INTERNATIONAL COUNCIL FOR THE
EXPLORATION OF THE SEA

ICES C.M. 1974/E : 13
Fisheries Improvement Committee

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Mia Kerkhoff, Netherlands Institute
for Fishery Investigations, Ymuiden,
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Netherlands Institute for Fishery Investigations,
Haringkade 1, Ymuiden, The Netherlands.

Introduction

In consequence of a ship-accident at Wemeldinge on December 11, 1973 80 tons of gas oil were spilt in the Oosterschelde. The oil pollution affected both water and sediment. The musselbeds were contaminated and mussels became unedible because of their oily taste.
The extent of the oil spread was investigated on December 21, 1973, by tasting mussels from several places (fig. 1). The highest concentration of oil was found near Wemeldinge, as could be expected. The oil did not only spread along the southern side of the Oosterschelde (Wemeldinge, Kattendijke, Zandkreek), but also to the northern side (Dortsman, Gouweveer) and over the Yerseke Bank.
No oil could be detected in mussels from the Hammen, the most western sampling place (table 1). Repetition of the investigation on January 2, 1974 showed about the same results, with the exception of mussels from Witte Tonne Vlije and the Brabantse Vaarwater, which also tasted of oil now. On January 11 the oil in the less polluted areas had virtually disappeared and only near Wemeldinge, the place of the accident, oil could be detected. At the end of that month the last traces of oil had disappeared and the normal harvesting of shellfish for the market could be resumed.
During and after the period of oil pollution we tried to connect both by organoleptical and by chemical means the effects observed in the mussels with the gas oil spill near Wemeldinge.

Organoleptical investigation

Introduction.

In an organoleptical test the impression obtained in the mouth is a total one, consisting of taste, tactile and olfactory sensations. The taste buds, located on the papillae of the tongue and the palate distinguish only four tastes: acid, bitter, salt and sweet. Most taste sensations are produced by a combination of taste and smell. The olfactory organ, situated in the upper portion of the nasal passage, forms the essential organ of smell and is of much more importance in perceiving the volatile flavour components in food than in the taste organs. For that reason the ability to taste oily products has to be associated with the appearance of volatile smell compounds in the oil.

During the refining process of crude oil the odour compounds are divided among the several distillate fractions. Light (gasoline) and heavy distillate fractions contain few, but the middle distillate fractions, like diesel oil, contain many of the smell compounds present in crude oil.
The nose is a very sensitive detector and can detect surprisingly low concentrations of mineral oil. Even 0.0005 ppm diesel oil in water can be detected, this in contrast to heavy fuel and crude oils, containing fewer odour compounds, which are only detected at 0.2-25 ppm (1).
Gas oil is a middle distillate fraction with many volatile smell compounds. Therefore human senses are able to detect very low concentrations of gas oil.

Methods and results

Since boiling made the smell more obvious, the presence of gas oil was determined by tasting boiled mussels.
In order to estimate the sensitivity of the organoleptical test clean mussels from unaffected areas were treated with gas oil-pentane solutions of different concentrations. We injected each mussel with 1 µl of a gas oil solution, after which the presence of oil was determined by tasting the boiled mussels. If 40 µg or more was added to a mussel of approximately 8 gr, an oily taste could be observed. At lower concentrations it was not possible to distinguish between the treated and untreated mussels. Therefore the organoleptically detectable level is about 5 ppm.

Chemical investigation

Introduction

Oil is a complex mixture of hydrocarbons and contains in addition small amounts of sulfur, oxygen and nitrogen compounds. Organoleptically the smell compounds were detected, but in the chemical investigation our attention was directed on the main constituent of mineral oil: the hydrocarbons.
Because of their great stability hydrocarbons are widespread in the environment. Most living organisms make their own hydrocarbons, which may spread through the environment. These biosynthesized hydrocarbons give problems in tracing hydrocarbon pollution. Fortunately, there are characteristic differences between the biosynthesized hydrocarbons and the hydrocarbons in mineral oils. Organisms produce a limited number of hydrocarbons such as pristane, 3, 6, 9, 12, 15, 18-heneicosahexaene and a few n-alkanes and n-alkenes (2). n-Alkanes with an odd number of carbon atoms predominate, although n-alkanes with an even carbon chain are present. The blue mussel (Mytilus edulis) contains approximately 1 mg of hydrocarbons per mussel (3). On the other hand oil contains a wide variety of hydrocarbons. Homologous series are present of n-alkanes and branched alkanes (isoprenoids). The ratio of odd to even carbon chains is about 1.0. In addition to the alkanes, cyclic and aromatic hydrocarbons are present, the latter being the most toxic fraction in oil pollution. Olefines are formed mainly in cracking processes. Gas oil is a destillate fraction of crude oil. The boiling range extends from 174-349°C and the n-alkanes ranges from dodecane, $C_{12}H_{26}$, to pentacosane $C_{25}H_{32}$.
Beside the distinction between natural and mineral oil hydrocarbons there are also other analytical problems, caused by physical, chemical and biochemical processes. During exposure to the air and seawater selective evaporation, dissolution, adsorption on

sediment, uptake by organisms, oxidation and microbial degradation may cause alterations in the chemical composition of the oil, which complicates the identification (4).

Fortunately in our investigation we observed no alterations in the chemical composition of the oil and therefore only corrections were necessary for the content of natural hydrocarbons.

Methods

Clean up

The flesh of 10 to 12 whole mussels, including the liquid, were combined for analysis. After homogenization in a Waring Blendor, the sample was ground with anhydrous sodium sulfate and extracted with n-pentane using a Soxhlet apparatus.
At first the extraction was carried out with a mixture of chloroform and methanol (3), but in this way it was not possible to avoid transesterification of lipid fractions and the extracts were contaminated with methylesters of fatty acids: e.g. methylpalmitate. Therefore n-pentane was used later, since interfering compounds were produced in a lesser degree with this solvent.

Column chromatography was used to separate hydrocarbons from lipids. The extracts were concentrated on a rotary film evaporator and transferred to a column (20 mm i.d.) of 15 gr. Al_2O_3 over 15 gr silica (both activated at 150°C and then partly deactivated with 5% (w,w) water. The hydrocarbons were eluted from the column using four column-volumes of n-pentane (5). After solvent evaporation the residues were redissolved in CCl_4 and the hydrocarbons could be analyzed.

Detection

The equipment used was a Packard/Becker gaschromatograph, type 419, provided with a flame ionization detector, F.I.D. The injection port was kept at 220°C, the detector at 250°C. The hydrogen and air velocity were respectively 30 ml/min. and 300 ml/min. A stainless steel column of 1,5 m length and 1/8" o.d. packed with 10% SE-30 on Gas-Chrom Q (80-100 mesh) was used at isothermal conditions (160°C) with carrier gas N_2 at a flow rate of 30 ml/min.
No method of detection available at the moment provides an accurate assessment of total oil concentration. The gas oil content could be estimated by comparing the sum of the peak areas of n- $C_{16}H_{34}$, n-$C_{17}H_{36}$ and n-$C_{18}H_{38}$ from the sample with the sum of the corresponding peak areas from the standard gas oil (Calpam), analyzed under the same conditions (6).
The peak areas were obtained by electronic integration.

Gas oil is composed of hydrocarbons in the carbon range C_{12} to C_{25}. The naturally occuring hydrocarbons in blue mussels are ranging from C_{16} to C_{26} (3), and therefore cause serious interferences.
So corrections are necessary and the method becomes less sensitive. Nevertheless it was possible to detect 20 mg gas oil per kg musselflesh.

The extracts were further analyzed by combined GC-MS on a Finnigan-9500 gaschromatograph coupled through a Cohlke separator to the Finnigan-3000 Quadrupole mass spectrometer. A glass column (1,8 m, 1/8" i.d.) packed with 3% OV-1 on Varaport 30 (80-100 mesh) was used. The carrier gas was He. The oven temperature was programmed from 110°C to 220°C (at 6°C/min.).

Results

Samples of sediment, affected and unaffected mussels were subjected to the extraction and clean up procedure, and then quantified by GLC. Although gaschromatography does not completely resolve all the many thousands of hydrocarbons, the unresolved complex signal gives much information about the composition of a hydrocarbon mixture.

The chromatogram of unaffected Wadden Sea mussels shows the presence of hydrocarbons, which occur naturally in the mussel (Mytilus edulis). On comparing this chromatogram with that of the polluted mussels from the Oosterschelde, it is obvious that in the latter many peaks are present, which do not occur in the former. In the chromatogram of sediment similar peaks are present. These peaks show a close relationship in retention indices and relative peak heights with those of a commercial gas oil (Calpam) (fig. 2).

In order to confirm the similarities of the hydrocarbons in Oosterschelde mussel extract and Calpam gas oil GLC-MS analysis was carried out (7). Total ion current chromatograms (fig. 3) and mass spectra (fig. 4) were obtained. The peaks in the chromatograms of mussel extract and gas oil with the same retention times gave very similar mass spectra. One example is given in fig. 4. The parent peak is m/e 254 ($C_{18}H_{38}$). The mass spectrum is characterized by groups of peaks spaced 14 mass units apart (corresponding to a difference of CH_2), of which the relative abundance decreases fairly regularly with increasing m/e ratio above $C_4H_9^+$ (m/e 57). The compound involved is n-octadecane. Peaks also occur two mass units below those associated with saturated carbonium ions C_nH_{2n+1} (27 below 29, 41 below 43, 55 below 57). They are due to alkenyl cations and are found in the spectra of most compounds containing long hydrocarbon chains. The other mass spectra demonstrate the presence of other n-alkanes: n-pentadecane, n-hexadecane, n-heptadecane, n-nonadecane, n-eicosane and n-heneicosane.

In all mass spectra peaks appear at m/e 91, 105, 119 and 133. The fragment at m/e 91 is the resonance stabilized tropilium ion ($C_7H_7^+$) and the other peaks come from homologous fragments (C_nH_{2n-7}) with intervals of 14 mass units. The fragmentation of alkylbenzenes gives rise to these ions (8) and so the mass spectrum of a complexly composed GLC peak demonstrates not only the presence of a n-alkane, but also the presence of aromatic hydrocarbons in polluted mussels.

The total ion current chromatogram of Oosterschelde mussel extract (fig. 3) shows the homologous serie of n-alkanes from n-tridecane, $C_{13}H_{28}$, to n-penteicosane, $C_{25}H_{52}$. Gas oil contains hydrocarbons in the carbon number range C_{12} to C_{25}, while for other distillate fractions of oil the ranges are different: gasoline extends from C_5 to C_{10}, kerosene from C_{10} to C_{14} and heavy distillate oil from C_{20} to C_{40}. A further indication of the presence of gas oil is the fairly equal distribution of odd and even numbered n-alkanes and the presence of other homologous series.

Mass spectra as well as gaschromatographic data confirm the organoleptical results: the mussels were polluted by gas oil.

The mussels probably took up the hydrocarbons by filtering the polluted water. Lee (3) observed that 10 to 15 mg of mineral oil was taken up by one mussel (Mytilus edulis) within 2 days and other authors found that as little as 0,01 ppm oil in the water would give an oily taste to some species of fish and shellfish (1,9).

Elimination of gas oil by mussel

In order to get information on the persistence of gas oil in the shellfish, polluted mussels were transferred into clean sea water with an average temperature of 4°C. Every day samples were taken and investigated both chemically and organoleptically. In the beginning 200 mg gas oil per kg (ppm) musselflesh was present. After one day of exposure to clean sea water only 20 to 30 ppm of oil could be detected and after three days distinction from the natural hydrocarbons was no longer possible (fig. 5).
The rate of excretion seems to be extremely fast: 90% of the oil has disappeared in one day (3). However, the oily tinge in the mussels was maintained during one to two months (10). Obviously the compounds of gas oil detected by GLC (hydrocarbons) could be eliminated rapidly, but those who cause an oily taste in mussels are retained longer.

Discussion

Comparing the organoleptical test and the gaschromatographic method it cannot be stated, that the former (detection limit: 5 ppm) is more sensitive than the latter (detection limit: 20 ppm), since completely different compounds are involved. The methods used here and most methods used by other investigators give only partial information. Only when all toxic compounds and those, giving an oily taste are identified and individually detectable a reasonable determination of the amounts of oil can be made. Untill then we can only describe the situation in terms of certain indicator compounds or groups of compounds.

Summary

In December 1973 the Oosterschelde estuary was polluted by 80 tons of gas oil. Mussels became inedible because of their oily taste. The gas oil was identified by gaschromatography and mass spectrometry in extracts of mussels and sediment. After transferring the mussels to clean sea water, they lost 90% of the gas oil in one day, but the oily taste persisted for one to two months.
The detection limits of the organoleptic and gaschromatographic methods are respectively 5 and 20 ppm.

Acknowledgement

I wish to thank Ir. H. Cats of the Keuringsdienst van Waren, Rotterdam for permission to use the gaschromatography/mass spectrometry system and Mrs. V. Schröder for her assistance in the service of this apparatus.

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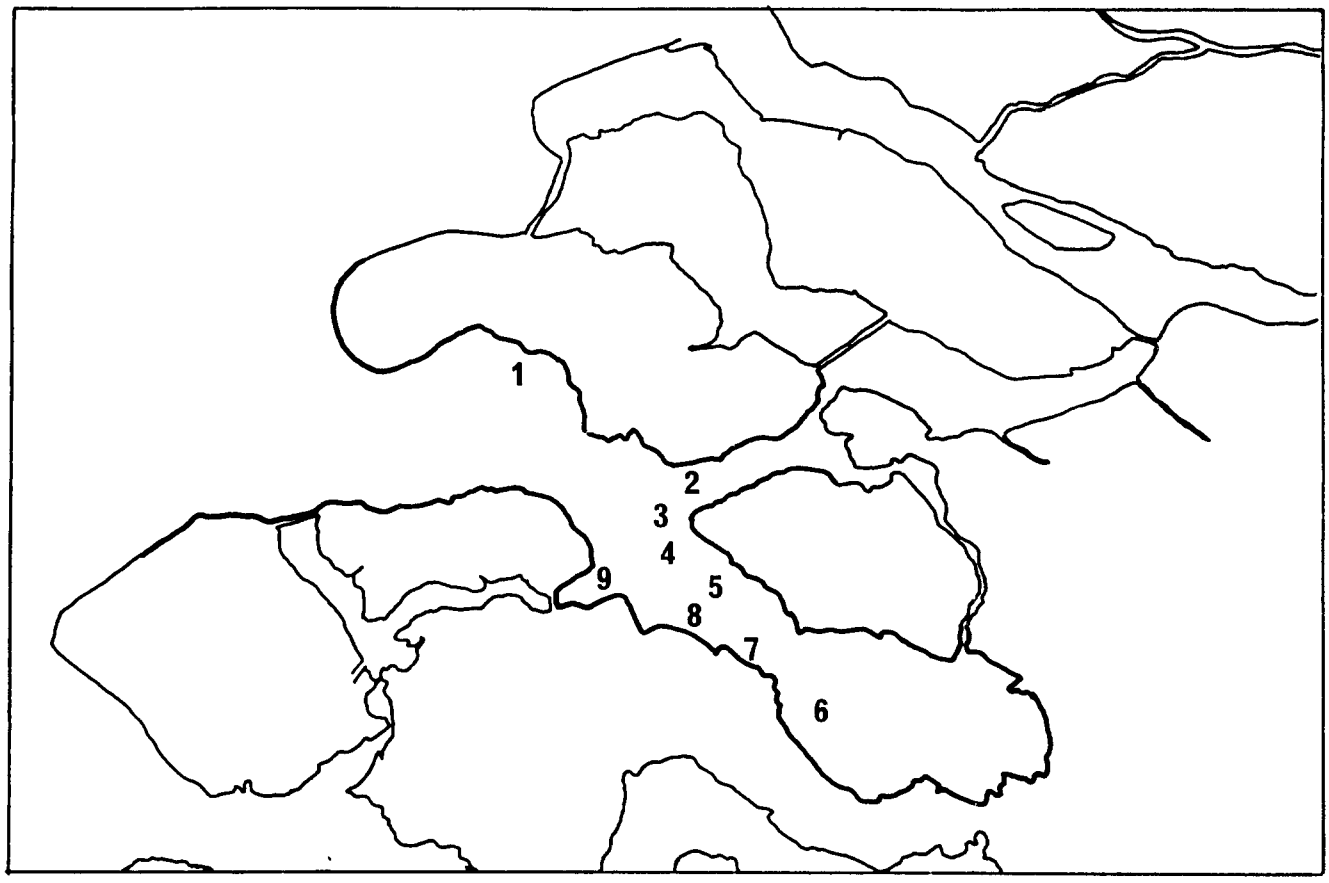

Fig.1 The Oosterschelde with several sampling places.

Tabel 1 The results of the organoleptical investigations.

		21/12	2/1	11/1
1	Hammen	−	−	−
2	Gouwe Veer	+	±	−
3	Witte Tonne Vlije	−	+	−
4	Brabantse Vaarwater	−	+	±
5	Dortsman	+	+	±
6	Yerseke Bank	+	±	±
7	Wemeldinge	+ +		+
8	Kattendijke	+	+	+
9	Zandkreek	+	+	+

\+ oil present.
± oil presence doubtful.
\- no oil detectable.

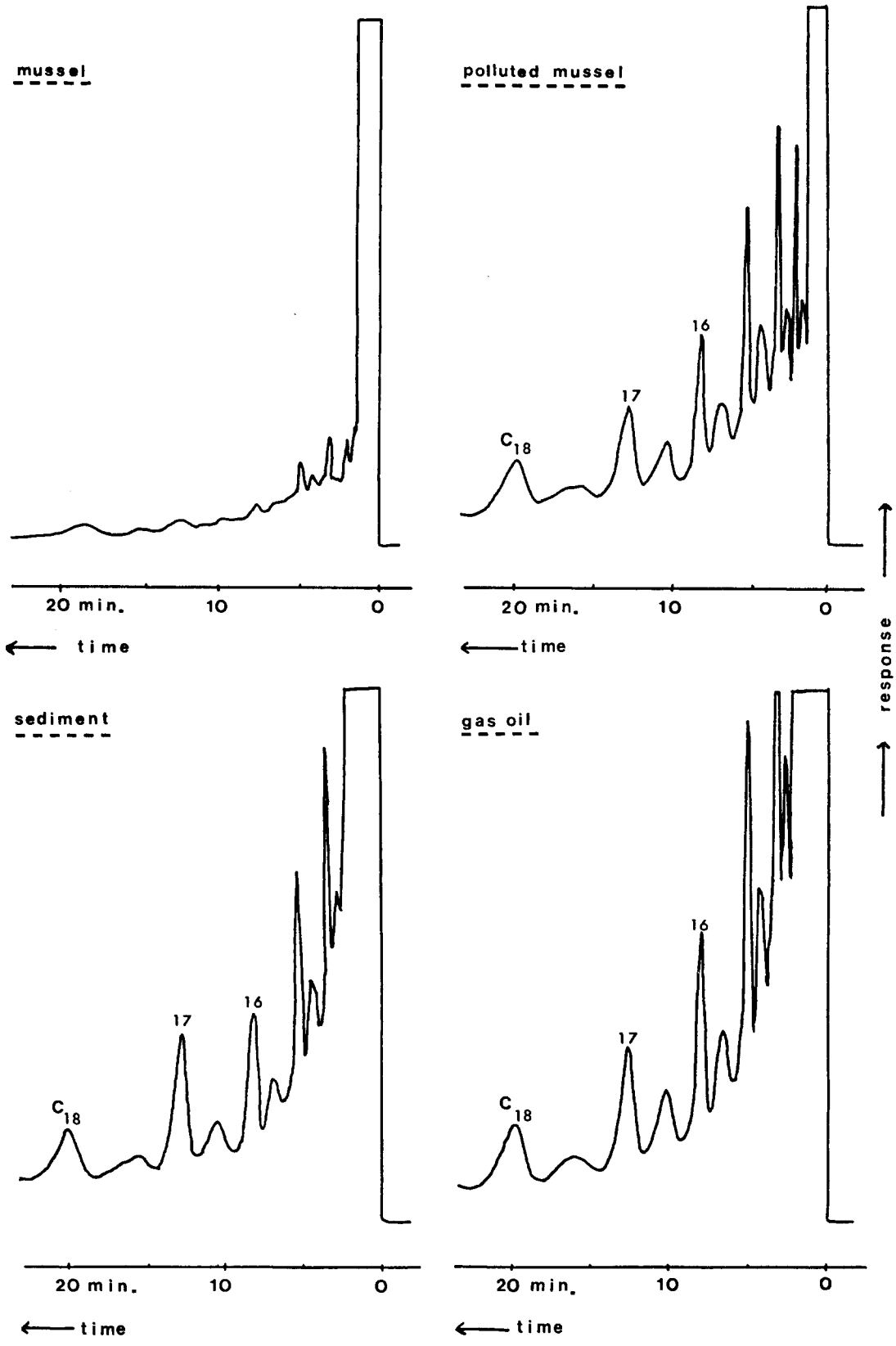

Fig. 2 Gaschromatograms of (Wadden Sea) mussels, polluted (Oosterschelde) mussels, sediment and gas oil standard (Calpam). Operating conditions are given in the text.

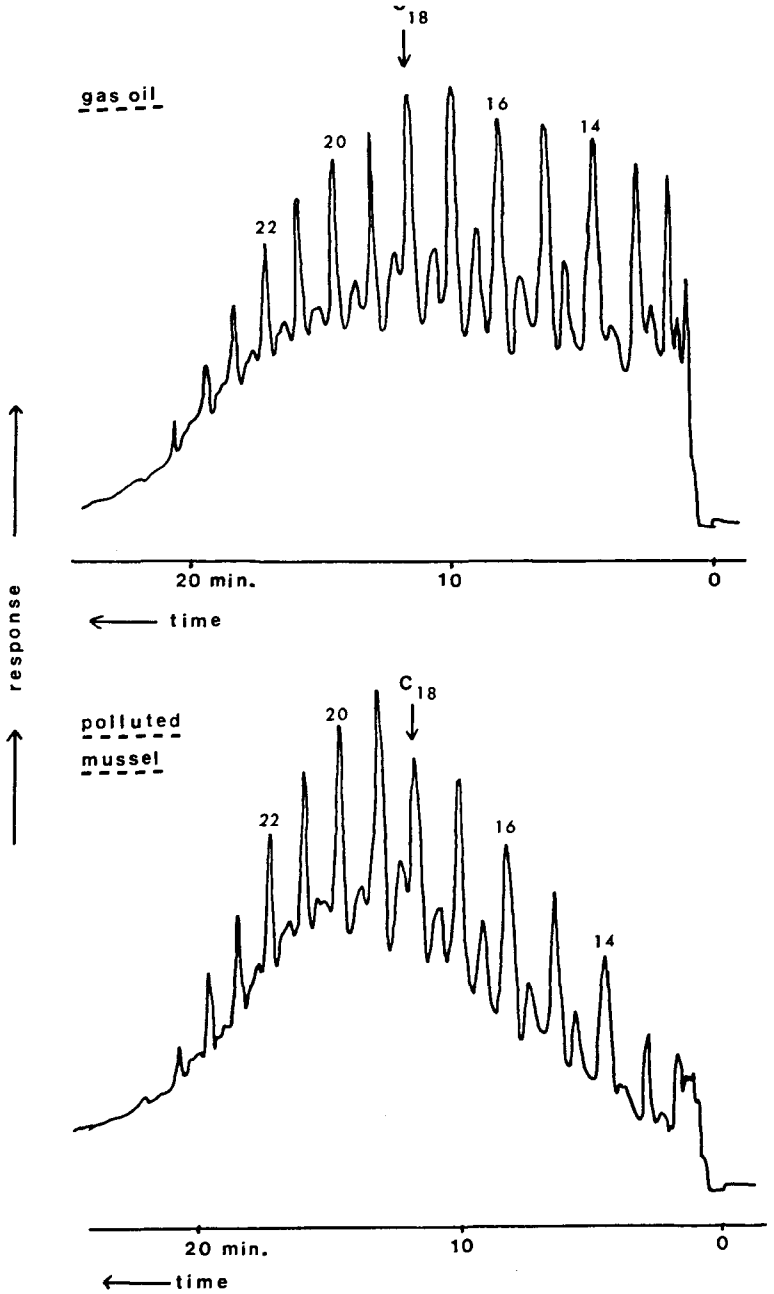

Fig. 3 Total ion current chromatograms of polluted mussels and gas oil standard (Calpam)
Operating conditions are given in the text.

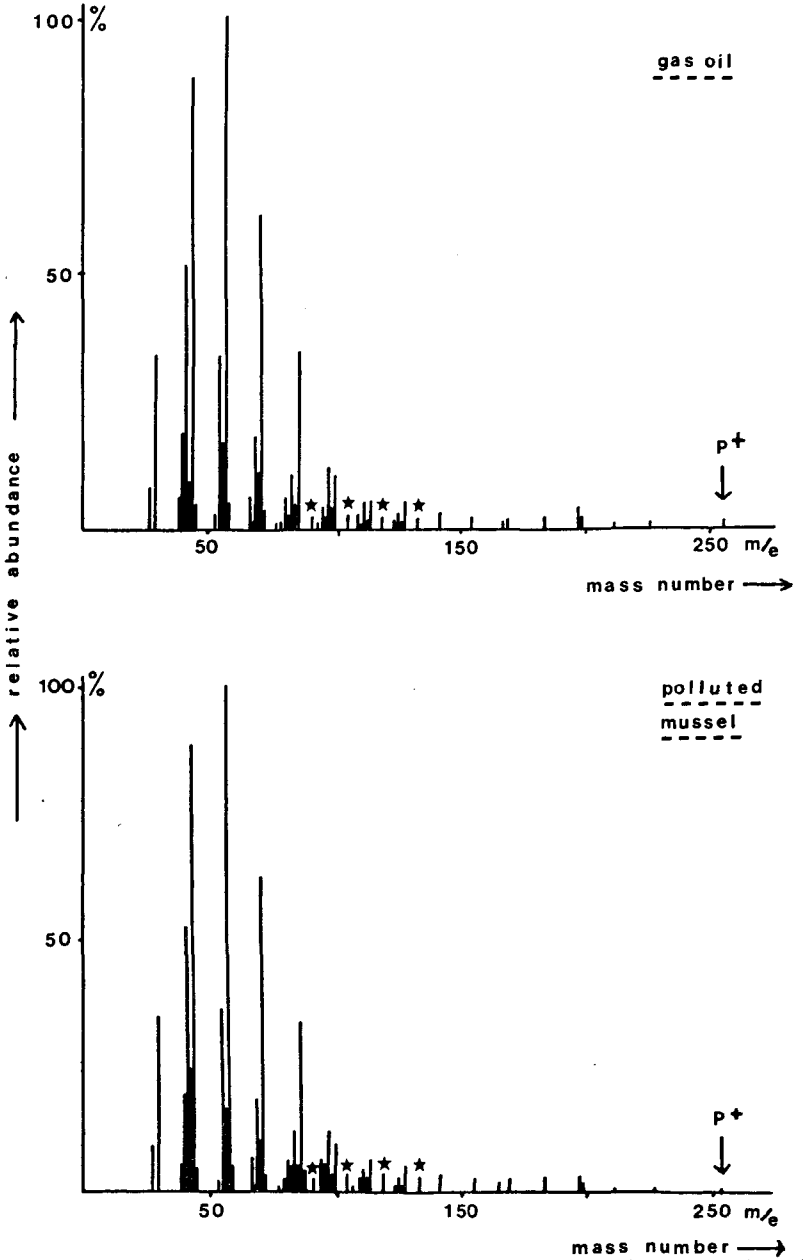

Fig. 4 Mass spectra of the peak C_{18} in the chromatogram of polluted mussels and gas oil standard (Calpam): n-octadecane and alkylbenzenes (*) are present. Operating conditions are given in the text.

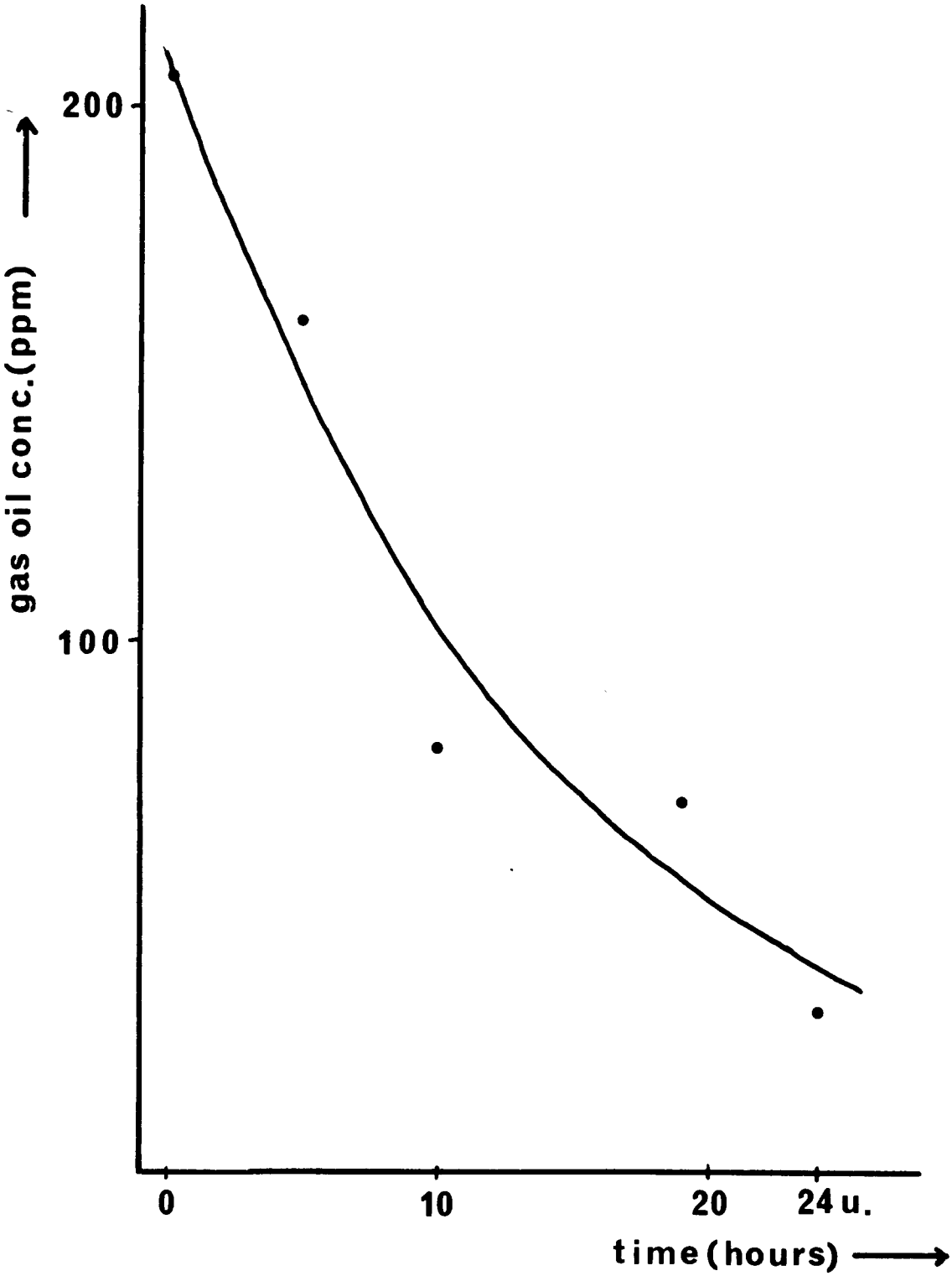

Fig. 5 The discharge of the gas oil concentration in polluted mussels transferred to clean sea water.