NOAA Technical Memorandum NMFS-SEFC-49

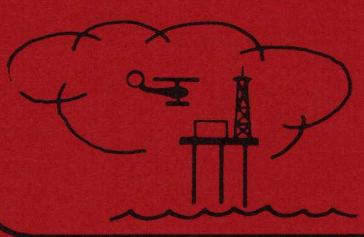
NOAA/NMFS MILESTONE REPORT TO EPA

Environmental Assessment of Buccaneer Gas and Oil Field in the Northwestern Gulf of Mexico, 1975-1980

A report to the Environmental Protection Agency on work conducted under provisions of Interagency Agreement EPA-IAG-D5-E693-EO during 1975-1980.

Volume III

BACTERIA



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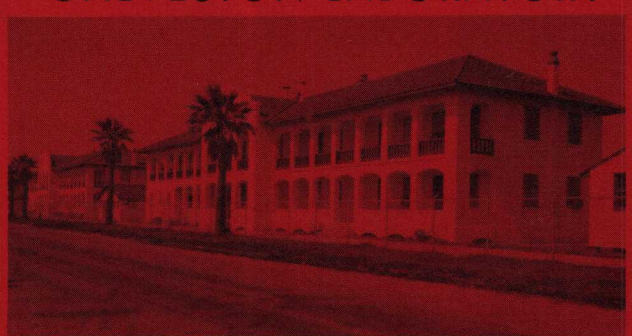

GALVESTON, TEXAS

NOVEMBER 1980

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Center
Galveston Laboratory
Galveston, Texas 77550

NOAA Technical Memorandum NMFS-SEFC- 49

Environmental Assessment of Buccaneer Gas and Oil Field In the Northwestern Gulf of Mexico, 1975-1980.

VOL. III-BACTERIOLOGY OF A GULF OF MEXICO GAS AND OIL FIELD

BY

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A report to the Environmental Protection Agency on work conducted under provisions of Interagency Agreement EPA-IAG-D5-E693-E0 during 1975-1980.

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J. Tillery

FOREWORD

Increased petroleum development of the outer continental shelf (OCS) of the United States is anticipated as the U.S. attempts to reduce its dependency on foreign petroleum supplies. To obtain information concerning the environmental consequences of such development, the Federal Government has supported major research efforts on the OCS to document environmental conditions before, during, and after oil and gas exploration, production, and transmission. Among these efforts is the Environmental Assessment of Buccaneer Gas and Oil Field in the Northwestern Gulf of Mexico, a project funded by the Environmental Protection Agency (EPA) through interagency agreement with the National Oceanic and Atomospheric Administration (NOAA) and managed by the National Marine Fisheries Service (NMFS), Southeast Fisheries Center (SEFC), Galveston Laboratory, in Galveston, Texas. Initiated in the autumn of 1975, the study was completed in 1980. Its major products have been annual reports disseminated by the National Technical Information Service, data files archived and disseminated by NOAA's Environmental Data and Information Service, and research papers written by participating investigators and published in scientific or technical journals. Results have also been made available through EPA/NOAA/NMFS project reviews and workshops attended by project participants, and various governmental (Federal and State), private, and public user groups. The final product are these milestone reports summarizing the findings of the major investigative components of the study.

Objectives of the project were (1) to identify and document the types and extent of biological, chemical and physical alterations of the marine ecosystem associated with Buccaneer Gas and Oil Field, (2) to determine specific pollutants, their quantity and effects, and (3) to develop the capability to describe and predict fate and effects of Buccaneer Gas and Oil Field contaminants. The project used historical and new data and included investigations both in the field and in the laboratory. A brief Pilot Study was conducted in the autumn and winter of 1975-76, followed by an extensive biological/chemical/physical survey in 1976-77 comparing the Buccaneer Gas and Oil Field area with adjacent undeveloped or control areas. In 1977-78, investigations were intensified within Buccaneer Gas and Oil Field, comparing conditions around production platforms, which release various effluents including produced brine, with those around satellite structures (well jackets) which release no effluents. In 1978-79, studies around Buccaneer Gas and Oil Field structures focused on (1) concentrations and effects of pollutants in major components of

the marine ecosystem, including seawater, surficial sediments, suspended particulate matter, fouling community, bacterial community, and fishes and macro-crustaceans, (2) effects of circulation dynamics and hydrography on distribution of pollutants, and (3) mathematical modeling to describe and predict sources, fate and effects of pollutants. The final year, 1979-80, of study continued to focus on items (1) and (2) and on preparation of the milestone reports which represented the final products of this study.

This project has provided a unique opportunity for a multi-year investigation of effects of chronic, low-level contamination of a marine ecosystem associated with gas and oil production in a long-established field. In many respects, it represents a pioneering effort. It has been made possible through the cooporation of government agencies, Shell Oil Company (which owns and operates the field) and various contractors including universities and private companies. It is anticipated that the results of this project will impact in a significant way on future decisions regarding operations of gas and oil fields on the OCS.

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1976-1977	Ichthyoplankton	78-0501
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INTRODUCTION

Location of Study Area

The area selected for study is the operational Buccaneer Gas and Oil Field located approximately 49.6 kilometers (26.8 nautical miles) south southeast of the Galveston Sea Buoy off Galveston, Texas (Figure 1). This field was selected in 1975 as the study area because: (a) the field had been in production for about 15 years, which time had allowed full development of the associated marine communities; (b) it was isolated from other fields which facilitated the selection of an unaltered area (for comparison) within a reasonable distance of the field; (c) it produced both gas and oil that represented sources of pollutants from marine petroleum extraction; (d) its location simplified logistics and reduced the cost of the research; and (e) the Texas offshore area had not been fully developed for gas and oil production but was expected to experience accelerated exploitation in the future.

Operation History of Buccaneer Field

Buccaneer Field was developed by Shell Oil Company in four offshore blocks leased in 1960 and 1968 as follows:

Year	Lease Number	Block Number	Acreage	Hectares
1960	G0709	288	2,790	1,129
1960	G0713	295	4,770	1,930
1960	G0714	296	4,501	1,821
1968	G1783	289	2,610	1,056

In development of the field, 17 structures were built; two are production platforms, two are quarters platforms, and 13 are satellite structures surrounding well jackets. Initial exploratory drilling began about mid-summer of 1960 with mobile drilling rigs. When (as the result of the exploratory drilling) proper locations for platforms were selected, the permanent production platforms were constructed.

There have been no reports of major oil spills from this field. There have been some reported losses of oil due to occasional mechanical failure of various pieces of equipment. The largest reported spill was three barrels in 1973. The reported oil spill chronology and quantity for Buccaneer Field is as follows:

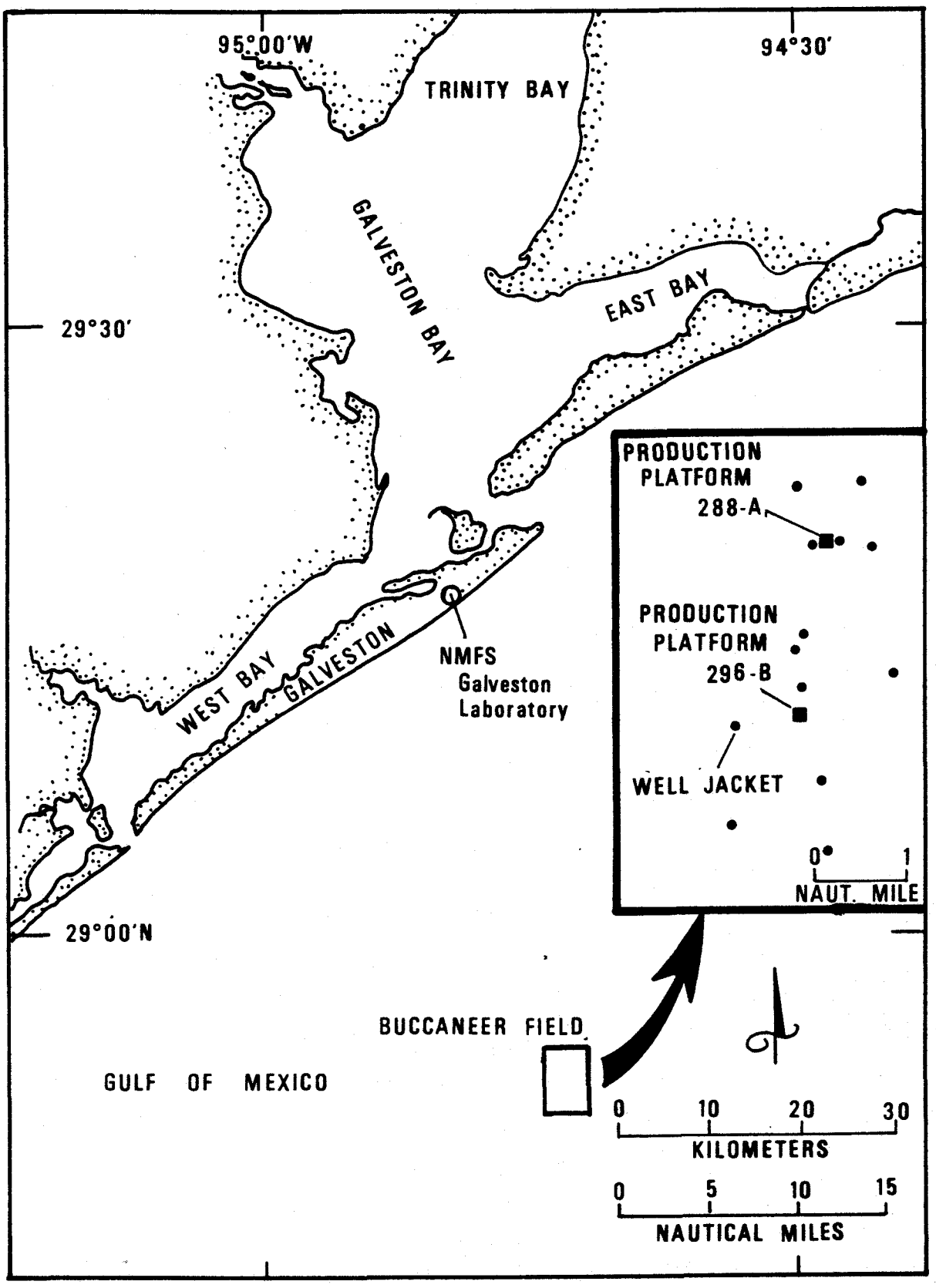


FIGURE 1. LOCATION OF BUCCANEER FIELD

Date	Source	Amount	
		Barrels	Liters
September 1973	Platform 296-B	0.5	79
November 1973	Unknown	3.0	477
July 1974	Platform 296-B	0.5	79
August 1974	Platform 296-B	1.7	265
September 1975	Platform 288-A	0.2-0.4	38-56
Totals		5.9-6.1	938-956

Buccaneer Field first began operations with the production of oil. Later, when significant quantities of gas were found, the field began producing both oil and gas and has continued to do so to date.

The production platforms and satellites (well jackets) are connected by a number of pipelines with a 50.8 centimeters (20-inch) diameter main pipeline connecting the field to shore. All of the pipelines that are 25.4 centimeters (10 inches) or greater in diameter are buried. The Blue Dolphin Pipeline Company was granted a pipeline permit (No. G1381, Blocks 288 and 296) in 1965 and has operated the pipeline since its construction.

Buccaneer Field occupies a limited area (about 59.3 km^2; 22.9 sq. statute miles) leased in the northwestern Gulf of Mexico. Four types of structures are located in Buccaneer Field: production platforms, quarters platforms, satellites (well jackets), and flare stacks. These are shown in Figure 2, which is an oblique aerial photograph of production platform 288-A and vicinity within Buccaneer Field. A map of Buccaneer Field, (Figure 3) depicts the locations of platforms and satellites within the field.

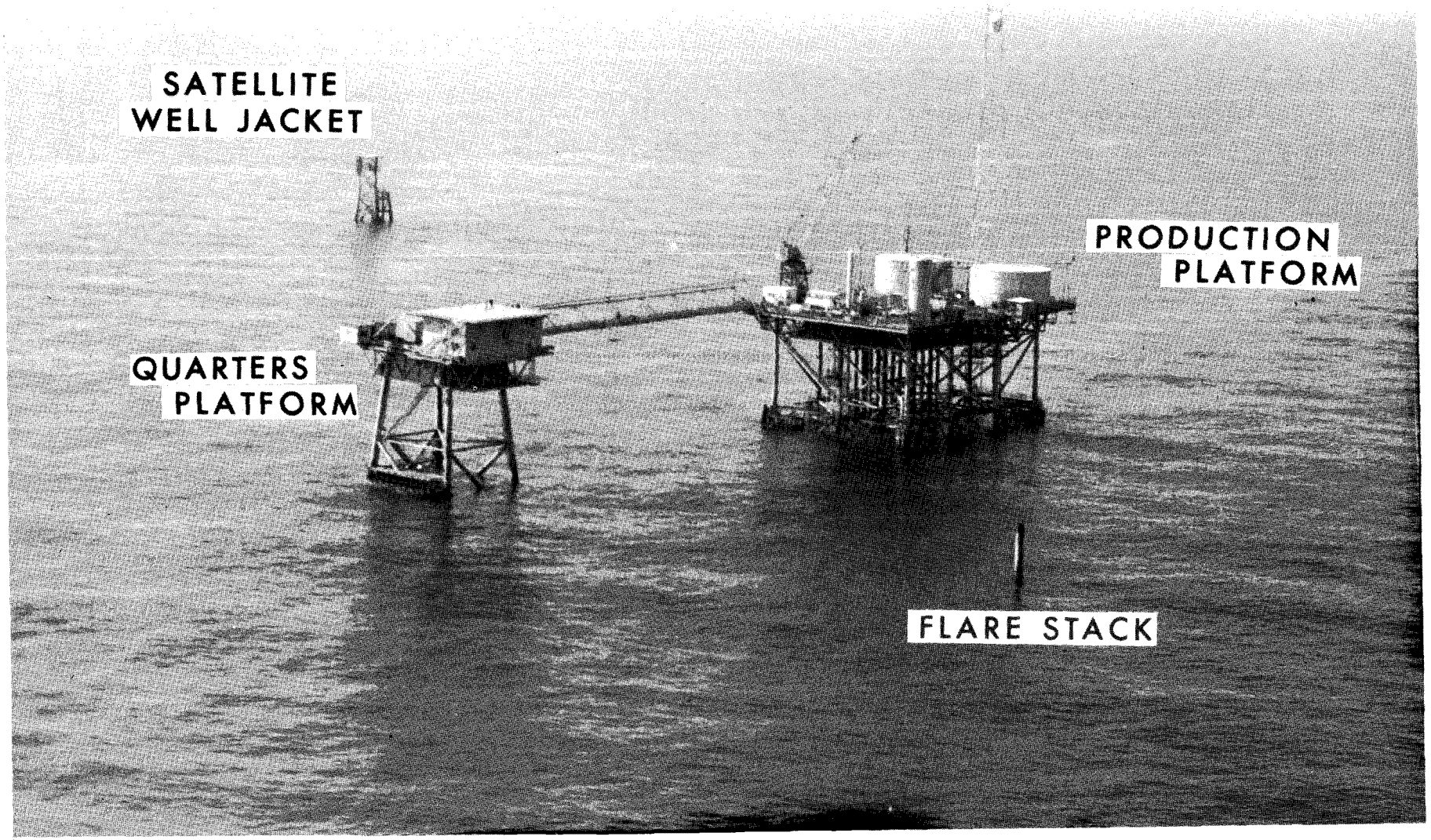


FIGURE 2. BUCCANEER FIELD STRUCTURES

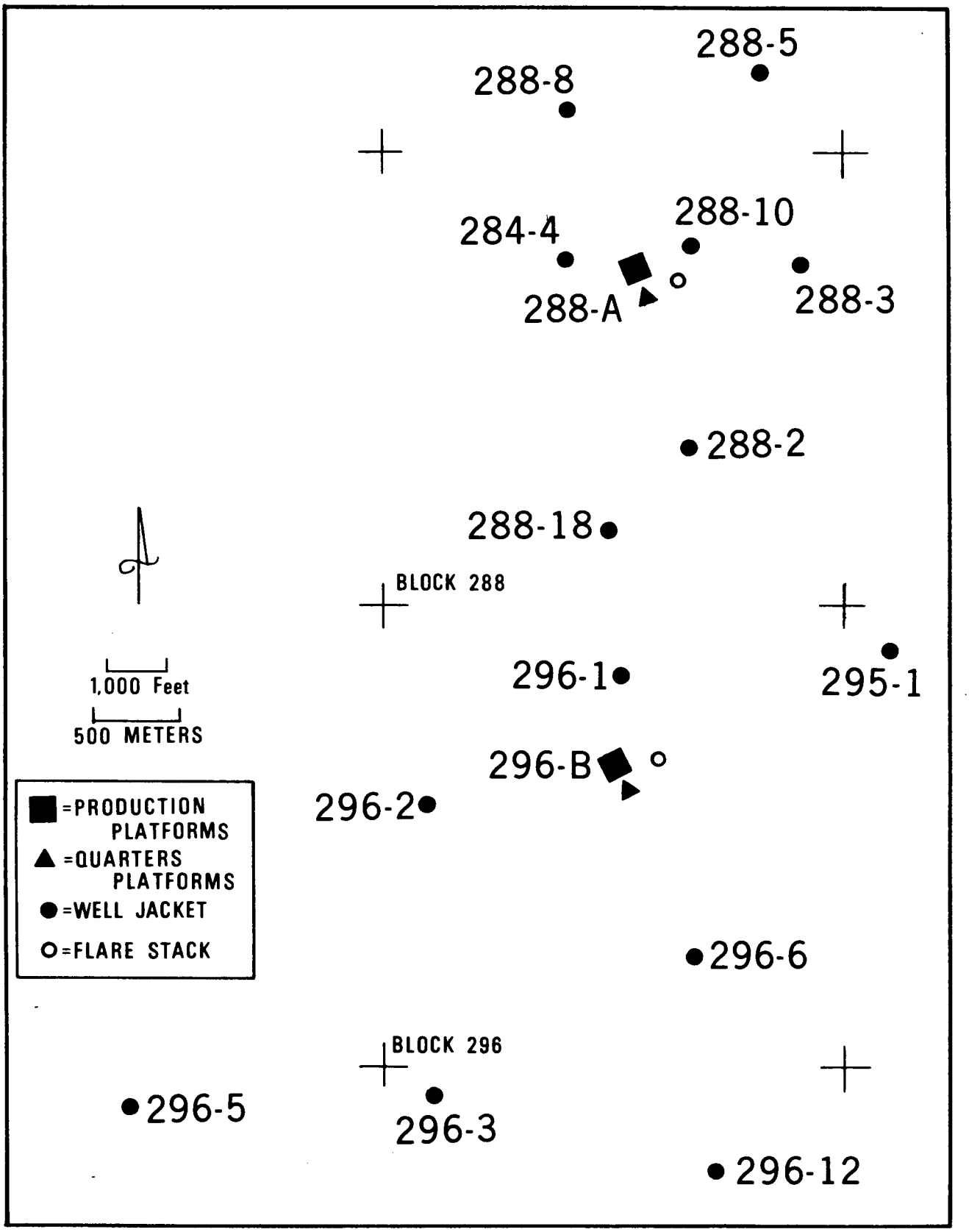


FIGURE 3. SHELL OIL COMPANY'S ALPHANUMERICAL IDENTIFICATION OF BUCCANEER GAS AND OIL FIELD STRUCTURES

DISCLAIMER

This report has been reviewed by the U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

A three year study of the bacteriology of a 20 year old active gas and oil field in the Gulf of Mexico has been completed. The bacterial community of the production platform area was in general identical to the control site. Taxonomic groups and genera diversity were essentially the same for the two sites.

The bacterial population around the platforms did show some adaptation, especially to the materials released from the platforms. A higher percentage of sulfur utilizing and oil degrading bacteria were found in the immediate area of the platform than at the control site. Bacterial strains from the platforms also appeared adapted to growth in the brine discharge released by the platform. Concentrations of brine discharge, which inhibit growth or activity of laboratory cultures and strains from the control site, did not affect bacteria from the platform site. Isolates from the platform area when suspended in diluted brine discharge grew, exhibited chemotatic responses, and attached to glass surfaces.

Brine discharge did not appear to be a significant bacterial nutrient in the platform area. Bacterial numbers and biomass were slightly lower in the platform area than at the control site. Sulfur oxidizing bacteria were generally Pseudomonas sp. rather than obligate autotrophic Thiobacillus sp. Bacterial strains which could utilize brine discharge hydrocarbons were found around the platform. Some strains and mixed cultures from the platform area could readily utilize most platform released hydrocarbons. However, these strains only represented a very small portion of the total bacterial populations.

In general, the differences between the bacteriology of the platform area and the control site were less than the difference seen between samples collected from different depths or seasons at the control site.

CONTENTS

FIGURES

INTRODUCTION

The bacteria represent the largest numerically population in most aquatic environments. Bacteria are active members of the marine ecosystem, many fulfilling the role of decomposers which recycle recalcitrant compounds. The rapid growth and great diversity of the bacteria insures that most naturally occurring materials will be subjected to attack by portions of the microbial population. This recycling capacity is tremendously important to the marine habitat because recalcitrant material often represent a significant portion of the nutrients available for microbial growth (Sieburth, 1979). Bacterial attack of these compounds often releases nutrients which can then be utilized by the microbial and non-microbial organisms. Furthermore, the bacterial growth which occurs during the breakdown of these compounds results in increased bacterial biomass which represents additional nutrients to organisms that feed on bacteria. In addition, some recalcitrant materials are toxic or detrimental to the marine habitat. Some of these compounds are subject to microbial attack which can decrease or remove their detrimental effects. Bacterial breakdown of these toxic materials may actually result in a net increase in biomass from the resulting nutrients released.

Petroleum products represent a class of generally recalcitrant pollutants which may have an adverse effect on portions of the marine ecosystem. Microorganisms, and especially bacteria are the major biological force responsible for the breakdown of petroleum in the marine environment (LaRock and Severance, 1973; Loughry, 1977). Recently much literature has dealt with two aspects of bacterial oil degradation. One type of study has concentrated on the careful examination of the capacity of individual bacteria to utilize specific hydrocarbons in the laboratory (for example, see Parekh et al., 1977). These studies have lead to the elucidation of the pathways involved in the breakdown of specific hydrocarbons and have identified strains capable of using specific hydrocarbons very effectively. These strains have been exploited and sometimes genetically manipulated to create rapid hydrocarbon utilizing bacteria. The in situ activity of these strains and the applicability of this type data to environmental assessment is questionable. The other type of study measures microbial response to major marine oil spills. These studies typically report changes in

the microbial population that have occurred after a major oil spill from a wrecked tanker (for example, see Loughry, 1977). These acute oil spills are the worst possible situations and do not represent the type of environmental changes expected around normally functioning off-shore oil production platforms.

Buccaneer gas and oil field offers a unique situation to study bacterial adaptation to petroleum pollution in the marine ecosystem. Chronic low levels of hydrocarbons and other petroleum related pollutants have been released into the Buccaneer field for a period of time sufficient (approximately 20 years) to permit optimal bacterial adaptation. Furthermore, the small amount of hydrocarbons released has not caused a dramatic change in the platform area such as would occur following a major oil spill. It is assumed that any effect from the discharge would be localized around the platform. This localization effect makes selection of a control site easier since an area of similar geological history, relatively close to the platform area can be used as a control site. The close proximity and assumed similarity between the platform study area and the control area permit detailed comparisons between the biota of the two sites in an attempt to pick up subtle differences between the two areas.

The initial year of study of the microbiology of the Buccaneer field by this work unit was 1977-1978. Since preliminary data on the bacteriology of the area was unavailable, an initial study was designed to determine if there were dramatic differences between control site bacteria, and bacteria of the area around the platforms. A sampling regime was chosen with sites at intervals on tract lines running two miles downstream from both platforms. Emphasis was given to examining the bacterial flora of the water column.

After the first year data became available, it was obvious that the bacterial flora of the platform area was not dramatically different than the control area. Therefore, in the subsequent second and third year of study the sampling regime and analytical techniques were modified to examine the small spatial differences in the bacterial population. Specifically, sampling sites were chosen much closer to the point source of discharge at the production platform. Also, when it became apparent that platform 288A was no longer routinely discharging material into the environment, emphasis was placed on platform 296B. Because of the transient nature of the water column around the platform, the emphasis in the second and third year of study was shifted to studying the bacteriology of the sediment.

After the first year of study, it also became apparent that the major pollutant released from the platform was

"brine discharge". Brine discharge is a multi-component suspension, composed of an aqueous solution containing all the materials (e.g. residue hydrocarbons, elemental sulfur, additives such as biocide which are used in the production process, etc.) arising from the well which are not removed by the equipment on the production platform. Since the production platforms are designed to remove hydrocarbons, the brine discharge contains only a small amount (see Middleditch et al., 1979) of petroleum hydrocarbons. In contrast, large amounts of elemental sulfur are contained in the brine discharge and are released by the platform into the environment. A major portion of this study was designed to determine the changes in bacterial flora which may have occurred in response to the release of these two materials in brine discharge. Also, a concerted analysis was made to determine the rate of bacterial attack on the hydrocarbons which are released in the platform discharge.

CONCLUSIONS

The Buccaneer field production platforms do not drastically change the bacteriology of the area. Only small differences between the platform area and the control site were noted. These differences appear to represent successful bacterial adaptation to the platforms.

Bacterial numbers and biomass were generally slightly lower in the platform area compared to the control site but this difference was smaller than the normal seasonal variation or between different depths at the same site. No differences were detected in the bacterial genera composition, taxonomic diversity, or "physiological profile" between the two study sites. The major detectable difference between the sites was that the platform area contained higher numbers of sulfur metabolizing and oil utilizing bacteria than the control site. Since sulfur and petroleum are known to be released by the production platforms, the increased number of bacteria capable of utilizing these nutrients is an indication of bacterial adaptation.

The discharge (i.e. brine discharge) from the platform had little effect on bacteria isolated from the Buccaneer field. Dilute brine discharge did not affect attachment, chemotaxis, or growth of bacterial strains. Laboratory stock cultures (e.g. Escherichia coli and Pseudomonas aeruginosa) and some of the isolates from the control site were affected by dilute brine discharge. Tolerance to dilute brine discharge therefore represents another type of bacterial adaptation to the production platforms. Full strength brine discharge as would be found immediately under the discharge point did affect Buccaneer field isolates. Isolates from the field generally did not grow or grew slowly in full strength brine discharge. Furthermore, samples collected from the brine discharge plume did not show the expected numbers of bacteria attached to suspended particulate material. This suggests that full strength discharge may inhibit normal bacterial attachment.

The bacterial population around the platform area shows adaptation to utilize the hydrocarbon and sulfur released by the platform. Obligate sulfur oxidizing bacteria of the genus Thiobacillus, faculative sulfur oxidizing Pseudomonas sp.,

and sulfate reducing bacteria were commonly found around the platforms. A number of species of oil degrading bacteria, most belonging to the genus Pseudomonas were isolated from around the platform. In the laboratory some of these isolates could dramatically increase oil loss from an experimental flask containing a seawater oil mixture. These oil degrading bacteria probably can utilize the majority of hydrocarbons released during normal platform operation but would not decrease the effect of a major oil spill in the area.

MATERIALS AND METHODS

Most of the techniques utilized in this study have been described in detail elsewhere (Sizemore, 1979; Sizemore and Olsen, in press) and are summarized in this report.

Samples for bacterial analysis were collected using sampling devices which are designed specifically to collect bacteria from the aquatic environment. Water samples were collected with a Niskin sterile bag sampler at a mid-water depth and from a depth approximately one to two meters above the bottom. Surface water samples were collected with a sterile glass bottle. Surface sediment samples were routinely collected with a Petite Ponar Grab. Subsamples of sediments were taken with a sterile spatula from the center of the least disturbed portion of the grab sample. All samples during the entire study were plated or fixed immediately upon recovery. This insured that any changes in bacterial population such as would occur after even brief storage were avoided.

Sampling sites included a variety of stations around platform 288A and 296B. During the first portion of the study, sites were sampled at intervals up to several kilometers from the rigs. However, as the study advanced it became obvious that any platform effects on bacterial communities would only be apparent in close proximity to the platform. Subsequent sampling sites were then chosen at intervals as close as feasible to the platform.

For the purpose of comparison, a control site five nautical miles due north of the platform site was chosen. This site is of a similar depth and bottom stratum as the platform area and is free from any petroleum production structures.

Bacterial enumeration utilized two techniques. Traditional plate counts were made using a broad spectrum medium, Modified Sea Water Yeast Extract (MSWYE) medium. This medium consists of 1 g of proteose peptone and 1 g of yeast extract dissolved in 1 ℓ of 3 salts solution at pH 7.2-7.4. If MSWYE agar was required, 20 g of Bacto agar was added to each liter of MSWYE broth before autoclaving. Three salts solution which is composed of 0.4 M NaCl, 0.028M Mg SO_4 and 0.01 M KCl was used as artificial seawater throughout this study. During the first year of study (1977-1978) samples were diluted with MSWYE broth

and plated in triplicate on MSWYE agar. To minimize sampling variance in the second and third year of this study (1978-1980), the subsampling scheme and plating technique of Kaper et al, (1978) was utilized. This technique required quadruplicate subsampling of the original sample and duplicate plating of each subsample. This technique was used primarily to obtain cultures and to provide total viable counts to compare with the selective media used. The other enumeration technique involved epifluorescent microscopic examination of acridine orange stained bacterial cells. This technique generally avoided problems inherent in the plating procedure and gives the highest and presumably the best estimate of bacterial population numbers (Hobbie et al., 1977). Biomass estimates were made by utilizing the bacterial numbers obtained and multiplying by the estimated (microscopically) average cell volume. This number was then multiplied by a carbon to mass correction factor (121 fg of carbon/μm^3 of bacterial cell volume) obtained from the literature (Watson et al., 1977).

Bacteria attached to suspended particulate material were enumerated during the first year of this study (1977-1978) by using selective filtration followed by spread plate counts on MSWYE agar. Freshly collected 100 ml water samples were filtered through sterile plankton netting with an average pore size of 50 µm on a sterile filter apparatus. The water which passed through the filter was collected in a sterile glass container and the bacteria were enumerated. The particulate material trapped on the filter was washed off by inverting the filter and flushing the filter with 100 ml of sterile 3 salts solution. The resulting solution was collected in a sterile container and blended in a sterile blender to remove any bacteria attached to the resuspended particulate material. The resulting solution was plated on MSWYE agar and the bacteria enumerated. To check the validity and precision of the filtration system, the counts of the bacteria trapped on the filter and passing through the filter were added and the sum compared to the total viable counts of untreated samples collected at the same time. The last two years of this study (1978-1980) bacteria attached to particulate material were enumerated using epifluorescence microscopy. Water samples were passed through a sequence of different pore size filters (8 µm, 5 µm, and 3 µm). These filters were treated as described above and the bacteria trapped on each filter were counted. The validity of the technique was tested by adding the number of bacteria trapped on each filter to the number passing through the last filter. This number was compared to the number generated by filtering a duplicate sample through a filter (0.22 µm) designed to trap all bacteria including unattached cells. If the totals for the two counts were similar the data was considered valid.

Oil and sulfur-utilizing bacteria were enumerated using a most probable number (MPN) extinction dilution technique. Triplicate tubes of the enumeration broths were inoculated with dilutions of freshly collected samples. Oil MPN tubes (composed of 0.1% KNO_3, 0.05% K_2HPO_4, 0.1% Buccaneer field crude oil, and 3 salts solution) were examined for turbidity after twenty-eight days of incubation at 25C. Random samples of the routine tubes were tested by steaking the positive tubes onto either oil silica gel plates or oil agar plates and checking for growth. Sulfur-oxidizing MPN's were enumerated by observing the pH change created by the bacterial oxidation of elemental sulfur to sulfuric acid (Postgate, 1967). Routinely, sulfur oxidizing MPN's were prepared by adding a pH indicator (bromthymol blue) to 3 salts solution (pH 7.4) which contained added phosphates and a nitrogen source . This medium was autoclaved and then a small amount of sterile elemental sulfur was added to each test tube. Sulfur loss was also measured gravimetrically in some experiments. In these experiments the amount of dry elemental sulfur added to flasks of MPN medium was carefully (to the nearest 0.01 gm) weighed. After inoculation and bacterial growth, the sulfur medium was filtered through tared Whatman No. 1 filter disc. The resulting filter discs were rinsed with distilled water and dried. The weight of dry sulfur on the filter was compared to the initial amount of sulfur added to each flask. Reduction in the weight of the sulfur collected from a flask was assumed to be due to bacterial oxidation of the sulfur. During the third year of this study (1979-1980) additional sulfur oxidizing test media were used. These media were similar in composition to the sulfur oxidizing medium described above, but contained extra nutrients (e.g. yeast extract) to detect more fastidious sulfur oxidizing bacteria.

Anaerobic sulfur metabolism was determined by using Kligler Iron Agar (KIA) prepared with 3 salts solution. During the first two years of this study (1977-1979) isolates from MSWYE agar plates were tested for anaerobic sulfur metabolism by inoculating individual colonies into KIA slants and observing the color change indicative of H_2S production. The third year of study utilized KIA plates which were inoculated with freshly collected samples on board ship and immediately placed in anaerobic growth chambers. This technique was used to decrease the loss of obligate anaerobic sulfur metabolizing bacteria.

During portions of this study bacterial isolates were checked for the presence of certain exoenzymes enabling them to utilize certain biological polymers. The presence of these enzymes were used to estimate what will be called in this report, "physiological diversity". The polymers which were tested include casein, chitin and Tween 80. Strains were also checked for the ability to grow on media lacking a salt water base

which suggests a non-marine origin of the strains.

Bacterial taxonomy was performed using isolates collected from either the non-selective medium (MSWYE) used for enumeration or from selective media (e.g. Oil MPN tubes) used to characterize specific types of bacteria. Isolates were identified using a simple dichotomous key. Some isolates were collected by swabbing the surface of healthy and diseased (as indicated by the presence of external lesions) fish with sterile cotton swabs and then streaking MSWYE agar plates with the swabs. The predominant colony type of these swab plates was selected and identified. Most isolates collected from fish were identified by utilizing API 20E strips. These strips are designed for clinical isolates and therefore could not identify all the marine strains. Where the strips didn't provide a positive identification, the test data from the strips was utilized in a dichotomous key.

Bacteria from the study area were examined for physiological diversity and the ability to tolerate discharge products from the production platforms. Bacteria from the platform and control sites as well as laboratory strains (Escherichia coli and Pseudomonas aeruginosa) were tested for their ability to grow, to attach to glass surfaces, and to exhibit normal chemotaxis in the presence of various concentrations of platform discharge. The concentrations used varied from full strength brine discharge to diluted discharge such as would be found in the area around the platform. Some brine discharge samples suspected to contain biocide were also used.

This study determined the capacity of bacteria from the study area to utilize hydrocarbon discharge from the platforms. These experiments utilized laboratory conditions which were designed to simulate optimal environmental conditions for oil degradation (i.e. optimal NO_3 + PO_4 concentrations). The hydrocarbon utilizing capacity of individual cultures and mixed cultures from the platform area were compared to isolates from the uncontaminated control site. During the first two years of study the technique and equipment used only permitted the examination of n-alkane degradation. In the last year of the study, model petroleum (Walker and Colwell, 1974) was utilized as a substrate in place of Buccaneer field petroleum. Model petroleum contains very few components relative to the number of components in crude oil and is thus different than the petroleum discharge actually found in the field. However, the utilization of model petroleum as a substrate for biodegradation permitted the identification and quantification of a variety of hydrocarbon components (e.g. aromatic compounds) with existing equipment. This was not possible and/or practical utilizing Buccaneer field brine discharge or crude oil.

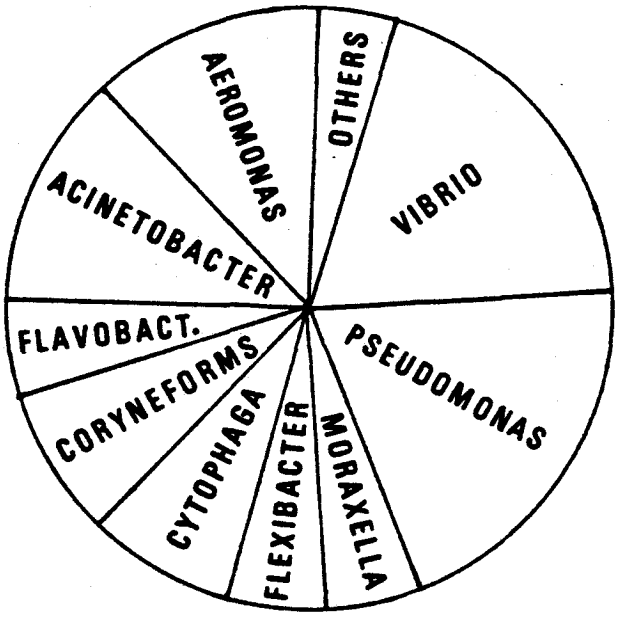
RESULTS AND DISCUSSION

Bacterial numbers and biomass tended to be slightly higher at the control site than at platform 296B (see Hollaway et al., 1980). For example, during the 1978-1979 study period bacterial counts in the water column around platform 296B averaged 465 bacteria per ml (as determined by plating) whereas the water in the control site averaged 564 bacteria per ml. Epifluorescence bacteria counts showed the same trends as total viable counts (plating) but were usually a thousand fold higher. Bacterial numbers in the sediment were also higher at the control site (4.1×10^6 bacteria per gm dry weight sediment) as opposed to sediment under platform 296B (3.4×10^6 bacteria per gm). During the 1977-1978 and 1979-1980 sampling periods this trend was constant. During the 1978-1979 period, the pattern was inconsistent and was hidden by the combination of data from platform 288A and platform 296B which at the time were assumed to have similar rates of petroleum production discharge (brine).

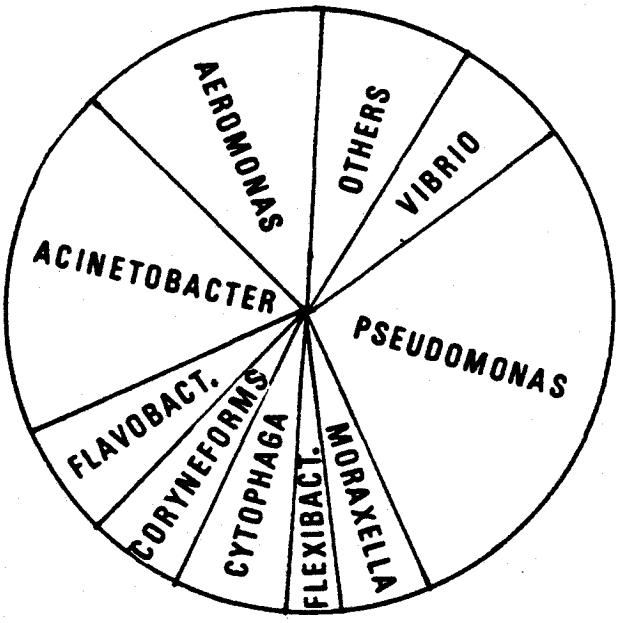
Throughout the latter two years of this study, platform 288A resembled closely the control site in bacterial numbers and biomass. This resemblance could be due to the cessation of routine discharge of brine from platform 288A in November, 1978. If only platform 296B was compared to the control site, differences in bacterial numbers were detectable by either the plating technique or the epifluorescence counts. The difference in numbers, however, were small (usually less than two fold) between the two sites and variation between sampling dates or different water depths at the same date and site were more dramatic (often 10 or 100 fold) than between sites.

A seasonal pattern in bacterial numbers was also seen. For example, in the 1978-1979 sampling period the highest bacterial numbers were found in the water of the February sampling cruise (e.g. 1123 bacteria per ml at platform 296B). These trends in bacterial numbers are discussed in detail elsewhere (Olsen and Sizemore, in press).

No taxonomic differences were noted during this study (see Fig. 1 and Fig. 2) in the bacterial population of the platform vs the control site in either the water column (Hollaway et al., 1980; Sizemore, 1979) or the sediment (Sizemore and Olsen, in press). The taxonomic diversity of both sites changed dramatically seasonally but the taxa and diversity were essentially the same for both sites.



PLATFORM SITE
296 B



CONTROL SITE

Fig. 1. A comparison of the major bacterial genera found at the two sampling sites on August 23, 1979. This sampling date had the highest taxon diversity of the study.

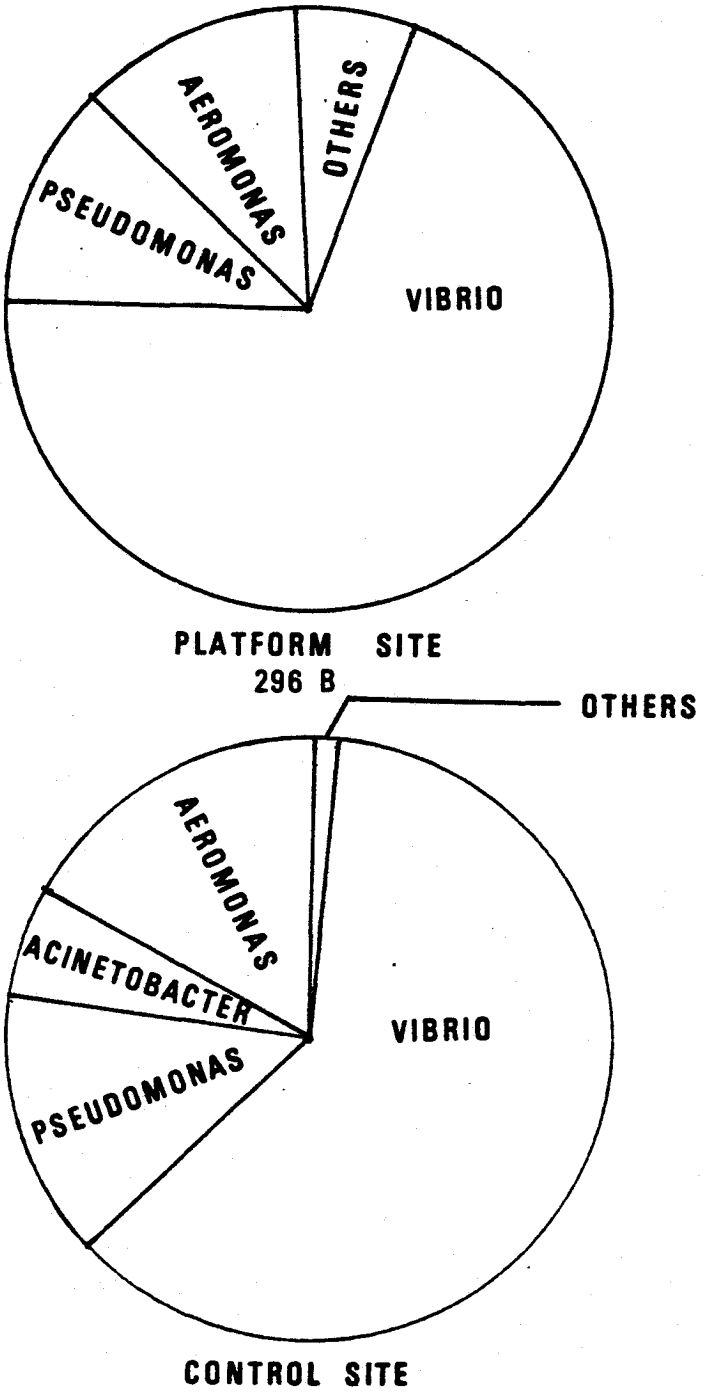


Fig. 2. A comparison of the major bacterial genera found at the two sampling sites on May 12, 1980. This sampling date had the lowest taxon diversity of the study.

The similarity between the platform site 296B and the control also held for the general physiological profile of the bacterial population (Hollaway et al., 1980).

The percentage of proteolytic, chitinoclastic, lipolytic and salt requiring bacteria were essentially identical for the platform site (296B) and the control site. Bacteria from the two sites were also compared for their ability to utilize two specific components of the discharge, oil and sulfur. The number of oil degrading bacteria and sulfur oxidizing bacteria was in general low at both sites. However, the platform site consistently contained a higher percentage of both types of bacteria than the control site (Fig. 3). Sulfate reducing bacteria were also found to be more prevalent in the sediment under the platform. This data suggests that the platform discharge has a very small hey detectable effect on the bacterial communities. Bacterial populations around the platform have adapted to the presence of the platform discharge products but this adaption has not required a dramatic change in the population.

Brine discharge is continuously being released from the "active" production platforms. At least three components are known to be in this discharge; sulfur, petroleum residue, and sporadic inclusion of biocides. The biocides are added to the brine discharge to limit bacterial growth within the tanks on the platform. The brine discharge was found to be inhibitory to the growth and functioning of some laboratory cultures. However, diluted brine discharge did not affect the growth of isolates from the platform area. Indeed, some strains grew more rapidly in the dilute discharge than the control medium. Full strength brine discharge did not support the growth of some platform strains but did not appear to be lethal to these strains. However, immediately below the platform, the suspended particulate material did not contain normal bacterial numbers (i.e. less than 1% of the total viable count). In all other water samples examined most (approximately 90%) bacteria were found to be attached to particles larger than 3 um. The lack of attached bacteria appears to be an artifact produced by particulate material released in the discharge. In the laboratory, platform isolates were able to attach to particles and could exhibit chemotaxis in dilute brine discharge. The lack of attached bacteria under the discharge point could be due to the effect of the high concentration of the discharge or an artifact of the composition of the discharge water itself. Biocide within the tank may kill the attached bacteria in the discharge before release. In the study no effect of biocide could be detected, however, there was some difficulty in

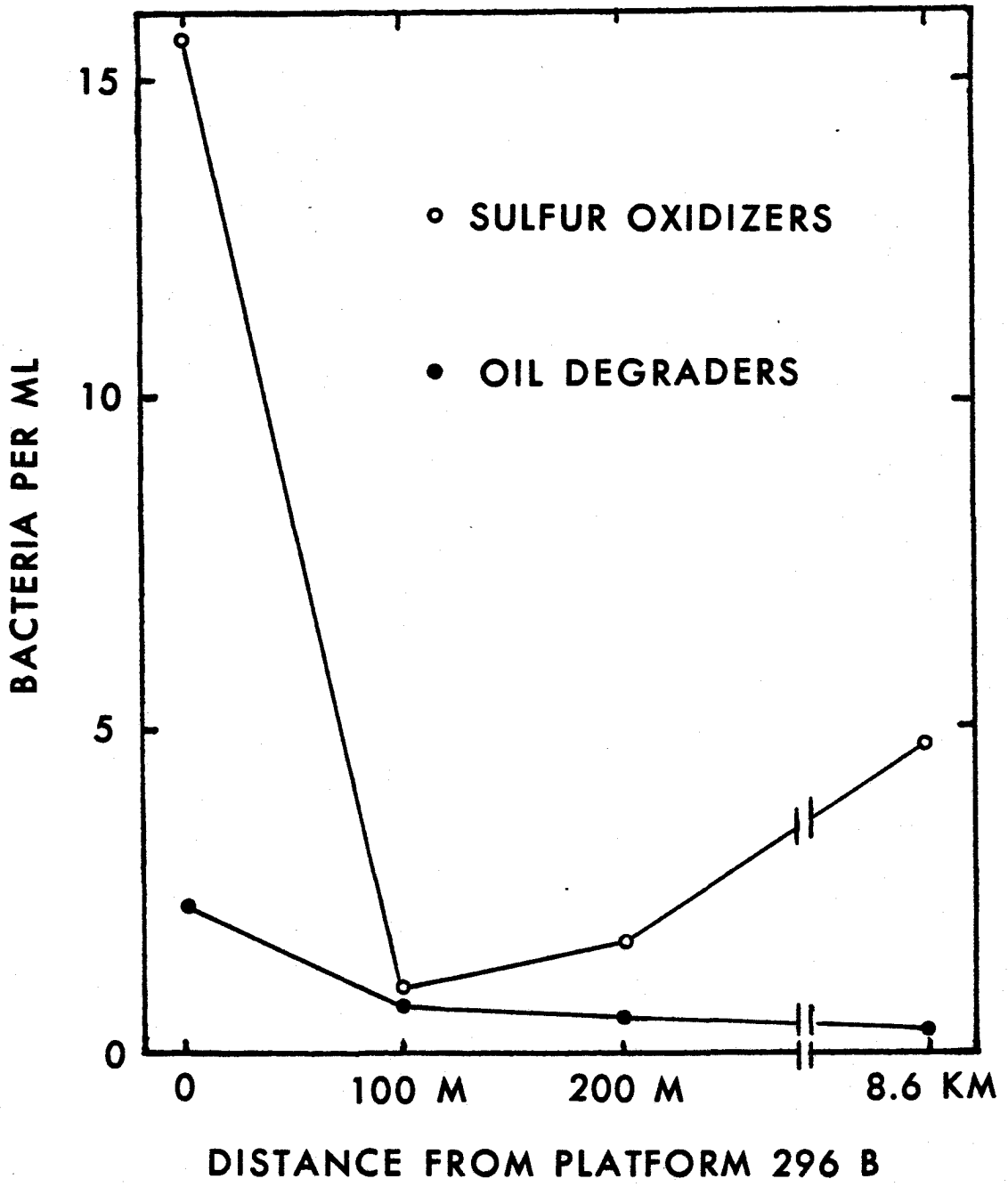


Fig. 3. Average number of oil degrading and sulfur oxidizing bacteria in the water column of 4 sampling sites collected during the period 8/78-8/79. The sample collected at a distance of 8.6 Km from the platform was in the control area.

obtaining fresh discharge samples known to contain biocides. A chemical examination of brine discharge which was thought to contain biocide showed no evidence of appreciable biocide levels (Middleditch et al., 1979). Thus the apparent lack of toxicity exhibited by brine discharge thought to contain biocide may be due to the absence or very low levels of biocide in the brine samples tested.

Two materials released in brine discharge, sulfur and hydrocarbons, were examined for their ability to serve as nutrients for bacterial growth. Sulfur was not utilized in detectable amounts in our experiments. Visual indication of sulfur oxidation was seen but gravimetric analysis did not detect sulfur loss. An examination of the taxonomic composition of the sulfur oxidizing bacteria suggests an explanation for this discovery. Obligate autotrophic sulfur oxidizing bacteria (i.e. genus Thiobacillus) were found in small numbers (less than 1% of the bacteria capable of attacking elemental sulfur) in the study area. Heterotrophic Pseudomonas sp. which can utilize sulfur as a supplemental nutrient (Tuttle et al., 1974) were more prevalent, comprising more than half of the sulfur oxidizing bacterial population. These strains preferentially utilize organic material for growth and would not be expected to utilize large quantities of sulfur. Sulfate reducers were common (32% of the bacterial population) in the sediment of the platform area but no attempt was made to measure their activity.

Petroleum hydrocarbons are continuously released in small quantities (Middleditch et al., 1979) from the production platforms. Measurements have been made of the potential of microorganisms from the platform area compared with strains from other sites to utilize different components of the discharge hydrocarbons. During the first two years of this study (1977-1979), only biodegradation of the n-alkane fraction of the hydrocarbons was studied. This portion of petroleum was readily utilized by a number of bacteria (Faw and Sizemore, manuscript in preparation-a). In general, mixed cultures prepared from water and sediment samples collected around the platform could utilize hydrocarbons (Faw and Sizemore, manuscript in preparation-a, Olsen and Sizemore, manuscript in preparation). The activity of these mixed cultures, combined with abiotic factors could result in loss of most of the n-alkane hydrocarbons within 7 to 28 days (Fig. 4). Individual pure cultures, usually belonging to the genus Pseudomonas, were also able to utilize n-alkanes. While most of the pure cultures did not utilize oil as quickly or thoroughly as mixed cultures, some isolates collected from around the platform appeared to be exceptional alkane utilizers. One strain isolated from the sediment under platform 296B was able to utilize the majority of n-alkanes in the study mixture in a 7 day period (Faw and Sizemore, manuscript in preparation-b).

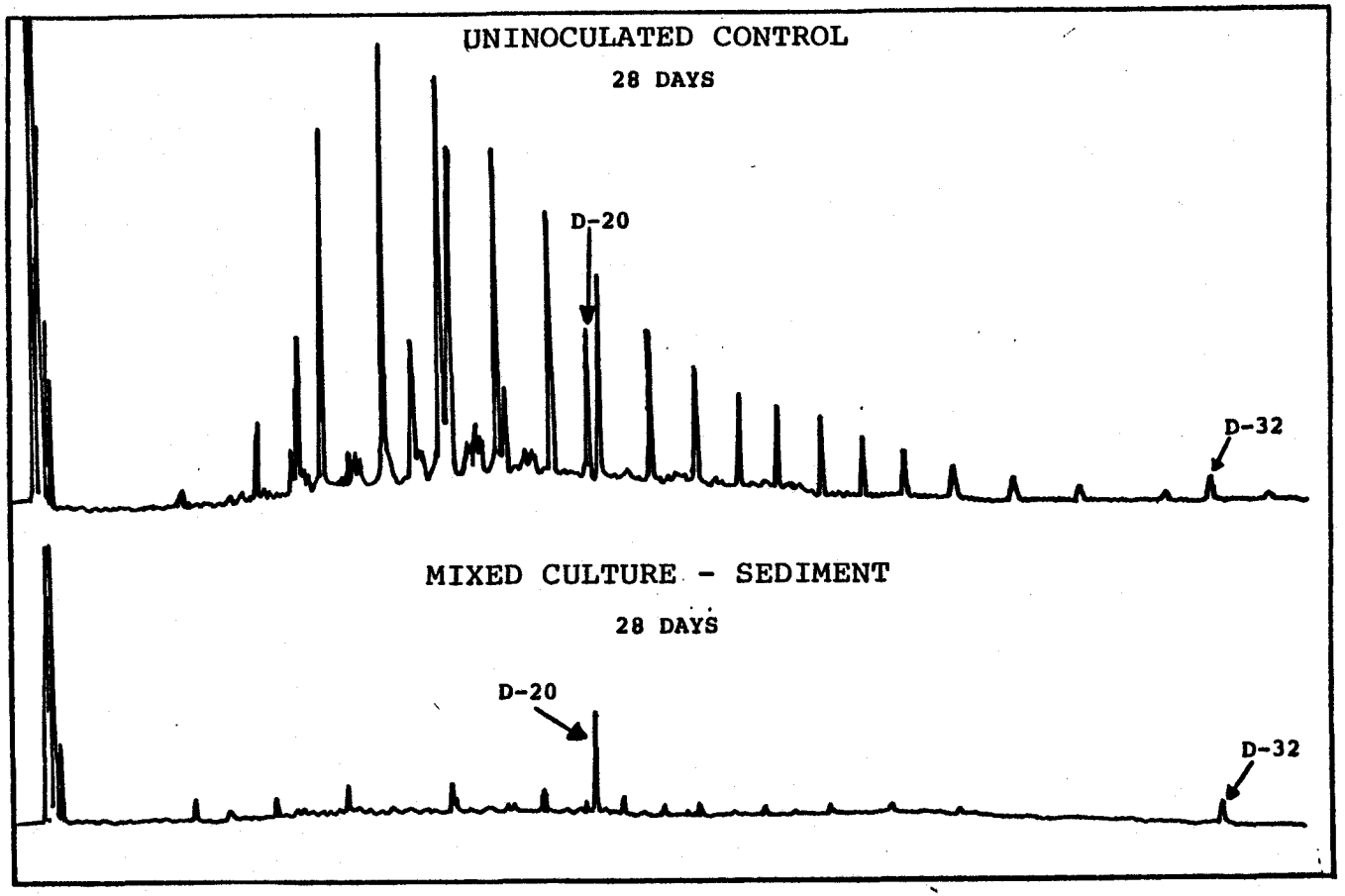


FIGURE 4. GAS-LIQUID CHROMATOGRAPHIC TRACING OF UNINOCULATED WEATHERED CONTROL (TOP) VS. BIODEGRADED SAMPLE (BOTTOM). D-20 AND D-32 ARE DEUTERATED INTERNAL STANDARDS ADDED AT A CONCENTRATION OF 2.5 PPM. (TAKEN FROM SIZEMORE AND OLSEN, IN PRESS)

Alkanes, however, are only a portion of the hydrocarbons found in petroleum. To study the biodegradation of other components of oil, a model or synthetic petroleum was used. This model petroleum contains n-alkanes but also included representative components of other fractions of crude oil (e.g. branched chain alkanes, aromatic hydrocarbons, polycyclic hydrocarbons, etc.). While this model petroleum does not approach the complexity of crude oil, its simplicity permits rapid gas-liquid chromatographic measures of each component. Therefore, utilizing the model petroleum, a number of bacterial cultures could be tested for biodegradation of a wide range of petroleum constituents in a brief period of time.

Mixed cultures prepared from sediment samples collected from around platform 296B were particularly effective in degrading the different classes of hydrocarbons in model petroleum. In general, aromatic compounds were degraded less rapidly than the aliphatic alkanes. Of the non-alkane hydrocarbons tested, cumene was the most readily degraded followed by napthalene, phenanthracene, 1,2 benzanthracene and perylene in respective order. Certain individual cultures were able to utilize some component of model petroleum almost exclusively. Figure 5 shows an oil field sediment culture which attacks napthalene.

In a separate study not sponsored by this contract, the genetic basis for adaptation to hydrocarbon utilization was studied (Hada and Sizemore, submitted for publication). Bacterial strains collected from the control site and platform site were examined for the presence of extrachromosomal plasmid DNA. The plasmid DNA represents an efficient way by which bacterial cultures adapt to new stresses or environments. The comparison of the two sites showed that strains from the oil field contained a higher percentage of plasmid containing strains than the control site. This higher incidence of plasmids may represent a mechanism by which the platform area bacteria adapt to the low level pollutants without exhibiting a large change in bacterial numbers or types. This type of adaptation represents a minimum of metabolic energy expenditures by the population but permits maximal genetic flexibility.

During the course of this study, Work Unit 2.3.5/2.3.8 reported apparent increased incidence of diseased fish around the platforms. A modest effort was made to examine the predominant bacterial flora of the diseased and healthy fish from the study area in an attempt to detect the presence of bacterial pathogens. A number of different members of the genera Vibrio and Aeromonas were found associated with diseased and apparently healthy fish. One bacterium, Aeromonas hydrophilia, a known fish pathogen was found associated with a few diseased fish. It is impossible to conclude from the small number of diseased fish examined that Aeromonas hydrophila is the casual agent. However,

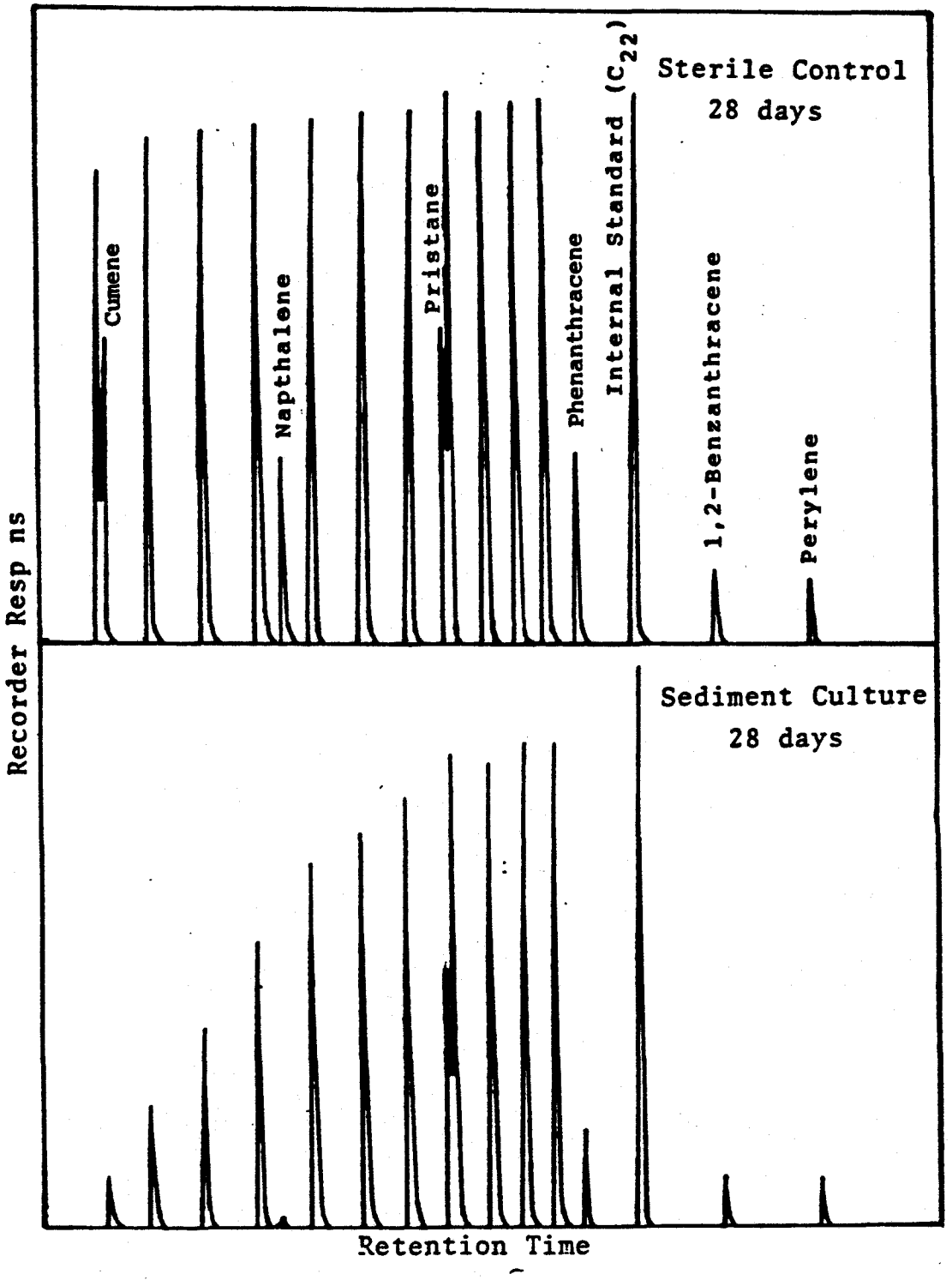


Fig. 5. Gas-liquid chromatographic tracing of 1% model petroleum without (top) and with (bottom) inoculum of a mixed sediment culture derived from Buccaneer sediment. Unlabeled peaks are straight chained alkanes from C_{10}-C_{20}. Peaks in the inoculated culture which are smaller than the control have been biodegraded.

Aeromonas hydrophilia has been linked with fish diseases normally triggered by polluted waters (Mitchell, 1980). This discovery is preliminary and an additional detailed study would be helpful.

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