Distribution of *n*-Parrafins in Selected Marine Benthic Organisms

Choo-Seng Giam, Hing S. Chan, and Grace S. Neff
Department of Chemistry, Texas A&M University
College Station, Tex. 77843

The South Texas Outer Continental Shelf in the Gulf of Mexico has recently been approved for leasing for oil exploration, thus providing an excellent area for studies on the effects of oil exploration and drilling on the marine environment before, during and after such activities. In order to determine the effects of this activity on the heavy hydrocarbon content of benthic organisms, we conducted a survey of the n-parrafin content in several species of benthic epifauna. Since there is little information on the hydrocarbon content and distribution of most species of marine organsims (MOORE et al., 1973), the data obtained from these analyses should not only be important for this study, but should also be useful in forming a data base for assessing whether organisms of the same species from other areas have been contaminated by oil.

For these studies, organisms representing three different phyla (squid, shrimp and fish) were analyzed for n-parrafins. The specimens were collected at a number of stations in the proposed lease area (see Figure 1) in January and April, 1974. The analyses were performed as described below using appropriate modifications of accepted procedures (GIAM and WONG, 1972; GIAM et al., 1975; FARRINGTON et al., 1972; FARRINGTON and MEDEIROS, 1975; WARNER, 1975).

EXPERIMENTAL

Materials*

Solvents used in the procedure were Mallinckrodt Nanograde^{R} and were used as received or re-distilled when required. Silica gel (Woelm, 70-230 mesh) was Soxhlet extracted with hexane and activated at 150° for at least 24 hr. before use. Hydrocarbon standards were obtained from Analabs, Inc.

Instrumentation

A Hewlett-Packard 5830 GC equipped with dual flame ionization detectors and a programmable integrator was used for analyses. It was equipped with 6' x 1/8" stainless steel columns of 5% FFAP or 3% SE-30 on Gas Chrom Q 100/120. The injector was at 270° and the detector at 350°. The column oven was temperature programmed from 100° to 260° at 6°/min.

*Trade names of reagents, solvents and equipment and addresses for sellers are included to facilitate recognition by interested users of what we happen to use; there is no implication that these are solely recommended.

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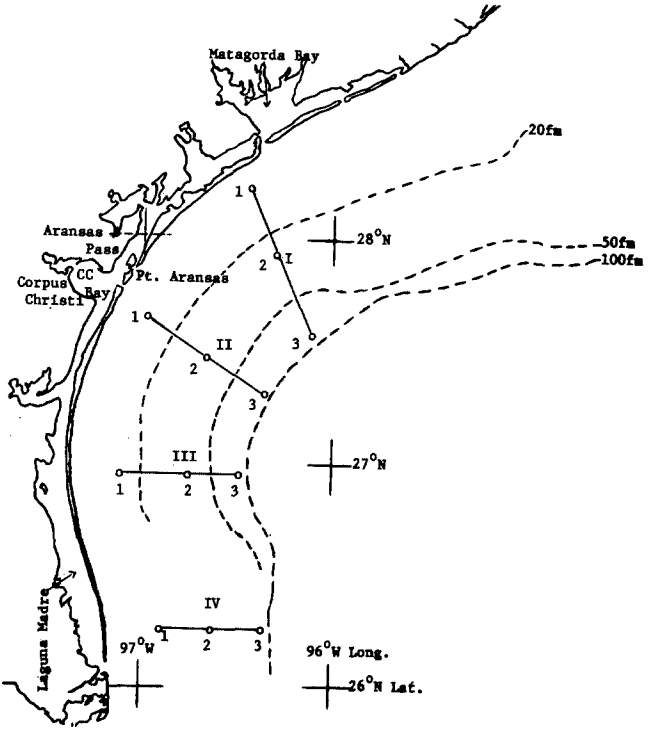

Figure 1. Station and Transect Locations for the Texas OCS Study.

Procedure

The procedure for analysis is outlined in Figure 2. Prior to analysis, all glassware and equipment was washed with Micro cleaning solution (International Products Corp.), rinsed with distilled tap water and acetone and heated at 320° overnight. Just prior to use, the glassware was rinsed twice with methanol and then with hexane. The final hexane rinse was concentrated and checked on the chromatograph. If any impurities were present, rinsing was repeated as needed to obtain an acceptable blank. Procedure blanks were also performed at intervals to insure the absence of contamination from reagents and solvents.

The samples, after defrosting for a short period (1-2 hr) were transferred to tared 250 ml round-bottom flasks. Small samples were used whole, while larger samples were cut into smaller pieces as needed for transfer into the flasks. After weighing, the samples were treated with potassium hydroxide (0.05 g/g tissue) and 50 ml. of methanol. The samples were then heated under reflux for 2 hr. At the end of this period, the contents were inspected and if the digestion of the tissue was not complete, heating was continued until no tissue remained.

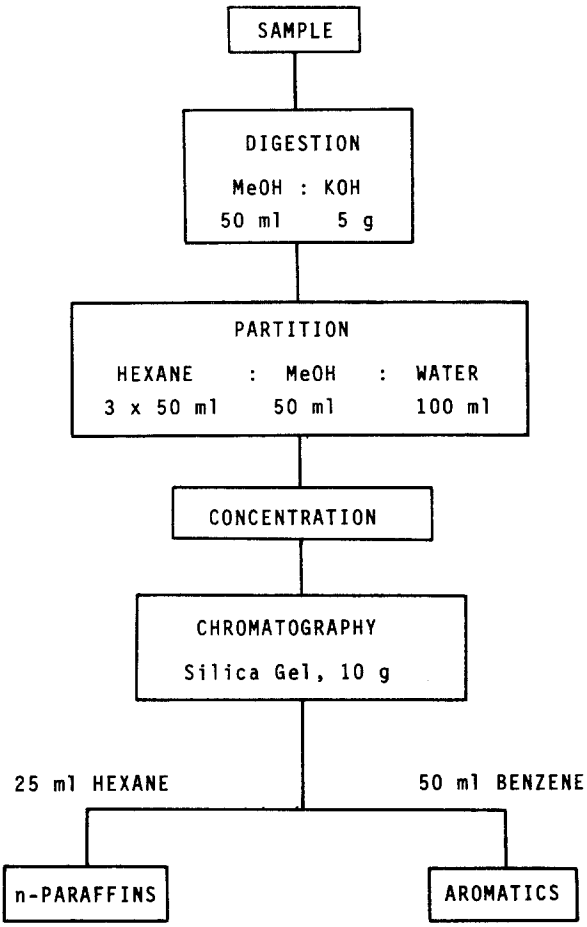

Figure 2. Analysis Scheme for n-Parrafins in Selected Benthic Organisms.

The methanolic hydrolysate was then transferred to a 250 ml. separatory funnel. The extraction flask was rinsed with 50 ml. of hexane which was transferred to the separatory funnel. Approximately 100 ml. of 5% NaCl in water was added to the funnel and the mixture shaken. After allowing for the separation of the hexane layer, the aqueous layer was drawn off and the hexane was transferred to a Kuderna-Danish concentrator. The aqueous layer was extracted with two more 50 ml. portions of hexane. The combined hexane extracts were then washed with salt water to remove methanol and concentrated to ca 5 ml. with steam.

The concentrated extract was then transferred to a silica gel column (10 g., 1.1 x 22 cm) that was covered with ca 1 g. of sodium sulfate and that had been washed with 50 ml. of hexane. The column was then eluted with 25 ml. of hexane to obtain the n-parrafins. The eluted extract was then concentrated as needed for gas chromatography by a stream of nitrogen.

The compounds present were determined by comparison of their retention times with authentic standards on columns of FFAP and of SE-30. Quantitation was performed by comparison of integrated areas with those obtained from standards.

RESULTS AND DISCUSSION

Prior to actual sample analyses, procedure blanks and recovery studies were performed. By the use of prechecked reagents and solvents and careful cleaning of all glassware and equipment, procedure blanks containing negligible quantities of hydrocarbons were obtained; (for a more detailed discussion on general decontamination procedures for the trace analyses of marine samples, see GIAM and WONG, 1972 and GIAM et al., 1975). An example is shown in Figure 3. Recovery studies were performed by adding known amounts of hydrocarbons to previously analyzed tissues; routine recoveries of 90 to 100% were attained.

Gas chromatography was used to quantitate the hydrocarbons present. Using the conditions described, the calibration curve shown in Figure 4 was determined. As opposed to a previous report (CLARK, 1974), a decline in sensitivity with increasing molecular weight of the hydrocarbons was not observed. However, this decreasing sensitivity was noted if the detector was allowed to become contaminated. The use of both FFAP and SE-30 columns not only provided confirmation of the compounds; SE-30 provided better quantitation of the higher n-parrafins while FFAP yielded a quantitatable separation of the n-C-17 hydrocarbon and pristane (Compare Figures 3a and 3b).

The results of the analyses are plotted in Figure 5 as carbon number versus % composition. The values plotted represent the highest and lowest % concentrations of the reported hydrocarbons (C_{14}-C_{34}) found in individual members of the species. The total hydrocarbon content varied from an average of 0.04 ppm for shrimp (muscle only) to 1.71 ppm for wenchmen with high variation within species. In spite of this variation, a number of important and useful generalizations can be observed from the data.

All of the organisms studied had relatively high concentrations of the C-15 and C-17 n-parrafins or of the C-31 compound or both. (Pristane was present in all samples in high concentrations and was not included in these results.) Shrimp were unique with respect to the C-15 and C-17 parrafins; these hydrocarbons were absent or in very low concentrations in shrimp but were present in the highest amounts in the other species studied. In squid, C-17 was generally found in higher concentrations than the C-15 n-parrafin while C-15 dominated in fish; however, these ratios did vary or invert for some individual samples and at present, the reasons for these variations (seasonal, physiological etc.) are not available. In contrast, all samples of wenchmen exhibited a higher percentage of C-15 than C-17.

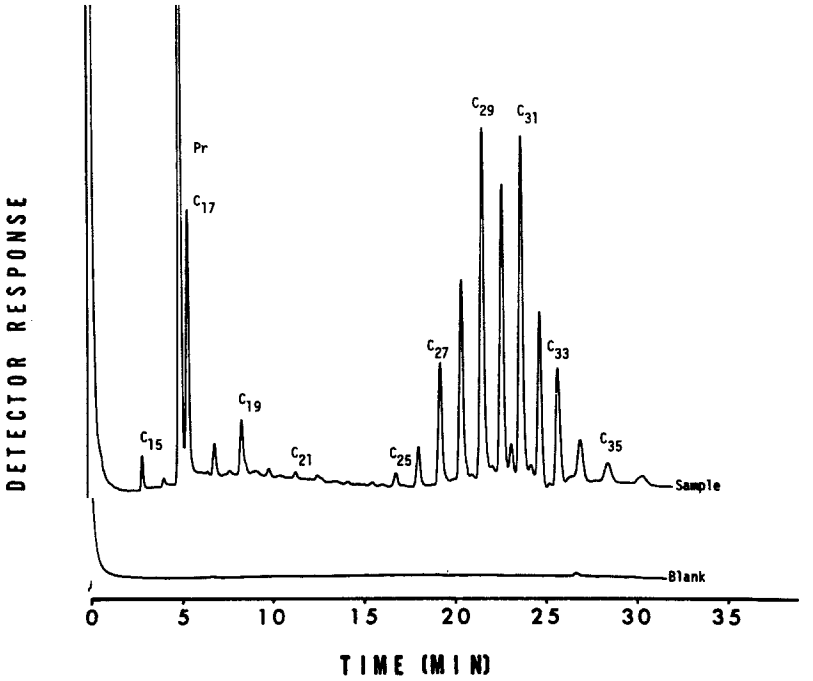

Figure 3a. Gas Chromatogram of Hexane eluate of Sand Trout (B38C) extract (n-paraffins) on 5% FFAP.

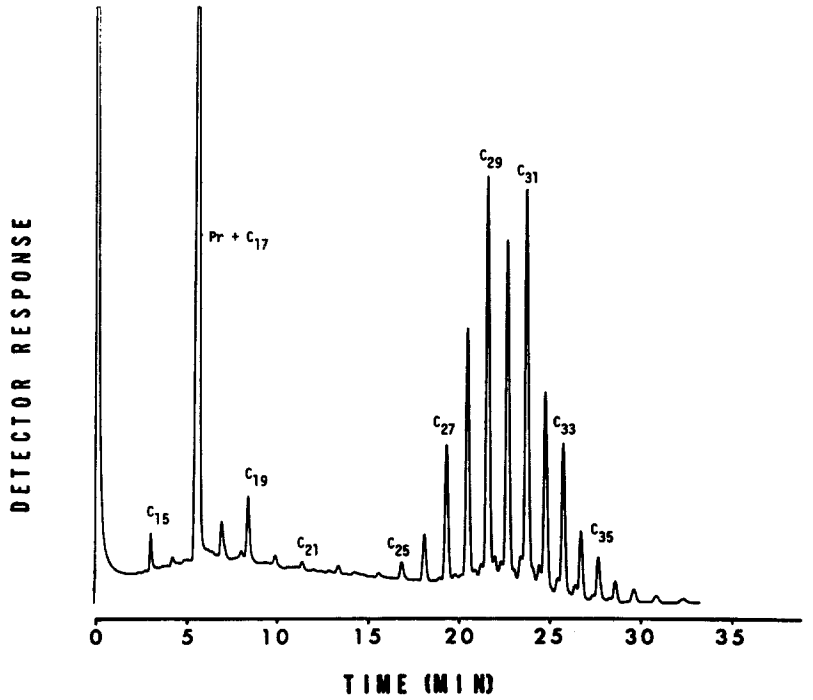

Figure 3b. Gas Chromatogram of Hexane eluate of Sand Trout (B38C) extract (n-paraffin) on 3% SE-30.

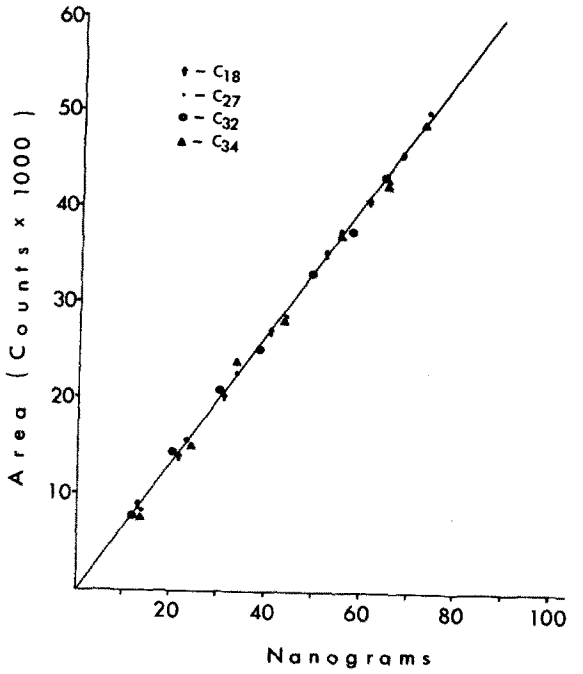

Figure 4. Calibration Curve.

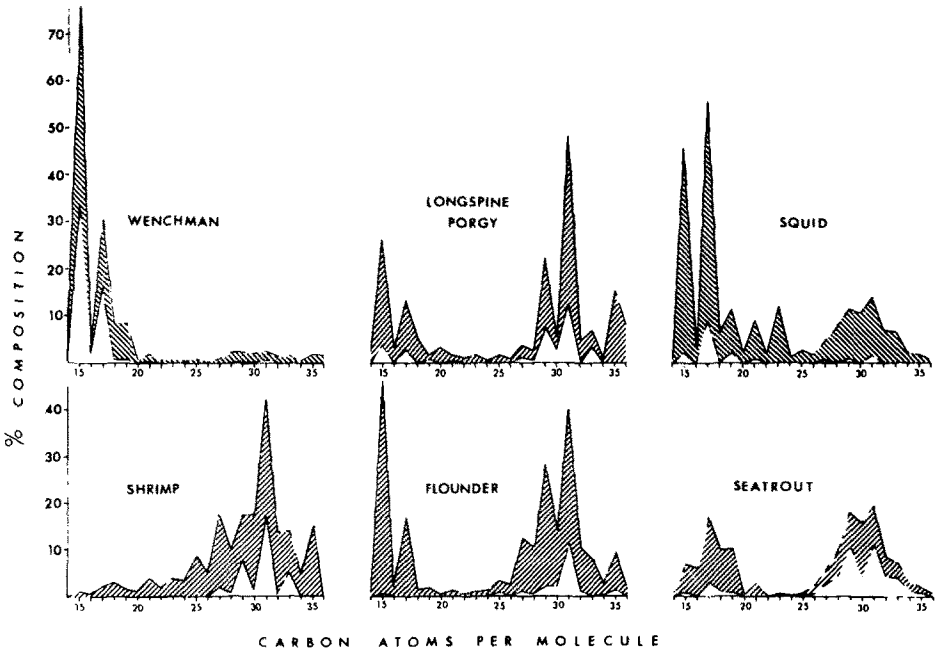

Figure 5. Hydrocarbon Distribution in Selected Benthic Organisms.

Shrimp and wenchmen samples appeared to exhibit the least intraspecies and seasonal variation relative to their distribution of n-parrafins. Thus, we recommend the use of the overall distribution in addition to the odd/even ratios of hydrocarbons for baseline monitoring of hydrocarbon content. These patterns also provided the "taxonomic paraffin fingerprint" for these organisms. We are investigating taxonomic fingerprints of other organisms for use as sentinal organisms for marine pollution. Thus, shrimp and wenchman should be very useful for the detection of petroleum hydrocarbon contamination as they provide consistent present baseline profiles for subtraction from future profiles.

ACKNOWLEDGEMENTS

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