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Studies on the Distribution, Abundance, and Activities of Heterotrophic and Petroleum Degrading Bacteria from Middle Atlantic Continental Shelf Waters and Sediments

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Special Report in Applied Marine Science
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CHAPTER 11

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CHAPTER 11

BACTERIOLOGY

H. I. Kator

INTRODUCTION

Degradation of Petroleum by Marine Bacteria

A significant role of bacteria on the continental shelf is that of degradation and regeneration of nutrients essential to biological productivity (Liston 1968). As biogenic hydrocarbons represent a natural substrate of marine bacteria (ca. 10^6 metric tons/yr. produced by phytoplankton alone), it is not surprising to find that bacterial populations can degrade hydrocarbons in petroleum. Unfortunately, petroleum is a substrate of such vast complexity and compositional variation (Kallio 1976), that a complete understanding of the microbiological fate of its components is not yet obtainable.

Despite the awesome task of unraveling the compositional particulars of bacterial degradation, marine bacterial populations appear to respond to petroleum spillage in a simple and reproducible fashion; elevated levels of petroleum or related compounds have been reported to result in elevated levels of bacteria which degrade hydrocarbons (ZoBell 1969; Atlas and Bartha 1973b; Walker and Colwell 1973; Seki 1976; Kator and Herwig 1977; Oppenheimer, Gunkel, and Gassman 1977; Walker and Colwell 1977). This increase in level also appears as an increase in value of the ratio of petroleum degrading bacteria to "total" heterotrophic bacteria (Walker and Colwell 1976; Kator and Herwig 1977).

Therefore, an important aspect of this study was to determine the distribution and populations of petroleum degrading and "total" heterotrophic bacteria in sediments and waters off the Middle Atlantic continental shelf. This region is now the site of proposed exploratory activities related to petroleum deposits. Assessment of bacterial populations prior to production activities would now provide "baseline" data on the distribution and levels of petroleum degrading bacteria.

While the ratio of petroleum degrading to "total" heterotrophs may be a useful indicator of petroleum pollution, this ratio does not yield information on the degradative potential of bacterial populations toward petroleum. To assess the degradative "potential" of bacterial populations from surface waters and sediments, a series of closed flask degradation experiments were performed utilizing ambient surface water and sediment-seawater homogenates as inocula. South Louisiana crude oil was added to these inocula under inorganically enriched and non-enriched regimes.

Changes in populations of "total" heterotrophs and petroleum degrading bacteria were determined over an extended incubation period at temperatures similar to the environment. Extent of degradation was measured by gravimetry of a chromatographically defined crude oil fraction (saturated hydrocarbons) and gas chromatography to determine patterns of n-paraffin

utilization. While cognizant of the uses and limitations inherent in the application of laboratory data to the environment, such studies can provide a measure of the relative "potential" for degradation by a given inocula.

A concept designated as "continuous" dilution was developed during this study as an ancillary methodology to closed flask or batch type degradation experiments. Essentially, a volume of sea water, contaminated with a small amount of petroleum, was continually diluted with fresh, ambient sea water. This technique attempts to simulate an oil slick which in the environment "sees" an increasing volume of sea water containing fresh inocula and nutrients.

In addition to experiments and field work dealing with the degradation of petroleum and the distribution and abundance of petroleum degrading bacteria, the following studies were performed to provide additional information on the effects of petroleum on bacterial growth and metabolism.

Chitin Degradation in the Presence of Petroleum

Chitin is one of the most ubiquitous biopolymers found in nature and is produced in copious quantities within the marine environment. Johnstone (1908) calculated that copepods alone can produce several billion tons of chitin annually. As this material does not accumulate in marine sediments, mineralization processes involving marine bacteria are effective in recycling chitin carbon and nitrogen in the marine ecosystem (ZoBell and Rittenberg 1938; Hood 1973).

The effects of petroleum on microbial degradation of chitin are unknown. Considering the importance of chitin as a reservoir of carbon and nitrogen in the environment, the increasing incidence of oil spillage in estuarine and coastal waters provides sufficient impetus to examine the effect(s) of oil on chitin degradation. If crude oil in a given environment becomes a competitive carbon source, or is directly toxic to chitinoclasts, it is possible that a reduction in chitin degradation would occur. Alternately, petroleum could either have no effect or actually enhance the activity of chitinoclastic bacteria, through development of a larger heterotrophic population. ZoBell and Rittenberg (1938) observed that although some bacterial cultures grew on chitin as a sole source of carbon and nitrogen, other cultures required additional forms of carbon or carbon and nitrogen for development of chitinolytic activity. Additionally, complex nutrients such as peptone have been shown to enhance and stimulate chitin decomposition (Chan 1970).

A series of preliminary experiments was performed to measure changes in populations of chitinoclastic marine bacteria and chitin degradation by mixed cultures of chitinolytic marine bacteria grown in the presence of South Louisiana crude oil.

Growth of Pure Cultures in the Presence of Petroleum

Marine bacteria, regarded primarily as oil degraders, have received little attention related to adverse effects of oil on their growth despite an abundance of literature dealing with the toxicity of petroleum to organ-

isms. Mitchell et al. (1972) reported that in the presence of low concentrations of aromatic hydrocarbons or petroleum, marine bacterial chemotactic responses toward glucose were totally repressed. In a subsequent study (Young and Mitchell 1973), it was shown that selected marine bacteria responded to sub-lethal concentrations of aromatic hydrocarbons by negative chemotaxis. Cobet and Guard (1973) evaluated the effects of a variety of petroleum hydrocarbons on isolates from a polluted beach and found that only a small percentage of isolates exposed were rendered non-viable after a 48 hour exposure period. Crude and fuel oils have been considered toxic for various groups of lytic estuarine bacteria (Walker et al. 1975).

Atlas (1975) reported that petroleums, particularly those of low specific gravity, contained toxic components which inhibited microbial degradation of these petroleums at low temperature (10°C). Aromatic hydrocarbons have been shown to reduce the growth rates of selected pure cultures of marine bacteria (Calder and Lader 1976). Such studies, along with various investigations on the toxic effects of petroleum hydrocarbons on phytoplankton (Pulich et al. 1974; Batterton et al. 1976), have indicated that the toxicity of petroleum can be associated with different fractions of crude or refined oils.

The purpose of this investigation was to determine the effects of unweathered and artificially weathered South Louisiana crude oil and soluble fraction thereof on the growth of pure cultures of marine bacteria in a dilute peptone-yeast extract seawater broth. Marine bacterial isolates were obtained from microlayer, water (1 m) and sediment samples collected from the Middle Atlantic continental shelf (BLM01B - 04B).

METHODS AND MATERIALS

Sampling

Microlayer

Surface microlayer samples were obtained under favorable sea conditions from selected stations (Figure 11-1). All samples were collected upwind of the research vessel using a self-propelled inflatable rubber boat. Replicate microlayer samples were obtained using a sterile screen (Nitex monofilament nylon, 6.5 mesh/cm) held in either a wooden or stainless steel frame to which a handle was attached. Alternate sampling methodologies allowing sterile techniques (filter paper, polycarbonate filters, etc.) evaluated under actual field conditions were inferior in terms of deployment/retrieval to the screen method.

Microlayer samples were collected in replicate by rapidly plunging the screen vertically through the water surface and smoothly raising the screen into the air parallel to the interface. After allowing water coating the frame to drain, the screen sample was collected in a sterile, calibrated test tube using a sterile funnel/support stand configuration and the volume recorded. Mean sample volume for the 40 samples collected during all cruises using the same screen was 11.6$\pm$ 1.8 ml.

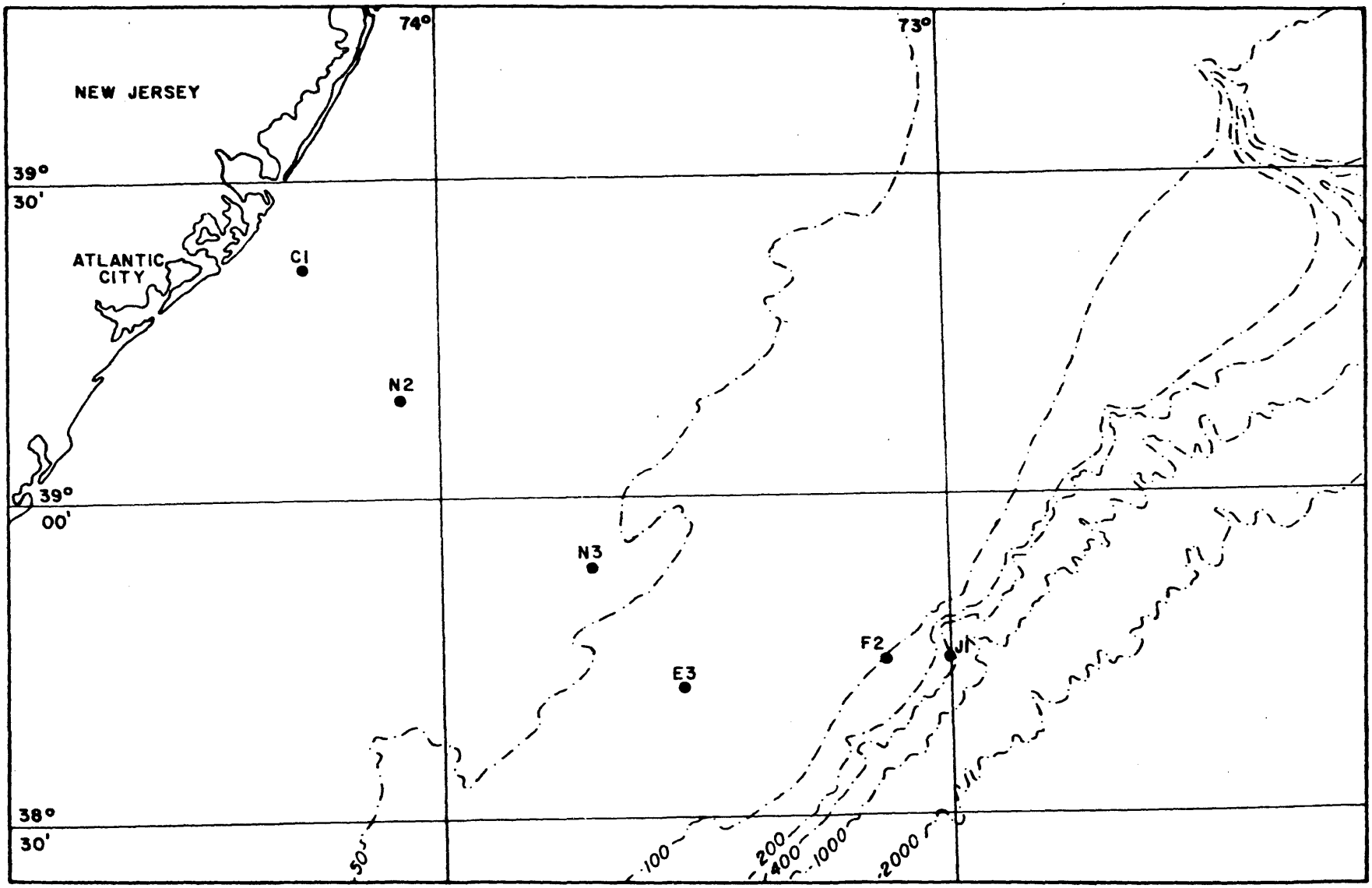


Figure 11-1. BLM 1st year - Bacteriology Surface and Water Sample Stations

Surface Water (1m)

Surface water samples were collected quarterly at selected stations (Figure 11-1) at 1 m depth using Niskin sterile bag samplers and weighted cable. Precautions were taken to prevent contamination by the vessel's bilges etc. Surface temperatures were measured at the time of sampling. Both microlayer and 1 m water samples were processed for enumeration of heterotrophic and petroleum degrading marine bacteria immediately on return to the vessel. Appropriate dilutions or concentrations on membrane filters were provided prior to enumeration.

Sediments

Sediment samples were collected quarterly at cluster stations (Figure 11-2) using a Smith-McIntyre grab. Additional sediment stations (along transects G, H, I, J, K, and L) were sampled only during the summer and winter seasons. Undisturbed central areas of the grab were sampled to obtain an uncontaminated sample using sterile "minicorers", plastic syringes with the luer end removed. "Mini-corers" were pushed into the sediment sample to a depth of about 5-6 cm yielding a sample volume of about 10 ml. Four replicate samples were obtained at each station, two for determination of heterotrophic and petroleum degrading bacterial populations and two for the determination of the ratio of sediment dry to wet weight. Sediment temperatures were also measured at this time by mercury thermometer and recorded.

Sediment samples for dry weight determinations were immediately frozen for processing at a later date. Replicate "mini-cores" for enumeration were then processed as follows. After weighing the "mini-corer" the sediment sample was extruded into 90 ml of sterile sea water in a Waring blender and homogenized (Stevenson, Millwood, and Hebeler 1974) for one minute. One ml volumes of this homogenate (1:10 dilution of sediment v/v) were diluted by appropriate powers of ten and used to inoculate media for enumeration of heterotrophic and petroleum degrading marine bacteria.

Enumeration

Inocula from dilution blanks or concentrated seawater samples on membrane filters (1m and microlayer), sediments, or experimental degradation studies were enumerated for "total" heterotrophic bacteria using a three tube MPN technique (Lewin 1974) in a heterotrophic medium (HM) modified after ZoBell's marine agar (2216). This medium consisted of 1 g/l peptone, 0.5 g/l yeast extract, 0.01 g/l ferric citrate, 0.1 g/l sodium glycerol phosphate, and 1000 ml of aged sea water. The final pH of this medium after autoclaving at 121°C for 15 minutes was 7.8. Petroleum degrading bacteria were enumerated using a three tube MPN technique (Gunkel 1968) employing a minimal salts enriched sea water (ESWB) containing 1 g/l $(NH_4)_2SO_4$ and 0.1 g/l K_2HPO_4 in 1000 ml aged sea water to which was added following inoculation, approximately 1% sterile unweathered South Louisiana crude oil as the sole added carbon source. Louisiana crude oil was sterilized by filtration through a polycarbonate membrane, and minimal salts enriched sea water (ESWB) was sterilized by autoclaving at 121°C for 15 minutes yielding a final pH of 7.8.

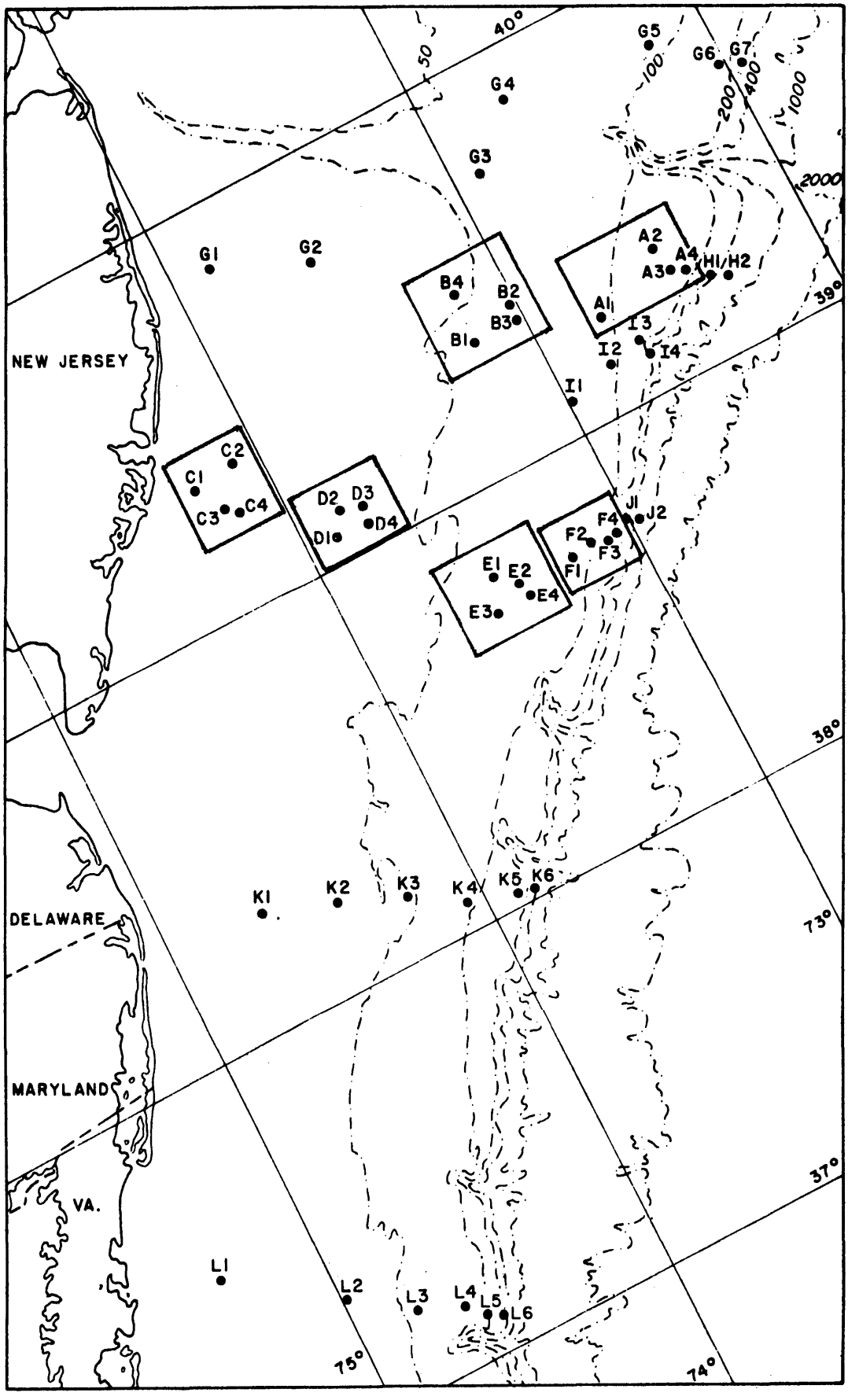


Figure 11-2. ☐ -Cluster stations sampled quarterly; ● - Stations sampled twice yearly.

HM enumeration tubes were incubated at 20-22°C for two weeks and read at weekly intervals. ESWB tubes were incubated at the same temperature on a rotary shaker (140 rpm) for one month and read at bi-weekly intervals. HM tubes were scored positive when turbid; ESWB tubes were scored positive when turbid, if the oil showed obvious signs of degradation with associated cellular debris, or a combination of both. The highest positive dilutions were retained for taxonomic analysis. MPN values were calculated using standard tables (APHA 1975) for three tube MPN distributions. Counts were expressed as bacterial units/ml sea water or bacterial units/g dry sediment. Sediment counts were corrected for dry weight, and a volume/weight conversion of the original 10 ml sediment sample was made.

Isolation of Chitinoclastic and Cellulytic Bacteria

Chitinoclastic bacteria were isolated by spread plating appropriate dilutions of water samples (1 m) and sediment homogenates on chitin containing bi-layer plate medium. This medium consisted of a lower layer of HM agar (ca. 20 ml) and a 10 ml chitin-agar overlayer (composed of 30 g/l "dissolved" chitin, 0.5 g/l yeast extract, 15 g/l agar, and 1000 ml aged sea water). Chitin (Calbiochem, unspecified purity from crab-shrimp) was ball milled at 4°C for 48 hours (Hood 1973), "dissolved" in 50% H_2SO_4, precipitated by the addition of large volumes of distilled water, and neutralized to a pH of 7.0. Chitinolytic bacterial colonies produced clear zones in the surrounding chitin agar.

Water samples and sediment homogenates were inoculated into a cellulose medium consisting of 1.0 g/l yeast extract, 1.0 g/l $(NH_4)_2SO_4$, 0.1 g/l K_2HPO_4, and 1000 ml aged sea water. Strips of cellulose (Whatman # 1 paper) extending well above the liquid surface were sterilized in this broth. Positive reactions were generally observed at the air-liquid interface as weakening and breakage of the paper or reduction of the paper to a gelatinous mass.

Taxonomic Analysis of Bacterial Isolates

Isolates were obtained from selected microlayer, water, and sediment stations using the highest positive dilutions of HM and ESWB MPN tubes. Three to five MPN tubes from dilutions were streaked on HM agar plates, the numerically dominant isolates chosen, subcultured to ensure purity, and placed on coded HM agar slants.

Isolates for taxonomic analysis were freshly streaked on HM agar plates to describe colony characteristics. Wet mounts of log phase HM broth cultures (generally 24 hour cultures) were examined by phase contrast microscopy for motility, size, shape, and cell arrangement. Gram staining was performed using the modification of Hucker (Hucker and Conn 1923).

Biochemical tests relevant to the classification scheme of Shewan (1963) were performed as follows. Oxidase was determined by the method of Kovacs (Kovacs 1956) using 48 hour HM agar slants or plates. Isolates were tested for glucose assimilation mode using Liefson's MOF medium (Liefson 1963). Closed or fermentative tubes were first sealed with Vaspar (50% v/v, paraffin oil and petrolatum) and then over-layered with

sterile paraffin oil. Without Vaspar, false positive fermentative reactions were more than infrequently observed. Growth, gas production, acid, alkaline, or "no reaction" were recorded at 1, 2, 4, 7, and 14 day intervals.

Additional tests such as catalase assay (1 drop of 15% H_2O_2 placed directly on 48 hour HM agar colonies), chitinase assay (spotting a loopful of a cell suspension on a chitin bi-layer plate) followed by examination of the resultant colonies for surrounding zones of hydrolysis or clearing, and the ability of isolates to degrade petroleum (inoculation into ESWB) were performed.

Antibiotic sensitivity profiles were determined using Sensi-Discs (BBL) for all pure isolates on freshly spread HM agar plates. Chloramphenicol, tetracyline, novobiocin, bacitracin, penicillin, sulfadiazine, colymycin, and 0/129 pteridine were used. HM agar plates were read at 1, 2, 4, and 7 day intervals for inhibitory response and size of inhibition zone.

Petroleum Degradation Experiments

Petroleum degradation experiments were performed using samples of sea water and sediment as inocula. Surface water (1m) stations selected for sampling during each season were located approximately along a west to east transect originating from Southern New Jersey (Figure 11-1). Samples were collected at a depth of 1 m using Niskin sterile bag samplers. Water collected at each station was used to fill sterile 250 ml Erlenmeyer flasks with 100 ml inocula. At the time of inoculation, water samples were also enumerated for petroleum degrading and heterotrophic marine bacteria. Replicate flasks for each station were treated as follows: one series was immediately autoclaved providing sterile controls, a second series received inorganic nutrient enrichment (1 mg $(NH_4)_2SO_4$ and 0.1 mg K_2HPO_4 as a sterile solution per each flask), and a final series was neither enriched nor autoclaved. All three series of replicate flasks then received 100 µl of membrane sterilized South Louisiana crude oil. During BLM cruise Ø4B an additional control in the form of an inoculated, non-enriched, oil-free flask was included to compensate for surface growth effects.

Sediment inocula were provided from sediment homogenates (Ø4B) used for enumeration of heterotrophic and petroleum degrading marine bacteria at selected benthic stations. Stations selected from the C, D, and F clusters (Figure 11-2) were used for sediment inocula. One ml volumes of the respective homogenates were added to a series of replicate 250 ml Erlenmeyer flasks containing 100 ml of sterile sea water. Experimental treatment of replicate flasks was the same as described for water inocula. Flasks were incubated on a rotary shaker (120 rpm) at average ambient water or sediment temperatures.

At selected intervals a flask from each series of treatments (i.e., sterile control, nutrient enriched, and non-enriched) was harvested. During Ø4B the oil free control was enumerated and replaced in the incubator. Flasks were described as to the condition of the oil, turbidity, and other evidence for bacterial degradation. Non-enriched, enriched, and oil-free flasks were enumerated after swirling using a three tube MPN technique for heterotrophs (Lewin 1974) and petroleum degrading bacteria

(Gunkel 1968). After enumeration, growth in enriched and non-enriched flasks was terminated by addition of 10 ml glass-distilled methylene chloride and the flasks sealed to prevent evaporation. Sterile control flasks were not enumerated but were tightly sealed following addition of 10 ml methylene chloride.

Residual crude oil was extracted from flasks following acidification by addition of 4 drops concentrated HCl and 25 ml methylene chloride. Flask contents were transferred to clean solvent rinsed separatory funnels, shaken, and after phase separation solvent phases were transferred to a second separatory funnel. Flasks were then rinsed with 25 ml of methylene chloride, and this was added to the aqueous phase in the first separatory funnel. Following phase separation, the organic solvent phases were combined and washed with 50 ml of acidified (pH 4.0) distilled water containing 3% NaCl. The washed solvent phase was dried by passage through pre-washed anhydrous sodium sulfate contained in a glass funnel lined with Whatman #54 hardened paper and collected in a tared flask.

Methylene chloride was removed by aspiration at 40°C using a water bath and monitoring the instant of solvent removal with a McLeod gauge. Residual crude oil extracts were then transferred to vials in 5 ml methylene chloride and stored at 4°C pending further analysis.

Crude oil extracts were fractionated into a saturated paraffin and an aromatic fraction using silica gel column chromatography. Silica gel (60-200 mesh), activated for at least 16 hours at 235°C, was packed in 1 cm diameter glass columns to a height of 17.5 cm and washes with four 10 ml portions of hexane. Extracts warmed to room temperature were placed on the column in 5 ml of hexane. After addition of 8 ml of hexane to the column, the first 5 ml of hexane eluting were discarded and the next 13 ml of hexane eluate containing saturated hydrocarbons collected (H2 fraction). Thirty-two ml of a hexane-benzene solvent mixture (60/40, v/v) were then added to the column. An initial 7 ml hexane-benzene eluate was discarded, and a second hexane-benzene eluate (HB 2) of 25 ml containing aromatic hydrocarbons was collected.

The H2 fractions were transferred to tared flasks and the solvent removed as previously described. H2 eluate residues were weighed and transferred in 0.4 ml hexane to vials for storage at -5°C. Appropriate procedural controls were routinely provided to check on the consistency of separations.

Gas chromatographic analysis of residual oil fractions was performed using a Tracor 560 gas chromatograph equipped with dual flame detectors. One microliter samples were injected on-column (6 ft x 4 mm i.d. glass columns packed with 3% OV-1 on Chromosorb HP 80/100 mesh) using an initial hold for 3 minutes at 70°C followed by programmed temperature increase to 300°C at 8°/min with a final hold at 300°C for 15 minutes. Injector and detector temperatures were set at 200°C and 325°C respectively, and carrier gas (N_2) flow was adjusted to 30 ml/min.

Chromatograms were evaluated for indications of degradation reflected as loss of specific normal saturated paraffins (nC-12 to nC-27, inclusive). Identification of n-paraffins was by comparison with retention times of

authentic standards. Changes in peak heights were expressed as the ratio of each n-paraffin to the naturally occurring isoprenoid hydrocarbon pristane (2, 6, 10, 14 -tetramethylpentadecane). Use of this compound as an internal standard during bacterial utilization of n-paraffins has been demonstrated (Kator 1972). Peak heights were measured to the shoulders of each n-paraffin at the unresolved baseline in a consistent manner as described by McNair and Bonelli (1968). A conservative estimate of the absolute concentration of pristane/unit weight crude oil was determined by the technique of standard addition (ibid.). Losses in non-enriched and enriched experimental flasks were compared to sterile controls to compensate for substrate evaporation.

Continuous Dilution Degradation Experiments

On selected cruises (BLM Ø2B and Ø3B) prototype experiments were performed designed to simulate weathering of oil in an "open" system under ambient nutrient conditions. Large sterile carboys (11 liters) were inoculated with 2 liters of sea water. Two ml of sterile unweathered South Louisiana crude oil were added to one bottle; the second bottle served as a non-oiled control. Populations of heterotrophic and petroleum degrading marine bacteria were determined at this time in the inoculum water.

Both carboys were covered and incubated at ambient temperature (20-22°C) with continuous stirring using Teflon coated stir bars. During a 10 day period following inoculation, 1 liter volumes of fresh sea water, collected along the surface (1m) station transect (Figure 11-1) were added to each carboy on a daily basis. At selected intervals, populations of heterotrophic and petroleum degrading bacteria were enumerated, and 250 ml volumes (or larger) of sea water were removed for ATP analysis (Strickland and Parsons 1972).

Oil Concentration Experiments

An experiment was performed to evaluate the effects of various concentrations of unweathered Louisiana crude oil on the levels and ratio of petroleum degrading to heterotrophic bacteria. Sterile 250 ml Erlenmeyer flasks were each inoculated with 100 ml of sea water collected at Station I1 (BLM Ø4B) and the following treatment conditions applied (one treatment condition/flask):

Treatment

A. 100 ml sea water, minus crude oil, minus inorganic nutrient amendment[1]
B. 100 ml sea water, 0.1% crude oil, minus inorganic nutrient amendment
C. 100 ml sea water, 0.1% crude oil, plus inorganic nutrient amendment
D. 100 ml sea water, 0.01% crude oil, minus inorganic nutrient amendment

[1] As described under Petroleum Degradation Experiments.

E. 100 ml sea water, 0.01% crude oil, plus inorganic nutrient amendment
F. 100 ml sea water, 0.001% crude oil, minus inorganic nutrient amendment
G. 100 ml sea water, 0.001% crude oil, plus inorganic nutrient

All flasks were sealed with foil and incubated on a rotary shaker (120 rpm) at an average seasonal temperature (21°C). At selected time intervals flasks were harvested, enumerated for petroleum degraders and heterotrophs, and the ratio of the populations calculated.

Chitin-Petroleum Degradation Studies

Dominant chitinolytic bacteria isolated from water or sediment samples during each cruise were combined to form, respectively, one mixed sediment and one mixed water culture for each season. These mixed cultures were maintained on a chitin-peptone-yeast extract seawater broth (CPY-broth) composed of 0.5 g/l peptone, 0.5 g/l yeast extract, 0.1 g/l ferric citrate, 0.1 g/l sodium glycerol phosphate, and 1000 ml aged sea water. Chitin (Calbiochem, Inc., undefined grade of shrimp-crab chitin), sieved to a size range of 250-500μ, was added to this medium to yield a final concentration of 2 g/l. CPY-broth, autoclaved at 121°C for 15 minutes, had a final pH of 7.8.

Mixed culture inocula for chitin-petroleum degradation studies were prepared from 18 hour CPY-broth cultures by centrifugation of cells and resuspension in sea water to an optical density of 0.1 (625nm). One ml volumes of washed suspensions were used to inoculate 150 ml bottles containing 50 ml of CPY-broth. Three series of replicate bottles for each mixed culture were treated as follows: one series received the mixed culture inoculum plus 50 μl sterile Louisiana crude oil (chitin + oil), the second received the mixed culture inoculum but no petroleum (chitin - oil), and the third received only 50 μl sterile Louisiana crude oil (sterile control). All bottles were sealed with gauze-cotton plugs to allow for aerobic growth. Initial levels of each seasonal water or sediment inoculum were enumerated for petroleum degrading, heterotrophic, and chitinoclastic marine bacteria as described in previous sections.

Following inoculation all bottles were incubated in the dark on a rotary shaker (120 rpm) at ambient temperature (20-22°C). At intervals corresponding to 2, 5, and 10 weeks, one bottle from each of three treatment conditions (i.e. chitin + oil, chitin - oil, sterile control) for each of the seasonal water and sediment cultures was randomly selected and inoculated bottles enumerated for the bacterial groups mentioned. Methylene chloride was then added to each bottle to terminate bacterial growth.

Recovery of residual chitin and crude oil was achieved by vacuum filtration of each bottle onto tared Whatman #54 hardened filter paper pre-washed with methylene chloride. Buchner funnels and filter flasks were also pre-washed with this solvent prior to use. Residual oil and filtered material were rinsed from the bottles using excess methylene chloride. The methylene chloride filtrate was held for fractionation and analysis by gas chromatography as previously described. Residual chitin was dried

in the presence of desiccant, allowed to equilibrate to weighing room moisture content, and weighed. Weight losses were expressed as a percentage of the uninoculated sterile controls. Observation of the filtered particles by low power microscopy was performed after weighing by rendering a portion of the filter transparent with paraffin oil.

Pure Culture Growth Experiments

Selected bacterial isolates obtained from enumeration of heterotrophic or petroleum degrading bacteria in microlayer, water (1 m), and sediment samples were utilized to examine the effects of petroleum on growth in a dilute nutrient broth. Isolates were maintained on HM agar and were passed three times in a dilute basal growth medium (BGM) prior to growth experiments. BGM consisted of 0.006% yeast extract and 0.02% peptone in aged Whatman filtered sea water diluted with glass distilled water to yield a salinity of 26 ppt. After autoclaving the basal medium at 121°C for 15 minutes, membrane sterilized glucose was added to obtain a concentration of 0.02% and the pH adjusted to 7.8 - 8.0 (if required).

Petroleum was added to BGM as unweathered South Louisiana crude oil, artificially "weathered" South Louisiana crude oil, and a water soluble fraction thereof. South Louisiana crude oil was selected for these growth experiments since there is no prior knowledge of the properties of yet undiscovered Middle Atlantic continental shelf crude oils and because it is being used as the enumeration substrate for petroleum degrading bacteria in BLM and American Petroleum Institute sponsored field studies performed by this laboratory.

"Weathered" crude oil and a water soluble fraction were prepared by addition of 20 ml of unweathered South Louisiana crude to one liter of aged sea water (26 ppt) in a glass carboy containing a Teflon stirbar. The bottle was left unsealed in the dark and the contents slowly stirred to avoid breakup of the oil layer for 48 hours at 20°C. After cessation of stirring for 10 minutes a soluble fraction was collected by draining the water beneath the quiescent oil layer. Residual "weathered" crude was then collected. All petroleum substrates were sterilized prior to use by membrane filtration (Millipore Micro-Syringe containing a Nuclepore membrane (0.4 microns) and prefilter). Nalgene membrane filtration units (0.45 microns) were used to sterilize the water soluble fraction.

Immediately prior to growth experiments, isolates were inoculated into BGM and incubated approximately 12-18 hours at 20°C. Cells were then harvested by centrifugation prior to resuspension in sea water to an absorbance of 0.1 (625nm). These suspensions were diluted 100 times and 0.1 ml of this suspension used to inoculate each of four tubes containing 5 ml of BGM and respectively: unweathered South Louisiana crude oil (ca. 1%, w/v), weathered South Louisiana crude oil (ca. 1%, w/v), the soluble fraction of South Louisiana crude oil (1%, v/v), and no crude oil (control). Cultures were incubated at 20°C on a laboratory shaker (50 rpm) at a 10° angle to the horizontal or on a New Brunswick Tissue Culture Rollerdrum (10-11 rpm). Growth was measured turbidimetrically at 2-4 hour intervals or longer (for slow growers) during the log phase of growth. Measurements were terminated when growth ceased in all tubes. Replicate experiments were occasionally performed to check on the reproducibility of the results

as well as observation by phase contrast microscopy to determine if variations in cell size due to crude oil occurred. As estimation of the standard error for the turbidometric assay was also determined for several isolates.

Data were plotted on semilog paper as time versus absorbance. Parameters routinely noted were growth yield, growth rate, and "lag" time. Of these "lag" time proved most useful in quantitating the effect(s) of petroleum on growth. "Lag" time was calculated by extending a straight line along the exponential portion of the growth curve to an absorbance value of 0.1. From this intersection a perpendicular was dropped to the time axis to determine the "lag" time required to reach an absorbance of 0.1. Growth experiments were performed on 269 isolates from water and sediment samples of which ca. 94% were subjected to taxonomic evaluation.

RESULTS

Distribution and Abundance of Heterotrophic and Petroleum Degrading Bacteria

Sediments

Petroleum degrading and heterotrophic marine bacteria were consistently isolated from Middle Atlantic continental shelf sediments. Typically, the levels of heterotrophic bacteria in sediments were ca. 2-3 log units greater than petroleum degrading bacteria. Bacterial data for all stations and seasons are found in Tables 11-1 through 11-4. Mean levels for sediments sampled quarterly are shown for each season in Table 11-5. Sediment stations were grouped into inner shelf (depth <50m), outer shelf (depth >50m <100m), and shelf break (depth >100m) for this table and other data presentations.

Table 11-5. Means of heterotrophic (HET) and petroleum degrading (HC) bacterial counts by season for inner shelf (depth < 50 m), outer shelf (depth > 50 < 100 m), and shelf break (depth > 100 m) for sediment stations (BLMØ1B-Ø4B).

Area	Bacterial Type	Season			
		Fall	Winter	Spring	Summer
Inner Shelf	HC	3.1±0.6*	2.3±0.6	2.5±1.1	1.8±1.0
	HET	5.5±0.4	5.4±0.3	6.2±0.5	6.0±0.3
	HC/HET	-2.4±0.8	-3.1±0.7	-3.7±1.0	-4.2±0.9
Outer Shelf	HC	1.9±0.5	2.0±0.4	1.6±0.4	1.8±0.6
	HET	5.2±0.3	5.4±0.5	5.6±0.4	5.8±0.3
	HC/HET	-3.3±0.3	-3.4±0.6	-4.0±0.4	-4.0±1.0
Shelf Break	HC	2.3±0.5	2.1±0.6	1.8±0.6	2.3±0.8
	HET	4.9±0.3	5.8±0.5	5.5±0.3	5.3±0.4
	HC/HET	-2.7±0.3	-3.7±0.4	-3.7±0.5	-3.0±0.7

* Log bacterial units/g dry sediment.

Table 11-1 BLM Microbiology Field Data Ø1B

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2		
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log HC/HET
A1	11-3-75	Sed.	83 m	16.5	12.5	1.8×10^5	4.9×10^2	-2.6
A2	11-3-75	Sed.	128 m	16.4	13.2	6.4×10^4	5.4×10^2	-2.1
A3	11-3-75	Sed.	137 m	16.5	13.5	5.1×10^4	8.0×10^1	-2.8
A4	11-3-75	Sed.	193 m	16.5	13.0	4.8×10^4	4.9×10^1	-3.0
B1	11-4-75	Sed.	63 m	15.2	12.0	5.7×10^4	3.2×10^1	-3.3 (1)
B2	11-4-75	Sed.	60 m	14.9	13.2	4.9×10^5	1.5×10^2	-3.5
B3	11-4-75	Sed.	70 m	15.0	12.0	3.9×10^5	1.2×10^2	-3.5
B4	11-4-75	Sed.	40 m	15.6	12.5	1.8×10^5	3.0×10^2	-2.8 (1)
C1	11-5-75	Sed.	14.5 m	16.0	16.0	4.8×10^5	7.7×10^3	-1.8
C1	11-5-75	Surface Film		16.0	---	9.7×10^4	2.1×10^2	-6.5 E -01 (s)
C1	11-5-75	Surface	1.0 m	16.0	---	9.3×10^4	4.3×10^2	-2.3 (1)
C2	11-5-75	Sed.	21 m	16.0	16.0	6.8×10^5	1.1×10^4	-8.0 E -01
C3	11-5-75	Sed.	25 m	16.0	16.0	2.0×10^5	1.5×10^3	-2.1
C4	11-5-75	Sed.	34 m	16.0	16.0	5.4×10^5	2.7×10^3	-2.3
D1	10-28-75	Sed.	36 m	16.4	15.8	3.8×10^5	2.7×10^2	-3.1
D2	10-28-75	Sed.	31 m	16.0	16.0	1.2×10^5	3.0×10^2	-2.6
D3	10-29-75	Sed.	38 m	15.9	15.5	4.0×10^5	2.7×10^2	-3.2
D4	10-29-75	Sed.	49 m	16.2	15.5	1.3×10^6	2.1×10^3	-2.8
E1	10-29-75	Sed.	70 m	14.0	12.0	2.4×10^5	1.1×10^2	-3.3
E2	10-29-75	Sed.	64 m	14.0	12.0	2.2×10^5	9.3×10^1	-3.4
E3	10-31-75	Sed.	64 m	14.4	13.0	1.9×10^2	6.1×10^1	-3.5
E3	10-31-75	Surface	1 m	14.4		9.3×10^5	9.3×10^{-2}	-4.0 (1)
E4	10-31-75	Sed.	75 m	14.0	12.5	2.2×10^5	3.6×10^2	-2.8
F1	10-31-75	Sed.	83 m	20.0	12.5	1.2×10^5	2.8×10^1	-3.6 (1)
F2	11-1-75	Sed.	112 m	20.5	15.5	2.4×10^4	7.8×10^2	-2.5
F2	11-1-75	Surface Film		20.5		2.4×10^4	1.1×10^4	-1.3 (1) (s)

Table 11-1 (Concluded)

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2		
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log $\frac{HC}{HET}$
F2	11-1-75	Surface	1 m	20.5		4.3×10^1	2.3×10^0	-1.3
F3	11-1-75	Sed.	152 m	20.7	15.5	8.4×10^4	1.7×10^2	-2.7
F4	11-1-75	Sed.	183 m	20.0	12.6	1.2×10^5	1.8×10^2	-2.8
I1	11-3-75	Surface	1 m	18.0		4.3×10^2(1)	2.3×10^0 (1)	-2.3
J1	11-2-75	Surface	1 m	20.7		2.3×10^1(1)	3.0×10^{-3} (1)	-3.9
J1	11-2-75	Surface Film		20.7		3.5×10^4(s)	2.2×10^3 (s)	-1.8
N2	11-5-75	Surface	1 m	17.5		9.3×10^3(1)	2.3×10^0 (1)	-3.6
N3	11-5-75	Surface	1 m	17.0		2.3×10^3(1)	4.3×10^{-1} (1)	-3.7

*MPN values indicated are geometric means of two replicates unless followed by (1) which indicates a single sample was used. Values followed by (s) are surface film counts and are expressed as an MPN/m^2.

Table 11-2 BLM Microbiology Field Data Ø2B

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2		
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log HC/HET
A1	3-4-76	Sed.	90	8.1	9.6	1.9×10^5	1.3×10^2	-3.2
A2	3-5-76	Sed.	127	9.7	11.4	1.4×10^6	5.7×10^2	-3.4
A3	3-15-76	Sed.	136	9.7	11.0	1.1×10^6	7.1×10^2	-3.2
A4	3-15-76	Sed.	196	9.8	10.9	2.7×10^5	3.3×10^1	-3.9
B1	3-4-76	Sed.	63	6.2	8.8	1.5×10^5	2.9×10^2	-2.7
B2	3-4-76	Sed.	61	6.7	8.5	1.0×10^6	2.0×10^2	-3.7
B3	3-4-76	Sed.	72	7.1	9.2	1.3×10^5	6.8×10^1	-3.3
B4	3-4-76	Sed.	41	5.7	7.1	3.8×10^5	1.8×10^2	-3.3
C1	2-20-76	Sed.	15	3.8	4.0	1.0×10^5	1.5×10^3	-1.8
C1(N1)	2-20-76	Surface	1	3.8		2.4×10^4	2.1×10^1 (1)	-3.1
C1(N1)	2-20-76	Surface Film		3.8		3.5×10^6	3.4×10^3 (s)	-3.0
C2	2-21-76	Sed.	25	3.5	4.0	2.1×10^5	2.3×10^3	-2.0
C3	2-21-76	Sed.	25	3.4	3.8	3.8×10^5	1.1×10^2	-3.5
C4	2-21-76	Sed.	33	3.4	3.2	3.0×10^5	8.3×10^1	-3.5
D1	2-21-76	Sed.	40	4.7	4.9	7.0×10^5	2.0×10^2	-3.5
D2	2-21-76	Sed.	32	4.4	4.5	1.2×10^5	1.4×10^2	-2.9
D3	2-21-76	Sed.	35	5.0	5.2	7.3×10^4	2.4×10^1	-3.5
D4	2-21-76	Sed.	49	4.8	5.5	3.8×10^6	1.1×10^3	-3.5
E1	3-3-76	Sed.	66	7.3	9.0	4.3×10^5	1.6×10^2	-3.4
E2	3-3-76	Sed.	73	7.0	8.9	2.4×10^4	3.7×10^1	-2.8
E3	3-2-76	Sed.	64	7.9	9.5	3.1×10^5	2.2×10^2	-3.1
E3	2-25-76	Surface	1	7.1		2.3×10^1	1.2×10^{-1} (1)	-2.3
E4	3-3-76	Sed.	77	7.0	9.2	3.4×10^5	2.5×10^1	-4.1

Table 11-2 (Continued)

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2 Heterotrophs (HET)	Petroleum Degraders (HC)	Log HC/HET
F1	3-18-76	Sed.	85	9.4	10.0	5.9×10^5	1.4×10^1	-4.6
F2	3-18-76	Sed.	110	10.1	11.0	1.4×10^6	8.9×10^1	-4.2
F2	2-25-76	Surface	1	9.3		4.3×10^2	1.5×10^1 (1)	-1.5
F3	3-18-76	Sed.	150	10.5	11.4	1.0×10^6	7.0×10^1	-4.2
F4	3-18-76	Sed.	183	10.2	11.5	8.7×10^4	3.3×10^1	-3.4
G1	3-8-76	Sed.	27	5.3	5.0	2.3×10^6	5.8×10^3	-2.6
G2	3-8-76	Sed.	37	5.5	6.0	9.3×10^5	4.6×10^1	-4.3
G3	3-8-76	Sed.	74	6.7	9.1	5.3×10^5	6.9×10^2	-2.9
G4	3-8-76	Sed.	55	6.2	8.2	2.7×10^5	2.5×10^2	-3.0
G5	3-9-76	Sed.	90	9.0	10.9	8.1×10^4	2.9×10^1	-3.4
G6	3-9-76	Sed.	167	9.3	11.1	3.5×10^5	1.9×10^2	-3.3
G7	3-9-76	Sed.	300	10.1	9.0	3.5×10^5	7.3×10^1	-3.7
H1	3-16-76	Sed. ca.	400	10.7	6.5	2.0×10^5	6.3×10^2	-2.5
H2	3-19-76	Sed.	720	10.5	7.0	1.5×10^4	3.4×10^2	-1.6
I1	3-14-76	Sed.	80	9.5	9.5	1.6×10^6	4.2×10^2	-3.6
I1	3-14-76	Surface	1	9.5		2.3×10^2	2.3×10^0 (1)	-2.0
I2	3-14-76	Sed.	94	8.0	10.0	3.9×10^5	2.8×10^1	-4.1
I3	3-15-76	Sed.	160	9.0	10.8	2.1×10^5	5.7×10^1	-3.6
I4	3-15-76	Sed.	440	9.4	7.0	9.4×10^5	4.0×10^2	-3.4
J1	3-20-76	Sed.	390	11.3	8.0	1.1×10^6	9.5×10^1	-4.1
J1	2-25-76	Surface	1	8.4		2.3×10^2	9.3×10^{-1} (1)	-2.4
J2	3-30-76	Sed. ca.	700	10.8	6.0	1.6×10^5	6.8×10^1	-3.4
K1	3-2-76	Sed.	29	7.2	8.5	1.5×10^5	1.2×10^2	-3.1
K1	3-2-76	Surface	1	7.2		1.1×10^4	4.3×10^0 (1)	-3.4
K2	3-12-76	Sed.	41	6.2	6.7	4.8×10^6	1.0×10^4	-2.7

Table 11- 2 (Concluded)

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2 Heterotrophs (HET)	Petroleum Degraders (HC)	Log HC/HET
K2	3-12-76	Surface	1	6.2		$2.4 \times 10^{3}_{5}$	$4.6 \times 10^{1}_{1}$ (1)	-1.7
K3	3-12-76	Sed.	53	7.5	7.5	1.3×10^{6}	4.8×10^{1}	-3.4
K4	3-12-76	Sed.	105	9.8	10.2	2.7×10^{5}	2.6×10^{3}	-3.0
K5	3-12-76	Sed.	151	11.7	11.6	7.4×10^{5}	1.8×10^{1}	-4.6
K6	3-21-76	Sed.	350	10.9	8.0	2.5×10^{5}	2.5×10^{2}	-3.0
L1	3-22-76	Sed.	26	7.9	8.8	5.2×10^{6}	2.3×10^{3}	-3.4
L2	3-22-76	Sed.	42	8.4	9.5	3.5×10^{6}	1.0×10^{3}	-3.5
L3	3-22-76	Sed.	58	8.4	11.0	4.6×10^{5}	6.0×10^{2}	-3.9
L4	3-22-76	Sed.	94	9.1	11.2	4.2×10^{6}	9.9×10^{1}	-3.6
L5	3-22-76	Sed.	180	9.5	11.5	1.9×10^{6}	1.0×10^{3}	-3.3
L6	3-22-76	Sed.	350	8.6	9.0	1.8×10^{5}	2.8×10^{2}	-2.8
N2	2-25-76	Surface	1	4.0		$1.1 \times 10^{4}_{6}$	9.3×10^{0} (1)	-3.1
N2	2-25-76	Surface Film		4.0		2.2×10^{2}	6.2×10^{2} (s)	-3.6
N3	2-25-76	Surface	1	5.5		1.5×10^{2}	7.5×10^{-2}	-3.3

*MPN values are geometric means of two replicates unless followed by (1) which indicates a single sample was used. Values followed by (s) are surface film samples and are expressed as an MPN/m^2.

Table 11 -3 BLM Microbiology Field Data Ø3B

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2 Heterotrophs (HET)	Petroleum Degraders (HC)	Log HC/HET	
A1	6-22-76	Sed.	92	19.0	12.0	2.1×10^5	6.3×10^1	-3.5	
A2	6-22-76	Sed.	132	19.2	13.0	6.0×10^5	2.6×10^2	-3.4	
A3	6-22-76	Sed.	139	19.5	13.0	3.6×10^5	4.0×10^2	-3.0	
A4	6-23-76	Sed.	196	19.8	12.0	4.6×10^5	3.1×10^1	-4.2	
B1	6-21-76	Sed.	65	18.8	9.6	9.9×10^5	4.3×10^1	-4.4	(1)
B2	6-21-76	Sed.	62	18.8	15.0	3.5×10^5	2.4×10^1	-4.2	
B3	6-21-76	Sed.	73	18.3	9.0	1.8×10^6	1.9×10^2	-4.0	
B3	6-21-76	Surface	1	18.3		1.5×10^3	4.3×10^{-1}	-3.5	(1)
B4	6-22-76	Sed.	42	18.9	9.4	2.4×10^5	2.1×10^1	-4.1	
C1	6-15-76	Sed.	17	16.2	11.1	9.0×10^5	2.3×10^4	-1.6	
C1(N1)	6-15-76	Surface	1	16.2		4.6×10^4	1.1×10^5	+0.4	(1)
C1(N1)	6-15-76	Surface Film		16.2		5.1×10^4	2.2×10^3	-1.6	(s)
C2	6-15-76	Sed.	26	16.8	14.3	3.1×10^6	2.0×10^3	-3.2	
C3	6-16-76	Sed.	25	17.0	14.1	1.4×10^6	6.1×10^1	-4.3	
C4	6-16-76	Sed.	36	17.0	14.5	8.0×10^6	4.4×10^3	-3.2	
D1	6-16-76	Sed.	32	16.7	11.0	8.5×10^5	9.4×10^1	-4.0	(1)
D2	6-16-76	Sed.	33	16.8	10.5	9.0×10^5	5.4×10^1	-4.2	
D3	6-17-76	Sed.	36	16.6	10.0	1.9×10^6	1.8×10^1	-5.0	
D4	6-17-76	Sed.	51	16.9	11.0	3.4×10^6	7.0×10^2	-3.7	
E1	6-18-76	Sed.	66	18.2	12.1	9.6×10^5	1.4×10^1	-4.8	
E2	6-18-76	Sed.	73	16.8	9.5	2.1×10^5	1.7×10^1	-4.1	
E3	6-18-76	Sed.	66	17.1	14.5	3.6×10^5	9.1×10^1	-3.6	
E3	6-18-76	Surface	1	17.1		1.5×10^2	2.3×10^{-2}	-3.8	(1)
E3	6-18-76	Surface Film		17.1		5.6×10^3	/ 3.0×10^1***	/ -1.8	(s) ***
E4	6-17-76	Sed.	80	16.7	8.0	4.2×10^5	4.2×10^1	-4.0	

Table 11-3 (Concluded)

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2			
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log $\frac{HC}{HET}$	
F1	6-19-76	Sed.	76	17.8	11.2	3.0×10^5	5.1×10^1	-3.8	
F2	6-19-76	Sed.	112	18.0	13.4	1.3×10^5	1.7×10^1	-3.9	
F2	6-19-76	Surface	1	18.0		9.3×10^2	2.3×10^{-2}	-4.6	(1)
F2	6-19-76	Surface Film		18.0		4.1×10^4	1.8×10^2	-2.4	(s)
F3	6-20-76	Sed.	160	17.9	12.0	2.2×10^5	1.8×10^1	-4.1	
F4	6-20-76	Sed.	184	17.8	12.0	1.8×10^5	9.0×10^1	-3.3	
I1	6-21-76	Surface	1	18.4		9.3×10^3	4.3×10^{-1}	-4.3	(1)
J1	6-20-76	Surface	1	17.8		1.5×10^3	4.3×10^{-2}	-4.5	(1)
J1	6-23-76	Surface	1	17.8		9.3×10^3	4.3×10^{-2}	-5.3	(1)
J1	6-23-76	Surface Film				5.7×10^4	2.3×10^2	-2.4	
N2	6-16-76	Surface	1	16.6		2.4×10^4	2.3×10^{-2}	-6.0	(1)
N3	6-17-76	Surface	1	17.0		9.3×10^2	9.3×10^{-1}	-3.0	(1)

*MPN values indicated are geometric means of two replicates unless followed by (1) which indicates a single sample was used. Values followed by (s) are surface film samples and are expressed as MPN/m^2.

**Minimum detectable value by technique.

Table 11 -4. BLM Microbiology Field Data Ø4B

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter²			
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log HC/HET	
A1	8-21-76	Sed.	92	19.7	15.0	7.9 E 05	2.3 E 01	-4.5	
A2	8-22-76	Sed.	128	19.5	13.0	4.6 E 05	1.6 E 03	-2.5	
A3	8-22-76	Sed.	136	19.8	13.0	2.0 E 05	1.7 E 01	-4.1	
A4	8-24-76	Sed.	198	22.3	12.0	3.7 E 04	2.1 E 01	-3.2	
B1	8-21-76	Sed.	63	19.5	9.5	3.1 E 05	5.4 E 01	-3.7	(1)
B2	8-21-76	Sed.	61	20.3	17.0	7.0 E 05	2.3 E 00	-5.5	(1)
B3	8-21-76	Sed.	72	19.0	13.0	3.2 E 06	1.0 E 02	-4.5	
B4	8-21-76	Sed.	41	21.2	14.0	6.6 E 05	2.9 E 00	-5.4	
C1	8-16-76	Sed.	16		18.5	1.4 E 06	6.7 E 03	-2.3	
(N1)	9-14-76	Surface Film		21.0		4.4 E 06	6.1 E 04	-1.9	(s)
(N1)	9-14-76	Surface	1	21.0		7.5 E 04	9.3 E 02	-1.9	(1)
C2	9-14-76	Sed.	21	19.4	15.2	8.4 E 05	2.4 E 01	-4.5	
C3	9-14-76	Sed.	24	19.4	12.0	9.6 E 05	1.9 E 01	-4.7	
C4	9-14-76	Sed.	33	19.2	10.5	1.9 E 06	7.9 E 02	-3.4	
D1	8-17-76	Sed.	31	22.5	14.0	9.8 E 05	5.7 E 01	-4.2	
D2	8-17-76	Sed.	33	22.5	13.0	1.9 E 06	3.3 E 01	-4.8	
D3	8-18-76	Sed.	34	22.7	16.0	3.0 E 05	1.0 E 01	-4.5	(1)
D4	8-17-76	Sed.	48	22.9	12.5	1.4 E 06	8.3 E 01	-4.2	
E1	8-17-76	Sed.	69	21.1	14.0	3.7 E 05	2.2 E 03	-2.2	
E2	8-18-76	Sed.	70	21.0	12.5	2.5 E 05	1.4 E 02	-3.3	
E3	8-18-76	Sed.	63	22.5	19.0	8.7 E 05	6.5 E 01	-4.1	
	9-14-76	Surface Film		21.1		5.4 E 04	6.8 E 01	-2.9	(s)
	9-14-76	Surface	1	21.1		4.3 E 03	2.3 E 00	-3.3	(1)
E4	8-18-76	Sed.	73	20.8	17.0	3.7 E 05	5.4 E 00	-4.8	

Table 11-4 (Continued)

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2			
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log $\frac{HC}{HET}$	
F1	8-20-76	Sed.	84	19.5	13.0	8.3 E 05	2.6 E 02	-3.5	
F2	8-20-76	Sed.	113	22.7	15.0	4.0 E 05	2.6 E 02	-3.2	
	9-14-76	Surface Film		21.0		2.5 E 05	3.1 E 02	-2.9	(s)
	9-14-76	Surface	1	21.0		1.5 E 03	4.3 E -01	-3.5	(1)
F3	8-20-76	Sed.	152	23.9	19.0	1.3 E 05	3.5 E 02	-2.6	
F4	8-21-76	Sed.	183	23.4	16.0	1.5 E 05	1.0 E 03	-2.2	
G1	8-26-76	Sed.	24	20.4	12.0	5.2 E 05	2.7 E 02	-3.3	
G2	8-27-76	Sed.	37	22.0	13.0	4.6 E 06	2.8 E 02	-4.2	
G3	8-27-76	Sed.	73	21.2	13.5	3.3 E 06	4.3 E 01	-4.9	
G4	8-27-76	Sed.	56	20.8	13.0	2.5 E 05	8.8 E 00	-4.5	(1)
G5	8-27-76	Sed.	92	20.9	13.0	3.6 E 05	1.1 E 01	-4.5	
G6	8-27-76	Sed.	170	21.1	11.8	1.9 E 05	2.5 E 00	-4.9	
G7	8-28-76	Sed.	296	21.2	9.5	1.1 E 05	4.4 E 00	-4.4	
H1	8-28-76	Sed.	396	21.6	7.5	9.6 E 04	1.2 E 01	-3.9	
H2	8-28-76	Sed.	750	21.8	6.0	2.5 E 04	2.5 E 01	-3.0	
I1	8-23-76	Sed.	75	20.1	14.0	2.9 E 05	1.2 E 01	-4.4	
I2	8-23-76	Sed.	93	20.4	13.0	5.7 E 04	2.2 E 00	-4.4	
I3	8-22-76	Sed.	179	20.2	16.0	1.4 E 05	9.2 E 00	-4.2	
I4	8-29-76	Sed.	444	21.5	7.3	2.7 E 05	5.5 E 01	-3.7	
J1	8-29-76	Sed.	354		8.0	1.4 E 05	7.4 E 00	-4.3	
	9-14-76	Surface Film		20.5		5.1 E 05	6.1 E 01	-3.9	(s)
	9-14-76	Surface	1	20.5		2.3 E 03	2.3 E -01	-4.0	(1)
J2	8-29-76	Sed.	768	23.5	6.8	2.7 E 05	4.1 E 01	-3.8	

Table 11- 4 (Concluded)

Station	Date	Sample Type	Sample Depth	Surface Water Temp °C	Sediment Temp °C	MPN Values*/gm dry sediment or ml or meter^2			
						Heterotrophs (HET)	Petroleum Degraders (HC)	Log $\frac{HC}{HET}$	
K1	8-23-76	Sed.	30	23.1	15.0	1.4 E 06	3.5 E 04	-1.6	
K2	8-23-76	Sed.	41	22.9	13.5	2.9 E 06	9.7 E 03	-2.5	
K3	8-23-76	Sed.	53	22.5	12.0	6.3 E 05	4.9 E 02	-3.1	
K4	8-31-76	Sed.	102	21.0	12.5	3.5 E 05	4.7 E 01	-3.9	
K5	8-31-76	Sed.	152	21.7	14.0	1.8 E 06	6.6 E 01	-4.4	
K6	8-31-76	Sed.	351	21.2	10.0	4.4 E 05	8.3 E 00	-4.7	
L1	9-1-76	Sed.	24	23.2	21.0	2.6 E 06	7.3 E 02	-3.6	
L2	9-1-76	Sed.	50	23.2	14.5	2.9 E 06	2.1 E 03	-3.1	
L3	9-1-76	Sed.	68	21.8	12.5	2.1 E 06	5.2 E 01	-4.6	
L4	9-1-76	Sed.	91	21.8	15.5	1.7 E 06	2.0 E 01	-4.9	(1)
L5	9-1-76	Sed.	199	22.4	14.0	1.4 E 05	6.6 E 01	-3.3	
L6	9-1-76	Sed.	340	22.4	8.0	2.5 E 06	6.7 E 01	-3.6	
N2	9-14-76	Surface Film		22.3		3.8 E 04	1.2 E 02	-2.5	(s)
	9-14-76	Surface	1	22.3		9.3 E 02	4.3 E -01	-3.3	
N3	9-14-76	Surface Film		22.3		3.3 E 04	8.7 E 01	-2.6	
	9-14-76	Surface	1	22.3		7.5 E 02	3.9 E -01	-3.3	

*MPN values indicated are geometric means of two replicates unless followed by (1) which indicates a single sample was used. Values followed by (s) are surface film samples and are expressed as MPN/m^2.

Considering variations due to locating stations, season, temperature, textural properties, bacterial distributions, and the variability inherent in the MPN enumeration technique, the mean levels indicated varied little from season to season. Small variations in these mean counts suggest stable bacterial sediment populations. Mean sediment temperatures for each season were as follows: fall, 13.9°C; winter, 7.9°C; spring, 11.8°C; and summer, 13.8°C. These mean values represent only an ca. 6°C range but the values are weighted toward warmer temperatures because of the more constant outer shelf and shelf break sediment temperatures. Larger variations (ranges) in temperature occurred at inner shelf sediment stations.

Data from transects sampled only twice yearly (Tables 11-2 and 11-4) showed similar constancy in heterotrophic levels but there appeared to be more variation in the levels of petroleum degraders during the summer. Additional data are required to determine if these changes are of significance.

Outer shelf and shelf break stations sampled quarterly appeared to be more stable than inner shelf sediments with respect to bacterial levels. Theoretically, bacterial populations from inner shelf sediments should be more susceptible to seasonal effects because of shallow depths, mixing, temperature effects, and terrestrial influences. In order to assess the relative stability of bacterial populations in sediments, a non-parametric test was used to determine the uniformity of counts as a function of season. Test results (Table 11-6, Column 1) indicated that the counts were not statistically uniform over all four seasons ($\alpha = 0.05$). The null hypothesis, H_0: distributions of counts were the same for all seasons, was rejected for both heterotrophic and petroleum degrading bacteria ($\alpha = 0.05$). When the sediment stations were divided into inner shelf, outer shelf, and shelf break regions, it was seen that inner shelf sediments (Table 11-6, Col. 3) were most likely to be non-uniform with season ($\alpha = 0.01$). Only heterotrophic bacterial populations caused rejection of the null hypothesis in the outer shelf region while bacterial populations in shelf break sediments were statistically similar during all seasons (HET and HC).

Overall patterns in heterotrophic and petroleum degrading bacterial populations as a function of depth (Figures 11-3 through 11-8) were generally similar from season to season. This similarity was probably related to the constancy of certain sediment texture properties, terrestrial influences, and contrasting topographic features. Heterotrophic bacteria from transects sampled quarterly did not manifest a well developed trend of counts with depth. However, repetitive maxima (and minima) in these curves generally occurred and corresponded to elevated levels in troughs D4, C4, and B3. In some graphs a slight trend toward decreasing counts with depth was apparent. "Plateaus," related to bacterial counts from clusters A and F, sometimes appeared elevated in value above outer shelf sediment stations. A and F cluster stations contained sediments with the highest % silt-clay for Southern New Jersey transect stations.

Patterns in the levels of petroleum degrading bacteria were more strongly developed than heterotrophs. Inner shelf sediments from cluster C usually exhibited the highest levels. These stations were most directly affected by terrestrial influences, which were reflected in the generally

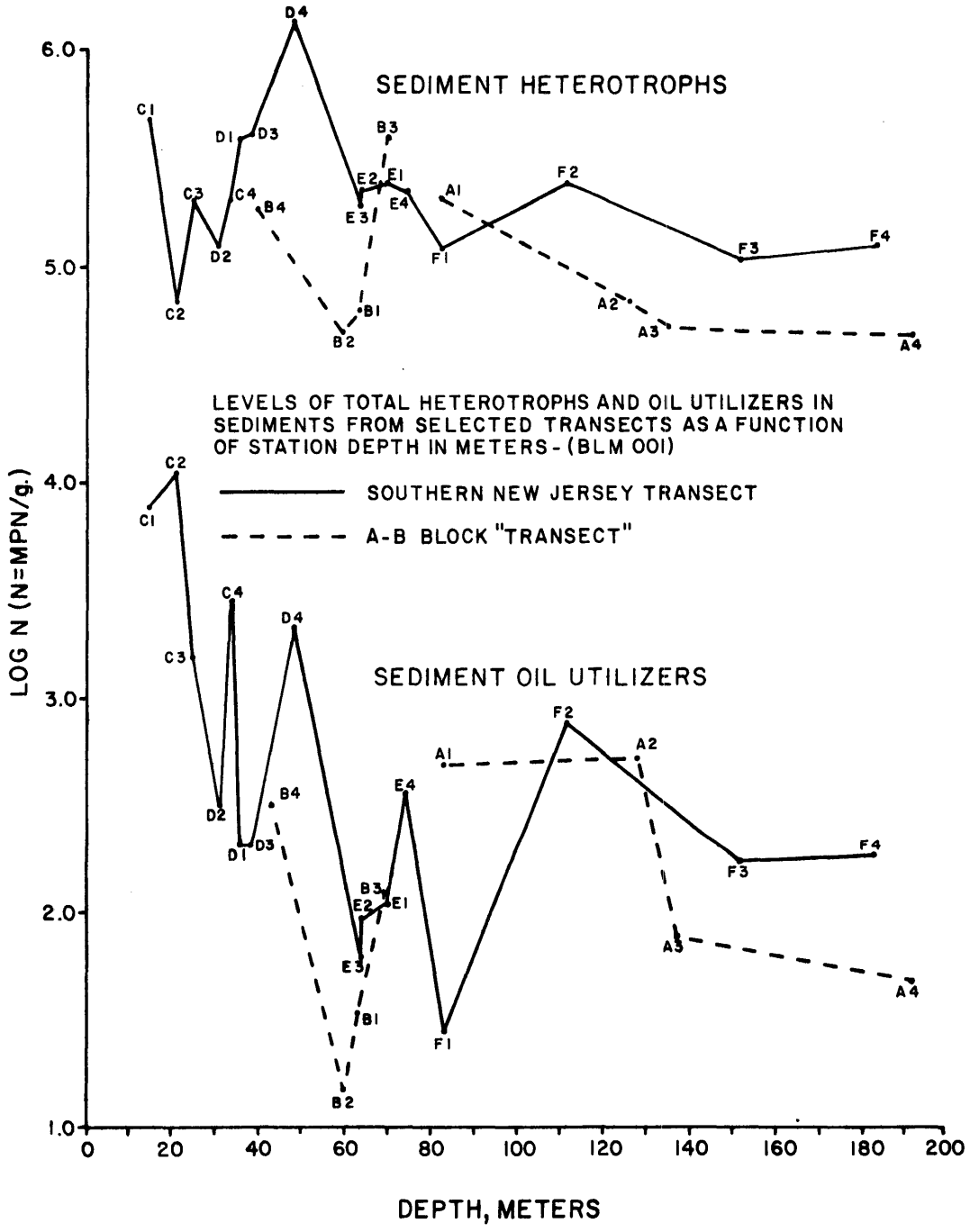


Figure 11- 3. Levels of petroleum degrading and heterotrophic marine bacteria in sediments from cluster stations sampled quarterly, Fall 1975.

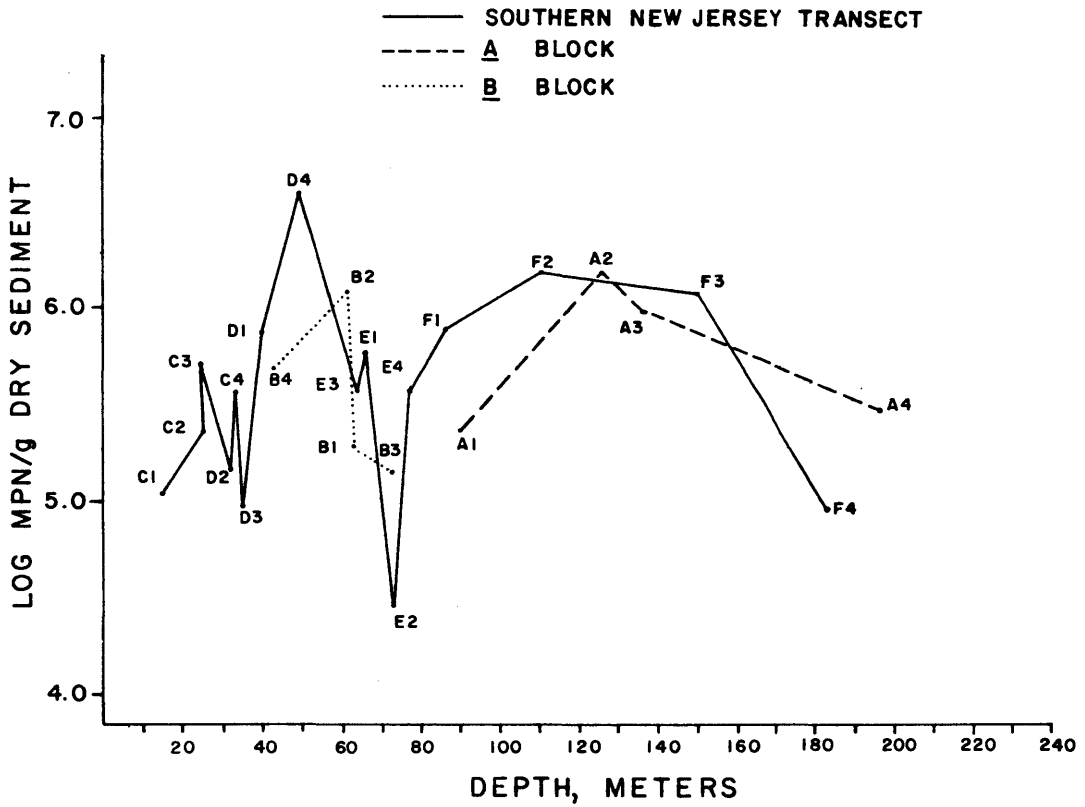


Figure 11- 4 . Levels of heterotrophic marine bacteria in sediments from cluster stations sampled quarterly, Winter 1976.

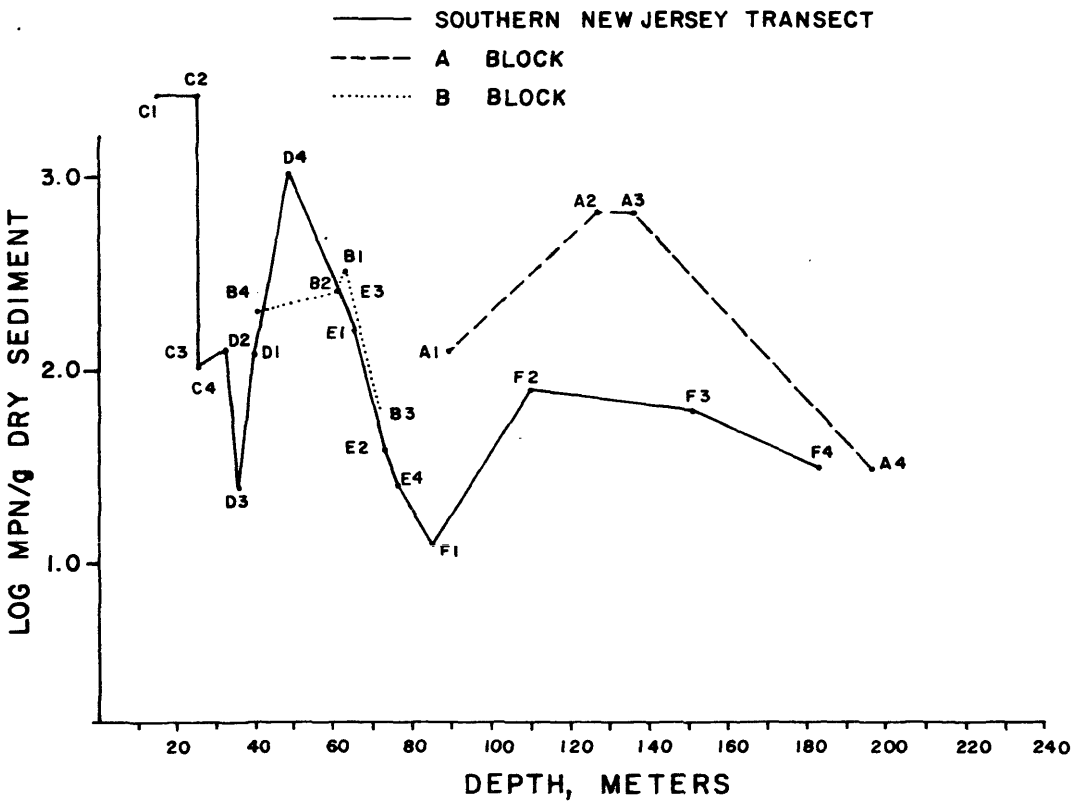


Figure 11-5. Levels of petroleum degrading marine bacteria in sediments from cluster stations sampled quarterly, Winter 1976.

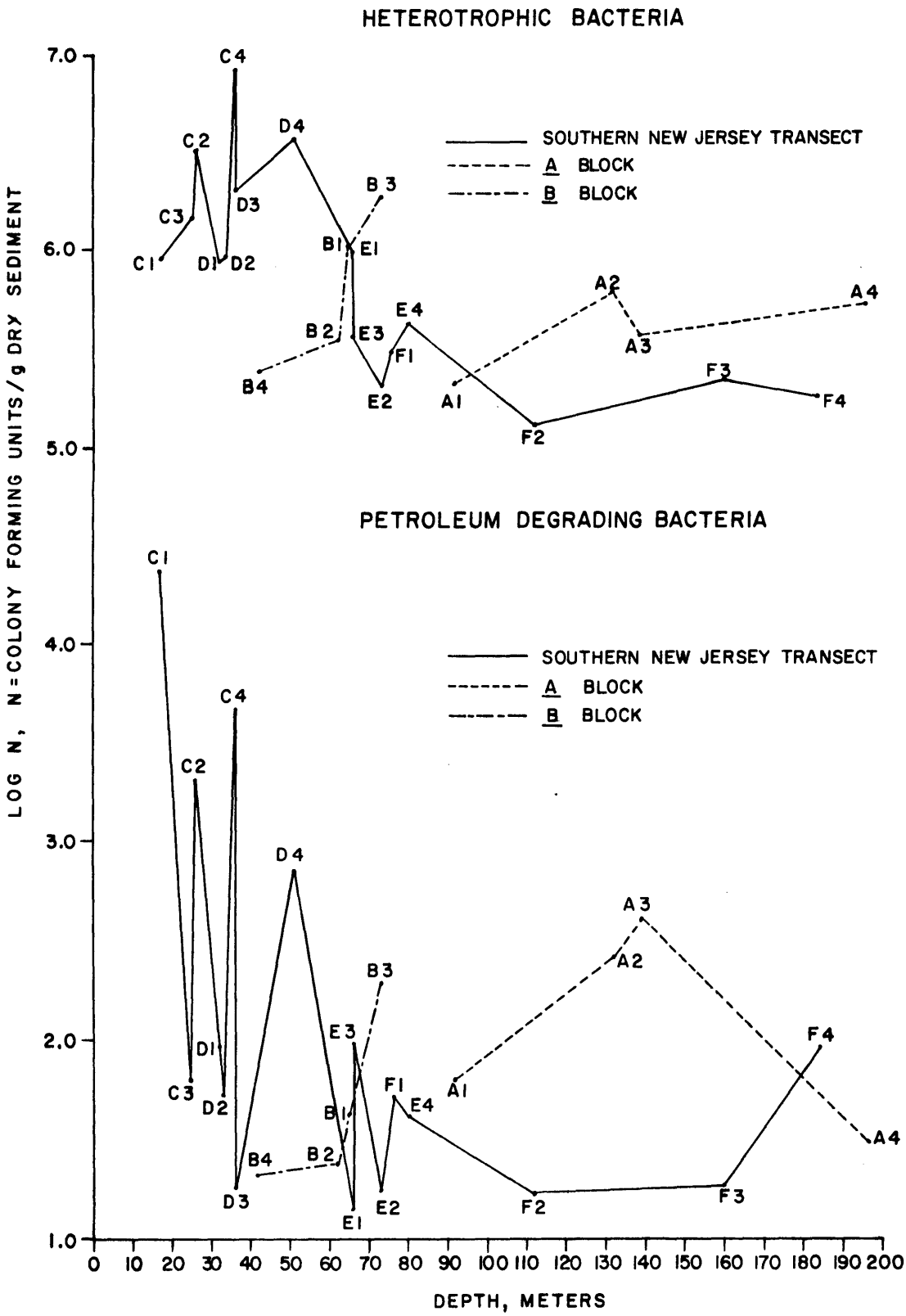


Figure 11-6. Levels of petroleum degrading and heterotrophic marine bacteria in sediments from cluster stations sampled quarterly, Spring 1976.

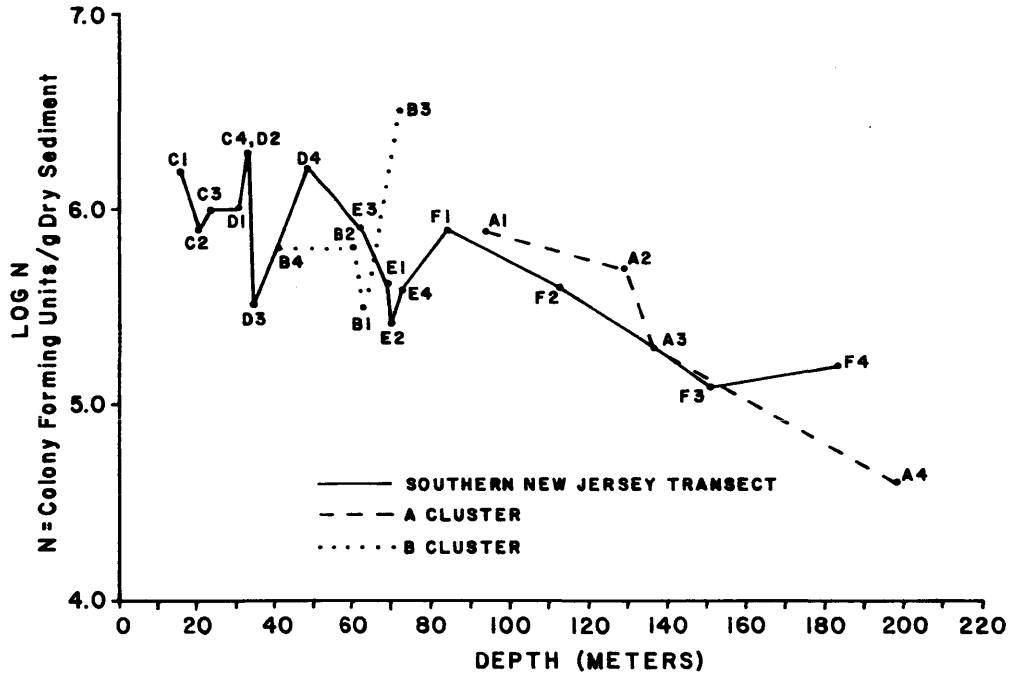


Figure 11-7. Levels of heterotrophic marine bacteria in sediments from cluster stations sampled quarterly, Summer 1976.

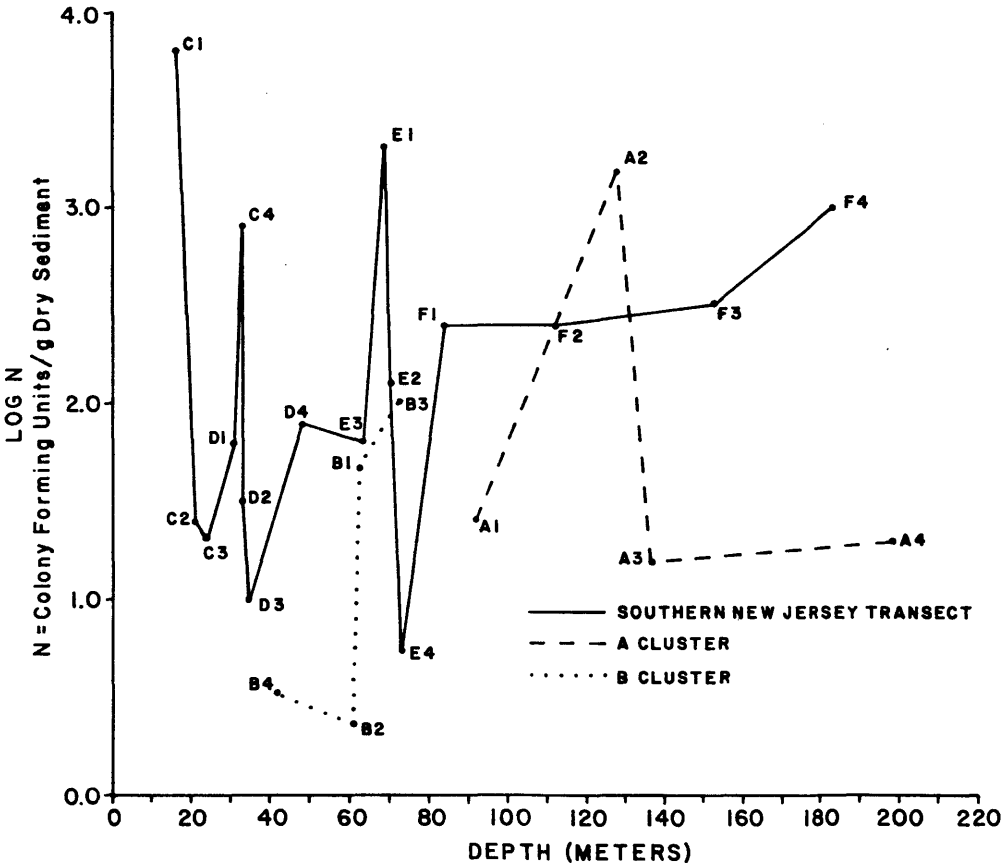


Figure 11- 8. Levels of petroleum utilizing marine bacteria in sediments from cluster stations sampled quarterly, Summer 1976.

Table 11- 6. Value of the Friedman Statistic[a] for the levels of petroleum degrading (HC) and heterotrophic (HET) marine bacteria, and the ratio HC/HET in sediments collected over all seasons (BLM Ø1B-Ø4B).

Bacterial Group	All Seasons All Stations[b]	All Seasons Inner Shelf Stations <50m	All Seasons Outer Shelf Stations 50-100m	All Seasons Shelf Break >100m
HC	9.33*	15.89**	0.63	3.85
HET	24.42**	15.87**	12.37**	7.40
HC/HET	24.39**	18.90**	6.93	10.85*

**Critical T value for alpha= 0.01 = 11.34
*Critical T value for alpha= 0.05 = 7.82
[a]Conover, W. J. 1971. Pratical Nonparametric Statistics. John Wiley and Sons, Inc. New York, N. Y.
[b]All Stations refers to stations sampled quarterly, i.e. clusters C, D, E, F, A, and B.

elevated levels of inorganic nutrients and organic carbon in the water column at C1 (N1). Additionally, a pattern of elevated counts in the troughs D4, B3, and C4 was frequently observed. Troughs contained larger amounts of silt-clay than corresponding ridge stations. As with heterotrophic bacteria, elevated counts were frequently observed for the A and F cluster stations. Mean levels (Table 11-5) of petroleum degrading bacteria were slightly larger during the fall in the inner shelf sediments and were significantly affected by season (Friedman Test, Table 11-6). In general, levels tended to decrease with depth in moving from the inner to the outer shelf and increased in the F and A cluster shelf break station sediments.

Patterns of bacterial populations with depth for sediment transects sampled twice yearly are shown in Figures 11-9 through 11-12. While the numbers of samples were too small for statistical treatment, trends in counts similar to those observed for the Southern New Jersey transect were evident. Heterotrophic bacterial counts did not exhibit well developed patterns with depth, and the mean levels (Table 11-7) were similar for both seasons as previously mentioned; heterotroph levels were ca. 2-3 log units greater than the levels of petroleum degrading bacteria. Higher numbers of petroleum degrading bacteria were noted in the inner shelf sediments compared to outer shelf, shelf break, or slope sediments. These patterns were similar to those observed for the Southern New Jersey transect.

Kendall correlation coefficients were calculated for bacterial populations against temperature, depth, % silt-clay, mean grain size, median grain size, and organic carbon for each season. The results of these computations are shown in Table 11-8. Overall, correlation coefficients were

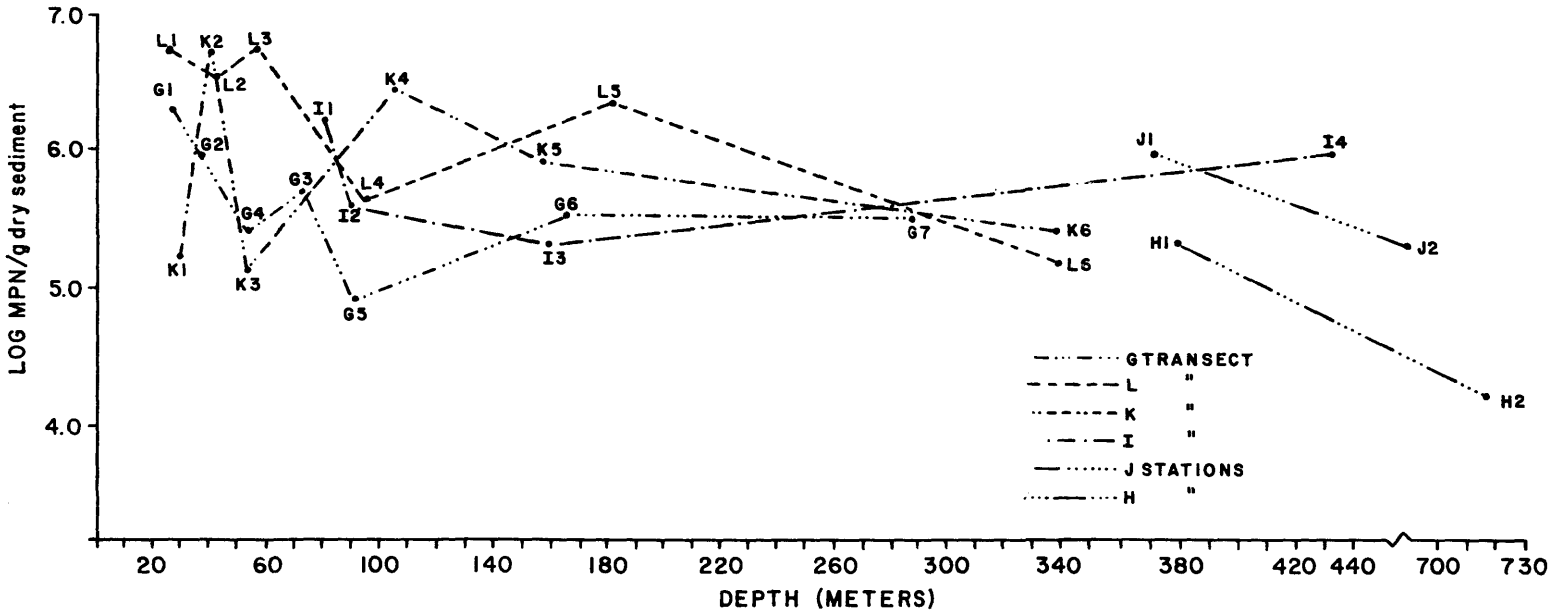


Figure 11- 9. Levels of heterotrophic marine bacteria in sediments from selected transect stations sampled twice yearly, Winter 1976.

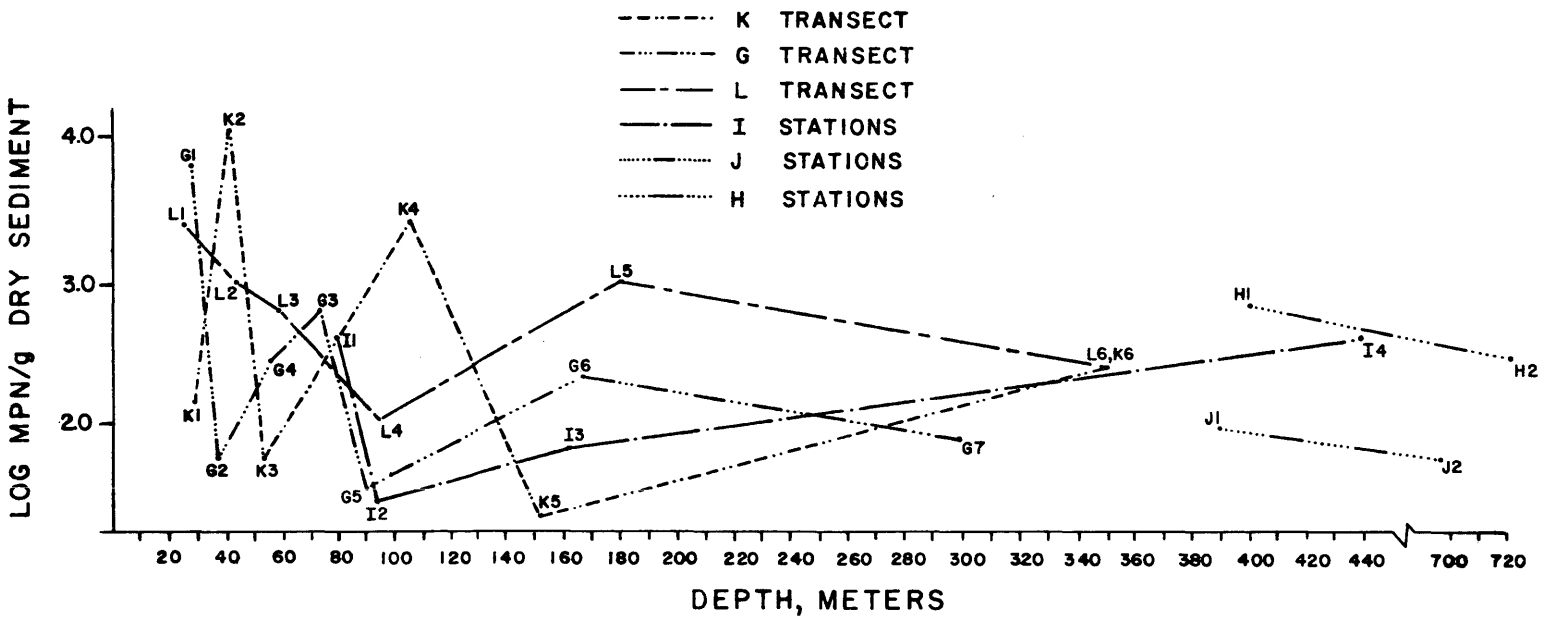


Figure 11-10. Levels of petroleum degrading marine bacteria in sediments from selected stations sampled twice yearly, Winter 1976.

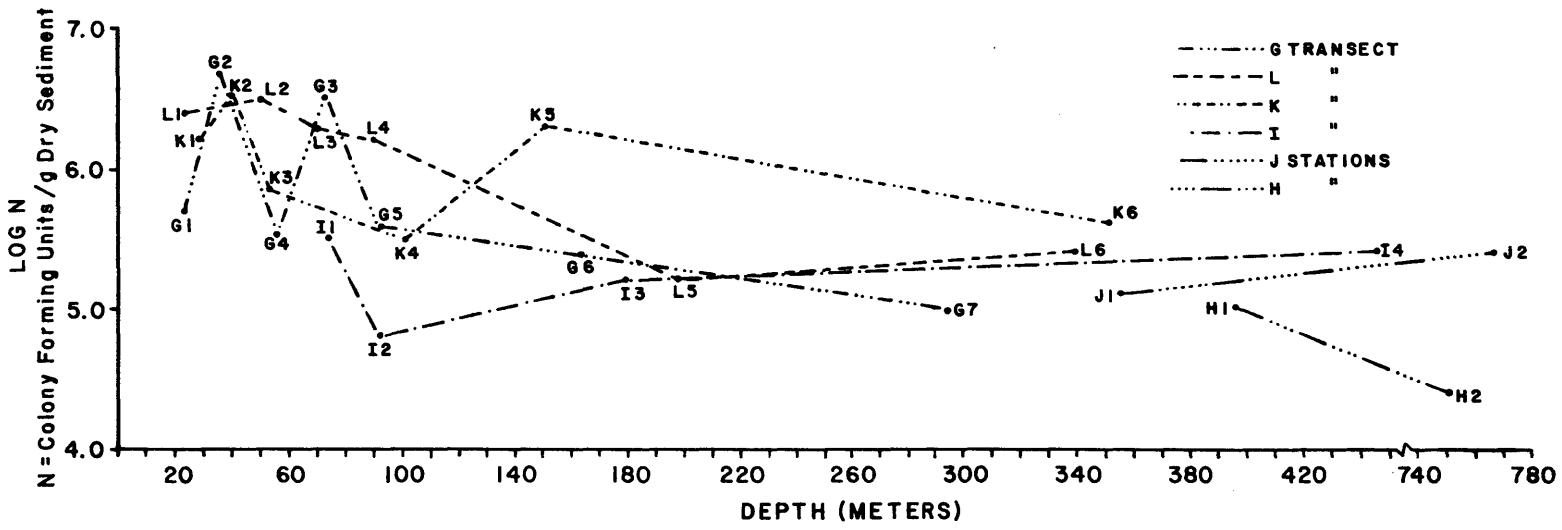


Figure 11-11. Levels of heterotrophic marine bacteria in sediments from transect stations sampled twice yearly, Summer 1976.

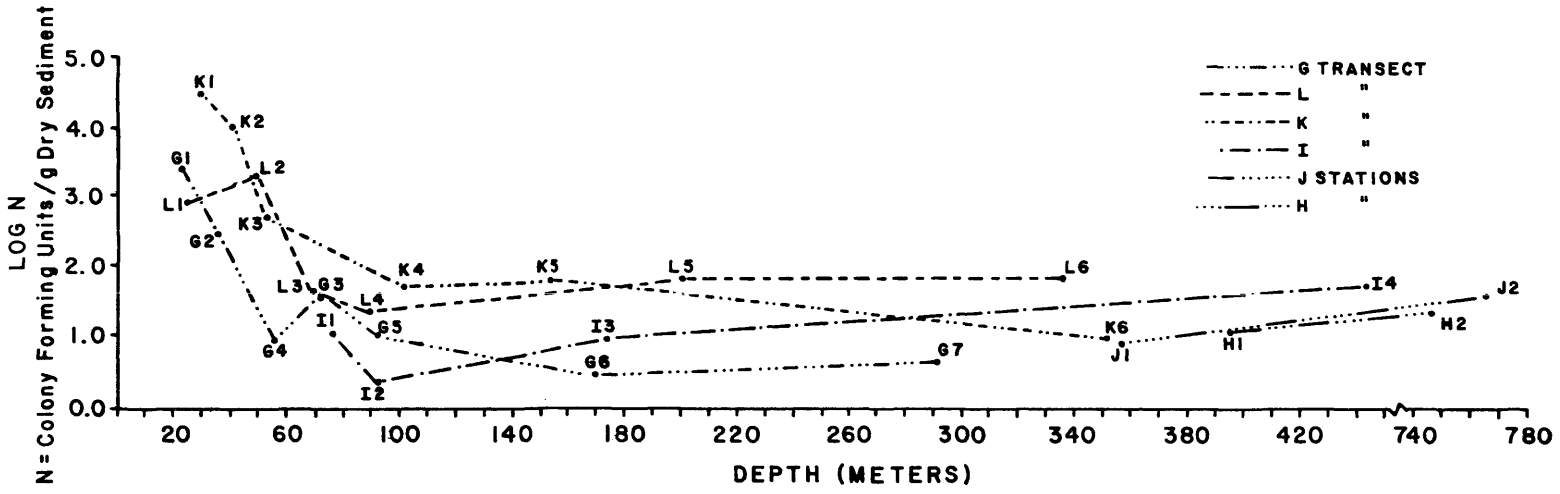


Figure 11-12. Levels of petroleum degrading bacteria in sediments from selected transect stations sampled twice yearly, Summer 1976.

Table 11-7. Mean levels of heterotrophic (HET) and petroleum degrading bacteria in sediments from transect stations sampled twice yearly.

Bacterial Group	Inner Shelf	Outer Shelf	Shelf Break and Slope
	WINTER		
HC	3.0±0.9 *	2.2±0.6	2.3±0.6
HET	6.2±0.6	5.7±0.6	5.6±0.6
	SUMMER		
HC	3.3±0.9	1.3±0.7	1.3±0.5
HET	6.3±0.3	5.6±0.6	5.3±0.4

* Bacterial units/g dry sediment

not significant, even though the signs were sometimes in the right direction. Correlation was generally better during the summer for petroleum degrading bacteria but no consistent pattern for any bacterial group with season was evident. The most consistent pattern of correlation was for bacterial counts with depth (heterotrophs). Petroleum degrading bacterial levels correlated with heterotrophic bacteria for only two of the four seasons.

Since the data in Table 11-8 were calculated considering all stations simultaneously, we felt that better correlation with certain parameters might be achieved by calculating Kendall coefficients for the three shelf areas mentioned before independently. Results for these calculations are shown in Table 11-9 for mean grain size and % silt-clay. The results were no more satisfactory than when all the stations were considered together.

Table 11-9. Kendall correlation coefficients for bacterial populations versus selected mean values of % silt-clay, total organic carbon, and mean grain size.

		Parameter							
		% Silt-Clay				Mean Grain Size			
Area	Bacterial Group	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
Inner Shelf	HC	+0.25	+0.14	+0.50	+0.50	-0.11	-0.14	+0.07	+0.36
	HET	+0.43	+0.05	+0.40	+0.36	+0.43	+0.21	+0.32	+0.29
Outer Shelf	HC	+0.38	-0.22	+0.36	+0.09	+0.07	-0.31	+0.22	+0.44
	HET	+0.22	-0.44*	+0.02	+0.11	+0.18	-0.27	+0.02	+0.24
Shelf Break	HC	-0.20	+0.13	+0.73*	+0.07	+0.07	+0.40	+0.47	-0.20
	HET	-0.47	+0.13	+0.73*	+0.20	-0.20	+0.47	+0.47	+0.47

* Significant correlation at α=0.05

Table 11-8. Kendall correlation coefficients[a] for bacterial sediment populations and selected parameters in mid-Atlantic continental shelf sediments sampled quarterly, BLM01B-04B. HC = petroleum degrading marine bacteria; HET = heterotrophic marine bacteria.

Cruise	Bacteria	HET	°C	Depth	% Silt-Clay	Mean Grain Size (phi)	Median Grain Size (phi)	Total Organic Carbon (mg/g)
Fall Ø1B	HC	0.37*	0.44*	-0.30	0.03	-0.11	-0.14	-0.04
	HET		0.30*	-0.31*	-0.12	0.06	0.01	0.05
	HC/HET		0.47*	-0.20	0.25	0.12	0.09	0.20
Winter Ø2B	HC	0.18	-0.17	-0.25	-0.16	-0.05	-0.14	-0.19
	HET		0.21	0.04	0.21	0.18	0.15	0.13
	HC/HET		0.23	-0.26	-0.24	-0.17	-0.12	-0.15
Spring Ø3B	HC	0.41*	0.05	-0.16	-0.11	0.12	0.07	0.14
	HET		-0.05	-0.41*	-0.16	-0.09	-0.15	-0.15
	HC/HET		0.19	0.11	0.23	0.24	0.22	0.25
Summer Ø4B	HC	0.08	-0.02	0.12	0.31*	0.38*	0.42*	0.23
	HET		-0.11	-0.49*	-0.25	-0.16	-0.10	-0.25
	HC/HET		-0.04	0.32*	0.37*	0.43*	0.42*	0.32*

* Correlation coefficient is significant at $\alpha = 0.05$.

[a]Conover, W. J. 1971. Practical Nonparametric Statistic. John Wiley and Sons, N. Y.

If the poor correlation between bacterial numbers and % silt-clay or mean grain size was due to small scale variability in sediment properties and bacterial sediment populations, a calculation based on contrasting extreme differences in % silt-clay (rather than small ones) might eliminate the effects of this variability. Therefore, % silt-clay, a parameter closely correlated with total organic carbon in sediments and invertebrate distributions, was contrasted using ridge-swale (trough) systems and by comparing A and F clusters with all other clusters sampled quarterly. (Troughs were eliminated from the latter calculations.) A and F clusters and troughs generally had sediments with the highest levels of silt-clay. Results using the Mann-Whitney test statistic for testing the null hypothesis H_o: stations with high % silt-clay values possessed larger bacterial populations than stations with low % silt-clay, are shown in Table 11-10. Note the null hypothesis was accepted at all ridge-swale pairs with the exception of C1 for petroleum degrading bacteria. Similarily, the ratio HC/HET was also rejected at C1. Comparison of A cluster populations with those transect clusters containing lesser amounts of silt-clay, indicated that H_o was accepted at all stations with the exception of the C cluster (Table 11-11). Stations C1 and C2 generally contained the highest populations of both heterotrophic and petroleum degrading bacteria. Again, only petroleum degrading bacteria at C1 was sufficiently elevated to reject H_o.

Table 11-10. Results of Mann-Whitney statistic T'* applied to bacterial populations sampled from ridge and swale stations (BLMØ1B-Ø4B). H_o : ridge (x) $\leq$ trough (y) and H_1 : ridge (x) > trough (y), where (x) and (y) are population distributions.

Bacterial Group	Ridge-Swale Stations			
	C1 - C4	D1 - D4	B2 - B3	E1 - E4
HC	$T' = 9 < T_c$ H_o rejected	$T' = 51 > T_c$ H_o accepted	$T' = 53.5 > T_c$ H_o accepted	$T' = 17.5 > T_c$ H_o accepted
HET	$T' = 49 > T_c$ H_o accepted	$T' = 52 > T_c$ H_o accepted	$T' = 44 > T_c$ H_o accepted	$T' = 24.5 > T_c$ H_o accepted
HC/HET	$T' = 8 < T_c$ H_o rejected	$T' = 39 > T_c$ H_o accepted	$T' = 51 > T_c$ H_o accepted	$T' = 30 > T_c$ H_o accepted

* T' = nm-T, $T_c = T_{critical} = 16$ for $\alpha = 0.05$. Reject H_o if $T' < T_c$.

Table 11- 11. Results of Mann-Whitney statistic T applied to bacterial populations sampled from cluster sediment stations (all seasons) with high % silt-clay versus low% silt-clay (BLM Ø1B-Ø4B) Where H_0: High % silt-clay (x) $\geq$ low % silt-clay (y)

H_1: High % silt-clay (x) < low % silt-clay (y)

Bacterial Group	Cluster High % (Silt-Clay)		Clusters (Low % Silt-Clay) B	C	D	E
HC	A	vs	T=134.5>61* H_0 Accepted	T=48<61 H_0 Rejected	T=115.5>61 H_0 Accepted	T=106.5>61 H_0 Accepted
HET	A	vs	T=84.5>61 H_0 Accepted	T=65>61 H_0 Accepted	T=75>61 H_0 Accepted	T-94.5>61 H_0 Accepted
HC/ HET	A	vs	T-137.5>61 H_0 Accepted	T=675>61 H_0 Accepted	T=133>61 H_0Accepted	T=116>61 H_0 Accepted
HC	F	vs	T-125.5>61 H_0 Accepted	T-44.5<61 H_0 Rejected	T-106>61 H_0 Accepted	T-106.5>61 H_0 Accepted
HET	F	vs	T=81>61 H_0 Accepted	T=63>61 H_0 Accepted	T=64>61 H_0 Accepted	T=707>61 H_0 Accepted
HC/ HET	F	vs	T=123>61 H_0 Accepted	T=61=61 H_0 Accepted	T=122>61 H_0 Accepted	T-97>61 H_0 Accepted

*$T_{Critical} = 61$, $\alpha = 0.05$

Ratio of Petroleum Degrading to Heterotrophic Bacteria in Sediments. Ratios of petroleum degrading to heterotrophic bacteria as a function of sediment depth are tabulated (Tables 11-1 through 11-4) and shown in Figures 11-13 through 11-17. Observations made for the counts of petroleum degrading bacteria can also be noted for this ratio since it appears the former value controls the ratio.

Generally, the ratio decreased from high values inshore (C cluster) to lower values in the outer shelf sediments. Maxima in the curves of inner shelf and outer shelf regions generally corresponded to troughs. Shelf break sediments generally possessed elevated values with respect to the outer shelf sediments, with the A cluster stations usually larger than the F cluster. Mean values of the ratio (Table 11-5) tended to decrease from fall to summer in the inner shelf region and thus were similar to counts of petroleum degrading bacteria. Furthermore, values of the ratio were significantly non-uniform. Shelf break values were lowest in the winter and spring but increased to almost fall levels in the summer. Examination of curves for petroleum degrading bacteria and the ratio of petroleum degrading to heterotrophic bacteria with depth revealed that the patterns of ratio curves are reflections of the levels of petroleum degrading bacteria.

Patterns of change in the ratio with depth for transects sampled twice yearly (Figures 11-15 and 11-17) exhibited trends essentially similar to those discussed above. Values of the ratio tended to decrease moving offshore from shallow waters, remained at somewhat lower values in the outer shelf region, and then increased at shelf break/slope stations. These latter stations possessed the highest amounts of silt-clay. Values in the former region were larger in the winter than in the fall. Additional data is required to determine the cause of this seasonal difference.

Results of calculations to correlate bacterial numbers and the ratio of HC/HET with hydrocarbon levels for sediments are shown in Tables 11-12 and 11-13. Data in Table 11-12 indicated that bacterial parameters in sediments sampled seasonally were usually not significantly ($p = 0.05$) correlated with USGS aliphatic hydrocarbon concentrations. While the majority of correlation coefficients were positive (cell counts increased with an increase in hydrocarbon concentration), only in the spring season were statistically significant results obtained for inner shelf sediments.

Similarly, values of correlation coefficients for aliphatic hydrocarbons in sediments (VIMS DATA) determined over the whole year without quarterly sampling, (Table 11-13) were positive and with one exception not statistically significant ($p = 0.05$).

Surface Water

Data indicating the levels of petroleum degrading and heterotrophic marine bacteria in surface water (1 m) and microlayer samples are listed in Tables 11-1 through 11-4 and 11-14 and shown in Figures 11-18 through 11-21.

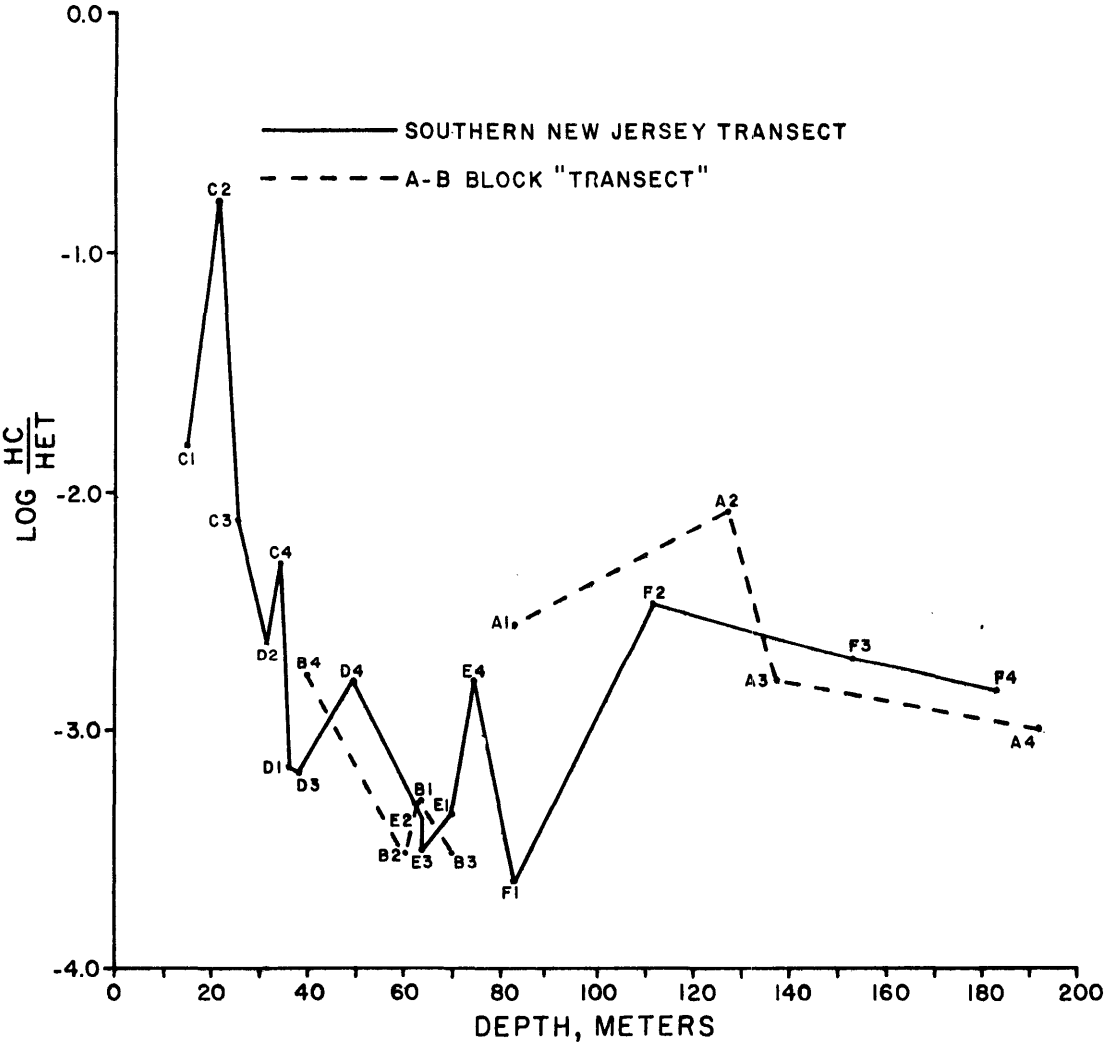


Figure 11-13. Ratio of petroleum degrading (HC) to heterotrophic (HET) bacteria in sediment samples, Fall 1976.

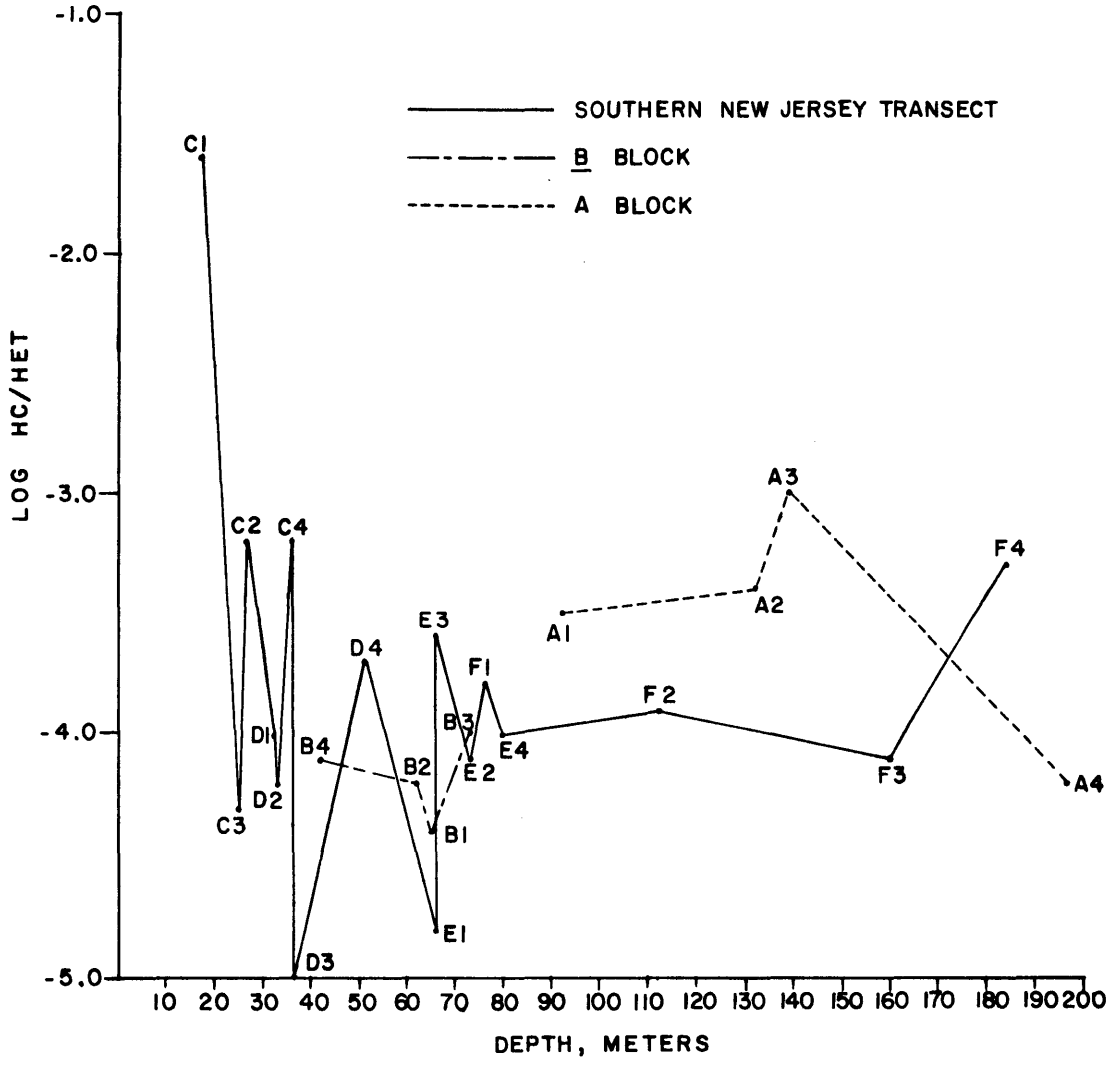


Figure 11-14. Ratio of petroleum degrading (HC) to heterotrophic (HET) bacteria in sediment samples, Spring 1976.

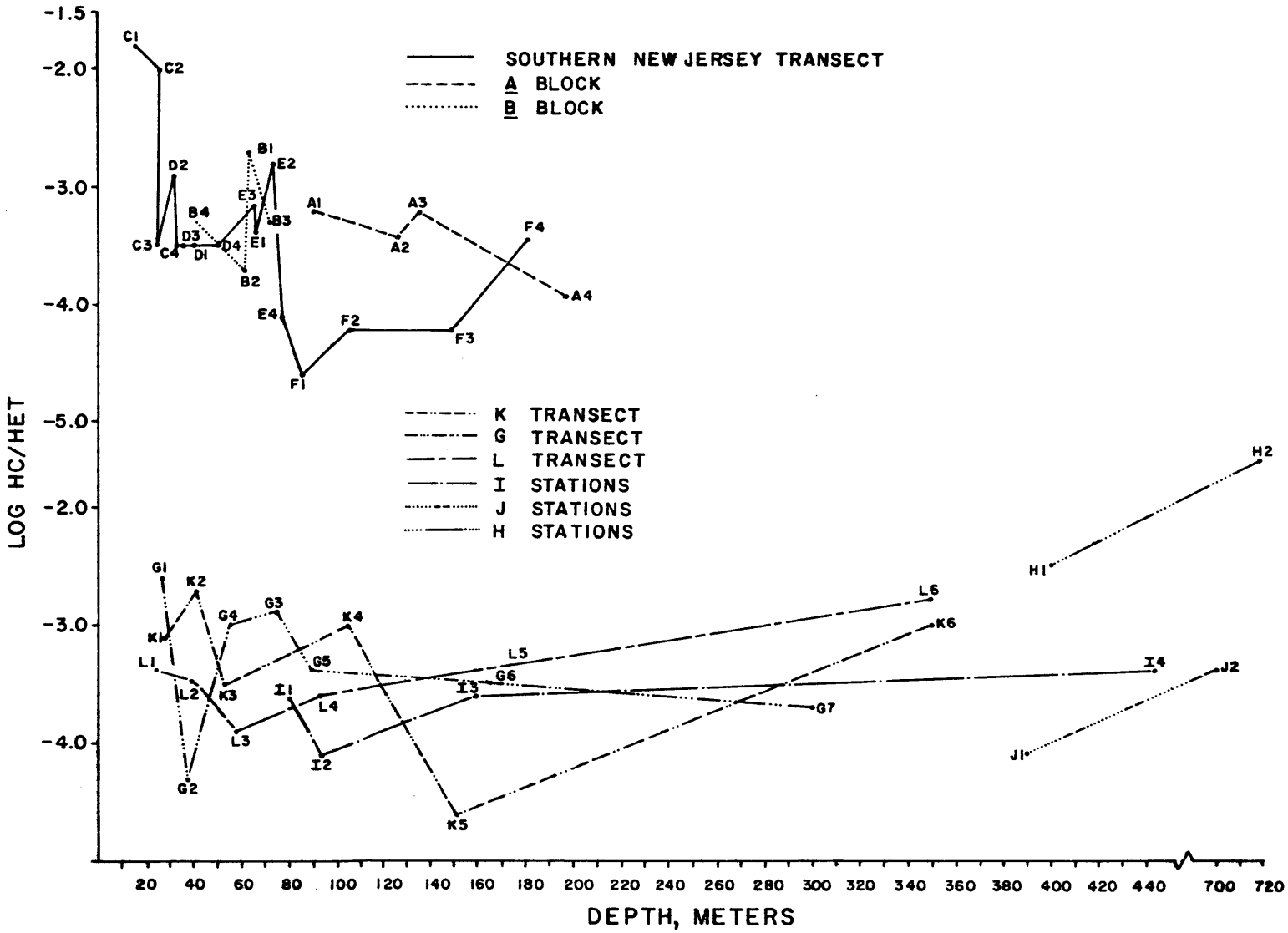


Figure 11-15. Ratio of petroleum degrading (HC) to heterotrophic (HET) bacteria in sediment samples, Winter 1976.

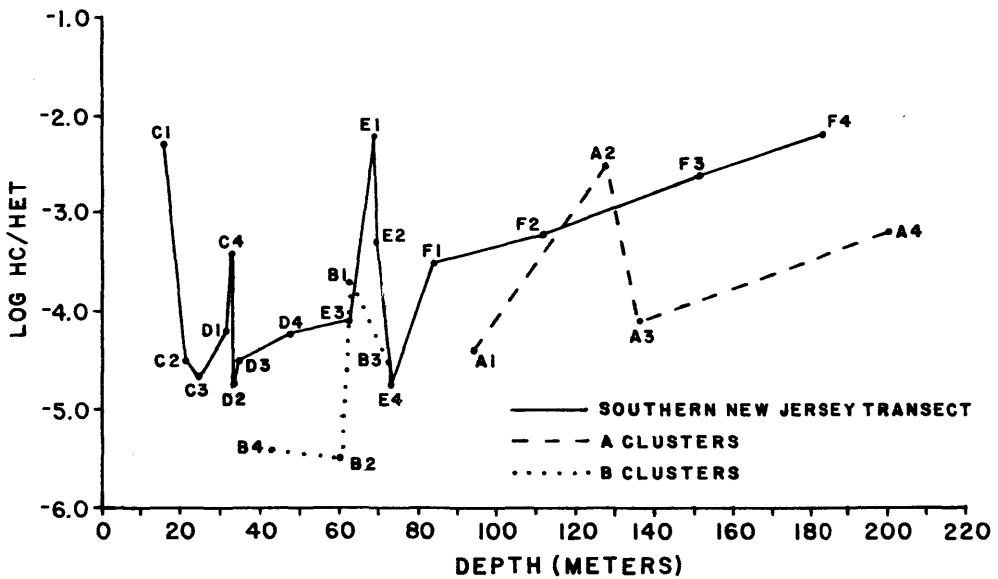


Figure 11-16. Ratio of petroleum degrading (HC) to heterotrophic (HET) bacteria in sediment samples, Summer 1976.

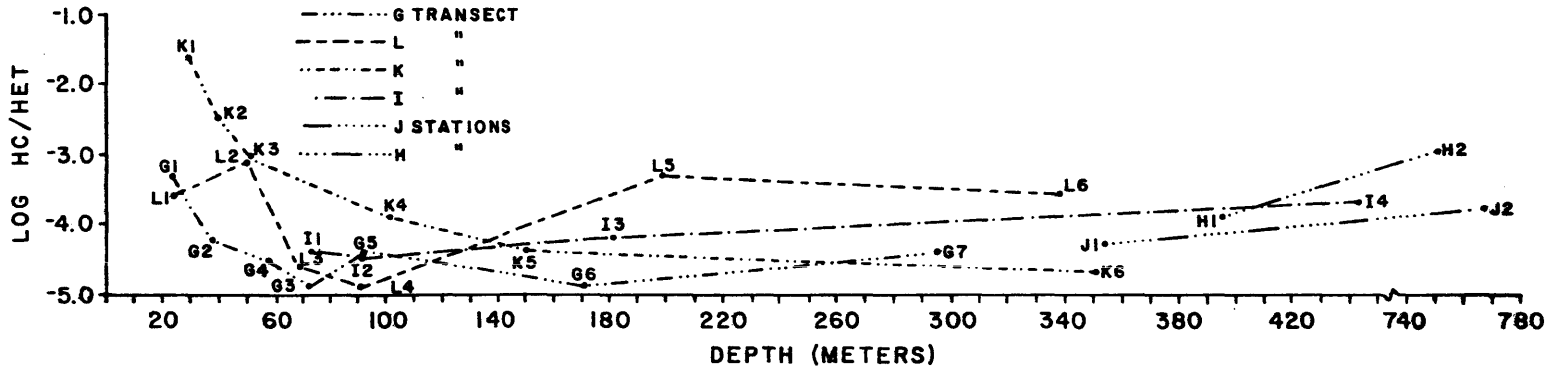


Figure 11-17. Ratio of petroleum degrading (HC) to heterotrophic (HET) bacteria in sediment samples, Summer 1976.

Table 11-12. Kendall correlation coefficients for bacterial populations and aliphatic hydrocarbons[a] in sediments collected during 01B-04B.

Area	Bacterial Parameter	Season			
		Fall	Winter	Spring	Summer
Inner shelf	HC	+0.39	+0.18	+0.79[b]	+0.43
	HET	+0.43	+0.79[b]	+0.54[b]	+0.43
	HC/HET	+0.14	-0.39	+0.68[b]	+0.29
Outer shelf	HC	-0.08	-0.20	+0.40	-0.47
	HET	-0.11	-0.47[b]	+0.22	+0.19
	HC/HET	-0.03	+0.36	+0.31	-0.69
Shelf break	HC	+0.07	-0.13	+0.47	+0.07
	HET	+0.20	-0.27	+0.73[b]	+0.47
	HC/HET	+0.13	-0.07	-0.07	+0.13

[a]Data: U.S.G.S.

[b]There is a significant correlation between bacterial counts and the aliphatic hydrocarbon concentration at p=0.05.

Table 11-13. Kendall correlation coefficients for bacterial populations and aliphatic hydrocarbons[a] in sediments.

Area	Bacterial Parameter	Kendall Tau
Inner shelf	HC	+0.07
	HET	+0.73[b]
	HC/HET	+0.07
Outer shelf	HC	+0.14
	HET	+0.07
	HC/HET	+0.20
Shelf break	HC	+0.43
	HET	+0.33
	HC/HET	+0.20

[a]VIMS Data, sediments not sampled quarterly.

[b]Value of tau significant at p=0.05, a significant correlation between bacterial counts and aliphatic hydrocarbon concentration.

Table 11- 14. Bacterial counts and selected parameters in 1 m surface water samples. HC: petroleum degrading bacteria, HET: heterotrophic bacteria.

Station	Log HC (MPN/ml)	Log Het (MPN/ml)	HC/Het	Temp. (°C)	Sal. (o/oo)	NO_3 (µgat/l)	PO_4 (µgat/l)	Organic Carbon (mg/l)
Cruise Ø1B				FALL				
N1	2.6	5.0	-2.34	16	29.81	2.16	0.64	1.7
N2	0.36	4.0	-3.60	17.5	30.87	0.12	0.52	1.1
N3	-0.37	3.4	-3.72	17.0	32.39	2.29	0.32	1.0
E3	-1.0	3.0	-4.0	14.4	32.96	1.12	0.34	0.9
F2	0.36	1.6	-1.28	20.5	35.24	0.20	0.09	1.4
J1	-2.5	1.4	-3.89	20.7	35.44	0.06	0.13	1.0
Cruise Ø2B				WINTER				
N1	1.3	4.4	-3.1	3.8	31.05	0.30	0.36	3.73
N2	0.97	4.0	-3.1	4.0	31.52	0.15	0.40	2.86
N3	-1.1	2.2	-3.3	5.5	32.54	0.03	0.26	1.95
E3	-0.92	1.4	-2.30	7.1	33.46	0.09	0.20	5.18
F2	1.2	2.6	-1.50	9.3	34.75	1.98	0.24	2.02
J1	-0.032	2.4	-2.40	8.4	34.89	0.05	0.04	3.94
Cruise Ø3B				SPRING				
N1	5.0	4.7	+0.4	16.2	32.02	1.55	0.24	2.14
N2	-1.6	4.4	-6.00	16.6	32.49	1.63	0.12	3.49
N3	-0.032	3.0	-3.00	17.0	32.20	1.52	0.04	5.67
E3	-1.6	2.2	-3.80	17.1	32.16	0.22	0.12	2.93
F2	0.30	3.0	-4.60	18.0	32.07	0.28	0.36	2.63
J1	-1.4	3.2	-4.50	17.8	31.78	0.09	0.04	2.22
Cruise Ø4BG				SUMMER				
N1	3.0	4.9	-1.91	21.0	31.76	0.10	0.48	4.55
N2	-0.37	3.0	-3.34	22.3	31.56	0.09	0.24	4.22
N3	-0.40	2.9	-3.28	22.3	32.19	0.05	0.20	2.56
E3	0.36	3.6	-4.27	21.1	31.10	0.08	0.16	4.12
F2	-0.37	3.2	-3.54	21.0	33.91	0.10	0.04	2.06
J1	-0.64	3.4	-4.00	20.5	33.93	0.10	0.04	2.46

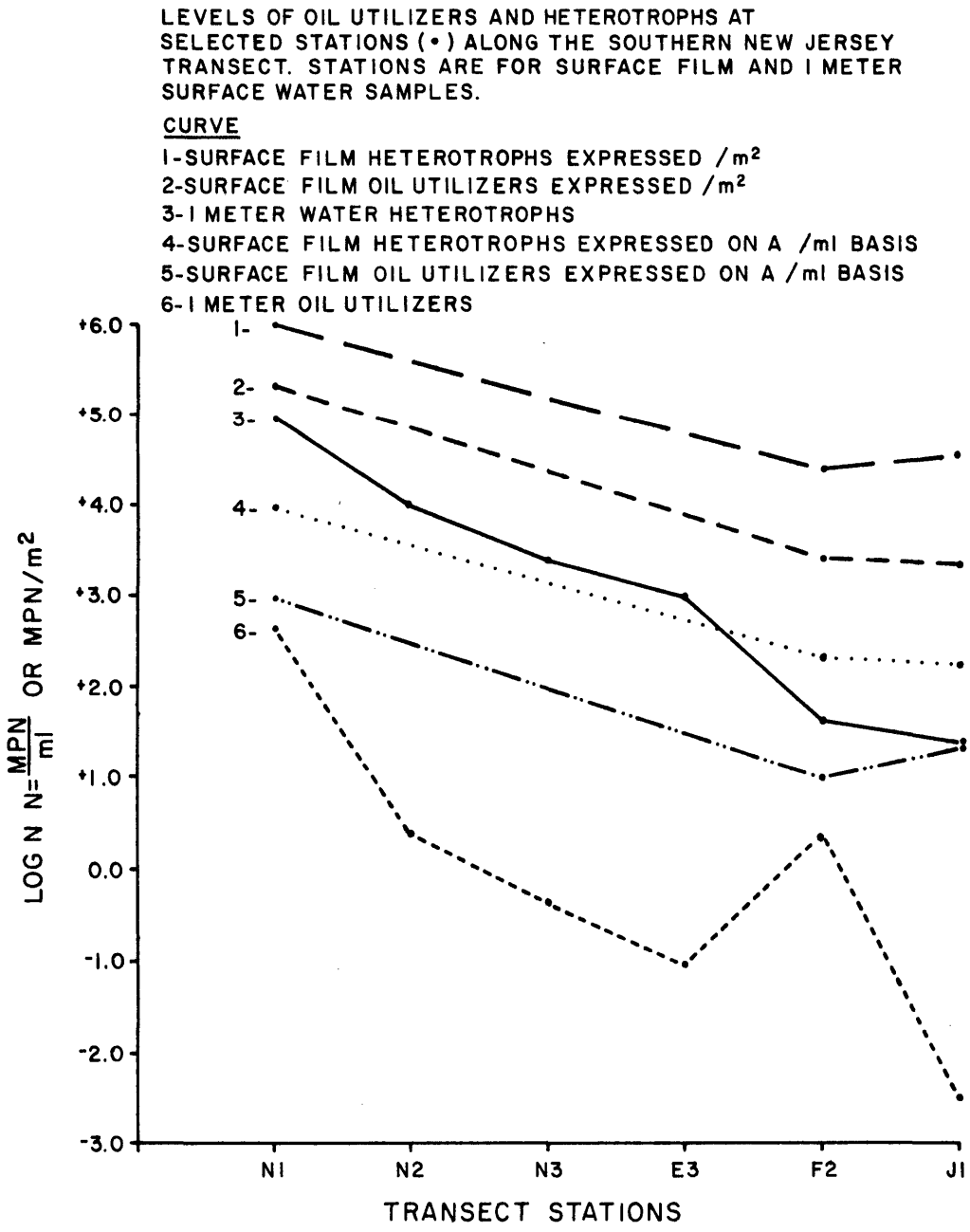


Figure 11-18. Levels of oil utilizers and heterotrophs at selected stations (•) along the southern New Jersey transect. Stations are for surface film and 1 meter surface water samples, Fall 1976.

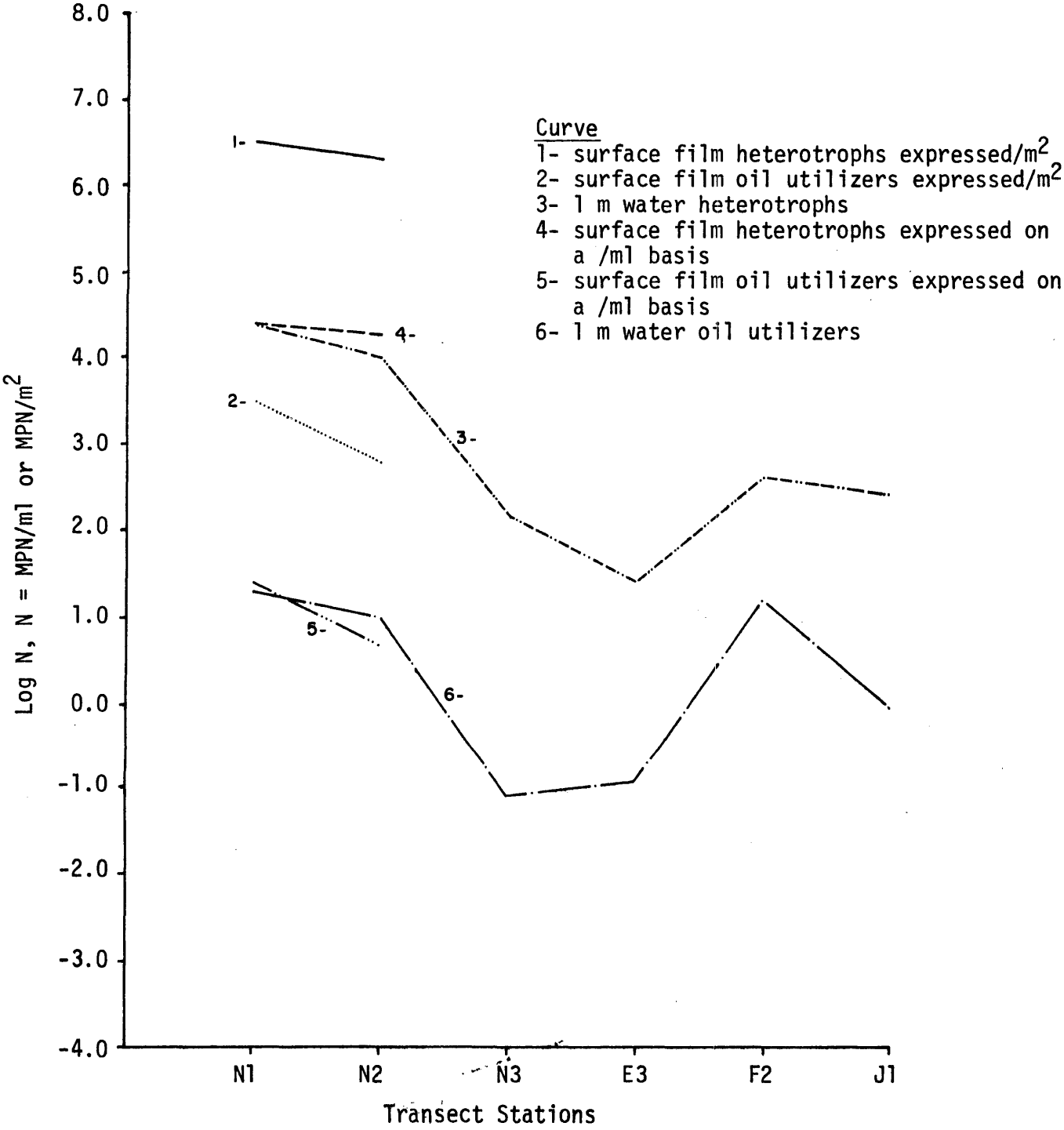


Figure 11-19. Levels of oil utilizers and heterotrophs in surface films and surface water (1 m) collected along a southern New Jersey transect during winter 1976.

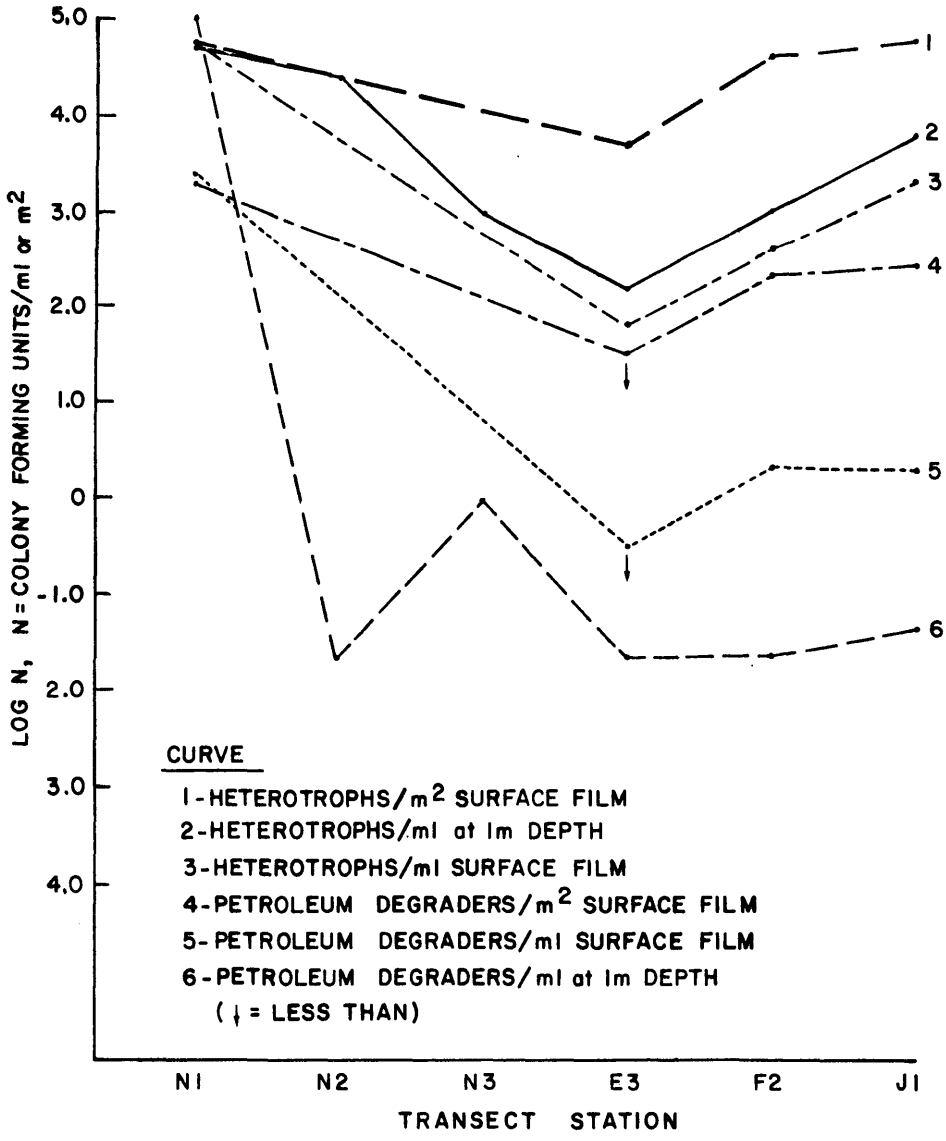


Figure 11-20. Levels of petroleum degrading and heterotrophic marine bacteria in surface films and surface water (1 m) collected along a southern New Jersey transect during spring 1976.

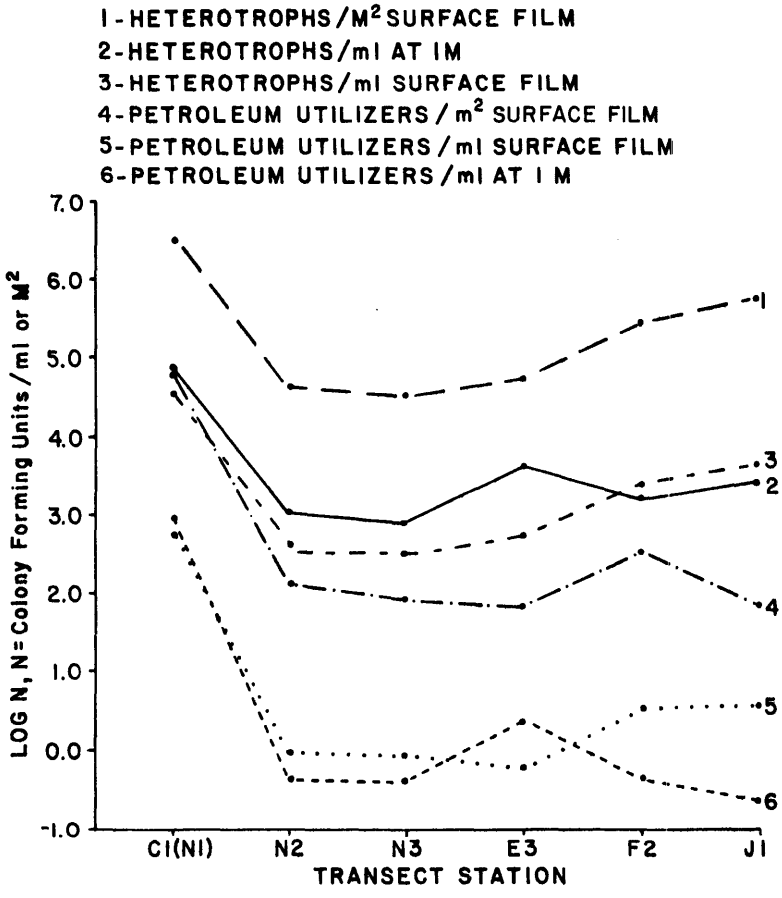


Figure 11-21. Levels of petroleum degrading and heterotrophic marine bacteria in surface films and surface water (1 m) collected along a southern New Jersey transect, summer 1976.

Heterotrophic bacterial populations in one meter water samples generally decreased with distance from shore for the first three transect stations, N1, N2 and N3. Continuing offshore, heterotroph counts at station E3 either continued this trend or increased (summer). Levels of heterotrophic bacteria at stations F2 and J1 were larger than station E3 in the winter and spring. Water at station N1 always exhibited the greatest number of heterotrophic bacteria regardless of season.

Mean values of heterotrophic bacteria for each station (all seasons) are shown in Table 11-15. These values substantiated the patterns mentioned above. Standard deviations were largest at those stations most susceptible to seasonal, terrestrial and hydrodynamic influences (N1, N2, and F2). This pattern displayed by the means was similar for all seasons, although heterotroph levels did decrease several log units during the winter.

Table 11-15. Mean (all seasons) populations (colony forming units/ml of petroleum utilizing and heterotrophic marine bacteria in seawater (1 m) along the southern New Jersey transect.

Bacterial Type	Station N1	N2	N3	E3	F2	J1
HC	2.5E04±5.0	3.0E00±4.3	4.6E-01±3.5	6.3E-01±1.1	5.1E00±7.2	8.7E-01±1.0
HET	6.4E04±3.3	1.2E04±1.0	1.1E03±1.0	1.3E03±1.8	7.6E02±6.8	1.1E03±1.2
Log $\frac{HC}{HET}$	3.9E-01	2.5E-04	4.2E-04	4.8E-04	6.7E-03	7.9E-04

Using the Friedman test statistic to examine all water transect bacterial populations for uniformity over all seasons, the null hypothesis was accepted ($\alpha = 0.05$) indicating factors which control bacterial distributions were independent of season. In fact, data from Table 11-14 indicate that the directions of change for water temperature and bacterial populations were in opposite directions, i.e. counts always decreased from land but temperatures increased or were uniform.

Petroleum degrading bacterial populations decreased with distance from land as did heterotrophic bacteria. However, departures from this pattern were noticeable as strongly delineated peaks in these curves (Figures 11-18 through 11-21). Peaks were observed at the shelf break station F2 (fall and winter), N3 (spring), or to a lesser extent at E3 (summer; although at this time a peak in the curve for microlayer petroleum degrading bacteria appeared at F2). Relatively elevated concentrations of dissolved organic carbon at these stations corresponded with these peaks. Occasionally, elevated NO_3^- concentrations also occurred at peak stations (winter and spring). Peaks at F2 probably resulted from increased biological activity owing to nutrient regimes produced by convergence zones in the shelf break region. Peaks at the inner shelf regions are more difficult to explain utilizing the data available at this time. Mean levels of

petroleum degrading bacteria are shown in Table 11-15. Note the decrease in level with distance from land and the fact that the standard deviations are largest at F2, the convergence zone station. Physical oceanographic data suggests that this convergence zone has a tendency to wander and therefore may be the reason for variations in seasonal counts at F2. Mean levels were lowest at Station J1 with less than 1 petroleum degrading bacterium per ml sea water.

Kendall correlation coefficients were calculated for bacterial levels with various physical and chemical parameters (Tables 11-16 and 11-17). All parameters but temperature, dissolved and particulate hydrocarbons and dissolved organic carbon were significantly ($\alpha = 0.05$) correlated with both heterotrophic and petroleum degrading bacterial populations in direction. Dissolved organic carbon values were somewhat bi-polar, being highest at both ends of the transect during several seasons (Table 11-14). This was undoubtedly related to the effect of the convergence zone at the shelf break. All bacterial groups were significantly correlated with distance from shore, and this effect was highlighted by excluding station F2 from the calculations.

Table 11-17. Kendall correlation coefficients[a] for bacterial levels and dissolved and particulate aliphatic hydrocarbons[b] collected during all seasons.

Bacterial Parameter	Aliphatic Hydrocarbons	
	Dissolved	Particulate
HC	0.03	0.05
HET	-0.16	-0.08
HC/HET	0.18	0.20

[a]Significant correlation at p=0.05 would be indicated by *.

[b]Data: VIMS.

The importance of the convergence zone in the shelf break region (F2) can be seen also in plots of the ratio of petroleum degrading to heterotrophic bacteria, HC/HET (Figures 11-22 through 11-25). During the fall, winter, and, to a lesser extent, spring, peaks in the curves of the ratio were detected. Data from the summer indicated that offshore stations were rather uniform with respect to the value of the ratio. Station N1 always exhibited the highest value for all seasons. As with the levels of petroleum degrading bacteria, peaks in plots of the ratio with depth corresponded with elevated levels of dissolved organic carbon. Thus, the levels of petroleum degrading bacteria tended to determine the values of the ratio. Mean of the ratio at each station (Table 11-15) revealed that these values decreased from a maximum at N1, were somewhat uniformly lower by ca. 3 log units in the inner and outer shelf regions, became elevated at F2 with

Table 11-16. Kendall correlation coefficients for selected parameters and bacterial counts from 1 m water samples (all seasons), BLM Ø1B-Ø4B. HC = petroleum degrading bacteria; HET = heterotrophic bacteria.

Bacteria	Salinity	Temp.	Depth	PO_4^{-3}	NO_3^{-1}	DOC	Relative Distance From Land	Relative Distance From Land (Excluding Station F2)
HC	-0.29*	-0.08	-0.26*	0.34**	0.25*	0.0	-0.29*	-0.44**
HET	-0.53**	-0.03	-0.49*	0.31*	0.32*	0.0	-0.44**	-0.54**
HC/HET	+0.03	-0.16	-0.20	0.12	0.08	0.11	-0.27*	-0.36*

* Correlation Significant at α=0.05, significant at α=0.01

** at α=0.01

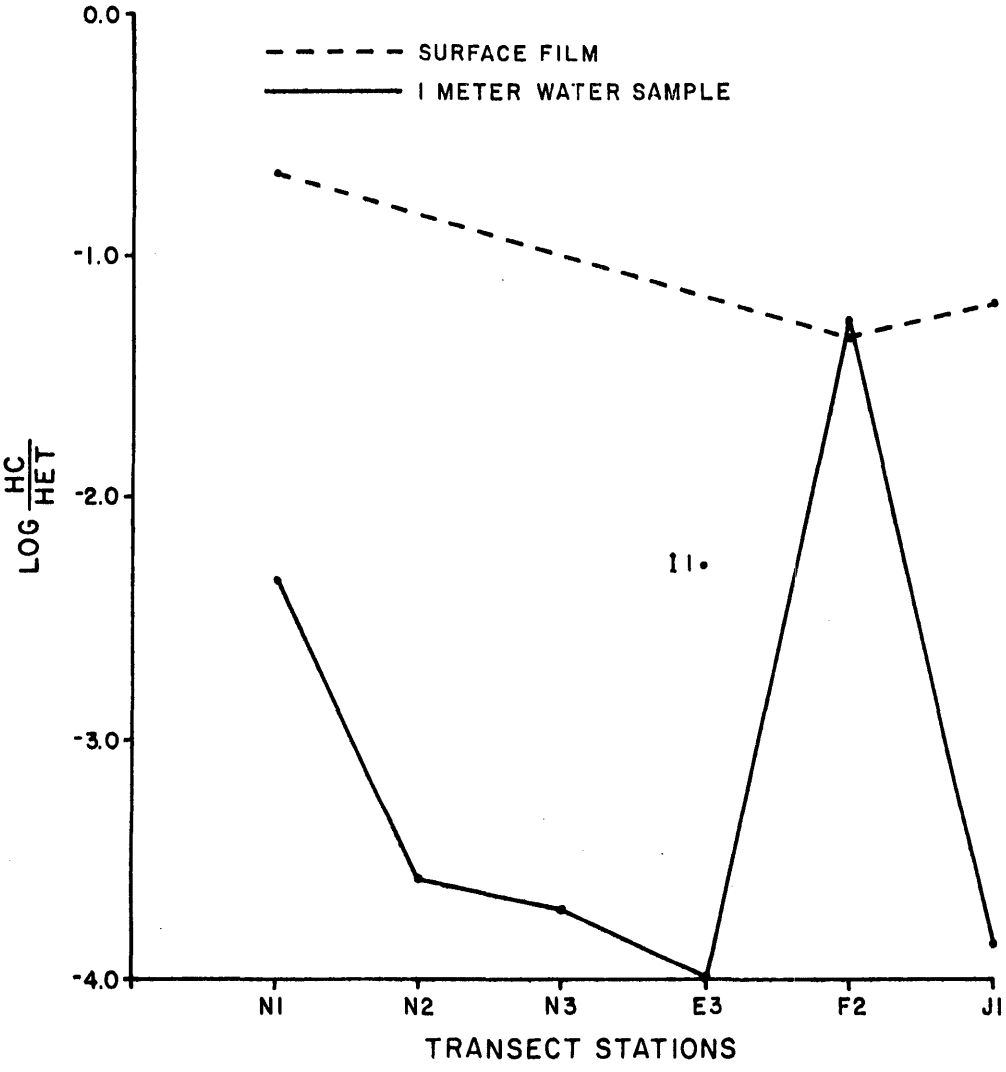


Figure 11-22. Ratio of petroleum degrading (HC) to total heterotrophic (HET) marine bacteria in surface films and surface water (1 m) samples collected mainly along the southern New Jersey transect during Fall 1975.

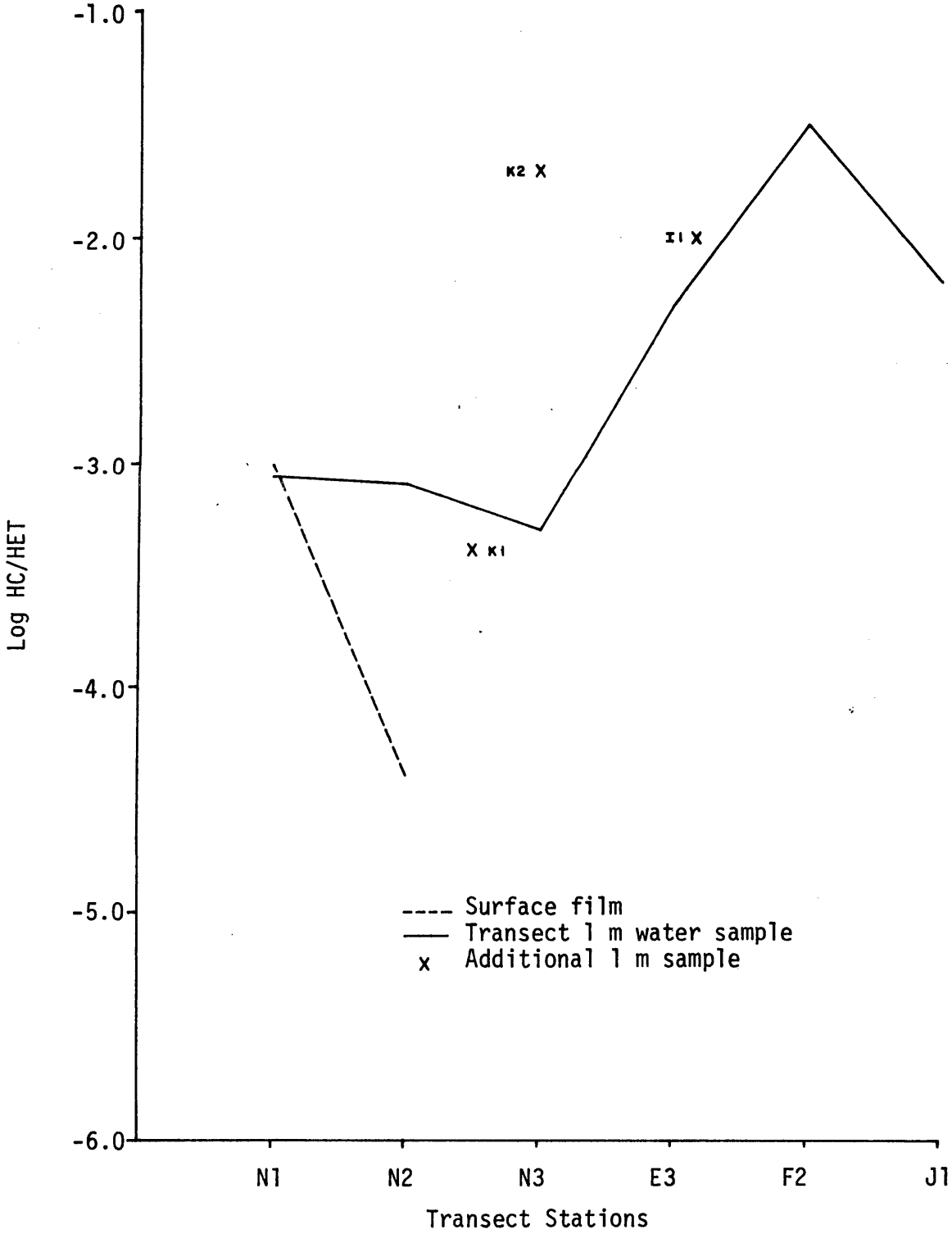


Figure 11-23. Ratio of petroleum degrading (HC) to total heterotrophic (HET) marine bacteria in surface films and surface water (1 m) samples collected mainly along the southern New Jersey transect during Winter 1976.

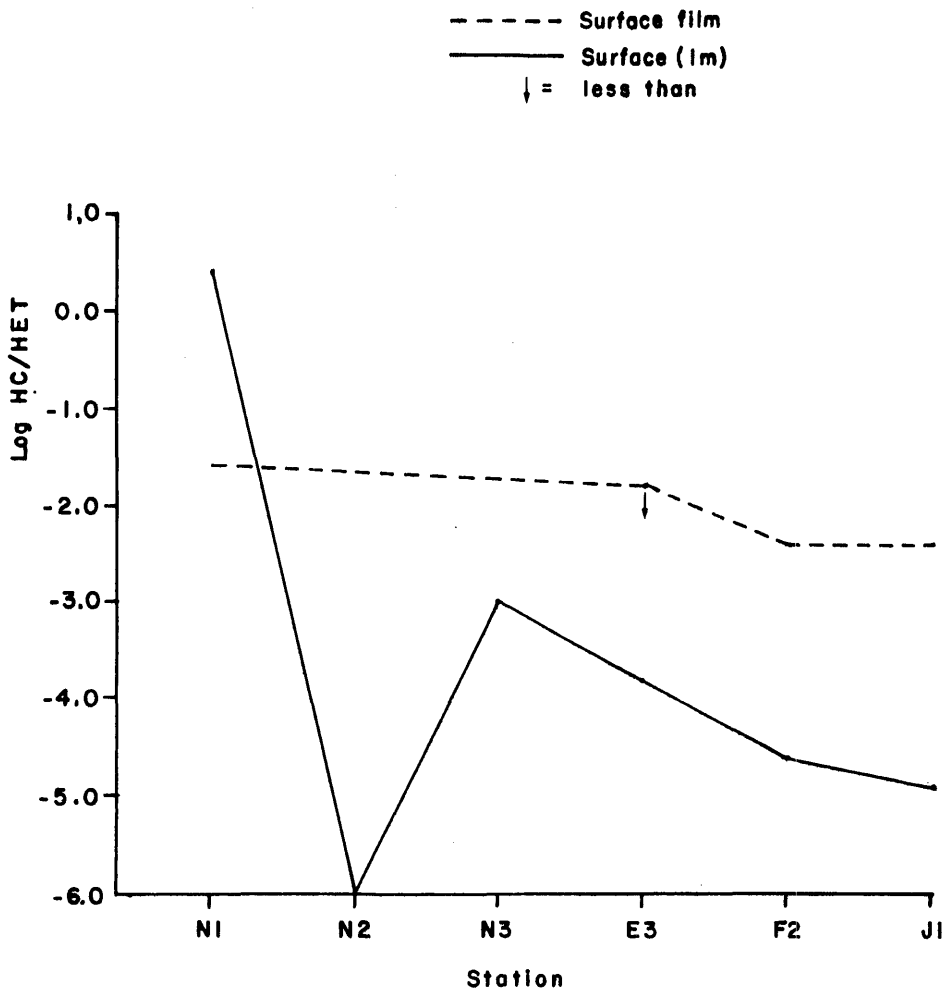


Figure 11-24. Ratio of hydrocarbon utilizing (HC) to total heterotrophic (HET) marine bacteria in surface films and surface water (1 m) samples collected along the southern New Jersey transect during Spring 1976.

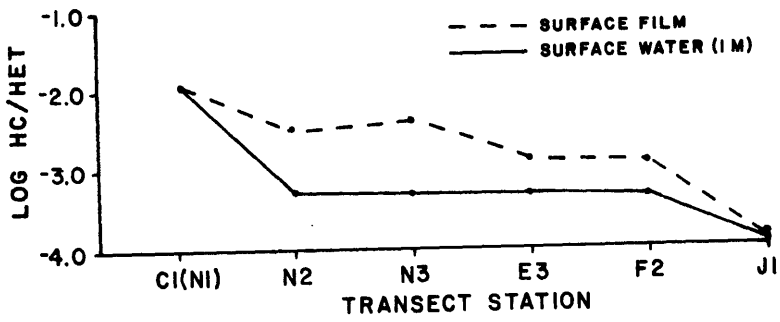


Figure 11-25. Ratio of petroleum degrading (HC) to heterotrophic (HET) marine bacteria in surface films and surface water (1 m) collected along a southern New Jersey transect, Summer 1976.

respect to all other stations (except N1), and were lowest at J1. Chemical analysis of water from Station J1 indicated the lowest mean levels for phosphate and nitrate of all stations.

Kendall correlation coefficients calculated for the relationship of the ratio with chemical and physical parameters indicated were not significant except for distance from land (Table 11-16). Data presented in Table 11-12 suggested the reasons for this apparent lack of correlation were that the ratio was varying with respect to two parameters, i.e. heterotrophic and petroleum degrading bacteria, and these populations were changing in similar directions, the net result being little change in the ratio. Correlation coefficients were always in the right direction (sign) but were not statistically significant.

Microlayer

Microlayer samples were collected whenever weather conditions permitted. Seasonally, conditions were usually most favorable for slick development during the summer. Fall, winter, and spring (to a lesser extent) cruises, were characterized by fronts with associated high winds at fairly regular intervals.

Bacterial populations from microlayer samples (Figures 11-18 and 11-21) were plotted as bacterial cells/m^2 or /ml sample. Although the data points were somewhat sparsely distributed among fall and winter seasons, concentrations of heterotrophic bacteria in the microlayer relative to 1 m water samples were not consistently demonstrated except perhaps at stations F2 and J1 during the fall and summer cruises. Additional seasonal data are required to substantiate this observation. Levels of microlayer heterotrophs were usually slightly lower than heterotrophs at 1 m but exhibited patterns of change in counts with distance from shore similar to the latter.

Levels of petroleum degrading bacteria in the surface microlayer were always lower than heterotroph counts in the microlayer. However, unlike heterotroph counts, there was evidence for enrichment of this group relative to the 1 m water samples. This was most evident during the summer and spring cruises and can be seen quite clearly in Figures 11-20 and 11-21. Plots of the ratio of petroleum degrading bacteria to heterotrophs also manifested this effects (Figures 11-24 and 11-25).

Isolation of Mixed Cultures of Cellulytic Bacteria

Cellulytic cultures of bacteria were isolated from undiluted sediment and water samples in relatively low numbers. Typical cell populations in surface waters were less than one cell/10 ml sea water, and, therefore, 25 ml plant volumes were employed. The low incidence of cellulytic bacteria in shelf sediments and waters is probably a reflection of the relative abundance of cellulose and its importance as a bacterial substrate. Liston (1968) noted the incidence of cellulytic bacteria was generally restricted to inshore sediments.

In the laboratory, mixed cultures of cellulytic bacteria suffered extraordinarily high mortalities. Of approximately 30 mixed cultures giving positive reactions, initially only two remained viable after the first

transfer. High mortalities and the lack of a consistent series of isolates on a seasonal basis precluded the use of cellulytic bacteria in experiments comparable to chitin-petroleum growth studies.

Laboratory Evaluation of Isolates

Isolate Characteristics

Streaked colonies were predominantly translucent to opaque and circular with an entire margin. Most white colonies were low convex to raised, while yellow and orange colonies were typically convex to pulmonate. Some colonies were visible after 1-3 day's incubation; ultimately growing to a diameter of 0.5-3 mm, while other isolates required 2 weeks to reach a colony diameter of 0.01 mm. Therefore, isolate selection and purification were performed at 2 week intervals.

The percentage of isolates which produced pigment varied with sample type and season. Approximately, twice as many pigmented isolates were obtained from HM tubes than from ESWB tubes. Pigmented isolates comprised 44, 32, and 40% of the initial microlayer, water, and sediment HM tube isolates, respectively.

Isolate mortality was a function of season, colony size, pigment production, and colony opacity. Approximately 25% of HM and 10% of ESWB isolates from fall, spring, and summer cruises were lost prior to the completion of taxonomic evaluation. Mortalities for winter isolates were 40% and 15% for HM and ESWB isolates, respectively. Each isolate was transferred at least four times during taxonomic evaluation, and often colony size would decrease with each successive transfer. Isolate mortality was related directly to decreases in colony diameter. Common micro-colonies (<0.5 mm diameter) were usually non-viable after 1-2 transfers.

Most of the pigmented isolates belonged to the genera *Pseudomonas* and *Flavobacterium*. *Pseudomonas* isolates typically formed translucent colonies while *Flavobacterium* isolates produced predominantly opaque colonies. Translucent colonies were typically rapid growers with high survival percentages. Opaque pigmented colonies tended to grow slowly with an observed mortality of 50%. More than 75% of these isolates from winter samples, classified as presumptive *Flavobacterium* sp., were lost. Translucent pigmented isolates from ESWB tubes suffered least mortality. Isolate mortality cannot be overlooked in assessing the dominance of genera in the original samples.

Taxonomic Evaluation

Table 11-18 is a list of the dominant genera to which isolates were assigned and the observed characteristics of each genus. Figure 11-26 presents the determinative scheme for identification of dominant genera as modified after Shewan (1963). In addition to this information, some additional comments are warranted.

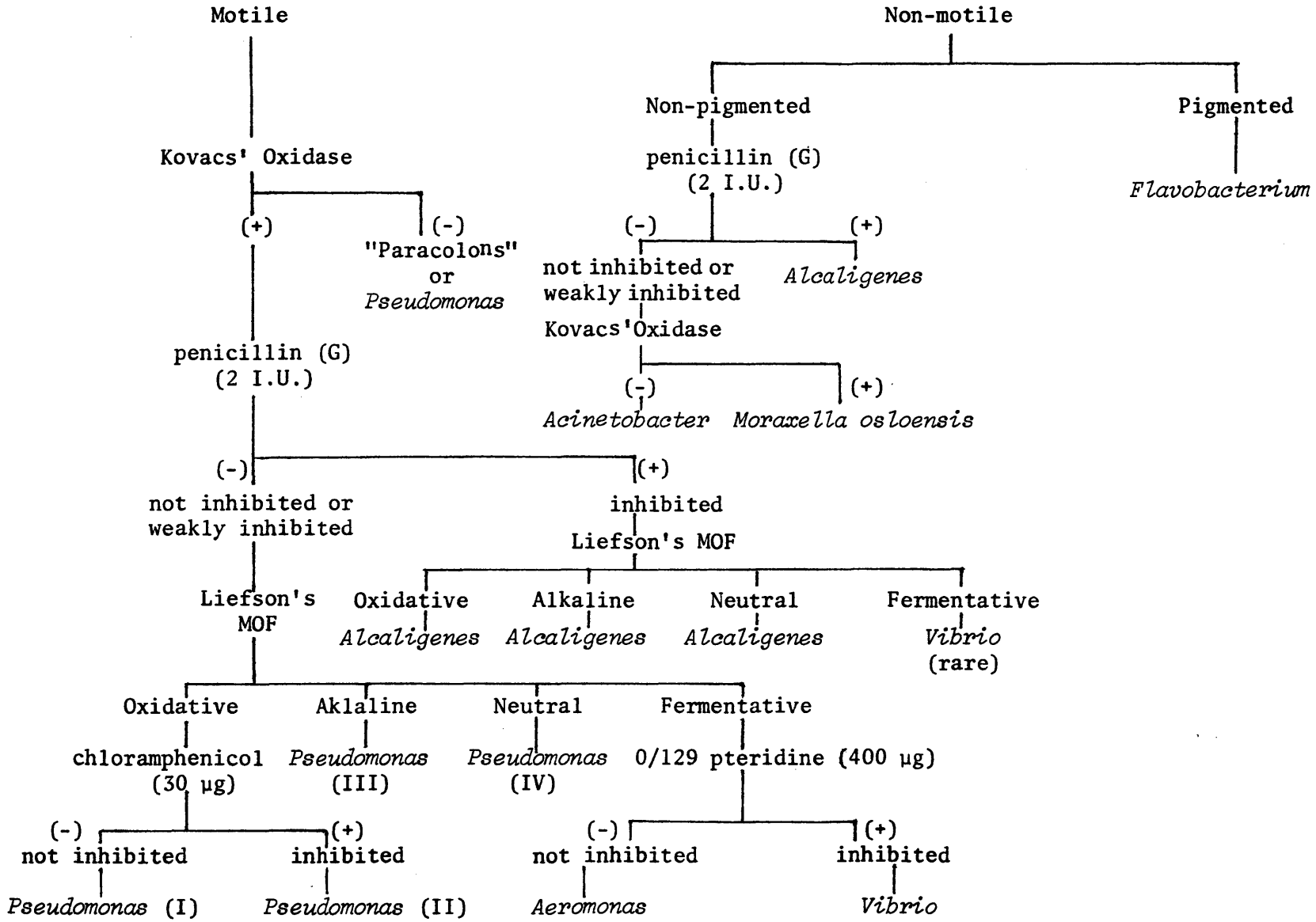
The proposed genus *Alteromonas* which Baumann et al. (1972) differentiated from *Pseudomonas* by % moles G + C was not recognized by us for this study. Non-motile pseudomonads were infrequently observed and were

Table 11-18. Observed characteristics of important genera used in identification of dominant isolates from water and sediment samples. + or - indicates character exhibited by 90% or more of isolates; V indicates variable characteristic.

Genera (Cell Morphology) Character	*Acinetobacter* (coccoid to short rods)	*Aeromonas* (rods)	*Alcaligenes* (coccoid to variable length rods)	*Brevibacterium*/ Coryneform (short rods, coryneform morphology)	*Flavobacterium* (coccoid to variable length rods)	*Micrococcus* (cocci)	*Moraxella*[1] (coccoid to short rods)	*Pseudomonas* (coccoid to variable length ends)	*Vibrio* (short rods to longer curved rods, occasional spheroplasts)
Gram Stain	- or v	-	-	+	-	+	-	-	-
Motile	-	+	v	v	-	-	-	+	+
Acid production									
Glucose aerobic	v	+	v	+	v	+	-	v	+
Glucose anaerobic	-	+	-	-	v	-	-	-	+
Kovacs' oxidase	-	+	+	v	+	-	+	+	+
Catalase	+	+	+	+ or -[2]	+	+	+	+	+
Pigmentation	-	-	-	v	+	v	-	v	v
Antibiotic sensitivity									
Penicillin (G) 2 i.u.	-	-	+	+	-	+	-	-	v
Penicillin (G) 10 i.u.	v	-	+	+	+	+	-	v	+
Cloramphenicol 30 µg	+	+	+	+	+	+	v	v	+
Colymycin 10 µg	-	v	+	-	v	-	+	v	v
Sulfadiazine 1 mg	-	-	v	-	-	-	-	-	-
Neomycin 30 µg	+	+	+	+	-	v	+	v	+
Streptomycin 10 µg	+	+	+	+	v	+	+	v	v
Tetracycline 30 µg	+	v	+	v	+	v	-	v	v
Novobiocin 30 µg	+	+	+	+	+	+	-	+	+
Bacitracin 10 µg	+	-	v	v	-	+	v	-	-
Pteridine 400 µg	-	-	+	v	v	v	-	v	+
Chitin hydrolyzed	-	v	-	-	v	-	-	v	v

[1] *Moraxella osloensis*
[2] *Brevibacterium* +, Coryneform -

Figure 11-26. Determinative Scheme for the Identification of Major Genera of Gram-Negative Marine Bacteria (after Shewan 1963).



classified as presumptive *Pseudomonas* sp. based on morphological, biochemical, and antibiotic profile similarities to motile pseudomonads. Similarly, oxidase negative isolates exhibiting characteristics similar to *Pseudomonas*, but which fell into Shewan's "Paracolon" group were also classified as presumptive *Pseudomonas* sp.

Members of the genus *Moraxella*, with the exception of *Moraxella osloensis*, are highly sensitive to pencillin (G) and require nutritionally complex media (Baumann et al. 1968a). Therefore, cultivation methods employed in this investigation eliminated these forms from consideration. Several isolates were identified as *M. osloensis* on the basis of their insensitivity to pencillin (G) and simple nutritional requirements. This species was added to the Shewan (1963) scheme.

Non-motile, oxidase-negative, gram variable coccoid-bacilli were assigned to the genus *Acinetobacter* and added to the Shewan (1963) scheme, Pagel and Seyfried (1976) separated this genus into two subgroups, one of which could ferment glucose. Several of our *Acinetobacter* isolates gave a very slow atypical fermentative MOF reaction, but most were unreactive or produced very weak acidic reactions in MOF tubes.

Genera assigned to numerically dominant isolates from microlayer, surface water, water values (1 m), and sediment samples are listed in Tables 11-19 through 11-22. Dominant genera are listed as a function of season and sampling location in Table 11-23 through 11-26. Genera from microlayer samples are not shown on these tables since microlayer samples could not be obtained with seasonal regularity.

Data in Tables 11-23 and 11-24 indicated that the genera from HM and ESWB tubes dominating in the water over all seasons were *Pseudomonas* and *Alcaligenes*. *Vibrio* was observed over all seasons but at fewer stations than either *Pseudomonas* or *Alcaligenes*. *Flavobacterium* was isolated at fewer stations during three seasons. All other genera were isolated during selected seasons, but the data are not adequate to suggest a seasonal correlation.

In sediments (Tables 11-25 and 11-26) *Pseudomonas*, *Alcaligenes*, and *Vibrio* were isolated every season from inner shelf, outer shelf, and shelf break stations. Compared to 1 m water samples, *Flavobacterium* and *Aeromonas* appeared more frequently in sediments. The remaining genera were isolated sporadically, and the data are not adequate to develop a seasonal correlation.

Comparison of genera obtained most frequently by season from HM and ESWB tubes indicated the *Pseudomonas* sp. was isolated more frequently from water and sediment ESWB tubes. Genera most frequently isolated by season in HM tubes were *Pseudomonas* sp. and *Alcaligenes* sp. However, if seasonal variations in isolation are ignored, dominant genera from both HM and ESWB tubes are markedly similar for all samples.

Petroleum Utilization in Pure Culture

The ability of isolates from ESWB tubes to utilize crude oil in pure culture was examined by reinoculating each isolate into ESWB tubes containing sterile South Louisiana crude oil. Results of these confirmation

Table 11-19. Dominant marine bacteria isolated from microlayer, water (1 m) and sediment samples from selected stations during 1975 fall cruise (Ø1B).

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Microlayer	C1	*Alcaligenes* *Flavobacterium*	*Pseudomonas*
	F2	*Flavobacterium*	*Flavobacterium* *Pseudomonas*
	J1	*Alcaligenes* *Micrococcus** *Pseudomonas*	*Alcaligenes* Coryneform group* *Flavobacterium* *Pseudomonas*
Water	C1	Coryneform group* *Micrococcus*	*Flavobacterium* *Pseudomonas*
	E3	*Pseudomonas*	*Flavobacterium* *Pseudomonas*
	F2	*Micrococcus* *Pseudomonas*	*Alcaligenes*
	I1	*Pseudomonas*	*Pseudomonas*
	J1	*Brevibacterium** *Pseudomonas* *Vibrio*	*Alcaligenes* *Vibrio*
	N2	*Alcaligenes*	*Alcaligenes* *Vibrio*
	N3	*Alcaligenes*	*Aeromonas* *Pseudomonas*
Sediment	A1	*Alcaligenes* *Pseudomonas*	*Alcaligenes* *Pseudomonas*
	A4	*Micrococcus**	*Pseudomonas* *Vibrio*
	B3	*Alcaligenes* *Micrococcus*	*Aeromonas* *Alcaligenes* *Pseudomonas*

Table 11-19. (concluded)

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Sediment	B4	*Brevibacterium**	*Alcaligenes* *Pseudomonas*
	C1	*Acinetobacter* *Aeromonas* *Brevibacterium**	*Aeromonas* *Vibrio*
	C4	*Alcaligenes*	*Alcaligenes* *Pseudomonas* *Vibrio*
	D1		*Alcaligenes* *Pseudomonas* *Vibrio*
	D4	*Micrococcus*	*Alcaligenes* *Pseudomonas*
	E3	*Flavobacterium* *Pseudomonas*	*Alcaligenes* *Flavobacterium* *Pseudomonas*
	E4	*Alcaligenes* *Pseudomonas*	*Pseudomonas*
	F2	*Alcaligenes*	*Alcaligenes* *Pseudomonas*
	F4		*Alcaligenes* *Pseudomonas*

*Presumptive Identification

Table 11-20. Dominant marine bacteria isolated from microlayer, water (1 m) and sediment samples from selected stations during 1976 winter cruise (Ø2B).

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Microlayer	C1	*Alcaligenes* *Pseudomonas*	*Flavobacterium* *Vibrio**
	N2	*Alcaligenes* *Flavobacterium*	*Alcaligenes* *Flavobacterium*
Water	C1	*Alcaligenes*	*Alcaligenes* *Pseudomonas*
	E3	*Pseudomonas*	*Aeromonas* *Vibrio*
	F2	*Alcaligenes*	*Pseudomonas*
	J1		*Pseudomonas*
	N2	*Alcaligenes*	*Alcaligenes* *Vibrio**
	N3	*Alcaligenes**	*Alcaligenes* *Vibrio*
Sediment	A1	*Pseudomonas*	*Alcaligenes* *Vibrio*
	A4	*Brevibacterium**	*Pseudomonas* *Vibrio*
	B3	*Vibrio*	*Flavobacterium* *Pseudomonas*
	B4		*Flavobacterium* *Vibrio*
	C1	*Alcaligenes*	*Alcaligenes* *Flavobacterium* *Pseudomonas*
	C4		*Pseudomonas* *Vibrio*
	D1	*Alcaligenes*	*Vibrio*

Table 11-20. (concluded)

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Sediment	D4	*Alcaligenes*	*Alcaligenes*
	E3	*Alcaligenes* *Pseudomonas* *Vibrio*	*Vibrio*
	E4		*Aeromonas* *Pseudomonas* *Vibrio*
	F2	*Flavobacterium* *Vibrio*	*Alcaligenes* *Pseudomonas*
	F4	*Pseudomonas*	*Pseudomonas*
	J1		*Vibrio*

*Presumptive Identification

Table 11-21. Dominant marine bacteria isolated from microlayer, water (1 m) and sediment samples from selected stations during 1976 spring cruise (Ø3B).

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Microlayer	C1	*Alcaligenes* *Pseudomonas*	
	E3	*Alcaligenes* *Micrococcus*	
	F2	*Alcaligenes* *Micrococcus*	*Pseudomonas* *Vibrio*
	J1	*Alcaligenes*	*Alcaligenes* *Brevibacterium** *Pseudomonas*
Water	C1	*Alcaligenes* *Flavobacterium*	
	E3	*Pseudomonas*	*Pseudomonas*
	F2	*Alcaligenes* *Vibrio*	*Pseudomonas*
	J1		*Acinetobacter* *Vibrio*
	N2	*Micrococcus*	*Pseudomonas*
	N3	*Flavobacterium*	*Pseudomonas* *Vibrio*
Sediment	A1	*Alcaligenes* Coryneform group* *Flavobacterium*	*Pseudomonas* *Vibrio*
	A4	*Aeromonas* *Pseudomonas* *Vibrio*	*Alcaligenes* *Pseudomonas*
	B3	*Flavobacterium*	*Alcaligenes* *Vibrio*

Table 11-21. (concluded)

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Sediment	B4	*Flavobacterium*	*Alcaligenes* *Flavobacterium* *Pseudomonas*
	C1	*Alcaligenes*	*Alcaligenes* *Vibrio*
	C4	*Alcaligenes* *Flavobacterium*	*Alcaligenes* *Pseudomonas*
	D1	*Alcaligenes*	*Vibrio*
	D4	*Alcaligenes*	*Alcaligenes* *Pseudomonas* *Vibrio*
	E3	*Flavobacterium*	*Pseudomonas*
	E4	*Alcaligenes* *Flavobacterium* *Pseudomonas**	*Pseudomonas*
	F2	*Vibrio*	*Vibrio*
	F4	*Alcaligenes* *Vibrio*	*Alcaligenes* *Pseudomonas*

*Presumptive Identification

Table 11-22. Dominant marine bacteria isolated from microlayer, water (1 m) and sediment samples from selected stations during 1976 summer cruise (Ø4B-Ø4G).

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Microlayer	C1	*Acinetobacter* *Flavobacterium* *Pseudomonas*	*Pseudomonas* *Vibrio*
	E3	*Moraxella* *Pseudomonas*	*Alcaligenes* *Flavobacterium*
	F2	*Alcaligenes* *Pseudomonas*	*Pseudomonas* *Vibrio*
	J1	*Alcaligenes* *Pseudomonas*	*Pseudomonas*
	N2	*Alcaligenes* *Vibrio*	*Alcaligenes* *Flavobacterium* *Pseudomonas*
	N3	*Aeromonas* *Pseudomonas* *Vibrio*	*Aeromonas* *Moraxella* *Pseudomonas*
Water	C1	*Flavobacterium*	*Vibrio*
	E3	*Vibrio*	*Pseudomonas*
	F2	*Pseudomonas*	*Pseudomonas*
	J1	*Alcaligenes* *Pseudomonas*	*Pseudomonas*
	N2	*Pseudomonas*	*Alcaligenes*
	N3	*Pseudomonas*	*Pseudomonas*
Sediment	A1	*Pseudomonas** *Vibrio*	*Alcaligenes* *Flavobacterium* *Pseudomonas*
	A4	*Alcaligenes* *Flavobacterium*	*Aeromonas* *Alcaligenes*
	B2	*Alcaligenes*	*Pseudomonas*
	B3	*Alcaligenes* *Flavobacterium*	*Acinetobacter* *Pseudomonas*

Table 11-22. (continued)

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Sediment	C1	*Acinetobacter* *Alcaligenes* *Flavobacterium*	*Aeromonas* *Pseudomonas* *Vibrio*
	C4	*Flavobacterium*	*Alcaligenes* *Flavobacterium* *Pseudomonas*
	D1	*Acinetobacter** *Vibrio*	*Pseudomonas* *Vibrio*
	D4	*Acinetobacter* *Aeromonas* *Alcaligenes* *Vibrio*	*Pseudomonas* *Vibrio*
	E1	*Flavobacterium* *Pseudomonas*	*Pseudomonas* *Vibrio*
	E4	*Pseudomonas*	*Pseudomonas*
	F1	*Alcaligenes* *Flavobacterium*	*Pseudomonas*
	F4	*Acinetobacter** *Pseudomonas** *Vibrio*	*Pseudomonas*
	G1	*Alcaligenes* *Vibrio*	*Flavobacterium* *Pseudomonas*
	G3	*Flavobacterium*	*Pseudomonas*
	G6	*Alcaligenes* *Flavobacterium* *Pseudomonas**	*Pseudomonas*
	H1	*Pseudomonas*	*Pseudomonas* *Vibrio*
	I2	*Alcaligenes* *Vibrio*	*Alcaligenes*

Table 11-22. (concluded)

Sample Type	Station	Taxonomic Groups Isolated	
		Heterotrophs	Petroleum Degraders
Sediment	I4	*Acinetobacter* *Pseudomonas*	*Aeromonas* *Pseudomonas*
	J1	*Vibrio*	*Alcaligenes* *Pseudomonas* *Vibrio*
	K1	*Flavobacterium*	*Alcaligenes* *Pseudomonas*
	K3	*Flavobacterium*	*Pseudomonas*
	K6	*Alcaligenes* *Vibrio*	*Alcaligenes* *Pseudomonas*
	L1	*Acinetobacter** *Alcaligenes*	*Aeromonas* *Alcaligenes* *Vibrio*
	L3		*Pseudomonas*
	L4	*Flavobacterium*	
	L5	*Flavobacterium* *Vibrio*	*Pseudomonas*

*Presumptive Identification

Table 11-23. Dominant bacterial genera isolated from highest dilution positive HET (Heterotrophic) MPN tubes from 1 m water samples. Dashes indicate no viable isolates.

Station	Seasonal Frequency	Fall	Winter	Spring	Summer
C1	2/4	--	*Alcaligenes* sp.	*Alcaligenes* sp.	--
	2/4	--	--	*Flavobacterium* sp.	*Flavobacterium* sp.
	1/4	Coryneform	--	--	--
N2	2/4	*Alcaligenes* sp.	*Alcaligenes* sp.	--	--
	1/4	--	--	*Micrococcus* sp.	--
	1/4	--	--	--	*Pseudomonas* sp.
N3	2/4	*Alcaligenes* sp.	*Alcaligenes* sp.	--	--
	1/4	--	--	*Flavobacterium* sp.	--
	1/4	--	--	--	*Pseudomonas* sp.
E3	3/4	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	--
	1/4	--	--	--	*Vibrio* sp.
F2	2/4	--	*Alcaligenes* sp.	*Alcaligenes* sp.	--
	2/4	*Pseudomonas* sp.	--	--	*Pseudomonas* sp.
	1/4	*Micrococcus* sp.	--	--	--
	1/4	--	--	*Vibrio* sp.	--
J1	2/4	*Pseudomonas* sp.	--	--	*Pseudomonas* sp.
	1/4	--	--	--	*Alcaligenes* sp.
	1/4	*Brevibacterium*	--	--	--
	1/4	*Vibrio* sp.	--	--	--

Table 11-24. Dominant bacterial genera isolated from highest dilution positive HC (Petroleum Degrading) MPN tubes from 1 m water samples. Dashes indicate no viable isolates.

Station	Seasonal Frequency	Fall	Winter	Spring	Summer
C1	2/4	*Pseudomonas* sp.	*Pseudomonas* sp.	--	--
	1/4	--	*Alcaligenes* sp.	--	--
	1/4	*Flavobacterium* sp.	--	--	--
	1/4	--	--	--	*Vibrio* sp.
N2	3/4	*Alcaligenes* sp.	*Alcaligenes* sp.	--	*Alcaligenes* sp.
	2/4	*Vibrio* sp.	*Vibrio* sp.	--	--
	1/4	--	--	*Pseudomonas* sp.	--
N3	3/4	*Pseudomonas* sp.	--	*Pseudomonas* sp.	*Pseudomonas* sp.
	2/4	--	*Vibrio* sp.	*Vibrio* sp.	--
	1/4	*Aeromonas* sp.	--	--	--
	1/4	*Alcaligenes* sp.	--	--	--
E3	3/4	*Pseudomonas* sp.	--	*Pseudomonas* sp.	*Pseudomonas* sp.
	1/4	--	*Aeromonas* sp.	--	--
	1/4	*Flavobacterium* sp.	--	--	--
	1/4	--	*Vibrio* sp.	--	--
F2	3/4	--	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
	1/4	*Alcaligenes* sp.	--	--	--
J1	2/4	--	*Pseudomonas* sp.	--	*Pseudomonas* sp.
	2/4	*Vibrio* sp.	--	*Vibrio* sp.	--
	1/4	*Alcaligenes* sp.	--	--	--
	1/4	--	--	*Acinetobacter* sp.	--

Table 11-25. Dominant bacterial genera isolated from highest dilution positive HET (Heterotrophic) MPN tubes from sediment samples collected quarterly (BLM Ø1B-Ø4B). Dashes indicate no viable isolates.

Station	Seasonal Frequency	Fall	Winter	Spring	Summer
Inner	4/4	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.
Shelf	2/4	*Acinetobacter* sp.	--	--	*Acinetobacter* sp.
Sediment	2/4	--	--	*Flavobacterium* sp.	*Flavobacterium* sp.
Stations	2/4	*Aeromonas* sp.	--	--	*Aeromonas* sp.
	1/4	*Micrococcus* sp.	--	--	--
	1/4	--	--	--	*Vibrio* sp.
	1/4	*Brevibacterium*	--	--	--
	1/4	Coryneform	--	--	--
Outer	4/4	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.
Shelf	4/4	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
Sediment	3/4	*Flavobacterium* sp.	--	*Flavobacterium* sp.	*Flavobacterium* sp.
Stations	2/4	--	*Vibrio* sp.	--	*Vibrio* sp.
Shelf	3/4	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.
Break	3/4	--	*Vibrio* sp.	*Vibrio* sp.	*Vibrio* sp.
Sediment	2/4	--	*Flavobacterium* sp.	--	*Flavobacterium* sp.
Stations	2/4	--	*Pseudomonas* sp.	*Pseudomonas* sp.	--
	1/4	--	--	*Aeromonas* sp.	--
	1/4	*Micrococcus* sp.	--	--	--
	1/4	--	*Brevibacterium* sp.	--	--
	1/4	--	--	Coryneform	--

Table 11-26. Dominant bacterial genera isolated from highest dilution positive HC (Petroleum Degrading) MPN tubes from sediment samples collected quarterly (BLM Ø1B-Ø4B). Dashes indicate no viable isolates.

Station	Seasonal Frequency	Fall	Winter	Spring	Summer
Inner	4/4	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.
Shelf	4/4	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
Sediment	4/4	*Vibrio* sp.	*Vibrio* sp.	*Vibrio* sp.	*Vibrio* sp.
Stations	2/4	*Aeromonas* sp.	--	--	*Aeromonas* sp.
	2/4	--	*Flavobacterium* sp.	--	*Flavobacterium* sp.
Outer	4/4	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.
Shelf	4/4	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
Sediment	3/4	*Flavobacterium* sp.	*Flavobacterium* sp.	--	*Flavobacterium* sp.
Stations	2/4	--	*Vibrio* sp.	*Vibrio* sp.	--
	1/4	--	--	--	*Acinetobacter* sp.
Shelf	4/4	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.
Break	4/4	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
Sediment	3/4	*Vibrio* sp.	*Vibrio* sp.	*Vibrio* sp.	--
Stations	1/4	--	--	--	*Aeromonas* sp.

assays are shown in Tables 11-27 and 11-28.

Percentages of total isolates utilizing petroleum varied from 6 to 36% on a seasonal basis. Genera most frequently isolated such as *Pseudomonas*, *Flavobacterium*, *Aeromonas*, and *Vibrio* exhibited proportions of confirming isolates between 10-40%. Interestingly, *Alcaligenes*, one genus frequently isolated from HM and ESWB tubes, exhibited the lowest proportion of confirmation. Taxonomic groups such as Coryneform, *Brevibacterium*, *Acinetobacter*, and *Moraxella*, although of much lower frequency of isolation and represented by fewer isolates, exhibited the largest percentages of petroleum utilization.

Total percentages of all confirming isolates were highest in the fall and summer seasons. Isolates from microlayer samples exhibited the highest percentages of confirming isolates followed by surface water and sediment samples.

Petroleum Degradation Experiments

Closed Flask Experiments

Closed Flasks, Water Inocula, Enumeration. Results of closed flask petroleum degradation experiments utilizing water inocula are summarized in Tables 11-29 through 11-34 and Figures 11-27 through 11-35. The following summarizes these results on a station to station basis. Unless otherwise stated, descriptions of changes in cell numbers refer to all four seasons.

I. Station N1(C1). Numbers of heterotrophic (HET) and petroleum degrading (HC) bacteria at Station N1 were consistently larger than all other water transect stations. Heterotrophic bacteria always increased in closed flasks under both enriched and non-enriched conditions. However, increases in flasks receiving nutrient amendment were larger than in non-enriched flasks. Generally the largest increase in heterotroph levels occurred at 3 days under both nutrient regimes. Heterotroph populations in the oil free flask (summer) exhibited population increases similar to those occurring in the non-enriched oil flask.

Petroleum degrading bacteria generally exhibited the largest increase in numbers at 3 days in non-enriched and enriched flasks. An exception was the non-enriched flask from spring where the initial populations were close to maximum values reached in other flasks after three days. Numbers in enriched flasks tended to be larger than in non-enriched flasks. Levels of petroleum degrading bacteria in the oil free control were comparable to non-enriched flasks. Values of the ratio HC/HET generally reflected changes in the levels of petroleum degrading bacteria and tended to be higher in enriched flasks. Values of HC/HET in oil free controls were essentially equivalent to those measured in both enriched and non-enriched flasks during the summer experiments.

II. Station N2. Heterotroph counts under both nutrient regimes generally manifested the greatest increase at 3 days of incubation.

Table 11-27. Proportion of isolates from ESWB tubes (all samples) by genera able to grow on South Louisiana crude oil in pure culture.

Genus	Season				Percentage Confirming
	Fall	Winter	Spring	Summer	
Pseudomonas	9/38	1/14	1/22	21/57	24.4 (n=131)
Alcaligenes	1/15	0/9	0/11	2/13	6.3 (n=48)
Vibrio	0/7	2/13	0/13	3/10	11.6 (n=43)
Acinetobacter	-	-	1/1	1/1	100 (n=2)
Flavobacterium	2/5	0/5	0/1	4/5	37.5 (n=16)
Aeromonas	1/4	0/2	-	1/5	18.2 (n=11)
Brevibacterium	-	-	1/2	-	50 (n=2)
Coryneform	1/1	-	-	-	100 (n=1)
Moraxella osloensis	-	-	-	1/1	100 (n=1)
Percentage of Total Isolates Confirming as Petroleum Degraders	20	7	6	36	

Table 11-28. Proportions of selected dominant isolates from ESWB tubes able to grow in pure culture on South Louisiana crude oil as a function of sample type.

Sample Type	Season				Mean Proportion
	Fall	Winter	Spring	Summer	
Microlayer	11/15	1/17	1/8	11/35	31/100
Surface Water (1m)	2/20	4/18	1/6	1/23	13/100
Sediment	1/40	2/48	1/16	32/167	8/100

Table 11-29. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments* using seawater collected at Station C1 as inocula, BLMØ1B-Ø4B. Log MPN in parentheses. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cruise	Days of Incubation	Oil Free Control			Non-Enriched + Oil			Enriched + Oil		
		HC MPN/ml	HET MPN/ml	Log HC/HET	HC MPN/ml	HET MPN/ml	Log HC/HET	HC MPN/ml	HET MPN/ml	Log HC/HET
Ø1B	0	$4.3x10^2$ (2.63)	$9.3x10^4$ (4.97)	-2.34						
	3				$4.6x10^5$ (5.66)	$1.1x10^6$ (6.04)	-0.38	$2.4x10^7$ (7.38)	$1.1x10^8$ (8.04)	-0.66
	6				$>2.4x10^7$ (7.38)	$4.6x10^6$ (6.66)	+0.72	$>2.4x10^8$ (>8.38)	$7.5x10^7$ (7.88)	+0.51
	12				$1.5x10^5$ (5.18)	$2.4x10^6$ (6.38)	-1.20	$4.6x10^7$ (7.66)	$9.3x10^7$ (7.97)	-0.31
	24				$9.3x10^4$ (4.97)	$4.6x10^6$ (6.67)	-1.70	$9.3x10^5$ (5.97)	$9.3x10^6$ (6.97)	-1.00
	48				$9.3x10^3$ (3.97)	$9.3x10^6$ (6.97)	-3.00	$7.5x10^5$ (5.88)	$2.4x10^8$ (8.38)	-2.51
Ø2B	0	$2.1x10^1$ (1.32)	$2.4x10^4$ (4.38)	-3.1						
	3				$3.9x10^4$ (4.59)	$2.4x10^5$ (5.38)	-0.79	$2.3x10^4$ (4.36)	$4.3x10^6$ (6.63)	-2.30
	6				$9.3x10^4$ (4.97)	$4.3x10^5$ (5.63)	-0.66	$4.3x10^5$ (5.63)	$2.3x10^6$ (6.36)	-0.73
	12				$2.3x10^4$ (4.36)	$2.4x10^6$ (6.38)	-2.00	$4.3x10^6$ (6.63)	$2.3x10^7$ (7.36)	-0.73
	24				$4.3x10^5$ (5.63)	$7.5x10^6$ (6.88)	-1.20	$2.4x10^7$ (7.38)	$4.6x10^7$ (7.66)	-0.28
	48				$2.1x10^5$ (5.32)	$3.9x10^5$ (5.59)	-0.27	$2.4x10^6$ (6.38)	$3.9x10^7$ (7.59)	-1.20
Ø3B	0	$1.1x10^5$ (5.04)	$4.6x10^4$ (4.66)	+0.4						
	3				$4.3x10^4$ (4.63)	$4.6x10^6$ (6.66)	-2.03	$2.4x10^6$ (6.38)	$2.3x10^6$ (6.36)	+0.02
	6				$2.3x10^5$ (5.36)	$9.3x10^5$ (5.97)	-0.61	$2.3x10^6$ (6.36)	$9.3x10^6$ (6.97)	-0.61
	12				$4.3x10^5$ (5.63)	$2.3x10^6$ (6.36)	-0.73	$2.4x10^6$ (6.38)	$4.6x10^8$ (8.66)	-2.00
	24				$2.3x10^5$ (5.36)	$2.3x10^6$ (6.36)	-1.00	$>2.4x10^8$ (>8.36)	$4.6x10^8$ (8.66)	-0.28
	48				$9.3x10^3$ (3.97)	$9.3x10^4$ (4.97)	-1.00	$9.3x10^7$ (7.97)	$2.3x10^7$ (7.36)	+0.61
Ø4B	0	$9.3x10^2$ (2.97)	$7.5x10^4$ (4.88)	-1.91						
	3	$9.3x10^4$ (4.97)	$2.4x10^6$ (6.38)	-1.41	$2.1x10^5$ (5.32)	$2.4x10^6$ (6.38)	-1.06	$>2.4x10^7$ (>7.38)	$2.4x10^7$ (7.38)	0.00
	6	$4.3x10^3$ (3.66)	$2.3x10^4$ (4.36)	-0.72	$2.1x10^5$ (5.32)	$4.3x10^5$ (5.63)	-0.31	$>2.4x10^6$ (>6.38)	$2.4x10^7$ (7.38)	-1.00
	24	$9.3x10^4$ (4.97)	$9.3x10^4$ (4.97)	0.00	$4.3x10^5$ (5.63)	$9.3x10^5$ (5.97)	-0.34	$>2.4x10^8$ (>8.38)	$4.3x10^7$ (7.63)	+0.75
	48	$9.3x10^4$ (4.97)	$2.3x10^5$ (5.36)	-0.39	$4.3x10^4$ (4.63)	$4.3x10^5$ (5.63)	-1.00	$2.3x10^7$ (7.36)	$2.4x10^8$ (8.63)	-1.02

*Incubation Temperature: 01B-22°C; 02B-8°C; 03B-15°C; 04B-21°C

Table 11-30. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments*using seawater collected at Station N2 as inocula, BLMØ1B-Ø4B. Log MPN in parentheses. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cruise	Days of Incubation	Oil Free Control			Non-Enriched + Oil			Enriched + Oil		
		HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$
Ø1B	0	$2.3(0.36)$	$9.3\times10^{3}(3.97)$	-3.60						
	3				$4.3\times10^{4}(4.63)$	$4.3\times10^{5}(5.63)$	-1.00	$\geq2.4\times10^{7}(7.38)$	$\geq2.4\times10^{7}(7.38)$	0.00
	6				$\geq2.4\times10^{4}(4.38)$	$4.6\times10^{7}(7.66)$	-3.28	$\geq2.4\times10^{7}(7.38)$	$2.4\times10^{8}(8.38)$	-1.00
	12				$9.3\times10^{1}(1.97)$	$4.3\times10^{4}(4.63)$	-2.66	$4.6\times10^{7}(7.66)$	$4.3\times10^{7}(7.36)$	0.04
	24				ND	$9.3\times10^{4}(4.97)$		$4.3\times10^{5}(5.36)$	$9.3\times10^{6}(6.97)$	-1.34
	48				$1.4\times10^{2}(2.15)$	$4.3\times10^{6}(6.63)$	-4.48	$1.5\times10^{7}(7.18)$	$2.4\times10^{8}(8.36)$	-1.20
Ø2B	0	$9.3\times10^{0}(0.97)$	$1.1\times10^{4}(4.04)$	-3.10						
	3				$4.3\times10^{2}(2.63)$	$2.3\times10^{5}(5.36)$	-2.70	$2.3\times10^{3}(3.36)$	$4.3\times10^{5}(5.63)$	-2.30
	6				$2.8\times10^{3}(3.45)$	$2.4\times10^{6}(6.38)$	-2.90	$1.5\times10^{4}(4.18)$	$7.5\times10^{6}(6.88)$	-2.70
	12				$9.3\times10^{3}(3.97)$	$9.3\times10^{5}(5.97)$	-2.00	$4.3\times10^{5}(5.63)$	$2.3\times10^{6}(6.38)$	-0.73
	24				$2.3\times10^{5}(5.36)$	$7.5\times10^{5}(5.88)$	-0.51	$2.4\times10^{7}(7.38)$	$2.4\times10^{7}(7.38)$	0.0
	48				$1.1\times10^{6}(6.15)$	$7.5\times10^{5}(5.88)$	+0.17	$2.4\times10^{7}(7.38)$	$2.3\times10^{7}(7.36)$	-0.98
Ø3B	0	$2.3\times10^{-2}(-1.6)$	$2.4\times10^{4}(4.38)$	-6.00						
	3				$3.6\times10^{0}(0.56)$	$4.3\times10^{5}(5.63)$	-5.08	$1.5\times10^{3}(3.18)$	$7.5\times10^{5}(5.88)$	-2.70
	6				$4.6\times10^{5}(5.66)$	$9.3\times10^{5}(5.97)$	-0.31	$4.3\times10^{5}(5.66)$	$2.3\times10^{5}(5.36)$	+0.27
	12				$7.5\times10^{3}(3.88)$	$9.3\times10^{5}(5.97)$	-2.09	$4.3\times10^{3}(3.66)$	$4.3\times10^{6}(6.66)$	-3.00
	24				$1.5\times10^{4}(4.18)$	$4.3\times10^{4}(4.63)$	-0.46	$1.5\times10^{7}(7.18)$	$9.3\times10^{6}(6.97)$	+0.21
	48				$4.3\times10^{5}(5.66)$	$4.3\times10^{4}(4.66)$	+1.00	$2.4\times10^{7}(7.38)$	$2.3\times10^{7}(7.36)$	+0.17
Ø4B	0	$4.3\times10^{-1}(0.37)$	$9.3\times10^{2}(2.97)$	-3.34						
	3	$9.3\times10^{-1}(-0.03)$	$9.3\times10^{5}(5.97)$	-6.00	$1.5\times10^{2}(2.18)$	$2.3\times10^{5}(5.36)$	-3.19	$1.6\times10^{3}(3.2)$	$1.5\times10^{6}(6.18)$	-2.97
	6	$<1\times10^{2}$	$3.9\times10^{5}(5.59)$		$1.3\times10^{3}(3.11)$	$2.3\times10^{5}(5.36)$	-2.25	$7.3\times10^{2}(2.86)$	$1.5\times10^{6}(6.18)$	-3.31
	24	$9.1\times10^{0}(0.95)$	$2.3\times10^{5}(5.36)$	-4.40	$2.0\times10^{2}(2.3)$	$2.3\times10^{5}(5.36)$	-3.06	$9.3\times10^{5}(5.97)$	$4.3\times10^{6}(6.63)$	-0.66
	48	$2.0\times10^{0}(0.30)$	$7.5\times10^{4}(4.88)$	-4.57	$9.3\times10^{2}(2.97)$	$2.3\times10^{5}(5.36)$	-2.39	$1.5\times10^{6}(6.18)$	$2.4\times10^{7}(7.38)$	-1.20

*Incubation Temperature: 01B-22°C; 02B-8°C; 03B-15°C; 04B-21°C

Table 11-31. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments* using seawater collected at Station N3 as inocula, BLMØ1B-Ø4B. Log MPN in parentheses. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cruise	Days of Incubation	Oil Free Control			Non-Enriched + Oil			Enriched + Oil		
		HC MPN/ml	HET MPN/ml	Log HC/HET	HC MPN/ml	HET MPN/ml	Log HC/HET	HC MPN/ml	HET MPN/ml	Log HC/HET
Ø1B	0	$4.3x10^{\bar{1}}$ (-0.63)	$2.3x10^{3}$ (3.36)	-3.72						
	3				$4.3x10^{3}$ (3.63)	$\geq 2.4x10^{6}$ (6.38)	-2.74	$2.3x10^{7}$ (7.36)	$1.1x10^{8}$ (8.04)	-0.66
	6				$2.4x10^{6}$ (6.38)	$2.4x10^{6}$ (6.38)	0.00	$\geq 2.4x10^{7}$ (7.38)	$4.3x10^{6}$ (6.63)	+0.75
	12				$4.3x10^{5}$ (5.63)	$3.9x10^{5}$ (5.59)	0.00	$2.1x10^{7}$ (7.32)	$2.4x10^{8}$ (8.38)	-1.06
	24				ND	$9.3x10^{4}$ (4.97)		$1.5x10^{6}$ (6.18)	$4.3x10^{6}$ (6.63)	-0.46
	48				$9.3x10^{4}$ (4.97)	$2.3x10^{5}$ (5.36)	-0.39	$2.1x10^{6}$ (6.32)	$7.5x10^{6}$ (6.88)	-0.55
Ø2B	0	$7.5x10^{\bar{2}}$ (-1.88)	$1.5x10^{2}$ (2.18)	-3.30						
	3				ND	$3.6x10^{3}$ (3.56)		ND	ND	
	6				$4.3x10^{3}$ (3.63)	$9.3x10^{4}$ (4.97)	-1.3	$4.3x10^{5}$ (5.63)	$7.5x10^{5}$ (5.88)	-0.24
	12				$4.3x10^{4}$ (4.63)	$4.3x10^{5}$ (5.63)	-1.00	$2.4x10^{6}$ (6.38)	$2.3x10^{6}$ (6.36)	+0.02
	24				$4.6x10^{5}$ (5.38)	$1.5x10^{6}$ (6.18)	-0.51	$2.4x10^{6}$ (6.38)	$2.4x10^{7}$ (7.38)	-1.00
	48				$4.3x10^{6}$ (6.63)	$1.5x10^{6}$ (6.18)	+0.46	$9.3x10^{6}$ (6.97)	$4.3x10^{7}$ (7.63)	-0.66
Ø3B	0	$9.3x10^{\bar{1}}$ (-0.03)	$9.3x10^{2}$ (2.97)	-3.00						
	3				$2.3x10^{2}$ (2.36)	$9.3x10^{5}$ (5.97)	-3.61	$2.3x10^{3}$ (3.36)	$2.1x10^{5}$ (5.32)	-1.96
	6				$4.3x10^{3}$ (3.63)	$2.3x10^{6}$ (6.36)	-2.72	$9.3x10^{5}$ (5.97)	$9.3x10^{5}$ (5.97)	0.00
	12				$2.3x10^{3}$ (3.36)	$7.5x10^{5}$ (5.68)	-2.51	$2.1x10^{4}$ (4.32)	$2.4x10^{7}$ (7.38)	-3.06
	24				$9.3x10^{2}$ (2.97)	$4.3x10^{5}$ (5.63)	-2.66	$1.1x10^{8}$ (8.04)	$2.3x10^{7}$ (7.36)	+0.68
	48				$4.3x10^{4}$ (4.63)	$1.5x10^{6}$ (6.18)	-1.54	$2.4x10^{8}$ (8.38)	$1.5x10^{7}$ (7.18)	+1.20
Ø4B	0	$3.9x10^{\bar{1}}$ (-0.59)	$7.5x10^{2}$ (2.88)	-3.28						
	3	$2.1x10^{3}$ (3.32)	$4.6x10^{5}$ (5.63)	-2.34	$2.0x10^{1}$ (1.30)	$9.3x10^{6}$ (6.97)	-5.67	$1.5x10^{4}$ (4.18)	$4.3x10^{5}$ (5.63)	-1.46
	6	$1.5x10^{1}$ (1.18)	$2.4x10^{6}$ (6.38)	-5.20	$3.4x10^{1}$ (1.54)	$7.5x10^{5}$ (5.88)	-4.34	$2.0x10^{1}$ (1.30)	$9.3x10^{5}$ (5.97)	-4.67
	24	ND	$4.3x10^{5}$ (5.63)		ND	$9.3x10^{5}$ (5.97)		ND	$4.6x10^{6}$ (6.68)	
	48	$3.0x10^{\bar{1}}$ (-0.52)	$9.3x10^{5}$ (5.97)	-5.49	$3.6x10^{1}$ (1.56)	$4.3x10^{4}$ (4.36)	-3.08	$2.3x10^{4}$ (4.36)	$9.3x10^{5}$ (5.97)	-1.61

*Incubation Temperature: 01B-22°C; 02B-8°C; 03B-15°C; 04B-21°C

Table 11-32. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments*using seawater collected at Station E3 as inocula, BLMØ1B-Ø4B. Log MPN in parentheses. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cruise	Days of Incubation	Oil Free Control			Non-Enriched + Oil			Enriched + Oil		
		HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$
Ø1B	0	$9.3\text{x}10^{2}$ (-1.03)	$9.3\text{x}10^{2}$ (2.97)	-4.00						
	3				$9.3\text{x}10^{5}$ (5.97)	$7.5\text{x}10^{4}$ (4.88)	+1.08	$\geq 2.4\text{x}10^{7}$ ($\geq$7.38)	$2.4\text{x}10^{7}$ (7.38)	0.00
	6				$\geq 2.4\text{x}10^{4}$ ($\geq$4.38)	$2.4\text{x}10^{4}$ (4.38)	0.00	$\geq 2.4\text{x}10^{7}$ ($\geq$7.38)	$9.3\text{x}10^{6}$ (6.97)	+0.41
	12				$2.3\text{x}10^{5}$ (5.36)	$2.4\text{x}10^{7}$ (7.38)	-2.02	$9.3\text{x}10^{5}$ (5.97)	$4.3\text{x}10^{5}$ (5.63)	+0.34
	24				ND	$9.3\text{x}10^{6}$ (6.97)		$9.3\text{x}10^{3}$ (3.97)	$9.3\text{x}10^{4}$ (4.97)	-1.00
	48				ND	$2.3\text{x}10^{4}$ (4.36)		$4.3\text{x}10^{3}$ (3.63)	$9.3\text{x}10^{5}$ (5.97)	-2.34
Ø2B	0	$1.2\text{x}10^{1}$ (-0.92)	$2.3\text{x}10^{1}$ (1.36)	-2.30						
	3				ND	$7.3\text{x}10^{3}$ (3.86)		ND	$9.1\text{x}10^{3}$ (3.95)	
	6				ND	$7.5\text{x}10^{4}$ (4.88)		$4.6\text{x}10^{6}$ (6.66)	$2.4\text{x}10^{7}$ (7.38)	-0.72
	12				$1.5\text{x}10^{4}$ (4.18)	$1.2\text{x}10^{6}$ (6.08)	-1.90	$1.1\text{x}10^{7}$ (7.04)	$2.4\text{x}10^{7}$ (7.38)	-0.34
	24				$4.6\text{x}10^{5}$ (5.66)	$2.3\text{x}10^{6}$ (6.36)	-0.70	$4.6\text{x}10^{5}$ (5.66)	$2.4\text{x}10^{7}$ (7.38)	-1.70
	48				$4.3\text{x}10^{5}$ (5.63)	$4.3\text{x}10^{5}$ (5.63)	0.00	$9.3\text{x}10^{6}$ (6.97)	$3.9\text{x}10^{7}$ (7.59)	-0.62
Ø3B	0	$2.3\text{x}10^{2}$ (-1.6)	$1.5\text{x}10^{2}$ (2.2)	-3.80						
	3				ND	$2.3\text{x}10^{5}$ (5.36)		ND	$2.3\text{x}10^{4}$ (4.36)	
	6				$2.4\text{x}10^{7}$ (7.38)	$1.5\text{x}10^{7}$ (7.18)	+0.20	$1.5\text{x}10^{7}$ (7.18)	$2.4\text{x}10^{7}$ (7.38)	-0.20
	12				$4.3\text{x}10^{4}$ (4.63)	$9.1\text{x}10^{4}$ (4.95)	-0.33	$4.6\text{x}10^{7}$ (7.66)	$4.6\text{x}10^{7}$ (7.66)	0.00
	24				$2.3\text{x}10^{4}$ (4.36)	$2.3\text{x}10^{5}$ (5.36)	-1.00	$4.6\text{x}10^{7}$ (7.66)	$4.6\text{x}10^{8}$ (8.66)	-1.00
	48				$4.3\text{x}10^{4}$ (4.63)	$2.3\text{x}10^{5}$ (5.36)	-0.73	$4.3\text{x}10^{7}$ (7.63)	$4.6\text{x}10^{8}$ (8.66)	-1.03
Ø4B	0	$2.3\text{x}10^{1}$ (-0.36)	$4.3\text{x}10^{3}$ (3.63)	-4.27						
	3	$1.5\text{x}10^{3}$ (3.18)	$2.3\text{x}10^{5}$ (5.36)	-2.19	$2.1\text{x}10^{3}$ (3.34)	$2.3\text{x}10^{5}$ (5.36)	-2.04	$4.3\text{x}10^{3}$ (3.63)	$2.3\text{x}10^{5}$ (5.36)	-1.73
	6	$1.5\text{x}10^{2}$ (2.18)	$2.3\text{x}10^{4}$ (4.36)	-2.19	$1.2\text{x}10^{2}$ (2.08)	$9.3\text{x}10^{5}$ (5.97)	-3.89	$1.5\text{x}10^{2}$ (2.18)	$4.3\text{x}10^{5}$ (5.36)	-3.46
	24	ND	$2.4\text{x}10^{6}$ (6.38)		$4.3\text{x}10^{2}$ (2.63)	$2.3\text{x}10^{5}$ (5.36)	-2.73	$2.3\text{x}10^{4}$ (4.36)	$1.5\text{x}10^{6}$ (6.18)	-1.81
	48	$1.5\text{x}10^{0}$ (0.18)	$2.3\text{x}10^{5}$ (5.36)	-5.18	$2.3\text{x}10^{2}$ (2.36)	$2.3\text{x}10^{5}$ (5.36)	-3.00	$2.1\text{x}10^{3}$ (3.32)	$4.3\text{x}10^{6}$ (6.63)	-3.31

*Incubation Temperature: 01B-22°C; 02B-8°C; 03B-15°C; 04B-21°C

Table 11-33. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments*using seawater collected at Station F2 as inocula, BLMØ1B-Ø4B. Log MPN in parentheses. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cruise	Days of Incubation	Oil Free Control			Non-Enriched + Oil			Enriched + Oil		
		HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$
Ø1B	0	$2.3\text{x}10^{0}$ (0.36)	$4.3\text{x}10^{1}$ (1.63)	-1.28						
	3				$9.3\text{x}10^{4}$ (4.97)	$3.9\text{x}10^{5}$ (5.59)	-0.62	$\geq 2.4\text{x}10^{7}$ ($\geq$7.38)	$4.3\text{x}10^{6}$ (6.63)	+0.75
	6				$\geq 2.4\text{x}10^{4}$ ($\geq$4.38)	$\geq 2.4\text{x}10^{5}$ ($\geq$5.38)	-1.00	$4.3\text{x}10^{6}$ (6.63)	$2.4\text{x}10^{7}$ (7.38)	-0.75
	12				$1.5\text{x}10^{4}$ (4.18)	$1.5\text{x}10^{5}$ (5.18)	-1.00	$7.5\text{x}10^{6}$ (6.88)	$2.1\text{x}10^{7}$ (7.32)	-0.44
	24				$1.5\text{x}10^{3}$ (3.18)	$4.3\text{x}10^{5}$ (5.63)	-2.46	$9.3\text{x}10^{5}$ (5.99)	$1.5\text{x}10^{7}$ (7.18)	-1.17
	48				$4.3\text{x}10^{0}$ (0.63)	$2.3\text{x}10^{5}$ (5.36)	-4.72	$4.6\text{x}10^{6}$ (6.66)	$4.6\text{x}10^{7}$ (7.66)	-1.00
Ø2B	0	$1.5\text{x}10^{1}$ (1.18)	$4.3\text{x}10^{2}$ (2.63)	-1.50						
	3				ND	$1.5\text{x}10^{4}$ (4.18)		ND	$9.1\text{x}10^{3}$ (3.96)	
	6				$2.3\text{x}10^{4}$ (4.36)	$9.3\text{x}10^{5}$ (5.97)	-1.60	$1.5\text{x}10^{5}$ (5.18)	$9.3\text{x}10^{6}$ (6.97)	-1.80
	12				$2.3\text{x}10^{4}$ (4.36)	$4.3\text{x}10^{6}$ (6.63)	-2.30	$2.7\text{x}10^{7}$ (7.43)	$4.6\text{x}10^{7}$ (7.63)	-0.23
	24				$9.3\text{x}10^{4}$ (4.97)	$4.3\text{x}10^{5}$ (5.63)	-0.66	$2.4\text{x}10^{6}$ (6.38)	$9.3\text{x}10^{6}$ (6.97)	-0.59
	48				$7.5\text{x}10^{4}$ (4.88)	$2.3\text{x}10^{5}$ (5.36)	-0.49	$4.6\text{x}10^{7}$ (7.66)	$2.3\text{x}10^{7}$ (7.36)	+0.30
Ø3B	0	$2.3\text{x}10^{-2}$ (-1.6)	$9.3\text{x}10^{2}$ (2.97)	-4.60						
	3				$4.3\text{x}10^{2}$ (2.63)	$2.3\text{x}10^{5}$ (5.36)	-2.73	$2.3\text{x}10^{3}$ (3.36)	$2.3\text{x}10^{5}$ (5.36)	-2.00
	6				$4.3\text{x}10^{4}$ (4.63)	$2.3\text{x}10^{5}$ (5.36)	-0.73	$1.5\text{x}10^{4}$ (4.18)	$2.3\text{x}10^{5}$ (5.36)	-1.19
	12				$2.3\text{x}10^{4}$ (4.36)	$2.3\text{x}10^{6}$ (6.36)	-2.00	$2.9\text{x}10^{6}$ (6.46)	$2.1\text{x}10^{7}$ (7.30)	-0.86
	24				$1.5\text{x}10^{5}$ (5.18)	$4.3\text{x}10^{5}$ (5.63)	-0.46	$9.3\text{x}10^{6}$ (6.97)	$4.3\text{x}10^{7}$ (7.63)	-0.66
	48				$1.5\text{x}10^{4}$ (4.18)	$9.3\text{x}10^{5}$ (5.97)	-1.79	$7.5\text{x}10^{7}$ (7.88)	$2.4\text{x}10^{8}$ (8.38)	-0.51
Ø4B	0	$4.3\text{x}10^{-1}$ (-0.37)	$1.5\text{x}10^{3}$ (3.18)	-3.54						
	3	$3.4\text{x}10^{0}$ (0.53)	$2.4\text{x}10^{5}$ (5.38)	-4.85	$2.9\text{x}10^{3}$ (3.46)	$4.3\text{x}10^{5}$ (5.63)	-2.17	$1.2\text{x}10^{5}$ (5.08)	$3.9\text{x}10^{5}$ (5.59)	-0.51
	6	$3.6\text{x}10^{2}$ (2.56)	$2.3\text{x}10^{5}$ (5.36)	-2.81	$1.5\text{x}10^{3}$ (3.18)	$9.3\text{x}10^{5}$ (5.97)	-2.79	$4.3\text{x}10^{3}$ (3.63)	$9.3\text{x}10^{5}$ (5.97)	-2.34
	24	$3.6\text{x}10^{1}$ (1.56)	$9.3\text{x}10^{3}$ (3.97)	-2.41	$2.9\text{x}10^{2}$ (2.46)	$9.3\text{x}10^{4}$ (4.97)	-2.51	$7.5\text{x}10^{3}$ (3.88)	$4.6\text{x}10^{7}$ (7.66)	-3.79
	48	ND	$4.3\text{x}10^{4}$ (4.63)		$2.1\text{x}10^{1}$ (1.30)	$4.3\text{x}10^{4}$ (4.63)	-3.31	$4.6\text{x}10^{6}$ (6.66)	$4.6\text{x}10^{7}$ (7.66)	-1.00

*Incubation Temperature: 01B-22°C; 02B-8°C; 03B-15°C; 04B-21°C

Table 11-34. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments*using seawater collected at Station J1 as inocula, BLMØ1B-Ø4B. Log MPN in parentheses. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cruise	Days of Incubation	Oil Free Control			Non-Enriched + Oil			Enriched + Oil		
		HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$
Ø1B	0	$3x10^{3}$ (-2.52)	$2.3x10^{1}$ (1.36)	-3.89						
	3				ND	$\geq 2.4x10^{5}$ (≥ 5.38)		$\geq 2.4x10^{6}$ (≥ 6.38)	$1.1x10^{7}$ (7.04)	-0.66
	6				ND	$1.1x10^{5}$ (5.04)		ND	$\geq 2.4x10^{5}$ (≥ 5.38)	
	12				$1.5x10^{4}$ (4.18)	$7.5x10^{4}$ (4.88)	-0.70	$1.2x10^{5}$ (5.08)	$4.3x10^{6}$ (6.63)	-1.55
	24				ND	$9.3x10^{5}$ (5.97)		$2.1x10^{2}$ (2.32)	$4.3x10^{5}$ (5.63)	-3.31
	48				ND	$2.3x10^{5}$ (5.36)		ND	$9.3x10^{5}$ (5.97)	ND
Ø2B	0	$9.3x10^{1}$ (-.04)	$2.3x10^{2}$ (2.36)	-2.40						
	3				ND	$4.3x10^{4}$ (4.63)		ND	$2.3x10^{4}$ (4.36)	
	6				$2.3x10^{4}$ (4.38)	$9.3x10^{4}$ (4.97)	-0.61	$9.3x10^{5}$ (5.97)	$9.3x10^{5}$ (5.97)	0.0
	12				$9.3x10^{4}$ (4.97)	$4.3x10^{5}$ (5.63)	-0.66	$1.5x10^{6}$ (6.18)	$2.4x10^{7}$ (7.36)	-1.20
	24				$4.6x10^{5}$ (5.66)	$2.3x10^{6}$ (6.36)	-0.70	$2.4x10^{6}$ (6.38)	$4.6x10^{7}$ (7.66)	-1.30
	48				$2.1x10^{5}$ (5.32)	$2.3x10^{5}$ (5.36)	-0.04	$4.3x10^{6}$ (6.63)	$4.3x10^{7}$ (7.63)	-1.00
Ø3B	0	$4.3x10^{2}$ (-1.4)	$1.5x10^{3}$ (3.18)	-4.50						
	3				$9.3x10^{2}$ (2.97)	$9.3x10^{5}$ (5.97)	-3.00	$2.3x10^{4}$ (4.36)	$9.3x10^{5}$ (5.97)	-1.61
	6				$9.3x10^{4}$ (4.97)	$4.3x10^{5}$ (5.63)	-1.66	$4.6x10^{6}$ (6.66)	$9.3x10^{6}$ (6.97)	-0.31
	12				$1.5x10^{4}$ (4.18)	$2.3x10^{5}$ (5.36)	-1.19	$2.3x10^{6}$ (6.36)	$3.9x10^{6}$ (6.58)	-0.23
	24				$4.6x10^{4}$ (4.66)	$2.1x10^{5}$ (5.39)	-0.66	$4.6x10^{7}$ (7.66)	$7.5x10^{7}$ (7.88)	-0.21
	48				$2.9x10^{4}$ (4.44)	$4.3x10^{4}$ (4.63)	-0.19	$2.3x10^{7}$ (7.36)	$2.4x10^{8}$ (8.38)	-1.02
Ø4B	0	$2.3x10^{1}$ (-1.36)	$2.3x10^{3}$ (3.36)	-4.00						
	3	ND	$4.6x10^{5}$ (5.66)		ND	$4.3x10^{4}$ (4.63)		ND	$1.1x10^{6}$ (6.04)	
	6	ND	$4.3x10^{5}$ (5.63)		ND	$2.3x10^{5}$ (5.36)		ND	$2.3x10^{6}$ (6.36)	
	24	ND	$4.3x10^{5}$ (5.63)		ND	$4.3x10^{4}$ (4.63)		ND	$2.3x10^{6}$ (6.36)	
	48	ND	$2.3x10^{5}$ (5.36)		$3.6x10^{0}$ (0.56)	$2.3x10^{4}$ (4.38)	-4.81	ND	$4.3x10^{5}$ (5.63)	

* Incubation Temperature: 01B-22°C; 02B-8°C; 03B-15°C; 04B-21°C

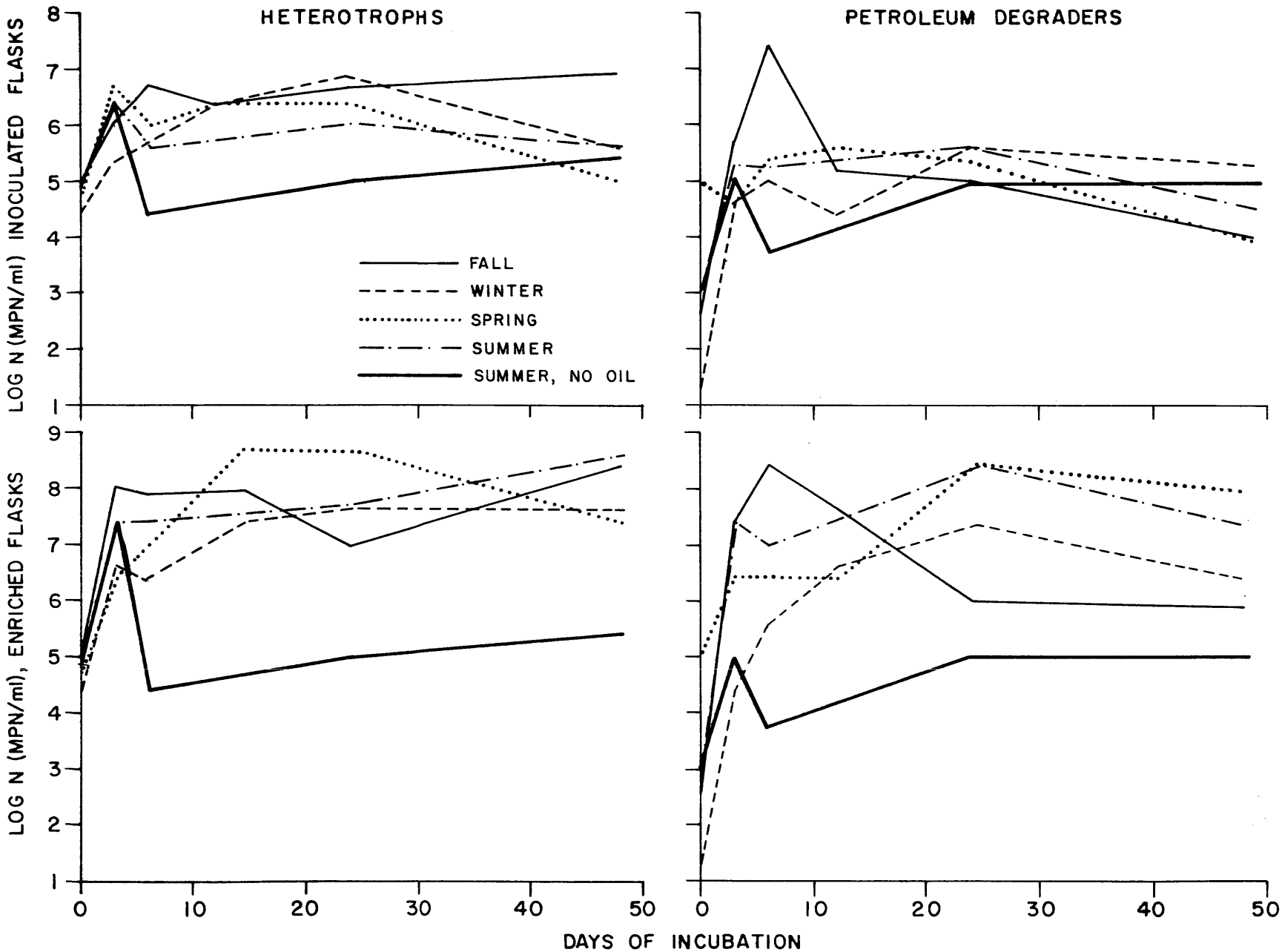


Figure 11-27. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments using seawater inoculum collected at Station C1, BLM01B-BLM04B.

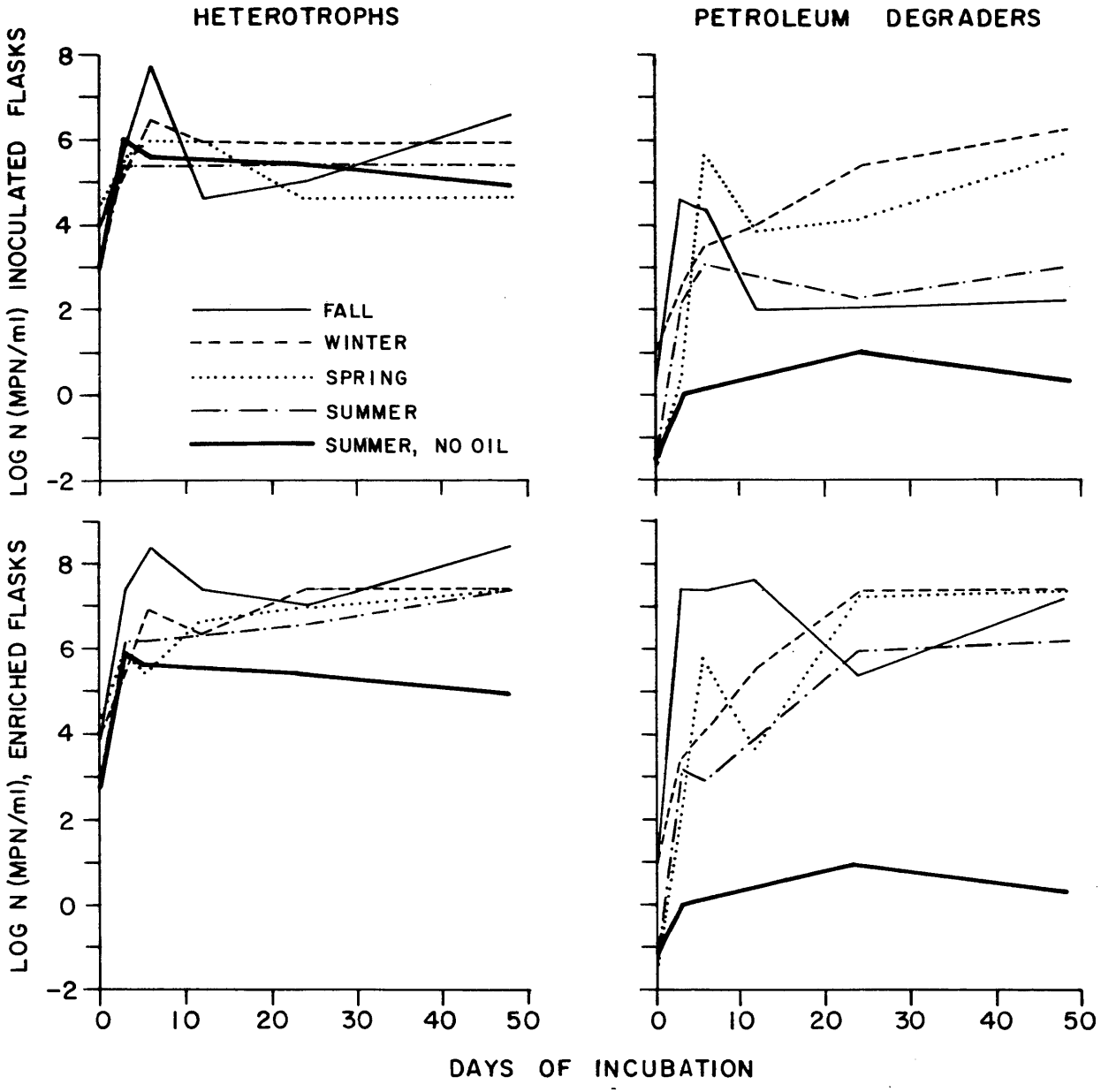


Figure 11-28. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments using seawater inocula collected at Station N2, BLM01B-BLM04B.

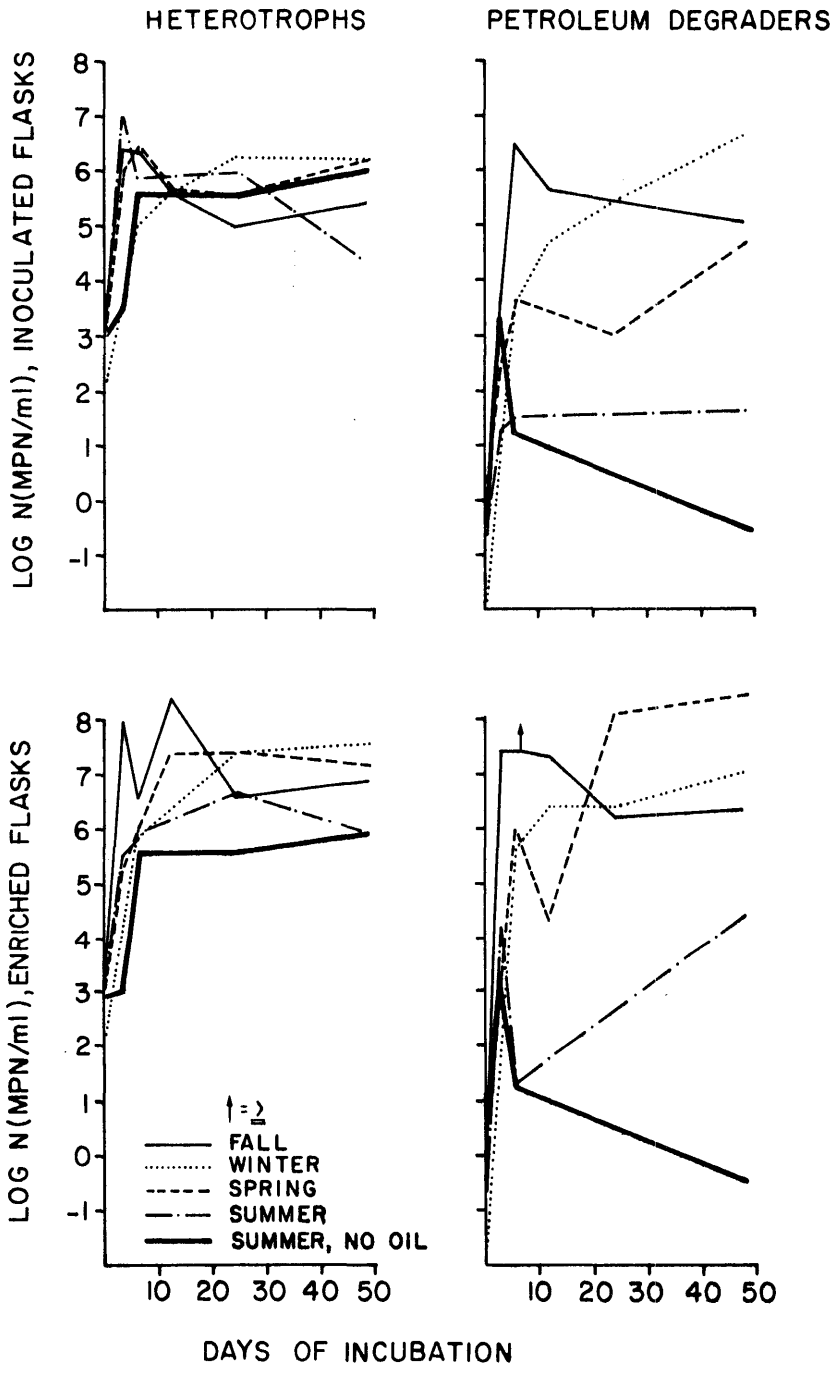


Figure 11-29. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments using seawater inocula collected at Station N3, BLM01B-BLM04B.

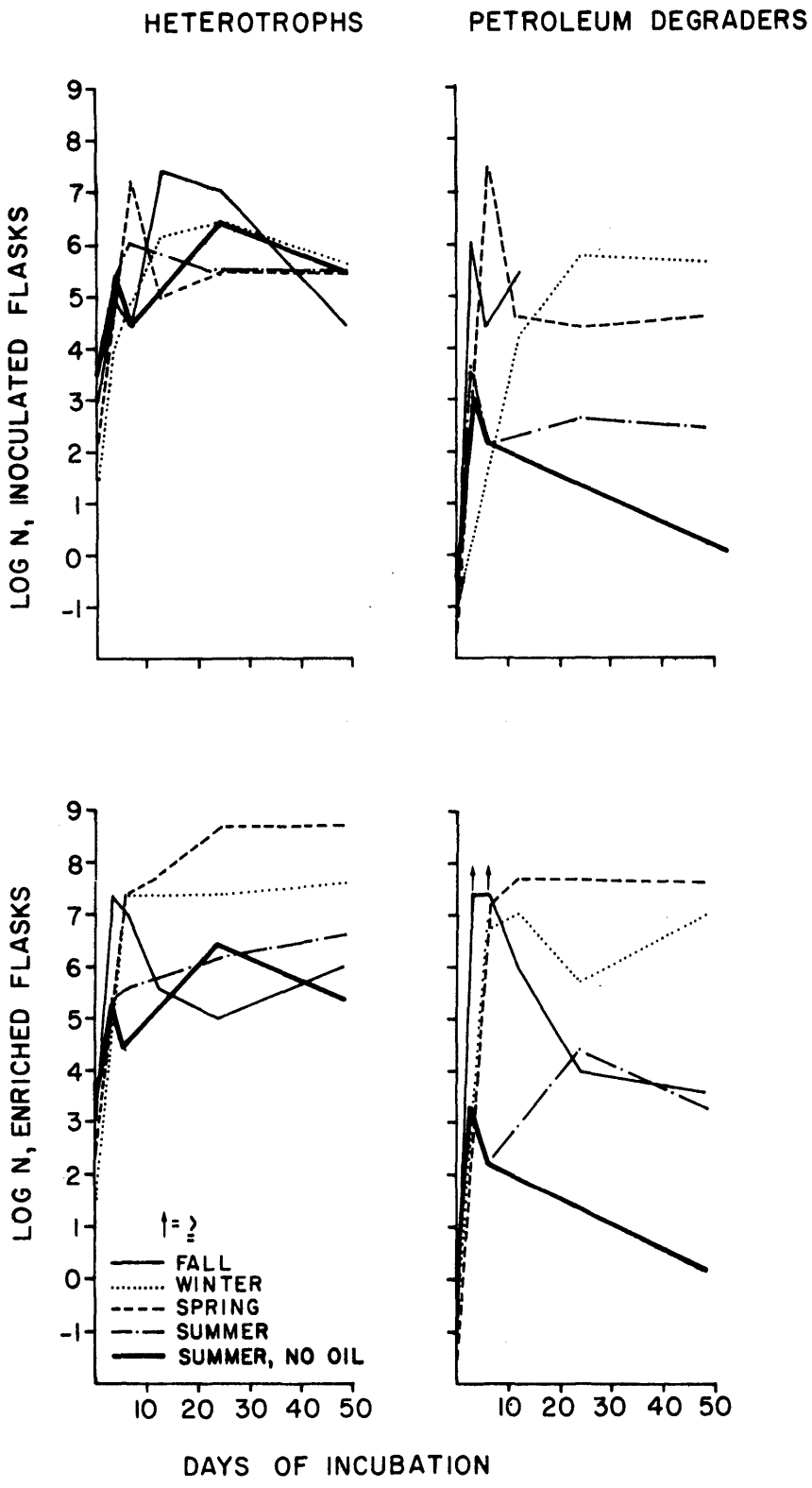


Figure 11-30. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments using seawater inocula collected at Station E3, BLM01B-BLM04B.

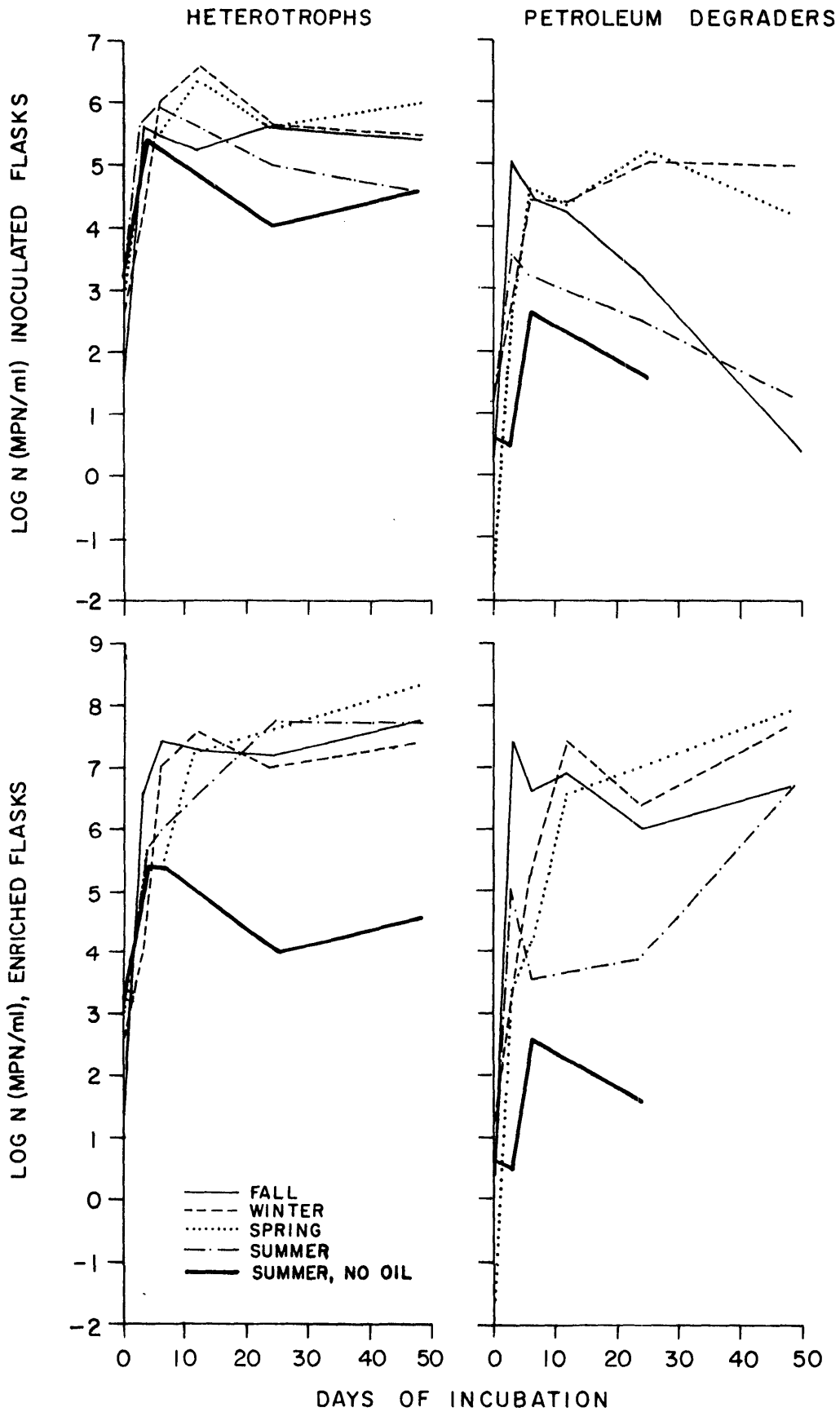


Figure 11-31. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments using sea water inocula collected at Station F2, BLM01B-BLM04B.

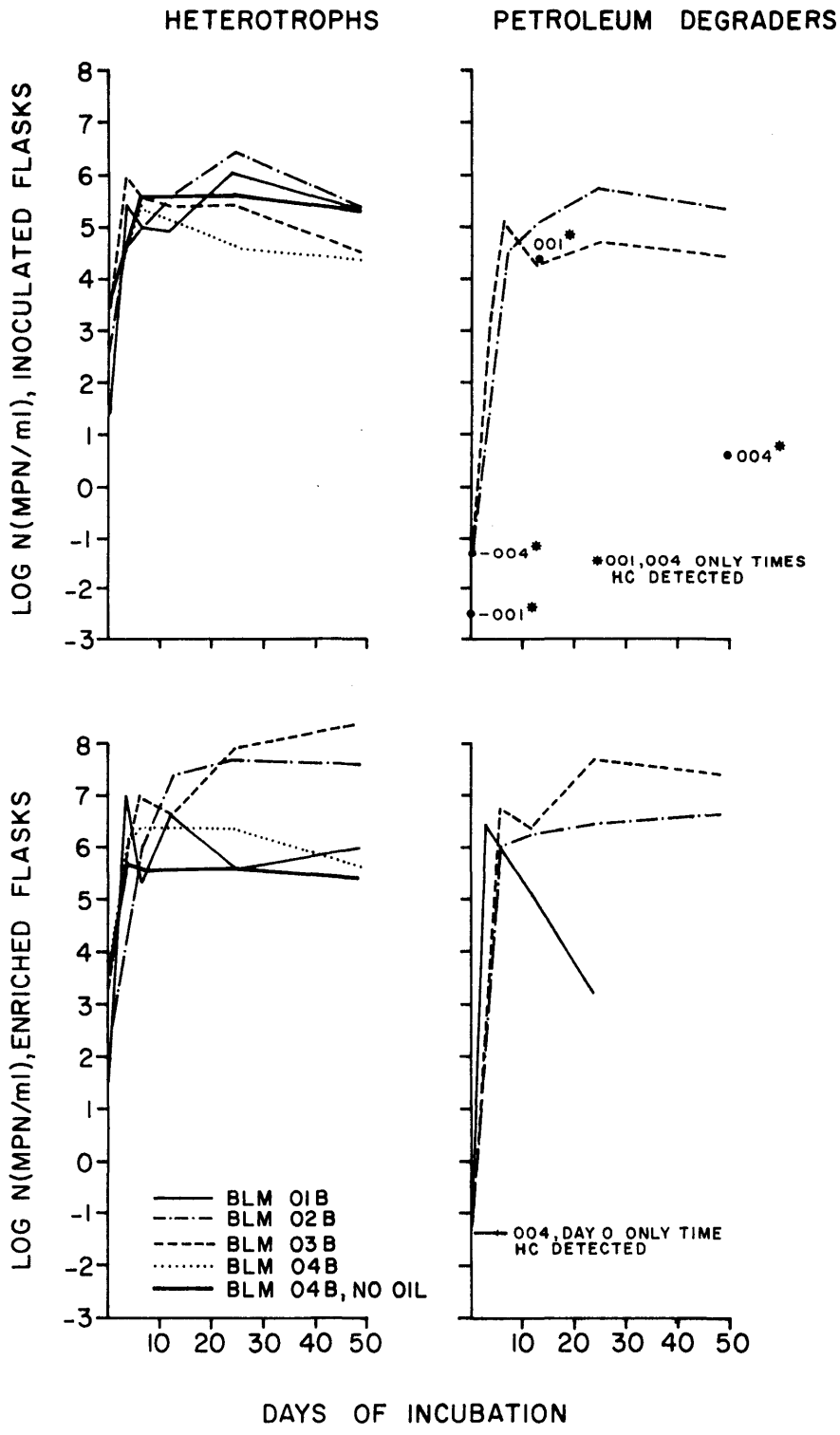


Figure 11-32. Changes in petroleum degrading and heterotrophic bacterial levels in closed flask oil degradation experiments using sea water collected at Station J1, BLM01B-BLM04B.

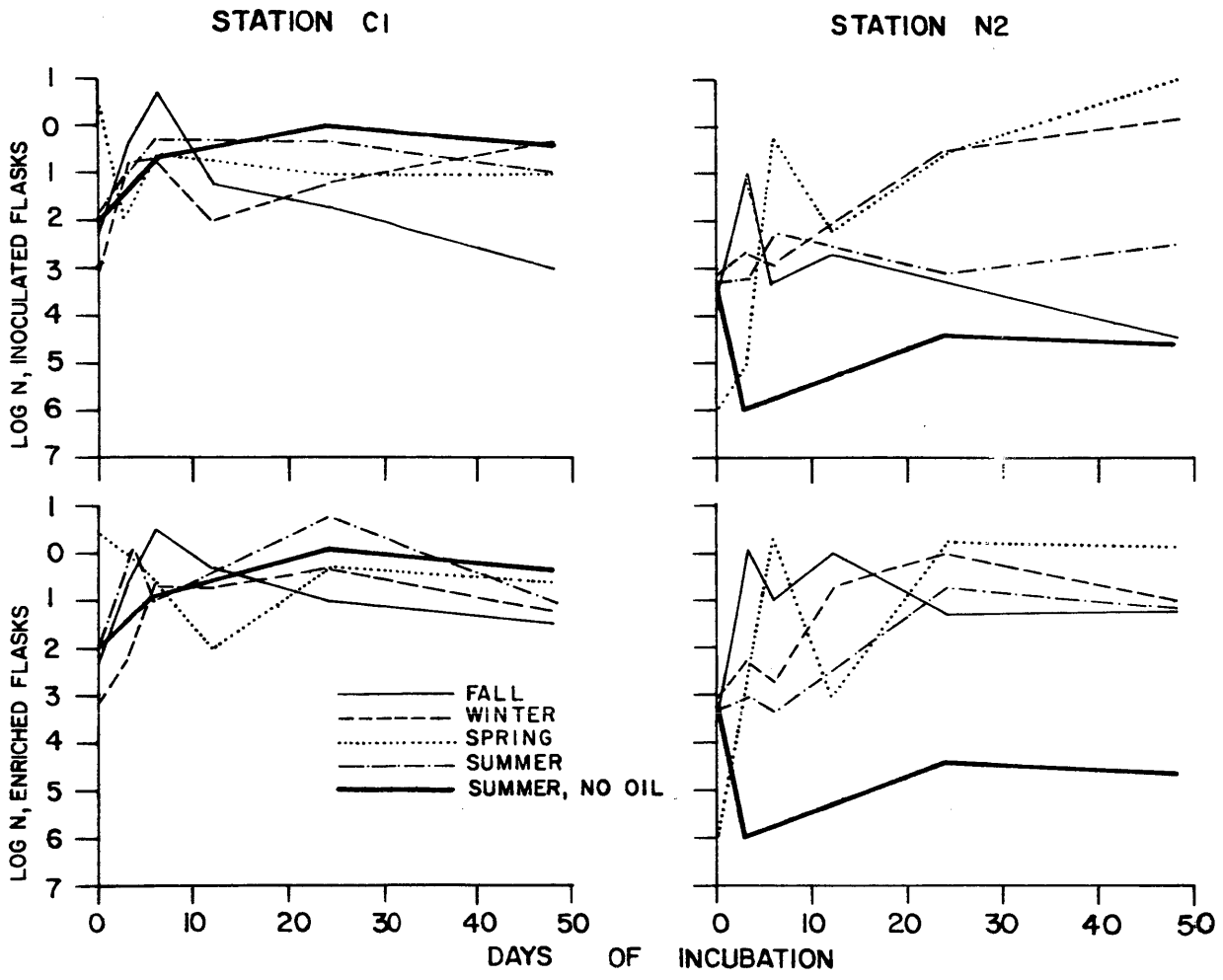


Figure 11-33. Changes in the ratio of petroleum degrading to heterotrophic bacteria in closed flask oil degradation experiments using sea water collected during BLM01B-BLM04B.

$$N = \frac{\text{MPN/ml Petroleum Degraders}}{\text{MPN/ml Heterotrophs}}$$

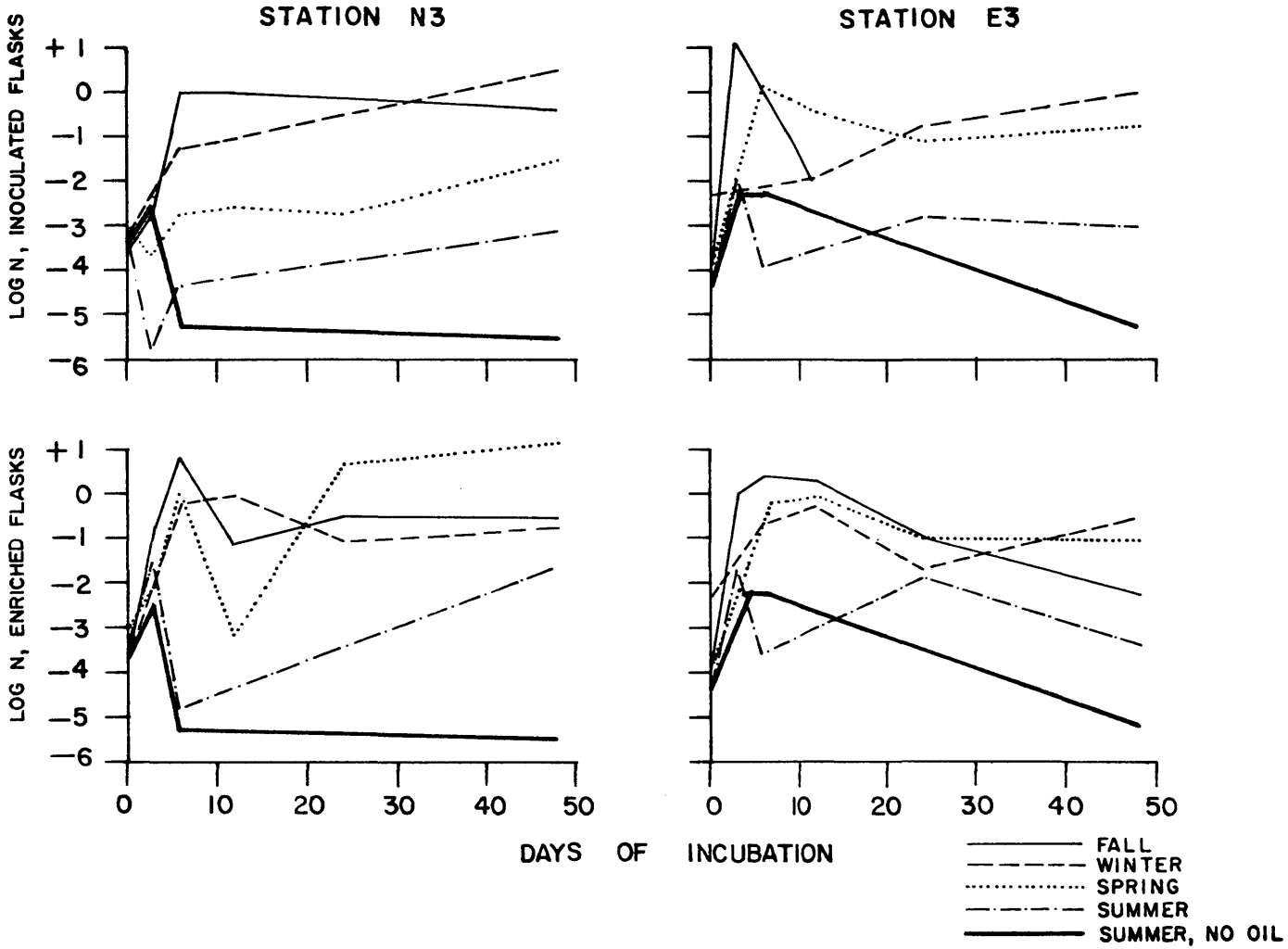


Figure 11-34. Changes in the ratio of petroleum degrading to heterotrophic bacteria in closed flask oil degradation experiments using sea water collected during BLM01B-BLM04B.

$$N = \frac{\text{MPN/ml Petroleum Degraders}}{\text{MPN/ml Heterotrophs}}$$

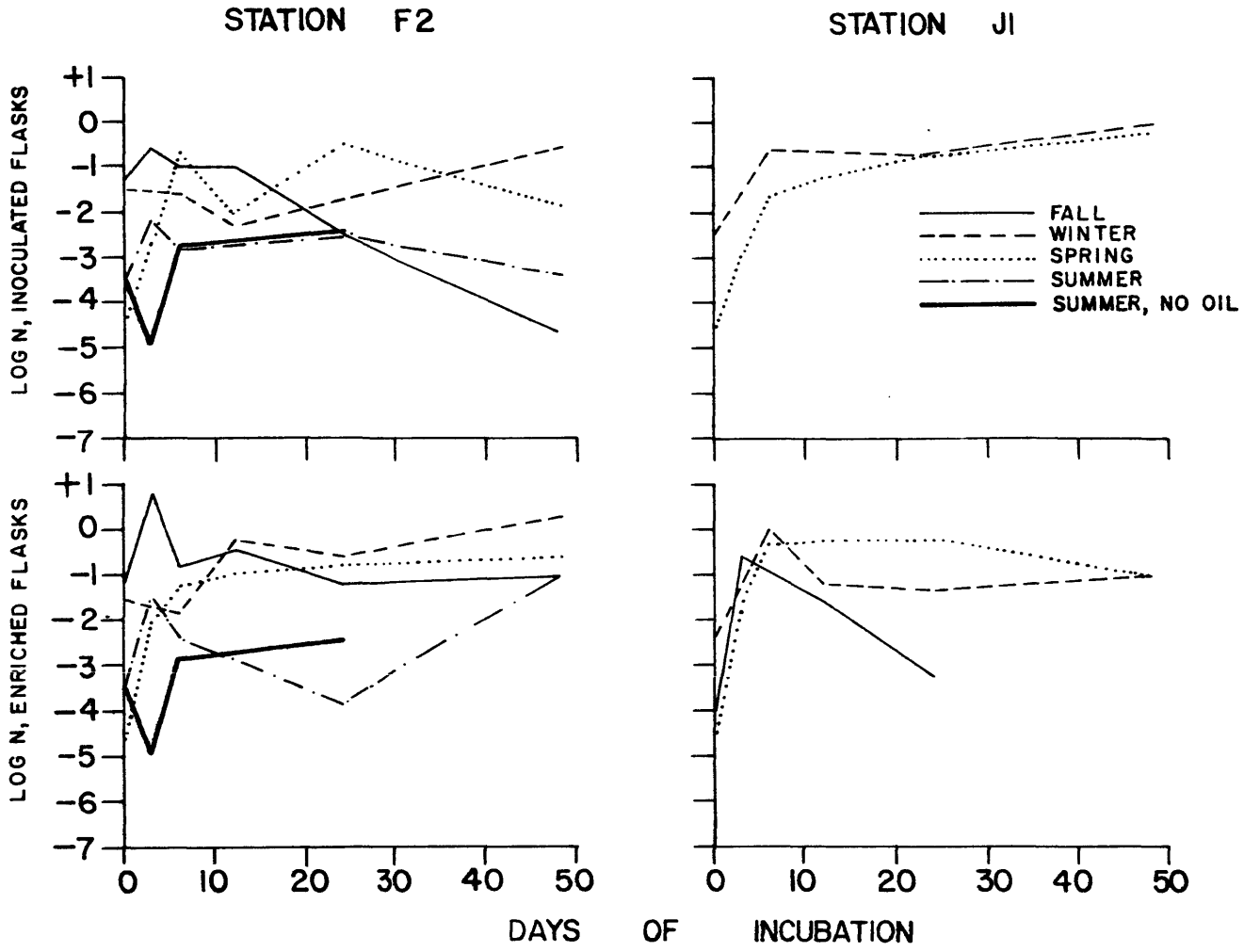


Figure 11-35. Changes in the ratio of petroleum degrading to heterotrophic bacteria in closed flask oil degradation experiments using sea water collected during BLM01B-BLM04B.

$$N = \frac{\text{MPN/ml Petroleum Degraders}}{\text{MPN/ml Heterotrophs}}$$

Heterotroph numbers were always larger under the enriched regime. Maximum levels achieved in the summer were lower than maximum levels for other seasons. Heterotroph levels in the oil free flask were about equivalent to levels in non-enriched flasks.

Numbers of petroleum degrading bacteria generally exhibited the largest increases at 3 days. Non-enriched flasks in spring exhibited the greatest increases between 3 and 6 days of incubation. Petroleum degrading bacterial levels were always larger in enriched flasks. Seasonally, numbers of these bacteria were lower in summer for both nutrient regimes. Counts in the oil free control were always lower than either enriched or non-enriched flasks. Changes in the value of HC/HET paralleled numbers of petroleum degrading bacteria and were generally higher in enriched flasks. The value of HC/HET in the oil free control was lower than either enriched or non-enriched flasks.

III. Station N3. Heterotroph counts were higher in enriched than non-enriched flasks except in the summer when the counts were similar. Numbers of heterotrophic bacteria in enriched and non-enriched flasks generally exhibited the greatest rate of increase at 3 days. Maximum rate of increase occurred at 6 days in the winter. Heterotroph levels in the oil free flask were similar to values in flasks of both nutrient regimes.

Numbers of petroleum degrading bacteria exhibited the greatest increase at 3 days with the exception of winter when the maximum increase occurred at 6 days. Higher levels of petroleum degrading bacteria were maintained in enriched flasks. Counts of petroleum degrading bacteria were about 2 log units lower in summer than those in other seasons under both enriched and non-enriched conditions. Petroleum degrading bacterial levels in oil free flasks were about equal to those in non-enriched flasks. Changes in the value of HC/HET paralleled changes in numbers of petroleum degrading bacteria under both nutrient regimes. Absolute values of this ratio in enriched and non-enriched flasks were similar during the fall and winter but were larger in enriched flasks during the spring and summer. Values of HC/HET in the oil free control were initially similar to non-enriched flasks but decreased to values lower than these flasks with time.

IV. Station E3. Heterotrophic bacteria exhibited the greatest rate of increase at 3 days in both enriched and non-enriched flasks, but generally larger levels were maintained in enriched flasks. Heterotroph levels in the oil free control were similar to those in non-enriched flasks.

Petroleum degrading bacteria manifested the greatest change in levels at 3 days of incubation during the fall and summer seasons. In the spring and winter, the greatest rate of increase occurred at 6 days. Maximum levels of petroleum degrading bacteria were several log units lower in the summer than other seasons under both nutrient regimes. Nutrient enrichment resulted in greater numbers of petroleum bacteria. Petroleum degrading bacterial levels in the oil free control were initially equivalent to the non-enriched flask but decreased

at later incubation times. Values of HC/HET paralleled changes of numbers of petroleum degrading bacteria under all nutrient regimes. Generally, enriched flasks exhibited greater values of HC/HET than non-enriched flasks. HC/HET values in the oil free flask were always less than values in the oil treated flasks during the summer.

V. Station F2. Enriched and non-enriched flasks exhibited the greatest increases in numbers of heterotrophic bacteria at 3 days. Heterotrophic bacterial levels were generally larger in enriched flasks. Counts in non-enriched flasks were essentially similar in the summer to those in the oil free flasks.

Numbers of petroleum degrading bacteria exhibited the largest rate of increase at 3 days under both nutrient regimes with the exception of winter where this occurred at 6 days. Counts of petroleum degrading bacteria were maintained at greater values in enriched flasks. In the summer, numbers of petroleum degrading bacteria were about 1.5 log units lower than other seasons under both nutrient regimes. Compared to oil treated flasks, numbers were lower in the oil free control at all times. The value of HC/HET paralleled changes in numbers of petroleum degrading bacteria and was generally larger in enriched flasks. Values of the ratio in the oil free control were always less than in oil-treated flask.

VI. Station J1. Heterotrophic bacterial levels in flasks of both nutrient regimes exhibited their greatest increase at 3 days. Slightly larger levels of heterotrophs were maintained in enriched flasks. Heterotroph counts in the oil free control were roughly equivalent to summer counts in oil treated flasks.

Numbers of petroleum degrading bacteria exhibited the greatest increase at 3 days in the spring and 6 days in the winter in both enriched and non-enriched flasks. Petroleum degrading bacteria were often too low to be detected in the fall and summer. When detected, levels of petroleum degrading bacteria were greater in enriched flasks. Values of the ratio HC/HET were greater in enriched flasks during the spring but essentially equivalent in value under both nutrient regimes during the winter.

Closed Flask, Water Inocula, Analysis of Residual Petroleum. Gravimetric analysis of the residual saturated hydrocarbon (H2) fraction of petroleum in enriched flasks inoculated with sea water collected during the spring and summer exhibited weight losses during the incubation periods (Tables 11-35 through 11-39). These losses were generally lower than those observed during the fall. Usually small and insignificant weight losses were noted in non-enriched flasks. This apparent degradation was most often due to losses of part of the sample during processing.

Gas chromatographic analysis of residual normal paraffins (nC_{12}-nC_{27}) supported gravimetric data (Tables 11-40 through 11-42, Figures 11-36 and 11-37). Some small weight losses indicated by gravimetry were not confirmed by subsequent gas chromatographic analyses. However, large losses in n-paraffins were paralleled by large weight losses in the H2 fraction.

Table 11-35. Weight loss in the saturated paraffin (H2) fraction of residual petroleum from closed flasks used in petroleum degradation experiments using sea water inocula collected during BLM Ø1.

Station	Treatment[B]	Days of Incubation 3[A]	6[A]	12[A]	24[A]	48[A]
C1	Control	0	0	0	0	0
	Inoculated	5	0	2	22	16
	Enriched	0	45	62	70	87
N2	Control	0	0	0	0	0
	Inoculated	0	20	0	28	17
	Enriched	12	43	9	57	70
N3	Control	0	0	0	0	0
	Inoculated	14	22	15	0	13
	Enriched	19	10	58	30	46
E3	Control	0	0	0	0	0
	Inoculated	5	0	2	0	0
	Enriched	0	17	0	6	17
F2	Control	0	0	0	0	0
	Inoculated	0	0	0	0	0
	Enriched	16	56	22	44	43
J1	Control	0	0	0	0	0
	Inoculated	7	0	0	0	40
	Enriched	0	0	0	9	45

A - % weight loss = $100\left(1-\frac{\text{weight of H2 fraction, treated flasks}}{\text{weight of H2 fraction, sterile control}}\right)$

B - Treatment: Control = sterile control + oil
Inoculated = sea water + oil
Enriched = sea water + oil + nutrient amendment

Table 11-36. Weight loss in the saturated paraffin (H2) fraction of residual petroleum from closed flasks used in petroleum degradation experiments using sea water inocula collected during BLM Ø2.

Station	Treatment[B]	Days of Incubation				
		3[A]	6[A]	12[A]	24[A]	48[A]
C1	Control	0	0	0	0	0
	Inoculated	8	3	16	0	0
	Enriched	19	16	22	36	44
N2	Control	0	0	0	0	0
	Inoculated	6	0	I.D.	0	7
	Enriched	21	9	3	0	15
N3	Control	0	0	0	0	0
	Inoculated	15	13	0	I.D.	I.D.
	Enriched	17	23	0	20	15
E3	Control	0	0	0	0	0
	Inoculated	4	4	0	0	3
	Enriched	3	16	0	0	30
F2	Control	0	0	0	0	0
	Inoculated	9	0	0	7	0
	Enriched	0	0	0	58	0
J1	Control	0	0	0	0	0
	Inoculated	0	10	0	11	5
	Enriched	0	0	1	7	25

A - % weight loss = $100\left(1-\frac{\text{weight of H2 fraction, treated flasks}}{\text{weight of H2 fraction, sterile control}}\right)$

B - Treatment: Control = sterile control + oil
Inoculated = sea water + oil
Enriched = sea water + oil + nutrient amendment

I.D. = Indeterminant, part of sample lost

Table 11-37 . Weight loss[a] in the saturated paraffin(H2) fraction of residual petroleum from closed flasks used in petroleum degradation experiments using seawater inocula collected during BLM Ø3B.

Station	Treatment[c]	Days of Incubation			
		3	6	24	48
C1	Control	0.0	0.0	0.0	0.0
	Inoculated	26.8	12.0	23.0	0.0
	Enriched	21.1	11.0	64.0	72.5
N2	Control	0.0	0.0	0.0	0.0
	Inoculated	0.0	65.0[b]	28.6[b]	17.0
	Enriched	0.0	26.0[b]	46.8	36.0
N3	Control	0.0	0.0	0.0	0.0
	Inoculated	24.0	0.0	7.0	0.0
	Enriched	26.0	22.0	18.0	9.8
E3	Control	0.0	0.0	0.0	5.0
	Inoculated	26.4	25.0	14.2	4.0
	Enriched	37.5	16.7	21.8	32.3
F2	Control	0.0	0.0	0.0	0.0
	Inoculated	3.7	0.0	6.0	0.0
	Enriched	0.0	10.5	8.0	11.0
J1	Control	0.0	0.0	0.0	0.0
	Inoculated	0.0	0.0	0.0	30.0[b]
	Enriched	2.0	0.0	34.0	0.0

[a] $\%\ \text{weight loss} = 100\ (1 - \frac{\text{weight of H2 fraction, treated flask}}{\text{weight of H2 fraction, sterile control}})$

[b] sample partially lost

[c] treatment: control = sterile control + sterile oil
inoculated + seawater + sterile oil
enriched = seawater = sterile oil + nutrient amendment

Table 11-38 . Weight loss[a] in the saturated paraffin (H2) fraction of residual petroleum from closed flasks used in petroleum degradation experiments using seawater and sediment inocula collected during BLM Ø4B.

Station	Treatment[b]	Days of Incubation			
		3	6	24	48
C1	Control	0.0[c]	0.0	0.0	0.0[e]
(Water)	Inoculated	0.0[c]	0.0	0.0	0.0[e]
	Enriched	0.0[c]	0.0	55.0	57.4[e]
N2	Control	0.0	0.0	0.0	0.0
(Water)	Inoculated	0.0	0.0	0.0	0.0
	Enriched	0.0	0.0	14.4	0.0
N3	Control	0.0	0.0	0.0	0.0
(Water)	Inoculated	0.0	0.0	0.0	0.0
	Enriched	0.0	22.5	50.0	2.7
E3	Control	0.0	0.0	0.0	0.0
(Water)	Inoculated	0.0	17.2	32.3	1.8
	Enriched	0.0	26.0	0.0	14.7
F2	Control	0.0	0.0	0.0	0.0
(Water)	Inoculated	16.9	15.0	7.8	11.0
	Enriched	18.7	14.7	27.0	32.1
J1	Control	0.0	0.0	0.0	0.0
(Water)	Inoculated	20.5	2.6	18.8	19.3
	Enriched	2.4	1.6	30.4	17.0
"C" Cluster	Control	0.0	0.0	0.0	0.0
(Sediment)	Inoculated	0.0	0.0	4.0	0.0
	Enriched	5.7	0.0	55.9	22.7
"D" Cluster	Control	0.0	0.0	0.0	0.0
(Sediment)	Inoculated	0.0	0.0	0.0	0.0
	Enriched	0.0	0.0	17.9	65.5
"F" Cluster	Control	0.0	0.0	0.0	0.0
(Sediment)	Inoculated	0.0	15.0[f]	6.1	0.0
	Enriched	32.3[d]	17.1[f]	37.4	64.0

[a] $\%\text{weight loss} = 100\ \left(1 - \frac{\text{weight of H2 fraction, treated flask}}{\text{weight of H2 fraction, sterile control}}\right)$

[b] treatment: control: sterile seawater + oil; inoculated = seawater + oil; enriched = seawater + oil + nutrient amendment

[c] 4 day incubation period

[d] sample partially lost

[e] 49 day incubation

[f] fractionated twice

Table 11-39. Change in summation of weights (μg) of crude oil n-paraffins (nC_{12} - nC_{27}) during incubation in closed flasks with seawater collected during BLM Ø1B. % weight loss[a] in parentheses.

Station	Treatment[B]	Days of Incubation 3	6	12	24	48
C1	Control	4639*	4178	4119	4120	3877
	Inoculated	4414(5)	4417(0)	4130(0)	3880(6)	3567(8)
	Enriched	3201(21)	396(91)	0(100)	0(100)	0(100)
N2	Control	4668	4676	4267	4458	4233
	Inoculated	4583(2)	4667(<1)	4551(0)	4646(0)	4299(0)
	Enriched	1482(68)	1289(72)	176(96)	0(100)	0(100)
N3	Control	4439	4785	4696	4704	4490
	Inoculated	4347(2)	4377(9)	4582(2)	4593(2)	4375(3)
	Enriched	2790(37)	1479(69)	1242(74)	0(100)	0(100)
E3	Control	4358	4236	4104	4538	4306
	Inoculated	4081(6)	4075(4)	4279(0)	4430(2)	4238(2)
	Enriched	1727(60)	1837(57)	486(88)	335(93)	397(91)
F2	Control	4324	4197	3923	4337	3953
	Inoculated	4414(0)	4283(0)	3910(<1)	4246(2)	4201(0)
	Enriched	1509(66)	500(88)	567(86)	200(95)	0(100)
J1	Control	4113	4393	4492	4593	4539
	Inoculated	4532(0)	4322(2)	4503(0)	4558(1)	4342(4)
	Enriched	3619(12)	4781(0)	1088(76)	3745(18)	339(93)

[a] % weight loss = $100 \left(1 - \frac{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{treated flasks}}{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{sterile control}}\right)$

[b] treatments: control - sterile seawater and sterile oil
inoculated - seawater + sterile oil
enriched - seawater + sterile oil + nutrient amendment

Table 11-40 . Change in summation of weights (μg) of crude oil n-paraffins (nC_{12} - nC_{27}) during incubation in closed flasks with seawater collected during BLM Ø2B. % weight loss[a] in parentheses.

Station	Treatment[b]	Days of Incubation				
		3	6	12	24	48
C1	Control	4345	4255	4187	4266	4205
	Inoculated	4236(2.5)	4270(0)	4351(0)	4255(0.3)	4182(0.5)
	Enriched	4254(2.1)	4104(3.5)	2328(44.4)	0(100)[c]	0(100)[c]
N2	Control	4520	4377	4298	4254	4527
	Inoculated	4355(3.6)	4071(7.0)	4245(1.2)	4313(0)	4225(6.7)
	Enriched	4419(2.2)	4154(5.1)	3170(26.2)	1635(61.6)	875(80.7)
N3	Control	4361	4190	4142	4267	4122
	Inoculated	4274(2.0)	4411(0)	4122(0.5)	4065(4.8)	4052(1.7)
	Enriched	4301(1.4)	3300(21.2)	3045(26.5)	706(83.5)	274(93.4)
E3	Control	4228	4068	4350	4190	4264
	Inoculated	4330(0)	4249(0)	4168(4.2)	4358(0)	4285(0)
	Enriched	4277(0)	3128(23.1)	2059(52.7)	2906(30.6)	456(89.3)
F2	Control	4217	4262	4230	4409	4164
	Inoculated	4192(0.6)	4305(0)	4161(1.6)	4344(1.5)	4169(0)
	Enriched	4068(3.5)	2748(35.5)	2473(41.5)	574(87.0)	987(76.3)
J1	Control	4232	4259	4328	4327	4125
	Inoculated	4378(0)	4186(1.7)	4333(0)	4257(1.6)	4046(1.9)
	Enriched	4365(0)	3635(14.7)	2912(32.7)	1763(59.3)	345(91.6)

[a] % weight loss = $100 \left(1 - \frac{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{treated flask}}{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{sterile control}}\right)$

[b] treatments: control - sterile seawater and sterile oil
inoculated - seawater + sterile oil
enriched - seawater + sterile oil + nutrient amendment

[c] no n-paraffins resolved

Table 11-41 . Change in summation of weights (µg) of crude oil n-paraffins (nC_{12} - nC_{27}) during incubation in closed flasks with seawater collected during BLM Ø3B. % weight loss[a] in parentheses.

Station	Treatment[b]	Days of Incubation 3	6	24	48
C1	Control	4184	4135	4081	4063
	Inoculated	4205(0)	4112(1.0)	4108(0)	3991(2.0)
	Enriched	3926(6.0)	2890(30.0)	0(100)[c]	0(100)[c]
N2	Control	4175	4236	4148	4104
	Inoculated	4423(0)	4390(0)	4003(3.0)	3891(5.0)
	Enriched	4130(1.0)	4243(0)	3971(4.0)	341(91.7)
N3	Control	4125	4100	3971	4194
	Inoculated	4122(0)	4218(0)	4070(0)	4066(3.1)
	Enriched	4108(0.4)	4089(0.3)	1288(67.6)	0[c](100)
E3	Control	4159	4197	4107	4263
	Inoculated	4180(0)	3990(4.9)	3913(4.7)	4040(5.2)
	Enriched	4239(0)	3805(9.3)	0[c](100)	0[c](100)
F2	Control	4228	4032	3986	4012
	Inoculated	4173(1.3)	4031(0)	3869(2.9)	3900(2.8)
	Enriched	4130(2.3)	4128(0)	1980(50.3)	660(83.5)
J1	Control	4273	4203	3893	4081
	Inoculated	4284(0)	4090(2.7)	4064(0)	3847(5.7)
	Enriched	4213(1.4)	3696(12.1)	0[c](100)	0[c](100)

[a] % weight loss = $100 \left(1 - \frac{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{treated flask}}{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{sterile control}}\right)$

[b] treatments: control = sterile seawater and sterile oil
inoculated = seawater + sterile oil
enriched = seawater + sterile oil + nutrient amendment

[c] no n-paraffins resolved

Table 11- 42. Change in summation of weights (μg) of crude oil n-paraffins (nC_{12} - nC_{27}) during incubation in closed flasks with seawater collected during BLM04B. % weight loss[a] in parentheses.

Station	Treatment[b]	Days of Incubation 3	6	24	48
C1	Control	4104	3382	3678	3407
	Inoculated	4062(1)	3977(0)	3300(9.9)	3550(0)
	Enriched	3433(16.4)	542(74)	0[c] (100)	0[c] (100)
N2	Control	4211	3803	3507	3625
	Inoculated	3818(9.4)	3524(7.4)	3692(0)	3597(0.8)
	Enriched	4304(0)	3096(18.7)	2799(20.2)	1715(52.7)
N3	Control	4109	3875	4012	3207
	Inoculated	3666(10.8)	3921(0)	3434(14.5)	3508(0)
	Enriched	4159(0)	3534(9)	1379(65.7)	967(69.1)
E3	Control	3460	3381	3522	3467
	Inoculated	3474(0)	3873(0)	3718(0)	3385(2.4)
	Enriched	4170(0)	3652(0)	2040(42.1)	818(76.5)
F2	Control	4156	3586	3537	3642
	Inoculated	3471(16.5)	368 (0)	3815(0)	3260(10.5)
	Enriched	3801(9.6)	3047(15)	2392(32.4)	0[c](100)
J1	Control	4036	3680	3653	3362
	Inoculated	4174(0)	3577(2.8)	3397(7)	3530(0)
	Enriched	4166(0)	3653(.8)	3747(0)	3356(0)

[a] % weight loss = $100 \left(1 - \frac{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{treated flask}}{(\Sigma\ \mu g\ \text{n-paraffins}\ nC_{12} - nC_{27})\ \text{sterile control}}\right)$

[b] Treatments: control = sterile seawater and sterile oil
inoculated = seawater + sterile oil
enriched = seawater + sterile oil + nutrient amendment

[c] No n-paraffins resolved.

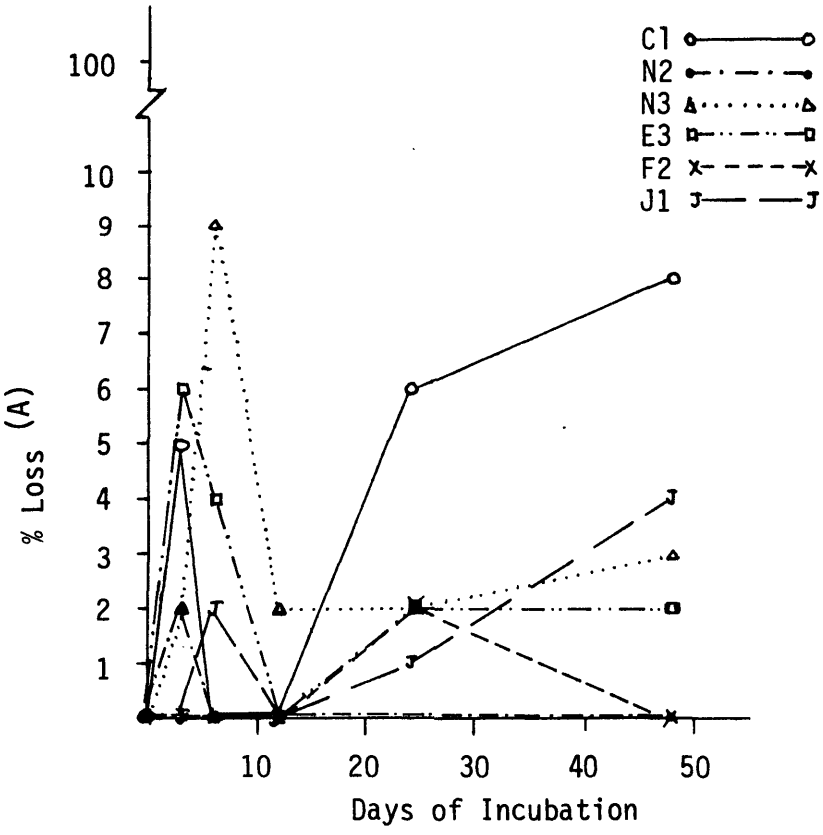


Figure 11-36. Weight loss of n-paraffins (nC_{12}-nC_{27}) in non-enriched closed flasks from petroleum degradation experiments using seawater inocula collected during BLM01B.

$$(A) = \% \text{ Loss} = 100\left(1 - \frac{\mu g \text{ n-paraffins } (nC_{12}\text{-}nC_{27}) \text{ non-enriched}}{\mu g \text{ n-paraffins } (nC_{12}\text{-}nC_{27}) \text{ sterile control}}\right)$$

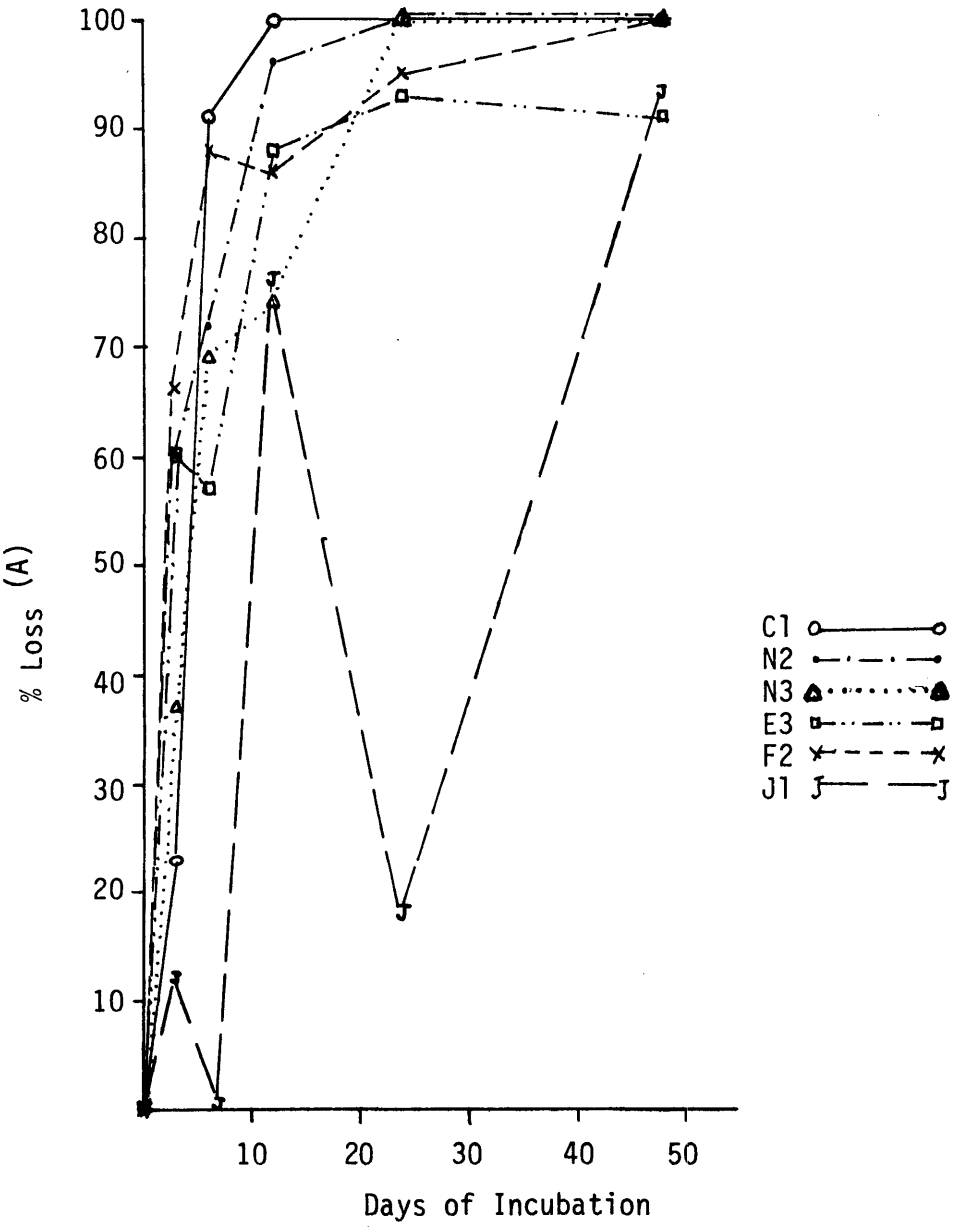


Figure 11-37. Weight loss of n-paraffins (nC_{12}-nC_{27}) in enriched flasks from petroleum degradation experiments using seawater inocula collected during BLM01B.

$$(A) = \% \text{ Loss} = 100 \left(1 - \frac{\mu g \text{ n-paraffins } (nC_{12}\text{-}nC_{27}) \text{ enriched}}{\mu g \text{ n-paraffins } (nC_{12}\text{-}nC_{27}) \text{ sterile control}}\right)$$

Such observations reveal the need to interpret gravimetric data very cautiously. No apparent preference for chain length was noted during any season. There was, however, a preferential utilization of the n-paraffins prior to degradation of isoprenoids which occurred only after n-paraffins could not be detected. A typical series of chromatograms illustrating patterns of n-paraffin degradation are shown in Figures 11-38 through 11-46.

Two methods were used to evaluate the rate of degradation of the n-paraffins. The first measured the rate of degradation based on µg of n-paraffins lost over selected incubation intervals (Tables 11-43 through 11-46). Rates of degradation were found to be non-linear and non-uniform over time. During each incubation period examined the rate changed. Maximum rates were reached after 3 to 6 days of incubation in the fall with 4 of 6 stations exhibiting maximum rates during the first three days of incubation. During no other season were the maximum rates obtained so rapidly. In general, maximum rates of degradation were reached after longer periods of incubation during the following seasons. This trend was most evident at the shelf break and slope stations F2 and J1. At these stations, the maximum rate of degradation occurred at increasingly longer incubation intervals during the year. Outer shelf stations (N3, E3) exhibited less pronounced seasonal variations. Station C1 varied only during the winter as all other seasons manifested maximum rates during the 3-6 days incubation interval.

The other method used to evaluate rates of degradation was the D_{50} and D_{100} measurement. D_{50} was defined as the days of incubation required for degradation of 50% or more of the n-paraffins (nC_{12}-nC_{27}) and, similarly, D_{100}, the days of incubation required for degradation of 100% of these n-paraffins (Table 11-47). D_{50} values for all stations were smallest in the fall. In general, D_{50} values were largest in the summer. D_{50} values were about equal during the winter and spring. A slightly different pattern emerged with the D_{100} values. During spring and fall seasons, 4 of 6 stations exhibited degradation of 100% of the n-paraffins within 48 days or less. During the winter, only C1 manifested degradation of 100% of the n-paraffins. Only two stations, C1 and F2, exhibited D_{100} values during the summer. Of all stations, C1 exhibited the most consistent seasonal degradative potential, exhibiting degradation of 100% of the n-paraffins and often the majority of isoprenoids within 24 days. It was unique in this respect. At the other extreme was J1, which exhibited degradation of 50% of n-paraffins only in spring and in summer and never reached degradation of 100% of the n-paraffins. Of the remaining stations, we could calculate D_{100} values only for F2 at 48 days for more than one season. Remaining stations exhibited only one D_{100} value either in spring or fall and D_{50} values for all seasons.

Comparisons of n-paraffin weight losses (nC_{12}-nC_{27}), numbers of petroleum degrading bacteria, and the values of HC/HET are shown for the fall experiment (Figures 11-47 through 11-52). Good agreement of n-paraffin losses with increased HC levels and HC/HET values in both enriched and non-enriched flasks was observed. Thus, the greatest increases in petroleum degrading bacteria and the HC/HET ratios were paralleled by the largest n-paraffin losses. It is of interest to note peaks consistently observed at early incubation periods in non-enriched flasks of n-paraffin losses and HC count increases. These may have reflected n-paraffin degradation

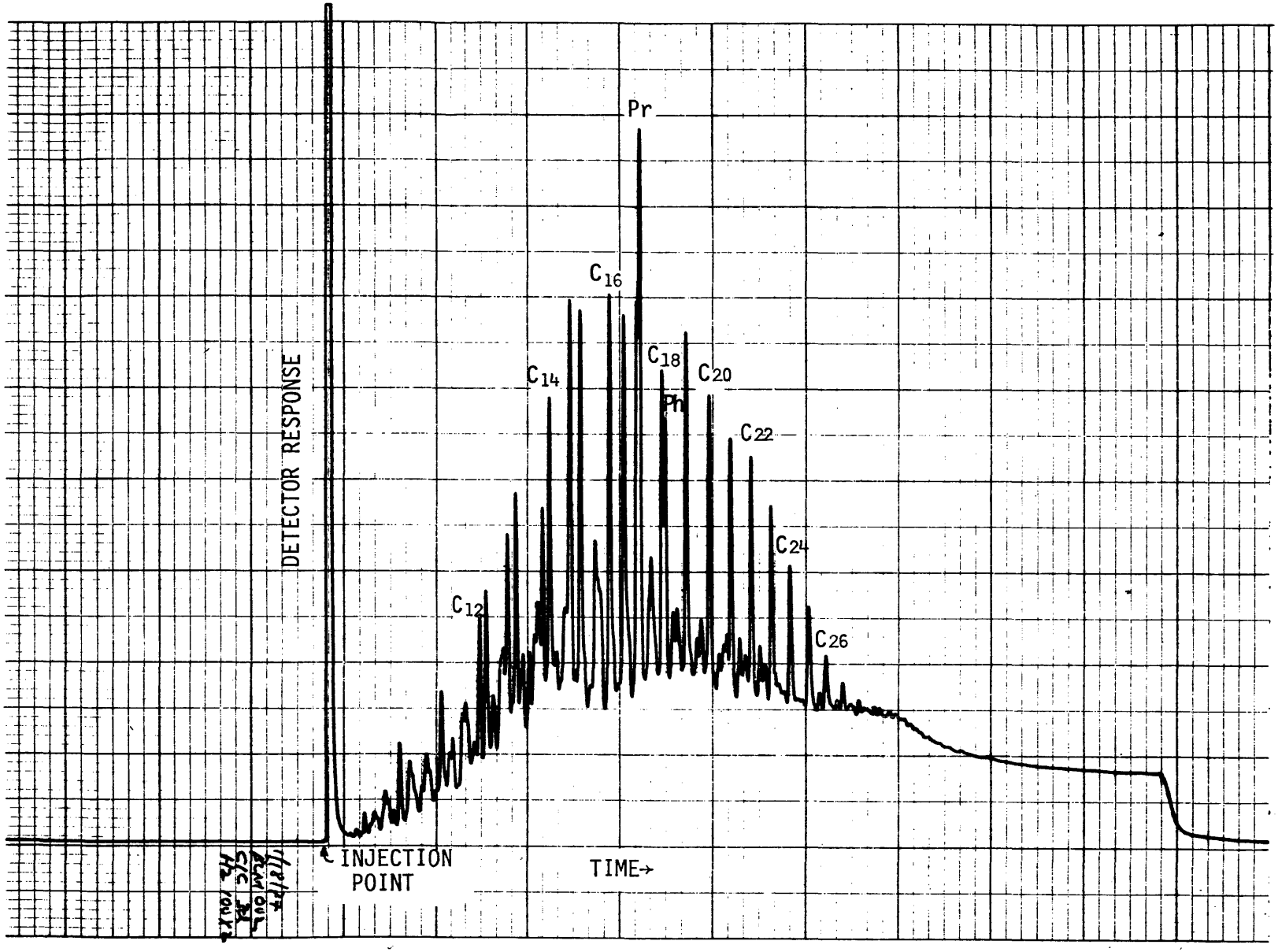


Figure 11-38. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 3 days of incubation. Sterile control, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

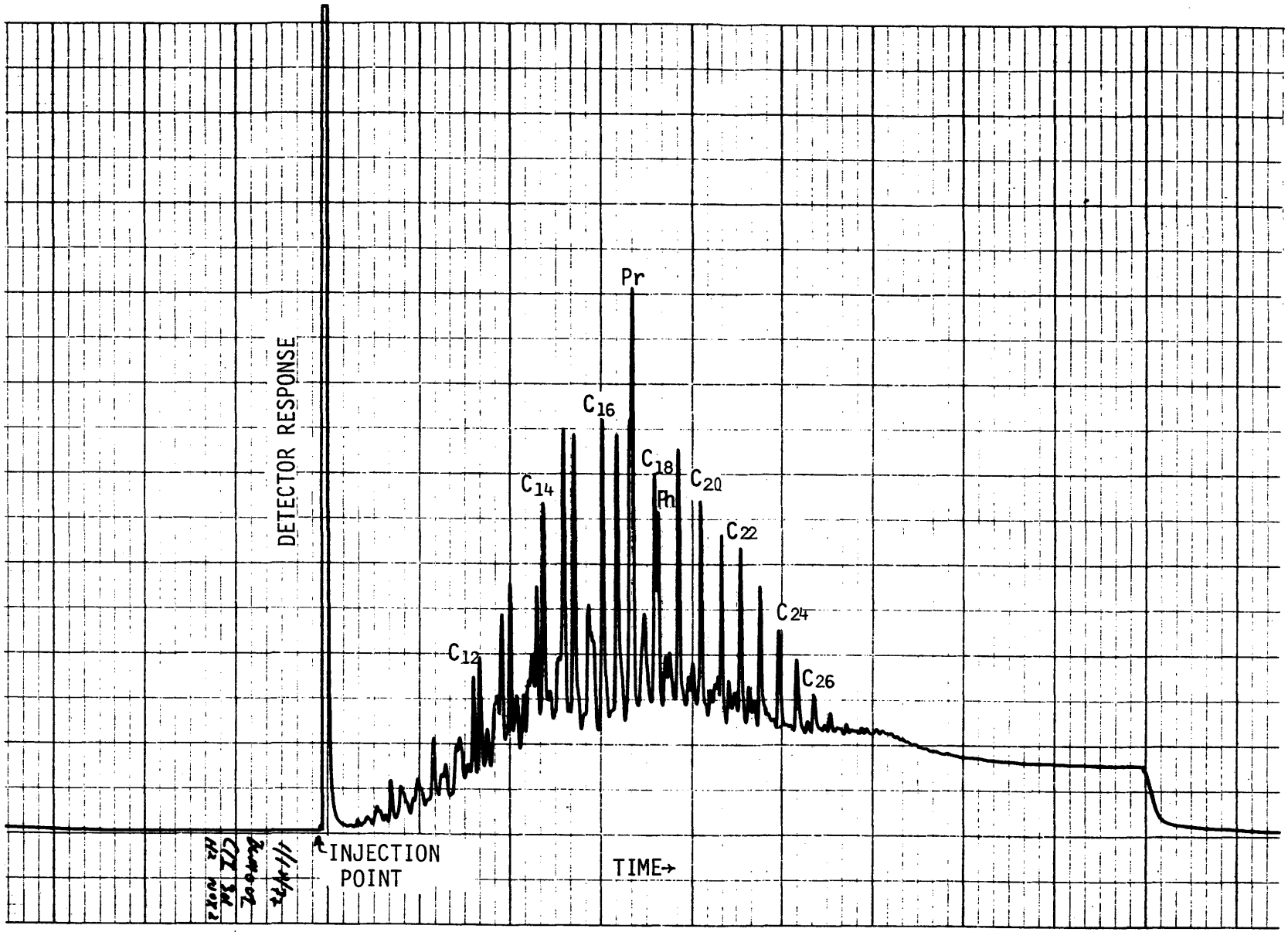


Figure 11-39. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 3 days of incubation. Non-enriched flask, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

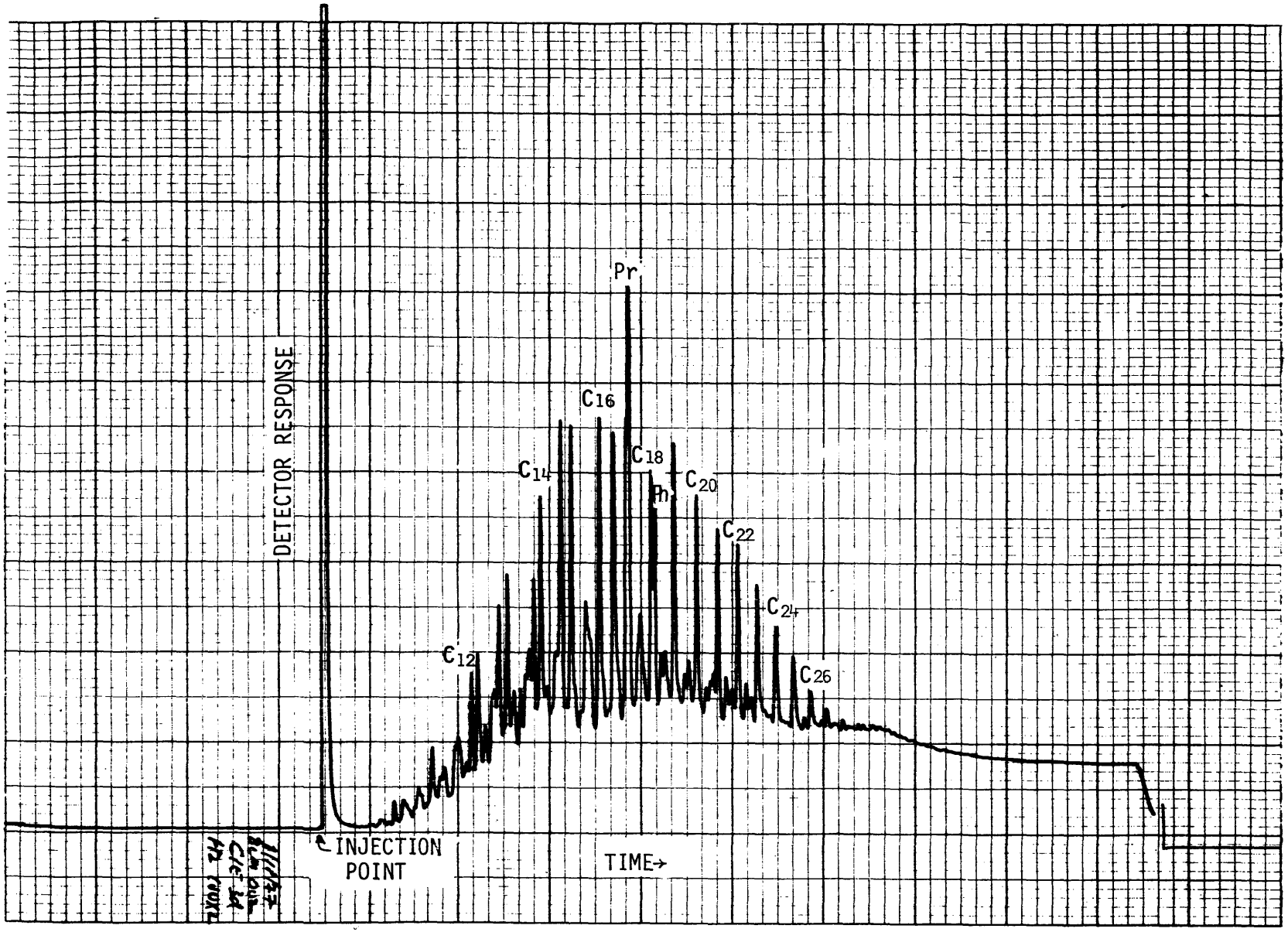


Figure 11-40. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 3 days of incubation. Enriched flask, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

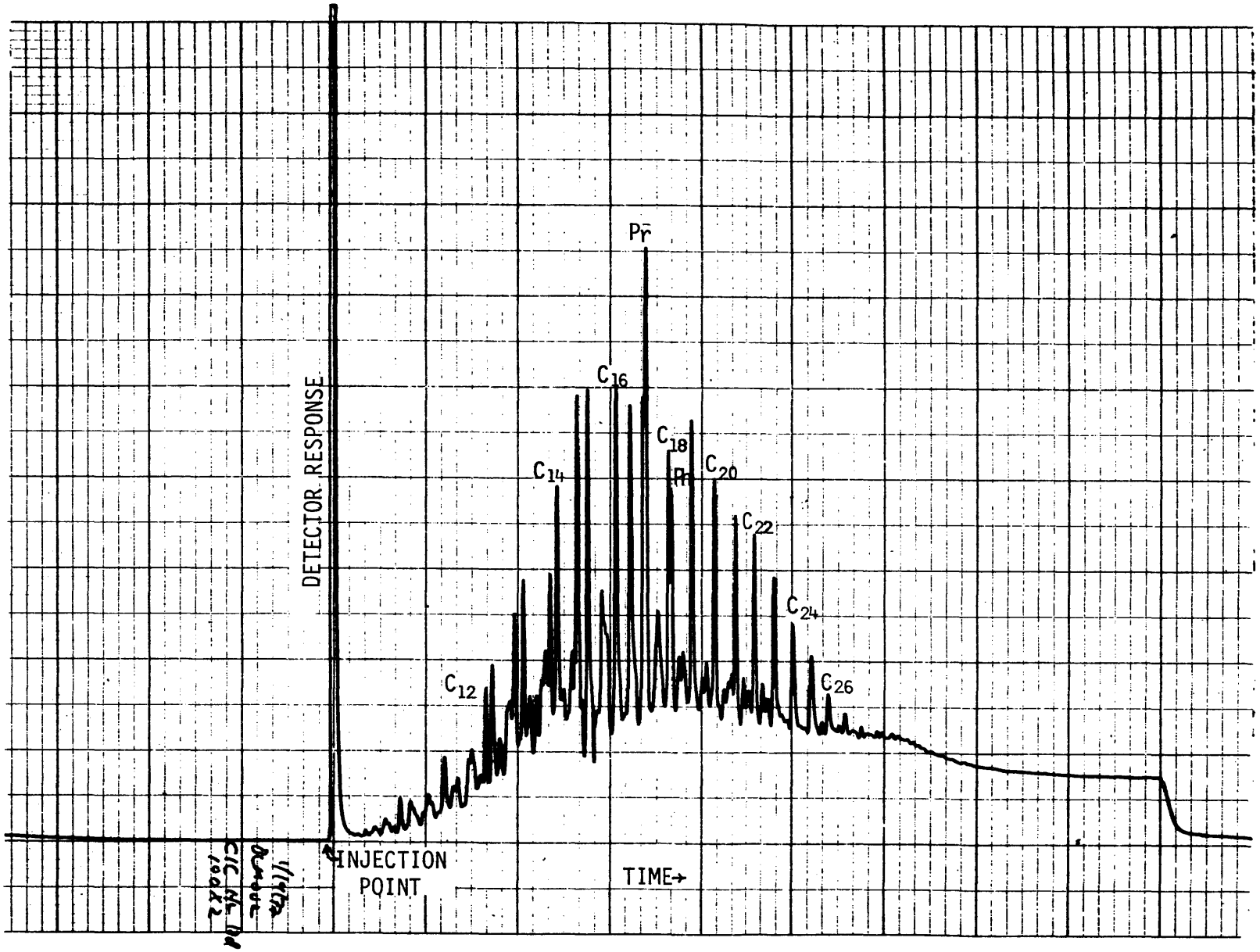


Figure 11-41. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 12 days of incubation. Sterile control, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

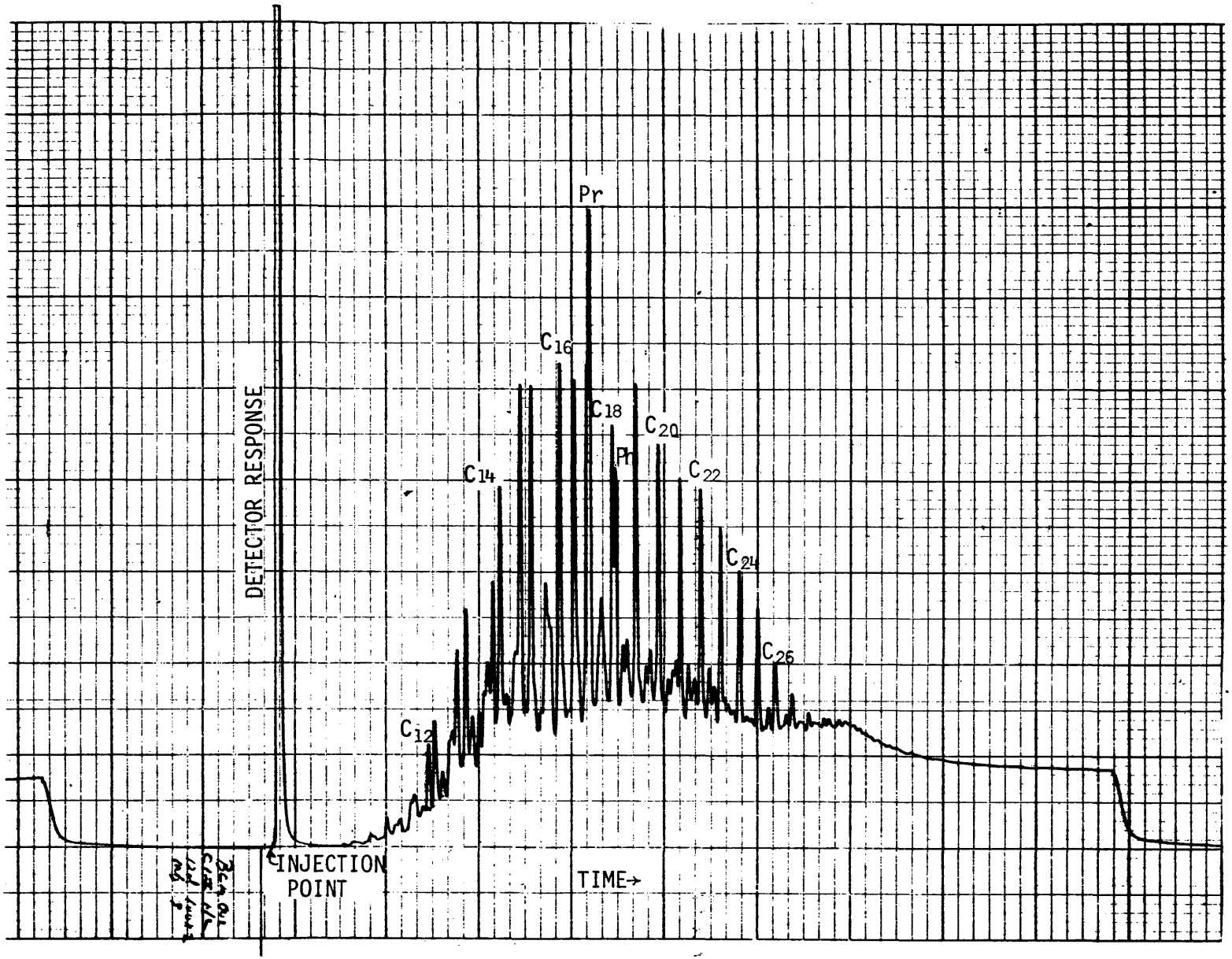


Figure 11-42. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 12 days of incubation. Non-enriched, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

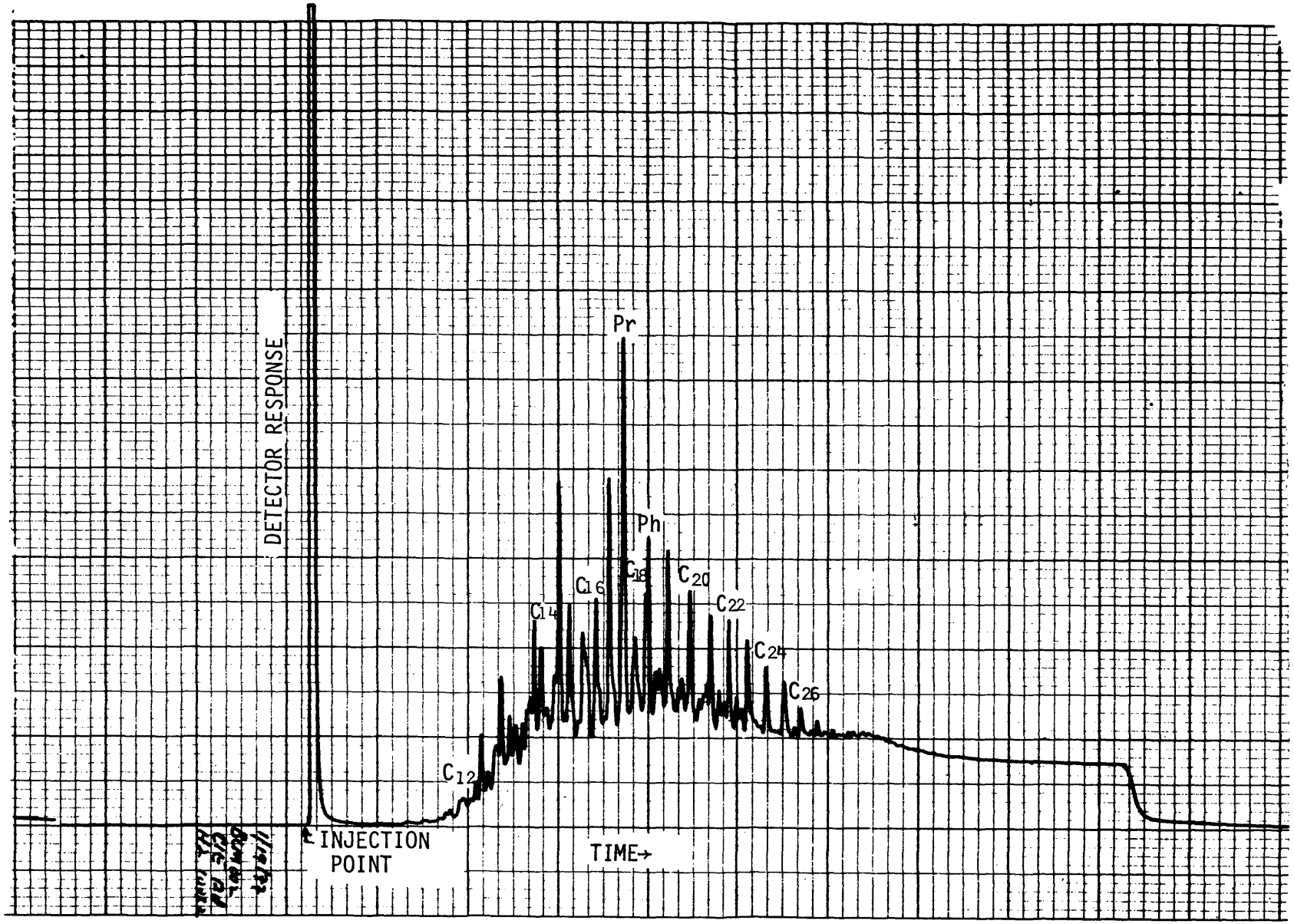


Figure 11-43. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 12 days of incubation. Enriched flask, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

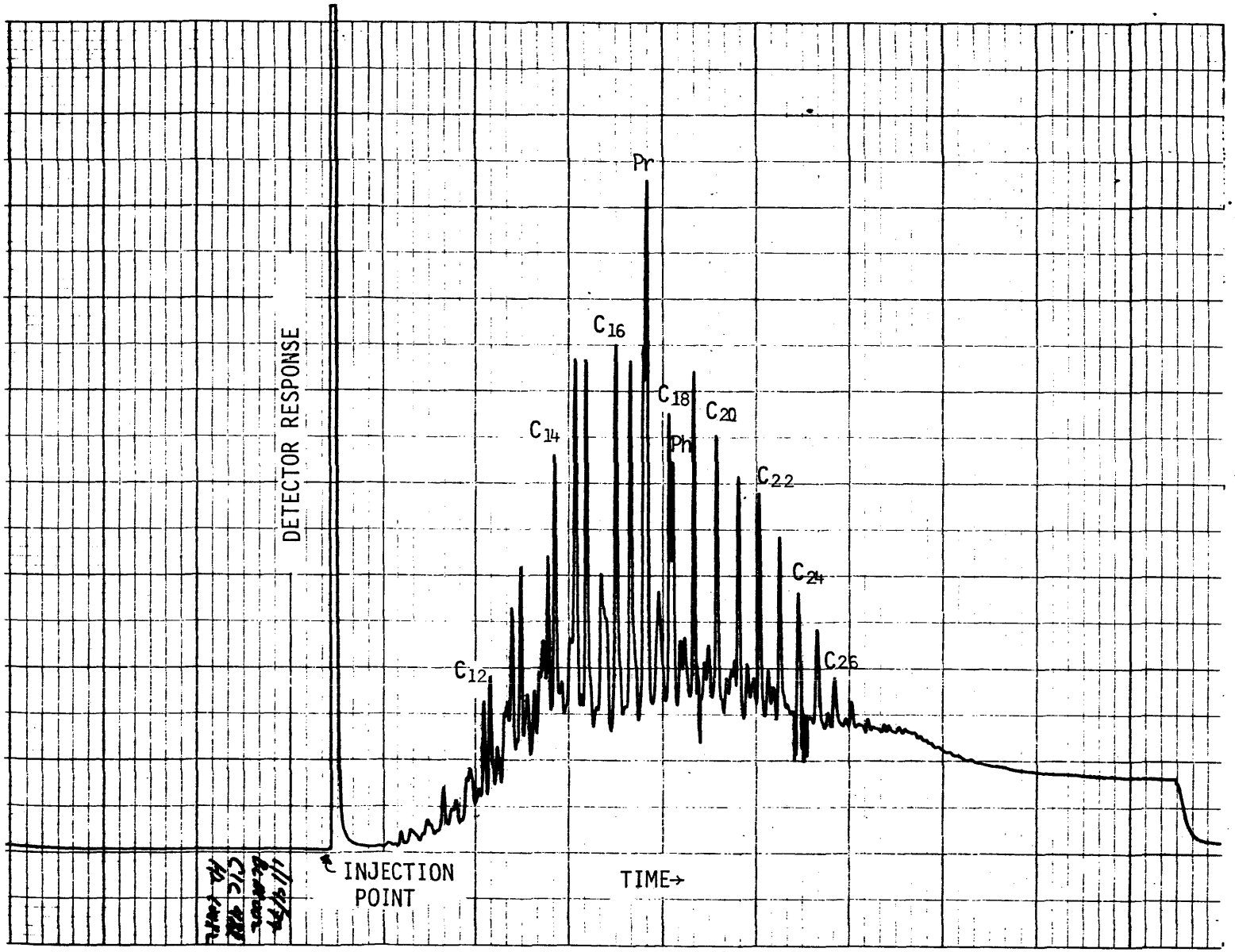


Figure 11-44. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 48 days of incubation. Sterile control, Station C1. Pr=pristane, Ph=phytane, C#=even numbered n-paraffins.

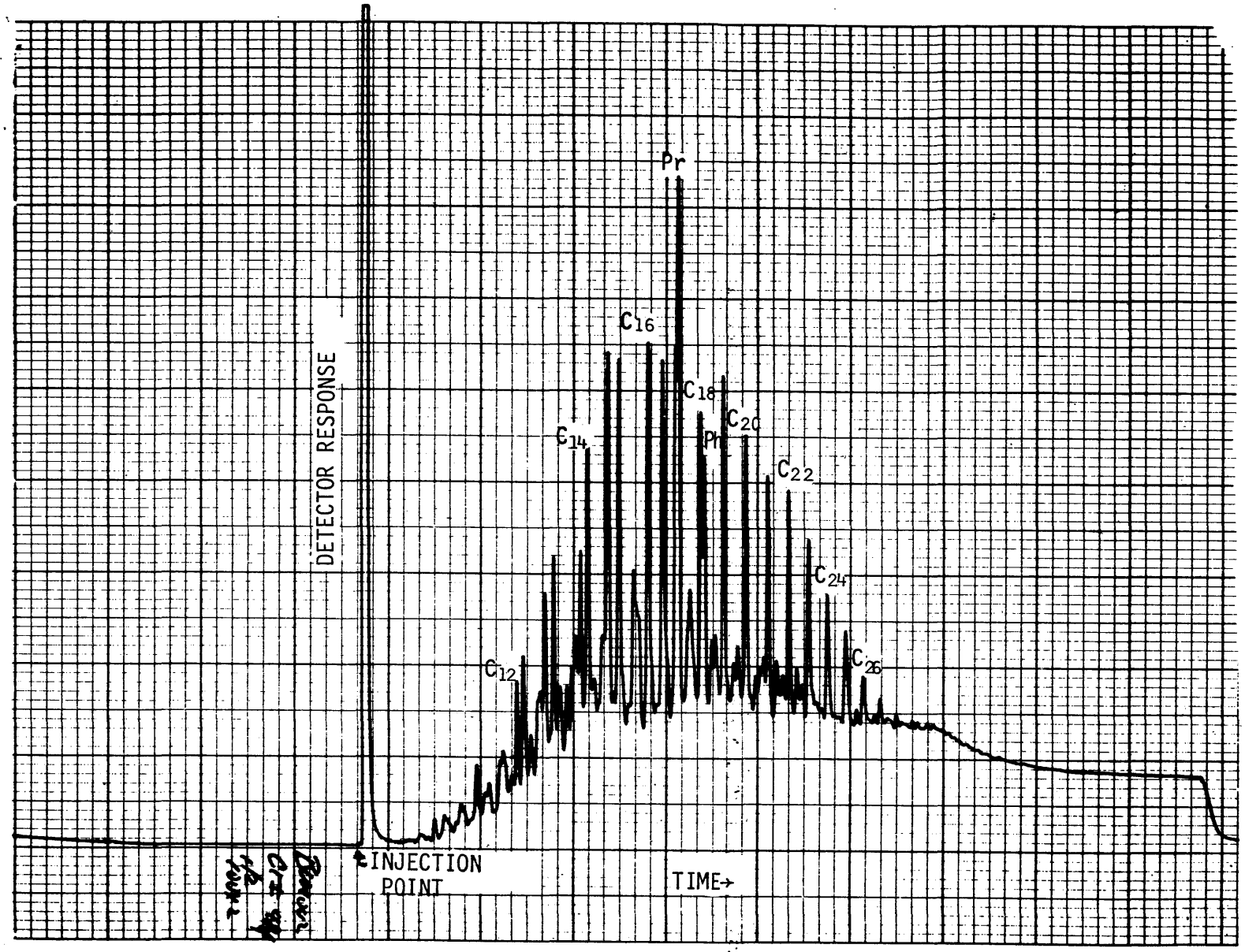


Figure 11-45. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 48 days of incubation. Non-enriched, Station C1. Pr= pristane, Ph=phytane, C#=even numbered n-paraffins.

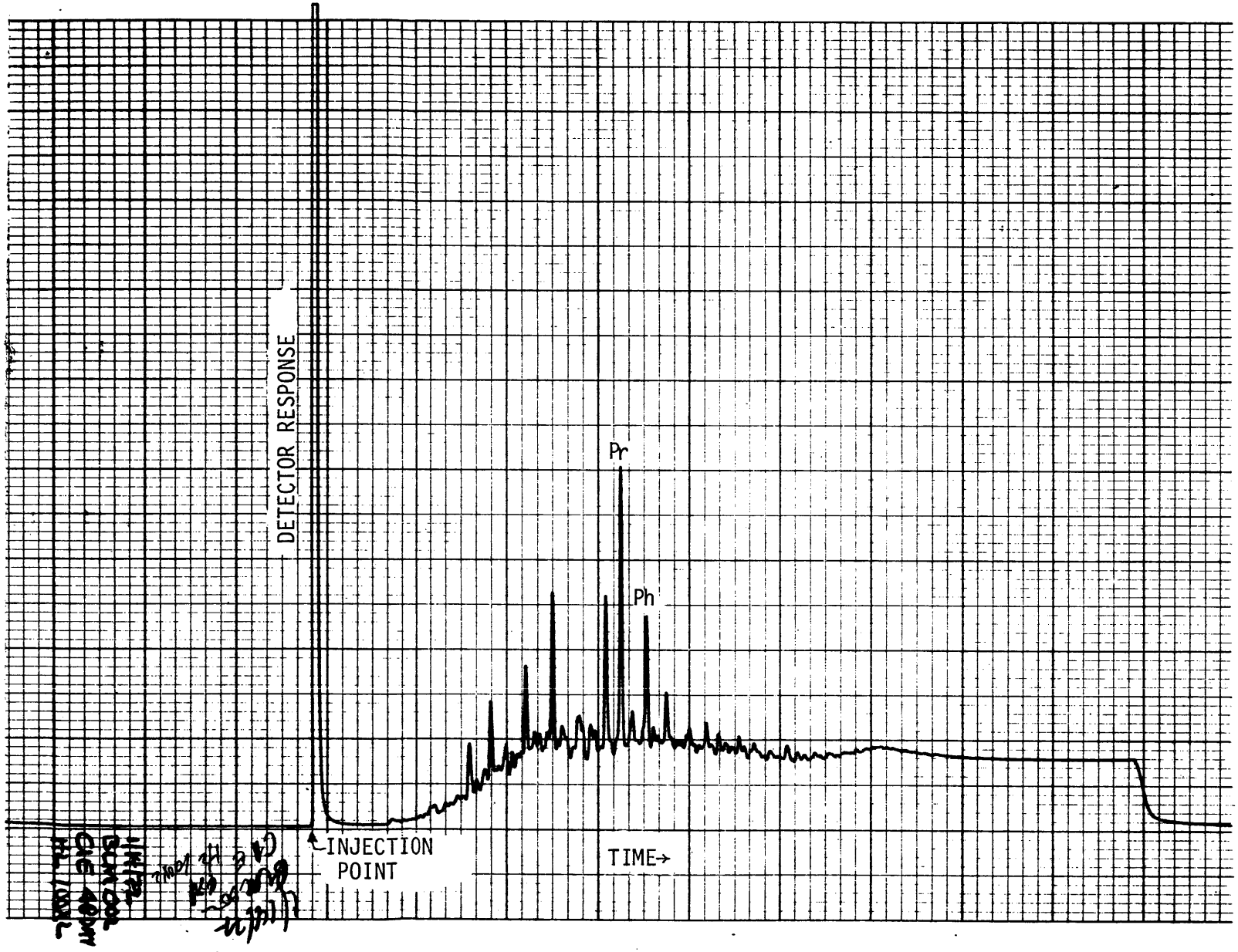


Figure 11-46. BLM02B flask studies, water inoculum. Gas chromatogram of H2 fraction of residual oil after 48 days of incubation. Enriched flask, Station C1. Pr=pristane, Ph=phytane.

Table 11-43. Rate of degradation[a] (µg/day) of n-paraffins (nC_{12}-nC_{27}) in closed flasks from petroleum degradation experiments using seawater inoculum collected during BLM Ø1B

Station	Treatment[b]	Days of Incubation 3	6	24	48
C1	I	0	0	0	0
	E	479.3	781.4	18.8	0
N2	I	0	0	0	0
	E	1062.0	67.0	59.5	0
N3	I	0	0	0	0
	E	549.7	552.3	77.7	0
E3	I	0	0	0	0
	E	877.0	0	74.9	0
F2	I	0	0	0	0
	E	938.3	294.0	29.4	2.7
J1	I	0	0	0	0
	E	164.7	0	16.9	139.7

[a] Rate of degradation = $\frac{\text{weight loss}_2 - \text{weight loss}_1}{\text{day}_2 - \text{day}_1}$

where: weight loss_1 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the first incubation period

weight loss_2 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the second incubation period

day_1 = the number of days in the first incubation period

day_2 = the number of days in the second incubation period

For this calculation only weight losses >10% were considered to to be due to bacterial degradation.

[b] Treatment: I = seawater + sterile oil
E = seawater + sterile oil + nutrient amendment.

Table 11-44. Rate of degradation[a] (µg/day) of n-paraffins (nC_{12}-nC_{27}) in closed flasks from petroleum degradation experiments using seawater inoculum collected during BLM Ø2B

Station	Treatment[b]	Days of Incubation 3	6	24	48
C1	I	0	0	177.8	0
	E	0	0	0	0
N2	I	0	0	0	0
	E	0	0	109.1	43.0
N3	I	0	0	0	0
	E	0	148.3	148.4	12.0
E3	I	0	0	0	0
	E	0	313.3	19.1	105.2
F2	I	0	0	0	0
	E	0	252.3	128.9	0
J1	I	0	0	0	0
	E	0	104	205.7	50.7

[a] Rate of degradation = $\frac{\text{weight loss}_2 - \text{weight loss}_1}{\text{day}_2 - \text{day}_1}$

where: weight loss_1 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the first incubation period

weight loss_2 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the second incubation period

day_1 = the number of days in the first incubation period

day_2 = the number of days in the second incubation period

For this calculation only weight losses >10% were considered to to be due to bacterial degradation.

[b] Treatment: I = seawater + sterile oil
E = seawater + sterile oil + nutrient amendment.

Table 11-45. Rate of degradation[a] (µg/day) of n-paraffins (nC_{12}-nC_{27}) in closed flasks from petroleum degradation experiments using seawater inoculum collected during BLM Ø3B

Station	Treatment[b]	Days of Incubation 3	6	24	48
C1	I	0	0	0	0
	E	0	207.5	154.0	0
N2	I	0	0	0	0
	E	0	0	0	78.4
N3	I	0	0	0	0
	E	0	0	111.8	63.0
E3	I	0	0	0	0
	E	0	0	171.1	0
F2	I	0	0	0	0
	E	0	0	83.6	56.1
J1	I	0	0	0	0
	E	0	84.5	188.1	0

[a] Rate of degradation = $\frac{\text{weight loss}_2 - \text{weight loss}_1}{\text{day}_2 - \text{day}_1}$

where: weight loss_1 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the first incubation period

weight loss_2 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the second incubation period

day_1 = the number of days in the first incubation period

day_2 = the number of days in the second incubation period

For this calculation only weight losses >10% were considered to to be due to bacterial degradation.

[b] Treatment: I = seawater + sterile oil
E = seawater + sterile oil + nutrient amendment.

Table 11-46. Rate of degradation[a] (µg/day) of n-paraffins (nC_{12}-nC_{27}) in closed flasks from petroleum degradation experiments using seawater inoculum collected during BLM Ø4B

Station	Treatment[b]	Days of Incubation 3	6	24	48
C1	I	0	0	0	0
	E	223.7	72.3	46.6	0
N2	I	0	0	0	0
	E	0	117.8	2.1	50.1
N3	I	0	0	6.4	0
	E	0	0	109.7	0
E3	I	0	0	0	0
	E	0	0	61.8	48.6
F2	I	0	0	0	0
	E	0	89.8	33.7	104.0
J1	I	0	0	0	0
	E	0	0	0	0

[a] Rate of degradation = $\frac{\text{weight loss}_2 - \text{weight loss}_1}{\text{day}_2 - \text{day}_1}$

where: weight loss_1 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the first incubation period

weight loss_2 = µg n-paraffins, control flasks - µg n-paraffins, treated flasks during the second incubation period

day_1 = the number of days in the first incubation period

day_2 = the number of days in the second incubation period

For this calculation only weight losses >10% were considered to to be due to bacterial degradation.

[b] Treatment: I = seawater + sterile oil
E = seawater + sterile oil + nutrient amendment.

Table 11-47. Days of incubation required for degradation[a] of 50% (D_{50})[b] and 100% (D_{100})[b] of n-paraffins (nC_{12} - nC_{27}) in closed flasks from petroleum degradation experiments using seawater inocula collected during BLM Ø1B-Ø4B.

Station	Treatment[c]	Cruise							
		Ø1B		Ø2B		Ø3B		Ø4B	
		D_{50}	D_{100}	D_{50}	D_{100}	D_{50}	D_{100}	D_{50}	D_{100}
C1	Inoculated								
	Enriched	6	12	24	24	24	24	6	24
N2	Inoculated								
	Enriched	3	24	24	NR	48	NR	48	NR
N3	Inoculated								
	Enriched	6	24	24	NR	24	48	24	NR
E3	Inoculated								
	Enriched	3	NR	12	NR	24	24	48	NR
F2	Inoculated								
	Enriched	3	48	24	NR	24	NR	48	48
J1	Inoculated								
	Enriched	12	NR	24	NR	24	24	NR	NR

[a] - Degradation based on weight loss in treated flask as compared to control.

[b] - D_{50} - Incubation period during which degradation was first observed to be 50% or more when compared to the control;
D_{100} - incubation period when degradation was first observed to be 100% of the control.

[c] - Inoculated = seawater + sterile oil
Enriched = seawater + sterile oil + nutrient amendment

NR - The amount of degradation never reached this level.

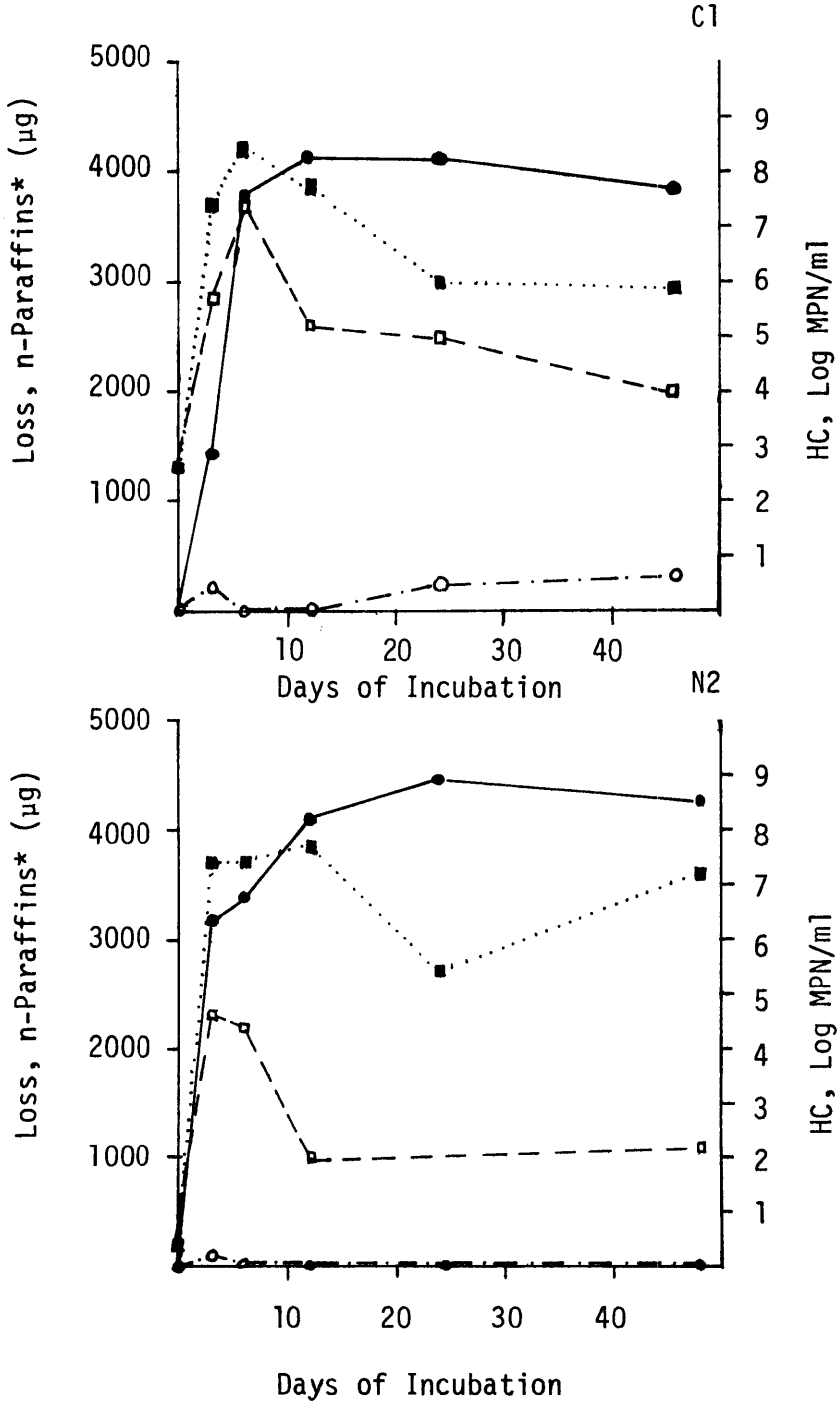


Figure 11-47. Losses in n-paraffins (nC_{12}-nC_{27}) and changes in the levels of petroleum degrading bacteria in closed flask petroleum degradation experiments using seawater inocula collected during BLM01B.

*Loss = µg n-paraffins (nC_{12}-nC_{27}) sterile control − µg n-paraffins (nC_{12}-nC_{27}) treated flask

●——● Enriched

○—·—·—○ Non-enriched

HC = Petroleum Degrading Bacteria, ■·····■ Enriched

□------□ Non-enriched

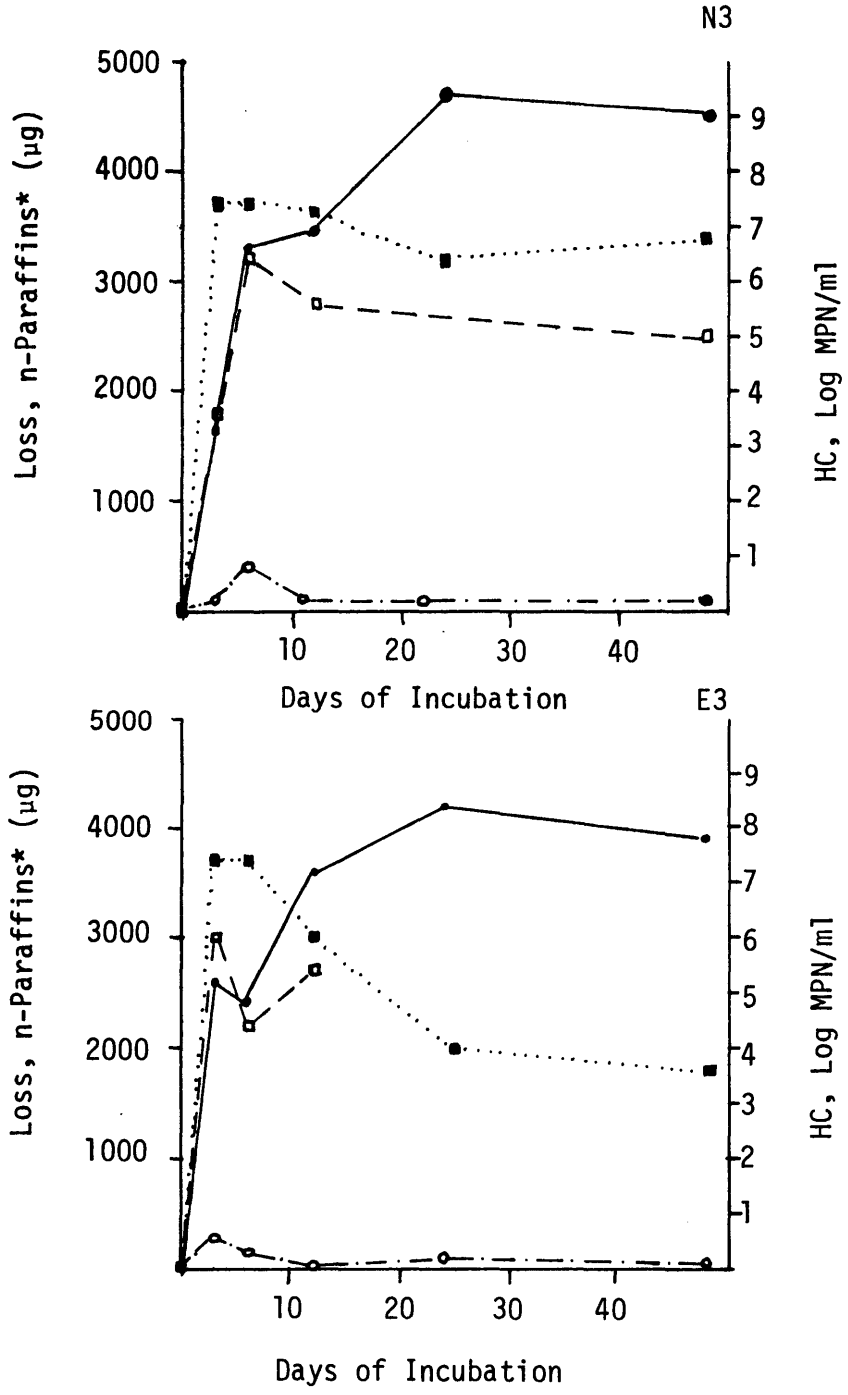


Figure 11-48. Losses in n-paraffins (nC_{12}-nC_{27}) and changes in the levels of petroleum degrading bacteria in closed flask petroleum degradation experiments using seawater inocula collected during BLM01B.

*Loss = µg n-paraffins (nC_{12}-nC_{27}) sterile control − µg n-paraffins (nC_{12}-nC_{27}) treated flask

●——● Enriched

○—·—·—○ Non-enriched

HC = Petroleum Degrading Bacteria, ■·····■ Enriched

▫------▫ Non-enriched

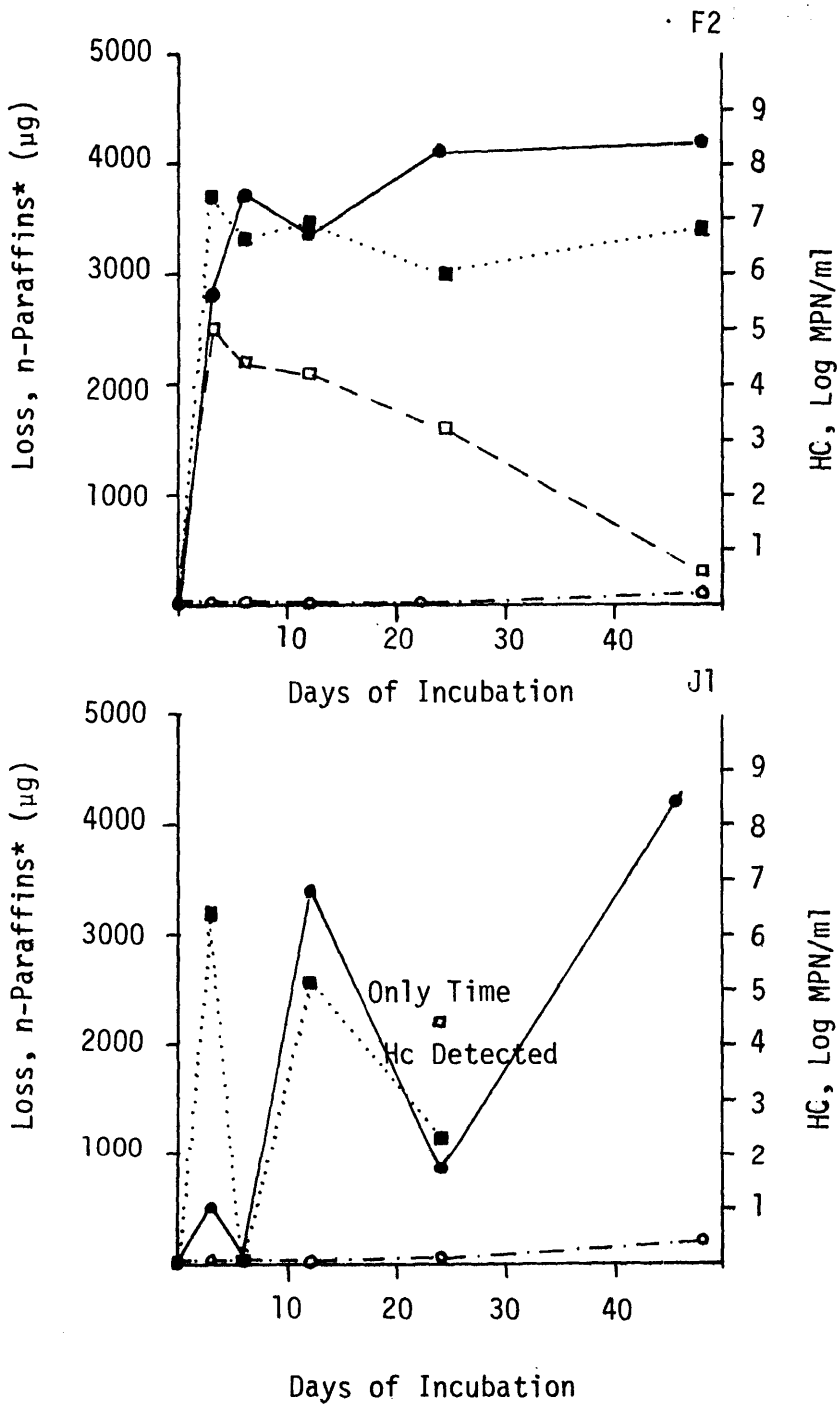


Figure 11-49. Losses in n-paraffins (nC_{12}-nC_{27}) and changes in the levels of petroleum degrading bacteria in closed flask petroleum degradation experiments using seawater inocula collected during BLM01B.

*Loss = µg n-paraffins (nC_{12}-nC_{27}) sterile control − µg n-paraffins (nC_{12}-nC_{27}) treated flask

●——● Enriched

○—·—·—○ Non-enriched

HC = Petroleum Degrading Bacteria, ■·····■ Enriched

□------□ Non-enriched

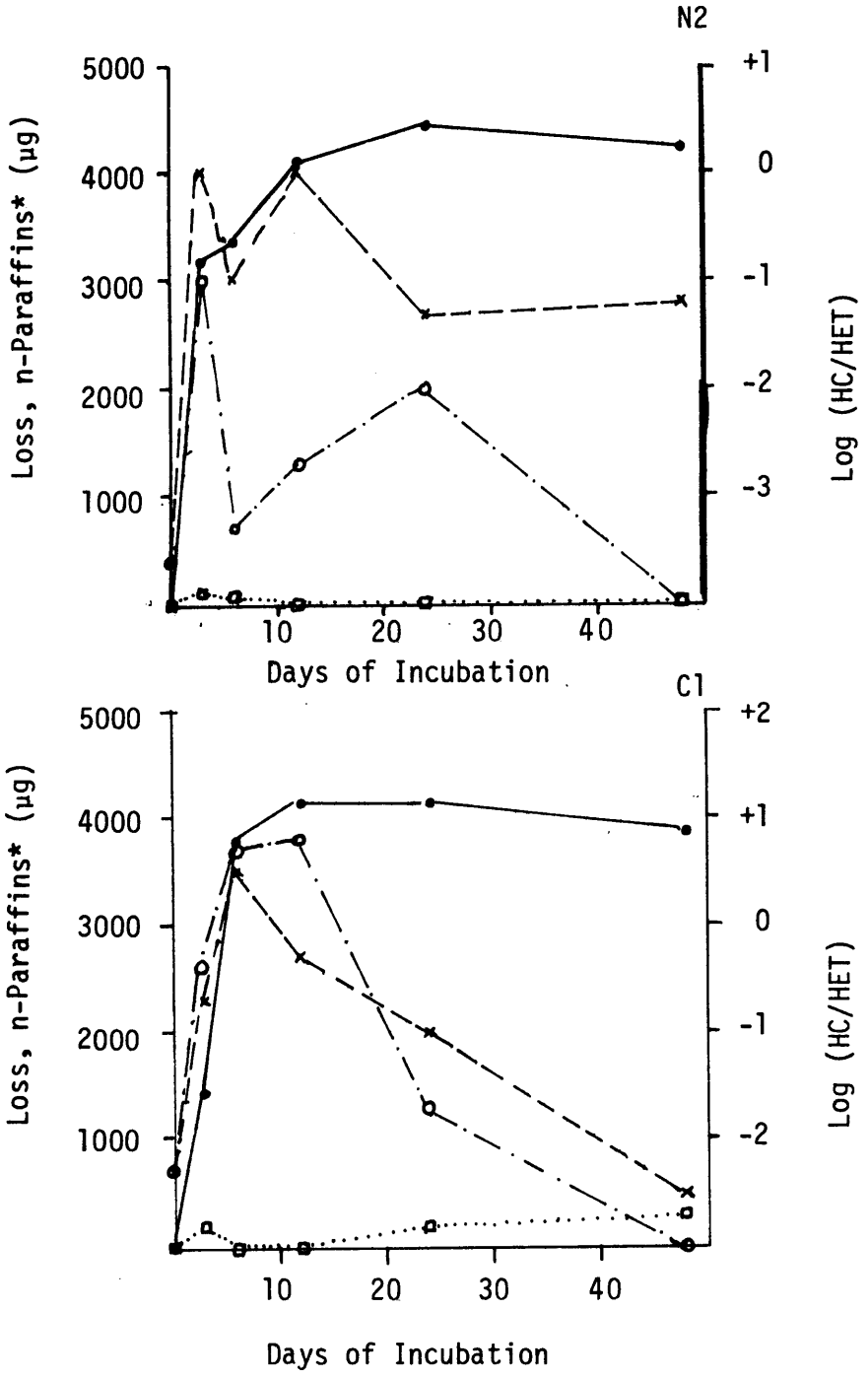


Figure 11-50. Losses of n-paraffins (nC_{12}-nC_{27}) and changes in the petroleum degrading to heterotrophic bacteria ratio in closed flask petroleum degradation experiments using seawater inocula collected during BLM01B.

*Loss = µg n-paraffins (nC_{12}-nC_{27}) control − µg n-paraffins (nC_{12}-nC_{27}) treated flask.

●——● Enriched

□·····□ Inoculated

$$HC/HET = \frac{\text{MPN/ml Petroleum Degrading Bacteria}}{\text{MPN/ml Heterotrophic Bacteria}}$$

×- - - × Enriched

○—·—·—○ Inoculated

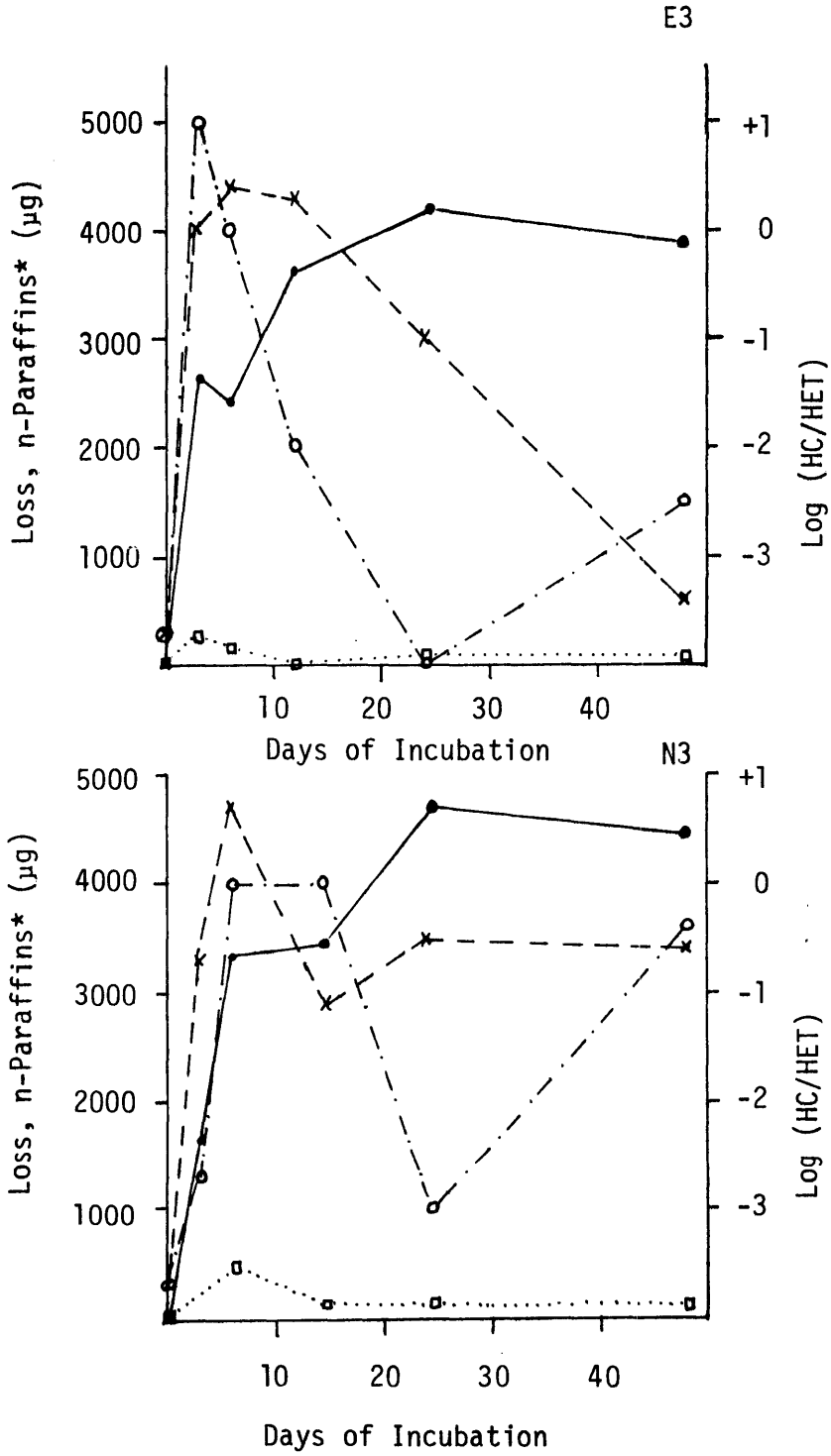


Figure 11-51. Losses of n-paraffins (nC_{12}-nC_{27}) and changes in the petroleum degrading to heterotrophic bacteria ratio in closed flask petroleum degradation experiments using seawater inocula collected during BLM01B.

*Loss = µg n-paraffins (nC_{12}-nC_{27}) control − µg n-paraffins (nC_{12}-nC_{27}) treated flask.

●——● Enriched

□·····□ Inoculated

$$HC/HET = \frac{\text{MPN/ml Petroleum Degrading Bacteria}}{\text{MPN/ml Heterotrophic Bacteria}}$$

×- - -× Enriched

○—·—·—○ Inoculated

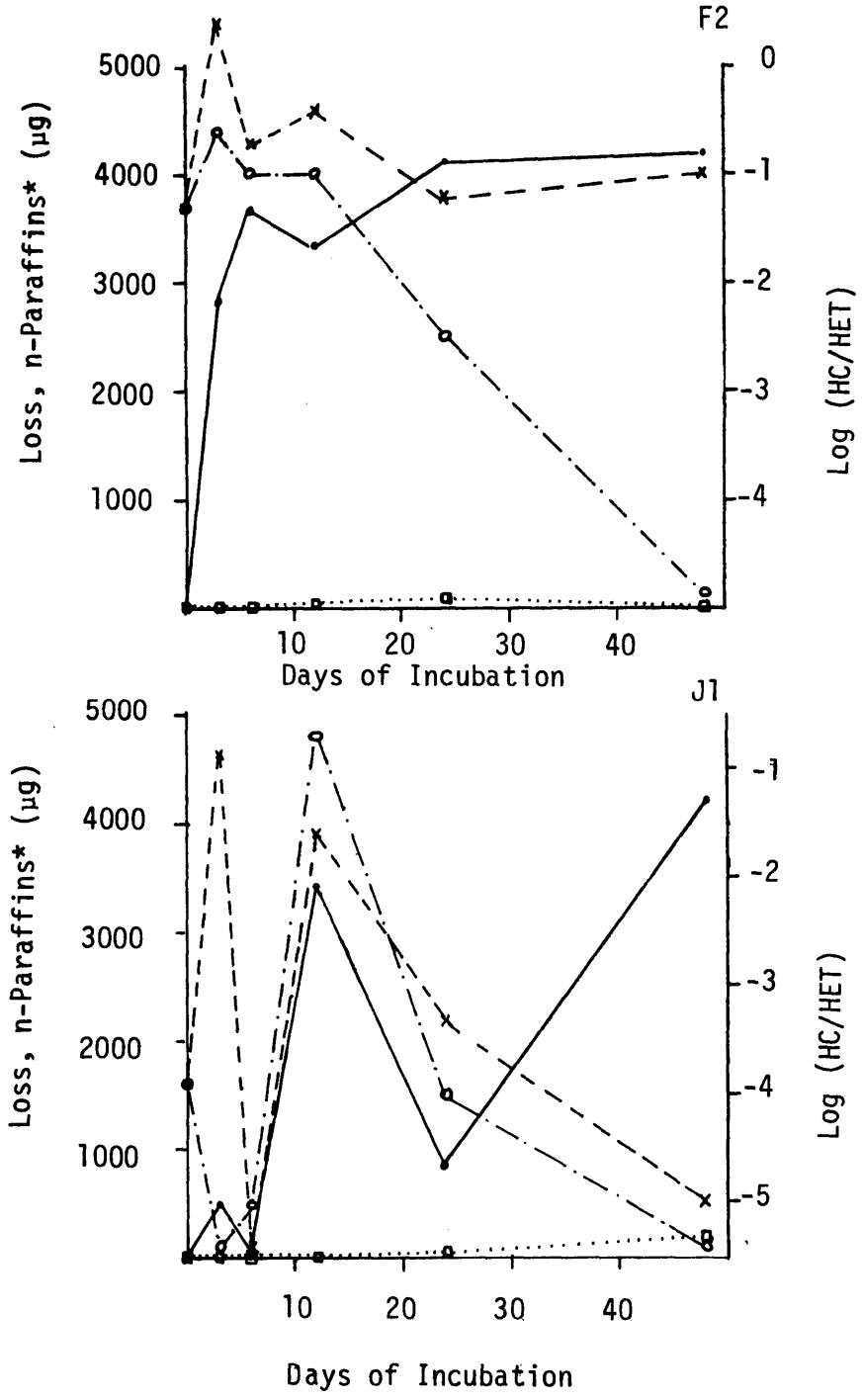


Figure 11-52. Losses of n-paraffins (nC_{12}-nC_{27}) and changes in the petroleum degrading to heterotrophic bacteria ratio in closed flask petroleum degradation experiments using seawater inocula collected during BLM01B.

*Loss = µg n-paraffins (nC_{12}-nC_{27}) control − µg n-paraffins (nC_{12}-nC_{27}) treated flask.

●——● Enriched

□·····□ Inoculated

$$HC/HET = \frac{\text{MPN/ml Petroleum Degrading Bacteria}}{\text{MPN/ml Heterotrophic Bacteria}}$$

×- - - × Enriched

o—·—·o Inoculated

at the expense of ambient nutrients.

Sediment Inocula, Enumeration. Results of this experiment are summarized in Table 11-48 and Figures 11-53 through 11-56. Heterotrophic bacterial counts increased from initial values in oil free, non-enriched, and enriched flasks for all station clusters examined. The greatest increases were observed in enriched flasks. Levels of petroleum degraders also increased under all treatment conditions for all station clusters. Greatest increases in these levels were observed under enriched conditions. Levels of petroleum degrading bacteria in non-enriched and oil free flasks with "F" cluster inocula decreased after initial increases. Values of HC/HET generally increased over the duration of the incubation period. However "F" cluster inocula values in oil free and non-enriched flasks decreased after 6 days of incubation. Statistical analysis of HC levels and HC/HET values indicated a significant difference ($p < 0.05$) in flasks inoculated with "F" cluster sediment homogenates (Table 11-49). Under enriched conditions, HC counts and HC/HET values were significantly higher than in either non-enriched or oil free conditions. There was no difference between HC counts and HC/HET values under the various treatments in flasks inoculated with "C" or "D" cluster sediment homogenates.

Table 11-49. Values of the Friedman test statistic comparing the responses of bacterial populations using sediment inocula from C, D, and F clusters.

	STATION		
	C-cluster	D-cluster	F-cluster
HC	3.5	3.3	8.0*
HET	1.1	3.9	5.3
HC/HET	2.0	3.3	6.1*

* Significant at the 5% level

Sediment Inocula, Analysis of Residual Petroleum. Gravimetric analysis of the residual degraded petroleum indicated that weight losses in the H_2 fraction from enriched flasks were similar for inocula from all the clusters (Table 11-38). These losses were comparable to those found with corresponding water inocula. Non-enriched flasks failed to exhibit significant degradation. Gas chromatographic analyses indicated the rates of degradation and weight losses of n-paraffins (nC_{12} to nC_{27}) were similar for all inocula (Tables 11-50 and 11-51).

Continuous Dilution Experiment

Changes in heterotrophic bacterial levels were similar in the two experiments performed. Heterotroph levels rose 1.5 to 2.5 log units above initial values in both oil treated and oil free containers. Elevated levels of heterotrophs were maintained over the entire incubation period under both nutrient regimes. Levels of petroleum degrading bacteria also increased in both containers during each experiment. The rate of increase for both bacterial groups was slower in the winter (maximum population reached in 12 days) than in the summer (maximum population reached in 2

Table 11-48. Levels of petroleum degrading (HC) and heterotrophic (HET) marine bacteria in closed flasks, inoculated with sediment homogenates collected during BLM Ø4B. HC = petroleum degrading bacteria; HET = heterotrophic bacteria; ND = none detected.

Cluster[b]	Days of Incubation	Oil Free Control			Inoculated			Enriched		
		HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$	HC MPN/ml	HET MPN/ml	$\text{Log}\frac{\text{HC}}{\text{HET}}$
C	0[a]	1.3×10^{2}	8.1×10^{4}	-2.79						
	3	9.1×10^{2}	4.3×10^{5}	-2.67	4.3×10^{4}	4.3×10^{5}	-1.00	2.3×10^{4}	4.3×10^{4}	-0.27
	6	4.3×10^{3}	2.3×10^{6}	-2.72	4.3×10^{4}	9.3×10^{5}	-1.34	$>2.4\times10^{7}$	2.3×10^{7}	0.018
	24	1.1×10^{5}	2.3×10^{5}	-0.32	4.3×10^{4}	4.3×10^{6}	-1.75	2.3×10^{6}	1.5×10^{7}	-0.81
	48	9.3×10^{5}	4.6×10^{6}	-0.69	4.6×10^{5}	9.3×10^{6}	-1.31	1.1×10^{7}	1.1×10^{8}	-1.00
D	0	5.0×10^{0}	9.2×10^{4}	-4.26						
	3	2.3×10^{4}	4.3×10^{5}	-1.27	1.5×10^{4}	2.3×10^{6}	-2.19	2.3×10^{3}	9.3×10^{5}	-2.61
	6	1.1×10^{3}	2.3×10^{5}	-2.32	2.4×10^{6}	9.3×10^{5}	0.41	6.4×10^{3}	9.3×10^{5}	-2.16
	24	4.3×10^{1}	2.4×10^{6}	-4.75	4.6×10^{5}	4.3×10^{5}	0.03	2.3×10^{5}	2.4×10^{7}	-2.02
	48	1.5×10^{4}	2.3×10^{5}	-1.19	9.3×10^{4}	4.3×10^{5}	-0.66	2.4×10^{6}	9.3×10^{6}	-0.59
F	0	1.7×10^{1}	1.1×10^{5}	-3.80						
	3	3.6×10^{2}	4.3×10^{4}	-2.08	4.3×10^{4}	9.3×10^{5}	-1.34	ND	ND	
	6	9.1×10^{3}	2.3×10^{6}	-2.40	2.3×10^{5}	1.5×10^{6}	-0.81	4.3×10^{5}	9.3×10^{6}	-1.34
	24	3.6×10^{2}	4.3×10^{5}	-3.08	1.5×10^{6}	2.3×10^{6}	-0.19	9.3×10^{6}	2.3×10^{7}	-0.39
	48	9.1×10^{-1}	9.3×10^{5}	-6.01	9.1×10^{2}	2.3×10^{6}	-3.40	1.5×10^{7}	2.3×10^{6}	0.81

[a]Day "0" Levels are means of all stations in the cluster (Replicate A was used for all inocula).
[b]Cluster Stations: C includes C1, C2, C3, C4; D includes D1, D2, D3, D4; F includes F1, F2, F3, F4.

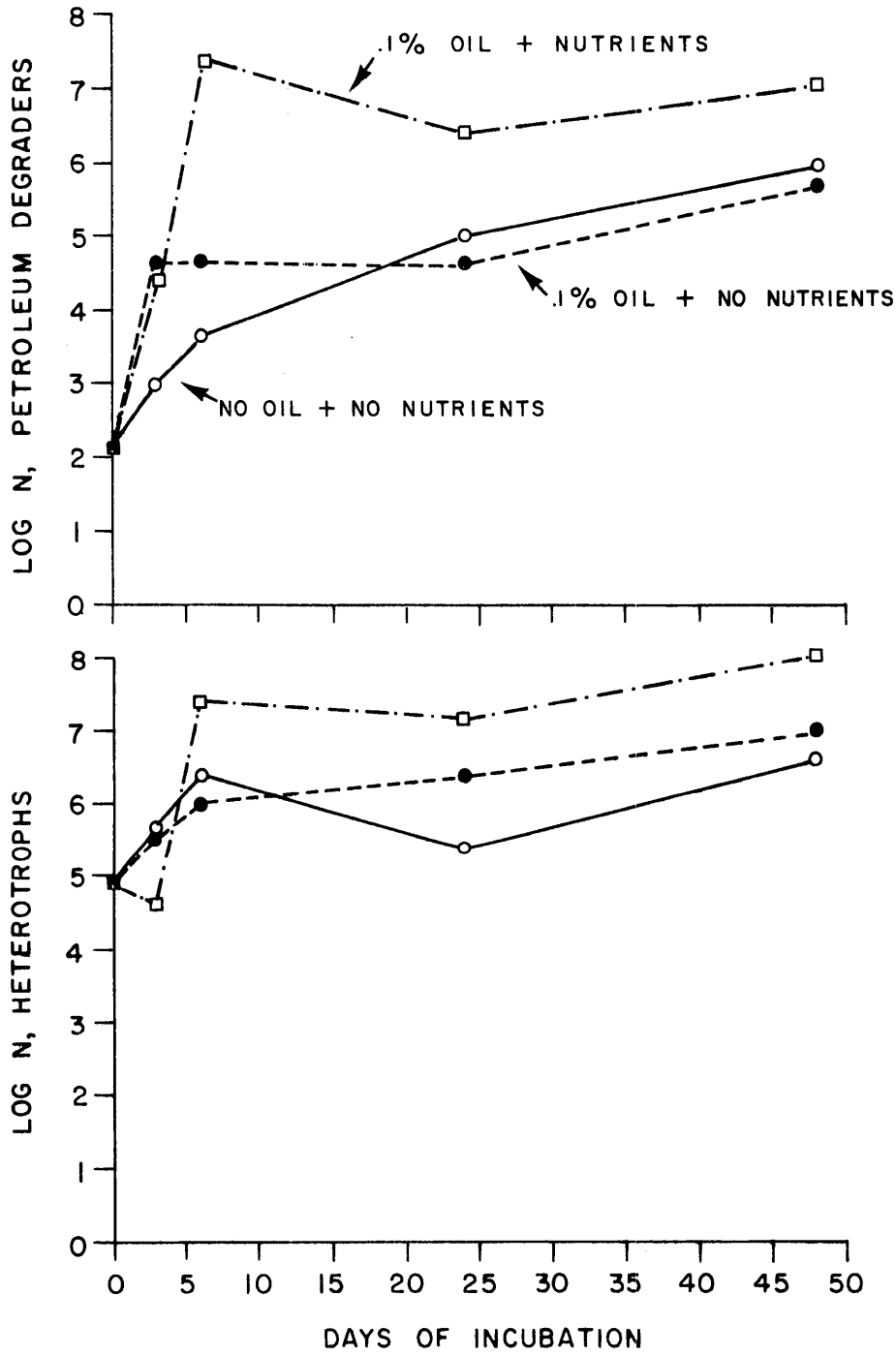


Figure 11-53. Change in levels of heterotrophic and petroleum degrading bacteria in closed flask petroleum degradation experiments utilizing sediment homogenates (From "C" Cluster) as inocula, BLM04B. N = colony forming units/ml.

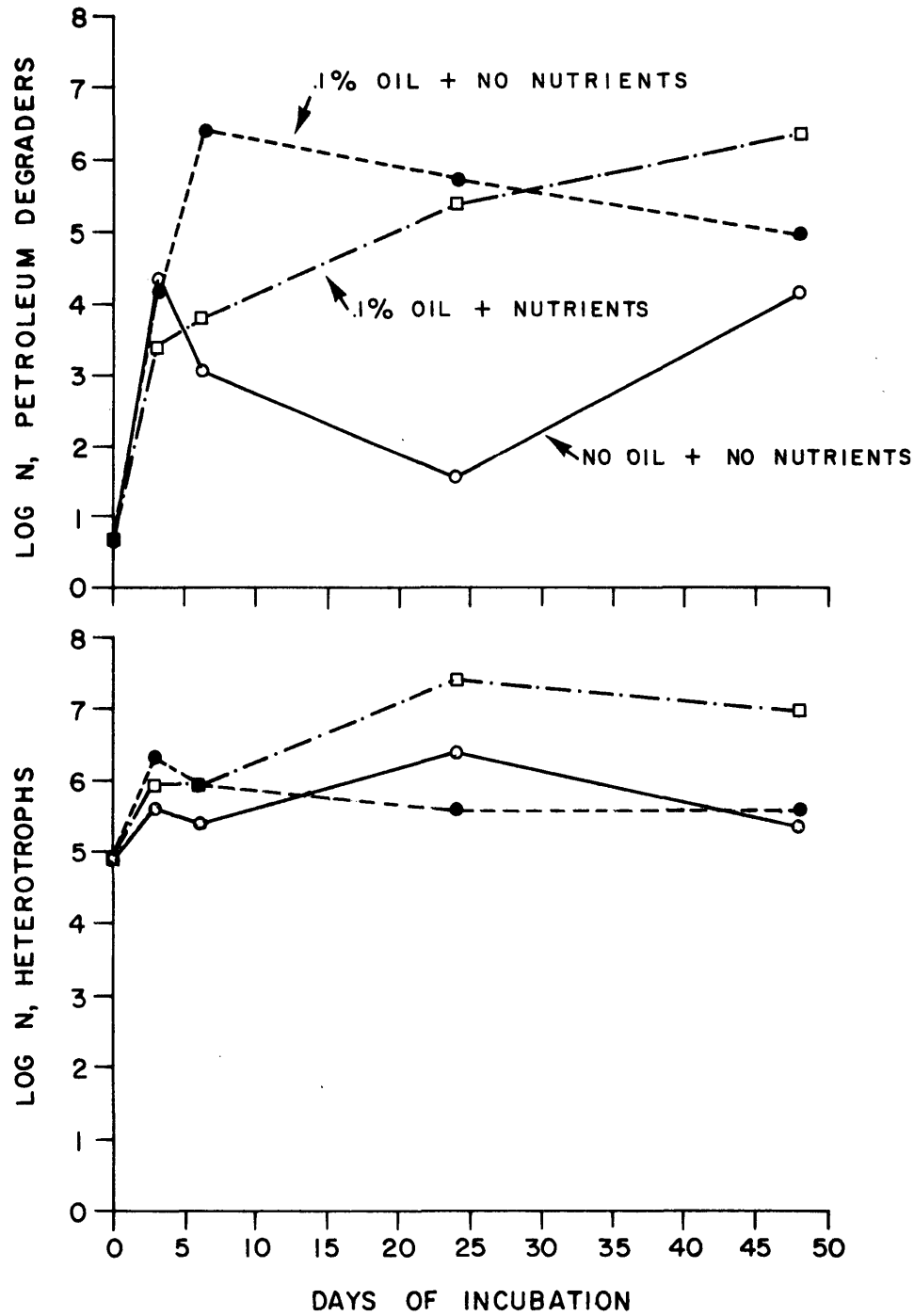


Figure 11-54. Change in levels of heterotrophic and petroleum degrading bacteria in closed flask petroleum degradation experiments utilizing sediment homogenates (From "D" Cluster) as inocula, BLM04B. N = colony forming units/ml.

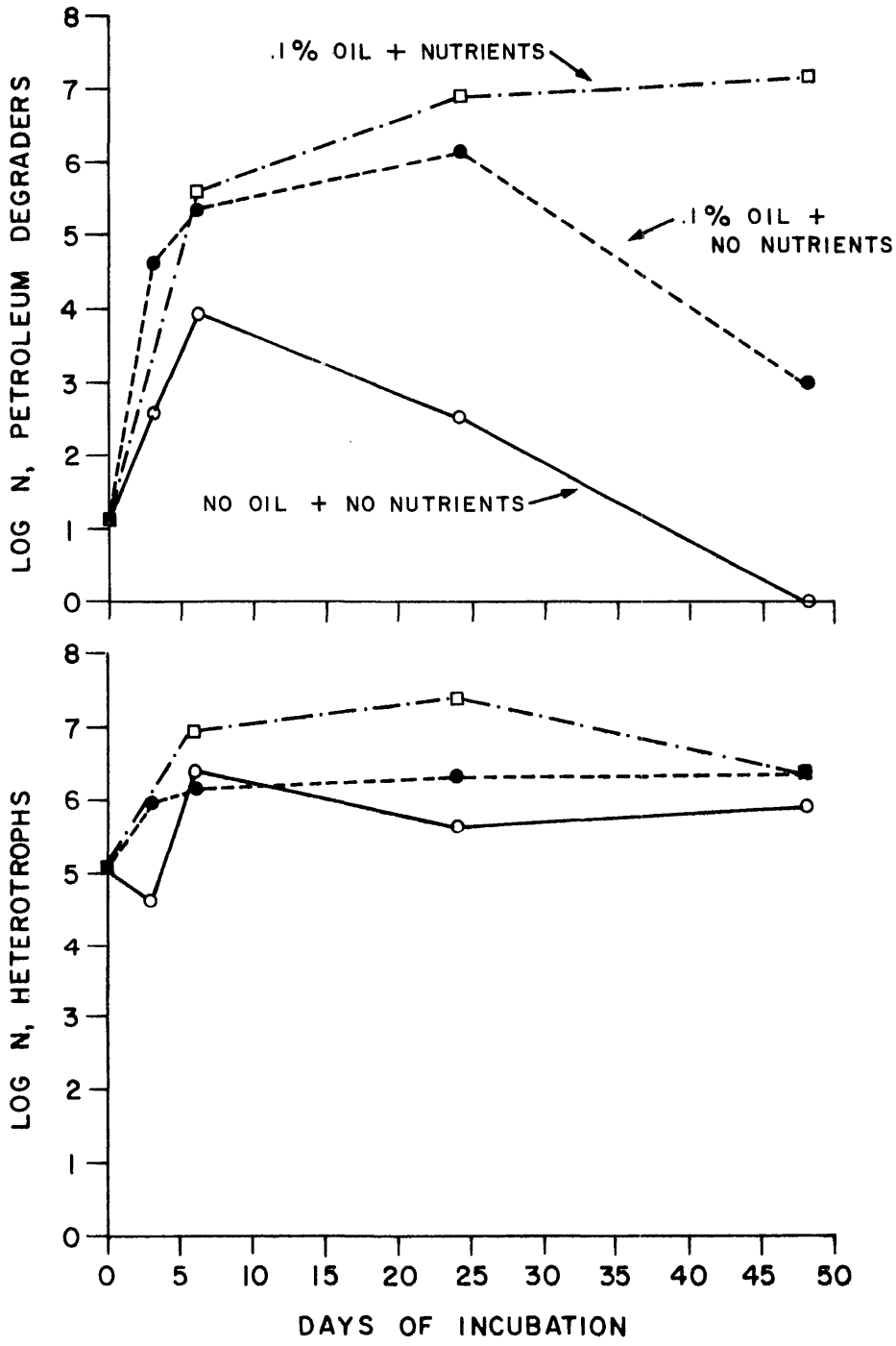


Figure 11-55. Change in levels of heterotrophic and petroleum degrading bacteria in closed flask petroleum degradation experiments utilizing sediment homogenates (From "F" Cluster) as inocula, BLM04B. N = colony forming units/ml.

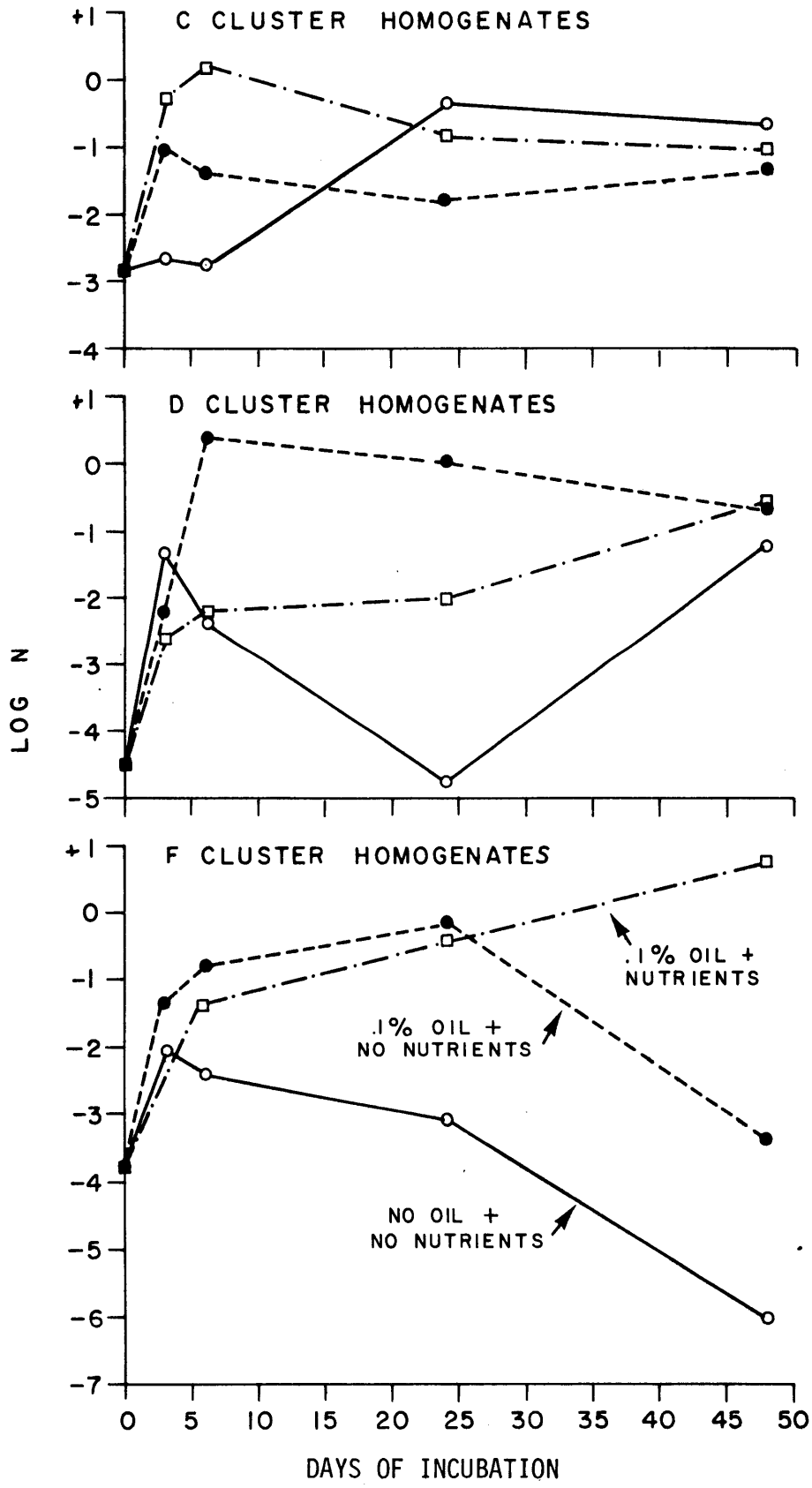


Figure 11-56. Change in the ratio of petroleum degrading to heterotrophic marine bacteria in closed flask petroleum degradation experiment utilizing sediment homogenates as inocula, BLM04B.

$$N = \frac{\text{colony forming units/ml Hydrocarbon Utilizers}}{\text{colony forming units/ml Heterotrophs}}$$

Table 11-50. Change in summation of weights (µg) of crude oil n-paraffins (nC_{12}-nC_{27}) during incubation in closed flasks with sediment homogenate inoculated seawater collected during BLM Ø4B. % weight loss[a] in parentheses.

Station	Treatment[b]	Days of Incubation 3	6	24	48
"C" Cluster	Control	3666	3409	3450	3522
	Inoculated	3866(0)	3326(2.6)	3398(1.5)	3517(0)
	Enriched	3500(4.5)	1809(47.0)	0 (100.0)	0 (100.0)
"D" Cluster	Control	3520	3326	3369	3180
	Inoculated	3648(0)	3198(3.9)	3234(4.0)	3487(0)
	Enriched	3458(1.8)	3421(0)	0 (100.0)	0 (100.0)[c]
"F" Cluster	Control	3392	3342	3198	3634
	Inoculated	3443(0)	3188(4.7)	3080(3.7)	3419(5.9)
	Enriched	3988(0)	3359(0)	0 (100.0)	0 (100.0)[c]

[a] % weight loss = $100\left(1-\frac{(\Sigma\mu g \text{ n-paraffins } nC_{12}\text{-}nC_{27}) \text{ treated flask}}{(\Sigma\mu g \text{ n-paraffins } nC_{12}\text{-}nC_{27}) \text{ sterile control}}\right)$

[b] treatments: control - sterile seawater and sterile oil
inoculated - seawater + sterile oil
enriched - seawater + sterile oil + nutrient amendment

[c] no n-paraffins or isoprenoids resolved.

Table 11-51. Days of incubation required for degradation[a] of 50% (D_{50})[b] and 100% (D_{100})[b] of n-paraffins (nC_{12}-nC_{27}) in closed flasks from petroleum degradation experiments using seawater inoculated with sediment homogenates collected during BLM Ø4B.

Station	Treatment	D_{50}	D_{100}
"C" Cluster	Inoculated	NR[d]	RR
	Enriched	24	24
"D" Cluster	Inoculated	NR	NR
	Enriched	24	24
"F" Cluster	Inoculated	NR	NR
	Enriched	24	24

[a] Degradation based on weight loss in treated flask as compared to control.

[b] D_{50} - Incubation period during which degradation was first observed to be 50% of the control; D_{100} - incubation period when degradation was first observed to be 100% of the control.

[c] Inoculated - sterile seawater + sediment homogenate + sterile oil.
Enriched - sterile seawater + sediment homogenate + sterile oil + nutrient amendment

[d] NR - never reached.

days). Temperatures were similar in both experiments (~ 20-22°C). Levels of petroleum degrading bacteria were greater in containers with oil in both experiments (Figures 11-57 through 11-59). The ratio HC/HET paralleled changes in the numbers of petroleum degrading bacteria. In both experiments, containers treated with oil exhibited greater values of HC/HET than the oil free containers (Figures 11-58 through 11-60).

Petroleum Concentration Experiment

Results of this experiment are summarized in Table 11-52 and Figures 11-61 and 11-62. Under all treatment conditions, heterotrophic bacterial levels increased at 3 days of incubation. Heterotroph levels in the non-enriched, oil free flask returned to initial values after an increase at 3 days while elevated counts were observed in all other flasks. Nutrient enriched flasks (all oil concentrations) exhibited higher heterotroph counts than non-enriched flasks.

Numbers of petroleum degrading bacteria increased in all flasks at 3 days. These levels decreased in the oil free flask but remained at elevated levels in all oil treated flasks. Enriched flasks contained greater populations of petroleum degrading bacteria for all concentrations of oil. The value of the ratio HC/HET generally paralleled the numbers of petroleum degrading bacteria. Thus, larger values of HC/HET were observed in enriched flasks than in non-enriched flasks. The values of HC/HET in the oil free flask were generally lower than similar values in oil treated flasks.

No effect of different concentrations of oil on petroleum degrading levels was observed under non-enriched conditions. With all concentrations of oil, petroleum degrading levels increased and were maintained at slightly elevated levels. However, enriched flasks exhibited a slightly different pattern. Petroleum degrading levels in flasks with 0.1% plus nutrient enrichment increased and were maintained at high counts. With 0.01% and 0.001% oil petroleum degrading levels increased and then decreased over the incubation period.

Chitin-Oil Degradation Experiments

Changes in the levels of chitinoclastic, heterotrophic, and petroleum degrading bacteria and filterable chitin as functions of time are shown in Figures 11-63 through 11-66 and Table 11-53. Chitin loss values have been corrected for procedural and incubation losses using triplicate controls.

These data indicated that the levels of chitinoclasts rose approximately two log units during the first two weeks of incubation, decreased during the next three weeks to initial values, and thereafter remained constant or exhibited a gradual decrease. With the exception of the Ø2B sediment inoculum (+ oil), chitin loss and chitinoclastic populations were generally related. Thus, the maximum chitin loss occurred concomitant with reaching the maximum population of chitinoclasts. Maximum degradation occurred during the initial two weeks of incubation with as much as 90% of the chitin being rendered non-filterable. Thereafter, chitin loss remained constant or gradually tapered off. Bottles inoculated with the mixed culture prepared from Ø2B sediments appeared to show reduced chitin

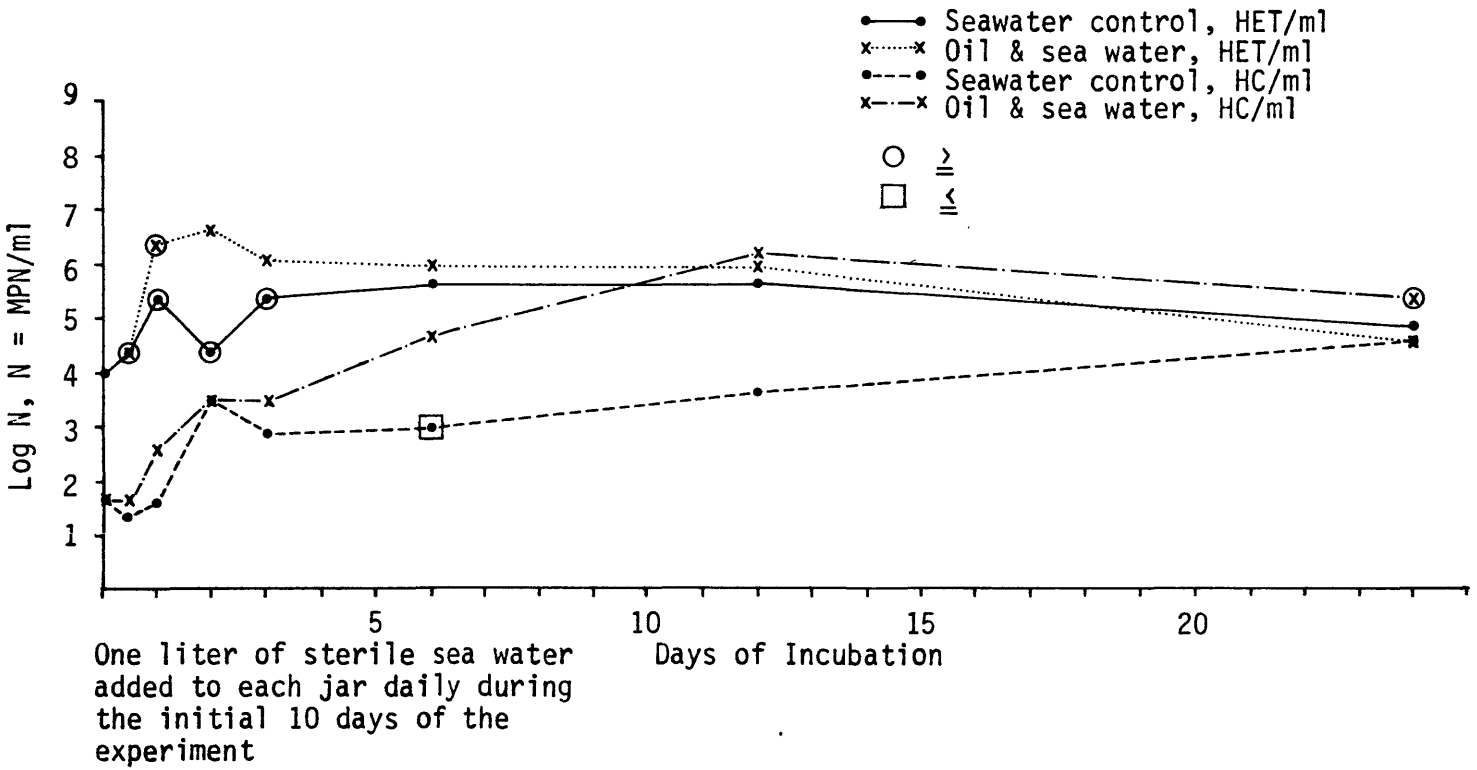


Figure 11-57. Changes in the populations of petroleum degrading (HC) and heterotrophic (HET) bacteria in a continuous dilution system, oil degradation experiment using sea water collected during BLM02B.

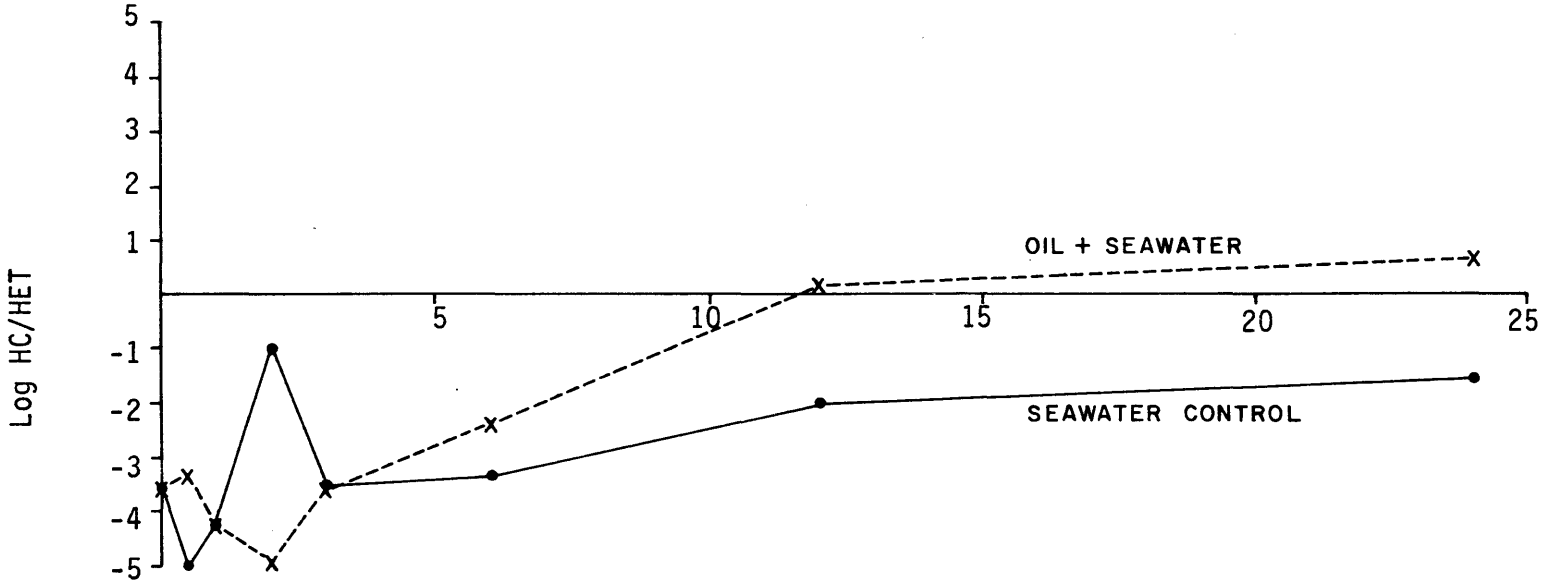


Figure 11-58. Changes in the ratio of petroleum degrading (HC) to heterotrophic (HET) bacteria in a continuous dilution system, oil degradation experiment using sea water collected during BLM02B.

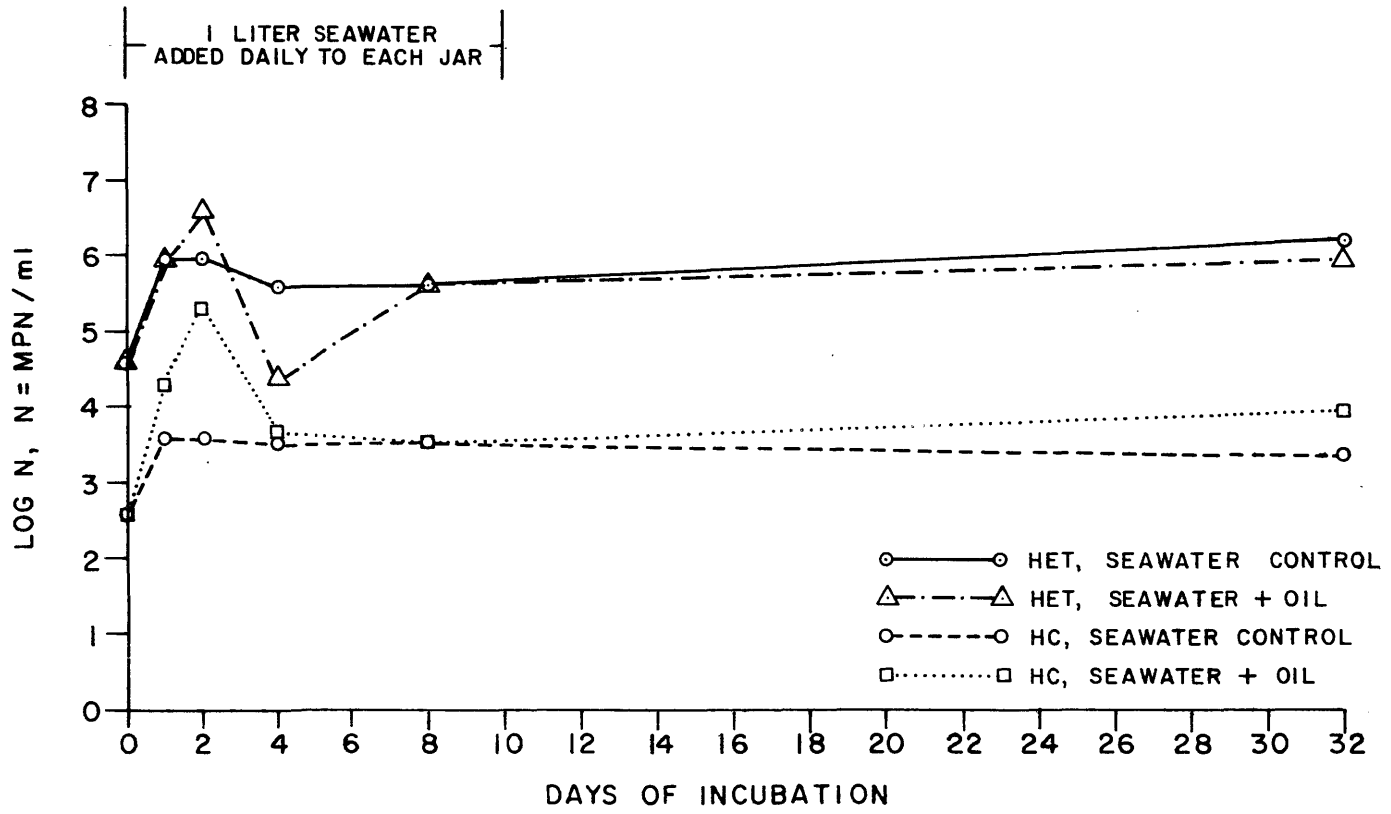


Figure 11-59. Changes in the populations of petroleum degrading (HC) and heterotrophic (HET) bacteria in a continuous dilution system, oil degradation experiment using sea water collected during BLM03B.

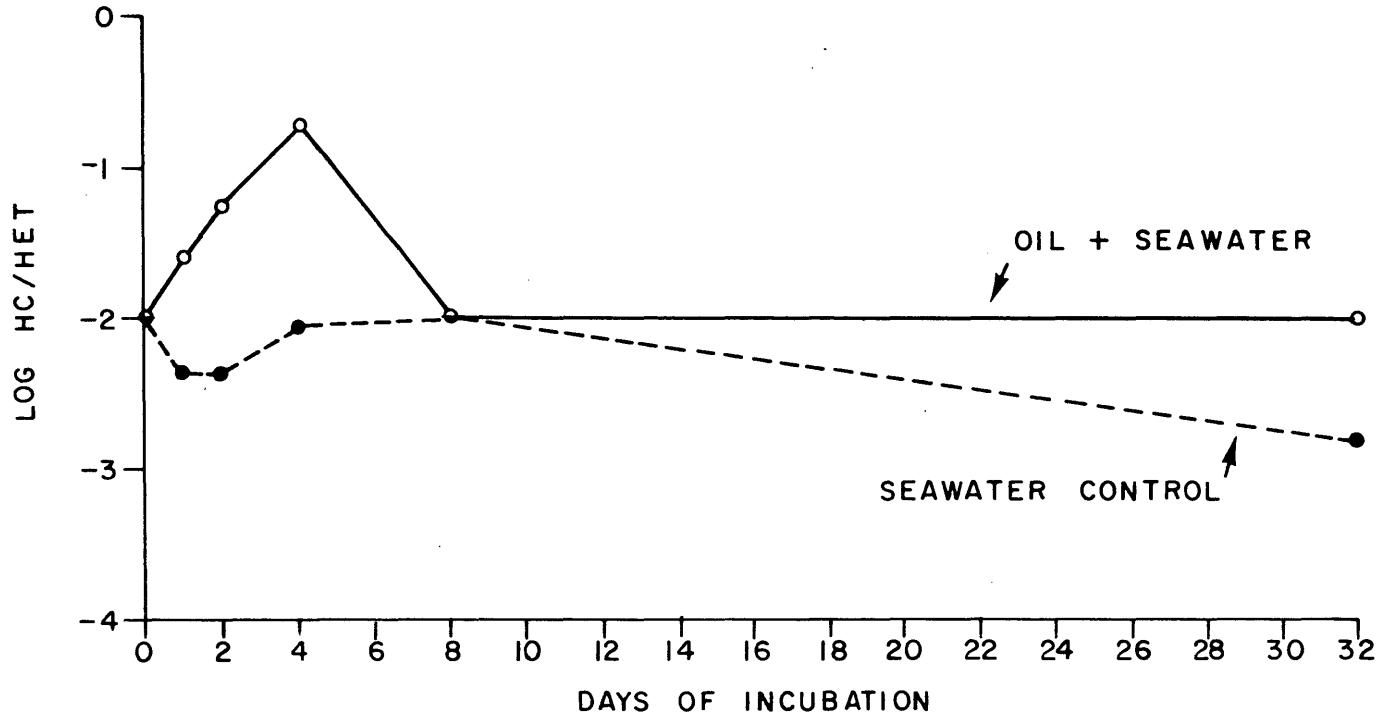


Figure 11-60. Changes in the ratio of petroleum degrading (HC) and heterotrophic (HET) bacteria in a continuous dilution system, oil degradation experiment using sea water collected during BLM03B.

Table 11-52. Effects of various concentrations of petroleum on bacterial populations in seawater under enriched and non-enriched conditions.

Sample	Days of Incubation	Log MPN, Petroleum Degraders (HC)	Log MPN, Heterotrophs (HET)	HC/HET
Seawater Control	0	3.04	4.18	-1.1
	3	4.97	5.36	-0.4
	6	3.63	4.63	-1.0
	24	3.63	4.36	-0.7
	48	2.63	4.18	-1.5
Seawater + 0.1% Oil	0	3.04	4.18	-1.1
	3	4.63	6.38	-2.0
	6	4.18	5.59	-1.4
	24	4.63	5.36	-1.0
	48	3.97	5.97	-2.0
Seawater + 0.1% Oil + Nutrient Amendment	0	3.04	4.18	-1.1
	3	$\geq$7.38	7.66	$\geq$-0.3
	6	$\geq$7.38	7.32	$\geq$+0.6
	24	7.38	7.38	0.0
	48	8.38	7.36	+1.0
Seawater + 0.01% Oil	0	3.04	4.18	-1.1
	3	2.97	5.97	-3.0
	6	3.81	5.36	-1.6
	24	3.97	4.63	-0.7
	48	4.63	5.63	-1.0
Seawater + 0.01% Oil + Nutrient Amendment	0	3.04	4.18	-1.1
	3	6.66	6.97	-0.3
	6	5.66	6.36	-0.7
	24	4.59	5.36	-0.8
	48	5.63	6.66	-1.0
Seawater + 0.001% Oil	0	3.04	4.18	-1.1
	3	4.59	5.97	-1.4
	6	3.97	5.36	-1.4
	24	4.63	4.97	-0.3
	48	4.63	5.18	-0.5
Seawater + 0.001% Oil + Nutrient Amendment	0	3.04	4.18	-1.1
	3	$\geq$6.38	5.97	+0.4
	6	6.04	5.36	+0.7
	24	4.63	6.66	-2.0
	48	4.97	5.63	-0.7

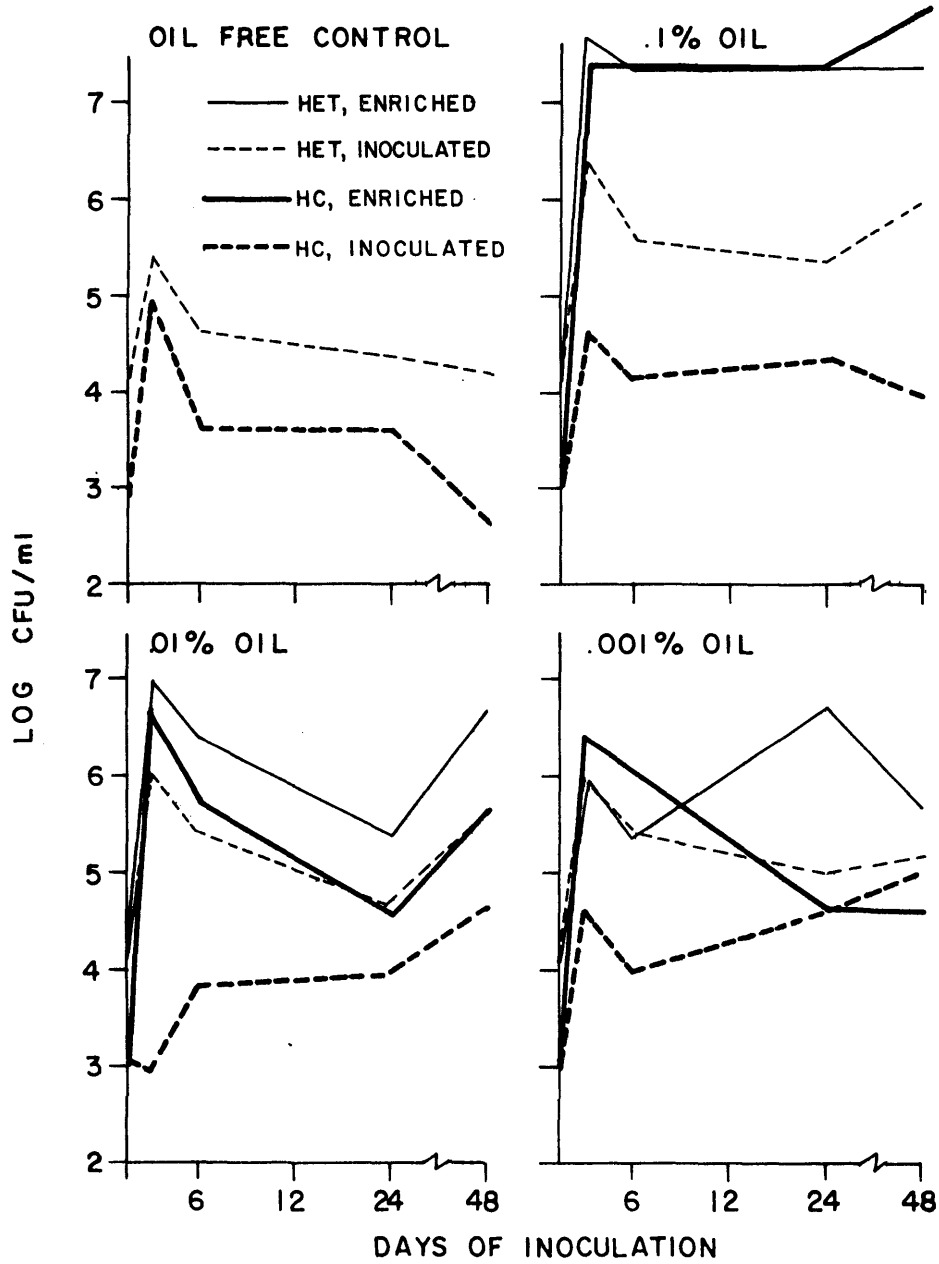


Figure 11-61. Effect of various concentrations of petroleum on populations of total heterotrophic (HET) and petroleum degrading (HC) bacteria in flasks inoculated with sea water obtained from Station I1 (BLM04B). CFU = colony forming units; Inoculated = flasks with no nutrient amendment; Enriched = Flasks with nutrient amendment.

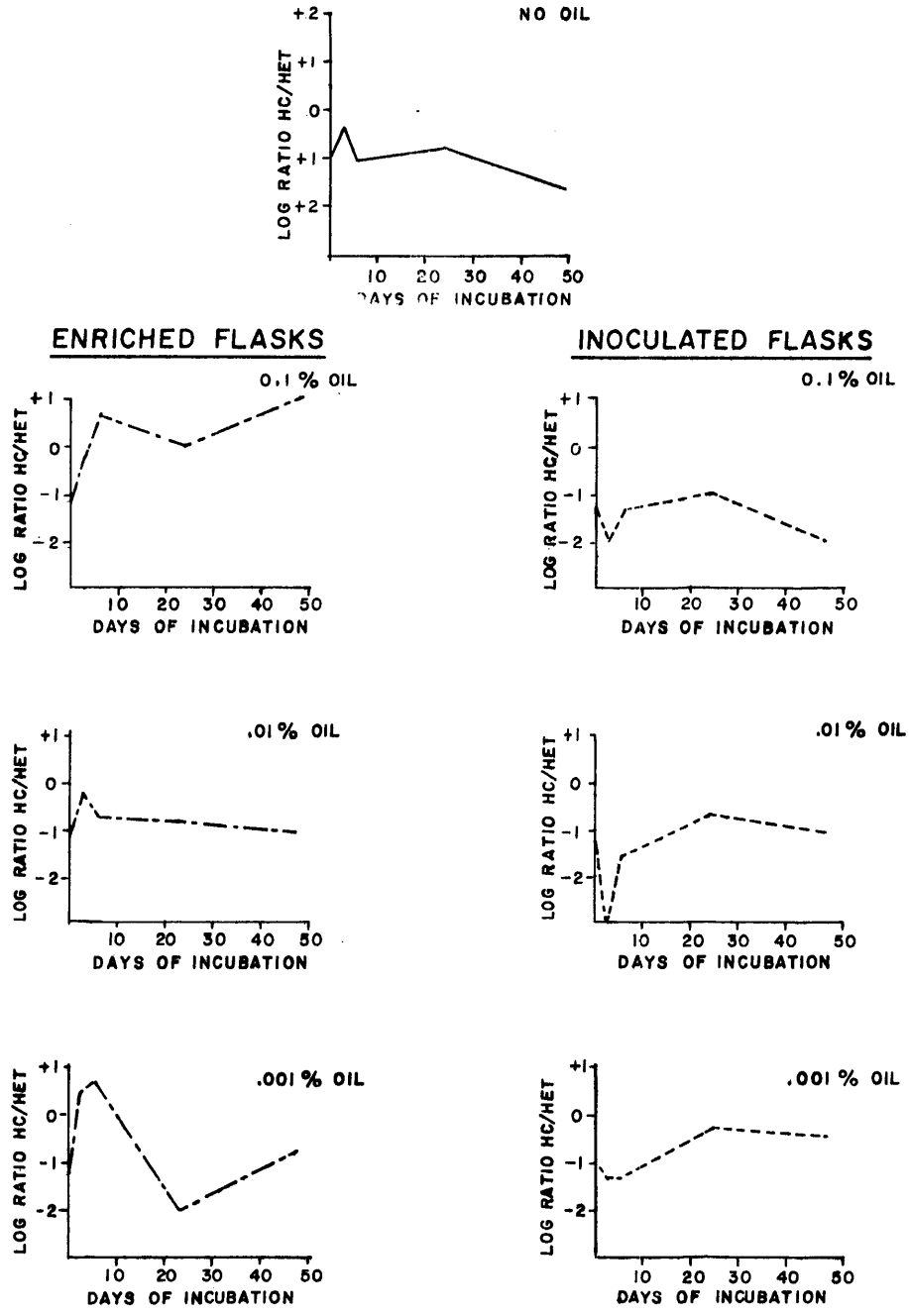


Figure 11-62. Effect of various concentrations of oil on the ratio of petroleum degrading to heterotrophic bacteria in flasks inoculated with sea water obtained from Station I1 (BLM 04B).

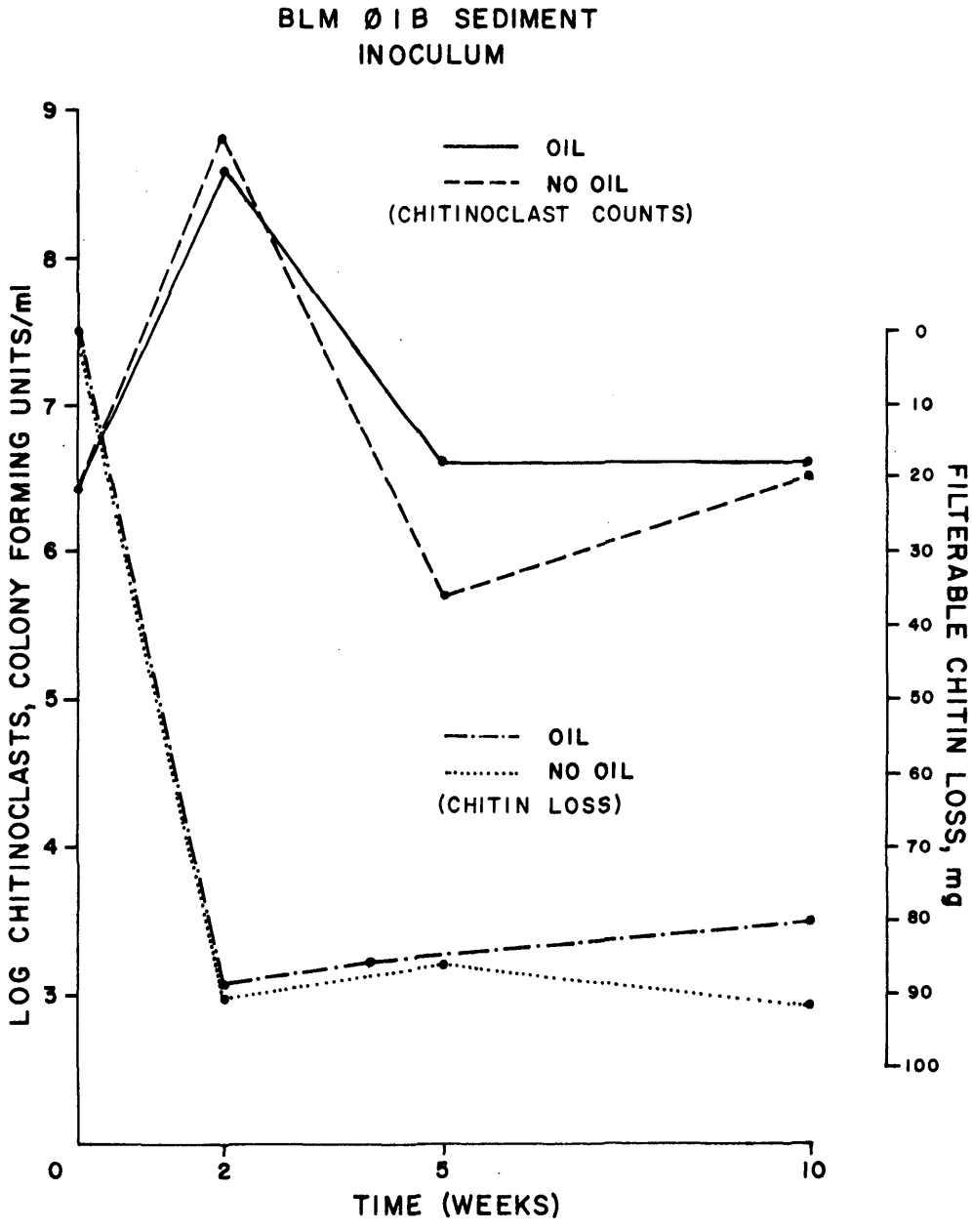


Figure 11-63. Change in amount of particulate chitin and chitinoclastic marine bacteria in a seawater-peptone broth with and without unweathered South Louisiana crude oil (0.1%).

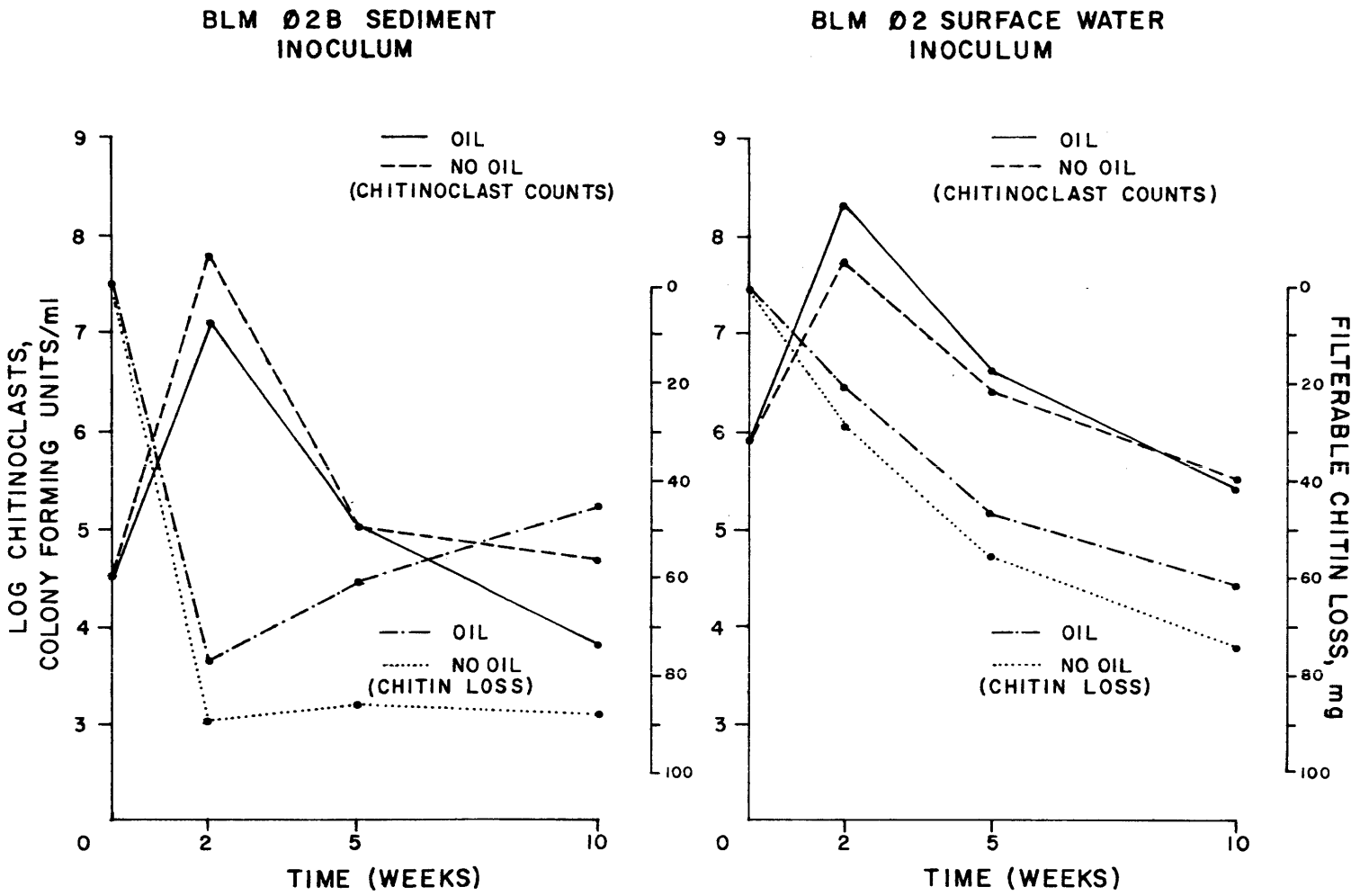
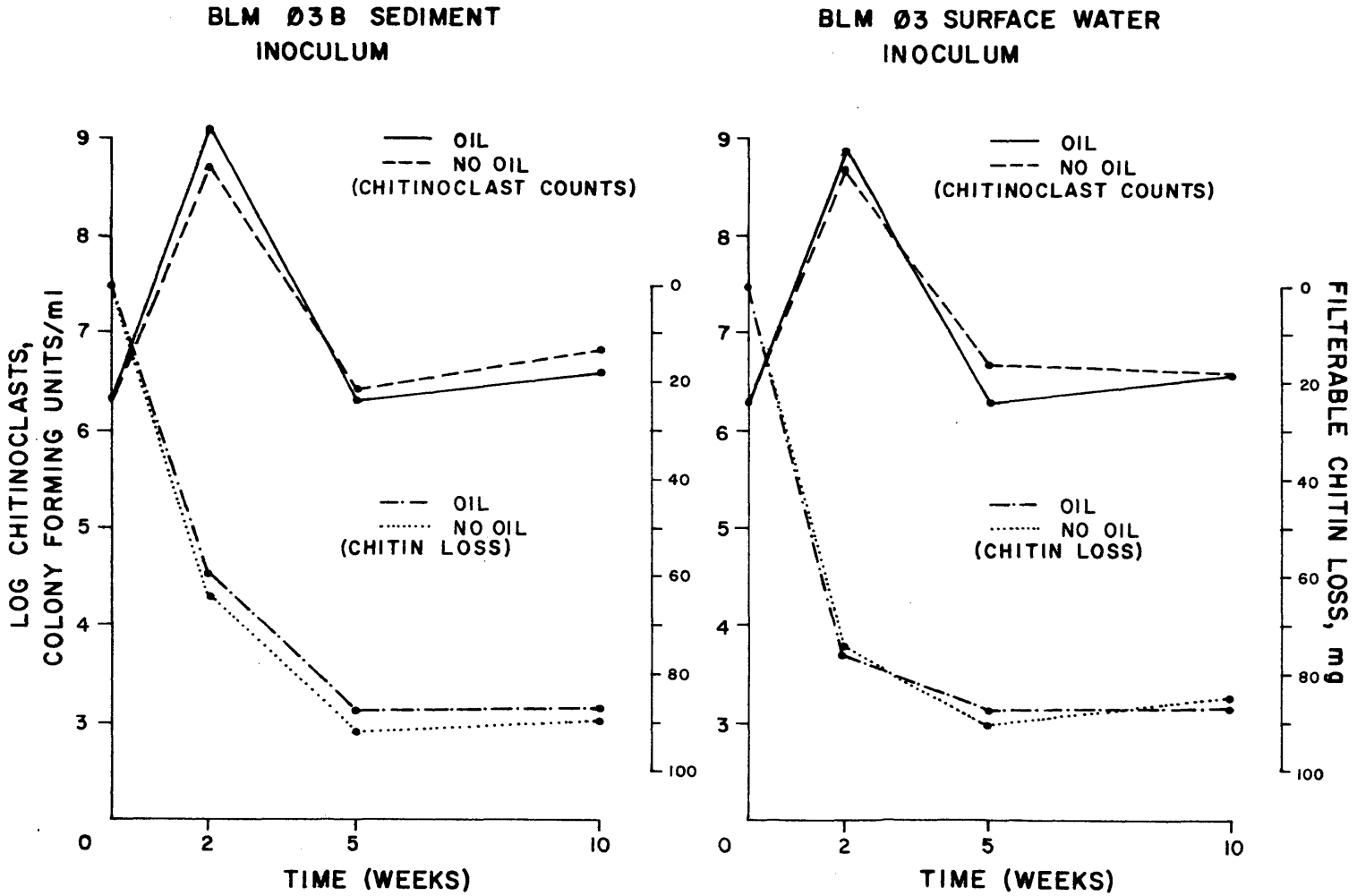


Figure 11-64. Change in amount of particulate chitin and chitinoclastic marine bacteria in a seawater-peptone broth with and without unweathered South Louisiana crude oil (0.1%).



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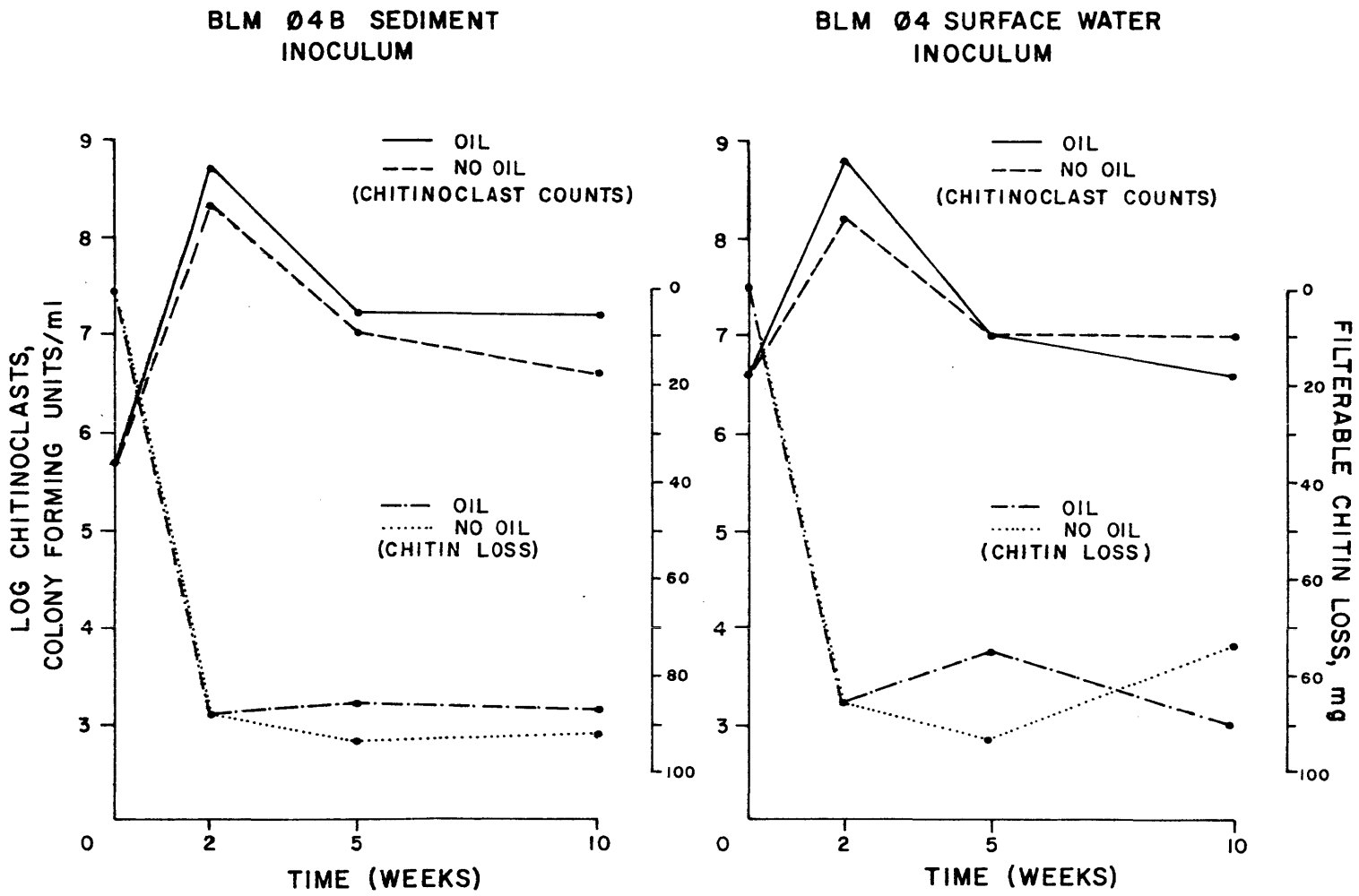

Figure 11-66. Change in amount of particulate chitin and chitinoclastic marine bacteria in a seawater-peptone broth with and without unweathered South Louisiana crude oil (0.1%).

Table 11-53. Change in levels (cells/ml) of Heterotrophic, Chitinoclastic and Petroleum degrading marine bacteria in closed flask chitin-oil degradation experiments using mixed cultures prepared from isolates obtained during BLM Ø1B-Ø4B. Log counts are in parentheses.

Time	Cruise	Type	Heterotrophs	Chitinoclasts	Petroleum Degraders
Initial Enumeration	001	Sed	$>4.8x10^7$ (>7.7)	$2.6x10^6$ (6.4)	ID
	002	Sed	$>4.8x10^7$ (>7.7)	$3.2x10^4$ (4.5)	ID
	002	H_2O	$>4.8x10^7$ (>7.7)	$7.2x10^5$ (5.9)	ID
	003	Sed	$>4.8x10^7$ (>7.7)	$1.9x10^6$ (6.3)	ID
	003	H_2O	$>4.8x10^7$ (>7.7)	$1.5x10^6$ (6.2)	ID
	004	Sed	$>4.8x10^7$ (>7.7)	$4.8x10^5$ (5.7)	ID
	004	H_2O	$>4.8x10^7$ (>7.7)	$3.8x10^6$ (6.6)	ID
Two Week Enumeration	001	Sed + Oil	$4.6x10^8$ (8.7)	$4.3x10^8$ (8.6)	0.0
	001	Sed - Oil	$4.6x10^8$ (8.7)	$6.3x10^8$ (8.8)	0.0
	002	Sed + Oil	$4.3x10^7$ (7.6)	$1.2x10^7$ (7.1)	$3.6x10^1$ (1.6)
	002	Sed - Oil	$2.3x10^7$ (7.4)	$5.9x10^7$ (7.8)	$5.6x10^1$ (1.7)
	002	H_2O + Oil	$9.3x10^7$ (8.0)	$2.0x10^8$ (8.3)	0.0
	002	H_2O - Oil	$9.3x10^7$ (8.0)	$4.5x10^7$ (7.7)	0.0
	003	Sed + Oil	$4.6x10^8$ (8.7)	$1.1x10^9$ (9.0)	$2.0x10^2$ (2.3)
	003	Sed - Oil	$4.6x10^8$ (8.7)	$5.1x10^8$ (8.7)	$1.5x10^2$ (2.2)
	003	H_2O + Oil	$>2.4x10^9$ (>9.4)	$8.5x10^8$ (8.9)	$1.1x10^5$ (5.0)
	003	H_2O - Oil	$1.1x10^9$ (9.0)	$4.7x10^8$ (8.7)	$2.3x10^2$ (2.4)
	004	Sed + Oil	$1.1x10^9$ (9.0)	$4.8x10^8$ (8.7)	$2.4x10^5$ (5.4)
	004	Sed - Oil	$2.4x10^8$ (8.4)	$2.2x10^8$ (8.3)	$2.1x10^3$ (3.3)
	004	H_2O + Oil	$4.6x10^8$ (8.7)	$6.5x10^8$ (8.8)	$2.1x10^3$ (3.3)
	004	H_2O - Oil	$4.6x10^8$ (8.7)	$2.3x10^8$ (8.4)	$2.1x10^3$ (3.3)

Table 11-53. (Concluded)

Time	Cruise	Type	Heterotrophs	Chitinoclasts	Petroleum Degraders
Five Week Harvest	001	Sed + Oil	1.5×10^7 (7.2)	3.8×10^6 (6.6)	9.6×10^1 (2.0)
	001	Sed - Oil	2.3×10^6 (6.4)	5.3×10^5 (5.7)	0.0
	002	Sed + Oil	4.3×10^5 (5.6)	$<10^5$ (<5.0)	6.3×10^1 (1.8)
	002	Sed - Oil	2.3×10^5 (5.4)	$<10^5$ (<5.0)	0.0
	002	H_2O + Oil	4.3×10^6 (6.6)	3.7×10^6 (6.6)	3.6×10^1 (1.4)
	002	H_2O - Oil	4.3×10^6 (6.6)	2.4×10^6 (6.4)	0.0
	003	Sed + Oil	2.3×10^6 (6.4)	2.0×10^6 (6.3)	3.6×10^1 (1.4)
	003	Sed - Oil	7.5×10^6 (6.9)	2.7×10^6 (6.4)	5.6×10^1 (1.7)
	003	H_2O + Oil	7.5×10^6 (6.9)	2.0×10^6 (6.3)	9.6×10^1 (2.0)
	003	H_2O - Oil	4.3×10^7 (7.6)	5.6×10^6 (6.7)	9.6×10^1 (2.0)
	004	Sed + Oil	2.3×10^7 (7.4)	1.5×10^7 (7.2)	2.8×10^1 (1.4)
	004	Sed - Oil	2.3×10^7 (7.4)	1.1×10^7 (7.0)	5.6×10^1 (1.7)
	004	H_2O + Oil	4.3×10^7 (7.6)	1.1×10^7 (7.0)	8.6×10^1 (1.9)
	004	H_2O - Oil	4.3×10^6 (6.6)	1.1×10^7 (7.0)	9.6×10^1 (2.0)
Ten Week Harvest	001	Sed + Oil	4.3×10^6 (6.6)	5.6×10^6 (6.7)	0.0
	001	Sed - Oil	9.3×10^6 (7.0)	3.2×10^6 (6.5)	0.0
	002	Sed + Oil	3.9×10^4 (4.6)	6.7×10^3 (3.8)	2.3 (0.4)
	002	Sed - Oil	9.3×10^4 (5.0)	5.4×10^4 (4.7)	0.0
	002	H_2O + Oil	2.3×10^6 (6.4)	2.8×10^5 (5.4)	0.0
	002	H_2O - Oil	1.5×10^6 (6.2)	3.3×10^5 (5.5)	0.0
	003	Sed + Oil	9.3×10^6 (7.0)	3.8×10^6 (6.6)	0.0
	003	Sed - Oil	1.5×10^7 (7.2)	6.5×10^6 (6.8)	0.0
	003	H_2O + Oil	4.3×10^6 (6.6)	3.9×10^6 (6.6)	0.0
	003	H_2O - Oil	4.3×10^7 (7.6)	4.1×10^6 (6.6)	0.0
	004	Sed + Oil	1.5×10^7 (7.2)	1.5×10^7 (7.2)	0.0
	004	Sed - Oil	9.3×10^6 (7.0)	4.2×10^6 (6.6)	0.0
	004	H_2O + Oil	4.3×10^7 (7.6)	4.0×10^6 (6.6)	$>2.4 \times 10^4$ (>4.4)
	004	H_2O - Oil	2.3×10^7 (7.4)	1.1×10^7 (7.0)	$>2.4 \times 10^4$ (>4.4)

utilization with time. This observation was attributed to the production of relatively large quantities of particulate cell debris which were recoverable on the filter paper with the residual chitin. The Ø2B sediment inoculum typically grew as a tightly bound cellular mass extremely difficult to disrupt. When grown without petroleum, methylene chloride and vigorous shaking tended to break up the mass of cells/debris and this was evidenced by the "typical" curve for chitin loss without oil present (Ø2B -oil). However, in the presence of crude oil, the cell mass resisted disruption with small clumps of debris retained on the filter. Examination of filters cleared with mineral oil failed to reveal significant amounts of particulate chitin and suggested that the observed weight increase was due to cell mass clumping, perhaps with incorporation of oil in this mass, or the production of some type of extracellular polymer.

Although plots of chitin weight loss versus time (Figures 11-63 through 11-66) suggest a trend favoring slightly smaller chitin losses in the presence of crude oil, statistical analysis of these data (Friedman test statistic) indicated no significant differences attributable to oil treatment ($\alpha = 0.05$).

Changes in the levels of petroleum degrading bacteria were more difficult to assess because of the reduction of particulate chitin (250-500 μ) to particles of colloidal dimensions. Such particles (visible under high power oil immersion) remained in suspension in inoculated ESWB enumeration tubes receiving no oil as well as in inoculated ESWB tubes receiving oil. Therefore, it was occasionally very difficult to obtain a reliable MPN value for the lowest dilutions. Thus, values reported in Table 11-53 were generally similar for non-oiled and oiled conditions but the significance of the integer values (not the order of magnitude) is questionable. However, while the data indicated that some chitinoclastic bacteria can also degrade petroleum and that changes in the levels of petroleum degrading bacteria tended to follow those of heterotrophs, gravimetric and gas chromatographic analyses of residual petroleum failed to reveal significant losses in oil treated flasks inoculated with water or sediment mixed cultures compared to sterile (chitin and oil) controls (Table 11-54).

Pure Culture - Petroleum Growth Studies

Results of experiments to examine the effects of unweathered, artificially weathered, and a water soluble fraction of South Louisiana crude oil on the growth of selected marine bacterial isolates are summarized in Table 11-55. Representative growth curves for the dominant genera are shown in Figures 11-67 through 11-78. Distributions of "lag" times for all isolates of a given genus for each treatment (i.e., control, soluble fraction, unweathered oil, weathered oil) are shown as histograms in Figures 11-79 through 11-84.

Plots of changes in optical density for each isolate without crude oil indicated that *Vibrio* sp. and *Aeromonas* sp. exhibited characteristically the shortest "lag" times (9 and 10 hours, respectively). These genera were followed by *Pseudomonas* sp. which required slightly longer (13 hrs) to reach the same optical density value. Contrastingly, "lag" times exhibited by isolates identified as *Alcaligenes* sp., *Acinetobacter* sp., and *Flavobacterium* sp. were considerably larger. The genera *Alcaligenes* and

Table 11-54. Change in summation of weights[a] (μg) of crude oil n-paraffins (nC_{12}-nC_{27}) during incubation in closed containers in a dilute peptone-seawater broth containing chitin and 0.1% (v/v) South Louisiana crude oil, and mixed pure culture inocula derived from sources indicated. % weight loss in parantheses.

Cruise	Inoculum Origin	Incubation Interval 2 Weeks	5 Weeks	10 Weeks
Ø1B	Sediment	1537(0)	1463(0)	1382(4.0)
Ø2B	Water	1540(0)	1459(0)	1310(9.0)
	Sediment	1537(0)	1502(0)	1517(0)
Ø3B	Water	1593(0)	1338(4.2)	1570(7.1)
	Sediment	1358(0)	1422(0)	1359(5.6)
Ø4B	Water	1520(0)	--[b]	1427(0.1)
	Sediment	1440(4.6)	1374(3.1)	1310(9.0)
Controls[c]		1508	1417	1437

[a] % weight loss = $100\ (1-\frac{\Sigma\mu g\ \text{n-paraffins}\ nC_{12}\text{-}nC_{27})\ \text{treated flask}}{\Sigma\mu g\ \text{n-paraffins}\ nC_{12}\text{-}nC_{27})\ \text{sterile control}}$)

[b] sample not chromatographed

[c] control values are the means of three replicates.

Table 11-55. Effects of unweathered, artificially weathered, and a soluble fraction of South Louisiana crude oil on the median "lag" times of isolates from representative genera of marine bacteria from Middle Atlantic continental shelf water and sediment samples.

Genus	Isolates Examined	Median Lag Time (hours) Control	Soluble Fraction	Unweathered Oil	Weathered Oil
Aeromonas	8	9	9	9.5	9
Vibrio	36	10	10	11	10
Pseudomonas	90	13	13	15	14
Acinetobacter	7	28	29	39	40
Alcaligenes	59	28	28	32	31
Flavobacterium	20	48	48	61	60

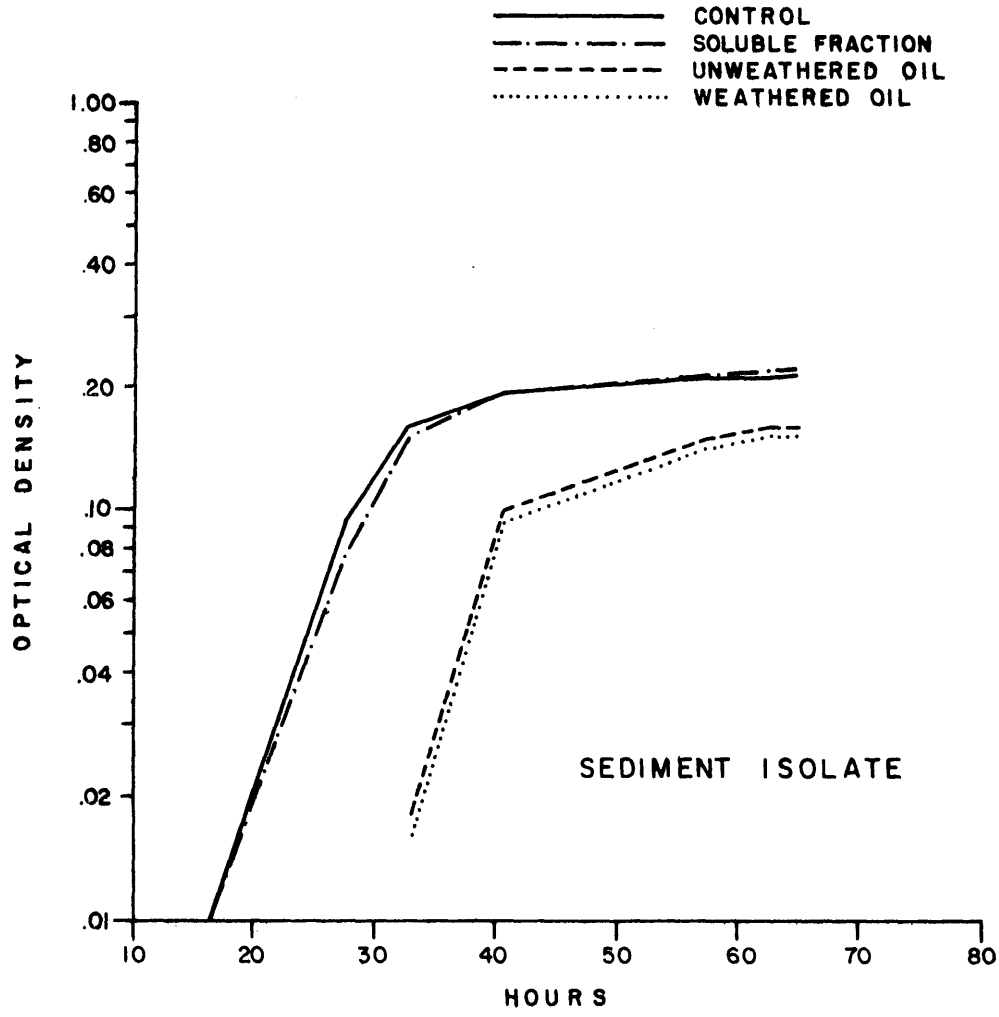

Figure 11-67. Representative growth curves for *Acinetobacter* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

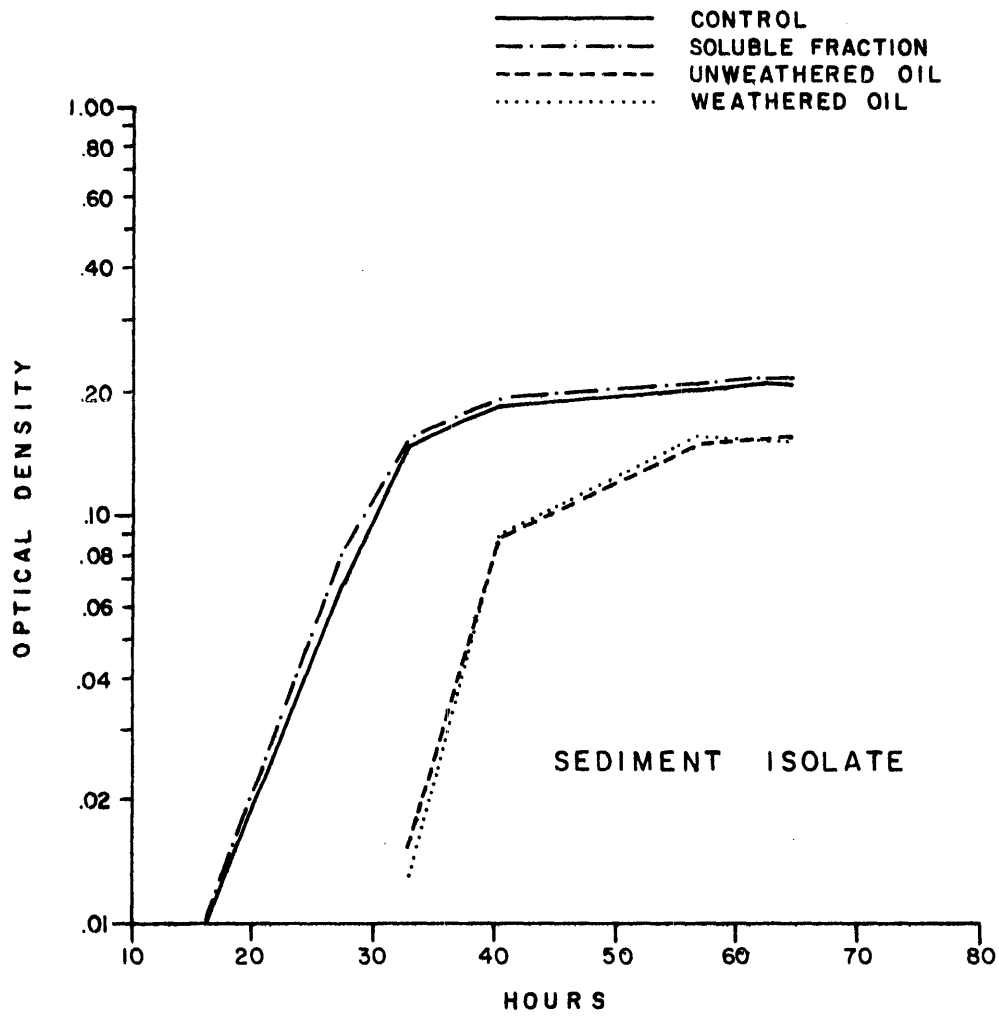

Figure 11-68. Representative growth curves for *Acinetobacter* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

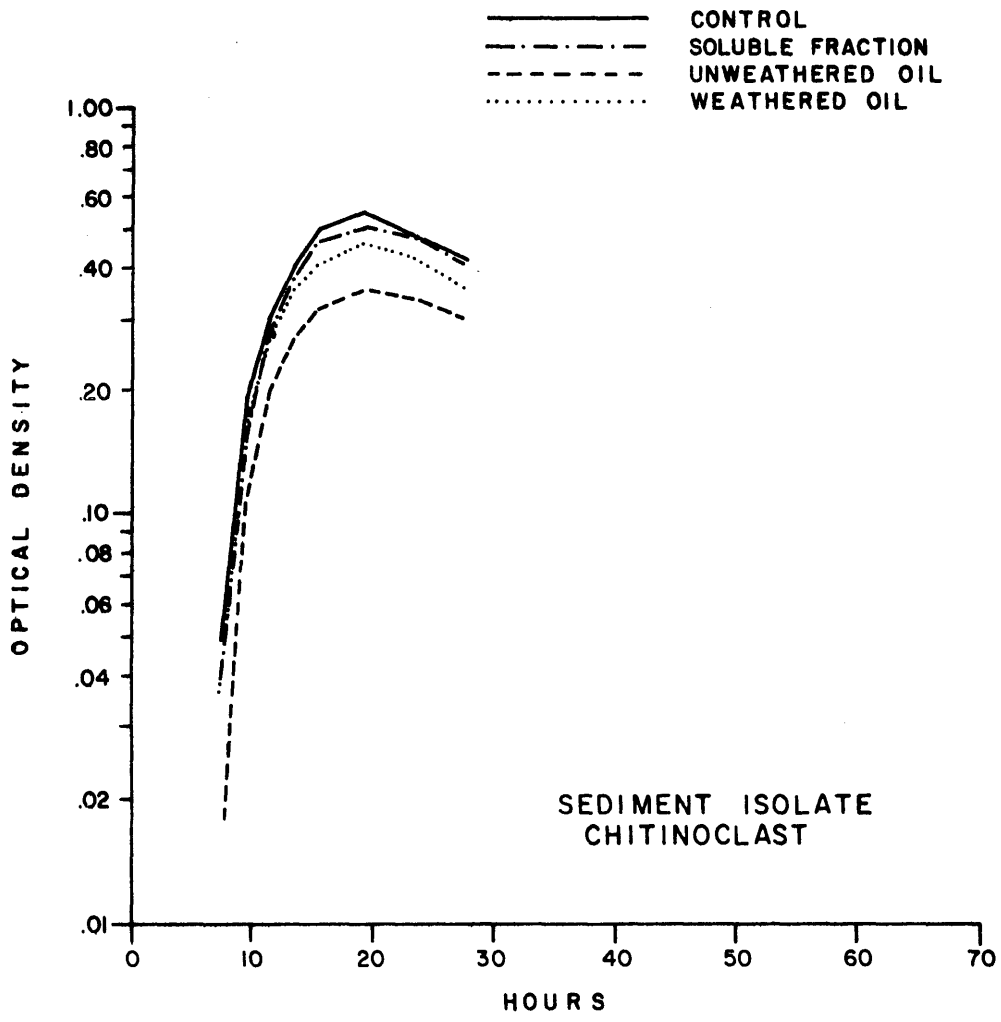

Figure 11-69. Representative growth curves for *Aeromonas* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

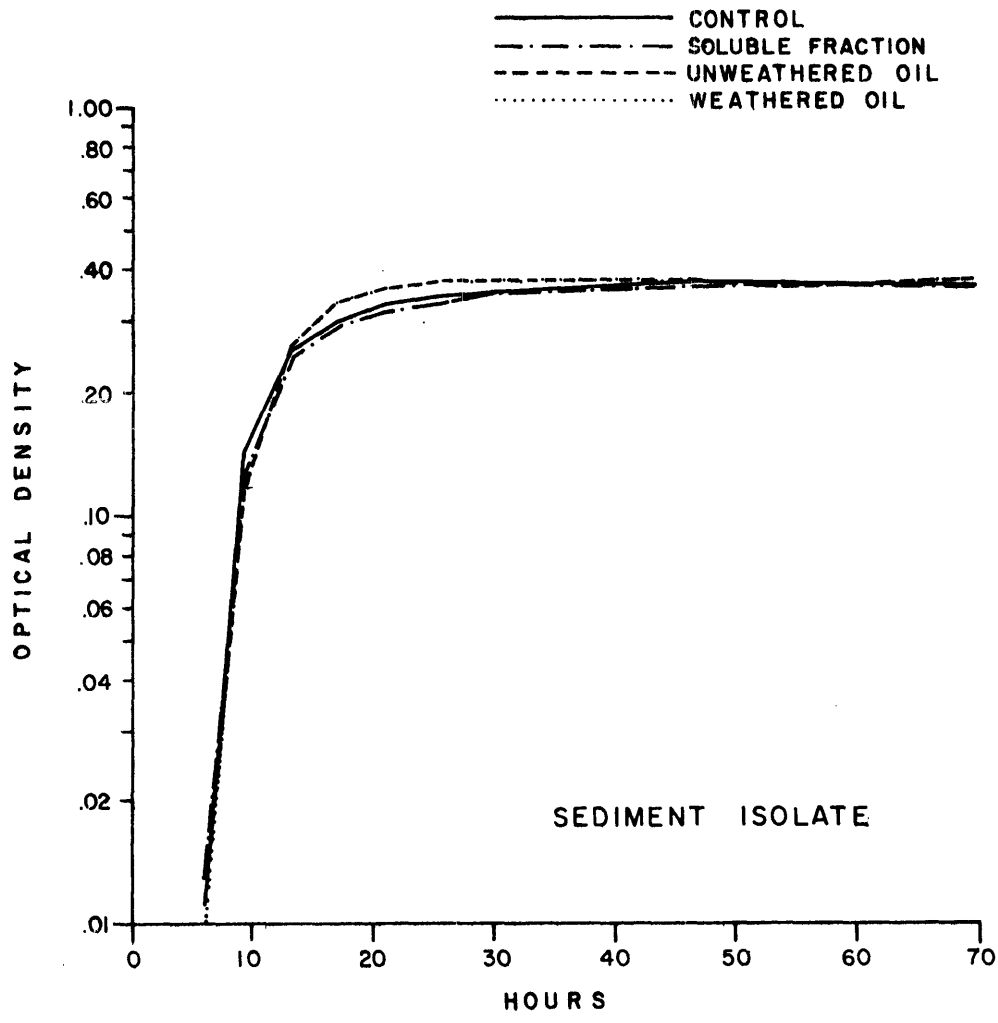

Figure 11-70. Representative growth curves for *Aeromonas* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

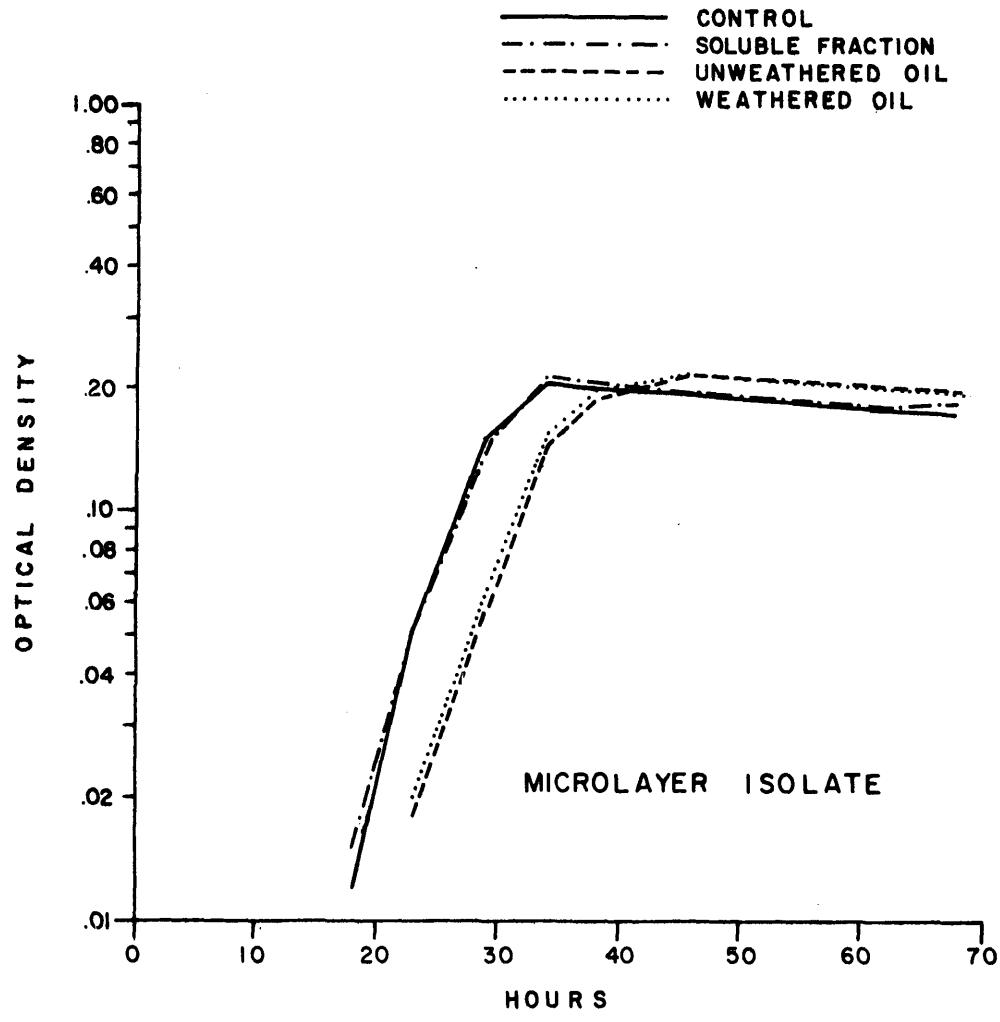

Figure 11-71. Representative growth curves for *Alcaligenes* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

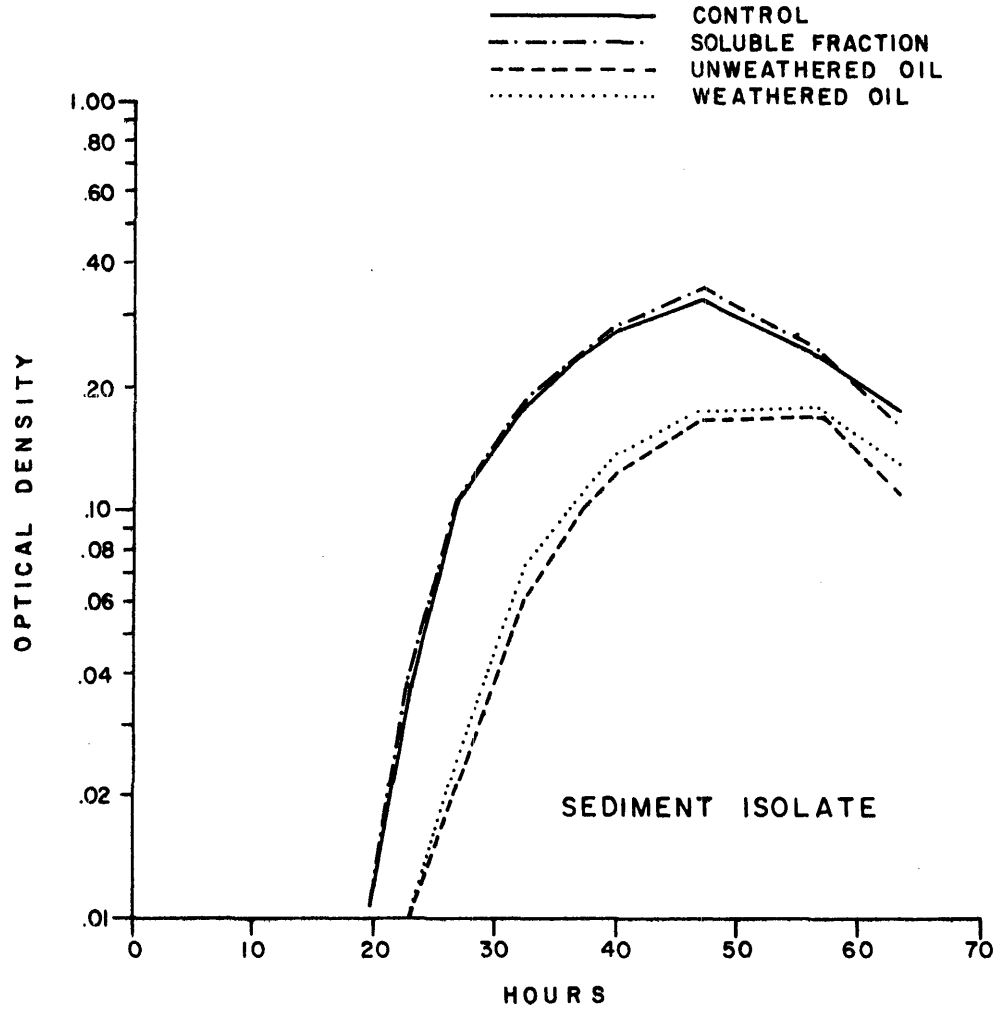

Figure 11-72. Representative growth curves for *Alcaligenes* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

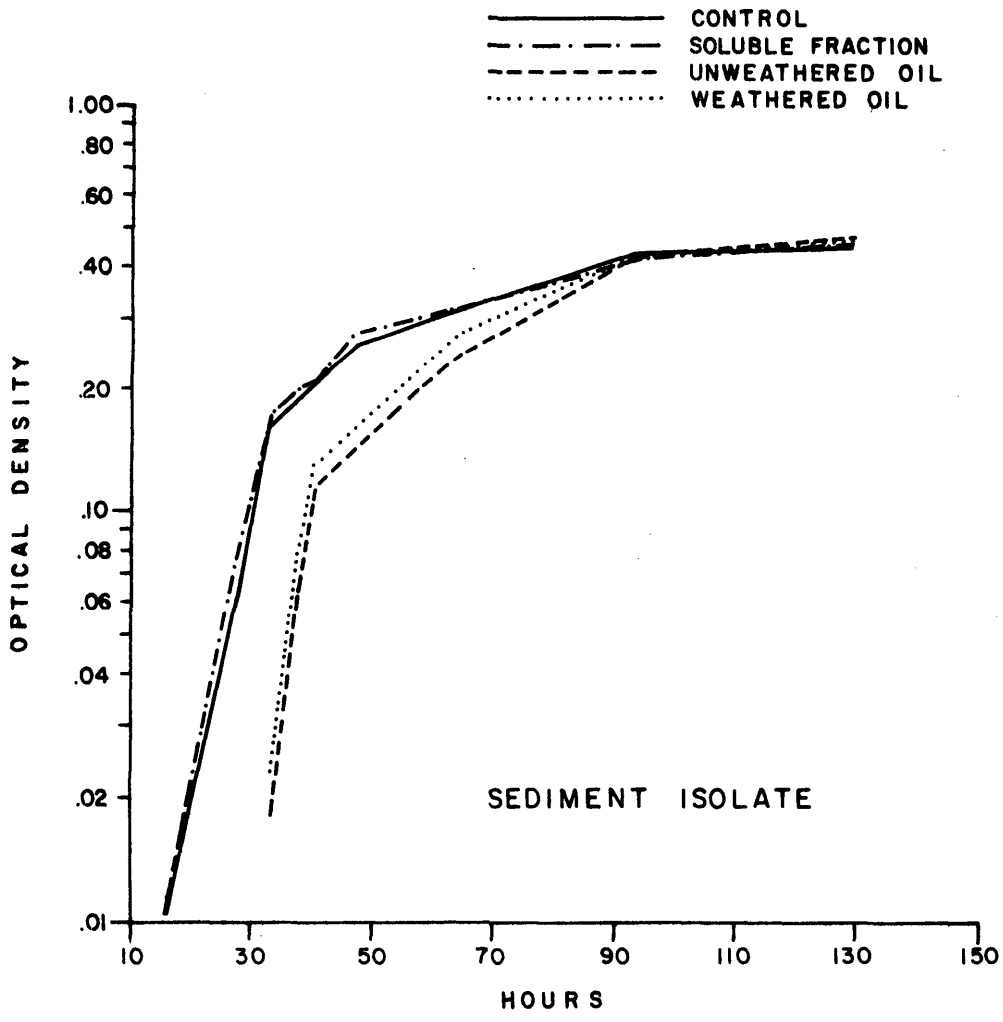

Figure 11-73. Representative growth curves for *Flavobacterium* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

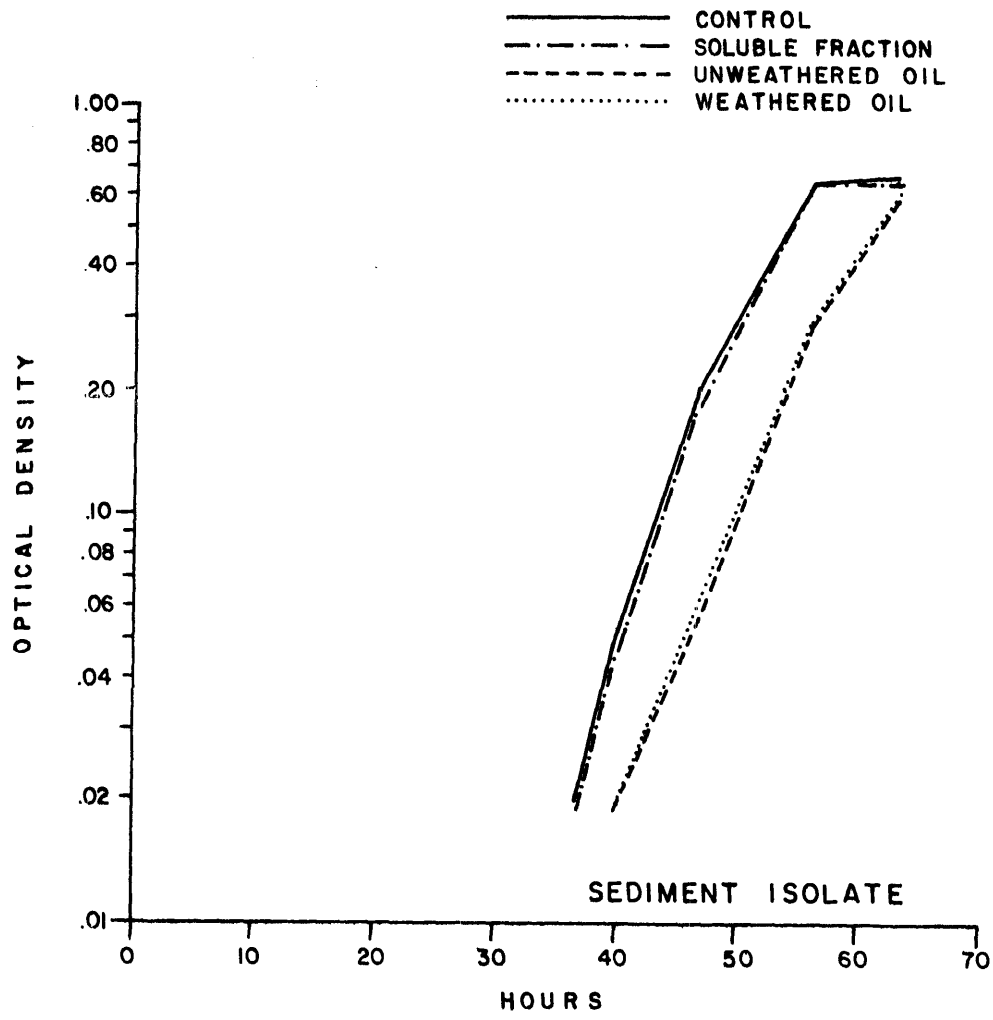

Figure 11-74. Representative growth curves for *Flavobacterium* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

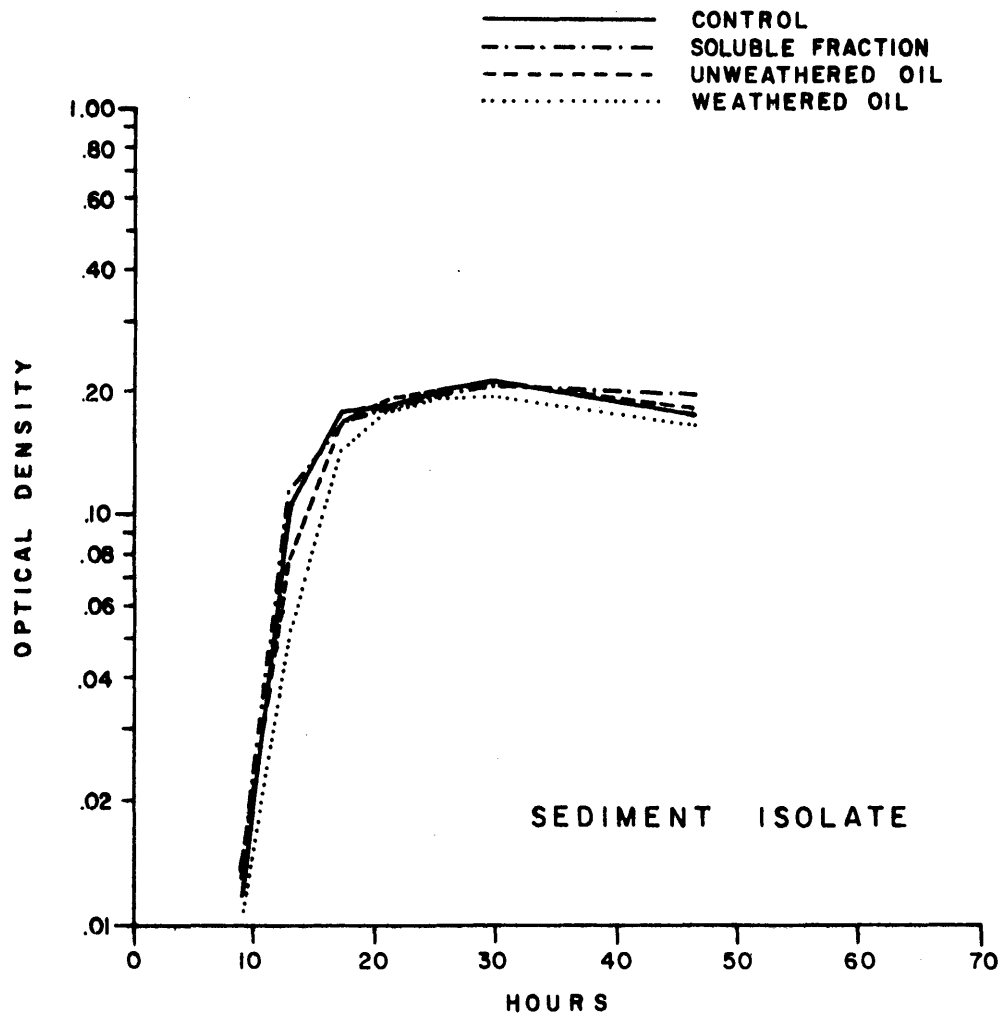

Figure 11-75. Representative growth curves for *Pseudomonas* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

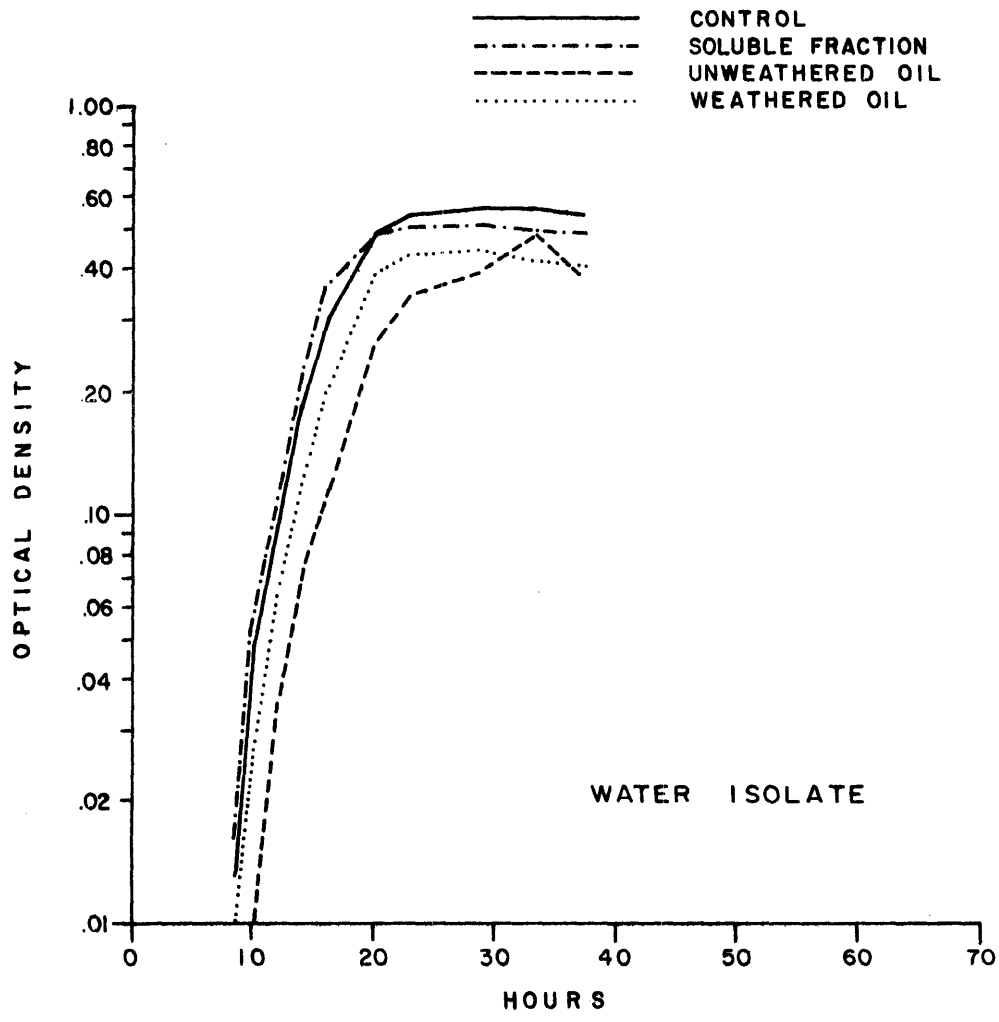

Figure 11-76. Representative growth curves for *Pseudomonas* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

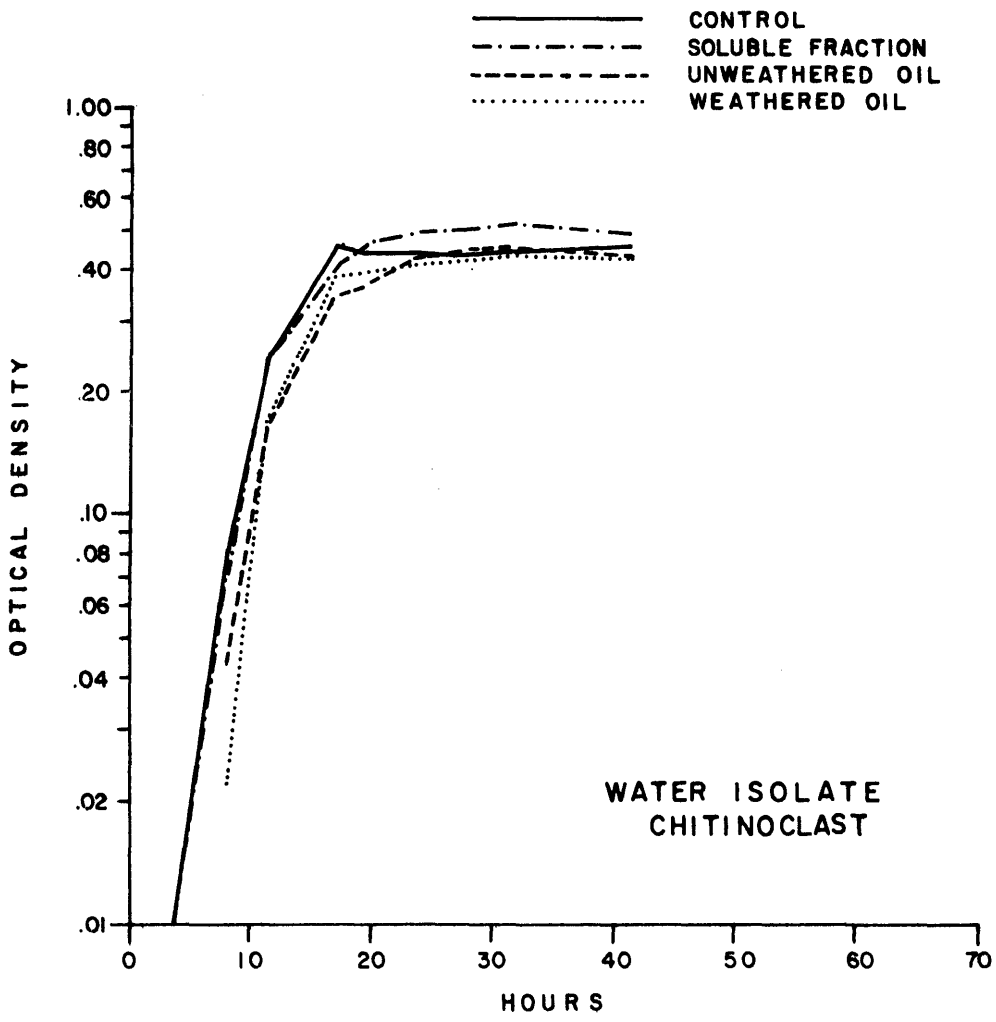

Figure 11-77. Representative growth curves for *Vibrio* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

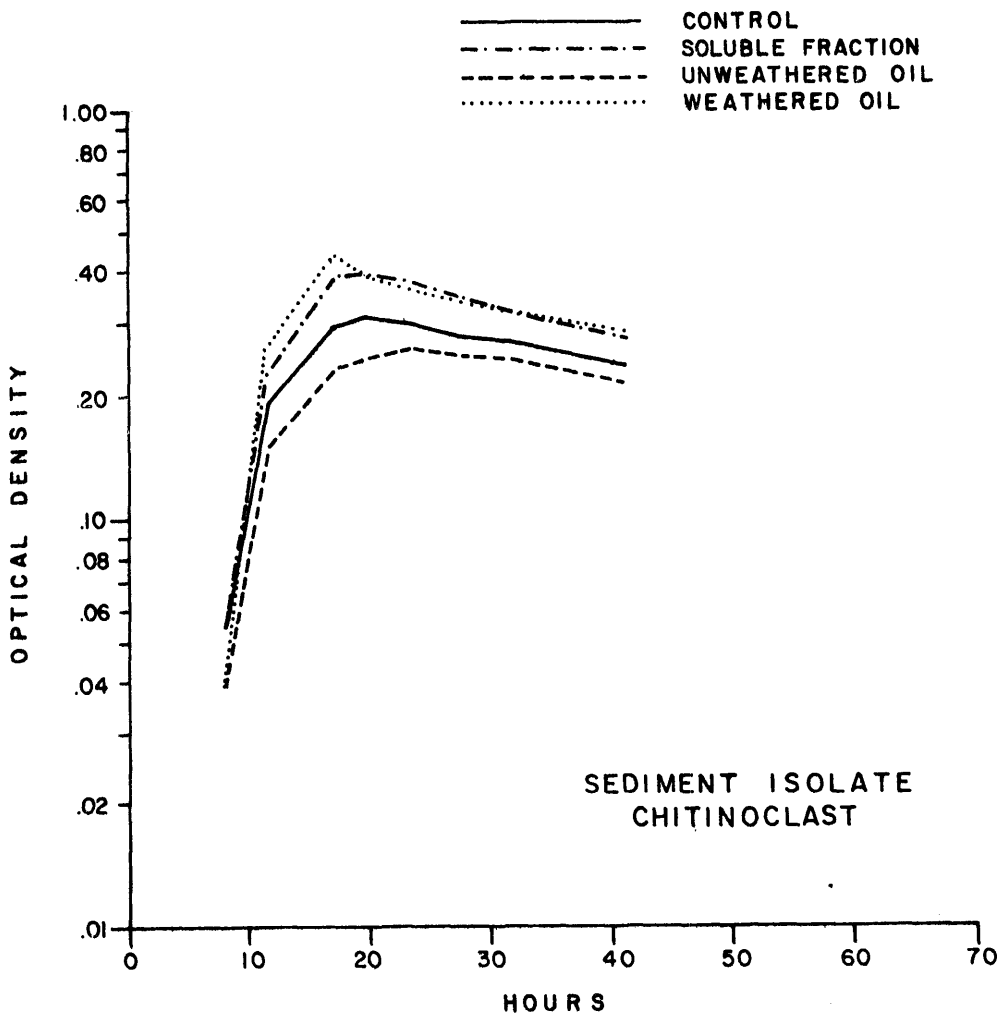

Figure 11-78. Representative growth curves for *Vibrio* sp. in a dilute peptone-seawater broth, and in a dilute peptone-seawater broth containing (a) a soluble fraction of South Louisiana crude oil, or (b) unweathered South Louisiana crude oil (ca. 1%), or (c) artificially weathered South Louisiana crude oil (ca. 1%).

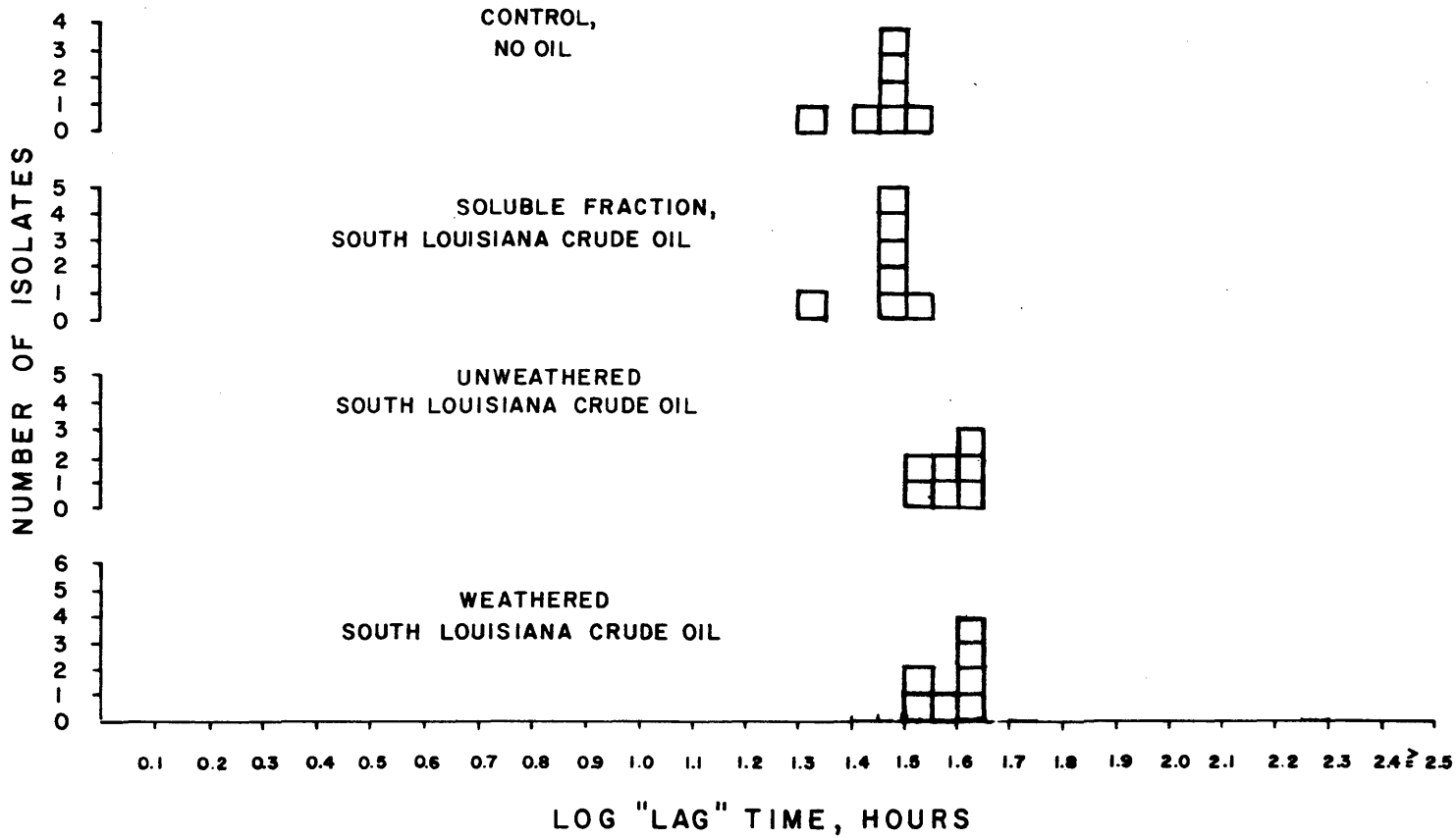

Figure 11-79. Effect of unweathered South Louisiana crude oil, artificially weathered South Louisiana crude oil, and a soluble fraction of South Louisiana crude oil on the growth ("Lag" Time) of seven isolates of *Acinetobacter* sp. in a dilute glucose-peptone seawater broth.

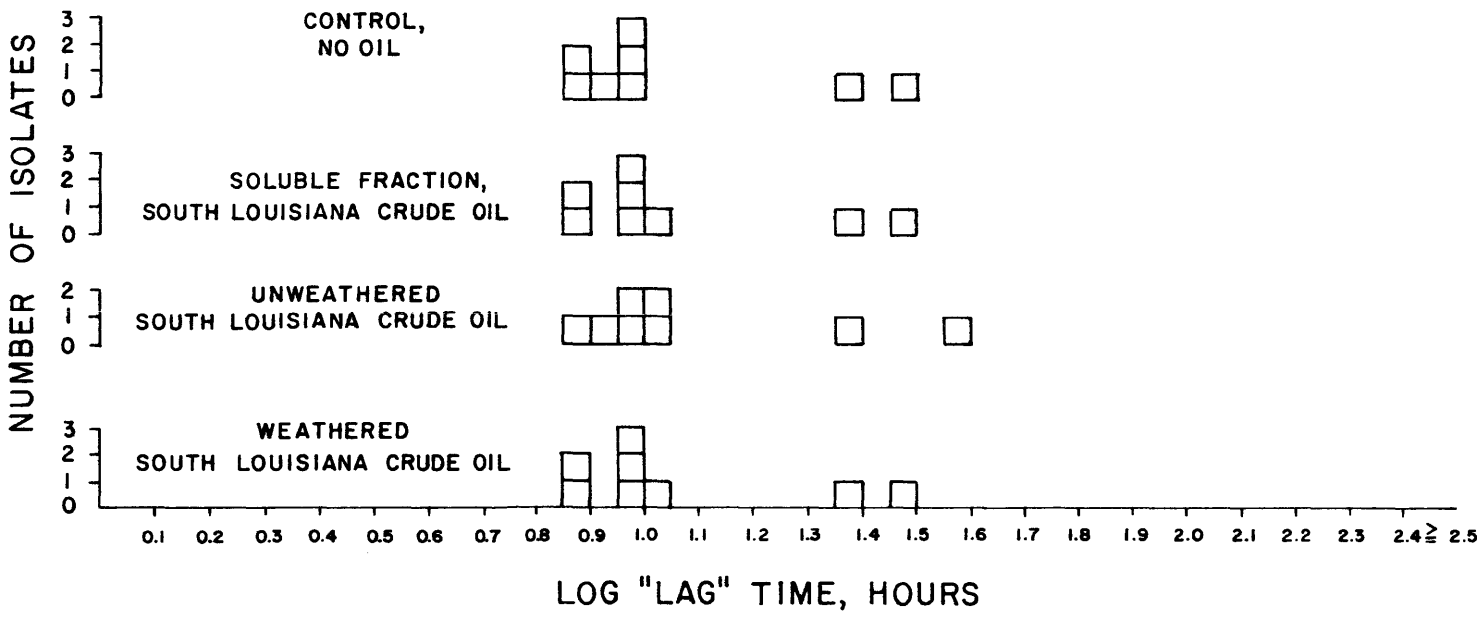

Figure 11-80. Effect of unweathered South Louisiana crude oil, artificially weathered South Louisiana crude oil, and a soluble fraction of South Louisiana crude oil on the growth ("Lag" Time) of eight isolates of *Aeromonas* sp. in a dilute glucose-peptone seawater broth.

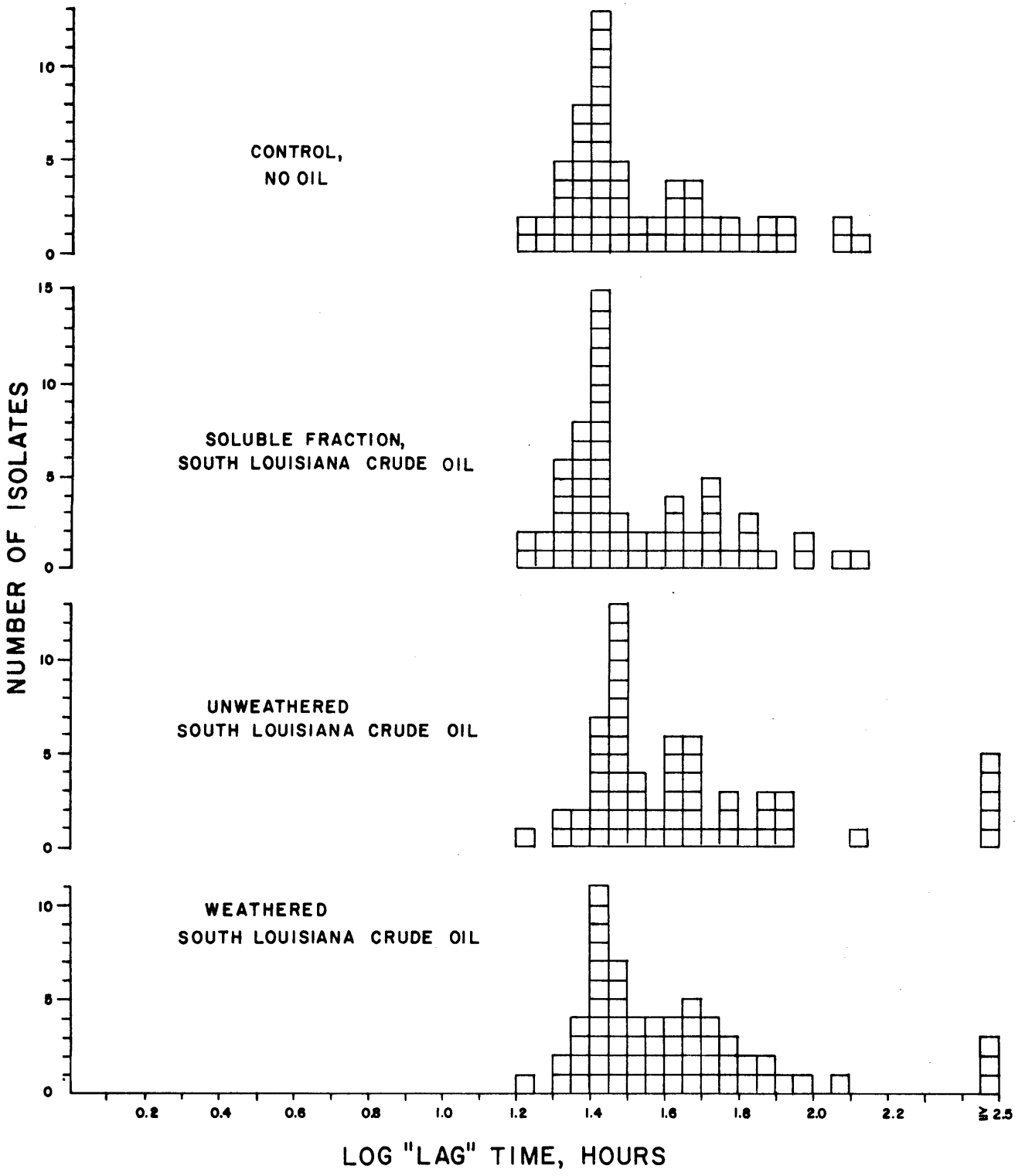

Figure 11-81. Effect of unweathered South Louisiana crude oil, artificially weathered South Louisiana crude oil, and a soluble fraction of South Louisiana crude oil on the growth ("Lag" Time) of fifty-nine isolates of *Alcaligenes* sp. in a dilute glucose-peptone seawater broth.

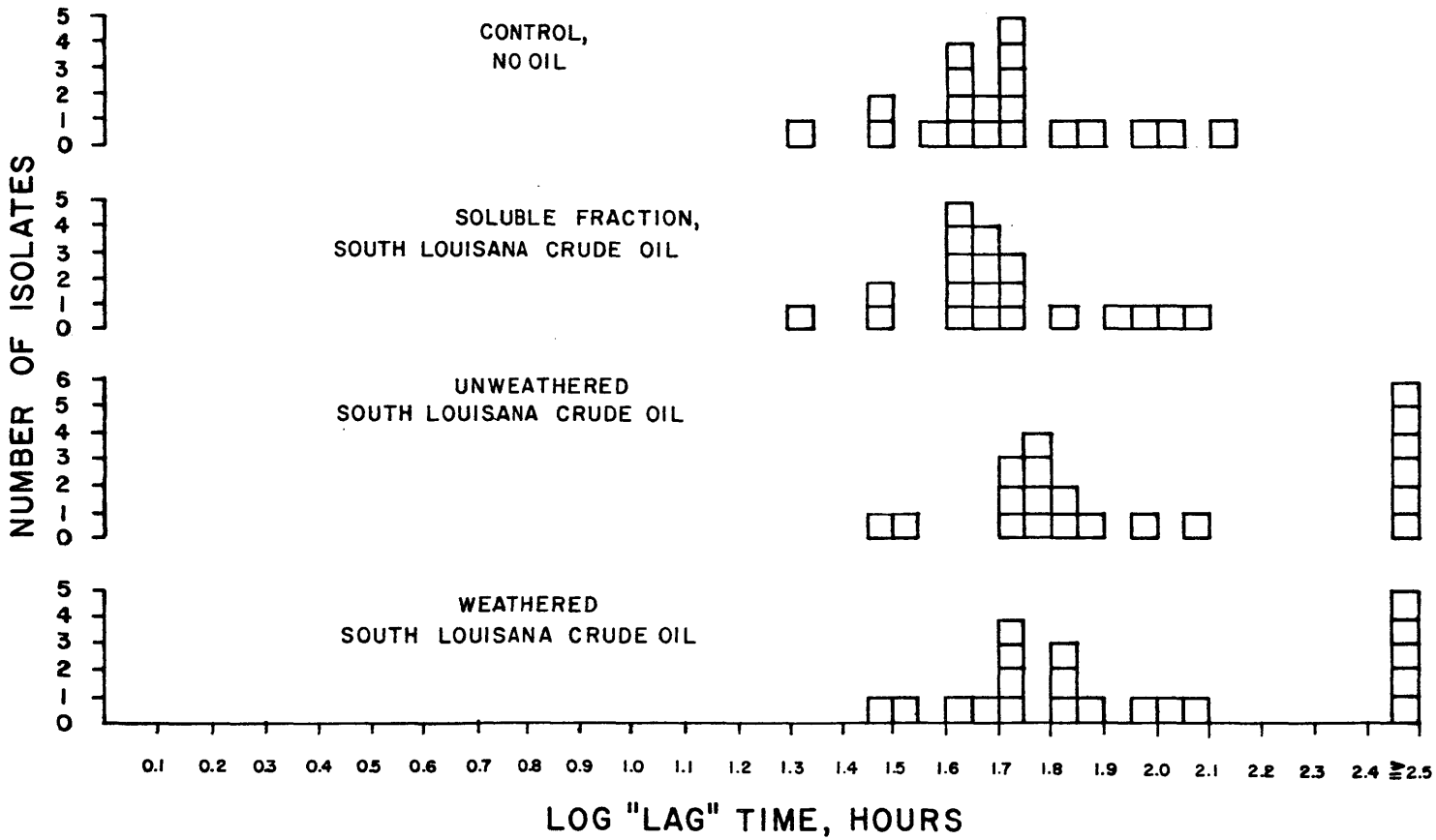

Figure 11-82. Effect of unweathered South Louisiana crude oil, artificially weathered South Louisiana crude oil, and a soluble fraction of South Louisiana crude oil on the growth ("Lag" Time) of twenty isolates of *Flavobacterium* sp. in a dilute glucose-peptone seawater broth.

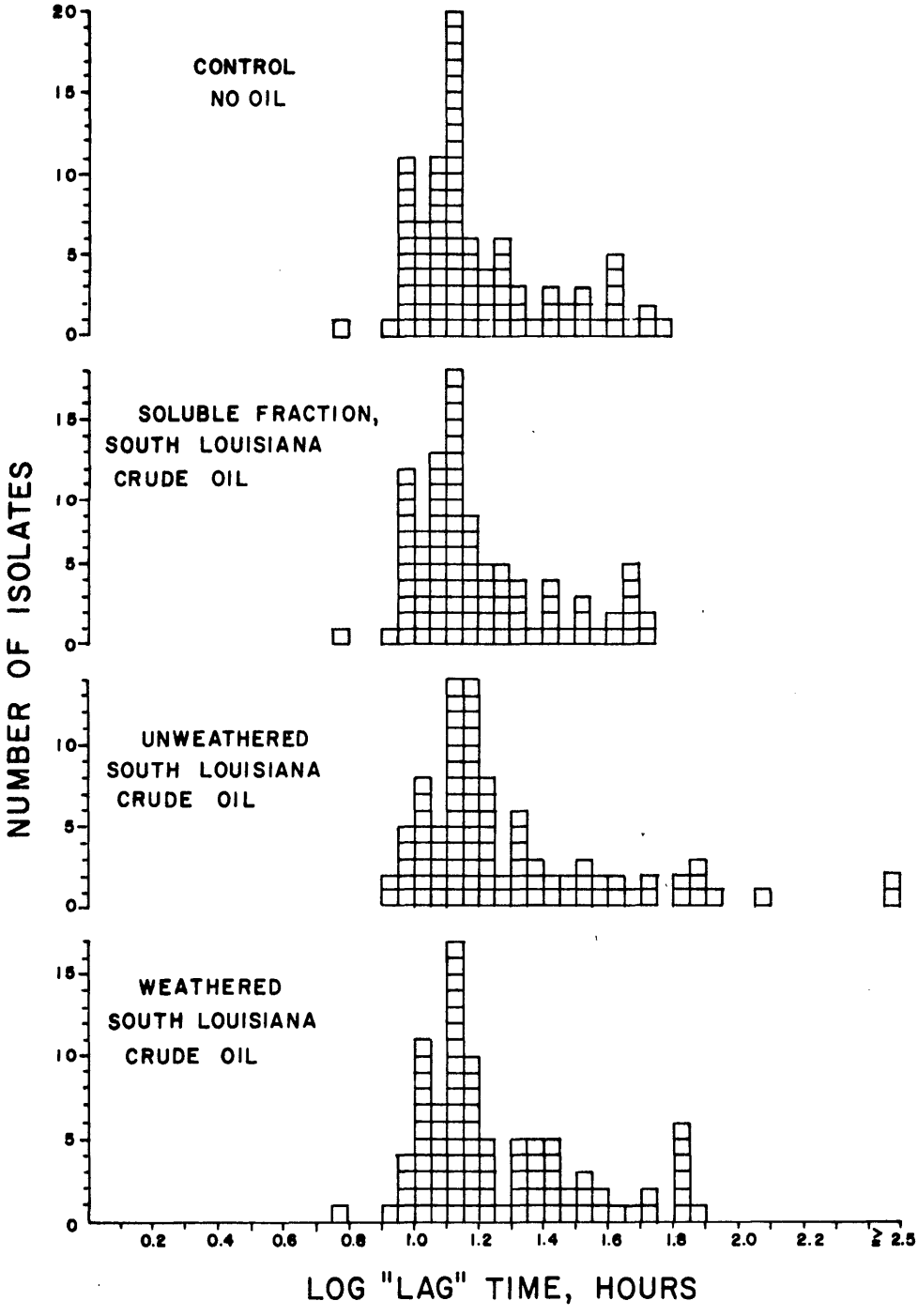

Figure 11-83. Effect of unweathered South Louisiana crude oil, artificially weathered South Louisiana crude oil, and a soluble fraction of South Louisiana crude oil on the growth ("Lag" Time) of ninety isolates of *Pseudomonas* sp. in a dilute glucose-peptone seawater broth.

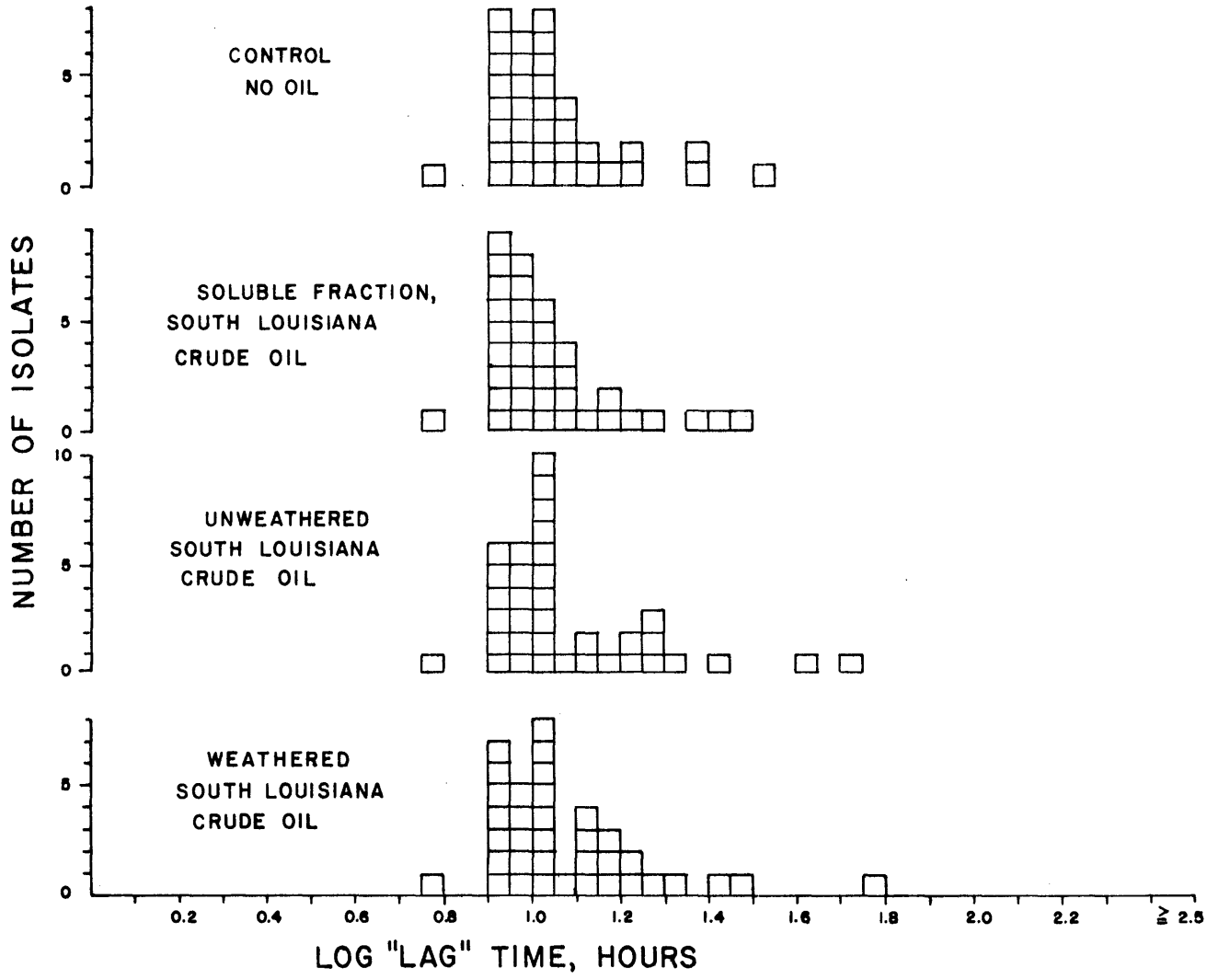

Figure 11-84. Effect of unweathered South Louisiana crude oil, artificially weathered South Louisiana crude oil, and a soluble fraction of South Louisiana crude oil on the growth ("Lag" Time) of thirty-six isolates of *Vibrio* sp. in a dilute glucose-peptone seawater broth.

Flavobacterium exhibited the most variation of "lag" time around their median values (Figures 11-81 and 11-82).

These results indicated that petroleum had no effect on the growth of *Aeromonas* isolates whereas *Vibrio* isolates evidenced a small but significant inhibition in the presence of unweathered crude oil (Table 11-56). "Lag" times for *Pseudomonas* isolates were also increased to a small but significant extent by both unweathered and artificially weathered crude oil. Cultures from the remaining genera were significantly inhibited showing increases in median "lag" time of 3-4; 11-12; and 12-13 hours for *Alcaligenes*, *Acinetobacter*, and *Flavobacterium* in the presence of unweathered and weathered crude oil (Tables 11-55 and 11-56). Water soluble fractions of Louisiana crude oil did not significantly affect the growth of "lag" times of the majority of isolates.

Table 11-56. Friedman T Statistic Calculated for "lag" Times Exhibited by Pure Isolates of Indicated Genera Grown in a Dilute Seawater-Peptone Medium and this same Medium Containing Either a Soluble Fraction of South Louisiana Crude Oil (1%), or Unweathered South Louisiana Crude Oil (1%), or Artificially Weathered South Louisiana Crude Oil (1%).

Genus	Number of Isolates Examined	Calculated T Value[a]	Critical T Value (α = 0.01) for Rejection of H_o	H_o: All Treatments Have Identical Effects
Acinetobacter sp.	7	14.6	11.3	Rejected
Aeromonas sp.	8	2.6	11.3	Accepted
Alcaligenes sp.	59	40.3	11.3	Rejected
Flavobacterium sp.	20	28.9	11.3	Rejected
Pseudomonas sp.	90	107.0	11.3	Rejected
Vibrio sp.	36	21.7	11.3	Rejected

[a] Friedman nonparametric test statistic. (Practical Nonparametric Statistics, W. J. Conover, John Wiley and Sons, Inc., 1971)

The susceptibility of isolates from HM and ESWB enumeration media from microlayer, surface (1M), and sediment samples as a function of degree of "lag" effect are shown in Table 11-57. Although the number of heterotrophic isolates drawn from microlayer samples was relatively small, these isolates were most strongly inhibited by unweathered and weathered crude oil. This is not surprising considering that the dominant isolates (Table 11-58) from microlayer samples belong to the most sensitive genera, *Flavobacterium* and *Alcaligenes*. In contrast, sediment hydrocarbon degrading bacteria were the least affected being composed of dominant genera least inhibited by petroleum (*Pseudomonas* and *Vibrio*).

Table 11-57. Percentages of cultures affected (relative to a non-oil treated control) and degree of effect of unweathered and weathered South Louisiana crude oil and the water soluble fraction thereof on growth of bacterial isolates from Heterotroph (HM) and Petroleum Degrading (ESWB) enumeration media from surface film, water (1 meter) and sediment samples (BLM Ø1B-Ø4B). Legend: (+)= enhancement, >1 hour decrease in "lag time"; (0)= no effect, <1 hour difference in "lag time"; (-)= 1 to 3 hour increase in "lag time"; (--)= 4-10 hour increase in "lag time"; (---)= >10 hour increase in "lag time".[1]

Culture Origin	No. Cultures Examined	% of cultures affected and degree of effect in growth media containing: Soluble Fraction (1%)					Unweathered Oil (1%)					Weathered Oil (1%)				
		+	0	-	--	---	+	0	-	--	---	+	0	-	--	---
Microlayer (HM medium)	22		68.2	27.3	4.5			9.1	4.5	22.7	63.6	4.5	13.6	4.5	18.2	59.1
Microlayer (ESWB medium)	28	10.7	75.0	10.7	3.6		3.6	17.9	21.4	39.3	17.9	3.6	28.6	25.0	25.0	17.9
Water (HM medium)	27	14.8	70.4	14.8			11.1	11.1	29.6	29.6	18.5	11.1	18.5	25.9	29.6	14.8
Water (ESWB medium)	38	2.6	78.9	5.3	5.3	2.6		15.8	42.1	23.7	18.4	2.6	21.1	50.0	7.9	18.4
Sediment (HM medium)	69	17.4	71.0	5.8	4.3	1.4	7.2	13.0	20.3	30.4	30.0	13.0	18.8	17.4	20.3	30.4
Sediment (ESWB medium)	85	5.9	84.7	7.1	2.4		3.5	41.2	34.1	9.4	11.8	2.4	49.4	29.4	10.6	8.2
TOTALS	269	9.3	77.3	9.3	3.3	0.7	4.5	22.3	27.5	23.0	22.7	6.3	29.4	26.4	16.7	21.2

[1]Standard deviation of "lag time" for replicate cultures of representative isolates = 0.01.

Table 11-58. Relative abundance of dominant genera as a function of sample type and enumeration media used for pure culture petroleum growth experiments.

	Sample Type					
	Microlayer		Surface (1m)		Sediment	
Genus	HC[1]	Het[2]	HC	Het	HC	Het
Acinetobacter						7/69
Aeromonas	1/28				6/85	1/69
Alcaligenes	4/28	8/22	8/38	4/27	15/85	20/69
Flavobacterium	3/28	4/22	2/38	3/27	1/85	7/69
Pseudomonas	12/28	3/22	17/38	10/27	41/85	7/69
Vibrio	1/28	1/22	7/38	3/27	17/85	7/69
Misc.	7/28	6/22	4/38	7/27	5/85	20/69

[1]ESWB medium for enumeration of petroleum degrading bacteria.
[2]HM-heterotroph medium.

Although the extent of inhibition as reflected in "lag" time delay was similar for unweathered and artificially weathered South Louisiana crude oils, the overall percentage of isolates significantly affected (i.e. 4 hour or greater delay in "lag" time) was larger for the unweathered crude oil (46%) than for the weathered crude oil (38%). Approximately 5-10% of the isolates were stimulated by the presence of petroleum hydrocarbons reflected as median "lag" time decreases of 5, 6.5 and 7.5 hours for the water soluble fraction, unweathered, and weathered crude oils, respectively.

DISCUSSION

Distribution and Abundance of Petroleum Degrading and Heterotrophic Bacteria

Populations of heterotrophic bacteria in Middle Atlantic continental shelf sediments sampled quarterly were rather uniform in numbers with mean values (all stations) which did not vary appreciably with season. Variations in numbers as a function of station could be related to the sediment textural property % silt - clay and indirectly, to topographic features. Populations of petroleum degrading bacteria, a sub-set of the heterotrophic bacterial population, tended to respond more distinctly to known pollutant inputs, textural and topographic features than heterotrophic populations. Thus, although heterotrophic bacterial numbers were somewhat higher in sediments from the station closest to Atlantic City, in troughs or those with higher percentages of silt-clay, populations of petroleum degrading bacteria strongly increased at these same stations. This "enrichment" was also manifested as an increase in the ratio of petroleum degrading to heterotrophic bacteria (HC/HET).

Observations of elevated levels of petroleum degrading bacteria in the vicinities of pollutant hydrocarbons have been made (Atlas and Bartha 1973b; Mulkins-Phillips and Stewart 1974; Seki 1976; Walker and Colwell 1977). Walker and Colwell (1977) stress the usefulness of the ratio of petroleum degrading bacteria to heterotrophs in detecting petroleum pol-

lution. Others (Atlas and Bartha 1973b; Seki 1976) have reported that elevated levels of petroleum degrading bacteria are themselves indicative of the presence of petroleum. Our data suggest that both approaches yield similar information, provided background information on the natural levels of petroleum degrading bacteria in non-polluted waters are known. Thus, while the numbers of petroleum degrading bacteria decreased moving offshore from Atlantic City, elevated values occurred in troughs and other stations with high amounts of silt-clay. Elevated levels were also expressed as an increase in the value of the ratio. However, values of the ratio were never as large as those found nearest Atlantic City (C1). These observations underline the importance of knowing where a sample of sediment came from with respect to topographic features, textural composition, as well as sampling reference locations free of suspected petroleum pollution.

Sediments rich in silt-clay may stimulate bacterial diagenetic activities because of surface effects (ZoBell 1946b). Similarly, the hydrodynamics of sediments accumulating silts may favor deposition of highly surface active particulates which already contain or scavenge hydrophobic substances from the water column. Finally, the benthic communities in sediments containing high concentrations of silt-clay may contribute lipoidal material which tends to be metabolized slowly because of the fine particulates and produces sustained elevated levels of petroleum degrading bacteria.

Attempts to statistically correlate bacterial sediment populations with various physical or chemical parameters failed to yield consistent results. Kendall non-parametric correlation coefficients, calculated by ranking mutually independent variables, were particularly sensitive to small variations in bacterial numbers which are commonly observed in field samples. The occurrence of variations in bacterial population distributions in water or sediments has been addressed frequently (ZoBell and Feltham 1934; Ashby and Rhodes-Roberts 1976). Attempts to reduce this variation usually rely on increasing the numbers or size of replicate samples, an approach which becomes prohibitive with large scale surveys. Despite this problem, standard deviations for mean counts of all Middle Atlantic shelf sediments sampled were about $\pm$ 0.55 log units. Considering the inherent variability of the MPN technique and that of sediment population distributions, this deviation is remarkably small. Liston (1968) observed a similar value of $\pm$ 0.5 log units for what he considered bacterial populations from stable shelf environments.

Variations related to technique and bacterial distributions were not the only possible sources of variability. As mentioned in Chapter 5, "despite the high overall correlation between silt-clay and organic carbon, correlations between these parameters with the replicates at any one station were generally poor. Significant ($p < 0.05$) correlations were found at less than 10% of the stations". Thus, variations in sediment properties, small but insignificant variations in bacterial populations, and the variability inherent in the MPN technique, precluded consistent correlation at significant values between bacterial populations and selected parameters.

Assuming that bacterial populations in Middle Atlantic continental shelf sediments should be inversely related to grain size distribution (ZoBell 1946b), percent clay-silt, in these relatively uniform sandy sediments, should be directly related to bacterial numbers. When stations

were segregated by grossly different amounts of silt-clay, significant ($p < 0.05$) "correlations" of bacterial populations with % silt clay were observed. Dale (1974) observed extraordinarily high correlation coefficient between bacterial populations from intertidal sediments and textural properties. However, he was working with a relatively simple system where tidal current energy produced a gradient of sediment characteristics.

In future surveys correlation of bacterial populations and physical-chemical parameters might be improved by analyzing replicates from the bacteriological grab using the "mini corer" to obtain similar penetration and sample size. Dilution of the upper, most active sediment layer, by varying amounts of sediments below, is also a source of variability for textural and chemical analyses.

Considering the absence of consistent and significant correlation of bacterial levels with other sediment-related properties, it is not surprising that a similar situation existed for aliphatic hydrocarbon concentrations. Aliphatic hydrocarbon concentrations in sediments were usually reported at concentrations at least 1000x smaller than corresponding total organic carbon values. It is plausible that we could not detect the response of bacterial populations to this small amount of hydrocarbon material using our methodologies. It is more likely that elevated bacterial counts or enrichment of petroleum degrading bacteria are only indirectly related to ambient hydrocarbon levels which are in turn a reflection of overall biological activities affected by topography, sedimentation, and granulometric properties. Thus, we find elevated levels of petroleum degrading bacteria in troughs which are also zones of heightened biological activity and relatively large amounts of clay-silt. Reports of other workers attempting to correlate bacterial parameters with sediment hydrocarbons (Walker and Colwell 1976; Buckley, Jones and Pfaender 1976) are difficult to compare and interpret due to differences in the levels of hydrocarbons involved and the incompatibility of analytical methods for hydrocarbon determination. It must be stated that at the levels of hydrocarbons encountered in BLM sediments, significant correlations of bacterial parameters with aliphatic sediment hydrocarbons were not observed.

Considering the consistency of bacterial data which indicated the largest populations of petroleum degrading bacteria were maintained at C stations, the lack of significant correlation with inner shelf sediment hydrocarbons (aliphatic) is somewhat surprising. Inner shelf stations represented a region of the largest gradient of bacterial decrease with distance from shore. Both TOC and hydrocarbon data were somewhat inconsistent in revealing a similar gradient. Possibly these inconsistencies were due to the rapid uptake activity of inner shelf bacterial populations which effectively remove and mineralize substrates to low steady-state levels. It can be calculated that a relatively small concentration of hydrocarbons can easily result in an increased bacterial population. Obviously, a single hydrocarbon measurement in time cannot yield meaningful information on a dynamic system. It should be noted that mean hydrocarbon values for sediments or for water samples were generally similar and small differences due to standard errors inherent in both bacteriological and chemical analyses could easily obscure correlation.

One recommendation is that bacterial and other sediment data (including hydrocarbon concentrations) be treated using partial correlation analysis

yielding statistical information on inter-relationships. Thus, bacterial parameters are only indirectly related to ambient hydrocarbon concentrations because these hydrocarbon levels only reflect controlling sedimentary or biological factors.

Petroleum degrading bacteria in surface water (1 m) samples were most abundant in the vicinity of Atlantic City (N1). Numbers dropped rapidly with distance from shore so that at N2 mean values (all stations) had decreased from 10^4 petroleum degrading bacterial units/ml to 3 petroleum degrading bacterial units/ml. With the exception of Station F2, mean populations for all other stations were less than one petroleum degrading bacterial unit/ml sea water. These minimal values have been reported as indicative of non-polluted water (Atlas and Bartha 1973b). Station F2, located in the vicinity of a frontal zone at the shelf break, exhibited markedly elevated levels of petroleum degrading bacteria during the fall and winter seasons. These elevated levels contrasted sharply with the numbers of petroleum degrading bacteria at other inner and outer shelf stations, especially during the winter. Elevated numbers of these bacteria sometimes occurred simultaneously with high concentrations of dissolved organic carbon. Hydrodynamic data indicated the dynamic nature of this frontal zone as one of mixing with associated upwelling. Upwelling, a process of nutrient replenishment of upper water layers, is known to stimulate productivity and the elevated levels of petroleum degrading at F2 may have resulted from enhanced mineralization activities associated with biological productivity. Determination of biomass (by ATP), hydrocarbon concentrations of the particulate phase and subsurface nutrient concentrations should help to elucidate this hypothesis. Finally, Station J1, located beyond the shelf break convergence zone, generally exhibited the lowest populations of petroleum degrading bacteria and inorganic nutrients.

The ratio HC/HET varied in direct proportion with the abundance of petroleum degrading bacteria. Levels of petroleum degrading bacteria and heterotrophic bacteria in 1 m water samples were significantly ($p < 0.05$) correlated with inorganic nutrient concentrations. This was not surprising since it is well known that petroleum degradation is limited by nitrogen and phosphorus concentrations in sea water (Atlas and Bartha 1972b).

Heterotrophic bacterial populations from Middle Atlantic shelf microlayer samples were not consistently enriched relative to the levels in bulk-subsurface water (1 m) samples. This observation contrasts with those of Sieburth (1963) and Tsyban (1971) who reported enrichment of heterotrophs in the microlayer as a fairly consistent observation. However, it would appear that the relative ability to detect enrichment would be a function of the difference between bacterial populations in the microlayer and bulk-subsurface water. Thus, Sieburth (1963) was able to detect enrichment in the microlayer because bacterial populations in the bulk-subsurface water were very low (mean=8 organisms/ml). In contrast, bacterial numbers in waters farthest from the shelf (J1) exhibited a mean value of 1100 bacterial units/ml. Obviously it becomes difficult to detect enrichment in the microlayer when bacterial levels in the bulk-subsurface water are at similar values.

In contrast to heterotrophic bacteria, a more consistent pattern of enrichment of petroleum degrading bacteria was observed in microlayer

samples. This observation is a logical one since it is known that lipoidal substances, such as hydrocarbons, accumulate at the air-water interface (Dietz and Lafond 1950; Garrett 1967; Jarvis et al. 1967; Seba and Corcoran 1969; Parker and Barsom 1970; Duce et al. 1972) and petroleum degrading bacteria would be capable of degrading such substrates. Enrichment of petroleum degrading bacteria appeared greater at the "oceanic" stations, i.e., stations in the region of the shelf break. It is possible that natural slick formation was greater in this region owing to enhanced productivity due to upwelling. Enrichment of petroleum degrading bacteria was also manifested by elevation of the ratio HC/HET relative to the subsurface layer.

Genera observed in microlayer samples supported Sieburth's (1963) contention that *Pseudomonas* sp. dominated. We also observed that *Pseudomonas* isolates in the microlayer exhibited the highest proportion of isolates confirming as petroleum degraders in pure culture.

If natural slick formation is related to biological productivity, it is possible that the ratio of petroleum degrading bacteria in the microlayer to those in the bulk-subsurface water could be larger in regions or periods of high productivity. Similarly, this ratio could be elevated if slicks produced by chronic oil discharge are present. These assumptions remain to be examined as additional seasonal data is obtained.

Laboratory Evaluation of Isolates

Isolate Characteristics

The majority of gram-negative isolates were identifiable at the generic level using a determinative scheme modified after Shewan (1965). However, difficulties were frequently encountered with isolates assigned to the genera *Alcaligenes* and *Flavobacterium* as well as certain non-motile, non-pigmented forms.

Various authors have placed motile marine bacteria with the characteristics of *Alcaligenes* into the genus *Pseudomonas* and non-motile forms in *Acinetobacter*, *Moraxella*, or *Neisserica*. Others simply lump non-motile strains into species *inceratae sedis*.

In this study, motile, non-fermentative isolates were placed in the genus *Alcaligenes* if they were relatively slow growing, biochemically inactive, highly sensitive to penicillin (G) and most other antibiotics tested, and tended to clump on gram stain preparations. The remaining non-fermentative isolates tended to grow rapidly, were biochemically active, insensitive to penicillin (G) and other antibiotics tested, and tended to appear as single cells or short chains on gram stain preparations. These non-fermentative motile isolates were phenotypically distinct from the preceding group and were usually classified as *Pseudomonas* sp. Differential sensitivities of *Alcaligenes* and *Pseudomonas* to penicillin (G) and ampicillin have been previously noted as a distinct biochemical feature of these genera (Shewan, Hodgkiss and Liston 1954; Shewan 1963; Stanier et al. 1966; Simon and Ridge 1974; Oberhoffer et al. 1977).

Identification of non-motile, non-fermentative, and non-pigmented isolates still remains a problem. Shewan (1963) placed all these isolates

in the genus *Achromobacter* (*Alcaligenes*). Others have placed similar isolates in *Acinetobacter* and/or *Moraxella* (Lewis 1973; Buchanan and Gibbons 1974). Kovacs' oxidase negative, penicillin (G) resistant strains have been considered as *Acinetobacter* (Baumann et al. 1968b; Pagel and Seyfried 1976). Oxidase positive strains have been placed in the genus *Moraxella* (Baumann et al. 1968a; Buchanan and Gibbons 1974). However, all but one species of *Moraxella* (*M. osloensis*) are sensitive to penicillin (G) and their extremely fastidious nutritional requirements eliminated them from consideration due to cultivation methods employed. Non-motile, penicillin (G) sensitive isolates with biochemically inert tendencies and characteristics similar to motile *Alcaligenes* sp. were designated as such.

No attempt was made to assign isolates to the genus *Achromobacter* because of its overall similarities with the genus *Alcaligenes*. The validity of *Achromobacter* as a taxonomic group has been seriously questioned (Baumann et al. 1972; Buchanan and Gibbons 1974).

Differentiation of non-motile *Flavobacterium* from *Cytophaga* is a major unresolved taxonomic problem (Mitchell et al. 1969; Weeks 1969; Buchanan and Gibbons 1974) and a reliable differentiation scheme is unavailable.

Seasonal Frequency

Seasonal frequencies of occurrence for dominant genera were such that there was either no change with season or the data were considered insufficient to assess the effect of season. *Alcaligenes*, the most frequently encountered genus in HM tubes, which along with *Pseudomonas* comprised the most frequently isolated genera from ESWB + oil tubes, were isolated during all seasons. Similarly, the lack of a consistent pattern of seasonal occurrence for *Vibrio* suggests no seasonal selection. It is probable that the seasonal occurrence of *Flavobacterium* has been under-estimated. Isolates considered presumptive *Flavobacterium* exhibited very high mortality rates prior to taxonomic evaluation. Variations in seasonal occurrences of the remaining genera suggested no consistent pattern and we consider it impossible at this time to assess the effects of season.

Dominance by *Alcaligenes* (as *Achromobacter*), *Vibrio*, *Flavobacterium* and *Pseudomonas* have been reported elsewhere in various parts of the world with essentially no variation with season (Sieburth 1967; Liston 1968; Ezura et al. 1974). Sieburth (1967) noted that although temperature selected for bacteria by "thermal type", it had no effect on the generic composition of the dominant isolates.

Isolates with the highest frequencies of seasonal occurrence were not the same when ESWB and HM tubes were compared. *Alcaligenes* sp. dominated in HM tubes for both water and sediment samples. In contrast, *Alcaligenes* sp. and *Pseudomonas* sp. were isolated during all seasons from ESWB + oil tubes. *Vibrio* sp. also tended to appear at a higher seasonal frequency in ESWB tubes. These differences must certainly be a reflection of the selectivity of the ESWB + oil medium for petroleum degrading cultures.

Variations in genera as a function of station location for both water and sediments were not apparent. Genera dominant offshore were also dominant inshore.

Petroleum Utilization in Pure Culture

Isolates belonging to genera most frequently obtained in ESWB tubes from water and sediments during this study have been reported to utilize petroleum or distillate products in mixed cultures under laboratory conditions (Jobson, Cook, and Westlake 1972; Cobet and Guard 1973; Buckley et al. 1976; Walker and Colwell 1976). In this regard, isolates of the genus *Pseudomonas* appear of primary importance although *Flavobacterium* sp., *Bacillus* sp., *Brevibacterium* sp., and presumptive *Micrococcus* sp. have been reported to degrade crude oil (Jobson, Cook, and Westlake 1972; Walker and Colwell 1976).

Results of assays for petroleum utilization by pure cultures of dominant genera from ESWB tubes showed that 24.4% of *Pseudomonas* isolates were capable of growing on petroleum as the sole added carbon source. However, of the isolates belonging to the genus *Alcaligenes*, the most frequently encountered group in all samples, only 6.3% could utilize petroleum in pure culture. Based on the high frequency of isolation of *Pseudomonas* sp. it must be considered the dominant genus capable of degrading petroleum in pure culture. The small percentage of *Alcaligenes* isolates confirming as petroleum utilizers, suggests that its abundance in ESWB + oil tubes is the result of synergistic and/or opportunistic growth. Variations in the degree of positive reactions in ESWB + oil tubes from field samples may be due to varied degrees of synergism. It is well known, for example, that mixed cultures degrade petroleum to a greater extent than pure cultures (ZoBell 1969).

The proportion of all dominant isolates capable of degrading petroleum in pure culture was lowest in the winter and spring seasons. It is possible that this reduction in proportion (not necessarily numbers of bacteria) was a reflection of reduced biological activity and availability of degradable material during these seasons. Additional data are necessary to determine the validity of this observation.

Microlayer samples yielded the largest percentages of isolates from ESWB + oil tubes confirming for petroleum utilization. The smallest percentage was found for sediments with water slightly larger. Enrichment in the microlayer of hydrophobic materials (Duce et al. 1972) may be responsible for the observed difference in percentages of isolates utilizing petroleum. Sieburth (1963) observed that 95% of the *Pseudomonas* sp. isolates, dominant in microlayer samples, were lipolytic.

Petroleum Degradation Experiments

While enumeration of bacterial populations in closed flask systems yields little information as to the degradative activity of bacteria, consistent responses of bacterial populations to petroleum were observed. Our data indicated correlation between elevated petroleum degrading bacteria levels and losses from the n-paraffin fraction in nutrient enriched flasks. Patterns of degradation observed in these studies (i.e. the sequence of paraffin utilization as a function of structure) have been reported by other workers (Miget et al. 1969; Kator et al. 1971; Atlas and Bartha 1972b; Walker et al. 1975; Olivieri et al. 1976). The extent of n-paraffin degradation in non-enriched flasks was never as great as that in enriched flasks although relatively high levels of petroleum degrading bacteria were found

in non-enriched flasks. The need for addition of inorganic nutrients to enhance oil degradation has been reported (Atlas and Bartha 1972a; Atlas and Bartha 1973a; Atlas et al. 1976; Olivieri et al. 1976). Increases in populations of petroleum degrading bacteria in non-enriched flasks may have been due to growth on some portion of the oil we did not assay by our methods or growth on such a small portion of the oil that losses are below detectability. More detailed and sensitive analysis using GC-MS would be required to determine the extent of degradation of hydrocarbons other than n-paraffins. Despite the low levels of ambient nutrients being inadequate for measurable degradation, elevated populations in these flasks (compared to oil-free controls) indicated utilization of some component of the petroleum.

Overall trends in the response of bacterial populations to petroleum were noted. In most experiments, levels of petroleum degrading and heterotrophic bacteria exhibited the greatest increase by 3 days of incubation under enriched, non-enriched and oil free conditions. This rapid increase may have been due to surface area effects associated with closed system (ZoBell 1946b). In winter for many stations the greatest increase occurred after 6 days and was probably due to reduction of growth rate at the lower incubation temperature. Heterotroph levels generally maintained elevated values over the entire incubation period in enriched, non-enriched and oil free flasks. Petroleum degrading bacterial counts, however, responded to the presence of oil. In oil free flasks petroleum degrading bacteria counts initially increased and then decreased to values well below those found in oiled flasks. Similar responses of petroleum degrading bacteria to oil in flask systems have been reported (Atlas and Bartha 1973b; Walker and Colwell 1975; Lee 1976; Walker and Colwell 1976). Flasks inoculated with sea water from station N1 were exceptions to these trends. Oil free flasks from Station N1 maintained elevated populations of petroleum degrading bacteria about equal to those in oiled flasks. The proximity of N1 to Atlantic City with its well developed maritime activities undoubtedly provide adequate sources of hydrocarbons to support bacterial growth in oil free flasks. Relatively high inorganic nutrient and organic carbon concentrations were detected at N1.

Seasonal and geographic variations in the rates and extent of n-paraffin degradation in closed flask seawater experiments were observed. Reasons for these variations require additional seasonal data but the following factors are hypothesized. At this writing, it appeared that the initial levels of HC bacteria in the original water sample and perhaps the genera composing that sample were of significance. Incubation temperature, while being a factor, (Kator et al. 1971; Westlake et al. 1974; Olivieri et al. 1976; Walker et al. 1976) did not appear to be the sole factor responsible for degradation rates. This was supported by the observation that summer degradation rates (even under enriched conditions) were lower and the extent of degradation was lowest for nearly all stations compared to other seasons despite the highest incubation temperature.

Station N1 exhibited the greatest degradative "potential" (overall rates and extent of n-paraffin degradation) during all four seasons. N1 always exhibited the highest levels of HC suggesting the bacterial population was adapted for petroleum/hydrocarbon degradation. At the other extreme, Station J1 showed the smallest degradative "potential." This correlated with the low levels of HC bacteria observed at J1. While it

appears that the initial numbers of the bacterial in the water column are an indication of degradative potential, a more detailed study of the importance of the generic composition of the closed flask system may explain variations in degradative potential.

The ratio HC/HET has been proposed as an indicator of oil pollution (Walker and Colwell 1976). Our studies indicated a clear response of the ratio to oil; HC/HET values increased in the presence of oil with and without nutrient amendment in nearly all experiments. Nutrient enrichment actually resulted in only slightly higher values. Often the oil free control exhibited increase in the ratio after 3 days of incubation followed by decreases to values well below those observed in oil flasks. This initial increase was probably due to surface area effects and growth on trace lipids (hydrocarbons) present in the inoculum.

Bacterial populations from sediment inocula exhibited responses similar to those observed with water inocula. Petroleum degrading bacterial populations and the HC/HET values increased in oil free controls with these inocula and were maintained at values about equal to those found in oiled flasks. Petroleum degrading bacterial populations of "F" cluster inocula appeared to respond to the presence of oil and nutrient enrichment and this response was reflected in HC/HET changes. It appears that petroleum degrading bacterial levels and/or HC/HET values would be valuable in assessing hydrocarbon contamination. No seasonal data was available concerning patterns or rates of degradation by sediment bacterial populations.

Data from petroleum concentration experiments indicated the sensitivity of petroleum degrading bacterial populations to oil as reflected in the change in HC/HET values in the presence of oil. Whenever oil was added, regardless of the concentration, petroleum degrading bacterial counts increased and remained at elevated values. The increase was greater with nutrient enrichment suggesting low levels of inorganic nutrients as the limiting factor to hydrocarbon utilization. With lower concentrations of oil, petroleum degrading bacterial populations were not maintained at values as high as with higher concentrations. This suggests the portion of oil utilized by the bacterial population was rapidly degraded at lower oil concentrations and subsequently the population decreased.

Previous work concerned with bacterial petroleum degradation has been deficient in certain areas. Most often pure or mixed cultures, rather than natural populations have been used to degrade petroleum or mixtures of pure hydrocarbons. Nearly all the studies have been performed in closed flask systems by analysis of the patterns of degradation using packed column gas-liquid chromatography. Little or no species composition information or evidence for bacterial succession are available. During the next year our work will attempt to develop methodology for experimentation in these deficient areas. Closed flask studies using inocula of sea water and sediments are being continued. A more sophisticated continuous dilution system has been devised and will be employed during each season. Analysis of residual petroleum from the flask studies will employ capillary column gas-liquid chromatography. Extensive taxonomic evaluation of genera present during incubation period is underway. These experiments and analyses should yield greater insight as to the responses and degradative potentials of Middle Atlantic continental shelf bacterial populations.

Chitin-Oil Degradation Experiments

Preliminary experiments indicated that mixed cultures of chitinolytic marine bacteria isolated from Middle Atlantic shelf sediments and waters grew in a dilute peptone-chitin seawater medium and degraded chitin equally well in the presence or absence of unweathered South Louisiana crude oil (ca. 1%). Changes in populations of chitinoclasts and chitin losses (as filterable chitin) followed a pattern not unexpected in an enriched batch type system. Results from gas chromatographic analysis of residual crude oil failed to indicate degradation of n-paraffins. Chitin and peptone were, therefore, the preferred substrates.

These preliminary experiments must be cautiously interpreted in relation to natural sediments or waters since the mixed cultures consisted solely of relatively high levels of chitinoclastic bacteria grown in a nutrient broth with a relatively large concentration of chitin. Therefore, it is possible that the chitin degrading potential of the inocula was optimized and effects of the crude oil on growth masked.

Walker et al. (1975) examined the effects of a petroleum and a fuel oil on populations of lytic groups of estuarine microorganisms in closed flask systems containing water and sediment inocula. Enrichment was not provided in either organic or inorganic form. Relative to non-oiled controls, increases in the viable counts of all lytic forms (including chitinoclasts) during the first three weeks of incubation were observed in petroleum treated flasks. However, by "normalizing" counts of chitinoclastic bacteria as a ratio of chitinoclasts to "total number of colonies" (chitinoclasts + non-chitinoclasts growing on each plate), it was concluded that both petroleum and fuel oil exhibited toxicities towards populations of lytic bacteria. It is difficult to assess the validity of this conclusion with respect to the reported increases in the absolute populations of chitinoclasts (and heterotrophs) in petroleum treated flasks. Chitin degradation was never actually measured as substrate loss and the absolute levels of bacteria (and lytic groups) can increase in closed flasks solely because of surface area effects. Our data thus furnish the first experimental evidence for degradation of chitin in the presence of oil.

Attempts to resolve the problems encountered in these preliminary experiments will be incorporated into the second year's chitin-oil experiments. Mixed cultures consisting of 3 chitinoclastic isolates from either water, inner shelf sediments, outer shelf sediments or shelf break sediments and 3 non-chitinolytic petroleum degrading bacterial isolates will be used as inocula. Using these inocula, it may be possible to demonstrate "competitive heterotrophy" as populations capable of degrading both substrates will be present.

Since peptone can greatly stimulate heterotrophic growth and chitin degradation, peptone (and yeast extract) concentrations will be reduced by a factor of five. This change should increase the importance of chitin and oil as substrates relative to the peptone and perhaps make effects or interactions more distinct.

Total heterotrophs will be enumerated using the spread plate method (HM agar) instead of the MPN technique. Chitinoclast counts can then be directly compared to heterotroph counts eliminating the problem of vari-

ability in count inherent in the MPN technique.

Additionally, an in situ chitin-oil flask experiment is planned for Ø7B. Sediment inocula from selected stations will be incubated in closed flasks containing chitin, oil, or chitin + oil as the sole sources of added carbon. Replicate sets of flasks will be prepared so that one set can be supplemented with low concentrations of $(NH_4)_2SO_4$-K_2HPO_4 nutrient solution. The second set will remain unenriched. Sterile controls containing chitin + oil as well as control flasks containing only sediment inocula (to account for surface growth effects) will be prepared.

Sediment homogenates will be allowed to settle and supernatants employed as flask inocula to reduce the addition of particulates to flasks. Sediment control flasks will be processed normally to determine the levels of particulates in the inocula. Flasks will be incubated for a three month period and harvested at regular intervals to determine bacterial levels, chitin weight loss, and degradation of oil.

Pure Culture - Petroleum Growth Studies

Cultures most sensitive to the presence of petroleum were most frequently detected in genera which possessed characteristically long "lag" times. Thus, the absence of marked inhibitory effects on the growth of *Aeromonas* sp. and *Vibrio* sp. due to unweathered or weathered crude oil may be related to their very short "lag" times. In future studies, the effects of oil on isolates from these two genera will be examined in a more dilute basal medium to retard their growth rates. Perhaps at a slower growth rate, oil induced cell damage, may not be compensated for by the cell as readily.

Although it was not anticipated that all isolates from a given genus would have identical "lag" times, greater variation was observed within the *Alcaligenes* and *Flavobacterium* genera than for the remaining taxonomic groups. "Lag" time variation may have been a function of the taxonomic heterogeneity within these genera (Shewan 1963; Baumann et al. 1972) or due to the tendencies of cultures within these genera to produce "rope" like or stringy cell masses and to clump. Despite heterogeneity of "lag" time within genera, it was possible to usually identify the genus of an unknown isolate by its characteristic "lag" time.

Of those isolates significantly sensitive to crude oil, *Alcaligenes* sp. was the most dominant genus isolated in HM broth MPN tubes from microlayer, water (1M) or sediment samples. *Flavobacterium* sp., which exhibited the greatest inhibition by crude oil, was also frequently isolated. These two genera comprised about 55% (Table 11-58) of the numerically dominant bacteria in the microlayer, the zone most immediately affected by an oil spill. *Alcaligenes* sp. and *Flavobacterium* sp. accounted for about 30% and 10%, respectively, of the dominant isolates from HM MPN tubes from sediment samples. Bacterial isolates from ESWB MPN tubes for sediment samples were comprised primarily of *Pseudomonas* sp. (48%) and *Vibrio* sp. (20%) genera least inhibited by the presence of the crude oil.

Mechanisms underlying petroleum toxicity towards marine bacteria are unknown. Mitchell et al. (1972) observed that low concentrations of selected aromatic hydrocarbons or a Kuwait crude oil inhibited a chemotactic

response of some marine bacteria through hypothetical blockage of chemoreceptors noting that motility rates were normal but random. This effect was reversible when exposed cells were washed free of the hydrocarbon(s). It is unlikely that our observations of delayed "lag" time would be related to such a phenomenon as substrates present in the basal medium are uniformly dissolved and chemotaxis need not occur. It is more likely that the inhibitory effects observed were the results of other mechanisms. Some cultures were unaffected or slightly affected yet others were completely inhibited in the presence of crude oil under normally optimum growth conditions. The latter such results indicate that utilization of the basal medium was prevented/inhibited and therefore could involve substrate transport or permease system inhibition by blockage of substrate receptor sites. However, since it was "lag" time and not growth rate which was consistently affected, it can be hypothesized that isolate exposed to petroleum synthesized different cellular components than non-exposed cells. Perhaps mixed function oxidase systems for hydrocarbon detoxification were induced. An extended lag phase can be related to requirements for the synthesis of certain components necessary for subsequent growth. Cells are most permeable during the lag or adjustment period of growth (Lamanna and Mallette 1965) and the inhibitory effects of toxic hydrocarbons would be most pronounced during the lag phase, possibly causing a reduction in effective inoculum size and/or cell metabolism-synthesis. Undoubtedly, variations in "lag" times for different genera exposed to crude oils are due to a complex interaction of mechanisms. Further research is required to elucidate the mechanisms of marine bacterial sensitivity to toxic petroleum hydrocarbons.

It was noted in the present study and has been reported elsewhere (Buckley et al. 1976; Cobet and Guard 1973; Walker and Colwell 1976) that some isolates of *Acinetobacter*, *Alcaligenes*, and *Flavobacterium* can grow on petroleum hydrocarbon in pure culture. Although our observations indicated that the "lag" times of isolates from these genera were usually increased in the presence of crude oil, occasionally cultures exhibited enhanced growth or a decrease in "lag" time. Regardless of the direction of the effect of crude oil on "lag" time, a "diauxic" type of stepping growth curve was never observed. This may have been due to the relative concentrations of glucose and petroleum components in the basal medium. Thus, a given isolate growing extensively on glucose (a preferred substrate), may have produced extracellular metabolites and cell populations which decreased the likelihood of secondary growth on petroleum hydrocarbons.

One previous study (Calder and Lader 1976) has demonstrated that selected examples of petroleum hydrocarbons can adversely affect the growth of marine bacteria. However, this study dealt with the effects of specific dissolved aromatic hydrocarbons on two cultures. Our studies based on the examination of 269 isolates show that low concentrations (ca. 1%) of South Louisiana crude oil can inhibit the growth of bacteria representing the dominant genera isolated from our samples. Additional research is required to determine if these inhibitory effects are temporary, i.e. would sensitive cultures transferred to a fresh medium without crude oil exhibit a delay in "lag" time. Additionally, growth studies using soluble fractions and crude oil weathered in the presence of sunlight (production of photo-oxidation products) would provide additional basic information concerning the toxicities of realistically weathered oil on bacterial growth.

Summary of Significant Findings

1. Petroleum degrading and heterotrophic marine bacteria were consistently isolated from Middle Atlantic continental shelf sediments. Levels of heterotrophs were typically 2-3 log units greater than petroleum degrading bacteria. Overall, viable counts of bacteria did not vary appreciably with season suggesting stable sediment populations over the monitoring period. Larger variations in viable counts occurred in inner shelf sediments which were more closely coupled to terrestrial influences and climatic effects. Heterotrophic bacterial populations varied little with depth when compared by season, and this was probably related to the stability of the sedimentary environment. Heterotrophic bacteria did not manifest a well developed trend with depth any given season. Maxima were sometimes observed in troughs or in the finer shelf break or slope sediments. Patterns in the levels of petroleum degrading bacteria were more strongly developed with inner shelf sediments generally exhibiting the highest levels. Troughs usually exhibited elevated levels compared to ridges. Generally, levels tended to decrease with depth when moving from inner shelf to outer shelf sediments but increased in shelf break sediments with high amounts of silt-clay.

2. Non-parametric correlation analyses for bacterial sediment populations with selected physical and chemical sedimentary properties failed to yield consistent and significant correlation coefficients. Calculations were performed using either all stations sampled during a given season or separately for inner shelf, outer shelf, and shelf break sediment regions. However, when ridge and trough sediments were contrasted, significantly larger populations of bacteria were detected in most troughs during all seasons. One exception was the "C" cluster where terrestrial effects at the ridge apparently obscure the concentration effect of the trough.

3. Values of the ratio of petroleum degrading to total heterotrophic bacteria generally tended to decrease from high values inshore to lower values in outer shelf sediments. Maximum values in inner and outer shelf regions usually occurred in sediment samples collected from troughs. Elevated values also were detected in shelf break sediments with the largest amounts of silt-clay. In general, elevated values of the ratio occurred because of elevated levels of petroleum degrading bacteria.

4. Significant correlation of bacterial populations with concentrations of aliphatic hydrocarbons in sediments was not observed.

5. Petroleum degrading bacteria in 1 m water samples were most abundant in the immediate vicinity of Atlantic City. Numbers dropped rapidly with distance from shore, usually 3-4 log units. With the general exception of Station F2, mean populations of petroleum degrading bacteria for other stations were less than one petroleum degrading bacterial unit/ml sea water. Station F2, usually located in the vicinity of a frontal zone at the shelf break, exhibited markedly elevated levels of petroleum degrading bacteria during the fall and winter seasons. These levels contrasted sharply with those measured

at other outer shelf stations, especially during the winter. Hydrodynamic data indicated that F2 is located in a convergence zone characterized by mixing and upwelling. Station J1, located beyond the shelf break convergence zone, generally exhibited the lowest populations of petroleum degrading bacteria.

6. The ratio HC/HET in 1 m water samples varied in direct proportion to the abundance of petroleum degrading bacteria. Furthermore, levels of petroleum degrading bacteria were significantly correlated with inorganic nutrient concentrations.

7. Heterotrophic bacterial populations from microlayer samples were not consistently enriched relative to the levels in the bulk-subsurface water (1 m). In contrast to heterotrophic bacteria, a more consistent pattern of enrichment was observed in these samples for petroleum degrading bacteria. Genera dominating microlayer samples included *Pseudomonas*, a genus known for its ability to degrade lipoidal substances.

8. Seasonally dominant bacterial genera isolated from heterotroph and petroleum degrading media were *Pseudomonas* and *Alcaligenes*. *Vibrio* was observed over all seasons but at fewer stations than the genera mentioned above. *Flavobacterium* was somewhat less frequently isolated than *Vibrio*. *Pseudomonas* was isolated most frequently from petroleum degrading medium whereas both *Pseudomonas* and *Alcaligenes* were most frequently isolated from heterotroph medium. No apparent effect of season on dominant genera isolated was observed.

9. Isolates from petroleum degrading medium were examined for confirmation of the ability to degrade petroleum in pure culture. Genera most frequently isolated such as *Pseudomonas*, *Flavobacterium*, *Aeromonas*, and *Vibrio* exhibited confirming percentages ranging from 10-40%. *Alcaligenes*, a genus frequently dominant in heterotrophic medium, had the lowest percentage of confirmation. Taxonomic groups such as Coryneform, *Brevibacterium*, *Acinetobacter*, and *Moraxella*, although of much lower frequency of isolation, exhibited the largest proportions of confirmation.

10. Closed flask degradation studies demonstrated consistent responses of bacterial populations to crude oil addition. There was a positive correlation between elevated levels of petroleum degrading bacteria and losses of n-paraffins from the saturated paraffin fraction. The extent of n-paraffin utilization in non-enriched flasks was never as great as in nutrient amended flasks.

11. Seasonal and geographic differences in petroleum degradation patterns and rates were observed using water inocula from 1 m samples. It appeared that the initial levels of petroleum degrading bacteria in the water samples directly correlated with the degree of degradation observed. N1, located closest to shore, exhibited the greatest degradation and had the largest populations of petroleum degrading bacteria. Conversely, Station J1, located at the shelf slope, showed the lowest populations of petroleum degrading bacteria and the poorest degradation "potential".

12. The ratio HC/HET manifested a clear response to the presence of crude oil both with and without inorganic nutrient amendment. Although oil free seawater controls exhibited increases in ratio values immediately after incubation, these increases were transient and usually followed by decreases to values well below those in oil treated flasks.

13. Preliminary experiments indicated that mixed cultures of chitinolytic marine bacteria isolated from Middle Atlantic shelf sediments and waters grew well in a dilute peptone-chitin-yeast extract broth and degraded particulate chitin equally well in the presence or absence of South Louisiana crude oil (1%, v/v). No degradation of n-paraffins from the saturated paraffin fraction of this oil in the presence of chitin was observed indicating metabolic preferences for chitin and peptone.

14. Pure cultures of bacterial isolates from sediment, water, and microlayer samples were grown in a dilute peptone-glucose-yeast extract medium in the absence and presence of unweathered, artificially weathered, and a soluble fraction of South Louisiana crude oil. Genera which typically exhibited the shortest generation times in this medium were least affected by the presence of petroleum. However, genera such as *Alcaligenes* and *Flavobacterium*, dominant genera in many field samples, exhibited greatly extended "lag" times in the presence of unweathered and artificially weathered crude oil. "Lag" time was defined as the time required to reach an optical density value of 0.1. Certain genera could be identified by their characteristic "lag" times. Isolates from petroleum degrading enumeration medium were composed of dominant genera least affected by the presence of crude oil.

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