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 Workshop 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.

A. D. McINTYRE
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THE ALKANES OF MARINE ORGANISMS FROM THE UNITED KINGDOM AND SURROUNDING WATERS

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At sites originally selected on the basis of expected hydrocarbon input, mixed plankton samples and various tissues from 11 species of invertebrates and 19 species of fish, representing some 255 samples in total, have been analysed for alkanes by capillary gas-liquid chromatography.

On a comparative weight-basis, the plankton samples had the highest *n*-alkane values, particularly at the coastal sites adjacent to industrial urban areas. The remaining animal samples fell within a range of means from 0·1 to 20 µg/g wet weight, the higher value usually found in fatty tissues. Different tissues exhibited different *n*-alkane distribution profiles. It was extremely difficult to determine the origin of the *n*-alkanes identified.

INTRODUCTION

The information available in the literature on the hydrocarbon content of marine organisms is far from complete. Emphasis seems to have been placed on the analysis of groups such as phytoplankton (Blumer et al., 1971), macro-algae (Youngblood et al., 1971) and zooplankton (Blumer et al., 1969; 1970) whilst there is paucity of data on higher organisms. Thus there is little information on the distribution, role and residence time of hydrocarbons in the marine ecosystem or their possible effects on man.

This paper reports data originating largely from a baseline survey for hydrocarbons conducted in the United Kingdom area and undertaken jointly between the laboratories of the authors. The scope and purpose of the survey has been described briefly in earlier papers in this volume (Hardy et al., 1977a; 1977b).

Where possible, representative marine organisms (mixed plankton, pelagic and demersal fish, and benthos) were collected at various stations. These were chosen to include reference or control sites that were thought to be relatively unpolluted as well as more heavily contaminated sites such as those near to refinery complexes and industrialised urban areas. As outlined earlier in this volume (Hardy et al., 1977a; 1977b) the relatively simple approach of alkane analysis was adopted and, for the most part, *n*-alkane levels are reported here.

METHODS

The methods and precautions taken in the collection and analysis of marine organisms have been described previously (Mackie et al., 1974) and elsewhere in this volume (Mackie et al., 1977). Single plankton tows were made at the stations shown in Figure 57. It should be noted, however, that the routine plankton sampling method (Mackie et al., 1974) also collects a variety of debris, particularly in estuarine waters. The larger elements of this — leaves, twigs, macro-algal slime, artefacts — were removed and the residue, mostly of zooplankton proportions, was extracted as the "mixed plankton sample". Thus, especially in coastal waters the sample is probably more representative of suspended matter and plankton rather than simply plankton. The fish and benthos analyses from the relevant stations were made on the bulked tissue of 6 animals or a representative sub-sample as appropriate.

RESULTS AND DISCUSSION

The geographical distribution of the United Kingdom sampling stations (Stn) is shown in Figure 57. The species analysed are given in Table 15, with the vertebrates arranged in order of the lipid content of the muscle tissue after Jacquot and Creac'h (1950) and Murray and Burt (1969). An attempt is made in Table 16 to group the stations into categories according to expected hydrocarbon input.

PLANKTON

The somewhat non-specific nature of the mixed plankton sample is best illustrated perhaps in Figure 58, where the *n*-alkane distribution profile of the sample from Stn. 3 is compared with the sediment analysis from

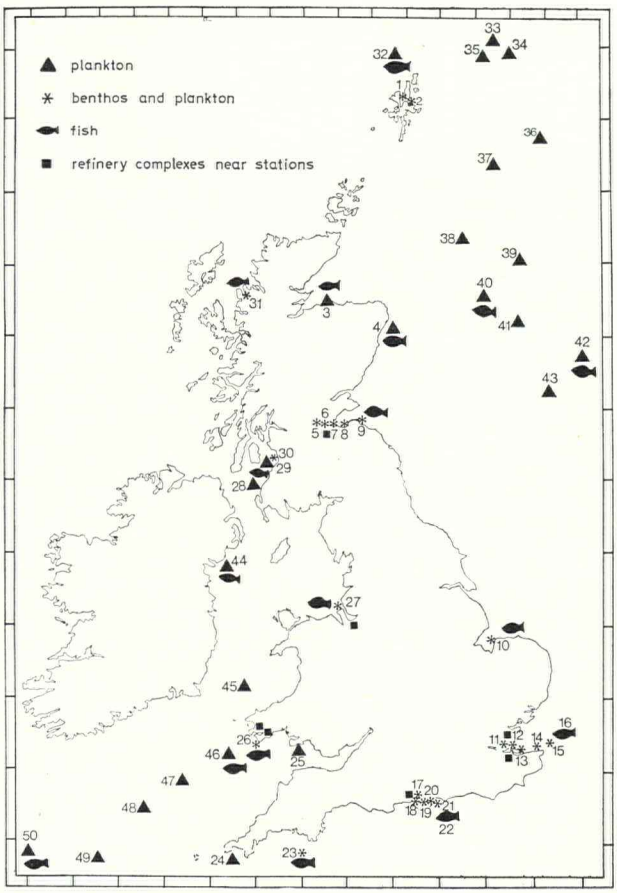

Figure 57. Sampling stations in the UK area hydrocarbon baseline survey. The numbers are the station designators used in the text, tables and subsequent figures. 1, 2, 31, 32 and 50 were selected because they were considered likely to be unpolluted.

the same site. They match almost completely which suggests that suspended sediment, stirred up locally from the bottom was entrapped in the plankton sample.

Table 16 compares the range of *n*-alkane (Σ C_{15}–C_{33}), pristane and phytane levels for 48 samples analysed from the coastal and open sea site groups. The coastal sites are sub-grouped according to the expected input of petroleum and petroleum derived components. A selection of *n*-alkane distribution profiles is shown in Figure 59.

The control group, being few in number and subject to wide variation is unsuitable as a reference for comparison. Indeed in each group or sub-group of analyses (Table 16) there is considerable scatter in the levels. In some cases, a particularly high value is present which has a marked influence on the group mean. This is especially noticeable for the pristane concentrations. The difference between the hydrocarbon levels in the coastal and open sea sites can be explained on the assumption that the exogenous hydrocarbon input is higher in coastal waters. Alternatively, the explanation may be either that the difference is a reflection of changing planktonic production in different environments or that it represents greater sample contamination in coastal waters. Support for an exogenous input or greater sample contamination can be inferred from a comparison of the coastal sub-groups which fall into two main categories, high and low expected inputs respectively. The *n*-alkanes are relatively high compared with pristane in the refinery/urban category in contrast to the control/coastal category where the concentrations are similar. Phytane levels tend to be higher in the former category rather than the latter. However, although some caution must be exercised in developing interpretations such as this since the scatter in the groups is large, it seems that analyses of the concentration of *n*-alkanes, pristane and phytane in sufficiently

Table 15. Species analysed in the United Kingdom hydrocarbon baseline survey

Survey species	Common name	% Lipid in muscle[1] Range	Median
Vertebrates			
Scomber scombrus	mackerel	1·5–23·5	12·3
Clupea harengus	herring	0·4–22·0	11·2
Lophius piscatorius	monk fish	7·5	
Trachurus trachurus	horse mackerel	0·4– 9·1	4·5
Morone labrax	bass	2·5	
Lepidorhombus wiffiagonis	megrim	1·0– 3·9	2·45
Pleuronectes platessa	plaice	1·1– 3·6	2·4
Microstomus kitt	lemon sole	0·5– 3·8	2·1
Solea solea	sole	1·8– 2·3	2·0
Trigla sp.	gurnard	1·1– 2·3	1·7
Merluccius merluccius	hake	0·4– 1·9	1·2
Zeus faber	John dory	0·6– 1·7	1·15
Limanda limanda	dab	0·5– 1·2	0·9
Raja sp.	skate	0·1– 1·6	0·8
Pollachius virens	saithe	0·6– 0·8	0·7
Gadus morhua	cod	0·1– 1·0	0·55
Merlangus merlangus	whiting	0·2– 0·6	0·4
Melanogrammus aeglefinus	haddock	0·1– 0·6	0·35
Ammodytes sp.	sand eel	0·3	
Invertebrates			
Pagurus sp.	hermit crab		
Carcinus maenus	shore crab		
Macropipus sp.	swimming crab		
Buccinum undatum	whelk		
Pandalus sp.	pink shrimp		
Mytilus edulis	mussel		
Nephrops norvegicus	scampi		
Crangon sp.	brown shrimp		
Chlamys opercularis	queen		
Pecten maximus	scallop		
Asterias rubens	common starfish		

[1] After Jacquot and Creac'h (1950) and Murray and Burt (1969).

Table 16. Alkane levels of mixed plankton samples at coastal and open sea sites, µg/g dry weight

Station groups	Σ *n*-alkanes Range	Σ *n*-alkanes *x*	Σ *n*-alkanes *n*	Pristane[1] Range	Pristane[1] *x*	Pristane[1] *n*	Phytane Range	Phytane *x*	Phytane *n*
Open Sea 32–50	0·3– 263	71	18	nd-17760 (644)	9992 (133)	18 (17)	nd-2·8	0·9	11
Coastal 1–31	1·4–1586	218	30	0·5–422	80	29	nd-28	4·3	23
Coastal sub groups Control/reference 1, 2, 31	1·4– 482	179	3	9·8–512	186	3	2·6–13·3	8·0	2
Miscellaneous coastal 3, 4, 9, 10, 14, 21, 23, 24, 28	5·7– 174	63	10	0·5–407	94	10	nd-3·3	0·9	7
Refinery 6, 12, 18, 26, 27	13 – 721	270	6	2·3–284	58	6	nd-28	9·0	5
Industrialised urban 5, 7, 8, 11, 13, 17, 19, 20, 25, 29, 30	3·2–1586	341	11	1·4–207	57	10	nd-28	4·5	9

[1] Figures in brackets are alternative values obtained on omitting the abnormally high pristane level (17760, Stn. 33).
Σ = total C_{15}–C_{33} *x* = arithmetic mean *n* = number of sites analysed nd = not detected.

well-selected "mixed plankton" samples could give a reasonable indication of comparative exogenous hydrocarbon input. For firmer interpretation a more rigorous analysis of the data is necessary than has been possible so far.

The most obvious difference between the distribution profiles of the coastal and open sea samples (Fig. 59) is the high incidence of the predominance of higher molecular weight odd carbon numbered *n*-alkanes (C_{25}—C_{31}) in coastal samples. Of course this can be due to the presence, locally, of suspended sediment although such a relationship was established clearly only for Stn. 3. It can also be due to land plant debris either directly, or indirectly, in the form of suspended particulates, carried downstream from river sources. In both groups, the profiles are quite varied in detail and, further as in Stn. 27 (Fig. 59), there is some evidence of recent petroleum contamination not only because of the relatively higher load of lower molecular weight *n*-alkanes but also because the distribution shows steadily decreasing concentrations with increasing carbon number across the full molecular weight range.

It has been possible also to analyse nine monospecific zooplankton samples including, *Pseudocalanus elongatus, Evadne nordmanii, Podon leuckarti, Temora longicornis, Acartia clausii* and *Calanus finmarchicus* collected from Stn. 31 in March/April. They show different alkane patterns (Fig. 58) which may be a reflection of differences in metabolism, in the level and type of feeding or in maturity. However, as reported later in this volume (Murray et al., 1977) short-term incubation of a mixed zooplankton culture with a ^{14}C-labelled algal diet did not show any evidence of the incorporation of ^{14}C into the *n*-alkane array of the zooplankton. Thus at least the intrinsic biosynthetic origin of the *n*-alkanes in the zooplankton species can be questioned.

FISH

Table 17 lists the average *n*-alkane levels of the muscle and liver tissues of 68 fish samples arranged in the order shown in Table 15. The majority of samples were obtained from the coastal stations and, although 7 species appear in both groups, the number of sites per species is smaller for the open sea samples.

Coastal samples. The amounts of the muscle tissue *n*-alkanes are low and fall mostly within the range of 0·1—1·0 µg/g tissue; instances of higher values usually are found either in the fatty species or hake, plaice, dab and whiting.

The liver tissue levels on the other hand mostly fall within the range 1—5 µg/g tissue. In the majority of cases within species, the muscle content is lower than that of the liver. Some samples of herring, mackerel, dab and whiting provide the exceptions to this generalisation. The last two examples are from the Thames and Mersey respectively, whilst the first two are species which can retain large quantities of neutral fat in the muscle. The highest amounts of *n*-alkanes in liver tissue are usually found in the fatty species, although one plaice value from the Thames estuary was about 120 µg/g tissue. Generally, the edible portion of the fish, the

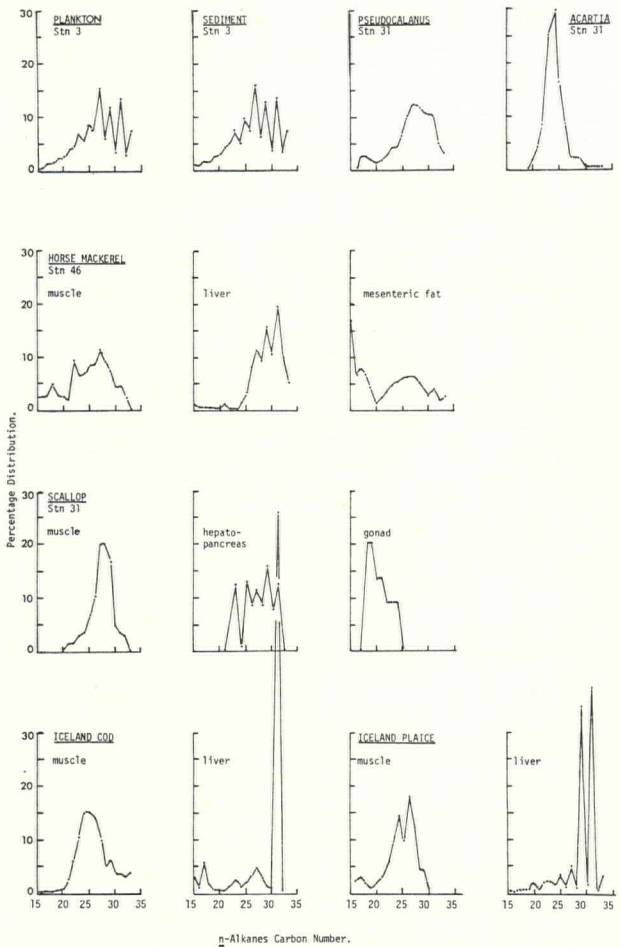

Figure 58. A variety of *n*-alkane profiles in tissues from marine organisms. The alkane profiles are plotted as percentage distribution over the carbon number range C_{15}–C_{33}. The lines joining the points are for diagrammatic display only. Stn = sampling station (see Fig. 57).

muscle, has a low load of *n*-alkanes being at least an order of magnitude less than the level in plankton on a comparative weight basis.

The distribution of species in the sampling programme is uneven and the scatter among the levels is high. Thus, it is difficult to make quantitative comparisons both within and between species from different areas on the basis of expected input. The urban/refinery areas do not show consistently higher values than the rest but, the occasional unusually high values are found most often in these areas.

Pristane, which generally is a minor component (averaging: 0·05 μg/g muscle in gadoids and flatfish), is a major alkane component in the tissues of herring, mackerel and horse mackerel (averaging: 8·6 μg/g muscle; 2·2 μg/g liver). Compared with liver tissues, levels are highest in the muscle tissues of herring and mackerel but not horse mackerel. Phytane was identified as a minor constituent in some muscle and liver tissue analyses but its presence and distribution was not consistent with the level of expected petroleum input.

The distribution of *n*-alkanes is quite different in the various tissues of fish. Profiles of the muscle, liver and mesenteric adipose tissue of horse mackerel are compared in Figure 58. Figure 60 compares *n*-alkane distribution profiles for the muscle and liver tissues of mackerel and cod from coastal and open sea stations. Comparison of all 108 profiles from the survey data plotted as percentage composition indicates that although different tissues have different profiles, these are not characteristic of species or even of groups such as teleosts and elasmobranchs. The most noticeable exception however, is that mackerel as a group show a different muscle profile (Fig. 60), apparently richer in the lower homologues.

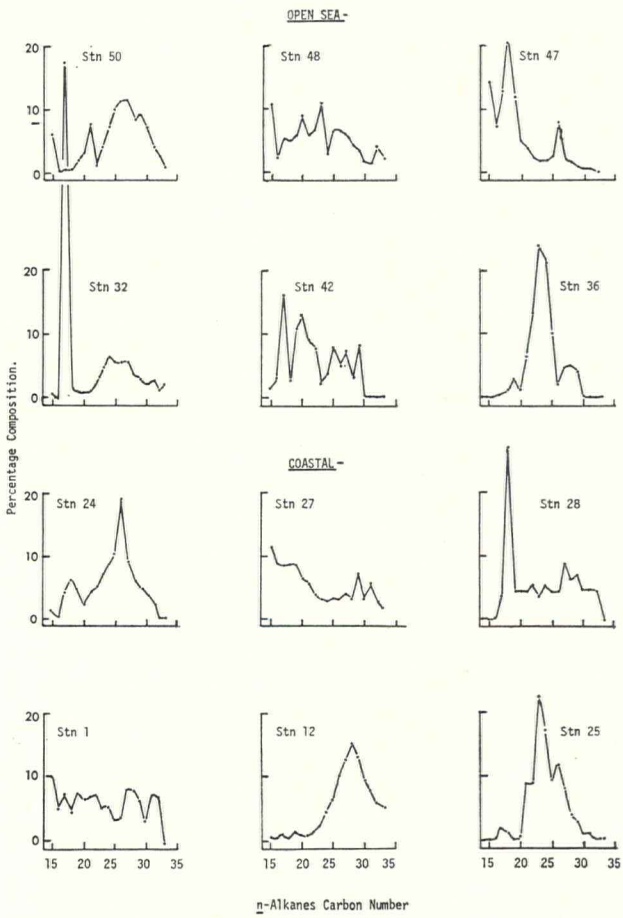

Figure 59. *n*-Alkanes in mixed plankton from open sea and coastal sites. The alkane profiles are plotted as percentage distribution over the carbon number range C_{15}–C_{33}. The lines joining the points are for diagrammatic display only. Stn = sampling station (see Fig. 57).

Table 17. Levels of *n*-alkanes in fish from UK coastal and open sea sites, μg/g wet weight in (a) muscle and (b) liver

Sample		Coastal				Open sea			
		Σ *n*-alkanes Range	*x*	*n*	Stations	Σ *n*-alkanes Range	*x*	*n*	Stations
Mackerel,	(a)	0·4– 2·8	1·2	5	3, 9, 23, 27, 31	0·6–1·4	1·0	3	32, 44, 46
	(b)	0·4–47·6	12·4	6	3, 9, 22, 23, 27, 31	1·3–6·3	4·1	3	
Herring,	(a)	7·6–11·8	9·7	2	15, 30	nc			
	(b)	4·8		1	15				
Monk,	(a)	0·3– 0·6	0·45	2	26, 27	nc			
Horse mackerel,	(a)	1·0		1	23	0·3–3·3	1·5	3	32, 46, 50
	(b)	4·8		1		1·7–7·5	5·3	3	
Bass,	(a)	0·6		1	22	nc			
	(b)	4·8		1					
Megrim,	(a)	nc				0·3–0·6	0·45	2	32, 50
	(b)					1·4–2·4	1·9	2	
Plaice,	(a)	0·01– 1·8	0·6	8	3, 4, 9, 22, 27, 31	1·5		1	42
	(b)	1·1 –118·0	19·0	7	4, 9, 12, 22, 27, 30, 31	8·9		1	
Lemon sole,	(a)	nc				0·2–0·3	0·25	2	40, 46
	(b)					1·7–5·1	3·4	2	
Sole,	(a)	0·3	0·3	2	11, 15	nc			
	(b)	4·5		1	15				
Gurnard,	(a)	0·3– 0·5	0·4	2	23, 27	0·2		1	32
	(b)	1·1–19·7	10·4	2		1·4		1	
Hake,	(a)	6·0		1	30	1·6		1	50
	(b)	nc				3·1		1	
John dory,	(a)	1·0		1	23				
	(b)	3·0		1					
Dab,	(a)	0·1–4·2	1·6	3	10, 15, 27	nc			
	(b)	1·5–9·5	4·2	3					
Skate,	(a)	0·2–0·5	0·35	2	10, 27	nc			
Saithe,	(a)	nc				0·1		1	32
	(b)					3·5		1	
Cod,	(a)	0·01–0·3	0·1	5	3, 9, 15, 16, 31	0·1–0·2	0·2	4	32, 40, 42, 44
	(b)	1·0 –3·1	1·6	4	3, 9, 15, 16	1·4–4·6	3·0	4	
Whiting,	(a)	0·01–3·1	0·9	6	10, 14, 15, 16, 23, 27	0·2	0·2	2	44, 46
	(b)	0·6 –6·0	2·5	5	14, 15, 16, 23, 27	1·6–3·9	2·8	2	
Haddock,	(a)	nc				0·3–1·7	1·0	2	32, 40
	(b)					2·8–3·9	3·3	2	
Sand eel,	(a)	tr		1	31	nc			
	(b)	9·0		1					

x = arithmetic mean Σ = total C_{15}–C_{33} *n* = number of samples analysed (each sample is a batch of 6 animals).
nc = not collected tr = < 0·01.

In liver tissues, a predominance of the odd carbon numbered higher molecular weight *n*-alkanes (C_{25}–C_{31}) is normally obvious but it is lacking in muscle tissues. There are individual exceptions, such as the cod muscle tissue from Stn. 3, which has a high odd carbon predominance and for which there is no explanation except, perhaps speculatively, as an indication of stress. Particularly in liver samples, a single, longer chain *n*-alkane may occur in much larger amounts than its nearest neighbours (×5 to ×10) for which we can offer no satisfactory explanation. It is worth noting however, that Cooper et al. (1974) have analysed sediment samples containing a predominant *n*-alkane although we cannot substantiate this finding from our survey data (Hardy et al., 1977b).

Open sea samples. The average *n*-alkane values for species from either the open sea or coastal groups each fall within the range of the other group; that range being smaller for the open sea samples and showing a slight tendency to be lower than coastal values. In all cases, the alkane concentration in the livers is higher than that in the muscle and, with the exception of

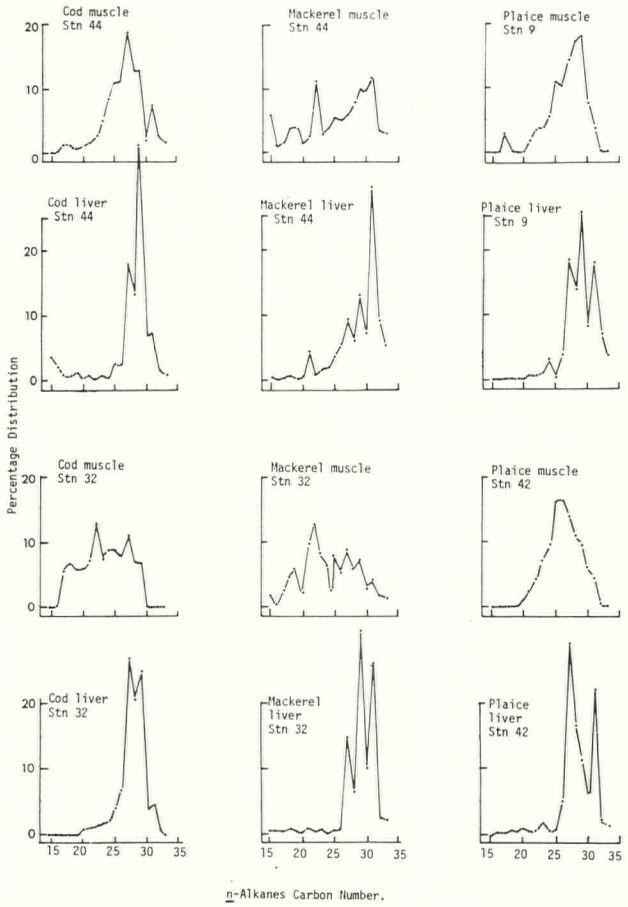

Figure 60. *n*-Alkanes in the tissues of fish. The alkane profiles are plotted as percentage distribution over the carbon number range C_{15}–C_{33}. The lines joining the points are for diagrammatic display only. Stn = sampling station (see Fig. 57).

mackerel (average, 12·4 µg/g tissue), this is also true of the pristane concentration (average 0·04 µg/g gadoids, 0·03 µg/g flatfish).

Examination of the *n*-alkane distribution profiles again shows that they are not characteristic of species although there are the gross differences noted earlier between liver and muscle tissues. However, the profiles do show the anomalous position of mackerel as in the coastal samples.

BENTHOS

Table 18 summarises the range and average of the total *n*-alkane levels for the 48 samples of the various benthic invertebrates collected. As with the fish, the distribution of species is very uneven and most of the samples are from coastal sites. Thus, comparisons between different environments are difficult but, where they are possible, the values from the reference stations tend to fall in the lower end of the range.

Table 18. Levels of *n*-alkanes in the survey benthos samples, µg/g wet weight[1]

Sample	Station	Σ *n*-alkanes Range	*x*	*n*
Hermit crab	13, 14, 15, 17 20, 21, 26, 31	0·5 –11·0	5·9	8
Shore crab	10, 11, 12, 14 15, 26, 27	0·7 – 6·2	3·1	7
Swimming crab	8, 10, 12, 13, 27	0·1 – 5·8	2·7	5
Whelk	1, 11, 13, 17, 21, 31	0·2 –15·6	2·8	6
Pink shrimp, head	10, 17	2·8 – 3·8	3·3	2
tail (muscle)		0·3 – 1·6	1·0	2
Brown shrimp, head	10, 11, 13, 17, 18, 19 26, 27	0·1 – 4·4	2·6	8
tail (muscle)	10, 11, 18, 19, 26, 27	0·3 – 0·8	0·5	6
Scampi, tail (muscle)	9, 30, 31	0·07– 0·7	0·5	3
Mussel	31	1·7		1
Queen, muscle	23	0·2		1
Scallop, muscle	31	0·3		1
Starfish	1, 8, 9, 15, 26, 27	0·1 – 4·2	1·7	6

[1] Whole organism (ex shell) unless indicated otherwise.
n = number of samples analysed (each sample is a batch of 6 animals).
x = arithmetic mean.
Σ = total C_{15}–C_{33}.

On a weight basis, the values are low in comparison with the plankton and are of the same order as the fish tissues. Within species, levels range within a half-decade of the mean except for the *Nephrops* "tail" or muscle. The muscle of the shellfish — the edible portion — consistently shows a lower concentration of *n*-alkanes.

As in fish, the various tissues of the invertebrates have different *n*-alkane distribution profiles (Fig. 58). A further selection of distribution profiles appear in Figure 61. In general, the profiles do not indicate any species specificity. The predominance of the odd carbon number *n*-alkanes, also shown in the analyses of whole organisms, does not correlate with sediment analyses from the same station although these were not strictly analyses of the very recent surface sediments which might have been picked up by the benthos. Perhaps we must seek a biochemical explanation for the predominance rather than simple acquisition from the sediment substrate.

CONCLUSIONS

Comparison of the *n*-alkane analyses between tissues and across the species suggests that concentrations are highest where the potential neutral lipid content is highest, such as liver tissues, shellfish heads and the muscle tissues of fatty fish. In general, the analyses show low levels of *n*-alkanes in organisms other than

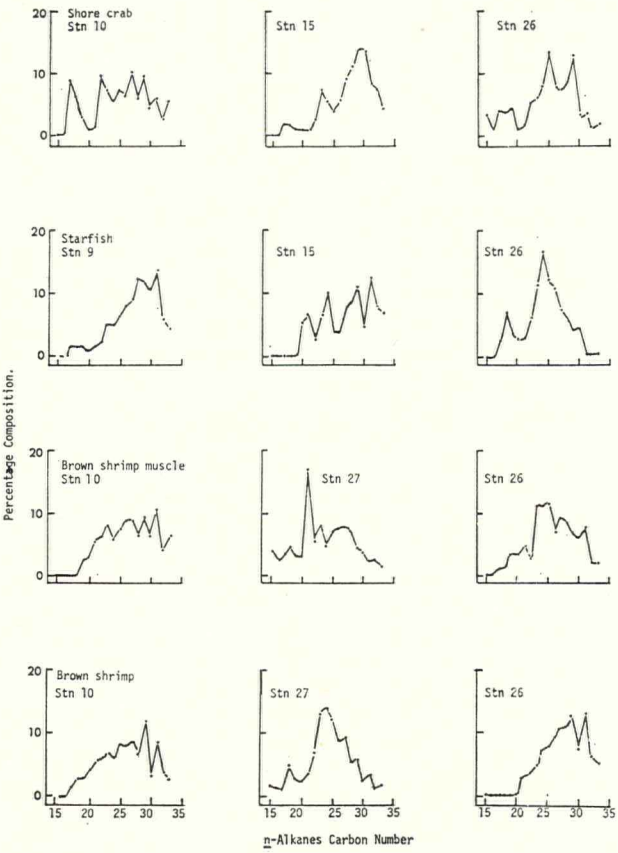

Figure 61. *n*-Alkanes in the tissues of benthos. The alkane profiles are plotted as percentage distribution over the carbon number range C_{15}–C_{33}. The lines joining the points are for diagrammatic display only. Stn = sampling station (see Fig. 57).

the mixed plankton samples and the values are similar to those found in samples from Icelandic fishing grounds (Whittle et al., 1974) or Antarctic waters (Mackie, Hardy and Platt, unpublished data). Incidentally, the latter also contain phytane at much the same level as that identified in some of the UK samples (0·01 µg/g liver in bass). The edible portion of the animals tend to have the lowest load of *n*-alkanes and there is no evidence of accumulation of these components at higher levels of the food chain on the basis of muscle tissue concentrations.

Unfortunately, no species are predominant candidates as indicator species of petroleum input on the basis of these *n*-alkanes analyses. Only the mixed plankton samples appear to fall into a pattern which may be related in some way to the expected input of petroleum derived *n*-alkanes but, from the wide scatter of the results, it is clear that a single measurement could give a misleading impression of the level of input at any given location.

None of the *n*-alkane distribution profiles in the higher organisms seem strictly analogous to the situation found in cod livers after these fish had been fed crude oil in the diet (Hardy et al., 1974). In this work the odd carbon predominance characteristic of the liver was masked by a more even distribution due to the assimilation of *n*-alkanes to the liver. No oil-derived alkanes were found in the muscle suggesting that cod muscle and perhaps that of fish with similar lipid metabolism may be insensitive to the input of exogenous hydrocarbons.

Clearly, one of the major difficulties in interpretation of the data is to determine the origin of the *n*-alkanes found. Are they dietary, absorbed from the surrounding environment or endogenous in origin?

ACKNOWLEDGEMENT

The monospecific zooplankton samples were isolated from plankton tows by Mr Duncan Seaton, Marine Laboratory, Aberdeen.

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