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BACTERIAL RESPONSE TO CRUDE OIL SPILLAGE IN A SALT MARSH

A Thesis

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Master of Arts

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

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DEDICATION

This work is dedicated to my two favorite women during my stay in Gloucester, Mrs. Edna 'Ma' Riley and my wife, Linda.

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LIST OF ABBREVIATIONS

AEW........................Artificial Estuarine Water

ATCC.......................American Type Culture Collection

ESWB.......................Enriched Seawater Broth

EW.........................Estuarine Water

HMA........................Heterotrophic Medium Agar

HMB........................Heterotrophic Medium Broth

MPN........................Most Probable Number

S_j.........................Similarity Coefficient of Jacard

VIMS.......................Virginia Institute of Marine Science

ABSTRACT

The purpose of this thesis was to evaluate the in situ responses of heterotrophic and petroleum-degrading bacteria populations in a salt marsh to controlled spillage of unweathered and weathered South Louisiana crude oil. Three transite enclosures were constructed at a marsh site for: A - spillage with unweathered oil, B - spillage with weathered oil, and C - control, no oil. Sampling areas and schedules were disignated, sediment and creek water zones were sampled. Aerobic heterotrophic bacteria were enumerated on a medium modified after ZoBell's 2216. Petroleum-degrading bacteria were enumerated by a three tube MPN technique. Taxonomic analysis included (1) isolation of pure cultures of heterotrophic and petroleum-degrading bacteria, (2) characterization of isolates by several taxonomic tests (Shewan's scheme), and (3) an intensive analysis of selected isolates using numerical taxonomic techniques.

Significant observations included: (1) The response of petroleum-degrading bacteria to the oil spill was a rapid increase in population within several days after the spill followed by maintenance of elevated populations for a one year period. (2) Significant change of the heterotrophic bacteria level was not observed in the polluted enclosures. (3) Tentative identification using Shewan's scheme showed a heterotrophic dominance by *Pseudomonas*, *Flavobacterium*, and *Alcaligenes* species, while *Pseudomonas* species dominated the petroleum-degraders. (4) The intensive analysis showed the distinct taxonomic character of the majority of the petroleum-degrading bacteria compared with the dominant heterotrophs, and confirmed the tentative generic names assigned to many of the isolates.

BACTERIAL RESPONSE TO CRUDE OIL SPILLAGE IN A SALT MARSH

INTRODUCTION

The real and potential toxicity of pollutant oils for freshwater, brackish, and marine organisms has been variously discussed after oil spills, and is the subject of increasing public attention. Publicized oil spills have occurred mostly in coastal areas characterized by rocky cliffs or beaches. In contrast, there are comparatively few studies on the effects of pollutant oils on salt marsh communities and their complex microbial ecosystems. The purpose of this thesis was to evaluate the effects of a controlled oil spill on the composition of heterotrophic and petroleum-degrading bacteria populations in a salt marsh.

Microorganisms which degrade hydrocarbons are widely distributed in soil and water. They are most numerous and the greatest variety occurs in places that have been subjected to chronic oil pollution either from natural seeps or by the activities of man (Walker and Colwell, 1975; Walker and Colwell, 1976a; Walker and Colwell, 1976b; Altas and Bartha, 1973; Atlas, Schofield, Morelli, Cameron, 1976). More than 200 species of bacteria, yeasts, and filamentous fungi have been shown to metabolize one or more kinds of hydrocarbons ranging from CH_4 to compounds containing more than 40 carbon atoms (Bushnell and Haas, 1941; ZoBell, 1946a; ZoBell, 1950; Beerstecher, 1954; Rogoff, 1961; Treccani, 1962; Davis, 1967; Klug and Markovetz, 1971; Floodgate, 1972; Friede, et al., 1972; Colwell and Walker, 1976a).

Hydrocarbon-degrading microorganisms are not necessarily associated only with petroleum pollution. Hydrocarbons are produced by numerous species of bacteria and algae, and a great variety of higher plants synthesize liquid and solid hydrocarbons (Borneff, et al., 1968; Davis, 1967; Oró, et al., 1967; ZoBell, 1971). Went (1960) estimated that millions of tons of terpenes per year are released by terrestial plants into the atmosphere where they undergo condensation and eventual precipitation in rain or snow to the oceans. Hence, biosynthesized hydrocarbons constitute natural substrates for hydrocarbon-degrading bacteria.

Despite the many data on the effects of oil on macroorganisms, the literature is practically devoid of parallel studies on microorganisms. Mitchell, et al. (1972) and Chet and Mitchell (1976) have provided laboratory evidence for the chemotactic inhibition of bacteria by petroleum hydrocarbons. Cobet and Guard (1973) randomly isolated bacteria from a beach following an oil spill. Walker and Colwell (1975), and Walker, Seesman, and Colwell (1975) concluded that oil appeared to be toxic to ecologically important bacteria involved in the cycling of nutrients in the estuary. However, the above experiments were performed under laboratory conditions, and the validity of this conclusion was not tested under actual field conditions.

Cobet and Guard (1973) studied the fate and effect of the residual bunker fuel oil following a Chevron spill in San Francisco. They found no significant changes in the diversity of bacterial genera with respect to depth and time. There were no measurable changes in the genera composition of the bacterial population.

Few studies have examined the in situ responses of marshland petroleum-degrading bacteria and aerobic heterotrophic bacteria to oil spillage. An experiment to introduce relatively large volumes of unweathered (fresh) and weathered crude oil into a *Spartina* salt marsh (Bender, et al., 1976), afforded an opportunity to quantitate the bacterial response as viable counts. Aerobic heterotrophic and petroleum-degrading bacterial levels were monitored for one year after the oil spillage, and the taxonomy of isolates selected on the basis of numerical dominance was studied.

MATERIALS AND METHODS

I. Experimental Area and Oil Spillage

Three experimental transite walled enclosures were constructed at a site on Cub Creek, which drains into the lower York River (Latitude 37°17'30", Longitude 76°37'32"). On 22 September 1975, three barrels of fresh South Louisiana crude oil were spilled within the creek area of one enclosure utilizing the flooding tide, and on 25 September, artificially weathered South Louisiana crude oil was spilled into another enclosure. Within several tidal cycles, the oils were transported into the enclosures, covering the sediments and *Spartina*.

Details of construction and experimental procedures involving the spills may be found in two papers (Bender, et al., 1976; Bieri, et al., 1976). Figure 1 shows the experimental site location.

II. Sample Collection

Transite enclosures were designated *A* (dosed with unweathered oil), *B* (dosed with weathered oil), and *C* (control-no oil) (Figure 2). For sampling purposes each enclosure was partitioned to yield 3 sets of duplicate sampling zones: creek and intertidal, mid-marsh, and back-marsh, of approximately equal area. *A* and *B* enclosures possessed a lengthwise divider actually constructed of transite. Each sampling zone was identified by a code which designated the particular enclosure (*A*, *B*, or *C*), a letter or number indicating the upstream (D,E,F) or downstream (1,2,3) side of the enclosure, and a letter indicating a sediment (-*S*) or water

Figure 1. Experimental site location.

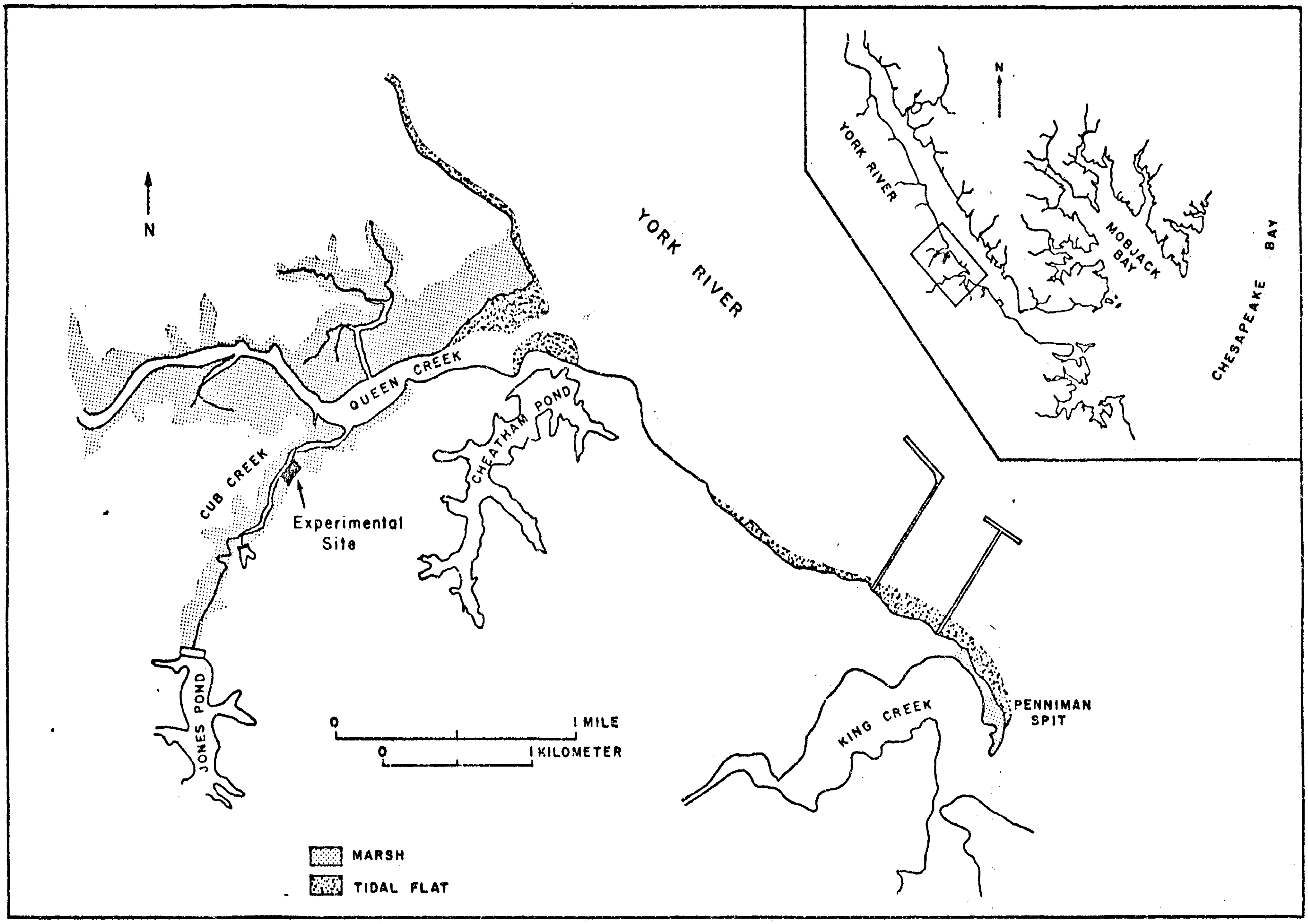
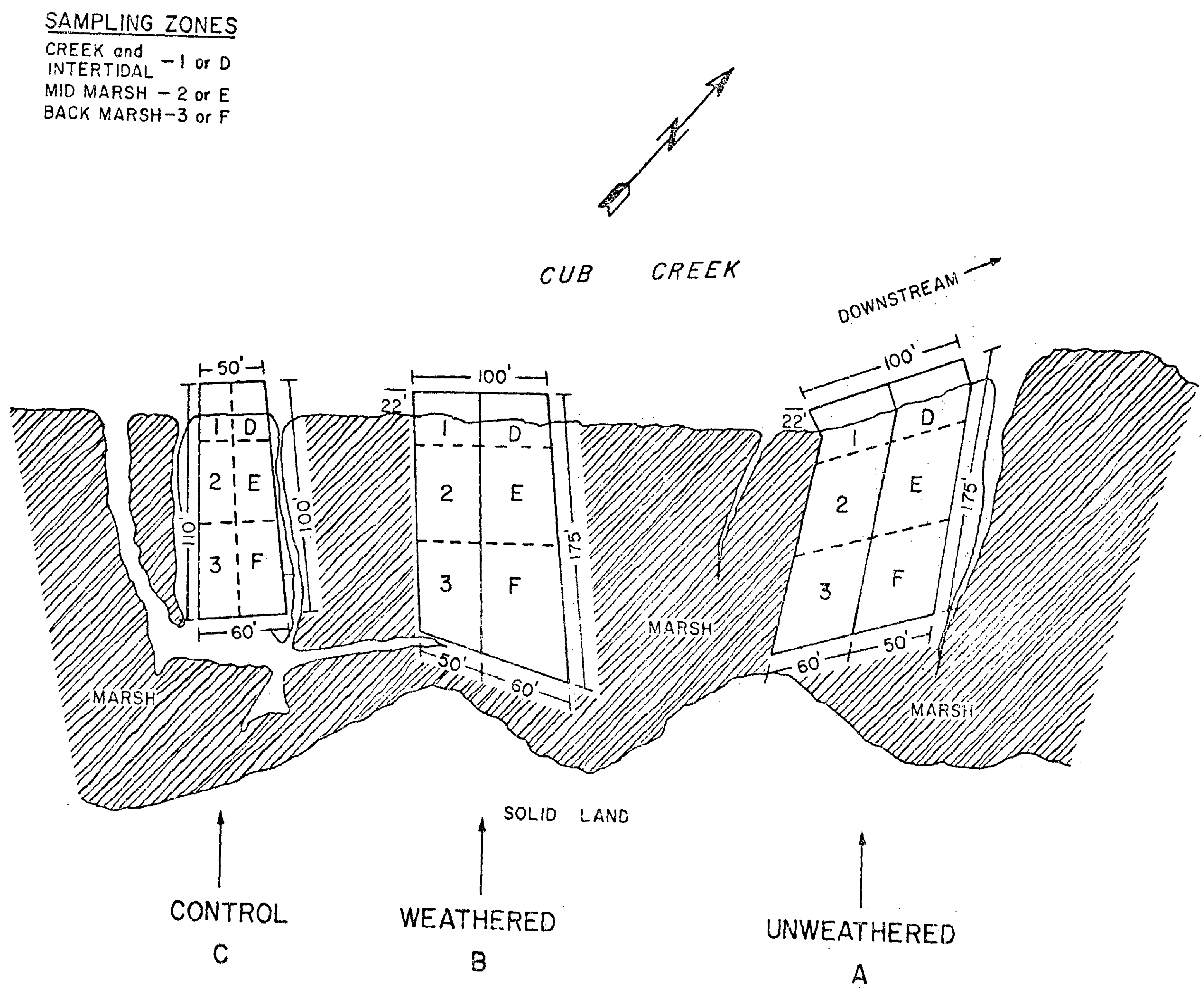

N
YORK RIVER
QUEEN CREEK
CUB CREEK
CHEATHAM POND
Experimental Site
JONES POND
0
1 MILE
0
1 KILOMETER
KING CREEK
PENNIMAN SPIT
MARSH
TIDAL FLAT
N
YORK RIVER
MOBJACK BAY
CHESAPEAKE BAY

Figure 2. Enclosures shown with creek, intertidal, mid-marsh, and back-marsh sampling zones.


SAMPLING ZONES
CREEK and INTERTIDAL – 1 or D
MID MARSH – 2 or E
BACK MARSH – 3 or F
CUB CREEK
DOWNSTREAM
50'
100'
110'
60'
22'
175'
1
D
2
E
3
F
MARSH
SOLID LAND
CONTROL
C
WEATHERED
B
UNWEATHERED
A

(-W) sample.

Different areas within each enclosure were subjected to periodic submergence due to tidal flooding. Intertidal zones were characterized by tall, dense Spartina stands and mud sediments. Mid- and Back-Marsh zones, subjected to tidal inundation of shorter duration, typically contained Spartina of shorter height and shallow pools of water with Fundulus sp. at low tide. Sediments in the Mid- and Back-Marsh, composed of fibrous plant detritus, rhizoidal root structures, and small qunatities of mud, possessed a very thin aerobic layer (ca. 2 cm) over an anaerobic layer blackened by precipitated sulfides and producing H_2S.

Sediment samples from the Spartina rhizoidal area were collected from three zones within each enclosure. Randomly selecting either the upstream or downstream side for each zone for sampling, five random sub-samples of 2 ml volume each were collected and pooled for each zone sampled, yielding a total volume of 10 ml. Sediment samples were collected in sterile plastic 10 or 50 cc syringes modified by the removal of the luer tip to produce a mini-corer. Intertidal zones were sampled by plunging the syringe in to a depth of 2 cm. Five subsamples were collected within this syringe. For sampling in the Mid- and Back-Marsh zones this mini-coring technique was not feasible. To sample these rooty and fibrous "sediments" a sterile spatula was used to remove 5 sediment subsamples from the upper 2 cm of the marsh sediment to give a final sample volume of 10 ml. Replicate, pooled sediment samples from each zone were obtained for sediment dry weight determinations. Water samples were collected from the Creek-Intertidal zones using sterile milk dilution bottles (150 ml) inverted below the creek surface. All samples were transported on ice to the laboratory for immediate processing. Metereological conditions were recorded at time of sampling.

Sampling was always performed at slack water before flood on days selected initially by a geometric time scale from the date of the oil spill (i.e. 0,1,2,4,8,16,32 and 64 days), and thereafter performed at approximately bi-monthly intervals.

III. Enumeration Media

Aerobic heterotrophic bacteria were enumerated on heterotroph medium agar (HMA) modified after ZoBell's 2216 medium (1946b). Modifications consisted of replacing ferric phosphate with ferric citrate (0.01 g/l) and sodium glycerol phosphate (0.1 g/l) to reduce inorganic precipitates, and lowering the concentration of peptone to 1.0 g/l.

Petroleum-degrading bacteria were enumerated by a three tube Most Probable Number (MPN) technique (Gunkel, 1967) using an enriched seawater broth (ESWB) which consisted of 1% (v/v) membrane filter sterilized crude oil as the sole added source of carbon in a mineral salts (1 g/l $(NH_4)_2SO_4$ and 0.1 g/l K_2HPO_4) aged estuarine water (EW) (Miget, et al., 1969). Unweathered Louisiana crude oil was used for the A and C enclosure enumerations and weathered Louisiana crude oil for the B enclosure.

All enumeration media were adjusted to a pH of 7.6 and a salinity of 12‰. Estuarine water from the York River was aged for 30 days in darkness and filtered through Whatman #1 paper immediately prior to use.

IV. Enumeration Procedures

All dilutions were performed using sterile aged and filtered EW (Salinity = 12‰). Sediment samples were weighed in the syringes, extruded into chilled Waring blenders containing 90 ml of chilled sterile EW, and blended for one minute. Empty syringes were weighed to determine the weight of the inoculum. Creek water and blended sediment suspensions were then serially diluted.

Aerobic heterotrophic bacteria were counted by spread plating (Buck and Cleverdon, 1960) 0.1 ml of selected dilutions on HMA in petri dishes. Most Probable Numbers of petroleum-degrading bacteria were determined by inoculating 1 ml of selected dilutions into ESWB tubes. MPN tubes were scored positive for petroleum degradation if there was visible turbidity at the oil/water interface, in the aqueous phase, or if the oil overlay had a "stringy" appearance.

Enumeration media were incubated at 20-22° C. The following incubation times were found to be optimal: heterotrophic bacteria, 2 weeks, petroleum-degrading bacteria, 4 weeks. Heterotrophic bacteria counts in sediments were expressed as C.F.U. (colony forming units)/g wet sediment because the water content in sediment samples was uniform within each zone. Water composed over 65% of the mass of most sediment samples. Heterotrophic bacterial counts in creek samples were expressed as C.F.U./ml.

V. Taxonomic Analysis of Isolates

Taxonomic analysis included three major steps: (1) isolation of pure cultures of heterotrophic and petroleum-degrading bacteria, (2) characterization of isolates by several standard taxonomic tests, and (3) an intensive analysis of selected isolates.

A. Isolation of pure cultures.

After the HMA plates had been counted, dominant heterotrophic bacteria, i.e., the most numerous types, were isolated from each sampled zone for each sampling period. Colonies were selected on the basis of morphology and pigmentation, picked, and streaked on HMA plates. Cultures were checked for purity by restreaking until pure. Isolated heterotroph cultures were maintained on HMA slants. Dominant petroleum-degrading bacteria were isolated by selecting 2 positive ESWB MPN tubes of the highest dilution from each sampled zone and streaking from these on to HMA plates. Dominant colony types were restreaked on HMA plates until pure cultures were obtained. Petroleum-degrading bacterial cultures were maintained on HMA slants.

B. Characterization of isolates by using selected standard taxonomic tests.

All of the isolated cultures, both heterotrophic and petroleum-degrading, were evaluated as follows:

1. Examination of wet mount of log phase culture by phase contrast microscopy (970x).

Purpose: motility, cell shape and size, arrangement.

2. Gram stain - Hucker's modification (Hucker and Conn, 1923).

Purpose: gram reaction.

3. Kovacs' oxidase test (Kovacs, 1956).

Purpose: detection of cytochrome C oxidase.

4. Liefson MOF Oxidation - Fermentation Medium (Liefson, 1963).

Purpose: oxidative or fermentative utilization of glucose with acid or alkaline reaction.

5. Pigmentation of colonies.

6. Antibiotic sensitivity.

Purpose: Sensitivity to pteridine 0/129 (Shewan, Hodgkiss and Liston, 1954), chloramphenicol, colistan, neomycin, penicillin, streptomycin, and tetracycline.

Tests were run on 24-48 hour cultures, or those that were in early stationary phase. Tests listed above were important because they are key tests in a scheme (Shewan, 1963) commonly used for the identification of some gram negative marine bacteria (Figure 3). Detailed descriptions of taxonomic tests and special media recipes used in this thesis are given in Appendix A.

C. Intensive analysis of selected isolates.

After preliminary testing had been completed the number of isolates was reduced to a more manageable size for the intensive analysis. As it was anticipated that the oil would have a significant impact on bacterial populations in the marsh sediments, two cultures were randomly selected from sediment stations from the 0, 1, 2, 4, and 8 day samples. Tests for intensive analysis were largely based on those utilized by Colwell and Wiebe (1970), whose study discussed characteristics to be used in classifying aerobic heterotrophic bacteria. Characteristics studied are listed in Table 1.

Figure 3. Shewan's (1963) scheme for the identification of some gram negative bacteria.

Figure 3. Shewan's (1963) scheme for the identification of some gram negative bacteria.

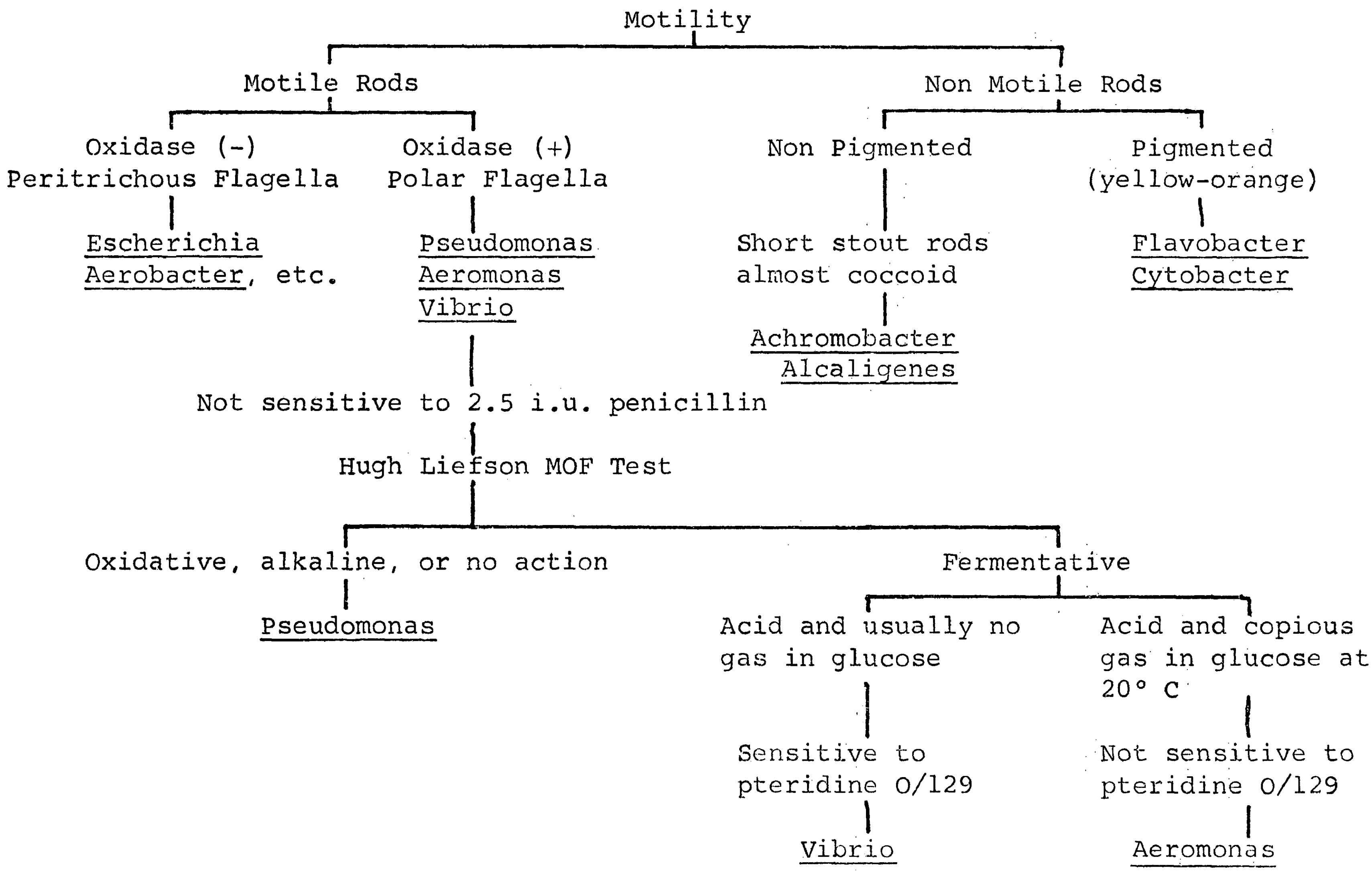


Table 1. Characteristics used in classifying selected heterotrophic and petroleum-degrading bacteria

Character no.	Character category and description
	(The following tests were performed at 20-22° C; media at salinity of 12‰ and final pH 7.6, unless otherwise indicated)
	<u>Cell Morphology and Arrangement</u>. Heterotrophic Medium Broth (HMB). Wet mount of log phase cultures, phase contrast microscopy.
	A. Length.
1	$< 1.0\mu$
2	$1.0 - 2.0\mu$
3	$2.1 - 3.0\mu$
4	$> 3.0\mu$
5	Variable, < 75% cells in above categories
	B. Width.
6	Length x Width 3:1 - Slender
7	2 - 3:1 - Short, stout
8	1:1 - Oval
9	Variable width
	C. Shape
10	Straight
11	Curved
12	Spiral
13	Coccal-Bacillary
14	Coccus (Sherical)
15	Branching
16	Pleomorphic
	D. End.
17	Square-ended
18	Round-ended
19	Tapered end
20	Fusiform end
21	End variable
22	Blank

Character no.	Character category and description
	E. Arrangement. (Predominant form in any category > 75%)
23	Single
24	Pair
25	Chain - short (≤5 cells/chain)
26	Chain - long (>5 cells/chain)
27	Coccal - clumping
28	Coccal - packet
29	Filaments - Length x Width 20:1
30	Variable
	Motility. HMB. Wet mount of log phase cultures, phase contrast microscopy.
31	Motile
	Gram Stain Reaction. (Hucker and Conn, 1923) Early stationary phase culture on HMA slant.
32	Gram positive
33	Gram negative
34	Gram variable
	Colony. Single colonies on HMA after 2 weeks growth.
	A. Size.
35	< 1 mm
36	1 - 3 mm
37	>3 mm
	B. Form.
38	Punctiform
39	Circular
40	Filamentous
41	Irregular
42	Rhizoid
43	Spindle
	C. Elevation.
44	Flat
45	Raised
46	Convex
47	Pulvinate
48	Umbonate
	D. Margin (Edge).
49	Entire
50	Undulate
51	Lobate
52	Erose

Character no.	Character category and description
53	Filamentous
54	Curled
	E. Density of Colony.
55	Transparent
56	Translucent
57	Opaque
58	Spreading on HMA plates
Pigment Formation.	
	A. Non-diffusable pigments. Colony pigmentation of stationary phase cultures on HMA plates.
59	White
60	Off-white to grey
61	Yellow
62	Orange
63	Pink
64	Red
65	Green
66	Brown
67	Blue
68	Violet-purple
69	Black
	B. Diffusable pigments.
70	Yellow — Pseudomonas F Agar (Difco) (King, et al., 1954)
71	Green — Pseudomonas F Agar (Difco) (King, et al., 1954)
72	Brown — Pseudomonas F Agar (Difco) (King, et al., 1954)
73	Brown — Pseudomonas P Agar (Difco) (King, et al., 1954)
74	Flourescent under ultraviolet light - Pseudomonas F Agar (Difco)
Growth in Liquid Medium. HMB tubes, cultures were shaken on Vortex mixer and turbidity was observed of cultures at 3, 7, 17, 21 days after inoculation. Maximum turbidity during the 21 days was recorded as absorbance (650 nm).	
75	Slight ($0.0 < \text{Absorbance} \leq 0.4$)
76	Moderate ($0.4 < \text{Absorbance} \leq 0.8$)
77	Heavy ($0.8 < \text{Absorbance} \leq \infty$)
78	Even turbidity
79	Flocculent turbidity

Character no.	Character category and description
80	Blank

Physiology.

A. Temperature of growth. HMB tubes. Cultures incubated at different temperatures. Positive result was visible within the following time periods: 1° C - 6 weeks; 10°C - 3 weeks; 22°C - 1 week; 35°C - 5 days; 45° C - 3 days.

81 1°C
82 10°C
83 22°C
84 35°C
85 45°C

B. pH growth range. pH adjusted HMB tubes for 4.5 - 8.5 pH range. For pH 9.5 and 10.5, excessive precipitation in HMB tubes resulted so the following was used: 1 g Yeast Extract, 1 g Proteose Peptone, 1000 ml Artificial Estuarine Water*. KOH and HCl were used for adjusting the pH. Positive result was growth within 5 days.

*Artificial Estuarine Water (AEW) - Modification of MacLeod (1968) artificial seawater, lowered salt concentrations to an equivalent 12‰ salinity.

8.00 g NaCl
0.23 g KCl
1.76 g $MgCl_2 \cdot 6H_2O$
2.33 g $MgSO_4 \cdot 7H_2O$
1000 ml distilled water

86 4.5
87 5.5
88 6.5
89 7.5
90 8.5
91 9.5
92 10.5

C. Salinity range. HMB tubes, aged Middle Atlantic Ocean seawater, diluted with distilled water to proper salinity. Positive result was growth within 5 days.

93 2 ‰
94 12 ‰
95 22 ‰

Character no.	Character category and description	
96	32‰	
	D. Cation requirements. Modification of Colwell and Wiebe (1970), reduction of cation concentrations to approximate estuarine (12‰) salinity. Medium: 0.1% Casamino Acids, 0.05% Yeast Extract, 1000 ml Distilled Water. Positive result was growth within normal time. Added $CaCl_2$, $MgSO_4$, KCl, and NaCl as follows:	
97	Na^+ requirement	Ca 0.0035 M Mg 0.0098 M K 0.0035 M
98	Ca^{++} requirement	Na 0.14 M Mg 0.0098 M K 0.0035 M
99	K^+ requirement	Na 0.14 M Ca 0.0035 M Mg 0.0098 M
100	Mg^{++} requirement	Na 0.14 M Ca 0.0035 M K 0.0035 M
101	Blank	

<u>Biochemistry</u>.

A. Liefson MOF (Liefson, 1963).

102 Aerobic acid
103 Aerobic gas
104 Anaerobic acid
105 Anaerobic gas

B. Acid and gas production from carbohydrates. Medium:

Casamino acids	1.0 g
Yeast extract	0.1 g
$(NH_4)_2SO_4$	1.0 g
K_2HPO_4	0.1 g
Phenol red	0.018 g
Estuarine water	1000 ml

Added inverted Durham tube. Autoclaved 15 lb., 15 min., pH 7.6. Added carbohydrate to 0.5% concentration.

106 Galactose acid
107 Galactose gas

Character no.	Character category and description
108	Glucose acid
109	Glucose gas
110	Lactose acid
111	Lactose gas
112	Mannitol acid
113	Mannitol gas
114	Ribose acid
115	Ribose gas
116	Sucrose acid
117	Sucrose gas
	C. Miscellaneous utilization of organic compounds.
118	Starch (Cowan and Steel, 1970)
119	Blank
120	Cellulose. Medium consisted of 0.1% $(NH_4)_2SO_4$, 0.01% Yeast Extract, 1000 ml EW. A strip of #1 Whatman filter paper was added to each tube. Positive result if, with gentile shaking, the paper strips could be broken at the air-water interface. Incubation period: 4 weeks.
121	Chitin. Modification after Hood (1973): chitin (Calbiochem - unspecified purity, San Diego, CA) was ball milled at 4°C for 48 h , "dissolved" in 50% H_2SO_4 and precipitated by the addition of large volumes of distilled water. This precipitate was centrifuged, washed with distilled water, and neutralized to a pH of 7.0. A bi-layer petri dish technique was employed with a base layer of HMA overlayed with 10 ml of medium consisting of 3% chitin, 0.05% yeast extract, 1.5% agar in EW.
122	Hexadecane. ESWB with 3 drops filter sterilized hexadecane as sole added carbon source.
123	Gelatin liquefaction - Frazier method
	D. Decarboxylase enzymes (Moeller,1955). AEW used.
124	Arginine
125	Lysine
126	Ornithine
	E. Nitrate reduction, Griess-Ilosvay method. Nitrate Broth (Difco), Durham tubes added to check for gas production, and Zn metal added after proper incubation period to check for nitrate presence.

Character no.	Character category and description
127	$NO_3 \rightarrow NO_2$
128	$NO_2 \rightarrow N_2$
	F. Lipolytic Activity (Sierra, 1957).
129	Tween 20 (polyoxyethylene (20) sorbitan monolaurate)
130	Tween 40 (polyoxyethylene (20) sorbitan monopalmitate)
131	Tween 60 (polyoxyethylene (20) sorbitan monostearate)
132	Tween 80 (polyoxyethylene (20) sorbitan monooleate)
Miscellaneous Microbiological Tests.	
133	Kovacs' oxidase test (Kovacs, 1956)
134	Catalase. 3% H_2O_2 dropped on early stationary phase colonies on HMA plates
135	Hydrogen sulfide production from sodium thiosulfate (Colwell and Quadling, 1962)
Antibiotic Sensitivities. HMA plates inoculated with 0.1 ml early stationary phase culture in HMB tubes. Read plates after visible growth was obtained. Zones 1 mm or greater from the edge of the disc were interpreted as positive sensitivity reactions.	
136	Pteridine O/129 (Shewan, Hodgkiss, and Liston, 1954), 400 mcg
137	Chloramphenicol, 30 mcg
138	Colistan (Coly-Mycin), 10 mcg
139	Neomycin, 30 mcg
140	Penicillin, 2 units
141	Penicillin, 10 units
142	Streptomycin, 10 mcg
143	Tetracycline, 30 mcg

Test results were coded on IBM computer cards into binary form using '+' for positive, '0' for negative, and 'b̸' for undetermined characters. Analysis of the data was performed on an IBM 370 computer using a numerical program, VOCD, developed at VIMS. Similarity coefficients using the coefficient of Jacard, S_j, in which negative matches were excluded from the calculations, were computed between every pair of strains. Subsequent clustering was derived by single linkage analysis using the S_j coefficient (Sneath and Sokal, 1973):

$$S_j = \frac{\text{number of positive matches}}{\text{number of comparisons - noncomparable characteristics}}$$

See Appendix B for a brief description of numerical taxonomy.

D. Bacterial strains.

Nineteen strains from the American Type Culture Collection (ATCC) were also completely analyzed for reference and for comparison of published characteristics with those determined under laboratory conditions. They were (genus, species, ATCC No.):

Acinetobacter calco-aeticus	23055
Aeromonas liquefaciens	14715
Aeromonas salmonicida	14174
Alcaligenes aquamarinus	14400
Alcaligenes cupidus	27124
Alclaigenes venustus	27125
Alteromonas communis	27118
Alteromonas macleodii	27126
Alteromonas marinopraesens	27127
Alteromonas vaga	27119
Cytophga marinoflava	19326

Flavobacterium tirrenicum	15997
Hyphomicrobium neptunium	15444
Pseudomonas bathycetes	23597
Pseudomonas coenobios	14402
Pseudomonas marina	25374
Pseudomonas nautica	27132
Serratia marinorubra	27614
Vibrio anguillarum	19264

The above strains represent a variety of genera that have been isolated from the marine environment.

RESULTS

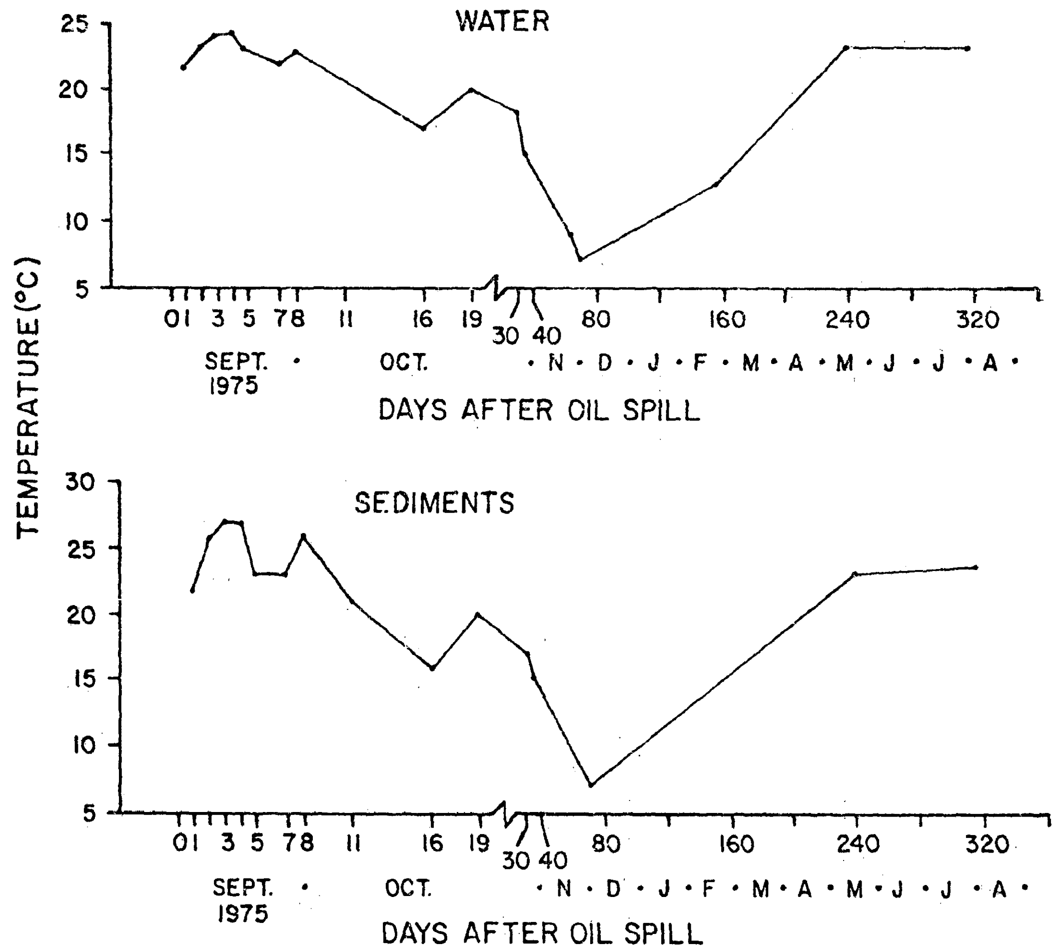
I. Field Data

Heterotrophic and petroleum-degrading bacteria levels in the marsh creek and sediments are tabulated in Appendix C. Calculations of the common logarithms of the ratio: (petroleum-degrading bacteria)/(aerobic heterotrophic bacteria) are also listed. Sediment and water temperatures for the sample period are shown in Figure 4.

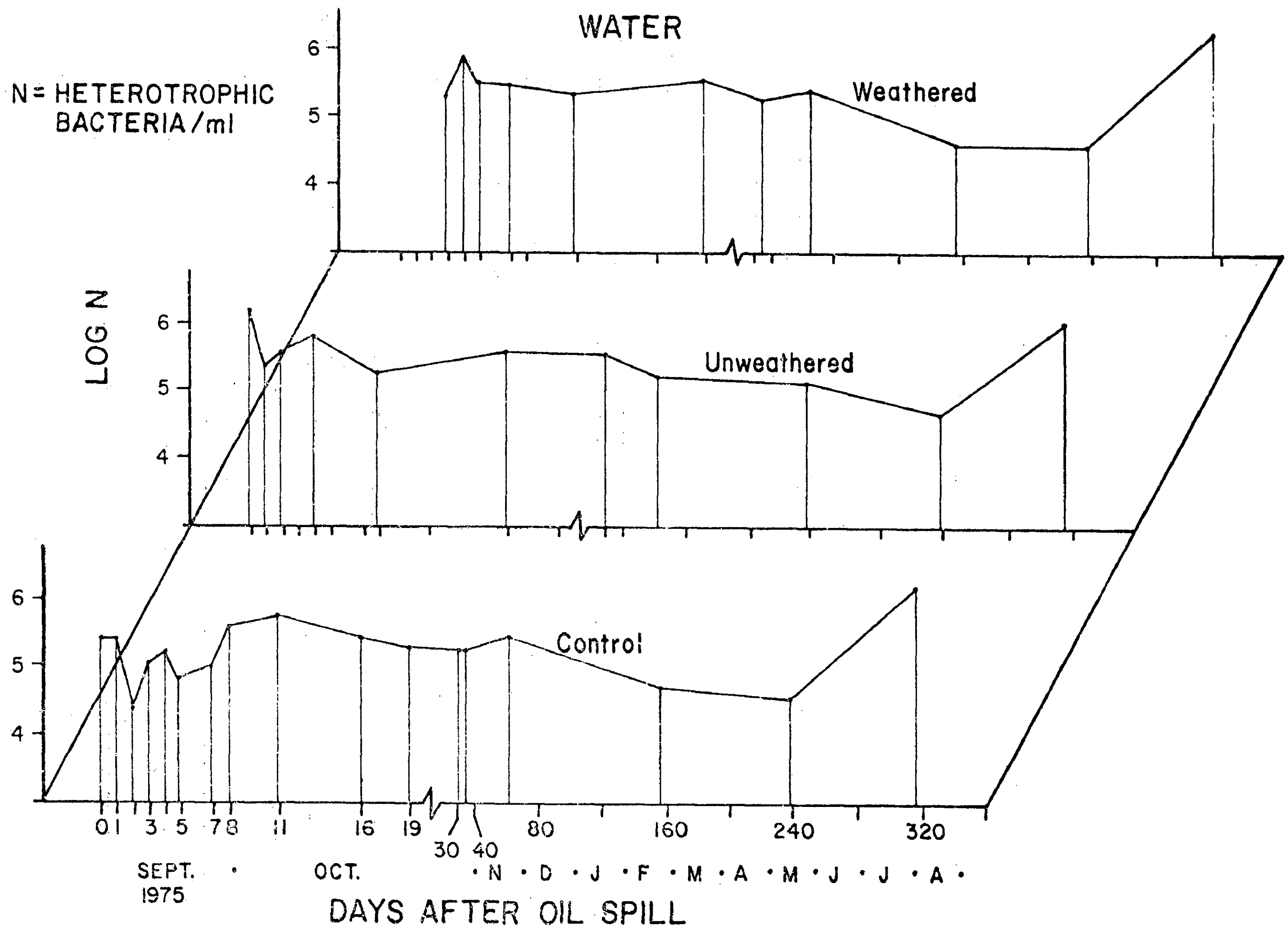
A. Heterotrophic bacteria.

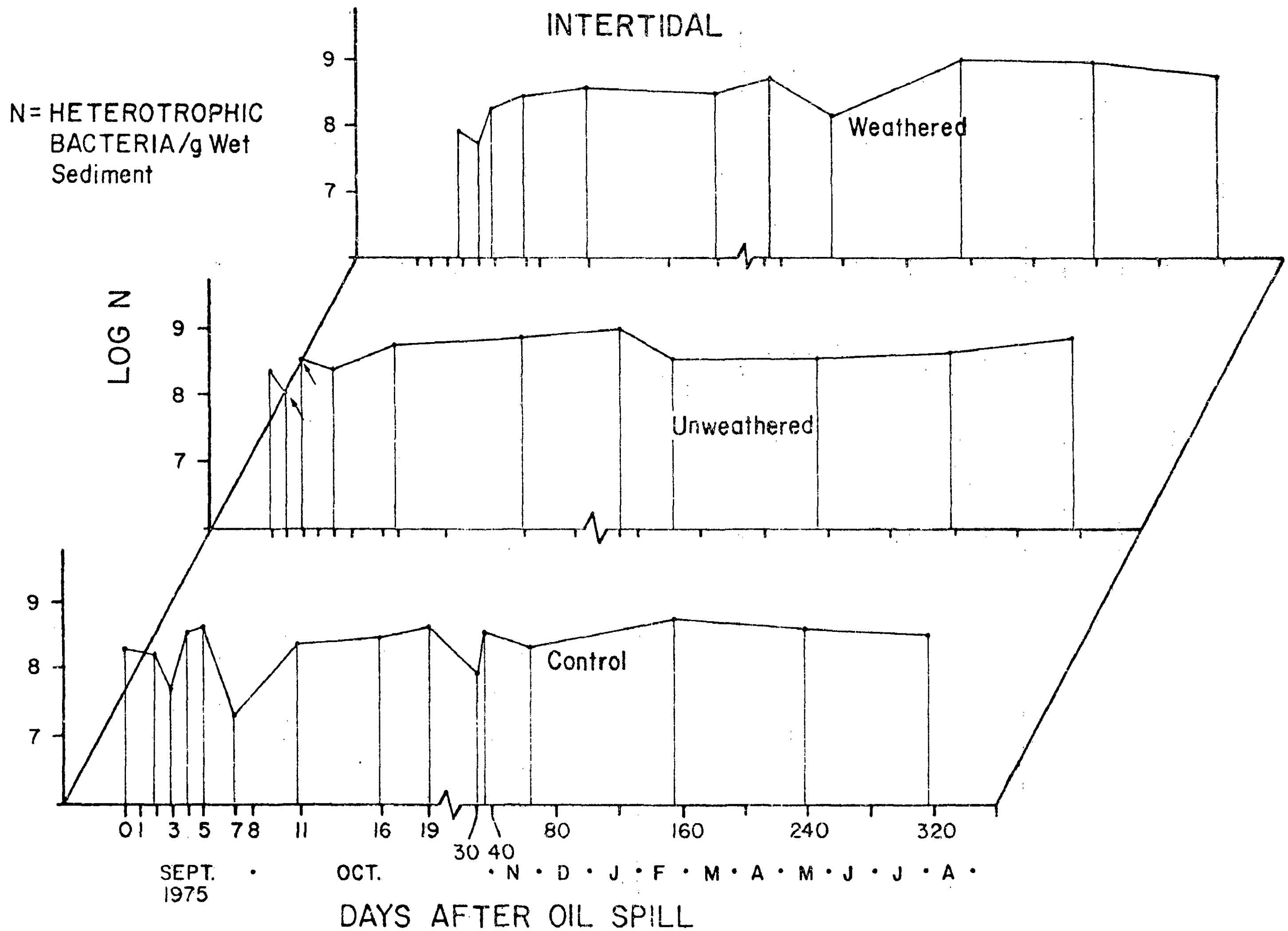
Heterotrophic bacterial levels following the oil spills are plotted for all zones in Figures 5 - 8. Creek values were 100-1000 times smaller than the 10^8 - 10^9 heterotrophic bacteria/g values found in the sediments. Fluctuations in the counts of heterotrophs immediately following the spill probably was due to a combination of complex, interacting processes as the marsh responded to the oil spillage. Counts in the creek water tended to decline after the minimum temperature of 7° C and then increase with warming. Populations within each enclosure were tested using the Kolmogorov-Smirnov statistic, D (Sokal and Rohlf, 1969), which is used to determine if deviations of observed continuous frequency distributions from expected ones are significant. Table 2 shows the results of this test for heterotrophs:

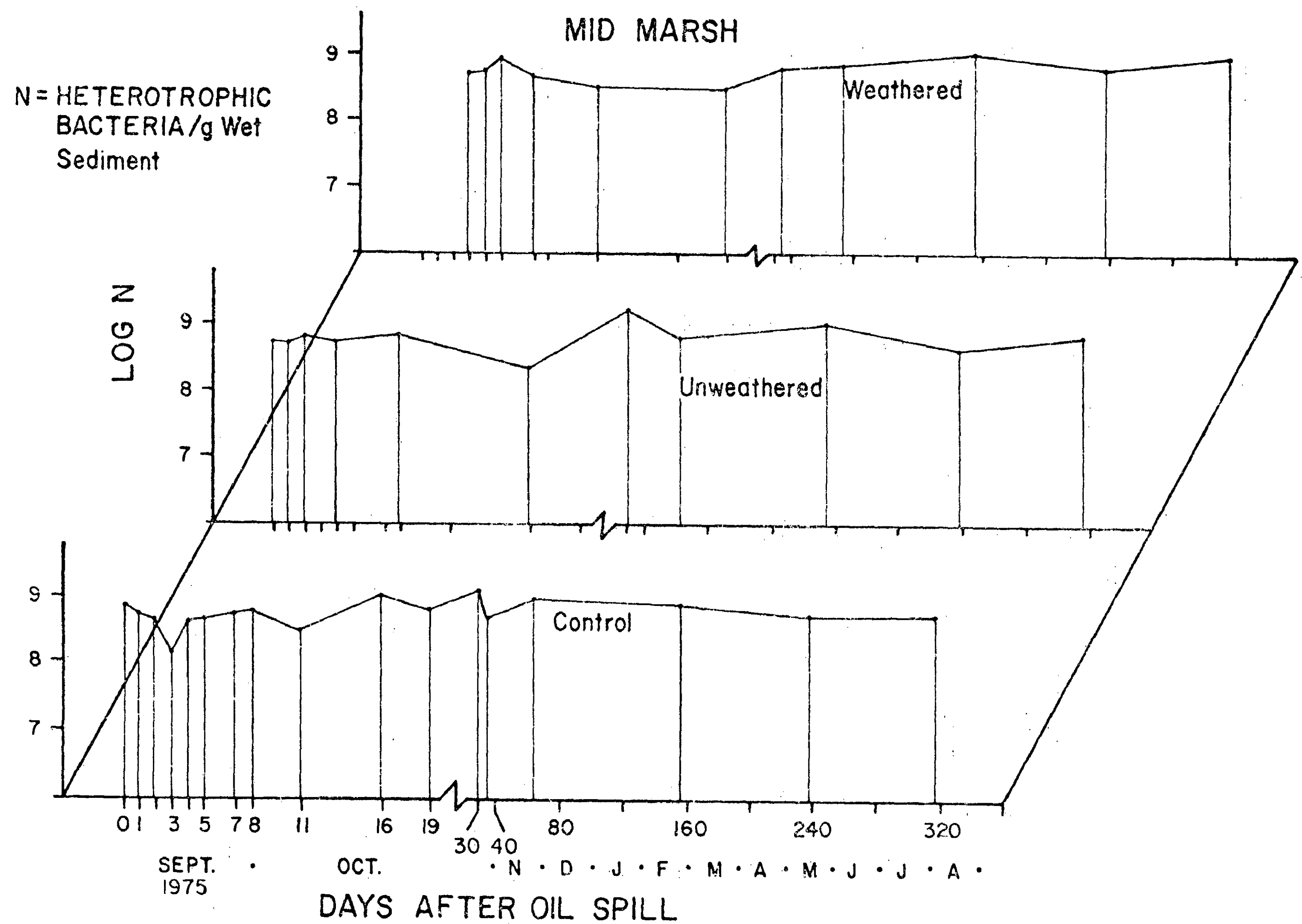
Figure 4. Water and sediment temperatures in the marsh recorded during sampling years 1975-1976.


WATER
SEDIMENTS
TEMPERATURE (°C)
DAYS AFTER OIL SPILL
SEPT. 1975
OCT.
N D J F M A M J J A

Figures 5, 6, 7, 8. Viable heterotrophic bacteria in tidal marsh water and sediment zones as a function of time after the oil spills. Note that oil spillage in the weathered enclosure occurred 3 days after oil spillage in the unweathered enclosure.


WATER
N= HETEROTROPHIC BACTERIA/ml
Weathered
LOG N
Unweathered
Control
6
5
4
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL


INTERTIDAL
N= HETEROTROPHIC BACTERIA/g Wet Sediment
LOG N
9
8
7
Weathered
Unweathered
Control
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL


MID MARSH
N = HETEROTROPHIC BACTERIA/g Wet Sediment
LOG N
Weathered
Unweathered
Control
9
8
7
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL

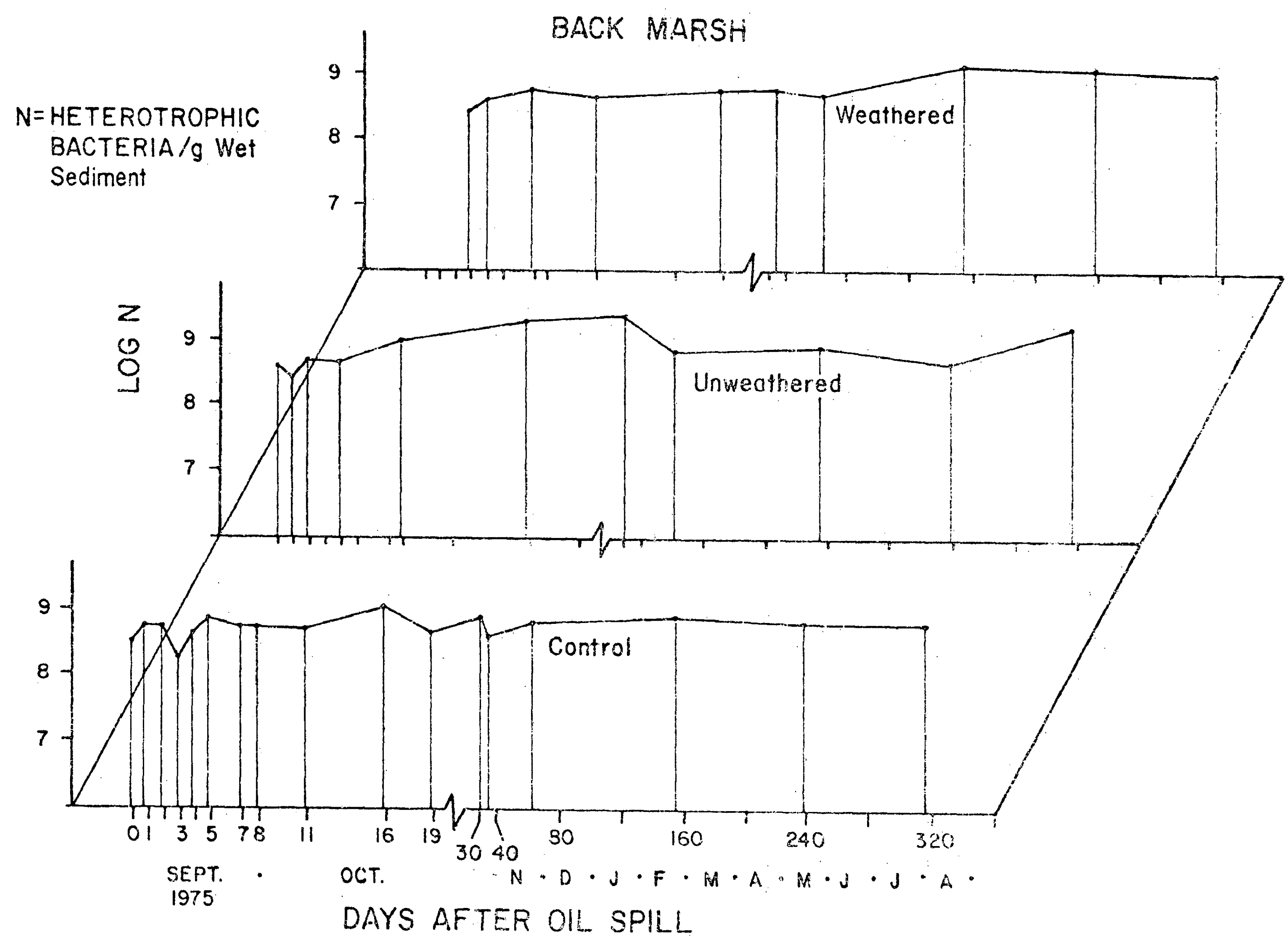

BACK MARSH
N= HETEROTROPHIC BACTERIA/g Wet Sediment
LOG N
9
8
7
Weathered
Unweathered
Control
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL

Table 2. Largest frequency difference for heterotrophic bacteria

Enclosure	N	Largest Frequency Difference
A (unweathered)	44	0.1687
B (weathered)	43	0.1248
C (control)	67	0.1325

No significant difference ($\alpha = 0.05$) between the observed continuous frequency distributions and the expected ones were found for heterotrophic population in any of the enclosures. Heterotroph field data were analyzed using a 2x2 factorial design with blocking (Sokal and Rohlf, 1969). This test was arranged to find if significant differences existed (1) between heterotroph populations in the enclosures (unweathered x weathered x control), (2) between the zones (creek x intertidal x mid-marsh x back-marsh), and (3) if interaction between the enclosures and the zones existed. As even sample sizes were required for this statistic, 24 heterotrophic control (C) data were not considered and only control values associated with the sampling periods of the unweathered (A) enclosure were used. Two missing data points were approximated by the method of Winer (1962). Degrees of freedom were adjusted for the anova table which had missing data. Data for 0, 1, 2, 4, 8, 16, 32 sampling dates for the weathered (B) enclosure were blocked together with corresponding 0, 1, 2, 4, 8, 16, 32 sampling dates for the unweathered (A) enclosure. Subsequent bimonthly sampling dates were blocked together yielding a total of eleven blocks for the eleven sampling periods. Table 3 shows the 2x2 factorial anova table for heterotrophic bacterial population levels following the oil spillage.

From the factorial design, significant (α = 0.05) difference was found in population levels between zones. Since data contained two estimated values, the heterotrophic population means from the four zones were tested using the Scheffe' test (Sokal and Rohlf, 1969). No significant (α = 0.05) differences were found between heterotrophic levels in intertidal, mid- and back-marsh sediments; and creek and intertidal zones. Significant differences were found between heterotroph levels in the creek and mid-marsh; creek and back-marsh zones.

B. Petroleum-degrading bacteria.

The response of petroleum-degrading bacteria to the oil spill was a rapid increase in population within several days after the oil spill (Figures 9-12). Generally, counts tended to reach their maximum values in the intertidal zones before the mid- and back-marsh zones. This probably reflected time differentials between zones receiving oil through tidal flooding. Elevated levels of petroleum-degrading bacteria detected in control creek samples suggested that either petroleum-degrading bacteria and/or oil, washed from the oil dosed enclosures during ebbing tides, was transported into the control enclosure during flood tide. Petroleum slicks were often observed near the C enclosure during the flood tide.

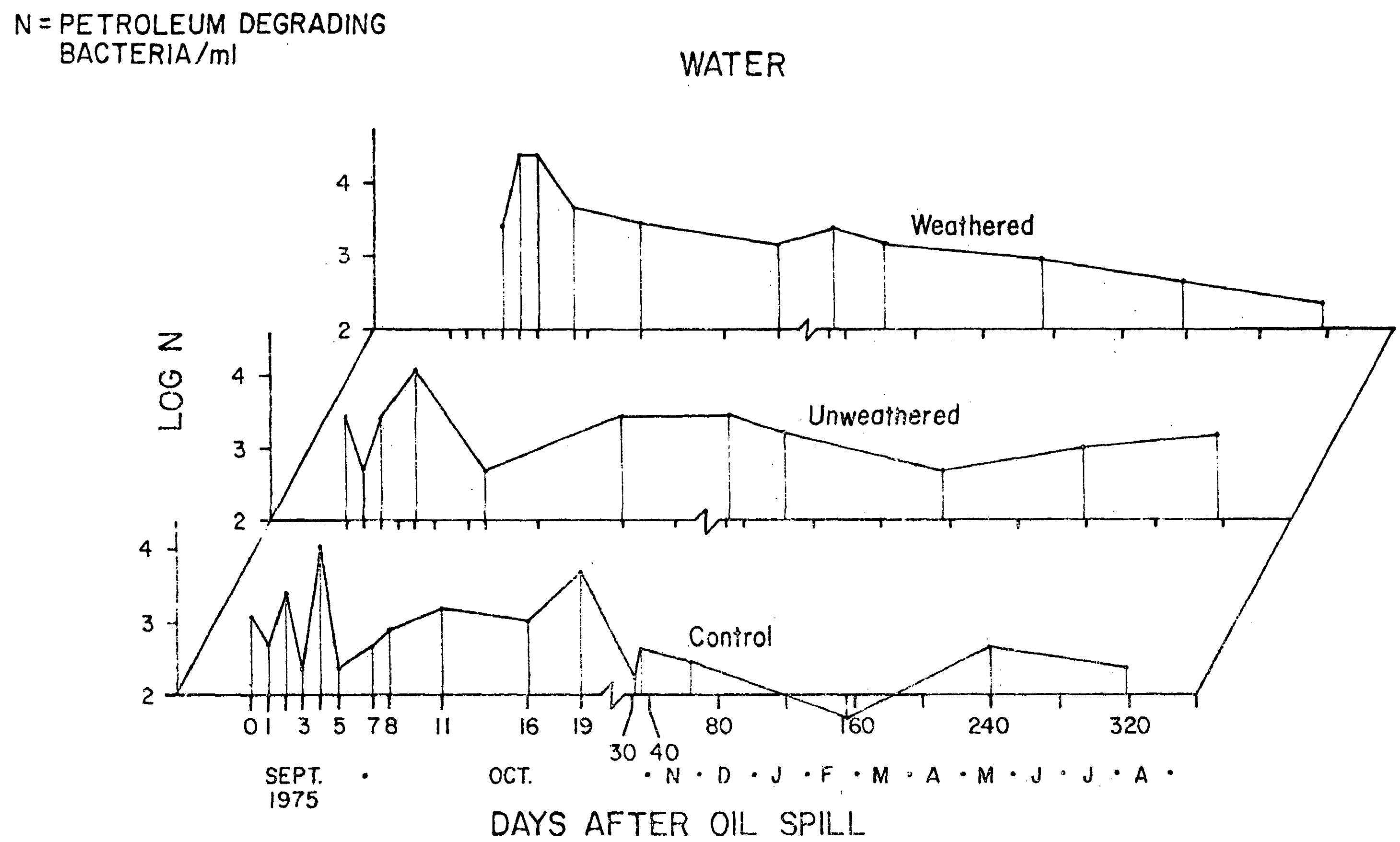
Using the Kolmogorov-Smirnov statistic, D, the petroleum-degrading population levels within each enclosure were tested for normality. The data were found to have significant (α = 0.05) deviation from the expected normal curve:

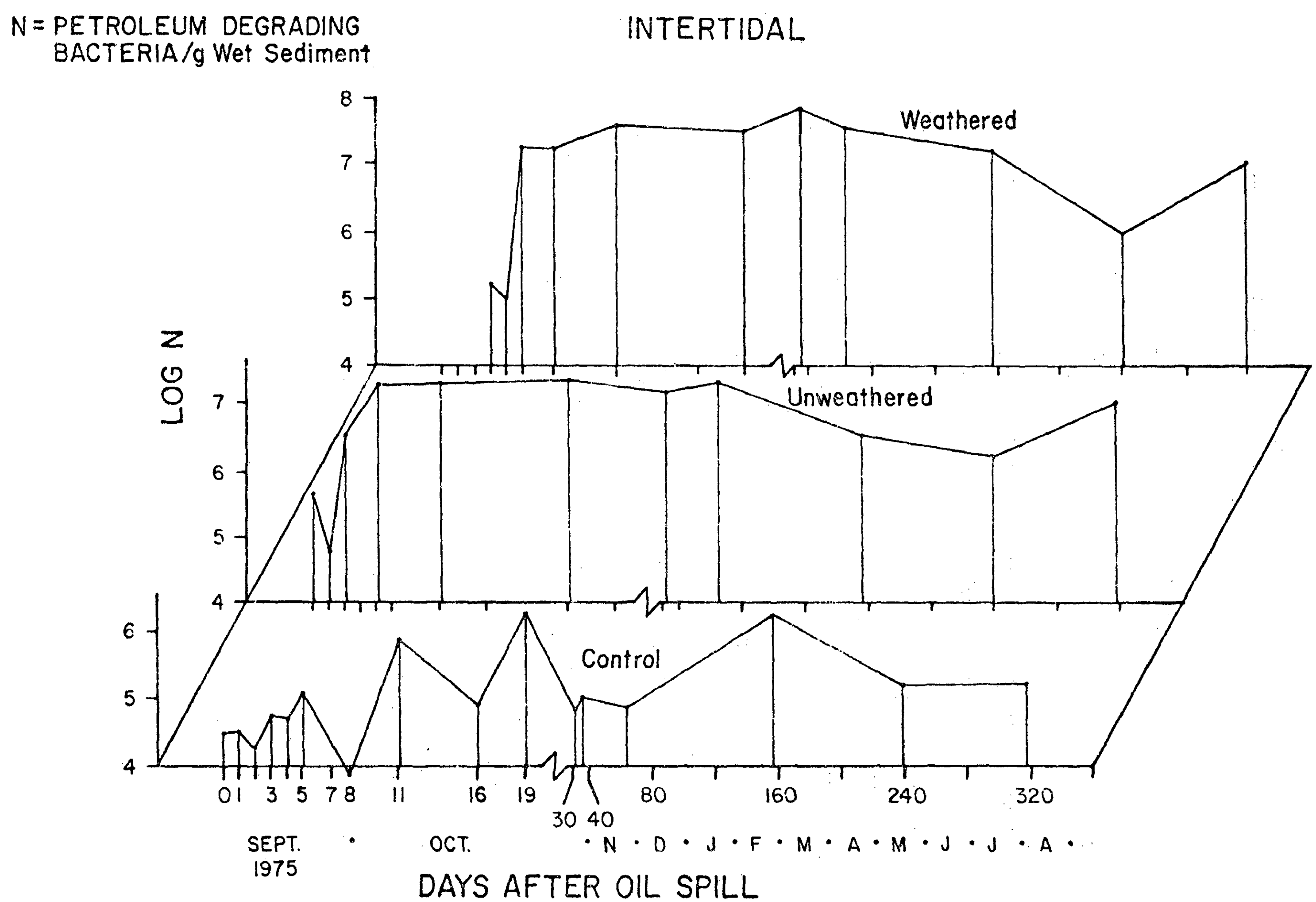
Table 3. 2x2 factorial anova for heterotrophic bacterial populations

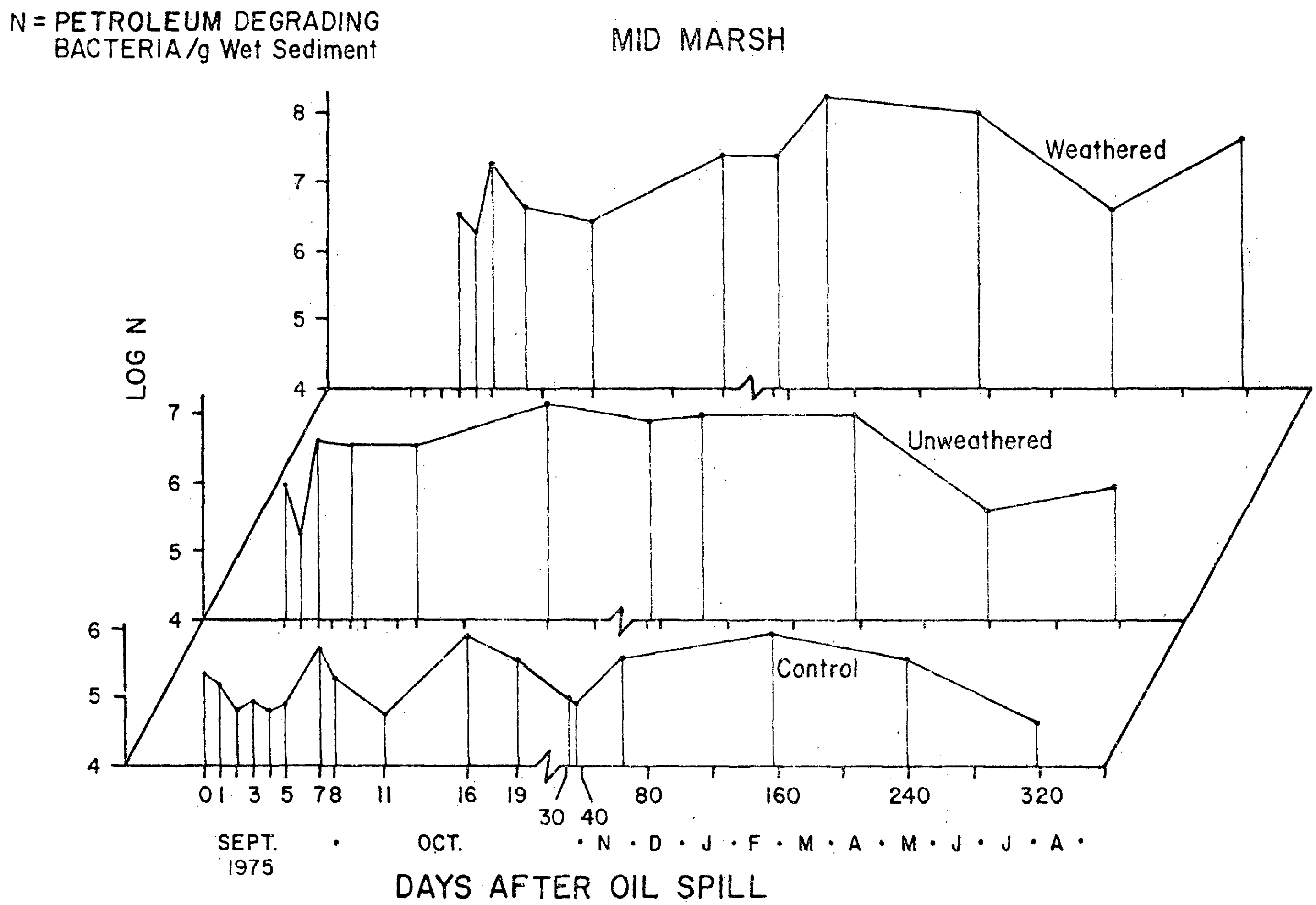
Source of Variation	DF	Sum Squares	Mean Squares	F
Blocks (Time)	10	2.548×10^{18}	2.548×10^{17}	
Enclosures	2	1.912×10^{17}	9.561×10^{16}	1.506
Zones	3	1.055×10^{19}	3.517×10^{18}	55.40*
Interaction	4	4.342×10^{17}	1.085×10^{17}	1.709
Error	110	6.984×10^{18}	6.349×10^{16}	
Total	129	2.071×10^{19}		

*significant $\alpha = 0.05$

Figures 9,10,11,12. Petroleum-degrading bacteria in tidal marsh water and sediment zones as a function of time after the oil spills. Note that oil spillage in the weathered enclosure occurred 3 days after oil spill in the unweathered enclosure.


N = PETROLEUM DEGRADING BACTERIA/ml
WATER
LOG N
Weathered
Unweathered
Control
2
3
4
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL


N = PETROLEUM DEGRADING BACTERIA/g Wet Sediment
INTERTIDAL
LOG N
Weathered
Unweathered
Control
8
7
6
5
4
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL


N = PETROLEUM DEGRADING BACTERIA/g Wet Sediment
MID MARSH
LOG N
Weathered
Unweathered
Control
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL

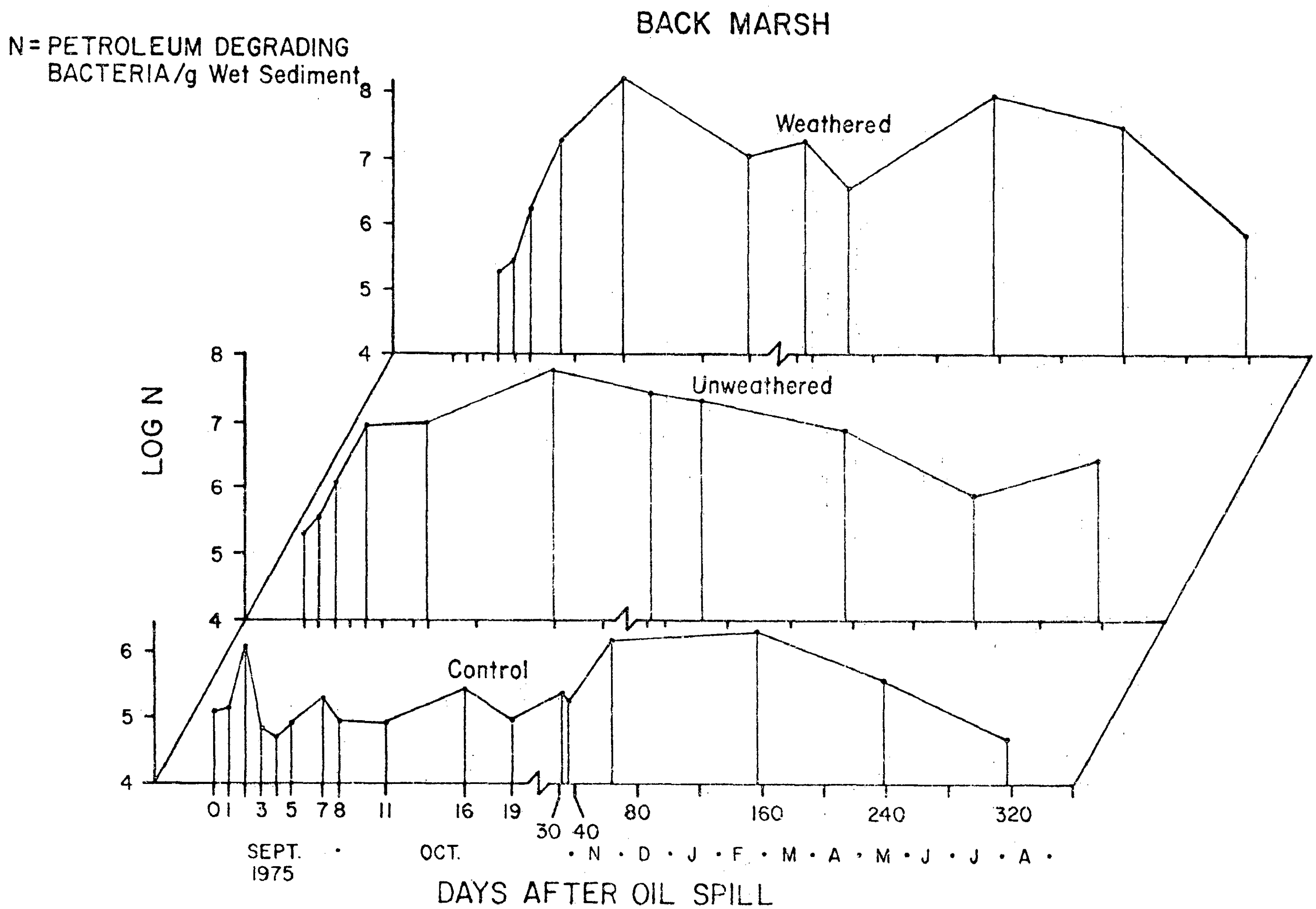

BACK MARSH
N = PETROLEUM DEGRADING
BACTERIA/g Wet Sediment
LOG N
Weathered
Unweathered
Control
8
7
6
5
4
O 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT.
1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL

Table 4. Largest frequency difference for petroleum-degrading bacteria

Enclosure	N	Largest Frequency Difference
A	44	0.2436*
B	44	0.2643*
C	67	0.3072*

*significant at $\alpha = 0.05$

A log transformation, $\log(x+1)$, was performed with the following result:

Table 5. Largest frequency difference for petroleum-degrading bacteria after log transformation of data

Enclosure	N	Largest Frequency Difference
A	44	0.1560
B	44	0.1546
C	67	0.2058*

*significant at $\alpha = 0.05$

Log transformations for the petroleum-degrading bacterial populations normalized the data for the A and B enclosures. Significant difference ($\alpha = 0.05$) from the expected cumulative frequency distribution was found in enclosure C, although the largest frequency difference was much smaller than for nontransformed data. For further statistical analysis, all petroleum-degrading bacterial population data was logarithmically transformed.

Submitting the petroleum-degrading bacterial data to a 2x2 factorial design with blocking yielded expected results (Table 6). As with the heterotrophic data, equivalent sample sizes were arranged for all blocks. Significant ($\alpha = 0.05$) differences in petroleum-degrading populations were found (1) between zones (creek x intertidal x mid-marsh x back-marsh), (2) between enclosures (unweathered x weathered x control), and (3) zone x enclosure interaction was found significant ($\alpha = 0.05$).

As significant differences existed for both statistic treatments (zones and enclosures) and interaction existed, the mean petroleum-degrading bacterial levels for each zone within the three enclosures were compared with each other using the Student-Newman Keuls test (Sokal and Rohlf, 1969). Results are shown in Table 7. Importantly, significant ($\alpha = 0.05$) differences between creek values for the enclosures were not found. Although the weathered enclosure sediments tended to maintain larger populations of petroleum-degrading bacteria compared with the unweathered enclosure, the differences were not statistically significant. Marsh sediment levels differed significantly from the creek population levels, and both oil polluted enclosures maintained population levels of petroleum-degrading bacteria that were significantly greater than the control sediment levels.

C. Ratio of petroleum-degrading bacteria to heterotrophic bacteria.

Logarithms of the ratio of petroleum-degrading bacteria to heterotrophic bacteria are plotted in Figures 13-16. Enrichment of petroleum-degraders is indicated as the ratio value increases approaching zero. An initial increase in the

Table 6. 2x2 factorial anova for petroleum-degrading populations

Variance	DF	Sum Squares	Mean Square	F
Blocks (Time)	10	17.72	1.772	
Enclosures	2	51.62	25.81	64.5*
Zones	3	246.3	82.11	205.3*
Interaction	6	8.458	1.410	3.525*
Error	110	43.99	.3999	
Total	131	368.1		

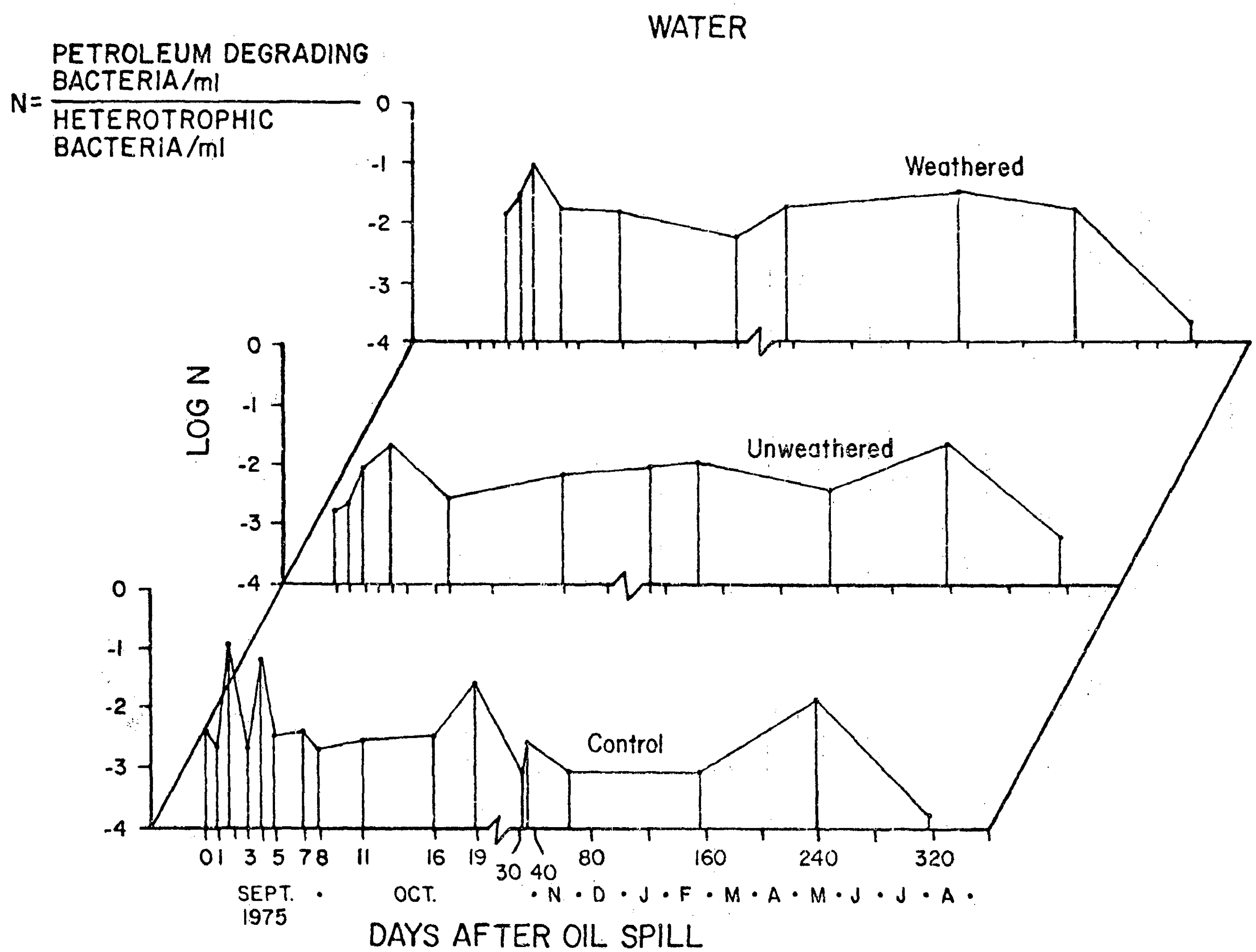
*significant $\alpha = 0.05$

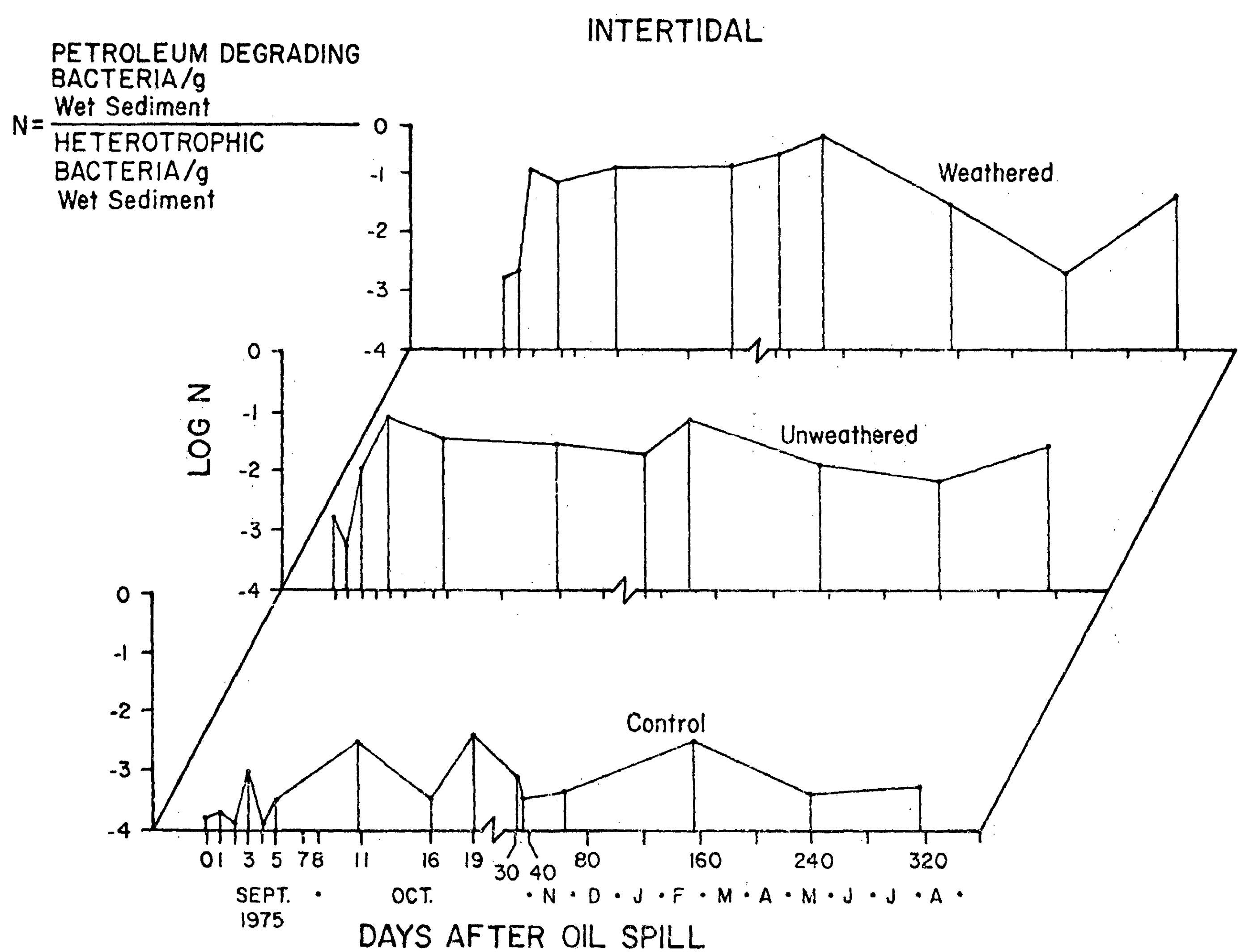
Table 7. Student-Newman Keuls test for petroleum-degrading bacterial populations

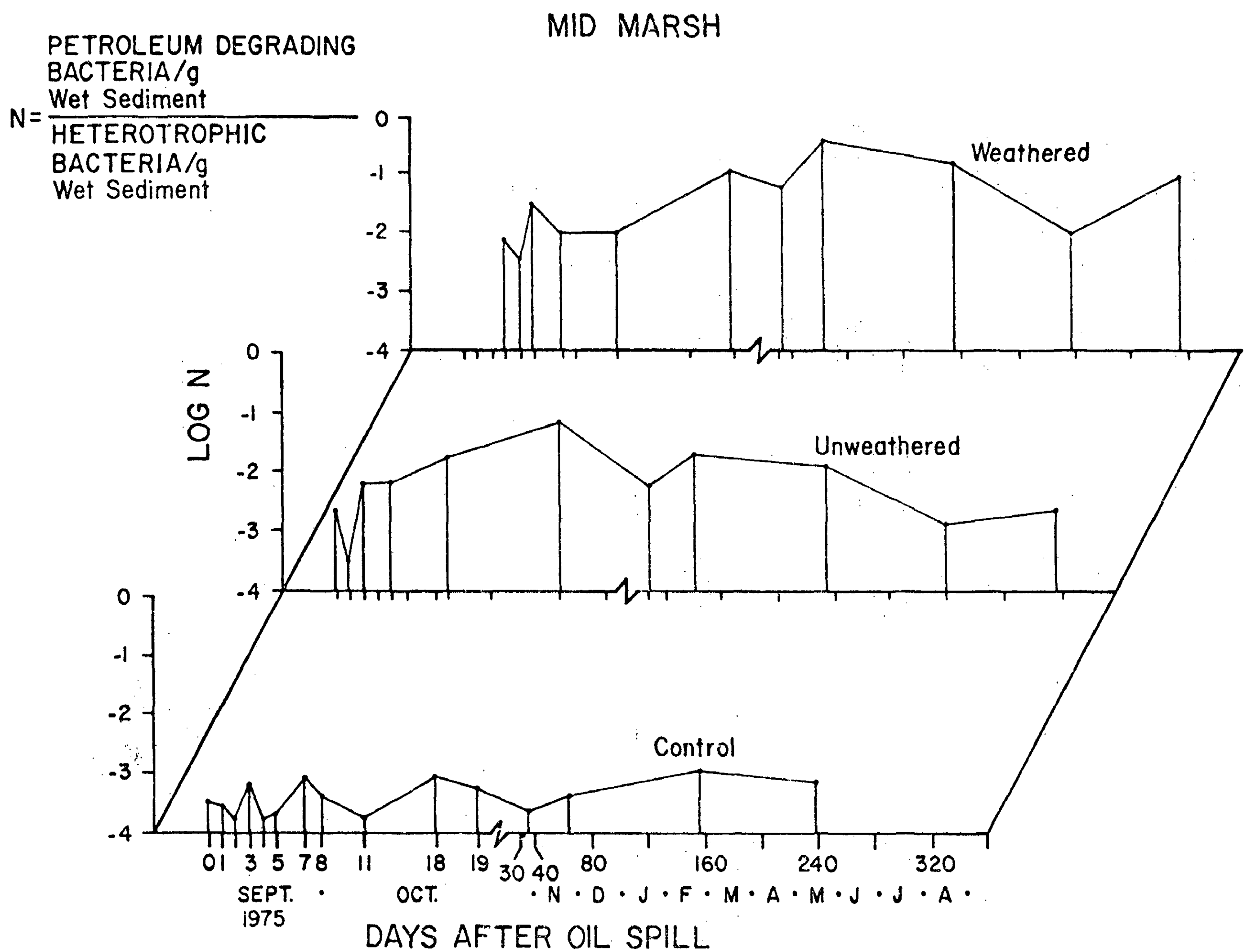
Arranging the treatment means in ascending order:

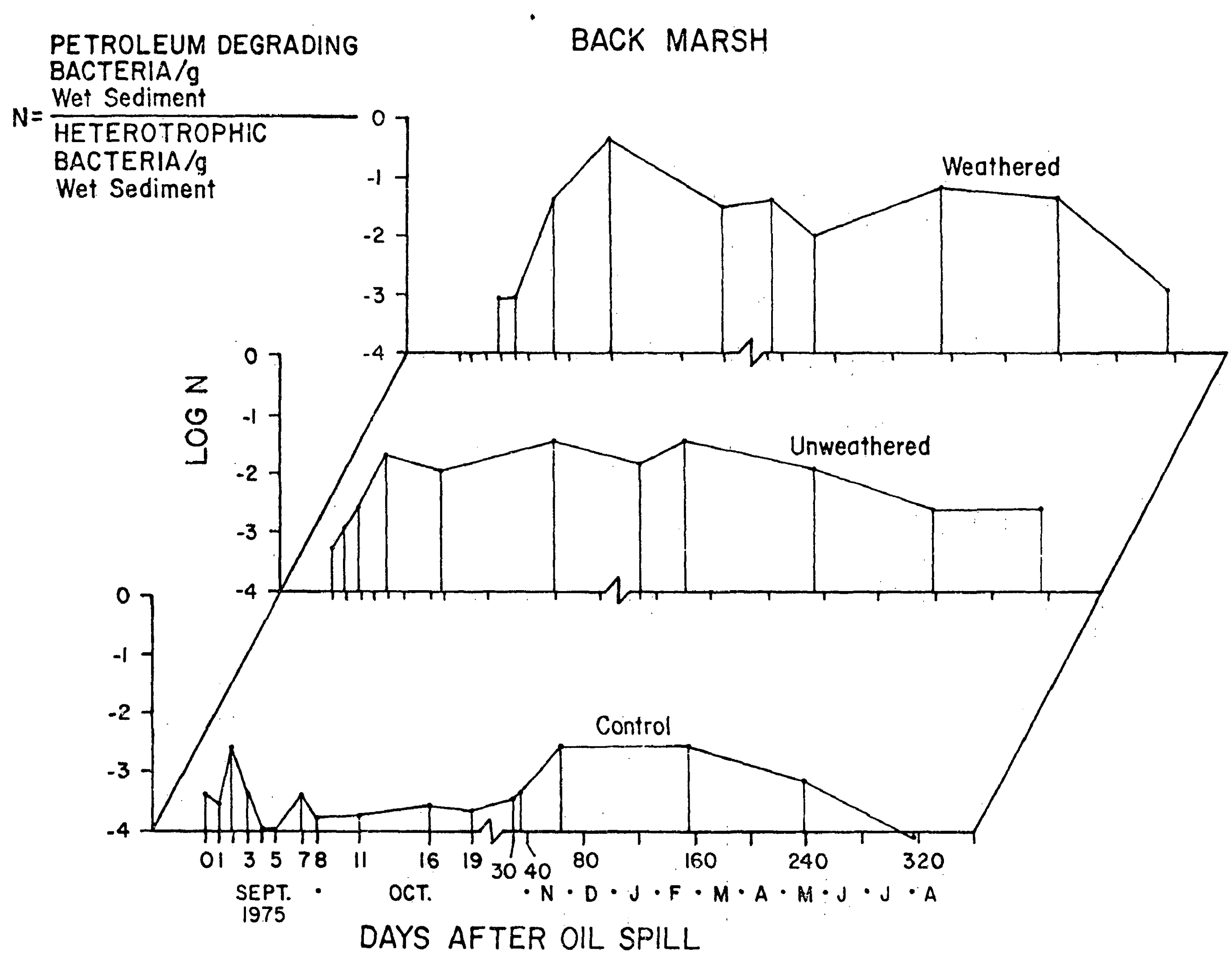
Enclosure	Zone	Mean $\left(\sum_{i=1}^{n} (\log x)/n\right)$	Significance Range
C	Creek	2.74	No significant difference
A	Creek	3.11	
B	Creek	3.36	
C	Intertidal	4.84	No significant difference
C	Mid-marsh	5.29	
C	Back-marsh	5.43	
A	Mid-marsh	6.42	No significant difference
A	Back-marsh	6.61	
A	Intertidal	6.63	
B	Back-marsh	6.81	
B	Intertidal	6.87	
B	Mid-marsh	7.11	

Figures 13,14,15,16. Log $\left(\frac{\text{Petroleum-degrading bacteria}}{\text{Heterotrophic bacteria}}\right)$ in marsh water and sediment zones as a function of time after the oil spills.


WATER
PETROLEUM DEGRADING BACTERIA/ml
N= HETEROTROPHIC BACTERIA/ml
LOG N
0
-1
-2
-3
-4
Weathered
Unweathered
Control
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975 • OCT. • N • D • J • F • M • A • M • J • J • A •
DAYS AFTER OIL SPILL


INTERTIDAL
N = PETROLEUM DEGRADING BACTERIA/g Wet Sediment / HETEROTROPHIC BACTERIA/g Wet Sediment
LOG N
0
-1
-2
-3
-4
Weathered
Unweathered
Control
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL


MID MARSH
N = PETROLEUM DEGRADING BACTERIA/g Wet Sediment / HETEROTROPHIC BACTERIA/g Wet Sediment
LOG N
0
-1
-2
-3
-4
Weathered
Unweathered
Control
01 3 5 78 11 18 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL


BACK MARSH
PETROLEUM DEGRADING BACTERIA/g Wet Sediment
N= HETEROTROPHIC BACTERIA/g Wet Sediment
LOG N
0
-1
-2
-3
-4
Weathered
Unweathered
Control
0 1 3 5 7 8 11 16 19 30 40 80 160 240 320
SEPT. 1975
OCT.
N D J F M A M J J A
DAYS AFTER OIL SPILL

ratio was seen the first week for sediment zones in the _B_ and _C_ enclosures. Values of the ratio in water suggested tidal transport of petroleum-degrading bacteria from oil dosed enclosures into the _C_ creek zone, but creek zones for the oil polluted enclosures (_A_ and _B_) did not show dramatic ratio increases as seen in the sediments. Ratio values in sediments appeared to increase faster in the intertidal zone, and were maintained at higher levels in the weathered oil enclosure relative to unweathered and control enclosures. At no time did the ratio become larger than -2 for the control sediment zones, while it exceeded -2 approximately 60% of the year, and -1 10% of the year in the oil polluted sediment zones.

II. Taxonomic Evaluation of Heterotrophic and Petroleum-Degrading Bacterial Isolates.

A. Preliminary analysis.

Taxonomic tests performed on dominant heterotroph and petroleum-degrading isolates suggested differences in the generic composition of these populations. Petroleum-degrading bacteria appeared more "hardy and adaptable" to laboratory conditions, i.e. withstood repeated transfers without as great a loss in viability as heterotrophs. Though the majority of cells in both populations were morphologically rod shaped, single cell arrangement was more predominant in petroleum-degrading isolates. Petroleum-degraders were more frequently motile and oxidase positive, less varied in pigmentation than heterotrophs. Generally, heterotrophic bacteria were more sensitive to antibiotics. Analysis of 378 heterotrophic and 315 petroleum-degrading isolates using a numerical taxonomy program (Lewis, 1973), indicated that petroleum-degrading bacteria had greater similarity with each other than the heterotrophs. Dominant heterotrophic isolates displayed greater phenotypic heterogeneity than petroleum-degrading isolates.

The Shewan (1963) scheme, designed for gram negative rods found in the marine environment, does not provide for the identification of gram positive bacteria. Gram positive motile rods were considered during the preliminary analysis to be probable <u>Bacillus</u> species, while many gram positive cocci fit the description of <u>Micrococcus</u> (Buchanan and Gibbons, 1974). <u>Spirillum</u> species were identified by purely morphological features, as were the Actinomycetes-type (which included cornyneform groups) (Buchanan and Gibbons, 1974).

Tentative identifications of all heterotrophic and petroleum-degrading bacterial isolates are listed in Appendix D. Results of the preliminary analysis indicated a heterotrophic population dominance by *Pseudomonas* species in the unweathered (39%) and weathered (32%) enclosures, while *Flavobacterium* species dominated in the control enclosure (32%) for the sampling year. Less than 10% of all heterotrophic isolates demonstrated fermentative abilities. For the three enclosures, gram positive bacteria constituted a maximal value of 11%. Table 8 lists the percentage generic heterotrophic composition found during the preliminary analysis.

Pseudomonas species overwhelmingly dominated the petroleum-degrading bacterial isolates. Over 60% of the isolates from the three enclosures were *Pseudomonas* spp., though many of the genera found during the heterotrophic analysis were present among the petroleum-degraders. Less than 3% were gram positive bacteria. Table 9 lists the percentage generic composition for the petroleum-degrading isolates.

B. Intensive analysis.

Data from the intensive analysis is represented in a dendogram cluster analysis (Figure 17). The dendogram was divided into 13 clusters at similarity levels of approximately 60%. Owing to the wide variety of organisms encountered, this level was selected for grouping rather than the higher 70-75% value used in several recent publications. These publications have usually concerned groups of bacteria that have been "preselected", i.e. enriched, and would be expected to have a high similarity to begin. Though each cluster appeared to be distinct, difficulty was found assigning traditional genera names to certain clusters. Some general characteristics for each cluster are found in Table 10.

Bergey's Manual of Determinative Bacteriology (Buchanan

Table 8. Percentage of heterotrophic genera of 3 enclosures from the preliminary taxonomic analysis

Tentative Identification	A(114)*	B(107)	C(157)
Pseudomonas	39	32	22
Flavobacterium	25	26	32
Alcaligenes	21	20	21
Enterobacteriaceae	4	3	3
Gram pos. rod (Bacillus)	3	9	10
Gram neg. cocci	3	1	1
Vibrio	2	4	1
Micrococcus	2	1	8
Actinomycetes-type	0	3	2
Spirillum	0	1	1
Aeromonas	0	1	0
Unknown	1	0	1

Table 9. Percentage of petroleum-degrading genera of 3 enclosures from the preliminary taxonomic analysis

Tentative Identification	A(86)*	B(77)	C(152)
Pseudomonas	63	60	73
Flavobacterium	6	8	6
Alcaligenes	18	23	7
Enterobacteriaceae	1	0	0
Gram pos. rod (Bacillus)	2	3	1
Vibrio	1	4	5
Micrococcus	1	1	0
Spirillum	7	0	4
Aeromonas	0	1	1
Unknown	0	0	4

*Number in parentheses indicates total number of isolates.

Figure 17. S_j dendrogram-cluster analysis for selected petroleum-degrading and heterotrophic bacterial isolates.

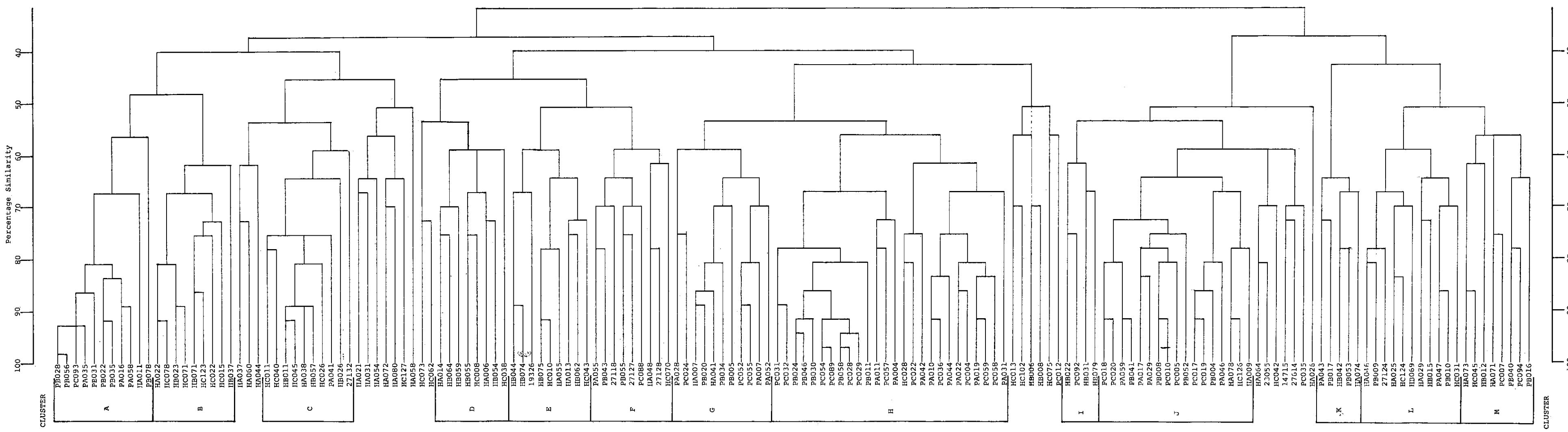

Percentage Similarity
40
50
60
70
80
90
100
CLUSTER
A
B
C
D
E
F
G
H
I
J
K
L
M

Table 10. Some general characteristics of petroleum-degrading and heterotrophic bacterial isolates based on a numerical taxonomic analysis

Cluster	A	B	C	D	E	F	G	H	I	J	K	L	M
Number in cluster	11	9	9	8	8	6	11	26	4	17	5	10	8
Character													
Single celled arrangement	10	0	8	1	5	2	11	24	4	17	1	10	5
Motile	0	1	1	3	1	4	10	26	3	16	4	6	4
Gram negative	11	4	9	7	8	5	11	26	1	16	5	10	8
Leifson MOF - aerobic acid	0	8	0	7	6	3	4	18	3	16	3	9	1
Leifson MOF - anaerobic acid	0	1	0	0	0	1	0	0	2	5	0	0	0
Galactose acid	10	1	0	5	5	2	4	9	4	16	2	6	1
Glucose acid	10	4	2	6	7	4	5	25	4	17	2	8	1
Mannitol acid	0	1	0	5	0	3	2	18	4	11	0	6	1
Ribose acid	9	1	0	3	2	5	7	8	2	14	1	6	0
Sucrose acid	2	5	1	7	2	5	4	8	4	9	1	4	4
Hexadecane	9	0	0	0	0	3	0	16	0	9	1	0	0
Arginine decarboxylase	8	6	9	6	6	6	7	25	4	11	5	8	7
Lysine	1	1	1	0	0	0	0	0	0	0	0	0	0
Ornithine	2	2	1	1	3	0	2	3	0	2	0	2	1
$NO_3 \rightarrow NO_2$	6	2	1	0	0	1	4	3	1	6	5	6	7
$NO_2 \rightarrow N_2$	0	0	1	1	0	0	1	0	1	3	1	1	1
Tween 80	6	7	5	2	7	4	7	25	2	17	5	0	2
Kovacs' oxidase	2	0	6	4	3	5	11	24	1	16	4	10	6
Pteridine sensitivity	5	5	4	5	7	4	0	2	0	3	2	0	4
Penicillin (10 units)	2	9	6	7	6	3	0	0	2	6	1	7	5

and Gibbons, 1974) was used as a guide for selecting genera names for each cluster of the dendogram. Names were chosen that would best represent the results of the intensive taxonomic testing for the majority of the members within each cluster. The following was considered:

Cluster A - nonmotile, gram negative rods, single cell arrangement, oxidase negative isolates, showing no acid production in MOF media, resistance to 10 units of penicillin, and utilizing hexadecane as sole added carbon source. Assigned: Acinetobacter sp.

Cluster B - nonmotile, a mixture of gram positive and negative isolates, showing chain formation, aerobic acid production in MOF media, oxidase negative, sensitive to 10 units of penicillin. Possible mixture including Micrococcus sp.

Cluster C - nonmotile, gram negative rods, single cell arrangement, showing little aerobic acid production from sugars, lipolytic, highly sensitive to penicillin. Assigned: Moraxella sp.

Cluster D - chaining morphology, nonmotile, gram negative, aerobically producing acid from sugars, sensitive to 10 units of penicillin. Cluster appears as saccharolytic Moraxella-like isolates.

Cluster E - nonmotile, gram negative, single cell arrangement, oxidase negative, colony pigments other than white. Assigned: Flavobacterium sp.

Cluster F - motile, gram negative rods, single cell morphology, oxidase positive, producing acid from sugars, lipolytic. Assigned: Pseudomonas sp.

Cluster G - motile, gram negative, single cell morphology, oxidase positive, some acid production from sugars, little sensitivity to all antibiotics, majority are lipolytic. Assigned: Pseudomonas sp.

Cluster H - the largest cluster, motile, gram negative, single sell arrangement, oxidase positive, aerobically producing acid from sugars, over 60% utilizing hexadecane as sole added carbon source, little sensitivity to all antibiotics, lipolytic. Assigned: Pseudomonas sp.

Cluster I - motile, gram positive, single cell morphology, acid production from sugars. Probable Bacillus sp.

Cluster J - second largest cluster, motile, gram negative, oxidase positive, produces acids from sugars, over half utilize hexadecane as sole added carbon source, and some isolates show fermentative capabilities, lipolytic. Tentatively identified as a mixture of Vibrio sp. and Pseudomonas sp.

Cluster K - motile, gram negative, chaining cell morphology, oxidase positive, nitrate reducing, lipolytic. Assigned: Pseudomonas sp.

Cluster L - majority are motile, gram negative, single cell morphology, oxidase positive, acid production from sugars, sensitive to penicillin. Assigned: Pseudomonas sp.

Cluster M - motile, gram negative, single cell morphology, oxidase positive, no acid production from sugars, sensitive to 10 units of penicillin. Assigned: Pseudomonas sp.

Source (enclosure) of the heterotrophic or petroleum-degrading bacteria appears to make little difference in the clustering analysis result (Table 11). Data suggest that the petroleum-degraders are a homogeneous group with 35% belonging to Cluster H (Pseudomonas) and an additional 50% found only in 4 other clusters (A,F,G,J). The heterotrophic isolates are a more diversified group scattered throughout several clusters of the dendogram. Distinct taxonomic differences between the petroleum-degrading population and the dominant heterotrophic organisms is demonstrated by the "exclusive" nature of several clusters, i.e., either the hetero-

Table 11. Number of petroleum-degrading and heterotrophic bacterial isolates from each enclosure within each cluster

Cluster	A	B	C	D	E	F	G	H	I	J	K	L	M
Number in cluster	11	9	9	8	8	6	11	26	4	17	5	10	8
<u>Heterotrophic bacteria</u>	2	9	8	8	8	2	2	1	3	3	2	7	4
Enclosure													
A	1	1	1	2	2	1	2			2	1	3	2
B	1	3	3	4	4				3		1	2	1
C		5	4	2	2	1		1		1		2	1
<u>Petroleum-degrading bacteria</u>	9	0	1	0	0	4	9	25	1	14	3	3	4
Enclosure													
A	3		1			1	3	8		4	1	1	
B	5					2	3	5		4	2	2	2
C	1					1	3	12	1	6			2

trophs or the petroleum-degraders will dominate within a phenon (cluster).

DISCUSSION

Few previous studies have examined the responses of bacterial populations to crude oil spillage in an estuarine ecosystem. Rapid in situ responses of microbial populations to hydrocarbon pollution were demonstrated in a freshwater study (Ward and Brock, 1976) in which mineral oil and hexadecane were added to lakewater. A lag phase of only 20 h was encountered before measurable hydrocarbon oxidation began, coincident with the development of bacteria on hydrocarbons. Data presented in this thesis indicated that spillage of a fresh and an artificially weathered crude oil in a *Spartina* salt marsh produced a rapid increase in the levels of autochthonous petroleum-degrading bacteria.

Levels of petroleum-degrading bacteria continued to be elevated within the polluted marsh sediments relative to an untreated control for almost one year following the spills, while petroleum-degrading populations in the polluted creek zones were not maintained at higher levels. No significant ($\alpha = 0.05$) difference was found comparing petroleum-degrading population levels in the unweathered and weathered enclosures, though the weathered oil tended to support greater levels of petroleum-degrading bacteria than the unweathered oil. Substantially more unweathered oil (i.e., substrate) may have been lost from the marsh owing to differential volatilization and greater mobility of unweathered oil compared to weathered oil, which tended to "stick" immediately to *Spartina* and detritus. Through tidal action, the oil slick was transported onto the marsh sediments and *Spartina*; through stream flow and mixing, the creek zone microflora were being constantly removed from the enclosures. Therefore, petroleum-degrading bacteria did not persist at elevated levels for the year within the creek zones of the oil polluted enclosures.

Previous laboratory experiments, usually in closed flask systems, have demonstrated that ecologically important bacteria isolated from natural estuarine and marine envirinments, were growth inhibited in the presence of petroleum hydrocarbons (Walker and Colwell, 1974; Walker, Seesman, and Colwell, 1976; Walker and Colwell, 1975). This thesis study indicated that spillage of crude oil did not affect in situ viable populations levels of heterotrophic bacteria in a salt marsh. Furthermore, a small portion of the heterotrophic bacterial population, the petroleum-degraders, significantly ($\alpha = 0.05$) increased in the polluted enclosures relative to the control enclosure.

Elevated populations of viable petroleum-degrading bacteria in the salt marsh must be assumed to reflect the presence of spilled crude oil as supplemental food source. Numbers of oil-degrading microorganisms in sea water samples have been suggested as a useful and sensitive indicator of low-level oil pollution (Mironov, 1970) which may escape routine gas chromatographic detection (Atlas and Bartha, 1973). Therefore based on data in this thesis one may hypothesize that since elevated levels of petroleum-degrading bacteria in the marsh sediments were present after one year, their substrate (hydrocarbons) must also be present. Since total heterotrophic population levels in sediments and creek zones were not significantly ($\alpha = 0.05$) altered by oil spillage, a useful indicator of oil pollution was found to be log (petroleum-degrading bacteria / heterotophic bacteria). Petroleum pollution as exemplified in this experimental system may be inferred by ratio values approaching 0.

Taxonomic analysis of dominant isolates from in situ microbial populations in the oil polluted and control salt marsh enclosures was performed. Preliminary evaluation using tests from Shewan's (1963) scheme showed the generic composition of heterotrophic and petroleum-degrading bacteria matching well with results from studies performed in other estuaries. For example, water samples taken in Long Island Sound revealed that gram negative rods constituted 99% of the isolates and 92% of the genera were identified as Flavobacterium, Pseudomonas, and Achromobacter; the remainder included Vibrio, Bacillus, Micrococcus, and Cytophaga (Murchelano and Brown, 1970). Petroleum-degrading microorganisms isolated from "model" petroleum and South Louisiana crude oil flask experiments containing water and sediment samples from Colgate Creek in Baltimore Harbor and Eastern Bay, Maryland were dominated by members of the genera Pseudomonas, Vibrio, Aeromonas, Acinetobacter, and coryneforms (Walker and Colwell, 1974; Walker, Colwell, and Petrakis, 1976).

Dramatic increases observed in petroleum-degrading populations within the polluted marsh enclosures may be partially explained by the response of the Pseudomonas component and commensal nonpetroleum-degrading bacteria. Atlas, Schofield, Morelli, and Cameron (1976) found that underlying crude oil slicks experimentally floated in Prudhoe Bay bacterial populations increased compared with the adjacent water. The increase of bacteria underlying the slicks reflected an incease of oil-degrading Pseudomonas sp. and of Staphylococcus epidermidis, a commensal nonhydrocarbon utilizing organism. A similar mutualistic relationship was suggested for many isolates from enumeration medium for petroleum degradation since 50% of these isolates were unable to utilize hexadecane as a sole added carbon source. However, other petroleum-degrading isolates may utilize components of the crude oil other than the n-paraffins as a sole carbon source.

In this thesis study petroleum spillages did not cause apparent shifts in heterotrophic bacterial composition in the marsh sediments and creek water, although other microbial populations may have changed. Meyers, et al. (1973) conducted a study in which Barataria Bay, Louisiana marsh areas were exposed to controlled additions of oil. Yeast populations were observed to shift toward an asexual hydrocarbonoclastic flora. Oil-induced yeast populations were not as metabolically active in regard to carbohydrate metabolism as the indigenous mycota, thus Meyers, et al. (1973) postulated a significant reduction in nutrient regeneration in the estuary, especially as the dominant indigenous yeast did not significantly assimilate crude oil.

Preliminary taxonomic analysis of the isolates provided genera which compared favorably with genera assigned using numerical taxonomic techniques. Shewan's (1963) scheme, originally developed for bacteriological analysis of fish, has found widespread acceptance for the initial identification of marine isolates. Modern taxonomy would place most of the achromobacters into Moraxella and Acinetobacter (Buchanan and Gibbons, 1974). However, Shewan's (1963) scheme provided a workable plan with a minimum number of media for the generic identification of both heterotrophic and petroleum-degrading isolates from the salt marsh. The major difficulty, as with other dichotomous identification schemes, is that the differences between genera or families may be one or two properties (Litchfield, 1976). For example in Shewan's (1963) scheme the pteridine (O/129) test differentiates Vibrio from other oxidase positive, gram negative rods. This test gave some equivocal results and several isolates that were non-motile showed high sensitivity to pteridine.

In contrast, for this thesis study numerical taxonomic analysis was superior since equal weight was given to all biochemical and cultural characteristics of an isolate. Identified phenons (clusters) in the intensive analysis were polythetic groups, i.e., groups of similar organisms in which not all representatives necessarily possess every characteristic of a group. Numerical taxonomy helped to accommodate the taxonomic variability of isolates from a complex microbial ecosystem found in the salt marsh. Though there was general agreement of generic names assigned in preliminary and intensive analysis, the intensive analysis demonstrated the existence of subtle differences among the isolates. For example, numerical taxonomy demonstrated distinct taxonomic differences between petroleum-degrading and dominant heterotrophic *Pseudomonas* isolates, and the confusing *Acinetobacter*-*Moraxella* organisms were deciphered to some degree.

Neither the fresh nor weathered South Louisiana crude oil used to pollute the marsh enclosures appeared to repress or elevate total heterotrophic bacterial populations. Increased levels of petroleum-degraders and the apparent disappearance of spilled petroleum (Bieri, et al., 1977) suggested increased metabolic activity by this subset of the heterotrophic population. The relationship of bacterial biomass to in situ microbial activity in a salt marsh is not well understood and must be interpreted with caution. Mitchell, et al. (1972) demonstrated total inhibition of bacterial chemoreception by low concentrations of hydrocarbons, and suggested that viable enumerations would not reveal harmful effects on the microflora since bacterial populations would not be killed. Chemotactic responses have been shown for selected marine bacteria, with utilization facilitated by the attraction of the micro-organism to its substrate (Fogel, Chet, and Mitchell, 1971). If significant concentrations of a pollutant inhibiting chemotaxis remain, a portion of the bacterial population may become less efficient in locating food sources and adaptation phenomena may occur (Walsh and Mitchell, 1973). In addition to possible chemotactic interference, petroleum pollutant may physically coat detrital particles blocking active sites for enzymatic attack (Meyers, et al., 1973) and microbial attachment.

Since nutrient cycling and transformation of marsh detritus to a protein rich substrate is mediated by micro-organisms, any deleterious effect on this population could have significant implications. Although taxonomic and enumerative bacterial data from this study suggested that the petroleum caused no detrimental effects on the in situ levels and taxa of aerobic heterotrophic bacteria, further research should be directed toward determination of the effects of crude oil apillage on in situ microbial metabolic activities.

APPENDIX A

Bacteriological Media Recipes

All media, unless otherwise indicated: pH 7.6, salinity 10-12‰, and autoclaved 15 lb., 15 minutes.

1. Artificial Estuarine Water (AEW)

NaCl	0.14 M
$CaCl_2$	0.0035 M
KCl	0.0035 M
$MgSO_4$	0.0098 M

2. Carbohydrate Broths

Casamino acids	1.0 g
Yeast extract	0.1 g
$(NH_4)_2SO_4$	1.0 g
K_2HPO_4	0.1 g
Phenol red	0.018 g
EW	1000 ml

Add inverted Durham tube; autoclave. Add filtered sterilized carbohydrate to 0.5% final concentration.

3. Cation Requirement Broths

Casamino acids	1.0 g
Yeast extract	0.5 g
Distilled water	1000 ml

Add $CaCl_2$, $MgSO_4$, KCl, NaCl in following concentrations:

$CaCl_2$	0.0035 M
$MgSO_4$	0.0098 M
KCl	0.0035 M
NaCl	0.14 M

using combinations of interest.

4. Cellulose Medium

Yeast extract	1.0 g
$(NH_4)_2SO_4$	1.0 g
K_2HPO_4	0.1 g

Strips of cellulose (No. 1 Whatman filter paper) in 9 ml of above liquid, portion of strip above the air-water surface.

5. Chitin Medium

Composed of bi-layer in a pour plate. Lower layer approximately 25 ml of HMA. Over-layer was 10 ml of:

"molecularized" chitin	30.0 g
Yeast extract	0.5 g
Agar	15 g
EW	1000 ml

Chitin (Calbiochem - unspecified purity, San Diego, CA) was ball milled at 4° C for 48 hr, "dissolved" in 50% H_2SO_4 and precipitated by the addition of large volumes of distilled water. This precipitate was centrifuged, washed with distilled water, and neutralized to a pH of 7.0.

6. Enriched Seawater Broth (ESWB)

$(NH_4)_2SO_4$	1.0 g
K_2HPO_4	0.1 g
EW	1000 ml

After inoculation, 3 drops membrane filter (0.45μ) sterilized oil.

7. Estuarine Yeast Extract Broth

Yeast extract	1 g
Proteose peptone	1 g
EW	1000 ml

8. Gelatin Liquefaction Agar - Frazier Method

Difco nutrient agar	23 g
Gelatin	4 g
EW	1000 ml

9. Heterotrophic Medium Agar (HMA)

"Bacto" peptone	1.0 g
Yeast extract	0.5 g
Ferric citrate	0.01 g
Sodium glycerol phosphate	0.1 g
Agar	15 g
EW	1000 ml

10. Heterotrophic Medium Broth (HMB)
    HMA, no agar.

11. H_2S Production Medium
    Basal medium:

Proteose peptone	10 g
EW	1000 ml
Sodium thiosulfate	10 g

Top of tube is plugged with strips of No. 1 Whatman filter paper, which have been soaked in a 5% lead acetate suspension, air-dried and autoclaved.

12. MOF Medium - Difco
    Distilled water.

13. Moeller Decarboxylase Base - Difco
Use AEW with either lysine, arginine, ornithine additions.
pH 6.2.

14. Nitrate Broth - Griess-Ilosvay Method

Beef extract	3.0 g
Peptone	5.0 g
KNO_3	1.0 g
EW	1000 ml

15. Pseudomonas F Agar - Difco

16. Pseudomonas P Agar

Bacto peptone	20.0 g
MgCl	1.4 g
K_2SO_4	10.0 g
Agar	15.0 g
Glycerol	10.0 ml
EW	1000 ml

17. Starch Medium

*Nutrient agar	23.0 g
Potato starch	10.0 g
EW	1000 ml

*Nutrient agar

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g

18. Tween 20, 40, 60, 80 Media

Yeast extract	1.0 g
Peptone	10.0 g
Agar	15.0 g
EW	1000 ml

For Tweens 20, 40, 60, 80, sterilize separately for 20 minutes at 120° C. Add 1 ml Tween of interest to 100 ml medium.

APPENDIX B

Numerical Taxonomy

Numerical taxonomy may be defined as the grouping by numerical methods of taxonomic units into taxa on the basis of their character states (Sneath and Sokal, 1973). Concepts of numerical taxonomy may be traced to Michel Adanson, a French botanist. The following is a summary of the concepts (Sneath and Sokal, 1973):

(1) The greater the content of information in the taxa of a classification and the more characters on which it is based, the better a given classification will be.

(2) A priori, every character is of equal weight in creating natural taxa.

(3) Overall similarity between any two entities is a function of their individual similarities in each of the many characters in which they are being compared.

(4) Distinct taxa can be recognized because correlations of characters differs in the groups of organisms under study.

(5) Classifications are based on phenetic similarity.

(6) Taxonomy is viewed and practiced as an empirical science.

In recent years, biological science has become more quantitative, and computers are now widely used. Increasing numbers of publications have described the successful application of numerical taxonomy to the problems of classification, particularly of the bacteria. Characters used in bacterial classification cannot logically be weighted on phylogenetic grounds, because evolutionary data are lacking. In consequence, microbial taxonomists frequently must utilize relatively large quantities of data to obtain satisfactory separation and definition of taxa. For this reason, numerical methods are particularly appropriate for studies of bacterial taxonomy.

Groupings of bacteria established by numerical taxonomy and those derived by more classical methods have a high degree of agreement. In many instances, numerical techniques have facilitated refinement and quantitative description of classical taxa. As a result, there is widespread use of the method, and its utility as a tool in classification is now broadly accepted among microbiologists.

The taxonomic procedure involves determining many properties of a number of bacterial cultures and recording these observations in a table in which each row represents a strain and characters are listed in the columns. Encoded descriptions of the properties of individual isolates are then compared to determine overall similarity between each pair. Usually this is done by determining the numbers of similar and dissimilar characters, and computing for each pair of bacterial isolates the numerical ratio of similar characters to the total characters compared. Sometimes only the characters positively present in both strains are considered similar, but in other widely used techniques negatively correlated characters are also included. Similarities between all pairs of strains within a study can be determined rapidly in an electronic computer. Isolates are then arranged into an array to delineate clusters or groups of strains and to measure the degree of homogeneity or relationship within and between groups. These methods thus provide for clustering individual organisms into groups on the basis of overall similarity by taking many properties into account. Often this appears to yield groupings which are internally more consistent than those provided by classification on the basis of only a few determinative characters.

APPENDIX C

Table C1. Heterotrophic and petroleum-degrading bacterial population levels, log (petroleum-degrading bacteria/ heterotrophic bacteria)

Date and Station	Heterotrophic Bacteria*	Petroleum-Degrading Bacteria**	Log $\frac{\text{Petroleum-Degraders}}{\text{Heterotrophs}}$
9/22/75			
AD-W	1.5×10^6	2.4×10^3	-2.8
AD-S	2.3×10^8	4.0×10^5	-2.8
AE-S	4.6×10^8	8.8×10^5	-2.7
AF-S	3.8×10^8	1.8×10^5	-3.3
CD-W	2.6×10^5	1.1×10^3	-2.4
CD-S	2.0×10^8	3.1×10^4	-3.8
C2-S	7.1×10^8	2.2×10^5	-3.5
C3-S	3.4×10^8	1.3×10^5	-3.4
9/23/75			
A1-W	2.3×10^5	4.6×10^2	-2.7
A1-S	1.0×10^8	5.1×10^4	-3.3
AE-S	4.8×10^8	1.7×10^5	-3.5
AF-S	2.7×10^8	3.7×10^5	-2.9
C1-W	2.5×10^5	4.6×10^2	-2.7
C1-S	1.8×10^8	3.2×10^4	-3.7
CE-S	5.3×10^8	1.5×10^5	-3.6
C3-S	5.4×10^8	1.4×10^5	-3.6
9/24/75			
A1-W	3.4×10^5	2.4×10^3	-2.1
A1-S	3.1×10^8	3.2×10^6	-2.0
AE-S	5.9×10^8	3.9×10^6	-2.2
A3-S	4.6×10^8	1.2×10^6	-2.6
C1-W	2.6×10^4	2.4×10^3	-1.0
C1-S	1.6×10^8	1.9×10^4	-3.9
CE-S	4.0×10^8	6.6×10^4	-3.8
CF-S	5.3×10^8	1.2×10^6	-2.6

Date and Station	Heterotrophic Bacteria*	Petroleum-Degrading Bacteria**	Log $\frac{\text{Petroleum-Degraders}}{\text{Heterotrophs}}$
9/25/75			
BD-W	1.9×10^5	2.4×10^3	-1.9
BD-S	8.0×10^7	1.6×10^5	-2.8
B2-S	4.9×10^8	3.3×10^6	-2.2
B3-S	2.5×10^8	1.9×10^5	-3.1
C1-W	1.1×10^5	2.1×10^2	-2.7
C1-S	5.0×10^7	5.7×10^4	-3.0
CE-S	1.4×10^8	8.8×10^4	-3.2
C3-S	1.8×10^8	7.1×10^4	-3.4
9/26/75			
AD-W	6.1×10^5	1.1×10^4	-1.7
AD-S	2.4×10^8	1.7×10^7	-1.1
A2-S	4.7×10^8	3.2×10^6	-2.2
AF-S	4.5×10^8	9.0×10^6	-1.7
B1-W	7.1×10^5	2.4×10^4	-1.5
B1-S	5.0×10^7	9.7×10^4	-2.7
BE-S	4.9×10^8	1.7×10^6	-2.5
BF-S	3.9×10^8	2.9×10^5	-3.1
CD-W	1.7×10^5	1.1×10^4	-1.2
CD-S	3.6×10^8	4.9×10^4	-3.9
CE-S	3.9×10^8	6.8×10^4	-3.8
C3-S	4.6×10^8	4.9×10^4	-4.0
9/27/75			
B1-W	3.0×10^5	2.4×10^4	-1.1
B1-S	1.8×10^8	1.8×10^7	-1.0
B2-S	8.2×10^8	1.9×10^7	-1.6
B3-S	N.A.	1.7×10^6	N.A.
CD-W	6.7×10^4	2.3×10^2	-2.5
CD-S	4.3×10^8	1.3×10^5	-3.5
CE-S	4.5×10^8	8.3×10^4	-3.7
CF-S	7.8×10^8	8.6×10^4	-4.0

Date and Station	Heterotrophic Bacteria*	Petroleum-Degrading Bacteria**	Log $\frac{\text{Petroleum-Degraders}}{\text{Heterotrophs}}$
9/29/75			
BD-W	2.9×10^5	4.6×10^3	-1.8
BD-S	2.5×10^8	1.7×10^7	-1.2
BE-S	4.6×10^8	3.9×10^6	-2.1
B3-S	5.2×10^8	2.0×10^7	-1.4
CD-W	1.0×10^5	4.3×10^2	-2.4
CD-S	2.0×10^7	N.A.	N.A.
CE-S	5.7×10^8	5.0×10^5	-3.1
CF-S	5.8×10^8	2.1×10^5	-3.4
9/30/75			
A1-W	1.7×10^5	4.3×10^2	-2.6
A1-S	5.2×10^8	1.8×10^7	-1.5
A2-S	6.4×10^8	9.4×10^6	-1.8
A3-S	9.0×10^8	1.0×10^7	-2.0
C1-W	4.1×10^5	7.5×10^2	-2.7
C1-S	N.A.	7.4×10^3	N.A.
CE-S	5.8×10^8	2.1×10^5	-3.4
CF-S	5.5×10^8	9.5×10^4	-3.8
10/3/75			
B1-W	2.1×10^5	2.9×10^3	-1.9
B1-S	3.4×10^8	3.7×10^7	-1.0
B2-S	3.0×10^8	2.6×10^6	-2.1
BF-S	4.1×10^8	1.7×10^8	-0.4
C1-W	6.1×10^5	1.5×10^3	-2.6
C1-S	2.4×10^8	8.2×10^5	-2.5
C2-S	3.2×10^8	5.7×10^4	-3.8
CF-S	5.3×10^8	8.6×10^4	-3.8
10/8/75			
AD-W	3.6×10^5	2.4×10^3	-2.2
AD-S	7.5×10^8	2.1×10^7	-1.6
AE-S	2.1×10^8	1.3×10^7	-1.2
AF-S	1.9×10^9	6.3×10^7	-1.5
C1-W	2.7×10^5	9.3×10^2	-2.5
C1-S	2.8×10^8	8.3×10^4	-3.5
CE-S	1.1×10^9	8.4×10^5	-3.1
C3-S	1.1×10^9	2.8×10^5	-3.6

Date and Station	Heterotrophic Bacteria*	Petroleum-Degrading Bacteria**	Log $\frac{\text{Petroleum-Degraders}}{\text{Heterotrophs}}$
10/11/75			
BD-W	$3.3x10^5$	$1.5x10^3$	-2.3
BD-S	$2.8x10^8$	$3.2x10^7$	-1.0
BE-S	$3.0x10^8$	$2.4x10^7$	-1.1
B3-S	$5.2x10^8$	$1.2x10^7$	-1.6
CD-W	$1.9x10^5$	$4.6x10^3$	-1.6
CD-S	$4.4x10^8$	$1.9x10^6$	-2.4
CE-S	$6.5x10^8$	$3.6x10^5$	-3.3
CF-S	$4.7x10^8$	$9.5x10^4$	-3.7
10/24/75			
A1-W	$3.3x10^5$	$2.4x10^3$	-2.1
A1-S	$8.9x10^8$	$1.5x10^7$	-1.8
A2-S	$1.5x10^9$	$7.1x10^6$	-2.3
AF-S	$2.1x10^9$	$2.9x10^7$	-1.9
CD-W	$1.8x10^5$	$1.5x10^2$	-3.1
CD-S	$8.4x10^7$	$7.0x10^4$	-3.1
C2-S	$1.3x10^9$	$9.9x10^4$	-4.1
CF-S	$7.4x10^8$	$2.6x10^5$	-3.5
10/27/75			
BD-W	$1.6x10^5$	$2.4x10^3$	-1.8
BD-S	$4.6x10^8$	$8.1x10^7$	-0.8
B2-S	$6.0x10^8$	$2.2x10^7$	-1.4
B3-S	$5.7x10^8$	$2.0x10^7$	-1.5
C1-W	$1.8x10^5$	$4.3x10^2$	-2.6
C1-S	$3.6x10^8$	$1.1x10^5$	-3.5
CE-S	$4.5x10^8$	$8.3x10^4$	-3.7
CF-S	$3.8x10^8$	$1.6x10^5$	-3.4
11/25/75			
A1-W	$1.6x10^5$	$1.5x10^3$	-2.0
A1-S	$3.3x10^8$	$2.0x10^7$	-1.2
AE-S	$5.6x10^8$	$8.7x10^6$	-1.8
A3-S	$6.2x10^8$	$2.2x10^7$	-1.5
CD-W	$2.7x10^5$	$2.3x10^2$	-3.1
CD-S	$2.1x10^8$	$7.6x10^4$	-3.4
C2-S	$9.2x10^8$	$3.9x10^5$	-3.4
CF-S	$6.3x10^8$	$1.7x10^6$	-2.6

Date and Station	Heterotrophic Bacteria*	Petroleum-Degrading Bacteria**	Log $\frac{\text{Petroleum-Degraders}}{\text{Heterotrophs}}$
11/25/75;	12/4/75***		
B1-W	2.2×10^{5}	1.5×10^{3}	-1.7
B1-S	1.3×10^{8}***	3.9×10^{7}	-0.5
B2-S	6.6×10^{8}***	1.7×10^{8}	-0.6
BF-S	4.8×10^{8}***	3.7×10^{6}	-2.1
2/25/76			
AD-W	1.3×10^{5}	4.3×10^{2}	-2.5
AD-S	3.1×10^{8}	3.4×10^{6}	-2.0
A2-S	9.5×10^{8}	9.2×10^{6}	-2.0
A3-S	7.4×10^{8}	8.1×10^{6}	-2.0
BD-W	3.5×10^{4}	9.3×10^{2}	-1.6
BD-S	8.4×10^{8}	1.7×10^{7}	-1.7
B2-S	1.0×10^{9}	1.0×10^{8}	-1.0
BF-S	1.3×10^{9}	9.6×10^{7}	-1.3
CD-W	5.9×10^{4}	4.3×10^{1}	-3.1
CD-S	5.6×10^{8}	1.9×10^{6}	-2.5
C2-S	7.8×10^{8}	8.6×10^{5}	-3.0
CF-S	7.8×10^{8}	2.1×10^{6}	-2.6
5/18/76			
A1-W	4.2×10^{4}	9.3×10^{2}	-1.7
A1-S	3.6×10^{8}	1.7×10^{6}	-2.3
A2-S	3.8×10^{8}	3.6×10^{5}	-3.0
A3-S	4.3×10^{8}	7.9×10^{5}	-2.7
BD-W	3.3×10^{4}	4.3×10^{2}	-1.9
BD-S	7.4×10^{8}	1.0×10^{6}	-2.9
BE-S	5.8×10^{8}	3.8×10^{6}	-2.2
BF-S	1.1×10^{9}	3.4×10^{7}	-1.5
CD-W	3.5×10^{4}	4.3×10^{2}	-1.9
CD-S	3.7×10^{8}	1.6×10^{5}	-3.4
C2-S	5.4×10^{8}	3.7×10^{5}	-3.2
CF-S	6.3×10^{8}	3.9×10^{5}	-3.2

Date and Station	Heterotrophic Bacteria*	Petroleum-Degrading Bacteria**	Log Petroleum-Degraders / Hetertrophs
8/3/76			
AD-W	$9.2x10^5$	$4.3x10^2$	-3.3
AD-S	$6.1x10^8$	$1.1x10^7$	-1.7
AE-S	$6.3x10^8$	$8.9x10^5$	-2.8
A3-S	$1.4x10^9$	$2.9x10^6$	-2.7
BD-W	$1.4x10^6$	$2.3x10^2$	-3.8
BD-S	$4.7x10^8$	$1.2x10^7$	-1.6
BE-S	$8.9x10^8$	$4.1x10^7$	-1.3
BF-S	$9.8x10^8$	$8.2x10^5$	-3.1
C1-W	$1.5x10^6$	$2.3x10^2$	-3.8
C1-S	$3.4x10^8$	$1.7x10^5$	-3.3
C2-S	$5.8x10^8$	$4.7x10^4$	-4.1
C3-S	$6.2x10^8$	$5.1x10^4$	-4.1

* counts expressed as C.F.U./ g wet sediment or C.F.U./ ml water.

** counts expressed as MPN/ g wet sediment or MPN/ ml water.

APPENDIX D1

Table 1. Heterotrophic bacterial isolates and results of the preliminary analysis

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
1	HA001	9/22/75	AD-W	Pseudomonas
2	HA002	9/22/75	AD-W	Pseudomonas
3	HA003	9/22/75	AD-W	Pseudomonas
4	HA004	9/22/75	AD-W	Flavobacterium
5	HA006	9/22/75	AD-S	Flavobacterium
6	HA007	9/22/75	AD-S	Pseudomonas
7	HA008	9/22/75	AD-S	Alcaligenes
8	HA009	9/22/75	AE-S	Vibrio
9	HA010	9/22/75	AE-S	Pseudomonas
10	HA011	9/22/75	AE-S	Pseudomonas
11	HA013	9/22/75	AF-S	Pseudomonas
12	HA014	9/22/75	AF-S	Alcaligenes
13	HA015	9/22/75	AF-S	Enterobacteriaceae
14	HA016	9/22/75	AF-S	Flavobacterium
15	HA018	9/23/75	A1-W	Pseudomonas
16	HA019	9/23/75	A1-W	Flavobacterium
17	HA020	9/23/75	A1-W	Alcaligenes
18	HA021	9/23/75	A1-S	Gram pos. rod
19	HA022	9/23/75	A1-S	Gram neg. cocci
20	HA023	9/23/75	A1-S	Unknown
21	HA025	9/23/75	AE-S	Pseudomonas
22	HA026	9/23/75	AE-S	Enterobacteriaceae
23	HA027	9/23/75	AE-S	Pseudomonas
24	HA028	9/23/75	AE-S	Enterobacteriaceae
25	HA029	9/23/75	AF-S	Pseudomonas
26	HA030	9/23/75	AF-S	Pseudomonas
27	HA031	9/23/75	AF-S	Flavobacterium
28	HA034	9/24/75	A1-W	Flavobacterium
29	HA037	9/24/75	A1-S	Pseudomonas
30	HA038	9/24/75	A1-S	Pseudomonas
31	HA039	9/24/75	A1-S	Alcaligenes
32	HA041	9/24/75	AE-S	Pseudomonas
33	HA042	9/24/75	AE-S	Flavobacterium
34	HA044	9/24/75	AE-S	Pseudomonas

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
35	HA045	9/24/75	A3-S	Alcaligenes
36	HA046	9/24/75	A3-S	Pseudomonas
37	HA048	9/24/75	A3-S	Pseudomonas
38	HA049	9/26/75	AD-W	Pseudomonas
39	HA054	9/26/75	AD-S	Gram neg. cocci
40	HA055	9/26/75	AD-S	Flavobacterium
41	HA057	9/26/75	A2-S	Flavobacterium
42	HA058	9/26/75	A2-S	Flavobacterium
43	HA059	9/26/75	A2-S	Micrococcus
44	HA060	9/26/75	A2-S	Alcaligenes
45	HA061	9/26/75	AF-S	Alcaligenes
46	HA064	9/26/75	AF-S	Alcaligenes
47	HA065	9/30/75	Al-W	Alcaligenes
48	HA066	9/30/75	Al-W	Flavobacterium
49	HA067	9/30/75	Al-W	Enterobacteriaceae
50	HA069	9/30/75	Al-S	Alcaligenes
51	HA070	9/30/75	Al-S	Pseudomonas
52	HA071	9/30/75	Al-S	Pseudomonas
53	HA072	9/30/75	Al-S	Alcaligenes
54	HA073	9/30/75	A2-S	Pseudomonas
55	HA074	9/30/75	A2-S	Flavobacterium
56	HA075	9/30/75	A2-S	Alcaligenes
57	HA076	9/30/75	A2-S	Alcaligenes
58	HA077	9/30/75	A3-S	Alcaligenes
59	HA078	9/30/75	A3-S	Vibrio
60	HA079	9/30/75	A3-S	Pseudomonas
61	HA080	9/30/75	A3-S	Flavobacterium
62	HA082	10/08/75	AD-W	Alcaligenes
63	HA084	10/08/75	AD-W	Flavobacterium
64	HA085	10/08/75	AD-S	Flavobacterium
65	HA086	10/08/75	AD-S	Pseudomonas
66	HA087	10/08/75	AD-S	Pseudomonas
67	HA088	10/08/75	AD-S	Pseudomonas
68	HA090	10/08/75	AE-S	Pseudomonas
69	HA092	10/08/75	AE-S	Gram neg. cocci
70	HA093	10/08/75	AF-S	Pseudomonas
71	HA095	10/08/75	AF-S	Pseudomonas
72	HA096	10/08/75	AF-S	Pseudomonas
73	HA097	10/24/75	Al-W	Pseudomonas
74	HA098	10/24/75	Al-W	Flavobacterium
75	HA099	10/24/75	Al-W	Pseudomonas
76	HA101	10/24/75	Al-S	Micrococcus

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
77	HA102	10/24/75	A1-S	Pseudomonas
78	HA105	10/24/75	A2-S	Pseudomonas
79	HA106	10/24/75	A2-S	Alcaligenes
80	HA107	10/24/75	A2-S	Flavobacterium
81	HA108	10/24/75	A2-S	Pseudomonas
82	HA109	10/24/75	AF-S	Pseudomonas
83	HA110	10/24/75	AF-S	Alcaligenes
84	HA111	10/24/75	AF-S	Pseudomonas
85	HA112	10/24/75	AF-S	Enterobacteriaceae
86	HA115	11/25/75	A1-W	Gram pos. rods
87	HA116	11/25/75	A1-W	Pseudomonas
88	HA119	11/25/75	A1-S	Flavobacterium
89	HA120	11/25/75	A1-S	Alcaligenes
90	HA121	11/25/75	AE-S	Flavobacterium
91	HA122	11/25/75	AE-S	Alcaligenes
92	HA125	11/25/75	A3-S	Alcaligenes
93	HA126	11/25/75	A3-S	Pseudomonas
94	HA127	11/25/75	A3-S	Pseudomonas
95	HA129	2/26/76	AD-W	Pseudomonas
96	HA130	2/26/76	AD-W	Pseudomonas
97	HA131	2/26/76	AD-W	Gram pos. rods
98	HA133	2/26/76	AD-S	Flavobacterium
99	HA134	2/26/76	AD-S	Flavobacterium
100	HA137	2/26/76	A2-S	Pseudomonas
101	HA138	2/26/76	A2-S	Flavobacterium
102	HA141	2/26/76	A3-S	Gram pos. rods
103	HA142	2/26/76	A3-S	Flavobacterium
104	HA145	5/18/76	A1-W	Pseudomonas
105	HA146	5/18/76	A1-W	Flavobacterium
106	HA147	5/18/76	A1-W	Flavobacterium
107	HA149	5/18/76	A1-S	Flavobacterium
108	HA150	5/81/76	A1-S	Flavobacterium
109	HA151	5/18/76	A1-S	Alcaligenes
110	HA152	5/18/76	A1-S	Alcaligenes
111	HA153	5/18/76	A2-S	Pseudomonas
112	HA158	5/18/76	A3-S	Alcaligenes
113	HA159	5/18/76	A3-S	Flavobacterium
114	HA160	5/18/76	A3-S	Alcaligenes
115	HB001	9/25/75	BD-W	Alcaligenes
116	HB002	9/25/75	BD-W	Alcaligenes
117	HB003	9/25/75	BD-W	Gram pos. rods
118	HB006	9/25/75	BD-S	Pseudomonas
119	HB008	9/25/75	BD-S	Pseudomonas

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
120	HB009	9/25/75	B2-S	Pseudomonas
121	HB010	9/25/75	B2-S	Pseudomonas
122	HB011	9/25/75	B2-S	Pseudomonas
123	HB012	9/25/75	B2-S	Pseudomonas
124	HB013	9/25/75	B3-S	Flavobacterium
125	HB014	9/25/75	B3-S	Actinomycetes-type
126	HB015	9/25/75	B3-S	Pseudomonas
127	HB018	9/26/75	B1-W	Flavobacterium
128	HB019	9/26/75	B1-W	Pseudomonas
129	HB021	9/26/75	B1-S	Flavobacterium
130	HB022	9/26/75	B1-S	Gram pos. rods
131	HB023	9/26/75	B1-S	Micrococcus
132	HB026	9/26/75	BE-S	Pseudomonas
133	HB027	9/26/75	BE-S	Flavobacterium
134	HB029	9/26/75	BF-S	Alcaligenes
135	HB030	9/26/75	BF-S	Pseudomonas
136	HB031	9/26/75	BF-S	Alcaligenes
137	HB032	9/26/75	BF-S	Flavobacterium
138	HB033	9/27/75	B1-W	Alcaligenes
139	HB034	9/27/75	B1-W	Pseudomonas
140	HB036	9/27/75	B1-W	Alcaligenes
141	HB037	9/27/75	B1-S	Gram pos. rods
142	HB041	9/27/75	B2-S	Pseudomonas
143	HB042	9/27/75	B2-S	Pseudomonas
144	HB043	9/27/75	B2-S	Pseudomonas
145	HB044	9/27/75	B2-S	Flavobacterium
146	HB049	9/29/75	BD-W	Alcaligenes
147	HB050	9/29/75	BD-W	Pseudomonas
148	HB051	9/29/75	BD-W	Vibrio
149	HB052	9/29/75	BD-W	Flavobacterium
150	HB054	9/29/75	BD-S	Flavobacterium
151	HB055	9/29/75	BD-S	Gram pos. rods
152	HB057	9/29/75	BE-S	Pseudomonas
153	HB059	9/29/75	BE-S	Pseudomonas
154	HB062	9/29/75	B3-S	Flavobacterium
155	HB064	9/29/75	B3-S	Pseudomonas
156	HB065	10/03/75	B1-W	Pseudomonas
157	HB066	10/03/75	B1-W	Pseudomonas
158	HB068	10/03/75	B1-W	Pseudomonas
159	HB069	10/03/75	B1-S	Pseudomonas
160	HB070	10/03/75	B1-S	Pseudomonas
161	HB071	10/03/75	B1-S	Flavobacterium
162	HB072	10/03/75	B1-S	Alcaligenes

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
163	HB073	10/03/75	B2-S	Flavobacterium
164	HB074	10/03/75	B2-S	Flavobacterium
165	HB075	10/03/75	B2-S	Pseudomonas
166	HB078	10/03/75	BF-S	Spirillum
167	HB079	10/03/75	BF-S	Gram pos. rods
168	HB081	10/11/75	BD-W	Pseudomonas
169	HB082	10/11/75	BD-W	Enterobacteriaceae
170	HB083	10/11/75	BD-W	Pseudomonas
171	HB085	10/11/75	BD-S	Gram pos. rods
172	HB086	10/11/75	BD-S	Alcaligenes
173	HB087	10/11/75	BD-S	Flavobacterium
174	HB088	10/11/75	BD-S	Gram pos. rods
175	HB089	10/11/75	BE-S	Pseudomonas
176	HB092	10/11/75	BE-S	Vibrio
177	HB094	10/11/75	B3-S	Gram neg. cocci
178	HB095	10/11/75	B3-S	Pseudomonas
179	HB097	10/27/75	BD-W	Flavobacterium
180	HB098	10/27/75	BD-W	Alcaligenes
181	HB099	10/27/75	BD-W	Aeromonas
182	HB101	10/27/75	BD-S	Pseudomonas
183	HB102	10/27/75	BD-S	Vibrio
184	HB103	10/27/75	BD-S	Flavobacterium
185	HB104	10/27/75	BD-S	Pseudomonas
186	HB106	10/27/75	B2-S	Alcaligenes
187	HB107	10/27/75	B2-S	Pseudomonas
188	HB109	10/27/75	B3-S	Pseudomonas
189	HB110	10/27/75	B3-S	Alcaligenes
190	HB111	10/27/75	B3-S	Alcaligenes
191	HB112	10/27/75	B3-S	Flavobacterium
192	HB117	12/04/75	B1-S	Enterobacteriaceae
193	HB121	12/04/75	B2-S	Flavobacterium
194	HB123	12/04/75	B2-S	Enterobacteriaceae
195	HB124	12/04/75	B2-S	Actinomycetes-type
196	HB125	12/04/75	BF-S	Flavobacterium
197	HB126	12/04/75	BF-S	Alcaligenes
198	HB127	12/04/75	BF-S	Flavobacterium
199	HB128	12/04/75	BF-S	Actinomycetes-type
200	HB129	2/26/75	BD-W	Flavobacterium
201	HB130	2/26/76	BD-W	Alcaligenes
202	HB137	2/26/76	B2-S	Alcaligenes
203	HB138	2/26/76	B2-S	Gram pos. rods
204	HB139	2/26/76	B2-S	Flavobacterium
205	HB140	2/26/76	B2-S	Gram pos. rods

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
206	HB141	2/26/76	BF-S	Vibrio
207	HB142	2/26/76	BF-S	Alcaligenes
208	HB145	5/18/76	BD-W	Alcaligenes
209	HB147	5/18/76	BD-W	Flavobacterium
210	HB149	5/18/76	BD-S	Alcaligenes
211	HB150	5/18/76	BD-S	Flavobacterium
212	HB151	5/18/76	BD-S	Alcaligenes
213	HB152	5/18/76	BD-S	Flavobacterium
214	HB153	5/18/76	BE-S	Flavobacterium
215	HB154	5/18/76	BE-S	Flavobacterium
216	HB155	5/18/76	BE-S	Pseudomonas
217	HB156	5/18/76	BE-S	Pseudomonas
218	HB157	5/18/76	BF-S	Gram pos. rods
219	HB158	5/18/76	BF-S	Alcaligenes
220	HB159	5/18/76	BF-S	Flavobacterium
221	HB160	5/18/76	BF-S	Flavobacterium
222	HC001	9/22/75	CD-W	Flavobacterium
223	HC003	9/22/75	CD-W	Flavobacterium
224	HC005	9/22/75	CD-S	Alcaligenes
225	HC006	9/22/75	CD-S	Gram pos. rods
226	HC007	9/22/75	CD-S	Alcaligenes
227	HC008	9/22/75	CD-S	Alcaligenes
228	HC010	9/22/75	C2-S	Flavobacterium
229	HC011	9/22/75	C2-S	Alcaligenes
230	HC013	9/22/75	C3-S	Alcaligenes
231	HC015	9/22/75	C3-S	Micrococcus
232	HC017	9/23/75	C1-W	Alcaligenes
233	HC018	9/23/75	C1-W	Micrococcus
234	HC019	9/23/75	C1-W	Pseudomonas
235	HC022	9/23/75	C1-S	Flavobacterium
236	HC023	9/23/75	C1-S	Alcaligenes
237	HC025	9/23/75	CE-S	Alcaligenes
238	HC026	9/23/75	CE-S	Flavobacterium
239	HC027	9/23/75	CE-S	Flavobacterium
240	HC028	9/23/75	CE-S	Pseudomonas
241	HC031	9/23/75	C3-S	Pseudomonas
242	HC033	9/24/75	C1-W	Achromobacter
243	HC034	9/24/75	C1-W	Flavobacterium
244	HC036	9/24/75	C1-W	Pseudomonas
245	HC038	9/24/75	C1-S	Flavobacterium
246	HC039	9/24/75	C1-S	Flavobacterium
247	HC040	9/24/75	C1-S	Flavobacterium
248	HC042	9/24/75	CE-S	Pseudomonas
249	HC043	9/24/75	CE-S	Flavobacterium

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
250	HC045	9/24/75	CE-S	Pseudomonas
251	HC046	9/24/75	CF-S	Flavobacterium
252	HC049	9/25/75	C1-W	Micrococcus
253	HC050	9/25/75	C1-W	Flavobacterium
254	HC051	9/25/75	C1-W	Micrococcus
255	HC053	9/25/75	C1-S	Pseudomonas
256	HC054	9/25/75	C1-S	Flavobacterium
257	HC059	9/25/75	CE-S	Flavobacterium
258	HC062	9/25/75	C3-S	Micrococcus
259	HC063	9/25/75	C3-S	Flavobacterium
260	HC066	9/26/75	CD-W	Flavobacterium
261	HC067	9/26/75	CD-W	Pseudomonas
262	HC068	9/26/75	CD-W	Flavobacterium
263	HC069	9/26/75	CD-S	Flavobacterium
264	HC070	9/26/75	CD-S	Flavobacterium
265	HC071	9/26/75	CD-S	Gram pos. rods
266	HC073	9/26/75	CE-S	Gram pos. rods
267	HC075	9/26/75	CE-S	Actinomycetes-type
268	HC076	9/26/75	CE-S	Pseudomonas
269	HC078	9/26/75	C3-S	Alcaligenes
270	HC081	9/27/75	CD-W	Pseudomonas
271	HC082	9/27/75	CD-W	Enterobacteriaceae
272	HC083	9/27/75	CD-W	Pseudomonas
273	HC086	9/27/75	CD-S	Pseudomonas
274	HC088	9/27/75	CD-S	Vibrio
275	HC089	9/27/75	CE-S	Alcaligenes
276	HC090	9/27/75	CE-S	Pseudomonas
277	HC091	9/27/75	CE-S	Alcaligenes
278	HC094	9/27/75	CF-S	Pseudomonas
279	HC095	9/27/75	CF-S	Gram pos. rods
280	HC098	9/29/75	CD-W	Flavobacterium
281	HC099	9/29/75	CD-W	Alcaligenes
282	HC100	9/29/75	CD-W	Pseudomonas
283	HC101	9/29/75	CD-S	Alcaligenes
284	HC102	9/29/75	CD-S	Enterobacteriaceae
285	HC103	9/29/75	CD-S	Gram pos. rods
286	HC105	9/29/75	CE-S	Micrococcus
287	HC107	9/29/75	CE-S	Flavobacterium
288	HC110	9/29/75	CF-S	Flavobacterium
289	HC111	9/29/75	CF-S	Flavobacterium
290	HC112	9/29/75	CF-S	Pseudomonas
291	HC114	9/30/75	C1-W	Flavobacterium
292	HC115	9/30/75	C1-W	Alcaligenes
293	HC116	9/30/75	C1-W	Gram pos. rods

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
294	HC121	9/30/75	CE-S	Alcaligenes
295	HC122	9/30/75	CE-S	Alcaligenes
296	HC123	9/30/75	CE-S	Micrococcus
297	HC124	9/30/75	CE-S	Pseudomonas
298	HC126	9/30/75	CF-S	Gram pos. rods
299	HC127	9/30/75	CF-S	Flavobacterium
300	HC129	10/03/75	C1-W	Alcaligenes
301	HC130	10/03/75	C1-W	Flavobacterium
302	HC131	10/03/75	C1-W	Flavobacterium
303	HC132	10/03/75	C1-W	Flavobacterium
304	HC134	10/03/75	C1-S	Pseudomonas
305	HC135	10/03/75	C1-S	Flavobacterium
306	HC138	10/03/75	C2-S	Flavobacterium
307	HC139	10/03/75	C2-S	Pseudomonas
308	HC140	10/03/75	C2-S	Gram pos. rods
309	HC141	10/03/75	CF-S	Pseudomonas
310	HC143	10/03/75	CF-S	Enterobacteriaceae
311	HC145	10/08/75	C1-W	Flavobacterium
312	HC147	10/08/75	C1-W	Gram pos. rods
313	HC150	10/08/75	C1-S	Gram pos. rods
314	HC151	10/08/75	C1-S	Micrococcus
315	HC153	10/08/75	CE-S	Flavobacterium
316	HC154	10/08/75	CE-S	Pseudomonas
317	HC155	10/08/75	CE-S	Pseudomonas
318	HC158	10/08/75	C3-S	Alcaligenes
319	HC159	10/08/75	C3-S	Flavobacterium
320	HC161	10/11/75	CD-W	Spirillum
321	HC162	10/11/75	CD-W	Pseudomonas
322	HC165	10/11/75	CD-S	Unknown
323	HC169	10/11/75	CE-S	Pseudomonas
234	HC170	10/11/75	CE-S	Pseudomonas
325	HC171	10/11/75	CE-S	Alcaligenes
326	HC173	10/11/75	CF-S	Flavobacterium
327	HC175	10/11/75	CF-S	Actinomycetes-type
328	HC176	10/11/75	CF-S	Alcaligenes
329	HC177	10/24/75	CD-W	Micrococcus
330	HC178	10/24/75	CD-W	Alcaligenes
331	HC179	10/24/75	CD-W	Enterobacteriaceae
332	HC182	10/24/75	CD-S	Alcaligenes
333	HC184	10/24/75	CD-S	Flavobacterium
334	HC185	10/24/75	C2-S	Alcaligenes
335	HC186	10/24/75	C2-S	Alcaligenes
336	HC187	10/24/75	C2-S	Alcaligenes
337	HC190	10/24/75	CF-S	Micrococcus

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
338	HC191	10/24/75	CF-S	Flavobacterium
339	HC192	10/24/75	CF-S	Pseudomonas
340	HC193	10/27/75	C1-W	Micrococcus
341	HC194	10/27/75	C1-W	Flavobacterium
342	HC195	10/27/75	C1-W	Micrococcus
343	HC196	10/27/75	C1-W	Pseudomonas
344	HC197	10/27/75	C1-S	Gram pos. rods
345	HC199	10/27/75	C1-S	Pseudomonas
346	HC200	10/27/75	C1-S	Pseudomonas
347	HC201	10/27/75	CE-S	Gram pos. rods
348	HC203	10/27/75	CE-S	Pseudomonas
349	HC204	10/27/75	CE-S	Pseudomonas
350	HC205	10/27/75	CF-S	Flavobacterium
351	HC206	10/27/75	CF-S	Gram neg. cocci
352	HC213	11/25/75	CD-S	Flavobacterium
353	HC214	11/25/75	CD-S	Alcaligenes
354	HC217	11/25/75	C2-S	Flavobacterium
355	HC219	11/25/75	C2-S	Actinomycetes-type
356	HC221	11/25/75	CF-S	Pseudomonas
357	HC223	11/25/75	CF-S	Gram pos. rods
358	HC225	2/26/75	CD-W	Flavobacterium
359	HC229	2/26/76	CD-S	Flavobacterium
360	HC233	2/26/76	C2-S	Flavobacterium
361	HC234	2/26/76	C2-S	Pseudomonas
362	HC235	2/26/76	C2-S	Gram pos. rods
363	HC237	2/26/76	CF-S	Pseudomonas
364	HC238	2/26/76	CF-S	Flavobacterium
365	HC239	2/26/76	CF-S	Gram pos. rods
366	HC241	5/18/76	CD-W	Alcaligenes
367	HC242	5/18/76	CD-W	Flavobacterium
368	HC245	5/18/76	CD-S	Enterobacteriaceae
369	HC246	5/18/76	CD-S	Alcaligenes
370	HC247	5/18/76	CD-S	Flavobacterium
371	HC248	5/18/76	CD-S	Alcaligenes
372	HC249	5/18/76	C2-S	Flavobacterium
373	HC250	5/18/76	C2-S	Alcaligenes
374	HC252	5/18/76	C2-S	Pseudomonas
375	HC253	5/18/76	CF-S	Flavobacterium
376	HC254	5/18/76	CF-S	Alcaligenes
377	HC254	5/18/76	CF-S	Flavobacterium
378	HC256	5/18/76	CF-S	Alcaligenes

APPENDIX D2

Table 2. Petroleum-degrading bacterial isolates and results of the preliminary analysis

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
1	PA001	9/22/75	AD-W	Pseudomonas
2	PA002	9/22/75	AD-W	Alcaligenes
3	PA003	9/22/75	AD-W	Alcaligenes
4	PA004	9/22/75	AD-S	Pseudomonas
5	PA007	9/22/75	AE-S	Pseudomonas
6	PA010	9/22/75	AF-S	Pseudomonas
7	PA011	9/22/75	AF-S	Pseudomonas
8	PA013	9/23/75	A1-W	Alcaligenes
9	PA014	9/23/75	A1-W	Pseudomonas
10	PA015	9/23/75	A1-W	Alcaligenes
11	PA016	9/23/75	A1-S	Alcaligenes
12	PA017	9/23/75	A1-S	Pseudomonas
13	PA019	9/23/75	AE-S	Alcaligenes
14	PA022	9/23/75	AF-S	Pseudomonas
15	PA025	9/24/75	A1-W	Alcaligenes
16	PA028	9/24/75	A1-S	Pseudomonas
17	PA029	9/24/75	A1-S	Alcaligenes
18	PA030	9/24/75	A1-S	Alcaligenes
19	PA031	9/24/75	AE-S	Pseudomonas
20	PA035	9/24/75	A3-S	Alcaligenes
21	PA037	9/26/75	AD-W	Pseudomonas
22	PA038	9/26/75	AD-W	Spirillum
23	PA039	9/26/75	AD-W	Spirillum
24	PA040	9/26/75	AD-S	Flavobacterium
25	PA041	9/26/75	AD-S	Flavobacterium
26	PA042	9/26/75	AD-S	Pseudomonas
27	PA043	9/26/75	A2-S	Alcaligenes
28	PA044	9/26/75	A2-S	Pseudomonas
29	PA046	9/26/75	AF-S	Vibrio
30	PA047	9/26/75	AF-S	Pseudomonas
31	PA049	9/30/75	A1-W	Pseudomonas
32	PA050	9/30/75	A1-W	Pseudomonas
33	PA051	9/30/75	A1-W	Alcaligenes

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
34	PA052	9/30/75	A1-W	Pseudomonas
35	PA055	9/30/75	A2-S	Pseudomonas
36	PA058	9/30/75	A3-S	Alcaligenes
37	PA059	9/30/75	A3-S	Pseudomonas
38	PA062	10/08/75	AD-W	Micrococcus
39	PA063	10/08/75	AD-W	Pseudomonas
40	PA064	10/08/75	AD-S	Pseudomonas
41	PA065	10/08/75	AD-S	Flavobacterium
42	PA066	10/08/75	AD-S	Pseudomonas
43	PA067	10/08/75	AE-S	Pseudomonas
44	PA068	10/08/75	AE-S	Pseudomonas
45	PA069	10/08/75	AE-S	Spirillum
46	PA070	10/08/75	AF-S	Pseudomonas
47	PA071	10/08/75	AF-S	Gram pos. rods
48	PA072	10/08/75	AF-S	Pseudomonas
49	PA073	10/24/75	A1-W	Pseudomonas
50	PA074	10/24/75	A1-W	Pseudomonas
51	PA076	10/24/75	A1-S	Alcaligenes
52	PA079	10/24/75	A2-S	Pseudomonas
53	PA081	10/24/75	A2-S	Pseudomonas
54	PA082	10/24/75	AF-S	Spirillum
55	PA083	10/24/75	AF-S	Pseudomonas
56	PA084	10/24/74	AF-S	Pseudomonas
57	PA088	11/25/75	A1-S	Pseudomonas
58	PA089	11/25/75	A1-S	Pseudomonas
59	PA090	11/25/75	A1-S	Gram pos. rods
60	PA091	11/25/75	AE-S	Spirillum
61	PA092	11/25/75	AE-S	Spirillum
62	PA094	11/25/75	A3-S	Pseudomonas
63	PA095	11/25/75	A3-S	Pseudomonas
64	PA096	11/25/75	A3-S	Pseudomonas
65	PA097	2/26/76	AD-W	Pseudomonas
66	PA098	2/26/76	AD-W	Pseudomonas
67	PA099	2/26/76	AD-W	Pseudomonas
68	PA099A	2/26/76	AD-W	Pseudomonas
69	PA100	2/26/76	AD-S	Pseudomonas
70	PA102	2/26/76	AD-S	Pseudomonas
71	PA103	2/26/76	A2-S	Pseudomonas
72	PA104	2/26/76	A2-S	Pseudomonas
73	PA106	2/26/76	A3-S	Pseudomonas
74	PA107	2/26/76	A3-S	Alcaligenes
75	PA108	2/26/76	A3-S	Pseudomonas
76	PA109	5/18/76	A1-W	Pseudomonas

Isolate No.	Culture Code	Source Date	Zone	Tentative Identification
77	PA110	5/18/76	A1-W	Pseudomonas
78	PA112	5/18/76	A1-S	Pseudomonas
79	PA113	5/18/76	A1-S	Pseudomonas
80	PA114	5/18/76	A1-S	Pseudomonas
81	PA115	5/18/76	A2-S	Pseudomonas
82	PA116	5/18/76	A2-S	Alcaligenes
83	PA117	5/18/76	A2-S	Flavobacterium
84	PA118	5/18/76	A3-S	Enterobacteriaceae
85	PA119	5/18/76	A3-S	Pseudomonas
86	PA120	5/18/76	A3-S	Flavobacterium
87	PB001	9/25/75	BD-W	Pseudomonas
88	PB003	9/25/75	BD-W	Pseudomonas
89	PB004	9/25/75	BD-S	Pseudomonas
90	PB005	9/25/75	BD-S	Alcaligenes
91	PB008	9/25/75	B2-S	Alcaligenes
92	PB009	9/25/75	B2-S	Alcaligenes
93	PB010	9/25/75	B3-S	Pseudomonas
94	PB011	9/25/75	B3-S	Pseudomonas
95	PB013	9/26/75	B1-W	Pseudomonas
96	PB014	9/26/75	B1-W	Pseudomonas
97	PB015	9/26/75	B1-W	Alcaligenes
98	PB016	9/26/75	B1-S	Pseudomonas
99	PB017	9/26/75	B1-S	Pseudomonas
100	PB019	9/26/75	BE-S	Pseudomonas
101	PB020	9/26/75	BE-S	Pseudomonas
102	PB021	9/26/75	BE-S	Pseudomonas
103	PB022	9/26/75	BF-S	Alcaligenes
104	PB023	9/26/75	BF-S	Gram pos. rods
105	PB024	9/26/75	BF-S	Pseudomonas
106	PB025	9/27/75	B1-W	Vibrio
107	PB026	9/27/75	B1-W	Vibrio
108	PB028	9/27/75	B1-S	Alcaligenes
109	PB029	9/27/75	B1-S	Alcaligenes
110	PB030	9/27/75	B1-S	Pseudomonas
111	PB031	9/27/75	B2-S	Alcaligenes
112	PB034	9/27/75	B3-S	Pseudomonas
113	PB035	9/27/75	B3-S	Alcaligenes
114	PB037	9/29/75	BD-W	Alcaligenes
115	PB038	9/29/75	BD-W	Alcaligenes
116	PB039	9/29/75	BD-W	Aeromonas
117	PB040	9/29/75	BD-S	Pseudomonas
118	PB041	9/29/75	BD-S	Pseudomonas
119	PB043	9/29/75	BE-S	Flavobacterium

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
120	PB044	9/29/75	BE-S	Alcaligenes
121	PB046	9/29/75	B3-S	Pseudomonas
122	PB049	10/03/75	B1-W	Alcaligenes
123	PB050	10/03/75	B1-W	Flavobacterium
124	PB052	10/03/75	B1-S	Pseudomonas
125	PB053	10/03/75	B1-S	Pseudomonas
126	PB054	10/03/75	B1-S	Pseudomonas
127	PB055	10/03/75	B2-S	Vibrio
128	PB056	10/03/75	B2-S	Alcaligenes
129	PB057	10/03/75	B2-S	Alcaligenes
130	PB058	10/03/75	BF-S	Pseudomonas
131	PB061	10/11/75	BD-W	Flavobacterium
132	PB062	10/11/75	BD-W	Pseudomonas
133	PB063	10/11/75	BD-W	Micrococcus
134	PB065	10/11/75	BD-S	Pseudomonas
135	PB066	10/11/75	BD-S	Pseudomonas
136	PB070	10/11/75	B3-S	Pseudomonas
137	PB073	10/27/75	BD-W	Pseudomonas
138	PB074	10/27/75	BD-W	Pseudomonas
139	PB075	10/27/75	BD-W	Pseudomonas
140	PB076	10/27/75	BD-S	Alcaligenes
141	PB077	10/27/75	BD-S	Pseudomonas
142	PB079	10/27/75	B2-S	Pseudomonas
143	PB080	10/27/75	B2-S	Pseudomonas
144	PB081	10/27/75	B2-S	Pseudomonas
145	PB082	10/27/75	B3-S	Pseudomonas
146	PB088	11/25/75	B1-S	Flavobacterium
147	PB089	11/25/75	B1-S	Pseudomonas
148	PB090	11/25/75	B1-S	Pseudomonas
149	PB091	11/25/75	B2-S	Flavobacterium
150	PB092	11/25/75	B2-S	Pseudomonas
151	PB097	2/26/76	BD-W	Alcaligenes
152	PB100	2/26/76	BD-S	Gram pos. rods
153	PB101	2/26/76	BD-S	Pseudomonas
154	PB103	2/26/76	B2-S	Pseudomonas
155	PB105	2/26/76	B2-S	Alcaligenes
156	PB107	2/26/76	BF-S	Pseudomonas
157	PB109	5/18/76	BD-W	Pseudomonas
158	PB110	5/18/76	BD-W	Pseudomonas
159	PB112	5/18/76	BD-S	Pseudomonas
160	PB113	5/18/76	BD-S	Pseudomonas
161	PB114	5/18/76	BD-S	Pseudomonas
162	PB115	5/18/76	BE-S	Flavobacterium

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
163	PB116	5/18/76	BE-S	Pseudomonas
164	PC001	9/22/75	CD-W	Pseudomonas
165	PC002	9/22/75	CD-W	Pseudomonas
166	PC003	9/22/75	CD-W	Pseudomonas
167	PC004	9/22/75	CD-S	Pseudomonas
168	PC005	9/22/75	CD-S	Vibrio
169	PC007	9/22/75	C2-S	Pseudomonas
170	PC010	9/22/75	C3-S	Pseudomonas
171	PC011	9/22/75	C3-S	Pseudomonas
172	PC012	9/22/75	C3-S	Pseudomonas
173	PC014	9/23/75	C1-W	Pseudomonas
174	PC017	9/23/75	C1-S	Pseudomonas
175	PC018	9/23/75	C1-S	Pseudomonas
176	PC019	9/23/75	CE-S	Pseudomonas
177	PC020	9/23/75	CE-S	Alcaligenes
178	PC022	9/23/75	C3-S	Pseudomonas
179	PC023	9/23/75	C3-S	Pseudomonas
180	PC024	9/23/75	C3-S	Flavobacterium
181	PC025	9/24/75	C1-W	Pseudomonas
182	PC026	9/24/75	C1-W	Pseudomonas
183	PC027	9/24/75	C1-W	Alcaligenes
184	PC028	9/24/75	C1-S	Pseudomonas
185	PC029	9/24/75	C1-S	Pseudomonas
186	PC031	9/24/75	CE-S	Pseudomonas
187	PC032	9/24/75	CE-S	Pseudomonas
188	PC034	9/24/75	CF-S	Pseudomonas
189	PC035	9/24/75	CF-S	Pseudomonas
190	PC036	9/24/75	CF-S	Pseudomonas
191	PC037	9/25/75	C1-W	Spirillum
192	PC038	9/25/75	C1-W	Spirillum
193	PC040	9/25/75	C1-S	Pseudomonas
194	PC042	9/25/75	C1-S	Pseudomonas
195	PC043	9/25/75	CE-S	Pseudomonas
196	PC044	9/25/75	CE-S	Pseudomonas
197	PC046	9/25/75	C3-S	Pseudomonas
198	PC047	9/25/75	C3-S	Pseudomonas
199	PC048	9/25/75	C3-S	Pseudomonas
200	PC049	9/26/75	CD-W	Alcaligenes
201	PC050	9/26/75	CD-W	Pseudomonas
202	PC052	9/26/75	CD-S	Pseudomonas
203	PC054	9/26/75	CD-S	Pseudomonas
204	PC055	9/26/75	CE-S	Pseudomonas
205	PC056	9/26/75	CE-S	Pseudomonas
206	PC057	9/26/75	CE-S	Unknown

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
207	PC058	9/26/75	C3-S	Pseudomonas
208	PC059	9/26/75	C3-S	Pseudomonas
209	PC061	9/27/75	CD-W	Pseudomonas
210	PC064	9/27/75	CD-S	Pseudomonas
211	PC067	9/27/75	CE-S	Pseudomonas
212	PC068	9/27/75	CE-S	Pseudomonas
213	PC069	9/27/75	CE-S	Vibrio
214	PC070	9/27/75	CF-S	Pseudomonas
215	PC071	9/27/75	CF-S	Pseudomonas
216	PC073	9/29/75	CD-W	Alcaligenes
217	PC074	9/29/75	CD-W	Pseudomonas
218	PC075	9/29/75	CD-W	Flavobacterium
219	PC076	9/29/75	CD-S	Pseudomonas
220	PC077	9/29/75	CD-S	Pseudomonas
221	PC078	9/29/75	CD-S	Pseudomonas
222	PC079	9/29/75	CE-S	Alcaligenes
223	PC080	9/29/75	CE-S	Pseudomonas
224	PC082	9/29/75	CF-S	Pseudomonas
225	PC083	9/29/75	CF-S	Pseudomonas
226	PC084	9/29/75	CF-S	Pseudomonas
227	PC085	9/30/75	C1-W	Alcaligenes
228	PC086	9/30/75	C1-W	Pseudomonas
229	PC088	9/30/75	C1-S	Pseudomonas
230	PC089	9/30/75	C1-S	Pseudomonas
231	PC091	9/30/75	CE-S	Pseudomonas
232	PC092	9/30/75	CE-S	Gram pos. rods
233	PC093	9/30/75	CE-S	Alcaligenes
234	PC094	9/30/75	CF-S	Pseudomonas
235	PC096	9/30/75	CF-S	Pseudomonas
236	PC097	10/03/75	C1-W	Pseudomonas
237	PC098	10/03/75	C1-W	Flavobacterium
238	PC099	10/03/75	C1-W	Pseudomonas
239	PC100	10/03/75	C1-S	Pseudomonas
240	PC101	10/03/75	C1-S	Alcaligenes
241	PC102	10/03/75	C1-S	Unknown
242	PC103	10/03/75	C2-S	Vibrio
243	PC104	10/03/75	C2-S	Pseudomonas
244	PC106	10/03/75	CF-S	Vibrio
245	PC107	10/03/75	CF-S	Vibrio
246	PC108	10/03/75	CF-S	Vibrio
247	PC108A	10/03/75	CF-S	Pseudomonas
248	PC109	10/08/75	C1-W	Pseudomonas
249	PC110	10/08/75	C1-W	Pseudomonas
250	PC111	10/08/75	C1-W	Pseudomonas

Isolate No.	Culture Code	Source Date	Source Zone	Tentative Identification
251	PC112	10/08/75	C1-S	Alcaligenes
252	PC113	10/08/75	C1-S	Pseudomonas
253	PC114	10/08/75	C1-S	Spirillum
254	PC115	10/08/75	CE-S	Pseudomonas
255	PC116	10/08/75	CE-S	Pseudomonas
256	PC117	10/08/75	CE-S	Pseudomonas
257	PC118	10/08/75	C3-S	Spirillum
258	PC119	10/08/75	C3-S	Pseudomonas
259	PC120	10/08/75	C3-S	Unknown
260	PC124	10/11/75	CD-S	Pseudomonas
261	PC125	10/11/75	CD-S	Flavobacterium
262	PC126	10/11/75	CD-S	Flavobacterium
263	PC127	10/11/75	CE-S	Pseudomonas
264	PC128	10/11/75	CE-S	Unknown
265	PC129	10/11/75	CE-S	Pseudomonas
266	PC130	10/11/75	CF-S	Pseudomonas
267	PC131	10/11/75	CF-S	Flavobacterium
268	PC132	10/11/75	CF-S	Pseudomonas
269	PC133	10/24/75	CD-W	Pseudomonas
270	PC134	10/24/75	CD-W	Vibrio
271	PC135	10/24/75	CD-W	Pseudomonas
272	PC136	10/24/75	CD-S	Pseudomonas
273	PC137	10/24/75	CD-S	Alcaligenes
274	PC138	10/24/75	CD-S	Spirillum
275	PC139	10/24/75	C2-S	Pseudomonas
276	PC142	10/24/75	CF-S	Pseudomonas
277	PC143	10/24/75	CF-S	Pseudomonas
278	PC144	10/24/75	CF-S	Pseudomonas
279	PC145	10/27/75	C1-W	Pseudomonas
280	PC146	10/27/75	C1-W	Flavobacterium
281	PC147	10/27/75	C1-W	Flavobacterium
282	PC147A	10/27/75	C1-W	Pseudomonas
283	PC148	10/27/75	C1-S	Pseudomonas
284	PC149	10/27/75	C1-S	Pseudomonas
285	PC150	10/27/75	C1-S	Flavobacterium
286	PC151	10/27/75	CE-S	Alcaligenes
287	PC152	10/27/75	CE-S	Pseudomonas
288	PC154	10/27/75	CF-S	Unknown
289	PC155	10/27/75	CF-S	Pseudomonas
290	PC156	10/27/75	CF-S	Pseudomonas
291	PC157	11/25/75	CD-W	Pseudomonas
292	PC158	11/25/75	CD-W	Pseudomonas
293	PC159	11/25/75	CD-W	Pseudomonas
294	PC160	11/25/75	CD-S	Pseudomonas

Isolate No.	Culture Code	Source		Tentative Identification
		Date	Zone	
295	PC161	11/25/75	CD-S	Pseudomonas
296	PC162	11/25/75	CD-S	Pseudomonas
297	PC163	11/25/75	C2-S	Pseudomonas
298	PC164	11/25/75	C2-S	Spirillum
299	PC165	11/25/75	C2-S	Pseudomonas
300	PC166	11/25/75	CF-S	Pseudomonas
301	PC169	2/26/76	CD-W	Pseudomonas
302	PC170	2/26/76	CD-W	Pseudomonas
303	PC172	2/26/76	CD-S	Pseudomonas
304	PC173	2/26/76	CD-S	Pseudomonas
305	PC174	2/26/76	CD-S	Pseudomonas
306	PC175	2/26/76	C2-S	Pseudomonas
307	PC176	2/26/76	C2-S	Pseudomonas
308	PC178	2/26/76	CF-S	Unknown
309	PC179	2/26/76	CF-S	Pseudomonas
310	PC183	5/18/76	CD-W	Pseudomonas
311	PC183	5/18/76	CD-S	Pseudomonas
312	PC187	5/18/76	C2-S	Pseudomonas
313	PC188	5/18/76	C2-S	Pseudomonas
314	PC190	5/18/76	CF-S	Aeromonas
315	PC192	5/18/76	CF-S	Pseudomonas

BIBLIOGRAPHY

Atlas, R.M., and R. Bartha. 1973. Abundance, distribution and oil biodegradation potential of microorganisms in Raritan Bay. Environ. Pollut. 4: 291-300.

Atlas, R.M., E.A. Schofield, F.A. Morelli, and R.E. Cameron. 1976. Effects of petroleum pollutants on Arctic microbial populations. Environ. Pollut. 10: 35-43.

Beerstecher, E. 1954. Petroleum Microbiology. Elsevier Press, New York, N.Y.

Bender, M.E., E.A. Shearls, R.P. Ayers, C.H. Herschner, and R.J. Huggett. 1976. Ecological effects of experimental oil spills on Eastern coastal plain estuarine ecosystems. In press.

Bieri, R.H., V. Stamoudis, M.K. Cueman. 1976. Chemical investigations of two experimental oil spills in an Eastern coastal plain estuarine ecosystem. In press.

Borneff, J., F. Selenka, H. Kunte, and A. Maximos. 1968. Experimental studies on the formation of polycyclic aromatic hydrocarbons in plants. Environ. Res. 2: 22-29.

Buchanan, R.E., and N.E. Gibbons, co-editors. 1974. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Co., Baltimore, Md.

Buck, J.D., and R.C. Cleverdon. 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. 5: 78-80

Bushnell, L.D., and H.F. Haas. 1941. The utilization of certain hydrocarbons by microorganisms. J. Bacteriol. 41: 653-673.

Chet, I., and R. Mitchell. 1976. Petroleum hydrocarbons inhibit decomposition of organic matter in seawater. Nature 261: 308-309.

Cobet, A.B., and H.E. Guard. 1973. Effects of a bunker fuel on the beach bacterial flora. pp. 815-819. In EPA, API, USCG, Prevention and Control of Oil Spills. API, Washington, D.C.

Colwell, R.R., and C. Quadling. 1962. Miniature tube tests in diagnostic bacteriology. Can. J. Microbiol. 8: 813-816.

Colwell, R.R., and W.J. Wiebe. 1970. "Core" characteristics for use in classifying aerobic, heterotrophic bacteria by numerical taxonomy. Bull. Georgia Academy Science 28: 165-185.

Cowan, S.T., and K.J. Steel. 1970. Manual for the Identification of Medical Bacteria. Cambridge University Press, London, England.

Davis, J.B. 1967. Petroleum Microbiology. Elsevier Pub. Co., Amsterdam, The Netherlands.

Floodgate, G.D. 1972. Biodegradation of hydrocarbons in the sea. pp. 153-171. In R. Mitchell, ed., Water Pollution Microbiology. Wiley-Interscience, New York, N.Y.

Fogel, S., I. Chet, and R. Mitchell. 1971. Chemotactic response of marine bacteria. Bacteriol. Proc. G 31.

Friede, J., R.K. Gholson, E. Gaudy, and A.F. Gaudy. 1972. Assessments of biodegradation potential for controlling oil spills on the high seas. U.S. Coast Guard, Transportation Dept., Project #DAT 204-72.

Gunkel, W. 1967. Experimentall-ökalogische Untersuchungen über die limitierenden Faktoren des microbiellen Ölabbaues im marinen Milieu. Helgolaender Wiss. Meeresunters. 15: 210-225.

Hood, M. 1973. Chitin degradation in the salt marsh environment. A Dissertation. The Louisiana State University, Baton Rouge.

Hucker, G.J., and H.J. Conn. 1923. Methods of gram staining. Tech. Bull. N.Y. St. Agric. Exp. Sta. no. 93.

King, E.O., M.K. Ward, and D.E. Raney. 1954. Two simple media for the demonstration of pyocyanin and floures-cin. J. Lab. Clin. Med. 44: 301-307.

Klug, M.J., and A.J. Markovetz. 1971. Utilization of aliphatic hydrocarbons by microorganisms. pp. 1-43. In Advances in Microbiol Physiology, Vol. 5. Academic Press, New York, N.Y.

Kovacs, N. 1956. Identification of *Pseudomonas pycanea* by the oxidase reaction. Nature 178: 703.

Leifson, E. 1963. Determination of carbohydrate metabolism of marine bacteria. J. Bacteriol. 85: 1183-1184.

Lewis, D.H. 1973. Predominant aerobic bacteria of fish and shellfish. TAMU-SG-73-401. Texas A&M University, College Station, Texas.

Litchfield, C.D., Editor. 1976. Marine microbiology. Dowden, Hutchinson, and Ross, Stroudsburg, Pa.

MacLeod, R.A. 1968. On the role of inorganic ions in the physiology of marine bacteria. Advan. Microbiol. Sea 1: 95-126.

Meyers, S.P., D.G. Ahearn, S. Crow, and N. Berner. 1973. The impact of oil on marshland microbial ecosystems. pp. 221-228. In S.P. Meyers, ed., The microbial degradation of oil pollutants. Center for Wetland Resources, LSU-SG-73-01.

Miget, R.J., C.H. Oppenheimer, H.I. Kator, and P.L. LaRock. 1969. Microbial degradation of normal paraffin hydrocarbons in crude oil. pp. 327-332. In Proc. API/FWPCA Joint Conference on Prevention and Control of Oil Spills. API, Washington, D.C.

Mironov, O.G. 1970. Role of microorganisms growing on oil in the self-purification and indication of oil pollution in the sea. Oceanology 10: 650-656.

Mitchell, R., S. Fogel, and I. Chet. 1972. Bacterial chemoreception: an important ecological phenomenon inhibited by hydrocarbons. Water Res. 6: 1137-1140.

Moeller, V. 1955. Simplified test for some amino acid decarboxylases and for the arginine dihydrolase system.

Acta Path. Microbiol. Scand. 36: 158.

Murchelano, R.A., and C. Brown. 1970. Heterotrophic bacteria in Long Island Sound. Mar. Biol. 7: 1-6.

Oró, J., T.G. Tornabene, D.W. Nooner, and E. Gelpi. 1967. Aliphatic hydrocarbons and fatty acids of some marine and freshwater microorganisms. J. Bacteriol. 93: 1811-1818.

Rogoff, M.H. 1961. Oxidation of aromatic compounds by bacteria. pp. 193-221. In Advances in Applied Microbiol., Vol. 3. Academic Press, New York, N.Y.

Shewan, J.M. 1963. The differentiation of certain genera of gram negative bacteria frequently encountered in marine environments. pp. 499-521. In C.H. Oppenheimer, ed., Syposium on Marine Microbiology. Charles C. Thomas, Pub.

Shewan, J.M., W. Hodgkiss, and J. Liston. 1954. A method for the rapid differentiation of certain non-pathogenic, asporogenous bacilli. Nature 173: 208-209.

Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substances. Antonie van Leeuwenhoek 23: 15-23.

Sneath, P.H.A., and R.R. Sokal. 1973. Numerical Taxonomy: the Principles and Practice of Numerical Classification. W.H. Freeman and Co., San Francisco.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco.

Treccani, V. 1962. Microbial degradation of hydrocarbons. pp. 2-33. In Progress in Industrial Microbiology, Vol. 4. Gordon and Breach, Science Pub., New York, N.Y.

Walker, J.D., and R.R. Colwell. 1974. Microbial degradation of model petroleum at low temperatures. Microbial Ecology 1: 63-95.

Walker, J.D., and R.R. Colwell. 1975. Some effects of petroleum on estuarine and marine microorganisms. Can. J. Microbiol. 21: 305-313.

Walker, J.D., and R.R. Colwell. 1976a. Enumeration of petreoleum-degrading microorganisms. Appl. Environ. Microbiol. 31: 198-207.

Walker, J.D., and R.R. Colwell. 1976b. Measuring the potential activity of hydrocarbon-degrading bacteria. Appl. Environ. Microbiol. 31: 189-197.

Walker, J.D., R.R. Colwell, and L. Petrakis. 1976. Biodegradation of petroleum by Chesapeake Bay sediment bacteria. Can. J. Microbiol. 22: 423-428.

Walker, J.D., P.A. Seesman, and R.R. Colwell. 1974. Effects of petroleum on estuarine bacteria. Mar. Pollut. Bull. 5: 186-188.

Walker, J.D., P.A. Seesman, and R.R. Colwell. 1975. Effects of South Louisiana crude oil and no. 2 fuel oil on growth of heterotrophic, lipolytic, chitinolytic, and cellulytic bacteria. Environ. Pollut. 9: 13-33.

Walsh, F., and R. Mitchell. 1973. Inhibition of bacterial chemoreception by hydrocarbons. pp. 275-278. In S.P. Meyers, ed., The microbial degradation of oil pollutants. Center for Wetland Resources, LSU-SG-73-01.

Went, F.W. 1960. Organic matter in the atmosphere, and its possible relation to petroleum formation. Proc. Nat. Acad. Sci. 46: 212-221.

Winer, B.J. 1962. Statistical principles in experimental design. McGraw-Hill, New York, N.Y.

ZoBell, C.E. 1946a. Action of microorganisms on hydrocarbons. Bact. Rev. 10: 1-49.

ZoBell, C.E. 1946b. Marine Microbiology. A Monograph on Hydrobacteriology. Chronica Botanica Co., Waltham, Mass.

ZoBell, C.E. 1950. Assimilation of hydrocarbons by microorganisms. pp. 443-486. In Advances in Enzymology, Vol. 10. Interscience Pub., New York, N.Y.

ZoBell, C.E. 1971. Sources and biodegradation of carcinogenic hydrocarbons. pp. 441-451. In API/EPA/USCG

Conference on Prevention and Control of Oil Spills.
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