ECOLOGICAL RELATIONSHIPS BETWEEN MARINE MICROORGANISMS AND HYDROCARBONS IN THE OEI STUDY AREA, LOUISIANA

by Carl H. Oppenheimer, Russell Miget, and Howard Kator

ABSTRACT

Seven cruise exercises were conducted as one component of the Offshore Ecology Investigation (OEI) to determine the relationships between microorganisms and hydrocarbons in surface waters. Techniques were developed to take surface samples and to determine bacterial numbers, hydrocarbon content, and the response of indigenous microorganisms to various added hydrocarbon molecules. The numbers of heterotrophic bacteria as determined by dilution techniques varied from 10 to 10,000 per ml. The hydrocarbon-oxidizing organisms varied from 10 to 100,000 per liter with maxima in the month of January. Hydrocarbon concentrations varied from 0.03 to 0.6 μg/l. There was no correlation between the numbers of oil degraders and hydrocarbon concentrations found. This may be the result of the low concentrations of hydrocarbons *in situ*, which would limit microbial response. The numbers of bacteria and concentrations of hydrocarbons in the OEI study area were similar to those found in control areas in the eastern and western parts of the Gulf. Biological oxygen demand (BOD) experiments conducted to measure the response of the microorganisms to various low and high molecular weight paraffinic and aromatic hydrocarbons

Carl Oppenheimer is with the Marine Science Institute, the University of Texas at Port Aransas, Port Aransas, TX. Russell Miget is with the Texas A&M University Research and Extension Center, Corpus Christi, TX. Howard Kator is with the Virginia Institute of Marine Science, Gloucester Point, VA.

indicated that all hydrocarbons were oxidized by some of the mixed indigenous microorganisms. These data suggest that an adequate inoculum was naturally present to respond to oil contamination in nature.

The dominant saturated hydrocarbons and isoprenoids ranged from C_{15} to C_{41}. Water from Timbalier Bay generally contained higher concentrations than offshore samples and exhibited a more pronounced preference of odd over even in the range C_{24} to C_{33}. Gas Chromatograph-Mass Spectrometer (GC-MS) analysis of the dominant paraffins revealed a characteristic bimodal distribution dominated by C_{17} and pristane and C_{25} to C_{35} with an unresolved envelope. No buildup of specific *n*-paraffin molecules was found, a finding supported by the BOD test results where hydrocarbons were added to indigenous microflora.

INTRODUCTION

The ecological significance of the occurrence and distribution of hydrocarbons in a marine environment cannot be considered without discussing the role of microorganisms. While such hydrocarbons in the form of crude oil have been commercially exploited from geological formations since the turn of the century, it was not until the advent of the gas chromatograph that hydrocarbons were found in recent sediments (Smith 1952). ZoBell (1945, 1946a, 1946b, 1950, 1952) and Oppenheimer (1965) have discussed the role of microorganisms in the formation and transformation of hydrocarbons in the marine environment and sediments. Stone and ZoBell (1952) first showed that up to 0.25% of the dry weight of petroleum was produced by several species of microorganisms. Hydrocarbons found in organisms such as fungi (Weete 1972), plants (Stransky et al. 1967), and marine phytoplankton (Blumer et al. 1971) clearly show that hydrocarbons are a common constituent of all living organisms at levels from 10 ppm to 1%.

Meinschein (1959, 1969) has reviewed the origin of hydrocarbons and the natural diversity of saturated, unsaturated, and aromatic species. A summary of world distribution of hydrocarbons in marine waters is given in Myers and Gunnerson (1976).

Up to the time of the OEI, little published work was available on the distribution of hydrocarbons and hydrocarbon-oxidizing microorganisms as related to any given ecological system.

ZoBell (1969) reported on the distribution of hydrocarbon bacteria in water and sediment in Barataria Bay, Louisiana, the Texas coast, and the California coast. The numbers of bacteria ranged from 0.1 to 10^8 per ml of water or mud. Other reports on oil-microbe distribution are pre-

sented in the Symposium on the Microbial Degradation of Oil Pollutants (Ahearn and Myers 1973) and table 9 of this paper.

The calculated yearly potential of hydrocarbon production by marine phytoplankton for the world's oceans, using a level concentration of 10 ppb, is approximately 8×10^7 bbls. Estimates of man's introduction by all sources to the oceans is 9×10^6 bbls per year. The report by Myers and Gunnerson cited above indicates a residual hydrocarbon concentration in the waters of the world's oceans to be about 10 ppb or 8.6×10^9 bbls. This is roughly 100 times the annual production from phytoplankton and 1,000 times the annual input from human sources.

It is generally accepted by microbiologists working in the field that the action of hydrocarbon-degrading microorganisms prohibits the accumulation of hydrocarbons in aquatic systems. Davies and Hughes (1968), Crow et al. (1974), Byrom and Beastall (1971), and others have demonstrated the mechanism and rate of degradation of crude oils by various microorganisms. Several reviews on the subject have been published, including those by Butler and Berkes (1972), Gholson et al. (1972), and McKenna and Kallio (1965).

Most of the experimental work on hydrocarbon-oxidizing microorganisms has been accomplished in the laboratory using cultures obtained from various areas historically exposed to oil. No known mechanisms have been shown for anaerobic decomposition; supporting the hypothesis that there is none is the persistence of large quantities of oil in ancient sediments.

This natural production and the accidental addition of hydrocarbons in the marine environment have been balanced by the oxidative activities of several species of microorganisms (Davis 1967). A review of the distribution of hydrocarbons in the waters of the world's oceans by Myers and Gunnerson (1976) suggests persistent levels at the threshold of microbial activity. The low levels of individual molecules, that is, 1 to 10 ppb, suggest that there has been no buildup through the ages of any persistent molecules. It is generally believed that microbial activities have alleviated accumulation of hydrocarbons at high levels except, of course, for the accumulation of crude oil and natural gas in ancient sediments.

The objective of the OEI investigation was to determine the relationship between the distribution of microorganisms and hydrocarbons (1) to detect and evaluate any differences between the offshore platforms and the control site and between the OEI area and areas remote from oil activities, and (2) to evaluate the responsiveness of the OEI area to biological degradation of crude oil contamination.

For most of the microbiological studies, samples were taken at depths of approximately 1.0 m. It was expected that the upper meter of

the water column would be well mixed and would probably contain higher concentrations of hydrocarbons than would be found at greater depth in the water column. Yet it was considered desirable to avoid using samples from the air-water interface because it was assumed that, although greater concentrations of hydrocarbons would occur there, the higher spatial and temporal variabilities would reduce the value of comparisons. Also, more detailed investigations of hydrocarbons occurring at the interface were being made under another OEI component investigation in order to determine possible platform-control differences. These "surface" samples were used, then, to enhance the probability of observing differences in the populations of hydrocarbon degraders and other heterotrophs that might be the result of higher hydrocarbon concentrations than could exist near the platforms. Of particular interest were possible increases in the population of hydrocarbon degraders with increasing oil concentration. These studies were supplemented with BOD determinations using samples taken from comparatively large areas of the interface (depths of less than a few cm). In addition to making the platform-control comparisons, we compared the populations of hydrocarbon degraders and other heterotrophs and concentrations of hydrocarbons in the OEI area with those in areas remote from oil activities, using our measurements and those reported by others. Laboratory studies of microbiological activity using water/oil/microbe samples taken from the OEI study area were conducted further to evaluate responsiveness to microbial degradation.

For comparions of hydrocarbon concentrations *per se*, measurements were made at depths of 1.0 and 4.0 m. These were compared with measurements for remote areas in the Gulf and in the world's oceans. The remote areas were far removed from oil drilling and production activities. Again, it was considered preferable to avoid the high spatial and temporal variabilities that would occur if surface samples were used.

Hydrocarbons, being hydrophobic and insoluble, will remain at the air-water interface until they are physically absorbed by heavier particles, reduced to more dense asphaltic compounds, emulsified in the water, or ingested by larger organisms. Devices were developed to take surface samples for both hydrocarbons and microorganisms. Analyses for microorganisms were conducted aboard the research vessel. BOD experiments to determine the response of indigenous microorganisms to added hydrocarbons were also conducted on board. Hydrocarbon analyses and the testing of degradation rates and oil changes by mixed cultures obtained from the enumeration studies were conducted at the Port Aransas Laboratory.

The research area selected for the OEI is one of the most prolific offshore oil-producing areas in the world. Development began in the late

1930s, and in August 1972 there were nearly 6,000 wells working from 1,900 platforms. Through the end of 1973, approximately 3 billion barrels of oil had been produced in the area. In addition, the area has been historically exposed to continuous tanker traffic. During World War II, sinkings in the area from May through July 1942 released an estimated 700,000 barrels of oil. The historical picture of oil production and exposure in the 400 square mile study area provides an excellent setting in which to determine the effects of oil production on a coastal environment.

This paper provides data on (1) the distribution and ecology of hydrocarbon-oxidizing and other heterotrophic microorganisms in the Louisiana coastal environment, and (2) the content and the molecular forms of hydrocarbons in the water column.

METHODS AND MATERIALS

Water sampling methods and stations

The locations of stations where water samples were taken for microorganisms and hydrocarbon analyses are presented in tables 1 and 2 and figures 1 and 2 (pp. 294-295).

Considerable emphasis was placed on obtaining Gulf control stations (50 to several hundred miles from the study sites) for comparative purposes. The designated control area, being only 5 miles from the platform site, might not accurately reflect baseline or background differences in microbial populations or metabolic activities free from the influence of oil drilling and production.

Water samples for hydrocarbons were collected at depths from 1 to 4 m from the surface. Hydrocarbons in surface films were collected with an apparatus described in Miget et al. (1974). All possible care was exercised in using techniques designed to prevent contamination from the ship or from handling.

Hydrocarbons in the water were sampled in a clean 5-gallon glass bottle in a metal frame. The device, attached to hydrographic wire, was dropped into the water over the side of the vessel and allowed to fill. On return to the surface, 100 ml of benzene were added to preserve the sample and a stopper coated with Teflon was used to close the bottle. The sample with benzene was mixed and not opened until extraction took place.

Surface water for BOD studies and bacterial enumeration was collected using a sterilized sampler developed as part of this investigation. The sampler, depending on the sea surface condition, collected the topmost 0.5 to 1.5 cm of the water column. The sampler

TABLE 1 - OEI MICROBIOLOGICAL PROGRAM SAMPLING STATIONS

YEAR	MONTH	STA. #	LOCATION	AREA	GURC LOC.
1972	June	1	28.34 N 93.00 W	N.W. Gulf coast	None
		2	28.32 N 90.18 W	OEI study area	P072
		3	27.37 N 88.30 W	N. Central Gulf	None
		4	27.11 N 85.40 W	N.E. Gulf	None
		5	27.20 N 84.11 W	E. Central Gulf	None
		6	24.46 N 83.16 W	Dry Tortugas area	None
		7	23.53 N 84.08 W	S.E. Gulf	None
		8	24.49 N 88.00 W	Central Gulf	None
1972	Sept.	1	28.51 N 92.10 W	S. Marsh Is. area	None
		3	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		4	28.53.13 N 90.19.30 W	Desig. control	H062
		6	28.48.30 N 90.27.30 W	S.W. Plat. 54A	K023
		8	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		9	28.53.13 N 90.19.30 W	Desig. control	H062
1973	Jan.	1	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		4	28.53.13 N 90.19.30 W	Desig. control	H062
		5	29.12.20 N 90.21.30 W	Tim. Bay	A079
		6	28.54 N 90.45 W	Ship Shoal area	None
		7	29.39 N 93.11 W	East of Sabine R.	None
		8	29.30 N 94.01 W	West of Sabine R.	None
1973	April	1	29.06 N 93.59 W	West of Sabine R.	None
		2	28.50 N 91.20 W	Ship Shoal area	None
		3	28.49.53 N 90.23.18 W	S. Tim 54A (upstrm)	K007
		5	28.49.53 N 90.23.18 W	S. Tim 54A (dnstrm)	K007
		6	29.13 N 90.00 W	11 mi off Grand Is.	C070
		7	28.49.53 N 90.23.18 W	S.T. 54A (Dnstrm)	K007
		8	28.53.13 N 90.19.30 W	Desig. control	H062
		16	29.12.20 N 90.21.30 W	Tim. Bay	A079
1973	July	1	28.55 N 95.05 W	Off Galveston, Tx.	None
		2	29.13 N 93.42 W	West of Sabine R.	None
		4	28.30 N 91.10 W	Ship shoal area	None
		5	28.49.53 N 90.23.18 W	S. Tim 54A	K007
		7	28.53.13 N 90.19.30 W	Desig. control	H062
		8	29.12.20 N 90.21.30 W	Tim. Bay	A079
1973	Oct.	1	29.07 N 92.52 W	East Cameron area	None
		4	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		7	29.12.20 N 90.21.30 W.	Tim. Bay	A079
		6	28.53.13 N 90.19.30 W	Desig. control	H069
1974	Jan.	1	28.53 N 94.06 W	N.W. Gulf	None
		4	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		5	28.53.13 N 90.19.30 W	Desig. control	H062
		6	29.12.20 N 90.21.30 W	Tim. Bay	A079

TABLE 2 - n-PARAFFINS IN SEAWATER (Depths average from 1 to 4 meters, water unfiltered)

Date	Station	Location	Concentration Total, g/l	Unresolved Envelope	Major Paraffins and Isoprenoids*
9-72	K040 S.E. 54A	28°50' 90°30'	0.35	present	C-25 to C-33 .
9-72	H062 Control	28°53' 90°19'	0.21	n.a.	C-15,16,17+pristane, 22,26,27, 28,29
9-72	K040 S.E. 54A	28°50' 90°30'	0.49	present	C-24 to C-33
9-72	K007 54A	28°49' 90'23'	0.34	present	17+pristane,26,27,28,29,30, 31,32
1-73	Timbalier Bay	29°12' 90°21'	0.46	present	C-25,27,28,29,30,31,32,33
1-73	Timbalier Bay	29°12' 90°21'	0.38	present	C-25,26,27,28,29,30,31,32,33
1-73	H062	28°53' 90°19'	0.05	present	C-22,25,26,27,28,29,30,31,32,33
1-73	H062	28°53' 90°19'	0.20	present	C-17+pristane, 18+phytane 19,20,28
1-73	E.Sabine R.	29°40' 93°11'	0.12	present	C-17+pristane, 18+phytane 19,20,21,22
1-73	W.Sabine R.	29°30' 94°01'	0.12	present	C-24,26,27,28,29,30,31,32,33
1-73	Ship Shoal	28°54' 90°45'	0.19	present	C-20,21,22,29,30,31,32,33,34,35
1-73	W.Sabine R.	29°30' 94°01'	0.22	present	C-18+phytane,20,21,22,23,24
1-73	K014 S.W.Plat. 54A	28°50' 90°30'	0.60	present	C-28,29,30,31,32,33,34,35
1-73	K014	28°50' 90°30'	0.19	present	C-17+pristane, C-18+phytane, C-19,20,28,29,30,31,32
1-73	K007 54 Plat.	28°49' 90°23'	0.13	present	C-17+pristane, C-18+phytane, C-19,20,21,22,23,28
4-73	Timbalier Bay	29°12' 90°21'	0.10	present	C-17+pristane, 18+phytane 19,20,22
4-73	Timbalier Bay	29°12' 90°21'	0.37	present	C-17+pristane, 19,27,29,31,33
5-73	P.Aransas 5 mi off-shore	27°46' 92°52'	0.07	absent	C-18+phytane, 28,29,30,31,32, 33,34
7-73	H062	28°53' 90°19'	0.05	absent	15,17+pristane, 18+phytane
7-73	29·03 95 52	29°03' 95°52'	0.09	absent	17+pristane, 18+phytane
7-73	W.Sabine R.	29°30' 94°01'	0.03	absent	17+pristane, 29,31,33,34,35,37
7-73	1.8 mi from Tim.Is.	- -	0.05	absent	17+pristane, 31,34,35,38
7-73	Browns-ville	26°10' 96°40'	0.09	present	15,17+pristane, 18+phytane
10-73	Timbalier Bay	29°12' 90°21'	0.20	present	15,17+pristane, 18+phytane, 19,21,23
1-74	Timbalier Bay	29°12' 90°21'	0.08	present	17+pristane, 18+phytane, 23,25,29,31
1-74	K007 54A	28°49' 90°23'	0.08	absent	17+pristane, 18+phytane, 19,28,29,30,31,32
1-74	H062	28°53' 90°19'	0.09	present	17+pristane
4-74	P.Aransas 5 mi off-shore	27°46' 92°52'	0.07	present	C-17+pristane, 18+phytane, 31,32,33,34,35

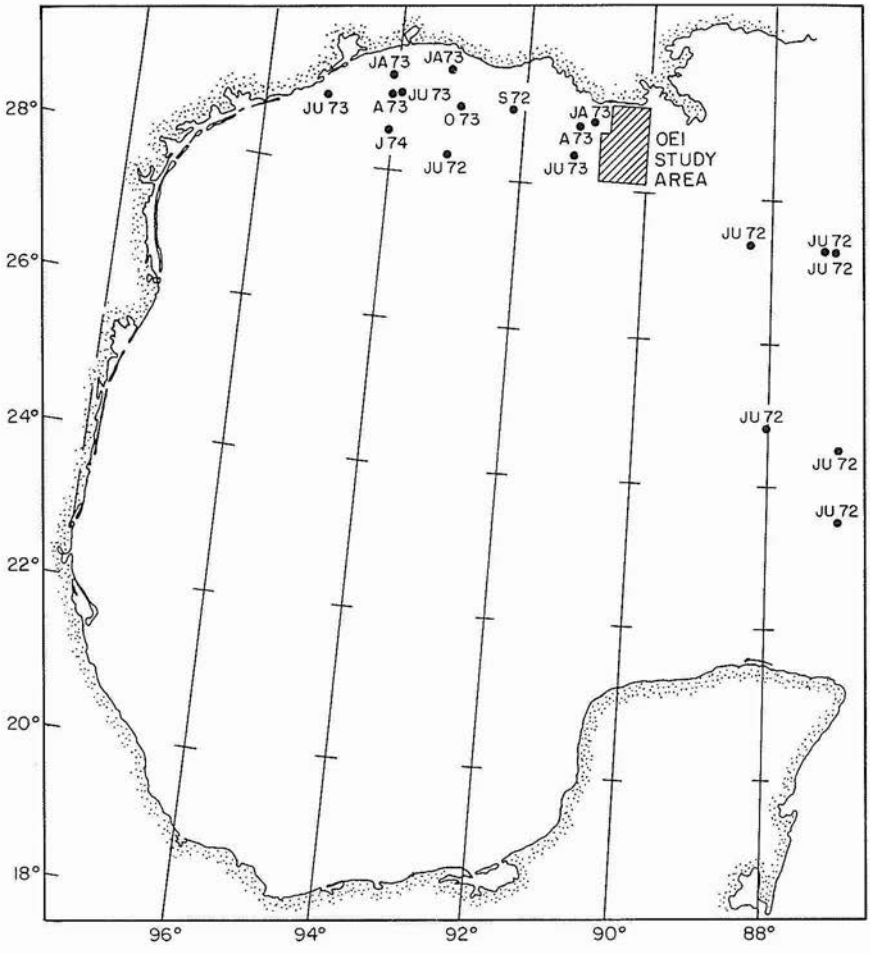


FIG. 1. MICROBIOLOGICAL GULF CONTROL STATIONS.

consists of a stainless steel funnel perforated around its upper perimeter with 0.2 mm diameter holes, which were sandwiched between two circular discs of epoxy-coated plywood that provided flotation at the surface. The apex of the funnel was connected to 50 feet of 1/4″ I.D. silicon tubing, which was connected to a vacuum flask aboard ship. A 1/16″ line connected to the sampler was attached to a boom amidships. It was then gently dropped onto the water and pulled to the end of the boom amidships. The ship was oriented so that the sampler was continuously pushed into clean water. When the floating sampler was at the desired position, a vacuum was pulled on the sterile sampling flask

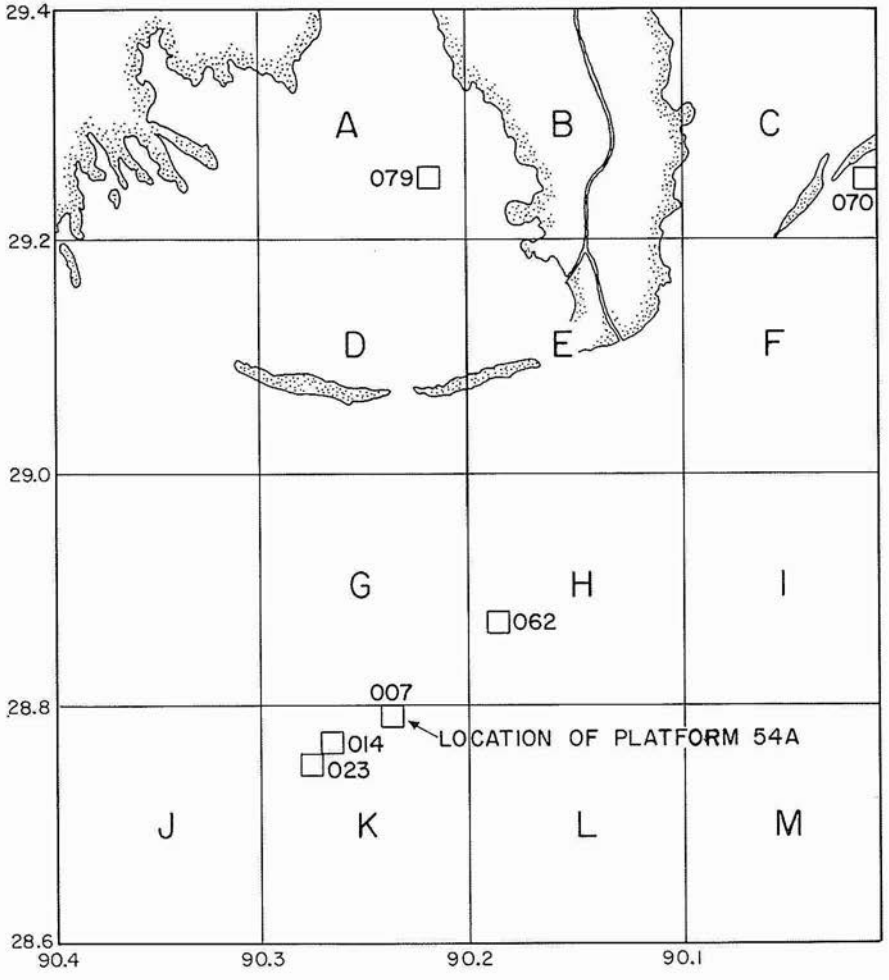


FIG. 2. MICROBIOLOGICAL STATIONS WITHIN OEI STUDY AREA. The numbered quadrants identify the areas where samples were collected.

for bacteria or the 20-liter bottle for BOD, causing the surface water to pass through the perforated top of the funnel between the plywood discs into the flask or bottle.

The same silicon tubing and vacuum flask arrangement was used for subsurface water sampling for bacteria. A sterile glass tube was inserted into the end of the tubing and a sterile weight (hydrocast messenger) was attached to the end of the glass tubing. A tension line was attached to the weight and tubing was dropped to the desired depth.

The tubing was flushed by discarding the first two liters of water, after which the desired volume of sample water was drawn into a sterile vacuum flask.

Hydrocarbon extraction techniques

Hydrocarbons were collected by a liquid-liquid extraction technique performed in the collection bottle. The mouth of the bottle was tapered for a Teflon collar. Heptane (300 ml) was added to the refluxing chamber and heat was applied. The vapors of the heptane passed up the column and down into the bottom of the mixed water. The heptane was released in small bubbles and mixed through the water. It slowly coalesced and floated to the top, whence it returned to the reflux chamber through a constant leveling device. Hydrocarbons were extracted during a 24-hr reflux period. Contamination from the air was prevented by a silica gel filter at the top of the reflux unit.

All solvents were double-distilled to remove contaminants. A 20 μl aliquot of a 0.1 μg/μl nC$_{36}$ internal standard solution was added to the sample. Upon completion of the extraction, the heptane in the extraction flask was evaporated to near dryness on rotary evaporator (40-50° C). The residual solvent was transferred to a 100 ml pear-shaped flask and a small volume of heptane (300 μl) was added. The extract was passed through a pre-washed chromatographic column containing 1 ml of activated silica gel (100-200 mesh, high purity grade, heated for 12 hrs at 200° C, and deactivated with 5% water). Saturated paraffins were eluted with 1.5 ml of heptane (3 column volumes) and aromatic-naphthenic hydrocarbons with 1.5 ml of benzene. Ninety to 95% of n-paraffins in a standard mixture with chain lengths greater than nC$_{18}$ were routinely recovered from seawater.

Hydrocarbon extracts were analyzed with a Finnigan 1015C GS-MS for compound identification. Using multiple channel ion monitoring, we selected ions for routine analysis: paraffin ions, 57 m/e and 71 m/e; isoprenoid ion, 113 m/e; olefin ion, 69 m/e; and tropylium ion, 91 m/e. This procedure is useful in the analysis of complex hydrocarbon extracts. Hydrocarbons are identified by a combination of their characteristic retention indices on columns of differing polarity with respect to authentic standards, multiple channel ion monitored mass chromatograms, and mass spectra when feasible.

Enumeration of oil-oxidizing marine bacteria and "total" heterotrophs in the field

Microbiological assays were performed aboard ship immediately after the water samples were collected. The "total" (aerobic-proteolytic) microbial population and the number of hydrocarbon-degrading bacteria

were enumerated. "Total" heterotrophs were determined on spread plates and/or membrane filters using 2216E marine medium (ZoBell 1946a). Colonies were counted after one week incubation in the dark at 22-24° C. Populations of hydrocarbon-degrading bacteria were enumerated using the following modified dilution technique: (1) Sample volumes of 0.01 ml or smaller were diluted serially (1:10) in sterile seawater and the appropriate dilution tubes were then added to 50 ml of sterile nutrient salts enriched seawater (ESW) contained in 125 ml incubation flasks (Miget et al. 1969); (2) sample volumes of 0.1 to 10 ml were added directly to 50 ml of ESW in incubation flasks; and (3) sample volumes greater than 10 ml were filtered through sterile 0.45 micron membrane filters, then the filters were cut in small pieces with sterile scissors and added to the incubation flasks containing 50 ml ESW. All flasks, including uninoculated controls, then received 0.5 ml of sterilized crude oil (obtained from a wellhead in ST 54), and were incubated on a gyrotary shaker at the ambient temperature on the ship (22-24° C) for four days. Positive growth (scored minus 4 to plus 4) was indicated by visible oil emulsification, or by increase in turbidity of the water accompanied by a physical change in the oil sheen—all relative to the appearance of the oil and water in the control flasks. Although positive flasks were scored on the completeness of emulsification, the number of oil-degrading microorganisms for each sample was determined by a dilution technique based on replicate dilution flasks showing any change in appearance of the oil or water compared with control flasks.

Evaluation of bacterial hydrocarbon degradation using the biochemical oxygen demand (BOD) technique

BOD studies were carried out as follows: 19-liter sterile glass carboys were filled with sample water from the surface at a given station, shaken vigorously to insure uniform oxygen dispersion, then carefully siphoned into sterile 300 ml standard BOD bottles. Nutrient salts [$(NH_4)_2SO_4$ at 1 g/l and K_2HPO_4 at 0.01 g/l] were added to each bottle from a sterile concentrated stock solution. Hydrocarbon substrates (either pure hydrocarbons or crude oils) were added to the replicate bottles in one of the following two ways: (1) Hydrocarbons that were solid at 20° C were dissolved in benzene and added to approximately 0.1 g of clean, silicon coated, sterile micro glass beads (590-840 micron diameter) contained in small pieces of solvent-cleaned aluminum foil. The solvent was allowed to evaporate in a fume hood and the coated beads were placed in BOD bottles immediately prior to filling with seawater; (2) hydrocarbons that were liquid at 20° C were added directly to filled bottles using a microliter syringe. Hydrocarbon substrates were rapidly injected into the nutrient-enriched water near the bottom of the bottle; the syringe was

withdrawn and the bottle was stoppered before the hydrocarbon floated to the surface. The bottles were incubated upside down. All ground glass stoppers were coated with a thin film of silicon grease to insure an airtight seal. Replicate BOD bottles of water from each carboy were immediately preserved and the oxygen concentration referred to as T+O values.

The oxygen concentration in each sample was determined after incubation in the dark by the Winkler method as described in the OEI handbook of procedures and methods (Menzies 1973).

RESULTS

Hydrocarbons in seawater

The concentrations and several salient features describing *n*-paraffins and selected isoprenoid hydrocarbons for the study period are listed in tables 2, 3, 4, 5, and 6 and in figures 3 and 4. Generally, *n*-paraffins were the predominant hydrocarbons found. The isoprenoid hydrocarbons, pristane and phytane, were detected in almost all samples. Despite differences in the distribution of *n*-paraffins and isoprenoids within any one sample, the total *n*-paraffin concentrations for offshore samples were within a relatively narrow range of 0.6 to 0.03 μg/l. Estimates of total saturated paraffins based on integration of the total signal area above the solvent baseline were around one order of magnitude larger than the total paraffins, or in the 5 μg/l range. The range of *n*-paraffins found in seawater was from nC_{15} to nC_{41}. These values are similar to those presented by Myers and Gunnerson (1976).

If any seasonal trend can be discerned, it is that the total *n*-paraffin concentrations decreased from the 9-72 collection period to a minimum in 7-73. This observation is derived from data both from samples in the study area and from relatively large distances away (see table 6 for Sabine River, Port Aransas, and Brownsville). The near-shore waters of the Gulf of Mexico appear to be rather homogeneous. Values for control and platform stations were very similar during the same sampling exercises. Hydrocarbon levels in Timbalier Bay (table 5) were generally higher than offshore. This is undoubtedly related to the high levels of suspended material (algae and organic detritus) in these waters. Carbon Preference Indices (nC_{21} to nC_{33}) reveal carbon preference of odd over even for hydrocarbons in the nC_{25} to nC_{33} region in water from Timbalier Bay (figure 3). A similar preference is observed in recently synthesized hydrocarbons derived from terrestrial marsh plants such as *Batis maritima* and *Salicornia bigelovii* (Lytle, Lytle, and Parker 1973). Crude

(text continues on p. 310)

TABLE 3 - TOTAL AMOUNT AND PERCENT COMPOSITION OF n-PARAFFINS ON DIFFERENT DATES OF COLLECTION PLATFORM 54A AREA

9-72 - K007 54A Platform 4M Total n-Paraffins 0.34 µg/l Minor unresolved envelope		1-73 - K007 54A 1M Total n-Paraffins 0.13 µg/l Broad envelope present		1-74 - K007 54A 1M Total n-Paraffins 0.08 µg/l No Envelope Present	
n-Paraffins	%	n-Paraffins	%	n-Paraffins	%
15	2.0	15	3.4	15	0.0
16	0.0	16	4.0	16	0.0
17+pristane	13.1	17+pristane	8.4	17+pristane	17.6
18+phytane	2.0	18+phytane	8.4	18+phytane	10.8
19	1.0	19	7.8	19	5.6
20	1.1	20	7.6	20	4.6
21	0.7	21	5.4	21	3.8
22	1.1	22	6.6	22	2.9
23	1.3	23	5.3	23	3.4
24	3.7	24	4.4	24	3.3
25	3.4	25	4.4	25	4.9
26	8.7	26	4.4	26	4.9
27	9.5	27	4.5	27	3.8
28	9.8	28	5.7	28	5.6
29	9.5	29	4.5	29	6.1
30	8.6	30	2.6	30	6.4
31	7.9	31	3.4	31	5.1
32	6.2	32	1.4	32	5.7
33	4.6	33	2.1	33	3.4
34	2.9	34	1.7	34	3.3
35	1.8	35	1.9	35	2.2
36 Internal Std.		36 Internal Std.		36 Internal Std.	
37	0.7	37	1.9		
38	0.5				

(continued on next page)

9-72 - K040 S.E. 54A 1M
Total n-Paraffins 0.49 µg/l
Peaked envelope from C-17 on

n-Paraffins	%
17+pristane	1.7
18+phytane	0.8
19	1.8
20	2.8
21	3.0
22	4.1
23	4.5
24	5.0
25	6.3
26	7.1
27	6.2
28	7.2
29	6.5
30	6.6
31	7.6
32	6.6
33	5.9
34	4.5
35	3.6
36 Internal Std.	
37	2.1
38	1.8
39	1.7
40	1.6
41	1.0

9-72 - K040 S.E. 54A 4M
Total n-Paraffins 0.35 µg/l
Broad envelope from C-17 to C-36

n-Paraffins	%
17+pristane	1.2
18+phytane	0.2
19	2.3
20	3.5
21	2.1
22	4.6
23	4.5
24	5.1
25	6.2
26	8.9
27	6.8
28	7.5
29	6.9
30	7.1
31	7.8
32	6.6
33	6.4
34	4.9
35	4.3
36 Internal Std.	
37	3.3

1-73 - K014 S.W. 54A 1M
Total n-Paraffins 0.60 µg/l
Envelope from C-16 to C-36

n-Paraffins	%
16	1.3
17+pristane	1.9
18+phytane	0.5
19	0.7
20	0.5
21	0.6
22	0.6
23	0.6
24	0.5
25	0.6
26	0.9
27	2.8
28	5.4
29	5.1
30	5.9
31	11.3
32	12.8
33	13.9
34	11.7
35	10.4
36 Internal Std.	
37	4.9
38	3.9
39	2.5
40	1.8

1-73 - K014 S.W. 54A 4M
Total n-Paraffins 0.19 µg/l
Envelope from C-17 to C-35

n-Paraffins	%
15	2.9
16	2.5
17+pristane	7.0
18+phytane	6.8
19	6.2
20	5.3
21	3.3
22	3.4
23	2.9
24	4.1
25	4.3
26	4.6
27	3.1
28	6.6
29	6.9
30	6.7
31	8.1
32	5.5
33	3.5
34	2.1
35	2.2
36 Internal Std.	
37	1.3
38	1.2

TABLE 4 - TOTAL AMOUNT AND PERCENT COMPOSITION OF n-PARAFFINS ON DIFFERENT DATES OF COLLECTION
CONTROL AREA H062

9-72	1M
Total n-Paraffins	0.21 µg/l
n-Paraffins	**%**
15	8.7
16	6.3
17+pristane	16.3
18+phytane	3.4
19	4.3
20	0.0
21	2.6
22	5.8
23	3.6
24	2.3
25	4.4
26	5.1
27	8.1
28	5.0
29	6.2
30	4.5
31	4.2
32	3.1
33	3.0
34	2.6
35	0.4
36 Internal Std.	

1-73	1M
Total n-Paraffins	0.20 µg/l
Broad envelope present from C-17 to C-36	
n-Paraffins	**%**
15	2.2
16	3.6
17+pristane	6.7
18+phytane	15.5
19	6.1
20	6.4
21	4.1
22	4.5
23	4.9
24	3.6
25	4.0
26	3.6
27	4.0
28	5.3
29	3.7
30	4.3
31	4.9
32	3.7
33	3.4
34	2.4
35	1.5
36 Internal Std.	
37	1.3
38	0.7

1-73	4M
Total n-Paraffins	0.05 µg/l
Broad envelope present from C-20 to C-35	
n-Paraffins	**%**
17+pristane	0.0
18	0.0
19	0.0
20	0.0
21	3.9
22	10.0
23	2.5
24	3.3
25	6.0
26	8.8
27	7.4
28	5.3
29	9.3
30	8.2
31	11.6
32	9.5
33	6.8
34	4.7
35	2.8
36 Internal Std.	

Table 4 continued.

7-73	1M
Total n-Paraffins	0.05 µg/l
No envelope	
n-Paraffins	%
15	21.4
16	0.0
17+pristane	35.0
18+phytane	12.7
19	4.2
20	2.6
21	2.2
22	2.6
23	1.6
24	1.2
25	1.1
26	1.0
27	1.8
28	1.6
29	1.4
30	1.8
31	2.0
32	1.6
33	1.6
34	1.8
35	1.2
36 Internal Std.	

1-74	1M
Total n-Paraffins	0.09 µg/l
Envelope nC-17 to C-26	
n-Paraffins	%
15	0.5
16	2.6
17+pristane	9.5
18+phytane	3.3
19	1.7
20	2.4
21	3.6
22	4.7
23	8.4
24	8.4
25	6.5
26	7.9
27	7.7
28	6.4
29	6.2
30	4.7
31	4.4
32	5.0
33	1.9
34	1.7
35	1.1
36 Internal Std.	
37	1.4

TABLE 5 - TOTAL AMOUNT AND PERCENT COMPOSITION OF n-PARAFFINS ON DIFFERENT DATES OF COLLECTION
TIMBALIER BAY

1-73 Total n-Paraffins 0.46 µg/l Broad envelope C-17 to C-30	
n-Paraffins	%
17+pristane	1.4
18+phytane	2.6
19	0.3
20	0.8
21	0.9
22	3.0
23	2.0
24	4.3
25	7.3
26	4.1
27	12.7
28	7.6
29	13.3
30	6.2
31	13.7
32	6.8
33	7.2
34	3.6
35	2.3
36 Internal Std.	

1-73 Total n-Paraffins 0.38 µg/l Envelope present	
n-Paraffins	%
15	2.2
16	
17+pristane	1.7
18+phytane	2.8
19	3.7
20	3.7
21	4.2
22	4.7
23	2.4
24	4.9
25	5.1
26	6.0
27	5.7
28	6.1
29	7.2
30	7.8
31	8.8
32	7.9
33	5.7
34	4.0
35	3.0
36 Internal Std.	
37	1.1
38	1.1

4-73 Total n-Paraffins 0.37 µg/l Broad envelope present	
n-Paraffins	%
15	1.7
16	3.2
17+pristane	6.7
18+phytane	3.4
19	5.2
20	3.6
21	4.0
22	3.2
23	2.1
24	2.2
25	3.6
26	3.4
27	5.7
28	3.4
29	9.4
30	2.0
31	9.4
32	2.5
33	6.1
34	1.7
35	1.3
36 Internal Std.	

Table 5 continued.

4-73
Total n-Paraffins 1.10 µg/l
Large envelope present
C-17 to C-38

n-Paraffins	%
15	2.7
16	5.1
17+pristane	12.3
18+phytane	7.6
19	6.7
20	6.0
21	4.0
22	5.2
23	4.6
24	4.4
25	4.6
26	4.0
27	4.3
28	3.0
29	4.4
30	2.5
31	4.4
32	2.4
33	3.4
34	1.8
35	2.6
36 Internal Std.	
37	2.6
38	1.3

10-73
Total n-Paraffins 0.20 µg/l
Envelope C-20 to C-28

n-Paraffins	%
15	18.8
16	1.3
17+pristane	29.0
18+phytane	7.2
19	5.6
20	4.3
21	5.1
22	4.7
23	5.1
24	3.0
25	1.8
26	1.7
27	1.8
28	0.0
29	1.9
30	1.5
31	1.8
32	1.1
33	1.1
34	0.6
35	1.2
36 Internal Std.	

1-74
Total n-Paraffins 0.08 µg/l
Broad envelope present

n-Paraffins	%
17+pristane	19.4
18+phytane	6.2
19	3.6
20	3.2
21	3.9
22	3.6
23	7.3
24	4.7
25	5.8
26	4.7
27	4.9
28	4.6
29	5.8
30	4.9
31	5.0
32	2.8
33	2.8
34	2.3
35	1.8
36 Internal Std.	
37	1.6
38	0.4

TABLE 6 - TOTAL AMOUNT AND PERCENT COMPOSITION OF n-PARAFFINS ON DIFFERENT DATES OF COLLECTION

SABINE RIVER, TEXAS

29 40; 93 11		24 30; 94 01	
1-73	1M	1-73	4M
Total n-Paraffins	0.12 µg/l	Total n-Paraffins	0.12 µg/l
Large envelope from C-15 to C-35		Broad envelope from C-17 to C-30	
n-Paraffins	%	n-Paraffins	%
15	1.1	17+pristane	3.2
16	4.5	18+phytane	2.5
17+pristane	11.4	19	4.2
18+phytane	11.1	20	3.7
19	12.4	21	3.2
20	10.9	22	4.6
21	7.0	23	4.1
22	6.9	24	5.3
23	4.8	25	1.0
24	3.7	26	6.0
25	4.5	27	6.6
26	2.9	28	9.0
27	2.3	29	7.8
28	3.9	30	6.5
29	2.0	31	9.0
30	1.3	32	5.3
31	2.7	33	6.0
32	1.4	34	3.7
33	1.6	35	3.3
34	2.1	36 Internal Std.	
35	1.6	37	2.3
36 Internal Std.		38	2.5

PORT ARANSAS, TEXAS

5-74	1M	5-73	1M
5 miles offshore from Marine Science Institute 27 46; 96 52		26 meter contour 5 miles offshore	
Total n-Paraffins	0.07 µg/l	Total n-Paraffins	0.07 µg/l
Minor envelope C-19 to C-28		No envelope	
n-Paraffins	%	n-Paraffins	%
15	2.9	17+pristane	1.7
16	4.3	18+phytane	8.2
17+pristane	22.2	19	1.7
18+phytane	9.5	20	2.1
19	3.8	21	2.1
20	2.0	22	3.0
21	2.2	23	3.5
22	1.1	24	3.0
23	0.9	25	3.6
24	0.9	26	2.7
25	0.9	27	3.2
26	0.6	28	6.6
27	1.1	29	6.5

(continued on next page)

Table 6 continued.

28		0.8	30		8.5
29		2.0	31		10.1
30		2.5	32		9.2
31		6.2	33		7.3
32		6.3	34		6.5
33		8.3	35		4.7
34		7.2	36	Internal Std.	
35		6.5	37		3.6
36	Internal Std.		38		3.9
37		3.2			
38		2.6			
39		0.9			
40		1.1			

BROWNSVILLE, TEXAS

7-73
Total n-Paraffins 0.09 µg/l
Small envelope C-20 to C-30

n-Paraffins		%
15		20.7
16		4.0
17+pristane		29.4
18+phytane		6.7
19		2.9
20		1.6
21		1.1
22		1.1
23		1.1
24		1.1
25		1.5
26		1.0
27		1.4
28		1.2
29		1.9
30		2.3
31		3.4
32		2.3
33		2.4
34		2.2
35		2.6
36	Internal Std.	
37		1.2
38		2.1
39		1.7
40		1.7
41		0.8
42		0.6

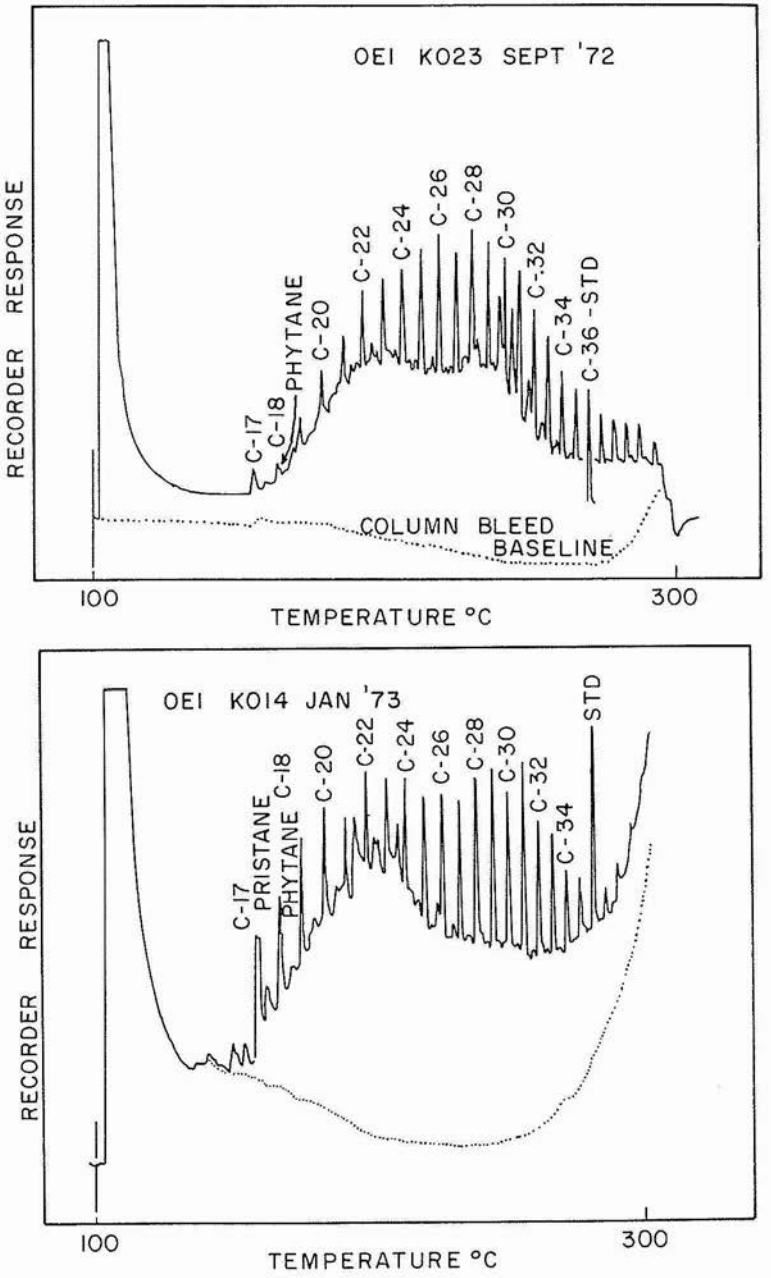


FIG. 3. CHROMATOGRAMS: HYDROCARBON COMPOUNDS IN TIMBALIER BAY (continued next page).

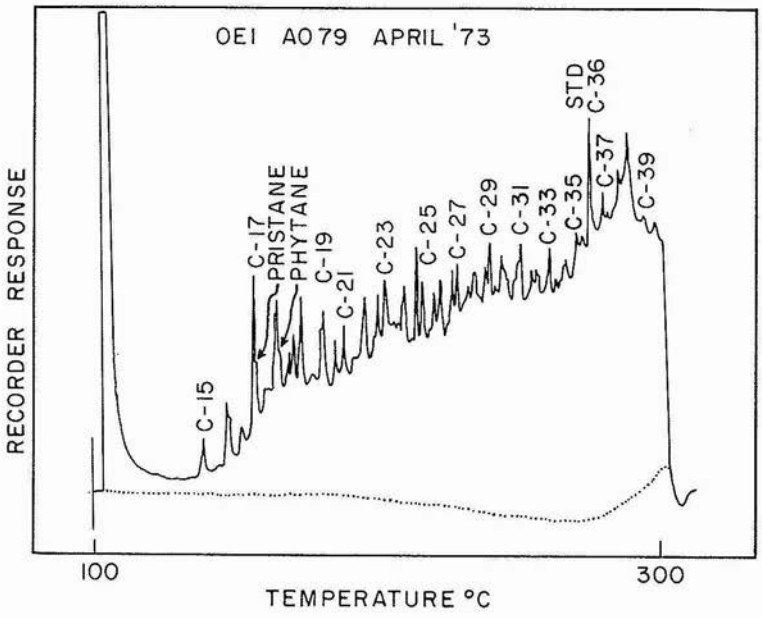


FIG. 3 continued.

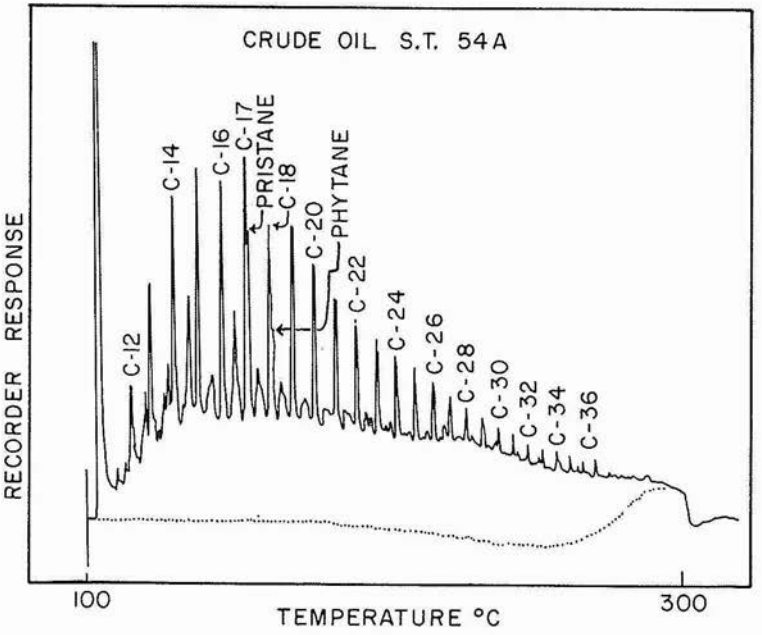
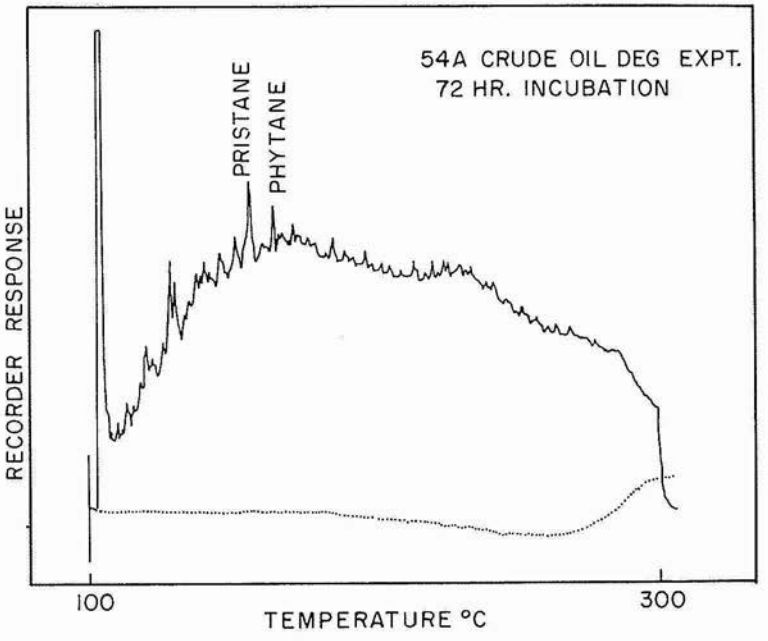


FIG. 4. CHROMATOGRAMS: MICROBIAL DEGRADATION OF CRUDE OIL FROM PLATFORM 54A (continued on next page).



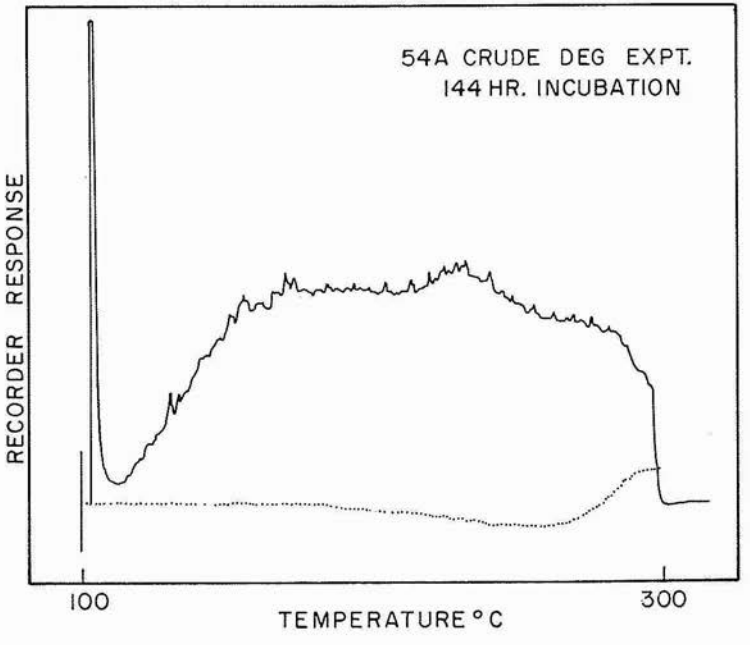


FIG. 4 continued.

oils and many of the other offshore water samples exhibit little or no odd-even carbon preference. Many of the samples possessed envelopes or unresolved baselines, indicating the presence of complex mixtures of hydrocarbons not resolved on packed columns. While this observation refers to almost all samples examined, the most complex chromatograms were generally encountered in Timbalier Bay samples. Figure 3 shows a chromatogram from a Timbalier Bay sample and reveals the presence of many overlapping peaks with retention indices intermediate of *n*-paraffins. Marsh plants are known to produce complex mixtures of branched as well as normal paraffins.

The previous chromatograms all possessed unresolved baseline envelopes, which indicate the presence of mixtures too complex to resolve with packed columns. Figure 4 shows a Louisiana crude oil (54A) which is typical of crudes produced in the area with its rather high *n*-paraffin content and recognizable *n*-paraffin fingerprint. Figure 4 illustrates the change in *n*-paraffins resulting from microbial degradation following 72 and 144 hours of incubation with mixed marine bacteria in nutrient-salts enriched seawater from the study area (Miget et al. 1969). All *n*-paraffins and major recognizable isoprenoids have been reduced to the level of an essentially undifferentiated baseline hump. Very little is known about degradation of these compounds, especially in the field where the nutrient regimes may not favor the rapid rates of degradation measured in the laboratory. It is therefore possible that the unresolved baseline encountered in hydrocarbon extracts from seawater in the Gulf of Mexico are derived from partially degraded crude oils or fuel oils and, if this is so, the widespread occurrence of this envelope in geographically diverse samples suggests that the near-shore Gulf is in some type of dynamic equilibrium with very complex mixtures of hydrocarbons such as those found in crude oils, petroleum-derived refined products, and tar balls.

The distributions of dominant *n*-paraffins and isoprenoid hydrocarbons in seawater extracts were generally similar. A bimodal distribution was typical with the nC_{17} (pristane), nC_{18} (phytane), nC_{19}, and nC_{20} paraffins composing the first dominant mode of the chromatogram and the paraffins nC_{25} through nC_{35} composing the second. Generally, a slight odd over even carbon chain length preference is exhibited in this latter region, suggesting the influence of terrestrially-derived hydrocarbons. Samples with a paraffin distribution dominated by nC_{17} + pristane, nC_{18}, nC_{19}, and nC_{20} (E. Sabine R. 1-73, K007 54A 1-73, Timbalier Bay 4-73, and K014 1-73) in approximately equal abundance suggest microbial degradation of a crude oil or a refined fuel. Finally, a large number of samples contain a C_{18} isoprenoid that is characteristically found in crude oils; certain workers consider the presence of

phytane as indicative of contamination by fossil fuels (Blumer and Snyder 1965).

While there are relatively few data on hydrocarbons in seawater, those values reported for *n*-paraffin concentrations in Gulf of Mexico and Caribbean samples (Lytle, Lytle, and Parker personal communication) are in the same range as the values obtained in this study. Estimates of total saturates based on integration of the total saturated paraffin signal also fall within the ranges reported by others (Brown et al. 1973; Levy 1971).

Distribution of bacteria

Figure 5 shows the results of the OEI microbiological survey from June 1972 through January 1974. Hydrocarbon content of water taken at the same sample site and time is also shown. The data are presented by cruise. The June 1972 data from a trip throughout the Gulf of Mexico on the Texas A&M ship, *Alaminos*, can be considered "baseline" data with which to compare subsequent values from the OEI study areas. With the exception of the two near-shore stations (1 and 2), the remainder of the open Gulf samples were consistent in the "total" heterotroph populations and hydrocarbon degraders—resulting in a 0.1% value for hydrocarbon-degrading bacteria. Water samples for hydrocarbon extraction were not collected on this cruise.

Subsequent trips to the OEI study areas offshore Louisiana and in Timbalier Bay showed correspondingly low percentages of hydrocarbon-oxidizing microorganisms. The percentage of hydrocarbon degraders not only did not vary significantly or consistently with location in the June 1972 open Gulf samples, but additional control samples ("Gulf" controls) taken en route to the study area from Port Aransas, Texas, failed to indicate a significant difference in the percentage of hydrocarbon degraders in near-shore, non-oil production areas.

The assumption that minor fluctuations in the low percentages of hydrocarbon degraders did not constitute significant differences was based largely on a comparison of values for the designated control and platform areas. These locations differ by only 5 miles, which, when offshore samples are being analyzed, is not a large difference. It can be seen that for most of the sampling trips these locations fluctuated in the percentage of hydrocarbon degraders as much as additional "Gulf" control stations taken en route hundreds of miles from the OEI study sites.

Except for the July 1973 samples, there was essentially no difference in the "total" heterotroph or oil-degrading populations at the surface and at 6 m depth. In July 1973 a massive plankton bloom, concentrated in the top few meters of the water column throughout the offshore study

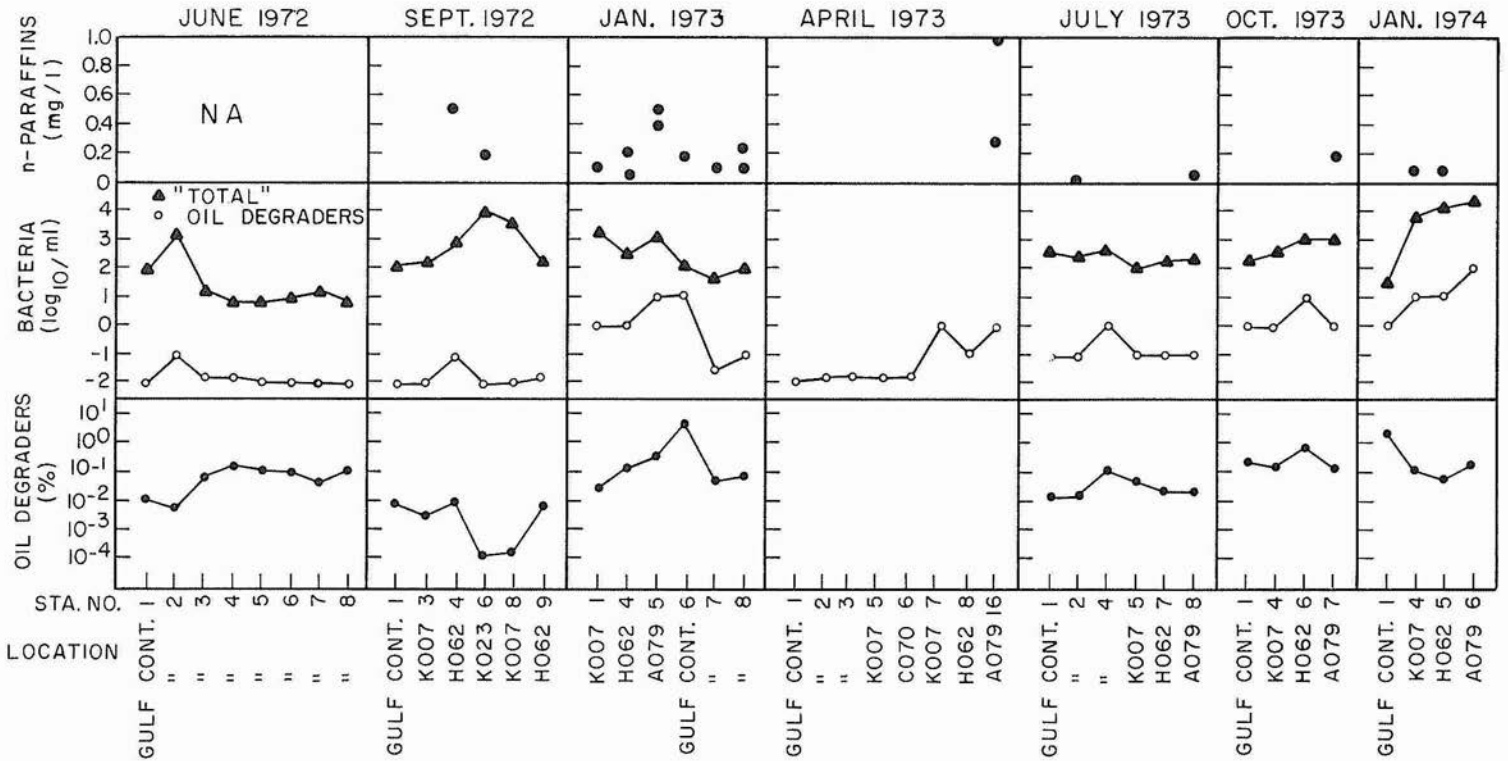


FIG. 5. BACTERIOLOGICAL SURVEY DATA.

area, was probably responsible for the order of magnitude difference observed in both "total" heterotrophs and hydrocarbon-degrading populations.

The microbial data, therefore, when considered along with the low values for hydrocarbons in the water (relative values of up to 0.8 g/l benzene extractable petroleum reported by Colwell et al. [1973]) would indicate that the OEI study area was not grossly contaminated with hydrocarbons and that the hydrocarbon-degrading populations of microorganisms were generally at a "Gulf baseline" level; i.e., they were comparable to those in non-oil production areas.

Two additional remarks concerning the microbial data are warranted. First, the numbers of "total" heterotrophs in the OEI study areas seemed anomalously low relative to organic carbon concentrations and plankton populations. For example, Jannasch and Oppenheimer (1962) found populations of 10^6 bacteria per ml ("total" heterotrophs) in near-shore waters off Port Aransas, Texas. Thus, the consistent enumeration of relatively low numbers of heterotrophs (generally 100 to 1000/ml) in the productive waters offshore Louisiana and in Timbalier Bay needs further study.

Second, as stated in the methods section, the numbers of hydrocarbon-degrading microorganisms shown in figure 5 were calculated based on changes in the appearance of either the oil or the water in replicate test flasks relative to control flasks. A re-examination of the dilution flask data showed that for certain sampling trips almost all of the flasks, including 300 ml filtered samples, showed only a "flaky" oil emulsification and/or cloudy water with no oil emulsification. During other times of the year nearly all of the flasks in a dilution series contained well emulsified oil, and only one or two "flaky" flasks as the sample volume was increased. Although a lack of replicate samples of both hydrocarbon-degrading microbial populations and hydrocarbon concentrations from the study areas prevented a statistical treatment of these two parameters, it should be noted that the "emulsifying" populations occurred in January 1973, April 1973, and January 1974, and to some extent in September 1972 and October 1973. All July 1973 samples exhibited the "flaky" type of oil degradation.

BOD studies

Table 7 shows BOD test data from samples collected in January 1974. These are typical of the BOD results and indicate the potential activities of microbial populations *in situ* in response to added hydrocarbons.

These data show that crude oil, paraffin oil, hexadecane, benzene,

TABLE 7 - JANUARY 1974 HYDROCARBON BOD DATA
(Platform 54A surface water)

SUBSTRATE[1]	INCUBATION (days)	O_2 REMAINING[2] (ml/l) X S.D.	X cont. - X test[3] (ml/l)
Control	10	3.83 + 0.21	--
Control	20	2.80 + 0.15	--
Control	43	2.55 + 0.11	--
Benzene	10	0.19 + 0.12	3.64
Toluene	10	0.14 + 0.08	3.69
Ethylbenzene	10	0.20 + 0.12	3.63
Propylbenzene	10	0.16 + 0.11	3.67
Hexadecane	10	0.48 + 0.39	3.35
Paraffin oil	20	1.30 + 0.39	1.50
54A crude oil	20	0.40 + 0.56	2.40
Isooctane	43	0.30 + 0.52	2.25
Cyclooctane	43	0.82 + 1.97	1.73
Anthracene	43	1.45 + 1.57	1.10
Triphenylene	43	0.80 + 0.96	1.75
Naphthalene	43	1.34 + 0.82	1.21
Fluoranthene	43	1.88 + 1.27	0.67
Phenanthrene	43	1.71 + 1.54	0.84
Benzanthrene	43	1.44 + 1.97	0.81

[1] Substrate concentrations were 10 µl/300 ml sample water for hydrocarbons liquid at room temperature and 100 mg/300 ml sample water for hydrocarbons solid at room temperature and dissolved in benzene as described in the Methods section.

[2] Determined chemically using the Winkler titration method as outlined in the Analysis of Seawater Handbook (Strickland and Parsons 1968).

[3] All values are reported even though the standard deviation sums exceeded the difference in oxygen concentration for some of the higher molecular weight aromatics. See text for justification.

and alkylbenzenes were metabolized by indigenous bacteria. On the basis of previous laboratory investigations using active hydrocarbon-degrading cultures, the rates of oxidation of the crudes were considered to be primarily a function of the composition of the crude oils and the solubilities of their more biologically labile components.

The heptane fraction of #8 crude oil (containing primarily *n*-paraffins) was oxidized at approximately the same rate as the whole crudes, indicating perhaps that the oxidation of *n*-paraffins was primarily responsible for oxygen utilization in these water samples. Biological oxidation of *n*-paraffins in crude oils has been previously reported from this laboratory (Miget et al. 1969). In order to verify this hypothesis, however, one of the most active mixed cultures isolated on a crude oil substrate from the study area offshore was grown on #8 crude oil in nutrient salts enriched seawater (ESW). The sequence of gas chromatograms of extracted hydrocarbons from replicate cultures after various periods of incubation shows the degradation of *n*-paraffins in whole crude oil (figure 4).

These data, however, do not necessarily imply a preferential utilization of *n*-paraffins in crude oils by naturally occurring microbial populations, as low boiling aromatic hydrocarbons (benzene and the alkylbenzenes) were also oxidized by indigenous microorganisms (table 7). It is obvious that these alkylbenzenes not only were non-toxic at this concentration (10 μl/300 ml water), but, in part because of their high solubilities relative to paraffins, were rapidly metabolized by the indigenous microorganisms.

The branched paraffin, isooctane, the cycloparaffin, cyclooctane, and all of the heavier molecular weight aromatic hydrocarbons tested showed greatly reduced biological oxidation.

Summarizing the BOD field data, we can say that microorganisms capable of metabolizing a variety of hydrocarbons (*n*-paraffins, crude oils, low molecular weight aromatics, and quite probably heavier polynuclear aromatics) were always present in the OEI study areas. All metabolic assays employed nutrient salts enriched seawater (ESW), so rates of metabolic activity of indigenous microorganisms reported are not comparable with *in situ* metabolism of the same hydrocarbons.

It should be re-emphasized that several of the low boiling aromatic hydrocarbons found in crude oil that have been shown to be highly toxic to marine organisms (Blumer 1969) were readily metabolized by the native microflora in the offshore Louisiana area. This is especially significant with regard to the "self-cleansing" potential of the areas, as GC-MS analyses of 30 offshore Louisiana crude oils obtained at the wellheads showed a composition averaging 20% benzene plus alkylbenzenes.

TABLE 8 - HYDROCARBONS IN SEAWATER LITERATURE REVIEW

INVESTIGATOR	SAMPLE LOCATION/NO.	VALUES OBTAINED
Brown et al. 1973	Atlantic (18)	Total - 1 to 12 µg/l Saturates - 5.7 µg/l Aromatics - 1.0 µg/l
GURC, 1973	Gulf of Mexico (16)	n-Paraffins - 0.30 µg/l Saturates - 3.7 µg/l
Levy, 1971	Nova Scotia area	Total - 2 to 13 µg/l
Parker et al. 1975	Gulf of Mexico (6)	n-Paraffins - 0.23 µg/l
Barbier et al. 1973	W. Central Atlantic	Total - 10 to 137 µg/l

DISCUSSION

A thorough literature review at the onset of this project suggested that total naturally occurring hydrocarbons in seawater are found at relatively low concentrations (table 8) and hydrocarbon-oxidizing bacteria are found in all habitats tested (table 9). We, therefore, developed an analytical technique that effectively extracted seawater for hydrocarbons at the nanogram/liter level and used for analyses a Finnigan GC-MS, an instrument of relatively high sensitivity.

As data accumulated, some tentative conclusions could be made. One conclusion was that one could reliably detect hydrocarbon components in any sample at the parts-per-trillion range, as shown by the similarity of replicate samples. Triplicate aliquots of the extracts from each 20-1 sample were analyzed. The analytical technique, then, permitted a routine sampling and analysis of a sufficiently large number of samples to provide statistically meaningful ecological data. Sampling distribution and frequency, rather than laboratory analysis, were the limiting factors.

Ecologically, the hydrocarbon data are quite interesting. In the water column we found the average total saturate (and normal hydrocarbon) content to be 2 $\mu g/l$. In the water extracts no relatively high peaks (e.g., high concentrations of specific compounds) were found by chromatography, etc., that would indicate any specific concentration of hydrocarbons from oil spills or their degradation products or from primary production. This was true, also, of the test and control areas off Port Aransas, Texas, which were used for additional comparisons. As one reviews the possible mechanisms of hydrocarbon oxidation, these determinations would indicate that the biota has maintained the

TABLE 9 - INDIGENOUS HYDROCARBON DEGRADING BACTERIAL POPULATIONS

LOCATION	"TOTAL" HETEROTROPHS/ml	HYDROCARBON DEGRADERS/ml	HYDROCARBON DEGRADERS	HYDROCARBONS IN WATER	COMMENT	REFERENCE
Moscow River						
1. Upstream refinery	NA	10 to 9×10^4	< 5	NA	Oil visible on water near refinery	Vorosilova, 1950
2. Downstream	NA	> 10^7	up to 12,800			
North Sea						
1. Helgoland Mar.St.	NA	0.9 to 460	0.5 to 16.8	NA	Data are averaged over a 14 mo. sampling period. No sig. diff. in # of H.C. degrading bacteria in surface film and at 1 m.	Gunkel, 1973
2. 25 mi. offshore from the mouth of the Elbe R.	90	3	3.3	NA		
Cook Inlet, Alaska	NA	Av. = 1.0 (n=32)	Av. = 10%	NA	No sig. diff. in # of H.C. degrading bacteria in the surface film and at 1 m.	Miget, et al. 1974
Raritan Bay, NJ						
1. Arthur Kill	NA	3.4	NA	NA	Classified as a "polluted" area by authors.	Bartha, 1973
2. Seaward fringe of Raritan Bay	NA	0.06	NA	NA	A seasonal fluctuation in # of H.C. degrading bacteria was noted.	

Table 9 continued.

Cheasapeake Bay										
1. East Bay	10-72 1.5×10^{3}	11-72 5.0×10^{1}	10-72 5.0×10^{1}	11-72 5.0×10^{0}	10-72 3.3	11-72 12.5	10-72 0.002%w/v	11-72 NA	Described as an unpolluted commercially productive area.	Colwell, et al., 1973
2. Colgate Creek	1.5×10^{4}	5.0×10^{5}	6.0×10^{2}	5.0×10^{4}	4.0	8.9	0.08%w/v	NA	Adjacent to Baltimore Harbor. Not commercially productive.	
Cornish Coast (Torrey Canyon)										
1. Sennen		8.5×10^{5}		5.0×10^{4}		6%		NA	No oil visible in area.	Gunkel, 1968
2. Trenow Cove		2.5×10^{8}		7.0×10^{8}		280%		NA	Oil was visible on water surface and in sediments.	
Gulf of Mexico										
1. Barataria Bay		NA		≥ 0.1		NA		NA	Over 300 stations sampled during a three-year period.	ZoBell, 1966
2. Texas coast Brownsville to Matagorda Bay		NA		≥ 0.1		NA		NA	Seven of 24 stations were negative for H.C. degrading bacteria in 10 ml samples.	

NA - Not Available

hydrocarbons in the water column at a very low level. In fact, the level appears to be too low for biological uptake; i.e., the combination of population, grazing range, and concentration are such that probability of uptake is very small. The probable, but still preliminary, conclusion is that the biota has decomposed the hydrocarbons down to an insignificant level in respect to their ecological effect.

Data for the distribution of hydrocarbon oxidation, rates of hydrocarbon degradation, and specific molecule uptake by BOD experiments support the low water concentrations and the fact that we were not able to detect any specific buildup of hydrocarbon molecules. It is pertinent to note that such compounds as benzene and toluene, considered by many to be toxic, are readily utilized by the indigenous microorganisms from the offshore Louisiana area. The hydrocarbons in Timbalier Bay organisms and water appear to reflect land influence, as our data show a general odd-even carbon ratio similar to hydrocarbons of land plants.

Data obtained for hydrocarbons $>C_{10}$ in the water column are also supported by other investigators (see table 8), who found comparable hydrocarbon levels in seawater in the Gulf of Mexico and in Atlantic oceanic water. It would appear that the values for open ocean waters would be lower than inshore waters, particularly in the vicinity of producing oil platforms, such as those in the OEI test area. The finding that levels in the OEI area are comparable to those in the open ocean appears to be consistent with the previous conclusion regarding biological oil grazing activities.

Information developed in this investigation also can be correlated with data obtained by other investigators in the OEI program. For example, Brent (1979) found total carbon in the water column to be in the range of 1 to 5 ppm. Total hydrocarbons reported in this investigation are approximately 0.1% to 1% of Brent's total organic matter. This value is also relevant to the concentrations of hydrocarbons in living systems that have been reported (from 0.001% to 1% by dry weight) in the literature.

Further, the data can be compared to the average daily rates of primary productivity reported by Fucik and El-Sayed (1979): 1.06 gC/m^2 at 54A and 1.03 gC/m^2 at the control station. If, of this carbon, 0.01% is hydrocarbon, then approximately 0.1 $mg/m^2/day$ are fixed (this percentage is based on laboratory experiments where algae grown in a hydrocarbon-free environment produce hydrocarbons). From the data it can be estimated that approximately one million barrels of oil-equivalent are produced annually by primary production in the Gulf of Mexico.

The total values and ratios of hydrocarbon-utilizing bacteria to total heterotrophs indicate a low hydrocarbon level. The rates of hydrocarbon

uptake by indigenous microbial populations in the test area also substantiate the conclusion that the grazing effect results in the very low hydrocarbon levels in the test area.

Laseter and Ledet (1979) reported that the air-water interface offshore had an average of 0.7 mg/m² of paraffins. They did not report any correlation between surface hydrocarbons at Platform 54A and the control area. They did, however, show that the hydrocarbons in the surface slick of the test area were higher than for north Florida waters. This difference could be due in part to the higher primary production and the hydrocarbons resulting from the Mississippi River drainage in addition to local spills from oil operations in the oil producing field. We have determined the relationship of Laseter's findings of surface oil concentrations to water column concentrations. If one assumes that Laseter's sampling technique would sample the top tenth of a millimeter of the surface, the value of 0.7 mg/m² is equivalent to 70 μg/1.

By using various OEI data, the content of 100 m² of water 10 m deep in the test area offshore can be calculated as follows:

Author	*Basis*	*Amount*
Laseter	Surface content paraffins (based on 0.70 mg/m²)	0.7 g
Kator	Content of paraffins (in water based on 1 μg/)	1.0 g
El-Sayed	Daily primary productivity (based on 1.0 g Carbon fixed/day/m²	100.0 g
Brent	Organic carbon (based on 5 mg/l)	5000.0 g

As Laseter's, El-Sayed's, and our work indicates, the distributions and types of hydrocarbons in seawater will be influenced by the normal productivity of photosynthesis, the spillage of hydrocarbons through production activities, and microbial degradation. In addition to these seasonal trends, however, there may be daily fluctuations. The BOD results show a rapid uptake of hydrocarbon, indicating the transitional nature of residuals and oxidation products.

Some correlations show interesting relationships. For example, Laseter gave data that the surface oil in the test area was 0.70 mg/m² compared to 0.18 mg/m² in the area offshore from north Florida. El-Sayed studied the same areas and found primary productivity in the water column at the test site to be approximately 85 mgC/m²/hr as compared to approximately 5 mgC/m²/hr off Panama City, Florida. Such a difference in productivity could explain the differences in surface hydrocarbons between the two

areas, as the data also indicate a higher primary productivity in the Fertile Crescent off Louisiana. Neither Laseter's nor our work definitely fingerprints the presence of crude oil in the carbon present in seawater. This suggests that it will be difficult to separate the input of hydrocarbons from the Mississippi River, from production operations, from primary productivity, and last, from the microbial degradation products of these materials.

CONCLUSIONS

The year-and-a-half study of the distribution of hydrocarbons and bacteria in surface and subsurface waters in the Louisiana area provided the basis for an environmental evaluation. The distribution pattern of low numbers of oil-degrading bacteria and low hydrocarbon concentrations, and the response of indigenous bacteria to added hydrocarbons, were indicative of a balanced ecosystem. Hydrocarbons released during oil production and ship operations, as well as from the Mississippi River, had not accumulated to concentrations greater than in other areas of the oceans. The low amount of hydrocarbons and numbers of bacteria suggest that in the previous months little contamination had been present. Otherwise, one would expect to find larger numbers of bacteria. The BOD data clearly indicate that the bacteria in the area are capable of oxidizing a wide variety of low and high molecular weight paraffinic and aromatic hydrocarbons. The rate of activity as related to oxygen uptake is indicative of a heterogeneous population, which has degraded contaminating oil to the very low level of a few parts per billion. There was also no evidence that specific molecules were more resistant to degradation, thus resulting in their concentration in water samples. Individual molecules occurred in the samples analyzed in the parts-per-trillion range.

Quantitative and qualitative data regarding hydrocarbons extracted from seawater and organisms collected both in the OEI study areas and in non-production Gulf control stations suggest that a large portion, if not all, of the Gulf of Mexico is uniformly exposed to hydrocarbons. which are probably derived from primary production or from petroleum. Perhaps the ubiquitous population of hydrocarbon-oxidizing microorganisms and the low concentrations of hydrocarbons found in the Gulf are a reflection of a dynamic system whereby hydrocarbons derived from petroleum, plants, and animals are introduced into the Gulf and are continuously reduced to a very low baseline level through physical, chemical, and biological processes.

ACKNOWLEDGMENT

This paper previously appeared as Contribution No. 385 in Marine Science, The University of Texas.

REFERENCES CITED

Ahearn, D. G. and S. P. Myers, eds. 1973. The Microbial Degradation of Oil Pollutants. Publication #LSU-SG-73-01. Baton Rouge, Louisiana: Louisiana State University.

Barbier, M., D. Joly, A. Saliot, and D. Tourres. 1973. Hydrocarbons from sea water. Deep-Sea Research **29**:305-314.

Bartha, R. and R. M. Atlas. 1973. Biodegradation of oil in seawater: Limiting factors and artificial stimulation. *In* D. G. Ahearn and S. P. Myers, eds., Microbial Degradation of Oil Pollutants. Publication #LSU-SG-73-01. Baton Rouge, Louisiana: Louisiana State University.

Blumer, M. 1969. Oil pollution of the ocean. *In* D. Hoult, ed., Oil on the Sea. New York: Plenum Press.

——— and D. W. Snyder. 1965. Isoprenoid hydrocarbons in recent sediments: Presence of pristane and probable absence of phytane. Science **150**:1588-1599.

———, R. R. L. Guillard, and T. Chase. 1971. Hydrocarbons of marine phytoplankton. Marine Biology **8**:183-189.

Brent, C. R., H. P. Williams, W. A. Bergin, J. L. Tyvoll, and T. E. Myers. 1979. Organic carbon, inorganic carbon, and related variables in offshore oil production areas of the Northern Gulf of Mexico. Rice University Studies **65**(4):245-264.

Brown, R. A., T. D. Searl, J. J. Elliot, B. G. Phillips, D. E. Brandon, and P. H. Monaghan. 1973. Distribution of heavy hydrocarbons on some Atlantic Ocean waters. *In* Proceedings of Joint Conference on Prevention and Control of Oil Spills. Washington, D.C.: American Petroleum Institute, pp. 505-519.

Butler, M. J. A. and F. Berkes. 1972. Biological aspects of oil pollution in the marine environment, A review. Marine Science Center Manuscript Report No. 22.

Byrom, J. A. and S. Beastall. 1971. Microbial degradation of crude oil with particular emphasis on pollution. *In* Institute of Petroleum, Microbiology, 1971 (A Symposium). Amsterdam: Applied Science Publishers, pp. 73-86.

Colwell, R. R., J. D. Walker, and J. D. Nelson, Jr. 1973. Microbial ecology and the problem of petroleum degradation in Cheasapeake Bay. *In* D. G. Ahearn and S. P. Myers, eds., Microbial Degradation of Oil Pollutants. Publication #LSU-SG-73-01. Baton Rouge, Louisiana: Louisiana State University, pp. 185-197.

Crow, S. A., S. P. Myers, and D. G. Ahearn. 1974. Microbiological aspects of petroleum degradation in the aquatic environment. Bulletin de la Société Franco-japonaise d'Oceanographie **12**(2):95-112.

Davies, J. A. and D. E. Hughes. 1968. The biochemistry and microbiology of crude oil degradation. The Biological Effects of Oil Pollution on Littoral Communities. Field Studies Council, July, pp. 139-144.

Davis, J. B. 1967. Petroleum Microbiology. New York: Elsevier.

Fucik, K. W. and S. Z. El-Sayed. 1979. Effect of oil production and drilling operations on the ecology of phytoplankton in the OEI study area. Rice University Studies **65**(4):325-353.

Gholson, R. K., P. Guire, and J. Friede. 1972. Assessment of Biodegradation Potential for Controlling Oil Spills on the High Seas. Report to the U.S. Coast Guard 4110.1/3.1 NTIS AD-759 848.

Gunkel, W. 1968. Bacteriological investigation of oil-polluted sediments from the Cornish coast following the Torrey Canyon disaster. *In* The Biological Effects of Oil Pollution on Littoral Communities (Suppl. to Field Studies, vol. 2). Field Studies Council.

———. 1973. Distribution and abundance of oil-oxidizing bacteria in the North Sea. *In* D. G. Ahearn and S. P. Myers, eds., Microbial Degradation of Oil Pollutants. Publication #LSU-SG-73-01. Baton Rouge, Louisiana: Louisiana State University, pp. 127-139.

Jannasch, H. and C. Oppenheimer. 1962. Bacterial populations in turbid and clear waters in shallow marine bays. Journal of the Institute of Marine Science **8**:56-60.

Laseter, J. L. and E. J. Ledet. 1979. Hydrocarbons and free fatty acids associated with the air/water interface, sediments, and beaches of the Timbalier Bay and offshore Louisiana area. Rice University Studies **65**(4):265-286.

Levy, E. M. 1971. The presence of petroleum residues off the east coast of St. Lawrence and the St. Lawrence River. Water Research **5**:723-733.

Lytle, T., J. Lytle, and P. Parker. 1973. A geochemical study of a marsh environment. Gulf Research Reports **4**:214-232.

———, J. Lytle, and P. Parker. Personal communication.

McKenna, E. J. and R. E. Kallio. 1965. The biology of hydrocarbons. *In* Annual Review of Microbiology. Palo Alto, California: Annual Reviews, Inc. **19**:183-208.

Meinschein, W. G. 1959. Origin of petroleum. American Association of Petroleum Geologists Bulletin **43**(5):925-943.

———. 1969. Hydrocarbons—Saturated, unsaturated and aromatic. *In* Organic Geochemistry. Berlin: Springer-Verlag, pp. 339-356.

Menzies, R. J. 1973. A Handbook on Procedures and Methods Employed in the Offshore Ecology Investigation (OEI). Gulf Universities Research Consortium. Report No. 121.

Miget, R., C. Oppenheimer, H. Kator, J. L. Laseter, and E. J. Ledet. 1974. New sampling device for the recovery of petroleum hydrocarbons and fatty acids from aqueous surface films. Analytical Chemistry **46**(8):1154-1157, reprinted in this volume, pp. 265-275.

———, C. Oppenheimer, H. Kator, and P. LaRock. 1969. Microbial degradation of normal paraffin hydrocarbons in crude oils. *In* Proceedings of the Joint Conference on the Prevention and Control of Oil Spills. New York: American Petroleum Institute, pp. 327-331.

Myers, S. P. and C. G. Gunnerson. 1976. Hydrocarbons in the Ocean. MESA Special Report. Washington, D.C.: U.S. Department of Commerce.

Oppenheimer, C. 1965. Bacterial production of hydrocarbon-like materials. Zeitschrift für Allgemeine Mikrobologie **5**:284-307.

Parker, P. L., J. K. Winters, and J. Morgan. 1975. A baseline study of petroleum in the Gulf of Naples. Unpublished IDOE Manuscript.

Smith, P. V., Jr. 1952. Preliminary note on the origin of petroleum. Tulsa, Oklahoma: American Association of Petroleum Geologists.

Stone, Robert W. and C. E. ZoBell. 1952. Bacterial aspects of the origin of petroleum. Industrial and Engineering Chemistry **44**(11):2564-2567.

Stransky, K., M. Streibl, and V. Herout. 1967. On natural waxes. VI—Distribution of wax hydrocarbons in plants at different evolutionary levels. Collection of Czechslovakian Chemical Communications **32**:3213-3220.

Strickland, J. and T. Parsons. 1968. A Practical Handbook of Seawater Analysis. Fishery Research Board of Canada Bulletin.

Vorosilova, A. A. and E. V. Dianova. 1950, 1952. Quoted in Gunkel 1973, cited above.

Weete, J. D. 1972. Aliphatic hydrocarbons of the fungi. Phytochemistry **11**:1201-1205.

ZoBell, C. E. 1945. The role of bacteria in the formation and transformation of petroleum hydrocarbons. Science **102**(2650):365-369.

———. 1946a. Marine Microbiology. Waltham, Mass.: Chronica Botanica Co.

———. 1946b. Functions of bacteria in the formation and accumulation of petroleum. The Oil Weekly, February 18, pp. 1-7.

———. 1950. Assimilation of hydrocarbons by microorganisms. Advances in Enzymology **10**:443-486.

———. 1952. The part played by bacteria in petroleum formation. Journal of Sedimentary Petrology **22**(1):42-49.

———. 1969. Microbial modification of crude oil in the sea. Joint Conference on the Prevention and Control of Oil Spills. New York: American Petroleum Institute, pp. 317-326.

——— and J. F. Prokop. 1966. Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Zeitschrift für Allgemeine Mikrobiologie **6**:143-162.