FINAL REPORT

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for the

OFFSHORE ECOLOGY INVESTIGATIONS

HYDROCARBONS IN SEAWATER AND ORGANISMS
and
MICROBIOLOGICAL INVESTIGATIONS

Submitted by:

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GENERAL INTRODUCTION

The active oil producing environment of coastal Louisiana offers an ideal field laboratory for studying the significance of oil production to an offshore area. Therefore the area from Timbalier Bay to the shelf was selected as a study area for an Offshore Ecology Investigation by a consortium of investigators representing most fields of marine science. The study was designed to determine the effects of offshore producing platforms on the ecology of the Gulf environment. In the area selected, nearly four billion barrels of oil have been produced from several hundred platforms during the past 30 to 35 years.

This report covers in two parts (I & II) one portion of the project to determine the distribution of hydrocarbons in water and living organisms, and the activity of hydrocarbon oxidizing microorganisms. Our program was integrated with the other programs through the coordination of time of sampling and location of sample areas. The procedures used are incorporated in a general Manual of Procedures that were developed by the team of investigators which will be published as a separate contribution.

During the visual observations on the first field collection period and through a literature search, it became obvious that the stations selected for general use of the team of investigators were going to be too uniform because of the general correlation and oil activities of the area. The data from the first field program supported the observation and therefore other control areas were added to the program. The control areas were located offshore at Brownsville, Port Aransas, and Sabine River and are indicated in the results.

The research project consisted of a study of the distribution of hydrocarbons in water and organisms and the distribution and activity of total and hydrocarbon oxidizing microorganisms. Thus the report is divided into two sections, each of which is complete for that portion of the study.

This project was developed to determine the ecological significance of hydrocarbons in the water and living organisms, relative to the Louisiana offshore development of oil producing platforms. These relationships are pertinent to the area of study, and to the overall program objectives; also, they complement other OEI investigations -- such as the estimates of primary production by Dr. El-Sayed, the distribution of nutrients by Dr. Burchfield, the distribution of organic carbon by Dr. Brent, the distribution and ratio of heterotrophic and hydrocarbon-utilizing microorganisms by Dr. Miget, and the total biomass of the study area as being determined by various investigators -- (such that there is mutual dependence in terms of the interpretation of the results of all these studies).

A thorough literature review at the onset of this project suggested that total naturally occurring hydrocarbons in seawater are found at relatively low concentration levels (Table 1). We, therefore, developed an analytical technique that effectively extracts seawater for hydrocarbons at the nanogram/liter level and uses for analysis a Finnigan GC-MS, an instrument of relatively high sensitivity.

As data accumulated, some tentative conclusions could be made. One was that one could obtain adequate reliability of type of compound detected at amounts in any sample at the part per trillion range, as shown by the similarity of replicate samples. Triplicate aliquots of the extracts from each 20 liter sample were analyzed. The analytical technique, then, permits a routine sampling and analysis of a sufficiently large number of samples to provide statistically meaningful ecological conclusions; hence, sampling distribution and frequency are the limiting factors rather than laboratory analysis.

The same analogy was used with the samples from living organisms. The amount of sample was usually quite small so that, although the amounts were in parts per million, it was still difficult to obtain more than a few micrograms per sample.

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

The data for rates of hydrocarbon degradation and specific molecule uptake by Biological Oxygen Demand experiments supports 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 benzine 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.

This tentative conclusion is supported by the analysis of hydrocarbon extracts from living organisms. Except for the C^{17}

TABLE 1

Hydrocarbons in Seawater Literature Review

Investigator	Sample Location	Values Obtained
Brown, et al.[a]	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[b]	Nova Scotia area	Total - 2 to 13 µg/l
Parker, et al.[c]	Gulf of Mexico (6)	n-Paraffins - 0.23 µg/l
Barbier, et al.[d]	East Central Atlantic	Total - 10 to 137 µg/l

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[a]Brown, R.A., T.D. Searl, J.J. Elliot, B.G. Phillips, D.E. Brandon, and P.H. Monaghan. 1973. Distribution of heavy hydrocarbons in some Atlantic Ocean waters. In: Proceedings of Joint Conference on Prevention and Control of Oil Spills. March 13-25, 1973. Washington, D.C.

[b]Levy,E.M. 1971. The presence of petroleum residues off the east coast of Nova Scotia, in the Gulf of St. Lawrence, and the St. Lawrence River. Water Research 5:723-733.

[c]Parker, P.L., J.K. Winters and J. Morgan. A base-line study of petroleum in the Gulf of Mexico. Unpublished IDOE manuscript.

[d]Barbier, M., D. Joly, A. Saliot and D. Tourres. 1973. Hydrocarbons in Sea Water. Deep Sea Research 20:305-314.

content (which is commonly reported to be very high in the analysis of plankton) the levels of individual compounds are at the part per billion range, which is within normal concentrations as found in living organisms reported in the literature. Data obtained for hydrocarbons $>C^{10}$ in the water column are also supported by other investigators (see Table 1) 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 as in the OEI test area. The findings of 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 can be correlated also with data obtained from complementary OEI investigations. For example, Dr. Brent finds the total carbon in the water column to be in the range of one to five parts per million. Total hydrocarbons reported in this investigation are approximately 0.1 to 1 percent of Brent's total organic matter. This value can be related to the concentration of hydrocarbons in living systems that have been reported between 0.001 and 1 percent by dry weight in the literature.

Further, the data can also be compared to the average daily rates of primary productivity reported by Dr. El-Sayed to be 1.06 gC/M^2 at 54A and 1.03 gC/M^2 at the Control station. If of this carbon, 0.01 percent 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 indicates a low organic hydrocarbon level as is shown by Dr. Brent and work reported here. 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 reported in Tampa that the air/water interface offshore had an average of 0.7 mg/M^2 of paraffins. He did not report any correlation between surface hydrocarbons between 54A and the control area. He did, however, show that the hydrocarbons in the surface slick of the test area were higher than for north Florida waters; 0.7 mg/M^2 in the test area compared to 0.18 in the 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 due to 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 interface, the value of 0.7 mg/M^2 is equivalent to 70 µg/l.

Other comparisons can be made of the data reported to date. The following assumptions can be made for the content of 100 square meters of water 10 meters deep in the test area offshore. The hydrocarbon and organic matter are as follows:

Laseter - Surface content paraffins based on 0.70 mg/M^2 -- 0.7 grams

Kator - Content of paraffins oil in water based on 1 µg/l -- 1.0 grams

El-Sayed - Daily primary productivity based on 1.0 gm of carbon fixed per day/M^2 -- 100 grams

Brent - Organic carbon based on 5 mg/l -- 5,000 grams

As information on local biomass is analyzed, we can attempt to describe the mass balance for hydrocarbons and organics in the area. As Laseter's, El-Sayed's, and our work indicate, the distributions and types of hydrocarbons will be influenced both by the normal productivity of photosynthesis and the spillage of hydrocarbons through production activities and microbial degradation. However, the relative quantities may show the daily primary productivity values to be most significant to hydrocarbon distribution. Also the microbiology results indicates 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/M^2 compared to 0.18 in the area offshore north Florida. El-Sayed also studied the same areas and found primary productivity in the water column at the test site to be approximately 85 mgC/M^2 per hour as compared to approximately 5 mgC/M^2 per hour off Panama City, Florida. Such a difference in productivity could explain the differences in surface hydrocarbons between the two areas as the data also indicates a higher primary productivity resulting in the Fertile Crescent off Louisiana. Neither Laseter's nor our work definitely fingerprint the presence of crude oil in the experimental area, although the data do indicate oil field hydrocarbon influence. This suggests that it will be difficult to separate the events of input of hydrocarbons from the Mississippi River, from production operations, from primary productivity and lastly the sequence of hydrocarbon and related products resulting from microbial degradation of the former materials.

Only the above generalizations with other institutions' results can be made at this time and they must be considered as preliminary until all our data can be examined concurrently with the complementary data of other investigators. As a result of a literature survey and discussions with other petroleum chemists, the techniques being used provide valid <u>ecological</u> results. The possibility of delving too deeply into the identity of minor constituents of the small samples has been intentionally avoided as

we believe this would detract from the original concept and overall objectives of the program by emphasizing analytical precision and details of hydrocarbon composition believed unnecessary to an ecological appraisal.

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PART I

The Distribution of Paraffinic Hydrocarbons in Seawater and Marine Organisms

Abstract

A liquid-liquid extraction technique has been used to determine the amounts and distributions of n-paraffins and selected isoprenoid hydrocarbons in seawater collected from nearshore areas in the northeastern and central Gulf of Mexico. The dominant saturated hydrocarbons in seawater were n-paraffins with chain lengths from nC-15 to nC-41 as well as the isoprenoid hydrocarbons pristane and phytane. Concentrations of n-paraffins plus isoprenoids ranged from 0.03 to 0.60 µg/l and were similar in value at nearshore study sites containing producing oil wells compared with "control" stations in the Gulf of Mexico displaced hundreds of miles from the study site. Seawater from Timbalier Bay generally contained higher concentrations of n-paraffins than the offshore samples and exhibited a more pronounced odd-even carbon preference in the range nC-25 to nC-33. This was attributed to the high load of particulate detritus presumably derived from terrestrial plants in Timbalier Bay water. Estimates of total saturated hydrocarbons in most samples were approximately 10 times larger than the concentrations of n-paraffins as determined by integration of the total paraffin signal.

GC-MS analysis of the dominant n-paraffins generally revealed a characteristic bimodal n-paraffin distribution dominated by nC-17 + pristane and nC-25 to nC-35 with an intermediate unresolved baseline envelope. The relative abundances of n-paraffins clustered around nC-17, the presence of an unresolved baseline envelope and pristane and phytane in almost all samples suggests the observed hydrocarbon distributions are derived from a variety of sources among which include petroleum or petroleum derived products.

Hydrocarbons extracted from plankton tows and selected organisms in the study area as well as "control" stations manifested similar bimodal distributions. The first region was dominated by pristane or nC-17 followed by a minimum leading to a second maximum in the nC-25 to nC-35 region. Again, the relative abundances of n-paraffins clustered around nC-17, a similarity in the distribution of certain major branched saturated paraffins encountered in crude oil and the zooplankton extracts, the ratio of phytane to nC-18, and the ubiquitous presence of an unresolved envelope suggest (1) the organisms are being exposed to petroleum or derived hydrocarbons, and (2) microbial degradation of n-paraffins is occurring.

Total saturated paraffins from marine organisms were generally higher than concentrations in analogous samples from other areas. It is not clear to what extent adsorption or the filtration of particulate detritus with the plankton (i.e. tar balls, plant detritus) have influenced these concentrations.

Odd-even carbon preferences (C.P.I. nC-21 to nC-33) were observed in extracts from seawater (Timbalier Bay) and plankton tows, in a foam line containing diesel fuel, and in polychaetes from Timbalier Bay. These observations serve to accentuate the contribution of terrestrially derived plant hydrocarbons as a very important source of hydrocarbons to the nearshore marine environment.

Introduction

Although hydrocarbons are natural constituents of the marine environment there is concern over the fate of what appear to be increasingly larger amounts of hydrocarbons introduced into the world ocean. In light of the dangerous properties of certain hydrocarbons or their derivatives (Blumer, 1969) it is significant to determine the effects of this load on the levels and kinds of hydrocarbons in seawater and the relationships of baseline hydrocarbons to marine organisms. An understanding of baseline hydrocarbons must take into account the hydrocarbons produced biogenically as well as those released through the industrial activities of man. We have measured the baseline concentrations of n-paraffins and selected isoprenoids in seawater and plankton in a highly productive area offshore Louisiana in close proximity to contemporary oil production activities. Baseline hydrocarbons are defined as those very small concentrations of hydrocarbons which can be extracted from seawater and represent hydrocarbons derived from biological processes, the industrial activities of man and natural geological processes such as petroleum seepages.

Methods

Seawater Extraction-A liquid-liquid extraction technique has been used for the extraction of hydrocarbons from seawater. The seawater is extracted in the same glass container in which it was collected to minimize potentially contaminating transfer operations. Samples were initially collected in pre-cleaned glass carboys (19 liter capacity) using a 4 meter section of cleaned 3/8" copper tubing through which seawater was drawn by means of a vacuum applied with an oil-free vacuum pump. For ease of collection and to further decrease sources of contamination, this method has now been replaced with the following technique. Seawater samples are now collected in glass carboys which are contained in a clean, weighted stainless steel cage. Bottles are lowered to the desired depth and allowed to fill by pulling a cable attached to a Teflon^{R} plug inserted in the bottle mouth. When the bottle has filled (bubbles no longer rise to the surface) the plug is allowed to fall back into the bottle mouth preventing any exchange of the sample with surface slicks or other foreign matter.

Seawater designated for hydrocarbon analysis is immediately poisoned with 50 ml of redistilled benzene to prevent degradation of the organic matter within during storage and sealed with Teflon^{R} lined screw caps. Laboratory studies designed to compare the extent of degradation of n-paraffins in a standard mixture contained in benzene poisoned and non-poisoned bottles reveal benzene as an effective

bacteriocide at the concentrations employed. The mixture of n-paraffins (nC-18,nC-20,nC-22,nC-24,nC-28,nC-32,nC-36, each at 0.1 ug/l) was completely utilized by the indigenous microbial populations in seawater collected offshore Port Aransas and stored for one month without the addition of benzene (Table 1). Essentially no change occurred in the n-paraffin mixture extracted from benzene poisoned bottles. In addition, microbiological assay for total heterotrophic bacteria using ZoBell's 2216E medium was negative in benzene poisoned bottles.

The liquid-liquid extraction apparatus is illustrated in Figure 1. The necks of the glass carboys have been ground to a standard taper to facilitate a leak-proof joint with a Teflon^{R} sleeve of the identical taper. The liquid-liquid extractor is joined to the carboy using the Teflon^{R} sleeve and a standard ground glass joint.

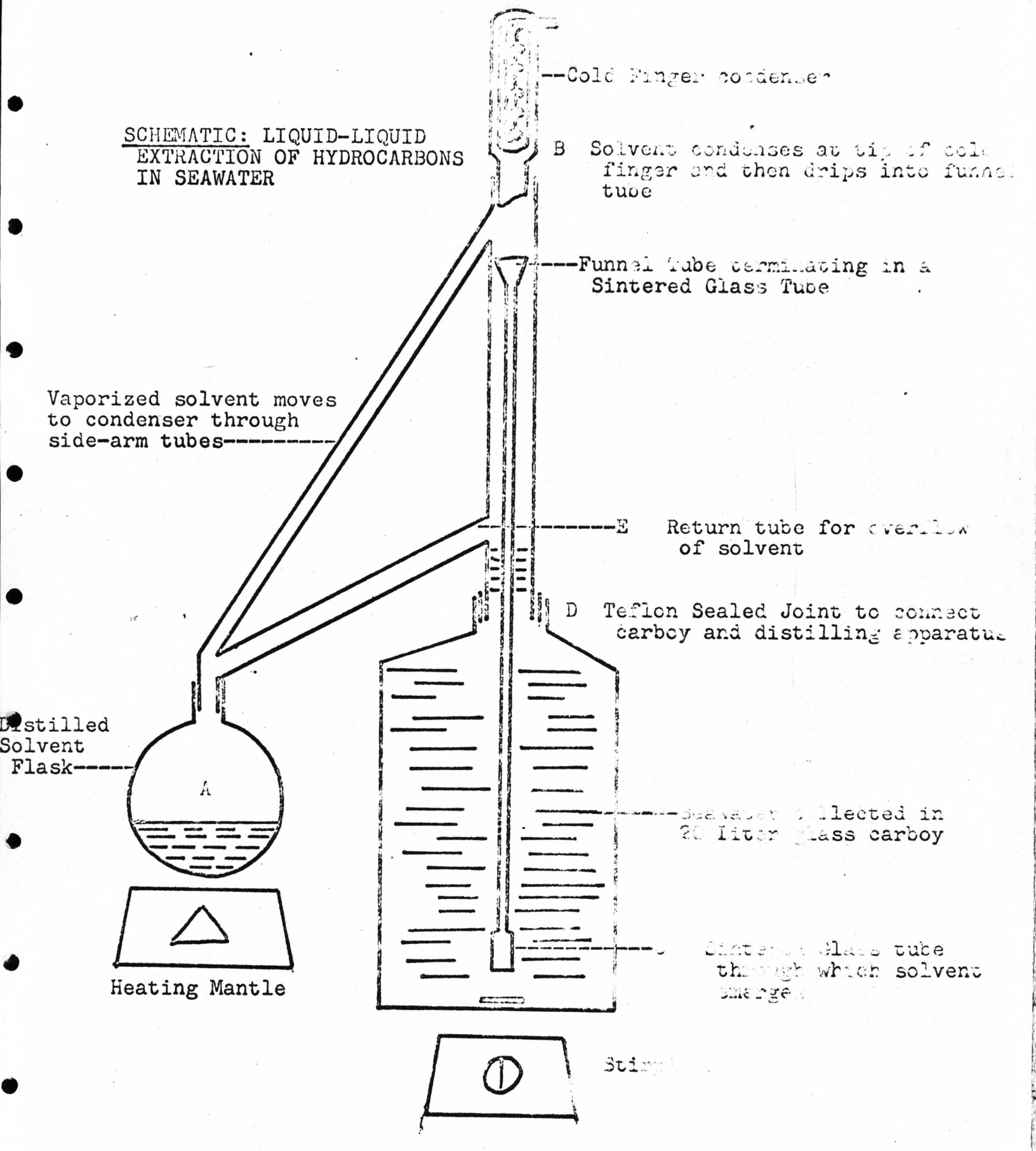
All glassware is cleaned using hot detergent solution, hot water, cold water, acidified distilled water and distilled or spectrograde methanol. The methanol is removed in a drying oven heated to 150 C and a final rinse in the extracting solvent is performed prior to use. Finally, we have found it imperative to clean cold finger condensers after each use as there is a very definite buildup of hydrocarbons, perhaps through steam distillation, on the cold fingers.

All solvents used are reagent grade quality or superior

Figure 1-

Figure 1 -- Liquid-Liquid extraction apparatus designed to extract hydrocarbons from seawater in the container in which it was collected. Redistilled solvent is heated at the boiling point in the Distilled Solvent Flask at A and distills off and condenses at B. Solvent then drips from B into the funnel tube which terminates in a sintered glass tube C. With sufficient head to overcome the water hydrostatus pressure the solvent emerges from the sintered glass in the form of small bubbles. These bubbles possess enormous surface area for extraction and revolve in the water sample due to the action of a stir bar at bottom of the bottle. Because of the density difference between heptane and water these bubbles of solvent rise past D until sufficient solvent accumulates to return to the distilling flask at E. In this fashion the seawater is continuously extracted with fresh solvent.

FIGURE 1


SCHEMATIC: LIQUID-LIQUID EXTRACTION OF HYDROCARBONS IN SEAWATER
--Cold Finger condenser
B Solvent condenses at tip of cold finger and then drips into funnel tube
---Funnel Tube terminating in a Sintered Glass Tube
Vaporized solvent moves to condenser through side-arm tubes---------
E Return tube for overflow of solvent
D Teflon Sealed Joint to connect carboy and distilling apparatus
Distilled Solvent Flask-----
A
Seawater collected in 20 liter glass carboy
Sintered glass tube through which solvent emerges
Heating Mantle
Stirrer

and redistilled before use. Initial and final cuts corresponding to 20% of the total volume are discarded. Solvents are distilled until the levels of contaminating substances are acceptable. Blank determinations were routinely performed with each fresh batch of solvent. The distillation apparatus was steam cleaned using distilled water following each solvent distillation.

Three hundred ml of redistilled heptane are added to the distilled solvent flask. Using a microliter syringe a 20 ul aliquot of a 0.1 ug/ul nC-36 internal standard solution is added to the sample by vortexing with a stir bar to facilitate rapid and complete mixing. The extractor is joined to the bottle and the distilled solvent flask sealed and heated for 24 hours. Upon completion of the extraction the heptane in the extraction flask is evaporated to near dryness on a rotary evaporator (40-50 C). The residual solvent is transfered to a 100 ml pear shaped flask using a disposable glass pipette and the flask and pipette rinsed with a small volume of fresh solvent. The combined volumes of heptane are reduced to dryness using aspiration at 40 C. A small volume of heptane (300 ul) is added and the extract placed on a pre-washed (4 column volumes of heptane) chromatographic column containing 1 ml of activated silica gel (100-200 mesh high purity grade heated for 12 hours at 200 C and deactivated with 5% water). Saturated paraffins are eluted with 1.5 ml of heptane (3 column volumes) and aromatic - naphthenic

hydrocarbons with 1.5 ml of benzene. Using these methods 90 to 95% of n-paraffins in a standard mixture with chain lengths greater than nC-18 are routinely recovered from seawater.

Hydrocarbon extracts are analyzed with a Finnigan 1015C Gas Chromatograph-Mass Spectrometer for compound identification. Multiple channel ion monitoring (McCoy, Porter and Ayers, 1971) is employed to allow some data processing in real time as the chromatograms emerge. Ions selected for routine analysis include: paraffin ions 57 m/e, 71 m/e, isoprenoid ion 113 m/e, olefin ion 69 m/e, tropylium ion 91 m/e. Although this technique is not as powerful as computerized data processing it is useful in the analysis of complex environmental hydrocarbon extracts in which the concentrations of individual compounds, the unresolved baseline and the similarity of ion fragmentation patterns for paraffinic hydrocarbons render manual spectrometry via the light beam oscillograph and subsequent data interpretation almost impossible. Therefore 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.

Stationary phases employed in the analysis of hydrocarbon extracts are 1% OV-1 on 100/120 Chromosorb W-HP and 5% FFAP on 80/100 Gas Chrom Q. Six or ten foot glass columns of 2 mm i.d. were maintained at a flow rate of 25 ml/min using

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zero grade helium.

Hydrocarbons from Organisms- Plankton samples were collected in the study area using a standard 1 meter net (#3) and making 6 to 10 vertical tows depending upon initial yield. Plankton were concentrated on pre-washed Whatman#50 paper and frozen prior to analysis. The relatively dry plankton are then scraped from the filter paper and placed in a H_2SO_4 cleaned sintered glass Soxhlet thimble. Entire organisms are cut or macerated to facilitate solvent penetration rather than emulsified since this latter step tended to clog the sintered glass. Tissue or plankton samples were extracted with 200 ml of a benzene:methanol solvent mixture (1:1,v/v). The sample is initially allowed to soak in about 50 ml of methanol for several hours to facilitate dessication. After this soak period the remaining solvents are added to the Soxhlet and the extraction allowed to proceed for 24 hours. At this time the solvent in the extraction flask is removed and replaced with 100 ml of fresh solvent mixture. The extraction is allowed to proceed for an additional 24 hours and the solvent extracts combined. The residual material in the thimble is weighed after the solvent evaporates and this value plus the total lipids extracted is used to calculate the weight of saturated hydrocarbons on a dry weight basis. The solvent extracted material is partitioned in a separatory funnel following the addition of heptane and water (25 ml of each), the aqueous layer separated and the organic layer washed. The wash and original aqueous layer are then re-extracted with fresh heptane(25 ml) and

layer are then re-extracted with fresh heptane (25 ml) and the heptane fractions combined and filtered through pre-washed anhydrous Na_2SO_4 . The dried extract is evaporated to near dryness on a rotary evaporator (40-50C) and then transfered to a tared pear shaped flask. The remaining solvent is removed and the total weight of extracted lipids determined prior to fractionation on activated silica gel. The weight of the extractables times 100 equals the volume (in ml) of gel required. After fractionation on silica gel as previously described the saturated paraffin fraction (heptane eluate) is taken to dryness, diluted to a known volume (50 or 100 ul) and aliquots removed for weighing on a Cahn Electrobalance. Extracts are analyzed using the same instruments and conditions previously described.

Absolute or relative amounts of paraffinic hydrocarbons were calculated by internal standardization or by summation of total peak areas. Peak areas were determined by planimetry or triangulation using the height times the peak width at one-half the peak height. The C.P.I. or carbon preference index (Cooper and Bray, 1963), which is an average ratio of C_{odd} to C_{even} n-paraffins is calculated for the range nC-21 to nC-33. The formula defining C.P.I. is

$$C.P.I. = 1/2 \left(\frac{\text{sum (nC-odd, n=21 to 33)}}{\text{sum (nC-even, n=20 to 32)}} + \frac{\text{sum (nC-odd, n=21 to 33)}}{\text{sum (nC-even, n=22 to 34)}} \right)$$

where sum refers to the abundances of n-paraffins of chain lengths n.

Sampling Program

The following tables list all stations where seawater or organisms were collected.

SAMPLE LOCATIONS

Seawater

Date	Location	Designation
9-20-72	28 50, 90 30	K040, S.E.54A Platform
9-20-72	28 53, 90 19	H062, Control
9-20-72	28 49, 90 23	K007, 54A Platform
1-10-73	29 12, 90 21	Timbalier Bay
1-9-73	28 53, 90 19	H062, Control
1-12-73	29 40, 93 11	E. Sabine River
1-12-73	29 30, 94 01	W. Sabine River
1-11-73	28 54, 90 45	Ship Shoal
1-9-73	28 50, 90 30	K014, S.W. 54A Platform
1-9-73	28 49, 90 23	K007, 54A Platform
4-6-73	29 12, 90 21	Timbalier Bay
5-5-73	27 46, 92 52	Port Aransas
7-11-73	28 53, 90 19	H062, Control
7-8-73	29 03, 95 52	Control
7-8-73	29 13, 93 42	W. Sabine River
7-8-73	29 00, 92 20	S. Timbalier Isle
7-21-73	26 10, 96 40	Brownsville
10-16-73	29 12, 90 21	Timbalier Bay
1-15-74	29 12, 90 21	Timbalier Bay
1-14-74	28 49, 90 23	K007, 54A Platform
1-16-74	28 53, 90 13	H062, Control
4-15-73	27 46, 92 52	Port Aransas

SAMPLE LOCATIONS Cont'd

Plankton and Organisms

Date	Location	Designation
9-20-72	28 48, 90 27	K023, S.W.54A
9-20-72	28 50, 90 30	G064, N. 54A
9-21-72	28 49, 90 23	K007, 54A Platform
9-21-72	28 49, 90 23	K007, 54A Platform
9-21-72	28 40, 90 23	K007, 54A Platform
9-20-72	28 50, 90 20	G064
1-9-73	28 50, 90 30	K014, S.W.54A
1-9-73	28 49, 90 23	K007, 54A Platform
4-2-73	29 06, 93 59	Control
4-3-73	28 49, 90 23	K007, 54A Platform
4-5-73	28 53, 90 19	H062, Control
4-5-73	28 56, 90 22	G039
4-3-73	28 51, 90 23	HORST-67
7-11-73	28 53, 90 19	H062, Control
7-8-73	29 03, 92 52	Control
10-17-73	28 53, 90 19	H062, Control
10-15-73	28 49, 90 23	K007, 54A Platform
10-7-73	29 09, 90 18	BC04, Timbalier Bay
1-13-74	28 41, 93 09	Control
1-14-74	28 49, 90 23	K007, 54A Platform
1-14-74	28 49, 90 23	K007, 54A Platform
4-15-74	27 48, 96 57	Control, Port Aransas

RESULTS AND DISCUSSION

Hydrocarbons in Seawater

The concentrations and several salient features describing n-paraffins and selected isoprenoid hydrocarbons are listed in Table 2. n-Paraffins are generally the predominant hydrocarbons in these extracts. 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 are within a relatively narrow range of 0.6 to 0.03 ug/l. Estimates of total saturated paraffins based on integration of the total signal area above the solvent baseline are around one order of magnitude larger than the total paraffins or in the 5 ug/l range. The range of n-paraffins found in seawater was from nC-15 to nC-41.

If any seasonal trend can be discerned it is that the total n-paraffin concentrations have decreased since the 9-72 exercise to a minimum in 7-73 and now maintained at a similar level. This observation is derived from both samples in the study area and from relatively large distances away (Sabine River, Port Aransas and Brownsville). In this respect the nearshore waters examined appear to be rather homogeneous in the Gulf of Mexico. Values for Control and Platform stations were very similar during the same sampling exercises. Hydrocarbon levels in Timbalier Bay 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 an odd-even carbon preference for hydrocarbons in the nC-25 to nC-33 region in sea water from Timbalier Bay. A similar preference is observed in recently synthesized hydrocarbons derived from terrestrial marsh plants such as Batis maritima and Salicornia bibelovii (Lytle, Lytle and Parker, 1973). Crude 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 which indicate the presence of complex mixtures of hydrocarbons not resolved on packed columns (Figures 2,3,4). While this observation refers to almost all samples examined the most complex chromatograms were generally encountered in Timbalier Bay samples. Figure 5 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 indicated the presence of mixtures too complex to resolve with packed columns. Figure 6 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. The next two Figures (7 and 8) reveal the crude oil profile following 72 and 144 hours of incubation with mixed marine bacteria from the study area in nutrient-salts enriched seawater (Miget et al., 1969). All n-paraffins and major recognizable isoprenoids

have been reduced to the level of an essentially undifferentiated baseline hump. This indicates that even under optimal growth conditions the compounds composing this unresolved baseline are not degraded. Very little is known about degradation of these compounds especially in the field where the nutrient regimes do 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 the nearshore Gulf is in some type of dynamic equilibrium with very complex mixtures of hydrocarbons such as 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 comprising the first dominant mode of the chromatogram with paraffins nC-25 through nC-35 comprising the second. Generally, a slight odd-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 4073, and K014 1-73) where these paraffins are in approximately equal abundance

suggests contamination by crude oil or a refined fuel. Table 23 shows that the ratios of the isoprenoids pristane and phytane to certain n-paraffins in seawater (K007 54A 1-74) recently sampled are essentially the same as in crude oil or a refined fuel. A reveiw of the literature has revealed that organisms rarely produce mixtures of paraffinic hydrocarbons where nC-17,nC-18,nC-19 and nC-20 are in nearly equivalent abundance and smoothly decrease towards higher chain lengths. Finally, a large number of samples contain a C-18 isoprenoid which is characteristically found in crude oils and 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-paraffins concentrations in Gulf of Mexico and Carribbean samples (Parker et al., Unpublished Manuscript) 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 with the ranges reported by others (Brown, et al., 1973; Levy, 1971).

TABLE 2

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

Date	Station	Concentration Total , ug/l	$\bar{X}$ ug/l	C.P.I. C-21 to C-33	Unresolved Envelope	Major Paraffins and Isoprenoids*
9-72	K040 S.E. 54A	0.35		0.94	present	C-25 to C-33
9-72	H062 Control	0.21		1.19	n.a.	C-15,16,17+pristane, 22,26,27,28,29
9-72	K040 S.E. 54A	0.49	$\bar{X}_{9-72}=$	0.99	present	C-24 to C-33
9-72	K007 54A	0.34	0.34±0.11	0.93	present	17+pristane,26,27 28.29,30,31,32
1-73	Timbalier Bay	0.46	$\bar{X}_{TimBay}=$	1.67	present	C-25,27,28,29,30, 31,32,33
1-73	Timbalier Bay	0.38	0.45±0.45	0.91	present	C-25,26,27,28,29 30,31,32,33
1-73	H062,	0.05		1.00	present	C-22,25,26,27,28 29,30,31,32,33
1-73	H062	0.20		0.99	present	C-17+pristane, 18+phytane,19,20 28
1-73	E.Sabine R.	0.12		0.96	present	C-17+pristane, 18+phytane,19,20, 21,22
1-73	W.Sabine R	0.12		0.93	present	C-24,26,27,28,29, 30,31,32,33
1-73	Ship Shoal	0.19		1.15	present	C-20,21,22,29,30, 31,32,33,34,35
1-73	W.Sabine R.	0.22		0.75	present	C-18+phytane, 20, 21,22,23,24
1-73	K014 S.W.Plat 54A	0.60		0.93	present	C-28,29,30,31,32, 33,34,35
1-73	K014	0.19	$\bar{X}_{1-73}=$	0.99	present	C-17+pristane, C-18+phytane, C-19,20,28,29,30,31,32
1-73	K007 54 Plat	0.13	0.24±0.16	1.37	present	C-17+pristane, C-18+phytane, 19,20, 21,22,23,28

*Greater than 5% of the total signal from n-paraffins+isoprenoids pristane and phytane

TABLE 2 Cont'd

4-73	Timbalier Bay	1.10	1.35	present	C-17+pristane, 18+phytane, 19, 20, 22
		$\bar{X}_{TimBay}$ = 0.73±0.51			
4-73	Timbalier Bay	0.37	2.06	present	C-17+pristane, 19, 27, 29, 31, 33
5-73	Port Aransas 5 mi offshore	0.07	0.98	absent	C-18+phytane, 28, 29, 30, 31, 32, 33, 34
7-73	H062	0.05	1.11	absent	15, 17+pristane, 18+phytane
7-73	29 03 95 52	0.09	1.34	absent	17+pristane, C18+ phytane
7-73	W.Sabine R.	0.03	1.15	absent	17+pristane, 29, 31, 33, 34, 35, 37
7-73	1.8 mi from Timbalier Isle.	0.05	1.17	absent	17+pristane, 31, 34, 35, 38
		$\bar{X}_{7-73}$ = 0.06±0.02			
7-73	Brownsville	0.09	1.17	present	15, 17+pristane 18+phytane
10-73	Timbalier Bay	0.20	1.30	present	15, 17+pristane, 18+phytane, 19, 21, 23
1-74	Timbalier Bay	0.08	1.27	present	17+pristane, 18+ phytane, 23, 25, 29 31,
1-74	K007 54A	0.08	1.18	absent	17+pristane, 18+ phytane, 19, 28, 29 30, 31, 32
1-74	H062	0.09	0.88	present	17+pristane
4-74	Port Aransas 5 mi offshore	0.07	1.32	present	C-17+pristane, 18+phytane, 31, 32, 33, 34, 35

FIG. 2

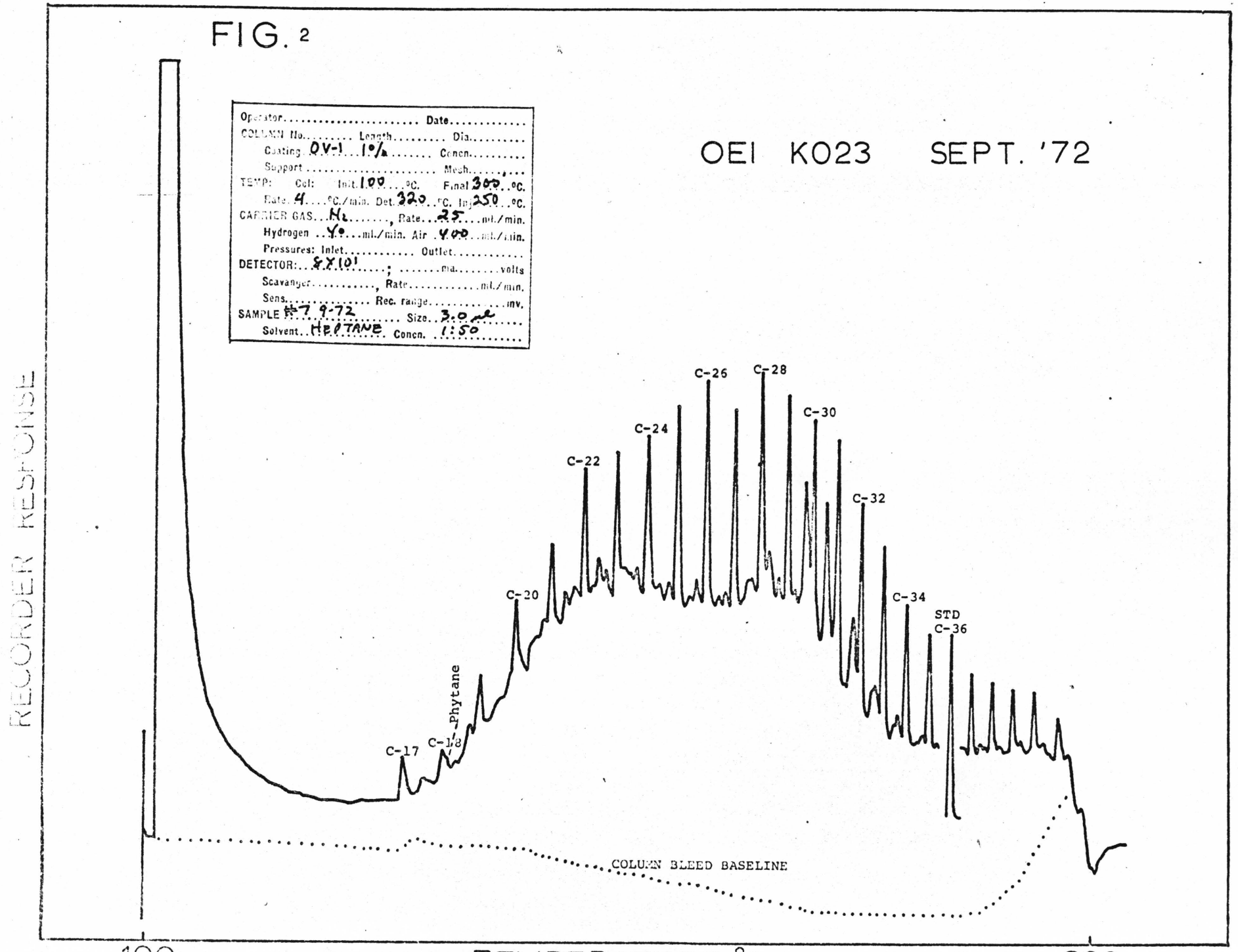

Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating OV-1 1% Concn.....
Support..... Mesh.....
TEMP: Col: Init. 100 °C. Final 300 °C.
Rate 4 °C./min. Det. 320 °C. Inj. 250 °C.
CARRIER GAS... H2, Rate... 25 ml./min.
Hydrogen ..40 ml./min. Air .400 ml./min.
Pressures: Inlet..... Outlet.....
DETECTOR:...FIX101...; .....ma.....volts
Scavanger....., Rate.....ml./min.
Sens..... Rec. range.....mv.
SAMPLE #7 9-72 Size..3.0 µl
Solvent..HEPTANE Concn. 1:50
OEI K023 SEPT. '72
RECORDER RESPONSE
C-17
C-18
Phytane
C-20
C-22
C-24
C-26
C-28
C-30
C-32
C-34
STD
C-36
COLUMN BLEED BASELINE
100
TEMPERATURE °C
300

FIGURE 4

19 Liters Port Aransas Texas 4-74
5 mi offshore

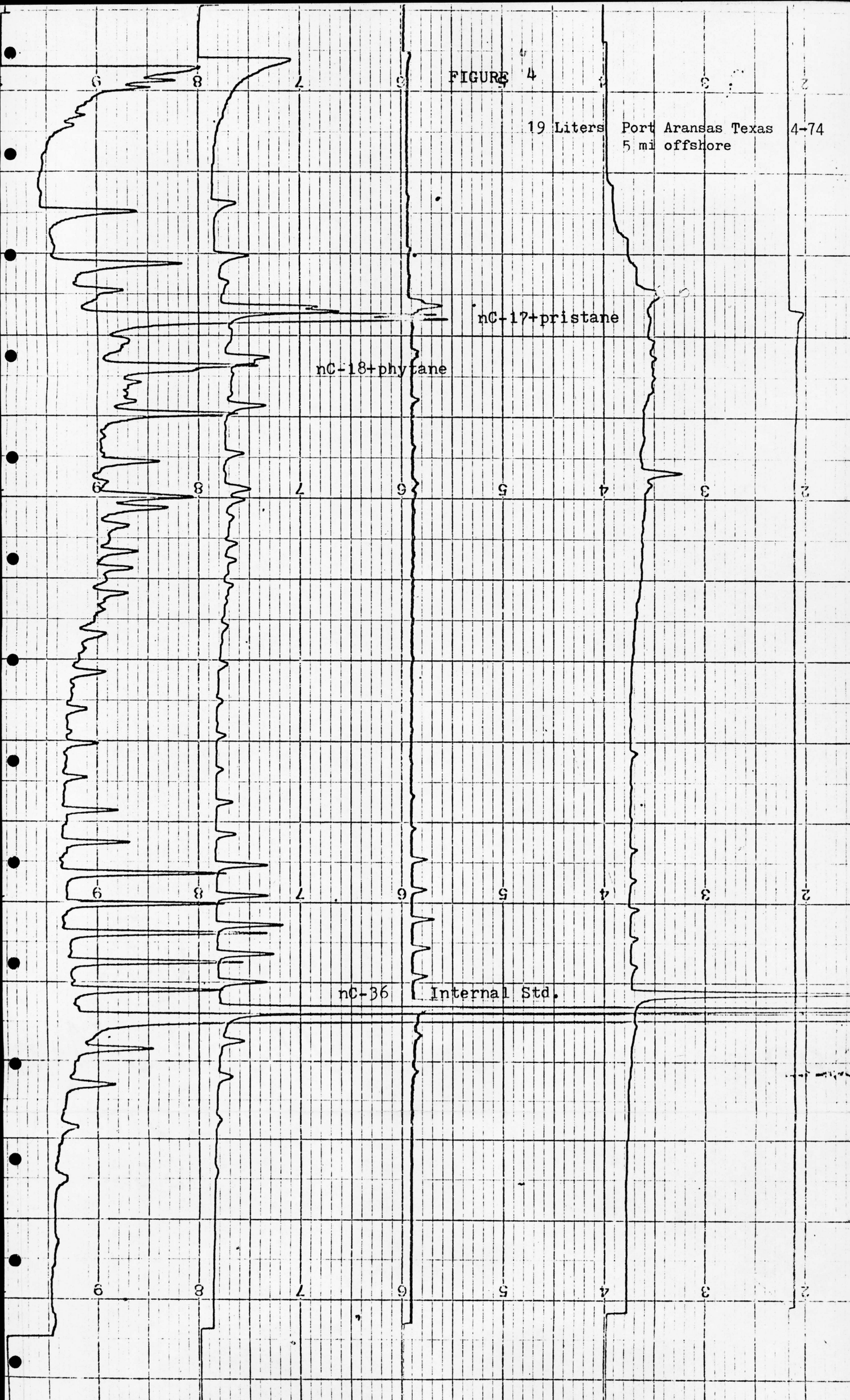


FIG. 3

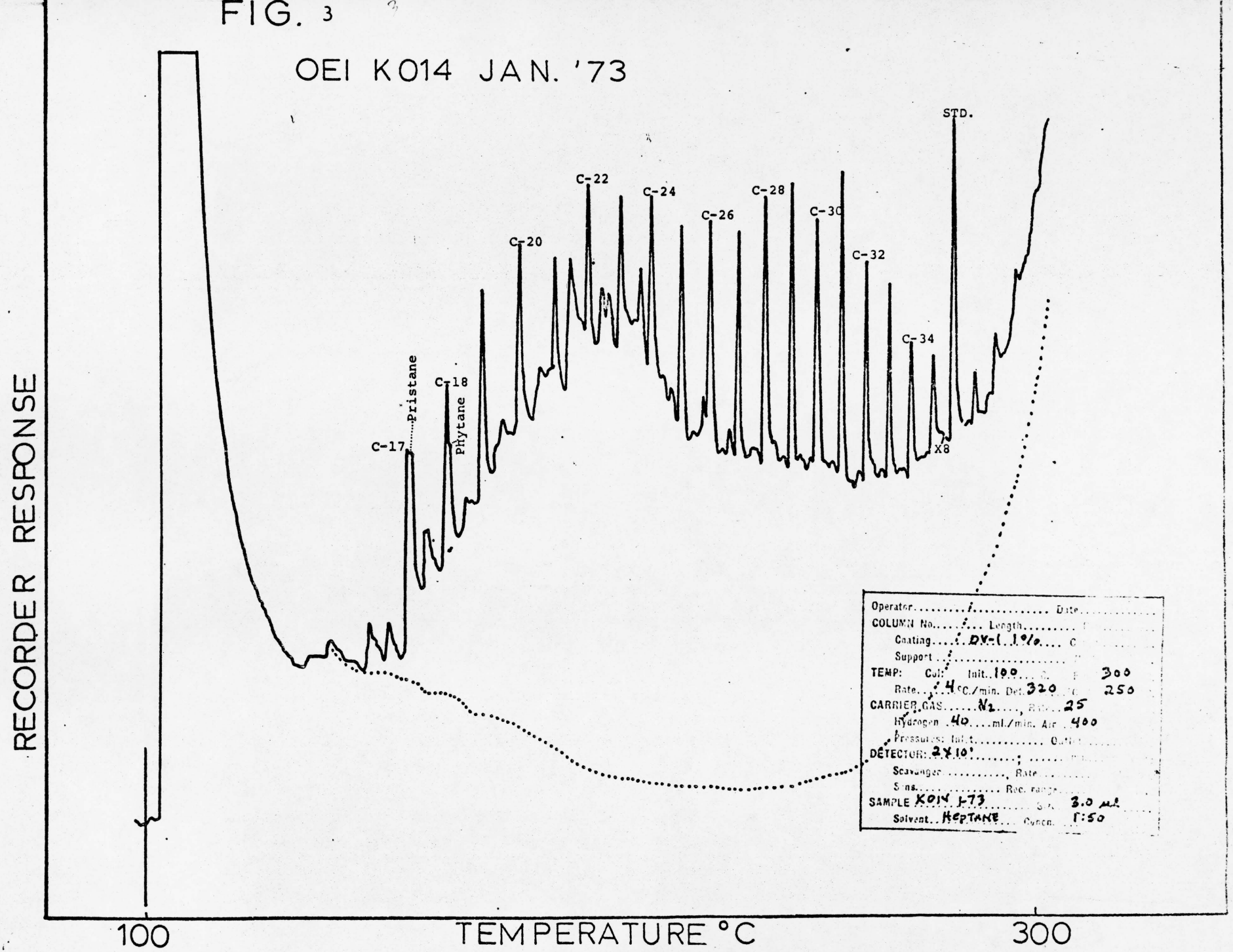

OEI K014 JAN. '73
RECORDER RESPONSE
TEMPERATURE °C
100
300
C-17
Pristane
C-18
Phytane
C-20
C-22
C-24
C-26
C-28
C-30
C-32
C-34
STD.
X8
Operator..... Date.....
COLUMN No..... Length.....
Coating.....DV-1 1%.....
Support.....
TEMP: Col: Init. 100 300
Rate.....4°C./min. Det. 320 250
CARRIER GAS.....N2....., Rate.....25
Hydrogen .40.....ml./min. Air .400
Pressures: Inlet..... Outlet.....
DETECTOR: 2X10.....
Scavenger....., Rate.....
Sens..... Rec. range.....
SAMPLE KOIN J-73..... Size 3.0 µl
Solvent..HEPTANE..... Concn. 1:50

FIG. 5

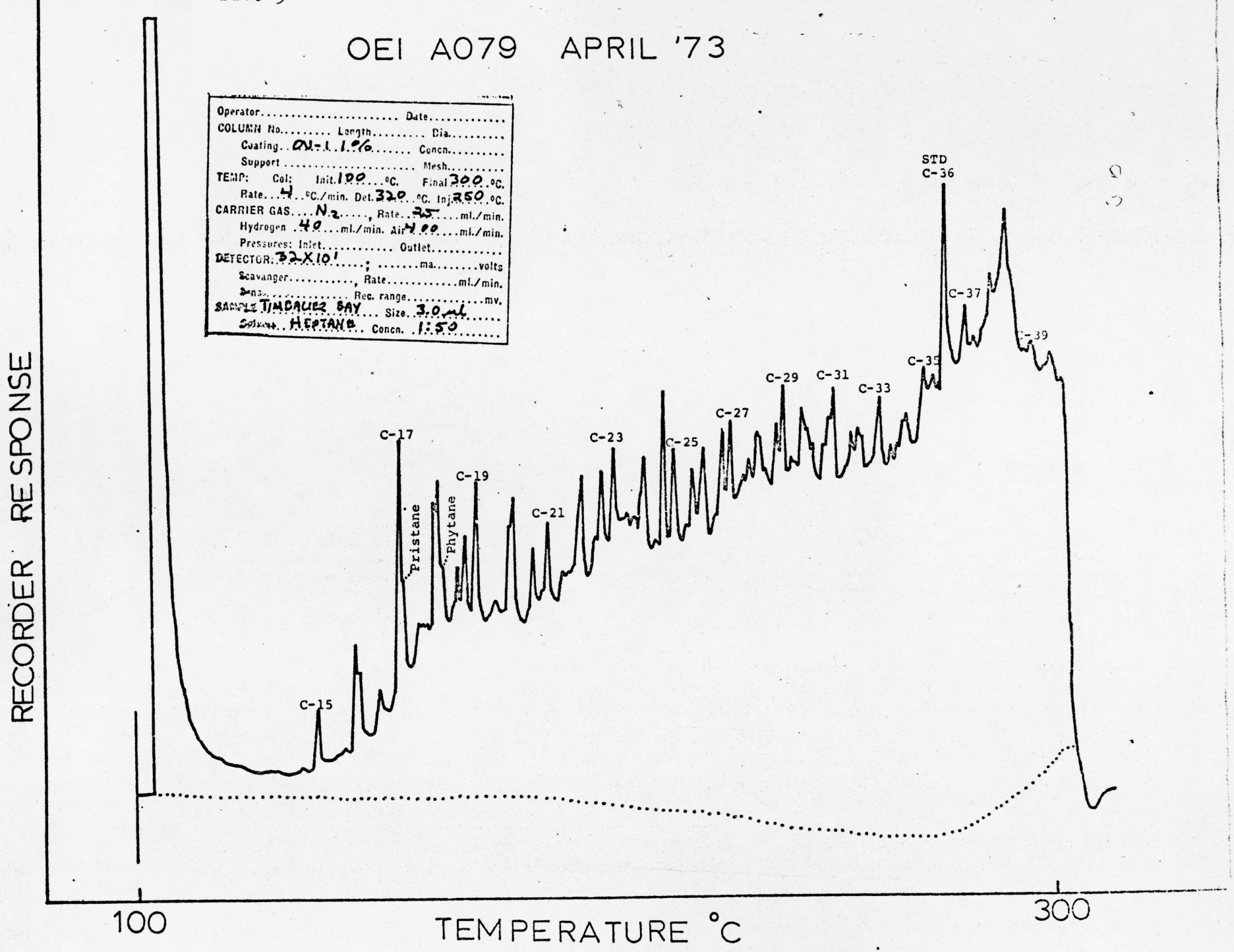

OEI A079 APRIL '73
Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating..OV-1 1.% ..... Concn.....
Support ..... Mesh.....
TEMP: Col: Init.100 ...°C. Final 300 °C.
Rate....4...°C./min. Det.320 °C. Inj.250 °C.
CARRIER GAS....N2....., Rate..25.....ml./min.
Hydrogen ..40...ml./min. Air 400 ...ml./min.
Pressures: Inlet..... Outlet.....
DETECTOR: 32X10'.....; .....ma.....volts
Scavanger....., Rate.....ml./min.
Sens..... Rec. range.....mv.
SAMPLE TINDALIER BAY... Size..3.0 µl.....
Solvent..HEPTANE.. Concn. 1:50.....
STD
C-36
C-37
C-39
C-35
C-33
C-31
C-29
C-27
C-25
C-23
C-21
C-19
C-17
C-15
Pristane
Phytane
RECORDER RESPONSE
100
300
TEMPERATURE °C

FIG. 6

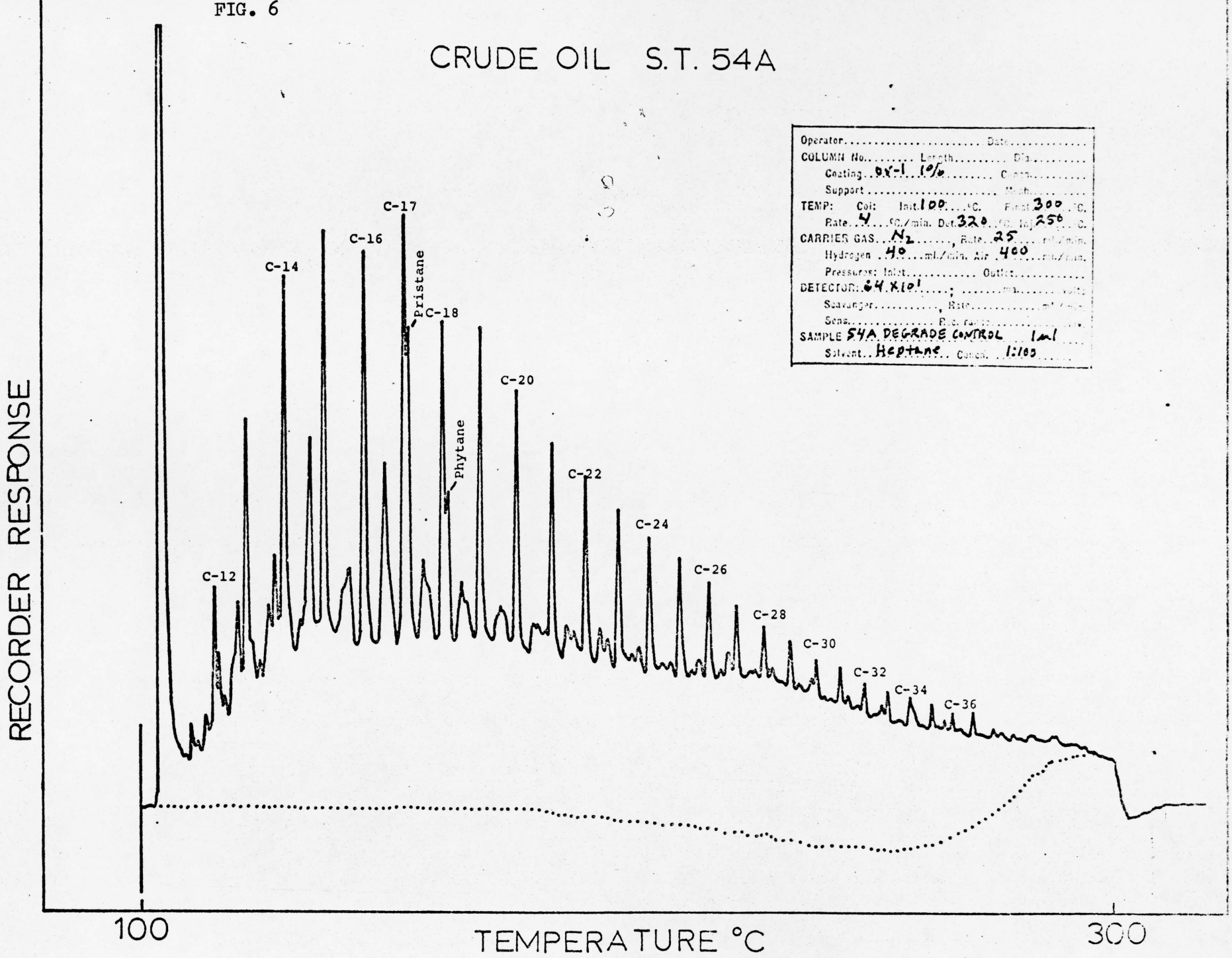

CRUDE OIL S.T. 54A
Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating..SE-1 10%..... Conc.....
Support..... Mesh.....
TEMP: Col: Init.100 °C. Final 300 °C.
Rate..4 °C./min. Det.320 °C. Inj.250 °C.
CARRIES GAS..N2, Rate..25 ml/min.
Hydrogen ..40 ml/min. Air 400 ml/min.
Pressures: Inlet..... Outlet.....
DETECTOR:.64 X10¹; ma. volts
Scavenger....., Rate..... ml/min.
Sens..... Recorder.....
SAMPLE 54A DEGRADE CONTROL 1ml
Solvent..Heptane Concn. 1:100
C-12
C-14
C-16
C-17
Pristane
C-18
Phytane
C-20
C-22
C-24
C-26
C-28
C-30
C-32
C-34
C-36
RECORDER RESPONSE
100
TEMPERATURE °C
300

FIG. 7

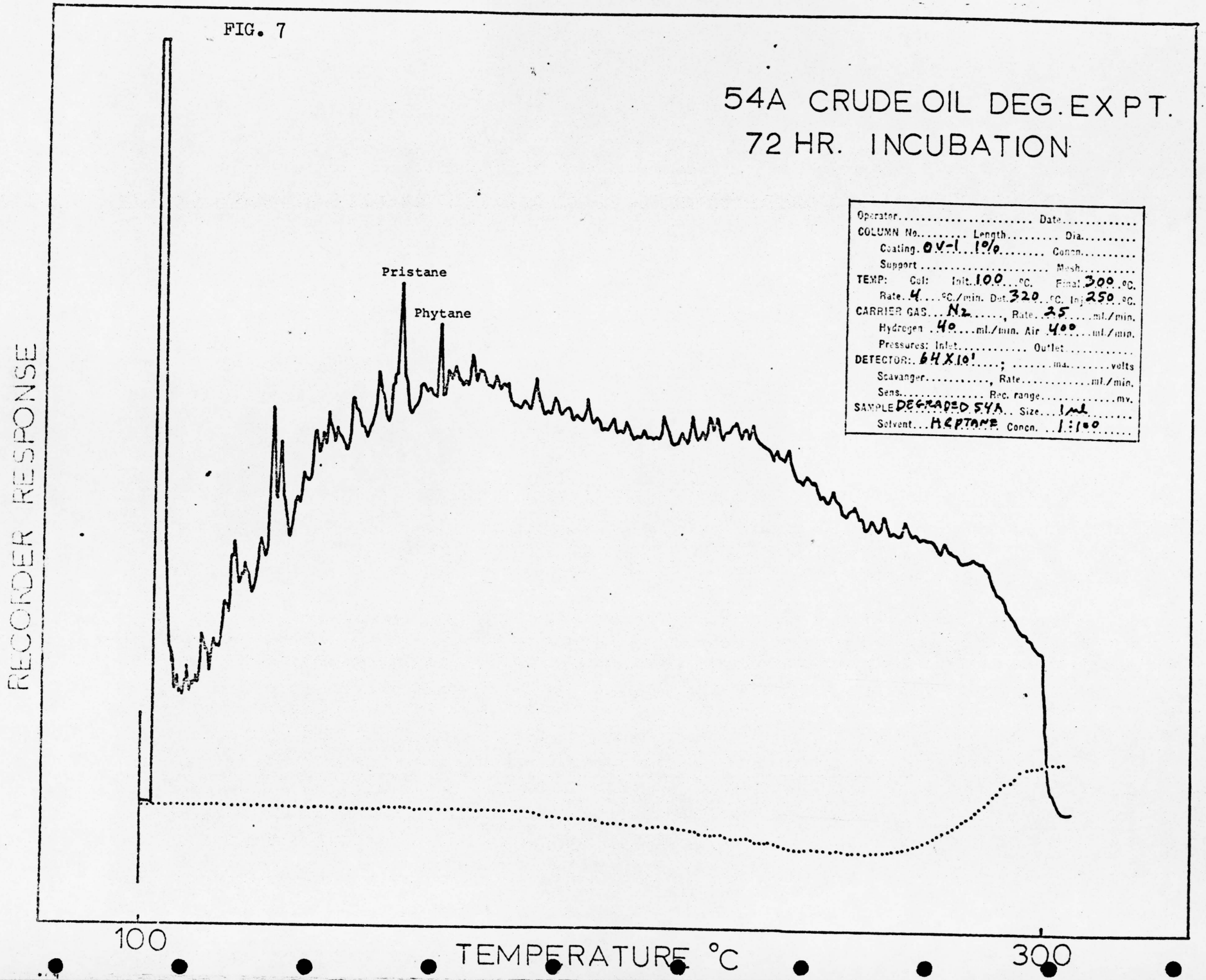
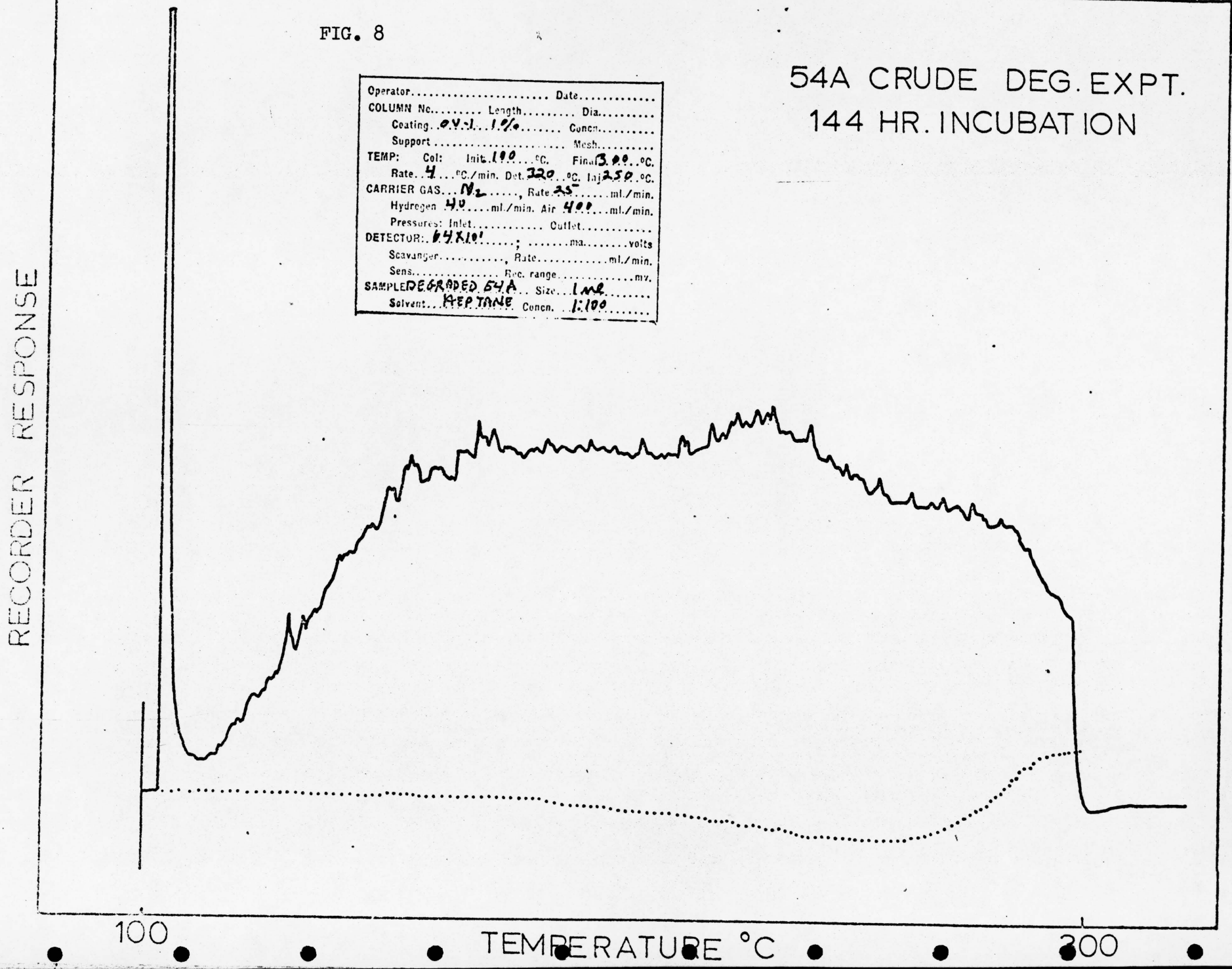

54A CRUDE OIL DEG. EXPT.
72 HR. INCUBATION
Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating. OV-1 1% ..... Concn.....
Support ..... Mesh.....
TEMP: Col: Init. 100 °C. Final 300 °C.
Rate. 4 °C./min. Det. 320 °C. Inj. 250 °C.
CARRIER GAS... N2 ....., Rate. 25 ml./min.
Hydrogen .40 ml./min. Air 400 ml./min.
Pressures: Inlet..... Outlet.....
DETECTOR:. 6H X 10' ....; ..... ma. ..... volts
Scavanger....., Rate..... ml./min.
Sens..... Rec. range..... mv.
SAMPLE DEGRADED 54A Size... 1 ml
Solvent... HEPTANE Concn. ..1:100
Pristane
Phytane
RECORDER RESPONSE
100
TEMPERATURE °C
300

FIG. 8

Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating..OV-1..1%..... Concn.....
Support..... Mesh.....
TEMP: Col: Init.100....°C. Final 300..°C.
Rate..4...°C./min. Det.320..°C. Inj 250 °C.
CARRIER GAS...N_2....., Rate.25.....ml./min.
Hydrogen 40....ml./min. Air 400...ml./min.
Pressures: Inlet..... Outlet.....
DETECTOR:..6.4×10^1.....; .....ma.....volts
Scavanger....., Rate.....ml./min.
Sens..... Rec. range.....mv.
SAMPLE DEGRADED 54A.. Size...1ml.....
Solvent...KEPTANE Concn. 1:100.....

54A CRUDE DEG. EXPT.
144 HR. INCUBATION

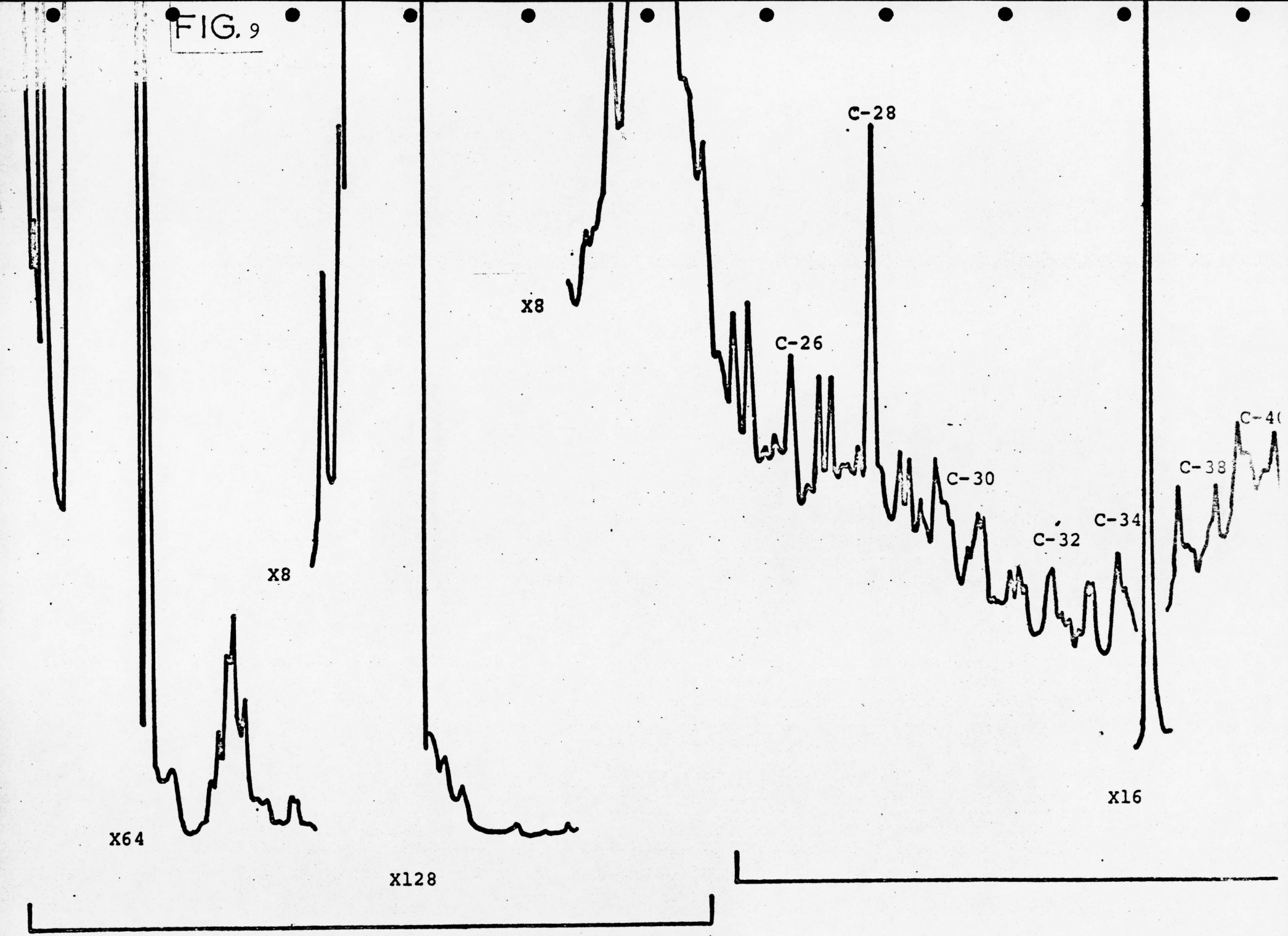


The results obtained during analysis of water samples collected during the April,May,July and October exercises merit special attention. Almost all these samples were contaminated with a suite of compounds which obscured the distribution of n-paraffins from nC-10 to nC-25 (Figure 9). Analysis of this contaminant with combined GC-MS revealed that certain of its components were chlorinated hydrocarbons with characteristic isotope clusters. A complete re-evaluation of our entire sampling procedure, analytical techniques and final work-up before analysis by MS-GC failed to turn up a source of contamination similar to the one encountered. A sample pooled from many water samples was sent to an outside laboratory for corroboratory analysis but had volatilized before arrival. We now find that certain samples can be re-analyzed with some degree of success as the chlorinated portion of the contaminant has evaporated. Attempts to remove the contaminant using column chromatography were not successful since the RF values were similar to hydrocarbons. The suggestion that construction activities in a new wing of the Marine Science Institute had poisoned the air conditioning system with plasticizers etc. appeared to have merit at one time. It was postulated that the contaminant entered the sample through the condenser vent during extraction. Extensive precautions to prevent the transfer of any organics through this vent were applied with little success. Finally, samples run in 1-74 through 4-74 which were sampled during the months indicated above still contained the contaminant in variable amounts while water samples extracted at the same time from Port Aransas or the 1-74 exercise show the sampling technique and the analytical scheme to be contaminant free. Our only conclusion is that the contaminant was not introduced into the water samples in the laboratory but was contained in the water. As previously mentioned exhaustive attempts to isolate the contaminant from some piece of equipment utilized in the sampling procedure were negative and according to Longhorn personel, the boat is a closed system incapable of contributing any contaminants. As mentioned in the Method section we have eliminated the

the acquisition of seawater samples using the copper tubing-vacuum technique for one which we feel is superior in ease of operation and eliminates potential sources of contamination possible with the copper tube method. The new technique was utilized for the 1-74 exercise with reasonable success for a prototype unit in sampling operations. All samples thus far extracted and analyzed have been free of contamination.

Detailed descriptions of each station sampled with respect to n-paraffin distribution are shown in Tables 3-21. In those instances where pristane and phytane were not completely resolved from the n-paraffins C-17 and C-18, the values are indicated as the sum of both the isoprenoid and its overlapping paraffin.

FIG. 9



CONTAMINANT

HYDROCARBONS

TABLE 3

Control Area H062

1-74

Total n-Paraffins 0.09 ug/l

C.P.I. 0.88

Envelope nC-17 to C-26

Percentage Composition 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

9-72

0.21 ug/l

1.19

Percentage Composition 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.	

TABLE 4

Control H062

1-73 4 Meters

Total n-Paraffins	0.05 ug/l
C.P.I. C-21 to C-33	1.00

Broad envelope present C-20 to C-35

Percentage Composition 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.	

1-73 1 Meter

	0.20 ug/l
	0.99

Broad envelope present from C-17 to C-36

Percentage Composition 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

TABLE 5

H062, 7-73 Control

1 Meter

Total n-Paraffins	0.05 ug/l
C.P.I.	1.11

No envelope

Percentage Composition 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.	

TABLE 6

K007 54A Platform 4m

9-72

Total n-Paraffins	0.34 ug/l
C.P.I.	0.93
Minor unresolved envelope	

Percentage Composition

n-Paraffins	
15	2.0
16	0.0
17+pristane	13.1
18+phytane	2.0
19	1.0
20	1.1
21	0.7
22	1.1
23	1.3
24	3.7
25	3.4
26	8.7
27	9.5
28	9.8
29	9.5
30	8.6
31	7.9
32	6.2
33	4.6
34	2.9
35	1.8
36 Internal Std.	
37	0.7
38	0.5

TABLE 7

54A Platform K007

1-73		1-74	
Total n-Paraffins	0.13 ug/l		0.08 ug/l
C.P.I.	1.37		1.18
Broad envelope present		No Envelope Present	

Percentage Composition n-Paraffins		Perc entage Composition n-Paraffins	
15	3.4	15	0.0
16	4.0	16	0.0
17+pristane	8.4	17+Pristane	17.6
18+phytane	8.4	18+phytane	10.8
19	7.8	19	5.6
20	7.6	20	4.6
21	5.4	21	3.8
22	6.6	22	2.9
23	5.3	23	3.4
24	4.4	24	3.3
25	4.4	25	4.9
26	4.4	26	4.9
27	4.5	27	3.8
28	5.7	28	5.6
29	4.5	29	6.1
30	2.6	30	6.4
31	3.4	31	5.1
32	1.4	32	5.7
33	2.1	33	3.4
34	1.7	34	3.3
35	1.9	35	2.2
36 Internal Std.		36 Internal Std.	
37	1.9		

TABLE 8

K040 S.E. 54A

9-72

Total n-Paraffins	0.35 ug/l
C.P.I.	0.94

Broad envelope from C-17 to C-36

Percentage Composition
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

K040 S.E. 54A

9-72

	0.49 ug/l
	0.99

Peaked envelope from C-17 on

Percentage Composition
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

TABLE 9

KO14 S.W. 54A

1-73 1 Meter

Total n-Paraffins 0.60 ug/l

C.P.I. C-21 to C-33 0.93

Envelope from C-16 to C-36

Percentage Composition 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

KO14 S.W. 54A

1-73 4 Meters

0.19 ug/l

0.99

Envelope from C-17 to C-35

Percentage Composition 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 10

Timbalier Bay

1-73	
Total n-Paraffins	0.38 ug/l
C.P.I.	0.91
Envelope present	

Percentage Composition

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	
0.37 ug/l	
2.06	
Broad Envelope Present	

Percentage Composition

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 11

Timbalier Bay

10-73

Total n-Paraffins	0.20 ug/l
C.P.I.	1.30

Envelope C-20 to C-28

Percentage Composition
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.	

1-73

	0.46 ug/l
	1.67

Broad envelope C-17 to C-30

Percentage Composition
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.	

TABLE 12

TIMBALIER BAY

1-74		4-73	
Total n-Paraffins	0.08 ug/l		1.10 ug/l
C.P.I.	1.27		1.35
Broad envelope present		Large envelope present	C17 to C-38
Percentage Composition		Percentage Composition	
n-Paraffins		n-Paraffins	
17-Pristane	19.4	15	2.7
18-Phytane	6.2	16	5.1
19	3.6	17-Pristane	12.3
20	3.2	18-Phytane	7.6
21	3.9	19	6.7
22	3.6	20	6.0
23	7.3	21	4.0
24	4.7	22	5.2
25	5.8	23	4.6
26	4.7	24	4.4
27	4.9	25	4.6
28	4.6	26	4.0
29	5.8	27	4.3
30	4.9	28	3.0
31	5.0	29	4.4
32	2.8	30	2.5
33	2.8	31	4.4
34	2.3	32	2.4
35	1.8	33	3.4
36 Internal Std.		34	1.8
37	1.6	35	2.6
38	0.4	36 Internal Std.	
		27	2.6
		38	1.3

TABLE 13

Sabine River
29 30
94 01 1-73 1 meter
Total n-Paraffins 0.22 ug/l
C.P.I. C-21 to C-33 0.75

Envelope present from C- 17 on

Percentage Composition
n-Paraffins

17+pristane	1.4
18+phytane	8.7
19	2.4
20	12.6
21	10.6
22	16.4
23	7.7
24	7.7
25	4.3
26	3.9
27	2.9
28	4.8
29	3.9
30	2.4
31	3.4
32	3.9
33	1.4
34	1.4
35	0.0
36 Internal Std.	

TABLE 14

Sabine River

29 40
93 11 1-73
E. Sabine River 1 Meter

Total n-Paraffins 0.12 ug/l
C.P.I. C-21 to C-33 0.96
Large envelope from C-15 to C-35

Percentage Composition
n-Paraffins

15	1.1
16	4.5
17+pristane	11.4
18+phytane	11.1
19	12.4
20	10.9
21	7.0
22	6.9
23	4.8
24	3.7
25	4.5
26	2.9
27	2.3
28	3.9
29	2.0
30	1.3
31	2.7
32	1.4
33	1.6
34	2.1
35	1.6
36 Internal Std.	

29 30
94 01 1-73
W. Sabine River .4 Meters

0.12 ug/l
0.93
Broad envelope from C-17 to C-30

Percentage Composition
n-Paraffins

17+pristane	3.2
18+phytane	2.5
19	4.2
20	3.7
21	3.2
22	4.6
23	4.1
24	5.3
25	1.0
26	6.0
27	6.6
28	9.0
29	7.8
30	6.5
31	9.0
32	5.3
33	6.0
34	3.7
35	3.3
36 Internal Std.	
37	2.3
38	2.5
39	1.1

TABLE 15

W.Sabine R. 7-73
29 13
93 42 1 m

Total n-Paraffins	0.03 ug/l
C.P.I. C-21 to C- 33	1.15

Percentage Composition
n-Paraffins

17+pristane	6.9
18+Phytane	4.0
19	2.6
20	2.6
21	3.0
22	3.3
23	3.6
24	3.0
25	4.3
26	3.0
27	4.0
28	3.6
29	5.3
30	4.0
31	5.3
32	4.0
33	5.0
34	5.0
35	10.32
36 Internal Std.	
37	5.6
38	4.6
39	3.3
40	2.6
41	1.3

TABLE 16

Ship Shoal
28 54
90 45 1-73

Total n-Paraffins	0.19 ug/l
C.P.I. C-21 to C-33	1.15
Broad envelope C-18 on	

Percentage Composition
n-Paraffins

16	0.7
17+pristane	1.1
18+phytane	0.7
19	3.0
20	6.2
21	8.1
22	5.1
23	2.7
24	1.3
25	3.1
26	3.9
27	4.9
28	4.7
29	8.3
30	9.6
31	9.9
32	7.4
33	6.4
34	5.1
35	6.0
36 Internal Std.	
37	0.0
38	1.8

TABLE 17

29 03
92 52 Control 7-73
1 Meter

Total n-Paraffins	0.09 ug/l
C.P.I. C-21 to C-33	1.34

No envelope

Percentage Composition
n-Paraffins

16	3.2
17+pristane	39.2
18+phytane	8.5
19	4.1
20	3.8
21	2.9
22	2.5
23	3.4
24	1.6
25	3.7
26	2.6
27	2.6
28	2.2
29	3.1
30	2.5
31	3.7
32	2.0
33	2.5
34	2.2
35	3.8
36 Internal Std.	

TABLE 18

Timbalier Isle. 1.8 Miles 7-73

Total n-Paraffins	0.05 ug/l
C.P.I. C-21 to C-33	1.17
No envelope	

Percentage Composition
n-Paraffins

17+pristane	15.1
18+phytane	2.5
19	0.0
20	2.0
21	2.0
22	2.9
23	4.1
24	2.9
25	0.0
26	3.3
27	4.1
28	4.1
29	4.5
30	3.3
31	7.8
32	4.5
33	4.5
34	5.3
35	8.6
36 Internal Std.	
37	4.5
38	5.7
39	4.1

Major hydrocarbons C-17+pristane, C-31, C-34, C-35, C-38

TABLE 19

Brownsville 7-73

Total n-Paraffins 0.09 ug/l
C.P.I. 1.17
Small envelope C20 to C-30

Percentage Composition
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

TABLE 20

Port Aransas, 5-73, 5 miles offshore, 26 meter contour

Total n-paraffins	0.07 ug/l
C.P.I. C-21 to C-33	0.98
No envelope	

Percentage Composition
n-Paraffins

17+pristane	1.7
18+phytane	8.2
19	1.7
20	2.1
21	2.1
22	3.0
23	3.5
24	3.0
25	3.6
26	2.7
27	3.2
28	6.6
29	6.5
30	8.5
31	10.1
32	9.2
33	7.3
34	6.5
35	4.7
36 Internal Std.	
37	3.6
38	3.9

TABLE 21

Port Aransas, Texas 5-74
5 miles offshore from Marine Science Institute, 27 46
96 52

Total n-Paraffins	0.07 ug/l
C .P.I. C-21 to C-33	1.32
Minor envelope C-19 to C-28	

Percentage Composition
n-Paraffins

15	2.9
16	4.3
17+pristane	22.2
18+phytane	9.5
19	3.8
20	2.0
21	2.2
22	1.1
23	0.9
24	0.9
25	0.9
26	0.6
27	1.1
28	0.8
29	2.0
30	2.5
31	6.2
32	6.3
33	8.3
34	7.2
35	6.5
36 Internal Std.	
37	3.2
38	2.6
38	0.9
40	1.1

Major hydrocarbons C-17+pristane, 18+phytane, 31, 32, 33, 34, 35.

Hydrocarbons in Organisms

Salient characteristics describing n-paraffin distributions in marine organisms and plankton are shovm in Table 22-23. n-Paraffin percentage composition data and C.P.I. values for these samples are found in Tables 25 through 46. In general, many of the characteristics noted for paraffiin hydrocarbons in seawater are also applicable to hydrocarbon distributions in organisms. Plankton n-paraffins are usually distributed bimodally with pristane and/or nC-17 dominant followed by a minimum in the distribution leading to a second maximum in the nC-25 to nC-35 region. Total saturated paraffins in plankton from the Gulf of Mexico are generally higher than those reported in the literature from other areas (Clark and Blumer, 1967; Youngblood et al., 1971, Brogden, 1968) and range from 81 to 5400 ug/gm, the latter sample being obtained in a recent plankton tow offshore Port Aransas (4-74, Table46, Figure 13). The relatively large saturated paraffin concentrations can be correlated with the presence of a very obvious unresolved envelope and/or to the production of large amounts of a single hydrocarbon such as pristane, nC-17 or nC-17:1. Figures 10,11,12 are examples of zooplankton and mixed plankton extracts. Figure 10 clearly illustrates the bimodal hydrocarbon distribution encountered in some samples. In this chromatogram the relative abundances of nC-16,nC-17,nC-18,nC-19,nC-20,nC-21 and nC-22 suggest contamination by a petroleum or refined product. There is no doubt that organisms in the area are being exposed to refined petroleum products owing to the activities of work boats etc. An extract of a foam line at G039 (4-73, 5 mi. N.54A)

was extraordinarily rich in what appears to be diesel fuel (Table 37). Particulate detritus in the foam line resulted in a marked odd-even carbon preference in the nC-25 to nC-33 region (C.P.I. = 5.72, Table 37). Figure 12 illustrates the pronounced hump found in many zooplankton extracts after 1-73. This begins at about nC-19 and then falls toward the baseline after peaking at nC-22. Branched paraffins are conspicuous in this hump region between nC-21 to nC-24. These paraffins possess the same retention indices as the major branched paraffins found in a molecular sieve treated 54A Louisiana crude oil (n-paraffin free) produced in the area. In some extracts it is possible to follow essentially the same branched paraffin fingerprint in the nC-19 to nC-25 region encountered in the 54A crude oil. Because of the dominance of branched paraffins in this region of the chromatograms we must surmise that planktonic organisms are being exposed to partially degraded mixtures of hydrocarbons which appear to originate from petroleum. Our laboratory studies (Kator, 1972) indicate that marine bacteria will selectively degrade n-paraffins before isoprenoids and other branched paraffins. It is tempting to suggest that the appearance of extracts with this nC-19 hump after 1-73 reflects the extent of degradation of petroleum which was released in the winter months and therefore subject to reduced rates of bacterial degradation. Hydrocarbons observed in Gulf of Mexico plankton are therefore a complex mixture resulting from the summation of diverse sources. Superimposed on the residual petroleum envelope are the dominant hydrocarbons produced by the plankton themselves, i.e. pristane, nC-15, nC-17 and the hydrocarbons obtained through the ingestion

of particulates rich in terrestrial plant hydrocarbons. Plankton in the study area generally exhibit an n-paraffin distribution in the nC-25 through nC-33 region which displays an odd-even carbon preference. This could be due to either ingestion or to physical incorporation within the tow of particulate detritus derived from terrestrial plants. Tar balls and crude oil from the area examined do not exhibit an odd-even carbon preference. In fact, although large tar balls were never observed in the study area during cruise exercises, one was isolated with great difficulty from a plankton tow (Oct 73, H062) with a diameter of 0.1 mm (and therefore we must presume have been present in other tows as well. The hydrocarbon distribution (Table 24, Figure 1) reveals the presence of the more resistant isoprenoids pristane and phytane and n-paraffins of relatively high molecular weight. The distribution suggests microbial degradation has occurred and is very similar to hydrocarbon distributions in seawater obtained in recent months from Port Aransas (Figure 4) and Timbalier Bay.

A striking example of the odd-even carbon preference found in some zooplankton extracts is seen in the hydrocarbon distribution from the polychaete Spiochaetopterus oculatus (50 samples) a dominant form in Timbalier Bay. A C.P.I. of 8.80 was calculated (Table 42) and must be correlated with the heavy load of organic plant detritus which falls to the bay sediments. Whether this paraffin distribution is dietary in origin or reflects particulate hydrocarbons incorporated in its tube is unknown but must be attributed to the influx of terrestrial hydrocarbons into the bay. The influence of terrestrial hydrocarbons on the

hydrocarbon distributions encountered in plankton and other organisms suggests the importance of terrestrial plants as a source of organic carbon in the nearshore zone. Thus the bimodal distribution encountered in zooplankton may be due to the ingestion and/or physical inclusion of particulate plant detritus into the plankton tow.

Both the sand trout , Cynoscion arenarius and the green algae Enteromorpha sp. possessed a homologous series of n-paraffins plus the isoprenoids pristane and phytane. While the dominant hydrocarbon in Enteromorpha was nC-17:1 as revealed by mass spectrometry and substantiated in the literature (Youngblood et al., 1971), trace amounts of n-paraffins from nC-17 to nC-37 were detected. This suite of hydrocarbons has not been observed in Enteromorpha compressa collected in Little River, Waquoit Bay, Mass (ibid.) The n-paraffin distribution in the sand trout revealed the presence of n-paraffins from nC-18 to nC-39 plus pristane. Unfortunately, comparative data on the n-paraffin distributions in fish are not available but the ratio of phytane/nC-18 suggests contamination by petroleum (Table 23). Surprisingly, Balanus sp., growing in close proximity to 54A as does Enteromorpha sp., does not contain a homologous series of n-paraffins. This is interesting if one considers that Balanus filters the water of zooplankton and other detritus. The paraffin distribution observed reveals pristane as the dominant saturated hydrocarbon with smaller amounts of phytane and nC-18. Smaller amounts of other n-paraffins were also

detected (Table 44). Perhaps Bàlanus sp. contains symbiotic microorganisms which degrade the n-paraffins contained in the zooplankton which it must ingest.

Finally, the data presented in Table 23 reveal that the ratios of pristane/nC-17 and pristane/phytane are rather variable and probably reflect compositional differences in the plankton tows, i.e., the relative abundance of zooplankton to phytoplankton. However, the ratio of phytane to nC-18 is very similar in all samples and in 54A crude oil and diesel fuel. Unless the constancy of this ratio is biologically controlled, this observation offers additional proof that fossil hydrocarbons are being released into the Gulf of Mexico and are either absorbed, adsorbed or ingested by marine organisms.

FIG. 10

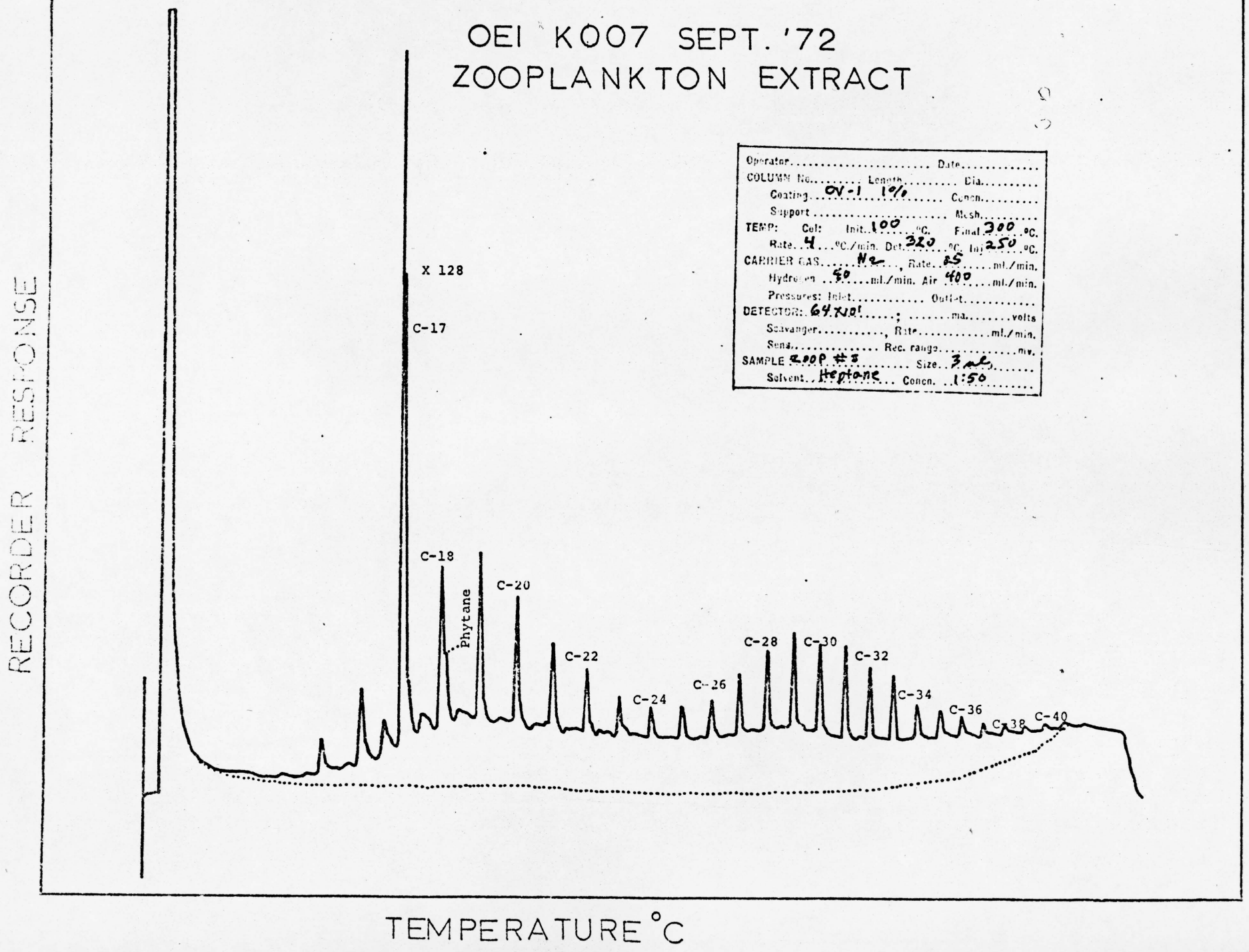

OEI K007 SEPT. '72
ZOOPLANKTON EXTRACT
Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating OV-1 1% Concn.....
Support..... Mesh.....
TEMP: Col: Init. 100 °C. Final 300 °C.
Rate 4 °C./min. Det. 320 °C. Inj 250 °C.
CARRIER GAS N2, Rate 25 ml./min.
Hydrogen 50 ml./min. Air 400 ml./min.
Pressures: Inlet..... Outlet.....
DETECTOR: 64 x 10; ..... ma. ..... volts
Scavanger....., Rate..... ml./min.
Sens..... Rec. range..... mv.
SAMPLE ZOOP #3 Size 3 ml
Solvent Heptane Concn. 1:50
RECORDER RESPONSE
X 128
C-17
C-18
Phytane
C-20
C-22
C-24
C-26
C-28
C-30
C-32
C-34
C-36
C-38
C-40
TEMPERATURE °C

FIG. 11

OEI K007 JAN. '73
ZOOPLANKTON EXTRACT

Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating...OV-1 1%... Concn.....
Support..... Mesh.....
TEMP: Col: Init. 100 °C. Final 300 °C.
Rate. 4 °C./min. Det. 320 °C. Inj 250 °C.
CARRIER GAS...N2..., Rate. 25 ml./min.
Hydrogen ..40.... ml./min. Air 400 ml./min.
Pressures: Inlet..... Outlet.....
DETECTOR: 8X10¹.....; .....ma.....volts
Scavanger....., Rate.....ml./min.
Sens..... Rec. range.....mv.
SAMPLE K007-200F ..... Size...1ml.....
Solvent. HEPTANE... Concn. 1:50.....

RECORDER RESPONSE

C-17
Pristane
Phytane
C-19
C-21
C-23
C-25
C-27
C-29
C-31
C-33
C-35
C-37
C-39
C-41

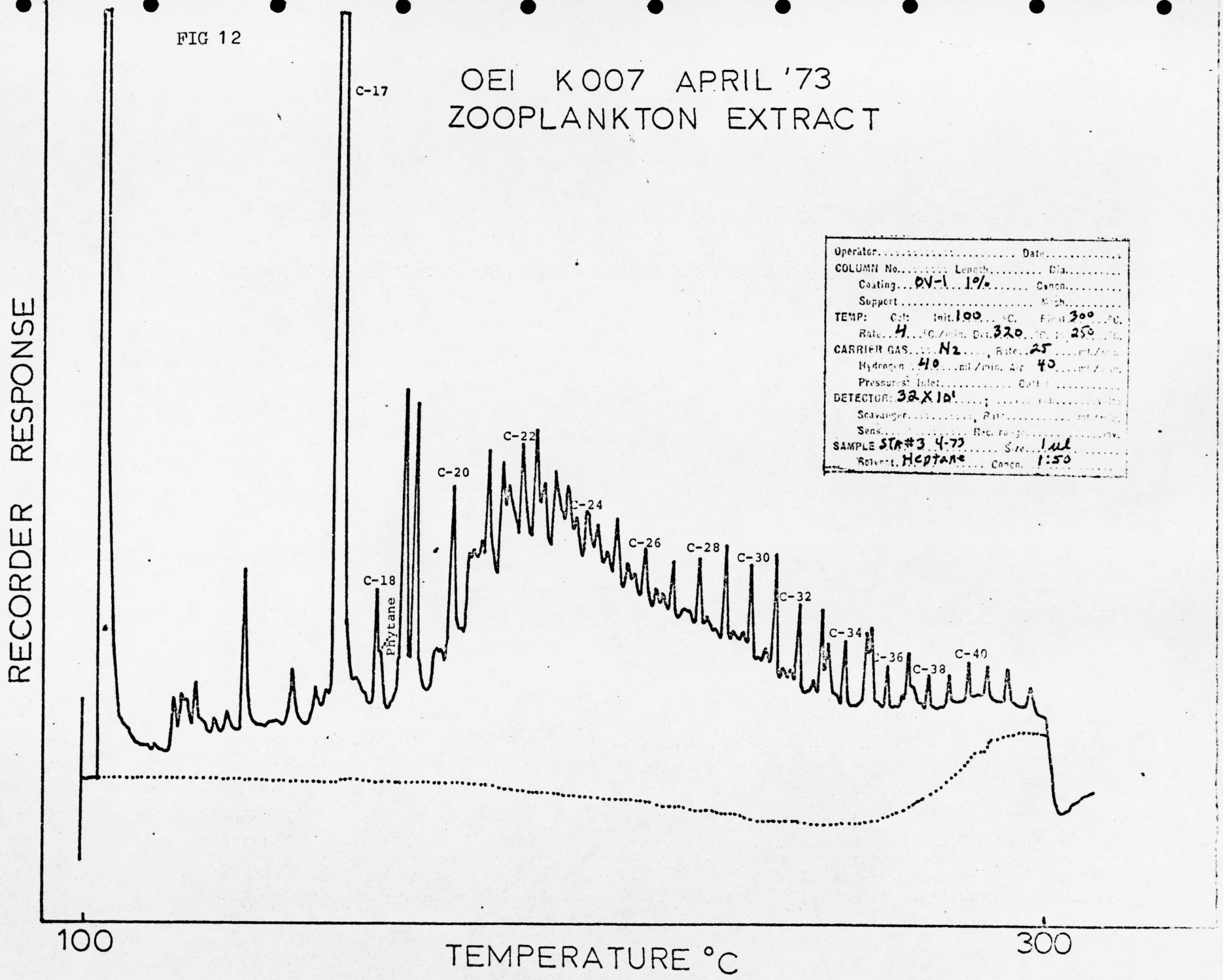
100

TEMPERATURE °C

300

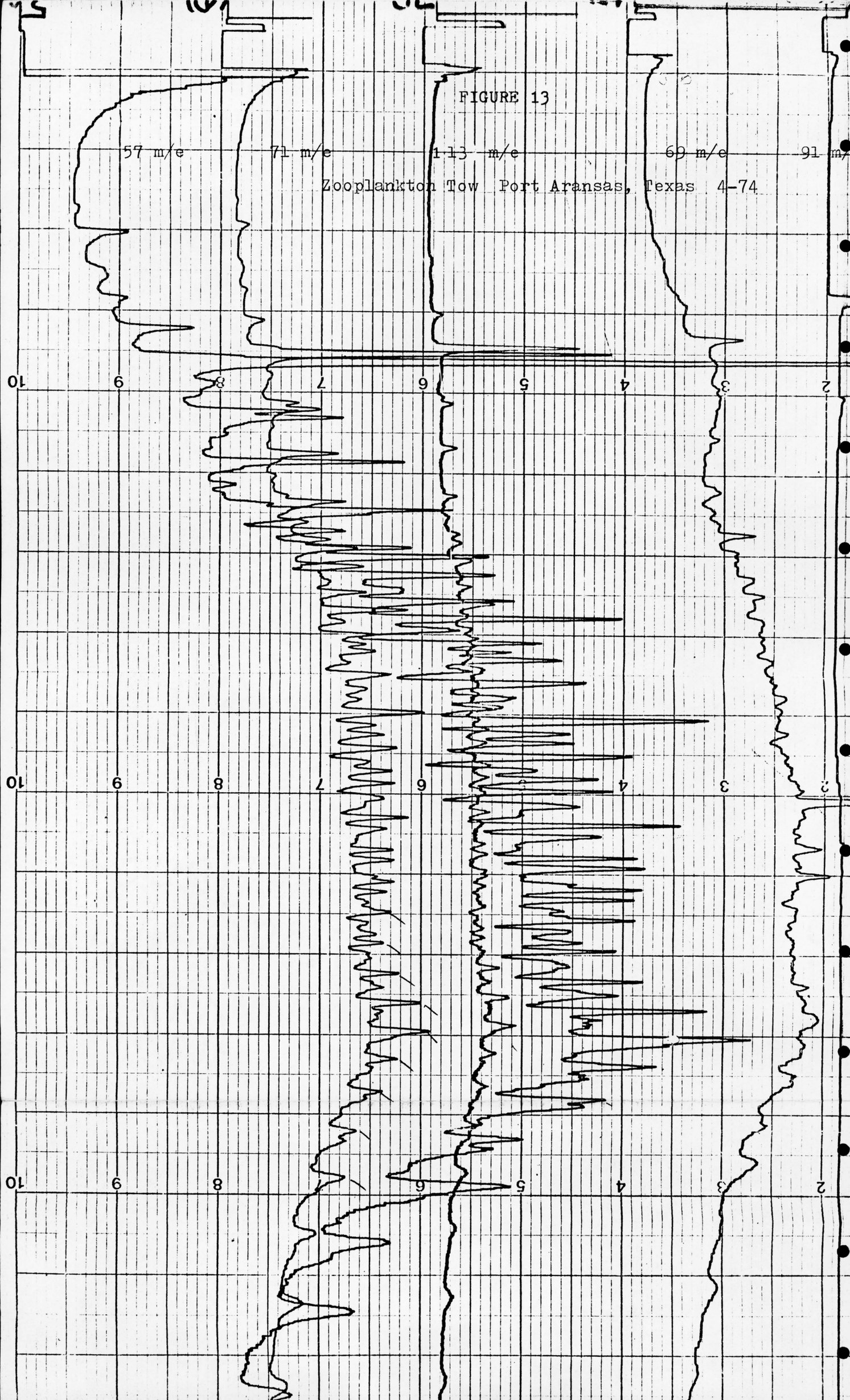
FIG 12

OEI K007 APRIL '73
ZOOPLANKTON EXTRACT



Operator..... Date.....
COLUMN No..... Length..... Dia.....
Coating...OV-1 1%..... Conc.....
Support..... Mesh.....
TEMP: Col: Init.100 °C. Final 300 °C.
Rate...4...°C./min. Det.320 °C. Inj. 250 °C.
CARRIER GAS...N2..., Rate...25 ml/min.
Hydrogen...40...ml/min. Air 40 ml/min.
Pressures: Inlet..... Outlet.....
DETECTOR: 32X10¹...; ..... °C.
Scavenger....., Rate..... ml/min.
Sens..... Rec. resp..... mv.
SAMPLE STA#3 4-73 ..... Size 1 µl
Solvent, Heptane ..... Concn. 1:50

FIGURE 13


57 m/e
71 m/e
113 m/e
69 m/e
91 m/e
Zooplankton Tow Port Aransas, Texas 4-74

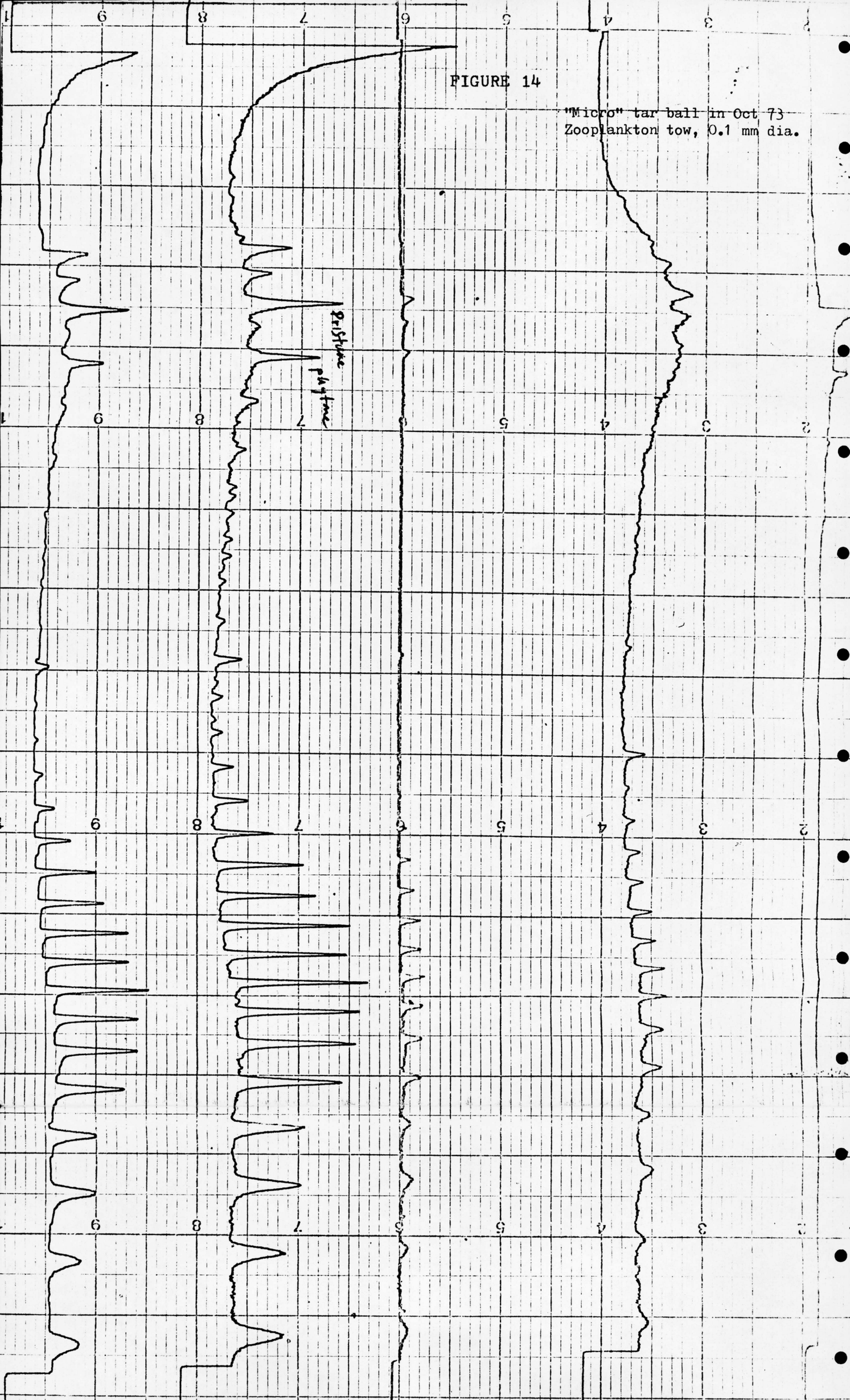


FIGURE 14

"Micro" tar ball in Oct 73
Zooplankton tow, 0.1 mm dia.

TABLE 22

Paraffins in Organisms

Date	Station	Concentration Saturated Paraffins ug/gm, Dry Weight	C.P.I. C-21 to C-33	Unresolved Envelope	Major* n-Paraffins and Isoprenoid Hydrocarbons
9-72	Zooplankton K023 S.W.54A	3714	1.03	absent	nC-17
9-72	Zooplankton G064 N.54A	490	1.23	present	pristane, nC-15, nC-16, nC-17, nC-18 nC-19
9-72	Macrocoleus sp. (Blue green algae) K007 54A	n.a.	---	absent	nC-17
9-72	Zooplankton K007 54A	n.a.	1.10	present	nC-17+pristane, nC-18+phytane, nC-19
9-72	Phytoplankton K007 54A	n.a.	1.20	absent	nC-17+pristane, nC-18+phytane, nC-19, nC-20, nC-21, nC-22
9-72	Mixed Plankton G086	3721	1.04	present	nC-17
1-73	Zooplankton K014 S.W.54A	414	1.21	present	pristane, nC-28
1-73	Zooplankton K007 54A	n.a.	1.01	present	nC-17, pristane, nC-24, nC-25, nC-26, nC-27, nC-28, nC-29
4-73	Zooplankton 29 06 93 59	1172	1.24	absent	pristane
4-73	Zooplankton K007 54A	356	1.20	present	pristane, nC-17, nC-19
4-73	Zooplankton H062 Control	189	1.65	present	pristane
4-73	Surface Foam G039	---	5.72	present	nC-14, nC-15, nC-16, nC-17, pristane, nC-18, phytane, nC-19

* Greater than 5% of the total signal from n-paraffins and the isoprenoids pristane and phytane.

TABLE 22 Cont'd

4-73	Cynoscion arenarius Sand Trout HORST-67	105	1.13	present	pristane
7-73	Zooplankton H062 Control	108	2.82	present	pristane,nC-17 nC-27,nC-29, nC-31
7-73	Zooplankton 29 03 92 52	81	2.39	present	pristane,nC-17 nC-31,nC-35
10-73	Zooplankton H062 Control	3074	1.16	present	pristane,nC-25
10-73	Zooplankton K007 54A	1620	1.39	present	pristane,nC-25
10-73	Spiochaetopterus oculatus polychaete, BC04 Timablier Bay	292	8.80	present	nC-23,nC-25, nC-27,nC-29
1-74	Zooplankton 28 41 93 09	1231	1.21	present	nC-15,nC-17, pristane
1-74	Enteromorpha sp., Buoy 54A	530	1.24	absent	nC-17:1,nC-17
1-74	Balanus sp.	37.9 mg/gm	---	----	pristane,nC-18 phytane
4-74	Zooplankton 27 48 96 57 Port Aransas	5.4 mg/gm	1.13	present	pristane,nC-39

TABLE 23

Ratios of Selected Isoprenoid and Normal Paraffin Hydrocarbons in Selected Samples

Sample	Pristane / nC-17	Pristane / Phytane	Phytane / nC-18
Zooplankton G064, 9-72	1.3	10.1	0.29
Zooplankton K007, 9-72	0.2	4.1	0.25
Zooplankton K023, 9-72	nC-17 Dominant hydrocarbon		
Phytoplankton K007, 9-72	0.1	2.3	0.17
Zooplankton K014, 1-73	20.1	150.1	0.31
Zooplankton K007, 4-73	3.7	94.0	0.30
Cynoscion arenarius HORST-67D 4-73	Pristane Dominant hydrocarbon		0.30
Surface Foam G039, 4-73	0.7	2.6	0.40
Zooplankton H062, 7-73	2.5	44.5	0.27
Zooplankton E.Cameron 7-73	7.1	52.0	0.25
Zooplankton H062, 10-73	2.8	74.5	0.42
Seawater Timbalier Bay 10-73	nC-17 Dominant hydrocarbon		0.17
Seawater K007, 1-74	0.5	3.0	0.28
Zooplankton W.Cameron 1-74	1.1	129.3	0.4
Seawater Timbalier Bay 1-74	0.1	19.4	0.2

TABLE 23 Cont'd

Zooplankton Port Aransas 4-74	5.2	9.3	0.8
54A Crude oil	0.6	2.4	0.3
Longhorn Diesel Fuel	0.8	3.6	0.4

TABLE 24

"Micro" Tar Ball (0.1 mm dia.) obtained from Plankton Tow K007 (54A) 10-73

C.P.I. = 0.93

Percent Composition n-Paraffins and Isoprenoids	
16	2.4
Pristane	1.8
Phytane	1.8
19	0.4
20	0.2
21	0.2
22	0.3
23	0.3
24	0.4
25	0.4
26	0.6
27	0.9
28	1.6
29	2.5
30	3.7
31	4.3
32	5.6
33	5.1
34	6.0
35	6.1
36	5.7
37	5.5
38	5.7
39	6.2
40	6.9
41	8.4
42	6.6
43	6.3
44	4.2

TABLE 25

Zooplankton Tow, K023 S.W.54A , Night, 9-72
Saturated Paraffins, Dry Weight Basis = 3714 ug/gm or .371%
C.P.I. 1.03
Envelope absent

Percentage Composition n-Paraffins	
15	0.28
16	1.02
17	96.76
18	0.28
19	0.22
20	0.03
21	0.03
22	0.06
23	0.08
24	0.08
25	0.10
26	0.10
27	0.11
28	0.11
29	0.13
30	0.11
31	0.13
32	0.17
33	0.10
34	0.03
35	0.03
36	0.02
37	0.01
38	0.01
39	0.01
40	0.02
41	0,02
42	0.02
43	0.01

Dominant Hydrocarbons = nC-17

Table 26

Zooplankton Tow , G064 N. 54A, 9-72
Saturated Paraffins, Dry Weight Basis = 490.01 ug/gm or 0.049%
C.P.I. 1.23
Envelope present from C-16 to C-36

Percentage Composition
n-Paraffins

15	6.8
16	5.8
17+pristane, pristane dominant	45.9
18+phytane	9.0
19	8.5
20	4.9
21	3.3
22	2.1
23	1.5
24	1.1
25	1.4
26	1.3
27	1.4
28	1.0
29	1.4
30	0.7
31	1.4
32	0.7
33	0.7
34	0.4
35	0.6

Dominant Hydrocarbons = pristane, C-15,16,17,18,19

TABLE 27

Macrocoleus sp. , K007 54A Platform, 9-72

Percentage Composition n-Paraffins	
15	0.2
16	0.1
17	69.5
18	1.3
19	0.6
20	26.3
21	0.3
22	0.4
23	0.2
24	0.1
25	0.1
26	0.1
27	0.2
28	0.2
29	0.1
30	0.1
31	0.1
32	0.1

Dominant Hydrocarbons = nC-17,

TABLE 28

Zooplankton Tow, K007 54A Platform, 9-72
Saturated Paraffins, Dry Weight Basis = n.a.
C.P.I. 1.10
Envelope present C-17 on

Percentage Composition n-Paraffins	
15	1.3
16	2.7
17+pristane	32.4
18+phytane	17.1
19	5.8
20	4.5
21	3.3
22	2.3
23	1.5
24	1.2
25	1.2
26	1.4
27	2.3
28	2.9
29	3.7
30	3.3
31	3.7
32	2.7
33	2.5
34	1.5
35	1.1
36	0.9
37	0.8
38	0.7
39	0.3
40	0.3
41	0.6
42	0.1

Dominant Hydrocarbons = C-17+pristane, 18+phytane, 19

TABLE 29

Phytoplankton Tow, K007 54A Platform, 9-72
Saturated Paraffins= n.a.
C.P.I. 1.20
No envelope

Percentage Composition
n-Paraffins

17+pristane, nC-17 dominant	30.3
18+phytane	12.6
19	10.6
20	10.9
21	8.7
22	6.7
23	4.1
24	2.4
25	4.0
26	1.4
27	1.0
28	1.1
29	1.0
30	0.9
31	1.3
32	0.7
33	0.9
34	0.6
35	0.8

Dominant Hydrocarbons = 17+pristane, 18+phytane, 19,20,21,22

TABLE 30

Mixed Plankton Tow, G086, 9-72
Saturated Paraffins, Dry Weight Basis = 3721 ug/gm or 0.372%
C.P.I. 1.04
Broad envelope C-19 on

Percentage Composition
n-Paraffins

16	1.80
17	95.00
18	0.60
19	0.50
20	0.08
21	0.05
22	0.05
23	0.05
24	0.06
25	0.08
26	0.08
27	0.11
28	0.11
29	0.12
30	0.12
31	0.15
32	0.16
33	0.14
34	0.11
35	0.11
36	0.09
37	0.09
38	0.09
39	0.08
40	0.08
41	0.08
42	0.06
43	0.03

Dominant Hydrocarbons = nC-17

TABLE 31

Zooplankton Tow, K014 S.W. 54A, 1-73

Saturated Paraffins, Dry Weight Basis = 414.4 ug/gm or 0.041%
C.P.I. 1.21
Pronounced envelope from C-19 to C-30

Percentage Composition
n-Paraffins

15	1.7
16	0.0
pristane	36.9
18+phytane	2.4
19	2.2
20	2.8
21	3.9
22	3.8
23	4.1
24	3.0
25	4.9
26	3.1
27	3.4
28	5.7
29	4.4
30	2.8
31	4.0
32	1.9
33	2.8
34	1.9
35	1.8
36	1.1
37	1.8

Dominant Hydrocarbons = pristane, C-28

TABLE 32

Zooplankton Tow, K007, 1-73
Saturated Paraffins, Dry Weight Basis= n.a.
C.P.I. 1.01

Percentage Composition
n-Paraffins

16	0.9
17+pristane, pristane dominant	8.4
18+phytane	2.0
19	3.9
20	2.7
21	2.3
22	2.7
23	3.5
24	5.8
25	7.5
26	8.2
27	7.9
28	6.8
29	5.5
30	4.3
31	4.6
32	3.5
33	3.2
34	2.4
35	2.3
36	2.0
37	2.0
38	1.9
39	2.0
40	2.2
41	1.6

Dominant Hydrocarbons =pristane+C-17, 24,25,26,27,28,29

TABLE 33

Zooplankton Tow, 29 06 , 4-73
93 59
Saturated Paraffins= 1171.8 ug/gm or 0.12%
C.P.I. 1.24

Percentage Composition
n-Paraffins

15	0.08
pristane	88.99
18+phytane	0.39
19	0.67
20	1.00
21	0.88
22	0.71
23	0.47
24	0.49
25	0.68
26	0.56
27	0.68
28	0.58
29	0.84
30	0.61
31	0.97
32	0.44
33	0.49
34	0.34
35	0.23

Dominant Hydrocarbons= Pristane

TABLE 34

Zooplankton Tow, K007 54A Platform, 4-73
Saturated Paraffins, Dry Weight Basis= 355.5 ug/gm or 0.035%
C.P.I. 1.20
Pronounced envelope from pristane to C-35

Percentage Composition
n-Paraffins

15	1.7
16	0.5
17+pristane, pristane dominant	41.3
18+phytane	4.7
19	6.5
20	3.6
21	3.5
22	2.3
23	1.9
24	1.6
25	1.9
26	1.3
27	1.7
28	1.9
29	2.9
30	2.8
31	3.8
32	2.6
33	2.7
34	2.1
35	2.0
36	1.2
37	0.9
38	0.9
39	0.6
40	0.7
41	0.8
42	0.8
43	0.4
44	0.4

Dominant hydrocarbons = C-17+pristane, C-19

TABLE 35

Zooplankton Tow, H062 Control, 4-73
Saturated Paraffins, Dry Weight Basis = 189.3 ug/gm or 0.019%
C.P.I. 1.65
Broad envelope present from pristane to C-38

Percentage Composition
n-Paraffins

15	0.3
16	0.4
pristane	89.7
18+phytane	0.9
19	0.8
20	0.7
21	0.6
22	0.4
23	0.4
24	0.3
25	0.6
26	0.5
27	0.6
28	0.5
29	0.7
30	0.3
31	0.8
32	0.3
33	0.8
34	0.2
35	0.2
36	0.1
37	trace
38	trace

Dominant Hydrocarbons = pristane

TABLE 36

Surface Foam, 28 56 23, G039, 4-73
90 22 45
C.P.I. 5.72
Envelope present C-13 to C-25

Percentage Composition
n-Paraffins

13	1.4
14	5.4
15	10.4
16	13.2
17	13.2
pristane	14.8
18	10.2
phytane	6.6
19	8.1
20	4.7
21	2.1
22	0.8
23	0.5
24	0.1
25	0.4
26	trace
27	1.2
28	trace
29	3.8
30	trace
31	1.9
32.	trace
33	0.9

Dominant Hydrocarbons = nC-14,15,16,17,pristane,18,phytane
19

TABLE 37

Cynoscion arenarius , HORST-67D, 4-73

Saturated Paraffins, Dry Weight Basis= 105. ug/gm or 0.011%
C.P.I. 1.13
Minor envelope Pristane to C-28

Percentage Composition
n-Paraffins

pristane	43.4
18+phytane	3.7
19	1.3
20	1.3
21	1.5
22	1.7
23	1.5
24	1.7
25	1.9
26	1.7
27	2.3
28	2.8
29	2.7
30	2.8
31	4.1
32	3.3
33	4.7
34	3.9
35	4.4
36	4.0
37	0.7
38	2.0
39	1.6

Dominant Hydrocarbons= pristane

TABLE 38

Zooplankton Tow, H062 Control 7-73
Saturated Paraffins, Dry Weight Basis = 108.0 ug/gm or 0.010%
C.P.I. 2.82
Pronounced envelope from C-19 to C-31

Percentage Composition
n-Paraffins

17+pristane, pristane dominant	25.5
18+phytane	2.4
19	2.1
20	2.2
21	3.0
22	2.3
23	2.3
24	2.1
25	4.7
26	2.3
27	5.3
28	2.6
29	10.4
30	2.3
31	10.6
32	1.4
33	4.7
34	1.4
35	10.9
36	0.4
37	0.4
38	0.3

Dominant Hydrocarbons =pristane, C-17,27,29,31

TABLE 39

Zooplankton Tow, Control 29 03, 7-73
92 52
Saturated Paraffins, Dry Weight Basis= 80.6 ug/gm or 0.008%
C.P.I. 2.39
Pronounced envelope present, pristane to C-30

Percentage Composition
n-Paraffins

pristane	33.7
18+phytane	4.8
19	2.6
20	2.6
21	3.8
22	2.9
23	2.9
24	2.1
25	4.9
26	2.4
27	4.5
28	3.1
29	9.0
30	2.1
31	7.7
32	1.0
33	3.4
34	0.6
35	5.8

Dominant Hydrocarbons = pristane, C-29, 31, 35

TABLE 40

Zooplankton Tow, H062 Control, Oct 73
Saturated Paraffins, Dry Weight Basis= 3074 ug/gm or 0.31%
C.P.I. 1.16
Major envelope present from C-20 to C-35

Percentage Composition
n-Paraffins

15	3.3
16	0.9
17+pristane, pristane dominant	31.6
18+phytane	0.7
19	1.2
20	2.6
21	4.3
22	4.8
23	4.3
24	4.1
25	5.3
26	4.1
27	4.1
28	3.2
29	4.3
30	2.9
31	4.7
32	2.3
33	1.9
34	4.6
35	4.9

Dominant Hydrocarbons Pristane, C-25

TABLE 41

Zooplankton Tow, K007 54A Platform, Oct. 73
Saturated Paraffins, Dry Weight Basis= 1620 ug/gm or 0.16%
C.P.I. 1.39
Major envelope present from C-17 to C-39

Percentage Composition
n-Paraffins

15	3.5
16	1.4
17+pristane, pristane dominant	28.1
18+phytane	4.2
19	1.4
20	4.2
21	4.2
22	3.9
23	3.5
24	2.1
25	7.8
26	2.5
27	1.0
28	2.3
29	4.0
30	3.3
31	3.9
32	2.2
33	2.8
34	2.4
35	1.5
36	1.5
37	1.5
38	1.5
39	1.5
40	1.5
41	0.7

Dominant Hydrocarbons = pristane, C-25

TABLE 42

Polychaete, BC04 Oct 73, Timbalier Bay
Spiochaetopterus oculatus
Saturated Paraffins, Dry Weight Basis= 292.4 ug/gm or 0.023%
C.P.I. 8.80
Envelope present rrom C-17 to C-26

Percentage Composition
n-Paraffins

15	0.7
16	0.9
17	2.1
Pristane	2.0
18	0.8
phytane	0.5
19	0.7
20	0.5
21	1.0
22	0.8
23	6.3
24	2.1
25	29.5
26	2.4
27	31.0
28	1.2
29	7.5
30	1.0
31	3.0
32	0.8
33	2.0
34	1.2
35	2.1

Dominant hydrocarbons C-23,25,27,29

TABLE 43

Zooplankton Tow, Control 28 41 1-74
93 09
Saturated Paraffins, Dry Weight Basis= 1231 ug/gm or 0.123%
C.P.I. 1.21
Envelope present, C-20 to C-40

Percentage Composition
n-Paraffins

15	5.4
16	0.6
17	23.0
Pristane	36.8
18	0.6
phytane	0.5
19	0.8
20	0.6
21	0.6
22	0.8
23	2.0
24	1.4
25	2.3
26	2.3
27	2.9
28	2.6
29	3.1
30	2.6
31	2.7
32	2.0
33	1.7
34	1.3
35	1.4
36	0.6
37	0.5
38	0.5
39	0.2
40	0.3

Dominant Hydrocabons= C-15, 17, pristane

TABLE 44

Balanus sp., K007 54A Platform, 1-74
Saturated Paraffins, Dry Weight Basis= 37.9 mg/gm or 3.79%
C.P.I. = n.a.
Atypical envelope present with complex branching indicated

Percentage Composition n-Paraffins	
14	0.3
15	2.2
16	---
17	trace
pristane	84.2
18+phytane	5.9
19	1.3
20	1.9
21	---
22	---
23	---
24	---
25	---
26	1.9
27	1.8
28	---
29	0.5

Dominant Hydrocarbons= pristane, 18+phytane

TABLE 45

Enteromorpha Sp. attached to line from Buoy 54A. (K007), 1-74

Saturated Paraffins, Dry Weight Basis= 530 ug/gm or 0.053%
C.P.I. 1.24
Pronounced envelope absent

Percentage Composition
n-Paraffins

15	0.70
16	0.00
17:1	83.60
17	13.70
18+phytane	0.20
19	0.10
20	0.10
21	0.07
22	0.07
23	0.08
24	0.09
25	0.13
26	0.09
27	0.14
28	0.14
29	0.14
30	0.10
31	0.12
32	0.07
33	0.10
34	0.04
35	0.06
36	0.06
37	0.01

Dominant hydrocarbons= 17:1, nC-17

TABLE 46

Zooplankton Tow, 27 48 (5 mi. offshore Port Aransas) 4-74
96 57
Saturated Paraffins, Dry Weight Basis= 5.44 mg/gm or 0.544%
C.P.I. 1.13
Pronounced broad envelope present from pristane to end of program (300 C)

Percentage Composition
n-Paraffins

15	0.69
17+pristane, pristane dominant	11.75
18	1.44
phytane	2.02
19	2.66
20	2.93
21	2.77
22	2.50
23	2.34
24	2.45
25	3.56
26	2.82
27	2.40
28	3.04
29	2.16
30	2.30
31	3.32
32	1.97
33	2.18
34	3.09
35	3.70
36	2.67
37	2.97
38	2.57
39	6.65
40	3.61
41	4.79
42	3.73
43	3.80
44	4.11
45	3.04

Summary

1-The absolute levels of baseline n-paraffins and selected isoprenoids in the study area and other regions of the Gulf of Mexico are in the range 0.60 to 0.03 ug/l.

2-Pristane and phytane are found in generally all seawater extracts.

3-Many extracts are characterized by a bimodal distribution of paraffin hydrocarbons in the nC-17 and nC-25 to nC-35 regions.

4-Unresolved envelopes indicating the presence of complex mixtures of hydrocarbons are found in almost all samples as well as lower molecular weight distributions of n-paraffins and isoprenoids which suggest the presence of petroleum.

5-While relatively higher concentrations of paraffinic hydrocarbons were observed during the initial months of the study concentrations are now lower in the study area and other nearshore waters in the Gulf of Mexico and have been that way for the last 9 months.

6-Relatively higher concentrations of n-paraffins generally appear in Timbalier Bay but there are no discernable differences between control and platform station hydrocarbon levels. All show equal evidence of petroleum as do samples from Port Aransas and Brownsville.

7-The chromatograms suggest that degradation of the intermediate n-paraffins (below nC-25) is occurring.

8-Comparison with other values of n-paraffins in seawater indicate the Gulf of Mexico values are of the same order of magnitude as other areas.

9-Plankton and selected organisms show evidence of contamination by petroleum derived hydrocarbons using many of the same

criteria discussed above.

10-It appears that hydrocarbons contributed to the marine environment by terrestrial plants are significant in determining the distribution of n-paraffins in the nC-25 to nC-35 region of zooplankton and a polychaete.

11-The ratio of phytane to nC-18 is very similar in the plankton examined to the ratio in crude oil and diesel fuel.

12-Based on all evidence it would appear that the nearshore Gulf of Mexico is uniformly exposed to hydrocarbons which probably are derived from petroleum and partially degraded remnants of the parent mixture can be found in seawater and associated with marine plankton and other organisms.

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II. MICROBIOLOGICAL INVESTIGATION

Abstract

A bacteriological survey carried out in conjunction with the OEI studies indicated that the percentage of hydrocarbon degrading microorganisms in the "total" population did not differ significantly between the OEI study areas (offshore Louisiana and in Timbalier Bay), and "Gulf" control stations several hundred miles away in non-oil production areas. It is hypothesized that the hydrocarbon concentrations in all water samples were too low to be reflected by a significant increase in the percentage of hydrocarbon degrading microorganisms. Thus the percentage values reported might be considered"baseline" data for the Gulf of Mexico.

Biochemical Oxygen Demand (BOD) data also indicated a ubiquitous population of microorganisms capable of oxidizing aromatic as well as paraffinic hydrocarbons. BOD field data, when considered with laboratory experimental data from simulated oil spills, indicate the widespread occurrence of facultative hydrocarbon degrading microorganisms (i.e. capable of oxidizing both hydrocarbon and non-hydrocarbon organics) throughout the Gulf of Mexico.

A bacterium has been isolated from the offshore study site which, in pure culture, rapidly emulsifies a variety of crude oils in closed flask systems. Laboratory studies have shown this organism to be strongly associated with oil droplets, adding credence to the hypothesis that certain of the indigenous hydrocarbon degrading microbial population are selectively attached to particulate material, and thus non-randomly distributed in the water column.

Introduction

In order to understand the fate of hydrocarbons released to the environment either through ongoing biological processes or by man's exploitation and utilization of fossil fuels we need to first know something of the ecology of microorganisms, i.e. their role in the natural environment. A first step in this direction is a compilation of the seasonal abundance and distribution of hydrocarbon degrading microorganisms along with environmental parameters such as temperature, salinity, depth, pH, total organic carbon, and hydrocarbon concentration so that correlations might be observed.

The Offshore Ecology Investigation program* afforded us an opportunity to begin such a microbiological investigation in the Gulf of Mexico while at the same time allowing us to contribute an important parameter to the overall assessment of the ecological impact of offshore drilling and production activities off the Louisiana coast.

One of the primary purposes of the bacteriological program was to determine the ratio of hydrocarbon degrading bacteria to "total" heterotrophs (aerobic-proteolytic bacteria) and compare these data with similar ecological surveys related to the biodegradation of hydrocarbons -- particularly in the marine environment. The determination of the % hydrocarbon degraders as a reflection of the amount (and types) of hydrocarbons in the water was based on the assumption that an indigenous bacterial

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* Administered through the Gulf Universities Research Consortium.

population will respond to an introduction of crude oil or refined petroleum hydrocarbons. If the quantity and/or type of hydrocarbon(s) introduced is not toxic,the number of bacteria capable of degrading hydrocarbons will increase since bacteria grow by dividing.

Table 1 shows a compilation of bacteriological survey data in which the number of hydrocarbon degrading bacteria were determined. The most dramatic percentages of hydrocarbon degrading bacteria occurred in visibly oil contaminated areas such as the Moscow River near a refinery and in Trenow Cove on the Cornish coast following the Torrey Canyon grounding.

Since few of the surveys reported additional parameters it is difficult to correlate the percentages of hydrocarbon degraders with other environmental parameters. However in one instance where hydrocarbon values are reported (Colwell, et.al.,1973) the authors feel that the increase in hydrocarbon degraders is a reflection of the increased concentration of hydrocarbons in the water at that location.

The second major aspect of the field microbiological program was to get some indication as to the potential of the study areas for "self-cleansing" of hydrocarbons through microbial degradation. Various modifications of the Biochemical Oxygen Demand technique were employed in determining the rates and ranges of hydrocarbon oxidation by indigenous microflora.

TABLE I

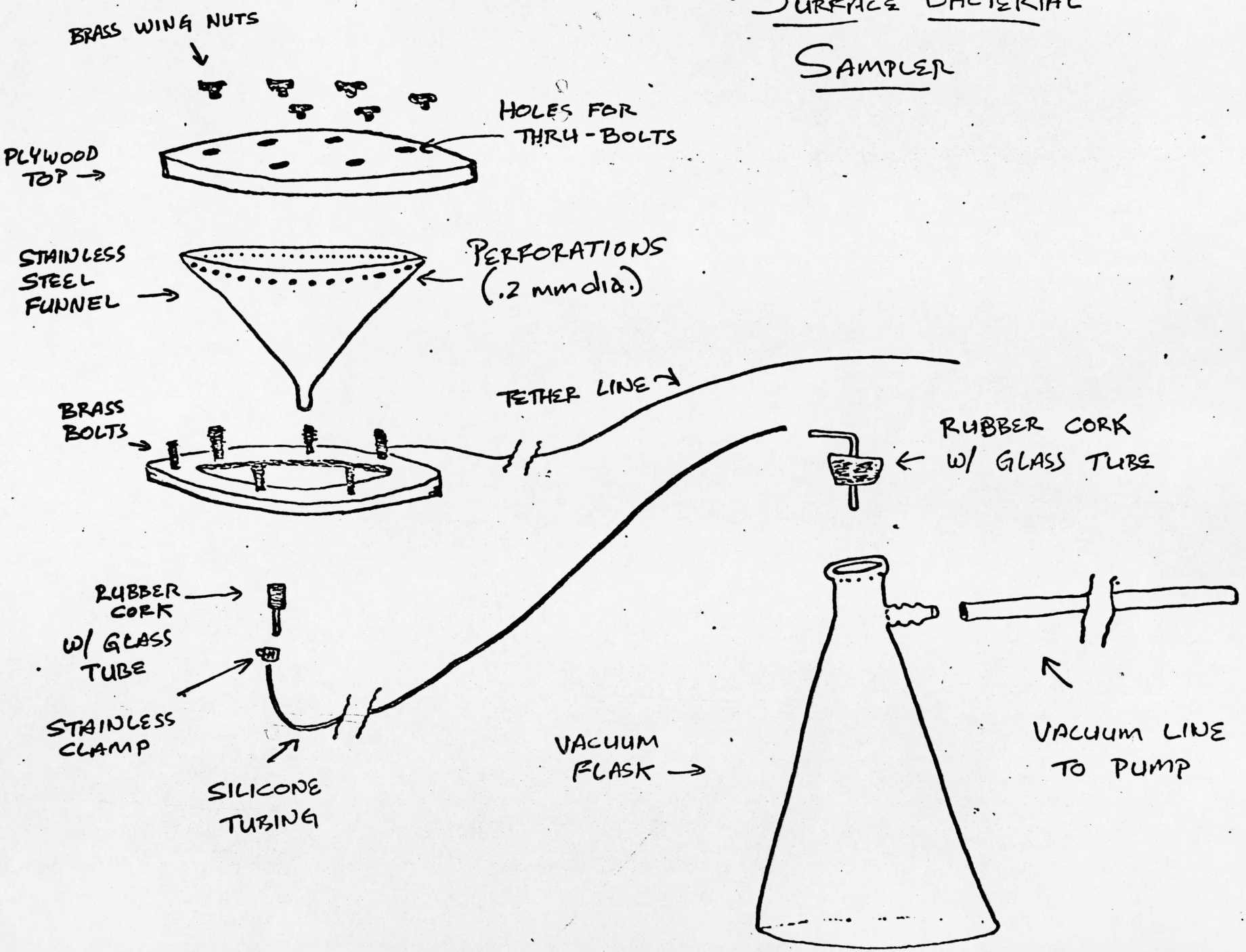
INDIGENOUS HYDROCARBON DEGRADING BACTERIAL POPULATIONS

LOCATION	"TOTAL" HETEROTROPHS/ml	HYDROCARBON DEGRADERS/ml	%HYDROCARBON DEGRADERS	HYDROCARBONS IN WATER	COMMENT	REFERENCE
Moscow River						13
1. Upstream refinery	NA	10 to 9×10^4	<5	NA	Oil visible on water near refinery.	
2. Downstream "	NA	$>10^7$	up to 12,800	NA		
North Sea						5
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 the surface film and at 1 meter.	
2. 25 mi. offshore from the mouth of the Elbe River	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 meter.	11
Raritan Bay, N.J.						1
1. Arthur Kill	NA	3.4	NA	NA	Classified as a "polluted" area by authors.	
2. Seaward fringe of Raritan Bay	NA	0.06	NA	NA	A seasonal fluctuation in the # of H.C. degrading bacteria was noted.	
Chesapeake Bay	10-72 / 11-72	10-72 / 11-72	10-72 / 11-72	10-72 / 11-72		3
1. East Bay	1.5×10^3 / 5.0×10^1	5.0×10^1 / 5.0×10^0	3.3 / 12.5	0.002%w/v / NA	Described as an unpolluted, commercially productive area.	
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 Balt. Harbor. Not commercially productive	
Cornish Coast (Torrey Canyon)						6
1. Sennen	8.5×10^5	5.0×10^4	6%	NA	No oil visible in the area.	
2. Trenow Cove	2.5×10^8	7.0×10^8	280%	NA	Oil was visible on the water surface and in the sediments.	
Gulf of Mexico						15
1. Barataria Bay	NA	≥ 0.1	NA	NA	Over 300 stations sampled during a three year period	
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

FIGURE

Surface Bacterial Sampler



FIELD METHODS

Aseptic collection of surface water samples for bacterial population determination.

Surface water was collected using a sampler developed at this laboratory for use in the GURC-OEI program for the offshore Louisiana study. Depending on the sea surface condition, the sampler collected the topmost 0.5 to 1.5 cm of the water column. The sampler, shown in Figure 1, consists of a stainless steel funnel perforated around its upper perimeter with a series of 0.2 mm diameter holes and sandwiched between two circular discs of epoxy-coated plywood which provide flotation at the proper level.

The apex of the funnel is connected to fifty feet of ¼" I.D. silicon tubing which is in turn connected to a vacuum flask aboard ship. A 1/16" tension line connected to the sampler is used to prevent stretching of the silicon tubing in heavy seas or strong currents.

In use, all components (including tubing) of the sampler were autoclaved except for the epoxy-coated plywood discs which were disinfected with 95% ethanol. The apparatus was assembled by an operator who had thoroughly washed and disinfected his hands. It was then gently dropped onto the water and permitted to float away from the ship. When the floating sampler was at the desired distance from the ship a vacuum was pulled on the sterile sampling flask and surface water, which overflowed through the perforated top of the funnel, was collected.

June, 1972, and September, 1972, values reported for both "total" heterotrophs and hydrocarbon degrading bacteria from offshore and all Timbalier Bay data are from water collected near the surface in sterile glass bottles.

Aseptic collection of subsurface water samples for bacterial population determination.

Following collection of surface water as described above, the same silicon tubing and vacuum flask arrangement was used for subsurface water sampling. 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 attached to the weight and tubing was dropped to the desired depth (indicated by markings on the tubing). 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.

Enumeration and cultivation of hydrocarbonoclastic and "total" heterotrophic marine bacteria in seawater.

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

Bacteriological assays were performed on board ship immediately after collecting water samples to determine the "total" (aerobic-proteolytic) microbial population and the number of hydrocarbon degrading bacteria. "Total" heterotrophs were determined on spread plates and/or membrane filters using 2216E marine medium (ZoBell, 1946). 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 - a serial dilution (1:10) of a given water sample was made in sterile seawater and the appropriate dilution tubes then added to 50 ml of sterile nutrient salts enriched seawater (ESW, Miget, et al., 1969) contained in 125 ml incubation flasks (Miget, et al.,

1969); (2) Sample volumes of 0.1 to 10 ml samples were added directly to 50 ml of ESW in incubation flasks; (3) Sample volumes greater than 10 ml - samples were filtered through sterile 0.45 micron membrane filters and the filters cut in small pieces with sterile scissors and added to incubation flasks containing 50 ml ESW. All flasks, including uninoculated controls, then received 0.5 ml of membrane filter sterilized crude oil (obtained from a wellhead in ST 54), and were incubated on a gyrotary shaker at ships' temperature (22-24°C) for four days. Positive growth (scored on a degree of + or a - basis) was indicated by visible oil emulsification, or an 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 a degree of emulsification basis, the number of oil degrading microorganisms for each sample was determined based on replicate flasks showing any change in appearance of the oil or water compared with control flasks.

ESW solidified with 1.5% Nobel agar (Difco) was used to isolate hydrocarbonoclastic bacteria. The same spread plate and membrane filter techniques used for the "total" heterotrophic population were employed after which the plates were incubated inverted over 1 ml of crude oil contained in the top of each petri dish.

Evaluation of bacterial hydrocarbon degradation using the Biochemical Oxygen Demand (BOD) technique.

BOD studies were carried out as follows: sterile 19 liter glass carboys were filled with sample water from the surface at a given station, shaken vigorously to insure uniform oxygen dis-

persion, then carefully siphoned into sterile 300 ml standard BOD bottles. Nutrient salts { $(NH_4)SO_4$ @ 1 gm/l and K_2HPO_4 @ 0.01 gm/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: A. Hydrocarbons which were solid at 20°C were dissolved in benzene and added to approximately 0.1 gm 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 respective BOD bottles immediately prior to filling with seawater. B. Hydrocarbons which were liquid at 20°C were added directly to filled bottles using a micro-liter syringe by rapidly injecting the substrate into the nutrient enriched water near the bottom of the bottle then withdrawing the syringe and stoppering the bottle before the hydrocarbon floated to the surface. A slight burr on the tip of the syringe needle caused the ejected hydrocarbon to come out in numerous small droplets which increased immediate dissolution into the water as well as allowing more time to stopper the bottle. 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=0 values.

April, 1973, BOD data were obtained using a self-stirring oxygen probe. Bottles were reaerated to a constant percent saturation value after each reading and the resulting oxygen consumption data reported as cumulative oxygen utilization per substrate vs.

time. Although certain hydrocarbons showed a relatively constant rate of oxygen utilization others were quite erratic. Further laboratory tests showed that loss of certain of the more volatile hydrocarbon substrates through repeated aeration was responsible for many erratic data. These data will therefore not be reported, but suffice to say that the extent and range of hydrocarbon substrate metabolism were similar to results which will be reported for later BOD experiments.

During the July, 1973, sampling trip to the study areas, the BOD techniques was modified in an attempt to maintain a higher substrate concentration over a longer incubation period. To accomplish this, bottles were not reaerated to a constant % saturation after each reading as in the April studies, but rather only after the oxygen tension fell below 15% saturation as determined with the self-stirring oxygen probe. It became apparent on the first reading after reaeration of the replicate samples, however, that the initial oxygen concentration greatly influenced the rate and extent of oxygen utilization by the indigenous microbial population. A representative example of this observation is illustrated by the replicate hepta-decylbenzene samples from Timbalier Bay. After 4 days incubation the samples read 28%, 23%, 21% and 8% saturation. Accordingly the 8% sample was reaerated to a constant value of 80% saturation. After an additional 5 days incubation the same samples read 7%, 8%, 5% and 15% respectively resulting in % saturation differences of 21, 15, 16, and 65. However, when all bottles were again reaerated the % saturation differences over the next 5 days incubation were more agreeable (33, 34, 40, and 38) indicating that populations in the four replicate bottles were comparably active but highly dependent on the oxygen tension for their rate of metabolism.

For this reason, results for substrates showing oxygen utilization greater than control samples (no hydrocarbon substrate) are reported only up to the first aeration. Values were not converted to absolute units but rather left as % saturation.

During the October sampling trip the BOD program was modified in the following manner: Only the Platform 54A site surface water was collected. Replicate BOD samples were enriched with nitrogen and phosphorus from a stock solution as previously described and the hydrocarbon substrates added in two concentrations (100 µg and 1000 µg per 300 ml sample for solid hydrocarbons and 1 µl and 10 µl for the liquid substrates). Except for the hepta-decylbenzene, no other light aromatics were used as substrates since the previous sampling trips had shown these to be rapidly oxidized by the native microflora. Samples were not intermittently reaerated but rather incubated in the dark for three weeks and the remaining oxygen in each sample chemically determined using the Winkler titration technique (Strickland and Parson, 1967).

In the January, 1974, trip to the study area additional replicate samples were collected for each substrate, the bottles were not reaerated, and the oxygen concentrations determined chemically.

April, 1974, BOD water was taken on an incoming tide from the Aransas Pass ship channel adjacent to the UT Marine Lab in Port Aransas, Texas, and treated identically to the January, 1974, water samples.

SAMPLING PROGRAM

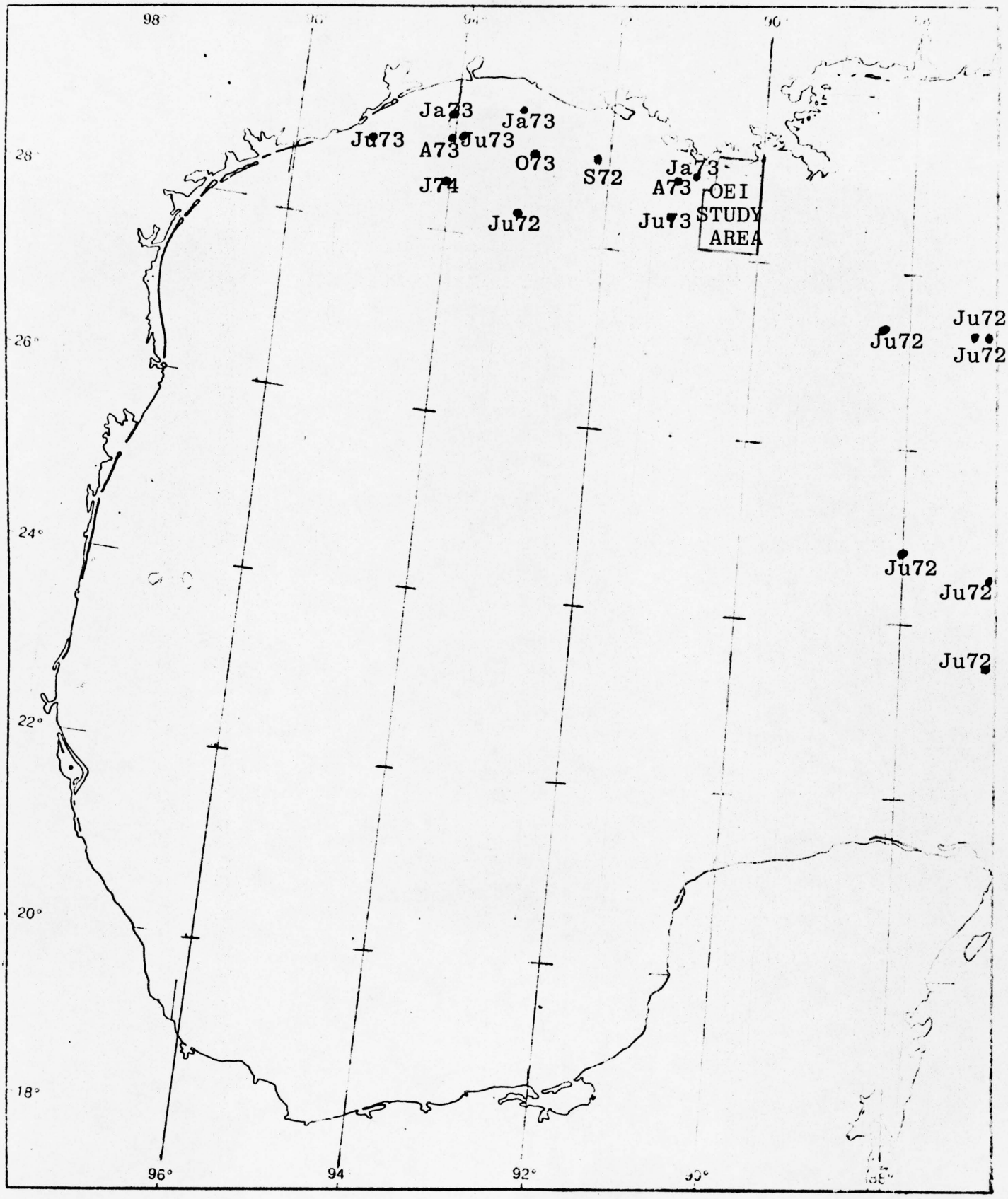
Sampling locations for the microbiological assays are listed in Table 2 and shown in Figures 2 and 3. It should be noted that considerable emphasis was placed on obtaining "Gulf" control stations (50 to several hundred miles from the study sites) for comparative purposes. It was felt that the designated control area, being only 5 miles from the Platform site, might not accurately reflect possible differences in microbial populations and/or metabolic activities as a result of oil production activities.

TABLE 2

GURC-OEI MICROBIOLOGICAL PROGRAM
SAMPLING STATIONS

YEAR	MONTH	DAY	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.	19	1	28.51 N 92.10 W	S. Marsh Is. area	NONE
		20	3	28.49.53 N 90.23.18 W	S.Tim. 54A	K007
		20	4	28.53.13 N 90.19.30 W	Desig. control	H062
		20	6	28.48.30 N 90.27.30 W	S.W. Plat.54A	K023
		21	8	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		21	9	28.53.13 N 90.19.30 W	Desig. control	H062
1973	Jan.	9	1	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		9	4	28.53.13 N 90.19.30 W	Desig. control	H062
		10	5	29.12.20 N 90.21.30 W	Tim. Bay	A079
		11	6	28.54 N 90.45 W	Ship Shoal area	NONE
		12	7	29.39 N 93.11 W	East of Sabine R.	NONE
		12	8	29.30 N 94.01 W	West of Sabine R.	NONE
1973	April	2	1	29.06 N 93.59 W	West of Sabine R.	NONE
		2	2	28.50 N 91.20 W	Ship Shoal area	NONE
		3	3	28.49.53 N 90.23.18 W	S. Tim. 54A(Upstm.)	K007
		3	5	" "	" (Dwnstm.)	K007
		4	6	29.13 N 90.00 W	11 mi. off Grande Is.	C070
		5	7	28.49.53 N 90.23.18 W	S.T. 54A(Dwnstm)	K007
		5	8	28.53.13 N 90.19.30 W	Desig. control	H062
		6	16	29.12.20 N 90.21.30 W	Tim. Bay	A079
1973	July	7	1	28.55 N 95.05 W	Off Galveston, Tx.	NONE
		8	2	29.13 N 93.42 W	West of Sabine R.	NONE
		8	4	28.30 N 91.10 W	Ship shoal area	NONE
		9	5	28.49.53 N 90.23.18 W	S. Tim. 54A	K007
		11	7	28.53.13 N 90.19.30 W	Desig. control	H062
		13	8	29.12.20 N 90.21.30 W	Tim. Bay	A079
1973	Oct.	14	1	29.07 N 92.52 W	East Cameron area	NONE
		15	4	28.49.53 N 90.23.18 W	S. Tim.54A	K007
		16	7	29.12.20 N 90.21.30 W	Tim. Bay	A079
		17	6	28.53.13 N 90.19.30 W	Desig. control	H062
1974	Jan.	13	1	28.35 N 94.06 W	N.W. Gulf	NONE
		14	4	28.49.53 N 90.23.18 W	S. Tim.54A	K007
		15	5	28.53.13 N 90.19.30 W	Desig. cont.	H062
		16	6	29.12.20 N 90.21.30 W	Tim. Bay.	A079

FIGURE 2



OEI MICROBIOLOGICAL "GULF" CONTROL STATIONS

TIMBALIER BAY STATION

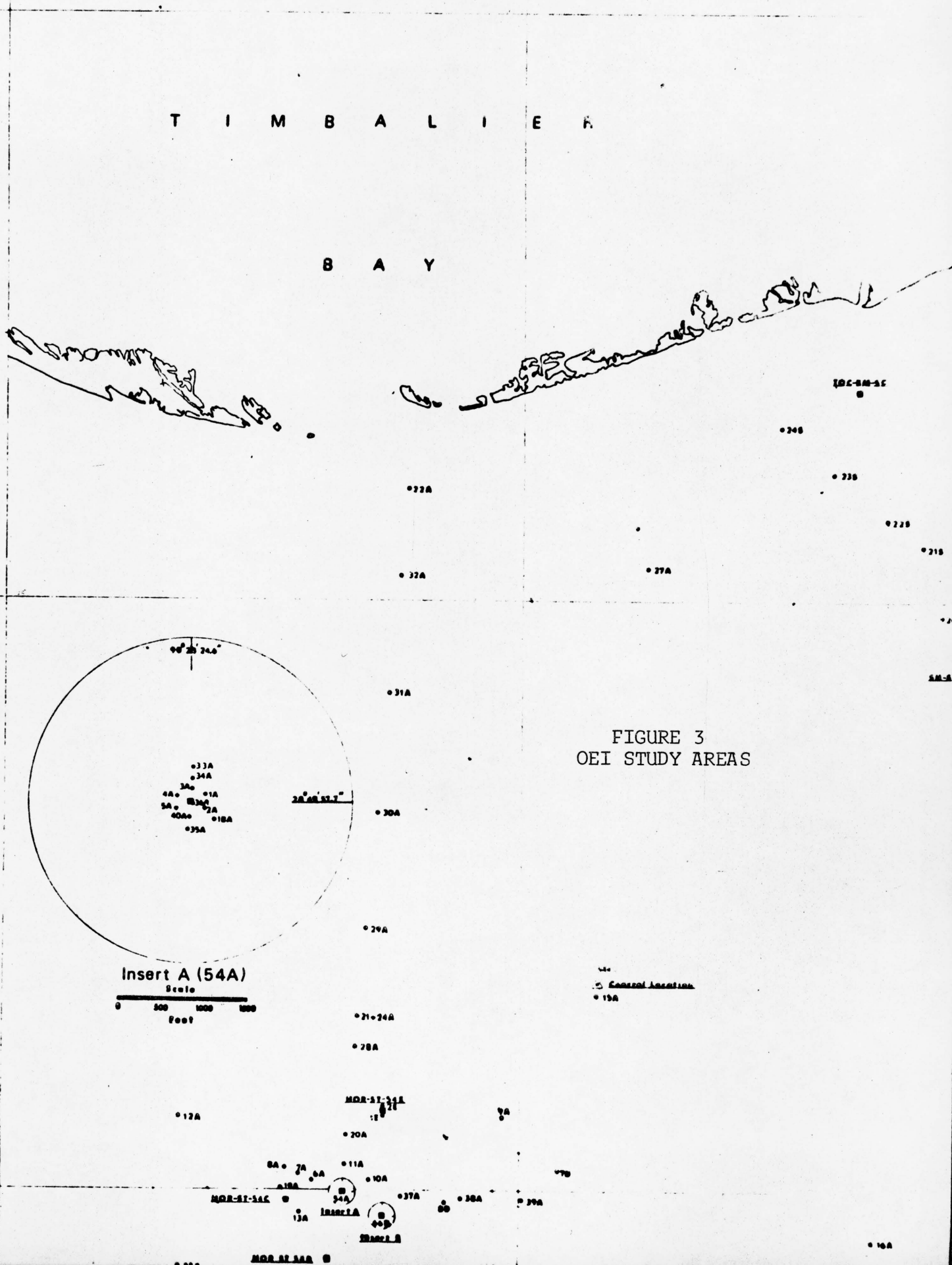


FIGURE 3
OEI STUDY AREAS

RESULTS AND DISCUSSION - FIELD DATA

Bacteriological Enumeration

Figure 4 shows the results of the OEI bacteriological survey from June, 1972, through January, 1974. The data are presented by cruise. The June, 1972, figures from a trip throughout the Gulf of Mexico on the Texas A&M ship ALIMINOS might be considered as "baseline" data with which to compare subsequent values from the OEI study areas. With the exception of the two nearshore stations (1 and 2) the remainder of 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 per location from the June, 1972, open Gulf values, but additional control samples ("Gulf" controls) taken enroute to the study area from Port Aransas, Texas, did not indicate a significant difference in the percent hydrocarbon degraders in nearshore 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

OEI-BACTERIOLOGICAL SURVEY DATA

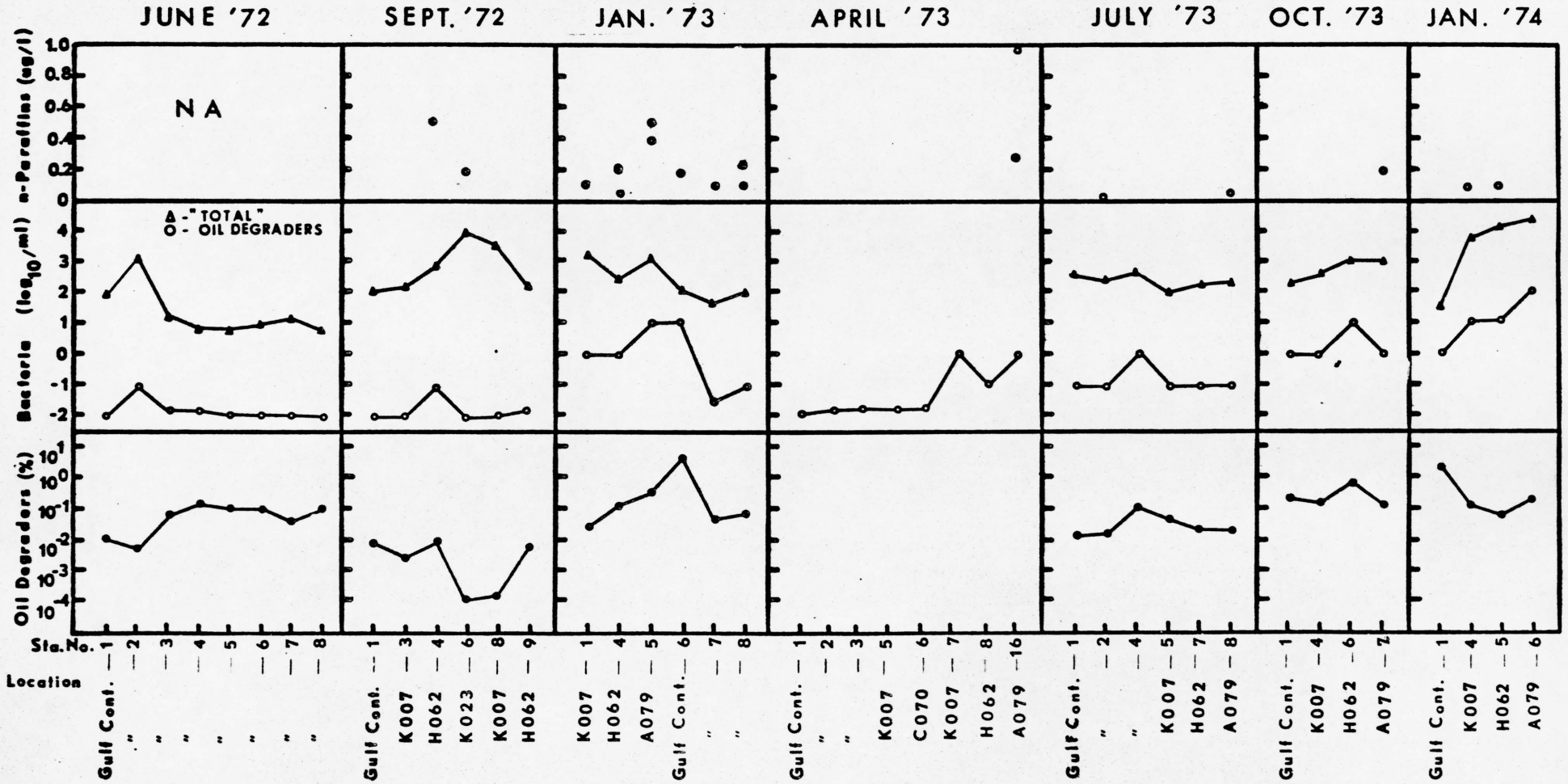


FIGURE 4

large difference. It can be seen that for most of the sampling trips these locations fluctuated in the percent of hydrocarbon degraders as much as additional "Gulf" control stations taken enroute hundres 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 6 meters. In July, 1973, a massive plankton bloom concentrated in the top few meters of the water column throughout the offshore study area was probably responsible for the order of magnitude difference observed in both "total" heterotrophs and hydrocarbon degrading populations. The April, 1973, data for "total" heterotrophs were not available due to the fact that the 2216E medium was inadvertently made up with fresh water rather than 75% seawater.

The bacteriological data, therefore, when considered along with the low values for hydrocarbons in the water (relative to values of up to 0.8 gm/l benzene extractable petroleum reported by Colwell, et al., 1973), would indicate that the OEI study areas were not grossly contaminated with adventitious hydrocarbons and that the hydrocarbon degrading population of microorganisms were generally at a "Gulf baseline" level; i.e. comparable to non-oil production areas. Laboratory studies designed to ascertain the minimum concentrations of crude oils and various pure hydrocarbons required to be reflected by a significant increase in the percent hydrocarbon degrading microbial population were initiated at the outset of the OEI investigations, but have thus far yielded inconclusive results.

Two additional remarks concerning the bacterial data are

warranted. First, the numbers of "total" heterotrophs in the OEI study areas seemed anomously low relative to organic carbon concentrations and plankton populations. For example, Jannasch and Oppenheimer (1962) found populations of 10^6 bacteria per milliliter ("total" heterotrophs) in nearshore 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 4 were calculated based on changes in the appearance of either the oil or 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 while at other times of the year nearly all of the flasks in a series showed either all the samples to contain well emulsified oil or one or two "flaky" flasks, then all emulsified flasks as the sample volume increased. Although a lack of replicate samples of both hydrocarbon degrading bacterial 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; January, 1974; and to some extent in September, 1972, and October, 1973. All July, 1973, samples exhibited the "flaky" type of oil degradation.

It is difficult at this time to speculate as to why these two parameters might correlate since we are only beginning to

understand the biochemical activities of various pure and mixed hydrocarbon degrading cultures isolated from the study area--as will be discussed later.

BOD Studies

The July, 1973, BOD samples (Table 3) are indicative of the general trend of hydrocarbon oxidation by indigenous microorganisms throughout the Gulf during the entire two year study. The data show that many of the low boiling aromatics found in crude oils were oxidized at rates comparable to n-paraffin hydrocarbons such as hexadecane. On this particular sampling trip none of the heavier molecular weight aromatic hydrocarbons were shown to be utilized by the native microflora. However, as stated in the methods section, the problems associated with the self-stirring probe technique used on this particular sampling trip would relegate these data to trends rather than absolute values.

In addition to rapid oxidation of benzene and alkylbenzenes by the indigenous microflora, two other considerations should be given the July data. First, since surface salinities offshore in July averaged around 15 ppt and a dense algal population was present in the top few meters of water, BOD sample water was collected at the surface and at 6 meters depth. Percent saturation values for the control samples, containing only naturally occurring organic and microorganisms but enriched with nitrogen and phosphorus, reflected the increased biomass near the top of the water column in the study area with surface samples utilizing approximately twice as much oxygen as 6 meter samples for the same incubation times.

Second, certain of the higher molecular weight aromatic compounds showed an average of less oxygen consumption than controls

TABLE 3

JULY, 1973, HYDROCARBON BOD DATA

LOCATION	SUBSTRATE[a]	INCUBATION (days)	Δ % O_2 SATURATION[b] TEST SAMPLES		Δ % O_2 SATURATION[b] CONTROL SAMPLES[c]		$\bar{X}_{test} - \bar{X}_{cont.}$ [d]
			$\bar{X}$	S.D.	$\bar{X}$	S.D.	
Plat.54A Sfc.	Benzene	8	63	8	26	5	37
" " 6 meter	"	8	47	7	12	4	35
Tim. Bay	"	4	73	1	50	2	23
Plat.54A Sfc.	Toluene	8	65	1	26	5	39
" " 6 meter	"	8	51	3	12	4	39
Tim. Bay	"	4	74	2	50	2	24
Plat.54A Sfc.	Ethyl-	8	60	1	26	5	34
" " 6 meter	benzene	8	50	4	12	4	38
Tim. Bay	"	4	74	2	50	2	24
Plat.54A Sfc.	Propyl-	8	65	2	26	5	39
" " 6 meter	benzene	8	45	3	12	4	33
Tim. Bay	"	4	73	3	50	2	23
Plat.54A Sfc.	Hepta-	8	55	12	26	6	29
" " 6 meter	decyl-	8	33	10	12	4	21
Tim. Bay	benzene	4	57	8	50	2	--
Plat.54A Sfc.	Cyclo-	34	31	22	35	11	--
" " 6 meter	octane	34	15	7	18	4	--
Tim. Bay	"	34[e]	32	5	146	16	--
Plat.54A Sfc.	Isooctane	34	32	8	35	11	--
" " 6 meter	"	34	19	7	18	4	--
Tim. Bay	"	14[e]	46	11	87	1	--

TABLE 3 (CONT.)

Plat.54A Sfc.	Paraffin	20	51	15	35	8	--
" " 6 meter	Oil	20	37	9	16	5	21
Tim. Bay	"	10[e]	50	6	70	1	--
Plat.54A Sfc.	Hexadecane	8	66	4	26	5	40
" " 6 meter	"	8	32	12	12	5	20
Tim. Bay	"	4	68	7	50	2	16
Plat.54A Sfc.	Anthracene	34	31	10	35	11	--
" " 6 meter	"	34	9	4	17	4	--
Tim. Bay		14[e]	30	22	87	1	--
Plat.54A Sfc.	Benzanthrene	34	28	10	35	11	--
" " 6 meter	"	34	4	4	18	4	--
Tim. Bay	"	14[e]	30	17	87	1	--
Plat.54A Sfc.	Chrysene	34	34	10	35	11	--
" " 6 meter	"	34	5	6	18	4	--
Tim. Bay	"	10[e]	40	19	70	1	--
Plat. 54A Sfc.	Coronene	34	37	10	35	11	--
" " 6 meter	"	34	4	5	18	4	--
Tim. Bay	"	10[e]	58	20	70	1	--
Plat.54A Sfc.	Fluoranthene	34	31	9	35	11	--
" " 6 meter	"	34	9	8	18	4	--
Tim. Bay	"	14[e]	34	13	87	1	--
Plat.54A Sfc.	Phenanthrene	34	34	8	35	11	--
" " 6 meter	"	34	7	8	18	4	--
Tim. Bay	"	14[e]	37	18	87	1	--
Plat.54A Sfc.	Triphenylene	34	50	6	35	11	--
" " 6 meter	"	34	12	9	18	4	--
Tim. Bay	"	10[e]	21	2	70	1	--

TABLE 3 (CONT.)

[a] Substrates solid at room temperature dissolved in benzene and added to glass beads (see Methods) at 100 ug/300 ml BOD water sample. Substrates liquid at room temperature added directly (see Methods) to filled bottles at 10 ul/300 ml BOD water sample.

[b] Determined electrochemically using a YSI self-stirring BOD Oxygen probe.

[c] Nutrient salts were added (see Methods) but no hydrocarbons.

[d] Reported only if the difference was greater than the sum of the standard deviations (S.D.)

[e] Control sample values include reaeration, test samples were not reaerated. (see Methods)

with no hydrocarbon substrates. These results might be interpreted as indicating a hydrocarbon toxicity to the indigenous microbial population. However, when the standard deviations are considered, most samples fall within the range of the controls. Two exceptions to this observation were the cyclooctane and isooctane samples which, even with Timbalier Bay water, did not require reaeration for 14 days (isooctane) and 34 days (cyclooctane).

Results of the October BOD studies are shown in Table 4. Ten microliter heptadecylbenzene and hexadecane samples showed a significantly higher oxygen utilization than control samples. Oxidation of these substrates was expected based on April and July data. However, 1 mg anthracene, 1 mg phenanthrene, and 100 µg fluoranthene substrate bottles also indicated significant oxygen consumption relative to controls over the three week incubation period.

In the January, 1974, BOD studies, utilization of several of the larger molecular weight aromatic hydrocarbons by indigenous microorganisms was evident over the extended incubation period of 43 days (Table 5). The large standard deviations associated with most of these substrates indicated either a population of somewhat less than one microorganism per 300 ml (volume of a BOD bottle) capable of degrading these compounds, (i.e. replicate bottles showed either extensive oxygen utilization or no utilization relative to controls), or, a somewhat larger population non-randomly distributed (i.e. attached to a detrital particle and thus reflected as a single organism). The small standard deviations for the low molecular weight aromatics would likewise indicate a substantially

TABLE 4

OCTOBER, 1973, HYDROCARBON BOD DATA

(Platform 54A surface water- 3 weeks incubation)

SUBSTRATE	CONCENTRATION (per 300 ml)	O_2 REMAINING[a] (ml/l)	$\bar{X}_{cont.} - \bar{X}_{test}$ [b] (ml/l)
CONTROL	--	3.51 ± 0.19	--
Plat. 54A crude oil	1 ul	3.48 ± 0.39	--
"	10 ul	2.29 ± 0.11	1.22
Heptadecylbenzene	1 ul	3.56 ± 0.48	--
"	10 ul	0.68 ± 0.31	2.83
Hexadecane	1 ul	3.68 ± 0.51	--
"	10 ul	1.28 ± 0.35	2.23
Naphthalene	0.1 mg	3.59 ± 1.22	--
"	1 mg	3.16 ± 0.72	--
1,2 Benzanthracene	0.1 mg	3.51 ± 0.25	--
"	1 mg	3.28 ± 0.32	--
Triphenylene	0.1 mg	3.65 ± 0.14	--
"	1 mg	4.02 ± 0.54	--
Anthracene	0.1 mg	3.37 ± 0.49	--
"	1 mg	2.32 ± 0.80	1.19
Phenanthrene	0.1 mg	3.30 ± 0.35	--
"	1 mg	0.94 ± 0.36	2.57
Fluoranthene	0.1 mg	2.18 ± 1.03	1.33 ?
"	1 mg	3.74 ± 0.37	--

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

[b] Reported only if the difference was greater than the sum of the standard deviations.

TABLE 5

JANUARY, 1974, HYDROCARBON BOD DATA

(Platform 54A surface water)

SUBSTRATE[a]	INCUBATION (days)	O_2 REMAINING[b] (ml/l) $\bar{X}$ ± S.D.	$\bar{X}_{cont.} - \bar{X}_{test}$[c] (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

a Substrate concentrations were 10 ul/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.

b Determined chemically using the Winkler titration method as outlined in the Analysis of Seawater Handbook (Strickland and Parsons, 1968)

c 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.

larger population of microorganisms capable of oxidizing these substrates.

Summarizing the BOD field data, it can be said 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 enumeration and metabolic assays employed nutrient salts enriched seawater (ESW), thus rates of metabolic activity of indigenous microorganisms reported would not be 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 which 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 area, as gas chromatograph-mass spectrometric analyses of 30 offshore Louisiana crude oils obtained at the wellheads showed a composition averaging 20% benzene + alkylbenzenes.

LABORATORY METHODS

Aquarium Studies

Two twenty liter aquaria containing raw seawater collected from the Aransas Pass Channel adjacent to the UT marine laboratory were set up in the microbiology laboratory. Sufficient aeration was provided to maintain a minimum of 80% saturation in each aquarium. The test aquarium received 5 ml of S.T. 54A crude oil while the control was left untreated. Bacterial populations in each aquarium were monitored several days preceeding the addition of oil and several days afterwards following the same enumeration methods outlined in the field methods section. In addition to quantitative enumeration, qualitative differences in colony types were noted for both peptone and ESW + oil plates.

RESULTS AND DISCUSSION - LABORATORY OBSERVATIONS

Aquarium Studies

The original intent of the aquarium experiments was to determine whether quantitative and/or qualitative changes in microbial populations developing when oil was introduced into an aqueous environment could be correlated with bacterial numbers and/or colony types from field surveys. In particular we were looking for indicator species of microorganisms which would be indicative of abnormally large amounts of hydrocarbons present in the study area at the time of sampling or having been there in the recent past. In addition, the percent hydrocarbon degraders in the total population was closely observed.

Thus far we have not found any specific microorganism(s) which we feel are indicative of crude oil hydrocarbons in the water, however the ratio of hydrocarbon degraders to total heterotrophs and the succession of microorganisms in oil treated aquaria have provided the initial data to allow speculation regarding the possible sequence of microbial activities taking place.

Table 6 shows the results of a typical aquarium experiment. These data show that the hydrocarbon degrading population in the test aquaria responded quantitatively and qualitatively to the introduced oil. For example, while the number of microorganisms and types of hydrocarbon degradation exhibited by the populations in both aquaria were about the same for the first three and one-half days of the experiment, after the addition of oil to the test aquarium the percent hydrocarbon degraders began to increase. There was no significant increase in the number of "total" heterotrophs in either aquarium nor in the population of hydrocarbon degraders in the control aquarium throughout the experiment.

The type of assay flask oil degradation in the test aquarium samples was as interesting as the numbers themselves. On day five (one and one-half days after oil was added) the first three flasks in the replicate dilution series showed a very fine oil emulsion (+4) with the next two flasks containing cloudy water but no emulsified oil (+1). On day 6½ however the first five dilution flasks showed a very fine emulsion with a sharp cutoff. Days 8½ and 11 still showed oil emulsion in the first five dilution flasks though not as fine as the day 6½ flasks.

Based on these observations one might speculate the following sequence of events in the test aquaria: Some of the indigenous

TABLE 6

LABORATORY AQUARIUM EXPERIMENT
NON-NUTRIENT ENRICHED SEAWATER PLUS CRUDE OIL

BACTERIAL POPULATIONS[a]

TIME (days)	TEST AQUARIUM[b] hydrocarbon deg. -2-3-4-5-6-7-8-9[c]									TEST AQUARIUM[b] peptone deg. -5-6-7-8-9[c]					CONTROL AQUARIUM[b] hydrocarbon deg. -2-3-4-5-6-7-8-9[c]									CONTROL AQUARIUM[b] peptone deg. -5-6-7-8-9[c]				
	5x10							1x10							5x10							1x10						
1	4	4	2	1	-	-	-	-		+	+	-	-	-	4	4	3	1	-	-	-	-		+	+	-	-	-
2	4	4	3	3	-	-	-	-		+	+	-	-	-	2	4	4	1	-	-	-	-		+	-	-	-	-
3½	4	3	2	3	-	-	-	-		+	+	-	-	-	3	1	4	-	-	-	-	-		+	-	-	-	-
OIL ADDED																												
5	4	4	4	1	1	-	-	-		+	+	-	-	-	3	3	2	-	-	-	-	-		+	+	-	-	-
6½	4	4	4	4	4	-	-	-		+	+	+	-	-	2	1	1	-	-	-	-	-		+	+	+	-	-
8½	4	3	3	3	3	-	-	-		+	+	+	-	-	3	1	1	-	-	-	-	-		+	+	+	-	-
11	4	3	2	2	2	-	-	-		+	+	+	-	-	2	1	1	1	-	-	-	-		+	+	-	-	-

[a] Samples were collected with a sterile pipet approximately 5 cm below the surface-oil was blown away from the surface before inserting the pipet into the test aquarium.

[b] Test aquarium received 5 ml oil immediately after the day 3½ assay. Control aquarium untreated.

[c] Numbers refer to the dilution series which were scored as follows: 1. ESW FLASKS- +4 very fine oil emulsion; +3 fine oil emulsion; +2 oil slightly emulsified but mostly on the surface as large droplets; +1 no oil emulsification but water phase cloudy; - no change relative to uninoculated sterile control flasks. 2. PEPTONE DILUTION TUBES- + visible turbidity; - no visible turbidity

mixed population of microorganisms either singly or in combination were capable of degrading various crude oil components. Looking at the dilution series scores, it might be assumed that either (1) the individual microorganisms, or the combination of microbes, necessary to produce the surfactants resulting in oil emulsification in closed flasks were not as numerous as the organisms (or populations) able to grow on certain oil components without emulsifying the oil; or (2) that the "emulsifying" organism(s) were as numerous but were not as randomly distributed throughout the water column, i.e., the "emulsifying" population was associated primarily with oil droplets. Based on the chemical analysis of various hydrocarbon oxidizing microorganisms showing a high lipid content in the cell membrane (Davis, 1967), as well as our own microscopic examination of both pure and mixed cultures of oil oxidizing microorganisms grown in shake flasks, it is reasonable to assume that many of these bacteria are hydrophobic and quite probably strongly associated with non-polar particulate material in the water.

Strong evidence for clumping of oil oxidizing bacterial in the OEI study areas was the observation that enumeration of hydrocarbon oxidizing bacteria on ESW + crude oil plates (see Methods) consistently showed a marked break in the serial dilution series at the transition between spread plates (for sample volumes of 0.1 ml and less) and membrane filter plates (for 1 ml and larger sample volumes), i.e., spread plates consistently indicated populations one or two orders of magnitude greater than would be estimated based on membrane filter samples. In addition, the colonies developing on the spread plates often exhibited only two or three

morphological types even when several hundred colonies were present. Membrane filter colonies were always more diverse. Thus it might be hypothesized that the mechanics of spreading broke up associations of bacteria attached to detrital particles resulting in higher counts than membrane filter plates in which entire populations on a particle would be manifested as a single colony.

Clumping of heterotrophic bacteria in the marine environment has been a topic of considerable discussion for some time. Original work by ZoBell (1946) and later by Jannasch and Jones (1959), and Jones and Jannasch (1959) indicated that enumeration techniques which did not break up bacterial clumps often showed one to several order of magnitude fewer microorganisms than did direct microscope counts.

Additional evidence for clumping of oil degrading microorganisms from the study area was indicated by the physical characteristics of a microorganism isolated from the offshore Louisiana study area. This bacterium, designated PC-6, rapidly emulsifies crude oil but does not grow to visible turbidity on a variety of non-hydrocarbon media (Table 7). The organism is a non-motile rod which when grown in ESW on 54A crude oil becomes closely associated with the oil phase to the extent that initially the cells cannot be separated by centrifugation. As the culture ages a small pellet is sometimes obtained, but the majority of cells remain associated with the oil phase as evidenced by microscopic examination.

When 54A crude oil was introduced into the test aquarium the potential for oil emulsification began to increase. The day 5 flasks, for example, began to show this potential superimposed over the increasingly abundant population capable of growth on, but

TABLE 7
GROWTH OF OIL EMULSIFYING PURE CULTURE (PC-6) ON VARIOUS SUBSTRATES

Substrate[a]	Growth[b]	Viability after 72 hours incubation[c]
1. Menhaden body oil	No	No
2. Menhaden body oil (Winterized)	No	No
3. Meat peptone 70 (GIBCO)	No	Yes
4. Difco peptone	No	Yes
5. Menhaden peptone (100% solubles)	Yes	Yes
6. Gelatin	No	Yes
7. Marine peptones	No	Yes
8. Dextrose	No	Yes
9. d-lactose	No	Yes
10. d-galactose	No	Yes
11. Fish protein hydrolysate	No	Yes
12. Casein hydrol. (peptone.50) (GIBCO)	No	Yes
13. d-maltose	No	Yes
14. Menhaden body oil-crude	No	No
15. Casein	No	Yes
16. 0.1% decanoic acid	No	Yes(?)

[a] All substrates except the decanoic acid were added 5 gms/l to nutrient salts enriched seawater(ESW), the pH adjusted to 7.8, and autoclaved.

[b] Visible turbidity after 72 hours incubation on a shaking table (160 rpm and room temperature 21-22C)

[c] After 72 hours flasks were read for growth then 0.5 ml of sterile crude oil added and the flasks were incubated on the shaking table for an additional 72 hours. Oil was finely emulsified in flasks containing viable cells.

not emulsification of, the oil. On day 6½, the oil emulsifying potential of the test aquarium population had become dominant but began to fade somewhat on days 8½ and 11.

Thus it might be hypothesized that in the aquarium study the water contained a relatively abundant population of indigenous microorganisms which metabolized the more soluble crude oil compounds (possibly benzene and the alkylbenzenes), resulting in cloudy water but no apparent oil emulsification in the assay flasks. Microorganisms, or associations of organisms, capable of oxidizing crude oil compounds that resulted in a fine oil emulsion (possibly through n-paraffin oxidation to corresponding fatty acids (Davis, 1968) may have been as numerous as the "non-emulsifying" population but were possibly selectively attached to oil droplets. Thus they did not show a dominance in the population until enough surfactant was produced in the aquarium to finely emulsify the oil, thereby increasing the probability of sampling this population at the 5 cm depth referred to in Table 6.

The fact that the "total" heterotroph population did not significantly increase as the number of "emulsifying" hydrocarbon degraders increased would indicate that a majority of this population might be metabolically similar to PC-6, i.e., growth on hydrocarbons but not on peptone.

Laboratory studies have shown that many of the microorganisms capable of oxidizing low molecular weight aromatic hydrocarbons are facultative hydrocarbon oxidizers (i.e., they can degrade both hydrocarbon and non-hydrocarbon organics). Raw seawater enriched with nitrogen and phosphorus (ESW) was supplied with various hydrocarbon substrates in respective flasks, incubated, and the resulting

populations plated out on 2216E medium. Dominant colony types growing on these plates were reinoculated into flasks containing sterile nutrient enriched seawater plus the respective test hydrocarbon substrate, resuling in visible turbidity in several of the flasks.

SUMMARY

In summary, the field studies and laboratory observations would suggest the following hypotheses regarding the degradation of hydrocarbons by native microflora in the Gulf of Mexico.

1. Microbiological enumeration data indicate a relatively uniform percentage of hydrocarbon oxidizing microorganisms throughout the Gulf which respond to the introduction of both crude oils and pure hydrocarbons. Perhaps this ubiquitous population of hydrocarbon oxidizing microorganisms is a reflection of a dynamic system whereby hydrocarbons derived from petroleum, plants, and animals are introduced into the Gulf and subsequently removed through physical, chemical, and biological processes. Due to the lack of true "baseline" data, it is impossible at this time to say whether this dynamic system is in equilibrium.

2. A population of microorganisms capable of rapidly degrading low molecular weight aromatic hydrocarbons was present throughout the study in the OEI areas off Louisiana and in non-production "Gulf" control areas. The ubiquity of these populations coupled with laboratory data showing the presence of facultative hydrocarbon degraders in the environment, suggest that perhaps these microorganisms were not obligate hydrocarbon degraders present in response to hydrocarbons in the water, but rather facultative hydrocarbon oxidizers which responded to the introduction of hydrocarbon substrates.

3. Emulsification of 54A crude oil, which was observed in enumeration dilution flask series, may have resulted from inoculation of aggregates of bacterial attached to non-polar organics in seawater samples. Laboratory investigations have suggested that a dominant

culture in this population may be metabolically similar to an isolate obtained from the study area offshore Louisiana. This bacterium, in pure culture, rapidly emulsifies crude oil in closed flasks, but does not grow to visible turbidity on a seawater-peptone medium. This type of microorganism, while not actively metabolizing non-hydrocarbon organics, might still have a competitive advantage over facultative hydrocarbon degraders in the environment due to its ability to adhere to polar organics.

It is hoped that the field observations regarding the distribution, abundance, and metabolic activities of hydrocarbon degrading bacteria in the marine environment, and the considerable speculation based on laboratory observations regarding the possible sequence of events occurring during microbial degradation of hydrocarbons will foster an exchange of ideas concerning the fate of hydrocarbons in the marine environment.

TENTATIVE CONCLUSIONS

Quantitative and qualitative data regarding hydrocarbons extracted from seawater and organisms collected in both 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 petroleum. Partially degraded remnants of Gulf crude oils have been found in seawater,and associated with marine plankton and other organisms.

Likewise microbiological data indicate a relatively uniform percentage of hydrocarbon oxidizing microorganisms throughout the Gulf which respond to the introduction of both crude oils and pure hydrocarbons. Perhaps this ubiquitous population of hydrocarbon oxidizing microorganisms is a reflection of a dynamic system whereby hydrocarbons derived from petroleum, plants, and animals are introduced into the Gulf and subsequently removed through physical, chemical, and biological processes. Due to the lack of true "baseline" data, i.e. before extensive oil production activities in Gulf bays and offshore, along with the recent advances in analytical techniques which make it possible to detect hydrocarbons in the tenth of a part per billion range, it is impossible at this time to say whether this dynamic system is in equilibrium.

We do not wish to speculate as to the origin of the petroleum derived hydrocarbons in the Gulf of Mexico at this time, but do want to reemphasize the finding that the OEI study areas do not differ appreciably in hydrocarbon concentrations or types relative to non-production "Gulf" control areas several hundred miles from the study sites.

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APPENDIX

Additional data collected on sampling trips to
the OEI Louisiana study area aboard the R/V LONGHORN

January, 1974, Physical Data
GURC-OEI LONGHORN Cruise

Station	Date	Depth (m)	Temp.(C)	Salinity(ppt)
1	1-13-74	Sfc.	19.2	26.7
		1	"	27.1
		2	"	27.1
		3	"	27.1
28.35N		4	"	27.1
		5	"	27.5
94.06W		6	"	"
		7	"	"
		8	"	"
"Gulf"		9	"	"
Control		10	"	"
		11	"	"
		12	19.5	27.7
		13	19.5	27.7
		14	19.6	28.8
		15	19.6	28.1
		16	"	"
		17	"	"
		18	"	"
		19	"	"
		20	19.7	"
		21	19.8	27.9
		25	19.8	27.9
4	1-14-74	Sfc.	17.1	18.3
		1	16.9	18.4
		2	16.5	18.5
		3	16.4	18.7
S.T. 54A		4	16.4	18.7
Study area		5	16.5	19.2
		6	17.0	19.5
		7	17.5	21.1
		8	17.5	21.3
		9	17.5	21.3
		10	17.9	21.3
		11	18.0	23.3
		12	18.5	22.8
		13	18.5	23.2
		14	19.0	24.1
		15	19.5	24.6
		16	19.8	24.8
		17	20.0	24.9
		18	19.9	25.0
		19	19.9	25.0
		20	19.9	25.0

Station	Date	Depth(m)	Temp.(C)	Salinity(ppt)
6	1-15-74	Sfc.	17.1	16.7
		1	17.0	16.8
Designated		2	16.9	17.0
Control		3	16.9	17.0
Area		4	16.8	16.9
		5	16.5	17.0
		6	16.7	17.0
		7	16.7	17.3
		8	16.8	17.6
		9	16.9	20.1
		10	18.5	22.8
		11	19.8	23.5
		12	20.2	24.6
		13	20.5	25.0
		14	20.2	25.2
		15	20.3	25.3
		16	20.3	25.3
		17	20.3	25.3
7	1-16-74	Sfc.	17.0	14.0
		1	16.8	14.5
		2	16.8	14.5
		3	16.5	15.0
S.T. 54A		4	16.4	15.4
Study area		5	16.5	15.8
		6	17.2	17.5
		7	17.9	19.1
		8	18.2	20.2
		9	19.0	21.3
		10	19.3	21.9
		11	19.5	22.2
		12	19.8	22.8
		13	20.0	23.1
		14	20.1	23.5
		15	20.3	23.9
		16	20.5	23.9
		17	20.5	24.0
		18	20.5	24.0

1973 OCTOBER GURC TDC

LOCATION	DEPTH(M)	TEMP.	SALINITY $^{o}/_{oo}$
	Air	27.0	-
	Sfc.	28.5	26.1
29 07 00 N	1	28.5	26.1
92 52 00 W	2	28.5	26.1
	3	28.5	26.1
"Control"	4	28.8	25.9
enroute to	6	28.8	25.9
Louisiana	8	28.8	25.9
	10	28.8	25.9
	12	28.8	25.9
	14	28.8	26.1
	16	28.8	26.1
	18	28.8	26.1
	20	28.8	26.1
Platform 54A	Sfc.	28.0	24.1
Approx. 1000 yd	1	28.0	24.1
downstream	2	28.0	24.1
	3	28.0	24.1
	5	28.0	24.1
	7	28.0	24.1
	8.5	28.0	24.1
	9	28.0	24.3
	10.5	28.0	24.5
	11	28.2	25.1
	12	28.2	25.1
	13	28.2	26.1
	14	28.3	26.1
	15	28.3	26.3
	16	28.3	27.2
	17	28.5	28.3
	18	28.8	28.6
	19	28.8	28.6
GU control	Air	24.0	-
RC area	Sfc.	26.8	23.9
	1	26.8	24.0
	2	26.8	24.0
	3	26.8	24.1
	4	26.8	24.2
	5	26.9	24.1
	6	26.9	24.1
	7	26.9	24.3
	8	26.9	24.3
	9	26.9	24.4
	10	27.0	24.5
	11	27.0	24.5
	12	27.0	24.6
	14	27.1	24.8
	16	27.4	25.3
	17	27.5	26.0
	18	27.8	26.3

Date, Time Station	Depth (Meters)	Temperature (°C)	Salinity (PPT)	Oxygen (% Sat.)*
9 July 73,1830 Platform 54A	0	32.0	15.3	
	1	31.5	15.7	
	2	31.5	15.7	
	3	31.0	16.2	
	4	30.0	21.0	
	5	29.1	25.9	
	6	28.8	26.7	
	7	28.2	27.7	
	8	28.0	28.3	
	9	28.0	29.0	
	10	26.2	29.2	
	11	25.3	29.7	
	12	25.0	29.9	
	13	24.0	30.2	
	14	23.8	30.3	
	15	23.5	30.2	
	16	23.0	30.4	
	17	22.8	30.5	
	18	22.8	30.5	
10 July 73, 1130 3 miles due East of Plat. 54 A	0	30.9	15.3	91
	1	30.8	15.3	
	2	30.8	15.3	
	3	30.8	15.3	
	4	30.8	15.3	
	5	30.8	15.0	91
	6	30.7	17.0	
	7	30.8	19.8	
	8	30.8	25.2	
	9	30.8	25.8	
	10	30.5	26.6	47
	12	29.8	26.9	
	14	28.5	27.5	
	16	28.0	27.9	
	18	27.8	28.2	3
11 July 73,0945 "Control Area"	0	30.0	16.8	105
	1	30.0	16.8	
	2	30.0	16.9	
	3	30.0	16.9	101
	4	30.2	25.4	
	5	30.5	26.9	
	6	30.5	27.6	
	7	30.5	23.1	
	8	30.2	28.4	
	9	29.5	28.2	
	10	28.8	28.5	71.5
	11	28.5	28.3	
	12	27.5	28.8	
	13	26.5	29.4	
	14	25.8	29.8	

Date, Time Station	Depth (Meters)	Temperature (°C)	Salinity (PPT)	Oxygen (%Sat.) *
"Control Area" Cont.	15	25.5	30.0	
	16	24.5	30.2	
	17	23.8	30.0	
	18	23.0	30.1	
	19	22.9	30.2	83
11 July 73, 1230 Platform 54 A	0	30.5	13.6	114
	1	30.5	13.6	
	2	30.5	13.6	
	3	30.5	13.9	112
	4	30.5	19.6	
	5	29.0	21.7	
	6	28.8	24.4	
	7	29.0	27.0	
	8	29.5	28.1	
	10	29.5	28.4	97
	12	26.5	29.7	
	14	25.8	30.0	
	16	25.8	30.0	
	18	25.5	30.0	36
11 July 73, 1400 3 miles due West of Platform 54A	0	30.5	14.4	114
	1	30.5	14.4	
	2	30.5	14.6	
	3	30.5	16.1	86
	4	29.5	20.9	
	5	29.3	23.2	
	6	28.1	27.5	
	7	28.2	28.8	
	8	27.5	29.2	
	10	26.3	29.4	74.5
	12	25.8	29.7	
	14	25.5	29.8	
	16	25.5	30.0	
	18	25.3	30.1	28
12 July 73, 0900 1.5 mi. offshore from Timbalier Island.	0	28.8	23.1	
	1	28.2	24.4	
	2	27.5	26.6	
	3	27.2	27.3	
	4	27.0	28.4	
A distinct foam line ran in a west to wsw dir. with clearer water nearer the beach.	5	27.1	28.7	
	6	27.1	29.2	
	7	24.5	30.1	
	8	24.2	30.1	
This station on the seaward, or murky, side of the foam line.				

Date, Time Station	Depth (Meters)	Temperature (°C)	Salinity (PPM)	Oxygen (% Sat.)
11 July 73, 1100	0.0	27.0	28.1	
0.8 Mi. offshore	0.5	26.5	28.7	
Timbalier Island	1.0	26.2	28.9	
	1.5	26.0	29.0	
Clear side of	2.0	25.8	29.1	
foam line, 500 ft.	2.5	24.5	29.9	
	3.0	24.5	29.9	
11 July 73, 1200	0.0	29.0	20.8	
0.9 mi. offshore	0.5	29.0	22.4	
Timbalier Is.	1.0	28.0	24.1	
	1.5	28.0	25.5	
Turbid side of	2.0	27.2	27.9	
foam line, 500 ft.	2.5	26.8	28.9	
	3.0	25.0	29.9	
11 July 73, 1300	0.0	27.1	28.7	
0.8 miles from	0.5	26.9	28.8	
beach off Timbalier	1.0	25.2	29.8	
Is. - 1 mi. west of	1.5	25.2	29.8	
previous station	2.0	25.2	29.8	
Clear side of	2.5	25.2	29.8	
foam line, 500 ft.	3.0	25.2	29.8	
	3.5	25.2	29.8	
11 July 73, 1345	0.0	28.0	25.3	
1.3 mile from beach	0.5	28.0	25.5	
off Timbalier Is.	1.0	27.8	26.6	
	1.5	27.5	27.0	
Turbid side of	2.0	27.2	27.1	
foam line, 0.5 mi.	2.5	27.5	27.1	
	3.0	27.3	27.2	
	3.5	27.3	27.8	
	4.0	27.2	27.8	
	5.0	27.0	28.7	
	6.0	24.2	30.1	
	7.0	24.0	30.2	
	8.0	24.0	30.2	
11 July 73, 1430	0.0	27.7	26.2	
3 miles south of	0.5	28.0	26.1	
Timbalier Is.	1.0	28.0	26.3	
	1.5	27.8	26.8	
	2.0	27.5	27.3	
	2.5	27.3	27.8	
	3.0	27.3	27.8	
	3.5	27.2	27.8	
	4.0	27.2	27.9	
	5.0	26.9	28.5	
	7.0	24.0	30.3	
	9.0	24.0	30.4	

Date, Time Station	Depth (Meters)	Temperature (°C)	Salinity (PPH)	Oxygen* (%Sat.)
11 July 73, 1500	0	28.8	23.5	104
5 miles south of	1	28.6	23.9	
Timbalier Is.	2	28.3	24.2	
	3	28.1	24.8	
	4	27.8	27.3	
	5	27.5	28.8	
	6	27.3	29.3	
	7	26.0	30.0	
	8	25.5	30.3	
	9	25.2	30.5	
	10	25.0	30.2	
	11	24.5	30.6	
	12	24.0	30.6	
	13	23.8	30.3	5
11 July 73, 1545	0	30.1	16.2	
7 miles due west of	1	29.9	16.2	
Platform 54 A	2	30.0	17.5	
	3	30.0	18.5	
	4	28.5	22.9	
	5	28.3	23.0	
	6	28.1	24.1	
	8	26.0	30.0	
	10	25.5	30.3	
	12	25.5	30.3	
	14	25.3	30.5	
	15	25.0	30.7	
	16	24.8	30.4	
	17	24.8	30.4	
11 July 73, 1630	0	30.2	12.5	
7 miles southwest	1	30.2	12.6	
of Platform 54A	2	30.2	12.6	
	3	30.2	13.1	
	4	30.2	16.8	
	5	30.2	19.3	
	6	29.3	22.9	
	7	28.2	25.6	
	8	27.2	27.6	
	9	27.0	29.4	
	10	26.8	29.6	
	11	26.0	30.4	
	12	25.0	30.7	
	13	25.0	30.7	
	14	24.8	30.8	
	15	24.8	30.8	
	16	24.8	30.8	
	17	25.0	31.0	

Date, Time Station	Depth (Meters)	Temperature (°C)	Salinity (PPM)	Oxygen (% Sat.) *
11 July 73, 1715 5 miles due south of Platform 54 A	0	31.5	13.94	
	1	31.5	13.94	
	2	30.5	13.94	
	3	30.2	14.03	
	4	30.2	14.34	
	5	30.2	15.58	
	6	30.3	16.2	
	7	29.8	21.4	
	8	28.8	24.1	
	9	28.5	24.9	
	10	28.0	26.0	
	11	27.0	29.1	
	12	26.5	29.7	
	13	26.0	30.2	
	14	26.0	30.3	
	15	25.3	31.0	
	16	25.0	30.8	
	17	25.0	30.8	
13 July 73, 0900 Philo Brice Is. in Timbalier Bay	0	28.0	13.2	
13 July 73, 1500 Platform 54 A	0	31.0	13.5	
	1	31.0	13.5	
	2	31.0	13.5	
	3	30.5	17.4	
	4	30.2	19.3	
	5	30.0	20.1	
	6	30.2	20.0	
	7	28.8	24.8	
	8	27.8	26.9	
	9	27.5	29.2	
	10	27.3	29.6	
	11	26.5	30.2	
	12	26.2	30.1	49
	13	25.5	30.3	31
	14	24.5	30.2	17.5
	15	24.0	30.6	6
	16	23.8	30.7	4.5
	17	23.5	30.9	5
	18	23.8	30.7	5
	19	23.8	30.7	4.5

* Oxygen values were determined with an oxygen electrode and are to be viewed comparatively per hydrocast. Winkler titration values for 100% calibration bottles appear to be too high, thus the values should not be used quantitatively. % saturation readings per water column do however show a dramatic decrease in oxygen near the bottom around 54A.

Russ Wright

SYNOPTIC RUN # 1

April 4, 1973

Begin run nearshore 29.13.20 N 89.59.30 W then run offshore on course of 150° for 10 miles, with stations at one mile intervals.

	MARTEC TDC DATA				WATER SAMPLES	
Station	Time	Depth(M)	T°C	Sal.°/oo	Depth(ft.)	% Oxygen Saturation
1	0905	sfc.	19.0	25.9	1	81.3
		4.2	19.0	25.9	5	84.2
		6.0	19.0	27.7	10	81.0
		6.4	19.0	-	16	79.0
					18 bottom	
2	1000	sfc.	19.1	20.8	1	90.5
		4	19.0	25.3	17	91.5
		7	19.0	27.7	22	92.2
		9	19.0	-	23	90.9
					25 bottom	
3	1057	sfc.	19.1	22.0	1	95.9
		4	19.0	22.4	20	88.6
		5	19.0	23.6	28	85.6
		5.5	19.0	27.7	29	85.8
		8.0	19.0	28.6	31 bottom	
		10.0	19.0	28.9		
4	1159	sfc.	19.0	22.7	1	98.0
		3.0	19.0	23.6	20	90.8
		4.0	19.0	26.9	31	77.7
		5.5	19.0	29.4	34	78.8
		8.0	19.0	29.9	35 bottom	
		9.0	19.1	30.0		
		10.0	19.2	31.4		
		13.0	19.2	30.4		
5	1245	sfc.	19.0	23.5	1	95.9
		3.5	19.0	24.4	30	84.5
		6.0	19.0	27.7	40	84.2
		7.0	19.1	30.5	42 bottom	
		11.0	19.5	32.3		
		13.5	19.6	32.2		

SYNOPTIC # 1 (cont.)

Station	Time	Depth(m)	T°C	Sal.°/oo	Depth (ft.)	% Oxygen Saturation
6	1322	sfc.	19.5	23.1	1	100.5
		3.5	19.4	23.0	35	88.2
		6.0	19.1	26.6	44	83.1
		9.2	19.1	30.9	46	82.6
		11.9	19.2	31.9	48 bottom	
		15.0	19.9	33.0		
7	1416	sfc.	19.9	22.8	1	98.5
		4.0	19.5	25.0	40	84.9
		6.5	19.3	27.2	52	78.7
		7.5	19.0	30.2	53	77.8
		9.5	19.0	31.0	55 bottom	
		12.5	19.6	32.6		
		16.0	20.0	33.6		
		17.6	20.0	33.7		
8	1455	sfc.	20.0	22.6	1	102.2
		4.0	19.9	23.9	45	81.4
		5.5	19.4	27.8	56	81.6
		6.0	19.1	28.8	58	80.5
		7.0	19.1	29.7	60 bottom	
		9.0	19.1	30.9		
		11.0	19.0	32.7		
		13.0	19.5	33.2		
		15.5	20.0	33.7		
		18.0	20.0	34.3		
9	1537	sfc.	19.9	22.2	1	106.6
		4.0	19.5	23.9	50	84.2
		6.0	19.0	28.6	63	81.3
		8.1	19.0	31.0	64	79.2
		12.0	19.1	32,8	66 bottom	
		13.7	19.9	33.2		
		16.5	20.0	34.4		
		19.5	20.0	34.4		

SYNOPTIC #1 (cont.)

Station	Time	Depth(m)	T°C	Sal.°/oo	Depth (ft.)	% Oxygen Saturation
10	1621	sfc.	19.8	22.4	1	109.0
		4.0	19.1	25.9	55	79.1
		5.5	19.1	26.2	68	77.9.
		8.0	18.9	31.2	70	76.7
		10.0	19.0	32.0	72 bottom	
		12.5	19.2	33.4		
		14.5	19.9	33.6		
		16.5	20.0	34.4		
		19.0	20.1	34.7		
		21.0	20.1	34.8		
		22.0	20.1	34.8		
11	1706	sfc.	19.6	22.6	1	115.1
		4.0	19.6	23.3	55	79.6
		6.0	19.0	28.4	74	77.6
		7.0	19.0	31.2	76	74.6
		9.0	18.9	31.6	78 bottom	
		11.5	19.0	32.6		
		14.5	19.4	33.4		
		17.5	20.0	34.4		
		21.0	20.2	35.0		
		24.0	20.2	35.0		

END OF SYNOPTIC # 1 April 4, 1973

SYNOPTIC RUN # 2

April 6, 1973

Begin run from offshore 28.57.30 N 90.00.30 W then run inshore on course of 310° for 10 miles, with stations at one mile intervals.

		MARTEC TDC DATA			WATER SAMPLES	
Station	Time	Depth(m)	T°C	Sal.°/oo	Depth(ft.)	% Oxygen Saturation
14	1115	sfc.	18.7	23.0	1	110.5
		5.0	18.9	23.1	70	102.9
		7.0	18.4	24.8	77	91.9
		9.8	18.5	25.6	82	87.3
		12.0	19.0	26.7	84 bottom	
		15.0	19.5	33.1		
		19.2	20.0	34.4		
		23.2	20.0	35.4		
		26.0	20.0	32.6		
15	1147	sfc.	18.4	23.1	1	110.4
		5.7	18.6	23.6	64	94.0
		8.9	18.7	24.7	72	81.5
		12.3	19.0	27.0	75	81.3
		14.6	19.8	33.0	77 bottom	
		17.3	19.8	34.4		
		19.2	20.0	34.8		
		21.5	20.0	35.4		
		23.2	20.0	35.5		
16	1230	sfc.	18.5	23.1	1	106.3
		5.0	18.5	23.1	53	85.5
		8.0	18.8	23.8	61	77.1
		10.0	18.9	24.7	65	77.7
		11.0	19.0	25.3	67 bottom	
		12.0	19.1	30.5		
		14.0	19.5	32.4		
		16.0	19.8	34.6		
		18.0	19.9	34.9		
		20	20.0	35.2		
		21.0	20.0	34.4		

SYNOPTIC # 2 (cont.)

Station	Time	Depth(m)	T°C	Sal.°/oo	Depth(ft.)	% Oxygen Saturation
17	1312	sfc.	18.2	23.1	1	102.7
		5.0	18.2	23.3	45	90.3
		8.0	18.8	24.0	57	91.0
		10.0	18.8	25.0	61	91.0
		11.0	19.0	27.3	63 bottom	
		12.0	19.0	30.6		
		13.0	19.0	31.5		
		14.0	19.3	32.5		
		16.0	19.8	34.2		
		19.0	19.8	34.6		
18	1350	sfc.	18.2	23.3	1	101.0
		5.0	18.3	23.2	41	85.9
		8.0	18.5	23.9	56	85.8
		10.0	19.0	26.9	57	85.1
		11.0	19.0	30.2	59 bottom	
		12.0	18.9	31.6		
		13.0	19.0	32.3		
		14.0	19.1	33.5		
		15.0	19.3	33.4		
		17.0	19.5	34.4		
19	1420	sfc.	18.0	22.8	1	110.0
		5.0	18.3	22.8	40	98.6
		8.0	18.5	24.4	51	85.0
		10.0	18.8	27.1	52	85.0
		11.0	18.9	27.8	54 bottom	
		12.0	19.0	30.3		
		13.0	19.0	30.7		
		14.0	19.2	32.1		
		15.0	19.5	33.2		
		17.0	19.5	33.2		
20	1455	sfc.	18.0	22.0	1	118.8
		5.0	18.2	23.2	33	104.0
		8.0	18.8	26.6	45	92.9
		10.0	18.5	28.5	48	92.9
		11.0	18.8	31.2	50 bottom	

SYNOPTIC # 2 (cont.)

Station	Time	Depth(m)	T°C	Sal.°/oo	Depth(ft.)	% Oxygen Saturation
20	1455	13.0	19.0	32.3		
		14.0	19.0	32.7		
		15.0	19.2	32.9		
21	1525	sfc.	18.1	22.5	1	121.0
		5.0	18.1	22.6	28	124.8
		8.0	18.8	24.3	40	88.5
		10.0	19.0	26.9	44	87.4
		11.0	18.9	30.8	46 bottom	
		12.0	19,0	31.1		
		13.0	19.1	31.4		
		14.0	19.1	32.6		
		15.0	19.0	33.2		
22	1604	sfc.	18.0	22.6	1	116.4
		5.0	18.0	23.0	22	131.7
		8.0	18.5	24.4	34	92.6
		10.0	19.0	30.6	38	92.6
		11.0	19.0	31.5	40 bottom	
		12.0	18.9	31.7		
23	1637	sfc.	18.0	22.6	1	107.6
		5.0	18.0	22.6	28	133.9
		8.0	18.5	24.4	34	136.0
		10.0	18.8	25.4	36 bottom	
		11.0	19.0	29.4		
		12.0	19.0	29.4		
24	1702	sfc.	18.0	23.4	1.	125.9
		4.0	18.2	23.7	18	134.3
		6.0	18.5	23.5	28	136.0
		8.0	18.5	25.6	30 bottom	
		10.0	19.0	29.4		

END OF SYNOPTIC # 2 April 6, 1973

GURC/OEI LONGHORN CRUISE January 8-14, 1973.

STATION	DEPTH (M)	TEMP. (C)	SALINITY (PPT)
#1	0 sfc.	15.0	26.1
Platform 54A	2.5	15.2	26.0
28 49 53 N	5.0	15.3	26.0
90 23 18 W	7.5	15.5	26.7
	10.0	17.2	29.6
	12.5	18.9	32.6
Jan. 9	15.0	19.1	34.0
	17.5	19.8	34.6
	20.0	19.9	34.6
	air	10.0	
#2	0 sfc.	14.5	25.8
Control area	2.5	14.5	25.8
28 53 13 N	5.0	14.7	25.8
90 18 30 W	7.5	15.1	27.8
	10.0	16.7	30.2
	12.5	18.5	30.8
Jan. 10	15.0	19.2	32.6
	17.5	19.4	33.4
	20.0	20.0	34.8
	air	8.0	
# 8	0 sfc.	8.8	24.7
Addn. control	0.5	8.8	24.7
29 30 20 N	1.0	8.8	24.7
94 01 20 W	1.5	8.8	24.3
	2.8	8.8	24.7
	4.0	8.8	24.7
	6.0	9.3	26.2
	8.0	10.3	28.6
	10.0	10.5	25.5
	11.8	10.7	24.3

GULF

Longhorn Cruise 9-18-72 to 9-23-72

92 10 20
28 50 30

Station 1 9-19-72 1400 hr. Clear Wind 5-8 k East

21 Meters deep Secci disc -11 meters

Temperature	Depth	Conductivity	Salinity
30.5	5 m	5.4	32.0
30.5	10 m	5.5	32.8
30.5	15 m	5.6	33.4
30.0	20 m	5.1	30.0

5 mi North from 54A

28 51 30
90 24 10

Station 2 9-20-72 0730 Clear Wind 1 k

Temperature	Depth	Conductivity	Salinity
30	0 m	5.2	30.8
30	5 m	5.5	32.8
30	10 m	5.0	29.4
30	12 m	5.6	33.4

54A
28°49'53"
90°23'18"

Station 3 (Platform- Station 1) 9-20-72 0930 hr. Pt. Cldy.
Approx. 500 yd. from living qtrs. on platform 54 A
20 meters deep Secci disc - 16.5 m

Temperature	Depth	Conductivity	Salinity	Oxygen
30	0	5.3	31.4	9.2
30	5	5.4	32.0	8.8
30	10	5.6	33.4	6.6
30	11	5.6	33.4	6.2
30	15	5.6	33.4	
30	19	5.7	34.0	

28°53'30"
90°18'30"

Station 4 (Control area) 9.20.72 1230 pH 8.38

28°53'30"
90°26'

Station 5 (Platform Station 2) 9.20.72 1500 hr. ✓

Temperature	Depth	Conductivity	Salinity	Oxygen
30.8	0	4.8	28.0	8.3
30	5	5.7	34.0	8.1
30	10	5.3	31.4	8.5
30	12	5.5	32.8	8.4

28°47'30"
90°27'

Station 6 (Platform area Sta. 3) 9.20.72 2000 hr. pH 7.95

Temperature	Depth	Conductivity	Salinity
30.8	0	5.2	30.8
30.2	5	5.4	32.0
30.0	10	5.6	33.4
30.0	11	5.6	33.4
30.8	20	5.6	33.4
			33.4

Station 7 (Platform station 4) 9.21.72 wind 2-4 k Sunny 0915

28°46'30"
90°20'30"

Temperature	Depth	Conductivity	Salinity
30.0	0 m	5.4	32.0
30.2	5	5.5	32.8
30.5	10	5.6	33.4
30.2	15	5.62	33.4
30.0	20	5.62	33.4

pH 8.05

Station 8 (Platform # 1 repeat) 9.21.72 1030

Temperature	Depth	Conductivity	Salinity
30.0	o m	5.2	30.8
30.1	5	5.2	30.8
30.1	10	5.6	33.4
30.0	15	5,7	34.0
30.0	20	5.7	34.0

pH 8.2

	Oxygen
0	8.9
1	8.7
2	8.5
3	8.5
4	8.7
5	8.7
6	8.7
7	8.1
8	8.4
9	8.2
10	8.3
11	7.5
12	7.5

Station 9 (control area repeat) 9.21.72 1200 hr

Temperature	Depth	Conductivity	Salinity
30.1	0 m	5.4	32.0
30.1	5	5.5	32.8
30.1	10	5.6	33.4
30.0	15	5.7	34.0
30.0	20=	5.1	30.0

	Oxygen
0	9.4
1	9.3
2	9.3
3	9.3
4	9.3
5	9.1
6	9.2
7	9.2
8	9.1
9	8.9
10	8.8
11	8.6
12	8.8