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Chitinoclastic bacteria from the Mid-Atlantic Shelf: Distribution, taxonomy, and effect of oil on growth and chitin degradation

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CHITINOCLASTIC BACTERIA FROM THE MID-ATLANTIC SHELF: DISTRIBUTION, TAXONOMY, AND EFFECT OF OIL ON GROWTH AND CHITIN DEGRADATION

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TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

HM	Heterotroph Medium
MPN	Most Probable Number
ESWB	Enriched Seawater Broth
CNB	Chitin Nutrient Broth
CH	Chitinoclasts
HC	Hydrocarbon(Petroleum)-utilizers
HET	Heterotrophs

ABSTRACT

The purpose of this study was to examine the distribution, taxonomy and effects of South Louisiana crude oil on chitinoclastic bacteria from the sediments and water column of the Mid-Atlantic continental shelf.

Chitinoclastic bacteria were found in every water and sediment sample examined. Levels in the water column varied directly with temperature and comprised from .08% to 50% of the total heterotroph population. Sediment levels were very erratic and ranged from .0066% to 7.5% of the total population. This variability may have been due to uneven deposition of chitinaceous debris in sediments.

Taxonomic evaluation of predominant chitinoclastic isolates showed 68.7% Vibrio, 21.7% Pseudomonas and 9.6% Aeromonas.

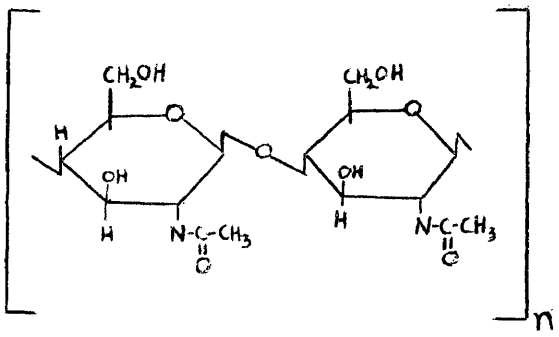
A series of closed flask chitin-oil degradation experiments was performed employing various culture conditions and inocula. Chitin degradation and chitinoclast levels were monitored over extended periods of incubation. With the possible exception of one experiment in which chitinoclasts could not be detected after two weeks, results of all experiments indicated that South Louisiana crude oil was not toxic to chitinoclastic marine bacteria under the conditions employed.

CHITINOCLASTIC BACTERIA FROM THE MID-ATLANTIC SHELF: DISTRIBUTION, TAXONOMY, AND EFFECT OF OIL ON GROWTH AND CHITIN DEGRADATION

INTRODUCTION

Chitin is one of the most abundant polysaccharides in nature and is predominantly found in the aquatic environment. According to an estimate by Johnstone (1908), copepods alone are responsible for the production of several billion tons of chitin annually. It is found in the exoskeleton of many molluscs, crustaceans, protozoans, insects and even in the cell wall of some fungi and algae. In spite of this vast abundance, chitin does not accumulate in estuarine and marsh sediments. Through mechanical erosion and microbial action, it is decomposed and thus serves as a major reservoir of carbon and nitrogen (ZoBell and Rittenberg, 1938).

Chitin is a very tough polymer of N-acetyl glucosamine units linked by B 1-4 bonds. It is an analog of cellulose with the N-acetylamino group replacing the hydroxyl on the 2nd carbon of glucose (White, et al, 1968).



Structure of chitin

In nature, chitin is not found in its pure form as represented above, but is found closely associated with inorganic salts and protein. The exact composition, however, varies with the animal,i.e. the hard exoskeleton of a crab contains more salts and more tightly bound protein than the soft cuticle of a shrimp (Hood, 1973).

Chitinoclastic (chitin decomposing) bacteria have been isolated from terrestrial and aquatic environments in many different parts of the world (ZoBell and Rittenberg, 1938; Campbell and Williams, 1951; Veldkamp, 1955; Seki and Taga, 1963; Chan, 1970; Hood, 1973). The exact mechanisms and end products of chitin decomposition have not been completely worked out, however, evidence suggests that an exocellular chitinase cleaves the B 1-4 linkages to form the dimer, chitobiose and the monomer, N-acetyl glucosamine. A chitobiase has also been detected that will split chitobiose into its two subunits (Reynolds, 1954; Berger and Reynolds, 1958). Clarke and Tracey (1956) observed chitinase activity in a medium containing no chitin, glucosamine or acetylglucosamine and, therefore, suggest that it is a constitutive enzyme. ZoBell and Rittenberg (1938) noted that the majority of their bacterial isolates utilizing chitin liberated ammonia, acetic acid and some butyric acid, indicating that acetylated amino groups were being hydrolyzed from the polymer. Some isolates were able to grow with chitin as the sole carbon source, but without depolymerization of the chitin. Apparently, these organisms were able to obtain sufficient carbon and nitrogen from the acetylated amino groups without cleaving C-O bonds critical to the integrity of the chitin polymer.

Most studies on the distribution of chitinoclastic bacteria

have occurred in bays, marshes and inlets. Seki (1965) and Seki and Taga (1963A) examined the occurrence of chitinoclasts throughout the water column and in the sediments of Aburatsubo Inlet, Japan. Chitinoclasts were found to make up less than 0.4% of the total bacterial population in the neritic region and there was a correlation between this percentage and the percentage of crustaceans in the plankton. A slight seasonality for viable counts in both water and sediment was noted and the percentage of chitinoclasts showed an inverse relationship with the COD (chemical oxygen demand) of the water. Hood (1973) sampled a salt marsh environment in Barataria Bay, Louisiana and found that over a one-year period, peak levels of chitinoclasts in the water column preceded peak levels in the sediment. She concluded that chitin containing animals act as a substrate for chitin utilizers in the water column. Upon death or molting, the exoskeleton and associated chitinoclasts are deposited in the sediment. ZoBell and Rittenberg (1938) examined a variety of sediments collected from nearshore to 200 miles offshore. No correlation between chitinoclastic populations and depth or distance from shore was observed. However, higher viable counts appeared to be associated with coarse sand where chitinous particles were concentrated. This observation contradicts Chan (1970) who found no correlation between the levels of chitinoclasts and the amounts of microscopically visible chitinous material in sediment samples.

The most recent and extensive taxonomic surveys of chitinoclastic bacteria are by Chan (1970) and Hood (1973). Each author used a different modification of a taxonomic scheme by Shewan et al. (1963) to group their isolates into genera. Chan (1970) found the majority of his isolates to be in the genus Vibrio and Hood (1973)

reported that the majority of her isolates belonged to the genus Beneckea. The distinction between these two genera is questionable and at present Beneckea is considered of uncertain taxonomic position (Buchanan, 1974). The other genera reported in these studies differed somewhat also, partly because their schemes were different, but mainly because of the controversial and continually changing state of marine bacterial taxonomy. Chitin utilizers isolated by Hock (1941), Campbell et al.(1951) and Kihara and Morooka (1962) were assigned to genera and species. Most of these species, however, are no longer recognized (Buchanan, 1974).

ZoBell and Rittenberg (1938) examined the effects of substrate composition on chitin utilization by individual cultures. They found that some grew on chitin alone while others needed either additional carbon or carbon plus nitrogen. They also observed a symbiotic relationship where two organisms growing together would utilize chitin, but either alone was incapable of chitin decomposition.

Chitin degradation rates, both in situ and in vitro, have been examined by several researchers (Hock, 1940, Seki and Taga, 1963B; Seki, 1965; Chan, 1970, Hood, 1973). Factors that affect chitin decomposition include temperature, substrate concentration, type of chitin (pure or native), particle size and salinity. Nutrients such as peptone supplied as a supplement to chitin will increase chitin decomposition. Native chitin was utilized faster than pure chitin and small particles with more surface area were degraded faster than larger ones. Lear (1963) found that chitinoclastic activity was inhibited by anaerobic conditions. Generally, any factor favoring heterotrophic bacterial growth will generally favor

chitin degradation as well.

Kim and ZoBell (1972) found that chitinase activity was increased by higher temperature, but was unaffected by an increase in hydrostatic pressure of up to 1000 Atm. Walker et al. (1975) reported that Louisiana crude oil and fuel oil No. 2 were toxic to chitinolytic bacteria.

The objectives of this study were to deal with the distribution of chitinolytic bacteria on the Mid-Atlantic shelf, the taxonomy of these forms and the effect of South Louisiana crude oil on chitin degradation.

With offshore drilling production operations now occurring on the shelf, there is even greater concern over the potential impact of oil spills on the biological systems of the waters and sediments. A major part of this research, therefore, involved experimental studies to evaluate the effect of oil on chitinoclasts and chitin degradation. As stated before, chitin is continually decomposed and consequently plays a major role in regeneration of carbon and nitrogen. Interference with chitin degradation or with the growth of chitinolytic microbes could ultimately affect this important cycling of nutrients.

METHODS AND MATERIALS

Study Location

A chart of the Mid-Atlantic continental shelf is represented in Figure 1. Water and/or sediment samples were collected on a seasonal basis at the indicated stations. Station depths ranged from 14 m at C1 to 410 m at L6. These depths varied somewhat from cruise to cruise due to shifting bottom topography and difficulties locating particular structures such as a small ridge or trough.

Sampling

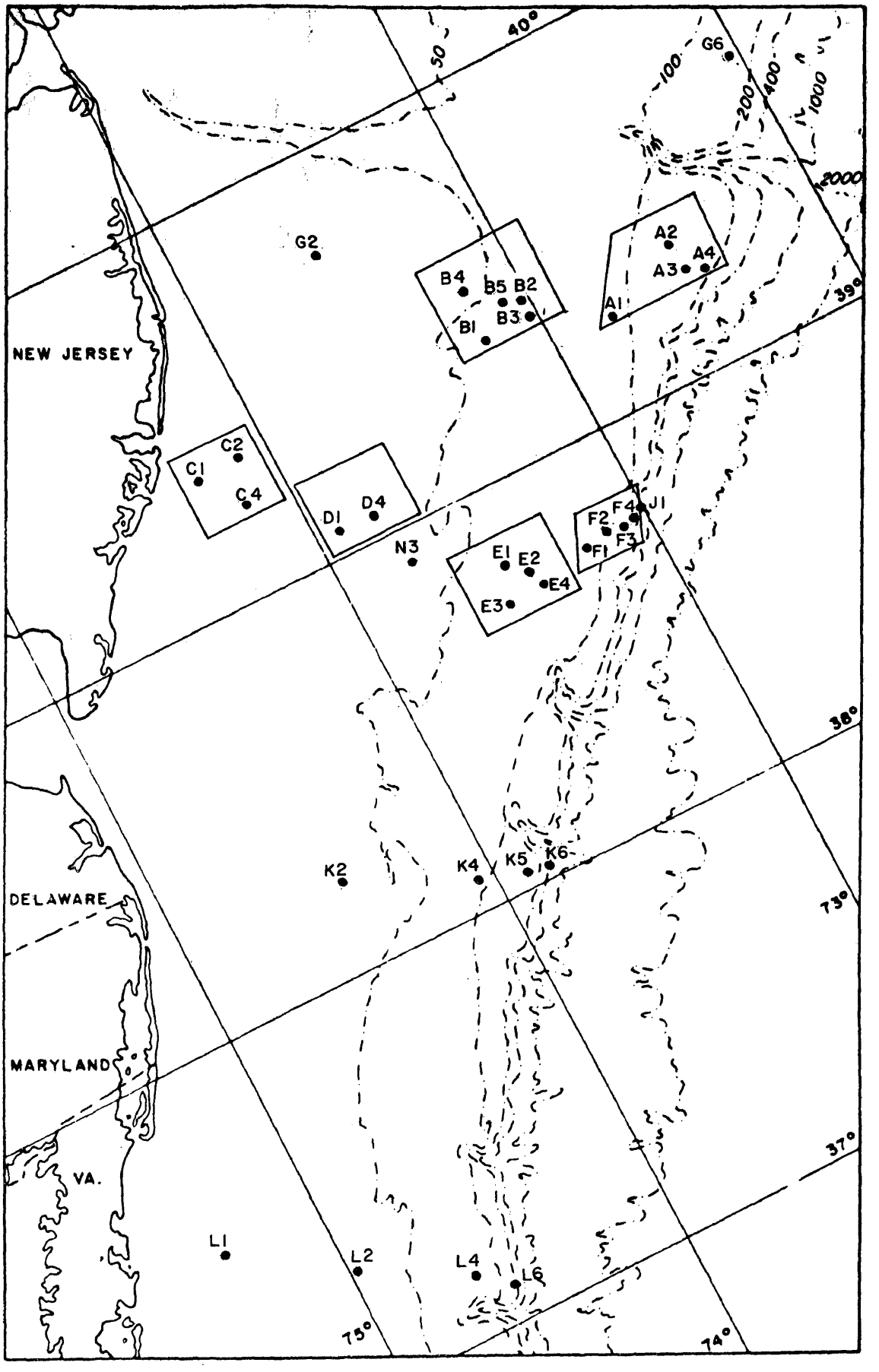
Water samples were collected at a depth of 1 meter with Niskin sterile bag samplers. Sediment samples were taken with a Smith-MacIntyre grab and subsamples for bacteriological analysis were removed aseptically using a 10 ml plastic syringe with the luer end removed. A 10 cc sample was blended for one minute with 90 ml of chilled sterile seawater and ten-fold serial dilutions of this homogenate were made using 9 ml sterile seawater dilution blanks.

Enumeration Procedure

Appropriate dilutions of sediment homogenate or water were spread onto bi-layer chitin plates. The lower layer was composed of a heterotroph medium (HM) consisting of 1g/l peptone, 0.5 g/l yeast extract, 0.01 g/l ferric citrate, 0.1 g/l sodium glycerophosphate, 15 g/l agar and 1000 ml aged seawater. HM is a modification of

Figure 1

Station Locations on Mid-Atlantic Continental Shelf


40°
50
100
200
400
1000
2000
G6
G2
A2
A3
A4
A1
B4
B5
B2
B3
B1
39°
NEW JERSEY
C2
C1
C4
D1
D4
N3
F2
F4
J1
F3
F1
E1
E2
E4
E3
38°
K2
K4
K5
K6
DELAWARE
73°
MARYLAND
37°
VA.
L1
L2
L4
L6
75°
74°

ZoBell's (1946) marine 2216 medium. The upper layer consisted of 0.5 g/l yeast extract, 15 g/l agar, 30 g/l "prepared" chitin and 1000 ml aged seawater. Chitin, an undefined grade of shrimp and crab shells (Calbiochem), was ball-milled for several days at 4°C. and sieved to a particle size of less than 250μ. Twenty five g ball-milled chitin was then slowly stirred into 300 ml of 50% sulfuric acid to shorten the molecular chain length. It was then reprecipitated in tap water, neutralized with KOH and centrifuged into a thick paste. Chitin preparation was based on techniques by Campbell et al. (1951) and Hood (1973). The presence of chitin resulted in an opaque layer and chitinoclastic colonies could be recognized by clearing zones around each colony where exocellular enzymes hydrolyzed the chitin polymer.

Three replicate plates were spread at each dilution and a geometric mean determined. Sediment values were converted to counts/g dry weight.

Total heterotroph counts were obtained on HM plates or by a three tube MPN (most probable number) technique (APHA, 1976) using HM without agar.

Petroleum utilizing bacteria were enumerated by a three tube MPN technique (Gunkel, 1968) using an enriched seawater broth (ESWB) consisting of 1g/l $(NH_4)_2SO_4$ and 0.1 g/l K_2HPO_4 in 1000 ml aged seawater. Approximately 0.1% filter sterilized South Louisiana crude oil was added to each tube after inoculation as a carbon source. ESWB tubes were incubated on a rotary shaker platform at room temperature for one month prior to counting. Tubes were considered positive if turbid or if the oil showed obvious signs of degradation.

All media were made using aged seawater filtered through Whatman #1 filter paper and autoclaved for 15 minutes at 121°C.; pH was adjusted to 7.8 and salinity to 30°/00.

Taxonomy

Isolates for taxonomic evaluation were obtained from spread plates inoculated in the field. Chitinoclastic colonies were re-streaked on chitin plates until obtained in pure form and were then maintained on slants of HM containing 15 g/l chitin.

The following criteria were used for classification.

1. Gram reaction - Hucker's modification, (Hucker and Conn, 1923)
2. Production of cytochrome oxidase - (Kovacs, 1956)
3. Oxidation or fermentation of glucose - Modified O/F medium, (Leifson, 1963)
4. Motility and cell shape - examination by phase contrast microscopy
5. Antibiotic sensitivity - Antibiotic discs - concentration per disc indicated

   -penicillin (2iu)

   -chloramphenicol (30µg)

   -neomycin (30µg)

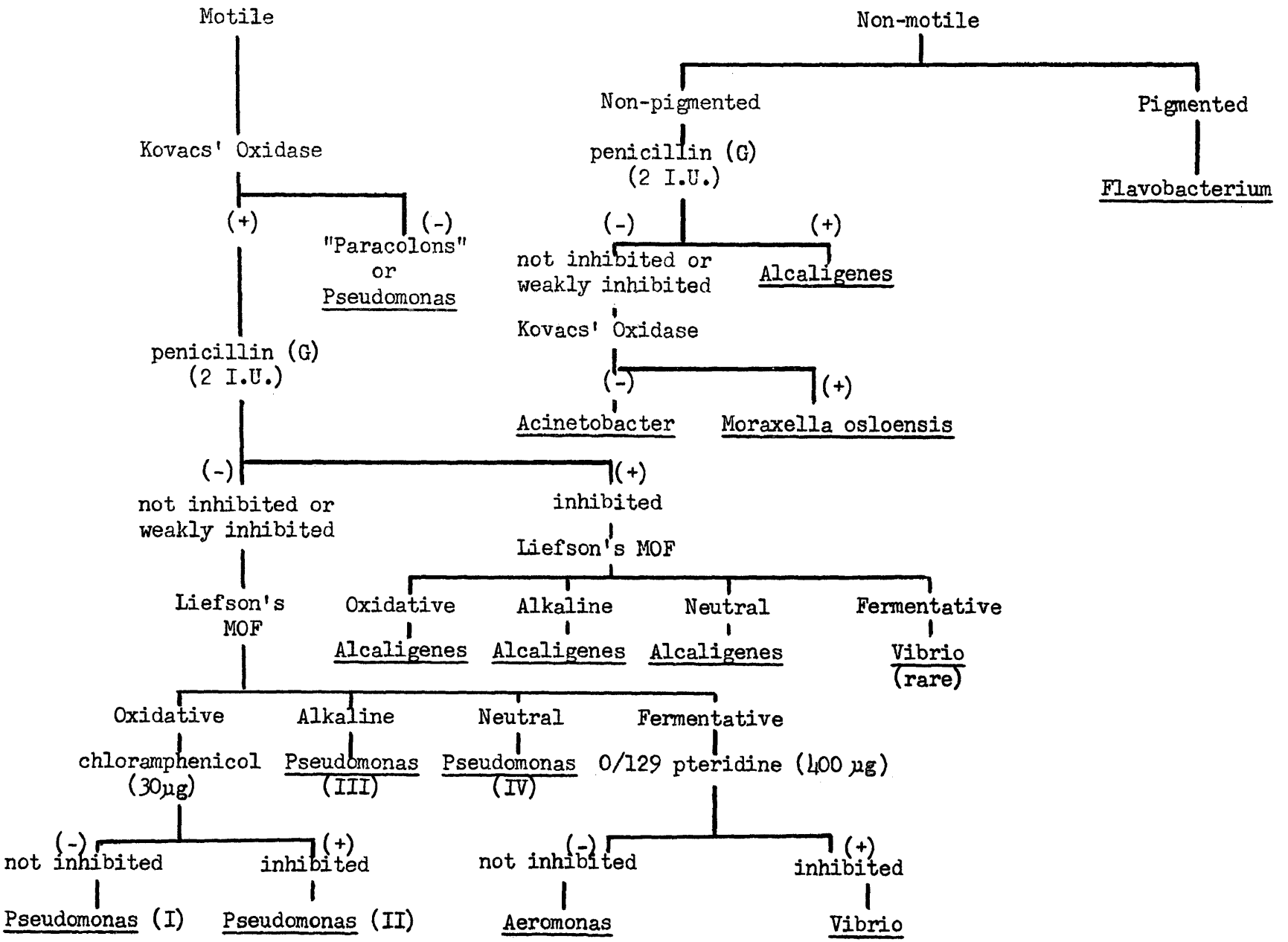
   -pteridine or vibriostatic agent 0/129 (400µg)

All isolates were identified according to a modification of a taxonomic scheme by Shewan (1963) shown in Figure 2.

Isolates were also tested for their ability to utilize crude oil by inoculation into ESWB + oil. Tubes were read after one month incubation at room temperature on a shaker table. Utilization of crude oil was not employed as a criterion in taxonomic evaluation.

Figure 2

Determinative scheme for the identification of major genera of gram-negative marine bacteria.


Motile
Non-motile
Non-pigmented
Pigmented
Flavobacterium
Kovacs' Oxidase
penicillin (G)
(2 I.U.)
(+)
(-)
"Paracolons"
or
Pseudomonas
(-)
(+)
not inhibited or
weakly inhibited
Alcaligenes
Kovacs' Oxidase
penicillin (G)
(2 I.U.)
(-)
(+)
Acinetobacter
Moraxella osloensis
(-)
(+)
not inhibited or
weakly inhibited
inhibited
Liefson's MOF
Liefson's
MOF
Oxidative
Alkaline
Neutral
Fermentative
Alcaligenes
Alcaligenes
Alcaligenes
Vibrio
(rare)
Oxidative
Alkaline
Neutral
Fermentative
chloramphenicol
(30µg)
Pseudomonas
(III)
Pseudomonas
(IV)
0/129 pteridine (400 µg)
(-)
(+)
not inhibited
inhibited
(-)
(+)
not inhibited
inhibited
Pseudomonas (I)
Pseudomonas (II)
Aeromonas
Vibrio

Pure culture chitin-petroleum degradation experiments using chitinoclasts isolated from water and sediment samples.

Sediment and water chitinoclasts isolated on four different cruises were combined to make one sediment and one water mixed culture from each cruise except fall, 1975 when no water isolates were available. In all, seven mixed cultures were prepared and used as inocula for chitin-petroleum degradation experiments. Cultures were grown up overnight in a chitin-nutrient broth (CNB) consisting of 0.5 g/l peptone, 0.5 g/l yeast extract, 0.01 g/l ferric citrate, 0.1 g/l sodium glycerophosphate, 1000 ml aged seawater and 2 g/l chitin sieved to a size range of 250-500 μ. Inocula were prepared by centrifugation and resuspension in sterile seawater to an optical density of 0.1 (625 nm). One ml aliquots of each mixed culture suspension were added to two replicate series of 150 ml milk dilution bottles containing 50 ml CNB-1. One series received 50 μl sterile South Louisiana crude oil while the other remained untreated. Uninoculated bottles with oil served as controls. All bottles were stoppered with cotton plugs to allow for aeration and shaken in the dark at 150 rpm. At two, five and ten-week intervals, one set of bottles for each of the seven cultures were harvested for enumeration of chitinoclasts, heterotrophs and petroleum utilizers. Ten ml methylene chloride was then added to all harvested bottles and to three sterile controls to terminate growth and extract residual oil.

Pure culture chitin-petroleum degradation experiments using chitinoclasts and petroleum utilizers.

Four experiments were performed for each season between fall, 1976

and summer, 1977. These experiments differed from the previous ones as the inocula consisted of three purified chitinoclasts from a designated area and either pure non-chitinolytic petroleum utilizers (fall) or a natural petroleum utilizing mixed culture (winter, spring, summer). Petroleum utilizers were introduced into the system so that chitinoclasts would not only be exposed to crude oil, but also its degradation by-products. The inocula consisted of chitinoclasts from water samples, inner shelf sediments (<50m), outer shelf sediments (50-100m) and shelf break sediments (>100m).

A second difference was that the peptone and yeast extract concentrations in the nutrient broth were reduced by a factor of 5 to increase the relative importance of chitin and oil as carbon sources and reduce the influence peptone may have had on heterotrophic growth and chitin degradation.

Incubation intervals also varied somewhat, but all other procedures regarding inocula preparation, incubation and harvesting were similar to those previously described.

"In-Situ" Experiments

In an attempt to more closely simulate chitin degradation in the natural environment, four experiments were performed during two different seasons to examine the effects of petroleum, chitin and nutrient interaction on natural bacterial populations.

Sediment homogenates were prepared as previously described and allowed to settle. One ml volumes of supernatant were added as inocula to milk dilution bottles containing 50 ml sterile seawater.

The following treatments were employed.

Set #1	Set #2	
Inoculum only - I(I)	Same as Set #1 except with nutrient amendment	E(I)
Inoculum + chitin - I(IC)		E(IC)
Inoculum + Oil - I(IO)		E(IO)
Inoculum + chitin + oil - I(ICO)		E(ICO)

Selected bottles received 0.1g chitin (particle size of 250-500μ determined on sieving) and 50μl sterile South Louisiana crude oil. One ml nutrient amendment consisting of 1.0 g/l $(NH_4)_2 SO_4$ and 0.1 g/l $K_2 HPO_4$ was added initially and at each harvest.

Bottles were shaken at 150 rpm in the dark at temperatures approximating the temperature of the sediment from which the inoculum was taken. Harvests were performed at appropriate intervals as previously described. Bottles containing chitin and oil and receiving sterile inoculum served as controls.

Two experiments were performed in the spring of 1977 using sediment inocula from ridge Station D1 and trough Station D4. Samples were incubated at 5°C and were harvested at 2, 5 and 12 weeks. The other two experiments were started in the summer of 1977 at ridge Station B2 and trough Station B3. These were incubated at 12°C and harvested at 2, 9 and 17 weeks.

Chitin Loss Determination

Chitin loss was determined gravimetrically by filtration of residual chitin onto tared Whatman #54 hardened filter paper. Glassware and filter paper were prewashed with methylene chloride to avoid

hydrocarbon contamination of the filtrate. Additionally, the filter paper was washed with methylene chloride after filtration to remove adsorbed oil. The filter and chitin were dried for one week at room temperature in the presence of silica gel and weighed after equilibration to room humidity. Control weights from uninoculated bottles were obtained to correct for losses resulting from the filtration procedure.

Extraction of residual oil

Oil was extracted from the filtrate with 10 ml methylene chloride after acidification with 4 drops concentrated HCL. The filtrate was then transferred to a separatory funnel pre-rinsed with solvent and combined with 20 ml methylene chloride used to rinse the culture bottle. After shaking, the organic phase was transferred to a second pre-rinsed separatory funnel and the aqueous phase was reextracted with 15 ml methylene chloride. The organic phases were then combined and washed with acidified water (pH 4.0) containing 3% NaCl. After phase separation, the organic phase was dried by passage through pre-washed anhydrous sodium sulfate in a piece of solvent-cleaned Whatman #54 hardened filter paper into a pre-weighed and solvent-rinsed evaporation flask.

Solvent Removal

Solvent was evaporated by aspiration at 40°C in tared pear shaped evaporation flasks. A McLeod gauge was used as an indicator of complete solvent removal. Residual oil was then weighed, trans-

ferred to vials in 1 ml of hexane and stored at 4°C.

Fractionation

Silica gel column chromatography was used to fractionate residual oil into saturated paraffins (H2 fraction) and aromatics (HB-2 fraction). Columns were packed to a height of 17.5 cm with activated silica gel (heated at 235°C for 18 hours) and washed with 40 ml hexane. Samples were put on the columns in 10 ml of hexane followed by an additional 13 ml. The first 5 ml eluted were discarded and the next 16 ml (H2) collected in solvent washed test tubes. Then 30 ml of a benzene/hexane (40/60) mixture were added and the elutriant collected as the HB-2 fraction. The paraffin fraction was transferred to pre-weighed evaporation flasks and the solvent was removed as previously described. After weighing, the fraction was dissolved in 5 ml hexane and stored at 0°C.

Gas chromatographic analyses for the first set of pure culture experiments were performed with a Tracor 560 gas chromatograph equipped with flame ionization detectors using a 6 ft. x 4 mm i.d. glass column packed with 3% OV-1 on Gas Chrom Q(100/120 mesh). One microliter samples were injected on column and programmed for a 3-minute hold at 70°C. The temperature was then programmed to 300°C at a rate of 8°/minute with a final hold of 15 minutes. Injector and detector temperatures were set at 200°C and 325°C respectively, and the carrier gas (N_2) flow rate was 30 ml/minute.

Paraffin fractions from the second set of pure culture experiments were analyzed with a Varian 2700 gas chromatograph using a glass capillary column (20 meters x 0.28 mm i.d. coated with 0.1% SE-52).

After a one-minute initial hold at 50°C, the oven temperature was programmed at a rate of 6°/min to 240°C with a 30-minute final hold. Flame ionization detectors and a Grob capillary injector were employed (Grob, 1972). The injector was set at 260° and the detector at 240°C. Carrier gas (He) flow rate was set at 30 ml/min and sample volumes ranged from 1.2-2.3 µl. The split was closed during injection and opened after 50 seconds.

"In-situ" experiments were analyzed with a Tracor 560 gas chromatograph using a glass capillary column and Grob injector as described above. The oven temperature was programmed from 45°C to 240°C at 4°C/min with a 10-minute final hold. The injector and detector were at 240°C and 325°C, respectively,with a carrier gas (He) flow of 2-3 ml/min. Sample volumes ranged from 2.4-2.6µl.

Degradation of n-paraffins (nC_{12}-nC_{27}) was determined from packed column chromatograms by measuring peak heights and comparing them with the isoprenoid pristane (2,6,10,14 - tetramethyl pentadecane). Pristane occurs naturally in South Louisiana crude oil and in this oil is only utilized by bacteria after n-paraffins reach baseline levels (Kator, 1971). As 50 µl of the oil utilized contained 330µg of pristane, determination of the ratio of paraffin peak height to pristane peak height yielded relative changes in micrograms of each paraffin. Summation values of nC_{12}-nC_{27} inclusive were then computed and compared to controls to account for evaporative loss.

Capillary column chromatograms were interpreted in the same way except that the area under each peak was automatically determined by an electronic integrator.

RESULTS

Distribution

Table 1 lists sediment and water chitinoclast counts from selected stations during the winter, spring and summer of the 1976-7 period. Sediment counts were very variable comprising from 0.0066% to 7.5% of the total heterotroph population. ZoBell and Rittenberg (1938) found a similar unevenness in numbers and attributed it to the uneven distribution of nutrient particles in sediments and the tendency of bacteria to colonize these particles.

Kendall's tau measure of rank correlation (Conover, 1971) was used to test relationships between sediment chitinoclast levels at Stations A1-F4 and various physical and chemical parameters such as sediment temperature, mean grain size, total organic carbon, % silt/clay content, depth and total nitrogen. No significant ($P = 0.05$) correlations were found except in the spring with mean grain size and in summer with % silt/clay and total organic carbon Table 2. It is difficult to say whether these correlations actually existed or were coincidental. Bacterial populations no doubt respond to a very complex interaction of all these variables. It was expected that chitinoclasts would vary according to the total heterotrophic population; however, a significant positive correlation was only found in the spring.

TABLE 1

Chitinoclast counts at selected sediment and water stations

SEDIMENT	Station	Winter, 1976		Spring, 1977		Summer, 1977	
	A1	1.7×10^2	2.2	2.8×10^4	4.4	5.3×10^3	3.6
	A4	7.3×10^2	2.9	2.0×10^3	3.3	1.7×10^4	4.0
	B2	2.1×10^1	1.3	2.6×10^2	2.4	3.9×10^3	3.6
	B3	1.7×10^3	3.2	4.7×10^3	3.7	1.6×10^3	3.2
	C1	1.3×10^2	2.1	3.9×10^4	4.6	6.4×10^2	2.8
	C4	3.1×10^3	3.5	2.0×10^4	4.3	2.9×10^5	5.5
	D1	2.3×10^1	1.4	4.2×10^2	2.6	2.7×10^1	1.4
	D4	3.6×10^4	4.6	5.2×10^3	3.7	1.4×10^3	3.1
	E1	1.1×10^4	4.0	7.4×10^2	2.9	2.6×10^3	3.4
	E4	4.9×10^3	3.7	7.0×10^2	2.8	6.9×10^3	3.8
	F1	5.8×10^3	3.8	1.0×10^3	3.0	7.6×10^3	3.9
	F4	3.8×10^3	3.6	2.2×10^3	3.3	1.7×10^3	3.2
	K2	1.4×10^3	3.1			5.5×10^3	3.7
	K4	4.1×10^3	3.6			9.1×10^3	4.0

TABLE 1 (Concluded)

SEDIMENT	Station	Winter, 1976		Spring, 1977		Summer, 1977	
	K5	1.1×10^{4}	4.0			9.4×10^{2}	3.0
	K6	7.3×10^{3}	3.9			1.2×10^{4}	4.1
WATER							
	A2	6.7×10^{0}	8.3×10^{-1}	1.0×10^{1}	1.0	7.3×10^{1}	1.9
	B5	6.7×10^{0}	8.3×10^{-1}	2.3×10^{1}	1.4	1.4×10^{2}	2.1
	C1	1.3×10^{1}	1.1	2.7×10^{1}	1.4	2.3×10^{2}	2.4
	D1	3.3×10^{0}	5.2×10^{-1}	< 3.3	$< 5.2 \times 10^{-1}$	2.8×10^{2}	2.4
	E3	1.0×10^{1}	1.0	6.5×10^{1}	1.8	7.4×10^{1}	1.9
	F2	3.3×10^{0}	5.2×10^{-1}	5.6×10^{1}	1.7	1.3×10^{2}	2.1
	K2	3.3×10^{0}	5.2×10^{-1}			3.7×10^{2}	2.6
	K4	1.3×10^{1}	1.1			4.5×10^{1}	1.7
	K5	6.7×10^{0}	8.2×10^{-1}			4.2×10^{1}	1.6
	K6	1.0×10^{1}	1.0			8.0×10^{1}	1.9

* 1st column = cells/ml H_2O or gm dry sediment 2nd column = log value

TABLE 2

Kendall correlation coefficients for sediment chitinoclast populations and selected parameters in mid-Atlantic continental shelf sediments sampled over three seasons during 1976-7.

Cruise	Het	C	Depth	% silt/clay	Mean Grain Size	Total Organic Carbon (mg/g)	Nitrogen
Winter	+.15	+.21	+.11	-.03	+.09	+.03	+.12
Spring	+.65**	-.09	-.03	+.33	+.52*	+.36	+.09
Summer	+.18	-.06	+.33	+.52*	+.23	+.50*	+.18

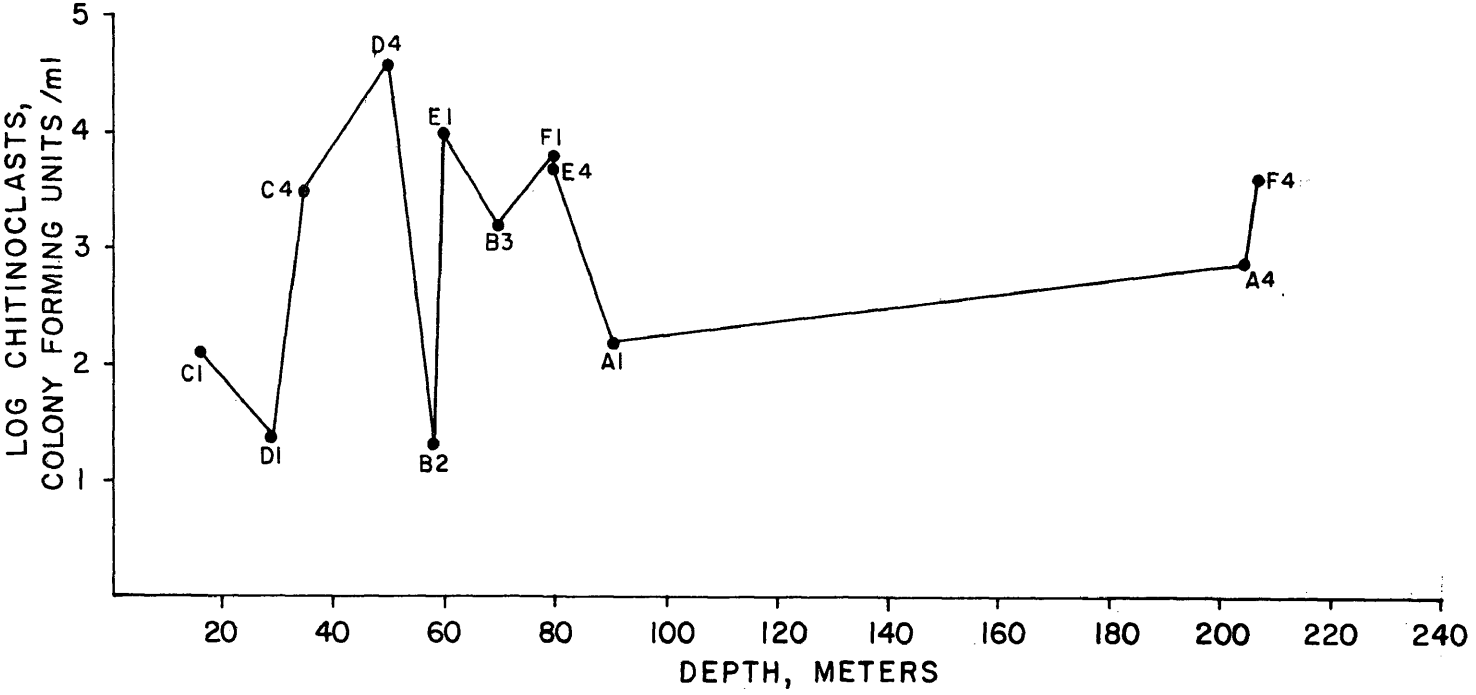
* Positive correlation P = 0.05

** Positive correlation P = 0.01

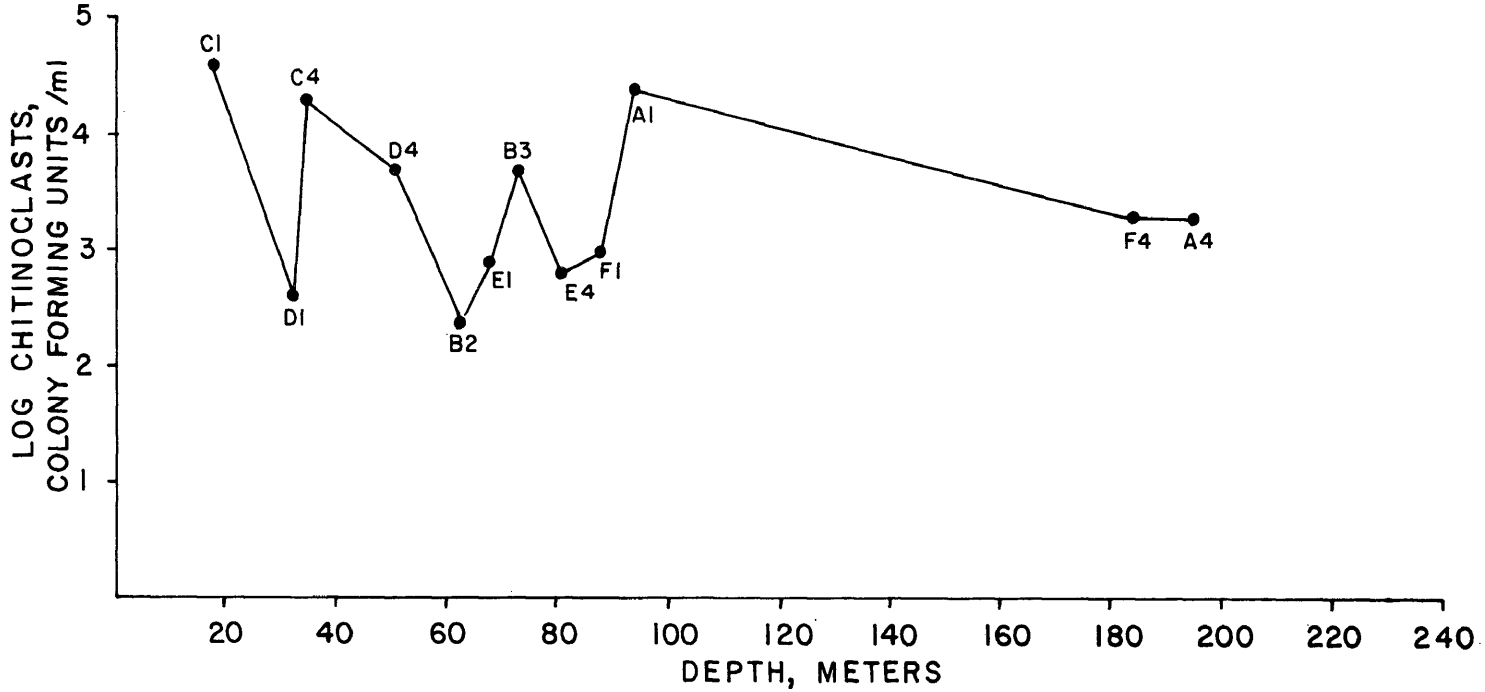
Figures 3 - 5

Levels of Chitinoclastic Marine Bacteria from Selected Sediment Stations, 1976 - 1977, showing relationships to depth.

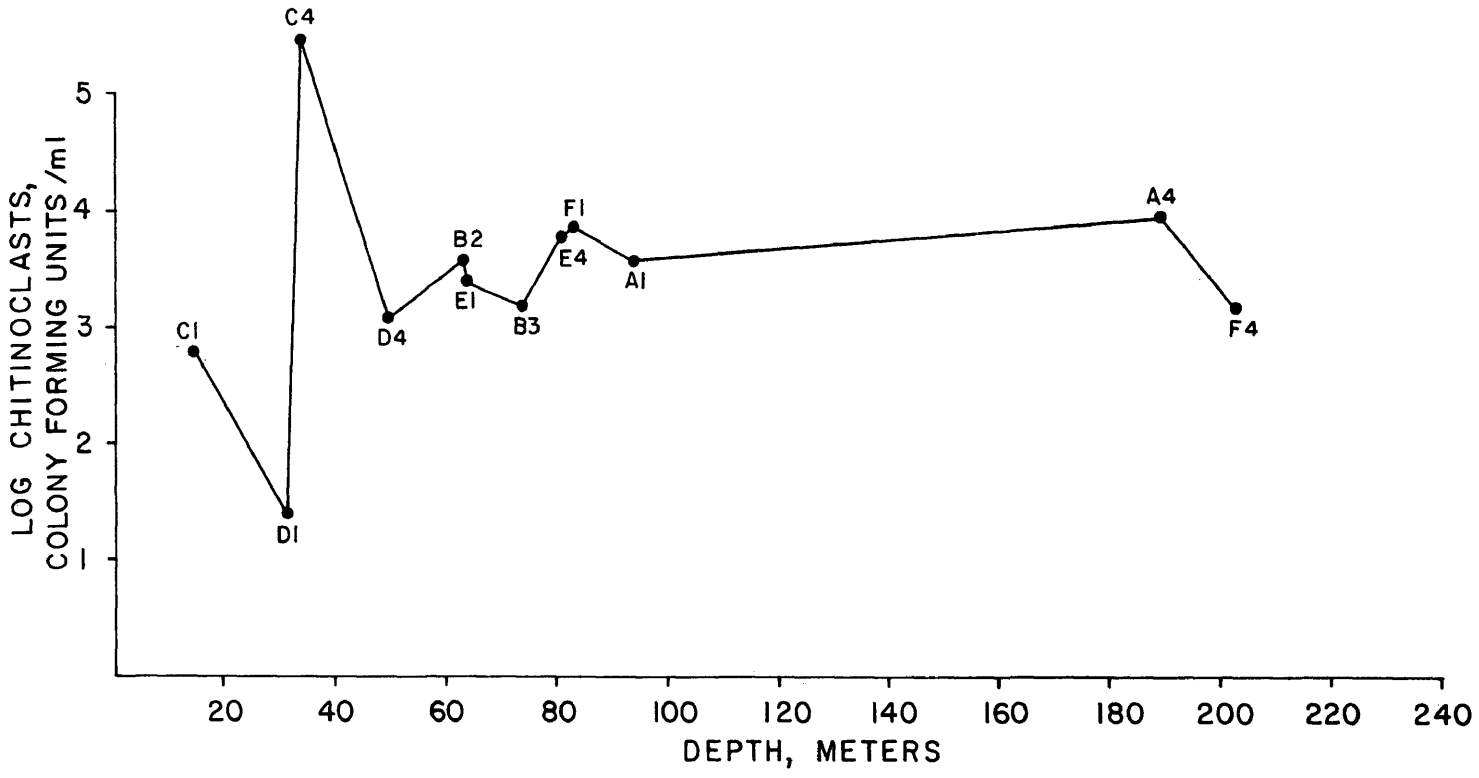
WINTER, 1976


LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
5
4
3
2
1
C1
D1
C4
D4
B2
E1
B3
F1
E4
A1
A4
F4
20
40
60
80
100
120
140
160
180
200
220
240
DEPTH, METERS

SPRING, 1977


LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
5
4
3
2
1
C1
C4
D4
B3
A1
F4
A4
D1
B2
E1
E4
F1
20
40
60
80
100
120
140
160
180
200
220
240
DEPTH, METERS

SUMMER, 1977


C4
C1
D1
D4
B2
E1
B3
F1
E4
A1
A4
F4
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
1
2
3
4
5
20
40
60
80
100
120
140
160
180
200
220
240
DEPTH, METERS

Selected sediment chitinoclast counts are plotted by depth in Figures 3 - 5. These show the large differences that often existed between ridge/trough pairs i.e. B2/B3, C1/C4, D1/D4 and E1/E4. Comparison of average counts for the four pairs using the Mann-Whitney test (Conover, 1971) showed a significant difference, $P = 0.1$.

K transect stations were enumerated in winter and summer to demonstrate changes, if any, due to depth or distance from land. No consistent patterns were found which is in keeping with the findings of ZoBell and Rittenberg (1938).

Chitinoclast counts from 1 m water stations ranged from one to four log units lower than sediment values comprising 0.08 to 50% of the total heterotroph populations. Seki and Taga (1963A) found that chitinoclasts made up less than 0.4% of the total population in the neritic region of Aburatsubo Inlet. Seki and Taga (1965), however, found that chitinoclasts comprised between 0 and 100% of the heterotrophic population in Sagami Bay and suggested that this patchiness could be due to their association with vertically migrating plankton.

As with the sediment values, chitinoclast counts did not vary in proportion to heterotrophs, however, they did vary directly with water temperature (Figure 6). Counts rose between 0.5 and 2 log units from winter to summer.

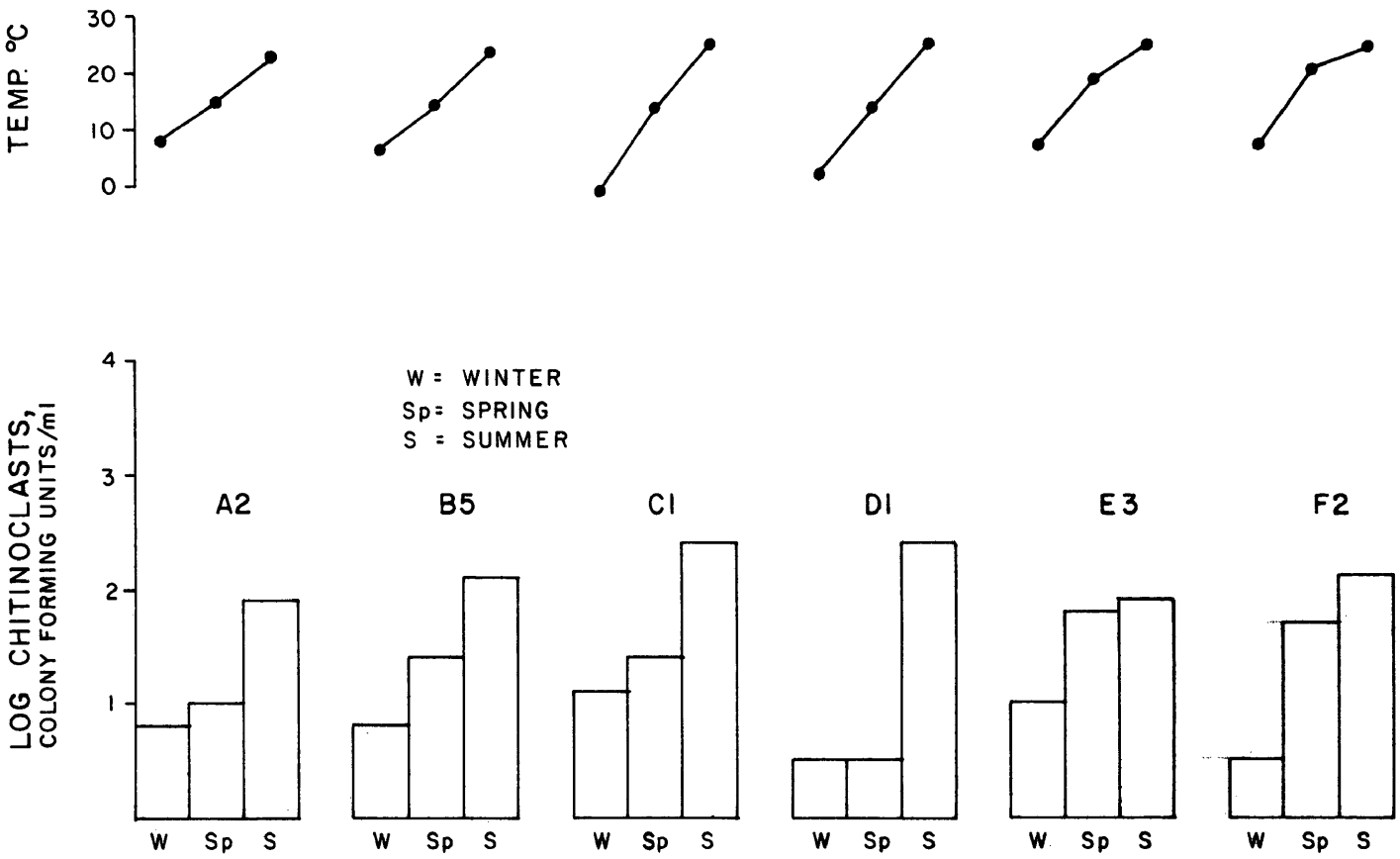
Taxonomy

A total of 332 sediment and water chitinoclastic isolates were identified. All isolates were gram negative, asporogenous, motile rods and all but two were cytochrome oxidase positive. The majority of isolates (68.7%) were identified as Vibrio followed by

Figure 6

Chitinoclast Counts and Water Temperatures

from Selected 1 meter Water Stations, 1976-1977


TEMP. °C
30
20
10
0
LOG CHITINOCLASTS,
COLONY FORMING UNITS/ml
4
3
2
1
W = WINTER
Sp= SPRING
S = SUMMER
A2
B5
C1
D1
E3
F2
W Sp S

21.7% Pseudomonas and 9.6% Aeromonas. Pseudomonas isolated were further classified as Type II, III or IV depending on their metabolism of glucose. Oxidase negative isolates were identified as Pseudomonas, but could not be assigned to one of the types. These findings support Chan (1970) who found 68% Vibrio, 15.6% Pseudomonas and 3.2% Aeromonas. He also isolated several other minor genera which were not encountered in this study; however, his work was done in Puget Sound and Burley Lagoon, Washington where some terrestrial influence would be expected.

Observed characteristics of chitinoclastic genera are given in Table 3 and a list of isolates, their source and classification can be found in Appendix E. Little seasonal variation was seen with the Pseudomonas population, however, the Vibrio and Aeromonas populations varied inversely with one another. Together they consistently comprised about 75-80% of the total population, but the percentage of Vibrios varied from 56.4 to 80.8%. This seasonal fluctuation may have been a function of the taxonomic scheme employed. Vibrio and Aeromonas could only be differentiated by pteridine sensitivity and in some cases the zones of inhibition were as small as 0.5 mm. These were considered positive but the zones may have been caused by a physical factor such as excess pteridine solution around the disc. Some researchers (Chan, 1970; Shewan, 1963) also use gas production by Aeromonas to distinguish them from Vibrio; however, not all Aeromonas species produce gas from glucose (Buchanan, 1974). In this study, no gas producing isolates were encountered.

Water and sediment isolates differed somewhat compositionally. Water isolates consisted of 60.2% Vibrio and 30.1% Pseudomonas while

TABLE 3

Observed characteristics of chitinoclastic genera from sediment and water. V indicates variable characteristic. Numbers in parentheses indicate number of isolates identified.

	Vibrio (228)	Aeromonas (32)	Pseudomonas II (55)	Pseudomonas III (7)	Pseudomonas IV (8)	Pseudomonas sp. (2)
Gram Stain	-	-	-	-	-	-
Motility	+	+	+	+	+	+
MOF						
Aerobic acid production	+	+	+	-	-	-
Anaerobic acid production	+	+	-	-	-	-
Alkaline reaction	-	-	-	+	-	-
No reaction	-	-	-	-	+	+
Kovacs Oxidase	+	+	+	+	+	-
Antibiotic Sensitivity						
Penicillin (G), 2 i.u.	V	V	V	-	V	-
Chloramphenicol, 30 µg	+	+	+	+	+	+
Pteridine, 400 µg	+	-	V	V	+	+
Neomycin, 30 µg	+	+	+	+	+	+
Petroleum Utilization	V	V	V	V	V	V

sediment isolates were composed of 72.4% Vibrio and 17.9% Pseudomonas. Aeromonas levels in water and sediments were nearly identical.

In general, very few brightly pigmented colonies were isolated and many of those that were initially isolated did not survive successive transfers. Most identified isolates were white, cream colored, light brown or grey.

Spheroplast production in very turbid broth cultures i.e. stationary or death phase, was a common occurrence for all genera.

Each isolate was reinoculated in ESWB plus oil to check for hydrocarbon use. Thirty-nine percent of 330 isolates checked were able to utilize South Louisiana crude oil as a carbon source. Percentages varied seasonally with 60% in fall, 58% in winter, 29% in spring and 26% in summer capable of utilizing oil.

Pure culture chitin-oil experiments using chitinoclasts only

Table A1 of Appendix A shows changes over time in levels of heterotrophs, chitinoclasts, petroleum utilizers and filterable chitin. Figures 7 and 8 show chitinoclast levels and chitin loss as a function of time. Chitin loss values were corrected for procedural error using replicate controls.

In all cases, the chitinoclast population rose two or more log units during the first two-week period, dropped to initial values at five weeks and continued to fall or leveled off at ten weeks. With the exception of the fall sediment inoculum plus oil, chitin loss was inversely related to growth. Maximum degradation occurred during the first two weeks with as much as 90% being utilized at a rate of 1.5-6.4 mg/day. From there, chitin loss either continued gradually or tapered off.

The fall sediment inoculum appeared to show reduced chitin utilization with time. This was attributed to the fact that large quantities of particulate cell debris were produced and recovered with the residual chitin. This particular culture typically grew as a tightly bound mass of cells that could not be broken up when grown in the presence of oil. When grown without oil, the mass could be broken up with methylene chloride and shaking. This is reflected by the typical curve for chitin loss without oil.

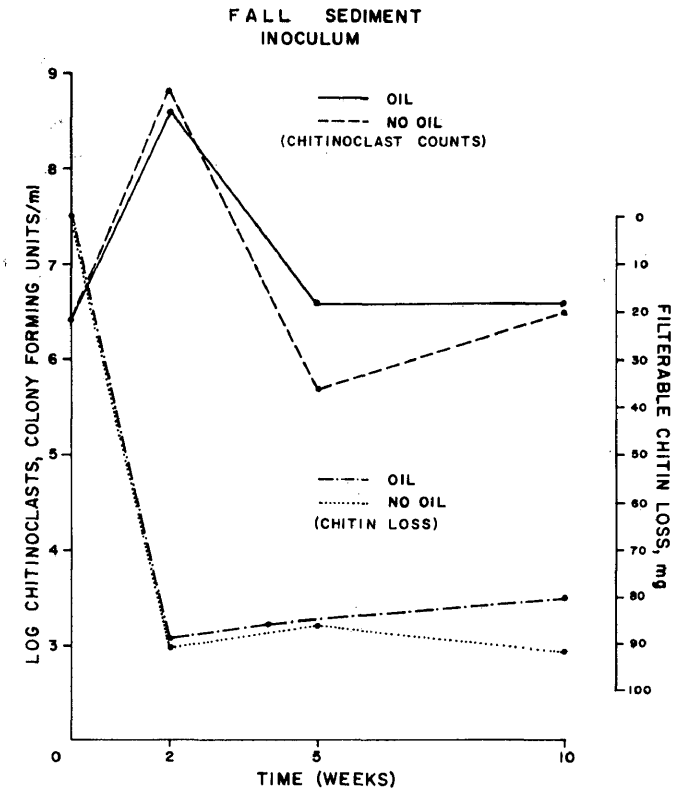
A Wilcoxin signed rank test (Sokal and Rohlf, 1969) was used to compare heterotroph and chitinoclast populations from oiled and unoiled bottles. No significant difference was found at the 5% level. A Friedman statistic (Conover, 1972) was used to compare chitin loss values so that differences due to procedural error could be taken into account. The procedural error was determined from six sterile controls to be $\pm$ 3 mg. Therefore, in the ranking process chitin loss values were considered ties unless they differed by more than 3 mg. Again, no significant differences were found at the 5% level.

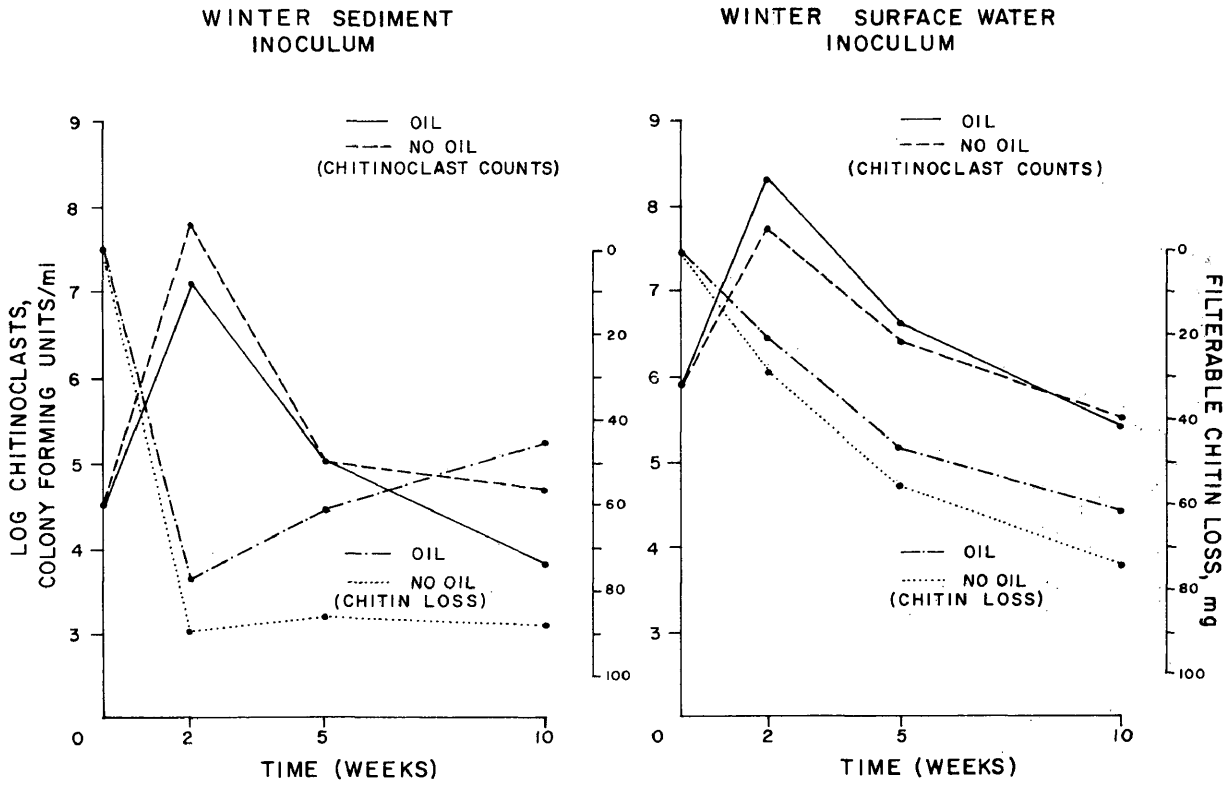
Some problems were encountered in trying to enumerate petroleum degraders because colloidal sized chitin particles which were transferred in the inoculum, tended to remain in suspension in ESWB tubes and caused oiled and unoiled tubes to be slightly turbid at lower dilutions. In spite of this, it was clear that some of the chitinoclast cultures were capable of utilizing oil and, in general, the petroleum-degrader counts followed the heterotrophs.

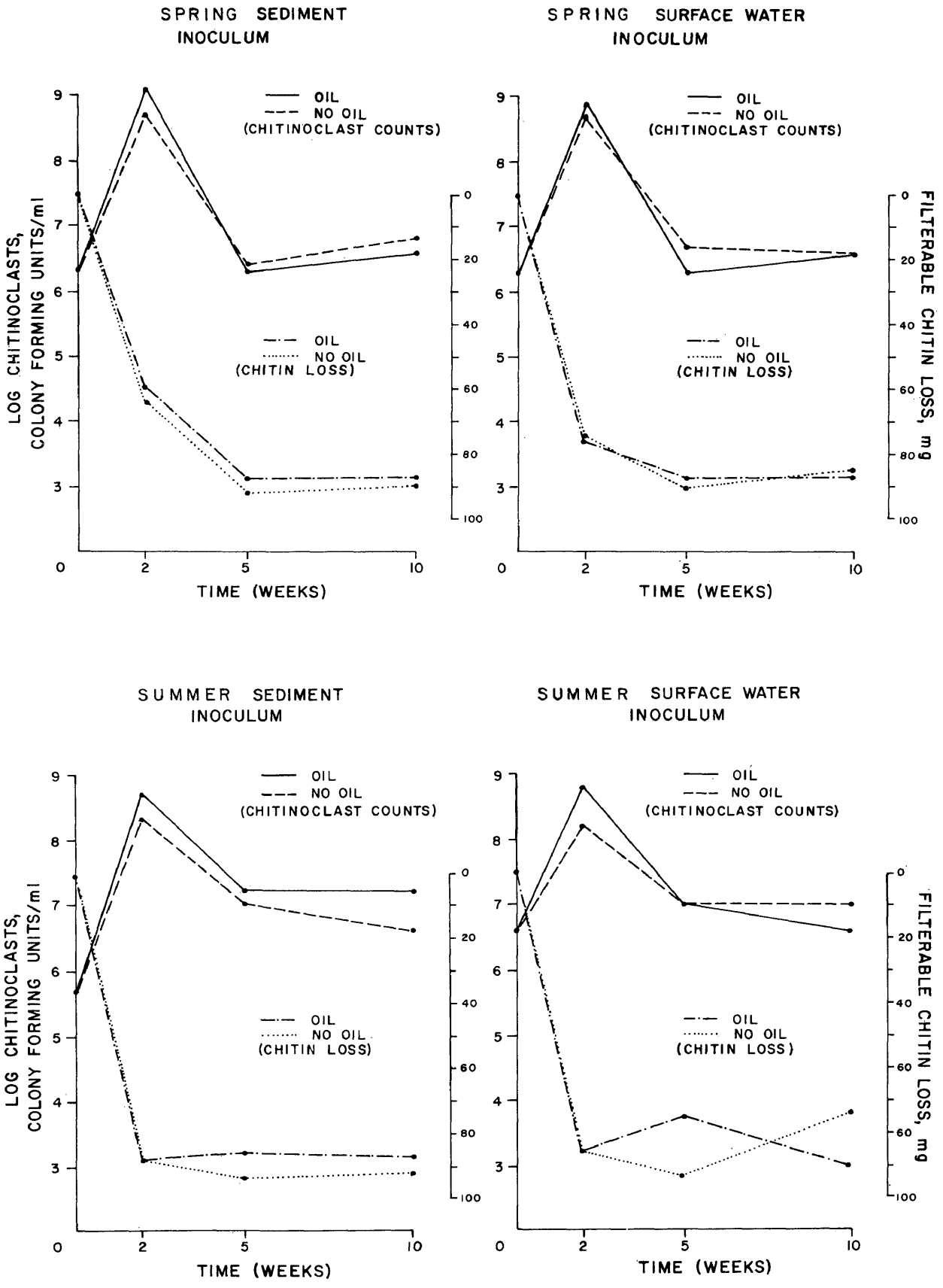
Gas chromatographic analysis of residual oil revealed little or no degradation of n-paraffins over the 10-week period for all inocula. Table B1 in Appendix B lists summation values (µg) for nC_{12}-nC_{27}.

Figures 7 - 8

Changes in amount of filterable chitin and chitinoclastic marine bacteria in a seawater-peptone broth with and without unweathered South Louisiana crude oil.


FALL SEDIMENT INOCULUM
OIL
NO OIL
(CHITINOCLAST COUNTS)
OIL
NO OIL
(CHITIN LOSS)
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
FILTERABLE CHITIN LOSS, mg
TIME (WEEKS)


WINTER SEDIMENT INOCULUM
WINTER SURFACE WATER INOCULUM
OIL
NO OIL
(CHITINOCLAST COUNTS)
OIL
NO OIL
(CHITIN LOSS)
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
FILTERABLE CHITIN LOSS, mg
TIME (WEEKS)


SPRING SEDIMENT INOCULUM
SPRING SURFACE WATER INOCULUM
SUMMER SEDIMENT INOCULUM
SUMMER SURFACE WATER INOCULUM
OIL
NO OIL
(CHITINOCLAST COUNTS)
OIL
NO OIL
(CHITIN LOSS)
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
FILTERABLE CHITIN LOSS, mg
TIME (WEEKS)
0
2
5
10
3
4
5
6
7
8
9
20
40
60
80
100

Eight controls were used to determine a procedural error of 3.4%. Degradation losses of less than this value were considered insignificant. No difference can be seen between representative control and inoculated chromatograms (Appendix D).

Pure Culture Chitin-oil Experiments Using Chitinoclasts and Petroleum Utilizers

Changes in levels of heterotrophs, chitinoclasts, petroleum utilizers and filterable chitin for pure culture experiments performed between fall, 1976 and summer, 1977 are listed in Table A2 of Appendix A and shown graphically in Appendix C. Results of the fall experiment (Figure 9) were similar to pure culture experiments using only chitinoclast inocula in that most chitin degradation occurred within the first two weeks of incubation. This period coincided with the highest levels of chitinoclasts. The presence of petroleum appeared to have no effect on chitinoclast populations or chitin degradation.

During the winter experiment (Figures 10 and 11) petroleum appeared to affect both chitinoclast counts and chitin degradation. In oiled flasks, chitinoclast counts usually did not reach maximum values before six weeks. Petroleum utilizers generally reached plateau values in two weeks, remained there for four weeks, and gradually tapered off. In oil-free flasks, chitinoclast counts rapidly increased to maximum values within two weeks and gradually decayed over the next nine weeks. Petroleum utilizers increased about 2-3 log units during the first two weeks, but then continued to slowly increase over the next six to nine weeks when chitinoclast levels were declining. This suggested diauxie-like growth where

petroleum utilizers were able to grow on lipoidal metabolic by-products or cellular components produced by chitinoclasts. Chitin degradation rates were greater in oil-free flasks during the first two weeks; however, total chitin losses at the end of the experiment were similar in oil-treated and oil-free flasks.

Similar patterns were observed for the spring shelf break (Figure 12) and summer inner and outer shelf (Figure 13) sediment inocula. The remaining experiments, however, showed little or no difference between oil-free and oil-treated flasks.

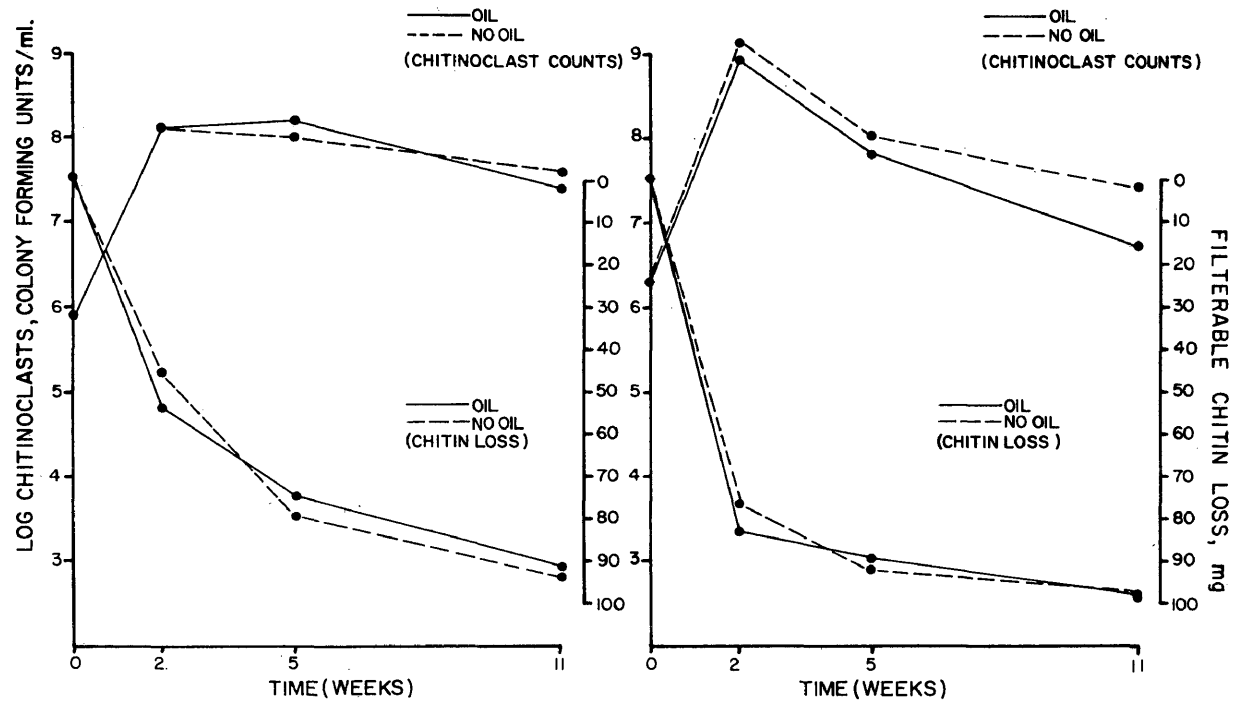
Results of gas chromatographic analysis are given in Table B2 of Appendix 3. In all cases 100% degradation of n-paraffins (nC_{13}-nC_{25}) occurred within two weeks. During the winter, spring and summer experiments, pristane, phytane and other unidentified branched compounds were also degraded. Representative chromatograms in Appendix D show a sterile control, 100% degradation of n-paraffins and 100% degradation of n-paraffins and isoprenoids.

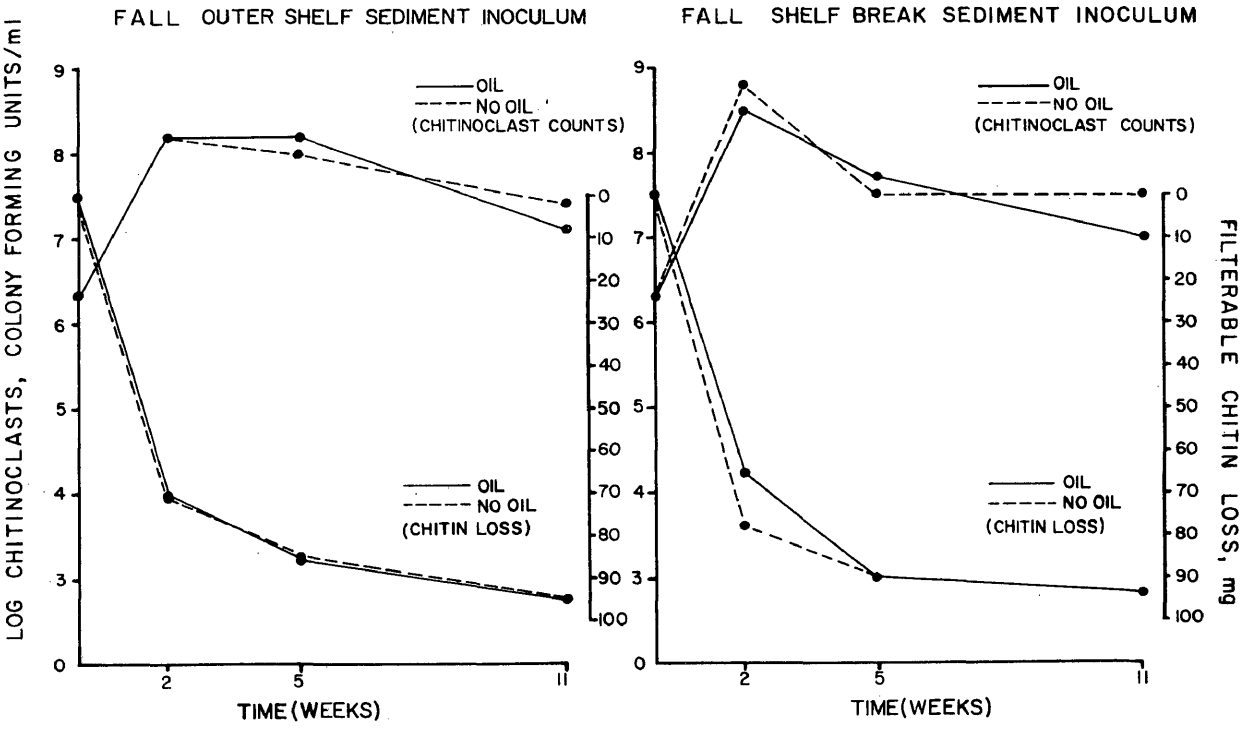
In-Situ Chitin Oil Degradation Experiments

Table A3 of Appendix A lists changes in levels of heterotrophs, chitinoclasts, petroleum utilizers and filterable chitin for in-situ chitin-oil experiments performed during spring and summer, 1977. Figures 14 to 16 illustrate the effects of oil alone, oil plus enrichment and enrichment alone on chitinoclast populations and chitin degradation. In some instances, there appeared to be less chitin utilized at the third harvest than at the second. This could usually be attributed to a build-up of particulate cellular detritus over extensive incubation and the initially larger chitin

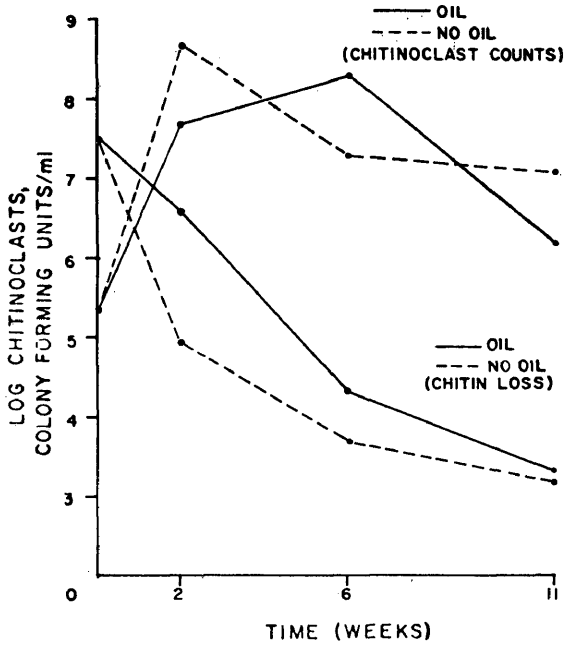
Figures 9 - 13

Changes in amount of filterable chitin and chitinoclastic marine bacteria in a dilute seawater-nutrient broth with and without South Louisiana crude oil.

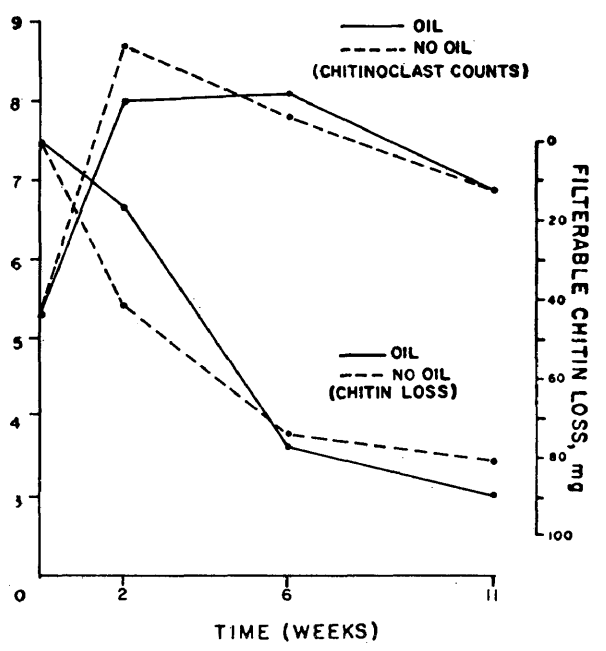

FALL H20 INOCULUM
FALL INNER SHELF SEDIMENT INOCULUM
OIL
NO OIL
(CHITINOCLAST COUNTS)
OIL
NO OIL
(CHITIN LOSS)
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml.
FILTERABLE CHITIN LOSS, mg
TIME (WEEKS)
0
2
5
11


FALL OUTER SHELF SEDIMENT INOCULUM
FALL SHELF BREAK SEDIMENT INOCULUM
OIL
NO OIL
(CHITINOCLAST COUNTS)
OIL
NO OIL
(CHITIN LOSS)
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
FILTERABLE CHITIN LOSS, mg
TIME (WEEKS)
0
2
5
11

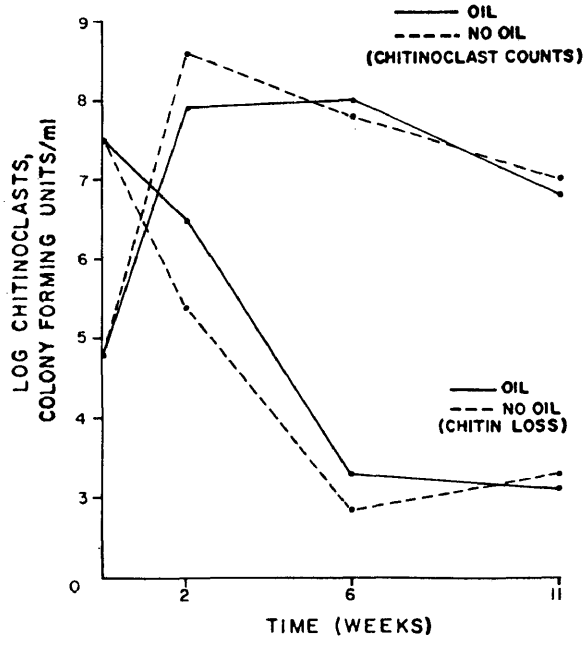
WINTER H20 INOCULUM



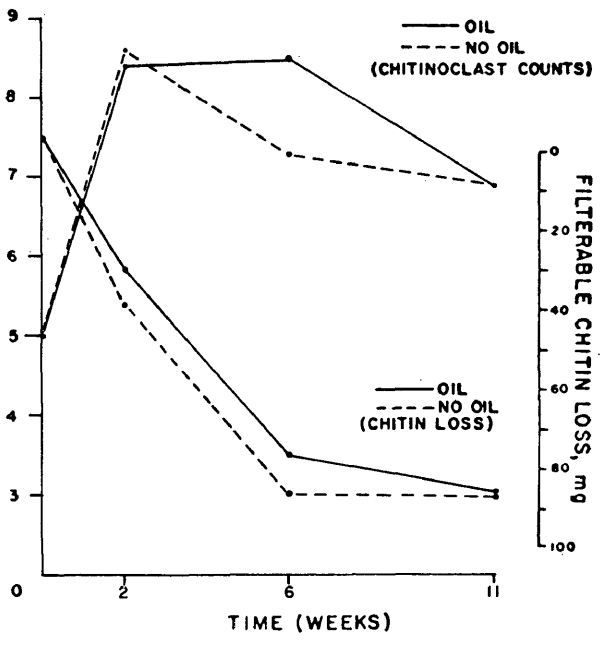
WINTER INNER SHELF SEDIMENT INOCULUM

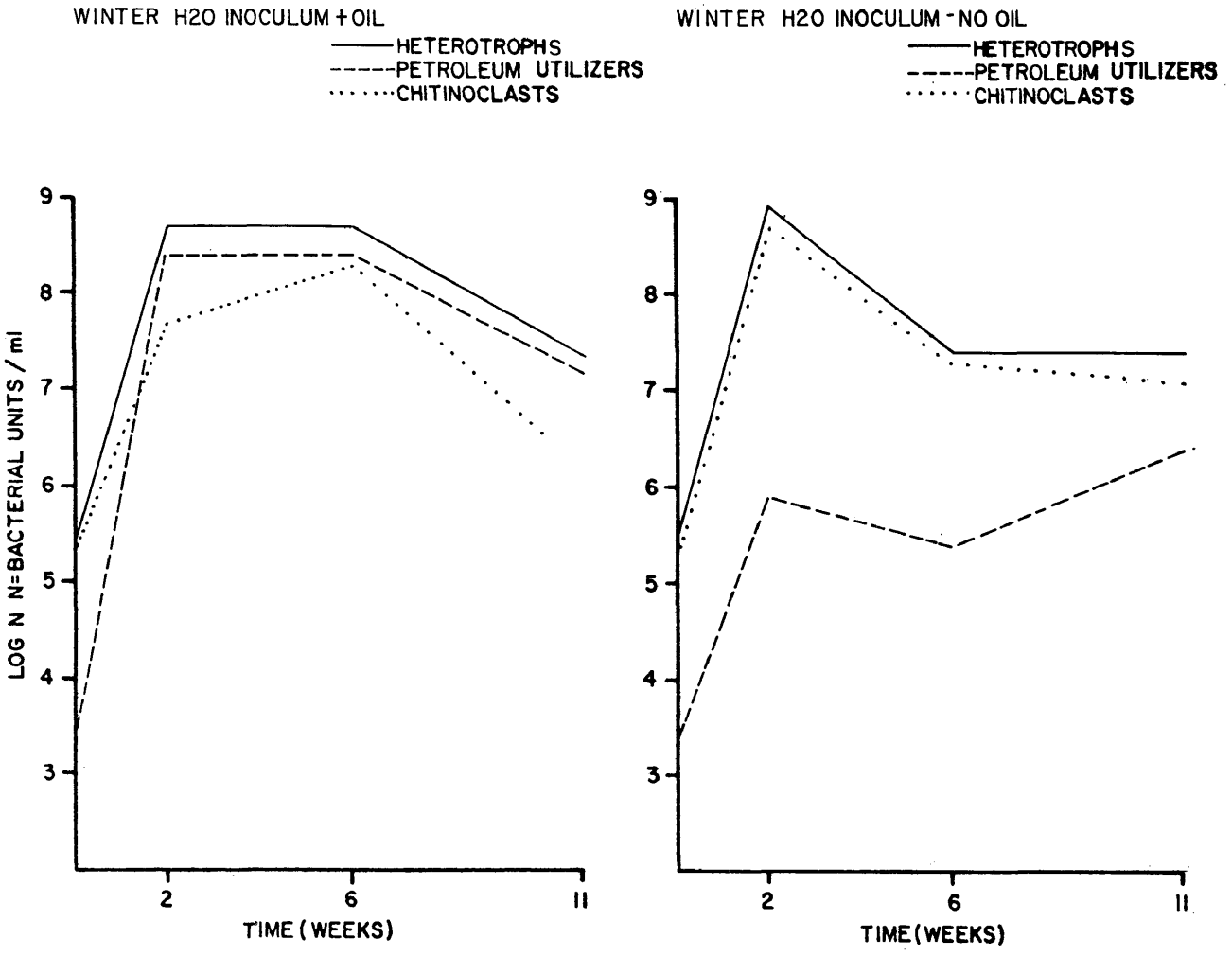


WINTER OUTER SHELF SEDIMENT INOCULUM

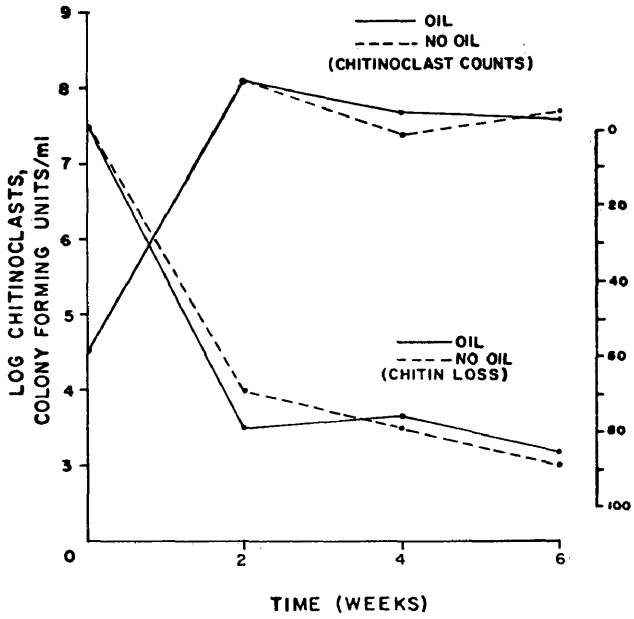


WINTER SHELF BREAK SEDIMENT INOCULUM

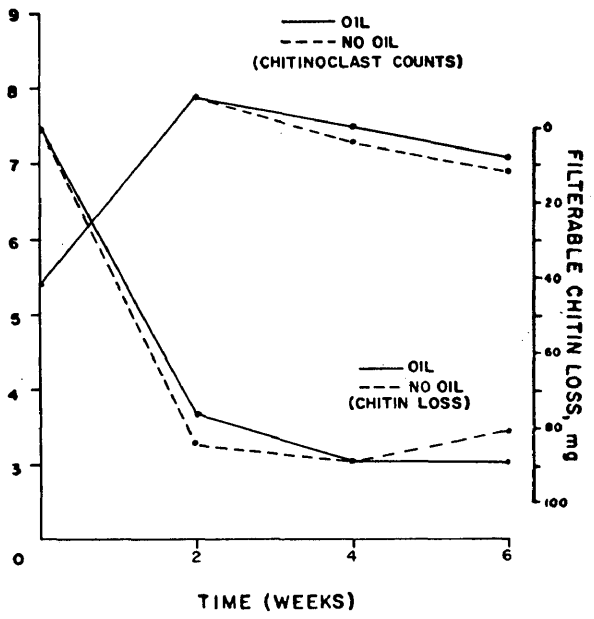



WINTER H2O INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS / ml
9
8
7
6
5
4
3
2
6
11
TIME (WEEKS)
WINTER H2O INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
9
8
7
6
5
4
3
2
6
11
TIME (WEEKS)

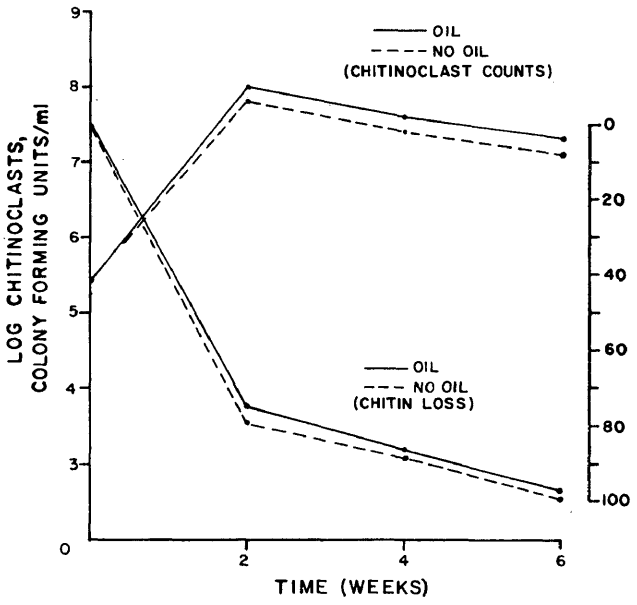
SPRING H2O INOCULUM



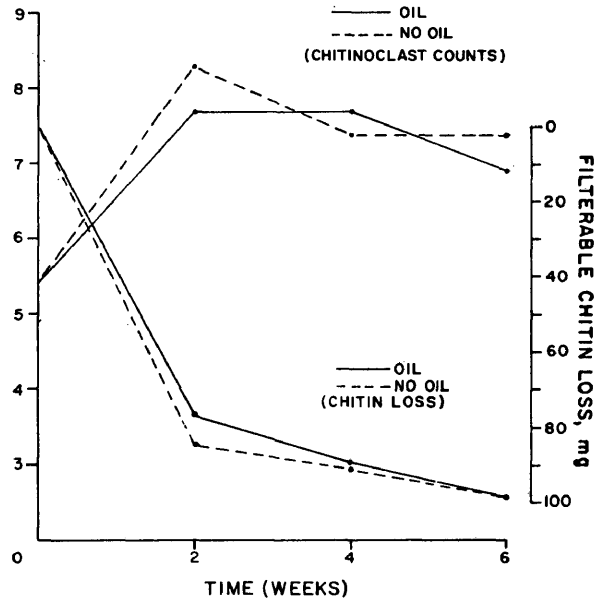
SPRING INNER SHELF SEDIMENT INOCULUM



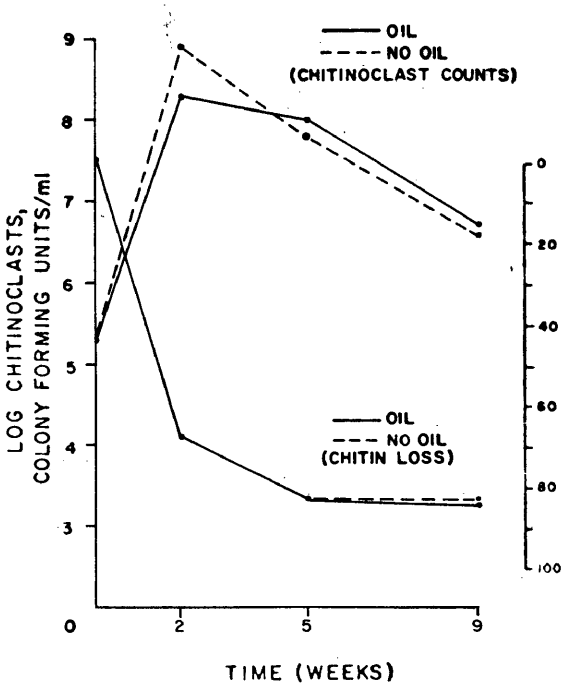
SPRING OUTER SHELF SEDIMENT INOCULUM



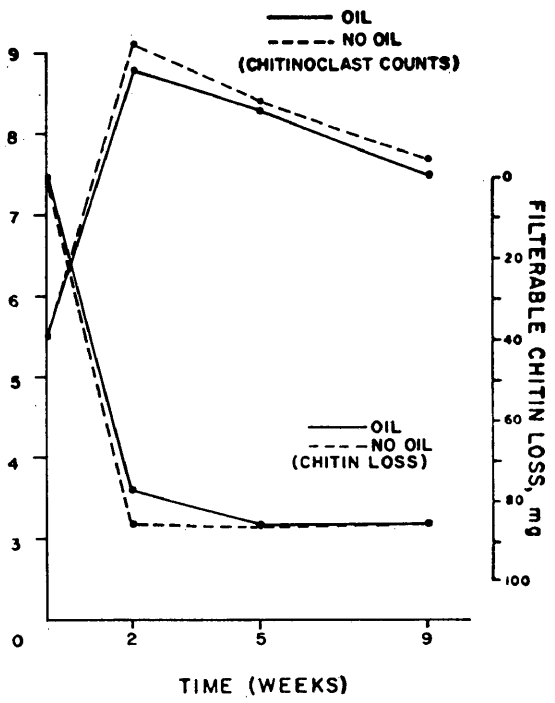
SPRING SHELF BREAK SEDIMENT INOCULUM



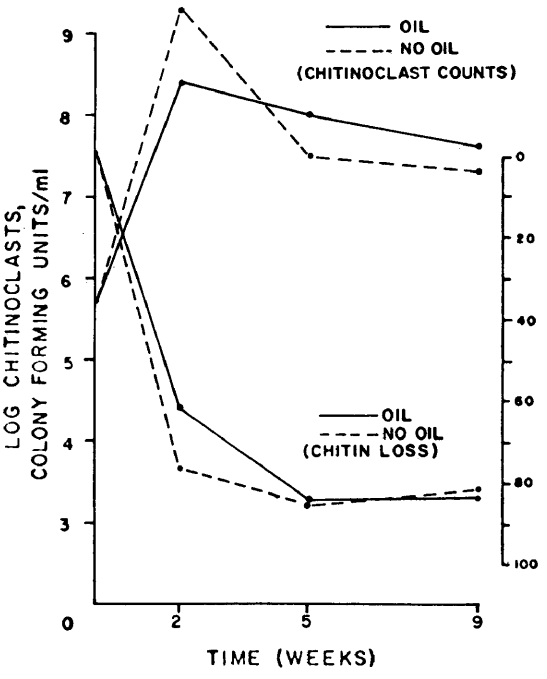
SUMMER H2O INOCULUM



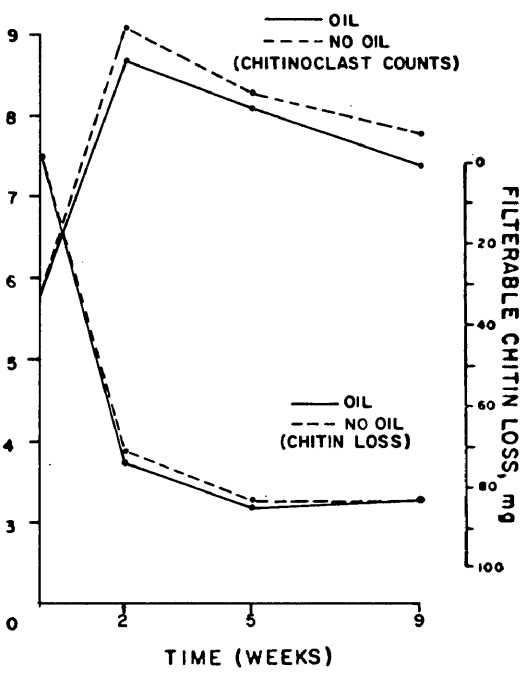
SUMMER INNER SHELF SEDIMENT INOCULUM



SUMMER OUTER SHELF SEDIMENT INOCULUM



SUMMER SHELF BREAK SEDIMENT INOCULUM



loss value was considered real. In the case of B2, chitin + enrichment (Figure 16), the % chitin loss value went from 51.9 at 9 wks to 11.7 at 17 wks. Since bacteria are known to clump and attach to particles, it is conceivable that this bottle received an inoculum deficient in chitinoclasts. This is supported by the fact that the dilution span normally used for chitinoclasts was too high in this case.

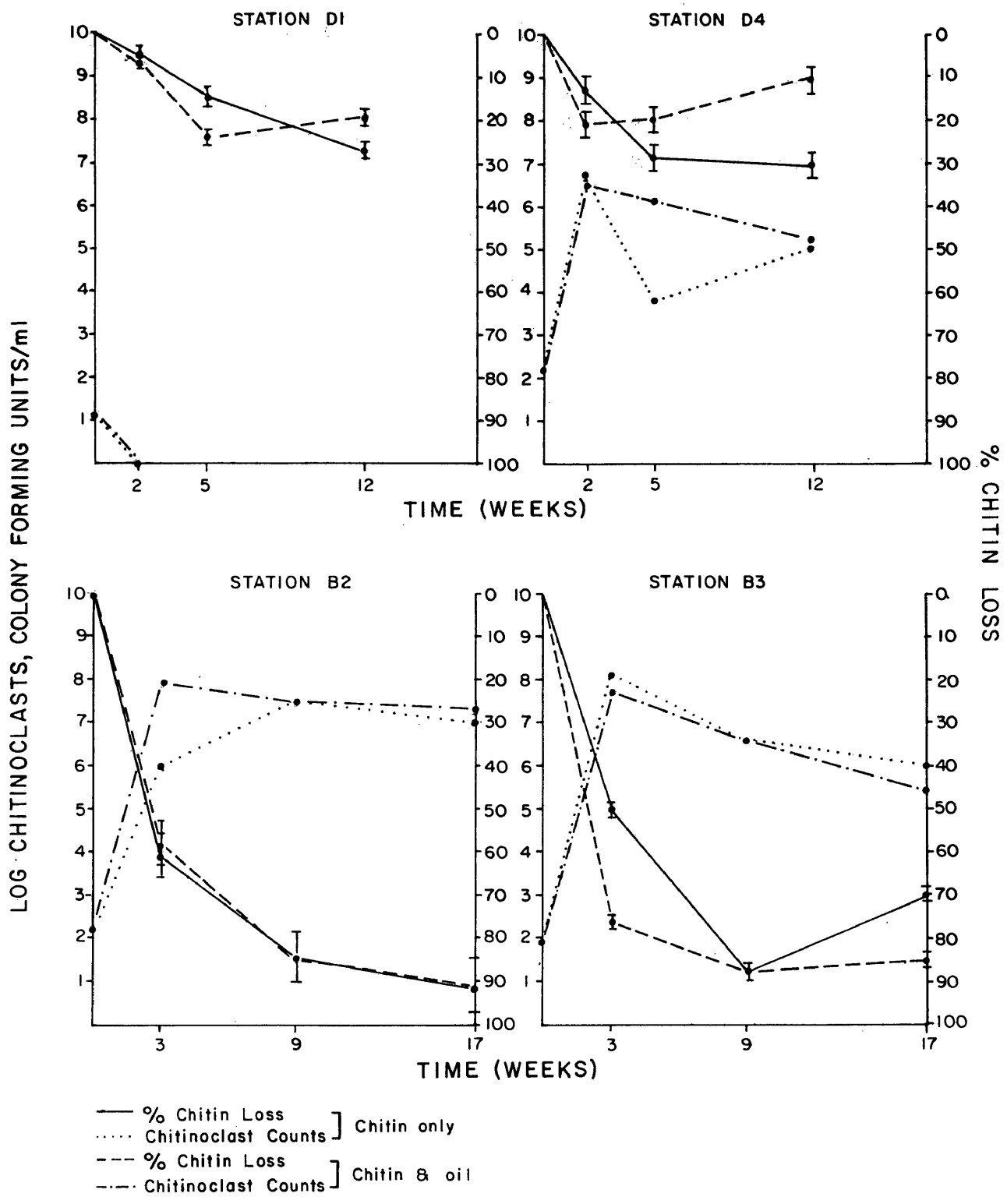
The D1 inoculum showed very little degradative activity for any of the treatments. Even though the total heterotroph population was substantial throughout the experiment, chitinoclast and petroleum utilizing subpopulations were very small or undetectable. Chitin degradation did appear to be somewhat reduced with oil and enrichment present.

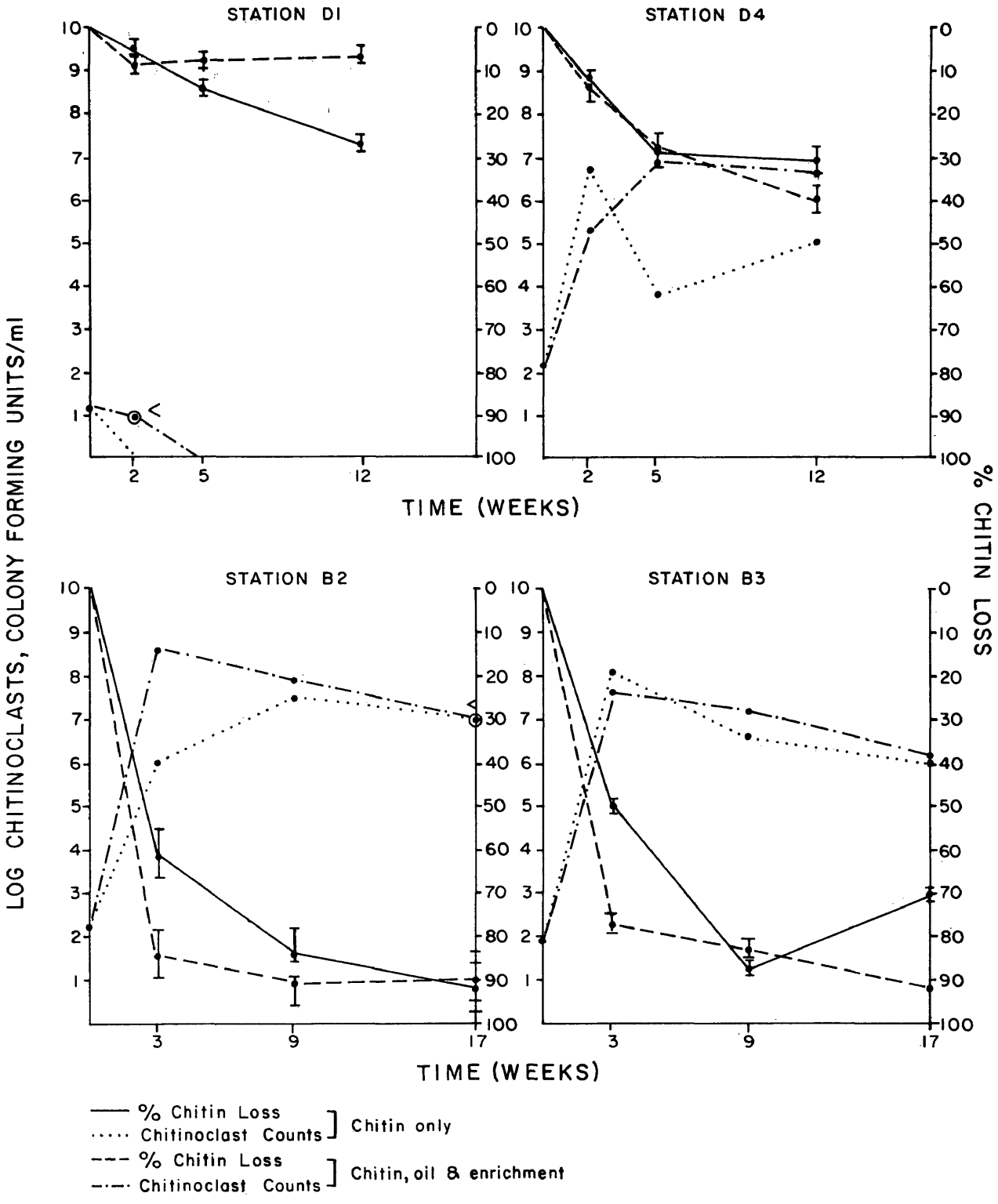
The D4 inoculum showed decreased degradation in the presence of oil, a slight increase with oil plus enrichment and a large increase with just enrichment. Enhanced chitin degradation due to enrichment is reflected by increased chitinoclast counts. For the other two treatments, the chitinoclast population was enhanced by the presence of oil even though chitin degradation was reduced or only slightly enhanced. Since many chitinoclasts were also able to use petroleum as a sole carbon source, it was possible in this case that the oil enhanced a population containing chitinoclasts which preferentially utilized petroleum. The chitinoclastic portion of the D4 inoculum could be supported for at least five weeks by oil alone.

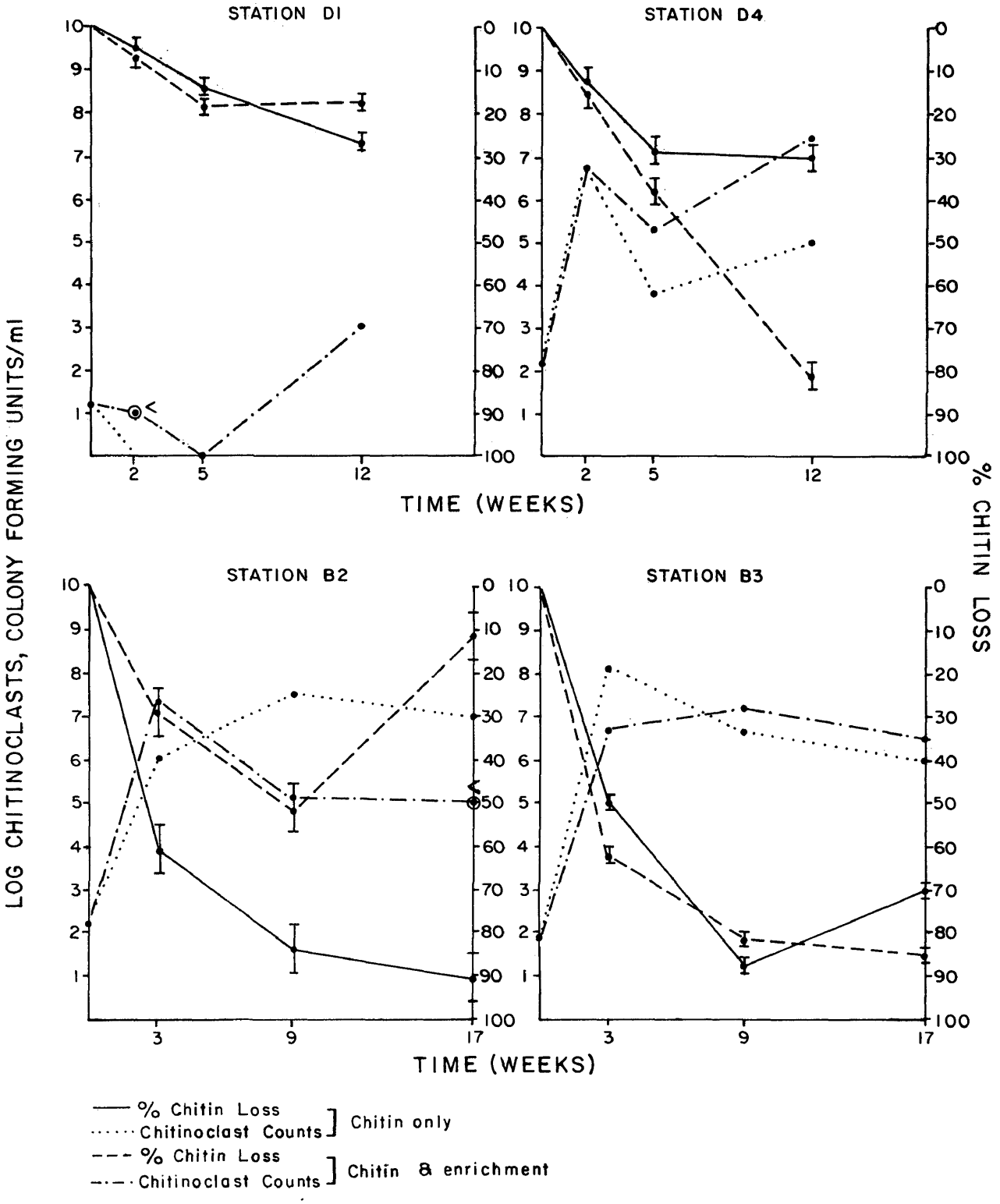
The B2 inoculum showed that oil had no effect on chitin degradation although the chitinoclast population at 3 wks was higher in the oil-treated flask. Oil plus enrichment initially enhanced the

Figures 14 - 16

Effects of oil alone, oil plus enrichment and enrichment alone on % chitin loss and chitinoclast counts in chitin-oil degradation experiments using sediment homogenate from the sources indicated.


STATION D1
STATION D4
STATION B2
STATION B3
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
% CHITIN LOSS
TIME (WEEKS)
TIME (WEEKS)
% Chitin Loss
Chitinoclast Counts
Chitin only
% Chitin Loss
Chitinoclast Counts
Chitin & oil


STATION D1
STATION D4
STATION B2
STATION B3
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
% CHITIN LOSS
TIME (WEEKS)
TIME (WEEKS)
% Chitin Loss
Chitinoclast Counts
Chitin only
% Chitin Loss
Chitinoclast Counts
Chitin, oil & enrichment


STATION D1
STATION D4
STATION B2
STATION B3
LOG CHITINOCLASTS, COLONY FORMING UNITS/ml
% CHITIN LOSS
TIME (WEEKS)
TIME (WEEKS)
% Chitin Loss
Chitinoclast Counts
Chitin only
% Chitin Loss
Chitinoclast Counts
Chitin & enrichment

chitinoclast population and resulted in increased chitin loss at 3 wks. By 9 wks, however, there was essentially no difference between treated and untreated flasks. Enrichment alone resulted in a reduced chitinoclast level and chitin loss.

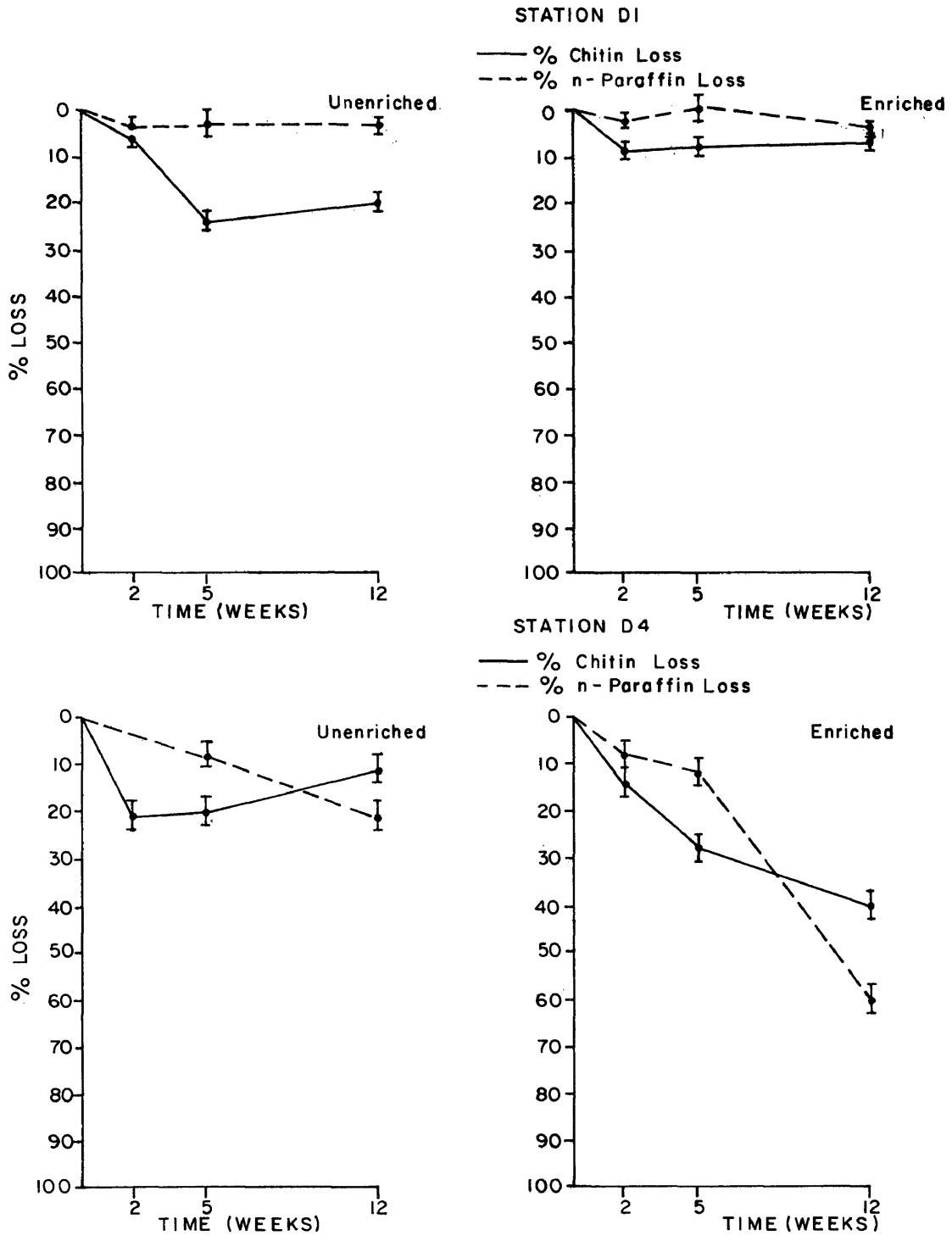
With B3, oil and oil plus enrichment resulted in greater chitin degradation at 3 wks, but by 9 wks there was no difference between treated and untreated flasks. Enrichment alone had a similar but less pronounced effect. For all three treatments, however, chitinoclast levels were either equivalent or lower in treated flasks than in untreated ones. It appeared the treatments increased the activity level i.e. enzyme production, of the existing population rather than increasing the number of chitinoclasts.

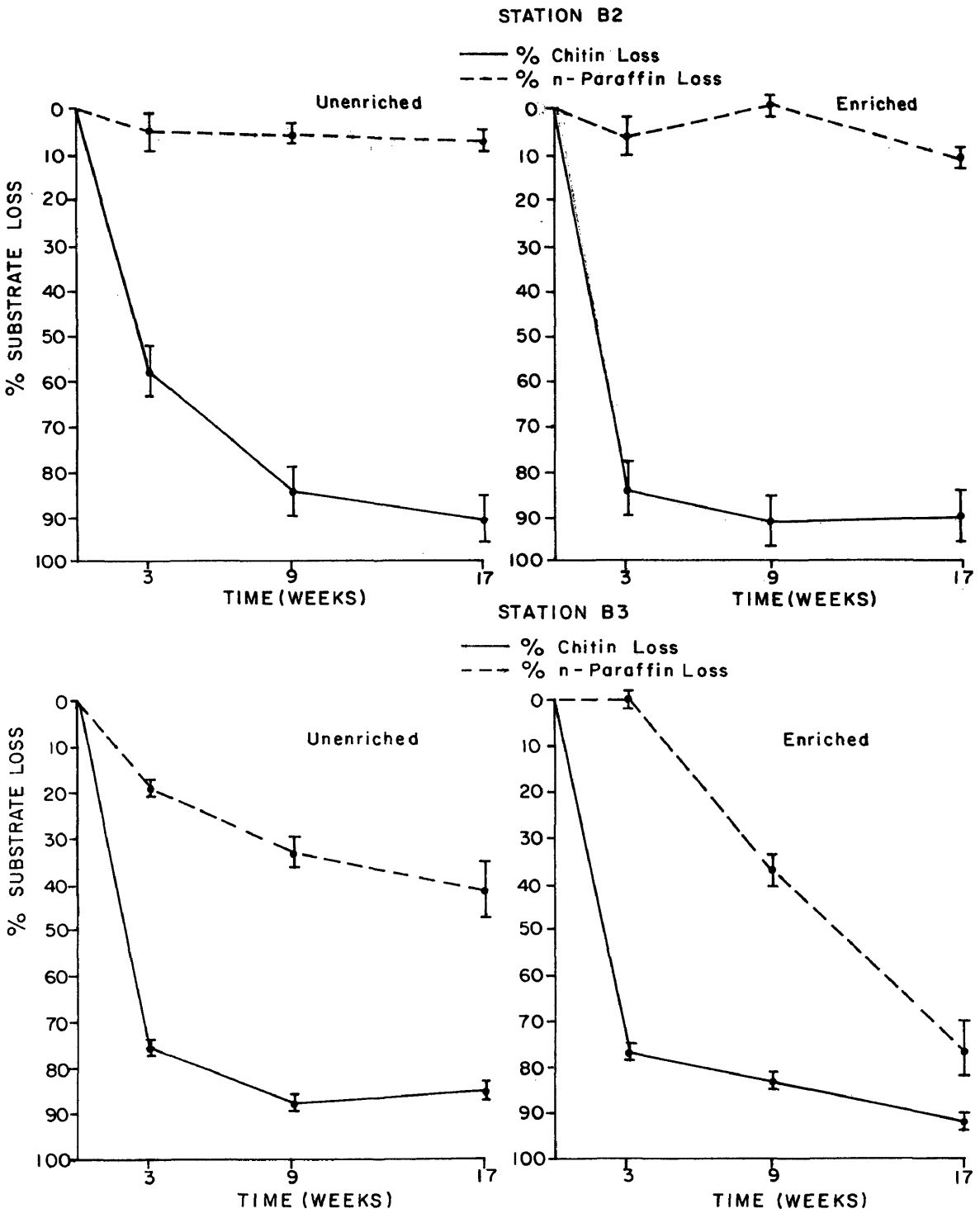
Changes in summation weights of crude oil n-paraffins for in-situ experiments are listed in Table B3 of Appendix B. Figures 17 and 18 illustrate the effects of nutrient enrichment on % chitin and % n-paraffin loss when both substrates were available. Although no n-paraffin loss was observed with D1, chitin use was slightly greater in the unenriched flask. Enrichment enhanced both chitin and n-paraffin use by the D4 inoculum. No n-paraffin losses occurred with the B2 inoculum and although chitin was initially utilized faster with enrichment, there was no significant difference in substrate loss at 17 wks. Enrichment did not affect chitin use by B3, but greatly enhanced petroleum use.

Figures 17 and 18 also show how temperature and the types of bacteria naturally occurring in sediments can affect substrate utilization. Stations D1 and B2 were located on ridges and such sediments were typically devoid of petroleum utilizers. For this reason, little

Figures 17 - 18

Per cent chitin and n-paraffin loss under enriched and unenriched conditions during closed flask incubation with sediment homogenate from the sources indicated.


STATION D1
% Chitin Loss
% n-Paraffin Loss
Unenriched
Enriched
% LOSS
0
10
20
30
40
50
60
70
80
90
100
2
5
12
TIME (WEEKS)
STATION D4
% Chitin Loss
% n-Paraffin Loss
Unenriched
Enriched
% LOSS
TIME (WEEKS)


STATION B2
% Chitin Loss
% n-Paraffin Loss
Unenriched
Enriched
% SUBSTRATE LOSS
0
10
20
30
40
50
60
70
80
90
100
3
9
17
TIME (WEEKS)
STATION B3
% Chitin Loss
% n-Paraffin Loss
Unenriched
Enriched
% SUBSTRATE LOSS
TIME (WEEKS)

petroleum degradation occurred in experiments employing inocula from these stations. D4 and B3 were trough stations and generally supported high levels of petroleum and chitin degrading bacteria. Degradation of both chitin and oil were lower at D4 relative to B3 because the D experiments were incubated at 5°C while the B experiments were incubated at 12°C.

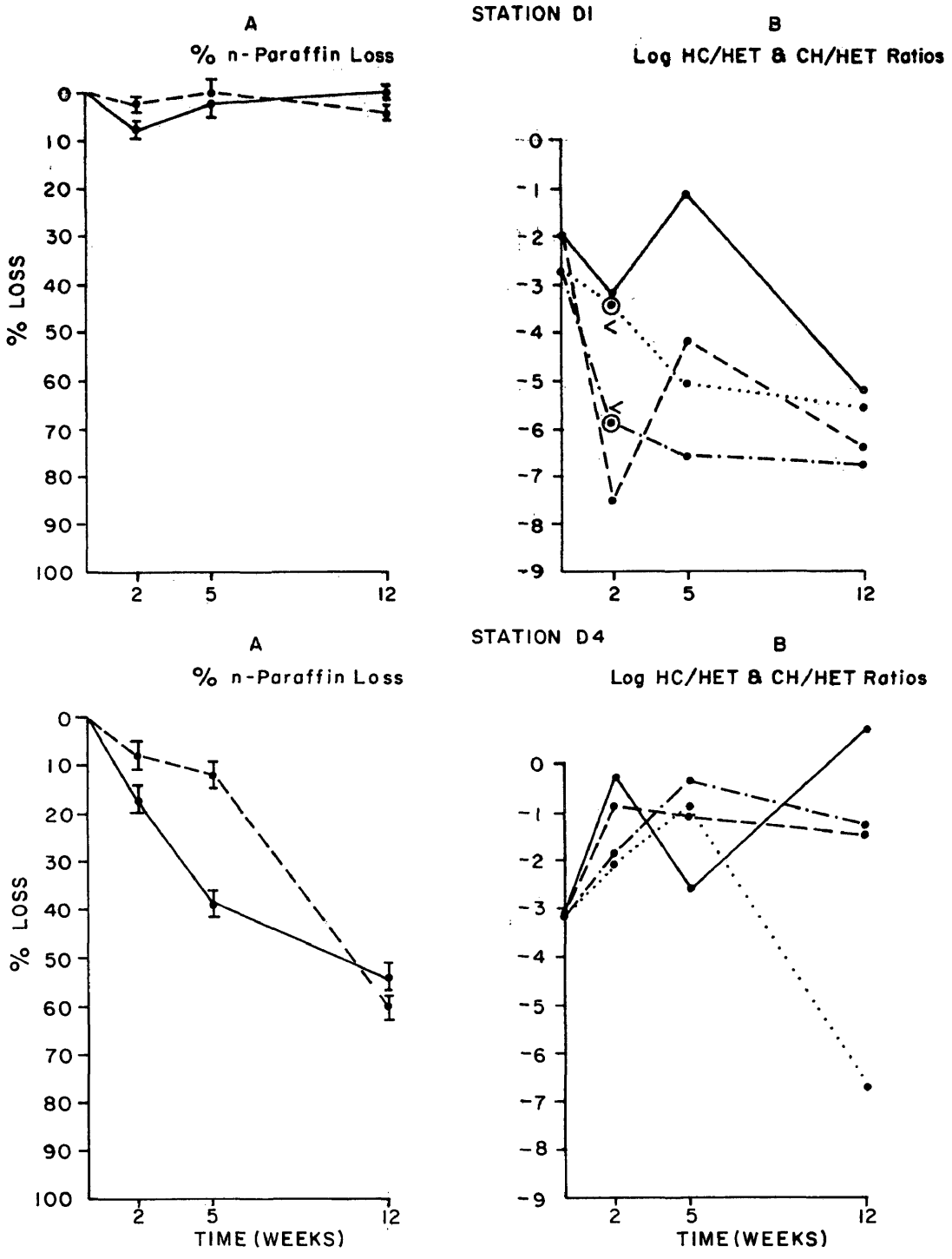
Figures 19 and 20 show how the presence of a second carbon source (chitin) influenced n-paraffin utilization when additional nutrients (nitrogen + phosphorus) were supplied. They also show ratios of petroleum-utilizers (HC) and chitinoclasts (CH) to total heterotrophs (HET). Station D1 showed no difference in n-paraffin loss due to chitin and station D4 showed a higher rate of utilization over the first five weeks without chitin, but by 12 wks there was no difference. B2 and B3 both showed increased n-paraffin loss in the absence of chitin, suggesting that chitin is the substrate preferred by these inocula. This is supported by data represented in Figure 15, previously discussed, which shows that chitin degradation by B2 and B3 inocula was actually enhanced by oil and enrichment. It appeared that oil and nutrients together acted to stimulate chitinoclast growth and chitin degradation and in doing so reduced the number of petroleum utilizers that actively degraded oil. Chitin was a metabolically preferred substrate for those bacteria which could utilize either petroleum or chitin.

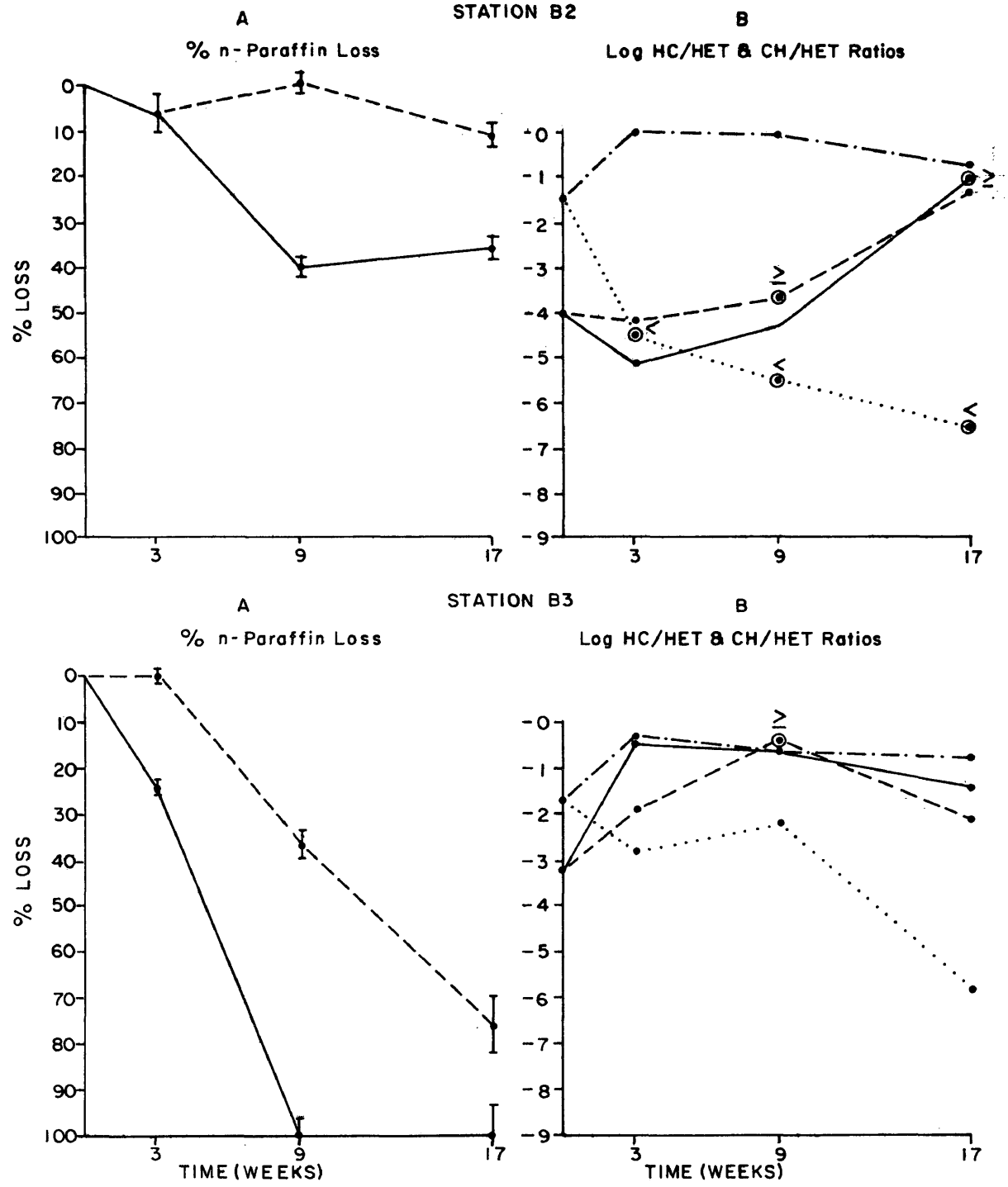
Representative chromatograms in Appendix D show a sterile control, approximately 50% degradation and 100% degradation of n-paraffins.

Figures 19 - 20

A. Per cent n-paraffin loss (nC_{12}-nC_{25}) with nutrient amendment and with (---) or without (——) chitin.

B. Ratio of petroleum utilizers (HC) and chitinoclasts (CH) to heterotrophs (HET) with nutrient amendment and with or without chitin. HC/HET w/o chitin (——); HC/HET with chitin (---); CH/HET w/o chitin (····); CH/HET with chitin (-·-).


STATION DI
A
% n-Paraffin Loss
% LOSS
0
10
20
30
40
50
60
70
80
90
100
2
5
12
B
Log HC/HET & CH/HET Ratios
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
2
5
12
STATION D4
A
% n-Paraffin Loss
% LOSS
0
10
20
30
40
50
60
70
80
90
100
2
5
12
TIME (WEEKS)
B
Log HC/HET & CH/HET Ratios
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
2
5
12
TIME (WEEKS)


STATION B2
A
% n-Paraffin Loss
B
Log HC/HET & CH/HET Ratios
% LOSS
0
10
20
30
40
50
60
70
80
90
100
3
9
17
-0
-1
-2
-3
-4
-5
-6
-7
-8
-9
STATION B3
A
% n-Paraffin Loss
B
Log HC/HET & CH/HET Ratios
% LOSS
TIME (WEEKS)
TIME (WEEKS)

DISCUSSION

Chitinoclastic bacteria were found in virtually every water and sediment sample examined and in many cases comprised a substantial portion of the total heterotrophic population. Viable counts of chitinoclasts in the water column varied directly with temperature, but sediment counts did not correlate with any single parameter. This is not surprising considering the complex interrelationships of physical and chemical variables affecting these organisms. The fact that chitinoclasts did not vary directly with the total heterotrophic population suggests that they may respond differently to certain environmental variables not measured such as the concentration of chitin in the sediment. In the water column, chitinoclasts were most likely associated with zooplankton populations which responded to phytoplankton populations. Uneven disposition of copepod carapaces in sediments would result in uneven sediment chitinoclast levels. Accumulation of chitinaceous material in troughs would explain the elevated counts at these stations.

Identification of sediment and water column chitinoclasts indicated the majority were Vibrio followed by Pseudomonas and Aeromonas. Pseudomonas isolates could be divided into three groups according to their ability to metabolize glucose. Difficulty in

differentiating Aeromonas from Vibrio was occasionally encountered, but the majority of isolates fell clearly under one of the three genera. Although marine bacterial taxonomy is still in a controversial and ever changing state, Shewan's scheme for gram negative marine bacteria is the most widely accepted one at present. The mortality rate was highest among pigmented forms and among isolates obtained during the winter. Fastidious forms may have been lost due to the isolation procedures employed. It is possible, therefore, that other chitinoclastic genera could be isolated from open ocean water and sediments if their special media needs were met. The results presented here are in agreement with those of Chan (1970) who conducted the most recent comparable taxonomic survey of chitinoclastic bacteria.

Pure culture chitin degradation experiments performed under high nutrient conditions revealed no change in chitinoclast growth or chitin degradation due to the presence of South Louisiana crude oil. These preliminary experiments held little relevance to a natural situation since the pure cultures consisted solely of chitinoclasts grown in a rich nutrient broth. It was possible that the chitin degrading potential of the inocula was maximized and the effects of oil on growth masked.

In an attempt to resolve these problems, the next set of pure culture experiments were modified. Mixed cultures consisted of both chitinoclasts and petroleum utilizers to better demonstrate "competitive heterotrophy" and to introduce petroleum degradation by-products into the system. Secondly, peptone and yeast extract levels were reduced to make chitin and oil the dominant carbon substrates available.

Most of these experiments yielded results similar to the first set of experiments in that no difference between oiled and unoiled bottles was observed. The winter experiments, however, showed significantly slower chitin degradation and lower chitinoclast counts during initial weeks of incubation in oil-treated flasks. Levels of petroleum degraders were consistantly greater (2-3 log units) in oil-treated flasks. Changes in levels of chitinoclasts and petroleum oxidizers suggested a diauxie-like growth phenomenon. Total heterotrophs were similar in treated and untreated flasks and appeared to have reached a maximum value within two weeks. In the presence of oil, the heterotroph population consisted of high levels of both chitinoclasts and petroleum-oxidizers, although petroleum-degraders were numerically dominant. In oil-free flasks chitinoclasts were clearly dominant. Similar patterns were seen with the spring shelf break and summer inner and outer shelf inocula.

For all experiments, the amount of chitin ultimately degraded was not significantly different between oil-treated and oil-free flasks. This suggests that although chitin degradation may have occurred more slowly in the presence of oil, neither South Louisiana crude oil nor its degradation by-products were toxic toward chitinoclasts under the experimental conditions employed. Walker et al. (1975) concluded that crude oil was toxic to chitinoclasts because the percentage of chitinoclasts to total heterotrophs was lower in the presence of oil even though the absolute values of chitinoclasts and heterotrophs were higher. It seems logical that when oil was introduced into the system, enhanced growth of petroleum oxidizers resulted in an increase in the total population. However, since chitin

was not provided as a substrate, chitinoclasts would not be expected to show a proportionate increase. Therefore if for the winter experiments, chitinoclast levels alone had been monitored for two weeks of incubation it could be concluded that petroleum inhibited chitinoclast growth when compared to oil-free flasks. However, by monitoring changes in total heterotrophs, chitinoclasts, petroleum utilizers and substrate utilization over a 2-3 month period, it was evident that petroleum was not toxic to chitinoclastic populations. It simply produced a shift in relative proportions of chitinoclastic and petroleum degrading bacteria in the total heterotrophic population.

The "in-situ" experiments were designed to more closely simulate natural conditions and each inoculum employed yielded a unique set of responses to the various treatments. For all three treatments (oil alone, oil + enrichment and enrichment alone), the responses ranged from reduced chitin degradation to enhanced chitin degradation. This illustrated the importance of inoculum source when evaluating the fate and effects of petroleum in the marine environment. With the exception of D4 (oil) and D1 (oil + enrichment), oil alone and with enrichment did not ultimately affect chitin degradation. In some cases, chitin degradation was greatly enhanced in oil-treated flasks during initial weeks of incubation. Although chitin utilization by D1 and D4 may have been slightly reduced, the chitinoclast populations were either unaffected or somewhat elevated throughout the experiment.

These experiments also demonstrated that nutrient amendment greatly enhanced degradation of n-paraffins while the presence of

chitin under enriched conditions tended to slow down n-paraffin loss indicating that chitin is the preferred carbon source. It was interesting to note, however, that under unenriched conditions, chitin could serve as a nutrient source and enhance n-paraffin degradation relative to flasks containing only oil. Therefore, depending on the availability of nutrients, chitin could either act as an alternate carbon source for certain versatile organisms or as a source of nitrogen for petroleum degraders lacking chitinase.

In conclusion, neither South Louisiana crude oil nor its degradation by-products appear to be detrimental to chitinoclastic bacterial populations from the sediments and waters of the Mid-Atlantic continental shelf region under a variety of experimental conditions.

APPENDIX A

Table A1 Change in levels (cells/ml) of bacterial populations and weights of filterable chitin in closed flask chitin-oil degradation experiments using mixed chitinoclast cultures prepared from isolates obtained during 1975-1976. Log counts are in parentheses.

Time	Cruise	Type	Heterotrophs	Chitinoclasts	Petroleum Degraders
Initial Enumeration	Fall	Sed	$>4.8\times10^{7}$ (>7.7)	2.6×10^{6} (6.4)	ID
	Winter	Sed	$>4.8\times10^{7}$ (>7.7)	3.2×10^{4} (4.5)	ID
		H_2O	$>4.8\times10^{7}$ (>7.7)	7.2×10^{5} (5.9)	ID
	Spring	Sed	$>4.8\times10^{7}$ (>7.7)	1.9×10^{6} (6.3)	ID
		H_2O	$>4.8\times10^{7}$ (>7.7)	1.5×10^{6} (6.2)	ID
	Summer	Sed	$>4.8\times10^{7}$ (>7.7)	4.8×10^{5} (5.7)	ID
		H_2O	$>4.8\times10^{7}$ (>7.7)	3.8×10^{6} (6.6)	ID
Two Week Enumeration	Fall	Sed + Oil	4.6×10^{8} (8.7)	4.3×10^{8} (8.6)	0.0
		Sed - Oil	4.6×10^{8} (8.7)	6.3×10^{8} (8.8)	0.0
	Winter	Sed + Oil	4.3×10^{7} (7.6)	1.2×10^{7} (7.1)	3.6×10^{1} (1.6)
		Sed - Oil	2.3×10^{7} (7.4)	5.9×10^{7} (7.8)	5.6×10^{1} (1.7)
		H_2O + Oil	9.3×10^{7} (8.0)	2.0×10^{8} (8.3)	0.0
		H_2O - Oil	9.3×10^{7} (8.0)	4.5×10^{7} (7.7)	0.0
	Spring	Sed + Oil	4.6×10^{8} (8.7)	1.1×10^{9} (9.0)	2.0×10^{2} (2.3)
		Sed - Oil	4.6×10^{8} (8.7)	5.1×10^{8} (8.7)	1.5×10^{2} (2.2)
		H_2O + Oil	$>2.4\times10^{9}$ (>9.4)	8.5×10^{8} (8.9)	1.1×10^{5} (5.0)
		H_2O - Oil	1.1×10^{9} (9.0)	4.7×10^{8} (8.7)	2.3×10^{2} (2.4)
	Summer	Sed + Oil	1.1×10^{9} (9.0)	4.8×10^{8} (8.7)	2.4×10^{5} (5.4)
		Sed - Oil	2.4×10^{8} (8.4)	2.2×10^{8} (8.3)	2.1×10^{3} (3.3)
		H_2O + Oil	4.6×10^{8} (8.7)	6.5×10^{8} (8.8)	2.1×10^{3} (3.3)
		H_2O - Oil	4.6×10^{8} (8.7)	2.3×10^{8} (8.4)	2.1×10^{3} (3.3)

Table A1 - (Continued)

Time	Cruise	Type	Heterotrophs	Chitinoclasts	Petroleum Degraders
Five Week Harvest	Fall	Sed + Oil	$1.5x10^7$ (7.2)	$3.8x10^6$ (6.6)	$9.6x10^1$ (2.0)
		Sed - Oil	$2.3x10^6$ (6.4)	$5.3x10^5$ (5.7)	0.0
	Winter	Sed + Oil	$4.3x10^5$ (5.6)	$<10^5$ (< 5.0)	$6.3x10^1$ (1.8)
		Sed - Oil	$2.3x10^5$ (5.4)	$<10^5$ (< 5.0)	0.0
		H_2O + Oil	$4.3x10^6$ (6.6)	$3.7x10^6$ (6.6)	$3.6x10^1$ (1.4)
		H_2O - Oil	$4.3x10^6$ (6.6)	$2.4x10^6$ (6.4)	0.0
	Spring	Sed + Oil	$2.3x10^6$ (6.4)	$2.0x10^6$ (6.3)	$3.6x10^1$ (1.4)
		Sed - Oil	$7.5x10^6$ (6.9)	$2.7x10^6$ (6.4)	$5.6x10^1$ (1.7)
		H_2O + Oil	$7.5x10^6$ (6.9)	$2.0x10^6$ (6.3)	$9.6x10^1$ (2.0)
		H_2O - Oil	$4.3x10^7$ (7.6)	$5.6x10^6$ (6.7)	$9.6x10^1$ (2.0)
	Summer	Sed + Oil	$2.3x10^7$ (7.4)	$1.5x10^7$ (7.2)	$2.8x10^1$ (1.4)
		Sed - Oil	$2.3x10^7$ (7.4)	$1.1x10^7$ (7.0)	$5.6x10^1$ (1.7)
		H_2O + Oil	$4.3x10^7$ (7.6)	$1.1x10^7$ (7.0)	$8.6x10^1$ (1.9)
		H_2O - Oil	$4.3x10^6$ (6.6)	$1.1x10^7$ (7.0)	$9.6x10^1$ (2.0)
Ten Week Harvest	Fall	Sed + Oil	$4.3x10^6$ (6.6)	$5.6x10^6$ (6.7)	0.0
		Sed - Oil	$9.3x10^6$ (7.0)	$3.2x10^6$ (6.5)	0.0
	Winter	Sed + Oil	$3.9x10^4$ (4.6)	$6.7x10^3$ (3.8)	2.3 (0.4)
		Sed - Oil	$9.3x10^4$ (5.0)	$5.4x10^4$ (4.7)	0.0
		H_2O + Oil	$2.3x10^6$ (6.4)	$2.8x10^5$ (5.4)	0.0
		H_2O - Oil	$1.5x10^6$ (6.2)	$3.3x10^5$ (5.5)	0.0
	Spring	Sed + Oil	$9.3x10^6$ (7.0)	$3.8x10^6$ (6.6)	0.0
		Sed - Oil	$1.5x10^7$ (7.2)	$6.5x10^6$ (6.8)	0.0
		H_2O + Oil	$4.3x10^6$ (6.6)	$3.9x10^6$ (6.6)	0.0
		H_2O - Oil	$4.3x10^7$ (7.6)	$4.1x10^6$ (6.6)	0.0
	Summer	Sed + Oil	$1.5x10^7$ (7.2)	$1.5x10^7$ (7.2)	0.0
		Sed - Oil	$9.3x10^6$ (7.0)	$4.2x10^6$ (6.6)	0.0
		H_2O + Oil	$4.3x10^7$ (7.6)	$4.0x10^6$ (6.6)	$> 2.4x10^4$ (> 4.4)
		H_2O - Oil	$2.3x10^7$ (7.4)	$1.1x10^7$ (7.0)	$> 2.4x10^4$ (> 4.4)

Table A1 - (Concluded)

Cruise		Incubation Interval	Filterable Chitin Loss, mg* + Oil	- Oil
Fall	Sed	2 wk	88.8	90.2
		5 wk	85.9	86.1
		10 wk	80.3	91.7
Winter	Sed	2 wk	77.3	90.0
		5 wk	61.4	86.6
		10 wk	46.6	88.3
	H_2O	2 wk	21.4	28.7
		5 wk	47.9	56.2
		10 wk	62.1	74.7
Spring	Sed	2 wk	59.6	64.3
		5 wk	87.7	92.0
		10 wk	87.7	89.6
	H_2O	2 wk	76.2	74.5
		5 wk	87.2	90.9
		10 wk	86.7	85.4
Summer	Sed	2 wk	88.2	88.7
		5 wk	86.4	93.9
		10 wk	87.4	92.4
	H_2O	2 wk	85.5	86.0
		5 wk	75.2	93.6
		10 wk	90.6	74.2

* Values corrected for processing error
Average of 6 controls = 98.6

Table A2 Changes in levels (cells/ml) of bacterial populations and weights of filterable chitin in closed flask chitin-oil degradation experiments using mixed cultures prepared from isolates obtained from sources indicated. Log counts in parentheses.

Season and Incubation Period	Inoculum Type	Heterotrophs	Chitinoclasts	Petroleum Oxidizers
FALL, 1976				
Initial	H_2O	1.6×10^6 (6.2)	8.6×10^5 (5.9)	3.0×10^4 (4.5)
	Inner Shelf Sed.	3.2×10^6 (6.5)	1.9×10^6 (6.3)	4.6×10^2 (2.7)
	Outer Shelf Sed.	2.1×10^6 (6.3)	2.1×10^6 (6.3)	4.6×10^4 (4.7)
	Shelf Break Sed.	2.0×10^6 (6.3)	1.8×10 (6.3)	4.6×10^4 (4.7)
2 wk	H_2O + Oil	2.7×10^8 (8.4)	1.3×10^8 (8.1)	$\geq 2.4 \times 10^8$ (≥ 8.4)
	H_2O - Oil	4.9×10^8 (8.7)	1.8×10^8 (8.1)	4.6×10^6 (6.6)
	Inner + Oil	9.7×10^8 (9.0)	8.3×10^8 (8.9)	$\geq 2.4 \times 10^8$ (≥ 8.4)
	Inner - Oil	1.0×10^9 (9.0)	1.2×10^9 (9.1)	9.3×10^6 (7.0)
	Outer + Oil	3.7×10^8 (8.6)	1.4×10^8 (8.2)	4.6×10^7 (7.7)
	Outer - Oil	2.1×10^8 (8.3)	1.8×10^8 (8.2)	9.3×10^6 (7.0)
	Shelf Break + Oil	3.1×10^8 (8.5)	3.0×10^8 (8.5)	4.6×10^7 (7.7)
	Shelf Break - Oil	1.2×10^9 (9.1)	6.5×10^8 (8.8)	2.4×10^7 (7.4)
5 wk	H_2O + Oil	1.2×10^8 (8.1)	1.6×10^8 (8.2)	2.4×10^7 (7.4)
	H_2O - Oil	1.4×10^8 (8.1)	9.6×10^7 (8.0)	4.6×10^7 (7.7)
	Inner + Oil	4.8×10^7 (7.7)	6.6×10^7 (7.8)	9.3×10^6 (7.0)
	Inner - Oil	7.1×10^7 (7.9)	9.2×10^7 (8.0)	2.4×10^7 (7.4)
	Outer + Oil	1.6×10^8 (8.2)	1.4×10^8 (8.2)	4.6×10^7 (7.7)
	Outer - Oil	1.6×10^8 (8.2)	1.0×10^8 (8.0)	2.4×10^7 (7.4)
	Shelf Break + Oil	5.8×10^7 (7.8)	5.1×10^7 (7.7)	4.3×10^6 (6.6)
	Shelf Break - Oil	1.1×10^8 (8.0)	3.0×10^7 (7.5)	2.4×10^7 (7.4)
11 wk	H_2O + Oil	2.4×10^7 (7.4)	2.3×10^7 (7.4)	9.3×10^5 (6.0)
	H_2O - Oil	8.0×10^7 (7.9)	4.5×10^7 (7.6)	9.3×10^7 (8.0)
	Inner + Oil	9.0×10^6 (7.0)	5.6×10^6 (6.7)	9.3×10^6 (7.0)
	Inner - Oil	3.5×10^7 (7.5)	2.5×10^7 (7.4)	2.3×10^7 (7.4)
	Outer + Oil	1.6×10^7 (7.2)	1.4×10^7 (7.1)	2.3×10^7 (7.4)
	Outer - Oil	4.1×10^7 (7.6)	2.6×10^7 (7.4)	9.3×10^7 (8.0)
	Shelf Break + Oil	2.7×10^7 (7.4)	1.1×10^7 (7.0)	9.3×10^7 (8.0)
	Shelf Break - Oil	5.2×10^7 (7.7)	3.3×10^7 (7.5)	4.3×10^7 (7.6)

Table A2 - (Continued)

Season and Incubation Period	Inoculum Type	Heterotrophs	Chitinoclasts	Petroleum Oxidizers
WINTER, 1977				
Initial	H_2O	2.6×10^5 (5.4)	2.0×10^5 (5.3)	1.9×10^3 (3.3)
	Inner Shelf Sed.	2.7×10^5 (5.4)	2.0×10^5 (5.3)	8.6×10^2 (2.9)
	Outer Shelf Sed.	2.0×10^5 (5.3)	6.0×10^4 (4.8)	4.6×10^2 (2.7)
	Shelf Break Sed.	2.9×10^5 (5.5)	9.0×10^4 (5.0)	4.2×10^2 (2.6)
2 wk	H_2O + Oil	5.1×10^8 (8.7)	4.7×10^7 (7.7)	2.4×10^8 (8.4)
	H_2O - Oil	8.5×10^8 (8.9)	4.8×10^8 (8.7)	7.5×10^5 (5.9)
	Inner + Oil	1.5×10^8 (8.2)	1.1×10^8 (8.0)	4.6×10^8 (8.7)
	Inner - Oil	7.1×10^8 (8.9)	4.9×10^8 (8.7)	4.3×10^5 (5.6)
	Outer + Oil	1.2×10^8 (8.1)	7.9×10^7 (7.9)	1.5×10^8 (8.2)
	Outer - Oil	6.3×10^8 (8.8)	4.2×10^8 (8.6)	9.3×10^5 (6.0)
	Shelf Break + Oil	7.1×10^8 (8.9)	2.8×10^8 (8.4)	1.1×10^9 (9.0)
	Shelf Break - Oil	6.7×10^8 (8.8)	3.8×10^8 (8.6)	7.5×10^5 (5.9)
6 wk	H_2O + Oil	5.4×10^8 (8.7)	1.8×10^8 (8.3)	2.4×10^8 (8.4)
	H_2O - Oil	2.7×10^7 (7.4)	2.2×10^7 (7.3)	2.3×10^5 (5.4)
	Inner + Oil	1.9×10^8 (8.3)	1.4×10^8 (8.1)	2.4×10^8 (8.4)
	Inner - Oil	6.7×10^7 (7.8)	6.9×10^7 (7.8)	4.3×10^6 (6.6)
	Outer + Oil	2.7×10^8 (8.4)	1.0×10^8 (8.0)	2.4×10^8 (8.4)
	Outer - Oil	7.6×10^7 (7.9)	6.0×10^7 (7.8)	9.3×10^6 (7.0)
	Shelf Break + Oil	8.7×10^8 (8.9)	3.1×10^8 (8.5)	4.6×10^8 (8.7)
	Shelf Break - Oil	2.2×10^7 (7.3)	1.9×10^7 (7.3)	2.3×10^6 (6.4)
11 wk	H_2O + Oil	2.6×10^7 (7.4)	1.5×10^6 (6.2)	1.5×10^7 (7.2)
	H_2O - Oil	2.3×10^7 (7.4)	1.3×10^7 (7.1)	2.3×10^6 (6.4)
	Inner + Oil	2.5×10^7 (7.4)	7.5×10^6 (6.9)	2.3×10^7 (7.4)
	Inner - Oil	1.1×10^7 (7.0)	8.6×10^6 (6.9)	2.3×10^6 (6.4)
	Outer + Oil	3.2×10^7 (7.5)	6.6×10^6 (6.8)	2.3×10^7 (7.4)
	Outer - Oil	1.6×10^7 (7.2)	9.5×10^6 (7.0)	4.3×10^6 (6.6)
	Shelf Break + Oil	2.6×10^7 (7.4)	8.8×10^6 (6.9)	9.3×10^6 (7.0)
	Shelf Break - Oil	1.2×10^7 (7.1)	8.0×10^6 (6.9)	9.3×10^6 (7.0)

Table A2 - (Continued)

Season and Incubation Period	Inoculum Type	Heterotrophs	Chitinoclasts	Petroleum Oxidizers
SPRING, 1977				
Initial	H_2O	2.6×10^4 (4.4)	3.4×10^4 (4.5)	.9 (-.04)
	Inner Shelf Sed.	2.8×10^5 (5.4)	2.4×10^5 (5.4)	.9 (-.04)
	Outer Shelf Sed.	3.2×10^5 (5.5)	2.7×10^5 (5.4)	4.2 (.6)
	Shelf Break Sed.	2.7×10^5 (5.4)	2.8×10^5 (5.4)	8.6 (.9)
2 wk	H_2O + Oil	1.2×10^9 (9.1)	1.4×10^8 (8.1)	1.1×10^9 (9.0)
	H_2O - Oil	1.2×10^8 (8.1)	1.3×10^8 (8.1)	4.3×10^6 (6.6)
	Inner + Oil	1.3×10^9 (9.1)	8.1×10^7 (7.9)	$\geq 2.4 \times 10^9$ ($\geq$9.4)
	Inner - Oil	8.4×10^7 (7.9)	8.4×10^7 (7.9)	1.5×10^7 (7.2)
	Outer + Oil	9.1×10^8 (9.0)	1.0×10^8 (8.0)	1.1×10^9 (9.0)
	Outer - Oil	7.4×10^7 (7.9)	6.2×10^7 (7.8)	9.3×10^6 (7.0)
	Shelf Break + Oil	1.1×10^9 (9.0)	4.9×10^7 (7.7)	$\geq 2.4 \times 10^9$ ($\geq$9.4)
	Shelf Break - Oil	2.5×10^8 (8.4)	2.1×10^8 (8.3)	7.5×10^6 (6.9)
4 wk	H_2O + Oil	3.6×10^8 (8.6)	5.6×10^7 (7.7)	2.1×10^8 (8.3)
	H_2O - Oil	3.2×10^7 (7.5)	2.3×10^7 (7.4)	9.3×10^6 (7.0)
	Inner + Oil	2.1×10^8 (8.3)	3.0×10^7 (7.5)	2.4×10^9 (9.4)
	Inner - Oil	5.2×10^7 (7.7)	1.8×10^7 (7.3)	4.6×10^7 (7.7)
	Outer + Oil	4.2×10^8 (8.6)	3.8×10^7 (7.6)	4.6×10^8 (8.7)
	Outer - Oil	6.0×10^7 (7.8)	2.3×10^7 (7.4)	1.5×10^7 (7.2)
	Shelf Break + Oil	3.3×10^8 (8.5)	4.7×10^7 (7.7)	2.4×10^8 (8.4)
	Shelf Break - Oil	5.4×10^7 (7.7)	2.7×10^7 (7.4)	2.4×10^7 (7.4)
6 wk	H_2O + Oil	1.2×10^8 (8.1)	3.7×10^7 (7.6)	4.3×10^7 (7.6)
	H_2O - Oil	8.3×10^7 (7.9)	4.8×10^7 (7.7)	1.5×10^7 (7.2)
	Inner + Oil	1.9×10^8 (8.3)	1.2×10^7 (7.1)	1.1×10^9 (9.0)
	Inner - Oil	4.3×10^7 (7.6)	7.4×10^6 (6.9)	2.4×10^7 (7.4)
	Outer + Oil	1.1×10^8 (8.0)	2.0×10^7 (7.3)	7.5×10^7 (7.9)
	Outer - Oil	4.6×10^7 (7.7)	1.4×10^7 (7.1)	9.3×10^6 (7.0)
	Shelf Break + Oil	7.8×10^7 (7.9)	7.8×10^6 (6.9)	9.3×10^7 (8.0)
	Shelf Break - Oil	5.3×10^7 (7.7)	2.8×10^7 (7.4)	4.6×10^7 (7.7)

Table A2 - (Continued)

Season and Incubation Period	Inoculum Type	Heterotrophs	Chitinoclasts	Petroleum Oxidizers
SUMMER, 1977				
Initial	H_2O	2.4×10^5 (5.4)	2.0×10^5 (5.3)	4.6×10^3 (3.7)
	Inner Shelf Sed.	3.0×10^5 (5.5)	3.0×10^5 (5.5)	8.6×10^2 (2.9)
	Outer Shelf Sed.	3.6×10^5 (5.6)	4.6×10^5 (5.7)	8.6×10^2 (2.9)
	Shelf Break Sed.	6.8×10^5 (5.8)	6.8×10^5 (5.8)	1.9×10^3 (3.3)
2 wk	H_2O + Oil	7.7×10^8 (8.9)	2.1×10^8 (8.3)	2.4×10^9 (9.4)
	H_2O - Oil	7.6×10^8 (8.9)	8.2×10^8 (8.9)	4.3×10^6 (6.6)
	Inner + Oil	1.6×10^9 (9.2)	7.0×10^8 (8.8)	9.3×10^8 (9.0)
	Inner - Oil	1.2×10^9 (9.1)	1.3×10^9 (9.1)	4.3×10^6 (6.6)
	Outer + Oil	5.6×10^8 (8.7)	2.7×10^8 (8.4)	4.3×10^8 (8.6)
	Outer - Oil	1.5×10^9 (9.2)	2.0×10^9 (9.3)	1.5×10^7 (7.2)
	Shelf Break + Oil	7.5×10^8 (8.9)	4.8×10^8 (8.7)	4.3×10^8 (8.6)
	Shelf Break - Oil	9.2×10^8 (9.0)	1.2×10^9 (9.1)	4.3×10^6 (6.6)
5 wk	H_2O + Oil	3.4×10^8 (8.5)	9.2×10^7 (8.0)	2.3×10^8 (8.4)
	H_2O - Oil	8.0×10^7 (7.9)	6.1×10^7 (7.8)	9.3×10^6 (7.0)
	Inner + Oil	3.5×10^8 (8.5)	2.2×10^8 (8.3)	2.9×10^8 (8.5)
	Inner - Oil	1.8×10^8 (8.3)	2.5×10^8 (8.4)	7.5×10^6 (6.9)
	Outer + Oil	2.4×10^8 (8.4)	1.1×10^8 (8.0)	2.3×10^8 (8.4)
	Outer - Oil	5.0×10^7 (7.7)	3.3×10^7 (7.5)	9.3×10^7 (8.0)
	Shelf Break + Oil	2.5×10^8 (8.4)	1.4×10^8 (8.1)	1.5×10^8 (8.2)
	Shelf Break - Oil	2.5×10^8 (8.4)	2.2×10^8 (8.3)	4.3×10^7 (7.6)
9 wk	H_2O + Oil	6.6×10^7 (7.8)	4.9×10^6 (6.7)	4.3×10^7 (7.6)
	H_2O - Oil	4.3×10^7 (7.6)	4.3×10^6 (6.6)	4.3×10^6 (6.6)
	Inner + Oil	9.1×10^7 (8.0)	3.2×10^7 (7.5)	4.3×10^7 (7.6)
	Inner - Oil	9.1×10^7 (8.0)	5.1×10^7 (7.7)	9.3×10^6 (7.0)
	Outer + Oil	8.9×10^7 (7.9)	4.0×10^7 (7.6)	4.3×10^7 (7.6)
	Outer - Oil	4.7×10^7 (7.7)	2.0×10^7 (7.3)	4.3×10^7 (7.6)
	Shelf Break + Oil	4.4×10^7 (7.6)	2.4×10^7 (7.4)	9.3×10^6 (7.0)
	Shelf Break - Oil	7.0×10^7 (7.8)	6.4×10^7 (7.8)	2.3×10^6 (6.4)

Table A2 - (Continued)

Filterable Chitin Loss, mg

FALL, 1976

	Incubation	+ Oil	- Oil		Incubation	+ Oil	- Oil
H_2O	2 wk	53.9	45.3	Outer Shelf	2 wk	69.6	70.5
	5 wk	74.8	79.3		5 wk	85.3	84.7
	11 wk	91.4	93.8		11 wk	94.8	95.0
Inner Shelf	2 wk	83.4	76.8	Shelf Break	2 wk	65.3	77.9
	5 wk	89.3	92.4		5 wk	90.0	89.3
	11 wk	98.0	97.2		11 wk	93.5	93.6

WINTER, 1977

	Incubation	+ Oil	- Oil		Incubation	+ Oil	- Oil
H_2O	2 wk	18.2	51.0	Outer Shelf	2 wk	20.3	41.8
	6 wk	63.2	75.9		6 wk	84.1	93.4
	11 wk	83.2	86.2		11 wk	87.9	84.2
Inner Shelf	2 wk	16.7	41.1	Shelf Break	2 wk	33.1	42.3
	6 wk	77.3	74.1		6 wk	80.0	89.9
	11 wk	89.3	80.7		11 wk	89.3	90.8

Table A2 - (Concluded)

Filterable Chitin Loss, mg

SPRING, 1977

	Incubation	+ Oil	- Oil		Incubation	+ Oil	- Oil
H_2O	2 wk	80.0	70.0	Outer Shelf	2 wk	74.4	79.0
	4 wk	76.9	80.0		4 wk	86.4	88.5
	6 wk	86.3	89.5		6 wk	96.8	99.2
Inner Shelf	2 wk	76.5	84.6	Shelf Break	2 wk	76.8	84.6
	4 wk	89.2	88.8		4 wk	89.1	90.9
	6 wk	89.7	81.0		6 wk	98.5	98.4

SUMMER, 1977

	Incubation	+ Oil	- Oil		Incubation	+ Oil	- Oil
H_2O	2 wk	67.9	68.1	Outer Shelf	2 wk	62.4	76.8
	5 wk	83.1	83.3		5 wk	84.5	85.7
	9 wk	84.8	83.7		9 wk	84.2	82.1
Inner Shelf	2 wk	77.9	86.2	Shelf Break	2 wk	75.4	72.5
	5 wk	86.2	86.6		5 wk	86.3	84.9
	9 wk	86.3	86.6		9 wk	84.4	84.8

Table A3 Change in levels (cells/ml) of bacterial populations and weights of filterable chitin in closed flask chitin-oil degradation experiments using sediment homogenate from the sources indicated. Log counts are in parentheses.
I = Inoculum only; IC = Inoculum + chitin; IO = Inoculum + oil; ICO = Inoculum + chitin + oil

Station D1			Heterotrophs		Chitinoclasts		Petroleum Utilizers	
Initial			8.0×10^{3}	(3.9)	1.5×10^{1}	(1.2)	8.6×10^{1}	(1.9)
Unenriched	2 wk	I	6.1×10^{5}	(5.8)	1.7×10^{5}	(5.2)	3.6×10^{-1}	(-4.4×10^{-1})
		IC	8.7×10^{7}	(7.9)	0.0	(0.0)	9.3×10^{0}	(9.6×10^{-1})
		IO	1.0×10^{6}	(6.0)	0.0	(0.0)	2.3×10^{0}	(3.6×10^{-1})
		ICO	5.4×10^{7}	(7.7)	0.0	(0.0)	2.3×10^{0}	(3.6×10^{-1})
	5 wk	I	2.6×10^{5}	(5.4)	0.0	(0.0)	2.3×10^{2}	(2.4)
		IC	2.8×10^{6}	(6.4)	0.0	(0.0)	0.0	(0.0)
		IO	6.4×10^{4}	(4.8)	0.0	(0.0)	1.2×10^{2}	(2.1)
		ICO	4.4×10^{5}	(5.6)	0.0	(0.0)	0.0	(0.0)
	12 wk	I	7.1×10^{4}	(4.9)	0.0	(0.0)	4.3×10^{2}	(2.6)
		IC	8.3×10^{6}	(6.9)	0.0	(0.0)	4.3×10^{0}	(6.3×10^{-1})
		IO	6.1×10^{5}	(5.8)	0.0	(0.0)	1.5×10^{3}	(3.2)
		ICO	3.9×10^{7}	(7.6)	0.0	(0.0)	0.0	(0.0)
Enriched	2 wk	I	6.3×10^{5}	(5.8)	0.0	(0.0)	2.3×10^{0}	(3.6×10^{-1})
		IC	7.5×10^{7}	(7.9)	$<1.0\times10^{2}$	(<2.0)	3.9×10^{4}	(4.6)
		IO	2.8×10^{5}	(5.4)	$<1.0\times10^{2}$	(<2.0)	1.5×10^{2}	(2.2)
		ICO	7.3×10^{7}	(7.9)	$<1.0\times10^{2}$	(<2.0)	2.3×10^{0}	(3.6×10^{-1})
	5 wk	I	2.2×10^{5}	(5.3)	0.0	(0.0)	2.3×10^{0}	(3.6×10^{-1})
		IC	1.6×10^{7}	(7.2)	0.0	(0.0)	0.0	(0.0)
		IO	1.4×10^{5}	(5.1)	0.0	(0.0)	9.3×10^{3}	(4.0)
		ICO	3.7×10^{6}	(6.6)	0.0	(0.0)	2.3×10^{2}	(2.4)
	12 wk	I	1.1×10^{5}	(5.0)	3.7×10^{2}	(2.6)	2.3×10^{3}	(3.4)
		IC	5.5×10^{6}	(6.7)	9.3×10^{2}	(3.0)	4.3×10^{0}	(6.3×10^{-1})
		IO	3.8×10^{5}	(5.6)	0.0	(0.0)	2.3×10^{0}	(3.6×10^{-1})
		ICO	6.2×10^{6}	(6.8)	0.0	(0.0)	2.3×10^{0}	(3.6×10^{-1})

Table A3 - (Continued)

Station D4			Heterotrophs		Chitinoclasts		Petroleum Utilizers	
Initial			2.4×10^5	(5.4)	1.6×10^2	(2.2)	1.9×10^2	(2.3)
Unenriched	2 wk	I	4.2×10^5	(5.6)	8.9×10^4	(4.9)	7.5×10^3	(3.9)
		IC	4.7×10^7	(7.7)	5.6×10^6	(6.7)	4.3×10^4	(4.6)
		IO	4.9×10^5	(5.7)	6.3×10^3	(3.8)	2.3×10^5	(5.4)
		ICO	1.0×10^8	(8.0)	3.2×10^6	(6.5)	$\geq 2.4 \times 10^7$	($\geq$7.4)
	5 wk	I	8.4×10^4	(4.9)	9.0×10^2	(3.0)	4.3×10^3	(3.6)
		IC	1.2×10^5	(5.1)	5.9×10^3	(3.8)	9.3×10^4	(5.0)
		IO	5.1×10^6	(6.7)	7.6×10^2	(2.9)	9.3×10^4	(5.0)
		ICO	6.9×10^6	(6.8)	1.2×10^6	(6.1)	9.3×10^4	(5.0)
	12 wk	I	1.1×10^4	(4.0)	0.0	(0.0)	1.5×10^4	(4.2)
		IC	4.0×10^6	(6.6)	1.1×10^5	(5.0)	7.5×10^5	(5.9)
		IO	2.7×10^5	(5.4)	0.0	(0.0)	4.3×10^6	(6.6)
		ICO	2.1×10^6	(6.3)	1.6×10^5	(5.2)	9.3×10^5	(6.0)
Enriched	2 wk	I	6.7×10^5	(5.8)	9.3×10^4	(5.0)	4.3×10^4	(4.6)
		IC	2.6×10^7	(7.4)	5.1×10^6	(6.7)	9.3×10^2	(3.0)
		IO	3.4×10^6	(6.5)	2.7×10^4	(4.4)	1.5×10^6	(6.2)
		ICO	1.6×10^7	(7.2)	2.1×10^5	(5.3)	2.1×10^6	(6.3)
	5 wk	I	6.9×10^4	(4.8)	2.4×10^3	(3.4)	9.3×10^3	(4.0)
		IC	1.5×10^6	(6.2)	1.8×10^5	(5.3)	2.3×10^6	(6.4)
		IO	6.1×10^6	(6.8)	8.2×10^5	(5.9)	1.5×10^4	(4.2)
		ICO	2.0×10^7	(7.3)	7.4×10^6	(6.9)	1.5×10^6	(6.2)
	12 wk	I	3.1×10^5	(5.5)	7.7×10^1	(1.9)	2.4×10^5	(5.4)
		IC	8.8×10^7	(7.9)	2.8×10^7	(7.4)	2.1×10^5	(5.3)
		IO	5.4×10^6	(6.7)	0.0	(0.0)	2.4×10^7	(7.4)
		ICO	8.2×10^7	(7.9)	3.8×10^6	(6.6)	2.3×10^6	(6.4)

Table A3 - (Continued)

Station B2			Heterotrophs		Chitinoclasts		Petroleum Utilizers	
Initial			5.6×10^{3}	(3.7)	1.6×10^{2}	(2.2)	4.6×10^{-1}	(-3.4×10^{-1})
Unenriched	3 wk	I	2.0×10^{5}	(5.3)	$\sim1.0\times10^{3}$	($\sim$3.0)	0.0	(0.0)
		IC	1.1×10^{8}	(8.0)	$\sim1.0\times10^{6}$	($\sim$6.0)	.3	(-.52)
		IO	4.4×10^{6}	(6.6)	$<1.0\times10^{2}$	(<2.0)	1.1×10^{6}	(6.0)
		ICO	5.2×10^{7}	(7.7)	8.0×10^{7}	(7.9)	3.5×10^{2}	(2.5)
	9 wk	I	5.7×10^{5}	(5.8)	2.6×10^{5}	(5.4)	3.9×10^{2}	(2.6)
		IC	4.5×10^{7}	(7.6)	3.4×10^{7}	(7.5)	$\geq 2.4\times10^{4}$	($\geq$4.4)
		IO	1.7×10^{6}	(6.2)	2.5×10^{3}	(3.4)	2.3×10^{4}	(4.4)
		ICO	8.0×10^{7}	(7.9)	3.3×10^{7}	(7.5)	$\geq 2.4\times10^{4}$	($\geq$4.4)
	17 wk	I	1.5×10^{5}	(5.2)	2.5×10^{2}	(2.4)	4.6×10^{3}	(3.7)
		IC	1.0×10^{8}	(8.0)	1.0×10^{7}	(7.0)	4.6×10^{6}	(6.7)
		IO	8.4×10^{5}	(5.9)	2.9×10^{2}	(2.5)	4.3×10^{2}	(2.6)
		ICO	$\sim2.0\times10^{7}$	($\sim$7.3)	$\sim2.0\times10^{7}$	($\sim$7.3)	1.1×10^{7}	(7.0)
Enriched	3 wk	I	4.6×10^{5}	(5.7)	$<1.0\times10^{2}$	(<2.0)	1.1×10^{5}	(5.0)
		IC	8.5×10^{7}	(7.9)	2.1×10^{7}	(7.3)	7.5	(.88)
		IO	2.9×10^{6}	(6.5)	$<1.0\times10^{2}$	(<2.0)	2.4×10^{1}	(1.4)
		ICO	4.7×10^{8}	(8.7)	4.2×10^{8}	(8.6)	2.9×10^{4}	(4.5)
	9 wk	I	1.4×10^{6}	(6.1)	$<1.0\times10^{1}$	(<1.0)	9.3×10^{2}	(3.0)
		IC	7.1×10^{7}	(7.9)	1.3×10^{5}	(5.1)	$\geq 2.4\times10^{4}$	($\geq$4.4)
		IO	3.2×10^{6}	(6.5)	$<1.0\times10^{1}$	(<1.0)	9.3×10^{1}	(2.0)
		ICO	1.1×10^{8}	(8.0)	8.6×10^{7}	(7.9)	$\geq 2.4\times10^{4}$	($\geq$4.4)
	17 wk	I	1.7×10^{5}	(5.2)	$<1.0\times10^{1}$	(<1.0)	4.3×10^{2}	(2.6)
		IC	2.7×10^{6}	(6.4)	$<1.0\times10^{5}$	(<5.0)	7.5×10^{4}	(4.9)
		IO	3.1×10^{7}	(7.5)	$<1.0\times10^{1}$	(<1.0)	$\geq 2.4\times10^{6}$	($\geq$6.4)
		ICO	6.5×10^{7}	(7.8)	$<1.0\times10^{7}$	(<7.0)	2.4×10^{6}	(6.4)

Table A3 - (Concluded)

FILTERABLE CHITIN LOSS *

	Chitin Only		Chitin + Nutrients		Chitin + Oil		Chitin + Oil + Nutrients	
	mg.	%	mg.	%	mg.	%	mg.	%
Station D1								
2 wk	5.4	5.2	7.8	7.4	6.6	6.3	9.6	9.2
5 wk	15.0	14.3	19.7	18.8	25.1	24.0	8.5	8.1
12 wk	28.4	27.1	18.9	18.0	20.8	19.8	7.8	7.4
Station D4								
2 wk	13.5	13.1	16.4	16.0	21.7	21.1	14.5	14.1
5 wk	29.9	29.1	39.8	38.7	20.7	20.1	28.8	28.0
12 wk	31.6	30.7	83.7	81.4	11.2	10.9	40.9	40.0
Station B2								
3 wk	61.0	61.0	29.6	29.6	58.1	58.1	83.9	83.9
9 wk	84.0	84.0	51.9	51.9	84.6	84.6	90.8	90.8
17 wk	91.1	91.1	11.7	11.7	90.9	90.9	89.7	89.7
Station B3								
3 wk	47.1	50.3	58.2	62.2	71.0	75.9	72.2	77.1
9 wk	81.9	87.5	76.3	81.5	82.6	88.2	78.0	83.3
17 wk	65.9	70.4	79.7	85.1	79.8	85.3	86.0	91.9

* Values corrected for processing error

APPENDIX B

Table B1 Change in summation weights (µg) of crude oil n-paraffins (nC_{12}-nC_{27}) and % loss of crude oil during incubation in closed containers in a chitin-peptone-seawater broth containing 0.1% (V/V) South Louisiana crude oil and mixed pure culture inocula derived from sources indicated. Control values are the means of three replicates.

Inoculum	Incubation Interval					
	2 Weeks		5 Weeks		10 Weeks	
	$\leq$ (µg)	% Loss	$\leq$ (µg)	% Loss	$\leq$ (µg)	% Loss
Fall Sed	1537	0.0	1463	0.0	1382	4.0
Winter Water	1540	0.0	1459	0.0	1310	9.0
Sed	1537	0.0	1502	0.0	1517	0.0
Spring Water	1593	0.0	1338	4.2	1570	7.1
Sed	1358	0.0	1422	0.0	1359	5.6
Summer Water	1520	0.0	ND	ND	1427	0.1
Sed	1440	4.6	1374	3.1	1310	9.0
Controls	$\bar{y}$ = 1508 S.D. = 19.9 S.D./$\bar{y}$ = 1.3%		$\bar{y}$ = 1417 S.D. = 26.9 S.D./ $\bar{y}$ = 1.9%		$\bar{y}$ = 1439 S.D. = 36.3 S.D./ $\bar{y}$ = 2.5%	

Table B2 Change in summation weights (µg) of crude oil n-paraffins (nC_{13}-nC_{25}) and % loss of crude oil during incubation in closed flasks with mixed cultures of chitinoclasts and hydrocarbon utilizers.

Inoculum	Σ (µg)	% Loss	Σ (µg)	% Loss	Σ (µg)	% Loss	
FALL	2 weeks		5 weeks		11 weeks		**
Control	1621.8		1753.7		1483.4		
Water	0.0	100.0	0.0	100.0	0.0	100.0	$\bar{y}$ = 1619.6
Inner Shelf	0.0	100.0	0.0	100.0	0.0	100.0	S.D. = 135.2
Outer Shelf	0.0	100.0	0.0	100.0	0.0	100.0	S.D./ $\bar{y}$ = 8.3%
Shelf Break	0.0	100.0	0.0	100.0	0.0	100.0	
WINTER	2 weeks		6 weeks		11 weeks		
Control	1307.1		1534.1		1261.2		** $\bar{y}$ = 1420.6
Water	0.0	100.0*	0.0	100.0*	0.0	100.0*	S.D. = 160.5
Inner Shelf	0.0	100.0*	0.0	100.0*	0.0	100.0*	S.D./ $\bar{y}$ = 11.3%
Outer Shelf	0.0	100.0*	0.0	100.0*	0.0	100.0*	
Shelf Break	0.0	100.0*	0.0	100.0*	0.0	100.0*	
SPRING	2 weeks		4 weeks		6 weeks		
Control	1616.7		1617.3		1785.0		** $\bar{y}$ = 1673.0
Water	0.0	100.0*	0.0	100.0*	0.0	100.0*	S.D. = 97.0
Inner Shelf	0.0	100.0*	0.0	100.0*	0.0	100.0*	S.D./ $\bar{y}$ = 5.8%
Outer Shelf	0.0	100.0*	0.0	100.0*	0.0	100.0*	
Shelf Break	0.0	100.0*	0.0	100.0*	0.0	100.0*	
SUMMER	2 weeks		5 weeks		9 weeks		
Control	1777.8		1615.8		1729.2		** $\bar{y}$ = 1707.6
Water	0.0	100.0*	0.0	100.0*	0.0	100.0*	S.D/ $\bar{y}$ = 4.9%
Inner Shelf	0.0	100.0*	0.0	100.0*	0.0	100.0*	S.D. = 83.1
Outer Shelf	0.0	100.0*	0.0	100.0*	0.0	100.0*	
Shelf Break	0.0	100.0*	0.0	100.0*	0.0	100.0*	

* Pristane, phytane and other unidentified branched compounds also degraded.
** Mean control value for entire incubation period.

Table B3 Change in summation weights (µg) of crude oil n-paraffins (nC_{12}-nC_{25}) and % loss of crude oil during incubation under various treatments with sediment homogenate inocula from the sources indicated. Inoc = no enrichment; Enr = with enrichment; IO = Inoculum + oil; ICO = Inoculum + chitin + oil.

Station D1

	Treatment	ΣnC_{12}-nC_{25}	Loss (µg)	% Loss	Controls	$\bar{X}$	S.D.	SD/ $\bar{x}$
2 wk	Inoc IO	2105.3	0.0	0.0	2109.7	2095.5	30.5	1.4%
	Enr IO	1939.2	156.3	7.5	2060.5			
	Inoc ICO	2010.2	85.3	4.0	2116.4			
	Enr ICO	2030.8	56.7	2.7				
5 wk	Inoc IO	2059.2	74.6	3.5	2066.2	2133.8	65.6	3.0%
	Enr IO	2088.9	44.9	2.1	2197.2			
	Inoc ICO	2066.2	67.6	3.2	2137.9			
	Enr ICO	2219.5	0.0	0.0				
12 wk	Inoc IO	2160.5	0.0	0.0	2123.0	2107.2	27.9	1.3%
	Enr IO	2218.6	0.0	0.0	2075.0			
	Inoc ICO	2030.0	77.2	3.7	2123.7			
	Enr ICO	2023.6	83.6	4.0				

Table B3 - (Continued)

Station D4

	Treatment	ΣnC_{12}-nC_{25}	Loss (µg)	% Loss	Controls	$\bar{X}$	S.D.	SD/ $\bar{x}$
2 wk	Inoc IO	2097.7	0.0	0.0	2039.5	2013.0	59.0	2.9%
	Enr IO	1673.4	339.6	16.9	2054.1			
	Inoc ICO	N.D.	N.D.	N.D.	1945.4			
	Enr ICO	1851.5	161.5	8.0				
5 wk	Inoc IO	1984.7	32.8	1.6	2066.0	2017.5	50.3	2.5%
	Enr IO	1232.9	784.6	38.9	2020.9			
	Inoc ICO	1850.2	167.3	8.3	1965.6			
	Enr ICO	1776.4	241.1	11.9				
12 wk	Inoc IO	2078.6	0.0	0.0	2011.8	2052.5	63.7	3.1%
	Enr IO	933.8	1118.7	54.5	2125.9			
	Inoc ICO	1614.6	437.9	21.3	2019.7			
	Enr ICO	821.6	1230.9	60.0				

Table B3 - (Continued)

Station B2

	Treatment	ΣnC_{12}-nC_{25}	Loss (µg)	% Loss	Controls	$\bar{X}$	S.D.	SD/ $\bar{x}$
3 wk	Inoc IO	2113.3	30.6	1.4	2060.2	2143.9	87.4	4.1%
	Enr IO	2003.9	140.0	6.5	2136.9			
	Inoc ICO	2025.4	118.5	5.5	2234.5			
	Enr ICO	2004.7	139.2	6.5				
9 wk	Inoc IO	1838.6	145.5	7.3	1935.6	1984.1	42.5	2.1%
	Enr IO	1175.4	808.7	40.8	2015.1			
	Inoc ICO	1865.8	118.3	6.0	2001.6			
	Enr ICO	1989.7	0.0	0.0				
17 wk	Inoc IO	1995.8	95.1	4.5	2078.3	2090.9	46.3	2.2%
	Enr IO	1329.9	761.0	36.4	2052.2			
	Inoc ICO	1934.8	156.1	7.5	2142.8			
	Enr ICO	1846.6	244.3	11.7				

Table B3 - (Concluded)

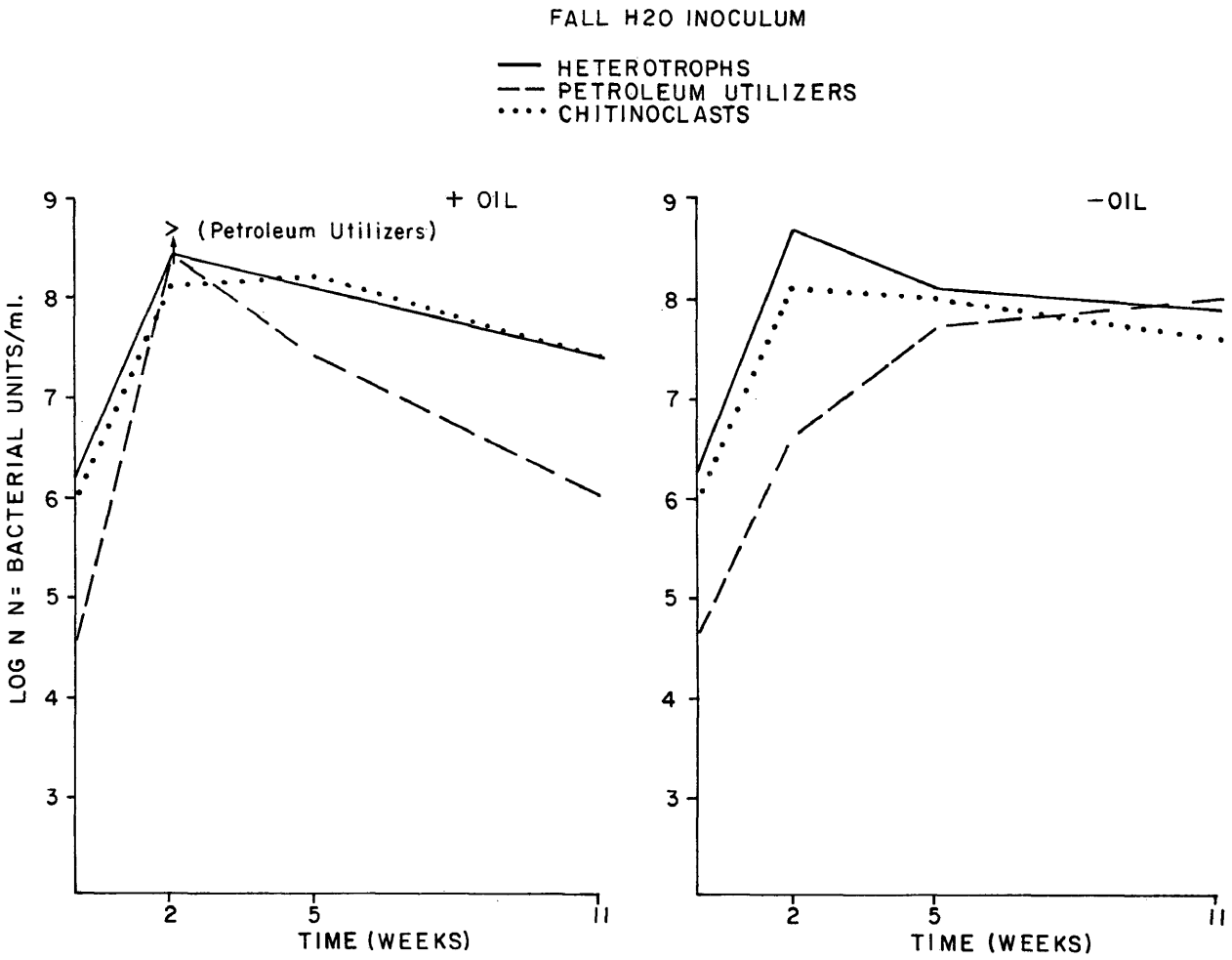
Station B3

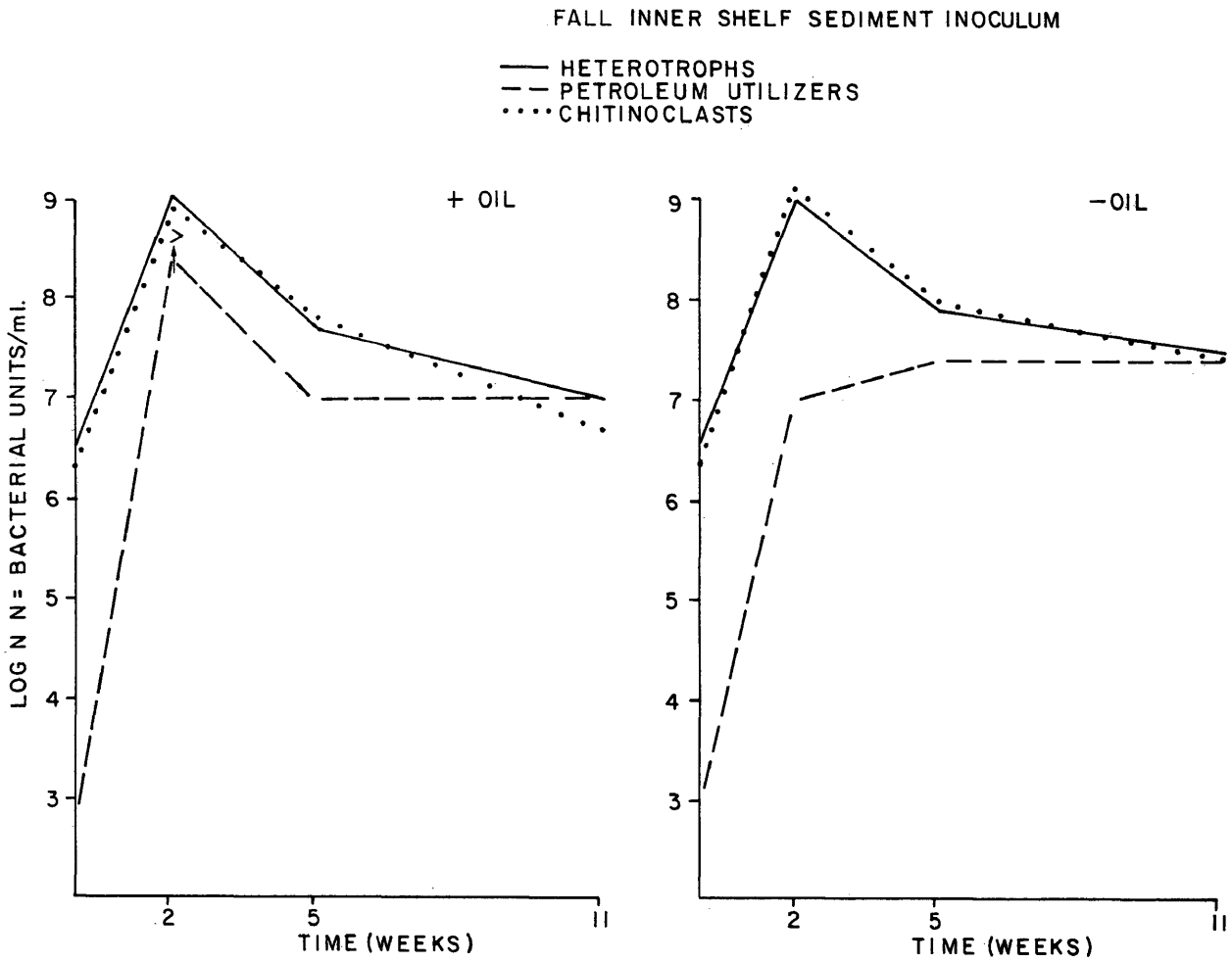
	Treatment	ΣnC_{12}-nC_{25}	Loss (µg)	% Loss	Controls	$\bar{X}$	S.D.	SD/ $\bar{x}$
3 wk	Inoc IO	1816.7	245.9	11.9	2060.6	2062.6	27.9	1.4%
	Enr IO	1556.7	505.9	24.5	2091.6			
	Inoc ICO	1662.5	400.1	19.4	2035.9			
	Enr ICC	2142.0	0.0	0.0				
9 wk	Inoc IO	1855.2	216.7	10.5	2139.7	2071.9	66.8	3.2%
	Enr IO	0.0	2071.9	100.0	2006.2			
	Inoc ICO	1373.5	698.4	33.7	2069.9			
	Enr ICO	1303.0	768.9	37.1				
17 wk	Inoc IO	1940.3	38.1	1.9	2102.5	1978.4	119.1	6.0%
	Enr IC	0.0*	1978.4	100.0	1967.7			
	Inoc ICO	1150.4	828.0	41.9	1865.0			
	Enr ICO	463.1	1515.3	76.6				

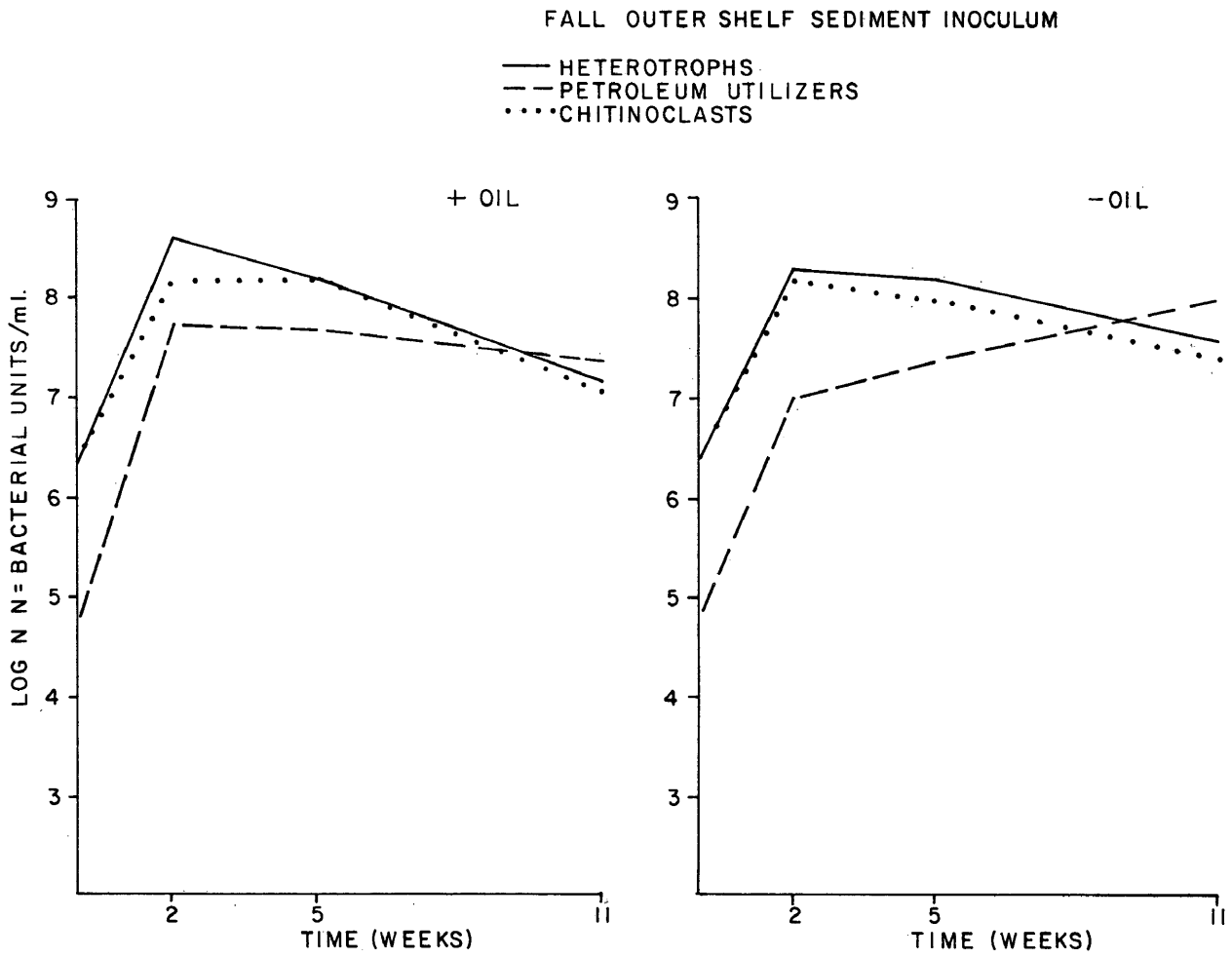
* Phytane/Pristane = 69.0%

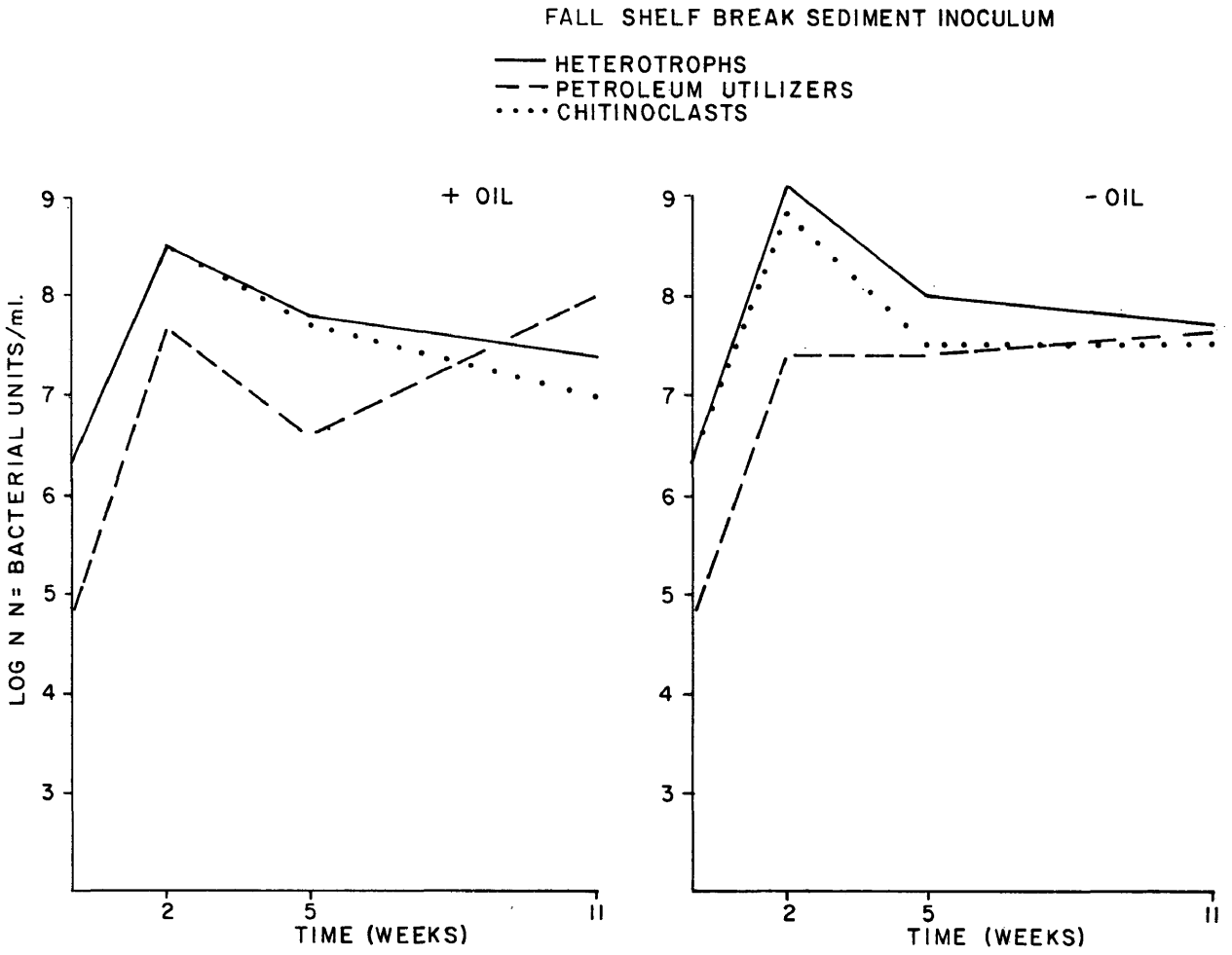
APPENDIX C

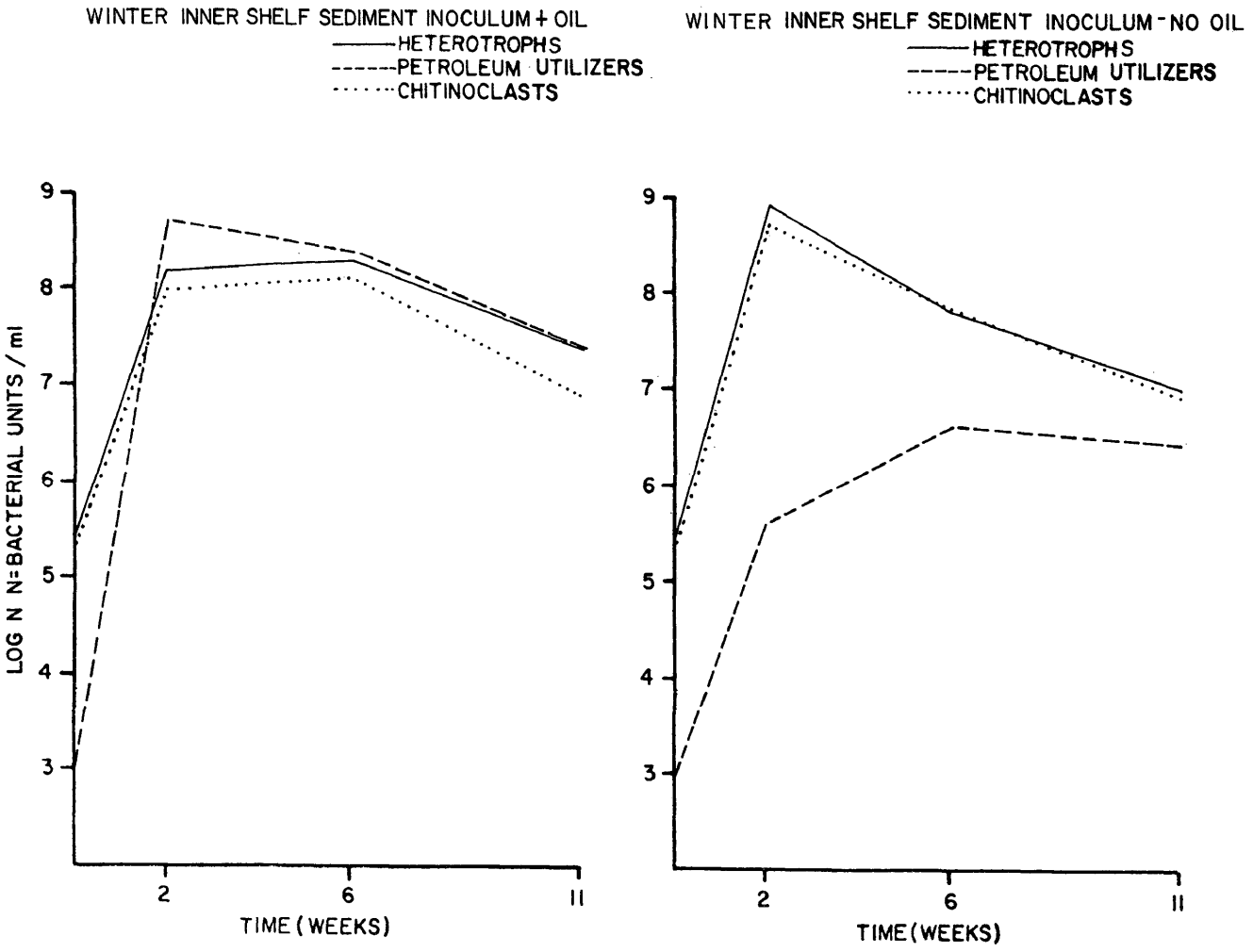
Figures representing viable counts of heterotrophic, chitinoclastic and petroleum degrading marine bacteria in closed flask chitin-oil degradation experiments using mixed cultures of chitinoclasts and hydrocarbon utilizers.

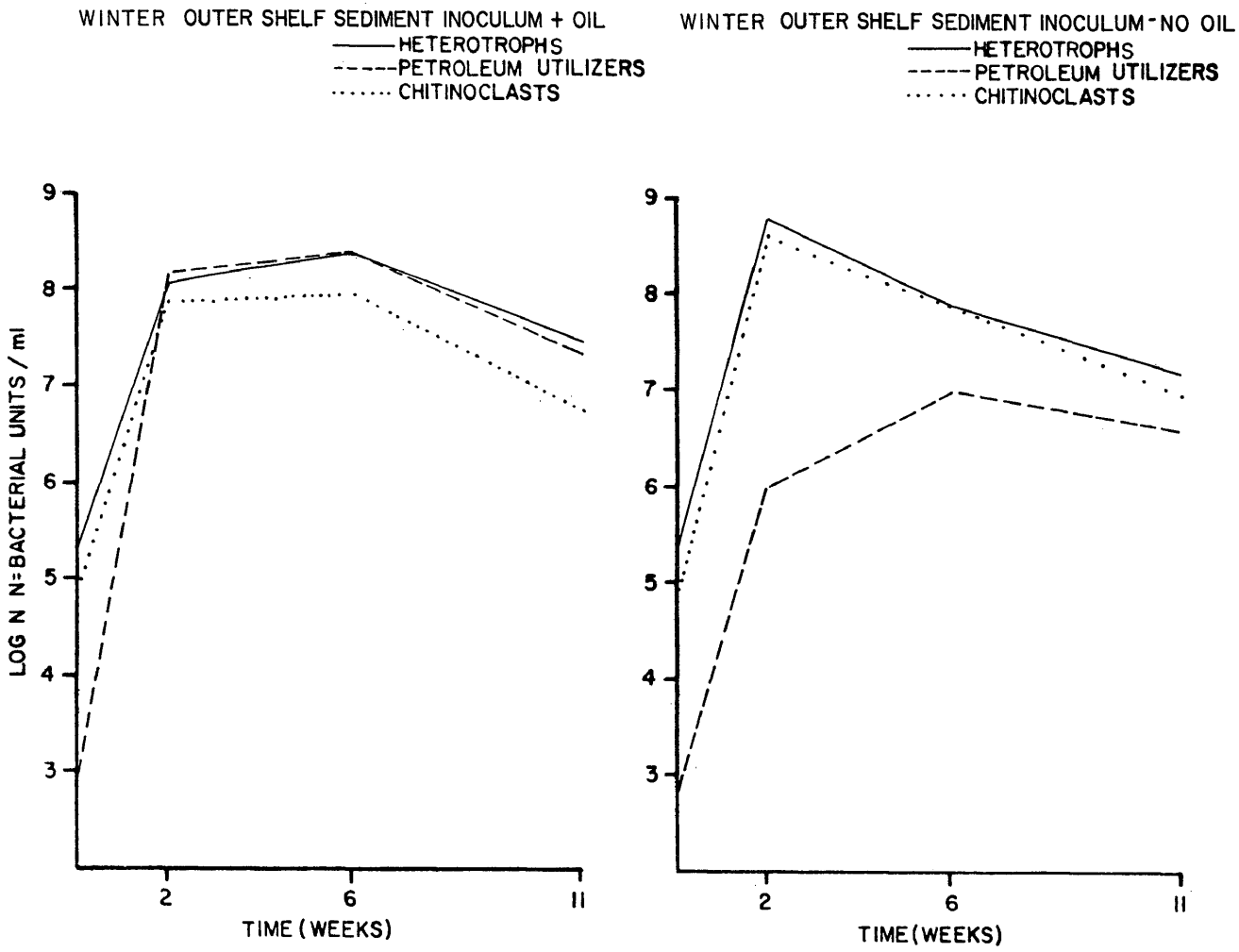

FALL H2O INOCULUM
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
+ OIL
(Petroleum Utilizers)
-OIL
LOG N N= BACTERIAL UNITS/ml.
9
8
7
6
5
4
3
2
5
11
TIME (WEEKS)

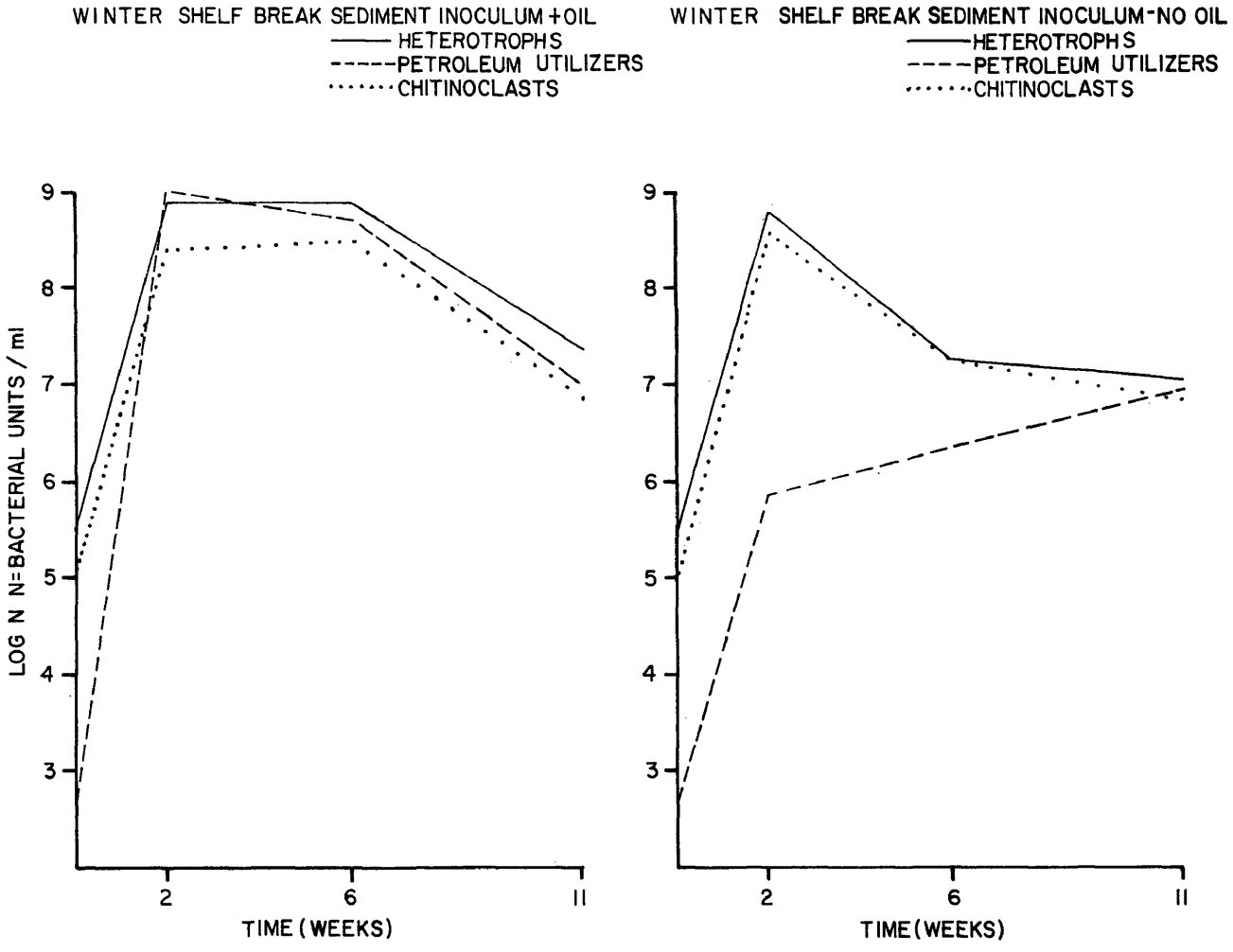

FALL INNER SHELF SEDIMENT INOCULUM
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
+ OIL
−OIL
LOG N N= BACTERIAL UNITS/ml.
9
8
7
6
5
4
3
2
5
11
TIME (WEEKS)

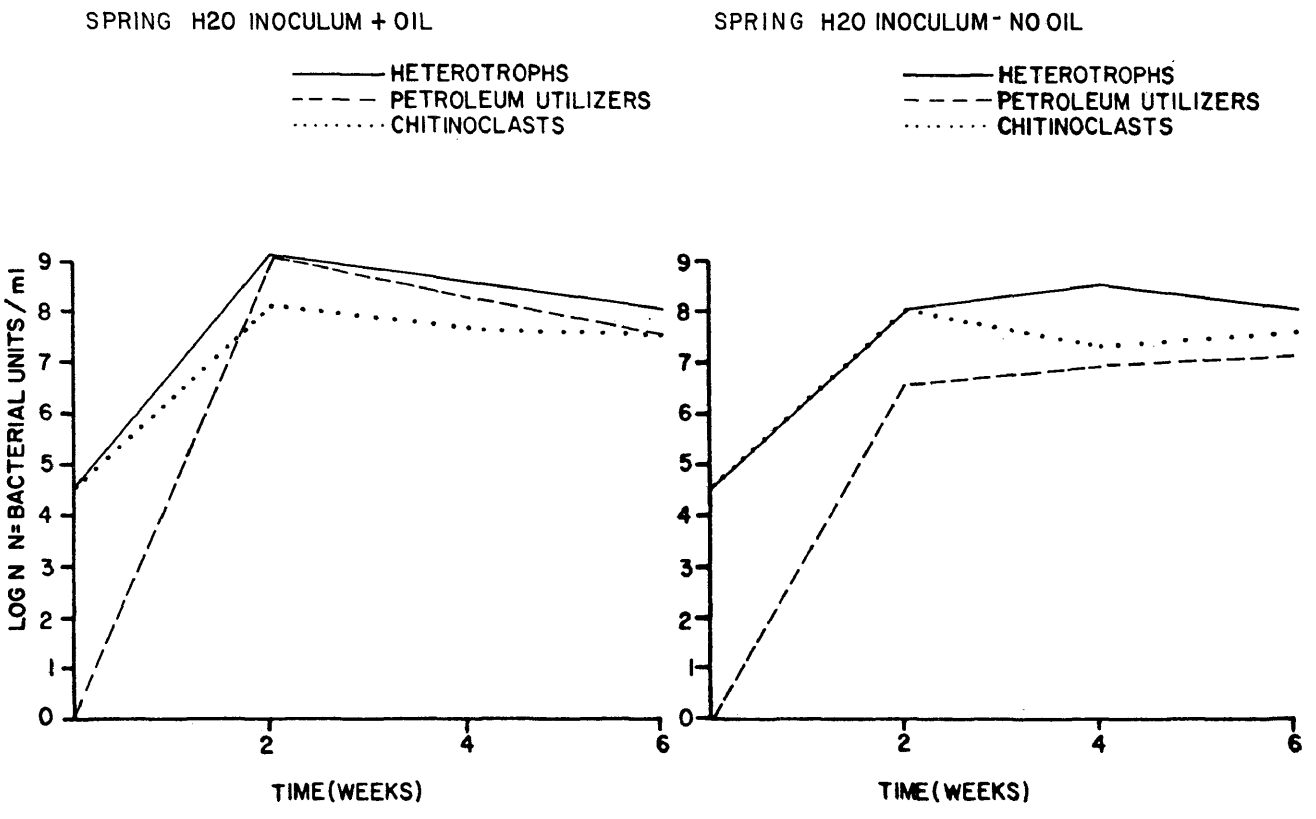

FALL OUTER SHELF SEDIMENT INOCULUM
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
+ OIL
-OIL
LOG N N= BACTERIAL UNITS/ml.
9
8
7
6
5
4
3
2
5
11
TIME (WEEKS)

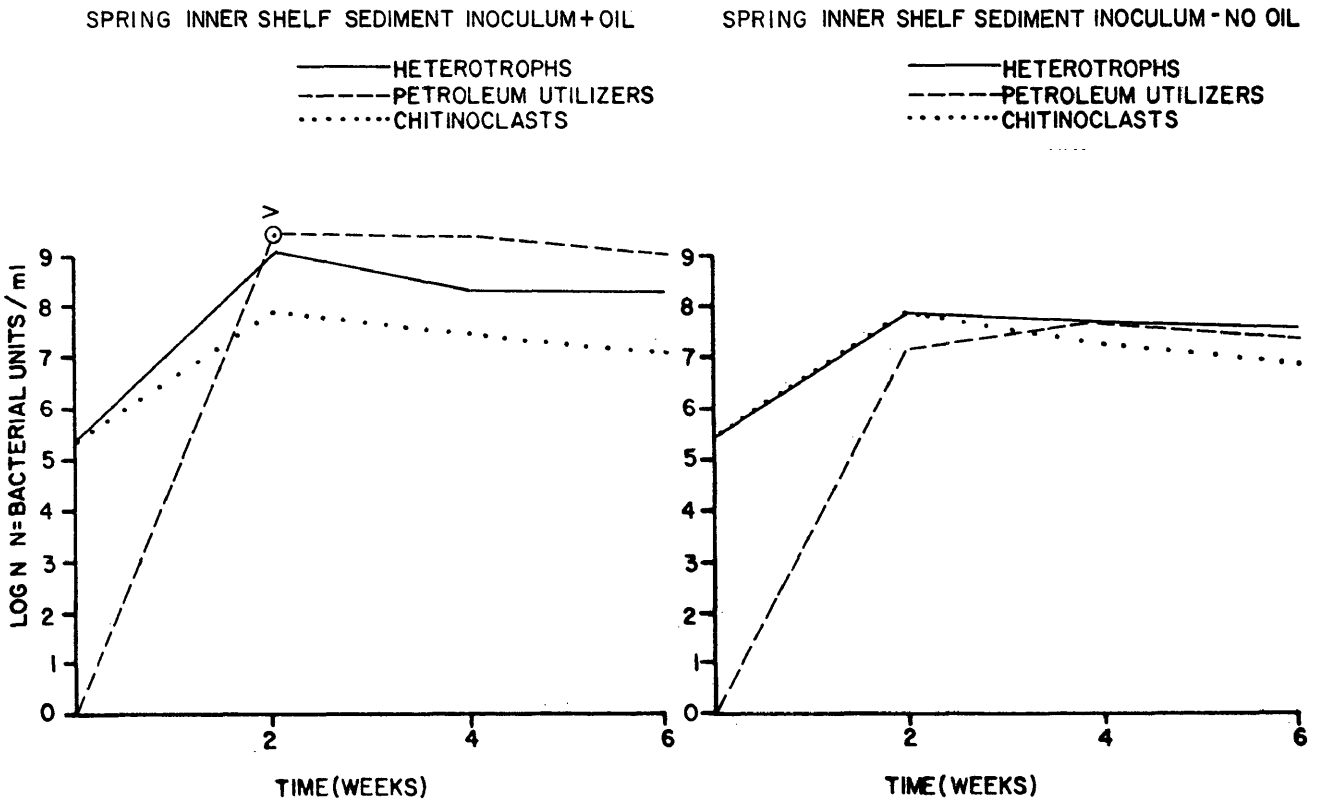

FALL SHELF BREAK SEDIMENT INOCULUM
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
+ OIL
- OIL
LOG N N= BACTERIAL UNITS/ml.
9
8
7
6
5
4
3
2
5
11
TIME (WEEKS)

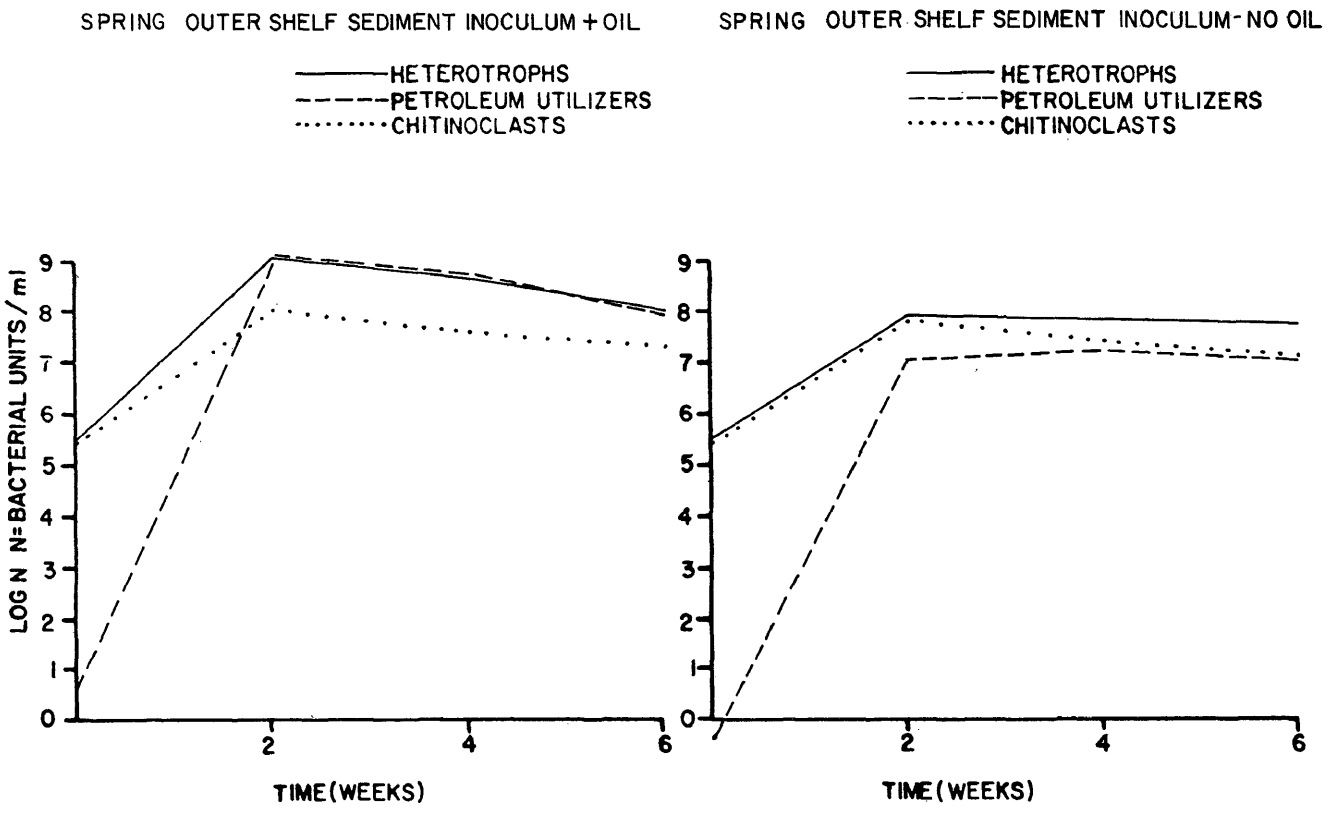

WINTER INNER SHELF SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS / ml
9
8
7
6
5
4
3
2
6
11
TIME (WEEKS)
WINTER INNER SHELF SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
9
8
7
6
5
4
3
2
6
11
TIME (WEEKS)


WINTER OUTER SHELF SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
WINTER OUTER SHELF SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS / ml
9
8
7
6
5
4
3
2
6
11
TIME (WEEKS)
TIME (WEEKS)


WINTER SHELF BREAK SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
WINTER SHELF BREAK SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS / ml
9
8
7
6
5
4
3
2
6
11
TIME (WEEKS)
TIME (WEEKS)


SPRING H2O INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS / ml
0
1
2
3
4
5
6
7
8
9
2
4
6
TIME(WEEKS)
SPRING H2O INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
0
1
2
3
4
5
6
7
8
9
2
4
6
TIME(WEEKS)


SPRING INNER SHELF SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N = BACTERIAL UNITS / ml
TIME (WEEKS)
SPRING INNER SHELF SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
TIME (WEEKS)


SPRING OUTER SHELF SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS / ml
0
1
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3
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5
6
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2
4
6
TIME(WEEKS)
SPRING OUTER SHELF SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
0
1
2
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9
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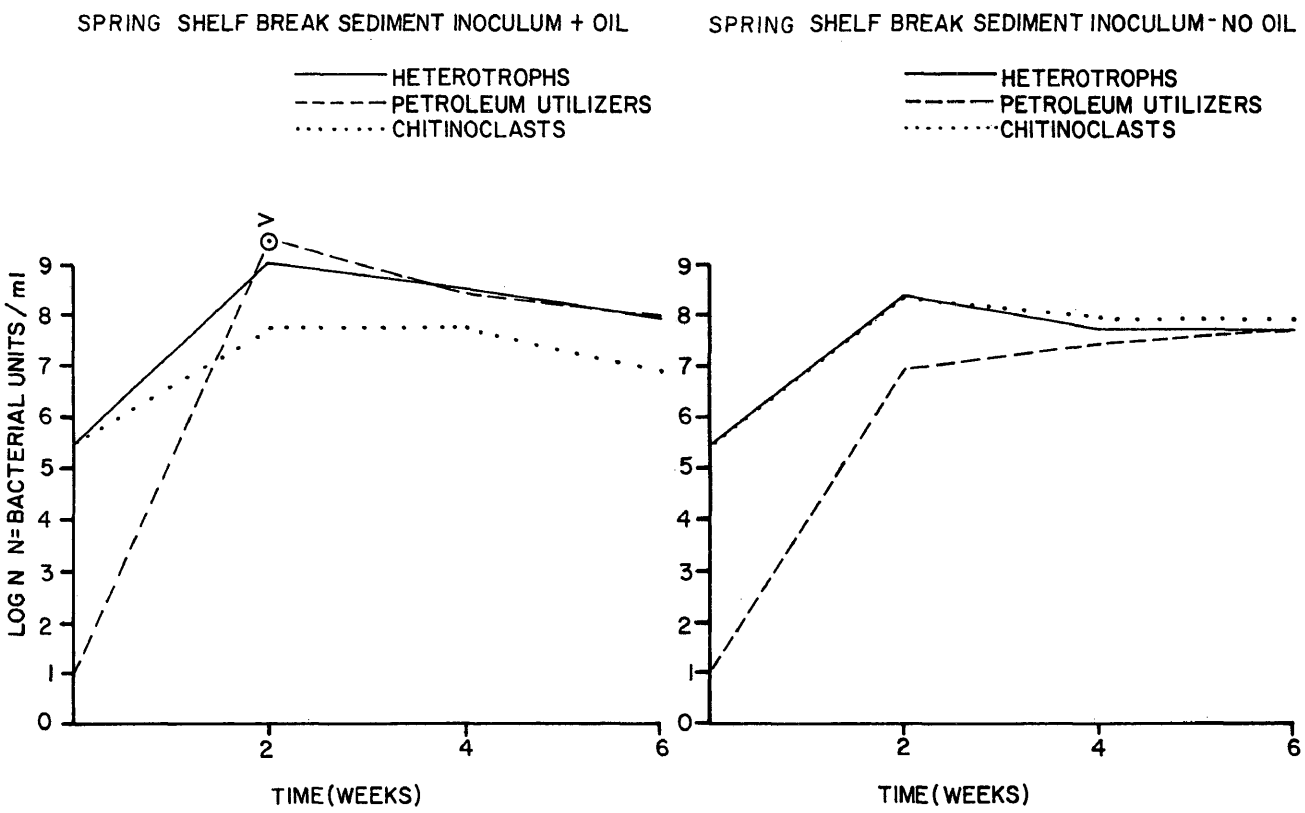
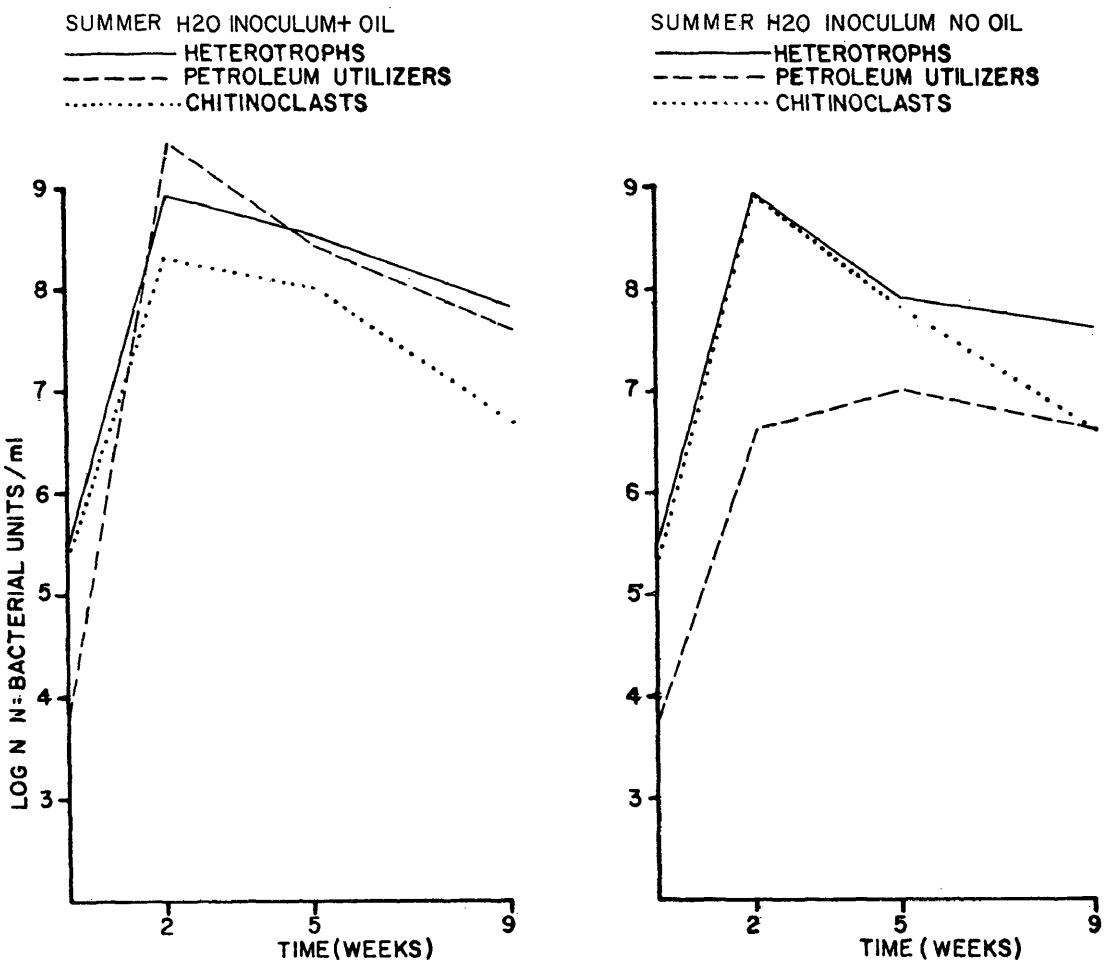
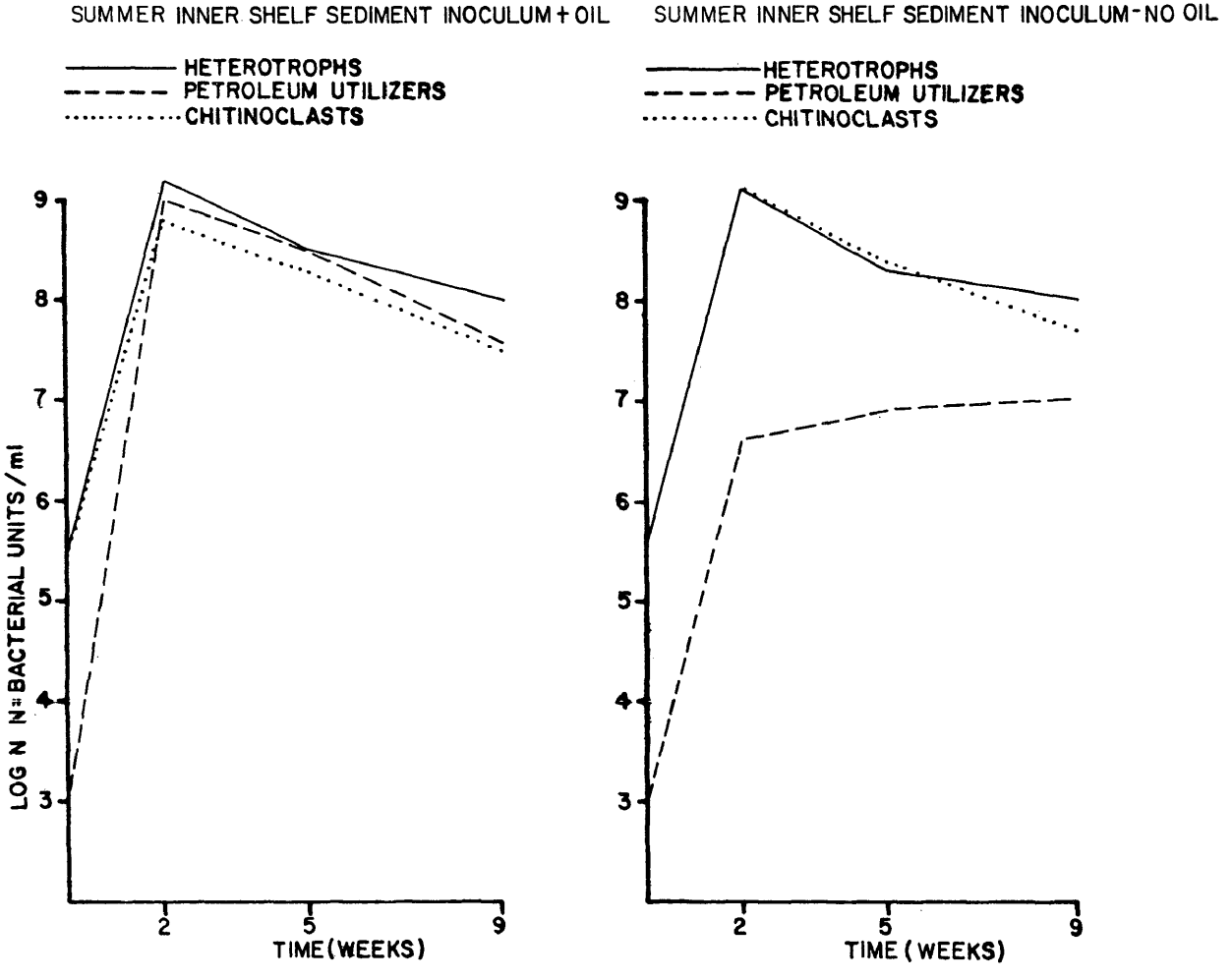
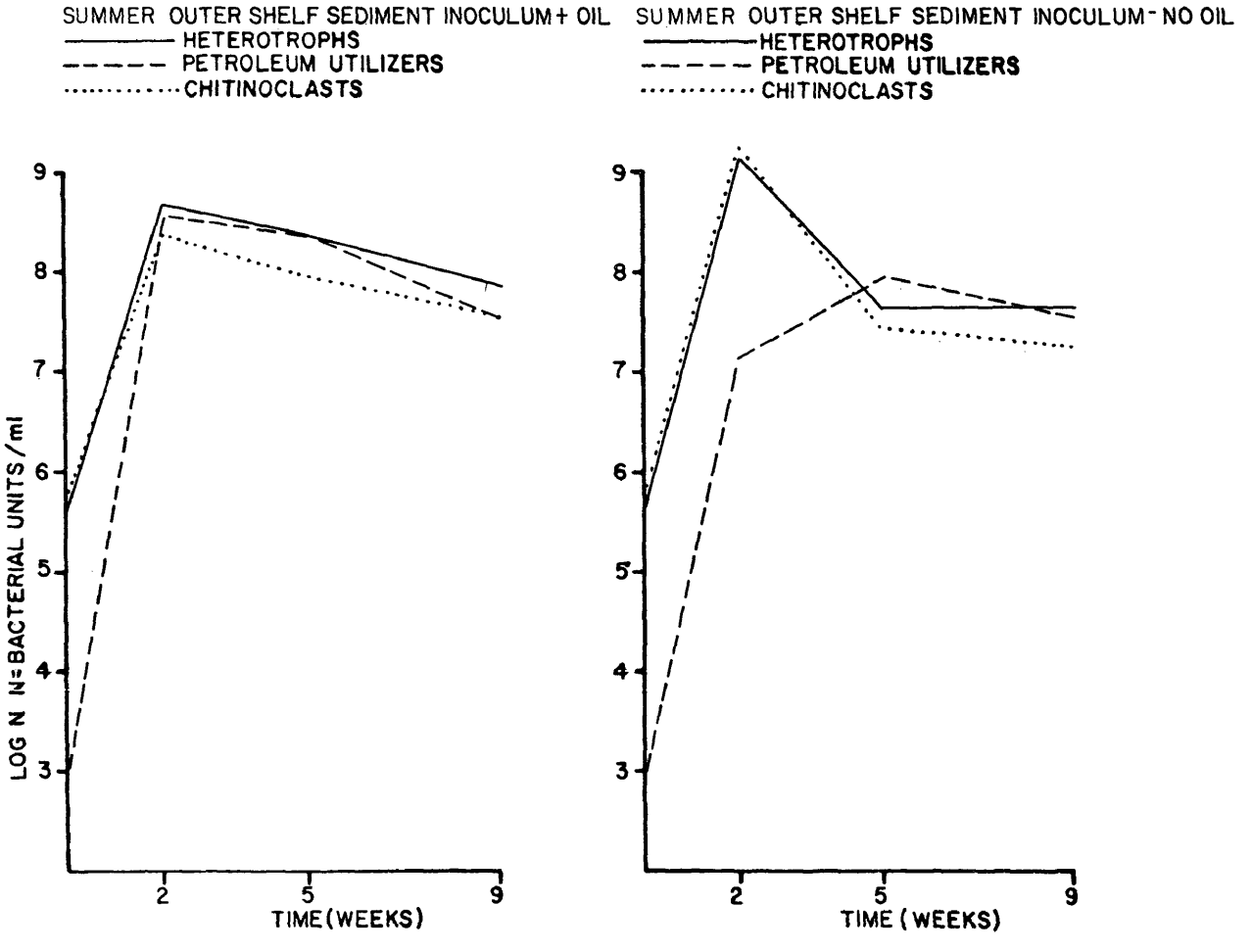
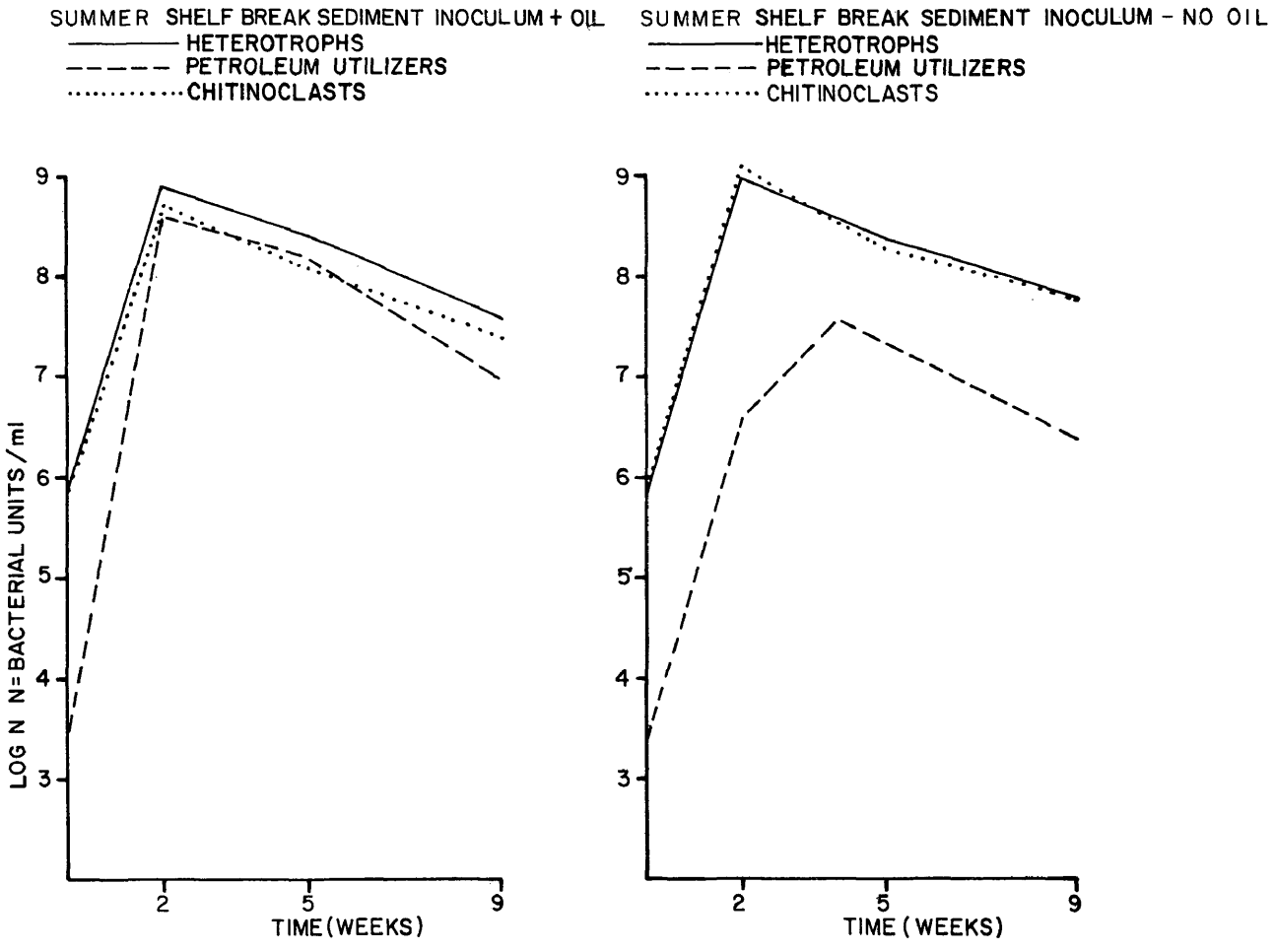
4
6
TIME(WEEKS)


SPRING SHELF BREAK SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS/ml
TIME(WEEKS)
SPRING SHELF BREAK SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
TIME(WEEKS)


SUMMER H2O INOCULUM+ OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N: BACTERIAL UNITS/ml
9
8
7
6
5
4
3
2
5
9
TIME (WEEKS)
SUMMER H2O INOCULUM NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
9
8
7
6
5
4
3
2
5
9
TIME (WEEKS)

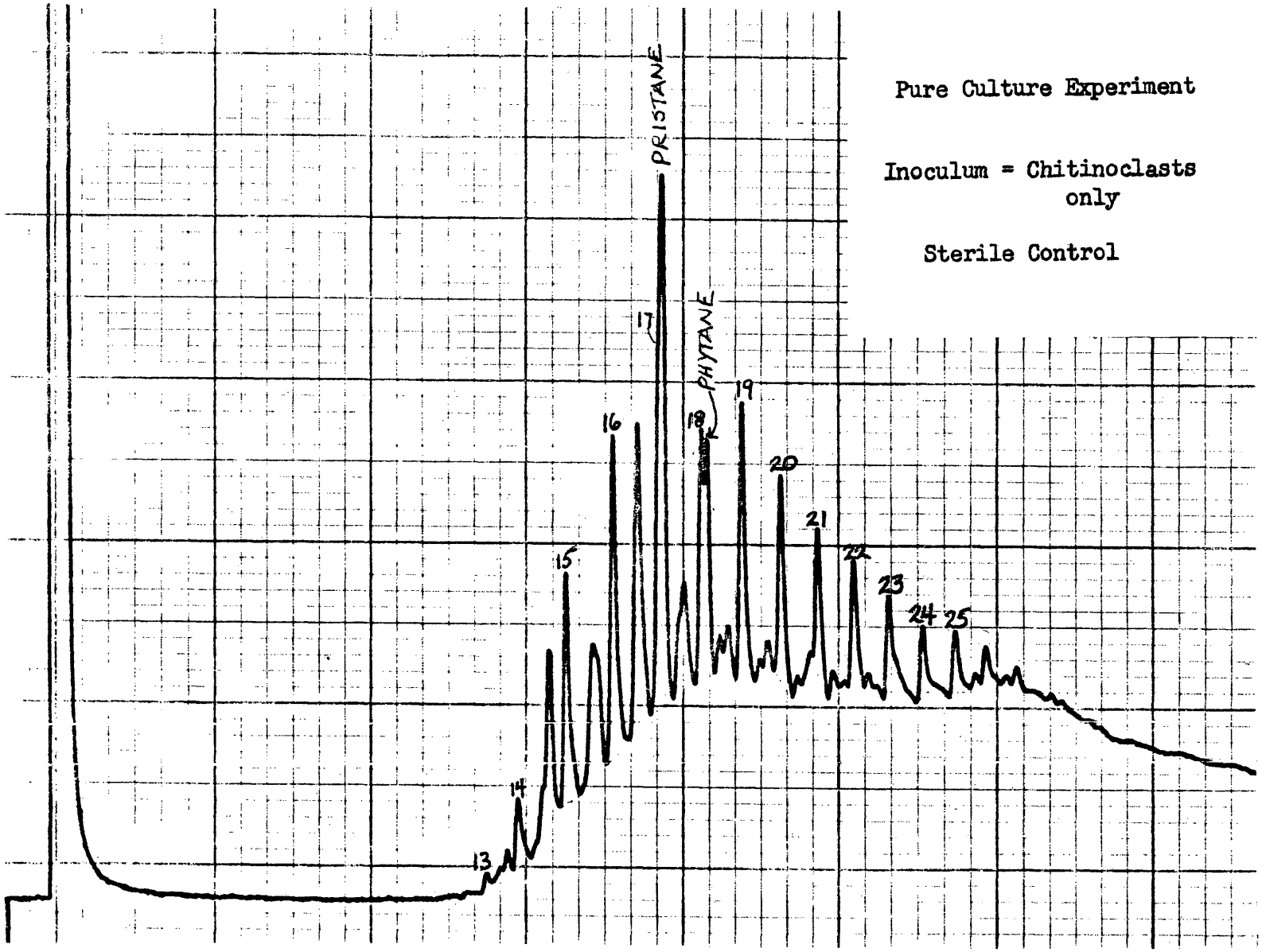

SUMMER INNER SHELF SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N= BACTERIAL UNITS/ml
9
8
7
6
5
4
3
2
5
9
TIME (WEEKS)
SUMMER INNER SHELF SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
9
8
7
6
5
4
3
2
5
9
TIME (WEEKS)


SUMMER OUTER SHELF SEDIMENT INOCULUM+ OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
SUMMER OUTER SHELF SEDIMENT INOCULUM- NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS/ml
9
8
7
6
5
4
3
2
5
9
TIME(WEEKS)
TIME (WEEKS)


SUMMER SHELF BREAK SEDIMENT INOCULUM + OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
SUMMER SHELF BREAK SEDIMENT INOCULUM - NO OIL
HETEROTROPHS
PETROLEUM UTILIZERS
CHITINOCLASTS
LOG N N=BACTERIAL UNITS/ml
9
8
7
6
5
4
3
2
5
9
TIME(WEEKS)
TIME (WEEKS)

APPENDIX D

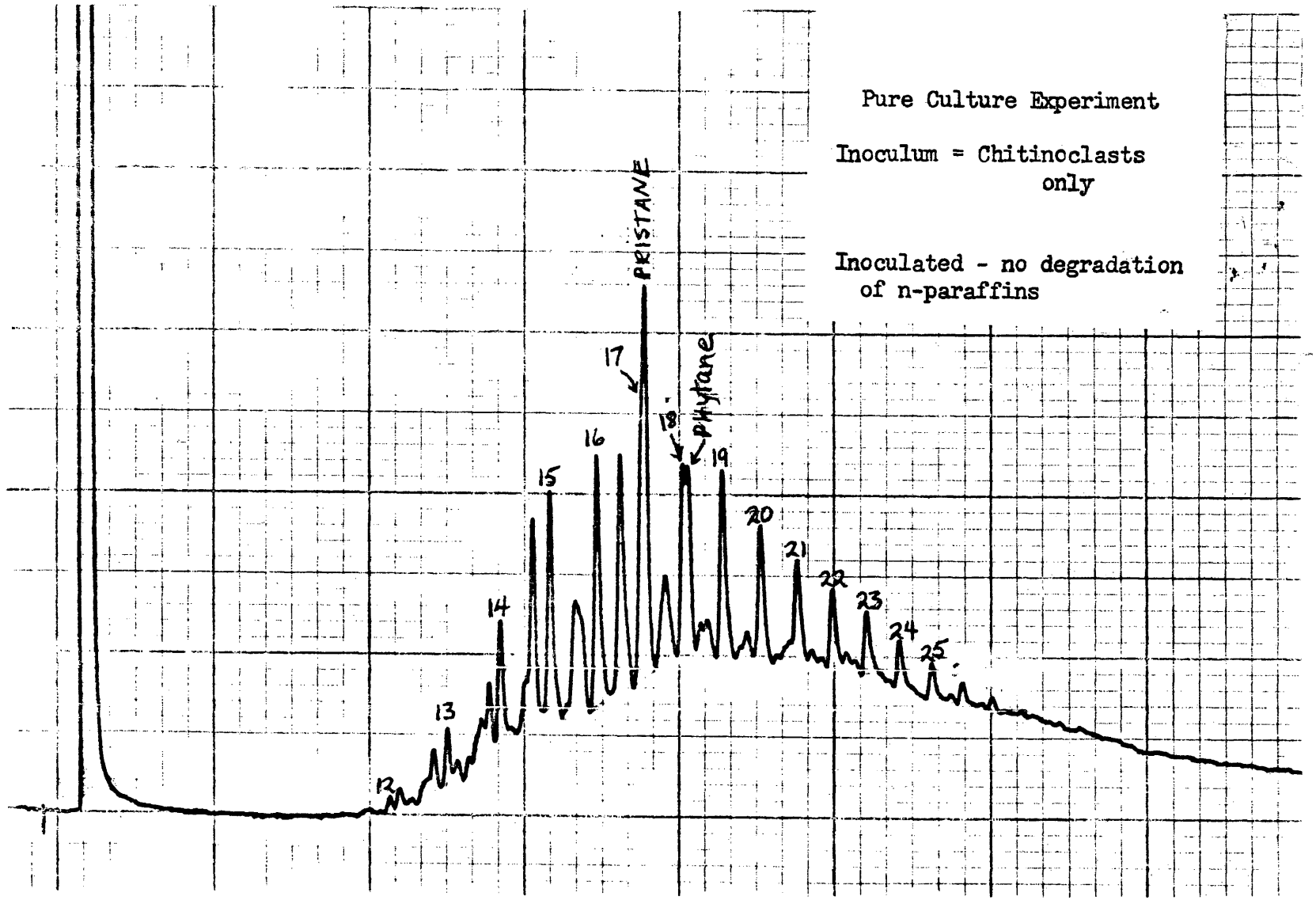
Representative Chromatograms

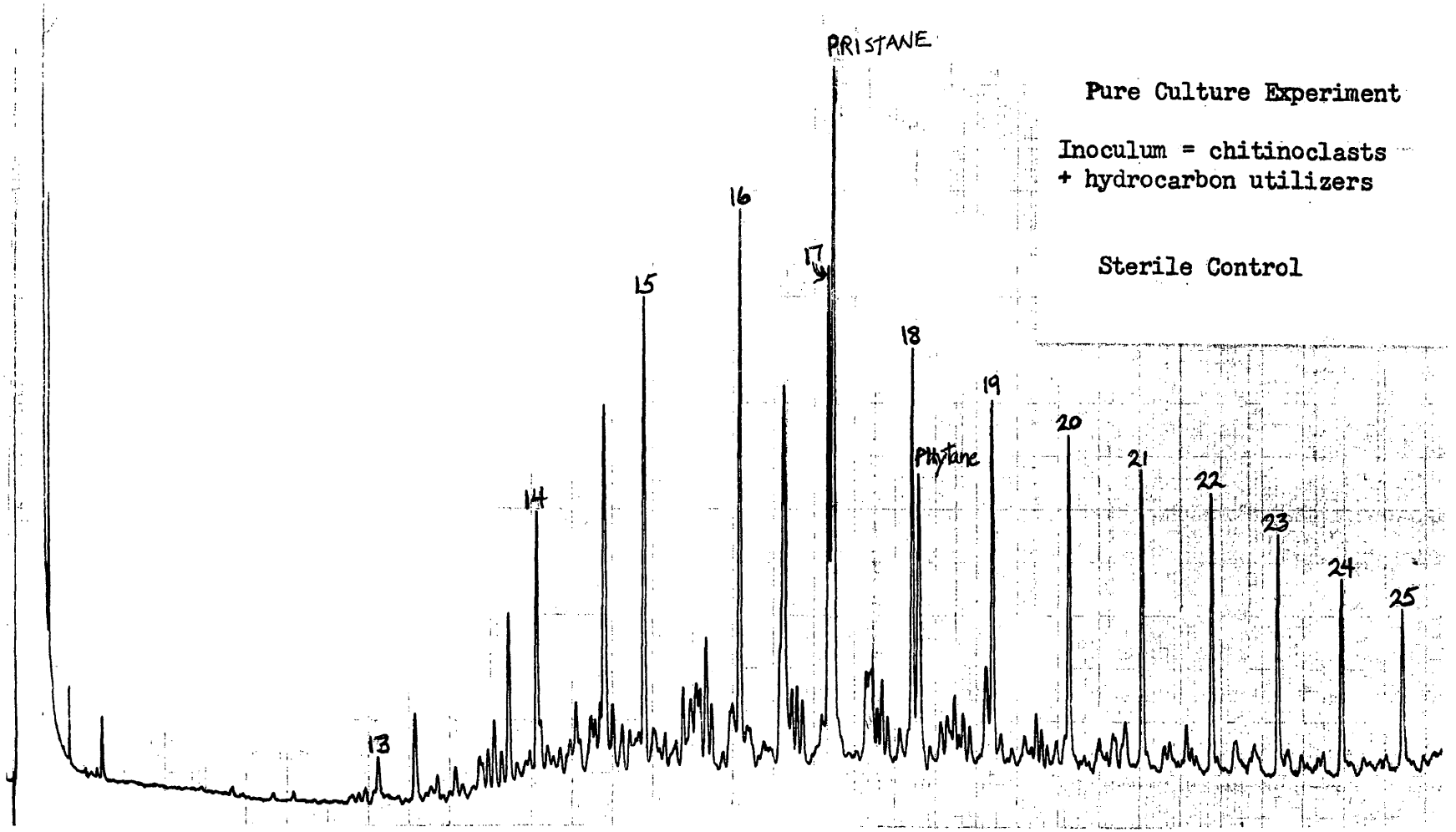


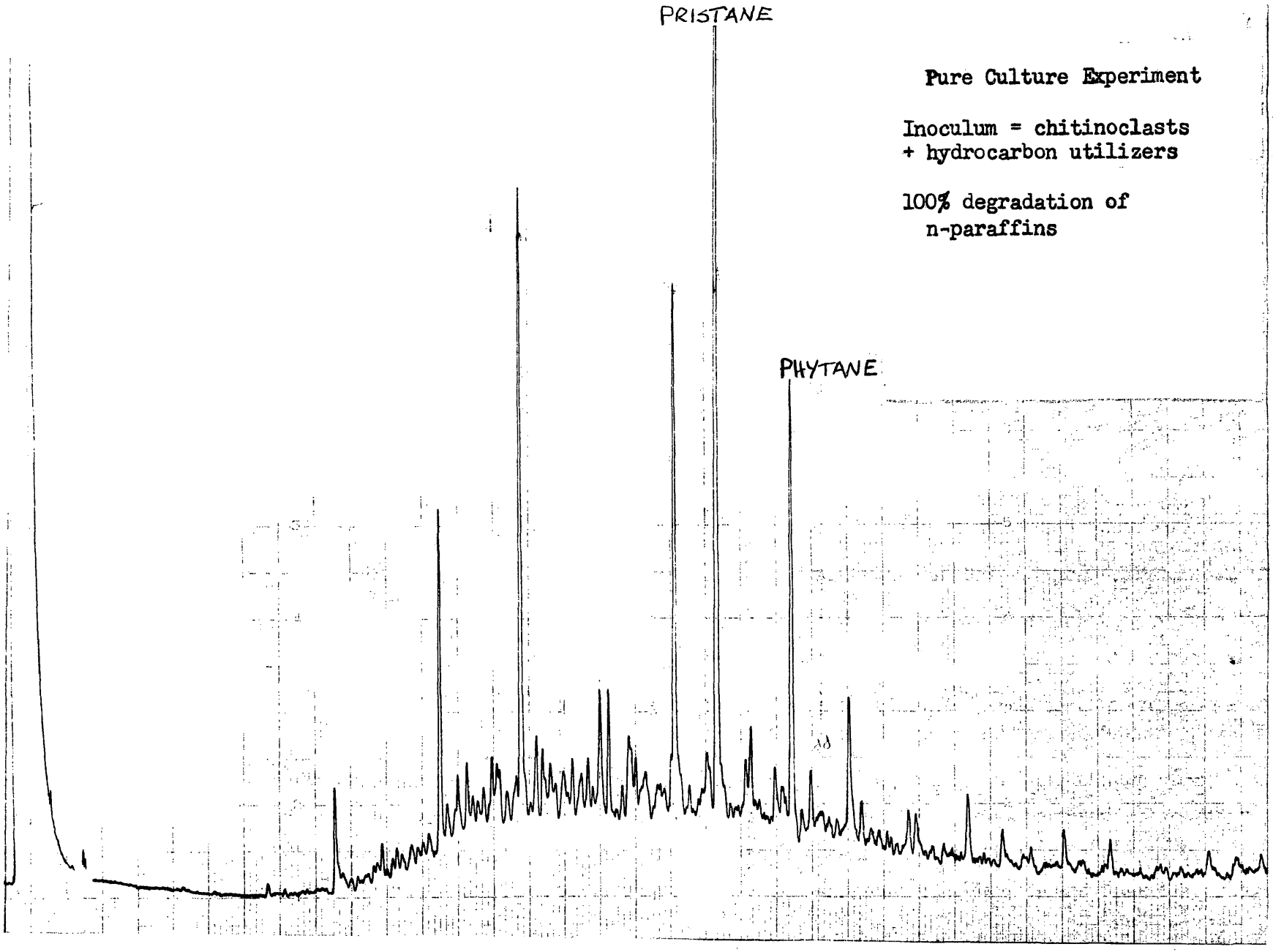
Pure Culture Experiment

Inoculum = Chitinoclasts only

Sterile Control


Pure Culture Experiment
Inoculum = Chitinoclasts only
Inoculated - no degradation of n-paraffins
PRISTANE
PHYTANE
12
13
14
15
16
17
18
19
20
21
22
23
24
25

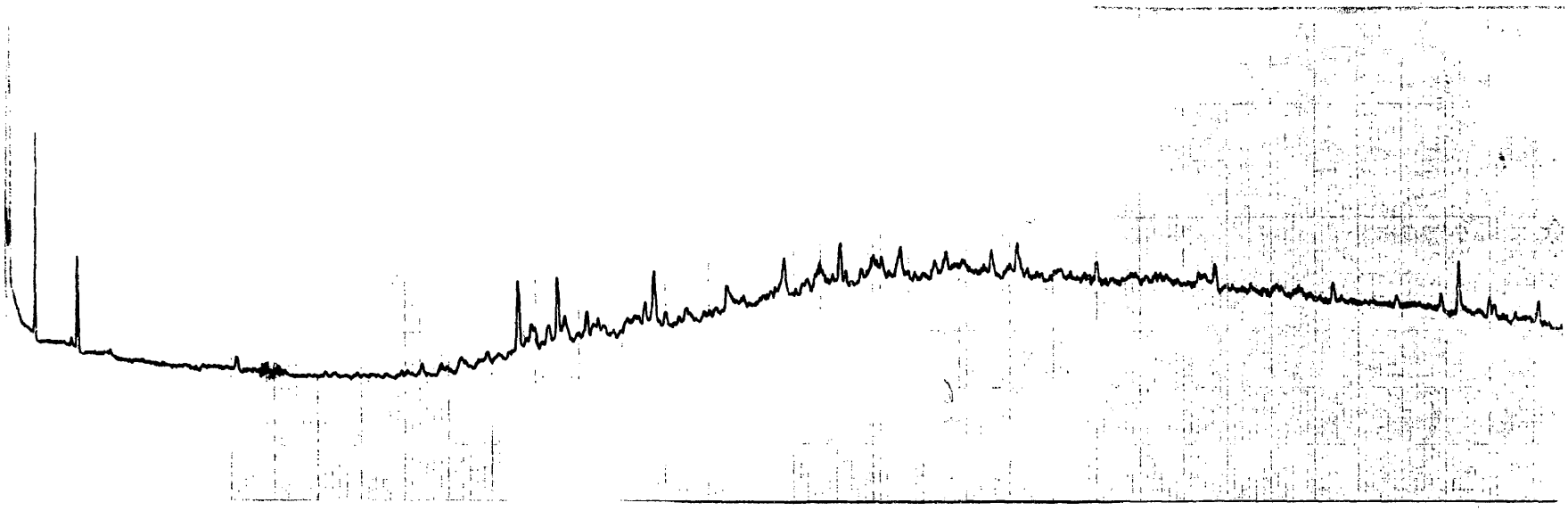

PRISTANE
Pure Culture Experiment
Inoculum = chitinoclasts
+ hydrocarbon utilizers
Sterile Control
13
14
15
16
17
18
Phytane
19
20
21
22
23
24
25


PRISTANE
PHYTANE
Pure Culture Experiment
Inoculum = chitinoclasts
+ hydrocarbon utilizers
100% degradation of
n-paraffins

Pure Culture Experiment

Inoculum = chitinoclasts
+ hydrocarbon utilizers

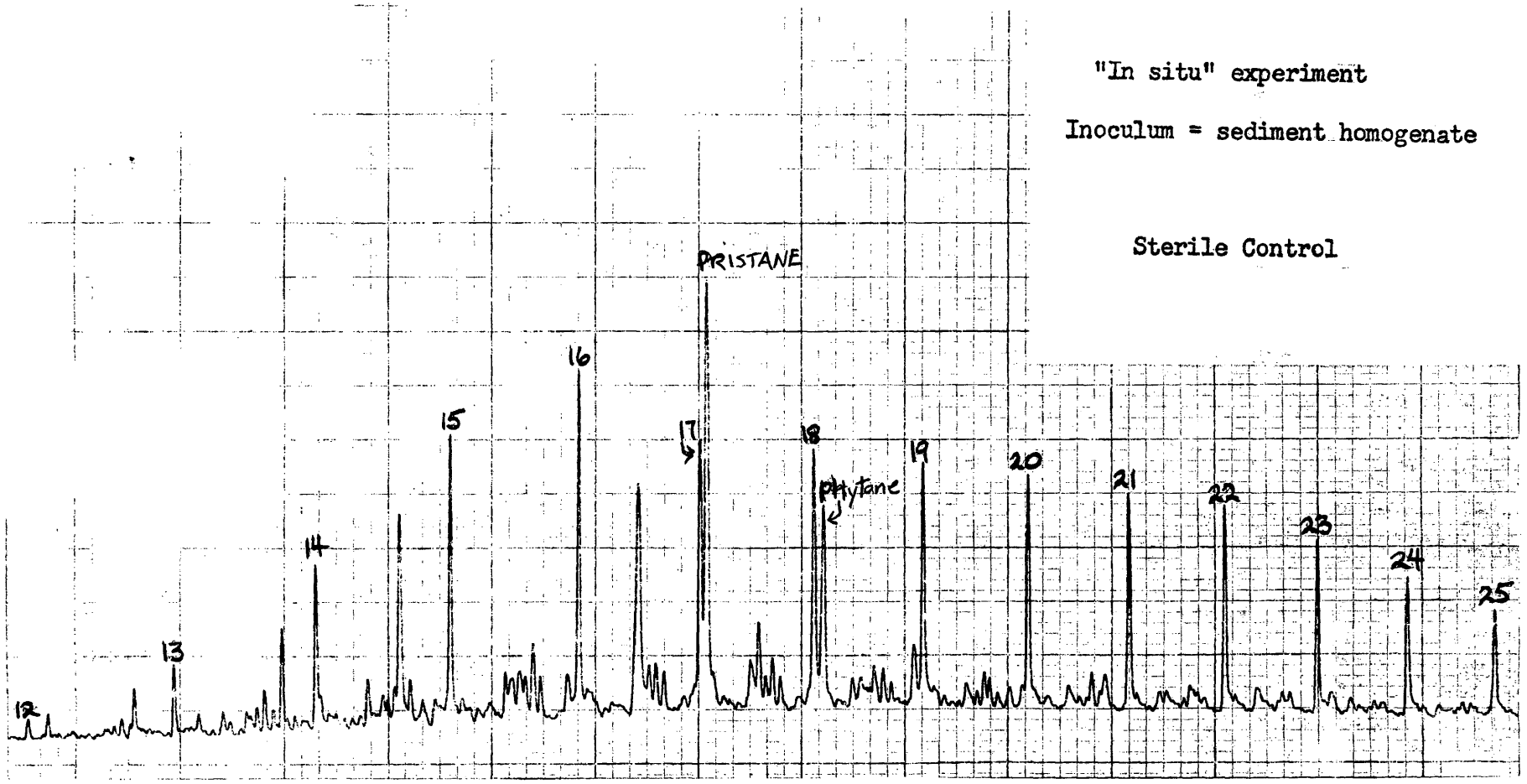
100% degradation of n-paraffins
and isoprenoids

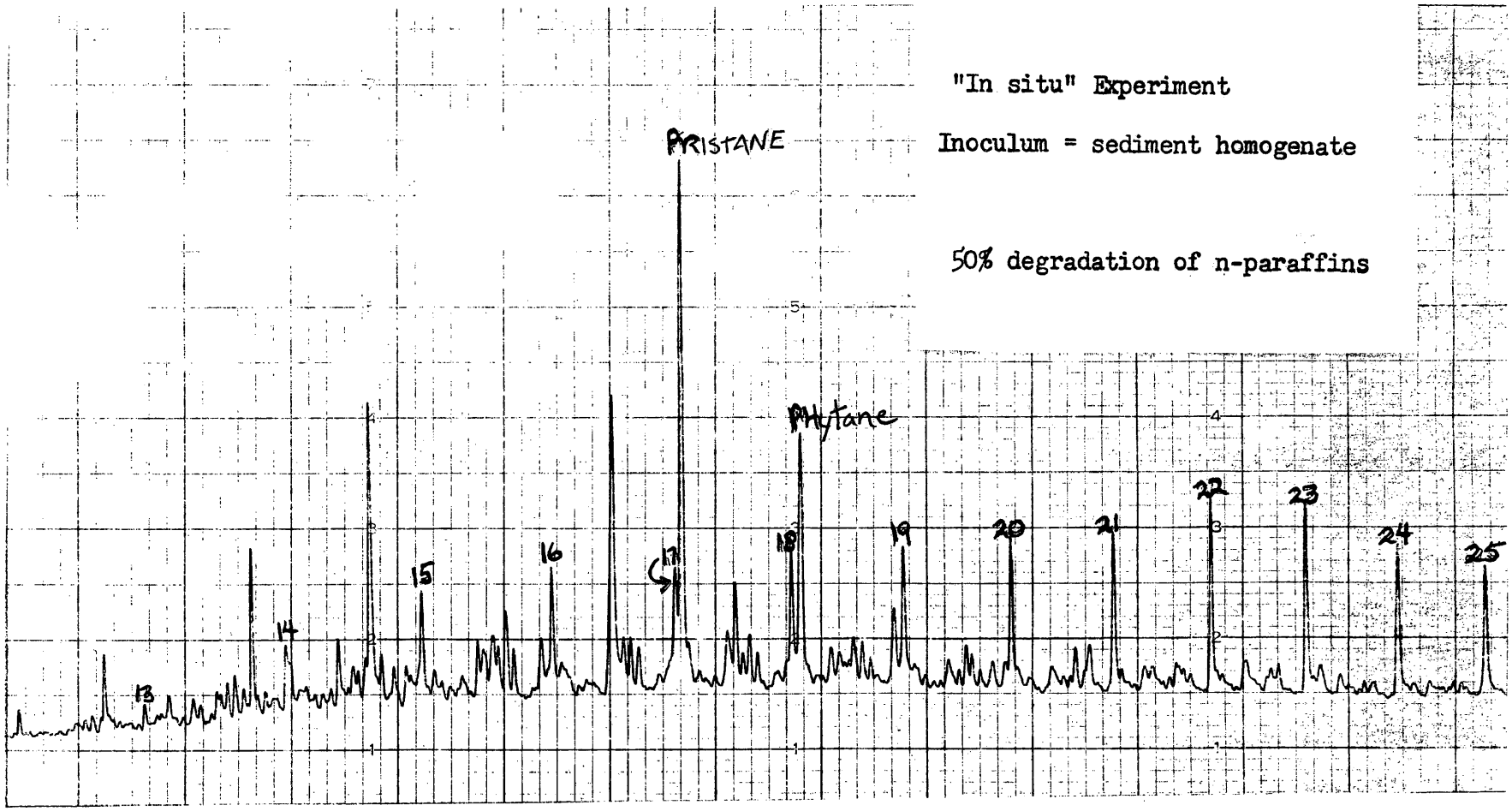


"In situ" experiment

Inoculum = sediment homogenate

Sterile Control

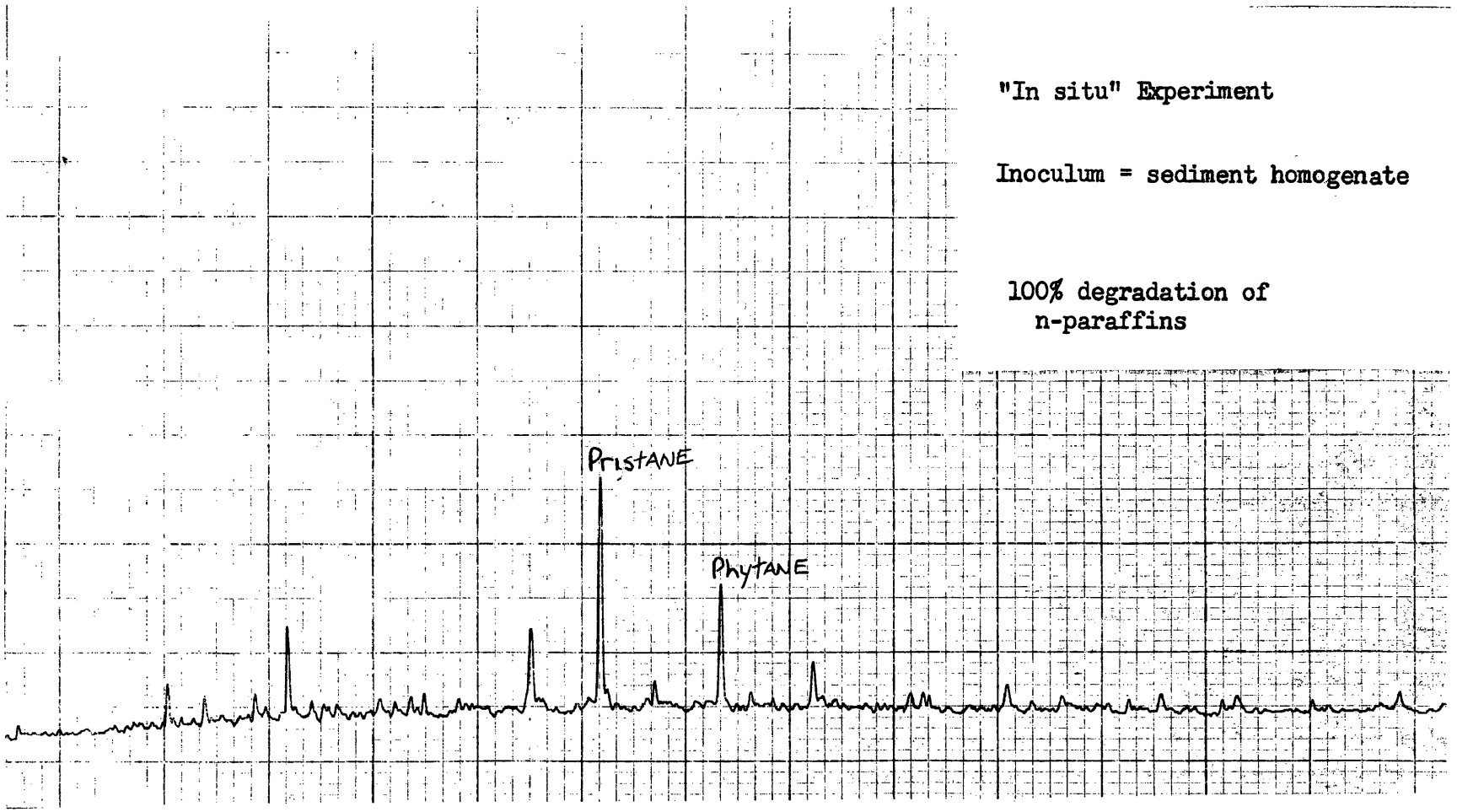



"In situ" Experiment
Inoculum = sediment homogenate
50% degradation of n-paraffins
PRISTANE
PHYTANE
13
14
15
16
17
18
19
20
21
22
23
24
25

"In situ" Experiment

Inoculum = sediment homogenate

100% degradation of
n-paraffins



Appendix E Chitinoclastic bacterial isolates and their classification. S = sediment, W = water

Isolate No.	Station	Source	Identification
Fall, 1976			
1	A1	S	Aeromonas
2	A2	W	Pseudomonas II
3	A2	S	Aeromonas
4	A2	S	Aeromonas
5	A3	S	Vibrio
6	A3	S	Aeromonas
7	A4	S	Pseudomonas III
8	A4	S	Pseudomonas III
9	B1	S	Vibrio
10	B2	S	Vibrio
11	B2	S	Vibrio
12	B2	S	Vibrio
13	B2	S	Vibrio
14	B3	S	Vibrio
15	B4	S	Vibrio
16	B4	S	Vibrio
17	B5	W	Vibrio
18	B5	W	Vibrio
19	C1	W	Pseudomonas IV
20	C1	W	Vibrio
21	C1	W	Pseudomonas II
22	C1	S	Vibrio
23	C1	S	Aeromonas
24	C2	S	Vibrio
25	C4	S	Aeromonas
26	D1	W	Vibrio
27	D1	W	Vibrio
28	D1	S	Pseudomonas II
29	D1	S	Pseudomonas II
30	D4	S	Vibrio
31	D4	S	Vibrio
32	E1	S	Vibrio
33	E2	S	Vibrio
34	E2	S	Vibrio
35	E3	W	Vibrio
36	E3	S	Aeromonas
37	E3	S	Pseudomonas II
38	E3	S	Vibrio
39	E3	S	Pseudomonas II
40	E4	S	Vibrio
41	E4	S	Vibrio
42	F1	S	Vibrio
43	F2	W	Vibrio
44	F2	W	Pseudomonas II
45	F2	S	Aeromonas

Appendix E - (Continued)

Isolate No.	Station	Source	Identification
46	F2	S	Aeromonas
47	F2	S	Vibrio
48	F3	S	Vibrio
49	F3	S	Pseudomonas III
50	F3	S	Aeromonas
51	F3	S	Pseudomonas III
52	F4	S	Vibrio
53	F4	S	Vibrio
54	L1	W	Vibrio
55	L1	W	Pseudomonas II
56	L2	W	Pseudomonas II
57	L2	W	Vibrio
58	L2	W	Vibrio
59	L4	W	Aeromonas
60	L4	W	Pseudomonas II
61	N3	W	Pseudomonas II
62	N3	W	Vibrio
Winter, 1976			
63	A1	S	Vibrio
64	A1	S	Vibrio
65	A1	S	Vibrio
66	A2	W	Vibrio
67	A2	S	Vibrio
68	A3	S	Vibrio
69	A3	S	Vibrio
70	A4	S	Pseudomonas II
71	A4	S	Vibrio
72	A4	S	Vibrio
73	B1	S	Vibrio
74	B1	S	Aeromonas
75	B1	S	Vibrio
76	B1	S	Vibrio
77	B2	S	Pseudomonas II
78	B3	S	Vibrio
79	B3	S	Vibrio
80	B4	S	Vibrio
81	B4	S	Pseudomonas II
82	B5	S	Vibrio
83	C4	S	Pseudomonas II
84	D1	W	Vibrio
85	D4	S	Vibrio
86	E1	S	Pseudomonas II
87	E1	S	Vibrio
88	E1	S	Vibrio
89	E2	S	Vibrio
90	E2	S	Vibrio

Appendix E - (Continued)

Isolate No.	Station	Source	Identification
91	E3	W	Vibrio
92	E3	S	Pseudomonas II
93	E4	S	Vibrio
94	E4	S	Pseudomonas II
95	E4	S	Vibrio
96	F1	S	Aeromonas
97	F1	S	Vibrio
98	F1	S	Vibrio
99	F1	S	Vibrio
100	F1	S	Pseudomonas IV
101	F2	S	Pseudomonas III
102	F3	S	Vibrio
103	F4	S	Pseudomonas IV
104	F4	S	Pseudomonas sp.
105	F4	S	Pseudomonas II
106	F4	S	Vibrio
107	F4	S	Vibrio
108	G2	S	Pseudomonas II
109	G2	S	Aeromonas
110	G2	S	Vibrio
111	G6	S	Vibrio
112	K2	S	Vibrio
113	K2	S	Vibrio
114	K4	W	Vibrio
115	K4	W	Vibrio
116	K4	S	Vibrio
117	K4	S	Vibrio
118	K4	S	Vibrio
119	K5	S	Pseudomonas II
120	K5	S	Vibrio
121	K6	W	Vibrio
122	K6	S	Vibrio
123	L2	S	Aeromonas
124	L2	S	Vibrio
125	L2	S	Pseudomonas IV
126	L4	W	Vibrio
127	L4	S	Vibrio
128	L4	S	Vibrio
129	L4	S	Vibrio
130	L4	S	Vibrio
131	L6	W	Vibrio
132	L6	W	Vibrio
133	L6	S	Vibrio
134	L6	S	Vibrio
135	N3	W	Aeromonas

Appendix E - (Continued)

Isolate No.	Station	Source	Identification
Spring, 1977			
136	A1	S	Vibrio
137	A2	S	Pseudomonas IV
138	A2	S	Vibrio
139	A2	S	Vibrio
140	A3	S	Vibrio
141	A4	S	Vibrio
142	A4	S	Vibrio
143	A4	S	Vibrio
144	B1	S	Vibrio
145	B2	S	Vibrio
146	B2	S	Vibrio
147	B3	S	Vibrio
148	B3	S	Vibrio
149	B3	S	Vibrio
150	B4	S	Vibrio
151	B5	W	Vibrio
152	B5	S	Vibrio
153	B5	S	Vibrio
154	B5	S	Vibrio
155	C1	W	Vibrio
156	C1	W	Vibrio
157	C1	S	Vibrio
158	C1	S	Vibrio
159	C2	S	Vibrio
160	D1	S	Vibrio
161	D1	S	Vibrio
162	E1	S	Vibrio
163	E1	S	Vibrio
164	E1	S	Vibrio
165	E2	S	Vibrio
166	E3	W	Vibrio
167	E3	W	Pseudomonas II
168	E4	S	Vibrio
169	E4	S	Vibrio
170	E4	S	Vibrio
171	F1	S	Vibrio
172	F1	S	Vibrio
173	F1	S	Vibrio
174	F1	S	Vibrio
175	F2	W	Pseudomonas II
176	F2	W	Pseudomonas II
177	F2	W	Vibrio
178	F2	W	Vibrio
179	F2	S	Vibrio
180	F2	S	Pseudomonas IV
181	F4	S	Vibrio

Appendix E - (Continued)

Isolate No.	Station	Source	Identification
182	F4	S	Pseudomonas IV
183	F4	S	Pseudomonas IV
184	J1	W	Pseudomonas II
185	L2	W	Vibrio
186	L6	W	Pseudomonas II
187	L6	W	Pseudomonas II
Summer, 1977			
188	A1	S	Vibrio
189	A2	W	Pseudomonas II
190	A2	W	Pseudomonas II
191	A2	S	Vibrio
192	A2	S	Vibrio
193	A3	S	Vibrio
194	A3	S	Vibrio
195	A3	S	Vibrio
196	A3	S	Vibrio
197	A4	S	Vibrio
198	B1	S	Vibrio
199	B1	S	Vibrio
200	B2	S	Pseudomonas II
201	B2	S	Pseudomonas II
202	B2	S	Vibrio
203	B2	S	Aeromonas
204	B3	S	Vibrio
205	B3	S	Aeromonas
206	B3	S	Vibrio
207	B3	S	Aeromonas
208	B3	S	Pseudomonas II
209	B5	W	Vibrio
210	B5	W	Vibrio
211	B5	W	Vibrio
212	B5	S	Vibrio
213	B5	S	Vibrio
214	B5	S	Vibrio
215	B5	S	Vibrio
216	B5	S	Pseudomonas II
217	B5	S	Vibrio
218	C1	W	Vibrio
219	C1	W	Vibrio
220	C1	W	Pseudomonas II
221	C1	S	Aeromonas
222	C2	S	Vibrio
223	C2	S	Pseudomonas III
224	C2	S	Vibrio
225	C2	S	Vibrio
226	C2	S	Pseudomonas II

Appendix E - (Continued)

Isolate No.	Station	Source	Identification
227	C4	S	Vibrio
228	C4	S	Vibrio
229	D1	W	Aeromonas
230	D1	W	Vibrio
231	D1	W	Pseudomonas sp.
232	D1	W	Vibrio
233	D1	W	Pseudomonas II
234	D1	W	Aeromonas
235	D1	W	Aeromonas
236	D1	S	Vibrio
237	D1	S	Vibrio
238	D4	S	Aeromonas
239	D4	S	Vibrio
240	D4	S	Vibrio
241	D4	S	Pseudomonas II
242	D4	S	Vibrio
243	E1	S	Vibrio
244	E1	S	Vibrio
245	E1	S	Vibrio
246	E1	S	Pseudomonas II
247	E2	S	Vibrio
248	E2	S	Vibrio
249	E2	S	Vibrio
250	E3	W	Vibrio
251	E3	W	Vibrio
252	E3	W	Pseudomonas II
253	E3	W	Vibrio
254	E3	W	Vibrio
255	E3	W	Pseudomonas II
256	E4	S	Vibrio
257	E4	S	Vibrio
258	E4	S	Vibrio
259	E4	S	Vibrio
260	F1	S	Vibrio
261	F1	S	Vibrio
262	F1	S	Vibrio
263	F1	S	Vibrio
264	F1	S	Vibrio
265	F2	W	Vibrio
266	F2	W	Vibrio
267	F2	W	Vibrio
268	F2	W	Pseudomonas II
269	F2	W	Vibrio
270	F2	S	Aeromonas
271	F2	S	Vibrio
272	F4	S	Vibrio
273	F4	S	Vibrio
274	F4	S	Vibrio

Appendix E - (Continued)

Isolate No.	Station	Source	Identification
275	G2	S	Vibrio
276	G2	S	Pseudomonas II
277	G2	S	Vibrio
278	G2	S	Pseudomonas II
279	G6	S	Vibrio
280	G6	S	Vibrio
281	G6	S	Vibrio
282	J1	W	Aeromonas
283	J1	W	Vibrio
284	J1	W	Pseudomonas II
285	J1	W	Vibrio
286	K2	W	Vibrio
287	K2	W	Vibrio
288	K2	W	Pseudomonas II
289	K2	W	Aeromonas
290	K2	W	Pseudomonas II
291	K2	W	Pseudomonas II
292	K2	W	Vibrio
293	K2	S	Vibrio
294	K2	S	Pseudomonas II
295	K2	S	Aeromonas
296	K2	S	Vibrio
297	K4	W	Pseudomonas II
298	K4	W	Vibrio
299	K4	S	Vibrio
300	K4	S	Pseudomonas II
301	K4	S	Pseudomonas II
302	K4	S	Pseudomonas II
303	K5	W	Pseudomonas II
304	K5	S	Vibrio
305	K6	W	Vibrio
306	K6	W	Vibrio
307	K6	W	Vibrio
308	K6	W	Vibrio
309	K6	W	Vibrio
310	K6	S	Vibrio
311	K6	S	Vibrio
312	L1	W	Aeromonas
313	L1	W	Vibrio
314	L2	W	Pseudomonas II
315	L2	W	Vibrio
316	L2	W	Vibrio
317	L2	S	Vibrio
318	L2	S	Vibrio
319	L4	W	Vibrio
320	L4	W	Pseudomonas II
321	L4	S	Vibrio
322	L4	S	Vibrio

Appendix E - (Concluded)

Isolate No.	Station	Source	Identification
323	I6	W	Vibrio
324	I6	W	Vibrio
325	I6	W	Aeromonas
326	I6	S	Aeromonas
327	N3	W	Pseudomonas III
328	N3	W	Aeromonas
329	N3	W	Vibrio
330	N3	W	Vibrio
331	N3	W	Vibrio
332	N3	W	Vibrio

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