

FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Work-

shop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

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A COMPARISON OF HYDROCARBONS IN ANIMALS AND THEIR BENTHIC HABITATS

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Studies in several estuarine and marsh areas have shown significant differences between the composition of petroleum hydrocarbons in benthic animals and their habitat. When subjected to an oil spill some animals take up a mixture of petroleum hydrocarbons with a composition similar to that of the spilled oil found in the substratum. Others develop the ability to metabolize or otherwise modify their hydrocarbon content possibly by selective uptake such that it approaches the pre-spill conditions.

When sediments are pulsed with petroleum hydrocarbons from an oil spill, degradation occurs at rates which are significantly different for different classes of compounds. Some classes persist for more than five years in significantly elevated concentrations. In contrast, biogenic hydrocarbons in relatively unpolluted sediments seem to be subjected to little degradation over 10 to 50 year periods after deposition.

INTRODUCTION

In the course of studies of natural and petroleum hydrocarbons in marine systems, we have observed significant differences between the compounds in benthic animals and the sediments on which they live and feed. We are interested in these differences for both biological and geochemical reasons. From the geochemical point of view we would like to know how the uptake of hydrocarbons from sediments or particulate matter influences the distribution of those compounds within the marine environment. How are hydrocarbons fractionated between organism and environment, and how long are they retained? To what extent are they modified by metabolism within the animal and in what forms are they released? Is the release primarily to the water or is it also directly to the sediment with faeces? There are obviously another series of questions that centre both about the rôles hydrocarbons play within the animals once they have been picked up from the sediment and, about the mechanisms by which they are accumulated.

In this paper we are concerned only with preliminary aspects of the problem: the differences we find in composition and concentrations of hydrocarbons between animals and sediments. We have looked at three different types of environments. First, coastal sediments where no known major oil spills have reached the bottom. These are the intertidal sediments of Sippewissett salt marsh and a muddy bottom about 15 m deep in neighbouring Buzzards Bay. Second, sediments from areas subject to chronic inputs of low levels of petroleum hydrocarbons from urban areas. These are Narragansett Bay which is chronically polluted in the mid-

bay region (Farrington and Quinn, 1973) where our sample was taken, and salt marshes in Quincy, Mass. The third set of samples comes from Wild Harbor river salt marsh which was pulsed with No. 2 fuel oil in a spill from a barge in September, 1969 (Blumer et al., 1970; Burns and Teal, 1971).

METHODS

Animal samples were collected by hand, clean dredge or in metal fish traps set unbaited for about one hour. Cores from intertidal areas were collected by hand in pre-washed stainless steel corers or in plastic core liners. Subtidal cores were taken with a 21 cm diameter sphincter corer with a fiberglass barrel. Where plastic was used the sediment that contacted liner or barrel was scraped off to avoid contamination. We obtained some surface and deep samples without measurable petroleum content indicating that the precautions taken were adequate.

Extractions were done by Soxhlet with methanol or methanol/benzene, partitioning into pentane, column chromatography on alumina over silica gel, and analysis by GLC. Methods have been given in detail in Farrington et al. (1973); Farrington and Quinn (1973); Farrington and Medeiros (1975); Farrington and Tripp (1975); and Burns and Teal (1973).

Most of the results in this paper deal only with the first fraction of hydrocarbons that are eluted with one column volume of pentane. These include the saturated alkanes and cycloalkanes, some alkenes and cycloalkenes, and exclude all but traces of the aromatic components.

Table 19. Comparison of alkanes found in cores from salt marsh and bay sediments in Massachusetts

Depth (cm)	n -alkanes 10^{-9} g/g dry wt												10^{-6} g/g dry wt			R/UCM	CPI
	20	21	22	23	24	25	26	27	28	29	30	31	Unres. complex mixture	calc	wt		
	Salt marsh																
0	90	430	280	1400	1700	6200	3600	4100	300	2400	200	690	6	30	88	3.9	2.2
25-30	72	645	246	1556	<100	950	440	2640	1800	4840	1360	950	28	73	81	1.6	2.8
45-50	54	250	100	1500	98	540	180	1100	490	2900	1500	1100	13	37	64	1.9	2.9
60-65	20	131	84	510	280	990	200	510	160	690	56	42	3.8	9.3	30	1.5	3.6
75-80	160	280	140	900	380	2500	350	1000	440	1800	310	470	8.6	18	56	1.1	4.0
	Buzzards Bay																
0-2	-	-	69	228	180	300	150	410	182	741	136	608	60	-	-	-	2.9
2-4	-	-	42	220	126	289	150	520	184	975	189	706	55	-	-	-	3.5
70-72	-	-	39	150	86	258	171	430	146	655	113	645	10	-	-	-	6.7
0-3	-	-	-	92	60	167	81	399	111	1070	58	584	15	-	-	-	3.0
39-43	-	-	-	71	35	123	72	400	103	1250	88	595	5	-	-	-	4.1

RESULTS

Both the absolute and relative amounts of n -alkanes in the unpolluted marsh and Buzzards Bay sediments are remarkably constant with depth, and also quite similar to each other (Table 19). Throughout the sam-

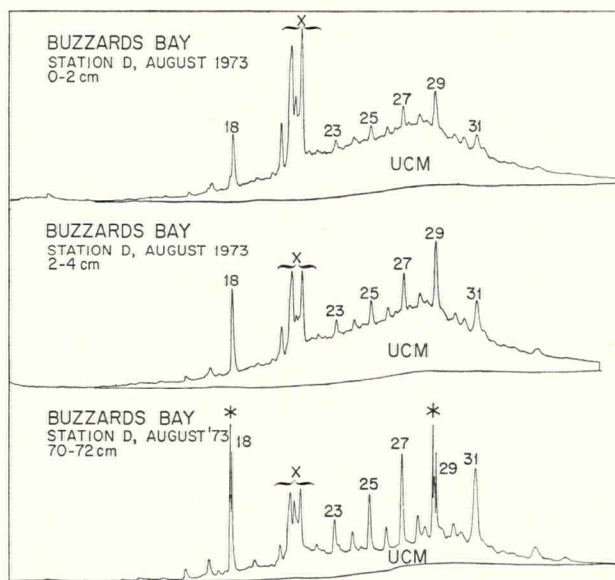


Figure 62. Gas chromatographs of alkanes, cycloalkanes, alkenes and cycloalkenes from sections of a sediment core from Station D, Buzzards Bay, Mass. $41^{\circ}32.2' N$, $70^{\circ}45.1' W$, August, 1973. SCOT OV-101 (Perkin Elmer Corp.) column with 15 000 theoretical plates temperature programmed from $75^{\circ} C$ to $275^{\circ} C$ at $6^{\circ}/min$, held at $275^{\circ} C$ until $n-C_{33}$ eluted. He carrier gas flow 4-6 ml/minute. 23, 25, 27—designate the number of carbon atoms in n -alkanes eluting at these peaks. 18- n -octadecane is an internal standard used to quantify the hydrocarbons. X designates a group of alkenes and cycloalkenes of unknown structure. UCM unresolved complex mixture. *automatic attenuation of peak.

ples, natural i.e. biogenic, odd chain hydrocarbons from marsh grasses predominate over even chain lengths. There is a slight indication of a relative loss in the longer chain n -alkanes with depth in cores that have been extracted with methanol, as were those in Table 19. When benzene extraction is used this relative diminution of the longer chains is reduced or disappears.

There is an indication of pollution hydrocarbons in the upper layers of the Buzzards Bay core as shown by the relatively large amount of the unresolved complex mixture (UCM) (Fig. 62), but this seems to have had little effect on the n -alkane distribution shown in the table. Gas chromatographs of petroleum and some petroleum products typically display this UCM. In our procedures, this is a mixture of naphthenes (Farrington and Quinn, 1973; Blumer et al., 1970).

The surface samples from Wild Harbour marsh pulsed with fuel oil show a regular change with time (Table 20). The n -alkanes disappeared most rapidly, as indicated by the decrease in n -alkane/isoprenoid ratio. By 3.5 years after the spill most of the straight chain

Table 20. Concentration of hydrocarbons extracted from surface sediments in Wild Harbor marsh after No. 2 fuel oil spill in Sept. 1969 (10^{-6} g/g dry weight)

Sample	Total by weighing	Total calc. from GLC	n -Alkanes isoprenoid	Phytane background
No. 2 fuel oil.....			2.7	1.5
Jan 1971.....	-	2 700	1.7	1.1
Jul 1972.....	2 045	1 960	0.6	1.2
Jul 1972.....	1 189	911	0.4	1.5
May 1973.....	2 660	-	0.1	0.7
Nov 1973.....	2 460	2 438	0.0	0.1

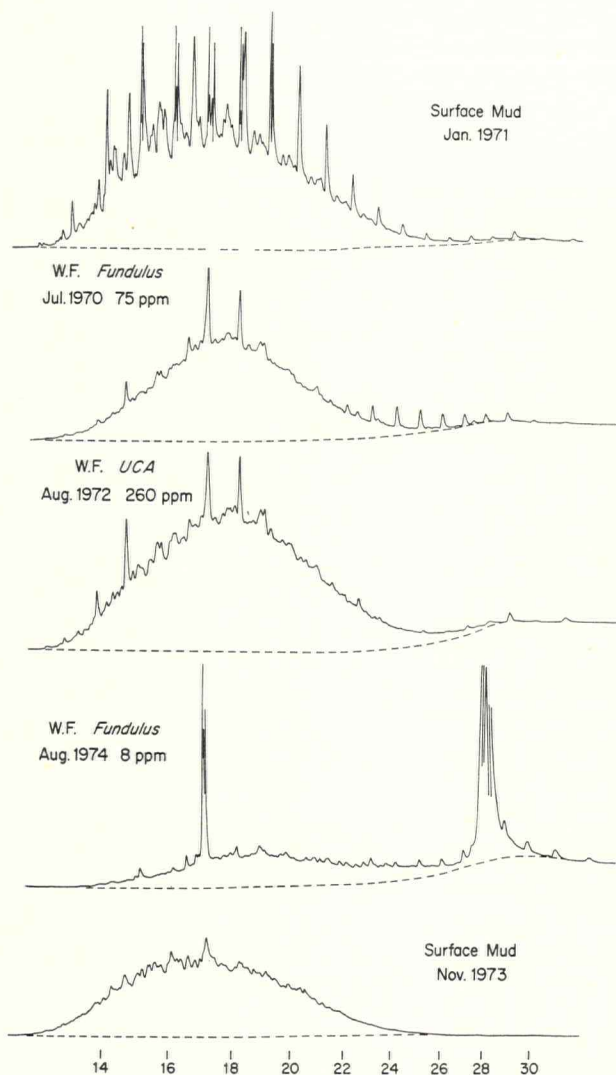


Figure 63. Gas chromatographs of hydrocarbons from sediments and animals from Wild Harbor salt marsh, Mass. Column and GLC conditions as in Figure 62 except held at 275° until n -C₃₁ was eluted, dashed line indicates level of column bleed. Abcissa shows chain length for n -alkanes determined by co-injection.

compounds from the spilled oil were gone. A few months later samples from the area showed little besides the UCM. In all Wild Harbor samples the natural n -alkanes found in the uncontaminated marsh sediments were overwhelmed in amount by the oil and were not measured.

The results of the comparison between animals and sediments are shown in Figures 63 and 64. In the uncontaminated marsh the animals contained hydrocarbons that very closely resembled those in the sediments. These gas chromatograms (GC) are not presented

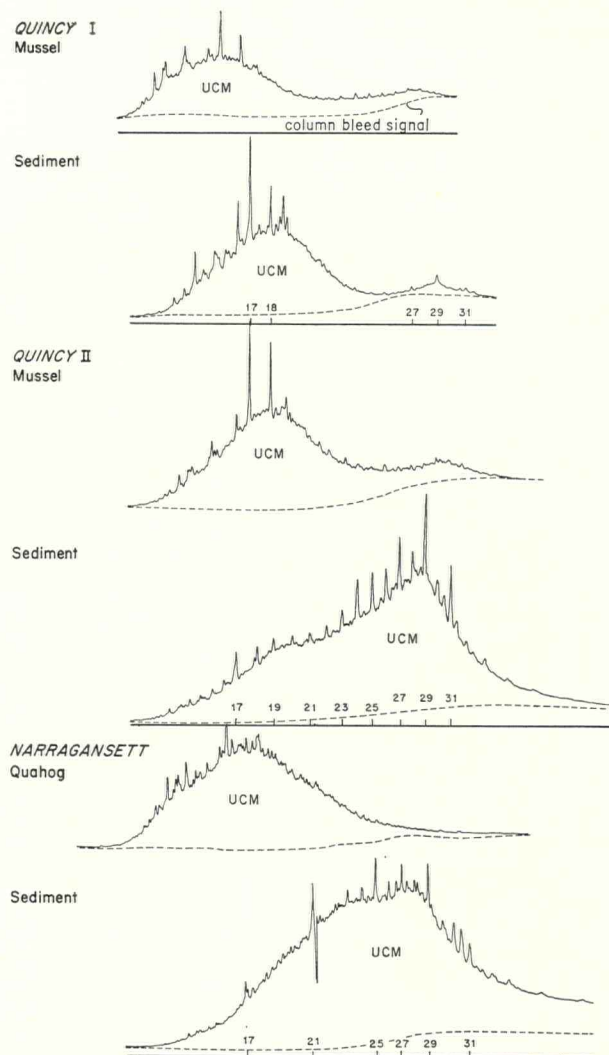


Figure 64. Gas chromatographs of alkanes, cycloalkanes, alkenes and cycloalkenes from sediment and shellfish. *Quincy I* and *Quincy II* Column was 3% SE-30 on chromosorb W 3 m × 0.6 cm o. d. Temperature programmed from 60 to 290° C at 6°/min. He carrier gas flow at 15 ml/min. 14, 17, 18, 25, . . . indicate elution positions of n -alkanes with those numbers of carbon atoms. *Narragansett* Column was SCOT OV-101 (Perkin Elmer Corp). 15 000 theoretical plates. Temperature programmed 75° C to 275° C at 6°/minute and held until n -C₃₃ eluted. He carrier gas 5-6 ml/minute. 14, 17, . . . same as above.

here but can be approximated from the 1974 *Fundulus* GC by reducing the unresolved complex mixture and so emphasizing the n -alkanes between C₂₀ and C₃₁.

In the first year, samples from the marsh pulsed with oil as well as both of the animals contained hydrocarbons that resembled degraded fuel oil (Fig. 62). The same was true for mussels and clams analyzed by Burns and Teal (1971) and by Blumer et al. (1970). The

Table 21. Hydrocarbons in the salt marsh minnow, *Fundulus heteroclitus*, and the marsh sediments on which they were living

Area	Date	Hydrocarbons, 10 ⁻⁶ g/g wet wt		
		Fish	Sediment	Ratio
Wild Harbor....	Jun 1970	75	1 500	0.030
Wild Harbor....	Aug 1974	8	2 500	0.003
Sippewissett.....	Aug 1974	7	15	0.047

fiddler crab, *Uca*, contained a virtually identical suite of hydrocarbons in 1974. But, by that time, the hydrocarbons in the minnow, *Fundulus*, were radically different, resembling those in uncontaminated fish. The quantity of hydrocarbon in the fish also had decreased to the pre-spill level (Table 21). Thus, the ratio of hydrocarbon in the fish to that in the sediment, unchanged immediately after the spill (0.030 compared to the ratio of 0.047 in fish from the control marsh), was only 0.0032 five years after the spill.

The animals from the chronically polluted areas, all pelecypods, prominently show the UCM characteristic of petroleum contamination (Fig. 64). In all cases, the distribution of these hydrocarbons is appreciably lower in retention time than those extracted from the sediments on which the animals were collected, i.e., unlike the fiddler crab, there has been a discrimination for lighter compounds in the processes by which the animals accumulate hydrocarbons from their surroundings.

DISCUSSION

The hydrocarbons apparently derived from marsh plants (i.e. *n*-C₂₁ to C₃₁) and deposited in the relatively unpolluted, shallow, marine sediments we have sampled, show little change with depth in the sediment, i.e., with time since they were deposited. We have found almost no change either in amount or distribution, although Johnson and Calder (1973) found that in a Florida salt marsh the odd-even ratio of the *n*-alkanes was reduced with depth and that the relative amounts of the longer chains also decreased.

We found a slight indication of the latter effect which disappeared when the sediment was more exhaustively extracted. This suggests that the longer chains are becoming more tightly bound into the sediments with time, perhaps in the humic substances in these organic rich muds. We cannot explain any further the differences between the Florida and our Massachusetts results without a more extensive investigation.

In spite of the differences in details, it is clear the hydrocarbons are refractory in surface sediments. They are found in benthic animals in unpolluted areas in about the same molecular weight distribution as found

in the sediments, the simplest explanation being that hydrocarbons are absorbed either from the sediment directly, from settling particles which also supply hydrocarbons to the sediments, or through intermediate solution or suspension in the bottom waters. Once in the animals, these hydrocarbons are not metabolized to an appreciable extent, at least not so that their relative abundances are changed.

If the sediment is pulsed with hydrocarbons from an oil spill, these normally resistant compounds are abundant enough to become a carbon source for organisms and begin to disappear. But, even under such circumstances, apparently only the more readily utilizable compounds are metabolized. The UCM, probably naphthenes, seem to be very long lasting. There is no indication that naphthenes are disappearing from the surface sediments of Wild Harbor marsh. The UCM is also found in the sites subject to chronic pollution although we cannot say in most cases whether other types of hydrocarbons ever reach these sediments at all.

The shellfish living on the polluted sediments contain hydrocarbons which must come from their surroundings since the compounds are not found in the same species from clean environments. But the range of compounds within the animals differs from that in the sediments. Since the shellfish are suspension feeders, it is possible that only the lighter fractions of the oils are released into the water and into the animals. It is equally possible to suppose from these data that the animals are discriminating, either by restricting their uptake to a subfraction of the compounds they encounter, or by selectively metabolizing a portion of the hydrocarbons that are assimilated into their bodies.

It is obvious that some animals have little ability to discriminate and, like the marsh fiddler crabs, always contain a mixture of naphthenes similar to that in their environment. The fact that the fiddlers contained little of the fuel oil *n*-alkanes even when these were present in the sediment is probably due to degradation by microorganisms since those living on uncontaminated sediments reflect the *n*-alkanes of their environment.

The minnows illustrate the most dramatic responses to the pulse of oil. At first they seemed to equilibrate with the petroleum to about the same extent as they had previously equilibrated with the much lower levels of natural hydrocarbons in the marsh. But within the course of a few years they had adapted to the point where their body burden was about the same in quality and quantity as it had been before the oil spill. Burns (1975) has shown that a major part of this adaptation results from the induction of enzymes capable of oxidizing hydrocarbons.

Interactions between animals and the sediments on which they live range from the extremes illustrated by those which contain mixtures virtually identical to those

in their surroundings to those which can discriminate almost completely against the hydrocarbons polluting their environment. The distribution and range of these responses among marine animals is important both to the distribution and the effects of hydrocarbons in marine ecosystems.

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REFERENCES

- Blumer, M., Souza, G. & Sass, J. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. *Mar. Biol.* 5: 195-202.
- Burns, K. A. 1975. Microsomal mixed function oxidases in an estuarine fish, *Fundulus heteroclitus*, and their induction as a result of environmental contamination. *Comp. Biochem. Physiol.* 53 B: No. 4, 443-46.
- Burns, K. A. & Teal, J. M. 1971. Hydrocarbon incorporation into the salt marsh ecosystem from the West Falmouth oil spill. Ref. Woods Hole Oceanogr. Inst. No. 71-69. (Unpublished manuscript).
- Burns, K. A. & Teal, J. M. 1973. Hydrocarbons in the pelagic *Sargassum* community. *Deep Sea Res.* 20: 207-11.
- Farrington, J. W. & Medeiros, G. C. 1975. Evaluation of some methods of analysis for petroleum hydrocarbons in marine organisms. *Proc. 1975 Conf. Prevention Control of Oil Poll. Amer. Pet. Inst. Washington, D. C.* p. 115-21.
- Farrington, J. W. & Quinn, J. G. 1973. Petroleum hydrocarbons in Narragansett Bay. I. Survey of Hydrocarbons in sediments and clams. *Estuar. & Coast. Mar. Sci.* 1: 71-9.
- Farrington, J. W., Teal, J. M., Quinn, J. G., Wade, T., & Burns, K. A. 1973. Intercalibration of analyses of recently biosynthesized and petroleum hydrocarbons in marine lipids. *Bull. Envir. Cont. Toxicology* 10: 129-36.
- Farrington, J. W. & Tripp, B. W. 1975. A comparison of analytical methods for hydrocarbons in surface sediments. *In Marine Chemistry with Coastal Environment*, Ed. by Thomas M. Church, Amer. Chem. Soc. Symp. Series No. 18, Washington, D.C., 267-84.