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MONITORING OF HYDROCARBONS IN BENTHIC CRUSTACEANS DURING OFFSHORE DRILLING AND PETROLEUM EXPLORATION

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Introduction

As part of a broad, multidisciplinary effort to monitor the impact of petroleum exploration activities on the outer continental shelf areas of the Gulf of Mexico, the local effects of a drilling rig on the benthic environment have been investigated. Petroleum hydrocarbons in or on seawater can become associated with suspended sediment particles[1] and be transported downward and incorporated into the bottom. In addition, cuttings settle around drilling sites. These processes can result in an alteration of sedimentary organic carbon characteristics of the type which has been documented in the vicinity of a petroleum production platform in the Gulf of Mexico[2] and in accumulation of non-biogenic hydrocarbons in the benthic environment. Crustaceans in this environment can rapidly take up petroleum hydrocarbons from the water or their food[3] and can be adversely affected as summarized by Hyland and Schneider.[4] For these reasons, the hydrocarbon contents of penaeid and stomatopod shrimp were determined in conjunction with exploratory drilling operations as a measure of possible petroleum contamination of the benthos in this locality.

Materials and Methods

Organisms were collected by trawling at distances of 100 m, 500 m, and 1000 m from a drilling rig located offshore of Mustang Island, Texas. Collection occurred during December 1975 prior to rig arrival, during January 1976 while exploratory drilling was being performed, and during March 1976 after removal of the rig. Identical sampling locations in four transects spaced 90° apart and originating at the drilling site were occupied in each of the three collection periods. No petroleum or gas was found at this drill site, and no production has occurred as a result of this exploration operation.

Seventy samples representing 5 species of crustaceans were obtained. Each sample represents a pooled group of conspecific animals collected at a particular location in the sampling grid. These 70 samples were made up of 22 from the predrilling period, 18 from the drilling period, and 30 from the postdrilling collection period. Two species of decapod shrimp, Penaeus setiferus and Trachypenaeus similis, are represented in all three periods. Although present in the trawl collections, not enough individuals of the decapod shrimp Penaeus duorarum nor of the stomatopods Squilla chydea or Squilla empusa were available to allow their analysis in every sampling period. Immediately after collection, animals were placed in solvent-rinsed glass jars topped with aluminum-foil-lined caps and frozen. Samples were maintained at -20°C or colder until analysis.

For analysis the entire pooled sample consisting of from 1 to 12 whole individuals was dried at 60°C to constant weight. The dried organisms were reduced to a homogeneous

powder by crushing and stirring. A solution of 0.5N KOH in benzene/methanol, 50/50, was added, and this mixture sonicated for 10 minutes. Saponification of lipid matter and extraction and isolation of hydrocarbons proceeded after published methods.[5,6] The non-saponifiable lipids were extracted with petroleum ether after addition of water to the cooled saponification mixture. Column chromatography using alumina over silica gel, 50/50, separated saturated from unsaturated plus aromatic hydrocarbons. Gas-liquid chromatography with a flame ionization detecter was employed to resolve and to quantify the various components of each hydrocarbon fraction. Both a non-polar column and a polar column were used. The non-polar column was 4 m x 2.1 mm ID 3% SP-2100 on 100-120 mesh Supelcoport and was operated from 150° to 325° C at 4° C/minute using nitrogen carrier gas at 15 ml/minute. The polar column was 2.5 m x 2.1 mm ID 10% SP-1000 mesh Supelcoport and was operated from 150° to 250° C at 8° C/minute using nitrogen at a flow rate of 15 ml/minute. These column packing materials are marketed by Supelco, Inc., Bellefonte, Pennsylvania. Retention times and integrated peak areas were entered into a computer program to calculate Kovats Indices[7] relative to authentic standards and concentrations for each peak. Quantification was based upon a known amount of n-docosane added to each hydrocarbon fraction after column chromatography and prior to gas chromatography. Areas of unresolved envelopes were measured by planimetry. Blank determinations on the entire procedure were routinely determined and were small. The reported results have been corrected for these, nonetheless.

Results and Discussions

Hydrocarbon compositions of nearly all 70 samples were quite similar. A certain amount of variability was observed among individual samples, but the amount of resemblance was considerably larger than the amount of variability. This was surprising because not only were five different species of Crustacea being investigated, but these species included members of two separate orders, Decapoda and Stomatopoda, within this class. Furthermore, there appeared to be only minor differences between organisms collected from different sampling periods.

Representative chromatograms obtained from the saturated hydrocarbon fractions of samples of two organisms, Penaeus duoraroum and Squilla chydea, are shown in Fig. 1.

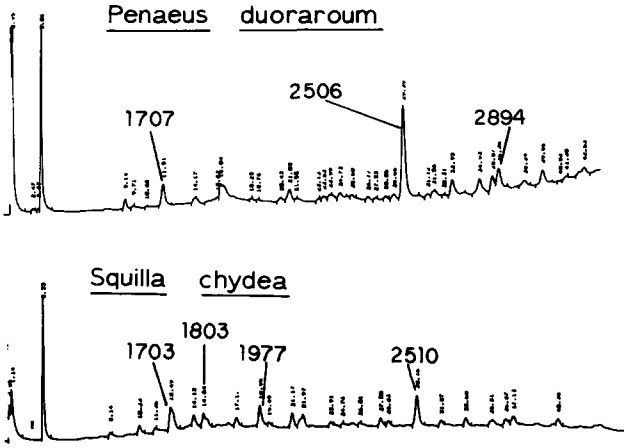

Figure 1. Representative Chromatograms of Saturated Hydrocarbons of Two Shrimps. Column conditions: 3% SP2100 on 100-120 mesh Supelcoport, 150-324°C at 4°/minute. Peaks labelled by Kovats Index.[7]

Although the organisms are from different orders of Crustacea and were collected during different sampling periods, their traces are very similar. Both represent a fairly simple mixture of hydrocarbons that is well-resolved by this chromatographic column. The major peak in both traces is at a Kovats Index of 2506-2510. A major peak is not found at 2500 when these fractions are rechromatographed on polar columns, so the hydrocarbon represented by this peak is not n-pentacosane. In fact, few normal alkanes are found in any of the samples, and the saturated hydrocarbon compositions of these animals appear to be composed mostly of branched compounds.

The unsaturated hydrocarbon compositions of these organisms also display a fairly simple pattern. Usually 4 to 6 peaks dominate the chromatograms obtained from both non-polar and polar columns. The major peaks of chromatograms of the saturated and unsaturated hydrocarbon fractions from representative samples of the 5 species are listed in Table 1 in terms of Kovats Retention Indices and weight percent contribution of each peak to the total fraction. Peaks from both polar and non-polar columns are listed. As shown

	Saturated Hydrocarbons				Unsaturated Hydrocarbons			
	Nonpolar Column		Polar Column		Nonpolar Column		Polar Column	
Organism	K.I.	Wt.Pct.	K.I.	Wt.Pct.	K.I.	Wt.Pct.	K.I.	Wt.Pct.
Penaeus setiferus	2248	15	2967	19	1935	14	2237	16
	2271	15	3013	13	2114	20	2453	14
	2508	23	3140	19	2143	11	2475	16
					2274	18	2843	14
Penaeus duoraroum	2506	34	2343	29	1950	19	2224	11
	2894	8	3145	26	2133	15	2244	11
					2300	18	2461	27
					2477	11	2810	16
Trachypenaeus similis	2506	31	2495	7	1921	14	2222	16
	2894	7	2798	8	2099	20	2430	11
			3144	26	2125	10	2455	18
					2256	18	2826	13
Squilla empusa	2244	11	2043	35	1933	20	2234	23
	2249	11	2253	27	2103	13	2438	12
	2507	16			2133	11	2463	11
					2263	13	2833	10
					2445	10		
Squilla chydea	1703	15	1699	8	1911	10	2219	14
	1803	8	1800	5	2005	8	2352	11
	1977	10	2202	11	2087	13	2449	11
	2510	16	3144	11	2237	8	2824	8

Table 1. Dominant Peaks in Chromatograms of Hydrocarbons from Uncontaminated Crustacean Samples. Peaks labelled by Kovats Index.[7] See text for column descriptions.

in this tabulation, hydrocarbon compositions are dominated by only a few peaks, and some peaks having the same Kovats Indices are common to all five crustaceans. The largest peak from the non-polar chromatograms of the saturated fraction has an index of 2506-2510 in all 5 samples and indeed in most of the 70 samples in the total study. However, a peak having an index around 2500 is not a major contributor to polar chromotographs of this fraction. Instead, the most common major peak in these latter distributions has a Kovats Index of 2140-3144. Major peaks comprising the unsaturated fractions of the

samples are grouped between indices of 1900 to 2500 on the non-polar chromatograms. A shift to indices between 2200 to 2850 on polar chromatograms is indicative of the relatively polar nature of these unsaturated hydrocarbons.

A number of criteria are available[8] to distinguish petroleum hydrocarbons from recently biosynthesized hydrocarbons in environmental samples such as these organisms. Three were employed in this study to determine petroleum contamination: (1) the presence of an unresolved complex mixture of saturated hydrocarbons, (2) the existence of a homologous series of n-alkanes, and (3) relatively high hydrocarbon concentrations. An unresolved complex mixture in hydrocarbons extracted from marine invertebrates has been considered diagnostic of petroleum in previous studies[5,6,9,10,11], as has a uniform abundance of n-alkanes in the C-15 to C-30 range.[9,10] In addition, animals contaminated by petroleum exhibit higher levels of total hydrocarbons than uncontaminated organisms normally contain.[9,11,12,13]

Nearly all of the 70 samples produced chromatograms which were free of an unresolved complex mixture of hydrocarbons and which lacked a homologous series of n-alkanes. Figure 1 is representative of the traces of this type. The only exceptions were two samples collected during drilling operations and two more collected in the postdrilling period. Chromatograms of the saturated and unsaturated fractions of one sample of Squilla chydea collected during the drilling period are presented in Fig. 2. The

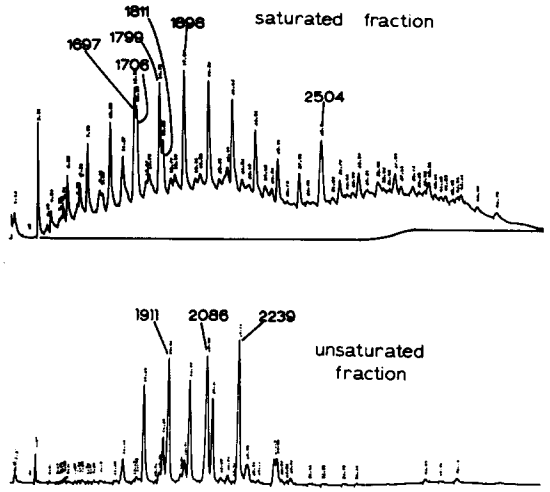

Figure 2. Chromatograms of Saturated and Unsaturated Hydrocarbons of Contaminated Sample of Squilla chydea. See Figure 1 for column conditions.

saturated fraction shows a large number of resolved peaks representing a homologous series of n-alkanes. A large envelope between these peaks and the baseline indicates the presence of a complex mixture of hydrocarbons unresolvable by this column. The unsaturated fraction exhibits only a limited number of peaks and no unresolved complex mixture in marked contrast to the saturated fraction. Similar patterns and differences between fractions were found in the other three anomolous samples.

Kovats Indices and weight percent contributions to the total hydrocarbon fraction of

the major peaks in two samples having unresolved envelopes are listed in Table 2. The

	Saturated Hydrocarbons				Unsaturated Hydrocarbons			
	Nonpolar Column		Polar Column		Nonpolar Column		Polar Column	
Organism	K.I.	Wt.Pct.	K.I.	Wt.Pct.	K.I.	Wt.Pct.	K.I.	Wt.Pct.
Trachypenaeus similis	1701	4	1661	4	1922	15	2230	17
	1710	4	1699	4	2099	20	2438	12
	1802	4	1768	3	2126	10	2464	18
	1815	3	1800	4	2256	17	2830	12
	1903	6	1900	5				
	2003	7	2002	6				
	2103	6	2100	5				
	2203	4	2199	6				
	2303	3	2300	4				
Squilla chydea	1498	5	1599	3	1800	10	2135	10
	1598	4	1659	5	1911	12	2215	12
	1697	5	1698	5	2004	11	2349	11
	1706	4	1766	4	2086	16	2446	12
	1769	5	1798	6	2239	16	2822	11
	1898	7	1899	6				
	1997	7	2000	6				
	2097	6	2098	5				
	2197	4	2197	5				
	2296	2	2297	3				
	2503	4	3143	2				

Table 2. Dominant Peaks in Chromatograms of Hydrocarbons from Contaminated Crustacean Samples. Peaks labelled by Kovats Index.[7] See text for column descriptions.

samples of Trachypenaeus similis and Squilla chydea were collected in the same trawl operation during January, 1976. The saturated fractions are composed of many peaks, none comprising more than 7% of the total fraction. Most of these peaks have retention indices corresponding to those of normal alkanes. This distribution is quite different from the listing of major peaks in Table 1. where a limited number of peaks are shown and few n-alkanes are indicated. In contrast to the saturated fractions, the unsaturated fractions of hydrocarbons of these two samples give only a few peaks. Furthermore, both the retention indices and weight percent contributions of the peaks are quite close to those of the same species listed in Table 1. This is true especially for S. chydea.

The data in Table 2. and the chromatographic traces in Figure 2 indicate hydrocarbon contamination of these samples by the presence of a homologous series of n-alkanes and an unresolved complex mixture of saturated hydrocarbons. It is interesting that no obvious change occurred in the unsaturated hydrocarbon fractions of these animals. However, this may indicate a difference in degree of effect rather than an actual lack of hydrocarbon contamination in the unsaturated fraction. Such a distinction arises because in all of the uncontaminated samples the concentrations of unsaturated hydrocarbons are two to three orders of magnitude greater than those of the saturated hydrocarbons. Thus, addition of equal amounts of contaminants of both fractions would have a greater effect upon the saturated fraction than on the unsaturated. Nonetheless, in this study it appears that the hydrocarbon contamination was primarily in the saturated fractions of the 4 samples which were anomalous.

This is shown in Table 3 in which concentrations of saturated and unsaturated frac-

tions of representative samples are given relative to the dry weights of the samples. In

Organism	Saturated Hydrocarbons		Unsaturated Hydrocarbons
	Peaks	UCM	
Penaeus setiferus	5	0	896
Penaeus duoraroum	3	0	3299
Trachypenaeus similis	4	0	915
Squilla chydea	3	0	233
Squilla empusa	2	0	840
Trachypenaeus similis	55	105	592
Squilla chydea	30	60	234

Table 3. Comparison of Hydrocarbon Concentrations in Uncontaminated and Contaminated Crustacean Samples. Concentrations given in µgm hydrocarbons per gram dry weight of whole animal. UCM = unresolved complex mixture (see ref. 5).

uncontaminated samples, the concentration of resolved peaks in the saturated fractions totals between 2 to 5 µgm/gm. No unresolved complex mixture is present in any of these samples. In contaminated samples, the concentrations of total resolved peaks are 10 times higher than in the natural populations, and unresolved complex mixtures which comprised about two-thirds of the total saturated hydrocarbons are present. However, no increases in unsaturated hydrocarbon concentration are found. In Squilla chydea, for example, this concentration is 233 µgm/gm in the pristine sample and 234 µgm/gm in the contaminated one. Furthermore, as indicated in Figure 1, no unresolved complex mixture is displayed in the chromatogram of the unsaturated fraction of this or any of the other 3 contaminated samples. Thus, it seems that most, if not all, of the contamination is composed of saturated hydrocarbons.

Conclusions

Analysis of the hydrocarbon compositions of these 70 samples of crustaceans revealed only minor differences related to exploratory drilling operation. The samples contained both amounts and types of hydrocarbons which were relatively invariant, with the exception of 4 samples. These 4 samples, two of which were collected together, indicated petroleum hydrocarbon contamination. Because such contamination does not correlate well with either presence or absence of the drilling rig, it is difficult to conclude that contamination of these samples arose directly from drilling operations. It may be that this contamination occurred as a result of release of petroleum products used as fuels either by the rig itself or by ships around the rig.

Regardless of the specific source of the petroleum hydrocarbons, it is interesting that contamination was not detected prior to arrival of the drilling rig yet was found subsequent to its departure. Evidently, the presence of the rig resulted in contamination of parts of the benthic community by petroleum. It is significant that such contamination was readily detectable some 6 weeks after the rig and its associated ship activity had left the area. This suggests that the petroleum hydrocarbons had been incorporated into the tissues of the affected organisms and were not coatings which could be washed away. Thus, these materials had entered the local benthic food web and potentially could have sublethal deleterious effects upon these shrimps and their predators.

This study has demonstrated the usefulness and efficacy of gas chromatography in identifying petroleum hydrocarbon contamination of organisms. It has also shown that in crustacean samples it is not feasible to monitor such contamination by determining hydrocarbon concentrations gravimetrically, because the amounts of saturated hydrocarbon contaminants found were very small relative to the total (saturated plus unsaturated) hydrocarbons in many of the samples. Finally, it must be observed that while gas chromatography is quite capable of detecting pollution, it would be scientifically highly desireable to identify the natural hydrocarbon components of samples such as those in this study by combined gas chromatography/mass spectrometry.

Acknowledgements

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