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A COMPARITIVE STUDY OF MONITORING TECHNIQUES TO ESTABLISH DISTRIBUTION AND BIOLOGICAL EFFECTS OF DRILLING MUDS AROUND OFF-SHORE INSTALLATIONS ON THE DUTCH CONTINENTAL SHELF (1986)

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CONTENTS

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SAMENVATTING

Dit rapport beschrijft het vervolgonderzoek naar de bruikbaarheid van monitoringstechnieken voor het vaststellen van de verspreiding en de biologische effecten van lozingen van boorgruis en aangehechte boorvloeistoffen rondom boorlokaties op het Nederlands Continentale Plat.
Het onderzoek werd verricht in opdracht van Rijkswaterstaat (Directie Noordzee), Ministerie van Economische Zaken (Staatstoezicht op de Mijnen) en Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer en begeleid door de werkgroep "Monitoring Mijnbouwinstallaties op het Continentale Plat".
De doelstellingen van het onderzoek waren:

- het vinden van de meest geschikte techniek voor het vaststellen van de verspreiding en de biologische effecten van boorgruislozingen;
- het uitvoeren van een baseline onderzoek waaraan later optredende effecten gerelateerd kunnen worden;
- onderzoek naar verschillen in effecten van Water Based Muds (WBM) en Oil Based Muds (OBM);
- onderzoek naar korte- en langetermijneffecten veroorzaakt door het gebruik van OBM.

In het kader van het onderzoek naar de meest geschikte techniek voor het vaststellen van de verspreiding van boorgruis en boorspoeling werd in het onderzoek van 1985 en in dit onderzoek een reeks van parameters in het sediment gemeten, te weten TOC, korrelgrootteverdeling, barium, olie (GC-methode) en biologische capaciteit tot afbraak van oliecomponenten.
Van de onderzochte parameters bleek barium het meest geschikt voor het vaststellen van de verspreiding van boorspoelingen; tot op 2000 m afstand van boorlokaties werden verhogingen in het sediment gevonden. Belangrijk is tevens het feit dat bariet zowel gebruikt wordt in OBM als WBM.
Het gebruik van de GC-methode voor het bepalen van olie maakte het mogelijk om een duidelijk verband te leggen tussen de in het sediment gevonden olie en de ter plekke gebruikte OBM.
Om mogelijke biologische effecten vast te stellen zijn twee methodieken toegepast. In de eerste plaats werd een Passieve Biologische Monitoring (PBM) uitgevoerd, daartoe werd tijdens de monitoringprogramma's het macro-

benthos verzameld en op oliecomponenten geanalyseerd. In de praktijk blijken echter de dichtheden van de voor olieanalyses geschikte organismen te gering te zijn.

De tweede toegepaste methode voor het vaststellen van biologische effecten is de Actieve Biologische Monitoring (ABM), daartoe worden mosselen (Mytilus edulis) afkomstig van een niet vervuilde lokatie getransporteerd naar plaatsen op verschillende afstanden van de boorlokaties. Na een expositie van 6-8 weken werden aan de mosselen, physiologische, cytochemische en biochemische stressparameters bepaald, waarbij deze metingen gerelateerd werden aan de geaccumuleerde oliecomponenten in de mosselen.

In 1986 en 1987 zijn twee ABM-experimenten uitgevoerd bij L-4-α, één gedurende de feitelijke boorfase en één zes maanden na afloop van het boren.

Tijdens het eerste experiment werden verhoogde olieconcentraties gevonden tot op 5000 m vanaf het boorplatform. Mogelijk is een nog groter gebied beïnvloed, maar hier zijn geen gegevens over. Zes maanden na afloop van de boringen werden alleen nog verhoogde olieconcentraties gevonden direct onder het platform, hetgeen erop wijst dat de mossel vooral geschikt is als indicator voor de verontreiniging van de waterfase. De olieconcentraties in de mosselen waren echter niet dusdanig verhoogd, dat ze aanleiding gaven tot meetbare verschillen in de resultaten van de stressparameterbepalingen.

Voor het vaststellen van een baseline werd bij L-4-α, vóór de start van de boringen met OBM, een monitoringsprogramma uitgevoerd. Hoewel er reeds een jaar verlopen was sinds de laatste boringen met WBM waren er nog duidelijk verhoogde Ba-concentraties in het sediment aanwezig. Er konden echter geen biologische effecten aangetoond worden (Mulder, Lewis, van Arkel, 1988).

Bij F-18-8 werden de effecten van een WBM-boring onderzocht. Hoewel er verhoogde Ba-gehalten in het sediment gevonden werden, resulteerden deze niet in aantoonbare effecten.

Voor het onderzoek naar de langetermijneffecten van het gebruik van OBM werd een monitoringsprogramma uitgevoerd bij K-12-α, op dezelfde lokaties waar in 1985 ook bemonsterd was. In vergelijking met de resultaten van 1985 bleek er geen verbetering te zijn opgetreden rondom dit platform, eerder een verslechtering.

SUMMARY

In 1986 and the beginning of 1987 a study was carried out by MT-TNO in cooperation with NIOZ-EON and the North Sea Directorate of the Ministry of Transport and Public Works on the Dutch Continental Shelf.
The aim of the study was to establish the usefulness of techniques for monitoring the distribution of drill cuttings and adhered drilling muds.
Furthermore Active Biological Monitoring (ABM) experiments with the common mussel (Mytilis edulis) were carried out to see if biological stress was detectable from such parameters as Scope for Growth (SFG), Lysosomal Stability (LS), Mixed Function Oxidase (MFO) etc.
The effects of drill cuttings on the macrofauna was investigated by NIOZ-EON and has been reported separately (Mulder, Lewis, van Arkel (1988).
This report describes the results of the experiments carried out by MT-TNO.

1. INTRODUCTION

Off-shore activities in the Dutch sector of the North Sea have increased considerably during the last decades. In drilling operations large quantities of drilling muds are used to lubricate the drilling bit, to remove cuttings from the hole, to stabilize the bore-hole and to counteract the pressure in the bore-hole. Drill cuttings which, after separation from the mud-system, are discharged to the sea-bed, contain a large quantity of mud which is adhered to the grain surfaces (Blackman and Law, 1981; Blackman et al., 1982).

Since the beginning of the 1980's the use of Oil Base Muds (OBM), in combination with Water Based Muds (WBM) has steadily increased. Originally the OBM was based on oil with a high content of aromatic hydrocarbons, but because of their high toxicity, nowadays "low toxicity" OBM with a lower aromatic content are being employed. Data presented at the 11th meeting of the "Working Group on Oil Pollution" of the Paris Commission showed an increase of the spillage of oil due to OBM from 7,426 ton in 1981 to 25,757 ton in 1985.

Results from studies in England and Norway (Dicks, 1981; Salzman, 1982; Davies et al., 1984; Hannam, 1987; Gillian et al., 1986; Matheson et al., 1986) showed effects on the benthic ecosystem. However, these results are not directly transferable to the Dutch situation, because of differences in for instance water-depths and sediment types.

In the light of these results and the recommandations of the North Sea countries to monitor pollution from off-shore installations (Paris Commission), the North Sea Directorate of the Ministry of Transport and Public Works (RWS) in cooperation with the Directorate General of the Ministry of Publich Health, Planning and Environment (VROM) and the State Supervision of Mines of the Ministry of Economical Affairs (EZ) commissioned TNO Division of Technology for Society (MT-TNO) and the Netherlands Institute for Sea Research, section Ecological Research North Sea and Waddensea (NIOZ-EON) to carry out a study of the usefulness of various monitoring techniques for assessing the distribution of discharged oil contaminated cuttings and their impact on the marine environment.

After a preliminary study in 1985 (Kuiper, Van het Groenewoud, 1986; Mulder, Lewis, Arkel, 1987) a comprehensive study started in 1986 with the following aims:

1. To find the best suitable technique for monitoring the distribution of drill cuttings and their biological effects.
2. To establish a baseline study to which effects could be related.
3. To study the differences in effects of WBM versus OBM.
4. To study the effects of OBM on a short and larger time-scale.

2. ORGANIZATION

The study was carried out by MT-TNO in cooperation with NIOZ-EON and RWS. RWS had the overall coordination and placed the research vessels M.V. "Holland" and M.V. " Small Agt" at the disposal of MT-TNO and NIOZ-EON. NIOZ-EON took care of the sampling of the sediment and analysed the composition and distribution of the macrobenthos. MT-TNO analysed the abiotic sedimentary parameters, the biodegradability of the oil components in the sediments and the bioaccumulation of oil components in the macrobenthos. Furthermore two ABM experiments with the common mussels (Mytilus edulis) were carried out to investigate the use of stress induced parameters in detecting the biological effects of the discharged drill cuttings and the bioaccumulation of oil components in the mussel tissues. The stress induced parameters, including Lysosomal Stability, "Mixed Function Oxidase" and induction of mallothioneïnen were analysed by MT-TNO in coorporation with the University of Ghent (Belgium). The metabolic rate and the end product ratio during anaerobiosis were analysed by the University of Utrecht, department of Experimental Zoology.
The Coast Guard vessels MV. "Terschelling" and M.V. "Breeveertien" handled the buoys and the concrete anchors which were used in the ABM experiments around platform L-4-α. The operator and owner of L-4-α, Petroland B.V. placed their facilities at our disposal and assisted in the sampling of the sediment underneath the platform.

3. ACKNOWLEDGEMENT

This study was commissioned by RWS, EZ and VROM under responsibility of Dr W. Zevenboom of RWS. The Working Group "Monitoring around Offshore installations (MONMY) formulated the aims of the study and met on a regular basis to discuss the results and adjust the aims. The composition of the Working Group is given in Appendix A.

The authors wishes to express their thanks to the crews of the research vessels M.V. "Holland" and M.V. "Small Agt" for their hospitality and their cooperation during the sediment sampling and the personnel of Petroland B.V. on board the platform L-4-α for their assistance.

The ABM experiments would not have been possible without the enthusiasm and skill of the crews of the Coast Guard vessels M.V. "Terschelling" and M.V. "Breeveertien", who solved all the technical problems related to the ABM experiments.

4. RESEARCH STRATEGY AND SELECTION OF SITES

In order to study the distribution and effects (chemical and biological) of the discharges of cuttings and muds three different approaches were taken:

- Chemical Monitoring of the sediment in the vicinity of platforms to assess the distribution of muds in the sediment.
- Passive Biological Monitoring of the macrobenthos to establish the accumulation of oil components in tissues due to the presence of OBM in the sediment.
- Active Biological Monitoring studies (ABM) with Mytilus edulis. Two experiments were carried out, one during the actual drilling to study the effects of discharges on the water compartiment and one several months after the drilling to study effects of the secondary release of oil from the oil present in the sediment.

The following platforms were selected by the Working Group:

Platform L-4-α : a platform were since January 1985 no drilling activities had taken place, up to this date only WBM was used. It is assumed that the ecosystem around the platform had sufficiently recovered to use it as a baseline for further studies (aim 2). Sampled in week 21-24, 1986. During 1986 two wells were drilled with "low tox" OBM (aim 1). Sampled in week 37-39, 1986.

Platform F-18-8 : an abandoned location where one recent drilling with WBM had been carried out (aim 3). Sampled in week 21-24, 1986.

Platform K-12-α : in 1983 the last of six wells was drilled. Some of these wells were drilled with a OBM with a high aromatic content (aim 4). Sampled in week 37-39, 1986.

The geographical positions of the sites are presented in Figure 1.
A summary of the relevant data of drilling activities at the test sites are given in Appendix B.

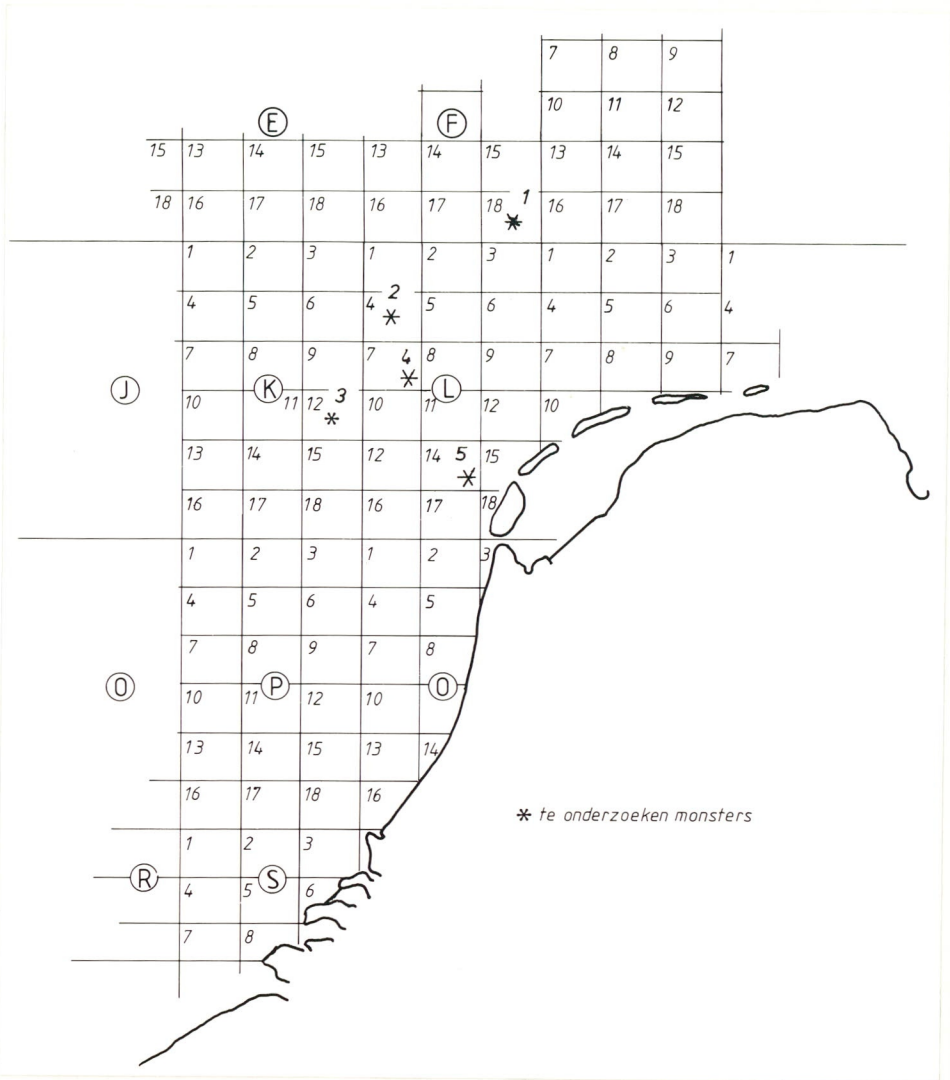


Fig. 1 Selected sites on the Dutch continental shelf.

5. MATERIALS AND METHODS

5.1 SEDIMENT

5.1.1 Sampling

At the sites two transects were sampled, one transect in the direction of the resulting current (R-transect) and one transect transverse to these line (T-transect). The stations at which sediment samples were collected are given in Figure 2.

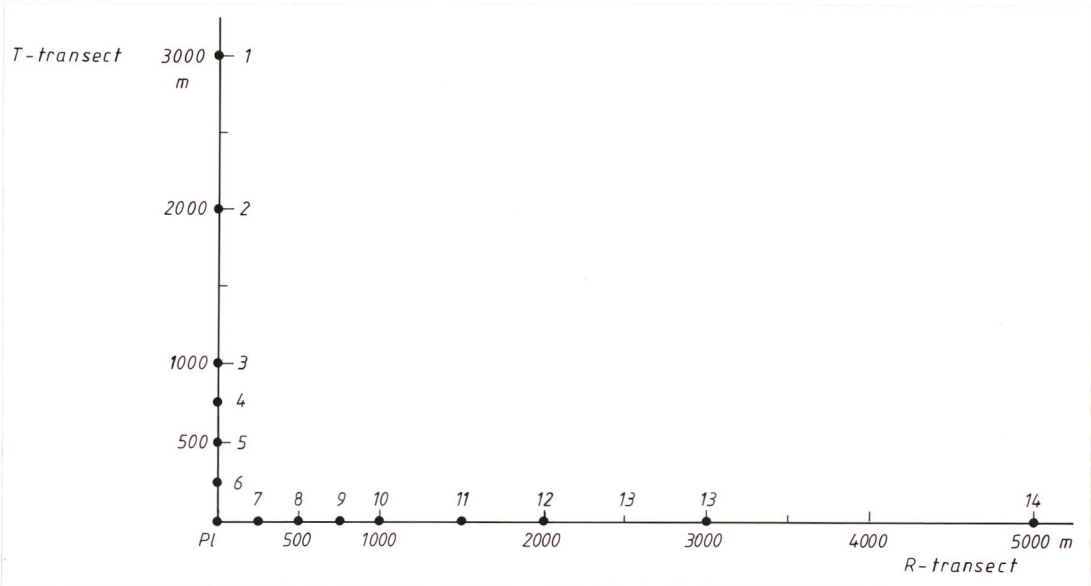


Fig. 2 Locations of stations.

Sediment samples were obtained using a Van Veen grab with a capacity of 50 l. With the exception of the stations "0" m and 100 m at L-4-α, ten samples per station were taken at all sites. On station "0" m at L-4-α only one sample and on the station 100 m (R-transect) two samples were collected. From each sample two subsamples were obtained using a core sampler with a diameter of 2.5 cm and a length of ca. 10 cm. The remaining sediment was washed and sieved over a 1 mm^2 mesh and the residue was preserved in a 6% buffered formaldehyde solution for determination of the macrobenthos composition. The subsamples were thoroughly mixed and divided over three jars,

of which two were directly frozen to -20°C until further analyses and the remaining jar was used for the analysis of the biodegradation of oil components in the sediment.

5.1.2 Selection of monitoring techniques

To establish the distribution of drill cuttings in the sea-bed the following sedimentary parameters were analysed: grain size distribution, oil components contents and barium content. Also an attempt was made to determine the biodegradability of oil components in the sediment.

The analyses of the grain size distribution gives an insight into the type and the variation of the sediment around the drilling sites, which is particularly important for the interpretation of the data of the macrobenthos composition. It was expected that due to the discharge of drill cuttings and their adhering mud a change in the grain size distribution should occur. The sediment was sieved over 65, 80, 125, 160 and 250 μm mesh and from the fraction <65 μm a partical size scan was made with a electronic particle counter. For the whole procedure see Chapter 1 of Appendix C.

The oil components content gives a good indication of the distribution of oil on the sea-bed, while the "fingerprint" of the oil gives a good idea of its likely origin. After extraction from the sediment the oil components were analysed with a gaschromatograph coupled to a flame-ionisation detector. The working procedure is given in Chapter 2 of Appendix C.

Barium is a good indicator of the distribution of drill muds in the sediment because large amounts of barite are employed in drill muds (400-600 ton per drill hole). Barium has the advantage that in contrast with oil no degradation occurs and it is also used in OBM as well as WBM. The analysis procedure is given in Chapter 3 of Appendix C.

The rate of oil degradation in the sediment was studied in order to get an impression of the capability of marine sediments to degrade the present oil and neutralize oil components. The rate of degradation depends on local conditions, for instance the presence of oxygen, nutrients etc., grain size, organic content.

The test procedure is given in Chapter 4 of Appendix C.

Results obtained in the preliminary study (Kuiper, van het Groenewoud, 1986) made it clear that it is meaningless to measure all the above listed parameters on all stations, therefore a selection was made.

5.2 MACROBENTHOS

5.2.1 Sampling

After subsampling the Van Veen grab as described in 5.1.1, the remaining sediment was washed and sieved over a 1 mm^2 mesh in order to collect the macrobenthos. The large species which could be used for analyses of oil accumulation were separately collected and immediately frozen at -20°C.
Only the species Cyprina islandica was present, albeit in small quantity at most of the stations, and was judged to be suitable for oil analyses because of their large body volume.

5.2.2 Sample preparation

After thawing of the frozen Cyprina sample, tissues were removed from the shells by mean of a titanium knife. Prior to the analyses the tissues were homogenised using a titanium modified Ultra Turrax homogeniser. The homogenates were used as stock from which subsamples were taken for separate dry- and ash weight determinations and oil analyses. The dry-and ash weight determinations were carried out as described by De Kock (1983). The procedure for oil analyses are given in Chapter 5 of Appendix C.

5.3 ACTIVE BIOLOGICAL MONITORING

5.3.1 General aspects

The term active biological monitoring implies the exposure of test organisms, collected from a clean environment, to monitor the conditions of a polluted or suspect area. In general, the bioaccumulation of selected contaminants in the transplanted test organisms is assessed, and connected to measurements of biological effects, which in the case of ABM experiments may be physiological, biochemical, cytological/cytochemical in nature.
After a preliminary ABM study in 1985 (Kuiper en Van het Groenewoud, 1986) two comprehensive ABM studies were carried out in 1986 and the beginning of 1987 with Mytilus edulis. The general aspects of the ABM are described by De Kock (1983, 1985, 1986) and Kuiper (1986). The common mussel Mytilus edulis is an exclusively epibenthic filter feeder, capable of accumulation of con-

taminants to a high degree and therefore a good indicator species for assessing the contamination of the water phase. The oil from the OBM can reach the water phase by two means. Firstly some components of the oil adhered to the cuttings dissolve instantly in the water during the discharge and secondly, after settling of the cuttings on the sea-bed a slow release of oil to the water can occur. To study the impact of both processes on mussels two ABM experiments were carried out at site L-4-α, one in the period September - November 1986 (ABM 1), during which drill activities took place with OBM and one in the period May - June 1987 (ABM 2), several months after the actual drilling.
Besides measurements on the accumulation of oil components by the mussel an attempt was made to study the experienced stress due to the discharge of cuttings and muds by means of the analyses of stress indicating parameters.

5.3.2 Technical design

ABM I

In September 1986 mussels of two different size classes (3-3.5 cm and 4-5 cm) were collected at the low tide level near Kats in the Oosterschelde. By means of random number tables, samples of 60 mussels in the 4-5 cm size class and samples of 30 mussels in the 3-3.5 cm size class were prepared. The samples were placed in plastic baskets and had been weathered in running seawater at the harbour of Den Helder prior to exposure at sea. On 19 September 1986 the samples were brought to the various stations with the Coast Guard vessel "Terschelling". One sample was kept behind to be analysed (Initial sample t = 0). The normal procedure for exposure of mussels to the marine environment is by means of cages which are tied to buoys and hang at a depth of 3 to 4 m below the surface. This time a new procedure was employed. At steel frame was constructed on top of the anchorstone of the buoy and the mussels in their baskets were fixed at a height of 50-100 cm above the stone and the sea-bed for exposition. This method has the following advantages: the risk of losing the sample due to bad weather conditions is greatly reduced and the mussels are exposed near one of the sources of pollution (sediment).
On buoy "Petro 18" (pos 53.34.22 N 04.01.36 E) a mussel sample was exposed using the normal procedure and was used as a reference station. This sample was unfortunately lost due to stormy weather condition in the exposure period.

On September 26, 1986 a sample was transported to the platform L-4-α and exposed at a depth of 3-4 m in the direction of the residual current. Table 1 and Figure 3 give the relevant data.

Per station 4 mussel samples were exposed:

1 sample of 60 mussels 4-5 cm for oil analyses
1 sample of 60 mussels 4-5 cm for SFG analyses
1 sample of 60 mussels 4-5 cm for stress induced parameters
1 sample of 60 mussels 3-3.5 cm for SFG analyses.

Table 1 General data on ABM experiments at L-4-α.

Experiment	Station	Start of exposure	End of exposure	Location
ABM 1	0 m R-transect	26-09-86	12-11-86	3 m under sea-level
	250 m R-transect	18-09-86	12-11-86	0.50 m above sediment
	500 m R-transect	18-09-86	12-11-86	0.50 m above sediment
	1000 m R-transect	18-09-86	12-11-86	0.50 m above sediment
	5000 m R-transect	18-09-86	12-11-86	0.50 m above sediment
	250 m T-transect	18-09-86	12-11-86	0.50 m above sediment
	Petro 18 (ref.)	18-09-86	lost	3 m under sea-level
	Initial sample t = 0	18-09-86	18-09-86	
ABM 2	0 m R-transect	07-04-87	19-05-87	0.50 m above sediment
	250 m R-transect	07-04-87	19-05-87	0.50 m above sediment
	500 m R-transect	07-04-87	19-05-87	0.50 m above sediment
	1000 m R-transect	07-04-87	19-05-87	0.50 m above sediment
	5000 m R-transect	07-04-87	19-05-87	0.50 m above sediment
	250 m T-transect	07-04-87	19-05-87	0.50 m above sediment
	VL-3 (ref.)	07-04-87	19-05-87	3 m under sea-level
	Initial sample t = 0	07-04-87	07-04-87	

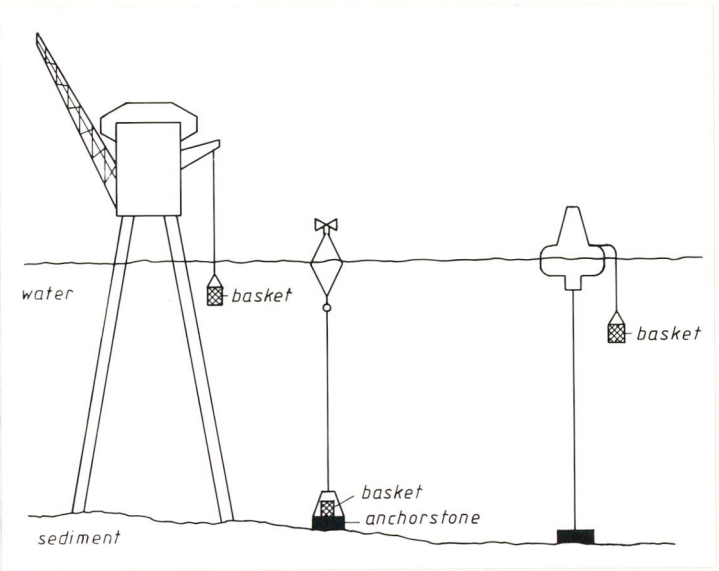


Fig. 3 Illustration of methods used in the ABM experiments.

ABM II

The technical procedure at the second ABM was virtually the same as the first experiment with the following exceptions; the buoy VL-3 (pos 53.11.00 N, 04.35.23 E) was used as a reference station and the mussels on the platform were lowered to 50-100 cm from the sea-bed (Table 1). In this experiment an attempt was made to expose a benthic deposit feeder, in this case Macoma balthica, to the sediment. For this purpose a stainless steel cage was constructed with a mesh of 1 cm^2 which was fastened in the centre of the anchorstones. It was hoped that due to the weight of the anchorstone the cage would partially sink into the sediment.

The mussels (4-5 cm) used in this experiment were collected from buoys which were stationed ca. 15 nautical miles north of the island of Texel, the Macoma balthica (1.5-2.5 cm) were collected on tidal flats in the Waddensea near Den Helder.

100 Mussels and 30 macoma's were exposed per station.

5.3.3 Sample preparation

After the exposure period the samples were collected by hauling the buoys and anchorstones on deck. Directly after sampling the mussels for oil determinations were frozen at -20°C and handled in the same way as the indigenous macrobenthic bivalve Cyprina islandica (see 5.2). The mussels to be used in the SFG determinations were kept in seawater during transport to the laboratory.
From the mussels used for cytochemical analyses (lysosomale activity, mixed function oxidase activity and metallothioneïnen production) the hepatopancreas was removed with a scalpel knife. After removal of the crystalline style, 5 hepatopancreases were placed on a 3 x 3 cm piece of cardboard and frozen in liquid nitrogen. They were then transported to the laboratory packed in dry ice. They were maintained at -80°C until the analyses. For the analyses of the biochemical stress parameters 6 subsamples of 8 animals were selected by means of random numbers. From 3 subsamples the large abductor muscle and the mantle was dissected and frozen in liquid nitrogen followed by freezedrying. The other 3 subsamples were kept, prior to this treatment, in water saturated air at 15°C (anaerobiosis).

5.3.4 Accumulation of oil components in mussel tissue

In order to establish the bioavailability of oil components a sample of the mussels used in the ABM experiments was analysed for oil content. Sample preparation and oil analysis are the same as used for the macrobenthos (5.2.2).

5.3.5 Stress parameters

5.3.5.1 General

Stress parameters, as the name suggests, attempt to quantify the stress experienced by a test organism, as a result of exposure to contamination. These sub-lethal measurements are designed to signalize biological effects of contamination levels substantially below lethal thresholds. As such, they are measurements of 'unseen' effects. Stress parameter measurement is generally restricted to the metabolic processes of individuals, but will ultimately have a bearing on the structure of populations and eventually at the community level of organization.

The cytochemical parameters chosen for the present research are lysosomale stability, mixed function oxidase activity and metallothioneins induction, while of the physiological level "scope for growth" has been applied.
The theory and methods used in these experiments are described in detail by Bains et al. (1985).

5.3.5.2 Physiological measurements

Scope for Growth

The Scope for Growth (SFG) is defined as the amount of energy an organism has available under specific circumstances per time interval for somatic growth and the production of gametes. The SFG measurements were carried out with 8 individual mussels per station.
To calculate the SFG it is necessary to analyse the food uptake (E_c = Energy consumed), the respiration (E_r = Energy respired), the excretion (E_{ex} = Energy excreted) and the assimilation efficiency (a). SFG is then calculated from the equation:

$$SFG = a.E_c - E_r - E_{ex} = E_a - E_r - E_{ex}$$

where

E_a = Energy absorbed = Part of the energy consumed that is digested and assimilated

a = Efficiency of food digestion (Conover ratio).

Prior to the analyses the mussels were adapted for at least 18 hours to the seawater conditions in the laboratory. The energy consumed (E_c) was measured by placing one mussel in a flow through cell for a period of 17 hours to which seawater with a known algae density (± 12,000 cells/ml Pheaodactilum tricornutum) was added at a constant rate. Over a period of 4 hours a seawater sample of the inflow and outflow was taken. In these samples the algal concentration was measured using a electronic particle counter.
By means of the flow through speed of the seawater, the decline of algal concentration and the ashfree dryweight of the used algae i.e. the E_c, was calculated. Next the E_r and E_{ex} were measured by closing the flow through cells for 1 hour and determining the concentrations of dissolved oxygen and ammonium in the seawater at the start of and the end of this incubation.

The faeces produced by the mussel during the whole experiment was collected and the ashfree dryweight percentages was measured in order to calculate the assimilation efficiency (a) according to Conover (1966).
Figure 4 gives a schematic summary of the SFG analyses.

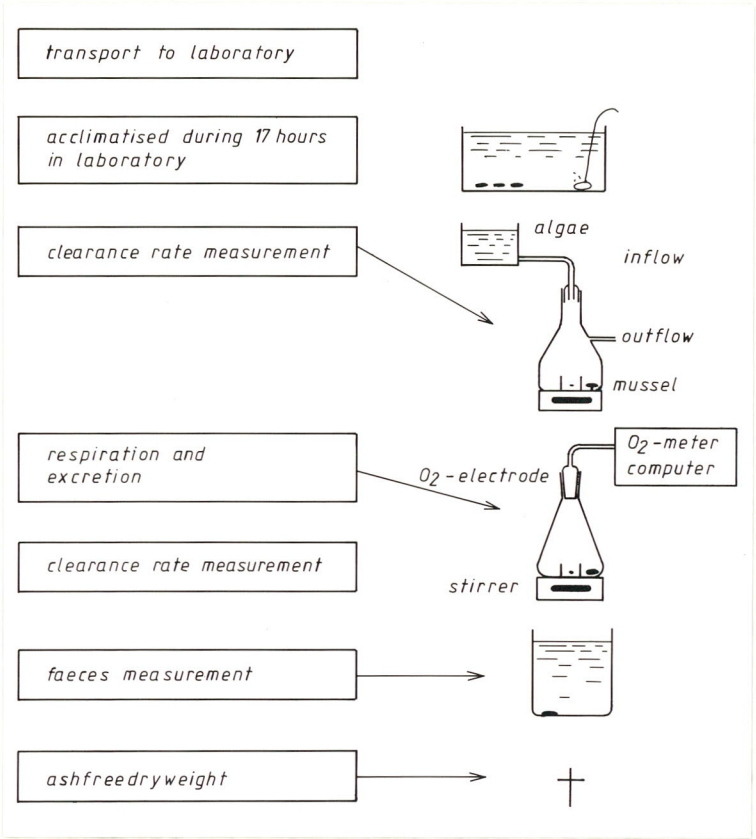


Fig. 4 Schematic overview of the measurements of SFG analysis.

5.3.5.3 Cytochemical measurements

Lysosomal stability

Lysosomes form a intercellulair digestive system which is capable of the catabolism of endogenous and exogenous substances. The lysosomes of bivalves contains 40-60 different catabolising enzymes and in the digestive tubelles of the bivalves many lysosomes are present and take up a great part of the volume of the cells. Many persistent xenobiotica (i.e. PAK, heavy metals) can accumulate in the lysosomes. A good functioning of the lysosomale membrane is very important for the cellular system. Under stress the membrane of the lysosomes can destabilize causing the catabolising enzymes

to come in contact with the cytoplasm and other organelles, thus disrupting the cellular system.
By inducing an artificial destabilization of the lysosomal membranes, by exposure to low pH levels, the condition of the membranes can be established.

Mixed function oxidase activity

The "Mixed Function Oxidase" MFO-system is capable of detoxication of organic xenobiotica for instance aromatic hydrocarbons, for which it requires NADPH and oxygen.
One of these MFO-components is the cytochrome P 450. The chemical Neotrazoliumchloride (NTR) is capable of accepting electrons from the cytochrome P 450 reductase and to substitute the natural acceptor cytochrome P 450. The reduction of NTR can be used as a standard for measuring the MFO-activity.

Induction of metallothioneïnen

A number of marine organisms can accumulate a large amount of heavy metals. The metals are detoxified in the cells of the organisms by binding to the protein metallothioneïn. Under stress induced by metals the organisms urge to synthesis of metallothioneines. The presence of metallothioneïnes are determined with the Shikata test (Shikata et al., 1974) which give an "all or nothing" response.

Timm sulfide test

A number of metals accumulate in the form of metalloprotein in old lysosomes in the digestive tubeles of mussels. The metalloproteines are coloured with Na_2S and $AgNO_3$.

Schmorl test

Compounds with an active SH group can be detected with this test and are an indication for general organic pollution. The presence of lipofusine is detected with this method.

Papanicolaou test

Sections of the hepatopancreas are coloured with Papanicolaou. Anatomic malformations of the sections, sex and possible infections with parasites (mytilicolas) are detectable.

5.3.5.4 Biochemical measurements

To study the possible effects of the discharge of drill cuttings on biochemical parameters, MT-TNO commissioned Dr. A. de Zwaan of the Department of Experimental Zoology, University of Utrecht, to carry out some analyses on mussel samples from the ABM I experiment.
The methods used and the theory of these analyses are given by Baines et al. (1985) and De Kock et al. (1986a, b). The following parameters were measured:

1. Specific activity of fosforfructokinase (PFK), pyruvatkinase (PK) and glutamate pyruvate transamine (GPT) in the great abductor muscle.
2. Concentrations of glycogen in the mantle.
3. Free amino acids in the mantle and the great abductor muscle.
4. Concentrations of metabolites in the abductor muscle on control and aerial exposed (anaerobiosis) animals.

6. RESULTS AND DISCUSSION

6.1 SEDIMENT

6.1.1 Sampling

In week 23 and 24 of 1986 the sediment at site F-18-8 was sampled. The direction of the R-transect was North East (40°) and the T-transect South East (130°) as seen from the platform. The sediment contained a fair amount of clay and in general the composition of the sediment was very much alike at the different stations of this site. No visual traces of drill cutting or drilling muds were found.

In week 21 and 24 of 1986 the first sampling (baseline) at site L-4-α took place. The directions of the R-transect and T-transect were respectively North East (45°) and South East (135°). No obvious differences were found in the sediment compositions between the stations and the subsamples per station. The site L-4-α was sampled for the second time in week 37 and week 39, 1986 after drill operations started. The sample from station "0" m was pitch black, smelled strongly of oil and during sieving an oil film was produced on the water surface. The samples from stations 100 m, 250 m and 500 m on the R-transect and 250 m on the T-transect smelled sometimes faintly of oil. There was no much difference at the other stations in sediment composition between the two sampling excercises.

The site K-12-α was sampled in week 37 and 39, 1986. The directions of the R-transect and T-transect were respectively 30° and 120°. On the R-transect the stations 100 m, 250 m and 500 m were black coloured and oil traces were visually detected. On the whole no differences were found between the subsamples at one station with the exception of station 250 m (R-transect). At this station, 9 subsamples were pitch black while one sample was sandy coloured. The drill cuttings and adhered drilling mud are therefore not always distributed homogenously on the sea-bed. The most striking visual observation at this site was the fact that the situation seemed to have deteriorated in comparison with the findings of the year before (Kuiper and Van het Groenewoud, 1986).

6.1.2 Measurements

Grain size distribution

The results of the grain size distributions of the sediment at the various stations are given in Figure 5. At site F-18-8 the sediment composition changed gradually away from the platform, at L-4-α_1 the composition is very much the same, except those at greater distances away from the platform, probably a natural variation. L-4-α "0" m showes an increase in the fraction >250 µm and at station K-12-α an increase was found in the fraction >250 µm and <63 µm at the stations 100 m and 250 m R-transect. Both phenomena may well be the result of the presence of drill cuttings (>250 µm) and drilling muds (<65 µm).

The <65 µm fraction was studied in detail with a Elzone Particle Data Counter in order to see if mud traces were detectable within the fraction <65 µm.

The results are given in Figure 6. There was a great similarity between the stations per site, however there seems to be an increase in the number of particles (2-6 µm) towards the platform.

Oil components in the sediment

The oil components were measured in the sediment from selected sites. A sample of the used OBM at L-4-α was obtained and an oil analysis was carried out on this sample.

The results of the analyses are given in Table 2 and Appendix D1.

In Table 2 the sum of the n-alkanes C8/C32, the fraction "others", which consists of compounds which are detectable with gaschromatography (isomers of alkanes, cyclo-alkanes, aromatic and polyaromatic substances) and the fraction UCM (Unresolved Carbon Matter) are given. UCM is calculated from the "hill" as seen in the chromatograms.

In Figure 7 chromatograms of sediment extract from site K-12-α are given. For comparison the results of the 1985 and 1986 experiments are given below for station K-12-α, 250 m, R-transect:

Year:	1985	1986	1986 (5000 m) ref.
n-alkanes (mg/kg)	65 (C8-C20)	4 (C8-C32)	1.4 (C8-C32)
others "	165	240	2.6
UCM "	230	133	1.9
total (incl. UCM)	460 mg/kg	377 mg/kg	5.9 mg/kg

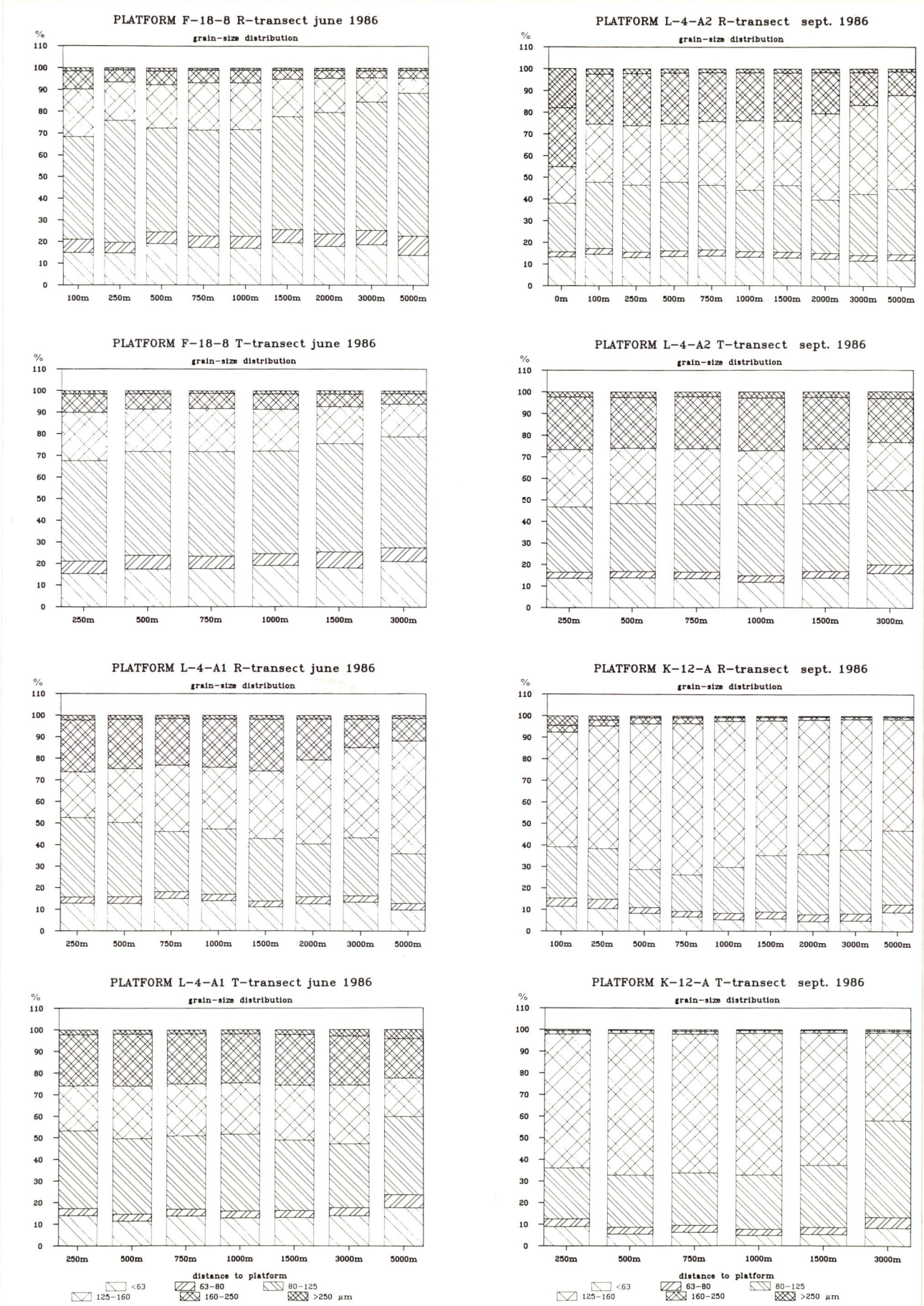


Fig. 5 Grain size distributions of the sediment at the various stations.

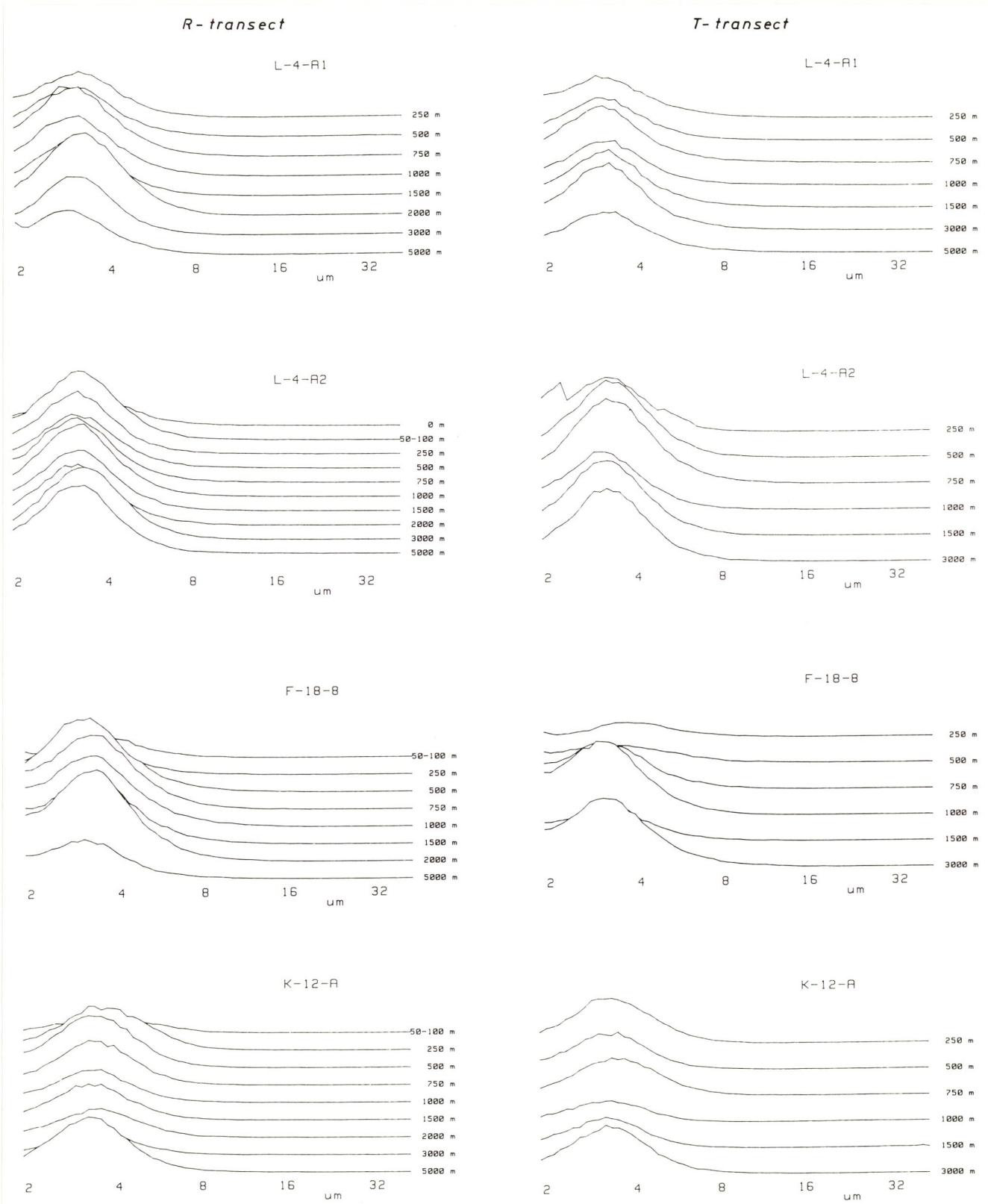
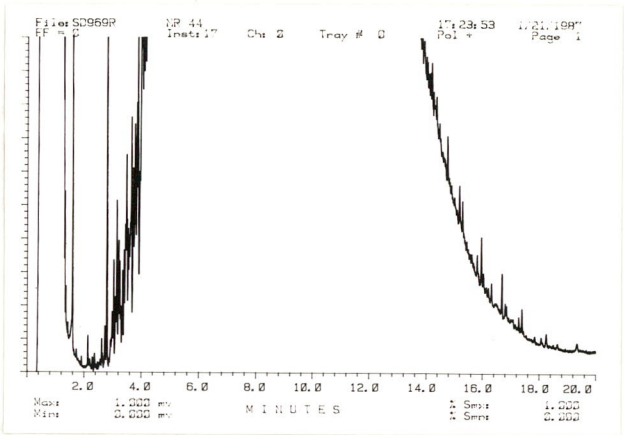


Fig. 6 Particle size scans of <63 µm fraction.
L-4-α_1: before drilling
L-4-α_2: during drilling

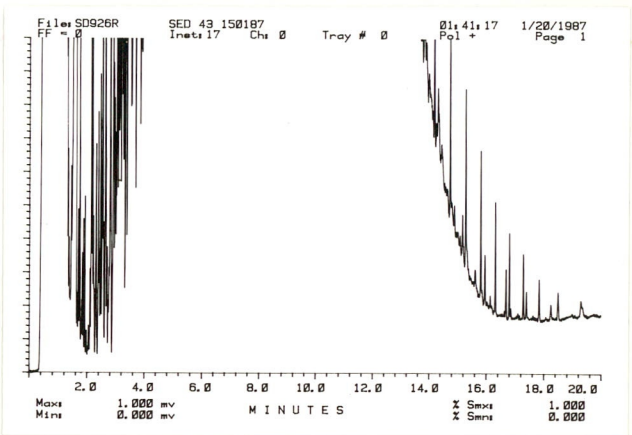
Table 2 Oil content of sediment (mg/kg) based on dryweight (September 1986).

Site		Station	Sum C8-C32	Others	UCM
K-12-α	R-Transect	100 m	21.73	340.02	12.78
"	"	250 m	4.02	239.89	132.68
"	"	500 m	4.20	3.86	3.78
"	"	750 m	3.04	1.65	0.13
"	"	1000 m	0.99	1.75	1.58
"	"	2000 m	1.11	1.45	0.13
"	"	5000 m	1.41	2.65	1.89
K-12-α	T-Transect	250 m	0.48	0.85	0.13
"	"	500 m	0.29	0.51	0.13
"	"	1000 m	1.15	1.16	0.13
L-4-α_2	R-Transect	0 m	1197.48	2319.87	1757.36
"	"	100 m	3.96	5.23	4.78
"	"	250 m	1.53	1.87	1.15
"	"	500 m	2.16	1.74	7.58
"	"	750 m	0.77	0.77	0.13
"	"	2000 m	1.64	1.10	0.13
"	"	5000 m	2.03	0.84	0.13
L-4-α_2	T-Transect	250 m	1.98	3.59	0.26
"	"	500 m	0.58	1.33	0.13
"	"	750 m	0.77	0.97	0.13
"	"	1000 m	2.89	1.89	0.13
OBM (in mg/kg)			4400.00	7289.00	5595.00

250 m R-transect '86



100 m R-transect '86



250 m R-transect '85

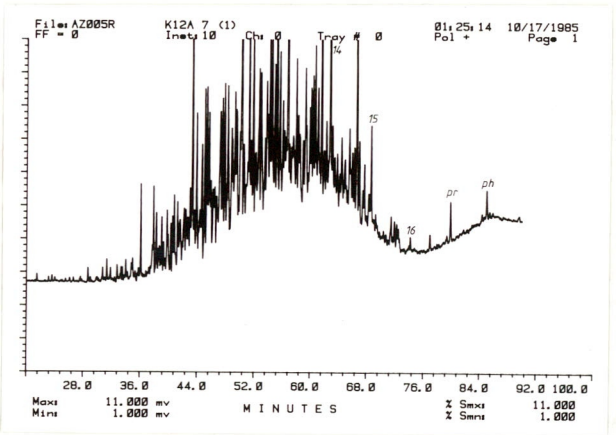


Fig. 7 Chromatograms of sediment extract from site K-12-α.

There seemed to be a change in the fractions UCM and "others". The calculation of the fractions "others" and UCM is strongly dependent, however, on the separating capacity of the capillary column at the point of analyses. With a high separating capacity it is possible that a given component can be included in the "other" fraction, while with low column separation it may fall under UCM. The n-alkanes however have almost completely vanished from the sediment, either by dissolving in the water phase or by biodegradation. In a laboratory experiment by De Kreuk and Van der Hoek (1987) to study the biodegradation of Clairsol 350 m the n-alkanes biodegraded within three months. In Figure 8 chromatograms of the sediment extract at L-4-α_2 and from the used OBM are given.

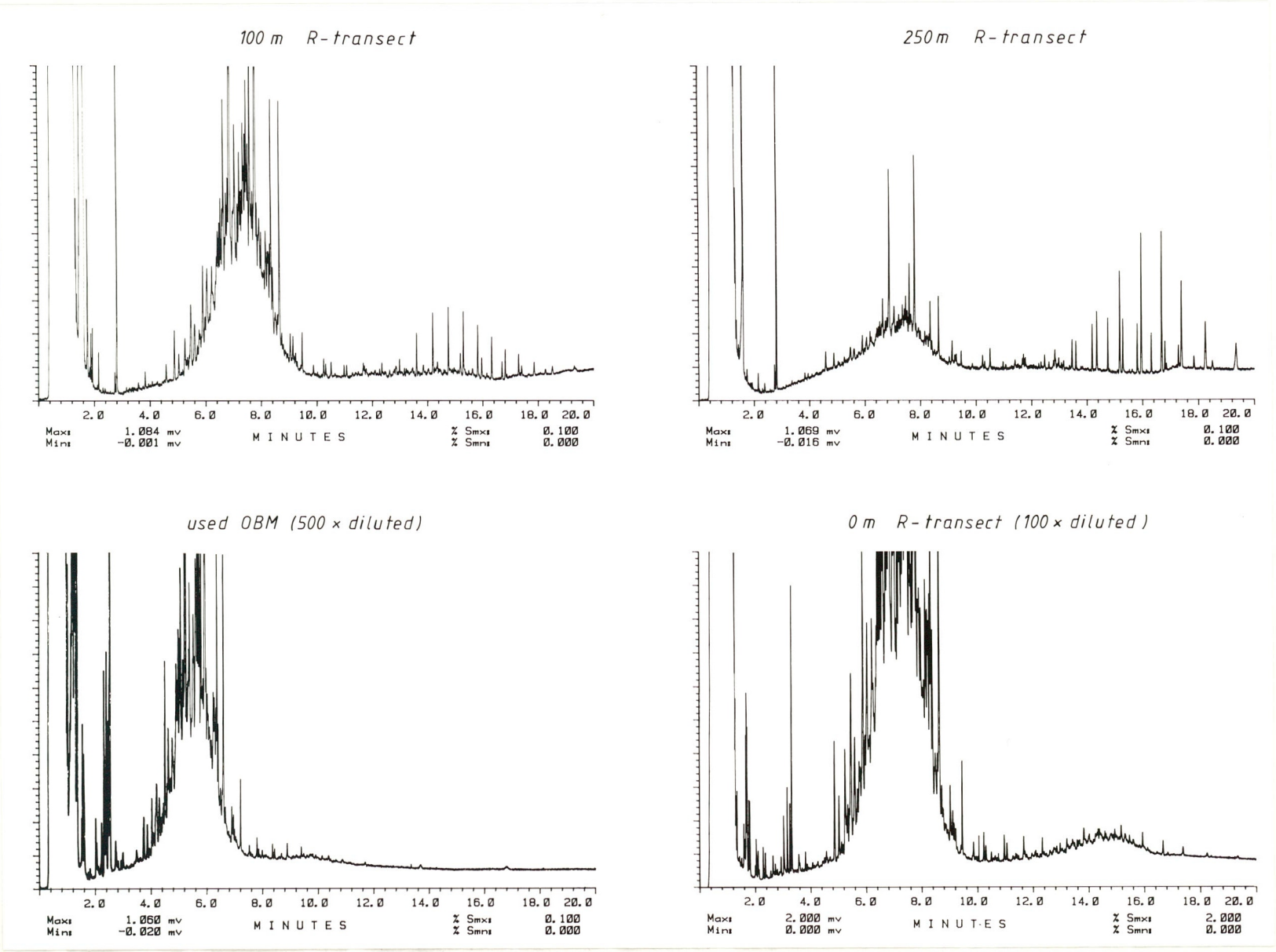


Fig. 8 Chromatograms of sediment extracts and used OBM at site L-4-α.

The chromatograms indicate clearly that the oil in the sediment originated from the used OBM at L-4-α_2. The oil in the sediment at station 0 m on the R-transect consisted of 22.7% n-alkanes, 44% "others" and 33.7% UCM, while the original OBM contained 22.4% n-alkanes, 42% others and 32.7% UCM, a clear indication that recently settled oil had been sampled. Otherwise, a change in general composition would have been expected.
The horizontal distribution of oil in the sediment at site L-4-α and K-12-α is shown in Figure 9. At K-12-α elevated oil concentrations were found up to 750 m on the R-transect, while at L-4-α elevated concentrations were found near the platform.

Barium

The barium content of the sediment was measured in order to investigate its value as a tracer for the distribution of OBM as well as WBM. The results of the measurements are given in Table 3 and Figure 10.
At site F-18-8 a elevated barium content (twice the background concentrations) was found as far out as 500 m on the R-transect. During the first sample excercise at L-4-α a distinct gradient in the barium content was found extending out to 2000 m on the R-transect and to 1000 m on the T-transect, assuming that the background concentration was about 50-60 mg/kg. The last drilling activities at this site had taken place at the end of 1984 and the beginning of 1985. After 1.5 year the presence of drilling muds in the sediment was still detectable. The selection of site L-4-α_1 as a baseline study was not so suitable although no biological effects due to the presence of barium were found (see Mulder, Lewis, Van Arkel, 1988). The barium content of the sediment sampled on the second sampling trip had not noticeably changed except at 100 and 250 m R-transect, which indicates that the drilling of the new well had just started.
The low content of barium in relation to the high oil content at station "0" m R-transect is remarkable, and an explanation is not easy to find.
At site K-12-α, strongly elevated barium contents were found at the stations examined, on the R-transect up to 1000 m and on the T-transect up to 250 m, although the last drilling took place in November 1983.
From the results we can conclude that barium is a useful parameter in establishing the distribution of drill cuttings and drilling muds around platforms.

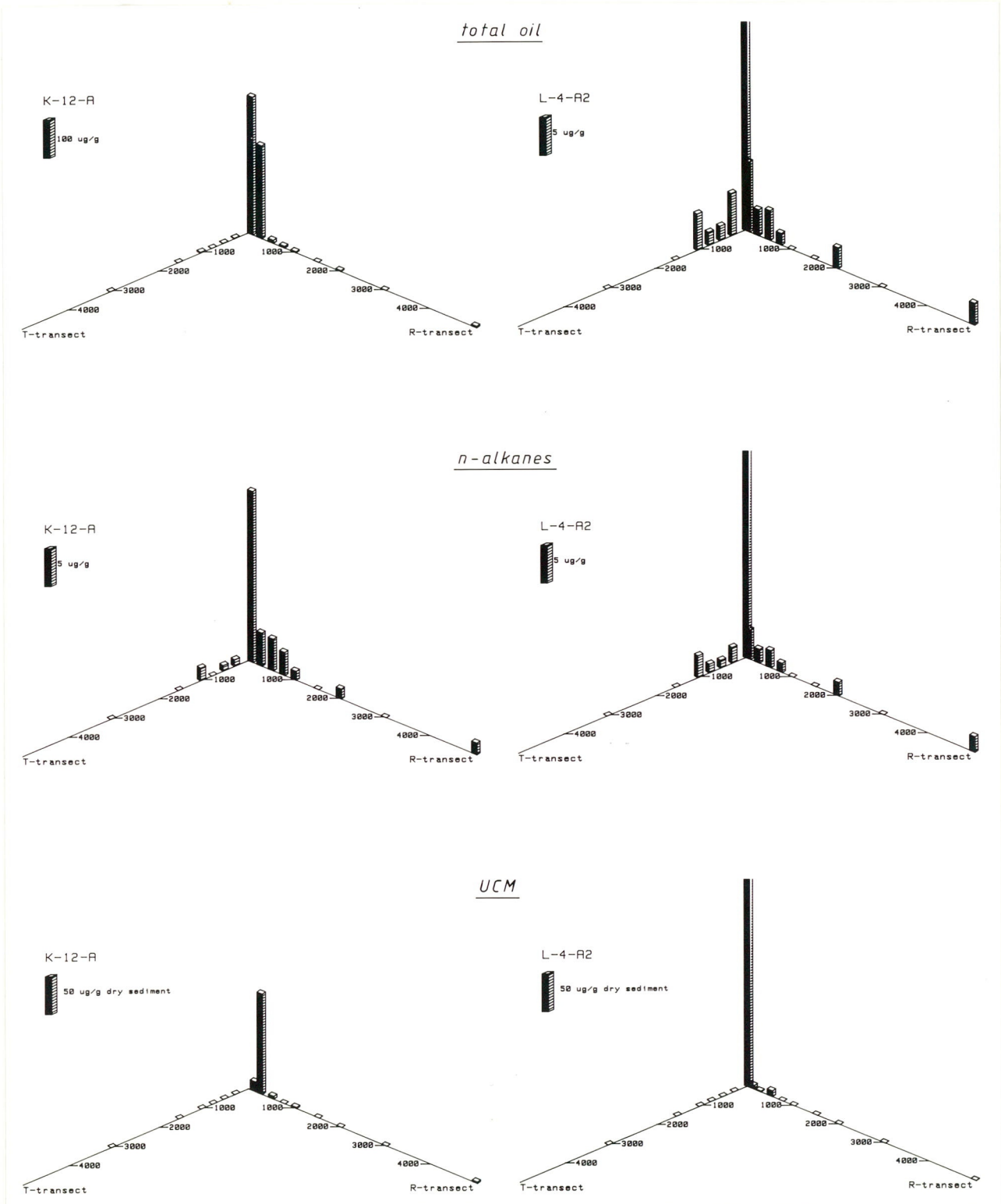


Fig. 9 Oil concentration in sediment.

Table 3 Barium content sediment (mg/kg) based on dry weight.

Site		Station	Barium
F-18-8	R-Transect	100 m	86.00
"	"	250 m	101.00
"	"	500 m	83.00
"	"	750 m	55.00
"	"	1000 m	55.00
"	"	1500 m	67.00
"	"	2000 m	63.00
"	"	3000 m	48.00
"	"	5000 m	55.00
F-18-8	T-Transect	250 m	54.00
"	"	500 m	54.00
"	"	750 m	33.00
"	"	1000 m	46.00
"	"	1500 m	52.00
"	"	3000 m	62.00
L-4-α_1	R-Transect	250 m	439.00
"	"	500 m	284.00
"	"	750 m	175.00
"	"	1000 m	87.00
"	"	1500 m	90.00
"	"	2000 m	91.00
"	"	3000 m	55.00
"	"	5000 m	53.00
L-4-α_1	T-Transect	250 m	262.00
"	"	500 m	140.00
"	"	750 m	104.00
"	"	1000 m	95.00
"	"	1500 m	77.00
"	"	2000 m	59.00
"	"	3000 m	66.00
K-12-α	R-Transect	100 m	6600.00
"	"	250 m	1700.00
"	"	500 m	176.00
"	"	1000 m	137.00
"	"	5000 m	38.00
K-12-α	T-Transect	250 m	88.00
L-4-α_2	R-Transect	0 m	201.00
"	"	100 m	950.00
"	"	250 m	455.00
"	"	500 m	125.00
"	"	1000 m	111.00
"	"	5000 m	46.00
L-4-α_2	T-Transect	250 m	202.00
"	"	500 m	116.00

L-4-α_1 sampled week 23-24, 1986;
L-4-α_2 sampled week 37-39, 1986.

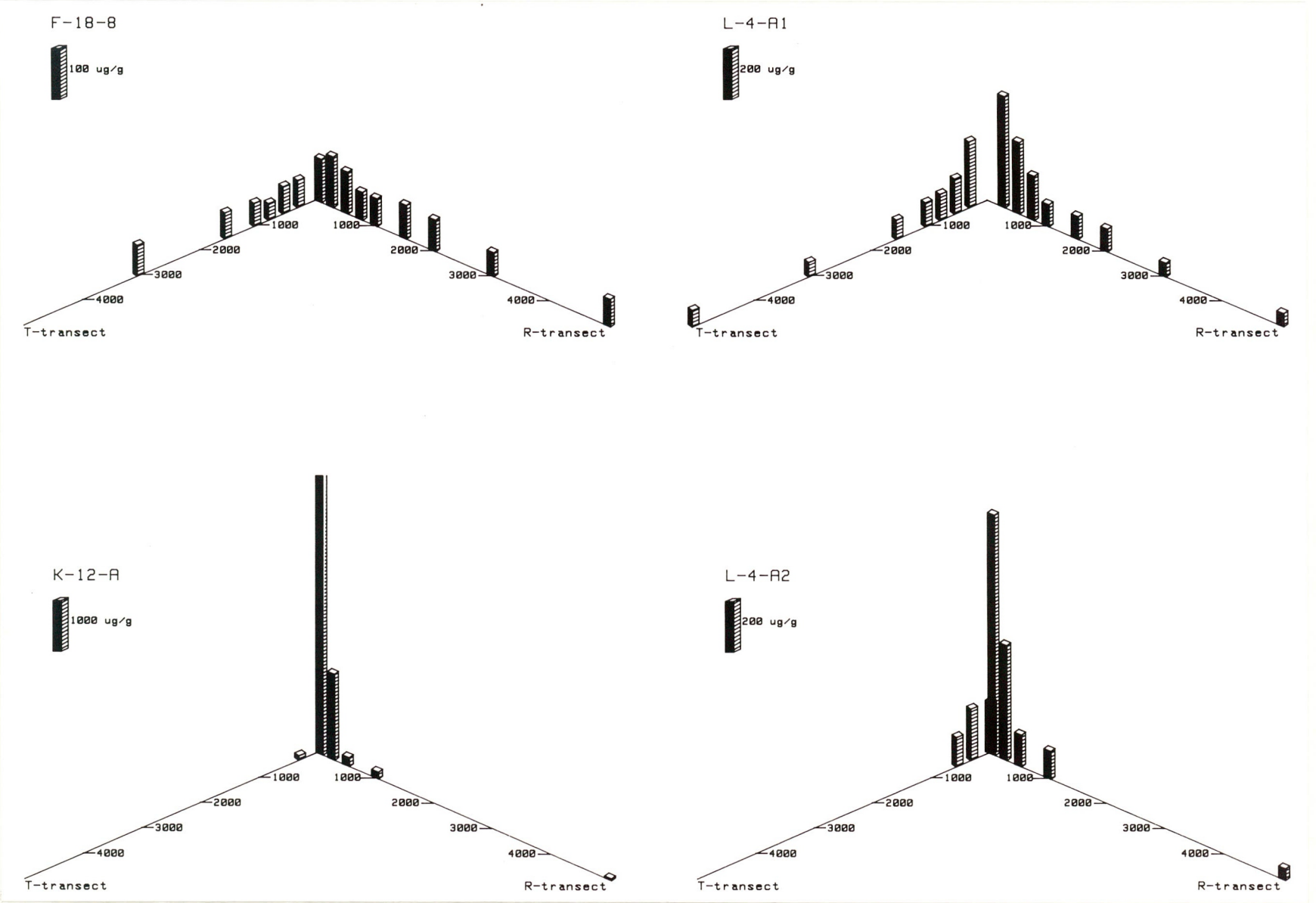


Fig. 10 Barium concentrations in sediment.

Biodegradability of oil components

The biodegradation of oil components in the sediment was studied with ^{14}C-labeled naphtalene, although naphtalene itself was not measured in this monitoring program. It was used because the results with naphtalene are more reliable than the results with for instance hexadecaan (Lahr, 1985).
The results of the measurements are given in Table 4.

Table 4 Biodegradation of naphtalene in µg/kg.day (dry sediment) September 1986.

Site		Station	Degradation µg/kg.day	oil in sediment mg/kg
K-12-α	R-transect	100 m	27.1	361
"	"	250 m	16.2	243
"	"	500 m	4.1	8.1
"	"	750 m	1.8	4.7
"	"	1000 m	9.6	2.7
"	"	5000 m	1.6	4.1
K-12-α	T-transect	250 m	1.6	1.3
L-4-α_2	R-transect	0 m	1556	3517
"	"	100 m	17.3	9.2
"	"	250 m	5.9	3.4
"	"	500 m	3.5	3.9
"	"	1000 m	3.1	n.a.
"	"	3000 m	0.7	n.a.
"	"	5000 m	0.3	2.8
L-4-α_2	T-transect	250 m	12.9	5.6
"	"	500 m	5.5	1.9

n.a. = not analysed.

The scintillation fluid used in this experiment was Lymnagel, which was coloured strongly under influence of some unknown compounds present in the sediment. The actual values are probably much higher than the values given in Table 4 due to quenching.
When interpretating these values it should be taken into account that the laboratory experiments are performed under optimum conditions e.g. sufficient oxygen and nutrients.
The high values found near the platform L-4-α along both R- and T-transect indicate the presence of many naphtalene degrading microorganisms, and a high concentration of naphtalene and OBM compounds which can degrade easily. The relatively low values at K-12-α indicate the presence of an OBM of older origin which does not degrade as readily. The ratios of phytane (C17) and phristane (C18) are considered a good indicator for biodegradation. A high ratio indicates that biodegradation has occurred. In Table 5 the ratios are calculated for those sediment samples which still contained measurable amounts of C17 and C18. It is obvious that no substantial biodegradation had taken place at station L-4-α.

Table 5 Ratio of oil components in sediment.

Ratio	1985 K-12-α 50 m	1985 K-12-α 250 m	1986 K-12-α 100 m	1986* L-4-α 0 m	1986* L-4-α 250 m
Phytane : C17	14.0	4.5	7.1	0.6	0.5
Phristane: C18	12.5	2.8	-	0.8	0.8
Ratio C17:C18	1.1	1.6	-	0.8	0.6

* September 1986 after OBM drilling.

6.2 MACROBENTHOS

At site L-4-α the species Cyprina islandica was found at various stations in sufficient quantities for the determination of bioaccumulated oil components. The results of the oil analyses are given in Table 6 and Appendix D2. No gradient was found, but because of the generally low oil content of the sediment this was not expected.

Table 6 Oil content of Cyprina islandica tissue (in mg/kg) based on ash-free dry weight.

Site		Station	C8/C32	Others	UCM
L-4-α_2	R-Transect	1000 m	34.13	37.82	0.92
L-4-α_2	T-Transect	250 m	51.90	39.22	1.15
"	"	750 m	34.13	48.89	0.92
"	"	1000 m	34.44	54.63	1.19
"	"	1500 m	59.55	32.26	1.24

In our opinion the use of the oil content accumulated in the macrobenthos as a passive biological monitoring technique for the distribution of drill cuttings and OBM is not feasible due to the impossibility to collect representative samples i.e. sufficient biomass of the one species, from all sites and stations.

6.3 ACTIVE BIOLOGICAL MONITORING

6.3.1 Sampling

On 12th November 1986 the ABM 1 mussels were collected at L-4-α with the aid of the Coast Guard vessel "Terschelling". The reference sample from bouy "Petro 18" was lost due to bad weather conditions in October 1986 (Table 1). Mussels from all stations seemed to be in a good condition. During the preparation of the mussels for stress parameter measurements, parasites were found in the hepatopancreas which were later determined as Mytilicola intestinales Steuer. Whether the presence of this parasite influenced the various analyses is hard to say. In Table 7 data on the mussels which were used for the oil analyses are presented. The ADW of the mussels of the ABM 1 experiment had increased during the exposure. The samples from ABM 2 were collected on 19th May 1987. The reference mussel sample was taken from bouy VL-3 and seem to be in good condition as was the rest of the mussel samples.

Table 7 Data on mussels and macoma samples of the ABM experiments.

	initial sample t = 0	reference sample	0 m	250 m	500 m	1000 cm	5000 m	250 m
			R-transect					T-transect
ABM 1 (mussel)								
number	58	n.a.	55	57	57	53	52	56
mean ADW (g)	0.29	n.a.	0.44	0.39	0.40	0.44	0.38	0.39
mean length (cm)	4.33	n.a.	4.36	4.30	4.34	4.34	4.31	4.34
infected with Mytilicola %	n.a.	n.a.	36	45	71	71	66	38
ABM 2 (mussel)								
number	60	55	46	42	55	46	53	44
mean ADW (g)	0.36	0.54	0.46	0.27	0.41	0.30	0.47	0.43
mean length (cm)	4.01	4.29	4.15	4.19	4.33	4.17	4.39	4.29
ABM 3 (Macoma)								
number	23	n.a.	8	8	13	11	20	15
mean ADW (g)	0.037	n.a.	0.033	0.045	0.049	0.050	0.048	0.041
mean length (cm)	1.83	n.a.	1.78	1.91	1.87	1.84	1.92	1.88

n.a. = not available.

The mussel samples were clearly in the last stage of the reproductive maternity, as most were seen to spawn during the first few days in the laboratory.

The Macoma balthica samples showed a high mortality, possibly caused by food stress, predation by starfish and/or the presence of contaminants in the sediment. Regrettably no data are available on ambient food conditions and accumulation of contaminants in these animals. The stainless steel cages in which the animals were exposed showed a dark colouring to ± 1 cm from the bottom indicating that the cages sunk ca. 1 cm into the sediment.

6.3.2 Bioaccumulation of oil components

The results of the oil analyses of mussel tissue are given in Table 8 and Appendix D3. In Figure 11 some chromatograms of the extracts are presented. No accumulation of n-alkanes was found in both the ABM experiments when compared to the initial samples (t = 0). The dominant alkane was C17. Regrettably, no clear explanation of this is available at present. It is possible that a natural tissue component gives a response in the chromatogram at the same place as C17 (see also the data of the macrobenthos (6.2). C17 is known to be a biogenic alkane produced by algae (Anonymous, 1985). In the first ABM experiment the contents of the fraction "others" at the different stations were increased compared with those of the initial (t = 0) up to 5000 m R-transect samples. The contents of the fraction "others" in tissue did not correlate with the contents of "others" in the sediment. In the second ABM experiment no accumulation of the fraction "others" had occurred.

The mussels in the first ABM experiment had a high internal concentration of the 'UCM' fraction. A distinct gradient was found on the R-transect (Figure 12). Even at 5000 m on the R-transect an highly elevated UCM content was found. The origin of the accumulated UCM was clearly the used OBM as can be seen from the chromatograms of the mussels (Figure 11) and of the oil in the OBM (Figure 8), which show the same "hill" at 6-10 min, representing the fraction UCM. While no direct correlation was found between the contents of the UCM fraction in the sediment and the tissues, and the exposure took place during the actual drilling, we assume that the soluble fraction of the discharge of cuttings is mainly responsible for the accumulation and not the oil components present in the sediment.

In the second ABM experiment UCM was found only at station "0" m R-transect. The chromatograms and the fact that to the best of knowledge no discharges took place during the exposure time indicates clearly that the UCM originated from the oil present in the sediment.

During the sediment sampling on the platform L-4-α on the 26th of September 1986, a mussel sample was recovered from the top of the sediment with a Van Veen sampler. The cluster of mussels had probably fallen from the legs of the platform. The oil content was much higher than the oil content of the mussel samples of the ABM 1 which were exposed at 50-100 m above the sediment (Table 8).

Table 8 Oil concentrations (mg/kg) in the common mussel (Mytilus edulis) at station L-4-α, based on ashfree dry weight.

	C8/C32	Others	UCM
ABM 1			
R-transect 0 m	29.2	145.4	189.7
" 250 m	16.2	130.9	166.3
" 500 m	20.6	79.1	80.8
" 1000 m	22.1	107.5	61.0
" 5000 m	45.2	89.0	19.5
T-transect 250 m	22.7	119.0	38.1
Initial sample t = 0	19.7	20.4	0.8
Reference sample t = 0	34.6	40.1	0.9
Platform sample	300.6	1520.8	3262.6
ABM 2			
R-transect 0 m	47.1	63.3	127.3
" 250 m	43.5	51.8	0.9
" 500 m	27.5	89.6	0.8
" 1000 m	15.8	64.2	0.9
" 5000 m	18.6	62.7	0.8
T-transect 250 m	43.0	52.8	0.8
Reference sample VL-3	41.0	63.8	0.7
Initial sample t = 0	24.3	56.5	0.7

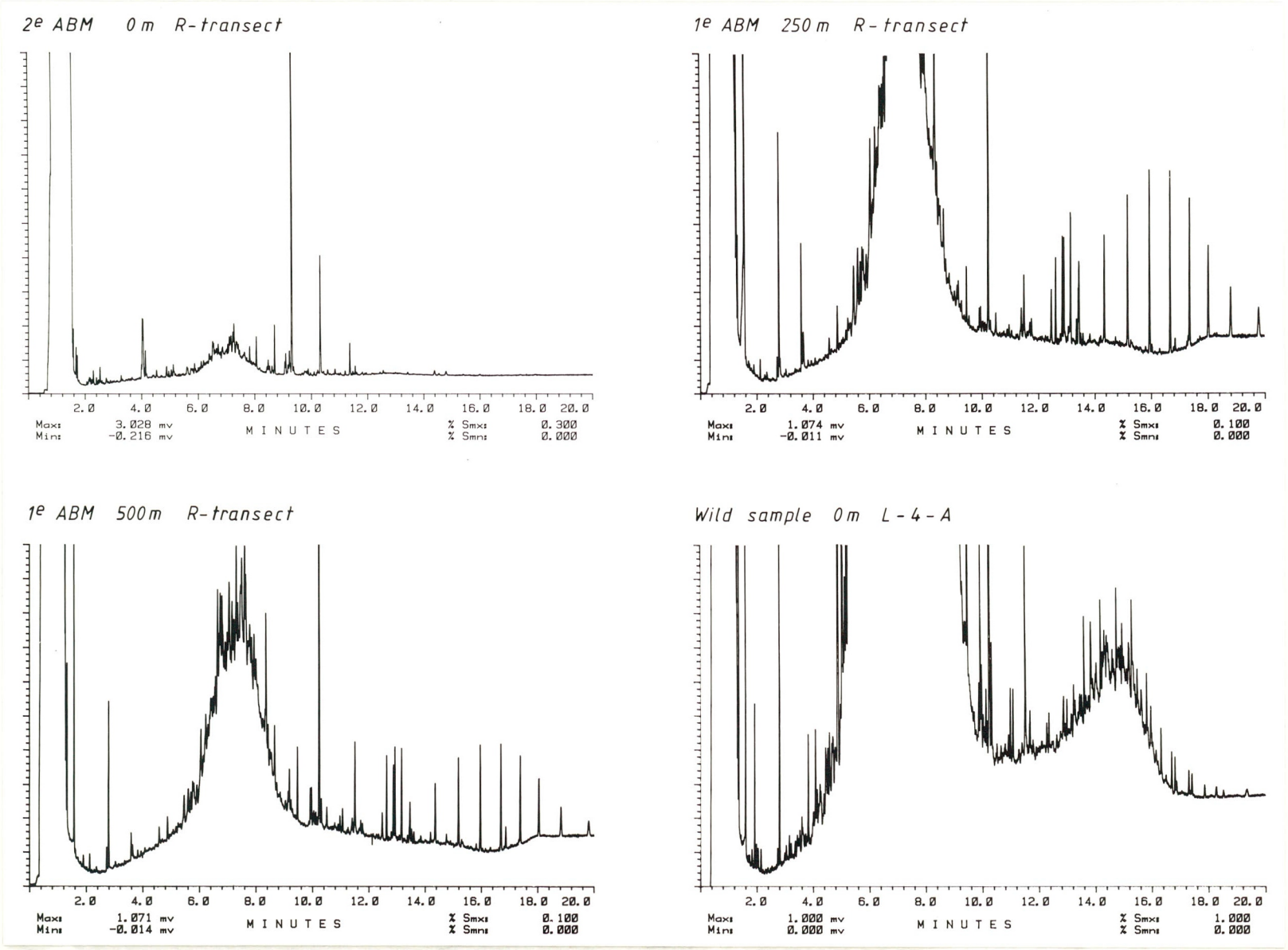


Fig. 11 Chromatograms of mussels.

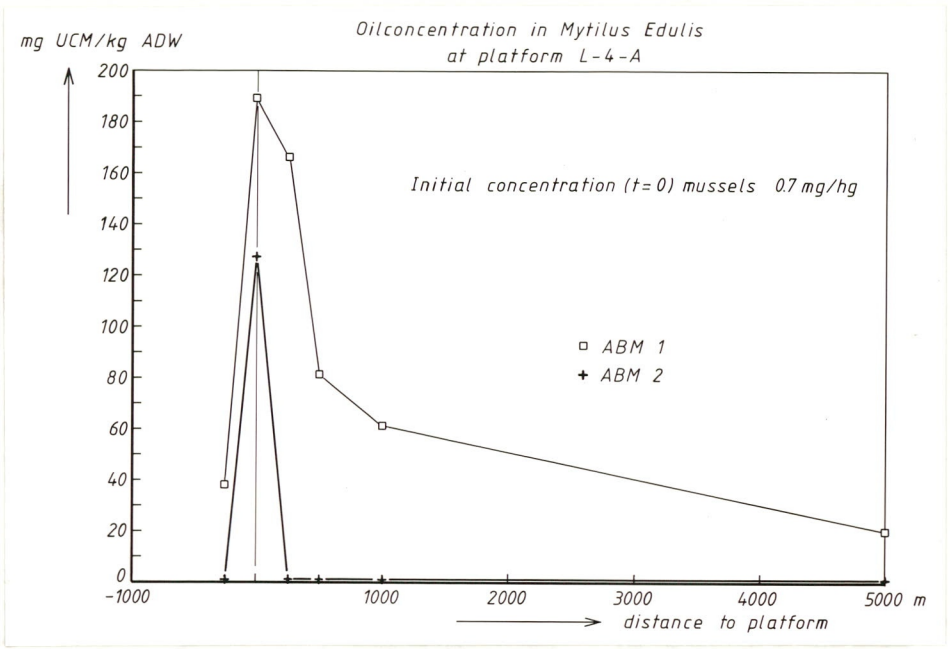


Fig. 12

6.3.3 Stress parameters

6.3.3.1 Physiological measurements

ABM 1

The mussel samples for SFG analysis were transported to the laboratory in seawater. In the laboratory they were kept in running seawater until analysis. Because only 8 measuring cells were available 2 mussels from 4 different stations were analysed daily. Thus it was possible to separate differences between stations from variances between measuring days and between measuring cells. In order to compare the data from different stations, the respired, excreted and assimilated energies were corrected to a "standard body size" of 1.0 gram ashfree dry weight (ADW) by means of the allometric equation.

$$E' = \frac{E}{ADW^b}$$

where

E = energy
ADW = ashfree dry weight in grams
b = experimentally established constant

The values of b for Energy absorbed (E_a), Energy respired (E_r) and Energy excreted (E_{ex}) were respectively 0.56, 0.70 and 0.72.
The results of the SFG measurements are presented in Table 9.

Table 9 Scope for Growth (SFG) corrected to "standard body size" of 1 gram in mussels from ABM 1 at site L-4-α.

Station	Mussels measured	E_a' J/h	E_r' J/h	E_{ex}' J/h	SFG J/h
R-transect 0 m	8	22.9 (3.8)	9.5 (3.0)	1.0 (0.6)	10.4 (3.5)
R-transect 250 m	14	23.1 (8.2)	9.2 (2.1)	0.8 (0.5)	13.1 (6.8)
R-transect 500 m	9	16.8 (8.7)	9.5 (1.5)	1.2 (1.6)	6.1 (9.2)
R-transect 1000 m	8	15.5 (5.1)	8.9 (1.6)	1.0 (0.8)	5.6 (6.2)
R-transect 5000 m	12	13.6 (10.2)	7.1 (2.4)	0.8 (0.4)	5.7 (9.9)
T-transect 250 m	9	18.0 (8.9)	9.3 (1.5)	0.9 (0.7)	7.8 (3.7)

( ) = standard deviation

The data were subjected to a non-parametric statistical test, the Krashal-Wallis test, in order to examine the differences between the stations.
It was proved that the values of the SFG of the different stations were not equal (-<0.5; H = 11.46; $X^2(5) = 11.08$). In addition an a-postiori multiple comparison test (STP: Sokal and Rohlf, 1969) was performed on the data, indicating that the SFG at stations 0 and 250 m R-transect were larger than those of the other stations ($p<.001$; ts = 3.31).
The same statistical methods were applied to the different forms of energy, and only the E'_a showed the same significant difference between stations mentioned for the total SFG (ts = 3.21; $p<0.001$). For the E_r and E_{ex} no significant differences were found between stations.

A possible cause of the presented pattern could be the elevated UCM content in the mussel tissue, although usually the SFG is reduced under influence of contaminants.

ABM 2

The mussel samples of the second experiment were treated the same way as those of the first experiment. The results of the SFG analyses are given in Table 10. The mussels were however in their last stage of reproductive maturity and reacted almost immediately to the changes in environment by spawning during the first few days.
In the period of six days, in which the SFG was measured, the ADW of the mussels diminished strongly due to the spawning (compare Table 7 and 10).
The eggs and sperm produced by the mussels consume a vast amount of oxygen, as was observed during earlier experiments, therefore influencing the measured E_r in the SFG analyses.
The loss of ADW and the consumption of oxygen influence the SFG measurements strongly and therefore no conclusions can be drawn from the SFG results of the second experiment.

6.3.3.2 Cytochemical measurements

For the determination of the lysosomal stability (LS), the mixed function oxidase (NTR) and the induction of metallothioneinen, ten mussels per sample of the first ABM were analysed. After preparing the sections by using a crysostat (-25°C) the sections were analysed by TNO and the University of Gent (RUG).
The Timmsufide test, the Schmorl test and the Papanicolaou test were carried out on the mussels from the second ABM. In order to establish the usefulness of Macoma balthica as a test organism in ABM experiments, the tests were also performed on one specimen of Macoma balthica per station. The samples of the ABM 2 were analysed by TNO.
The results are represented in Table 11. The data of the LS of the first ABM demonstrate that the animals were in excellent condition. The low NTR values and the absence of metallothioneinen indicate that the mussels were not suffering from stress due to organic and heavy metal contaminants. The results produced by TNO and RUG compared well with each other.

Table 10 Scope for Growth (SFG) corrected to "standard body size" of 1 gram in mussels from ABM 2 at site L-4-α.

Station		Mussels measured	ADW g	E_a' J/h	E_r' J/h	E_{ex}' J/h	SFG J/h
VL-3		8	0.403	18.5 (8.3)	18.8 (3.4)	1.2 (0.7)	-1.5 (12.5)
R-transect	0 m	8	0.250	31.8 (10.2)	15.4 (2.5)	1.4 (0.9)	15.0 (9.2)
R-transect	250 m	8	0.246	32.6 (3.9)	17.9 (3.8)	1.5 (0.9	13.2 (3.8)
R-transect	500 m	8	0.235	20.8 (13.1)	14.9 (2.3)	1.2 (0.6)	4.7 (11.8)
R-transect	1000 m	6	0.248	39.6 (10.2)	14.9 (2.1)	1.1 (0.4)	23.6 (12.1)
R-transect	5000 m	7	0.245	34.7 (4.6)	18.8 (2.9)	1.7 (0.8)	14.1 (4.3)
T-transect	250 m	8	0.244	35.1 (10.0)	14.9 (4.2)	1.6 (0.8)	18.6 (8.8)

( ) = standard deviation

Table 11 Cytochemical parameters measured in Mytilus edulis and Macoma balthica in ABM experiments at site L-4-α.

Experiment	Organism	Station	L.S.	N.T.R.	Metallothioneïnen	Timm-sulfide test	Schmorl-test	Papanicolaou test
ABM 1	Mytilus edulis	0 m R-transect	23 (22)	2.3 (2.4)	- (-)	n.a.	n.a.	n.a.
		250 m R-transect	20 (20)	2.8 (2.4)	- (-)	n.a.	n.a.	n.a.
		500 m R-transect	25 (25)	2.5 (2.1)	- (-)	n.a.	n.a.	n.a.
		1000 m R-transect	20 (19)	2.5 (2.1)	- (-)	n.a.	n.a.	n.a.
		5000 m R-transect	24 (23)	2.4 (1.7)	- (-)	n.a.	n.a.	n.a.
		250 m T-transect	21 (20)	1.4 (1.3)	- (-)	n.a.	n.a.	n.a.
ABM 2	Mytilus edulis	0 m R-transect	2	1	-	-	0	o.k.
		250 m R-transect	2	2	-	-	-	o.k.
		500 m R-transect	2	2	-	-	-	o.k.
		1000 m R-transect	2	<2	-	-	-	o.k.
		5000 m R-transect	2	2	-	-	0	o.k.
		250 m T-transect	2	1-2	-	-	0	o.k.
		VL-3 (reference)	2	2	-	-	0	o.k.
	Macoma balthica	0 m R-transect	2	0	+	++	0	o.k.
		250 m R-transect	5	1	0	++	-	o.k.
		500 m R-transect	2	0	0	++	+	o.k.
		1000 m R-transect	2	0-1	+	++	-	o.k.
		5000 m R-transect	5	0-1	+	++	+	o.k.
		250 m T-transect	5	0	+	++	-	o.k.
		range	25 → 2	0 → 5	- → +	- → ++	- → +	

( ) = analysed by RUG
n.a. = not analysed
range = (good - bad)

The mussels of the ABM 2 had a very low lysosomal stability. However in all the other tests no stress due to organic or metal contaminants were detected. In all the sections of the mussels full gonads were found. Therefore the low lysosomal stability can only be explained by the fact that the mussels were in their last stage of reproductive maternity. In the Papanicolaou test some mussels were found to be infected with mitilicolas. The test with Macoma balthica showed a very low lysosomal stability. The NTR values were low, but the test on the presence of metallothioneinen and metalloproteins were positive. The observed low lysosomal stability can therefore be explained by metal contamination and possibly some food stress occurred although no data are available to verify this. The cause for the metal contamination could be the presence of metals in the sediment but it is also possible that anaerobic conditions in the sediment stimulated the release of metals from the stainless steel cages which were used to expose the Macoma. In all cytochemical tests no correlation was found with the distribution of drill cuttings in the sediment.

6.3.3.3 Biochemical measurements

In order to make an assessment of the usefulness of biochemical measurements to detect stress induced by the distribution of drill cuttings in the seabed, Dr. A. de Zwaan of the University of Utrecht was asked to carry out some measurements on mussel samples of the ABM 1 experiment. The report of the measurements is presented in Appendix E (Dutch version). The codes used in the appendix corresponds with the following stations:

Code		Station
71	=	L-4-α 0 m R-transect
65	=	L-4-α 250 m R-transect
66	=	L-4-α 500 m R-transect
67	=	L-4-α 1000 m R-transect
68	=	L-4-α 5000 m R-transect
69	=	L-4-α 250 m T-transect

The main conclusions of the report is that there is a great similarity between the samples from the various stations, and no indications were found of a reduced resistance to anoxia due to natural or anthropogenic stress.
The enzyme activity in mussel tissues is stimulated under influence of organic contamination. In the mussel samples no significant differences were found between the samples from the different stations (see Table E.1 of Appendix E). In bivalve molluscs the ratio of taurine: glycine and the sum of threonine and serine decreases under the influence of stress. In the experiment with the mussel samples of the ABM 1 no significant differences were found (see Tables E.3a and b, Appendix E) between the samples.
The end product ratio (see Table E.4b last column, Appendix E) varied from 0.00 to 0.18. These low values are caused by a relative high succinate accumulation in comparison with the pyruvate derivatives (octopine, strombine and lactate), which indicates that the glucose flux is very low during the anoxia. This low flux was confirmed by the calculated ATP turnover speed values (Table E.5, Appendix E). The values found for end product ratio and ATP turnover speed are the lowest even found or calculated from the literature. This indicate that the mussels adapted very well to the anoxia stress.
From the fact that the Asp: Suc + Prop ratio (Table E.5, second column, Appendix E) is very much smaller than 2.0 we may conclude that the mussels switched very soon from "initial" to "steady state" anaerobiosis during the 24 hours anoxia. This confirms the good adaption of the anoxia.
The contents of glycogen in the mantle tissues of the mussels (Table E.2, Appendix E) showed some differences. The mussels from station 500 m on the R-transect had the highest content and were therefore the healthiest while the mussels from 5000 m on the R-transect and 250 m on the T-transect showed the lowest contents. The differences were probably caused by variations in food supply.

7. CONCLUSIONS

- Of all sedimentary parameters measured in the experiments in 1985 (Kuiper, Van het Groenewoud, 1986) and 1986, barium is the most suitable for monitoring the distribution of muds around drilling sites, "a history marker" (6.1.2).

- The n-alkanes disappear rapidly from the sediment; when monitoring the presence of OBM in the sediment over a longer period the n-alkanes are not the most suitable indicator component, the fraction "others" and UCM are! (6.1.2).

- Oil found in the sediment near L-4-α originated from the used OBM at this site (5.2.1).

- The methode used for the determination of biodegradability gives a good indication of the presence of bacteria which are capable of degrading naphtalene and possible other oil components.

- Because the distribution of drill cuttings in the sediment can be patchy (6.1.2), it is necessary to collect sufficient subsamples in order to get a representative sample.

- The grain-size distributions of the sediment around platforms were very much alike, with the exception of the stations very near the platforms were the presence of drill cutting and mud were detectable (5.1.2).

- L-4-α was not a suitable site for carrying out a baseline study.

- Because of the difficulty in collecting representative macrobenthos samples, the oil content in macrobenthos sampled in the field is not suitable for passive monitoring purposes (6.2).

- ABM experiments with mussels and macoma are technically feasible.

- The oil accumulation in mussel tissues in the first ABM experiment is assumed to be caused by the oil fraction dissolved in the water.

- Active chemical monitoring in mussels in ABM experiments during drilling operations is a more sensitive, more notable assessment of the contamination with e.g. oil than the chemical monitoring of sediments (effects detectable up to 5000 m from drilling site).

- The mussel is not a sensitive indicator for monitoring biological effects, the high accumulation did not result in significant changes in stress parameters (6.3.3).

- Due to the slow decrease of the oil in the sediment (see K-12-α), possible biological effects can be expected to occur over a long period.

8. LITERATURE

Anonymus (1985)
Oil in the sea: inputs, fates and effects
National Academic Press, Washington.

Bayne e.a. (1985)
The effects of stress and pollution on marine animals
Preager special studies.

Conover, R.J. (1966)
Assimilation of organic matter by zooplankton
Limnol. Oceanogr. 11: 338-354.

Davies, J.M., J.M. Addy, R.A. Blackman e.a. (1984)
Environmental effects of the use of oil based drilling muds in the North Sea
Mar. Pollut. Bull. 15: 363-370.

Department of Energy (1985)
Development of the oil and gas resources of the United Kingdom
HMSO London 84 pp. + cherts.

Dicks, B. (1982)
Monitoring the biological effects of North Sea platforms
Mar. Pollut. Bull. 13: 221-227.

Gillan, A.H., K. O'Carroll and J.N. Wardell (1986)
Biodegradation of the oil adhering the drill cuttings
Proc. Cong. Oil Bas Drilling Fluids, Trondheim, Febr. 1986.
NIVA Oslo, pp. 123-126.

Hannam, M.D., J.M. Addy, B. Dicks (1987)
Ecological monitoring of drill cuttings of the seabed in the Thistle Oilfields
Paper presented at the conference "Fate and effects of oil in marine ecosystems", Amsterdam 23-27 Febr. 1987.

Kock, W.Chr. de (1983)
Accumulation of cadmium and polychlorinated biphenyls by Mytilus edulis transplanted from pristine waters into pollution gradients
Can. J. Fish. Aquat. Sci. 40 (suppl. 2): 282-294.

Kock, W.Chr. de, M. Scholten, A. de Zwaan e.a. (1986)
Onderzoek naar een aantal stressindicerende parameters in Mytilus edulis, langs een verontreinigingsgradiënt in de Westerschelde
MT-TNO report R 86/289 47 pp. + appendix.

Kock, W.Chr. de (1986)
Monitoring bio-available marine contaminants with mussels (Mytilus edulis) in the Netherlands
Environmental Monitoring and Assessment 7: 209-220.

Kock, W.Chr. de, M. Scholten (1986)
Preliminary measurements of marine pollution effects in the Rhine-Meuse outlet area using stress indicating parameters in Mytilus edulis
TNO-report R 87/118.

Kreuk, J.F. de, E. van der Hoek (1987)
Ontwikkeling van toetsmethoden ter vaststelling van de schadelijkheid voor het mariene milieu van "spoelingen op oliebasis"
MT-TNO rapport R 86/288 15 pp. + bijlagen.

Kuiper, J., H. van het Groenewoud (1986)
Monitoren van de verspreiding en de biologische effecten van olie rond platforms op de Noordzee
MT-TNO report R 85/350a 53 pp. + appendix.

Kuiper, J. (1986)
Monitoring of the fate and effects of oil in the marine environment
Environmental Monitoring and Assessment 7: 221-232.

Lahr, J. (1986)
De biodegradatie van ruwe olie in zeewater en marien sediment
Doctoraalscriptie, Vakgroep waterzuivering, Landbouw Hogeschool, Wageningen.

Mathesen, I., P.F. Kingston, C.S. Johnson, M.J. Cribson (1986)
Stratfjord field environmental study
In: Proc. Conf. in oil based drilling fluids Trondheim, Febr. 1986
NIVA, Oslo, pp. 3-16.

Mulder, M., W.E. Lewis, M.A. van Arkel (1987)
Effecten van oliehoudende boorgruis op de benthische fauna rond mijnbouw-installaties op het Nederlands continentaal plat
NIOZ report 1987-3 22 pp. + appendix.

Mulder, M., W.E. Lewis, M.A. van Arkel (1988)
Biological effects of the discharges of contaminated drill cuttings and water-bases drilling fluids in the North Sea
NIOZ report 1988-3 39 pp. + appendices

Saltzmann, H.A. (1982)
Biodegradation of aromatic hydrocarbons in marine sediment of three North Sea oil fields
Mar. Biol. 72: 17-26.

Shikata e.a. (1974)
Japanese Journal of Exp. Med. 44: 25-36.

Sokal, R.R., F.J. Rohlf (1969)
"Biometry"
Publ. Freeman and Company.

APPENDIX A PARTICIPANTS WORKING GROUP "MONITORING AROUND OFFSHORE INSTALLATIONS"

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APPENDIX B GENERAL DATA ON DRILLING ACTIVITIES AT THE STUDIED SITES

Well	Drilling period	Type mud	Discharges drill cuttings (m^3)	Bariet consumption (ton)	Used OBM (ton)
L-4-α-2	16-10-81/26-10-82	WBM	539	435	-
L-4-α-3	16-10-81/25-11-82	WBM	567	555	-
L-4-α-4	20-07-84/07-10-84	WBM	533	640	-
L-4-α-4	18-11-84/22-01-85	WBM	549	516	-
L-4-α-5	September, 1986	WBM+OBM	?	?	117
L-4-α-6	November , 1986	WBM+OBM	?	?	61
F-18-8-1	1985	WBM	?	622	?
K-12-α-1	?	WBM+OBM	?	?	?
K-12-α-2	?	WBM+OBM	?	?	?
K-12-α-3	?	WBM+OBM	?	?	?
K-12-α-4	?	WBM+OBM	?	?	?
K-12-α-5	?	WBM+OBM	?	?	?
K-12-α-6	November, 1983	WBM+OBM	?	?	?

APPENDIX C WORKING PROCEDURES

C.1 GRAIN SIZE DISTRIBUTION

In order to determine the grain size distribution, 100-150 g of wet sediment was sieved over 63 µm, using water. From the <65 µm fraction a subsample of 50-500 ml was filtered over a dried and weighed filter (Schleiger und Schull Schwarzband), dried for 24 hours at 90°C and weighed on a Mettler H30 balance.
In another subsample (20 ml) the particle size distribution was determined by means of an electronic particle data counter (Particle Data Inc. Celloscope), using a 120 µm orifice tube. The residue that remained on the 63 µm sieve was dried for one day at 90°C and placed in the sieves of a mechanical sieve shaker (80-125-160-250 µm) for 15 minutes. The resultant fractions were weighed using a Mettler PK300 balance.

C.2 METHOD FOR MEASURING OIL IN SEDIMENT

In order to perform the oil analyses, a 20 gram subsample of each sediment sample was (simultaneously) steam-distilled during 12 hours and extracted with 10 ml hexane. The extracted sediment was separated, dried at 105°C and weighed. Four ml of extract was taken to perform a clean-up. Hexane (nanograde) was used as the eluent and aluminium oxide five aq as solid state phase. The pure clean extract was injected onto the gas chromatograph (GC) and detected by means of a flame ionisation detector. The GC was equipped with a glass-capillary column (length 50 mm, inside diameter 0.7 mm), coated with SE-30.
After measuring for 5 minutes at 40°C, the temperature was raised by 2°C per minute, until 220°C was reached.
The detected components were identified on the basis of retention time and quantified by means of wellknown standards.
The total amount of oil was calculated by adding up all peaks in the spectrum, leaving out the UCM (Unresolved Carbon matter).

C.3 BARIUM CONTENT

About 10 g of sediment was dried during 2 hours at 105°C, after which 2 g was homogenized and destructed by means of sulphuric acid and hydrogen peroxide. After settling, the barium content of the destruate was determined using inductive coupled plasma-atomic-emission sepctrometry (ICP-AES).

C.4 BIOLOGICAL DEGRADABILITY OF OIL

The biological degradability of oil in sediment was determined using a method developed by L. Lahr (1985), by means of ^{14}C-labeled naphtalene.
In a 250 ml conical flask 100 ml filtered (0.45 µm filter) seawater was brought, directly after sampling.
For every sampling location 5 subsamples were collected, from which 2 served as a blank. To the blanks 10 ml of 50% HPO_4 solution was added, before addition of the sediment. This resulted in a pH < 1, causing all biological activity to be stopped. To each subsample 1 ml sediment was added by means of a syringe with a cut off end. Dry weight was determined by weighing different portions of 1 ml sediment samples.
A tube containing 1 ml 10N NaOH was placed in every flask to collect the $^{14}CO_2$ that was formed by the degradation of ^{14}C labeled naphtalene (see Figure C.1). Finally, 0.1 ml of a ^{14}C labeled naphtalene solution was added to every flask, after which the flasks were closed with an air-tight screw-cap.
The naphtalene solution contained 9.9 mg naphtalene and 2.6 mg ^{14}C-naphtalene in 100 ml DMSO (dimethyl sulphoxide), an inert organic solvent.
After naphtalene addition samples were incubated for 24 hours at a temperature of 10-12°C, after which 10 ml 50% HPO_4 solution was added to the non-blank subsamples. By acidifying, the biological acitivity was stopped, while the low pH enabled the bicarbonate, formed by the degradation, to evade from the water as CO_2.
The subsamples were placed in the incubator for another 15 hours to achieve a nearly total adsorption of the CO_2 formed into the sodium hydroxide.

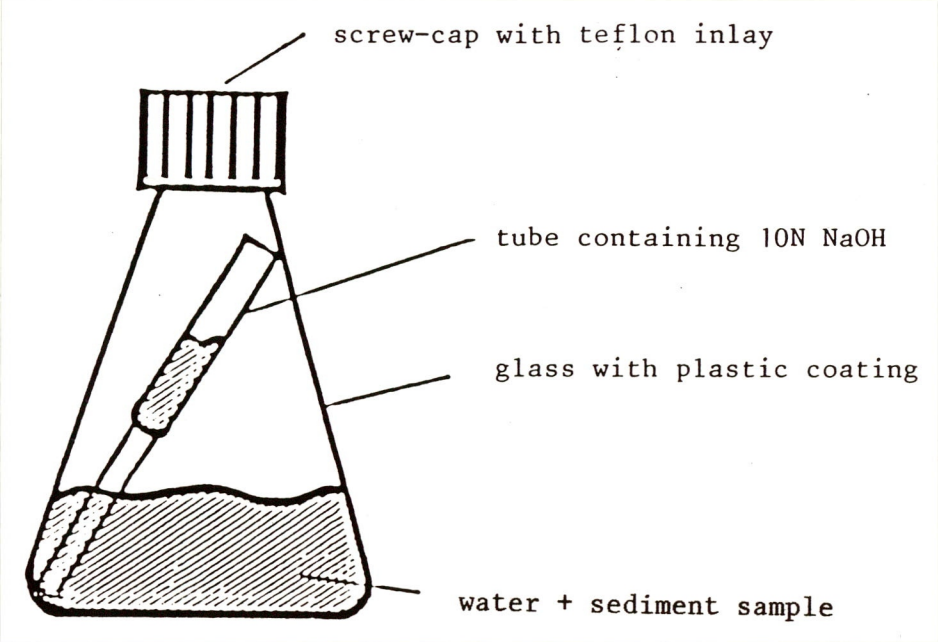

Fig. C.1 Conical flask, used to establish the degradability of ^{14}C-naphtalene.

Thereafter the total content of the NaOH containing tube was transferred to 15 ml scintillation liquid (10 ml lumagel + 5 ml methanol).
The total activity in the scintillation bottle was measured with a Packard Tricarb Liquid Scintillation Counter. The biological degradability of the naphtalene is calculated using the formula:

$$\text{degradability } (\mu g.kg^{-1}.dag^{-1}) = \frac{\text{Act - Act blanc}}{\text{Spec. Act x amount sediment (kg) x time (days)}}$$

where:

Act = average activity in NaOH tube of the sample to be measured in dpm (desintegrations per minute)

Act blanc = average activity in NaOH tube of the blanc measurement in dpm

Spec. Act = specific activity of the naphtalene in dpm/µg (= 17600 dpm/µg naphtalene

C.5 METHOD FOR MEASURING OIL IN ORGANISMS

About 5 g of homogenized material was taken for analyses.
The sample was destructed, using a pepsine solution, by stirring in a water-bad at 37°C for one night.
After destruction the sample was treated as described in C.2.

Oil content of sediment (mg/kg) based on dryweight

Site	station	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	Prist	C-18	Phyth	C-19	C-20	C-21	C-22	C-23	C-24	C-26	C-27	C-28	C-29	C-30	C-32
K-12-A R-Transect	100 m	0.00	0.85	0.00	2.61	0.78	0.00	0.66	2.57	3.43	0.78	5.55	0.00	4.34	0.63	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.03	0.06	0.15	0.12
" "	250 m	0.00	0.69	0.00	0.69	0.89	0.00	0.55	0.59	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.03	0.07	0.16	0.12
" "	500 m	0.15	0.74	0.03	0.03	0.02	0.00	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.08	0.21	0.31	0.58	0.41	0.66	0.27	0.30	0.19	0.11
" "	750 m	0.00	0.50	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.10	0.23	0.28	0.47	0.31	0.46	0.19	0.20	0.14	0.07
" "	1000 m	0.09	0.63	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.03	0.02	0.12
" "	2000 m	0.01	0.35	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.05	0.07	0.14	0.10	0.15	0.07	0.07	0.05	0.11
" "	5000 m	0.02	0.60	0.01	0.02	0.02	0.02	0.03	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.10	0.08	0.13	0.06	0.07	0.04	0.12
K-12-A T-Transect	250 m	0.00	0.34	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.03	0.07	0.16	0.12
" "	500 m	0.00	0.26	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.03	0.06	0.16	0.12
" "	1000 m	0.02	1.06	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.07	0.16	0.12
L-4-A2 R-Transect	0 m	0.01	1.54	5.45	33.29	58.28	476.88	461.08	120.01	14.36	4.95	2.90	3.98	3.35	3.48	4.44	2.74	2.34	0.01	0.01	0.01	0.03	0.03	0.07	0.16	0.12
" "	100 m	0.11	0.59	0.03	0.08	0.11	0.90	0.86	0.24	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.03	0.07	0.09	0.17	0.11	0.19	0.04	0.09	0.06	0.03
" "	250 m	0.12	0.71	0.02	0.03	0.03	0.21	0.21	0.07	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.15	0.12
" "	500 m	0.00	0.61	0.02	0.02	0.01	0.05	0.05	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.10	0.15	0.27	0.18	0.28	0.11	0.12	0.07	0.12
" "	750 m	0.11	0.55	0.02	0.02	0.00	0.02	0.02	0.02	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.03	0.07	0.16	0.12
" "	2000 m	0.00	0.67	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.03	0.07	0.10	0.18	0.13	0.20	0.08	0.08	0.05	0.12
" "	5000 m	0.08	0.80	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.03	0.07	0.11	0.21	0.15	0.23	0.09	0.11	0.06	0.03
L-4-A2 T-Transect	250 m	0.00	0.67	0.01	0.02	0.03	0.19	0.19	0.06	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.05	0.07	0.12	0.10	0.16	0.08	0.09	0.06	0.12
" "	500 m	0.01	0.43	0.01	0.01	0.01	0.04	0.04	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.03	0.07	0.16	0.12
" "	750 m	0.02	0.62	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.07	0.16	0.12
" "	1000 m	0.11	0.68	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.02	0.05	0.10	0.17	0.36	0.29	0.45	0.19	0.21	0.13	0.06
OBM (mg/kg wet sediment)		0.01	11.3	8.1	50.2	193.0	1712.0	1743.0	515.0	64.0	21.0	10.0	15.0	12.0	17.0	15.0	13.0	0.01	0.01	0.01	0.01	0.04	0.04	0.10	0.24	

Oilcontent of Cyprina islandica tissue (in mg/kg) based on ashfree dryweight

Site	Station		C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	Prist	C-18	Phyt	C-19	C-20	C-21	C-22	C-23	C-24	C-26	C-27	C-28	C-29	C-30	C-32
L-4-A2	R-Transect	1000 m	0.83	5.81	0.28	0.28	0.18	0.09	0.18	0.28	0.37	6.27	0.09	0.18	0.09	0.18	0.18	0.92	2.12	2.49	4.06	2.03	2.86	1.20	1.48	2.21	1.66
L-4-A2	T-Transect	250 m	0.23	5.88	0.12	0.12	0.12	0.58	0.58	0.46	0.23	3.34	0.12	0.12	0.12	0.12	0.23	1.04	2.31	3.34	5.77	4.15	5.54	3.23	4.15	3.23	6.34
"	"	750 m	1.48	7.38	0.46	0.55	0.37	0.18	0.18	0.74	0.92	15.41	0.09	0.37	0.09	0.28	0.09	0.37	0.65	0.83	1.38	0.65	0.83	0.37	0.92	2.21	1.66
"	"	1000 m	0.83	9.38	0.24	0.36	0.24	0.12	0.36	0.48	0.71	9.98	0.24	0.24	0.12	0.12	0.12	0.48	1.07	1.19	1.43	0.48	0.71	0.24	5.11	2.85	2.14
"	"	1500 m	0.50	7.44	0.12	0.25	0.12	0.25	0.50	0.62	0.37	4.47	0.37	0.50	0.37	0.50	0.62	1.24	2.36	2.98	4.71	3.47	5.46	4.96	6.45	5.83	5.83

Oilconcentrations (mg/kg) in the common mussel (Mytilus Edulis)
at station L-4-A, based on ashfree dryweight.

		C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	Prist	C-18	Phyt	C-19	C-20	C-21	C-22	C-23	C-24	C-26	C-27	C-28	C-29	C-30	C-32
ABM-1																										
R-Transect	0 m	0.45	4.95	0.97	1.12	0.75	1.12	1.27	0.60	0.52	5.55	0.30	0.15	0.15	0.22	0.07	0.30	1.05	2.02	1.50	2.70	1.20	1.50	0.75	1.80	1.35
"	250 m	0.15	4.31	0.15	0.92	0.08	0.92	1.15	0.62	0.62	5.77	0.23	0.15	0.08	0.15	0.08	0.08	0.23	0.15	0.15	0.08	0.08	0.15	0.77	1.85	1.39
"	500 m	0.34	3.53	0.17	0.52	0.34	0.52	0.77	0.77	0.95	10.06	0.43	0.26	0.34	0.17	0.09	0.09	0.26	0.17	0.34	0.17	0.09	0.17	0.86	2.06	1.55
"	1000 m	0.46	4.65	0.23	0.46	0.38	0.30	0.53	0.99	1.30	12.50	0.08	0.23	0.08	0.08	0.08	0.08	0.15	0.08	0.08	0.08	0.08	0.15	0.76	1.83	1.37
"	5000 m	0.78	5.69	0.23	0.39	0.31	0.23	0.31	1.17	1.72	16.07	0.08	0.31	0.08	0.23	0.23	0.86	2.18	2.57	4.13	2.11	3.20	1.33	0.78	1.87	1.40
T-Transect	250 m	0.27	5.36	0.09	0.27	0.18	0.27	0.36	0.64	0.73	7.81	0.18	0.18	0.09	0.27	0.09	0.09	0.91	1.45	0.73	1.18	0.36	0.91	0.91	2.16	1.63
Original sample		0.24	5.58	0.31	0.31	0.16	0.08	0.08	0.86	0.94	9.98	0.08	0.24	0.08	0.08	0.08	0.08	0.24	0.16	0.24	0.08	0.08	0.16	0.79	1.89	1.42
Original sample		0.36	6.02	0.27	0.27	0.09	0.09	0.09	1.09	1.28	13.40	0.09	0.36	0.09	0.18	0.18	0.64	1.19	1.28	2.19	1.46	2.28	1.00	1.19	2.19	1.64
Wild sample	0 m	4.51	10.70	3.01	23.43	13.70	83.02	59.24	17.86	6.54	49.69	2.03	1.24	1.24	0.71	0.62	0.97	2.65	2.12	3.98	2.12	3.27	1.41	1.59	2.12	1.59
ABM-2																										
R-Transect	0 m	0.00	5.15	0.07	1.10	0.88	0.96	1.18	1.69	2.72	34.51	0.59	0.37	0.15	0.22	0.07	0.37	0.15	0.15	0.15	1.25	0.07	0.15	0.07	1.77	1.32
"	250 m	0.00	6.38	0.09	1.20	1.11	0.46	1.39	2.22	2.78	30.80	0.09	0.37	0.09	0.28	0.09	0.46	0.19	0.19	0.28	1.39	0.09	0.19	0.93	2.22	1.67
"	500 m	0.00	3.06	0.08	1.10	0.47	0.31	1.34	1.18	1.41	17.83	0.71	0.24	0.24	0.31	0.08	0.47	0.16	0.08	0.08	1.26	0.08	0.16	0.79	1.89	1.41
"	1000 m	0.00	1.58	0.09	0.35	0.26	0.18	1.23	0.70	0.79	8.18	0.44	0.18	0.09	0.26	0.09	0.53	0.18	0.18	0.26	2.29	0.09	0.18	0.88	2.11	1.58
"	5000 m	0.00	2.01	0.08	0.62	0.31	0.23	1.24	0.77	0.93	11.30	0.62	0.23	0.15	0.39	0.08	0.39	0.08	0.08	0.15	1.01	0.08	0.15	0.77	1.86	1.39
T-Transect	250 m	0.00	2.71	18.01	0.75	0.38	0.30	1.28	1.73	2.64	32.78	0.53	0.38	0.15	0.30	0.08	0.45	0.08	0.08	0.08	1.58	0.08	0.15	0.75	1.81	1.36
Ref. sample	VL-3	0.00	4.56	1.24	1.69	1.82	0.39	0.78	1.50	1.89	32.25	0.26	0.33	0.07	0.13	0.07	0.13	0.07	0.07	0.07	0.07	0.07	0.13	0.65	1.56	1.17
Original sample		0.00	0.07	0.07	0.21	0.21	0.21	0.79	0.64	0.64	4.36	1.14	0.36	0.72	0.43	0.29	0.93	1.57	1.93	2.86	1.79	1.79	1.57	1.79	1.72	1.29

APPENDIX E BIOCHEMICAL SUPPLEMENT

Onderzoek naar effecten op de anaërobe koolhydraatstofwisseling bij mosselen ten gevolge van naburige olieplatform-activiteiten.

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(in opdracht van TNO-Den Helder).

E.1 INLEIDING

De hier beschreven resultaten hebben betrekking op een gecombineerd experiment van actieve biomonitoring en het vaststellen van intracellulaire storingen op de (anaërobe) koolhydraatstofwisseling bij mosselen ten gevolge van expositie in mogelijk verontreinigde gebieden.
De theoretische achtergrond met betrekking tot de keuze van de bepaalde biochemische parameters wordt voor het koolhydraatmetabolisme in TNO rapportnr. R 86/289 gegeven en is voor de vrije aminozuren uiteengezet in het boek "The effects of stress and pollution on marine animals" (B.L. Bayne, editor). Tevens geven bijlagen E.1 en E.2 een beknopte samenvatting voor wat betreft de theoretische achtergrond van de in de discussie besproken begrippen en bepalingen.

E.2 MATERIAAL EN METHODEN

Voor de biochemische analysemethoden wordt verwezen naar TNO rapportnr. R 86/289.
Direct na het verzamelen op de locaties werden van 3 subgroepen van 8 dieren de mantel en grote sluitspier uitgeprepareerd en in vloeibare stikstof gedompeld, gevolgd door vriesdrogen. Drie andere subgroepen werden voorafgaande aan deze opwerkingen gedurende 24 uur drooggelegd in met waterdamp verzadigde lucht bij 15°C. Dit zijn de z.g. anaërobe groepen. De subgroepen werden steeds afzonderlijk geanalyseerd op de gerapporteerde parameters. De gegevens in alle tabellen zijn daarom gemiddelde waarden (van 3 subgroepen samengesteld uit 8 mosselen ieder) ± standaarddeviaties.
Voor de eindprodukten en de aminozuren wordt het weefsel geëxtraheerd met een ethanol: water (80:20 v/v) mengsel. Voor het berekenen van opwerkingsverliezen werd voor de aminozuren norleucine (0.3 mM) en voor de vluchtige vetzuren valeriaanzuur (2.5 mM) als interne standaard toegevoegd.

E.3 RESULTATEN

De resultaten zijn in de volgende 5 tabellen samengevat.

Tabel E.1 De specifieke activiteit van fosfofructokinase (PFK), pyruvaat kinase (PK) en glutamaat pyruvaat transaminase (GPT) in adductor spier (activiteit in µmole substraat omgezet per min en gram drooggewicht).

Code	PFK	PK	GPT
65	2,81±0,51	21,54±0,28	15,92±1,33
66	3,47±0,28	22,66±3,60	17,23±0,39
67	3,38±0,89	20,98±3,04	14,42±1,20
68	3,89±0,36	21,72±0,75	16,11±1,31
69	3,47±0,73	22,29±1,19	15,73±1,96
71	3,66±1,40	21,53±3,83	15,55±2,16

Tabel E.2 Het glycogeengehalte in mantel. Concentraties zijn gegeven in µmole glycosyl units/g drooggewicht.

Code	glycogeen
65	1114,5±11,5
66	1454,9±67,7
67	1291,8±62,5
68	965,5±44,6
69	982,0±54,1
71	1175,1±76,2

Tabel E.3a De concentraties aan vrije aminozuren in mantel en adductor spier (µmole per gram drooggewicht).

Code	Mantel								
	TAU	ASP	THR	SER	ASN	GLU	GLN	GLY	ALA
65	210,71± 7,39	24,59±0,94	12,61±0,36	32,98±1,52	8,41±1,53	29,80±4,59	17,84±1,58	148,61±19,05	39,59± 2,29
66	208,76±15,99	29,44±2,56	19,92±4,64	38,08±3,59	8,95±1,24	27,14±2,41	26,26±5,87	105,76± 4,73	41,55±11,51
67	196,49± 7,38	26,65±1,74	16,53±2,78	37,18±3,96	7,94±0,25	26,24±3,13	20,82±3,93	105,24± 5,56	43,83±3,39
68	223,04± 8,89	32,11±3,21	15,66±1,24	32,65±3,94	8,43±1,99	28,74±0,15	21,76±3,53	130,61±37,03	34,07±3,00
69	205,80± 1,18	26,07±0,75	13,73±4,81	30,23±3,29	6,73±0,47	27,69±2,05	18,14±1,38	124,63±12,93	41,78±5,63
71	200,83± 6,27	23,64±0,81	17,95±3,20	28,59±1,06	8,45±0,15	25,73±2,44	21,08±1,94	113,88±10,82	33,71±6,71

Code		Spier							
		TAU	ASP	THR	SER	GLU	GLN	GLY	ALA
65	0	269,07± 7,58	20,16±1,93	6,80±1,10	14,00±2,72	13,19±1,43	8,09±0,49	147,94±11,36	15,85±2,13
66	0	258,98±11,99	21,77±0,56	6,89±0,54	11,86±1,66	13,23±2,07	8,49±1,06	150,95±16,11	12,30±0,95
67	0	264,02±13,74	21,69±1,47	5,96±0,39	12,30±1,57	13,37±0,36	8,89±0,81	130,57±11,63	15,79±0,43
68	0	267,91± 8,39	23,05±1,56	7,74±1,02	14,90±2,83	14,84±0,81	9,06±0,63	133,65± 4,45	16,08±0,86
69	0	259,92± 2,44	18,69±2,09	6,58±0,83	12,30±0,99	14,51±0,43	10,76±1,09	147,88± 9,71	18,85±2,20
71	0	282,89± 9,20	23,73±2,58	7,90±1,69	11,71±2,29	13,74±0,19	10,49±0,89	121,38±14,75	15,43±0,47
65	24h	283,49±17,78	12,43±0,06	8,22±1,99	12,11±1,94	19,19±1,44	8,27±2,29	151,75±18,05	28,25±2,47
66	24h	265,80± 7,87	13,99±0,61	7,51±0,70	15,05±3,30	17,24±0,99	7,14±0,88	154,32±23,35	25,86±2,56
67	24h	259,69± 5,44	14,02±1,30	6,21±0,28	9,46±1,23	16,95±0,88	7,06±0,49	142,35± 7,82	23,86±1,48
68	24h	248,56±25,01	12,95±2,29	5,98±0,83	8,98±0,41	16,98±0,18	8,29±0,40	139,20± 6,21	25,42±1,77
69	24h	265,02± 2,68	12,80±1,05	6,10±0,15	11,43±1,32	17,80±0,94	6,57±0,88	138,33± 8,76	25,27±1,98
71	24h	290,97± 8,63	16,61±2,58	8,68±0,26	12,43±1,26	19,62±1,73	10,26±1,16	163,84±10,77	25,64±2,26

0 : na verzamelen op zee; 24h : na 24h luchtblootstelling (anaërobiose) bij 15°C.

Tabel E.3b De concentratieveranderingen (µmole.g^{-1} drooggewicht) in de aminozuren glutamaat (GLU), aspartaat (ASP) en alanine (ALA) in de adductorspier na 24 uur luchtblootstelling bij 15°C, alsmede de som van threonine en serine (THRE + SER) in de taurine-glycine ratio (TAU/GLY) in de adductorspier en mantel direct na verzamelen op zee.

Code	Spier			Spier		Mantel	
	δGLU	δASP	δALA	THRE+SER	TAU/GLY	THRE+SER	TAU/GLY
65	6,00	- 7,73	12,40	20,80	2,33	45,59	1,42
66	4,01	- 7,78	13,56	18,78	1,75	58,00	1,97
67	3,58	- 7,67	8,10	18,26	2,00	53,71	1,87
68	2,14	-10,55	9,34	22,64	2,02	48,31	1,71
69	3,29	- 5,89	6,42	18,88	1,71	43,96	1,65
71	5,88	- 7,12	10,21	19,61	1,82	46,54	1,76

Tabel E.4a De concentraties van anaërobe eindprodukten in de sluitspier van controlegroepen (0: direct na het verzamelen op zee) en van groepen nadat ze 24 uur bij 15°C aan de lucht waren blootgesteld (24h). Waarden in µmole per gram drooggewicht.

Code	D-lactaat		Octopine		Strombine	
	0	24h	0	24h	0	24h
65	0,28±0,30	0,01±0,02	0,80±0,51	0,28±0,18	6,87±0,38	7,54±0,73
66	0,01±0,02	0,13±0,14	0,20±0,07	0,22±0,20	6,02±0,13	8,59±1,41
67	0,08±0,07	0,01±0,02	0,13±0,21	0,00±0,00	5,92±0,70	7,58±0,29
68	0,00±0,00	0,07±0,12	0,16±0,27	0,10±0,17	4,78±0,84	6,61±0,44
69	0,02±0,04	0,08±0,09	0,77±0,36	0,11±0,13	5,71±0,37	8,52±0,97
71	0,11±0,09	0,30±0,12	0,45±0,40	0,08±0,14	6,03±0,08	8,07±0,69

Code	Succinaat		Acetaat		Propionaat	
	0	24h	0	24h	0	24h
65	1,66±0,14	13,78±0,24	1,28±0,35	1,55±0,35	0,03±0,05	0,87±0,07
66	0,93±0,03	12,66±0,17	0,76±0,18	1,42±0,24	0,05±0,09	1,32±0,35
67	0,88±0,53	12,83±0,19	1,34±0,14	1,61±0,26	0,06±0,02	1,82±0,29
68	0,91±0,12	12,44±0,95	1,47±0,16	1,76±0,09	0,00±0,00	1,81±0,37
69	1,18±0,23	10,60±0,99	1,38±0,25	1,69+0,20	0,06±0,07	2,70±0,08
71	1,49±0,19	13,00±0,58	2,79+0,27	2,62±0,23	0,23±0,04	2,39±0,39

Tabel E.4b De concentratieveranderingen (μmole.g^{-1} drooggewicht) in succinaat (SUC), propionaat (PROP) en de pyruvaat derivaten strombine, octopine en lactaat (STR, OCT, LAC). De laatste kolom geeft de z.g. eindprodukt ratio.

Code	δSUC	δPROP	δ(SUC+PROP)	δSTR	δOCT	δLAC	δ(STR+OCT+LAC)	$\delta\frac{(STR+OCT+LAC)}{(SUC+PROP)}$
65	12,12	0,84	12,96	0,67	-0,52	-0,27	-0,12	0,00
66	11,73	1,27	13,00	2,57	0,02	0,12	2,71	0,21
67	11,95	1,76	13,71	1,66	0,00	-0,07	1,59	0,12
68	11,53	1,81	13,34	1,83	-0,06	0,07	1,84	0,14
69	9,42	2,64	12,06	2,81	-0,66	0,06	2,21	0,18
71	11,51	2,16	13,67	2,04	-0,37	0,19	1,86	0,14

Tabel E.5 De ratio van aspartaatverbruik en (1) de toename van alanine of (2) de toename in de som van succinaat en propionaat. De ATP-turnover snelheid ($\dot{M}$ ATP) is berekend uit de geaccumuleerde eindprodukten en geeft het ATP-verbruik in μmole per gram droog weefsel als gemiddelde waarde over 24 uur blootstelling aan de lucht bij 15°C (anaërobiose).

Code	δASP/δALA (1)	δASP/δ(SUC+PROP) (2)	$\dot{M}$ ATP d.g.	n.g.
65	0,6	0,60	0,025	0,0051
66	0,6	0,60	0,028	0,0057
67	0,9	0,56	0,029	0,0058
68	1,1	0,79	0,028	0,0057
69	0,9	0,49	0,027	0,0053
71	0,7	0,84	0,029	0,0058

E.4 DISCUSSIE

Met uitzondering van de glycogeengehalten in de mantel (Tabel E.2) valt ten aanzien van de bepaalde biochemische parameters de grote onderlinge vergelijkbaarheid op tussen de mosselen afkomstig van de 5 verschillende locaties. Dit geldt voor:

1) de enzymactiviteiten,
2) de concentraties van de vrije aminozuren in de sluitspier en de mantel, en
3) de ophoping van anaërobe eindprodukten tijdens blootstelling van de mosselen na verzamelen aan de lucht (anaërobiose).

ad 1.

Met name voor organische microverontreinigingen zijn voor het fosfofructokinase uit zeemosselweefsels stimulerende effecten op de specifieke activiteit beschreven (zie Bayne et al., 1985). De hier verkregen resultaten laten echter voor geen van de drie enzymen, inclusief fosfofructokinase, significante verschillen zien tussen de mosselen van verschillende herkomst (Tabel E.1).

ad 2.

Voor bivalve mollusken is diverse malen gevonden dat de taurine: glycine ratio en de som van threonine en serine reageren op tal van natuurlijke en antropogene stressors. Beide worden ze gehanteerd als algemene index voor stress. De aminozuren ratio stijgt en de aminozurensom daalt bij stress. Het kan gaan om aanzienlijke veranderingen tot een drievoudige stijging of daling van de waarde van de controle (zie Bayne et al., 1985). In ons experiment laten beide grootheden echter marginale veranderingen zien (≦ 30% van de controle), terwijl ze onderling niet gecorreleerd zijn (tabellen E.3a en 3b).

ad 3.

De eindproduktratio (Tabel E.4b, laatste kolom) varieert van 0,00 tot 0,18. De lage waarden zijn het gevolg van de relatief hoge succinaataccumulatie ten opzichte van de pyruvaatderivaten (octopine, strombine en lactaat), hetgeen indicatief is voor een lage glycolyseflux tijdens de opgelegde anoxie

stress. Deze lage flux wordt bevestigd door de berekende ATP-turnover snelheden ($\dot{M}$ ATP in Tabel E.5) welke binnen zeer nauwe grenzen varieert (0,0051-0,0058 µmole per gram natgewicht, min). Zowel de eindproduktratio's als de $\dot{M}$ ATP-waarden behoren tot de laagste waarden die we ooit hebben gevonden of berekend uit literatuurgegevens. Zij wijzen op een ongestoord verloop van het anaërobe metabolisme en op een optimale aanpassing aan de opgelegde anoxie stress.

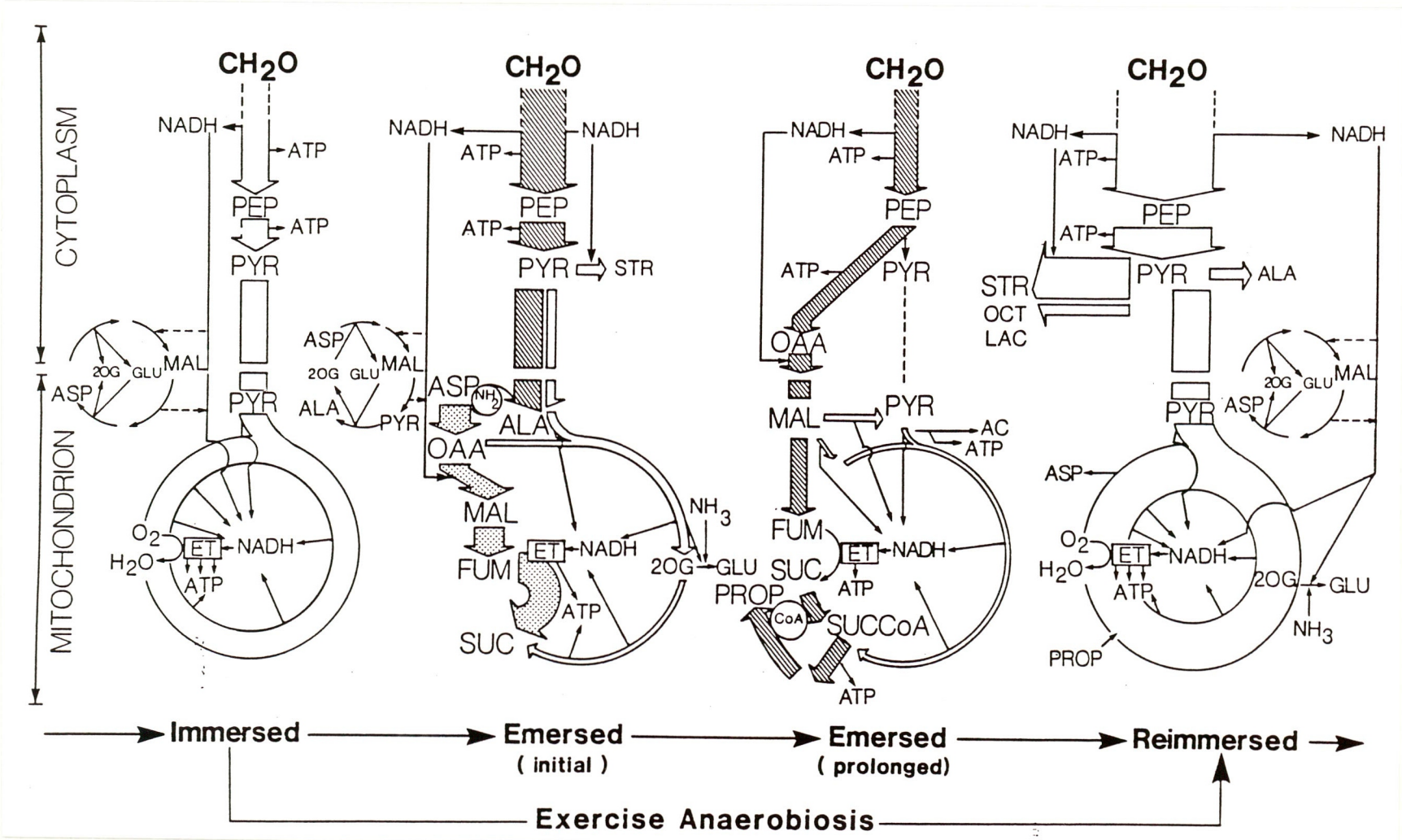
Uit het feit dat de ASP: SUC+PROP ratio (Tabel E.5, 2e kolom), veel kleiner is dan 2 kan worden afgeleid dat er binnen de 24 uren van experimentele anoxie reeds spoedig een switch moet hebben plaatsgevonden van "initial" (aspartaat is koolstofbron van succinaat) naar "steady state" anaërobiose (glycogeen is koolstofbron van succinaat). Deze switch is geïllustreerd in Figuur E.1 in de twee binnenste panelen (initial-prolonged). Ook dit bevestigt de goede aanpassing aan de opgelegde anoxie.

Ten aanzien van de aanwezige glycogeenvoorraden in de mantel op het moment van terughalen van de korven is er echter wel degelijk verschil tussen de locaties; locatie 66 springt er als gunstigste uit en de locaties 68 en 69 als meest ongunstig. Dit gegeven staat echter los van de overige bepaalde biochemische gegevens en is waarschijnlijk het gevolg van verschillen in voedselaanbod.
In zijn totaliteit laten de verkregen resultaten vooral overeenkomsten tussen de locaties zien en is er geen aanwijzing gevonden dat er externe factoren op het energiemetabolisme hebben ingewerkt die de weerstand voor natuurlijke anoxie stress hebben verminderd.

Lit. ref. B.L. Bayne et al. (1985). The effects of stress and pollution on marine animals. Praeger special studies. Praeger scientific. UK.

BIJLAGE E.1

De verschillende afbraakroutes van glycogeen (en aspartaat) in de sluitspier van de zeemossel in relatie tot de getijdenbewegingen van het zeewater. Bij eb zullen de hoog in het littoraal gelegen mosselen droogvallen en glycogeen fermenteren in alanine onder obligate koppeling van de aspartaat ($\rightarrow CO_2$ + alanine) voor waterstoftransport door de mitochondrium membraan. De stoichiometrie is: 1 glycosyl + 2 aspartaat → 2 alanine + 2 (succinaat + propionaat) (2e paneel, emersed, initial). Na verloop van tijd (de duur wordt bepaald door deels nog onbekende factoren) vindt er een switch plaats op het niveau van fosfoenolpyruvaat (PEP) waardoor glycogeen wordt omgezet in succinaat en propionaat. De glucolyseflux heeft nu zijn minimale waarde bereikt (3e paneel, "prolonged"). Bij reïmmersie (laatste paneel) treedt er een sterke toename op in de glycogeenafbraak (Pasteur effect) dat deels anaëroob (pyruvaatderivaten als strombine) en deel aëroob (CO_2 + H_2O) wordt afgebroken. De simultane ATP-generatie uit fermentatie en respiratie treedt ook op bij z.g. "exercise" anaërobiose wanneer het dier veel energie verbruikt. (De diameter van de staven is een relatieve weergave van de koolstoffen door het betreffende deel van het ketenproces).


CYTOPLASM
MITOCHONDRION
CH2O
NADH
ATP
PEP
PYR
STR
ASP
2OG
GLU
MAL
ALA
OAA
NH2
NH3
O2
H2O
ET
FUM
SUC
PROP
CoA
SUCCoA
AC
OCT
LAC
Immersed
Emersed
(initial)
Emersed
(prolonged)
Reimmersed
Exercise Anaerobiosis

RIJKSUNIVERSITEIT UTRECHT UTRECHT, THE NETHERLANDS

The development of a general biochemical stress index

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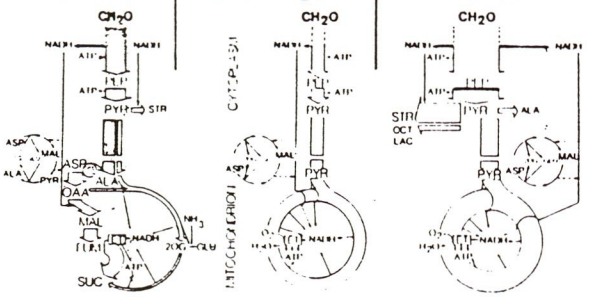
Theoretical background

Anaerobic pathways

Blue mussels exploit different pathways at low and high output mode of anaerobic metabolism.

Environmental anaerobiosis (low output mode)	Aerobiosis	Exercise anaerobiosis (high output mode)
SUC > STR	$CO_2 + H_2O$	STR



	MATP (μmole.g⁻¹fresh weight.min)		
	environmental (>10h)	aerobic (rest)	exercise (<2min)
MOLLUSCA	0.0065	0.498	11.32
ANNELIDA	0.0380	0.420	2.74
CRUSTACAEA	0.0380	0.372	24.64

(summary of literature research)

End product ratio as stress indicator

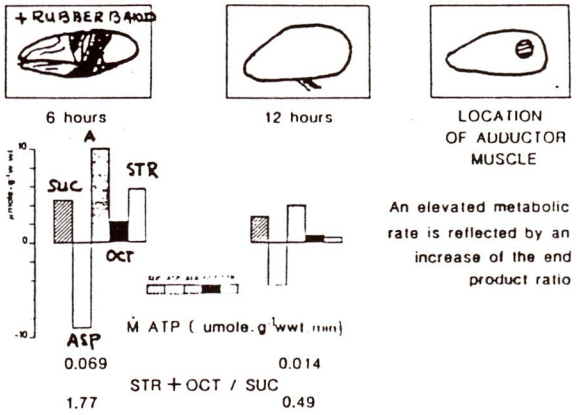
A main strategy in surviving anaerobic conditions is by energy conservation as a consequence of metabolic arrest. This is shown by a steep drop in the ATP turnover rate. When anaerobic survival goes along with a low metabolic rate the pathway terminates mainly in malate derivatives. In the blue mussel *Mytilus edulis* succinate is the major end product which after a temperature dependent lag period can be further converted in propionate.

Another group of end products, the pyruvate derivatives lactate and the so-called opines, are dominating when the metabolic rate is relatively high (see right side). In the sea mussel it concerns strombine and octopine. This is for instance the case when fermentation processes augment energy production in situations that the energy exceeds the aerobic scope (e.g. burst activity).

Pollution and stress in general cause an elevated metabolic rate. This will make blue mussels more vulnerable for anoxia stress (see right side) as they will conserve less energy due to a reduced scope for metabolic arrest. Since mussels exploit different pathways at low and high output mode of anaerobic metabolism (see left side) a shift in the scope for metabolic arrest (Ṁ ATP) will be reflected in the ratio in which the corresponding end products accumulate (see right side).

In a field project combining active biomonitoring with measurements on stress indicator parameters we took the opportunity to study the suitability of the end product ratio as a stress parameter (lower part).

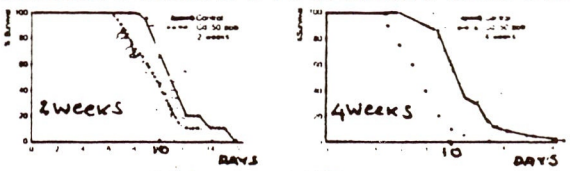
Effect of stress on the metabolic rate and the end product ratio during anaerobiosis



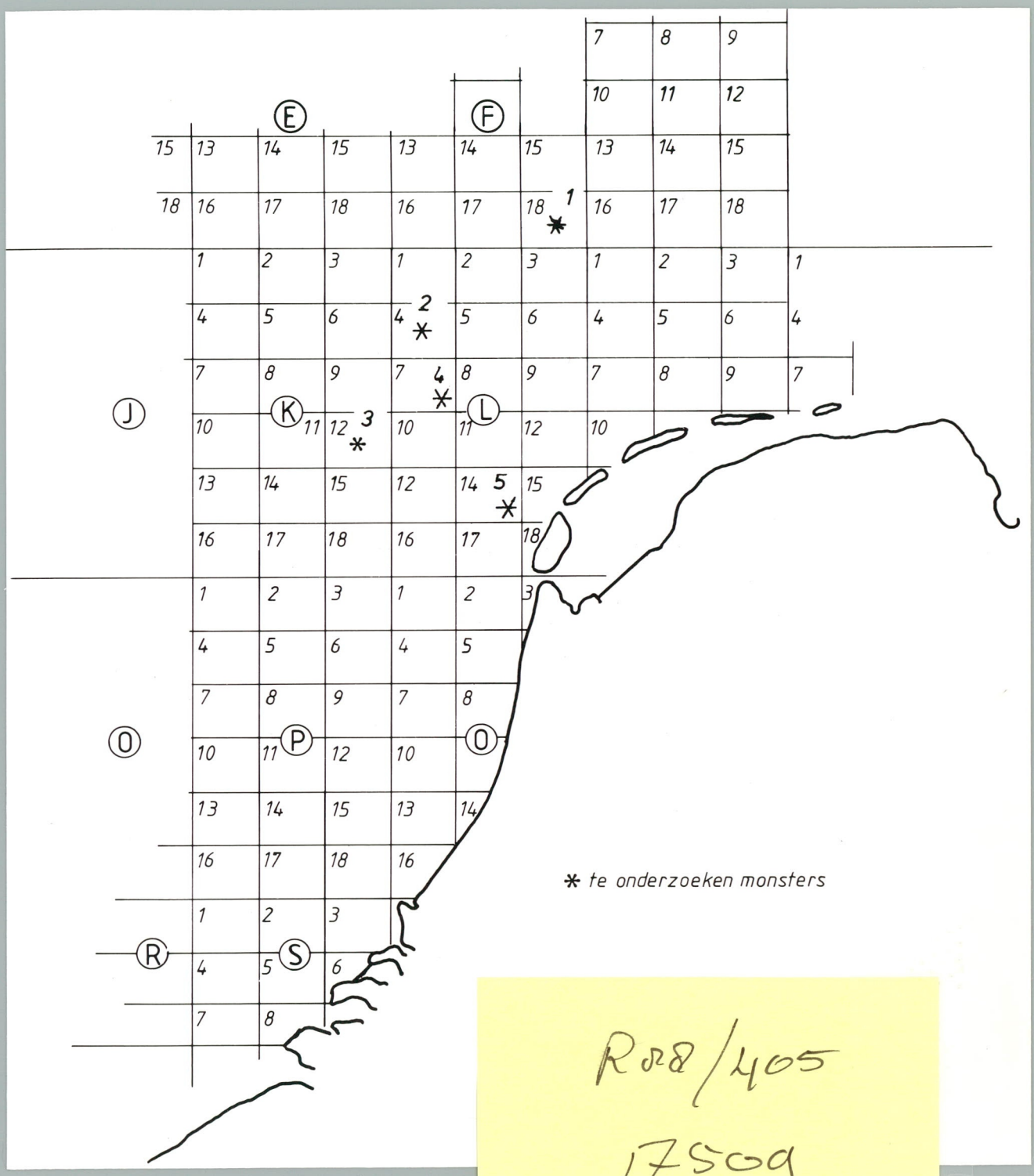
An elevated metabolic rate is reflected by an increase of the end product ratio

(Pollution) stress makes animals more vulnerable to anoxia stress

preincubation in seawater of 12 °C in the presence or absence of Cd



Results obtained by P.J. den Besten and M.B. Veldhuizen-Tsoerkan



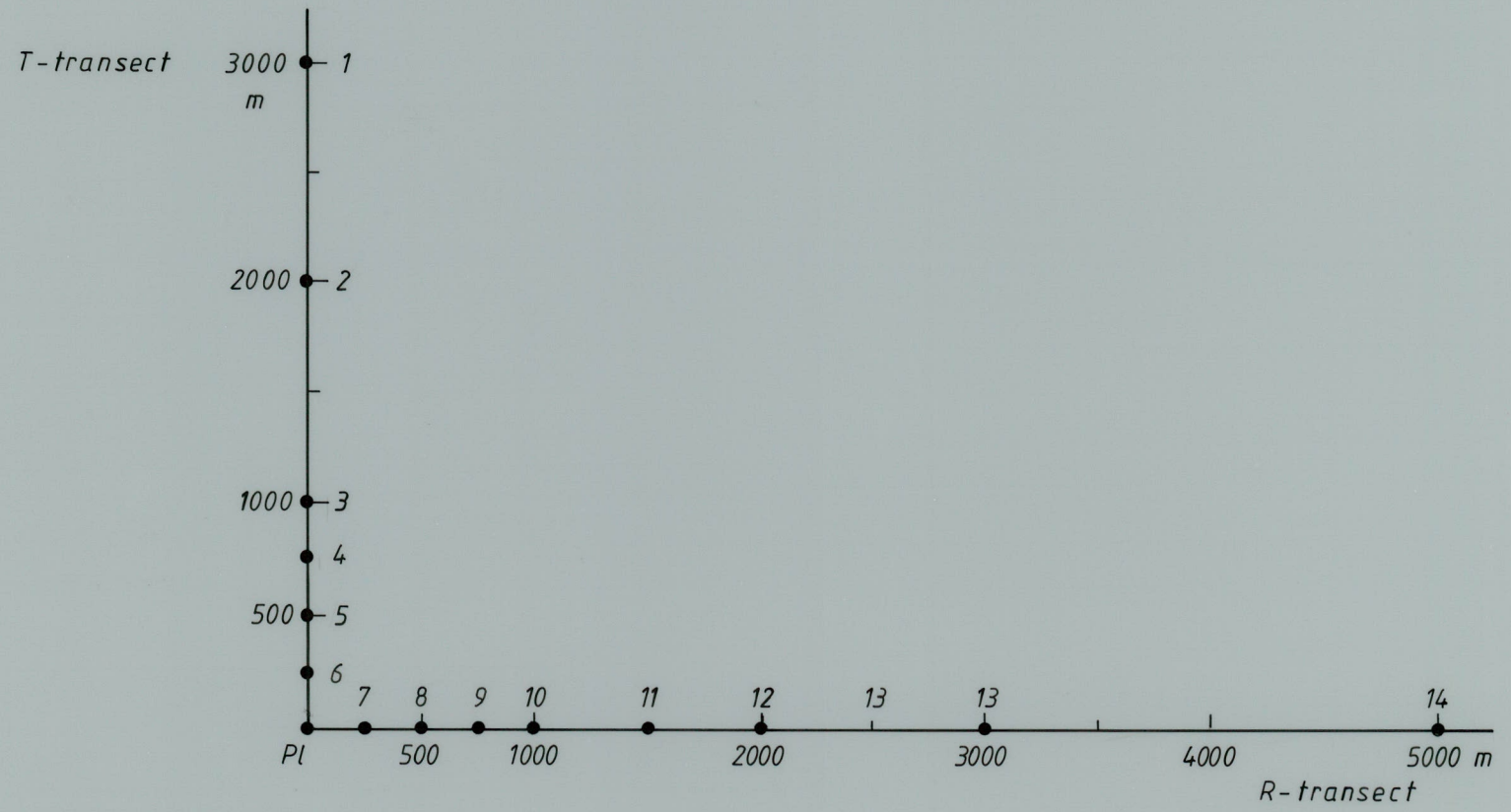
R 28/405

1750g

H.v.h. Groenewoud

Tek.

1
Tek


T-transect
3000
m
1
2000
2
1000
3
4
500
5
6
7
8
9
10
11
12
13
13
14
Pl
500
1000
2000
3000
4000
5000 m
R-transect

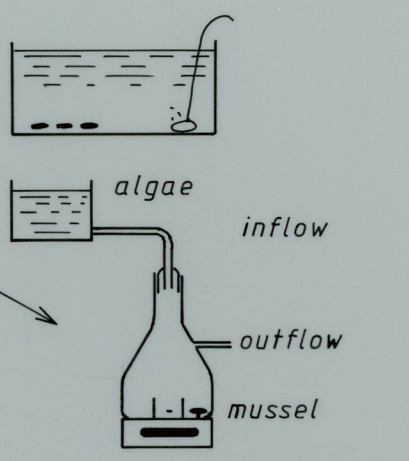
2
7016

Transect-A
m
500
1000
2000
3000
4000
5
6
7
8
9
10
11
12
13
14
Transect-T
m
3000
2000
1000
500
1
2
3
4

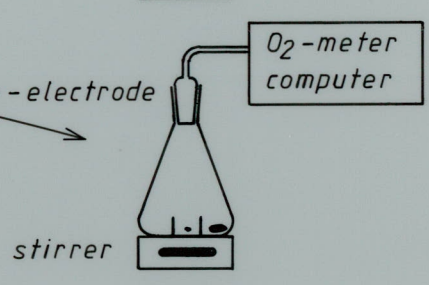
transport to laboratory

acclimatised during 17 hours in laboratory

clearance rate measurement

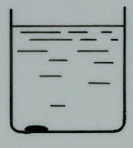


respiration and excretion



clearance rate measurement

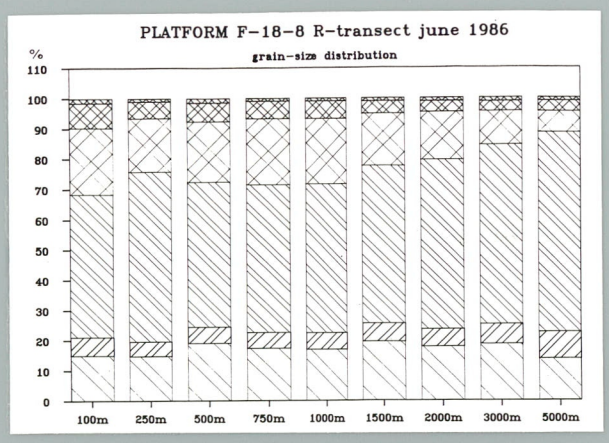
faeces measurement

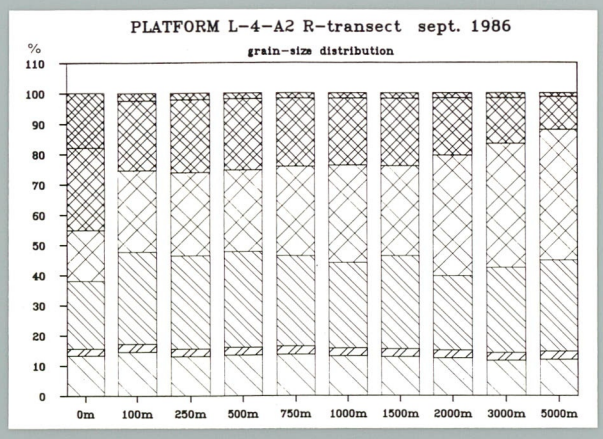


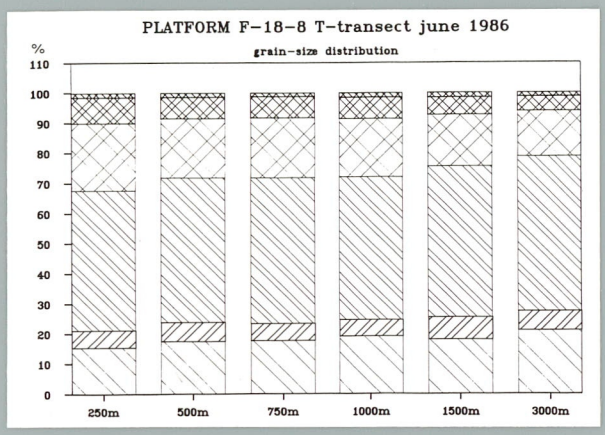
ashfreedryweight

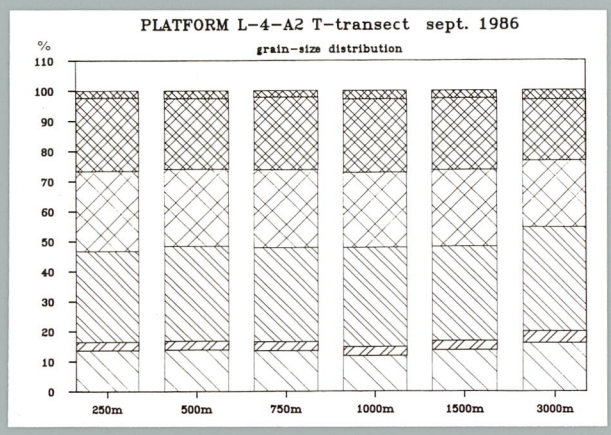
4 o/o 70/0

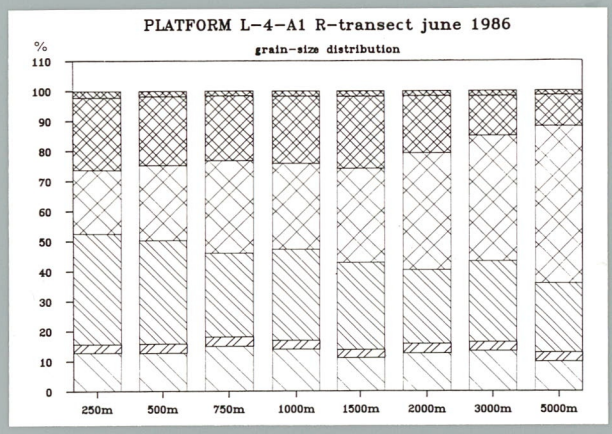
transport to laboratory
acclimatised during 12 hours in laboratory
clearance rate measurement
algae
inflow
outflow
mussel
O2-meter computer
O2-electrode
respiration and excretion
clearance rate measurement
stirrer
faeces measurement
ashfreedryweight

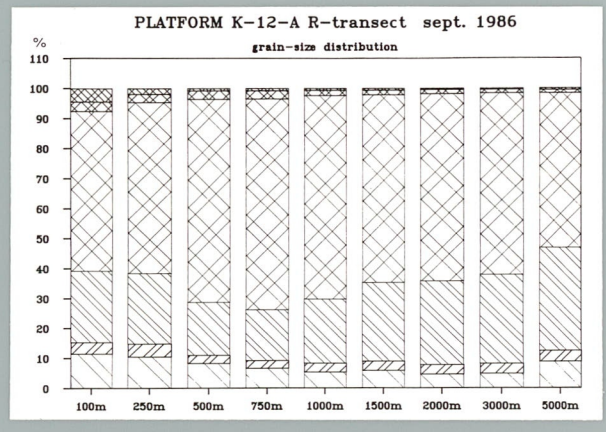

PLATFORM F-18-8 R-transect june 1986
grain-size distribution
%
100m 250m 500m 750m 1000m 1500m 2000m 3000m 5000m

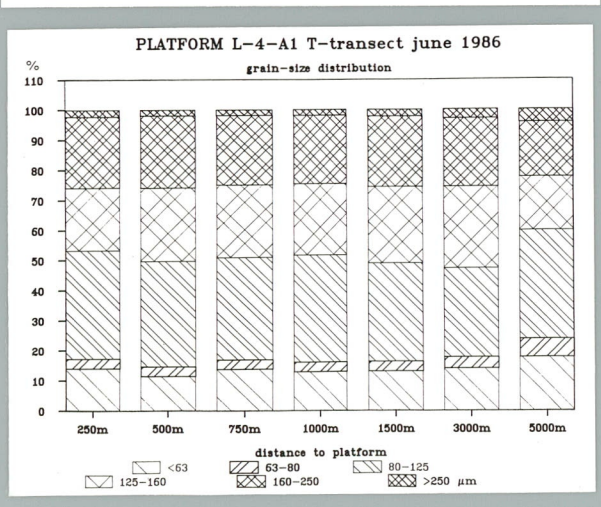

PLATFORM L-4-A2 R-transect sept. 1986
grain-size distribution
%
0m 100m 250m 500m 750m 1000m 1500m 2000m 3000m 5000m

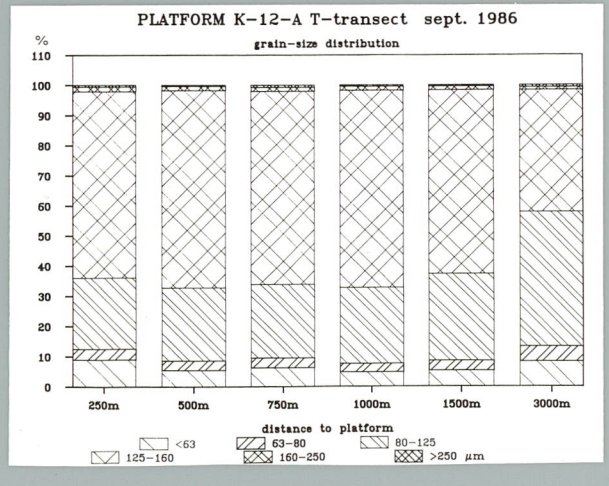

PLATFORM F-18-8 T-transect june 1986
grain-size distribution
%
250m 500m 750m 1000m 1500m 3000m


PLATFORM L-4-A2 T-transect sept. 1986
grain-size distribution
%
250m 500m 750m 1000m 1500m 3000m


PLATFORM L-4-A1 R-transect june 1986
grain-size distribution
%
250m 500m 750m 1000m 1500m 2000m 3000m 5000m


PLATFORM K-12-A R-transect sept. 1986
grain-size distribution
%
100m 250m 500m 750m 1000m 1500m 2000m 3000m 5000m


PLATFORM L-4-A1 T-transect june 1986
grain-size distribution
%
250m 500m 750m 1000m 1500m 3000m 5000m
distance to platform
<63 63-80 80-125
125-160 160-250 >250 µm


PLATFORM K-12-A T-transect sept. 1986
grain-size distribution
%
250m 500m 750m 1000m 1500m 3000m
distance to platform
<63 63-80 80-125
125-160 160-250 >250 µm

5
at/m h

5e

5a

5f

5b

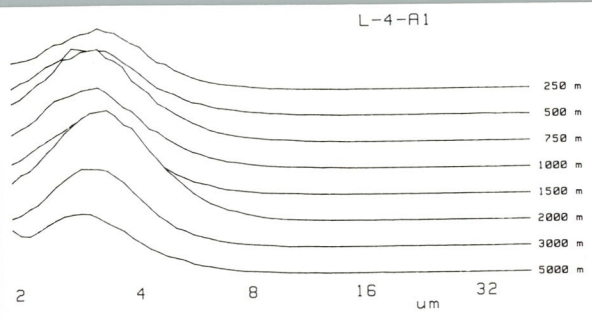
5g

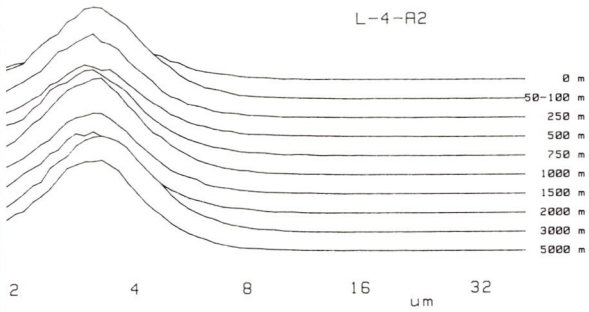
5c

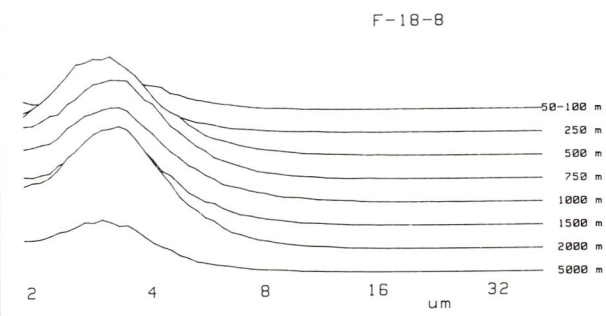
5h

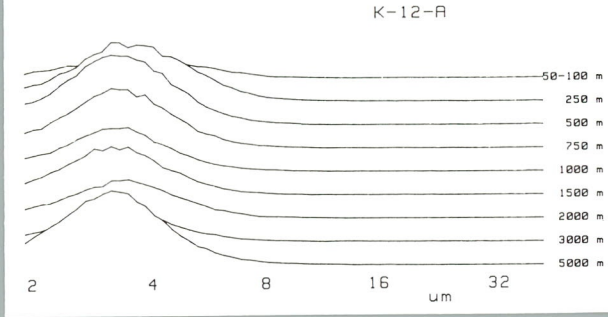
5d

R- transect

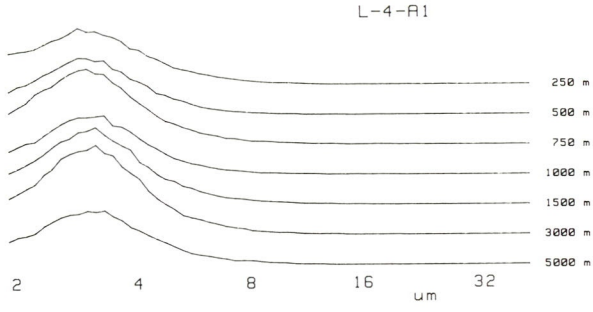


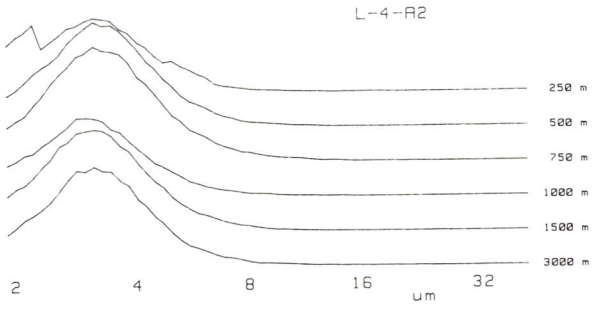


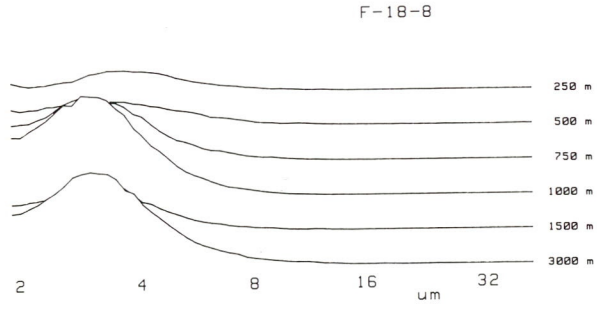


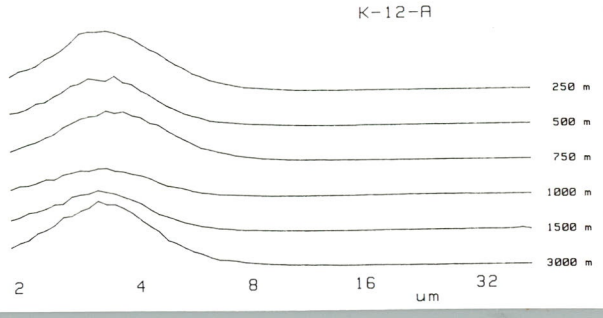


T- transect

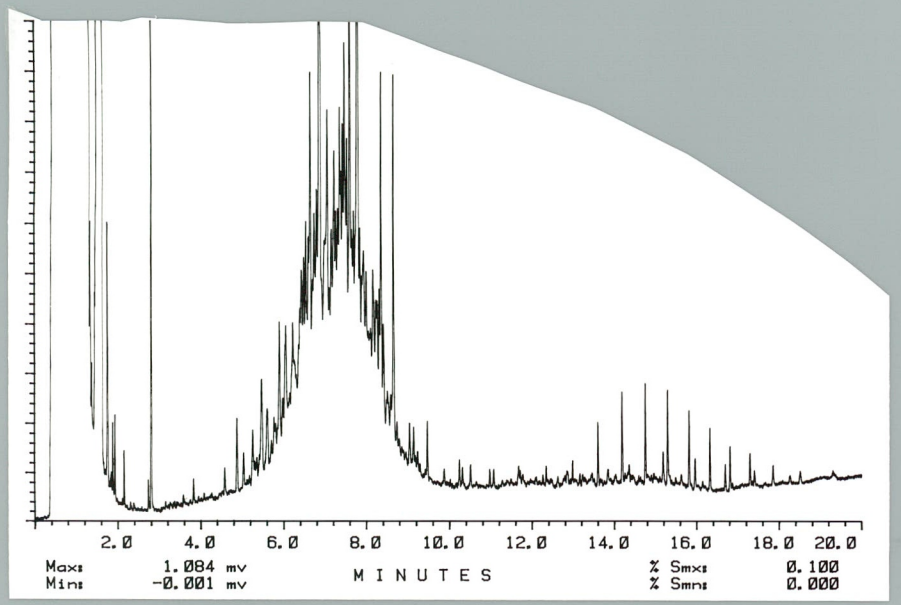




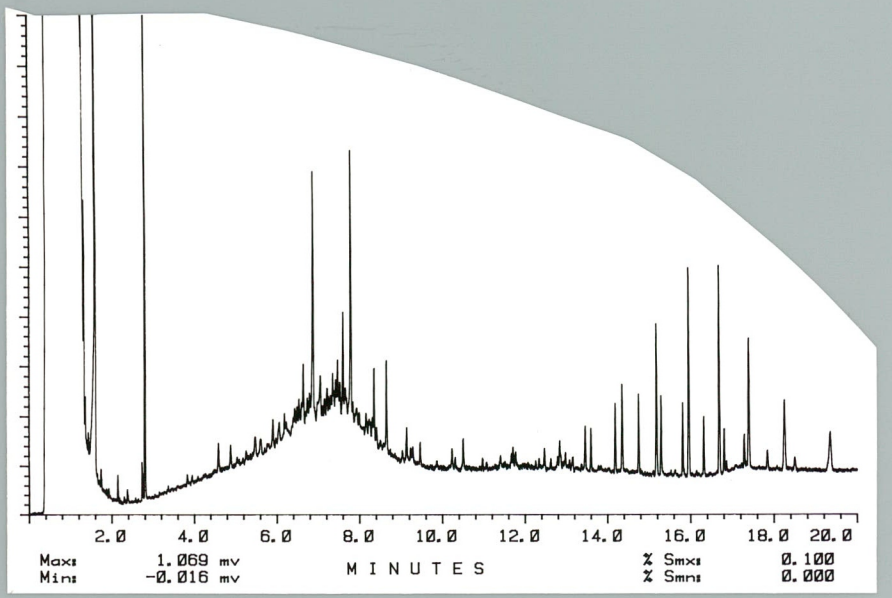




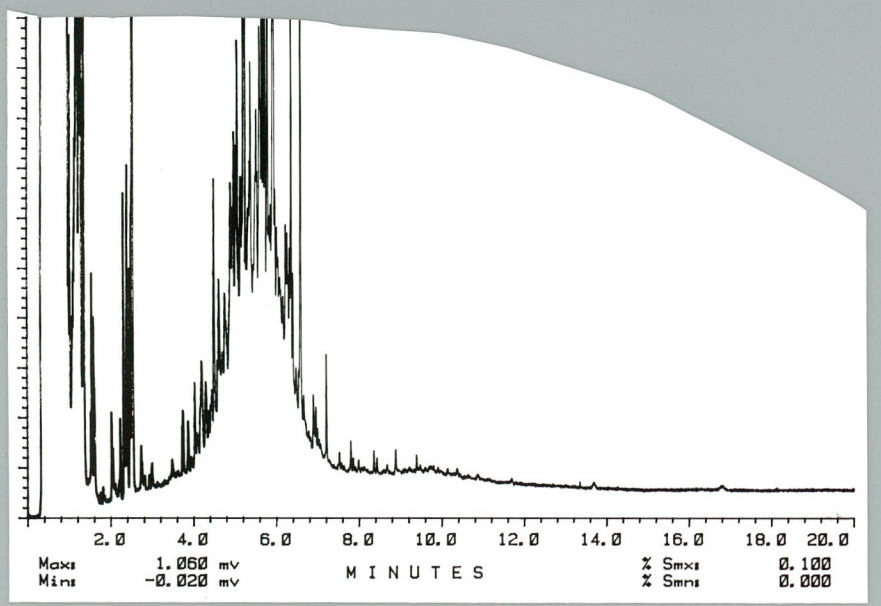
100 m R-transect



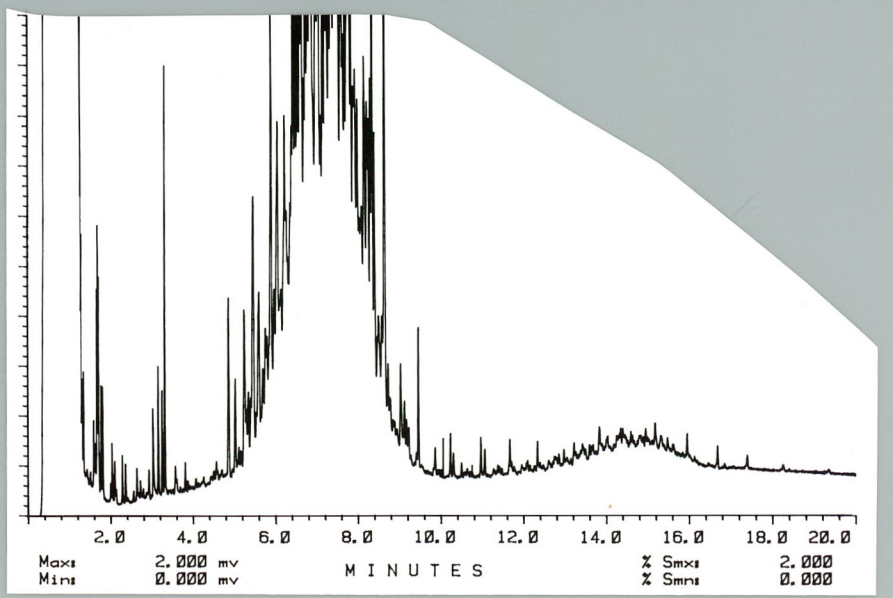
250 m R-transect



used OBM (500 x diluted)



0 m R-transect (100 x diluted)



135 → 150

18

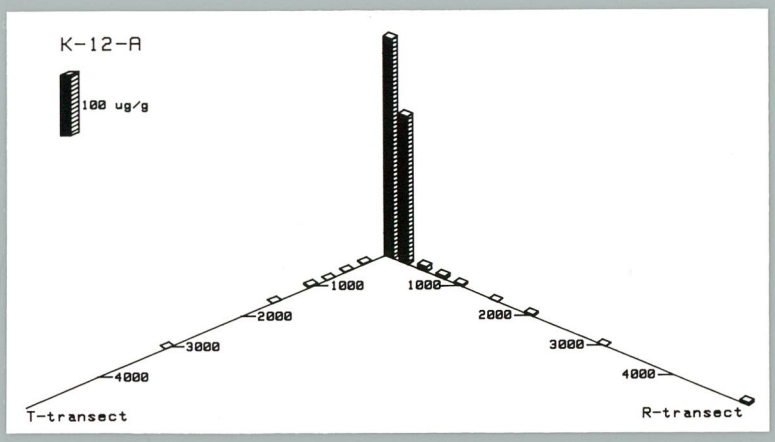
250m R-transect

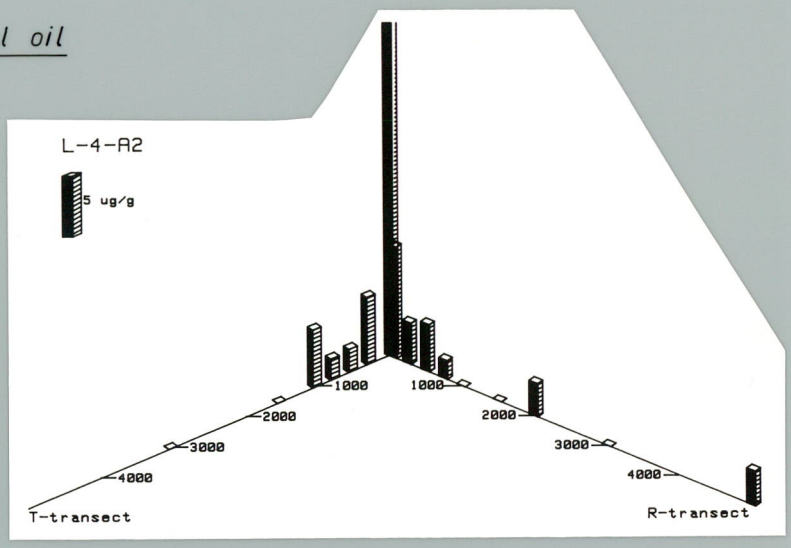
0m R-transect (100 × diluted)

100m R-transect

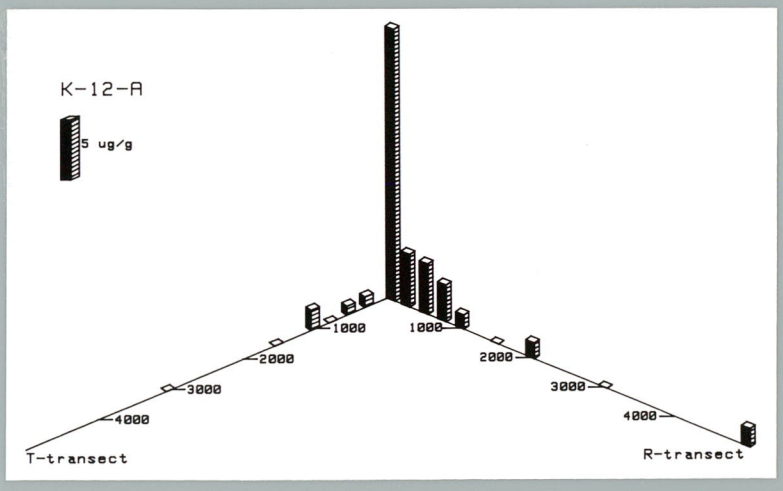
used OBW (200 × diluted)

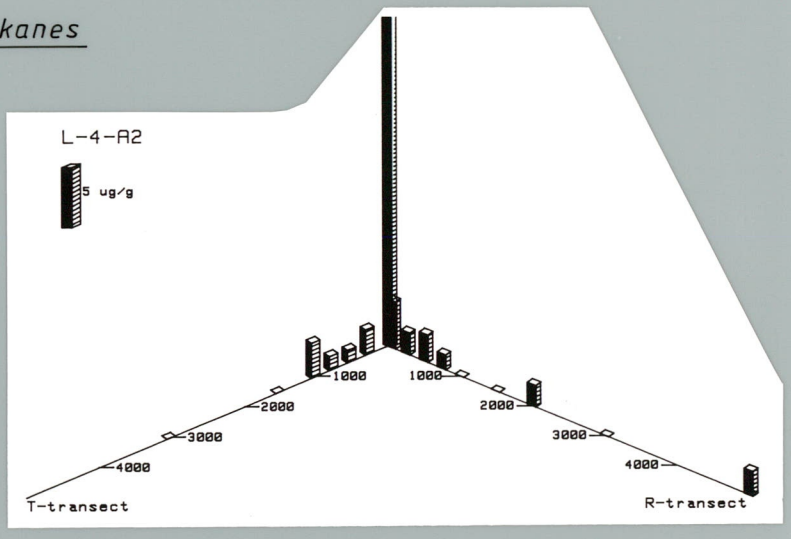
total oil



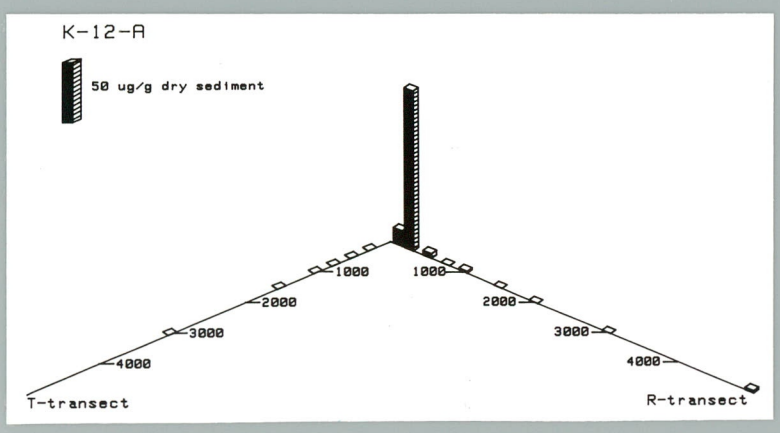


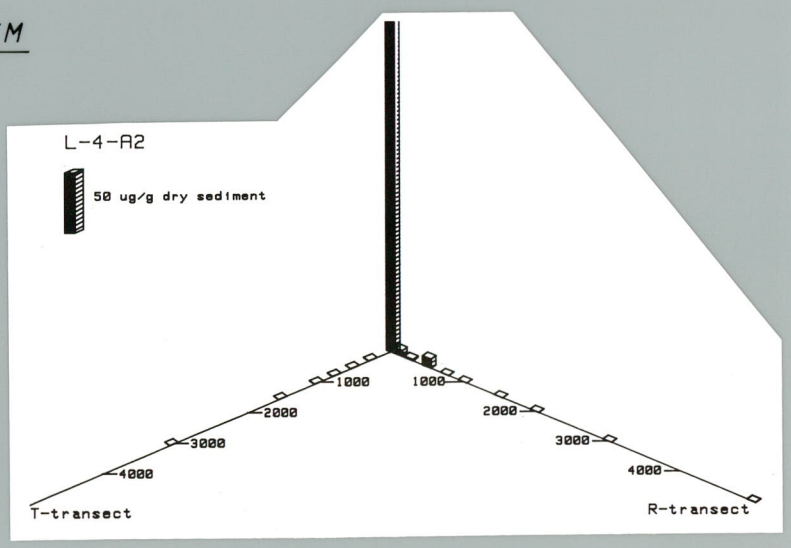
n-alkanes





UCM





199→160 9

total oil

oil total

g

oil total

g

n-alkanes

n-alkanes

g

n-alkanes

g

UCM

UCM

g

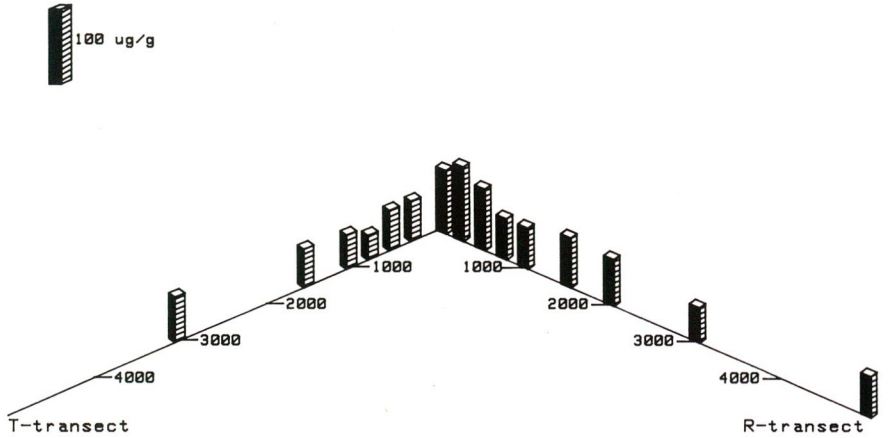
UCM

g

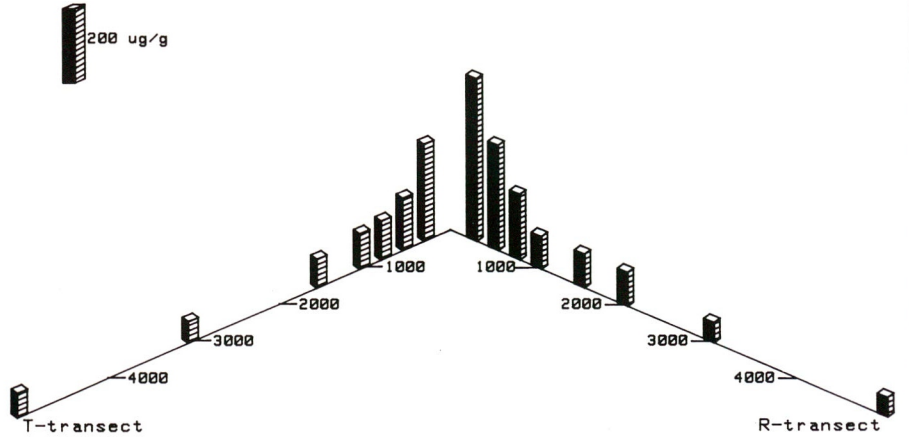
3

add → ppb

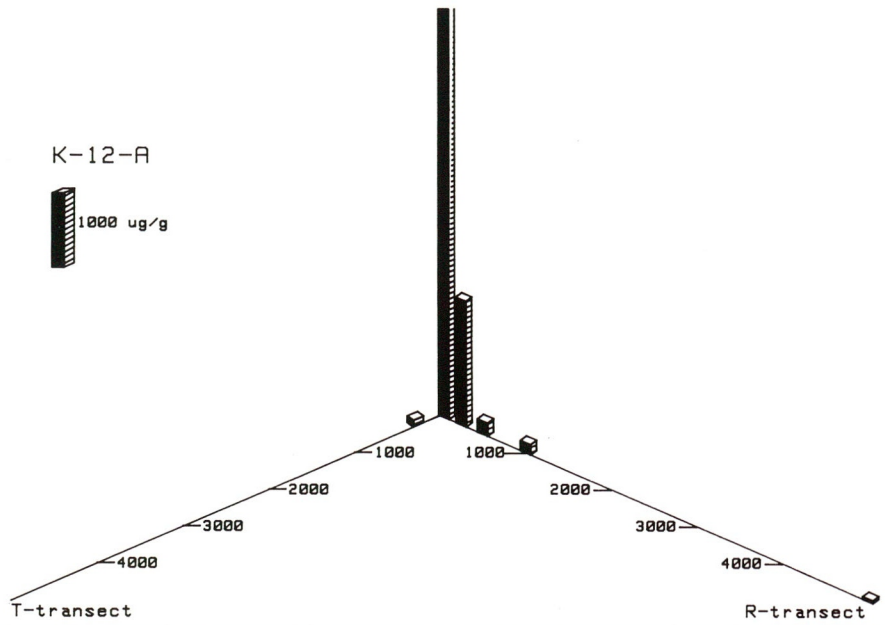
F-18-8



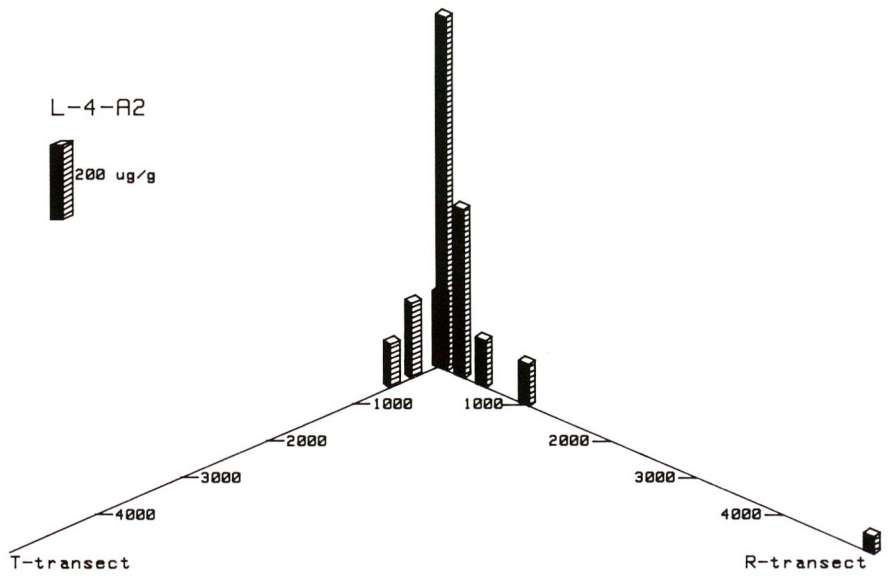
L-4-A1



K-12-A



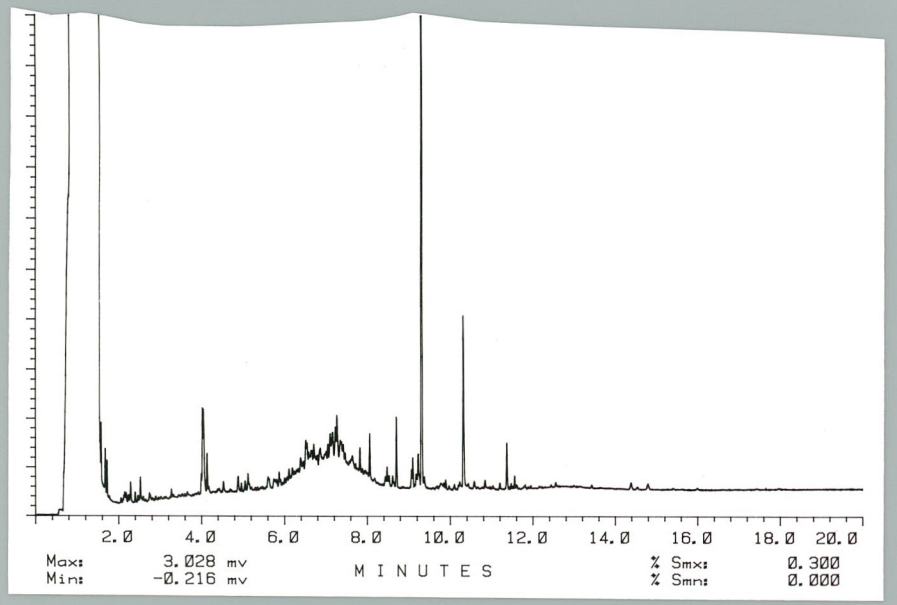
L-4-A2



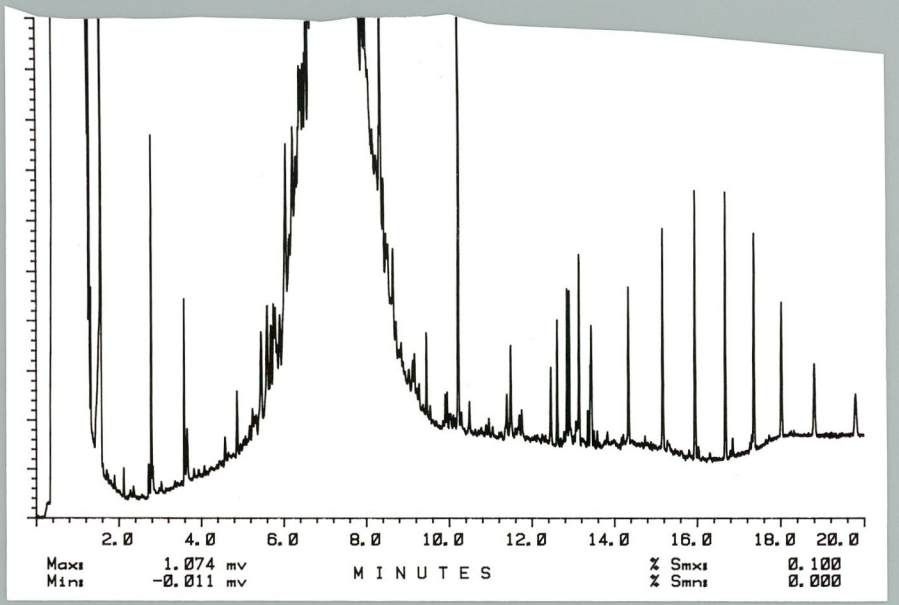
167→150 10

01 02174-FV1

2e ABM 0 m R-transect



1e ABM 250 m R-transect



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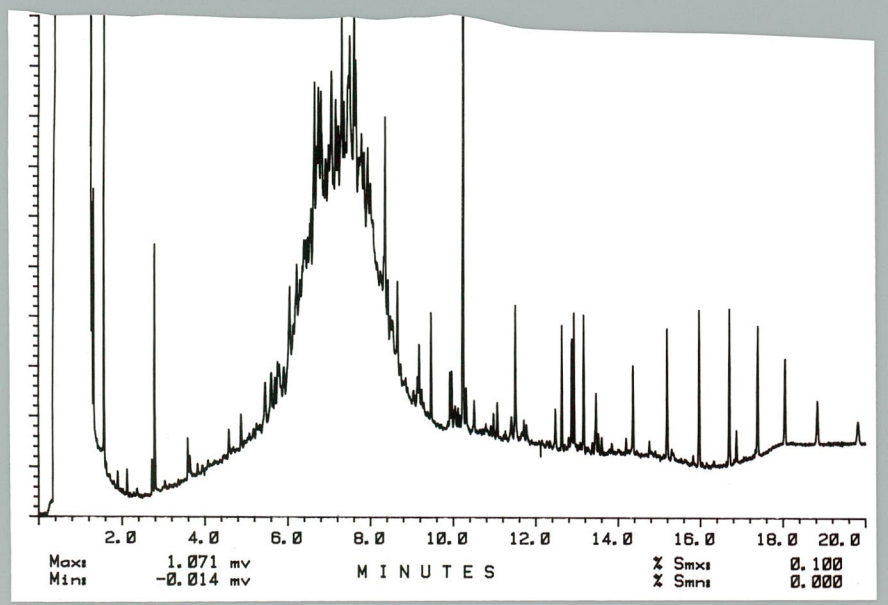
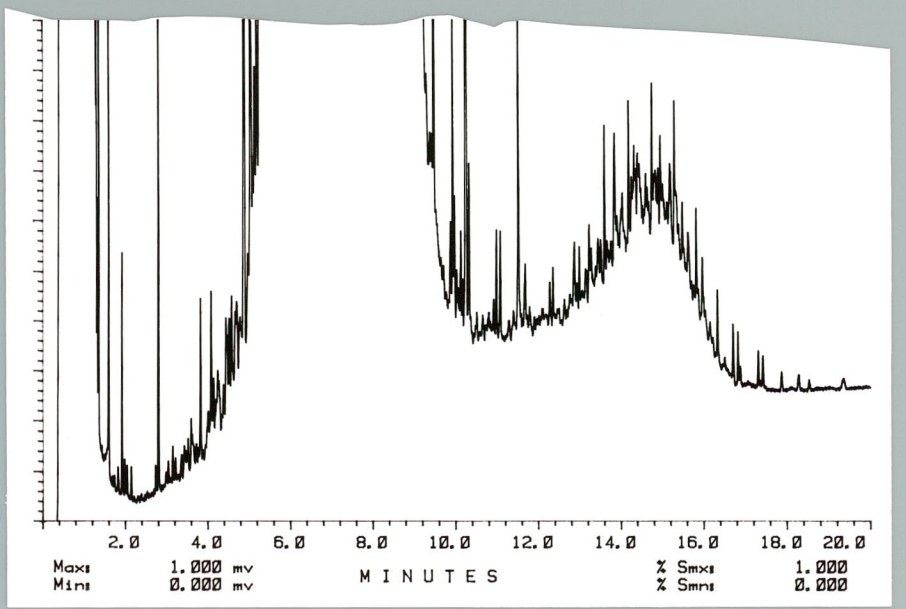


Wild sample 0m L-4-A



175 → 150

11

1e ABM 500m R-transect

Wild sample 0m L-4-A

2e ABM 0m R-transect

1e ABM 250m R-transect