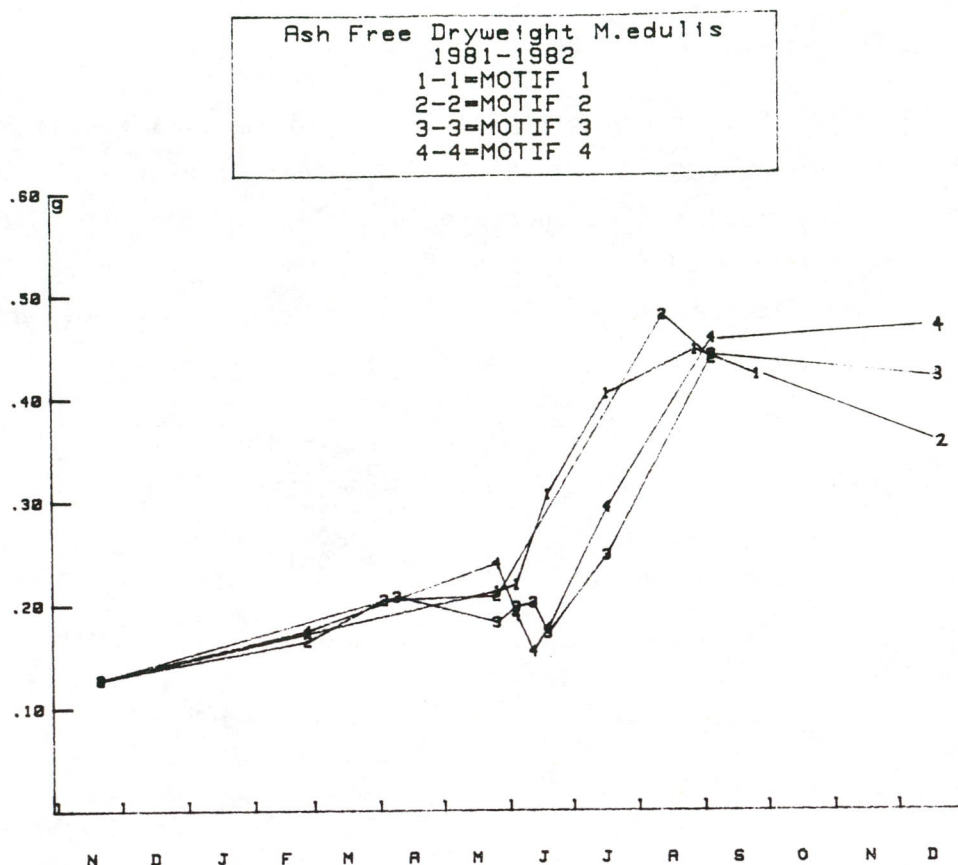


Fig. 27 The ash-free dry weight (ADW) per individual of *M. edulis* in the different MOTIFs during OPEX.

Cerastoderma

The numerical densities of the cockle populations are shown in Fig. 28. Five hundred animals of year-class 1979 with a length of 30-35 mm were introduced into each MOTIF, giving an initial density of 28 per m².

Due to the sampling, 79 cockles were removed from MOTIF 1, 83 from MOTIF 2, 55 from MOTIF 3 and 79 from MOTIF 4. Eighty-one empty shells were found on the surface of MOTIF 1, and 50 on that of MOTIF 2. These numbers are, however, an underestimate of the natural mortality, as part of the succumbed animals will have remained hidden in the sediment or have been buried by bioturbative action by *Arenicola*.

At the end of the experiment the uncontaminated MOTIFs 1 and 2 had therefore suffered losses in the order of 26-32% of their initial population, whereas the losses in the contaminated MOTIFs 3 and 4 were about 48-52%.

Although the numbers in Fig. 28 generally tend to decline with time, the monthly variations in numbers are so large that any significant conclusion on the effect of oil is disputable. It can, however, be concluded that the sampling procedure was apparently inadequate. A non-destructive method, for example by estimation of the number of active visible siphons or by location of the cockles by touch, could be considered.

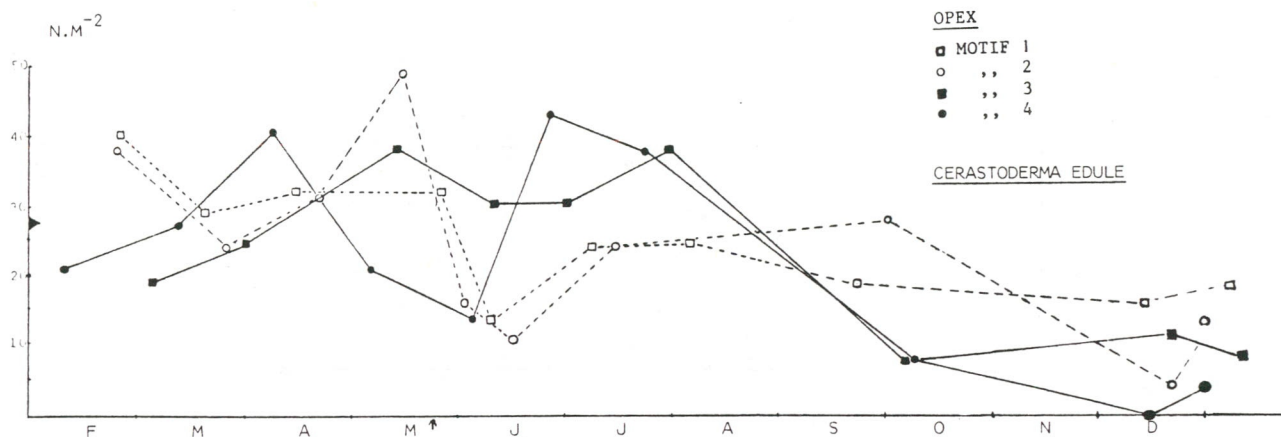


Fig. 28 Numbers of *C. edule* in the different MOTIFs (results from sediment sampling) during OPEX.

Fig. 29 gives the cumulative numbers of dead cockles recovered in each MOTIF versus time. A rapid effect of the oil at the end of May (arrow) was not observed. Towards the end of August (3 months after the oil mousse was added), however, a postponed mortality occurred in MOTIFs 3 and 4, indicating a significant negative effect of oil contamination on the intertidal ecosystem.

A direct cause of this postponed mortality is speculative, but can perhaps be related to reproduction in summer. Delayed mortality in *Cerastoderma*, caused by another stress (severe winters) has been described by Kristensen (1957). Another explanation of the postponed mortality might be accumulation of oil components to critical levels.

Ash-free dry weight (g) of the soft parts of the cockle is presented in Fig. 30. In early spring this weight is low (~ 0.3 g) as a result of a period of continuous emaciation in autumn and winter. Only a limited growth is seen in the first few months of the experiment. Growth generally was slow in adult cockles and poor feeding conditions for this typical filter feeder, may have been responsible for this.

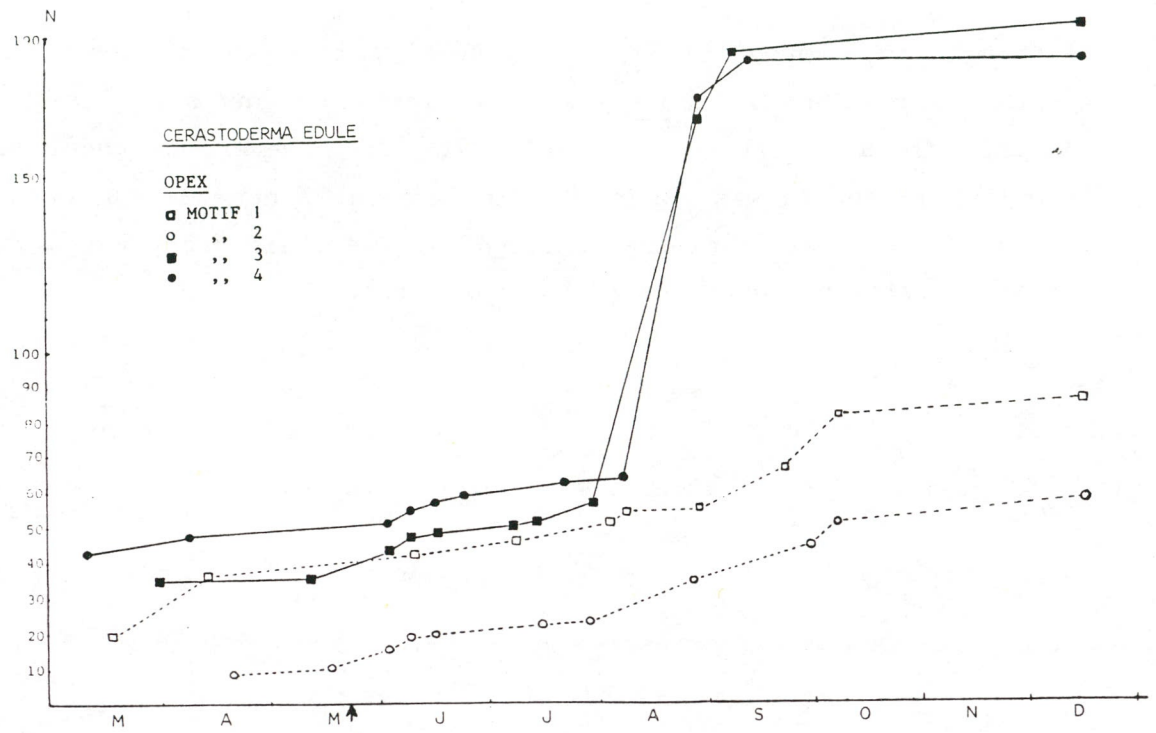


Fig. 29 Cumulative of *C. edulis* found dead on top of the sediments in the different MOTIFs during OPEX.

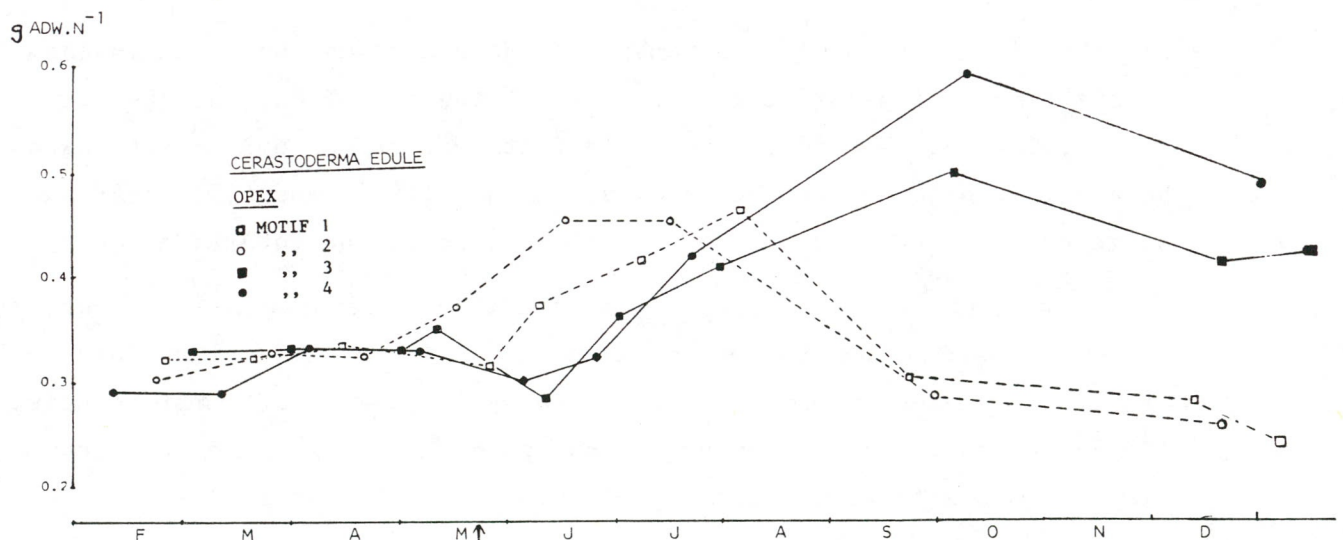


Fig. 30 The ash-free dry weight (ADW) per individual of *C. edulis* in the different MOTIFs during OPEX.

In May and June considerable growth occurred in MOTIF 1; MOTIF 2 followed one month later and this continued in July. The origin of this difference is not clear. After July, a weight loss was seen in MOTIFs 1 and 2. A prolonged period of emaciation in autumn and winter is a known phenomenon for the cockle, but the extent of the weight loss observed is remarkable. Low food availability might be one of the causes. The polluted animals in MOTIFs 3 and 4 responded directly to the application of the oil, showing weight loss in June, a recovery took place in July and continued until October.

Improved feeding conditions may have caused rapid growth in summer; and the mass mortality in part of the population, which eliminated the meagrest specimens may have flattered growth of the remaining animals.

The variations in the average condition index, $W.l^{-3}$ (given in Appendix I) in *Cerastoderma*, throughout the course of the experiment generally reflected the results given in Fig. 30, but also allow further speculation. Low condition indices point to the liberation of the gametes from the contaminated cockles in June and from the contaminated animals in July; this may indicate a delay in spawning under influence of the oil contamination.

Macoma balthica

As in the case of the cockle, the population density of adult baltic tellins was determined by the decrease in numbers caused by the sampling programme itself and by mortality. The initial population density was 100 specimens per m^2 (= 1800 in total). In total 313, 286, 184 and 233 specimens (= 17, 16, 10 and 13%) were removed by sampling from MOTIF 1-4 respectively. Mortality during the whole period of observation, as judged from the numbers of empty shells collected, amounted to 35 in MOTIF 1, 39 in MOTIF 2, 402 in MOTIF 3 and 250 in MOTIF 4. This reveals a considerable difference between the mortality of about 2% of the initial number in the uncontaminated MOTIFs 1 and 2, and that of 22 and 14% in the contaminated MOTIFs 3 and 4. Except for a slight deviation early in June, the numbers of animals recovered in MOTIFs 1 and 2 show the expected gradual decline caused by sampling and, to a lesser extent, by mortality (Fig. 31).

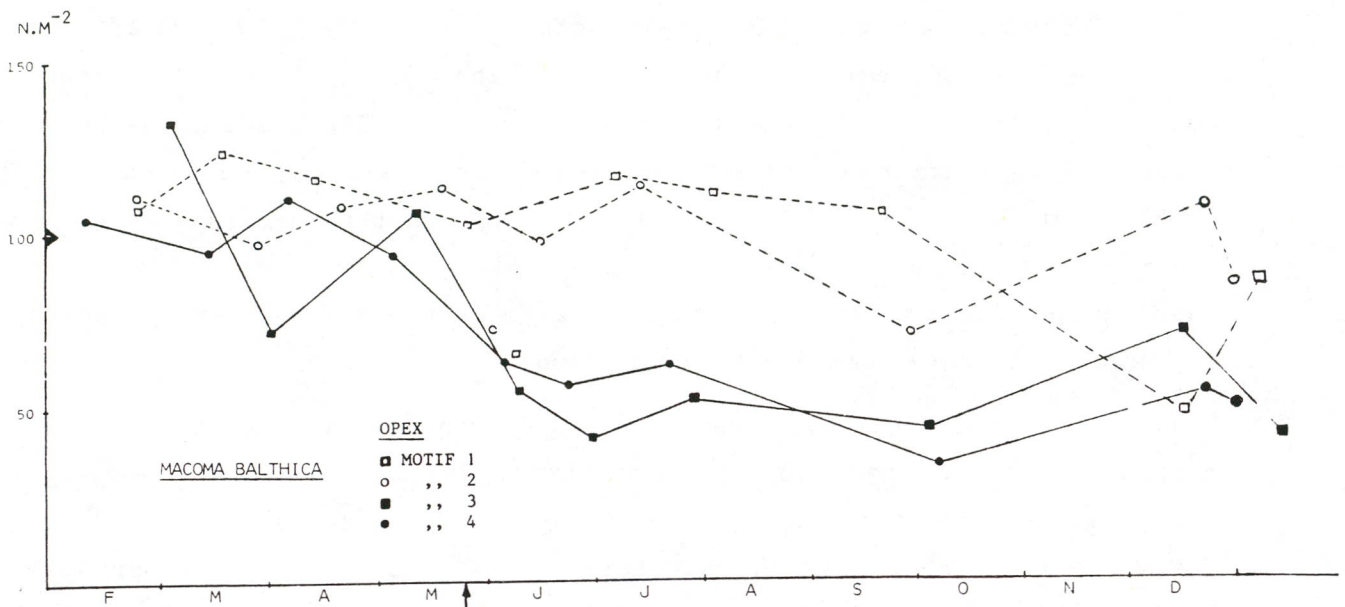


Fig. 31 Numbers of *M. balthica* in the different MOTIFs (results from sediment sampling) during OPEX.

The contaminated MOTIFs 3 and 4 suffered total losses due to sampling and observed mortality of 32 and 28% respectively, mortality being responsible for the major part. The numbers of empty shells collected represents a considerable underestimate for mortality, as the relatively small-sized and deeper-living shells will easily be lost in the sediment. The low numbers (well under 50 *Macoma*'s per m²) recovered in MOTIFs 3 and 4 early in October are, therefore, thought to be realistic, which was confirmed in December.

Fig. 32 shows the cumulative number of collected empty shells in the MOTIFs. In MOTIFs 1 and 2 a gradual and slow increase of these numbers point to natural mortality, commonly observed in populations of adult *Macoma*'s. The lines for MOTIF 3 and 4 show a quite different pattern. An increased number of dying and dead animals was detected shortly after the exposure, with mass mortality occurring, in the second week after exposure; 300 dead *Macoma*'s being found in MOTIF 3. Towards the end of June a normal level of mortality comparable to that in MOTIFs 1 and 2 was reestablished. A direct cause for the increased mortalities in June is not clear. Adult *Macomas* are known to be resilient animals, able to cope with a multitude of unfavourable environmental conditions, in particular when compared with the cockle. *Macoma* is predominantly a boreal species which thrives best at a temperature of

5-15°C. The combination of the extremely high summer temperatures in June (the temperature of the upper sediment layer in the oil-contaminated MOTIFs rose to over 30°C at low water), and the oil exposure was probably fatal. The temperature of the sediment in the uncontaminated MOTIFs were up to 5°C lower than those in the contaminated MOTIFs. In addition, in the controls *M. balthica* avoided higher temperatures by burrowing more deeply (~ 10 cm) in the sediment than in the contaminated MOTIFs where they were found at 2 cm depth.

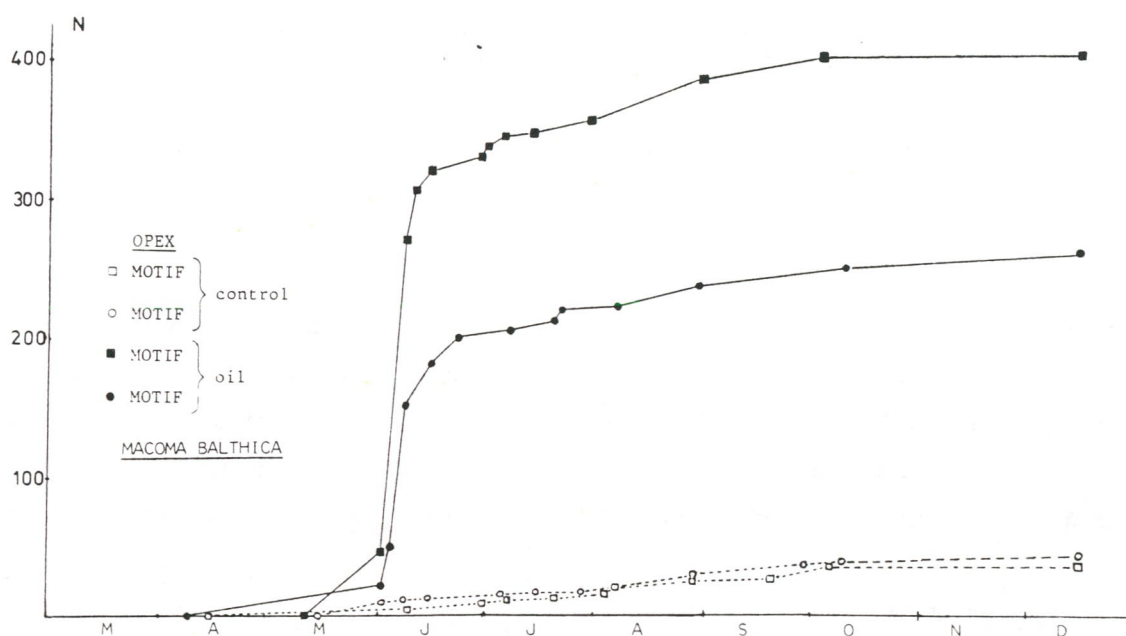


Fig. 32 Cumulative numbers of *M. balthica* found dead on top of the sediment in the different MOTIFs during OPEX.

The mean ADW of the soft parts per animal are given in Fig. 33. The initial ADW value early in March was about 90 mg. In spring the populations showed a slow weight increase. In June and July a rapid growth in the order of 300-400 mg per animal was seen in MOTIFs 1 and 2. This was followed by loss of weight in late summer and autumn. The oil cover in the MOTIFs 3 and 4 negatively effects growth as shown by a significant weight loss in June. Following a slight recovery phase in July, emaciation took place in the remainder of the year.

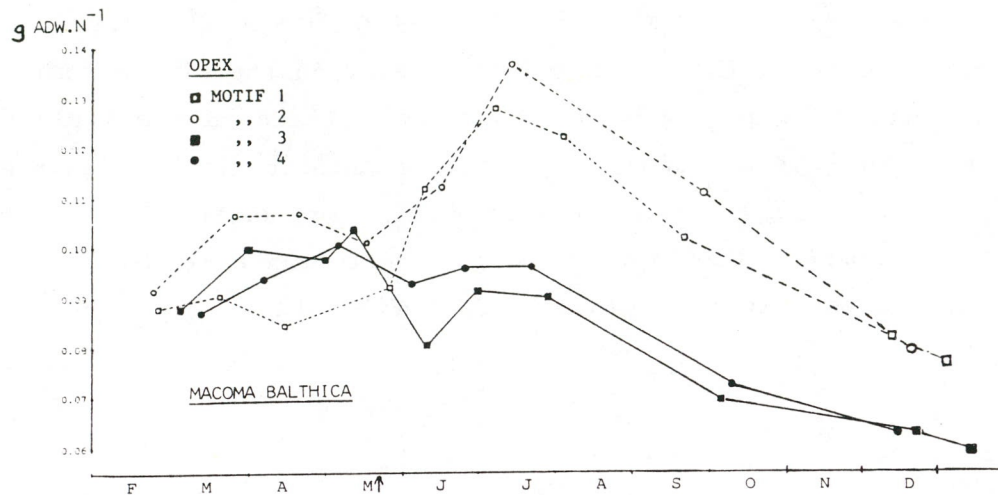


Fig. 33 The ash-free dry weight (ADW) per individual of *M. balthica* in the different MOTIFs during OPEX.

In natural populations of *M. balthica* in the Wadden Sea growth starts as early as March, maximum weight being reached towards the end of June, after which an extended period of gradual emaciation is found. The prolonged growth of adult *Macoma*'s in the uncontaminated MOTIFs is remarkable and may be related to deposit of more than usual organic material in the sediment.

Nereis diversicolor

As previously mentioned a number ragworms were introduced into the MOTIFs with the sediment. Their initial numbers, however, fell beneath the detection limits of the sampling applied, and their presence could only be concluded from the presence of feeding tracks on the sediment surface. The first juvenile *Nereis* were observed in small numbers in the second half of June (Fig. 34). These numbers rapidly increased in July, both in the contaminated and the uncontaminated MOTIFs. Maximum numbers being found in the contaminated MOTIFs. *Nereis diversicolor* thrives best at relatively high ambient temperature and is apparently not sensitive to oil exposure.

The high mortalities of *Macoma* and *Cerastoderma* in the MOTIFs 3 and 4 will have probably influenced the *Nereis* populations in a positive sense, by providing living space and easy accessible food to these carnivorous worms.

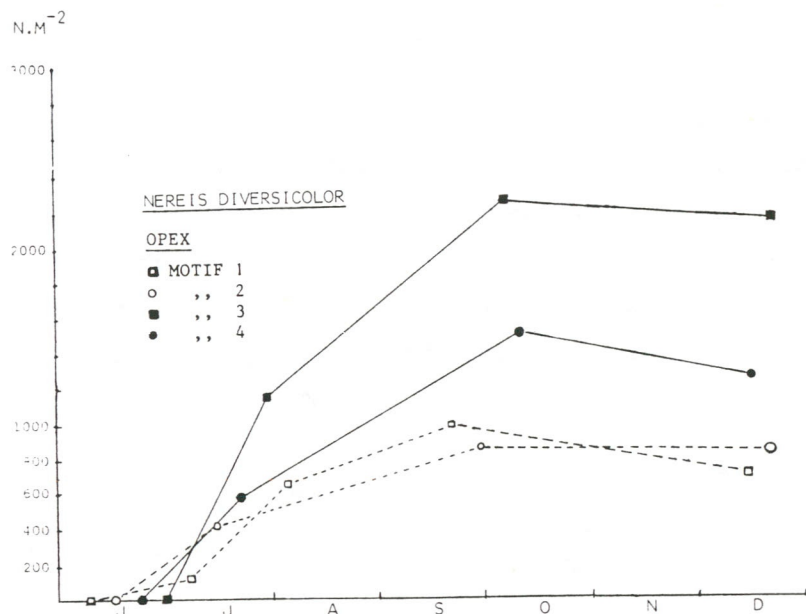


Fig. 34 Numbers of *N. diversicolor* in the different MOTIFs during OPEX.

Fig. 35 shows the average individual ash-free dry weights in the 4 populations. Early in autumn the average weights in MOTIFs 1, 2 and 3 was about 7.5 mg. Population densities in the contaminated MOTIF 3 was, however, twice as high as the densities in MOTIFs 1 and 2. Better feeding conditions in the contaminated environment of MOTIF 4 are reflected by lower population density and larger average weight.

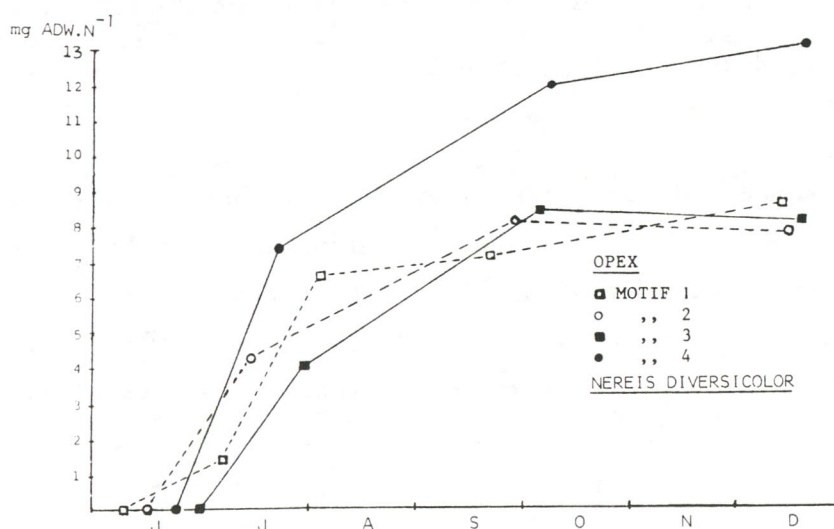


Fig. 35 The ash-free dry weight (ADW) per individual of *N. diversicolor* in the different MOTIFs during OPEX.

Arenicola marina

Fig. 36 shows the population density of lugworm in MOTIFs 1-4; 25 worms per m^2 where initially introduced into each MOTIF. The animals were easily damaged by the sampling method used, and 10 parts of lugworms were counted as one complete animal. During the whole period of observation, 47.7 animals as defined above were recovered from MOTIF 1. This was thought to correspond to about 80 sacrificed lugworms, or about 18% of the number introduced. For MOTIF 2 these data are 52.7-80-18%, for MOTIF 3: 52.9-80-18%, and for MOTIF 4: 57.7-90-20%.

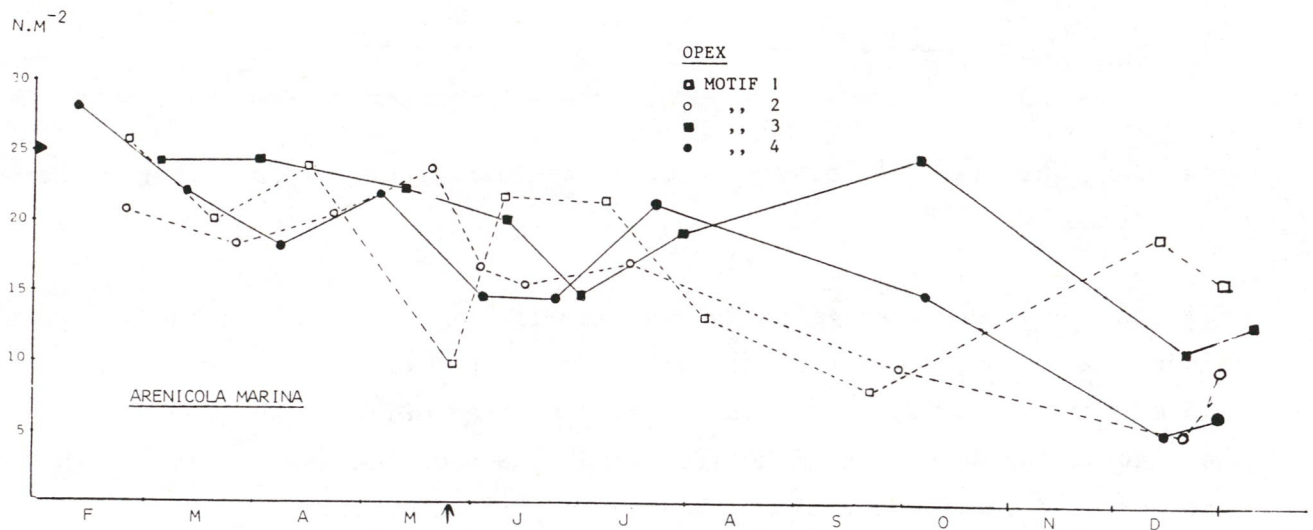


Fig. 36 Numbers of *A. marina* in the different MOTIFs during the experiment. Data of December represent the numbers of casts per m^2 obtained at the final sampling.

Although weakened and succumbing lugworms were observed at the sediment surface now and then, these were not systematically collected for an estimate of natural mortality. Foraging *Nereis* detect worms in this condition and draw them into their burrows. Although an unknown number of worms was eliminated in this way, the lines are still thought to present realistic population dynamics. The similar gradual declines in numerical densities do not indicate any significant effect of oil on the lugworm populations.

The sampling procedure adopted (sediment coring and sieving) may be even less adequate for an estimate of the numerical density in this large macro-fauna species, than in the case of the cockle. Extreme values, such as those found in October in MOTIF 3, are therefore doubtful.

The annual variation in weight of adult lugworms mean ADW per animal is clearly shown in Fig. 37. Considerable growth is observed early in the season and continues into July. Loss of weight takes place after mid summer; this being a normal phenomenon in lugworms. The rapid weight decline and large variations in weight in autumn may be partly explained by differences in spawning time.

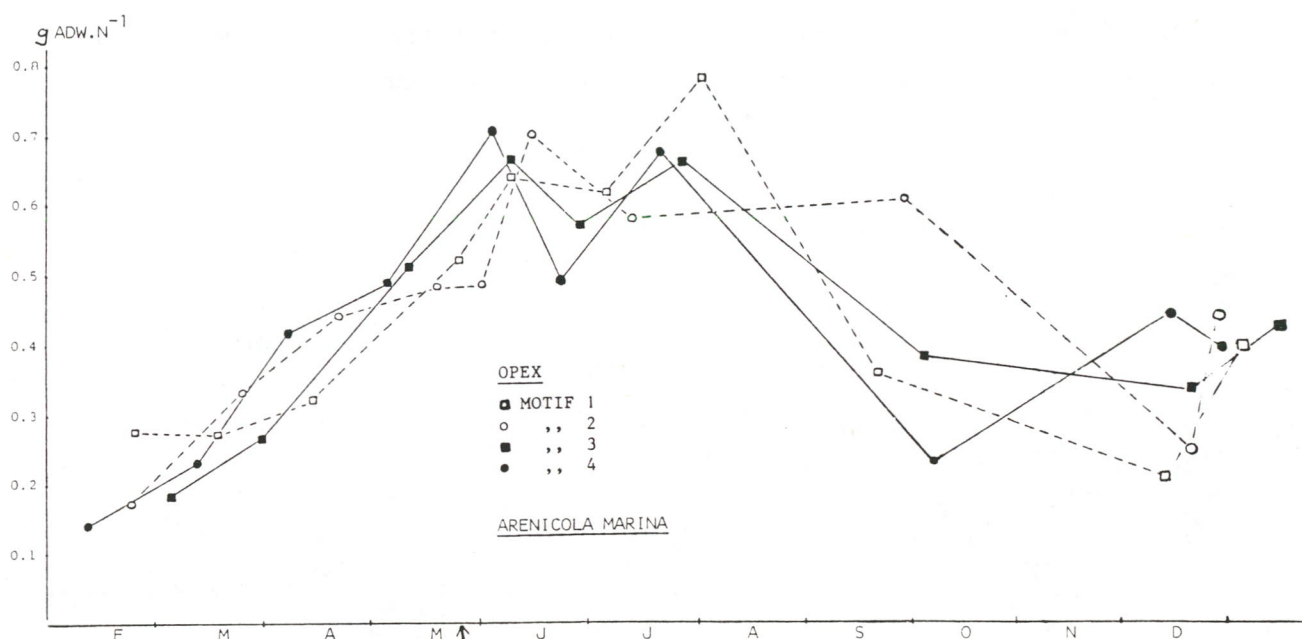


Fig. 37 The ash-free dry weight (ADW) per individual of *A. marina* in the different MOTIFs during OPEX.

Although visual observations of the oil contaminated MOTIFs, particularly MOTIF 4, sometimes suggested large localized mortalities and restricted (feeding) activities, the data presented in Fig. 37 show hardly any significant differences between the 4 populations.

Pygospio elegans

Fig. 38 shows the population densities of one of the smaller tube-building polychaetes. *Pygospio* belongs to the group of relatively short-living summer annuals. As they were not observed shortly after the start of the experiment, they probably entered the systems with the supply water as larvae or juveniles. Small numbers were already present in late winter and these gradually increased during spring. Maximum numbers were reached early in June (MOTIF 1) or July (MOTIF 2, 3 and 4). Numerical densities gradually reduced during summer and autumn. Significant differences between contaminated and uncontaminated MOTIFs were not found. The considerable variation in numbers in MOTIF 1 and 2 towards the end of June is questionable, but could perhaps be explained by size differences between individuals of the two populations, the larger animals being just retained by a mesh size of 1 mm.

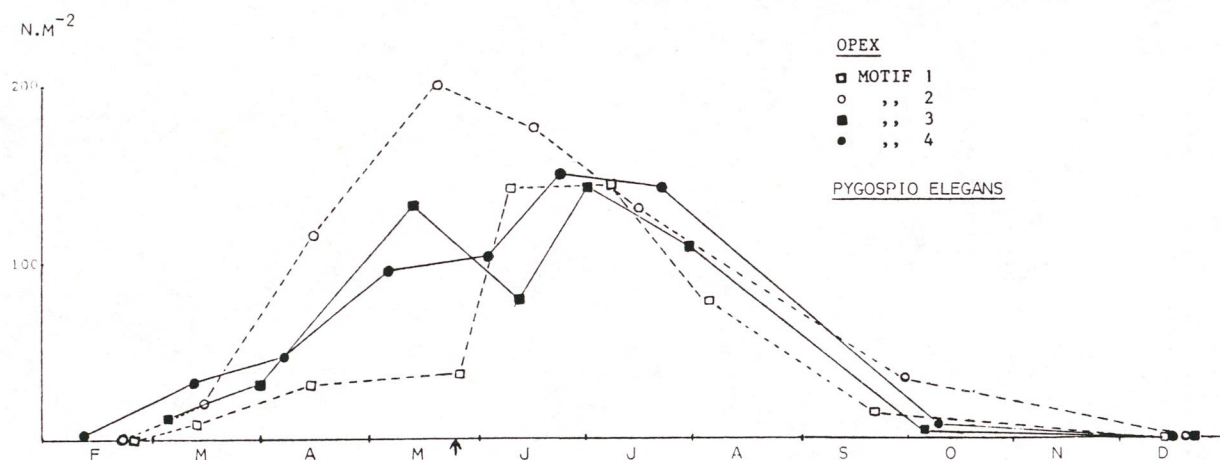


Fig. 38 Numbers of *P. elegans* in the different MOTIFs during OPEX.

Hydrobia ulvae

The common mudsnail *Hydrobia ulvae* was observed in small numbers at the start of the experiment and must have been introduced with the sediment. From March to mid-June the population densities were similar at about 100-200 specimen per m² in all MOTIFs (Fig. 39).

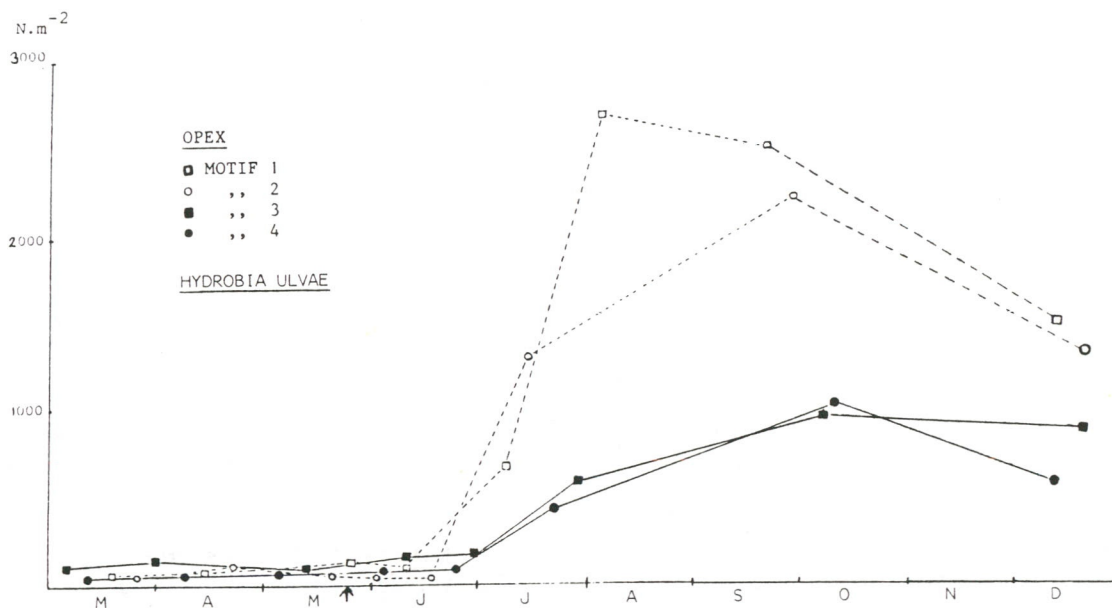


Fig. 39 Numbers of *H. ulvae* in the different MOTIFs during OPEX.

In July the numbers in the uncontaminated MOTIF 1 and 2 rapidly increased, reaching maximum values of 2000-3000 in summer. Numerical increase of the population in the contaminated MOTIFs occurred much more slowly; maximum numbers of only 1000 per m² being found in October. Settlement and/or reproduction of *Hydrobia* are negatively effected by the presence of oil in MOTIFs 3 and 4.

Gammarus spec.

The presence of this small crustacea - also a summer annual - in the intertidal flats of the Wadden Sea is highly dependent on the presence and abundance of thallos and filamentous macro-algae (*Ulva* and *Enteromorpha*). Macro-algae were present in the uncontaminated MOTIFs in June and July.

In these MOTIFs *Gammarus* rapidly increased in numbers and size in June (Fig. 40). Considerable differences in maximum numbers found between MOTIFs 1 and 2 in July can be explained by clustering of *Gammarus* caused by the presence of algal pockets which were inadequately sampled by the method applied. The wane of the algae brought about a concomitant decline of the *Gammarus* stock. In the contaminated MOTIFs 3 and 4 only very small populations of *Gammarus* were found in July. *Gammarus* occasionally was found in the zooplankton samples from the uncontaminated MOTIFs, but hardly any species were found in the contaminated MOTIFs. This *Gammarus* species is directly or indirectly negatively effected by oil.

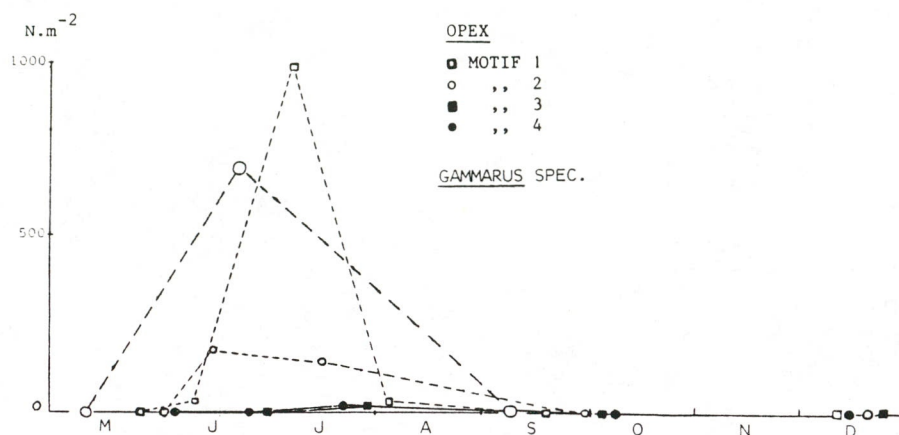


Fig. 40 Numbers of *Gammarus* sp. in the different MOTIFs during OPEX. The upper line for MOTIF 2 represents numbers retained by a 400 µm sieve, the other lines represent numbers retained by a 1000 µm sieve.

Corophium volutator

Fig. 41 shows the development of *C. volutator* in the MOTIFs.

Corophium is a common and extremely abundant crustacean which reaches dense populations in summer and autumn in near shore mud-flats. Increasing numbers were observed from June onwards in the uncontaminated MOTIFs. The large numbers of juveniles collected from sediment cores sieved over a 400 µm screen indicate that the increasing numbers in the MOTIFs were caused by settlement of larvae carried with the supply water.

Settlement of larvae and juveniles in the contaminated MOTIFs 3 and 4 could not be observed; no *Corophium* being found, even five months after the adding of oil mousse. *Corophium*, a very important food item for commercially important juvenile flat-fish species, shrimps and some birds in natural ecosystems is completely unable to settle in oil-contaminated environments.

Littorina littorea

Fivehundred adult periwinkles were placed in each MOTIF on 19 February. These were sampled only once, in October. They were carefully picked from the walls by hand and transferred to the laboratory, where the maximum length of the snails was measured in mm with a sliding calipers, or (for the smallest animals) under a stereomicroscope fitted with a mm grid.

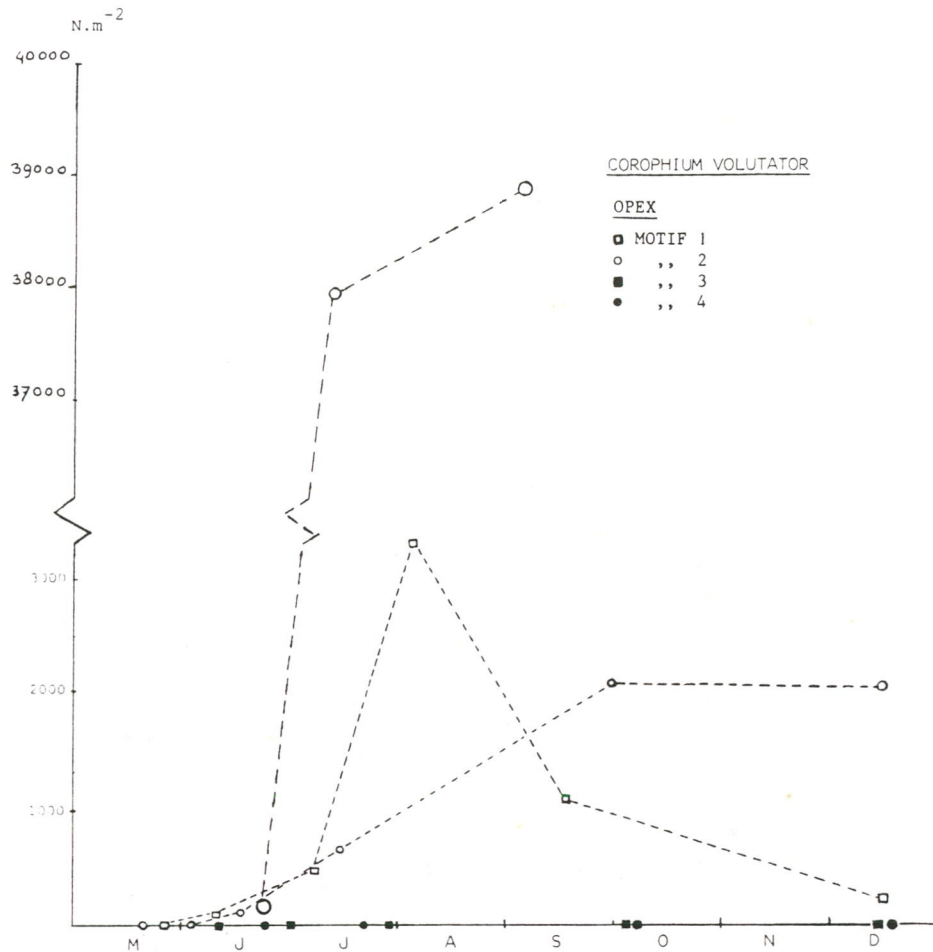


Fig. 41 Numbers of *C. volutator* in the different MOTIFS during OPEX.

Fig. 42 shows the length frequency distribution of the 4 populations in October. The larger individuals (larger than 15 mm) represent the surviving members of the initial stock. Survival was highest in the uncontaminated MOTIF 1, and lowest in contaminated MOTIF 3. Survivals in MOTIFS 2 and 4 were similar, and intermediate to those of MOTIFS 1 and 3. The smaller individuals (less than 15 mm) are young and show the reproduction success of *Littorina*, either derived from the parent stock in the MOTIF or derived from larvae imported from outside with the supply water. It is clear that new generations are almost absent in the contaminated MOTIFS whereas considerable numbers of juvenils are found in the uncontaminated MOTIFS 1 and 2. Differences in the populations in these latter MOTIFS cannot be explained, but may be related to minor technical faults in the supply water flow. Oil is extremely harmless to the periwinkle population, as it prevents reproduction and/or larval settlement.

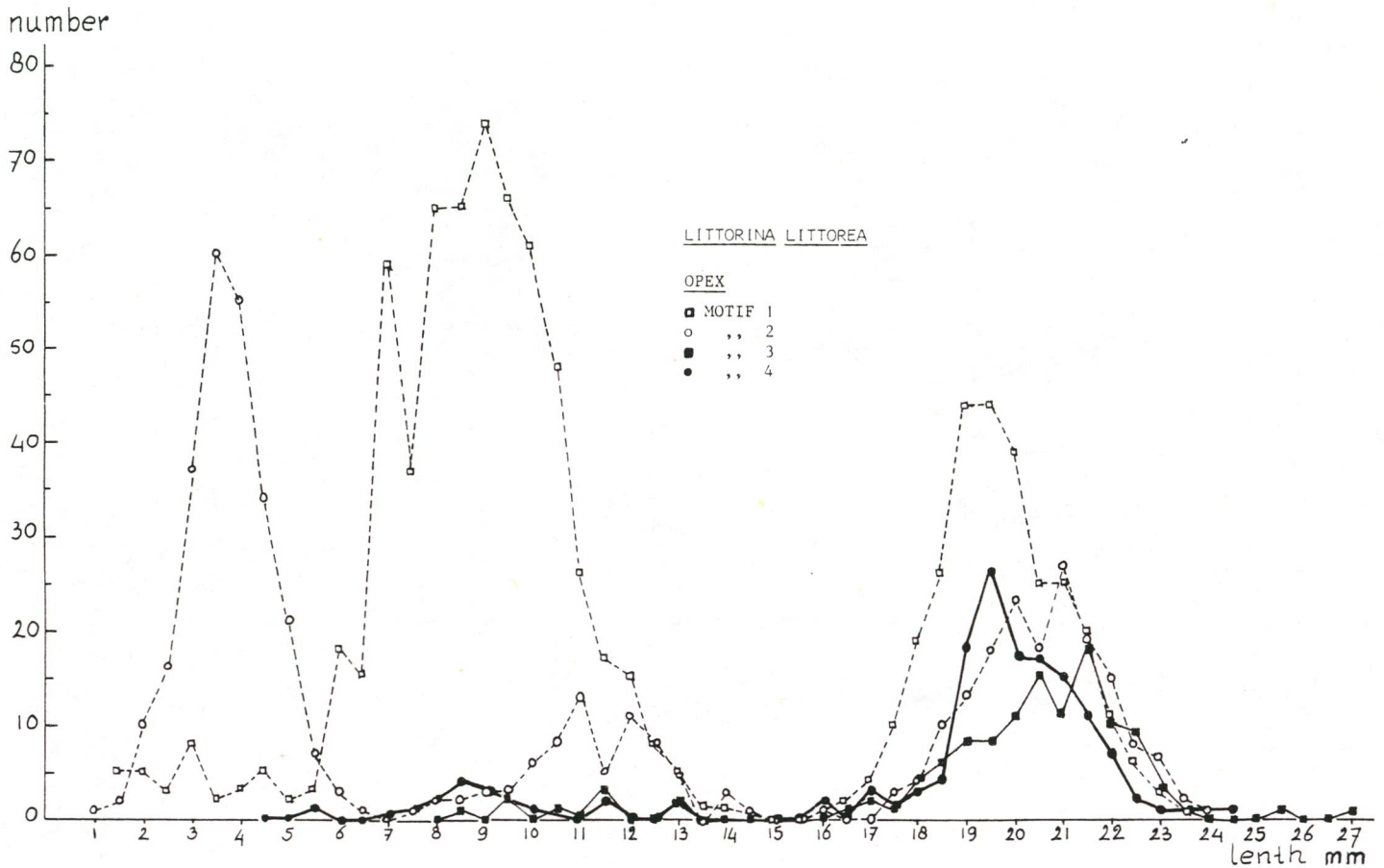


Fig. 42 Length-frequency distributions of *L. littorea* in the different MOTIFS in October 1982.

Heteromastus filiformis

The medium sized, deep living polychaete worm *Heteromastus filiformis* was observed for the first time in considerable numbers (100-200 specimens per m²) in September in the uncontaminated MOTIFS 1 and 2, whereas numbers in the contaminated MOTIFS 3 and 4 remained low. This situation continued into December (Fig. 43). With 3-4 g ADW/m² *Heteromastus* made an important contribution to the total biomass of the unpolluted systems. Settling and/or reproduction of *Heteromastus filiformis* is negatively affected by the oil addition.

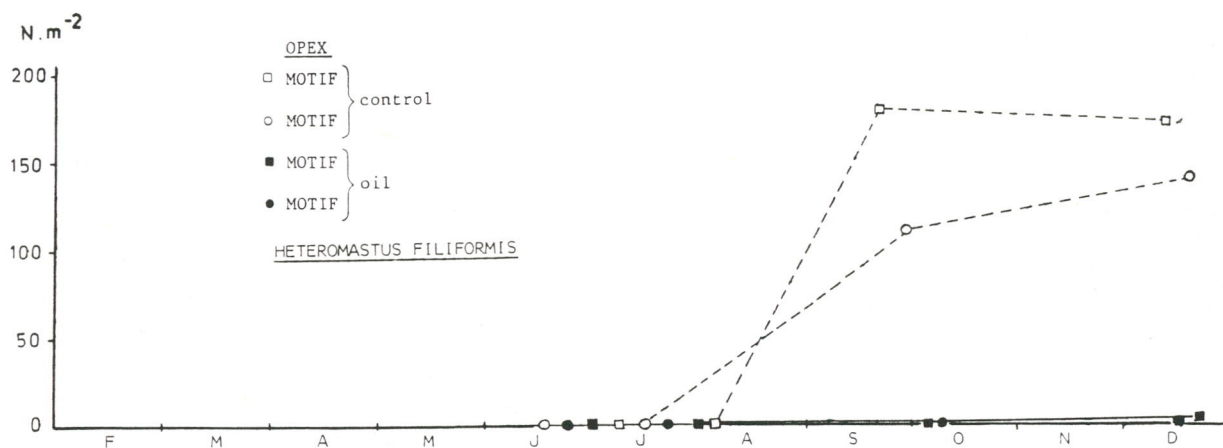


Fig. 43 Numbers of *Heteromastus filiformis* in the different MOTIFs during OPEX.

Other species

During the intensive final sampling programme in December 1982 species occurred in the samples which had not been found in the previous, smaller, samples. Firstly the bivalve *Mya arenaria* was found in the uncontaminated MOTIFs 1 and 2 in population densities of 1.1-2.8 per m². Apparently they had grown from larvae introduced to the MOTIFs with the supply water from the Wadden Sea. This species was not found in the oil contaminated MOTIFs, so that it can be concluded that the development of *Mya* was negatively influenced by the oil addition.

During the final sampling also adult fish, *Gobius minutus*, was found in the controls, but not in the oil contaminated MOTIFs.

One adult common shrimp, *Crangon crangon*, was found in control MOTIF 2, this one specimen shows that shrimps can be introduced, and can live in the MOTIFs, but does not allow any conclusion about the effect of the added oil.

Finally the very large polychaete, *Nereis virens* (about 30 cm), was found in all MOTIFs, with exception of MOTIF 3.

3.2.7 Development of the biota in the watercolumn

3.2.7.1 Phytoplankton and nutrients

Fig. 44 shows the development of the phytoplankton biomass, measured as chlorophyll concentrations, in the water of the MOTIFs. The results from both pairs of MOTIFs (1 and 2, 3 and 4) are pooled because the same water was pumped between the two MOTIFs of each pair.

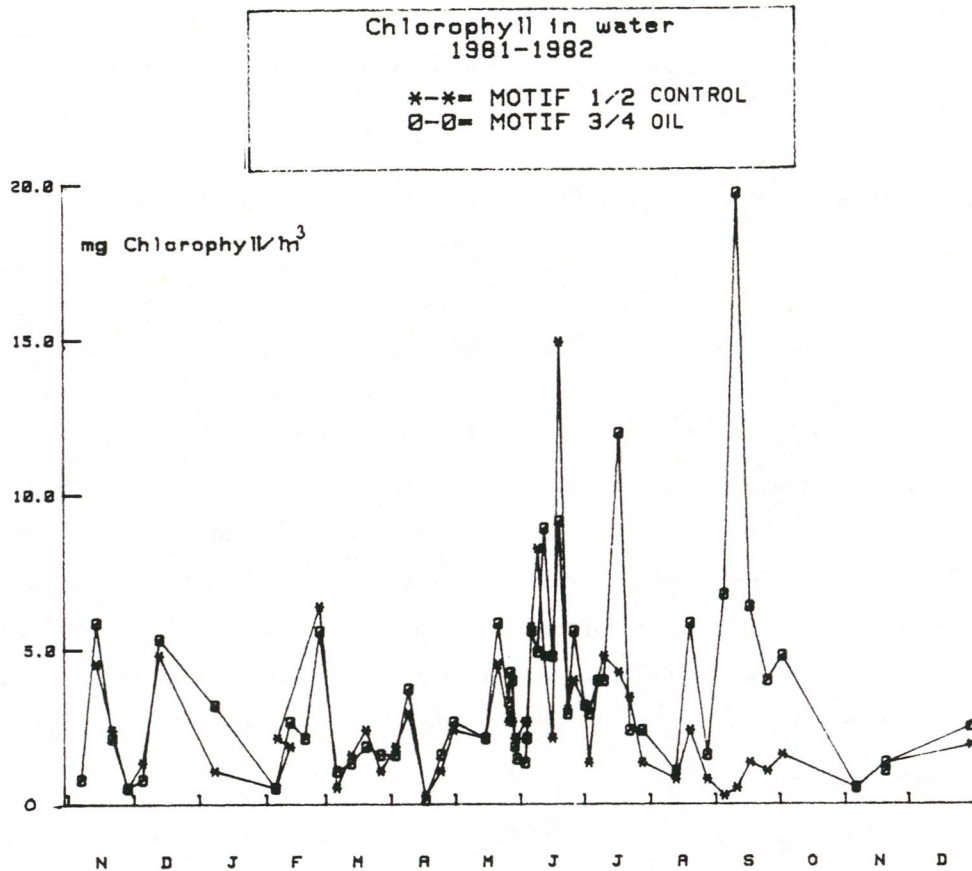


Fig. 44 Chlorophyll concentrations in the water of the MOTIFs during OPEX.

The results show that the phytoplankton density was generally low. Chlorophyll maxima were observed in November and December 1981 and February 1982, these maxima were caused mainly by diatoms. The development of the phytoplankton up to the addition of the oil, followed very similar patterns in all MOTIFs.

From March to May, the phytoplankton species composition was studied in detail by Scholten (1983). Results, given in Table 9, show firstly that the species composition was very similar in the different MOTIFs. After the early spring diatom peak the importance of diatoms decreased and that of flagellates increased. Pennate diatoms such as *Naviculaceae* and *Nitzschiaceae* were found throughout this period with maximum numbers around the middle of March. The numbers of Centrales increased up to the start of April. Dominant species followed each other in time, resulting in a clear succession: *Melosira nummuloides* up to mid March, *Thalassiosira decipiens* from the end of March - start of April, *Skeletonema costatum* generated a large peak in the first week of April, and *Rhizosolenia* spp. formed a new peak in early May. The large numbers of pennate diatoms are characteristic for the shallow waters of the Wadden Sea.

For two days after the addition of the oil, chlorophyll concentrations in the contaminated MOTIFs were 50-100% higher than those in the uncontaminated MOTIFs (Table 10). Between 3 and 9 days after oil addition, concentrations were lower than in the uncontaminated MOTIFs. Thereafter (Fig. 44), chlorophyll concentrations were again higher in the contaminated MOTIFs for long periods, with some blooms of phytoplankton (during July, August and October) which did not occur in the uncontaminated MOTIFs.

Primary production was measured after the addition of oil. Fig. 45 shows the gross primary productivity (increase of oxygen concentrations per mg chlorophyll during the incubation period) in the different MOTIFs. Production is expressed on a chlorophyll basis to exclude the influence of differences in biomass. Directly after the addition of the oil, primary productivity was higher in the contaminated than in the uncontaminated MOTIFs. This caused the higher chlorophyll concentrations found in the contaminated MOTIFs immediately after the oil addition. Primary productivity was inhibited, one day after the addition, and this remained true until the end of the measurement period (September). The higher phytoplankton biomass found in the contaminated MOTIFs (Fig. 44) cannot therefore be attributed to stimulation by oil compounds, but rather to less removal by grazing. The initial stimulation followed by the inhibition of primary productivity by the oil compounds is also shown by the pH of the water (Fig. 46).

Table 9 Species composition of the phytoplankton in the water of the MOTIFS during the period March-May.

The density per sampling date is indicated by:

X = < 10	cells.ml ⁻¹	5 = 5-10x 10 ² cells.ml ⁻¹
R = 10-25	" "	6 = 1-2.5x10 ³ " "
1 = 25-50	" "	7 = 2.5-5x10 ³ " "
2 = 50-10 ²	" "	8 = 5-10 x10 ³ " "
3 = 1-2.5x10 ²	" "	9 = > 10 ⁴ " "
4 = 2.5-5x10 ²	" "	

Table 9

Date	4.3	11.3	18.3	25.3	1.4	7.4	15.4	22.4	29.4	6.5	13.5
MOTIF no.	2 4	1 3	2 4	1 3	2 4	1 3	2 4	1 3	2 4	1 3	2 4
Species											
<u>Centricae</u>											
Melosira nummuloides	R	R 2	1 R	X R	X X	1	R	1 R	R X		
M. varians		R	X	X X		1			R		
Skeletonema costatum	R R	R R	1 1	2 2	4 4	6 6	3 3	X 1	R R	R X	1 R
Thalassiosira decipiens	R	1	1 R	1 1	2 2	1			R R	X	
Th. nordens kiöldii			X X						R X		
Leptocylindrus danicus											R R
Rhizosolenia delicatula	R R	X	1 1	R R					R R	X X	3 3
Rh. shrubsolii		R		X	X R	X			X X	X X	R R
Rh. setigera					X X					X X	1 R
Chaetoceros spp.	X		X X			X	X R				
Biddulphia aurita	X	X		X	X	1	X				
B. regia							X				
Cerataulina bergonii			X	X	X X	X					
<u>Pennatae</u>											
Phagiogramma brockmannii								X R	R	1 1	
Thalassionema nitzschioides	R X	X	1 R	R R	1 1	R X	X	X X			
Asterionella kariana	X	R R	X X	1 R	1 1						
A. japonica					R						
Nitzschiaceae	3 6	5 5	4 6	2 3	2 3	3 3	2 2	2 2	2 1	2 2	2 2
Naviculaceae	4 3	4 4	6 5	5 5	4 4	4 5	4 4	3 3	4 4	4 4	3 4
Flagellates	6 5	5 5	5 5	5 4	5 5	6 6	7 7	7 7	8 7	7 6	7 7
µ-flagellates	6 6	7 6	7 7	6 6	6 6	7 7	7 7	6 6	8 8	9 9	9 9
<u>Fresh water algae</u>											
Scenedesmus spp.	X	1	X R	R R		R	X	X X	R R		
others	X	R R	X	X X	X X					R	

Table 10 Chlorophyll concentrations in the water of the MOTIFs. Oil was added on day 0.

date	day	uncontaminated	contaminated	ratio uncontaminated : contaminated
24.5	0	3.34	3.12	0.93
25.5	1	2.94	4.27	1.45
26.5	2	2.01	4.14	2.06
27.5	3	2.14	2.07	0.97
28.5	4	2.01	1.96	0.98
1.6	8	2.21	1.27	0.57
2.6	9	2.47	1.94	0.79
4.6	11	5.34	5.28	0.99

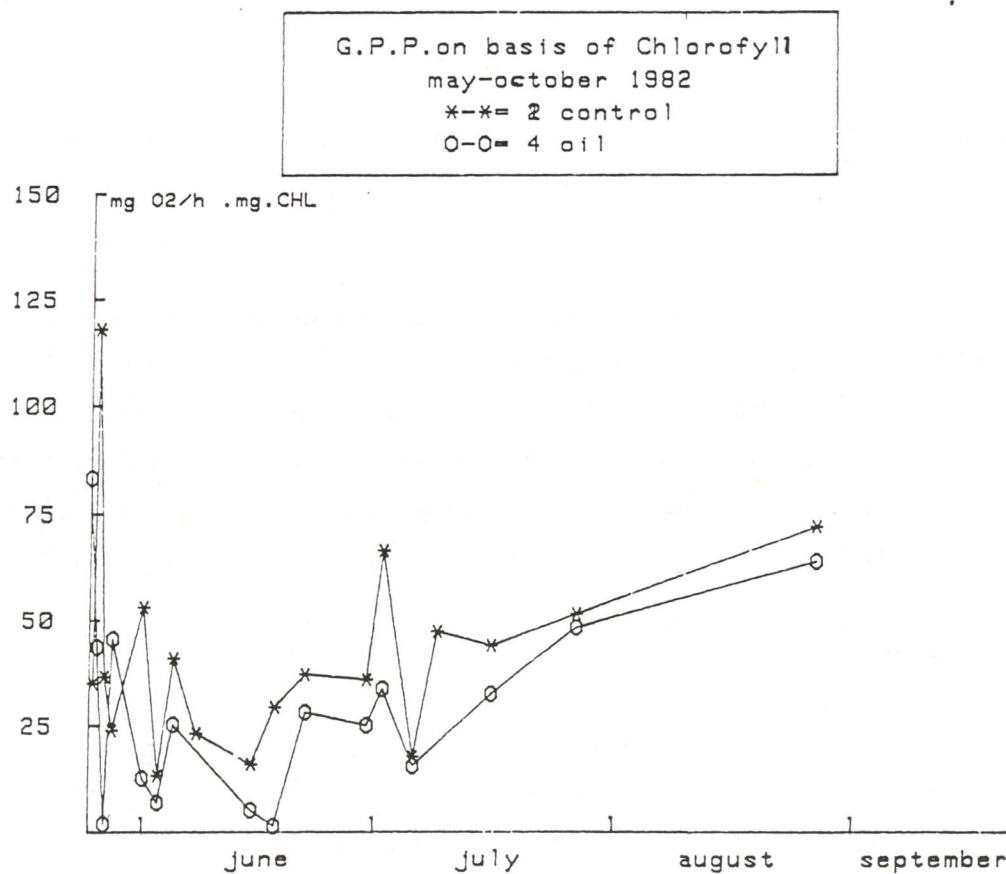


Fig. 45 Gross primary productivity in the water of the different MOTIFs during the period June-September 1982.

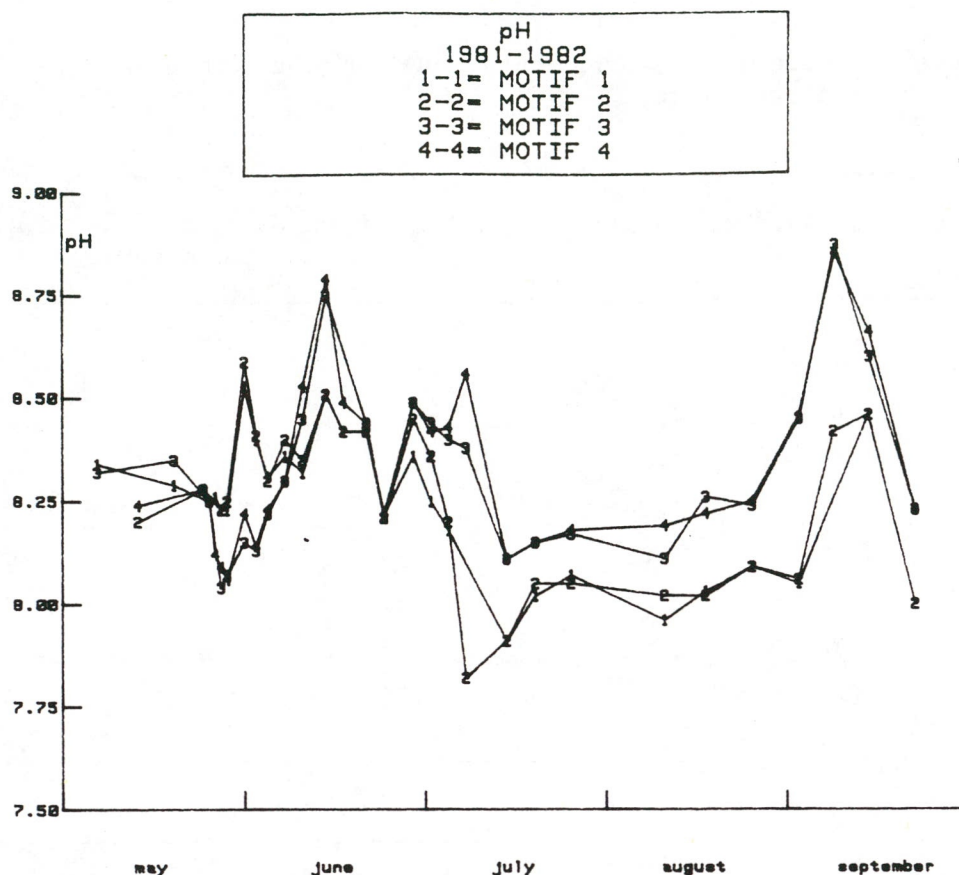


Fig 46 The pH of the water in the MOTIFs during the period May-September 1982.

After the oil addition, only a few phytoplankton samples were inspected for species composition determination. Results are given in Table 11. In August and September, numbers of flagellates were much higher in the contaminated MOTIFs than in uncontaminated MOTIFs, due to growth of a species not present in the latter. The numbers of μ -flagellates were also much higher in the contaminated MOTIFs. In September Nitzschiaceae and Naviculaceae formed blooms in the uncontaminated, but not in the contaminated, MOTIFs. This reduction of diatom numbers due to oil exposure was also found for other species (*Phagiogramma* sp. end May, *Chaetoceros* spp. July-September, *Rhizosolenia* spp. May-July, September, *Skeletonema costatum* September). Only Naviculaceae were found in any numbers in the contaminated MOTIFs. The shift in the species composition in the contaminated MOTIFs in favour of the flagellates is shown by the F/D ratio (numbers of flagellates divided by the number of diatoms) in Table 11.

Table 11 Species composition of the phytoplankton in the MOTIFs after the oil addition. The density index of Table 9 is used. F/D ratio is the number of flagellates divided by the number of diatoms; averaged values for the pairs of MOTIFs are given.

Date	24.5		27.5			2.6			8.7		18.8		23.9	
MOTIFS	2	3	2	3	4	2	3	4	2	3	2	3	2	3
species														
<u>Centricae</u>														
Melosira sp.									R					
Skelotonema costatum						4	5	4	4				1	
Rhizosolenia delicatula						R			R	R			1	
Rh. shrubsolii			R			R			1	R				
Chaetoceros sp.									1		R	R	3	
<u>Pennatae</u>														
Phagiogramma brockmannii			3	2	1									
Nitzschiaceae	2	1	R	1	R	1	1	R	3	3	1		3	R
Naviculaceae	1	1	2	2	1	3	3	2	1	1	1	1	2	1
Flagellates	6	6	6	7	6	6	6	6	7	7	5	9	5	8
μ-flagellates	9	8	7	8	7	7	8	8	8	9	7	8	7	8
	24.5		27.5			2.6			8.7		18.8		23.9	
F/D ratio														
control	175		19			9			16		48		9	
oil contaminated	122		61			16			31		455		27	

Fig. 47a-e shows the concentrations of ammonia, nitrate, nitrite, phosphate and silicate in the water of the MOTIFs during the experiment. With the exception of a few outliers, the development of nutrient concentrations was very similar in the different MOTIFs in the period up to the oil addition. Nitrate concentrations were very high in winter and spring. The phytoplankton/phytobenthos spring bloom caused a steep decrease of the nitrate concentrations in April, and concentrations remained low in May and June. Nitrate concentrations increased again in the uncontaminated MOTIFs in July-October. Concentrations remained lower in the contaminated MOTIFs. A similar pattern was shown by the nitrite concentrations.

Variations in ammonia concentrations were stronger, with highest concentrations at the end of May, following the decline of the phytobenthos maxima in spring. After the addition of oil, differences were found between contaminated and uncontaminated MOTIFs, but the interpretation of these differences is not easy at the moment.

Phosphate concentrations were around $2 \mu\text{mol.l}^{-1}$ from November to May 1982 and increased thereafter, indicating a large mineralization activity. Important differences in phosphate concentrations were not observed in the different MOTIFs directly after the oil addition, but later (August-October) concentrations were lower in the contaminated MOTIFs, possibly as a result of lower mineralization activity or of the higher phytoplankton standing stock. Silicate concentrations were also lower in the contaminated MOTIFs in this period.

3.2.7.2 Zooplankton

Fig. 48 shows the development of the main groups of the zooplankton in the different MOTIFs. Calanoid copepods decreased in numbers during the winter months and increased in spring reaching maximum numbers at the end of April. The numbers of adults remained very low. After May calanoid copepods were rarely found in any of the MOTIFs. The species found were typical for Dutch coastal waters (*Temora longicornis*, *Acartia clausi*, *Pseudocalanus/Paracalanus*, *Centropages* spp., *Eurytemora* sp.).

Numbers of harpacticoid copepods in the water column increased considerably in all MOTIFs from April onwards, reaching maxima at the end of May. Harpacticoids remained in the systems until the end of the experiment.

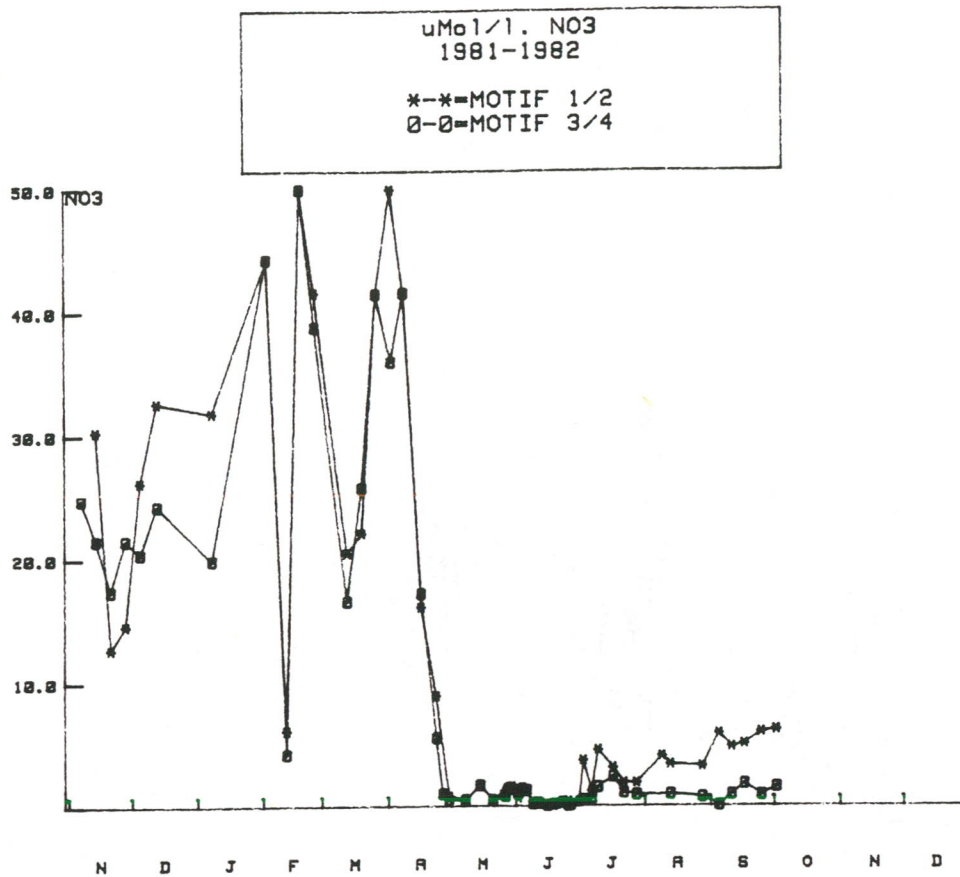


Fig. 47a

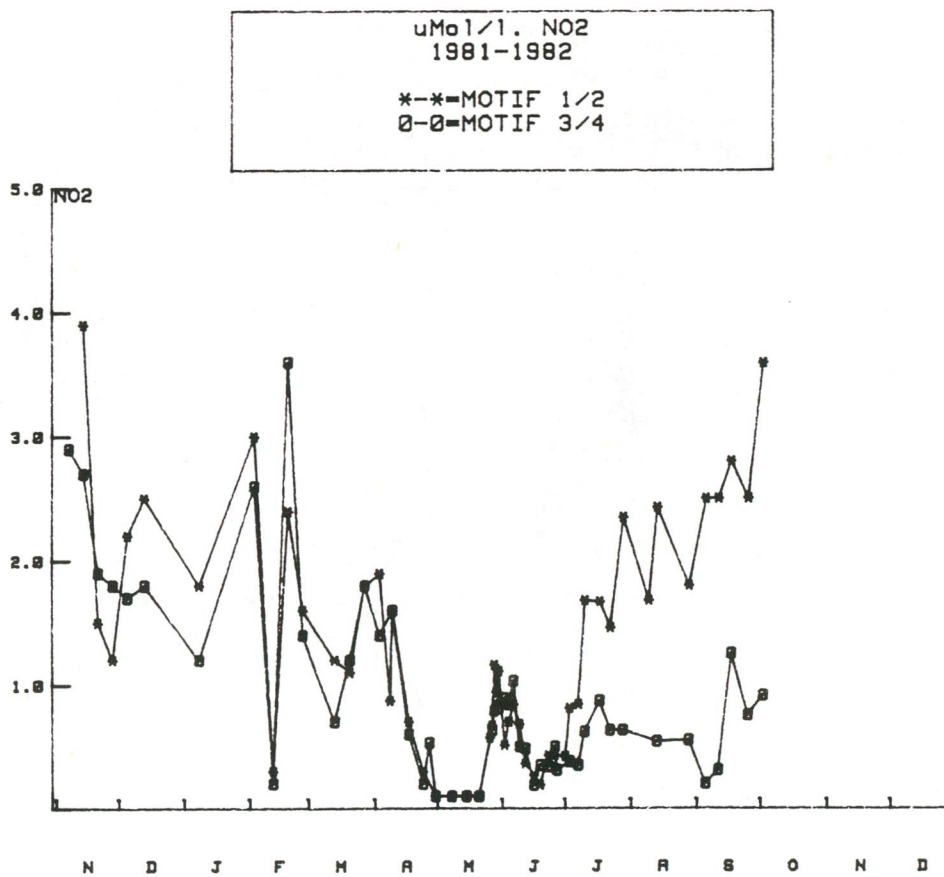


Fig. 47b

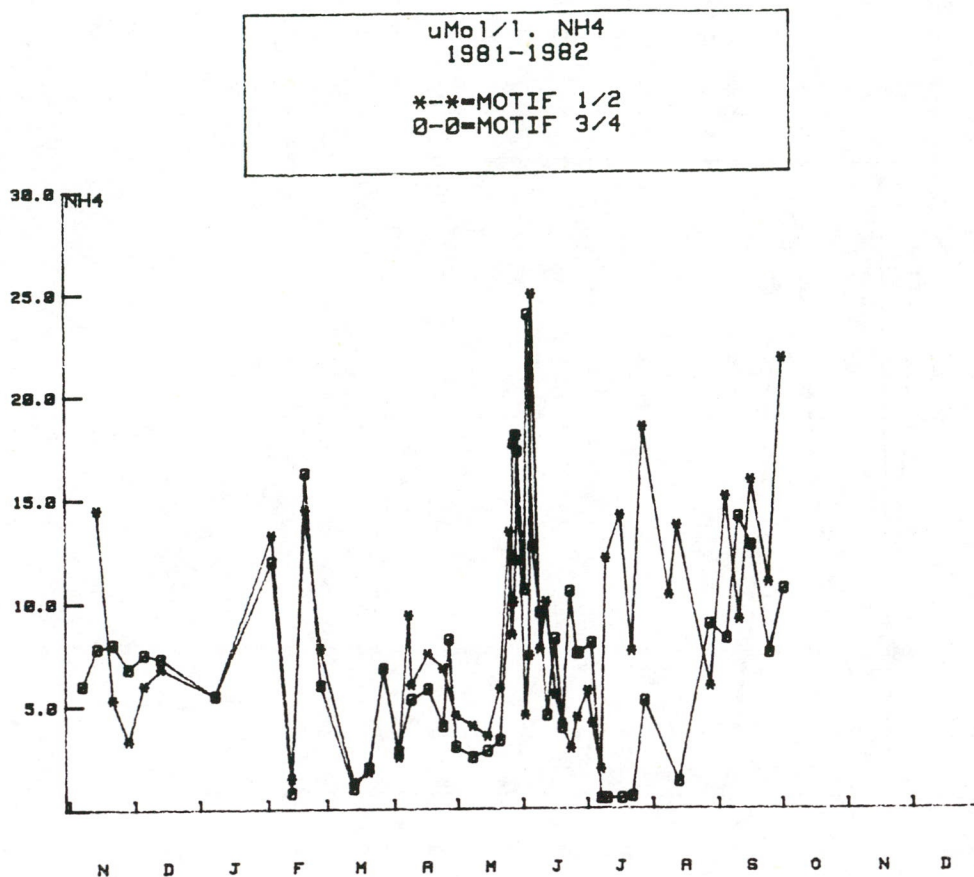


Fig. 47c

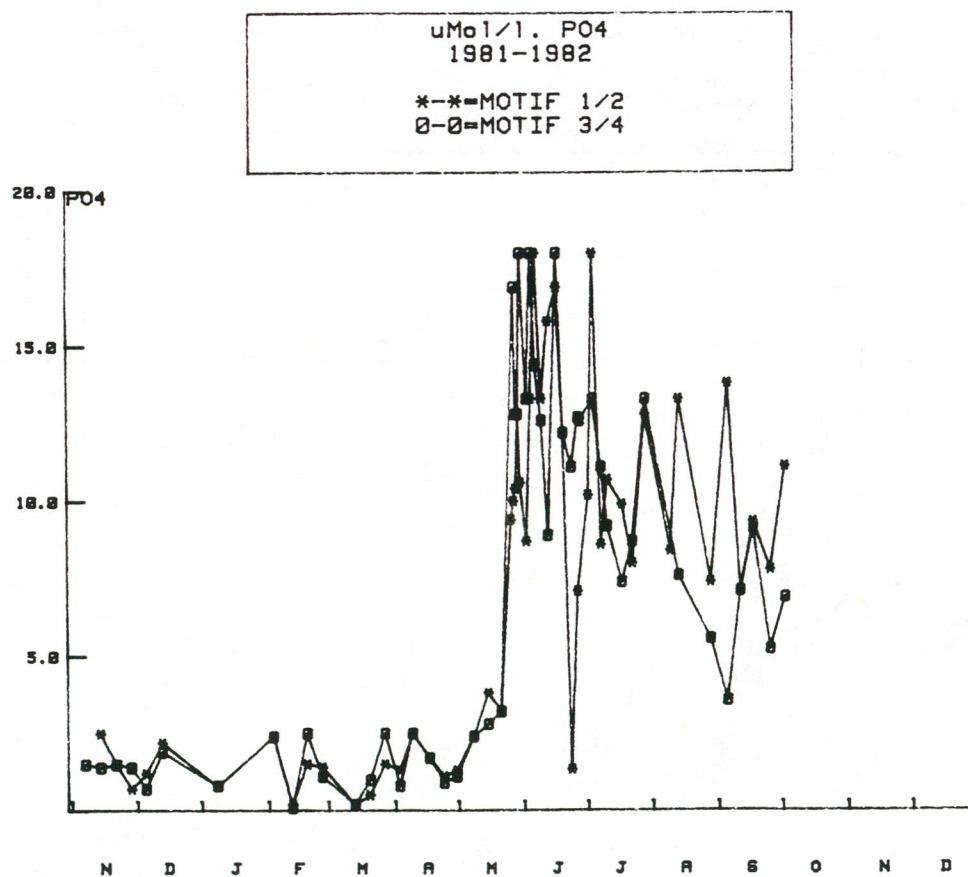


Fig. 47d

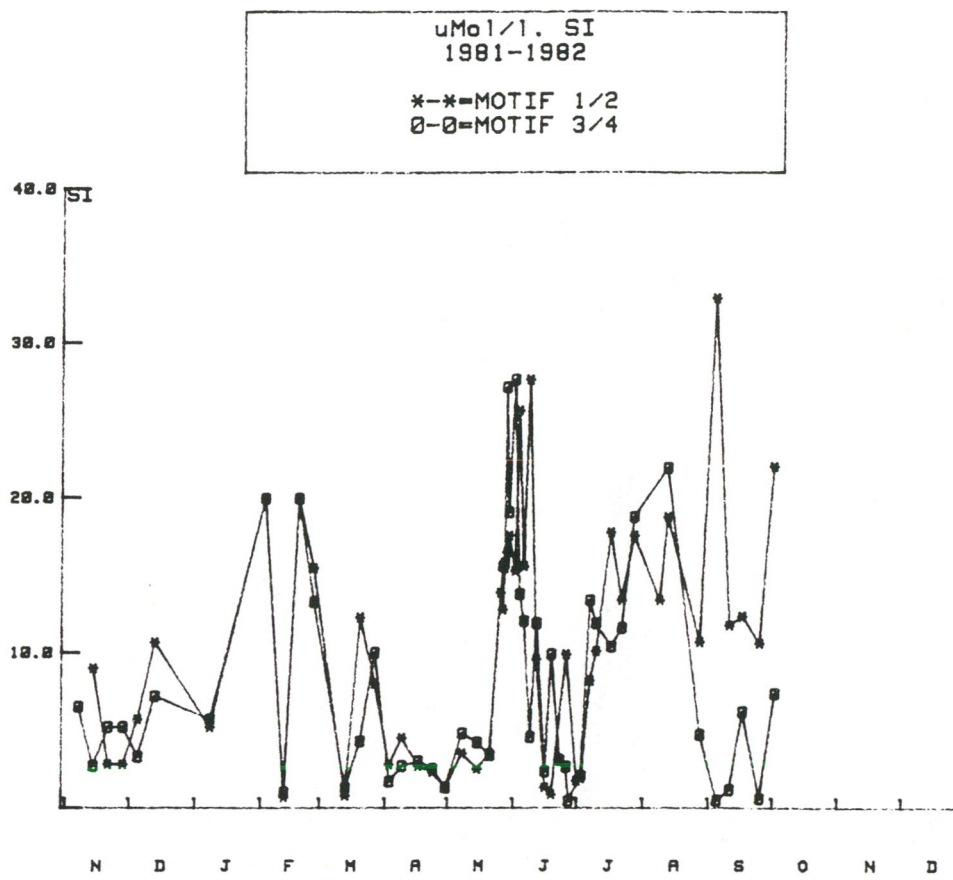


Fig. 47e

Fig. 47 Concentrations of ammonia (a), nitrate (b), nitrite (c), phosphate (d) and silicate (e) in the water of the MOTIFs during OPEX.

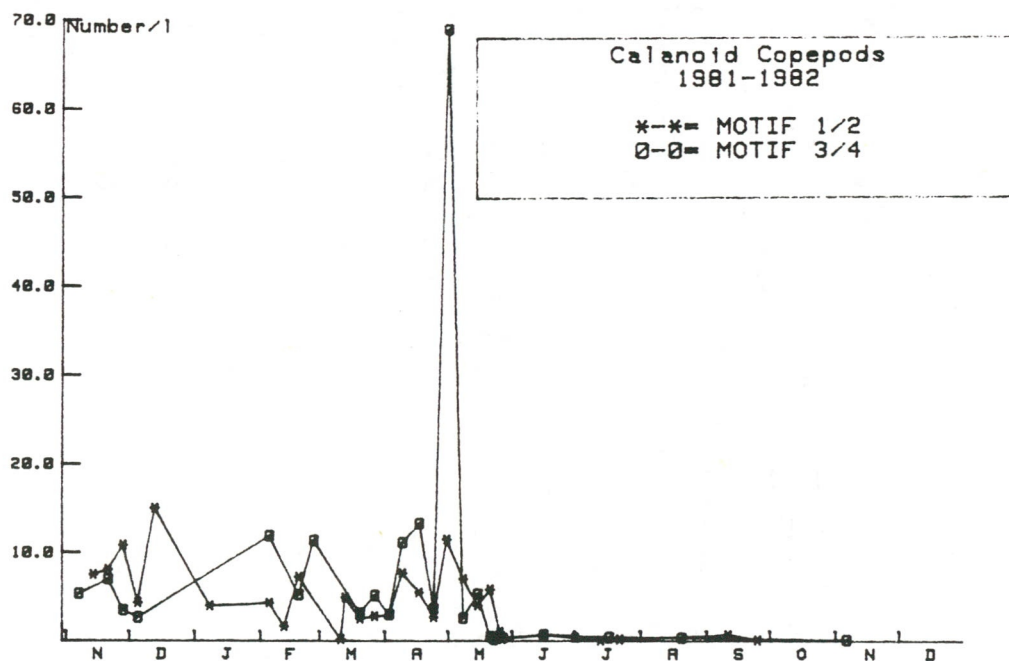


Fig. 48a

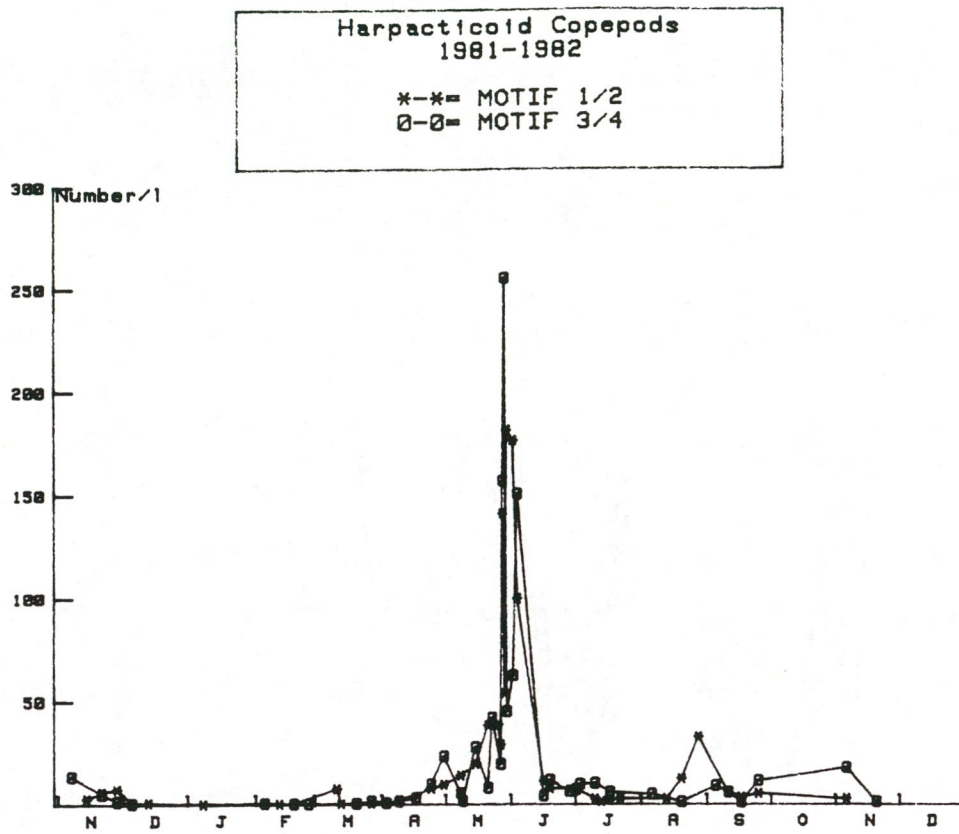


Fig. 48b

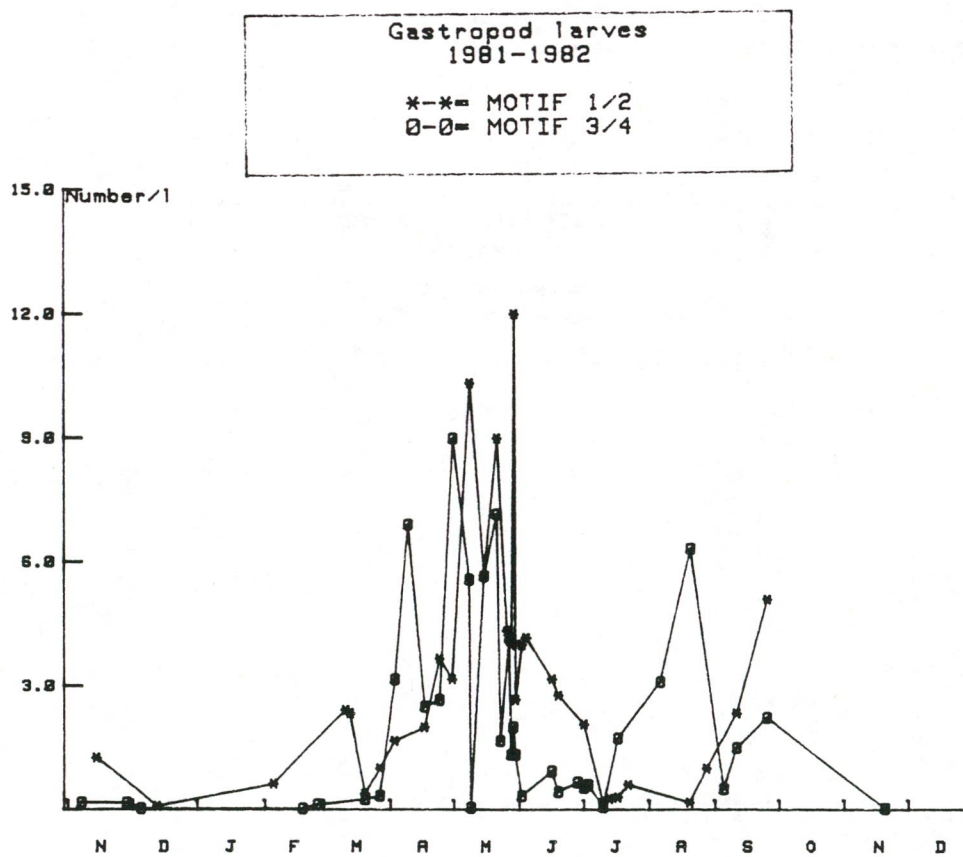


Fig. 48c

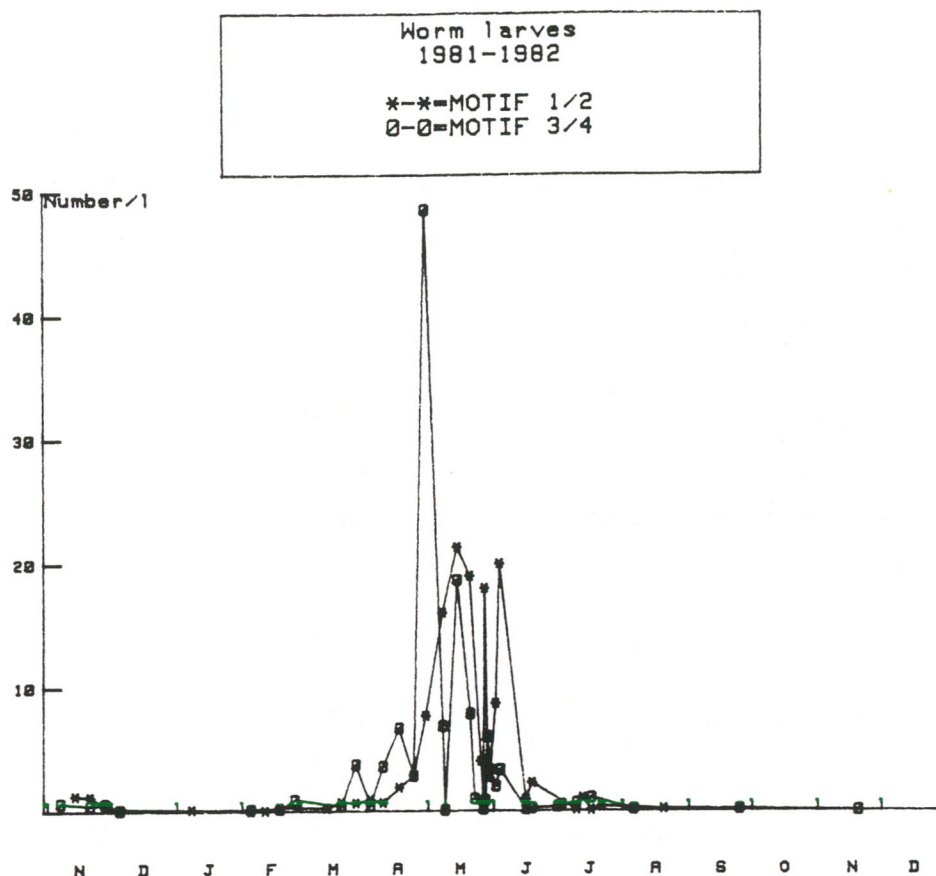


Fig. 48d

Fig. 48 Numbers of calanoid copepods (a), harpacticoid copepods (b), gastropod larvae (c) and larvae of worms (d) in the water of the MOTIFs during OPEX.

Larves of worms were also found throughout the experiment, reaching maximum numbers in May. Eggs and veligers of gastropods were found from February to September. They were not identified at species level.

Addition of the oil at the end of May resulted in lower numbers of harpacticoid copepods in June, although numbers in all MOTIFs are low by then. Calanoid numbers were too low to detect differences between the different MOTIFs. The numbers of larvae of gastropods were lower in the contaminated MOTIFs in June, a peak was found in the contaminated MOTIFs in July, but not in the uncontaminated. Interpretation of these results is not very clear.

3.2.7.3 Bacteria

Directly after the start of the experiment a very large bloom of bacteria developed in all MOTIFs reaching a maximum of more than 10^8 bacteria per ml around the middle of November (Fig. 49). This bloom collapsed (or was washed out of the system), and from the end of November 1981 to the end of the experiment bacterial numbers were between $1-8 \times 10^6$ per ml. These numbers are comparable to those found in the Wadden Sea.

From March to May numbers increased, probably as a reaction to the increased production of the phytoplankton and the phytobenthos. The addition of the oil mousse did not result in detectable effects on the total numbers of bacteria in the water.

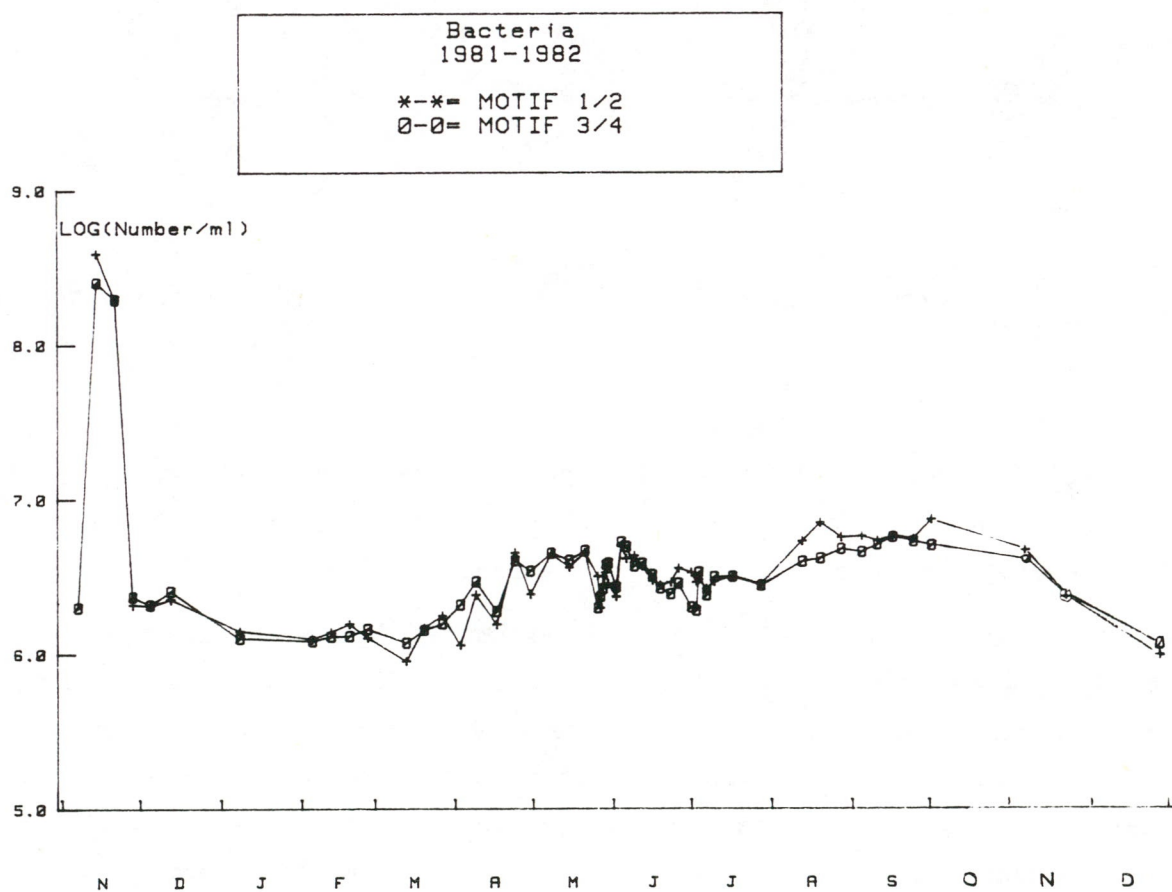


Fig. 49 Numbers of bacteria in the water of the different MOTIFs during OPEX.

4. DISCUSSION

4.1 DEVELOPMENT IN NON-CONTAMINATED MOTIFS

4.1.1 Development in replicate MOTIFS

From October 1981 to May 1982 the four MOTIFS were treated similarly. The variations in the different biotic and abiotic parameters between the four systems were generally small. After May, two duplicate MOTIFS were sampled until December 1982, and again most of the parameters measured developed very similarly in the duplicate systems. Some parameters showed sometimes large differences between replicate MOTIFS, but it is not clear at the moment if these occasional differences were only caused by sampling errors.

Information available, although limited due to derivation from only a limited number of MOTIFS, indicates that the variation between replicate MOTIFS is relatively small. This means that larger differences between uncontaminated MOTIFS can be attributed to the addition of the oil.

4.1.2 Comparison of the MOTIFS with the field situation

In this section the development of the MOTIFS is compared with what is known of natural tidal flats in the Wadden Sea from literature and current research in the area by the investigating institutes.

It is interesting to compare the development in all 4 MOTIFS up to end of May, and that later in the uncontaminated MOTIFS 1 and 2 with that of a natural mud-flat ecosystem.

During autumn the animal populations in the MOTIFS were still negligible, consisting of small numbers of herbivores (zooplankton *Pygospio* and *Hydrobia*), some carnivorous *Nereis* and unknown but probably less numerous meiobenthos organisms. Some primary production by microalgae, and the decaying organic material and associated micro-organisms will have provided a sufficient source of food. *Nereis* could have survived by predation on *Pygospio* and oligochaetes, or by the ingestion of plant material, which latter possibility has been observed frequently in the indoor NIOZ tidal flat systems. Early in November *Macoma* was introduced, and most individuals rapidly dug into the sediment. The characteristic grazing marks on the surface layer of the sediment indicated that normal feeding activity was taking place. A period of frosty weather in December did not have any negative

effects. Cockles and lugworms were introduced early in January, after the end of the December frosts. These animals burrowed rapidly into the sediment.

The extending syphos of *Cerastoderma* and the numerous casts of *Arenicola* suggested normal behaviour of these organisms. A second and more severe period of cold weather, causing a solid ice cover in January was also withstood well by the systems. Anoxic conditions and consequent mortalities did not occur. After the thaw, the mud flat surfaces appeared healthy.

In February periwinkles were introduced; those settled easily on the walls of the MOTIFs. They showed normal mobile and feeding activity and did not aggregate above the high water level, a phenomenon which points to unfavourable conditions.

As in the normal situation on a natural tidal flat, microphytobenthos developed in the MOTIFs in spring. Enhanced feeding activities of the macrobenthos organisms, and particularly the bioturbative action of *Arenicola* prevented the development of algal mats on the mudflat; the mudflats thus maintained their open character. Of considerable importance was the fact that a floating film of small particles (sandgrains, microalgae and bacteria) did not develop during high tide. Such layers caused difficulties in the indoor systems of the NIOZ, but wind action probably prevented their formation in the outdoor MOTIFs.

The adult *Macomas* and *Cerastodermas* showed only slight growth, in comparison with that under natural conditions. The growth of the shells of the mussels was also slightly less than expected, but the growth and decline of the individual ADW of the different bivalves was similar to that under natural conditions. Growth of the lugworm, however, was normal and well comparable to growth in natural populations. The settling of very small numbers of juvenil lugworms was observed in May. The mortalities of adult bivalves and lugworms were small and of similar magnitude to mortality in the natural populations in the Wadden Sea.

Growth of filamentous algae started in MOTIFs 1 and 2 in June. Local growth of macro-algae is also a normal phenomenon on natural flats during the summer. The epigrowth of macro-algae in the MOTIFs was occasionally thinned out by hand, but by July the stands of macro-algae vanished almost completely, mainly due to the grazing activities of the numerically dense *Gammarus* populations. This early disappearance contrasts with the wane of these algae in the Wadden Sea, which are only removed by autumn gales.

The settlement of larval and juveniles of all kind of benthic organisms during the summer is of outstanding importance in natural mudflat ecosystems. Particularly the so-called summer annuals reach numerically high densities. Considerable stocking with larvae and juveniles, either imported from outside or derived from reproduction of the parent stocks, was observed in the MOTIFs. Settlement by juvenile mussel, cockle, *Macoma* and lugworm was, however, small compared to that of natural tidal flats. This can be explained by the long and relatively complicated route of the suppletion water to the MOTIFs, during which the majority of the larvae and juveniles will have settled elsewhere. Part of the juveniles may also have been damaged by the centrifugal pumps; juvenile shrimps and shore crabs, certainly juvenile flatfishes (plaice and flounder) and gobiids will therefore have a very low chance of entering the MOTIFs. Some dense clusters of mussel spat were, however, found in the suppletion water piping.

On the other hand, large numbers of the summer annuals *Hydrobia*, *Corophium*, *Gammarus* and various oligochaete species occurred. Moreover *Nereis* and *Litorina* showed a considerable reproduction. During late summer and autumn the majority of the smaller macrobenthos species present in MOTIFs 1 and 2 declined, as normally occurs in natural flats. Towards the end of 1982 MOTIFs 1 and 2 were still in a healthy condition.

Other important processes in natural functioning tidal flat systems such as bioturbation, mineralization and primary production were well developed in the MOTIFs. In contrast to the natural situation in the Wadden Sea, the water was relatively clear most of the time.

The turbulence in the MOTIFs is much less than that under natural conditions. This lower turbidity probably resulted in a relatively higher primary production.

Although some parameters indicated differences between development of the MOTIFs and that of natural tidal flat systems (in particular the intensity of processes such as growth and larval settlement of the bivalves) it appears that the development of the MOTIFs was generally similar to that of the natural system in many ways.

4.2 FATE OF THE ADDED OIL MOUSSE

The various analytical techniques used in this project are evaluated by Compaan (1982). Fluorescence appeared to be a useful screening technique, high performance liquid chromatography (HPLC) and gas chromatography (GC) gave valuable additional information on the fate of various oil compounds. Infra-red (IR) analysis did not add much to the information gathered with the other techniques.

Visual inspection indicated that no floating oil compounds were transported through the pump generating the tides in the MOTIFs. During the first week a maximum of 8% of the added oil mousse stuck to the walls. After this week a maximum of around 1% (mostly weathered oil) remained on the walls. In October - November only 14 mg oil was found per gram of the thin algal layer on the walls. These results indicate that the walls of the MOTIFs did not seriously disturb the experiment.

Short-chained alkanes disappeared rapidly from the mousse, probably as a result of evaporation. Conditions for evaporation were very favourable because of the high temperatures (up to 36°C in the mousse!).

A detailed review of the literature on the possible fate of oil in intertidal systems will not be given here. Excellent reviews have been published recently (Bergman, 1982; Connell and Miller, 1980; CNEXO, 1981). A number of points which may be important to the authorities responsible for combating possible oil after a spill in the Wadden Sea area will, however, be made.

A most important result on the fate of the added oil is that part of the added oil was buried in the sediment by bioturbation activities of *Arenicola marina*. During the 14h of ebb that the oil laid on the tidal flat, 2-5 l (i.e. 10-30% of the added oil) was taken up into the sediment. Most of the oil remained in the top 2-5 cm, but oil concentrations were also higher than in the controls at greater depths after a few months.

Samples for detailed analysis of the vertical distribution of the oil were taken at locations where high concentrations of oil could be seen on the surface, because it was expected that analytical problems would be smallest here.

Bioturbation activity was also most limited at these locations, so that concentrations in deeper layers remained lower than at less contaminated locations in the MOTIFs. In the absence of bioturbation activity, the oil at the most contaminated locations will remain in the system longer and expose orga-

nisms in the surrounding longer to increased oil concentrations.

The tidal flats in the MOTIFs were of a non-percolating type. Based on experience from limited field experiments in the western Wadden Sea (de Boer, 1976, 1977), and on the assumption that percolation is prerequisite for oil entry into sediments (Bergman, 1982) it was expected that the oil layer would be completely lifted from the sediment by the rising tide. Fresh sediment deposited by bioturbation on top of the mousse during ebb partially prevented this oil lift off. This finding is confirmed by work in other intertidal areas (e.g. Bergman, 1982; Little et al., 1980; Fricke et al., 1981). In subtidal systems large part of oil can also be transported to the sediments (e.g. Davies et al., 1981; Linden et al., 1979; Elmgren and Fritzsche, 1982).

The burial of part of the oil in the sediment is important from an ecological point of view, because oil components can be delivered to the intertidal and overlying water from this pool long after that the floating oil has been removed. These higher oil concentrations in the water for very long periods give rise to prolonged higher concentrations in organisms which accumulate oil components and to long-term effects of the oil spill on the ecosystem.

From this experiment, only results from oil analysis in *Mytilus edulis* are available; these show intense accumulation of oil components during the experiment (c.f. Farrington et al., 1982). All other macroinvertebrates (and other organisms) in the MOTIFs, however, probably also accumulated oil (Connell and Miller, 1980; Neff and Anderson, 1981; Bergman, 1983).

There are no reasons to expect that the mechanism by which the oil was buried in the sediments in the MOTIFs does not occur also in the real field situation. It is therefore probable that, in particular during periods of intense bioturbation activities on tidal mud flats (spring, summer) significant amount of oil can be retained by the sediments, even after very short exposure periods. The experiments of De Boer (1976, 1977) were probably too limited to show this effect (too short, oil layer too thick, inadequate sampling technique).

4.3 EFFECTS OF THE ADDED OIL MOUSSE

Oil spills must be regarded as a normal consequence of the immense oil consumption in the industrialized part of the world. The larger spills, such as those of the Torrey Canyon, Amoco, Cadiz, Metula and Argo Merchant, are well known, and have resulted in losses of 50.000 to 220.000 tons of oil each. All these huge spills had grave environmental consequences (Chassé, 1978; CNEXO, 1981; Bergman, 1982). Spills of much lower quantities, however, occur daily (RWS, 1982; Kerkhof et al., 1981) and these smaller spills may also have serious consequences (e.g. Tsesis spill; Linden et al., 1979; Elmgren and Fritschen, 1982).

In the MOTIFs a moderate oil spill, in which the systems were directly exposed for a relatively short period (1 mm of oil on the flat for 1 week) was simulated. This one week direct exposure to the oil mousse resulted in several acute effects on organisms in the systems. A summary of effects observed directly following the spill (May-June) and after a longer time period (July-December) is given in Table 12.

A detailed comparison with the huge amount of literature data on effects of oil compounds on organisms and ecosystems, will not be given here since excellent reviews are available (e.g. Neff and Anderson, 1981; CNEXO, 1981; Bergman, 1982); only some general trends will be highlighted.

The acute short term effect observed might have partly been predicted from laboratory toxicity tests. Stimulation and inhibition of phytoplankton, effects on the benthic crustaceans *Corophium* and *Gammarus* and decrease in ash-free dry weights of all the bivalve species following the spill are not surprising. In fact, some of the observed short-term effects on the biota were less serious than expected. Acute mortality in the macrofauna was only observed with *Macoma balthica*, and in this case it seems probable that the presence of oil alone was not the cause of the mortality. Temperatures under the oil mousse were up to 5°C higher than those in the controls (cf. Erasmus and de Villiers, 1982). Moreover, *M. balthica* in the controls avoided higher surface temperature by burrowing more deeply (up to ca 10 cm) in the sediment. This escape reaction was not observed in the contaminated MOTIFs, *M. balthica* being found at shallow depths (ca 2-3 cm) and increased mortality was probably due to a combination of oil and high temperatures.

Table 12 Summary of short-term (May-June) and long-term (July-December) effects of the oil addition on different organisms in the MOTIFs.
+ = higher value of the parameter in the contaminated MOTIFs than in the uncontaminated MOTIFs; - = lower value in the contaminated MOTIFs; 0 = no detectable difference between contaminated and uncontaminated MOTIFs.

organisms	parameter	short term	long-term
phytobenthos	chlorophyll	+	0
phytobenthos	O ₂ -production per chlorophyll	(-?)	-
bacteria benthos	Electron Transport System	+	+
<u>Meiofauna</u>			
Oligochaet 1	numbers	0	0/-
Paranais/Capitella	numbers	0	+
Nematods	numbers	-	-
Harpacticoids	numbers	+	0
<u>Macrofauna</u>			
Mytilus edulis	total biomass	-	0
Mytilus edulis	numbers	0	0
Mytilus edulis	ash-free dry weight	-	0(?)
Cerastoderma edule	numbers	0	-
Cerastoderma edule	ash-free dry weight	-	+
Macoma balthica	numbers	-	-
Macoma balthica	ash-free dry weight	-	-
Mya arenaria	numbers	(?)	-
Hydrobia ulvae	numbers	0	-
Littorina littorea	reproduction	(?)	-
Arenicola marina	numbers	0	0(-*)
Arenicola marina	ash-free dry weight	0	0
Nereis diversicolor	numbers	0(?)	+
Nereis diversicolor	ash-free dry weight	0	0
Pygospio elegans	numbers	0	0
Heteromastus filiformis	numbers	0	-
Corophium volutator	numbers	-	-
Gammarus spp.	numbers	-	not present
<u>Zooplankton and fish</u>			
copepods	numbers	0	0
larvae	numbers	-	(-?)
Gobius minutus	numbers	(?)	-
<u>Phytoplankton</u>			
	chlorophyll	-	+
	species composition	different	different
	O ₂ -production per chlorophyll	+/-	-
Bacteria water	numbers	0	0

* Arenicola was absent at locations with the highest oil concentrations.

Model ecosystem and field experiments are very useful to show such indirect effects caused by interactions between organisms and abiotic and/or biotic factors. A beautiful example of the influence of such interactions is given by Pearson et al. (1981), who showed that littleneck clams were more easily consumed by Dungenese crabs in oil sediments than in non-contaminated controls.

The increased numbers of harpacticoid copepods found in the contaminated MOTIFs is probably also an indirect effect of this sort, food conditions being better because less is consumed by competitors.

The long-term effects are much more important from an ecological point of view than the short-term effects. As a result of the burial of 10-30% of the added oil, dissolved compounds were delivered to the overlying water long after the floating oil mousse had been removed. Water concentrations of oil components were at least 10-20 times higher than in the controls for the rest of the experiment. Concentrations in the interstitial water were probably even higher. These higher concentrations caused various long-term effects on the biota in the MOTIFs (see Table 12).

In addition to the effects on organisms already living in the systems (e.g. mortality of *Cerastoderma edule* starting three months after most of the oil had been removed, inhibition of *Littorina littorea* reproduction) significant differences were also recorded between the settlement of larvae in uncontaminated and contaminated MOTIFs. The worms *Capitella capitata*, *Paranais* sp. and *Nereis diversicolor* were found in higher numbers in the contaminated systems than in the uncontaminated systems; on the other hand numbers of nematodes, *Heteromastus filiformis*, *Hydrobia ulvae* were much lower in the contaminated systems. These organisms were not introduced into the MOTIFs deliberately, but larvae were introduced with the suppletion water from the Wadden Sea or perhaps produced in the MOTIFs by small populations of organisms introduced with the sediment at the start of the experiment.

The mortality of several species and the large differences in settlement success resulted in large differences in the species composition of the macrofauna community between the contaminated and the uncontaminated MOTIFs. These differences persisted to the end of the experiment and were clearly visible (e.g. Fig. 50).

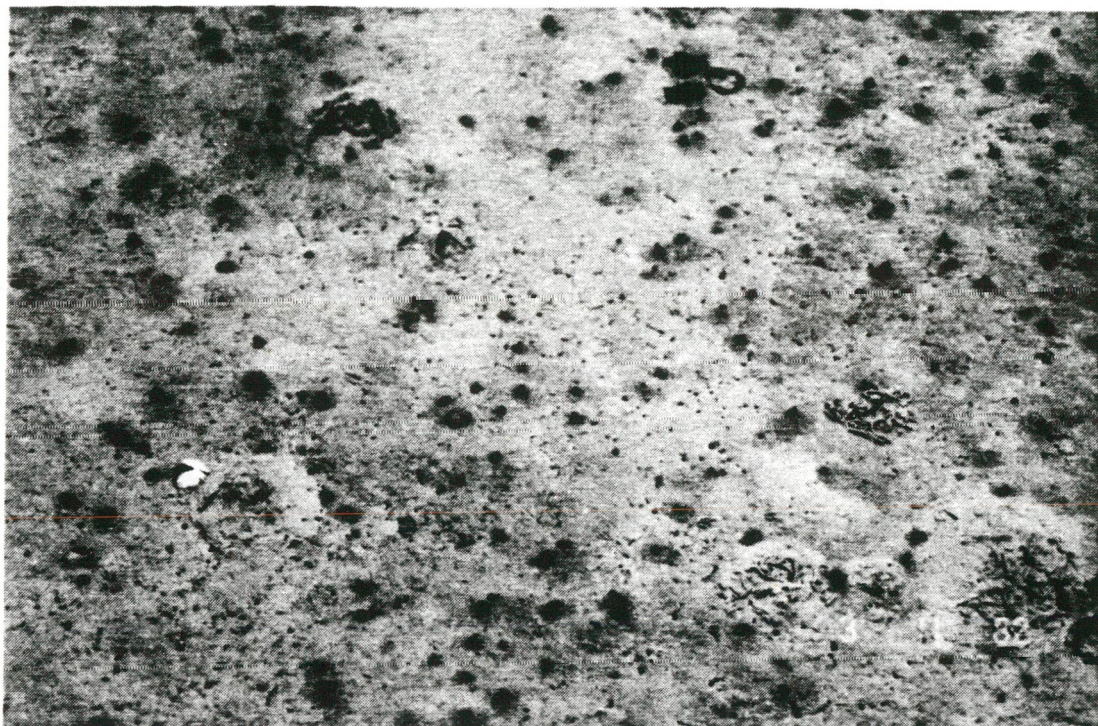


Fig. 50a

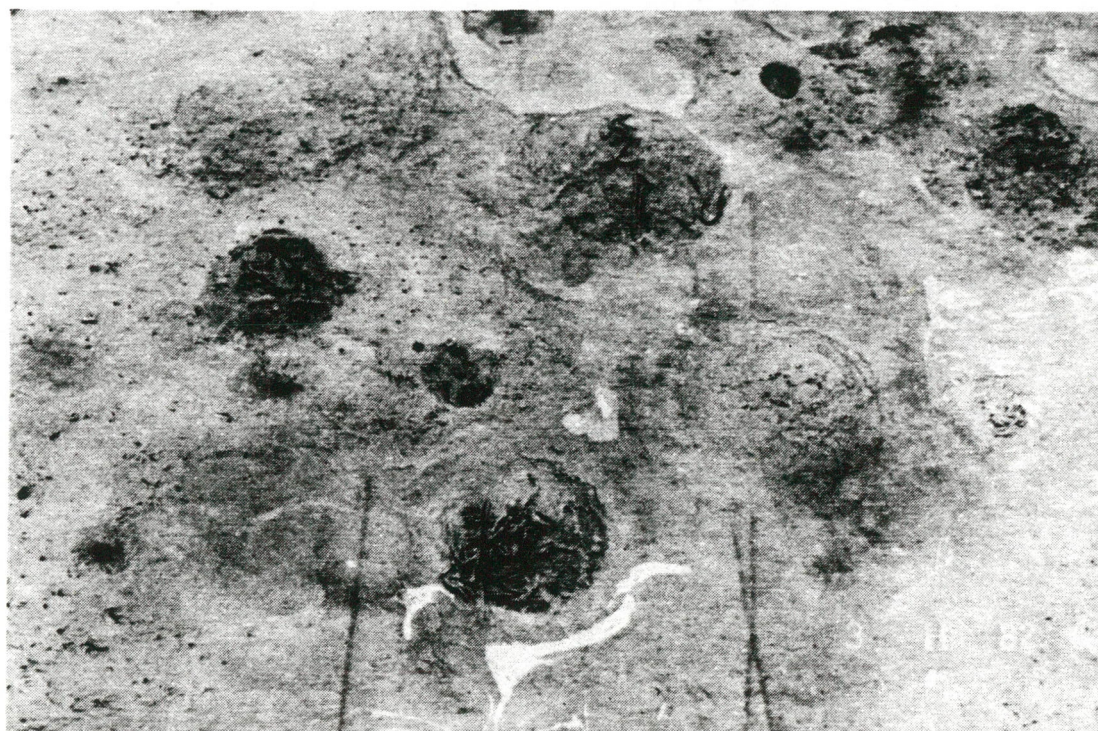


Fig. 50b

Fig. 50 An overview of the sediment surface in (a) the uncontaminated and (b) the contaminated MOTIFs in November 1982. Pits and casts of *Arenicola marina* are clearly visible, the many small holes are formed mainly by *Nereis diversicolor*, the many black casts in the uncontaminated sediment are formed by *Heteromastus filiformis*. A surface film of floating oil can be observed in the contaminated MOTIF.

The structure of the community in the water-column was also changed by the addition of the oil, and long-term effects were also recorded here, even though the water was permanently refreshed with water from the Wadden Sea. The average chlorophyll values were higher in the contaminated MOTIFs, in particular during August-October and blooms of diatoms were found in the uncontaminated, but not in the contaminated, MOTIFs. Small flagellates were relatively more important in the contaminated MOTIFs. These effects are probably related to differences in grazing and/or mineralization processes; direct inhibition of several species can also not be excluded, because primary production per unit of chlorophyll was found to remain lower than in the uncontaminated MOTIFs during the whole period in which these measurements were made (May-September). A change in the species composition of the phytoplankton in favour of small flagellates has been found by many authors (e.g. Parsons et al., 1976; Lee et al., 1978) and an increase in small flagellates relative to other groups was also observed in other model plankton ecosystems experiments using the water soluble fraction of the oil applied in the MOTIFs (Scholten, 1983).

Not all the effects in Table 12 can be regarded as direct effects of the added oil. For example, the higher biomass of *Nereis diversicolor* and the higher phytoplankton biomass in the contaminated systems are probably indirect effects resulting from changed interactions between the different species (less competition for space and food for *Nereis*, less predation on the phytoplankton) and not from direct stimulation of these organisms by oil compounds. In fact primary productivity by the phytoplankton was probably inhibited for a very long time by the oil, since oxygen production per unit of chlorophyll was lower than in the uncontaminated systems for as long as these measurements were made (May-September). Primary production only appeared to be directly stimulated immediately after the addition of the oil.

A similar initial stimulation, followed by an inhibition of primary productivity was also found in a model plankton ecosystem experiment with the water soluble fraction of the oil used in the MOTIFs (Scholten, 1983). Stimulation of phytoplankton activity by low concentrations of oil has been found by many authors (e.g. Parsons et al., 1976; Bergman, 1983) and is probably a general reaction of many organisms to pollutants (Stebbing, 1981). An increase of phytoplankton biomass as a result of decreased grazing pressure was also observed after oil spills in the field (Lannergren, 1978; Linden et al., 1979).

The experiment showed that, although the MOTIFs were only directly exposed for a relatively short period to most of the oil, long term effects (direct or indirect) could be observed as a result of burial of part of the oil into the sediments. At the end of the experiment (December 1982), 7 months after the removal of the floating oil mousse, a large amount of oil was still present in the sediments of the contaminated MOTIFs.

Information gathered after spills in the field indicates that the removal of oil from this type of tidal flat system can take many years (Bergman, 1982). Effects on the development of the biota in following seasons are therefore probable, or at least cannot be excluded. In the real field situation, recovery of an area will depend on the dimensions of the contaminated area and on the possibilities for recolonization from refuge areas. The structure of the ecosystem in the contaminated MOTIFs had changed considerably, and changes in following seasons will probably be influenced by this changed structure. Unfortunately the experiment had to be terminated in December 1982. At that time no indications were present that a recovery of the system, back to the situation in the uncontaminated system, had already started.

5. RECOMMENDATIONS FOR FURTHER RESEARCH

The feasibility study reported here was planned as a basis for a project to study the optimum methods to assess and combat oil pollution resulting from an oil spill in or near the Wadden Sea or other similar estuarine areas. The unexpected results on the fate of the oil in the MOTIFs stress the importance of an efficient oil combat strategy.

The one year study showed that it is possible to build and maintain model ecosystems representing a tidal mud flat ecosystem. It has also been shown that the physico-chemical behaviour of oil in the MOTIFs, is quite similar to that under natural conditions, and that significant effects could be observed after the single oil addition in May. The development in the four replicate MOTIFs up to May was very similar and the differences between contaminated and uncontaminated MOTIFs were generally so large that they could be regarded as effects of the oil addition.

Before the MOTIFs can be used for other pollution studies (oil or other chemicals) the "natural" variations between identically treated MOTIFs should be known. The study of this natural variation in untreated MOTIFs is the main aim of a project to be carried out in 8 MOTIFs in 1983. Within the framework of this project technical improvements will be made and sampling methodology (e.g. for *Arenicola* and *Cerastoderma*) will be further optimized.

If the variation between identically treated MOTIFs is small or predictable, then a model ecosystem is available which can be used as a efficient tool to simulate various problems concerning the fate and effects of pollutants or changes in the management of tidal flat areas.

An overview of possible applications of the MOTIFs to environmental problems is given by Kuiper et al. (1983). In relation to problems concerning the threat to the Wadden Sea by oil pollution, scientific information on the means to combat an oil spill in or near the area is needed: the MOTIFs can be used to gather this information. The effects of treating an oil spill with detergents or other chemicals can be studied, as can the consequences of mechanical removal of oil from tidal flats using heavy material such as bulldozers, mechanical shovels, tractors, etc.

Although the influence of the walls of the MOTIFs seemed relatively small, this influence can be minimized by using removable walls (e.g. plastic

sheeting) during the periods of direct exposure to floating oil.

In addition to the threat caused by the risk of oil spills from damaged tankers, rigs or pipelines, the chronic exposure to low levels of oil compounds and other chemicals (resulting from land run-off, rivers, dumpings of process water, chemical wastes, dredging sludge, etc.) may be even more important. The MOTIFs can be a useful tool, forming a link between laboratory and field studies, to investigate the short- and long-term fate and effects of chemicals in sediment-water ecosystems.

6. CONCLUSIONS

6.1 DEVELOPMENT OF THE MOTIFs

The question: "is it possible to build an experimental ecosystem in large outdoor basins, which is sufficiently representative for a natural tidal flat system typical for the Wadden Sea?", could be answered affirmatively.

The structure of the community living in the MOTIFs was similar to that from natural tidal flat ecosystems in many aspects, such as species composition and species population densities. The functioning of the system was also comparable in many ways, such as rates of primary production, mineralization, bioturbation, growth and decline of many organisms. Growth and larval settlement of the bivalves *M. balthica* and *C. edule* appeared to be somewhat smaller than under natural conditions.

It appeared to be possible to sample the systems without disturbing the systems too much by this sampling.

Four MOTIFs were similarly treated from October 1981 to May 1982. The variation in the various biotic and abiotic parameters between the four systems were small. After May, two duplicate MOTIFs were sampled until December 1982 and most parameters measured developed very similarly in these duplicate systems. Some parameters sometimes showed large differences between MOTIFs, but it is not clear at the moment if these occasional differences were caused by sampling errors. Information available shows that the variation between replicate MOTIFs seems acceptably small. This point will be studied in more detail in a separate project in 1983.

6.2 FATE OF THE ADDED OIL MOUSSE

Mineral oil could be added to the MOTIFs in such a way that its physico-chemical behaviour was similar to that under field conditions. The oil was added as a water-in-oil emulsion (mousse) and during the weeklong exposure it weathered as expected. Part of the oil (e.g. short-chained alkanes and other volatile compounds) evaporated. The wall effect was relatively small; less than 8% of the oil stuck to the walls during the first week; later, less than 1% of weathered oil could be found on the walls.

Contrary to expectations, part of the oil was buried in the sediments. This was mainly caused by bioturbation activity of organisms, such as *Arenicola*

marina in the sediment. The oil pool in the sediment delivered oil to the interstitial and overlying water long after the floating oil mousse had been removed from the MOTIFs, giving rise to oil concentrations (100-200 µg/l) in the water 10-20 times higher than those in the uncontaminated MOTIFs. Concentrations as high as 10,000-50,000 mg per gram dry sediment were found in the top layers of the sediment and concentrations were also higher than in the uncontaminated system in deeper layers (up to 30 cm).

The nature of the mechanism involved (bioturbation by very common species) means that it can be expected that part of the oil resulting from accidental spills in the field will also be buried on tidal flats. This is ecologically important, because prolonged exposure to oil compounds can result in long term effects in the ecosystem.

6.3 EFFECTS OF THE ADDED OIL MOUSSE

The addition of the oil resulted in several well defined effects on the organisms in the MOTIFs. Table 12 (p. 86) summarizes the short term (one month after the addition of the oil) and long term (up to six months) effects. Long term effects were probably caused by continuing exposure to increased concentrations of oil compounds, resulting from the continuing delivery of oil from the sediments.

The phytoplankton was stimulated during the first day of exposure to the oil. Thereafter phytoplankton and phytobenthos activity per unit of biomass was lower in the contaminated than in the uncontaminated systems. The biomass of the phytoplankton and phytobenthos in the contaminated MOTIFs was higher or similar to that in the uncontaminated system, indicating that grazing was less in the contaminated systems. The species composition of the phytoplankton changed as a result of the exposure to oil in favour of small flagellates.

Macoma balthica was the only macroinvertebrates which suffered acute mortality, probably as a result of exposure to oil in combination with higher temperatures in the contaminated systems than in the controls.

The settlement success of many species was negatively influenced by the presence of oil, and the reproduction of some organisms (e.g. *L. littorea*) was also inhibited by the single oil addition. Prolonged exposure to elevated oil concentrations resulted in long term mortality effects in many species (*Arenicola*, *Cerastoderma*, a.o.).

The direct and indirect effects of the oil addition resulted in large differences in structure and function between contaminated and uncontaminated MOTIFs. Some species, common in the controls, were hardly found in the contaminated systems, whilst other species which could stand the oil stress, increased in numbers in the contaminated MOTIFs, probably as a results of less competition for food and space.

It is concluded that these differences between contaminated and uncontaminated MOTIFs will probably remain in following seasons, because the oil content of the sediments was still very high at the end of the experiment. Unfortunately the experiment had to be terminated 7 months after the simulated oil spill.

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APPENDIX I

SUMMARY OF MEASUREMENTS NOT PRESENTED IN THE RESULTS SECTIONS

This appendix contains the results of various measurements made during OPEX, but not dealt with in the main report.

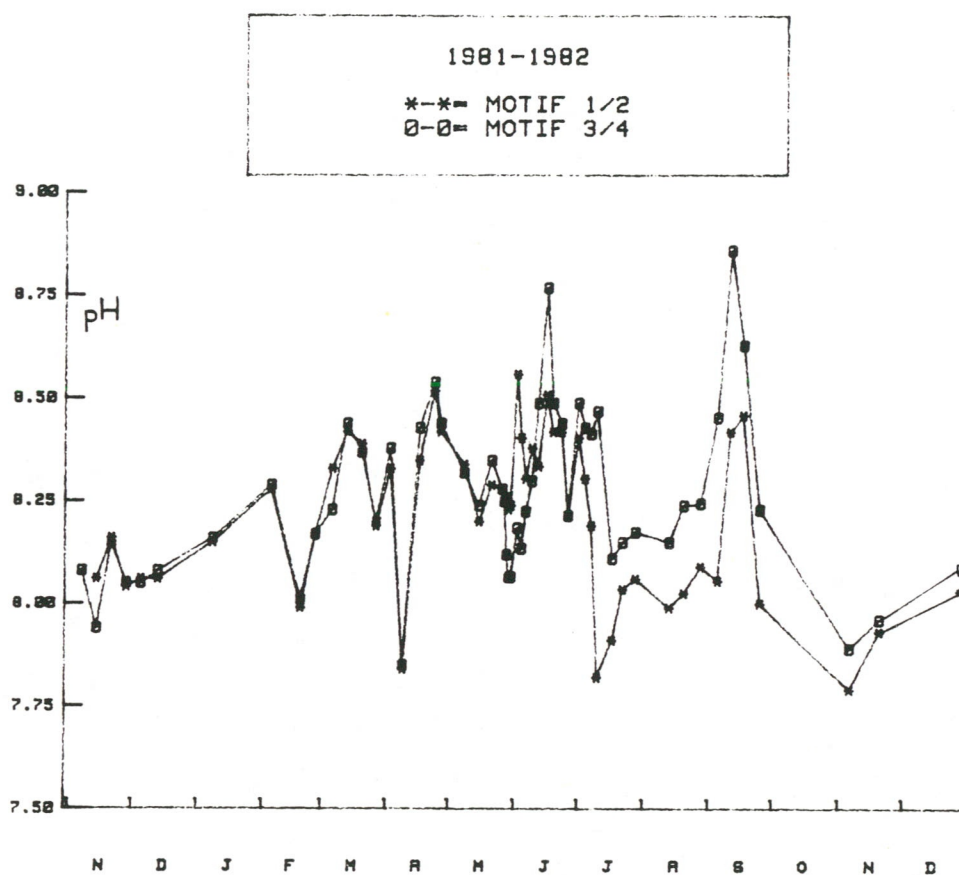


Fig. I.1 pH of the water in the different MOTIFs during OPEX.

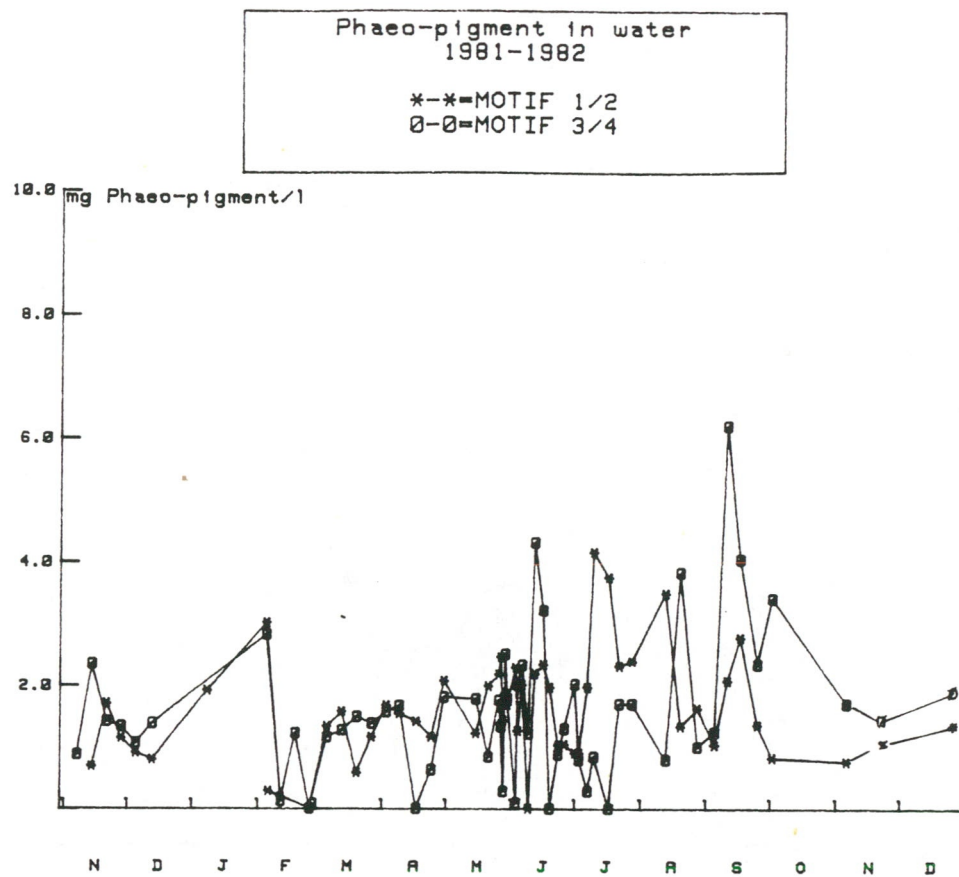


Fig. I.2 Concentrations of phaeopigments in the water of the different MOTIFs during OPEX.

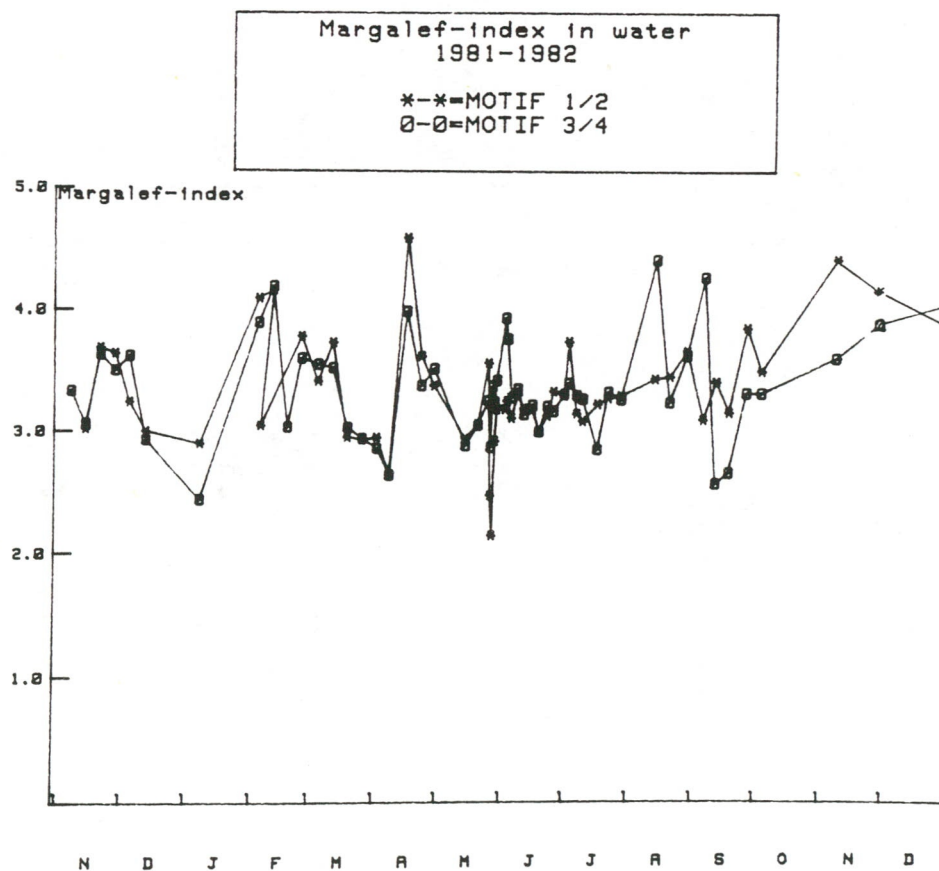


Fig. I.3 Pigment index in the water of the different MOTIFs during OPEX.

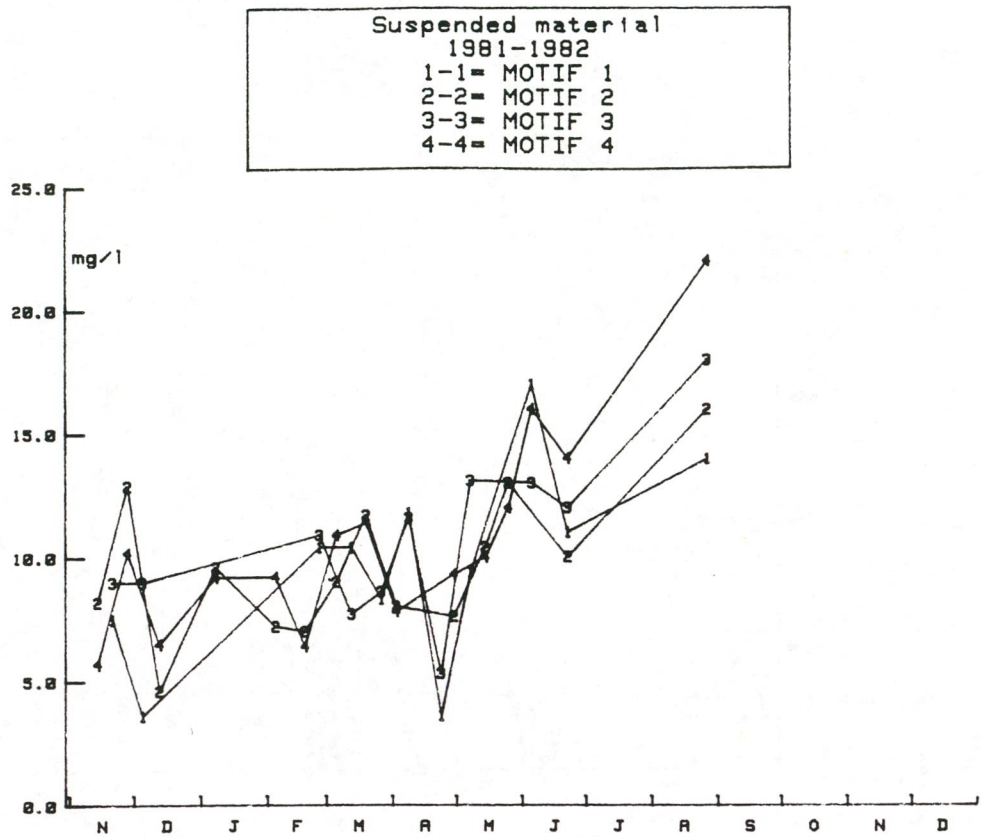


Fig. I.4 Concentrations of suspended matter in the water of the different MOTIFs during OPEX.

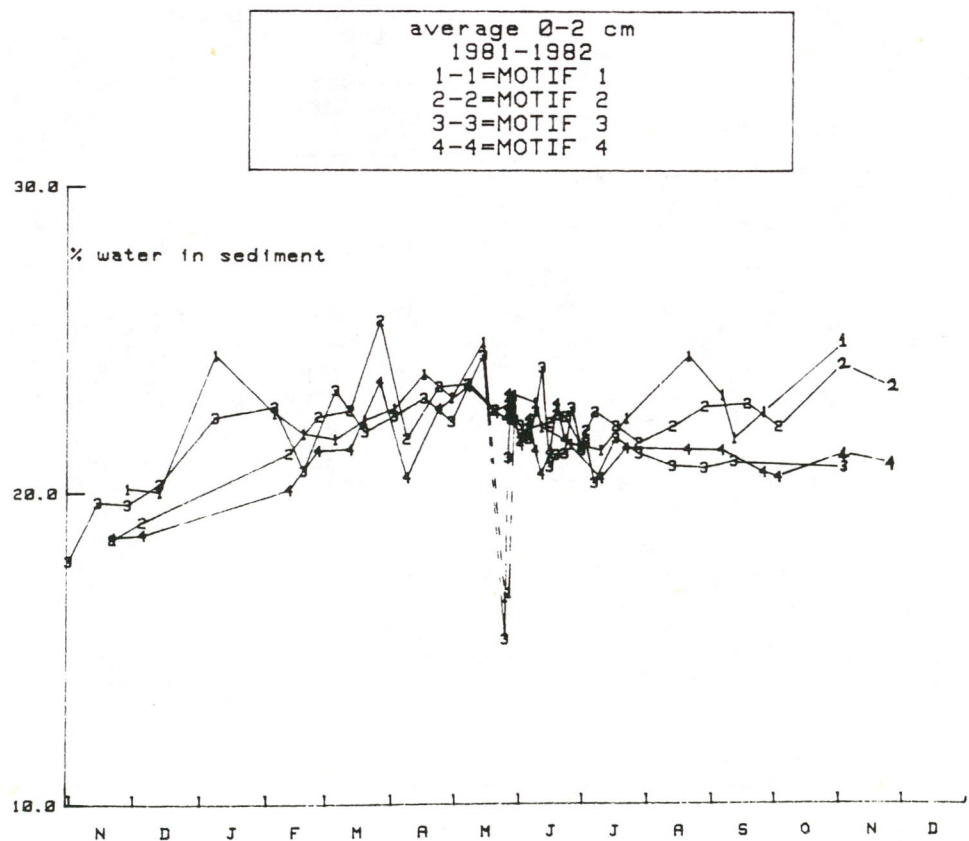


Fig. I.5 The water content of the upper 2 cm of the sediment in the different MOTIFs during OPEX.

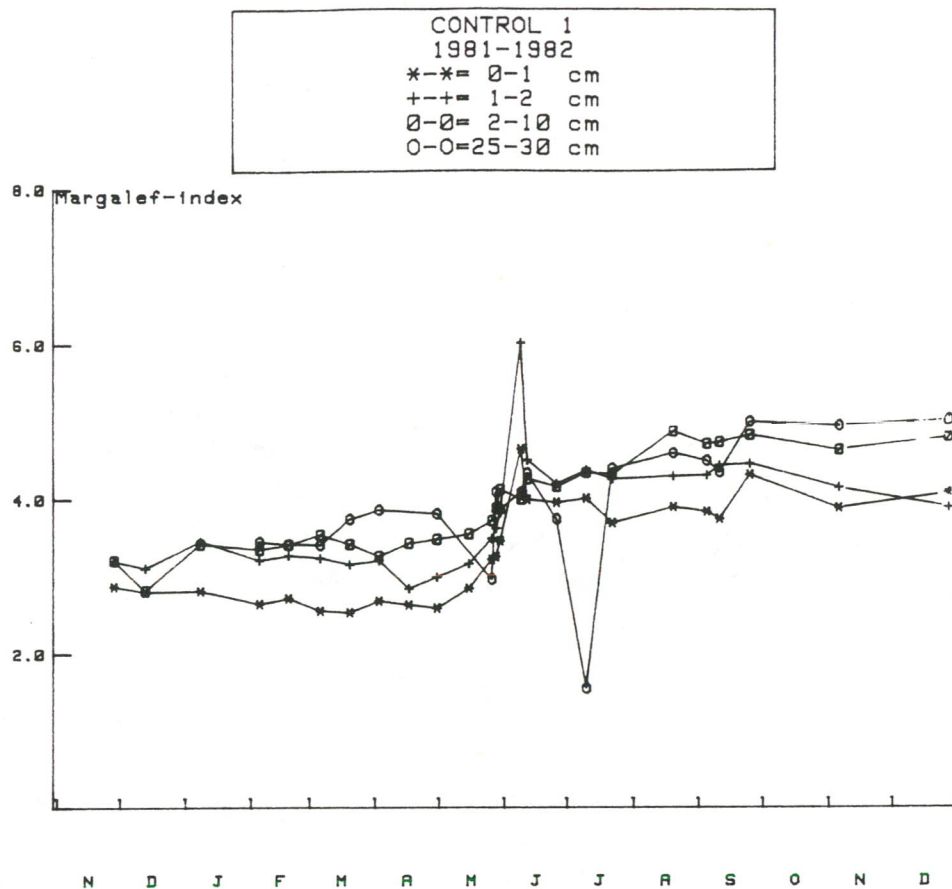


Fig. I.6 Pigment index at different depths in the sediment of MOTIF 1 during OPEX.

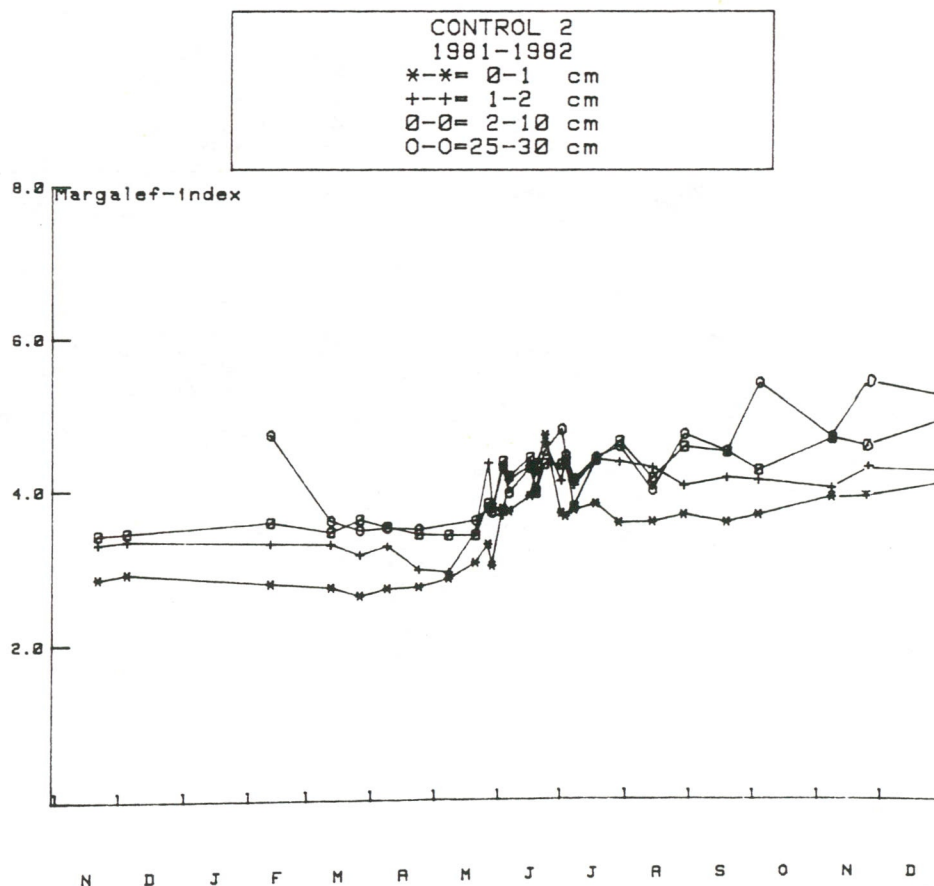


Fig. I.7 Pigment index at different depths in the sediment of MOTIF 2 during OPEX.

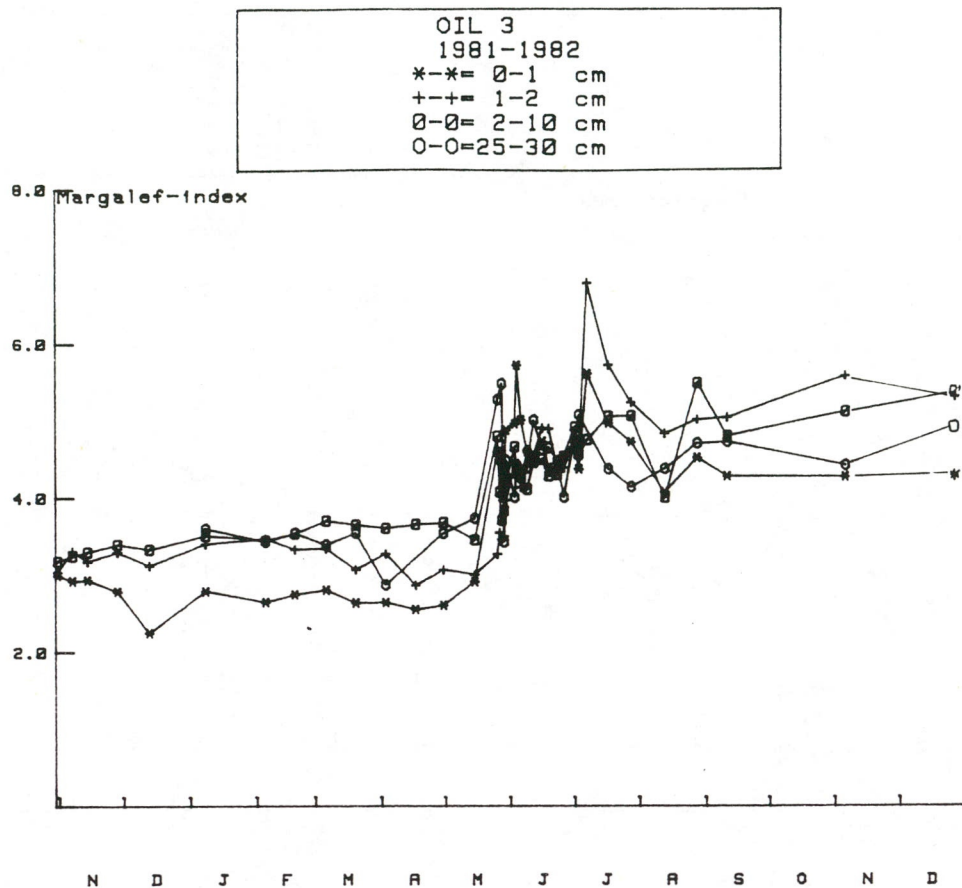


Fig. I.8 Pigment index at different depths in the sediment of MOTIF 3 during OPEX.

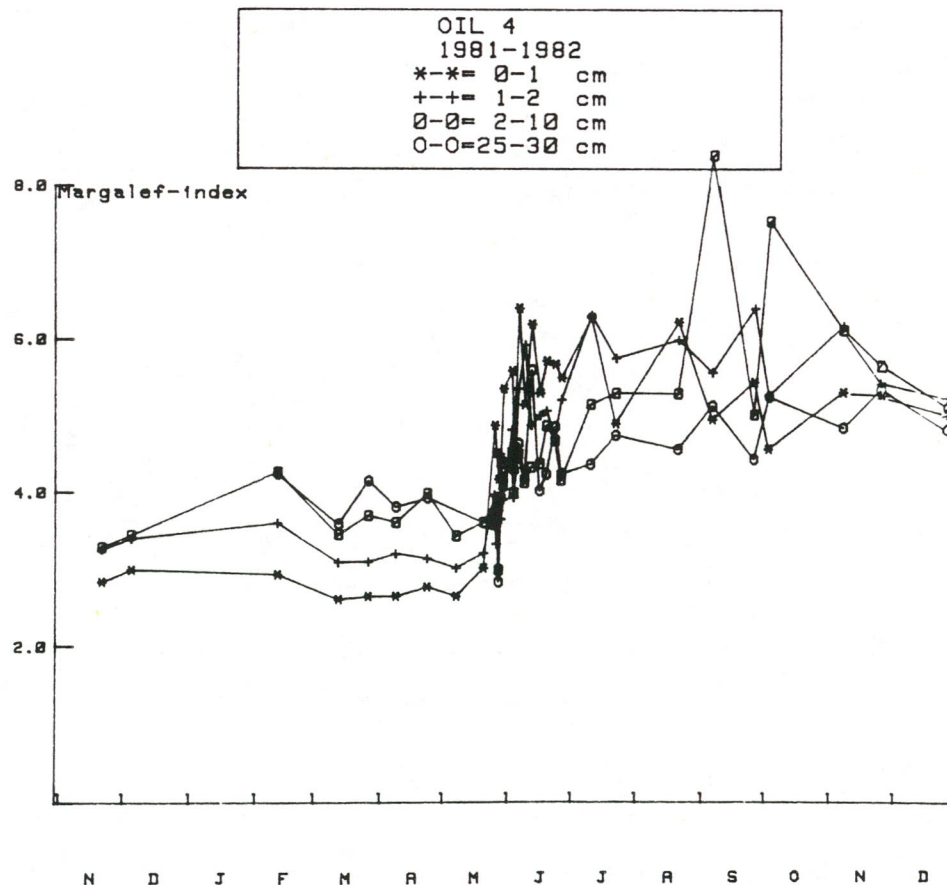


Fig. I.9 Pigment index at different depths in the sediment of MOTIF 4 during OPEX.

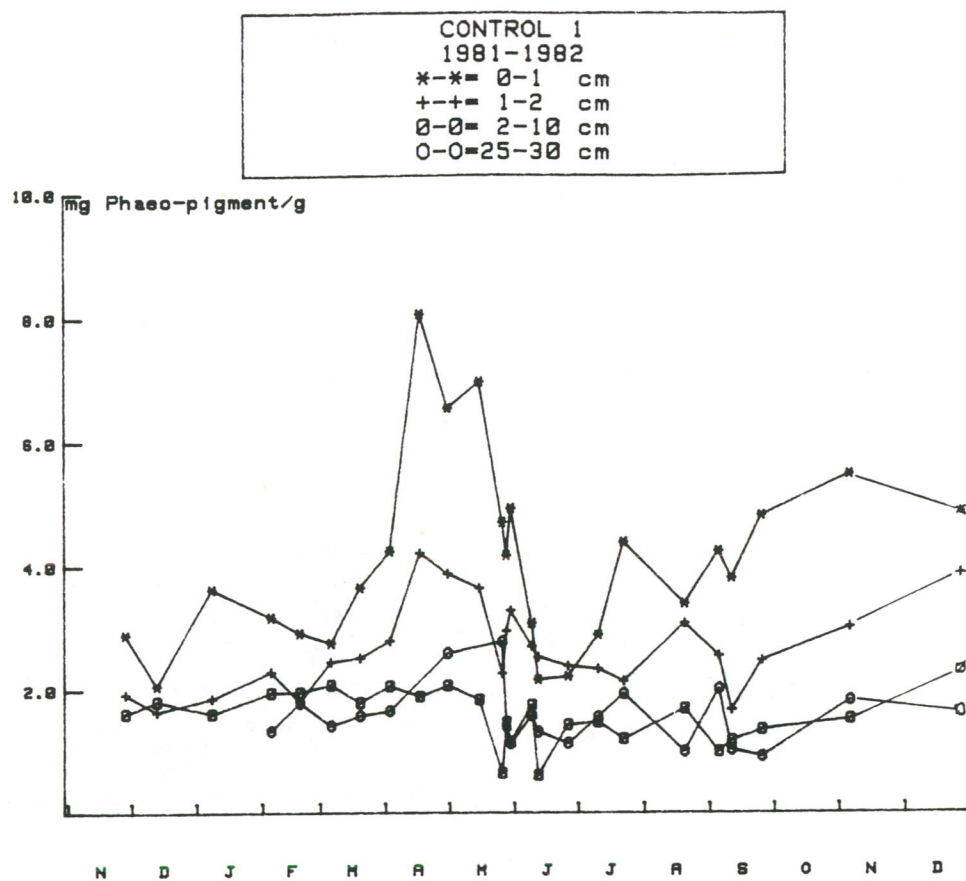


Fig. I.10 Concentrations of phaeopigments at different depths in the sediment of MOTIF 1 during OPEX.

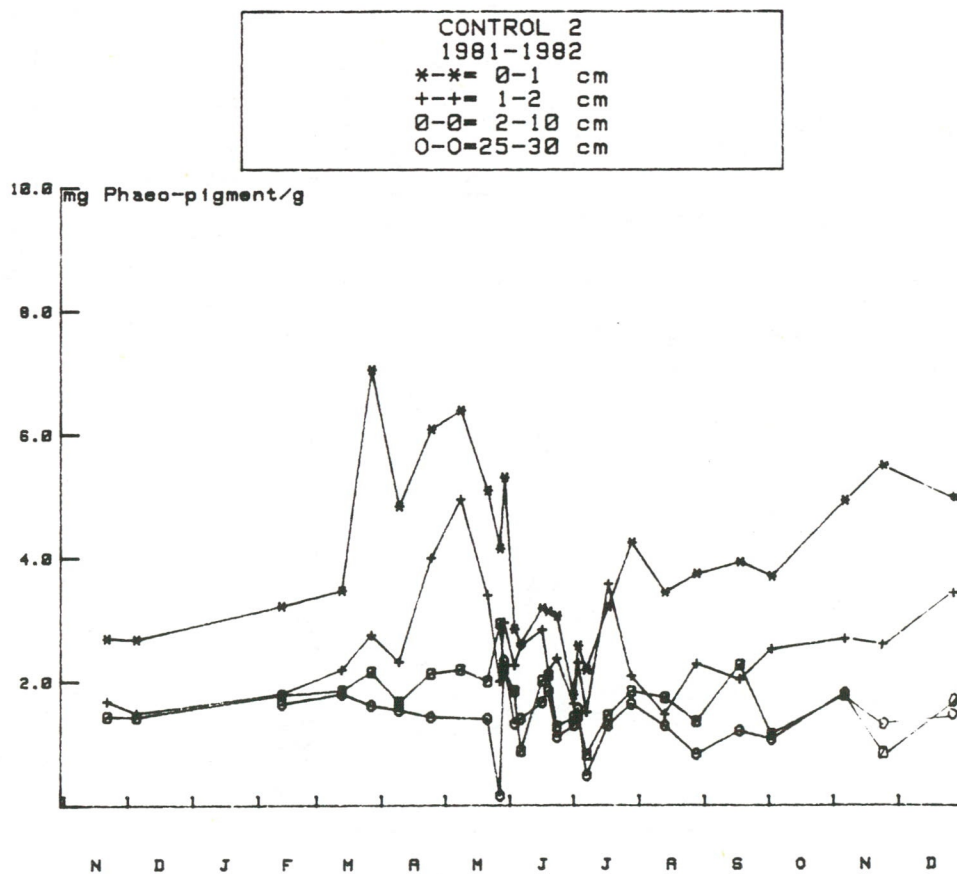


Fig. I.11 Concentrations of phaeopigments at different depths in the sediment of MOTIF 2 during OPEX.

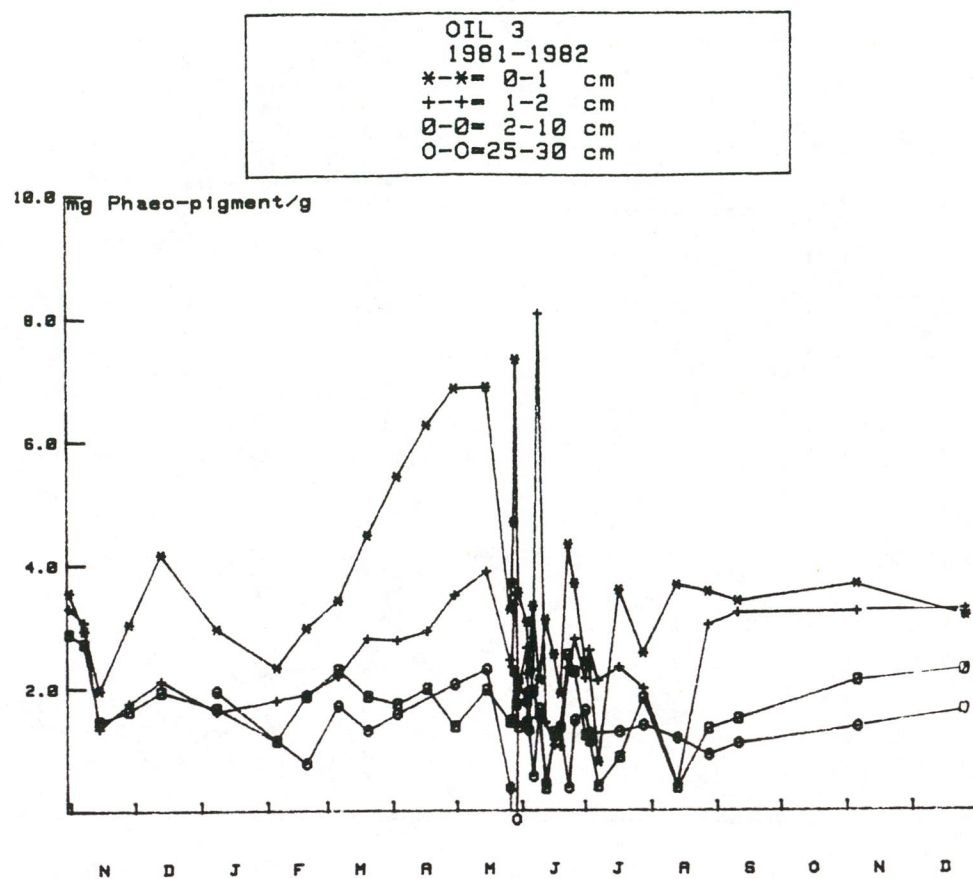


Fig. I.12 Concentrations of phaeopigments at different depths in the sediment of MOTIF 3 during OPEX.

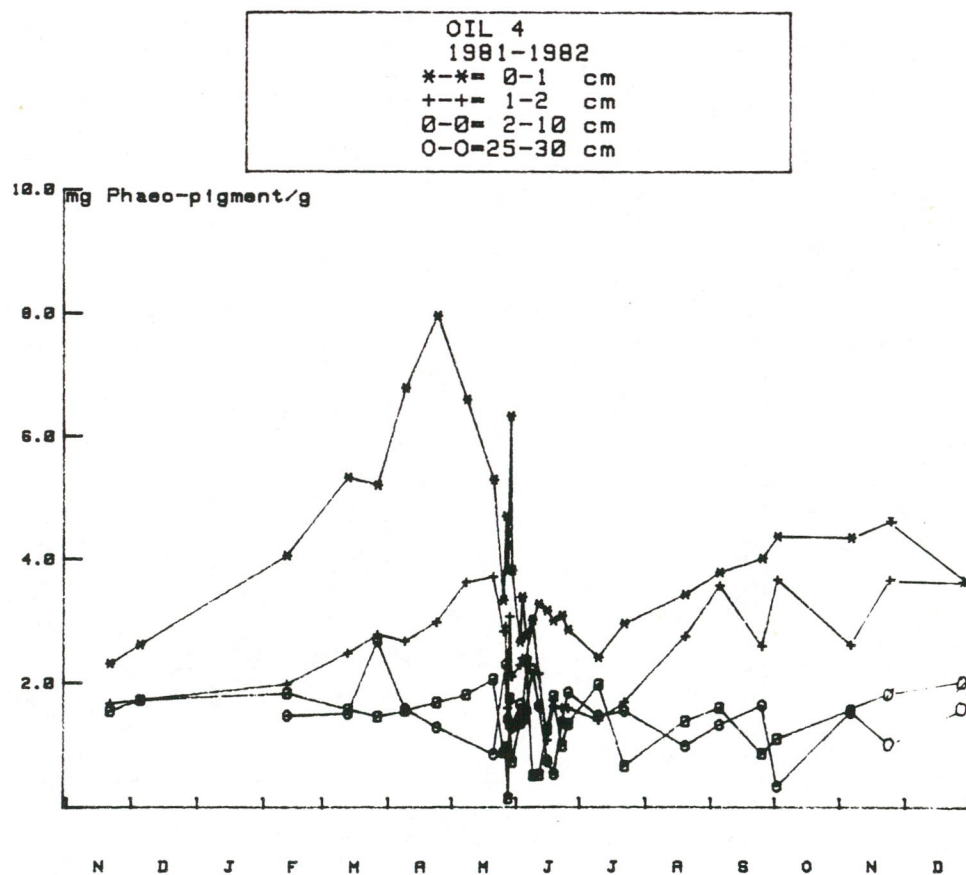


Fig. I.13 Concentrations of phaeopigments at different depths in the sediment of MOTIF 4 during OPEX.

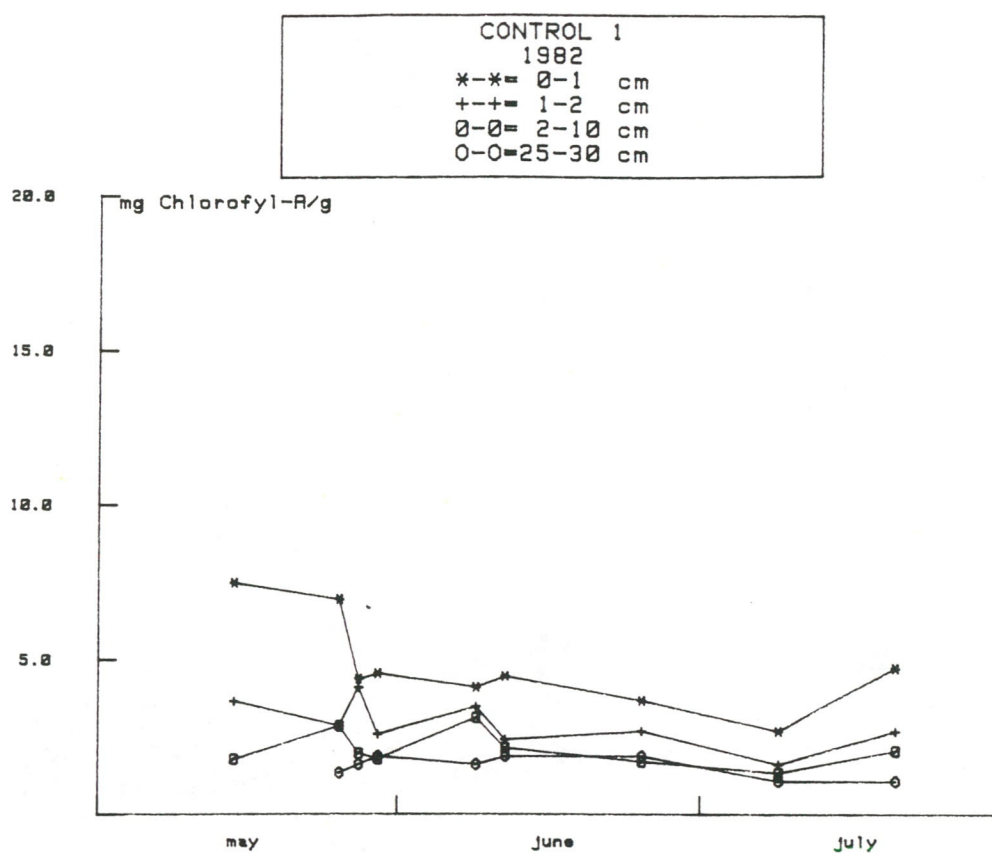


Fig. 1.14 Chlorophyll concentrations at different depths in the sediment of MOTIF 1 during the period May-July (OPEX).

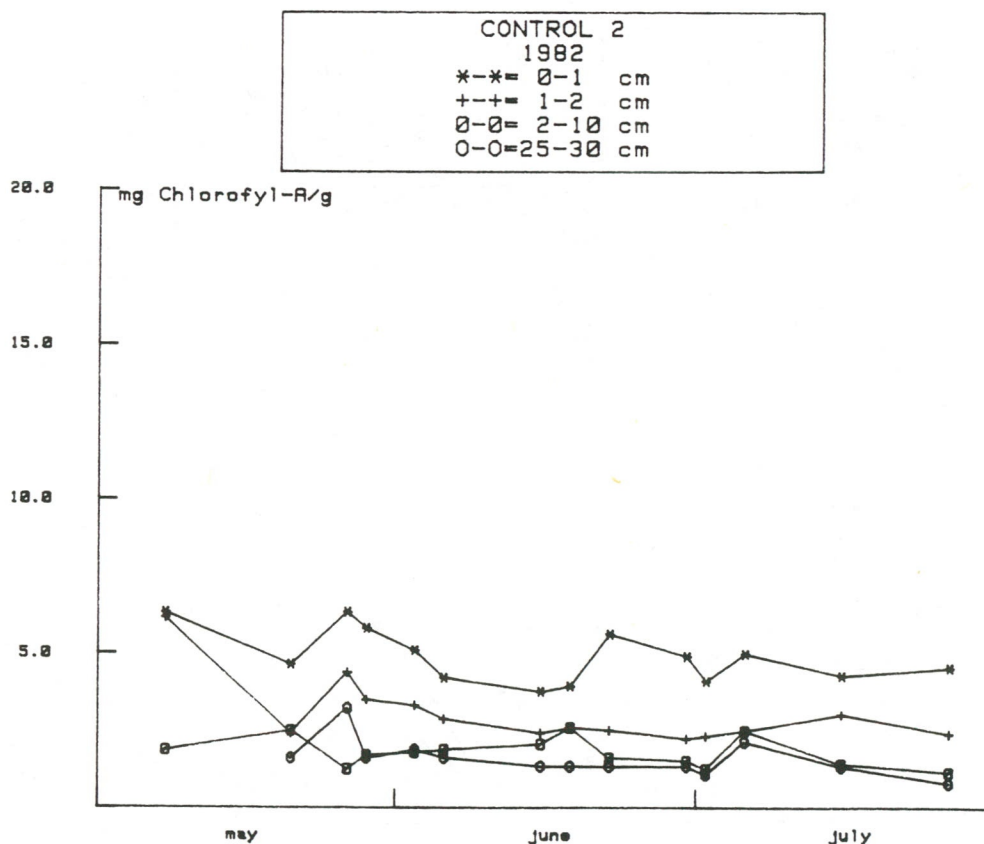


Fig. I.15 Chlorophyll concentrations at different depths in the sediment of MOTIF 2 during the period May-July (OPEX).

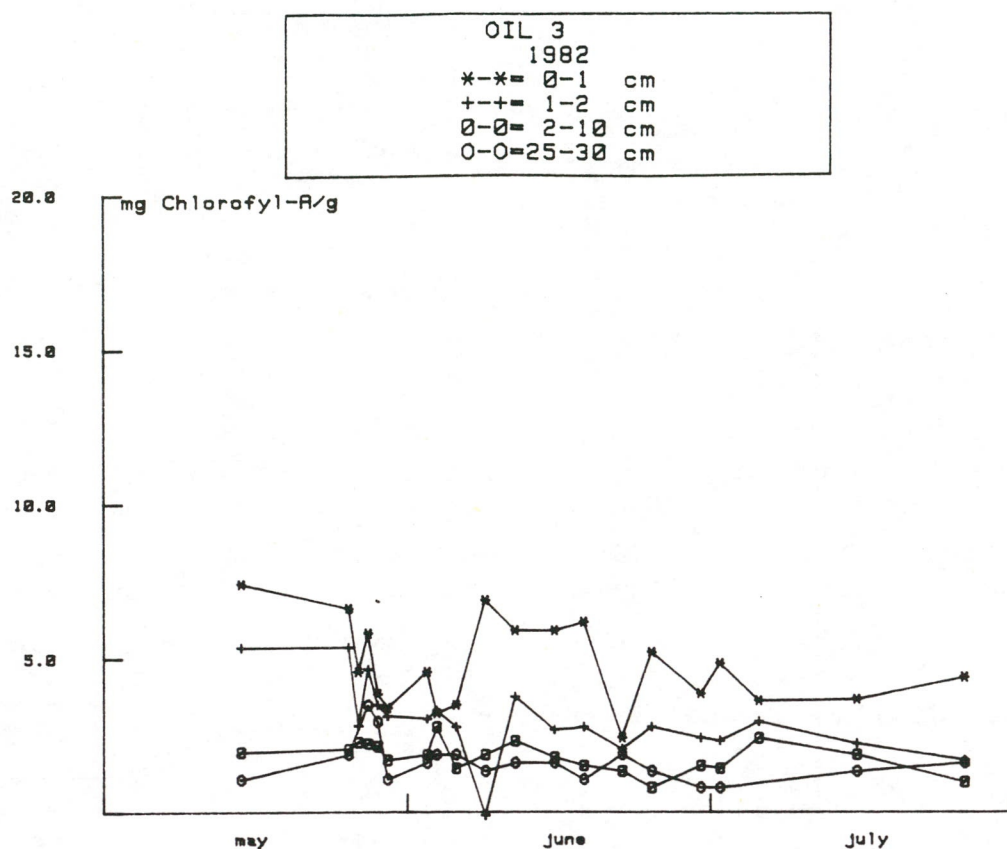


Fig. I.16 Chlorophyll concentrations at different depths in the sediment of MOTIF 3 during the period May-July (OPEX).

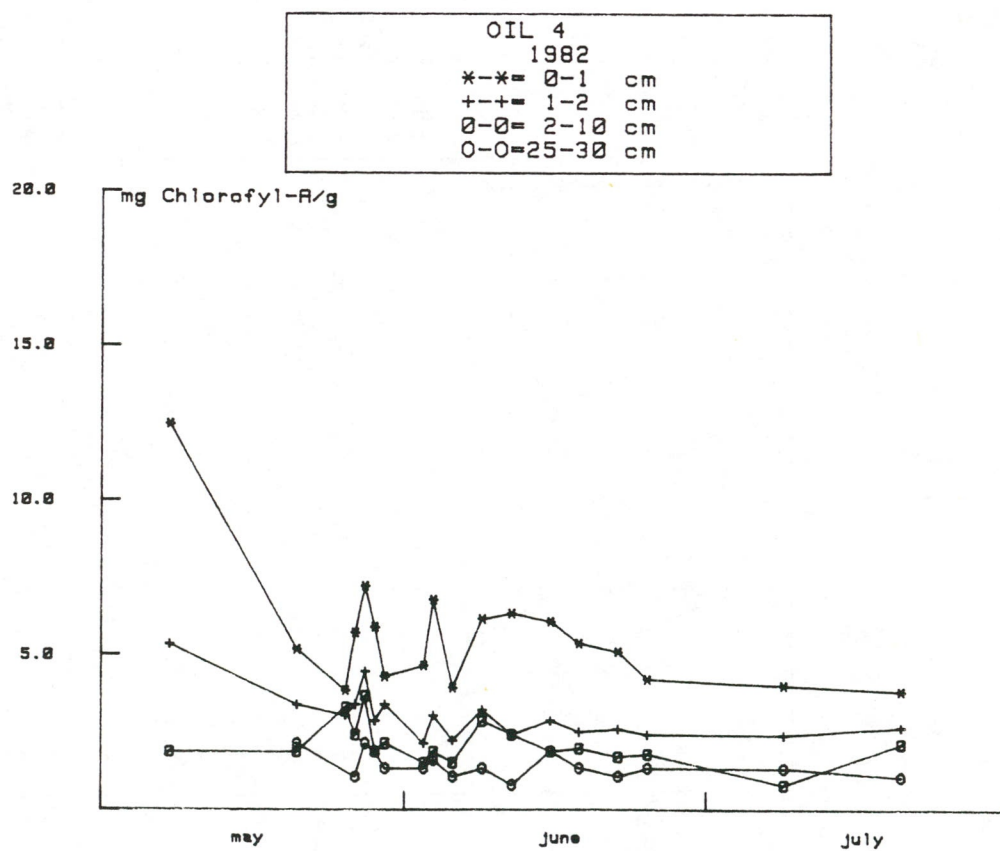


Fig. I.17 Chlorophyll concentrations at different depths in the sediment of MOTIF 4 during the period May-July (OPEX).

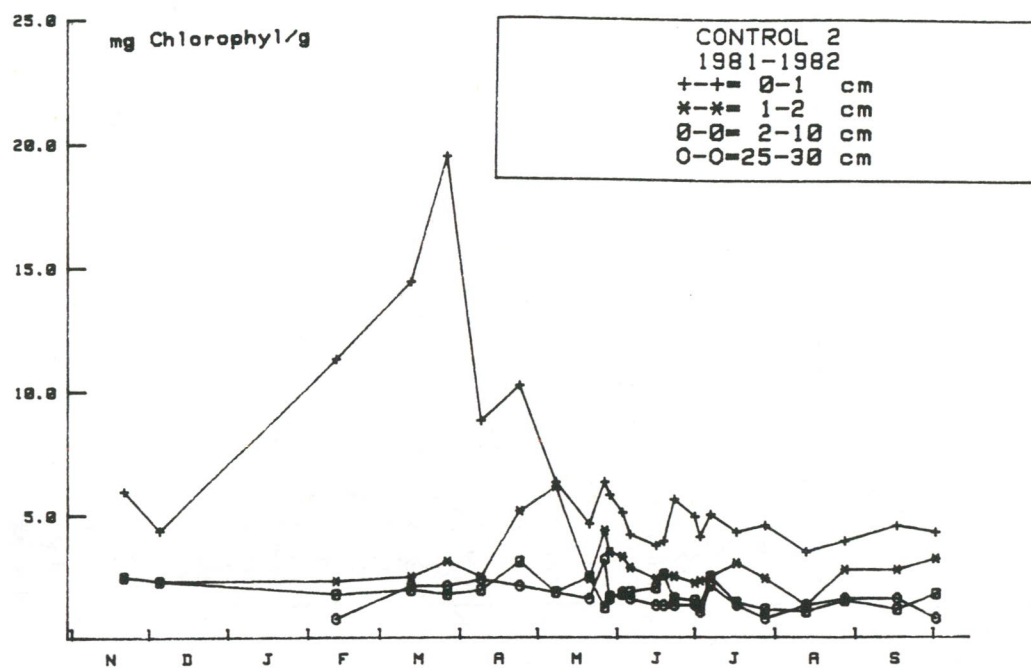


Fig. I.18 Chlorophyll concentrations at different depths in the sediment of MOTIF 2 during OPEX.

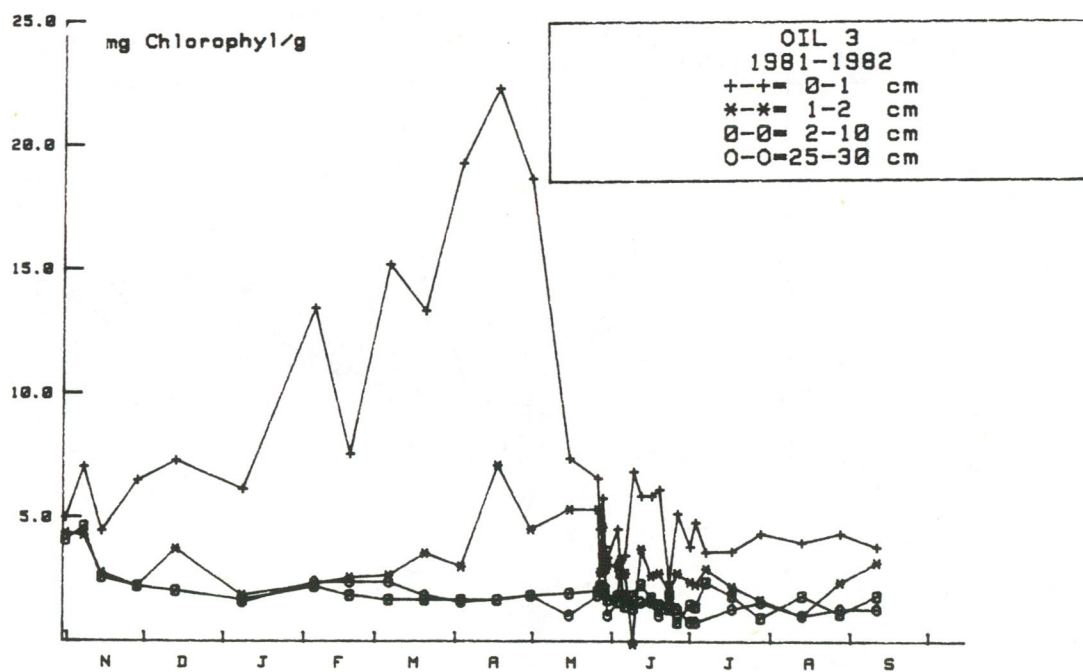


Fig. I.19 Chlorophyll concentrations at different depths in the sediment of MOTIF 3 during OPEX.

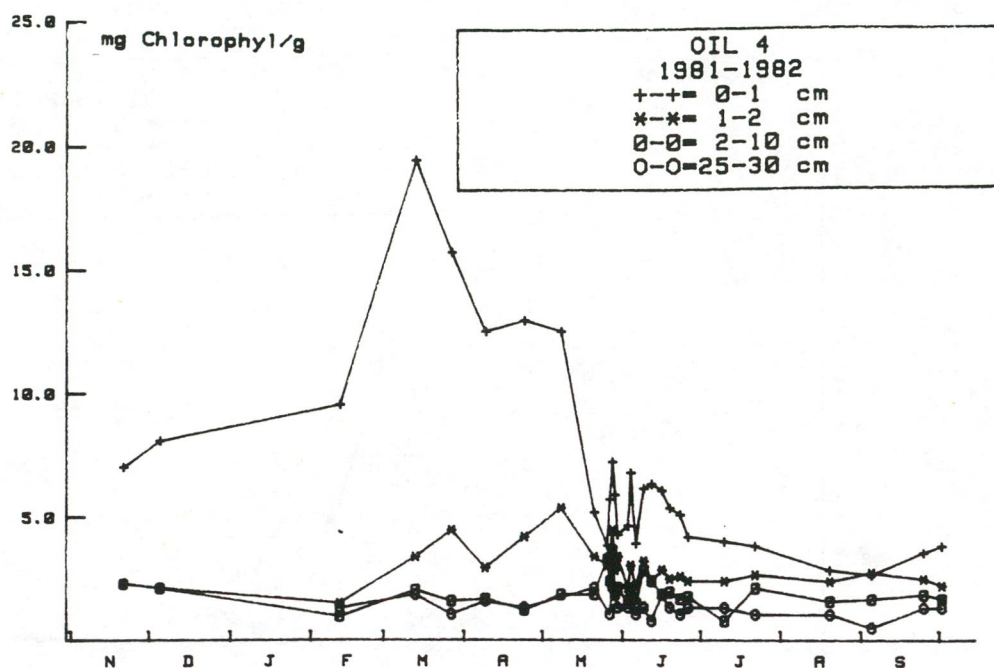


Fig. I.20 Chlorophyll concentrations at different depths in the sediment of MOTIF 4 during OPEX.

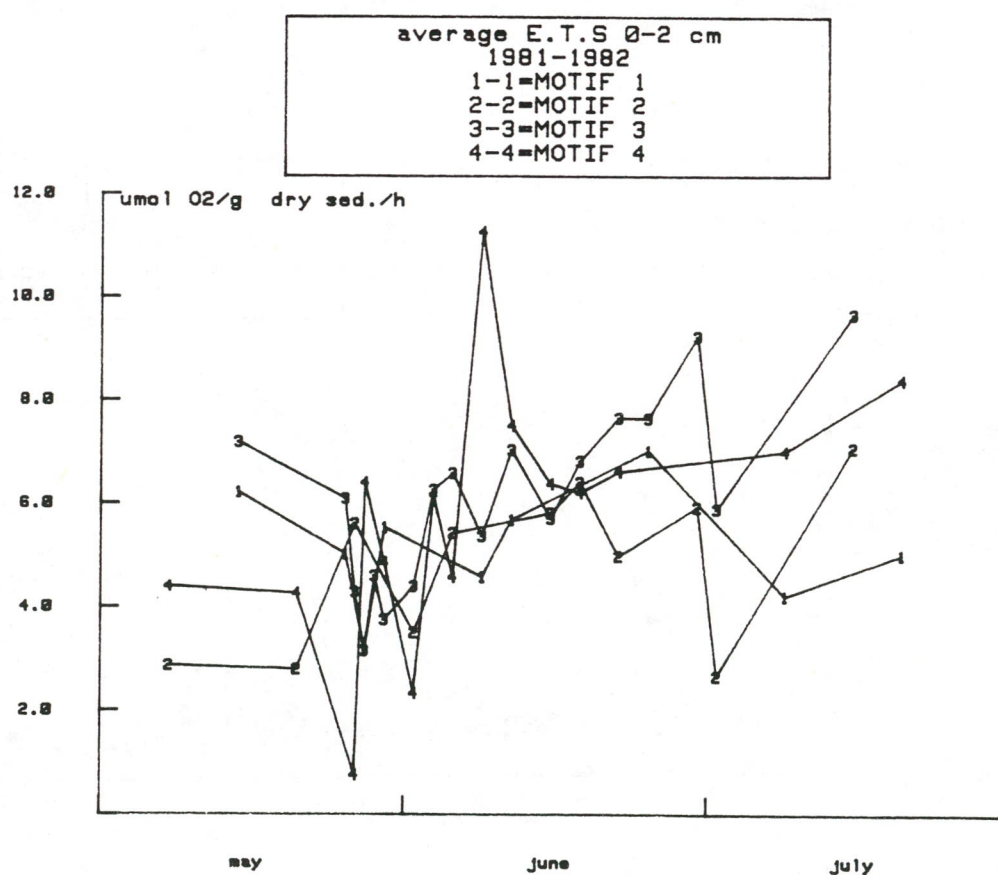


Fig. I.21 The average ETS (Electron Transport System) activity in the upper 2 cm of the sediment in the different MOTIFS in the period May-July (OPEX).

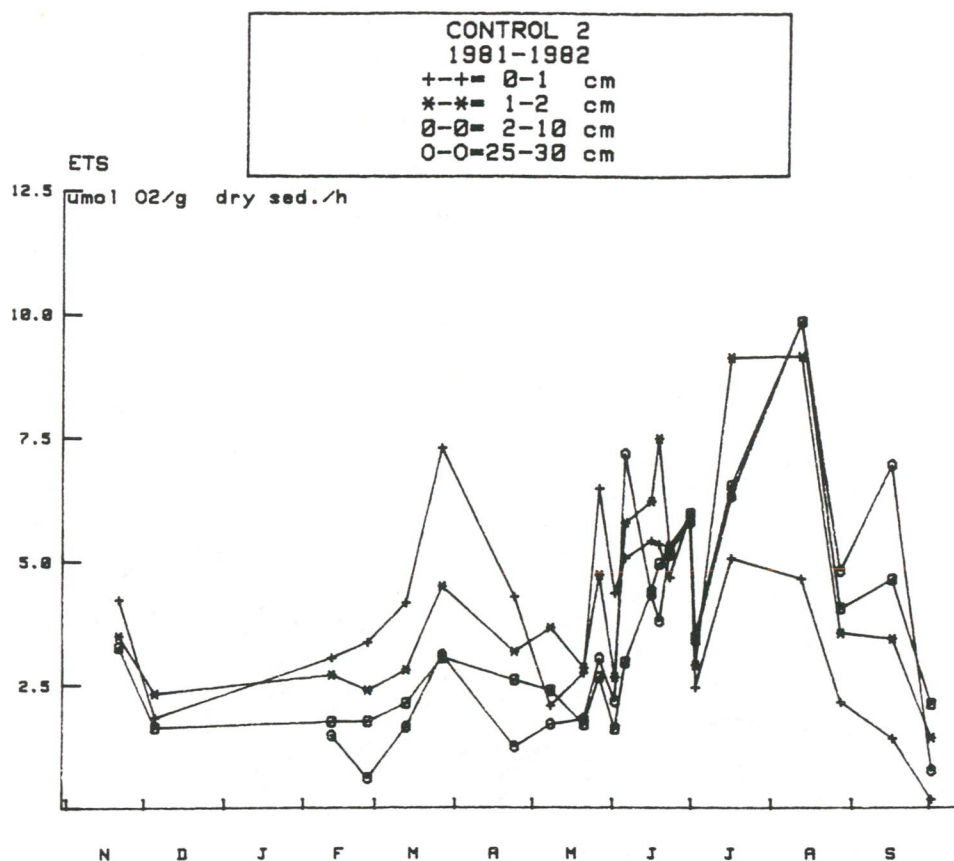


Fig. I.22 ETS activity at different depths in the sediment of MOTIF 2 during OPEX.

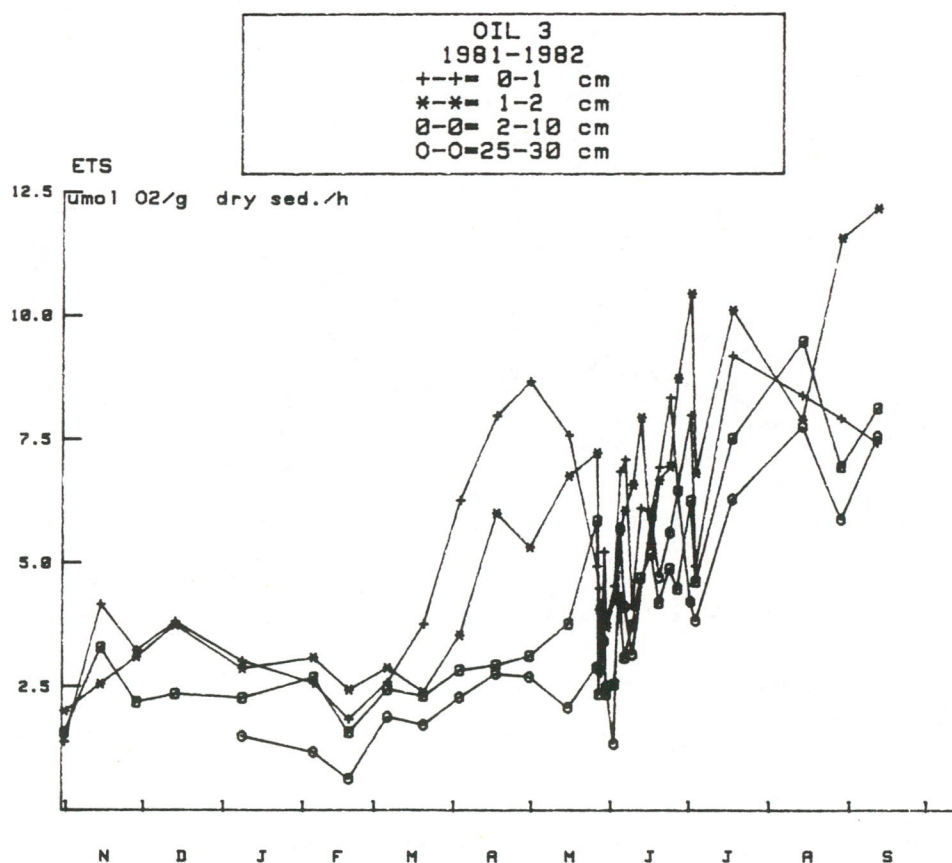


Fig. I.23 ETS activity at different depths in the sediment of MOTIF 3 during OPEX.

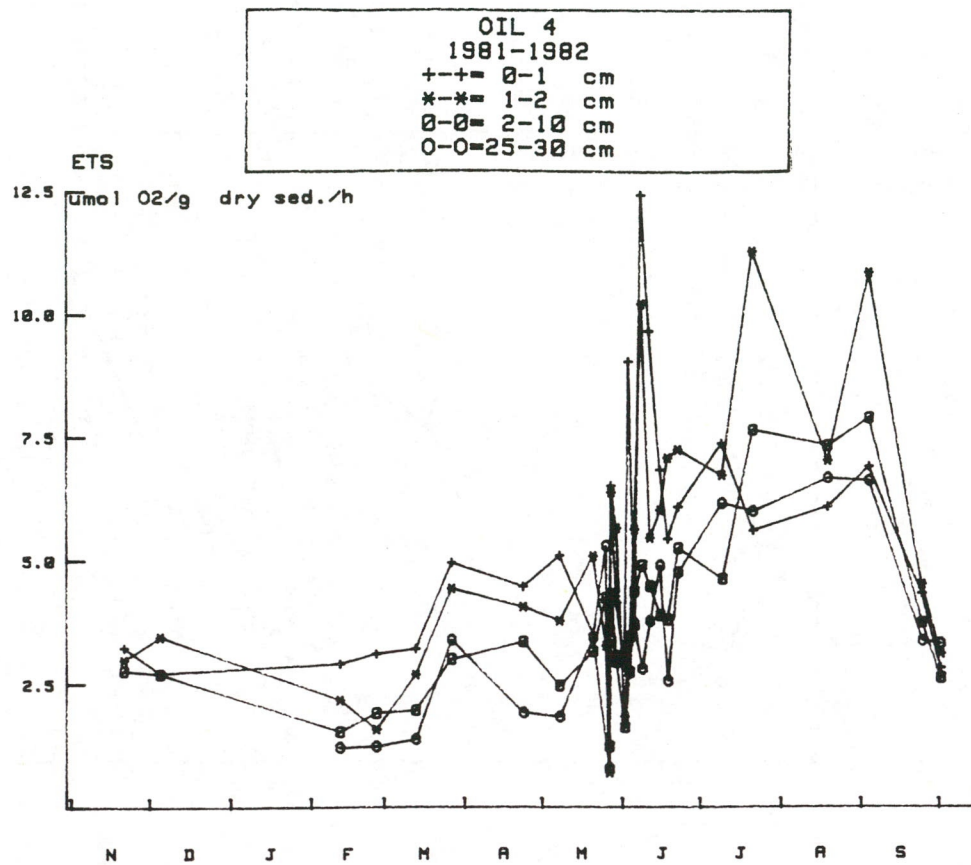


Fig. I.24 ETS activity at different depths in the sediment of MOTIF 4 during OPEX.

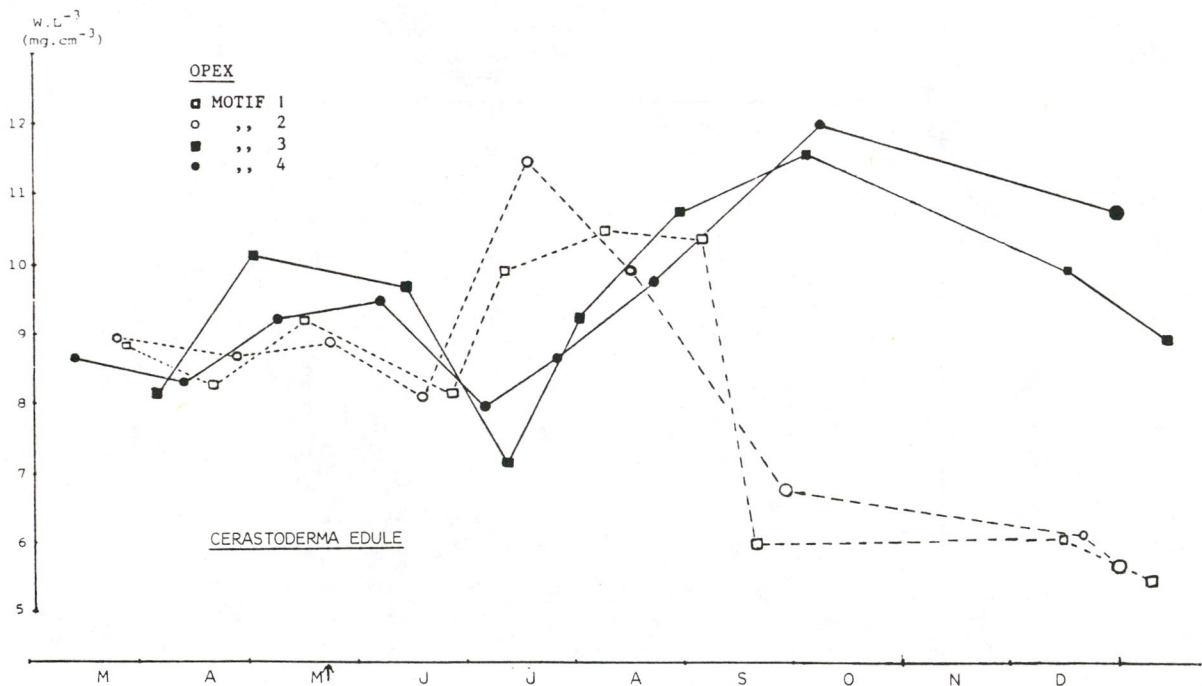


Fig. I.25 The condition index of *Cerastoderma edule* in the different MOTIFs during OPEX.

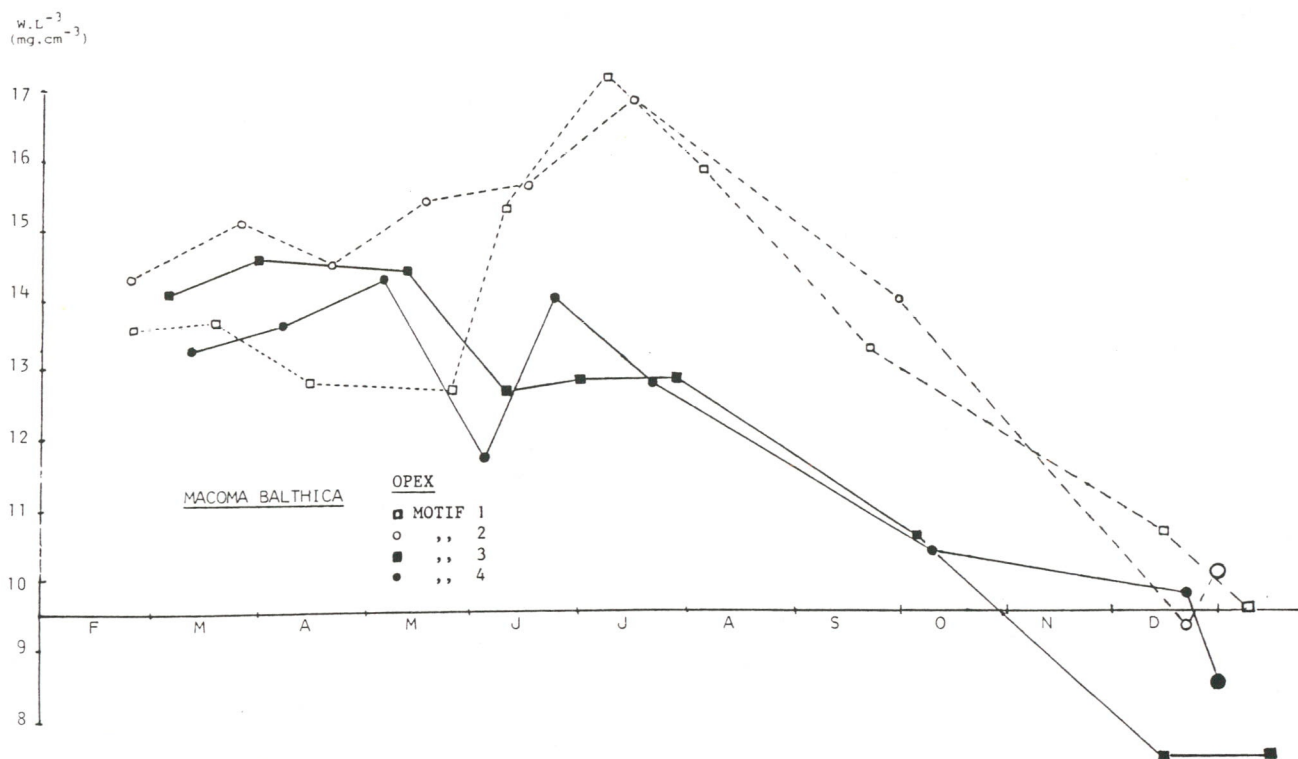


Fig. I.26 The condition index of *Macoma balthica* in the different MOTIFs during OPEX.

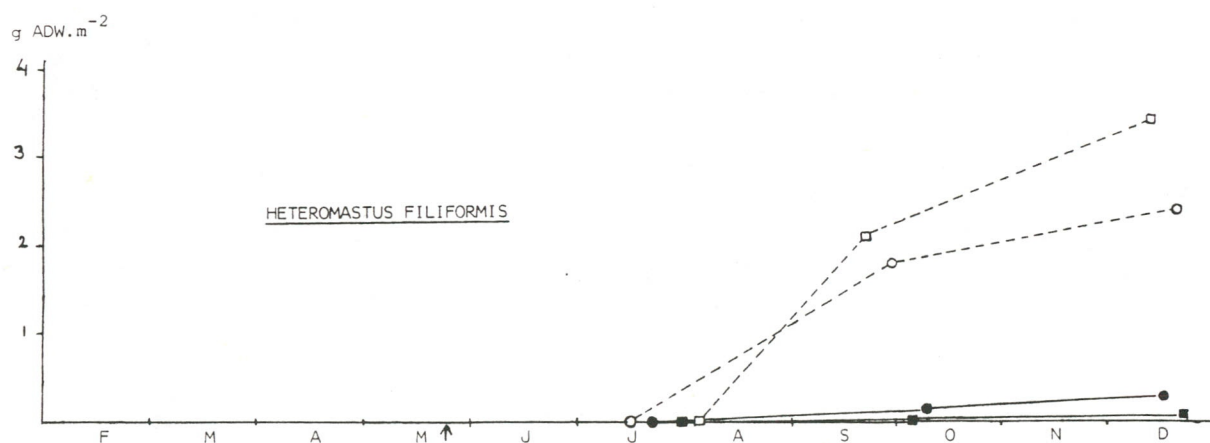


Fig. I.27 The ash-free dry weight (ADW) of *Heteromastus filiformis* per m² in the different MOTIFs during OPEX.

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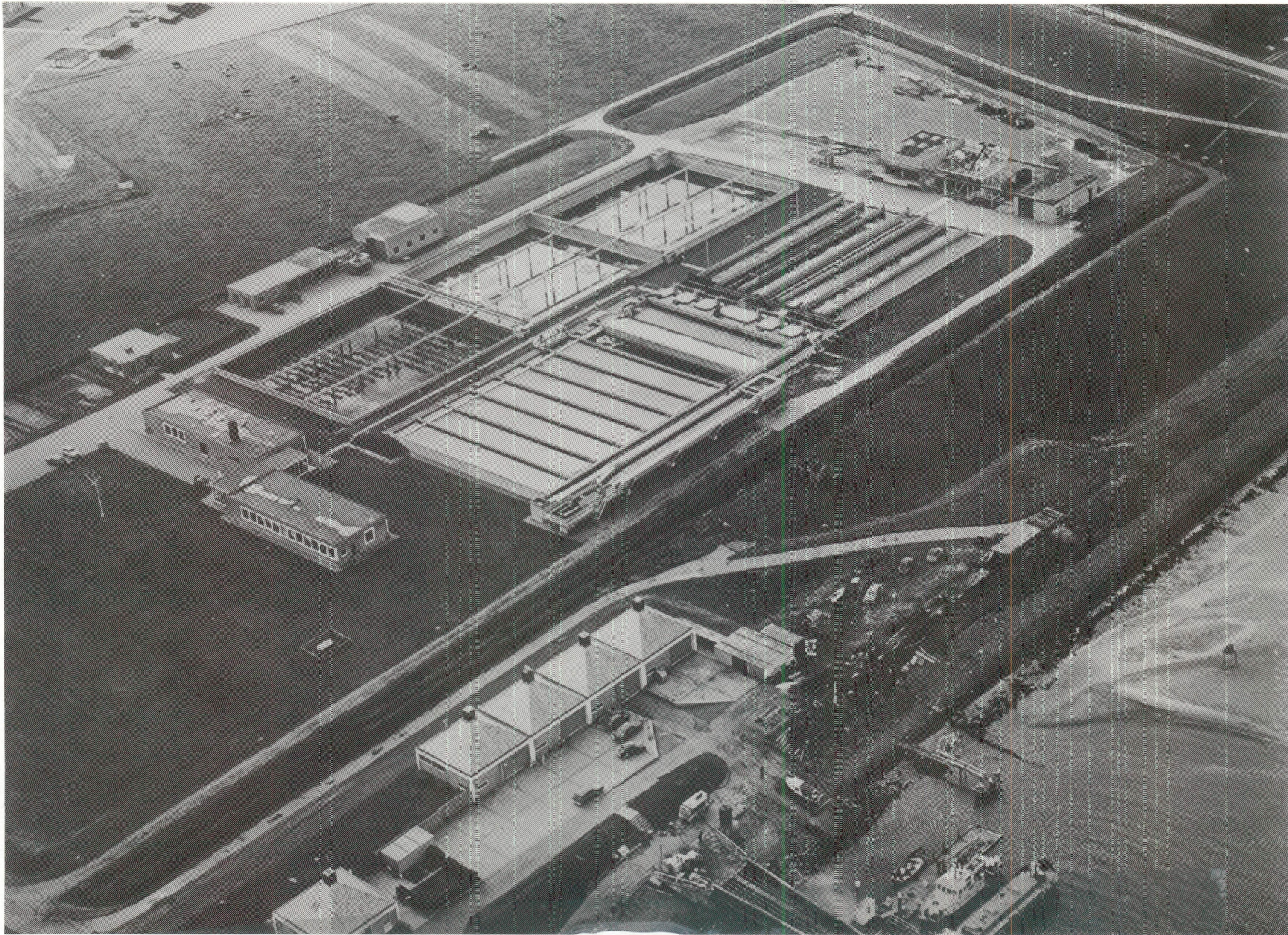


Fig. 1 Aerial photograph of experimental facilities of the Research Institute for Nature Management at 't Horntje, Texel.



Fig. 2 An overview of the four MOTIFs.

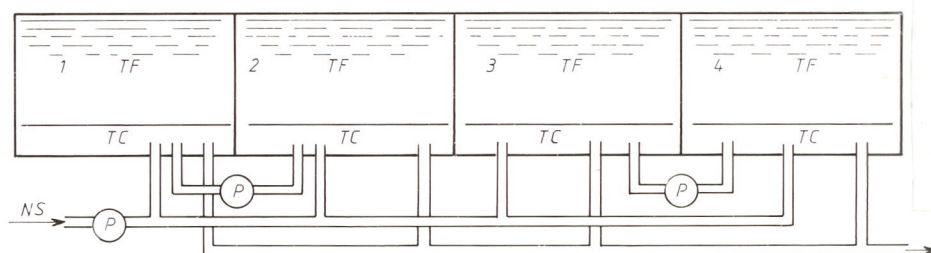


Fig. 3 Diagram of the MOTIFs.

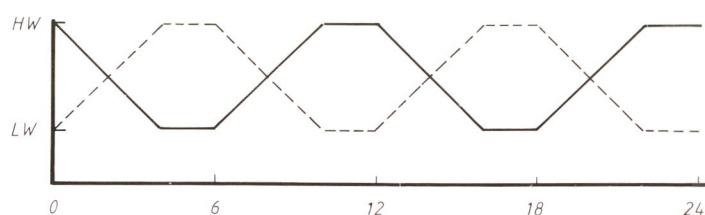


Fig. 4 Tidal cycle of the MOTIFs.

HW = high water;
LW = low water.

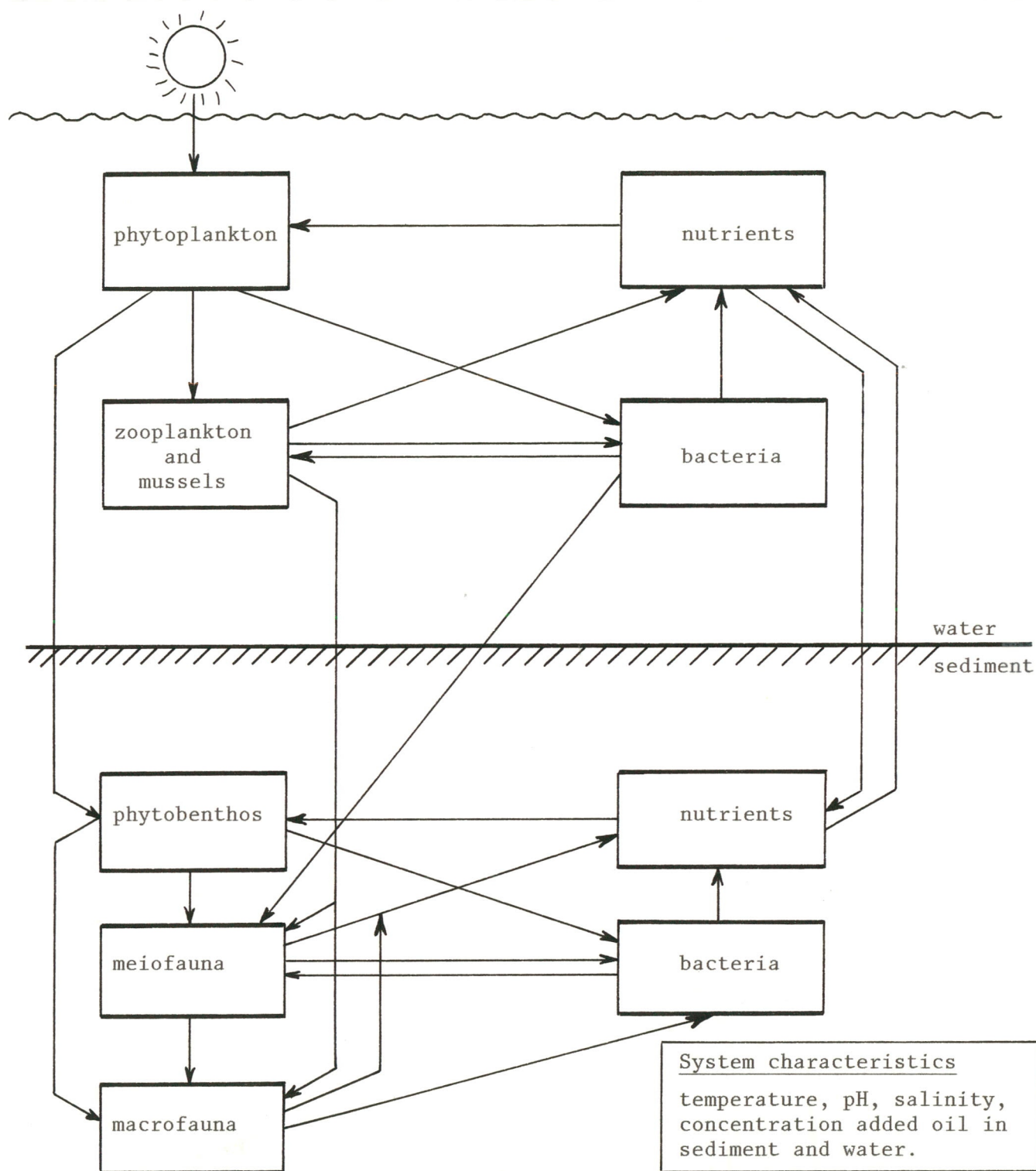


Fig. 5 Simplified diagram of the interrelations between the different biotic compartments and the abiotic factors influencing the development of the organisms in the compartments of the community in a MOTIF.

After the ice had melted, water movement (tidal flow, suppletion of water) was restored.

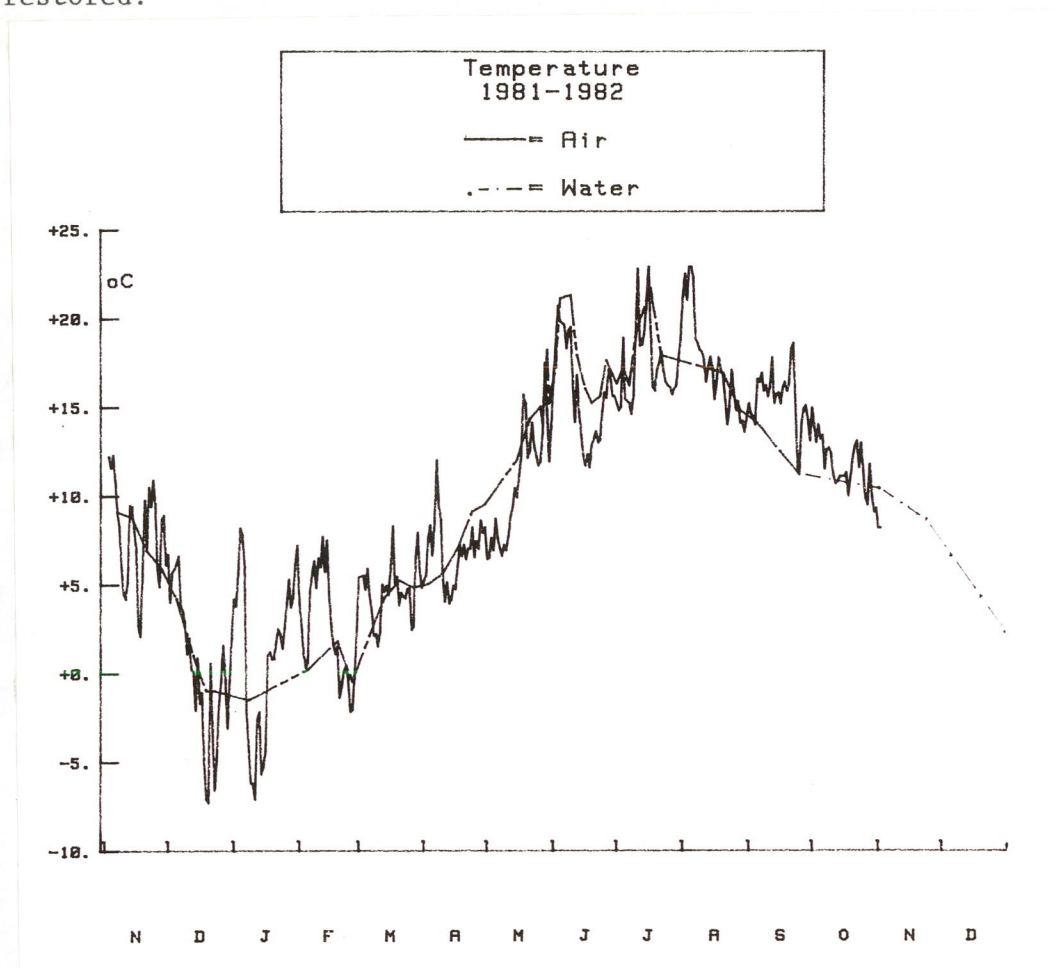


Fig. 6 Water and air temperatures in the MOTIFs during OPEX.

Fig. 7 shows the salinity in the different MOTIFs during the experiment. The salinities were generally within the normal range for the western Wadden Sea, although low values were measured sometimes, which cannot easily be explained. The salinity was generally similar in all basins, at any time, showing that the water had similar residence times, and that differences in other parameters such as nutrients and chlorophyll must be attributed to measurement errors or to differences in development between the different MOTIFs.

The concentrations of suspended particulate matter in the MOTIFs were around 10 mg/l throughout the year. This is low in comparison with concentrations found in the Wadden Sea, which is sediment laden (range 30-200 mg/l) because of the strong currents.

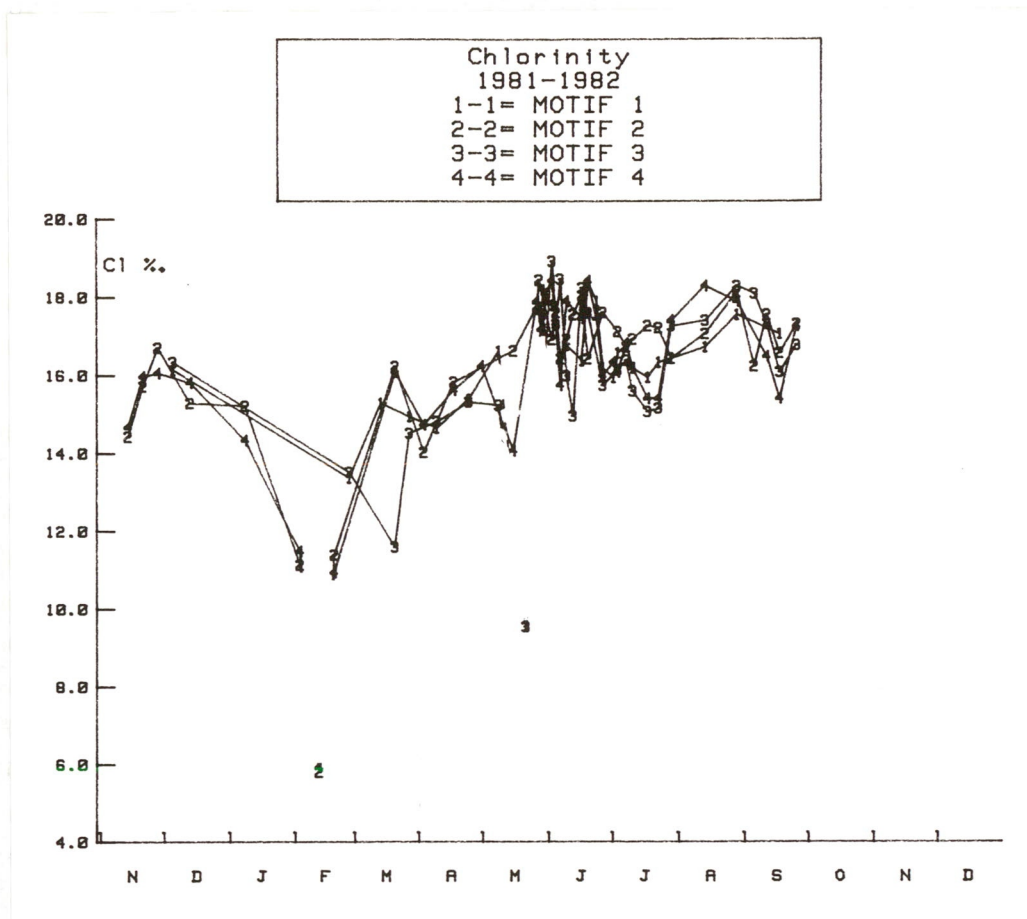


Fig. 7 Chlorinity in the MOTIFs during OPEX.
(salinity = $1.805 \times \text{chlorinity} + 0.03$).

Fig. 8 shows the global radiation as measured at the meteorological station at Den Helder. Measurements made in Den Helder do not differ by more than 10% from those on Texel (Cadee pers. comm.). In addition to the seasonal fluctuation, it can be seen that very much light was available in the period after the oil addition. This sunny period is also reflected in the high air and water temperatures (Fig. 6).

A few remarks on the texture of the sediment can be made here.

The compaction of the sediment increased after the start of the experiment. However, after the introduction of the lugworm *Arenicola marina*, bioturbation increased considerably and the water content of the top layers of the sediment increased again. In the controls this situation lasted for the rest of the experiment, although bioturbation activity decreased from September to the end of the year. After addition of the oil in May *A. marina* disappeared from the most contaminated locations in the MOTIFs (as a result of mortality or migration) and bioturbation was very low at these spots.

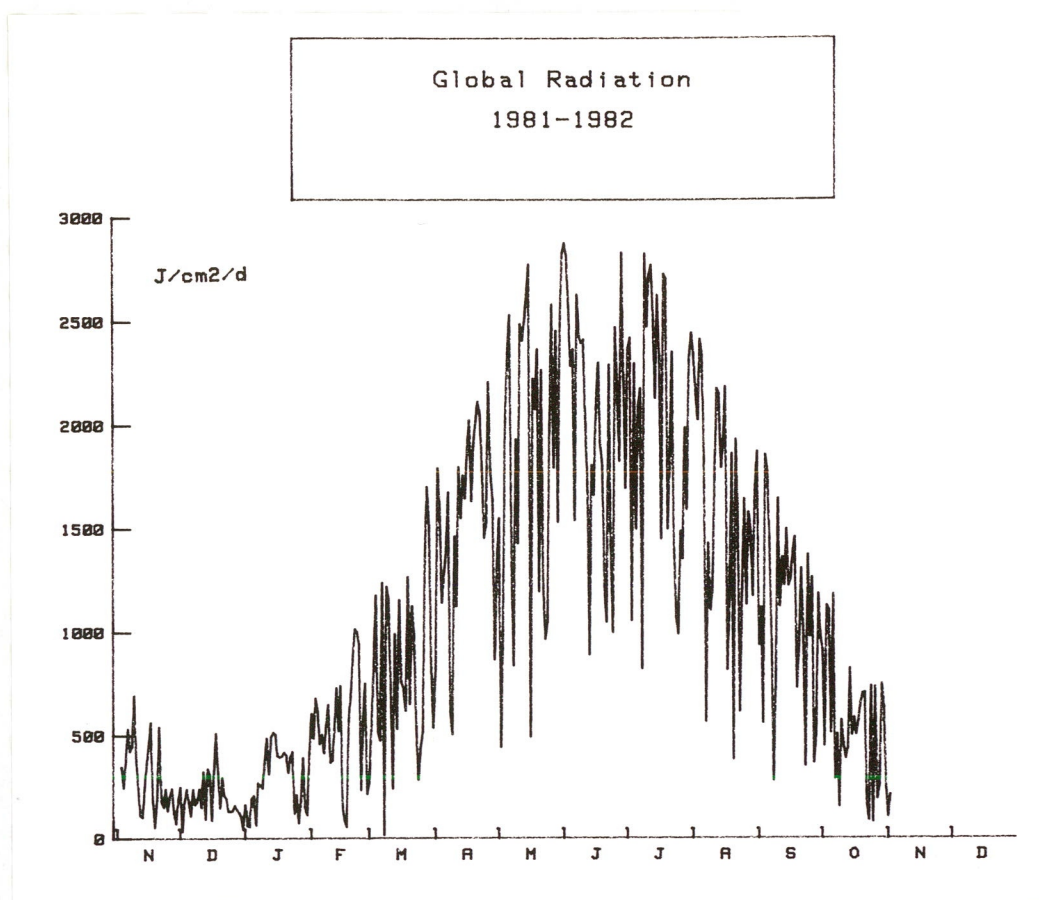


Fig. 8 Global radiation during OPEX at the meteorological station at Den Helder.

This decreased bioturbation activity resulted in low water content and a very compact sediment, into which it was difficult to push a core sampler. The water content of the sediment was less in the contaminated MOTIFs than in the controls for the rest of the experiment, indicating an overall decrease of bioturbation activity in the oil contaminated systems.

The results of the pH measurements and the concentrations of nutrients will be given in the section on the phytoplankton development (§ 3.2.7).

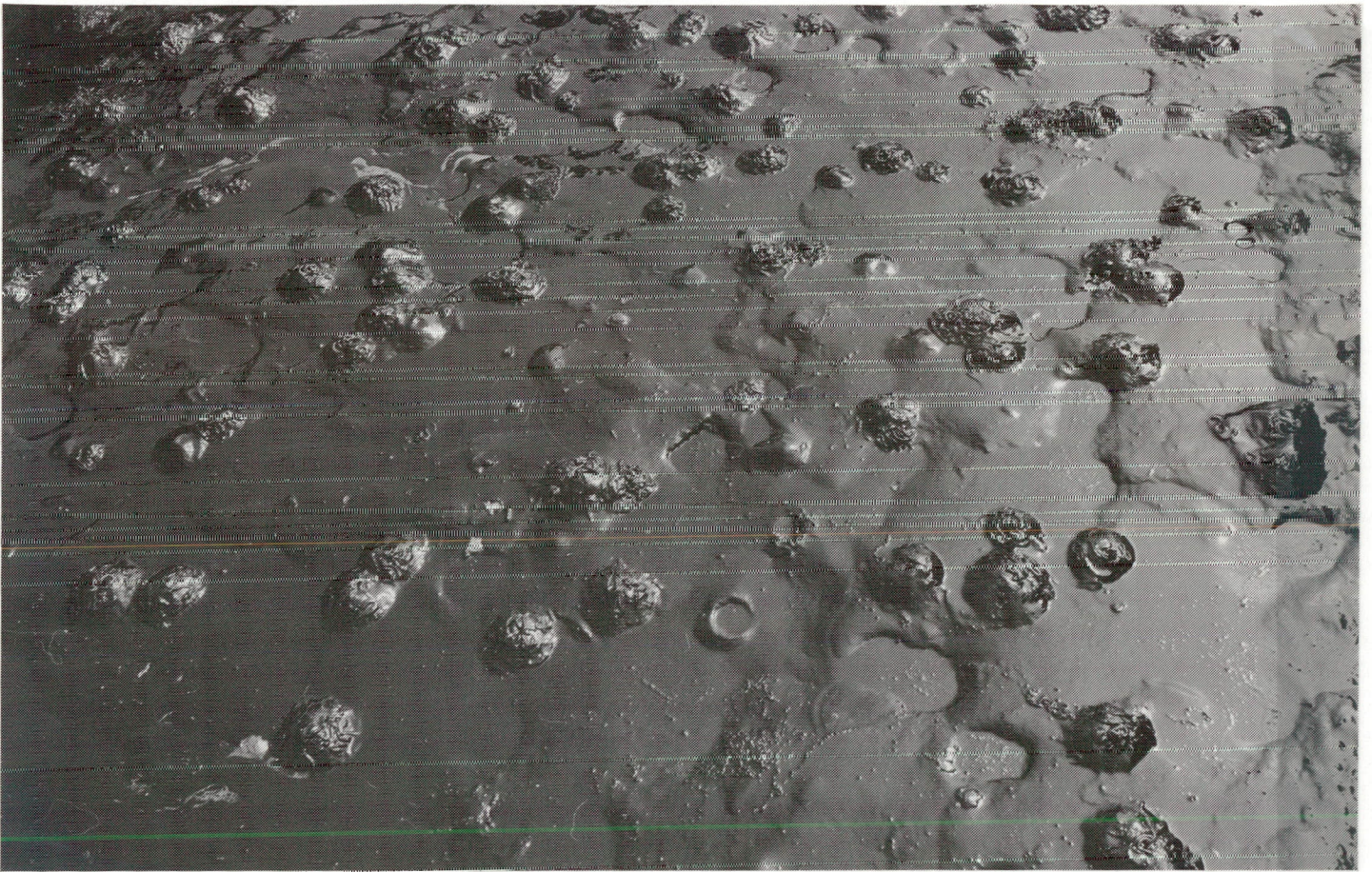


Fig. 9a The oil mousse covering the sediment at ebb; the pits and casts of *A. marina* can be clearly seen.

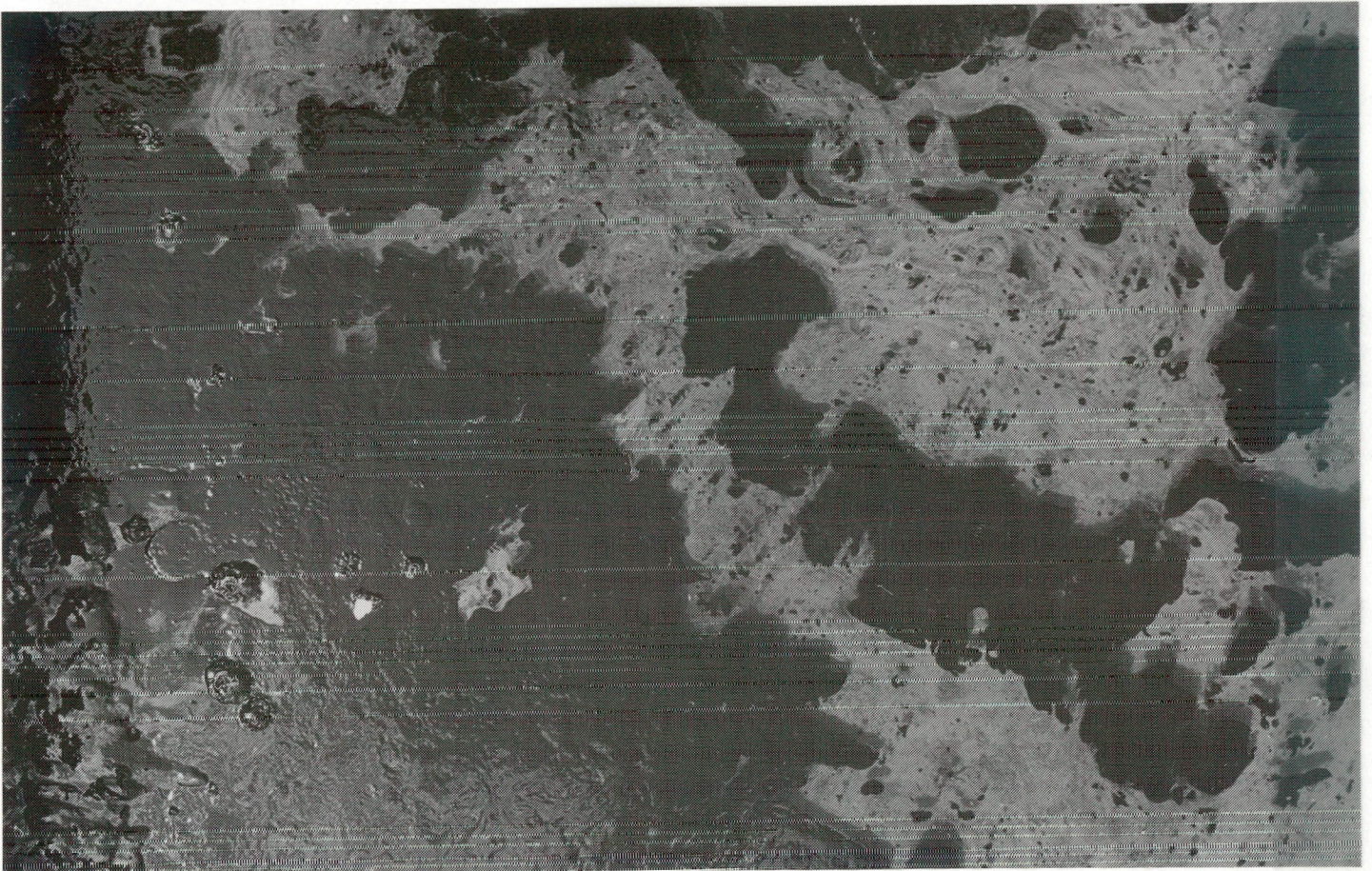


Fig. 9b Part of the mousse is lifted off the sediment by the rising tide.

3.2.2.2 Measurements on the mouse

IR analyses had indicated that the added oil contained ca 31% of aromatic compounds. The colour of the floating mousse changed during the week of exposure from dark brown to nearly black. The viscosity increased with time. The water content of the mousse was not monitored, but destabilization of the mousse probably occurred.

GC analyses of mousse samples taken from the floating slick during the first week showed that the lower alkanes (C8 - C12) disappeared in two days, and that also higher alkanes (C13 - C15) disappeared to a large extent during the week. This was probably a result of evaporation.

3.2.2.3 Oil in the watercolumn

Fig. 10 shows the concentration of oil in the water of the MOTIFs as measured with the fluorescence technique. Concentrations quickly rose after the addition of the oil mousse, and reached a maximum of around 400 $\mu\text{g/l}$ at the end of the week of exposure to the mousse. After this maximum concentrations remain relatively high throughout the rest of the experiment (around 100 $\mu\text{g/l}$), indicating that oil components were constantly released from the sediment to the overlying watercolumn. In the controls concentrations of around 10 $\mu\text{g/l}$ (having a different fluorescence spectrum) were recorded. The fluorescence spectrum in the contaminated systems did not change very much in time, indicating that the composition of the oil in the water also did not change very much with time. This was confirmed by compound analyses using HPLC and GC techniques. The IR analyses of the water samples did not give much information: oil concentrations being below the detection limit (0.2 mg/l) in all cases.

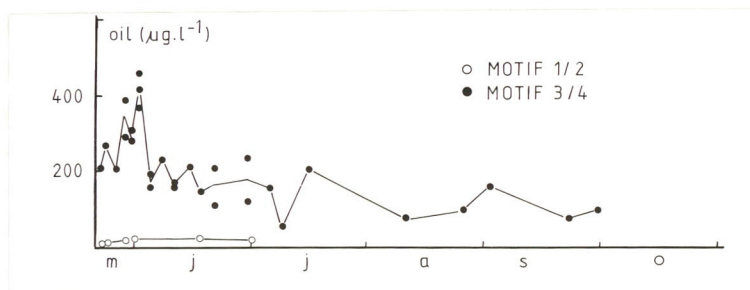


Fig. 10 Total oil concentrations in the water of the MOTIFs during OPEX (measured with fluorescence technique)

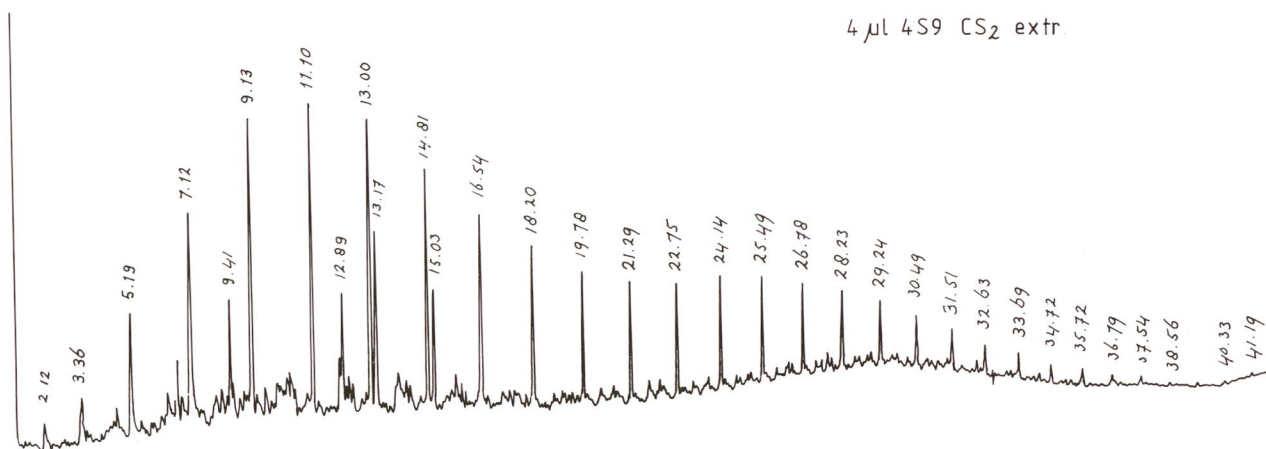


Fig. 11 Typical GC (gaschromatography) results of oil analysis during OPEX.

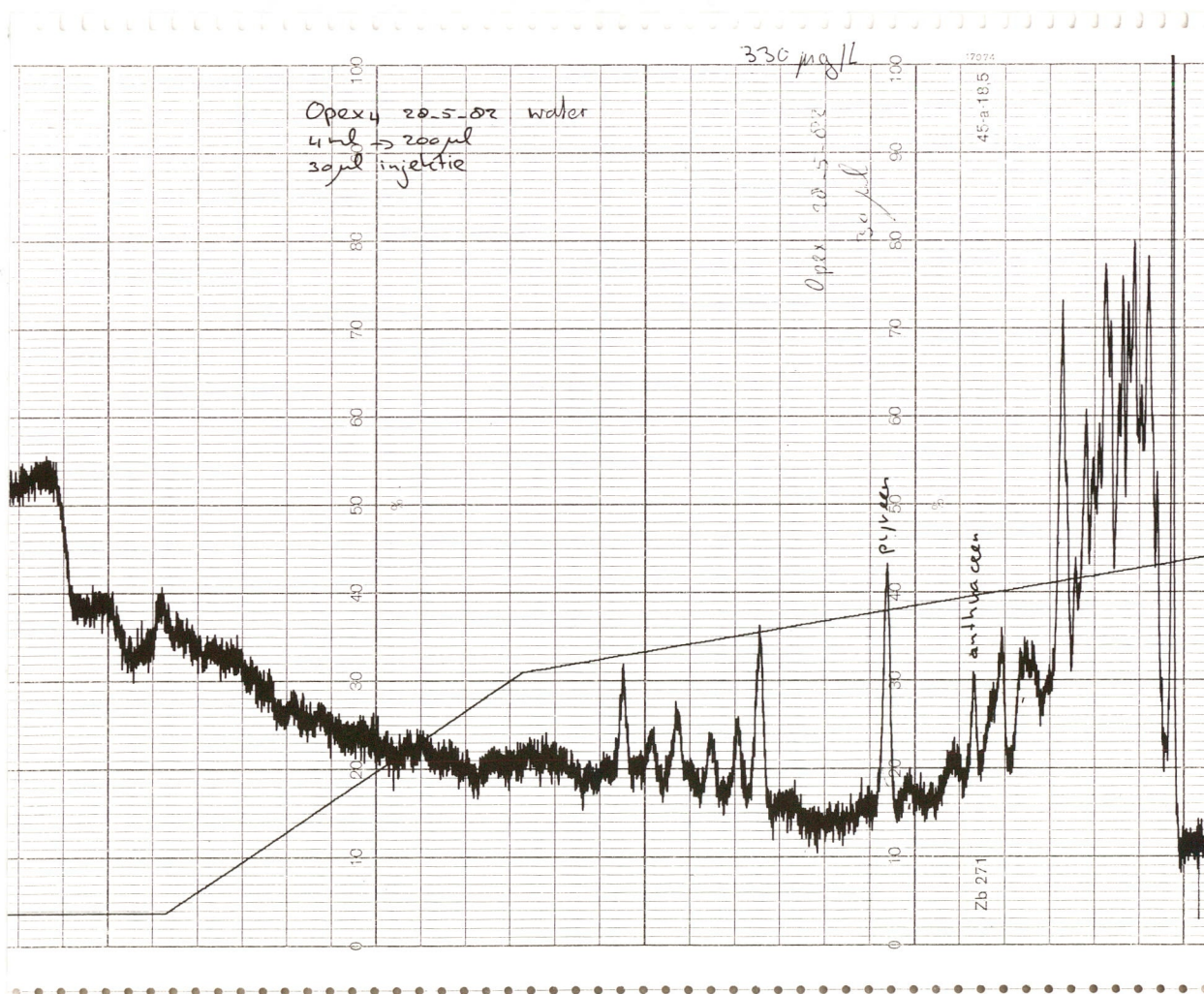


Fig. 12 Typical HPLC (High Performance Liquid Chromatography) results of oil analysis in water during OPEX.

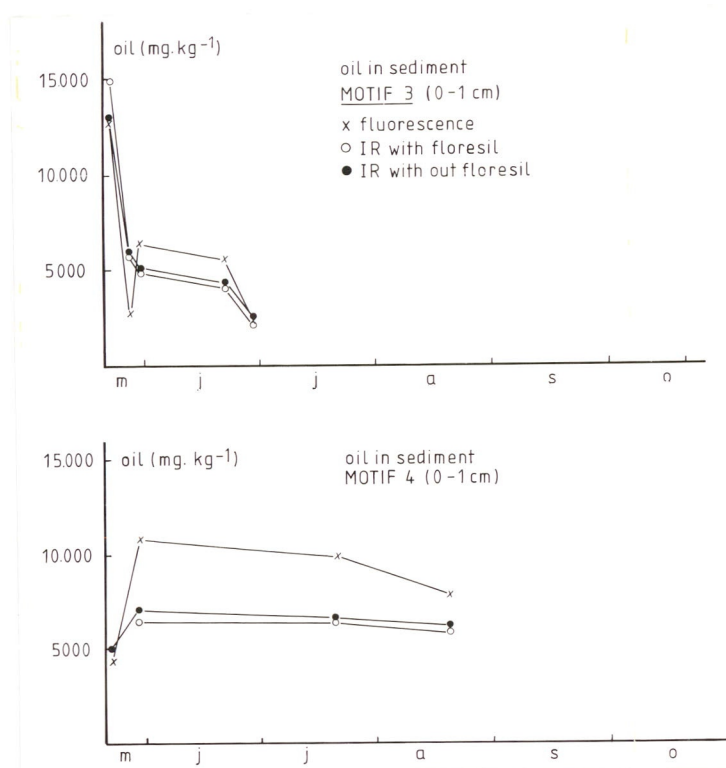


Fig. 13 Total oil concentrations (as measured with IR (Infra-red) and fluorescence techniques) in the upper cm of the sediment in the two contaminated MOTIFs (samples consisted of 20 subsamples, giving an average concentration for the whole tidal flat).

Fig. 14 shows the distributions of the oil with depth on a few selected days. Oil concentrations decreased strongly with depth. At the start of the experiment most of the oil was concentrated in the top mm, but after a few months the oil was distributed through the top few cm. On August 18, 1982 concentrations in the layer between 1 and 2 cm were even higher than those in the top cm, indicating that clean sediment from lower depths had been brought to the surface by bioturbation activity. Results shown in Fig. 14 refer to mixed samples from the whole basin. To study the vertical distribution of the oil in detail, samples were also taken on a few selected locations in the basins using a carefully cleaned sampler. Some of the results of these samples are shown in Fig. 15, in which the strong decrease with depth is also apparent. The samples from MOTIF 3 on 29 June, and from MOTIF 4 on 14 June, were taken at heavily oiled spots where bioturbation activity was much reduced, and therefore most oil remained in the top millimeters.

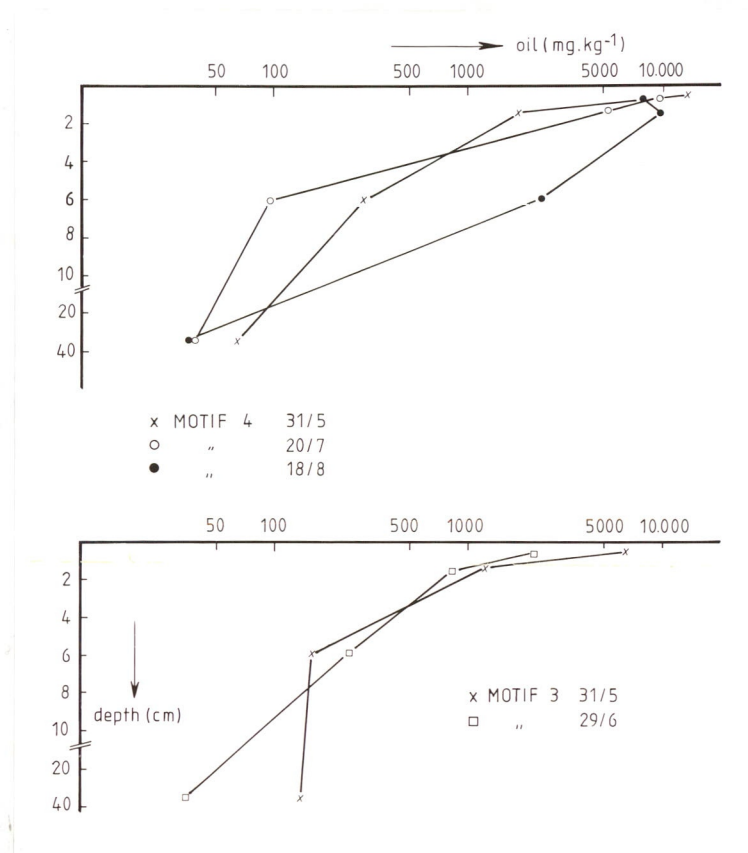


Fig. 14 Vertical distribution of total oil concentrations (measured by fluorescence) in the sediments of the oil contaminated MOTIFs on selected days. (samples consisted of 20 subsamples, giving an average concentration for the whole tidal flat).

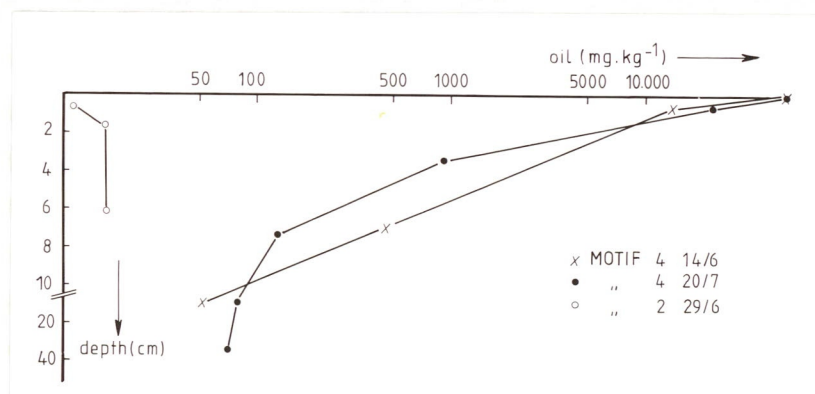


Fig. 15 Vertical distribution of total oil concentrations (measured by fluorescence) in some selected sediment samples.

3.2.3 Development of the phytobenthos

Fig. 16 shows the development of the phytobenthos biomass as measured by chlorophyll concentrations in the different layers sampled in the sediment in one of the controls (no. 1). Since no important differences were found between the upper, middle and lower reach of the tidal flat, results were pooled, so that every value is the average of three analyses (with the exception of the 25-30 cm layer, which was only sampled in the middle reach).

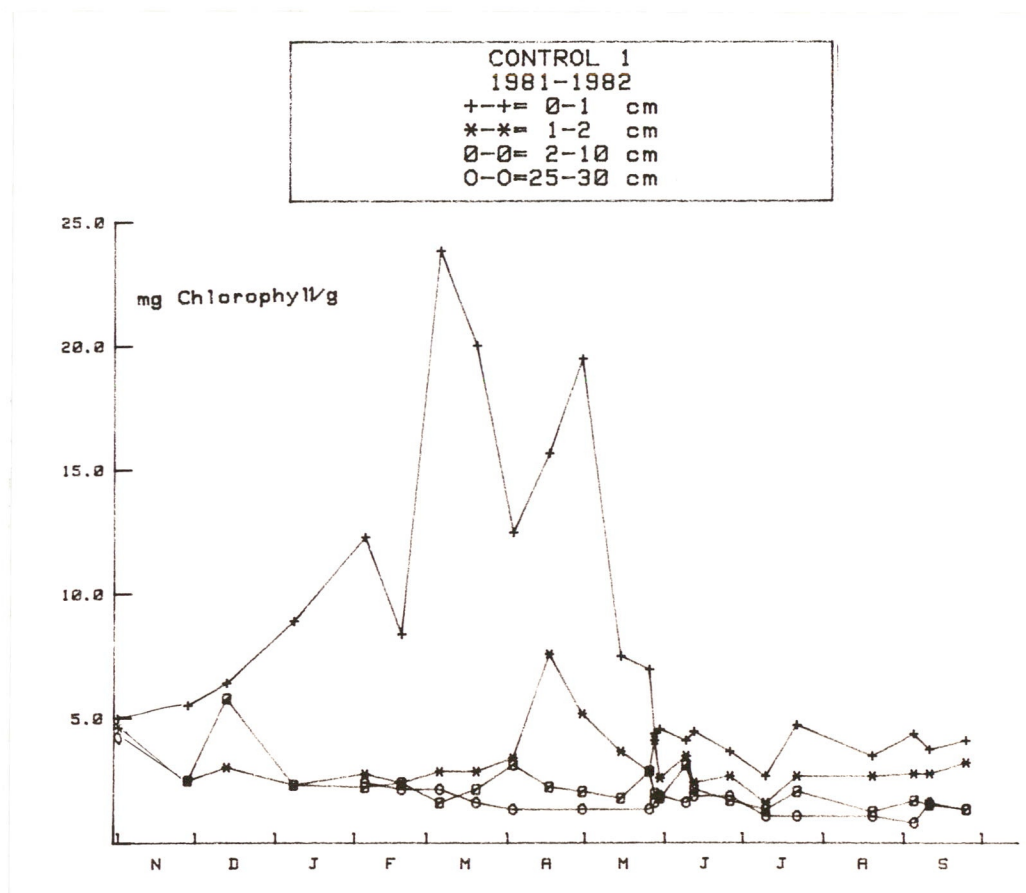


Fig. 16 Chlorophyll concentrations at different depths in the sediment of MOTIF 1.

A similar pattern was found in the other MOTIFs. In October 1981, at the start of the experiment, chlorophyll was homogenously distributed through the sediment at a concentration of 5 mg chlorophyll/g sediment. Directly after the start a stratification developed. At depths of 25-30 cm concentrations decreased from 5 to around 1.5 mg chlorophyll/g sediment.

Table 7 Potential primary productivity ($\mu\text{mol O}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) as measured in the community respiration experiments.

date	control	oil contaminated
middle May	1038	1114
end June	980	575
start September	781	595

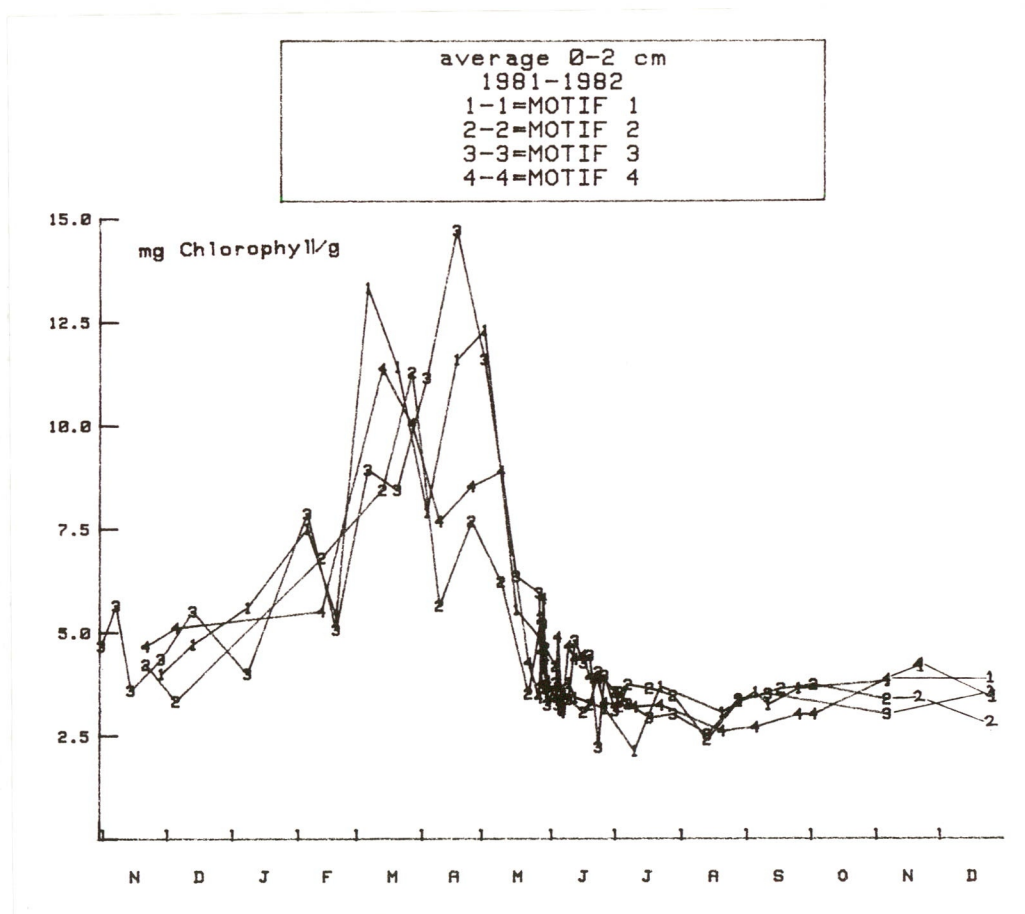


Fig. 17 Average chlorophyll concentrations in the upper 2 cm of the sediment in the different MOTIFS during OPEX.

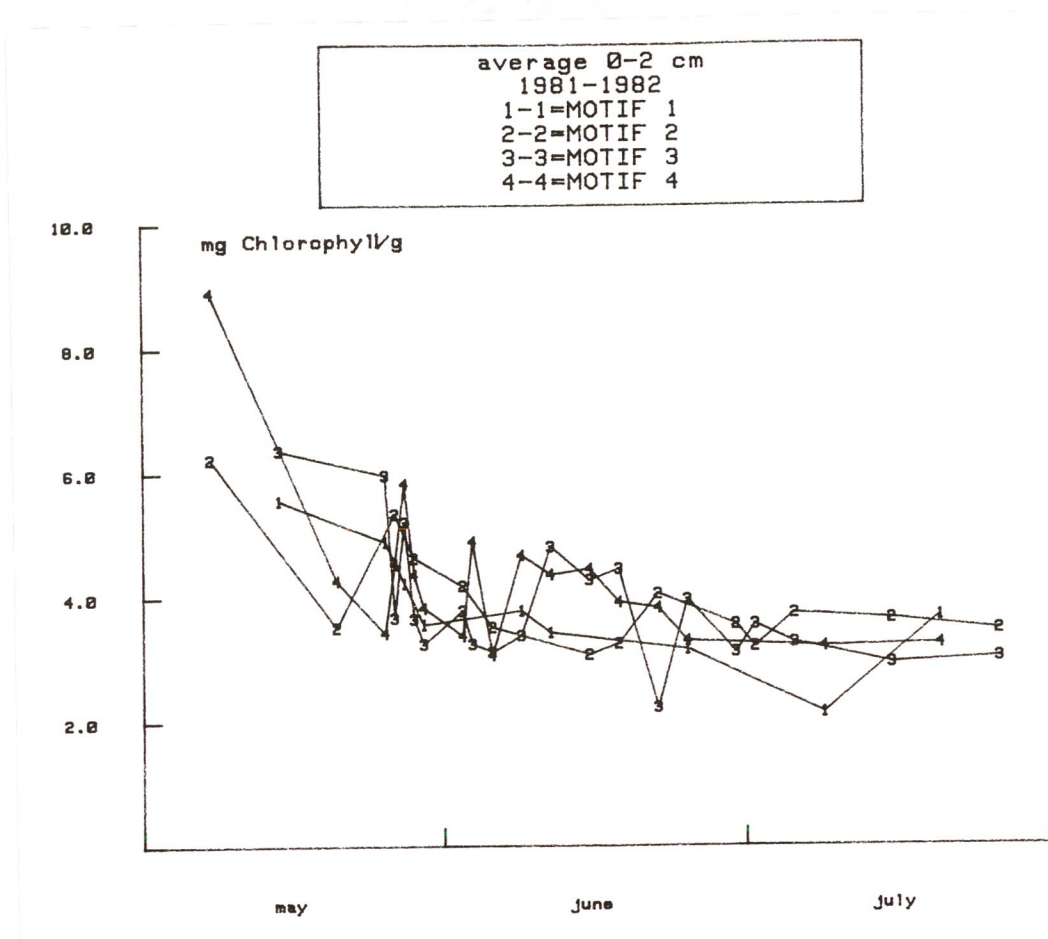


Fig. 18 Average chlorophyll concentrations in the upper 2 cm of the sediment in the different MOTIFS during the period May-July 1982.

In May very similar values were obtained in both MOTIFS (2 and 4). In June and September the values in the oil contaminated MOTIF no. 4 were lower than in the control. In June higher chlorophyll amounts were found in the oil contaminated system, in September chlorophyll values were similar to that in the control. The lower oxygen production of the phytobenthos per unit chlorophyll therefore indicates that the phytobenthos production was limited by the presence of the oil.

Fig. 19 shows the development of the pigment index of the phytobenthos. Very similar values were observed in the different MOTIFS before addition of the oil mousse. After the addition, values were constantly higher in the oil contaminated systems. This result is another indication that the phytobenthos activity was inhibited in the oil contaminated MOTIFS for the rest of the experiment.

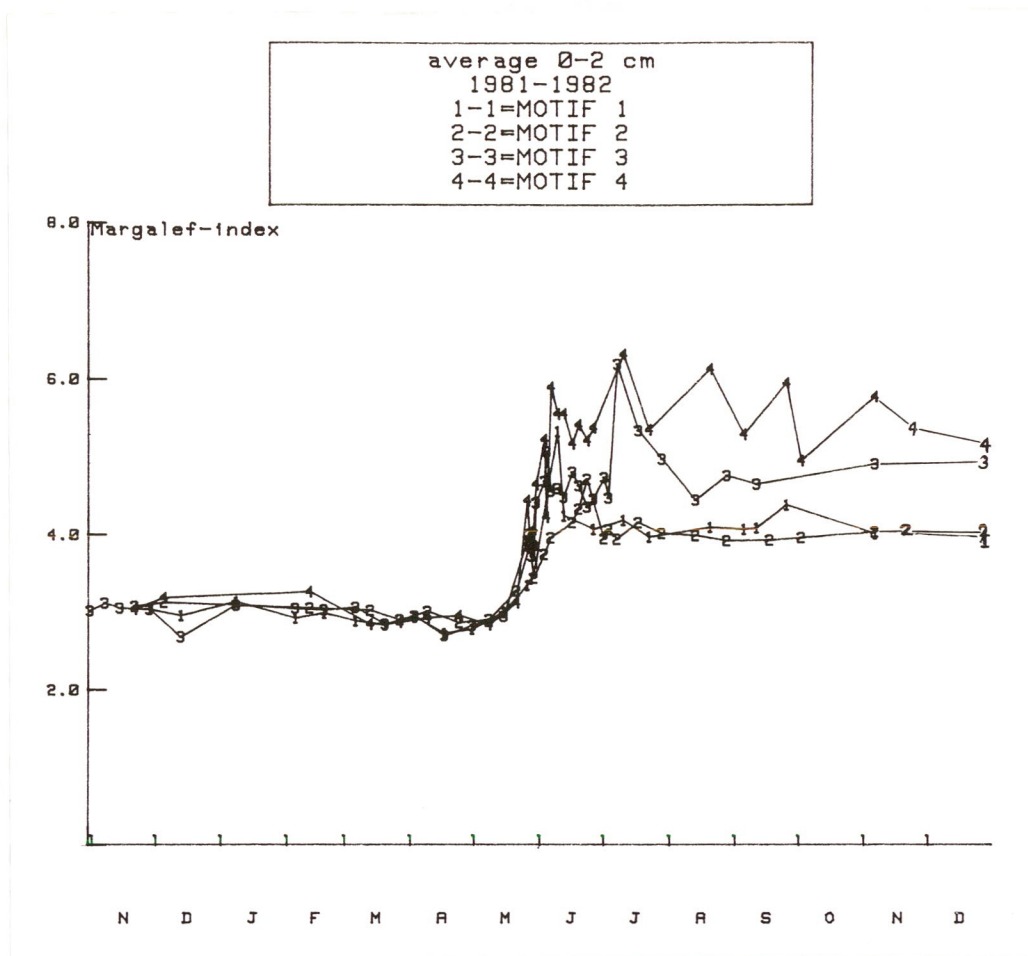


Fig. 19 Pigment index in the different MOTIFs during OPEX in the upper 2 cm of the sediment.

3.2.4 Development of the Electron Transport System (ETS) activity and community metabolism

Fig. 20 shows the development of the ETS activity in the different layers of the sediment sampled in one of the controls (no. 1). From the start of the experiment until March 19, 1982 the ETS activity ranged from 2-4 $\mu\text{mol O}_2/\text{g}$ dry sediment/h, being highest in the top 2 cm. ETS activity increased in this top layer simultaneously with the increasing phytobenthos populations. In May-June ETS activity increased in the deeper layers of the sediment in all MOTIFs, while there was a small decrease in the upper layer. From June until September ETS in the control remained high at depths of 1-30 cm, in the top cm somewhat lower values were recorded. From September to the end of the experiment decreasing ETS activities were found.

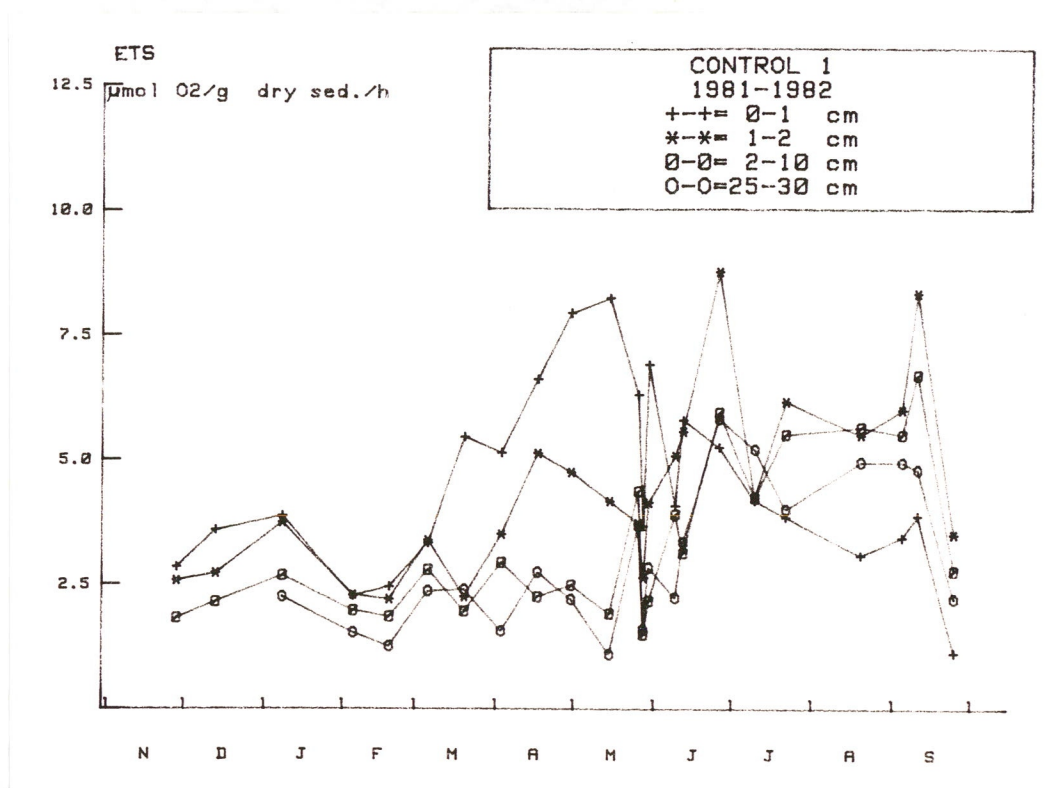


Fig. 20 Development of ETS (Electron Transport System) activity on different depths in the sediment of MOTIF 1.

Fig. 21 shows the average ETS activity in the 0-2 cm layer, in all MOTIFS. During the first six months of the experiment, the same trends were seen in all systems, although the timing of the first peak in April-May differed between systems, giving rise to large variations between the MOTIFS in May. After the oil addition, ETS activity showed very large variations in the oil contaminated systems. In the period July-September ETS activity was somewhat higher in these systems than in the control systems, but the variation between controls was of the same order of magnitude during this period.

The large variability directly after the addition of the oil may have been partly due to measurement errors caused by the presence of oil in the samples, but the high spatial variability of the oil concentrations in the sediment in the contaminated systems was probably also responsible.

Table 8 gives various results obtained from the incubated bottles in May, June and September; control MOTIF 2 is compared with contaminated MOTIF 4 for May (prior to exposure), June and September (after exposure to the oil mousse). As described above (§ 2.4.2) the contents of all of the incubation bottles were sieved over 400 μm nylon gauze and then meticulously inspected.

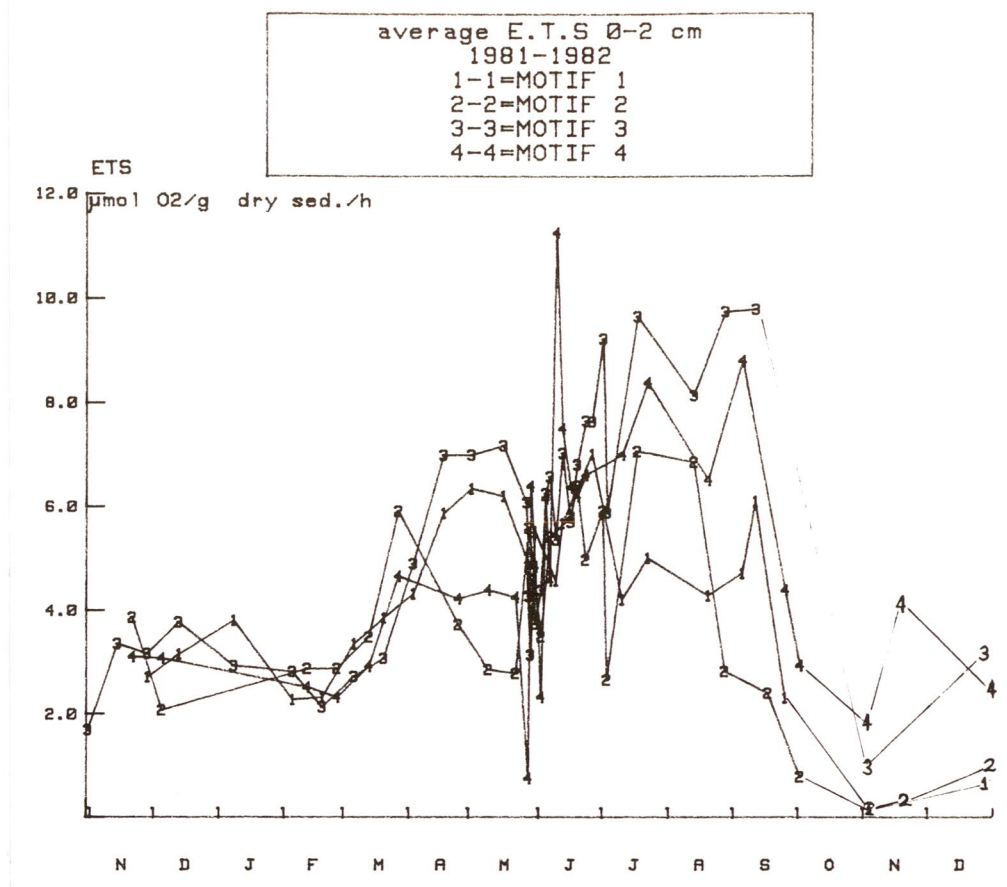


Fig. 21 Average ETS-activity in the upper 2 cm of the sediment in the different MOTIFS during OPEX.

This procedure provides much better information regarding average densities and weight for the majority of the small species than the normal sampling procedure. On the other hand, larger errors can be made in determining the population density of the larger organisms *Macoma*, *Cerastoderma* and *Arenicola*, than in the normal macrobenthos sampling procedure.

The average biomass per m² (= the weight of the total fauna assemblage expressed in grams ash-free dry weight (ADW) per m²) is a more useful parameter than the total number of organisms per m² (n.m⁻² in Table 8), because the organisms can range from macro- and mesofauna to extremely numerous meiofauna.

Table 8 shows the oxygen consumption of the complete community (= community metabolism, 3rd row from under, O₂-consumption per m² per gram (ADW) (2nd row from under), relative O₂-production by the incubated community under light conditions (bottom row). These relative O₂-production values give insight into the potential primary production of the benthic algae and can be related to the phytobiomass present (see 3.2.3).

Table 8 Results from the community respiration incubation experiment. The remarks in the right hand column indicate which parameter (n, ADW, etc.) in which months (J = June, S = September) is used to estimate the effect of the oil addition (for further explanation: see text).

Species	middle of MAY						end of JUNE						start of SEPTEMBER						Effect oil	Remarks
	MOTIF 2			MOTIF 4			MOTIF 2			MOTIF 4			MOTIF 2			MOTIF 4				
	n.m. ⁻²	g biomass.m ⁻²	mg ADW	n.m. ⁻²	g biomass.m ⁻²	mg ADW	n.m. ⁻²	g biomass.m ⁻²	mg ADW	n.m. ⁻²	g biomass.m ⁻²	mg ADW	n.m. ⁻²	g biomass.m ⁻²	mg ADW	n.m. ⁻²	g biomass.m ⁻²	mg ADW		
Macoma ☆)	149	16.3	109.0	149	14.1	94.6	116	16.7	144.2	149	10.6	72.9	50	4.8	95.9	50	3.6	72.1	-	ADW in J
Cerastoderma ☆)	0	0	0	17	6.5	391.3	33	8.5	256.0	0	0	0	50	8.6	172.2	0	0	0	-	n in J, S
Hydrobia	182	0.2	0.8	82	<0.1	0.7	580	0.2	0.3	133	<0.1	0.4	1243	2.1	1.7	1027	2.0	2.0	?	
Corophium	17	0	0	0	0	0	133	<0.1	0.2	0	0	0	38857	2.1	<0.1	166	<0.1	0.1	-	n in J, S
Gammarus	0	0	0	0	0	0	713	0.4	0.6	0	0	0	0	0	0	0	0	0	-	n in J
Arenicola ☆)	17	4.4	265.5	22	4.8	224.4	12	1.7	145.0	5	0	0	0	0	0	0	0	0	?	
Nereis	0	0	0	17	0	0	133	2.1	0.4	928	0.4	0.4	1690	8.9	5.3	2320	18.9	8.1	+	n in J, S; ADW in S
Heteromastus	0	0	0	0	0	0	0	0	0	0	0	0	215	0.9	4.1	33	<0.1	1.4	-	n in S; ADW in S
Anaitides/Eteone	0	0	0	0	0	0	14	0.1	7.8	50	0.2	3.2	50	0.1	2.7	66	0.2	3.6	?	
Pygospio	514	0.5	0.9	514	0.5	0.9	414	0.5	1.3	530	0.2	0.4	133	<0.1	0.3	66	<0.1	0.5	?	
Capitella	0	0	0	0	0	0	795	0.2	0.3	2651	0.4	0.2	0	0	0	0	0	0	+	n in J
Paranais	0	0	0	0	0	0	21375	0.7	<0.1	38541	1.1	<0.1	3927	0.4	0.1	12576	0.9	0.1	+	n in J, S
Oligochaet I	2088	0.5	0.2	2601	0.6	0.2	2502	0.2	0.1	1740	0.2	0.1	2486	0.2	0.1	878	0.2	0.2	-	n in S
(large) Nematodes	5833	0.2	<0.1	5866	0.2	<0.1	5766	0.2	<0.1	6495	0.2	<0.1	3413	<0.1	<0.1	3960	0.2	<0.1	?	
total numbers	8800			9270			32590			51222			52114			21140			?	
total biomass		22.1			26.8			29.6			13.4			28.3			26.30		?	
total number excluding☆)	8634			9181			32425			51070			52014			21090				
total biomass excl.☆)		1.4			1.4			2.7			2.8			14.9			22.7		+	biomass in S
O ₂ -consumption in µgat.h ⁻¹ .m ⁻² (= community metabolism)		1588			1510			2340			2150			4316			4312		?	
O ₂ -consumption in µgat.h ⁻¹ .m ⁻² .g ⁻¹ (ash-free dry weight)		72.5			56.5			79.0			160.0			153.5			165.2		-	O ₂ -consumption per g in J
relative O ₂ -production in µgat O ₂ .h ⁻¹ .m ⁻²		1038			1114			980			575			781			595		-	O ₂ -production in J and S

Paranaís is apparently a relatively insensitive species to oil, and perhaps the oil-contaminated sediment creates a favourable environment for reproduction and growth of this species.

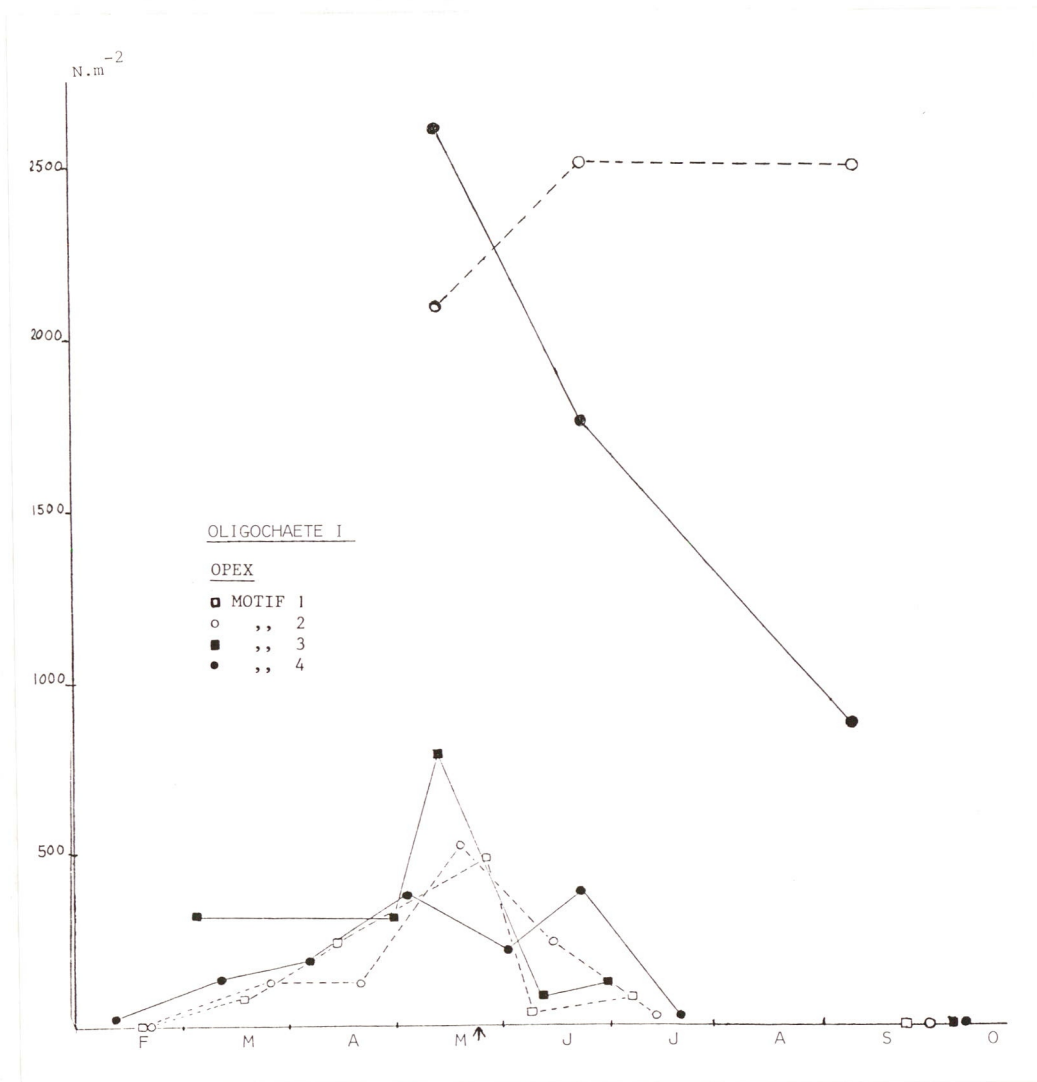


Fig. 22 Numbers of oligochaete I in the different MOTIFs. Lower lines represent numbers retained by a 1000 µm sieve; upper lines represent numbers retained by a 400 µm sieve.

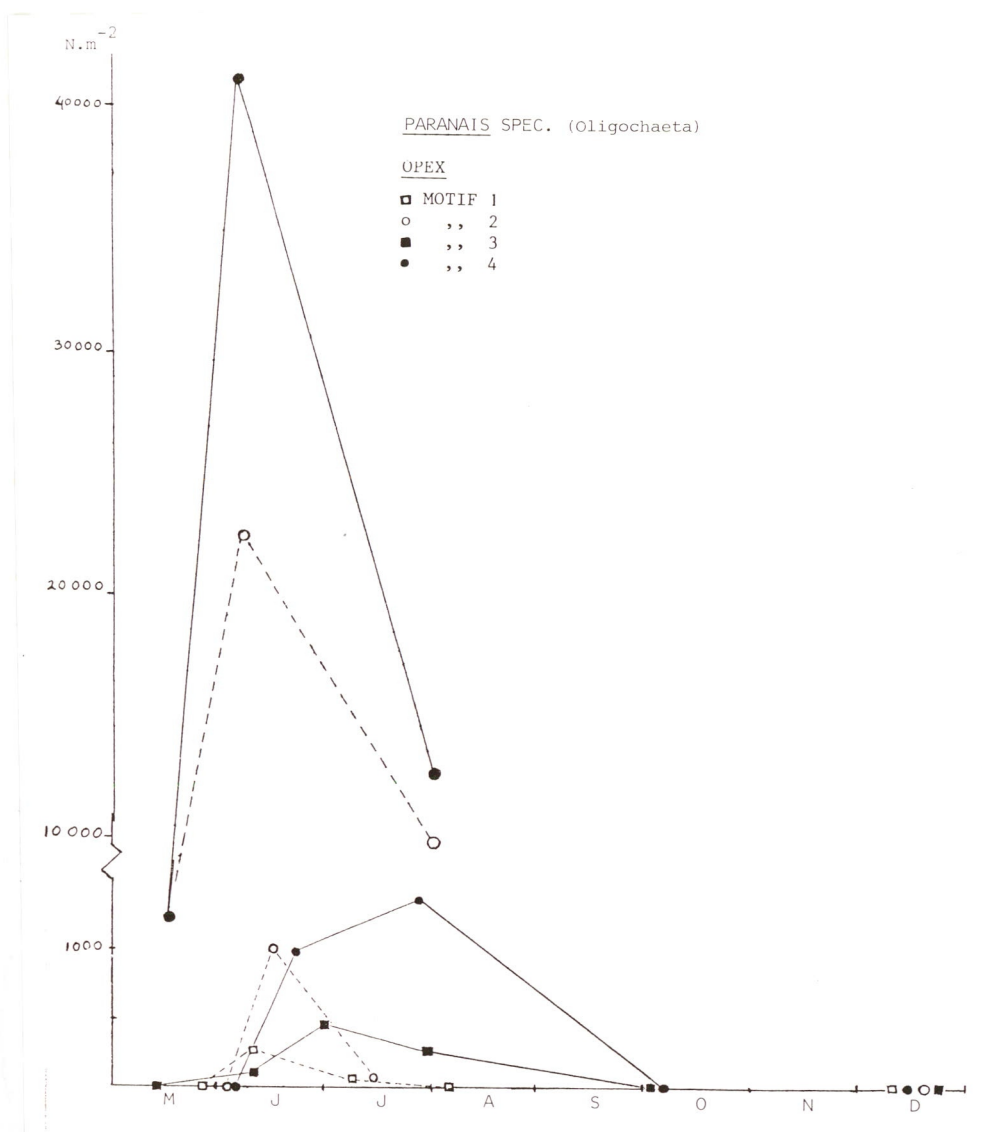


Fig. 23 Numbers of *Paranais* sp. in the different MOTIFs. Lower lines represent number retained by a 1000 µm sieve; upper lines represent numbers retained by a 400 µm sieve.

Nematodes

Some 200 species of nematodes are known to occur in the Wadden Sea, and they form by far the most important group of meiofauna organisms proper in the mud-flats. The largest species can reach a body length of about 10 mm and populations of this group in the MOTIFs 2 and 4, sampled with a 400 µm sieve are shown in the lower lines of Fig. 24. Although these numbers certainly do represent an underestimate, no significant differences were observed between MOTIF 2 and 4. The upper lines of Fig. 24 show the numbers of the complete nematode assemblage (small and large) obtained by elutriation.

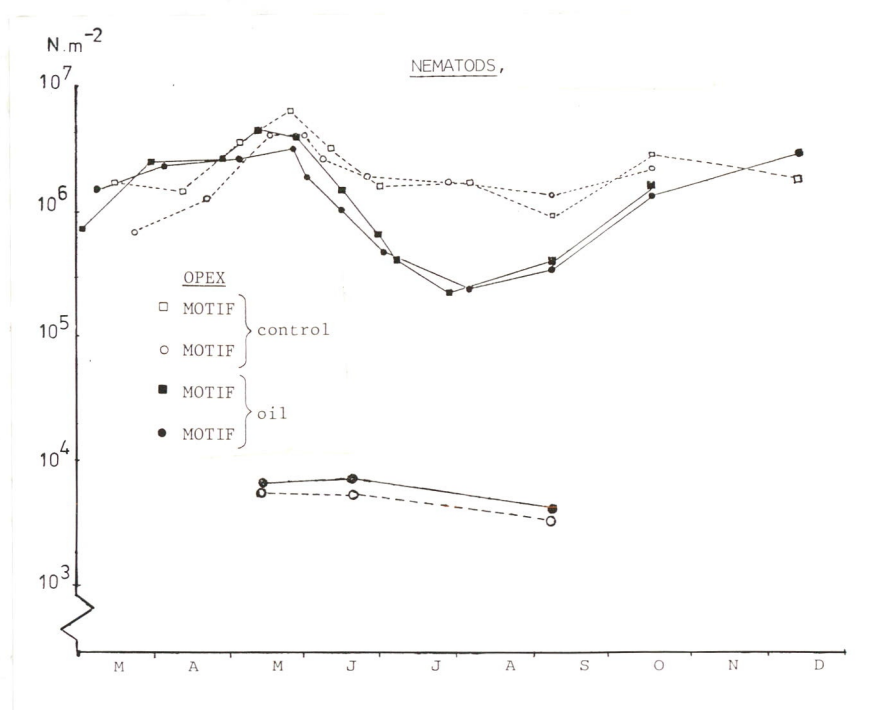


Fig. 24 Numbers of nematodes in the different MOTIFs. Lower lines represent numbers retained by 400 μm sieve (larger nematodes), the upper lines represent numbers obtained by elutriation of the samples.

In spring the nematode populations increased and reached maximum values of about 6×10^6 nematodes per m^2 in May with a fall in June and July. This fall was, however, much more pronounced in the oil contaminated MOTIFs 3 and 4 with minimum values of about 0.5×10^6 per m^2 , than in the uncontaminated MOTIFs 1 and 2 with values of about 1.0×10^6 per m^2 . The populations in MOTIF 3 and 4 showed a recovery in September and October.

Harpacticoid copepods div. spec.

The lines in Fig. 25a, b show the population dynamics of harpacticides, adults and juveniles being plotted separately.

In spring, all the populations rapidly increased in numbers up to values of about 1.5×10^6 per m^2 . A sudden fall in numbers was observed early in June in the uncontaminated MOTIFs 1 and 2 whereas most remarkably numbers in the contaminated MOTIFs 3 and 4 continued to rise, with a decrease in July.

In autumn the picture became still more confusing. The numbers of adults recovered in MOTIF 1, 3 and 4, whilst the number in MOTIF 2 here in September was very low. The behaviour of the number of juveniles in autumn was also perplexing: MOTIFs 1 and 3 showing increases and MOTIFs 2 and 4 showing decreases.

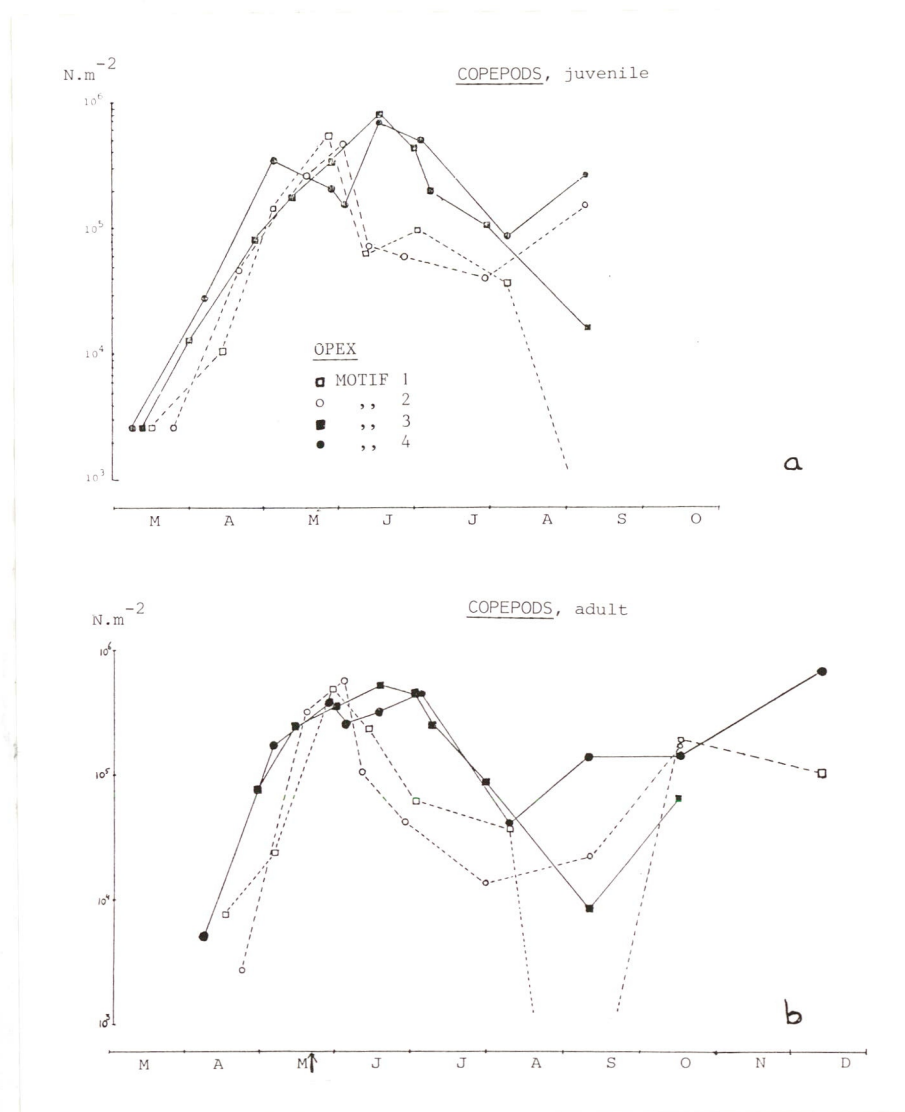


Fig. 25 Numbers of juvenile (a) and adult (b) harpacticoid copepods in the different MOTIFs.

3.2.6 Development of the macrofauna

In this section the development of the macroinvertebrates present in the MOTIFs will be presented per species. In addition to data on the species deliberately introduced at the start of the experiment (*Mytilis edulis*, *Cerastoderma edule*, *Macoma balthica*, *Littorina littorea* and *Arenicola marina*, data will also be given on other species which developed in the systems. The populations of these species may have developed from small stocks of individuals introduced with the sediment at the start of the experiment, from larvae introduced with the suppletion water.

Mytilus edulis

23-25 of the 25 specimens originally placed in each sample were recovered. Mortality was negligible in both the uncontaminated and the contaminated systems. Fig. 26 shows the increase in shell length in the different MOTIFs. The mussels increased in length by approximately 1 cm in all MOTIFs, no significant differences being found between uncontaminated and contaminated systems.

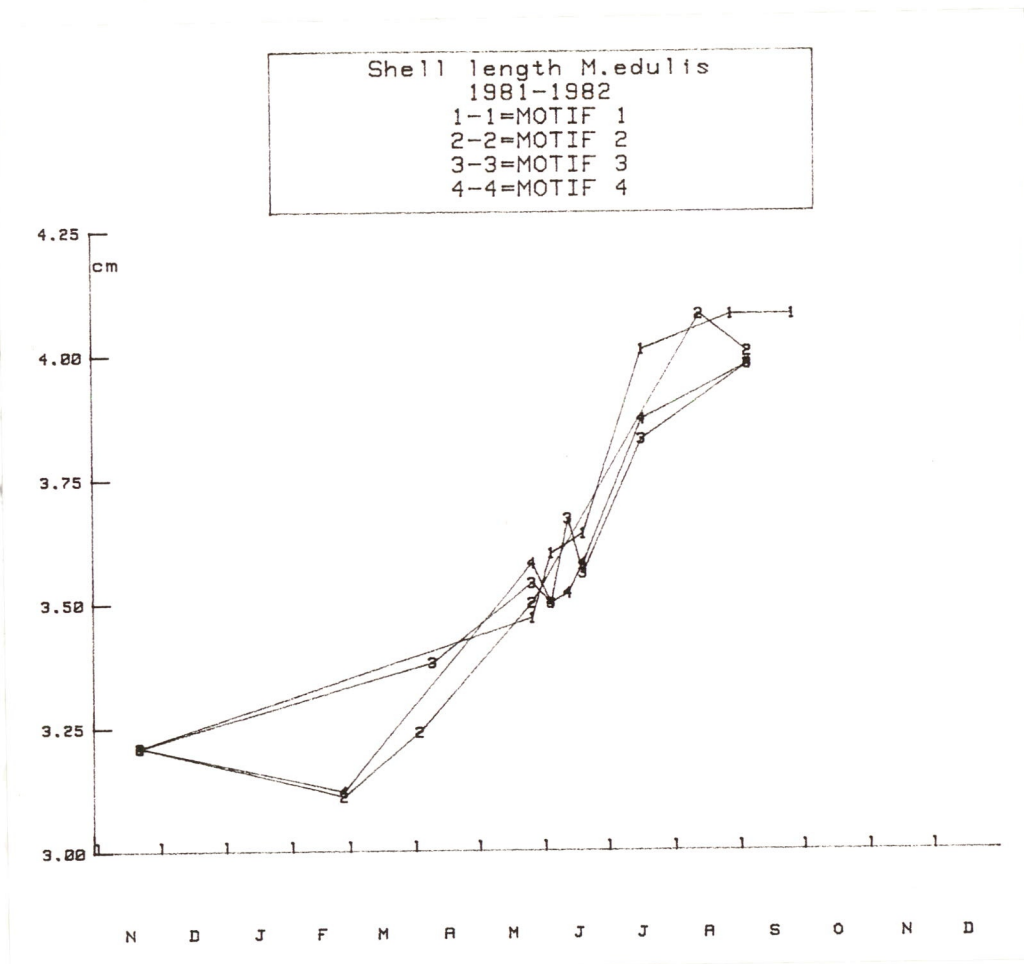


Fig. 26 Development of the length of shells of *M. edulis* in the different MOTIFs.

Fig. 27 shows the average ash-free dry weight (ADW) of the soft parts per mussel throughout the experiment. The increase in ADW after the winter was similar to that found under field conditions, with a maximum in September. ADW decreased in the oil contaminated MOTIFs following exposure, but this seemed to recover later.

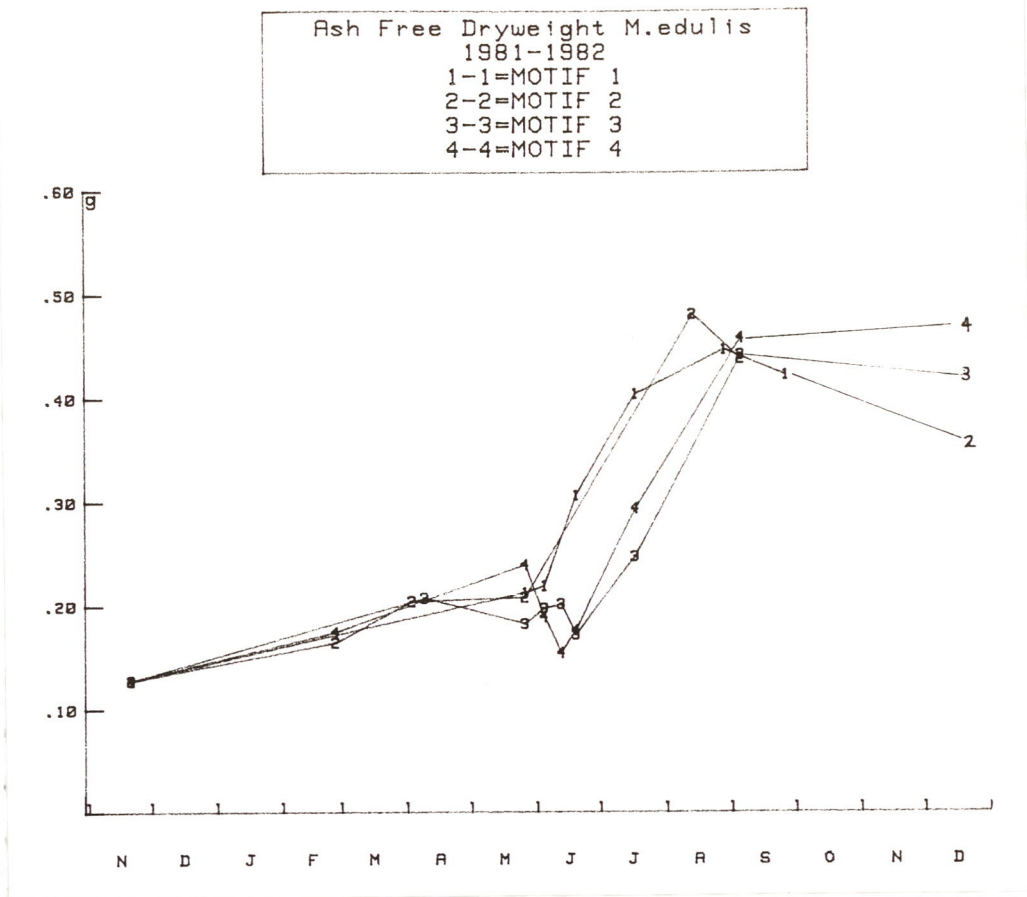


Fig. 27 The ash-free dry weight (ADW) per individual of *M. edulis* in the different MOTIFs during OPEX.

Cerastoderma

The numerical densities of the cockle populations are shown in Fig. 28. Five hundred animals of year-class 1979 with a length of 30-35 mm were introduced into each MOTIF, giving an initial density of 28 per m².

Due to the sampling, 79 cockles were removed from MOTIF 1, 83 from MOTIF 2, 55 from MOTIF 3 and 79 from MOTIF 4. Eighty-one empty shells were found on the surface of MOTIF 1, and 50 on that of MOTIF 2. These numbers are, however, an underestimate of the natural mortality, as part of the succumbed animals will have remained hidden in the sediment or have been buried by bioturbative action by *Arenicola*.

At the end of the experiment the uncontaminated MOTIFs 1 and 2 had therefore suffered losses in the order of 26-32% of their initial population, whereas the losses in the contaminated MOTIFs 3 and 4 were about 48-52%.

Although the numbers in Fig. 28 generally tend to decline with time, the monthly variations in numbers are so large that any significant conclusion on the effect of oil is disputable. It can, however, be concluded that the sampling procedure was apparently inadequate. A non-destructive method, for example by estimation of the number of active visible siphons or by location of the cockles by touch, could be considered.

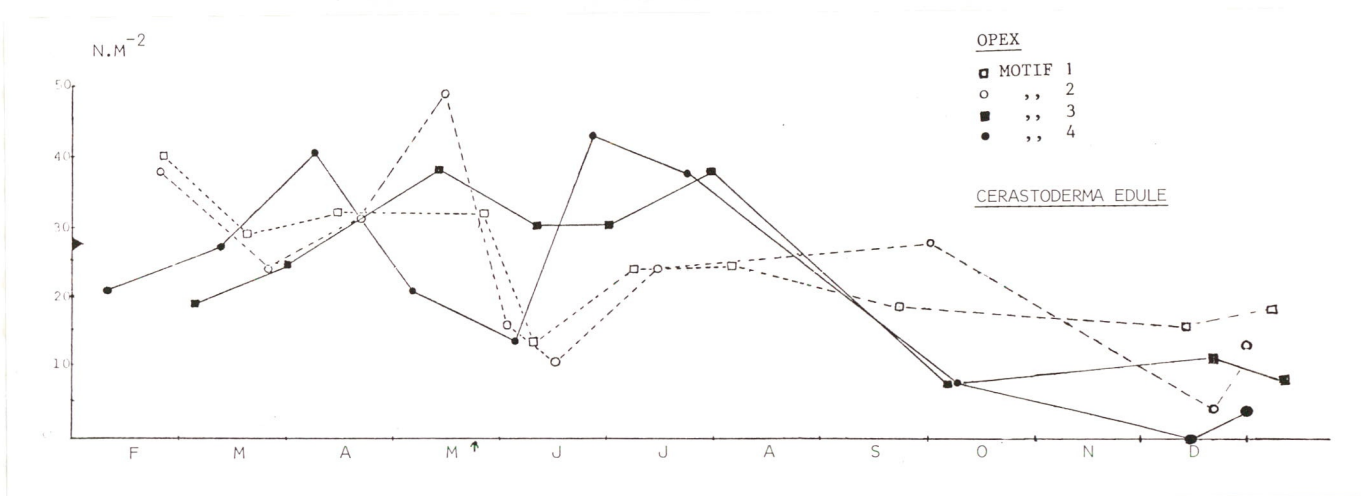


Fig. 28 Numbers of *C. edule* in the different MOTIFs (results from sediment sampling) during OPEX.

Fig. 29 gives the cumulative numbers of dead cockles recovered in each MOTIF versus time. A rapid effect of the oil at the end of May (arrow) was not observed. Towards the end of August (3 months after the oil mousse was added), however, a postponed mortality occurred in MOTIFs 3 and 4, indicating a significant negative effect of oil contamination on the intertidal ecosystem.

A direct cause of this postponed mortality is speculative, but can perhaps be related to reproduction in summer. Delayed mortality in *Cerastoderma*, caused by another stress (severe winters) has been described by Kristensen (1957). Another explanation of the postponed mortality might be accumulation of oil components to critical levels.

Ash-free dry weight (g) of the soft parts of the cockle is presented in Fig. 30. In early spring this weight is low (~ 0.3 g) as a result of a period of continuous emaciation in autumn and winter. Only a limited growth is seen in the first few months of the experiment. Growth generally was slow in adult cockles and poor feeding conditions for this typical filter feeder, may have been responsible for this.

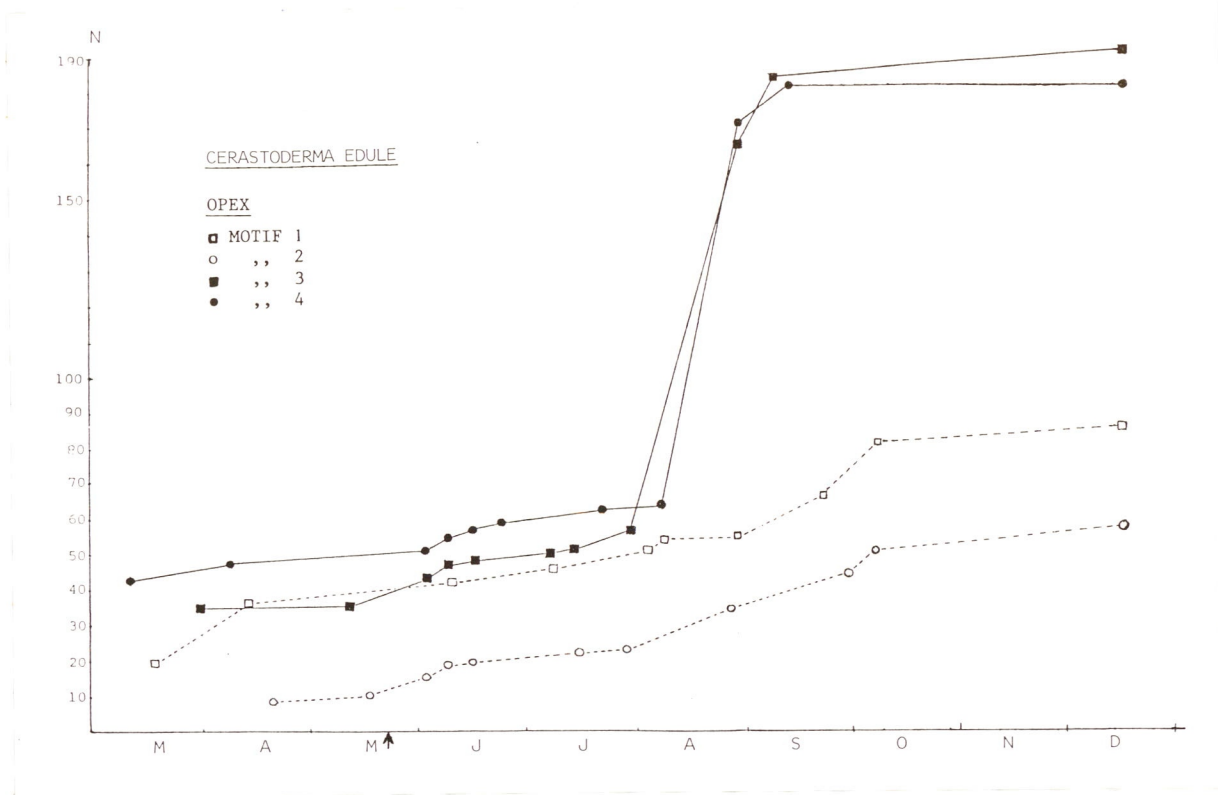


Fig. 29 Cumulative of *C. edulis* found dead on top of the sediments in the different MOTIFs during OPEX.

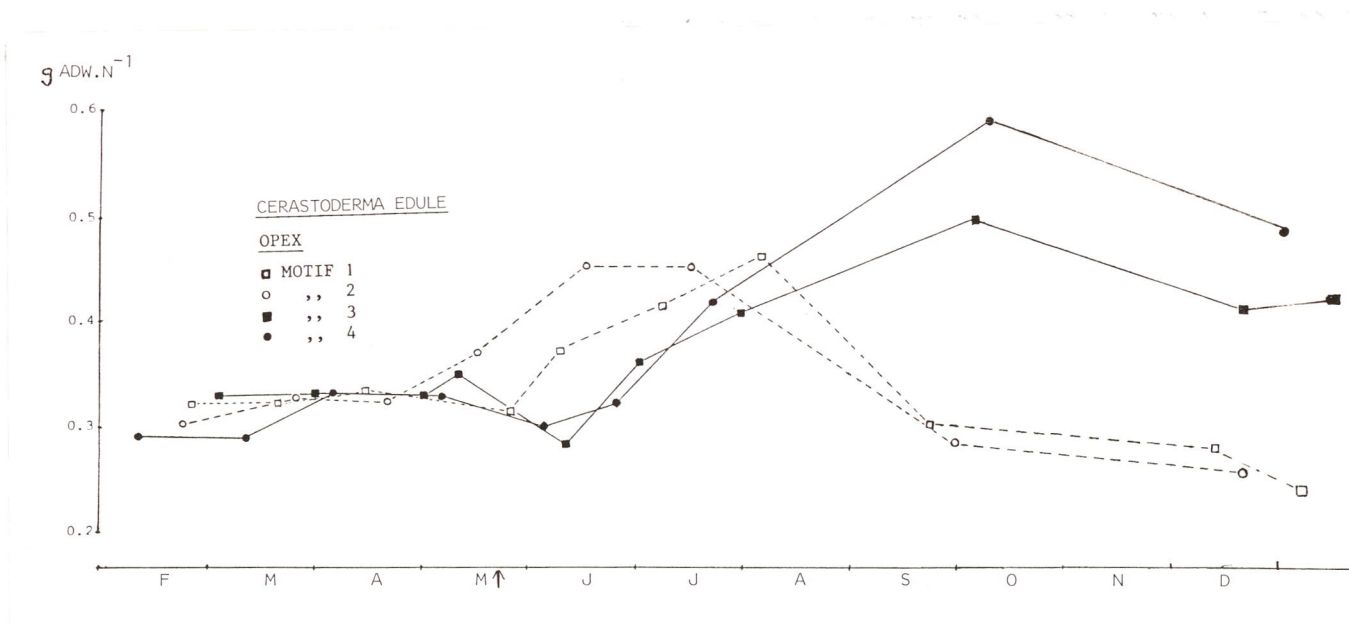


Fig. 30 The ash-free dry weight (ADW) per individual of *C. edulis* in the different MOTIFs during OPEX.

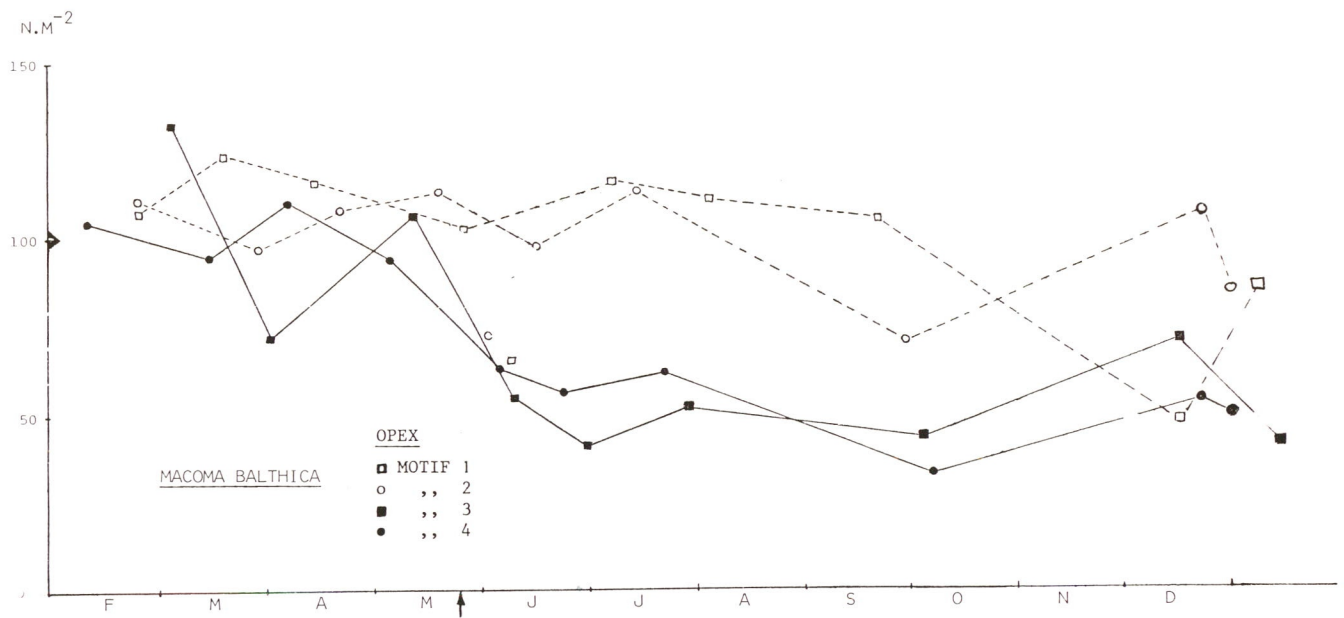


Fig. 31 Numbers of *M. balthica* in the different MOTIFs (results from sediment sampling) during OPEX.

The contaminated MOTIFs 3 and 4 suffered total losses due to sampling and observed mortality of 32 and 28% respectively, mortality being responsible for the major part. The numbers of empty shells collected represents a considerable underestimate for mortality, as the relatively small-sized and deeper-living shells will easily be lost in the sediment. The low numbers (well under 50 *Macoma*'s per m²) recovered in MOTIFs 3 and 4 early in October are, therefore, thought to be realistic, which was confirmed in December.

Fig. 32 shows the cumulative number of collected empty shells in the MOTIFs. In MOTIFs 1 and 2 a gradual and slow increase of these numbers point to natural mortality, commonly observed in populations of adult *Macoma*'s. The lines for MOTIF 3 and 4 show a quite different pattern. An increased number of dying and dead animals was detected shortly after the exposure, with mass mortality occurring, in the second week after exposure; 300 dead *Macoma*'s being found in MOTIF 3. Towards the end of June a normal level of mortality comparable to that in MOTIFs 1 and 2 was reestablished. A direct cause for the increased mortalities in June is not clear. Adult *Macomas* are known to be resilient animals, able to cope with a multitude of unfavourable environmental conditions, in particular when compared with the cockle. *Macoma* is predominantly a boreal species which thrives best at a temperature of

5-15°C. The combination of the extremely high summer temperatures in June (the temperature of the upper sediment layer in the oil-contaminated MOTIFs rose to over 30°C at low water), and the oil exposure was probably fatal. The temperature of the sediment in the uncontaminated MOTIFs were up to 5°C lower than those in the contaminated MOTIFs. In addition, in the controls *M. balthica* avoided higher temperatures by burrowing more deeply (~ 10 cm) in the sediment than in the contaminated MOTIFs where they were found at 2 cm depth.

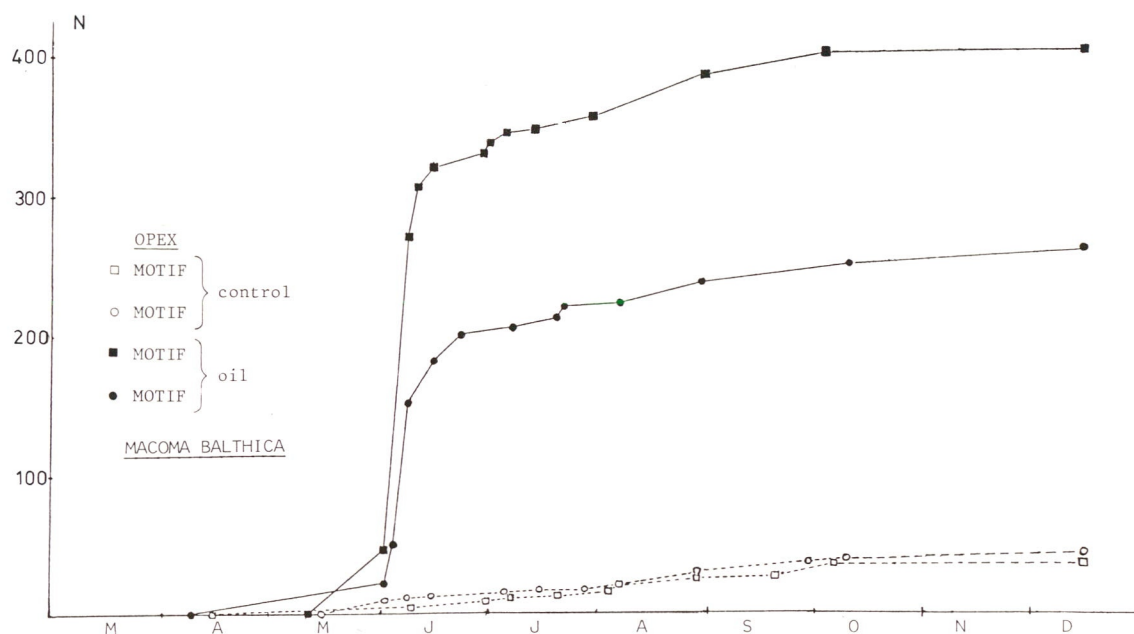


Fig. 32 Cumulative numbers of *M. balthica* found dead on top of the sediment in the different MOTIFs during OPEX.

The mean ADW of the soft parts per animal are given in Fig. 33. The initial ADW value early in March was about 90 mg. In spring the populations showed a slow weight increase. In June and July a rapid growth in the order of 300-400 mg per animal was seen in MOTIFs 1 and 2. This was followed by loss of weight in late summer and autumn. The oil cover in the MOTIFs 3 and 4 negatively effects growth as shown by a significant weight loss in June. Following a slight recovery phase in July, emaciation took place in the remainder of the year.

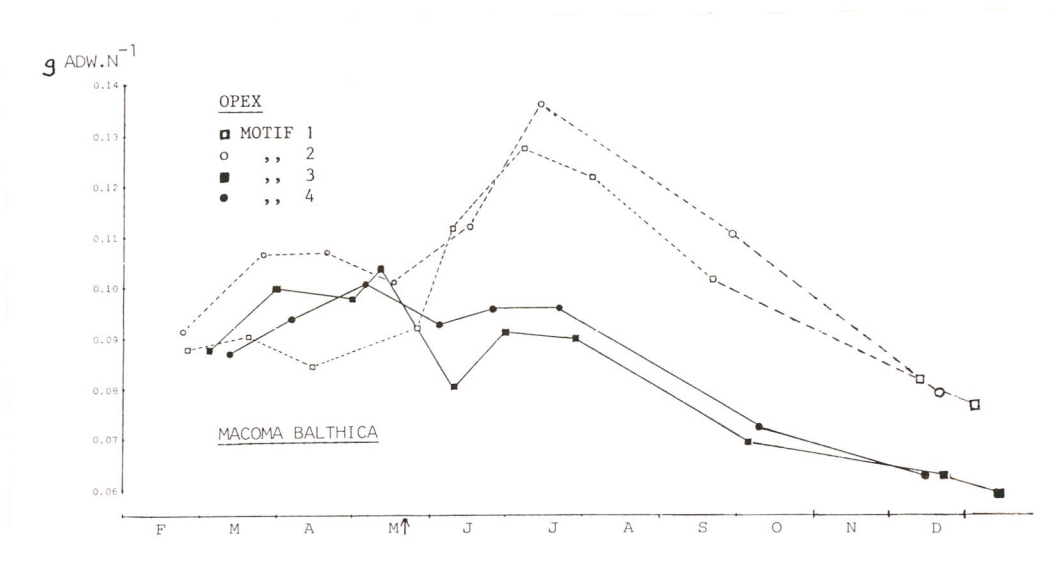


Fig. 33 The ash-free dry weight (ADW) per individual of *M. balthica* in the different MOTIFs during OPEX.

In natural populations of *M. balthica* in the Wadden Sea growth starts as early as March, maximum weight being reached towards the end of June, after which an extended period of gradual emaciation is found. The prolonged growth of adult *Macoma*'s in the uncontaminated MOTIFs is remarkable and may be related to deposit of more than usual organic material in the sediment.

Nereis diversicolor

As previously mentioned a number ragworms were introduced into the MOTIFs with the sediment. Their initial numbers, however, fell beneath the detection limits of the sampling applied, and their presence could only be concluded from the presence of feeding tracks on the sediment surface. The first juvenile *Nereis* were observed in small numbers in the second half of June (Fig. 34). These numbers rapidly increased in July, both in the contaminated and the uncontaminated MOTIFs. Maximum numbers being found in the contaminated MOTIFs. *Nereis diversicolor* thrives best at relatively high ambient temperature and is apparently not sensitive to oil exposure.

The high mortalities of *Macoma* and *Cerastoderma* in the MOTIFs 3 and 4 will have probably influenced the *Nereis* populations in a positive sense, by providing living space and easy accessible food to these carnivorous worms.

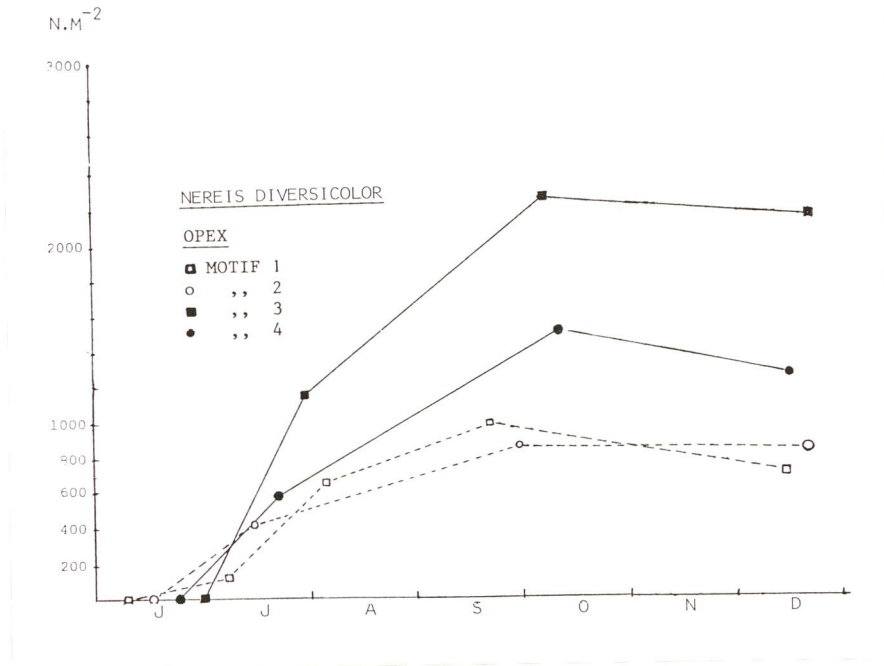


Fig. 34 Numbers of *N. diversicolor* in the different MOTIFs during OPEX.

Fig. 35 shows the average individual ash-free dry weights in the 4 populations. Early in autumn the average weights in MOTIFs 1, 2 and 3 was about 7.5 mg. Population densities in the contaminated MOTIF 3 was, however, twice as high as the densities in MOTIFs 1 and 2. Better feeding conditions in the contaminated environment of MOTIF 4 are reflected by lower population density and larger average weight.

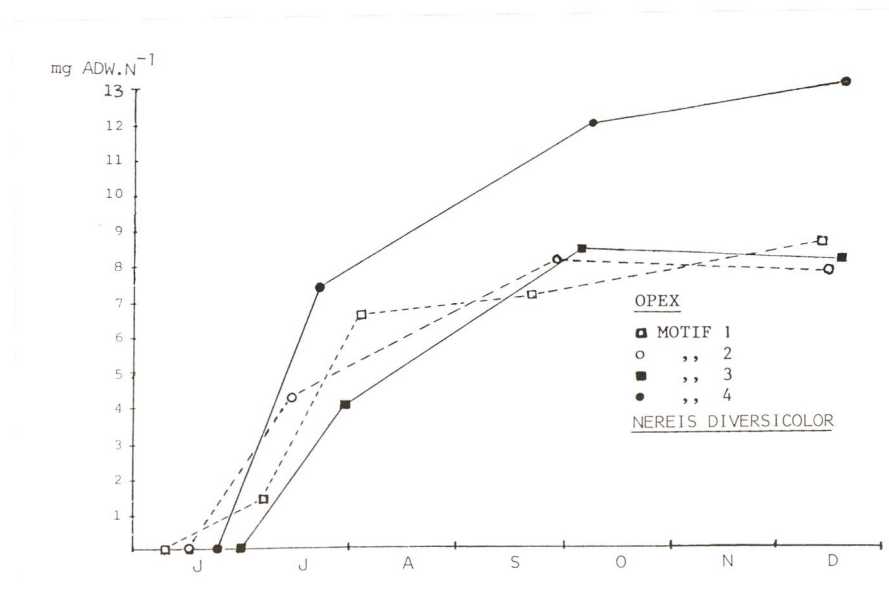


Fig. 35 The ash-free dry weight (ADW) per individual of *N. diversicolor* in the different MOTIFs during OPEX.

Arenicola marina

Fig. 36 shows the population density of lugworm in MOTIFs 1-4; 25 worms per m^2 where initially introduced into each MOTIF. The animals were easily damaged by the sampling method used, and 10 parts of lugworms were counted as one complete animal. During the whole period of observation, 47.7 animals as defined above were recovered from MOTIF 1. This was thought to correspond to about 80 sacrificed lugworms, or about 18% of the number introduced. For MOTIF 2 these data are 52.7-80-18%, for MOTIF 3: 52.9-80-18%, and for MOTIF 4: 57.7-90-20%.

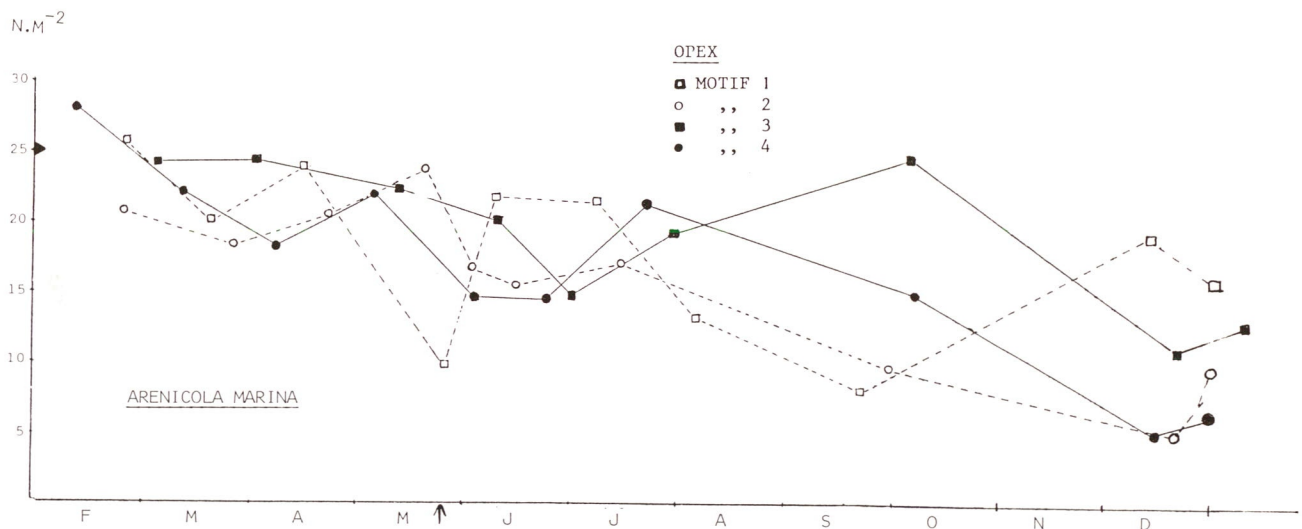


Fig. 36 Numbers of *A. marina* in the different MOTIFs during the experiment. Data of December represent the numbers of casts per m^2 obtained at the final sampling.

Although weakened and succumbing lugworms were observed at the sediment surface now and then, these were not systematically collected for an estimate of natural mortality. Foraging *Nereis* detect worms in this condition and draw them into their burrows. Although an unknown number of worms was eliminated in this way, the lines are still thought to present realistic population dynamics. The similar gradual declines in numerical densities do not indicate any significant effect of oil on the lugworm populations.

The sampling procedure adopted (sediment coring and sieving) may be even less adequate for an estimate of the numerical density in this large macrofauna species, than in the case of the cockle. Extreme values, such as those found in October in MOTIF 3, are therefore doubtful.

The annual variation in weight of adult lugworms mean ADW per animal is clearly shown in Fig. 37. Considerable growth is observed early in the season and continues into July. Loss of weight takes place after mid summer; this being a normal phenomenon in lugworms. The rapid weight decline and large variations in weight in autumn may be partly explained by differences in spawning time.

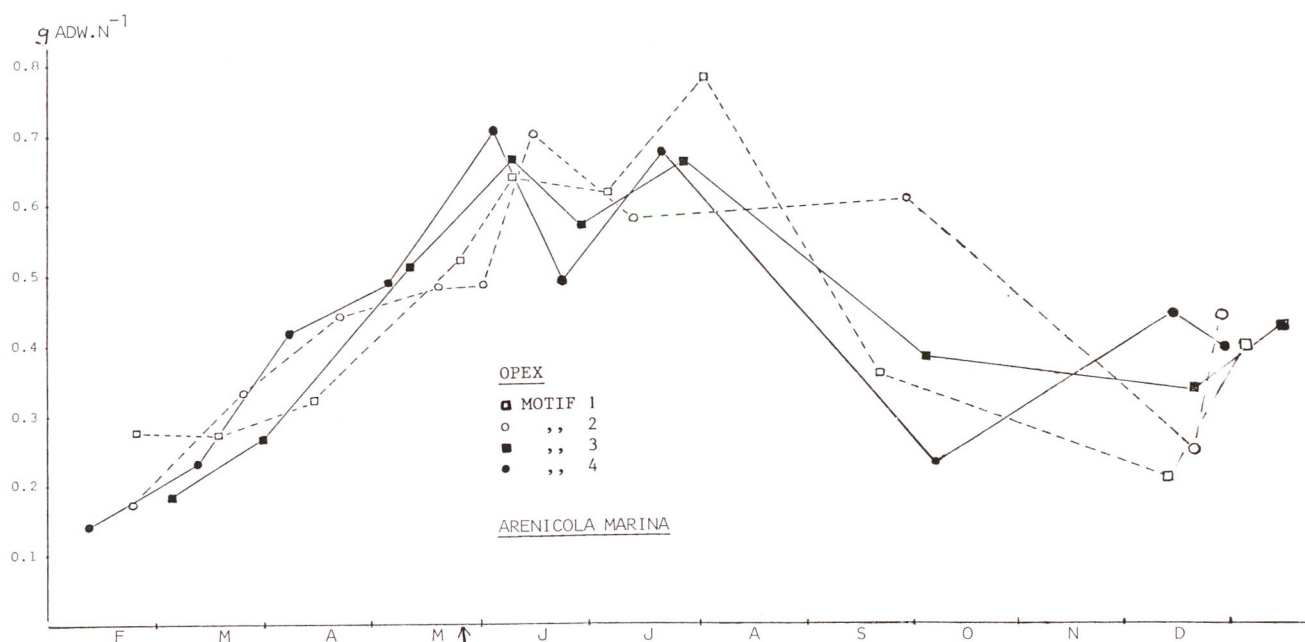


Fig. 37 The ash-free dry weight (ADW) per individual of *A. marina* in the different MOTIFs during OPEX.

Although visual observations of the oil contaminated MOTIFs, particularly MOTIF 4, sometimes suggested large localized mortalities and restricted (feeding) activities, the data presented in Fig. 37 show hardly any significant differences between the 4 populations.

Pygospio elegans

Fig. 38 shows the population densities of one of the smaller tube-building polychaetes. *Pygospio* belongs to the group of relatively short-living summer annuals. As they were not observed shortly after the start of the experiment, they probably entered the systems with the supply water as larvae or juveniles. Small numbers were already present in late winter and these gradually increased during spring. Maximum numbers were reached early in June (MOTIF 1) or July (MOTIF 2, 3 and 4). Numerical densities gradually reduced during summer and autumn. Significant differences between contaminated and uncontaminated MOTIFs were not found. The considerable variation in numbers in MOTIF 1 and 2 towards the end of June is questionable, but could perhaps be explained by size differences between individuals of the two populations, the larger animals being just retained by a mesh size of 1 mm.

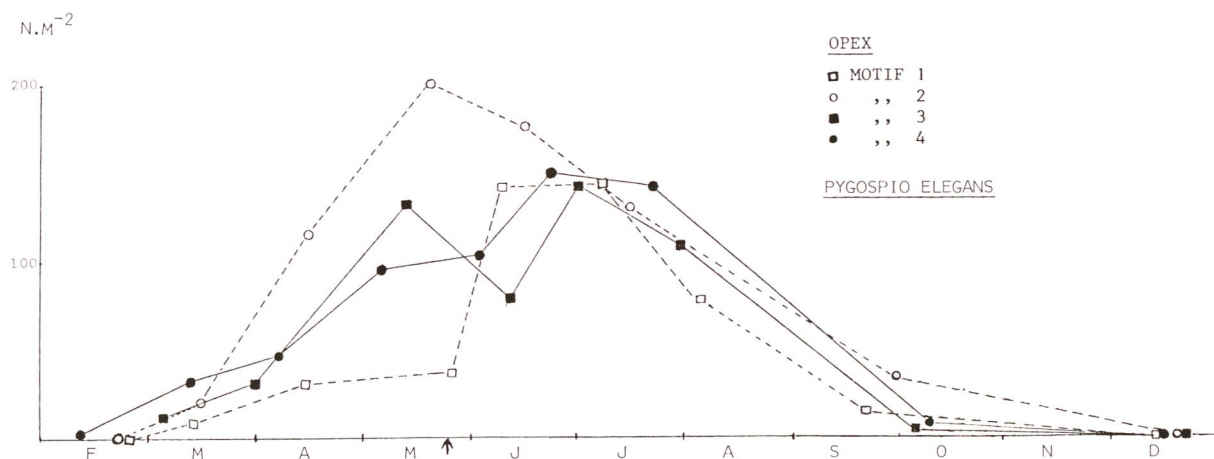


Fig. 38 Numbers of *P. elegans* in the different MOTIFs during OPEX.

Hydrobia ulvae

The common mudsnail *Hydrobia ulvae* was observed in small numbers at the start of the experiment and must have been introduced with the sediment. From March to mid-June the population densities were similar at about 100-200 specimen per m² in all MOTIFs (Fig. 39).

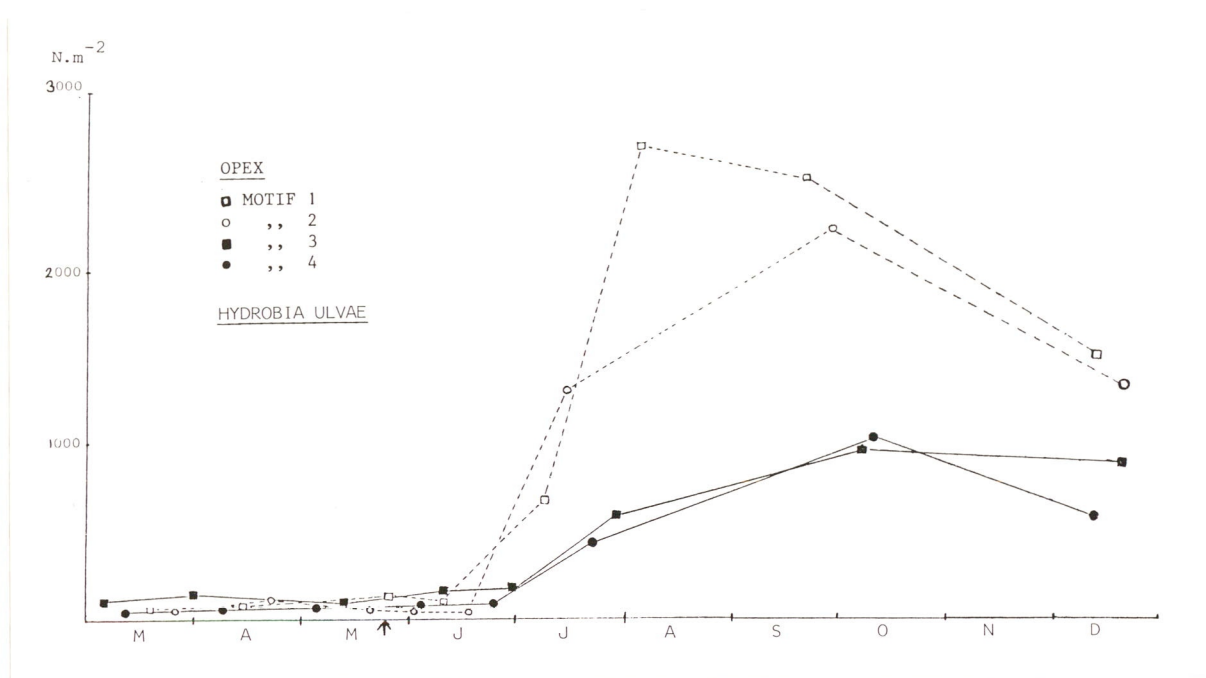


Fig. 39 Numbers of *H. ulvae* in the different MOTIFs during OPEX.

In July the numbers in the uncontaminated MOTIF 1 and 2 rapidly increased, reaching maximum values of 2000-3000 in summer. Numerical increase of the population in the contaminated MOTIFs occurred much more slowly; maximum numbers of only 1000 per m² being found in October. Settlement and/or reproduction of *Hydrobia* are negatively effected by the presence of oil in MOTIFs 3 and 4.

Gammarus spec.

The presence of this small crustacea - also a summer annual - in the intertidal flats of the Wadden Sea is highly dependent on the presence and abundance of thallos and filamentous macro-algae (*Ulva* and *Enteromorpha*). Macro-algae were present in the uncontaminated MOTIFs in June and July.

In these MOTIFs *Gammarus* rapidly increased in numbers and size in June (Fig. 40). Considerable differences in maximum numbers found between MOTIFs 1 and 2 in July can be explained by clustering of *Gammarus* caused by the presence of algal pockets which were inadequately sampled by the method applied. The wane of the algae brought about a concomitant decline of the *Gammarus* stock. In the contaminated MOTIFs 3 and 4 only very small populations of *Gammarus* were found in July. *Gammarus* occasionally was found in the zooplankton samples from the uncontaminated MOTIFs, but hardly any species were found in the contaminated MOTIFs. This *Gammarus* species is directly or indirectly negatively effected by oil.

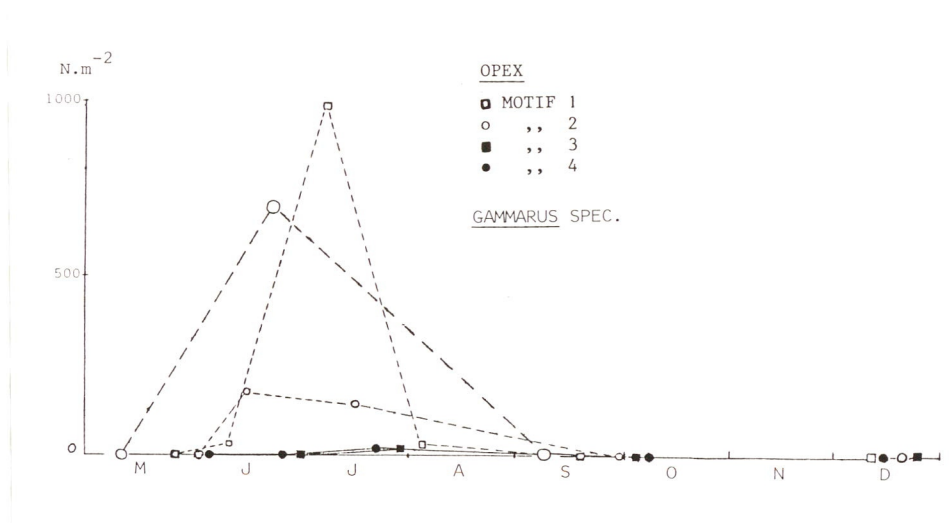


Fig. 40 Numbers of *Gammarus* sp. in the different MOTIFs during OPEX. The upper line for MOTIF 2 represents numbers retained by a 400 μ m sieve, the other lines represent numbers retained by a 1000 μ m sieve.

Corophium volutator

Fig. 41 shows the development of *C. volutator* in the MOTIFs.

Corophium is a common and extremely abundant crustacean which reaches dense populations in summer and autumn in near shore mud-flats. Increasing numbers were observed from June onwards in the uncontaminated MOTIFs. The large numbers of juveniles collected from sediment cores sieved over a 400 μ m screen indicate that the increasing numbers in the MOTIFs were caused by settlement of larvae carried with the supply water.

Settlement of larvae and juveniles in the contaminated MOTIFs 3 and 4 could not be observed; no *Corophium* being found, even five months after the adding of oil mousse. *Corophium*, a very important food item for commercially important juvenile flat-fish species, shrimps and some birds in natural ecosystems is completely unable to settle in oil-contaminated environments.

Littorina littorea

Fivehundred adult periwinkles were placed in each MOTIF on 19 February. These were sampled only once, in October. They were carefully picked from the walls by hand and transferred to the laboratory, where the maximum length of the snails was measured in mm with a sliding calipers, or (for the smallest animals) under a stereomicroscope fitted with a mm grid.

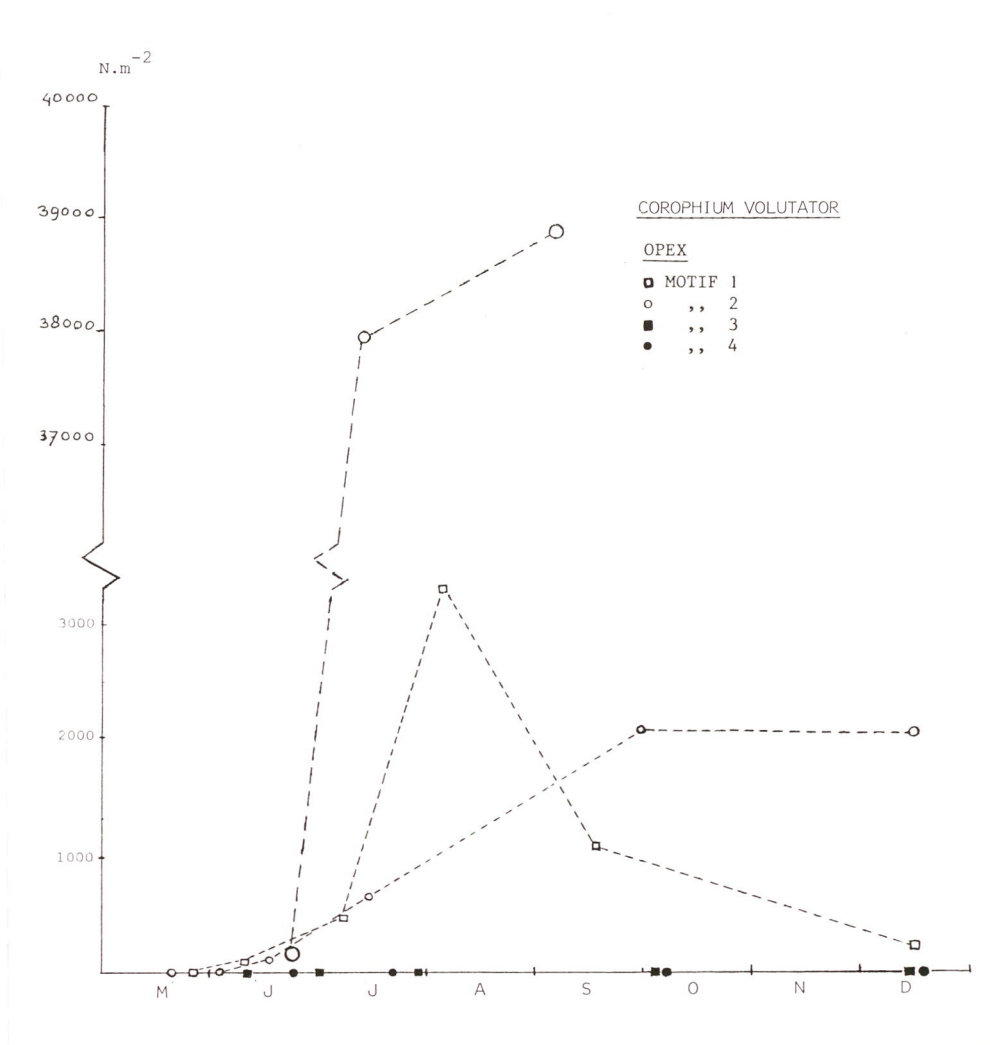


Fig. 41 Numbers of *C. volutator* in the different MOTIFs during OPEX.

Fig. 42 shows the length frequency distribution of the 4 populations in October. The larger individuals (larger than 15 mm) represent the surviving members of the initial stock. Survival was highest in the uncontaminated MOTIF 1, and lowest in contaminated MOTIF 3. Survivals in MOTIFs 2 and 4 were similar, and intermediate to those of MOTIFs 1 and 3. The smaller individuals (less than 15 mm) are young and show the reproduction success of *Littorina*, either derived from the parent stock in the MOTIF or derived from larvae imported from outside with the supply water. It is clear that new generations are almost absent in the contaminated MOTIFs whereas considerable numbers of juvenils are found in the uncontaminated MOTIFs 1 and 2. Differences in the populations in these latter MOTIFs cannot be explained, but may be related to minor technical faults in the supply water flow. Oil is extremely harmless to the periwinkle population, as it prevents reproduction and/or larval settlement.

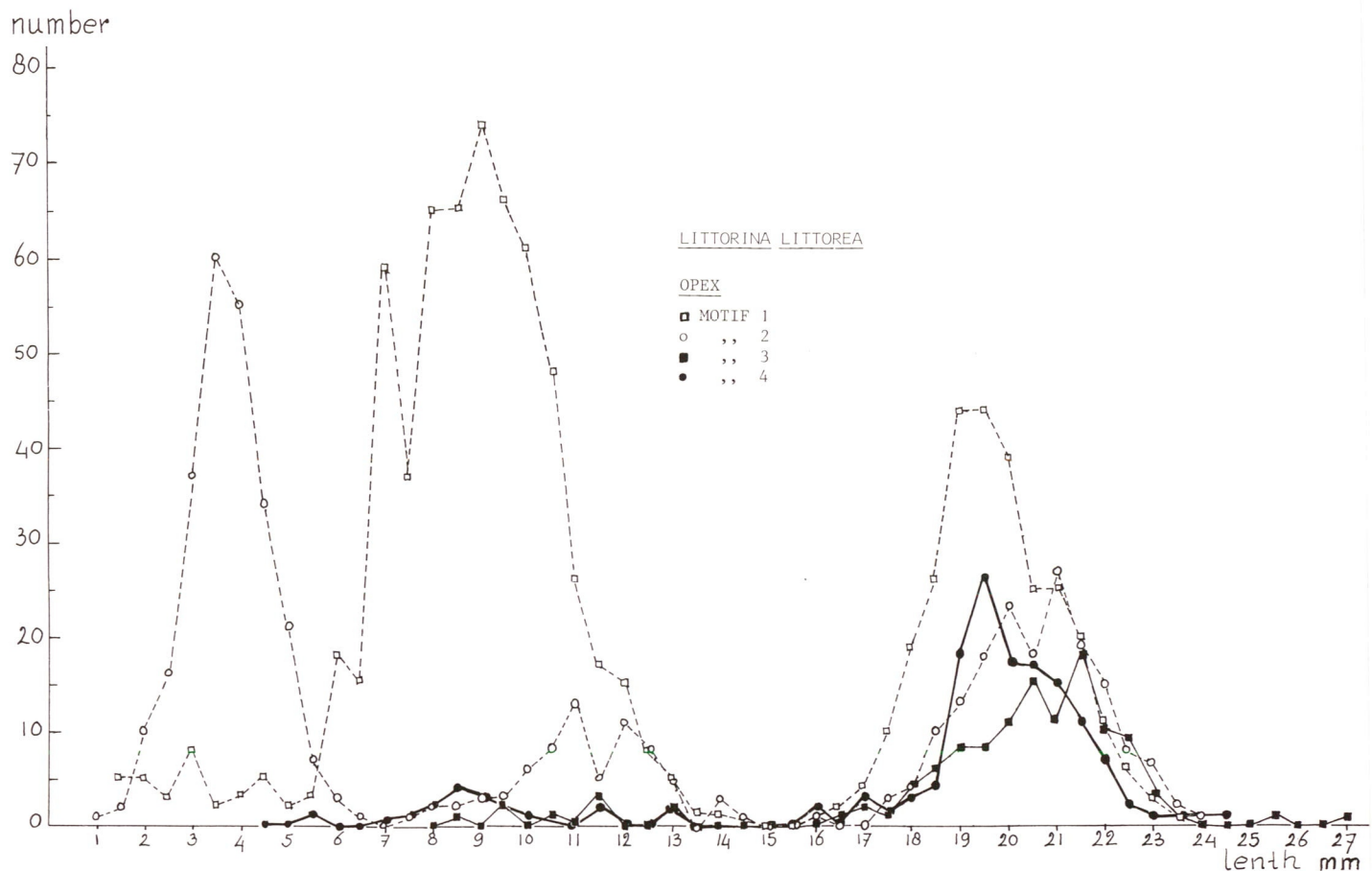


Fig. 42 Length-frequency distributions of *L. littorea* in the different MOTIFs in October 1982.

Heteromastus filiformis

The medium sized, deep living polychaete worm *Heteromastus filiformis* was observed for the first time in considerable numbers (100-200 specimens per m²) in September in the uncontaminated MOTIFs 1 and 2, whereas numbers in the contaminated MOTIFs 3 and 4 remained low. This situation continued into December (Fig. 43). With 3-4 g ADW/m² *Heteromastus* made an important contribution to the total biomass of the unpolluted systems. Settling and/or reproduction of *Heteromastus filiformis* is negatively affected by the oil addition.

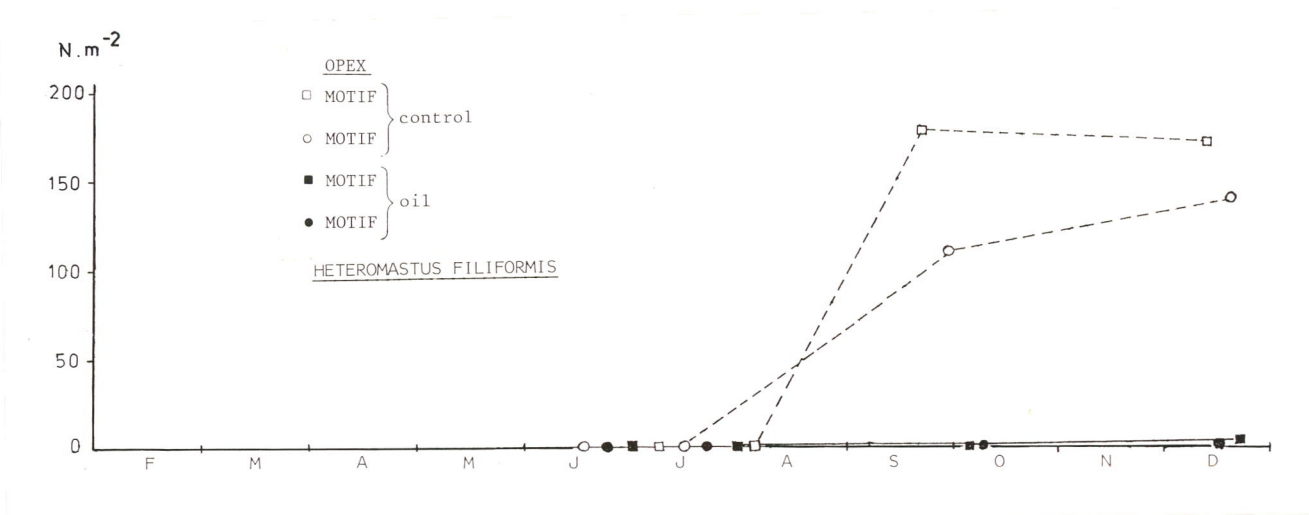


Fig. 43 Numbers of *Heteromastus filiformis* in the different MOTIFs during OPEX.

Other species

During the intensive final sampling programme in December 1982 species occurred in the samples which had not been found in the previous, smaller, samples. Firstly the bivalve *Mya arenaria* was found in the uncontaminated MOTIFs 1 and 2 in population densities of 1.1-2.8 per m². Apparently they had grown from larvae introduced to the MOTIFs with the supply water from the Wadden Sea. This species was not found in the oil contaminated MOTIFs, so that it can be concluded that the development of *Mya* was negatively influenced by the oil addition.

During the final sampling also adult fish, *Gobius minutus*, was found in the controls, but not in the oil contaminated MOTIFs.

One adult common shrimp, *Crangon crangon*, was found in control MOTIF 2, this one specimen shows that shrimps can be introduced, and can live in the MOTIFs, but does not allow any conclusion about the effect of the added oil.

Finally the very large polychaete, *Nereis virens* (about 30 cm), was found in all MOTIFs, with exception of MOTIF 3.

3.2.7 Development of the biota in the watercolumn

3.2.7.1 Phytoplankton and nutrients

Fig. 44 shows the development of the phytoplankton biomass, measured as chlorophyll concentrations, in the water of the MOTIFs. The results from both pairs of MOTIFs (1 and 2, 3 and 4) are pooled because the same water was pumped between the two MOTIFs of each pair.

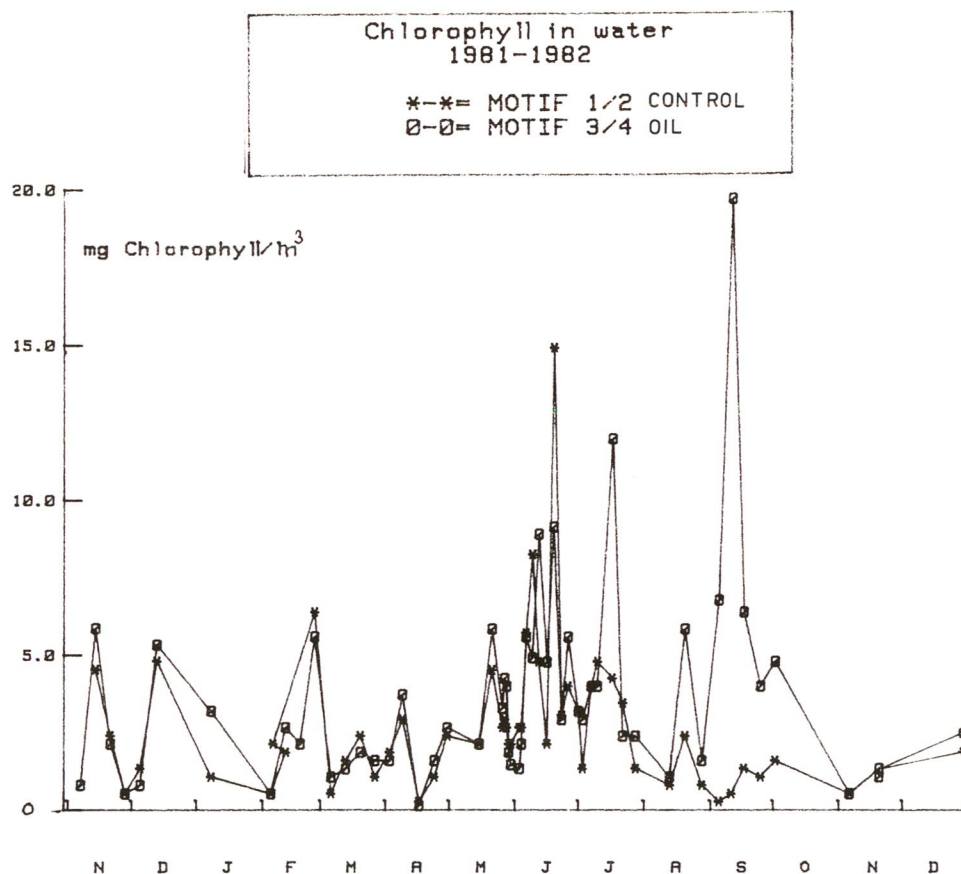


Fig. 44 Chlorophyll concentrations in the water of the MOTIFs during OPEX.

The results show that the phytoplankton density was generally low. Chlorophyll maxima were observed in November and December 1981 and February 1982, these maxima were caused mainly by diatoms. The development of the phytoplankton up to the addition of the oil, followed very similar patterns in all MOTIFs.

Table 9 Species composition of the phytoplankton in the water of the MOTIFs during the period March-May.

The density per sampling date is indicated by:

X = < 10	cells.ml ⁻¹	5 = 5-10x 10 ² cells.ml ⁻¹
R = 10-25	" "	6 = 1-2.5x10 ³ " "
1 = 25-50	" "	7 = 2.5-5x10 ³ " "
2 = 50-10 ²	" "	8 = 5-10 x10 ³ " "
3 = 1-2.5x10 ²	" "	9 = > 10 ⁴ " "
4 = 2.5-5x10 ²	" "	

Table 9

Date	4.3	11.3	18.3	25.3	1.4	7.4	15.4	22.4	29.4	6.5	13.5
MOTIF no.	2 4	1 3	2 4	1 3	2 4	1 3	2 4	1 3	2 4	1 3	2 4
<u>Species</u>											
<u>Centricae</u>											
Melosira nummuloides	R	R 2	1 R	X R	X X	1	R	1 R	R X		
M. varians		R	X	X X		1			R		
Skeletonema costatum	R R	R R	1 1	2 2	4 4	6 6	3 3	X 1	R R	R X	1 R
Thalassiosira decipiens	R	1	1 R	1 1	2 2	1			R R	X	
Th. nordens kiöldii			X X						R X		
Leptocylindrus danicus											R R
Rhizosolenia delicatula	R R	X	1 1	R R					R R	X X	3 3
Rh. shrubsolii		R		X	X R	X			X X	X X	R R
Rh. setigera					X X					X X	1 R
Chaetoceros spp.	X		X X			X	X R				
Biddulphia aurita	X	X		X	X	1	X				
B. regia							X				
Ceretaulina bergonii			X	X	X X	X					
<u>Pennatae</u>											
Phagiogramma brockmannii								X R	R	1 1	
Thalassionema nitzschioides	R X	X	1 R	R R	1 1	R X	X	X X			
Asterionella kariana	X	R R	X X	1 R	1 1						
A. japonica					R						
Nitzschiaceae	3 6	5 5	4 6	2 3	2 3	3 3	2 2	2 2	2 1	2 2	2 2
Naviculaceae	4 3	4 4	6 5	5 5	4 4	4 5	4 4	3 3	4 4	4 4	3 4
Flagellates	6 5	5 5	5 5	5 4	5 5	6 6	7 7	7 7	8 7	7 6	7 7
µ-flagellates	6 6	7 6	7 7	6 6	6 6	7 7	7 7	6 6	8 8	9 9	9 9
<u>Fresh water algae</u>											
Scenedesmus spp.	X	1	X R	R R		R	X	X X	R R		
others	X	R R	X	X X	X X					R	

Table 10 Chlorophyll concentrations in the water of the MOTIFs. Oil was added on day 0.

date	day	uncontaminated	contaminated	ratio uncontaminated : contaminated
24.5	0	3.34	3.12	0.93
25.5	1	2.94	4.27	1.45
26.5	2	2.01	4.14	2.06
27.5	3	2.14	2.07	0.97
28.5	4	2.01	1.96	0.98
1.6	8	2.21	1.27	0.57
2.6	9	2.47	1.94	0.79
4.6	11	5.34	5.28	0.99

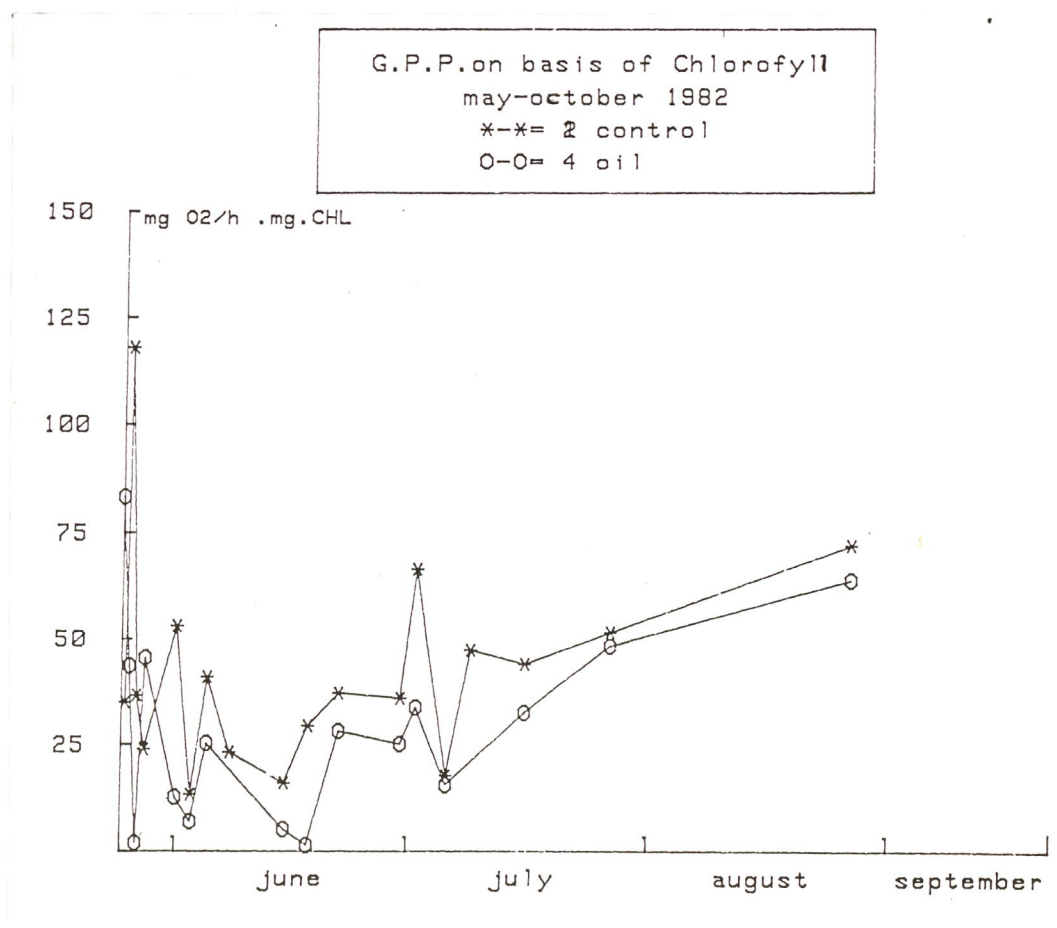


Fig. 45 Gross primary productivity in the water of the different MOTIFs during the period June-September 1982.

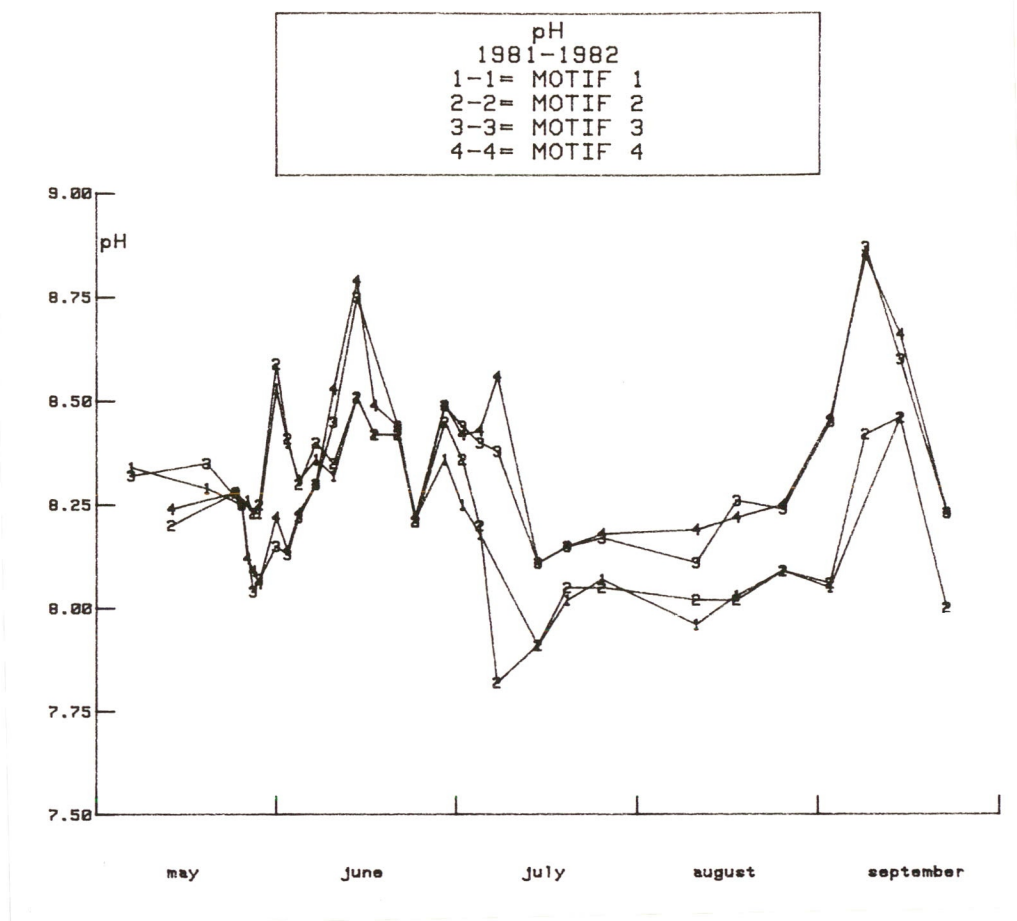


Fig 46 The pH of the water in the MOTIFs during the period May-September 1982.

After the oil addition, only a few phytoplankton samples were inspected for species composition determination. Results are given in Table 11. In August and September, numbers of flagellates were much higher in the contaminated MOTIFs than in uncontaminated MOTIFs, due to growth of a species not present in the latter. The numbers of μ -flagellates were also much higher in the contaminated MOTIFs. In September Nitzschiaceae and Naviculaceae formed blooms in the uncontaminated, but not in the contaminated, MOTIFs. This reduction of diatom numbers due to oil exposure was also found for other species (*Phagiogramma* sp. end May, *Chaetoceros* spp. July-September, *Rhizosolenia* spp. May-July, September, *Skelotonema costatum* September). Only Naviculaceae were found in any numbers in the contaminated MOTIFs. The shift in the species composition in the contaminated MOTIFs in favour of the flagellates is shown by the F/D ratio (numbers of flagellates divided by the number of diatoms) in Table 11.

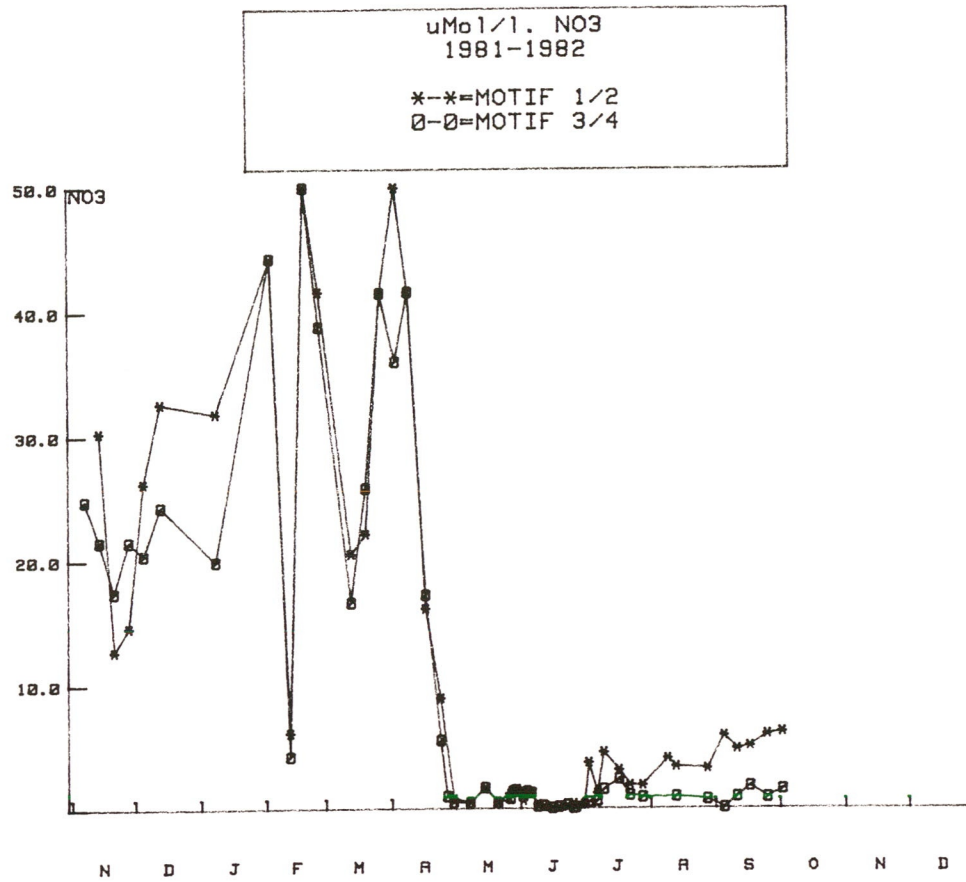


Fig. 47a

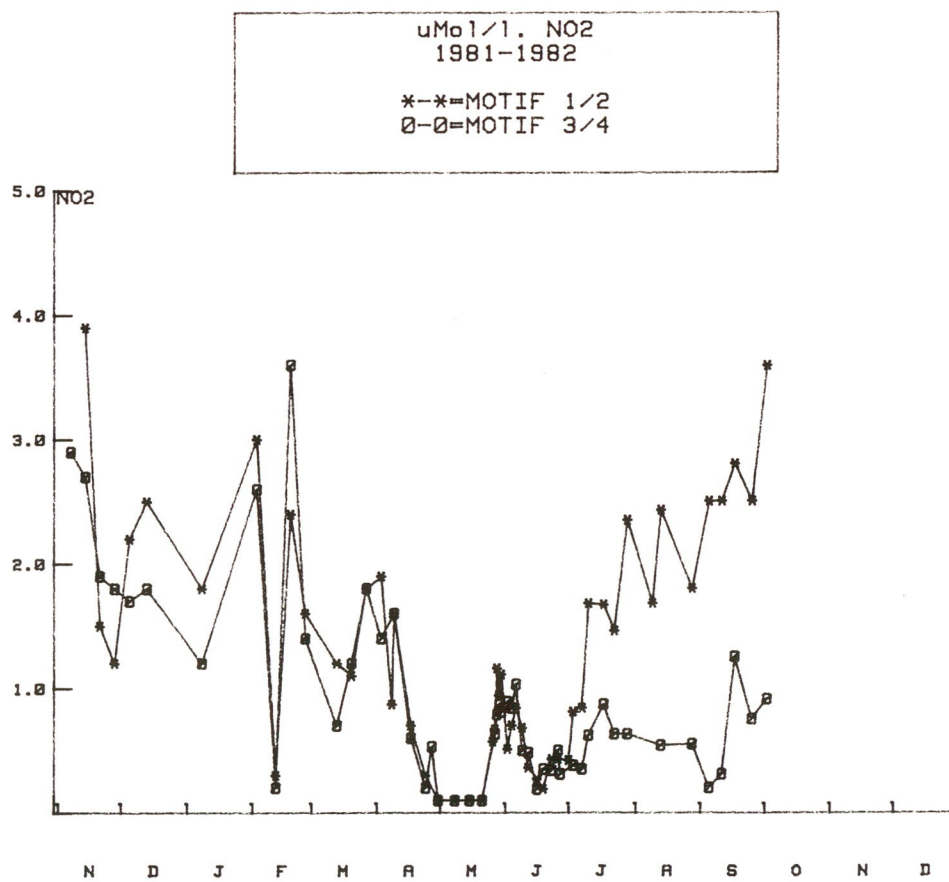


Fig. 47b

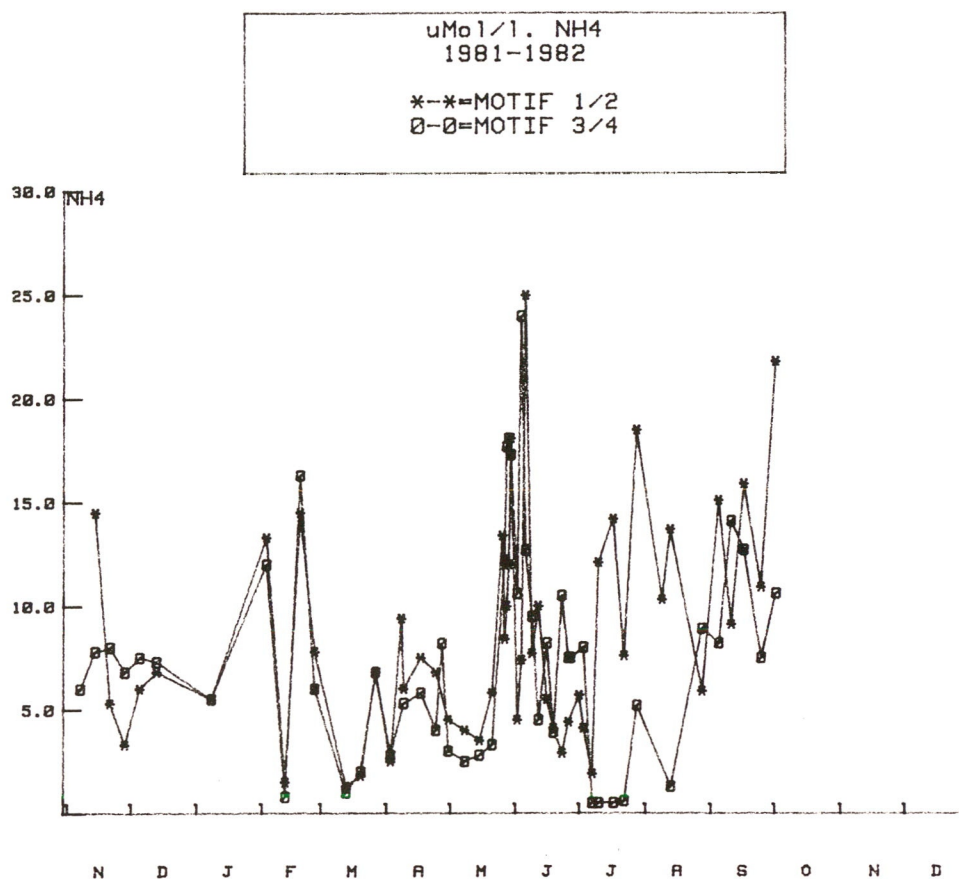


Fig. 47c

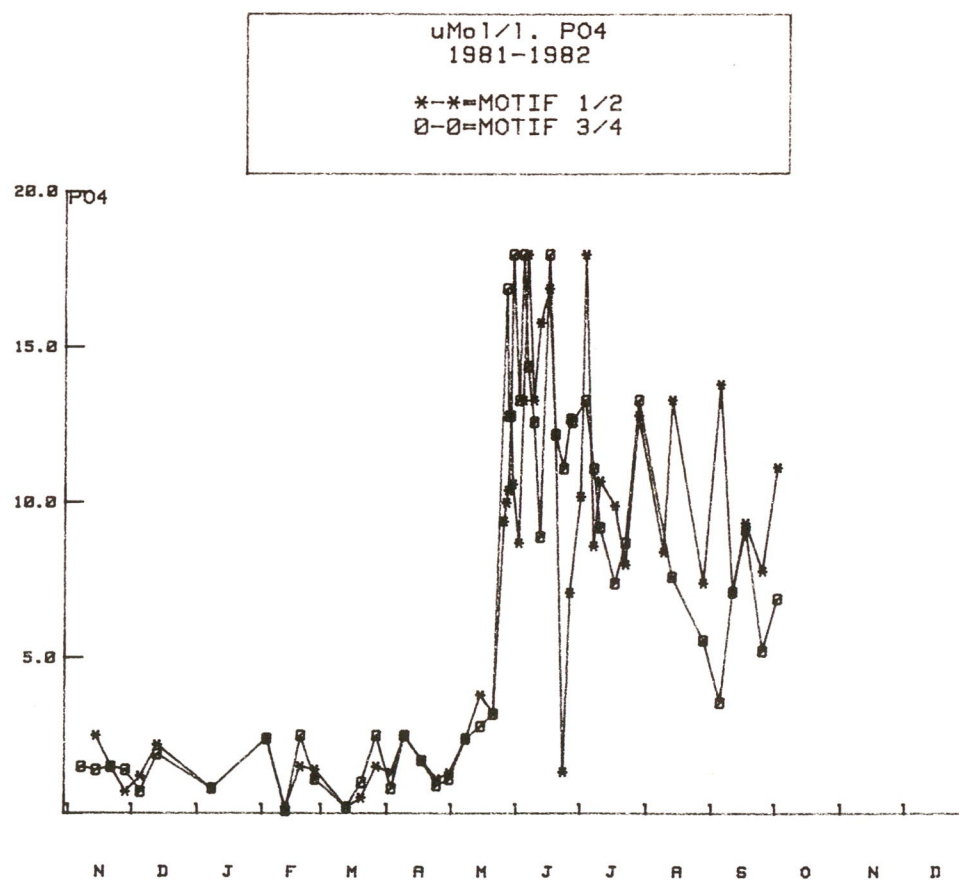


Fig. 47d

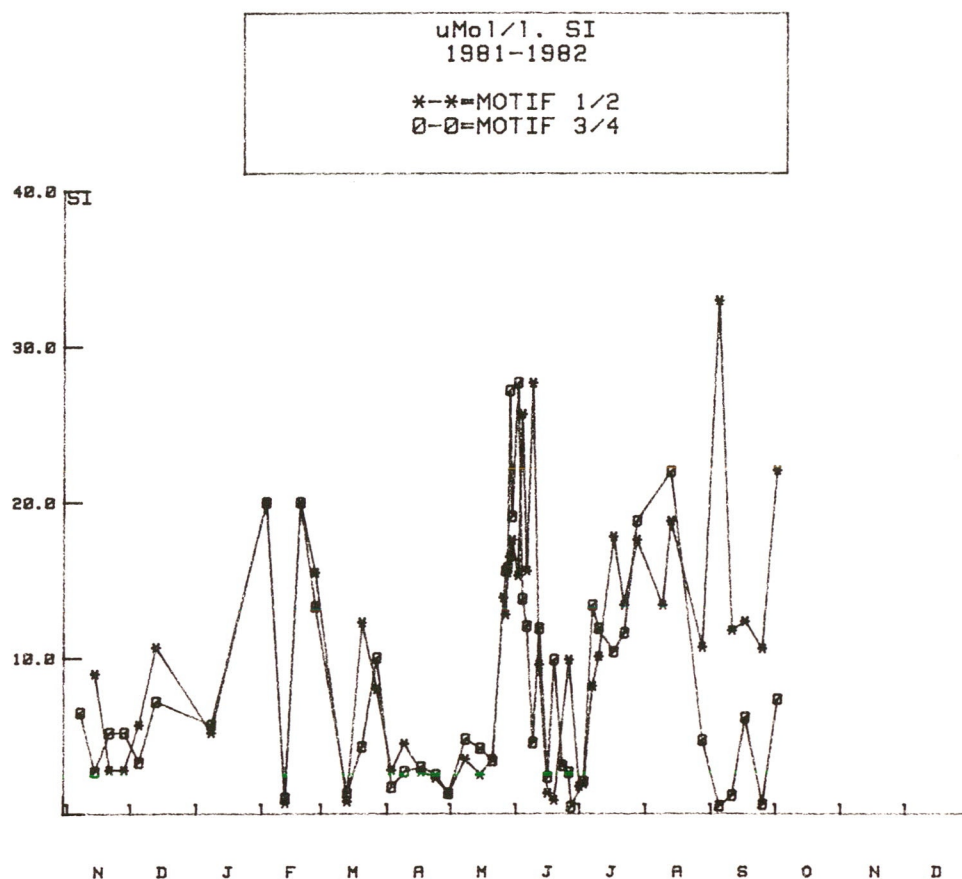


Fig. 47e

Fig. 47 Concentrations of ammonia (a), nitrate (b), nitrite (c), phosphate (d) and silicate (e) in the water of the MOTIFs during OPEX.

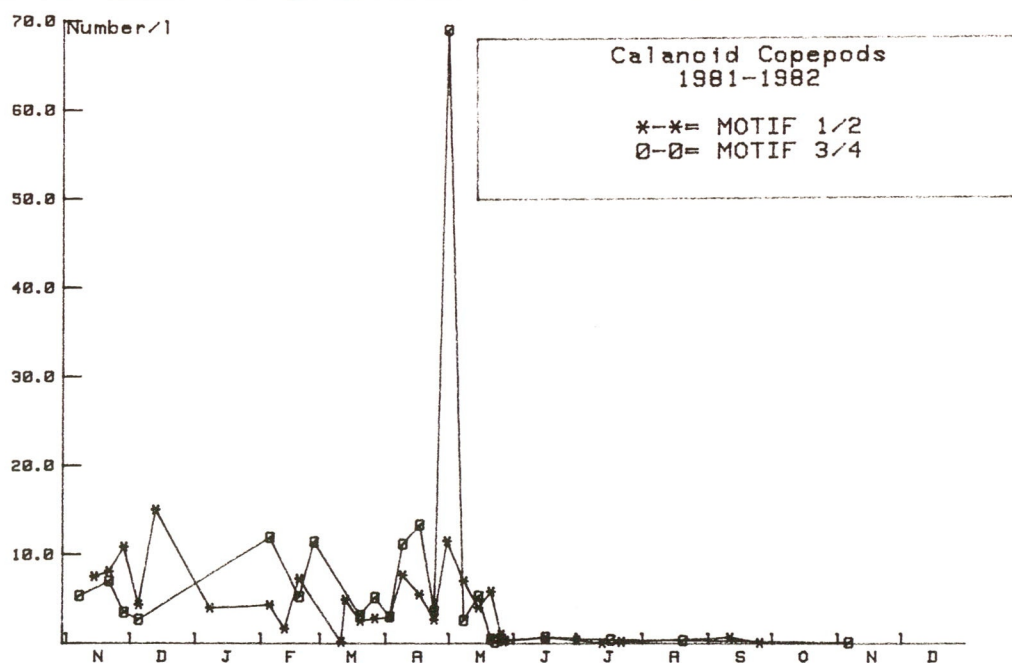


Fig. 48a

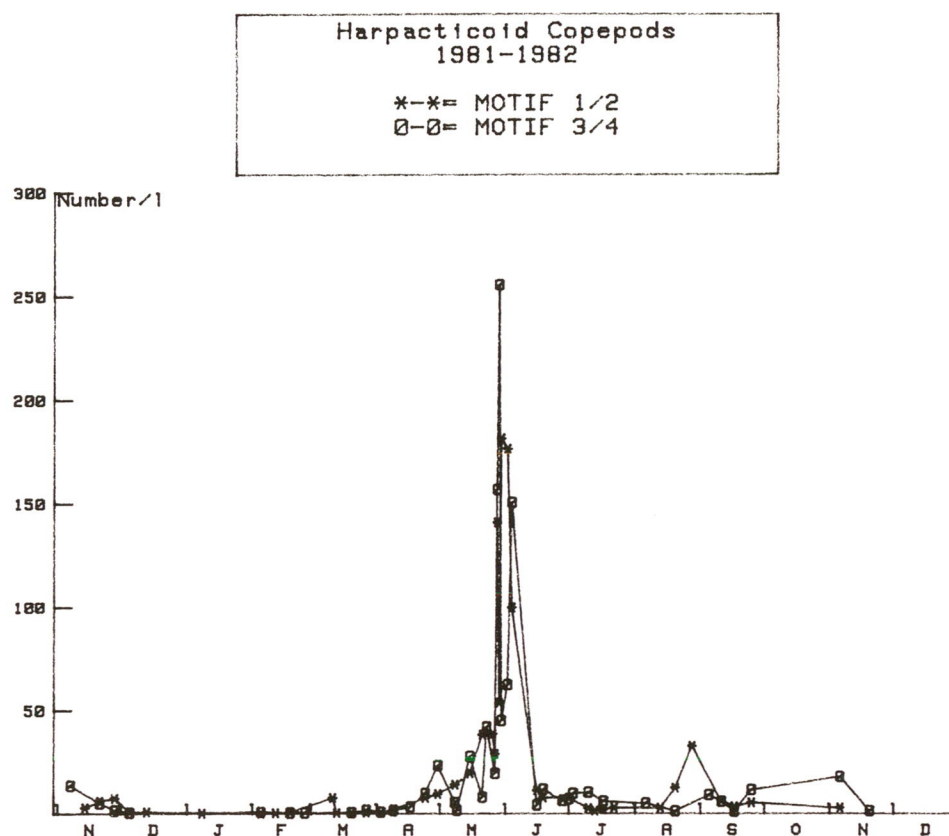


Fig. 48b

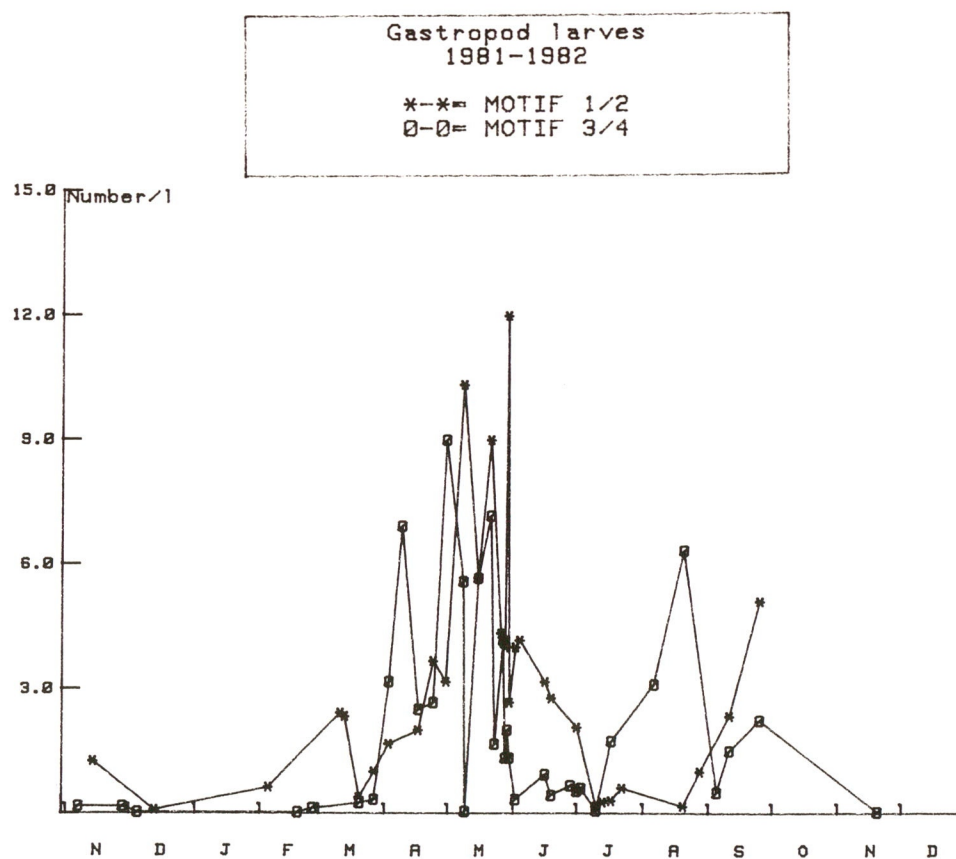


Fig. 48c

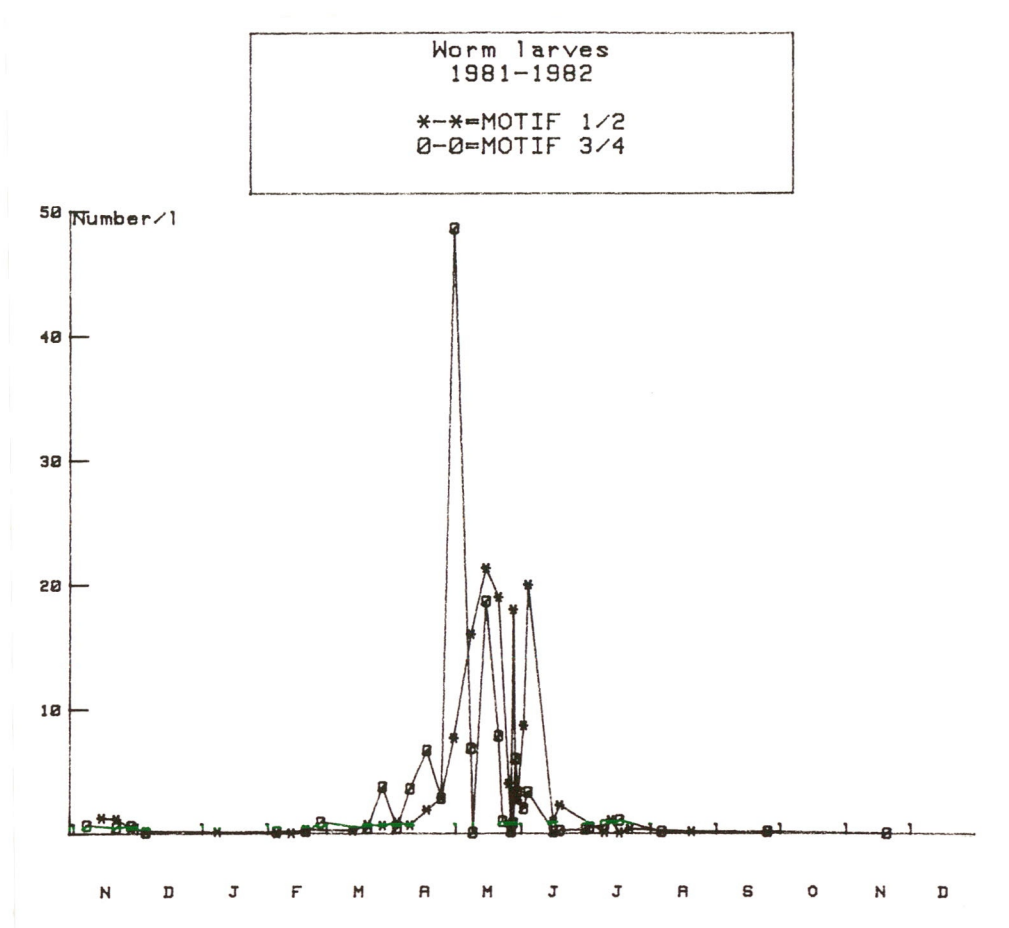


Fig. 48d

Fig. 48 Numbers of calanoid copepods (a), harpacticoid copepods (b), gastropod larvae (c) and larvae of worms (d) in the water of the MOTIFs during OPEX.

Larves of worms were also found throughout the experiment, reaching maximum numbers in May. Eggs and veligers of gastropods were found from February to September. They were not identified at species level.

Addition of the oil at the end of May resulted in lower numbers of harpacticoid copepods in June, although numbers in all MOTIFs are low by then. Calanoid numbers were too low to detect differences between the different MOTIFs. The numbers of larvae of gastropods were lower in the contaminated MOTIFs in June, a peak was found in the contaminated MOTIFs in July, but not in the uncontaminated. Interpretation of these results is not very clear.

3.2.7.3 Bacteria

Directly after the start of the experiment a very large bloom of bacteria developed in all MOTIFs reaching a maximum of more than 10^8 bacteria per ml around the middle of November (Fig. 49). This bloom collapsed (or was washed out of the system), and from the end of November 1981 to the end of the experiment bacterial numbers were between $1-8 \times 10^6$ per ml. These numbers are comparable to those found in the Wadden Sea.

From March to May numbers increased, probably as a reaction to the increased production of the phytoplankton and the phyto-benthos. The addition of the oil mousse did not result in detectable effects on the total numbers of bacteria in the water.

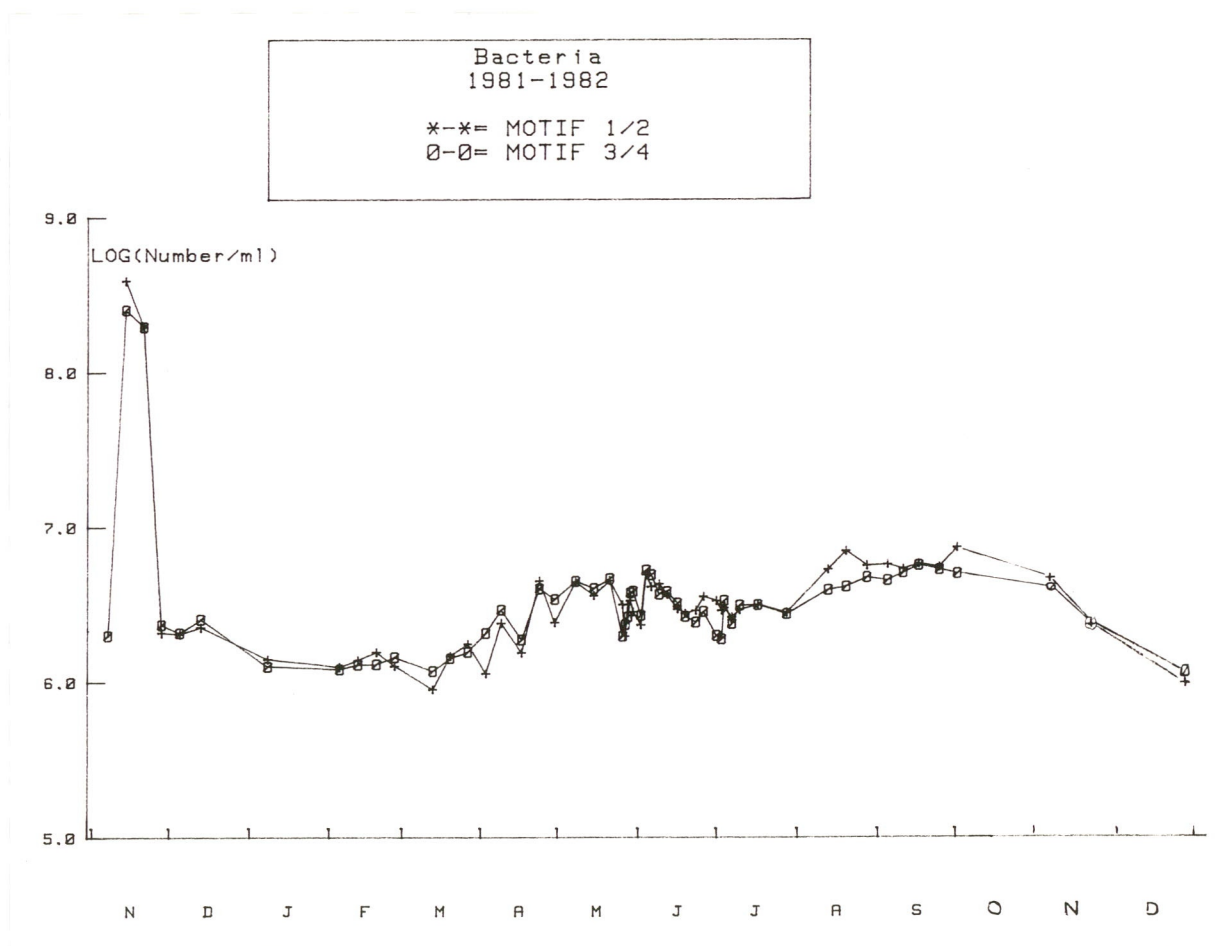


Fig. 49 Numbers of bacteria in the water of the different MOTIFs during OPEX.

Table 12 Summary of short-term (May-June) and long-term (July-December) effects of the oil addition on different organisms in the MOTIFs.
+ = higher value of the parameter in the contaminated MOTIFs than in the uncontaminated MOTIFs; - = lower value in the contaminated MOTIFs; 0 = no detectable difference between contaminated and uncontaminated MOTIFs.

organisms	parameter	short term	long-term
phytobenthos	chlorophyll	+	0
phytobenthos	O ₂ -production per chlorophyll	(-?)	-
bacteria benthos	Electron Transport System	+	+
<u>Meiofauna</u>			
Oligochaet 1	numbers	0	0/-
Paranaïs/Capitella	numbers	0	+
Nematods	numbers	-	-
Harpacticoids	numbers	+	0
<u>Macrofauna</u>			
Mytilus edulis	numbers	0	0
Mytilus edulis	ash-free dry weight	-	0(?)
Cerastoderma edule	numbers	0	-
Cerastoderma edule	ash-free dry weight	-	+
Macoma balthica	numbers	-	-
Macoma balthica	ash-free dry weight	-	-
Mya arenaria	numbers	(?)	-
Hydrobia ulvae	numbers	0	-
Littorina littorea	reproduction	(?)	-
Arenicola marina	numbers	0	0(-*)
Arenicola marina	ash-free dry weight	0	0
Nereis diversicolor	numbers	0(?)	+
Nereis diversicolor	ash-free dry weight	0	0
Pygospio elegans	numbers	0	0
Heteromastus filiformis	numbers	0	-
Corophium volutator	numbers	-	-
Gammarus spp.	numbers	-	not present
<u>Zooplankton and fish</u>			
copepods	numbers	0	0
larvae	numbers	-	(-?)
Gobius minutus	numbers	(?)	-
<u>Phytoplankton</u>			
	chlorophyll	-	+
	species composition	different	different
	O ₂ -production per chlorophyll	+/-	-
Bacteria water	numbers	0	0

* Arenicola was absent at locations with the highest oil concentrations.

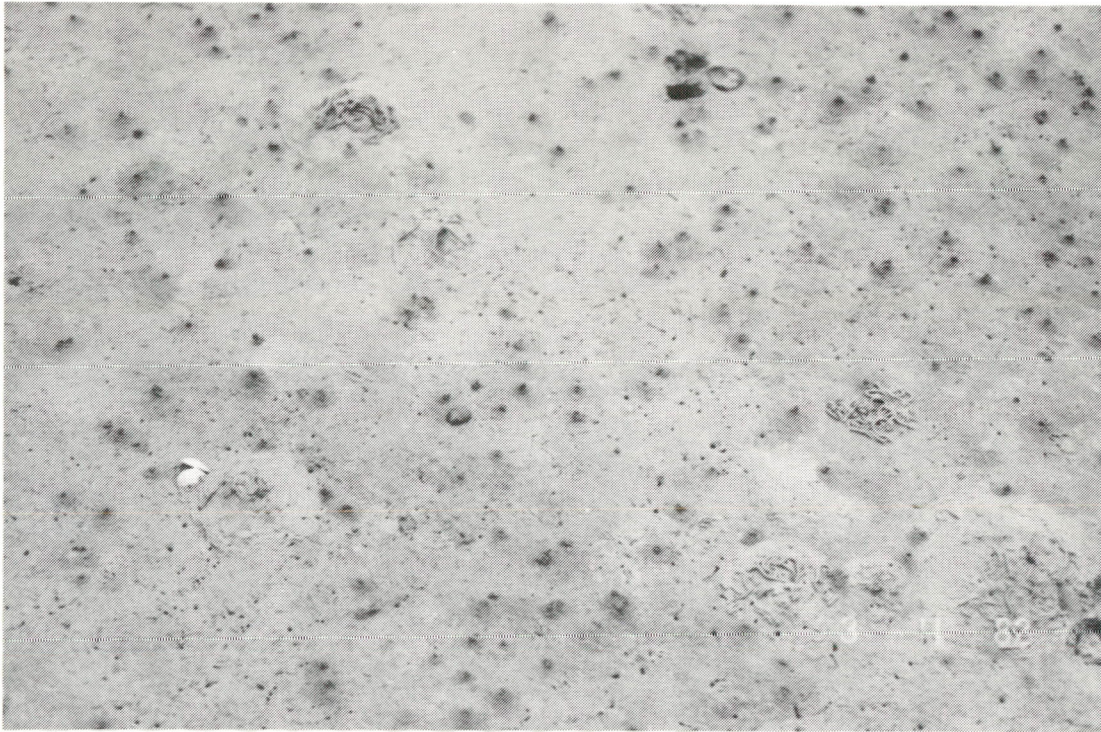


Fig. 50a

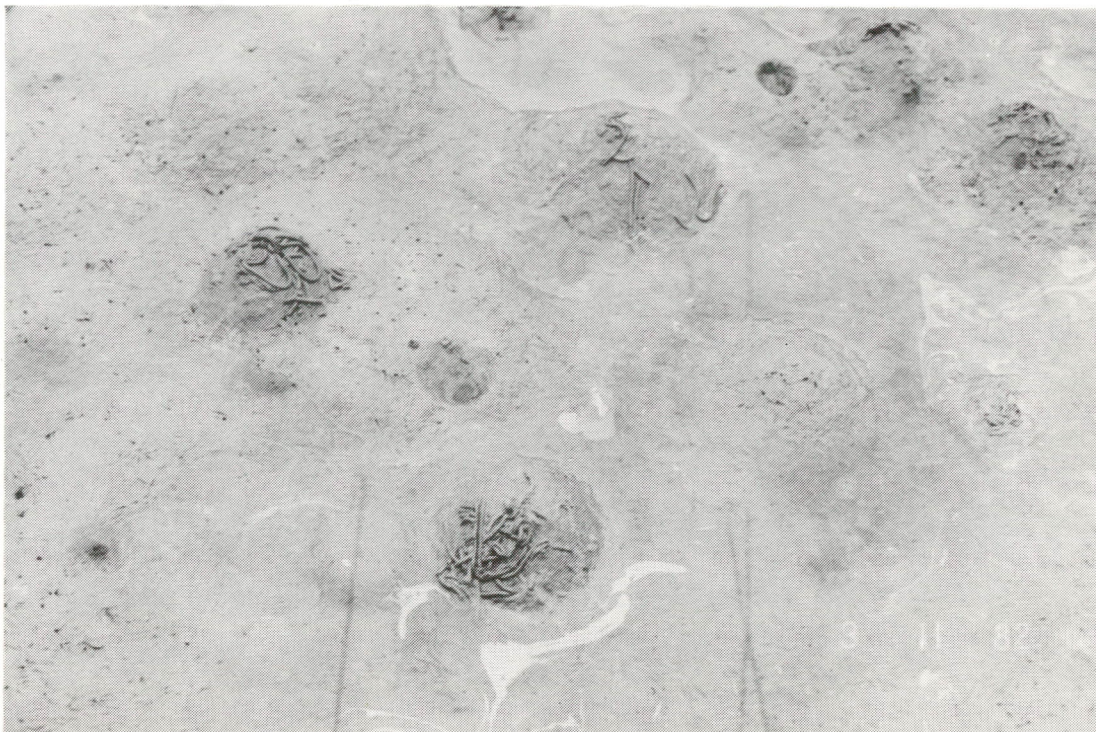


Fig. 50b

Fig. 50 An overview of the sediment surface in (a) the uncontaminated and (b) the contaminated MOTIFs in November 1982. Pits and casts of *Arenicola marina* are clearly visible, the many small holes are formed mainly by *Nereis diversicolor*, the many black casts in the uncontaminated sediment are formed by *Heteromastus filiformis*. A surface film of floating oil can be observed in the contaminated MOTIF.

APPENDIX I SUMMARY OF MEASUREMENTS NOT PRESENTED IN THE RESULTS SECTIONS

This appendix contains the results of various measurements made during OPEX, but not dealt with in the main report.

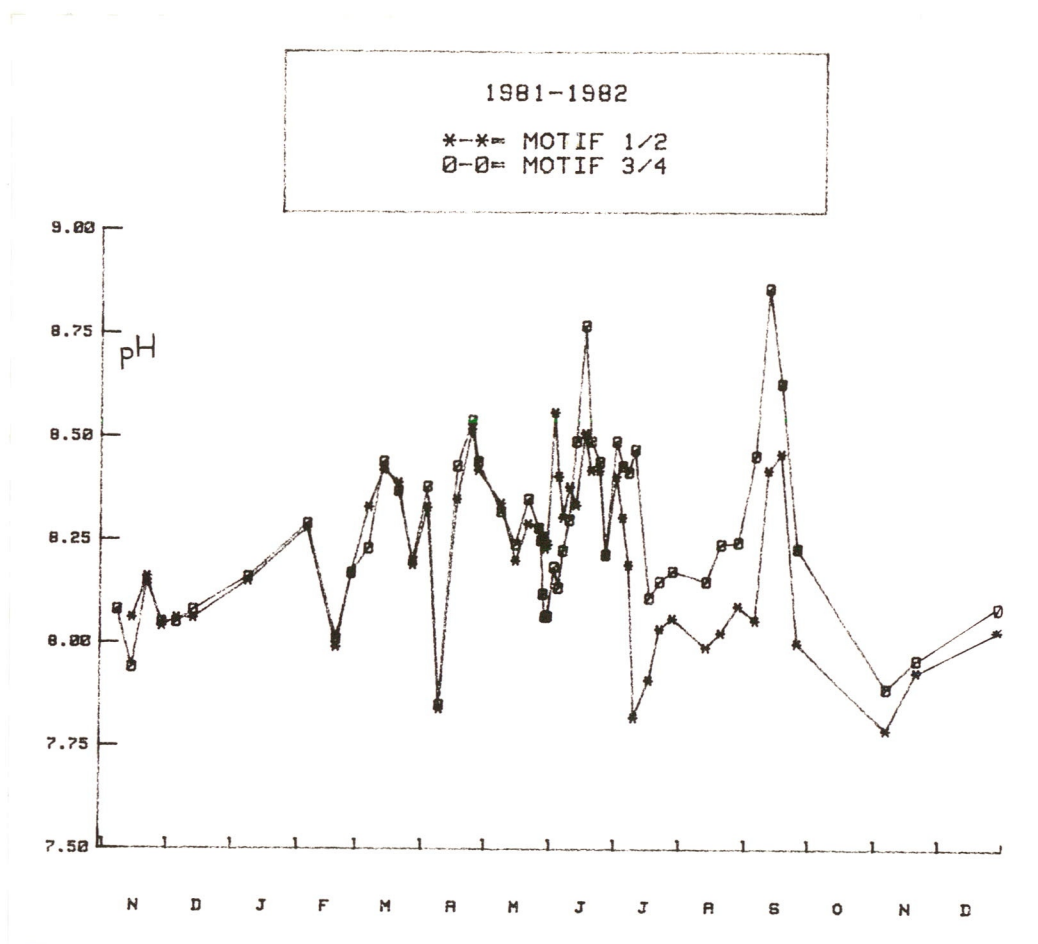


Fig. I.1 pH of the water in the different MOTIFs during OPEX.

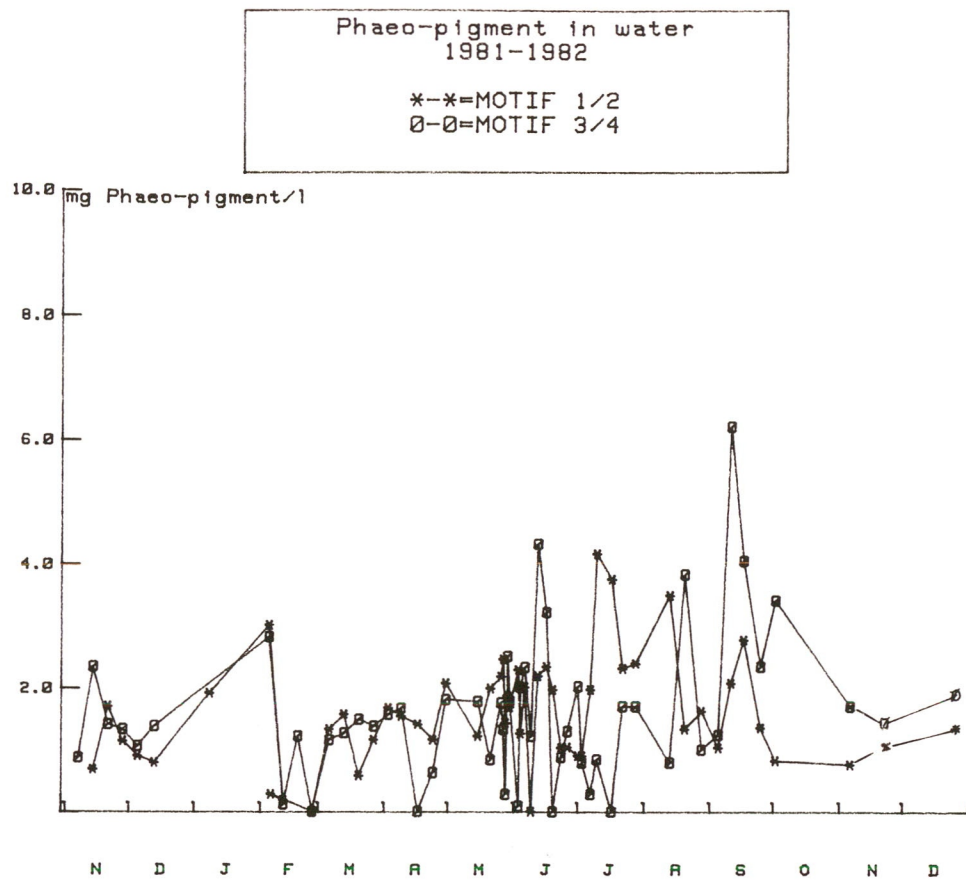


Fig. I.2 Concentrations of phaeopigments in the water of the different MOTIFs during OPEX.

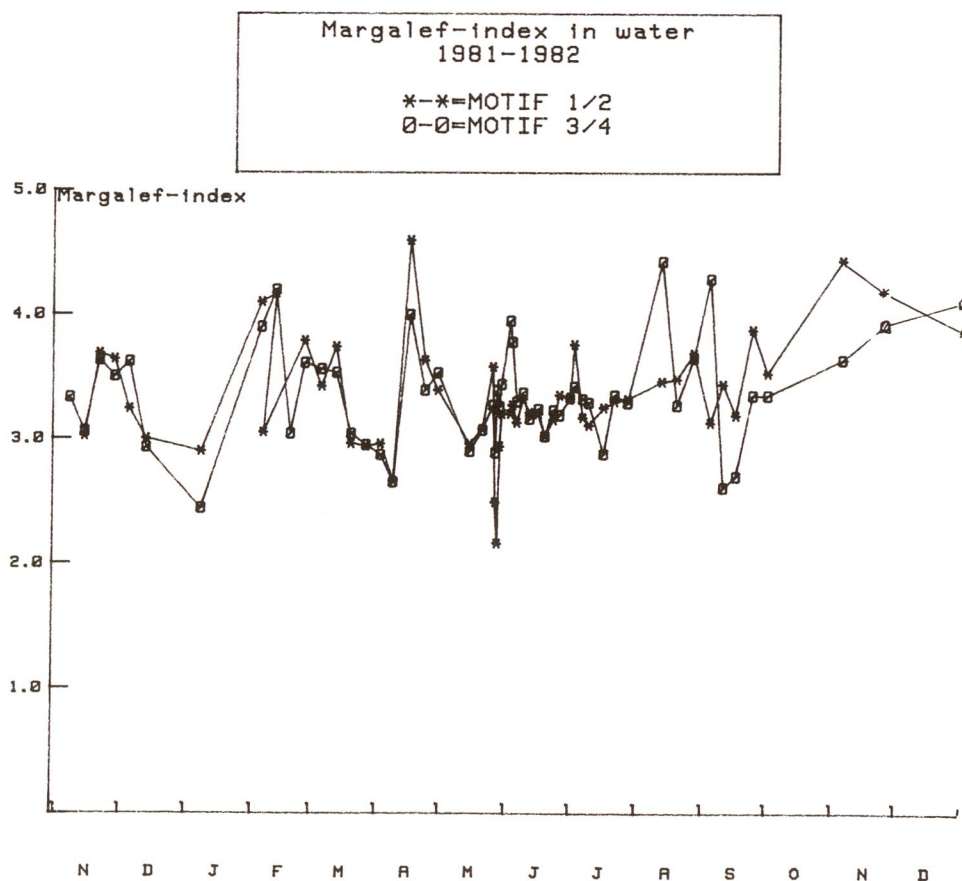


Fig. I.3 Pigment index in the water of the different MOTIFs during OPEX.

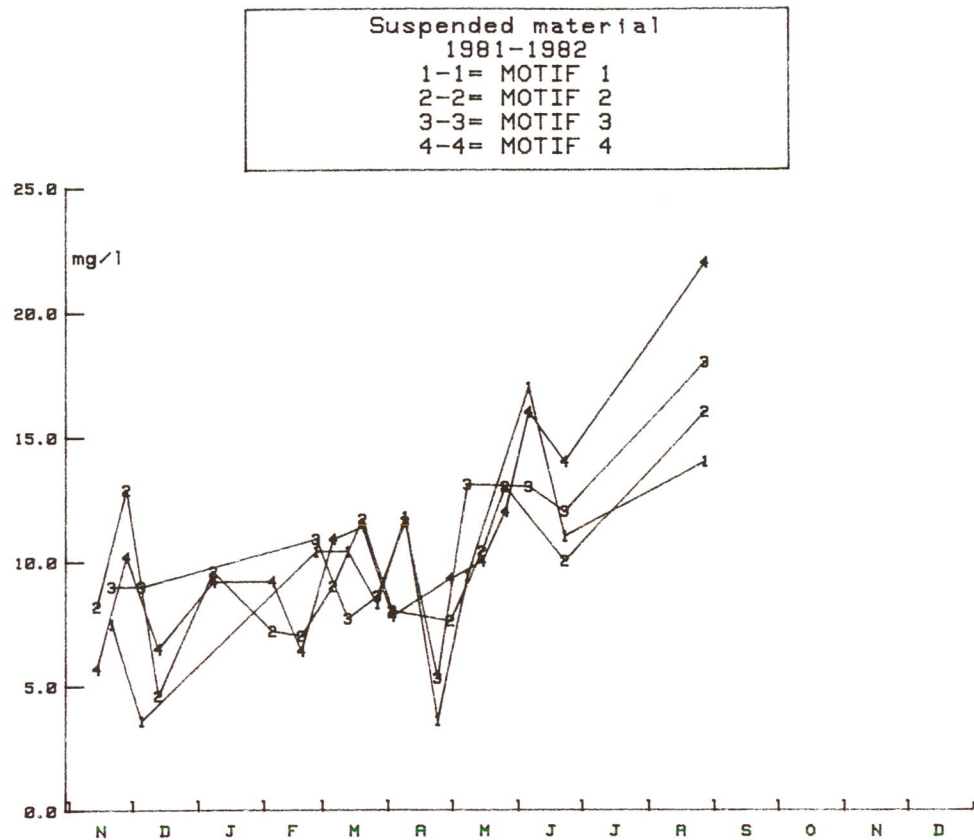


Fig. I.4 Concentrations of suspended matter in the water of the different MOTIFs during OPEX.

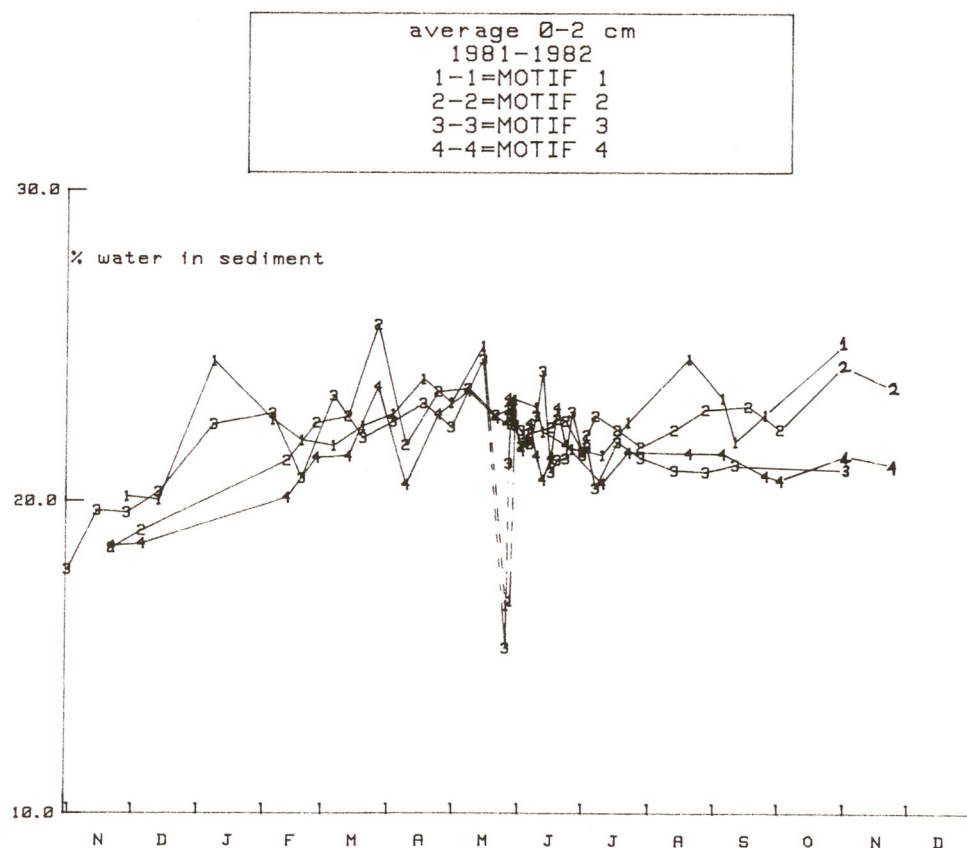


Fig. I.5 The water content of the upper 2 cm of the sediment in the different MOTIFs during OPEX.

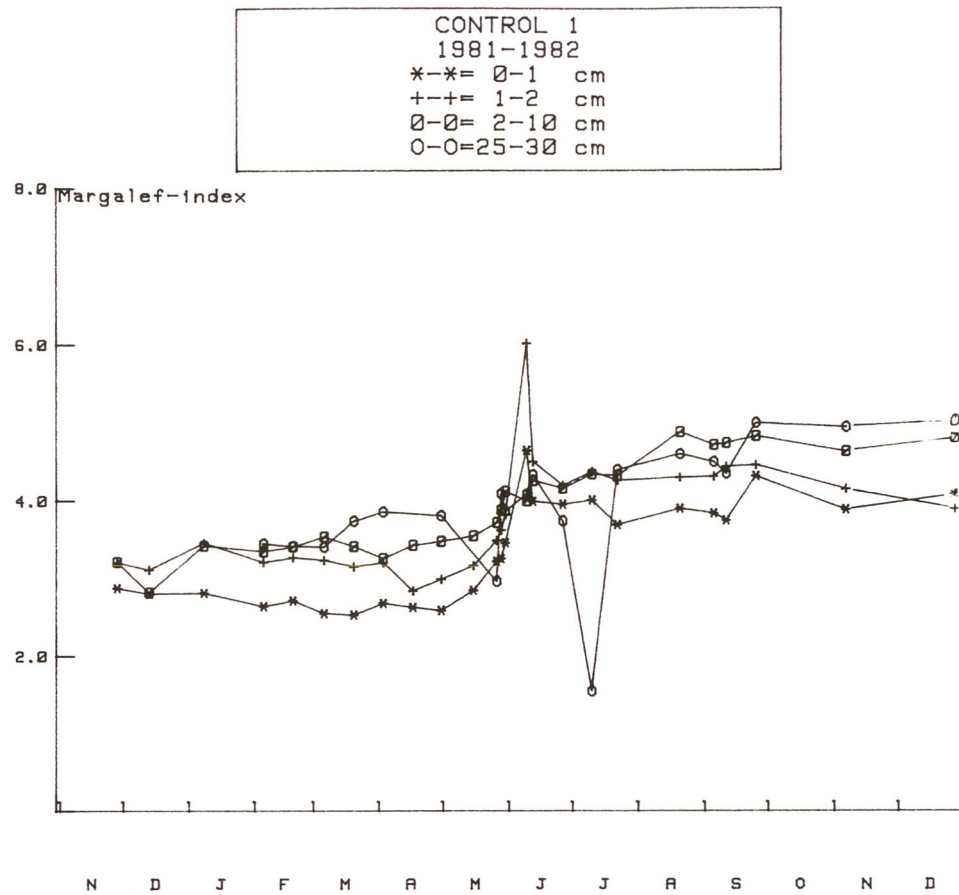


Fig. I.6 Pigment index at different depths in the sediment of MOTIF 1 during OPEX.

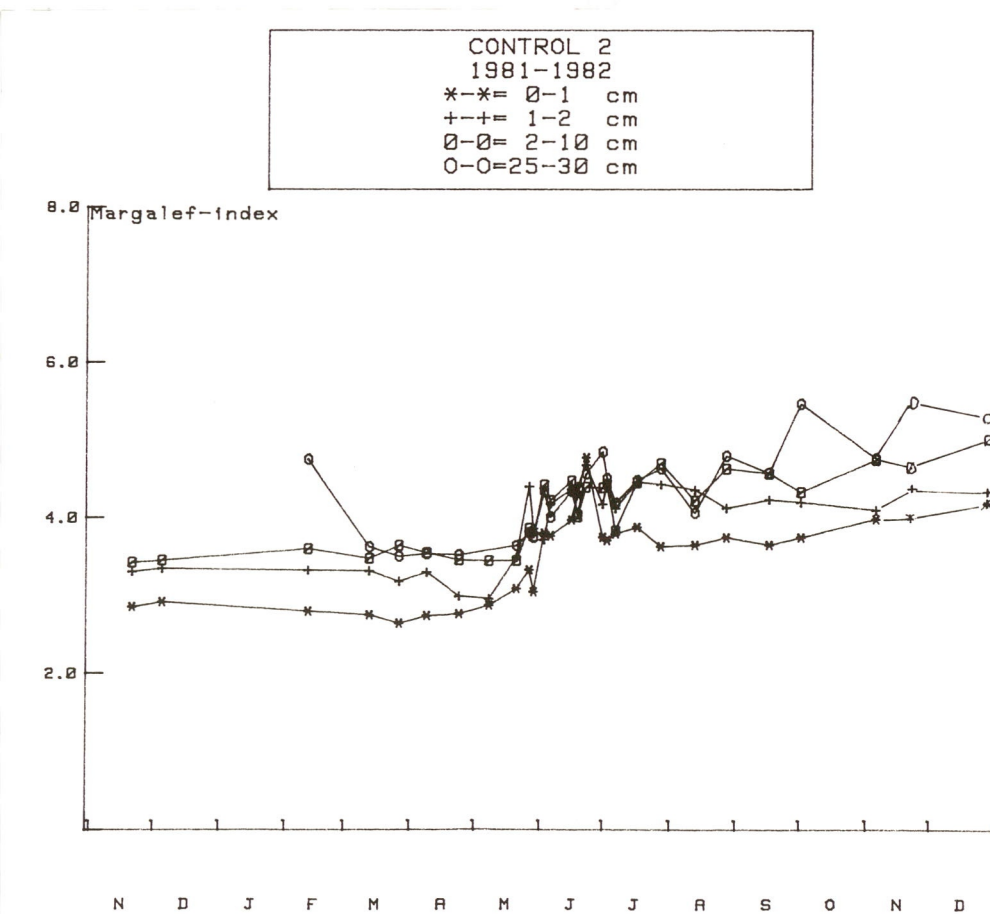


Fig. I.7 Pigment index at different depths in the sediment of MOTIF 2 during OPEX.

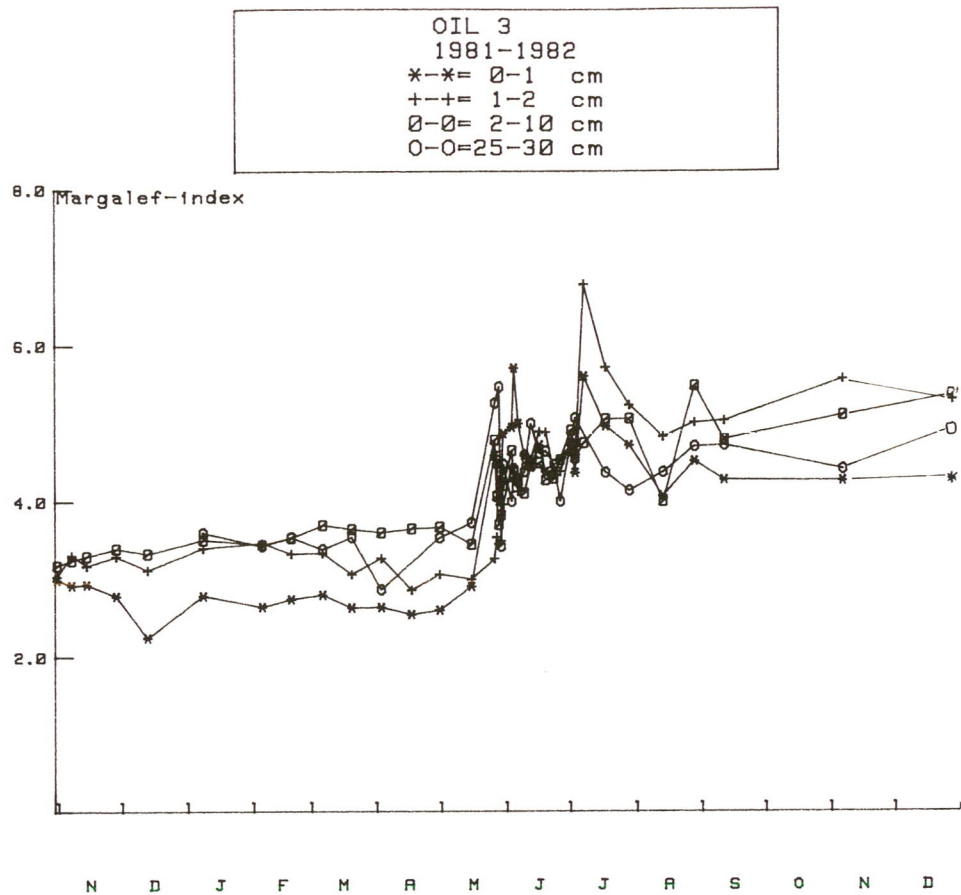


Fig. I.8 Pigment index at different depths in the sediment of MOTIF 3 during OPEX.

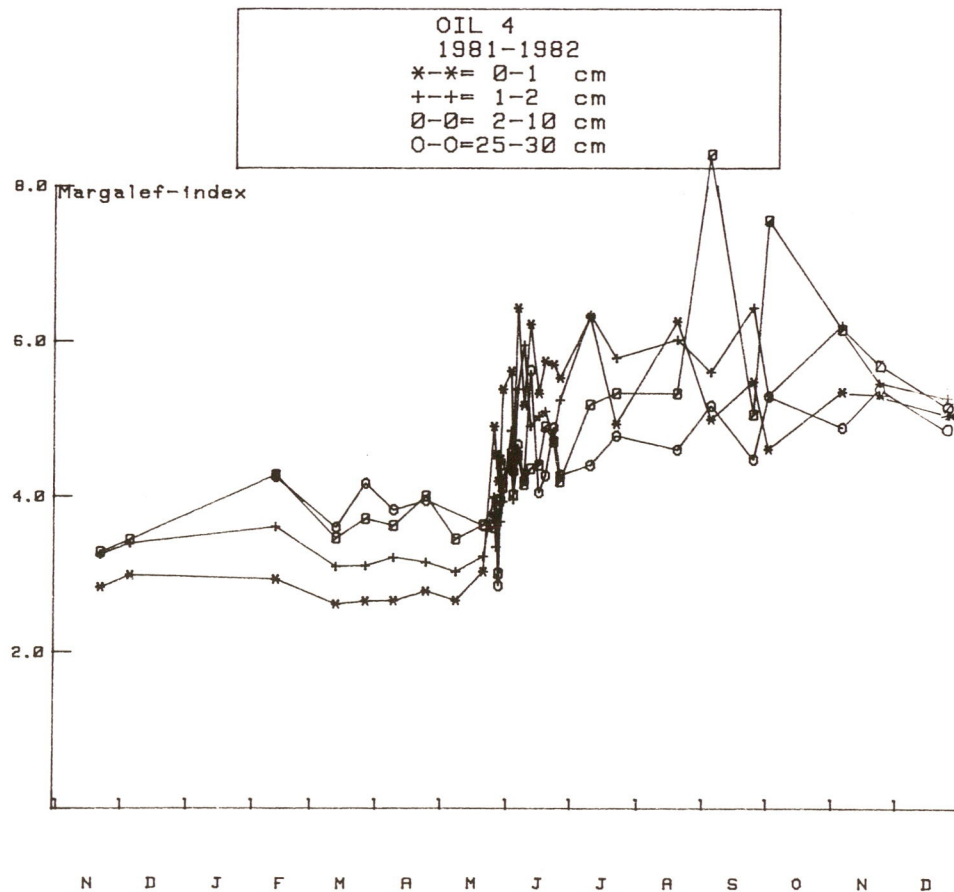


Fig. I.9 Pigment index at different depths in the sediment of MOTIF 4 during OPEX.

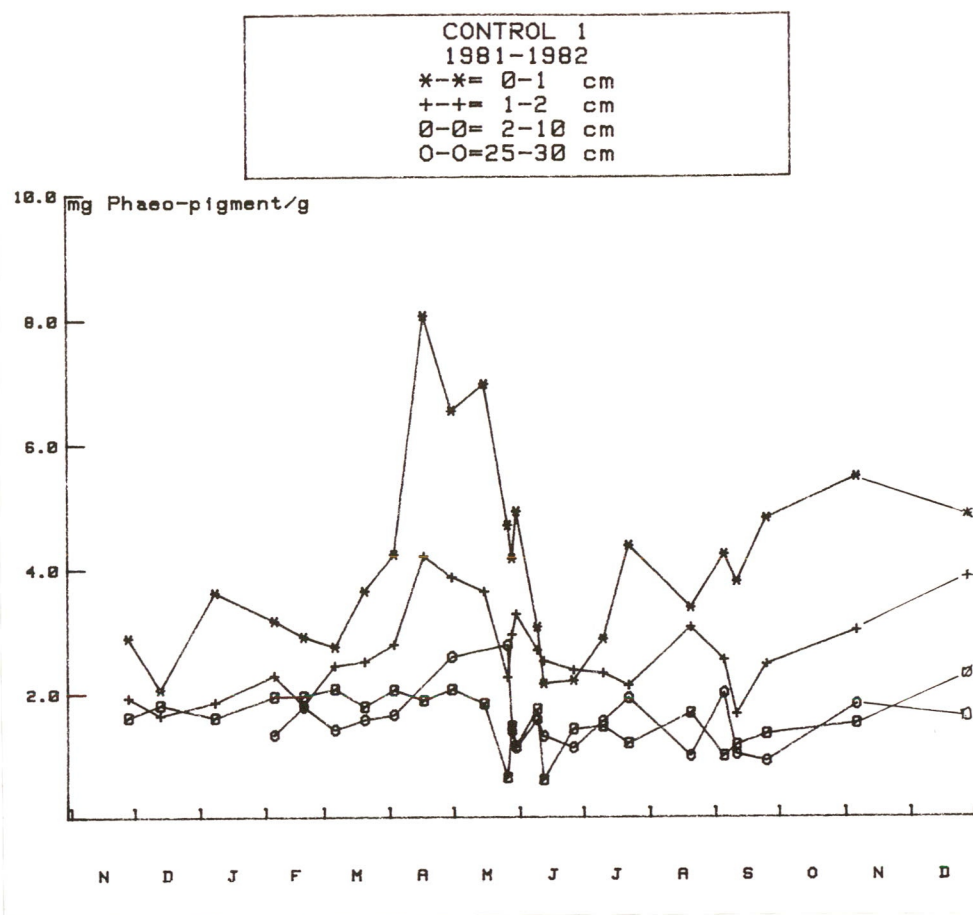


Fig. I.10 Concentrations of phaeopigments at different depths in the sediment of MOTIF 1 during OPEX.

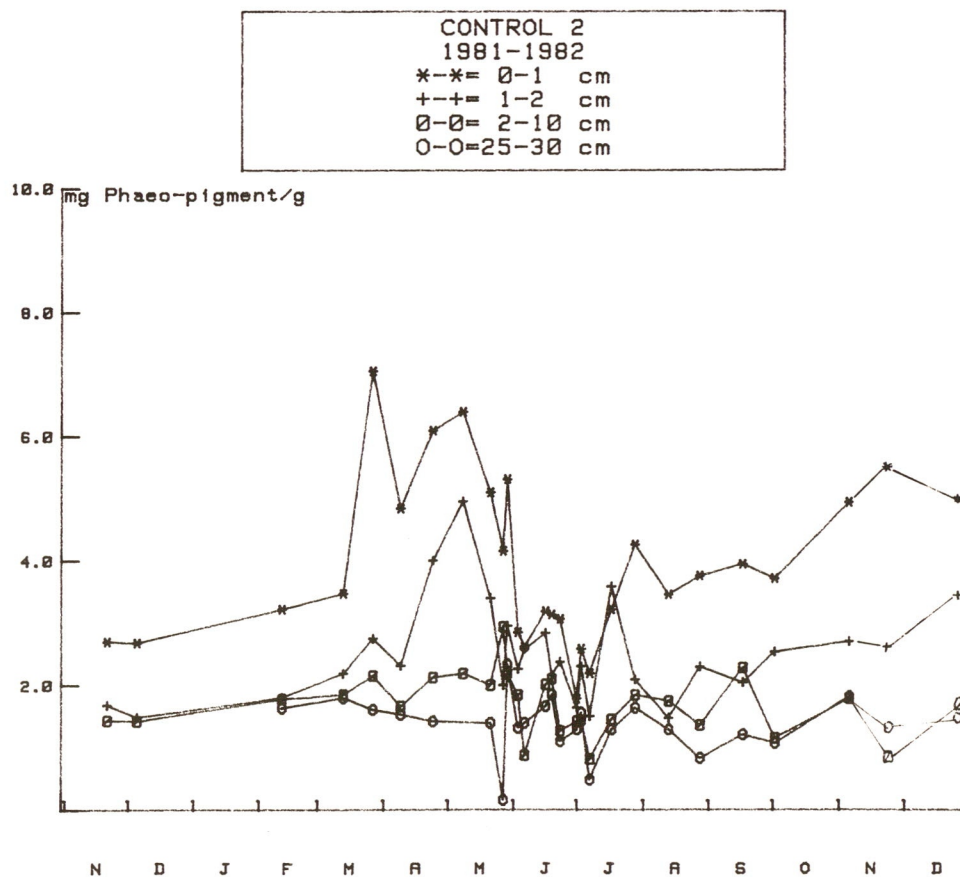


Fig. I.11 Concentrations of phaeopigments at different depths in the sediment of MOTIF 2 during OPEX.

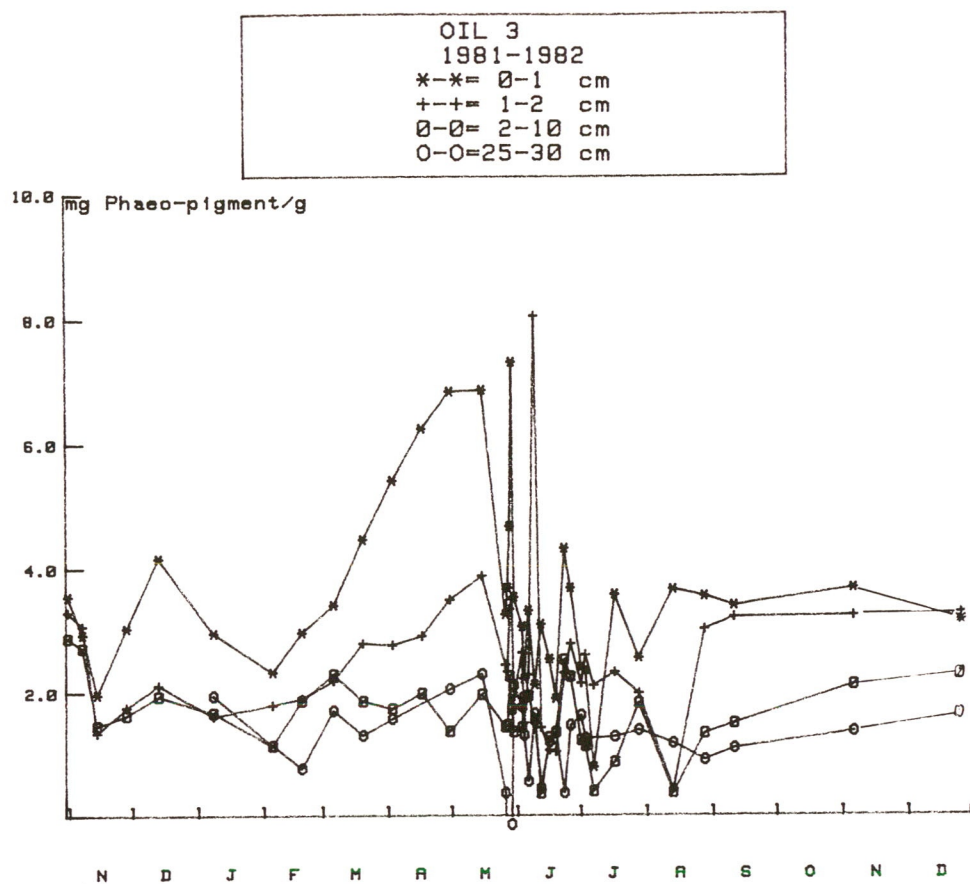


Fig. I.12 Concentrations of phaeopigments at different depths in the sediment of MOTIF 3 during OPEX.

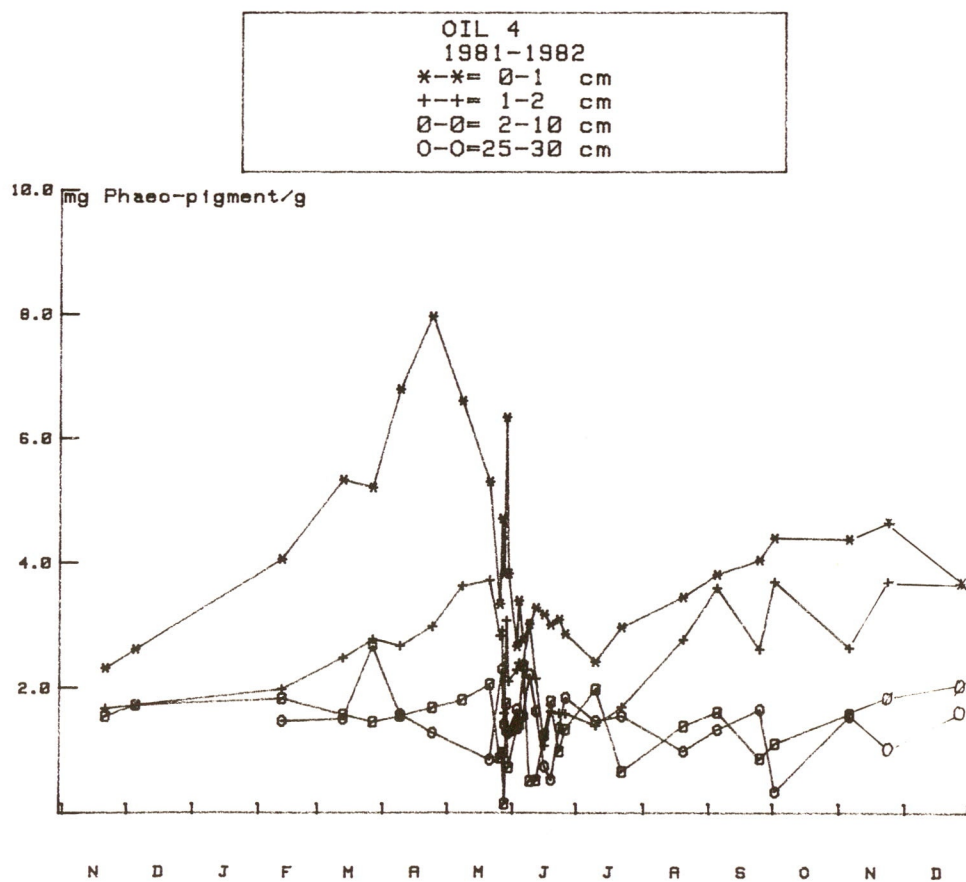


Fig. I.13 Concentrations of phaeopigments at different depths in the sediment of MOTIF 4 during OPEX.

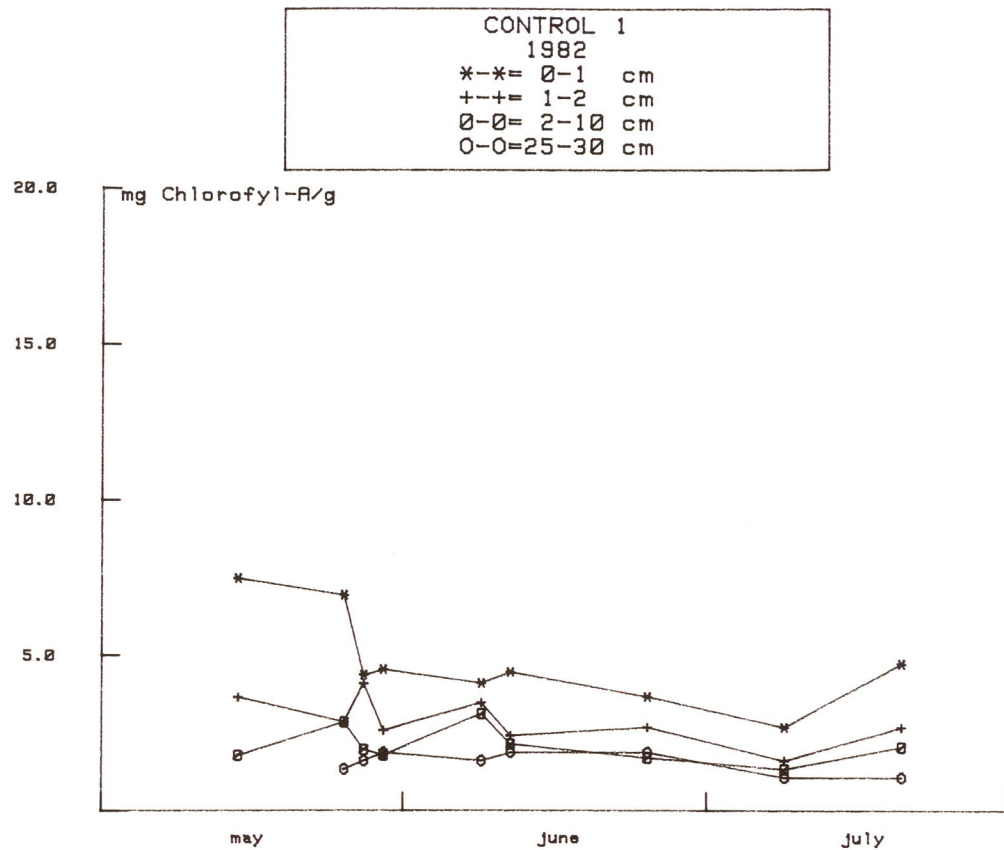


Fig. 1.14 Chlorophyll concentrations at different depths in the sediment of MOTIF 1 during the period May-July (OPEX).

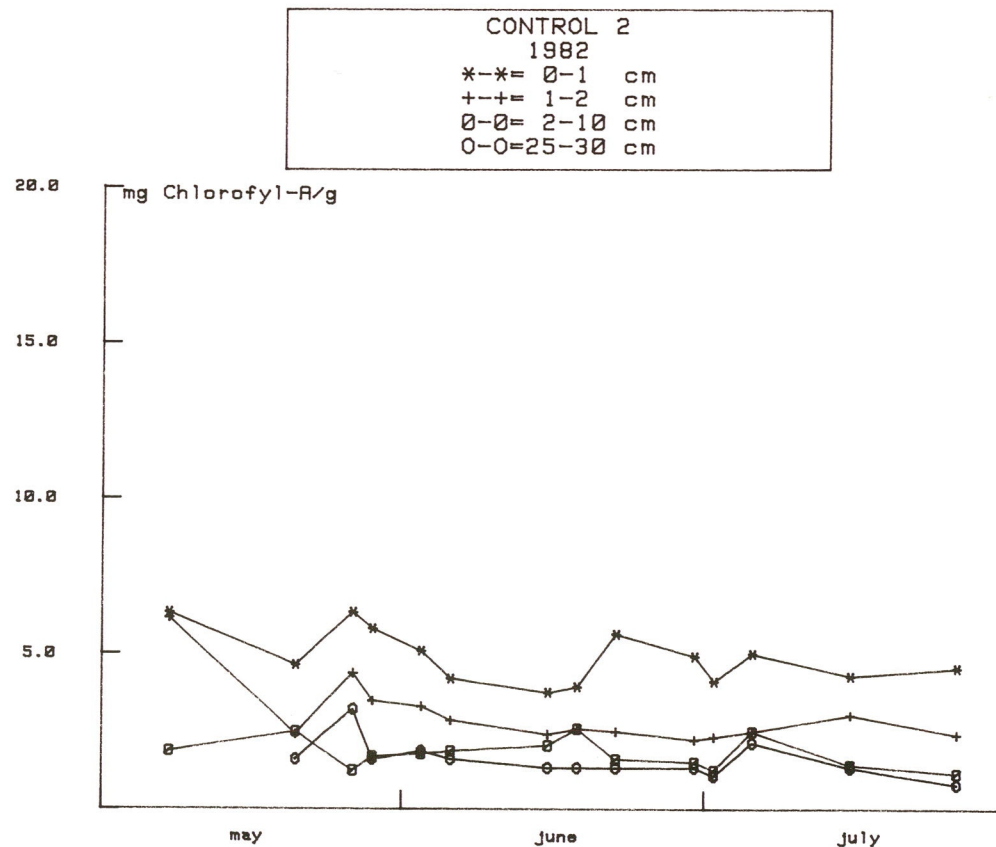


Fig. I.15 Chlorophyll concentrations at different depths in the sediment of MOTIF 2 during the period May-July (OPEX).

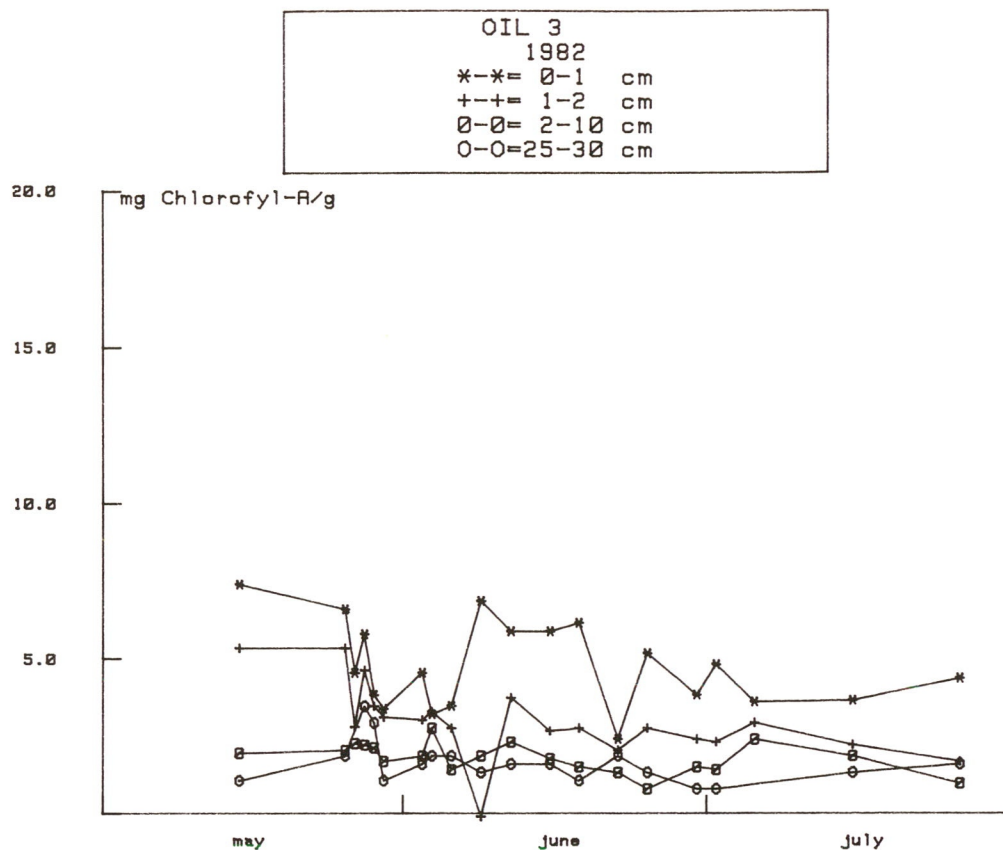


Fig. I.16 Chlorophyll concentrations at different depths in the sediment of MOTIF 3 during the period May-July (OPEX).

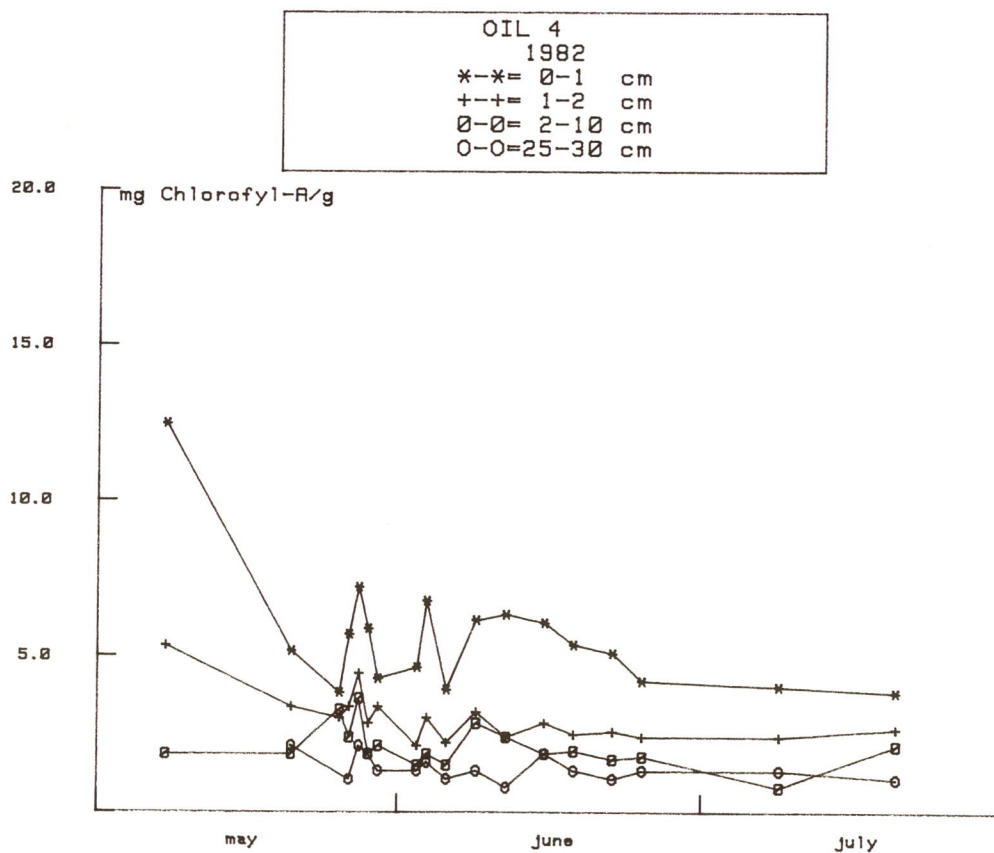


Fig. I.17 Chlorophyll concentrations at different depths in the sediment of MOTIF 4 during the period May-July (OPEX).

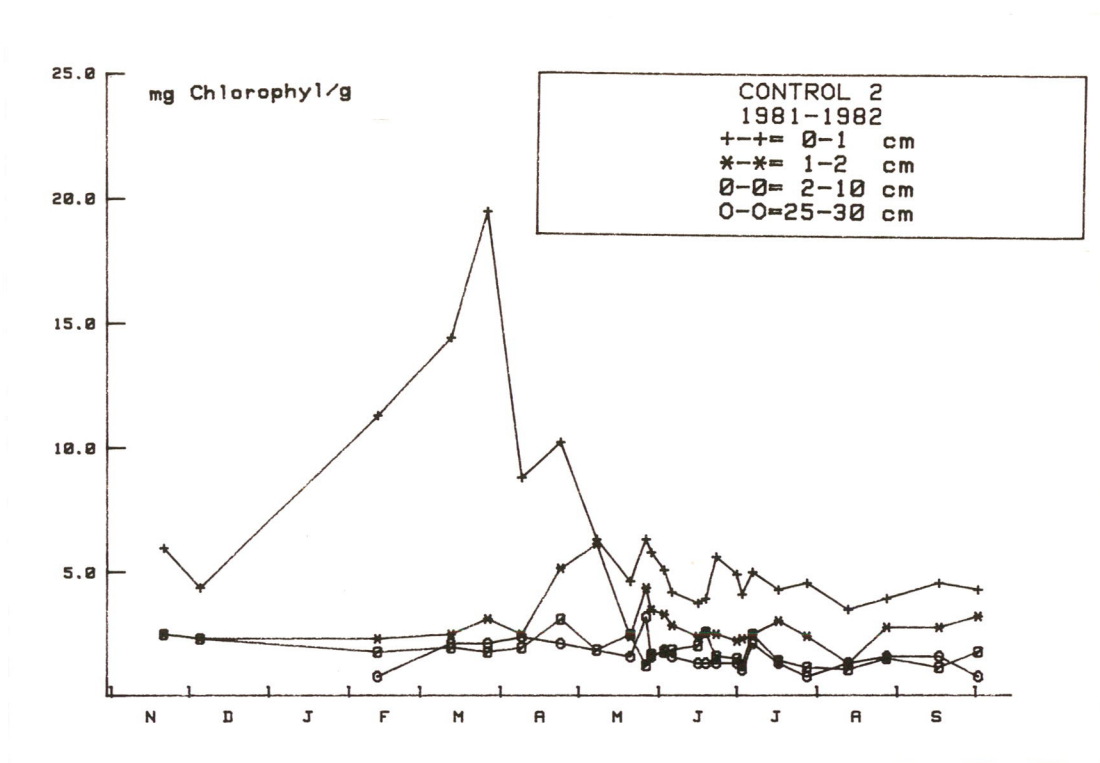


Fig. I.18 Chlorophyll concentrations at different depths in the sediment of MOTIF 2 during OPEX.

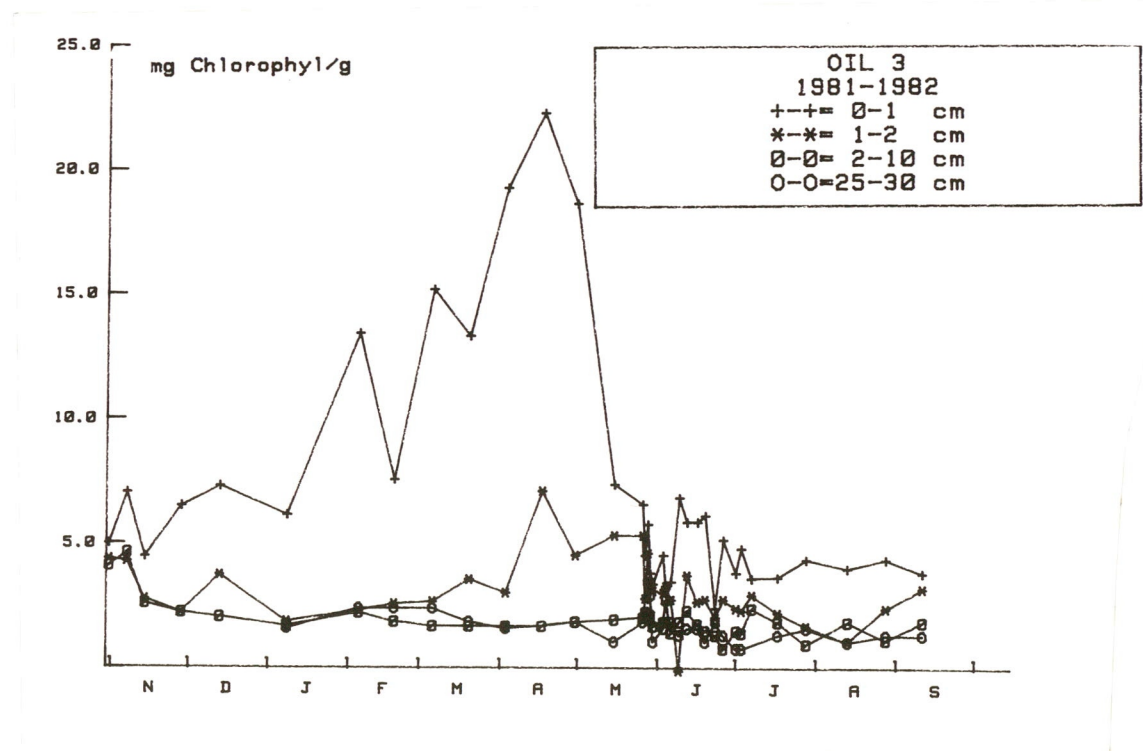


Fig. I.19 Chlorophyll concentrations at different depths in the sediment of MOTIF 3 during OPEX.

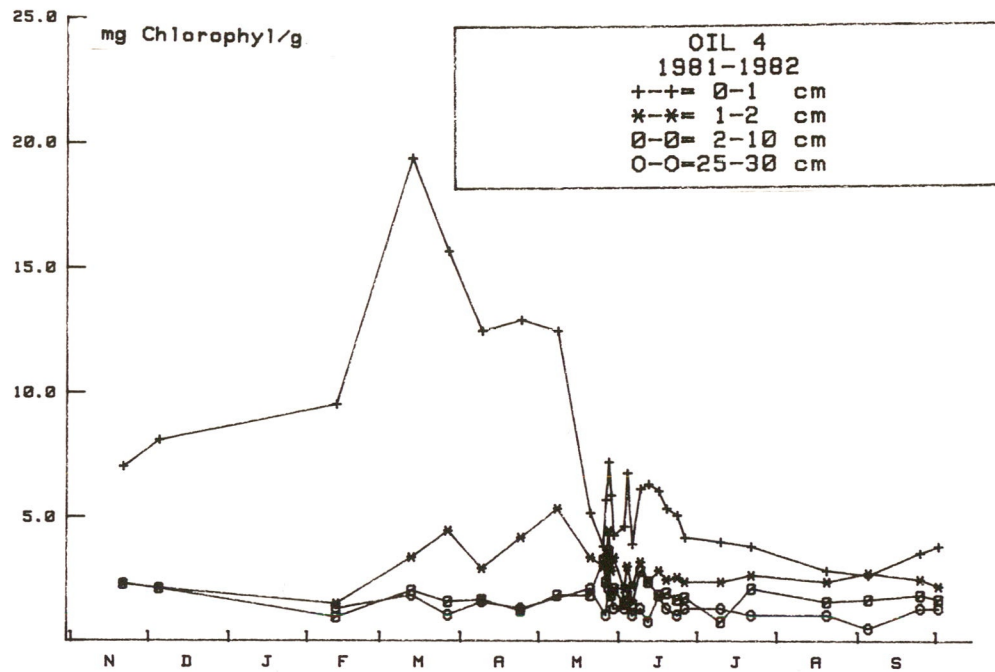


Fig. I.20 Chlorophyll concentrations at different depths in the sediment of MOTIF 4 during OPEX.

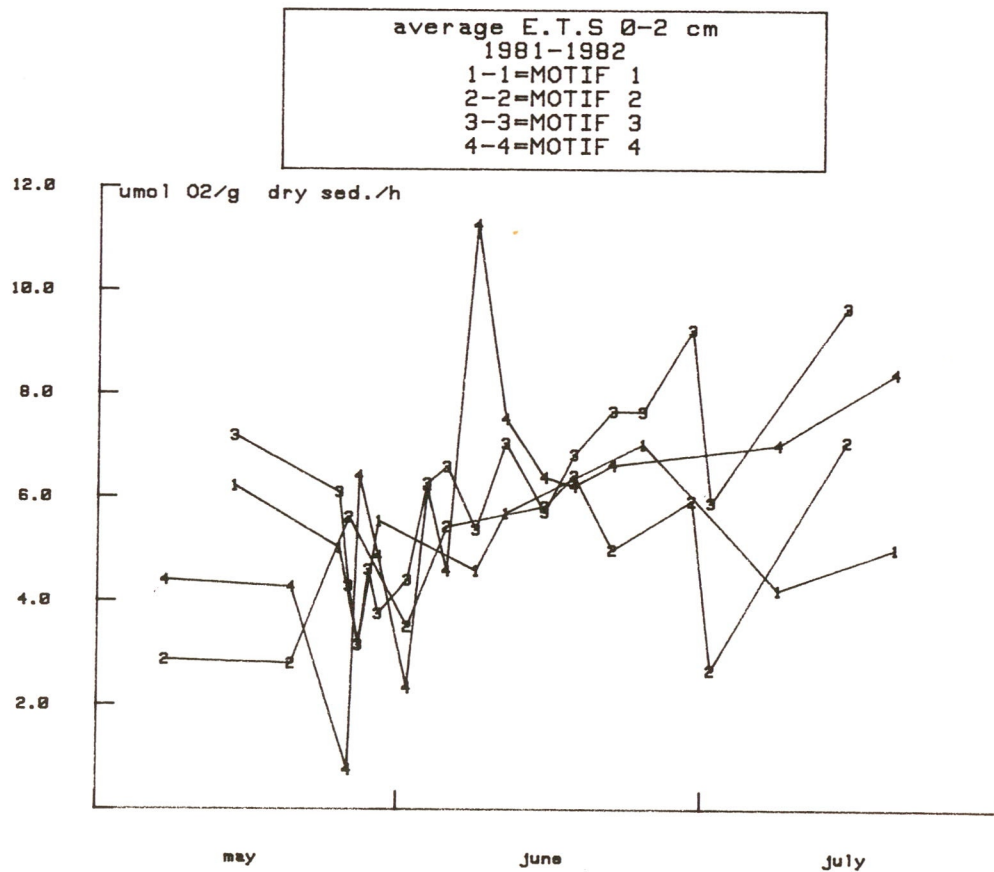


Fig. I.21 The average ETS (Electron Transport System) activity in the upper 2 cm of the sediment in the different MOTIFs in the period May-July (OPEX).

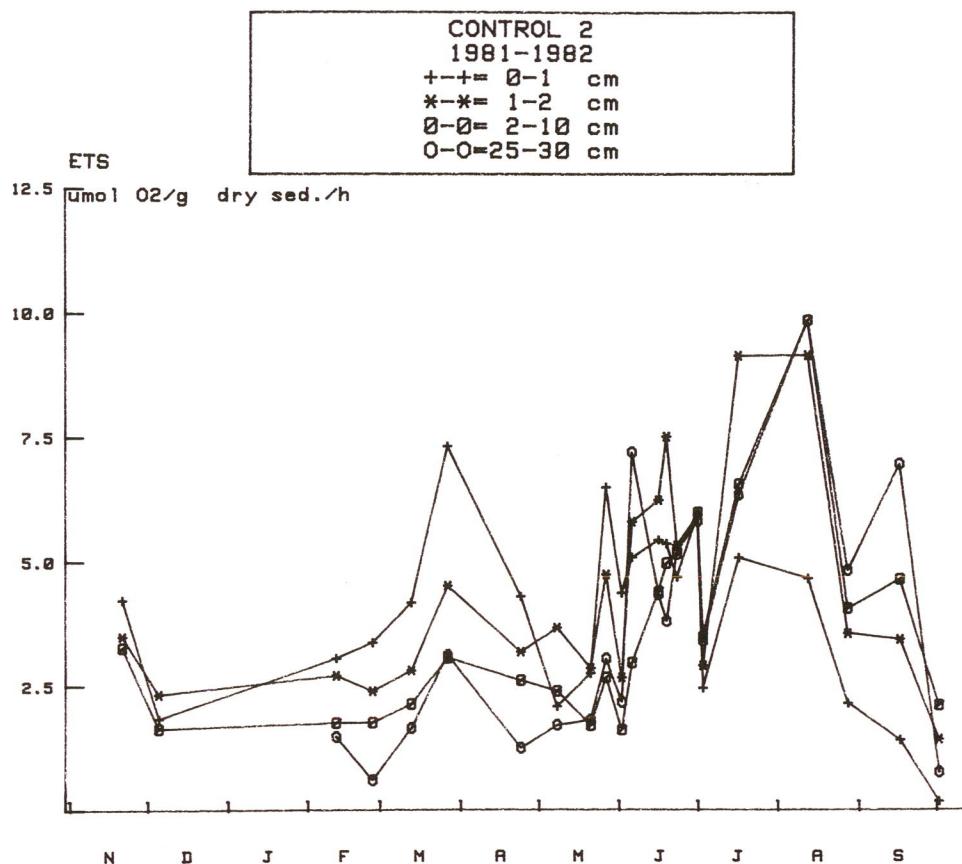


Fig. I.22 ETS activity at different depths in the sediment of MOTIF 2 during OPEX.

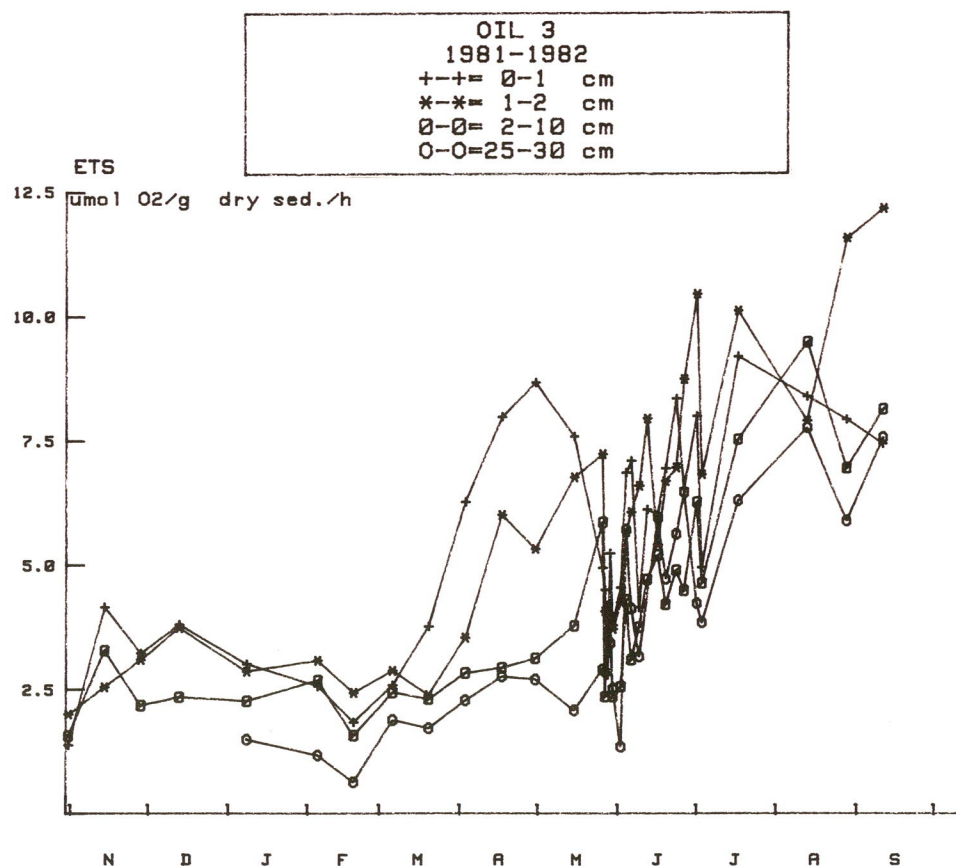


Fig. I.23 ETS activity at different depths in the sediment of MOTIF 3 during OPEX.

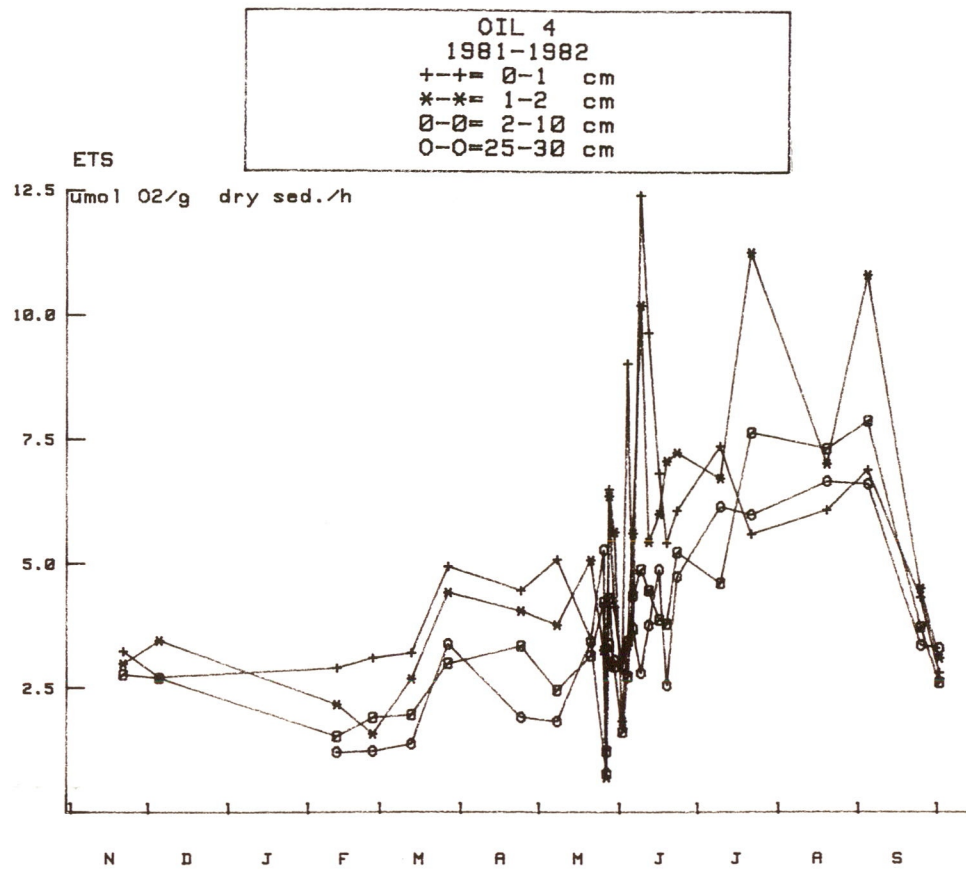


Fig. I.24 ETS activity at different depths in the sediment of MOTIF 4 during OPEX.

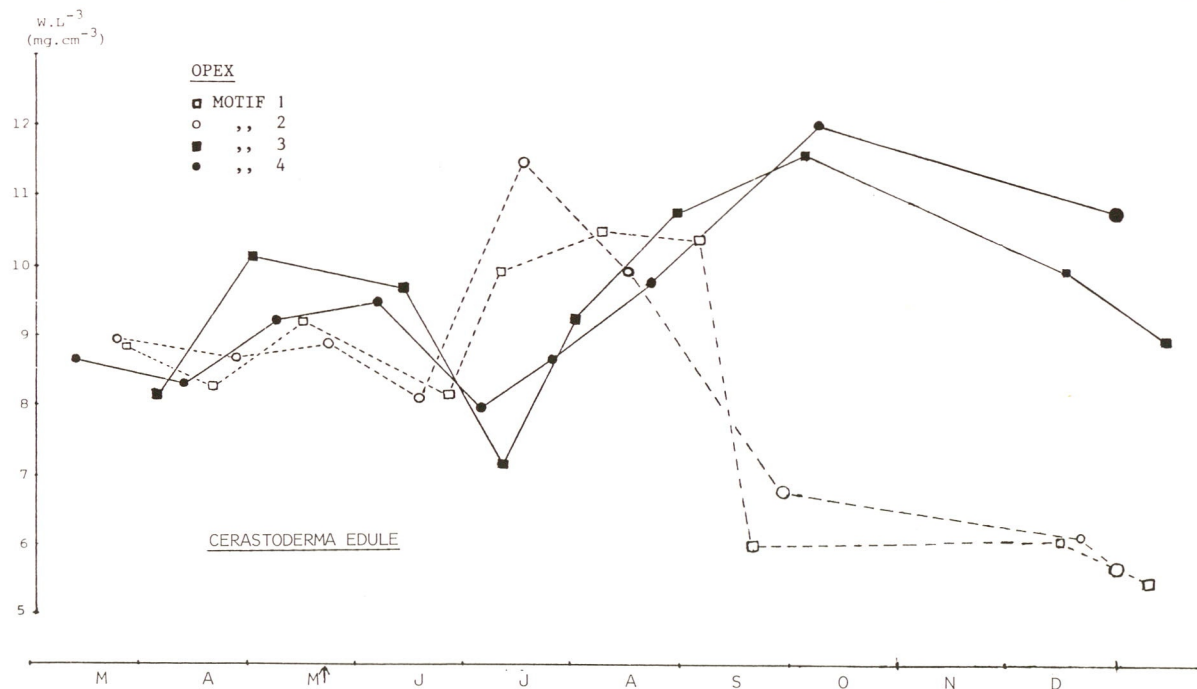


Fig. I.25 The condition index of *Cerastoderma edule* in the different MOTIFs during OPEX.

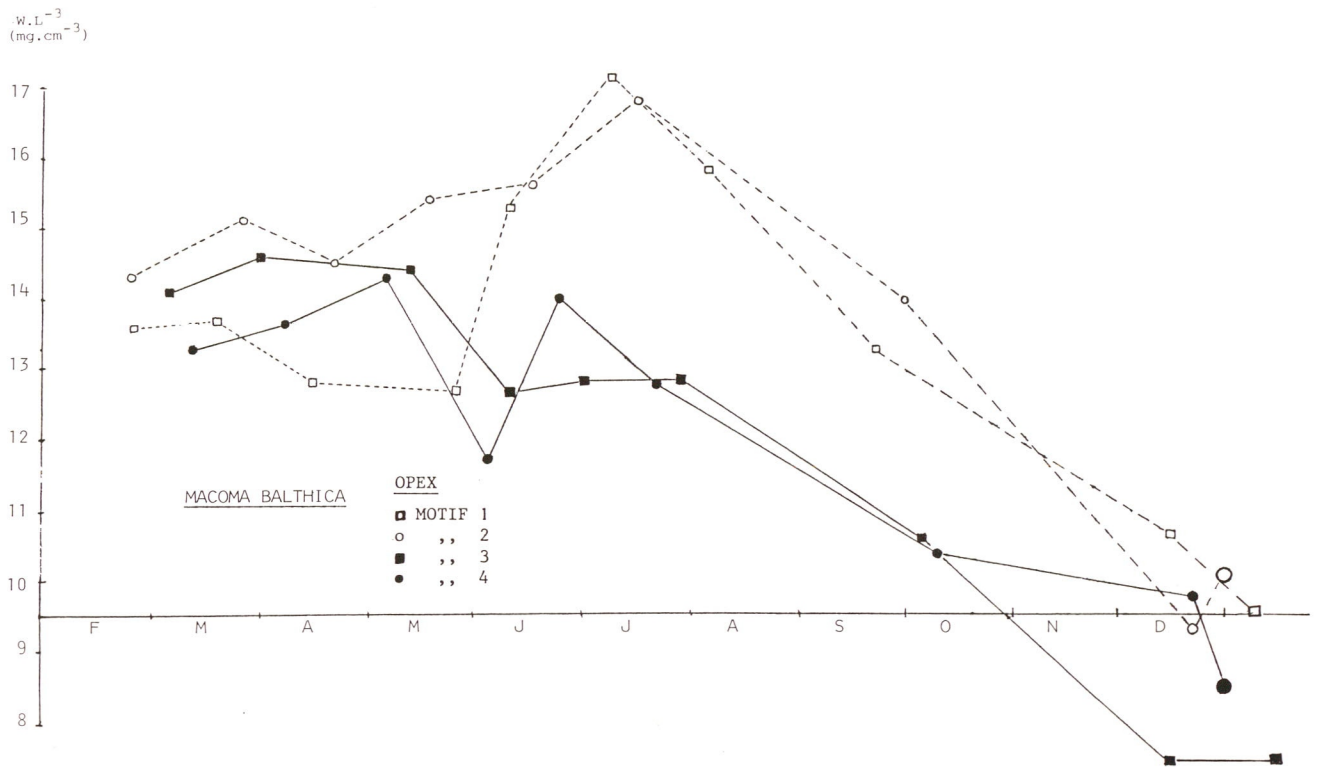


Fig. I.26 The condition index of *Macoma balthica* in the different MOTIFs during OPEX.

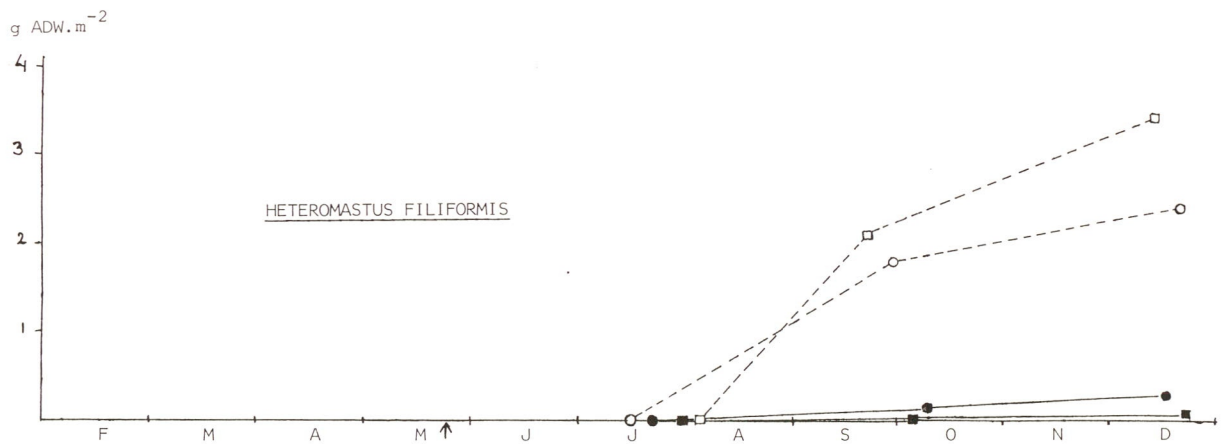


Fig. I.27 The ash-free dry weight (ADW) of *Heteromastus filiformis* per m² in the different MOTIFs during OPEX.

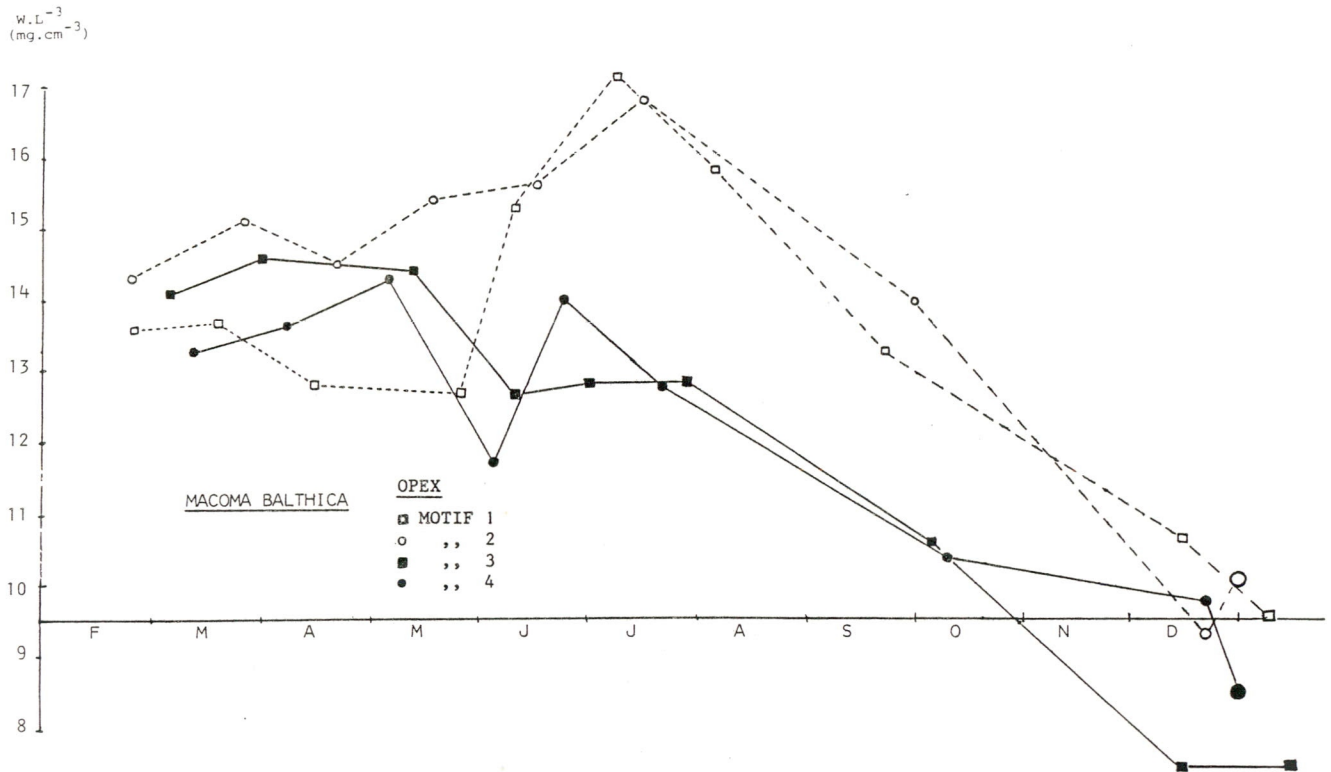


Fig. I.26 The condition index of *Macoma balthica* in the different MOTIFs during OPEX.

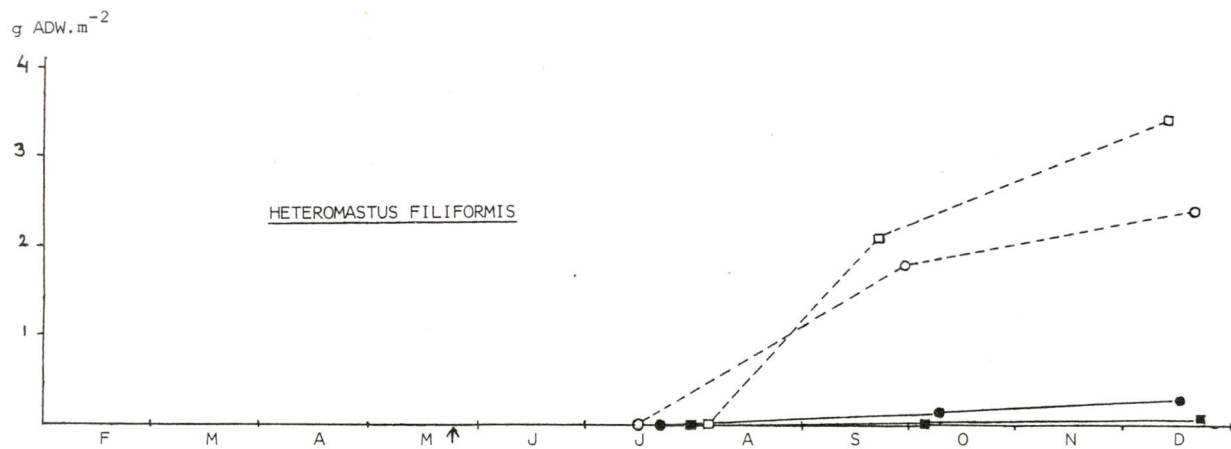
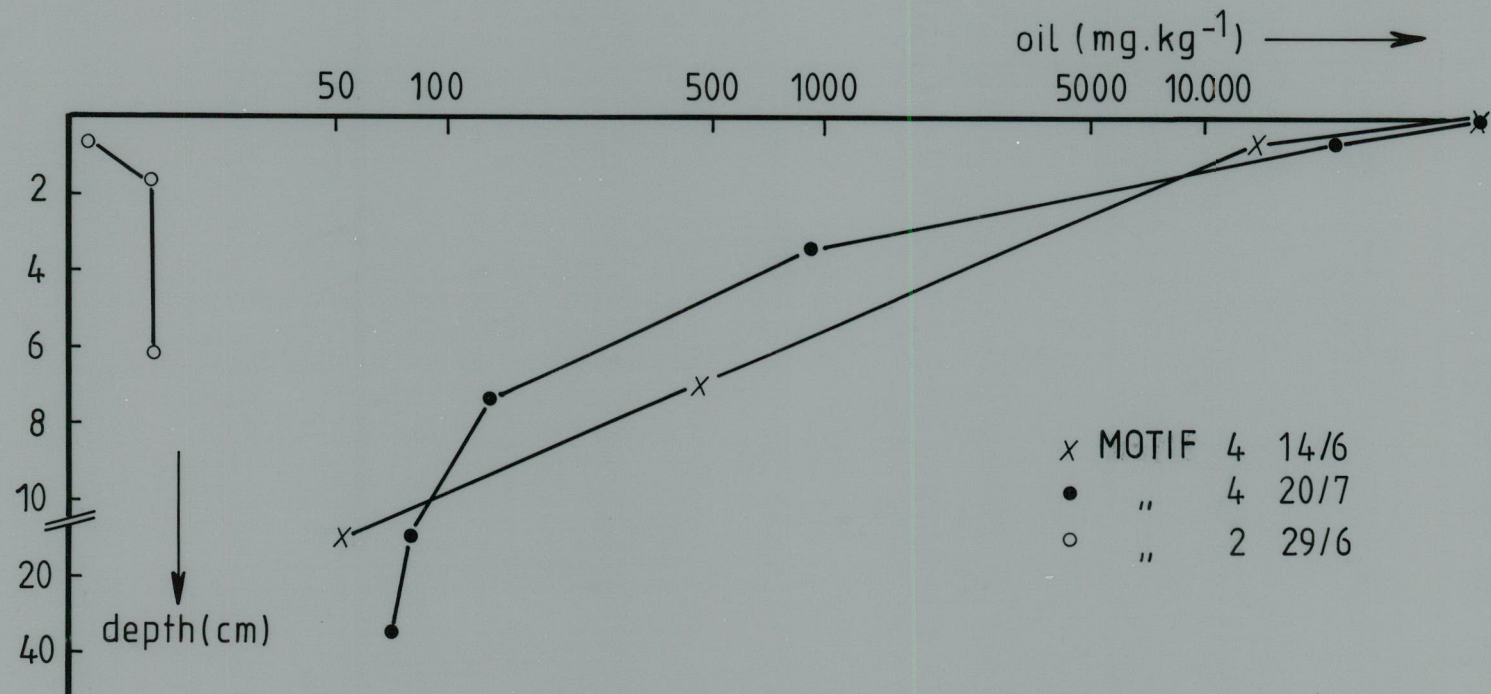
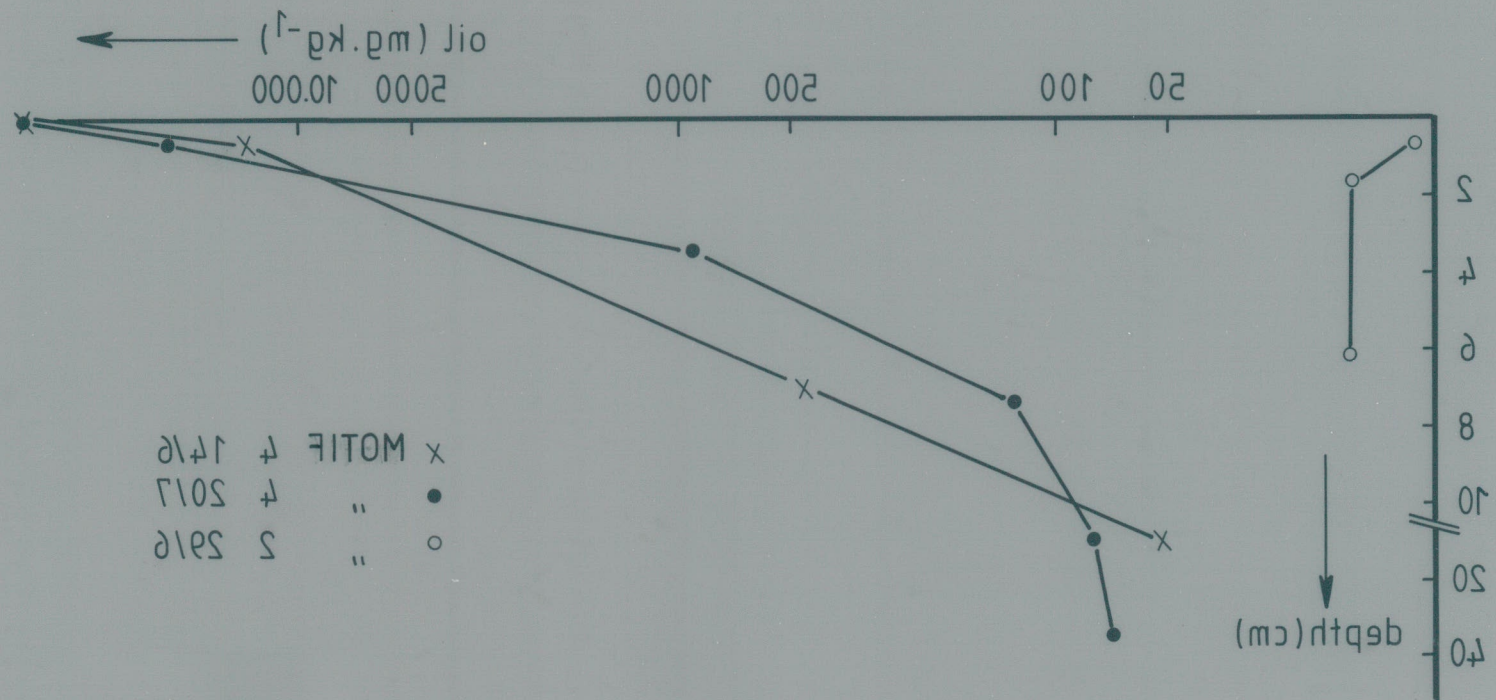


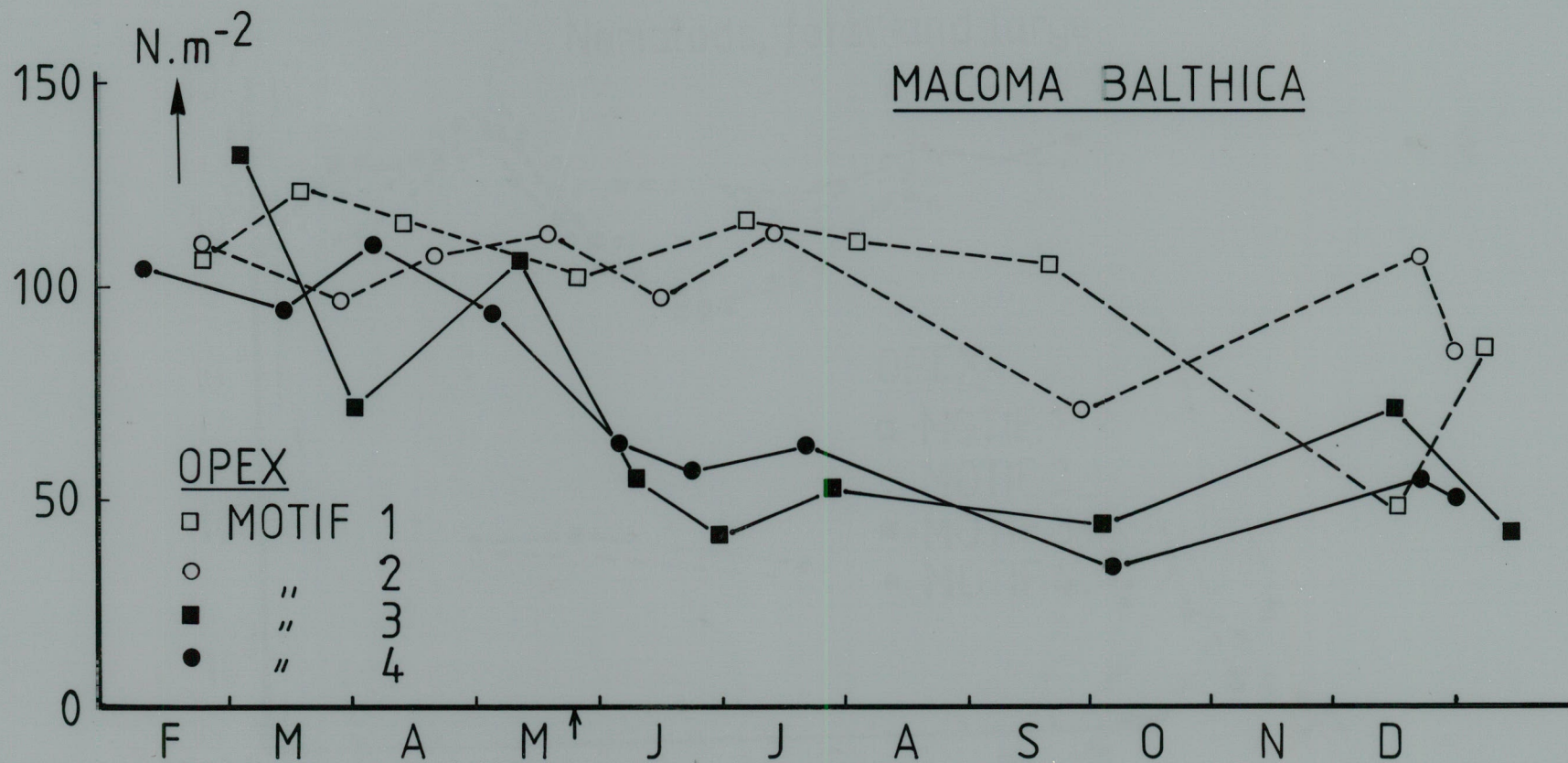
Fig. I.27 The ash-free dry weight (ADW) of *Heteromastus filiformis* per m² in the different MOTIFs during OPEX.

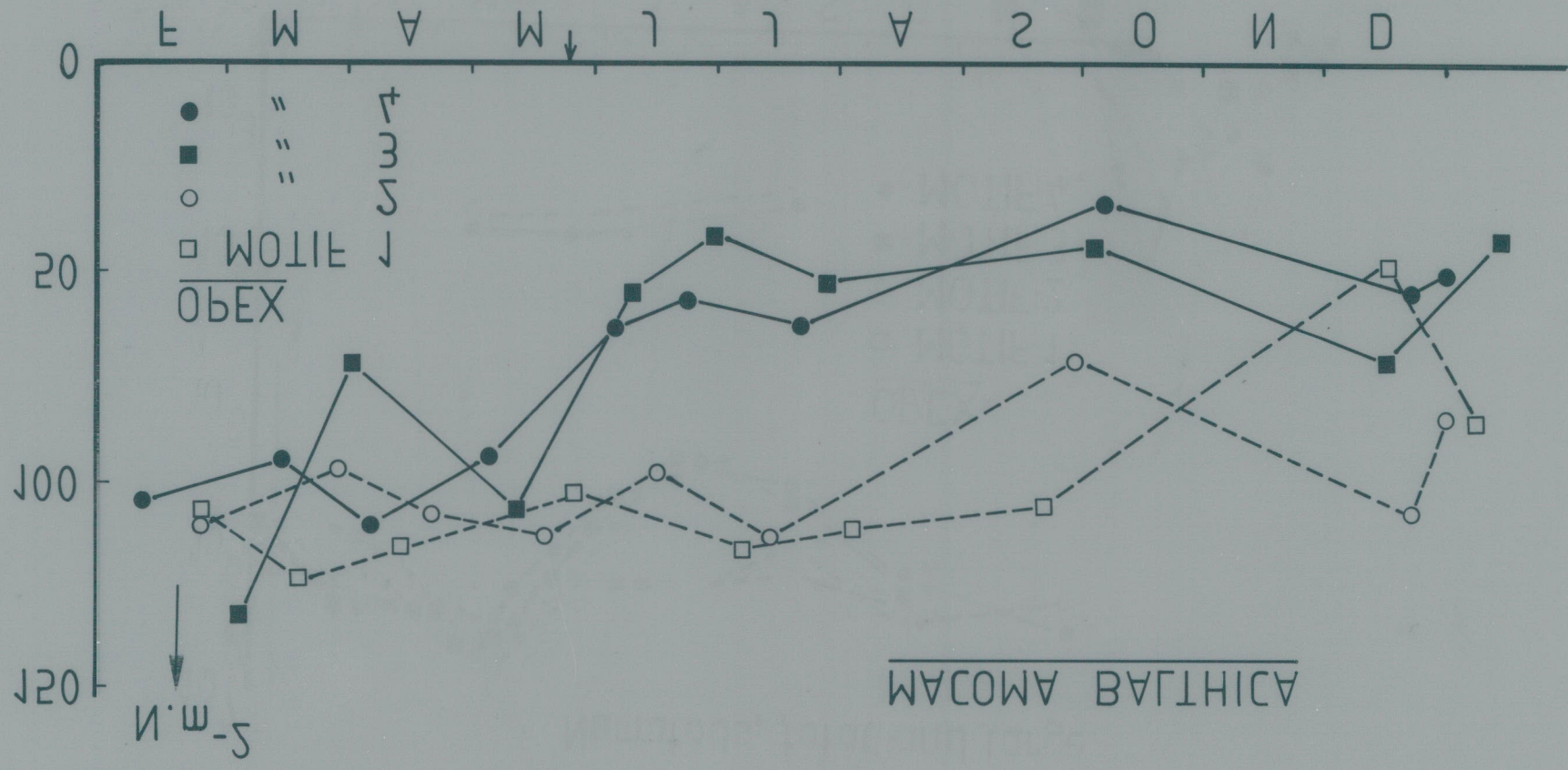
Table 8

Species	middle of MAY						end of JUNE					
	MOTIF 2			MOTIF 4			MOTIF 2			MOTIF 4		
	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW
Macoma *)	149	16.3	109.0	149	14.1	94.6	116	16.7	144.2	149	10.6	72.9
Cerastoderma *)	0	0	0	17	6.5	391.3	33	8.5	256.0	0	0	0
Hydrobia	182	0.2	0.8	82	<0.1	0.7	580	0.2	0.3	133	<0.1	0.4
Corophium	17	0	0	0	0	0	133	<0.1	0.2	0	0	0
Gammarus	0	0	0	0	0	0	713	0.4	0.6	0	0	0
Arenicola *)	17	4.4	265.5	22	4.8	224.4	12	1.7	145.0	5	0	0
Nereis	0	0	0	17	0	0	133	2.1	0.4	928	0.4	0.4
Heteromastus	0	0	0	0	0	0	0	0	0	0	0	0
Anaitides/Eteone	0	0	0	0	0	0	14	0.1	7.8	50	0.2	3.2
Pygospio	514	0.5	0.9	514	0.5	0.9	414	0.5	1.3	530	0.2	0.4
Capitella	0	0	0	0	0	0	795	0.2	0.3	2651	0.4	0.2
Paranais	0	0	0	0	0	0	21375	0.7	<0.1	38541	1.1	<0.1
Oligochaet I	2088	0.5	0.2	2601	0.6	0.2	2502	0.2	0.1	1740	0.2	0.1
(large) Nematodes	5833	0.2	<0.1	5866	0.2	<0.1	5766	0.2	<0.1	6495	0.2	<0.1
total numbers	8800			9270			32590			51222		
total biomass		22.1			26.8			29.6			13.4	
total number excluding*)	8634			9181			32425			51070		
total biomass excl.*)		1.4			1.4			2.7			2.8	
O ₂ -consumption in µgat.h ⁻¹ .m ⁻² (= community metabolism)		1588			1510			2340			2150	
O ₂ -consumption in µgat.h ⁻¹ .m ⁻² .g ⁻¹ (ash-free dry weight)		72.5			56.5			79.0			160.0	
relative O ₂ -production in µgat O ₂ .h ⁻¹ .m ⁻²		1038			1114			980			575	

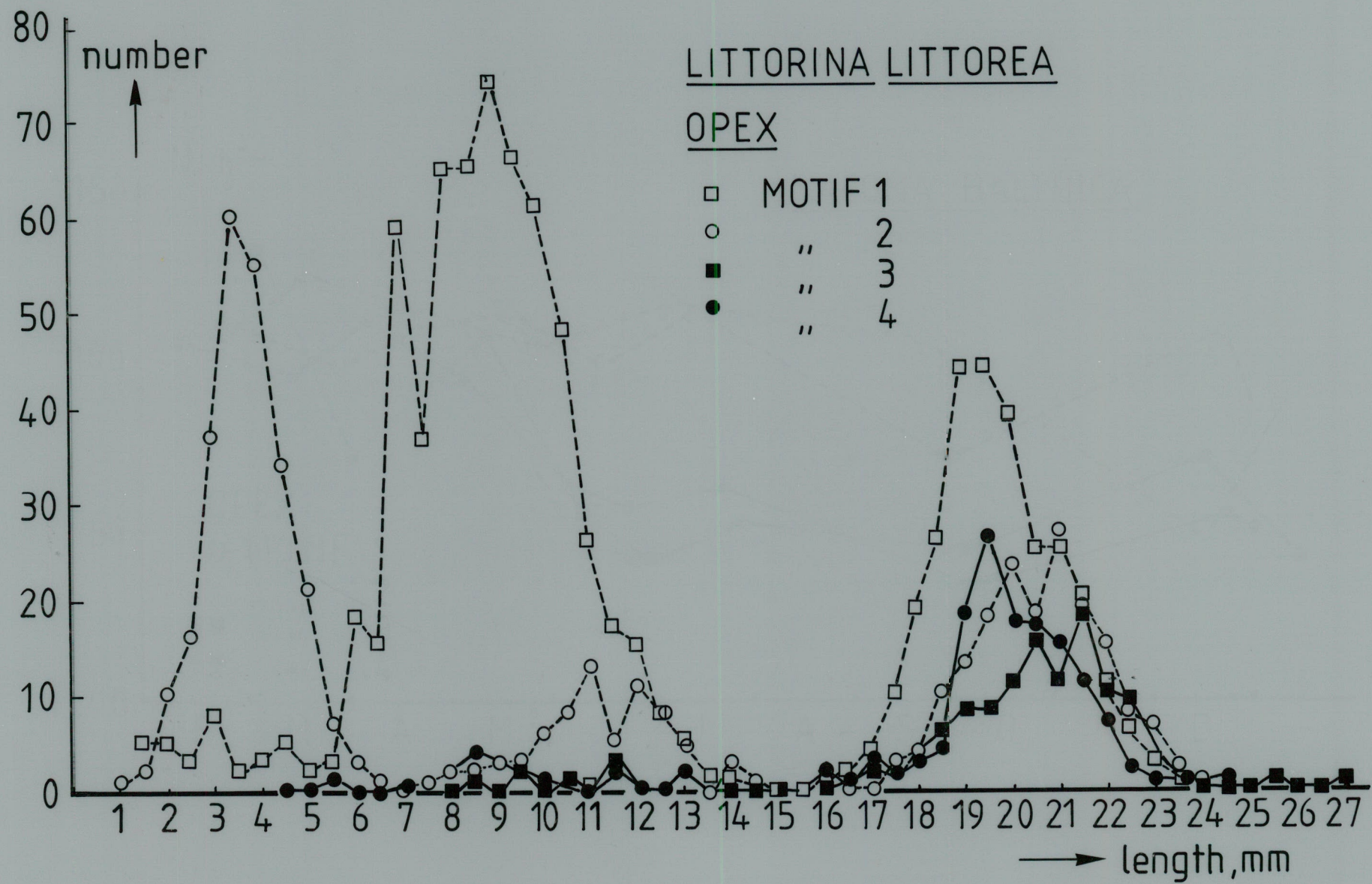


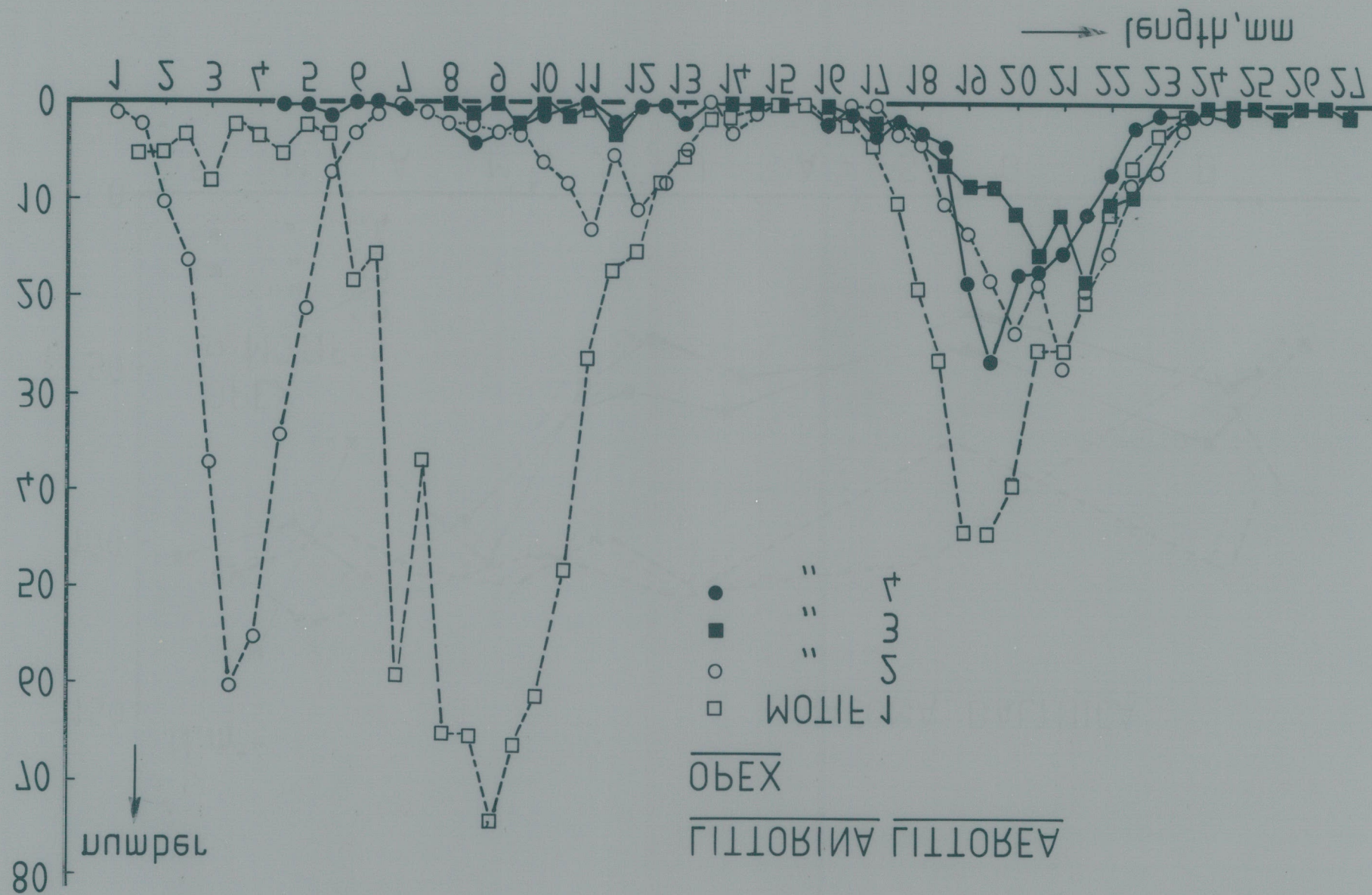


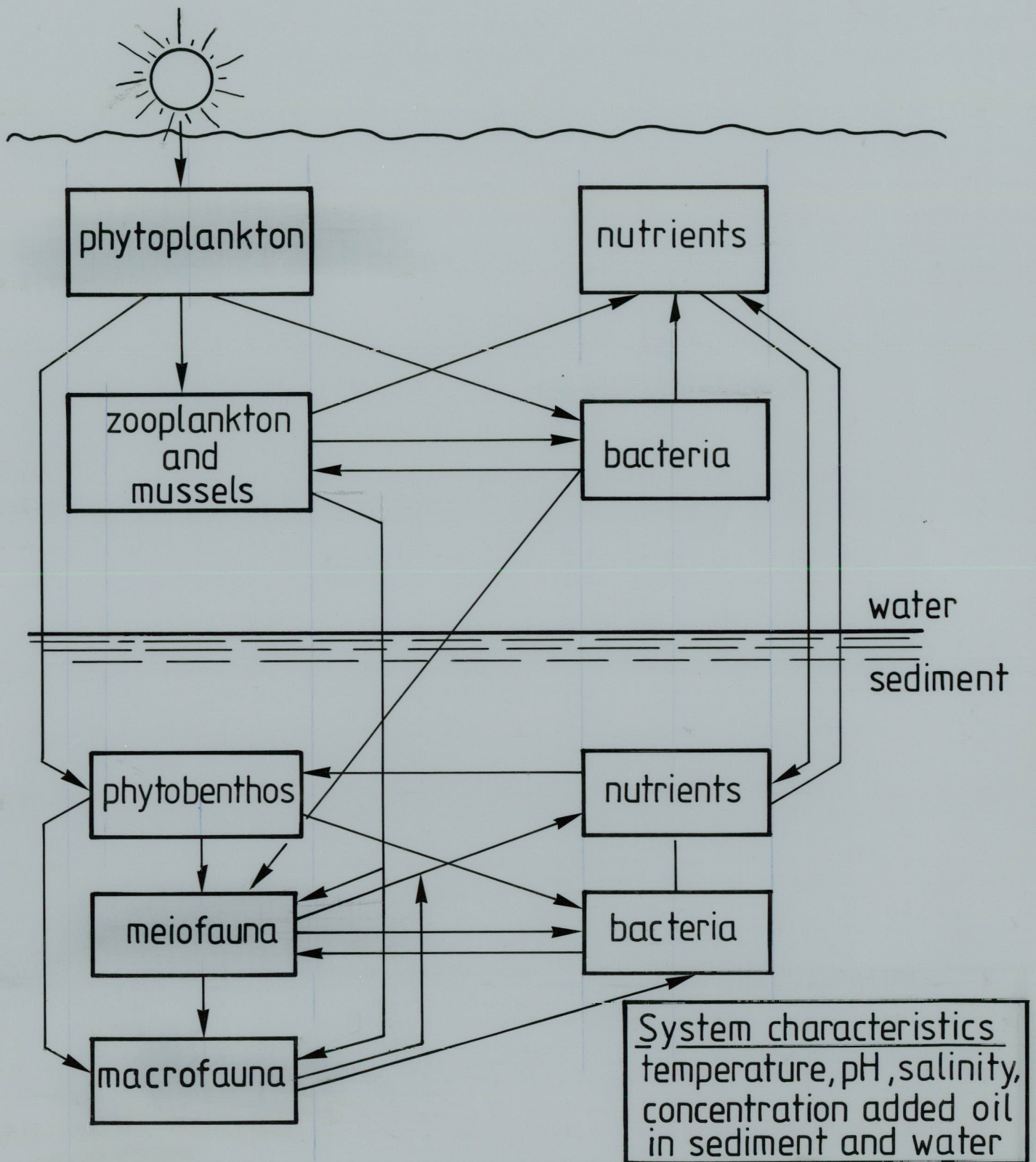


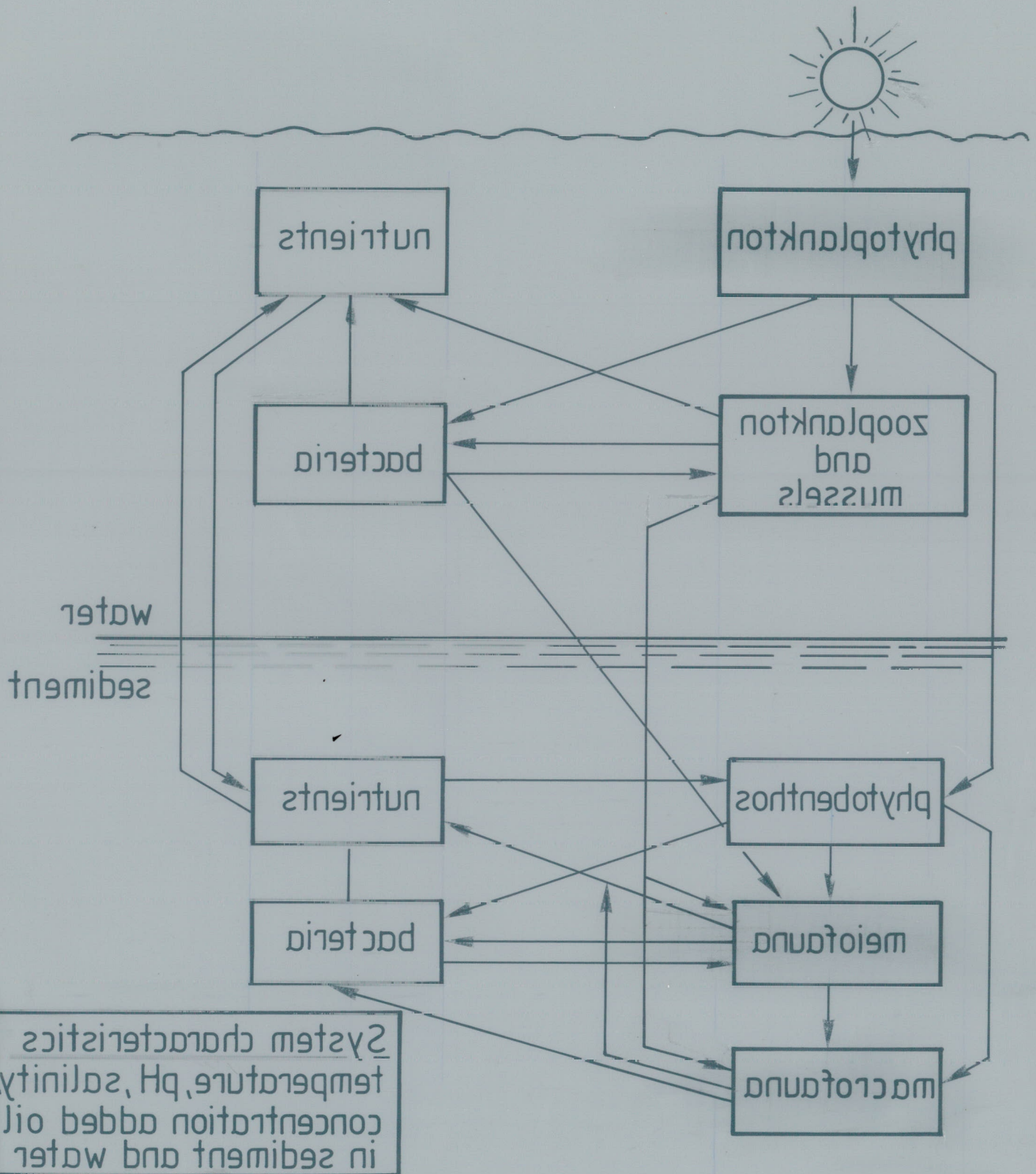


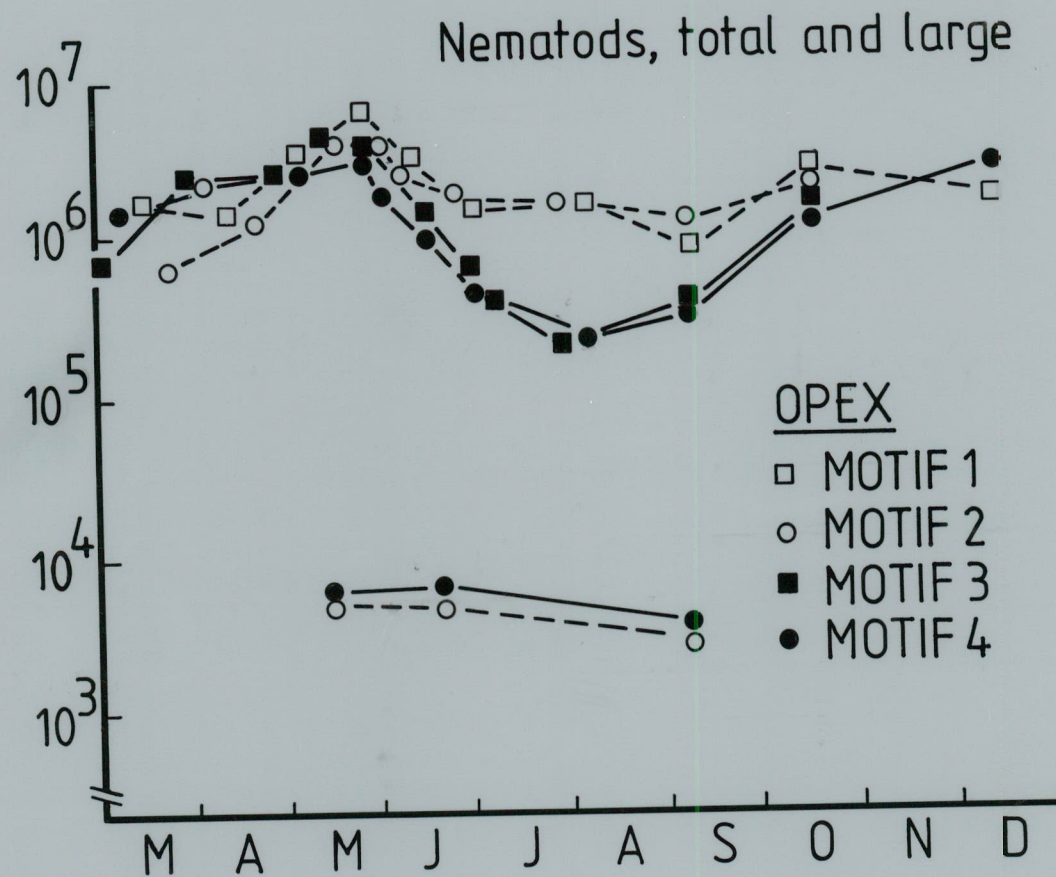
MACOMA BATHICA

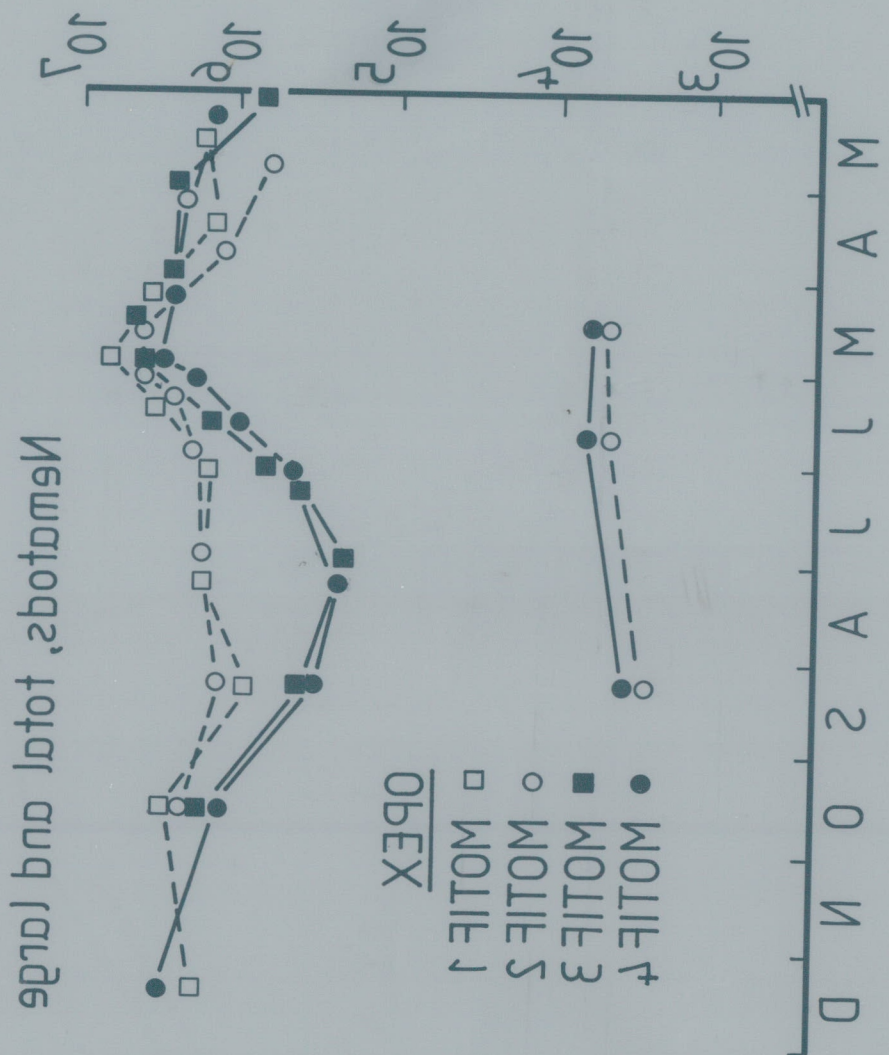


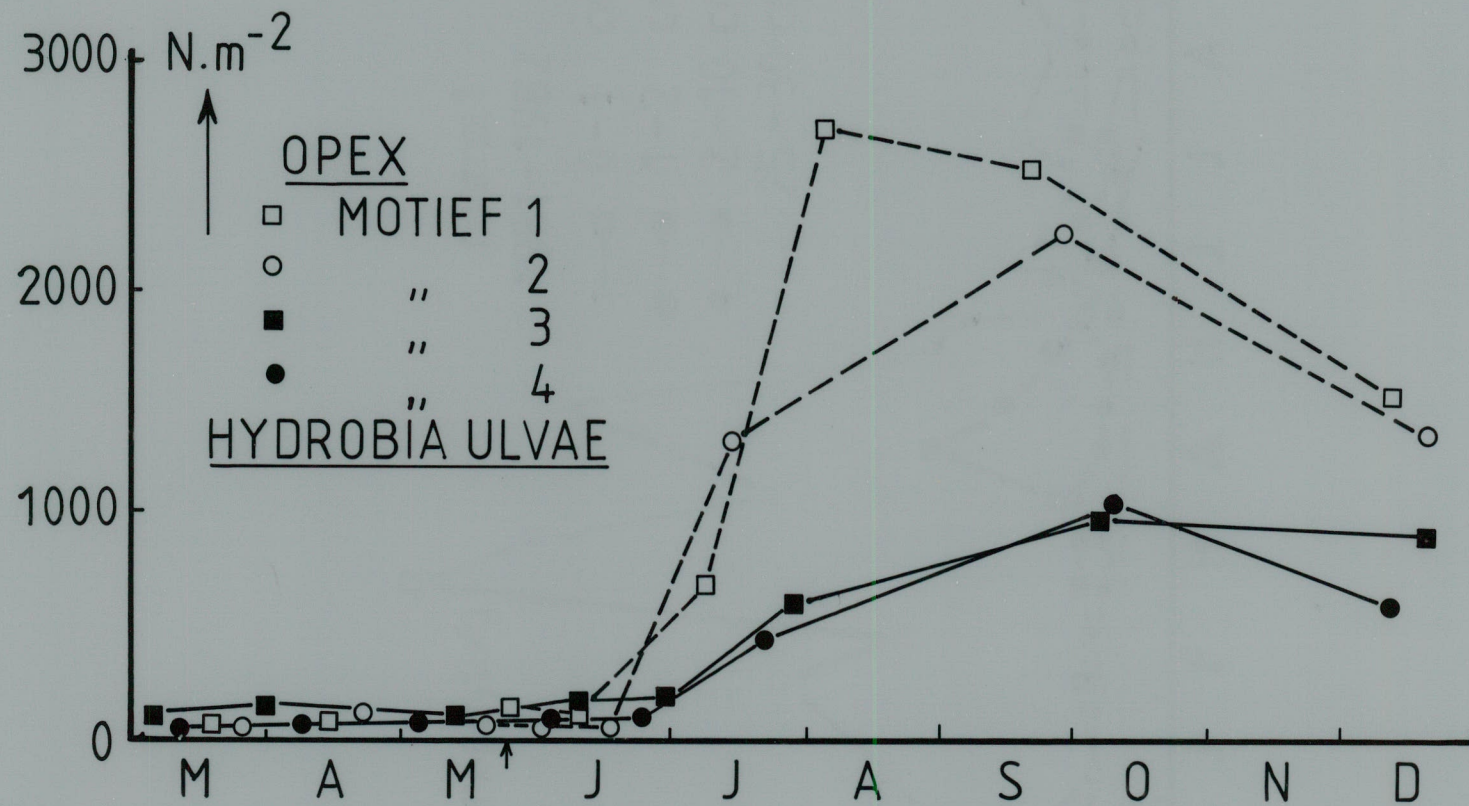


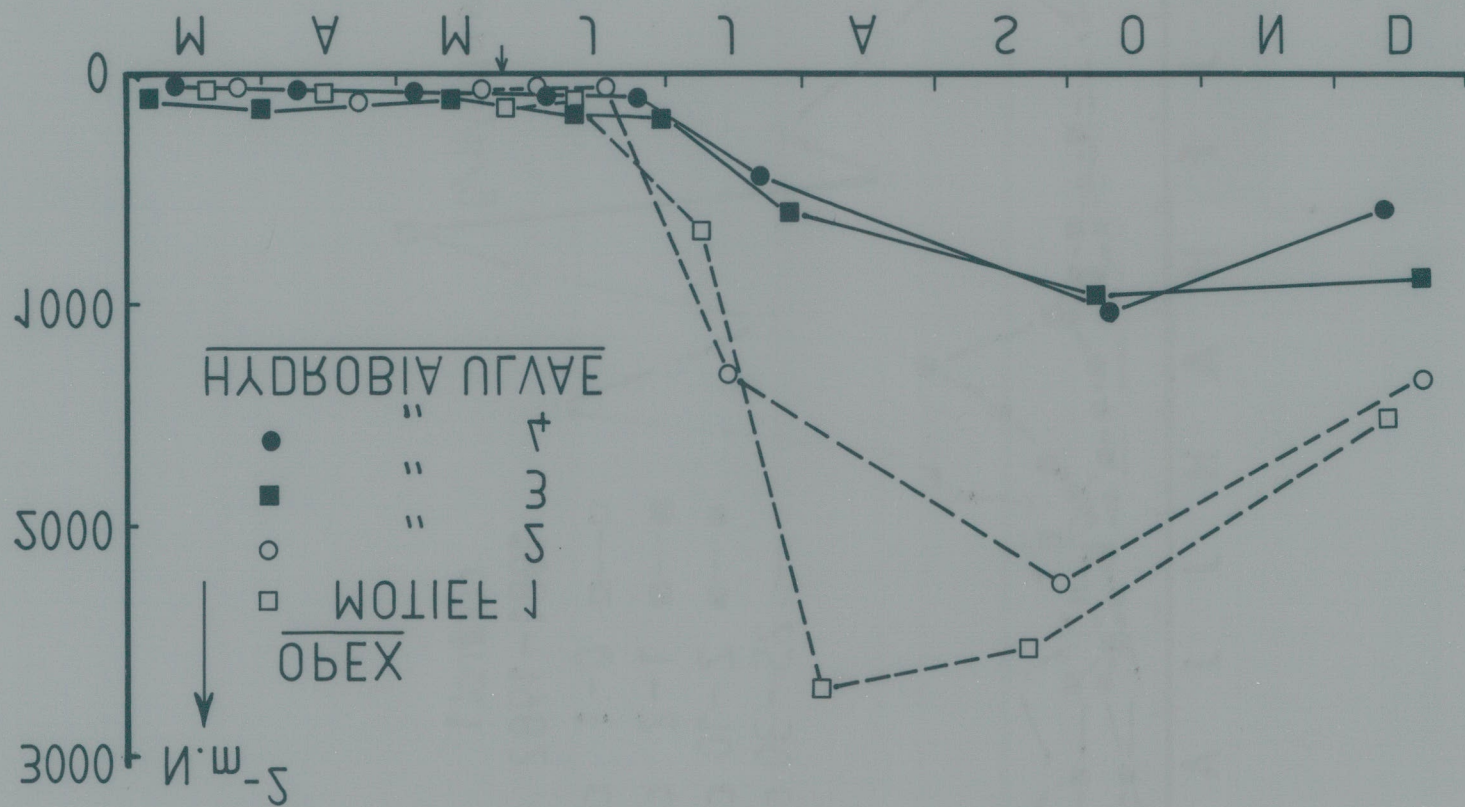


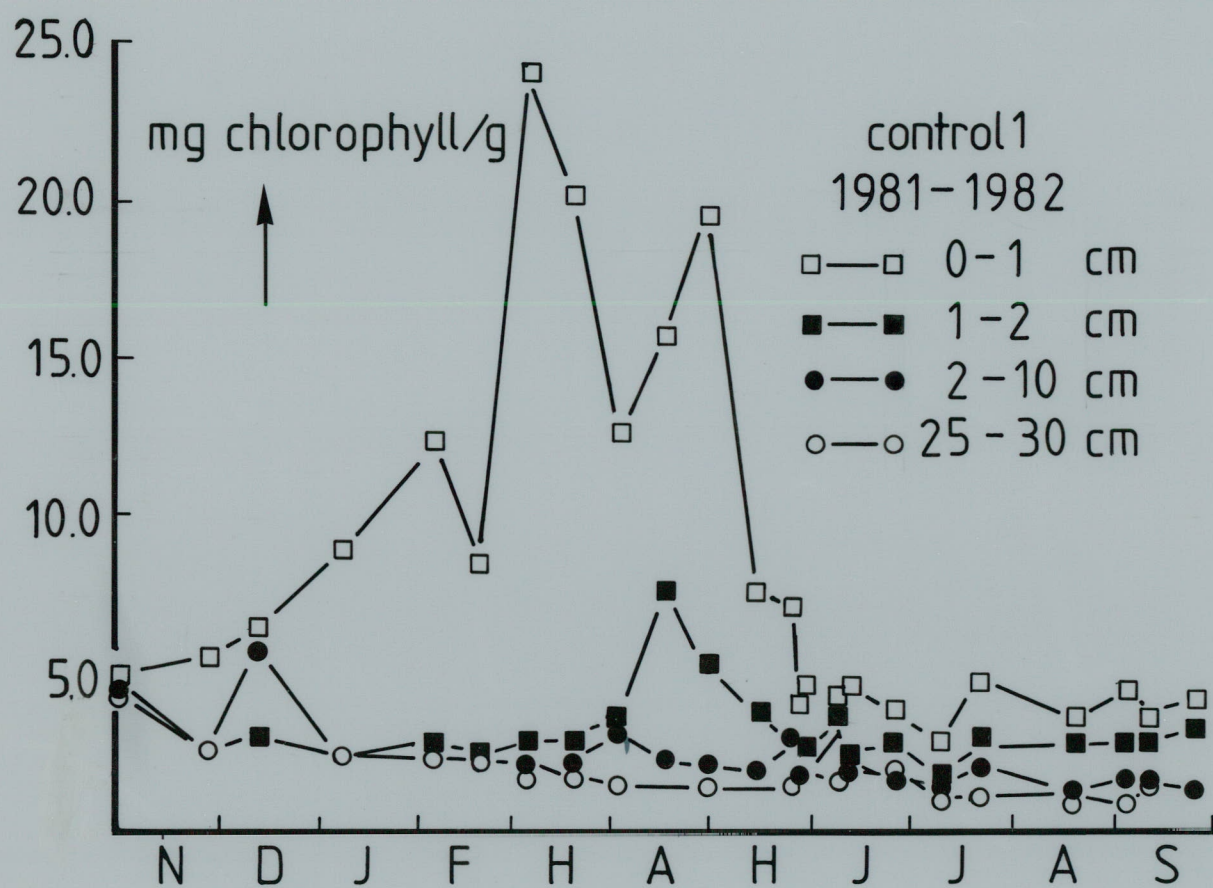


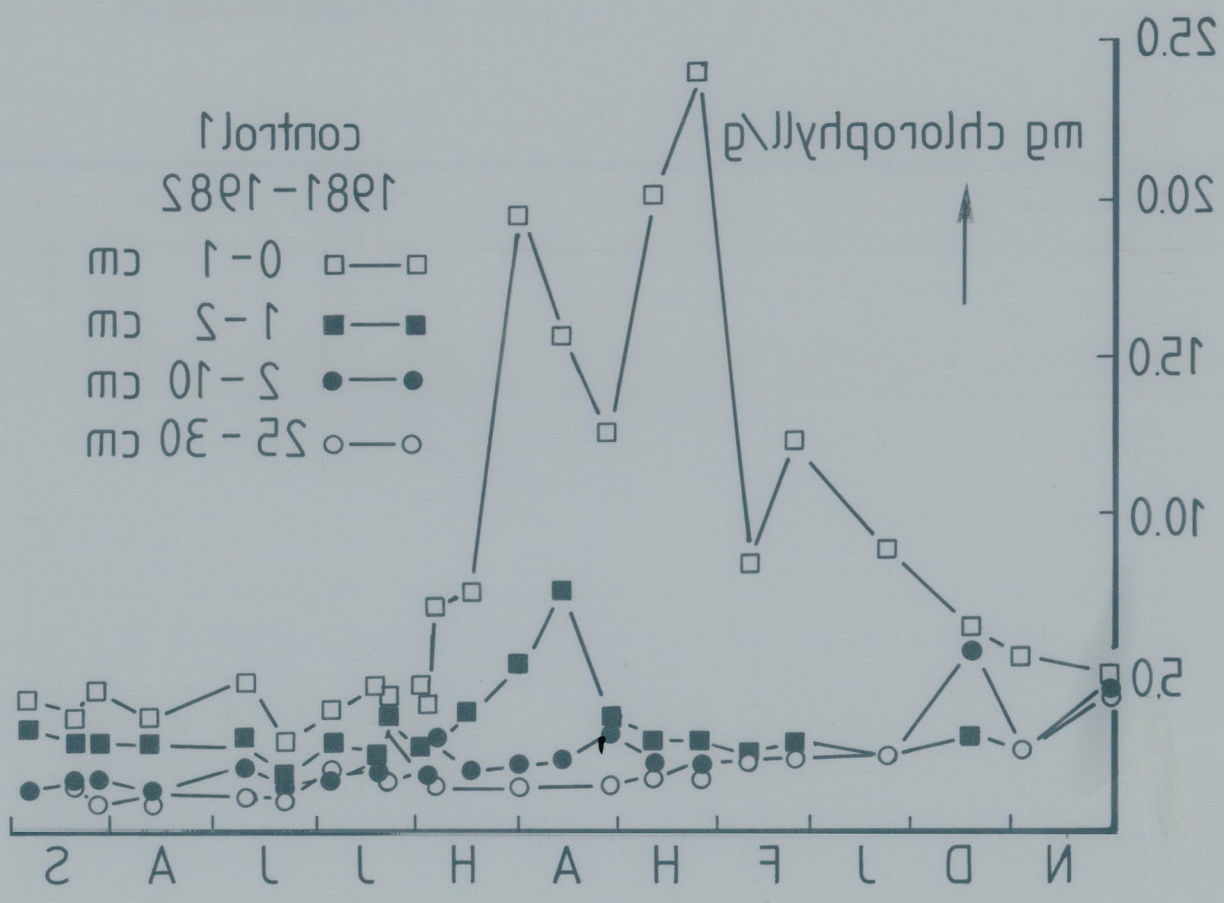


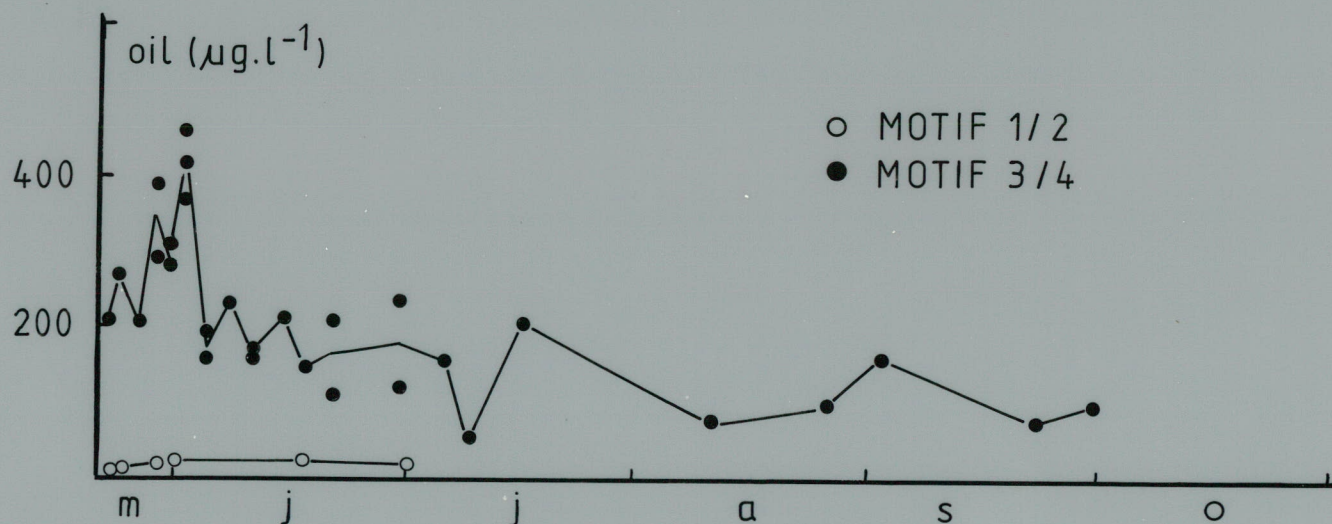
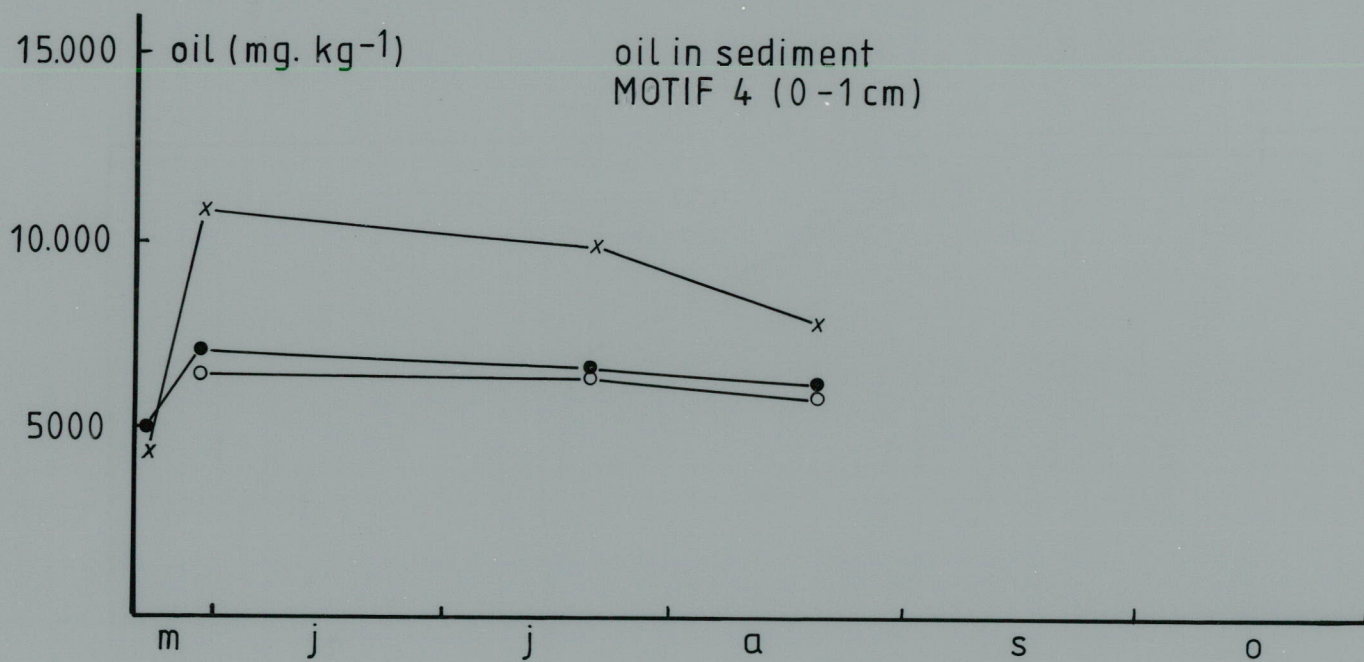
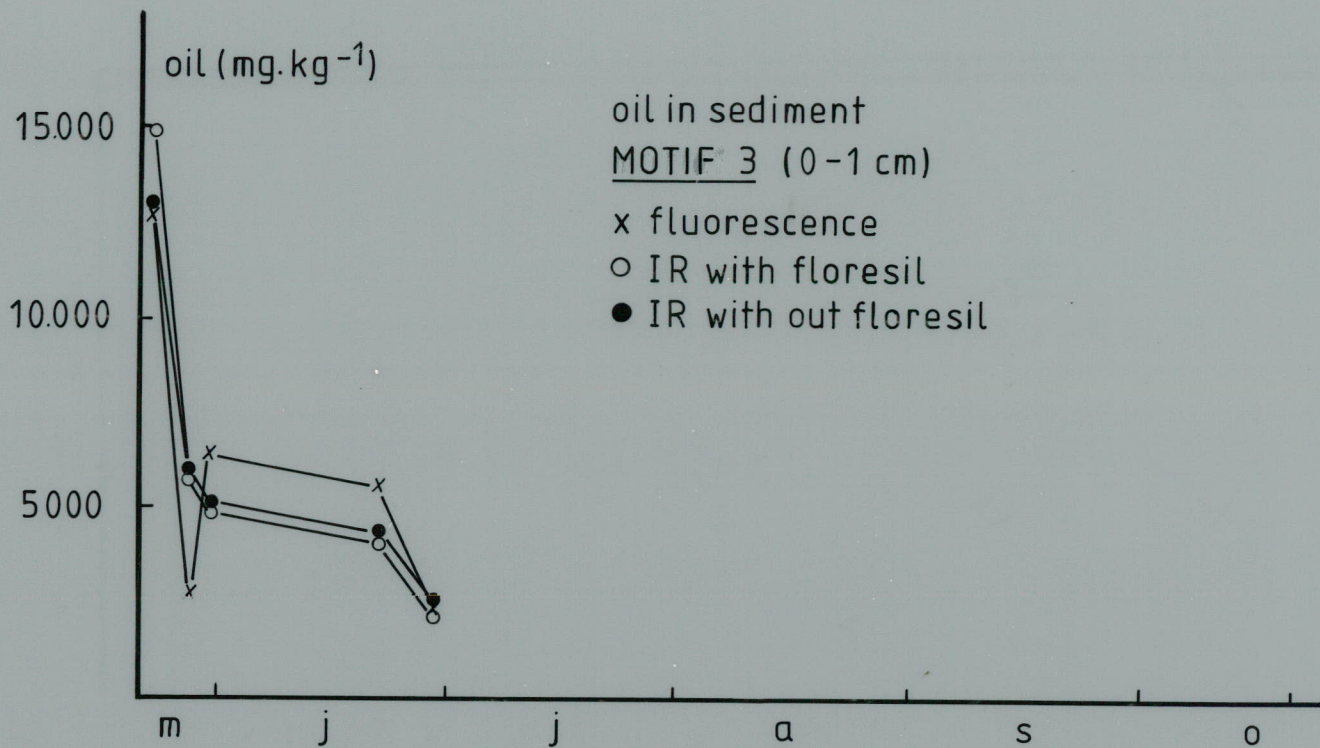


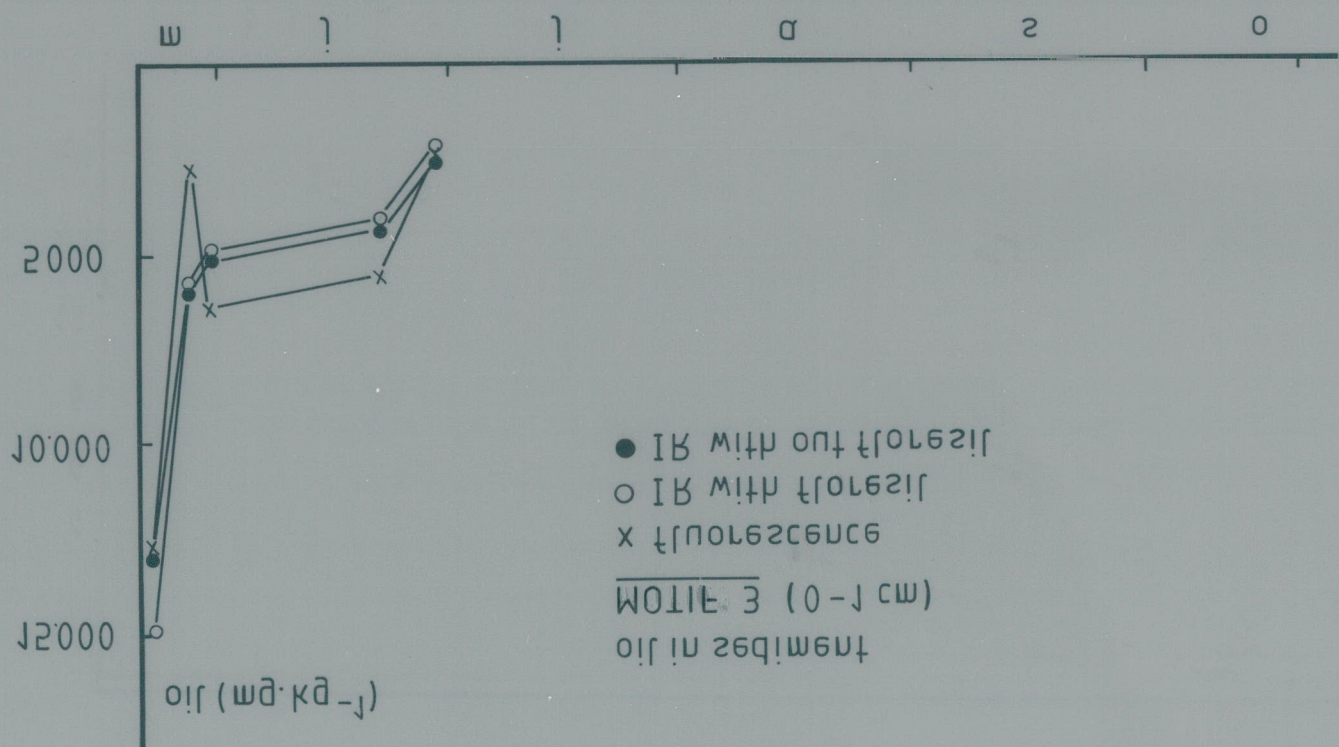
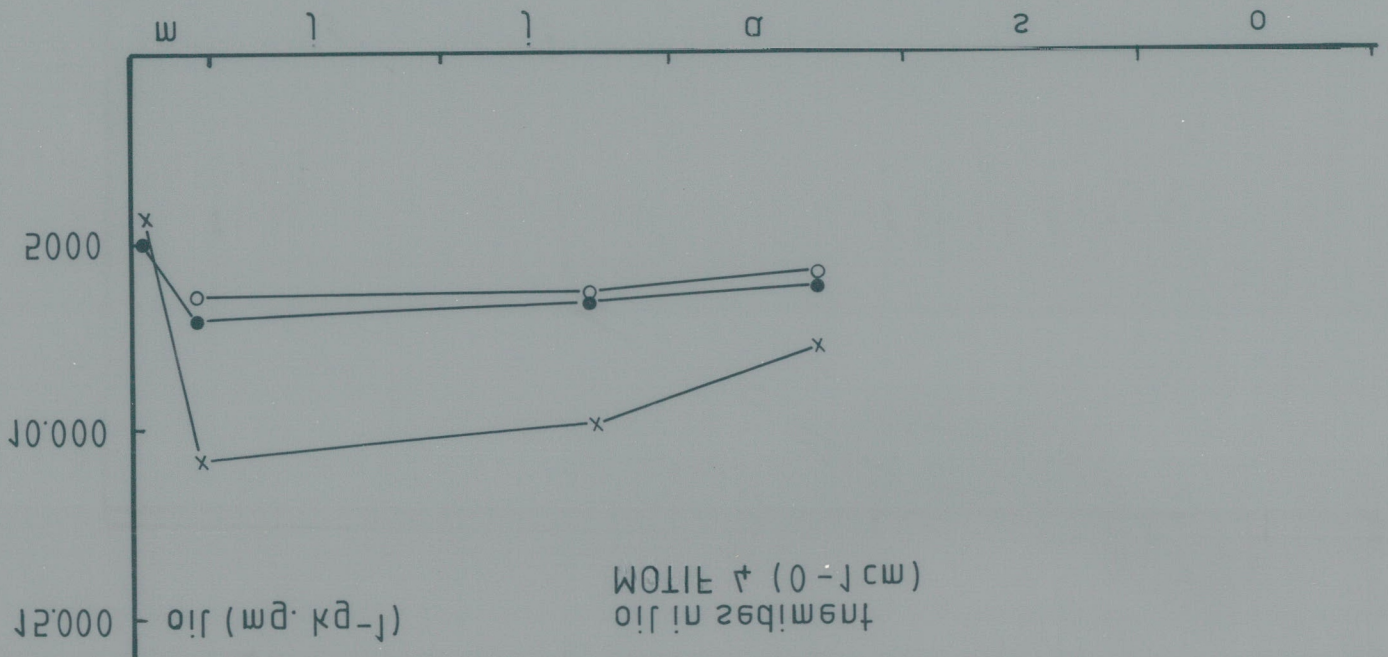
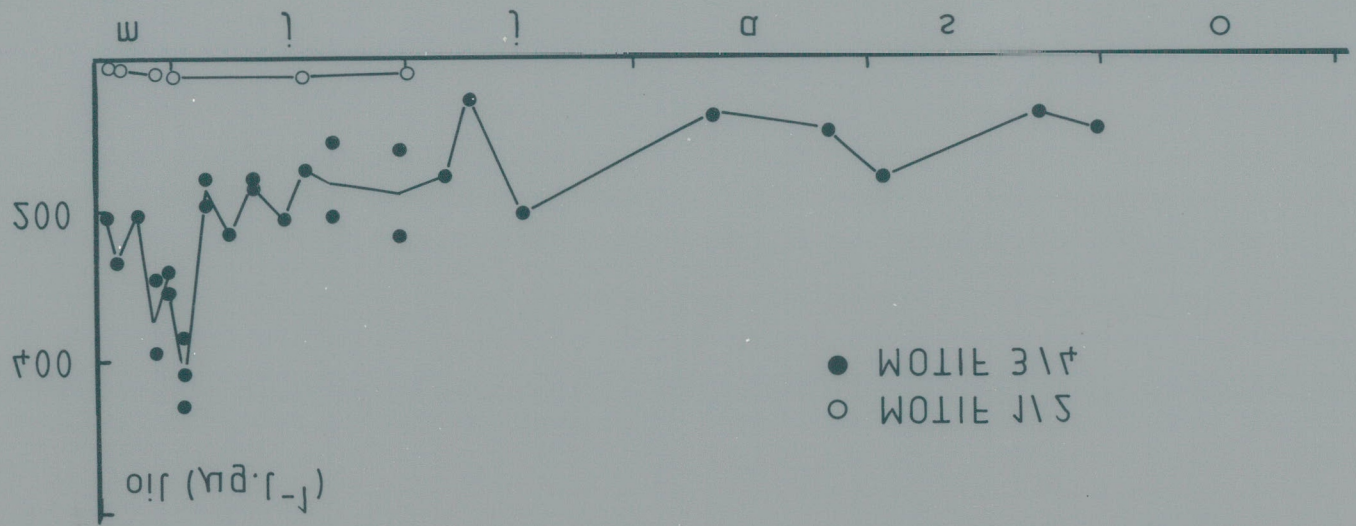


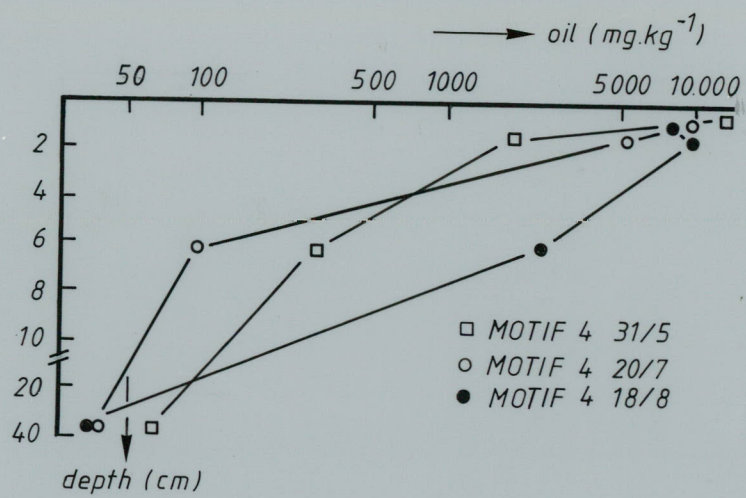












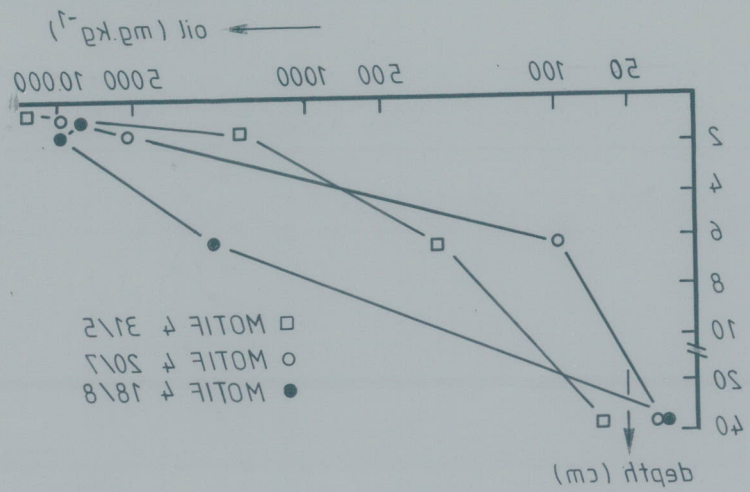


Table 9

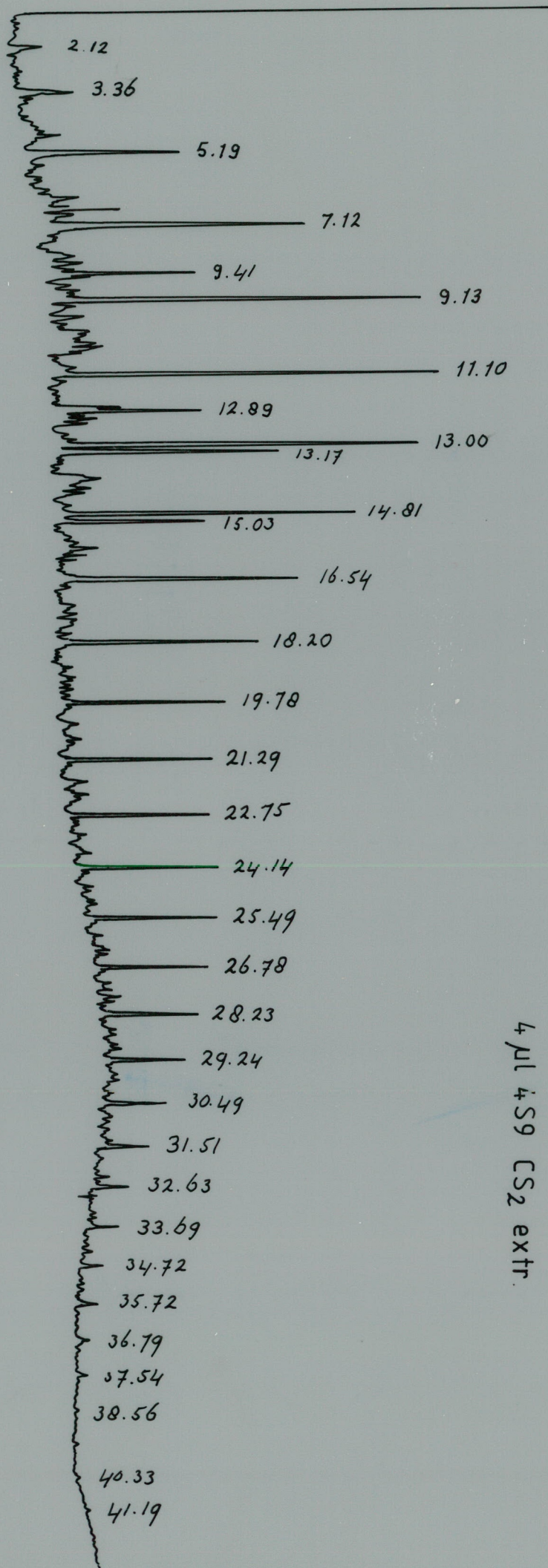
Date	4.3	11.3	18.3	25.3	1.4	7.4	15.4	22.4	29.4	6.5	13.5
MOTIF no.	2 4	1 3	2 4	1 3	2 4	1 3	2 4	1 3	2 4	1 3	2 4
Species											
<u>Centricae</u>											
Melosira nummuloides	R	R 2	1 R	X R	X X	1	R	1 R	R X		
M. varians		R	X	X X		1			R		
Skeletonema costatum	R R	R R	1 1	2 2	4 4	6 6	3 3	X 1	R R	R X	1 R
Thalassiosira decipiens	R	1	1 R	1 1	2 2	1			R R	X	
Th. nordenskiöldii			X X						R X		
Leptocylindrus danicus											R R
Rhizosolenia delicatula	R R	X	1 1	R R					R R	X X	3 3
Rh. shrubsolii		R		X	X R	X			X X	X X	R R
Rh. setigera					X X					X X	1 R
Chaetoceros spp.	X		X X			X	X R				
Biddulphia aurita	X	X		X	X	1	X				
B. regia							X				
Cerataulina bergonii			X	X	X X	X					
<u>Pennatae</u>											
Phagiogramma brockmannii								X R	R	1 1	
Thalassionema nitzschioides	R X	X	1 R	R R	1 1	R X	X	X X			
Asterionella kariana	X	R R	X X	1 R	1 1						
A. japonica					R						
Nitzschiaceae	3 6	5 5	4 6	2 3	2 3	3 3	2 2	2 2	2 1	2 2	2 2
Naviculaceae	4 3	4 4	6 5	5 5	4 4	4 5	4 4	3 3	4 4	4 4	3 4
Flagellates	6 5	5 5	5 5	5 4	5 5	6 6	7 7	7 7	8 7	7 6	7 7
µ-flagellates	6 6	7 6	7 7	6 6	6 6	7 7	7 7	6 6	8 8	9 9	9 9
Fresh water algae											
Scenedesmus spp.	X	1	X R	R R		R	X	X X	R R		
others	X	R R	X	X X	X X					R	

organisms	parameter	short term	long-term
phytobenthos	chlorophyll	+	0
phytobenthos	O ₂ -production per chlorophyll	(-?)	-
bacteria benthos	Electron Transport System	+	+
<u>Meiofauna</u>			
Oligochaet 1	numbers	0	0/-
Paranais/Capitella	numbers	0	+
Nematods	numbers	-	-
Harpacticoids	numbers	+	0
<u>Macrofauna</u>			
Mytilus edulis	numbers	0	0
Mytilus edulis	ash-free dry weight	-	0(?)
Cerastoderma edule	numbers	0	-
Cerastoderma edule	ash-free dry weight	-	+
Macoma balthica	numbers	-	-
Macoma balthica	ash-free dry weight	-	-
Mya arenaria	numbers	(?)	-
Hydrobia ulvae	numbers	0	-
Littorina littorea	reproduction	(?)	-
Arenicola marina	numbers	0	0(-*)
Arenicola marina	ash-free dry weight	0	0
Nereis diversicolor	numbers	0(?)	+
Nereis diversicolor	ash-free dry weight	0	0
Pygospio elegans	numbers	0	0
Heteromastus filiformis	numbers	0	-
Corophium volutator	numbers	-	-
Gammarus spp.	numbers	-	not present
<u>Zooplankton and fish</u>			
copepods	numbers	0	0
larvae	numbers	-	(-?)
Gobius minutus	numbers	(?)	-
<u>Phytoplankton</u>			
	chlorophyll	-	+
	species composition	different	different
	O ₂ -production per chlorophyll	+/-	-
Bacteria water	numbers	0	0

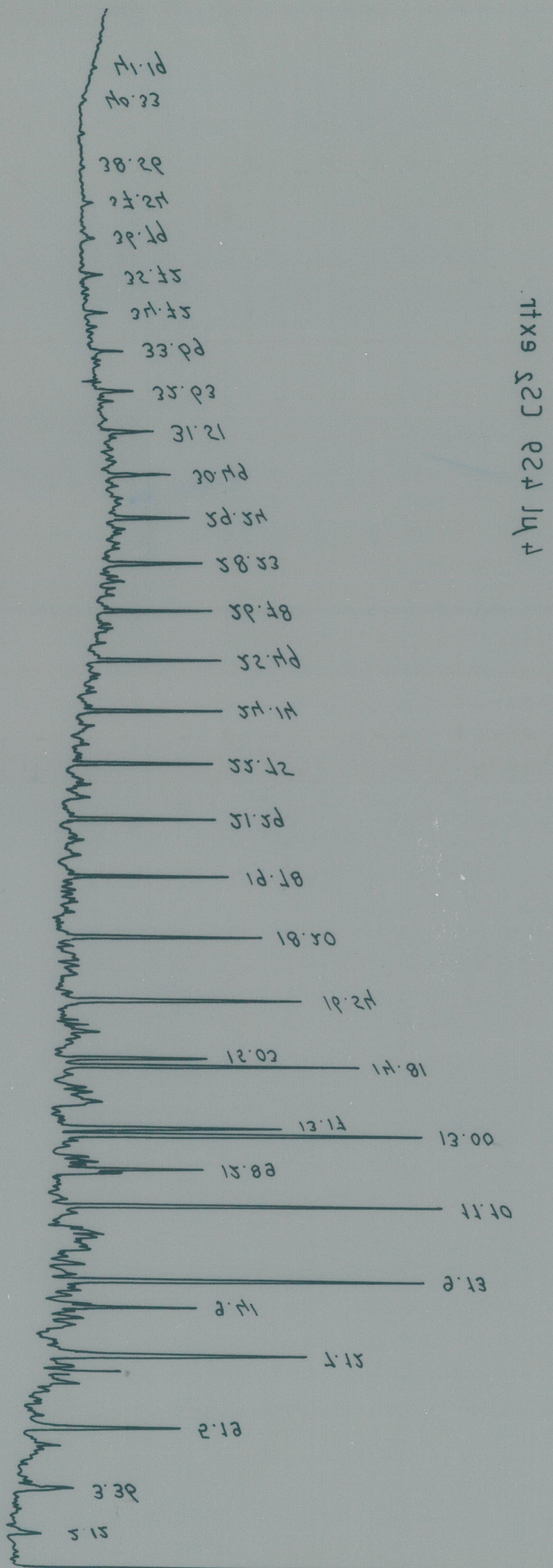
* Arenicola was absent at locations with the highest oil concentrations.

Table 8(vervolg)

start of SEPTEMBER						Effect oil	Remarks
MOTIF 2			MOTIF 4				
n.m ⁻²	g biomass.m ⁻²	mg ADW	n.m ⁻²	g biomass.m ⁻²	mg ADW		
50	4.8	95.9	50	3.6	72.1	-	ADW in J
50	8.6	172.2	0	0	0	-	n in J, S
1243	2.1	1.7	1027	2.0	2.0	?	
38857	2.1	<0.1	166	<0.1	0.1	-	n in J, S
0	0	0	0	0	0	-	n in J
0	0	0	0	0	0	?	
1690	8.9	5.3	2320	18.9	8.1	+	n in J, S; ADW in S
215	0.9	4.1	33	<0.1	1.4	-	n in S; ADW in S
50	0.1	2.7	66	0.2	3.6	?	
133	<0.1	0.3	66	<0.1	0.5	?	
0	0	0	0	0	0	+	n in J
3927	0.4	0.1	12576	0.9	0.1	+	n in J, S
2486	0.2	0.1	878	0.2	0.2	-	n in S
3413	<0.1	<0.1	3960	0.2	<0.1	?	
52114			21140			?	
	28.3			26.30		?	
52014			21090				
	14.9			22.7		+	biomass in S
	4316			4312		?	
	153.5			165.2		-	O ₂ -consumption per g in J
	781			595		-	O ₂ -production in J and S



4 μ l 4S9 CS₂ extr.

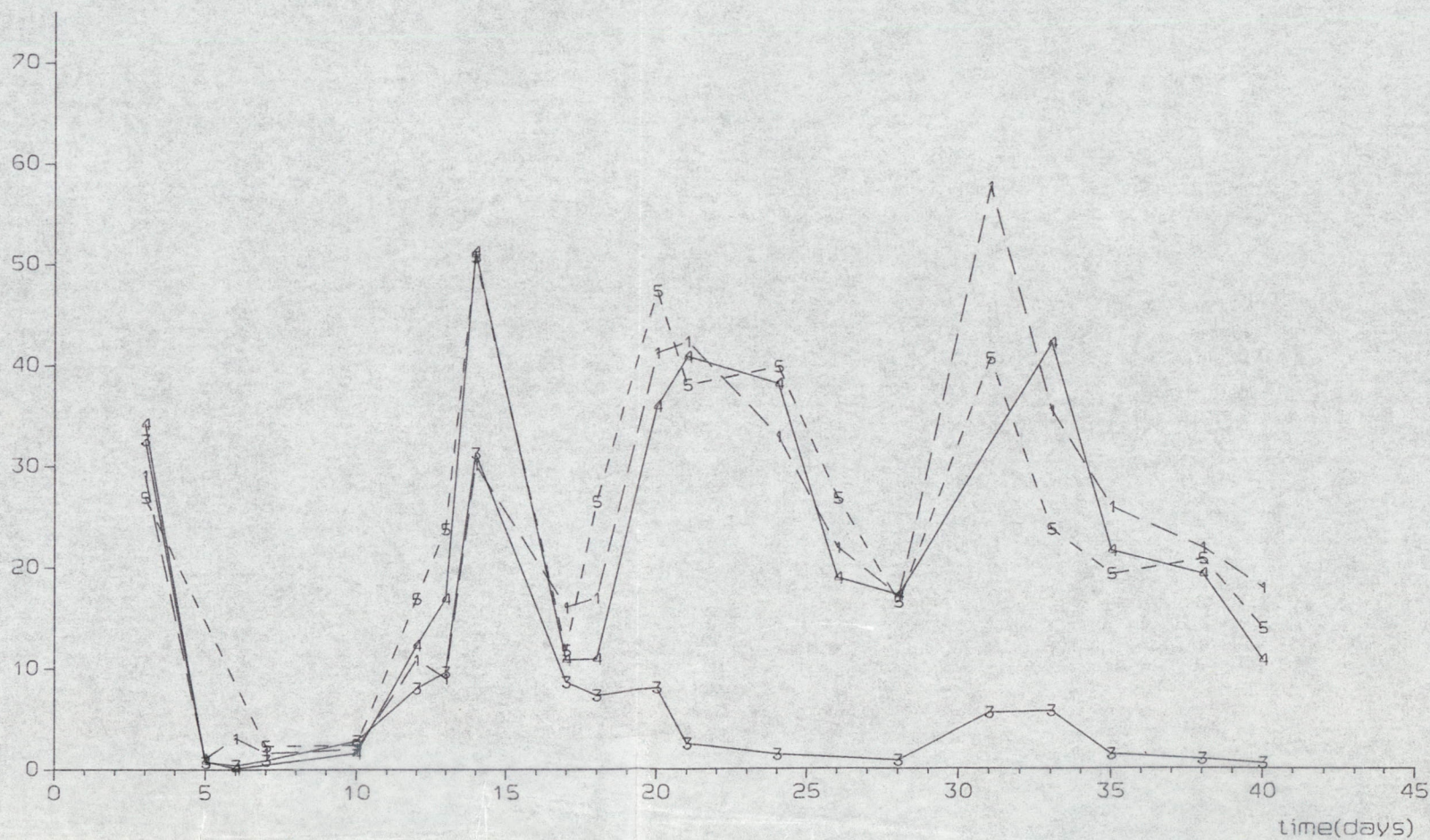


71.10 70.33 38.29 25.25 29.10 32.35 25.35 33.00 35.03 31.21 30.70 50.51 58.53 59.38 52.70 51.11 55.12 51.50 10.10 18.80 10.21 12.02 10.81 13.13 13.00 15.80 11.10 11.0 14.0 11.15 2.10 3.30 5.15

I-45

carbon assimilation
mg Cm⁻³

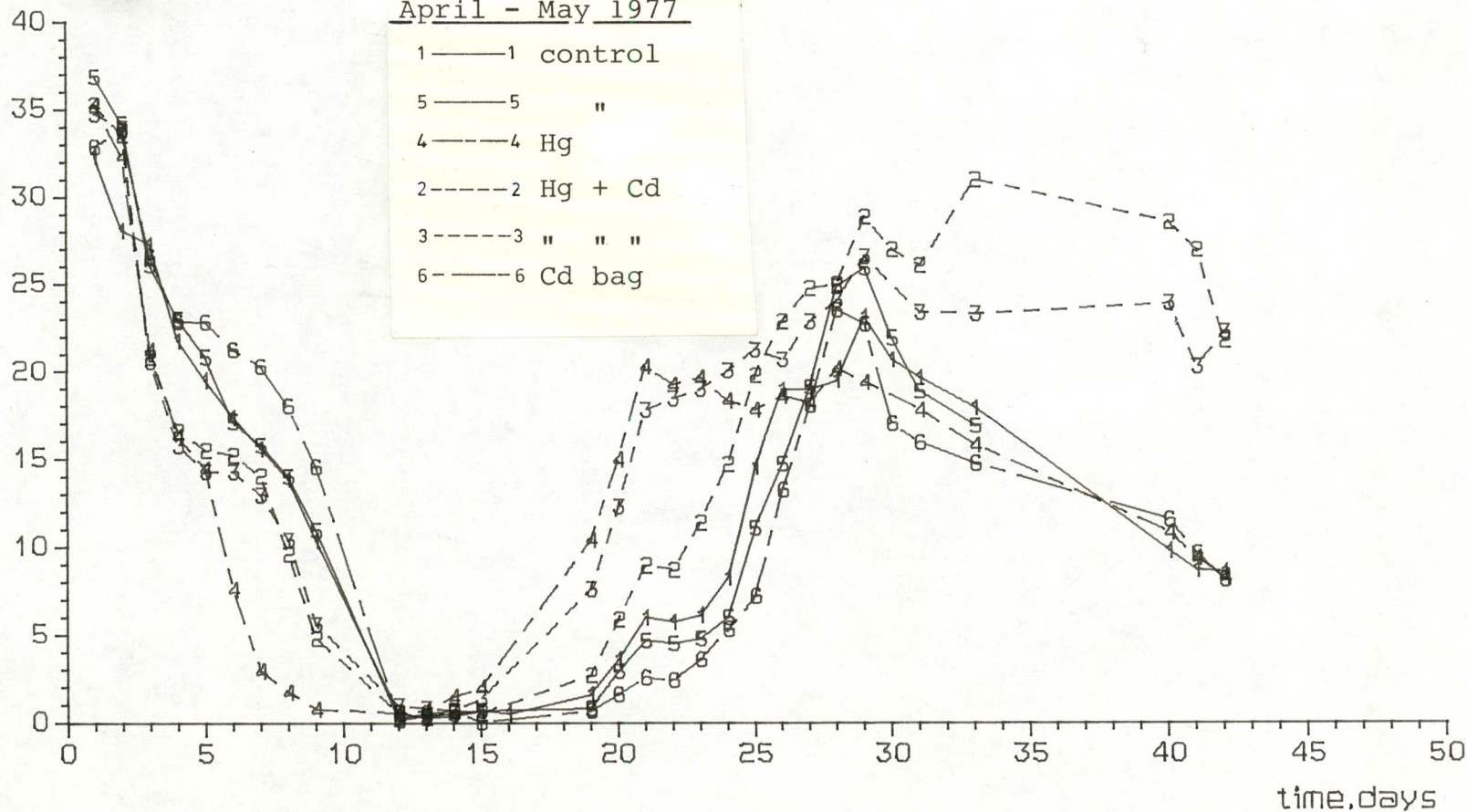
MAY-JUNE 1978
samples 2.0 m



chlorophyll,
 mg. m^{-3}

April - May 1977

- 1 — 1 control
5 — 5 "
4 — 4 Hg
2 — 2 Hg + Cd
3 — 3 " " "
6 — 6 Cd bag



1005(42742)



1005(42742) 0.2 5 w
1005(42742) 0.2 5 w

kopieën van geplakte exemplaren

83/14

