Research and Development

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Alaska Oil Spill Bioremediation Project

Science Advisory Board Draft Report
Sections 7 through 13 and Appendices

ALASKA OIL SPILL BIOREMEDIATION PROJECT

SCIENCE ADVISORY BOARD DRAFT REPORT

SECTIONS 7 THROUGH 13 AND APPENDICES

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SECTION 7
PASSAGE COVE FIELD RESULTS

VISUAL OBSERVATIONS

Original oil contamination in Passage Cove was considered moderate. Following complete physical washing, oil was well distributed over most of the surface of all cobble and all gravel under the cobble. The oil was black, dry, and dull in appearance with considerable stickiness. It was spread as a thin layer over the beach material. Relatively few patches of pooled oil or mousse were present, but where they were, the oil was thick and viscus. Oil was also found at depth in the beach, generally 30 to 40 cm below the surface. It was well distributed within the beach material.

Approximately two weeks following the combined application of oleophilic and granular fertilizer (Tern Beach), it became apparent that the treated beach was considerably cleaner relative to the untreated control plots. In contrast to the observations at Snug Harbor, the rock surfaces looked cleaner and the oil under the rocks in the mixed sand and gravel plots within the beaches had disappeared to a greater extent. In another two weeks, oil was found only in isolated patches, and at 10 cm and below in the subsurface. At no time were oil slicks or oily material seen leaving the beach area. During this time, no visual disappearance of oil from the surface of cobble on the untreated control beach (Raven) occurred.

Unexpectedly, the beach treated with fertilizer solution from the sprinkler system (Kittiwake beach) behaved in a very similar manner to the oleophilic/granular-treated plot: extensive disappearance of the oil occurred compared to the untreated control plots (Figures 7.1 and 7.2). The only difference was that it lagged behind the oleophilic/granular-treated beach by approximately 10 to 14 days. By the end of August, both beaches (Kittiwake and Tern) looked equally clean. In contrast, the untreated control beach appeared very much as it did in the beginning of the field study. Oil in the subsurface still remained in all plots within the beaches. However, in the fertilizer-treated plots within the beaches, oil was visually apparent only below a depth of 20 to 30 cm.

Disappearance of oil from the rock surfaces on the beach treated with the fertilizer solution provided the definitive proof that biodegradation (and not chemical washing) was responsible for the oil removal, as there was no other reasonable mechanism to explain this fertilizer-mediated loss of oil.

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Figure 7.1. Kittiwake Beach at Passage Cove, Treated with Fertilizer Solution from the Sprinkler System, Showed Extensive Disappearance of Oil Compared to Untreated Control Plots (Figure 7.2).

Figure 7.2. Oiled Untreated Control Plots.

NUTRIENT CONCENTRATIONS

Data on the nutrient concentrations in tidal waters following fertilizer application at Passage Cove were lost by the contractor (SAIC) responsible for performing the analysis.

OIL CHEMISTRY

Oil Residue Weight

Cobble Surface Samples

Figures 7.3 to 7.5 show changes (decay curves), for the three test beaches (Tern, Raven and Kittiwake), in residue weights of oil on the cobble surface. The residue weights are normalized to a kilogram weight of cobble. Given the relatively smooth surface of the cobblestones, it was assumed that a consistent relationship between rock surface area and rock weight existed. Variability in the residue weights was a function of both the sampling of heterogenous beach material and the uneven distribution of oil on the beach.

Highly variable oil residue weights at all sampling times (two orders of magnitude variation in some cases) are conspicuous in the figures. Extensive decreases in oil residue weights through time occurred on all beaches. The percent change over time in the median residue weight is shown in Figure 7.6 and Table 7.1.

A statistical comparison of the residue weights for the cobble samples was conducted using the nonparametric Mann-Whitney test (Zar, 1984). The results from this test show that at the 95% confidence level, significantly less oil residue was present on the fertilizer solution-treated beach (Kittiwake) than on the untreated control beach (Raven) at all sampling times except $t=0$ days (Table 7.1). Thus, initially these two beaches had statistically similar amounts of oil residue on the beach cobble, but with time more oil was lost from the fertilizer solution-treated beach. By the last sampling period in September, approximately four times more oil residue remained on the surface of the untreated control beach than on the fertilizer solution-treated beach.
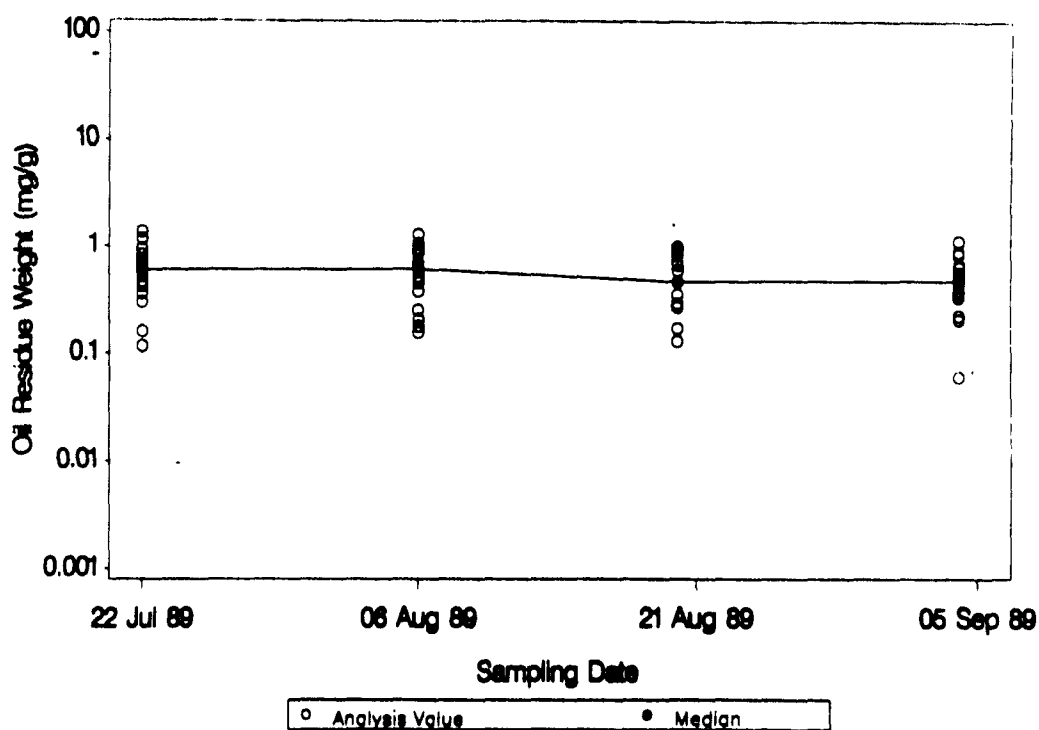
Figure 7.3. Change In Oil Residue Weight Through Time for Raven Beach (Untreated Control) at Passage Cove (Cobble Surface).
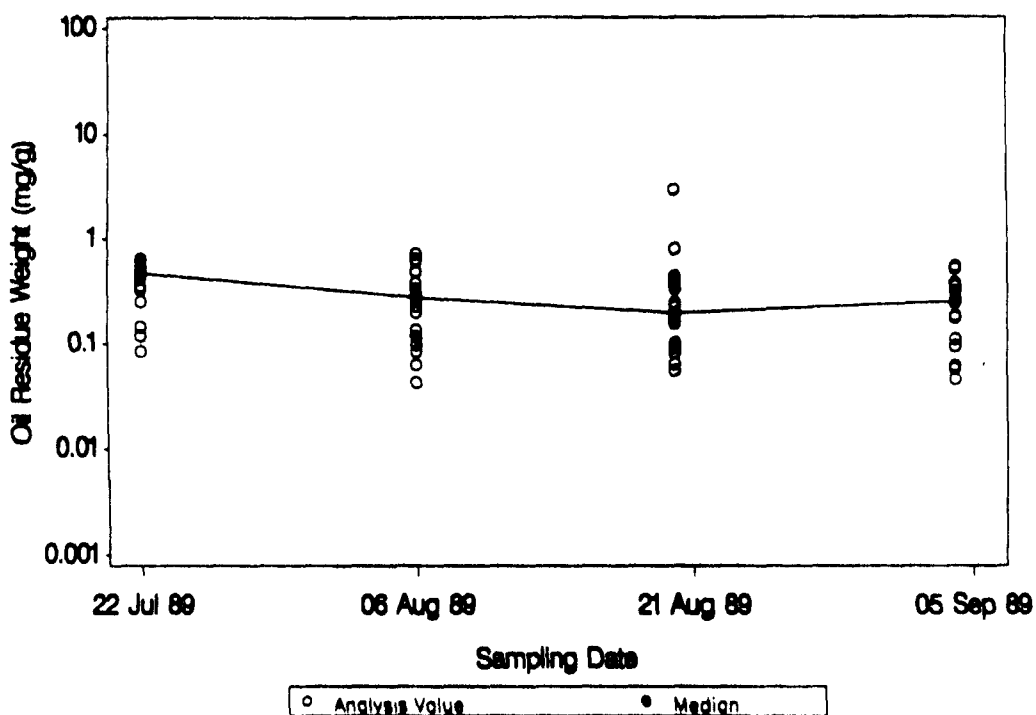

Figure 7.4. Change In Oil Residue Weight Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Cobble Surface).
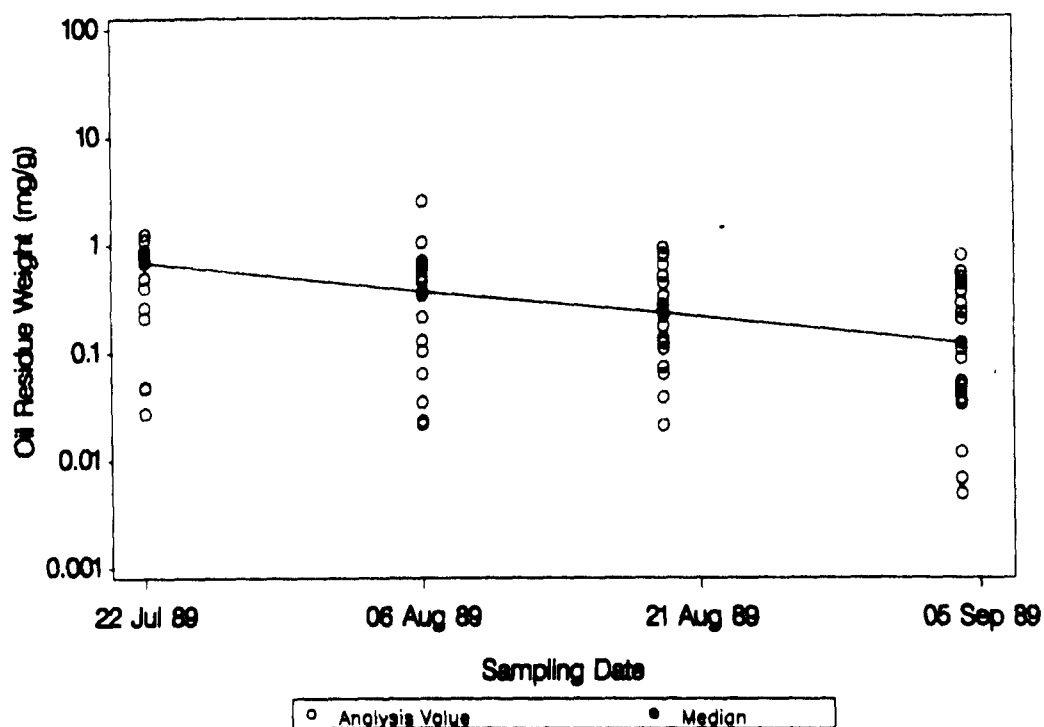
Figure 7.5. Change In Oil Residue Weight Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Cobble Surface).
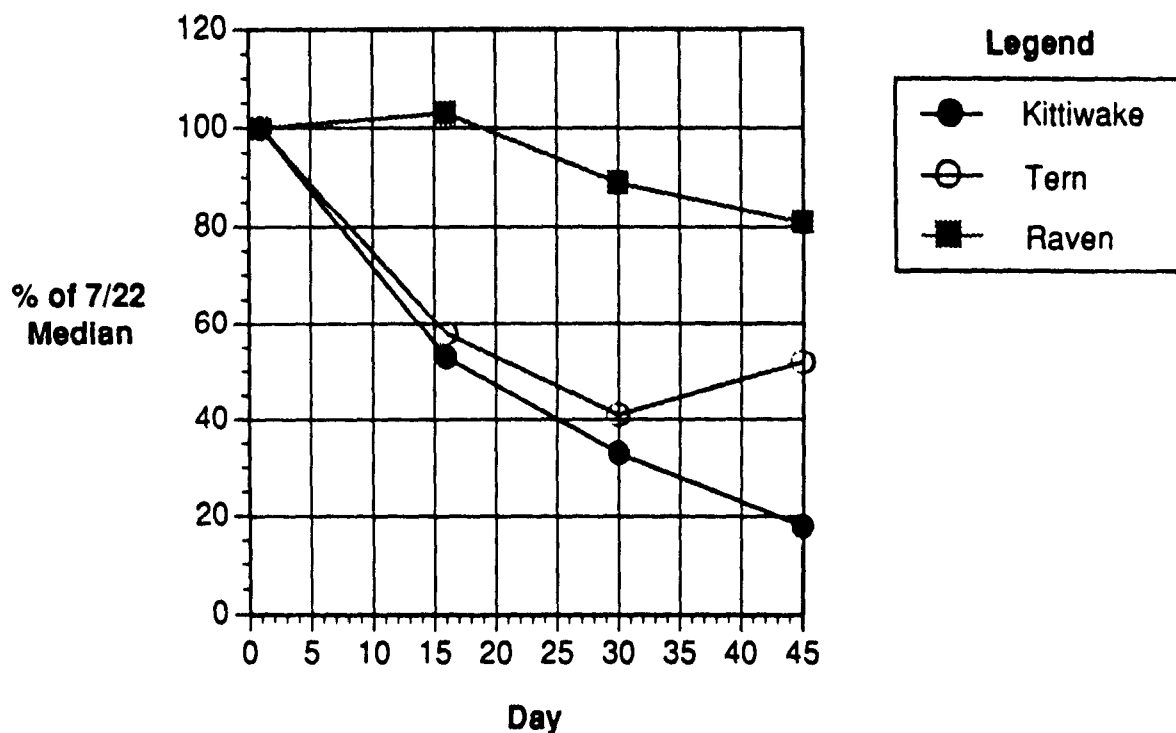

Figure 7.6. Change In the Median Residue Weight, Expressed as Percent of the 7/22 Median Over Time for Kittiwake, Raven, and Tern Beaches at Passage Cove.

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TABLE 7.1. MEDIAN VALUES AND STATISTICAL COMPARISONS OF OIL
RESIDUE WEIGHTS FROM DIFFERENT BEACH TREATMENTS[a]

Median Values (Percent of 7/22/89 Median)

Sampling Date	Days	Kittiwake	Tern	Raven
7/22/89	0	0.684	0.476	0.596
8/06/89	15	0.364 (53%)	0.274 (58%)	.611 (103%)
8/20/89	29	0.228 (33%)	0.194 (41%)	0.530 (89%)
9/05/89	44	0.120 (18%)	0.249 (52%)	0.481 (81%)

Mann-Whitney Test Results[b]

Sampling Date	Kittiwake vs. Tern	Kittiwake vs. Raven	Tern vs. Raven
7/22/89	SAME	SAME	TR < RV
8/06/89	SAME	KW < RV	TR < RV
8/20/89	SAME	KW < RV	TR < RV
9/05/89	SAME	KW < RV	TR < RV

[a]Raven = no fertilizer treatment; Tern = combined fertilizer treatment with INIPOL and CUSTOMBLEN; Kittiwake = treatment with fertilizer solution.
[b]95 Percent Confidence Level

The striking effect of the fertilizer solution application strongly supports that oil disappearance was the direct result of biodegradation and not chemical-enhanced removal, as might be argued for the application of the INIPOL fertilizer. The only reasonable explanation for the effect of the fertilizer solution was that it enhanced biodegradation through the provision of nutrients, and biodegradation was responsible for the removal of the oil. Loss of oil residue through time, therefore, was most likely due to biodegradation and not a nonbiological fate process. It can be concluded that oil biodegradation in Prince William Sound is limited by the availability of nutrients and not carbon. In addition, the results imply that reapplication of nutrients (the extreme in this case) is probably important. The sprinkler system provided the most controlled and concentrated introduction of nutrients to the oil-degrading microbial communities.

Further data analysis demonstrated a statistically significant difference in the residue weights at the 95% confidence interval between the INIPOL/CUSTOMBLEN-treated beach (Tern) and the untreated control beach (Raven) included the t=0 sampling date (Table 7.1). Thus, the amount of oil residue on each beach was initially different, and through time this difference was preserved. Therefore, enhanced oil disappearance (relative to the untreated control) as a result of fertilizer application on Tern beach cannot be established from this analysis.

Statistical analysis of the oil residue weight decay rates was also conducted. Regression analysis of the natural log-transformed residue weights versus sampling time suggested that a linear first-order approximation was sufficient to explain the decay curves for Kittiwake and Raven beaches, but not for Tern beach. Examination of the data from Tern beach shows that the median oil residue weight on the last sampling date actually increased. Plotting the percent changes in the medians shows this rather dramatically (Figure 7.6). This increase could not be attributed to any anomalies in the data (the residue weights for the last two sampling dates on Tern are not statistically different), although some reoiling of the beach could have occurred.

It is also possible that the decay curve for Tern beach reflects an oil biodegradation rate limitation by both substrate and nutrients because the nutrient release capabilities of the INIPOL/CUSTOMBLEN fertilizers became spent by the last sampling date (at Kittiwake beach nutrient supply was sustained at initial levels throughout). Accordingly, since the last two sampling dates on Tern are not statistically different, it is possible that the biodegradation rate of the oil leveled-off very suddenly.

Comparisons of the first-order decay constants (slopes of the lines) using a least squares fit are shown in Table 7.2 (Tern results are based on only three sampling dates, as the September 5 results are not consistent with the first order behavior seen in the first three sampling periods). Only the slope for Kittiwake beach (fertilizer solution-treated) was significantly different from zero; p=0.0009 is much less than the 0.05 level for the traditionally used 95% confidence level. The slope for Tern beach (INIPOL/CUSTOMBLEN-treated), however, is very close to being significantly different from zero (93.8% confidence level), suggesting a potential significant effect of the fertilizer application. The slope for Raven was significantly different from zero at a lower confidence level (<90%).

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TABLE 7.2. RATE ANALYSIS OF NATURAL LOG-TRANSFORMED OIL RESIDUE
WEIGHTS ON COBBLE SURFACES FOR TEST BEACHES IN PASSAGE COVE

Beach[a]	Fertilizer Treatment	Slope (Std. Dev.)	Significance of Slope Greater than Zero			Half Life, days	Time to Remove 90%, days
			N	T-value	p		
KW	Fertilizer Solution	-0.030 (0.0087)	80	-3.45	0.0009	23.1	76
T	INIPOL/ CUSTOMBLEN	-0.016 (0.0087)	60	-1.90	0.062	43.3	143
R	None	-0.006 (0.0041)	82	-1.54	0.127	115.5	383

Beaches Compared	Ratio of Slopes	t Value	Degrees of Freedom	Probability Level
KW/R	4.7	2.51	158	<0.01
T/R	2.6	1.16	138	>0.10
KW/T	1.8	0.79	136	>0.25

[a]KW - Kittiwake Beach; T - Tern Beach; R - Raven Beach.

Ratios of the slopes suggest that the addition of fertilizer solution to Kittiwake increased the decay rates as much as 4.7 times over the untreated control beach. Addition of INIPOL/ CUSTOMBLEN produced a decay rate 2.6 times greater than the untreated control beach. However, only the Kittiwake/Raven comparison was statistically significant at the 95% confidence interval. The Tern/Raven comparison was significant at the 90% confidence level.

Table 7.2 also gives the calculated half-lives for the decay of the oil residue weights on each beach. It was assumed that the decay rate remained constant over an extended period (into winter months), although there is no evidence to support this assumption. Assuming that the first-order

decay rate constant remains the same, the time to remove 90% of the starting material was calculated; the values are 76, 143, and 383 days for Kittiwake, Tern, and Raven, respectively. From this we would predict that by the following spring (1990), all of the oil on the fertilizer solution-treated beach should be gone, but oil might still be found on the other beaches. The other beaches have large uncertainties in their slopes, making predictions very uncertain.

The oil residue weight decay curves were also analyzed as zero-order rates by determining the least squares fit of nontransformed medians to a straight line. From inspection it was noted that the data from the last sampling date on Kittiwake and Tern (September 5) was not following a linear trend. The Tern median was above the third sampling date (an anomalous increase) and the Kittiwake median did not decrease enough for a linear rate of change (significantly different behavior from the assumed zero-order decay). Thus, only the first three sampling dates were included in the analysis. The results are shown in Table 7.3.

TABLE 7.3. RATE ANALYSIS OF NONTRANSFORMED OIL RESIDUE WEIGHTS (ZERO-ORDER) ON COBBLE SURFACES IN PASSAGE COVE

Beach	Fertilizer Treatment	Slope[a] (mg/kg/day)	Std. Dev.[a]	T-Value	Significance	Estimated Time (days) for complete removal
Raven	None	-7.2	1.3	-5.5	0.03	32
Tern	INIPOL/ CUSTOMBLEN	-13.2	2.4	-5.5	0.11	36
Kittiwake	Fertilizer Solution	-21.3	0.7	-29.0	0.02	82

[a]Linear regression fit.

The slopes and standard deviations are in units of mg oil residue lost per kg of beach material per day. As expected, with only one degree of freedom per median point, the significance is not very good, particularly for Tern beach. However, because each median represents the location of a distribution of approximately 20 samples and the distribution is skewed, the derived slopes are

probably more representative of the change in the central values of the oil residue than the significance values would indicate. Direct extrapolation of this rate information to cleanup times must, therefore, be considered with caution. Accordingly, the rate analysis would suggest that much of the oil on the cobble surfaces from the fertilizer-treated beaches should have disappeared in four to five weeks. This agrees with the visual observations. Oil on the cobblestone surface from the untreated control beach should have disappeared much more slowly, and as observed, oil was still present 11 to 12 weeks following initiation of the field study.

It can be confidently concluded that the application of fertilizer solution promoted the loss of oil residue four to five times faster than no treatment, and with slightly less confidence, approximately two to three times faster with the INIPOL/CUSTOMBLEN treatment.

Mixed Sand and Gravel Samples

Analysis of oil disappearance rates in the mixed sand and gravel samples showed that only the fertilizer solution-treated beach (Kittiwake) had a slope significantly different from zero (Figures 7.7 to 7.9). The first-order decay rate constant based on regression analysis of log transformed medians was -0.035 per day (std. dev. = 0.009, t = -3.73, significance = 0.0004), or a half-life of 20 days. Oil disappearance in the mixed sand and gravel on Kittiwake beach, therefore, was as fast as the cobble surface. The absence of any significant effect of fertilizer on Tern beach is difficult to explain. It does not appear to be an effect of different oil concentrations; residue weights per unit of beach material are generally the same. Availability of nutrients to the bacterial populations and to the oil are unlikely to be much different from cobble surfaces, as the mixed sand and gravel was very porous. However, because of the fertilizer release characteristics, it is possible that lower concentrations of nutrients reached the mixed sand and gravel on Tern beach. It is also possible that greater retention of oil residues within the matrix of the mixed sand and gravel may have occurred. Tidal action should have had less of a physical scouring effect in this matrix relative to the surface of the cobblestone.
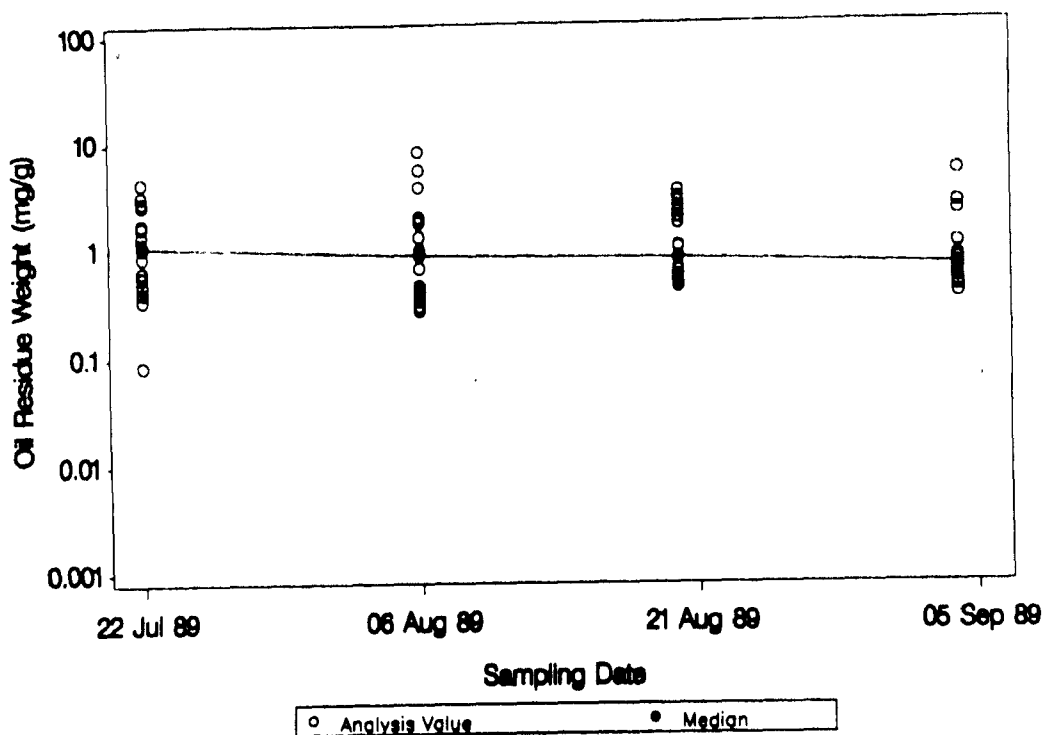
Figure 7.7. Change In Oil Residue Weight Through Time for Raven Beach (Untreated Control) at Passage Cove (Mixed Sand and Gravel).
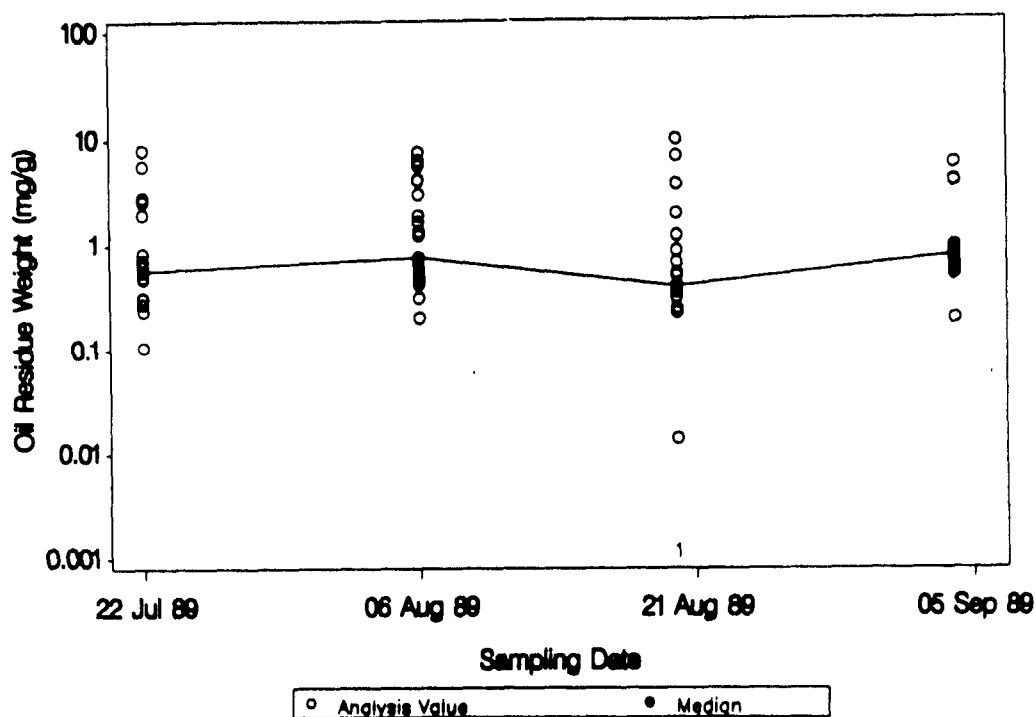

Figure 7.8. Change In Oil Residue Weight Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.
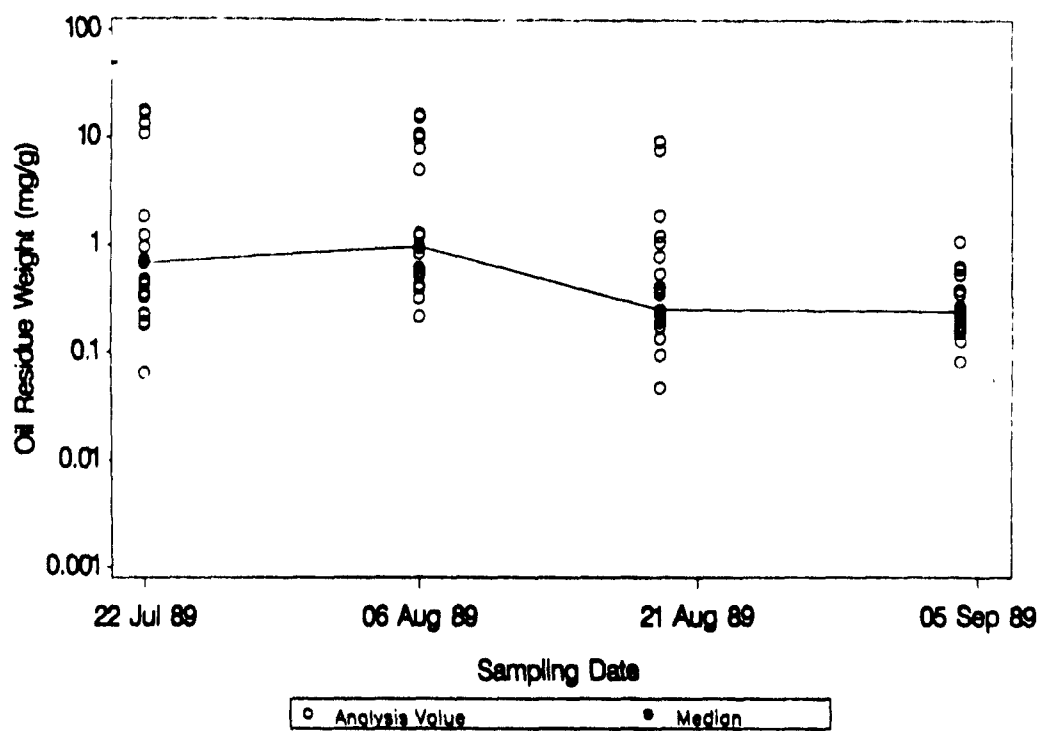
Figure 7.9. **Change in Oil Residue Weight Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Mixed Sand and Gravel).**

Oil Composition

Cobble Samples

Changes through time in the concentration of nC18, the sum of normal alkanes nC18 to nC27, and phytane in cobble samples lend strong support the role of biodegradation. Decay curves for each of these hydrocarbons are shown in Figures 7.10 to 7.18. All values of hydrocarbon concentration are normalized to the weight of oil in the extracted sample. The normal alkanes nC18 to nC27 were chosen because correlation analysis showed that these hydrocarbons tracked each other consistently. In all cases, values below detection limits were treated as zeros.

For comparative purposes Tables 7.4 and 7.5 summarize the percent change in the medians of individual hydrocarbons, and the number of samples showing a hydrocarbon concentration of zero (below detection limits) with each sampling period, respectively. Figure 7.19 also provides a graphical representation of the percent change in the medians.

By qualitatively assessing the extent of compositional change, several important trends were identified. Overall, the results mirror trends observed with the oil residue weights: a substantial decrease in hydrocarbon concentration through time occurred for both fertilizer-treated and untreated control beaches. However, Kittiwake beach was consistently and distinctly different from the other two beaches. Percent change in the medians for Kittiwake was always the largest and the quickest to occur, and many more samples had hydrocarbon concentrations below detection limits. As observed with the residue weights, there was little question that the application of the fertilizer solution on Kittiwake beach affected changes in oil composition that were substantially greater than those observed in samples from the untreated control beach (Raven).

Effects resulting from the application of INIPOL/CUSTOMBLEN to Tern beach are less clear and distinct. In general, it appeared that changes in hydrocarbon composition were in-between those observed for Kittiwake and Raven. Tern beach appeared to behave differently on many sampling dates compared to Raven beach: percent change in the medians and the number of below detection limit values were considerably greater than Raven beach in many cases. These differences were most prominent on the August 6 and August 20 sampling dates.
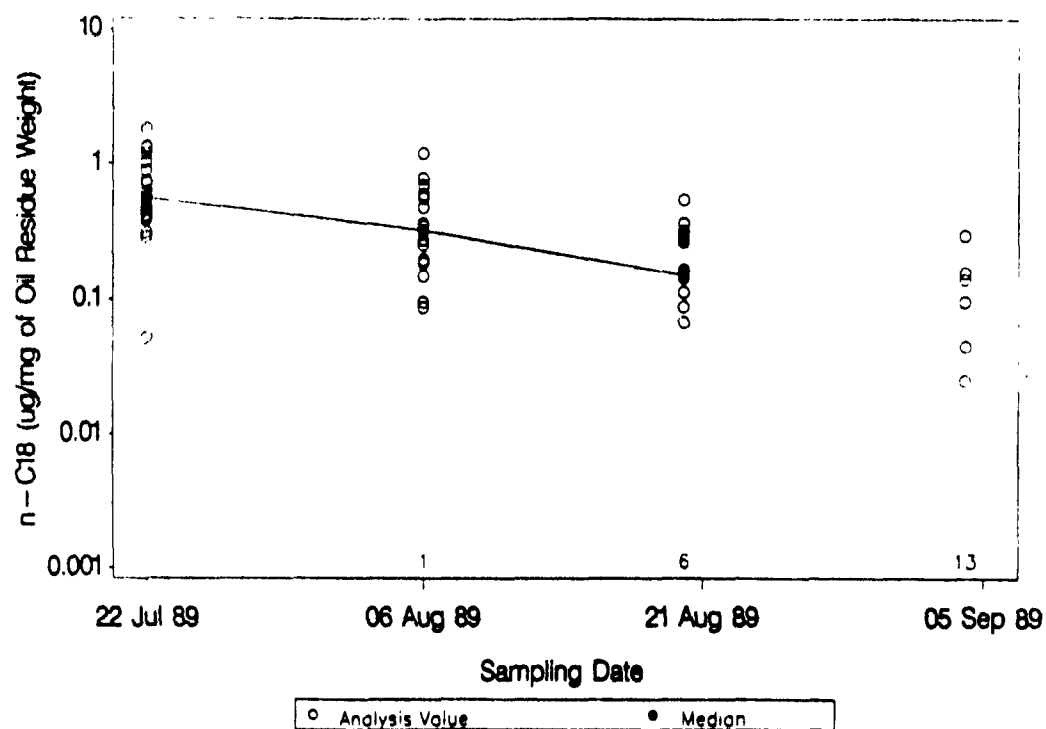
Figure 7.10. Change In nC18 Alkane Concentration Through Time for Raven Beach (Untreated Control) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
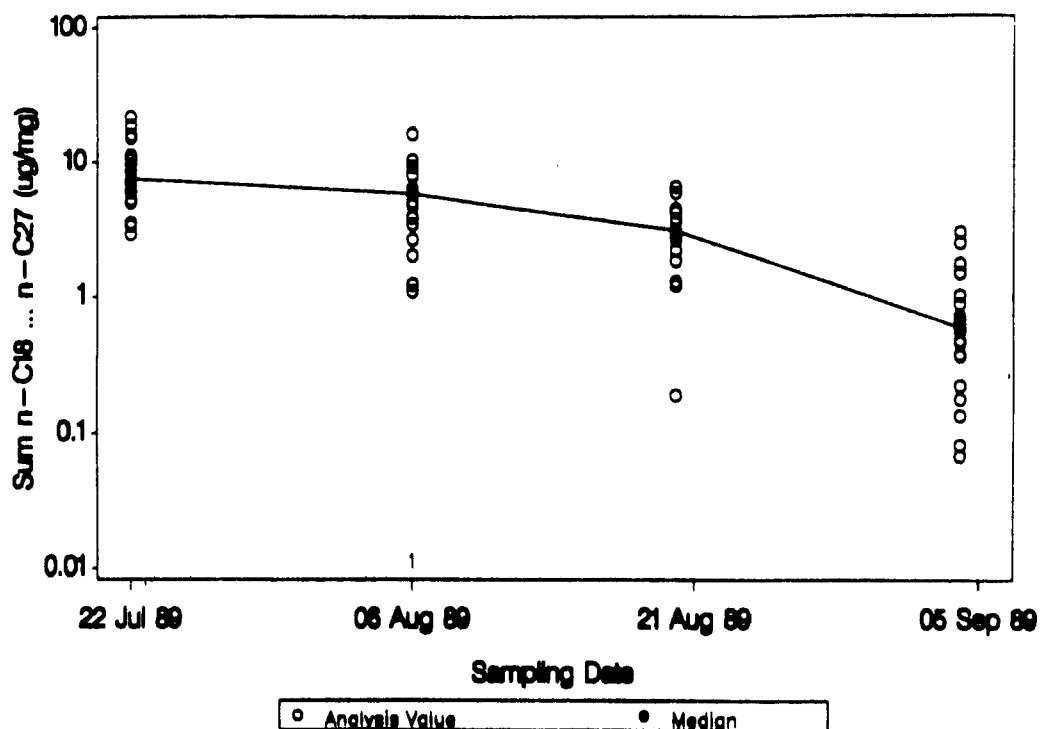
Figure 7.11. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Raven Beach (Untreated Control) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
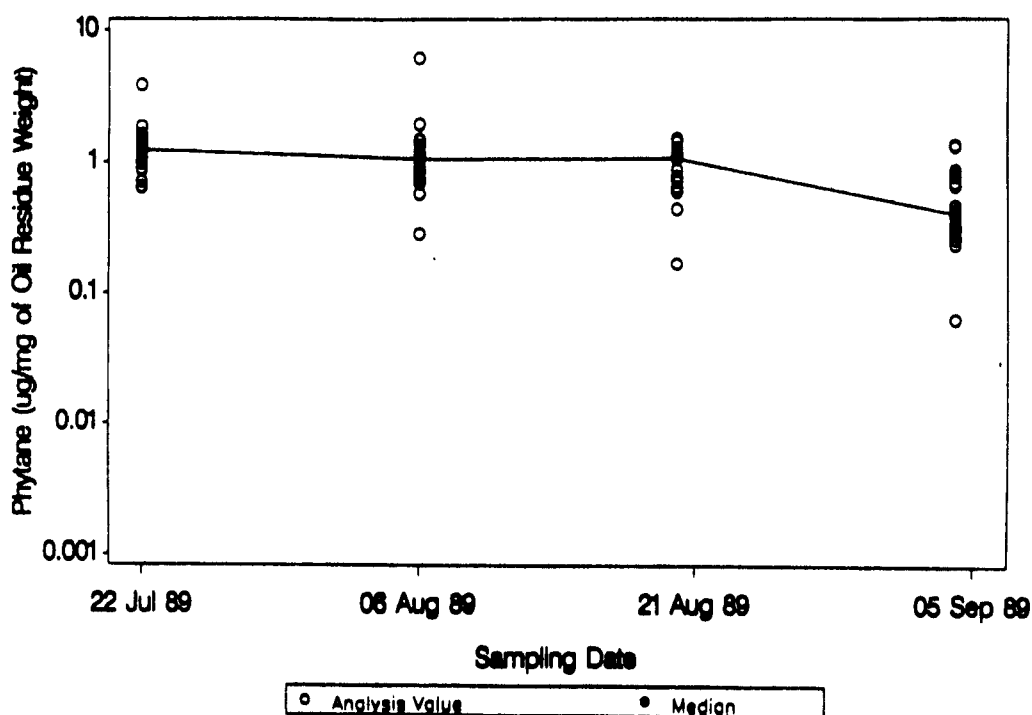

Figure 7.12. Change In Phytane Concentration Through Time for Raven Beach (Untreated Control) at Passage Cove (Cobble Surface).
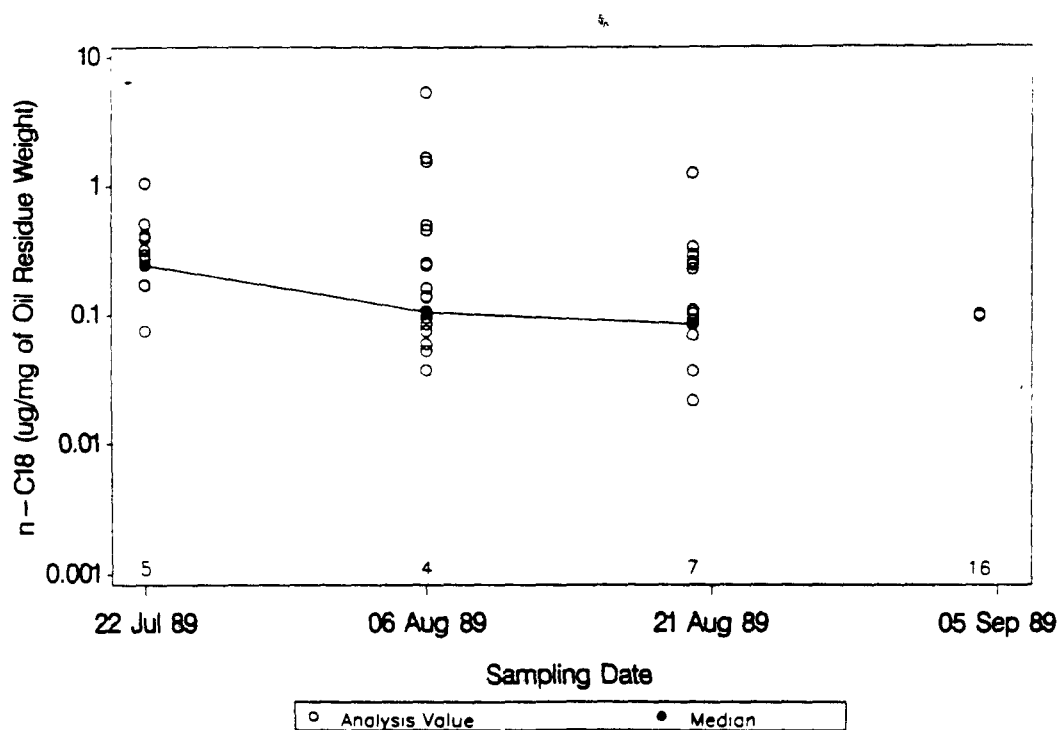
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Figure 7.13. Change in nC18 Alkane Concentration Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

Figure 7.14. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.

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Figure 7.15. Change in Phytane Concentration Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
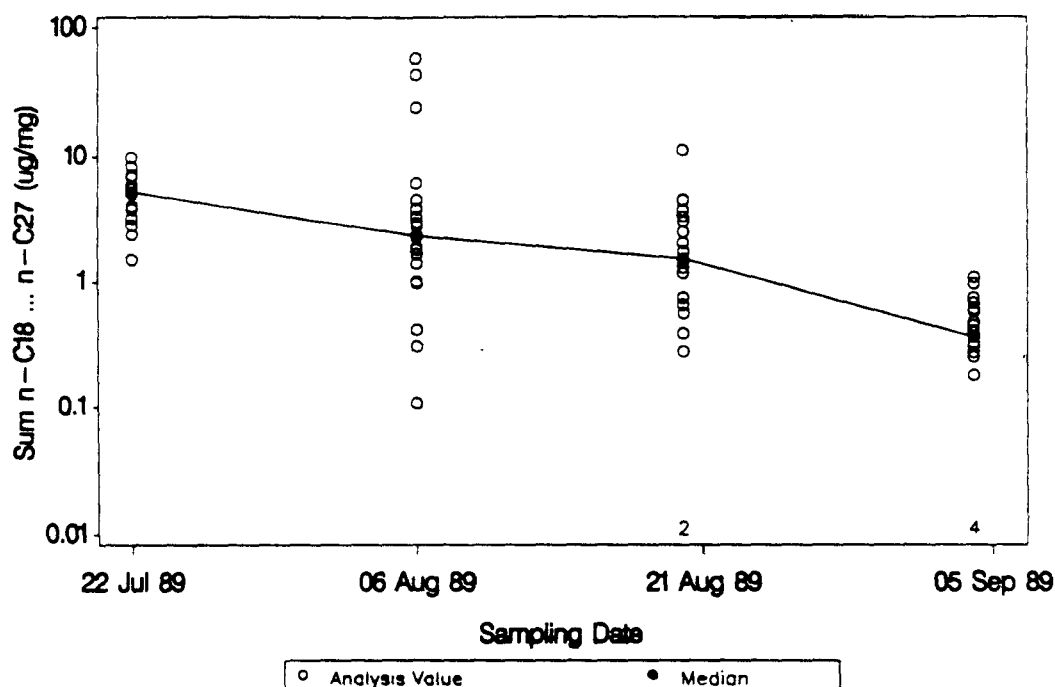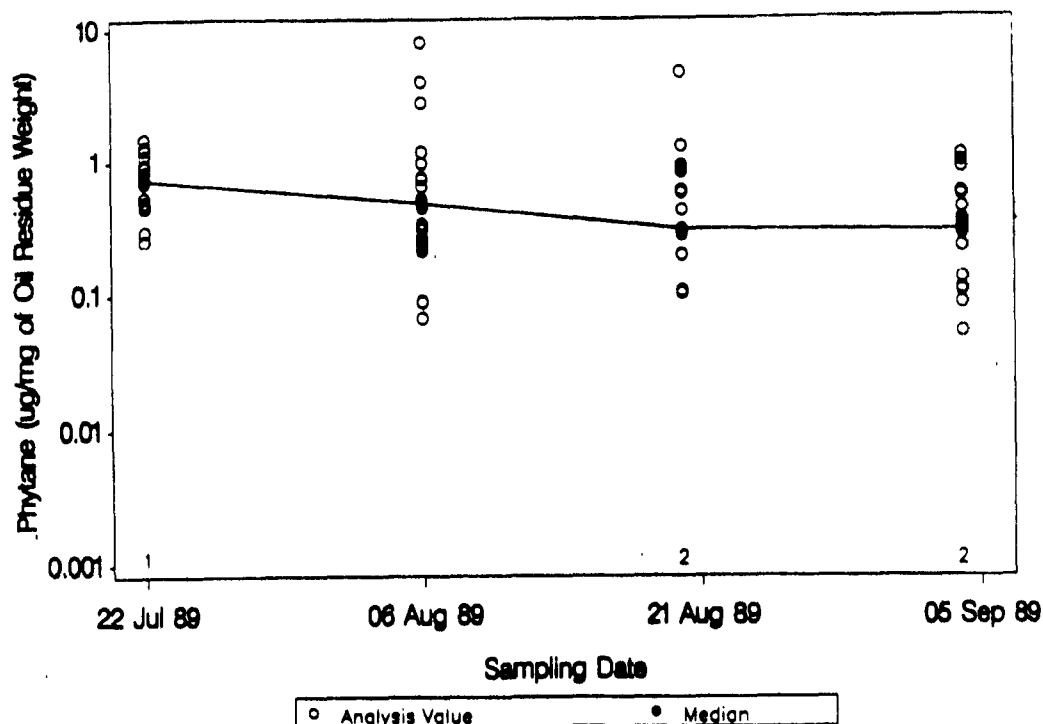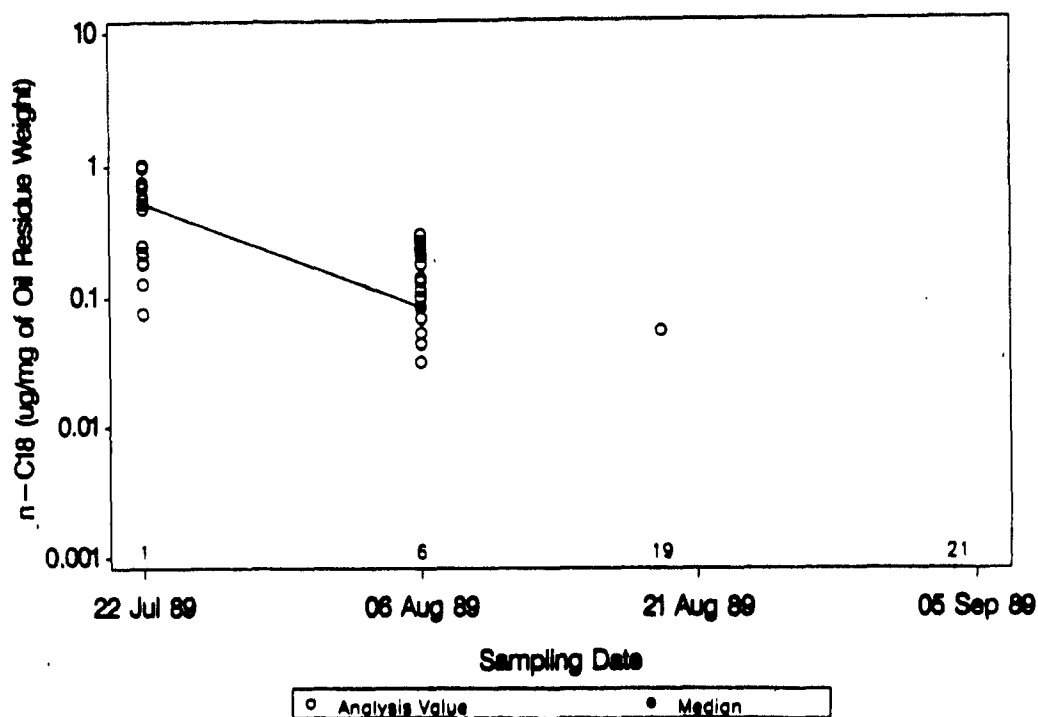

Figure 7.16. Change in nC18 Alkane Concentration Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 7.17. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 7.18. Change in Phytane Concentration Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
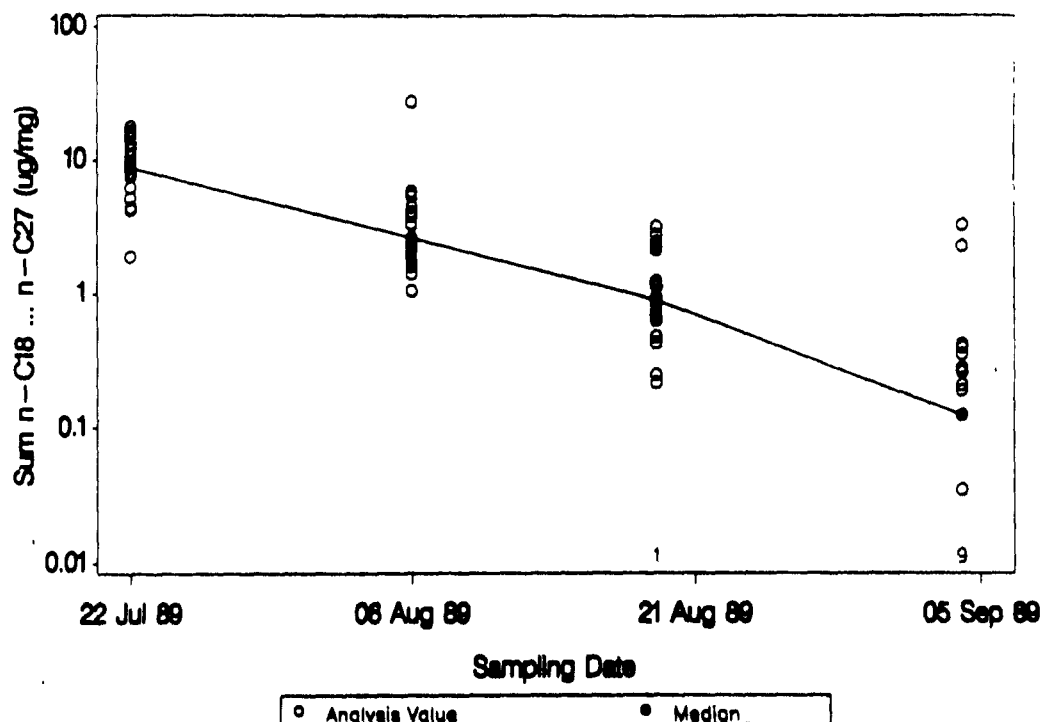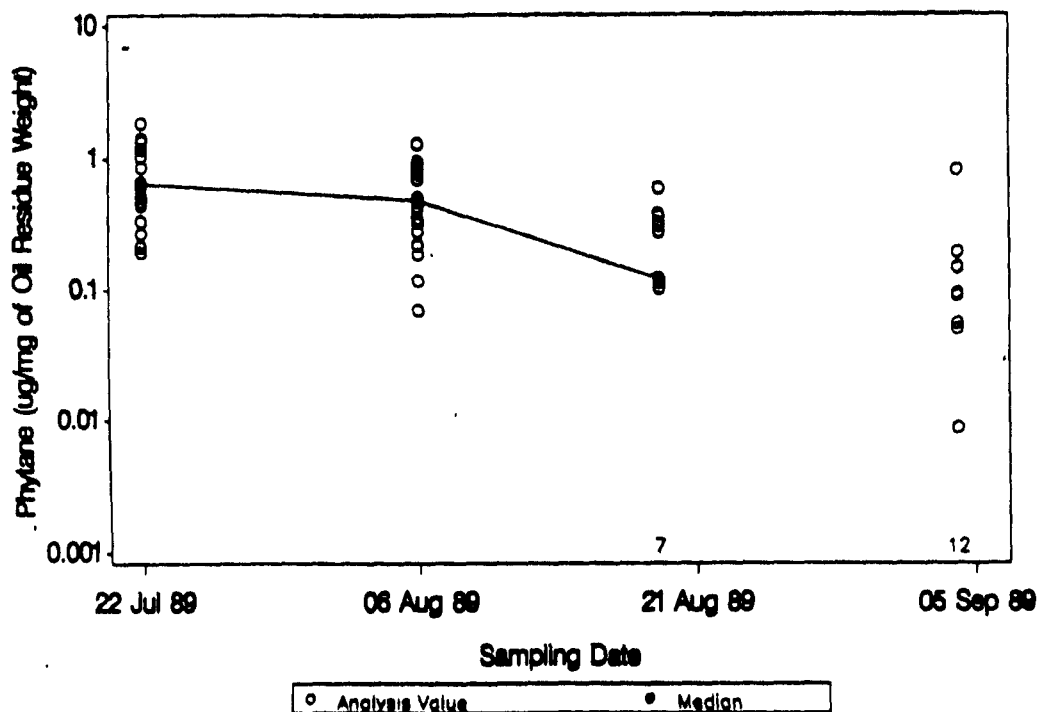
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TABLE 7.4. CHANGE IN HYDROCARBON COMPOSITION THROUGH TIME
EXPRESSED IN PERCENT OF THE MEDIAN CONCENTRATION OF
INDIVIDUAL HYDROCARBONS ON THE 7/22 SAMPLING[a]

Alkane	Beach Code	8/6	8/20	9/5
nC18	K	11	0	0
	T	43	34	0
	R	60	27	0
nC19	K	18	0	0
	T	54	33	13
	R	52	34	11
nC20	K	27	0	0
	T	41	23	0
	R	68	24	6
nC21	K	24	0	0
	T	39	15	0
	R	59	40	1
nC22	K	32	11	0
	T	47	27	0
	R	59	41	7
nC23	K	23	10	0
	T	35	32	12
	R	83	34	14
nC24	K	31	0	0
	T	44	28	0
	R	92	46	9
nC25	K	59	0	0
	T	41	33	0
	R	92	56	12
nC26	K	43	6	0
	T	53	40	0
	R	75	59	6
nC27	K	43	37	0
	T	57	51	0
	R	82	55	0
nC18 to nC27	K	30	10	1.4
	T	44	29	7
	R	77	44	8
Phytane	K	76	19	0
	T	67	41	41
	R	85	86	33

[a]R=Raven (untreated control beach); T=Tern (INIPOL/CUSTOMBLEN-treated beach); K=Kittiwake (fertilizer solution-treated beach).

TABLE 7.5. OF APPROXIMATELY 21 SAMPLES, NUMBER OF SAMPLES TAKEN AT
EACH SAMPLING TIME WITH ALKANE CONCENTRATION BELOW DETECTION LIMIT*

Alkane	Beach Code	7/22	8/6	8/20	9/5
nC18	K	1	6	19	21
	T	5	4	7	16
	R	0	1	6	13
nC19	K	0	0	13	17
	T	2	3	6	9
	R	0	1	1	6
nC20	K	1	0	12	17
	T	1	0	4	15
	R	0	1	0	7
nC21	K	0	0	13	14
	T	0	2	5	13
	R	0	1	1	10
nC22	K	0	0	5	13
	T	0	2	3	11
	R	0	1	1	9
nC23	K	0	0	6	19
	T	0	2	2	7
	R	0	1	2	1
nC24	K	1	1	14	20
	T	0	2	6	17
	R	0	1	1	8
nC25	K	1	1	13	20
	T	1	3	5	15
	R	0	1	1	5
nC26	K	1	2	10	20
	T	1	2	5	14
	R	0	2	1	9
nC27	K	1	0	2	15
	T	1	2	3	14
	R	0	1	3	13
nC18 to	K	0	0	1	9
nC27	T	0	0	2	4
	R	0	1	0	0
Phytane	K	0	0	7	12
	T	1	0	2	2
	R	0	0	0	0

*R=Raven (untreated control beach); T=Tern (INIPOL/CUSTOMBLEN-treated beach); K=Kittiwake
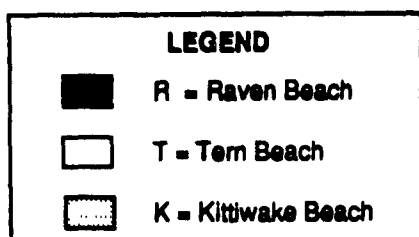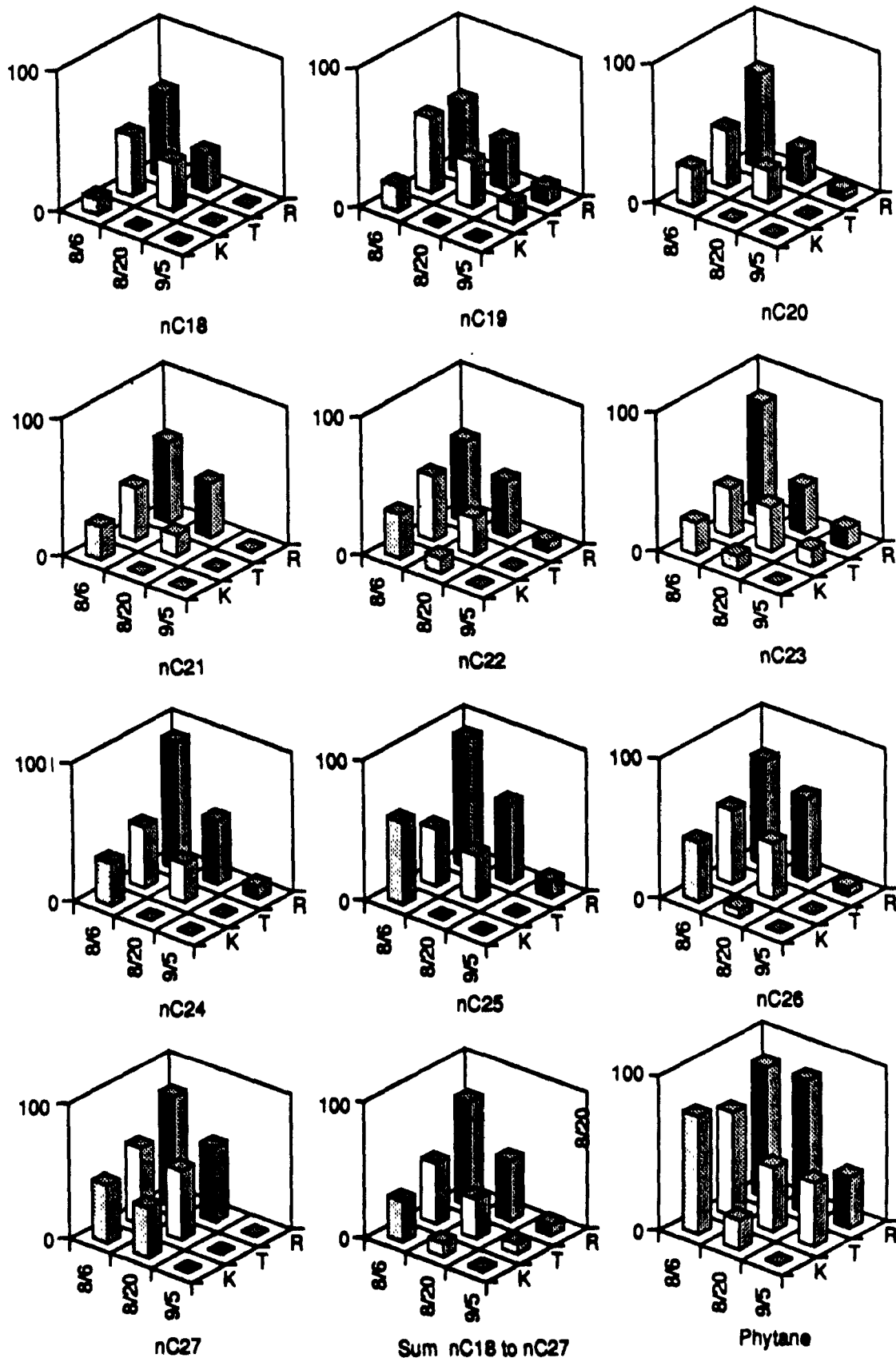(fertilizer solution-treated beach).

Figure 7.19. Change in Composition Expressed as Percent of the Median Concentration of Individual Hydrocarbons on the 7/22/89 Sampling for Raven, Tern, and Kittiwake Beaches at Passage Cove

LEGEND
R = Raven Beach
T = Tern Beach
K = Kittiwake Beach

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These qualitative differences are further enhanced in Figures 7.20 and 7.21. On the August 6 sampling day, it is apparent that for each of the individual hydrocarbons, Kittiwake samples consistently showed the greatest percent changes in the medians, Raven samples the least, and Tern samples in-between. Samples from both Kittiwake and Raven also show a general upward trend in the percent change in the medians, suggesting, as one might expect, that the lower molecular weight alkanes degrade more rapidly than the higher molecular weight alkanes. It is interesting that the upward trend is not apparent in the Tern samples. It is tempting to argue that initially the INIPOL/CUSTOMBLEN fertilizer application caused the higher molecular weight alkanes to be degraded as rapidly as the lower molecular weight alkanes. This is also reflected in the percent change of the phytane medians for this sampling date (data not plotted) in which the greatest change occurred in the samples from Tern beach. Svem, 1987, speculated that the mechanism of action for INIPOL might involve increased bioavailability of oil hydrocarbons, and this could affect the biodegradation rate of the higher molecular weight alkanes.

Results from the August 20 sampling day again show that the extent of the oil compositional change on Tern beach was generally in-between Raven and Kittiwake. However, in the samples from Tern it appeared that further decreases of the high molecular weight alkanes were small in contrast to the lower molecular alkanes, thus generating a pattern of compositional change similar to the other test beaches. This could be related to a waning of the INIPOL effect, and consequently a "catching up" by degradation in the absence of fertilizer.

Historically, the definitive indication that changes in hydrocarbon composition are due to biodegradation has been based on examining the weight ratio between a hydrocarbon that is known to readily biodegrade (generally the nC17 or nC18 alkanes) and those that are slower to biodegrade (generally the branched alkanes, pristane and phytane, which chromatograph very close to the nC17 and nC18 alkanes, respectively). This is based on the concept that most nonbiological fate processes (physical weathering, volatilization, photolysis, etc.) do not produce differential losses of aliphatic hydrocarbons that have similar gas chromatographic and chemical behavior. Consequently, any decrease in the ratio can be confidently assigned to the process of biodegradation. The changes in the nC18/phytane ratio are shown in Figures 7.22 to 7.24. The nC17/pristane ratios are not shown, but they provide essentially the same information.
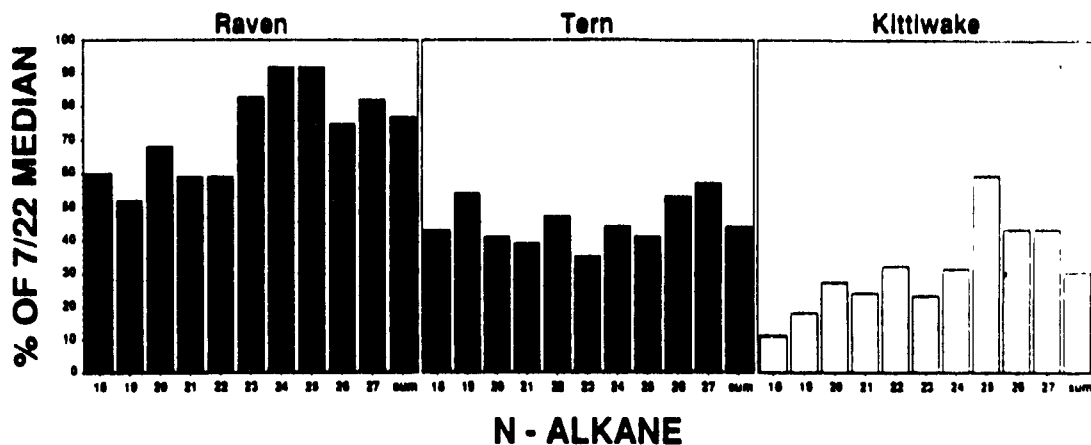
Figure 7.20 Hydrocarbon Composition on August 6, 1989, Expressed as Percent of the Median Concentration of Individual Hydrocarbons on the 7/22/89 Sampling for Raven, Tern, and Kittiwake Beaches at Passage Cove.
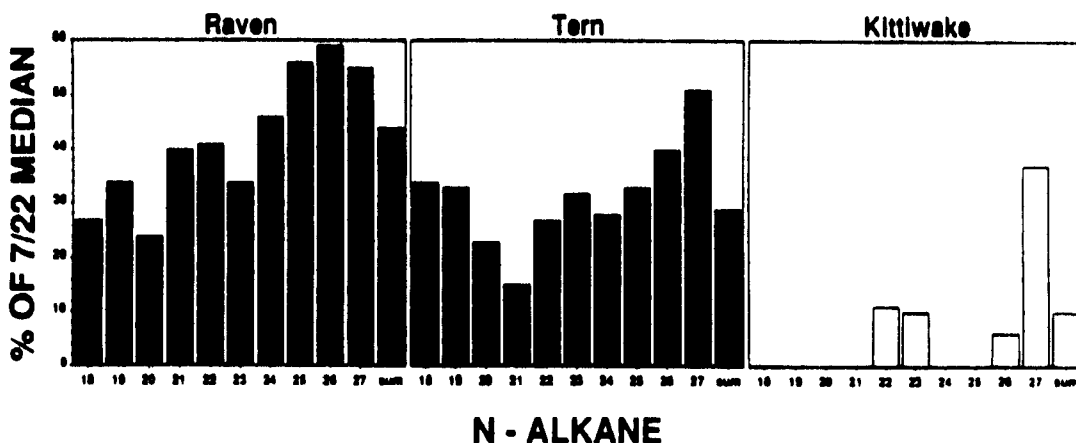

Figure 7.21. Hydrocarbon Composition on August 20, 1989, Expressed as Percent of the Median Concentration of Individual Hydrocarbons on the 7/22/89 Sampling for Raven, Tern, and Kittiwake Beaches at Passage Cove.
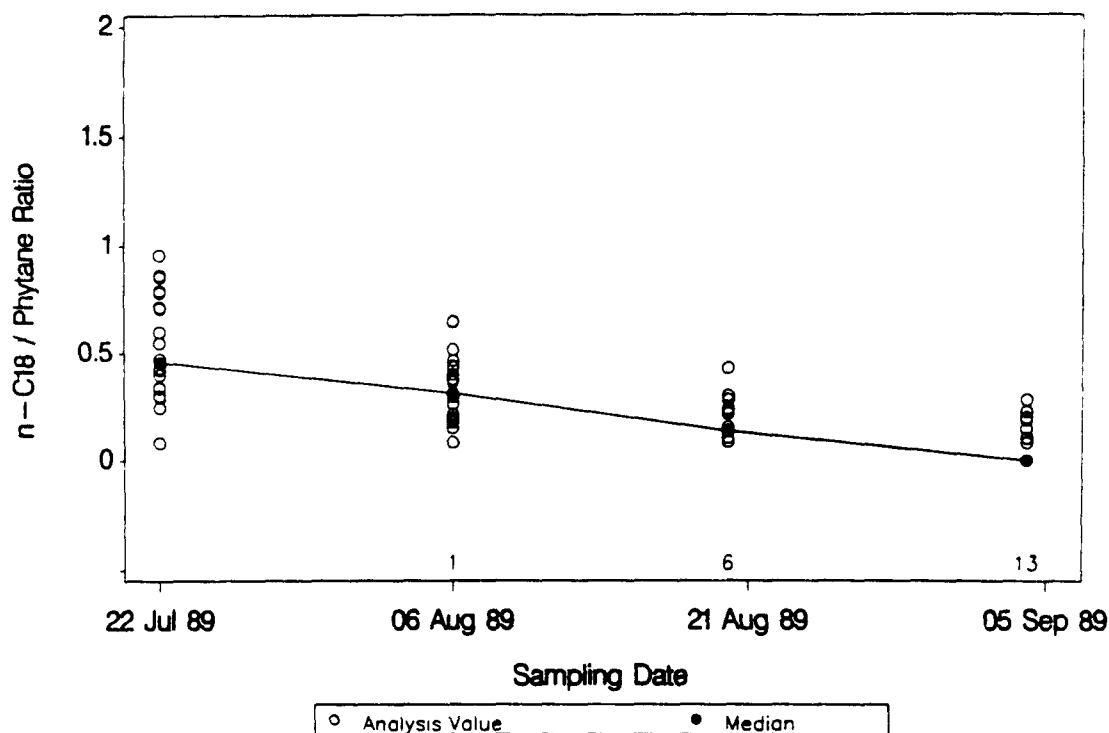
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Figure 7.22. Change In the nC18/phytane Ratio Through Time for Raven Beach (Untreated Control) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.
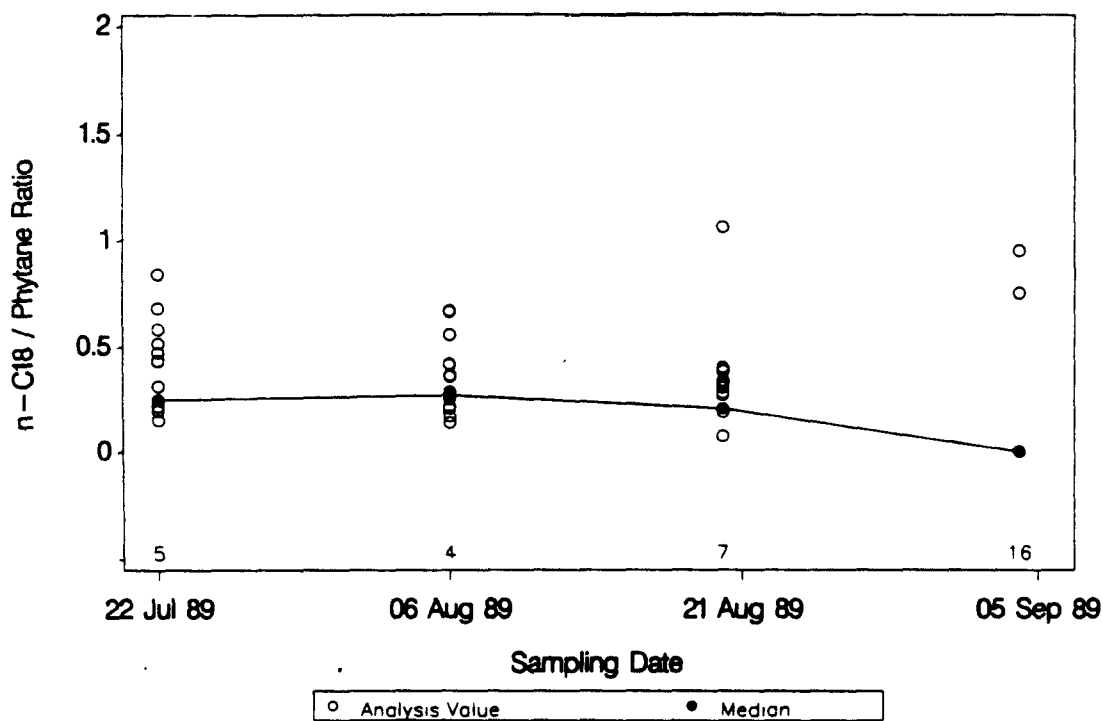

Figure 7.23. Change In the nC18/phytane Ratio Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit Is Shown Above the Sampling Date.
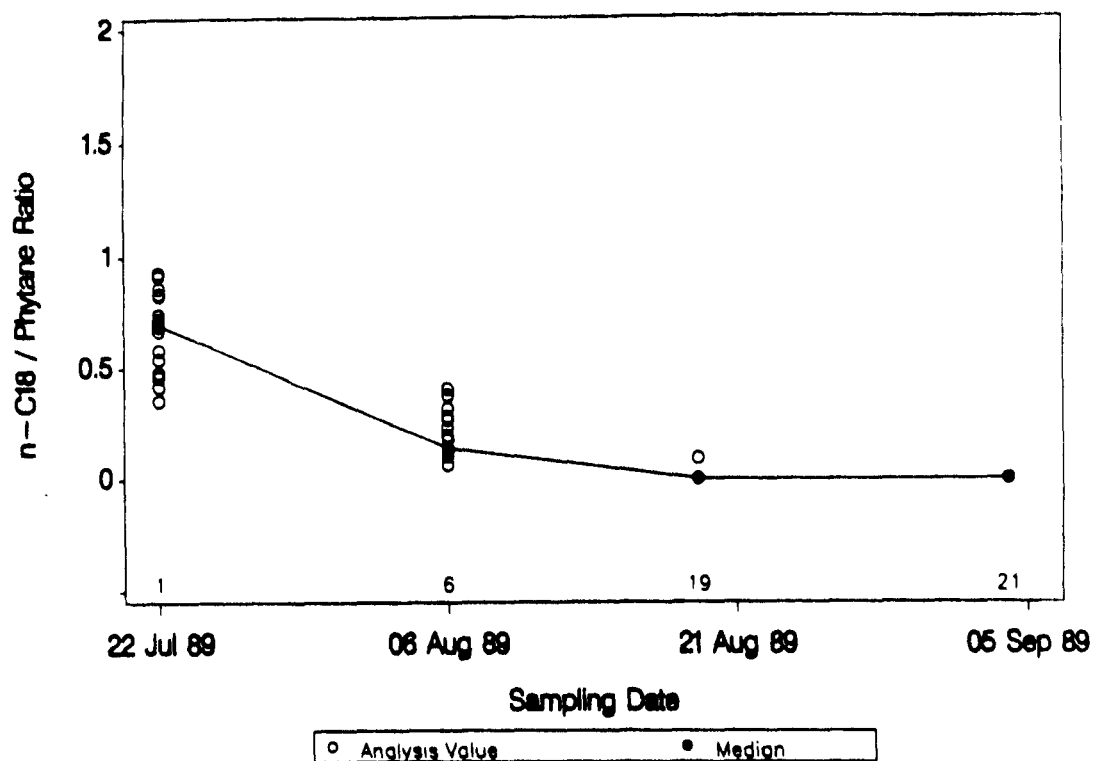
Figure 7.24. Change in the nC18/phytane Ratio Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Cobble Surface). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Several important pieces of information can be derived from these graphs. First, the initial ratios indicate that the oil samples taken from all beaches were significantly biodegraded. The ratio of unweathered Prudhoe Bay crude oil should be around 2.0. Median ratios were beow 1.0 at the initiation of the test. Thus, oil on the beaches was more than 50% biodegraded in terms of the aliphatic hydrocarbons. The extent of biodegradation was not statistically different between the three test beaches.

Under the assumption that oil on the beaches at the initiation of the fertilizer application test was generally uniformly colonized by oil-degrading bacteria, it is important to know if the variability in oil residue weights among beach samples was a function of the initial heterogenous distribution of the oil, or a function of the amount of biodegradation that had already taken place. In other words, was biodegradation prior to fertilizer application sufficient to remove significant amounts of oil residue from the beach material. Figures 7.25 and 7.26 show there was a very weak correlation (r square = 0.36 and 0.26, respectively, at the 95% confidence level) between standing oil residue weight and the nC18/phytane ratio at t=0 and t=0 plus one sampling. Thus, the variability in both the extent of biodegradation of the oil and the amount of oil on the beach at t=0 was essentially random.

Second, it is clear that the ratios decreased significantly on all beaches during the first two sampling periods, with the fertilizer solution-treatment showing the most effect. Considering just the medians on the second sampling (August 6), the percentage decrease in the ratios relative to the July 22 sampling was 85% for Kittiwake, and 33% for Tern and Raven. In many of the later samples, as the nC18 fell below detection limits, the ratio could not be calculated. In addition, significant decreases in the concentration of phytane occurred on all beaches (Figures 7.12, 7.15, and 7.18). It is assumed that this decrease is the result of biodegradation, although there is little direct proof other than it is easy to isolate bacteria from oiled beach samples that grow on phytane as a sole source of carbon and energy. The results further support that fertilizer-enhanced biodegradation does not result in a preference by the natural microbial community to degrade only the easily degradable hydrocarbons. In fact, just the opposite appears to be true; enhanced biodegradation, as evidenced on Kittiwake beach, perhaps leads to a more extensive degradation of oil.
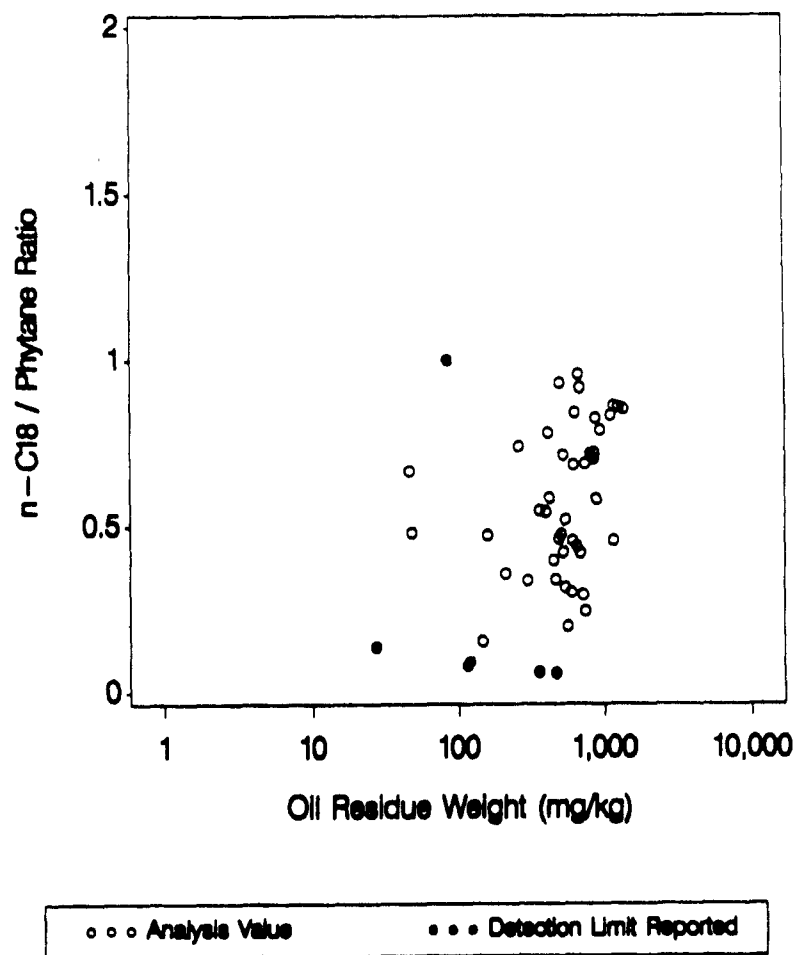
Figure 7.25. Relationship Between the nC18/phytane Ratio and Oil Residue Weight for All Beaches at Passage Cove (Cobble Surface) at Time Zero. R Value Day 1=0.36.
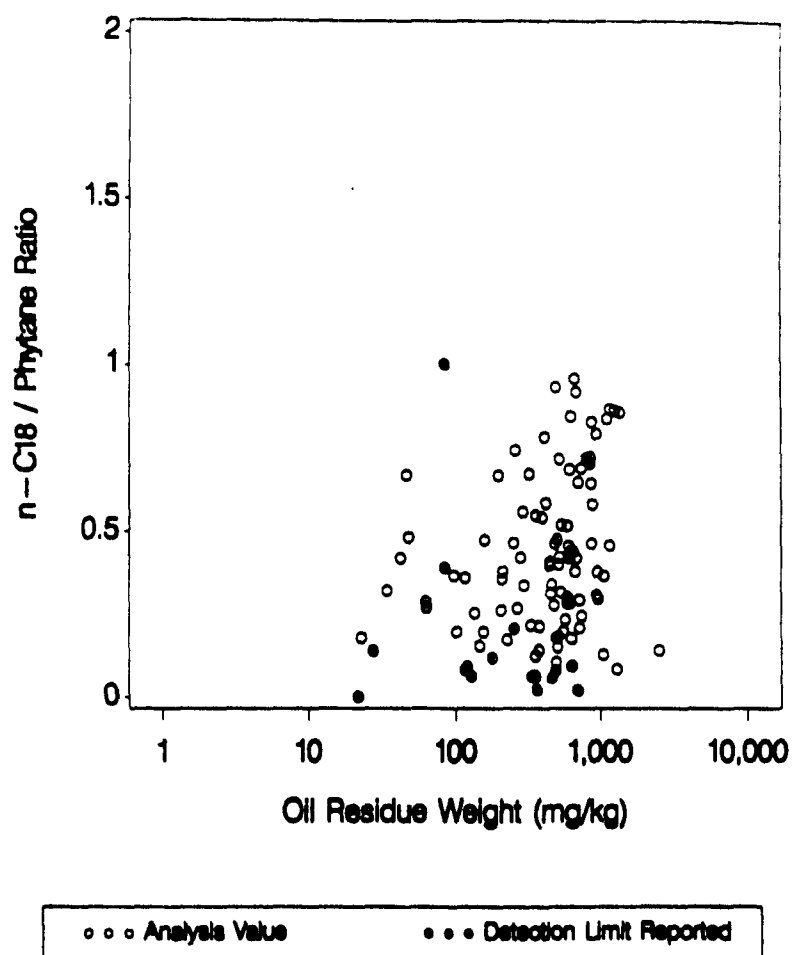
Figure 7.26. Relationship Between the nC18/phytane Ratio and Oil Residue Weight for All Beaches at Passage Cove (Cobble Surface) at Time Zero + Time One Sampling. R Value for Days 1 and 2=0.26.

Third, if changes in phytane concentration were due to biodegradation, then the persistence of phytane is quite different between the three beaches as shown in Figure 7.27. Up to and including the August 20 sampling, phytane in samples from the untreated control beach had changed very little, showing only a 15% decrease in the median concentration. The large change subsequent to this date is unexplained. Phytane concentrations were substantially reduced on the fertilizer-treated beaches, completely absent in samples from Kittiwake on the last sampling date, and reduced approximately 60% on Tern beach by the August 20 sampling. On Tern, reductions in median phytane concentrations leveled-off by the last sampling, suggesting perhaps a possible depletion of the active ingredient in the INIPOL/CUSTOMBLEN fertilizer materials. The difference in phytane reduction between Tern and Raven beaches (Figure 7.27) suggests that the nC18 ratios are misleading, and, in fact, there is probably considerably more oil biodegradation occurring on Tern beach than is indicated. However, we can not rule out the possibility that the INIPOL/CUSTOMBLEN fertilizer application was physically or chemically removing both phytane and nC18 together, resulting in no greater change in the ratios than that seen on Raven beach.

However, for the fertilizer solution-treated beach and the untreated control, where the question of chemical or solvent effects can be ignored, based on the ratios it appears that extensive biodegradation of the oil occurred, and that the degradation was significantly increased by the addition of nutrients. Therefore, it is argued that all observed changes in hydrocarbon composition discussed above were also due to biodegradation. The relationship of these measures of biodegradation to the losses of oil residue weight are portrayed in Figures 7.28 to 7.30. The results are very interesting. Changes in hydrocarbon composition for Raven beach, as modeled by changes in the summed alkanes, were generally weakly dependent on the loss of oil residue weight (Figure 7.28). Over time there appears to be, on average, much more change in hydrocarbon composition than in oil residue weight. However, it can be argued that the enhancement of biodegradation by the application of the fertilizer solution (greater rate of decay in the nC18/phytane ratio and in the summed alkanes relative to the untreated control), caused concomitant or corresponding decreases in oil residue weights (Figures 7.29 and 7.30). The results from Tern beach, as seen so many times, fell in-between the results of Raven and Kittiwake. Thus, the visual removal of oil from the fertilizer treated-beaches is a direct result of enhanced biodegradation, and it can be confidently assumed that measurements of changes in oil residue weight are good indicators of the extent of oil biodegradation.
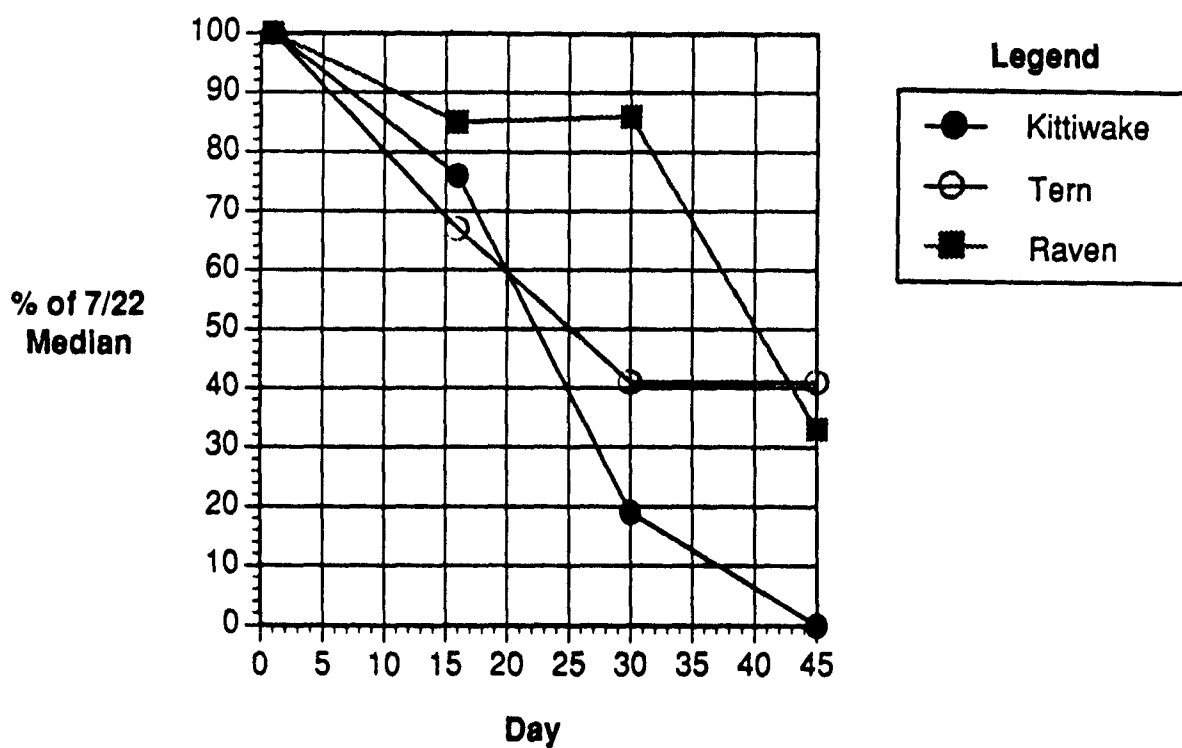
Figure 7.27. **Change in Phytane, Expressed as Percent of the 7/22/89 Median Over Time for Kittiwake, Raven, and Tern Beaches at Passage Cove.**
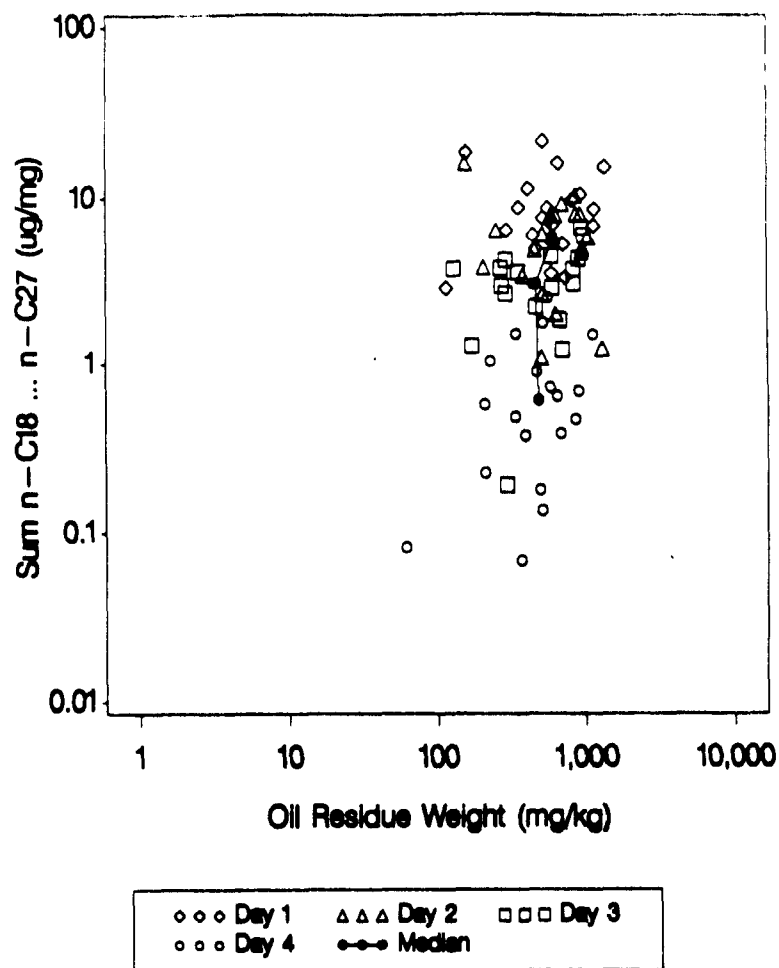
Figure 7.28. Relationship Between Oil Residue Weight and the Sum of Alkane Concentration nC18 to nC27 for Raven Beach (Untreated Control) at Passage Cove (Cobble Surface). Values Less than Detection Limit Were Set to Zero. R Value for Day 1=0.16, Day 2=-0.18, Day 3=0.30, Day 4=0.41.
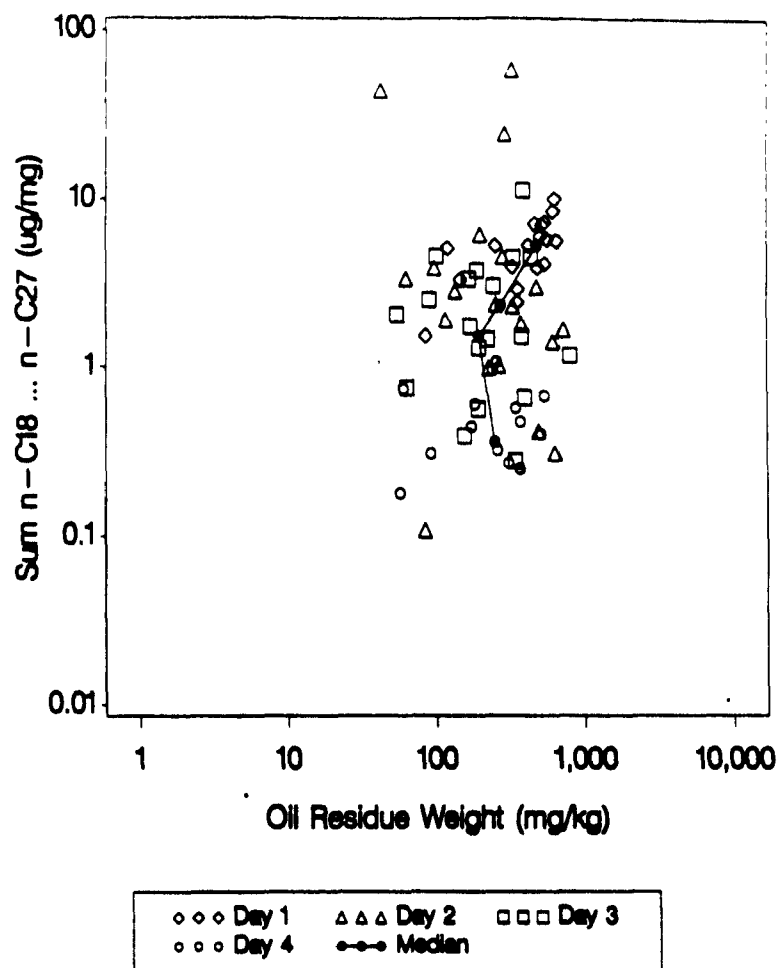
Figure 7.29. Relationship Between Oil Residue Weight and the Sum of Alkane Concentration nC18 to nC27 for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Cobble Surface). Values Less than Detection Limit Were Set to Zero. R Value for Day 1=0.66, Day 2=-0.23, Day 3=0.03, Day 4=0.19.
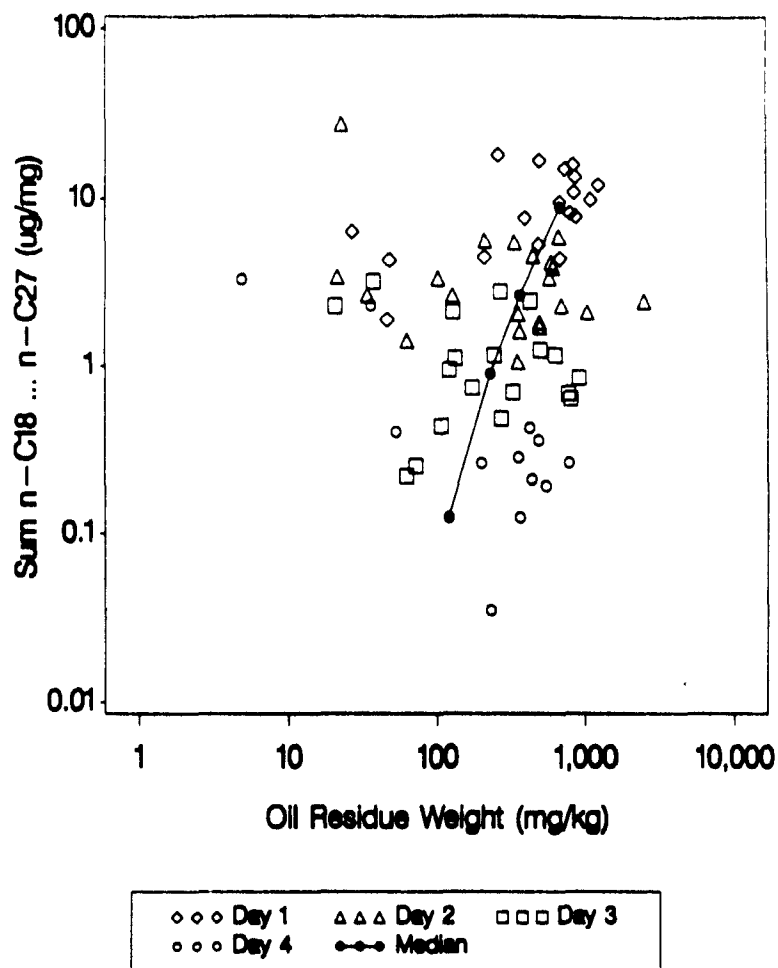
Figure 7.30. Relationship Between Oil Residue Weight and the Sum of Alkane Concentration nC18 to nC27 for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Cobble Surface). Values Less than Detection Limit Were Set to Zero. R Value for Day 1=0.60, Day 2=-0.31, Day 3=-0.05, Day 4=-0.72.

Internal Markers

Finally, many of the beach samples are currently being analyzed by selective ion mass spectroscopy to further establish the extent of compositional change. Not enough analyses have been performed to establish statistically significant trends at this time. However, it is clear that in many samples changes in the composition of the alkanes were accompanied by substantial changes in other hydrocarbons. This is illustrated in Figure 7.31.
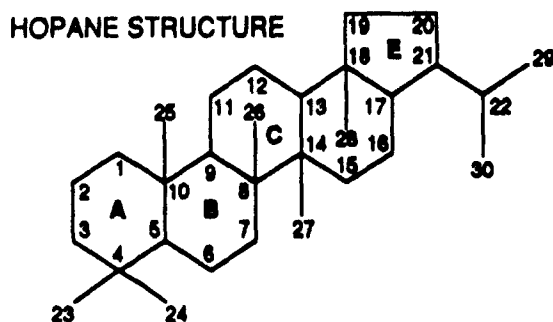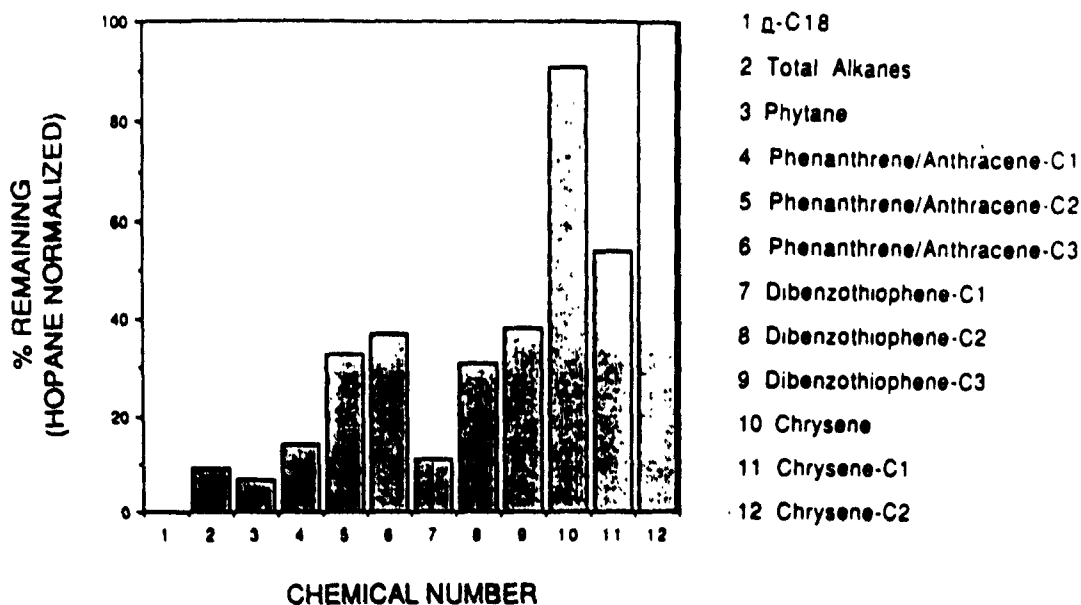

Figure 7.31.   Percent Remaining (Hopane Normalized) of different Hydrocarbons at Kittiwake Beach at Passage Cove on 8/20/89.

The analysis is based on hopane, an internal marker hydrocarbon. As mentioned above, phytane, the persistent branched alkane, has been used in the past as a stable internal marker. However, bioremediation appears to accelerate the biodegradation of this hydrocarbon. Hopanes are pentacyclic molecules derived geologically from bacteria. Through diagensis of the bacterial kerogen, hopanes are modified, eventually producing specific isomers that are uniquely characteristic of different oils. Prudhoe Bay crude oil has significant quantities of 17a,21b-hopane (structure shown in Figure 7.31). Cyclic alkanes in general are very difficult to biodegrade, and we believe that the multi-ring cyclic alkanes are almost totally recalcitrant to biodegradation. Thus, 17a,21b-hopane is a good internal marker.

However, unlike the nC18/phytane ratio, which is based on two hydrocarbons that chemically behave very similarly, use of hopane ratios has to be carefully considered because of very different chemical behavior. Most crucial in this regard is the assumption that as oil is degraded and portions of residue are sloughed off, the hopane will not leave, but instead becomes enriched in the remaining "biodegraded" oil. All of the samples analyzed to date from Kittiwake beach, where the most oil degradation was observed, were substantially enriched for hopane. The standard in this case is Prudhoe Bay crude oil that has been artificially weathered (evaporated at 521°C to remove 30% of the original weight) to represent the oil that washed up on the beaches in Prince William Sound after the spill. Our assumption, therefore, appears to be correct in a very conservative way (i.e., some hopane is undoubtedly lost as oil residue is removed from the beaches).

Any change in the mass ratio of a specified hydrocarbon to hopane represents differential decay of hydrocarbons and we assume, based on the recalcitrance of hopane and other aspects of nonbiological decay, that this can only occur through biodegradation. Accordingly, Figure 7.31 shows that very extensive biodegradation of the straight chain alkanes is accompanied consistently by substantial biodegradation of many other hydrocarbon groups. Thus, we can be assured that fertilizer-enhanced biodegradation is doing more than simply removing the readily degradable alkanes.

This can be further illustrated by examination of different groups of hydrocarbons in the residual oil. For example, if the phenanthracene/anthracene group is compared with the dibenzothiophene group in undegraded oil, it can be shown in the mass spectral analysis that the different substituted isomers in each grouping relative to hopane, C-1's, C-2's, and C-3's, respectively, were present in decreasing concentrations. C1 was the highest. As oil is degraded, the

respective concentrations become reversed; the isomer groupings are found with increasing concentrations. C3 was the highest. This change in degraded oil is shown in Figures 7.32a and 7.32b. Differential removal of the lower molecular weight materials first (presumably more soluble, leachable, and volatile), could theoretically be due to either biological or nonbiological processes. However, because of the substantially different chemical behavior of the benzothiophenes relative to the phenanthracenes/anthrancenes, as evidenced by their gas chromatographic behavior, it is highly unlikely that this reverse concentration gradient will occur in the oil sample simultaneously if the process is due strictly to chemical or physical processes. Biodegradation, on the other hand, which could involve the simultaneous degradation of these chemical groups by bacteria with different substrate specificities, is a much more reasonable explanation of simultaneous reversal of these concentration gradients. Thus, in the samples analyzed to date, changes in alkane composition by biodegradation were almost always accompanied by simultaneous reversals in the concentration gradients for the phenanthracene/anthracene and dibenzothiophene groups. These results leave little doubt that biodegradation of oil is quite comprehensive and simply does not reflect a selective use of the most easily degradable hydrocarbons.

Assessing the rates of hydrocarbon composition change for cobble samples was also complicated. Because the concentration of certain alkanes in many samples was zero (below detection limits), indications of significance were disproportionately affected at the latter sampling dates where fewer data points were available for analysis (with log transformations, any value set to zero automatically drops out of the analysis). However, plots of the summed normal alkanes, nC18 to nC27, could be analyzed statistically to some extent because during the course of the test the total mass of these hydrocarbons did not fall below detection limits even though individual hydrocarbons within the summed hydrocarbons did. In essence, this approach creates the second bias of producing artificially low values for the summed alkanes.

Regression analyses of the median values of the summed alkanes (nC18 to nC27) plotted on linear and logarithmic axes are shown in Figures 7.33 and 7.34. Depending on the treatment, it is clear that a zero-order model provides a better fit to the data in some cases, while a first-order model is better in others. For example, Kittiwake decay rates appear to be first-order, Raven appears to be zero-order, and Tern somewhere in-between. In fact, Tern appears to be biphasic; on the logarithmic display the median of the last sampling date was significantly different from a regression line drawn through the median for the first three sampling dates. The same is also true for Raven beach. Thus, some unexplained change in decay rate appears to have occurred between the third and
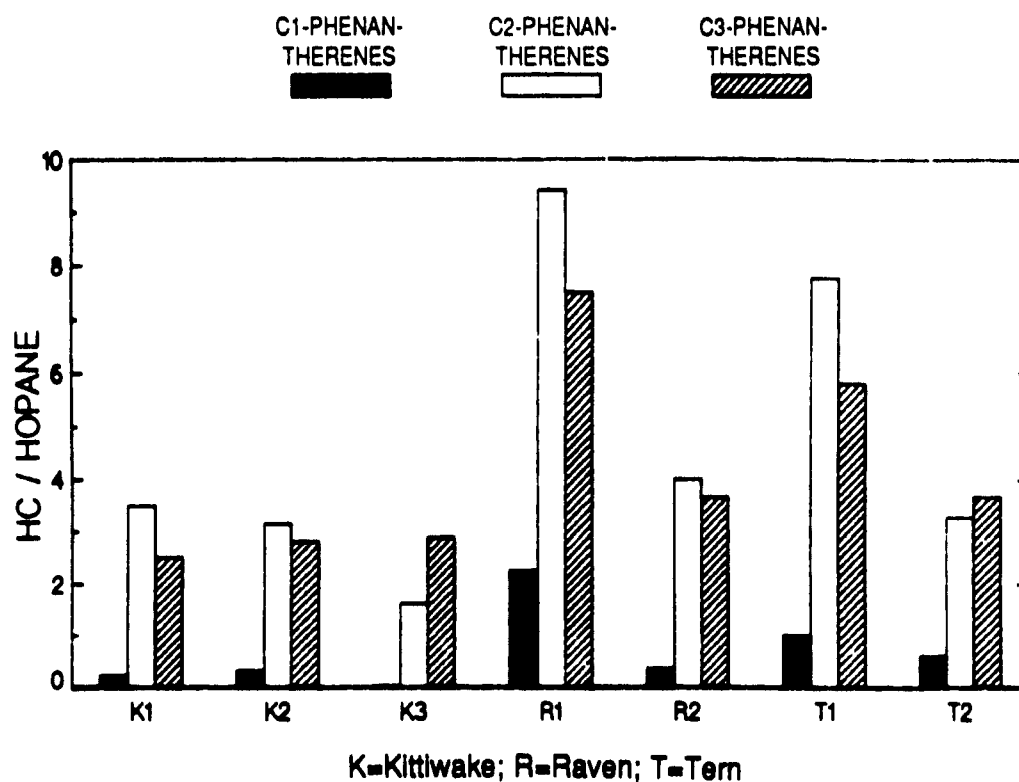
Figure 7.32a. Aromatic Homologs Relative to Hopane (Phenanthracene/ Anthracene Group). All samples are 50 mg/mL Oil Residue.
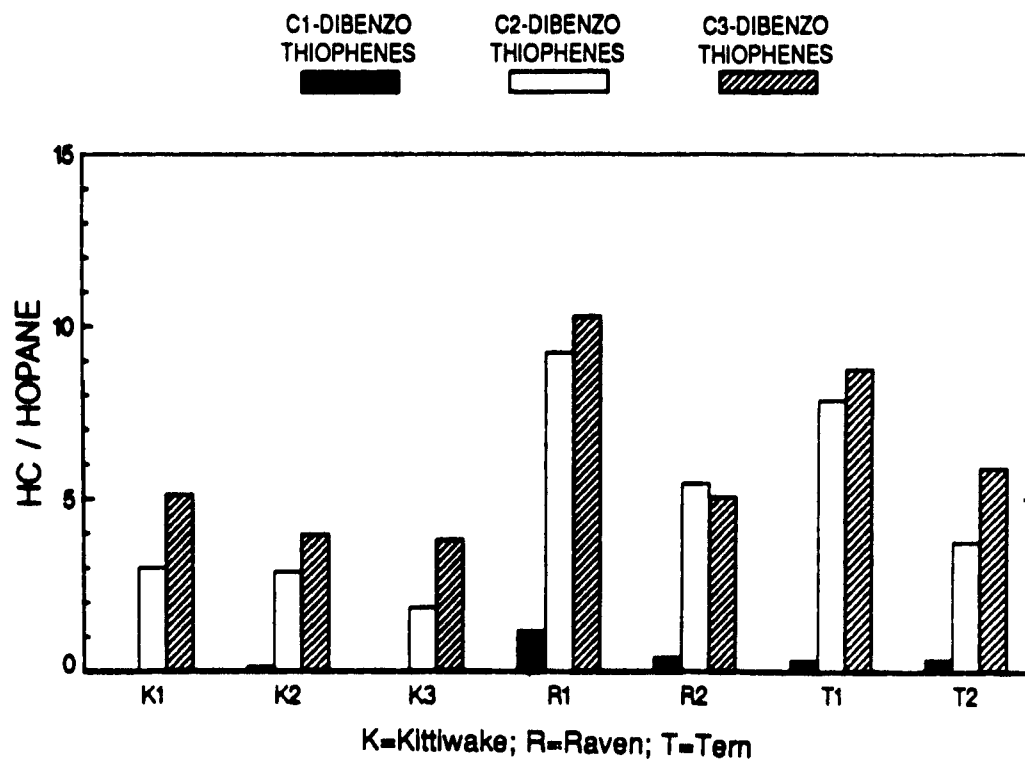
Figure 7.32b. Aromatic Homologs Relative to Hopane (Dibenzothiophene Group). All samples are 50 mg/mL Oil Residue.

Figure 7.33. Change in the Median Concentration of Summed Alkanes nC18 to nC27 (Arithmetic Scale) Over Time, for Kittiwake, Raven, and Tern Beaches at Passage Cove.

Figure 7.34. Change in the Median Concentration of Summed Alkanes nC18 to nC27 (Log Scale) Over Time, for Kittiwake, Raven, and Tern Beaches at Passage Cove.

final samplings on these two beaches. Again, it is quite possible that the effect of INIPOL application was short-lived (nutrient depletion or degradation of the added carbon).
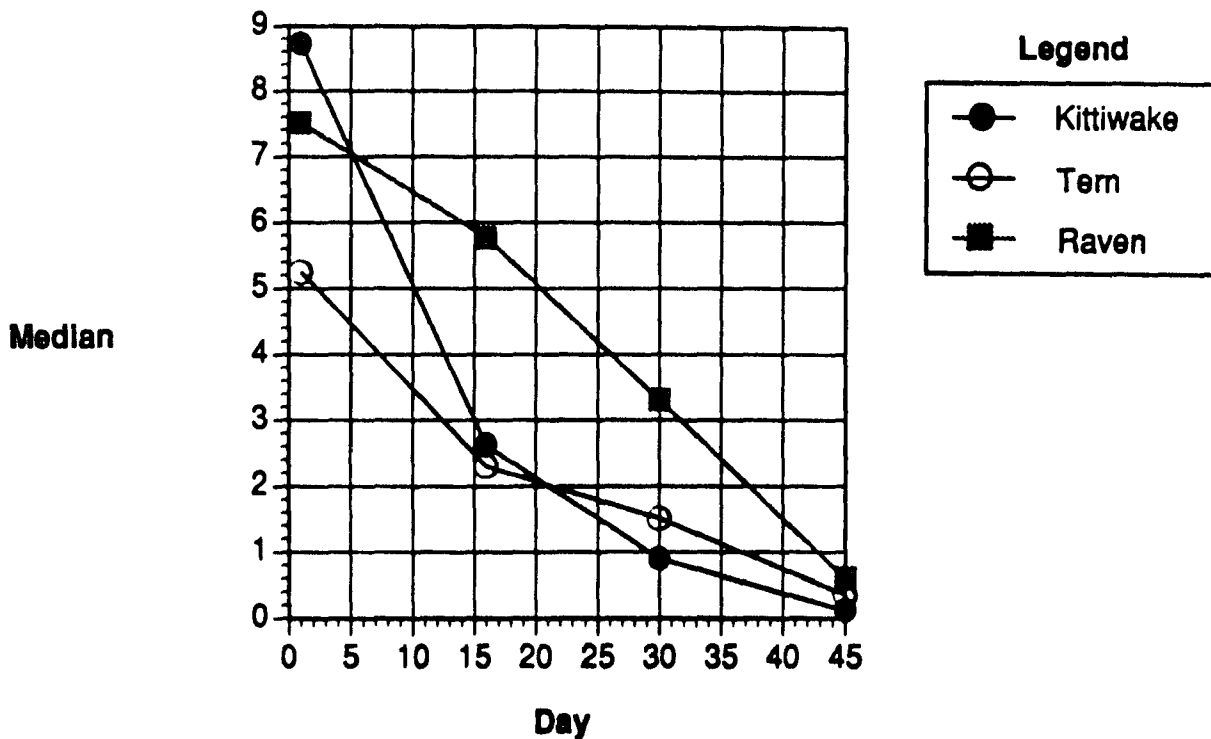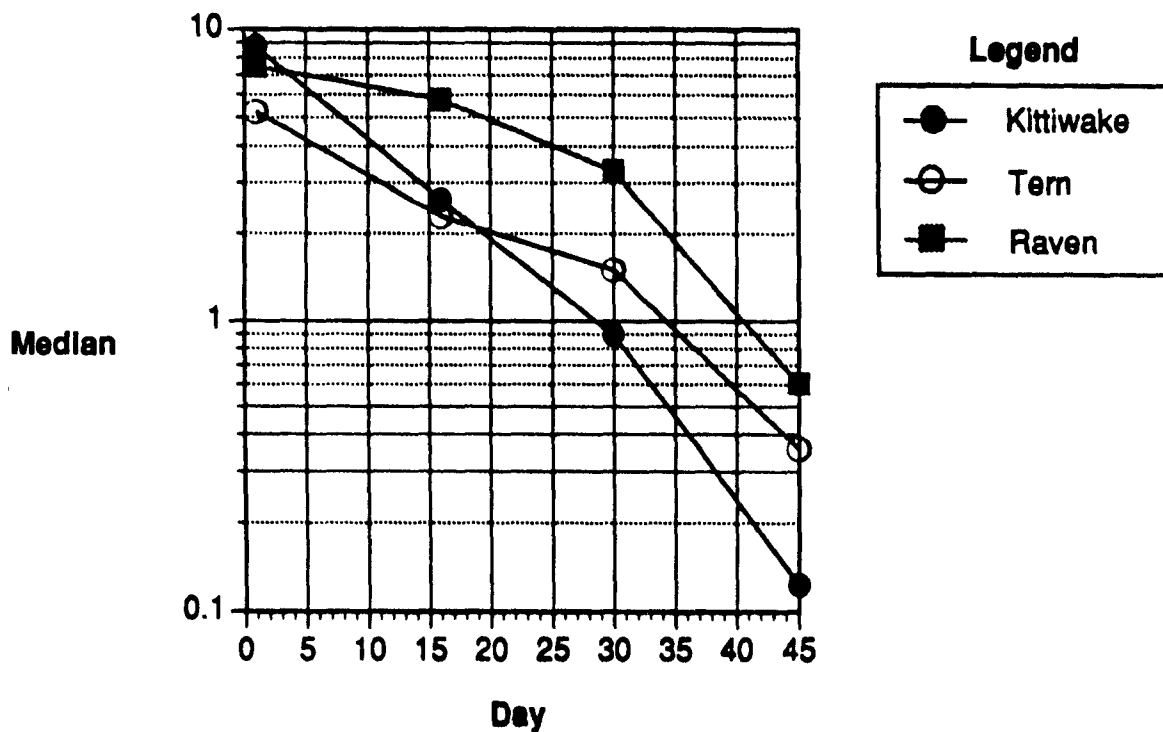
A comparison of first-order decay constants (slopes of the linear regressions of log transformed medians) using all sampling dates in one case and excluding the last sampling time in another case, is shown in Table 7.6. In the former case decay rate constants were not significantly different between Tern and Raven; both were significantly less than Kittiwake. From these calculations it can be seen that the enhancement effect of fertilizer solution application on the rate of hydrocarbon composition change, as measured by the summed alkanes nC18 to nC27, was approximately 1.2 times. This contrasts dramatically with the enhancement of rates observed with the residue weights (4.7 times). However, if the last sampling date is excluded, the rate enhancement effect of fertilizer application was 2.1 times for the fertilizer solution. There was no enhancement for the INIPOL/ CUSTOMBLEN application. The differences in rates are a little more consistent with the oil residue weights.

Mixed Sand and Gravel Samples

Data on hydrocarbon composition for the mixed sand and gravel samples are shown in Figures 7.35 to 7.46. Significant changes occurred only in samples from Kittiwake beach. Less reduction occurred in the mixed sand and gravel samples than in samples from the cobble surface. Decreases in the nC18/phytane ratios were also smaller.

Analysis of the relationship between the summed alkanes nC18 to nC27, and the oil residue weight for mixed sand and gravel samples shows several interesting trends (Figures 7.47 to 7.49). Pearson's correlation coefficients were generally higher than those observed in samples from the cobble surface. Again, there appears to be substantial differences between beaches. Raven beach, which showed no significant decreases in either oil residue weight or the concentration of the summed alkanes, had the highest correlation coefficients with each sampling time. If the two outliers on the first sampling are excluded, the correlation coefficient improves to 0.77, and is then consistent with the other sampling dates for Raven beach (Figure 7.47). Fertilizer treatment causes the correlations to be considerably reduced with the greatest effect seen with the fertilizer solution treatment (Kittiwake). INIPOL/CUSTOMBLEN treatment was in-between. The correlation breaks down because oil residue weight appears to have decreased, while the concentration of the summed alkanes remained relatively unchanged. In fact, a relatively strong inverse relationship was observed with the

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TABLE 7.6. COMPARISON OF ZERO-ORDER AND FIRST-ORDER RATES
(LINEAR REGRESSION FIT TO MEDIANS) OF CHANGE IN
HYDROCARBON COMPOSITION, BASED ON DECREASES THROUGH TIME IN
HYDROCARBON CONCENTRATIONS OF THE SUMMED ALKANES, nC18 TO nC27

Beach	Fertilizer Treatment	Slope (standard deviation)		
		First-Order-I[a]	First-Order-II[b]	Zero-Order[c]
Kittiwake	Fertilizer Solution	-0.032 (.0027)	-0.033 (0.004)	-0.19 (0.0-6)
Tern	INIPOL/CUSTOMBLEN	-0.022 (0.0033)	-0.016 (0.005)	-0.11 (0.02)
Raven	None	-0.025 (0.0025)	-0.016 (0.003)	-0.16 (0.01)

Beaches Compared	Ratio of Slopes (standard deviation)		
	First-Order-I	First-Order-II	Zero-Order
KW/R	1.27 (A)[d]	2.1(A)	1.2
T/R	0.85	1.0	0.7
KW/T	1.50(A)	2.1(A)	1.8

[a] I = All sampling dates used; units = days^{-1}
[b] II = Last sampling date (9/5) excluded; units = day^{-1}
[c] All sampling dates used; units = mg/kg/day
[d] (A) = Statistically different (95% confidence level).

Figure 7.35. Change in nC18 Alkane Concentration Through Time for Raven Beach (Untreated Control) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 7.36. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Raven Beach (Untreated Control) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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Figure 7.37. Change in Phytane Concentration Through Time for Raven Beach (Untreated Control) at Passage Cove (Mixed Sand and Gravel).

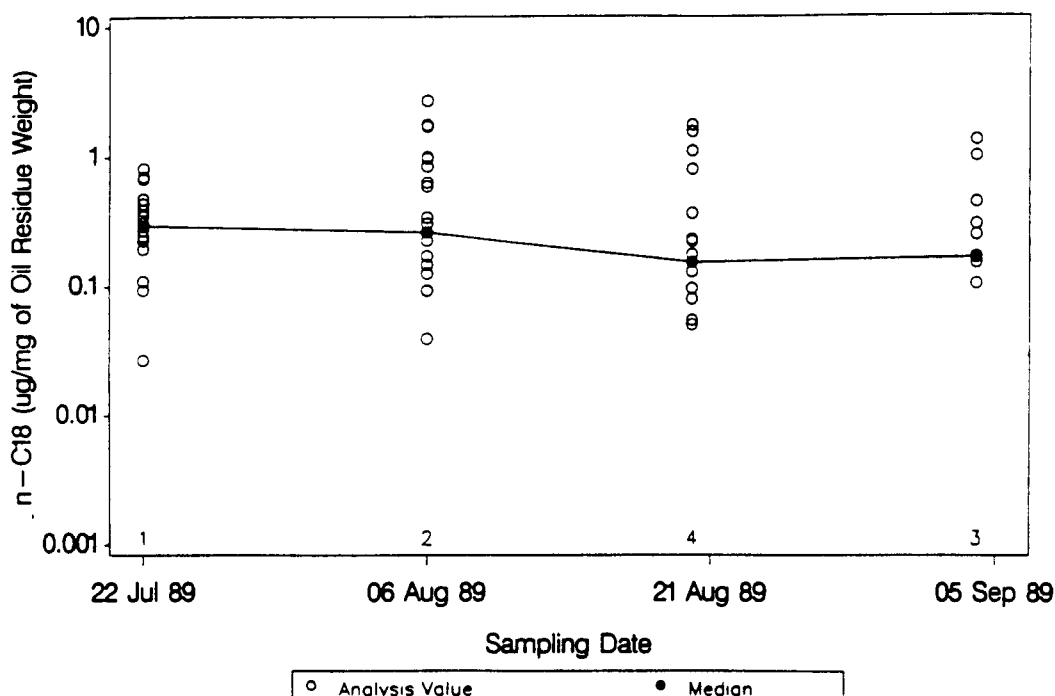
Figure 7.38. Change in nC18 Alkane Concentration Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

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Figure 7.39. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
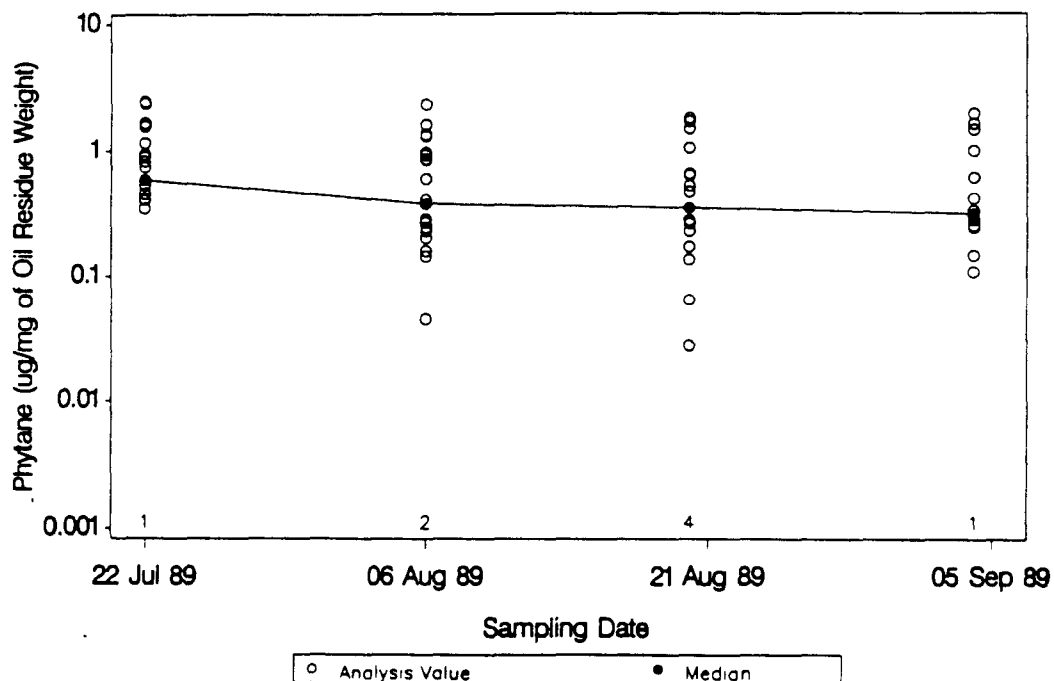
Figure 7.40. Change in Phytane Concentration Through Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
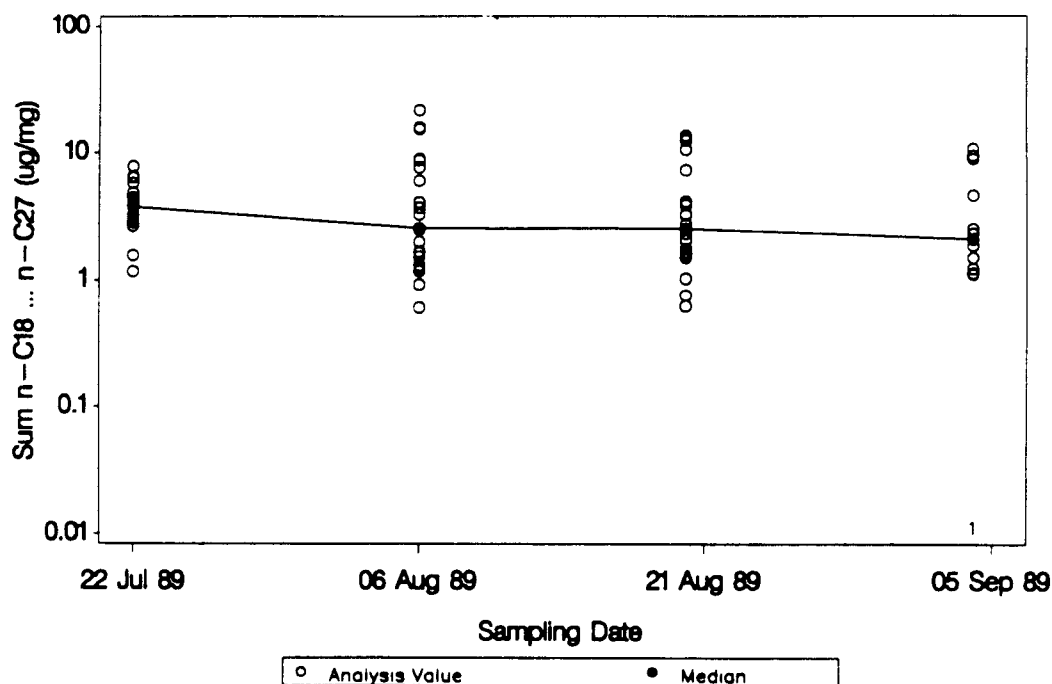
293
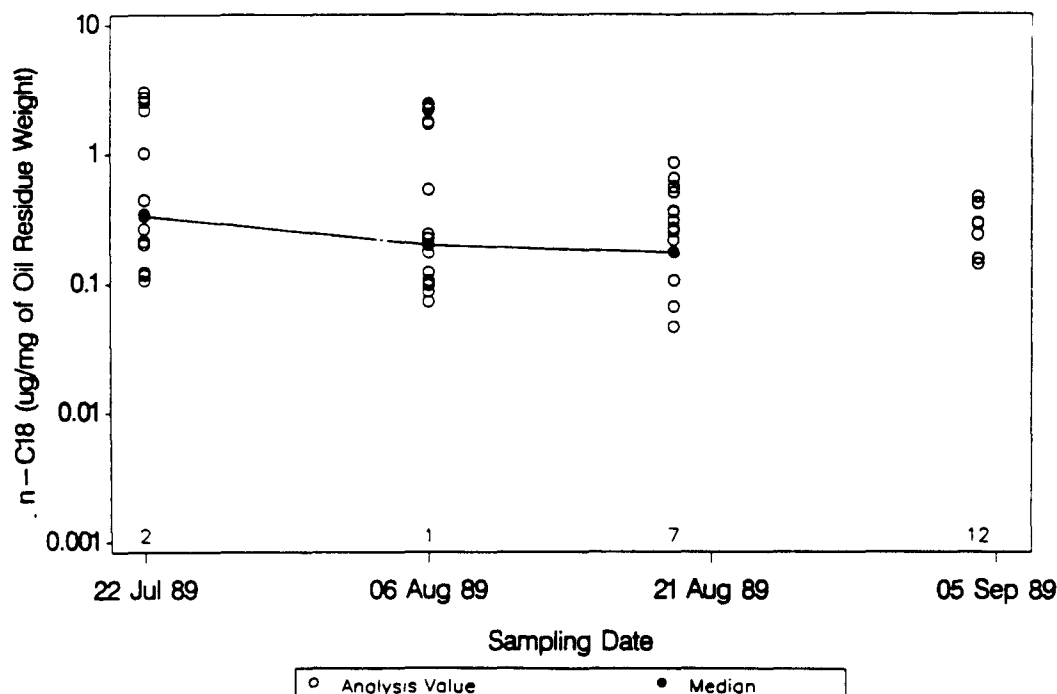
Figure 7.41. Change in nC18 Alkane Concentration Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.

Figure 7.42. Change in the Sum of Alkane Concentration nC18 to nC27 Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
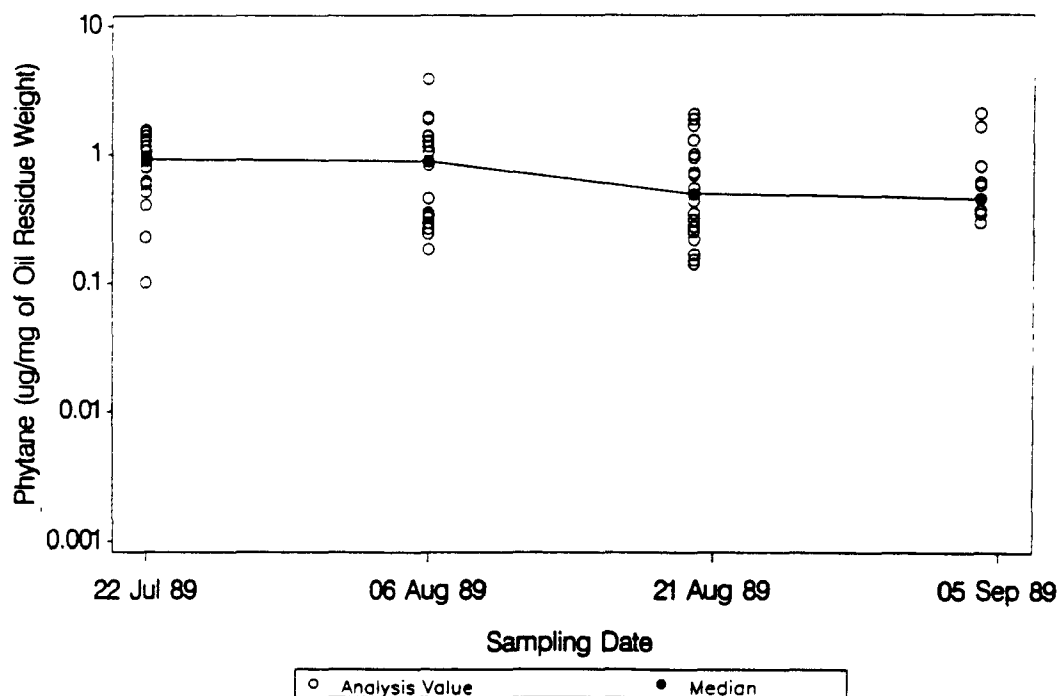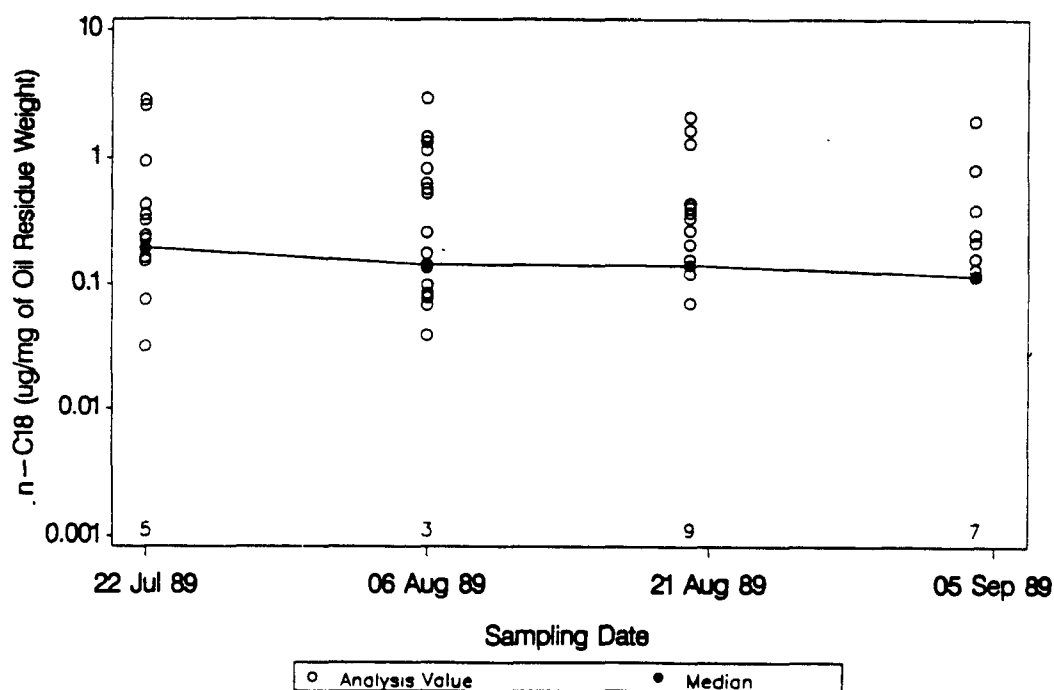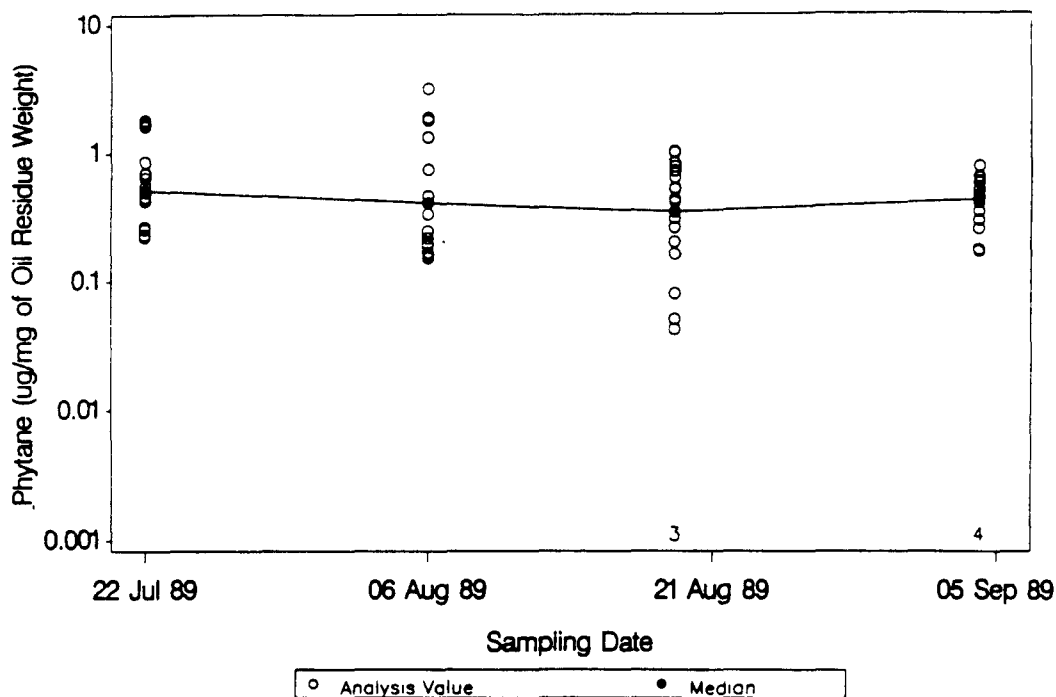
Figure 7.43. Change in Phytane Concentration Through Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
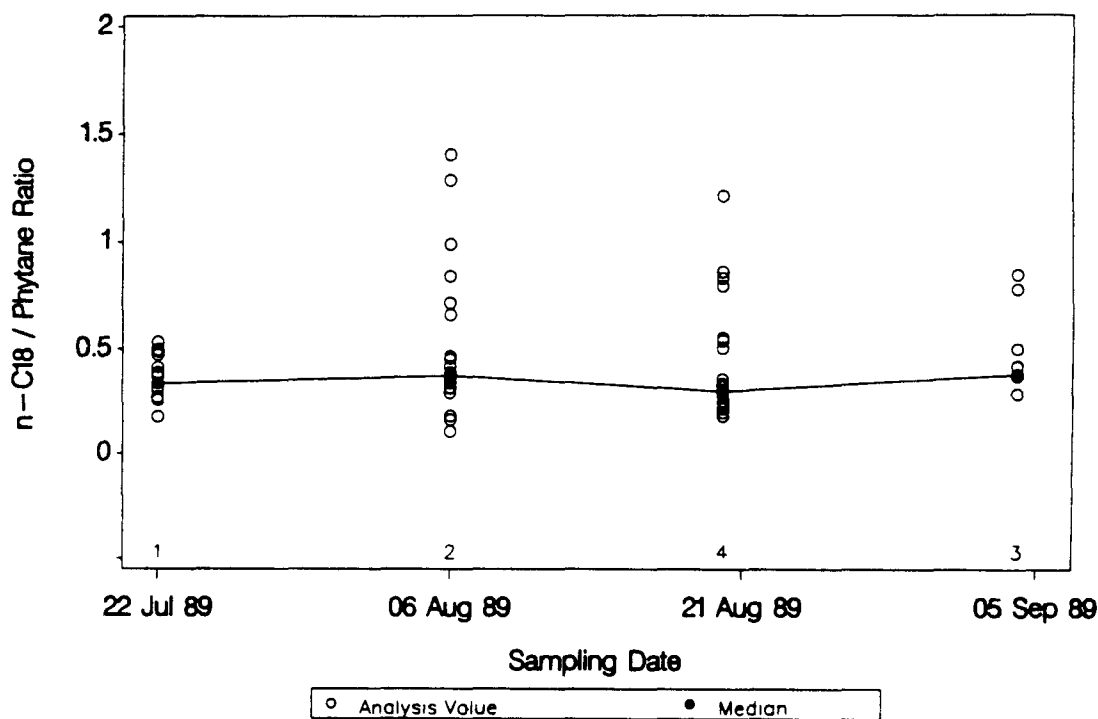
Figure 7.44. Change in the nC18/phytane Ratio Through Time for Raven Beach (Untreated Control) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
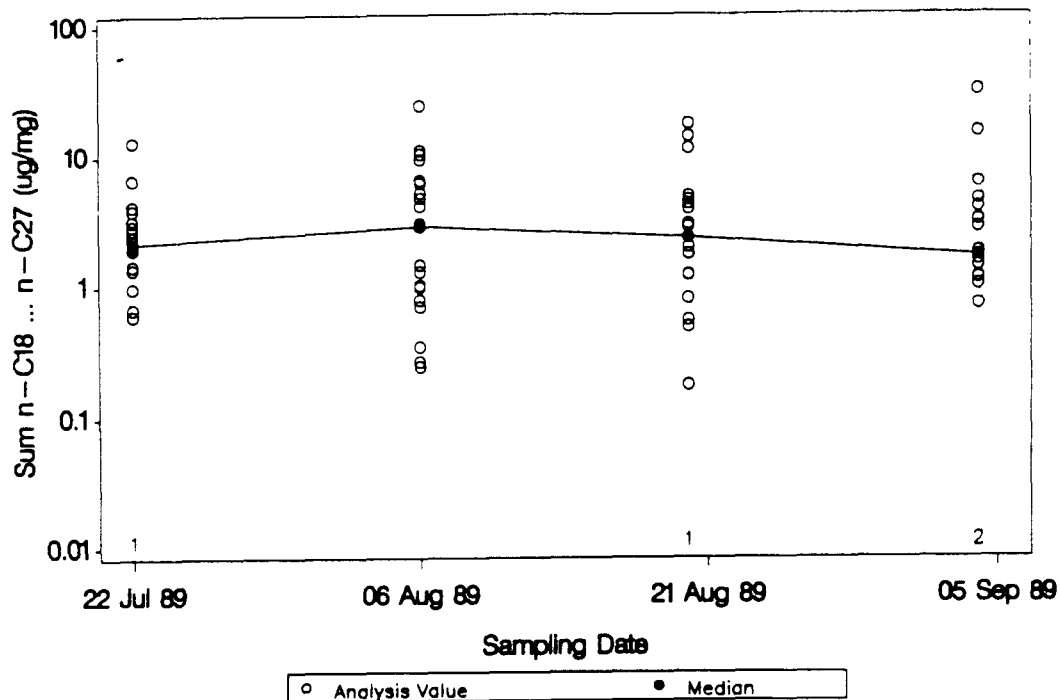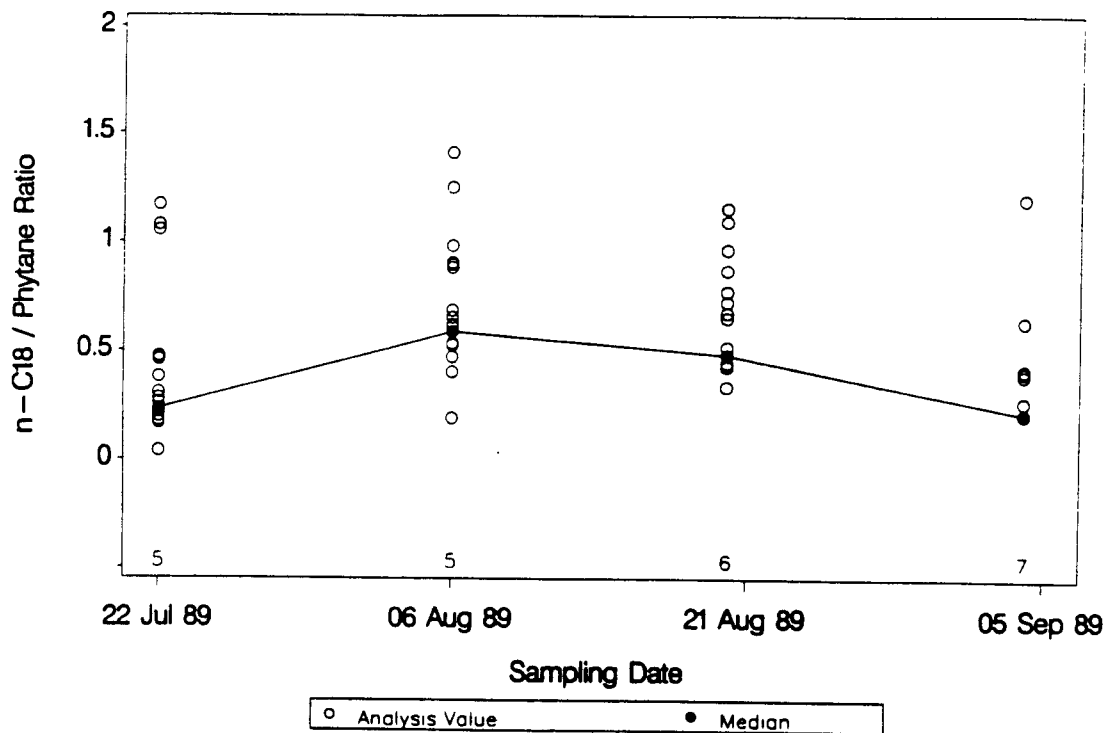
Figure 7.45. Change in the nC18/phytane Ratio Over Time for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
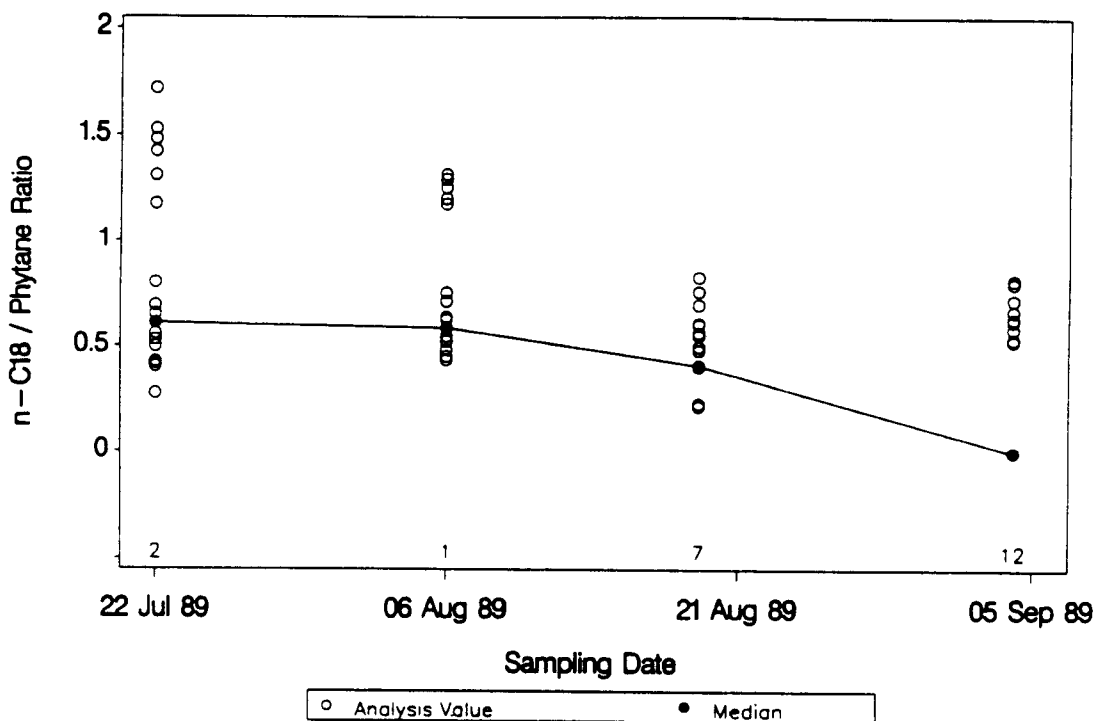
Figure 7.46. Change in the nC18/phytane Ratio Over Time for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Mixed Sand and Gravel). The Number of Samples Showing Concentrations Below Detection Limit is Shown Above the Sampling Date.
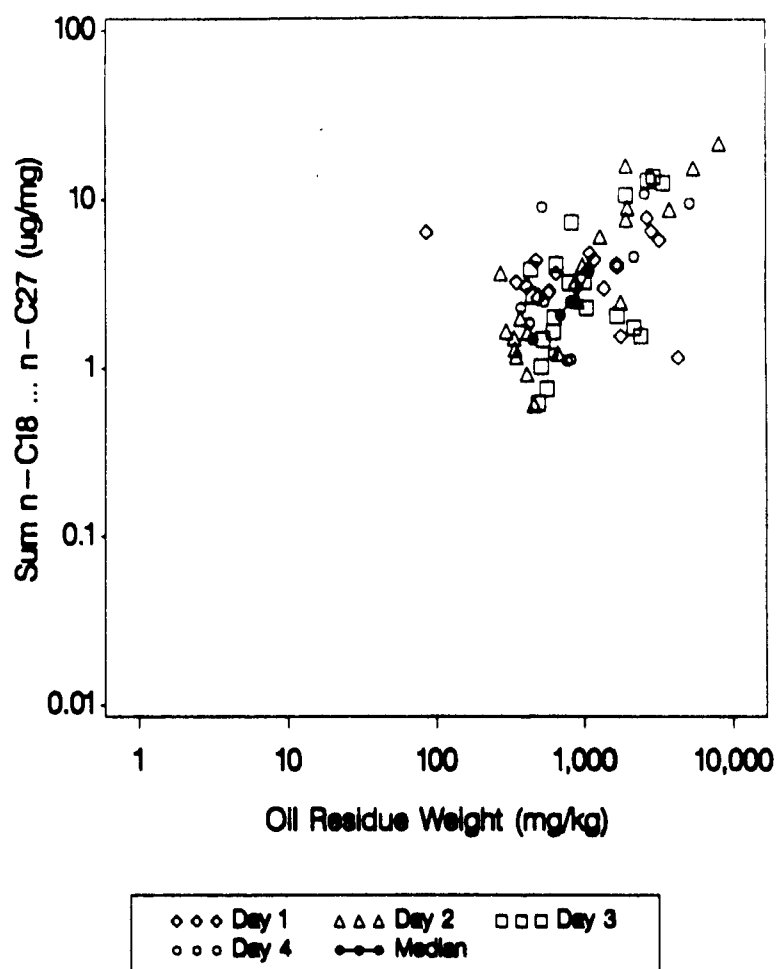
Figure 7.47. Relationship Between the Sum of Alkane Concentration nC18 to nC27 and Oil Residue Weight for Raven Beach (Untreated Control) at Passage Cove (Mixed Sand and Gravel). Values less than Detection Limit were set to Zero. R Value Day 1=-0.11, Day 2=0.86, Day 3=0.61, Day 4=0.63.
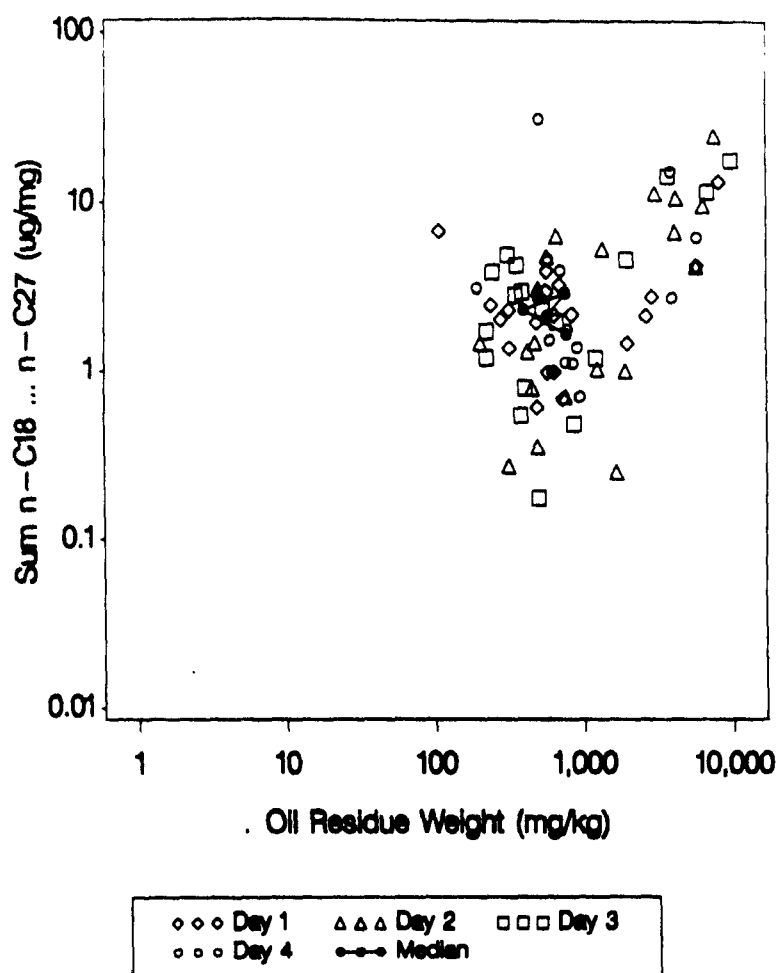
Figure 7.48. Relationship Between the Sum of Alkane Concentration nC18 to nC27 and Oil Residue Weight for Tern Beach (INIPOL + CUSTOMBLEN) at Passage Cove (Mixed Sand and Gravel). Values less than Detection Limit were set to Zero. R Value Day 1=0.28, Day 2=0.62, Day 3=0.57, Day 4=0.22.
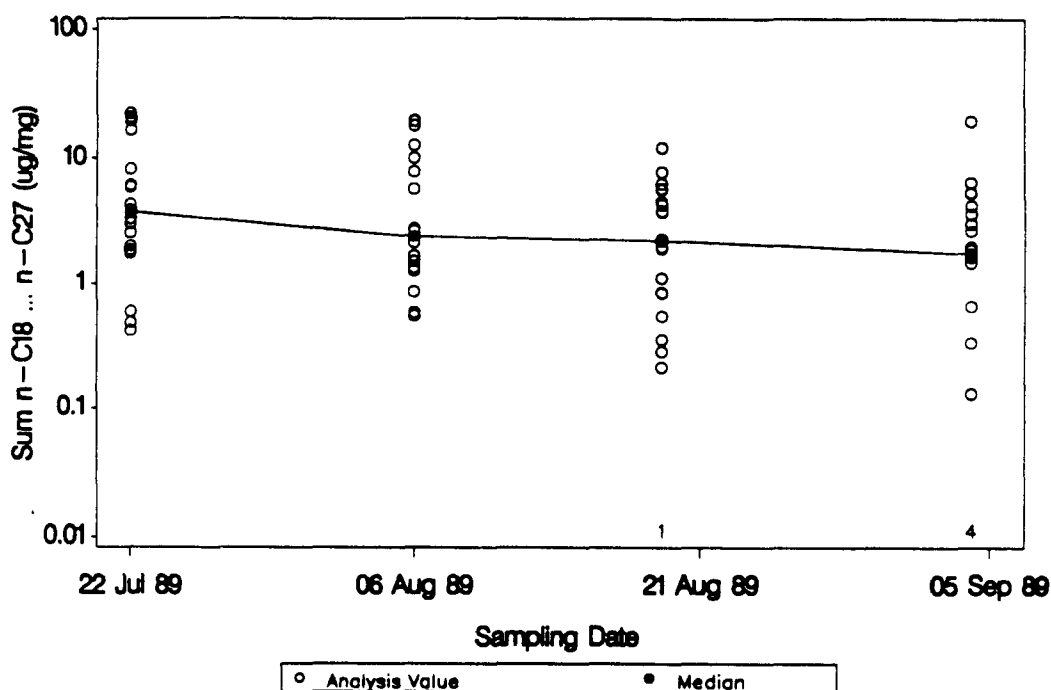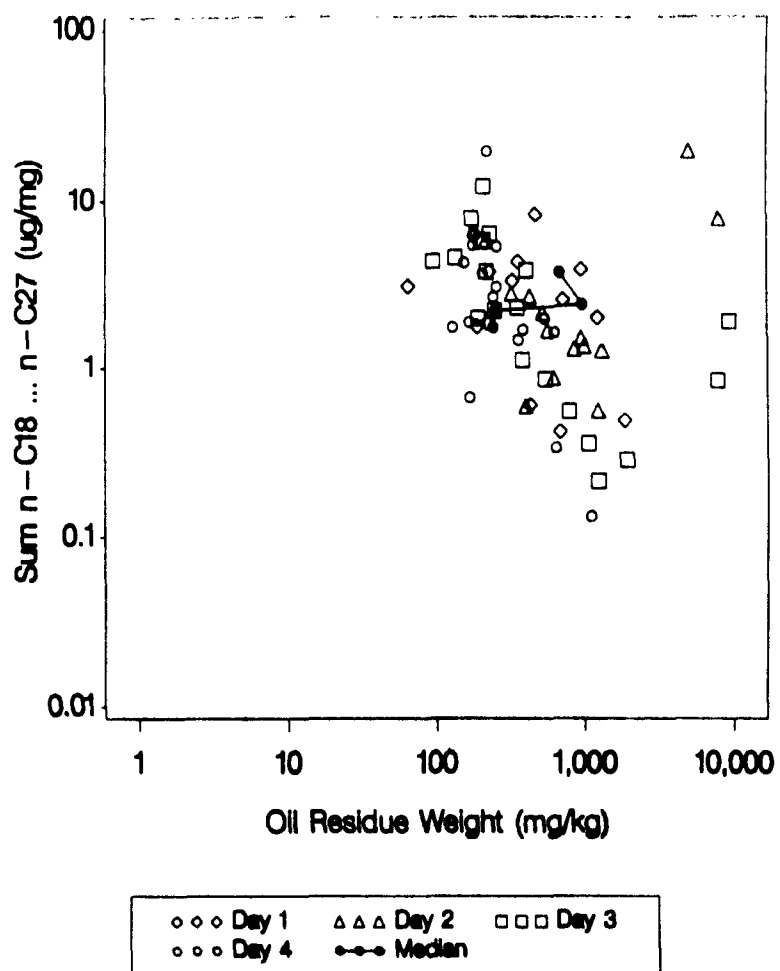
Figure 7.49. Relationship Between the Sum of Alkane Concentration nC18 to nC27 and Oil Residue Weight for Kittiwake Beach (Fertilizer Solution) at Passage Cove (Mixed Sand and Gravel). Values less than Detection Limit were set to Zero. R Value Day 1=0.56, Day 2=0.74, Day 3=-0.63, Day 4=-0.63.

last two samplings on Kittiwake. Similar responses were also seen in samples from the cobble surface, and the most pronounced effect was always seen on Kittiwake beach. It is possible that this is an artifact of scaling the results to reflect the sample size (samples were very small), and the effect of samples falling below detection limits.

Thus, fertilizer treatment of oil in the mixed sand and gravel under the cobble caused oil residue to be lost without large decreases in the readily biodegradable alkanes. The only reasonable explanation to account for the results is an effect due directly to biodegradation. Physical scouring in the mixed sand and gravel should be greatly reduced, and chemical washing can be eliminated as a factor because the effect was greatest with the fertilizer solution application, which was essentially seawater.

Significant decay of the summed alkanes occurred only on Kittiwake beach. The decay rate from the slope of a first-order plot was -0.0072 per day, or approximately 4 times less than what was observed on the cobble surface. This contrasts with the oil residue weight comparisons in which decay rates in mixed sand and gravel and cobble surface samples were the same. Thus, as noted above, significant changes in oil residue weights in mixed sand and gravel samples from the fertilizer solution-treated beach were accompanied by considerably less change in oil composition than oil samples from the cobble surface. We currently have no explanation for this difference.

WINTER 1989/1990 SAMPLING

On November 19, 1989 the beaches at Passage Cove were sampled a fifth time. Despite winter conditions, it was apparent that there was very little oil remaining on the cobblestone surface of the two treated beaches (Tern and Kittiwake). The untreated control beach, Raven, was still visually oiled, although the oil was very patchy. The amount of oil on the surface, however, was much less than the previous sampling in early September. In the mixed sand and gravel below the cobble, it was very clear that many samples taken on the treated beaches were visually void of oil. This was not the case on the untreated control beach. Oil on the treated beaches appeared to be reduced to approximately the same extent; there was no visual evidence that one fertilizer worked better than another. Samples from the beach subsurface (approximately 15 to 18 cm) generally showed the same pattern; patchy oil distribution, but visually much less oil on the two treated beaches as compared to the untreated control.

Concentrations of oil residue, nC18, nC27, the summed alkanes nC18 to nC27, phytane, and differences in the nC18/phytane ratio are shown in Figures 7.50 to 7.61. Sampling was conducted in the same plots used during the summer sampling. Due to short winter days and windows of good weather, sampling was performed only in every other block starting at the edge of each plot. Samples were taken from the upper (blocks 1 to 7) and lower (blocks 15 to 21) tide zone. The data were not combined because of significant differences between the two areas (see below); this spatial effect was not apparent in the summer sampling.

A general overview of all the data clearly portrays the long-term effects of enhancing oil biodegradation by the addition of fertilizers. In most cases, the sampling showed significantly less oil residue and more change in hydrocarbon composition on the treated beaches than on the untreated control beach. Where the statistical significance of INIPOL/CUSTOMBLEN enhancement of oil biodegradation was difficult to establish during the summer sampling, there is little question, based on the winter sampling results, that the enhancement effect was real.

Cobble Surface Samples

The amount of oil remaining on the cobble surface was generally very low, showing concentrations approximately ten fold less than concentrations at the time of initial fertilizer application. This remaining material probably represents the fraction of oil that is slowest to biodegrade. The distribution of the remaining oil varied between the two sampling zones(Figures 7.50 and 7.56); in the low tide zone oil concentrations on all beaches were approximately the same (Figure 7.50), regardless of fertilizer application. This may reflect less resolution of differences because of the relatively small number of samples taken, or it could be a true spacial effect, possibly related to greater wave energy or more consistent coverage by tidal waters on the lower part of the beach.

Based on the half-lives calculated for the decay rate of oil on each beach (Table 7.2), the degree of reduction in oil concentration seen in the winter sampling was less than would have been predicted, suggesting that the winter conditions slowed the decay rates.

Changes in hydrocarbon composition were best interpreted by examination of the summed alkanes, nC18 to nC27, and phytane; concentrations of nC18 and nC27 were not as useful because many samples had concentrations below detection limits. In general, the summed alkanes and phytane were reduced to the greatest extent in the low tide zone of the beaches (Figures 7.53 and 7.54). It was

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C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface
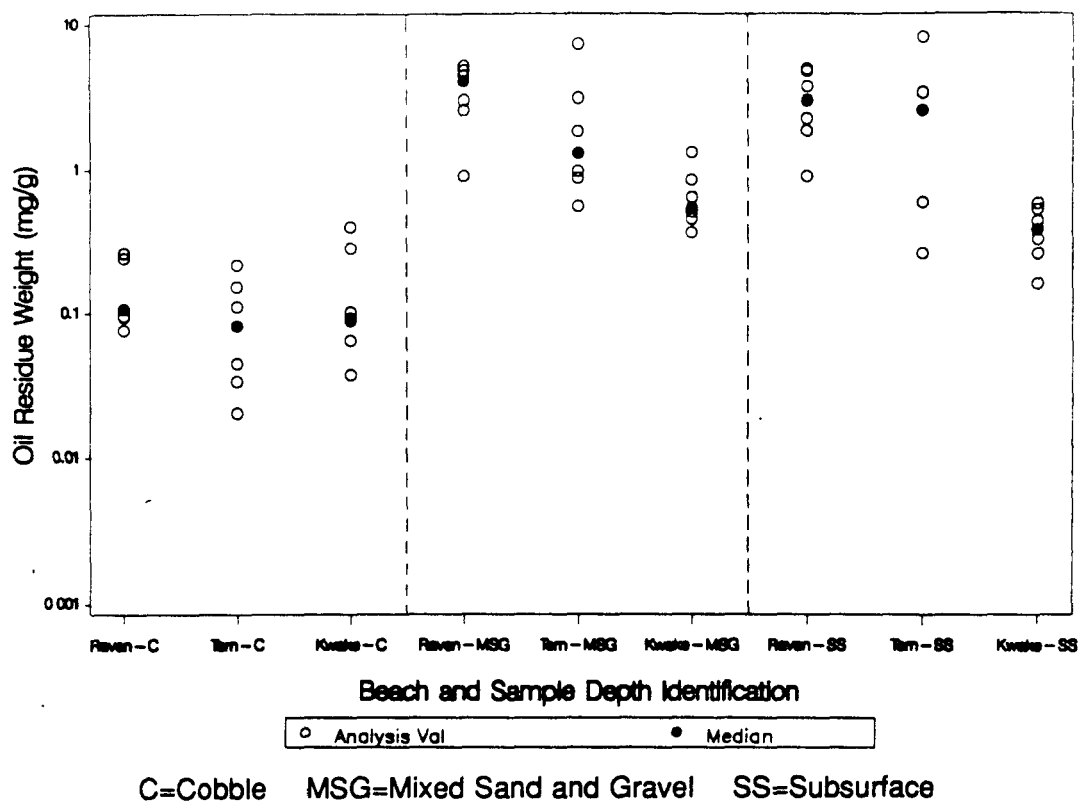
Figure 7.50.   Oil Residue Weights (Log Scale) for the Low Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface
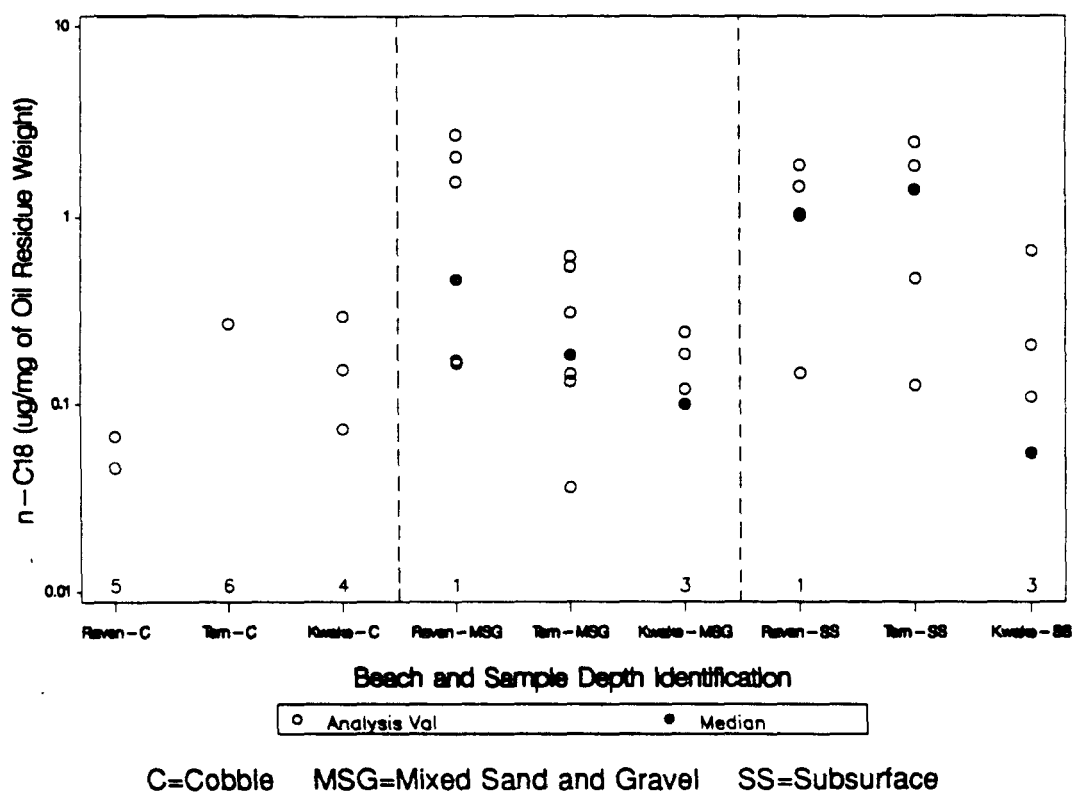
Figure 7.51.   nC18 Concentration (Log Scale) for the Low Tide Zone at Passage Cove During the Winter of 1989.
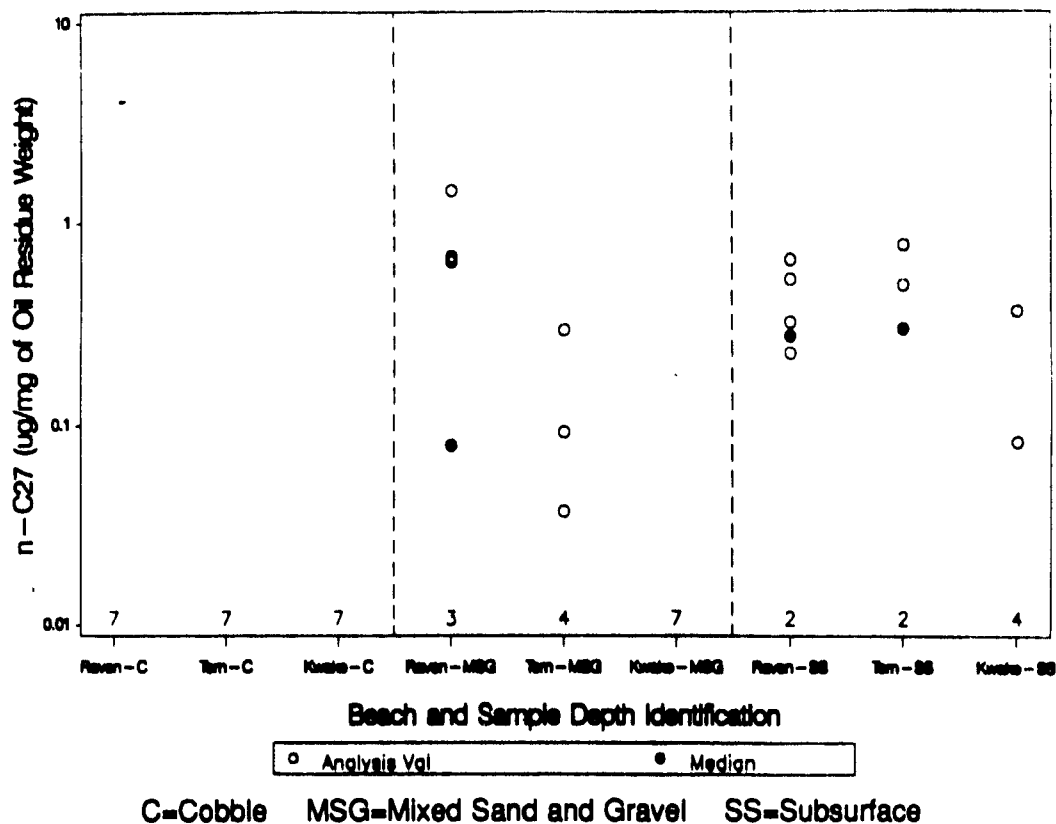
Figure 7.52. nC27 Concentration (Log Scale) for the Low Tide Zone at Passage Cove During the Winter of 1989.
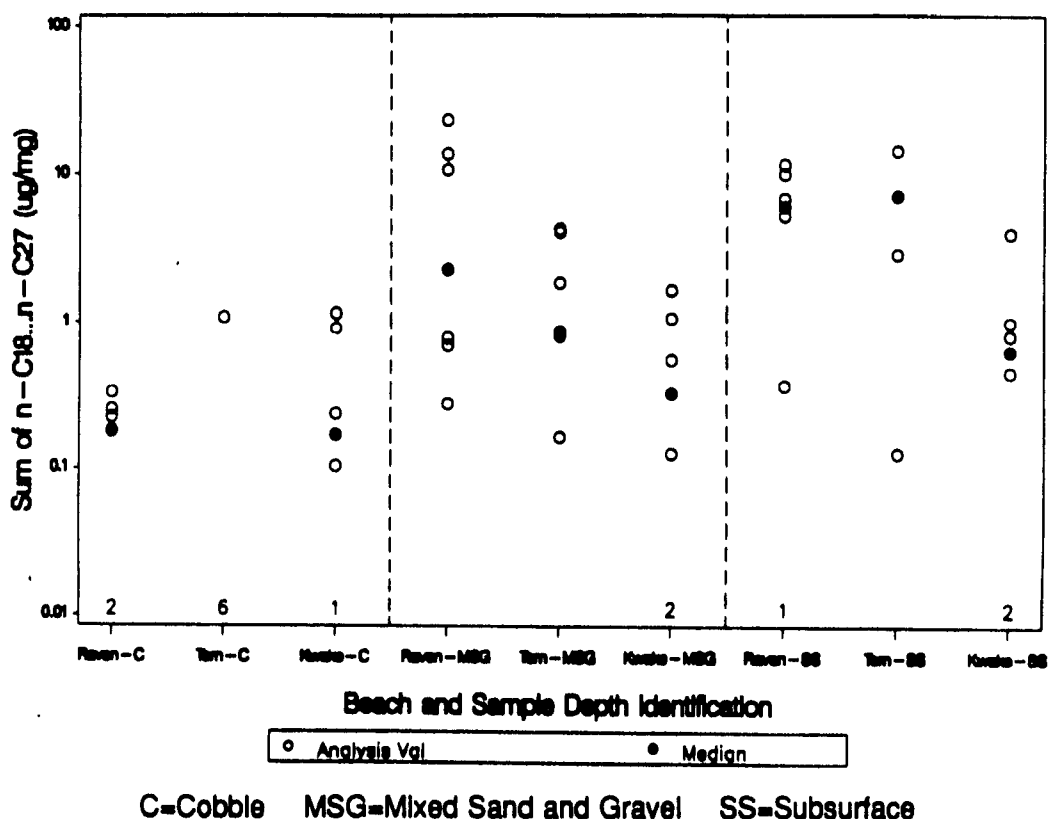
Figure 7.53. Sum of Alkane Concentration nC18 to nC27 (Log Scale) for the Low Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface
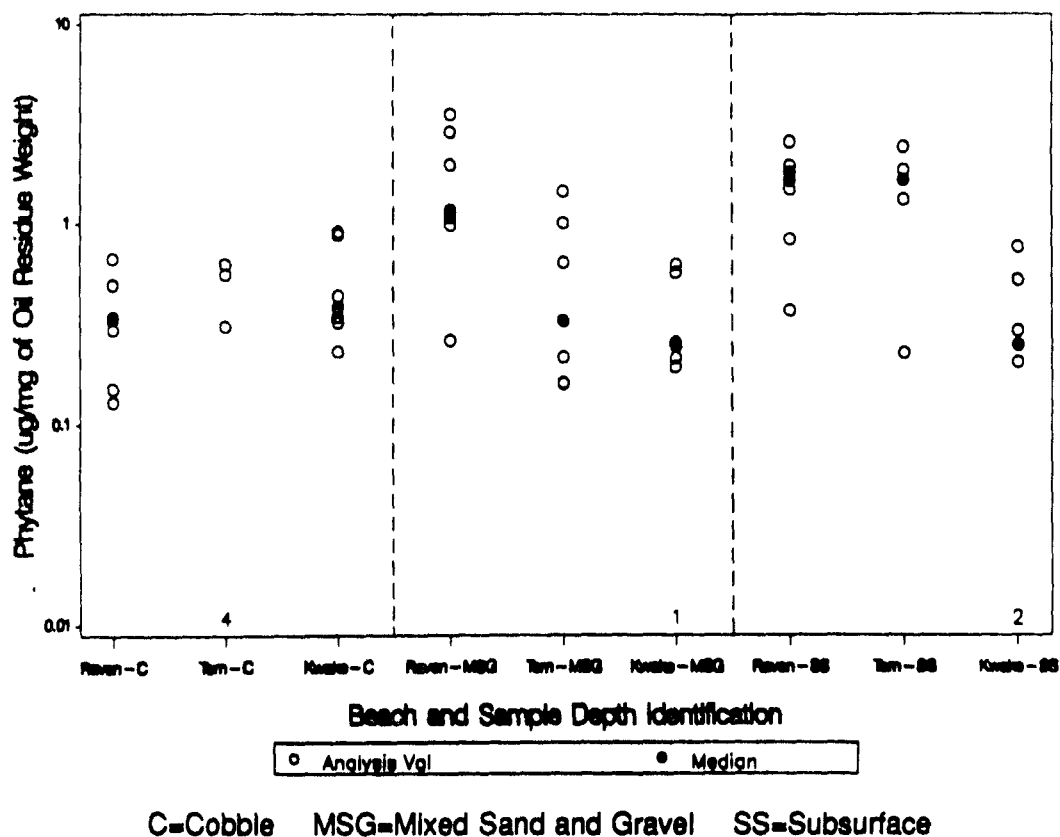
Figure 7.54.   Phytane Concentration (Log Scale) for the Low Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface
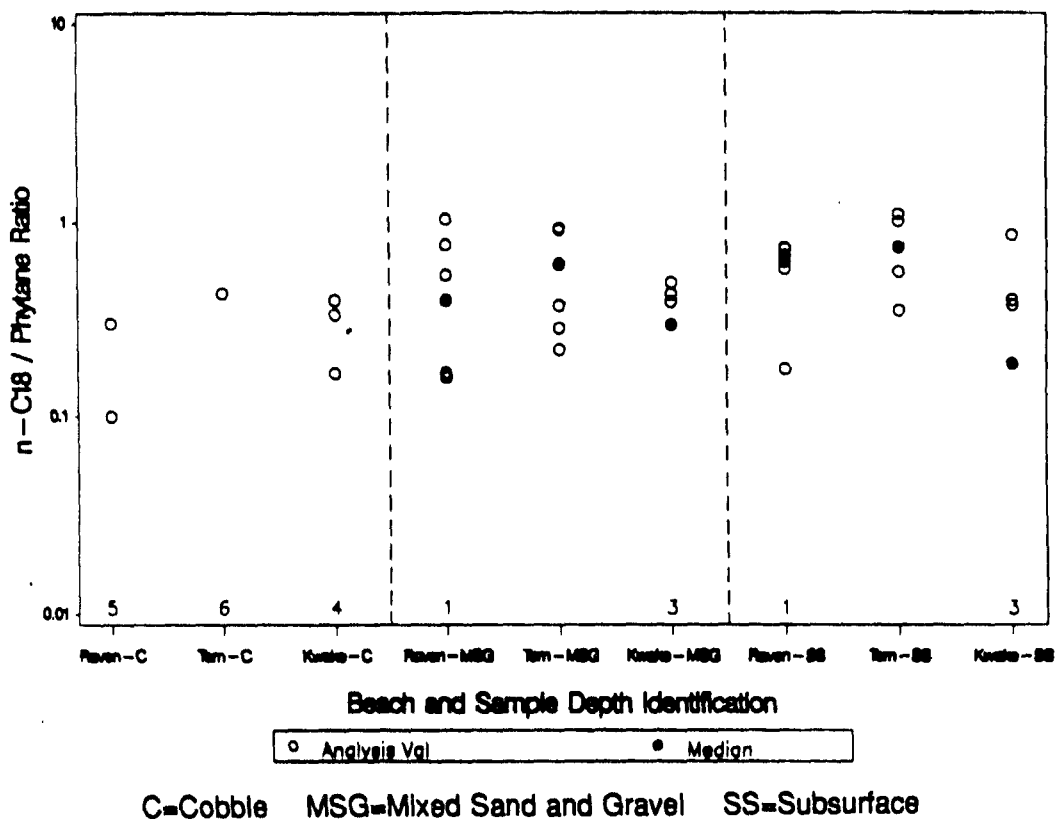
Figure 7.55.   nC18/phytane Ratio (Log Scale) for the Low Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface
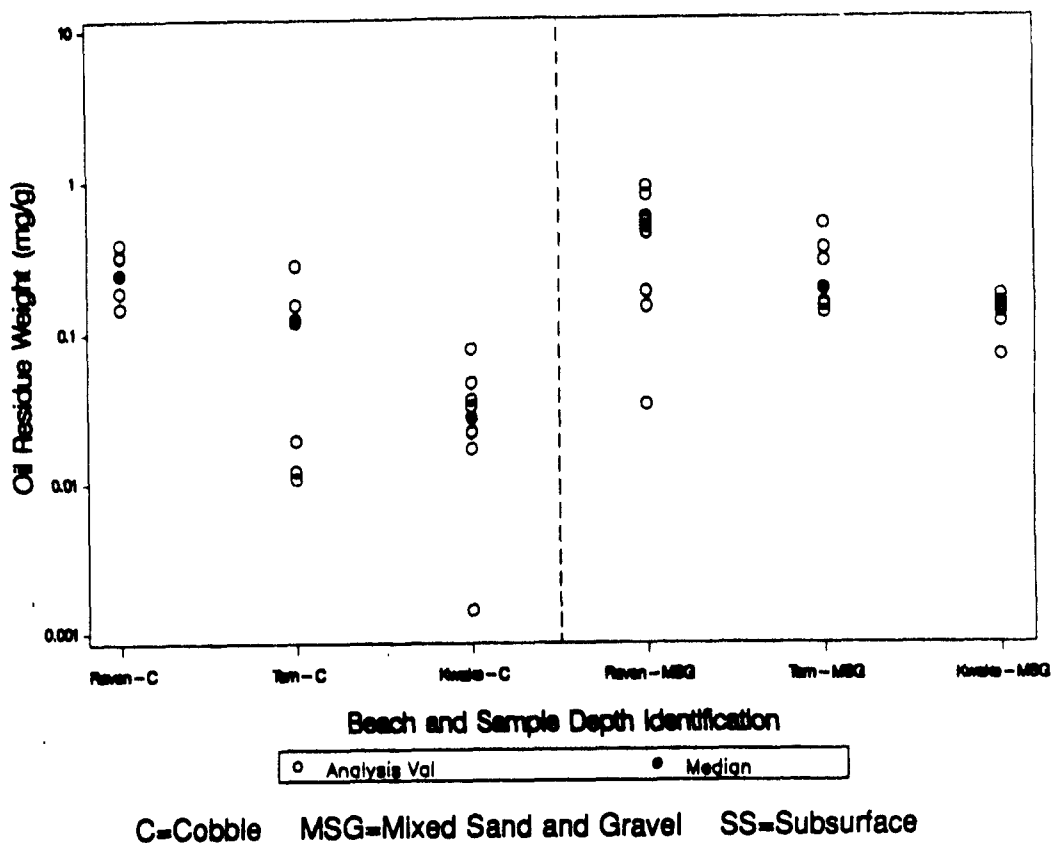
Figure 7.56.   Oil Residue Weights (Log Scale) for the High Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface

Figure 7.57.   nC18 Concentration (Log Scale) for the High Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface

Figure 7.58.   nC27 Concentration (Log Scale) for the High Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface

Figure 7.59.   Sum of Alkane Concentration nC18 to nC27 (Log Scale) for the High Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface

Figure 7.60.   Phytane Concentration (Log Scale) for the High Tide Zone at Passage Cove During the Winter of 1989.

C=Cobble   MSG=Mixed Sand and Gravel   SS=Subsurface

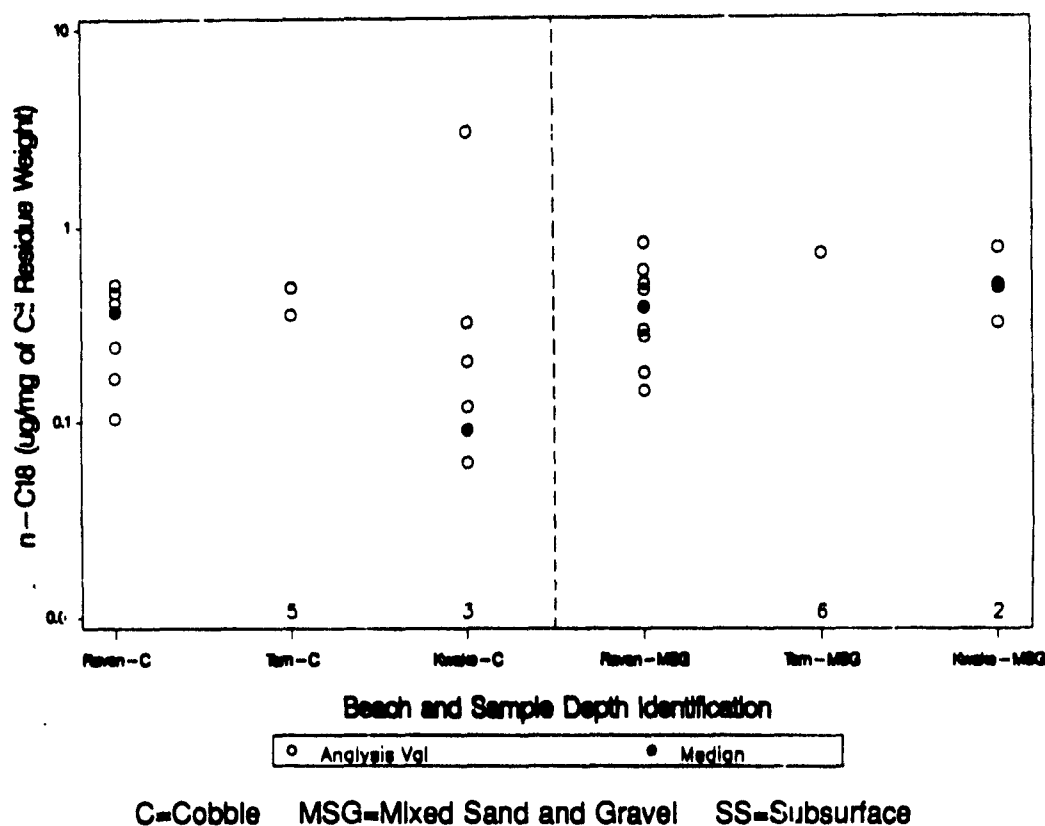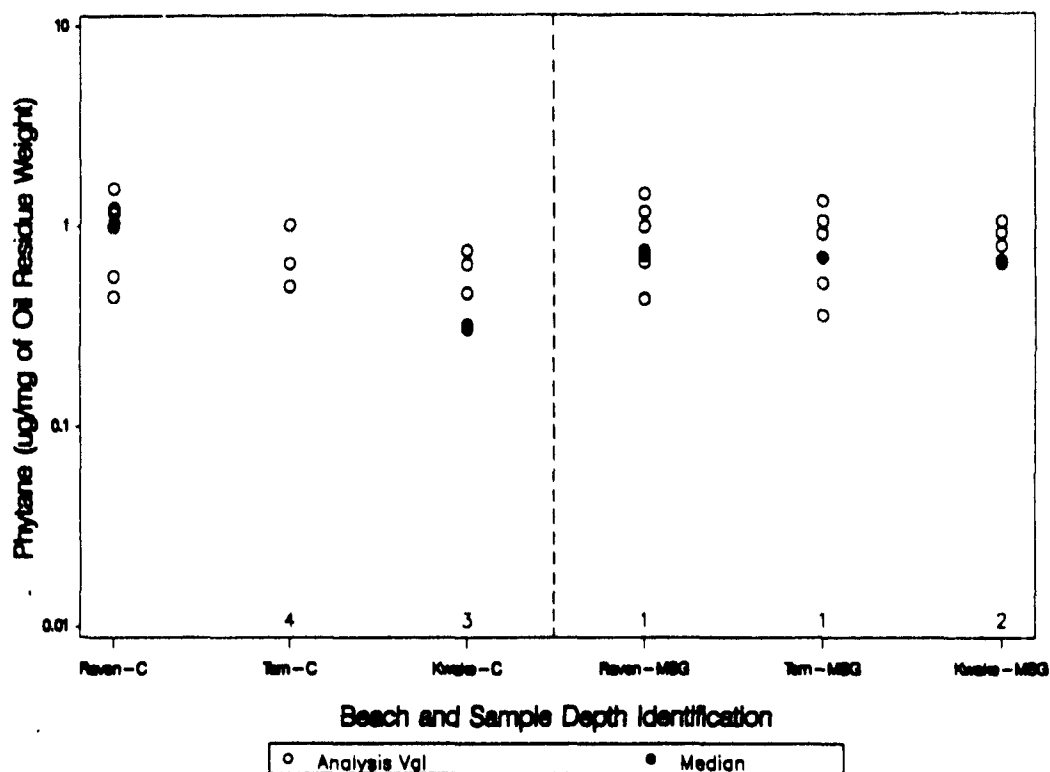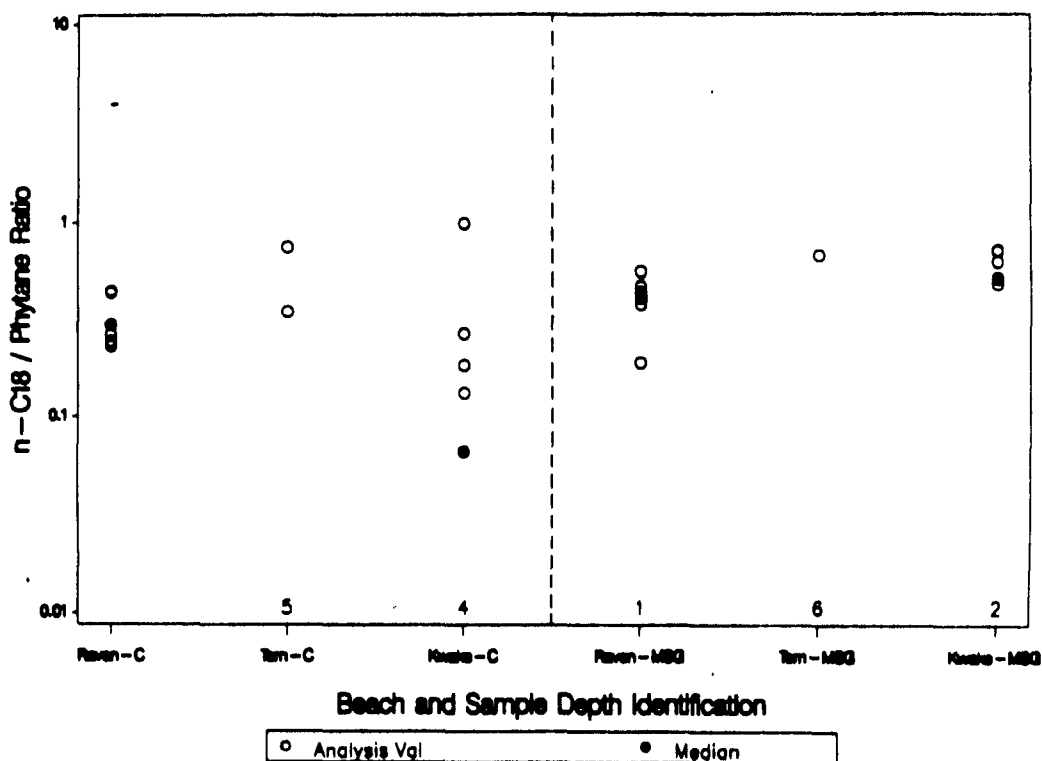**Figure 7.61.   nC18/phytane Ratio (Log Scale) for the High Tide Zone at Passage Cove During the Winter of 1989.**

unexpected that in this area of the beach many samples from Tern had values below detection limits; this suggests that INIPOL/CUSTOMBLEN may have had a greater long-term effect than the fertilizer solution application. For samples taken from the high tide zone, the effect of fertilizer was very pronounced. Significantly greater reductions at the 95% confidence level in the summed alkanes (Figure 7.59) and phytane (Figure 7.60) were apparent as a result of fertilizer application. In addition, the effect of the fertilizer solutions was greater than the effects of the INIPOL/CUSTOMBLEN. However, as noted above, there were many cases where samples from the INIPOL/CUSTOMBLEN-treated beach had concentrations below detection limits and, thus, over time, the INIPOL/CUSTOMBLEN fertilizer may have been as effective as the fertilizer solution.

Mixed Sand and Gravel Samples

Examination of the oil concentration in the mixed sand and gravel below the cobble clearly depicts the long-term effects of fertilizer application and enhanced oil biodegradation. At the end of the summer sampling, only the fertilizer solution application had significantly affected concentrations of oil residue in the mixed sand and gravel. It is clear from the winter data that the INIPOL/CUSTOMBLEN application was also exerting an effect, but to a lesser extent (Figure 7.53). Therefore, the effect was only visible after an extended incubation period. This contrasts dramatically with the observed residual oil concentrations on Raven beach, which were only slightly different from those observed at the initial sampling on July 22, 1989. The importance of enhancing oil biodegradation by fertilizer addition before the winter conditions slowed biological activity is clearly illustrated in these results.

These trends occurred despite residue weights in the low tide zone of the beach (Figure 7.50) that were approximately 10 times higher than those recorded in the high tide zone (Figure 7.56). This difference in oil residue weights may have been due to consistently colder temperatures resulting from more coverage by tidal waters over time in the low tide zone. Otherwise, there is no reasonable explanation for the difference.

Changes in hydrocarbon composition are again best expressed in terms of the summed alkanes (Figure 7.53) and phytane (Figure 7.54). The most pronounced effects of the fertilizers are shown in samples taken from the low tide zone. Again, the greatest compositional change occurred on the treated beaches with Kittiwake consistently demonstrating greater change than Tern beach. This trend is also reflected in the nC18 alkane as well in Figure 7.51 (fewer samples below detection

limits). The trend is not as evident in samples from the high tide zone; alkane concentrations were not significantly different between Kittiwake and Raven beaches. However, all of the samples with hydrocarbon concentrations below the detection limits were from the fertilizer-treated beaches (particularly the INIPOL/CUSTOMBLEN treatment), and, thus, the absence of these data points (set to zero) in the statistical analysis creates a bias that is conservative in terms of fertilizer effect.

Subsurface Samples

Beach samples taken at a depth of 15 to 18 cm below the beach surface provide the first evidence that fertilizer-enhanced biodegradation was also occurring in the beach subsurface. Unfortunately, only the low tide zone was sampled. The results clearly show that penetration of nutrients to the subsurface and the concomitant enhancement of oil biodegradation was most effective with fertilizer solution application. All measurements shown in Figures 7.50 to 7.55 (oil residue weight, individual alkanes, summed alkanes, phytane, and the nC18/phytane ratio), were significantly different at the 95% confidence level from the untreated control. The application of INIPOL/CUSTOMBLEN fertilizer did not appear to have an effect at this time. It is therefore concluded that oil in the subsurface was adequately colonized by oil-degrading microorganisms, but the oil biodegradation was nutrient limited. The ineffectiveness of the INIPOL/CUSTOMBLEN application was probably the result of insufficient total nutrient release; in other words, there were only enough nutrients provided in a single application to significantly affect oil biodegradation below the beach surface.

SPRING 1990 SAMPLING

Following the field demonstration during the summer and late fall, 1989, subsequent sampling was conducted in the early summer, 1990. Oil residue weight results from these samplings are shown in Figure 7.62.

On June 12, 1990, visual examination of the Passage Cove beaches showed there was no oil on the cobble surface or in the mixed sand and gravel below the cobble on any beach, including the untreated control beach. However, after excavating many holes to a depth of approximately 15 to 18 cm below the beach surface throughout the high, mid, and low tide zones, it was visually obvious that only the untreated control beach, Raven, still contained oil. There were no visual quantities of oil on the two treated beaches. Although many areas of Raven beach had become free of oil in the
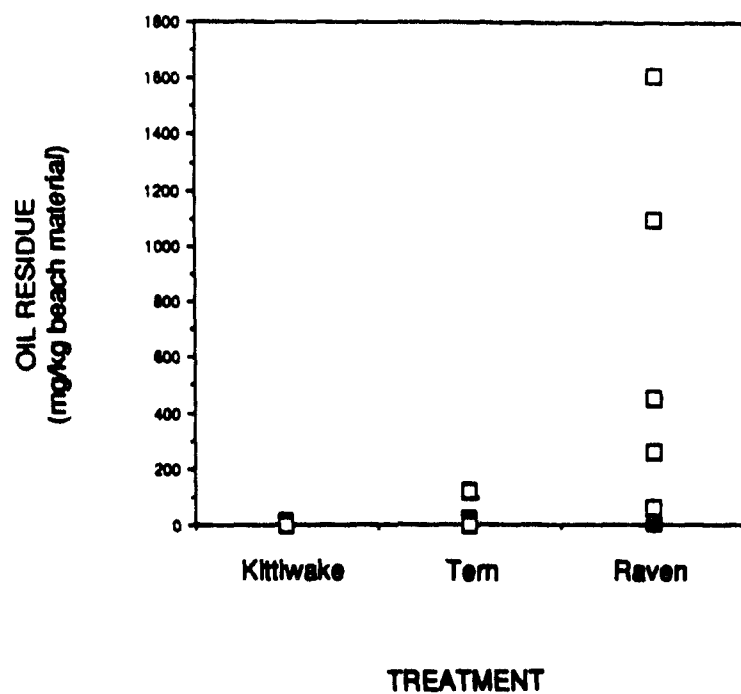
Figure 7.62. Oil Residue Weight in the Subsurface for the Three Treatments in the Spring of 1990.

subsurface over the winter, 5 to 6 large pockets of oil-contaminated beach material remained. It is possible that these areas were those heavily contaminated with oil the previous summer.

Determination of oil residue weight in samples from the June 12, 1990 sampling verified that subsurface samples on Kittiwake and Tern beaches contained essentially no oil (Figure 7.62). Subsamples on Raven, where oil was observed, had significant concentrations of oil, some as high as the average starting concentration the previous summer.

Revisiting Passage Cove in August, 1990, showed that very little oil could be found on Raven beach in the subsurface. Thus, it required essentially another summer of natural biodegradation activity to adequately remove all oil contamination from Raven beach. Fertilizer application probably accomplished this end point in the very early spring, if not sooner. It is therefore believed that by general extrapolation bioremediation, as a secondary cleanup process, potentially reduced beach cleanup time by as much as 50%.

MICROBIOLOGY

Numbers of Oil-Degrading Bacteria

The number of oil-degrading bacteria present on beach materials was also determined for Passage Cove. Samples of beach material were taken from grids 1, 3, 5, 7, 8, 10, 12, 14, 15, 17, 19, and 21. Numbers of degraders were assessed by using modification of the dilution to extinction method used for Snug Harbor. Five replicate dilution series were prepared from the initial 1:10 dilution. The relative numbers of bacteria in each sample were an average of the five replicate dilution series.

Results from these studies are shown in Table 7.7. The values reported are the $\log_{10}$ normal mean and standard deviation of 11–12 dilution series for each mixed sand and gravel sample. Results suggested no consistent increase in oil-degrading microorganisms occurred as a result of fertilizer application. This means that even in the plot treated with nutrient solutions from a sprinkler system, where nutrient exposure to the bacteria should be optimized, an increase in oil-degrading microorganisms did not occur. This could be due to a relatively constant sloughing of microbial biomass from the surfaces of the beach material, perhaps caused by tidal flushing action. Grazing by protozoans could also keep the microbial numbers at a specific density. The presence of high

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numbers of oil-degrading microorganisms was much greater than that observed for unimpacted beaches (Table 7.8). It is clear that the number of oil degraders in uncontaminated areas was 1,000 to 100,000 times lower than in contaminated areas. Thus, the presence of oil causes a significant enrichment of oil-degrading microorganisms.

TABLE 7.7. RELATIVE CONCENTRATION (LOG_{10} OF THE CELL NUMBER/G OF BEACH MATERIAL) OF OIL-DEGRADING MICROORGANISMS IN PASSAGE COVE

Sampling Date	Fertilizer-Treated Plots		
Before Application	Untreated Control	Water-Soluble	Oleophilic + Water-Soluble
07/22/89	6.44 ±1.44	6.31 ±1.36	6.44 ±1.33
After Application			
08/06/89	5.32 ±1.12	5.78 ±1.45	5.71 ±0.67
08/19/89	6.60 ±1.83	5.47 ±1.34	5.66 ±0.35

TABLE 7.8. RELATIVE CONCENTRATION (LOG_{10} OF THE CELL NUMBERS/G OF BEACH MATERIAL AND STANDARD DEVIATION) OF OIL-DEGRADING MICROORGANISMS IN SAMPLES FROM BEACHES THAT WERE NOT IMPACTED BY OIL.

Site	High Tide	Mid Tide	Low Tide
Tatitlek	2.41 ±.58	4.31 ±1.14	6.11 ±2.05
Fish Bag	(1.51	(1.31	(2.71
Snug Corner Cove	2.31 ±.54	2.51 ±.55	(1.11
Hell's Hole	(2.11	2.51 ±.89	(.91
Commander Cove	4.51 ±1.14	(1.31	3.11 ±.45

Microbial Activity

Oiled Versus Unoiled Beaches

To compare oiled with unoiled beaches, samples were collected from Raven beach, an oiled beach that served as a control in the Passage Cove field test, and from a beach in Hell's Hole, an area not impacted by the oil spill. The activity of bacterial populations was assessed by analyzing the evolution of $^{14}CO_2$ from radiolabeled hexadecane, naphthalene, and phenanthrene. Each compound was incubated in 50 mL of artificial sea water medium with 5.0 g of sample for periods up to 120 hours.

Hexadecane was mineralized at the same rate by both samples (Figure 7.63). Naphthalene was also mineralized in both samples but at a lower rate in samples from Hell's Hole. Phenanthrene was mineralized only in the samples from Raven beach. Degradative activity, indicative of the presence of bacteria capable of using the compound as substrate, was clearly present at Raven. Activity in samples from the unimpacted site may indicate either that the site had a prior history of oil exposure (perhaps from oil navigational traffic) that resulted in an acclimated bacterial population, or that unexposed microbial communities may have broad enough metabolic capabilities to utilize oil hydrocarbons without a lengthy acclimation period. If the former were the case, all of the compounds should have been degraded at both sites.

Given that hexadecane appears to be more readily degradable than naphthalene or phenanthrene, it is likely that indigenous bacteria can utilize straight chain hydrocarbons more readily than aromatics. Both phenanthrene and hexadecane were used as test compounds in subsequent experiments, but phenanthrene may be a better indicator of adapted populations.

Passage Cove

For the Passage Cove tests only mineralization of radiolabeled hexadecane and phenanthrene was measured in material from the test beaches (Kittiwake, Tern, Raven) three days prior to the application of the fertilizer treatments, and four and six weeks after application. Comparison of tidal regions for pre-application samples had demonstrated that the mid-tide zone was representative of mean mineralization activity for a beach. Therefore, in post-application samples it was assumed that this would also be true. Due to the lengthy incubation times needed to determine mineralization rates
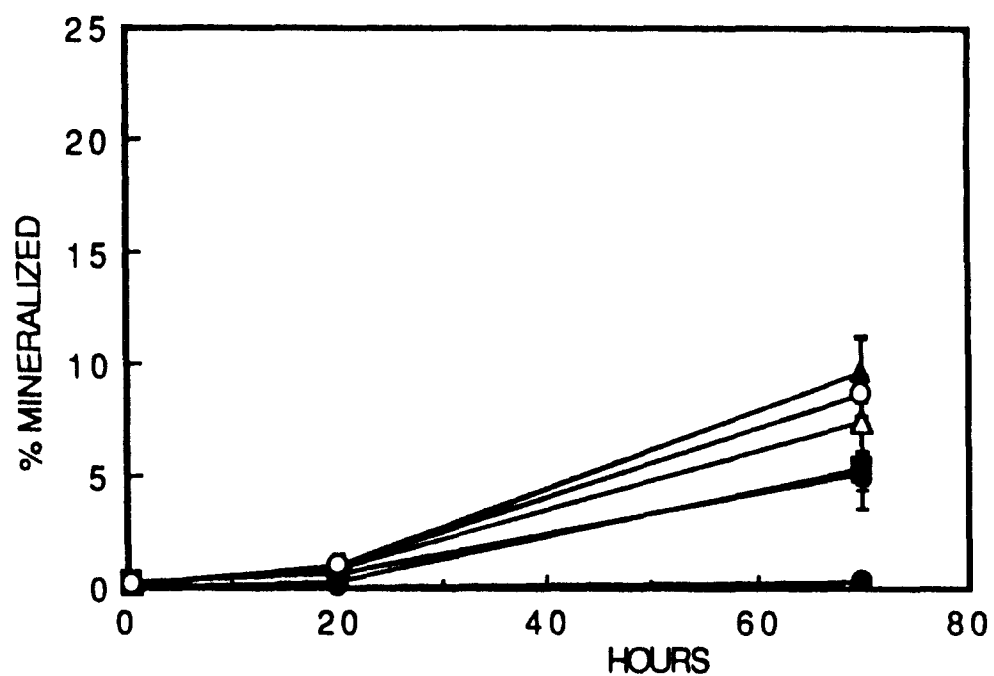
Figure 7.63. Mineralization of Radiolabeled Phenanthrene (•,○), Naphthalene (□,■), and Hexadecane (△,▲), in Samples From Oiled (Open Symbols) and Unoiled (Closed Symbols) Beaches.

and the limited number of samples that could be processed daily, it was felt that mineralization measured for several time points using a few samples was more accurate than using single time points for numerous samples. The concentration of radiolabeled substrate incubated with beach material was therefore reduced, reflecting the decrease in the amount of these compounds present in the oil, as determined by gas chromatography.

In the pre-fertilizer application samples, mineralization commenced before the first sampling time (20 hours), indicating little or no lag period. The mineralization curves were S-shaped, which is typical of a growing population. A representative curve is shown in Figure 7.64. No significant differences in the rates of mineralization were detected among the three beaches (Table 7.9). In addition, the extent of mineralization was somewhat lower in the high tide zone.

TABLE 7.9. MINERALIZATION OF 10 μG ^{14}C-PHENANTHRENE PER G PASSAGE COVE BEACH MATERIAL PRIOR TO APPLICATION OF FERTILIZER (7/22/89)

Treatment[a]	Sample Zone	Lag Time[b] (h)	Max Rate %/g/h	Max Rate ng/g/h	Extent %
Water-	Low	42 ± 5[c]	1.0 ± 0.2	103 ± 16	42 ± 8
Soluble	Mid	18 ± 9	2.1 ± 0.4	205 ± 41	50 ± 2
(KW)	High	37 ± 7	2.3 ± 0.5	230 ± 50	40 ± 5
Untreated	Low	NS[d]	NS	NS	NS
Control	Mid	11 ± 1	1.6 ± 0.1	160 ± 7	50 ± 4
(R)	High	18 ± 7	1.7 ± 0.2	169 ± 21	44 ± 6
Oleophilic	Low	16 ± 8	1.8 ± 0.4	183 ± 40	45 ± 9
(T)	Mid	19 ± 17	1.5 ± 0.8	147 ± 75	31 ± 3
	High	19 ± 10	1.9 ± 0.9	186 ± 91	39 ± 5

[a]KW = Kittiwake Beach; R = Raven Beach; T = Tern Beach
[b]Time required for 10% mineralization of phenanthrene
[c]Mean and standard deviation of four samples
[d]No sample analyzed
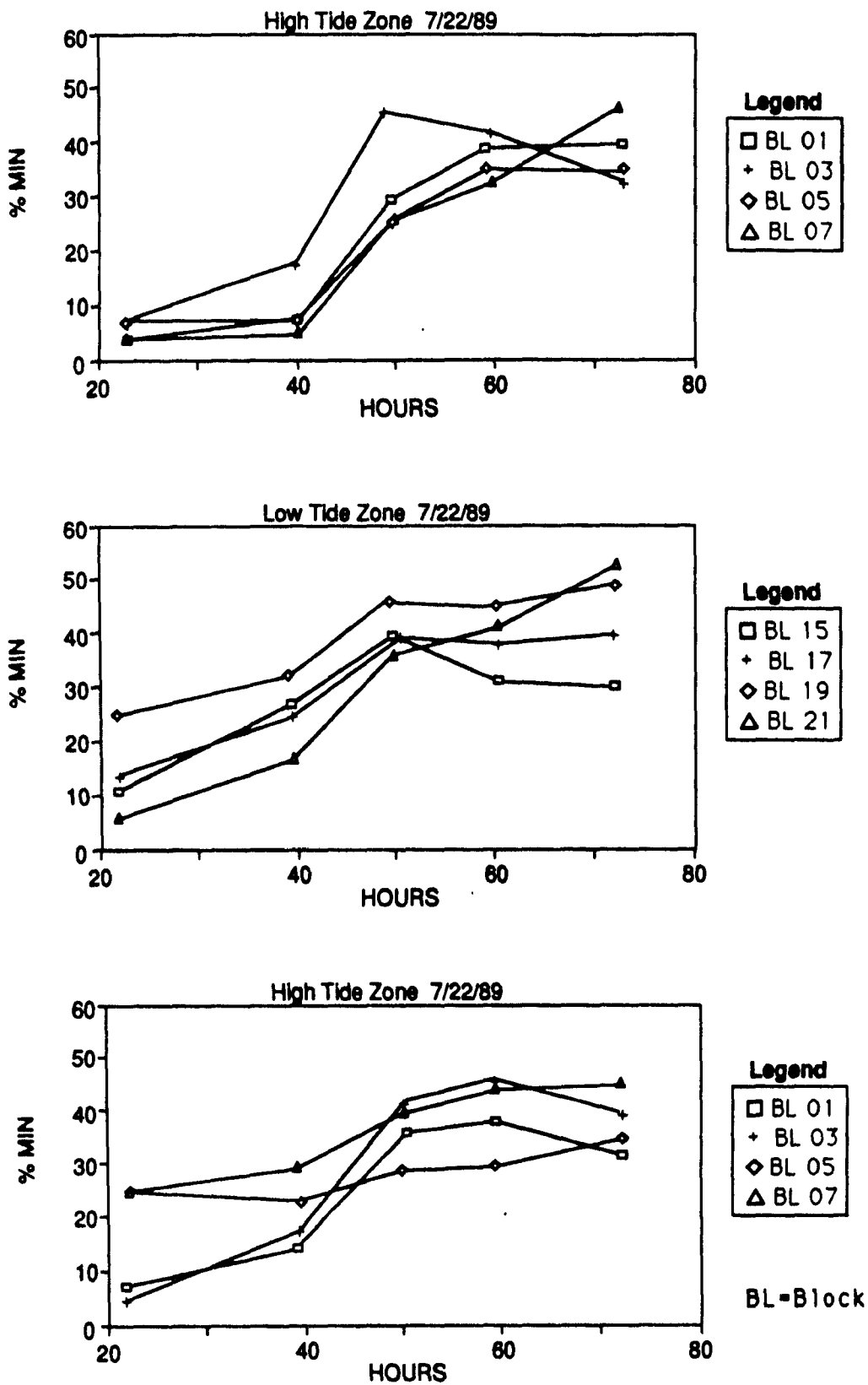
Figure 7.64. Mineralization of Phenanthrene in Samples From (A) Water-Soluble Fertilizer-Treated; (B) Untreated Control; and (C) Oleophilic Beaches at Passage Cove.

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At four and six weeks following application of the fertilizer, the rate of mineralization was lower than the pre-application rate when expressed on a mass basis (Table 7.10), but similar when expressed as percentage rate. This suggested that the mass transfer of the compound may be rate-limiting. At four weeks post-application, the mineralization of phenanthrene on the untreated control beach (Raven) was greater than on the treated beaches (Kittiwake and Tern). No difference was detected between the water-soluble and oleophilic beach. By six weeks, the previously noted difference between the treated beaches and the untreated control beach had disappeared.

TABLE 7.10. MINERALIZATION OF 0.32 μG OF ^{14}C-PHENANTHRENE AND 0.44 μG ^{14}C-HEXADECANE PER G PASSAGE COVE BEACH MATERIAL

No. of Weeks Post Application[a]	Cmpd	Lag (h)	Max Rate %/g/l	Max Rate ng/g/h
Water-Soluble (KW) Treatment[b]				
4 (8/22/89)	Hex	> 50	1.9 ± 0.8	8.4 ± 3.4
4 (8/22/89)	Phe	> 50	0.8 ± 0.2	2.8 ± 0.6
6 (9/5/89)	Hex	>100	1.4 ± 0.4	4.4 ± 1.3
6 (9/5/89)	Phe	>100	2.4 ± 0.8	7.6 ± 2.4
Untreated Control (R)				
4 (8/22/89)	Hex	39 ± 1	5.6 ± 0.1	24.6 ± 4.4
4 (8/22/89)	Phe	42 ± 10	6.4 ± 0.5	21.2 ± 1.6
6 (9/5/89)	Hex	>100	1.4 ± 0.4	4.4 ± 1.3
6 (9/5/89)	Phe	>100	2.3 ± 0.9	7.5 ± 2.8
Oleophilic (T)				
4 (8/22/89)	Hex	> 50	1.8 ± 0.4	8.0 ± 1.8
4 (8/22/89)	Phe	> 50	2.6 ± 0.9	8.6 ± 3.0
6 (9/5/89)	Hex	>100	1.8 ± 0.3	5.7 ± 0.9
6 (9/5/89)	Phe	>100	2.3 ± 0.6	7.8 ± 2.0

[a]KW = Kittiwake Beach; R = Raven Beach; T = Tern Beach
[b]Four blocks from mid-tide zone

The observed differences between the extents of mineralization for the pre- and post-fertilizer application samples can not be readily explained. The abundance of bacteria in the water column at Passage Cove declined during this interval, although no differences in population size were reflected in the numbers of hydrocarbon degraders in the beach material. However, not all of the hydrocarbon degraders are necessarily phenanthrene degraders.

A radiolabeled MPN technique indicated that the number of phenanthrene degraders was $4.95\pm0.30 \times 10^5$, which is less than the total number of hydrocarbon degraders (approximately 10^6 per gram of beach material). It is possible that as the more labile components of the oil mixture on the beaches were degraded, the fraction of the bacterial population adapted to the utilization of those compounds declined, and thus there was less mineralization activity with time. An alternative explanation is that the bacteria may utilize the compound at different rates or with different efficiencies at different concentrations. Either cometabolism or a high percentage of incorporation into cell material might explain the relatively small fraction of labeled compound converted to $^{14}CO_2$ at the lower concentration.

Regardless of the reason for the differences in the rate and extent of mineralization before and after fertilizer application at Passage Cove, it can be concluded that organisms capable of degrading hexadecane and phenanthrene existed on the beach prior to treatment, and that the application of either fertilizer treatment did not significantly alter the rate and extent of mineralization compared to the untreated control beach.

Mineralization in Microcosms

Microcosms were designed to test the efficacy of bioremediation for the treatment of subsurface oil. Clean beach material from an unaffected beach at Hell's Hole and oiled beach material from Raven beach at Passage Cove was used to construct the microcosms. Containers were filled by either layering oily material over clean material or vice versa. The material from Hell's Hole was tested prior to the construction of the microcosms and no mineralization activity was detected.

Each set of microcosms was treated with water-soluble fertilizer, oleophilic fertilizer (INIPOL), or no fertilizer (untreated controls). All of the microcosms were flushed with sea water in accordance with the tidal cycles. Twenty-four microcosms were constructed, allowing duplicate microcosms to be sacrificed on two sampling dates. During sampling, the contents of each microcosm was

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fractionated into thirds, giving clean, oily, and interface layers. Subsamples were collected from the well-mixed material derived from each layer and were analyzed for numbers of oil-degrading organisms, chemical composition of the oil, and bacterial activity. Bacterial activity was measured as mineralization of phenanthrene and hexadecane.

The results for both compounds were similar, so only the phenanthrene data is presented. Mineralization of phenanthrene was measured for incubation periods of approximately 120 hours. The compound was 5 to 20 percent mineralized in all samples except for the top layer in the oleophilic-treated microcosms where clean material overlay oily material (Figure 7.65). No mineralization was detected in these samples, and this was true for the replicates and both sampling times. Bacterial activity is vertically translocated either up or down from oily to clean material for all treatments except for the case noted above. The oleophilic fertilizer would be a possible treatment for a beach only containing subsurface oil because fertilizer will enhance activity in the subsurface if nutrients filter down into the subsurface material. When oleophilic fertilizer is applied to an oily surface, however, bacterial activity is evident throughout the beach material.

Biodegradation rates, as determined by mineralization of radiolabeled substrates in natural samples, are subject to interpretation, especially in light of the amount of residual hydrocarbon in the sample and the processes that affect the rate of availability of the compound to bacteria. The rate of mineralization of the labeled substrate is proportional to the total rate of mineralization of all the substrates utilized by a given population or metabolic pathway. Therefore, caution must be exercised in extrapolating such rates to unknown concentrations of compounds and to periods of time over which both concentrations and bacterial populations may change. Similarly, radiolabeled compound added to a sample is almost certainly more available to bacteria than an in situ compound which may be limited by insolubility or the presence of a complex matrix. On the other hand, the absence of mineralization of labeled substrate is a strong indication of the absence of acclimated bacteria.

Mineralization of radiolabeled phenanthrene and hexadecane in samples of beach material demonstrate the activity of acclimated populations of hydrocarbon-degrading bacteria prior to the application of fertilizers. Rates of mineralization were lower after fertilizer application, although the decrease of rates at the control and treated beaches suggests this was a general phenomenon instead of a response to fertilizer. The depletion of labile substrate and the attendant shift in bacterial activity over time would explain both the overall decrease in rates and the lower rates observed for treated beaches one month after fertilizer application. Thus, a lower instantaneous rate may reflect a higher activity in the preceding interval.
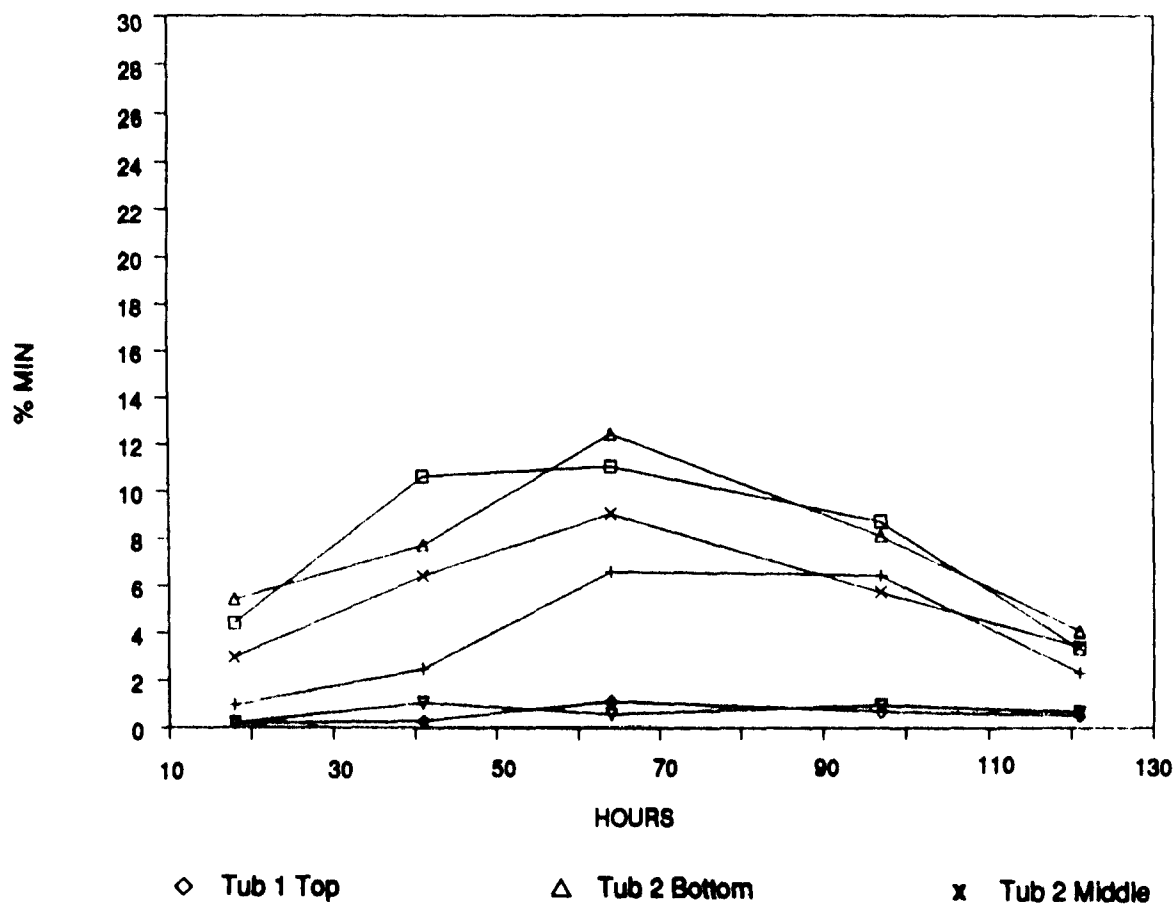
Figure 7.65. Mineralization of Phenanthrene in Oleophilic-Treated Microcosm with Oiled Layer Over Clean Layer, Showing Lack of Bacterial Activity in Upper Layer.

ECOLOGICAL MONITORING

The same environmental parameters monitored at Snug Harbor were monitored at Passage Cove, but a somewhat modified strategy was used for sample site location. Sample stations were located along the central axis of the embayment and along three nearshore areas where fertilizers were applied (see Sections 3 and 8). Reference sites for the Passage Cove study were established outside the embayment along the eastern shore of northern Knight Island. Water from the central sites of Passage Cove was sampled at 0.5 m and 5 m depths, while the nearshore stations (1 m offshore of low tide) were sampled at 0.5 m depths. Fertilizers were applied on July 25 and 26, 1989, to selected plots along the shoreline. Samples were collected prior to application of fertilizer along the shoreline, 3 days after application, and then at weekly intervals for 6 weeks after application.

Nutrients

Data from analyses of water samples from Passage Cove for ammonia, nitrite, nitrate, and phosphorus in water off the test beaches were unfortunately lost.

Chlorophyll Analysis

Phytoplankton chlorophyll data showed a slight increasing trend over the course of the study period for all treated and control stations (Figure 7.66). No trends consistent with nutrient effects were observed. The differences through time were not significant except for an increase observed on August 27 in the 0.5 m sample from all mid-channel stations and the reference site. These results are consistent with expected range of plankton chlorophyll for Prince William Sound and seasonal increases leading to a fall plankton bloom.

Phytoplankton Primary Productivity

Results from the pre-treatment sample (July 21), Day 3 (July 28), and Weeks 1 (July 31) and 2 (August 7) are presented in Figure 7.67. These data, and results from other dates not presented, show no trends for Passage Cove stations with greater primary productivity as a result of nutrient additions, except for the results from July 31. Primary productivity estimates on this date show greater values for stations 5, 6, and 7, the nearshore stations along the treated shoreline. This trend was not observed one week later, nor was it borne out in the chlorophyll data. If primary productivity was

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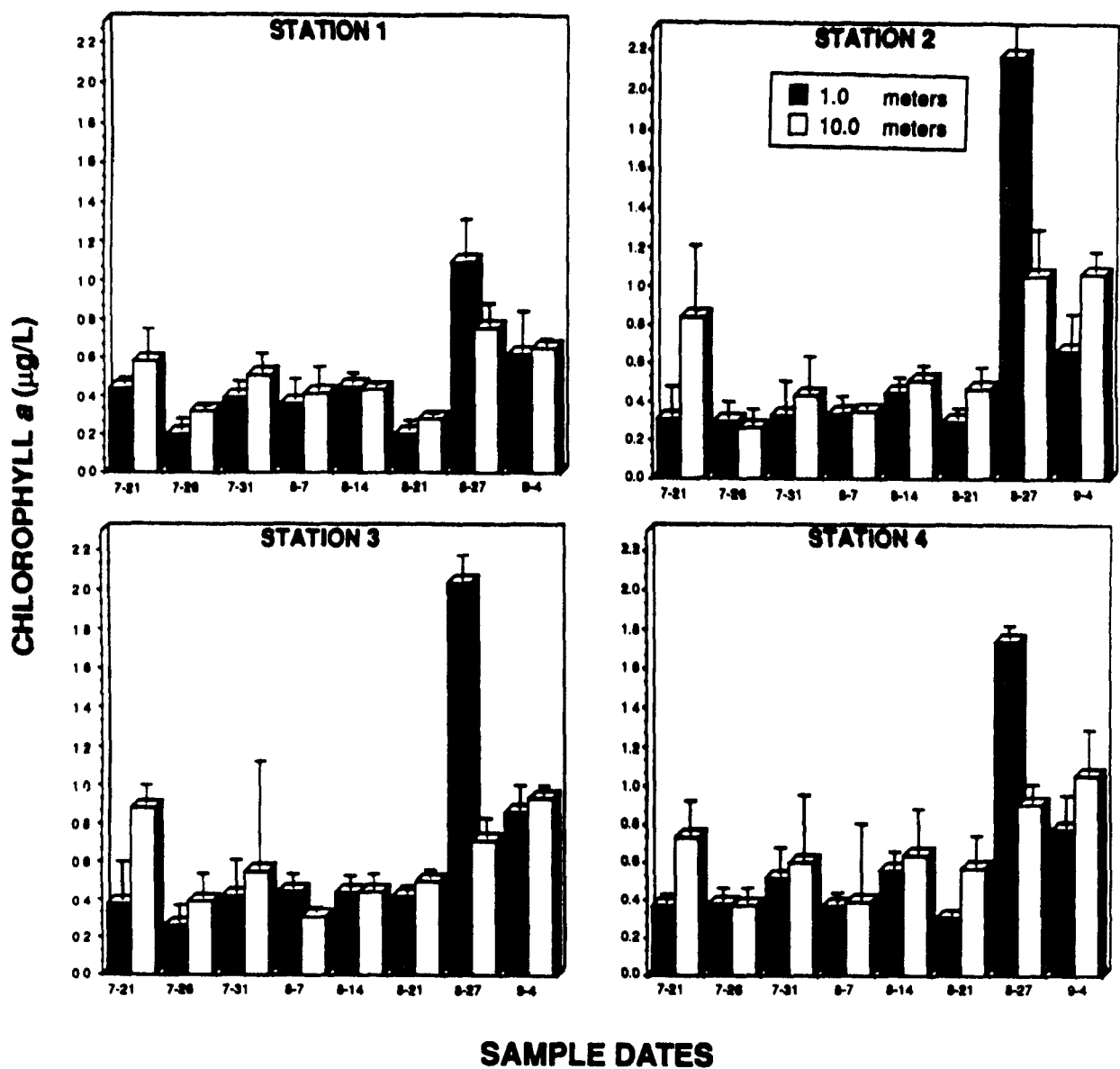
Figure 7.66. Mean Chlorophyll a/L Measurements (+ SD) from 4 Replicate Plankton Samples Taken at Passage Cove Study Site Following July 25, 1989, Fertilizer Applications to Shorelines. Values are Means (+ SD) of 4 Replicates; Dark Bars for Samples Collected at 0.5 m, Open Bars for Samples Collected at 5 m. Refer to Figure 3.3 for Sample Locations.

Figure 7.66. (Continued)
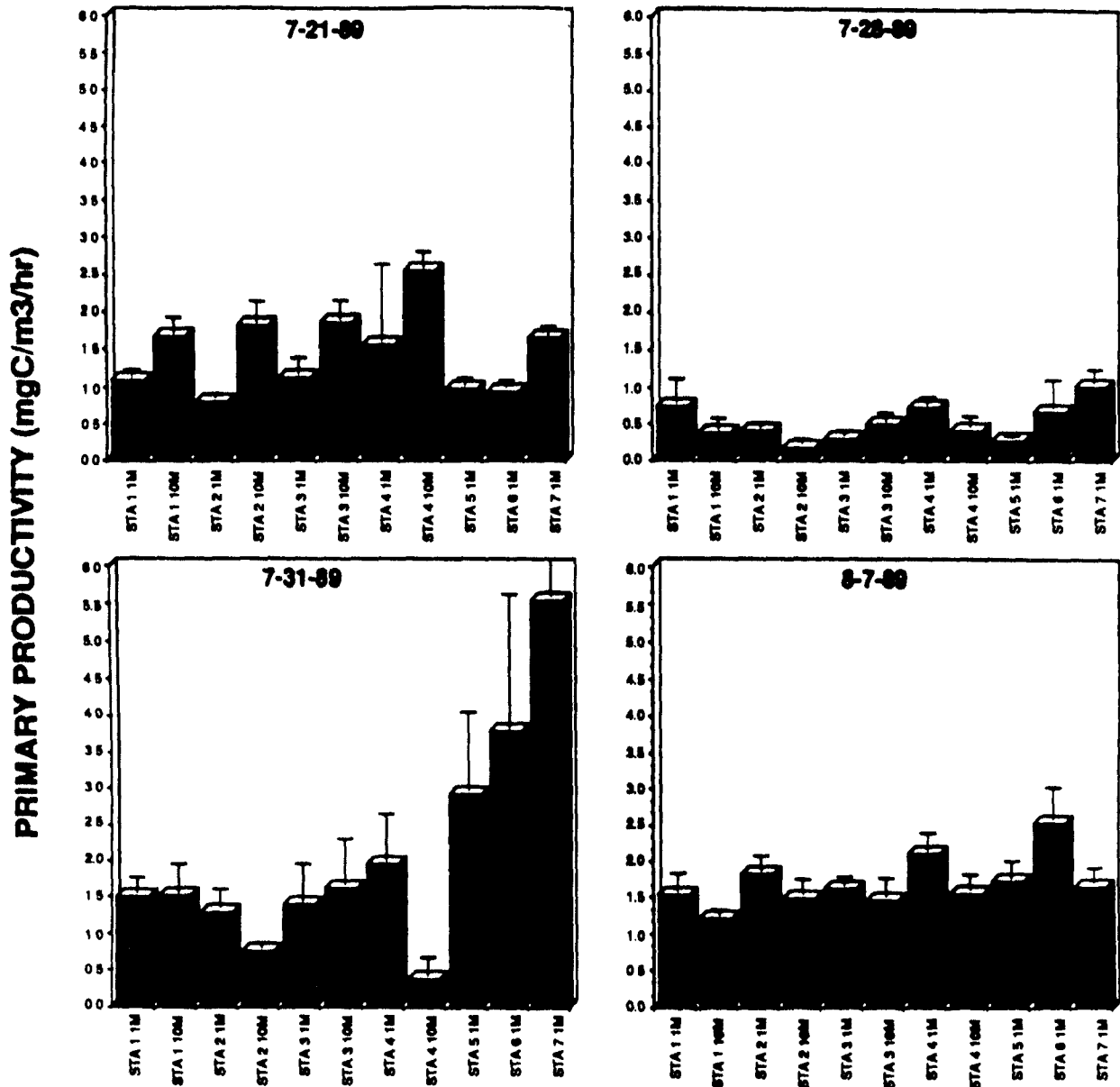
Figure 7.67. Mean Primary Productivity Measurements (+ SD) From
^{14}C-Uptake (mg C/m^3/hour) From 4 Replicate Plankton
Samples Taken at Passage Cove Study Site Following July
25, 1989, Fertilizer Applications to Shorelines. Refer
to Figure 3.3 For Sample Locations.

enhanced along the shoreline because of nutrient input, the effect on plankton growth was not sufficient to overcome dilution and transport that resulted from tidal exchange (i.e., there was no concomitant increase in plankton chlorophyll).

Bacterial Abundance

The mean number of bacterial cells per liter of water at Passage Cove sample sites ranged from approximately 0.4 to 1.2 x 10^9 over the seven-week sample period (Figure 7.68). All stations followed the same general pattern of greater numbers on the first three sample dates and lesser abundance thereafter. No trends were observed for nearshore and offshore comparisons, treated versus untreated control comparisons, or 0.5 m to 5.0 m sample comparisons. Fertilizer additions had no stimulatory effect on bacterial numbers.

Bacterial Productivity

The functional measure of bacterial incorporation of tritiated thymidine demonstrated considerable variability between sample dates with no consistent trends through time or with fertilizer treatments (Figure 7.69). The prominent samples with increased productivity usually occurred on the same dates for all samples, with similar trends at upper and lower depths. There were no trends consistent with effects of nutrient addition.

Caged Mussels

Only three of the samples had total PAH concentrations above the 0.20 μg/g detection limit. One of the replicate samples from the Passage Cove untreated control site (station 10) on August 8 and August 14, and from station 3 on August 14 had total PAH residues of 0.27, 0.23, and 0.25 μg/g wet weight, respectively. The other replicates from these stations on these sample dates did not have detectable PAHs. These results are given in Table 7.11. Residues of this order would not be unexpected, given the magnitude of boat traffic and residual oil in the study area. The overall results show no trend of enhanced hydrocarbon release and mussel bioaccumulation attributable to fertilizer additions. Problems in analysis were encountered using a small sample size (3 mussels/sample collected) and should have been larger (10 mussels/sample collected). In addition, the number of "time zero" mussels collected should have been equal to the number of mussels set out in all the test sites at the beginning of the test. This would have ensured enough tissue for analytical method
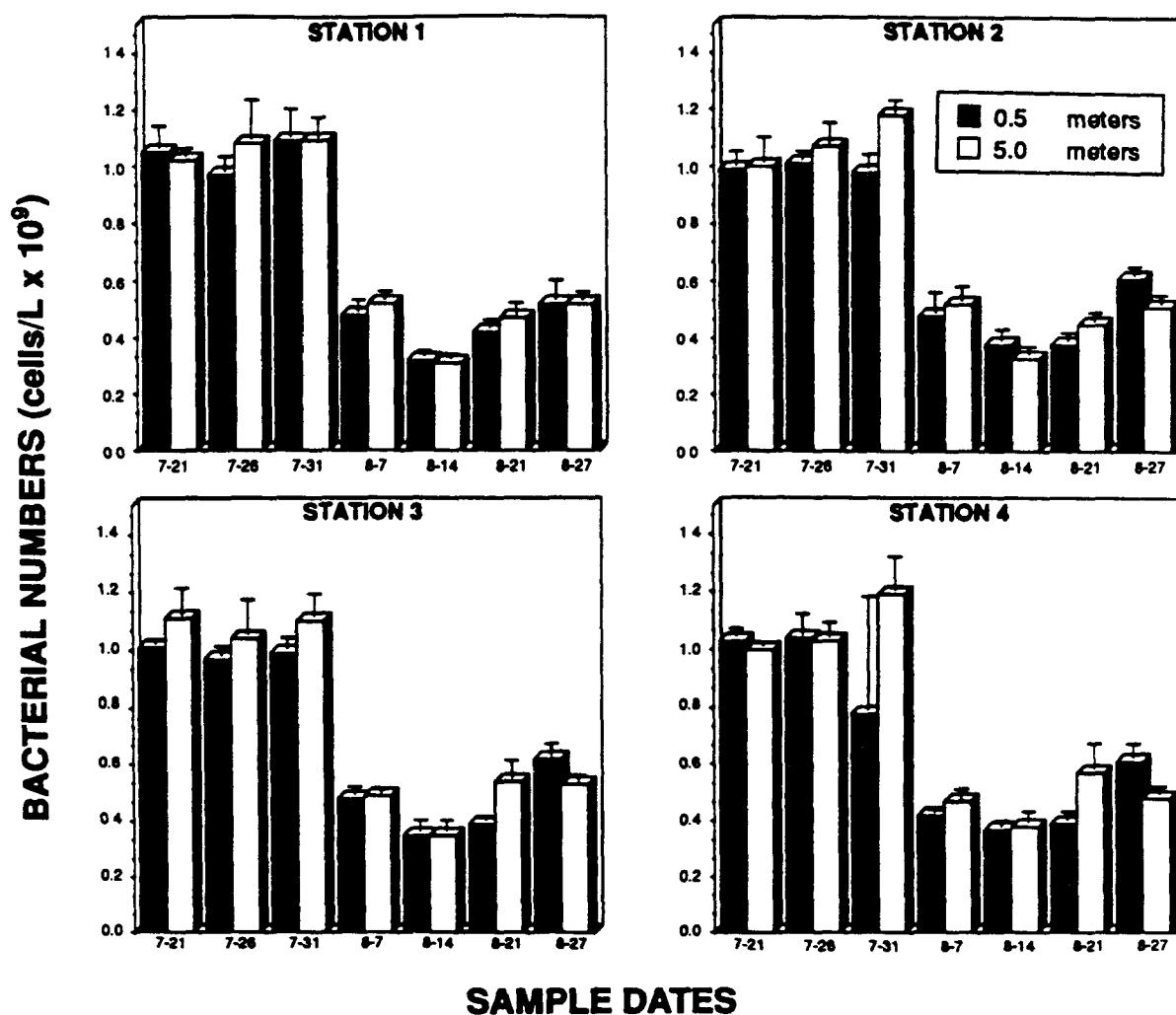
Figure 7.68. Abundance of Bacteria (cells x10⁹/L) From Water Samples Collected at the Passage Cove Study Site. Fertilizer was Applied on July 25, 1989. Values are Means (+ SD) of 4 Replicates; Dark Bars For Samples Collected at 0.5 m, Open Bars For Samples Collected at 5 m. Refer to Figure 3.3 For Sample Locations.
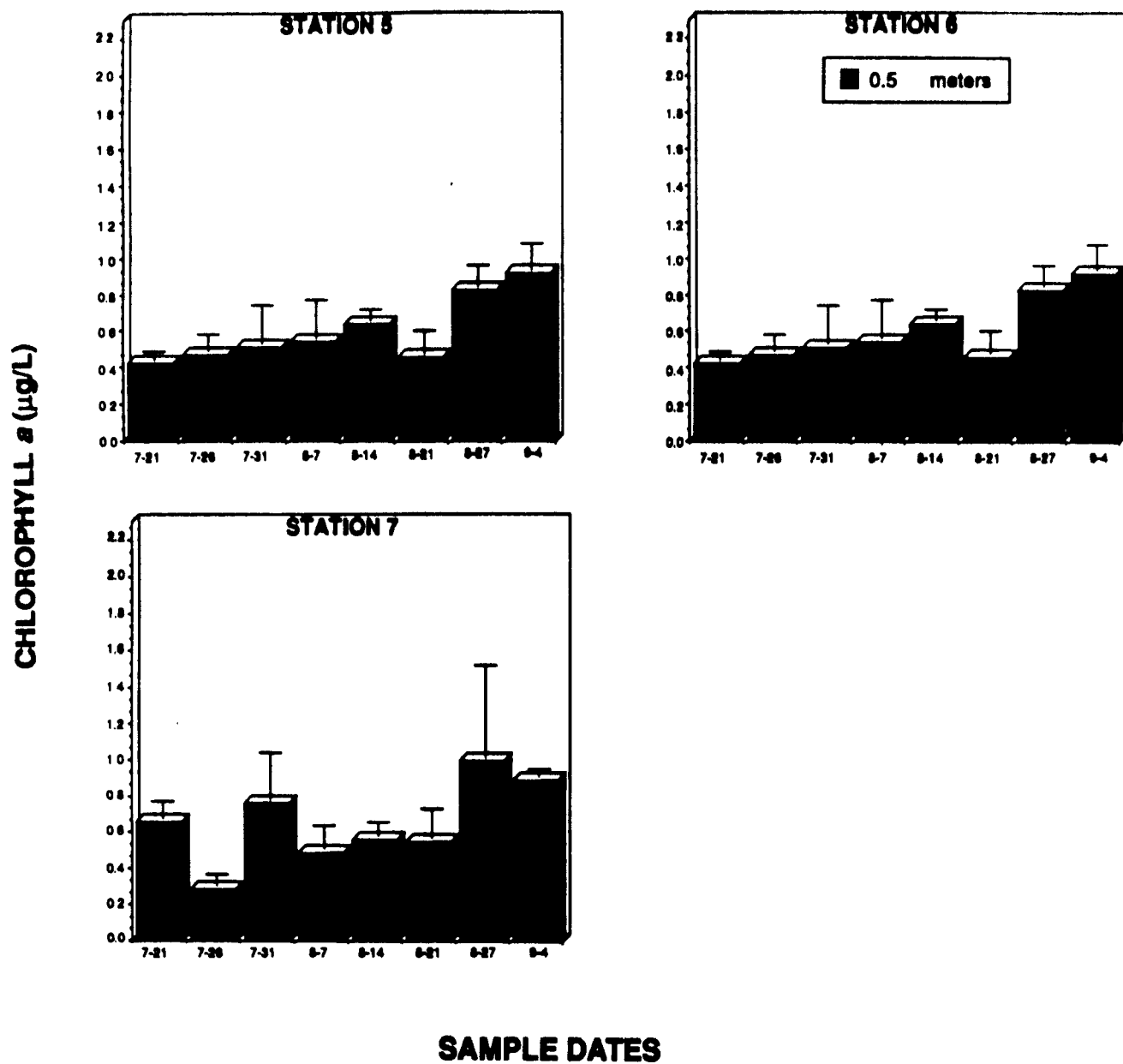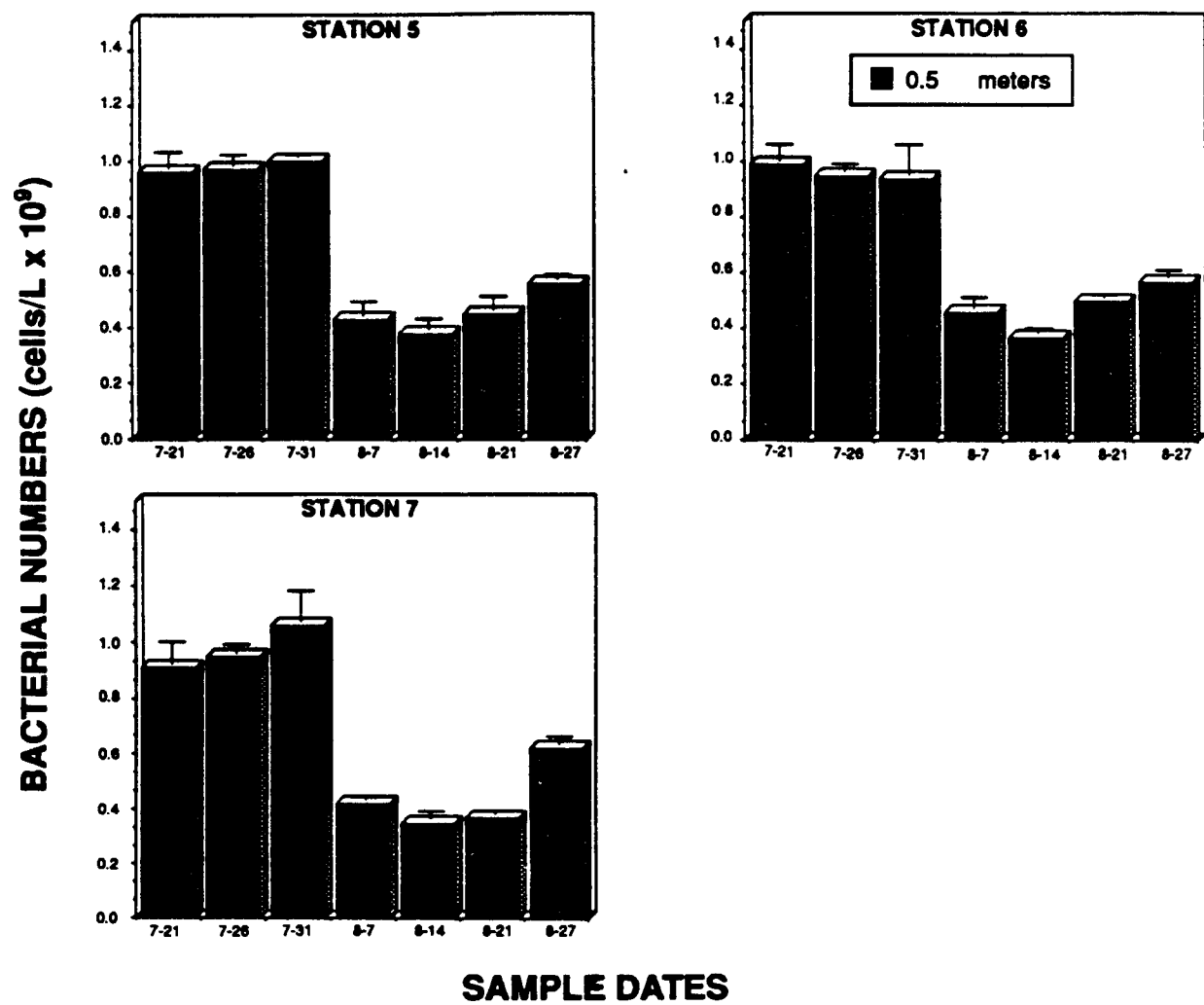
Figure 7.68. (Continued)
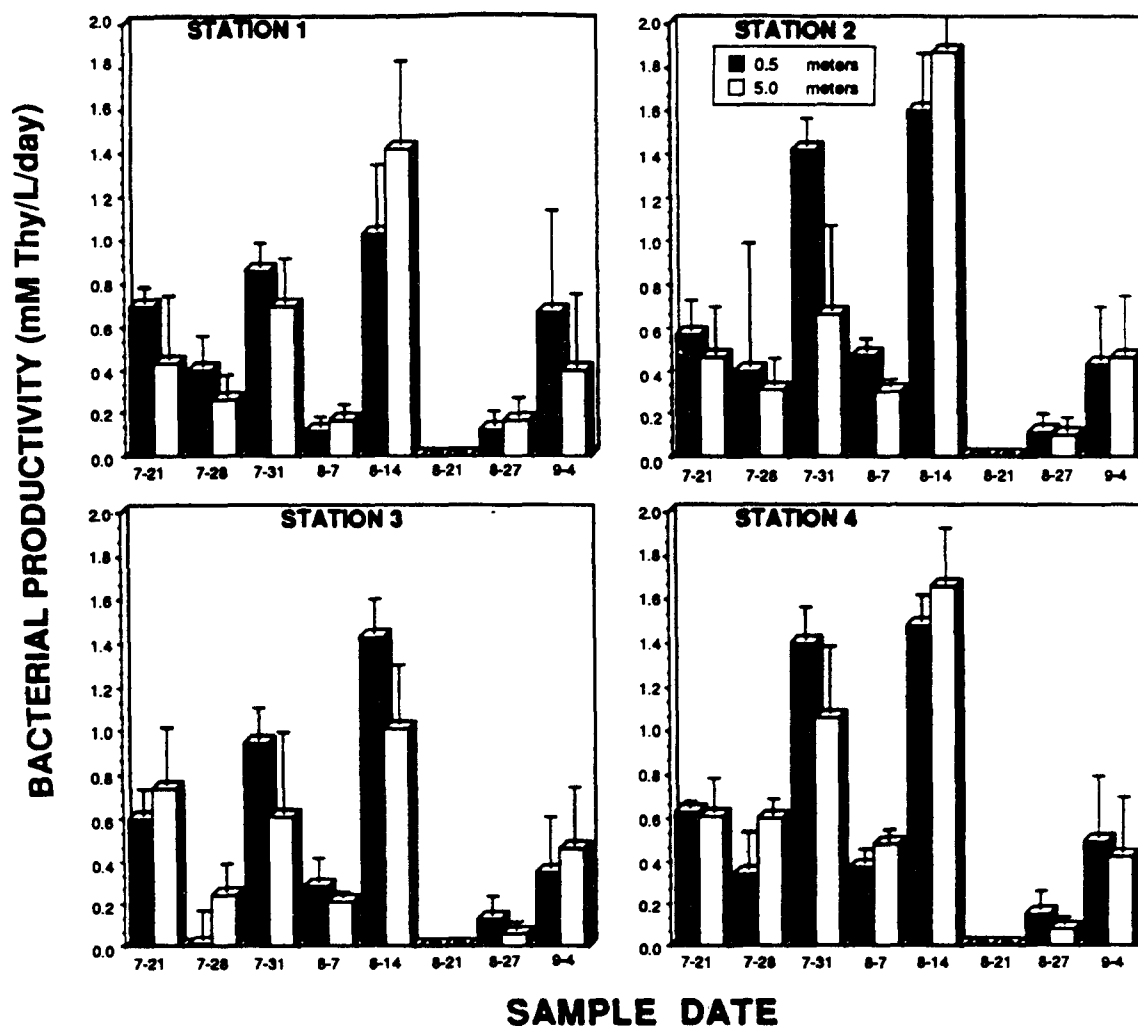
Figure 7.69. Bacterial Productivity Measurements From Tritiated Thymidine Uptake (mM Thymidine/L/day) in Water Samples Collected at Various Sites in Passage Cove Following Nutrient Application to Shorelines on July 25, 1989. Values are Means (+ SD) of 4 Replicates; Dark Bars for Samples Collected at 0.5 m, Open Bars for Samples Collected at 5 m. Refer to Figure 3.3 for Sample Locations.

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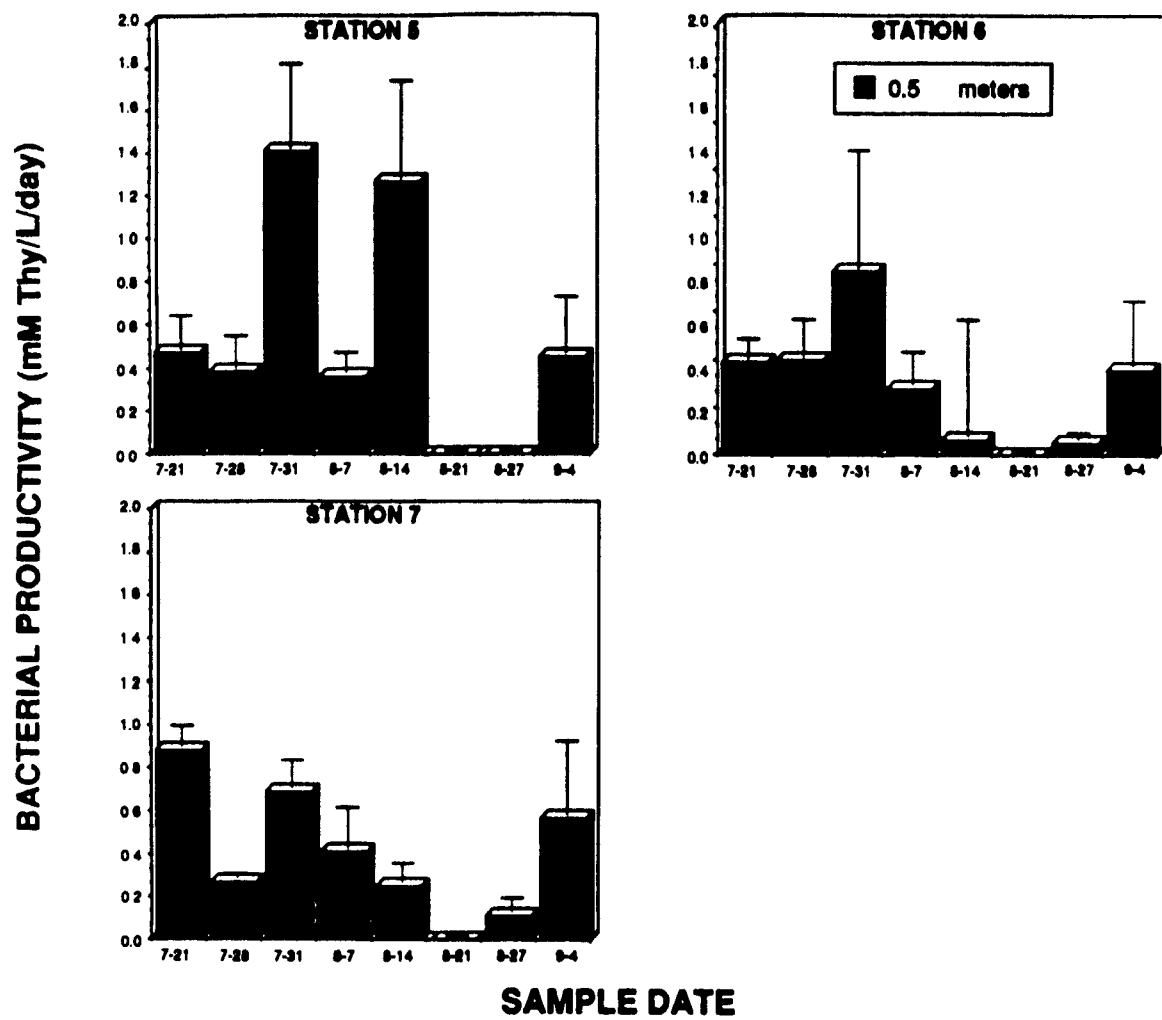
Figure 7.69. (Continued)

development and validation at the beginning of the test, and enough for quality assurance analysis for the duration of the test.

TABLE 7.11. TOTAL PAH'S (μG/G) IN CAGED MUSSELS AT PASSAGE COVE
AT 5 STATIONS OVER TIME

Date	10	30	Station 50	60	70	# Replicates Analyzed
8/5	ND	ND	ND	ND	ND	4
8/8	0.27, 3 ND	ND	ND	ND	ND	4
8/14	0.23, 3 ND	0.25, 3 ND	ND	ND	No sample	4
8/21	ND	ND	ND	ND	No sample	4
8/27	ND	ND	ND	ND	No sample	4
9/3	ND	ND	ND	ND	No sample	4

Detection limit = 0.20 μg/g (wet wt.)
ND = None detected
All values are reported as wet weight

Field Toxicity Tests

A risk assessment indicates that application of INIPOL poses a potential toxic risk to marine biota if water concentrations approach 15 mg/L, the LC50 for the most sensitive species tested in laboratory toxicity tests. To characterize the extent to which toxic concentrations might develop during or immediately after application to oiled shorelines, a series of toxicity tests were conducted with field water samples using a testing scheme similar to that used to test acute toxicity of industrial effluents. These data provide some insight into the rate at which INIPOL enters the marine environment and the dilution required to mitigate toxic effects.

Water samples collected at specified intervals before and after INIPOL application were sent to a consulting laboratory for 48-hour toxicity tests with oyster larvae, *Crassostrea gigas*. Endpoints

monitored for these tests were larval survival to test termination, and percentage of larvae that exhibited abnormal development. The results for these tests are tabulated in Table 7.12.

TABLE 7.12. 48-HOUR SURVIVAL AND DEVELOPMENT OF LARVAE TESTED WITH 100% SITE WATER (UNDILUTED EXCEPT FOR SALINITY ADJUSTMENT)

Sample Designation	Survival	Abnormal	Tidal Stage
Lab Seawater Control	92%	9.5%	
Hypersaline Control (28 ppt)	75%	7.8%	
Field Control (10 a.m.)	70%	8.4%	2-hour pre-low
Pre-Application (Time 0, 10 a.m.)	74%	10.4%	2-hour pre-low
Application 10 a.m. - 2 p.m.			
1-hour Post Application (3 p.m.)	62%	14.2%	3-hour post-low
3-hour Post Application (5 p.m.)	87%	16.1%	near high tide
6-hour Post Application (8 p.m.)	77%	10.5%	mid-tide, outgoing
12-hour Post Application (2 a.m.)	58%	10.1%	mid-tide, incoming
18-hour Post Application (8 a.m.)	39%	31.4%	mid-tide, outgoing

Test acceptability criteria dictate that for each series tested, control survival must be greater than 70% with abnormality equal to or less than 10%. All laboratory control, field control, and pre-application samples met these criteria. The greater survival of larvae in laboratory seawater controls relative to hypersaline controls, field controls, and pre-application samples may be related to minor toxic components in field samples or the brine solution. These differences were not statistically significant when compared by Dunnett's procedure. The percentage of abnormal larvae varied little among the four control samples.

Tests with water samples collected at the field site after INIPOL application resulted in survival of less than 70% and rates of abnormal development greater than 10%, suggesting the presence of toxic components. Because the survival of larvae was greater than 50% for all water samples except the 18-hour sample, an LC50 could only be computed for the 18-hour sample. Toxicity associated with the other samples was assessed through the use of Dunnett's procedure to determine if observed effects were significantly greater than mortality or abnormal development rates for the field control and pre-application samples, which are the proper samples for comparisons with test site treatments.

None of the values for post-application samples, except the 18-hour test samples, were significantly different from the field control survival (70%) and pre-application survival (74%). In addition, none of the percentages of abnormally developed larvae for these samples were significantly different from those of the field control sample (8.4%) and pre-application sample (10.4%). Comparison with laboratory seawater controls showed significant effects for several samples, but these comparisons combine INIPOL toxicity with residual toxicity in site water at Passage Cove before fertilizer additions.

The water sample taken at 18-hour post treatment killed 61% of the oyster larvae during the toxicity test. Using the dilution series of 100% site water, 56%, 32%, 18% and 10%, a 48-hour LC50 of 58% of full-strength water was calculated. In other words, when full-strength site water was diluted to 58% of its original concentration, it would kill 50% of the oyster larvae during a 48-hour test. The 95% confidence interval for the LC50 is 46% to 75%. The full-strength site water collected at 18 hours had significantly greater numbers of abnormal larvae compared to site and laboratory controls. Dilution to 56% of the full strength concentration produced abnormal larvae at a rate not significantly different from the field control and pre-application samples.

Toxic effects from potential over-application of INIPOL or immediate release of INIPOL from the shoreline during initial tidal flooding were not seen. Test results indicate that application of INIPOL to oiled shorelines at the Passage Cove test site resulted in water concentrations that caused abnormal development and mortality of oyster larvae only during the sampling that occurred 18 hr after application. The 48-hour LC50 for this sample was 58% of full-strength site water. The increase in abnormal development associated with this sample was mitigated by dilution to 56% of full-strength.

Apparently, more toxicity was associated with the second flooding of the INIPOL-treated shoreline than with the initial flooding. This is unexpected, and may be related to a different degree of wave action or to additional floating oil that moved into the test area. However, no unusual weather or oil movements were observed following the INIPOL application. In the absence of INIPOL additions, test site water produced survival and abnormality rates that were marginally above acceptance criteria. This may demonstrate residual toxicity problems that exist along oiled shorelines unless definitive clean-up actions are taken.

If we attribute all the observed toxicity to release of INIPOL from the treated shoreline upon re-flooding by incoming tides, then the release rate can be estimated.

a) Using the application rate of 293 g INIPOL/m^2, a concentration of 4,500 mg/L would be expected if we assume 100% of the applied INIPOL is released into water immediately over the treated beach, with minimal dilution.

b) The LC50 for the most toxic sample, the 18-hour post application sample, was 58% of full-strength, i.e., an exposure resulting in 50% mortality from the field sample.

c) Using 50 mg/L as the LC50 for oyster larvae and INIPOL, any field sample that caused 50% mortality should have 50 mg INIPOL/L. Thus, we can assume that a 58% dilution of 18-hour water would lower concentrations to 50 mg/L.

d) Thus, the initial concentration in the 18-hour sample may have been 90 mg/L INIPOL (dilution to 58% gave 50 mg/L). This concentration is 2% (90 mg/L divided by 4,500 mg/L) of the "no-dilution and 100% release" assumption.

e) This crude estimate of the release rate (2%) is within the expected range for initial releases of INIPOL following application.

SUMMARY AND CONCLUSIONS

The following conclusions can be drawn from the Passage Cove study (beaches previously washed) conducted during the latter part of the summer of 1989:

a) The visual reduction in oil due to application of the oleophilic/granular fertilizer combination was similar to Snug Harbor results. This visual reduction became apparent approximately two to three weeks following application of the fertilizers; the untreated control beach, on the other hand, essentially did not change visually. The effect was perhaps more dramatic in Passage Cove since oil from both the cobble surface and the subsurface mixed sand and gravel visually disappeared in a shorter timeframe. It is possible that when the beaches in Passage Cove were physically washed, oil was distributed over a large surface area, subsequently creating improved conditions for the biological degradation of oil.

b) Application of fertilizer solutions from a sprinkler system also caused oil to visually disappear in approximately the same general timeframe as Snug Harbor results (3 to 4 weeks). This observation provided definitive proof that biodegradation (and not chemical washing was likely responsible for the oil removal, since there was no other reasonable mechanism to explain this effect of nutrient addition to the oil. The application of fertilizer solutions, therefore, proved to be the most efficient system for exposing oil-degrading microorganisms to nutrients in a controlled and reproducible manner.

c) Application of the fertilizer solution produced a statistically significant enhancement of oil biodegradation relative to the untreated control beach. Rates of total oil residue loss were greater than four-fold faster than rates of removal on the untreated control beach. The loss of oil residues was accompanied by extensive changes in oil composition. This included large decreases in the nC18/phytane ratio. Thus, enhanced biodegradation was probably responsible for changes in oil residue and composition.

d) Results from the fertilizer solution treatment further support that oil biodegradation in Prince William Sound was limited by the availability of nutrients and not by the availability of the oil itself. In addition, reapplication of nutrients (the extreme in the case of the fertilizer solution) is probably important for sustaining enhanced biodegradation.

e) Application of the INIPOL/CUSTOMBLEN combination also substantially enhanced oil biodegradation. At a slightly lower degree of statistical confidence (90% instead of 95%), the INIPOL/CUSTOMBLEN fertilizer produced a significant two to three-fold enhancement in the removal of total oil residues relative to the untreated control beach. This was accompanied by an extensive change in the composition of the oil as well.

f) Mechanistically, there is no evidence to suggest that the application of INIPOL/CUSTOMBLEN worked differently than the application of the fertilizer solution; each process provided enough nutrients to the oil-degrading microbial populations to enhance biodegradation. Results from changes in oil composition during the initial two weeks following fertilizer application suggest that the oleophilic fertilizer uniquely caused simultaneous degradation of the higher and lower molecular weight hydrocarbons. Results from the untreated control beach and the fertilizer solution-treated beach showed that during the same time period a more typical response was observed; that is, the lower

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molecular weight hydrocarbons degraded faster than the higher molecular weight hydrocarbons. It is also believed that the eventual greater response from the fertilizer solution application was due to higher nutrient concentrations sustained over a longer period. Reapplication of the INIPOL/ CUSTOMBLEN fertilizer combination every three to four weeks might produce the same effect observed with the fertilizer solution application.

g) Although it is difficult to prove at this time, we believe that the physical washing process provided conditions that greatly enhanced the effectiveness of oil bioremediation. In addition, we believe that after a certain amount of biodegradation, the physical consistency of the oil changes, making it less likely to adhere to surfaces. As a result, the turbulence created by tidal action is sufficient to remove the degraded oil residues from the beach material, thus producing visually cleaner beaches.

h) Further monitoring of the fertilizer-treated beaches through early summer 1990, revealed that even subsurface oil (to a depth of approximately 0.3m) was virtually completely removed within approximately 10 months. However, significant, but patchy amounts of oil remained on the untreated control beach after this time period. This suggests that bioremediation greatly reduced beach cleanup time.

i) Due to high variability in the numbers of oil-degrading bacteria in each sample, it was not possible to show statistically significant increases in the oil-degrading microbial populations as a result of the fertilizer addition.

j) No widespread or persistent adverse ecological effects were observed from the monitoring program that was designed to measure toxic responses, eutrophication, and bioaccumulation of oil residues. Ammonia, the only component in the oleophilic fertilizer that was potentially toxic to indigenous species, never reached toxic concentrations outside the immediate zone of application (as inferred from the toxicity test results). Measurements of chlorophyll, primary productivity, and bacterial production indicated eutrophication did not occur. The absence of oil residues in caged mussels, held just offshore of the fertilizer-treated areas, supported the tenet that oil was not released from the beaches into the water column as a result of the fertilizer treatment.

SECTION 8
DISK ISLAND FIELD RESULTS

NUTRIENT RELEASE TEST

To assure that the application of fertilizer granules would release nutrients into a defined plot size, a short test was performed on an uncontaminated cobble beach. Three plots of different sizes were established and subsurface sampling wells were placed in the middle of each of four quadrants within the plots. Thus, distance between wells was scaled to the plot size. In addition, on the first incoming tide interstitial water was also sampled with root feeders, the same method used to monitor nutrients in beach material during the summer of 1989. This was done to compare the sampling efficiency of this method with the well method. Fertilizer granules were applied to each plot at a rate of 100 g/m^2. The results showed significant nutrient release occurred on each sampling day, but higher concentrations of ammonia and phosphate were present in the interstitial water during the outgoing tide than the incoming tide (Figures 8.1 to 8.14). Thus, it would appear that some "soaking" of the granules was required to obtain the greatest nutrient release.

Other than a few outliers, it appeared that nutrient release was reasonably uniform and not affected by scaling; in other words, nutrients in the wells were generally similar (not statistically different) on all plot sizes. This means that all sampling baskets placed within a plot will likely experience the same nutrient exposure. The sporatically high concentrations of ammonia and phosphate in certain wells is not readily explained, but could represent channeling effects in the beach material. However, since the higher levels were not consistent from well to well or day to day, any channeling would have been temporary, such as that created and removed by tidal action.

Compared to the well method, the use of root feeders to sample interstitial water appeared to be equally effective. In fact, in some cases, higher nutrient concentrations were seen in root feeder samples, suggesting that nutrient-containing water was drawn from points distal to the immediate sampling area.

This test showed something of the nutrient release pattern expected from the fertilizer granules: a large, short-lived, pulse within the first 24 to 48 hours after application, followed by further slow release to give nutrient concentrations barely above background levels. Based on studies at Elrington Island (Section 9), this pulse effect may be the primary way in which oil biodegradation rates are enhanced by this method of fertilizer application.

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Figure 8.1. Ammonia Concentrations in Wells in Each Plot on the Incoming Tide for the Scaling Experiment on 6/19/90.

Figure 8.2. Ammonia Concentrations in Wells in Each Plot Using Root Feeders on the Incoming Tide for the Scaling Experiment on 6/19/90.

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Figure 8.3. Ammonia Concentrations in Wells in Each Plot on the Outgoing Tide for the Scaling Experiment on 6/19/90.

Figure 8.4. Ammonia Concentrations in Wells in Each Plot on the Incoming Tide for the Scaling Experiment on 6/20/90.

Figure 8.5. Ammonia Concentrations in Wells in Each Plot on the Outgoing Tide for the Scaling Experiment on 6/20/90.

Figure 8.6. Ammonia Concentrations in Wells in Each Plot on the Incoming Tide for the Scaling Experiment on 6/21/90.

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Figure 8.7. Ammonia Concentrations in Wells in Each Plot on the Outgoing Tide for the Scaling Experiment on 6/21/90.

Figure 8.8. Phosphate Concentrations in Wells in Each Plot on the Incoming Tide for the Scaling Experiment on 6/19/90.

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Figure 8.9. Phosphate Concentrations In Wells In Each Plot Using Root
Feeders on the Incoming Tide for the Scaling Experiment
on 6/19/90.

Figure 8.10. Phosphate Concentrations In Wells In Each Plot on the
Outgoing Tide for the Scaling Experiment on 6/19/90.

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Figure 8.11. Phosphate Concentrations in Wells in Each Plot on the Incoming Tide for the Scaling Experiment on 6/20/90.

Figure 8.12. Phosphate Concentrations in Wells in Each Plot on the Outgoing Tide for the Scaling Experiment on 6/20/90.

Figure 8.13. Phosphate Concentrations In Wells In Each Plot on the
Incoming Tide for the Scaling Experiment on 6/21/90.

Figure 8.14. Phosphate Concentrations In Wells In Each Plot on the
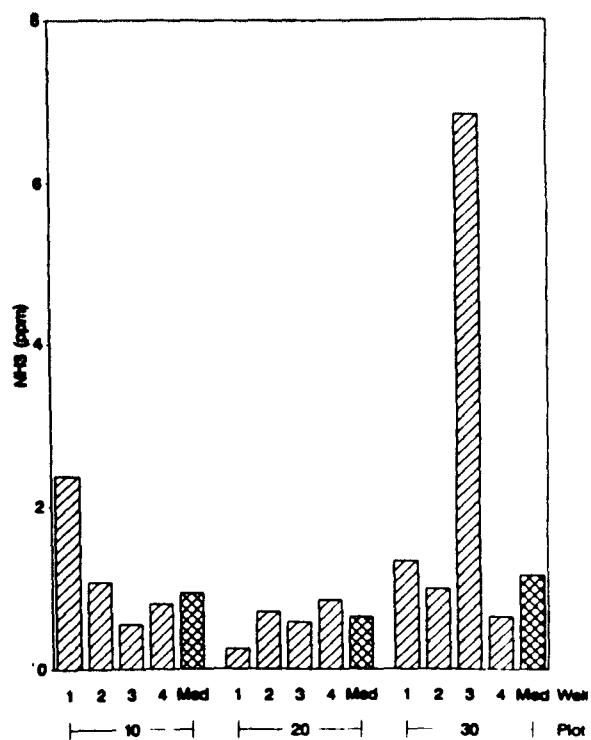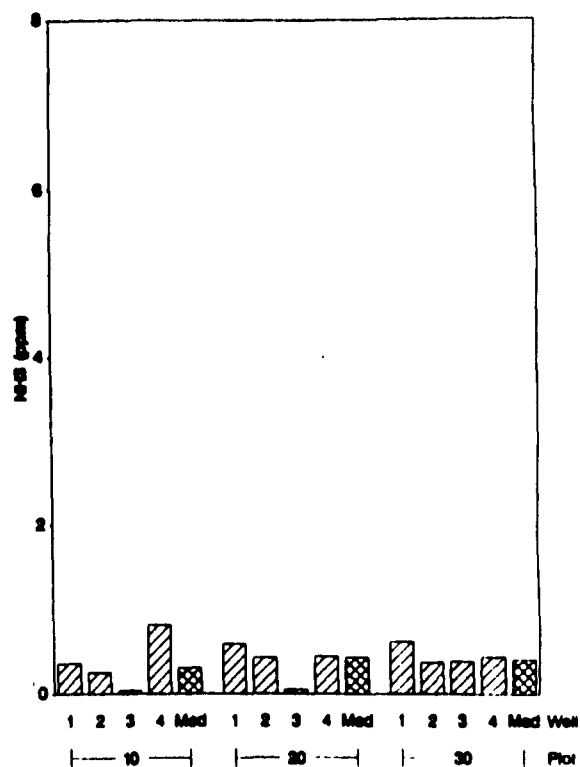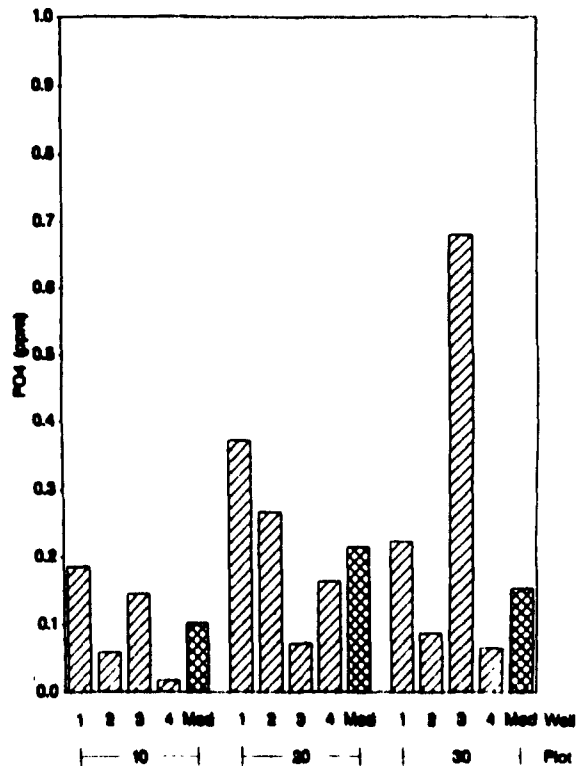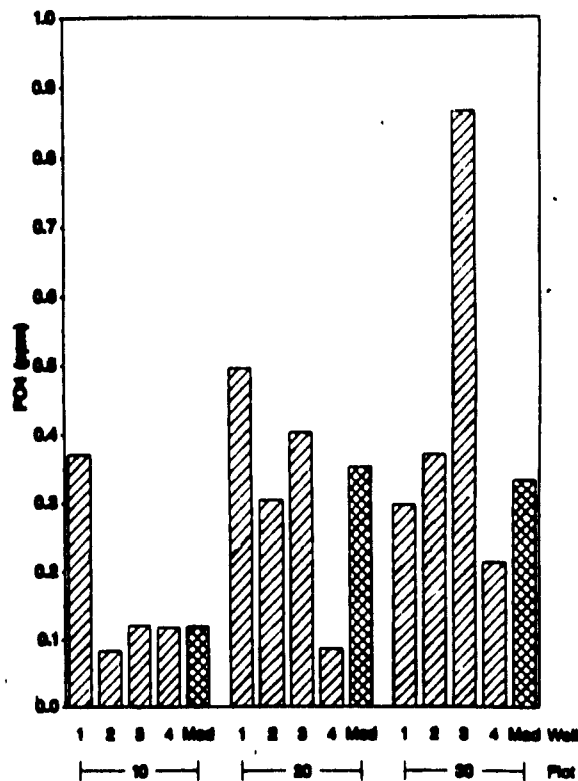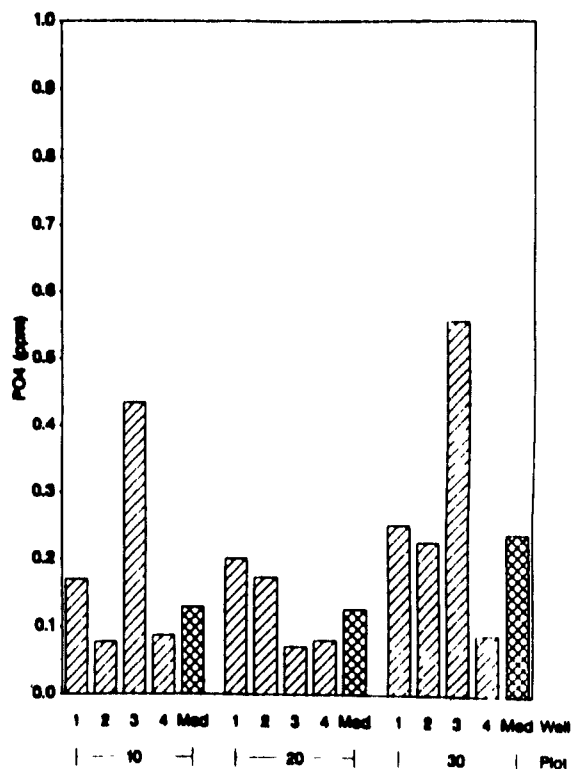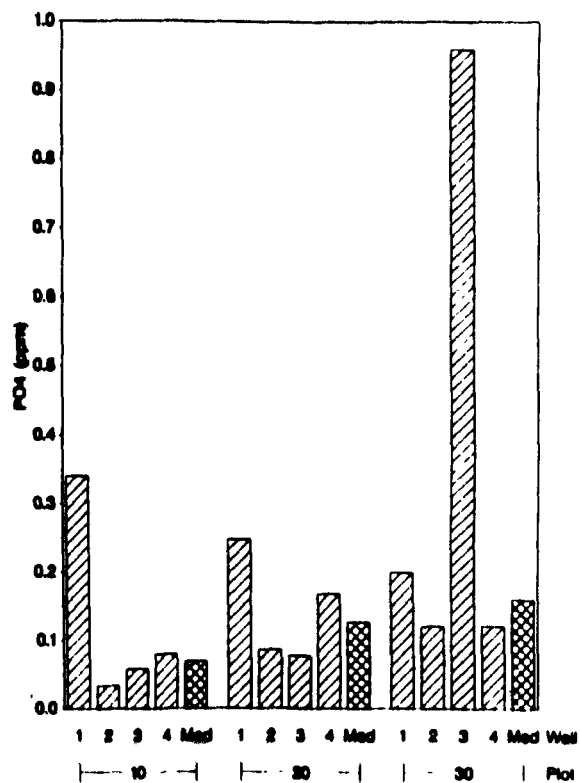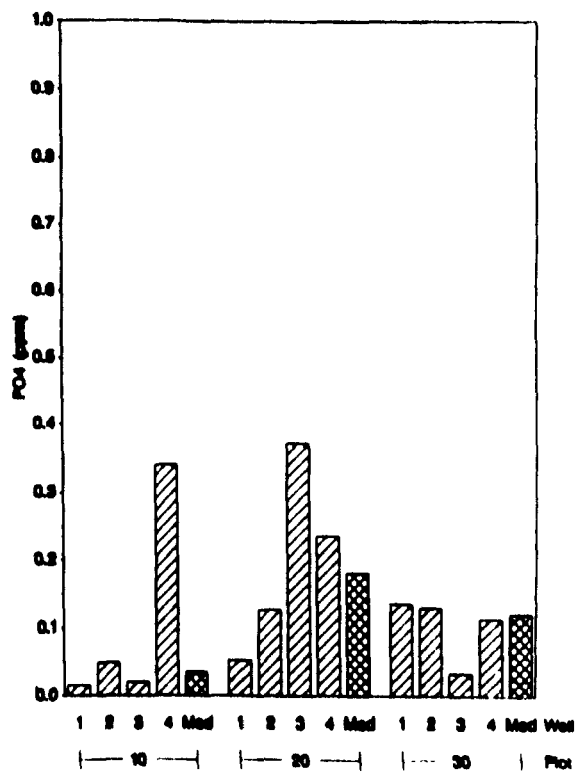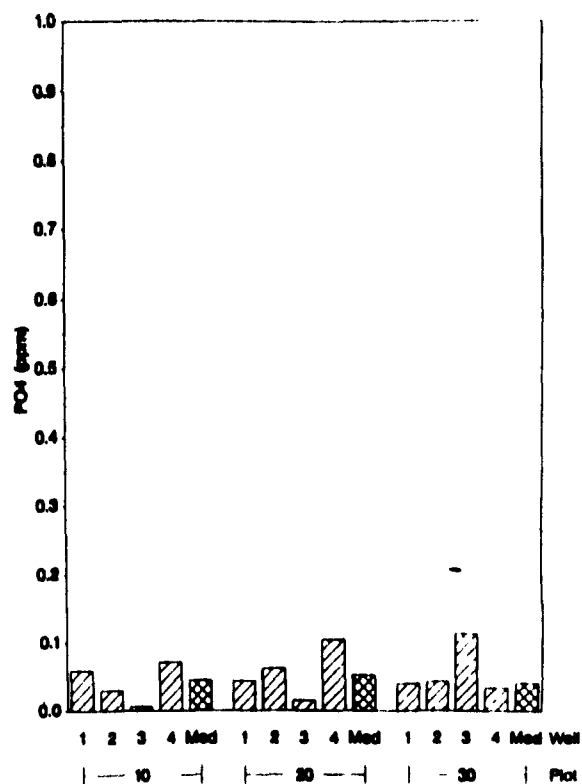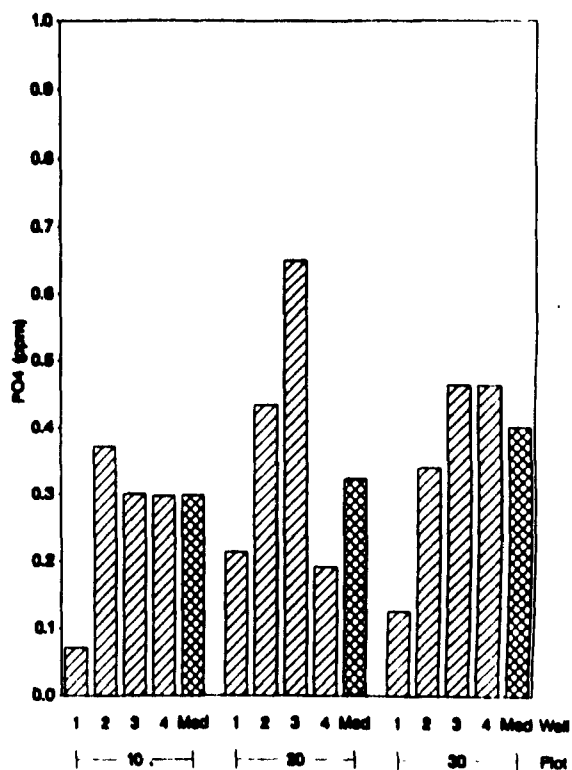Outgoing Tide for the Scaling Experiment on 6/21/90.

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NUTRIENT CONCENTRATIONS

The lack of any fertilizer effect on oil degradation cannot be attributed to a lack of nutrient exposure to the microbial communities. The pattern of nutrient concentrations (Figures 8.15 to 8.19) was very similar to the scaling test; nutrients were readily detected in the beach subsurface, and the application resulted in an initial pulse of nutrients followed by the maintenance of concentrations slightly above background levels over a 21-day period, especially on the beaches receiving the higher fertilizer loadings.

For ammonia release, the incoming tide generally produced higher interstitial water concentrations than the outgoing tide (Figures 8.15 and 8.16). Concentrations above toxic thresholds (approximately 10 ppm NH_4) did not occur. Following the initial release, phosphate concentrations were actually maintained at higher levels than ammonia over the remainder of the test (Figures 8.17 and 8.18). This verifies, rather convincingly, the long-term release characteristics of the fertilizer granules. The much lower concentration of ammonia over the 20-day test period suggests that either it was all released during the initial burst (rough calculations show this is not unreasonable) or that it was rapidly taken up by the oil-degrading microbial community in the beach subsurface (algal uptake is also a possibility).

Although fewer data points were taken for nitrate, nitrate release also appeared to mimic phosphate in terms of the longer-term release characteristics (Figure 8.19). Initial burst concentrations were higher than ammonia. If it is assumed that nitrate is as toxic as ammonia, then the combined nitrate/ammonia concentrations at the highest fertilizer application approached the toxicity threshold of approximately 10 ppm. This does not consider that the exposure was very short. At the next highest fertilizer application (500 g/m^2), total concentrations were more acceptable and, thus, normal application rates of fertilizer granules (100 g/m^2) could be increased five-fold without any ecological effect.

OIL CHEMISTRY

In an attempt to determine the specific activity of fertilizer-enhanced biodegradation, or the extent of rate enhancement per quantity of nutrients released, sampling baskets containing homogenized, oiled beach material were placed in six beach plots and exposed to different concentrations of fertilizer. Baskets were sampled periodically and subsamples analyzed for changes in oil chemistry as a function of four different fertilizer application rates. Two untreated control

Figure 8.15. Change in Ammonia Concentration Over Time for the Incoming Tide for all plots for the Disk Island Fertilizer Application Rate Study.
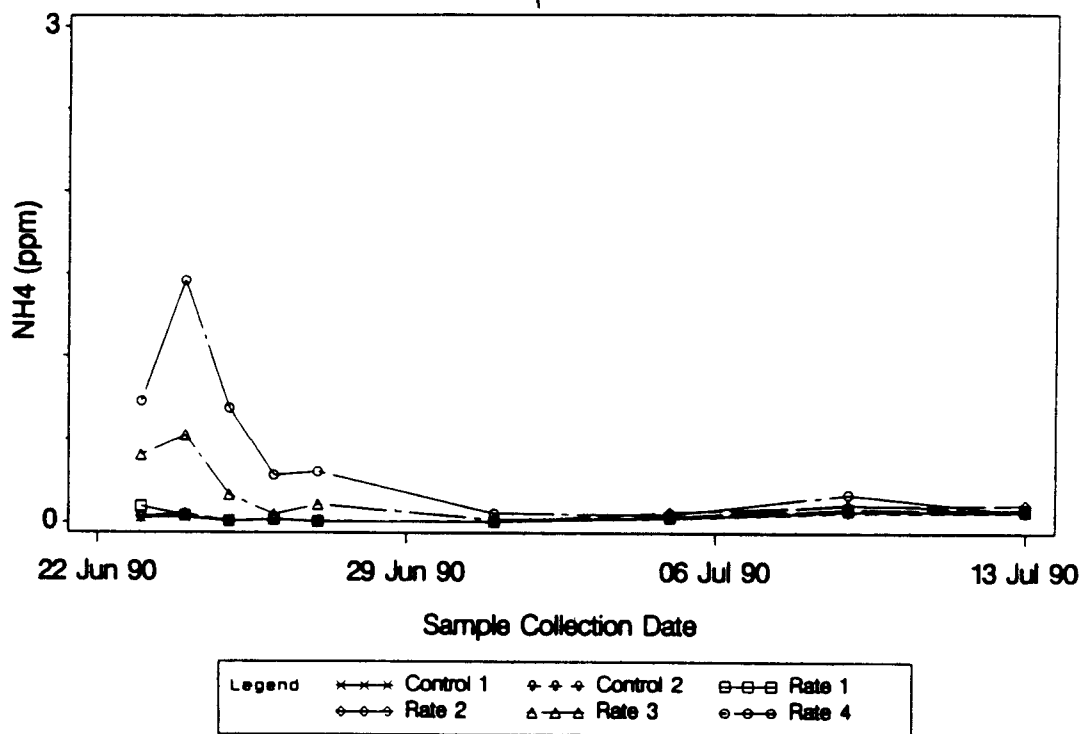
Figure 8.16. Change in Ammonia Concentration Over Time for the Outgoing Tide for all plots for the Disk Island Fertilizer Application Rate Study.

Figure 8.17. Change in Phosphate Concentration Over Time for the Incoming Tide for all plots for the Disk Island Fertilizer Application Rate Study.
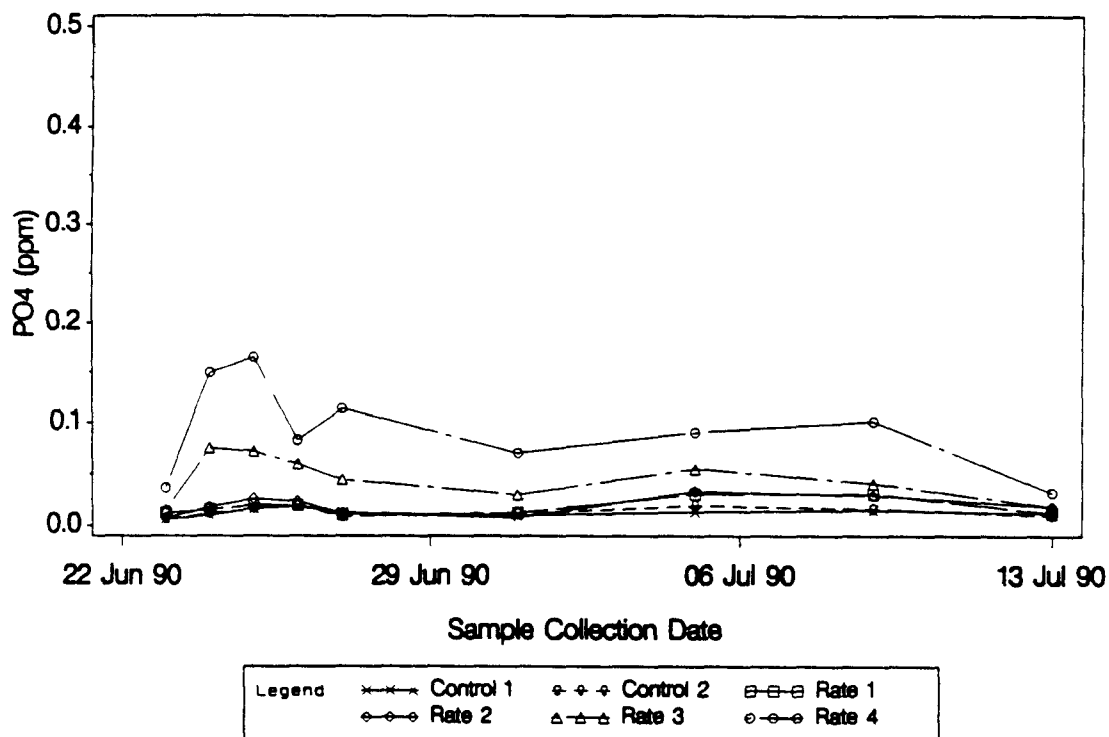
Figure 8.18. Change in Phosphate Concentration Over Time for the Outgoing Tide for all plots for the Disk Island Fertilizer Application Rate Study.
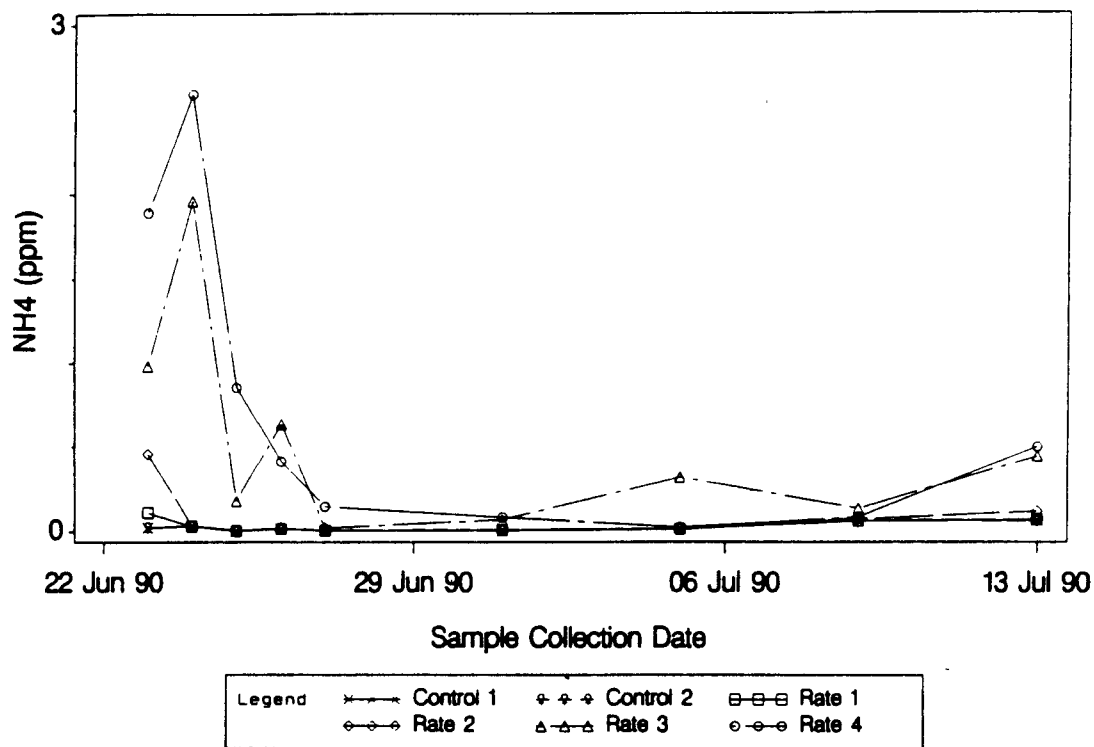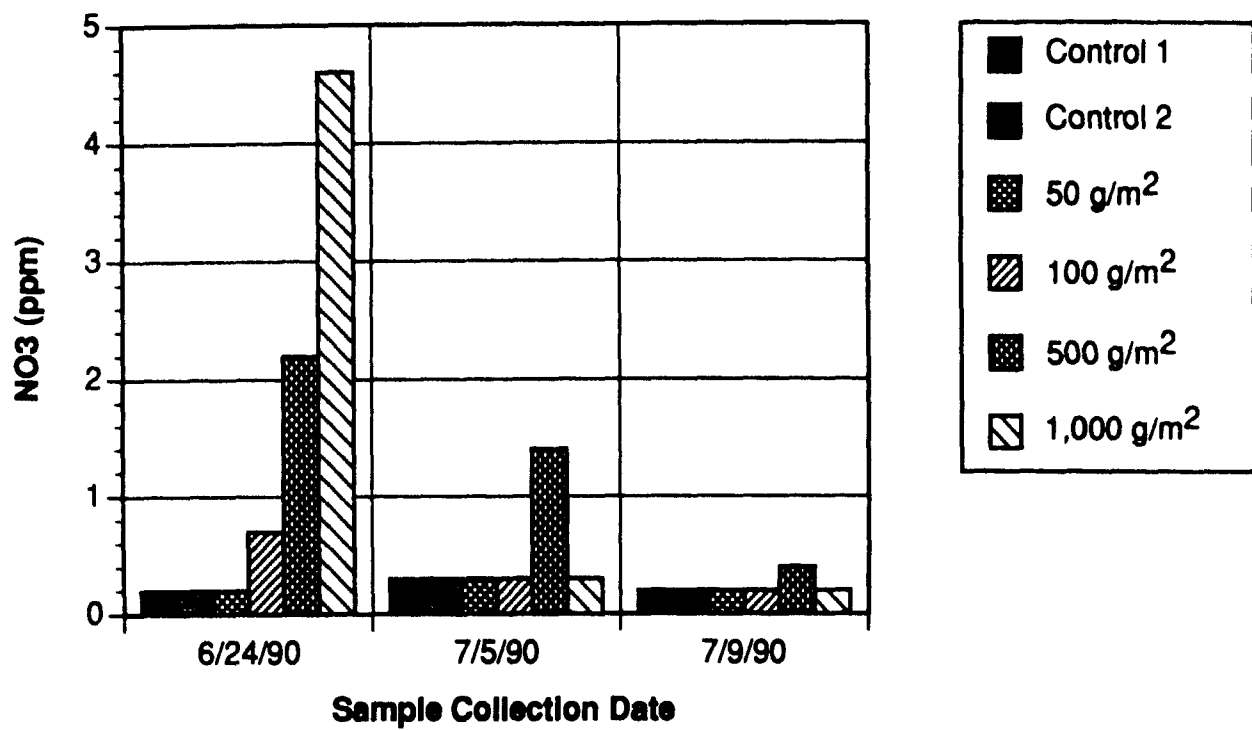
Figure 8.19. Change in Nitrate Concentration Over Time for the Incoming Tide for all Plots for the Disk Island Fertilizer Application Rate Study.
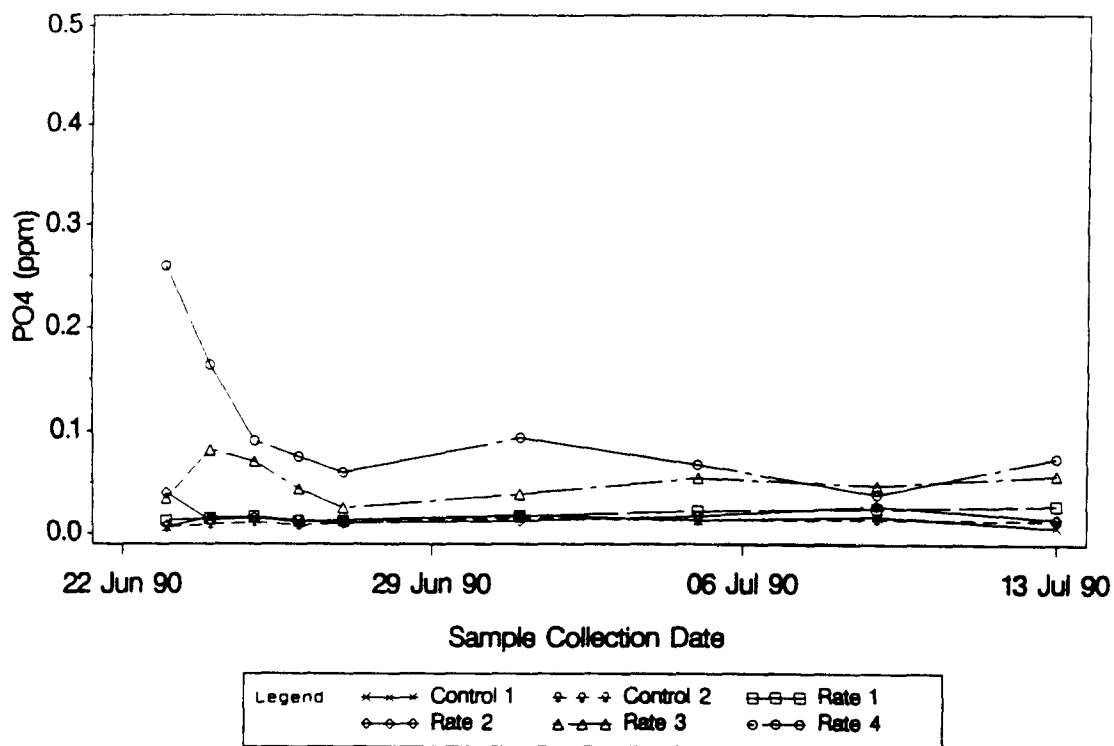
plots within the beach were used to assess background oil degradation rates and any possible beach effects.

Changes in oil residue weights and oil composition (concentration decreases in nC18, nC27, the summed alkanes nC18 to nC27, phytane, and the nC18/phytane ratio) are shown in Figures 8.20 to 8.55. A broad overview of the data shows that unfortunately, the experiment did not work. Losses of oil residue were generally insignificant and compositional changes were not different from the controls.

Trends in the oil residue weight decay over time (Figures 8.20 to 8.25) were confusing and complex. On the two untreated control plots, triplicate baskets removed from the beach on the same date showed relatively small variations in oil residue weight. Variation between sampling dates, however, was quite large, showing a very fluctuating pattern. While a regression line through the median values for untreated control plot number one indicated a slight, but not statistically significant, decrease in residue weights through time, untreated control plot number two showed virtually no decrease.

The most reasonable explanation for these results is that oil physically moved within the entire beach, perhaps as a function of different tidal or weather conditions, thus producing this high variability. Groundwater surges due to heavy rains could have caused oil to move up or down in the beach material. This would have occurred uniformally across each of the untreated control beach plots because the differences between sampling baskets was low. Visual observations of the oil revealed it was considerably more "fluid" than on other beaches and consequently seemed to be removed from the beach material quite easily. Any water accumulating in holes dug on the beach invariably had very pronounced oil slicks. After disturbance of the beach material following insertion of the sampling baskets, a large slick accompanied the incoming tide line as it passed over the disturbed area. These observations contrasted sharply with Elrington Island where slicks were never seen following physical reworking of the beach material. In addition, oil on this beach was drier and thicker in consistency, with more glacial till incorporated. It is not clear what conditions preventing oil on the Disk Island test site (DI-067a) from becoming the more typical consistency of oil on Elrington Island. It may be due to the lower energy of the beach, it's relative shallow slope, or the presence of humic material throughout the beach material.
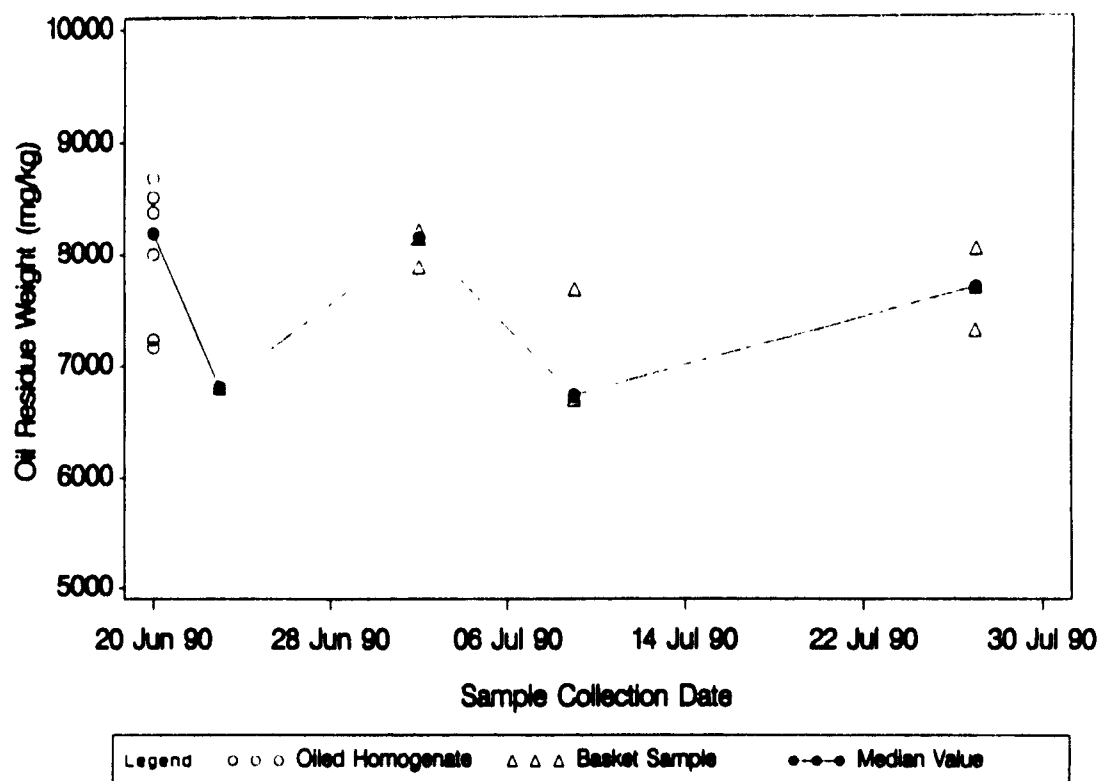
Figure 8.20. Change in Oil Residue Weight Over Time for Untreated Control Plot #1 for the Disk Island Application Rate Study.
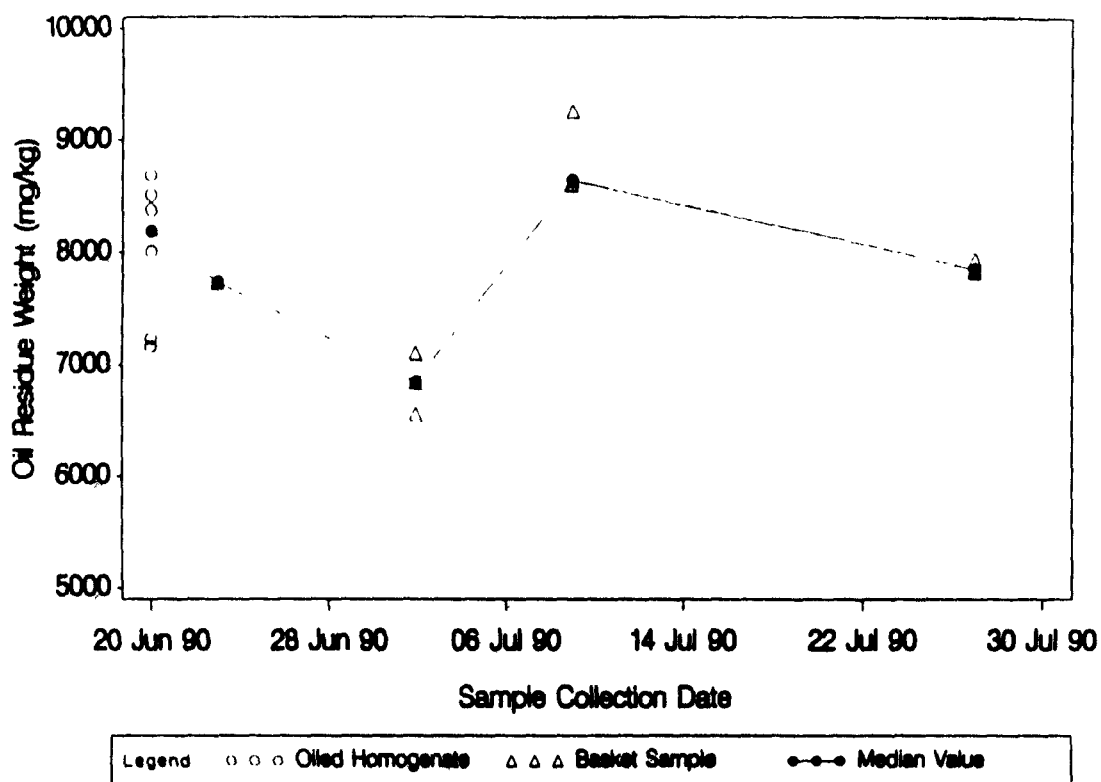
Figure 8.21. Change in Oil Residue Weight Over Time for Untreated Control Plot #2 for the Disk Island Application Rate Study.

Figure 8.22. Change in Oil Residue Weight Over Time for the 50 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.
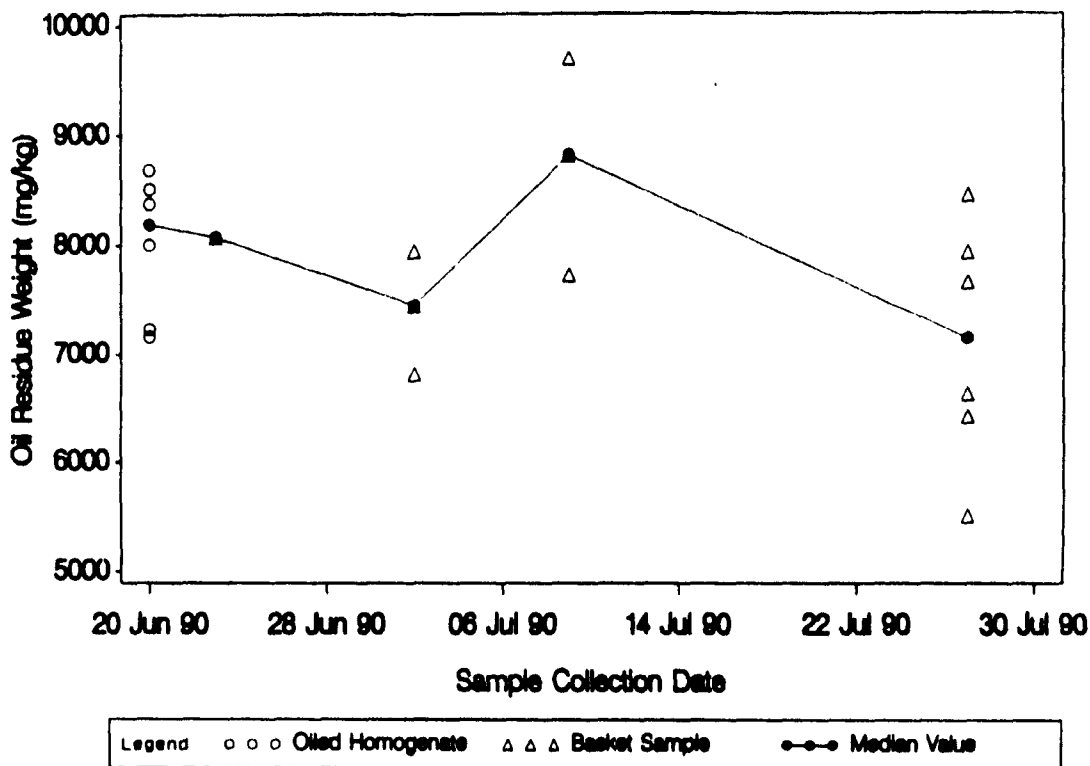
Figure 8.23. Change in Oil Residue Weight Over Time for the 100 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.

Figure 8.24. Change in Oil Residue Weight Over Time for the 500 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.

Figure 8.25. Change in Oil Residue Weight Over Time for the 1,000 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.

Figure 8.26. Change in nC18 Concentration Over Time for Untreated Control Plot #1 for the Disk Island Application Rate Study.

Figure 8.27. Change In nC18 Concentration Over Time for Untreated Control Plot #2 for the Disk Island Application Rate Study.

Figure 8.28. Change in nC18 Concentration Over Time for the 50 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.

Figure 8.29. Change in nC18 Concentration Over Time for the 100 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.

Figure 8.30. Change In nC18 Concentration Over Time for the 500 g/m² Fertilizer Application for the Disk Island Application Rate Study.

Figure 8.31. Change In nC18 Concentration Over Time for the 1,000 g/m² Fertilizer Application for the Disk Island Application Rate Study.
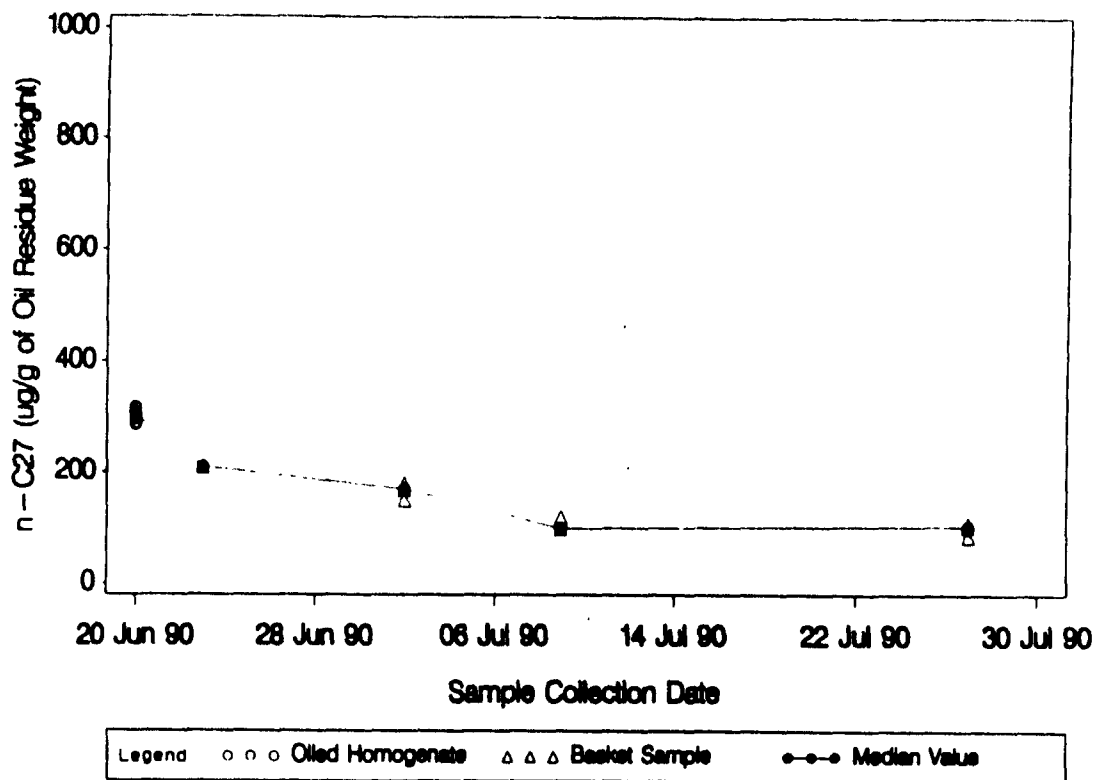
Figure 8.32. Change In nC27 Concentration Over Time for Untreated Control Plot #1 for the Disk Island Application Rate Study.
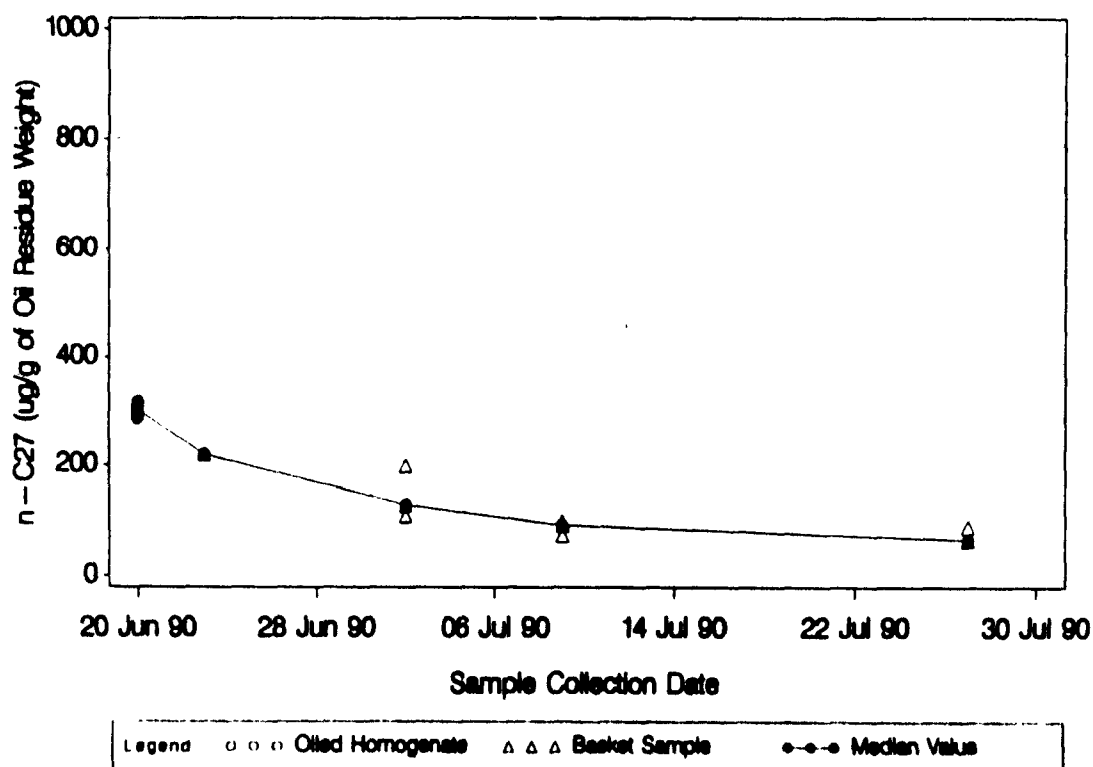
Figure 8.33. Change In nC27 Concentration Over Time for Untreated Control Plot #2 for the Disk Island Application Rate Study.
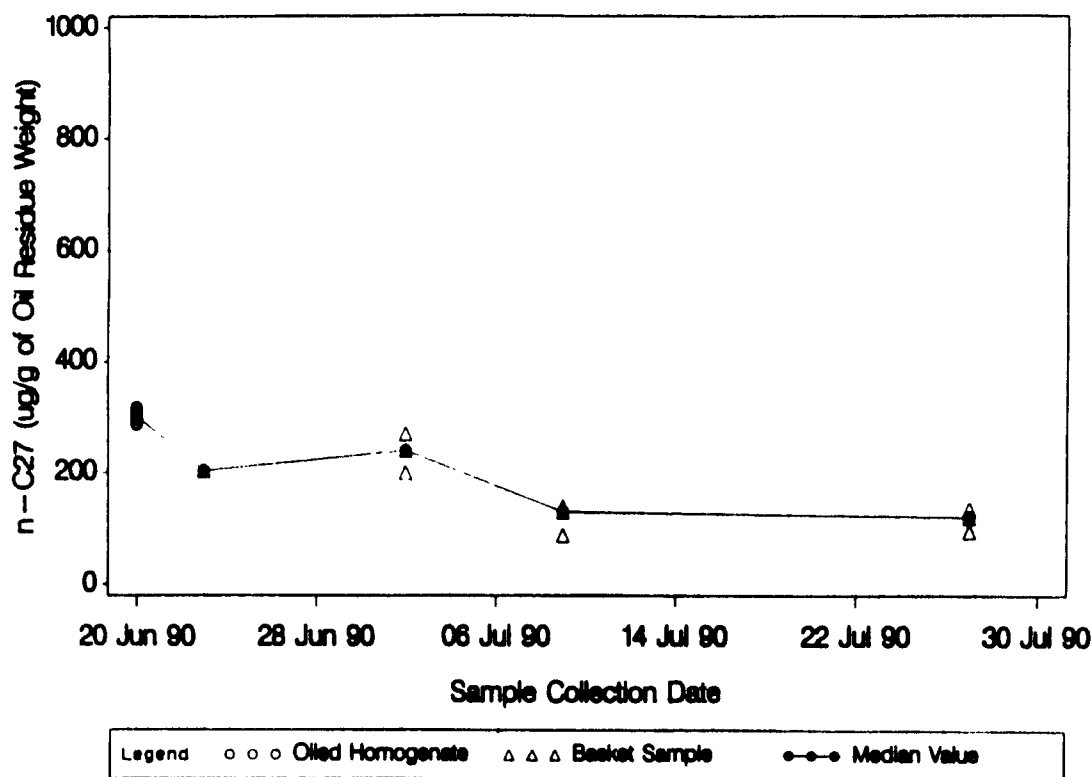
Figure 8.34. Change In nC27 Concentration Over Time for the 50 g/m² Fertilizer Application for the Disk Island Application Rate Study.
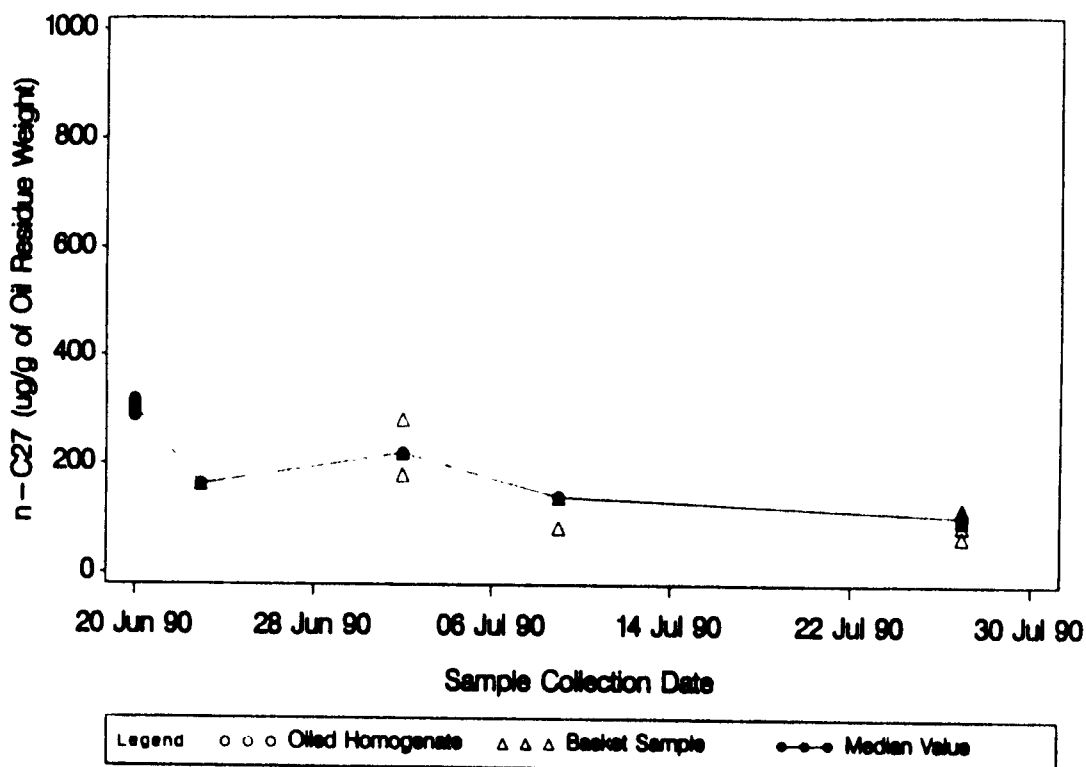
Figure 8.35. Change In nC27 Concentration Over Time for the 100 g/m² Fertilizer Application for the Disk Island Application Rate Study.
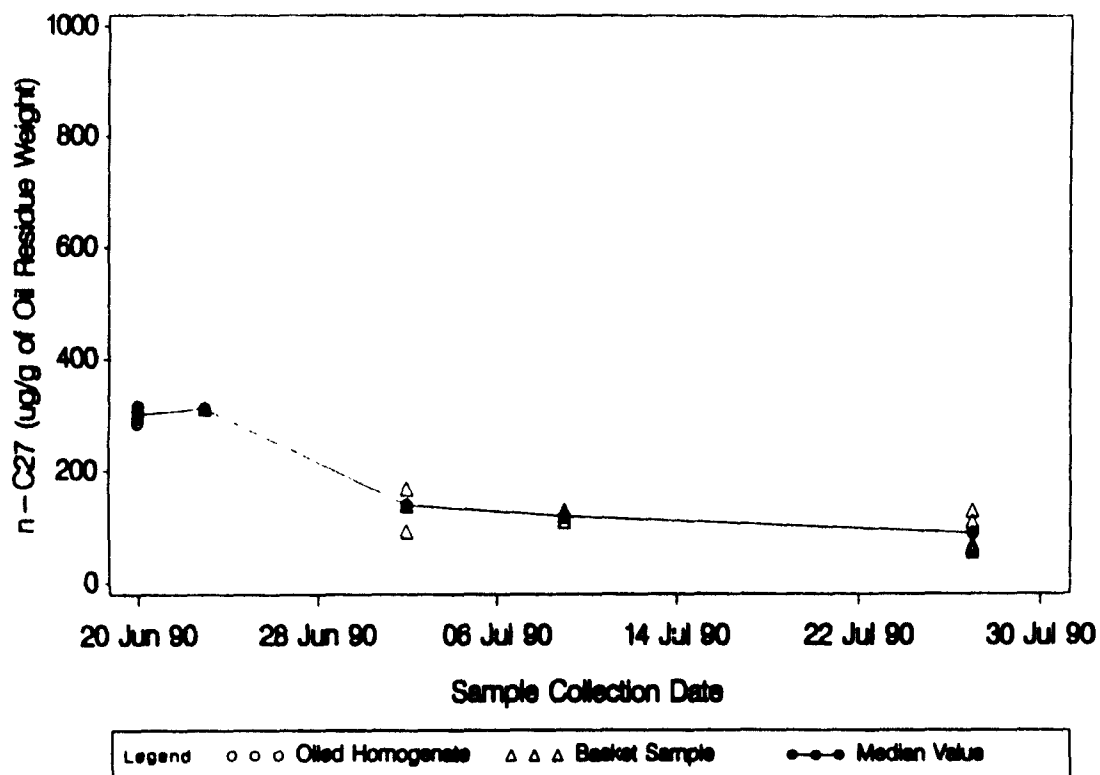
Figure 8.36. Change in nC27 Concentration Over Time for the 500 g/m² Fertilizer Application for the Disk Island Application Rate Study.
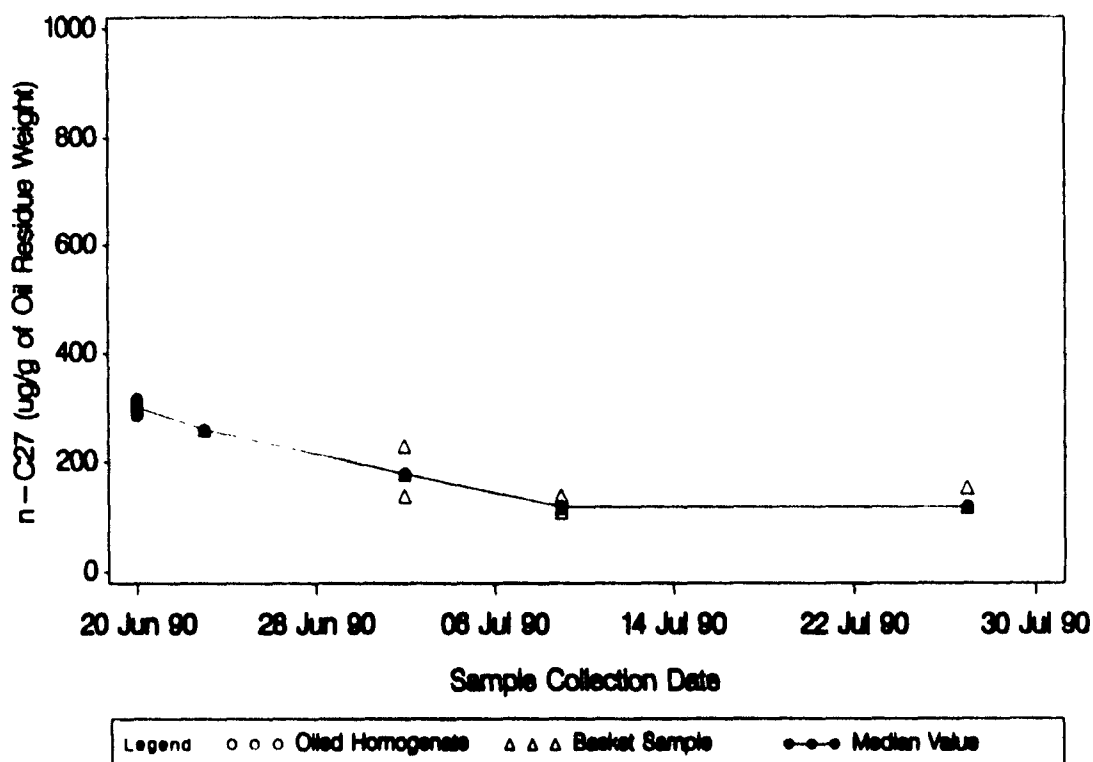
Figure 8.37. Change in nC27 Concentration Over Time for the 1,000 g/m² Fertilizer Application for the Disk Island Application Rate Study.
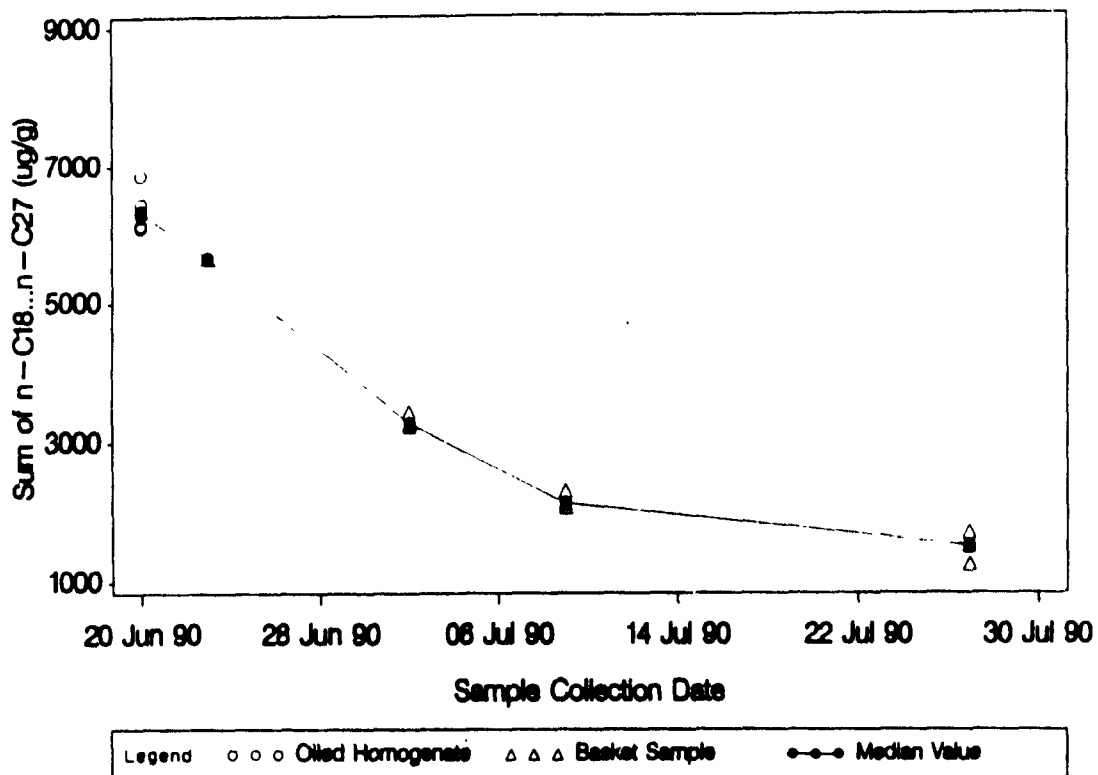
Figure 8.38. Change In the Sum of the Alkane Concentration nC18 to nC27 Over Time for Untreated Control Plot #1 for the Disk Island Application Rate Study.
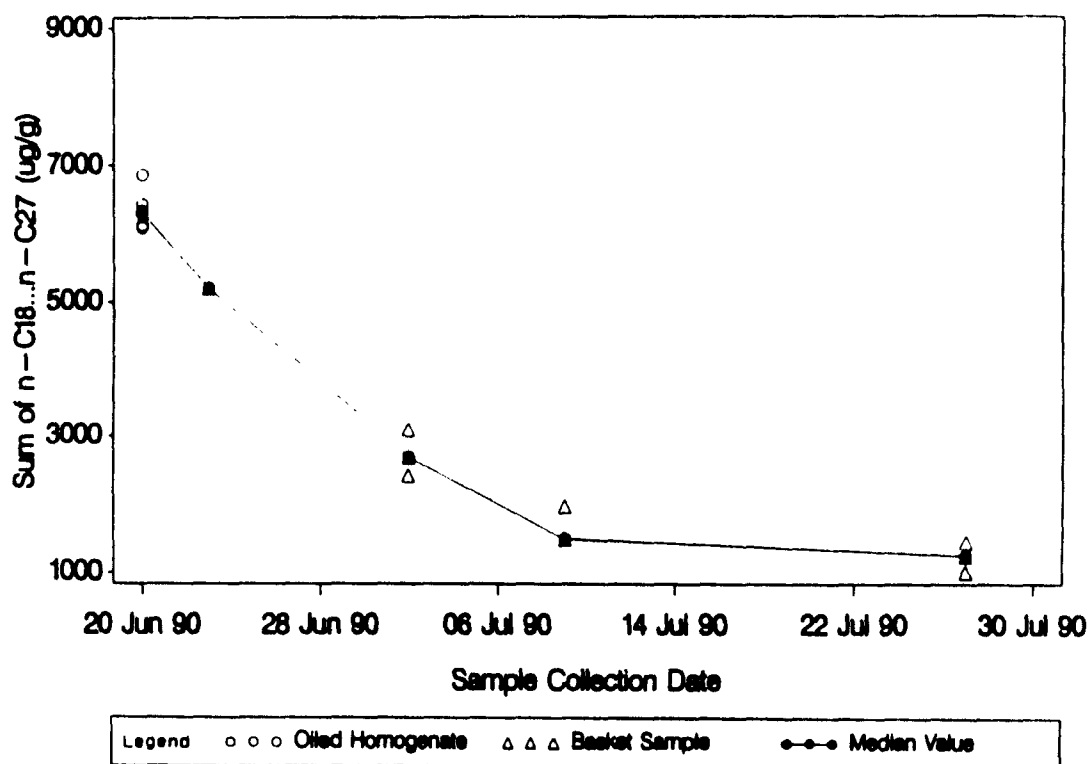
Figure 8.39. Change In the Sum of the Alkane Concentration nC18 to nC27 Over Time for Untreated Control Plot #2 for the Disk Island Application Rate Study.
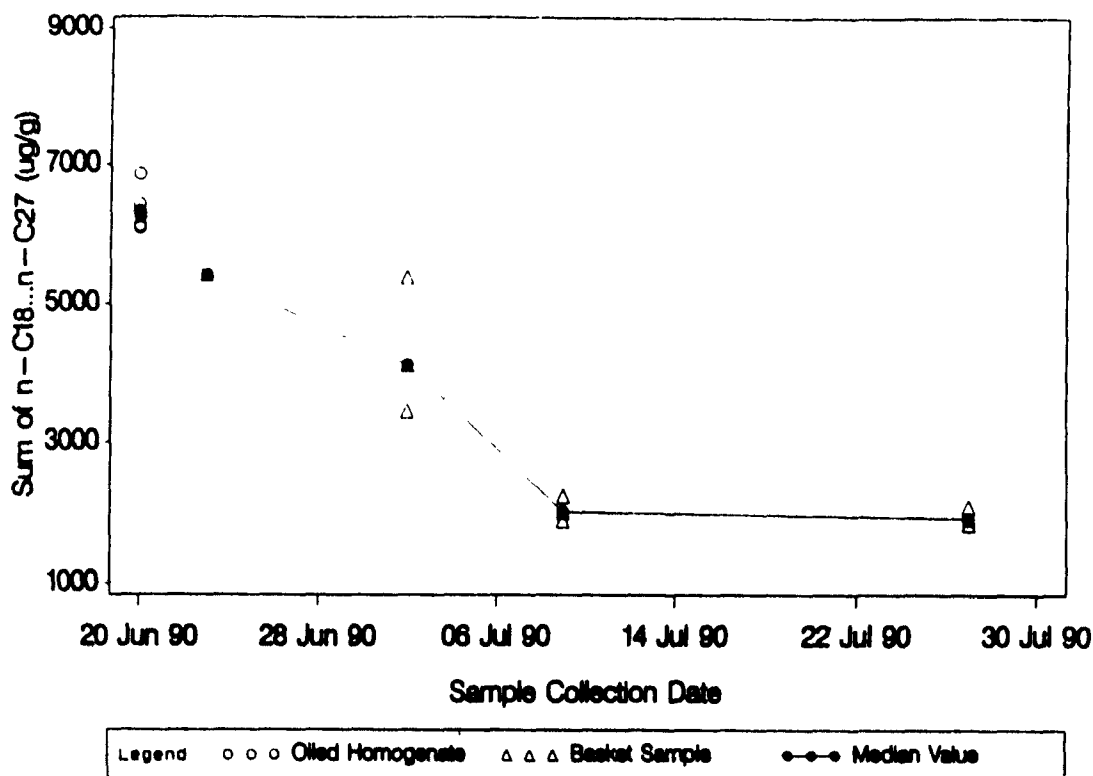
Figure 8.40. Change in the Sum of the Alkane Concentration nC18 to nC27 Over Time for the 50 g/m² Fertilizer Application for the Disk Island Application Rate Study.
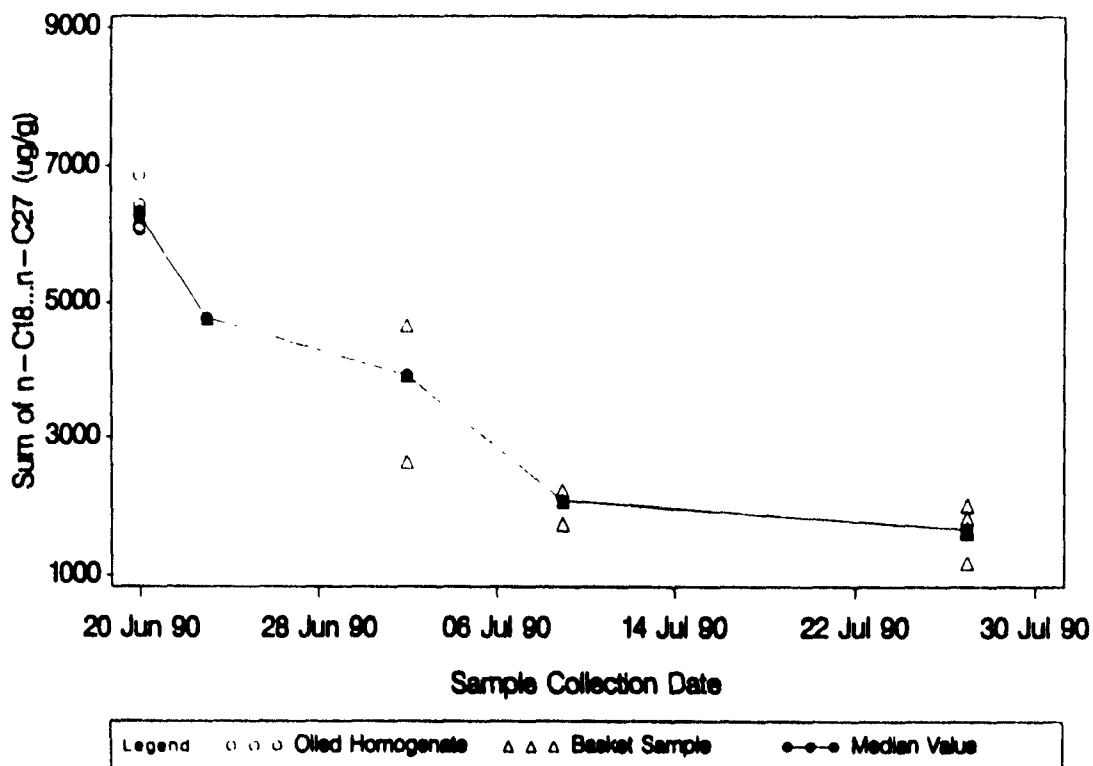
Figure 8.41. Change in the Sum of the Alkane Concentration nC18 to nC27 Over Time for the 100 g/m² Fertilizer Application for the Disk Island Application Rate Study.
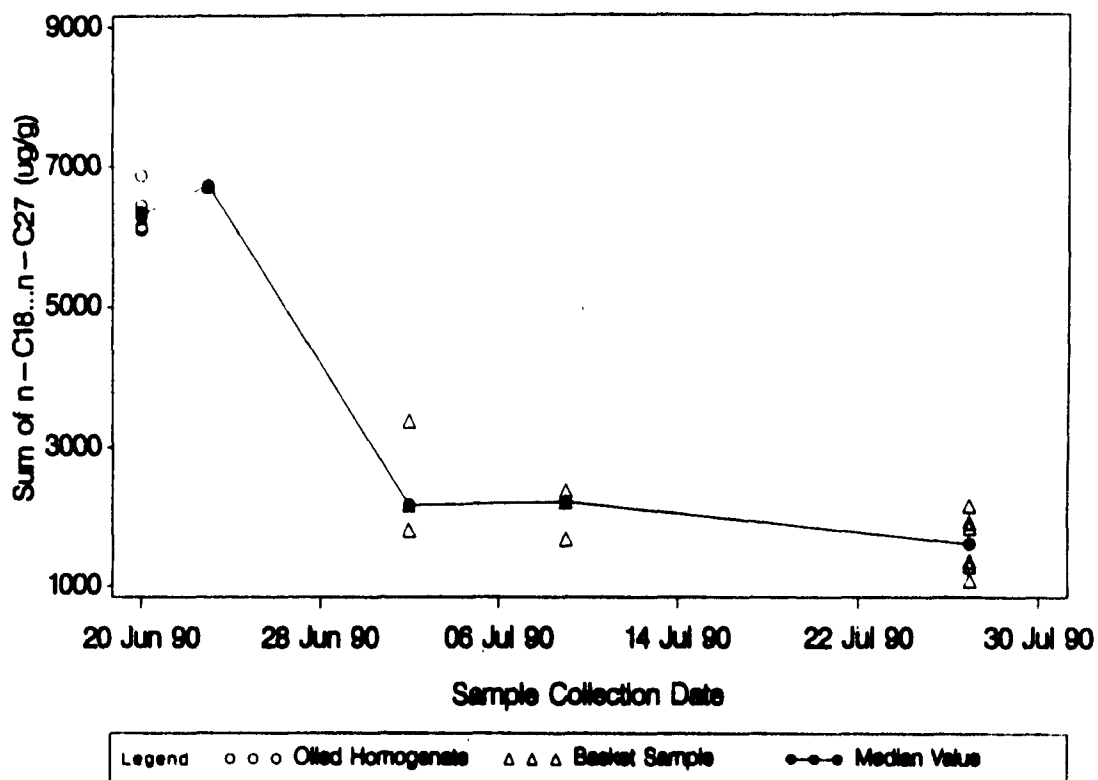
Figure 8.42. Change in the Sum of the Alkane Concentration nC18 to nC27 Over Time for the 500 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.
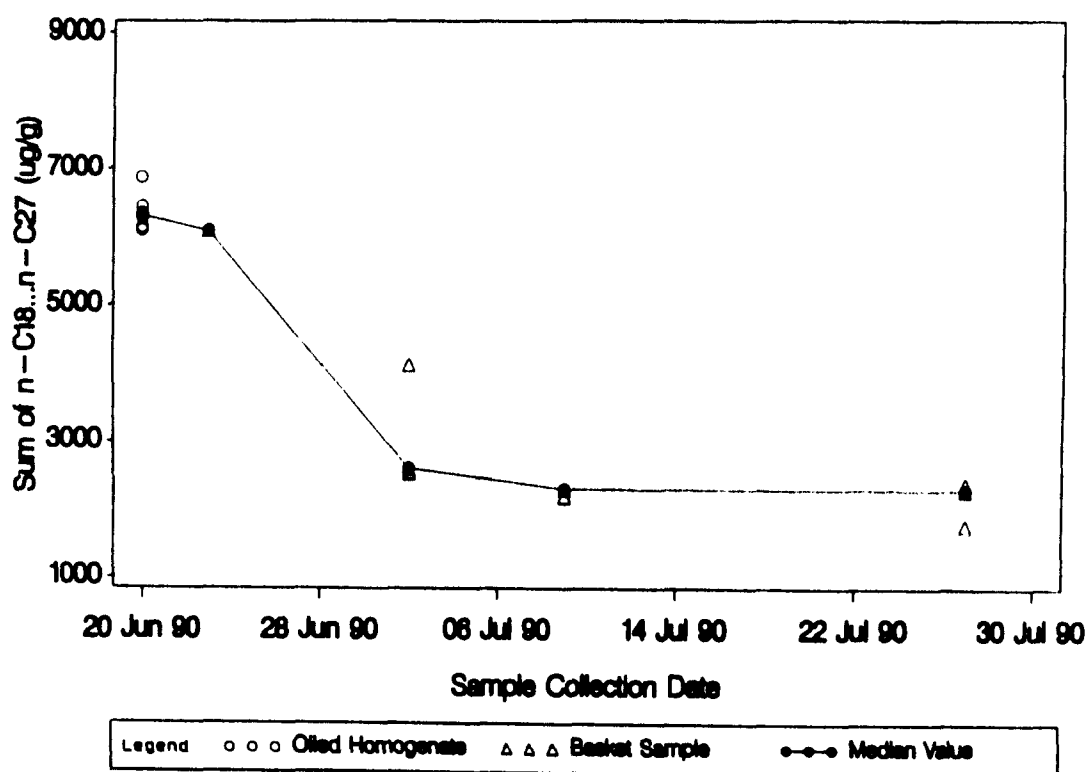
Figure 8.43. Change in the Sum of the Alkane Concentration nC18 to nC27 Over Time for the 1,000 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.
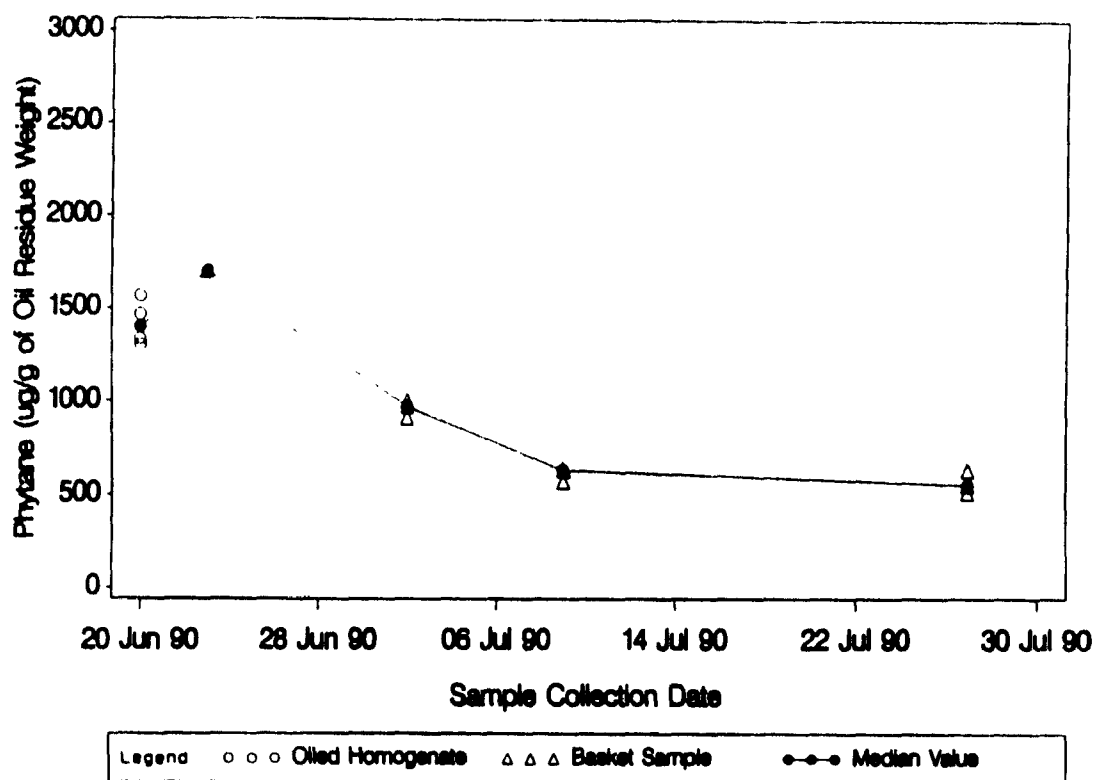
Figure 8.44. Change In Phytane Concentration Over Time for Untreated Control Plot #1 for the Disk Island Application Rate Study.
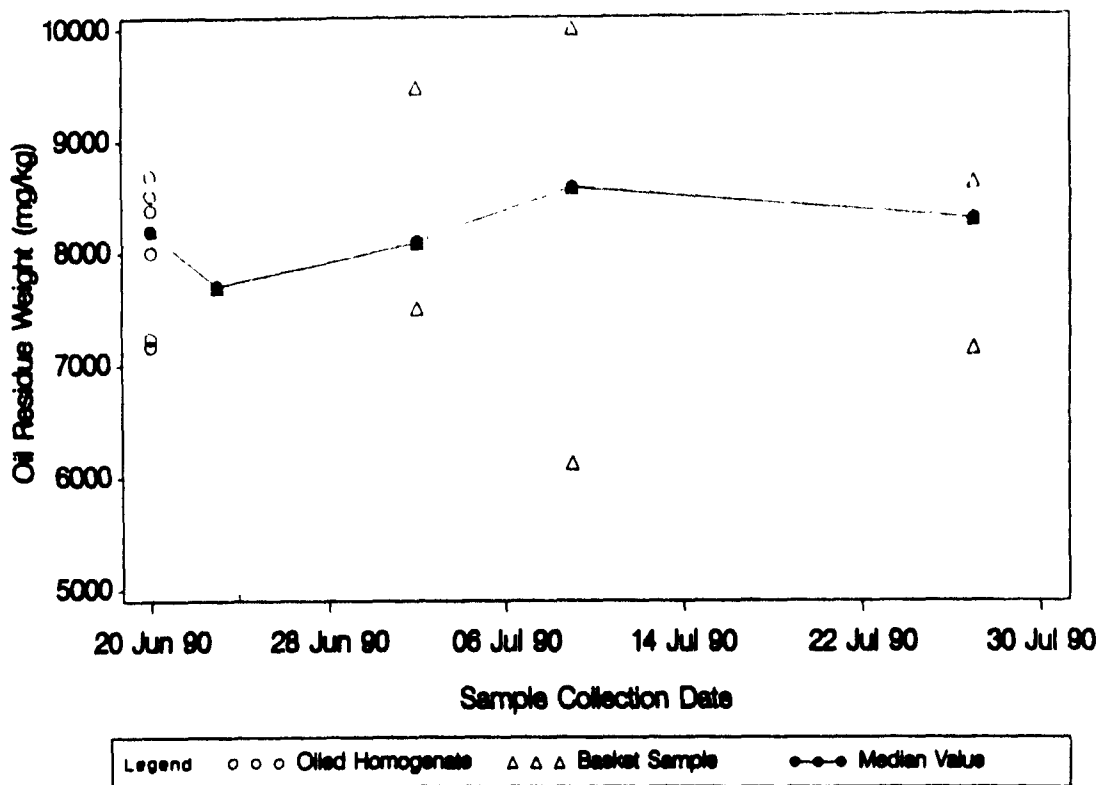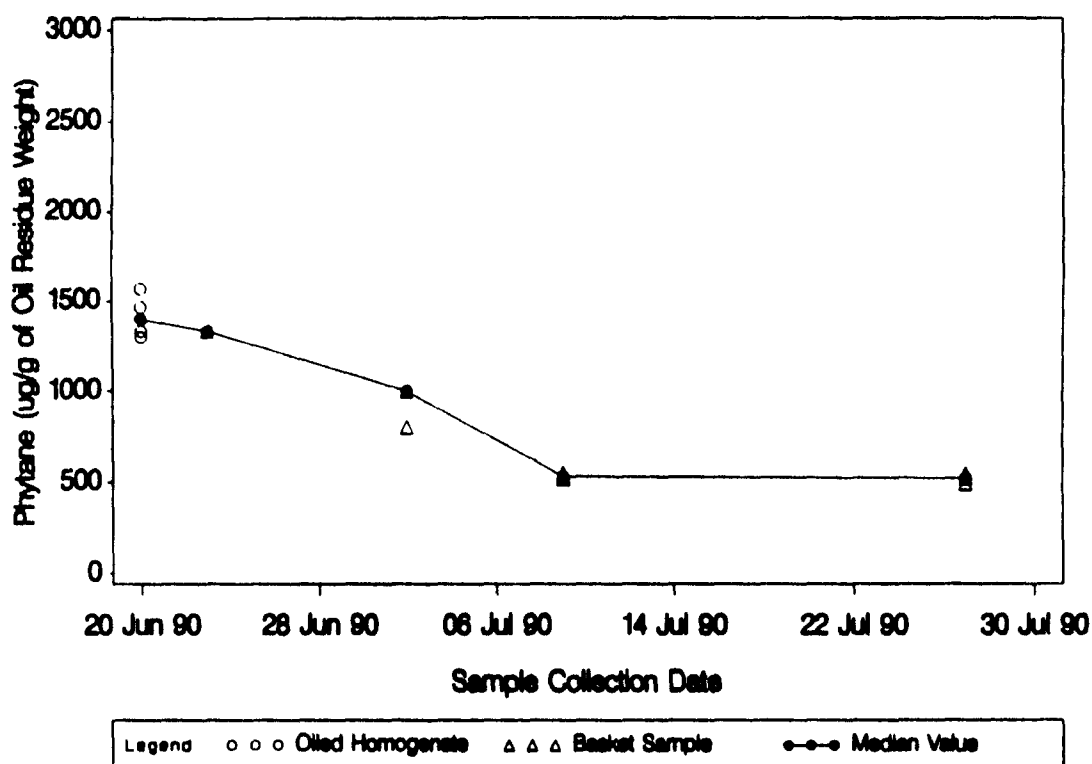

Figure 8.45. Change In Phytane Concentration Over Time for Untreated Control Plot #2 for Untreated Disk Island Application Rate Study.
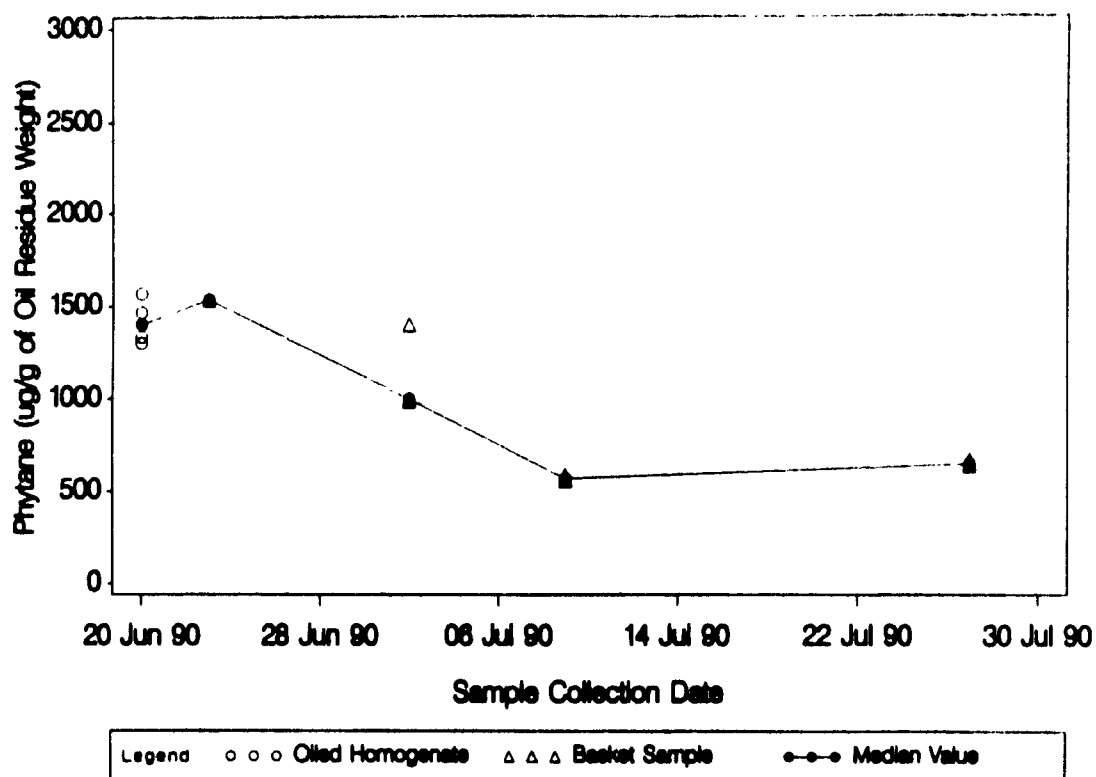
Figure 8.46. Change In Phytane Concentration Over Time for the 50 g/m² Fertilizer Application for the Disk Island Application Rate Study.
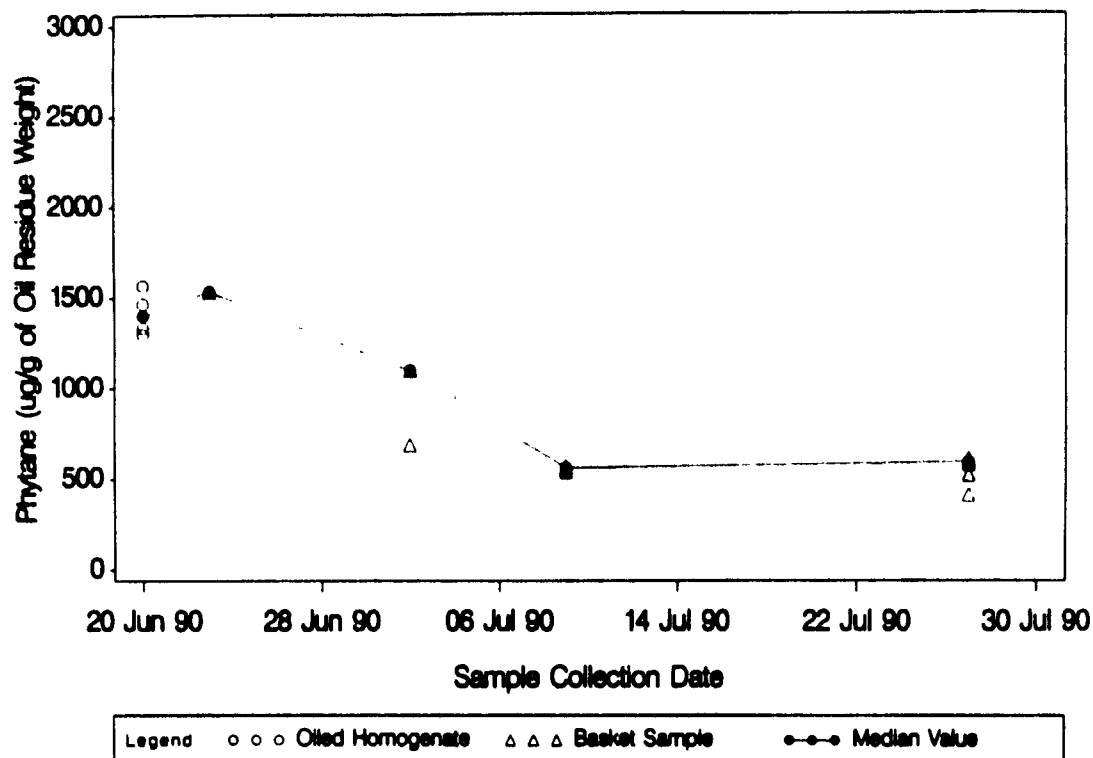
Figure 8.47. Change In Phytane Concentration Over Time for the 100 g/m² Fertilizer Application for the Disk Island Application Rate Study.
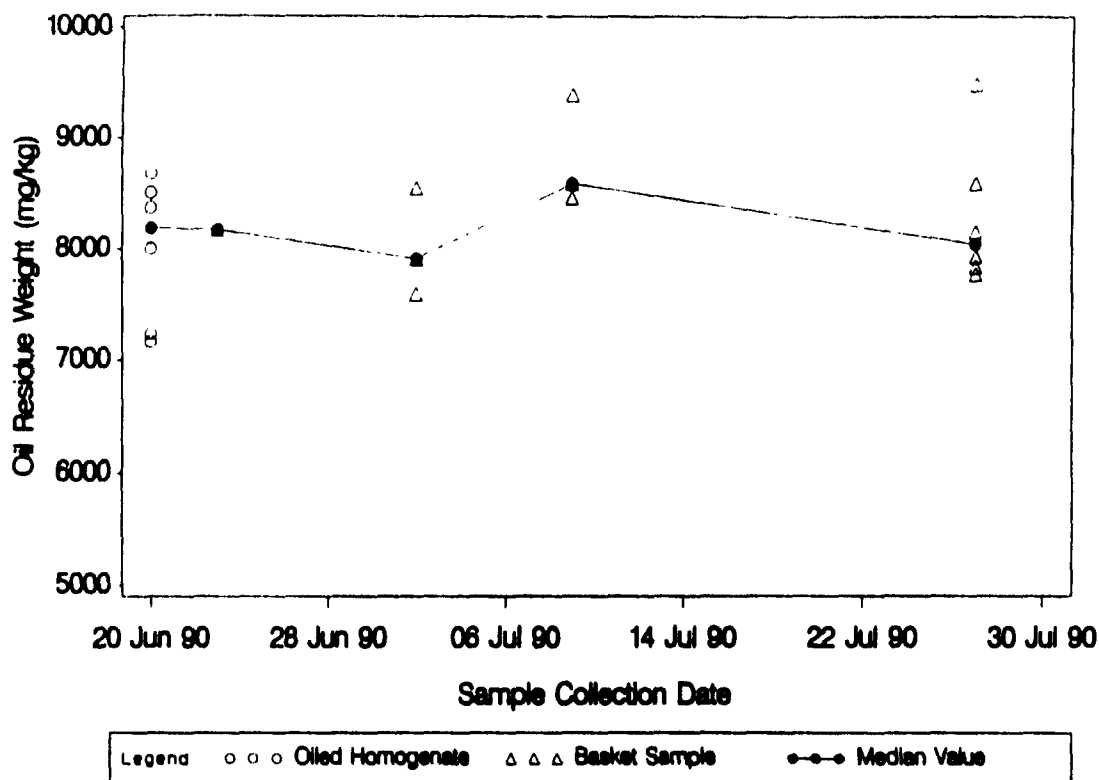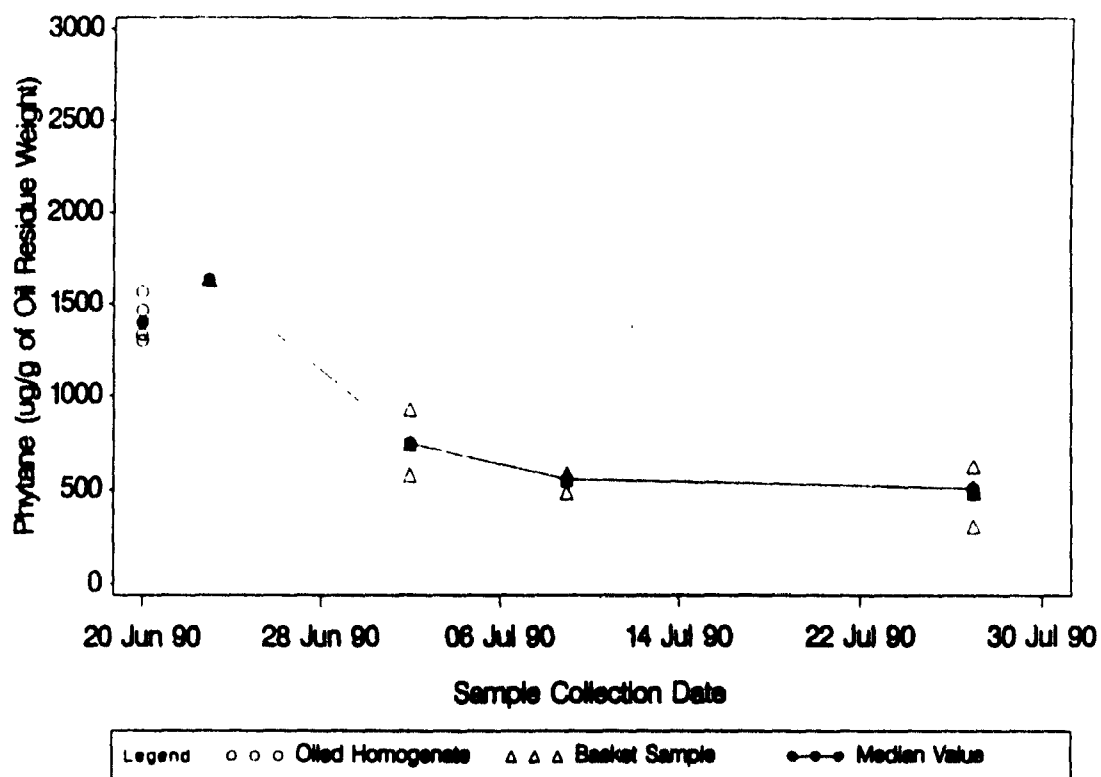
Figure 8.48. Change in Phytane Concentration Over Time for the 500 g/m² Fertilizer Application for the Disk Island Application Rate Study.
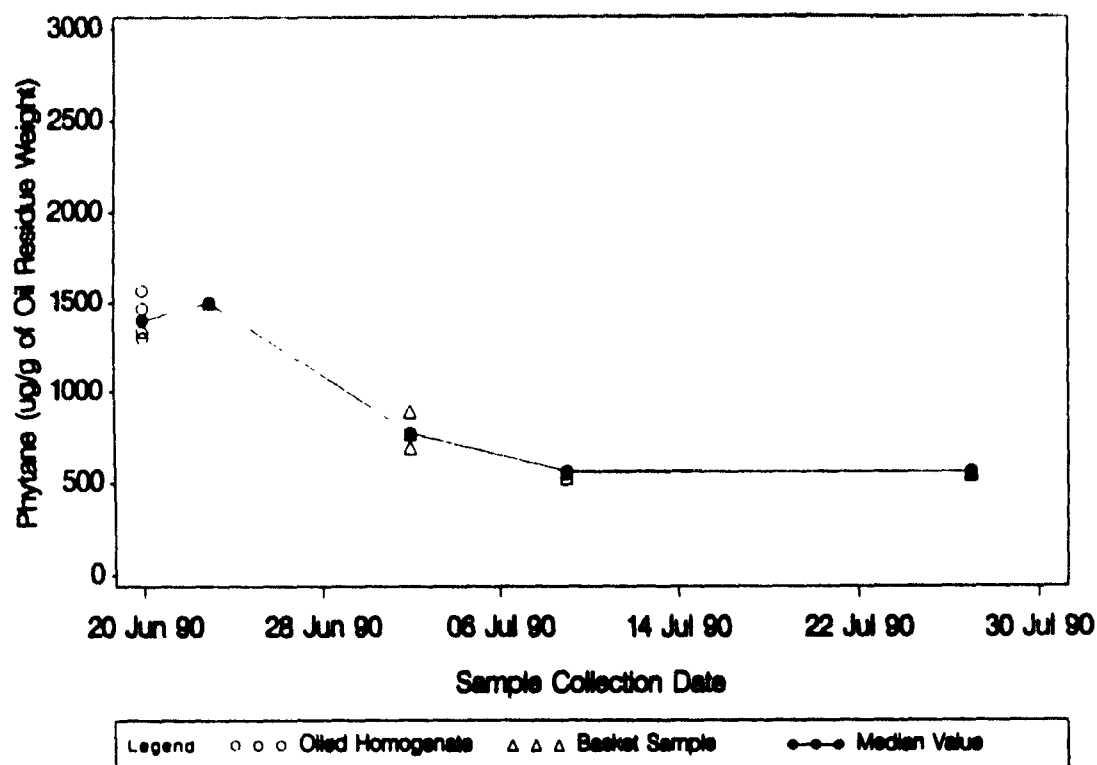
Figure 8.49. Change in Phytane Concentration Over Time for the 1,000 g/m² Fertilizer Application for the Disk Island Application Rate Study.
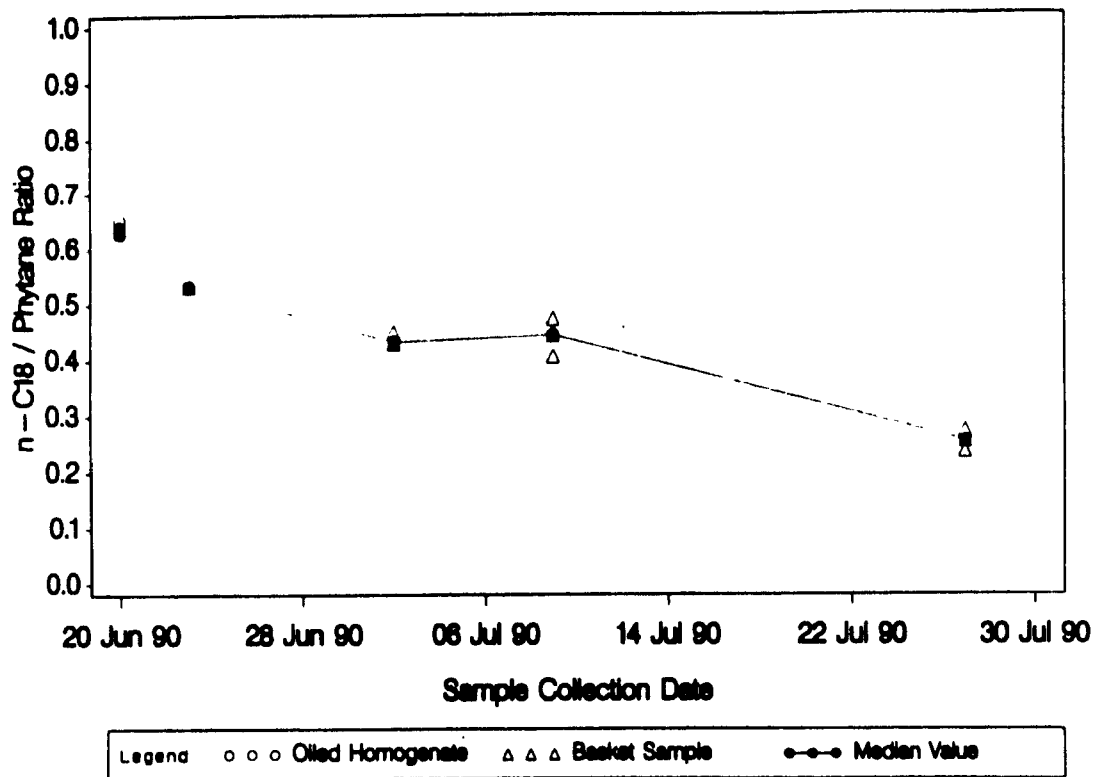
Figure 8.50.   Change in the nC18/phytane Ratio Over Time for Untreated
Control Plot #1 for the Disk Island Application Rate Study.
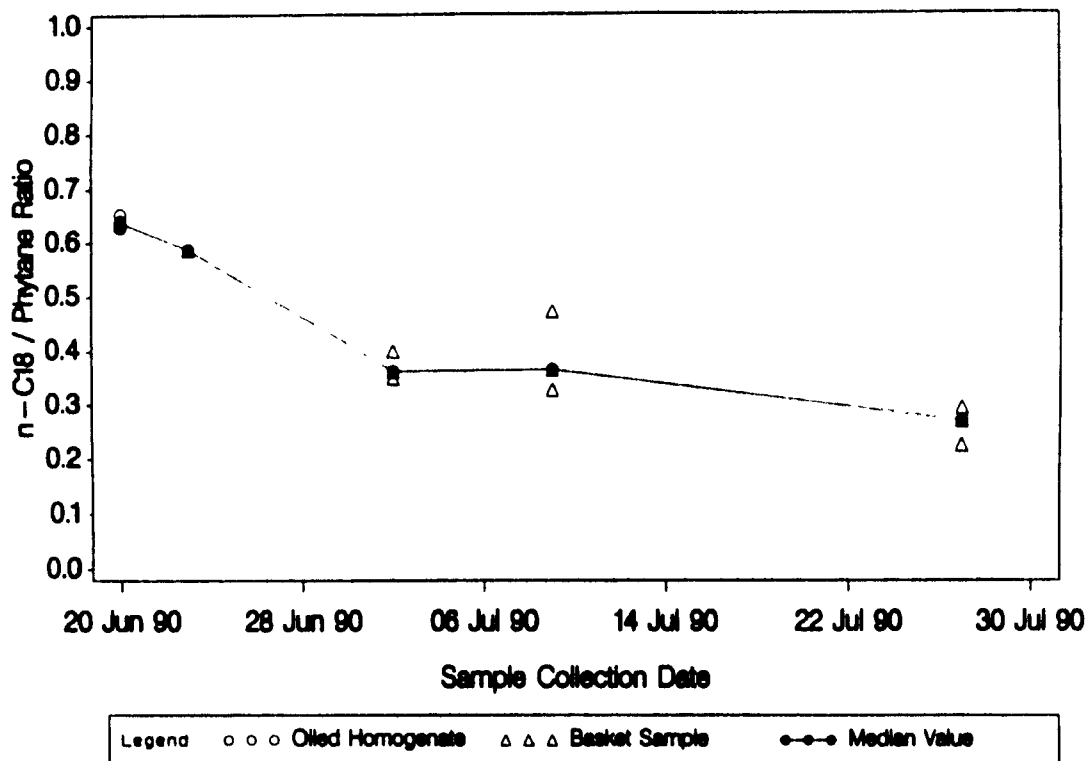
Figure 8.51.   Change in the nC18/phytane Ratio Over Time for Untreated
Control Plot #2 for the Disk Island Application Rate Study.
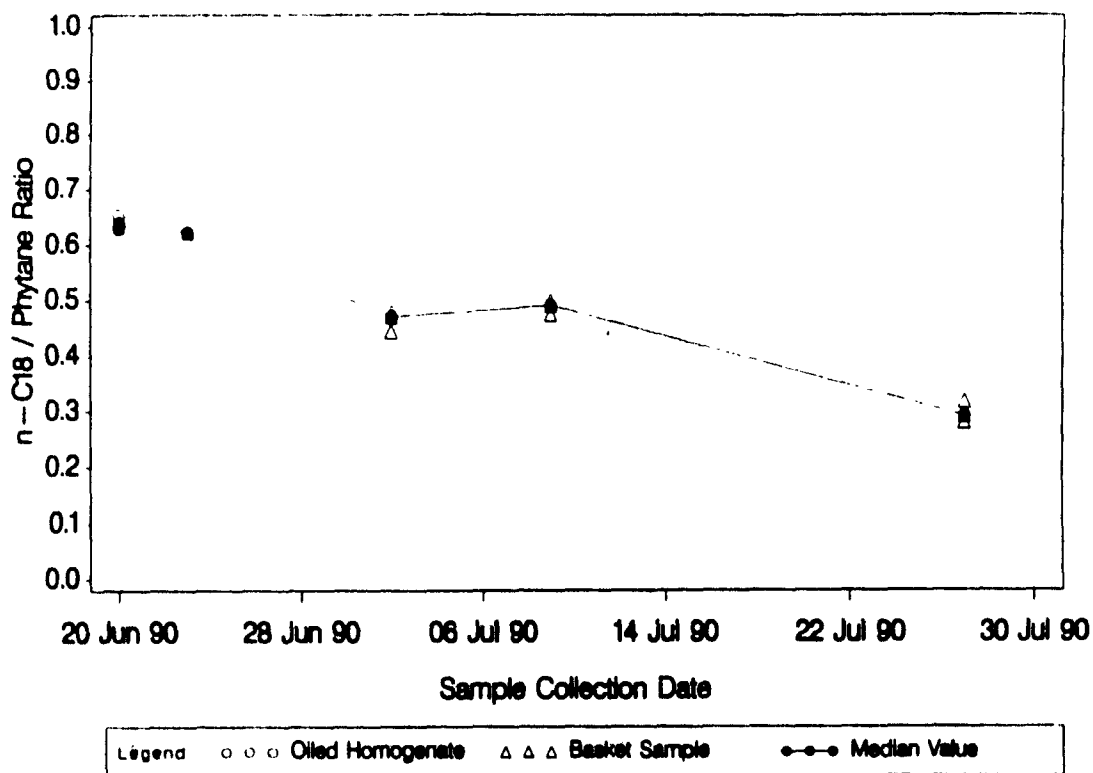
Figure 8.52. Change in the nC18/phytane Ratio Over Time for the 50 g/m² Fertilizer Application for the Disk Island Application Rate Study.
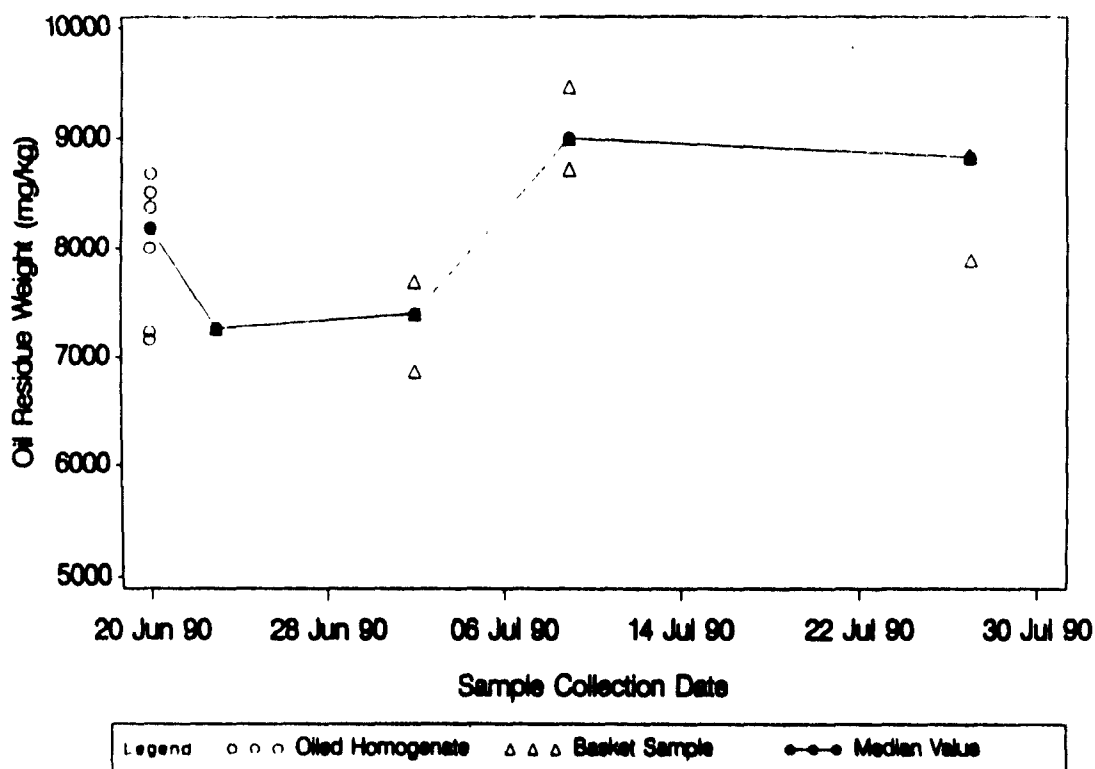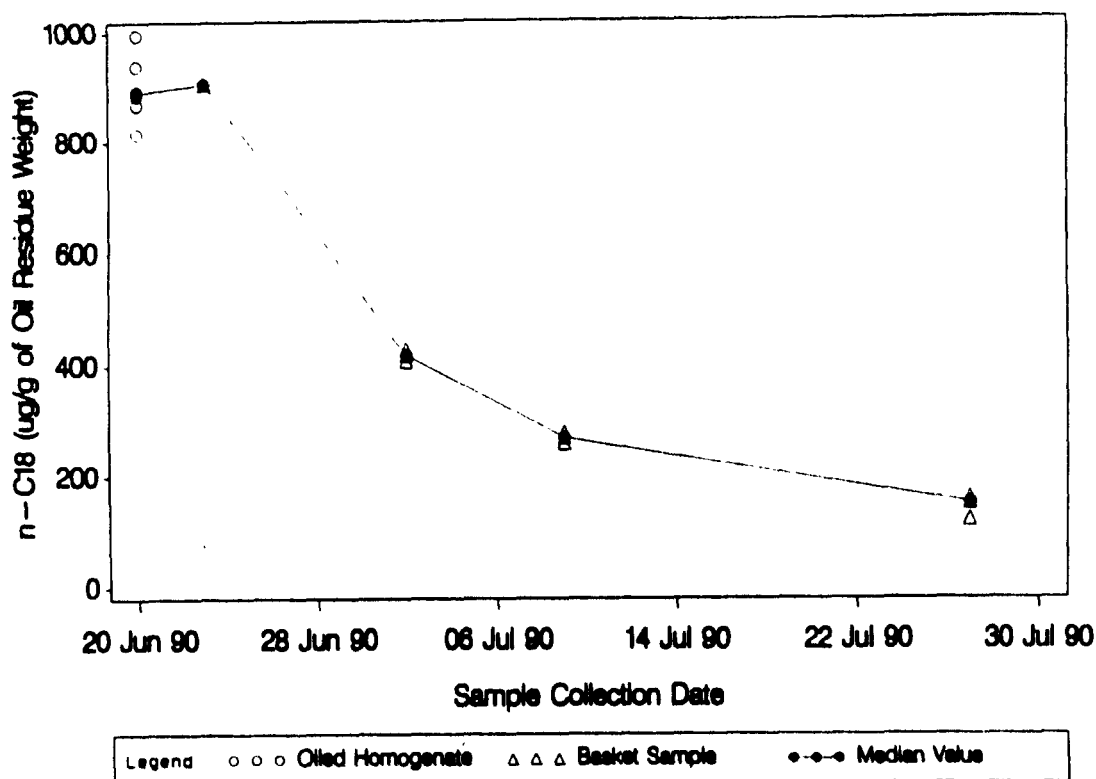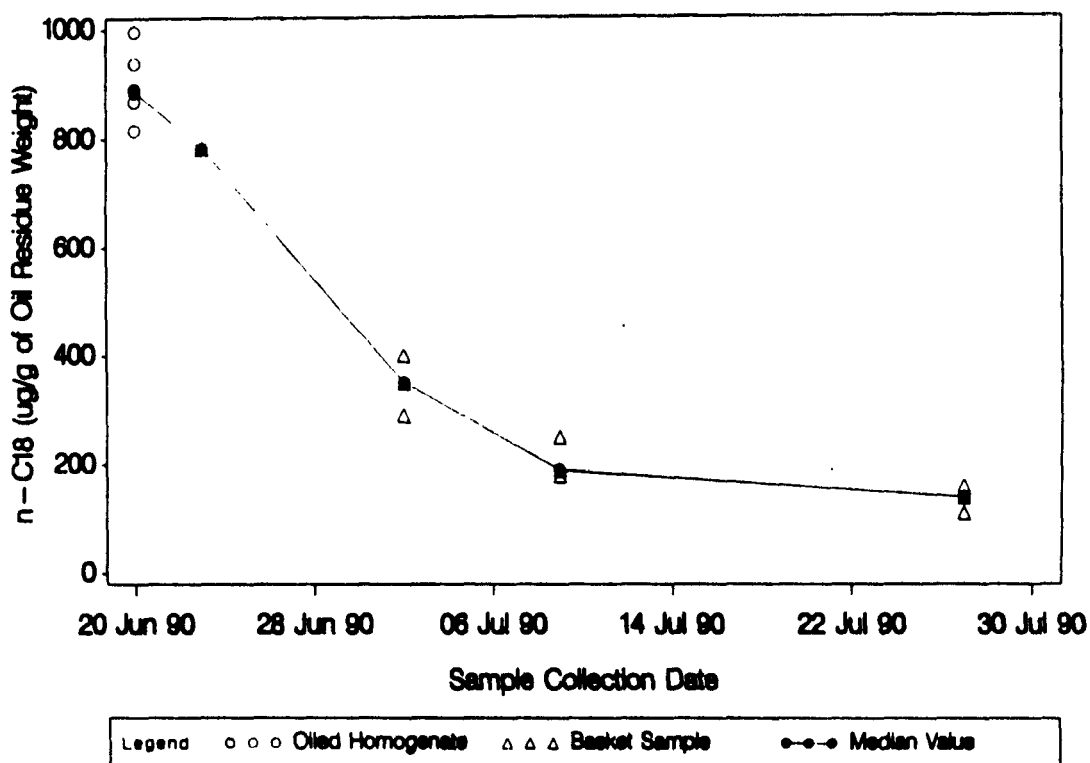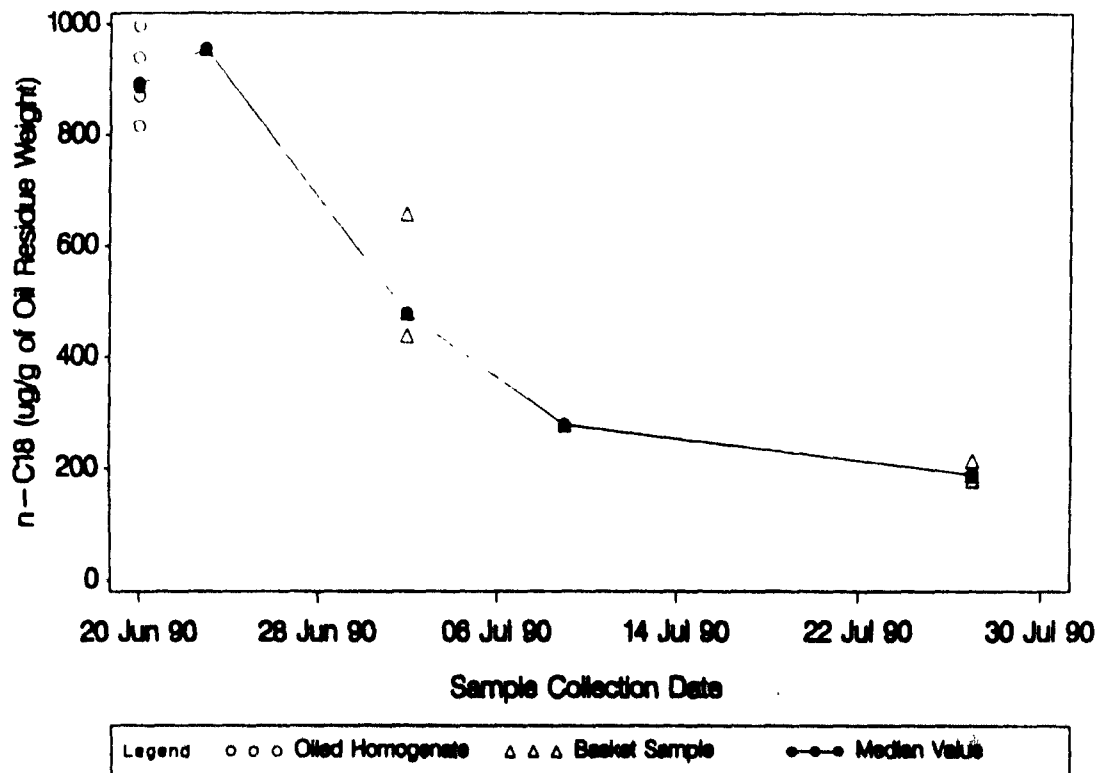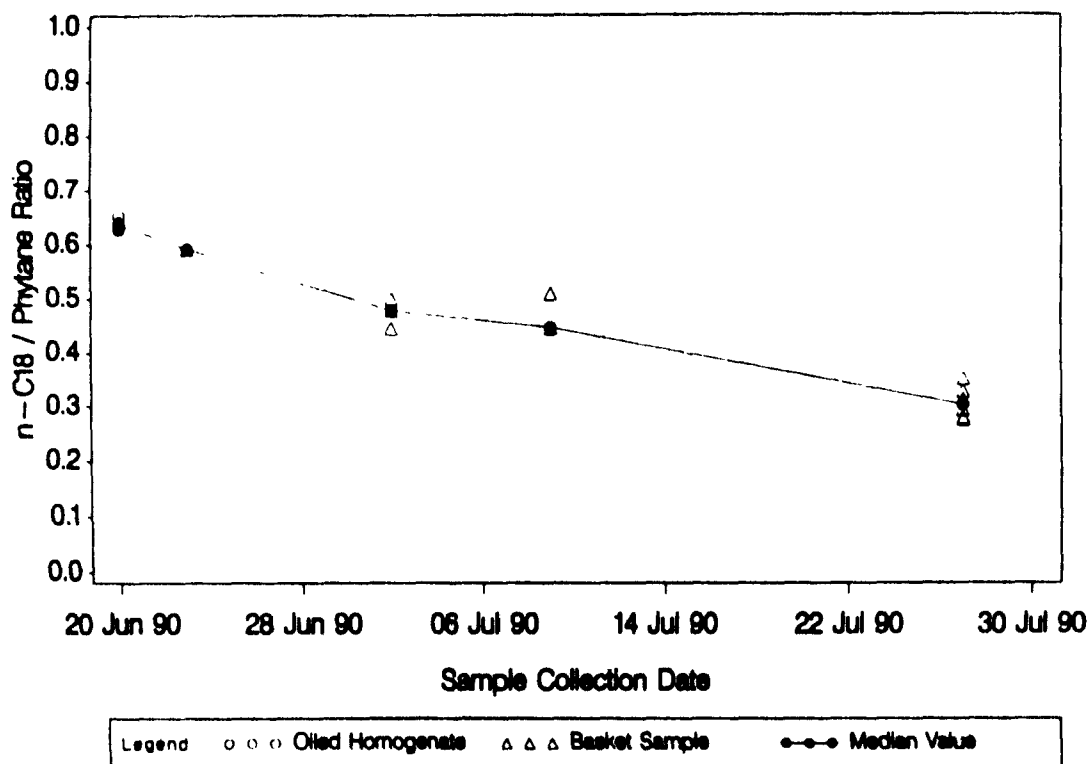

Figure 8.53. Change in the nC18/phytane Ratio Over Time for the 100 g/m² Fertilizer Application for the Disk Island Application Rate Study.
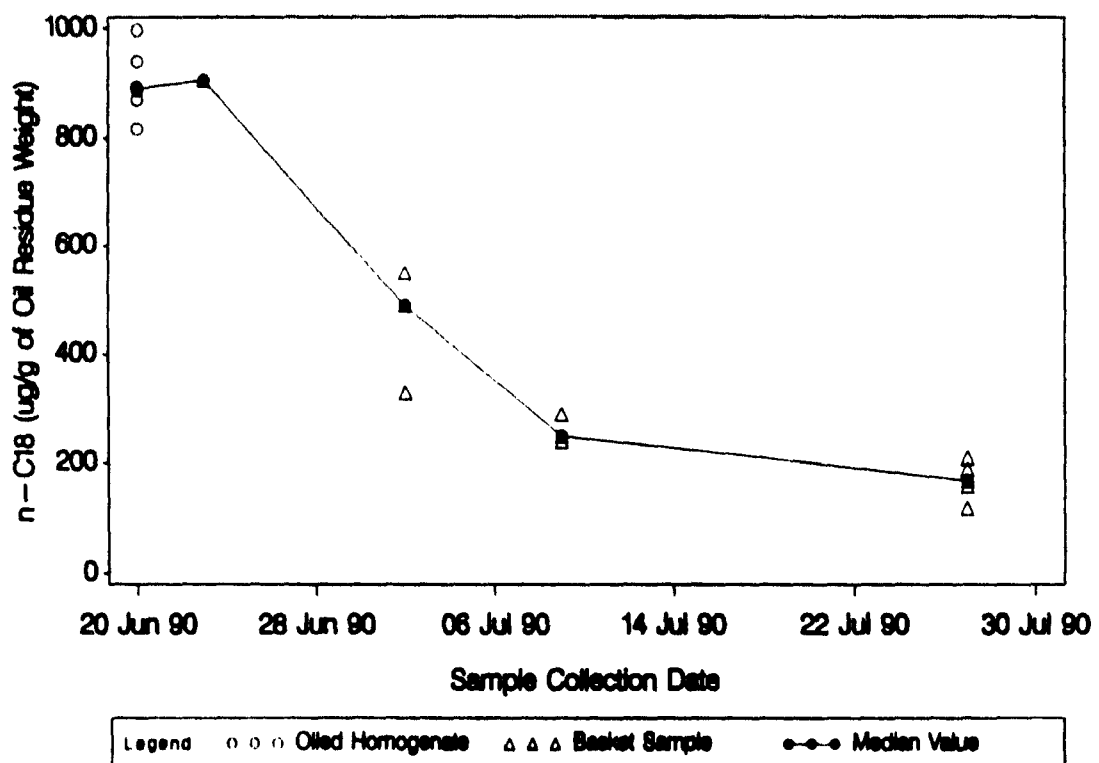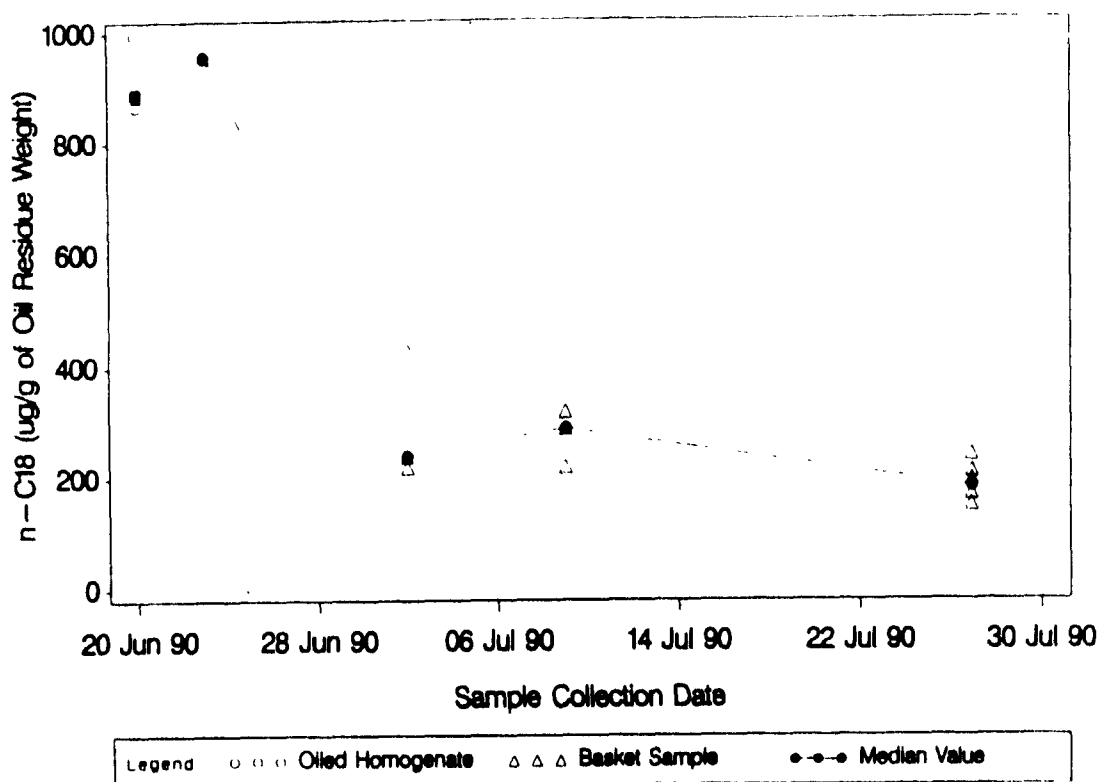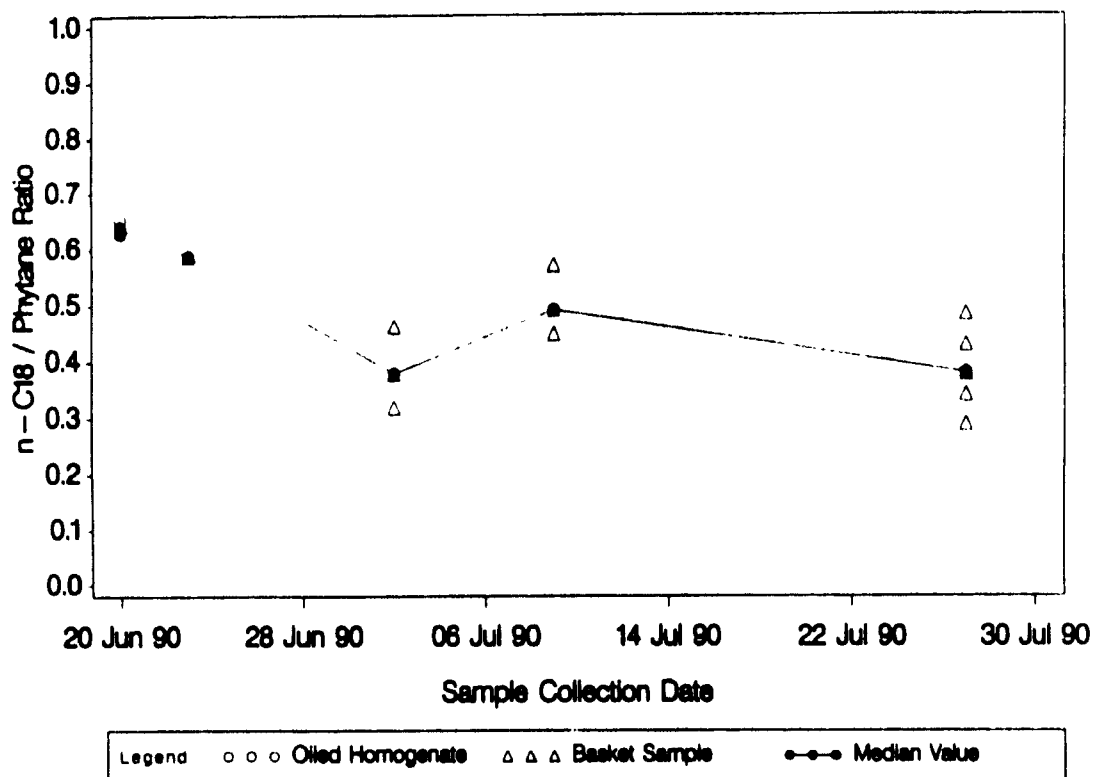
Figure 8.54. Change in the nC18/phytane Ratio Over Time for the 500 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.
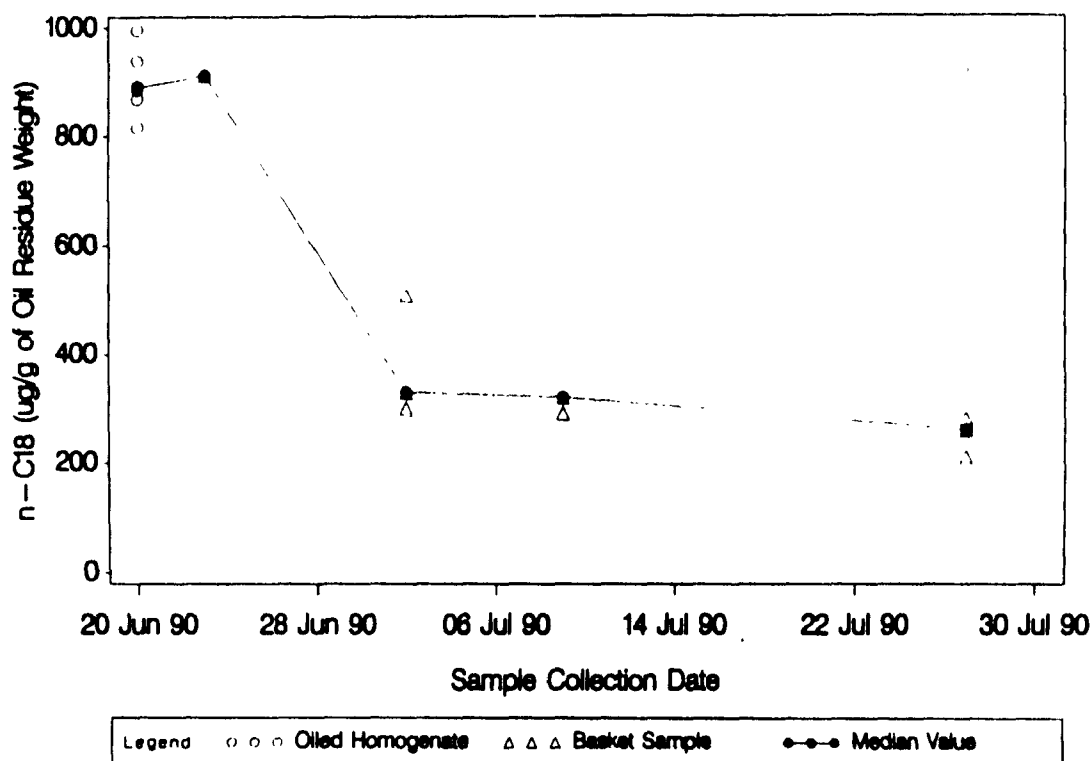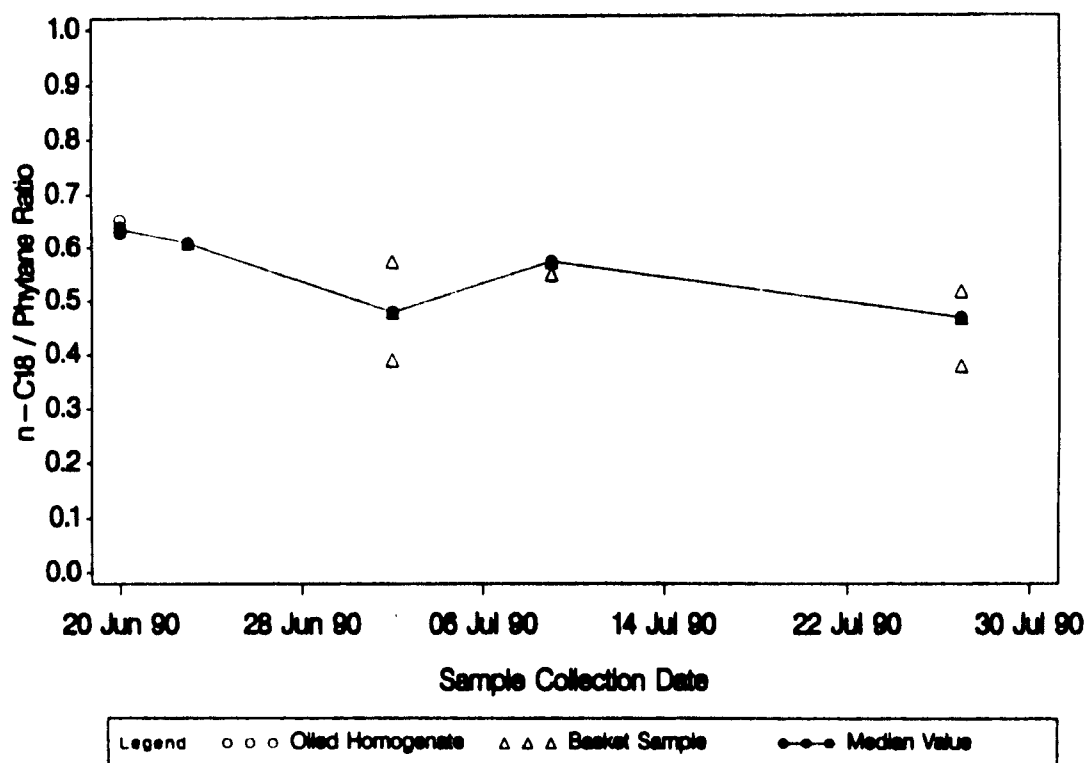

Figure 8.55. Change in the nC18/phytane Ratio Over Time for the 1,000 g/m^2 Fertilizer Application for the Disk Island Application Rate Study.

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On the fertilizer-treated plots, trends in oil residue decay were obscured by the large variability between the sampling dates, and in some cases between the sampling baskets. Variability between sampling baskets was particularly apparent on the plots receiving the 50 g/m^2 and 100 g/m^2 application of granules. When comparing day zero with the last sampling date, the only fertilizer-treated beach which showed a net loss of oil residue was the 100 g/m^2 application. The lack of any net change on the other beaches would suggest that this decrease was not real but due more to the observed variability in oil concentrations.

Changes in the nC18/phytane ratio strongly suggest that oil biodegradation was occurring on each test plot, although slowly (Figures 8.26 to 8.49). The nC18/phytane ratios showed slow but steady decreases through time despite relatively low values initially (i.e., significant hydrocarbon degradation previous to this test) (Figures 8.50 to 8.55). Decreases were generally seen with all other n-alkanes and to a smaller extent with the branched alkane, phytane. However, most importantly, it appeared that the fertilizer application had virtually no effect on the hydrocarbon decay rates and therefore on biodegradation. The only possible effect was seen with the nC18 alkanes and the summed alkanes (nC18 to nC27), where a somewhat faster initial drop in concentration of these hydrocarbons occurred at the 500 g/m^2 application. We would conclude that biodegradation was not nutrient limited on this Disk Island beach. It is possible that the low energy conditions and/or the packed nature of the beach material on this beach (more sand than gravel) could have caused oxygen to become the limiting factor. This condition could have been compounded by the presence of additional oxygen demand from humic and/or other organic materials which were visually apparent in the beach matrix.

It is also important to note the considerably reduced variation in the hydrocarbon concentrations between sampling baskets (low variation in replicates) and between sampling dates (relatively consistent downward trend) compared to that seen in oil residue weights. Since hydrocarbon concentrations were normalized to the oil residue weights, the results suggest that percentage composition of the oil was very consistent, regardless of the amount of oil present. If the methylene chloride was extracting other organic matter besides the oil (a possible explanation for the variable residue weights), this should also affect the hydrocarbon percentage composition. In fact, it does not appear that this was the case. Thus, it is concluded that oil on this Disk Island test beach was quite uniform in terms of the extent of biodegradation, but very heterogeneous in terms of distribution over time and space.

MICROBIOLOGY

As samples were brought back to the laboratory for chemical analysis, subsamples were taken to measure rates of total CO_2 production, or oil mineralization by microbial communities. In addition, following incubation for the CO_2 analysis, the samples were spiked with ^{14}C-phenanthracene and the amount of radiolabeled CO_2 was also measured. In all cases incubations were performed in biometer flasks with a 12:12 low:high tidal cycle. Results from these studies are shown in Figure 8.56. It is quite evident that considerable oil biodegradation was occurring in these samples. Production of large amounts of radiolabeled CO_2 from phenanthracene strongly suggests that this was due to biodegradation of oil and not other types of organic matter. The amount of total CO_2 produced was also in the same range as samples taken from Elrington beach (Section 9), where oil chemistry results showed more rapid oil degradation than on the Disk Island test beach.

The effect of fertilizer application rate on oil biodegradation, as measured by the CO_2 mineralization, was fairly consistent. Adding fertilizer generally caused significant increases in mineralization rates relative to the untreated control plots, and the greatest stimulation occurred with the 500 g/m^2 application (approximately 2 to 5 times greater than the untreated controls). The highest application of 1,000 g/m^2 actually seemed to inhibit mineralization to some extent. Graphing a double reciprocal plot of the mineralization rate versus the fertilizer application rates (Figure 8.56, insert) showed a generally linear relationship, at least up to the 500 g/m^2 concentration of granules. Based on this relationship it would appear that background mineralization rates were very high, and to approximately double the oil degradation rate a six-fold increase in fertilizer granules was required.

It appears that application of almost any amount of fertilizer below 500 g/m^2 will stimulate oil mineralization. However, similar studies performed during the winter of 1989/90 (Section 10) where oiled beach material was exposed to different fertilizer concentrations in shake flasks showed that biodegradation rates could be stimulated at nitrogen concentrations at least 10-fold higher (35 ppm) than those actually measured in the interstitial water at the Disk Island test site (approximately 3 ppm). It is possible that oxygen availability in the field became a limiting factor, which would not be the case in the idealized conditions of a shake flask test. Therefore, extrapolation of laboratory data on fertilizer specific activity to the field must be considered carefully, as it will be very dependent on beach conditions.
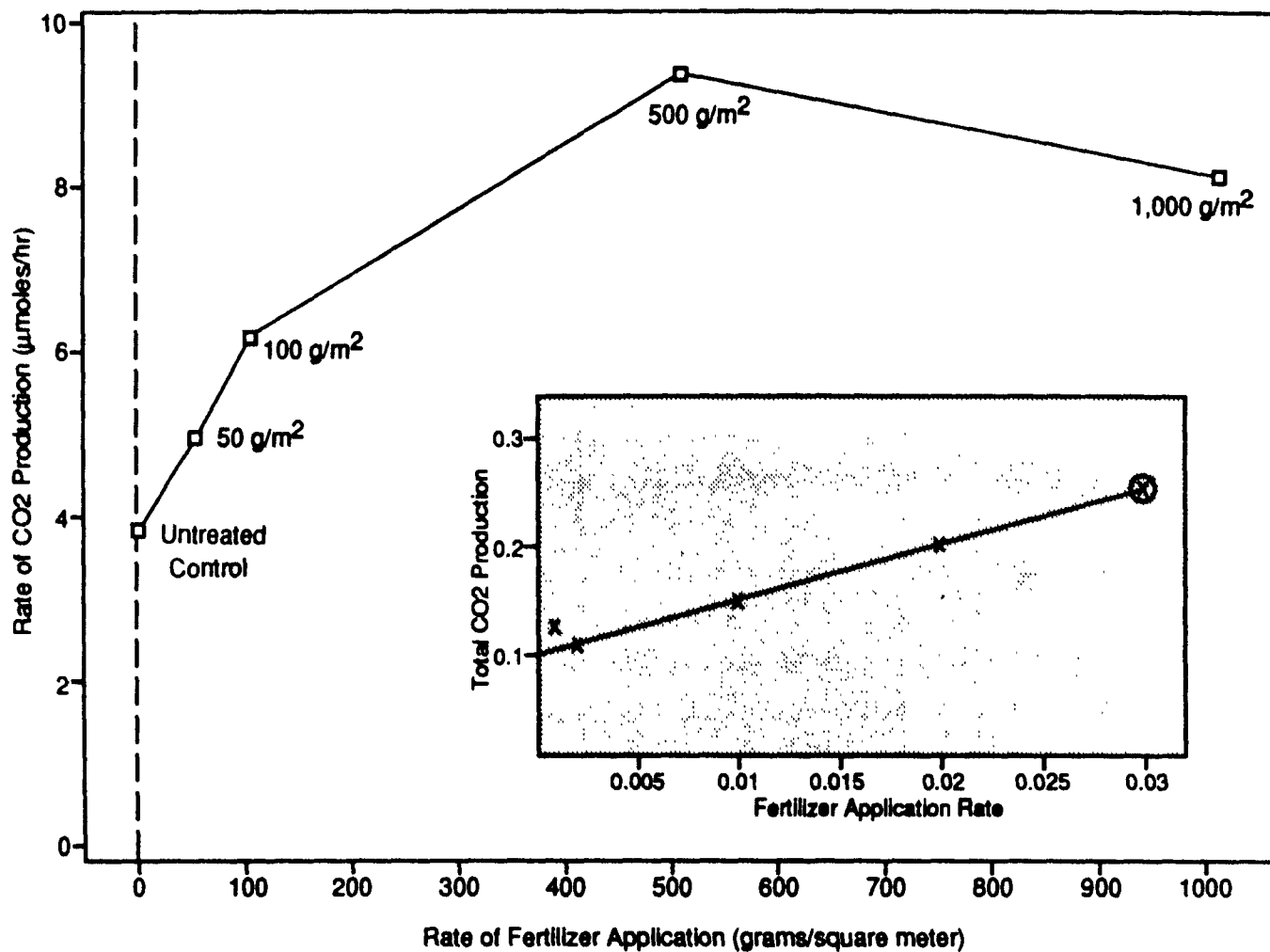
Figure 8.56. Plot of Rate of CO$_2$ Production Versus Rate of Fertilizer Concentration.

It is possible that the mineralization study results were due to the carryover of fertilizer granules from the lab to the flasks. Despite attempts to remove subsamples from the sampling baskets that were free of the granules, their exclusion could not be guaranteed (to avoid the granules, subsamples were removed from below the surface of the beach material in the baskets). However, since nutrients in the fertilizer granules are depleted over time, we would expect that mineralization differences between beach plots would become less with time if they were due strictly to carryover of fertilizer. This, in fact, was not the case.

Mineralization of radiolabeled hexadecane (performed by the ADEC Oil Spill Response Laboratory and the University of Alaska, Fairbanks) also showed similar effects of the fertilizer application (Figures 8.57 and 8.58). A summary of the combined results is shown in Figure 8.59. The samples showed increases in mineralization relative to fertilizer application in the field. The highest application rates, 500 and 1,000 g/m^2, gave the greatest effect with the peak occurring between day 4 and 12. The 1,000 g/m^2 application did not seem to have the inhibitory effect seen with measurements of total CO_2 production and phenanthracene mineralization. Unfortunately, samples taken prior to fertilizer application were lost.

Finally, measurements of total heterotrophic and oil-degrading bacterial populations are shown in Figure 8.60. Other than some anomalous results shortly after fertilizer application (first sampling), there was a strong indication that the number of oil degraders increased substantially on the fertilizer-treated plots, especially at the two highest application rates. On the 19th day after fertilizer application, the number of oil degraders on the treated beaches (500 and 1,000 g/m^2) were significantly higher than the untreated control plots (at the 95% confidence level). The difference was greater than 10-fold. There was also some indication that total heterotrophs may have also increased on the 1,000 g/m^2 plot. It is interesting that as the effectiveness of fertilizer release from the granules presumedly diminished through time, the enrichment effect also diminished with essentially no statistically difference in oil degrader numbers on the last sampling date. This suggests that the oil-degrader populations were quite dynamic, responding quickly to the presence or absence of fertilizer.
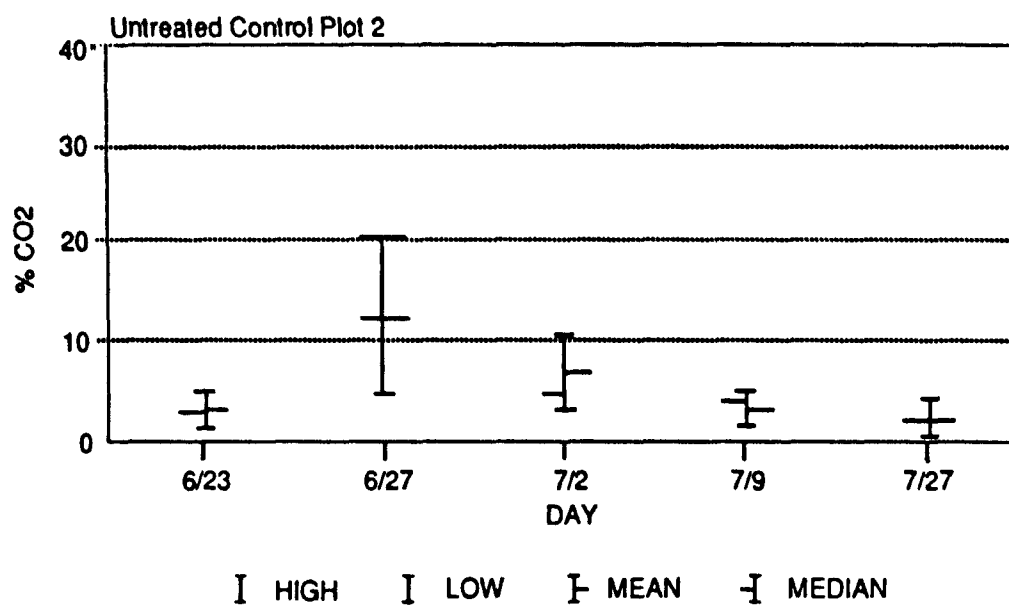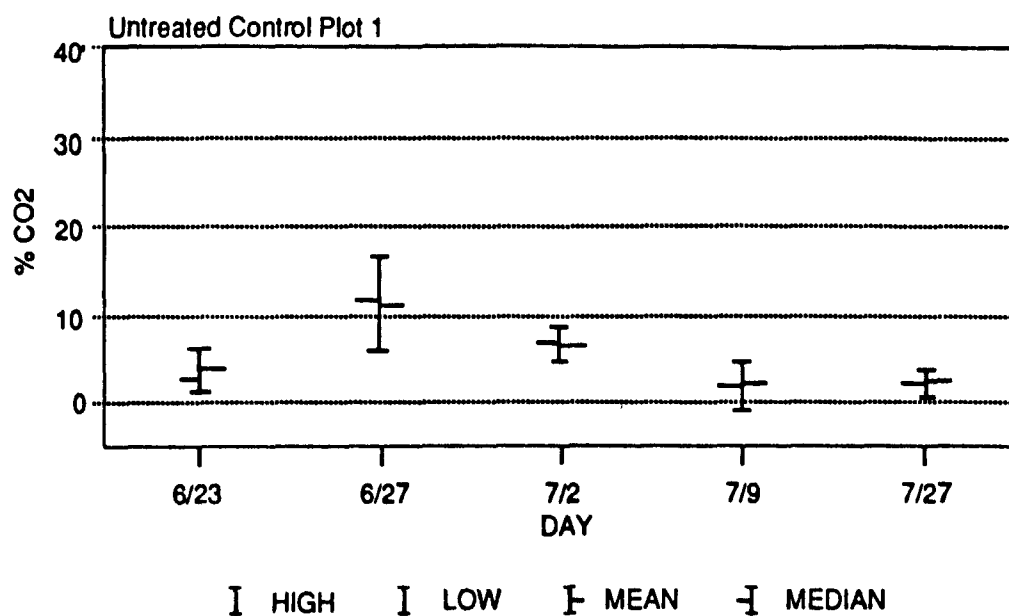
Figure 8.57. Radiolabled Hexadecane Mineralization Over Time (Standard Deviation) for both Untreated Control Plots at Disk Island.
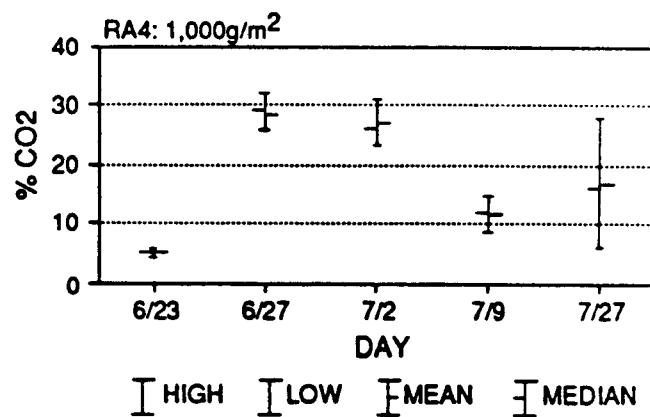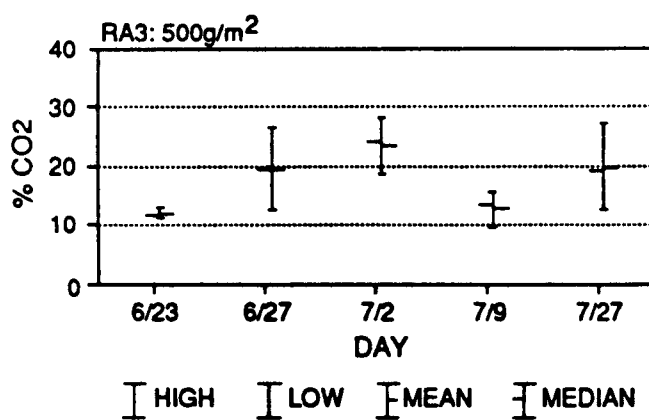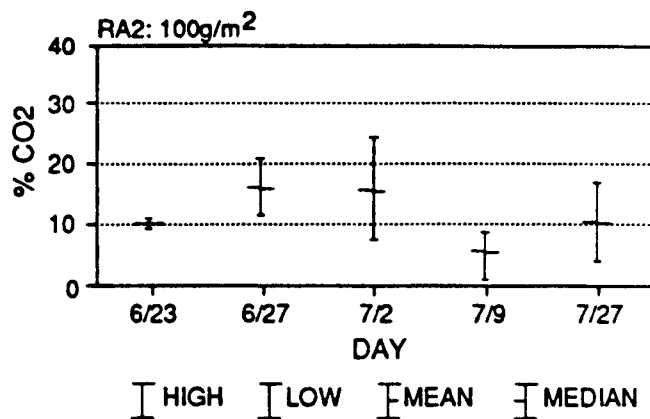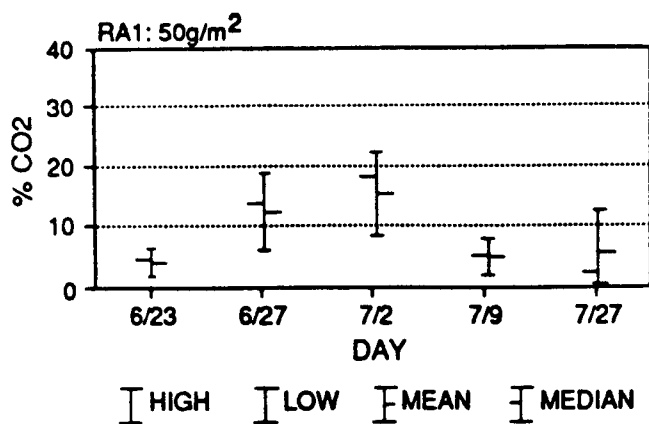
Figure 8.58. Radiolabeled Hexadecane Mineralization Over Time (Standard Deviation) for the Four Treated Plots at Disk Island.

DISK ISLAND DOSE RESPONSE STUDY
HEXADECANE MINERALIZATION

%CO2

30
25
20
15
10
5
0

RA4
RA3
RA2
RA1
CTR2
CTR1

0   4   9   16   34

DAYS AFTER FERTILIZER APPLICATION
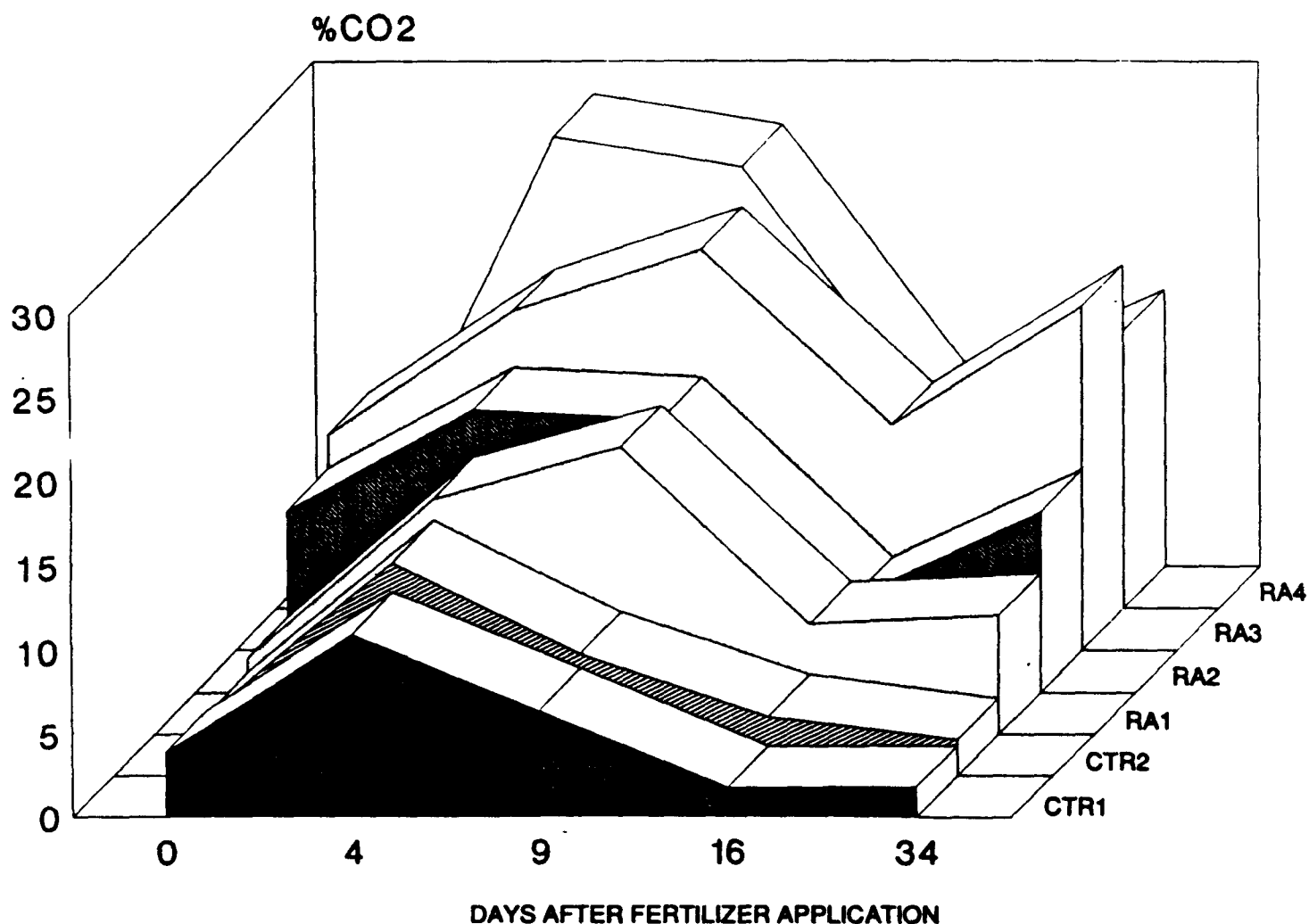
CUSTOMBLEN DOSE	
RA1	$50g/m^2$
RA2	$100g/m^2$
RA3	$500g/m^2$
RA4	$1000g/m^2$

Figure 8.59. Summary of Radiolabeled Hexadecane Mineralization Over Time for All Plots at Disk Island.
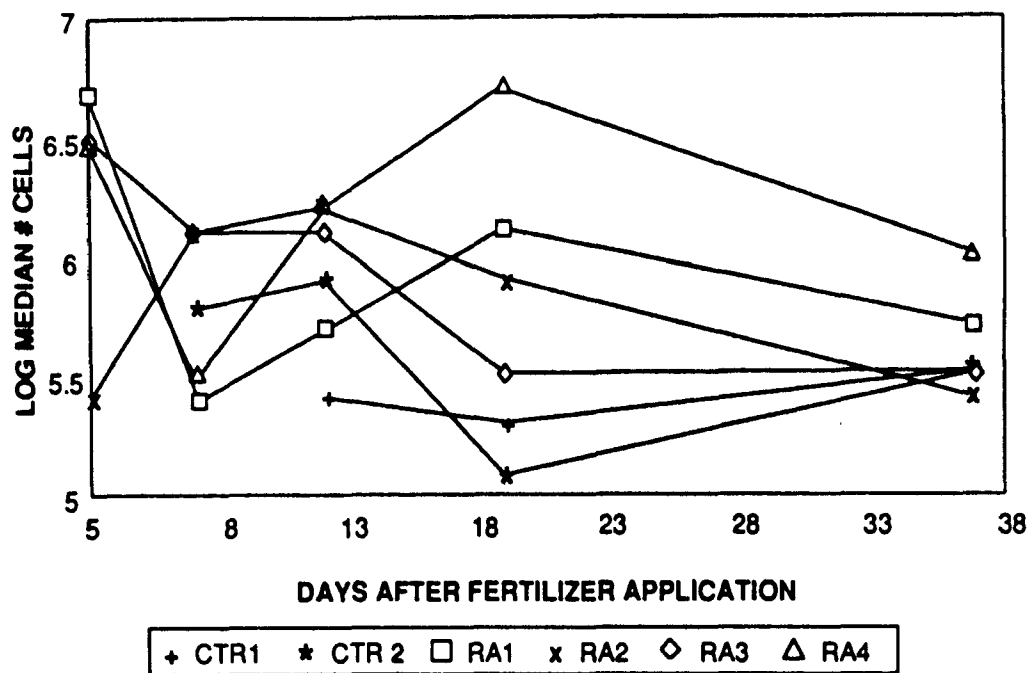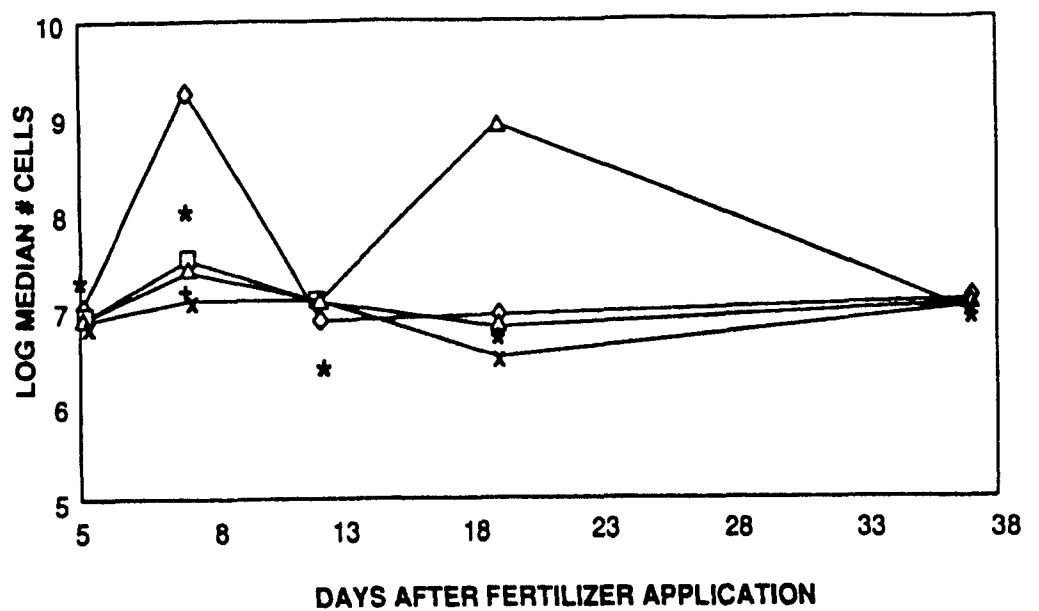
LEGEND

CTR1 =	Untreated Control Plot 1	RA2 = 100g/m² CUSTOMBLEN
CTR2 =	Untreated Control Plot 2	RA3 = 500g/m² CUSTOMBLEN
RA1 =	50g/m² CUSTOMBLEN	RA4 = 1000g/m² CUSTOMBLEN

Figure 8.60. Measurements of Total Heterotrophic and Oil-Degrading Bacterial Populations Over Time at All Plots at Disk Island.

SUMMARY AND CONCLUSIONS

The following conclusions can be drawn from the Disk Island study conducted during the summer of 1990:

a) Effects of different fertilizer concentrations could not be assessed because of an overall lack of general oil biodegradation. Field samples did show increased mineralization (total CO_2 production) in proportion to increased fertilizer application, but oil chemistry results showed essentially no effect.

b) Analysis of ammonia, nitrate and phosphate in interstitial beach water showed nutrient release was generally proportional to fertilizer application rate. However, nutrient release represented more of a pulsed high concentration during the first 2 to 3 days following application rather than a slow release over time.

c) Monitoring of wells in the test area revealed that nutrient release from fertilizer granules was not totally slow-release. Highest nutrient concentrations occurred shortly after fertilizer application and then decreased to background levels 2 to 3 days later.

d) Analytical chemistry results showed that the addition of fertilizer failed to enhance oil biodegradation, regardless of the application rate. Changes in oil composition occurred, although slowly, but were similar for both treated and untreated plots. It is not known what conditions at the Disk Island site could have precluded enhanced oil biodegradation. Disk Island may represent a type of beach (low energy, low slope profile, less porous beach material) that is not amenable to oil bioremediation because of insufficient oxygen availability and/or high natural organic matter content (peat deposits).

e) Measurements of oil mineralization, based on total CO_2 production or $^{14}CO_2$ released from radiolabeled hydrocarbons (phenanthrene and hexadecane), did show effects due to fertilizer application rate. It was quite evident that considerable oil biodegradation was occurring in the beach samples. Production of large amounts of radiolabeled CO_2 from phenanthracene and hexadecane strongly suggested that this was due to biodegradation of oil and not other types of organic matter (humic materials). Adding fertilizer generally caused significant increases in mineralization rates relative to the untreated control plots,

with the greatest stimulation occurring with the 500 g/m^2 application (approximately 2 to 5 times greater than the untreated controls). The largest application (1,000 g/m^2) actually seemed to inhibit mineralization to some extent. A calculated dose response indicated that a six-fold increase in fertilizer granule application produced a two-fold increase in oil degradation rates. In addition, oil-degrading bacteria increased following fertilizer application. Concentrations were almost 10-fold greater than the untreated controls on the plots receiving the two highest fertilizer concentrations.

SECTION 9
ELRINGTON ISLAND FIELD RESULTS

VISUAL OBSERVATIONS

Section ER 20 on the north end of Elrington Island represented a beach with typical subsurface oil contamination. The surface was relatively free of oil but extensive amounts of oil were found approximately .2 m to .3 m below the surface. Three test beaches were established and implanted with sampling baskets containing a constructed subsurface oil layer designed to simulate the normal subsurface condition of the beach. A trench was excavated that was long enough to allow all of the baskets to be simultaneously placed in a line. During excavation it was obvious that oil was very heterogeneously distributed in the subsurface. In some areas, oil was coagulated in large globs containing varying degrees of mixed sand and gravel, in other areas the oil was relatively dispersed, coating most of the surface of the mixed sand and gravel. The oil consistency was dry rather than runny, gritty to the touch (because of the presence of glacial till), and it did not produce an oil sheen when disturbed and covered with water.

Excavated beach material was used to fill in the area around the baskets. This, of course, made it difficult to reestablish the oiled subsurface layer next to the basket. However, due to the oil consistency and the considerable heterogeneity of the beach material, it appeared that the filling operation did not affect the movement of oil into or out of the sampling baskets. Baskets were covered with clean beach material, giving a visual consistency with the surrounding beach areas. As the tides moved in following the placement of the baskets, very little sheen was observed on the water surface.

The presence of bed rock just below the beach surface on the Bath beach required that baskets be placed higher on the beach than intended. As a result, water did not cover the area containing the baskets during some tidal cycles.

For beach sampling, a set of baskets was removed from the beaches and transported back to Valdez. Care was taken in the laboratory to remove subsamples of the constructed oil layer, the unoiled layers (top and bottom), and the interface between the oiled and unoiled layers (top and bottom). At all samplings, the oiled layer in the baskets remained distinct, and there was no visual difference over time in the amount of oil seen in each oiled layer from the different treatments. Also,

the consistency of the oil inside the baskets looked very much like the oil coating the beach material outside and surrounding the baskets.

NUTRIENT CONCENTRATIONS

Nutrient concentrations in the interstitial water from the monitoring wells of the Sprinkler beach and the untreated Control beach are shown in Figures 9.1 to 9.3 (see Section 3 for positioning of the wells on the beach). No nutrient monitoring was conducted on the Bath beach. Ammonia, nitrate, and phosphate concentrations on the untreated Control beach were all below 0.1 ppm. No cross-contamination from the Sprinkler beach was apparent. Concentrations of ammonia on the Sprinkler beach increased substantially as a result of fertilizer application. The particularly high values following the July 10, 1990 application resulted from the accidental use of fertilizer solution with a higher concentration of nutrients. In all cases, the spike concentrations were rapidly diluted by the tidal cycles. However, a residual concentration of ammonia in the interstitial water did occur, with concentrations of 1 to 2 ppm over a seven day period. Based on the beach dynamic studies during the summer of 1989, this residual was not surprising.

OIL CHEMISTRY

Oil Residue Weight

Changes in the oil residue weights over time are shown in Figures 9.4 a-c. Data from the middle of the oiled subsurface layer are shown. Examination of the figures reveals that by using the sampling basket procedure, variability in the data points was very small--a significant improvement over the sampling procedure used at Passage Cove. The oil concentration on the Bath and Sprinkler beaches at t = 0 was estimated because the sampling baskets were placed in the beach at different times prior to fertilizer application (the result of logistical complications). Therefore, starting oil concentrations were calculated based on the oil decay rate of the untreated Control beach and extrapolated from data obtained between the time of placement of the baskets and initiation of fertilizer application.

The subsequent decay curves for all beaches appeared to be zero-order. This suggested that the oil biodegradation rate was not limited by substrate availability. However, it is presumed that, with time, the rate would become first-order, possibly when the concentration reached 1,000 to 2,000
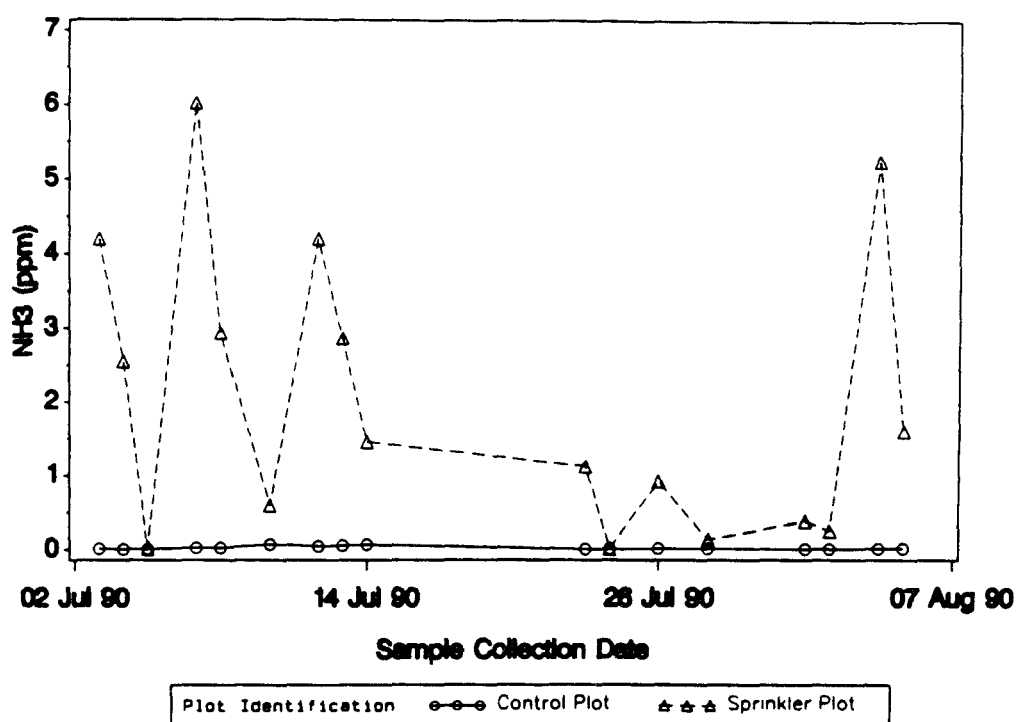
Figure 9.1. Change in Ammonia Concentration Through Time for the Incoming Tide at the Untreated Control Beach and Sprinkler Beach at Elrington Island.
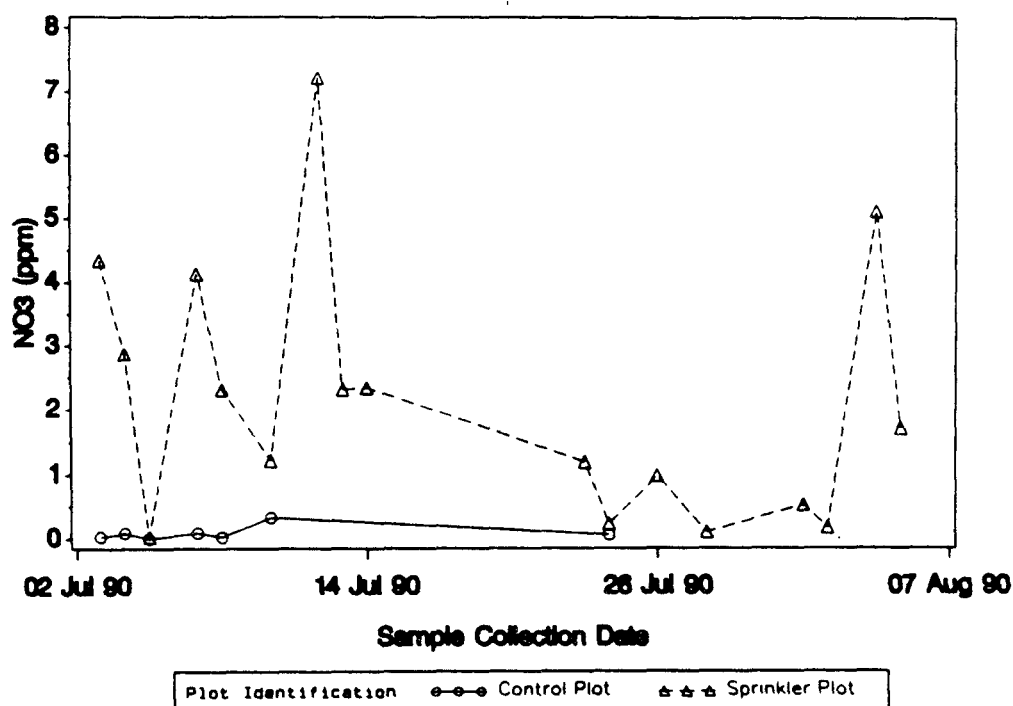
Figure 9.2. Change in Nitrate Concentration Through Time for the Incoming Tide at the Untreated Control Beach and Sprinkler Beach at Elrington Island.
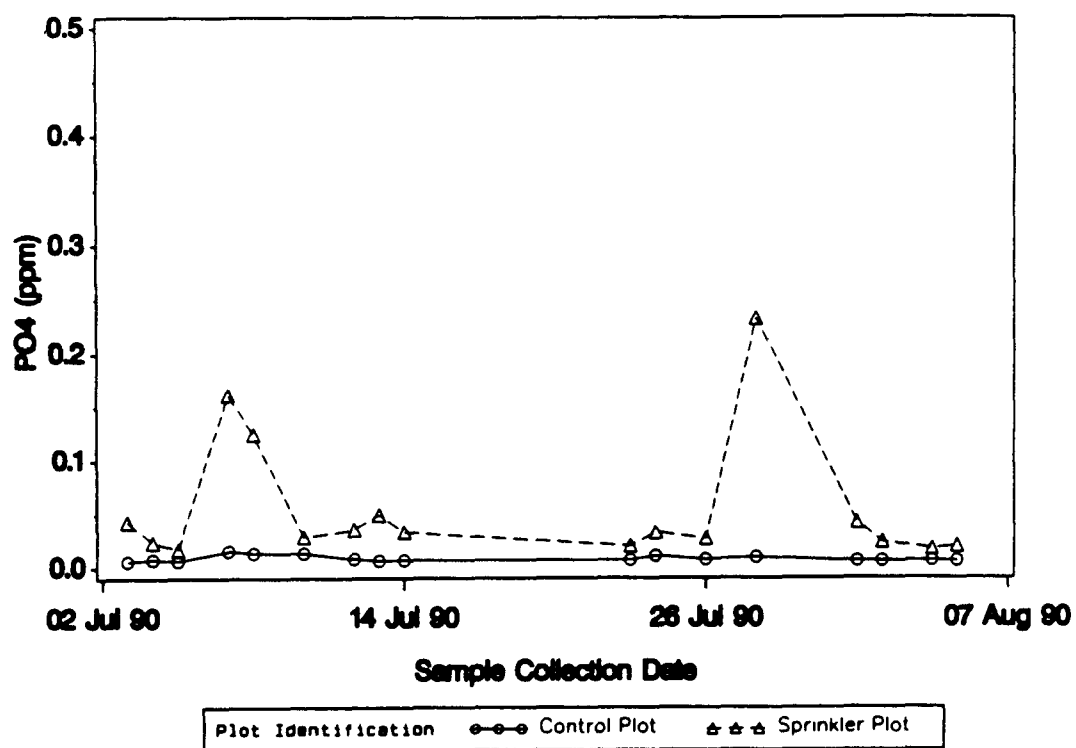
Figure 9.3. Change in Phosphate Concentration Through Time for the Incoming Tide at the Untreated Control Beach and Sprinkler Beach at Elrington Island.
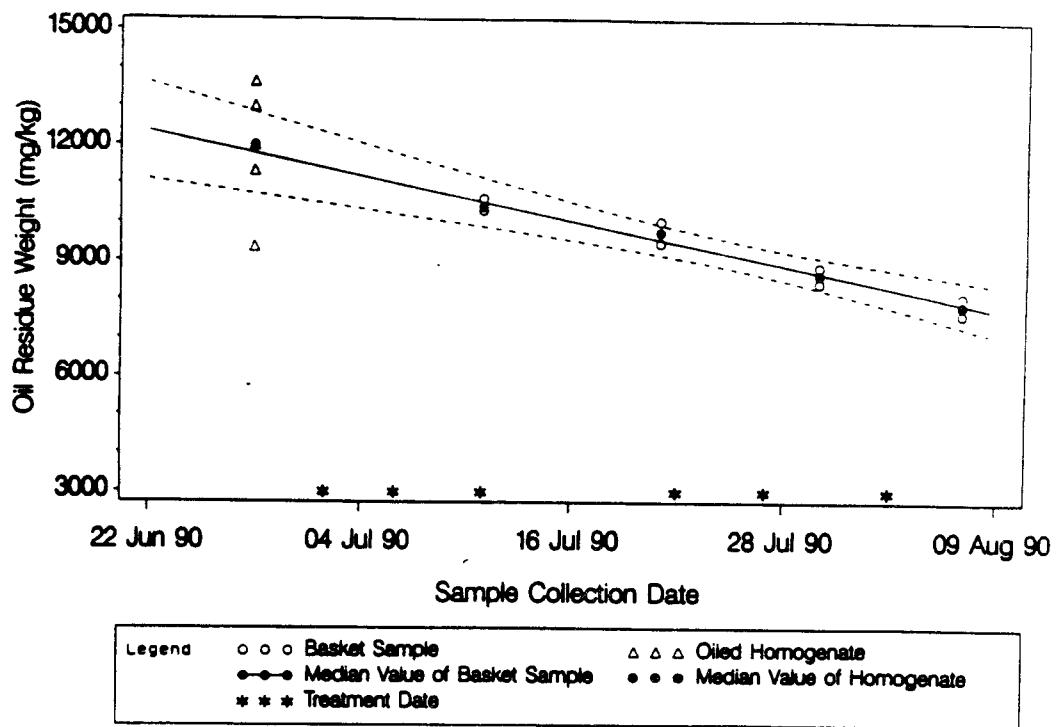
Figure 9.4a. Change in Oil Residue Weight Through Time for the Sprinkler Beach Oiled Subsurface Layer at Elrington Island.
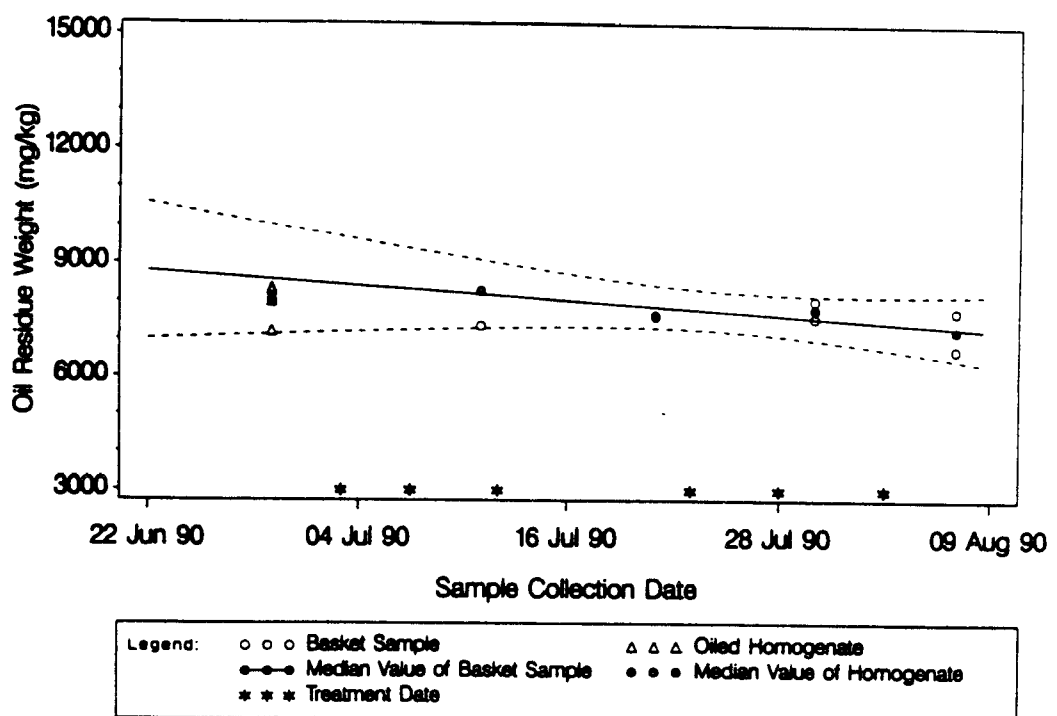
Figure 9.4b. Change in Oil Residue Weight Through Time for the Untreated Control Beach Oiled Subsurface Layer at Elrington Island.

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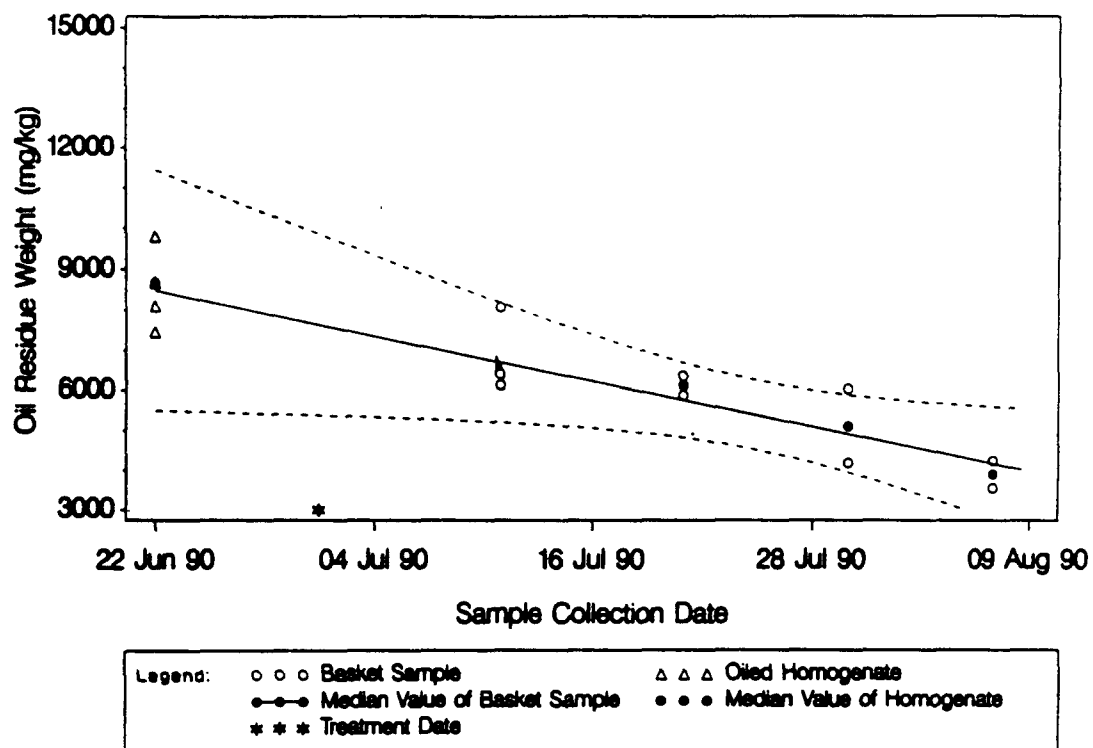
Figure 9.4c. Change in Oil Residue Weight Through Time for the Bath Beach Oiled Subsurface Layer at Elrington Island.

mg/kg (Passage Cove results showed good first-order kinetics with starting oil concentrations of approximately 1,000 mg/kg). Since less than a single half-life of decay for the most active beach had occurred during the test period, it is also possible that first-order kinetics were not yet distinguishable.

Using a zero-order model, the difference in decay rates between the treated and untreated beaches is very pronounced. A statistical analysis of the decay curves (regression line) is shown in Table 9.1. At the 95% confidence level, the slopes of the decay curves for the treated beaches were significantly different from the untreated Control, but were not significantly different from each other. The slope of a linear regression on these decay curves produced rates of 118 mg of oil/kg of beach material/day for the Bath beach, and 103 mg/kg/day for the Sprinkler beach. These rates were approximately 6 to 7 times faster than the rate calculated for the untreated Control beach (approximately 16 mg/kg/day), representing the largest fertilizer-enhanced oil removal yet observed.

TABLE 9.1. RESIDUE DECAY RATES FOR ELRINGTON ISLAND

Beach Plot Treatment	Rate (mg/kg/day)	Standard Deviation	Significance
Bath	-118	8	0.0008
Sprinkler	-103	6	0.0003
Control	-16	7	0.09

For the Bath and Sprinkler beaches, values for the treatment day are extrapolated from pretreatment values based on rate for the untreated Control beach.

Comparing the information with results from the Summer 1989 study in Passage Cove, the rate of oil disappearance on the untreated Control beach was 2.3 times the value (7 mg/kg/day) obtained from a similar untreated test plot in Passage Cove. However, the Passage Cove results were for oil residue on the cobble subsurface. The decay rate for oil in the mixed sand and gravel under the cobble was essentially zero during the Summer 1989 test period, thus indicating an even further difference between the two summer seasons. Possibly the physical mixing of the beach material prior to placement in the sampling baskets, or greater colonization of the oil by oil-degrading microorganisms could account for this considerable difference. We tend to believe the former. Any

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mechanical mixing of the beach material would almost certainly increase oil biodegradation rates because of increased availability of oil to the microorganisms. On the other hand, considering that the rates remained relatively constant throughout the test duration, it suggests that the initial mixing provided a continuously favorable situation for biodegradation (which would not be expected).

Despite the high background of oil residue decay in the untreated Control beach samples, the effect of applying fertilizer was considerable. A single pulse application of fertilizer was as successful as multiple pulse applications, at least over a period of approximately 3 to 4 weeks. This verifies similar results observed in laboratory studies (see Section 10). The mechanism responsible for the effective single pulse is not known, but we speculate that perhaps it involves a process of luxury nutrient uptake by the oil-degrading microorganisms, followed by a recycling of the nutrients within the matrix of the microbial communities. Whether this phenomena occurred during the summer of 1989 is currently unclear. It may have taken an entire season for the colonization of the oil by bacteria (and their potential nutrient limitation) (i.e., more oil carbon per bacterial cell, but no corresponding increase in nutrients), to reach a point where a pulse of nutrients was effective. On the other hand, much of the success of the bioremediation during the summer of 1989, particularly in August, could be attributed to this pulse phenomena (i.e., if the fertilizer formulations used on the beaches released much of their nutrients soon after application, the bioremediation response we observed may have been more controlled by this initial pulse rather than a sustained nutrient release).

Oil residues in the "unoiled" layers of the sampling baskets (we were aware at the beginning of the field test that the "unoiled" beach material did contain small amounts of oil) and at the interface between the oiled and unoiled layers were also examined. The results from the "unoiled" layers are shown in Figures 9.5 and 9.6. Samples from the layer interfaces were quite variable in oil concentration due to the difficulty in sampling uniformally at the interface, and are not presented.

Concentrations of oil in the "unoiled" layers (top and bottom) ranged from 400 to 1,000 mg/kg, or approximately 4 to 8% of the oil concentration in the middle oiled layer. Interestingly, there was a trend toward increasing concentrations in the bottom layer with time, particularly for the treated beaches. Concentrations on the last sampling date were very high for the Bath beach. The trend was not present in the top layer. The possibility of oil entering only the bottom layer baskets from the surrounding oiled beach material is unlikely. Movement of oil from the oiled layer (middle layer) is more reasonable, but it is not related to the sprinkling operation, as the effect is most pronounced in samples from the Bath beach which received only a single initial application of nutrients. Somewhat
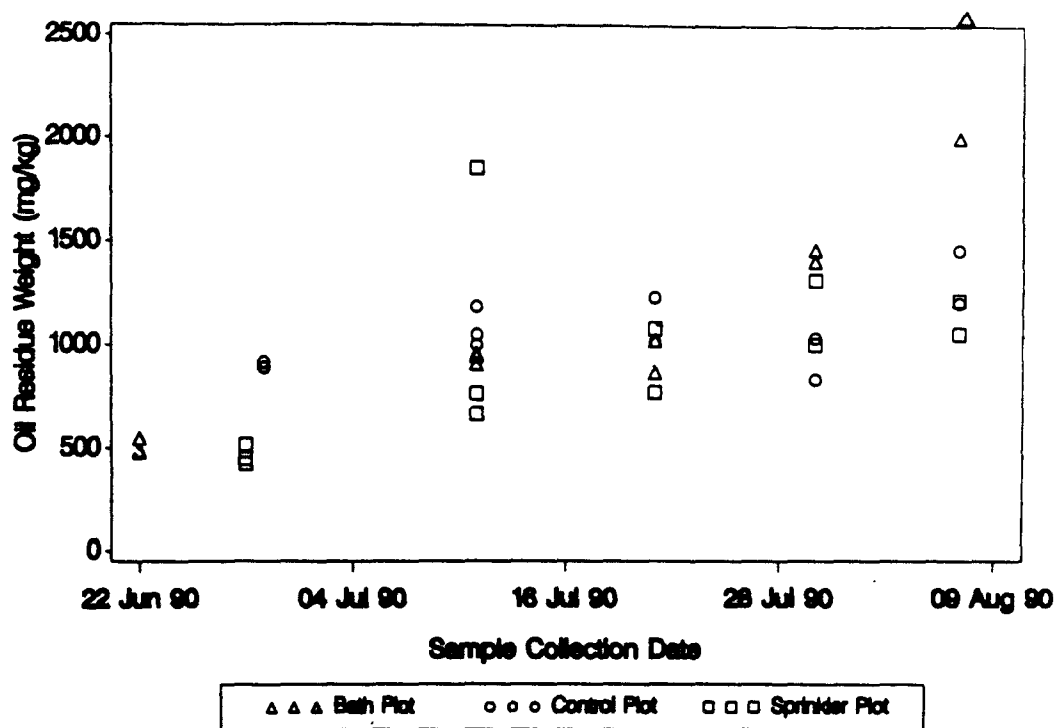
Figure 9.5. Change in Oil Residue Weight Through Time for the Bottom Unoiled Layer for All Beaches at Elrington Island.
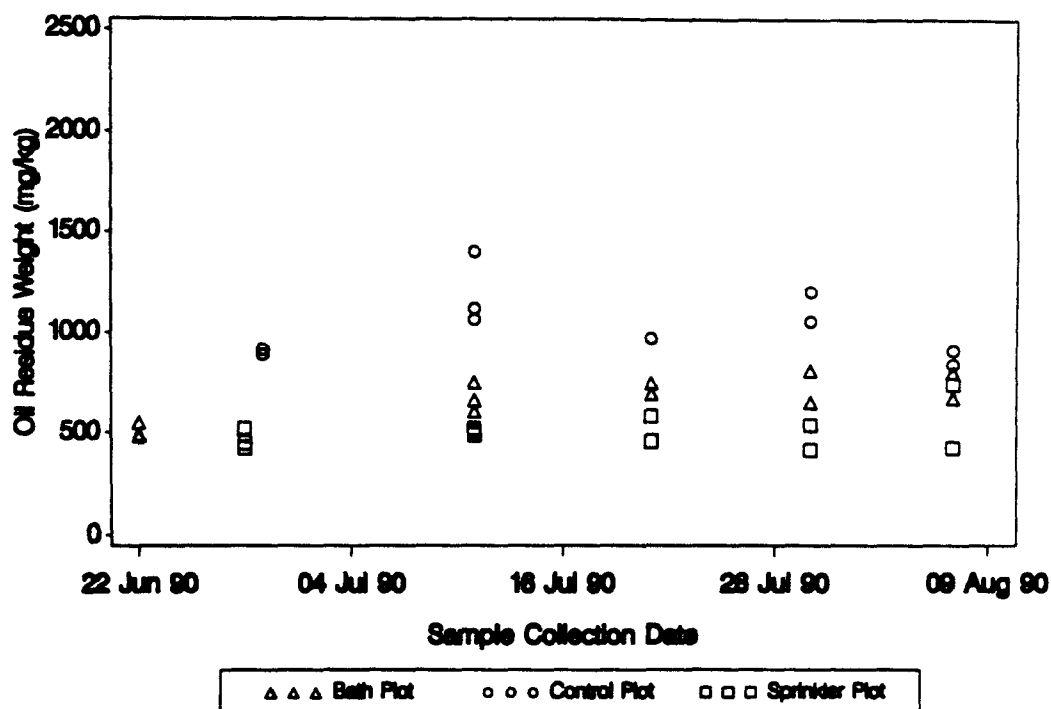
Figure 9.6. Change in Oil Residue Weight Through Time for the Top Unoiled Layer for All Beaches at Elrington Island.

smaller sampling baskets were used on the Bath beach, but it is unlikely this affected either subsampling or physical separation of the layers. However, the effect could be related to biodegradation of the oil, if it is assumed that oil loses its sticky qualities after extensive degradation and is then free to migrate in the beach material (such as through the physical action of gravity and tidal movement). This would mean that the hydrocarbon composition of the oil in the bottom layer could change significantly, even though oil residues increased. This possibility is currently under investigation.

The fact that oil residues in the top layer remain relatively constant is also interesting since there are probably as many oil degraders in this layer as there are in the middle oiled layer, and yet there is little evidence of oil decay from biodegradation. The oil concentrations, however, may be too low to see significant changes over the short time period of this field test.

Oil Composition

To verify that loss of oil residue weight from the sampling baskets was accompanied by changes in oil composition (biodegradation), the loss of selected alkanes over time was examined. Changes in the concentrations of individual alkanes nC18 to nC27, the sum of alkanes nC18 to nC27, phytane, and the nC18/phytane ratio, are shown in Figures 9.7 to 9.19 for the three test beaches. Note that the oil composition at t=0 was estimated for each beach because the sampling baskets were placed in the beaches at different times prior to fertilizer application (the result of logistical complications). Therefore, starting oil compositions were based on the hydrocarbon decay rate of the untreated Control beach and extrapolated from the data obtained between the time of basket placement and initiation of fertilizer application. The responses are quite interesting. On all beaches large decreases in the nC18/phytane ratios were observed (Figure 9.19), strongly suggesting effects due to biodegradation (see Section 8). Table 9.2 gives the mean nC18/phytane ratios at each sampling time and compares statistical differences between beaches. Initial ratios were not statistically different from each other. Thus, even though the sampling baskets on each beach started with a different oil concentration, the oil was equally degraded in all cases.

Changes in the nC18/phytane ratios from treated beaches was quite dramatic--decreasing an order of magnitude on the Bath beach in the first 7 days, and almost an order of magnitude on the Sprinkler beach in the first 17 days. The greater change on the Bath beach was statistically significant, as were the changes on the treated beaches relative to the untreated control. The extent

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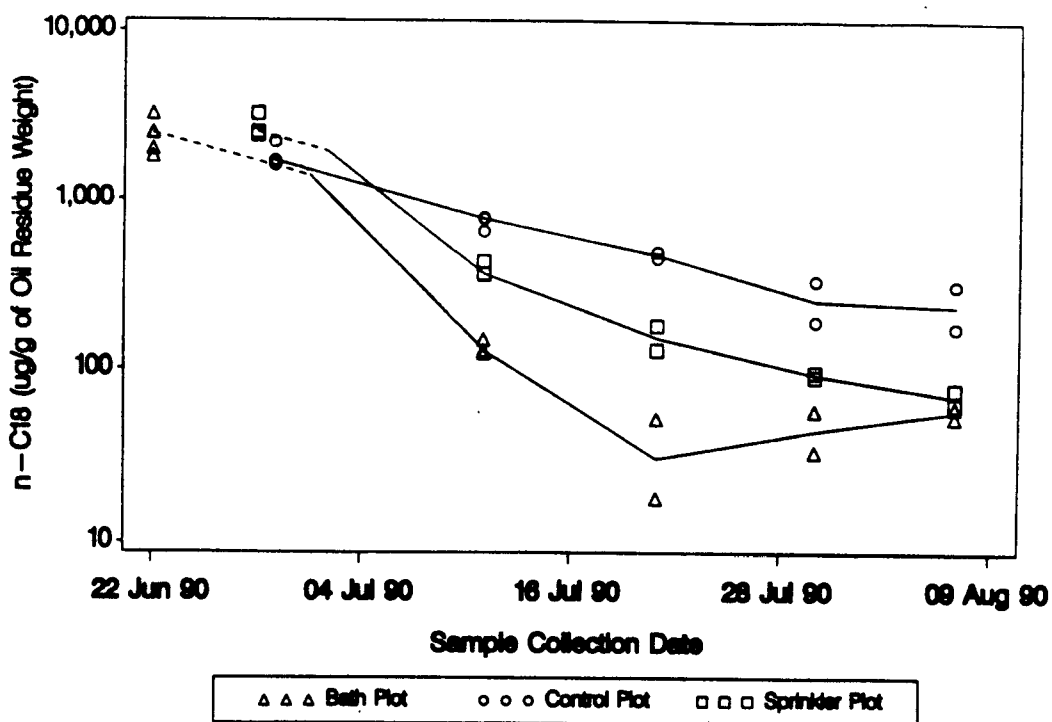
Figure 9.7.   Change in the nC18 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
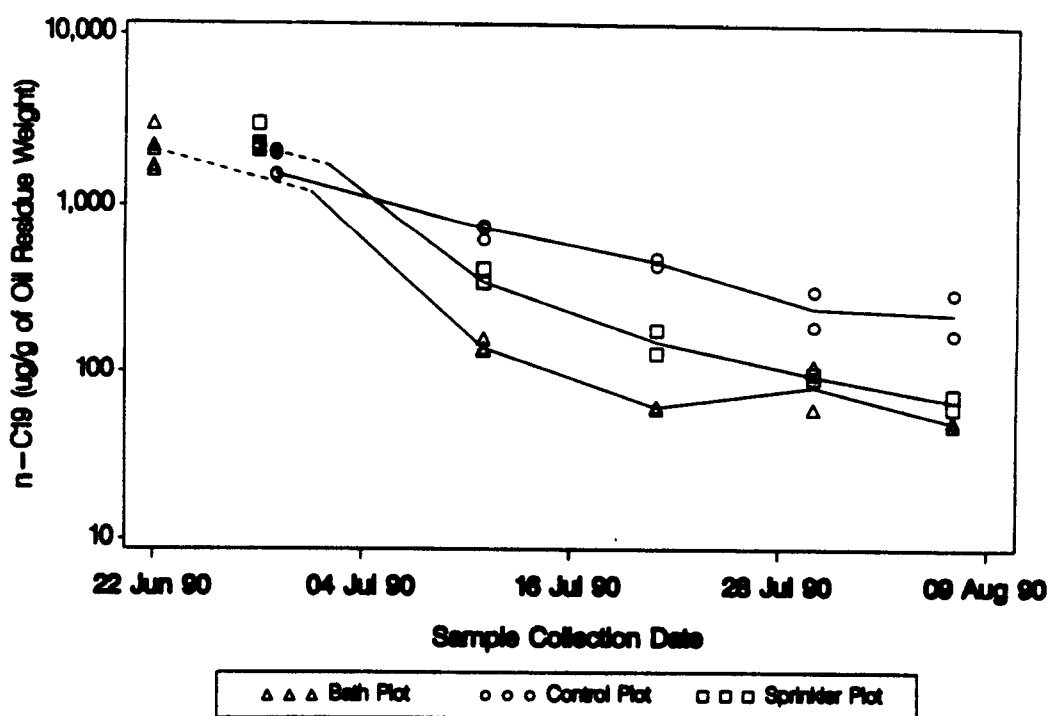
Figure 9.8.   Change in the nC19 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
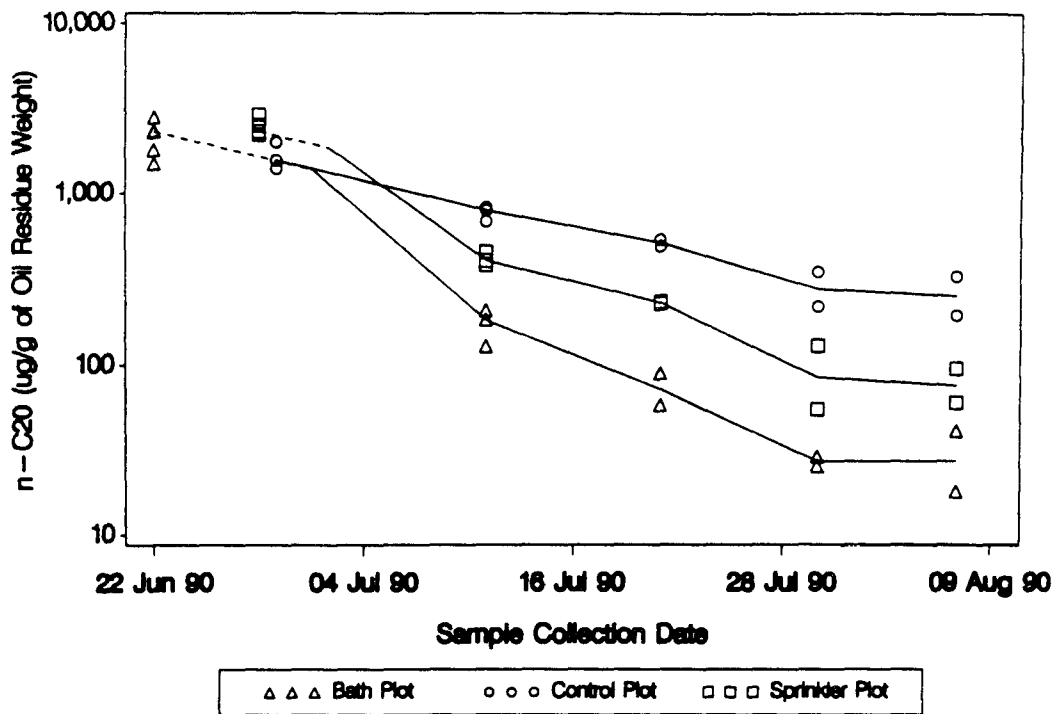
Figure 9.9. Change in the nC20 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
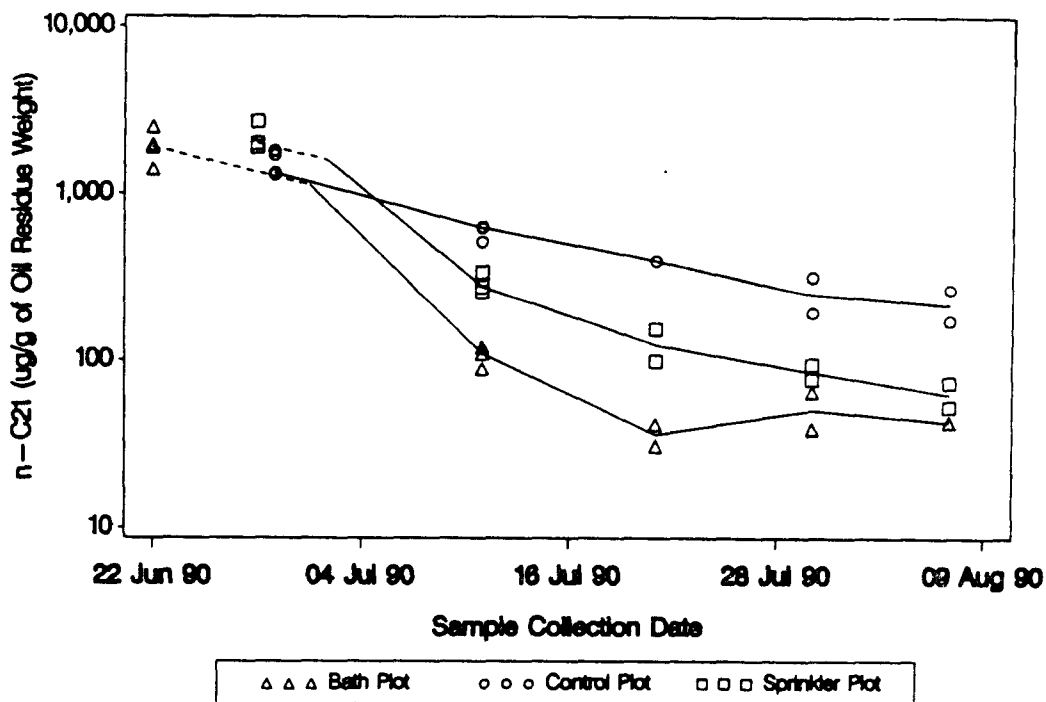
Figure 9.10. Change in the nC21 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
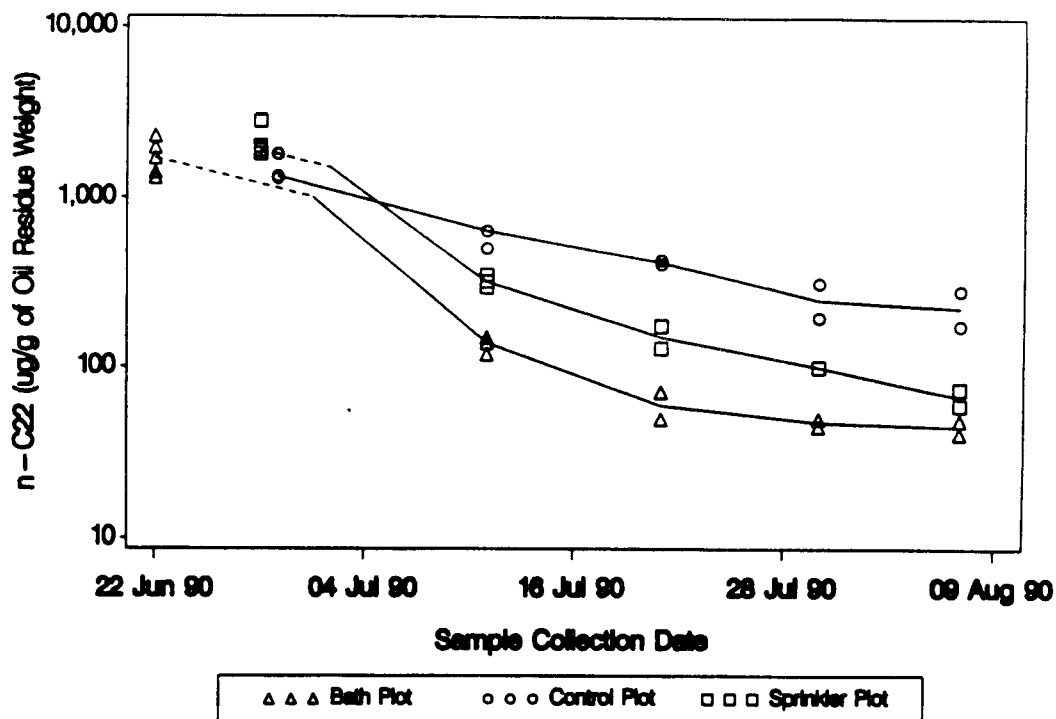
Figure 9.11. Change in the nC22 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
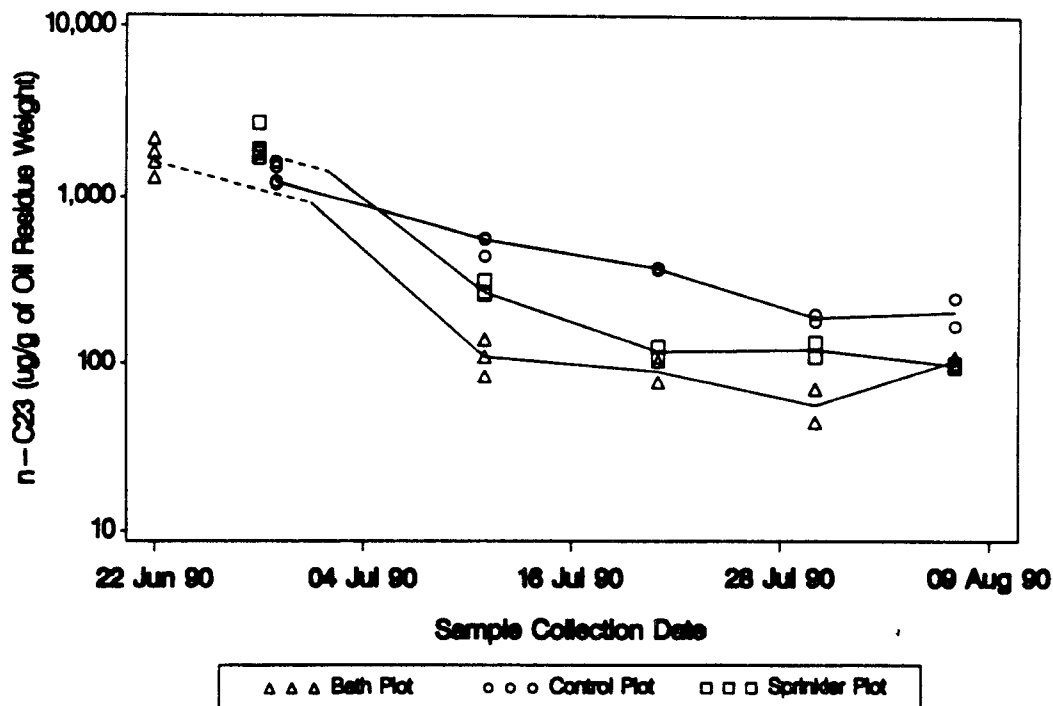
Figure 9.12. Change in the nC23 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
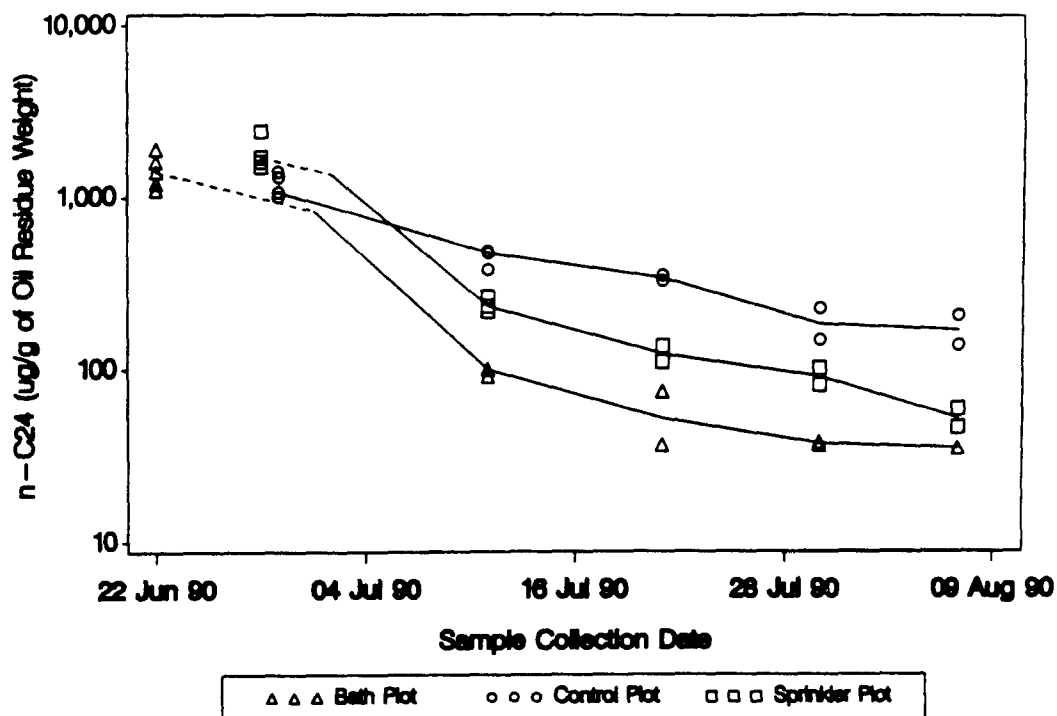
Figure 9.13. Change in the nC24 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
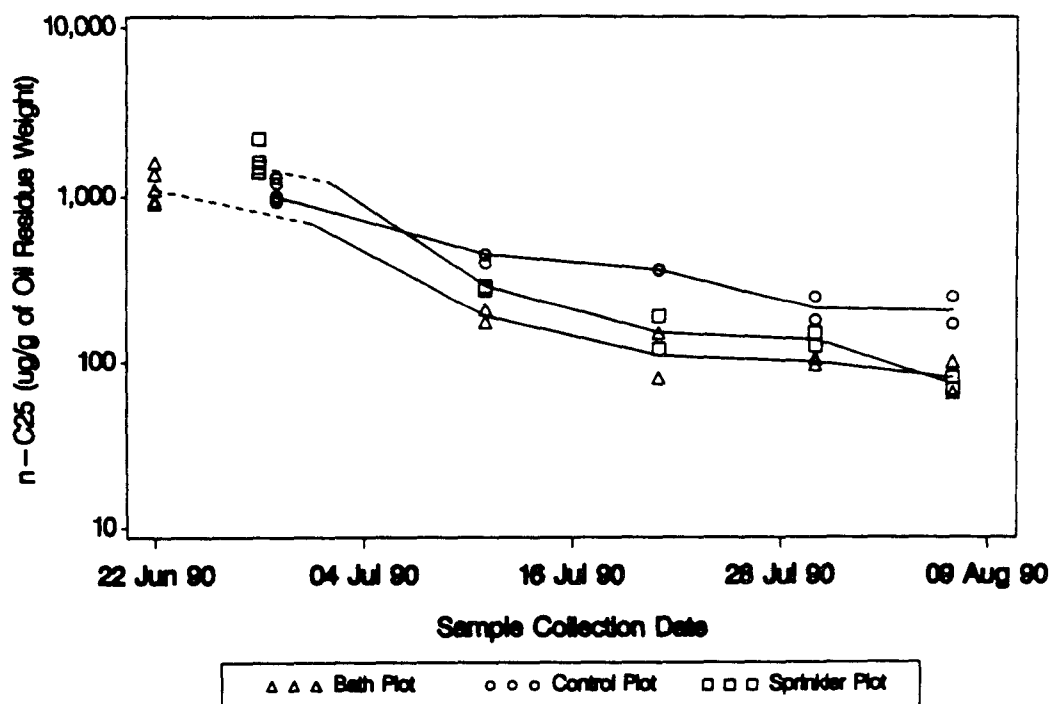
Figure 9.14. Change in the nC25 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
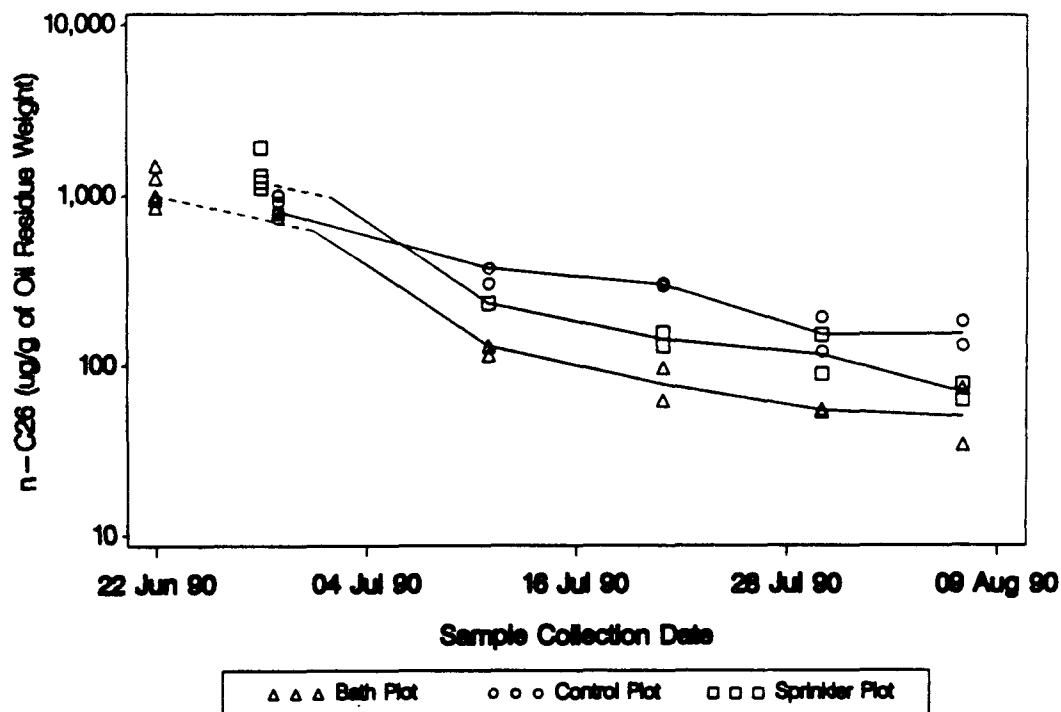
Figure 9.15. Change in the nC26 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
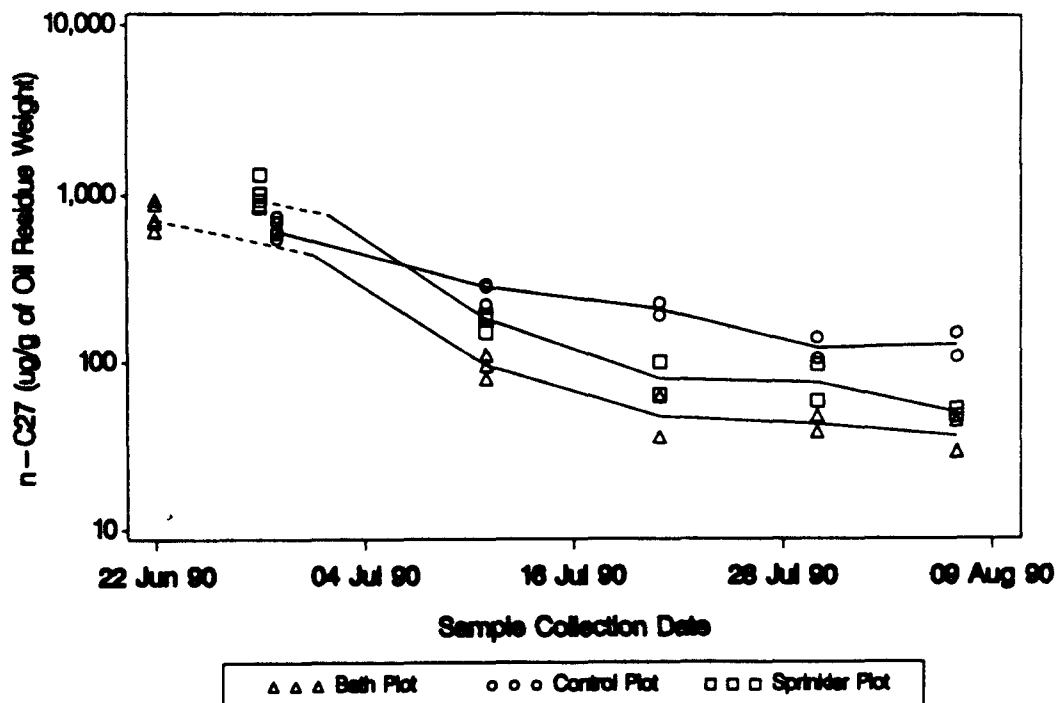
Figure 9.16. Change in the nC27 Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).

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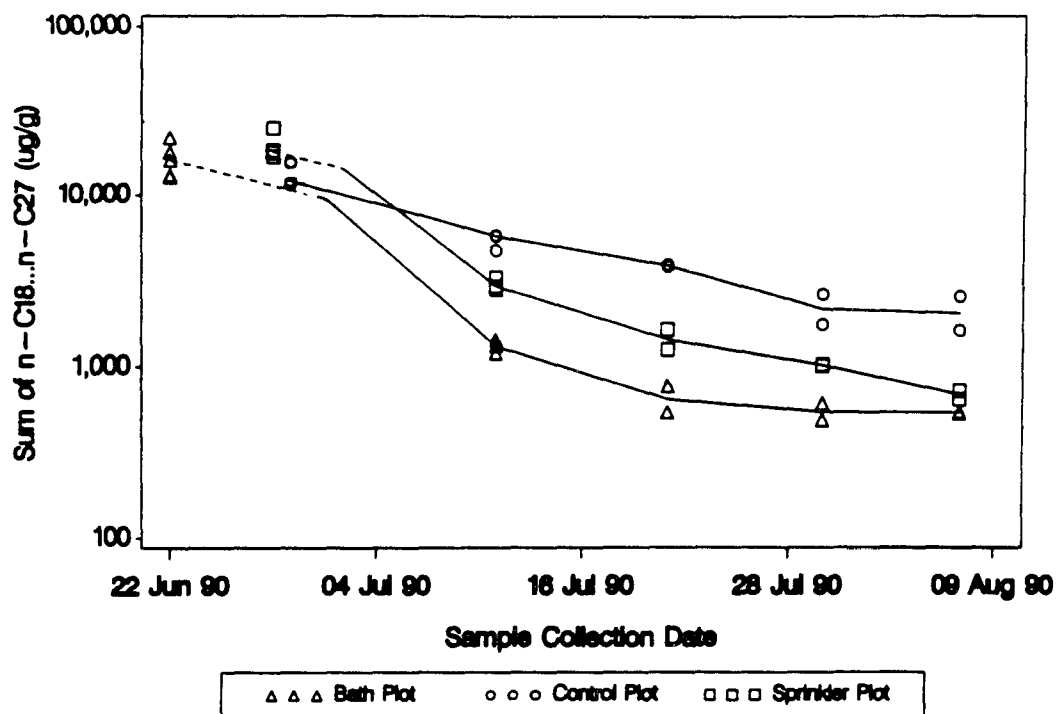
Figure 9.17. Change in the Sum of the Alkane Concentration for nC18 to nC27 Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
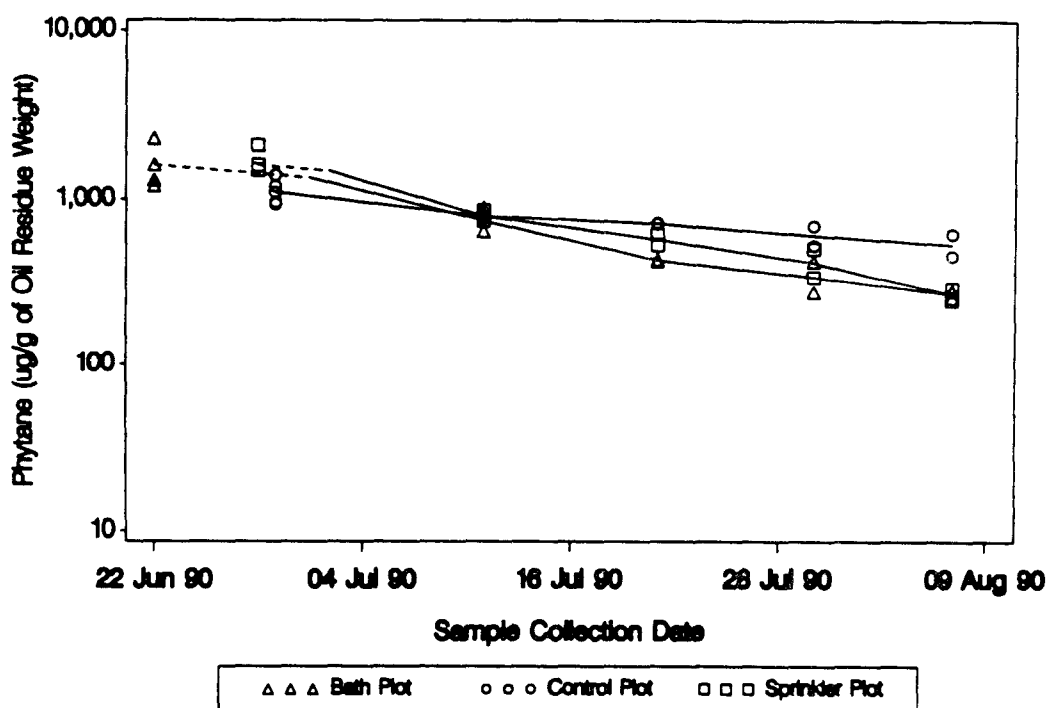
Figure 9.18. Change in the Phytane Concentration Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).
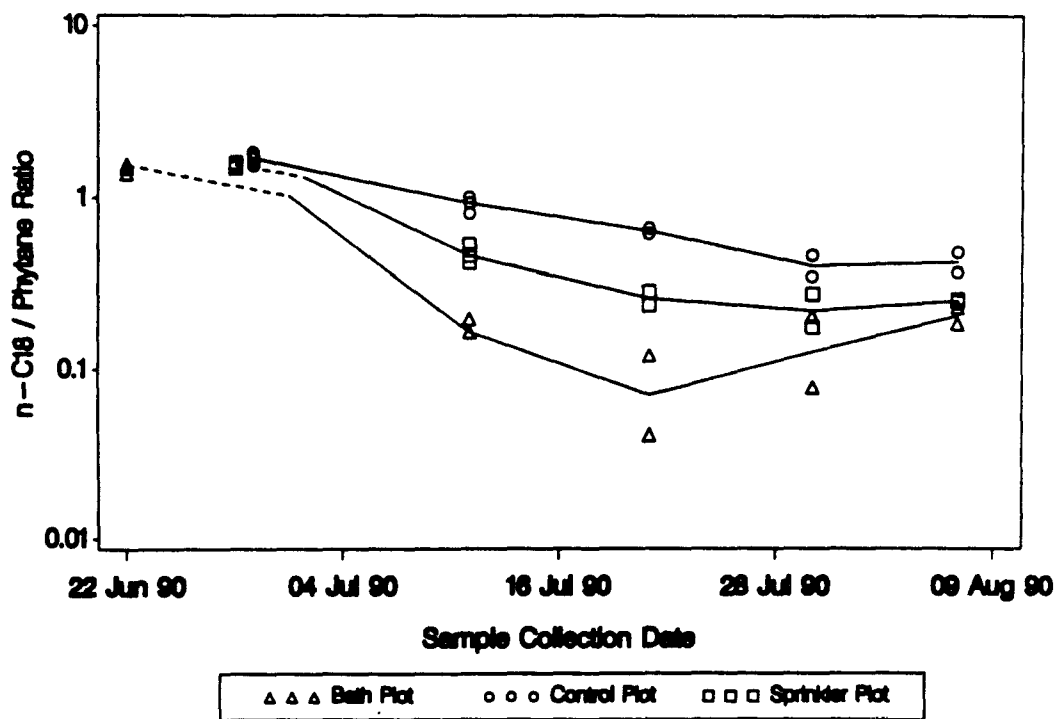
Figure 9.19. Change In the nC18/phytane Ratio Over Time for the Oiled Subsurface Layer for All Beaches at Elrington Island (Dashed Lines Extrapolate Control Plot Decay Rate for Treated Beaches to Date of Fertilizer Application).

TABLE 9.2. ANALYSIS OF BEACH PLOT DIFFERENCES BY DATE FOR ELRINGTON ISLAND BASED ON nC18/PHYTANE RATIO

Beach	Mean	Std. Dev.	Comparison	t-value	Sig. level 0.95	0.90
Date: Pretreatment			Degrees of freedom: 8			
Bath	1.506	0.068	Spr.-Bath	0.536	–	–
Sprinkler	1.548	0.039	Control-Bath	1.263	–	–
Control	1.678	0.118	Control-Spr.	1.046	–	–
Date: 11-July-1990			Degrees of freedom: 4			
Bath	0.180	0.017	Spr.-Bath	4.763	*	*
Sprinkler	0.477	0.060	Control-Bath	7.621	*	*
Control	0.923	0.096	Control-Spr.	3.940	*	*
Date: 21-July-1990			Degrees of freedom: 2			
Bath	0.080	0.057	Spr.-Bath	2.766	–	*
Sprinkler	0.265	0.035	Control-Bath	8.976	*	*
Control	0.650	0.028	Control-Spr.	8.590	*	*
Date: 30-July-1990			Degrees of freedom: 2			
Bath	0.145	0.092	Spr.-Bath	0.731	–	–
Sprinkler	0.230	0.071	Control-Bath	2.239	–	*
Control	0.415	0.078	Control-Spr.	1.754	–	–
Date: 07-August-1990			Degrees of freedom: 2			
Bath	0.210	0.028	Spr.-Bath	1.559	–	–
Sprinkler	0.255	0.007	Control-Bath	2.715	–	*
Control	0.435	0.078	Control-Spr.	2.298	–	–

Critical values for a one-sided t-test at 95% and 90% confidence levels.

n	alpha (0.05)	alpha (0.10)
8	1.860	1.397
4	2.132	1.533
2	2.920	1.886

of change in the ratios also seemed to maximize at different times--the Bath beach maximized at the first sampling, the Sprinkler beach at the second sampling, and the untreated Control beach at the third sampling. At the third sampling, ratios were statistically indistinguishable at the 95% confidence level. Thus biodegradation of the alkanes was greatly enhanced by the fertilizers and ultimately a single pulse of fertilizer at the higher nutrient concentration produced the best effect.

We will assume for further discussion that changes in other hydrocarbons are also caused by biodegradation. Similar extents of biodegradation are reflected in the data shown in Table 9.3 and 9.4. At the 95% confidence interval, starting concentrations of nC18 and the summed alkanes, nC18 to nC27, were not statistically different, again suggesting similar extents of initial alkane degradation in oil from sampling baskets. As with the nC18/phytane ratio, hydrocarbon concentrations decreased very rapidly on treated beaches relative to the untreated control, and the single pulse application was more effective than multiple pulse applications. However, the effect of the Bath application appeared to maximize early, thus allowing the effect of sprinkler application to catch up--that is, by the last sampling, hydrocarbon concentrations on both beaches were not statistically different.

Comparing the biodegradation rate of individual hydrocarbons was complicated, due to large differences in the decay curve response. Similar types of hydrocarbon decay curves were seen in the Passage Cove study. Rates of biodegradation from the Elrington Island study appeared first-order only for the untreated Control beach. Treated beaches were decidedly mixed-order, indicating that biodegradation rate constants were becoming limited by more than just substrate. The much more slowly degraded phytane was an exception. It appeared first-order on all beaches. We believe that the mixed-order response on the treated beaches was probably caused by an extremely rapid degradation of the initial normal alkane concentration in the first 10 to 15 days following fertilizer application, with a slower degradation once the hydrocarbon concentrations approached detection limits. That is, by the first sampling and certainly by the second sampling, hydrocarbon concentrations had been reduced in most cases approximately ten-fold, bringing them very close to detection limits. Concentrations reported below 100 μg/g are based on very small peaks within the gas chromatography profiles and may not necessarily be the hydrocarbon in question. The ability to see further significant changes in concentration was unlikely, due to the small remaining concentrations.

TABLE 9.3. ANALYSIS OF BEACH PLOT DIFFERENCES BY DATE FOR ELRINGTON ISLAND BASED ON nC18 LINEAR ALKANE

Beach	Mean	Std. Dev.	Comparison	t-value	Sig. level 0.95	0.90
Date: Pretreatment			Degrees of freedom: 8			
Bath	3.370	0.097	Spr.-Bath	0.380	–	–
Sprinkler	3.412	0.053	Control-Bath	-2.861	–	–
Control	3.268	0.068	Control-Spr.	-1.670	–	*
Date: 11-July-1990			Degrees of freedom: 4			
Bath	2.129	0.042	Spr.-Bath	7.570	*	*
Sprinkler	2.589	0.043	Control-Bath	12.022	*	*
Control	2.869	0.045	Control-Spr.	4.579	*	*
Date: 21-July-1990			Degrees of freedom: 2			
Bath	1.486	0.326	Spr.-Bath	2.050	–	*
Sprinkler	2.185	0.100	Control-Bath	3.627	*	*
Control	2.672	0.026	Control-Spr.	4.713	*	*
Date: 30-July-1990			Degrees of freedom: 2			
Bath	1.644	0.169	Spr.-Bath	1.930	–	*
Sprinkler	1.972	0.018	Control-Bath	3.150	*	*
Control	2.399	0.170	Control-Spr.	2.498	–	*
Date: 07-August-1990			Degrees of freedom: 2			
Bath	1.757	0.040	Spr.-Bath	1.166	–	–
Sprinkler	1.843	0.062	Control-Bath	3.380	*	*
Control	2.367	0.176	Control-Spr.	2.808	–	*

Critical values for a one-sided t-test at 95% and 90% confidence levels.

n	alpha (0.05)	alpha (0.10)
8	1.860	1.397
4	2.132	1.533
2	2.920	1.886

TABLE 9.4. ANALYSIS OF BEACH PLOT DIFFERENCES BY DATE FOR ELRINGTON ISLAND BASED ON THE SUM OF THE LINEAR ALKANES FROM nC18 TO nC27

Beach	Mean	Std. Dev.	Comparison	t-value	Sig. level 0.95	0.90
Date: Pretreatment			Degrees of freedom: 8			
Bath	4.205	0.097	Spr.-Bath	-0.642	–	–
Sprinkler	4.281	0.068	Control-Bath	-0.721	–	–
Control	4.120	0.067	Control-Spr.	-1.687	–	*
Date: 11-July-1990			Degrees of freedom: 4			
Bath	3.118	0.039	Spr.-Bath	6.745	*	*
Sprinkler	3.476	0.036	Control-Bath	9.699	*	*
Control	3.733	0.050	Control-Spr.	4.170	*	*
Date: 21-July-1990			Degrees of freedom: 2			
Bath	2.810	0.108	Spr.-Bath	2.556	–	*
Sprinkler	3.155	0.081	Control-Bath	7.156	*	*
Control	3.585	0.008	Control-Spr.	5.283	*	*
Date: 30-July-1990			Degrees of freedom: 2			
Bath	2.736	0.070	Spr.-Bath	3.847	*	*
Sprinkler	3.006	0.005	Control-Bath	4.183	*	*
Control	3.328	0.123	Control-Spr.	2.616	*	*
Date: 07-August-1990			Degrees of freedom: 2			
Bath	2.729	0.007	Spr.-Bath	2.886	–	*
Sprinkler	2.832	0.035	Control-Bath	4.124	*	*
Control	3.303	0.139	Control-Spr.	3.286	*	*

Critical values for a one-sided t-test at 95% and 90% confidence levels.

n	alpha (0.05)	alpha (0.10)
8	1.860	1.397
4	2.132	1.533
2	2.920	1.886

In the untreated Control beach, biodegradation remained first-order throughout the test period, presumably because degradation was slower. Concentrations of hydrocarbons never reached detection limits during the duration of the test, and thus decay rates could be calculated based on all the sampling periods. The same can also be said for phytane.

For comparative purposes, decay rates for all three test beaches were examined only during the first sampling period (day 0 to 7). Table 9.5 shows the estimated rates for nC18, the summed alkanes nC18 to nC27, and phytane. The difference between treated and untreated beaches was approximately a factor of three. This is considerably different from the residue weight results in which differences were 6 to 7. Interestingly, the data from Passage Cove also showed much less of a difference in rate of hydrocarbon composition change, compared to changes in residue weight, between treated and untreated beaches. However, Passage Cove rates were approximately 50 to 100 times slower (compare with Table 7.6).

TABLE 9.5. ESTIMATED DECAY RATES IN MG/KG/DAY FOR SELECTED HYDROCARBONS BASED ON CONCENTRATION CHANGES BETWEEN t = 0 and t = 7 DAYS

	Untreated Control Beach	Sprinkler Beach	Bath Beach
nC18	1.3	3.8	3.1
Summed Alkanes (nC18 to nC27)	9.0	28.0	20.3
phytane	0.010	0.037	0.035

The higher absolute rates of compositional change on the Sprinkler beach relative to the Bath beach differed from the results above. This result was generated by normalization to oil residue weights on each beach, and shows that percent change in hydrocarbon composition was much greater at the Sprinkler beach.

In summary, changes in hydrocarbon composition in samples taken from the treated and untreated beaches at Elrington Island reflected the same trends in biodegradation enhancement as seen with measures of the residue weight changes. Fertilizer addition enhanced oil biodegradation considerably. This enhancement was more apparent in measures of oil residue weight than measures of hydrocarbon compositional change. In addition, the effect of the single pulse application was less effective than the multiple pulse applications, when gauged with changes in hydrocarbon composition normalized to oil residue weights. Thus, multiple pulse applications of the fertilizer solutions led to a greater percent degradation in hydrocarbon composition.

Interestingly, the biodegradation of the normal alkanes was apparently not directly coupled to decreases in oil residue weight; reduction in oil residue continued long after most of the normal alkanes had been degraded. Phytane degradation actually provided a better correlation with changes in oil residue weight.

MICROBIOLOGY

Numbers of Oil-Degrading Bacteria

Numbers of oil degraders were assessed using the MPN sheen screen test developed by researchers at the University of Alaska, Fairbanks, and the ADEC Oil Spill Response Support Laboratory in Valdez. Results are shown in Table 9.6. As observed in other studies in Prince William Sound, the number of oil degraders was approximately 10^5 to 10^6 cells per gram of beach material and were quite variable. There was no consistent effect of the fertilizer application on the degrader numbers. The large numbers of oil degraders in the oiled layer, however, suggests that the high concentration of oil in the beach material does not prevent colonization by oil-degrading microorganisms. Determination of total culturable heterotrophic bacteria was also performed on the oiled samples, and the oil degraders represented approximately 1 to 10% of this population.

TABLE 9.6. NUMBER OF OIL DEGRADERS (MPN'S X 10^4) IN SAMPLES
FROM ELRINGTON ISLAND

Beach	Basket Layers[a]	July 11	July 21	July 30	August 7
Untreated Control	Interface (Top)	18.1	7.7	88.3	23.0
	Oiled	14.6	18.5	18.9	357.4
	Interface (Bottom)	14.0	5.3	182.2	32.3
Bath	Interface (Top)	11.4	11.3	5.2	17.5
	Oiled	11.5	3.4	256.2	52.7
	Interface (Bottom)	0.9	134.3	163.7	228.6
Sprinkler	Interface (Top)	N.R.[b]	17.5	13.7	34.2
	Oiled	15.2	13.9	183.2	35.3
	Interface (Bottom)	14.1	11.6	316.1	83.1

[a]Oiled = Oiled layer; Interface = Material between the oiled and unoiled layer, top and bottom of the oiled layer.
[b]N.R. = Not reported.

Microbial Activity

In addition to chemical analysis of the samples taken from the sampling baskets, measures of microbial activity were also performed using several approaches. In one case, 100 g beach samples were placed in special biometer flasks, and the total amount of carbon dioxide produced over a three-day period was measured. No nutrients or fertilizer were added to the flasks. CO_2 production measured in contaminated beach material taken from sampling baskets removed from the beach on July 11, July 21, July 30, and August 7 (10, 20, 29 and 37 days after fertilizer application respectively), is shown in Figures 9.20a-d. Cumulative CO_2 production was approximately linear over the 72-hour incubation period. The differences in total CO_2 production mimic the oil chemistry results: the Bath beach showed the highest activity but only slightly greater than the Sprinkler beach. Both treated beaches were substantially more active than the untreated control.
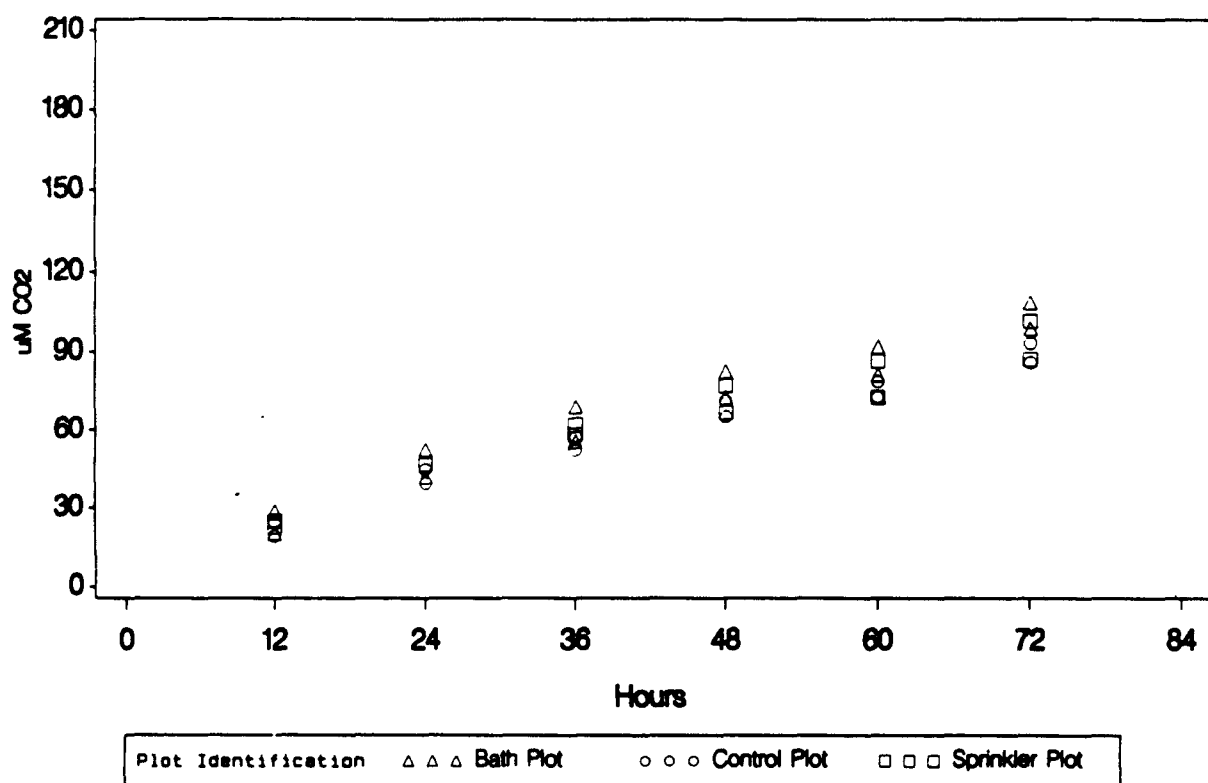
Figure 9.20a. Change in the CO₂ Concentration Over Time for all Beaches at Elrington Island on July 11, 1990.
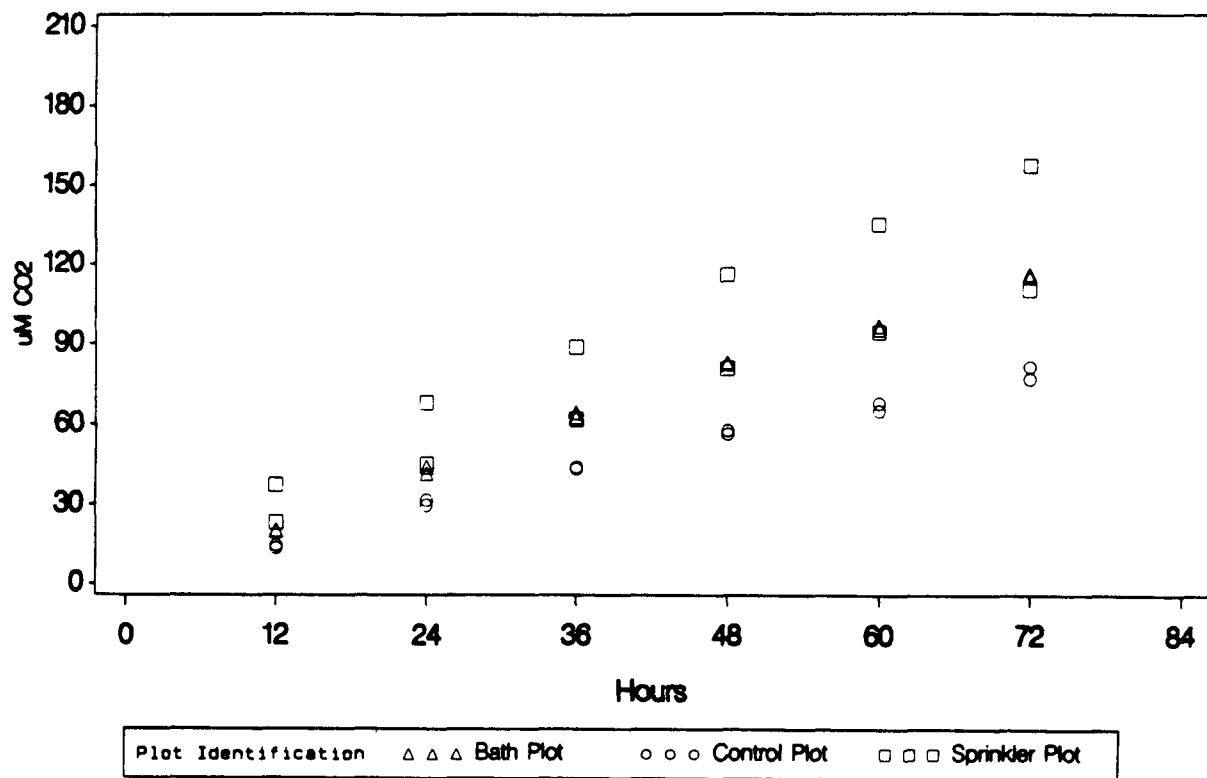
Figure 9.20b. Change in the CO$_2$ Concentration Over Time for all Beaches at Elrington Island on July 21, 1990.
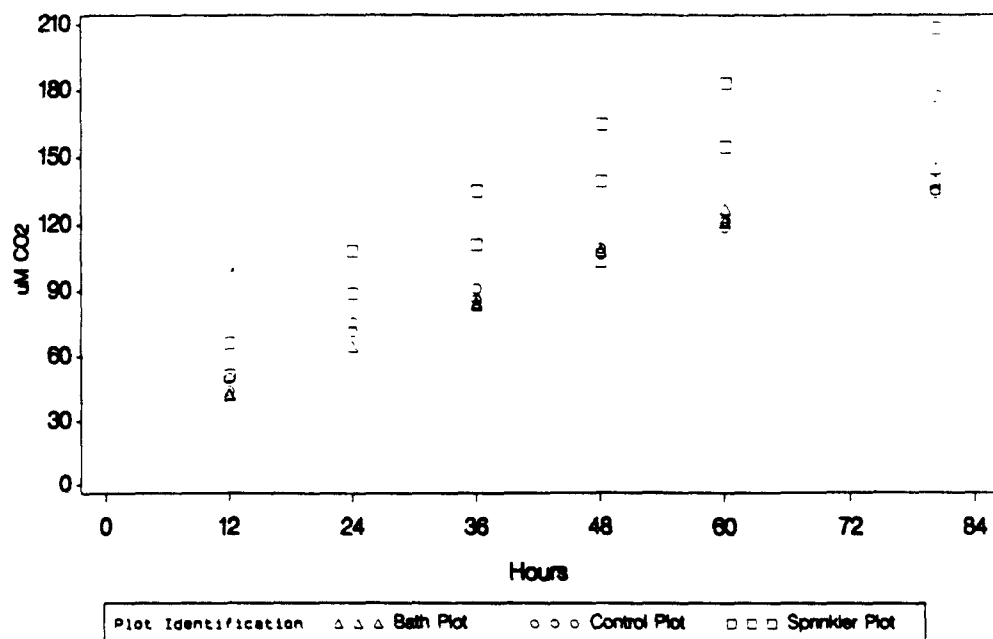
Figure 9.20c. Change in the CO_2 Concentration Over Time for all Beaches at Elrington Island on July 30, 1990.
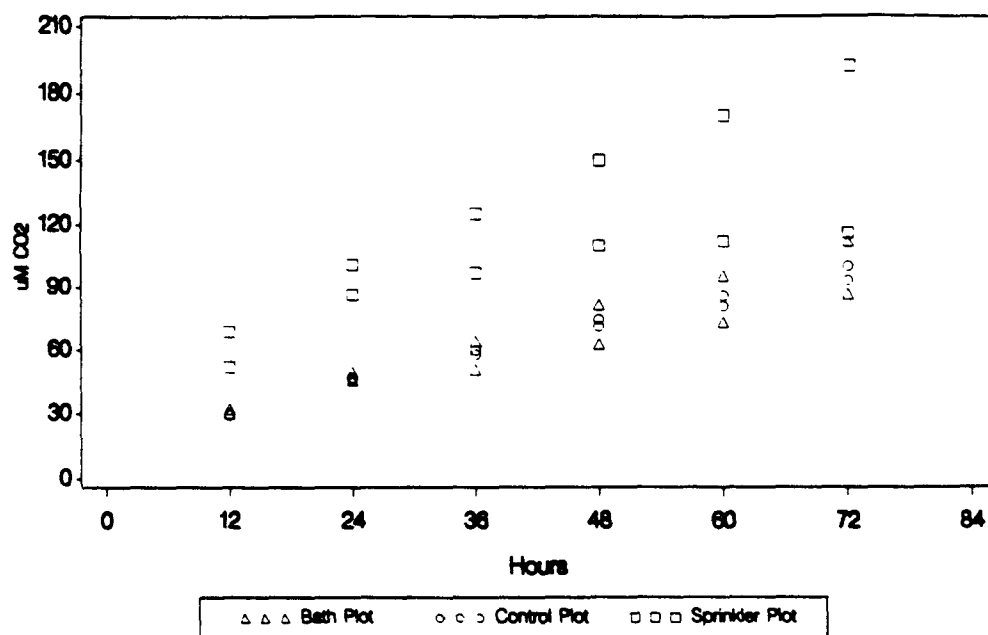
Figure 9.20d. Change in the CO_2 Concentration Over Time for all Beaches at Elrington Island on August 7, 1990.

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Table 9.7 summarizes the rates of CO_2 production (slopes of regression lines fit to the data points) for the four sampling periods. The substantial CO_2 production reflects the vigor with which biodegradation occurred, and strongly supports biodegradation as the primary factor affecting changes in oil chemistry. Comparison of the mineralization rates between beaches at t = 0 (data not shown) indicated that rates were very similar, suggesting that preparation of the beach material for the sampling baskets on different days resulted in excellent homogeneity in initial microbial activity. With time, the difference in mineralization rates (CO_2/day/100 g of beach material) between the fertilizer-treated beaches and the untreated control increased, and were statistically significant at the 95% confidence level. Note the mineralization rates on the third sampling (July 30) for all three beaches appear to be unusually high, and the high mineralization rates on the Sprinkler beach seemed to be maintained through the final sampling. This was not true for the Bath beach where rates appeared to drop to untreated Control beach levels. It is possible that the effect of the single pulse fertilizer application had waned on the Bath beach.

TABLE 9.7. MINERALIZATION RATES FROM TOTAL CO_2 PRODUCTION
(μ MOLES/100 g/hour) AND CALCULATED OIL DEGRADATION (mg/kg/day)[a]
IN OILED BEACH MATERIAL TAKEN FROM SAMPLING BASKETS ON ELRINGTON ISLAND

Sampling Date	Untreated Control Beach CO_2	Oil	Sprinkler Beach[b] CO_2	Oil	Bath Beach[c] CO_2	Oil
7/11	5.69	16.4	5.89	16.9	6.53	18.8
7/21	5.47	15.7	8.73	25.1	7.96	22.9
7/30	7.58	21.8	11.55	33.3	8.31	23.9
8/7	5.41	15.6	7.29	20.9	5.62	16.1

[a] Based on oil as hexadecane or 85% carbon.
[b] Fertilizer application occurred on 7/2, 7/6, 7/11, 7/22, 7/27, and 8/3, 1990.
[c] Fertilizer application occurred on 7/1/90.

When the CO_2 production rates are converted to oil carbon degraded, values for the untreated Control beach were generally in close agreement with changes in the oil residue weight (Table 9.1). This suggests that oil biodegradation was largely responsible for the changes in residue weight. Moreover, the carbon turnover rates were almost twice as fast as that for the degradation of the summed alkanes (Table 9.5). To account for this additional CO_2, one would have to assume that natural biodegradation of the oil involves more than just metabolism of the straight chain alkanes.

In a similar manner carbon turnover rates, as determined by CO_2 mineralization in samples from the fertilizer-treated beaches, were approximately one-fifth the rates for changes observed for the oil residue weights. On the other hand, they are in the same range as the initial degradation rates for the summed alkanes. It is concluded at this time that fertilizers significantly enhanced biodegradation of the alkane fraction of the oil, but may have had a disproportionate positive effect on the degradation of total oil residues. Thus, as argued above, extensive biodegradation of the oil leads to a change in oil consistency and stickiness that then allows degraded residues to be scoured from the beach material by tidal action.

A second type of measurement involved the mineralization of ^{14}C labeled phenanthrene, a substrate indicative of the biodegradation of PAHs in the oil. The beach material used for the measurement of total carbon dioxide production in the biometer flasks was amended with the labeled substrate (see Section 4, Methods) and the production of radiolabeled CO_2 was measured over a four day period. Figures 9.21a-c show that phenanthrene biodegradation was immediate and extensive, indicating active PAH biodegradation in the subsurface beach material. CO_2 production curves are hyperbolic, indicating depletion of the substrate with time. In all cases the greatest extent of phenanthrene mineralization occurred in samples from the Bath beach, followed by the Sprinkler and untreated Control beaches, respectively. Thus, fertilizer application also significantly enhanced PAH biodegradation. With time, the difference between samples from treated and untreated beaches increased. This further supports the effectiveness of a pulse application of fertilizer, and suggests that enhanced biodegradation at the Bath beach (higher concentration of nutrients) was due to the amount of fertilizer applied at one time, as compared to more frequent applications at lower concentrations.
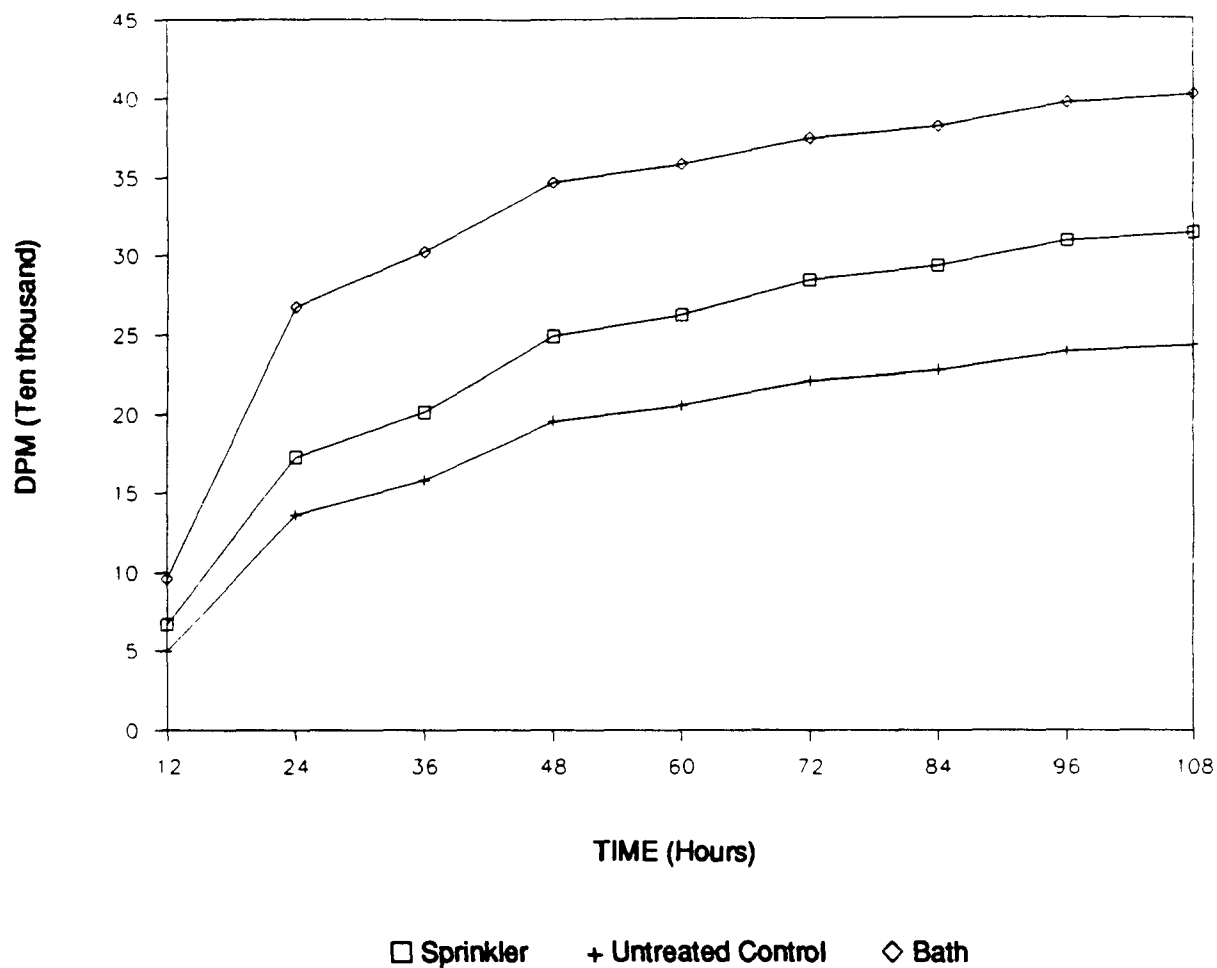
Figure 9.21a. Production of Radiolabeled CO_2 from Phenanthrene Through Time in Oiled Beach Samples Taken From the Sampling Baskets (Layer 3) on 7/11/90, for the Sprinkler, Untreated Control, and Bath Beaches at Elrington Island.

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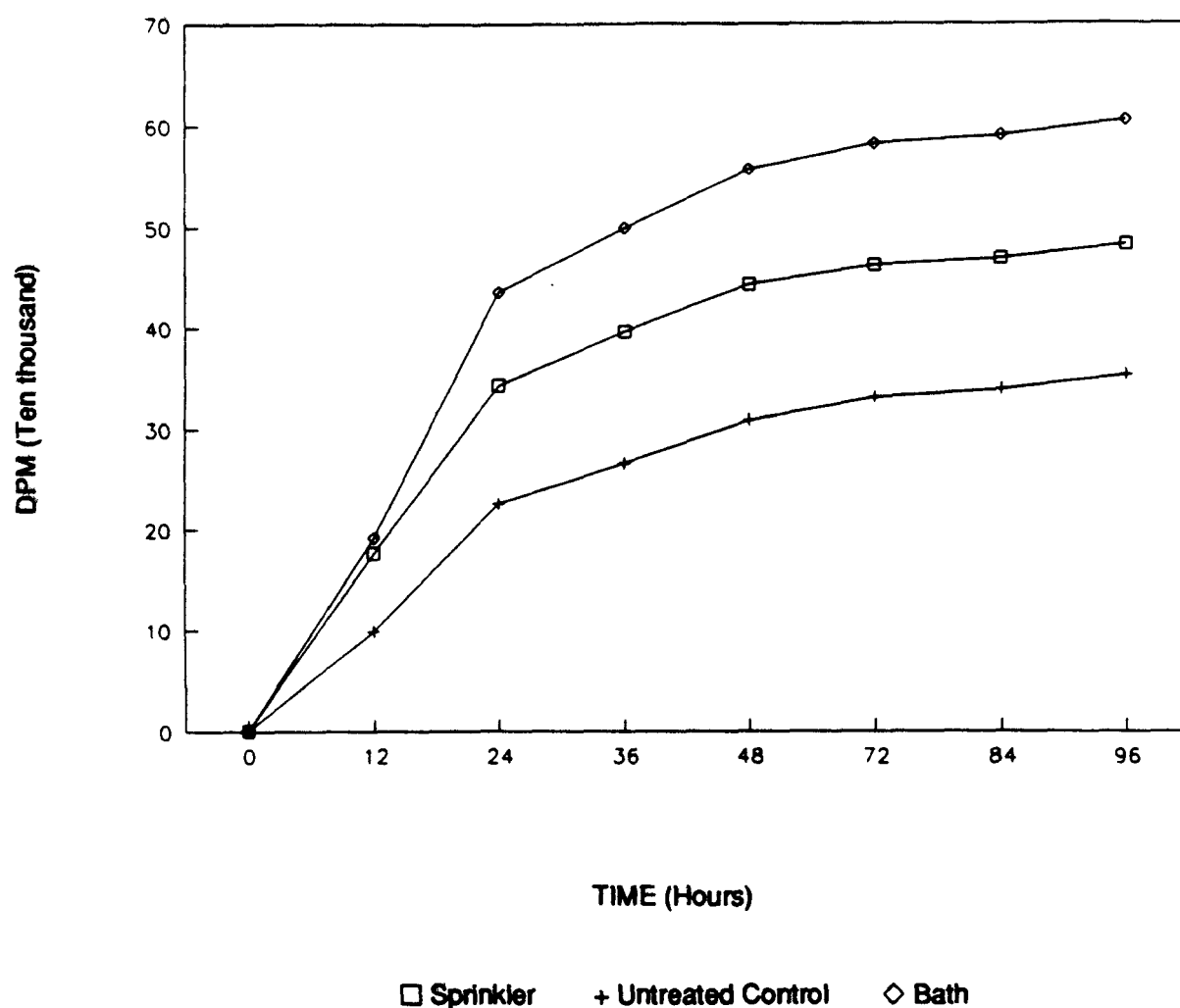
Figure 9.21b. Production of Radiolabeled CO_2 from Phenanthrene Through Time in Oiled Beach Samples Taken From the Sampling Baskets (Layer 3) on 7/21/90, for the Sprinkler, Untreated Control, and Bath Beaches at Elrington Island.

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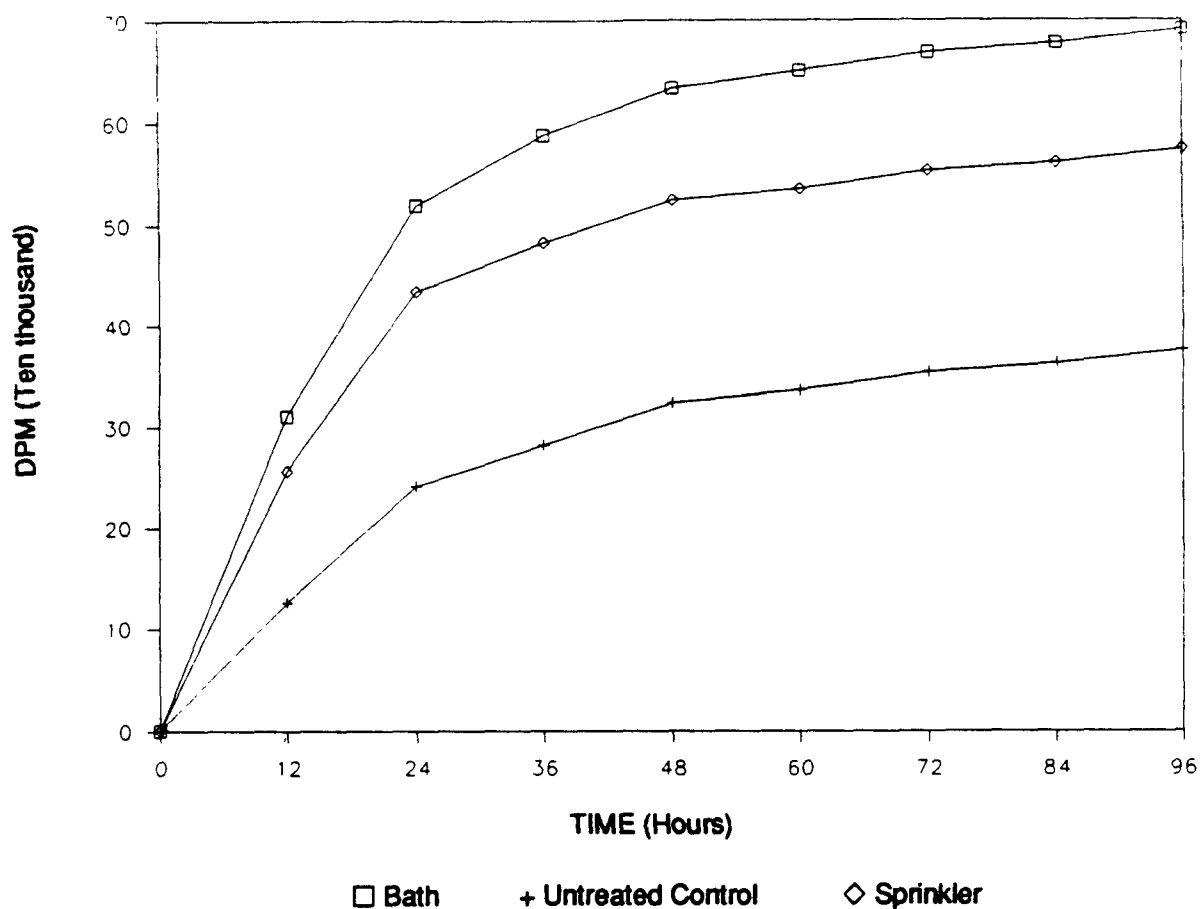
Figure 9.21c. Production of Radiolabeled CO_2 from Phenanthrene Through Time in Oiled Beach Samples Taken From the Sampling Baskets (Layer 3) on 7/30/90, for the Sprinkler, Untreated Control, and Bath Beaches at Elrington Island.

Mineralization of ^{14}C-hexadecane was measured in the same beach samples by the ADEC Oil Spill Response Support Laboratory in Valdez. The mineralization data are shown in Figure 9.22 (data for samples taken prior to the fertilizer application unfortunately are not available). Although the percent hexadecane mineralized was relatively low, the results again show the effect of a single pulse application with a high concentration of fertilizer. On the first sampling date (10 days after fertilizer application) mineralization activity was approximately three times greater on the Bath beach than the other beaches. The mineralization activity on the Sprinkler beach (two fertilizer applications) was essentially the same as the untreated control. Ten days later, the Sprinkler beach (three fertilizer applications) was showing increased activity, while activity on the Bath beach had decreased. By the end of the test period, activity on the Sprinkler beach (six fertilizer applications) was the highest observed overall, and the Bath beach was essentially the same as the untreated Control beach.

This information for the Sprinkler beach differs with observed changes in the oil composition (Figures 9.7-9.19). In the latter case, the straight chain alkanes were rapidly degraded during the initial 20 days of the test on the Sprinkler beach, suggesting that highest mineralization should have occurred in the initial part of the test. The fact that it did not may be a function of isotope dilution; that is, initially the bacteria populations utilized more of the unlabeled hexadecane in the oil. On the Bath beach, hydrocarbon degradation must have occurred so rapid initially that isotope dilution was attenuated, giving higher initial mineralization rates of ^{14}C-hexadecane. Such an attenuation could only be explained by very rapid increases in biomass specific activity, giving basically zero-order degradation kinetics.

Dissolved Oxygen and Nutrient Uptake

Specialized sampling baskets were placed in the Elrington beaches to allow measurements of oxygen uptake as seawater passed through the oiled layer of beach material in treated and untreated beaches (see Section 4). Two sampling wells were placed in the baskets; one well trapped incoming tidal water before it passed through the oiled layer, while the other trapped water after it passed through the oiled layer. With dissolved oxygen probes in each well, the concentration of oxygen was monitored as the tidal water moved up and through the bottom of the basket and eventually over the top of the wells.
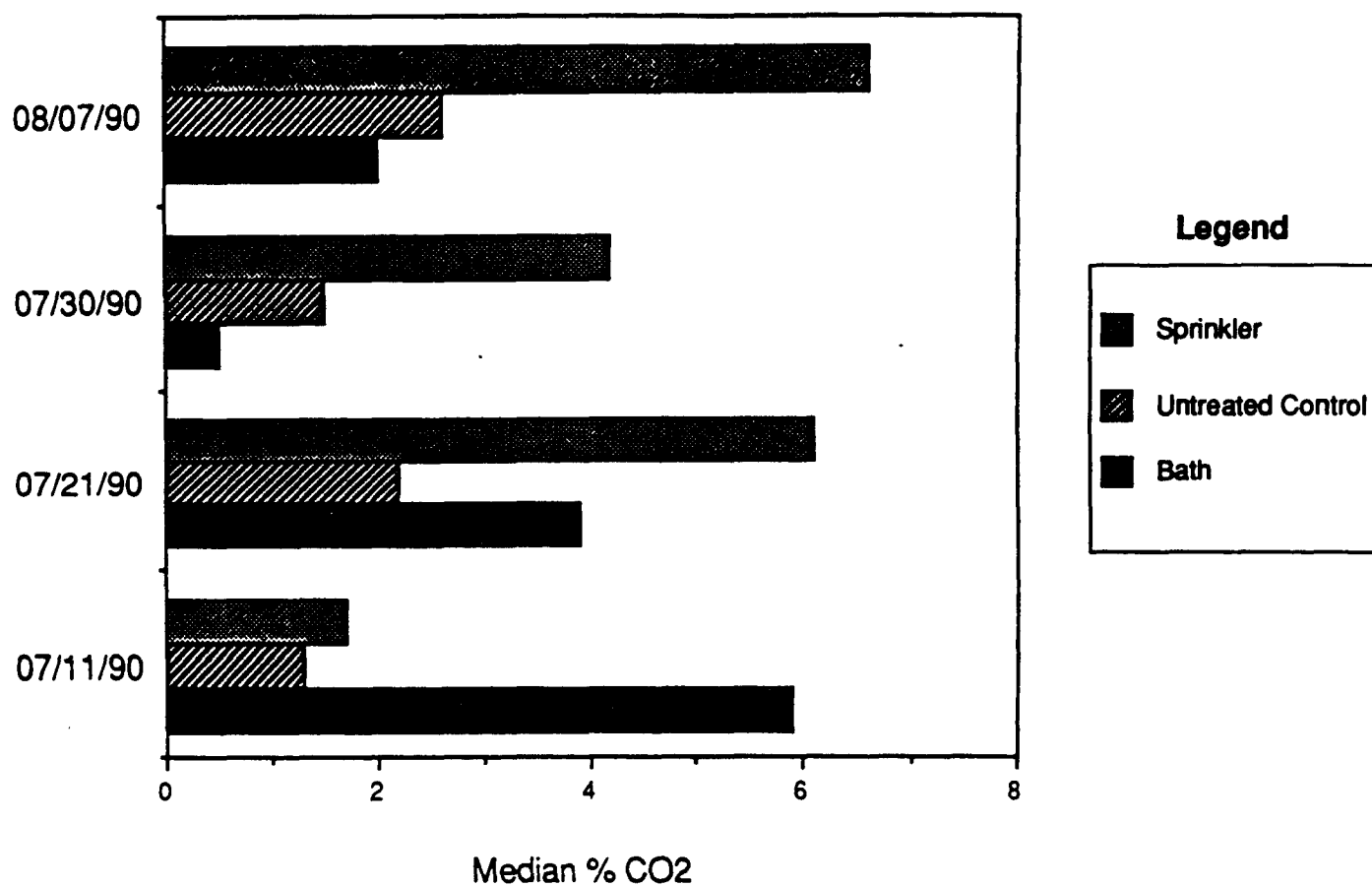
Figure 9.22. Median Percent Total CO_2 Production (Triplicate Samples) Over Time for the Sprinkler, Untreated Control, and Bath Beaches at Elrington Island.

Changes in dissolved oxygen concentrations shortly after fertilizer application are shown in Figures 9.23 to 9.25, and approximately 30 days later in Figures 9.26 to 9.28. Despite the crudeness of the *in situ* test basket, the effect of fertilizer-enhanced biodegradation in the subsurface oil layer could be observed by the differential oxygen uptake, comparing the change in oxygen levels from the time water entered the wells to its lowest level after that time. Tidal water passing through the oil layer had a greater oxygen demand than water isolated from it, and the demand was only apparent in the fertilizer-treated beaches. The general absence of oxygen uptake in the untreated control as water passed through the oil layer is interesting, since the oil chemistry results showed that some biodegradation was occurring in the sampling baskets on this beach. Since measurements of oxygen uptake were performed 4 to 6 days after initial fertilizer application, the response to the added nutrients was quite rapid. Furthermore, similar degrees of oxygen uptake also occurred 30 days later, suggesting the effect of fertilizer application was sustained, particularly in the single pulse application on the Bath beach. This data provides another strong indication that fertilizer-enhanced changes in oil chemistry are linked to enhanced microbial activities in the beach subsurface.

Samples of seawater were also taken from the in situ test baskets to determine if nutrient uptake accompanied the dissolved oxygen uptake. The samples were taken just before the wells were totally filled by the tide, and the concentrations of ammonia, nitrate, and phosphate were measured. If nutrients were truly linked to enhanced biodegradation of the oil, water that passed through the oiled layer should have shown fewer nutrients compared to water that did not pass through the oiled layer. These data are shown in Table 9.8. Any value with a ratio greater than one indicates that lower nutrient concentrations (NH_4, PO_4, NO_3, and NO_2) were found in the wells receiving water that had passed through the oiled layer. As a result of passing through the oiled layer, nutrient uptake occurred on all beaches. However, there were several cases where uptake on the fertilizer-treated beaches appeared to be greater than the untreated control, particularly for the July 2, 1990 ammonia analysis on the Bath beach (the Bath beach was only sampled twice because a major storm dislodged the *in situ* basket), and the July 26,1990 and August 4,1990 ammonia analysis on the Sprinkler beach. Some of the same trends were seen with the nitrate analysis. It is concluded that there is some evidence of a connection between enhanced oil degradation and nutrient uptake. This uptake seems to be related to the large pulse of fertilizer added with the Bath application, and possibly to the accumulated benefit of several applications at low nutrient concentrations on the Sprinkler beach. In several cases high ratios were obtained, and the highest ratios occurred on the treated beaches.
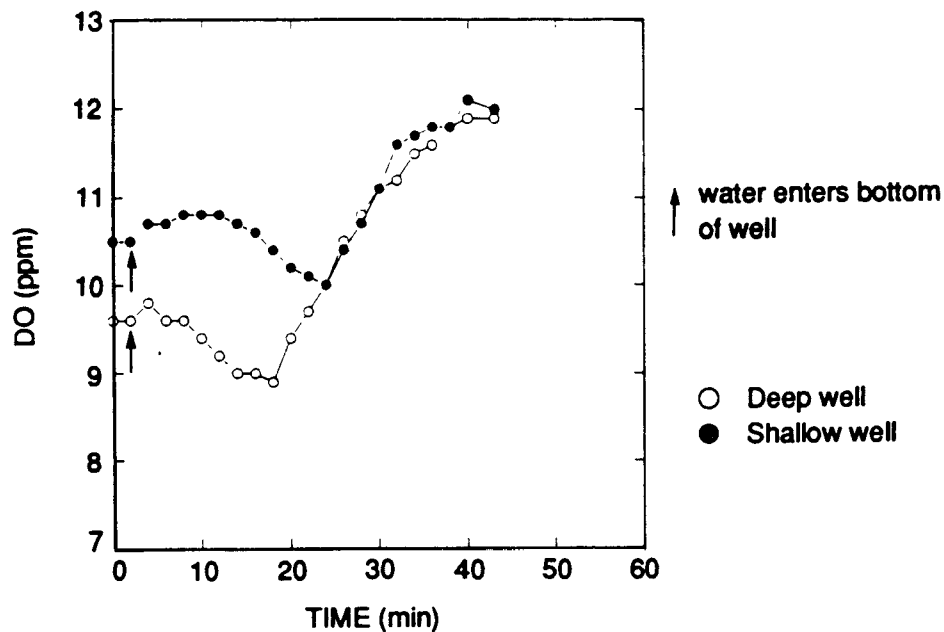
Figure 9.23. Change in Dissolved Oxygen Concentration Over Time for the Untreated Control Beach at Elrington Island on July 6, 1990.
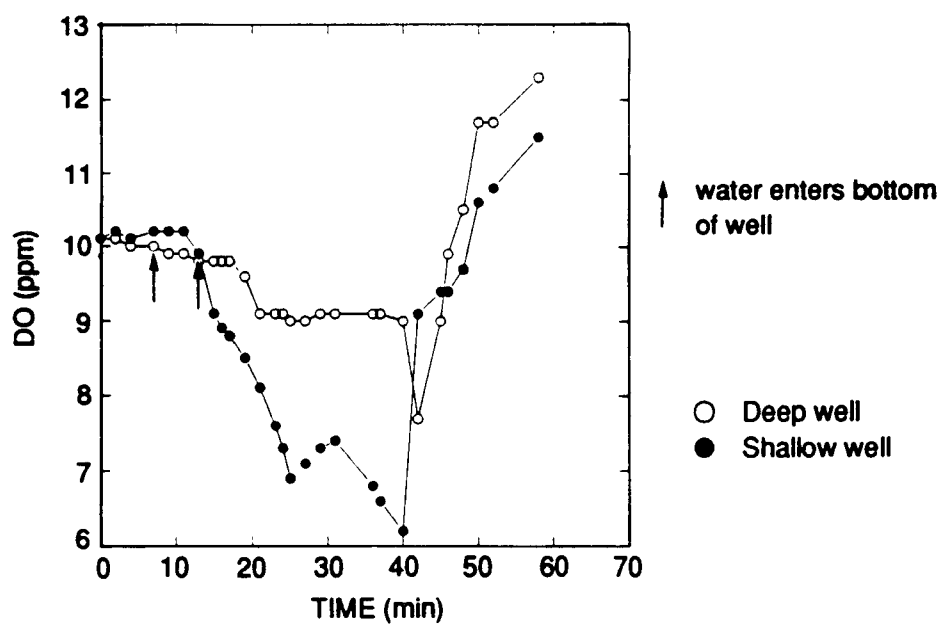
Figure 9.24. Change in Dissolved Oxygen Concentration Over Time for the Sprinkler Beach at Elrington Island on July 3, 1990.
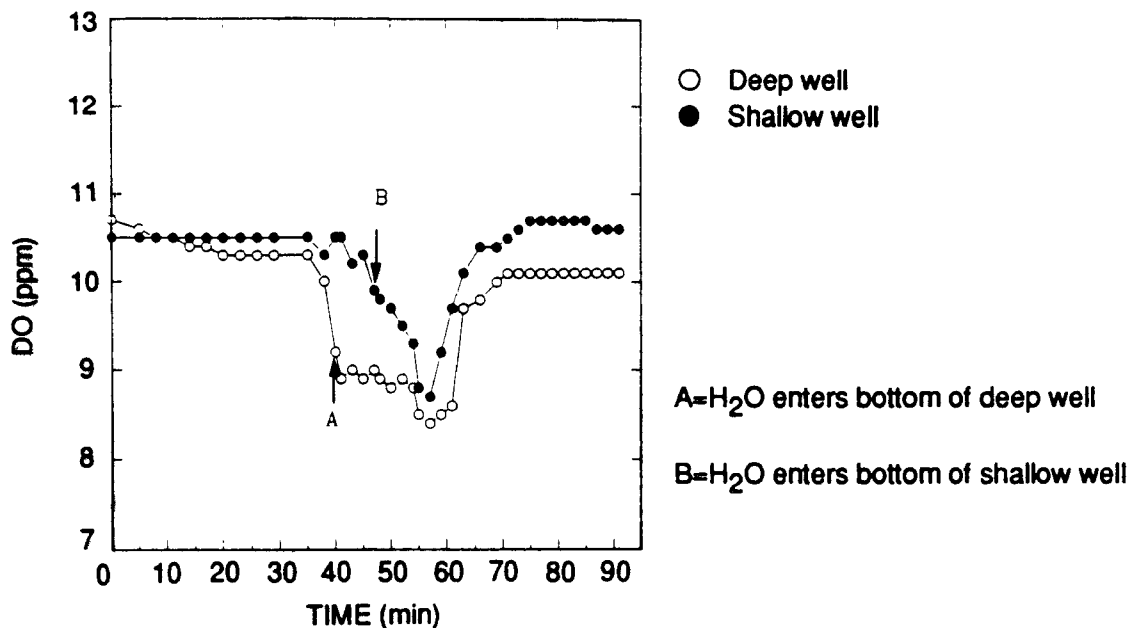
Figure 9.25. Change in Dissolved Oxygen Concentration Over Time for the Bath Beach at Elrington Island on July 7, 1990.
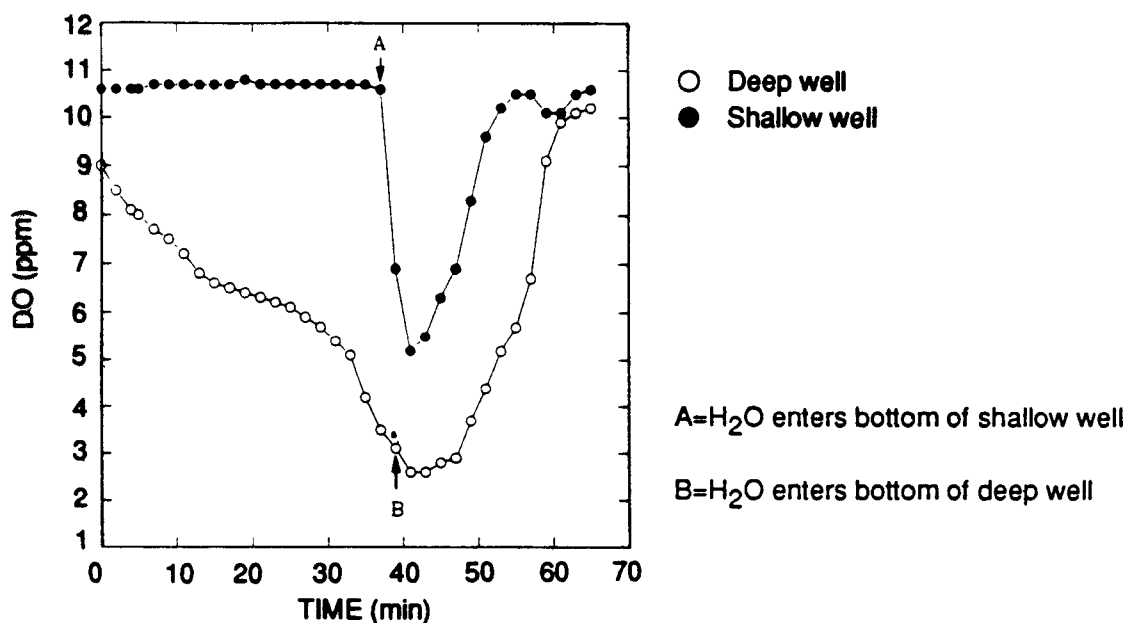
Figure 9.26. Change in Dissolved Oxygen Concentration Over Time for the Untreated Control Beach at Elrington Island on August 3, 1990.
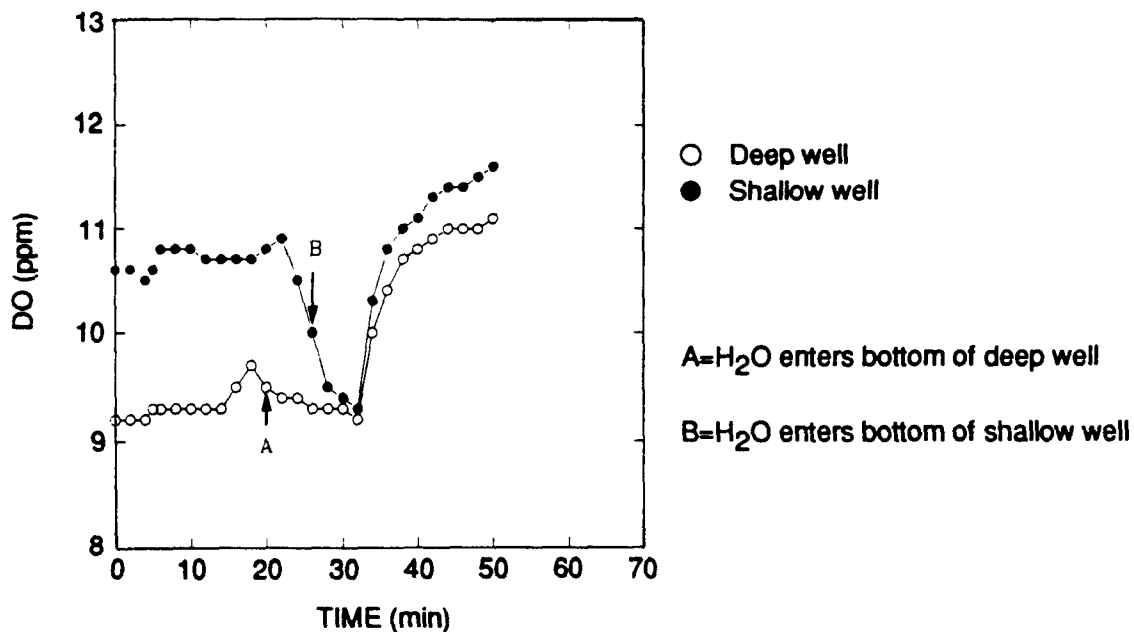
Figure 9.27. Change in Dissolved Oxygen Concentration Over Time for the Sprinkler Beach at Elrington Island on August 5, 1990.
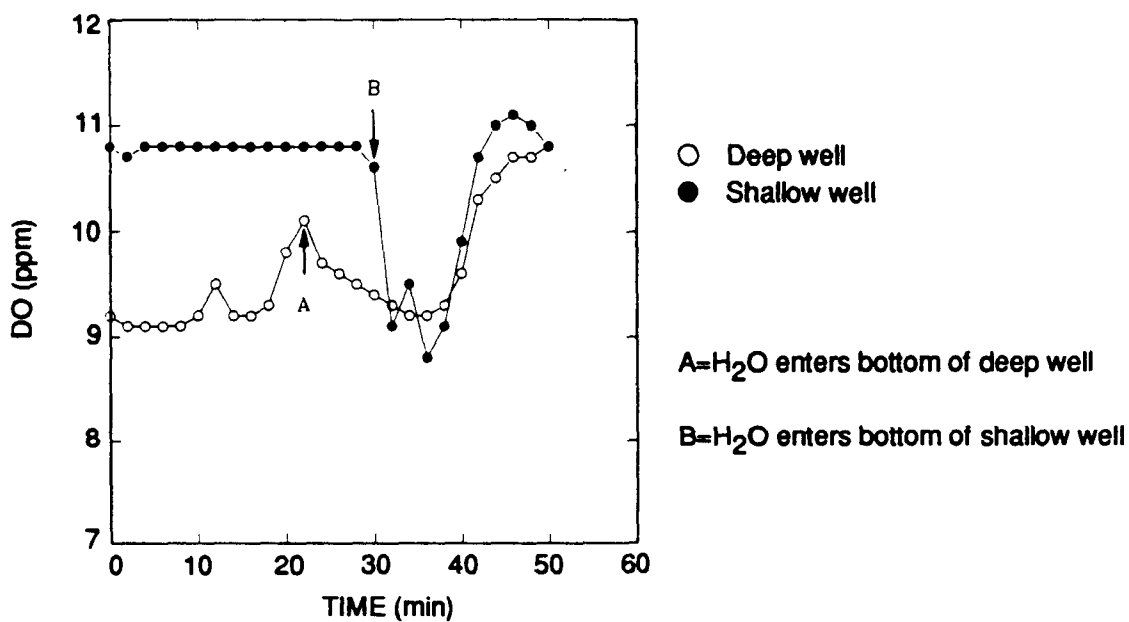
Figure 9.28. Change in Dissolved Oxygen Concentration Over Time for the Bath Beach at Elrington Island on August 3, 1990.

TABLE 9.8. RATIO OF NUTRIENT CONCENTRATIONS BEFORE AND AFTER EXPOSURE
TO OILED BEACH MATERIAL IN SPECIAL SAMPLING BASKETS
FROM ELRINGTON ISLAND BEACHES

Beach	Sampling Date	NH_4	PO_4	NO_3	NO_2
Control	June 30	1.33	0.74	0.45	2.00
Control	July 4	1.00	1.17	1.20	1.00
Control	July 5	1.50	1.43	1.00	1.00
Control	July 14	1.08	1.50	2.00	0.50
Control	July 26	0.53	0.35	1.37	0.80
Control	August 4	0.78	1.22	NDA[a]	1.25
Bath	June 30	0.80	1.13	0.08	0.50
Bath	July 2	11.20	3.10	8.50	4.50
Sprinkler	June 30	2.00	0.86	1.94	1.00
Sprinkler	July 3	0.86	0.96	0.95	1.25
Sprinkler	July 4	1.06	1.26	1.81	0.67
Sprinkler	July 5	1.06	0.88	0.37	0.50
Sprinkler	July 14	1.25	2.00	84.00	7.00
Sprinkler	July 26	7.56	0.93	4.35	1.38
Sprinkler	August 4	3.11	0.84	1.17	1.20

[a]NDA = No data available

MICROCOSM STUDIES

For this project, flow-through aquatic microcosms provided the opportunity to study bioremediation in a more realistic simulated field setting than shake flasks. Although a shake flask may indicate maximized effects of nutrient additions by increasing oiled beach material contact with a homogeneous solution, the flow-through microcosms are better at simulating field conditions by allowing exposure of a column of beach material to two simulated tidal cycles per day, each slowly rising from the bottom and falling from the top.

The primary objective of the flow-through microcosm studies was to test the effect of nutrients on oil biodegradation for comparison with field sprinkler application (Elrington Beach) and laboratory (shake-flask) test results. The systems were used to determine the difference in oil degradation between simulated "high" and "low" tide conditions.

TOC analyses indicated no significant products of incomplete degradation or bioemulsification in the effluent water from either treatment. Figure 9.29 indicates the cumulative carbon dioxide produced by duplicate columns, after subtracting background values from control columns 1 and 2. Both the "bath" and the "sprinkled" nutrient treatments show accelerated carbon dioxide production over time compared to the no nutrient microcosm CO_2 production rate.

Tidal cycle phase did not seem to have a significant effect on average daily oil mineralization, as indicated in Figure 9.30. This figure also indicates that replicate columns generated approximately the same quantity of carbon dioxide. The "bath" nutrient treatment appeared to enhance oil mineralization by approximately 2-fold over the control without nutrients, and the "sprinkled" treatment by about 3-fold (Figure 9.31). The relative difference in the mineralization rates was greater than that seen in samples taken directly from the beach (Table 9.6). In addition, the total CO_2 production in the microcosms was only one fourth of that seen in samples taken directly from the beaches. These differences could be due to the absence of turbulence in the microcosm; the flask tests were operated with slow mixing, which would have produced some turbulence.

Oil residues appeared to decline somewhat over the 2-week test period. Samples of oiled beach material at the beginning of the test contained 6363 mg oil/kg (n = 3, s.d. = 168). At the end of the test, control columns 3 and 4 contained 6667 mg oil/kg beach material (n = 2, s.d., = 0), "bath" treated columns 5 and 6 had 6129 mg/kg (n = 2, s.d = 81) and the "sprinkled" treatment columns 7 and 8 contained 5284 mg oil/kg (n = 2, s.d. = 907). This gave a rate of oil residue weight change for the sprinkled treatment (83 mg/kg/day) that was in the same range as seen in the field (110 mg/kg/day, Table 9.1). If oil residue weight change is a good indicator of oil degradation, then we would surmise from these microcosm studies that oil carbon was either being converted to bacterial biomass or to degraded oil residues (at least initially). Since TOC of microcosm effluents was not elevated, we would conclude that the biomass and oil residues remained associated with the oiled beach material. In the field, the turbulence of tidal action could have caused the biomass and oil residues to be sloughed out of the beach material.

Effect of Oil Concentration

The high rates of oil degradation on the treated Elrington beaches (>100 mg oil residue/kg/day) compared to the previous summer in Passage Cove (10 to 20 mg oil residue/kg/day) raised the question of the effect of oil mass on the biodegradation rate; that is, does a direct relationship exist
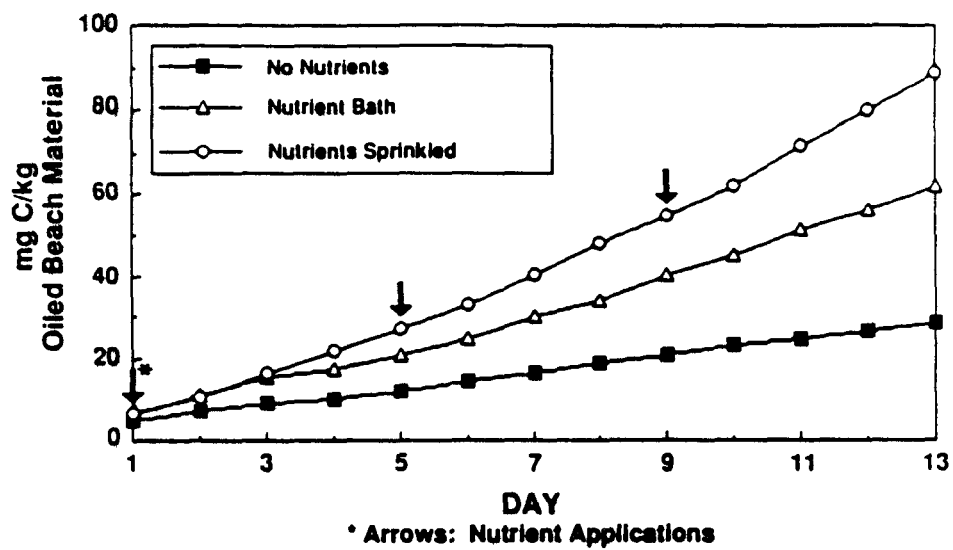
Figure 9.29. Cumulative Mineralization of Oil Carbon from Flow-Through Column Microcosms.
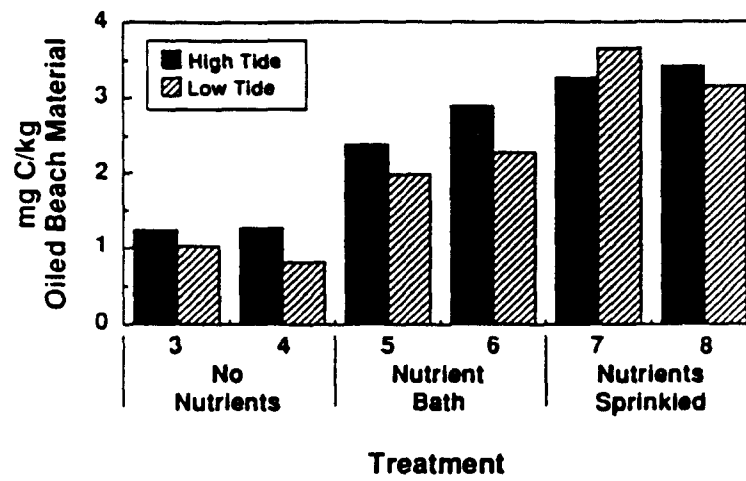
Figure 9.30. Average Daily Oil Carbon Mineralization During High and Low Tidal Cycles of Replicate Column Microcosms.

AVERAGE CO_2 PRODUCTION PER DAY
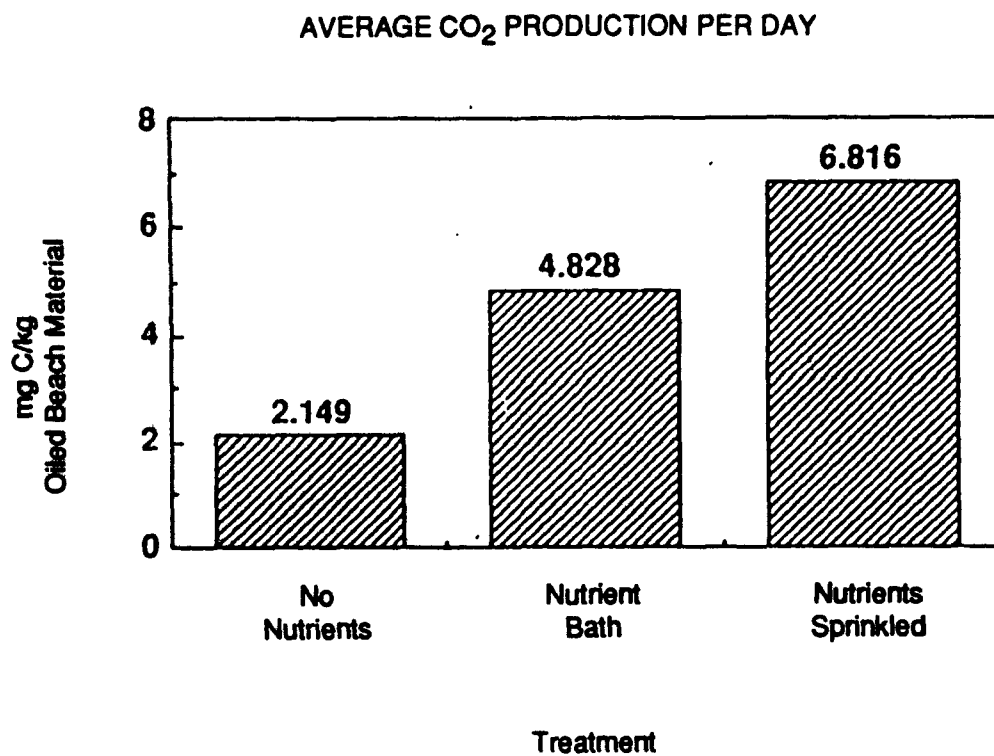
Figure 9.31. Average Daily Oil Carbon Mineralization in Column Microcosms for Each Nutrient Treatment.

between higher oil concentrations (i.e., Elrington Beach) and faster oil degradation rates. To test this idea, samples of oiled beach material were randomly collected from Elrington Island, placed in the biometer test flasks, and total CO_2 production measured. The contents of the flasks were flooded and drained of seawater amended with N and P nutrients every 12 hours to simulate the effect of tides. By randomly selecting samples from the beach, it was assumed that oil concentrations would be quite heterogeneous and, thus, the mineralization rate, as a measure of the oil degradation rate, could be correlated with the different oil residue weights.

Figure 9.32 shows that mineralization was linear and did indeed vary. However, if the mineralization rate is normalized to the total oil residue (Table 9.9), then there appears to be little reduction in the variability of the rates. Thus, rates are not affected by initial oil weights. It also appears that biodegradation of the oil is not first-order with respect to oil mass and that many other factors limit the rate. This is reasonable if one considers that as a hydrophobic substance, oil will not be totally available to the oil degrader biomass at any point in time.

TABLE 9.9. RELATIONSHIP BETWEEN OIL MINERALIZATION RATES
AND OIL CONCENTRATION IN BEACH MATERIAL FROM ELRINGTON ISLAND

Sample Designation	Mineralization Rate (ppm/day)	Residue Weight of oil (mg)	Normalized Rate (ppm CO_2/mg oil/day)
1	71.88	0.49	146.7
2	59.55	0.65	91.6
3	56.00	0.19	294.7
4	55.00	0.28	196.4
5	54.00	0.46	117.4
6	51.88	0.19	273.1
7	38.13	0.48	79.4

WINTER SAMPLING

On December 7, 1990, Elrington Island was sampled a final time. We were unaware that Exxon had applied INIPOL and CUSTOMBLEN to the test beaches at the end of the summer. Thus, the winter sampling information must be weighed against the effects of the fertilizer application.
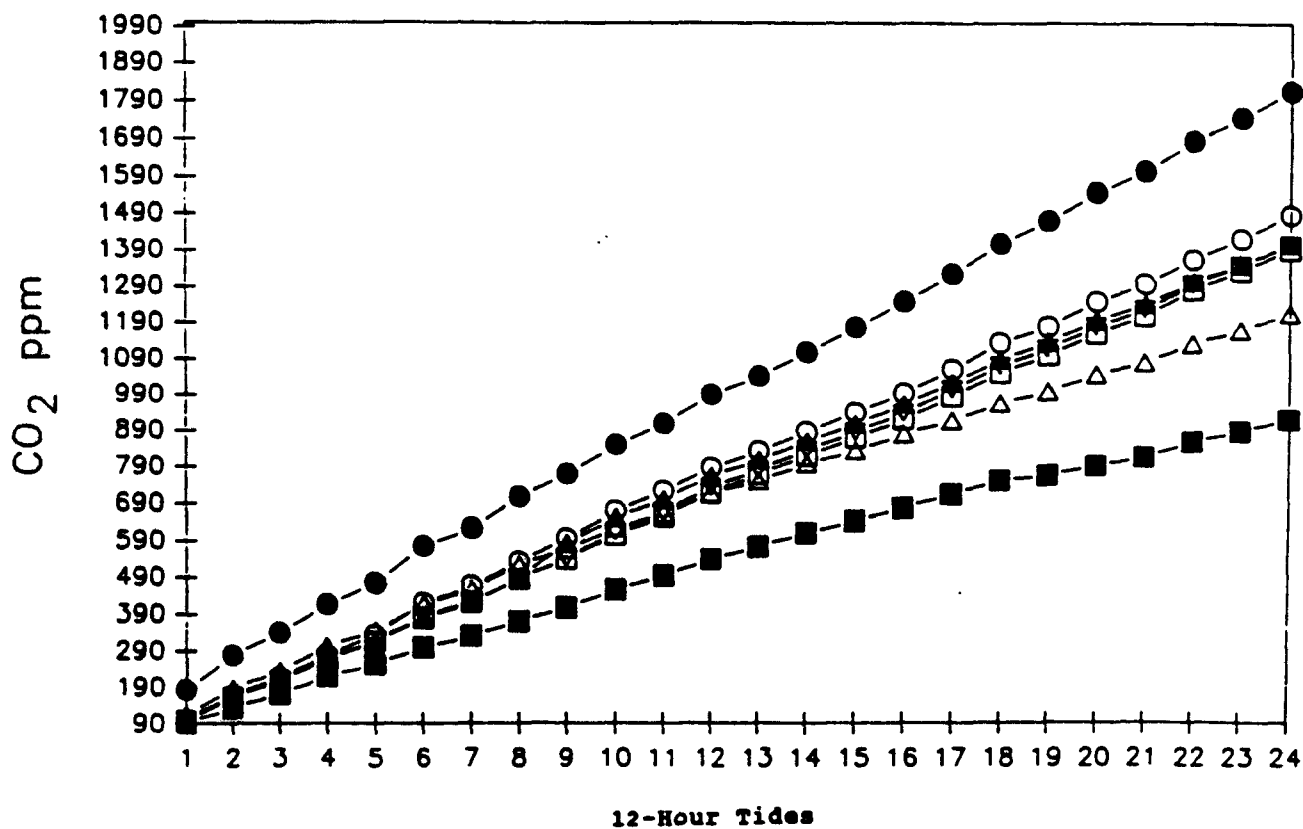
Figure 9.32. Change in CO$_2$ Concentration Over Time for Variable Oil Concentrations at Elrington Island.

Samples were taken along a transect parallel to the water line on the untreated Control, Bath, and Sprinkler beaches. Samples were analyzed for oil residue weights, changes in oil chemistry, microbial activity, and numbers of oil-degrading microorganisms. The chemistry data is not yet available but initial analyses of the microbiology data are presented. Numbers of oil degraders on the different test beaches are shown in Figure 9.33. Eleven to twelve samples were analyzed from each beach. Numbers of degraders are only slightly less than observed on the same beaches during the summer. Thus, winter storm activities do not appear to have substantially affected the oil-degrading microbial populations. In general, however, there do not appear to be differences between the beaches.

On the other hand, measurements of hexadecane mineralization activity indicated that some of the enhanced activity from the summer was still present (Figure 9.34). In other words, a number of samples from the beaches treated with the fertilizer solution showed the highest level of activity (no statistical analysis was performed) (keep in mind that INIPOL and CUSTOMBLEN were applied to all treated and untreated Control beaches at the end of the summer). In addition, samples from the treated beaches appeared to show more heterogeneity than those from the untreated control. Thus, it is possible that the enhanced biodegradation activity that occurred in the summer may have been carried over into the winter.

SUMMARY AND CONCLUSIONS

The following is concluded from the Elrington Island study conducted during the summer of 1990:

a) Bioremediation of subsurface oil is reasonable if sufficient quantities of nitrogen and phosphorus nutrients can be supplied. This was accomplished by using fertilizer solution.

b) A single pulse application of fertilizer (4 hours, once at low tide) enhanced oil biodegradation for as long as 3 to 4 weeks. This application was as effective, if not more so, than a multiple dose application. These results raise the question of whether the effects of fertilizer application during the summer of 1989, which also consisted of large initial pulses followed by a gradual release over time of nutrients at lower concentrations, may have been related more to the extent of nutrient exposure to the microbial communities at one time than the length of exposure. We believe microbial communities may concentrate nutrients and recycle them within the biological matrix of the community.

LOG MEAN MPN/GRAM DRY WEIGHT SEDIMENT

LOG MEAN MPN/GRAM DRY WEIGHT SEDIMENT
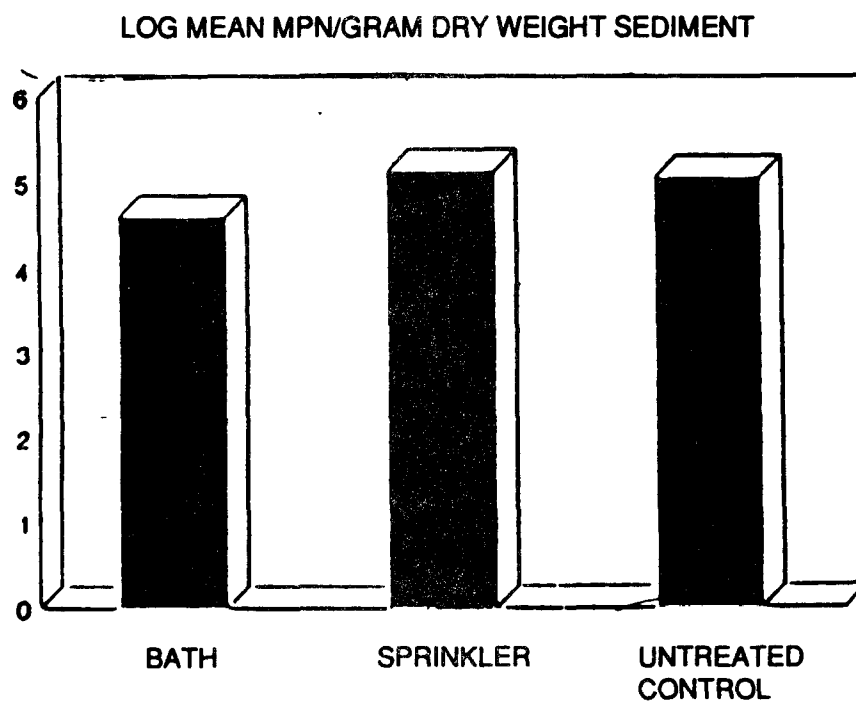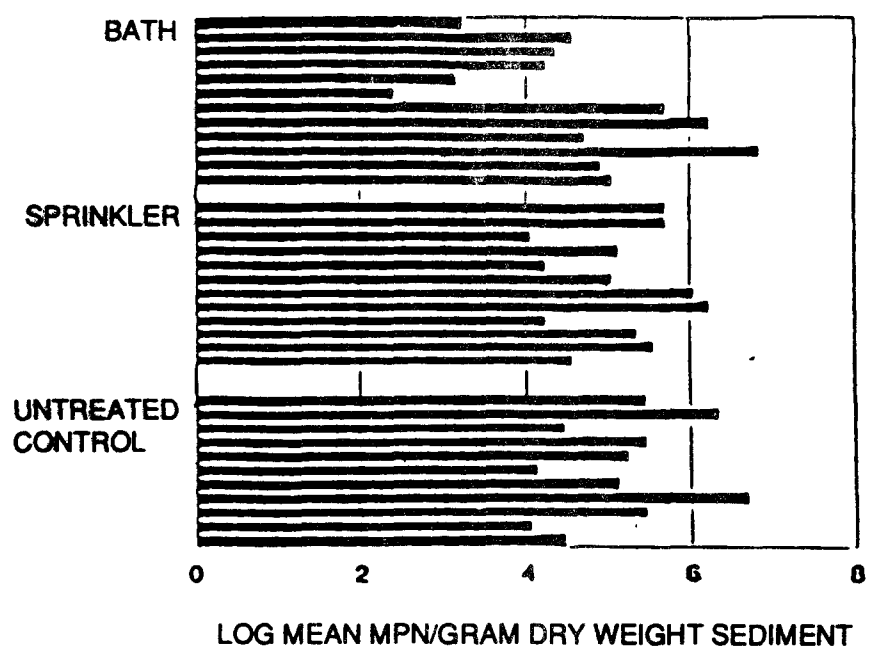
Figure 9.33. Number of Oil Degraders at the Bath, Sprinkler, and Untreated Control Beaches at Elrington Island.

OF VALUES

% HEXADECANE TO CO2

Legend

☐	Untreated Control
▨	Sprinkler
■	Bath
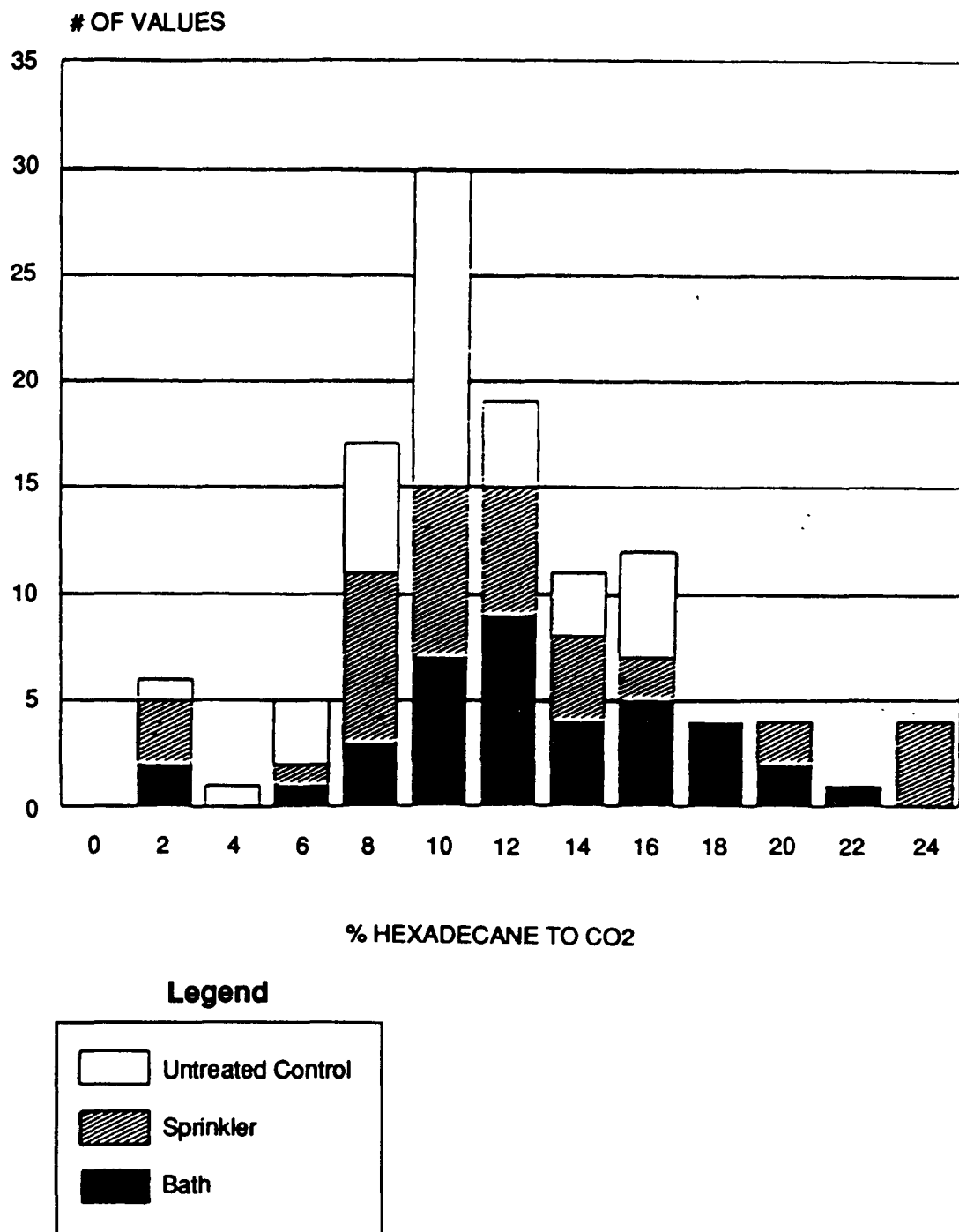
Figure 9.34.   Hexadecane Mineralization Activity for the Untreated Control, Sprinkler, and Bath Beaches at Elrington Island.

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c) Fertilizer-enhanced oil biodegradation rates on Elrington Island were the highest recorded in the field demonstrations. These rates were approximately 100 mg/kg of beach material/ day, almost six-fold higher than the untreated Control beach. The enhancement was statistically significant. This occurred despite the fact that rates on the untreated Control beach were approximately three-fold higher than rates reported at Passage Cove the previous summer. This increase in effectiveness of fertilizer application could be due to extensive colonization of the subsurface oil by oil-degrading microorganisms, and/or increased availability of oil to the bacteria by virtue of impregnation with glacial till (greater exposed surface area).

d) Measurement of oil mineralization rates in the field and the laboratory using total CO_2 production, oxygen uptake, and nutrient assimilation generally coincided with changes in oil concentration and composition. This supports the use of oil chemistry as a true measure of oil biodegradation. Relative to oil chemistry analysis, mineralization measurements could therefore provide simpler procedures for assessing the effect of fertilizer-enhanced biodegradation in future field studies.

e) The Elrington Island study clearly validated the use of laboratory oil degradation information from flask and microcosm studies to predict bioremediation events in the field.

f) The use of sampling baskets containing homogenized beach material proved to be a reliable method for assessing the biological fate of oil, especially in terms of the relative effects of different fertilizer application strategies. This method considerably reduced sampling variability, provided information that would have required a much more intensive and costly sampling effort using standard beach sampling, and provided information that was representative of the beach it modeled.

SECTION 10

SUPPLEMENTAL LABORATORY STUDIES

SHAKE FLASKS

In a series of experiments carried out by Exxon researchers, it was found that microorganisms indigenous to Prince William Sound have an ability to degrade weathered crude oil if provided adequate nutrients. Initial experiments in shake flasks demonstrated that organisms in both Prince William Sound seawater and water from the Alyeska ballast water treatment facility were able to substantially degrade artificially weathered crude oil in the presence of high levels of nitrogen and phosphorus (3.5 and 4.1% with respect to oil, 0.03% N and 0.04% P by weight of water) (Figure 10.1). There was a substantial decrease in the amount of dichloromethane extractable material, and substantial degradation of both the resolvable fractions and the unresolvable fractions on GC analysis. Very little organic carbon remained in the aqueous phase after dichloromethane extraction once the organisms were allowed to settle out.

Biodegradation proceeded much more effectively at warmer temperatures, but there was significant biodegradation at 5°C in the presence of water-soluble fertilizers (Figure 10.2). Oleophilic fertilizer shows a sharper temperature dependency than water-soluble fertilizers, and at 20°C there was very little biodegradation when oleophilic fertilizer was used alone (Figure 10.3).

Oleophilic fertilizer stimulated the biodegradation of crude oil. Flask experiments revealed that the extent of biodegradation increased with increased fertilizer concentration (Figure 10.4). Water-soluble and oleophilic fertilizers had at least an additive, and perhaps a synergistic, effect on biodegradation (Figure 10.5).

Oleophilic fertilizer also stimulated the biodegradation of oil on Prince William Sound beach material (Figure 10.6). Rocks treated with INIPOL and then incubated at 15°C became significantly cleaner after 14 days; all the resolvable peaks had disappeared in the GC analysis, and 50% of the total dichloromethane-extractable material had disappeared. The rocks were also clean to the touch. This biodegradation was not accompanied by a detectable lowering of interfacial tension between oil and brine, indicating that the microorganisms were not producing significant amounts of surfactants under the conditions tested.

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Bushnell-Haas Broth
(3.5% N, 4.1% P)
15°C, 16 days

Alyeska Inoculum

15.0 30.0 45.0 60.0 75.0 90.0 105.0 120.0 135.0 150.0

MINUTES

No Inoculum

15.0 30.0 45.0 60.0 75.0 90.0 105.0 120.0 135.0 150.0

MINUTES

Seawater Inoculum

15.0 30.0 45.0 60.0 75.0 90.0 105.0 120.0 135.0 150.0

MINUTES
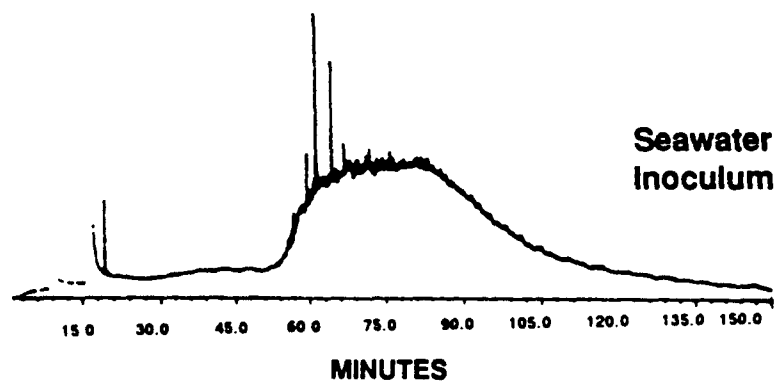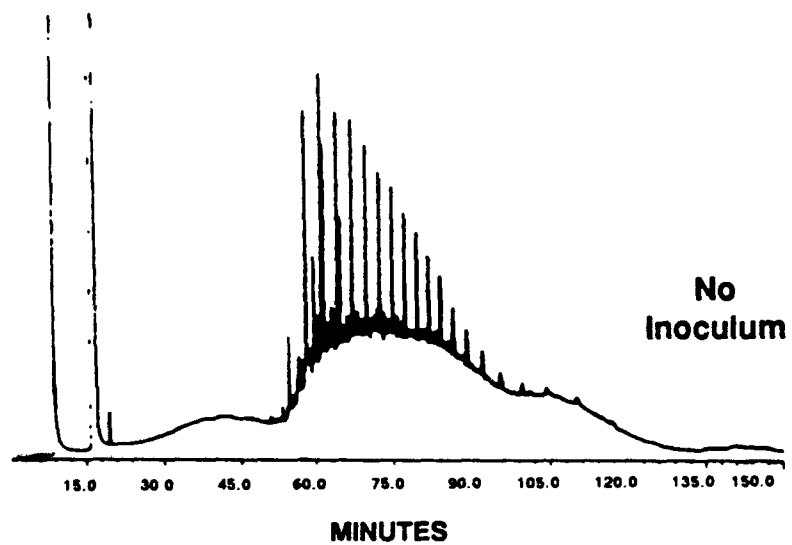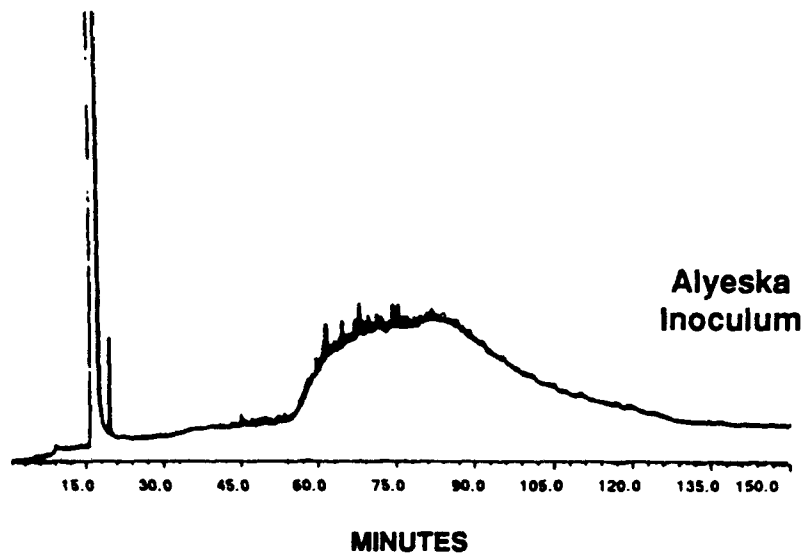
Figure 10.1. Gas Chromatographic Profiles Showing the Effect of Different Inocula on Degradation of Artificially Weathered Prudhoe Bay Crude Oil.

428

**Bushnell-Haas Broth
(3.5% N, 4.1% P)
15°C, 16 days**

5°C

15°C
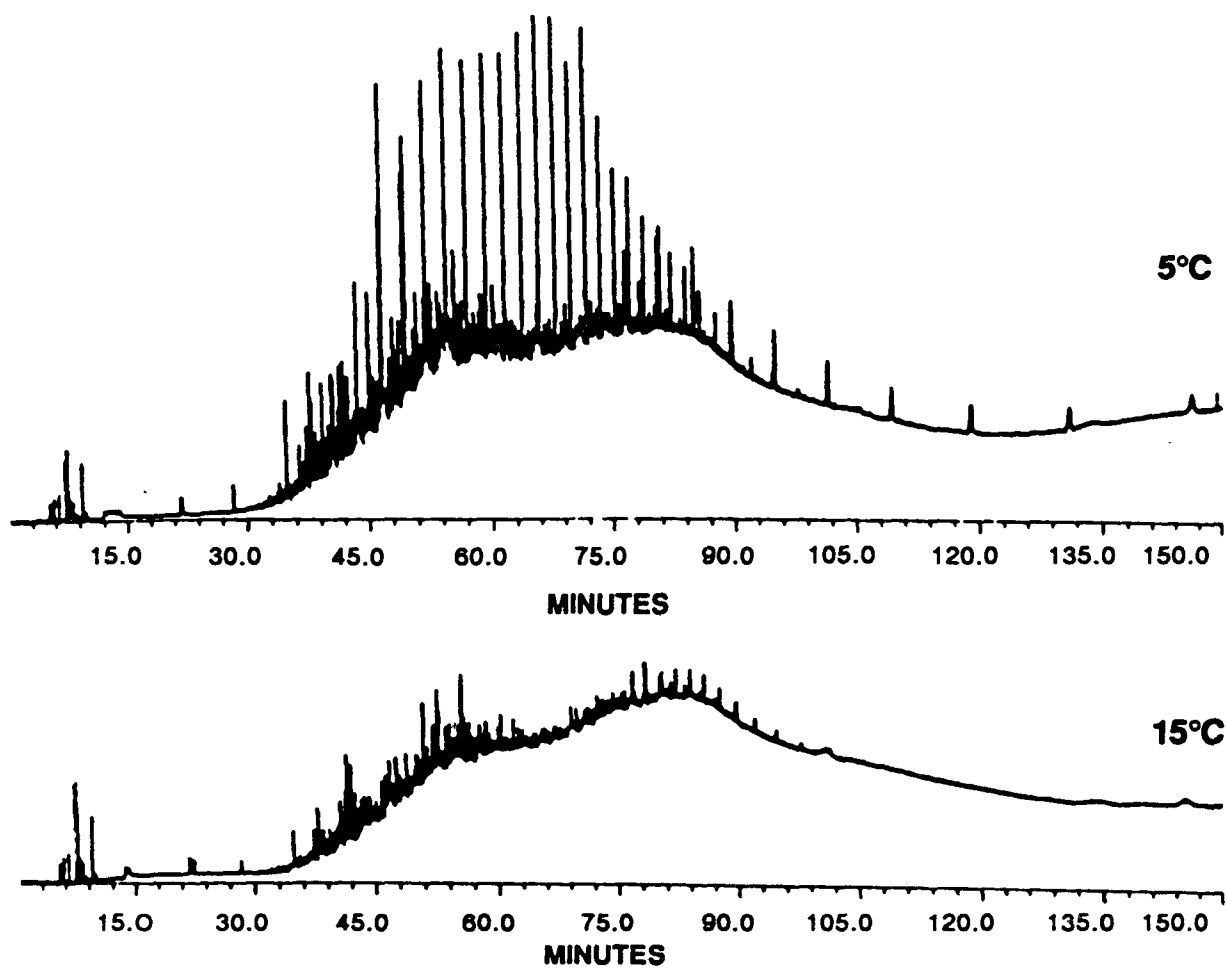
Figure 10.2. Gas Chromatographic Profiles Showing the Effect of Temperature on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil.

429

Artificial Seawater, 10% INIPOL, 38 days
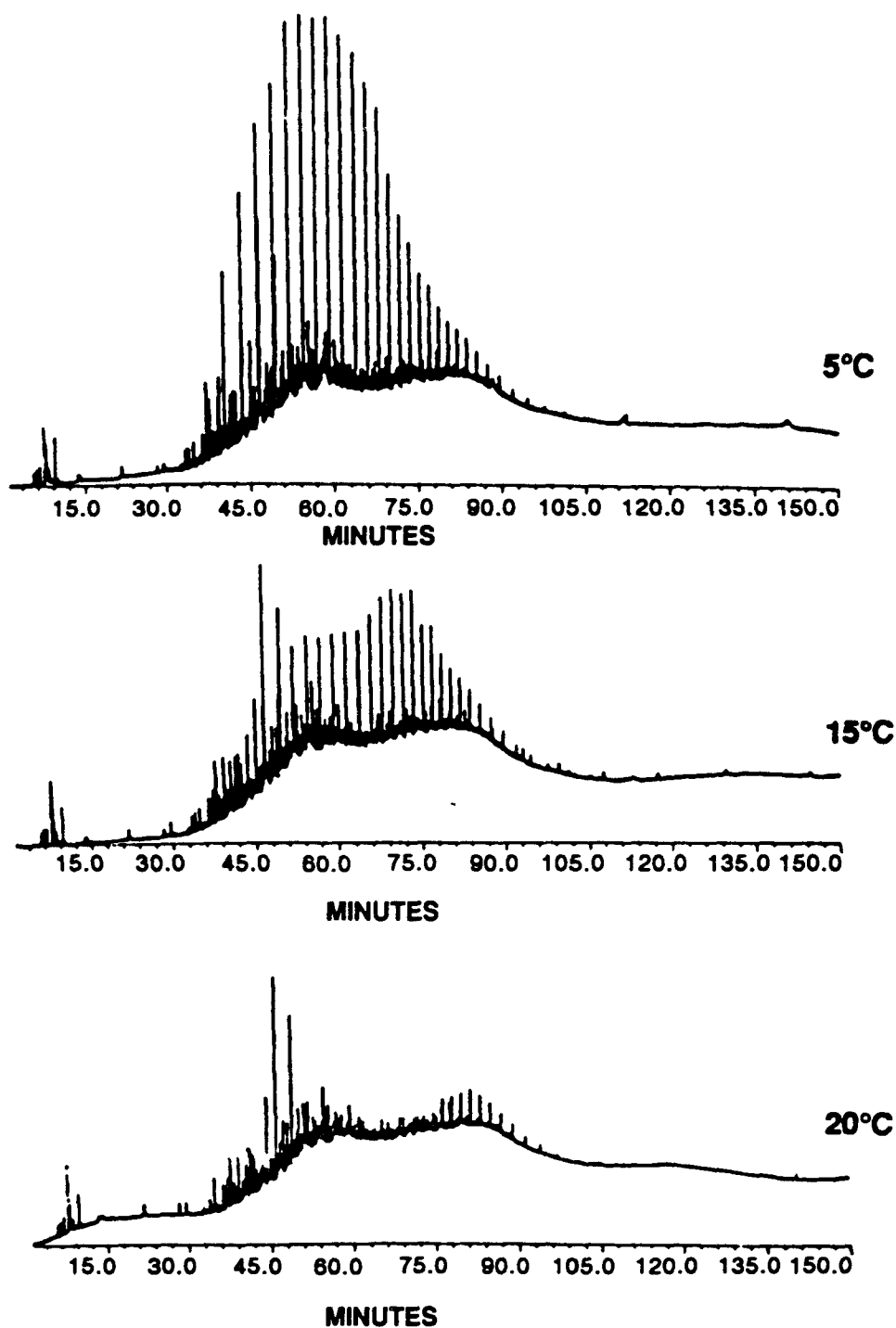
Figure 10.3. Gas Chromatographic Profiles Showing the Effect of Temperature on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil Treated with INIPOL.

430

Artificial Seawater, 15°C, 16 Days
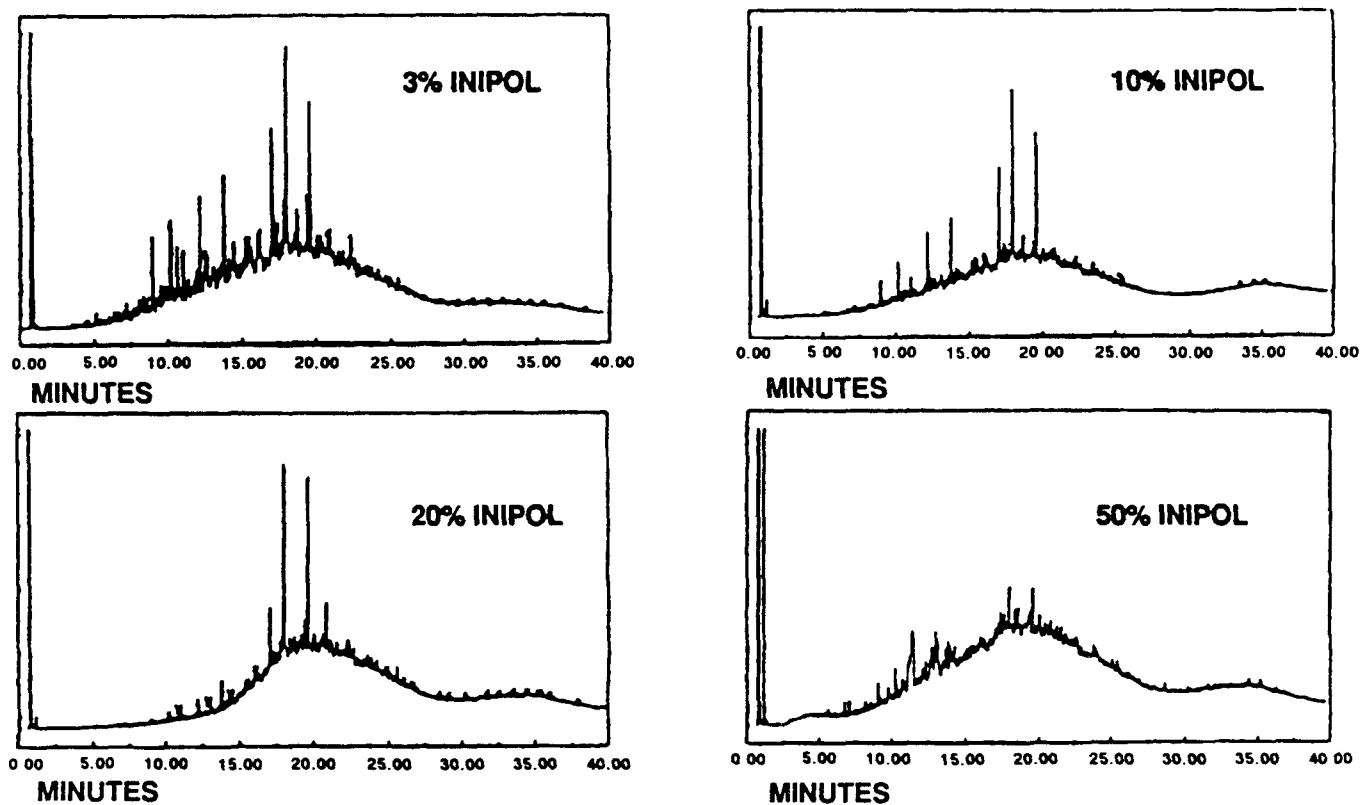
Figure 10.4. Gas Chromatographic Profiles Showing the Effect of Different Concentrations of INIPOL (% of Oil Concentration) on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil.

Figure 10.5. Gas Chromatographic Profile Showing the Effect of Different Fertilizers, Under Poisoned and Unpoisoned Conditions, on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil.

Artificial Seawater, Active, 15°C, 16 days

Oil + WOODACE Briquettes
(0.4% N, 0.08% P)

MINUTES

Oil + 10% INIPOL
(0.7% N, 0.06% P)

MINUTES

Oil + INIPOL and WOODACE Briquettes
(1.1% N, 0.14% P)

MINUTES

433
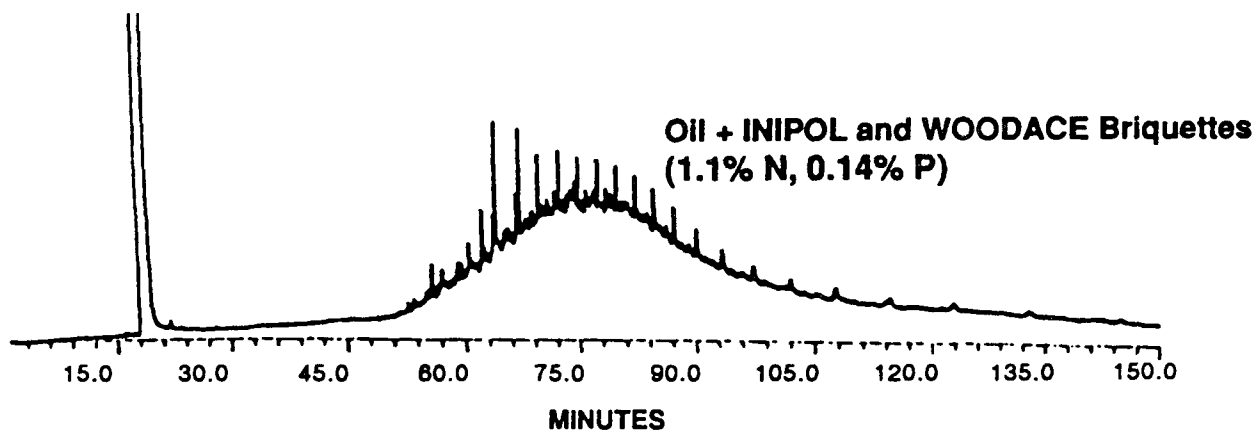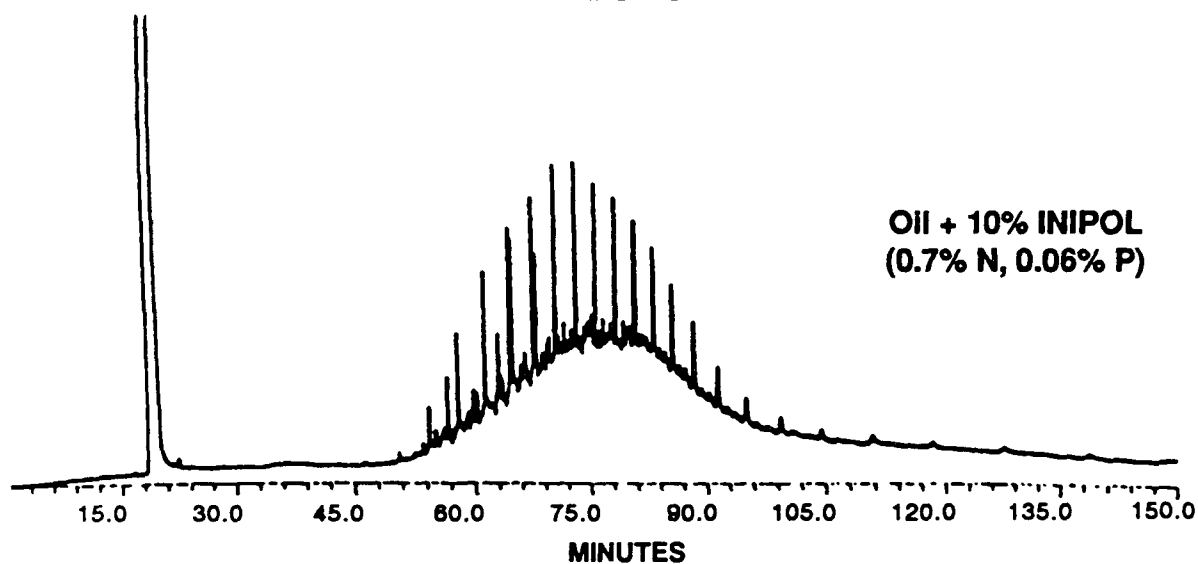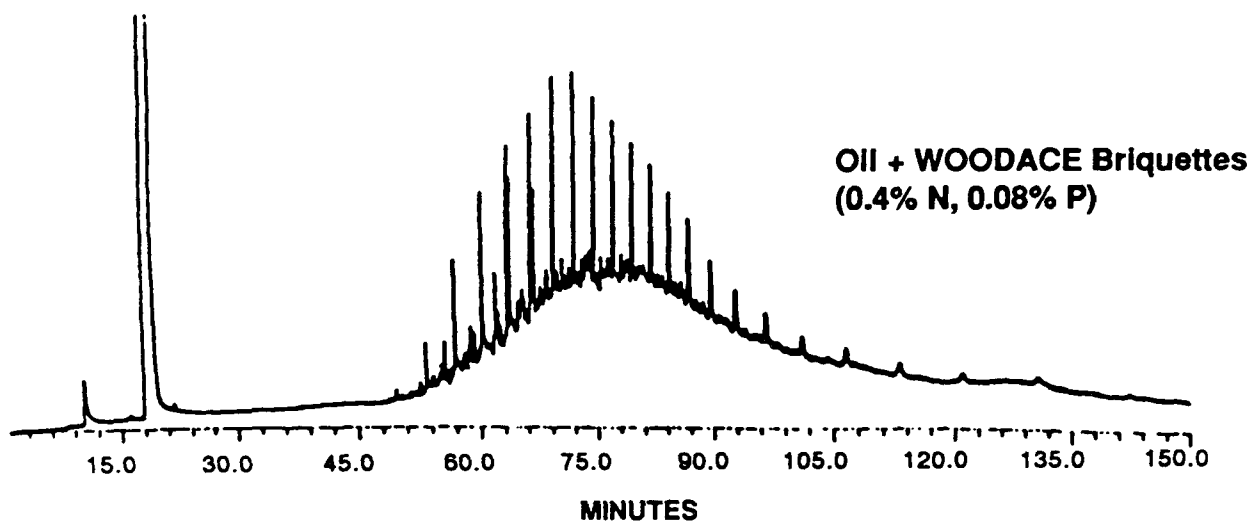
Figure 10.5. (Cont.)
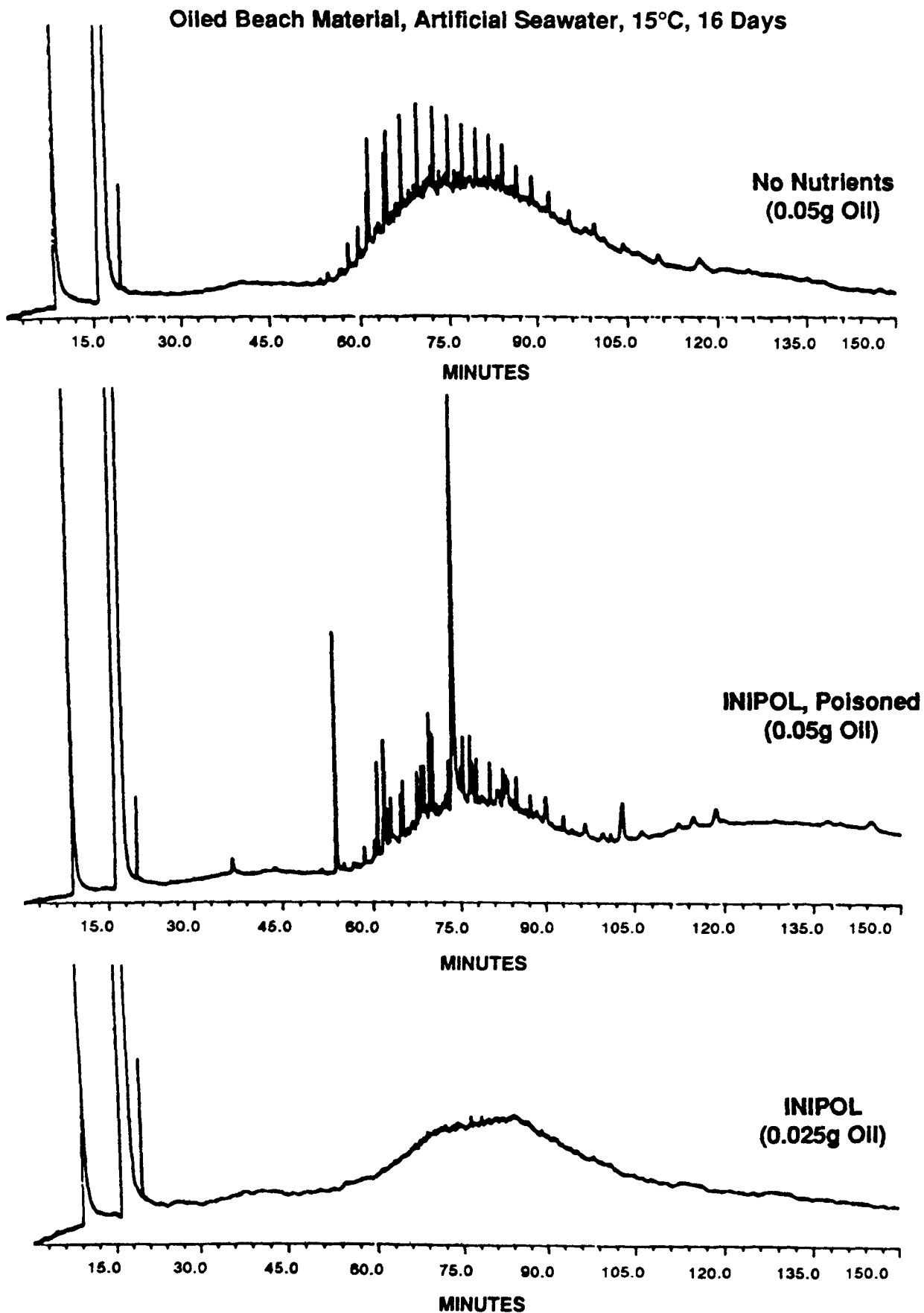
Figure 10.6. Gas Chromatographic Profiles Showing the Effect of INIPOL, Under Poisoned and Unpoisoned Conditions, on the Degradation of Oil on Beach Material Taken from Prince William Sound.

RESPIROMETRY

In a corollary set of shake flask experiments carried out at EPA Cincinnati, analytical respirometry was used to assess the effects of fertilizer on oil biodegradation. The results are summarized in Figure 10.7. Cumulative oxygen uptake as a function of time is shown in the respirometric vessel containing 1000 mg/L oil and INIPOL (5% by weight of oil) and in the respirometric vessel containing only oleophilic fertilizer (INIPOL). Oxygen uptake began in both vessels after only a 1.5 day lag period. Maximum uptake of oleophilic fertilizer occurred by the 9th day, then leveled-off at approximately 150 mg/L. The oxygen uptake rate on weathered oil with oleophilic fertilizer added was multi-phasic: the first 10 days exhibited the highest uptake rate, followed by a slower rate for the next 16 days, a somewhat faster rate for the next 4 days, and a much slower rate after the 30th day. Endogenous oxygen uptake (vessel without oleophilic fertilizer or oil) was always close to background (data not shown).

The vessel containing oil, oleophilic fertilizer, and the Alyeska ballast water biomass exhibited an oxygen uptake curve that was almost superimposable on the curve for oil plus oleophilic fertilizer (data not shown). Thus, in the closed environment of the respirometric vessel, no enhancement of oil degradation by an external source of oil-degrading organisms was detected.

Shake flasks run in conjunction with the analytical respirometry flasks were periodically sacrificed following initial application of nutrients, and the oil was extracted for chemical analysis. Figure 10.8 summarizes the results of GC/FID scans of the alkane hydrocarbons from three sets of flasks: control (containing 10,000 mg/L weathered crude oil and no nutrients); oleophilic fertilizer-treated (containing 10,000 mg/L oil and 500 mg/L oleophilic fertilizer); and defined minimal salts-treated (containing 10,000 mg/L oil and OECD). In the control some minor changes in the alkane fractions were evident after 6 weeks incubation. Some of these changes may have been due to biodegradation resulting from background levels of N and P present in the seawater, oil, or beach material; adsorption to the flask walls; sampling error; or a combination of the above. Whatever the cause, the magnitude of the changes was relatively insignificant.

Flasks containing oil plus oleophilic fertilizer exhibited complete removal of all aliphatic components within six weeks. Even the pristane and phytane fractions were reduced to undetectable levels. The flask containing the minimal-salts solution also exhibited complete removal of the straight chain aliphatics. However, there were still measurable amounts of pristane and phytane remaining
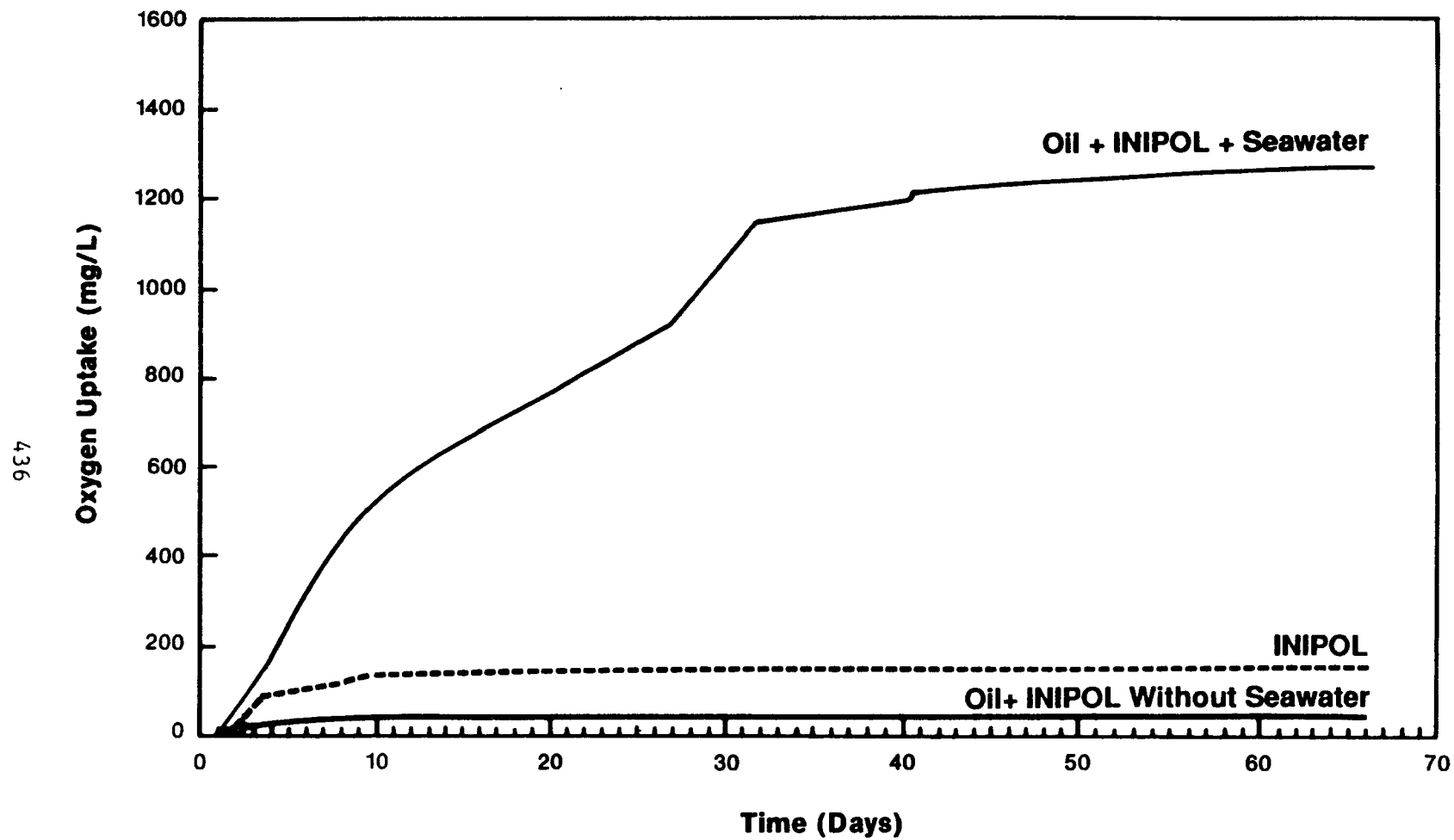
Figure 10.7. Cumulative Oxygen Uptake on Weathered Prudhoe Bay Crude Oil.
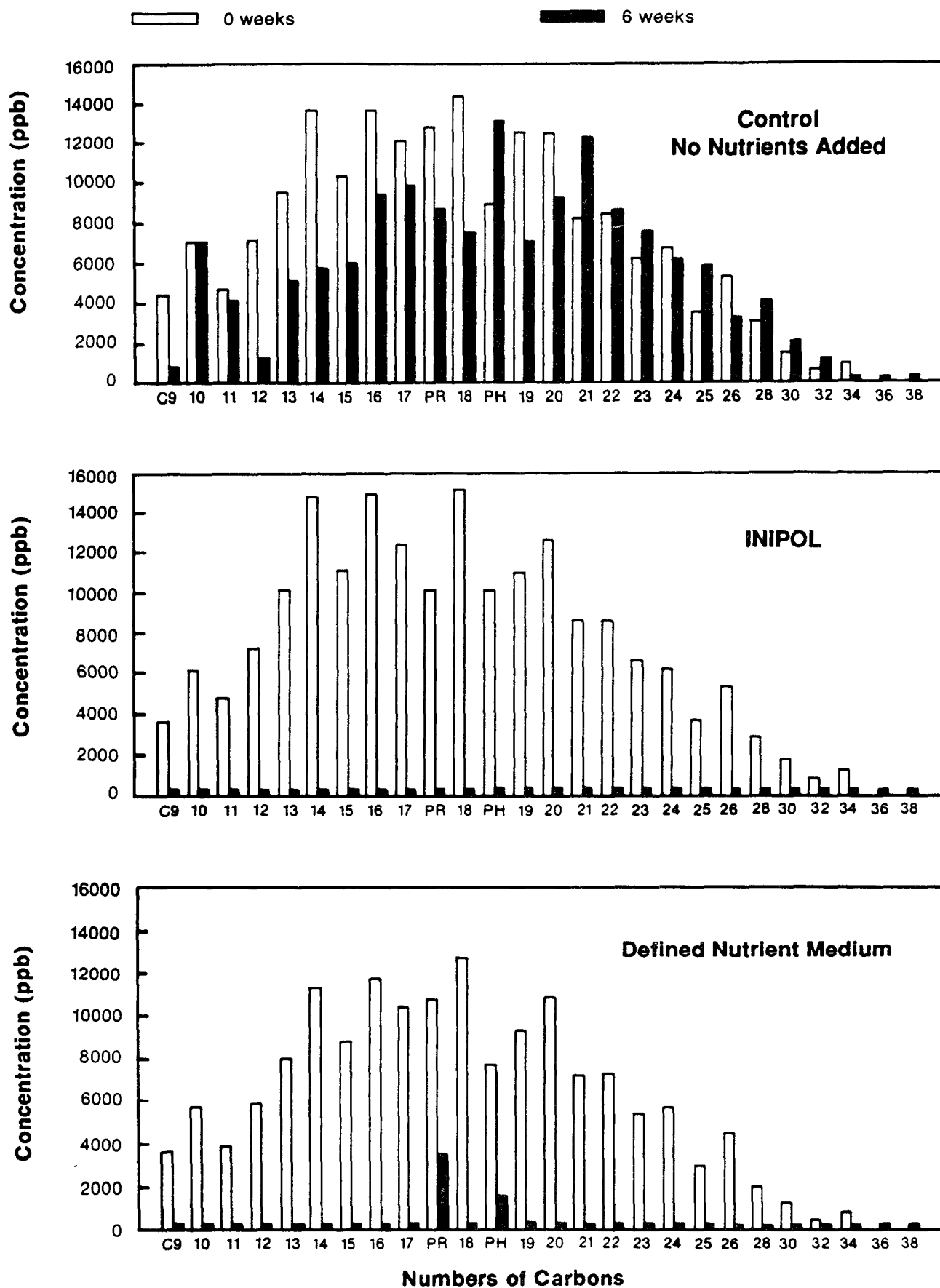
Figure 10.8. Gas Chromatographic Profiles of Alkanes at 0 and 6 Weeks After Initiation of Flask Studies.

at six weeks, although the levels were significantly reduced from the controls. These results suggest that the oleophilic fertilizer and minimal-salts solution may have enriched different types of microbial populations. The oleophilic fertilizer-enriched organisms were not only able to break down straight chain components at a very rapid rate but also branched-chain components. The organisms enriched by the minimal-salts solution were also able to degrade the branched chain aliphatics, but at a reduced rate or after a longer lag period.

The GC/MS traces of the aromatic fractions are presented in Figure 10.9 (tabular representation of the data is given in Table 10.1, as well as the compound names for the alphanumeric designations in Figure 10.9). In the control several components were reduced to undetectable levels after six weeks (note fractions H, Q, S, and T, corresponding respectively to dibenzothiophene, C3-fluorenes, naphthylene, and C1-naphthylene). The traces from the oleophilic fertilizer and minimal-salts solution flasks exhibited virtually complete removal of all aromatic fractions after six weeks incubation.

Results indicate rapid and virtually complete biodegradation of all aliphatic and aromatic components of the weathered oil contaminating Alaskan beaches occurred in nutrient enriched respirometer vessels and flasks. Oxygen uptake started after only a 1.5-day lag period and disappearance of aliphatic and aromatic components occurred within 6 weeks. Different microbial populations appear to have been enriched by the two types of nutrient solutions (oleophilic fertilizer and a minimal-salts solution). This suggests a combination of oleophilic fertilizer and a water-soluble source of nutrients may ultimately be the best way to stimulate rapid bioremediation of crude oil contaminating Alaskan beaches. Results from ballast water biomass enrichments suggest that external sources of microbial populations would not enhance biodegradation, and massive inoculations may not be warranted, at least in the Alaskan bioremediation effort. The respirometric data will eventually be quantitatively analyzed to calculate the kinetics of oil biodegradation.
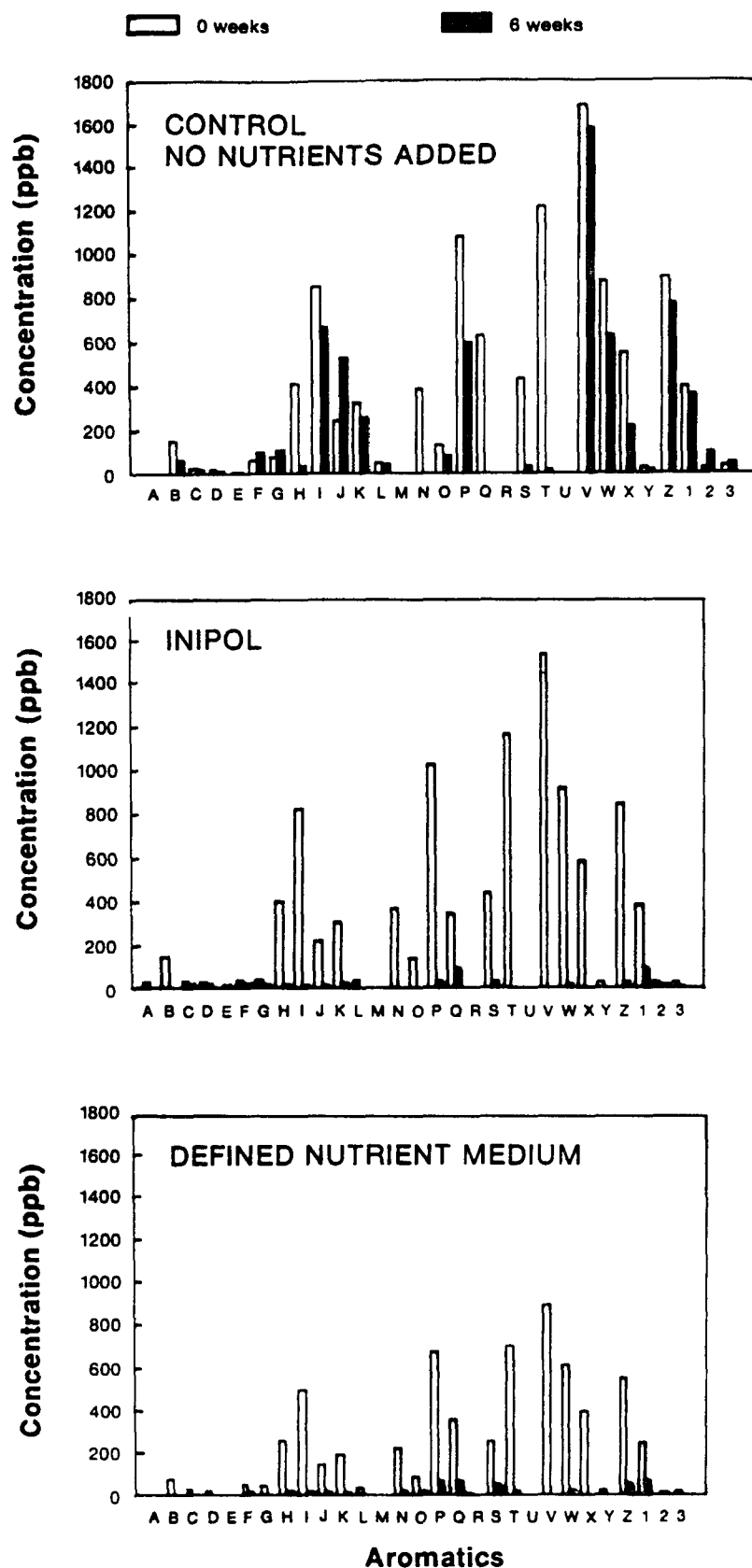
Figure 10.9. Gas Chromatographic Profiles of Aromatics at 0 and 6 Weeks After Initiation of Flask Studies.

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TABLE 10.1. SHAKER FLASK STUDIES: BIODEGRADATION OF AROMATICS (PAHs)

I. FLASKS WITH OIL + INIPOL	0 wks incubation		6 wks incubation	
	SI[a,b]	SIR[a,b]	SI[a,b]	SIR[a,b]
A. Acenaphthene	19.6	24.1	< 5.0	< 5.0
B. Acenaphthylene	165.0	120.0	< 5.0	< 5.0
C. Benzo(a)pyrene	37.2	30.3	12.0	8.99
D. Benzo(b)fluoranthene	18.9	15.2	6.83	< 5.0
E. Benzo(g,h,i)perylene	< 3.0	< 3.0	< 3.0	< 3.0
F. Chrysene/Benzo(a)anthracene	49.0	30.7	15.9	14.8
G. C1-Chrysenes	56.2	35.1	18.9	19.0
H. Dibenzothiophene	463.0	330.0	12.8	12.2
I. C1-Dibenzothiophenes	993.0	675.0	11.9	8.70
J. C2-Dibenzothiophenes	269.0	179.0	8.83	8.72
K. C3-Dibenzothiophenes	378.0	235.0	31.4	24.3
L. Fluoranthene	55.9	37.0	3.57	3.80
M. C1-Fluoroanthenes/Pyrenes	< 3.0	< 3.0	< 3.0	< 3.0
N. Fluorene	412.0	319.0	< 5.0	< 5.0
O. C1-Fluorenes	155.0	128.0	< 5.0	< 5.0
P. C2-Fluorenes	1,267.0	820.0	38.4	30.3
Q. C3-Fluorenes	419.0	265.0	102.0	77.6
R. Indeno(1,2,3-cd)pyrene	< 3.0	< 3.0	< 3.0	< 3.0
S. Naphthalene	494.0	378.0	27.1	29.2
T. C1-Naphthalenes	1,401.0	965.0	< 5.0	< 5.0
U. C2-Naphthalenes	< 3.0	< 3.0	< 5.0	< 5.0
V. C3-Naphthalenes	1,621.0	1,510.0	< 5.0	< 5.0
W. C4-Naphthalenes	1,141.0	743.0	11.9	15.8
X. Phenanthrene/Anthracene	695.0	489.0	6.79	< 5.0
Y. C1-Phenanthrenes/Anthracenes	34.4	26.2	< 5.0	< 5.0
Z. C2-Phenanthrenes/Anthracenes	1,066.0	676.0	38.0	34.4
1. C3-Phenanthrenes/Anthracenes	445.0	307.0	102.0	90.1
2. C4-Phenanthrenes/Anthracenes	34.6	17.2	14.3	18.6
3. Pyrene	25.6	28.7	5.41	2.96

[a] Histogram values were the average values from the above duplicate flasks
[b] Concentration in μg/L (ppb)

TABLE 10.1. (CONT.)

II. FLASKS WITH OIL + MINIMAL SALTS (OECD)	0 wks incubation		6 wks incubation	
	S9[a,b]	S9R[a,b]	S9[a,b]	S9R[a,b]
A. Acenaphthene	< 3.0	< 3.0	< 3.0	< 3.0
B. Acenaphthylene	88.2	75.5	< 5.0	< 5.0
C. Benzo(a)pyrene	24.4	21.6	< 5.0	6.90
D. Benzo(b)fluoranthene	11.1	10.3	< 5.0	< 5.0
E. Benzo(g,h,i)perylene	< 3.0	< 3.0	< 5.0	< 5.0
F. Chrysene/Benzo(a)anthracene	50.3	37.2	< 5.0	10.5
G. C1-Chrysenes	47.8	44.5	< 5.0	10.8
H. Dibenzothiophene	277.0	252.0	12.0	12.6
I. C1-Dibenzothiophenes	538.0	491.0	8.36	14.0
J. C2-Dibenzothiophenes	159.0	141.0	< 5.0	< 5.0
K. C3-Dibenzothiophenes	209.0	195.0	12.6	10.5
L. Fluoranthene	32.6	30.6	< 5.0	< 5.0
M. C1-Fluoroanthenes/Pyrenes	< 3.0	< 3.0	< 5.0	< 5.0
N. Fluorene	255.0	221.0	< 5.0	< 5.0
O. C1-Fluorenes	87.1	76.1	< 5.0	< 5.0
P. C2-Fluorenes	743.0	660.0	28.6	96.1
Q. C3-Fluorenes	388.0	346.0	41.4	97.5
R. Indeno(1,2,3-cd)pyrene	< 3.0	< 3.0	< 3.0	< 3.0
S. Naphthalene	279.0	230.0	33.8	50.9
T. C1-Naphthalenes	776.0	663.0	6.22	19.3
U. C2-Naphthalenes	< 3.0	< 3.0	< 3.0	< 3.0
V. C3-Naphthalenes	969.0	869.0	< 5.0	< 5.0
W. C4-Naphthalenes	674.0	578.0	16.5	20.3
X. Phenanthrene/Anthracene	423.0	587.0	< 5.0	< 5.0
Y. C1-Phenanthrenes/Anthracenes	18.6	17.0	< 5.0	< 5.0
Z. C2-Phenanthrenes/Anthracenes	581.0	538.0	30.9	87.3
1. C3-Phenanthrenes/Anthracenes	263.0	259.0	37.7	90.5
2. C4-Phenanthrenes/Anthracenes	17.2	14.7	7.97	20.3
3. Pyrene	25.6	24.9	< 5.0	< 5.0

[a] Histogram values were the average values from the above duplicate flasks
[b] Concentration in μg/L (ppb)

TABLE 10.1 (CONT.)

III. Control: Oil Only	0 wks incubation	6 wks incubation
	SC 27[a]	SC 27[a]
A. Acenaphthene	< 3.0	< 3.0
B. Acenaphthylene	143.0	89.4
C. Benzo(a)pyrene	28.3	19.9
D. Benzo(b)fluoranthene	15.2	12.2
E. Benzo(g,h,i)perylene	< 3.0	< 3.0
F. Chrysene/Benzo(a)anthracene	62.3	100.0
G. C1-Chrysenes	76.4	105.0
H. Dibenzothiophene	398.0	385.0
I. C1-Dibenzothiophenes	843.0	788.0
J. C2-Dibenzothiophenes	239.0	222.0
K. C3-Dibenzothiophenes	317.0	252.0
L. Fluoranthene	49.0	43.8
M. C1-Fluoroanthenes/Pyrenes	< 3.0	< 3.0
N. Fluorene	382.0	327.0
O. C1-Fluorenes	130.0	82.8
P. C2-Fluorenes	1,038.0	1,015.0
Q. C3-Fluorenes	618.0	585.0
R. Indeno(1,2,3-cd)pyrene	< 3.0	< 3.0
S. Naphthalene	431.0	412.0
T. C1-Naphthalenes	1,206.0	1,135.0
U. C2-Naphthalenes	< 3.0	< 3.0
V. C3-Naphthalenes	1,691.0	1,570.0
W. C4-Naphthalenes	876.0	631.0
X. Phenanthrene/Anthracene	556.0	477.0
Y. C1-Phenanthrenes/Anthracenes	31.7	23.8
Z. C2-Phenanthrenes/Anthracenes	896.0	778.0
1. C3-Phenanthrenes/Anthracenes	406.0	367.0
2. C4-Phenanthrenes/Anthracenes	38.9	105.0
3. Pyrene	41.9	58.8

[a] Concentration in μg/L (ppb)

TOXICITY OF OLEOPHILIC FERTILIZER

Laboratory Bioassays

Toxicity tests were conducted with oleophilic fertilizer (INIPOL) in the summer of 1989 to develop definitive acute toxicity values for fishes, invertebrates, and algae. Tests with silver salmon smolts, herring fry, juvenile sticklebacks, mussel larvae, oyster larvae, mysids, and pandalid shrimp plus oleophilic fertilizer in seawater have been completed, as well as tests utilizing fertilizer plus weathered Prudhoe Bay Crude oil in seawater.

Results show that larvae of mussels, oysters and juvenile mysids were two orders of magnitude more sensitive than salmon, and more sensitive than herring and sticklebacks by a factor of 2 to 6. When mixed with oil, the toxicity of oleophilic fertilizer to salmon was reduced four-fold. The mixture was slightly more toxic to most other test species, although the differences were within the variability of test repeatability.

The data in Table 10.2 were provided to the Shoreline Committee and advisory groups in Valdez, Seward, and Homer to assist in the evaluation of potential toxic effects associated with large-scale application of the fertilizer as a clean-up technique. In addition, a risk assessment procedure was suggested as a means to establish a benchmark no-acute-effect concentration for comparison with possible environmental concentrations following shoreline treatment. This method was modified by the Shoreline Committee in Valdez and Homer, and was used to assist in establishing shoreline segments approved for fertilizer application.

Laboratory toxicity tests were conducted at ERL/GB to determine the toxicity of ammonia and laural phosphate to sensitive marine biota. A shrimp-like crustacean, *Mysidopsis bahia*, and an estuarine fish, the silverside, *Menidia beryllina*, were tested using standard acute toxicity test methods (ASTM, 1988). These standard test animals are representative surrogates for invertebrates and fish in marine systems. In the course of testing, an article by Miller et al. (1990) was published, providing definitive data on ammonia toxicity for these two standard test species over a range of temperature,

TABLE 10.2. RESULTS OF LABORATORY TOXICITY TESTS WITH
THE OLEOPHILIC FERTILIZER, INIPOL, AND VARIOUS MARINE SPECIES[a]
(VALUES ARE 96-HOUR LC50 ESTIMATES UNLESS NOTED OTHERWISE)

	Oleophilic Fertilizer	Fertilizer Plus Oil
Fish		
Salmon smolts	1,500 ppm	6,000 ppm
Herring	100 ppm[b]	---[c]
Sticklebacks	100 ppm[b]	110 ppm
Invertebrates		
Mussel larvae	63 ppm (72 hour)	55 ppm (72 hour)
Oyster larvae	41 ppm (48 hour)	9.0 ppm
Mysids	15 ppm	8.5 ppm
Pandalid shrimp	360 ppm	92 ppm.

[a] Oil concentration used was below toxic concentrations; oil was layered on the surface water at the start of the test, sprayed with oleophilic fertilizer, and mixed once.

[b] Best estimate from non-definitive test

[c] Data are not available

salinity and pH conditions. Results of both acute toxicity tests (96-hour) and chronic estimator tests (7-day) were published, and are presented below for temperatures of 25°C, 31 ppt salinity, and pH of 8.0, test conditions for which both acute and chronic tests were conducted:

	Acute LC50	Chronic Toxicity Value
Mysids	1.7 ppm	0.232 ppm
Silversides	1.3 ppm	0.061 ppm

This publication also addressed the effect of lower temperatures on ammonia toxicity to fish. Both silversides and sheepshead minnows (*Cyprinodon variegatus*) were slightly more sensitive when tested at temperatures less than 25°C (18°C for silversides and 13°C for sheepshead minnows), indicating that the acute LC50s listed above are representative of ammonia toxicity at temperatures encountered in Alaska.

INIPOL also contains tri-laureth phosphate, a common component in cosmetic products. Because a source of this material could not be located, it was not tested with aquatic animals.

Results of chronic estimator toxicit tests are presented in the following table:

Estimate of Chronic Toxicity of INIPOL
to Two Species of Estuarine Fish

No-Effect Concentrations at 7 Days

Laboratory	Test species	7-Day LC50	Survival	Growth
Contract Lab	*Menidia beryllina*	69 ppm	50 ppm	25 ppm
ERL/GB	*Menidia beryllina*	50 ppm	30 ppm	30 ppm
	Cyprinodon variegatus	69 ppm	48 ppm	48 ppm

These test results place the potential for chronic effects to fish at exposure concentrations greater than the 15 to 50 ppm concentrations previously reported to be acutely toxic to invertebrates (i.e., mussel or oyster larvae, juvenile mysids) in laboratory tests. Exposures to INIPOL at concentrations ≥50 ppm in the field for 7 days is extremely unlikely. The results do not change the previous recommendations and assessments that INIPOL can be safely applied at the recommended rates to oiled shorelines, after consideration of the potential for the development of acutely toxic conditions for nearshore invertebrates for very short time periods immediately after application.

Under routine application procedures and specified environmental conditions, no acute toxic effects are expected for marine fishes. The likelihood of toxic effects to marine invertebrates is minimal and transient. This is based on knowledge of (1) the relative sensitivity of fishes and invertebrates tested under laboratory conditions; (2) the minimal input of oleophilic fertilizer into marine waters from unintentional over-spraying of marine waters during application; (3) the minimal release of oleophilic fertilizer from shoreline into the bay following application; and (4) tidal mixing, dilution, and transport of bay waters contaminated with oleophilic fertilizer that will continue to decrease fertilizer concentrations in marine waters.

Toxicity of oleophilic fertilizer to marine biota may be associated with release of fertilizer from the shoreline into the bay immediately after application. A worst-case example would involve protected embayments with minimal tidal exchange and maximum shoreline-to-water ratios (long, narrow bays with constricted openings). INIPOL was applied to oiled shorelines at the rate of 293 g/m^2 (0.06 lb/ft^2). Applied to 100 m of shoreline in a 10-m swath marked from the low-tide line to the upper storm berm, a total of 293,000 g would be used.

If all of the oleophilic fertilizer reached the water in a pulse and: 1) a generalized, 100 m stretch of nearshore environment from the tide line to 10 m offshore, with an average depth of 1 m (1,000 m^3) is used; and 2) water is assumed to be completely mixed, a "worst-case" expected environmental concentration of 293 ppm can be calculated. This value is considerably less than the 96-hour LC50 value for salmon and comparable to the LC50 for herring. Toxicity to marine invertebrates residing in this nearshore area is possible at these concentrations, should unrealistic application conditions result.

Any exposure resulting from shoreline applications would be mitigated by tidal mixing, dilution, and transport out of the system into Prince William Sound. The initial concentrations should decrease by orders of magnitude within 1 to 2 days, to levels considerably less than concentrations acutely toxic in 2 to 4 days in laboratory tests. Thus, the prospect of sustaining acutely lethal concentrations for any biota is very unlikely.

Since there are no proven analytical methods to quantify INIPOL in seawater, no measured environmental concentrations of INIPOL are available from previous field trials to compare with worst-case predictions. However, a more likely estimate of daily input into nearshore waters is in the range of 1% to 10% of the applied material, based on: (1) visual observations of the colored film

present after spraying; (2) our ability to maintain nutrient-enriched pore water in the intertidal zone; and (3) the lack of measured nutrient increases in the nearshore zone. When a more realistic input estimate (assume 10%) is diluted with a nearshore (10 m) volume of water averaging 2 m (a depth consistent with the steep slope of most shorelines in Prince William Sound), the estimated environmental concentration is between 3 and 30 ppm. These values indicate that environmental concentrations less than the laboratory LC50 values of INIPOL and oil mixtures for invertebrates could develop immediately after application, subject to subsequent tidal dilution and transport. When considered in this light, the potential for toxic effects appears to be minimal.

Oleophilic fertilizer and oil mixtures that may leave the treated shoreline should have minimal ecological impact, based on their propensity to degrade and the dilution potential of surrounding waters. The enhanced microbial biomass and available nutrients associated with mixtures of oleophilic fertilizer and oil will result in their rapid degradation. In their mixed form, both oil and INIPOL are less toxic to marine biota, as demonstrated by the reduced toxicity of INIPOL in laboratory tests where it was mixed with oil.

Wildlife Toxicity Issues

Decisions to apply oleophilic fertilizer (INIPOL) or other fertilizer formulations to enhance biodegradation must consider potential for toxicological effects on terrestrial and aquatic life. Information about toxicity of INIPOL to aquatic organisms has been obtained directly from laboratory and field tests with invertebrates and fish. Toxicity of water-soluble fertilizer formulations was assessed using toxicity data available on ammonia in the U.S. Environmental Protection Agency Water Quality Criteria Document. Ammonia is the principle toxic component for water-soluble fertilizers.

Toxicity of several INIPOL components has been sufficiently quantified for small mammals to allow some realistic assessments of likely events that could lead to lethal or toxic exposures for mammalian and avian wildlife. Inhalation of toxic vapors of ammonia or 2-butoxy-ethanol poses a negligible risk to wildlife. Direct ingestion of contaminants from licking treated surfaces poses some risk if the animals inhabit these areas within a couple of days of treatment. In addition, the potential for ingestion of contaminants during cleaning behavior of mammals and birds should be considered for exposures to 2-butoxy-ethanol, laureth phosphate, or a total dose of INIPOL. Skin irritation and eye irritation are possibile toxic effects to wildlife resulting from exposures to some INIPOL constituents. The potential for toxic effects lessens with time after application.

LABORATORY STUDIES

This review was conducted to obtain information on the toxicity of INIPOL and its constituents in order to evaluate more fully the potential for adverse effects on wildlife that might enter oiled shorelines after bioremediation treatments during 1990 oil spill clean-up activities. Toxicity of fertilizers to small and large mammals and birds was assessed in a cursory manner during the summer of 1989 by extrapolating available human health information on chemical toxicity and by using the incidental knowledge of experts working with the Prince William Sound Interagency Shoreline Clean-up Committee. The purpose of this review was to supplement that information with a systematic review of what is known about toxicity to mammals and birds for nutrient amendments used in bioremediation programs.

The data evaluated were those providing exposure (dose)-response information upon which direct effects to wildlife survival, growth, or reproductive success could be inferred. A considerable number of abstracts were found in the databases on the biochemical and physiological effects of atmospheric or aqueous exposures of test animals to ammonia or urea. Most of these reports discussed metabolic pathways or physiological responses and did not relate effects to survival, growth, or reproductive success of the animal. Metabolic or physiological studies do not provide a basis for meaningful extrapolations to direct ecological consequences of exposure. These metabolic and physiological publications were not included in the evaluation.

Effects data are summarized in Tables 10.3 to 10.7 that group human, other mammalian, and avian data separately. Not all chemicals had data for each grouping. For chemicals without data on toxicity to birds, hazards to avian wildlife must be extrapolated from mammalian data. No toxicity data were retrieved for oleophilic fertilizer (INIPOL); however the use of INIPOL for the oil spill in Spitsbergen was listed in the Toxline database. Since there were no toxicity data for laureth phosphate in the databases, information on lauryl sulfate is presented for comparative purposes.

Atmospheric Ammonia

Concentrations of ammonia in air ≥ 10 ppm pose potential toxicity problems for birds, based on the lowest effect exposure noted in Table 10.3. Lethal concentrations of ammonia in air for short-term exposures (hours) to small mammals appear to be in the thousands of ppm. Sensitivities of humans and small mammals appear to be the same order of magnitude based on the data in Table 10.3. Ammonia has induced mutagenic changes in bacteria at high doses; a similar effect has not been reported in mammals or birds.

TABLE 10.3. SUMMARY OF TOXICITY DATA FOR AMMONIA. MOST LETHAL OR EFFECTS EXPOSURES WERE REPORTED AS PARTS-PER-MILLION (PPM) IN AIR OR AS MG NH$_3$ PER CUBIC METER OF AIR (1 MG/M^3 = 1.414 PPM). SOME RESULTS WERE CONVERTED TO TOXIC DOSES EXPRESSED AS MG NH$_3$/KG TEST ANIMAL. TCLO=LOWER LEVEL OF TOXIC EXPOSURE, LCLO=LOWER LEVEL OF LETHAL CONCENTRATIONS, LC50=CONCENTRATION LETHAL TO 50% OF TEST POPULATION

Human Data

Inhalation	TCLo	20	ppm (duration unspecified)
Inhalation	LCLo	5000	ppm for 5 minutes
Inhalation	LDLo	132	mg/kg

Non-human Mammalian Data

Inhalation for Rat	LC50	37,200	ppm for 1 hour
Inhalation for Rat	LC50	2,000	ppm for 4 hours
Inhalation for Mouse	LC50	4,230	ppm for 1 hour
Inhalation for Cat	LC50	7,000	mg/m^3 for 1 hour
Inhalation for Rabbit	LC50	7000	mg/m^3 for 1 hour
Ingestion by Guinea Pig	LD75	900-1200	mg/kg

Bird Data

Inhalation for Pullets: 200 ppm for 17 days; Toxic effects noted as decreases in food intake, growth, egg production and increased mortality.

Inhalation for Broiler Chicks: 75 or 100 ppm for 4 days; Toxic effects noted as respiratory and circulatory damage.

Inhalation for Turkeys: 10 or 40 ppm (duration not specified). Toxic effects noted as respiratory damage.

Other toxicity data noted

Mutagenic in bacteria (*Escherichia coli*) at 1500 ppm for 3 hours in atmosphere over culture disc.

Cytogenic effects noted in rats exposed to 19.8 mg/m^3 for 16 weeks.

TABLE 10.4. SUMMARY OF TOXICITY DATA FOR AQUEOUS AMMONIA. MOST LETHAL
OR EFFECTS EXPOSURES WERE REPORTED AS PARTS-PER-MILLION (PPM) NH_3
IN WATER OR AS MG NH_3 PER LITER OF WATER. SOME RESULTS WERE CONVERTED
TO TOXIC DOSES EXPRESSED AS MG NH_3/KG TEST ANIMAL. TCLO=LOWER LEVEL OF
TOXIC EXPOSURE, LCLO=LOWER LEVEL OF LETHAL CONCENTRATIONS,
LC50=CONCENTRATION LETHAL TO 50% OF TEST POPULATION, LDLO=LOWER LEVEL
OF LETHAL DOSES, LD50=DOSE LETHAL TO 50% OF TEST POPULATION

Human Data

Oral Dose	LDLo	43	mg/kg
Inhalation	LCLo	5000	mg/kg (fumes derived from solution)
Inhalation	TCLo	408	ppm (fumes derived from solution)

Non-human Mammalian Data

Oral Dose to Rat	LD50	350	mg/kg
Oral Dose to Cat	LDLo	750	mg/kg
Injected into Mouse	LDLo	160	mg/kg (beneath skin)
Injected into Rabbit	LDLo	200	mg/kg (beneath skin)
Injected into Mouse	LD50	91	mg/kg (into veins)
Injected into Rabbit	LDLo	10	mg/kg (into veins)

Eye irritation to rabbit	0.75	mg causes severe reaction
Eye irritation to rabbit	0.044	mg causes severe reaction
Eye irritation to rabbit	1.0	mg in 30 second rinse causes severe reaction

No Bird Data Available for Review

Other Toxicity Data Noted

Mutation in Bacteria (*Escherichia coli*) at 10 mg/growth disc

TABLE 10.5. SUMMARY OF TOXICITY DATA FOR UREA. TOXIC DOSES ARE EXPRESSED AS MG UREA/KG TEST ANIMAL. LDLO=LOWER LEVEL OF LETHAL DOSES, LD50=DOSE LETHAL TO 50% OF TEST POPULATION

Human Data

Skin irritation - 22 mg applied intermittently over 3 days caused mild irritation
No other toxicity data were available for review

Non-Human Mammalian Data

Oral Dose Rat	LD50	8,471	mg/kg
Oral Dose Mouse	LD50	11,000	mg/kg
Oral Dose Goat and Sheep	LDLo	511	mg/kg
Injection Rat	LD50	8,200	mg/kg (beneath skin)
Injection Mouse	LD50	9,200	mg/kg (beneath skin)
Injection Dog	LDLo	3,000	mg/kg (beneath skin)
Injection Rabbit	LDLo	3,000	mg/kg (beneath skin)
Injection Rat	LD50	5,300	mg/kg (into vein)
Injection Mouse	LD50	4,600	mg/kg (into vein)
Injection Dog	LDLo	3,000	mg/kg (into vein)
Injection Rabbit	LDLo	4,800	mg/kg (into vein)

Bird Data

Injection Pigeon	LDLo	14,800	mg/kg (beneath skin)

Other Toxicity data noted

Urea has caused reproductive effects in humans and monkeys when injected into placentas during the 16th or 18th week of pregnancy. Tumorigenic effects in rats and mice have resulted with one year exposures. Tests with cell cultures of human or other mammal tissues have shown positive results for mutation screens.

TABLE 10.6. SUMMARY OF TOXICITY DATA FOR 2-BUTOXY-ETHANOL. TOXIC DOSES ARE EXPRESSED AS MG 2-BUTOXY-ETHANOL/KG TEST ANIMAL. EXPOSURES BY INHALATION ARE PPM IN AIR OR MG 2-BUTOXY-ETHANOL/M^3. LDLO=LOWER LEVEL OF LETHAL DOSES, TDLO=LOWER LEVEL OF TOXIC DOSES, LD50=DOSE LETHAL TO 50% OF TEST POPULATION, LC50=CONCENTRATION IN AIR LETHAL TO 50% OF TEST POPULATION

Human Data

Oral Dose (one woman)	TDLo	600	mg/kg
Inhalation	TCLo	195	ppm for 8 hours
Inhalation	TCLo	100	ppm (duration unspecified)

Non-human Mammalian Data

Oral Dose Rat	LD50	470	mg/kg
Oral Dose Mouse	LD50	1,230	mg/kg
Oral Dose Rabbit	LD50	300	mg/kg
Oral Dose Guinea Pig	LD50	1,200	mg/kg
Inhalation for Rat	LC50	2,900	mg/m^3
Inhalation for Mouse	LC50	700	ppm in air for 7 hours
Injection Rat	LD50	220	mg/kg (into abdomen)
Injection Mouse	LD50	536	mg/kg (into abdomen)
Injection Rabbit	LD50	220	mg/kg (into abdomen)
Injection Rat	LD50	340	mg/kg (into vein)
Injection Mouse	LD50	1,130	mg/kg (into vein)
Injection Rabbit	LD50	280	mg/kg (into vein)
Injection Mouse	LDLo	500	mg/kg (beneath skin)
Absorbed by Skin Rabbit	LD50	220	mg/kg
Absorbed by Skin Guinea Pig	LD50	230	mg/kg
Eye Irritant Rabbit		500	mg caused mild irritation
Eye Irritant Rabbit		18	mg caused some irritation
Eye Irritant Rabbit		100	mg over 24 hours caused moderate irritation

Bird Data

No Bird data were available for review

Other Toxicity Data noted

This chemical has caused reproductive effects in tests with rats, mice, or rabbits using extended exposures or exposures at TCLo concentrations reported above.

TABLE 10.7. SUMMARY OF TOXICITY DATA FOR SODIUM LAURYL SULFATE.
TOXIC DOSES ARE EXPRESSED AS MG SODIUM LAURYL SULFATE/KG TEST ANIMAL.
LD50=DOSE LETHAL TO 50% OF TEST POPULATION

Human Data

Skin Irritant, 250 mg caused mild irritation in 24 hours
Skin Irritant, 25 mg caused mild irritation in 24 hours
No other toxicity data were available for review.

Non-human Toxicity Data

Oral Dose Rat	LD50	1,288	mg/kg
Injection Rat	LD50	210	mg/kg (into abdomen)
Injection Mouse	LD50	250	mg/kg (into abdomen)
Injection Rat	LD50	118	mg/kg (into vein)
Injection Mouse	LD50	118	mg/kg (into vein)
Skin Irritant Mouse		25	mg, moderate irritation in 24 hours
Skin Irritant Dog		25	mg, mild irritation in 24 hours
Skin Irritant Pig		25	mg, mild irritation in 24 hours
Skin Irritant Guinea Pig		25	mg, mild irritation in 24 hours
Skin Irritant Rabbit		50	mg, severe irritation in 24 hours
Skin Irritant Rabbit		25	mg, moderate irritation in 24 hours
Skin Irritant Rabbit		250	mg, moderate irritation in 24 hours
Skin Irritant Rabbit		10	mg, no degree reported for 24 hours
Skin Irritant Rabbit		50	mg, mild irritation in 24 hours

No Bird Data were Available for Review

Other Toxicity Data Noted

Mutagenic effects noted for bacterial and fungal cultures at very high exposure concentrations when tested in the early 1900's. Results of modern tests at extremely high exposures conducted with bacterial cultures were negative for mutagenicity (Hazardous Substances Data Base).

Because application of fertilizers produces ammonia from slow-release solubilization of urea or controlled dissolution of ammonium-containing reagents, release of ammonia to the atmosphere is expected to be a very indirect process regulated by dissolution of gas from water into the air. It is expected that this process will yield atmospheric concentrations much less than 10 ppm in air immediately above treated shorelines. Dilution by directional breezes and thermal air currents will quickly diminish any atmospheric concentrations of ammonia to background levels. No short-term

or chronic exposure problems are expected for wildlife with respect to atmospheric ammonia for shorelines treated with fertilizers. Although quantitative monitoring data were not obtained, this assertion would appear to be supported by the fact that teams applying fertilizers during 1989 did not report fumes of ammonia in treated areas nor did they appear to suffer from acute exposure to ammonia.

Aqueous Ammonia

Ammonia dissolved in water is toxic to small mammals if ingested in sufficient quantities to obtain exposures in the hundreds of mg per kilogram of body weight (Table 10.4). Body burdens obtained by injecting ammonia into animal bodies produced lethal effects at similar or slightly lower dose rates. No avian toxicity data were available for review.

Maximum aqueous concentrations of ammonia associated with fertilizer additions from the bioremediation study were reported for subsurface (interstitial) samples. Peak concentrations in interstitial water ranged from 100 to 400 μg/L. A small mammal would have to drink over one hundred liters of subsurface seawater to obtain a toxic dose of ammonia. In actuality, wildlife would be exposed to nearshore surface waters where concentrations of ammonia would be considerably less compared to subsurface water. For example, water samples collected immediately offshore from treated areas within a day of treatment were always at background concentrations of <1 to 3 μg ammonia/L or less, demonstrating the rapid dilution potential from tidal mixing and nearshore currents. For wildlife frequenting shorelines treated with fertilizers, the potential hazard of ammonia poisoning from intentional or incidental ingestion of surface waters is negligible.

Aqueous solutions of ammonia can cause eye irritation. Laboratory tests conducted by adding drops to the eyes of rabbits (a standard procedure for determining eye irritants) determined that as little as 0.044 mg could cause severe eye irritation. If this quantity was contained in a mL of water, it would be the equivalent of a concentration of 44 mg/L. None of the surface water samples analyzed the summer of 1989 approached such concentrations, although some subsurface water samples did. Eye irritation from ammonia exposures in water would seem unlikely for shoreline wildlife.

Urea

Urea is lethal to mammals and birds at body burdens in the thousands of mg/kg, even though exposures vary from ingestion to subcutaneous injections (beneath the skin) to intravenous injection (into a vein) (Table 10.5). There is a single report where 500 mg/kg was lethal to goats and sheep. Urea was a mild skin irritant for humans when 22 mg was applied over 3 days.

The application rate of oleophilic fertilizer (INIPOL) during the summer of 1989 was approximately 300 g/m^2, equivalent to 48 g of urea/m^2 because INIPOL is 16% urea. A small animal (1 kg) would have to eat all the INIPOL applied to an area 0.25 m by 0.25 m (0.0625 square meters, or an area approximately .3 m by .3 m) in order to obtain a lethal dose of urea (using 3,000 mg/kg as an LD50). Effects of skin irritation could be possibile for animals active on recently treated shorelines.

2-butoxy-ethanol

Information on the toxicity of 2-butoxy-ethanol to mammals is shown in Table 10.6. Body burdens of 200 to 500 mg/kg from ingestion, injection, or absorption through the skin are lethal. Mild eye irritation in rabbits occurs at doses of 18 to 100 mg. Reproductive effects in mammals have been reported for a range of doses administered to pregnant females. A human exposure limit of 25 ppm in air has been established to protect workers. No avian toxicity data were available.

Monitoring by Exxon industrial hygiene personnel during initial oleophilic fertilizer (INIPOL) applications during the summer of 1989 indicated that exposure limits of 25 ppm were not exceeded. As a result, workers in the field were not required to wear personal protection equipment during applications. Inhalation exposures for wildlife would likewise seem to pose no toxic threat.

The 2-butoxy-ethanol comprises 11% of the formulated INIPOL, providing an application rate of 30 g/m^2 for this chemical. Acute toxicity could be expected for 1 kg animals that ingest freshly applied INIPOL (licking treated substrate, chewing coated sticks, ingesting coated gravel) from an area approximating 0.01 m^2 (0.1 m by 0.1 m, or 4 inches by 4 inches) to obtain a 300 mg/kg dose. Ingestion during cleaning behavior also should be considered, because wildlife could contaminate their feet, fur, or feathers with 2-butoxy-ethanol if the animals venture onto a treated shoreline immediately after application. Transfer of mg quantities of butoxy-ethanol from feet or fur into the

eyes could occur during cleaning or sleep, causing eye irritation. As butoxy-ethanol is volatile and should quickly evaporate from beach surfaces, substantial body contamination would be likely only for animals utilizing shorelines treated with INIPOL within hours of application.

Lauryl sulfate (surrogate for laureth phosphate)

A considerable amount of data exists on the skin irritant capabilities of sodium lauryl sulfate (Table 10.7) because it is a common ingredient in soap. Laureth phosphates could be expected to act similarly. Skin exposure to $\geq$25 mg of lauryl sulfate can cause irritation. No effects were seen with an application of 10 mg to rabbits. Body doses $\geq$100 mg/kg by injection or ingestion can be lethal to small mammals. No avian data were available for review.

The prospects of adverse effects to wildlife attributable to laureth phosphate following oleophilic fertilzier (INIPOL) applications are intermediate of those posed by urea and 2-butoxy-ethanol. This constituent is neither volatile nor readily absorbed across the skin, so toxicity risks are considerably less than those of 2-butoxy-ethanol for body burdens. Ingestion appears to be the only likely route of exposure. If the rat oral LD50 of 1288 mg/kg for lauryl sulfate is used for calculation purposes, an acutely toxic dose of laureth phosphate for a 1 kg animal would be contained in the INIPOL applied to approximately 0.02 m^2 (or an area .1 m by .1 m), as this component comprises approximately 25% of the INIPOL. Potential for toxic effects from ingestion of treated surfaces or from cleaning contaminated feet, fur, of feathers should be considered. Incidental eye irritation is possible for animals visiting treated shorelines shortly after application.

If necessary, some of the uncertainty about the availability of applied INIPOL could be quantified or characterized qualitatively during monitoring programs. Collection of samples from shoreline surfaces could be scheduled on a daily basis for quantification of grams of nitrogen or μg of butoxy-ethanol per square meter through time after treatment to measure residual concentrations of these components. Such data would assist in quantifying exposure potential for animals visiting the shoreline over several days after fertilizer application.

As with other environmental risks associated with clean-up activities, the potential for wildlife effects following fertilizer application must be compared to and balanced with risks posed to wildlife by the residual oil along the shoreline.

TOXICITY OF CUSTOMBLEN PELLETS TO BIRDS

The EPA Environmental Research Laboratory at Corvallis conducted a screening-level acute toxicity test with CUSTOMBLEN and adult (9 month) bobwhite quail. Quail were dosed with CUSTOMBLEN pellets using a dosing gun to push the pellets down their throat. Birds were dosed at the rate of 5 g, 1 g or 0.2 g per bird (birds weighed approximately 200 g). The 5 g/bird dose (20 g/kg) caused acute mortality within 8 hours; 1 g/bird (4 g/kg) dose caused mortality within 36 hours for half the test population; 0.2 g/bird (1 g/kg) caused no mortality among the test population. When quail were offered CUSTOMBLEN pellets mixed with their food, they did not discriminate between the two; the pellets were not preferentially ingested, nor were they avoided. The acute LD50 calculated from these screening tests converts to the consumption of 40 to 60 pellets for a 200 g adult quail. Subsequent feeding tests with black-legged stilts, a heron-like bird that normally feeds on live invertebrates inhabiting the shoreline, showed that they did not ingest the CUSTOMBLEN pellets and preferred live food.

No definitive toxicity tests with birds were conducted because of limited bird availability. This was a low priority issue in light of these data, reviewed literature, and the lack of reports of bird mortality or consumption of CUSTOMBLEN pellets in the field. The screening level tests indicate that seed-eating birds are at greater risk than live-feeders. Since shoreline and aquatic birds are not seed eaters, their risks of mortality from consumption of CUSTOMBLEN pellets are minimal.

BIOMETER STUDIES

During the winter of 1989/1990 and early spring 1990, further laboratory experimentation was conducted in an attempt to fully optimize oil degradation in the field using refined and improved methods for fertilizer application.

Fertilizer Specific Activity

When treated with a single application of inorganic nutrient solutions containing varying amounts of nitrogen and phosphorus followed by subsequent additions of 3% NaCl, microorganisms associated with oiled beach material from Disk Island responded with increased respiratory activities. These increases in activity were directly related to the amount of N and P added to each system, with 10-fold dilutions of these solutions producing proportional decreases in the microbial activities

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observed (Figure 10.10). Over the 3-day incubation period, material treated with the level 1 nutrient solution produced approximately 400 M CO_2 (cumulative) while consuming close to the same amount of oxygen (460 M) for an average respiratory coefficient of about 1.1. Since crude oil represented the primary source of carbon in these systems, this activity was presumably due to the stimulation of oil-degrading microorganisms.

Material treated with various dilutions of the level 1 solution exhibited proportional decreases in activity. Both the lowest level of nutrient addition (level 4) and unamended Prince William Sound water exhibited similar responses, suggesting that the level of available N and P in natural waters was roughly equivalent to 35 and 0.07 g/L, respectively (nutrient analysis of this water determined N and P concentrations of 35 and 9 g/L, respectively). These responses are compared to those obtained with the 3% NaCl control. Preliminary studies demonstrated that this treatment was equivalent to a poisoned control (formalin), but did not produce some of the undesirable effects associated with acidification and the addition of formaldehyde, which precipitated during chemical extraction procedures (data not shown). Therefore, 3% NaCl was used as the control in subsequent studies.

The stair-step response in CO_2 production data, most notably evident with the level 1 treatment, was a result of intermittent sampling during both high- and low-tide periods. During high-tide periods (0 to 12, 24 to 36, and 48 to 60 hour), the rate of CO_2 production appeared to be much slower due to the ability of the tide waters (pH=8.1) to adsorb evolved CO_2. The presence of tide waters did not affect oxygen this way.

Following 3 days of measuring microbial respiration rates, radiolabeled phenanthrene was used as a reporter chemical to further demonstrate the ability to stimulate the activity of indigenous oil-degrading microorganisms through the addition of inorganic nutrients (Figures 10.11 and 10.12). Radiolabeled substrate was added during the first high-tide period, which lasted for 6 hours, and systems were monitored for 60 hours. During the first low-tide period (6 hours of incubation), there was a dramatic increase in the amount of $^{14}CO_2$ produced in all systems except the 3% NaCl control. With continued incubation, specific treatment effects were pronounced: level 1>level 2>level 3>level 4=Prince William Sound. These radiorespirometric data correlate well with data presented in Figure 10.10.
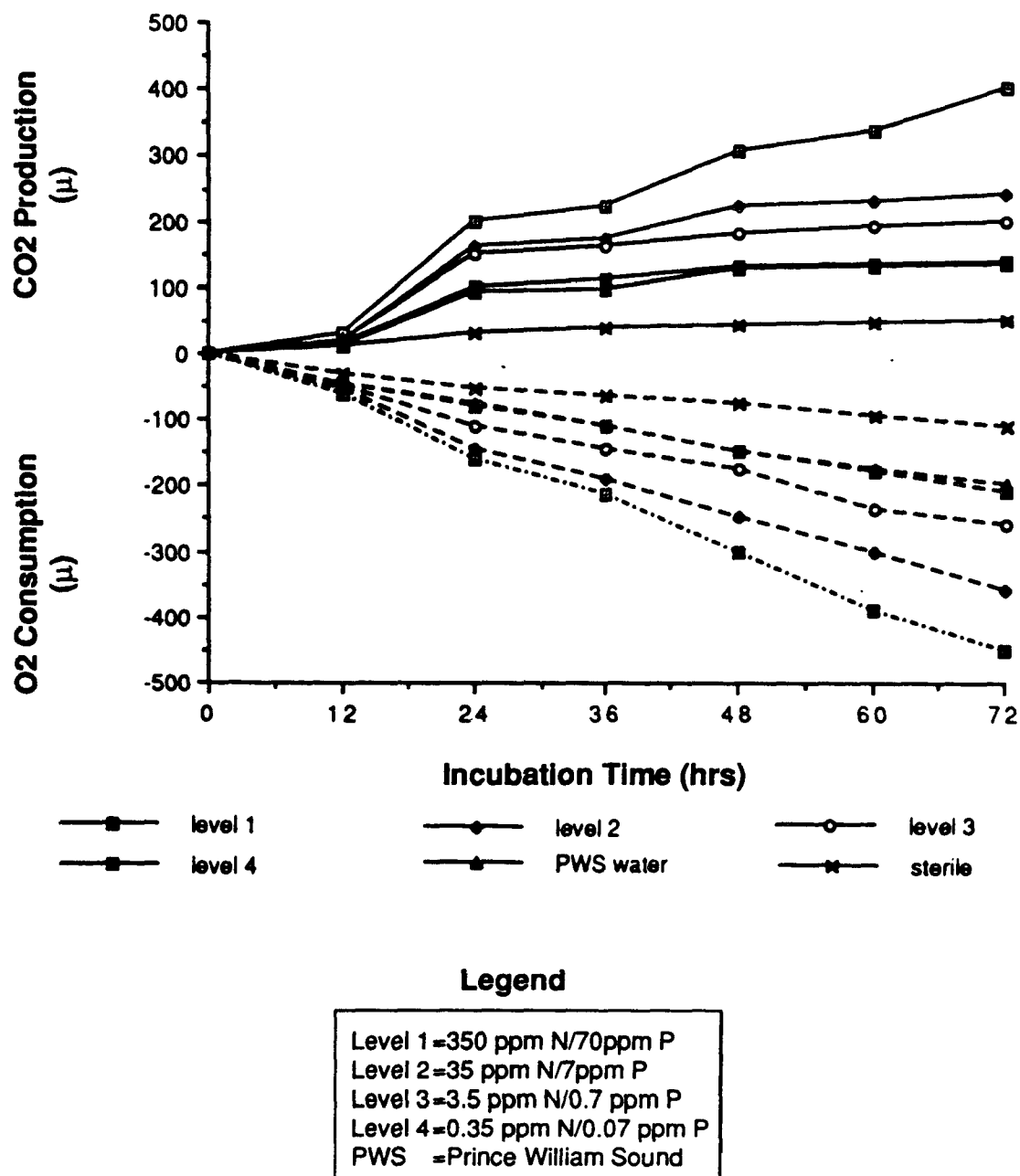
Figure 10.10. Fertilizer Specific Activity (O_2 Consumption, CO_2 Production) for Six Treatments Over Time.
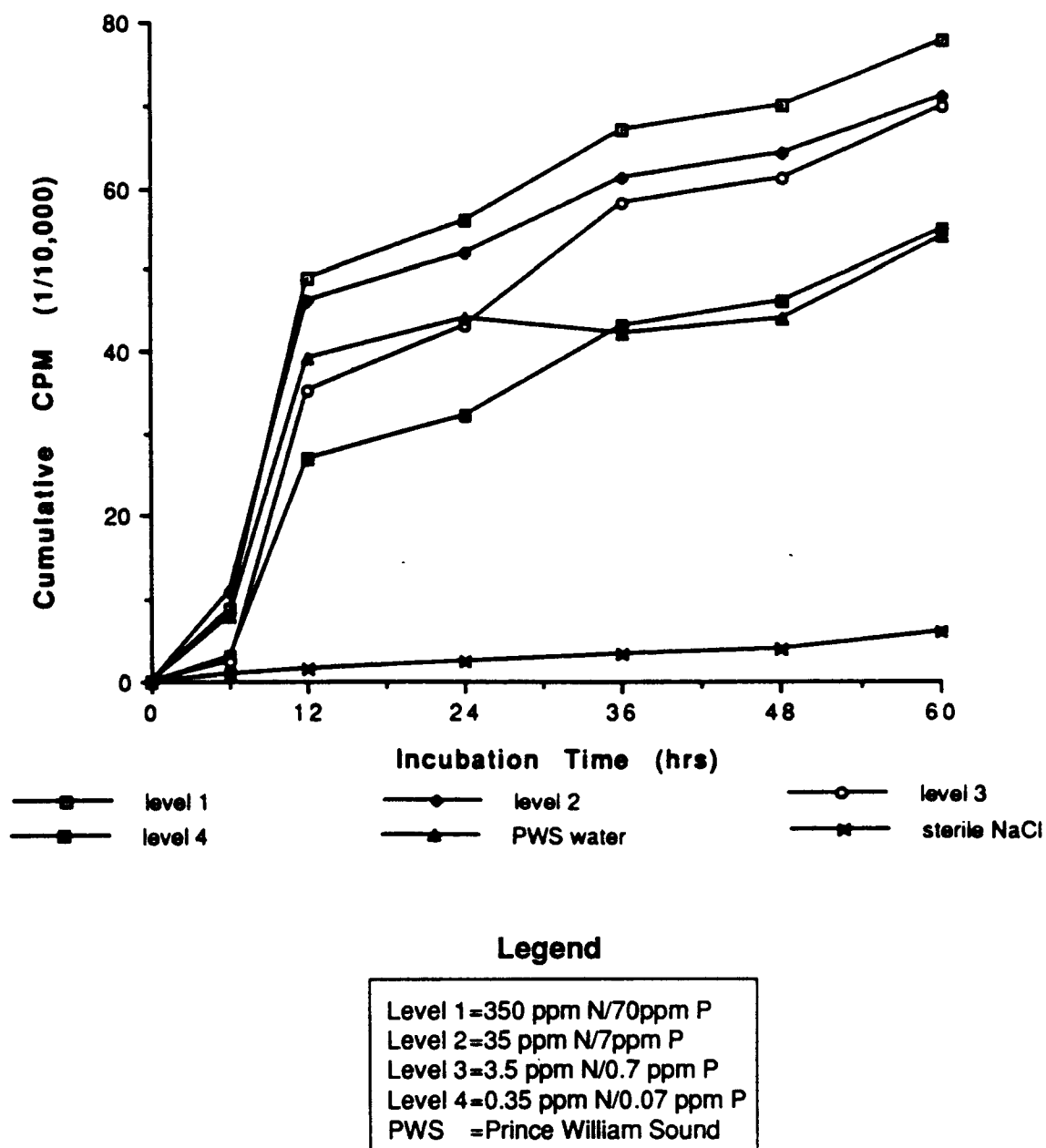
Figure 10.11. Mineralization of Radiolabeled Phenanthrene Over Time As Influenced by Fertilizer Application.
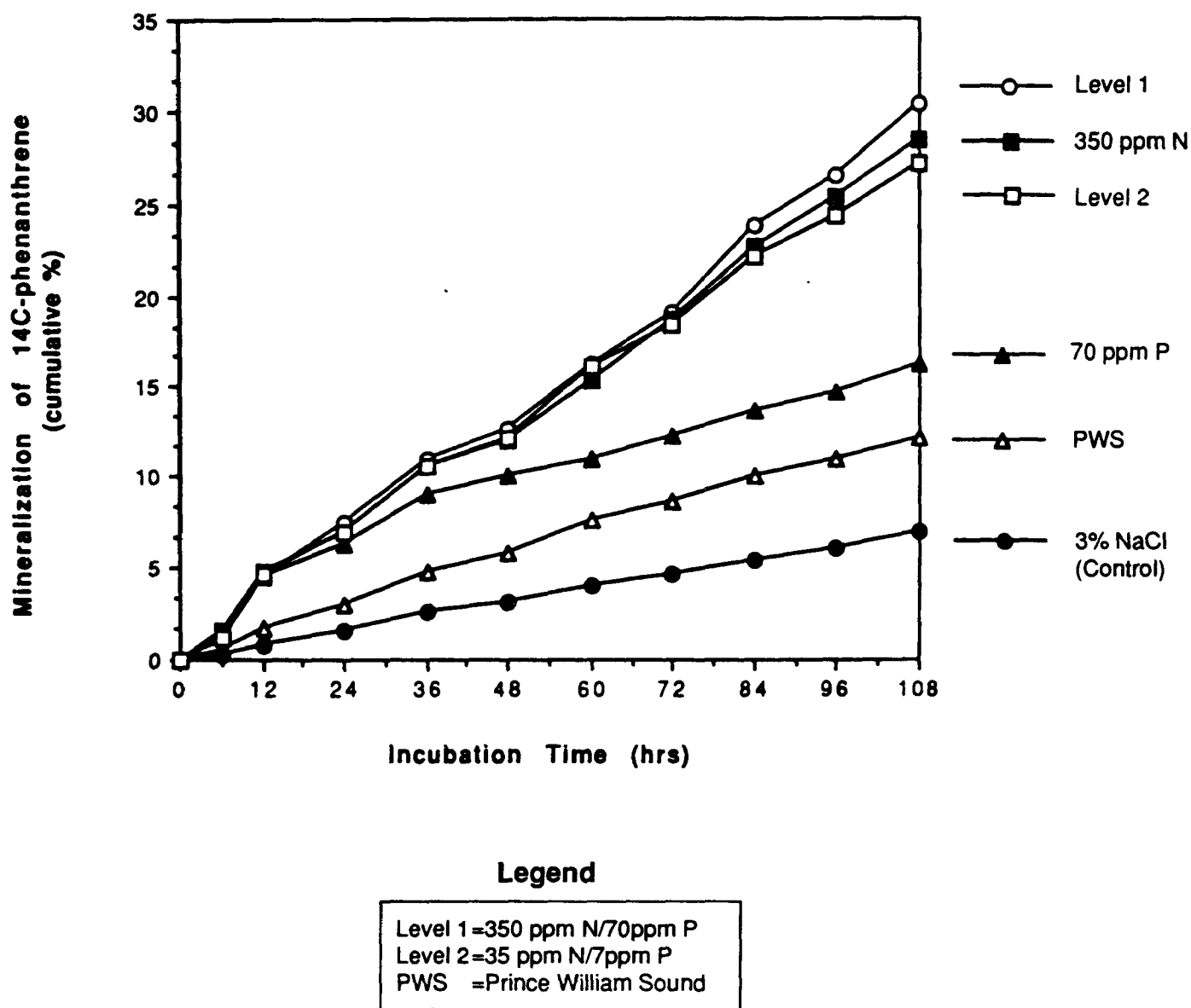
Figure 10.12. Stimulation of Oil-Degrading Microflora with Inorganic Nutrient Supplementation Over Time.

The observed relationship between N concentration and the activity of oil-degrading microorganisms is summarized in Figure 10.13. Based on these data, the optimum concentration of N, the primary factor limiting the biodegradation of crude oil, was determined to be approximately 35 ppm.

Corresponding changes in the amounts of oil present in each system further demonstrated the ability to stimulate the activity of oil-degrading microorganisms through the addition of inorganic nutrients (Table 10.8). Following 6 days of incubation after initial treatment, beach material treated with level 1 nutrients contained 202 mg hexane-soluble and 212 mg hexane-insoluble oil constituents.

TABLE 10.8. CHANGES IN OIL CONCENTRATION AND DISTRIBUTION
DURING BIODEGRADATION WITH VARIOUS
NUTRIENT AMENDMENTS

Treatment	Oiled Rocks			Tide Waters		
	Hexane-Soluble	Hexane-Insoluble	Total peak area	Hexane-Soluble	Hexane-Insoluble	Total peak area
	mg	mg	$\log_{10}$	mg	mg	$\log_{10}$
Level 1	202	212	5.7	47	73	6.0
Level 2	224	223	5.8	32	47	5.8
Level 3	204	214	5.9	42	62	5.7
Level 4	213	228	5.9	32	50	6.1
PWS water	227	244	6.0	36	62	6.0
Control	273	298	6.2	75	52	6.5

Oiled rocks extracted after 6 days incubation;
Tide waters combined for extraction;
Fertilizer treatments applied on day 1 only (initial high-tide);
Control = level 1 plus 3.7% formaldehyde added at each high tide.
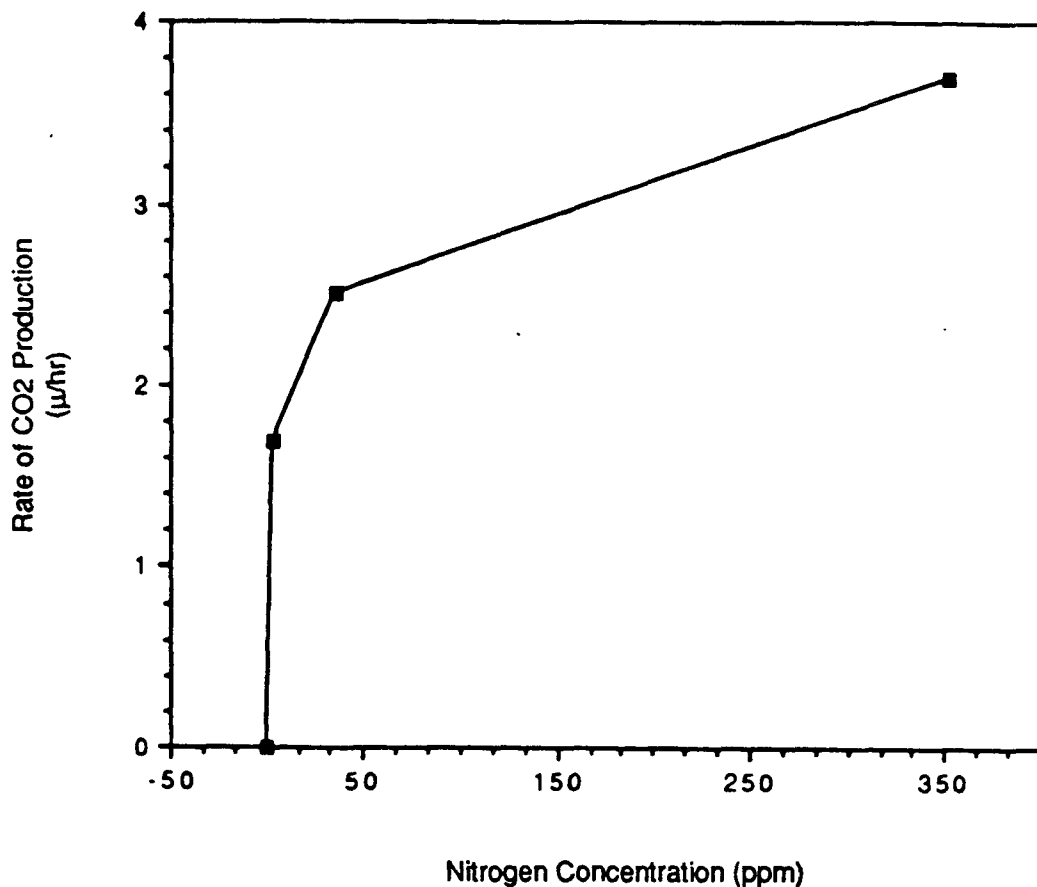
Figure 10.13. Specific Relationship Between N Concentration and the Activity of Oil-Degrading Microflora.

When compared to the values of 273 mg hexane-solubles and 298 mg asphaltenes for the control, the ability to enhance the extent of oil biodegradation through nutrient additions was clearly demonstrated. Moreover, total peak area (calculated from gas chromatographic analyses) was less with the level 1 treatment than with any other treatment. These further support the ranking of treatments for their ability to accelerate the rate and extent of oil biodegradation as described above.

Changes in the amount and composition of oil recovered from tide waters was less definitive. For all parameters measured (hexane-soluble weights, asphaltene weights and total peak area) there were no noticeable differences between treatments.

Using CO_2 evolution data as a representative measurement of relevant microbial activities, the importance of nitrogen concentration on the activity of oil-degrading microorganisms was demonstrated. This relationship is shown in Figure 10.13 where the rate of CO_2 production is plotted as a function of N concentration. From these data, it was determined that the most effective concentration of soluble nitrogen was between 350 and 35 ppm N. Therefore, these two concentrations were used in subsequent studies.

Agitation Rates

The effects of physical agitation on the removal of crude oil from contaminated beach material were determined using radiolabeled phenanthrene as a reporter chemical. Generally, increased agitation stimulated the activity of oil-degrading microorganisms (Figure 10.14). However, when agitation was excessive (125 rpm), the amount of labeled substrate physically removed from the system was high (Figures 10.15 and 10.16; Table 10.9). Since most of the oil was washed away under these conditions, the amount of labeled phenanthrene available for biodegradation was reduced.

Fertilizer Application Strategies

Oleophilic fertilizer application to beach material in biometer flasks was based on field remediation activities where fertilizer was applied at a rate of 20% (weight) of the oil present. Preliminary studies showed that beach material from Passage Cove was contaminated with 4.5% (weight) partially weathered, Prudhoe Bay crude oil (450 mg oil per 100 g beach material). Therefore, a single application of 80 L oleophilic fertilizer was applied to 100 g of oiled beach material, resulting in the addition of 19 mg laureth phosphate and 12.6 mg urea per 100 g oiled beach material.

When compared with level 1 (350 ppm N, 70 ppm P) and level 2 (35 ppm N, 7 ppm P) inorganic nutrient solutions, the effect of oleophilic fertilizer application was proportional to its N concentration effect (126 ppm N) (Figure 10.17). However, the stimulatory effect of oleophilic fertilizer on the activity of oil-degrading microorganisms appeared to decrease 4 days after fertilizer application.

After 6 days of incubation, radiolabeled phenanthrene or oleic acid was added to each flask and the production of $^{14}CO_2$ was monitored for 24 hours (Figures 10.18 and 10.19). Within the 12 hour high-tide incubation period, only 10 to 14% of the added phenanthrene was removed from the system
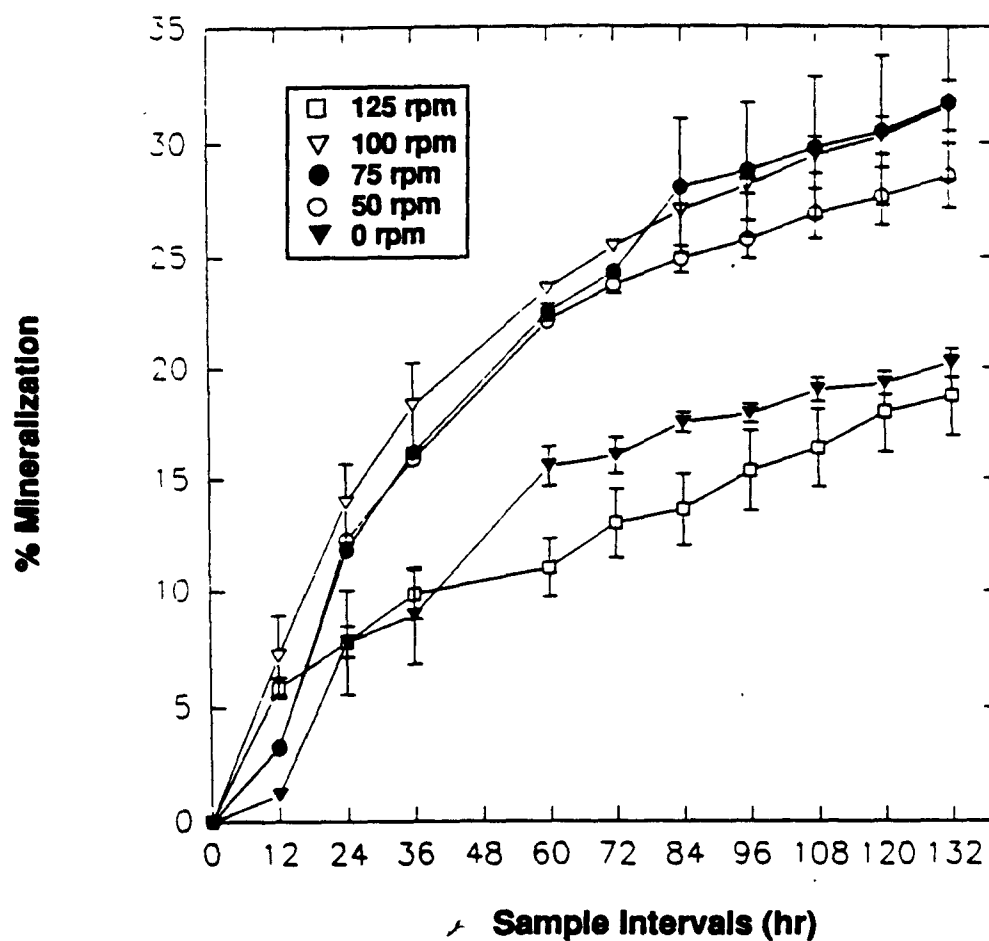
Figure 10.14. Cumulative Percent Mineralization of ^{14}C Phenanthrene Over Time.
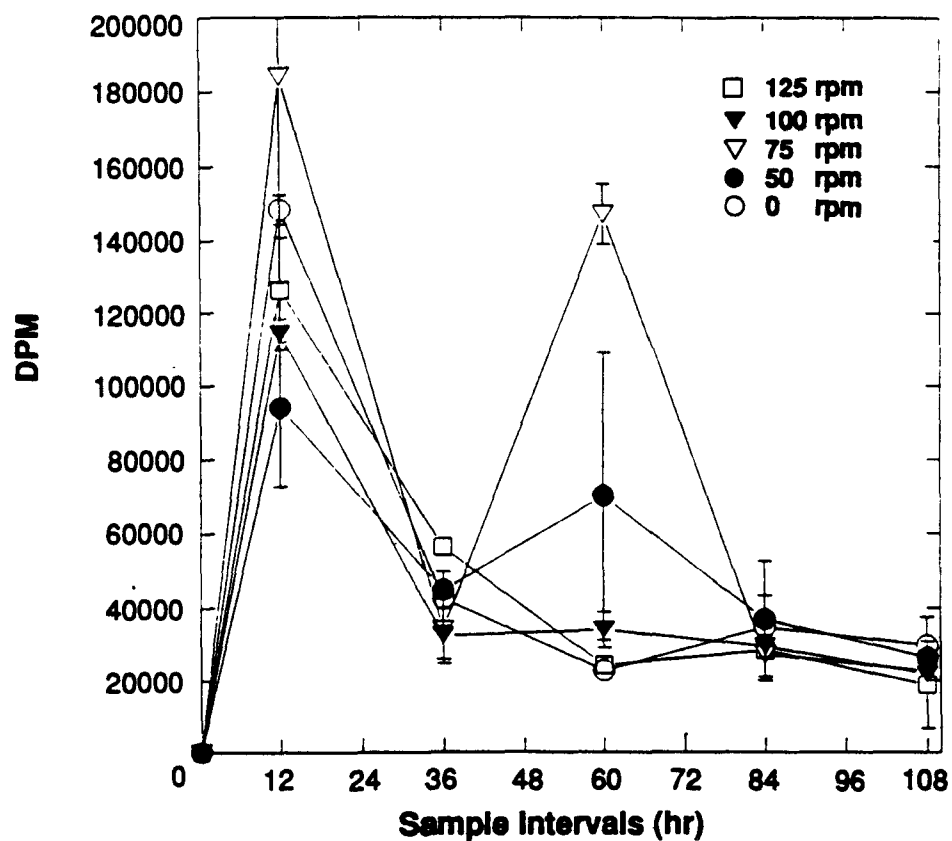
Figure 10.15. "Tidalnates" Counts per Sample Interval.
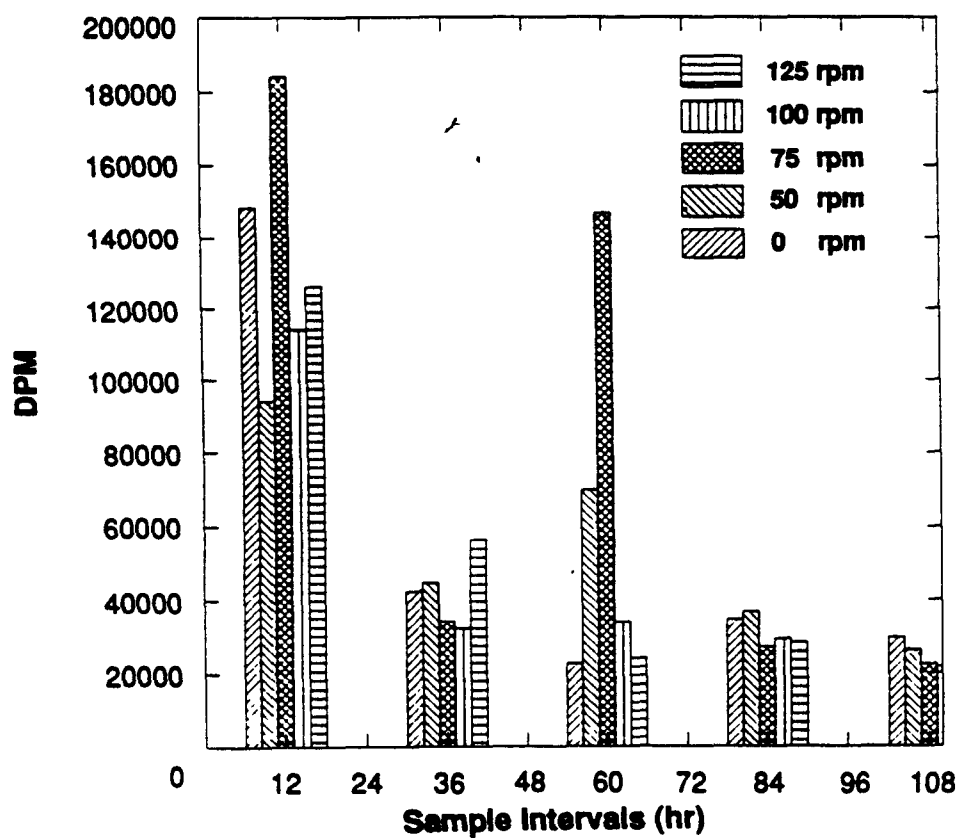
Figure 10.16. "Tidalnates" Counts per Sample Interval.

TABLE 10.9. TOTAL PEAK AREAS: GC ANALYSIS OF OIL RESIDUE
FROM SHAKER EXPERIMENT

Condition	Rocks Total Peak Area	% 0 rpm Control
0 rpm	3.05×10^6	100.0
50 rpm	2.65×10^6	87.0
75 rpm	2.34×10^6	76.8
100 rpm	2.40×10^6	78.6
125 rpm	2.35×10^6	77.1
TO Control (no "tidal" cycle)	3.32×10^6	109.0

Condition	"Tides" Total Peak Area	% 0 rpm Control
0 rpm	6.69×10^5	100.0
50 rpm	1.16×10^6	173.6
75 rpm	2.22×10^6	331.8
100 rpm	22.06×10^6	308.4
125 rpm	2.31×10^6	345.5

TO Control: 100 g oiled beach material that was not subjected to simulated "tidal" cycling or shaking.
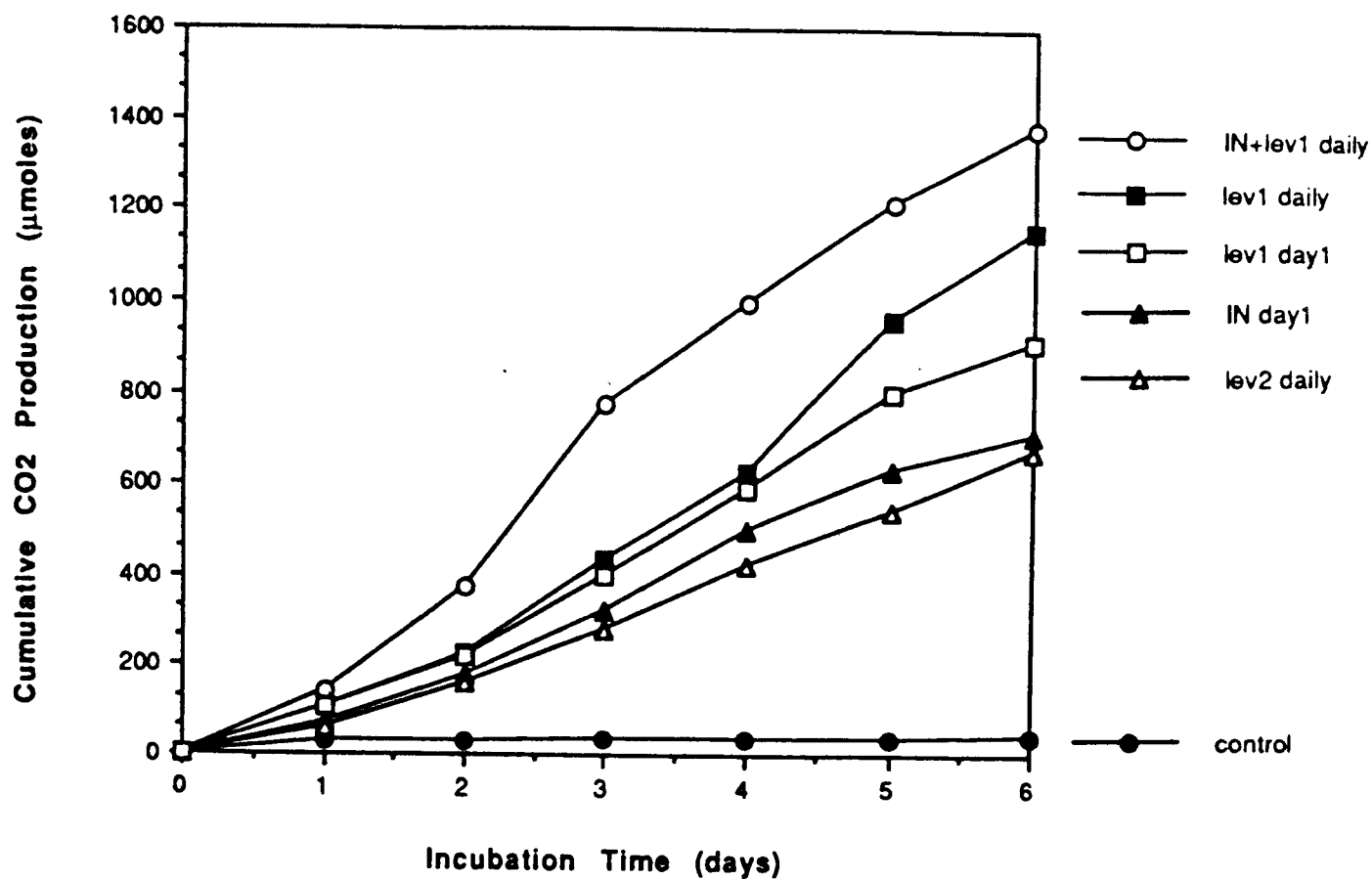
Figure 10.17. Effect of Various Treatments on the Activity of Indigenous, Oil-Degrading Microorganisms.
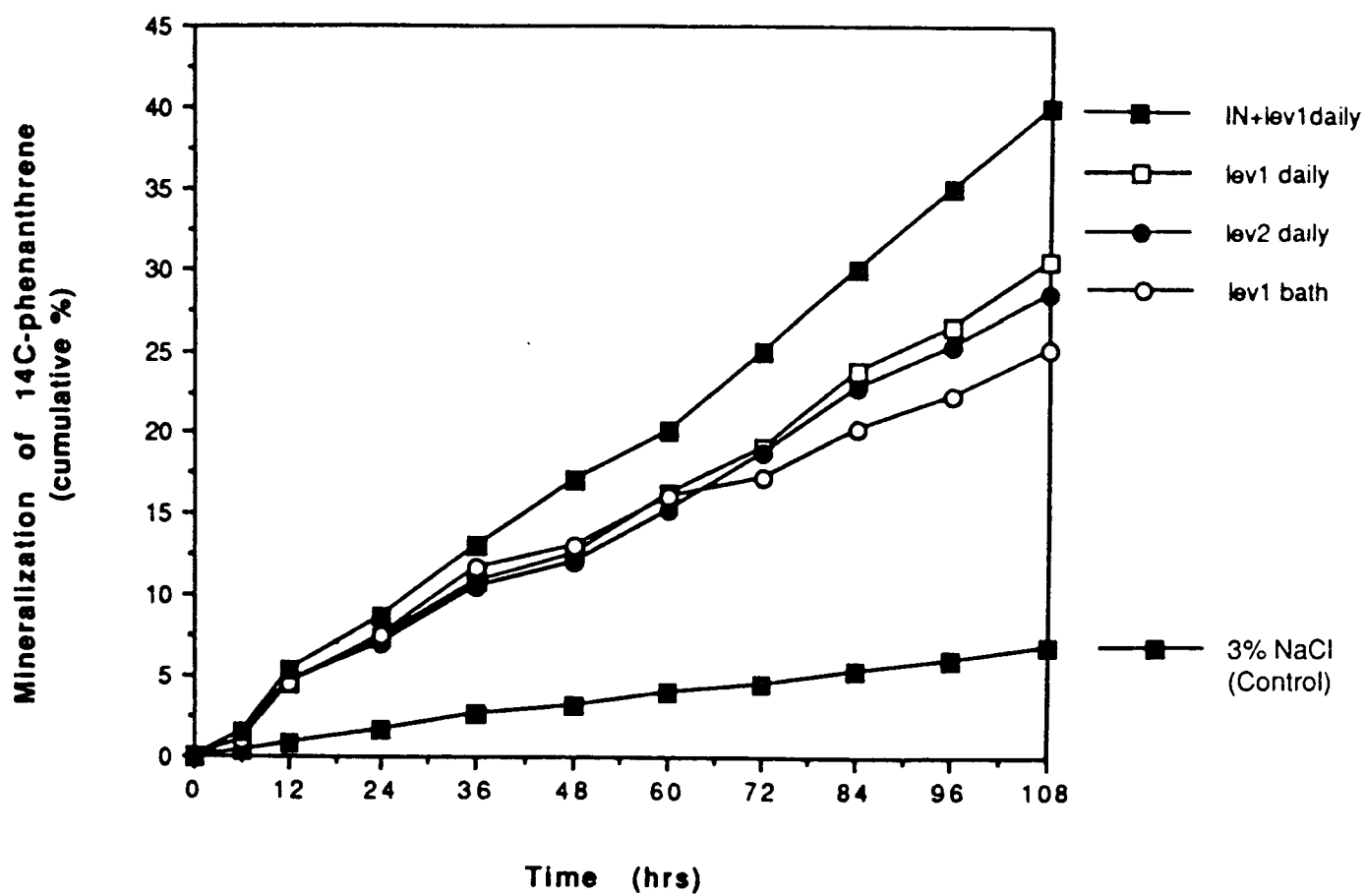
Figure 10.18. Effect of Various Treatments on the Activity of Indigenous, Oil-Degrading Microorganisms.
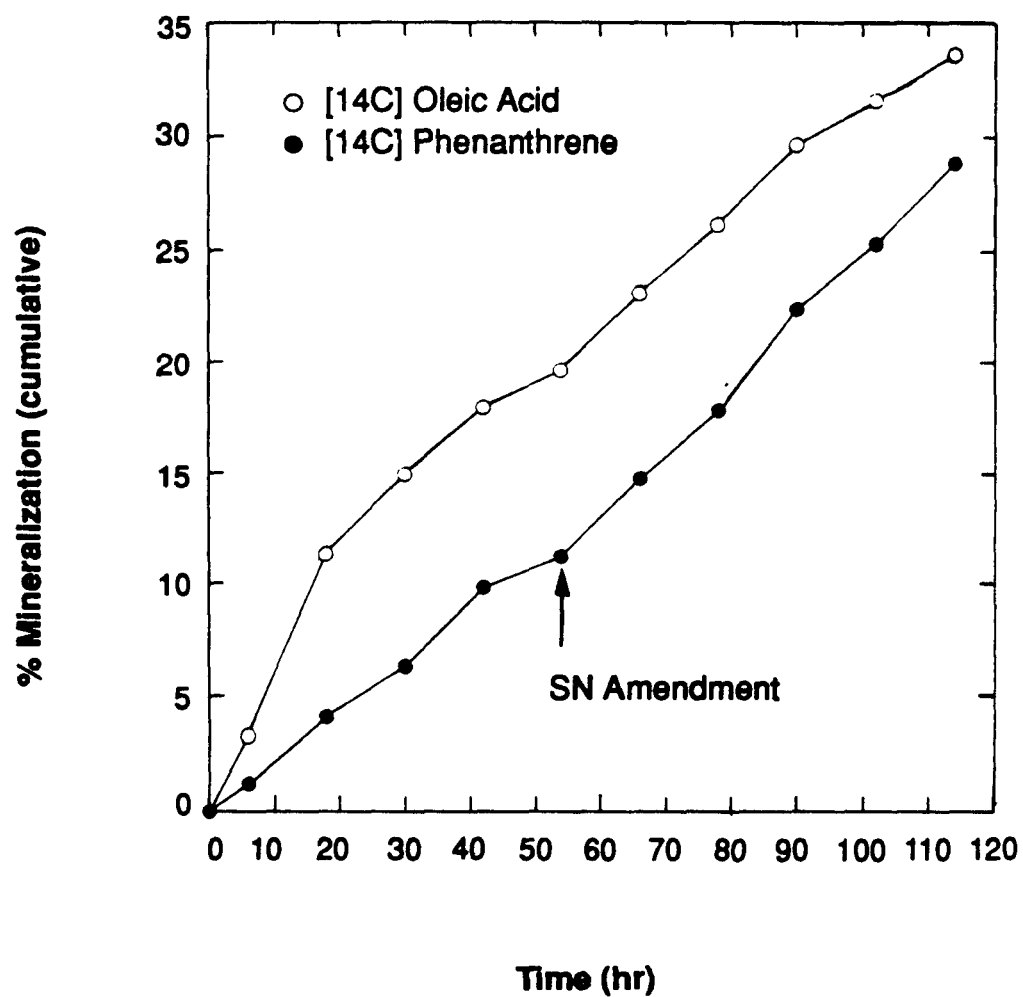
Figure 10.19. Effects of INIPOL and Soluble Nutrients on ^{14}C Oleic Acid and ^{14}C Phenanthrene Mineralization Over Time.

with the "out-going tide". The extent of phenanthrene mineralization also depended on the amount of inorganic nutrients present. With the level 1 and 2 nutrient solutions, 41 and 21% of the radiolabel was mineralized, respectively. Treatment with oleophilic fertilizer alone, 6 days prior to the addition of radiolabel, resulted in 17% phenanthrene mineralization. When treated with level 1 and oleophilic fertilizer together, the effect was equivalent to level 1 alone. These values were much higher than the 3% NaCl control (<3% mineralization over 24 hours).

Considering these data, it was postulated that some of the CO_2 production data presented in Figure 10.17 may have been due to the biodegradation of oleophilic fertilizer constituents (i.e., oleic acid, laureth phosphate). Utilizing radiolabeled oleic acid as a reporter chemical, oleophilic fertilizer biodegradation was indeed shown to be a potential contributing factor (Figure 10.19). Within the 24 hour incubation period that followed the 6 day period during which microbial respiration was recorded, approximately 23% of the added ^{14}C-oleic acid was mineralized when pretreated with oleophilic fertilizer. However, approximately the same amount of oleic acid biodegradation was observed when beach materials were treated solely with inorganic nutrients. Regardless of the treatment applied, the amount of either ^{14}C-oleic acid and ^{14}C-phenanthrene mineralization was greater than that observed with the untreated control.

Corresponding changes in the amount of oil in each system and its physical distribution further demonstrated the ability to accelerate the rate and extent of oil biodegradation through the addition of inorganic nutrients (Table 10.10). Since previous studies showed little difference in the amount of oil in removed tide water, these data are not presented. In terms of the amount of oil biodegraded within the 7 day incubation period, the same trend was again observed: level 1 inorganic nutrient solution was most effective followed by level 1 + INIPOL, level 2, and INIPOL alone; all were more effective than no treatment (3% NaCl control). Chemical profiles from gas chromatographic analysis of hexane extracts clearly reflect this response.

When treated daily with a solution containing only nitrogen at a concentration of 350 ppm N, the response of oil-degrading microorganisms (as determined by phenanthrene mineralization) was equal to those treated with level 1 or level 2 inorganic nutrient solutions (Figures 10.20 and 10.21). Conversely, the effect of phosphorus alone (70 ppm P) was much less. Therefore, the stimulatory effect of inorganic nutrient amendments was primarily a function of nitrogen addition.

TABLE 10.10. CHANGES IN OIL CONCENTRATION AND OIL DISTRIBUTION
AFTER 6 DAYS INCUBATION AND TREATMENT WITH INIPOL
AND/OR INORGANIC NUTRIENT SOLUTIONS

	Oiled Rocks		
Treatment	Hexane-soluble	Hexane-insoluble	Total peak area
	mg	mg	$\log_{10}$
INIPOL + high-level soluble nutrients	434	31	5.8
High-level soluble nutrients	423	39	5.6
Pulse high-level soluble nutrients	429	38	5.7
INIPOL	434	34	6.1
Low-level soluble nutrients	430	39	6.1
3% NaCl (control)	435	38	6.3

Gravimetric measurements obtained following 7 days of incubation to record respirometric responses to treatments;
INIPOL applied on day 1 only;
Soluble nutrients added daily with each high-tide.

When oil contaminated beach material was treated with a single application of level 1 nutrient solution (pulse application), response in terms of oil-degrading ability was equivalent to daily treatment with the same solution. The initial stimulatory response persisted for 3 to 4 days. Field data collected over a 30-day time period demonstrated that the stimulatory effects of the pulse application strategy persisted for 10 to 14 days (see Elrington Island results in Section 9). Therefore, the requirement for inorganic nitrogen by indigenous, oil-degrading microflora may be fulfilled relatively quickly. Once this requirement is satisfied, these microorganisms appear to be active for extended periods of time before nitrogen again becomes an essential limiting factor for oil biodegradation.

Figure 10.20. Distribution of ^{14}C from Radiolabeled Phenanthrene.
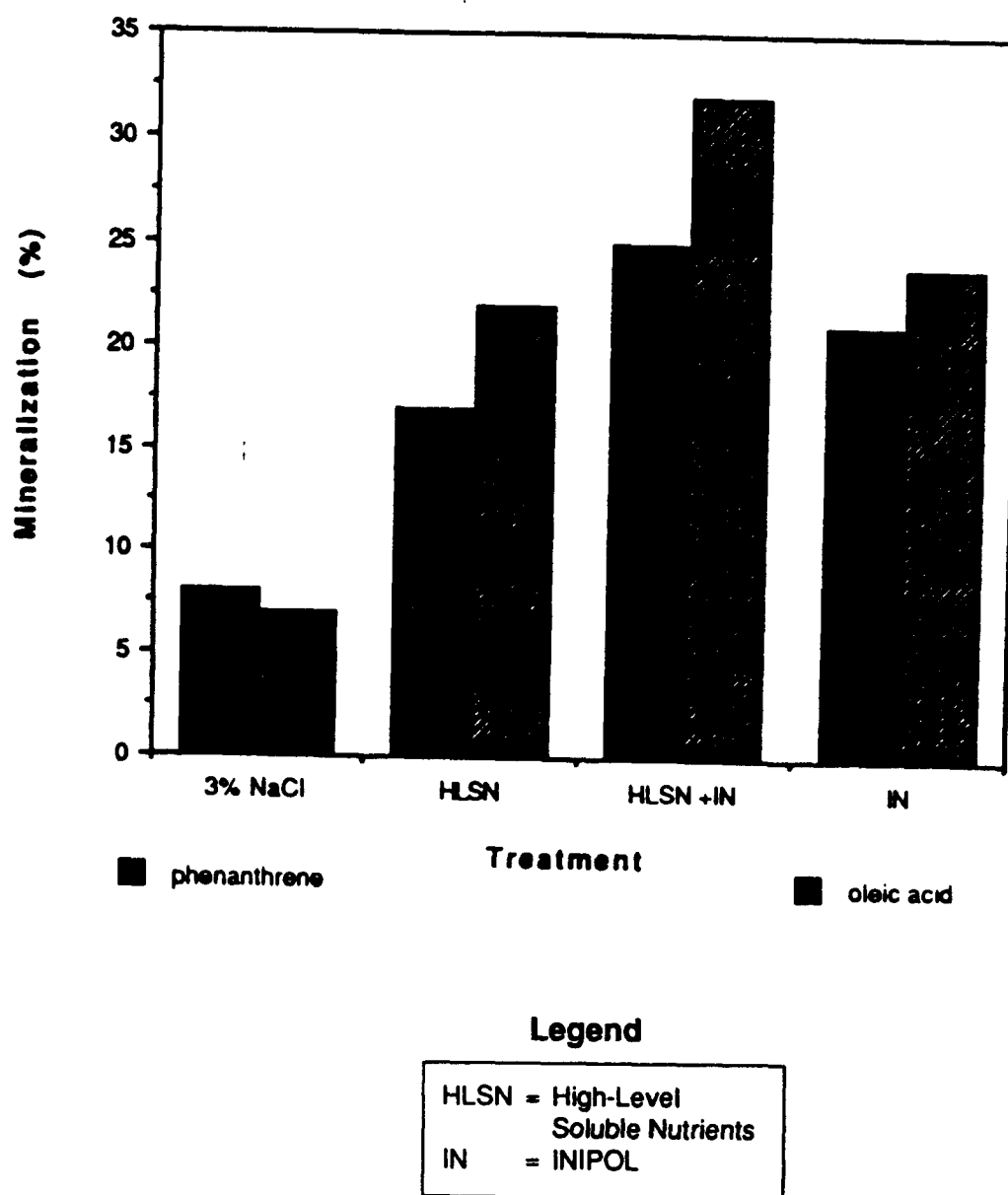
Figure 10.21. Accelerated Biodegradation of ^{14}C-Phenanthrene and ^{14}C-Ole Acid.

Bioaugmentation Studies

The possibility of enhancing the rate and extent of oil biodegradation through the addition of oil-degrading microorganisms was evaluated. Microbial inoculum of specially-selected, oil-degrading marine isolates indigenous to Prince William Sound are described in Table 10.11. Of the various organisms available, a mixed-culture community (DI/EI 1-5a) and a pure bacterial culture (strain EI 2V) were selected for further study based on their catabolic abilities and observed growth rates in the presence of Prudhoe Bay crude oil.

When oiled beach materials were exposed to the action of these organisms, the amount of oil biodegradation was enhanced (Figures 10.22 and 10.23, and Table 10.12). Therefore, under controlled conditions, the potential of enhancing the bioremediation process through bioaugmentation was demonstrated. Nutrient supplementation was again effective in stimulating the activity of indigenous, oil-degrading microorganisms. By no means, however, do these data state that a similar response would be observed if these organisms were applied in situ.

Oleophilic Fertilizer Mode of Action

Laboratory studies designed to discern the mode of action of oleophilic fertilizer were basically inconclusive. While the fertilizer increased microbial activities above background levels, the most significant response was related to the addition of soluble nutrients (Figure 10.24). Moreover, the effect of oleophilic fertilizer did not appear to be directly related to the presence of laureth phosphate or oleic acid (Figure 10.24). Conversely, the addition of nitrogen, as urea in the case of INIPOL or NH_4NO_3 for soluble nutrient additions, showed the most significant response.

Mineralization of radiolabeled phenanthrene (Table 10.13) and oil chemistry data (Figure 10.26) supported microbial respiration data. The addition of soybean oil, an alternative source of triglycerides, was ineffective (Figure 10.25; Table 10.14). Therefore, the unique effectiveness of INIPOL appeared to be due to a synergistic effect of constituents resulting from its novel formulation (i.e., synergistic interaction between INIPOL constituents).

TABLE 10.11. ORGANISMS ISOLATED FROM OILED BEACH MATERIAL IN PRINCE WILLIAM SOUND, ALASKA

Organism Designation	Enriching Substrate	Genus[a]	Carbon source tested as growth substrates					
			C8	C16	C26	Pristane	Naph	Phen
DI/EI #1	crude oil	*Rhodococcus* sp.	+	+	+	+	-	-
DI/EI #2	crude oil	*Rhodococcus* sp.	+	+	+	+	-	-
DI/EI #3	crude oil	*Pseudomonas* sp.	+	-	-	-	-	-
DI/EI #5a	crude oil	*Rhodococus* sp.	+	+	+	+	-	-
Hexadecane #2	nC16	*Rhodococus* sp.	-	+	+	+	-	-
Hexacosane #1	nC26	*Acinetibacter* sp.	-	+	+	-	-	-
Hexacosane #2	nC26	*Rhodococcus* sp.	-	+	+	-	-	-
Pristane #2	pristane	*Rhodococcus* sp.	-	+	+	+	-	-
AK-Naph-B	PAHs+ naph	*Pseudomonas* sp.	-	-	-	-	+	-
AK-Phen-6	crude oil + phen	*Pseudomonas* sp.	-	-	-	-	-	+
EI 2V	crude oil	*Rhodococcus* sp.	+	+	+	+	-	-

[a] Bacterial identification was determined by analysis of fatty acid profiles (Microbial ID, Inc., Newark, DE)
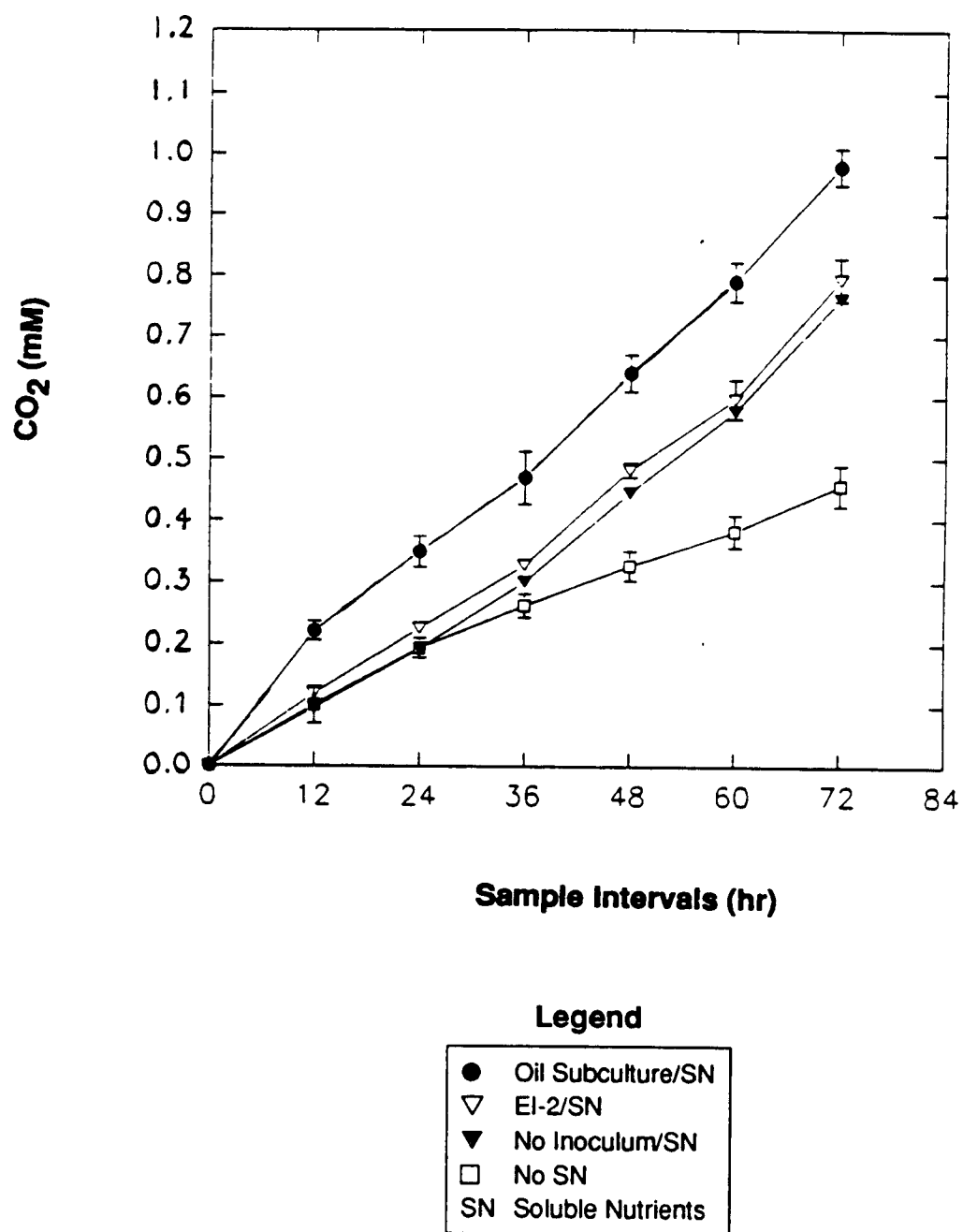
Figure 10.22. Cumulative Total CO_2 Production Over Time.
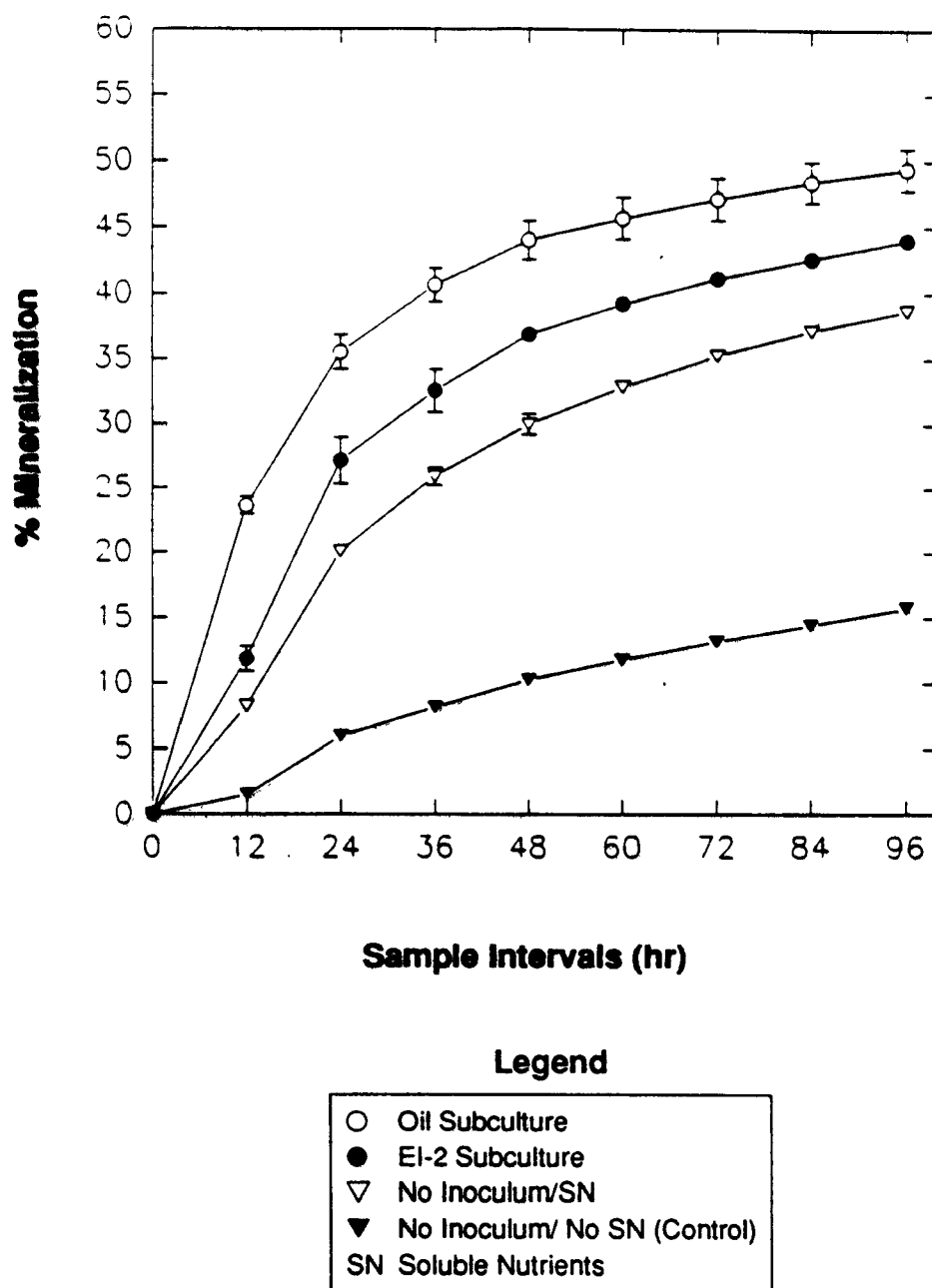
Figure 10.23. Cumulative Percent Mineralization of ^{14}C-Phenanthrene Over Time.

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Figure 10.24. Effect of INIPOL and Inorganic Nutrients on the Activity of Oil-Degrading Microflora Over Time.
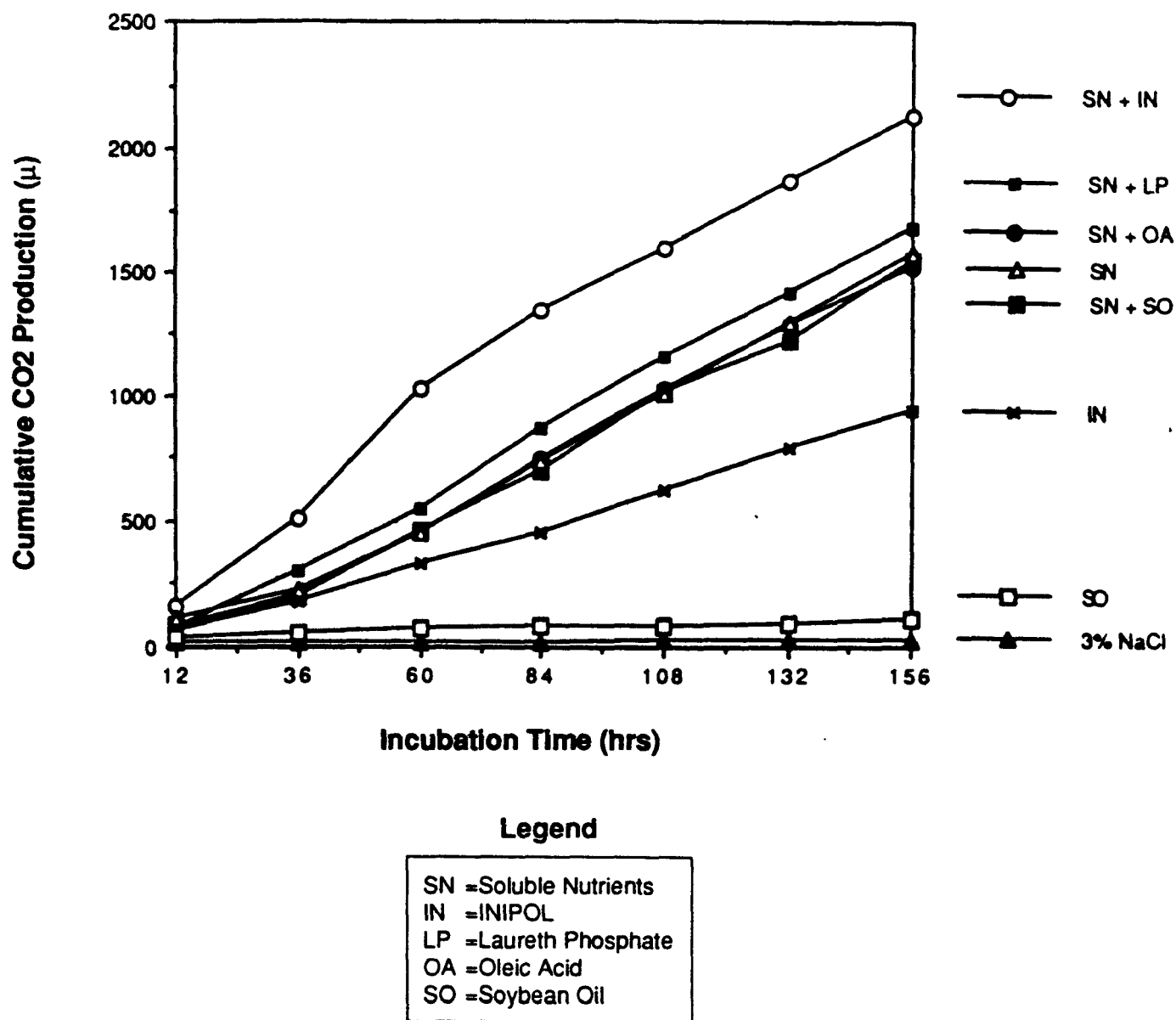
Figure 10.25. Effect of INIPOL and its Constituents on Microbial Activities Over Time.
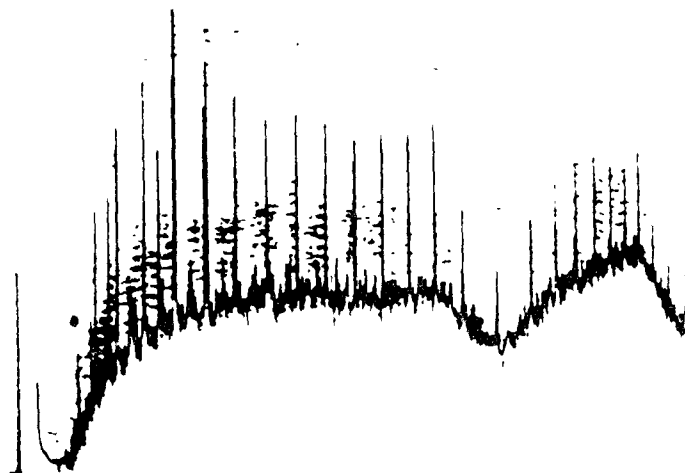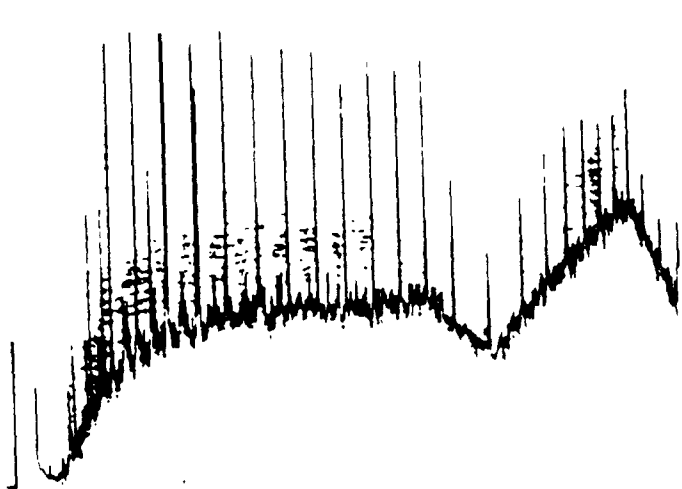
INIPOL Alone Time 0
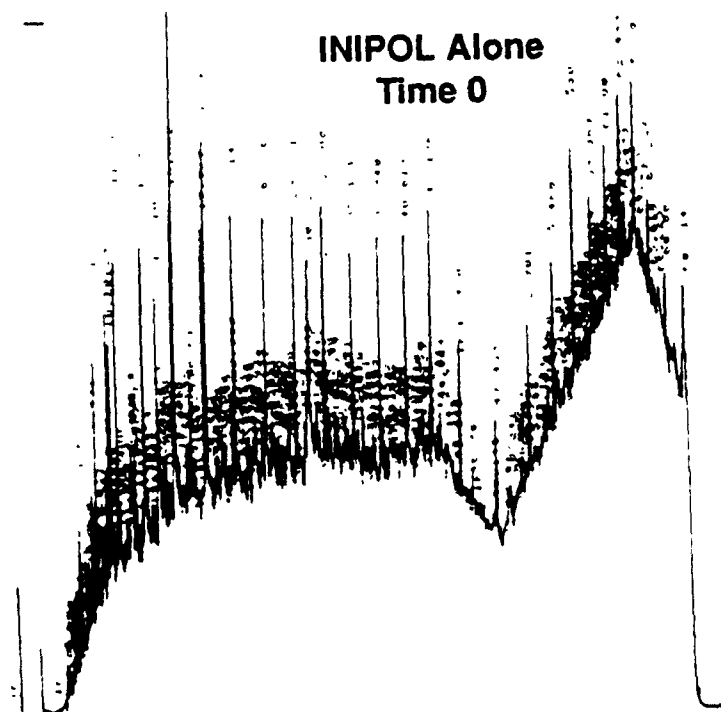
Soluble Nutrients (Daily)
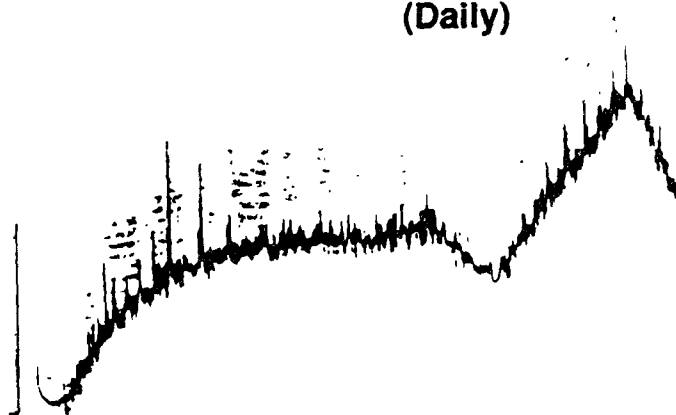
Soluble Nutrients + INIPOL
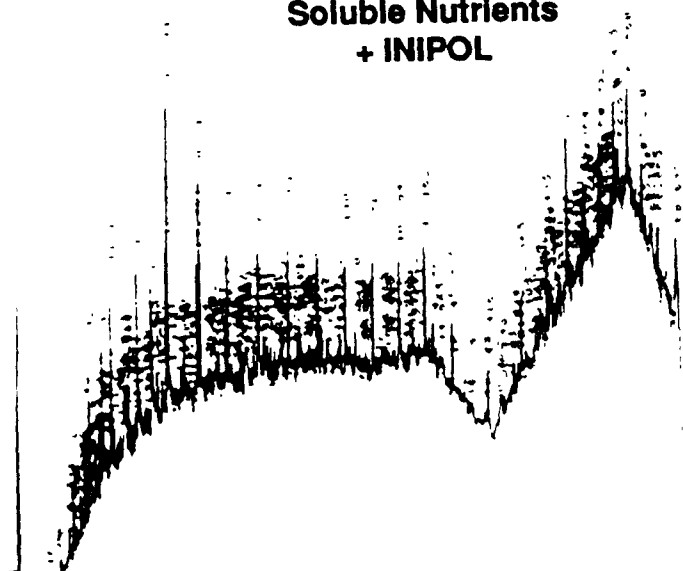
Soybean Oil Alone (T=0)
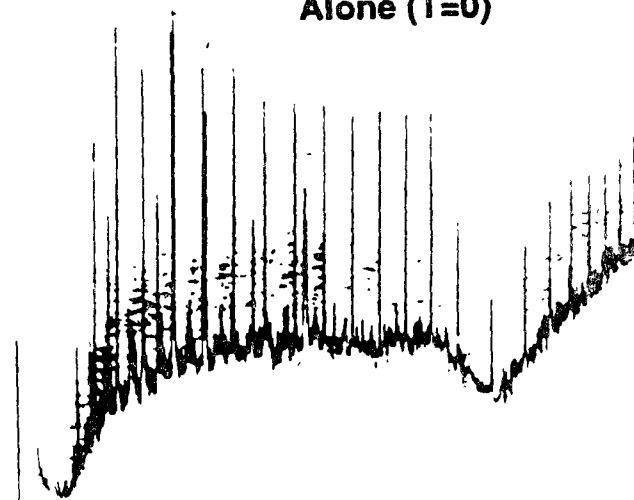
Figure 10.26. Oil Chemistry Data.

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TABLE 10.12. COMPARISON OF GC PROFILE TOTAL PEAK AREAS FROM MICROBIAL INOCULATION BIOMETER STUDY

Condition	Mean Peak Area	% Control	% TO
Oil Subculture with SN	1.59×10^6	90.9	68.7
EI-2, Mixed Isolate Culture with SN	1.55×10^6	88.4	66.8
No Inoculum with SN	1.61×10^6	92.0	69.6
Control, No Inoculum, No SN	1.75×10^6	100.0	75.6
TO Extraction	2.31×10^6	132.2	100.0

SN: Soluble Nutrients, 35.7 mMol N/8.07 mMol P
Filtered Prince William Sound, AK "tide"
TO Extraction: 100 g oiled beach material that was not subjected to "tidal" cycling

TABLE 10.13. MINERALIZATION OF 9-^{14}C-PHENANTHRENE (24 HOUR INCUBATION) FOLLOWING 7 DAYS OF TREATMENT EXPOSURE

Treatment	Mass Balance of Radioactivity		
	Counts in "tidalnate"	Counts as CO_2	Counts absorbed or assimilated (calculated)
	%	%	%
Sterile SN	9.6	< 0.01	90.4
3% NaCl	15.0	4.7	80.3
Soybean Oil	19.1	5.5	75.4
INIPOL	8.8	15.1	76.1
SN + Soybean Oil	15.9	18.6	65.5
SN + INIPOL	14.1	36.5	49.4
SN + OA	9.3	38.1	52.6
SN	13.2	40.7	46.1
SN + LP	14.6	42.1	43.3

SN = Soluble Nutrients
OA = Oleic Acid
LP = Lauryl Phosphate

TABLE 10.14. CHANGES IN OIL CONCENTRATION AND OIL DISTRIBUTION AFTER 7 DAYS INCUBATION

Treatment	Oil residue analysis		
	CH_2Cl_2-soluble	Hexane-soluble	Total peak area
	mg	mg	$\log_{10}$
Sterile SN	421.3	397.8	6.34
3% NaCl	451.3	425.6	6.25
Soybean Oil	413.7	418.7	6.34
INIPOL	511.0	447.2	6.48
SN + Soybean Oil	377.7	375.8	6.34
SN + INIPOL	428.7	434.3	6.23
SN + OA	488.4	427.5	6.23
SN	429.4	422.2	5.81
SN + LP	435.7	436.5	6.32

SN = Soluble Nutrients
OA = Oleic Acid
LP = Lauryl Phosphate

SUMMARY AND CONCLUSIONS

Treatment with inorganic nutrient solutions increased the rate and extent of oil biodegradation 2 to 3 fold. This stimulatory effect was primarily due to nitrogen addition with phosphorus amendment exhibiting little effect on the microbial activities measured. Moreover, a pulse application strategy appears to offer an efficient means of stimulating the rate and extent of oil biodegradation by microorganisms indigenous to Prince William Sound. Application of these findings may enhance the efficiency of future oil spill bioremediation efforts.

SECTION 11

STABLE ISOTOPES

Stable isotopes were used in the oil spill bioremediation project to evaluate the assimilation of carbon from oil carbon into microbial food chains, and trace nitrogen fertilizer used for bioremediation into bacteria and other organisms on the beach. Studies were conducted on beaches at Snug Harbor, Passage Cove, Elrington Island, and Disk Island. At these sites algae, seagrasses, and heterotrophic consumers were sampled to examine the assimilation of nitrogen fertilizer into organisms other than bacteria. Microcosms were used to study bacteria assimilation of nitrogen fertilizer and the effect of the nitrogen on bacteria assimilation of oil.

FOOD CHAIN STUDIES

Food Chain Studies at Snug Harbor and Passage Cove, Summer 1989

Potential damage (other than from oil) to the ecological balance of intertidal and shallow subtidal communities in Prince William Sound through addition of large quantities of fertilizer to contaminated beaches was possible. Stable carbon and nitrogen isotopes in algae and higher consumers were used in the summer of 1989 to trace assimilation of nitrogen in bioremediation treatments on beaches in Prince William Sound.

The stable carbon and nitrogen isotope ratios of primary producers and consumers are located in Table 11.1. The δ^{15}N for *Laminaria* spp only ranged from +6.3 to +9.9 ‰. The δ^{13}C values for *Uruspora* spp., *F. distichus* and *Laminaria* spp. were consistent with previous measurements of marine C3 plants (Fry and Sherr, 1984). The δ^{13}C values for *Z. marina* were as expected for C4 plants (Fry and Sherr, 1984). Seston, which in seawater environments contains primarily algal material and detritus, exhibited the most negative carbon isotope measured in these samples (δ^{13}C = -23.7), with the exception of an unidentified terrestrial plant spp, (δ^{13}C = -28.4 ‰).

The range of δ^{13}C and δ^{15}N for different primary producers and consumers observed in Snug Harbor and Passage Cove are shown in Figure 11.1. Although not all species are represented at both sites, there wasn't a significant difference among the ranges within the two environments. There was a progressive enrichment in δ^{15}N among limpets, periwinkles, *M. edilus*, *Balanus* spp, and whelks. The values for consumers were equal to or more positive than those for the primary producers. Only seston contained δ^{15}N as enriched in δ^{15}N as the consumers. In contrast, consumers were depleted in

TABLE 11.1. ISOTOPIC DATA FOR SAMPLES TAKEN FROM SELECTED BEACHES OF PRINCE WILLIAM SOUND, AK BETWEEN JUNE AND AUGUST, 1989

Sample	Date	Location	$\delta^{15}N$	$\delta^{13}C$
Urospora spp	6/13/89	Tatitalek Is.	+8.5	-19.1
Urospora spp	6/17/89	Snug Harbor	–	–
Urospora spp	8/10/89	Snug Harbor	+4.9	-21.7
Urospora spp	8/10/89	Snug Harbor	+8.3	-19.4
Urospora spp	8/10/89	Snug Harbor[2]	+5.6	-16.0
Urospora spp	8/13/89	Passage Cove[1]	+4.6	-14.6
Urospora spp	8/20/89	Passage Cove[1]	+6.5	-18.9
Urospora spp	8/13/89	Passage Cove[2]	+2.3	-18.1
Urospora spp	8/20/89	Passage Cove[2]	+3.3	-18.4
Fucus distichus	6/13/89	Tatitalek Is.	+8.5	-15.4
Fucus distichus	6/17/89	Snug Harbor	+5.9	-20.0
Fucus distichus	8/10/89	Snug Harbor	+7.6	-16.2
Fucus distichus	8/10/89	Snug Harbor	+7.9	-18.9
Fucus distichus	8/10/89	Snug Harbor[2]	+5.8	-18.8
Fucus distichus	8/10/89	Snug Harbor[2]	+7.4	-16.6
Fucus distichus	8/13/89	Passage Cove[1]	+6.3	-19.5
Fucus distichus	8/20/89	Passage Cove[1]	+7.0	-15.2
Fucus distichus	8/13/89	Passage Cove[2]	+6.7	-17.4
Fucus distichus	8/20/89	Passage Cove[2]	+4.6	-16.0
Laminaria spp	6/13/89	Tatitalek Is.	+9.9	–
Laminaria spp	6/17/89	Snug Harbor	+7.5	-18.4
Laminaria spp	8/10/89	Snug Harbor[1]	+8.4	-21.2
Laminaria spp	8/10/89	Snug Harbor[2]	+7.6	-19.3
Laminaria spp	8/20/89	Passage Cove[1]	+7.5	–
Laminaria spp	8/20/89	Passage Cove[2]	+6.3	-22.4
Zostera marina	6/13/89	Tatitalek Is.	+1.8	-10.6
Zostera marina	6/17/89	Snug Harbor	+4.5	-9.5
Zostera marina	6/17/89	Snug Harbor	+4.5	-9.7
Zostera marina	8/10/89	Snug Harbor[1]	+7.4	-11.0
Zostera marina	8/10/89	Snug Harbor[1]	+7.3	-10.4
Zostera marina	8/10/89	Snug Harbor[2]	+6.9	-11.6
Zostera marina	8/10/89	Snug Harbor[2]	+6.7	-11.2
Zostera marina	8/13/89	Passage Cove[1]	+8.1	-11.3
Zostera marina	8/20/89	Passage Cove[1]	+6.7	-9.3
Zostera marina	8/13/89	Passage Cove[2]	+8.1	-13.9
Zostera marina	8/20/89	Passage Cove[2]	+7.3	-8.9

[1]Samples collected directly from untreated control plots
[2]Samples collected directly from fertilized plots

TABLE 11.1. CONTINUED

Sample	Date	Location	$\delta^{15}N$	$\delta^{13}C$
Seston	7/16/89	Snug Harbor	–	-22.9
Seston	8/13/89	Passage Cove[1]	+8.2	-22.2
Seston	8/22/89	Passage Cove[1]	+9.1	-23.2
Seston	8/13/89	Passage Cove[2]	+5.7	-21.6
Seston	8/22/89	Passage Cove[2]	+6.6	-23.7
Collisella pelta	8/13/89	Passage Cove[1]	+7.7	-20.1
Collisella pelta	8/20/89	Passage Cove[1]	+7.8	-15.5
Notoacmea scutum	8/13/89	Passage Cove[2]	+7.4	-22.6
Notoacmea scutum	8/20/89	Passage Cove[2]	+7.3	-19.4
Littorina spp	8/10/89	Snug Harbor[2]	+7.6	-21.0
Littorina sitkana	8/13/89	Passage Cove[1]	+7.9	-18.3
Littorina spp	8/20/89	Passage Cove[1]	+8.4	-18.0
Littorina sitkana	8/13/89	Passage Cove[2]	+8.3	-17.2
Littorina sitkana	8/20/89	Passage Cove[2]	+7.7	-17.8
Mytilus edilus	6/8/89	Tatitalek Is.	+9.1	–
Mytilus edilus	6/17/89	Snug Harbor	+7.8	-21.5
Mytilus edilus	7/11/89	Snug Harbor[3]	–	-18.9
Mytilus edilus	7/11/89	Snug Hárbor[3]	+8.5	-21.4
Mytilus edilus	7/11/89 ·	Snug Harbor[3]	–	-20.3
Mytilus edilus	8/10/89	Snug Harbor[1]	+7.8	-20.3
Mytilus edilus	8/10/89	Snug Harbor[1]	+8.3	-20.4
Mytilus edilus	8/10/89	Snug Harbor[2]	+8.4	-20.2
Mytilus edilus	8/10/89	Snug Harbor[2]	+7.9	-21.4
Mytilus edilus	8/13/89	Passage Cove[1]	+8.2	-20.1
Mytilus edilus	8/20/89	Passage Cove[1]	+8.3	-20.9
Mytilus edilus	8/13/89	Passage Cove[2]	+8.5	-20.1
Mytilus edilus	8/20/89	Passage Cove[2]	+8.7	-20.7
Balanus glandula	6/13/89	Tatitalek Is.	+9.9	-19.0
Balanus glandula	6/17/89	Snug Harbor	–	-20.2
Balanus glandula	8/10/89	Snug Harbor[1]	+8.9	-19.0
Balanus rastratus	8/10/89	Snug Harbor[1]	+9.9	-19.8
Balanus glandula	8/10/89	Snug Harbor[2]	+9.9	-20.9
Balanus glandula	8/10/89	Snug Harbor[2]	+9.2	-20.8
Balanus glandula	8/13/89	Passage Cove[1]	+9.9	-19.5
Balanus glandula	8/20/89	Passage Cove[1]	+9.5	-20.9
Balanus glandula	8/13/89	Passage Cove[2]	+9.9	-17.7
Balanus glandula	8/20/89	Passage Cove[2]	+10.1	-19.4

[1]Samples collected directly from untreated control plots
[2]Samples collected directly from fertilized plots
[3]Samples collected from experimental cages

TABLE 11.1. CONTINUED

Sample	Date	Location	$\delta^{15}N$	$\delta^{13}C$
Nucella lima	8/13/89	Passage Cove[1]	+10.4	-18.1
Searlesia dira	8/20/89	Passage Cove[1]	+11.4	-16.2
Nucella lima	8/13/89	Passage Cove[2]	+10.3	-20.3
Nucella lima	8/20/89	Passage Cove[2]	+10.0	-17.4
Root feeder parti	8/13/89	Passage Cove[2]	+3.4	-28.4
Root feeder parti	8/20/89	Passage Cove[2]	+6.0	-23.9
Z. marina epiphytes	6/13/89	Tatitalek Is.	–	-10.8
Z. marina epiphytes	6/17/89	Snug Harbor	–	-12.4
Brown algal spp	6/13/89	Tatitalek Is.	+6.8	-21.4
Mollusk spp	6/13/89	Tatitalek Is.	+10.2	-15.3
Terr plant spp	6/13/89	Tatitalek Is.	–	-28.4
Brown algal spp	8/10/89	Snug Harbor[1]	+6.5	-20.0
Red algal spp	8/10/89	Snug Harbor[1]	+8.4	-18.6
Ammonia, Root Feeder	7/16/89	Snug Harbor	–	N.A.

[1]Samples collected directly from untreated control plots
[2]Samples collected directly from fertilized plots

the heavier isotope of carbon, $\delta^{13}C$, compared with primary producers, but were more positive than the seston. Limpets had the largest carbon isotope variability among consumers. Finally, a progressive enrichment pattern was observed for $\delta^{13}C$ among *M. edilus*, *Balanus* sp, and whelks.

Fertilizer Nitrogen Assimilation by Food Chains on Beaches, Summer 1989

At Snug Harbor (August 10) and Passage Cove (August 13; August 20; August 22), biological samples and organisms were taken directly from either untreated control or fertilized plots within the beach. In both cases, the area was contaminated by oil. INIPOL, the oleophilic fertilizer used in the test sites, had an $\delta^{15}N$ of +2.0 ‰. The $\delta^{13}C$ of INIPOL was -29.9 ‰, while crude oil from the Alyeska Terminal used to fill the *Exxon Valdez* had a $\delta^{13}C$ of -30.1 ‰. Both the nitrogen and carbon isotope ratios of the fertilizer and oil-contaminant are isotopically lighter than values measured for biological samples and seston (Table 11.1). Mean $\delta^{13}C$ and $\delta^{15}N$ for different species and seston from untreated control and fertilized plots within the beach are shown in Figure 11.2 (In most cases, n>3, refer to
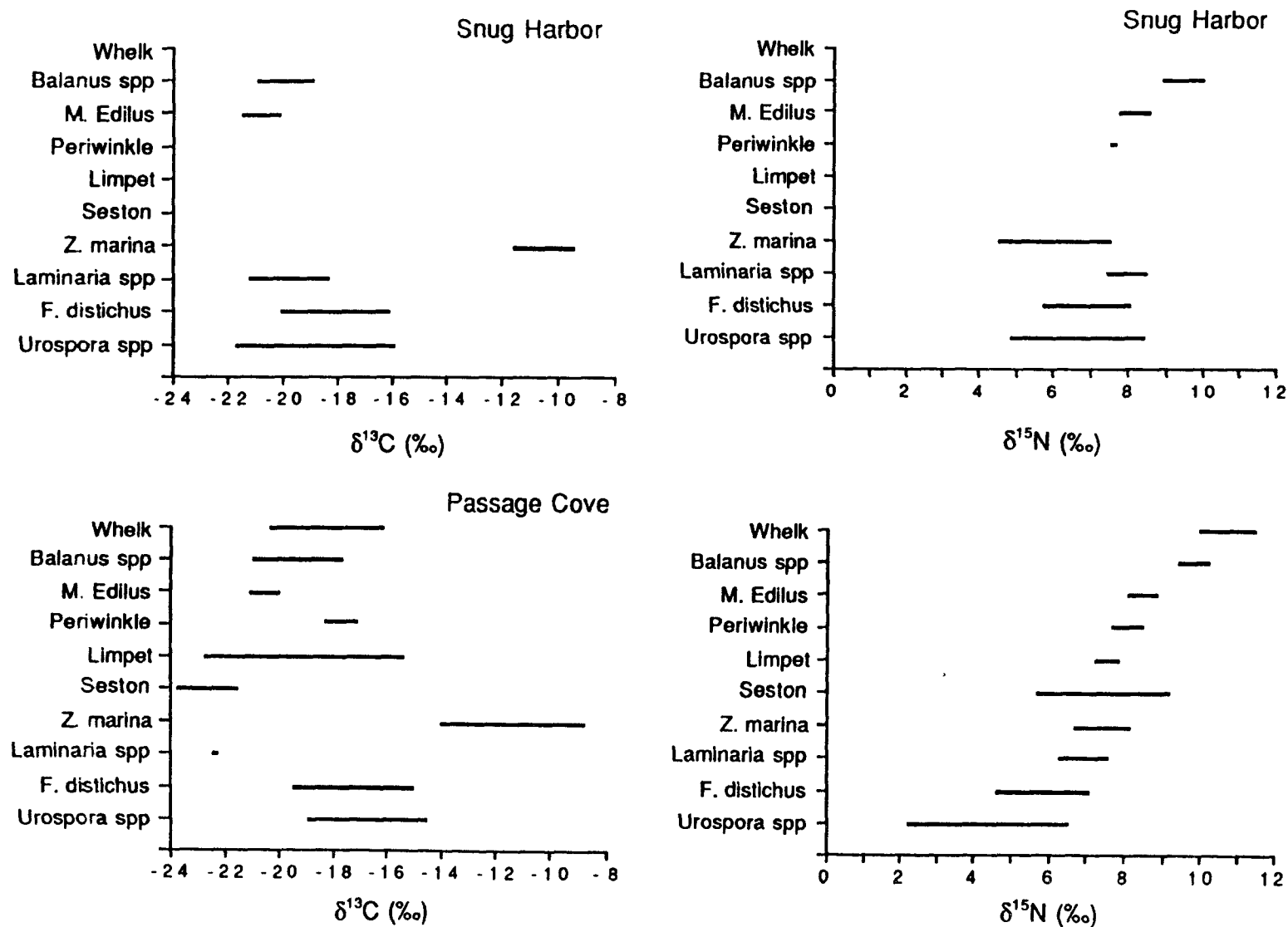
Figure 11.1. The $\delta^{13}C$ and $\delta^{15}N$ of Plants, Animals, and Seston Collected from Snug Harbor (6/13/89, 7/16/89, 8/10/89) and from Passage Cove (8/13/89, 8/20/89, 8/22/89). The Bar Indicates the Range of Measured Values (Refer to Table 11.1).
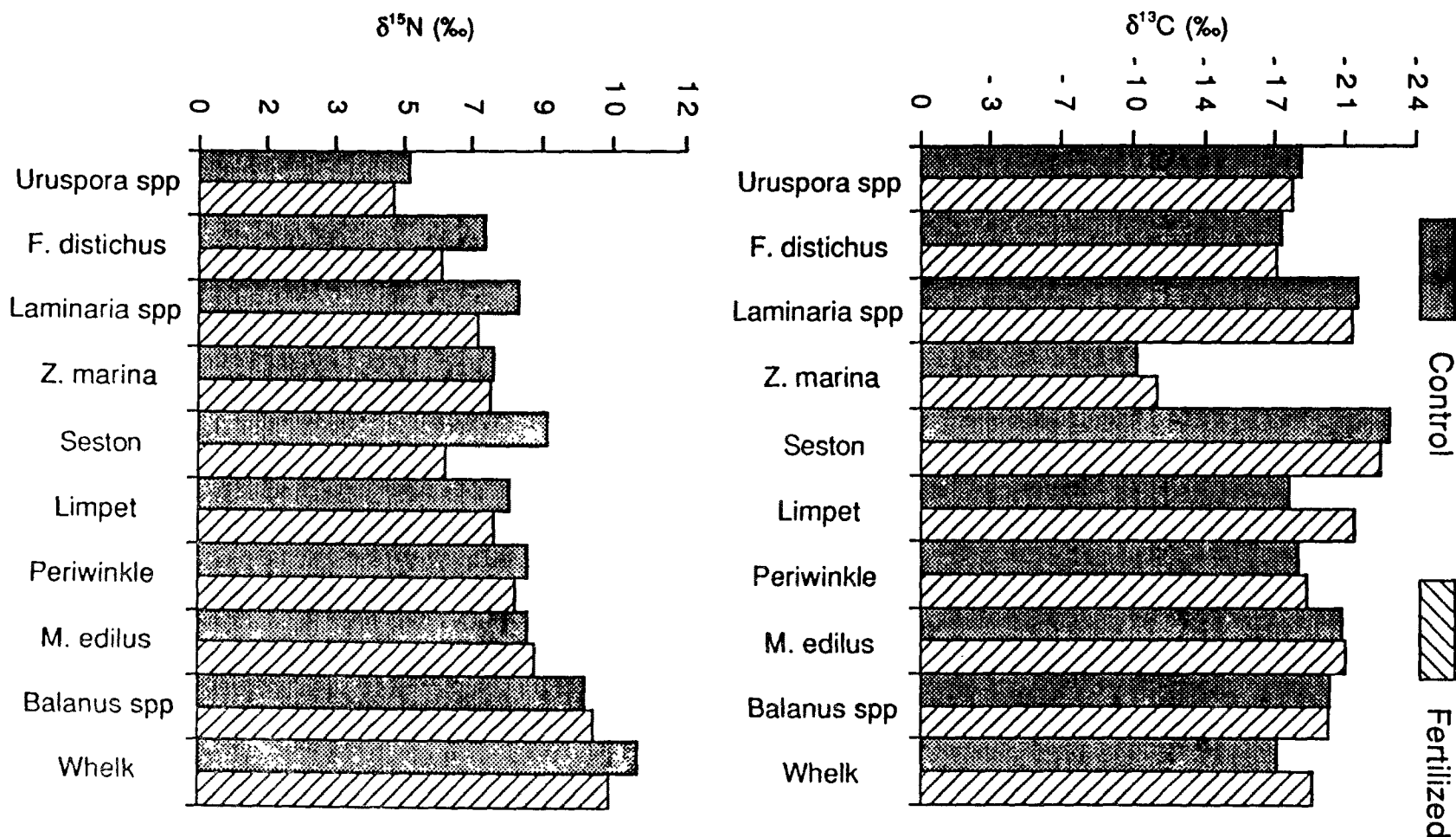
Figure 11.2. Mean $\delta^{13}C$ and $\delta^{15}N$ of Plants, Animals, and Seston Collected Directly from Untreated Control and Fertilized Plots.

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Table 11.1). Lack of a major difference among samples from untreated control and fertilized plots for within the beach either carbon or nitrogen isotope ratios suggested that in the short period after application of the fertilizer, utilization of fertilizer carbon or nitrogen and transfer up the food chain was not significant. There was, however, a small decrease in the $\delta^{15}N$ in seston, *F. distichus*, and *Laminaria* spp, which might indicate that fertilizer nitrogen was beginning to be incorporated into algae.

Limpets feed mostly on microscopic algae. Periwinkles feed on films of diatoms, blue green algae, filamentous algae, and occasionally on small pieces of macroscopic plants. *M. edilus* and *Balanus* spp are filter feeders. Whelks are carnivores, and feed primarily on snails and limpets. With a trophic level transfer, there generally is a small positive shift in the carbon isotope ratio (up to 1 ‰), and a slightly larger shift in the nitrogen isotope ratio (up to 3 ‰) (Fry and Sherr, 1984; Peterson and Fry, 1987). Thus, the carbon isotope ratio of these consumers should be similar to that of its food source, and the nitrogen isotope ratio should be slightly enriched in $\delta^{15}N$ (more positive).

The isotopic data shows that herbivores were primarily feeding on algae and not on seagrasses (Figure 11.3). Similarly, the filter-feeders *M. edilus* and *Balanus* spp were utilizing suspended organic matter derived from marine algal material. The isotopic difference between the filter-feeders and seston demonstrated that these organisms were selectively removing material from suspension. In addition, there was a difference between the food source ingested by *M. edilus* and *Balanus* spp. Finally, the similarity among carbon isotope ratios and the clear trophic level shift in the nitrogen isotope ratio between whelks and the limpets and periwinkles indicated the latter were a likely food source for the carnivore.

The following preliminary conclusions can therefore be drawn: 1) there is no clear evidence for the immediate incorporation of fertilizer carbon or nitrogen into the food-web of affected intertidal beaches, and 2) the isotopic evidence does not indicate a shift from the expected trophic level interactions among the organisms that were sampled in this study. In addition, it was demonstrated that there was no immediate (weeks to months) effect of the addition of fertilizers on the food-web structure in Prince William Sound.

Preliminary analysis from the summer of 1989 data indicated some assimilation of nitrogen from the fertilizer. The sampling frequency through the summer, however, was not great enough to establish a relationship between stable nitrogen isotope ratios in organisms and the fertilizer. The summer 1990 ecological monitoring study was designed to confirm the trends observed during the summer of 1989.
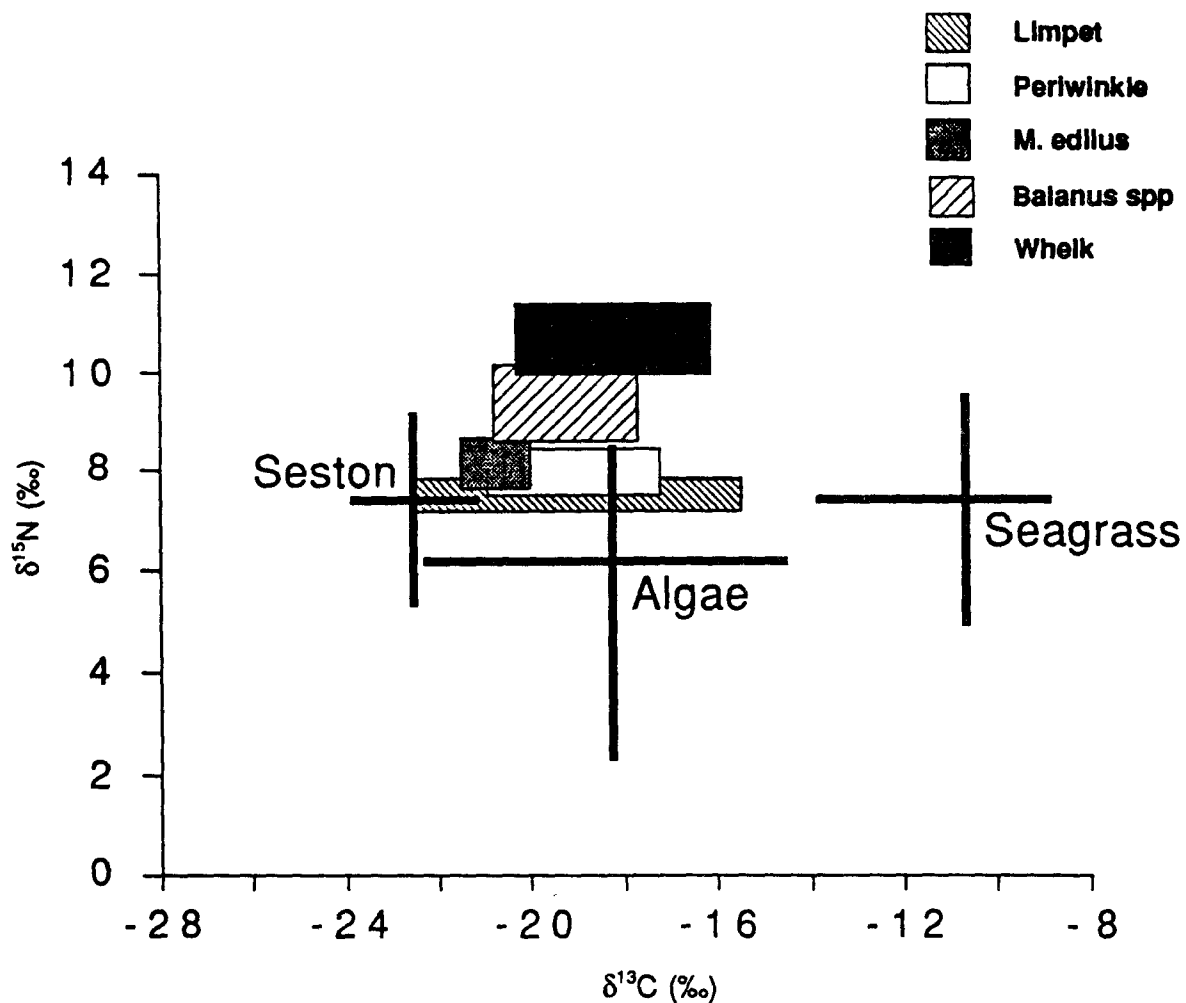
Figure 11.3. Limpet, Periwinkle, *M. edilus, Balanus* spp, and Whelk Data Showing $\delta^{15}N$ Plotted as a Function of $\delta^{13}C$. The Boxes Indicate the Range of Values Measured for These Respective Consumers. The Crosses are Averages and Ranges of $\delta^{15}N$ and $\delta^{13}C$ for Algae, Seagrasses, and Seston (Refer to Table 11.1; Values from Tatitalek Island not included)

The position of organisms in the food chain may be established by plotting the range of stable nitrogen or carbon isotope values for each trophic level in an ascending trophic status. It is important to establish these relationships to understand the assimilation of fertilizer nitrogen into algae and consumers. Fractionation of isotopically heavy carbon isotopes is not significant between trophic levels; therefore, a direct correspondence between the trophic levels indicates a dietary dependence by the higher tropic level.

Food Chain Studies at Elrington and Disk Island, Summer 1990

On Disk Island stable carbon isotope values ($\delta^{13}C$) indicated that the brown and/or green algae plus seston were important carbon sources for barnacles (Figure 11.4A). Limpets and periwinkles relied on the same carbon sources; however, red algae was another possible food source. In addition, the wide range in $\delta^{13}C$ of limpets and periwinkles suggested a greater number of carbon sources, probably relating to the feeding strategy. Sources of carbon for eel blennys and whelks appeared to be organisms that feed on brown and/or green algae. With some interesting exceptions, similar results were noted on Elrington Island.

On Elrington, red algae did not appear to be a significant source of carbon for higher trophic levels (Figure 11.4B). In fact, the $\delta^{13}C$ of the Odonthalia on the two islands was significantly different, suggesting different species were analyzed. Another interesting difference was a substantially narrower range in $\delta^{13}C$ of limpets and periwinkles on Elrington. The difference in feeding exposure for these organisms on the islands may be related to the physical structure of the beaches, since the slope of the beach on Disk Island was considerably shallower than Elrington. The steeper slope may create more distinct ecological niches for organisms resulting in a smaller number of species of algae that provide organic matter for the limpets and periwinkles.

Food chain studies have demonstrated that stable nitrogen isotopes are generally fractionated by 3‰ for each trophic step. Considering this fractionation, data from Elrington and Disk Islands suggest that eel blennys may prey on all of the lower trophic consumers (Figure 11.5A and B). Also, whelks, periwinkles, limpets and barnacles may depend on any of the primary producers as food sources. Stable carbon isotopes provided an indication of the transfer of carbon sources from lower to higher trophic levels. Nitrogen isotopes were used to place the organisms that were studied in the food chain in an appropriate order. Furthermore, it is clear from the nitrogen data that the normal status of the food chains were not altered by the addition of nitrogen.
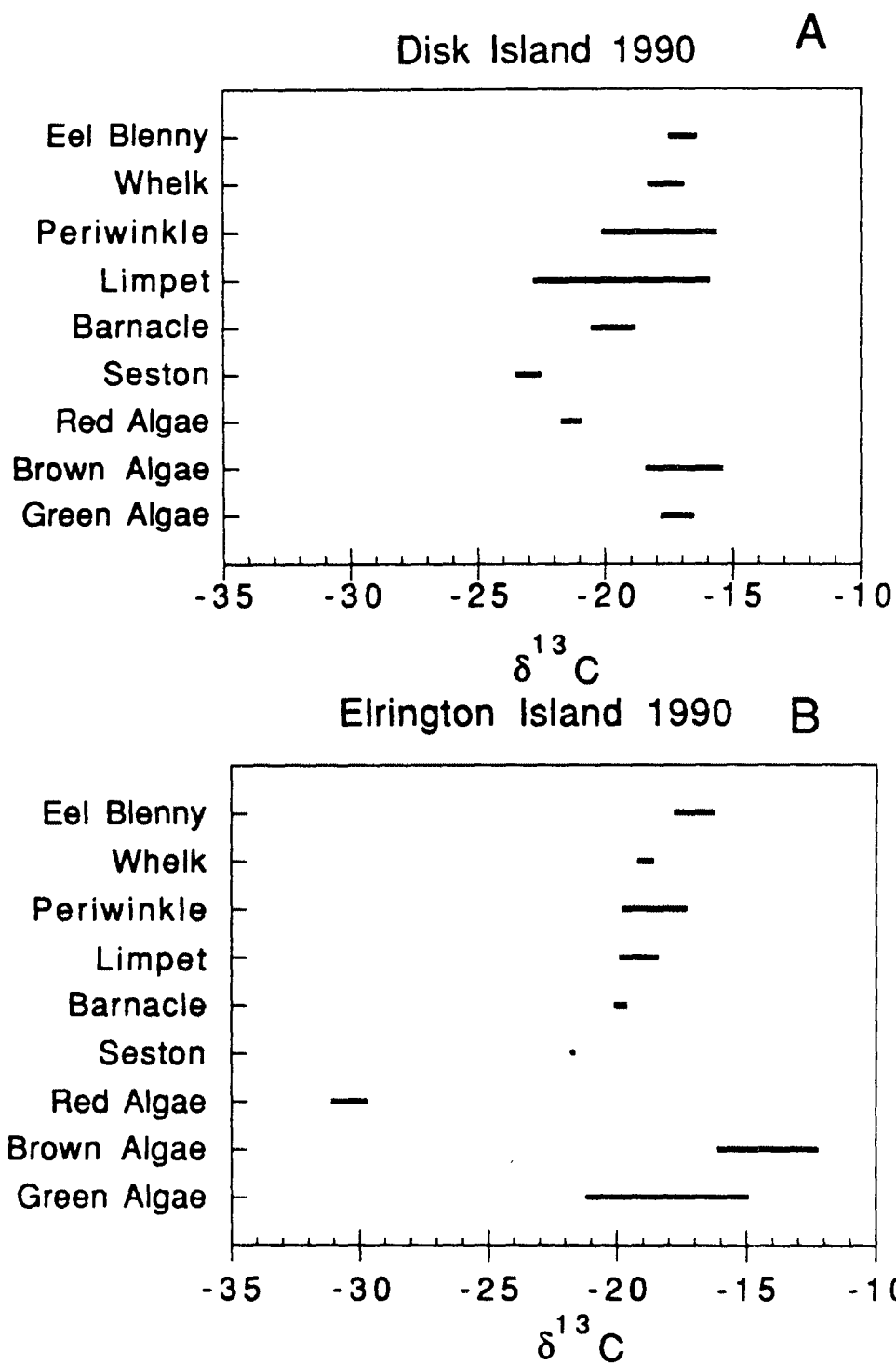
Figures 11.4A, 4B. Stable Carbon Isotope Values for Ecological Samples Collected at Disk Island and Elrington Island.
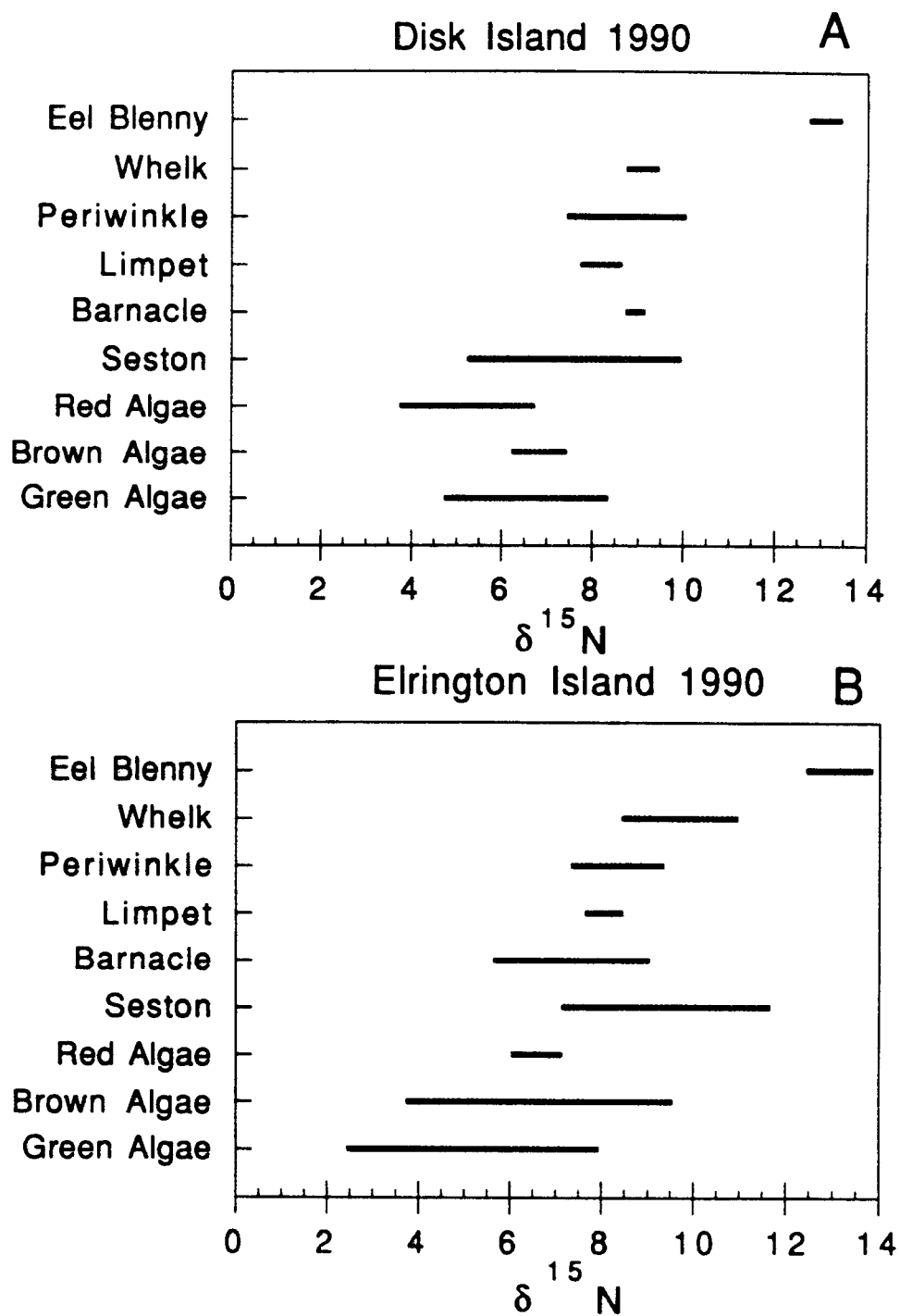
Figures 11.5A, 5B. Stable Nitrogen Isotope Values for Ecological Samples Collected at Disk Island and Elrington Island.

Fertilizer Nitrogen Assimilation by Food Chains on Beaches, Summer 1990

The stable nitrogen isotope ratio $\delta^{15}N$ of the fertilizer was approximately 0.0 ‰, while isotope ratios of organisms on the beach were substantially higher, ranging from approximately 8.0 ‰ to 12.0 ‰. In contrast, $\delta^{15}N$ of NH_4^+ and NO_3^- measured in pore waters from Disk Island were -1.4 ‰ and -2.3 ‰, respectively. Therefore, declining $\delta^{15}N$ values indicated that organisms on the beach assimilated nitrogen from the fertilizer. Figure 11.6 presents $\delta^{15}N$ for algae and consumers from Elrington Island from June to August 1990. Only brown algae appeared to assimilate fertilizer nitrogen; $\delta^{15}N$ started to decline soon after fertilizer application was initiated. It appears that the uptake of fertilizer nitrogen is related to the ecological niche of the algae in the rocky intertidal zone. Brown algae, especially Fucus, are resistant to drying and thus would be found closest to the fertilizer-treated beaches. Red and green algae are not as resistant to drying and would be found in the lower intertidal and subtidal zones.

The transfer of fertilizer nitrogen from algae to consumers depends on the feeding strategies of the organisms. Trends in the stable nitrogen isotope values in the consumer organisms suggested that fertilizer nitrogen was transferred to barnacles, whelks, and eel blenny biomass (Figure 11.6B). Barnacle $\delta^{15}N$ values appeared to respond dramatically to fertilizer nitrogen while more gradual changes were evident in whelk and eel blenny isotope ratios. Barnacle stable nitrogen isotope values declined rapidly because these organisms are immediately dependent on algae as a food source. Stable nitrogen isotope values in the eel blennys and whelks declined more gradually because the isotope ratios of organisms in higher trophic levels are more buffered from rapid changes than in the lower trophic levels.

On Disk Island brown and red algae became isotopically lighter through June and July, suggesting the uptake of nitrogen in the fertilizer applied to the beaches (Figure 11.7A). It is possible that red algae consumed fertilizer nitrogen on Disk and not on Elrington because different species of Odonthalia were examined and each was located in a different area of the intertidal zone. Another interesting result was that $\delta^{15}N$ in seston samples taken from waters a couple of hundred meters of the beach from Disk Island did not vary through the summer (Figure 11.7A). It was expected that seston and the algae on the beaches would have the same nitrogen source. The data, however, suggest two nitrogen sources. This supports our findings that organisms on the beaches assimilated fertilizer nitrogen. In contrast to beaches on Elrington Island, consumer organisms did not appear to assimilate nitrogen on Disk Island (Figure 11.7B). This may also be related to the physical differences between the beaches. If ecological niches are more distinct on steeper beaches, then the higher trophic levels on the shallower beaches of Disk Island would have more sources of substrate and, as a result, $\delta^{15}N$ signals from fertilizer nitrogen could be diluted.

A

B
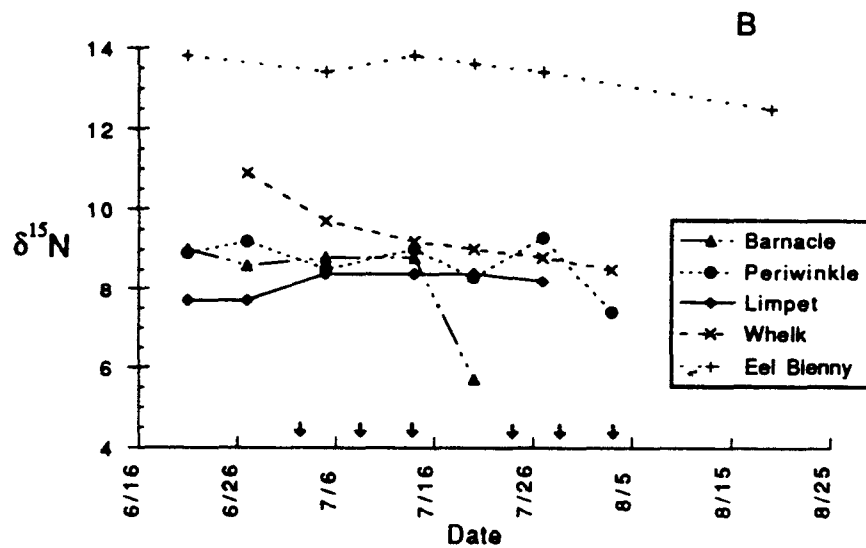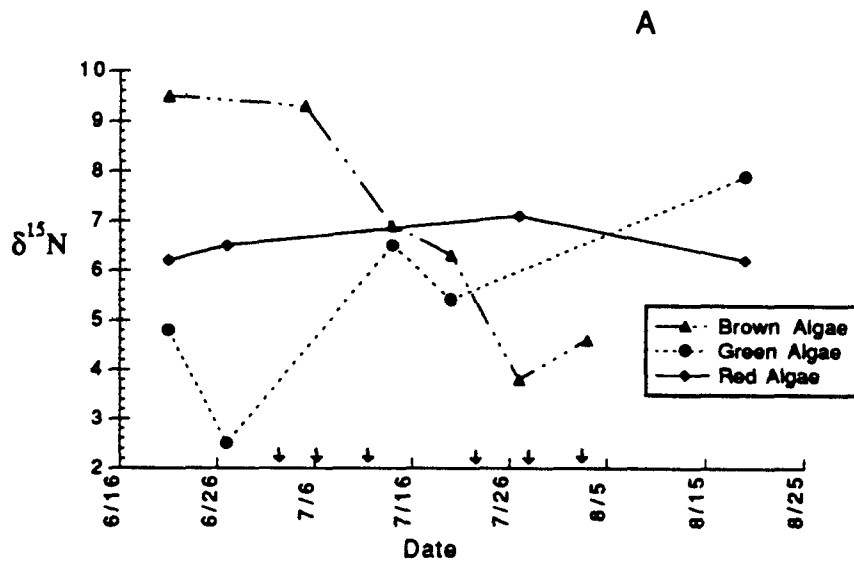
Figures 11.6A, 6B. Stable Nitrogen Isotope Values Over Time for Ecological Samples Collected at Elrington Island.

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A

B
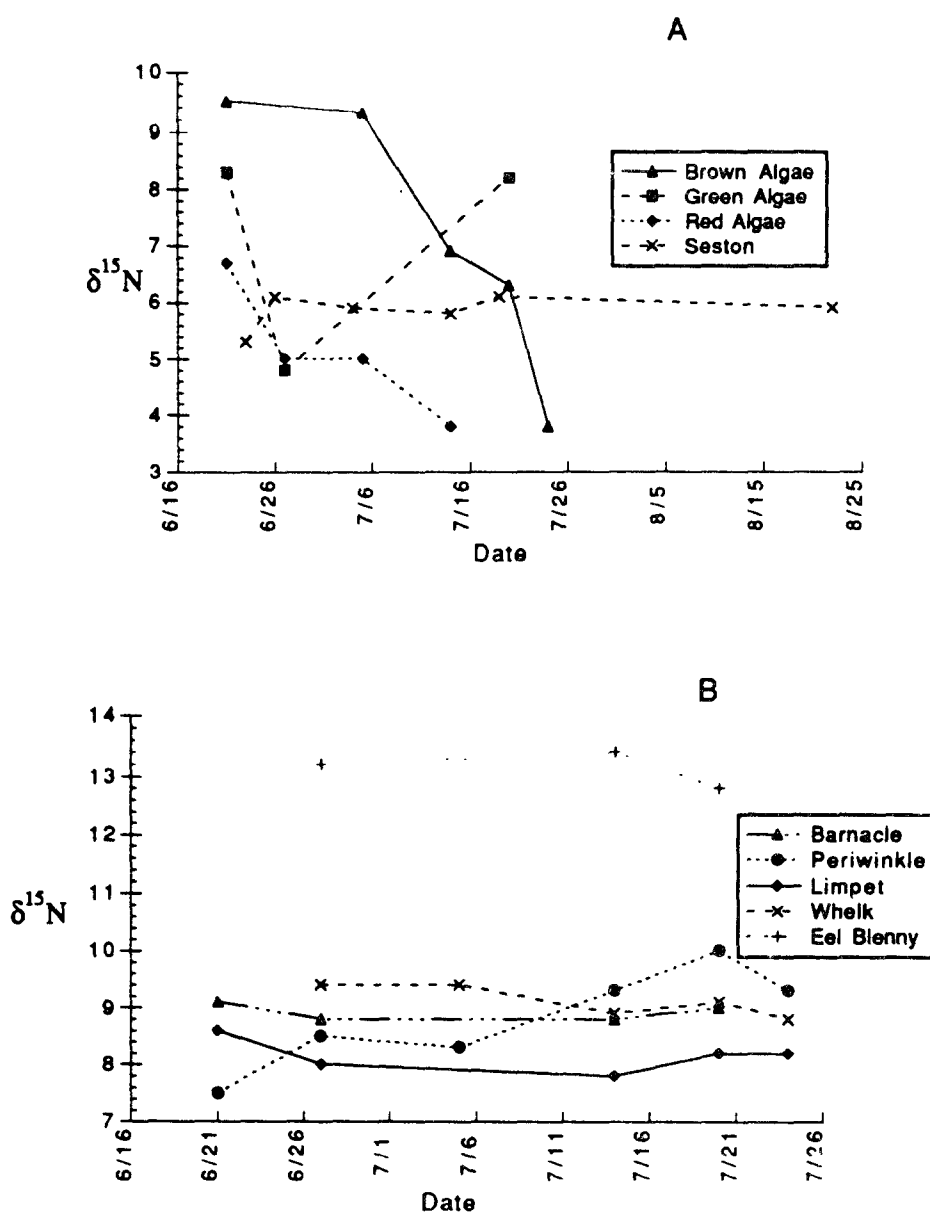
Figures 11.7A, 7B. **Stable Nitrogen Isotope Values Over Time for Ecological Samples Collected at Disk Island.**

Stable nitrogen isotope values indicated that fertilizer nitrogen was assimilated into intertidal food chains on Elrington and Disk Islands. However, both stable carbon and nitrogen isotope values indicated that the structure of the food chains were not altered by the application of fertilizer. It is hyphothesized that the slope of the beaches on each island controls spatial distribution of the organisms and, therefore, the distance from fertilizer application. Isotope ratios indicated that on Disk Island (the beach with a shallower slope), limpets and periwinkles had a greater diversity of food sources. As a result, nitrogen appeared to be assimilated only by the organisms located close to fertilizer treated areas. Furthermore, assimilation of nitrogen into the heterotrophic food chain was not observed on Disk Island, and may be due to dilution. These results support the use of stable isotopes as tracers of elements in aquatic food chains.

ANCILLARY FIELD DATA, SUMMER 1990

The stable carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) measurements of samples taken on Disk Island are shown in Table 11.2. The nitrogen isotope data from Disk Island shows that although suspended particulate matter (SPM) in the water removed from the well in the CUSTOMBLEN fertilized plot within the beach (100 g/m^2) was more positive (+7.5 ‰), it was similar to SPM from intertidal and cove waters, +5.8 ‰ and +5.7 ‰, respectively. Finally, the $\delta^{15}N$ of bacterial assays and nucleic acid extracts were all greater than 6 ‰. Table 11.3 presents stable carbon and nitrogen isotope values of carbon and nitrogen added to microcosms.

Carbon isotope values were similar to SPM from fertilized (-23.7 ‰) and unfertilized (-24.3 ‰) wells, but more negative than SPM from intertidal and cove waters (-21.7 ‰). The $\delta^{13}C$ data of bacteria from bacterial bioassays conducted in intertidal and cove waters (-22.0) was similar to the SPM recovered from these waters. In contrast, the $\delta^{13}C$ data from bacterial bioassays conducted with well water (-26.5 ‰) were more negative compared to the SPM, and, in addition, were significantly more negative than bacteria incubated in intertidal and cove water. However, the $\delta^{13}C$ of nucleic acid extracted from intertidal waters (-20 ‰) was similar to the $\delta^{13}C$ extracted from well water (-19.9 ‰).

MICROCOSM EXPERIMENT

The microcosm study was designed to determine if bacteria growing on different carbon and nitrogen sources could be distinguished isotopically. The experimental design consisted of four treatments of oiled gravel: 1) fertilizer; 2) fertilizer and seagrass detritus; 3) seagrass detritus; and 4) an untreated control. Table 11.3 presents stable carbon and nitrogen isotope values of carbon and nitrogen added to the microcosm.

TABLE 11.2. STABLE CARBON AND NITROGEN ISOTOPE DATA FROM DISK ISLAND AND RELATED BIOASSAY EXPERIMENTS

Location	Location	$\delta^{13}C(‰)$	$\delta^{15}N(‰)$
Disk Island SPM	Intertidal and cove water	$-21.7\pm1.0(5)$	$+5.8\pm0.3(6)$
Disk Island SPM	Untreated control plot	$-24.3\pm0.9(5)$	$+7.5\pm1.7(6)$
Disk Island SPM	Fertilized plot	$-23.7\pm0.7(4)$	$+5.7\pm1.4(6)$
Disk Island Bioassay	Intertidal and cove water	$-22.0\pm2.0(2)$	$+8.4\pm0.1(2)$
Disk Island Nucleic Acid	Intertidal and cove water	$-20.0(1)$	$+6.8,\ 14.8$
Disk Island Bioassay	Porewater	$-26.5(1)$	$+8.0(1)$
Disk Island Nucleic Acid	Porewater	$-19.9(1)$	$+9.1(1)$
Disk Island Bioassay	Porewater + fertilizer	$-27.4(1)$	$-7.4(1)$

SPM = Suspended particulate matter

TABLE 11.3. STABLE CARBON AND NITROGEN ISOTOPE DATA OF CARBON AND NITROGEN SOURCES IN THE MICROCOSM EXPERIMENT

Source	$\delta^{13}C(‰)$	$\delta^{15}N(‰)$
Oil	-30.1	-
Seagrass Detritus	-16.8	$+13.5$
Fertilizer Nitrate	-	-2.4
Fertilizer Ammonium	-	$+0.5$

The acridine orange direct counts (AODC) of bacteria, organic carbon content (PC), elemental carbon to nitrogen ratio $(C:N)_{wt}$, and isotopic data are shown in Figures 11.8-11.12. Cubic splines have been drawn through the data to facilitate comparisons among treatments, and are not meant to interpolate between data points. The solid dark line indicates the untreated control (oiled gravel without fertilizer or seagrass).

In all treatments a maximum in bacterial abundance was observed within the first 5 days of the experiment, followed by a sharp decline (Figure 11.8). Both fertilizer treatments had a secondary, although smaller, maximum occurring between days 7 and 11. Organic carbon concentrations decreased uniformly during the first 5 days of the experiment, and then for the unfertilized treatments remained relatively constant (Figure 11.9). In contrast, PC in the fertilized treatments increased after day 9. Except for the untreated control, elemental carbon to nitrogen ratios by weight $((C:N)_{wt})$ were in the range expected for bacteria at the beginning of the experiment (4 to 6; Figure 11.10). As the experiment continued, however, $(C:N)_{wt}$ increased in all treatments. The $(C:N)_{wt}$ variation among treatments was also greater at the end of the experiment compared to the beginning of the experiment.

The $\delta^{15}N$ of the seagrass was +13.5, while the $\delta^{15}N$ of NO_3^- and NH_4^+ from the CUSTOMBLEN fertilizer was -2.4 and +0.5, respectively (Table 11.3). Throughout the experiment, the nitrogen isotope ratio of the untreated control remained relatively constant $(+8.1\pm1.4$ ‰), while all the treatments decreased in comparison to the untreated control (Figure 11.11). This decrease was much more evident in the fertilized treatments where values at the end of the experiment were similar to the fertilizer.

The $\delta^{13}C$ of the oil and seagrass were -30.1 and -16.8, respectively (Table 11.3). As observed with the $\delta^{15}N$ of the untreated control, the $\delta^{13}C$ of the untreated control was also relatively constant $(-25.8\pm0.8$ ‰). In contrast, the values for the treated tanks changed significantly during the course of the experiment (Figure 11.12). Both fertilizer treatments attained the lowest values at the end of the experiment. The seagrass treatment without fertilizer, however, was higher than the untreated control at the end of the experiment.

A comparison of the carbon isotope data measured on GF/F filter concentrates of bacteria with carbon isotope data of nucleic acid extracts is given in Table 11.4. The latter data are less likely to include carbon or nitrogen from non-bacterial sources. The $\delta^{13}C$ of nucleic acids decreased during the experiment in all treatments. For the untreated control, the initial $\delta^{13}C$ value of nucleic acid was
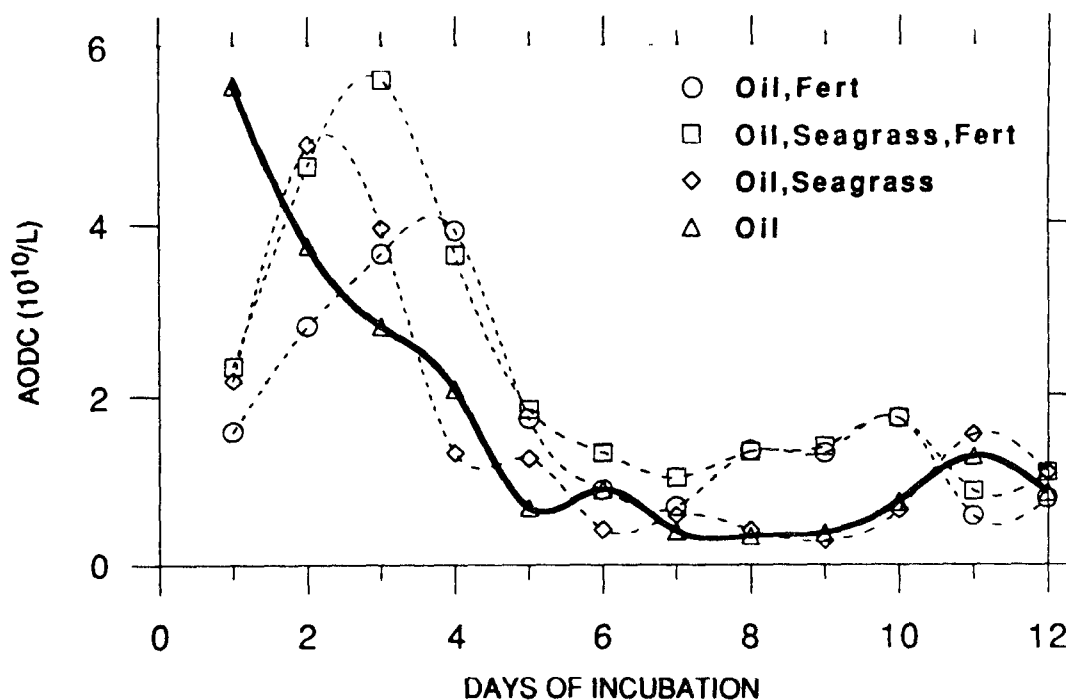
Figure 11.8. Acridine Orange Direct Counts (AODC) of Bacteria for the Four Microcosm Treatments Over Time.
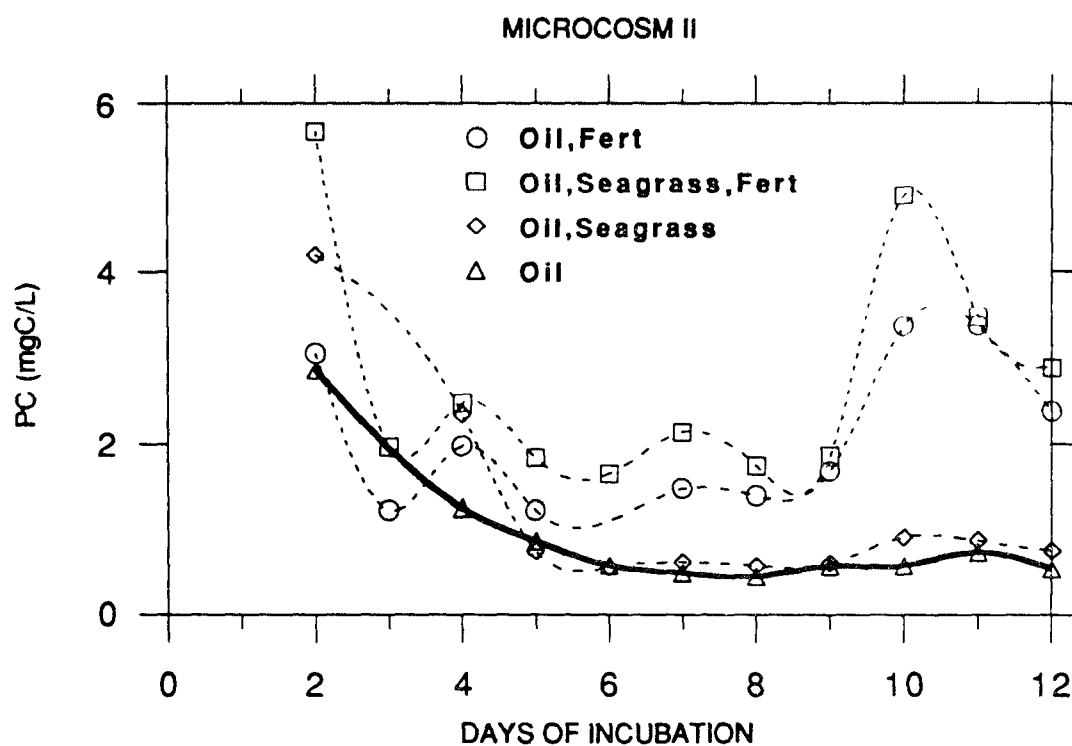
MICROCOSM II

Figure 11.9. Organic Carbon Content (PC) for the Four Microcosm Treatments Over Time.
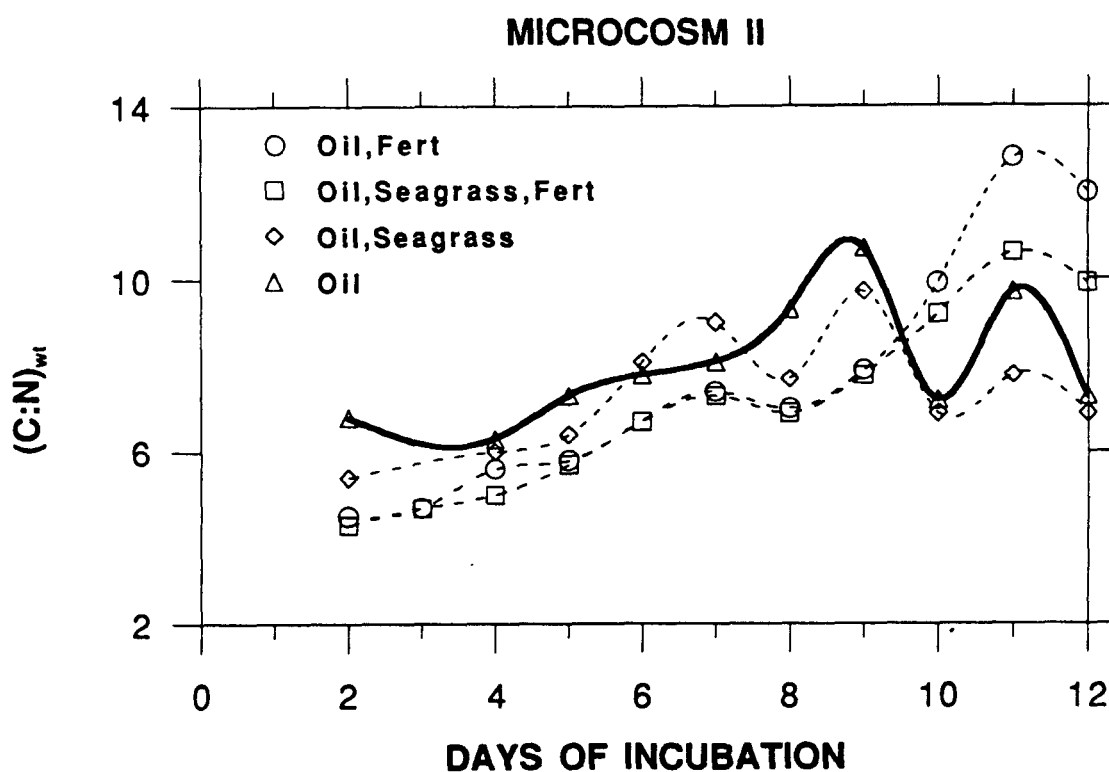
MICROCOSM II

Figure 11.10. Elemental Carbon to Nitrogen Ratio $(C:N)_{wt}$ for the Four Microcosm Treatments Over Time.
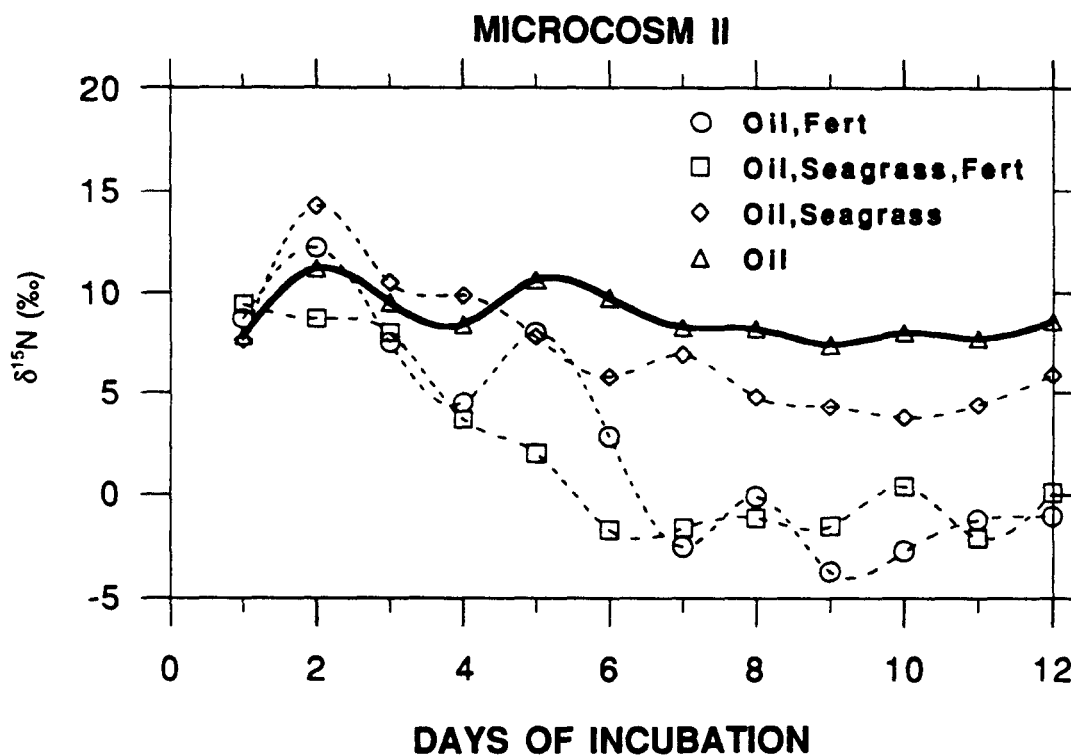
MICROCOSM II

Figure 11.11. $\delta^{15}N$ for the Four Microcosm Treatments Over Time.
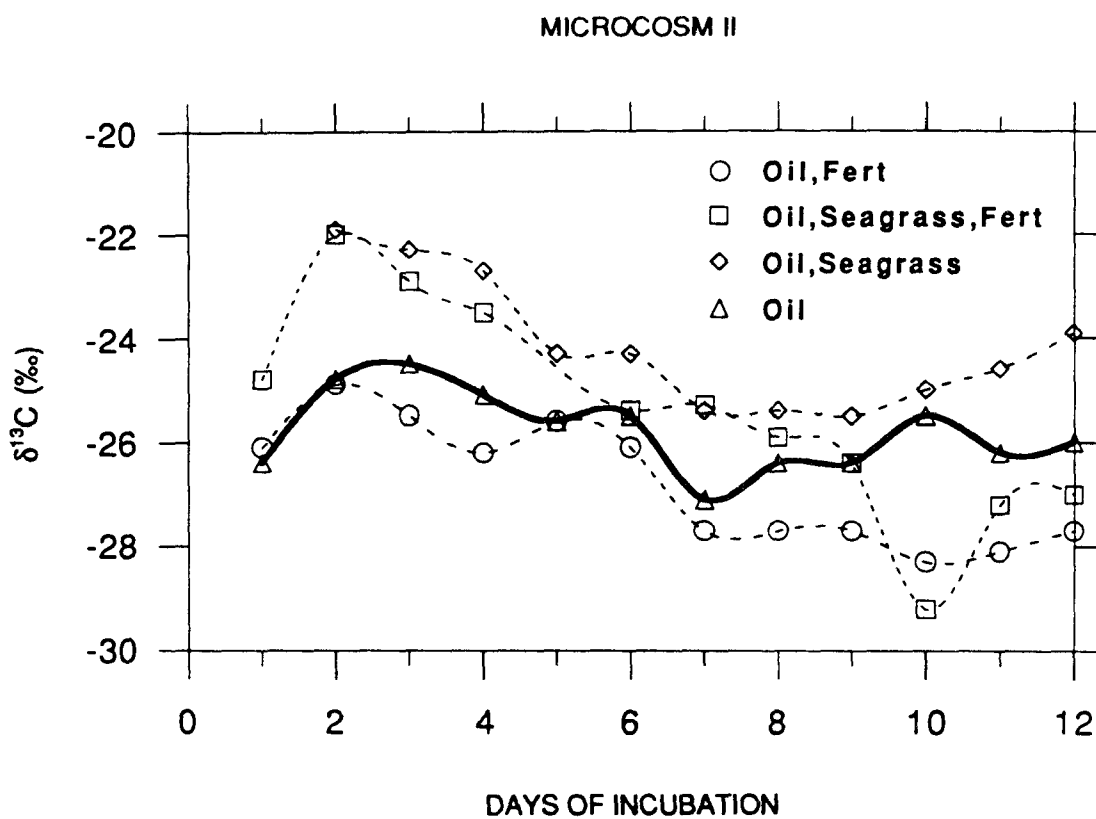
Figure 11.12. $\delta^{13}C$ for the Four Microcosm Treatments Over Time.

TABLE 11.4. STABLE CARBON ISOTOPE DATA OF MATERIAL COLLECTED ON GF/F FILTERS, NOMINALLY CONSIDERED TO BE BACTERIA, AND NUCLEIC ACID EXTRACTS FROM THE MICROCOSM EXPERIMENT

Sample	Date	$\delta^{13}C$ (‰) GF/F	$\delta^{13}C$ (‰) Nucleic Acid
Oil, Fertilizer	8/13/90	-26.1	-29.4
Oil, Fertilizer	8/16/90	-26.2	-29.7
Oil, Fertilizer	8/20/90	-27.7	-33.3
Oil, Fertilizer, Seagrass	8/13/90	-24.8	-18.9
Oil, Fertilizer, Seagrass	8/16/90	-23.5	-24.2
Oil, Fertilizer, Seagrass	8/20/90	-25.9	-32.7
Oil, Seagrass	8/13/90	-	-18.6
Oil, Seagrass	8/16/90	-23.5	-24.2
Oil, Seagrass	8/20/90	-25.9	-32.7
Oil, Seagrass	8/25/90	-23.9	-31.6
Oil	8/13/90	-26.4	-26.8
Oil	8/16/90	-25.1	-30.9
Oil	8/20/90	-26.4	-33.4

similar to the filter, but as the experiment continued the $\delta^{13}C$ of nucleic acids became more negative than the filter concentrates. Initially, both seagrass treatments had heavy $\delta^{13}C$ of nucleic acids. These values subsequently decreased, becoming more negative than the filter concentrates. This can be contrasted to the oil and fertilizer treatment (more negative $\delta^{13}C$ of nucleic acids) compared to filter concentrates throughout the experiment.

The isotope ratios of organic matter less than 1 μ that was recovered from the microcosms and a GF/F filter (termed filter concentrates of bacteria), varied between treatments. Although AODC and C:N data were consistent with a bacterial source of organic matter at the beginning of the experiment, another source with a higher C:N was present at the end of the experiment on these filter concentrates. In general, the nucleic acid carbon isotope data suggested the other organic sources were isotopically heavier (see Table 11.4). The most positive $\delta^{13}C$ of nucleic acids was observed at the start of the experiment in the seagrass treatments. This suggests the bacteria were using seagrass carbon as a substrate source (compare Tables 11.3 & 11.4). All treatments reached light $\delta^{13}C$ values at the end of the experiment. Petroleum hydrocarbons, which have more negative carbon isotope values (-28 to -30 ‰), could be the source of carbon resulting in the lighter values.

The nitrogen isotope ratio at the end of the experiment was lighter in all treatments compared to the untreated control (oiled-gravel only) and was similar to the CUSTOMBLEN in the fertilizer treatment. This suggests fertilizer nitrogen was utilized during the metabolism of available carbon substrates.

The data from the field do not resemble the microcosms data. The fertilized plot within the beach on Disk Island did not reach the lighter nitrogen or carbon isotope ratios. Values similar to those observed in the microcosm were found only when CUSTOMBLEN was added to a bioassay, where high fertilizer concentrations were available to bacteria (see Table 11.2). This result suggests that the bacteria in the fertilized plot within the beach were not subjected to the same concentration of nutrients as the bacteria in the microcosm.

SECTION 12
MODELING OIL BIOREMEDIATION

APPROACH AND ANALYSIS

A structured protocol was formulated to devise a predictive methodology to determine the time needed to clean up the oil contamination in Prince William Sound beaches using bioremediation. A corollary objective was to quantify the impact on clean-up time that various beach material amendments or treatments (the use of fertilizers) have on shortening the period required for oil decontamination using bioremediation.

The overall strategy was to formulate a predictive algorithm calibrated using data collected generated using bench-scale laboratory microcosm systems. This particular strategy was more economical and less time consuming for evaluating the effectiveness of various treatments on enhancing bioremediation rates. Ideally, it is desirable to quantify the impact of the rate enhancement on lessening clean-up time.

To develop the methodology, the following five tasks were implemented:

- A model was devised utilizing accepted procedures for biological systems that can predict clean-up times.

- Key modeling parameters were identified.

- Existing laboratory and field data were analyzed using modeling (i.e., attempt to use the laboratory data to predict field results).

- What would be needed to improve the model was determined.

- Additional field and laboratory efforts for improving predictive capability and streamlining batch data collection and analysis were suggested.

The four major areas relevant to development of a methodology for predicting bioremediation rates at the Prince William Sound beaches are as follows:

- Review and synopsis of existing predictive modeling procedures for biological waste treatment systems.

- Proposed modeling approach for the Prince William Sound bioremediation systems.

- Review of laboratory and field data collected to date.

- Methodology to calibrate the bioremediation model.

It was deemed appropriate to develop the predictive methodology based on analogous work which involved the predictive modeling of biological wastewater systems. For this work, respirometric techniques provided the means to calibrate the model cost-effectively.

Modeling Procedures for Biological Waste Treatment Systems

The effluent quality for a full-scale biological wastewater treatment plant was predicted by calibrating a model by using kinetic coefficients that were obtained via analysis of respirometric measurements of plant biomass (Colvin et al., 1990).

Modeling procedures for biological waste treatment systems are usually based on being able to quantify and hopefully control or influence the specific growth rate of microorganisms that are degrading target waste components. As an example, consider the modeling of a simple chemostat system depicted in Figure 12.1. A steady-state mass balance for cell concentration, X, shows that the growth rate is controlled by the dilution rate, which is a product of the influent substrate flow rate divided by the reactor volume (F/V). Since cell growth rate is also a function of substrate concentration, S, then specific output substrate concentrations can be selected by adjusting the flow rate, which in turn controls the growth rate.

This basic procedure was used to formulate a predictive modeling approach for activated sludge systems which are used for treating municipal and industrial wastewaters (Gaudy and Gaudy, 1980). In this case, predictive equations are derived by writing mass balances for both substrate (waste) and biomass concentrations around the reactor. An appropriate equation is used to relate growth rate to substrate concentration and the equations are solved to obtain predictors for substrate and biomass concentrations. This approach is enhanced by using respirometric (oxygen uptake) measurements of the biomass on the target waste to quantify the relationship between growth rate and waste concentration; in other words, the respirometric technique affords a relatively rapid and cost-effective calibration of the process model for the activated sludge system. Such an approach was successfully employed to obtain accurate predictions of a full-scale activated sludge system treating a municipal waste that had received a heavy industrial contribution. A field study showed that

Flow

Substrate

Flow

Substrate, Biomass

$$\mu = \frac{\text{Flow rate}}{\text{reactor volume}}$$
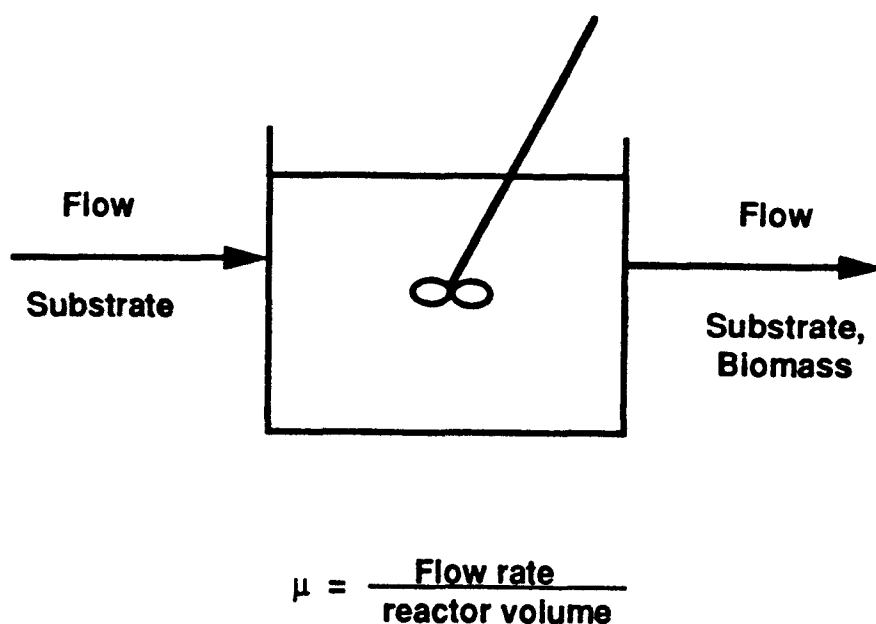
Figure 12.1. Chemostat Reactor.

predicted and measured values for effluent quality were in agreement and that a process model was calibrated using separate bench-scale respirometric measurements is a reliable predictor for activated sludge systems.

Modeling Approach for Bioremediation

Both biological waste treatment systems and bioremediation techniques are dependent on microbial growth and subsequent utilization of target waste components. This feature enables one to assume with a degree of certainty that a reasonable modeling approach for bioremediation systems should be based on the growth rate characteristics of the biomass. Consequently, a similar mass balance approach can be employed to obtain a predictive model.

The bioremediation model for the Prince William Sound beaches was derived by using the diagram shown in Figure 12.2. The mass balances for substrate and biomass are written around the control volume of beach material.

$$M(dX/dt) = \mu XM - F_{s} \tag{1}$$

$$M(dS/dt) = - \mu XM/Y_{t} \tag{2}$$

X represents biomass concentration in the beach material in mg/kg; S is the oil concentration in mg/kg; M is the mass of beach material in kg; μ is the specific growth rate in days^{-1}; F_{s} is the sloughing rate of biomass in mg/day; Y_{t} is the cell yield in mg/mg; and t is time in days.

It is assumed that biomass does not accumulate and is relatively constant because of sloughing that occurs due to tidal washing of the beach material. Using this assumption, dX/dt equals 0 and X is assumed to be at a relatively constant value.

$$\mu XM = F_{s} \tag{3}$$

$$dS/dt = - \mu X/Y_{t} \tag{4}$$

The substrate mass balance equation can now be solved using integration and the initial condition that at t=0, S=S$_{i}$, which is the initial oil concentration in the control volume. This procedure provides a predictive equation for S, the oil concentration in the beach material as a function of time.

$$S = S_{i} - \mu Xt/Y_{t} \tag{5}$$

It is appropriate at this point to compare and contrast this equation with the predictive equations employed in biological wastewater treatment systems (e.g., the chemostat). With the sloughing assumption, it is also assumed that the growth rate of the cells is effectively constant. This is analogous to a chemostat at steady-state, since these reactors attain steady growth rates when influent flow rates are constant.
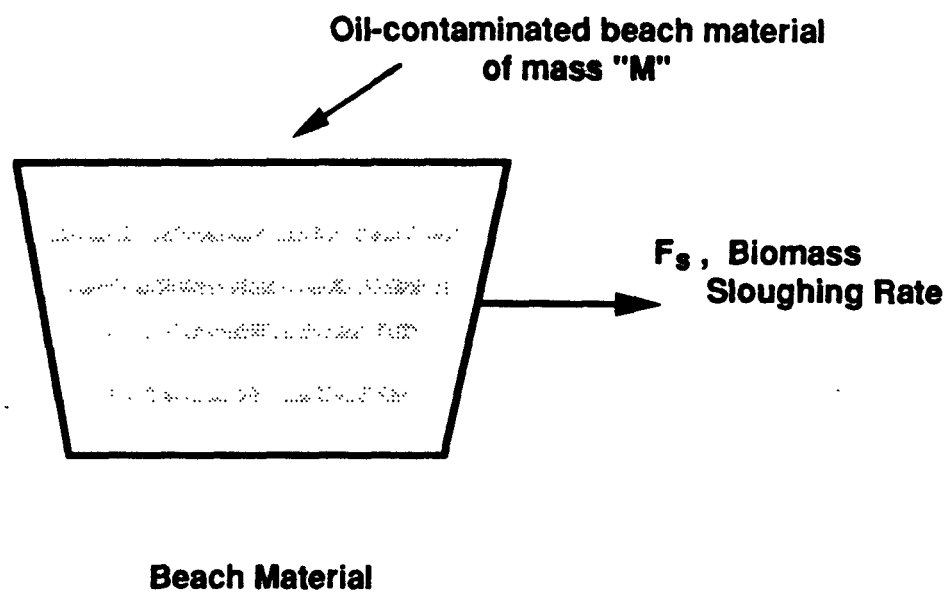
Figure 12.2. Schematic for Modeling the Beach Bioremediation System

The question of model calibration must be addressed. For a biological wastewater treatment system, this is accomplished by quantifying the relationship between specific growth rate and substrate concentration. This approach is unlikely to be applicable for an oil bioremediation application because substrate concentration will not be the limiting factor. In other words, the rate at which the target organics solubilize will control the availability of the carbon source to the microbial populations. Consequently, an alternative calibration strategy was warranted. This strategy consisted of utilizing bench-scale laboratory methods to determine the growth rates the biomass will achieve under various conditions. For example, flasks can be set-up to determine growth rate potential for control conditions, for various amendments or soil additives which are employed, or for extremes in weather conditions (e.g., cold temperatures). The laboratory approach simulated the target microcosm and then measured the resulting microbial growth rate, presumably using a surrogate indicator such as oxygen uptake or carbon dioxide evolution to calculate a cell growth rate.

This modeling approach affords separate consideration for the influence of microbial growth rate and biomass concentration. If one merely uses laboratory bench-scale carbon dioxide evolution or oxygen uptake data to compute predicted oil degradation rates, an aggregate oil degradation rate which is the resultant product of the interactive effect of biomass growth rate and biomass concentration can not be predicted. If the predictive methodology is incapable of meeting these criteria, it will be difficult to extrapolate results and identify design or operational modifications which will enable engineers and scientists to optimize and refine bioremediation systems. Specifically, if growth rate is optimized and the cells are achieving maximum rates, the model indicates that an increase in biomass concentration will enhance oil degradation rates. The question then is how to engineer an increase in biomass concentration in a bioremediation system. This aspect is discussed in greater detail in another part of this section.

Review of Field and Laboratory Data Collected the Summer of 1990

Existing data were reviewed and assessed for applicability to modeling. Both field and laboratory data were collected by EPA (see Sections 8 and 9). The primary field data collected consisted of:

- Weight of oil in beach material

- Biomass in beach material using a modified most probable number (MPN) technique for hydrocarbon degraders

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• Hydrocarbon degradation activity in beach material using radiolabeled phenanthrene as a reporter chemical

The bulk of the bench-scale laboratory data in the summer of 1990 consisted of microcosm studies. In addition to residual oil and biomass data, oxygen uptake and carbon dioxide evolution measurements were taken as a means to indicate microbial activity.

Microcosm studies were initially performed in a batch mode. Beach material was added to a flask and the various parameters were monitored with time. These studies were later modified to reflect the impact of tidal action on the bioremediation rates. This modification changed the reactor configuration of the bench-scale system to a mode that ameliorated biomass retention and prevented biomass accumulation.

Methodology to Calibrate Bioremediation Model

The most direct way to calibrate the bioremediation model is to calculate microbial growth rates by measuring increases in cell numbers. This approach was utilized in shake flask batch methods where a relatively easily measured parameter such as absorbance was employed as a surrogate indicator of microbial numbers. The most practical way to determine microbial numbers in oil bioremediation systems is the modified MPN technique that measures the number of hydrocarbon degraders. This methodology is relatively labor intensive when considering the number of data points needed to compute a growth rate. Alternatively, oxygen uptake or carbon dioxide evolution data can be used as a surrogate indicator of microbial growth and "translated" into cell numbers or mass. The assumption is that cell replication is occurring simultaneously with oxygen uptake or carbon dioxide evolution. As discussed previously, an analogous approach was successfully employed to calibrate biological wastewater treatment models. It is recommended that the bench-scale systems which are currently used can be employed for generating the data needed for calibrating a bioremediation model. Modifications may be necessary to streamline or enhance the testing methodology.

The field data were analyzed using the proposed bioremediation model as represented by equation 5. To apply the model the values of a number of parameters had to be assumed. The value of cell yield was assumed to be 0.5 mg cell/mg oil COD (chemical oxygen demand) and the mass of oil was converted into an oxygen demand equivalent using a conversion factor of 2.8 mg COD/mg oil. Biomass concentration estimates represented a problem since most of the data were collected

using an MPN method and the results were reported in logarithms of numbers of organisms (e.g., 10^6, 10^7, etc.). This means that the smallest increment between biomass concentration readings is one logarithm. In terms of the MPN test this is reasonable, but in terms of modeling this is problematic. Consequently, when both field- and bench-scale data were analyzed, different values of biomass concentration were assumed for calculating growth rates or for predicting oil degradation rates. MPN values were based on the results of measurements made by the University of Alaska (Fairbanks), and they also provided input to assumptions for biomass concentrations ranges for both the bench and field work.

For the bench-scale work, equation 5 was employed to calculate microbial growth rates based on oxygen uptake or carbon dioxide evolution data. For analysis of the field data, the growth rates that were measured in the batch tests were used in equation 5 to predict oil removal rates in the field. To calculate batch growth rates and to apply the model for predicting field results, a number of assumptions had to be made and are detailed below.

Modeling Assumptions

The first major assumption is that it is reasonable to use carbon dioxide evolution data or oxygen utilization data as a surrogate indicator of cell growth; in other words, it is assumed that cell replication or proliferation is occurring simultaneously with substrate or oil degradation. The next step is to "transform" these data into growth data. This is accomplished by using the mass balance principle as given in the equation below:

$$\text{Substrate} + O_2 = CO_2 + \text{Cells} \tag{6}$$

The substrate in this case is oil. It is assumed that the oil has a chemical oxygen demand (COD) value of 2.8 mg COD/mg oil; it is also assumed that the oil contains 0.8 mg C (carbon)/mg oil. Carbon dioxide contains 0.273 mg C/ mg CO_2 (12/44).

The cell yield value, Y_t, is taken as 0.3 mg X/ mg COD or 1.05 mg X/ mg C ((0.3)(2.8)/0.8). COD and C values for biomass are computed assuming a cell stoichiometry of $C_5H_7O_2N$; these are 1.42 mg COD/ mg X and 0.57 mg C/ mg X.

Equation 5 is manipulated to compute growth rate from the bench-scale data:

$$\mu = Y_t(S_i - S)/(Xt) \tag{7}$$

In equation 7, the term "S_i - S" represents the amount of oil degraded; this will be calculated or estimated using oxygen uptake or carbon dioxide evolution data. The oxygen uptake or CO_2 evolution data is converted into oil degradation information by using the aerobic balance principle for microbial systems:

$$\Delta COD = O_2 \text{ uptake} + \Delta COD \text{ cells} \tag{8}$$
$$\textbf{OR}$$
$$\Delta TOC = CO_2 \text{ evolved} + \Delta TOC \text{ cells} \tag{9}$$

These equations merely state that the degraded substrate (ΔCOD or ΔTOC) is accounted for in new cells (ΔCOD cells or ΔTOC cells) and oxygen uptake or carbon dioxide evolution (provided that there are insignificant physical removal or chemical oxidation mechanisms operative). Our model assumption is that with both the beach and bench-scale reactors, the biomass concentration is at steady state due to the effects of sloughing. This means that the ΔCOD cells and ΔTOC cells terms are equal to the mass of COD or TOC which is incorporated into biomass production. Equation 7 can then be rewritten to allow computation of growth rates directly from the bench-scale oxygen uptake or carbon dioxide evolution data.

The first step is to write expressions for the ΔCOD cells and ΔTOC cells terms in terms of biomass production:

$$\Delta COD \text{ cells} = 1.42\mu Xt \tag{10}$$

$$\Delta TOC \text{ cells} = 0.53\mu Xt \tag{11}$$

These terms represent the mass of COD or TOC which is incorporated into cell material; these are used in conjunction with the COD and TOC balance equations to provide an expression for the S_i-S term in equation 7.

$$\Delta COD = (S_i - S) = O_2 \text{ uptake} + 1.42\mu Xt \tag{12}$$

$$\Delta TOC = (S_i - S) = CO_2 \text{ evolved} + 0.53\mu Xt \tag{13}$$

These equations are now inserted into equation 7:

$$\mu = (Y_t/Xt)(O_2 \text{ uptake} + 1.42\mu Xt) \tag{14}$$

$$\mu = (Y_t/Xt)(CO_2 \text{ evolved} + 0.53\mu Xt) \tag{15}$$

These equations can now be solve to provide expressions for μ:

$$\mu = O_2 \text{ uptake}/((1/Y_t - 1.42)Xt) \tag{16}$$

$$\mu = CO_2 \text{ evolved}/((1/Y_t - 0.53)Xt) \tag{17}$$

Regarding other model parameters, X represents the biomass concentration on the beach material; X is calculated by using an MPN value and by assuming that one cell weighs 10^{-12} grams. Two or three MPN values will be assumed when calculating growth rates from the bench-scale data. Time is represented by "t".

For predicting field results, equation 5 is used as a predictor for oil concentration in the beach material:

$$S = S_i - \mu Xt/Y_t \tag{5}$$

S_i represents the initial oil concentration in the beach material. This equation predicts the time it takes to achieve oil clean-up levels in the beach material.

It is appropriate at this point to discuss the utility of equation 5 and the modeling approach in general. The overall strategy is to use the bench-scale tests to quantify the growth rates which the cells can achieve while degrading the oil. If a particular amendment or additive enhances the growth rate, and consequently the oil degradation rate, equation 5 will predict the impact on clean-up time.

In other words it will quantify the reduction in clean-up time that can be expected by using various additives to enhance bioremediation rates.

The biomass term in equation 5 demonstrates that the overall rate of oil degradation is a combined result of biomass growth rate and biomass concentration. This means that merely increasing microbial growth rates represents a relatively limited technique for increasing oil degradation rates. Specifically, much faster oil degradation rates can be achieved if the biomass concentration on the beach material is increased. Practically speaking, this is an engineering question. For example, consider the extended aeration activated sludge process where biomass is deliberately retained to achieve relatively high reactor degradation rates by maintaining high biomass concentrations. For bioremediation projects such as the one on the Prince William Sound beaches, one way to increase biomass concentration is to add an additional carbon source that enables the beach to support a higher biomass concentration that may be more resistant to sloughing.

From an analytical perspective, the significance of the biomass term in equation 5 is somewhat negative, since it points out that accurate bench estimates of growth rate are contingent on the ability to quantify biomass concentrations in the bench-scale systems. Similarly, accurate beach material biomass concentrations are needed to apply the current version of the model for predicting oil degradation rates. In view of the accuracy of the existing biomass concentration data (+ or - one logarithm), it was decided to generate growth rate information by analyzing the bench-scale data by assuming two or three different biomass concentrations representative of current MPN data. This results in producing two or three different growth rates which are predicted by each set of batch data. These growth rates are, in turn, used to predict oil degradation rates at the various test plots. Different growth rate and biomass concentration combinations are employed in the field data predictive modeling effort and the predictive results can be compared with actual field data.

Bench-Scale Data Analysis

To evaluate the efficacy of using the bench-scale data to calibrate the predictive model, it is appropriate to use equation 16 or 17 (for O_2 and CO_2 data, respectively) to compute growth rates using the Micro-Oxymax data. Growth rate calculations were made for different biomass, x, concentrations.

Results of bench-scale evaluation of the microbial activity on the oil adhering to the beach material were analyzed using the current modeling approach. Typical computational results are presented in Tables 12.1 through 12.4. These data present calculated growth rates for oxygen uptake data that were reported elsewhere (USEPA, 1990). As expected, the impact of varying the values of the biomass concentration by one logarithm produces a tenfold variation in the values of the calculated growth rates. For most biokinetic work, the quantification of biomass concentration or the time course variation in biomass or cell number concentration is an essential component of the data collection effort. This does not, however, diminish the significance of the results presented in these tables. **This is stated** because the relatively rough method of analysis (i.e., lack of more refined biomass concentration data, cell yield values, data on COD and TOC content of oil and cells, etc.) of the bench-scale data produced computational results for growth rate which are reasonable; calculated μ values range from 0.005 to 0.10 hours^{-1} for Tables 12.1 and 12.2. It should be noted that this variation does contain a comparison of a control and a fertilizer amended sample of beach material.

A comparison of the kinetic calculations in Tables 12.1 and 12.2 makes a good point regarding the potential significance and effectiveness of fertilizers and other bioremediation amendments. The comparison of growth rates shows that the populations which were growing on the amended beach material achieved growth rates that were approximately double those of the untreated control systems. This can be concluded because it is reasonable to assume that both systems had approximately the same biomass concentration. As shown by equations 14 and 15, an observed doubling of the rate of oxygen uptake or carbon dioxide evolution realizes a doubling of the cell growth rate; knowledge of the biomass concentration is required in order to compute the value of the growth rate in each bench-scale reactor system. However, it is safe to conclude that the comparative bench evaluations provide a **quantitative relative** comparison (e.g., 10%, 50%, etc.) of the impact of fertilizers and other amendments on cell growth rates on the beach material. For quantitatively extrapolating the bench data for predicting field results, more refined biomass estimates are needed for both bench and field activities.

Field Data Analysis

Two sets of corresponding bench and field data collected the summer of 1990 for studies at Elrington Island were analyzed and the results are given in Tables 12.3 and 12.4. The average of the computed bench-scale growth rates were used in conjunction with equation 5 to predict oil degradation rates in the test plots and these analytical results are given in Tables 12.5 and 12.6.

TABLE 12.1. GROWTH RATE CALCULATIONS FOR 350 PPM NITROGEN FLASK

Time (hours)	O_2 Uptake[a] per 100 g Beach Material in Moles	Calculated μ[b] (h^{-1})	
		X=10^7 MPN per g Beach Material	X=10^8 MPN per g Beach Material
12	65	0.09150	0.0092
24	155	0.109	0.00109
36	225	0.1050	0.0105
48	300	0.1050	0.0105
60	375	0.1050	0.0105
72	445	0.1040	0.0109

[a] Data obtained from Figure 6b (USEPA, 1990)

[b] $$\mu = \frac{O_2 \text{ uptake}}{\left(\frac{1}{Yt} - 1.42\right) Xt} \quad \text{(Equation 16)}$$

TABLE 12.2. GROWTH RATE CALCULATIONS FOR 0.35 PPM NITROGEN FLASK

Time (hours)	O_2 Uptake[a] per 100 g Beach Material μ Moles	Calculated μ[b] (h^{-1})	
		X=10^7 MPN per g Beach Material	X=10^8 MPN per g Beach Material
12	50	0.0690	0.0069
24	85	0.060	0.0060
36	120	0.0560	0.0056
48	155	0.0543	0.0054
60	200	0.0560	0.0056
72	240	0.0560	0.0056

[a] Data obtained from Figure 6b (USEPA, 1990)

[b] $$\mu = \frac{O_2 \text{ uptake}}{\left(\frac{1}{Yt} - 1.42\right) Xt} \quad \text{(Equation 16)}$$

TABLE 12.3. GROWTH RATE CALCULATIONS FOR ELRINGTON ISLAND CO_2 EVOLUTION DATA, FERTILIZER-TREATED BEACH MATERIAL[a]

Time (hours)	μ CO_2 per 100 g Beach Material	Calculated μ[b] (h^{-1})	
		X=10^7 MPN per g Beach Material	X=10^8 MPN per g Beach Material
12	150	0.356	0.0356
24	270	0.320	0.0320
36	370	0.292	0.0292
48	480	0.284	0.0284
60	580	0.275	0.0275

[a] Data obtained from Figure 4a (USEPA letter, August 7, 1990)

[b] $$\mu = \frac{CO_2 \text{ evolved}}{\left(\frac{1}{Yt} - 0.53\right) Xt}$$

TABLE 12.4. GROWTH RATE CALCULATIONS FOR ELRINGTON ISLAND CO_2 EVOLUTION DATA, UNTREATED CONTROL BEACH MATERIAL[a]

Time (hours)	μ CO_2 per 100 g Beach Material	Calculated μ[b] (h^{-1})	
		X=10^7 MPN per g Beach Material	X=10^8 MPN per g Beach Materal
12	70	0.165	0.0165
24	155	0.185	0.0183
36	220	0.174	0.0174
48	280	0.166	0.0166
60	325	0.154	0.0154

[a] Data obtained from Figure 4b (USEPA letter, August 7, 1990)

[b] $$\mu = \frac{CO_2 \text{ evolved}}{\left(\frac{1}{Yt} - 0.53\right) Xt}$$ (Equation 17)

TABLE 12.5. PREDICTED S (RESIDUAL OIL) VALUES, MG OIL/KG FOR
ELRINGTON ISLAND UNTREATED CONTROL[a]
FOR DIFFERENT μ AND MPN VALUES[b]

		$\mu = 0.170$ h^{-1} Assumed X, MPN			$\mu = 0.017$ h^{-1} X, MPN			
Date	Time (days)	10^6	10^7	10^8	10^6	10^7	10^8	Actual
6/29/90	0	--	--	--	--	--	--	8,000
7/12/90	13	7,937	7,368	1,679	7,994	7,937	7,368	8,000
7/19/90	22	7,893	6,930	0	7,989	7,893	5,930	7,700

[a] Actual data obtained from Figure 3b (USEPA Letter, August 7, 1990)
[b] Predictions made using equation (5)

TABLE 12.6. PREDICTED S (RESIDUAL OIL) VALUES, MG OIL/KG FOR
ELRINGTON ISLAND FERTILIZER TREATED BEACH MATERIAL[a]
FOR DIFFERENT μ AND MPN VALUES[b]

		$\mu = 0.31$ h^{-1} X, MPN			$\mu = 0.031$ h^{-1} X, MPN			
Date	Time (days)	10^6	10^7	10^8	10^6	10^7	10^8	Actual
6/29/90	0	--	--	--	--	--	--	12,000
7/12/90	13	11,884	10,847	470	11,988	11,885	10,847	10,500
7/19/90	22	11,805	10,049	0	11,980	11,805	10,050	10,000

[a] Actual data obtained from Figure 3c (USEPA Letter, August 7, 1990)
[b] Predictions made using equation (5)

The results in Tables 12.5 and 12.6 are noteworthy because several of the growth rate and biomass combinations resulted in yielding reasonable predictions using equation 5. Based on previous knowledge of microbial kinetics and other supporting field data, there would be a tendency to believe that the combination of the lower growth rate values and the MPN values of 10^8 tend to be more realistic. Growth rates in excess of 0.10 hours^{-1} are somewhat high for organisms in natural environments, especially organisms that must contend with difficult-to-degrade substrates such as petroleum hydrocarbons. In addition, results of field analyses indicate that MPN numbers of 10^8 are reasonable for hydrocarbon degraders. These facts and the favorable results of the predictive modeling exercise suggest that the approach advocated herein merits further consideration.

These results are especially positive considering that the predictions of oil degradation rates were largely performed using data collected from bench-scale systems. In other words, the estimates of growth rate potential, as quantified by the measured μ values, for the control and fertilizer remediation conditions were used to calibrate the predictive model.

SUMMARY AND CONCLUSIONS

The following recommendations are suggested for future work regarding predictive model development:

- To devise a rigorous field test of the modeling approach, it is not necessary to totally simulate conditions at the beaches to obtain verification of, or the data to modify, the modeling approach presented. For example, it is suggested that a modified field and associated bench-scale testing regimen be implemented which eliminates the impact of the tidal washing effect. This program can be performed by isolating a substantial portion of sieved beach material and confining it to a location where the tidal effect is nullified. Separate samples can be removed from this test area for respirometric (O_2 uptake or CO_2 evolution) testing to obtain kinetic parameters (i.e., model calibration). Different test areas can be devised to evaluate and model the impact of different treatments or amendments on bioremediation rates. The advantages of this approach are that it simplifies the system model and permits focus on evaluating and/or modifying the modeling approach. Additionally, the "biomass retention hypothesis" (i.e., the idea that enhancing biomass retention in the beach bioremediation systems will accelerate overall bioremediation rates), can be tested in a more structured and controlled manner.

● Additional work for improving and/or modifying the modeling approach should target issues such as the refinement of system biomass estimates (+ or - one logarithm is simply not acceptable for model testing purposes) and defining other parameters such as the cell yield and cell carbon content. Consideration should also be given to the use of a substrate measurement parameter which is easily convertible to COD or TOC readings. This will enable analysts to check mass balance calculations during batch respirometric tests.

● Grazing of bacteria may play a significant role in the modeling procedure. If data are available which enables computation of grazing rates (which are presumed to be commensurate with a decay rate), then it should be relatively straightforward to compute the aggregate effect of grazing and ascertain whether or not it warrants inclusion in the modeling algorithm. For example, if the number of protozoa per gram of beach material and the associated "grazing rate" are known, a biomass attrition rate attributable to grazing can be computed. This rate can be compared to current estimated values for growth rates and the associated biomass concentrations to determine the significance of the potential impact of grazing.

SECTION 13

COMMERCIAL PRODUCTS TESTING

THE FOLLOWING EXPERIMENTS WERE NOT FUNDED OR SUPPORTED BY THE EXXON CORPORATION. THIS WORK WAS CONDUCTED BY EPA'S RISK REDUCTION ENGINEERING LABORATORY IN CINCINNATI, OHIO WITH SUPPORT FROM THE NATIONAL ENVIRONMENTAL TECHNOLOGY APPLICATIONS CORPORATION (NETAC), FOR PROPOSAL SELECTION AND FIELD ANALYTICAL SUPPORT. IT HAS BEEN INCLUDED IN THIS REPORT FOR INFORMATIONAL PURPOSES ONLY.

The commercial products testing effort conducted by EPA's Risk Reduction Engineering Laboratory (RREL) in Cincinnati, Ohio, with support from NETAC, involved: 1) laboratory screening of ten commercial products; and 2) field testing of those commercial products in Prince William Sound that met EPA's three criteria for field testing. The results of these two phases are described below.

LABORATORY STUDIES

After the EPA oil spill bioremediation project showed in the summer of 1989 that bioremediation of oil-polluted beaches was enhanced by fertilizer addition, the question then arose whether further enhancement was possible with the addition of microbial inocula prepared from oil-degrading populations not indigenous to Alaska. Seeding experiments have been conducted in previous studies with mixed results (Leahy et al., 1990). In a recent study, Dott et al., 1989, compared fuel oil degradation rates of activated sludge microorganisms with nine different commercial bacterial cultures in separate laboratory flasks. They found that the rate and extent of n-alkane and total hydrocarbon degradation by the diverse populations in activated sludge were significantly higher than any of the highly adapted commercially available cultures. Most success with biodegradation enhancement by allochthonous microbial cultures has been achieved when chemostats or fermentors were used to control conditions or reduce competition from indigenous microflora (Wong et al., 1988).

In February, 1990, EPA issued a public solicitation for proposals to the bioremediation industry to test the efficacy of commercial microbial products for enhancing degradation of weathered Alaskan crude oil. The Agency commissioned NETAC, a non-profit organization dedicated to the commercialization of environmental technologies, to convene a panel of experts to review the proposals and choose those that offered the most promise for success in the field. Forty proposals

were submitted, and 11 were selected for the first phase of a two-tiered testing protocol (only 10 were tested because one company did not participate). The laboratory testing consisted of electrolytic respirometers set up to measure oxygen uptake over time, and shake flasks to measure oil degradation and microbial growth. If one or more products were found effective, the second tier would take place, consisting of small field plots on an actual contaminated beach in Prince William Sound in the summer of 1990. The first phase of testing, the laboratory batch flask and respirometric evaluations, are discussed below.

The objective of the laboratory protocol was to determine if commercial bioremediation products could enhance the biodegradation of weathered crude oil to a significantly better degree than that achievable by simple fertilizer application. Testing was conducted in a controlled and closed environment designed to give quick results under ideal conditions. It was not meant to simulate the open environment of the oiled beaches of Prince William Sound, where conditions are in a constant state of flux with respect to tidal cycles and washout, temperature variation, climatic changes, freshwater/saltwater interactions, etc. The organisms inside the respirometer vessels were in continuous contact with the oil, seawater, and nutrients that were added initially. The seawater was not replenished every 12 hours as is the case in nature. The test was merely a screening procedure designed to determine if there was sufficient enhancement by the commercial additives to justify proceeding to the next tier of testing. To proceed to the field phase, three lines of evidence were used for decision-making: rapid onset and high rate of oxygen uptake, substantial growth of oil degraders, and significant degradation of the aliphatic and aromatic hydrocarbon fractions of the weathered Prudhoe Bay crude oil.

The 10 companies participating in the laboratory testing phase were (in alphabetical order): Alpha Environmental, Bioversal, Elf Aquitaine, ERI-Microbe Masters, Imbach, Microlife Technics, Polybac, Sybron, WasteMicrobes, and Woodward Clyde. Of the 10 products tested, 8 were microbial and 2 were non-microbial formulations (i.e., fertilizers and/or dispersants).

METHODS

Electrolytic Respirometry

The studies were conducted using four automated continuous oxygen-uptake measuring Voith Sapromats (Model B-12), which consist of a temperature-controlled water bath containing measuring

units, and a recorder for digital indication and direct plotting of the oxygen uptake curves. The measuring units comprise 12 reaction vessels each with a carbon dioxide absorber mounted inside, 12 oxygen generators each connected to its own reaction vessel by tubing, and 12 pressure indicators connected electronically to the reaction vessels. The measuring units are interconnected by tubing, forming an air-sealed system, so that the atmospheric pressure fluctuations do not adversely affect the results.

Depletion of oxygen by microbiological activity creates a vacuum, which is sensed by the pressure indicator. The oxygen generator is triggered to produce just enough oxygen to counterbalance the negative pressure. The electrical current used to generate the oxygen is measured by the digital recorder, and the data are converted directly into mg/L oxygen uptake. The CO_2 produced by microbial activity is absorbed by soda lime. The oxygen generators of the individual measuring units are electrolytic cells that supply the required amount of oxygen by electrolytic decomposition of copper sulfate/sulfuric acid solution.

A recorder/plotter constructs an oxygen uptake curve as a function of time and displays it on the computer screen while digitally saving the data on disc. For frequent recording and storage of oxygen uptake data, the Sapromat B-12 recorders are interfaced to an IBM-AT personal computer via the Metrabyte interface system. A software package, Labtech Notebook version 2.8 (Laboratory Technologies Corp., Wilmington, MA) allows the collection of data at 15 minute intervals.

Experimental Design

All commercial products were tested in duplicate at the concentration recommended by the manufacturer. Each 500 mL respirometer flask was charged with the following materials in the order listed: 250 mg weathered crude oil, 250 mL seawater from Prince William Sound, and commercial product. Seawater was prepared as follows: 25 g of oiled rocks from a contaminated beach in Prince William Sound were placed in a 4-L flask; 2 L of seawater were added; and the mixture was shaken for approximately 30 minutes to wash off all microbial inoculum from the rocks. The flask contents were allowed to settle, and the supernatant was mixed with more seawater for use in the respirometer vessels. Table 13.1 presents the summarized experimental design showing all control and experimental flasks.

TABLE 13.1. EXPERIMENTAL DESIGN FOR RESPIROMETRIC STUDIES

Reaction Vessel	Nutrients	Weathered Oil	Commercial Product	Seawater	TOTAL
TEST FLASKS:					
T_{Pn}	+	+	+	+	20
F_1, F_2	+	+	−	+	2
CONTROL FLASKS:					
C_{Pn}	+	−	+	+	20
CF_1, CF_2	+	−	−	+	2
C_1-inoculum	−	−	−	+	2
C_2-no nutrients	−	+	−	+	2
TOTAL					48

TPn = duplicate commercial product flasks (n = 10)
F_1, F_2 = fertilizer flasks (mineral N and P nutrients)
C_{Pn}, CF_1, CF_2 = no-oil controls for products and fertilizer,respectively
C_1, C_2 = inoculum and no-nutrient controls

Flasks F_1 and F_2 represented simple inorganic fertilizer application and contained the following ingredients (mg/L final concentration): (6.33) KH_2PO_4; (16.19) K_2HPO_4, (24.86) Na_2HPO_4; (38.5) NH_4Cl; (45) $MgSO_4.7H_2O$; (55) $CaCl_2$; and (2.5) $FeCl_3.6H_2O$. A small amount of yeast extract (0.15 mg/L final concentration) was added to each flask to provide vitamins and trace elements. The above formulation was identical to a minimal salts medium developed previously (OECD), except the concentration of NH_4Cl and the phosphate salts were reduced to more closely simulate the concentrations used in the field.

All respirometer flasks were incubated at 15° C in the dark and continuously stirred at 300 rpm by magnetic stirrers. The first set of control flasks (C_{Pn}, C_{F1}, C_{F2}) represented background oxygen uptake of the product and nutrient-supplemented seawater without oil. Results from these flasks were subtracted from the appropriate test flasks to obtain the net oxygen uptake on the weathered oil. The inoculum control represented the endogenous oxygen uptake of the organisms from the washed beach material and the seawater without oil or nutrients. The no-nutrient control represented the oxygen uptake of the organisms from the washed beach material and seawater on weathered oil without any external source of nutrient addition (i.e., background nutrient levels from Prince William Sound).

Shake Flasks

Shaker flasks duplicating the respirometer flasks were used to assess the quantitative changes in oil composition by chromatographic separation of the individual components. Although it was possible to remove samples from the respirometer flasks, it was deemed more prudent not to disturb the respirometric runs, but to instead use the shake flasks with proportionately higher levels of oil, commercial products, etc., to facilitate sampling and precision/accuracy of the analytical chemistry. Table 13.2 summarizes the shaker flask experimental design.

The test flasks corresponded exactly to the 22 test flasks listed in Table 13.1 but with the following modifications: flask size, 250 mL; seawater, 100 mL; weathered oil and commercial products, 10 times the final concentrations used in the respirometer flasks; and mineral nutrients, same final concentration used in the respirometer flasks. The higher concentration of weathered oil was used to improve the final sensitivity of the chemical analyses.

In addition to the 22 test flasks, 18 supplemental flasks were set up. These reactors represented 9 sterile product controls, which determined whether the enhancement was due to the microorganisms or to the nutrients or metabolites in the product, and 9 sterile background controls (i.e., sterile oil and seawater, but non-sterile product) to evaluate, by comparison to non-sterile flasks, the effect of competition from naturally occurring organisms (one of the 10 products did not receive these sterile treatments). Sterilization of materials was accomplished by autoclaving at 121° C for 15 minutes.

TABLE 13.2. EXPERIMENTAL DESIGN FOR THE SHAKER FLASK STUDIES

Reaction Vessel	Nutrients	Weathered Oil	Commercial Product	Seawater	TOTAL
TEST FLASKS:					
T_{Pn}	+	+	+	+	20[a]
S_{Pn}	+	+	sterile	+	9[a]
$T_{Pn}S_b$	+	sterile	+	sterile	9[a]
F_1, F_2	+	+	—	+	2[a]
CONTROL FLASKS:					
C_{Pn}	+	—	+	+	10[b]
C_{F1}	+	—	—	+	1[b]
C_1-inoculum	—	—	—	+	1[b]
C_2-no nutrients	—	+	—	+	2[a]
TOTAL					54

T_{Pn}	= duplicate commercial products (n = 10), non-sterile system
S_{Pn}	= sterile products in non-sterile seawater/oil, non-duplicated
$T_{Pn}S_b$	= non-sterile products in sterile seawater/oil, non-duplicated
F_1, F_2	= fertilizer (mineral N and P nutrients) in non-sterile system
C_{Pn}, C_{F1}	= no-oil controls for products and fertilizer, respectively
C_1, C_2	= inoculum and no-nutrient controls
a	= microbiological and chemical analysis
b	= microbiological analysis only

Sampling

There were three sampling events for analytical chemistry and microbiology, days 0, 11, and 20. These events were determined by the shape of the oxygen uptake curves from the respirometry experiments. Each shaker flask was sacrificed at the indicated sampling time by mixing the contents with methylene chloride and performing the extraction on the entire mixture. Before sacrificing a flask, a small aliquot was removed for determination of microbial density changes.

Nutrient Analysis

The nitrogen species NH_3-N, NO_2-N, and NO_3-N were determined by U.S. EPA Methods [EPA 600/4-79-020]. The NH_3-N method was No. 350.1 and the NO_2-N/NO_3-N method was No. 353.1.

Oil Chemistry

The oil constituents were analyzed by measuring the aliphatic and aromatic fractions of the methylene chloride extracts. The extracts were concentrated and passed through a silica gel fractionation column to separate the alkanes and the polycyclic aromatic hydrocarbons (PAHs). The column was first eluted with hexane to collect the alkane fraction and then with a 1:1 mixture of hexane and benzene to collect the aromatic fraction. Any polar compounds remaining in the extract stayed bound to the silica gel column. Aliphatic fractions were measured by gas chromatography using a flame ionization detector. The PAH fractions were characterized by gas chromatography/mass spectrometry (GS/MS).

Microbiology

Growth of oil-degraders was measured by spread plates on oil agar (Bushnell–Haas medium supplemented with Prudhoe Bay crude oil as the carbon source). Plates were incubated at 15° C for 21 days prior to counting.

COMMERCIAL PRODUCTS

RESULTS

Respirometry

The net oxygen uptake curves (oxygen uptake in product flasks with oil minus oxygen uptake in flasks without oil) for all 10 products (curves with symbols) compared with the curve for mineral nutrients (curve with no symbols) are summarized in Figures 13.1a and 13.1b. In Figure 13.1a products F and G showed significantly higher alkane degradation and also exhibited higher net oxygen consumption than mineral nutrients. The final plateau in total oxygen uptake was slightly less than 500 mg/L for both Products E and G compared with about 340 mg/L for the mineral nutrient flasks. The acclimation lag period for products E and G was approximately 2 and 4 days, respectively, compared with 5 days for mineral nutrients. Product A gave the highest maximum net uptake (630 mg/L compared with 340 for mineral nutrients) but the lag period was almost 10 days. Products B and D exhibited O_2 uptake characteristics that were not different from the nutrient control.

In Figure 13.1b only products J and C gave higher overall net O_2 consumption than mineral nutrients, although product F exceeded the control after 27 days. The lag period for both products J and C was only 1 day. The shape of the product F curve was multi-phasic, suggesting the organisms' were consuming different substrates at different rates and at different times (diauxie). Very little net oxygen consumption was observed with product I.

Nutrient Concentrations

Product flasks requiring nutrient addition, as specified by the product manufacturer, received the same level of mineral nutrients as the fertilizer flasks. The concentrations of ammonia nitrogen measured in each product flask at day 0 are summarized in Table 13.3.

Statistical Analysis of Alkane Degradation Data

The percent reductions of the resolved aliphatic constituents of the weathered oil (nC12 through nC34 plus the isoprenoid hydrocarbons pristane and phytane) were computed at day 11 for each product flask and the results compared with the percent reduction computed for the mineral nutrient flasks. Table 13.4 summarizes the statistical differences observed using Tukey's Studentized Range

TABLE 13.3. NH$_3$-N LEVELS IN EACH PRODUCT FLASK
AT THE START OF THE EXPERIMENT

PRODUCT	NH$_3$-N mg/L	NUTRIENTS ADDED
A	8.0	YES
B	2.1	NO
C	1080.0	NO
D	11.8	YES
E	11.3	YES
F	10.0	YES
G	24.9	YES
H	426.0	NO
I	0.5	NO
J	1.5	NO
FR*	6.9	YES

*FR = mineral fertilizer

TABLE 13.4. TUKEY'S STUDENTIZED RANGE TEST FOR DETECTING DIFFERENCES IN
MEAN PERCENT REMOVAL OF ALKANES BY PRODUCTS IN 11 DAYS

PRODUCT	% REMOVAL	SIGNIFICANTLY DIFFERENT FROM INORGANIC NUTRIENTS*
E	94.5	YES
G	93.6	YES
B	87.9	NO
A	75.9	NO
D	74.2	NO
FR	68.4	NO
C	67.8	NO
J	59.9	NO
H	49.5	NO
F	33.3	YES
I	27.9	YES

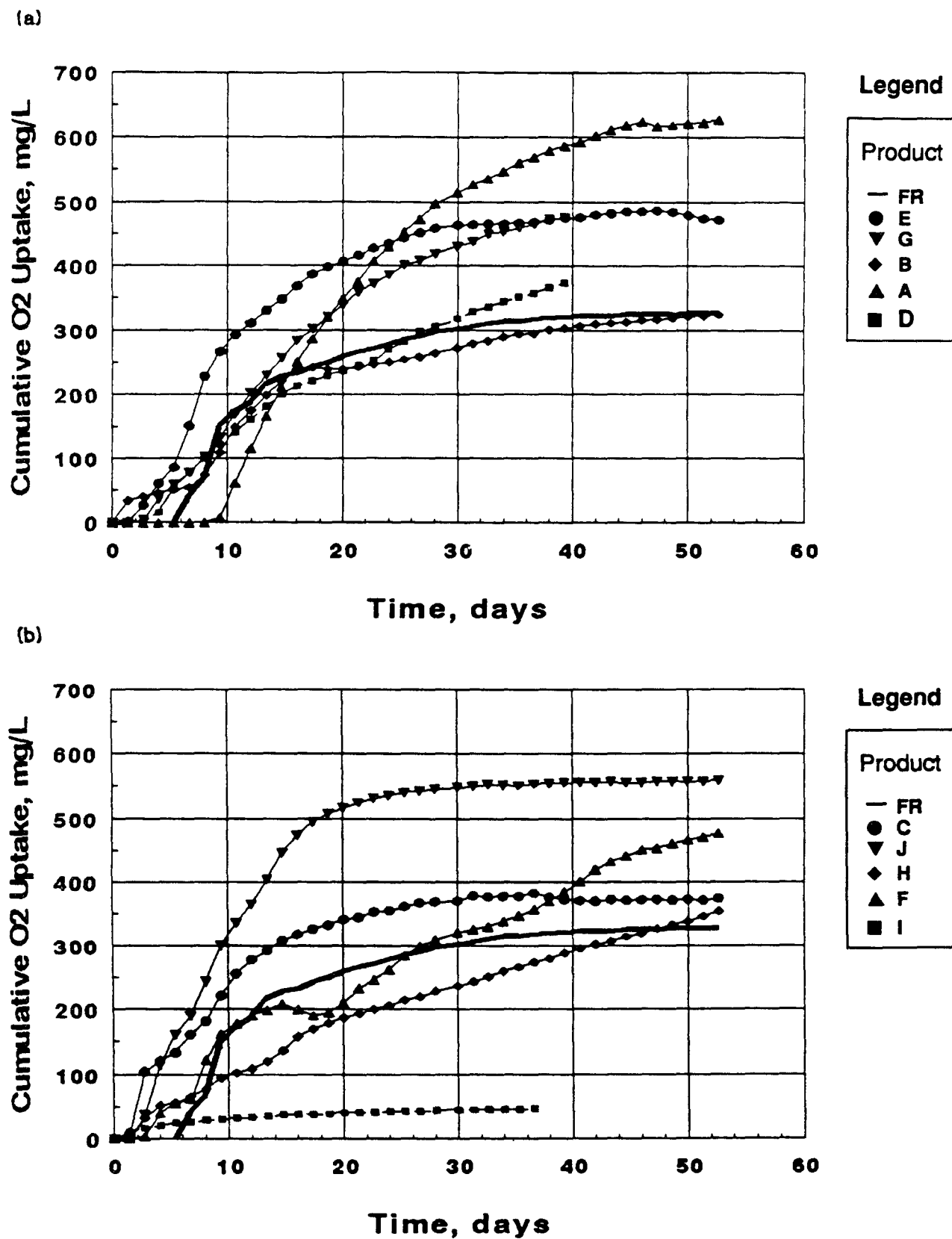* Minimum Detection Difference = 21.3% at 5% Significance Level

Figure 13.1. Net Oxygen Uptake Curves for Products and Mineral Nutrients: a) Products E, G, B, A, and D; (b) Products C, J, H, F, and I.

Test (Tukey, 1953). The products are arranged in descending order of significance. Only Products E and G gave significantly higher removals (p < 0.05) than inorganic fertilizer after 11 days. Six of the other products gave results that were not different from mineral nutrients, while two actually gave significantly lower removals. The latter results suggest that the products may have been inhibitory to the biomass at the levels used in the closed flasks.
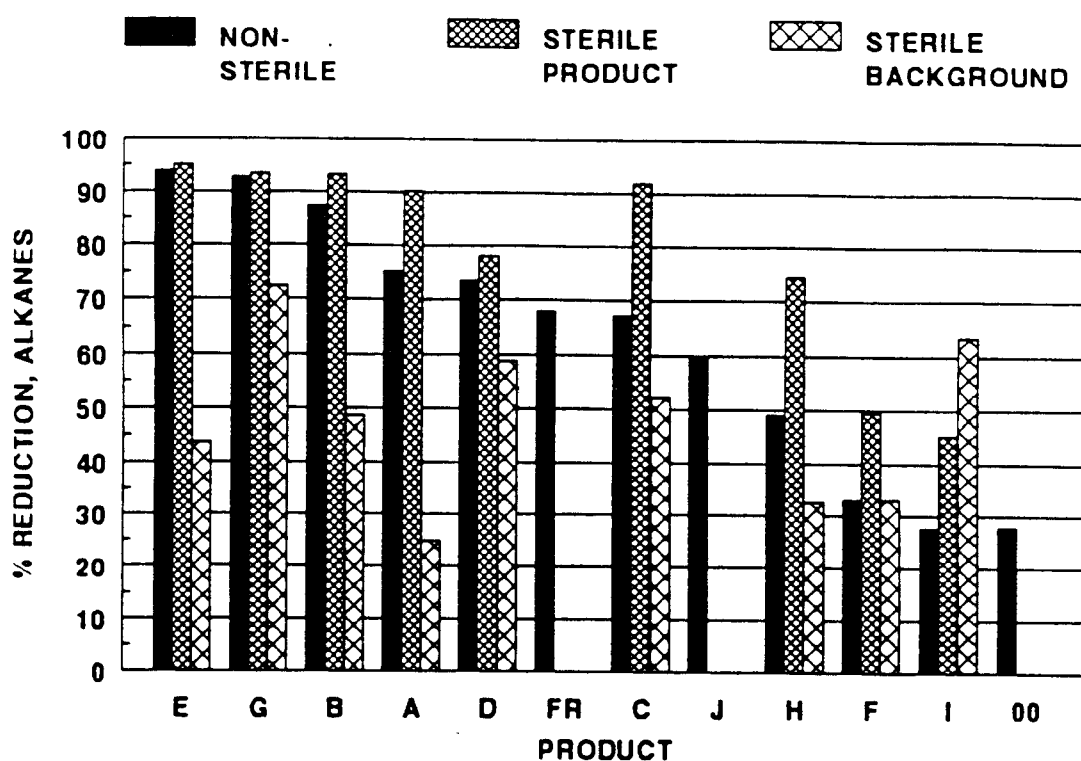
Total Alkane Reduction

The total alkane degradation data from the product flasks and the corresponding sterile controls at days 11 and 20 are summarized in Figure 13.2. The products are arranged on the x-axis in the order determined by the statistical analysis (this same ordering has been made on all figures). At day 11 (top half of Figure 13.2), better degradation was observed in every case when the commercial products were first sterilized, suggesting that the indigenous Alaskan populations were doing most, if not all, of the bioremediation. In contrast, less degradation occurred in every case except Product I when the background (seawater and oil) was first sterilized. This suggests that when left alone, the product organisms were less able to degrade the alkane fraction than the indigenous organisms. In the non-sterile treatments, enhancement was observed for Products E and G compared with mineral nutrients, suggesting that the products exhibiting the enhancement were providing metabolites or some other form of nutritional benefit that was lacking in the mineral nutrient flask. By day 20 (bottom half of Figure 13.2), all products except Products F and I caught up, giving greater than 85% reduction in the total alkane levels in the flasks. However, most of the flasks containing oil and seawater that were first sterilized still significantly lagged behind the non-sterile systems.

Typical Chromatographic Profiles

Typical day 11 chromatographic profiles of the individual alkane components for three of the products are shown in Figure 13.3. The three profiles shown were selected to represent products giving significantly better (Product E), equivalent (Product A), and lower (Product I) alkane removals than mineral nutrients after 11 days. The solid bars in the figure are the non-sterile systems (i.e., as they would be used in the field). The small-hatched bars are the flasks containing non-sterile oil and seawater inoculated with sterile product, while the large-hatched bars are those containing sterile seawater and oil inoculated with non-sterile products. The no-nutrient controls are represented by solid lines with symbols to facilitate comparisons.

NON-
STERILE

STERILE
PRODUCT
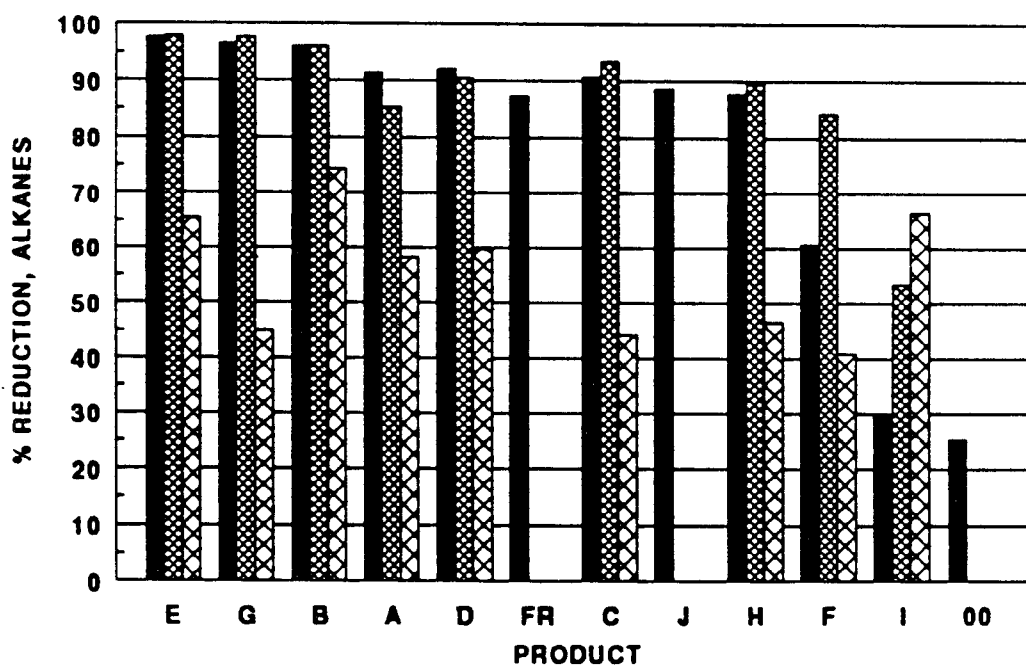
STERILE
BACKGROUND

(b) DAY 20

Figure 13.2. Total Alkane Reduction in the Product Flasks: (a) day 11, (b) day 20.

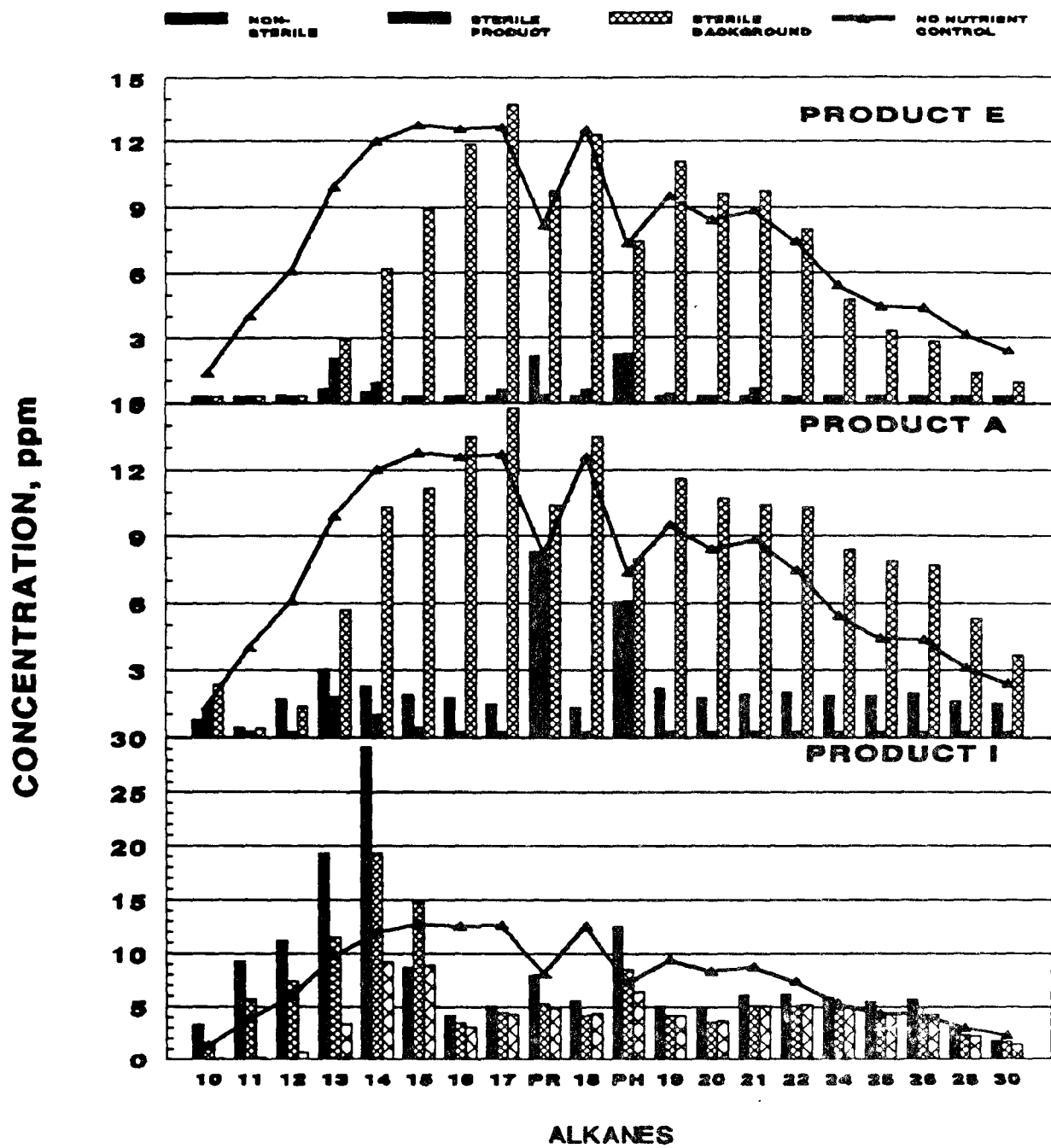**Figure 13.3.** Chromatographic Profile of the Alkane Data at Day 11 for Products E, A, and I.

In the case of Product E, virtually all of the normal alkanes in both the non-sterile and sterile product flasks were at or below detectable limits. Even the branched chain aliphatics were substantially reduced. In the flasks containing sterile seawater and oil with non-sterile product, changes were not observed in the normal alkanes nC15 and above, while some decreases occurred in the lower molecular weight fractions. In the case of Product A, the concentrations of normal alkanes in the non-sterile flasks were higher than the corresponding Product E flasks. Levels of the branched chain alkanes (pristane and phytane) were unchanged. In the flasks containing sterile product, most of the normal alkanes were nearly undetectable, while the pristane and phytane remained close to starting levels. In the sterile background flasks, levels of all constituents were similar to the corresponding Product E flasks. Finally, in the case of Product I, most of the alkanes were unchanged in all flasks, regardless of the sterile nature of the controls. The exceptions were the normal alkanes nC16 through nC22, which averaged approximately 53% lower than starting levels.

Total PAH Reduction

A summary of the total PAH reduction data at days 11 and 20 is presented in Figure 13.4. Differences are less clear among the products, although Products C, F, H, and I gave total reductions considerably less than mineral nutrients. By day 20, PAH reduction by Product C was somewhat closer to the others, while Products H, F, and I substantially lagged. Excellent removal of aromatics was observed in all other flasks.

Typical Mass Spectral Profiles

Typical day 11 mass spectral profiles of the individual PAHs for the same three products are shown in Figure 13.5. Mass spectral analyses of the fractionated extracts from the sterile controls were not performed. Day 0 and day 11 PAH levels for each of the non-sterile product flasks are shown on the figure. Differences in the PAH levels among the three product flasks are clearly evident. Higher concentrations of PAHs, especially in the substituted naphthalene group, occurred in the Product A flasks and even higher in the Product I flasks. The dibenzothiophene group was also more resistant to degradation in the Product I flasks than the other two.
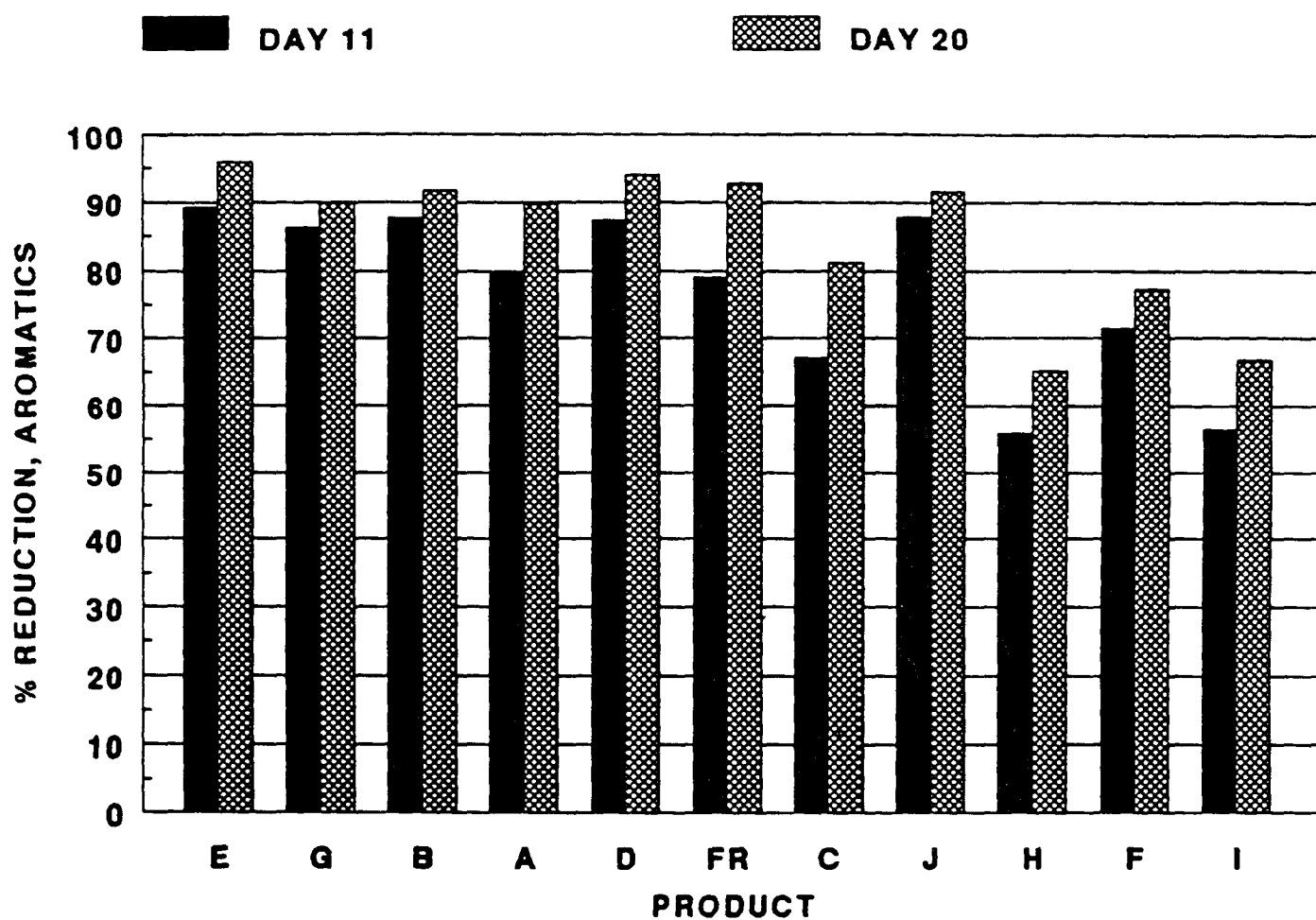
Figure 13.4. Total Aromatic Reduction in the Product Flasks at Days 11 and 20.

Figure 13.5. GC/MS Profile of the Aromatic Data at Day 11 for Products E, A, I.

A Acenaphthene
B Acenaphthylene
C Benzo(a)anthracene
D Benzo(a)pyrene
E Benzo(b)fluoranth
F Benzo(g,h,i)perylene
G Chrysene
H C1-Chrysenes

I C2-Chrysenes
J C3-Chrysenes
K Dibenzo(a,h)anthr.
L Dibenzothiophene
M C1-Dibenzothiophs
N C2-Dibenzothiophs
O C3-Dibenzothiophs
P Fluoranthene

Q Fluorene
R C1-Fluorenes
S C2-Fluorenes
T C3-Fluorenes
U Indeno(1,2,3-cd)pyr
V Naphthalene
W C1-Naphthalenes
X C2-Naphthalenes

Y C3-Naphthalenes
Z C4-Naphthalenes
1 Phenanth./anthra.
2 C1-Phenanth./anths
3 C2-Phenanth./anths
4 C3-Phenanth./anths
5 C4-Phenanth./anths
6 Pyrene

538

Microbiology

Virtually all changes in oil-degrader densities occurred by day 11. The populations leveled off in all flasks thereafter. Consequently, the growth of oil-degraders has been summarized for days 0 and 11 only, and the results from all flasks, including sterile controls, are presented in Figures 13.6 and 13.7. Figure 13.6 depicts the yield of oil-degraders for all products at day 11, and Figure 13.7 shows the $\log_{10}$ change in oil-degraders in 11 days. Data absent from the figures were caused by missed dilutions.

Products E and G, which gave the best alkane degradation of all the products (Table 13.4 and Figure 13.2) and displayed net oxygen uptake characteristics superior to most (Figure 13.1a), also exhibited excellent yield and growth of oil-degraders in 11 days. Products C, J, and F yielded high levels of oil-degraders and good oxygen uptake curves, but alkane degradation was not better than the populations growing in simple mineral nutrients. Oil-degrader populations actually declined in the Product B and A flasks, and the increase in oil-degraders in the flasks containing Products D and I was minimal.

Flasks containing sterile seawater and oil inoculated with non-sterile products (large-hatched bars in Figure 13.6) gave higher oil-degrader counts in all cases except Products C and F. The organisms in the latter two products were less able to grow on the weathered crude oil than the indigenous populations. If Products C and F were first sterilized, however, the indigenous populations grew better than either the non-sterile systems or the sterile background control flasks. This suggests that either there was an antagonism between the indigenous populations and product organisms, or the products contained one or more inhibitors that prevented the indigenous bacteria from achieving their ultimate biodegradative potential.

Flasks containing Products B and A also gave better growth when the products were first sterilized (Figure 13.7, small-hatched bars), again suggesting either a microbial antagonism or the presence of a heat-labile inhibitory substance. Product D gave the best growth when the indigenous populations were first sterilized. Products E, G, and J gave good final yields and density increases, but Product J did not perform well with respect to oil constituent degradation.
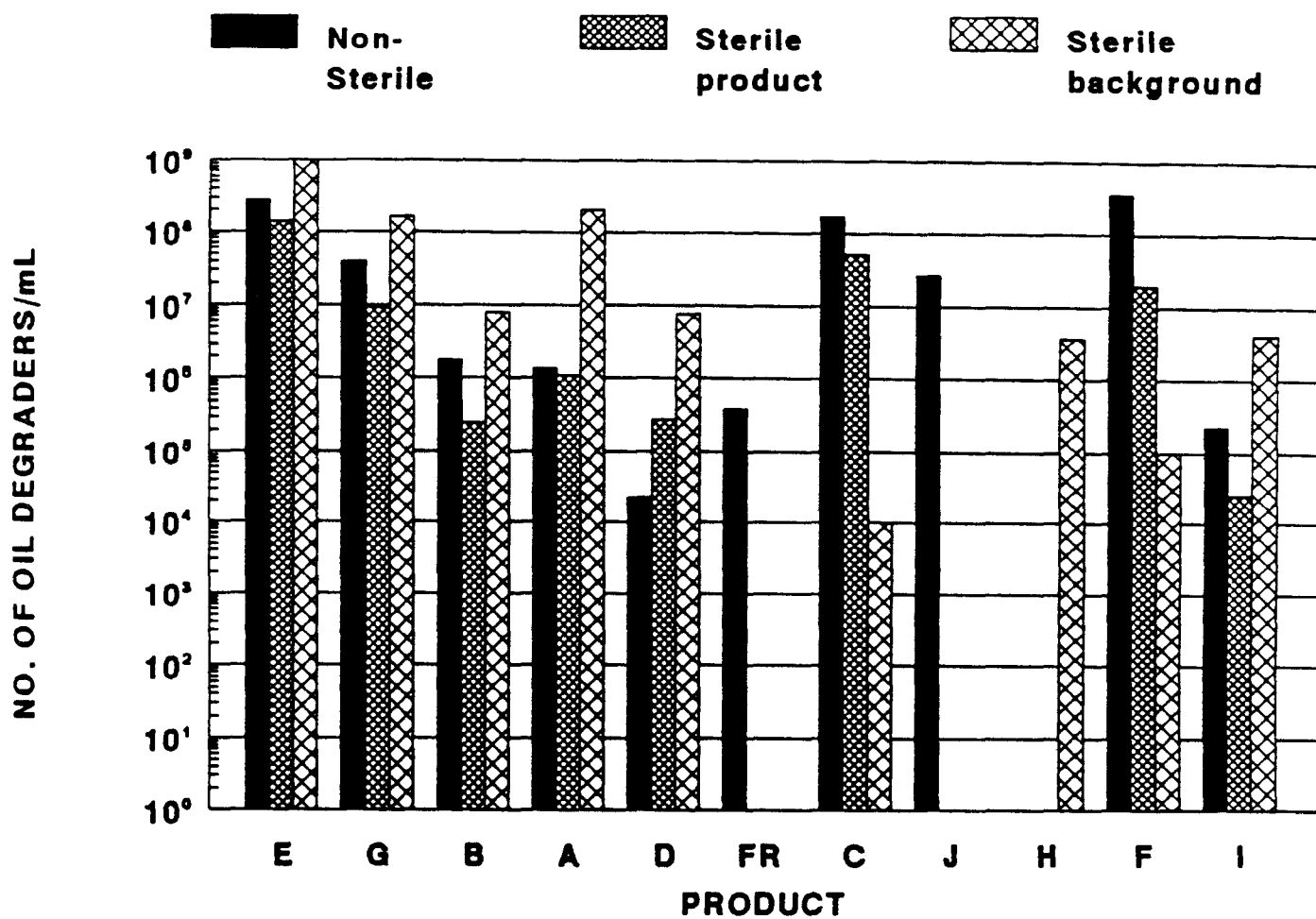
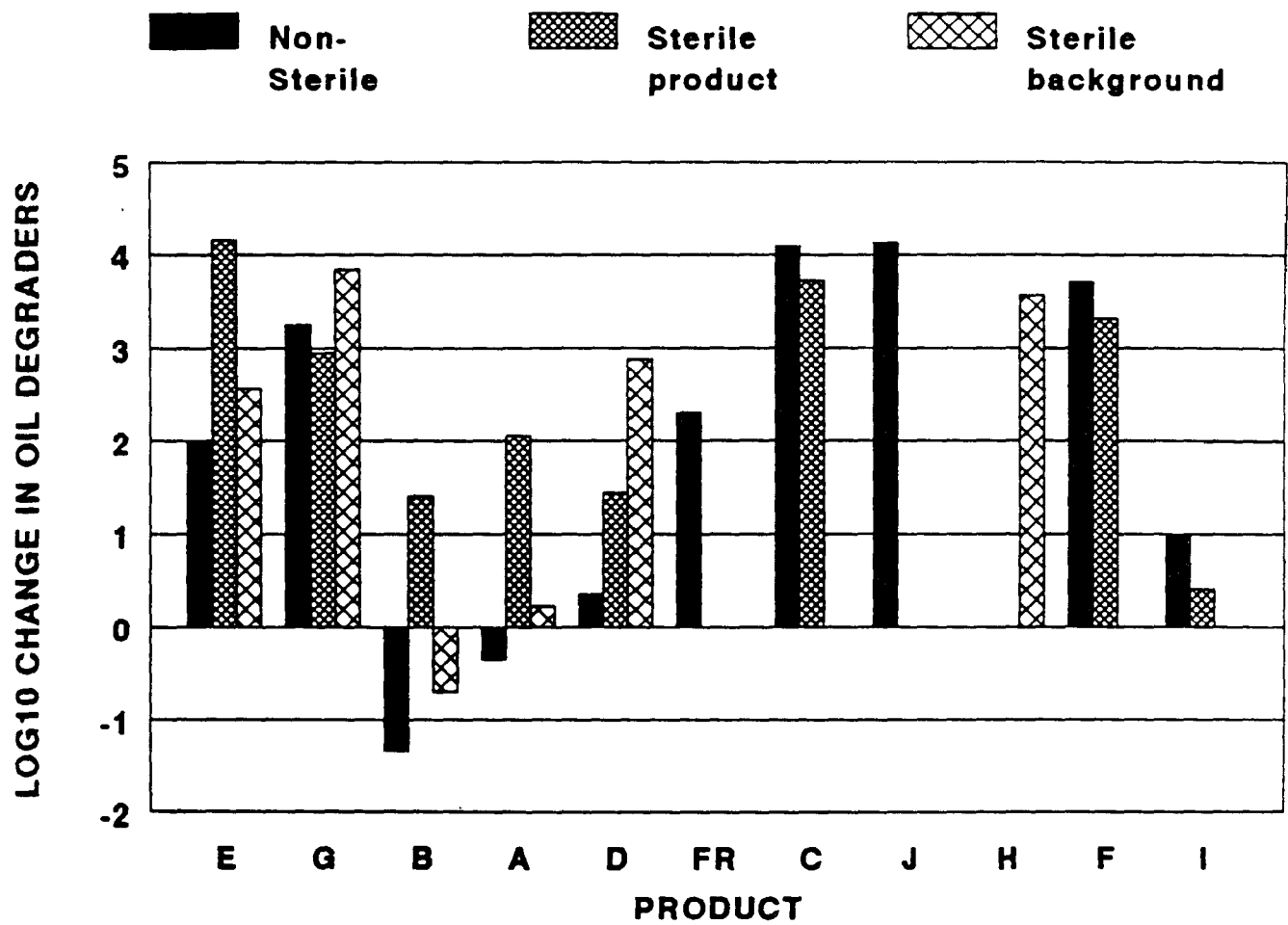Figure 13.6. Yield of Oil-Degraders for All Products at Day 11.

Figure 13.7. Log Increase in Oil-Degraders for All Products in 11 Days.

COMMERCIAL PRODUCTS

SUMMARY AND CONCLUSIONS

The objective of this laboratory experiment was to determine if weathered crude oil could be degraded faster when natural microbial populations were supplemented with exogenous oil-degraders and excess nutrients than excess nutrients alone. Oil degradation chemistry, oxygen uptake in respirometer flasks, and microbial density changes were used to decide which product(s) would proceed to field testing.

Of all the products tested, the two that provided the most consistent results in all three tests were products E and G. Both gave higher oxygen uptake with more rapid onset, greater growth of oil-degraders, and superior alkane degradation than mineral nutrients. Products C and J showed good growth of oil-degrader populations and gave excellent net oxygen uptake curves, but were not better oil-degraders than indigenous populations supplied with simple mineral nutrients. Product F yielded the highest oil-degrading population of all, yet it's oxygen uptake curve was not better than the mineral nutrient curve until after day 27, and alkane degradation was relatively poor. Product A gave the best overall net oxygen consumption, but the change in oil-degraders and the relative alkane degradative capability at day 11 were unsubstantial, perhaps because of the extended acclimation lag period as noted in the oxygen uptake curve for Product A. Flasks containing products B, D, H, and I also produced only minor changes in oil-degrading populations and unenhanced oil-degradative capability.

The sterile controls revealed that the indigenous Alaskan oil-degrading populations were performing most, if not all, of the biodegradative activity. The organisms present in products E and G did not appear to contribute significantly to such activity. This suggests that a co-metabolite, a nutrient, or some other unknown factor exists in these two products and stimulates the indigenous microorganisms to degrade the crude oil constituents at faster rates than is possible with simple nutrient addition. Further work needs to be done to define the enhancement factor(s) in these products.

Correlations have not yet been made between weathered crude oil degradation and oxygen uptake, nor have carbon balances been performed. Work is being planned to measure carbonaceous metabolic end products, CO_2 production, and total biomass yield, and then to correlate this information with the oxygen consumption data. If such correlations can be established, then use of oxygen consumption data for estimating biodegradation efficacy as part of a screening protocol will

be made possible. The respirometric technique requires much less effort than conventional shake flask studies because data gathering is automated and computerized, and it is not necessary to collect samples manually during the course of a biodegradation experiment. All that is required, assuming the proper correlations have been established, is the careful measurement of initial substrate and biomass values followed by the measurement of the residual soluble product value at the plateau of the uptake curve (Grady et al., 1989). From the analysis of this information, treatment decisions can be facilitated.

It appears from all the available evidence that the indigenous Alaskan microorganisms were primarily responsible for the biodegradation in the closed flasks and respirometer vessels, and that any enhancement provided by products E and G might have been due simply to metabolites, nutrients, or co-substrates fortuitously present in the product. The NETAC panel reviewed the results of the tests and agreed with the recommendation for further testing of two products that exceeded the performance of inorganic nutrient addition. Results from all three lines of evidence (respirometry, microbiology, and oil chemistry), supported the decision to only field test products E and G. The field testing of these two products at Disk Island in Prince William Sound is described below.

FIELD STUDIES

In addition to application of nutrients, bioremediation in the field may be enhanced by inoculation with allochthonous microorganisms. Cultures and cultural products have been added to different environments to stimulate biological removal of contaminants. Some of the investigations have demonstrated enhancement, while others have not (Leahy and Colwell, 1990). Lehtomaki and Niemela, 1975, found that addition of brewers' yeast to oil-contaminated soil enhanced oil removal by factors of 2- to 10-fold. This was most likely due to the supply of critical nutrients, vitamins, or cofactors naturally lacking in the soil. Christianson and Spraker (1982), reported a series of case histories of refinery wastewater treatment plants using commercial cultures to overcome various specific problems, such as foaming, toxic loads, low biomass, etc. Most success with biodegradation enhancement by allochthonous microbial cultures has been achieved when chemostats or fermentors were used to control conditions or reduce competition from indigenous microflora (Wong and Goldsmith, 1988).

COMMERCIAL PRODUCTS

The results of the field testing of the two selected products (E and G) are presented below. The objective was to determine if commercial microbiological products were able to enhance bioremediation of an oil-contaminated beach in Prince William Sound to a greater extent than that achievable by simple fertilizer application. The two companies that participated in the testing were Sybron, Inc. and ERI-Waste Microbes, Inc.

METHODS

Plot Description

A schematic representation of the experimental layout is depicted in Figure 13.8. The experiment was a randomized complete block design. Four beach segments ("blocks"), each 20 m wide (labeled 1 through 4) were staked out in the intertidal zone on Disk Island (DI-67a). Within each block were 4 treatment plots, labeled A through D, 2 m wide by 5 m long (top-to-bottom). The plots were separated by a 3 m buffer zone. Plot A was the no-treatment control; plot B was the nutrient-only treatment; plot C received nutrients plus Sybron's product; and plot D received nutrients plus ERI's product. The treatment plots within each block were randomly distributed.

Each plot was subdivided horizontally into three equal segments 2 m wide by 1.67 m long, as shown schematically in Figure 13.9. In each of the three segments, four bags made of fiberglass screening material were filled with approximately 750 to 1000 g of oiled gravel, buried approximately 5 to 10 cm below the surface, and covered with mixed sand and gravel. To obtain the gravel for each bag, oiled gravel from Disk Island was first sieved through a 25 mm coarse screen to remove large stones and then through a 4.75 mm sieve to remove the small sand granules that compact the beach material. The gravel was mixed manually with shovels and hoes in a large wooden container to achieve reasonable homogeneity with respect to oil contamination and rock size. The four bags corresponded to the four sampling events planned for the experiment. A surveyor's ribbon was attached to each bag for easy identification. The 12 samples within each block were numbered 1 through 4 in the top third, 5 through 8 in the middle third, and 9 through 12 in the bottom third.
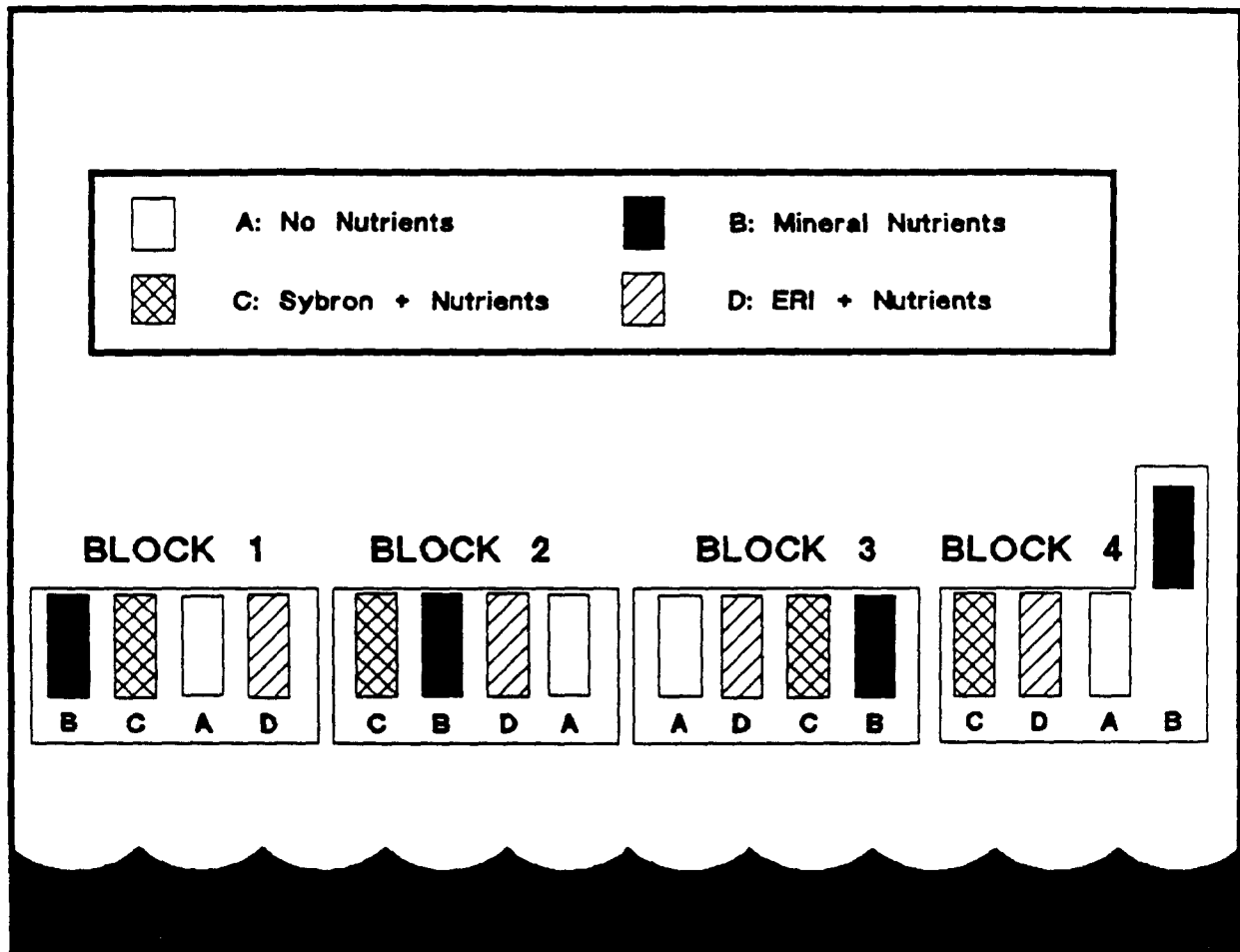
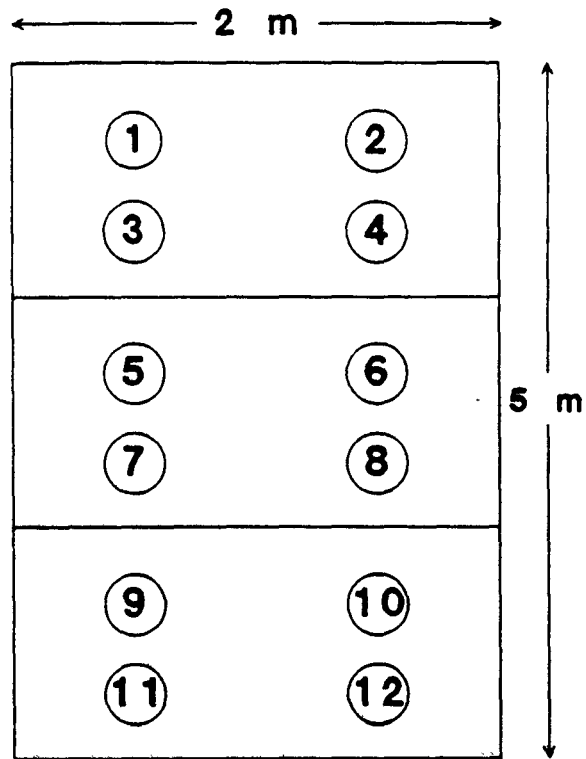**Figure 13.8. Schematic Diagram of the Experimental Plot Layout on Disk Island.**

Figure 13.9. Schematic Diagram of a Typical Beach Plot Showing Dimensions and Location of Sampling Bags.

Sampling

On a given sampling day, triplicate samples from each plot within a block were collected according to a random schedule. One sample was randomly taken from each of the three identical sectors of each plot. Some of the gravel was transferred into 500 mL I-Chem jars, labeled, and placed in a cooler to be carried back to Valdez for freezing and shipment via Federal Express to the analytical chemistry laboratory located in Pittsburgh, PA. The rest of the gravel was archived in aluminum foil and frozen. Thus, 48 samples were collected on each of the four sampling days, giving a total of 192 samples for the entire experiment.

Nutrients

To track the fate of the added nutrients with time, wells were installed in some of the plots for collecting subsurface water samples. Well points extending approximately 60 cm below the surface were driven into the center of the four control plots and the four plots receiving only nutrients. Subsurface water from these 8 wells, which served as samples for nutrient analysis, were collected according to a pre-determined sampling schedule (see below).

Nutrient Application

The source of nitrogen was ammonium nitrate. Each 2 m x 5 m plot received 200 g of N (20 g/m^2). At 35% N, the amount of NH_4NO_3 containing 200 g of N was 570 g or 1.25 lb per plot. This amount, less approximately 40 g to account for the N in the product containing the phosphate salt (see next paragraph), was added to 6 gallons of seawater and the contents stirred until dissolved. A 2-gallon plastic sprinkling can was filled with the solution and the entire contents poured onto the top third of a plot earmarked for nutrients. The sprinkling can was again filled and the contents poured onto the middle third. The procedure was repeated for the bottom third.

The source of phosphorus was an Ortho product named "Upstart," which had an N-P-K analysis of 3-10-3. At 10% P_2O_5, the amount of Upstart used was 450 g (1 lb) per plot. This corresponded to a phosphorus loading of 20 g P per plot (2 g P/m^2). The 450 g of Upstart was added to the 6 gallons of seawater above (after the NH_4NO_3 had been dissolved) before applying to each plot. Note that this product contained 3% N in the form of NH_4NO_3. The amount of N in Upstart had already been accounted for in the above 530 g computation of NH_4NO_3 needs.

COMMERCIAL PRODUCTS

Schedule

The experiment lasted only 27 days because severe Alaskan winter weather precludes field activities beyond the month of August. Day 0 occurred on Sunday, July 29, 1990. Nutrients and commercial products were applied on days 0, 4, 8, 12, 16, 20, and 24. One extra application (day 2), was used for an additional commercial product application, as specified by the two vendors. After nutrients and products had been delivered to the appropriate plots, randomly assigned triplicate sampling bags were removed from the plots for time 0 sediment chemistry and microbiology analysis. The other triplicate sampling bags were collected on days 9, 18, and 27. Nutrient sampling took place on days 1, 2, 3, 4, 17, 18, 19, and 20. This allowed determination of nutrient concentrations throughout the four-day interval between applications at two different times in the experiment.

Sediment Chemistry

All samples were analyzed for oil residue weight by methylene chloride extraction followed by evaporation to dryness and weighing on an analytical balance. After weighing, each sample was reconstituted with methylene chloride, passed through a silica gel fractionation column, and analyzed for the normal alkanes nC12 through nC34, plus the isoprenoid hydrocarbons pristane and phytane by gas chromatography using a flame ionization detector. The aliphatic fraction was eluted from the silica gel column with hexane prior to GC injection.

Microbiology

Sub-samples from the 8 plots of blocks 2 and 3 were analyzed for oil-degrading bacteria by standard plate count, using Bushnell-Haas medium supplemented with Prudhoe Bay crude oil as the carbon source. Only one of the three triplicates from those 8 plots was analyzed for microbial numbers. The plates were incubated at 15° C for 21 days and the colonies counted.

Data Analysis

The data were analyzed by analysis of variance and individual contrast methodology using SAS Software Release 6.06.

RESULTS

Persistence of Nutrients

Figures 13.10-13.12 summarize the average changes in nutrient levels over time in each block on Disk Island. Figure 13.10 shows the ammonia-N data, Figure 13.11 the nitrate-N data, and Figure 13.12 the phosphate-P data.

Persistence of ammonia-N was the most erratic. In block 1 the levels of NH_3-N in the nutrient-treated plot were measured at 1.1 and 4.0 mg/L one and two days after application, respectively, and in block 2 the NH_3-N was 1.7 mg/L one day after application. Little NH_3-N was measured in any of the control plots at any time except in block 4, where 0.1 mg/L was measured after one day and almost 1.0 mg/L after four days. The source of the high NH_3-N spike in the control plot of the fourth block may have been caused by carry-over of nutrients from the nutrient-treated plot onto the control plot. The nutrient-treated plot had to be placed above the control plot (see Figure 13.8) because of the presence of compacted peat on the extreme right end of the beach. There was a surface flow of water from a saltwater lagoon located approximately 50 m above the test area that flowed across the nutrient-treated plot onto the control plot. This stream was not noticed when the plots were first delineated. Although this explains the higher levels of NH_3-N measured one day after application, it does not explain why such a high spike was observed on the fourth day.

The nitrate and phosphate data indicate significant but decreasing levels of nutrients in the nutrient-treated plots as time progressed to four days after application (Figures 13.11 and 13.12). Again, high levels of NO_3-N and measurable levels of PO_4-P appeared in the control plot of the fourth block four days after application.

Numbers of Oil-Degrading Microorganisms

Oil-degrader counts in all plots of blocks 2 and 3 are shown in Figure 13.13. Although the levels of oil degraders were high in each of the plots, there were no significant changes or differences in any of the plots after 27 days of field testing.
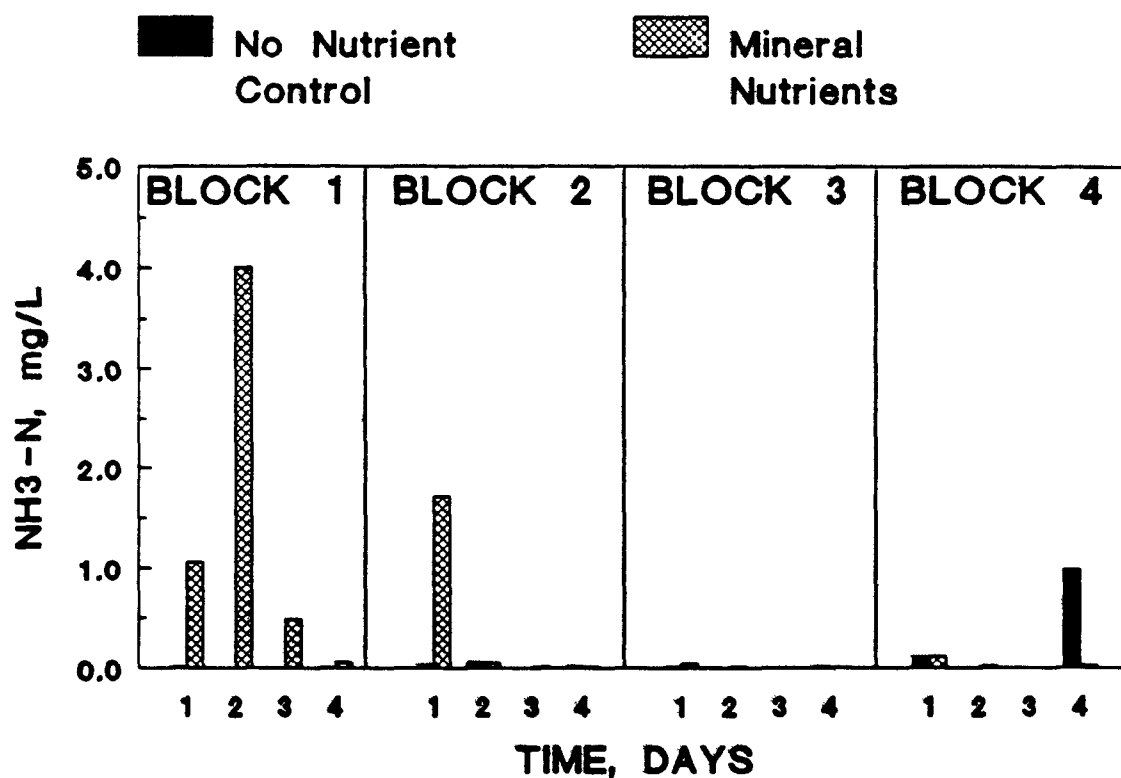
Figure 13.10. Average Changes in Ammonia-N Levels in the Four Days Between Fertilizer Applications.
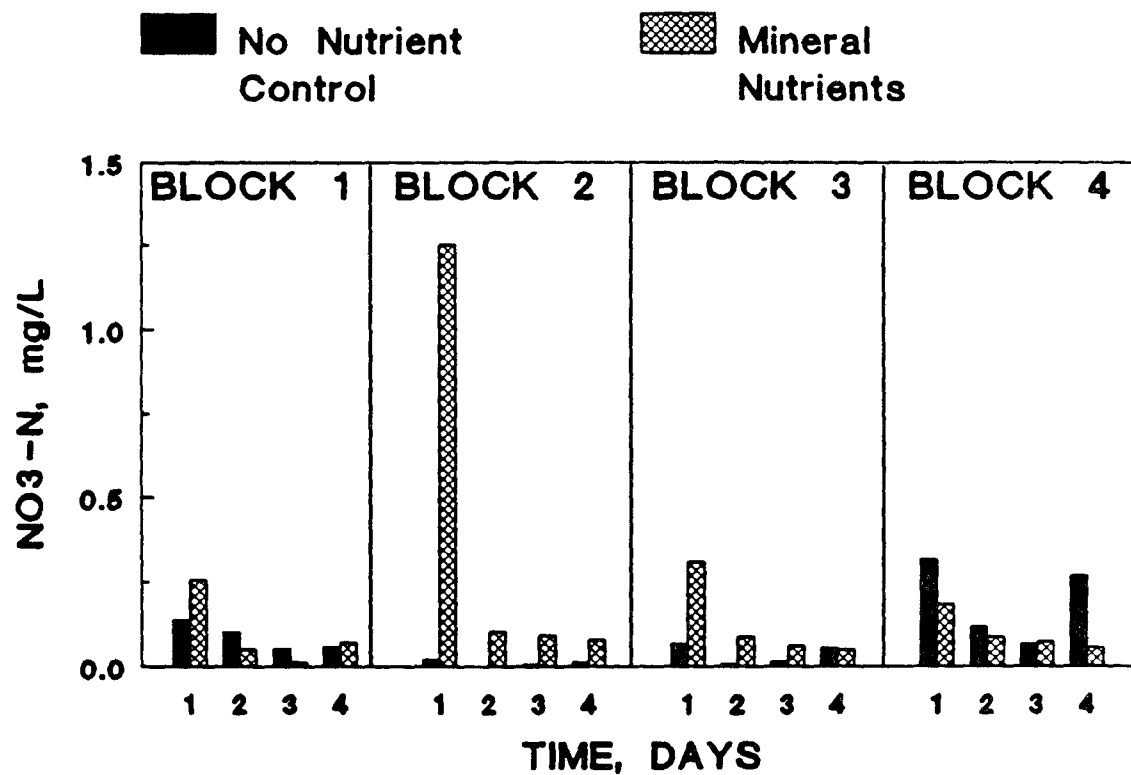
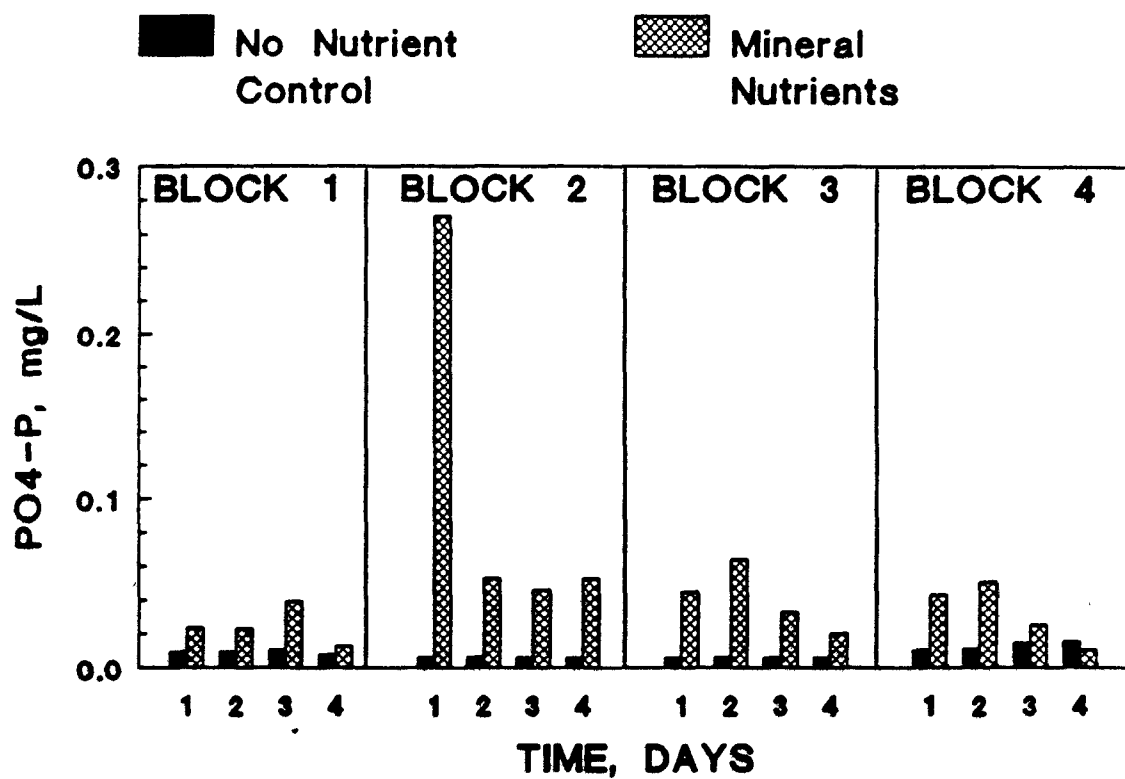Figure 13.11. Average Changes in Nitrate-N Levels in the Four Days Between Fertilizer Applications.

Figure 13.12. Average Changes In Phosphorus Levels In the Four Days Between Fertilizer Applications.
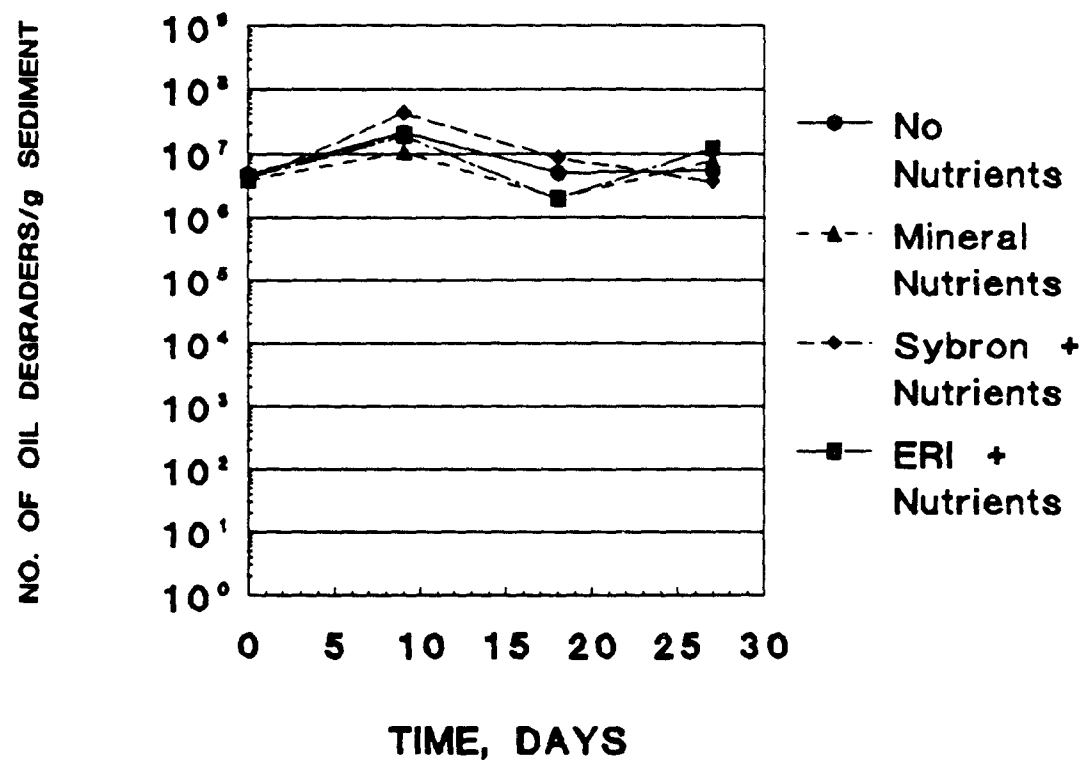
Figure 13.13. Oil-Degrader Counts in All Plots of Blocks 2 and 3 as a Function of Time.

Oil Residue Weight

Changes in oil residue weight, averaged over all four blocks, are summarized in Figure 13.14 as a function of time. The points on the connected curves are the mean residue weights for each of the four treatments, and the error bars depict one standard deviation unit above and below the means. These error bars represent the variation in oil residue weight among the four blocks and are indicative of the overall experimental error.

Visual inspection of the data from the plots treated with mineral nutrients alone and mineral nutrients supplemented with Sybron's product indicates a decrease in oil residue weight of approximately 33% at the end of the experimental period, compared with no net change in the no-nutrient control plot and a slight increase in the ERI plot. When the data were subjected to analysis of variance, however, there were no statistically significant differences among any of the four treatments at the 5% significance level. This was true even after the data were log transformed to stabilize the variance.

Note the broad error bars on Figure 13.14 at the day 0 sampling time compared with the other three sampling times. Despite the effort to control the heterogeneity of rock size and contamination by the sieving and mixing techniques, there was still substantial variation in oil residue weight between plots and between blocks at day 0. To ascertain the source of this variation, a breakout of plot oil residue weights by block was conducted. Results are shown in Figure 13.15.

Examination of these data reveals the differences in the distribution of oil between plots. Note that very little change took place in any of the treatments in blocks 2 and 4. Oil residue weights in the no-nutrient control plot and the nutrient-only plot of block 1 and the Sybron plot of block 2 appeared to decline markedly within the first 9 days and then level-off for the remainder of the experimental period. The oil residue weights in the ERI plots of blocks 1 and 3 actually showed an increase between days 18 and 27.

The error bars shown on this figure are the standard deviations of the triplicate samples within each plot and are indicative of the total sampling and analysis error. At day 0 the agreement of the triplicate samples averaged within each plot (Figure 13.15) was better than the agreement of identical plots averaged over blocks (Figure 13.14). This suggests that the cause of the variation among plots was consistent within each of the plots.
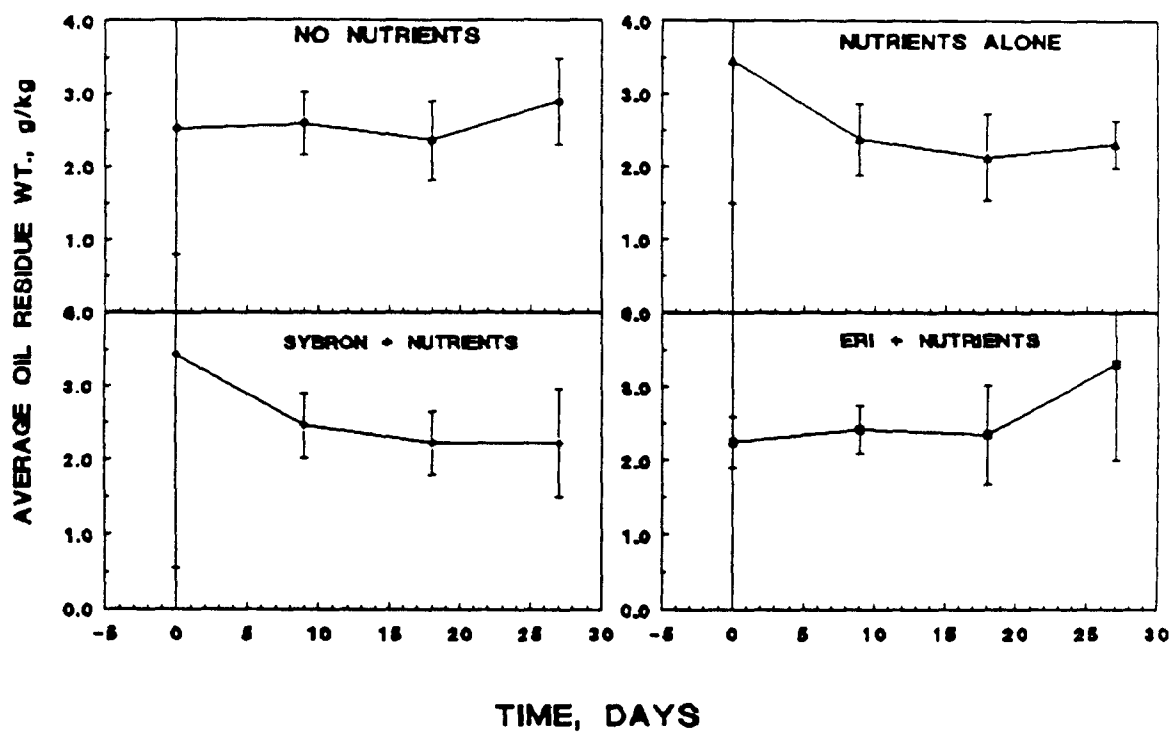
Figure 13.14. Changes in Oil Residue Weight Averaged Over All Four Blocks as a Function of Time.
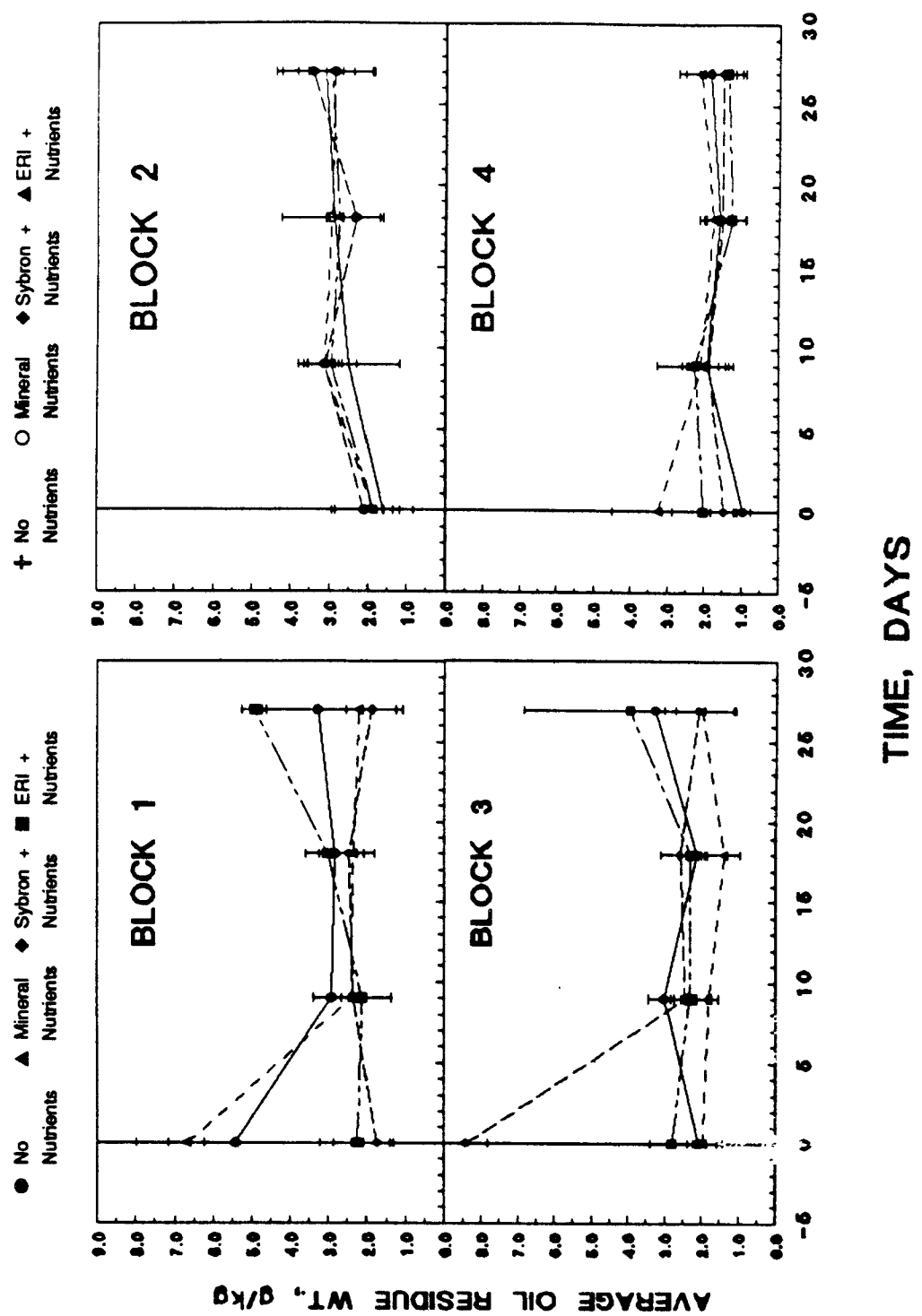
Figure 13.15. Changes in Oil Residue Weight in Each Block as a Function of Time.

Total Resolvable Alkanes

All samples were subjected to GC analysis to determine the changes in the aliphatic profiles of the oil among the various treatments. The concentrations of all the normal alkanes and the isoprenoid alkanes pristane and phytane resolvable by GC/FID were summed together for each treatment, averaged over all four blocks, and plotted as a function of time. The data with associated error bars are shown on Figure 13.16.

Except for the day 0 data, the error bars in Figure 13.16 were generally higher than the corresponding residue weight error bars (Figure 13.14). Although a downward trend in resolvable alkane concentrations was perceptible in all of the treatments after 27 days, the analysis of variance revealed no significant differences among the treatments (p<0.05). This agrees with the findings of no significance among treatments in the oil residue weight data.

Figure 13.17 was constructed to examine the behavior of the GC data in the individual plots within each block. A downward trend in alkane hydrocarbon levels is generally perceptible in the control, nutrient-only, and Sybron plots of blocks 1 to 3 and the ERI plot of block 1. Temporal changes in the alkane levels from the ERI plot of block 2 are highly variable, showing an increase at day 9 followed by decreases at days 18 and 27, while in block 3 increases are observed successively after day 9.

The error bars represent the sampling error associated with the triplicate samples in each plot. The error bars are higher overall than the corresponding oil residue weight data (Figure 13.15). The sampling errors associated with the GC data appear to be no better than the overall experimental error, which contrasts somewhat with the residue weight data.

Total Resolvable Alkanes as a Percent of the Residue Weight

The previous two figures depicted observed temporal changes in total resolvable alkanes normalized to sediment weight but not to the weight of the oil. Since there might have been significant differences in the extent of sediment contamination among samples, the measured resolvable alkanes were normalized to oil residue weight within each sample and the results plotted in Figures 13.18 and 13.19. Figure 13.18 shows the changes in the total resolvable alkanes as a percent of the residue weight, averaged over all four blocks for each treatment, and plotted as a function of

Figure 13.16.   Changes in Total Resolvable Alkanes Averaged Over All
Four Blocks as a Function of Time.

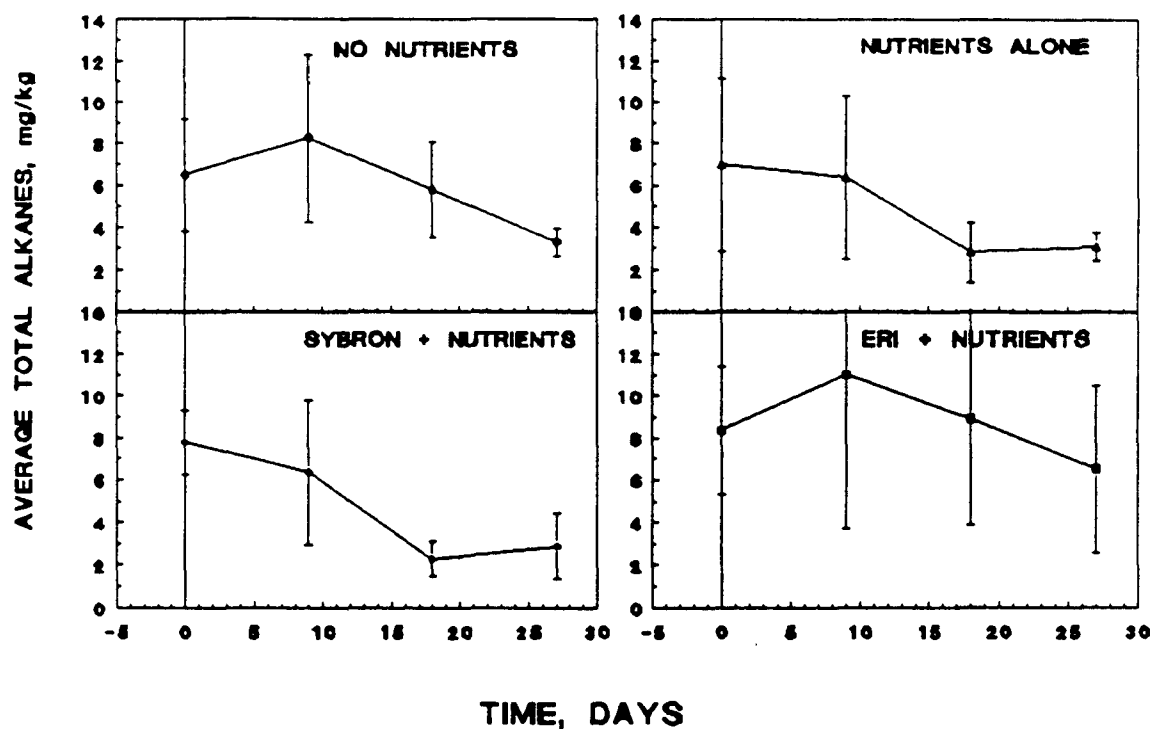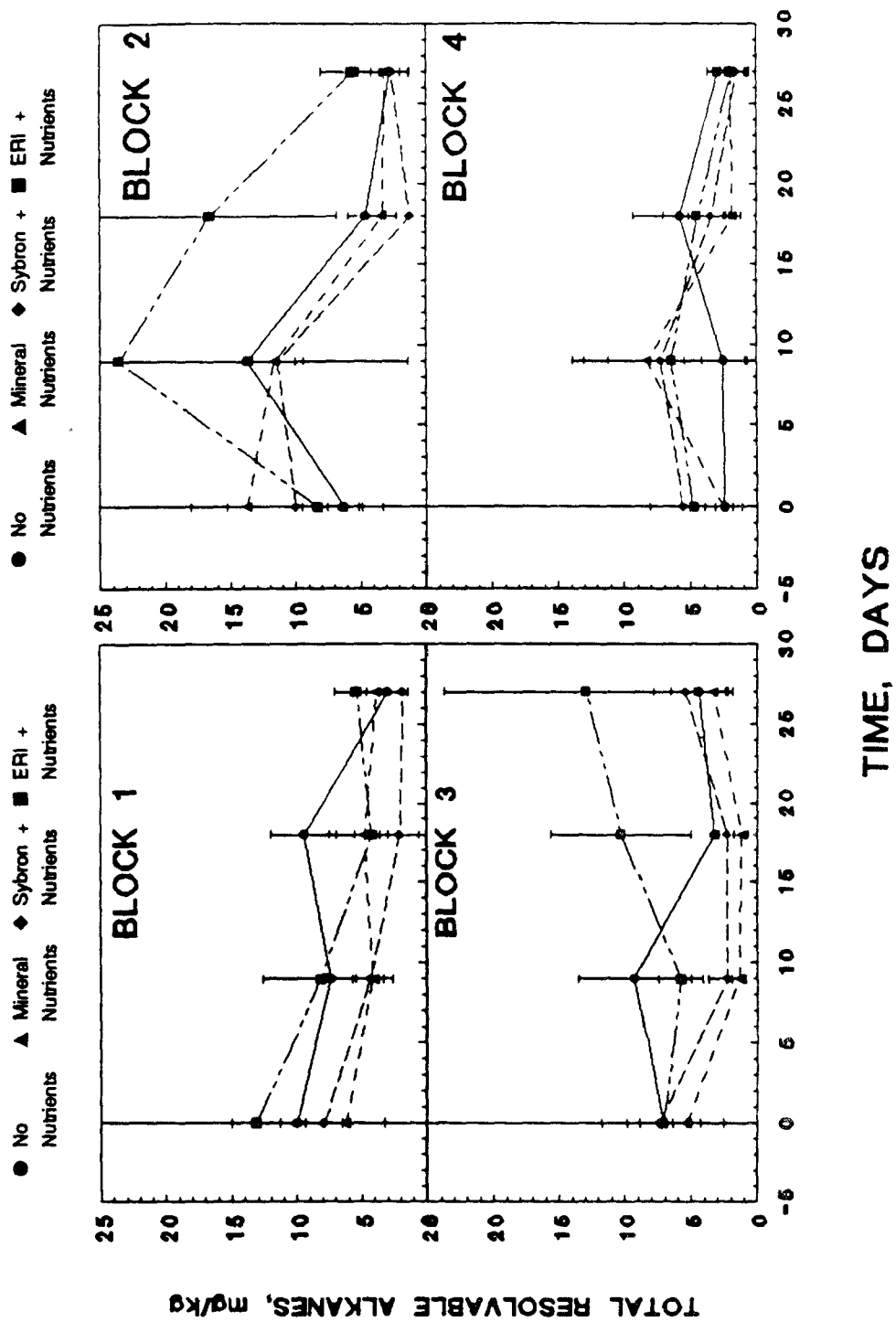**Figure 13.17.   Changes In Total Resolvable Alkanes In Each Block as a Function of Time.**
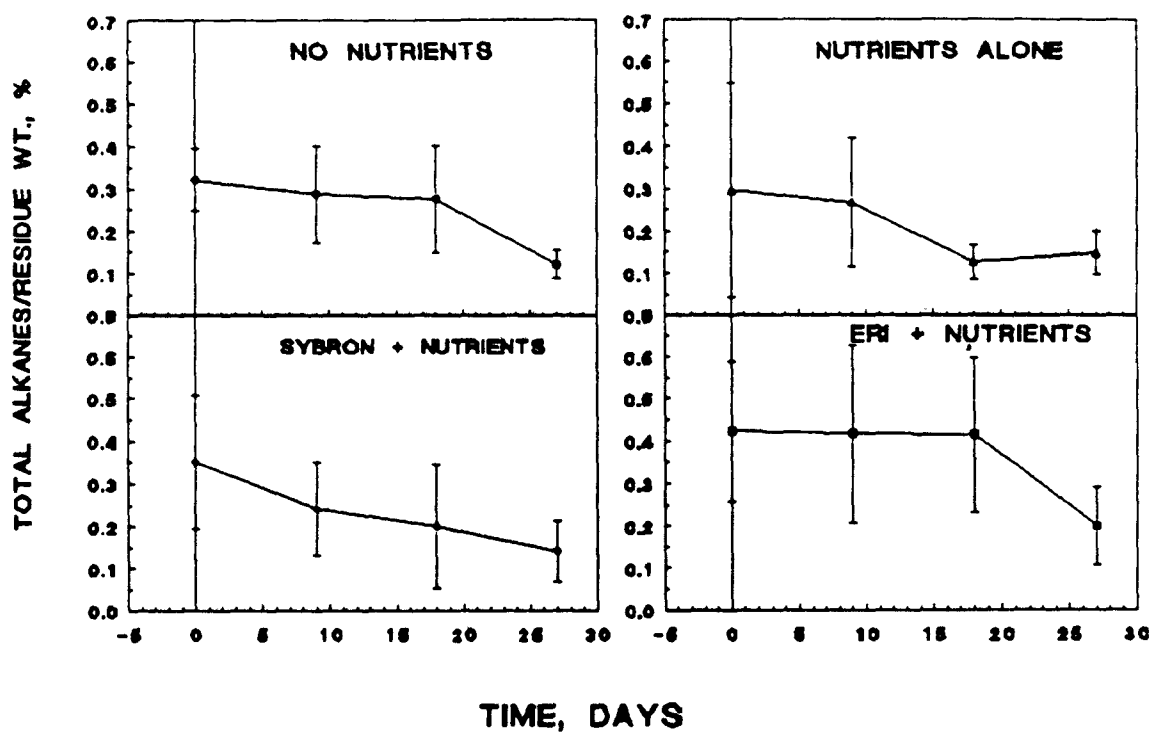
559

Figure 13.18. Changes in Total Resolvable Alkanes as a Percent of Oil Residue Weight Averaged Over All Four Blocks as a Function of Time.
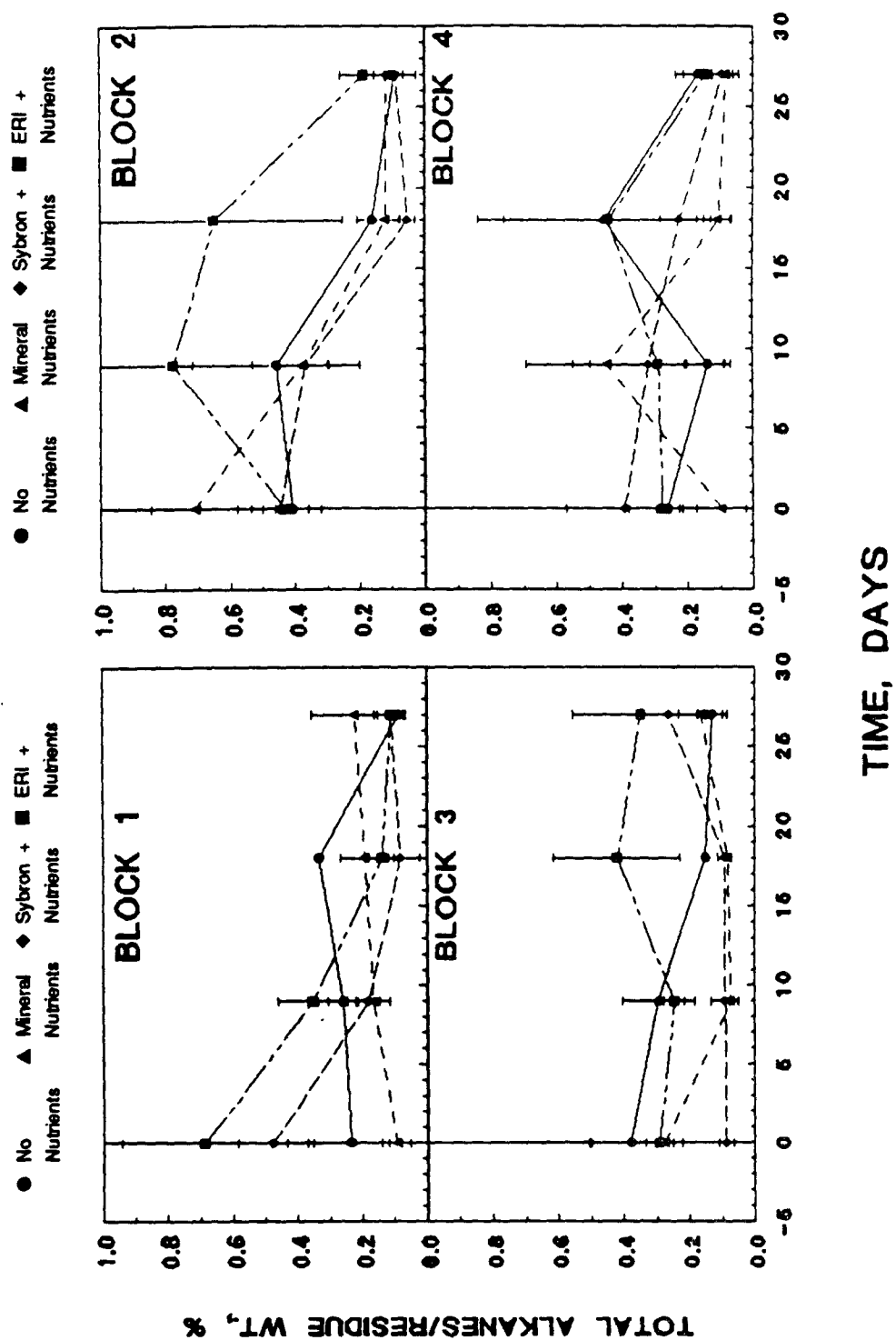
Figure 13.19.   Changes in Total Resolvable Alkanes as a Percent of Oil Residue Weight in Each Block as a Function of Time.

time. Figure 13.19 shows the changes within each block. Again, there were no significant differences among the four treatments. Note that the general behavior of the residue weight-normalized curves in Figures 13.18 and 13.19 is similar to the corresponding behavior of the sediment weight-normalized curves in Figures 13.16 and 13.17. This suggests that the contamination of the sediment samples was moderately homogeneous.

An important observation from Figure 13.18 is the magnitude of the total alkane/residue weight ratio. The total alkane hydrocarbons resolvable by GC/FID are less than 0.5% of the total oil residue weight. In other words, over 99.5% of the oil remaining on Disk Island 1.5 years after the spill is not resolvable by conventional gas chromatography. The compounds comprising this persistent fraction are likely the tars and asphaltines that degrade slowly with time.

SUMMARY AND CONCLUSIONS

The conclusions reached in this field study were based on three sources of information: nutrient persistence, microbiology, and sediment chemistry. The nutrient data clearly demonstrated that nitrogen and phosphorus persisted at measurably higher levels in the treated plots compared with the control plots throughout the four days between applications. These measurements were taken approximately 60 cm below the surface of the beach, suggesting that nutrients were in constant contact with the subsurface sediment layers for relatively long periods of time.

The microbiology data clearly demonstrated no net increase in oil-degrader populations in any of the plots after 27 days, and no differences among the four treatments at any time during the 27 day period. The oil-degrader populations were initially high and were maintained with or without the presence of excess nutrients. Either the oil-degraders were dormant or, more likely, they were sufficiently able to sustain their activity with the oligotrophic levels of nutrients present in the ambient environment.

Sediment chemistry revealed the most definitive information because it was the basis of the statistical analyses conducted. No significant differences were found among the four treatments at the 5% significance level either from the standpoint of oil residue weight, total resolvable alkane hydrocarbons, or total resolvable alkanes as a percent of oil residue weight. The relatively high variation observed in the day-0 residue weight data clearly indicates the necessity of replicating treatments when conducting field experiments.

One possible explanation for the apparent downward trend in residue weights and alkane hydrocarbons in both the control and treated plots is the perturbation of the beach material as a result of the sieving and mixing techniques prior to experimental initiation. Although day 0 occurred 2 days after the rocks were prepared and buried, that might not have been enough time for stabilization of the perturbed oil on the bagged rocks. Thus, the apparent disappearance of oil might have simply been mobilization due to tidal action.

Most of the readily biodegradable compounds in the aliphatic fraction of the oil had disappeared in the 1.5 years since the spill took place. This is the most likely explanation for the lack of any significant enhancement by either nutrient addition alone or nutrient addition supplemented with commercial microbial cultures. Further evidence supporting this conclusion derives from examining the n-alkane/isoprenoid alkane ratios. These ratios have been used in past literature to indicate extent of biodegradation; the lower the ratio, the more extensive the biodegradation. The average nC17/pristane and nC18/phytane ratios on day 0 for all the plots on Disk Island were 0.18 and 0.27, respectively. This compares to approximately 1.5 to 1.8 for unweathered Prudhoe Bay crude oil. Thus, the remaining oil present on Disk Island will likely degrade very slowly from now on because of the recalcitrant nature of the substrate. If either nutrient application or commercial inoculation can accelerate this rate, the time period must extend significantly beyond the 27 days allotted for this study or the trial must be conducted on beaches with fresher oil contamination.

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APPENDIX A
PROJECT ORGANIZATION AND RESPONSIBILITIES

The Oil Spill Bioremediation Project was directed by a team of scientists from EPA, with support from EPA laboratory contractors, the State of Alaska, the University of Alaska, and Exxon. The project was a team effort and personnel assisted where needed. C. Costa, Division Director at EPA/EMSL Las Vegas, and P.H. Pritchard, Branch Chief at EPA/ERL Gulf Breeze, shared overall responsibility for the project. C. Costa handled logistics, administration, and other nontechnical areas of project management; H. Pritchard served as the Principal Investigator with responsibility for the scientific aspects of the program. Visits from Headquarters personnel provided oversight of program operations, objectives, and management. Project staff were drawn from the following organizations:

- EPA ORD Headquarters in Washington, D.C.

- EPA Environmental Research Laboratories - Gulf Breeze, Florida; Athens, Georgia; and Ada, Oklahoma

- EPA Risk Reduction Engineering Laboratory - Cincinnati, Ohio

- EPA Environmental Monitoring Systems Laboratories - Las Vegas, Nevada, and Cincinnati, Ohio

- EPA Health Effects Research Laboratory - Research Triangle Park, North Carolina

- EPA Center for Environmental Research Information - Cincinnati, Ohio

- University of Alaska

- Alaska Department of Environmental Conservation

- Exxon Research and Engineering - Annandale, New Jersey

- Exxon Production Research - Houston, Texas

- Exxon Biomedical Services - East Millstone, New Jersey

The organizational structure for the Oil Spill Bioremediation Field Project is shown in Figures A.1 and A.2. Principal scientists and support staff for the project are listed in Table A.1.
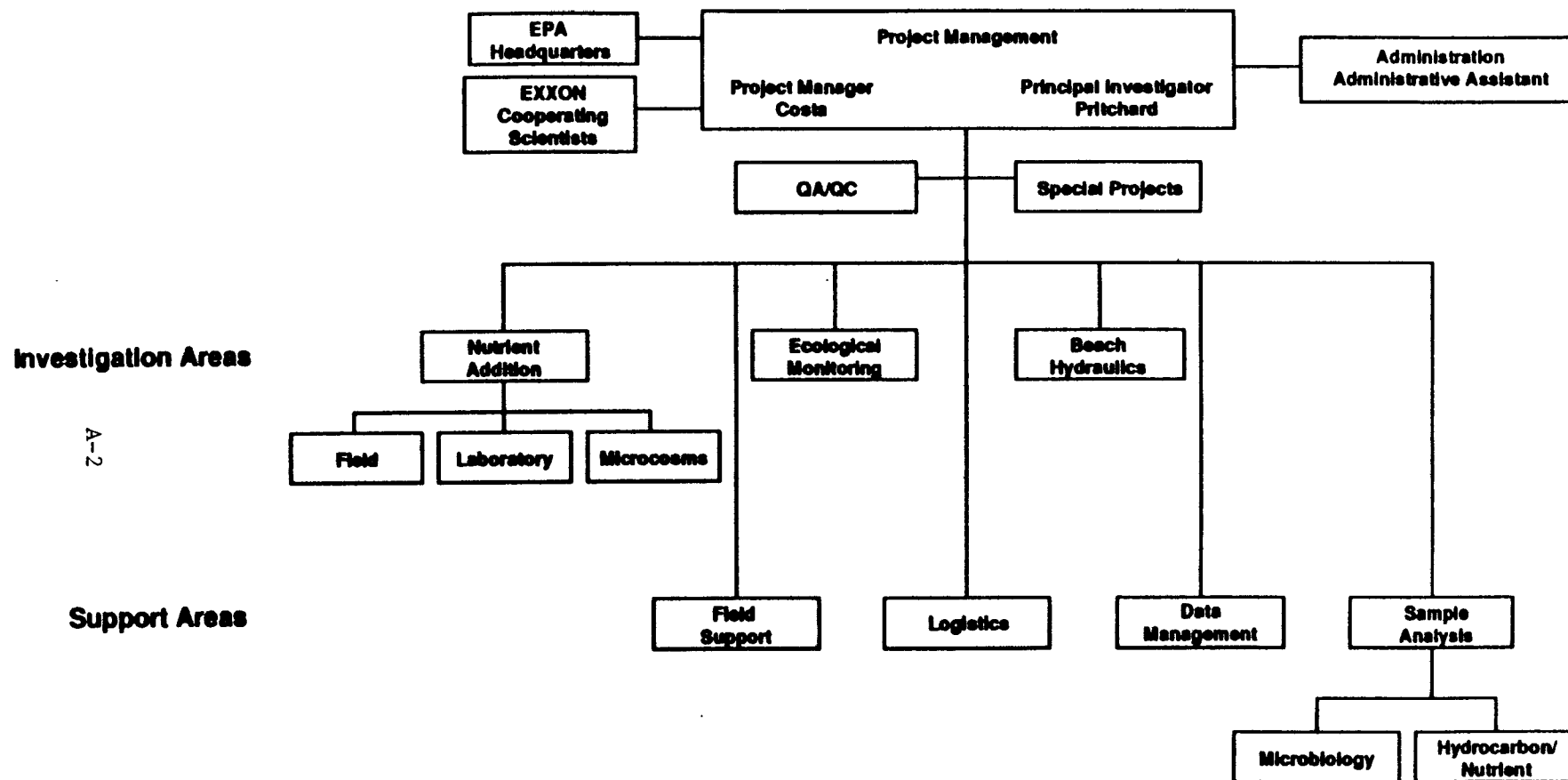
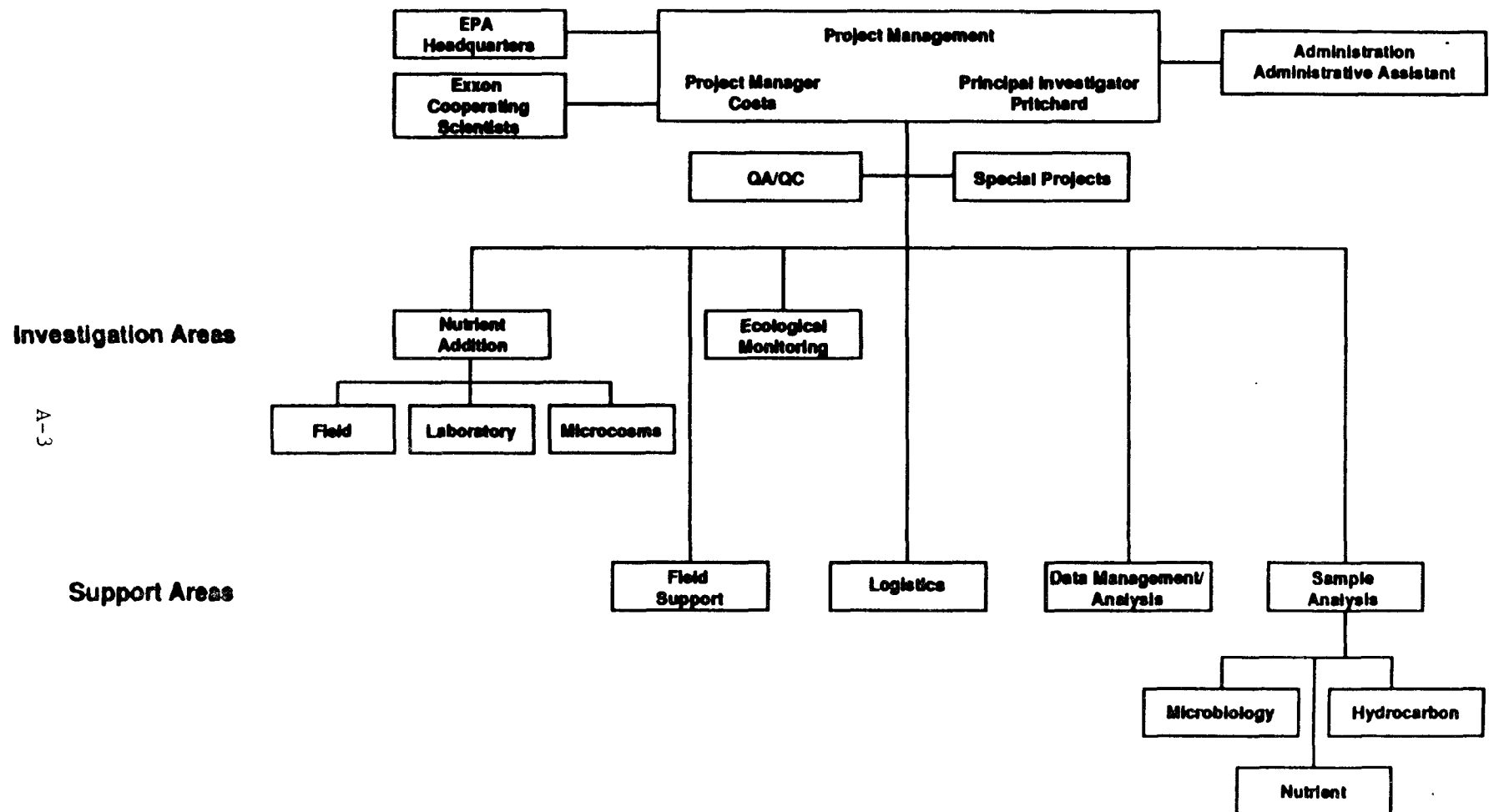**Figure A.1 Project Organization Chart for Summer 1989**

Figure A.2 Project Organization Chart for Summer 1990

TABLE A.1. EPA BIOREMEDIATION PROJECT STAFF

Responsibility	Name	Affiliation	Location
Project Management	H. Pritchard[1,2,3]	EPA/ERL	Gulf Breeze, FL
	C. Costa[1,2,3]	EPA/EMSL	Las Vegas, NV
EPA Headquarters	J. Skinner[1,2,3]	EPA/ORD	Washington, DC
	D. Valentinetti[1]	EPA/ORD	Washington, DC
	T. Baugh[2,3]	EPA/ORD	Washington, DC
Special Projects and Administration	E. Sullivan[1]	TRI	Rockville, MD
	E. Gray[1]	TRI	Seattle, WA
	K. Brown[3]	TRI	Rockville, MD
	S. Doherty[3]	TRI	Gulf Breeze, FL
	D. Rosenblatt[3]	TRI	Rockville, MD
	L. Suit[3]	TRI	Rockville, MD
	V. Furlong[1,2,3]	EPA/EMSL	Las Vegas, NV
	R. Shoemaker[1]	EPA/EMSL	Las Vegas, NV
	E. Clay[1]	EPA/EMSL	Las Vegas, NV
	W. Barlow[1]	EPA/EMSL	Las Vegas, NV
	T. Morton[1]	EPA/EMSL	Las Vegas, NV
Nutrient Addition	A. Venosa[1,2]	EPA/RREL	Cincinnati, OH
	J. Glaser[1]	EPA/RREL	Cincinnati, OH
	F. Kremer[1,2]	EPA/CERI	Cincinnati, OH
	J. Haines[1]	EPA/RREL	Cincinnati, OH
	E. Opatken[1]	EPA/RREL	Cincinnati, OH
	S. Safferman[1]	EPA/RREL	Cincinnati, OH
	A. Horowitz[1]	Indep. Consultant	Shaker Heights, OH
Ecological Monitoring	J. Clark[1,2,3]	EPA/ERL	Gulf Breeze, FL
	L. Claxton[1]	EPA/HERL	Res. Tri. Park, NC
	R. Parrish[1,3]	EPA/ERL	Gulf Breeze, FL
	R. Coffin[1,2,3]	TRI	Gulf Breeze, FL
	L. Cifuentes[1,2,3]	TRI/Texas A&M EPA/ERL	College Sta., TX
	J. Macauley[1]	TRI	Gulf Breeze, FL
	J. Hoff[1]	EPA/ERL	Gulf Breeze, FL
	J. Patrick[1]	EPA/ERL	Gulf Breeze, FL
	R. Stanley[1]	TRI	Gulf Breeze, FL
	G. Primrose[1]	TRI	Gulf Breeze, FL
	T. Heitmuller[1]	TRI	Gulf Breeze, FL
	B. Dorn[3]		Gulf Breeze, FL

[1]Participated during the Summer of 1989.
[2]Participated during the Winter of 1989/1990.
[3]Participated during the Summer of 1990.

TABLE A.1 (CONT'D)

Responsibility	Name	Affiliation	Location
QA/QC	D. Heggem[1,2,3]	EPA/EMSL	Las Vegas, NV
	M. Papp[1,3]	Lockheed	Las Vegas, NV
	L. Stetzenbach[1]	EPA/UNLV	Las Vegas, NV
	J. Pollard[1,3]	Lockheed	Las Vegas, NV
	W. Beckert[1]	EPA/EMSL	Las Vegas, NV
	D. Chaloud[3]	Lockheed	Las Vegas, NV
	T. Chiang[3]	Lockheed	Las Vegas, NV
Data Management/	W. Hom[1]	SAIC	San Diego, CA
Analysis	B. Damiata[1]	SAIC	Golden, CO
	B. Gerlach[2,3]	Lockheed	Las Vegas, NV
	J. Pollard[1]	Lockheed	Las Vegas, NV
	A. Neale[2,3]	Lockheed	Las Vegas, NV
	A. Borders[3]	EPA/EMSL	Las Vegas, NV
Field Support	J. Baker[1]	Lockheed	Las Vegas, NV
	W. Kinney[1]	EPA/EMSL	Las Vegas, NV
	F. Kremer[1]	EPA/CERI	Cincinnati, OH
	D. Miller[1]	EPA/ERL	Ada, OK
	R. Wright[1]	SAIC	San Diego, CA
	M. Dillon[1,2,3]	SAIC	Valdez, AK
	J. Wilson[1]	EPA/ERL	Ada, OK
	K. Cabble[3]	Lockheed	Las Vegas, NV
	E. Eschner[3]	Lockheed	Las Vegas, NV
	R. Dushek[3]	Lockheed	Las Vegas, NV
	G. Merritt[3]	Lockheed	Las Vegas, NV
	N. Halsell[3]	Lockheed	Las Vegas, NV
Logistics	B. Shokes[1]	SAIC	San Diego, CA
	K. Schmidt[1,2,3]	SAIC	San Diego, CA
	D. Peres[3]	Lockheed	Las Vegas, NV
	M. Sweeney[3]	Lockheed	Las Vegas, NV
Microbiology	J. Rogers[1]	EPA/ERL	Athens, GA
Analysis	R. Araujo[1,2,3]	EPA/ERL	Athens, GA
	S. Montgomery[1]	TRI	Gulf Breeze, FL
	C. Robinson[1]	TRI	Gulf Breeze, FL
	M. Shields[1]	TRI	Gulf Breeze, FL
	L. Bosworth[1]	TRI	Gulf Breeze, FL
	J. Mueller[1,2,3]	So. Bio.	Gulf Breeze, FL
	S. Baraket[1]	TRI	Gulf Breeze, FL
	S. Resnick[3]	TRI	Gulf Breeze, FL

[1]Participated during the Summer of 1989.
[2]Participated during the Winter of 1989/1990.
[3]Participated during the Summer of 1990.

TABLE A.1 (CONT'D)

Responsibility	Name	Affiliation	Location
Microbiology Analysis (Cont.)	E. Brown[3,a]	U. of AK	Fairbanks, AK
	G. Winter[3]	ADEC	Valdez, AK
	E. Pritchard[3]	U. of AK	Fairbanks, AK
	L. Pritchard[3]	U. of AK	Fairbanks, AK
	A. Rozich[3]	ERM	Exton, PA
	J. Kittigawa[3]	ADEC	Valdez, AK
	R. Cripe[1,2,3,a]	EPA/ERL	Gulf Breeze, FL
Microcosms	D. Dalton[3]	TRI	Gulf Breeze, FL
	S. Friedman[3]	TRI	Gulf Breeze, FL
	T. Mandeville[3]	TRI	Gulf Breeze, FL
	M. Shelton[3]	TRI	Gulf Breeze, FL
	J. Morin[3]	TRI	Gulf Breeze, FL
	J. Rogers[1,3]	EPA/ERL	Athens, GA
	M. Dillon[1,3]	Lockheed	Las Vegas, NV
Hydrocarbon/ Nutrient Analysis	J. Payne[1]	SAIC	San Diego, CA
	D. McNabb[1]	SAIC	San Diego, CA
	R. Sims[1,3]	SAIC	San Diego, CA
	M. McCabe[1]	SAIC	San Diego, CA
	J. Evans[1]	SAIC	San Diego, CA
	J. Stiefvater[1,3]	SAIC	San Diego, CA
	E. Smiley[1]	SAIC	Anchorage, AK
	J. Lopez[1]	SAIC	San Diego, CA
	T. Fogg[1]	SAIC	San Diego, CA
	M. Hart[1,3]	SAIC	San Diego, CA
	M. Peters[3]	SAIC	San Diego, CA
	C. Timm[3]	SAIC	San Diego, CA
	J. Clayton[1,3]	SAIC	San Diego, CA
	G. Salata[1,3]	SAIC	San Diego, CA
	W. Hom[1,3]	SAIC	San Diego, CA
Exxon Cooperating Scientists	R. Prince[3,a]	Exxon	Annandale, NJ
	R. Atlas[3]	U. Ky.	Lexington, KY
	D. Elmendorf[3,a]	Exxon	Annandale, NJ
	R. Bare[3,a]	Exxon	Annandale, NJ
	M. Grossman[3]	Exxon	Annandale, NJ
	J. O'Bara[3]	Exxon	Florham Pk, NY
	R. Chianelli[1,2,3]	Exxon	Annandale, NJ
	S. Hinton[1,a]	Exxon	Annandale, NJ
	J. Wilkinson[2,3]	Exxon	Anchorage, AK

[1]Participated during the Summer of 1989.
[2]Participated during the Winter of 1989/1990.
[3]Participated during the Summer of 1990.
[a]Oil biodegradation studies.

ON-SITE ORGANIZATION AND RESPONSIBILITIES

On-site personnel provided the following support:

- The **special projects staff** handled helicopter and boat schedules, housing and transportation issues, travel, EPA timecards, weekly reports, office management and support, and equipment deposition.

- The **nutrient addition staff** was responsible for nutrient application and monitoring for Snug Harbor in 1989. This involved staking plots, loading of briquettes into bags, excavation of monitoring wells, and monitoring of well samples.

- The **ecological monitoring staff** collected water and biological samples for ecological effects testing and prepared the samples for shipment. Water samples were collected from the field experiment beaches and Prince William Sound. Biological samples, including aquatic vegetation and fauna, were collected from the experimental beaches, identified, and frozen for analyses.

- The **QA/QC staff** designed the QA/QC program, prepared the QA Plans, monitored QA/QC data, resolved data quality issues, and prepared an assessment of overall program data quality. The QA Plans provided the written documentation of the QA program. QA staff monitored data quality and performed informal, internal audits of program operations.

- The **data management/analysis staff** was responsible for software development and testing, development and input of field data forms, sample tracking and archiving, and data analysis. Plots and statistical outputs of the data were generated by the staff as requested by the Principal Investigator, QA staff, and program scientists.

- The **field crew** set up beaches for field experiments, collected field samples and data, and removed all installed equipment following completion of an experiment. Activities included staking plots, preparing oiled homogenates and sample baskets, installing monitoring wells, and applying fertilizer.

- The **logistics staff** was responsible for ordering and tracking all equipment and supplies for the program staff. An inventory of all equipment and supplies was maintained, and the staff oversaw removal and proper distribution of materials from Valdez at project completion in accordance with EPA regulations and guidelines.

- The **microbiology staff** performed field and laboratory-based experiments to measure numbers of oil degraders, and used biometers to monitor microbial activity. Analyses included MPN viable count, carbon dioxide, TOC, and radiolabeled carbon analyses. The staff also input data and prepared plots for data analysis and interpretation.

- The **microcosm staff** conducted experiments using three types of microcosms: jar, tank, and column. These systems used oiled beach material from Prince William Sound. The jar and column microcosm studies were performed in a laboratory in Valdez, while the tank microcosm study was performed aboard the motor vessel AUGUSTINE in 1989.

● The analytical laboratory conducted nutrient and oil chemistry analyses, performed QC checks, and prepared data for transfer to the data base management system. Additional responsibilities included transfer of samples to the sample archive, and set-up and removal of the laboratory.

● The cooperating scientists from Exxon provided a linkage between EPA and Exxon dealing with scientific issues, experimental designs, data analysis and interpretation, and logistics. Individuals were also located in Valdez to perform their experiments and provide scientific expertise and assistance to the Exxon/EPA/ADEC monitoring program.

CHRONOLOGY OF EVENTS

An overview of the events that took place since the initiation of the oil spill project is presented.

MAY 1989

15 • EPA command center for the Bioremediation Project established in Valdez.
16 • Investigation of potential fertilizers for field tests initiated.
19 • Snug Harbor selected as the initial test site.
   • Efforts initiated to establish test plots within the beaches and set up a staging operation.
   • Fishing vessel AUGUSTINE available for use.
20 • Studies on the movement of groundwater and nutrients in the beaches initiated.
23 • Methods for adequately sampling contaminated beach material developed, a sampling design established, and methods for fertilizer application to the beaches finalized.
25 • Snug Harbor background data on the extent of oil contamination on the test beaches collected.

JUNE 1989

2 • Collection of background ecological monitoring data from Snug Harbor initiated.
4 • Microcosm design and construction initiated the first week of June (placed aboard the AUGUSTINE).
   • Due to the length of time to establish a microbiology laboratory, only one type of method established to measure the number of oil degraders.
8 • Following delays due to complications with logistics and weather, nutrient field application began. Stable isotope study initiated.
12 • A major rainstorm washed much of the oleophilic fertilizer off the beaches, so it was reapplied.
15 • A workshop on "Beach and Nearshore Hydraulics" was held in Seattle, Washington. (See Appendix B)
20 • Visual loss of oil from rock surfaces of the cobblestone beaches treated with oleophilic fertilizer apparent.

JULY 1989

1 • First status report on the field demonstration project submitted to Exxon.
18 • A recommendation for large-scale application of fertilizers to beaches in PWS submitted to Exxon.
24 • Passage Cove selected for additional nutrient and beach hydraulic investigations.
25 • Application of INIPOL and slow-release CUSTOMBLEN granules to Tern Beach at Passage Cove.
27 • Sampling wells for beach hydraulic study installed on Kittiwake Beach.

APPENDIX B

AUGUST 1989

2 • Began operation of sprinkling system on Kittiwake Beach at Passage Cove.
10 • Passage Cove beaches treated with oleophilic and slow-release fertilizer visually showed marked disappearance of oil relative to the reference beaches.
18 • Disappearance of oil evident from the Passage Cove beach treated with fertilizer solution from a sprinkler system.

SEPTEMBER 1989

5 • Final sampling at Passage Cove.
12 • Final sampling at Snug Harbor.
15 • Addendum to the July 1 Status Report submitted to Exxon.

NOVEMBER 1989

8 • Workshop held in Gulf Breeze, FL, to discuss data analysis, interpretations, and significant findings.
12 • Winter sampling on Passage Cove test beaches.

DECEMBER 1989

13 • Workshop held in Washington, D.C. to develop winter research plan for oil bioremediation.

APRIL 1990

• EPA, Exxon, NOAA, UAF, and ADEC scientists met to develop strategies for the summer research.
• Joint monitoring program conceived and developed.
• Research plan for testing the effects of fertilizer concentrations and fertilizer applications developed by EPA scientists.

MAY 1990

22 • Peer scientists met in Anchorage to review research plans; plan revised and submitted to Exxon.

JUNE 1990

• Sampling baskets constructed and tested on Disk Island.
• Test plots on Disk Island for fertilizer-specific activity established.
• A small test plot with monitoring wells established at Snug Harbor to assess scaling effects of fertilizer granule application.
11 • Elrington Island beach selected for testing fertilizer
to solution application through a sprinkler system.
15 • Oil chemistry analysis laboratory established at Prince William Sound Community College; microbiology laboratory established at ADEC lab.
18 • Experimental plots and well points installed at Disk Island, and also at Snug Harbor for
to scaling experiment.
22 • Motor vessel INSPECTOR made available by Exxon for research support.

20 • Sampling baskets prepared and installed at Disk Island.
23 • CUSTOMBLEN fertilizer applied at Disk Island and monitoring of nutrient concentrations initiated.
30 • Sampling baskets prepared and installed on the Bath beach at Elrington Island.

JULY 1990

1 • Sampling baskets prepared and installed on the Sprinkler beach at Elrington Island.
  • Fertilizer applied on the Bath beach.
2 • Sampling baskets prepared and installed on the Control beach at Elrington Island.
  • Initial run of the sprinkler system conducted at the Sprinkler beach.
9 • QA/QC audits of field and laboratory operation conducted.
15 • Alaska Department of Natural Resources barred beach access to Elrington Island pending resolution of the site permit.
20 • Site permit problem for Elrington Island resolved.
21 • Fertilizer application on the Sprinkler beach resumed. Experiment to detect nutrient enrichment initiated.
23 • EPA Administrator William Reilly visited various beach sites in Prince William Sound.
27 • Field activities completed for the Disk Island fertilizer application rate study.
30 • Ecological monitoring initiated on Elrington Island.

AUGUST 1990

6 • External QA audit conducted.
to • Meeting held with representatives of Exxon to discuss results of the Disk Island and
10 Elrington Island experiments.
7 • Final sampling (basket removal) and removal of all remaining field items at Elrington Island.
13 • Samples upon which to base winter sampling collected at Elrington Island along a transect from the three treatment areas.

DECEMBER 1990

12 • Winter sampling at Elrington.

FEBRUARY 1991

19 • Meeting with EPA, Exxon, ADEC, U. of Alaska held in Las Vegas, NV to discuss results
to of the oil spill project and plan for the summer of 1991.
21