

**INTERIM REPORT**  
**OIL SPILL BIOREMEDIATION PROJECT**

**Prepared by**

**U.S. Environmental Protection Agency**  
**Office of Research and Development**

**February 28, 1990**

INTERIM REPORT  
OIL SPILL BIOREMEDIATION PROJECT

P.H. Pritchard, Ph.D.; R. Araujo, Ph.D.; J.R. Clark, Ph.D.;  
L.D. Claxton, Ph.D.; R.B. Coffin, Ph.D.; C.F. Costa;  
J.A. Glaser, Ph.D.; J.R. Haines, Ph.D.; D.T. Heggem;  
F.V. Kremer, Ph.D.; S.C. McCutcheon, Ph.D., P.E.;  
J.E. Rogers, Ph.D.; A.D. Venosa, Ph.D.

U.S. Environmental Protection Agency  
Office of Research and Development



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

MAR 15 1980

OFFICE OF  
RESEARCH AND DEVELOPMENT

Rear Admiral David Ciancaglini  
Federal On Scene Coordinator  
United States Coast Guard  
Key Bank Building  
601 West 5th Avenue, Suite 500  
Anchorage, Alaska 99501

Dear Admiral Ciancaglini:

At the Alaska bioremediation planning meeting in February, Captain Dave Zawadzki of your organization requested that the Environmental Protection Agency (EPA) define its position on bioremediation. Enclosed please find, Interim Report: Oil Spill Bioremediation Project, which summarizes our conclusions to date.

Based upon visual observations of our demonstration plots and analyses of the field and laboratory data from both EPA and Exxon efforts, we conclude that the application of nitrogen and phosphorus fertilizers enhances biodegradation of oil from the contaminated beaches. The absence of adverse ecological effects observed from fertilizer application further supports bioremediation as a feasible clean-up procedure.

In summation, our findings indicate that fertilizer addition to enhance biodegradation of oil is effective and environmentally safe. Should you have any questions or comments regarding bioremediation, please address them to Dr. John H. Skinner, Acting Deputy Assistant Administrator for Research and Development, at (202) 382-7676.

Sincerely yours,

*Erich W. Bretthauer*  
Erich W. Bretthauer  
Assistant Administrator  
for Research and Development

Enclosure

## CONTENTS

	Page
Executive Summary . . . . .	xv
Acknowledgment . . . . .	xix
1. Introduction . . . . .	1
2. Background . . . . .	5
3. Overview of Activities . . . . .	11
Organization . . . . .	11
Chronology of Events . . . . .	15
4. Site Selection and Characteristics . . . . .	19
Snug Harbor . . . . .	19
Passage Cove . . . . .	21
5. Fertilizer Selection and Characteristics . . . . .	27
Background . . . . .	27
Selected Fertilizer Formulations . . . . .	27
Nutrient Release Characteristics -	
Methods . . . . .	31
Static Tests . . . . .	31
Intermittent Submersion Tests . . . . .	31
Field Tests . . . . .	32
Nutrient Release Characteristics - Test	
Results . . . . .	32
Woodace IBDU Briquettes . . . . .	32
IBDU Granules . . . . .	35
Osmocote Briquettes . . . . .	39
MAGAMP Briquettes . . . . .	39
Sierra Chemical Granules . . . . .	43
Inipol EAP 22 . . . . .	43
Discussion and Conclusions . . . . .	48
6. Field Test Design and Methods . . . . .	51
Test Plot Sampling Design . . . . .	51
Sampling Procedure . . . . .	51
Sampling Method . . . . .	53
Fertilizer Application . . . . .	55
Slow-Release Water-Soluble	
Fertilizers . . . . .	55
Oleophilic Fertilizer . . . . .	60
Sprinkler System . . . . .	61
Analytical Procedures . . . . .	61
Oil Chemistry . . . . .	61
Nutrient Analysis . . . . .	63
Microbiological Analysis . . . . .	64



Ecological Monitoring . . . . .	65
Caged Mussels . . . . .	67
Field Toxicity Tests . . . . .	67
7. Field Test Results - Snug Harbor . . . . .	69
Visual Observations . . . . .	69
Nutrient Concentrations . . . . .	70
Changes in Oil Residue Weight and Composition . . . . .	77
Microbiology . . . . .	109
Ecological Monitoring . . . . .	111
Nutrients . . . . .	111
Chlorophyll Analyses . . . . .	111
Phytoplankton Primary Production . . . . .	117
Bacterial Abundance . . . . .	117
Bacterial Productivity . . . . .	117
Microflagellate Abundance . . . . .	121
Dissolved Organic Carbon, Particulate Carbon, Particulate Nitrogen . . . . .	121
Stable Isotope Ratios of Carbon and Nitrogen . . . . .	121
Caged Mussels . . . . .	121
Discussion and Conclusions . . . . .	124
8. Field Test Results - Passage Cove . . . . .	127
Visual Observations . . . . .	127
Nutrient Concentrations . . . . .	127
Changes in Oil Residue Weight and Composition . . . . .	127
Microbiology . . . . .	139
Ecological Monitoring . . . . .	142
Nutrients . . . . .	142
Chlorophyll Analyses . . . . .	142
Phytoplankton Primary Production . . . . .	142
Bacterial Abundance . . . . .	146
Bacterial Productivity . . . . .	146
Caged Mussels . . . . .	146
Field Toxicity Tests of Oleophilic Fertilizer at Passage Cove . . . . .	146
Discussion and Conclusions . . . . .	154
9. Supporting Studies . . . . .	155
Microcosms . . . . .	155
Background . . . . .	155
Methods . . . . .	155
Results . . . . .	157
Discussion and Conclusions . . . . .	158
Laboratory Biodegradation Screening Evaluation . . . . .	165
Background . . . . .	165
Methods . . . . .	165
Results . . . . .	166

Discussion and Conclusions . . . . .	168
Respirometric Analysis of Biodegradation .	175
Background . . . . .	175
Methods . . . . .	175
Results . . . . .	176
Discussion and Conclusions . . . . .	180
Mechanism of Action of Inipol-Enhanced Oil	
Degradation . . . . .	183
Background . . . . .	183
Methods . . . . .	183
Results . . . . .	185
Discussion and Conclusions . . . . .	185
Chemical Effect of Oleophilic Fertilizer .	188
Background . . . . .	188
Methods . . . . .	188
Results . . . . .	188
Discussion and Conclusions . . . . .	188
Toxicity of Oleophilic Fertilizer . . . . .	189
Background . . . . .	189
Methods . . . . .	189
Results . . . . .	191
Discussion and Conclusions . . . . .	191
Beach Hydraulics . . . . .	192
Background . . . . .	192
Methods . . . . .	192
Results . . . . .	193
Discussion and Conclusions . . . . .	221
Mutagenicity Tests . . . . .	222
Background . . . . .	222
Methods . . . . .	222
Results . . . . .	223
Discussion and Conclusions . . . . .	223
Reference . . . . .	224

## Tables

	Page
Table 2.1.    Calculated Ratios of C17/Pristane and C18/ Phytane . . . . .	10
Table 3.1.    EPA Bioremediation Project Staff . . . . .	13
Table 4.1.    Description of Demonstration Plots at Snug Harbor . . . . .	22
Table 4.2    Analysis of oil extracted from mixed sand and gravel samples taken from Otter Beach on May 28, 1989, two weeks prior to fertilizer application . . . . .	23
Table 4.3.    Description of Fertilizer Treatment Demonstration Plots at Passage Cove . . . . .	25
Table 5.1.    Inipol EAP 22 Chemical Composition . . . . .	30
Table 5.2.    Total Kjeldahl Nitrogen (TKN) Released From IBDU Granular Fertilizer in Static Water Conditions . . . . .	40
Table 5.3.    Release of Ammonia, Total Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP) from Inipol EAP 22 During Intermittent Submersion Experiment . . . . .	49
Table 7.1.    Ammonia Concentrations in Interstitial Water Samples . . . . .	71
Table 7.2.    Nitrate/Nitrite Concentrations in Interstitial Water Samples . . . . .	75
Table 7.3.    Relative Concentrations ( $\log_{10}$ of the Cell Numbers/g of Beach Material) of Oil Degrading Microorganisms in Snug Harbor Mixed Sand and Gravel Test Plots . . . . .	110
Table 7.4.    Ammonia Nitrogen ( $\mu\text{M N/l}$ ) from Nearshore Water Over Gravel Beaches at Snug Harbor. Mean of four replicates (standard deviation). (Method detection limit = $0.13 \mu\text{M N/l}$ .) . . . . .	112
Table 7.5.    Ammonia Nitrogen ( $\mu\text{M N/l}$ ) from Nearshore Water Over Cobble Beaches at Snug Harbor. Mean of four replicates (standard deviation). (Method detection limit = $0.13 \mu\text{M N/l}$ .) . . . . .	113

Table 7.6.	Phosphate ( $\mu\text{M P/l}$ ) From Nearshore Water Over Gravel Beaches at Snug Harbor. Mean of four replicates (standard deviation). (Method detection limit = $0.20 \mu\text{M P/l}$ for sample date 6/10/89, $0.02 \mu\text{M P/l}$ thereafter.) . . . . .	114
Table 7.7.	Phosphate ( $\mu\text{M/Pl}$ ) from Nearshore Water Over Cobble Beaches at Snug Harbor. Mean of four replicates (standard deviation). Method detection limit = $0.20 \mu\text{M P/l}$ for sample date 6/10/89, $0.02 \mu\text{M P/l}$ thereafter.) . . . . .	115
Table 8.1.	Relative Concentration ( $\text{Log}_{10}$ of the cell number/g of beach material) of oil-degrading microorganisms in Passage Cove . . . . .	140
Table 8.2.	Relative Concentration ( $\text{Log}_{10}$ of the cell number/g of beach material) of oil-degrading microorganisms in samples from Beaches that were not impacted by oil . . . . .	141
Table 8.3.	Larval Survival and Development After 48 hours in Salinity-Adjusted Prince William Sound Water .	152
Table 9.1.	Chemical Analysis of Mixed Sand and Gravel Microcosms Sampled 17 Days After Initiation of Fertilizer Application . . . . .	159
Table 9.2.	Residue Weight of Oil in Cobble Microcosms Analyzed 26 Days After Fertilizer Application . . . . .	160
Table 9.3.	Ratios of Hydrocarbons in Oil From Cobble Microcosms Analyzed 26 days After Fertilizer Application . . . . .	161
Table 9.4.	Comparison of C17/Pristane Ratios and C17/Norhopane Ratios as Measures of Oil Degradation in Samples Taken From Cobble Microcosm 42 Days After Initiation Of Fertilizer Application . . . . .	162
Table 9.5.	Use of Dibenzothiophene Peaks/Norhopane Ratios as Relative Measures of the Degradation of Aromatic Components in Oil Sampled From Cobble Microcosms 42 Days After Initiation of Fertilizer Application . . . . .	163
Table 9.6.	Experimental Design for Respirometric Studies . . . . .	177
Table 9.7.	Experimental Design of Flask Studies . . . . .	178

Table 9.8.	Experimental Design for Laboratory Microcosm Study . . . . .	184
Table 9.9.	Results of Laboratory Toxicity Tests with Oleophilic Fertilizer, Inipol EAP 22, and Various Marine Species. (Values are 96-hour LC50 estimates unless otherwise noted.) . . . . .	190
Table 9.10.	Passage Cove Beach Hydraulics: August 6, 1989; 4:30 a.m.; High Tide . . . . .	196
Table 9.11.	Passage Cove Beach Hydraulics: August 6, 1989; 7:30 a.m.; Falling Tide . . . . .	197
Table 9.12.	Passage Cove Beach Hydraulics: August 6, 1989; 10:00 a.m.; Low Tide . . . . .	198
Table 9.13.	Passage Cove Beach Hydraulics: August 6, 1989; 1:00 p.m.; Rising Tide . . . . .	199
Table 9.14.	Passage Cove Beach Hydraulics: August 6, 1989; 5:10-7:30 p.m.; High-Falling Tide . . . . .	200
Table 9.15.	Passage Cove Beach Hydraulics: August 6, 1989; 9:00 p.m.; Low Tide . . . . .	201
Table 9.16.	Passage Cove Beach Hydraulics: August 7, 1989; 6:00 a.m.; High Tide . . . . .	202
Table 9.17.	Passage Cove Beach Hydraulics: August 20, 1989; 7:20 a.m.; Falling Tide . . . . .	203
Table 9.18.	Passage Cove Beach Hydraulics: August 20, 1989; 10:00 a.m.; Low Tide . . . . .	204
Table 9.19.	Passage Cove Beach Hydraulics: August 20, 1989; 12:50 p.m.; Rising Tide . . . . .	205
Table 9.20.	Passage Cove Beach Hydraulics: August 20, 1989; 4:15 p.m.; High Tide . . . . .	206
Table 9.21.	Passage Cove Beach Hydraulics: August 20, 1989; 9:15 p.m.; Falling Tide . . . . .	207
Table 9.22.	Passage Cove Beach Hydraulics: August 21, 1989; 6:30 a.m.; Falling Tide . . . . .	208
Table 9.23.	Passage Cove Beach Hydraulics: August 21, 1989; 10:00 a.m.; Low Tide . . . . .	209
Table 9.24.	Passage Cove Beach Hydraulics: August 21, 1989; 12:00 noon; Rising Tide . . . . .	210



Table 9.25.	Passage Cove Beach Hydraulics: August 21, 1989; 3:45 p.m.; Rising Tide . . . . .	211
Table 9.26.	Passage Cove Beach Hydraulics: August 21, 1989; 5:00 p.m.; Falling Tide . . . . .	212
Table 9.27.	Passage Cove Beach Hydraulics: September 10, 1989; 12:20 noon; Falling Tide . . . . .	213
Table 9.28.	Passage Cove Beach Hydraulics: September 10, 1989; 3:10 p.m.; Low Tide . . . . .	214
Table 9.29.	Passage Cove Beach Hydraulics: September 10, 1989; 6:15 p.m.; Rising Tide . . . . .	215
Table 9.30.	Passage Cove Beach Hydraulics: September 10, 1989; 8:45 p.m.; Rising Tide . . . . .	216
Table 9.31.	Passage Cove Beach Hydraulics: September 11, 1989; 8:10 a.m.; Rising Tide . . . . .	217
Table 9.32.	Passage Cove Beach Hydraulics: September 11, 1989; 11:45 a.m.; High Tide . . . . .	218
Table 9.33.	Passage Cove Beach Hydraulics: September 11, 1989; 3:00 p.m.; Falling Tide . . . . .	219
Table 9.34.	Passage Cove Beach Hydraulics: September 11, 1989; 6:30 p.m.; Low Tide . . . . .	220

## Figures

	Page
Figure 2.1. Unfractionated Prudhoe Bay Crude Oil . . . . .	7
Figure 2.2. Prudhoe Bay Crude Oil, Aliphatic Fraction . . . . .	8
Figure 2.3. Prudhoe Bay Crude Oil, Aromatic Fraction . . . . .	9
Figure 3.1. Project Organization Chart . . . . .	12
Figure 4.1. Snug Harbor, Knight Island . . . . .	20
Figure 4.2. Passage Cove, Knight Island . . . . .	24
Figure 5.1. Cumulative Release Rate From IBDU Briquettes . . . . .	33
Figure 5.2. Daily Nutrient Release Rate From IBDU Briquettes (mg/l/day) . . . . .	34
Figure 5.3. Ammonia Release From IBDU Briquettes at 9 and 21 Degrees Centigrade in 3 Different Water Sources . . . . .	36
Figure 5.4. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) From IBDU Fertilizer Granules Contained in Bags . . . . .	37
Figure 5.5. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) From IBDU Granules in Static Flask Experiments . . . . .	38
Figure 5.6. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from Osmocote Briquettes in Static Flask Experiments . . . . .	41
Figure 5.7. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from MAGAMP Briquettes in Static Flask Experiments . . . . .	42
Figure 5.8. Sampling Point Location for Magnesium Ammonia Phosphate Fertilizer Field Test . . . . .	44
Figure 5.9. Magnesium Ammonium Phosphate Fertilizer Test . . . . .	45
Figure 5.10. Cumulative Release of Ammonia and Nitrate from Sierra Chemical Granules in Static Flask Experiments . . . . .	46

Figure 5.11.	Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) from Inipol EAP 22 in Static Flask Experiments . . . . .	47
Figure 6.1.	Placement of the Bags of Fertilizer Briquettes on Otter and Seal Beaches . . . . .	56
Figure 6.2.	Repositioning of the Bags of Fertilizer Briquettes on Otter and Seal Beaches . . . . .	57
Figure 6.3.	Tidal Fluctuations for High Tides, Snug Harbor, June 6-30, 1989 . . . . .	58
Figure 6.4.	Tidal Fluctuations for Low Tides, Snug Harbor, June 6-30, 1989 . . . . .	59
Figure 7.1.	Mean Residue Concentration at Snug Harbor Mixed Sand and Gravel Plots, All Zones . . . . .	78
Figure 7.2.	Mean Residue Concentration at Snug Harbor Mixed Sand and Gravel Plots, Mid and Low Tide Zones . . . . .	79
Figure 7.3.	Mean Residue Concentration at Snug Harbor Cobble Plots, All Zones . . . . .	80
Figure 7.4.	Median Residue Concentration at Snug Harbor Cobble Plots . . . . .	81
Figure 7.5.	Mean C17/Pristane Ratio at Snug Harbor Mixed Sand and Gravel Plots, All Zones . . . . .	83
Figure 7.6.	Mean C17/Pristane Ratio at Snug Harbor Cobble Plots, Top, All Zones . . . . .	84
Figure 7.7.	Mean C17/Pristane Ratio at Snug Harbor Cobble Plots, Bottom, All Zones . . . . .	85
Figure 7.8.	Mean C18/Phythane Ratio at Snug Harbor Mixed Sand and Gravel Plots, All Zones . . . . .	86
Figure 7.9.	Mean C18/Phytane Ratio at Snug Harbor Cobble Plots, Top, All Zones . . . . .	87
Figure 7.10.	Mean C18/Phytane Ratio at Snug Harbor Cobble Plots, Bottom, All Zones . . . . .	88
Figure 7.11a	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Surface of Cobble Two Weeks Following Application of Oleophilic Fertilizers . . . . .	91

Figure 7.11b	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Surface of Cobble Four Weeks Following Application of Oleophilic Fertilizers . . . . .	92
Figure 7.12a	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Mixed Sand and Gravel Under the Cobble Prior to Application of Oleophilic Fertilizer . . . . .	93
Figure 7.12b	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Mixed Sand and Gravel Under the Cobble Two Weeks following Application of Oleophilic Fertilizer . . . . .	94
Figure 7.12c	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Mixed Sand and Gravel Under the Cobble Four Weeks following Application of Oleophilic Fertilizer . . . . .	95
Figure 7.12d	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Mixed Sand and Gravel Under the Cobble prior to Application of Water Soluable Fertilizer . . . . .	96
Figure 7.12e	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Mixed Sand and Gravel Under the Cobble Two Weeks Following Application of Water Soluble Fertilizer . . . . .	97
Figure 7.12f	Recreated Gas Chromatographic Profiles from Samples of Oil Extracted from the Mixed Sand and Gravel Under the Cobble Four Weeks Following Application of Water Soluble Fertilizer . . . . .	98
Figure 7.13.	Mean Weight of Alkanes (mg) Normalized to the Total Oil Residue Weight (mg) Extracted from the Beach Material; Control Mixed Sand and Gravel Beaches . . . . .	100
Figure 7.14.	Mean Weight of Alkanes (mg) Normalized to the Total Oil Residue Weight (mg) Extracted from the Beach Material; Oleophilic Fertilizer Treated Mixed Sand and Gravel . . . . .	101
Figure 7.15.	Mean Weight of Alkanes (mg) Normalized to the Total Oil Residue Weight (mg) Extracted from the Beach Material; Water Soluble Fertilizer Treated Cobble Beaches . . . . .	102

Figure 7.16.	Median of Total Concentration of Oil on Treated and Untreated Cobble Plots at Snug Harbor, All Zones . . . . .	103
Figure 7.17.	C17/Pristane Ratio versus Log10 Residue Weight Two Weeks Before Fertilizer Application . . . . .	105
Figure 7.18.	C18/Phytane Ratio versus Log10 Residue Weight Two Weeks Before Fertilizer Application . . . . .	106
Figure 7.19.	C17/Pristane Ratio versus Log10 Residue Weight at Time Zero of Fertilizer Application . . . . .	107
Figure 7.20.	C18/Phytane Ratio versus Log10 Residue Weight at Time Zero of Fertilizer Application . . . . .	108
Figure 7.21.	Phytoplankton Chlorophyll data from Water Samples Collected at Snug Harbor Following June 7 and 8, 1989, Fertilizer Additions to Gravel Shorelines . . . . .	116
Figure 7.22.	Primary Productivity Estimates (as $^{14}\text{C}$ uptake) for Phytoplankton Samples from Snug Harbor at Various Sample Dates Following the June 7 and 8, 1989, Fertilizer Additions . . . . .	118
Figure 7.23.	Bacterial Productivity, as Measured by Tritiated Thymidine Uptake, for Bacterial Samples Collected on Various Sample Dates Adjacent to Gravel Shorelines at Snug Harbor . . . . .	120
Figure 7.24.	Abundance of Bacterial Cells ( $\times 10^9$ ) from Water Samples Taken along Gravel Shorelines on Various Sample Dates Following the June 7 and 8, 1989, Fertilizer Additions to Snug Harbor Shorelines . . . . .	122
Figure 7.25.	Bacterial Productivity, as Measured by Tritiated Thymidine Uptake, for Bacterial Samples Collected on Various Sample Dates Adjacent to Cobble Shorelines at Snug Harbor . . . . .	123
Figure 8.1.	Median Residue Concentration of Oil on Passage Cove Cobble Beaches . . . . .	129
Figure 8.2.	Median of C17/Pristane Ratio Passage Cove Cobble, Top, All Zones . . . . .	130
Figure 8.3.	Median of C18/Phytane Ratio Passage Cove Cobble, Top, All Zones . . . . .	131



Figure 8.4a.	Gas Chromatographic Profiles of Oil Residue Weights Before Plots Were Sprinkler Irrigated with Water-Soluble Fertilizer . . . . .	133
Figure 8.4b.	Gas Chromatographic Profiles of Oil Residue Weights Two Weeks After Sprinkler Application of Water-Soluble Fertilizer Began . . . . .	134
Figure 8.4c.	Gas Chromatographic Profiles of Oil Residue Weights Three Weeks After Sprinkler Application of Water-Soluble Fertilizer Began . . . . .	135
Figure 8.5a.	Gas Chromatographic Profiles of Oil Residue Weights on Untreated Plots - Raven Beach . . . . .	136
Figure 8.5b.	Gas Chromatographic Profiles of Oil Residue Weights on Untreated Plots - Raven Beach - Two Weeks After Previous Profiling . . . . .	137
Figure 8.6.	Median Total Aliphatic Hydrocarbon Concentrations on Passage Cove Plots . . . . .	138
Figure 8.7.	Mean Chlorophyll Measurements (+ SD) From 4 Replicate Plankton Samples Taken at Passage Cove Study Sites Before and After July 25, 1989, Fertilizer Applications to Shorelines . . . . .	143
Figure 8.8.	Mean Primary Productivity Activity Measurements (+ SD), as <sup>14</sup> C-Uptake From 4 Replicate Plankton Samples Taken at Passage Cove Study Sites Before and After July 25, 1989, Fertilizer Applications to Shorelines . . . . .	145
Figure 8.9.	Abundance of Bacterial Cells (x10 <sup>9</sup> ) From Water Samples Collected at Passage Cove Study Sites Before and After Fertilizer Application on July 25, 1989. Values Are Means (+ SD) of 4 Replicates . . . . .	147
Figure 8.10.	Bacterial Productivity Measurements From Tritiated Thymidine Uptake by Water Samples Collected at Passage Cove Before and After Nutrient Application to Shorelines on July 25, 1989. Values are means (+ SD) of 4 replicates . . . . .	149
Figure 9.1.	Schematic Diagram of the Microcosms . . . . .	156
Figure 9.2.	Gas Chromatographic Profiles Showing the Effect of Different Inocula on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil . . . . .	167

Figure 9.3.	Gas Chromatographic Profiles Showing the Effect of Temperature on the Degradation of Artificially Weathered Prudhoe Crude Oil . . . . .	169
Figure 9.4.	Gas Chromatographic Profiles Showing the Effect of Different Concentrations of Inipol (% of Oil Concentration) on the Degradation of Artificially Weathered Prudhoe Crude Oil . . . . .	170
Figure 9.5.	Gas Chromatographic Profiles Showing the Effect of Different Fertilizers, Under Poisoned and Unpoisoned Conditions, on the Degradation of Artificially Weathered Prudhoe Crude Oil . . . . .	171
Figure 9.6.	Gas Chromatographic Profiles Showing the Effect of Temperature on the Degradation of Artificially Weathered Prudhoe Crude Oil Treated with Inipoll . . . . .	173
Figure 9.7.	Gas Chromatographic Profiles Showing the Effect of Inipol (Poisoned and Unpoisoned Conditions) on the Degradation of Oil on Beach Material Taken from Prince William Sound . . . . .	174
Figure 9.8.	Cumulative Oxygen Uptake on Weathered Prudhoe Bay Crude Oil . . . . .	179
Figure 9.9.	Gas Chromatographic Profiles of Alkanes at 0 and 6 Weeks After Initiation of Flask Studies . . . . .	181
Figure 9.10.	Chromatographic Scan of Aromatic at 0 and 6 Weeks After Initiation of Flask Studies . . . . .	182
Figure 9.11.	Effect of Inipol on the Relative Number of Oleic Acid-degrading Bacteria in Jars Containing Oiled Rocks and Seawater, Defined Nutrient Medium, or Saline Solution . . . . .	186
Figure 9.12.	Effect of Inipol on the Relative Numbers of Oil-degrading Microorganisms in Jars Containing Oiled Rocks and Seawater, Defined Nutrient Medium, or Saline Solution . . . . .	187
Figure 9.13.	Location of Wells for Beach Hydraulics Experiment at Passage Cove . . . . .	194
Figure 9.14.	Casing Configuration . . . . .	195

## EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency's Alaska Bioremediation Project was initiated in the aftermath of the March 24, 1989, EXXON VALDEZ oil spill. The objective of the project was to demonstrate an alternative cleanup method for oil-contaminated shorelines based on enhancing natural biodegradation of the oil through the addition of nitrogen and phosphorus nutrients. This enhancement process is a well-recognized and scientifically sound approach to bioremediation but had never been tested on a large scale in marine environments. The project was managed by EPA's Office of Research and Development with financial, scientific, and logistical support from the Exxon Company USA under the authority of the Federal Technology Transfer Act.

After planning, mobilizing staff and facilities, and selecting test sites in Prince William Sound, Alaska, nutrient application began on June 8, 1989. Nitrogen- and phosphorus-rich nutrients were added to the oil-contaminated shoreline sites as three types of fertilizers: (1) a slow-release formulation in which water-soluble nutrients leaching from point sources were distributed over the contaminated beaches by tidal actions; (2) an oleophilic formulation designed to "dissolve" nutrients into the oil on the surface of the beach substrata; and (3) a fertilizer solution in which inorganic nitrogen and phosphorus were added to seawater and the solution applied to the beaches at low tide using a sprinkler system. The liquid oleophilic fertilizer was sprayed onto the test plots from a hand-pumped backpack sprayer. The slow release formulations were in the form of either briquettes or granules. Application of these formulations was accomplished by placing netbags of briquettes on the shoreline surface in a designated pattern or by broadcasting the granules on the beaches using a commercial fertilizer spreader.

Test plots were established on Knight Island in Prince William Sound, one in Snug Harbor that was tested early in the summer, and the other in Passage Cove that was tested late in the summer. Snug Harbor, a moderately oiled beach, was selected to simulate conditions considered typical of a beach following physical washing of the beach material (the primary cleanup procedure used by Exxon). Passage Cove, a heavily oiled beach that had been physically washed, served as the definitive test beach to show effectiveness of the large-scale application of fertilizers ultimately performed by Exxon. The shoreline surfaces were both mixed sand and gravel, and cobblestone. Beach materials were sampled before and after application of the fertilizers and the results compared to untreated reference beaches. Samples were processed to determine changes in the quantity and composition of oil residues following fertilizer

application. Visual changes in the amount of oil remaining on rock surfaces were also noted. In addition, monitoring for any potential adverse environmental effects was performed. This included measurements of algal growth (eutrophication) due to any nutrient accumulation in seawater adjacent to the treated beaches and any toxicity of the fertilizer to marine species. Laboratory and microcosm experiments were conducted to examine nutrient-enhanced oil degradation under more controlled conditions.

Following careful discussion of visual observations, analysis of the field and laboratory data, and two formal workshops to discuss interpretations and significant findings, we conclude that the application of nitrogen and phosphorus fertilizers did enhance biodegradation of the oil and the clean-up of oil from the contaminated beaches. Since there were no adverse ecological effects observed from the fertilizer application, bioremediation is recommended as a feasible cleanup procedure.

Specific conclusions and interpretations of the demonstration project are as follows:

- \* Visual inspection of beaches treated with the inorganic nutrients (using the sprinkler system) showed that within three weeks following fertilizer application, considerably less oil was observed on the rock surfaces than in the untreated control beaches. This condition became more pronounced with time and remained visually apparent through the end of the summer season (five weeks).
- \* No oil slicks were observed in the near-shore seawater following application of the inorganic fertilizer, indicating oil was not released.
- \* Samples of the oil taken from the beach surfaces when the oil was visually beginning to disappear showed changes in composition indicating extensive biodegradation.
- \* Visual disappearance of the oil as a result of inorganic nutrient application (sprinkler system) can only be attributed to enhanced biodegradation.
- \* Similar visual oil disappearance was also observed 10 days following application of the oleophilic fertilizer. Again, no oil slicks were observed, and oil sampled from the rock surfaces when oil was beginning to disappear showed changes in composition indicating extensive biodegradation.
- \* Laboratory studies with contaminated Prince William Sound beach material showed that the oleophilic fertilizer was not a chemical rock washer and that it enhanced the extent and

rate of oil degradation as compared to untreated beach material.

- \* As observed in laboratory studies, enhanced oil biodegradation from fertilizer addition was accompanied by significant changes in the physical consistency of the oil. A flaky, particulate material consisting of degraded oil, degradation products, and microbial cells was produced. This process occurs 1 to 2 weeks following incubation.
- \* A more rapid and extensive disappearance of oil from Passage Cove as compared to Snug Harbor suggested that physical washing of the beaches spread a very thin layer of oil over a large surface area of rock and gravel, facilitating bacterial degradation.
- \* Samples from untreated oil-contaminated beaches in Prince William Sound showed 1,000 to 100,000 times more oil-degrading bacteria than uncontaminated beaches. Thus, biodegradable carbon from the oil was sufficient to enrich for high numbers of oil-degrading bacteria. Because of these naturally elevated bacterial numbers, it was difficult to demonstrate significant increases in oil-degrading bacteria resulting from fertilizer application. The high bacterial numbers are probably maintained due to an equilibrium condition between the growth of bacteria on the oil, protozoan predation, and physical sloughing.
- \* Oil clean-up in the field was the direct result of enhanced biodegradation coupled with washing of the degraded materials from the rock surfaces by tidal action.
- \* Chemical analysis of field samples over time showed that changes in oil residue weight and oil composition were greater in the fertilizer-treated beaches than in the untreated reference beaches. However, due to several confounding factors, statistical verification of these results has not been possible. These factors include: (1) surprisingly high rates of natural oil biodegradation due to significant natural concentrations of nutrients in seawater and freshwater; (2) high variability in oil concentration and distribution in beach material; and (3) extensive degradation of pristane and phytane, two branched chain hydrocarbons normally used as conserved internal standards to measure changes in oil composition.
- \* Chemical analysis of oil from the two untreated reference plots used in this study showed that on some shorelines there were high rates of natural oil degradation that were comparable to that observed in the treated plots. However there is no guarantee that other untreated beach areas would respond in the same way (variable concentrations in nitrogen



and phosphorus). The fact that oil still remains on some Alaskan shorelines is evidence that natural degradation rates are not uniformly high. Therefore fertilizer addition assures oil degradation in a controlled and predictable manner during the short "time window" when water temperatures are high enough for optimal microbial activity.

- \* Addition of fertilizer to oiled shorelines did not cause any increases in planktonic algae or bacteria or any measurable nutrient accumulation in adjacent embayments.
- \* The concentration of oleophilic fertilizer and ammonia that is toxic to various marine species has been established. They were both mildly toxic. Seawater collected directly over the beaches just treated with a combination of the oleophilic and water-soluble fertilizer (worst case situation) were toxic to the most sensitive marine species (oyster larvae). A 50% dilution of this seawater, which would occur through tidal mixing within a few feet of the treated shoreline, reduced the concentrations to levels where toxicity is zero.
- \* No oil was detected in tissues taken from mussels that had been placed (in flow-through plastic containers) just offshore of the fertilizer-treated beaches.
- \* Samples taken from the fertilizer-treated beaches and tested in the standard Ames mutagenicity test showed that as the oil was biodegraded, the slight mutagenicity of fresh Prudhoe Bay crude was eliminated. Thus, no mutagenic byproducts result from enhanced biodegradation.

Overall, our findings indicate that fertilizer addition to enhance biodegradation of the oil is effective and environmentally safe. Projects initiated this winter, in conjunction with the Exxon Company USA were designed to continue and complement the research effort. Examples of projects allowing more insight into the biodegradation of spilled oil include the mechanism of action of the oleophilic fertilizer; and identification of oil components that could act as internal standards for evaluating the extent of degradation. These projects as well as several others are providing valuable information for further, more effective scale-up bioremediation this summer and for future oil spills.

## ACKNOWLEDGMENTS

For any field work, the success of the study is dependent to a great extent upon the support and dedication of the field crew. We have been very fortunate to have an outstanding field team who has consistently demonstrated its commitment to this project. After many long and hard hours, and in some cases, work around the clock, the team members have maintained their spirits and brought this study to fruition. Our sincere gratitude is extended to John R. Baker, Martin Dillon, Wesley L. Kinney, Dennis E. Miller, and Richard Wright.

Deepest appreciation is expressed to the crew of the F/V AUGUSTINE, Garth, Phyllis, and Jaris Tyler, Tyler Morgan, and Vern Boyd, and to the crew of the F/V CARMEN ROSE, Brian King, Dave Kuntz, Stuart Carter, and George Covell, for their support to the onsite staff at Snug Harbor and Passage Cove, respectively; to Dennis Thacker and Ken Lobe, pilots of the aircraft N756AF, for their continued safe transport of personnel, equipment, and samples; and to Don Carlson and Ken Broker, Norcon Inc. carpenters, for demonstrating superior craftsmanship in the construction of the two field laboratories.

Gratitude is also extended to Russell Chianelli, Ph.D.; Stephen M. Hinton, Ph.D.; and Roger C. Prince, Ph.D. at Exxon Research and Engineering Co. in Annandale, New Jersey and to Sara J. McMillen and Richard Requejo, Ph.D. at Exxon Production Research Co. in Houston, Texas. Their work on this project, particularly in the Laboratory Biodegradation Screening Evaluations was crucial.

## SECTION 1

### INTRODUCTION

On March 24, 1989, the EXXON VALDEZ went aground in Prince William Sound, Alaska, releasing approximately 11 million gallons of Prudhoe Bay crude oil. After learning of the magnitude of the spill, the EPA Assistant Administrator for the Office of Research and Development (ORD) convened a meeting of nationally and internationally recognized scientists in the field of oil biodegradation to evaluate the feasibility of using bioremediation to assist in cleanup operations. Members of the Biosystems Scientific Steering Committee, a research group within ORD that is developing the technology of bioremediation, organized and implemented the meeting. The meeting was chaired by Dr. Hap Pritchard, a senior scientist in the ORD Environmental Research Laboratory in Gulf Breeze, Florida. After intensive discussion, scientists at the meeting recommended that ORD plan and conduct a field demonstration project to evaluate the use of fertilizers for accelerating natural biodegradation of the spilled oil.

Specifically, the recommendation was based on the following conclusions:

- The presence of readily degradable hydrocarbons from the spilled oil will enrich for naturally occurring oil-degrading bacteria.
- Oil biodegradation in Prince William Sound waters is probably limited by the availability of nitrogen and phosphorus; therefore, fertilizing the beaches with these nutrients will enhance natural degradation of the oil.
- Past studies have shown convincingly that the enhancement of oil biodegradation by nutrient addition readily occurs. Further verification of these studies by laboratory experiments are unnecessary.
- Successful bioremediation will require consideration of the logistics and mechanics of long-term nutrient application and the physical agitation of oil.
- An oleophilic fertilizer, such as that produced by Elf Aquitaine Chemical Company, may be the only way to assure extended contact of the nutrients with the oil-contaminated beach material.
- Bioremediation should be used as a finishing step for any cleanup program; that is, removing the bulk oil, regardless

of method, will allow bioremediation to clean up the residual.

- Treatment of the beaches with fertilizer will not necessarily remove the black oil residues (i.e., visually unchanged) but will considerably reduce, if not eliminate, ecological availability of the oil.
- Inoculation of oil-contaminated beaches with hydrocarbon-degrading microorganisms enriched from Prince William Sound waters was not the best initial approach but should be considered in an experimental context or for future spills.

Depending on the outcome of a demonstration project, recommendations for the use of bioremediation to help clean up oil-contaminated beaches on a larger scale would be made to Exxon.

A detailed oil spill bioremediation research plan was then developed. The major objectives of this plan were to:

- Examine the extent to which natural biodegradation of oil on the contaminated beaches was occurring.
- Determine if the rates of oil biodegradation on contaminated beaches could be enhanced by the addition of nutrients in the field.
- Develop methods for long-term application of nutrients to contaminated beaches.
- Establish methods for monitoring potential ecological effects resulting from nutrient addition.
- Develop information on the movement of nutrients in beach substrata (beach mechanics).
- Examine the possibility of inoculation as a means to enhance oil biodegradation.

The research plan was based on the use of two types of fertilizers: (1) a slow-release formulation in which nutrient leaching from point sources would be distributed over the contaminated beaches by tidal actions and (2) an oleophilic formulation in which nutrients would be "dissolved" into the oil on the surface of the beach substrata. Biodegradation of the oil would be followed through time using analytical chemistry and microbiological techniques. An ecological monitoring program would be implemented to check for possible adverse ecological effects resulting from the direct toxicity of the fertilizer or from eutrophication. The bioenhancement (inoculation of bacteria) part of the plan was designed as a small program to

check feasibility in laboratory microcosm studies. In addition, development of a detailed quality assurance plan was also initiated.

The research plan was reviewed by a special committee of EPA's Scientific Advisory Board. The committee recommended that the plan be implemented with minor modification.

Following development of the research plan, a decision was made to approach Exxon and propose a cooperative effort under the Federal Technology Transfer Act of 1986. On June 2, 1989, the two parties reached an agreement, and the project was formally initiated. Exxon agreed to provide all logistical support (transportation from Valdez to test sites, field laboratory facilities, and subsistence) and \$1.6 million for direct support of the field demonstration project. EPA provided \$1.6 million for management personnel, scientific expertise, quality assurance, and operations technical support.



## SECTION 2

### BACKGROUND

The site of the Alaskan oil spill is a harsh and diverse environment with poor accessibility. The shoreline is geologically young, is composed largely of metamorphic rock, and ranges from vertical cliffs to boulder and pebble beaches. High-energy beaches are common, with tides that vary from +4 to -1 m. In some areas, glacial and snow melt introduce large amounts of fresh water to nearshore water of the Prince William Sound. Prince William Sound has a considerable population of seals and sea otters. The area also has extensive herring spawning areas and significant numbers of seabirds and shorebirds. There is a substantial migration of birds that feed at beaches and intertidal areas.

The spilled oil spread over an estimated 350 miles of shoreline in Prince William Sound. Major contaminated shoreline areas include Knight Island, Eleanor Island, Smith Island, Green Island, and Naked Island. Knight Island, the largest and one of the most heavily polluted of these islands, has restricted tidal flushing action in some bays and coves.

The oil settled into the beach gravel and on rock surfaces and the faces of vertical cliffs. Contamination occurred primarily in the intertidal zone.

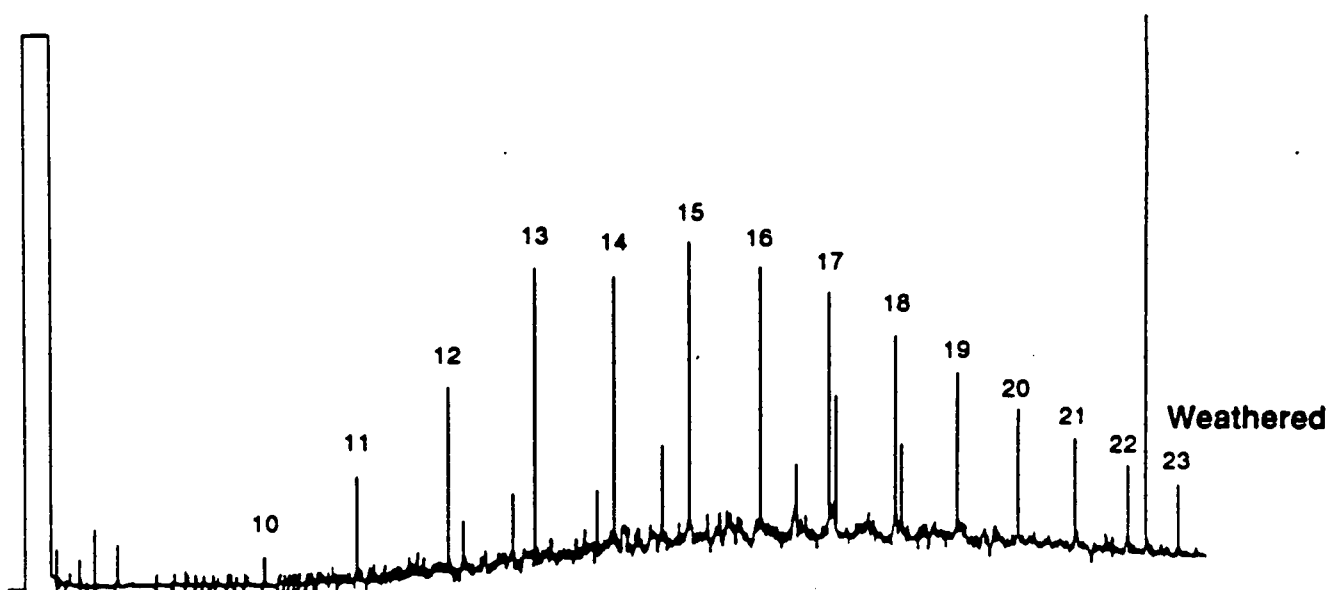
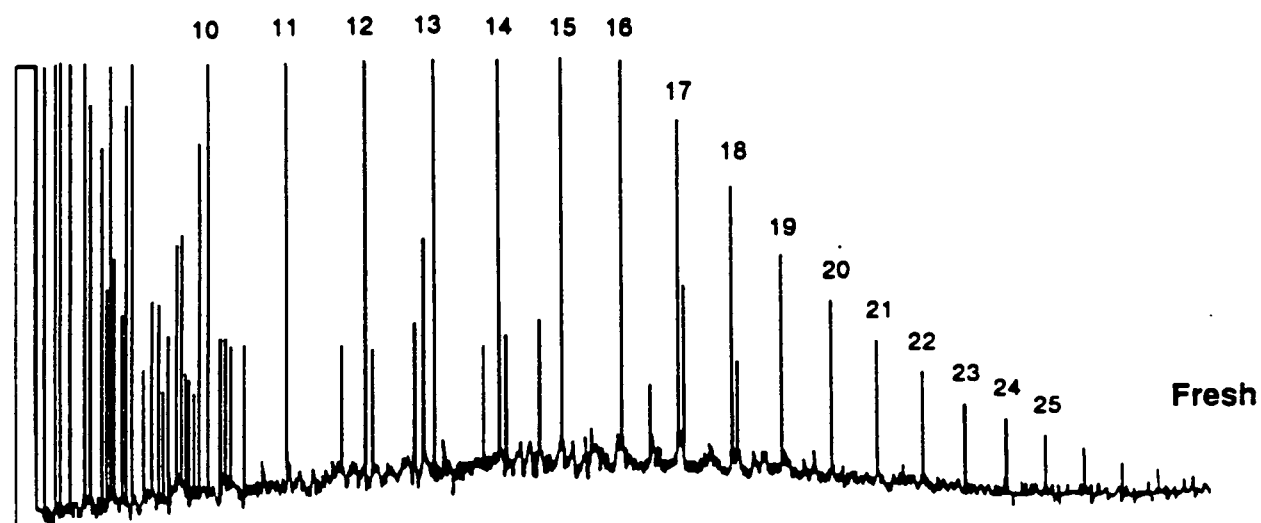
Initial weathering of the oil resulted in a loss of approximately 15% to 20% of the oil by volatilization. Volatilized components included normal aliphatic hydrocarbons of less than 12 carbon atoms and aromatic hydrocarbons such as benzene, toluene, xylene, and some methyl-substituted naphthalenes. The resulting residue consisted of approximately 40% to 50% high-molecular-weight waxes and asphaltenes. On most beaches in Prince William Sound the weathered oil was black and viscid rather than brown and mousse-like.

Beaches were physically cleaned by a combination of flooding and the application of water under high and low pressure and/or high temperature. Vacuum extraction and physical skimming were used to remove the released oil from the water surface. The cleaning process partially removed oil from the surface of rocks and beaches, particularly the pools of oil, but did not effectively remove the oil trapped in and below the matrix of gravel and cobble. However, the washing process spread a thin layer of oil over a much greater surface area of rock and gravel. The extent of physical washing was dependent upon the degree of contamination.

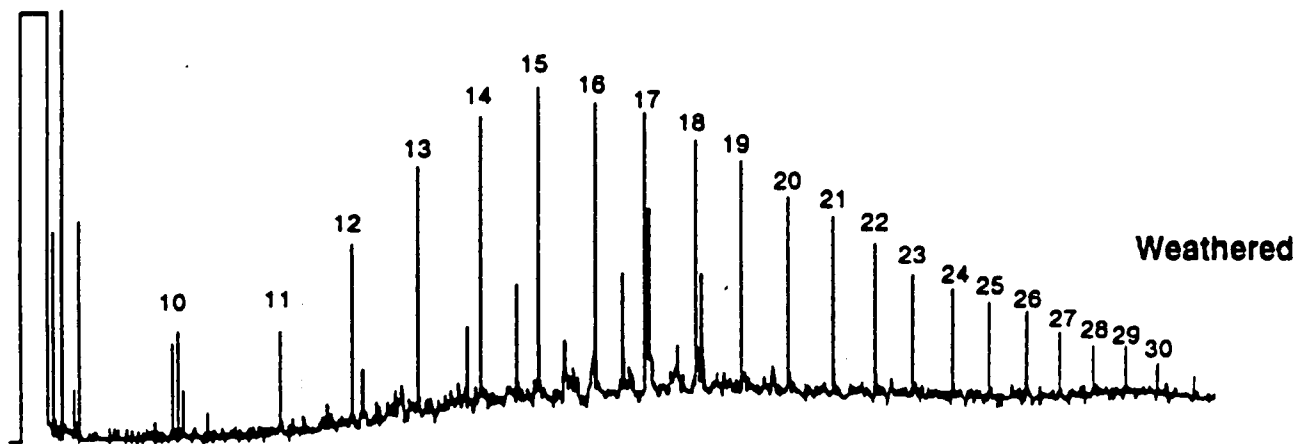
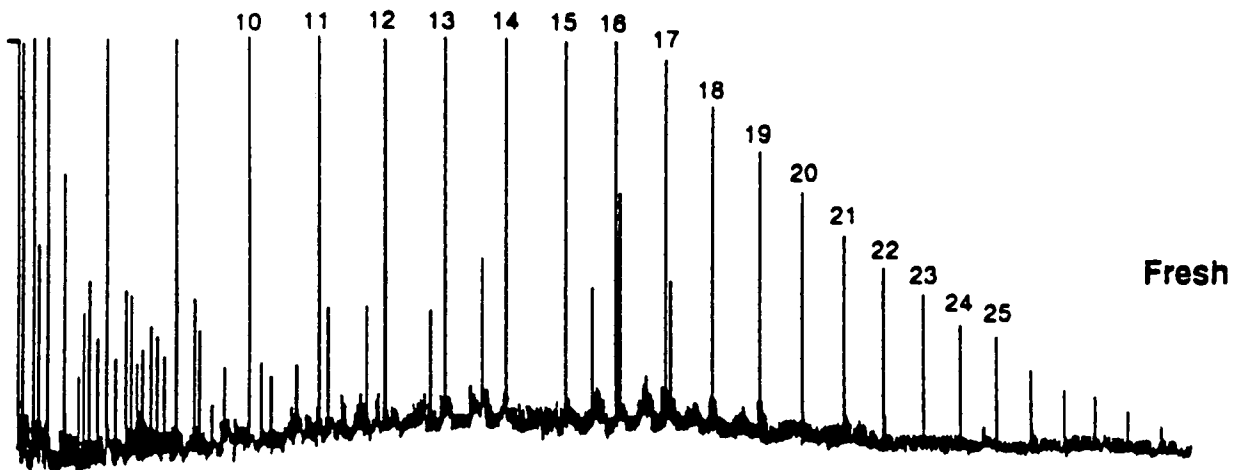
The biodegradation of oil has been extensively studied over the last 20 years. As a result, the fate and microbial decomposition of oil in aquatic environments is well understood. Studies have shown that oil degradation can occur in cold-water environments.

Oil degradation is commonly measured by extracting oil from beach material and then analyzing oil composition using gas chromatography. A typical gas chromatogram of fresh and weathered Prudhoe Bay crude oil is shown in Figure 2.1. The weathered Prudhoe Bay crude oil was taken from a Prince William Sound beach (Northwest Bay) in the late spring. The major peaks represent detector responses for the normal alkanes; the annotated numbers are the carbon lengths of the appropriate alkane. Normal aliphatic hydrocarbons of 12 carbons or less are absent in the weathered oil. Large quantities of biodegradable hydrocarbons (C13-C28) remain. Gas chromatograms for oil samples fractionated into the aliphatic and aromatic components are shown in Figures 2.2 and 2.3. These fractionated samples of oil showed the presence of small quantities of aromatic hydrocarbons in the weathered oil, but hydrocarbons up to the methyl naphthalene were absent.

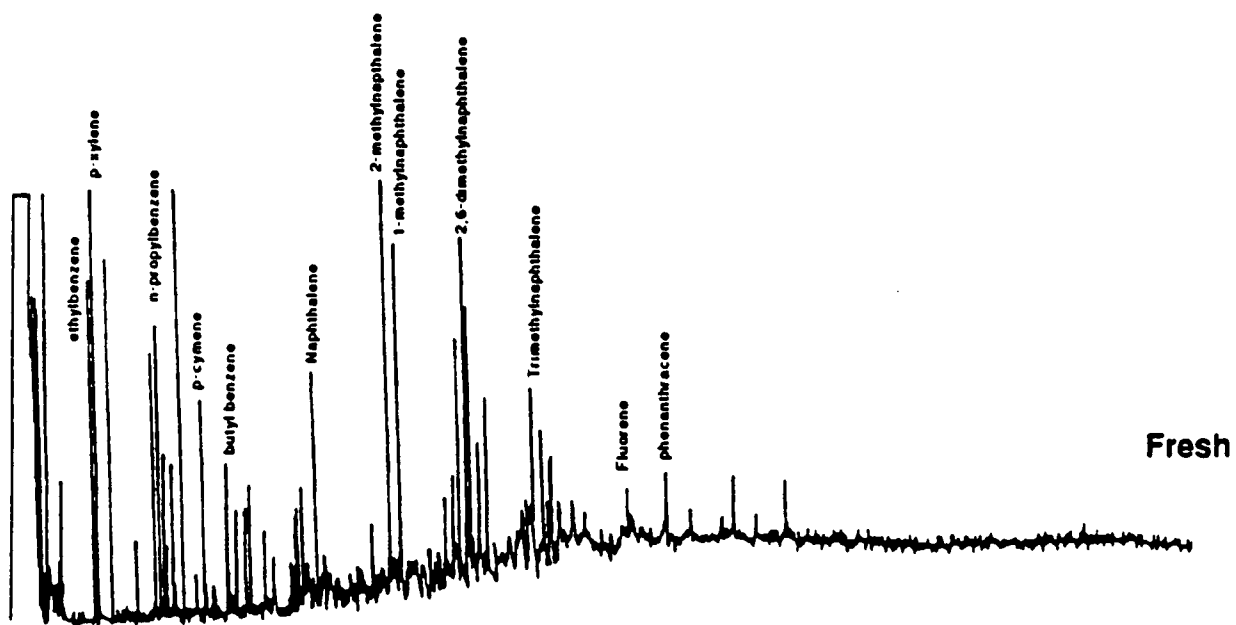
Pristane and phytane, branched alkanes, are slow to biodegrade and have been used as conserved internal standards. Therefore, changes in the ratios of hydrocarbon concentration for the linear alkanes relative to the branched alkanes can be used to indicate biodegradation. Table 2.1 gives the calculated ratios of C17 linear alkane to pristane and C18 linear alkane to phytane for samples taken from Prince William Sound on April 4 to May 2, 1989. The sample from Disk Island (gravel) is the only one with a significant difference in these ratios relative to fresh Prudhoe Bay crude oil. This suggests that natural biodegradation was occurring at this beach.



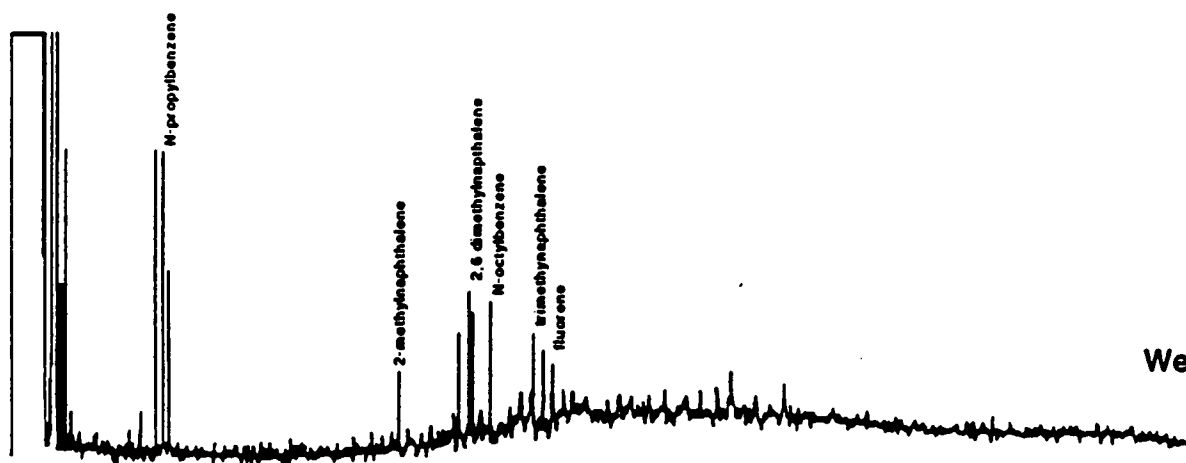
**Figure 2.1. Unfractionated Prudhoe Bay Crude Oil (Number Indicates Carbon Atoms of Alkane).**



**Figure 2.2. Prudhoe Bay Crude Oil, Aliphatic Fraction (Number Indicates Carbon Atoms of Alkane).**



Fresh



Weathered

Figure 2.3 Prudhoe Bay Crude Oil, Aromatic Fraction.

Table 2.1. Calculated Ratios of C17/Pristane and C18/Phytane

<u>SAMPLE</u>	<u>N-C17/ Pristane</u>	<u>N-C18/ Phytane</u>
Fresh Prudhoe Bay Crude Oil	1.7	2.0
Eleanor Island		
Northwest Bay		
Surface	1.5	1.9
Surface Control <sup>a</sup>	<0.47	--
6" Depth	1.4	1.7
6" Depth Control <sup>a</sup>	<0.45	--
Seal Island	1.6	2.1
Smith Island	1.5	1.9
Disk Island		
Gravel	0.8	1.0
Fresh Oiled Rock	1.4	1.7
Weathered Oiled Rock	1.8	2.0

<sup>a</sup> Sample taken from an uncontaminated beach area.

## SECTION 3

### OVERVIEW OF ACTIVITIES

#### ORGANIZATION

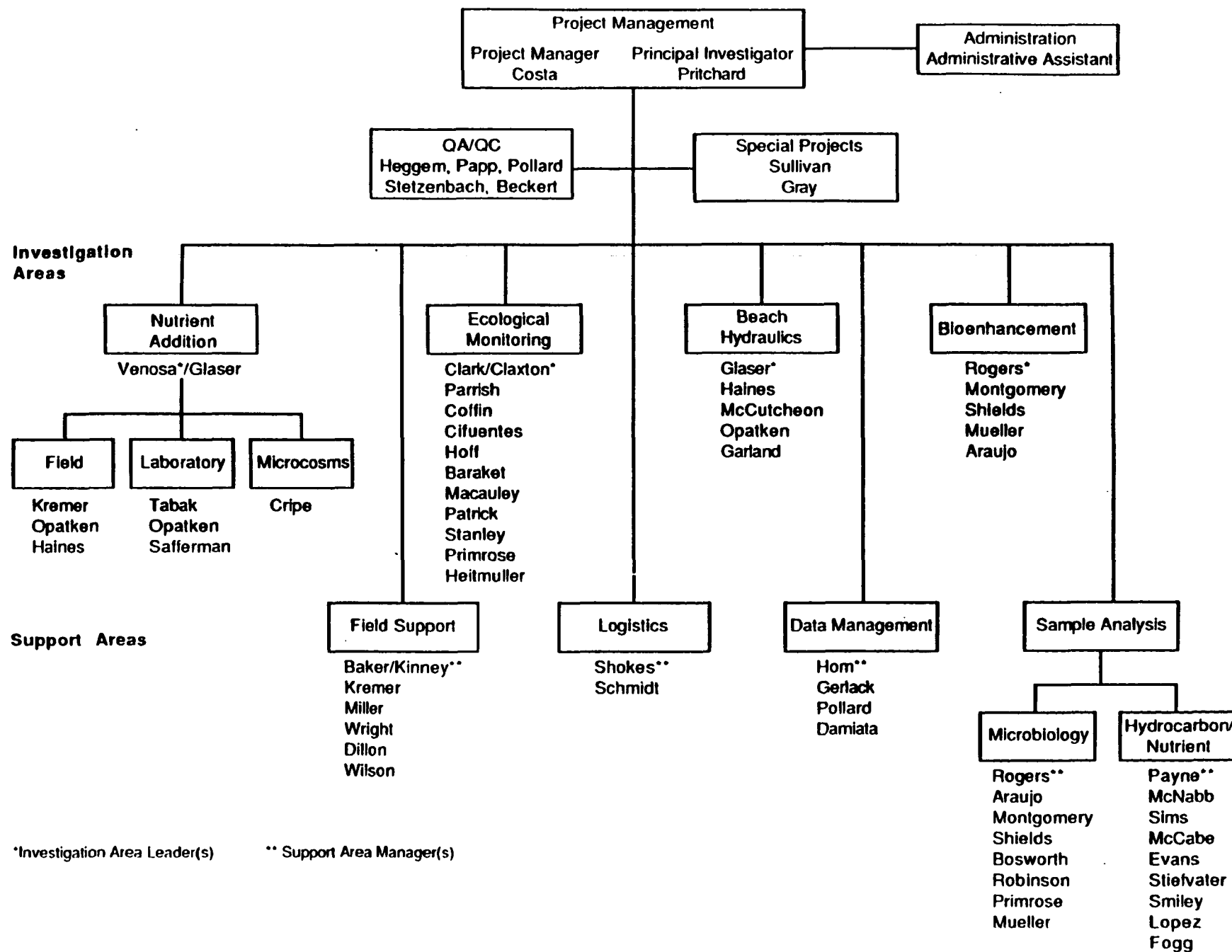
The organizational structure for the Oil Spill Bioremediation Field Project is shown in Figure 3.1. The following organizations contributed their expertise to the project:

- 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
- Exxon Research and Engineering - Annandale, New Jersey
- Exxon Production Research - Houston, Texas
- Exxon Biomedical Services - East Millstone, New Jersey

Principal scientists and support staff for the project are listed in Table 3.1.

Existing EPA contracts with several companies were expanded to facilitate logistics, analytical services, and administrative support. The contractors are as follows:

- Analytical support (oil and nutrient analysis): Science Applications International Corporation (SAIC)
- Personnel and administrative support: Technical Resources, Incorporated (TRI)
- Quality assurance planning: Engineering Science Company (Lockheed)
- Data management and logistical support: SAIC
- Field team support and logistics: Lockheed and SAIC



**Figure 3.1 Project Organization Chart.**



Table 3.1. EPA Bioremediation Project Staff

	<u>Name</u>	<u>Affiliation</u>	<u>Location</u>
Management	Hap Pritchard Chuck Costa	EPA/ERL EPA/EMSL	Gulf Breeze, FL Las Vegas, NV
Headquarters	William Reilly Erich Bretthauer John Skinner Dick Valentinetti	EPA/ORD EPA/ORD EPA/ORD EPA/ORD	Washington, DC Washington, DC Washington, DC Washington, DC
Special Projects	Eulalie Sullivan Ellen Gray	TRI TRI	Rockville, MD Seattle, WA
Administration	Valerie Furlong Robin Shoemaker Evelyn Clay Wendy Barlow Terry Morton	EPA/EMSL EPA/EMSL EPA/EMSL EPA/EMSL EPA/EMSL	Las Vegas, NV Las Vegas, NV Las Vegas, NV Las Vegas, NV Las Vegas, NV
Nutrient Addition	Al Venosa John Glaser Fran Kremer John Haines Ed Opatken Henry Tabak Steve Safferman Rick Cripe	EPA/RREL EPA/RREL EPA/CERI EPA/RREL EPA/RREL EPA/RREL EPA/RREL EPA/ERL	Cincinnati, OH Cincinnati, OH Cincinnati, OH Cincinnati, OH Cincinnati, OH Cincinnati, OH Cincinnati, OH Gulf Breeze, FL
Ecological Monitoring	Jim Clark Larry Claxton Rod Parrish Rick Coffin Luis Cifuentes John Macauley Jerry Hoff Sana Baraket Jim Patrick Roman Stanley Ginny Primrose Tom Heitmuller	EPA/ERL EPA/HERL EPA/ERL TRI TRI/Texas A&M EPA/ERL TRI TRI EPA/ERL EPA/ERL TRI	Gulf Breeze, FL Res. Tri. Park, NC Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL Gulf Breeze, FL

Table 3.1. Continued

	<u>Name</u>	<u>Affiliation</u>	<u>Location</u>
Bioenhancement	John Rogers	EPA/ERL	Athens, GA
	Stacy Montgomery	TRI	Gulf Breeze, FL
	Rochelle Araujo	EPA/ERL	Athens, GA
	Jim Mueller	EPA/ERL	Gulf Breeze, FL
	Malcolm Shields	TRI	Gulf Breeze, FL
QA/QC	Dan Heggem	EPA/EMSL	Las Vegas, NV
	Mike Papp	Lockheed	Las Vegas, NV
	Linda	EPA/UNLV	Las Vegas, NV
	Stetzenbach	Lockheed	Las Vegas, NV
	Jim Pollard	EPA/EMSL	Las Vegas, NV
	Werner Beckert		
Data Management	Wilson Hom	SAIC	San Diego, CA
	Brian Damiata	SAIC	Golden, CO
	Bob Gerlack	Lockheed	Las Vegas, NV
	Jim Pollard	Lockheed	Las Vegas, NV
Field Support	John Baker	Lockheed	Las Vegas, NV
	Wes Kinney	EPA/EMSL	Las Vegas, NV
	Fran Kremer	EPA/CERI	Cincinnati, OH
	Dennis Miller	EPA/ERL	Ada, OK
	Rick Wright	SAIC	San Diego, CA
	Marty Dillon	SAIC	Valdez, AK
	John Wilson	EPA/ERL	Ada, OK
Logistics	Bob Shokes	SAIC	San Diego, CA
	Kurt Schmidt	SAIC	San Diego, CA
Microbiology Analysis	John Rogers	EPA/ERL	Athens, GA
	Rochelle Araujo	EPA/ERL	Athens, GA
	Stacy Montgomery	TRI	Gulf Breeze, FL
	Christy Robinson	TRI	Gulf Breeze, FL
	Malcolm Shields	TRI	Gulf Breeze, FL
	Lorna Bosworth	TRI	Gulf Breeze, FL
	Ginny Primrose	TRI	Gulf Breeze, FL
	Jim Mueller	EPA/ERL	Cincinnati, OH
Hydrocarbon/ Nutrient Analysis	Jim Payne	SAIC	San Diego, CA
	Dan McNabb	SAIC	San Diego, CA
	Rusty Sims	SAIC	San Diego, CA
	Mike McCabe	SAIC	San Diego, CA
	John Evans	SAIC	San Diego, CA
	Jeff Stiefvater	SAIC	Seattle, WA
	Elizabeth Smiley	SAIC	Anchorage, AK
	Juliet Lopez	SAIC	San Diego, CA
	Tom Fogg	SAIC	San Diego, CA

- Beach hydraulics: Battelle Northwest Laboratories
- Toxicity testing: Battelle and E.V.S. Consultants

#### CHRONOLOGY OF EVENTS

After finalizing the Research Plan, an implementation strategy was developed. It was imperative to initiate the field demonstration as quickly as possible to provide enough time during the summer for large-scale application if results were favorable. Considering the extensive logistical problems that were faced, a target date for field application of the nutrients was set for June 6, 1989. The following is an overview, in approximate chronological order, of the different stages in the project.

- May 15 - A command center for the Bioremediation Project was established in Valdez through the dedicated help of a local citizen, Bill Wyatt. The center was used by both EPA and Exxon personnel.
- May 16 - A search for information on the characteristics of different fertilizer preparations that might be applied at the test sites was initiated. Laboratory studies to determine nutrient release rates were also begun. These studies are described in Section 5.
- May 19 - A beach survey team was assembled to select appropriate test sites. Snug Harbor (southeast Knight Island) was selected as the initial test site. Efforts were initiated to establish test plots and set up a staging operation. Description of this site is provided in Section 4. Staging was greatly facilitated by the 93-foot fishing vessel (F/V) AUGUSTINE (under Exxon contract), which provided space for microcosms, microbiological and nutrient analyses, and sample-handling facilities as well as berthing space for field personnel.
- May 20 - Studies to provide information on the movement of groundwater and nutrients in the beaches were initiated once the staging operation was in place. Results from these studies are provided in Section 9.
- May 23 - Methods for adequately sampling contaminated beach material were developed at this time. A sampling design was established, and methods for application of the fertilizers to the beaches were finalized. Information on these aspects is given in Section 6.

- May 25 - Background information on the extent of oil contamination on the test beaches in Snug Harbor was collected, and chemical analyses were subsequently conducted. These results are given in Section 7.
- Jun 2 - Extensive efforts were undertaken to develop an ecological monitoring program to look for potential toxicological and eutrophication effects. Collection of background data from the site was started. These activities are summarized in Section 7 of this report.
- Jun 4 - Microcosm studies were designed to model the nutrient treatments on the test plots. These studies had the best potential for determining weight changes in the oil as a result of biodegradation and nutrient enhancement. The design and construction of microcosms that could be accommodated aboard the F/V AUGUSTINE was initiated in the first week of June. A summary of the microcosm studies is given in Section 9.
- Jun 4 - Because of a greater than expected time requirement in establishing a microbiology laboratory, only one type of method could be established to measure the number of oil degraders. Microbiological media to perform these tests were prepared at the EPA Environmental Research Laboratory in Athens, Georgia, and shipped to Valdez for the first sampling. Results from the microbiology analyses studies are given in Section 7 of this report.
- Jun 8 - Following delays due to complications with logistics and weather, field application of the nutrients was begun. Details of this application are given in Section 6 of this report.
- Jun 12 - A major rainstorm washed much of the oleophilic fertilizer off the beaches. This fertilizer was reapplied.
- Jun 15 - A workshop on "Beach and Nearshore Hydraulics" was held in Seattle, Washington, to determine the effects of tides, waves, and groundwater flow on the transport and transformation of nutrients in the porous, steep beaches in Prince William Sound.

- Jun 20 - Visual loss of oil from the rock surfaces of the cobble beaches treated with oleophilic fertilizer was noticed. The clearing remained apparent for approximately 3 weeks, at which time reoiling occurred reducing the contrast with untreated areas. The untreated control plots had not changed.
- Jul 1 - The first status report on the field demonstration project was submitted to Exxon.
- Jul 18 - A recommendation for large-scale application of fertilizers to beaches in Prince William Sound was submitted to Exxon.
- Jul 24 - A site at Passage Cove, on the north end of Knight Island, was selected for additional nutrient and beach hydraulics investigations on a beach that had been physically cleaned of spilled oil.
- Jul 25 - Application of the Oleophilic and slow release granular fertilizers was performed on Tern Beach. The sprinkling system on Kittiwake was also set into operation.
- Jul 27 - Wells were dug and the well equipment installed on the Kittiwake Beach plot in Passage Cove.
- Aug 10 - Passage Cove beaches treated with oleophilic and slow-release fertilizer showed marked removal of black oil residues relative to the reference beaches.
- Aug 18 - Clearing of oil from the beach at Passage Cove treated with fertilizer solution from a sprinkler system was evident.
- Sep 4 - Final sampling at Passage Cove.
- Sep 8 - Final sampling at Snug Harbor.
- Sep 15 - September 15 Addendum to the July 1 Status Report.
- Nov 8 - Workshop held in Gulf Breeze, FL, to discuss data analysis, interpretations, and significant findings.
- Dec 22 - Followup workshop held in Washington, D.C., in which the outline for a winter research plan was established.

## **SECTION 4**

### **SITE SELECTION AND CHARACTERISTICS**

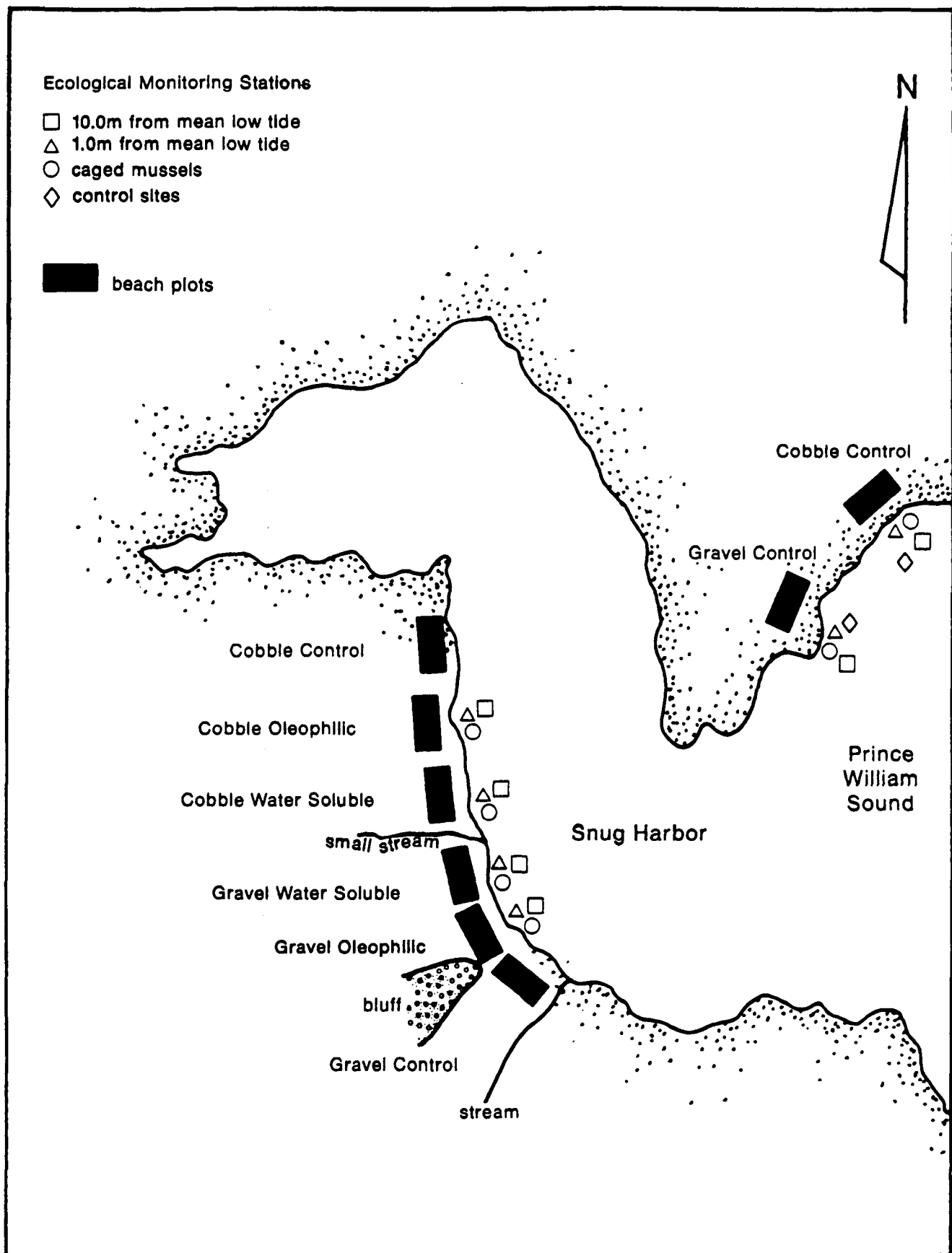
Two test sites were selected for the field demonstration project, Snug Harbor and Passage Cove. Snug Harbor was selected to serve as a beach with oil contamination that approximated the degree of contamination remaining after a heavily oiled beach had been physically washed. Physical washing was the major cleanup procedure used by Exxon. Physically washed beaches were not available for testing early in the summer. In July, a second site was selected that had been physically washed by the Exxon operations. This site, Passage Cove, served as the main reference beach for the large-scale application of fertilizers and as a means to evaluate a sprayer system for fertilizer application.

Criteria for the selection of the test sites were based on the following:

- Typical shoreline of Prince William Sound; i.e., mixed sand and gravel and cobblestone beaches
- Sufficient area with fairly uniform distribution of sand, gravel, and cobble for the test plots
- Protected embayment with adequate staging areas and sufficient size to support several test and control plots
- Uniform oil contamination
- Minimal impact from freshwater inputs
- Shoreline with a gradual vertical rise

#### **SNUG HARBOR PROJECT SITE**

Snug Harbor is located on the southeastern side of Knight Island. The shoreline utilized for the demonstration is located on the western side of this harbor (Figure 4.1). The area is surrounded by mountains, reaching an elevation of approximately 2,000 feet, with steep vertical ascents. Major sources of freshwater runoff are from precipitation and snowmelt, which is typical of islands in Prince William Sound. Although other shorelines in Snug Harbor were heavily contaminated with oil, it appeared that little oil was being released to the water, thus minimizing the prospect of reoiling on the beaches chosen for treatment and reference plots.



**Figure 4.1. Sampling Locations at Snug Harbor, Knight Island, in Prince William Sound, Alaska.**

Table 4.1 identifies the beach types, dimensions, and treatment. Each plot was divided into 21 blocks, with 7 blocks in 3 tidal zones: high, intermediate, and low tidal areas.

Oil contamination in the test area represented a continuous band along the length of the beach. This band was approximately 15 to 20 meters wide and corresponded roughly with the average boundaries of the high and low tides observed in Snug Harbor. To determine the approximate distribution of oil on the beach, samples of beach material from one of the designated mixed sand and gravel plots were taken on May 25, 1989. The samples were extracted, and the oil weight and chemical composition were determined. Methods for the sampling and analysis are given in Section 6. The oil residue weights and ratios of C17/pristane and C18/phytane at two different depths are shown in Table 4.2. It is clear that oil concentrations varied considerably, ranging from a high of 67,000 mg/kg of beach material to a low of 37 mg/kg of beach material. In general, higher concentrations were found in the top 10 cm of the beach. Changes in the ratios, relative to fresh Prudhoe Bay crude, were also apparent in some samples, indicating biodegradation of the oil. Changes were quite variable, but it does appear that biodegradation was occurring at the lower depths.

#### PASSAGE COVE PROJECT SITE

Passage Cove is located on the northwestern side of Knight Island. This site was originally heavily contaminated with oil and was subjected to physical washing by Exxon. Even after physical washing, considerable amounts of oil remained at this site, mostly spread uniformly over the surface of rocks and in the beach material below the rocks. Pools of oil and mousse-like material were minimal on the surface. Contamination was apparent to about 50 cm below the beach surface. The shoreline area and the designated beaches in Passage Cove are shown in Figure 4.2. All of the beach areas tested were cobblestone set on a mixed sand and gravel base. Table 4.3 lists Passage Cove beach designations and plot dimensions and fertilizer treatments.



Table 4.1. Description of Fertilizer Treatment Demonstration Plots at Snug Harbor

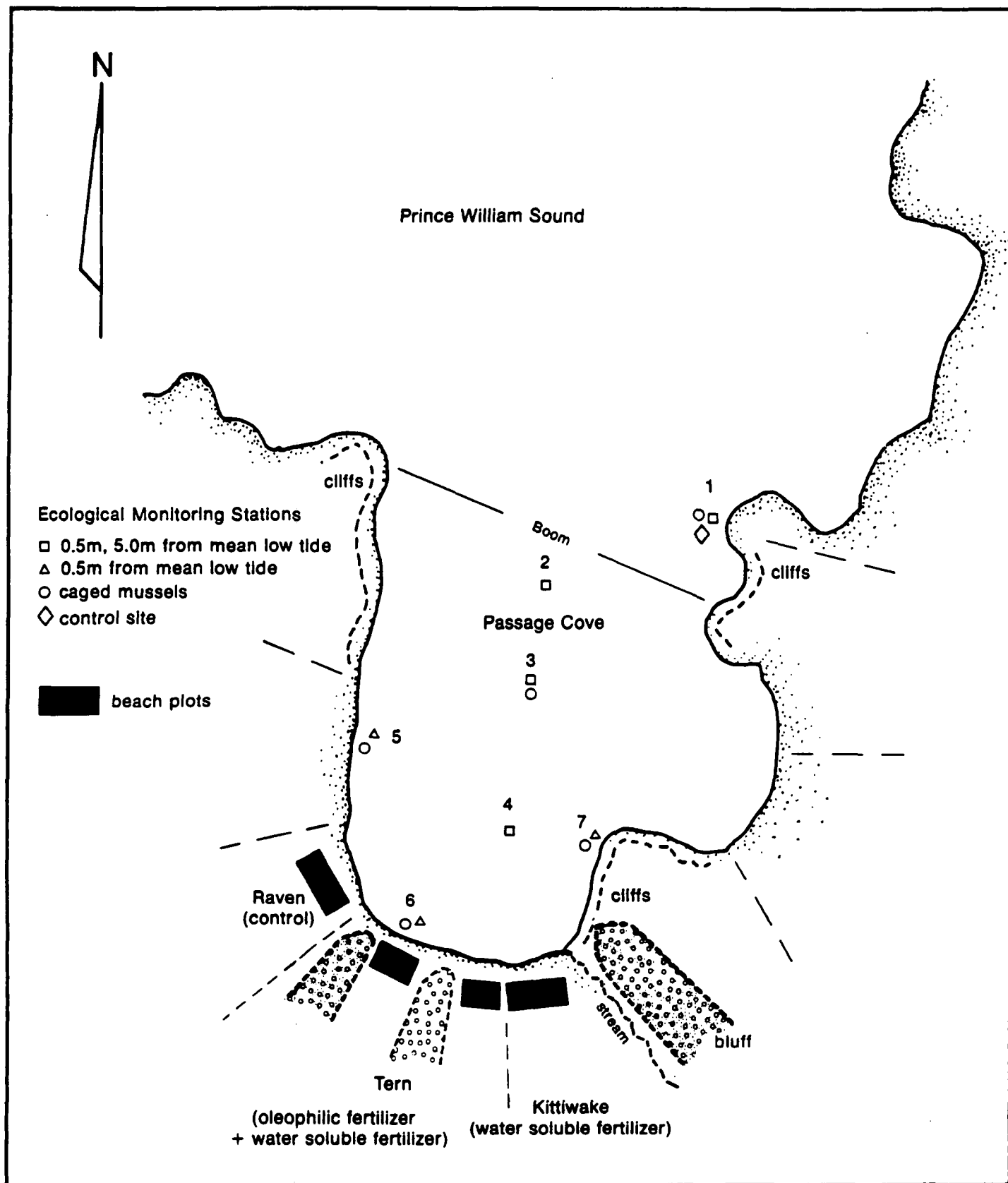
<u>Beach Name</u>	<u>Beach Type</u>	<u>Fertilizer Treatment</u>	<u>Length (m)</u>	<u>Depth (m)</u>
Eagle	Sand, gravel	None-reference	21	12
Otter	Sand, gravel	Oleophilic fertilizer	21	12
Otter	Sand, gravel	Water-soluble fertilizer	35	12
Seal	Cobble	Water-soluble fertilizer	28	12
Seal	Cobble	Oleophilic fertilizer	28	12
Seal	Cobble	None-reference	21	8

Table 4.2. Analysis of Oil Extracted from Mixed Sand and Gravel Samples Taken from Otter Beach on May 28, 1989, Two Weeks Prior to Fertilizer Application<sup>a</sup>

Block No.	TOP (0-10 cm)			BOTTOM (10-20 cm)		
	Residue wt. (mg/kg)	C17/ Pristane <sup>b</sup>	C18/ Phytane <sup>b</sup>	Residue wt. (mg/kg)	C17/ Pristane	C18/ Phytane
1	100	0.8	1.0	253	0.9	0.8
2	29,000	1.6	1.9	18,300	1.6	2.0
	30,100	1.5	1.7			
3				296	1.0	1.2
4	6,070	1.5	1.8	2,600	1.5	1.9
5	2,030	1.2	1.5	37	0.8	1.0
6	6,600	1.2	1.7			
7	1,440	1.1	1.4			
8	1,030	0.8	1.1			
9	7,600	1.4	1.7			
10				97	1.1	1.3
				365	0.8	1.1
				469	0.9	1.1
				412	0.9	1.1
11	9,820	1.5	1.8	512	1.2	1.5
12	658	1.5	1.9	8	1.0	1.2
13	67,200	1.6	1.8	9,280	1.6	1.8
				9,620	1.5	1.8
				8,100	1.6	1.9
14				45	0.9	0.9
15				538	1.3	1.6
16				80	0.9	1.3
17						
18				622	1.1	1.4
19				125	0.9	1.3
20	1,560	1.0	1.3			
21	<u>8,190</u>	<u>1.6</u>	<u>1.7</u>	<u>1,790</u>	<u>1.4</u>	<u>1.6</u>
Mean	12,242	1.3	1.6	2,169	1.1	1.4
Std Dev	+/- 18,556	+/- 0.3	+/- 0.3	+/- 4,842	+/- 0.3	+/- 0.3

<sup>a</sup> The Otter Beach plot was divided into three equal zones lengthwise across the beach to represent high, mid, and low tide areas. Each zone was divided into 7 equal blocks and blocks were numbered from left to right consecutively, starting with the high tide zone.

<sup>b</sup> C/17 Pristane and C18/Phytane ratios in fresh Prudhoe Bay crude oil are approximately 1.7 and 2.0, respectively.



**Figure 4.2. Sampling Locations at Passage Cove, Knight Island, in Prince William Sound, Alaska.**

Table 4.3. Description of Fertilizer Treatment Demonstration Plots at Passage Cove

<u>Beach Name</u>	<u>Beach Type</u>	<u>Nutrient Applications</u>	<u>Length (m)</u>	<u>Depth (m)</u>
Raven	Cobble over mixed sand and gravel	None-reference	28	21
Tern	Cobble over mixed sand and gravel granules	Oleophilic and slow-release	35	21
Kittiwake	Cobble over mixed sand and gravel	Nutrient solution sprinkler system	28	21
Guillemot	Mixed sand and gravel with patchy cobble	Oleophilic and granules slow-release	21	7

## **SECTION 5**

### **FERTILIZER SELECTION AND CHARACTERISTICS**

#### **BACKGROUND**

An important aspect of this project was the selection of fertilizers for the field test. The goal was to find fertilizer formulations which would release nitrogen and phosphorous nutrients over extended time periods or would keep nutrients in contact with surface microbial communities over extended time periods. In addition, consideration was given to formulations that were amenable to practical and inexpensive application to contaminated shorelines, keeping in mind the possibility of large-scale applications in the future. Three types of fertilizer were selected:

- 1) Solid, slow-release fertilizer, in which nutrients would be released slowly from a point source and tidal action would distribute the nutrients over the beach surface.
- 2) Liquid oleophilic fertilizer, in which nutrients would "dissolve" into the oil covering the rock and gravel surfaces. Nutrient distribution over the beach material would be accomplished in the original fertilizer application.
- 3) Fertilizer solutions, in which inorganic nitrogen and phosphorus would be dissolved in seawater and distributed via fixed sprinkler systems.

Several commercially available fertilizer formulations that satisfied these requirements were selected and their nutrient-release characteristics determined. A small study was also conducted to see how specific solid fertilizer formulations physically behaved under field conditions.

#### **SELECTED FERTILIZER FORMULATIONS**

The fertilizers selected are described below.

An oleophilic fertilizer was incorporated for testing under recommendation by participants in the original April workshop held to evaluate the feasibility of bioremediation. It was suggested that the oleophilic application might be suited for application to cobble and rocky beaches where the application of soluble nutrients might prove difficult or ineffective.

### "Woodace" Briquettes

This fertilizer formulation contains isobutylidene diurea (IBDU),<sup>c</sup> a chemical that spontaneously hydrolyzes into isobutyl aldehyde and urea when released from the briquette matrix into water. This process is responsible for the slow release of nitrogen that is characteristic of the product. Hydrolysis is temperature dependent, being slower at lower temperatures but still significant. The source of phosphorus is Linstar, a citric acid soluble phosphatic fertilizer developed by Mitcubichi Chemical Corp. Each briquette weighs approximately 17 grams and has a specific gravity of 1.5 to 1.8. This fertilizer has a N:P:K ratio of 14:3:3.

### IBDU Granules

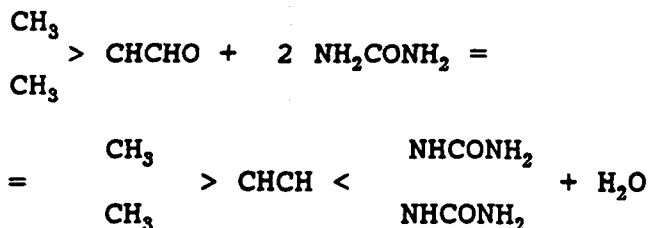
This fertilizer is a granular 24:4:12 (N:P:K) fertilizer formulated to give an immediate and sustained release of nutrients. The product used was Par Ex, produced by Estech, Inc. All of the nitrogen is derived from ammonium phosphate, urea, and IBDU. A minimum of 45% of the nitrogen is derived from the IBDU. The available phosphorus is derived from potassium magnesium phosphate. Iron is also present as ferrous sulfate. The granules have a specific gravity of 1.3.

### "Osmocote" Briquettes

Manufactured by Sierra Chemicals, this fertilizer contains urea formaldehyde as the nitrogen source in a slow release formulation created by thermoplastic resin encapsulation. The urea formaldehyde released from the briquettes must be biologically hydrolyzed to produce ammonia. This fertilizer has an N:P:K ratio of 20:10:5. Phosphorus is present as calcium phosphate. Iron is also present as ferrous sulfate. Each briquette weighs 21 grams.

---

<sup>c</sup>Isobutylidene Diurea (IBDU) is a condensation product of urea and iso-butyraldehyde. The reaction can be carried out both in aqueous solution and between solid urea and liquid aldehyde as follows:



### "MAGAMP"

This fertilizer formulation contains magnesium ammonium phosphate (MAGAMP), which is sparingly soluble in water. It is made by Martin Marietta Magnesia Specialties. The chemical congeals when wetted and can then be cast into different shapes and dried into solid forms (granules, briquettes, bricks) of varying densities, which will slowly release ammonia and phosphate when submersed. This fertilizer has a N:P:K ratio of 9.2:27:0. This fertilizer is available in briquettes that weigh 209 gm each and bricks of 8 or 40 lbs.

### "Sierra Chemical" Granules

This fertilizer formulation consists of inorganic nutrient sources (ammonium nitrate, calcium phosphate and ammonium phosphate) contained in a vegetable oil coating (polymerized by reaction with a diene). The coating gives the fertilizer its slow-release characteristic. The N:P:K ratio is 28:8:0. The granules have a specific gravity of 1.8.

### Oleophilic Fertilizer

The only oleophilic fertilizer that was available in sufficient amounts to use in a scaled-up operation was the Elf Aquitaine (France) product, Inipol EAP 22. This is a mixture of nutrients encapsulated by oleic acid (the external phase). Oleic acid and surfactants in the fertilizer formulation cause the nutrients to become sequestered to the oil phase, preventing rapid release of the nutrients into the aqueous phase and subsequent washout. Inipol EAP 22 is a clear liquid with a specific gravity of 0.996, a viscosity of 250 cSt, a pour point of 11°C, and a flash point of >100°C. The N:P:K ratio is 7.3:2.8:0.

The main ingredients in Inipol EAP 22 are oleic acid and urea, along with chemicals to maintain them in a microemulsion. The chemical composition of Inipol is given in Table 5.1. The product is designed to initially stimulate oleic acid degrading bacteria. The quantity of nitrogen and phosphorus present is sufficient to allow the natural oleic acid degraders found in the receiving environment to consume all of the oleic acid carbon present in the Inipol. Once the added oleic acid is consumed, and the numbers of oleic acid degraders have increased substantially, oil biodegradation is thought to commence. It is not exactly clear why degradation of oil hydrocarbons occurs in this way, but many oleic acid-degrading bacteria are known to

Table 5.1. Inipol EAP 22 Chemical Composition

<u>CHEMICAL</u>	<u>CHEMICAL FORMULA</u>	<u>PURPOSE</u>
Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Oleophilic Phase (Continuous)  Primary Carbon Nutrient
Lauryl Phosphate	$\text{C}_{12}\text{H}_{25}\text{PO}_4$	Phosphate Nutrient Surfactant
2-Butoxy-1 Ethanol	$\text{HO}-\text{C}_2\text{H}_4-\text{O}-\text{C}_4\text{H}_9$	Co-Surfactant Emulsion Stabilizer
Urea	$\text{NH}_2-\text{C}-\text{NH}_2$	Nitrogen Nutrient
Water	$\text{H}_2\text{O}$	Hydrophilic Phase



degrade petroleum hydrocarbons. Elf Aquitaine representatives have suggested that the oleic acid degrading microorganisms may die once they reach a certain density, creating a natural recycling of nutrients through mineralization of this dead biomass.

Oleophilic fertilizer has been shown to work well on sandy beaches, but similar testing has not been done with rock and cobble beaches. Therefore, its use on the rock and cobble beaches found in Prince William Sound represents a new application.

## **NUTRIENT RELEASE CHARACTERISTICS - METHODS**

### **Static Tests**

A specific weight of slow-release soluble fertilizer was placed in cloth bags in a beaker and covered with 1400 mls of artificial seawater (Instant Ocean, supplied by Aquarium Systems, Inc.) Granules were contained in cloth bags. The beakers were incubated at 15°C without mixing. According to an established schedule, water was decanted out of the beaker and replaced with fresh seawater. The amounts of ammonia (EPA method 350.1), nitrate (EPA method 353.1), total phosphorus (EPA method 365.4), and total Kjeldahl nitrogen (TKN) (EPA method 365.4) were measured in the decanted water.

Flask studies, to determine the effect of microbial activities on the release of ammonia from the TKN leached out of the IBDU briquettes, were conducted by covering briquettes with three types of water: deionized, sterile (filter sterilized - 0.22 $\mu$ ) seawater, and non-sterile seawater. The amount of ammonia released over time was determined. The experiments were also run at two different temperatures (9° and 21°C).

The oleophilic fertilizer was applied to the surface of oil-contaminated rocks obtained from Prince William Sound. The rocks were then covered with seawater. Varying amounts of fertilizer were applied to determine the best application rate for retention of Inipol on the oiled rocks. Excess fertilizer, when not adsorbed to the oiled surfaces, loses its solution properties on contact with water and releases urea very rapidly.

### **Intermittent Submersion Tests**

A rocker table equipped with a 19" long, 4" wide pipette tray was used to simulate tidal action and the intermittent submersion of the different fertilizer types. A nutrient bag or nutrient briquettes were buried in clean Alaskan beach material at one end of the tray, or the oleophilic fertilizer was sprayed directly on beach material and artificial seawater added. The device was maintained in a cold room at a temperature of 15°C.

The rocker table was started and samples were taken after 5, 15, 30, and 60 minutes. The table was then stopped, the water drained, and the beach material allowed to remain undisturbed for 4 hours. The rocks were covered again with water, the table was operated for another hour, and sampling was repeated.

### Field Tests

To test nutrient release characteristics of MAGAMP under field conditions, 8 lb and 40 lb MAGAMP bricks, were placed on a sand and gravel beach at Snug Harbor and samples were taken at different locations down the beach from the bricks. Sampling points were placed to measure the downward and lateral spread of nutrients released from the bricks during tidal cycle. The weight of the bricks was expected to minimize their movement. Nutrient samples were collected 12, 24, and 96 hours after placing the bricks on the beach.

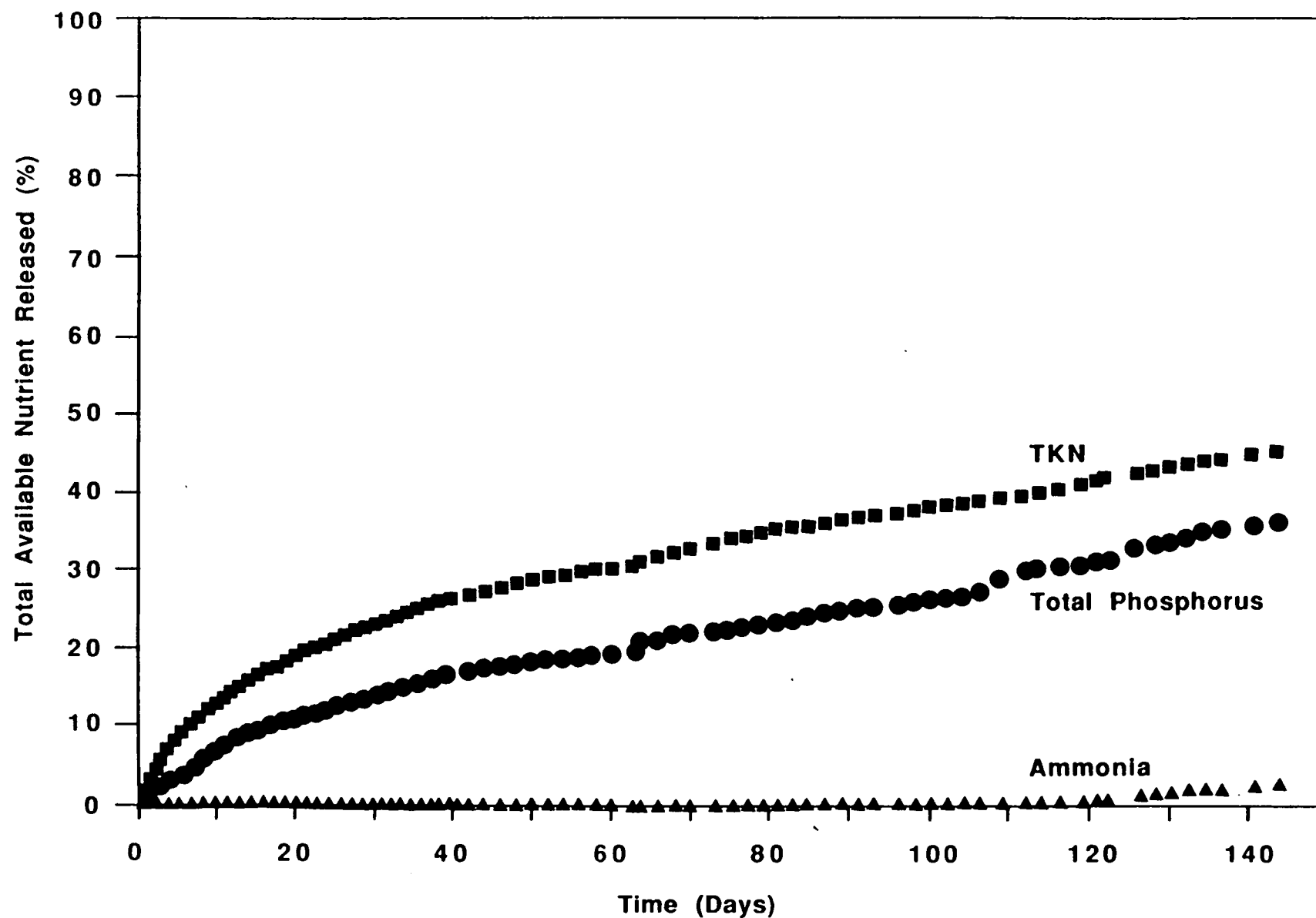
### **NUTRIENT RELEASE CHARACTERISTICS - TEST RESULTS**

#### "Woodace" IBDU Briquettes

The cumulative nutrient release pattern for ammonia, total phosphorus, and TKN from static tests with the IBDU briquettes is shown in Figure 5.1. Nitrate was formulated into the briquettes. Release rates per day for each nutrient are shown in Figure 5.2. Rates for ammonia release were generally constant, excluding the initial rapid release. Release rates for TKN dropped dramatically over the 60-day test period. Release rates for total phosphorus, although somewhat variable, were constant over time. The variability was probably associated with analytical error. Overall, release rates were slightly increased for all nutrients if the frequency of water exchanges rates was increased 5% (data not shown).

It is apparent that small amounts of ammonia and phosphorus are released with each 24-hour soaking of the briquettes. The average amount of ammonia released per day is approximately 100-fold higher than background levels in Snug Harbor waters (see Section 8: Nutrients). However, considering the rapid dilution of ammonia that will occur in the field following release from briquettes, it would be unlikely to measure any increased concentrations of ammonia (discarding contributions from the TKN) in the field as a result of fertilizer application. The total amount of ammonia released is only a small fraction of the total nitrogen available in the formulation.

Large amounts of TKN were released, accounting for as much as 17% of the total available nitrogen after 17 days, 31% after 60-days, and 45% after 140 days. The TKN is probably urea since the IBDU will rapidly hydrolyze on release from the briquettes. Urea, in the absence of bacteria would not be hydrolyzed to  $\text{NH}_4^+$ .



**Figure 5.1. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from Woodace IBDU Briquettes in Static Flask Experiments.**

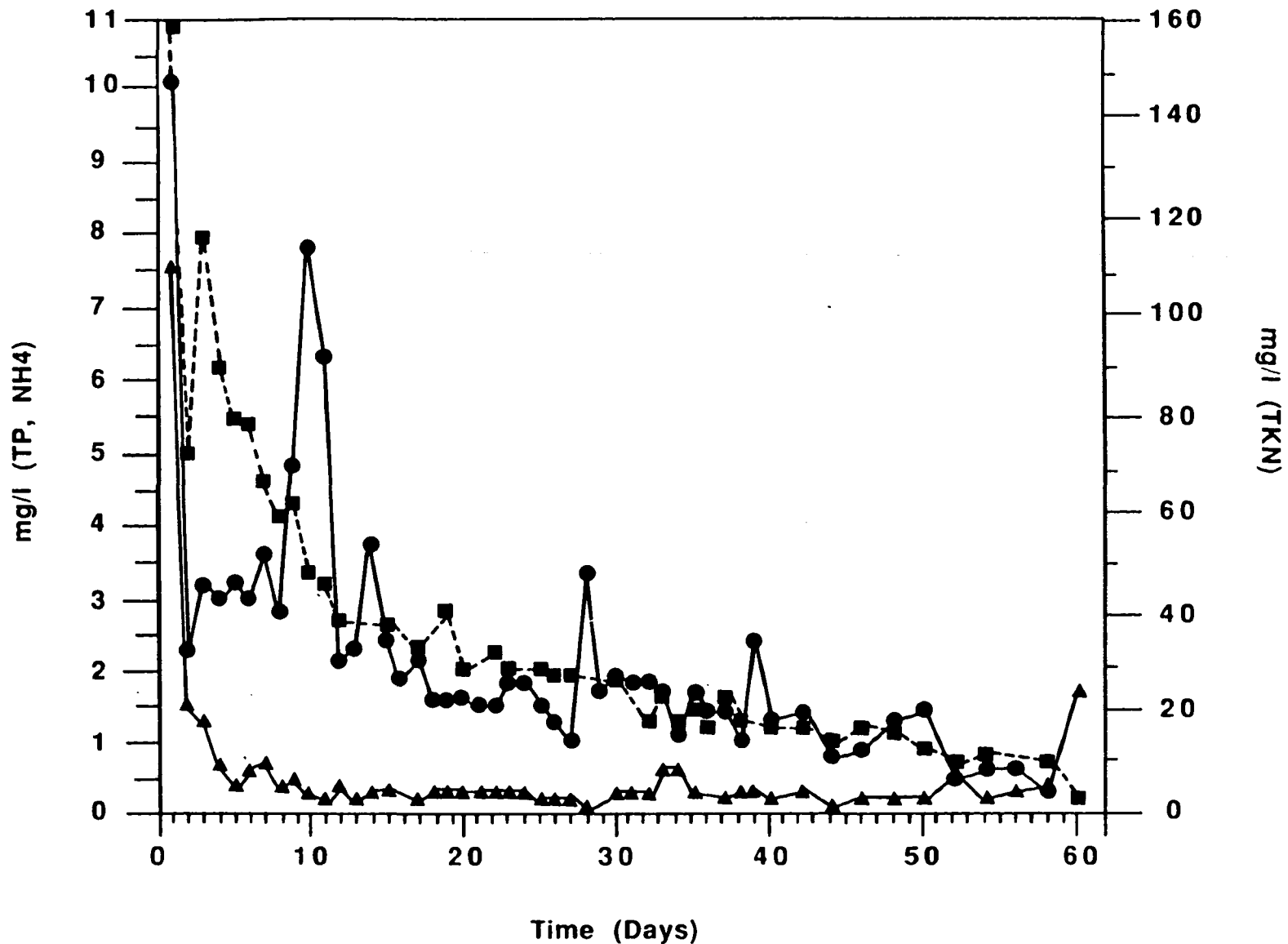


Figure 5.2 Daily Nutrient Release Rate of Ammonia ( $\text{NH}_4$ ), Total Phosphorus (TP), and Total Kjeldahl Nitrogen (TKN) from IBDU Briquettes.

To determine if the TKN was a source of ammonia under natural conditions, briquettes were soaked for 3 successive 1-hour periods (water was changed for each period) using 2 different temperatures and 3 different sources of water. The results are shown in Figure 5.3. Significant amounts of ammonium were released under all conditions. The lowest amount of ammonia released occurred with filtered seawater (containing no microorganisms). Since this was less than that released in the presence of deionized water, it suggests that there was an ionic strength effect on the ammonia release. Temperature had little effect on release rates in these media. However, with unfiltered seawater, considerably more ammonia was released, particularly at the higher temperatures. This indicated a possible biological effect on ammonia release, presumably through the microbial breakdown of the TKN fraction.

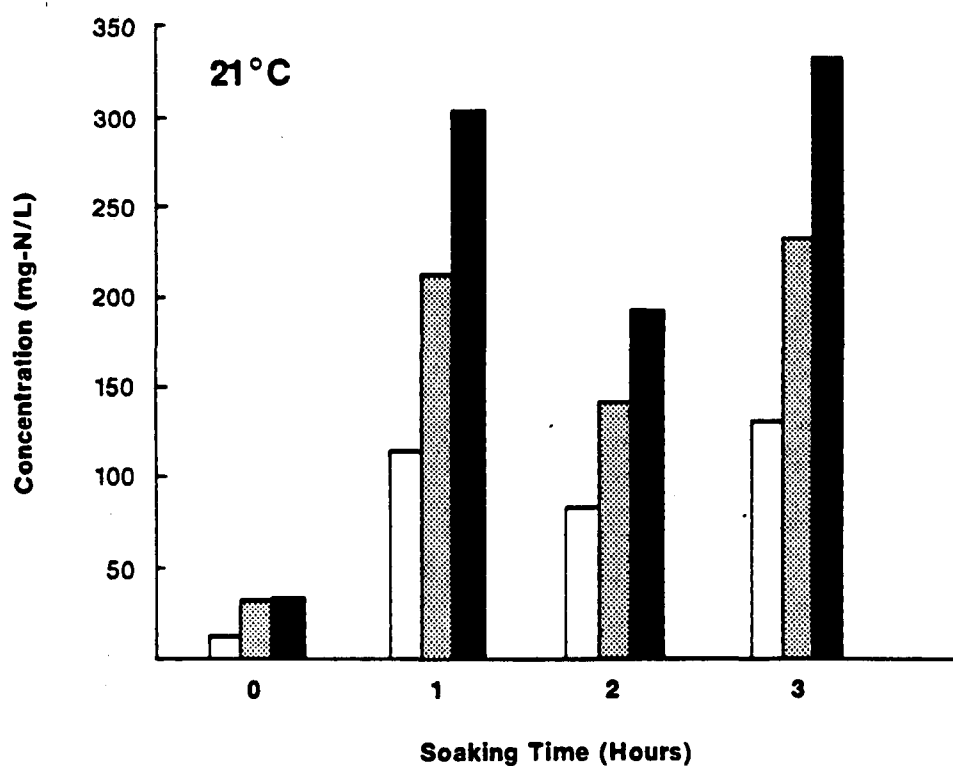
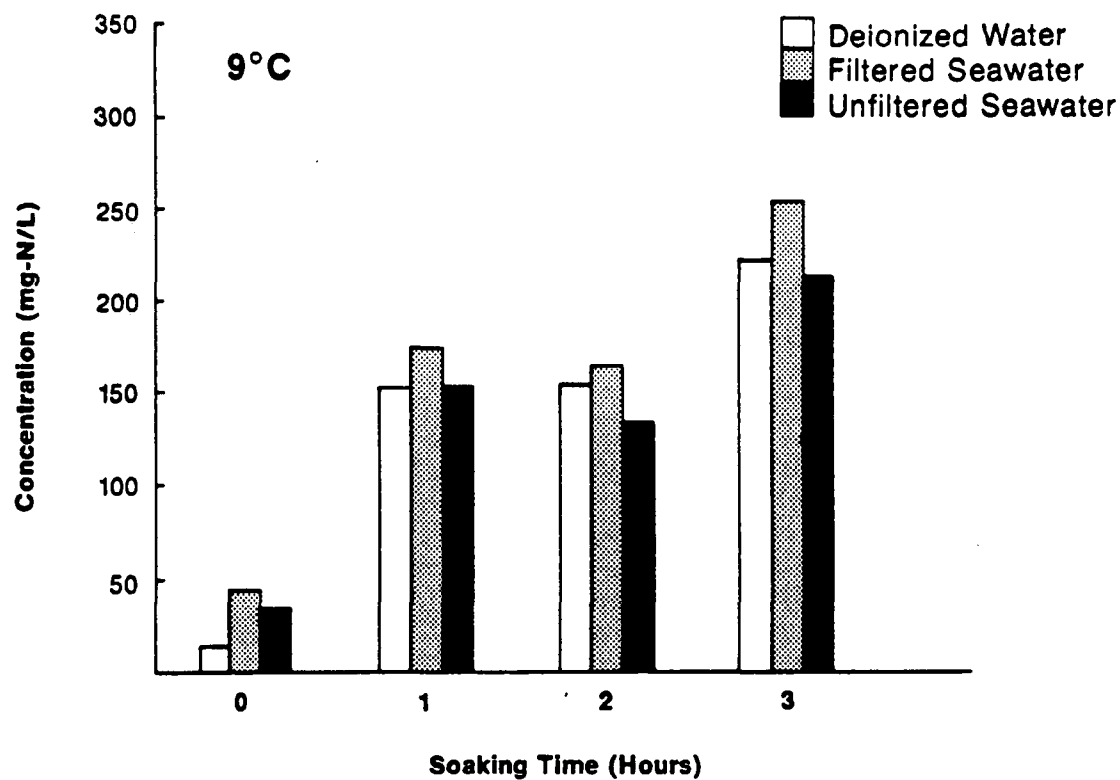
Nutrient release from the IBDU briquettes was also tested using the rocker table system. In general, concentrations of the nutrients and TKN released were similar to those observed in the static tests.

Under all conditions, the physical integrity of the IBDU briquettes was excellent with very little change in shape and consistency occurring after one month of submersion in water. A simple freeze/thaw experiment was also conducted on the Woodace briquettes. The experiment consisted of alternately freezing and thawing submerged and non-submerged briquettes, then weighing and visually observing changes. Preliminary results indicated good durability. The briquettes appeared to be a good choice for field application.

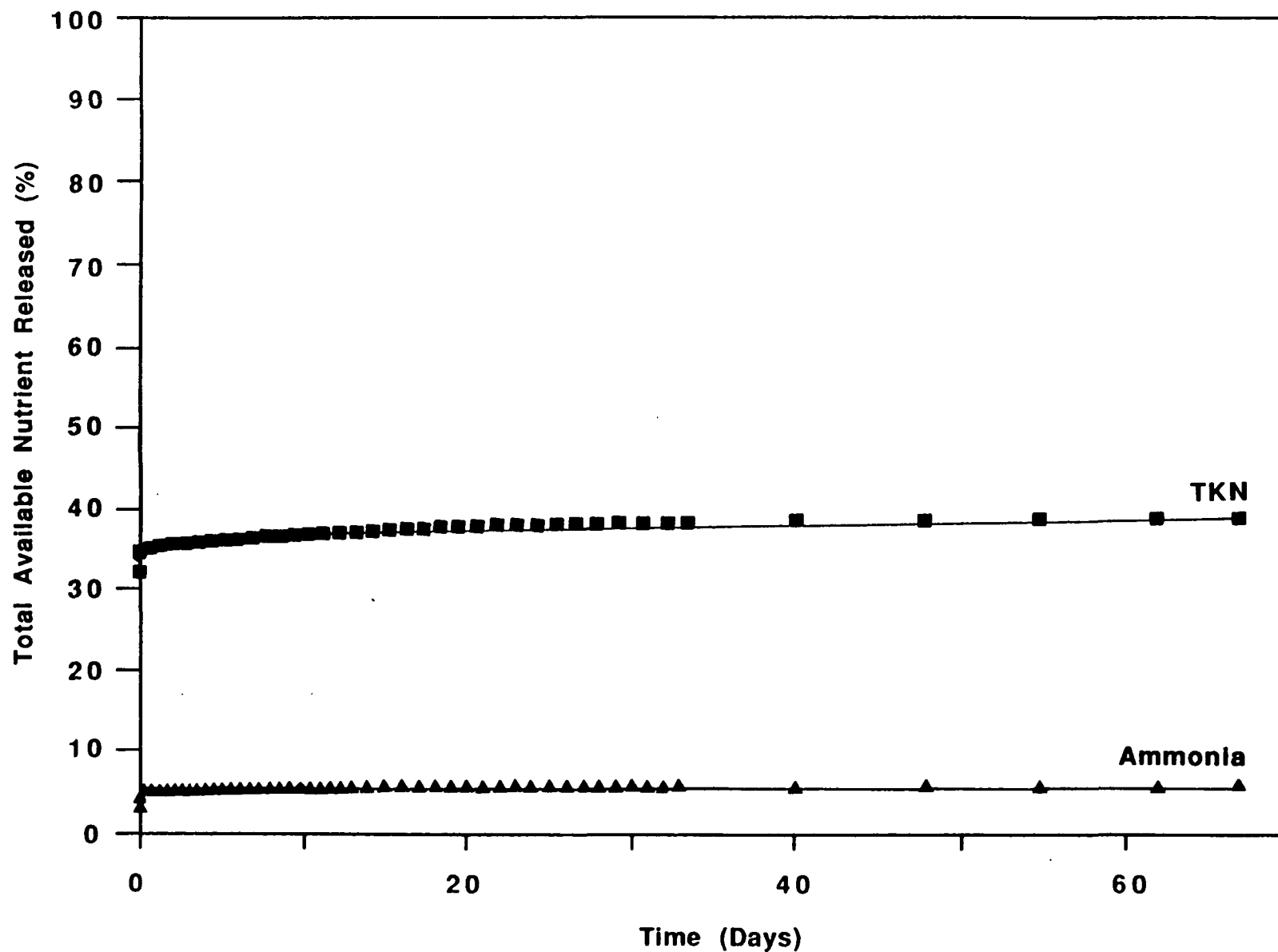
Studies were also performed on the movement of briquettes broadcast on the beach. Results showed that unconfined briquettes will not retain this position distributed after several tide cycles. Greater redistribution occurred on sand and gravel beaches as compared with cobble beaches. Due to the rapid redistribution of briquettes on sand and gravel beaches, this form of fertilizer application is best applied in containers which will hold the briquettes in place. Unconfined briquettes may be of some limited use on sheltered cobble beaches, where wave action may have less influence on the beach.

#### IBDU Granules

The cumulative nutrient release pattern for ammonia, phosphate, and TKN from IBDU granules in bags using static tests is shown in Figure 5.4. Typically high amounts of nutrient were released initially, followed by a very slow release. If the experiment was repeated with the granules layered on the bottom of the beaker (i.e., no bag to contain the granules), a slower and more gradual nutrient release pattern was observed. This is shown in Figure 5.5. The greater initial release when the



**Figure 5.3. Ammonia Release From IBDU Briquettes at 9 and 21 Degrees Centigrade in 3 Different Water Sources.**



**Figure 5.4. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) from IBDU Fertilizer Granules Contained in Bags in Static Flask Experiments.**

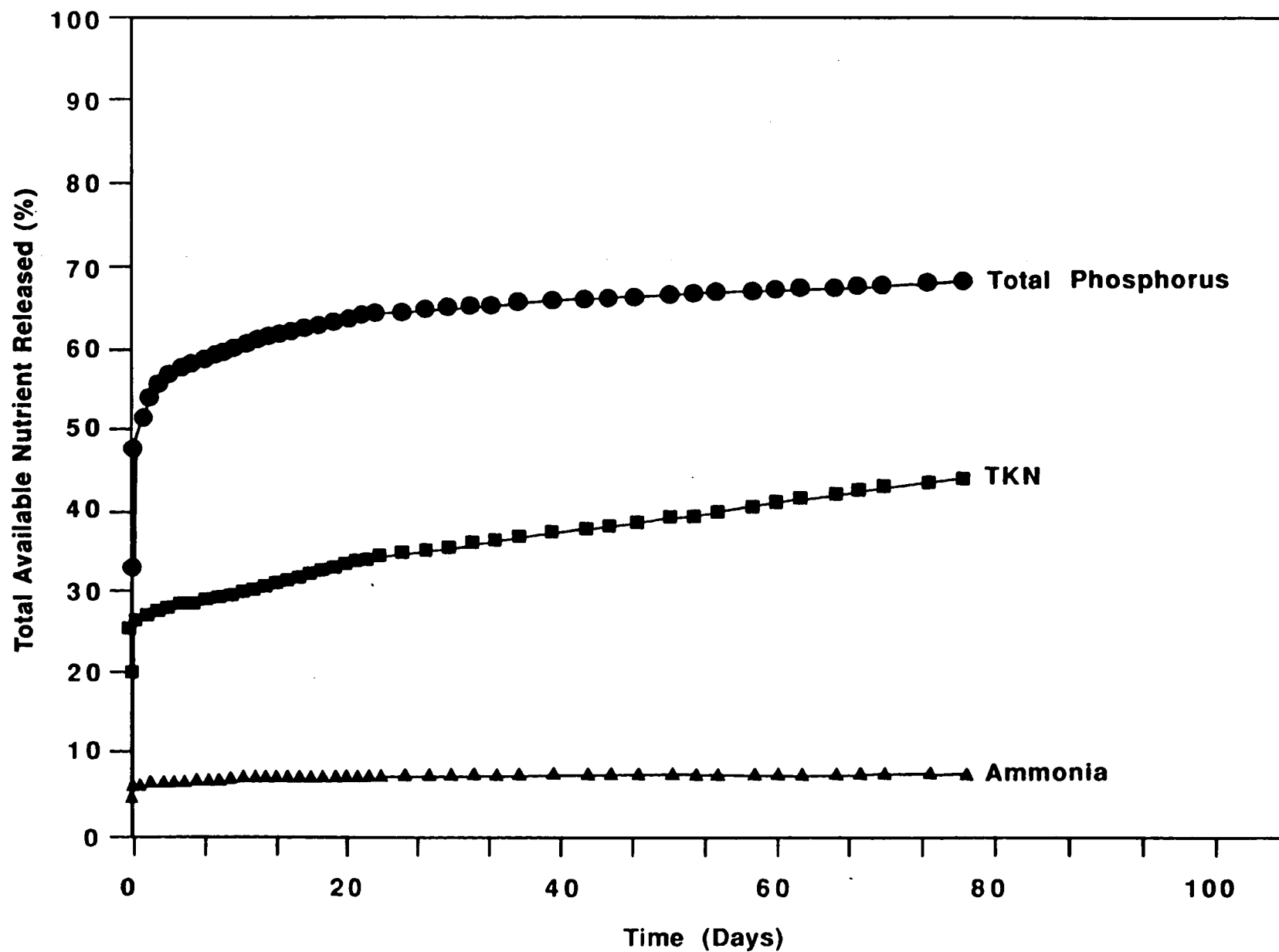


Figure 5.5. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from IBDU Granules in Static Flask Experiments.



granules were contained in bags was probably due to either the methods of exchanging water in these experiments (unbagged IBDU granules were drained through a fine mesh, whereas bagged granules were drained through very fine mesh cloth) or increased water contact experienced by the unbagged granules. In the later case, unbagged granules were loosely packed in the bottom of the flasks, and the water was stirred both before and after each water exchange. Granules in bags were tightly packed, and the water was not stirred when it was exchanged.

Additional studies also showed that if the granule bag volume is reduced relative to the bag surface area, slightly more nutrient release occurred (Table 5.2). Thus, the more water passing over the granules, the higher the release rate.

#### "Osmocote" Briquettes

The cumulative nutrient release pattern for ammonia, phosphate, and TKN for static tests is shown in Figure 5.6. After 2 months of testing, approximately 25% of the available nitrogen was released, primarily as TKN, where as almost 60% of the phosphate was released over this time period. The physical form of the briquette was unstable, flaking soon after initial submersion and further decomposing over time. A dye within the briquette turned the water green with each water exchange. These briquettes, despite their good nutrient release characteristics, appeared unsuitable for long-term use in the field.

#### MAGAMP Briquettes

When high-density and low-density MAGAMP briquettes were tested, the low-density briquette (about half the weight of the high density) disintegrated almost immediately upon submersion in the defined nutrient medium, and consequently was not tested. Release of ammonia, TKN, and phosphate from the high-density briquettes was slow and constant (Figure 5.7). After 10 days, only 1.5% of the available nitrogen was released. At 75 days, approximately 5% to 6% had been released. The release rate appeared to be independent of the number and volume of water exchanges. The high-density briquettes appeared to be very durable. When MAGAMP powder was tested, it congealed to a putty-like consistency soon after the experiment was started. Accumulative release of ammonia was about the same as the briquettes.

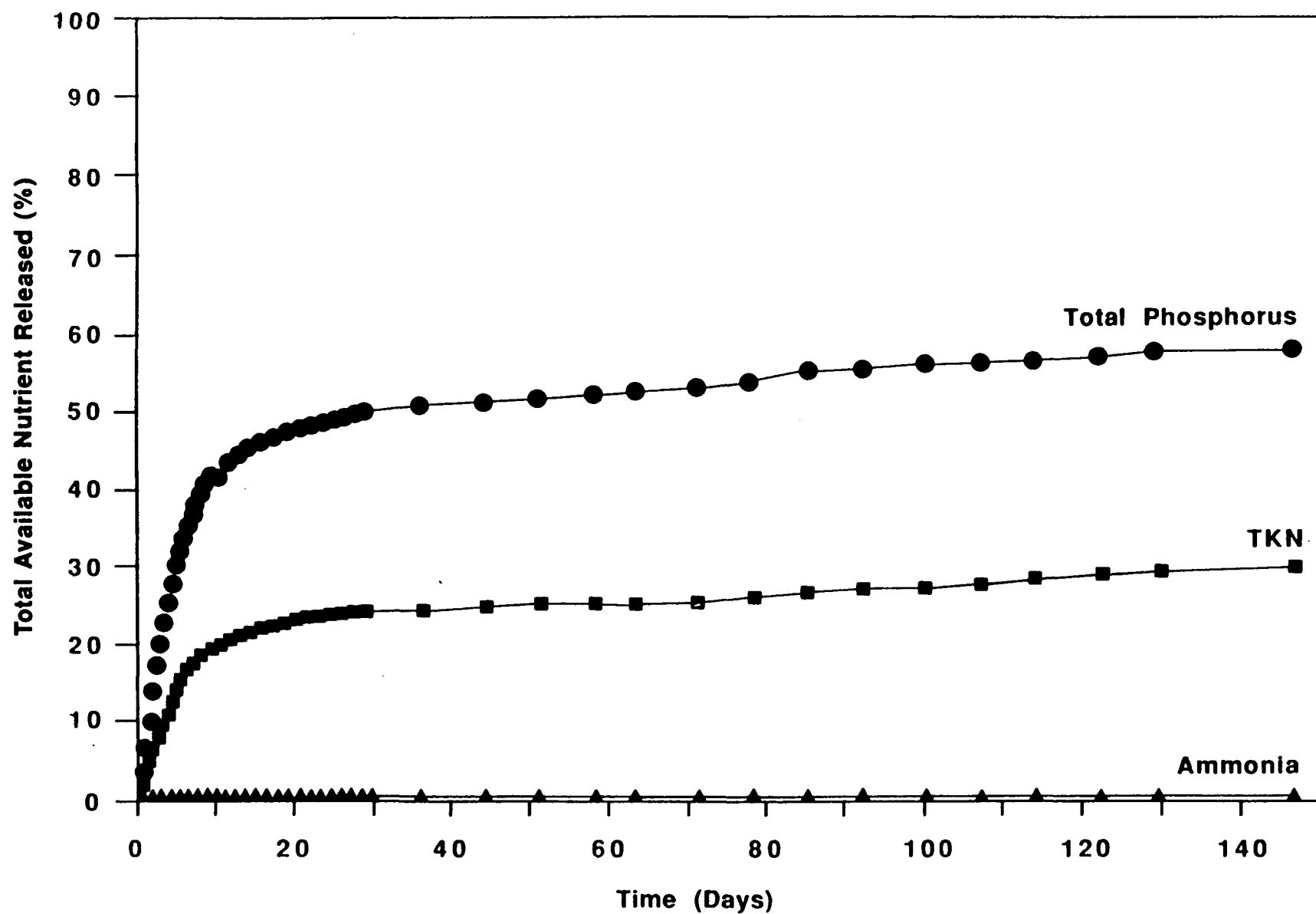
Results from the intermittent submersion tests showed the same low release rates. A high burst of ammonia seen within the first half hour of the test was explained by initial flaking of the briquettes. Flaking at later times did not occur.

Table 5.2. Total Kjeldahl Nitrogen (TKN) Released from IBDU Granular Fertilizer in Bags, Static Water Conditions

<u>Bag Volume/ Surface Area (cubic cm/square cm)</u>	<u>Fertilizer Weight (g)</u>	<u>Seawater Volume (ml)</u>	% of Cumulative Available Nitrogen (TKN) Released in:	
			<u>24 hours</u>	<u>45 Days</u>
1.8	893	4,800	34	38
1.3	256	1,400	36	42
1.3 <sup>a</sup>	258	1,400	39	46
0.6	32	450	41	49

---

<sup>a</sup>Prince William Sound beach material.



**Figure 5.6. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from Osmocote Briquettes in Static Flask Experiments.**

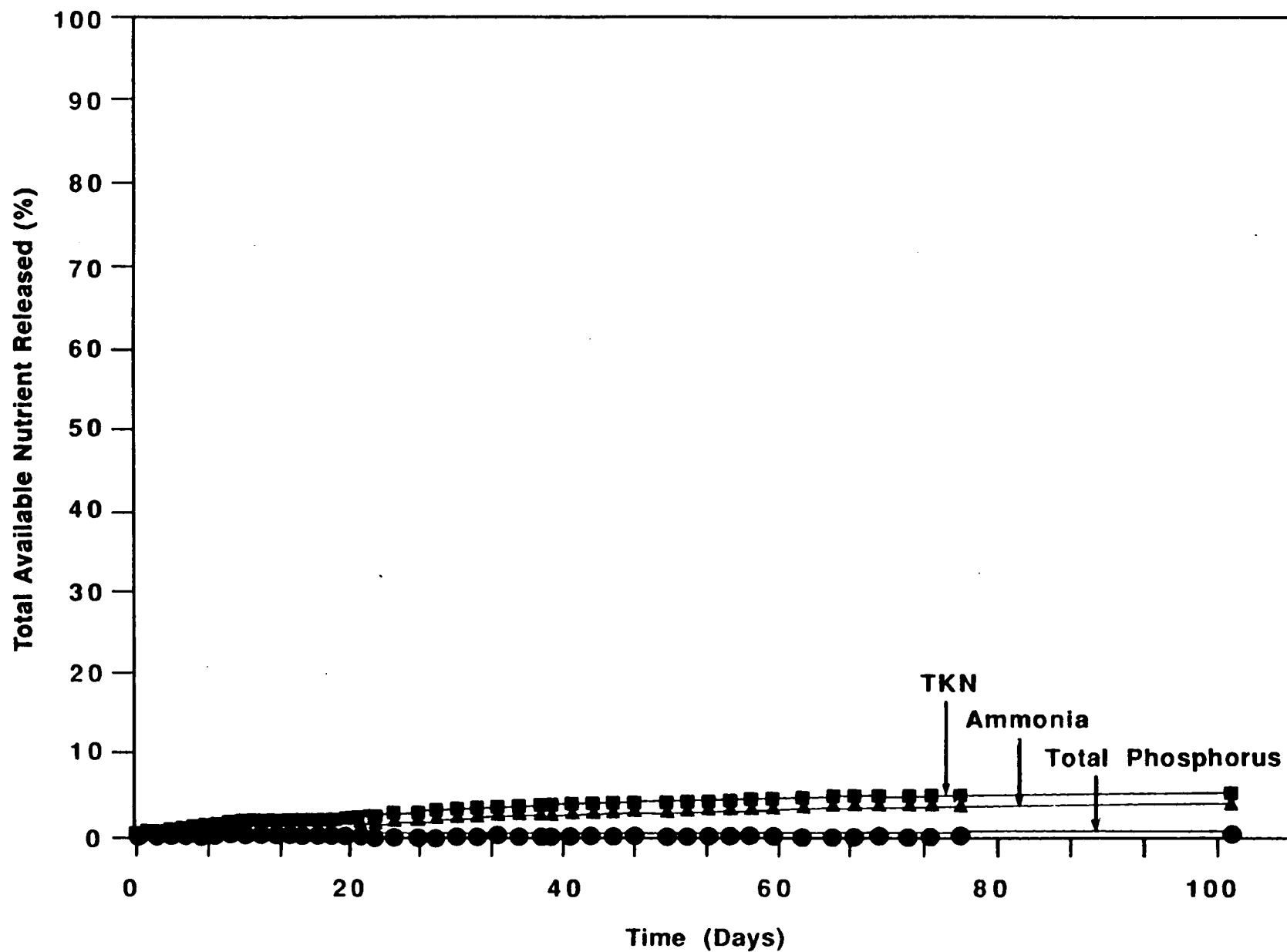


Figure 5.7. Cumulative Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus from MAGAMP Briquettes in Static Flask Experiments.

MAGAMP can be formulated into dense bricks. Bricks weighing 8 and 40 lbs were field tested as an alternative physical form for fertilizer application. The usefulness of these bricks in the field comes from their positional stability on the beach without an anchoring device. However, these bricks could not be produced in large quantity and were, therefore, unavailable for use in any of the fertilization studies. However, because of their potential promise as an alternative physical form of fertilizer, separate beach mechanics studies were conducted to evaluate the nutrient release and distribution characteristics of these bricks. The very slow release of ammonia from MAGAMP made it necessary to determine, under controlled field conditions, if nutrient release could be detected in the field.

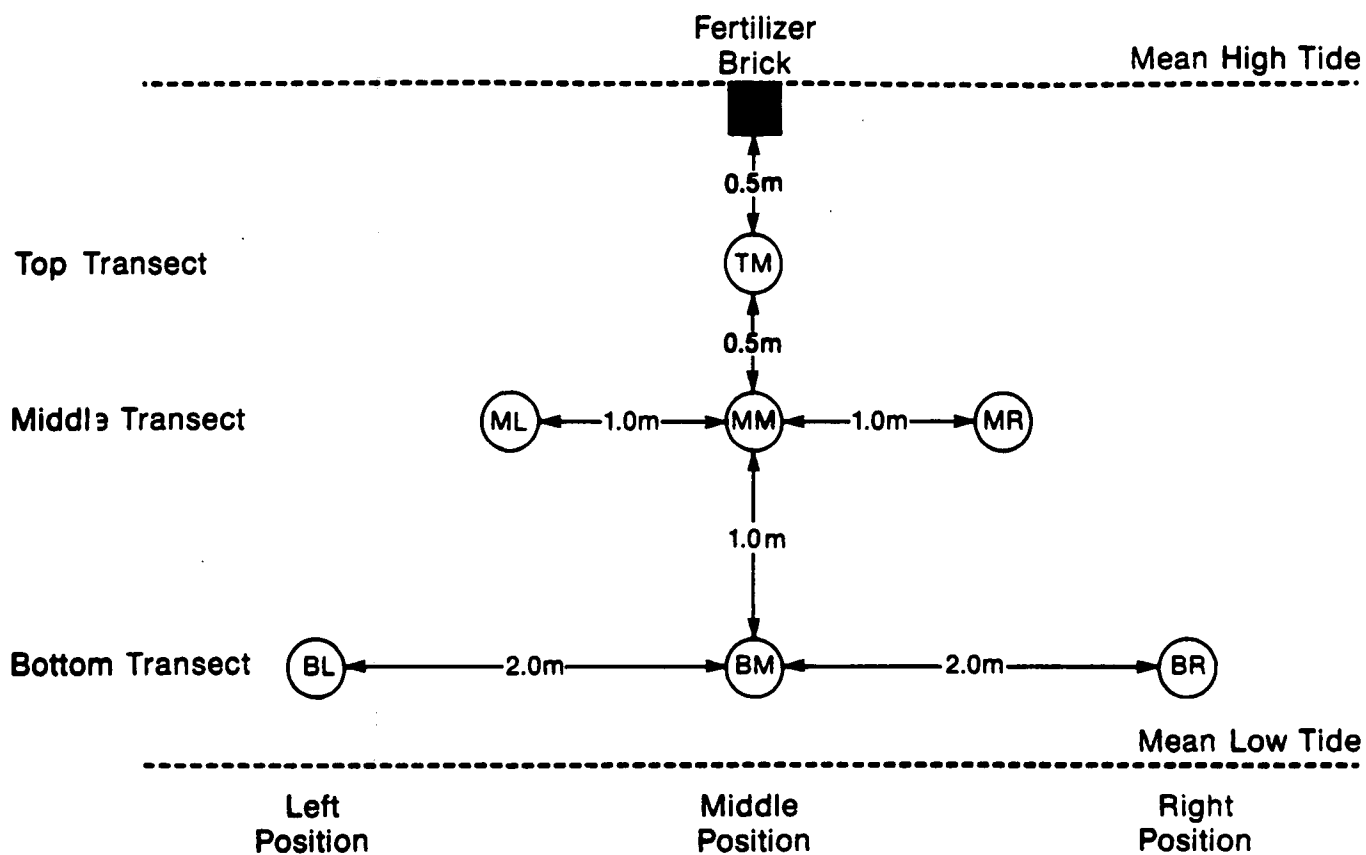
Beach pore water sampling stations were placed down-gradient from MAGAMP bricks as shown in Figure 5.8. Samples for ammonia analysis were collected 12, 24, and 96 hours after initial placement of the bricks. The data are shown in Figure 5.9. The 40 lb brick released up to 138  $\mu\text{M}$  of nitrogen as ammonia, with the highest concentrations directly down-gradient from the block. Significant quantities of ammonia were observed up to 2 m from the bricks at low tide. Ammonia also appears to be well distributed around the brick. The data suggest that this type of point source for fertilizer application could be quite useful in the future.

#### Sierra Chemical Granules

Two tests were conducted to study the effect of water exchange rate on nutrient release from this fertilizer. Figure 5.10 shows the cumulative nutrient release pattern with a variable exchange rate (5 exchanges on the first day, 2 exchanges per day thereafter through the 10th day, daily thereafter through the 40th day, and every other day thereafter. The amount of nitrogen (ammonia and nitrate) released after 80 days was 77% of the total available nitrogen. When this frequency of water exchanges was doubled, 95% of the total nitrogen (approximately half ammonia and half nitrate) was released after 80 days. The shape of the release curves were similar. This effect of water exchanges was probably due to the mechanical agitation of the system prior to each exchange and to the possible abrasive action of the fine mesh screen used to drain the granules.

#### Inipol EAP 22

The results from static tests with this fertilizer are shown in Figure 5.11. All of the nitrogen (>100%) was released within the first few water exchanges. The release of more nitrogen than was theoretically thought to be in the Inipol formulation suggests that manufacturer's specifications for this batch of Inipol were incorrect.



**Figure 5.8. Sampling Point Locations for Magnesium Ammonia Phosphate Fertilizer Field Test**

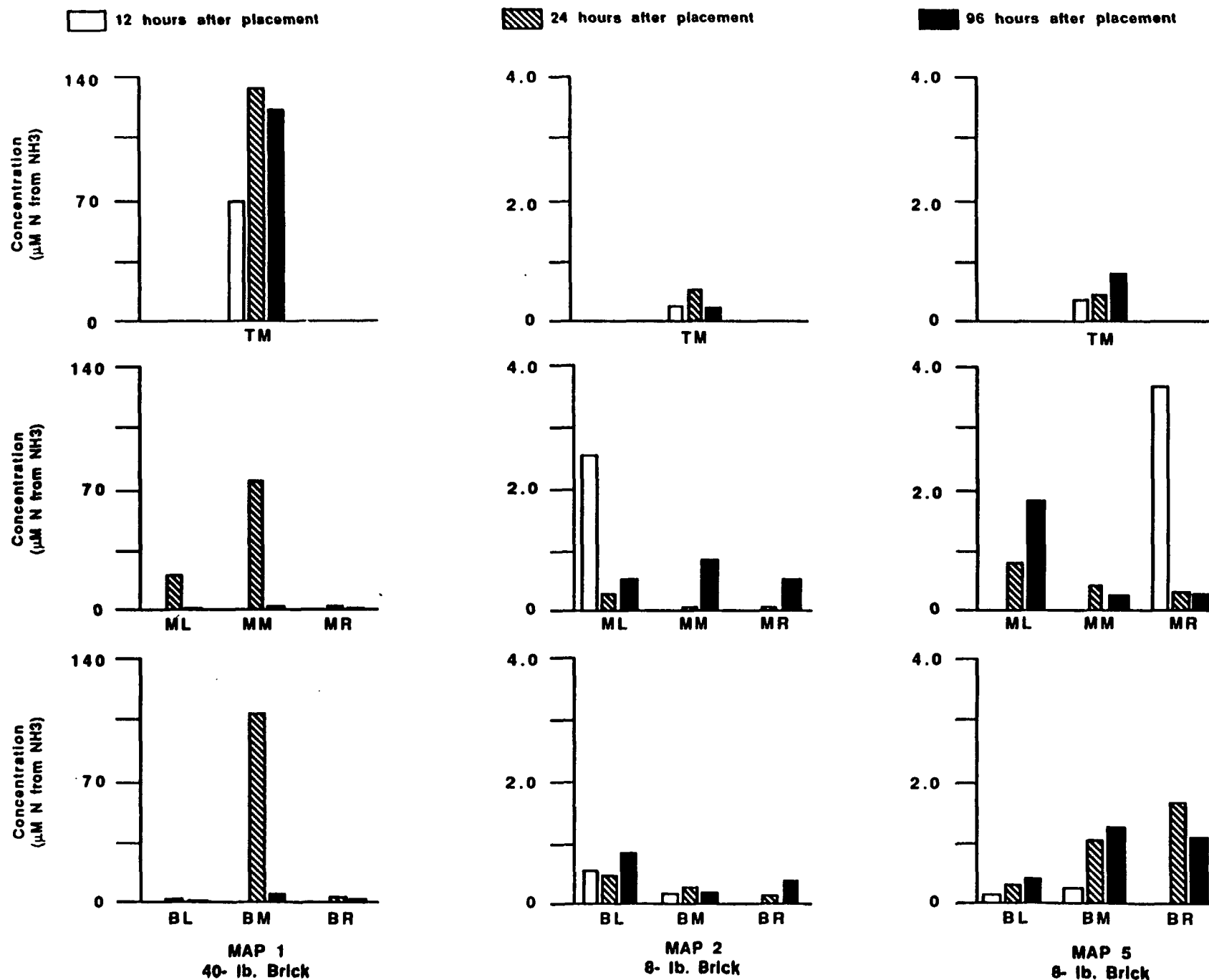


Figure 5.9. Magnesium Ammonium Phosphate Fertilizer Test: Ammonium Concentration in Beach Pore Water at 12, 24, and 96 Hours After Placement of Fertilizer (Sampling Locations Given in Figure 5.8)

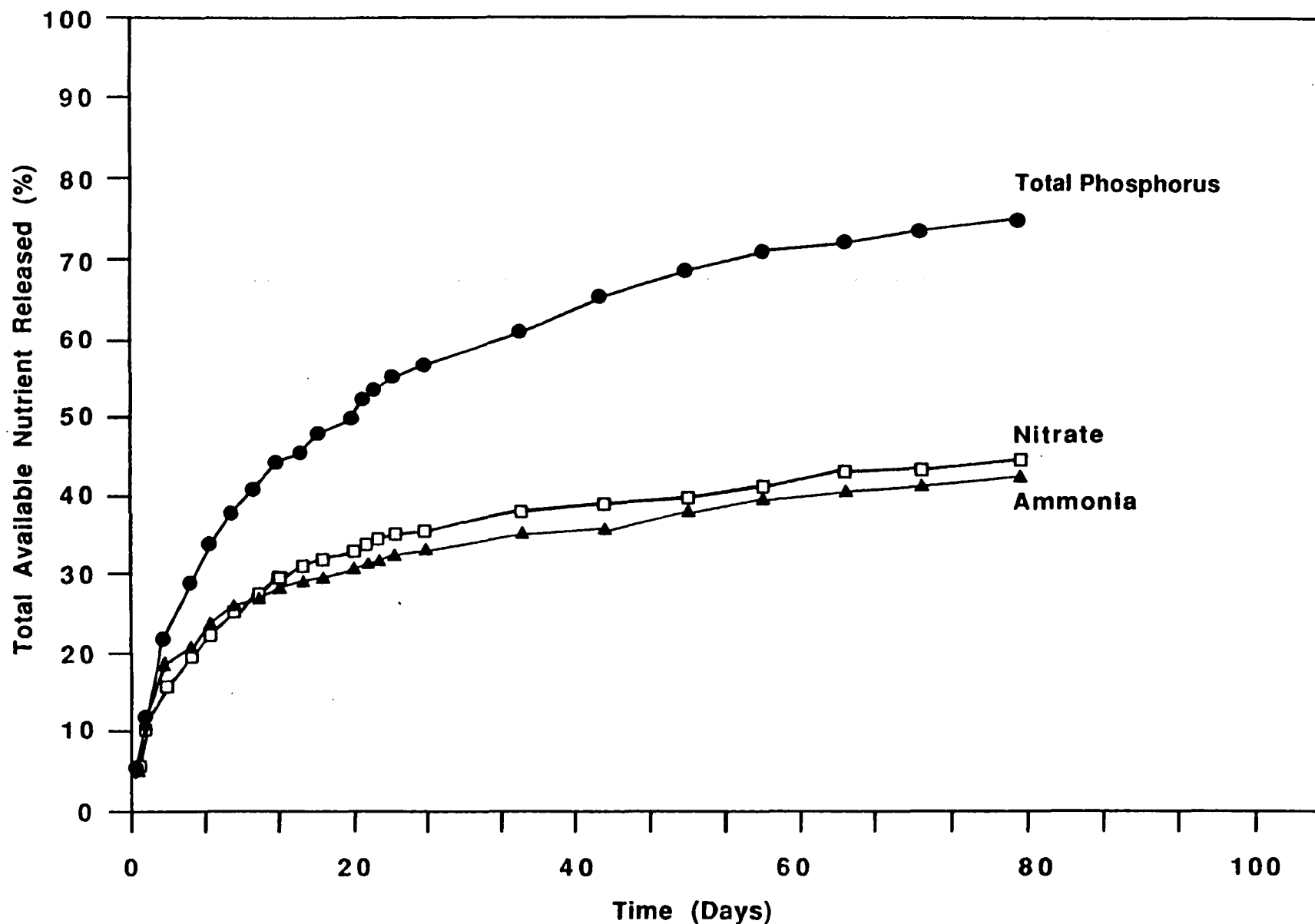


Figure 5.10. Cumulative Release of Ammonia and Nitrate from Sierra Chemical Granules in Static Flask Experiments



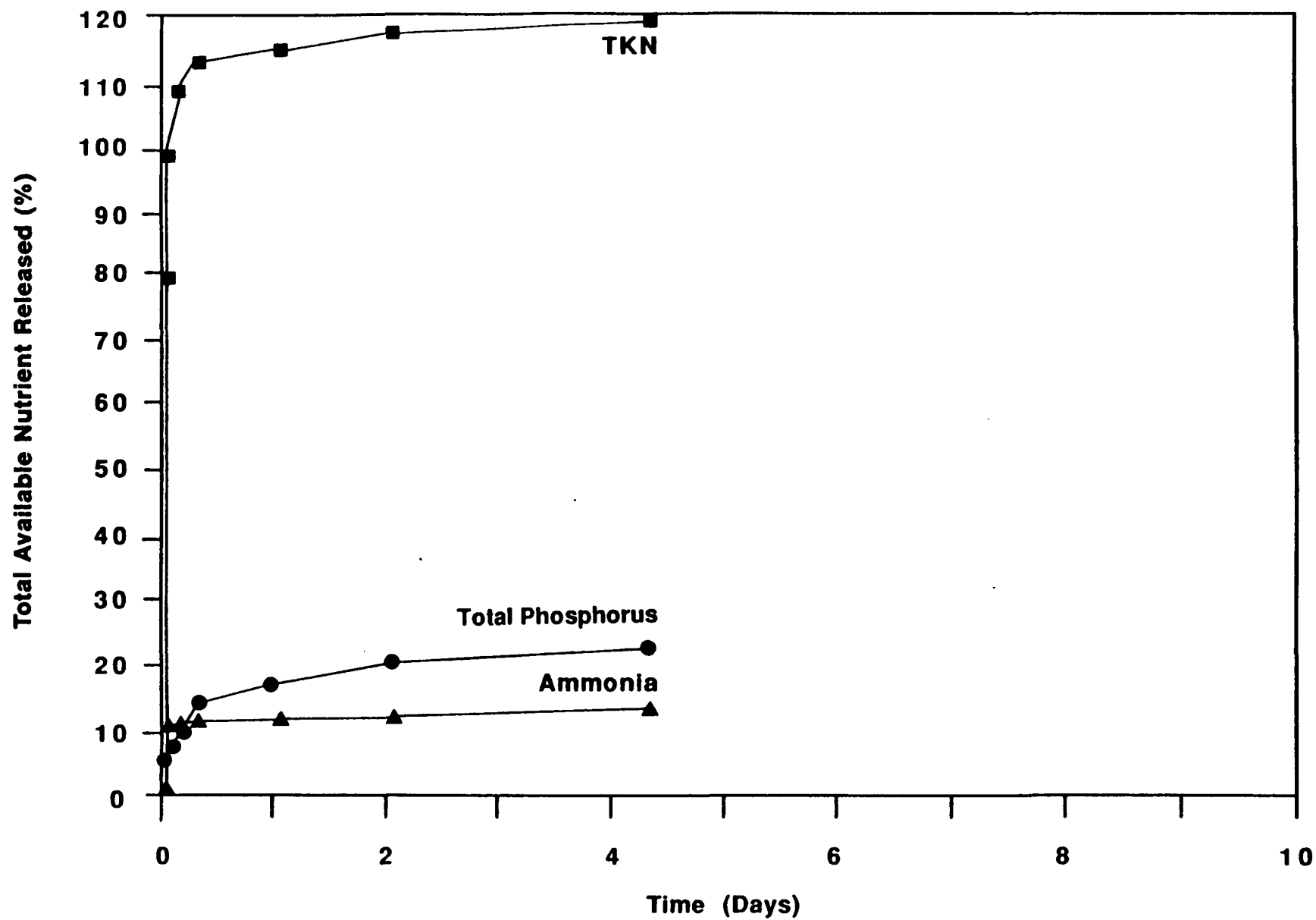


Figure 5.11. Cumulative Release of Ammonia and Total Kjeldahl Nitrogen (TKN) from Inipol EAP 22 in Static Flask Experiments.

An intermittent submersion test was run on the oleophilic fertilizer applied to oil-covered Prince William Sound beach material. The data are shown in Table 5.3. Within 5 minutes after Inipol-treated oiled rocks were covered with seawater, over 60% of the available nitrogen was released as TKN. However, following this initial burst, TKN appeared to be released more slowly, i.e., very little increase in TKN occurred over the next 115 minutes. After decanting the water off the beach material, allowing it to sit unsubmerged for 6 hours and recovering the rocks with water, only 8.3% of the available nitrogen was further released as TKN. Concentrations of ammonia and phosphate released were quite low, but generally followed the same pattern as the TKN.

Allowing the fertilizer to remain in contact with the oil for 6 hours prior to the addition of water did not change the nutrient release patterns. This suggests that the amounts of nutrient which sequester with the oil (i.e., not washed off) are incorporated very soon after fertilizer application.

In addition, mixing the beach material as the Inipol was applied, or warming the Inipol to 25°C before application, did not significantly change the amount of nitrogen released in the first few minutes.

#### **DISCUSSION AND CONCLUSIONS**

From these studies it was concluded that bags of "Woodace" fertilizer briquettes would be used in the initial field demonstration for slow-release fertilizer. This fertilizer had good nutrient release characteristics, excellent durability in the field, and ready availability. Also, given the time constraints of the bioremediation field demonstration project, this fertilizer was a reasonable first choice.

Recognizing that bagged briquettes could not be produced in sufficient quantities for large-scale application, slow-release fertilizer granules (Sierra Chemicals) were selected for the second field test, as this material could be easily broadcast over the beach surface in a large-scale operation. The granules had good nutrient release characteristics but were not as long lasting or durable as the briquettes.

Tests with the oleophilic fertilizer, Inipol EAP 22, showed that it retained nutrients on the surface of oil, although approximately half of the available nitrogen was lost in the first minutes following application. Except for the gel point (11°C) being high enough to require warming the fertilizer in cold weather, this liquid fertilizer was potentially very suitable for large-scale application.

Table 5.3. Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus (TP) from Inipol EAP 22 During Intermittent Submersion Experiment

Min. from Start of Experiment	5 Min. Contact Time <sup>a</sup>	6 Hour Contact Time <sup>a</sup>
Ammonia Released (mgN/L) <sup>b</sup>		
5	1.1	0.5
15	1.1	0.4
30	1.4	0.5
60	1.3	0.7
120	1.4	0.7
510 <sup>c</sup>	0.2	
540	0.1	
600	0.0	
TP Released (mgP/L)		
5	1.3	1.4
15	1.2	1.2
30	1.0	1.1
60	1.5	1.3
120	1.0	1.0
510 <sup>c</sup>	1.1	
540	0.9	
600	0.9	
TKN Released (mgN/L) <sup>b</sup>		
5	24.6	29.8
15	26.1	34.8
30	27.2	35.5
60	32.5	34.3
120	29.4	32.3
510 <sup>c</sup>	4.6	
540	4.6	
600	4.3	

<sup>a</sup> Time between fertilizer application and initial submersion.

<sup>b</sup> Initial concentration of nitrogen = 57 mg/L.

<sup>c</sup> Water drained; beach material remained unsubmerged for 6 hours; seawater replaced.

## SECTION 6

### FIELD TEST DESIGN AND METHODS

#### TEST PLOT SAMPLING DESIGN

##### Sampling Procedure

The beach sampling design was formulated to generate scientifically defensible conclusions relative to the success of bioremediation. Each test site was divided into a series of plots. The plots were generally 30 m long and 12 m wide running the length of the beach. Plot size was controlled by the available beach (i.e., sections of relative uniformity), the extent of beach covered by the oil, and the prominence of certain topographical features. Buffer zones of at least 5 m separated the plots. Larger buffer zones (>20 m) were established between treated and reference plots to minimize cross contamination. Cross contamination of nutrients between plots was not expected because of a small tendency for lateral movement along the beaches and extensive dilution.

Approximately equal sampling effort was used in three intertidal zones; high, mid, and low. Zonal sampling was used to uncover any effect due to length of time of ocean coverage, rainfall, and freshwater runoff or temperature (exposure to sun, air, ocean, etc.) that sampling intensity was intended to be great enough that may have influenced biological and physical degradation. Sampling intensity was intended to be great enough that if active biodegradation occurred in only one tidal zone, then a sufficient number of samples per zone still would be available for analysis. If degradation occurred in all three zones, three points could be utilized to discover trend from high to low tide and to explain changes in biodegradation rates.

From each intertidal zone, blocks were derived by dividing the beach plot length into seven equal segments, thus creating a total of 21 blocks. It was recognized that certain sampling points on the beach were not representative of the entire beach. For instance, stream runoff flows over one section of the beach might have been caused by an underlying solid rock outcrop near the surface of the beach. Having seven samples for each beach stratum allowed for the existence of a nonrepresentative sample, or for the possibility of a sample with an obvious gross error due to a flaw in sampling or analysis. In essence, several samples were insurance against a host of potential problems. In addition, several samples were needed to ensure adequate power of statistical tests.

Each block was divided into 1 m x 1 m sampling grids. Therefore, although the number of blocks within plots did not

vary with beach size, the number of sampling grids within a block for a particular plot did vary. Each plot was laid out using rope secured to rebar stakes to identify the boundaries of the blocks. Squares of rebar (1 m x 1 m) were used to delineate sampling grid cells.

For each designated sampling time, a sample was taken from one grid cell within each block for all analyses. The sampling grid selection procedure included the following steps:

- The sampling crew began at the upper-left-hand corner of block 1 or 15 and from a random number table picked two numbers that fell within the confines of the block. That is, if the block size for the particular plot was 5 m in length and 3 m in width, the table was used to pick a number from 1 to 5 to designate the distance along the beach from the starting point. A second number from 1 to 3 was picked to designate the distance toward the low-tide mark. Squares of 1 m X 1 m rebar were used to locate the sampling grids. The intersection of the two randomly selected points was the upper-left-hand corner of the selected sampling grid. The same sampling grid location was used for all blocks in a single plot during a single sampling event.
- A 1 m x 1 m frame was placed on the beach in the designated grid cell and samples were collected from the center of the frame.
- If a sample could not be taken at the center of the grid cell, a random number between 1 and 12 was chosen. These numbers represented positions on the face of a clock, in which 12 pointed to high tide. The sampler then moved away from the center of the frame toward the indicated clock position until an appropriate site was found within the sampling frame. The sampling crew used judgment in many situations, e.g., if a large boulder was encountered, the site was discarded and step 3 was repeated.

This procedure was repeated for each block until completion of the beach plot sampling. For all analyses of the samples, except nutrients, site selection was the same.

All sampling was performed at low tide. Two days were required to sample all plots at each test site. Consequently, only one-half of the control plots were sampled each day.

For chemical measurements, a 25% error was assumed for the sampling system, and changes of 30% to 50% were suggested as significant changes in the measured variables. For testing a hypothesis at the 95% confidence level with a power of 0.90, 6 replicates are needed to detect a 30% change in the measured variable and 3 are needed to detect a 50% change. For a power of

0.95, 8 and 3 replicates are needed to detect a 30% and a 50% change, respectively.

For biological measurements, a change of 50% in population value was considered as a significant response. Experience suggested that measurement variability could range from 25% to 75%. For hypothesis testing as described above, sample sizes of 3, 9, or 20 are needed to detect 25, 50, or 75% change in the measured variable with a power of 0.90, and sample sizes of 3, 11, or 25 are needed to detect 25, 50, or 75% changes with a power of 0.95.

This analysis assumes the data all follow a normal distribution. Unfortunately, environmental populations often are not normally distributed. In the present case, differences in the length of time sample sites were underwater, inhomogeneous drainage of freshwater across the beach, drift, and other factors affected the variability of beach conditions, and therefore sampling system errors. All these concerns tended to inflate the number of samples needed to ensure adequate power of statistical analyses.

The overall design of beach sampling efforts was non-optimal in a statistical sense. The major limitation arose from the lack of duplicate beaches for each treatment (and reference). Measured effects were attributable to both nutrient treatment effects and beach effects. It could not be determined statistically whether an increased bioremediation rate at a site was due to either the treatment or to a fortuitously good location, since these two variables were confounded. When only one treated beach was successful, low confidence should be assigned to the result; however, because two types of beaches and two types of treatments were used, when one or both treatments were successful on both types of beaches, confidence in the results may be high.

#### Sampling Method

On mixed sand and gravel beaches, samples were taken by placing a metal pail with the bottom removed onto the beach surface and working the bucket down into the substratum. As small rocks were encountered that prevented the pail from going further into the beach material, the material around the pail was excavated and the rock removed. If 50% of the rock was inside the perimeter of the pail, it was added to the pail and included in the sample. If 50% or more was on the outside, it was excluded from the sample. All large rocks (approximately 4 cm or larger in any dimension) were discarded from the sample, since the amount of oil covering their surface was insignificant relative to oil in the entire sample, and exclusion of these rocks reduced variability in substrate characteristics of the sample.

Once the pail was worked down into the beach material to a depth of approximately 13 to 14 cm (using marks on the inside of the pail), all of the beach material down to 10 cm, including small rocks that protruded more than 50% above that mark, were included in the sample. Rocks that did not protrude more than about 50% above the mark were left behind. All beach material removed from the sampler was placed in new paint cans that had been previously washed with a detergent solution and thoroughly rinsed. The contents of the paint can were then thoroughly mixed with a steel spoon. A subsample of material sufficient to fill a 400 ml wide-mouth jar was taken from the mixed sample. The jar and its contents were subsampled for microbiology analysis and then frozen.

Cobblestone beaches were sampled by removing all the rocks from the sampling area covered by the bottomless pail and placing them in a paint can. Enough sample of the underlying mixed sand and gravel to fill a 400 ml wide mouth jar was then collected. Samples in both the jar and the paint can were frozen for subsequent analysis.

In each treatment and control plot, in situ jars were inserted into the beach material. These jars served as a consistent source of beach material in which the oil concentration and composition was well defined. The jars were straight-sided, high-density polyethylene containers 10" high and 8" in diameter with screw cap lids. The jars were perforated with 1/16" wide and 2" long slits at 2" spacings in the walls, cap, and bottom to allow adequate percolation of beach interstitial water through the contained beach material. These jars contained a known amount of oil-contaminated beach material. To fill the jars, a large amount of contaminated beach material was collected and thoroughly mixed in a large plywood box for 30 minutes. Subsamples were used to completely fill each jar, and the jars were implanted into the beach material 4 inches below the beach surface. Subsamples were also taken and frozen for T=0 chemical analysis. Duplicate jars were placed in the sediment, with a spacing of 4 inches horizontally between any two containers, and the lid placed up-gradient. A total of 40 containers were placed in the plot treated with oleophilic fertilizer, 42 in the plot with the water-soluble fertilizer, and 18 in the reference plot. The differences in numbers were functions of the plot size and the availability of containers.

These containers were sacrificed when significant biodegradation occurred as evidenced by a reduction in the pristane/phytane ratio from samples taken from the beach material in the plot. Subsamples were frozen for subsequent analysis of changes in residue weight and composition of the oil. Enough jars were available to allow two samplings.

The in situ sampling jars were placed along a transect between the high-tide and mid-tide zones and between the mid-tide and low-tide zones. Along these transects, two jars were placed in each block, equidistant from the corners of the block, except in control plots, where pairs of jars were placed in alternate blocks.

## **FERTILIZER APPLICATION**

### **Slow-Release Water-Soluble Fertilizers**

Slow-release fertilizers used in this project were briquettes that were applied in mesh bags and granules that were broadcast. The following paragraphs describe the methods used to place these fertilizers.

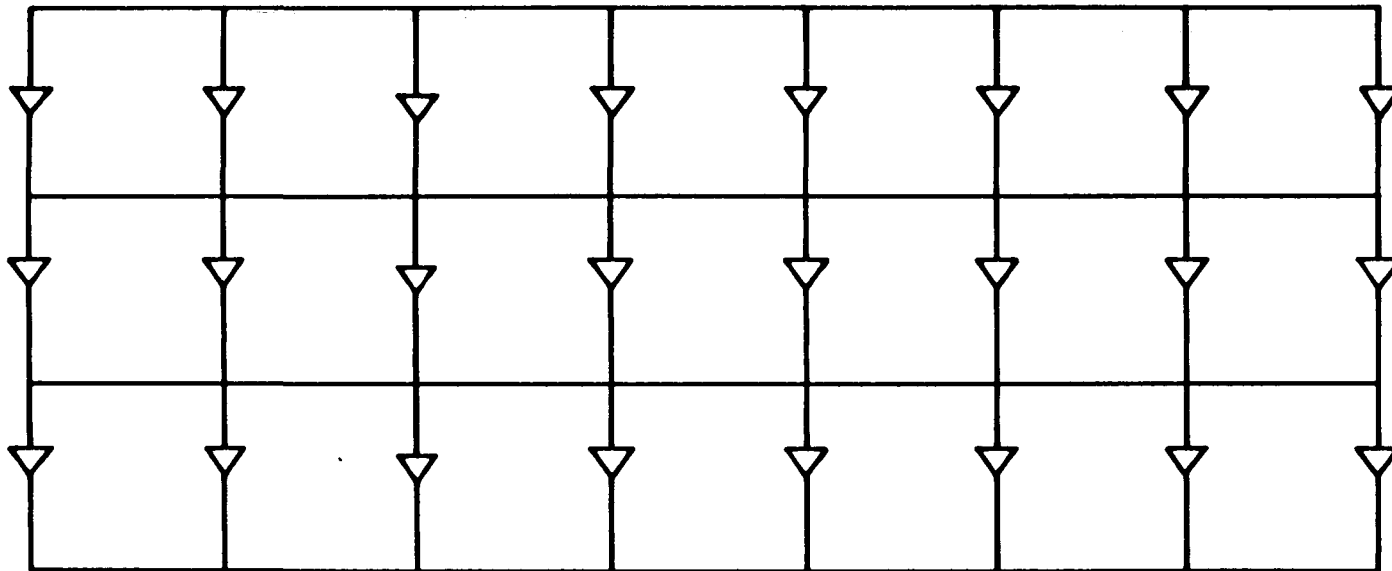
Herring-seine net bags filled with slow-release fertilizer briquettes (Woodace) were placed on the beach in a manner that was intended to provide complete exposure of the beach material to nutrients leaching from the bags. Each bag contained approximately 33 pounds of briquettes. Application of the briquette bags occurred on June 11, 1989. The total quantity of briquettes applied to the 35 m x 12 m plot (Otter Beach) was 800 pounds, representing approximately 100 pounds nitrogen and 24 pounds phosphorus (as  $P_2O_5$ ). The bags were tethered to 3-foot sections of 1-1/8 inch diameter steel rods that were buried 6 inches below the surface of the beach. Figure 6.1 indicates the positioning of the 24 bags in the experimental plot. Three rows of eight bags were placed at 2 m, 6 m, and 10 m from the top of the plot.

On June 20 and 21, 1989, the bags were repositioned according to the layout in Figure 6.2, as the bags located at the 2 m row were not being submerged consistently by the high tide (see below). Additionally, preliminary data indicated that the nutrients were being channelled vertically down the beach. Four more bags were added to the previous 24 bags for a total of 28 bags, resulting in 920 pounds of fertilizer (130 pounds N).

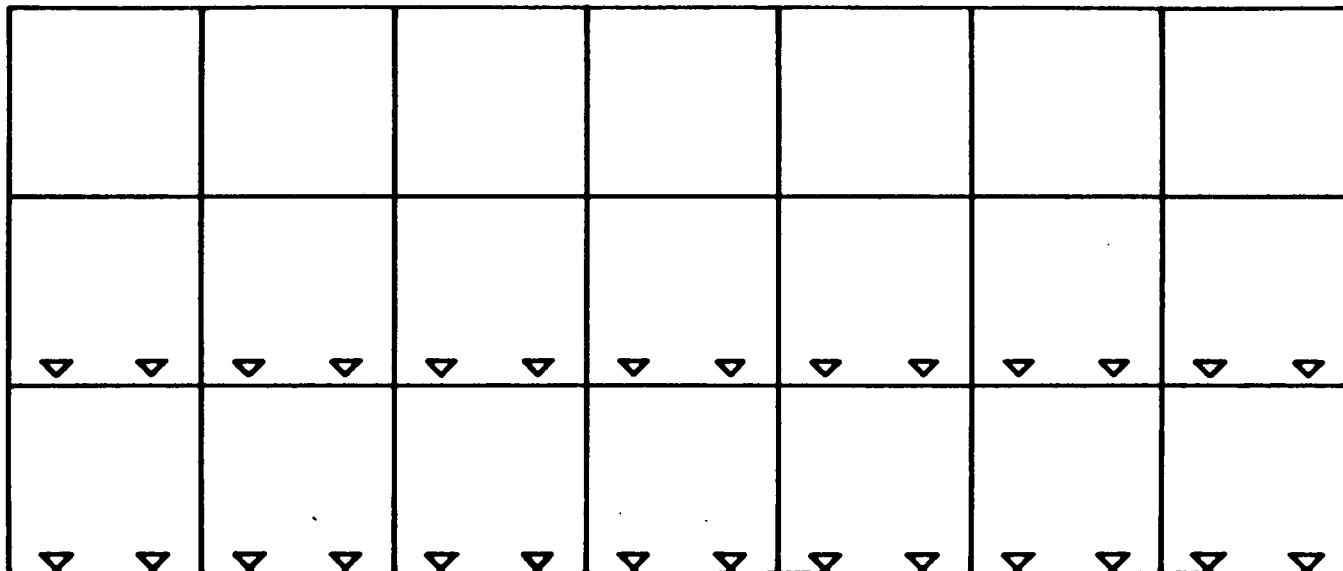
The same arrangement and repositioning was used for the briquette bags on Seal Beach. This beach was smaller (28 m wide rather than 35 m) and, thus, the weight of briquettes applied per bag was 26 pounds (rather than 33 pounds) for a total of 620 pounds, increasing to 730 pounds after the four new bags were added.

Figures 6.3 and 6.4 represent the significant tidal fluctuations typical of Snug Harbor. These tidal fluctuations affected the amount of time each zone was underwater and that nutrients were being dissolved and transported. For example, in the sand and gravel plot treated with the fertilizer briquettes, the top row of fertilizer bags were placed at a relative tidal height of





**Figure 6.1. Placement of the Bags of Fertilizer Briquettes on Otter and Seal Beaches (See Figure 4.1. for beach locations).**



**Figure 6.2. Repositioning of the Bags of Fertilizer Briquettes on Otter and Seal Beaches (See Figure 4.1. for beach locations).**

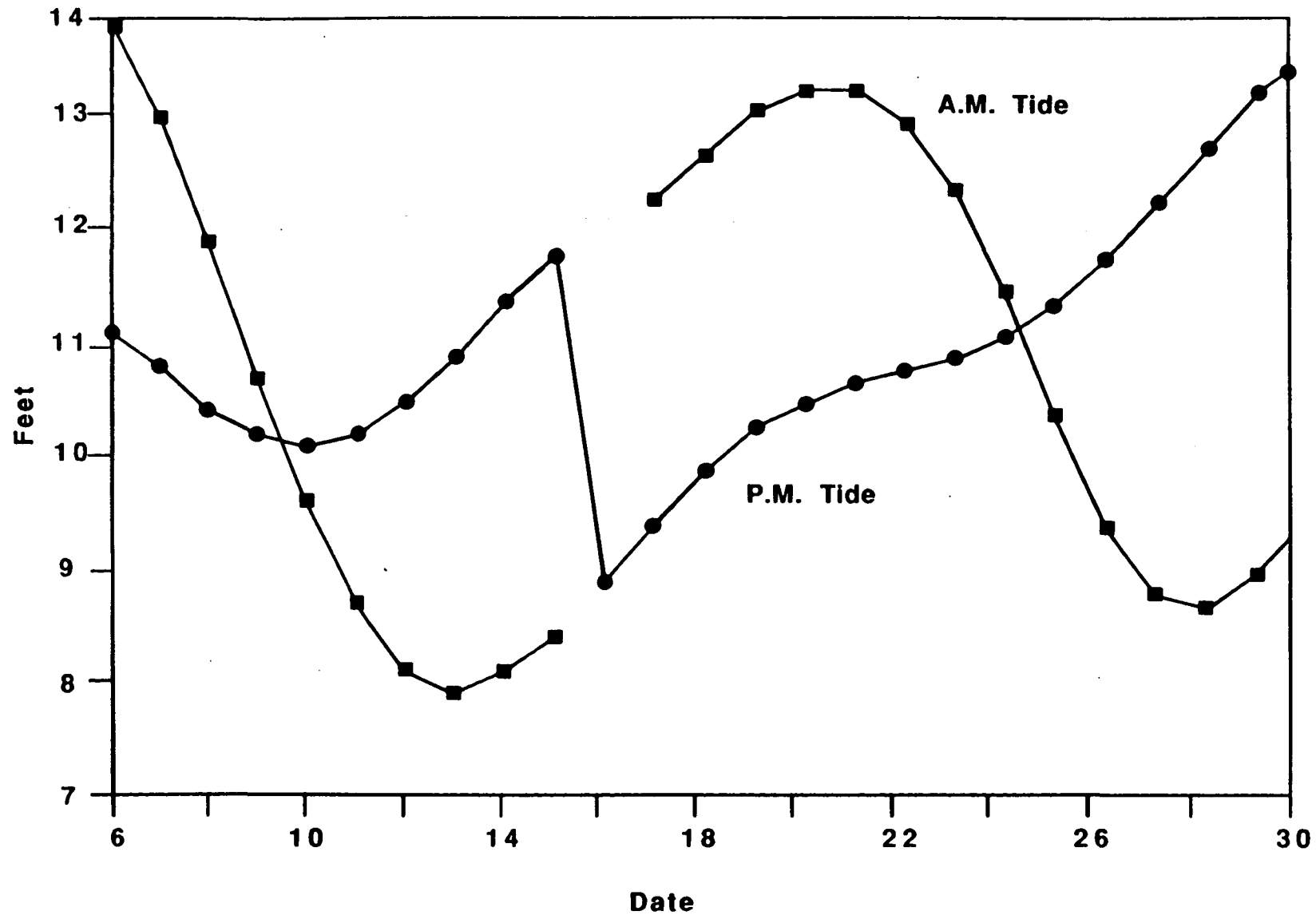


Figure 6.3. Tidal Fluctuations for High Tides, Snug Harbor, June 6-30, 1989.

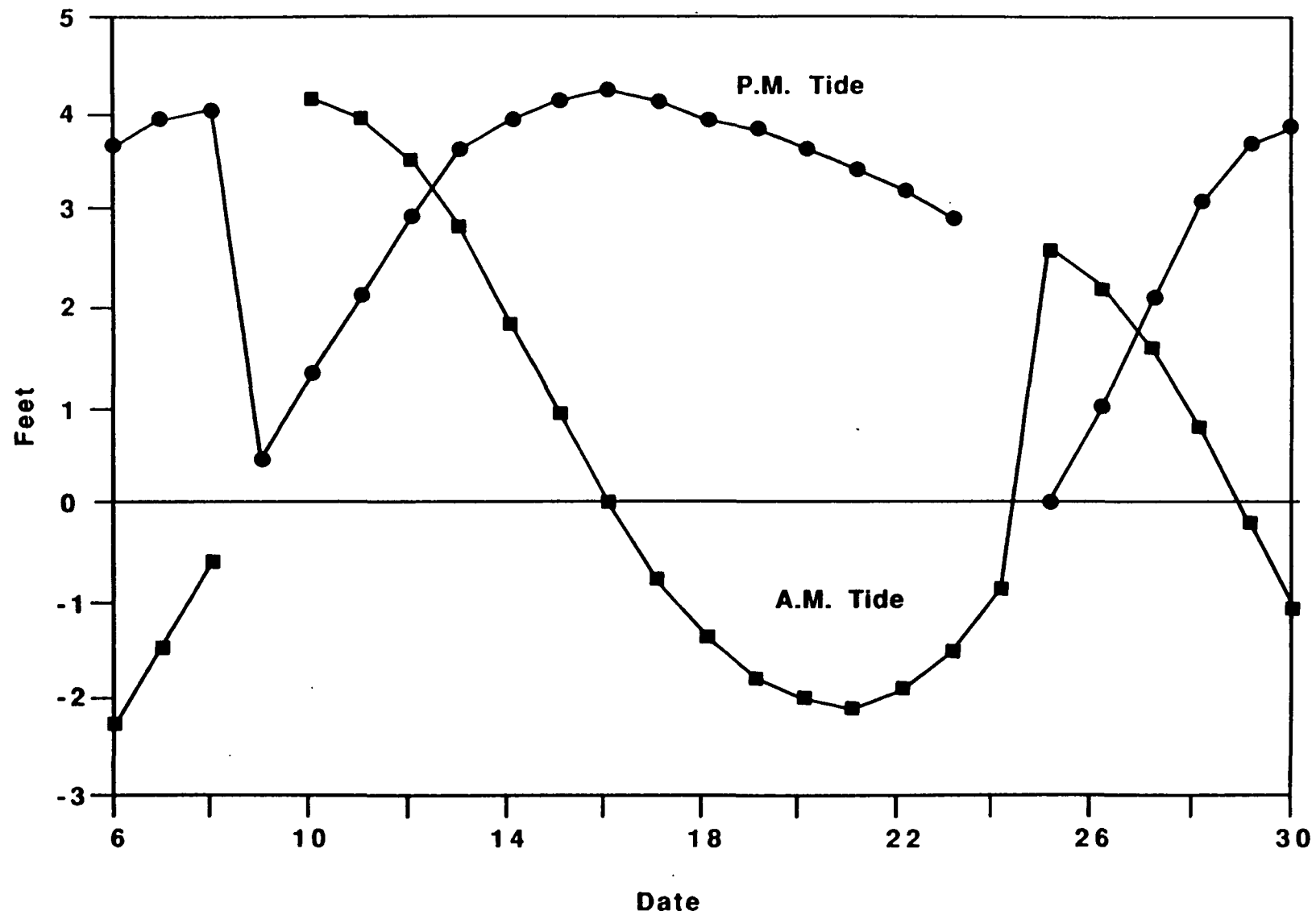


Figure 6.4. Tidal Fluctuations for Low Tides Snug Harbor, June 6-30, 1989.

13 feet. As shown in Figure 6.3, the top row of bags were only underwater approximately one-fourth of the days in June. Consequently, precipitation was a primary factor in controlling the dissolution and transport of the nutrients in this zone. This high-tide zone, which was contaminated with oil in Snug Harbor, was representative of other oil-contaminated zones in the Prince William Sound.

Slow-release granules were applied to Tern Beach in Passage Cove using a commercial broadcast fertilizer spreader, at a rate of approximately 0.0033 lbs/ft<sup>2</sup>. The total application of nitrogen and phosphorus by slow-release granules in Passage Cove was approximately 400 lbs and 40 lbs, respectively. The granules stuck to the oil on the rock surfaces and were therefore not easily displaced from the beach or redistributed by the tidal action.

#### Oleophilic Fertilizer

Oleophilic fertilizer (Inipol EAP 22) was first applied to Otter Beach in Snug Harbor (mixed sand and gravel) on June 8, 1989. A total of 10 gallons (83 pounds) was applied, which represented approximately 5% of the estimated weight of the oil on the treated beach. The following computations were made to determine the application rate:

- Plot was  $20 \text{ m} \times 12 \text{ m} = 240 \text{ m}^2 = 2,600 \text{ ft}^2$
- Assumptions:
  - 6 inch oil depth:  $2,600 \text{ ft}^2 \times 0.5 \text{ ft} = 1,300 \text{ ft}^3$
  - 20% void volume: total rock volume =  $1,300 \text{ ft}^3 \times 0.8 = 1,000 \text{ ft}^3$
  - Specific gravity of rock = 2.6 or 160 pounds/ft<sup>3</sup>
  - Weight of rock =  $160 \text{ pound/ft}^3 \times 1,000 \text{ ft}^3 = 160,000 \text{ pounds}$
  - Oil = 1% of weight of rock = 1,600 pounds
- Specific gravity of Inipol = 1.0
- Based on a 5% loading rate of the Inipol/oil,  $1,600 \text{ pounds of oil} \times 0.05 = 83 \text{ pounds Inipol or 10 gallons.}$

A second application 10.5 gallons of Inipol was made on June 17, 1989, to the Otter Beach plot based on recommendations from Elf Aquitaine representatives.

The first oleophilic application at Seal Beach in Snug Harbor (cobble) was on June 9 at a rate of 13 gallons. The second application of 14 gallons occurred on June 18th.

Oleophilic fertilizer was applied on the plots as the tide was going out in the evening. Application was initiated beginning at the top of the beach, an hour after the tide was past the lowest zone in the plot. The fertilizer was applied using a backpack sprayer with a capacity of four gallons. The fertilizer was initially warmed, to ensure uniform application and to prevent clogging of the spray nozzle.

The weather during the first applications on June 8 and 9 was rainy and cool. During the second application, both days were clear and sunny, with temperatures around 60°F. Examination of the plots the day after the second application indicated a noticeable gelatinous sheen on the surface of the sediment and rocks where the fertilizer was applied. The sheen lasted for two days. Wave action was minimal over this period. This sheen was not seen with the first application.

#### Sprinkler System

Kittiwake Beach in Passage Cove was used to evaluate the effectiveness of application of nitrogen and phosphorus via spray irrigation. Nitrogen and phosphorus fertilizers dissolved in seawater were sprayed onto the beach daily. The spray irrigation system used sprinkler heads typical of lawn sprinklers. The fertilizer solution was pumped by a gasoline-driven well pump to four sprinkler heads set on each side of the plot. Each sprinkler swept a 180° arc across the plot during application. Typical applications were about 0.4 inch of water per day. Application rates were established to supply 6 µg/l of nitrogen and 3 µg/l of phosphorus to pore water in the saturated beach material to a depth of 2 m.

#### **ANALYTICAL PROCEDURES**

Detailed information on the standard operating procedures are given in the Quality Assurance Plan, which is available upon request from Dan Heggem at the Environmental Monitoring Systems Laboratory in Las Vegas, NV. Only brief accounts of the analytical procedures will be given here.

#### Oil Chemistry

Beach samples consisted of either mixed sand and gravel frozen in 400 ml I-Chem jars or cobblestones wrapped in aluminum foil and frozen. The mixed sand and gravel was thawed immediately prior to the initiation of oil analysis, and the contents were mixed thoroughly. A weighted 100 gm subsample was removed and mixed thoroughly with 300 mls of methanol in a

separatory funnel. The slurry was shaken for five minutes, and the methanol was decanted into a 2 L separatory funnel. The samples were similarly re-extracted two times with 300 ml of pesticide - or HPLC grade methylene chloride. The three organic fractions were combined and back-extracted with 100 ml of 3% aqueous sodium chloride. The phases were separated and the aqueous portion was extracted with 50 mL of fresh methylene chloride. This aqueous extraction in methylene chloride was added to the combined organic fraction.

The combined organic fraction and 3 or 4 clean boiling chips were placed into a 1 L round bottom flask fitted with a three-ball Snyder column. The volume of solvent was reduced until the color was approximately the color of dilute weathered oil (ca 15 mg/2 ml methylene chloride). The final volume of the extract was measured with a syringe having an appropriate graduated cylinder, and an aliquot was transferred to a GC autosampler vial.

All of the cobblestones were extracted using the same procedure (methanol, followed by methylene chloride), except that shaking was replaced by gentle swirling to remove oil from the rock surfaces.

Gas chromatographic (GC) analysis was accomplished with an instrument capable of reproducible temperature programming with a flame ionization detector and a reliable autosampler. The GC conditions were:

Column: DB-5, 30 m X 0.25 mm, film thickness 0.25 um  
Initial Temperature: 45°C, 5 min. hold  
Temperature Rate: 3.5°C/min  
Final Temperature: 280°C, 60 min. analysis  
Injector: splitless, 1 in valve closure  
Injector Temperature: 285°C  
Injection: 2.0 µl  
Detector: FID, 350°C

Those samples that demonstrated significant evidence of biodegradation were fractionated to allow separate determination of aliphatics and aromatics. Aliquots of the sediment or oil extracts selected for fractionation were solvent exchanged to hexane under a stream of dry nitrogen. A volume of 50 µl of hexamethylbenzene (80 ng/µl) and 25 µl of n-decyclohexane (1 µg/µl) was added to each sample extract prior to fractionation. The fractionation was accomplished using a 10 mm X 23 cm glass column that was slurry packed (with hexane) with 60/200 mesh silica gel activated at 210 C for 24 hours. The aliphatic fraction was eluted with 30 ml of hexane and the aromatic fraction was eluted with 45 ml of hexane/benzene (1:1). Both the aliphatic and the aromatic fractions were analyzed using the GC methods described above.

Subsamples of the final concentrated extract were subjected to mass spectral analysis using a Hewlett-Packard Gas Chromatography/Mass Spectrometry (GC/MS) system provided by the U.S. Coast Guard Mobile Analytical Laboratory. The analytical procedure is given in their Fucus oil analysis protocols (Hildebrand, 1989).

Subsamples (5-15 mls) of the final concentrated extract were also removed, filtered through sodium sulfate, and placed in tared watch glasses. After passive evaporation of the solvent, the oil residue weight was determined.

Changes in oil composition were determined using three data analysis procedures:

- The branched hydrocarbons pristane and phytane were used as internal standards, under the assumption that they were slow to degrade, and weight ratios of C17:Pristane and C18:Phytane were calculated as indicators of biodegradation.
- The total weight of all alkanes appearing on the chromatograph, normalized to the total residue weight of oil, were compared on a sample by sample basis.
- Assuming that hopane and norhopane were not biodegraded, weight ratios with other identifiable hydrocarbons were calculated.

### Nutrient Analysis

Water samples taken for nutrient analysis were filtered (Whatman glass fiber filter) and then placed in 150 ml plastic screw capped bottles. The bottles were immediately frozen with a dry ice-antifreeze solution. Water samples taken offshore were collected with a clean bucket and subsamples were taken for nutrient analysis. Water samples from the beach were collected behind or in front of an ebbing or flooding tide, using a commercial root feeder. The root feeder was outfitted with rubber tubing and a peristaltic pump to allow interstitial pore water to be sucked into the feeder tube and sampled at the top of the feeder tube. The feeder was inserted approximately 20 cm into the mixed sand and gravel. Pore water was flushed through the feeder for one minute prior to sampling.

Nutrient concentrations were determined using the following standard methods:

#### Nitrate--

Nitrate was determined by reduction to nitrite followed by a colorimetric assay for nitrite (see below). Nitrate was reduced to nitrite by passage through a column containing copperized cadmium filings. The resulting solution contained total nitrite



that was equivalent to the sum of the initial nitrate and nitrite in the sample; nitrate was determined by difference. The procedure for nitrate was derived from the non-automated technique described in Parsons, Maita, and Lalli (1984). Detection limits for nitrate and nitrite were expected to be about 0.05 and 0.01  $\mu\text{M}$ , respectively. An estimate of the precision for the nitrate measurements at the 20  $\mu\text{M}$  level in the samples was calculated as the mean of n determinations  $\pm 0.5$  (mean/n<sup>2</sup>) in  $\mu\text{M}$ .

#### Nitrite--

Nitrite was determined by the Geiss reaction in which sulfanilamide and N-(1-Naphthyl) ethylenediamine dihydrochloride (NNED) react with nitrite in an aqueous acidic solution to form an intensely pink diazo dye with an adsorption maximum at 540-543 nm.

#### Ammonium--

Ammonium was determined by the Berthelot reaction in which hypochlorite and phenol react with ammonium in an aqueous alkaline solution to form indophenol blue, an intensely blue chromophore with an absorption maximum at approximately 637-640 nm. Based on the information in Parsons, Maita, and Lalli (1984) and Whittedge, Malloy, Patton, and Wirick (1981), the detection limit for ammonium was expected to be approximately 0.1  $\mu\text{M}$ . An estimate of precision at the 1  $\mu\text{M}$  level was calculated as the mean of n determinations  $\pm 0.1$  (mean/n<sup>2</sup>) in  $\mu\text{M}$ .

#### Phosphate--

Phosphate (i.e., orthophosphate) was determined as phosphomolybdic acid, which has an absorption maximum at 880-885 nm in its reduced form in the presence of antimony (Parsons, Maita, and Lalli 1984). The detection limit for phosphate was expected to be about 0.03  $\mu\text{M}$ . An estimate of the precision at the 3  $\mu\text{M}$  level was calculated as the mean of n determinations  $\pm 0.03$  (mean/n<sup>2</sup>) in units of  $\mu\text{M}$ .

#### Total Kjeldahl Nitrogen (TKN)--

TKN was measured by heating the sample in a sulfuric acid solution containing  $\text{K}_2\text{SO}_4$  and  $\text{HgSO}_4$  and comparing colorimetrically with standards and blanks using a Technicon AutoAnalyzer (EPA method 365.4).

#### Microbiological Analysis

Numbers of oil-degrading microorganisms were measured by extinction to dilution procedure using oil as the carbon source. The samples for microbiological analysis were a subset of the samples taken for analytical analysis. A 5 g portion of the analytical sample for the sand and gravel beach was transferred to a pre-weighed sterile dilution bottle.

The defined nutrient medium (DNM) used in these tests contained (per liter of distilled water): NaCL, 24 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0 g; KCL, 0.7 g;  $\text{KH}_2\text{PO}_4$ , 2.0 g;  $\text{Na}_2\text{HPO}_4$ , 3.0 g; and  $\text{NH}_4\text{NO}_3$ , 1.0 g. The pH of the medium was adjusted to 7.4 with 1.0 N NaOH following autoclaving. The DNM was distributed in 4.5 ml portions to sterile dilution tubes. The initial dilutions were prepared by adding 5.0 g wet weight of sand and gravel subsample to the prepared dilution bottles containing 50 ml autoclaved DNM. Following vigorous mixing (the sample was rapidly shaken by hand for 15 seconds), a 0.5 ml sample of the initial dilution was used to prepare a dilution series from  $10^2$  to  $10^{10}$ . Each tube was then amended with 20  $\mu\text{l}$  of weathered Prudhoe Bay crude oil collected from an oil-contaminated beach in the Prince William Sound. The tubes were scored at 21 days of incubation. Tubes that showed visible microbial turbidity or changes in the physical form of the oil (oily droplets converted to stringy and flaky particulate material) were considered positive. The tubes were scored independently by two individuals. Numbers of oleic-acid degrading bacteria were determined using standard plate counting procedures on minimal-salts agar medium supplemented with 1% oleic acid.

#### Measurements of Microbial Activity--

Evolution of  $^{14}\text{CO}_2$  from phenanthrene-9- $^{14}\text{C}$ , hexadecane-1- $^{14}\text{C}$ , and naphthalene-1- $^{14}\text{C}$  was used to measure the activity of indigenous petroleum-degrading microorganisms as influenced by the addition of Inipol and water-soluble fertilizers. Duplicate 5.0 g samples of beach material (1-5 mm diam.) obtained from oiled beaches with and without Inipol or water-soluble fertilizer treatments were added to 10 ml artificial salt-water medium (ASWM) in clean, sterile 100 ml Wheaton bottles. Each bottle was spiked with 0.1  $\mu\text{Ci}$  of radiolabeled substrate and crimp sealed with a Teflon-lined septum. Following 0, 12, 24 and 48 hr incubation in the dark at ambient temperature (ca.  $15^\circ\text{C}$ ), vessels were sacrificed and the amount of radiolabeled  $\text{CO}_2$  released from acidified medium was determined. Medium was acidified to  $\text{pH} < 3.0$  with HCL, the headspace was flushed for 10 min., and  $\text{CO}_2$  was trapped in 5.0 ml of 1 N NaOH. Subsamples (0.5 ml) of NaOH trapping solution were added to 10.0 ml Ready-safe liquid scintillation cocktail, and the amount of radioactivity present was determined by liquid scintillation. Trapping efficiency was determined by recovery of  $^{14}\text{Na}_2\text{CO}_3$  from acidified medium. Quench was accounted for internally.

#### Ecological Monitoring

Water samples collected offshore in cubitainers were transported to the laboratory in Valdez and analyzed for several parameters that might be affected by bioremediation research efforts. Analysis included measurements reflecting possible eutrophication, release of oil from the beaches, toxic effects

from the fertilizers themselves, and the presence of mutagenic oil residues. Procedures for these measurements are as follows:

#### **Eutrophication Measurements--**

Seven biological and chemical indicators of eutrophication were monitored routinely through the fertilizer addition periods:

**Chlorophyll**--One-liter water samples were filtered through glass fiber filters, and the filters were extracted with a solution of 90% acetone and 1 N NaOH. After overnight incubation in the refrigerator, samples were centrifuged and the optical density of the supernatant was determined at 750 nm (total absorbance) and 665 nm (chlorophyll a). Phaeophytin was determined by rereading the optical densities after the addition of 10% HCl.

**Primary Productivity**--Photosynthetic productivity by phytoplankton was estimated by incorporation of  $^{14}\text{C}$ -bicarbonate. Plankton samples collected in the field were transported to the Valdez laboratory, incubated in BOD bottles in an outside waterbath, filtered, and frozen. Prior to July 5, 1989, samples were then sent to the U.S. EPA Environmental Research Laboratory ERL/Gulf Breeze for analysis using a liquid scintillation counter. Once the liquid scintillation counter was operational at the Valdez laboratory, on July 5, primary productivity samples were counted there.

**Bacterial Abundance**--Estimates of the numbers of bacteria per ml of water in the water column were determined using acridine orange direct counting with fluorescent microscopy (Hobbie et al., 1977). Water samples were filtered through black Nucleopore 0.2  $\mu$  pore size filters and stained with buffered acridine orange solution (Fisher chemical). A minimum of 200 bacterial cells were counted in 5 to 10 grid fields in the microscope.

**Bacterial Productivity**--The thymidine incorporation method of Fuhrman and Azam (1982) was used to measure bacterial productivity. Triplicate water samples were spiked with 5  $\mu\text{l}$  of  $^3\text{H}$ -methyl thymidine (1.1  $\mu\text{Ci}$ ; 2.86 nM final concentration), incubated for 20 minutes and then extracted with 5 ml of cold 10% trichloroacetic acid (TCA). Samples were filtered through 0.22  $\mu\text{m}$  Millipore filters, washed with cold TCA, and the radioactivity on the filter was measured in a liquid scintillation counter.

**Microflagellate Abundance**--Microflagellate abundance was estimated with epifluorescence direct counts using the method described by Caron (1983).

**Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen**--Ten ml water samples for dissolved organic carbon (DOC) analysis were filtered through precombusted glass-

fiber filters (Whatman GF/F). Filtrates were sealed in glass ampoules, frozen and stored until they were analyzed at ERL/Gulf Breeze. To remove inorganic carbon, 1 ml water samples are acidified with 5  $\mu$ l concentrated phosphoric acid and bubbled with N<sub>2</sub> gas for 10 minutes. DOC was measured in an Ionics model 555 high combustion temperature TOC analyzer equipped with a platinum catalyst.

Seawater samples (500 ml to 1000 ml) were filtered through pre-combusted glass-fiber filters (Whatman GF/F) for subsequent analysis of particulate carbon and nitrogen. Filters were dried at 50°C and shipped to ERL/Gulf Breeze for analysis. Particulate carbon and nitrogen were measured simultaneously with a Carlo Erba Model NA 1500 CHNS analyzer.

#### Stable Isotope of Carbon and Nitrogen--

For analysis of stable isotopes of carbon and nitrogen in seawater, filters were prepared as was described above for particulate carbon and nitrogen analyses. These filters, benthic algae, and mussels were collected and shipped to Texas A&M for analysis. Samples were dried and then combusted in quartz tubes with cupric oxide at 900°C. CO<sub>2</sub> and N<sub>2</sub> gases were isolated by cryogenic distillation. Stable carbon and nitrogen isotopes were measured by mass spectroscopy.

#### Caged Mussels

At each station designated for mussel monitoring, four cages filled with 25 mussels (*Mytilus edulis*) each were deployed to measure the uptake of petroleum hydrocarbons that might be released into the water column following application of fertilizers on the beaches. The mussels were collected from Tatitlek Narrows, an area of Prince William Sound that was not affected by the oil spill. The mussels were sampled weekly from the cages throughout the summer. At each sampling, 3 mussels from each cage were sacrificed and the tissues were removed from the shell and frozen. The frozen tissues were returned to the laboratory, where the tissues from all 3 mussels from a single cage were extracted by homogenizing and spiking approximately 20 g of tissue with appropriate surrogates, digested with 6 N KOH at 35°C for 18 h. The sample was then serially extracted with ethyl ether. The eluate was dried with sodium sulfate, concentrated, and cleaned using the EPA Method 3611 alumina column cleanup procedure to remove matrix interferences. The combined saturated and aromatic fractions collected from the cleanup column were concentrated and optionally split in aliquots for analysis.

#### Field Toxicity Tests

Application of fertilizer poses a potential toxic risk to marine biota if water concentrations of oleophilic fertilizer or ammonia approach 50 mg/l, the LC50 for the most sensitive species

tested in the laboratory toxicity tests. To characterize the extent to which toxic concentrations might develop in the course of, or following, application of fertilizers to oiled shorelines, toxicity tests were conducted using field water samples and a testing scheme similar to that used to test acute toxicity of industrial effluents. The data provided insight into the rate at which fertilizers entered the marine environment from test beaches and the amount of dilution required to mitigate toxic effects.

Water samples were collected at specified intervals before and after application of Inipol and the Sierra Chemicals slow-release granules to shorelines in Passage Cove. These samples were sent to a consulting laboratory for 48 hr 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.

One water sample (field control) was collected at the field reference site, immediately outside of the test area, just before the initiation of fertilizer application. At the beach where fertilizer was applied (a 100 m stretch of shoreline), water was collected at 0.5 m depth (just above the bottom) immediately offshore. Water samples were collected immediately before fertilizer application (pre-application, which was 2 hours before low tide), following the completion of application (2 hours after low tide), and again after 1 hr, 3 hr, 6 hr, 12 hr, and 18 hr intervals. Sampling stopped at this time in order to return samples for shipping. All water samples were maintained at 4°C until toxicity tests began.

Oyster larvae toxicity tests were conducted with a standard dilution series (used for effluent toxicity tests: 100%, 56%, 32%, 18%, and 10%) prepared for each water sample collected after application. Because the salinity of site water was 26 ppt, field samples were adjusted to 28 ppt by addition of 90 ppt brine solution before test dilutions were prepared. The salinity adjustment accounted for approximately 3% dilution and was selected as the minimum change necessary to ensure that salinity was sufficient to sustain normal development of oyster larvae. (This dilution was not accounted for in the subsequent reporting of sample concentrations.) The same brine was diluted to 28 ppt and tested as a "hypersaline control" to characterize the adequacy of the brine mixture as a test solution. Laboratory seawater was diluted from 32 ppt to 28 ppt and tested as a seawater control.

## SECTION 7

### FIELD TEST RESULTS - SNUG HARBOR

#### VISUAL OBSERVATIONS

Test beaches at Snug Harbor were moderately contaminated. Visually, the cobble plots had a thin coating of dry, sticky, black, oil covering rock surfaces and gravel areas under the cobble. Oil did not penetrate more than a few centimeters below the gravel surface. In mixed sand and gravel plots, oil was well distributed over exposed surface areas and commonly found 20-30 cm below the surface. In many areas of the test plots, small patches of thick oil and mousse could be found. This material was very viscous and mixed with extensive amounts of debris.

Approximately 8-10 days following oleophilic fertilizer application to the cobble beach plot, reductions in the amount of oil on rock surfaces were visually apparent. It was particularly evident from the air where the contrast with oiled areas surrounding the plot was dramatic, etching a clean rectangle on the beach surface. The contrast was also impressive at ground level; there was a precise demarkation between fertilizer-treated and untreated areas.

Close examination of this treated cobble plot showed that much of the oil on the surface of the rocks was gone. There were still considerable amounts of the oil under rocks and in the mixed gravel below these rocks. The remaining oil was not dry and dull as was the oil in other areas of the beach, but appeared softened and more liquid. It was also very sticky to the touch, with no tendency to come off the rocks. At the time of these observations, no oil slicks or oily materials were observed leaving the beach during tidal flushing.

The mixed sand and gravel beach treated with oleophilic fertilizer also appeared to have reduced amounts of oil in 8-10 day period. However, differences between treated and untreated plots were not as dramatic as on the similarly treated cobble beach. Loss of subsurface oil in treated areas was also visually apparent. Reduction of oil contamination was particularly evident at sampling times, as noticeably less oil remained on sampling equipment used on this beach plot.

At this time, all other plots appeared as oiled as they did at the beginning of the field study. There were essentially no visual indications of oil removal on plots treated with slow-release fertilizer briquettes.

Over the next two to three weeks, the cleaned rectangle on the cobble beach remained clearly visible. Oil below the rocks remained but was less and less apparent and untreated reference plots appeared relatively unchanged. The oleophilic-treated mixed sand and gravel plot actually showed a greater loss of oil, appearing increasingly cleaner.

Six to eight weeks after fertilizer application the contrast between the treated and untreated areas on the cobble beach narrowed. This was due to reoiling from subsurface material concurrent with the slow removal of oil on the beach material surrounding the plot. However, it was evident that the total amount of oil on the treated plots had decreased substantially relative to reference plots. The corresponding mixed sand and gravel plot was also reoiled but to a lesser extent. All other plots still had observable oil contamination but generally less than that seen at the beginning of the study.

Toward the end of the summer season the area used for the bioremediation study became steadily cleaner, including most of the areas surrounding the test plots. This was attributed to several storms and more frequent rainfall. A heavily contaminated area to the south which was never treated, remained heavily contaminated by all visual criteria.

#### **NUTRIENT CONCENTRATIONS**

Table 7.1 shows the ammonia concentrations found in interstitial water for the treatment and reference plots. The initial background ammonia concentrations ( $T=0$ ) were low, and uniform throughout the plots. One to two days after application ( $T=1$ ) of the fertilizers, an increase in the ammonia concentrations was evident only in the plots treated with the oleophilic fertilizer. However, concentrations within the zones were highly variable. Based on the literature and laboratory nutrient release experiments described in Section 6, a pulse of ammonia was expected following application.

In contrast, ammonia concentrations in the plots treated with the slow-release briquettes remained at background levels. This is not unreasonable, because nutrient release studies with the briquettes showed nitrogen was released entirely as TKN, probably as urea. The absence of elevated  $NH_4$  concentrations suggests that, on the beaches, hydrolysis of urea by microorganisms leads to immediate uptake of the resulting ammonia by bacteria or algae.

Eight to 10 days after application of the fertilizers ( $T=2$ ), ammonia concentrations were above background only in the sand and gravel plot treated with oleophilic fertilizer. Ammonia concentrations in plots treated with briquettes were comparable

Table 7.1. Ammonia Concentrations in Interstitial Water Samples

NH<sub>3</sub> (uM N)

T=0 (before application)

<u>Tide Zone</u>	<u>Block</u>	<u>ESR</u>	<u>6/08/89 OSO</u>	<u>6/10/89 OSW</u>	<u>6/10/89 SCW</u>	<u>6/9/89 SCO</u>	<u>SCR</u>
High	1	NS	NS	NS	2.8	NS	NS
	3	NS	NS	NS	2.5	NS	NS
	5	NS	NS	NS	2.7	NS	NS
	7	NS	NS	NS	2.6	NS	NS
	Avg	NS	NS	NS	2.6	NS	NS
Mid	8	NS	2.0	2.1	3.0	2.3	NS
	10	NS	2.1	2.3	2.6	2.3	NS
	12	NS	2.0	2.3	2.5	2.2	NS
	14	NS	1.9	2.1	2.7	2.2	NS
	Avg	NS	2.0	2.2	2.7	2.2	NS
Low	15	NS	2.1	2.1	2.6	2.0	NS
	17	NS	2.4	2.0	2.7	2.4	NS
	19	NS	2.3	2.2	2.7	2.1	NS
	21	NS	2.1	2.0	2.6	2.6	NS
	Avg	NS	2.2	2.0	2.6	2.3	NS

T=1 (1-2 days post application)

<u>Tide Zone</u>	<u>Block</u>	<u>ESR</u>	<u>6/09/89 OSO</u>	<u>6/12/89 OSW</u>	<u>6/12/89 SCW</u>	<u>6/10/89 SCO</u>	<u>SCR</u>
High	1	NS	NS	NS	1.2	57.0	NS
	3	NS	NS	NS	DL	300.0	NS
	5	NS	NS	NS	0.5	9.9	NS
	7	NS	NS	NS	0.4	3.8	NS
	Avg	NS	NS	NS	0.5	92.7	NS
Mid	8	NS	2.6	0.4	0.2	410.0	NS
	10	NS	92.0	0.7	2.2	61.0	NS
	12	NS	8.5	0.2	DL	2.8	NS
	14	NS	4.8	0.2	0.3	6.5	NS
	Avg	NS	27.0	0.4	0.7	120.0	NS
Low	15	NS	22.0	1.1	0.8	190.0	NS
	17	NS	460.0	0.9	0.6	2.9	NS
	19	NS	9.4	0.5	0.5	2.4	NS
	21	NS	2.4	1.0	0.4	3.0	NS
	Avg	NS	123.4	0.9	0.6	48.8	NS



Table 7.1. (Continued)

T=2 (8-10 days post application)

<u>Tide</u> <u>Zone</u>	<u>Block</u>	<u>ESR</u>	6/18/89 <u>OSO</u>	6/18/89 <u>OSW</u>	6/18/89 <u>SCW</u>	6/19/89 <u>SCO</u>	6/19/89 <u>S C R</u>
High	1	NS	NS	NS	DL	ND	ND
	3	NS	NS	NS	DL	ND	ND
	5	NS	NS	NS	DL	ND	ND
	7	NS	NS	NS	DL	ND	ND
	Avg	NS	NS	NS	DL	ND	ND
Mid	8	DL	36.0	DL	DL	ND	ND
	10	DL	30.0	0.3	DL	ND	ND
	12	DL	30.0	0.3	DL	ND	ND
	14	DL	2.8	DL	DL	ND	ND
	Avg	DL	24.7	0.2	DL	ND	ND
Low	15	0.5	19.0	DL	1.3	ND	ND
	17	1.1	29.0	0.3	0.3	ND	ND
	19	1.1	3.9	0.6	0.6	ND	ND
	21	0.8	0.9	DL	0.5	ND	ND
	Avg	0.8	13.2	0.2	0.7	ND	ND

T=3 (30 days post application)

<u>Tide</u> <u>Zone</u>	<u>Block</u>	7/7/89 <u>ESR</u>	7/7/89 <u>OSO</u>	7/7/89 <u>OSW</u>	7/7/89 <u>SCW</u>	7/7/89 <u>SCO</u>	7/7/89 <u>SCR</u>
High	1	0.6	0.3	0.5	1.0	0.4	1.4
	3	0.4	0.4	0.6	0.4	0.4	0.2
	5	0.5	0.3	0.9	0.6	0.6	0.8
	7	0.7	0.5	0.4	0.6	0.5	0.2
	Avg	0.6	0.4	0.6	0.6	0.5	0.6
Mid	8	0.4	0.3	0.5	0.6	1.0	0.9
	10	0.4	0.2	0.5	0.8	1.0	1.4
	12	0.4	0.4	0.4	0.8	0.6	0.5
	14	0.7	0.4	0.5	0.8	0.6	1.0
	Avg	0.5	0.3	0.5	0.8	0.8	1.0
Low	15	0.6	0.5	0.5	4.2	0.8	0.5
	17	0.4	0.4	0.5	1.0	0.9	0.6
	19	0.4	0.4	0.4	0.8	0.6	1.0
	21	0.6	0.4	0.6	0.8	0.7	1.2
	Avg	0.5	0.4	0.5	1.7	1.8	0.8

Table 7.1. (Continued)

T=4 (6 weeks post application)

<u>Tide</u> <u>Zone</u>	<u>Block</u>	7/17/89 <u>ESR</u>	7/16/89 <u>OSO</u>	7/16/89 <u>OSW</u>	7/16/89 <u>SCW</u>	7/16/89 <u>SCO</u>	7/17/89 <u>SCR</u>
High	1	1.0	NS	NS	NS	NS	1.5
	3	1.2	NS	NS	NS	NS	1.4
	5	1.3	NS	NS	NS	NS	1.2
	7	1.0	NS	NS	NS	NS	1.5
	Avg	1.1	NS	NS	NS	NS	1.4
Mid	8	1.3	0.9	0.8	1.3	1.8	1.3
	10	1.0	1.2	0.7	1.2	1.0	1.3
	12	0.8	1.0	1.0	1.3	1.0	1.0
	14	1.0	0.9	0.8	1.3	1.5	1.2
	Avg	1.0	1.0	0.8	1.3	1.3	1.2
Low	15	1.0	0.9	1.0	1.3	1.3	1.1
	17	1.0	1.1	1.0	1.3	1.3	1.2
	19	1.3	0.8	1.1	1.2	1.3	1.4
	21	1.7	0.9	1.0	1.3	1.3	1.3
	Avg	1.2	0.9	1.0	1.3	1.3	1.2

ESR = Control Mixed Sand and Gravel

SCR = Control Cobble

OSO = Oleophilic Fertilizer-Treated Mixed Sand and Gravel

SCO = Oleophilic Fertilizer-Treated Cobble

OSW = Water-Soluble Fertilizer-Treated Mixed Sand and Gravel

SCW = Water-Soluble Fertilizer-Treated Cobble

NS = No Sample Taken

DL = Detection Limit

ND = No Data Available

to the reference plot. At approximately 4 and 6 weeks after the fertilizer application (T=3 and T=4, respectively), no substantial difference in the ammonia concentrations was apparent between the treatment and the reference plots.

Table 7.2 shows nitrate/nitrite concentrations found in interstitial water for the treatment and reference plots. One to 2 days following application, notable concentrations of nitrate were found in samples taken from the briquette-treated beaches. Eight to 10 days after application (T=2), sand and gravel beaches treated with oleophilic fertilizer showed substantially higher levels of nitrate/nitrite nutrients than did the reference plots. Plots treated with water-soluble fertilizer showed only slightly elevated concentrations. One month after fertilizer application (T=3), nitrate/nitrite levels in the treated plots were only slightly higher than in the reference plots, particularly for the cobble beach treated with briquettes. Neither the Inipol or the briquettes contain nitrate or nitrite. Thus, the presence of these nutrients have been the result of ammonia conversion to nitrite by nitrification.

Samples taken in July from streams near Eagle and Otter Beaches showed measurable levels of inorganic nutrients. The stream to the south of Eagle Beach had 5.2  $\mu\text{M}$  nitrogen as nitrate. Stream samples taken adjacent to Otter Beach contained an average of 4.8  $\mu\text{M}$  nitrogen as nitrate. A sample of snow collected from a snow pile 300 yards southeast of Eagle Beach (a result of a winter avalanche) contained 2.8  $\mu\text{M}$  of nitrogen as ammonia, 0.54  $\mu\text{M}$  of phosphorus as phosphate, and 1.1  $\mu\text{M}$  of nitrogen as nitrate. Although these concentrations were relatively low, they indicate that snow-melt and runoff may serve as important sources of nutrients for limited sections of the shoreline, particularly in the spring and early summer. Even though some of the test plots were located near the streams, nutrient concentrations in the plots were probably unaffected. This was an unlikely source of the nitrate found in the treated beaches, as no elevated nitrate/nitrite was detected in reference beaches having equal exposure to the freshwater. Also, no nitrate/nitrite was found at T=0 in any of the plots.

On June 19, the briquette bags were repositioned, and all the bags were placed in the mid- and low-tide zones of the plots. This resulted in the fertilizer being submerged a longer time, enhancing nutrient transport in these zones. In general, this repositioning did not have a detectable impact on nutrient distribution on the beaches; i.e., nutrient concentrations in the zones showed no new trends. It was still apparent that minimal dispersion of the nutrients was occurring from the briquettes in areas of the shoreline not subjected to routine tidal washing. Precipitation during the month of June was probably insufficient to effectively transport nutrients released from the bags of briquettes located in the high-tide zone.

Table 7.2. Nitrate/Nitrite Concentrations in Interstitial Water Samples

Total Concentrations of  $\text{NO}_3 + \text{NO}_2$  ( $\mu\text{M N}$ )

T=1 (1-2 days before application)

<u>Tide Zone</u>	<u>Block</u>	<u>ESR</u>	6/08/89 <u>OSO</u>	6/10/89 <u>OSW</u>	6/10/89 <u>SCW</u>	6/9/89 <u>SCO</u>	<u>SCR</u>
High	1	NS	NS	NS	7.8	ND	NS
	3	NS	NS	NS	1.2	ND	NS
	5	NS	NS	NS	1.3	ND	NS
	7	NS	NS	NS	8.5	ND	NS
	Avg	NS	NS	NS	4.7	ND	NS
Mid	8	NS	ND	13.6	20.8	ND	NS
	10	NS	ND	16.3	35.8	ND	NS
	12	NS	ND	18.8	36.0	ND	NS
	14	NS	ND	9.2	38.7	ND	NS
	Avg	NS	ND	14.5	33.1	ND	NS
Low	15	NS	ND	6.6	48.2	ND	NS
	17	NS	ND	5.1	29.4	ND	NS
	19	NS	ND	67.5	56.5	ND	NS
	21	NS	ND	18.0	42.6	ND	NS
	Avg	NS	ND	23.3	44.2	ND	NS

T=2 (8-10 days post application)

<u>Tide Zone</u>	<u>Block</u>	6/18/89 <u>ESR</u>	6/18/89 <u>OSO</u>	6/18/89 <u>OSW</u>	6/19/89 <u>SCW</u>	6/19/89 <u>SCO</u>	<u>SCR</u>
High	1	NS	NS	NS	DL	ND	ND
	3	NS	NS	NS	1.8	ND	ND
	5	NS	NS	NS	2.2	ND	ND
	7	NS	NS	NS	7.0	ND	ND
	Avg	NS	NS	NS	2.8	ND	ND
Mid	8	2.8	12.0	5.7	8.6	ND	ND
	10	1.5	17.0	1.4	14.0	ND	ND
	12	0.6	29.0	0.5	24.0	ND	ND
	14	4.1	14.2	8.3	19.0	ND	ND
	Avg	2.2	18.0	4.0	16.4	ND	ND
Low	15	1.9	11.0	2.5	14.7	ND	ND
	17	1.4	25.0	4.3	17.7	ND	ND
	19	1.7	6.1	2.4	16.4	ND	ND
	21	5.7	5.3	2.4	19.5	ND	ND
	Avg	2.7	11.8	2.9	17.1	ND	ND

Table 7.2. (continued)

T=3 (30 days post application)

<u>Tide</u> <u>Zone</u>	<u>Block</u>	7/7/89 <u>ESR</u>	7/7/89 <u>OSO</u>	7/7/89 <u>OSW</u>	7/7/89 <u>SCW</u>	7/7/89 <u>SCO</u>	7/7/89 <u>SCR</u>
High	1	0.6	0.7	0.2	0.6	3.1	0.2
	3	DL	0.7	DL	0.9	2.7	0.7
	5	0.6	0.5	0.6	1.7	7.1	2.2
	7	DL	1.0	3.6	1.4	1.4	3.7
	Avg	0.3	0.7	1.0	1.2	3.6	1.7
Mid	8	0.1	2.5	0.6	1.7	4.3	4.0
	10	DL	2.8	0.4	9.6	4.5	3.4
	12	1.1	0.5	0.3	11.0	2.8	1.9
	14	1.3	0.6	0.4	11.0	3.1	2.2
	Avg	0.6	1.6	0.4	8.3	3.7	2.9
Low	15	DL	2.7	0.8	4.4	4.4	3.6
	17	0.1	4.1	1.7	7.2	7.2	3.2
	19	1.3	1.7	1.5	2.9	2.9	2.7
	21	0.3	1.6	0.1	2.9	2.9	4.1
	Avg	0.4	2.5	1.0	4.4	4.4	3.4

---

ESR = Control Mixed Sand and Gravel

SCR = Control Cobble

OSO = Oleophilic Fertilizer-Treated Mixed Sand and Gravel

SCO = Oleophilic Fertilizer-Treated Cobble

OSW = Water-Soluble Fertilizer-Treated Mixed Sand and Gravel

SCW = Water-Soluble Fertilizer-Treated Cobble

NS = No Sample Taken

DL = Detection Limit

ND = No Data Available

## CHANGES IN OIL RESIDUE WEIGHT AND COMPOSITION

Data analysis for oil residue weight and chemistry in samples taken from beach plots in Snug Harbor has not yet been completed. Over 1100 samples have been analyzed and the resulting information is being incorporated into the data base. Six different approaches for analyzing trends in the data are being used. These involve analysis through time of the following;

- Oil residue weights (methylene chloride extractable material),
- Ratios of C17/pristane and C18/phytane,
- Gas chromatographic profiles of aliphatic hydrocarbons,
- Total concentrations of aliphatic hydrocarbons,
- Average individual aliphatic hydrocarbon concentrations and,
- Relationship of degradation extent to oil residue weight.

For oil residue weights and ratios of C17/Pristane and C18/Phytane, data is presented as mean values. All data from the cobble beaches has a top and bottom component. Top refers to the oil extracted from the surfaces of the cobblestones and bottom refers to the mixed sand and gravel below the cobble.

As expected, oil distribution in the beach plots was heterogeneous. Sampling procedures, although carefully standardized, could not guarantee a constant volume or surface area of sample each time, particularly in cobble beaches. Standard deviations around the means are therefore large in most cases, making determinations of statistical significance difficult. Several statistical approaches are currently being evaluated to assist in interpretation of the data. Some general statements, however, can be made at this time.

### Residue Weight

Changes in the mean residue concentrations through time for all plots are shown in Figures 7.1 to 7.4. Each data point on the figures is the mean of the number of samples available at this time (maximum samples equals 21 for any sampling time). Where a small number of samples has been included, standard deviations may be quite large.

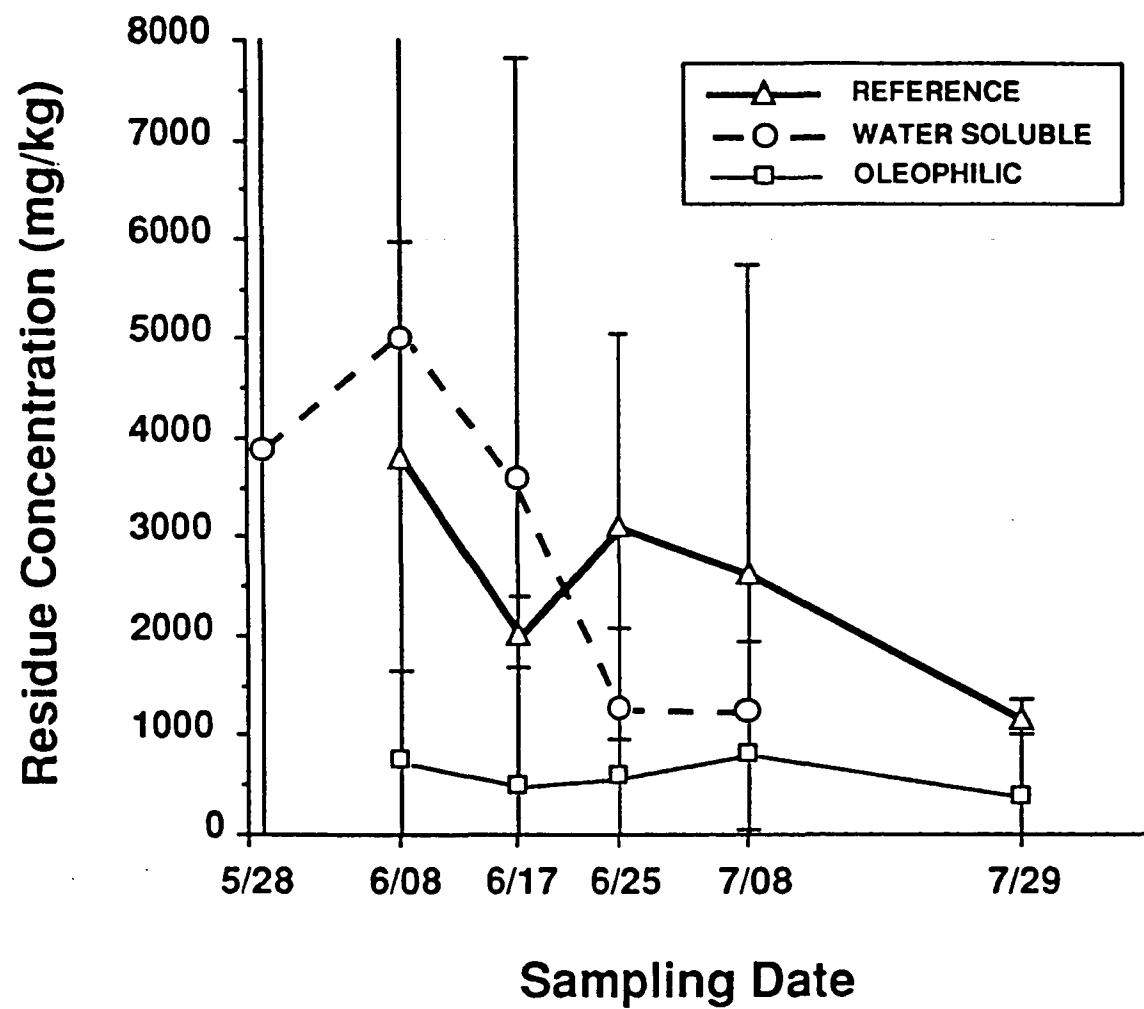


Figure 7.1 Mean Residue Concentration at Snug Harbor Mixed Sand and Gravel Plots, All Zones.

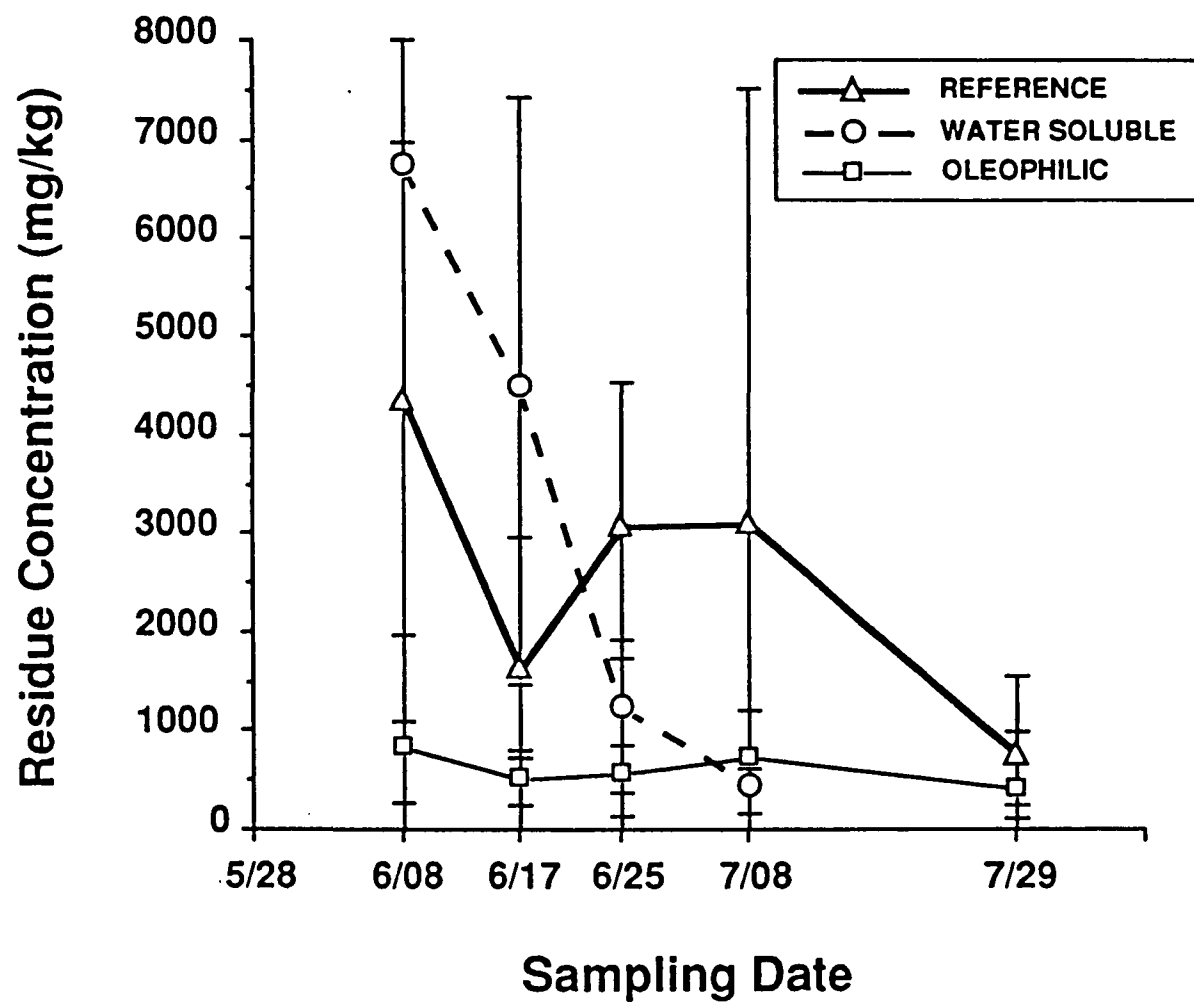


Figure 7.2 Mean Residue Concentration at Snug Harbor Mixed Sand and Gravel Plots, Mid and Low Tide Zones.



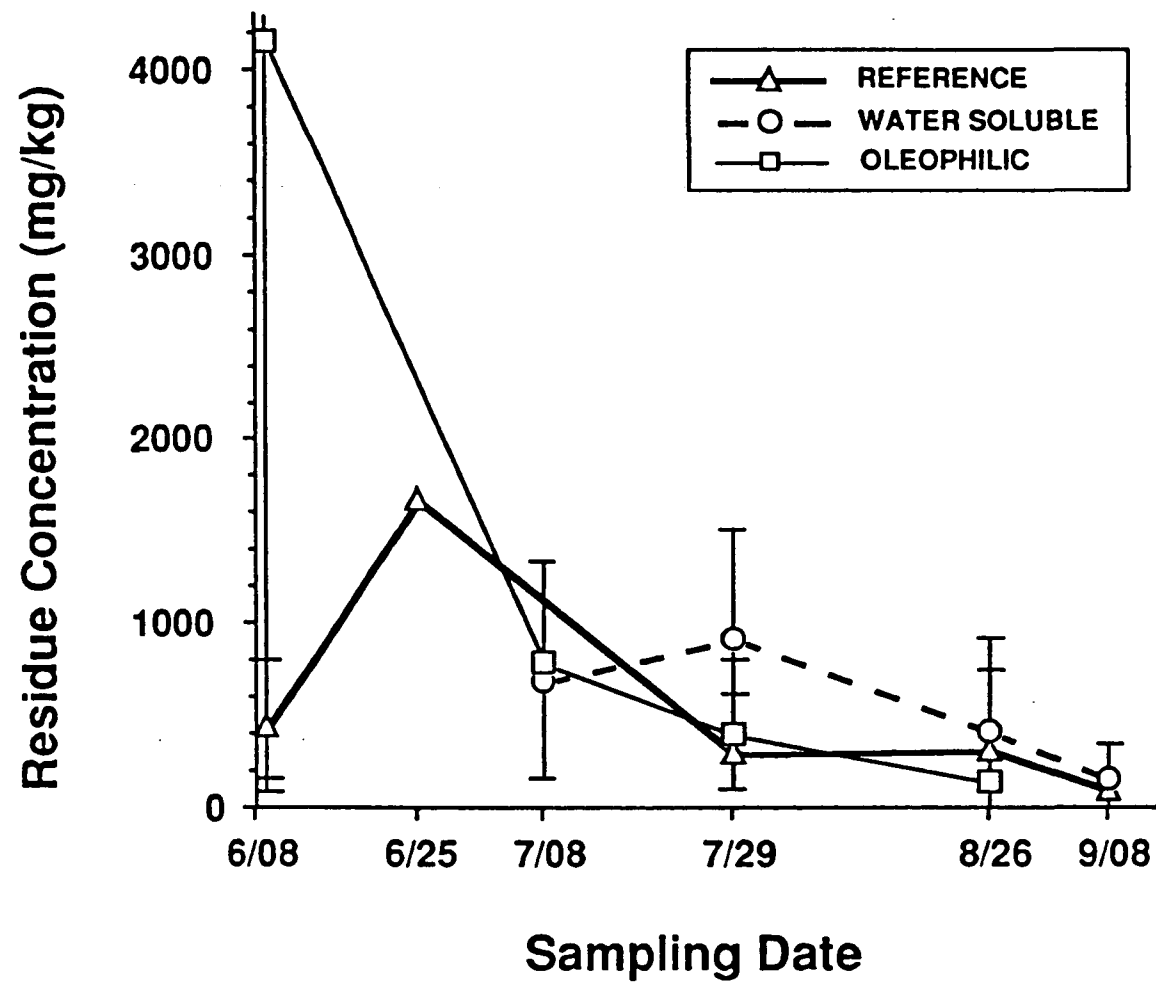


Figure 7.3 Mean Residue Concentration at Snug Harbor Cobble Plots, Top, All Zones.

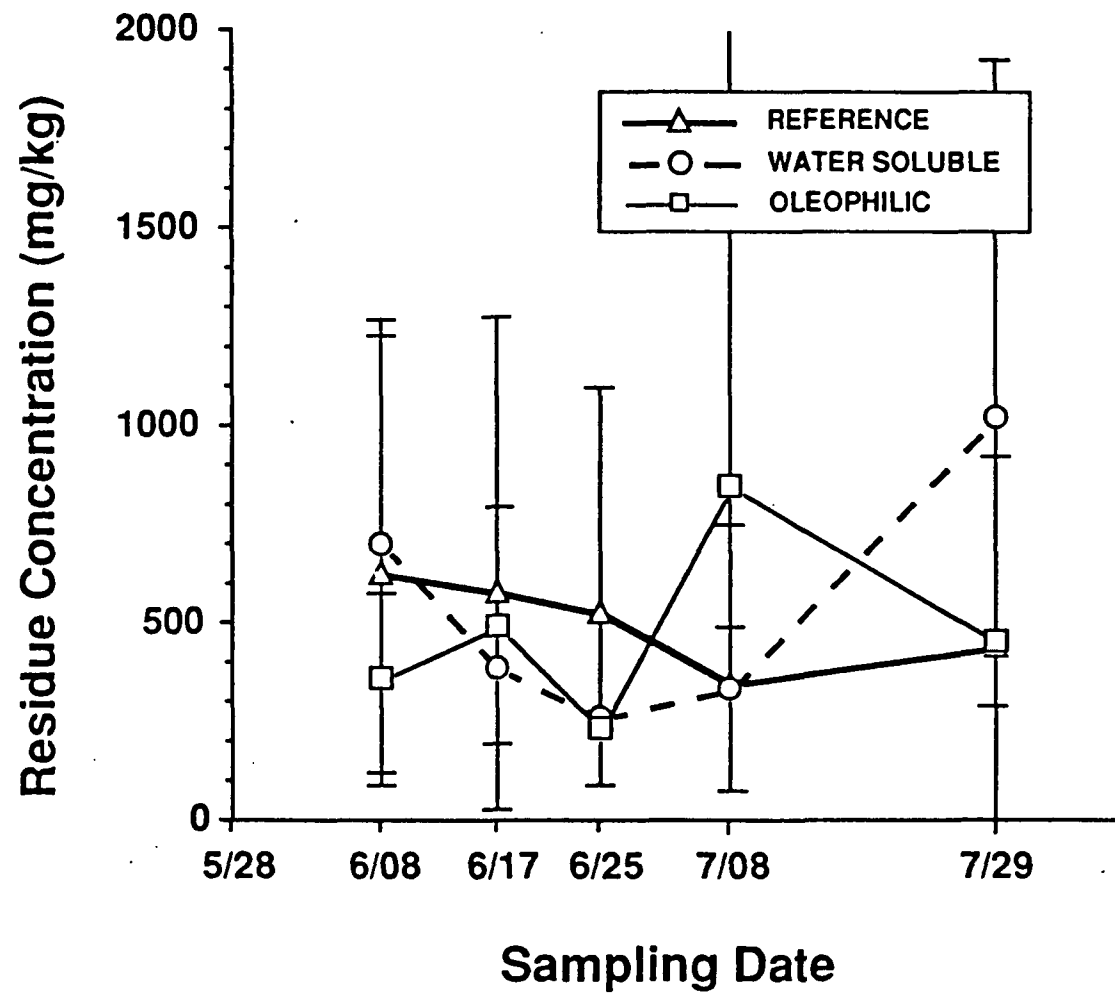


Figure 7.4 Mean Residue Concentration at Snug Harbor Cobble Plots, Bottom, All Zones.

In the mixed sand and gravel plots (Figure 7.1), residue weights showed a decreasing trend over time for the reference plot and the briquette-treated plot. The data is variable. As much as a 5-fold decrease in residue weight was apparent in the briquette-treated plot with some indication that the overall rate of decrease was more rapid than in the reference plot. This difference may be attributable to nutrient addition but it could not be verified by statistical analysis. Although several factors could control changes in oil residue weights, the large decreases may indicate extensive oil degradation.

The residue weights in the mixed sand and gravel beach treated with oleophilic fertilizer were very low at the time of fertilizer application and did not appear to decrease substantially thereafter. The initial concentration of oil in this plot was in the same range as that seen in the other treated and untreated plots toward the end of July.

When the mid and low tide zones of the mixed sand and gravel plots are considered (Figure 7.2), the same general trends are apparent. However, the relative difference in rate of residue weight loss in the briquette-treated plot compared to the others is even more pronounced.

Data for the residues of oil on the cobble rock surfaces are shown in Figure 7.3. Unfortunately, information from several sampling periods are not yet complete. However, it would appear that oil residue weights decreased dramatically over the two weeks following application of the oleophilic fertilizer. This corresponds with the visual observation. Information on the reference plot during this time period is not available and therefore it is not known if decreases in residue waste were as extensive.

In the cobble plots, oil concentrations in the mixed sand and gravel under the cobble was initially very low (Figure 7.3). Essentially no change in oil residue weights was apparent in any plot or in any zone within a plot.

#### Ratios of branched and straight chain hydrocarbons

Changes in the C17/pristane and C18/phytane ratios through time for all plots are shown in Figures 7.5 to 7.10. Each data point on the figures is the mean of the number of samples available (maximum samples equals 21 for any sampling time). Where a small number of samples has been included, standard deviations may be quite large.

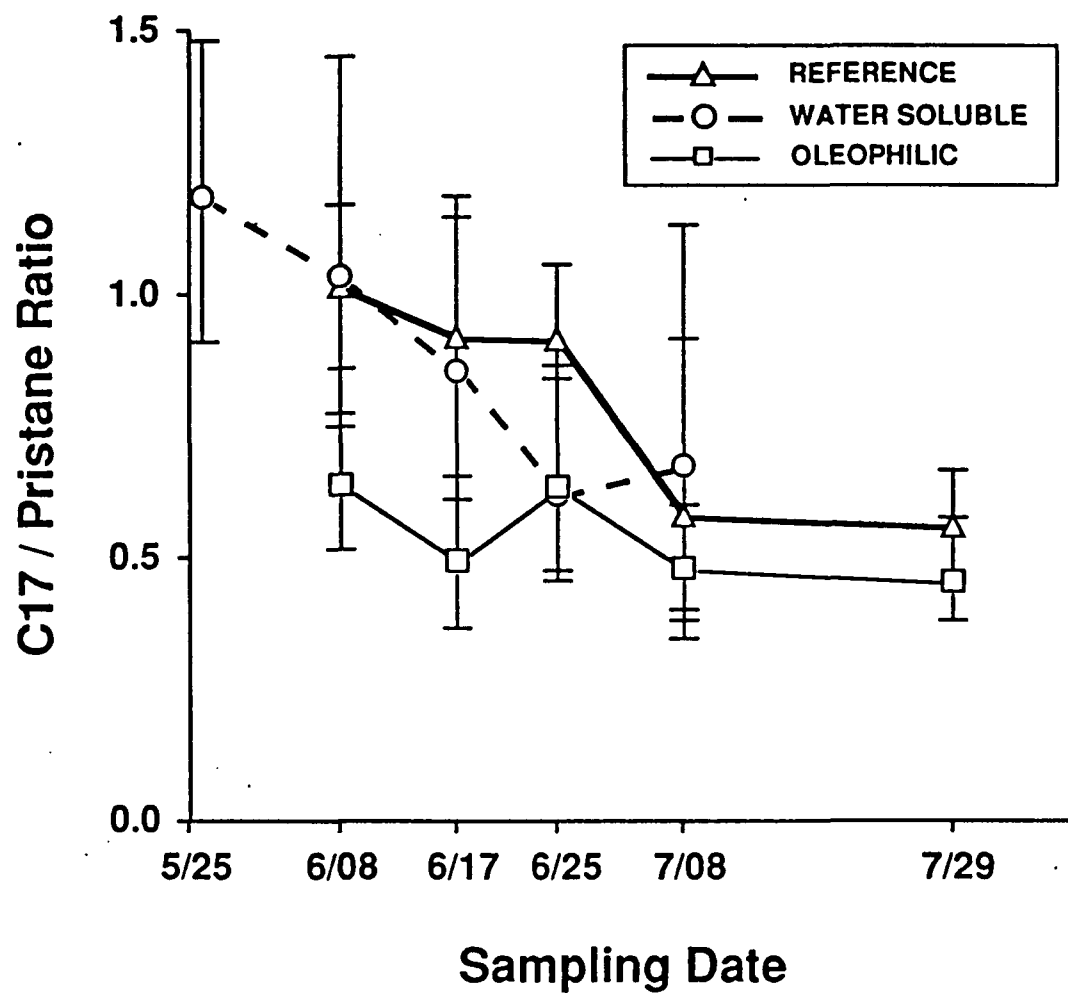


Figure 7.5 Mean C17 / Pristane Ratio at Snug Harbor Mixed Sand and Gravel Plots, All Zones.

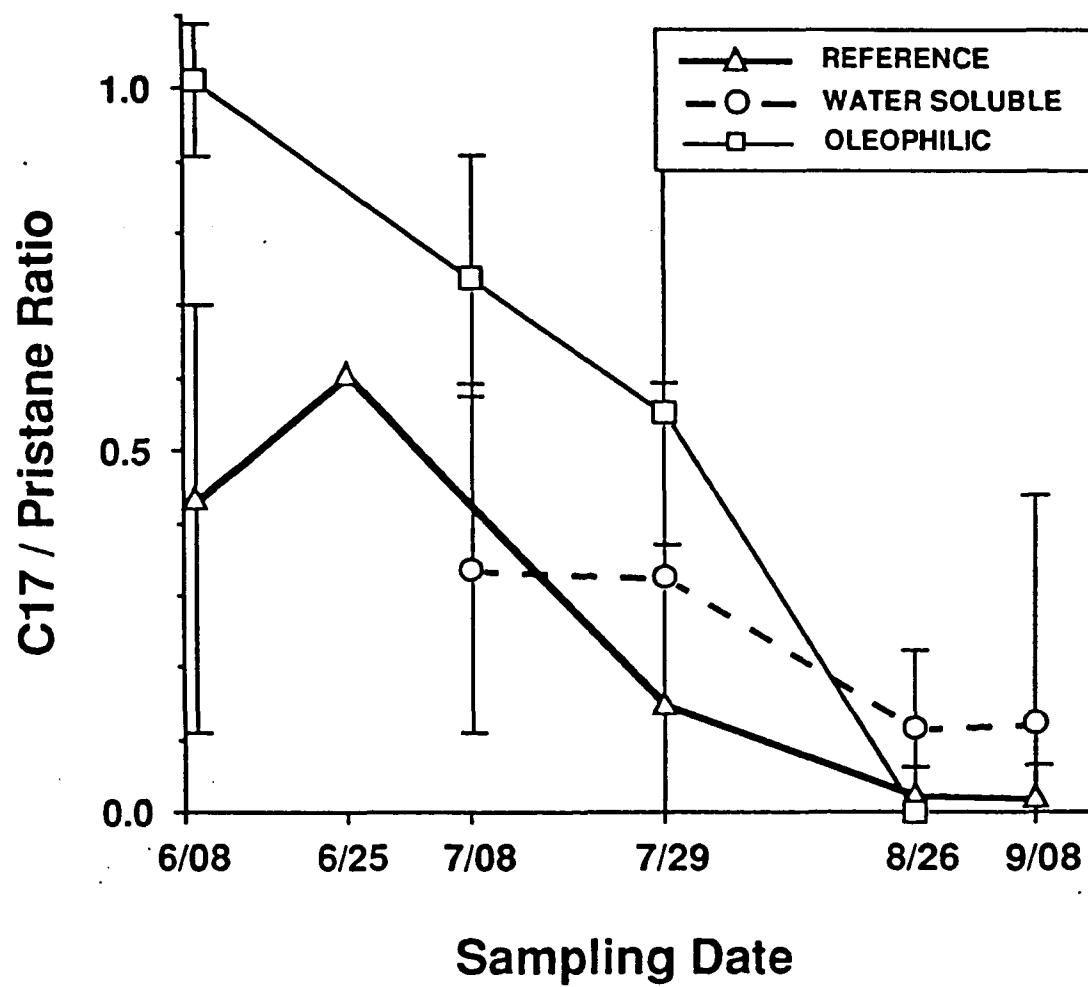


Figure 7.6 Mean C17 / Pristane Ratio at Snug Harbor Cobble Plots, Top, All Zones.

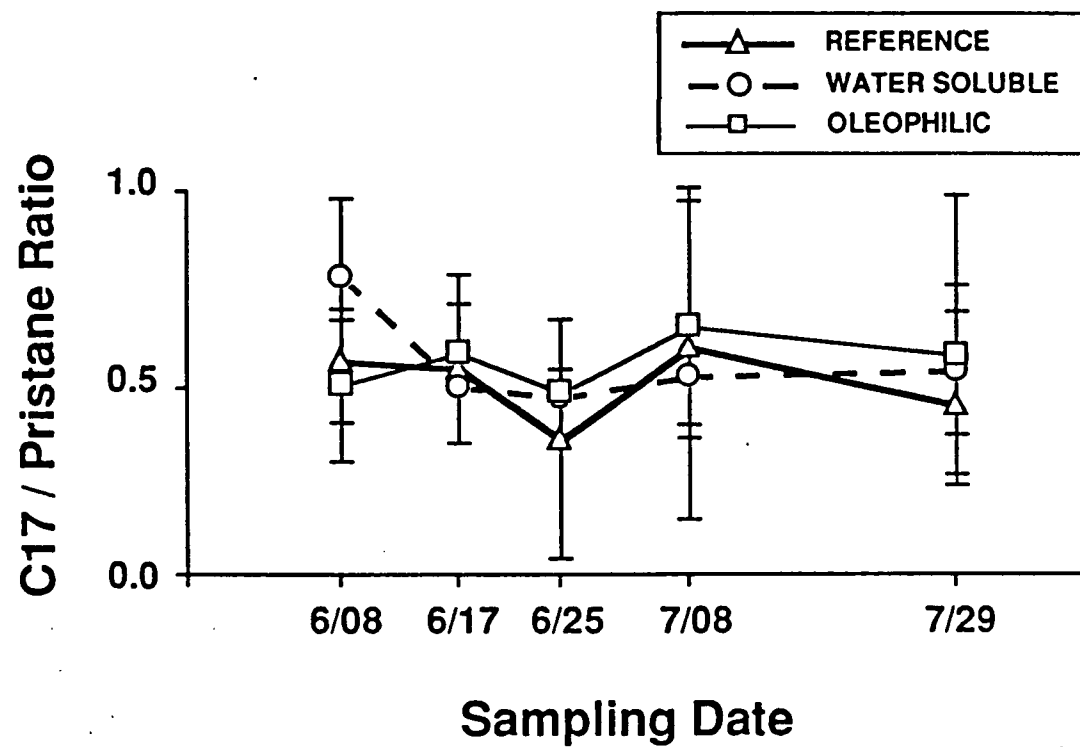


Figure 7.7 Mean C17 / Pristane Ratio at Snug Harbor Cobble Plots, Bottom, All Zones.

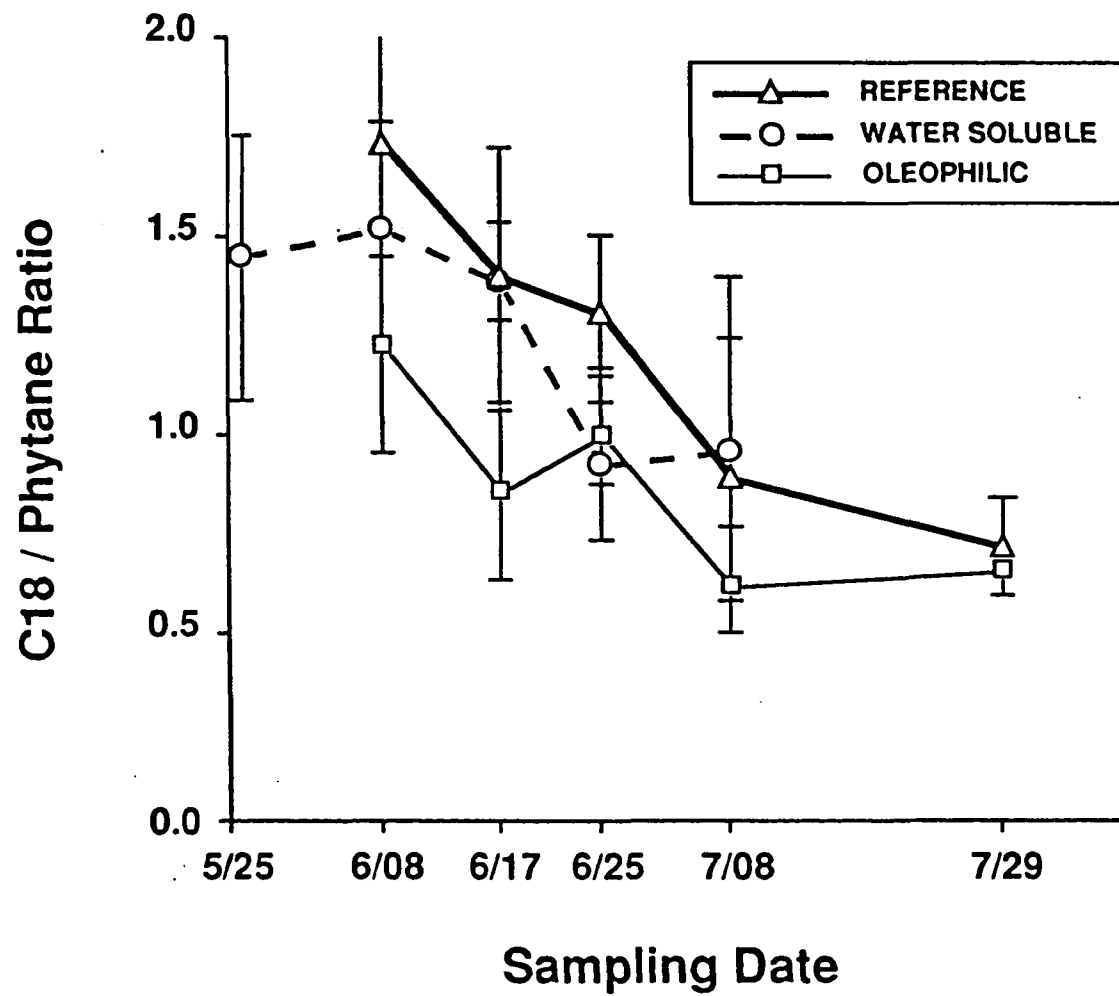


Figure 7.8 Mean C18 / Phytane Ratio at Snug Harbor Mixed Sand and Gravel Plots, All Zones.

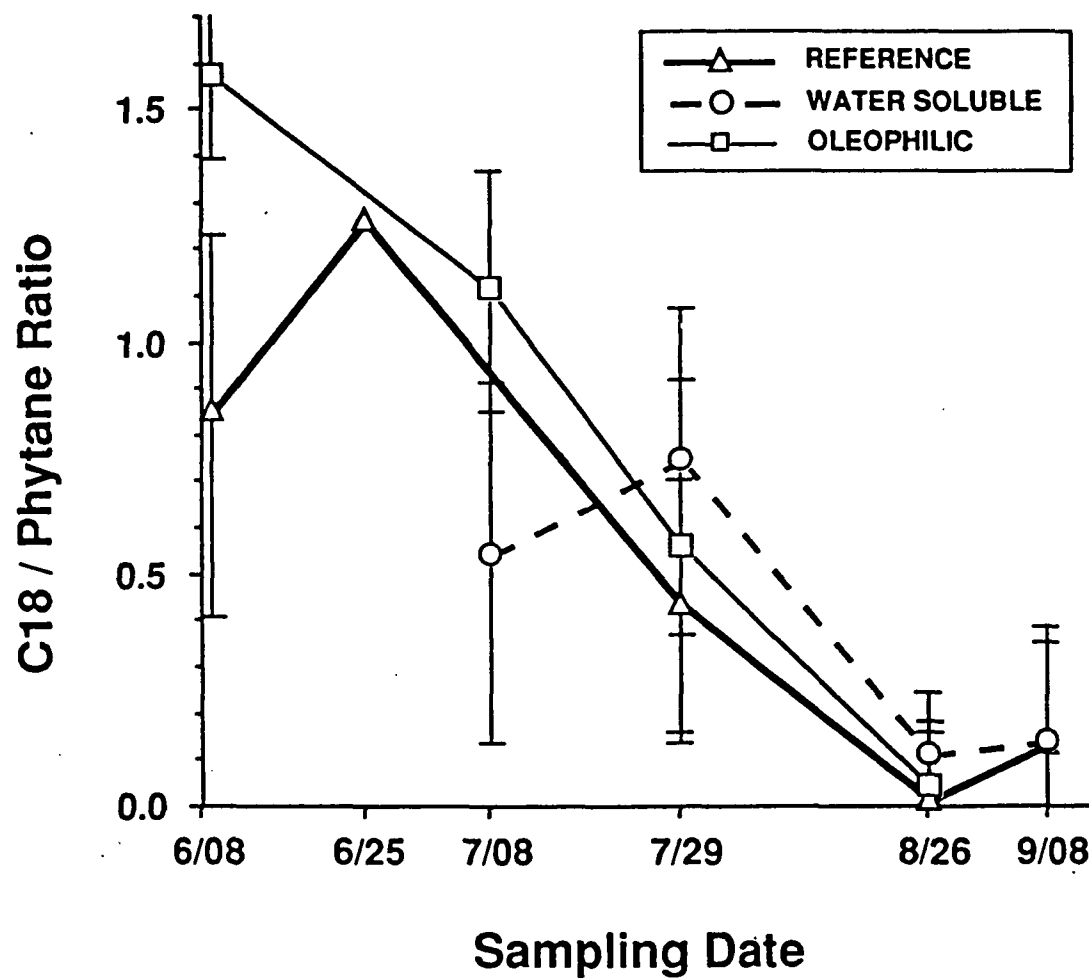


Figure 7.9 Mean of C18 / Phytane Ratio at Snug Harbor Cobble Plots, Top, All Zones.



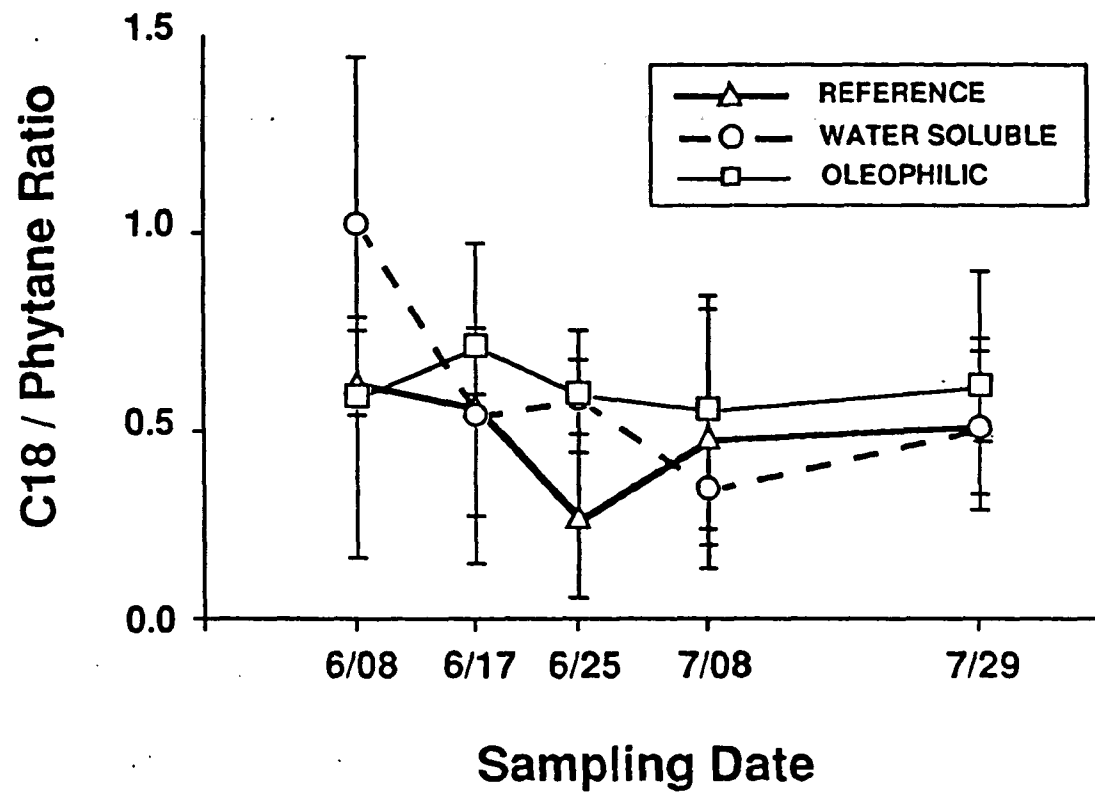


Figure 7.10 Mean C18 / Phytane Ratio at Snug Harbor Cobble Plots, Bottom, All Zones.

In mixed sand and gravel plots (Figures 7.5 and 7.8), the ratios showed a general decrease through time for the reference plot and the briquette-treated plot. Decreases in these ratios are traditionally considered good predictors of biological degradation of oils. As much as a 2-fold decrease in the ratio was apparent in the briquette-treated plot with some indication that the overall rate of decrease was more rapid than in the reference plot. Examination of the low and mid tide zones taken together (data not shown) reflected the same trend. This difference may be attributable to nutrient addition but the effect could not be verified by statistical analysis.

At the July 8 sampling, the ratios appeared to have increased. Reoiling of the beaches or, more likely, degradation of the internal standards, pristane and phytane, could be responsible for this increase.

Data for the C17/pristane and C18/phytane ratios on the cobble rock surfaces are shown in Figure 7.6 and 7.9. As mentioned above, information for some sampling periods is not complete at this time. It would appear that in all plots, ratios decreased through time. Nonetheless it was evident that biodegradation of the oil (i.e., significant change in the ratios) was occurring in the oleophilic-treated cobble plot at about the time visual loss of oil from the beaches was observed (week 2 to 4). Since a similar decrease in ratios was occurring in the reference cobble plot, yet no visual loss of the oil was apparent in the field, it would appear that the oleophilic fertilizer was having a more extensive effect on the biodegradation processes that is, as yet, undefined.

In the oleophilic-treated mixed sand and gravel plots (Figures 7.5 and 7.8), the ratios decreased as well but seemingly at a much slower rate. Data from the mixed sand and gravel under the cobble (Figures 7.7 and 7.10) showed little change in the ratios through time. The ratios were initially very low compared to ratios measured in oil samples from other plots. This is surprising; i.e., despite very low concentrations of oil in these samples (see Figure 7.1), it was not expected that the ratios would necessarily be low as well. This may reflect a more rapid biodegradation of the oil due to its low concentration and its distribution over a large surface area (see section below which provides data for this relationship). The C18/phytane ratio in samples from the briquette-treated plot showed a decrease through time but again this has not yet been statistically verified.

## Gas Chromatographic Profiles

For the C17/pristane ratios there was a possible faster change in the oleophilic-treated plot relative to the reference plot, although this is not the case for the C18/phytane ratio. Note, however that the data point at the 6:25 sampling represents information from only one block. Other measures of degradation and data analysis were examined to further explain striking visual differences of oil disappearance. Examination of gas chromatographic profiles provided a qualitative indication of important changes that could be later verified through more quantitative measures.

Gas chromatographic profiles were therefore recreated by computer. Representative examples of these illustrations are shown in Figure 7.11a and b. All lines on the profiles represent concentrations of aliphatic hydrocarbons (approximately 12 through 28) normalized to the oil residue weight. As a floating concentration scale was used in the initial analysis to accommodate all profiles (Figure 7.11a,b), changes in relative concentrations for the hydrocarbons can be visualized by comparing the overall profile of the peaks to a profile typical of a relatively undegraded but weathered oil. This is shown as the solid "mountain" line in the figure. However, comparing absolute peak height is not meaningful. Blank graphs indicate that data for that block was unavailable.

Data from two sampling times, two and four weeks after application of the oleophilic fertilizer to the cobble plot, are shown in Figure 7.11. The gas chromatographic profiles are for oil extracted from the surface of the rocks. These profiles attest to the heterogeneity of oil composition within a plot. Further, low molecular weight aliphatic hydrocarbons have decreased, indicating significant biodegradation has occurred. This is important as this degradation corresponds with the observed loss of oil from the oleophilic treated plots in the field. Visual impressions of differences between the tidal zones (top line - high tide zone; middle line - mid tide zone; bottom line = low tide zone) can also be examined. Samples analyzed from the low tide zone of the cobble plots receiving the oleophilic fertilizer (Figure 7.11b) may indicate more loss of hydrocarbons than samples from the other zones.

The recreated profiles can also be illustrated without the floating concentration axis (i.e., all the same scale). Examples comparing the mixed sand and gravel plots treated with oleophilic and slow release fertilizers are shown in Figures 7.12a through f. These figures show analysis of samples taken prior to fertilizer application, and 2 to 4 weeks following fertilizer application. It is apparent that there is considerably less oil and more degradation of the alkanes (change in the peak profile) in the samples taken from the oleophilic-treated plots in the 4 week

## Snug Harbor - Cobble Surface - Oleophilic Fertilizer - 2 Weeks After Application

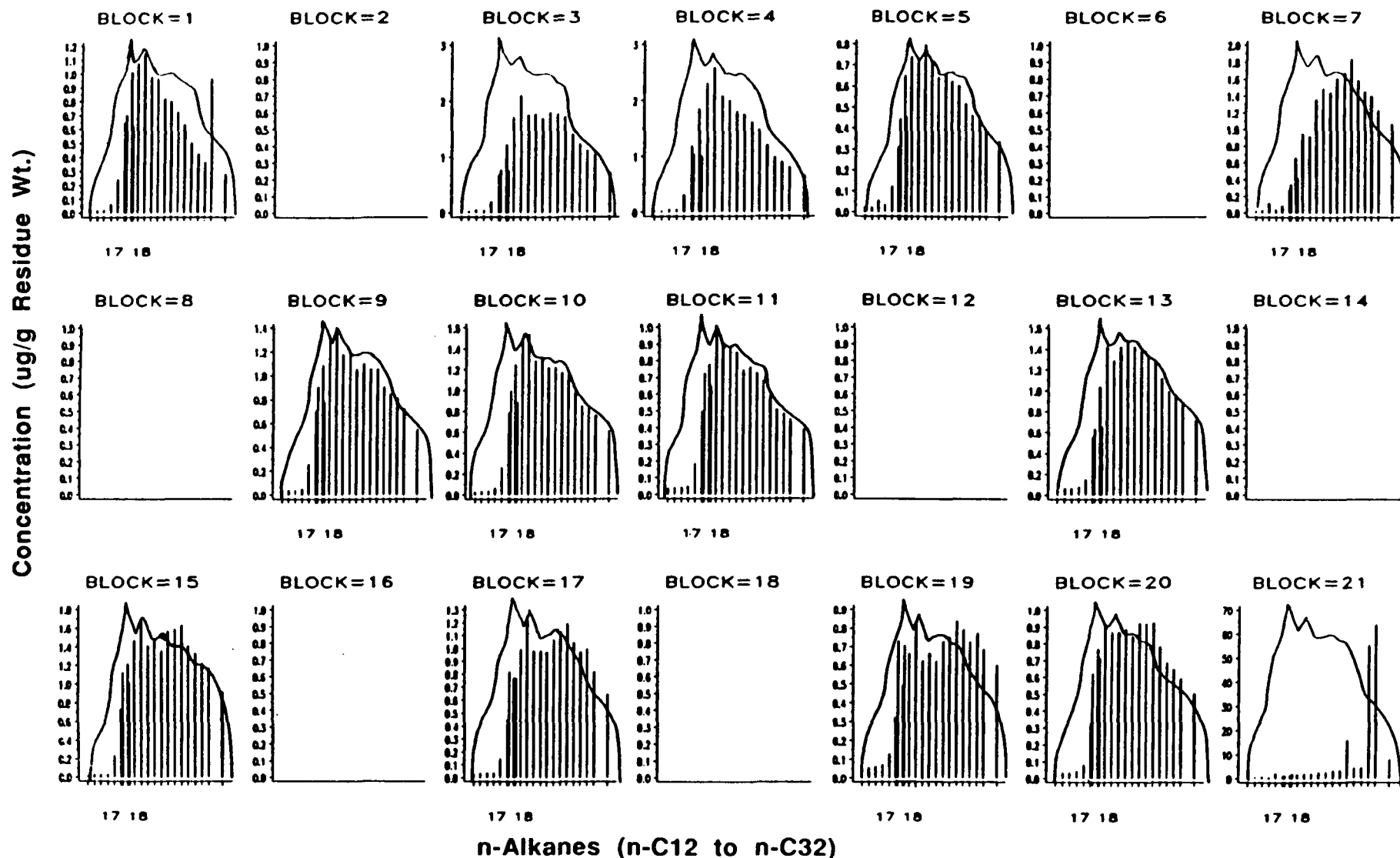
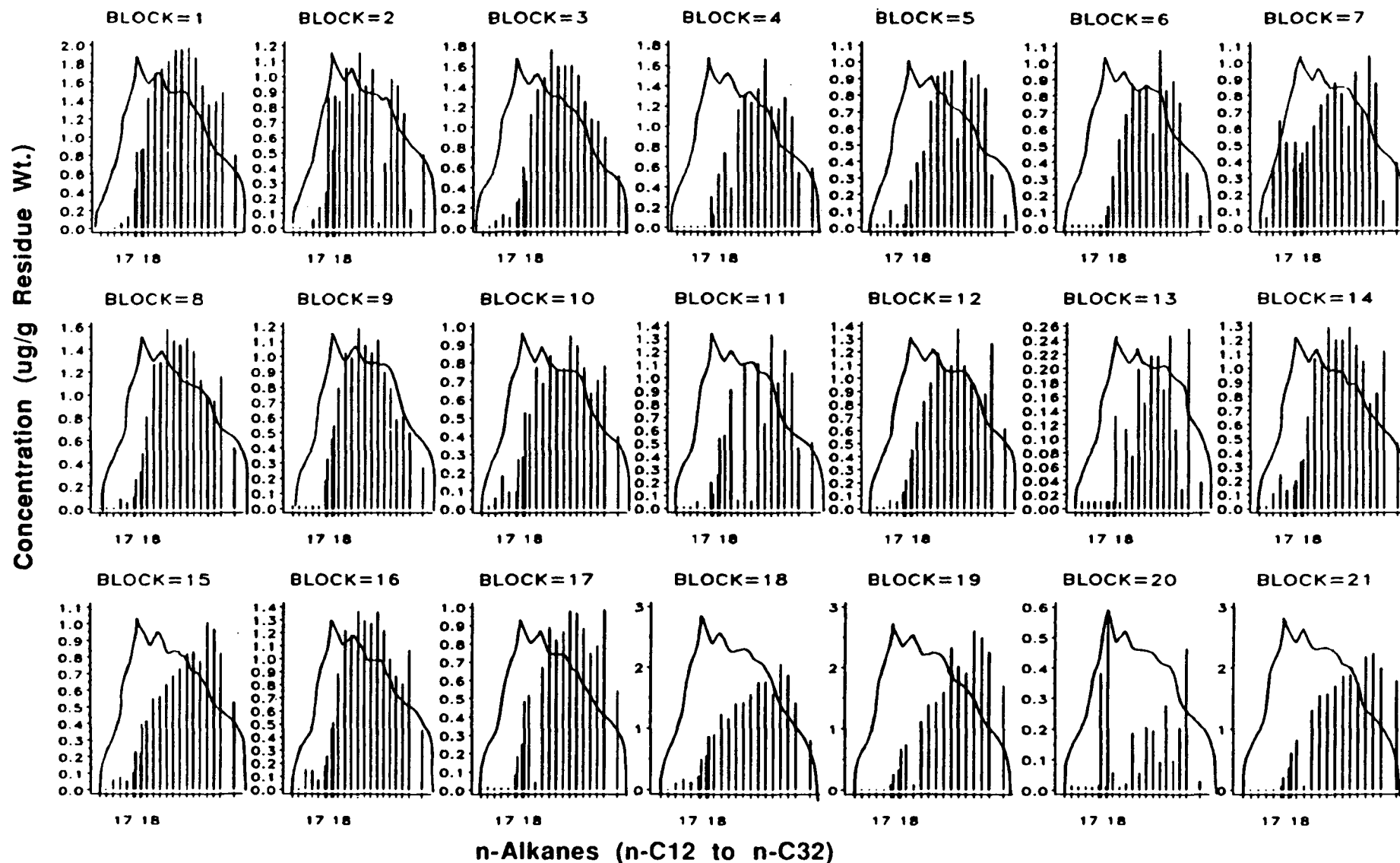


Figure 7.11a. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble two weeks following application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. Solid line profile estimates peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.

## Snug Harbor - Cobble Surface - Oleophilic Fertilizer - 4 Weeks After Application



**Figure 7.11b. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble four weeks following application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. Solid line profile estimates peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.**

## 93



## Snug Harbor - Below Cobble - Oleophilic Fertilizer - 2 Weeks After Application

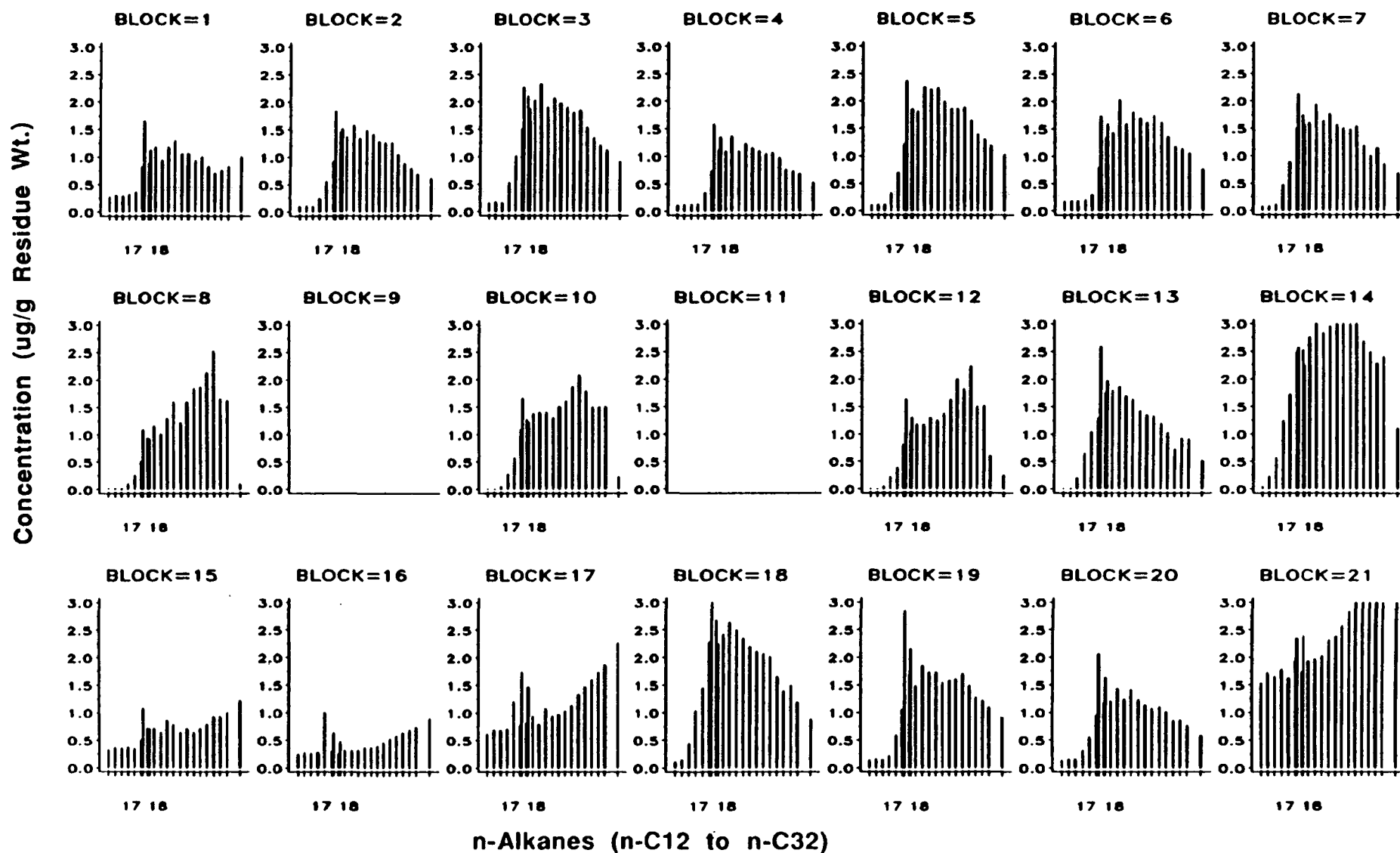
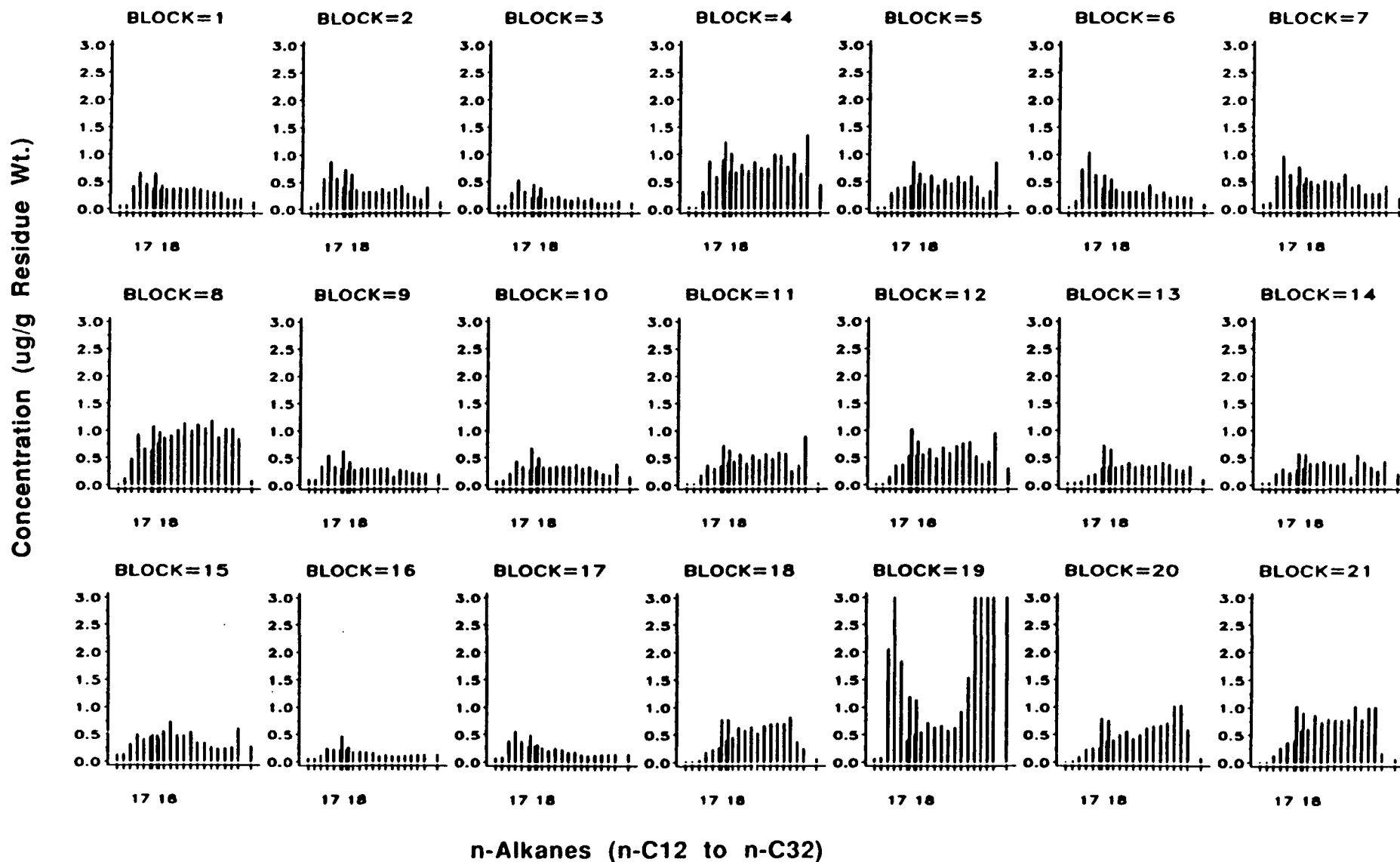


Figure 7.12b. Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble two weeks following application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. Note all concentrations are on the same scale.

## Snug Harbor - Below Cobble - Oleophilic Fertilizer - 4 Weeks After Application



**Figure 7.12c.** Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble four weeks following application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. Note all concentrations are on the same scale.



# Snug Harbor - Below Cobble - Water Soluble Fertilizer - Before Application

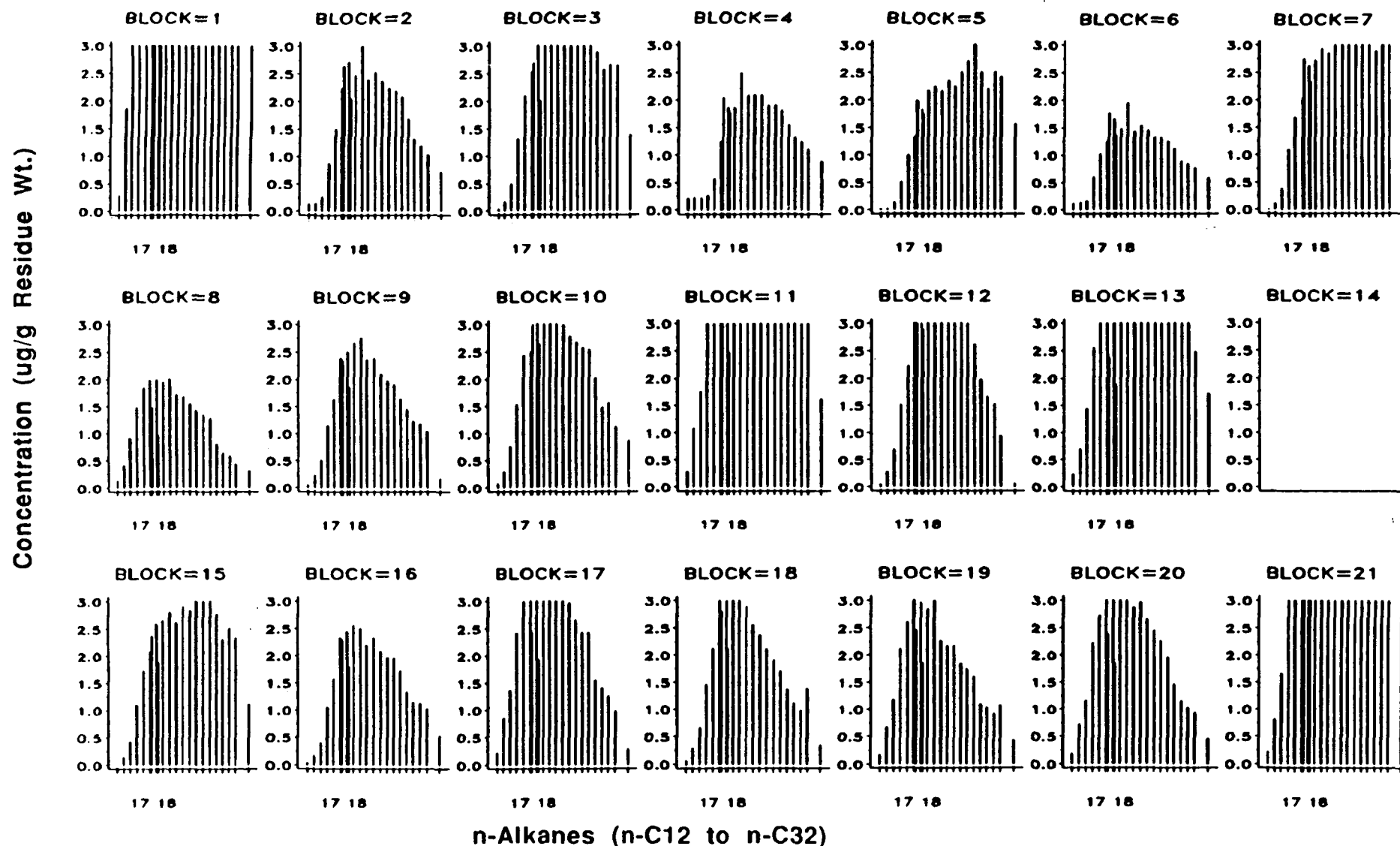


Figure 7.12d. Recreated gas chromatographic profiles normalized to oil residue weight from samples of oil extracted from the mixed sand and gravel under the cobble prior to application of water soluble fertilizer briquettes at Snug Harbor. Blanks indicate data not available. Note all concentrations are on the same scale.

# Snug Harbor - Below Cobble - Water Soluble Fertilizer - 2 Weeks After Application

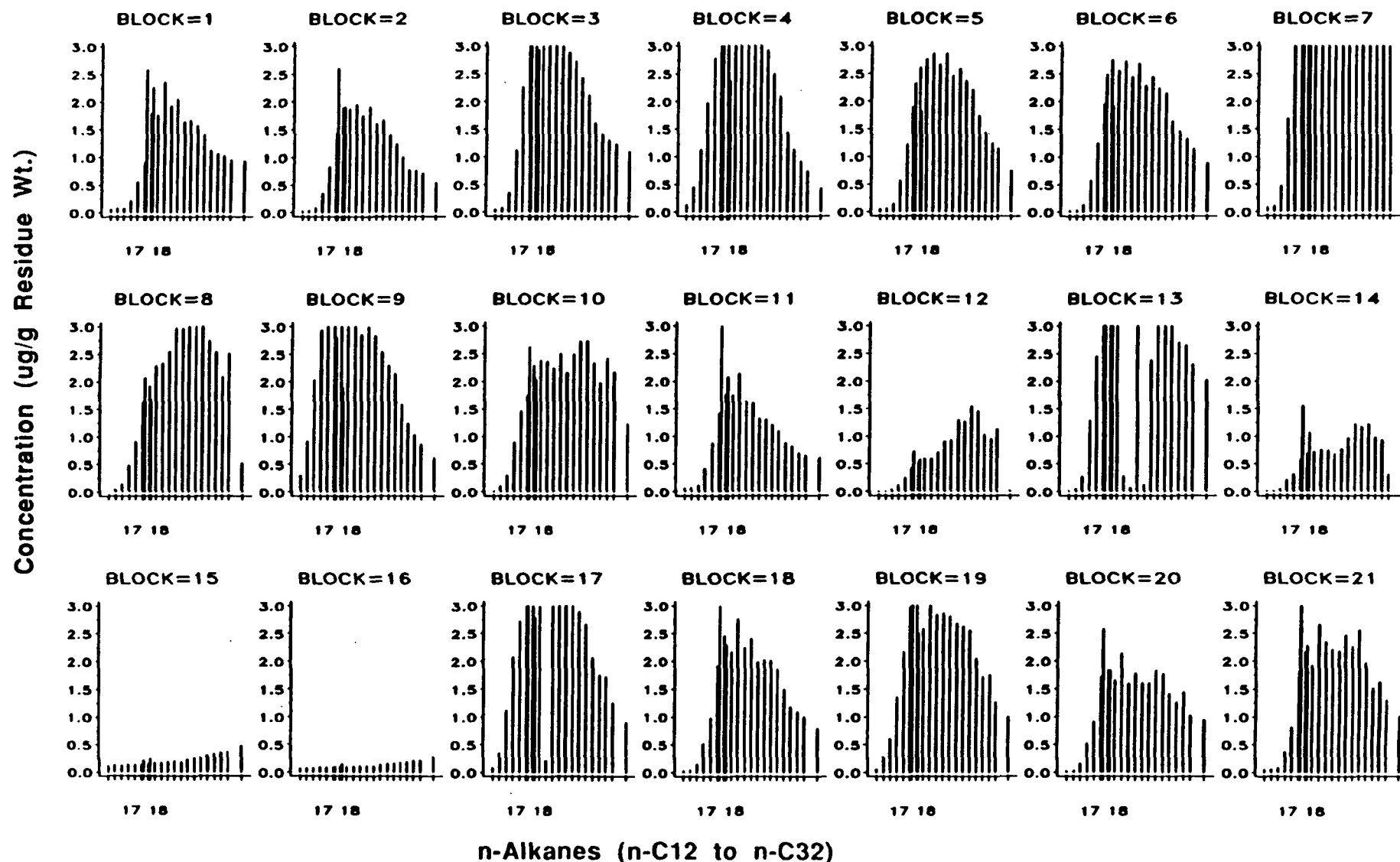
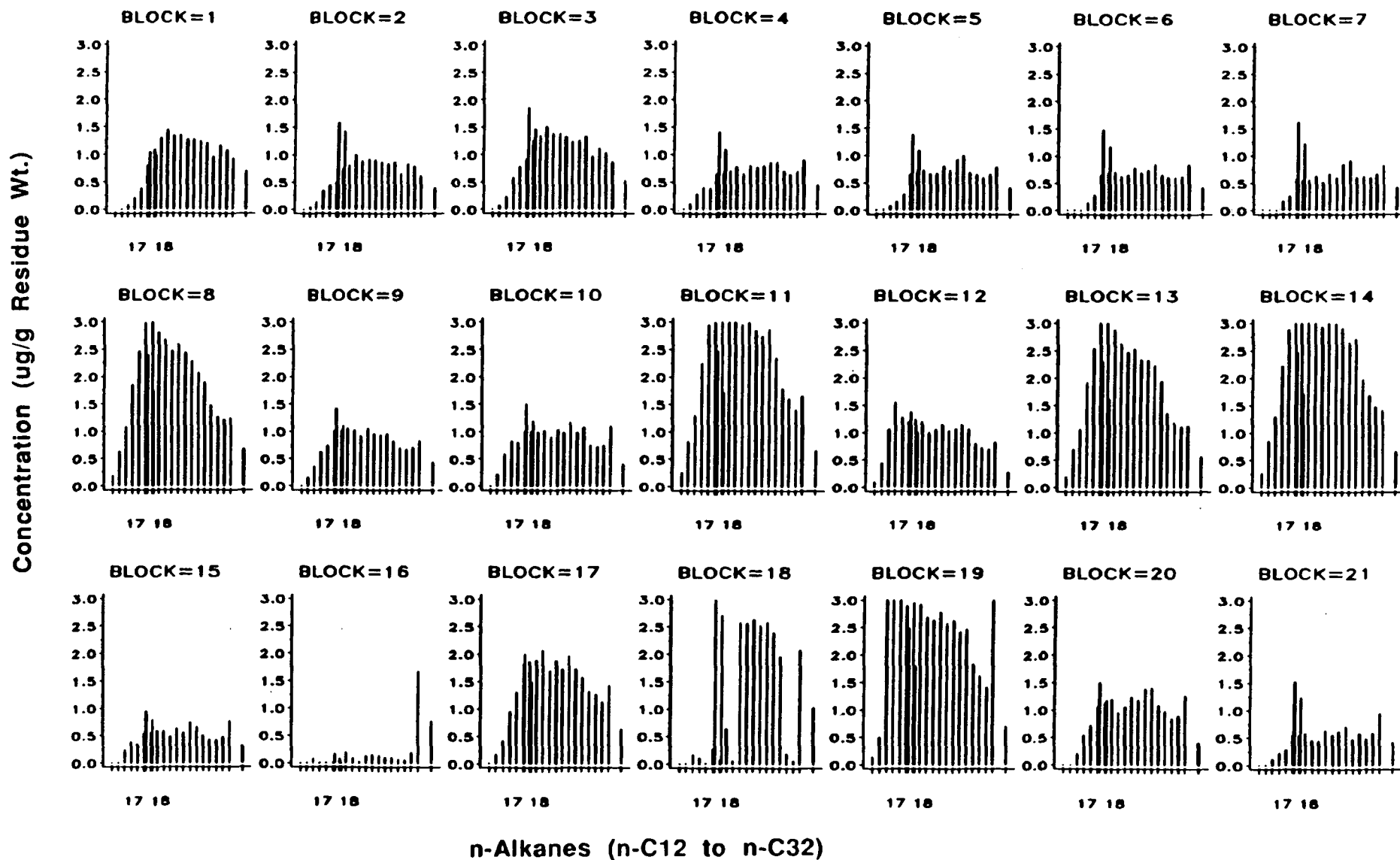


Figure 7.12e. Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble two weeks following application of water soluble fertilizer briquettes at Snug Harbor. Blanks indicate data not available. Note all concentrations are on the same scale.



**Figure 7.12f. Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble four weeks following application of water soluble fertilizer briquettes at Snug Harbor. Blanks indicate data not available. Note all concentrations are on the same scale.**

sampling period. There was a similar change in the hydrocarbon profiles from samples taken in the briquettes-treated beach. Note also that the pristane and phytane were decreasing as fast as the C17 and C18 alkanes respectively indicating the use of these hydrocarbons as conserved internal standards was more highly suspect. Minimal changes in the ratios may not therefore be indicative of extensive overall degradation. As the data is further analyzed, more of these profiles will become available for examination.

#### Average Individual Aliphatic Hydrocarbon Concentration

Another approach for data analysis is to examine the mean individual aliphatic hydrocarbon concentrations for all blocks within a sampling period. Figures 7.13 through 7.15 present bar charts of mean n-alkane and pristane and phytane concentrations normalized to the extractable oil residue weight.

Several interesting trends are apparent when the three sets of figures are compared. For the reference beach (Figure 7.13 a-d), the relative concentration of pristane and phytane appeared to be somewhat unchanged over time, whereas C17 and C18 alkanes decreased approximately 50% in the last two sampling periods. The high variability and the limited amount of available data provide only a very rough estimation.

For the oleophilic fertilizer treated beach plot (Figure 7.14 a-d), both the pristane and phytane and the individual hydrocarbons decreased over time. The decrease for the individual hydrocarbons was similar to that observed in the reference plot. Interestingly, the possible decrease in pristane and loss of phytane may indicate that oil biodegradation in the oleophilic fertilizer treated plots was more extensive, including branched hydrocarbons. Preliminary results from the slow release fertilizer-treated cobble beaches (Figure 7.15 a,b) mirrored the degradation pattern of the reference beach.

Removal of the marker compounds (pristane and phytane) makes sole reliance on C17/pristane and C18/phytane ratio data tenuous. Therefore, additional analyses of available data and limited GC/MS analyses of selected extracts for residual aromatics and other marker components (e.g., norhopane and hopane) must be completed before final evaluation of all treatment processes.

#### Total Aliphatic Hydrocarbon Residues

Relative differences in the concentrations of aliphatic hydrocarbons can be further analyzed by examining the total (summed) aliphatic hydrocarbon residues. Figure 7.16 shows the total hydrocarbon residues (median values) through time for the cobble plots, treated (oleophilic and slow release) and untreated

# Snug Harbor-Mixed Sand and Gravel-Untreated Beach

Figure 13a

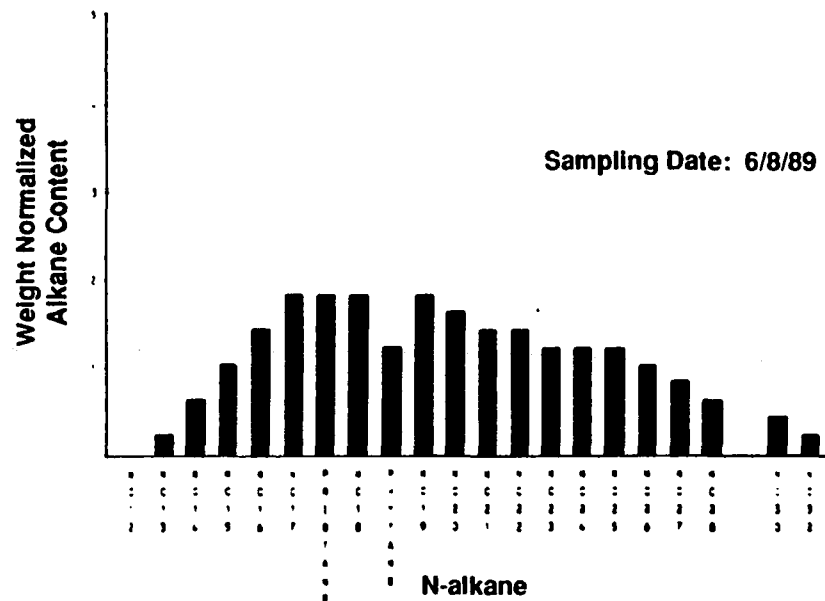


Figure 13c

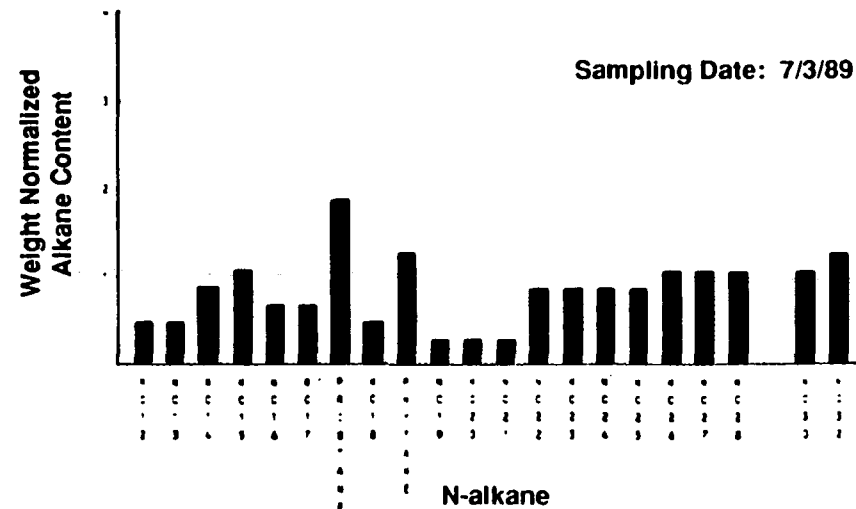


Figure 13b

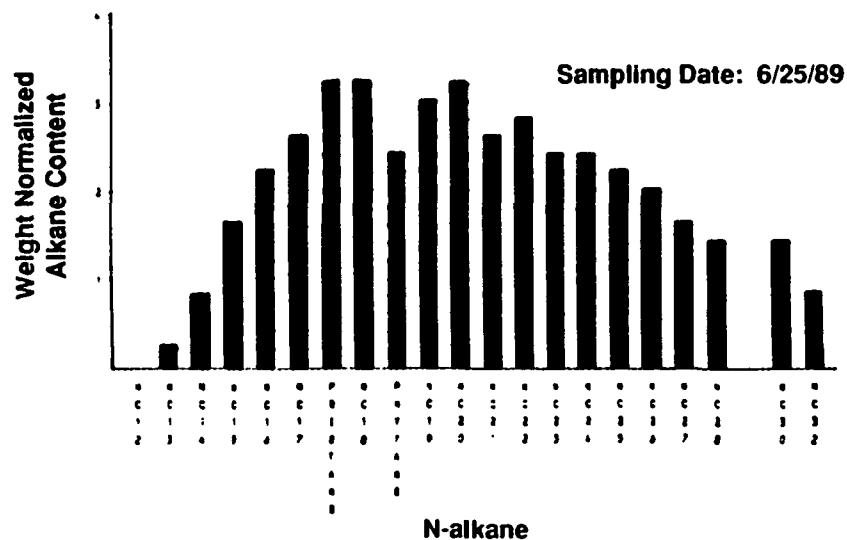


Figure 13d

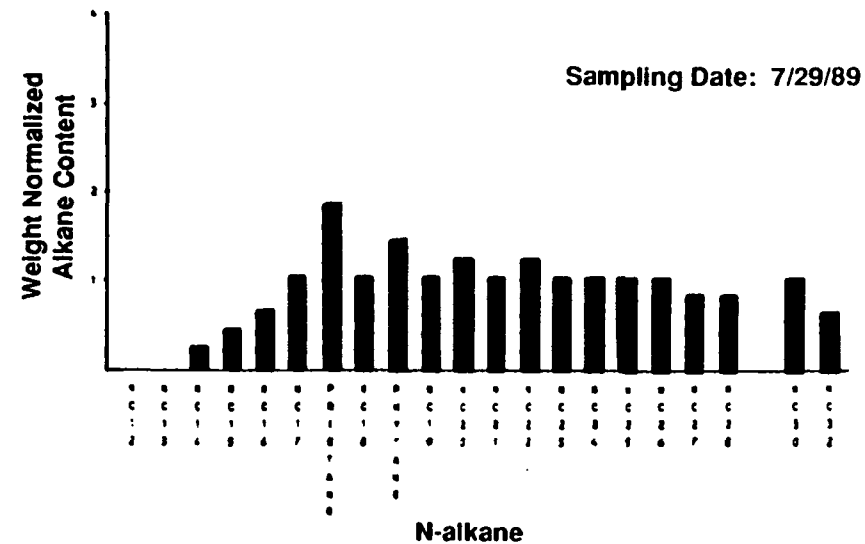


Figure 7.13a-d. Mean weight of alkanes normalized to the total oil residue weight extracted from the beach material; control mixed sand and gravel beaches.

## Snug Harbor-Mixed Sand and Gravel-Oleophilic Fertilizer

Figure 14a

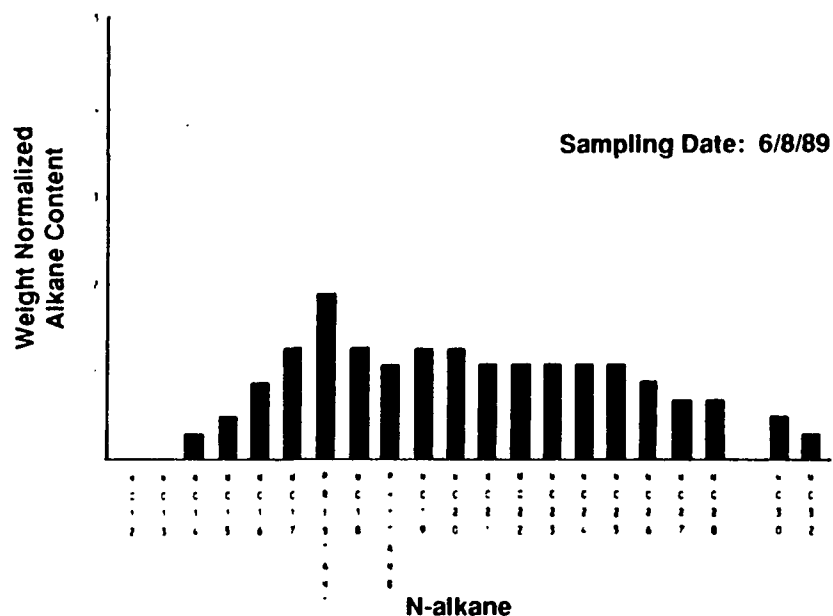


Figure 14c

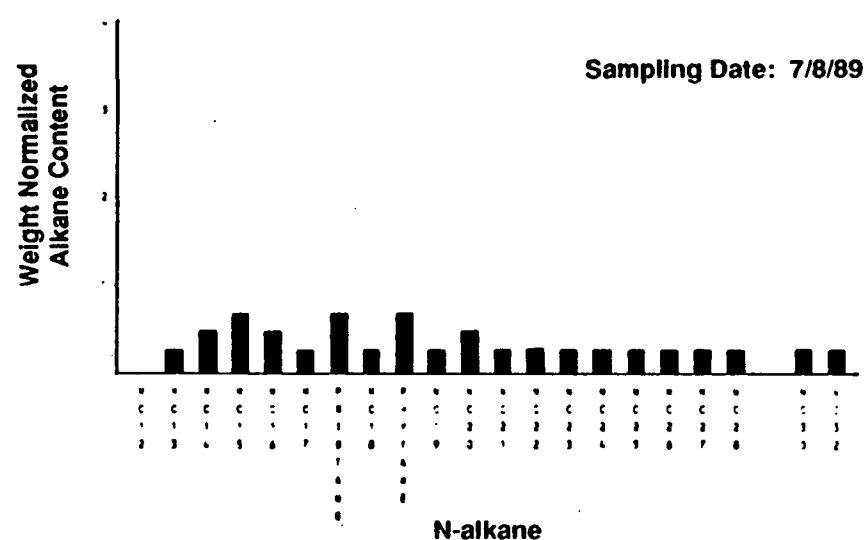


Figure 14b

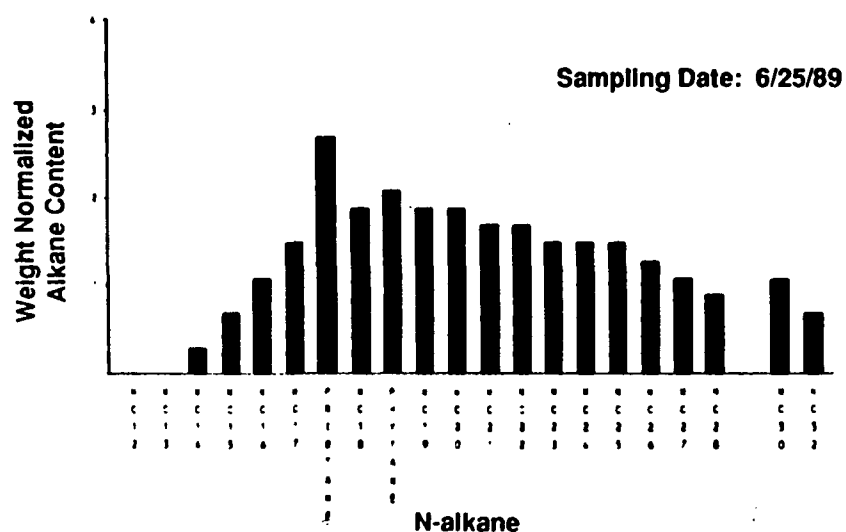
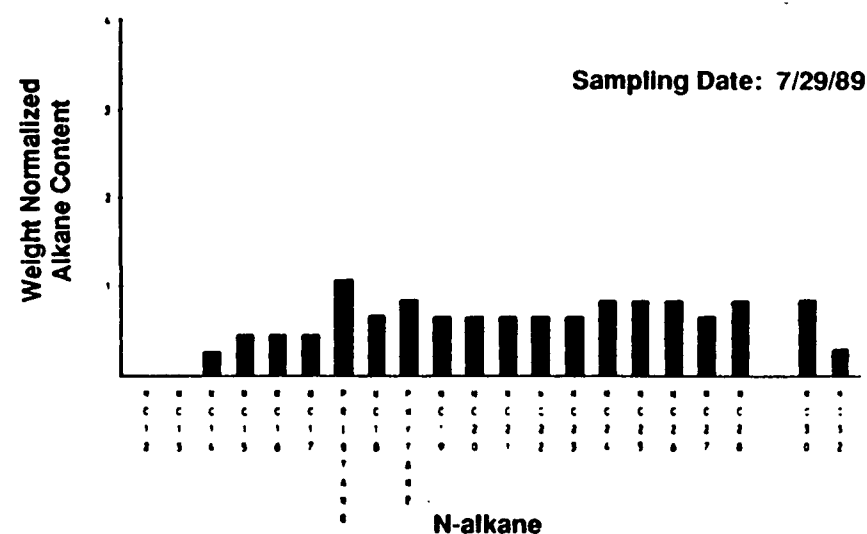


Figure 14d



**Figure 7.14a-d. Mean weight of alkanes normalized to the total oil residue weight extracted from the beach material; oleophilic-fertilizer-treated mixed sand and gravel beaches.**

## Snug Harbor-Cobble-Water Soluble Fertilizer

Figure 15a

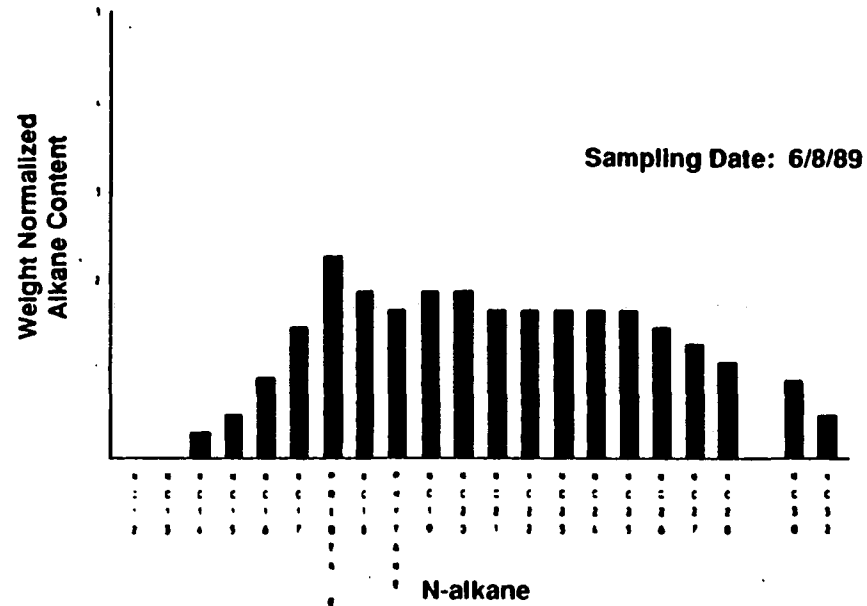


Figure 15b

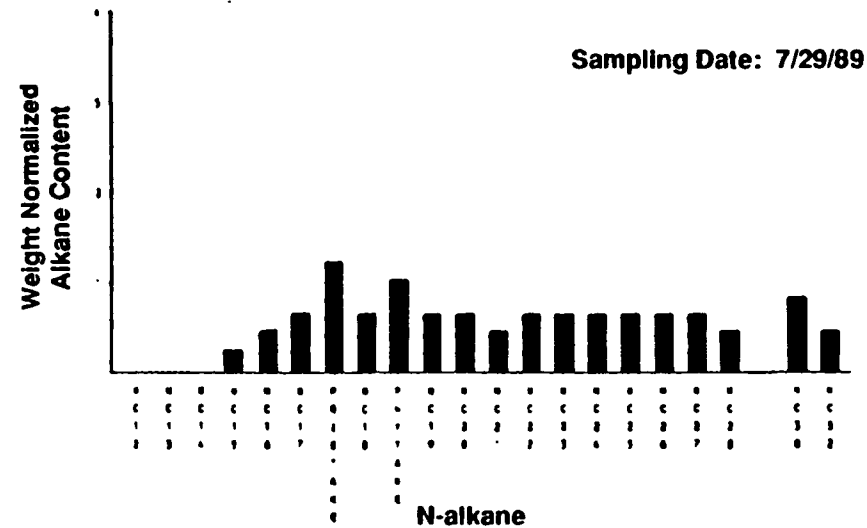


Figure 7.15a-b. Mean weight of alkanes normalized to the total oil residue weight extracted from the beach material; water soluble-fertilizer treated (fertilizer briquettes) cobble beaches.

## SNUG HARBOR Cobble Top

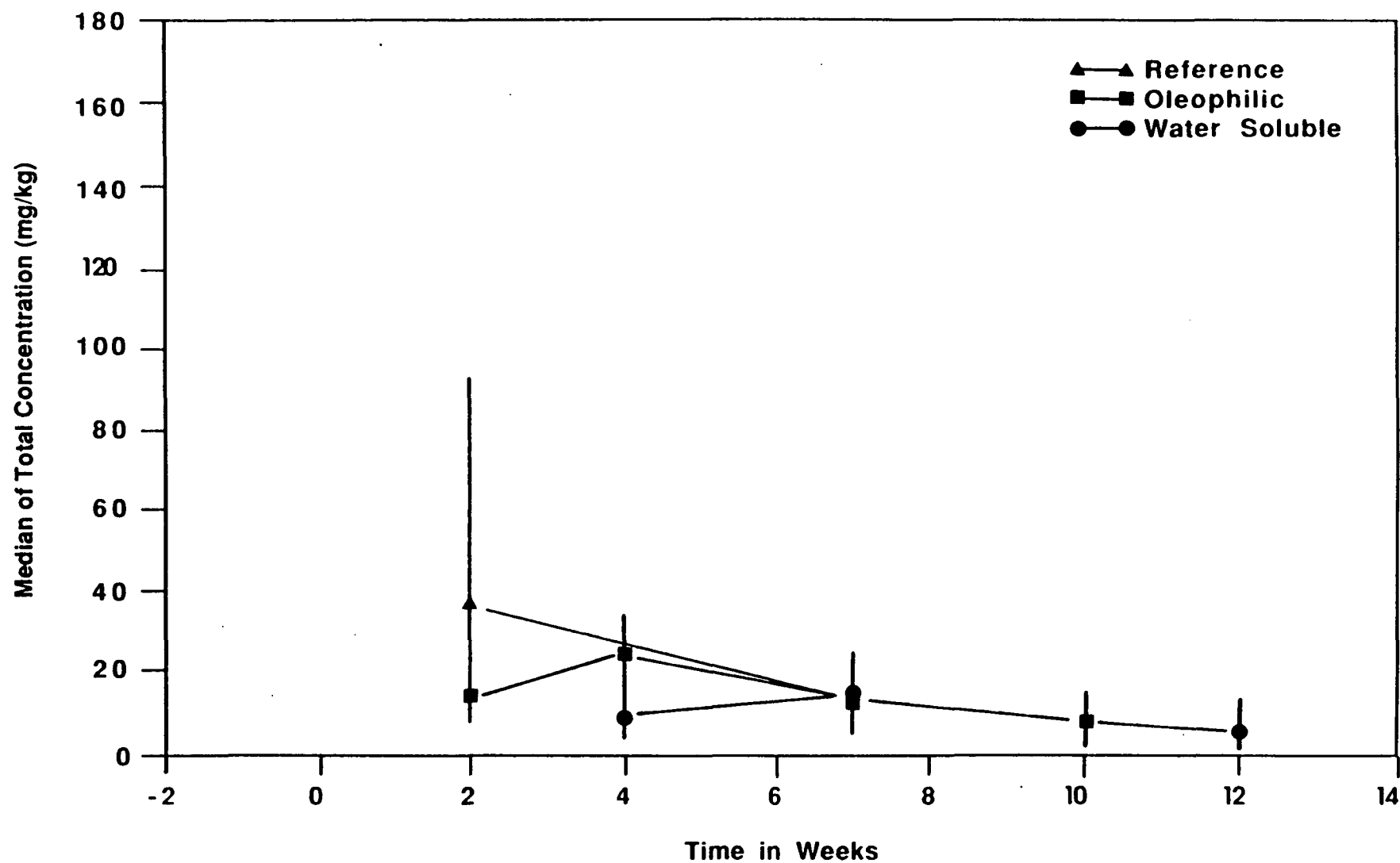


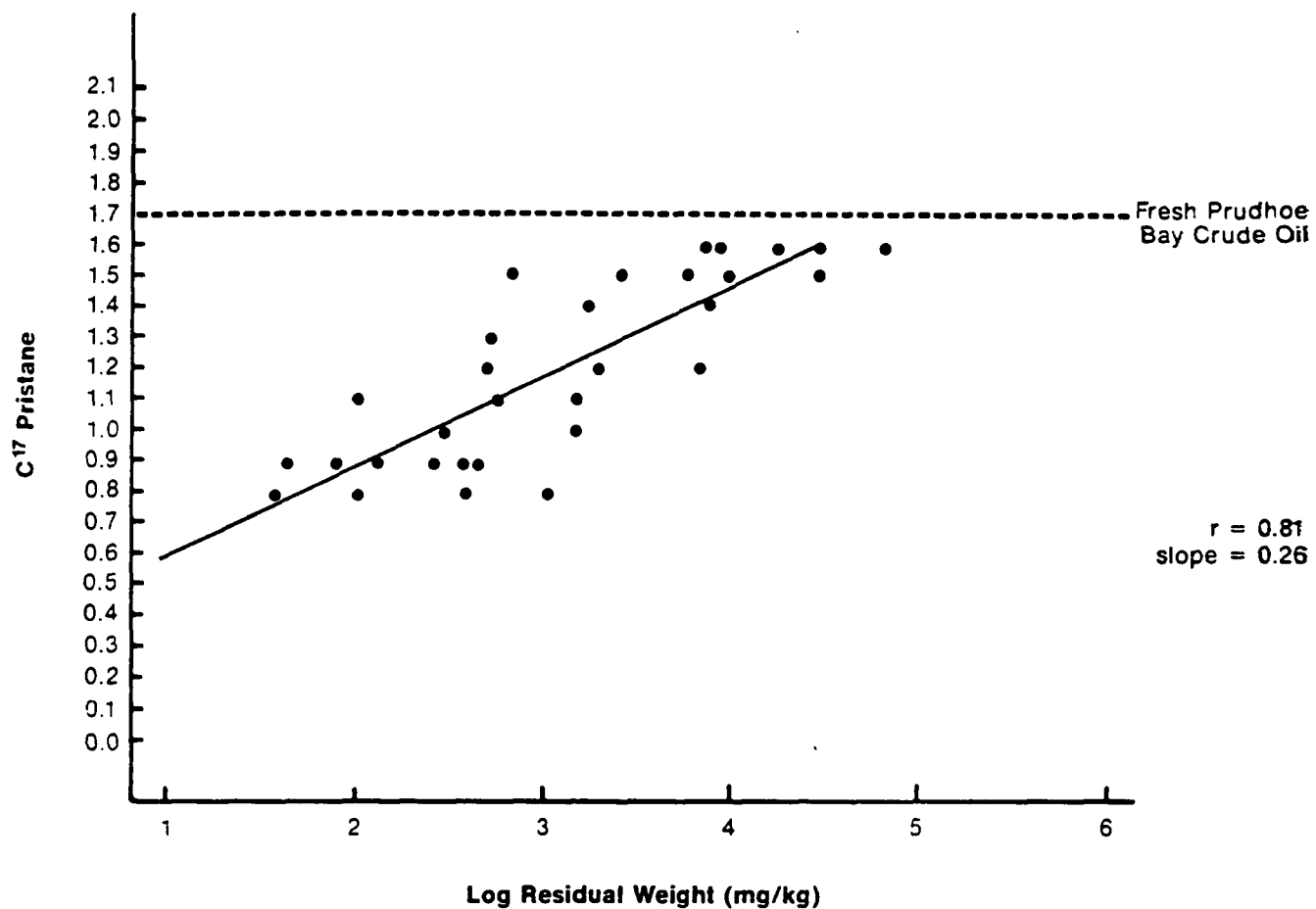
Figure 7.16. Median of Total Concentration of Oil on Treated and Untreated Cobble Plots at Snug Harbor, All Zones



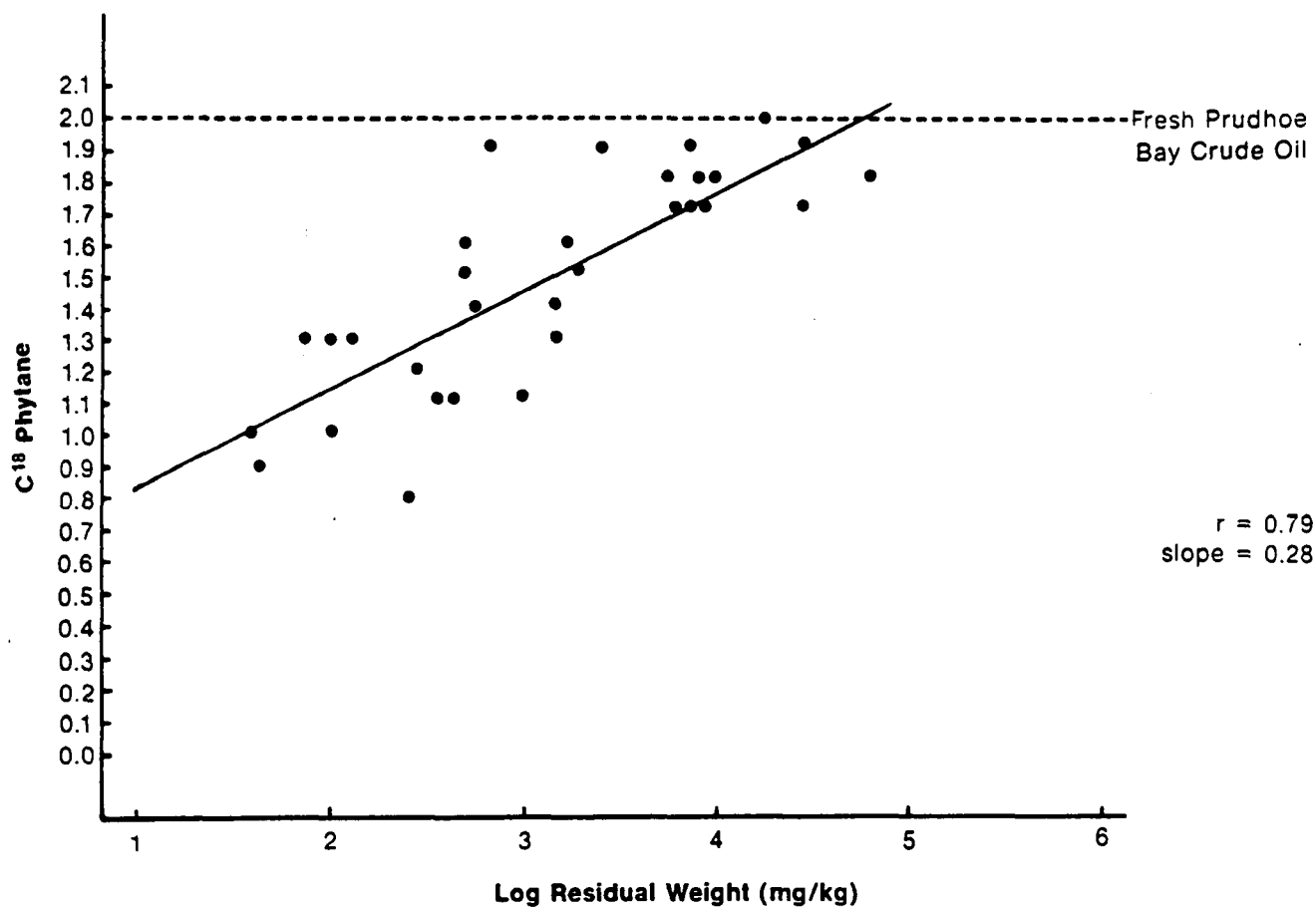
(reference). The data are for oil extracted from rock surfaces. These hydrocarbons as a group showed a decrease in concentration through time but little can be concluded at this time because of a lack of data points in the earlier sampling times. However, the data analysis technique does have promise in helping to evaluate the effect of the fertilizers.

#### Degradation Extent/Oil Residue Weight Relationships

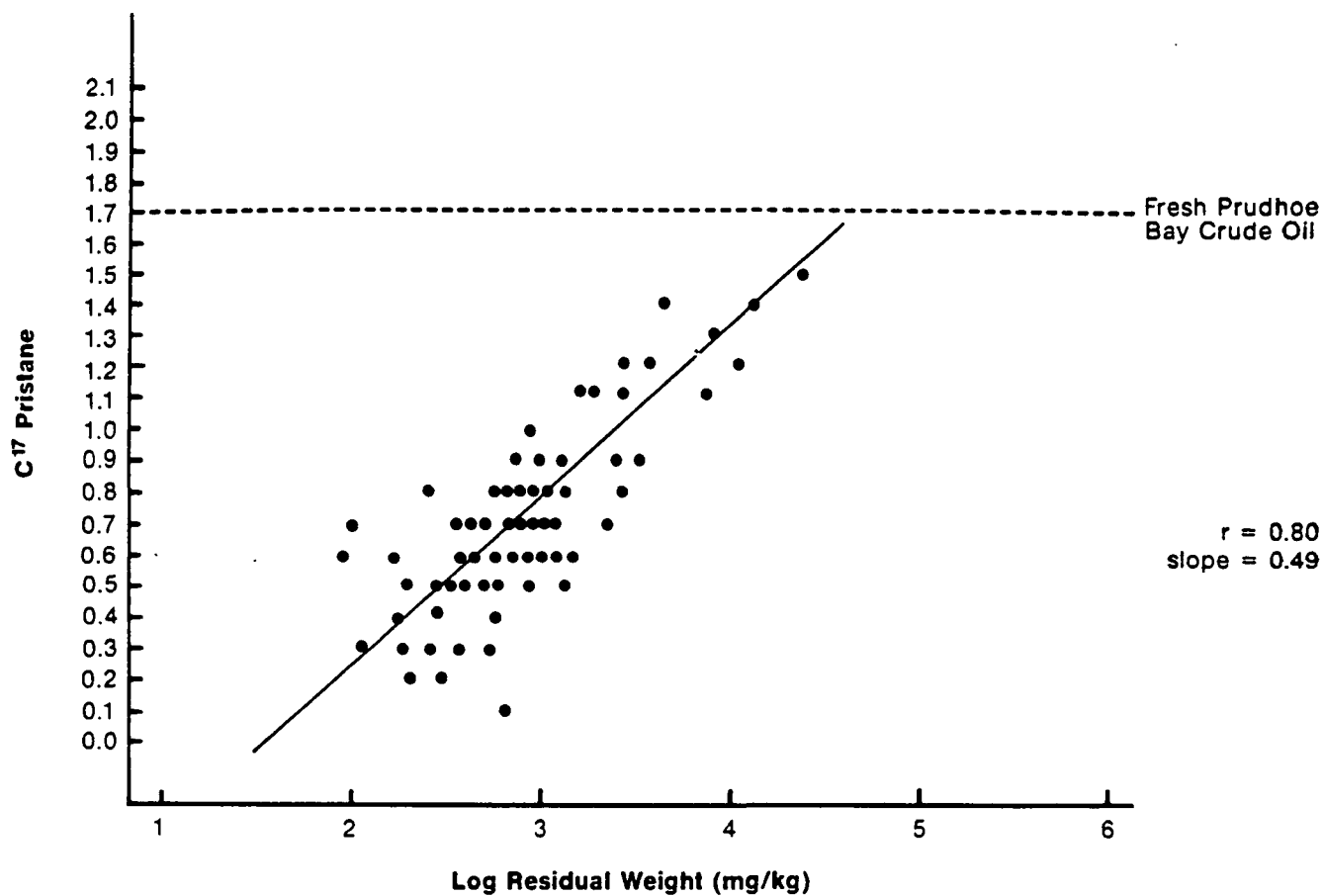
During sampling of the beaches it was obvious that globs of viscous, sticky oil were present in some areas. Where these globs were encountered, there was concern that spike concentrations of undegraded oil would mask evidence of degradation. Examination of the data indicated that changes in the C17/pristane and C18/phytane ratios were most apparent in the samples containing less total oil. This is reasonable if one realizes that at low concentrations, the surface area-to-oil residue weight ratio is large, as it is when oil is dispersed into the beach material as small droplets or films. Effectiveness of biodegradation will increase as the oil surface area increases. With higher concentrations of oil, the same degradation rate is probably occurring, but the surface area-to-oil amount is much less. Because the oil is in bigger globs, the degraded oil on the surface is diluted by the undegraded oil during sampling and homogenization. If this observation is valid, it should be possible to normalize the extent of degradation to the amount of oil present. Figures 7.17 through 7.20 show that when the C17/pristane and C18/phytane ratios are plotted against their respective residue weights, a direct relationship exists. This data is from plots prior to fertilizer treatment. Regression analysis of the data gave r-values around 0.8 ( $\alpha = 0.0001$ ). By comparing slopes of this relationship from two different sampling periods, the effect of biodegradation can be seen. The slopes increased by 2 and 3 fold in the space of 2 weeks. With more degradation the slope will continue to steepen to a limit where the data points begin to cluster closer to the origin. This relationship may have application in further analyzing data from treated and untreated plots. Initial attempts to normalize the ratios with the oil residue weight to reduce variability of the data have, to date, been ineffective. The approach, however, seems promising and further work will evaluate its usefulness.



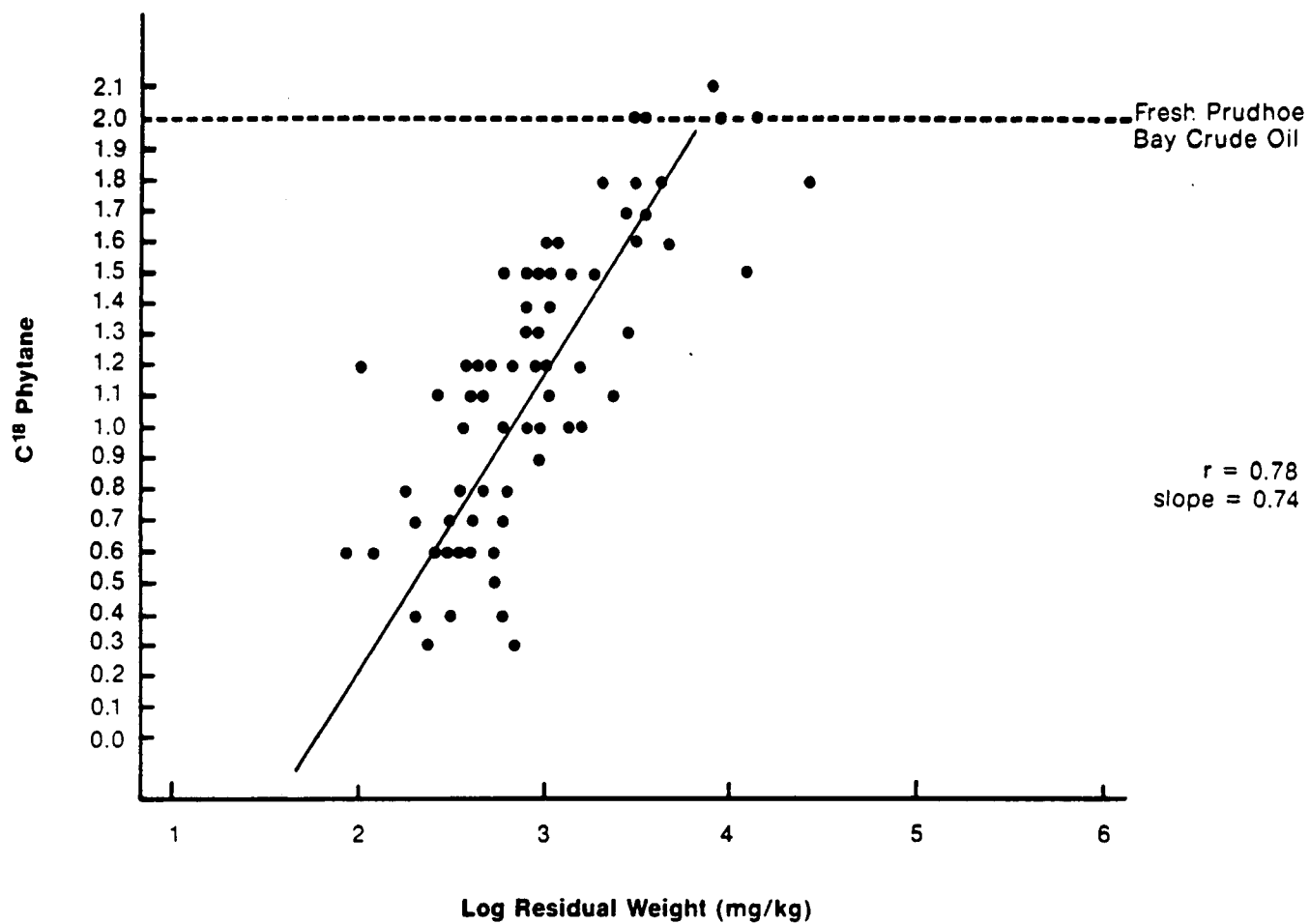
**Figure 7.17. C17/Pristane Ratio versus Log10 Residue Weight  
Two Weeks Before Fertilizer Application (5/28/89)**



**Figure 7.18.  $C^{18}$ /Phytane Ratio versus Log<sub>10</sub> Residue Weight  
Two Weeks Before Fertilizer Application (5/28/89)**



**Figure 7.19. C17/Pristane Ratio versus Log10 Residue Weight at Time Zero of Fertilizer Application (6/8/89)**



**Figure 7.20.  $C^{18}$ /Phytane Ratio versus Log10 Residue Weight at Time Zero of Fertilizer Application (6/8/89)**

## MICROBIOLOGY

Determinations of numbers of oil-degrading bacteria present in beach materials were made at each sampling of Snug Harbor, using all 21 sediment samples taken from each test plot for sediment chemistry. Numbers of degraders were assessed by serially diluting each sample in a minimal salts medium containing ammonium and phosphate, adding a small quantity of oil to each dilution, and incubating the dilutions for 21 days. The highest dilution series showing degradation is then scored, and a calculation is made based on dilution to extinction of the total oil degraders in the undiluted sample. Although similar in design to a single tube MPN procedure, the dilution to extinction procedure should not be mistaken for such.

Results from these studies are shown in Table 7.3. The values reported are the  $\log_{10}$  mean and standard deviation of 18 to 21 dilution series for each mixed sand and gravel plot (no cobble beach material was analyzed). When a control plot was sampled on 2 separate days, the results represented 8 to 10 dilution series per day. Table 7.3 has been keyed to indicate the number of determinations within a plot in which every dilution in the series was positive for oil degradation. The greater the number of positive dilutions, the greater the underestimation of the relative oil-degrading population.

Results suggested that an increase in oil-degrading microorganisms occurred within the oleophilic fertilizer-treated plots between the 0 time and 9 days after application. The results from the water-soluble treatment showed the same trend, but the differences in both cases were not statistically supportable. For unexplained reasons, oil degraders increased more than 100- to 200-fold on day 31 in control and water-soluble fertilizer-treated plots.

It was concluded from the available data that an increase in oil-degrading microorganisms may have occurred as a result of fertilizer application but, it could not be statistically varified. The apparent increase in organism populations in the fertilized plots at day 9 corresponds to the high level of nutrients seen immediately following the application of nutrients. In these tests, the presence of high numbers of oil-degrading bacteria in the control beaches made differences in the numbers of degrading organisms between treatments subtle and difficult to detect.

Table 7.3. Relative Concentrations ( $\text{Log}_{10}$  of the Cell Numbers/g of Beach Material) of Oil-Degrading Microorganisms in Snug Harbor Mixed Sand and Gravel Test Plots<sup>a</sup>

<u>Sampling Date<sup>b</sup></u> <u>Before Application</u>	<u>Days</u>	<u>Fertilizer</u>		
		<u>Control</u>	<u>Water Soluble</u>	<u>Oleophilic</u>
6/8/89	0	6.58		5.95
			±1.00	+/-1.29
6/11/89	1	6.16	5.80	
		±0.89	±0.91	
<u>After Application</u>				
6/17/89	9	6.24*	6.62*	6.91**
		±1.53	±1.19	+/-1.21
6/24/89	16	5.96	5.86	5.96
		±0.83	±1.15	+/-1.10
7/8/89	30	6.61		5.86
		±1.34		+/-0.67
7/9/89	31	8.47*	9.39	
		±1.33	±1.12	

<sup>a</sup> No. of dilution series positive in all dilution tubes (0-25%);  
\*(25-50); \*\*(50-75).

<sup>b</sup> Samples on 6/8/89 and 6/11/89 are preapplication of the fertilizer.

## ECOLOGICAL MONITORING

The monitoring component of the project was designed to identify ecological effects of nutrients added to the shore zone on planktonic microorganisms. Sampling stations were established in nearshore locations next to both treated and untreated (reference) beaches in Snug Harbor (see Sections 5 and 6) and at locations outside of Snug Harbor. Samples were collected on 9 occasions; once prior to the addition of fertilizer, 2 days after addition, and 1, 2, 3, 4, 5, 6, and 8 weeks after addition. After week 5, the stations 10 m from shore were no longer sampled in order to accommodate the workload from an additional study site. Data analyzed after week 5 indicated no significant loss in assessment capability resulted from this decision.

### Nutrients

Ammonia, nitrite, nitrate, and phosphate analyses have been completed on water samples taken through week 7. Nutrient concentrations showed no increases in waters adjacent to treated shorelines as illustrated by ammonia and phosphorus data in Tables 7.4 through 7.7. These data provide evidence that fertilizers applied to the Snug Harbor shoreline either remained within the beach matrix as applied, were taken up by microbial biomass, or have been diluted to background concentrations within 1 m of the shoreline. In any event, the potential for stimulating plankton biomass from nutrient enrichment along the shoreline was not evident from these data.

### Chlorophyll Analyses

Chlorophyll analyses of phytoplankton samples were used to monitor for changes in the abundance of algae. Increased chlorophyll concentrations would indicate nutrients had washed from the beach and had been incorporated into algal biomass, if nutrient enrichment stimulated algal growth in Snug Harbor. None of the chlorophyll data indicated that algal populations within Snug Harbor were stimulated by fertilizer applications beyond the extent of variability observed in week to week sampling (Figure 7.21). Problems with obtaining sufficient extract volumes to obtain optical densities appropriate for the spectrometer, and procedural problems in quantifying extinction values contributed to a great deal of variability in the first three data sets. Pending additional analyses and evaluation of QA data, results stated herein should be considered preliminary. However, results to date demonstrate that nearshore concentrations were similar to those offshore, and there were no consistent differences between samples collected near treated beaches and reference areas. Although statistically significant differences were observed between treated and untreated samples on some dates, these differences were not greater than those observed at control sites week to week (i.e., the normal ecological variability).



Table 7.4. Ammonia Nitrogen ( $\mu\text{M N/l}$ ) from Nearshore Water Over Gravel Beaches at Snug Harbor. Mean of Four Replicates (standard deviation). (Method detection limit =  $0.13 \mu\text{M N/l}$ .)

Sample Date	Control (Rodney Beach)		Oleophilic (Otter Beach)		Water Soluble (Otter Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	1.5 (0.05)	1.5 (0.08)	1.6 (0.05)	1.7 (0.06)	1.5 (0.22)	1.8 (0.17)
6/14/89	0.68 (0.10)	0.65 (0.05)	0.52 (0.09)	0.58 (0.10)	0.61 (0.08)	0.58 (0.10)
6/21/89	0.92 (0.03)	1.02 (0.06)	0.74 (0.03)	0.83 (0.05)	0.73 (0.03)	0.74 (0.06)
6/28/89	0.21 (0.06)	0.15 (0.02)	0.13 (0.00)	0.20 (0.10)	0.13 (0.00)	0.20 (0.14)
7/5/89	0.51 (0.11)	0.52 (0.03)	0.56 (0.09)	0.57 (0.10)	0.74 (0.16)	0.53 (0.09)
7/12/89	0.80 (0.32)	0.73 (0.19)	0.57 (0.11)	0.50 (0.05)	0.63 (0.08)	0.96 (0.57)
7/23/89	0.13 (0.00)	-- <sup>a</sup> --	0.13 (0.00)	-- --	0.13 (0.00)	
8/9/89	0.13 (0.00)	-- --	0.13 (0.00)	-- --	0.13 (0.00)	

<sup>a</sup> -- = Sample not collected.

Table 7.5. Ammonia Nitrogen ( $\mu\text{M N/l}$ ) from Nearshore Water Over Cobble Beaches at Snug Harbor. Mean of Four Replicates (standard deviation). (Method detection limit =  $0.13 \mu\text{M N/l}$ .)

Sample Date	Control (Fred Beach)		Oleophilic (Seal Beach)		Water Soluble (Seal Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	2.1 (0.12)	1.8 (0.00)	1.5 (0.05)	1.5 (0.10)	1.4 (0.27)	1.4 (0.08)
6/14/89	0.73 (0.03)	0.70 (0.08)	0.45 (0.06)	0.55 (0.12)	0.64 (0.06)	0.48 (0.06)
6/21/89	0.99 (0.08)	0.91 (0.06)	0.96 (0.04)	0.82 (0.03)	0.87 (0.09)	0.88 (0.10)
6/28/89	0.24 (0.06)	0.35 (0.26)	0.22 (0.13)	0.13 (0.00)	0.22 (0.11)	0.18 (0.07)
7/5/89	0.61 (0.12)	0.65 (0.19)	0.52 (0.03)	0.50 (0.05)	0.44 (0.21)	0.47 (0.05)
7/12/89	0.62 (0.18)	0.70 (0.20)	0.79 (0.16)	0.75 (0.14)	0.86 (0.17)	0.78 (0.08)
7/23/89	0.13 (0.00)	-- <sup>a</sup>	0.13 (0.00)	--	0.13 (0.00)	--
8/9/89	0.13 (0.00)	--	0.13 (0.00)	--	0.13 (0.00)	--

<sup>a</sup> -- Sample not collected.

Table 7.6. Phosphate ( $\mu\text{M P/l}$ ) From Nearshore Water Over Gravel Beaches at Snug Harbor. Mean of Four Replicates (standard deviation). (Method detection limit =  $0.20 \mu\text{M P/l}$  for sample date 6/10/89,  $0.02 \mu\text{M P/l}$  thereafter.)

Sample Date	Control (Rodney Beach)		Oleophilic (Otter Beach)		Water Soluble (Otter Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	0.20 (0.00)	0.20 (0.00)	0.34 (0.27)	0.20 (0.00)	0.20 (0.00)	0.26 (0.12)
6/14/89	0.10 (0.00)	0.13 (0.03)	0.18 (0.04)	0.15 (0.00)	0.15 (0.04)	0.12 (0.05)
6/21/89	0.44 (0.00)	0.40 (0.03)	0.29 (0.04)	0.28 (0.11)	0.34 (0.03)	0.35 (0.04)
6/28/89	0.25 (0.00)	0.25 (0.00)	0.15 (0.03)	0.17 (0.02)	0.20 (0.00)	0.16 (0.00)
7/5/89	0.27 (0.04)	0.27 (0.04)	0.36 (0.04)	0.23 (0.03)	0.37 (0.05)	0.28 (0.04)
7/12/89	0.23 (0.03)	0.29 (0.03)	0.22 (0.04)	0.32 (0.03)	0.25 (0.03)	0.22 (0.00)
7/23/89	0.08 (0.00)	-- <sup>a</sup> --	0.08 (0.00)	-- --	0.10 (0.03)	-- --

<sup>a</sup> -- = Sample not collected.

Table 7.7. Phosphate ( $\mu\text{M}/\text{P/l}$ ) from Nearshore Water Over Cobble Beaches at Snug Harbor. Mean of Four Replicates (standard deviation). Method detection limit =  $0.20 \mu\text{M P/l}$  for sample date 6/10/89,  $0.02 \mu\text{M P/l}$  thereafter.)

Sample Date	Control (Fred Beach)		Oleophilic (Seal Beach)		Water Soluble (Seal Beach)	
	1 m	10 m	1 m	10 m	1 m	10 m
6/10/89	0.22 (0.03)	0.20 (0.00)	0.20 (0.00)	0.20 (0.00)	0.22 (0.03)	0.20 (0.00)
6/14/89	0.16 (0.02)	0.15 (0.00)	0.15 (0.00)	0.12 (0.03)	0.14 (0.04)	0.14 (0.06)
6/21/89	0.36 (0.02)	0.31 (0.03)	0.35 (0.04)	0.25 (0.03)	0.26 (0.00)	0.27 (0.04)
6/28/89	0.18 (0.02)	0.28 (0.03)	0.16 (0.04)	0.15 (0.03)	0.20 (0.04)	0.24 (0.05)
7/5/89	0.29 (0.05)	0.30 (0.04)	0.32 (0.03)	0.25 (0.03)	0.34 (0.03)	0.30 (0.07)
7/12/89	0.38 (0.00)	0.34 (0.03)	0.25 (0.03)	0.23 (0.05)	0.25 (0.06)	0.22 (0.00)
7/23/89	0.10 (0.03)	-- <sup>a</sup> --	0.09 (0.01)	-- --	0.09 (0.01)	-- --

<sup>a</sup> -- = Samples not collected.

## SNUG HARBOR

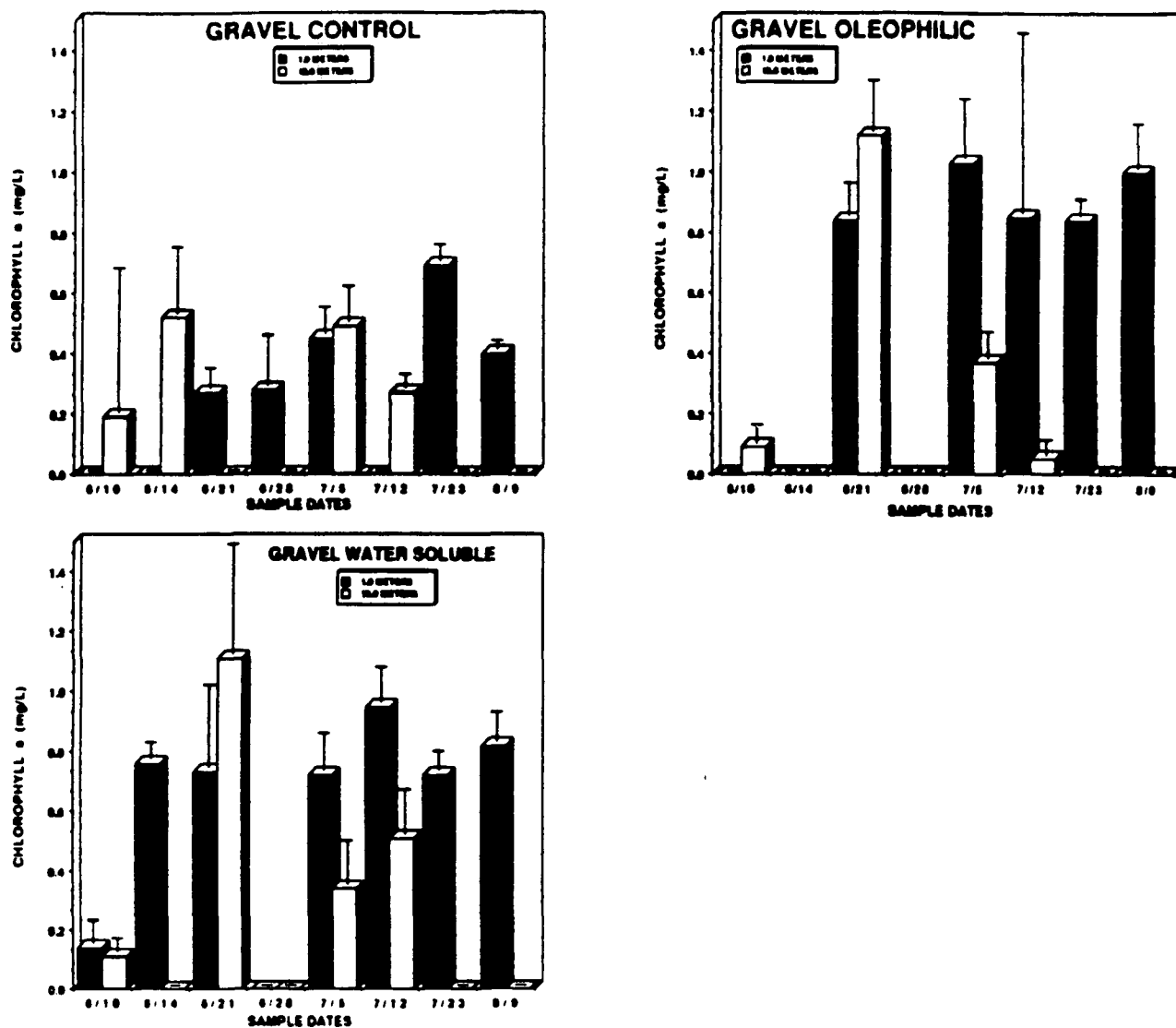


Figure 7.21 Phytoplankton Chlorophyll Concentrations (Mean + SD) in 4 Replicates of Snug Harbor Water Samples Collected After June 7 and 8, 1989, Fertilizer Additions to Gravel Shorelines

Differences between nearshore (1 m) and offshore (10 m) samples and fertilized and reference shoreline samples were within the expected day-to-day variation common for phytoplankton data.

#### Phytoplankton Primary Productivity

Phytoplankton productivity was used as a functional measure of the photosynthetic activity of algal cells. It allowed an evaluation of whether the algal population sampled was viable and active, nutrient limited or enriched. Comparisons of photosynthetic rates obtained on different sampling dates are not valid as the light conditions during incubation could have been different enough to significantly affect productivity estimates. Only treatment-versus-treatment and treatment-versus-reference comparisons were valid for each sampling date. Overall, differences between treated and reference samples appear small, inconsistent, and within the range of expected ecological variability (Figure 7.22). Samples from 6/21 and 6/28 showed a consistent increase in productivity for treated shoreline samples compared to reference samples, however, all samples were within a factor of two. If elevated primary productivity was caused by nutrient addition, the absence of a change in nearshore chlorophyll concentration suggested that biomass was not increasing faster than dilution and transport by tidal exchange was depleting it.

#### Bacterial Abundance

Mean bacterial abundances in water column samples from Snug Harbor varied from 0.51 to  $2.49 \times 10^9$  cells per liter, reported in Figure 7.23. Due to sampling error, data were lost for all samples collected prior to fertilizer addition. One week after nutrient additions, bacterial numbers near fertilized shorelines were higher than the second day after fertilizer application. Bacterial numbers near reference shorelines did not change. Bacterial numbers near treated beaches returned to background levels 1 week after treatment. Because bacterial numbers near treated beaches were no greater than numbers near reference beaches, the increase from day 1 to week 1 were not considered ecologically significant. Changes of this magnitude reflect natural system variability. Other than a decrease from slightly elevated bacterial numbers in early June, no trends in bacterial abundance were associated with shoreline treatments or time over the monitoring period.

#### Bacterial Productivity

Bacterial productivity was estimated by the incorporation of  $^3\text{H}$ -thymidine in water samples transported, prepared, and incubated at the ecology laboratory at Valdez. Because the abundance of cells alone may not represent the viability of

## SNUG HARBOR

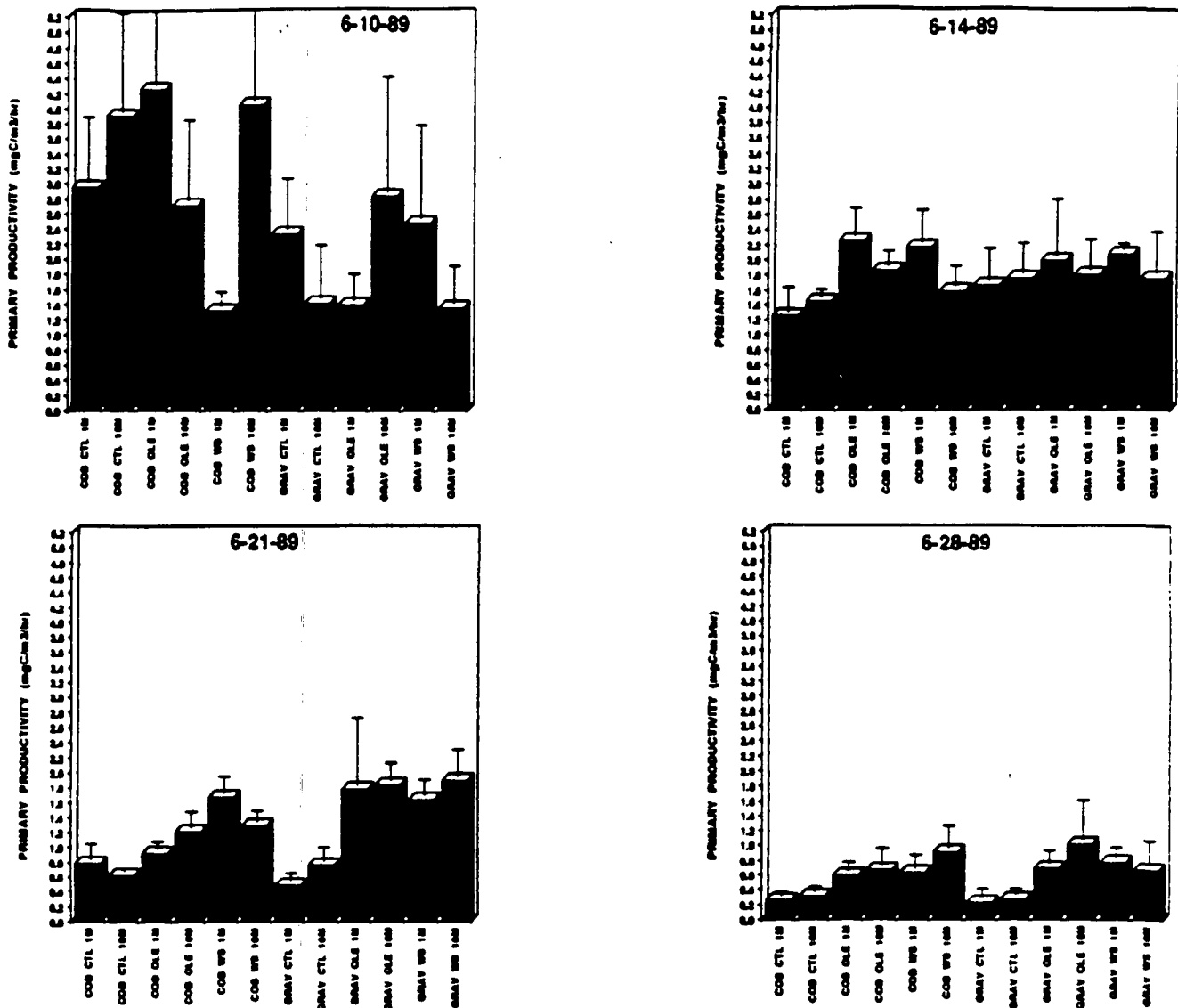


Figure 7.22 Primary Productivity Estimates (as <sup>14</sup>C Uptake) (Mean + SD) for 4 Phytoplankton Replicates from Snug Harbor Collected After June 7 and 8, 1989, Fertilizer Applications to Shorelines

# SNUG HARBOR

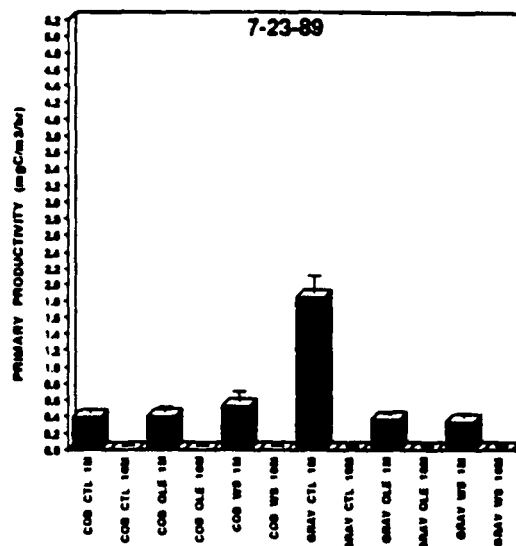
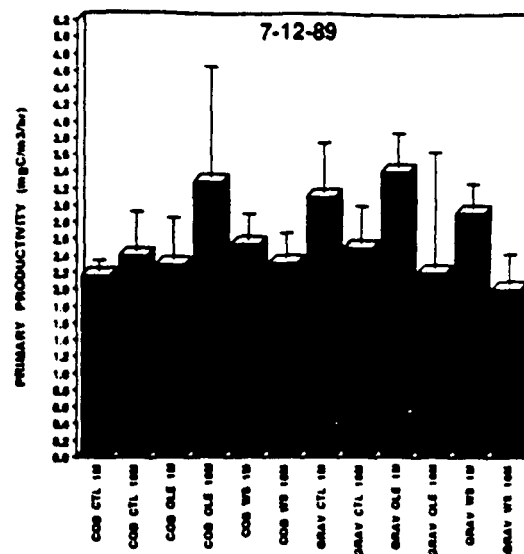
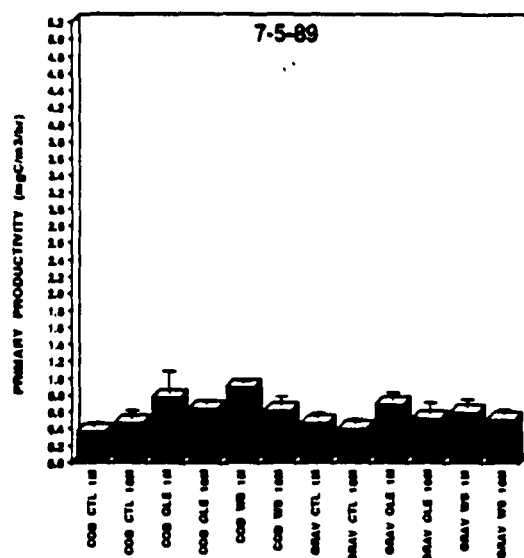


Figure 7.22 (Continued)



## SNUG HARBOR

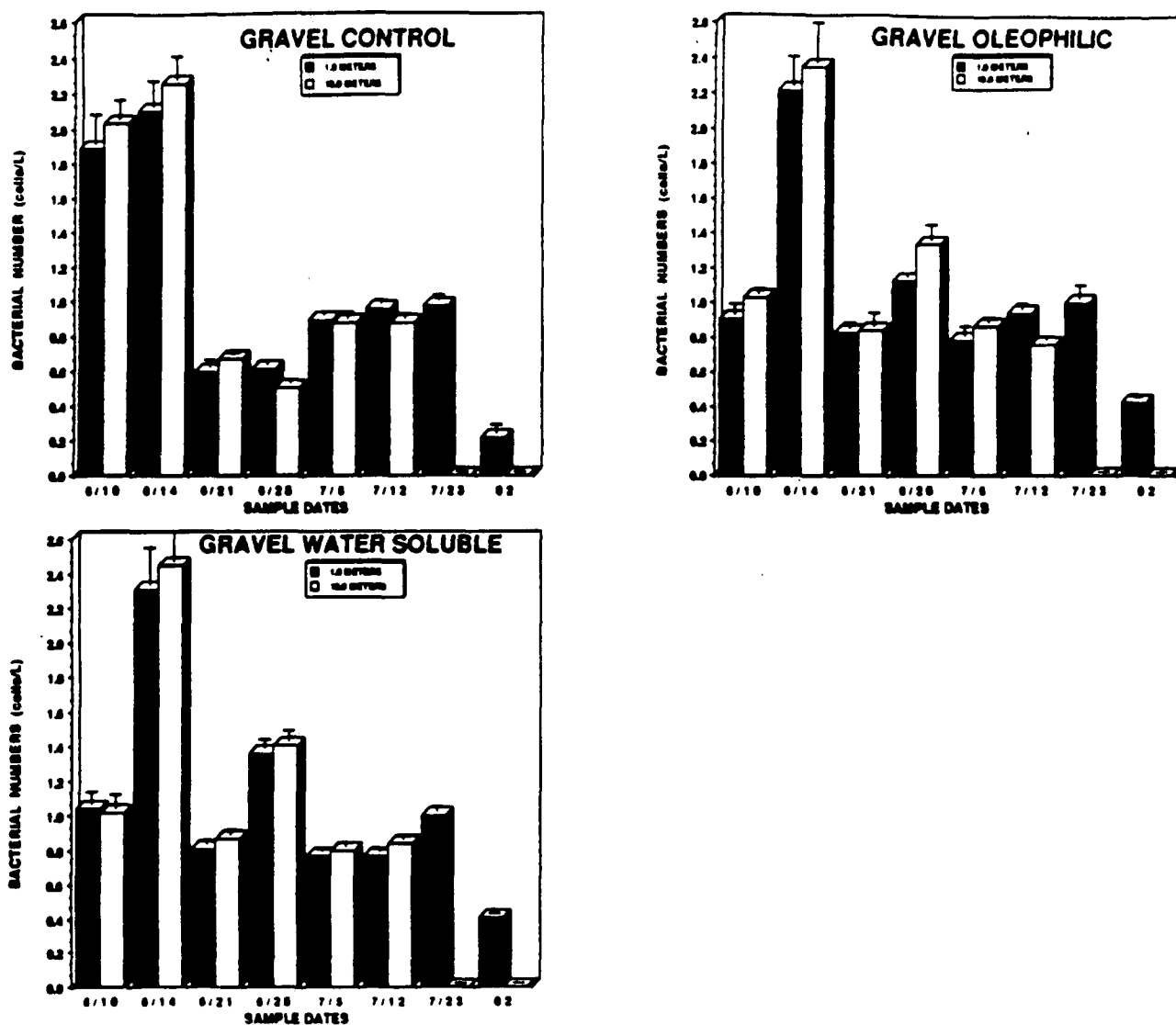


Figure 7.23 Primary Productivity Estimates (as  $^{14}\text{C}$  Uptake) (Mean + SD) for 4 Phytoplankton Replicates from Snug Harbor Collected After June 7 and 8, 1989, Fertilizer Applications to Shorelines

planktonic microbes, bacterial productivity was estimated to allow an evaluation of functional activity of this community and the effect of nutrient enrichments.

Bacterial productivity data revealed no consistent changes or trends associated with fertilizer application to the shoreline (Figures 24 and 25). Although data showed greater productivity during the first two sampling periods compared to subsequent sampling, this difference was seen in samples from reference sites as well as treated sites. This data probably represented a seasonal trend rather than a treatment effect. None of these differences appeared to be ecologically significant.

#### Microflagellate Abundance

Samples for microflagellate abundance were used as an estimate of the population of grazers that consume bacteria in microbiological food chains. Increases in their abundance at the fertilizer-treated sites would indicate that bacterial biomass was being directly incorporated into the next step in the food chain at a rate reflective of nutrient enrichment. None of the initial samples indicated any measurement effect from the fertilizer applications; therefore, analyses of additional microflagellate samples was stopped to minimize costs and streamline sample processing.

#### Dissolved Organic Carbon, Particulate Carbon, Particulate Nitrogen

Water samples for these analyses have been processed through the EPA Valdez laboratory and shipped to US EPA ERL, Gulf Breeze for analysis. Only preliminary analyses have been completed as of this date.

#### Stable Isotope Ratios of Carbon and Nitrogen

Biological samples for stable isotope analyses were sent to Texas A&M University for analysis.

#### Caged Mussels

Analyses of mussel tissues is still proceeding, only 20% of the samples have been analyzed to date. These samples represent a cross-section of the stations and times sampled at the Snug Harbor study site. An inspection of available results to date indicate that slightly more than half of the samples analyzed had no detectable PAH residues ( $<0.05$  ug/g) and, when present, total PAH concentrations were always less than  $1 \mu\text{g/g}$  (PPM). The predominant PAH present in samples with residues was benzo(a)pyrene. This compound is not prominent in Prudhoe Bay crude oil and is more likely an indicator of the presence of diesel combustion products from the myriad of vessels working in

## SNUG HARBOR

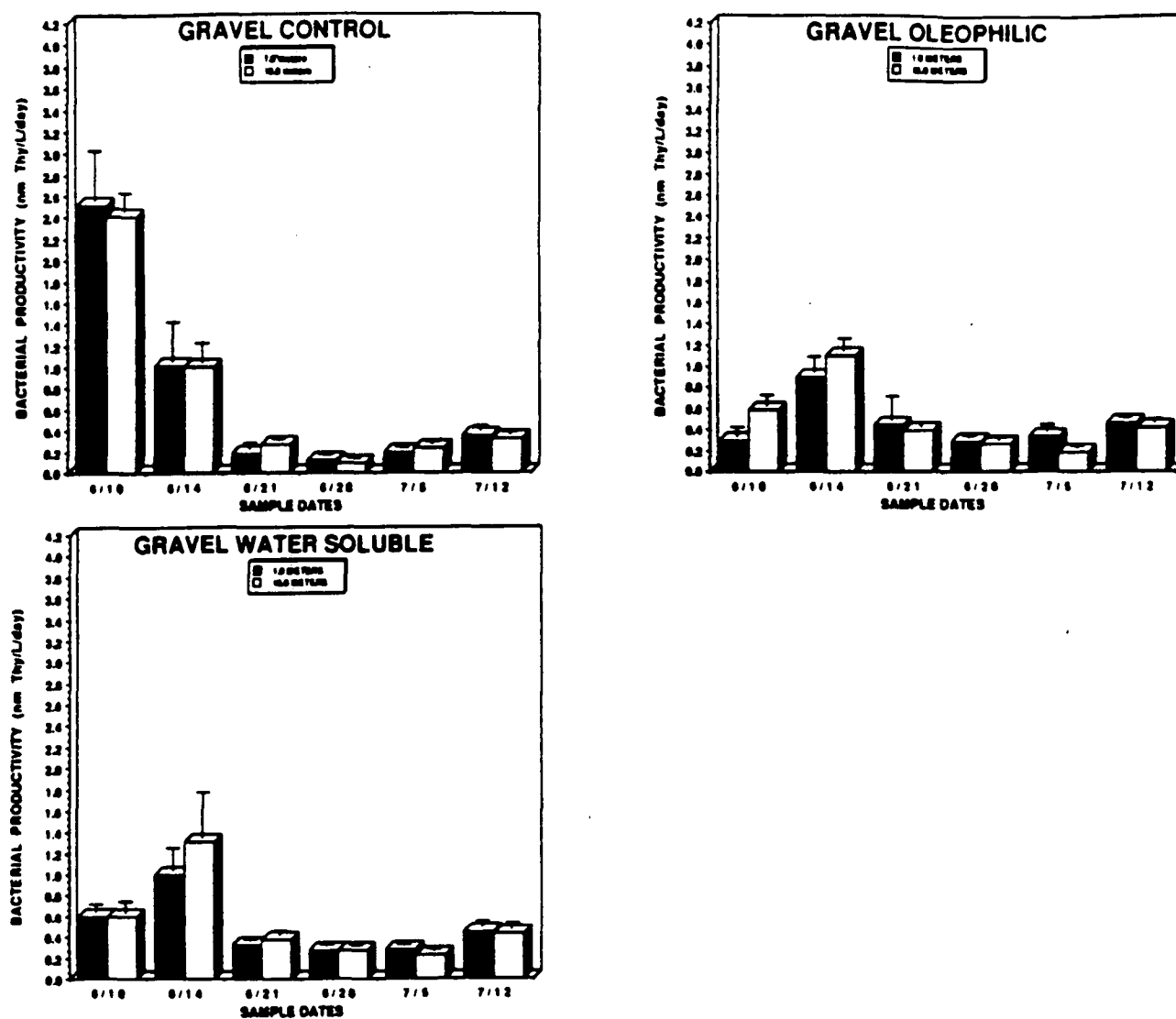


Figure 7.24 Abundance of Bacterial Cells ( $\times 10^9$ ) (Mean + SD) from 4 Replicates of Snug Harbor Water Collected on Various Dates After June 7 and 8, 1989, Fertilizer Applications to Shorelines

## SNUG HARBOR

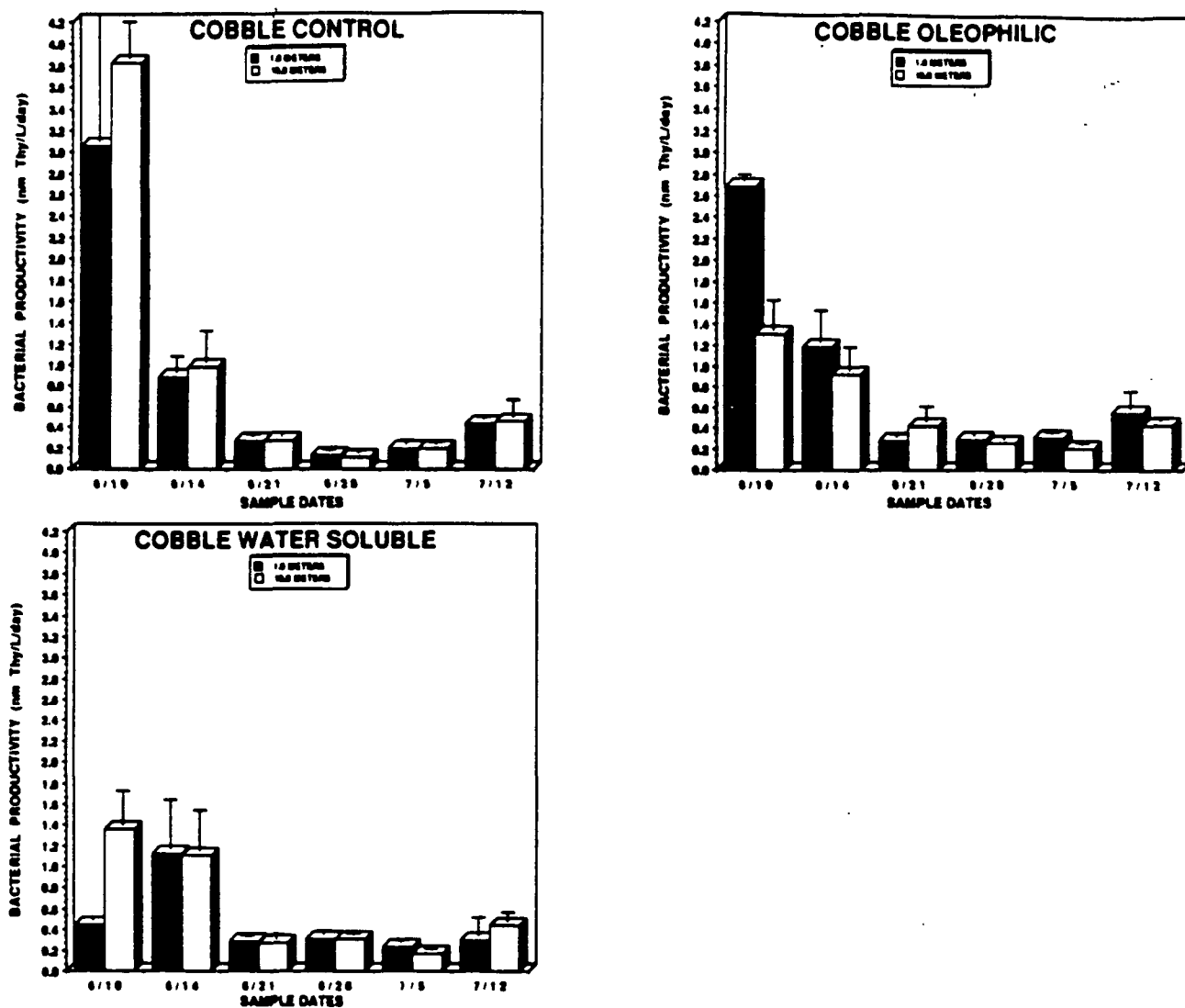


Figure 7.25 Bacterial Productivity (Mean + SD) as Measured by Tritiated Thymidine Uptake from 4 Replicates of Snug Harbor Water Collected on Various Dates After June 7 and 8, 1989, Fertilizer Applications to Shorelines

Prince William Sound on oil spill clean-up efforts. None of the mussel tissue data to date indicates any enhanced residues from bioremediation activities. A definitive assessment must await completion of analytical work on a greater number of samples.

## **DISCUSSION AND CONCLUSIONS**

Data from the bioremediation field demonstration in Snug Harbor have been collected and are being processed and analyzed. Although all evaluations are not yet complete, the following general discussion and conclusions can be made.

- Visual inspection of beaches treated with oleophilic fertilizer showed that in approximately 2 to 3 weeks oil was removed from the treated shorelines. The effect was most apparent on cobble beaches, where initially much of the surface oil was removed. No visible decrease in the oil occurred on the beaches treated with the slow-release fertilizer briquettes or the reference beaches. This removal continued on oleophilic-treated plots, eventually leading to the disappearance of oil from the surfaces of all beach material.
- No oil slicks or oily materials were observed in the seawater following application of the fertilizers. Based on the analyses to date, no oil or petroleum hydrocarbons have been detected in mussels contained in cages just offshore from the fertilizer treated beaches. Thus removal of oil from the beaches did not appear to be chemically mediated.
- Analysis of oil extracted from reference beach plots showed that loss of oil residue weight and changes in chemical composition of the oil were substantial and progressed steadily through time. This suggested that natural biodegradation of the oil occurred at a surprising rate. Indeed, nutrient analysis of tidal and fresh water that washed test beaches showed the presence of significant quantities of ammonia, nitrate and phosphate. Thus, if biodegradation rates (and possibly the extent of biodegradation) are limited by the availability of nitrogen and phosphorous in the Prince William Sound, natural processes are doing an effective job of bioremediation by the continual low level supply of these essential nutrients.
- Analyses of oil extracted from beach samples taken from plots treated with oleophilic and slow-release fertilizer briquettes showed that decreases in oil residue weight and changes in oil composition (as measured by a variety of approaches) may have been stimulated by the fertilizer addition. This is reasonable given the detection of the above ambient concentrations of ammonia and nitrate following fertilizer application. However, due to the very

heterogenous distribution of oil on the beaches, imprecise methods for sampling unconfined gravel and cobble, and high amounts of natural oil biodegradation, it has been difficult to statistically verify that nutrient addition caused enhanced biodegradation. Since only a portion of the total data set has been analyzed, many options are under investigation to explain and interpret field test results.

- Preliminary indications suggest that the standard measures of biodegradation, changes in the ratios of specific branched and straight-chained alkanes may be inadequate. This is because pristane and phytane, which were thought to degrade slowly, were in fact readily degraded in some cases, thus making them very unpredictable as conserved internal standards. Alternate measures of biodegradation will have to be developed, including the use of chemical analyses for different fractions of the crude oil.
- Samples of the oil from fertilizer-treated beaches, particularly from cobble surfaces, taken at about the time when the oil was visually disappearing, showed substantial changes in hydrocarbon composition, indicating extensive biodegradation. This suggests that biodegradation was affecting removal of the oil, both through direct decomposition and possibly through the production of biochemical products (bioemulsifiers) known to be produced by bacteria as they consume oil and hydrocarbons as sources of food.
- Extensive monitoring studies indicated that the addition of fertilizer to oiled shorelines caused no ecologically significant increases in planktonic algae or bacteria or any measurable nutrient enrichment in adjacent embayments.

## **SECTION 8**

### **FIELD TEST RESULTS - PASSAGE COVE**

#### **VISUAL OBSERVATIONS**

Original oil contamination in Passage Cove was heavy. Following complete physical washing, oil was well distributed over most of the surface of all cobble and all gravel under the cobble. The oil in appearance was black, dry, and dull 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 present, the oil was thick and viscous. 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.

Within approximately two weeks following application of oleophilic fertilizer and slow release granular fertilizer, it became apparent that the treated beach was considerably cleaner relative to the reference plots. In contrast to the observations at Snug Harbor, not only did the rock surfaces look cleaner but the oil under the rocks and on the gravel below was also disappearing. In another two weeks, oil could be 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 loss of oil from the rock surfaces was apparent in the reference plot.

The beach treated with fertilizer solution from the sprinkler system behaved in a very similar manner to the oleophilic/granule-treated plot; that is, it became clean. The only difference was that it lagged behind the oleophilic/granular-treated beach by about a 10-14 days. By the end of August, both beaches--the oleophilic and fertilizer solution treated--looked equally clean. In contrast, the reference plot appeared very much as it did in the beginning of the field study. Oil in the subsurface still remained in all plots. However, visually in the fertilizer-treated plots oil was apparent only below 20-30 cm of depth.

#### **NUTRIENT CONCENTRATIONS**

At the time this interim report was completed, nutrient data from Passage Cove was still being processed and therefore, could not be included.

#### **CHANGES IN OIL RESIDUE WEIGHT AND COMPOSITION**

Data analysis for oil residue weight and chemistry in samples taken from beach plots in Passage Cove has not yet been

completed. Over 600 samples have been analyzed and the resulting information is being incorporated into the data base. Approaches for analyzing trends in the data are the same as those used for Snug Harbor (see Section 7).

Data for oil residue weights and ratios of C17/Pristane and C18/phytane, have been analyzed only for oil extracted from the surface of the cobbles (referred to as top), not for oil from gravel under the cobble. Some general statements, however, can be made at this time.

#### Residue Weight

Changes in the mean residue concentration with 75th and 25th quartiles through time for all plots are shown in Figure 8.1. Each data point is the mean of the number of samples available at this time (maximum samples equals 21 for any sampling time). The reference plot showed a slow decrease in oil residue weights over the first three weeks followed by a somewhat more rapid decrease. The plot treated with fertilizer solution applied by a sprinkler system started at approximately the same oil concentration as the reference plot but over time dropped rapidly and then leveled off. Oil in the oleophilic granule-treated plot showed a slow steady decrease through time, perhaps with a noticeable drop between week 1 and 3.

Although none of these trends have yet been satisfactorily verified, results do appear to show a notable effect of the fertilizer solution. It corresponds with the visual observation and with similar observations in microcosms (see Section 9) where controlled nutrient addition was also maintained. The lack of a large decrease in oil residue weight in the oleophilic fertilized-treated beach contrasts with the visual observations.

#### Ratio of Branched and Straight Chain Hydrocarbons

Information on changes in the C17/pristane and C18/phytane ratios is available only for the oil extracted from the cobble surface. The data is presented in Figures 8.2 and 8.3. The results generally mirror trends observed with oil residue weights. Ratios typically decreased through time. The plot treated with the fertilizer solution applied by sprinkler system showed the most dramatic change in ratios with a 2-3 fold decrease in the first week. Ratios from the reference plot decreased steadily over the two week period, the change in C18/phytane ratio being more pronounced than the C17/pristane ratio (note initial values for the C17/pristane ratio were very low). The increase was probably not due to reoiling, as oil residue weights did not increase during the same time period. It is possible that degradation of the pristane and/or phytane occurred, rendering this measure of degradation inadequate.



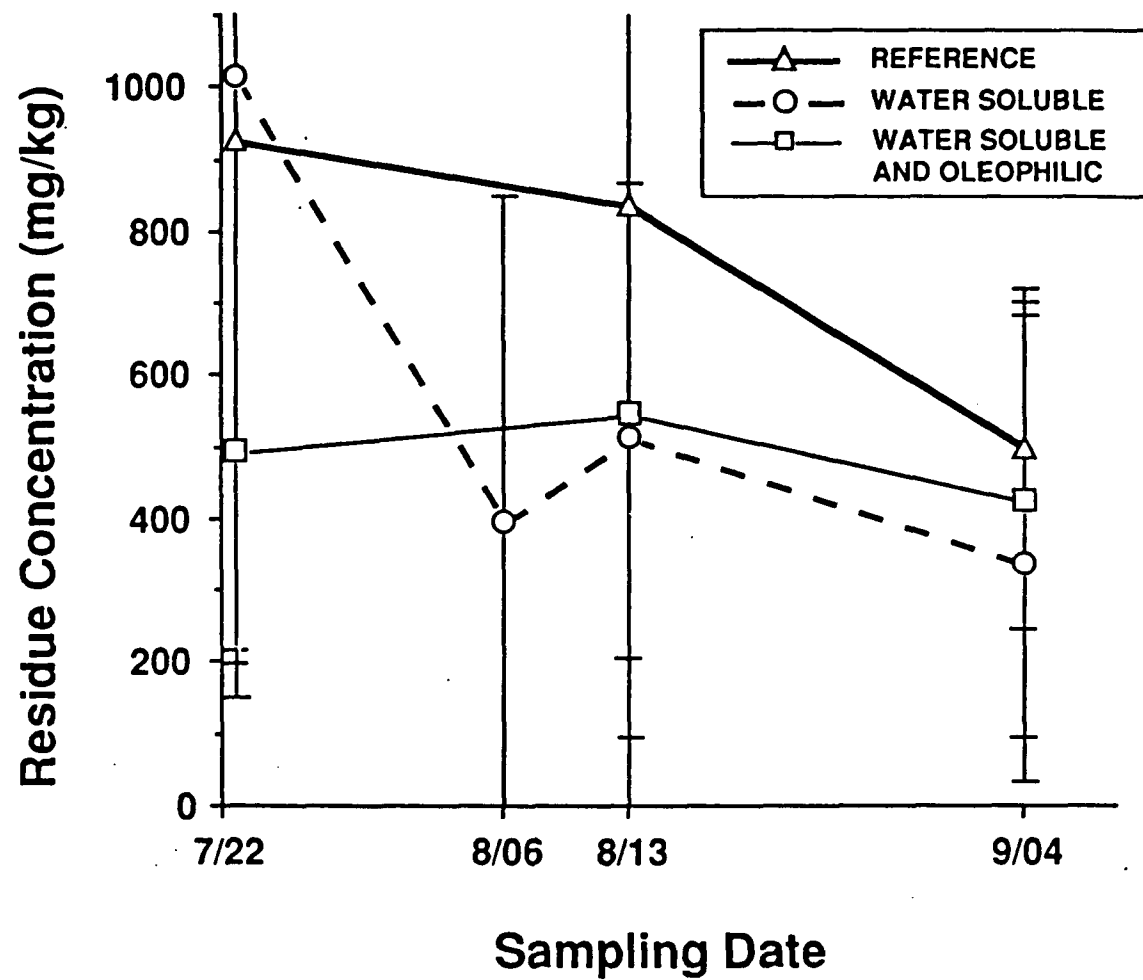


Figure 8.1 Mean Residue Concentration at Passage Cove Cobble Plots, Top, All Zones.

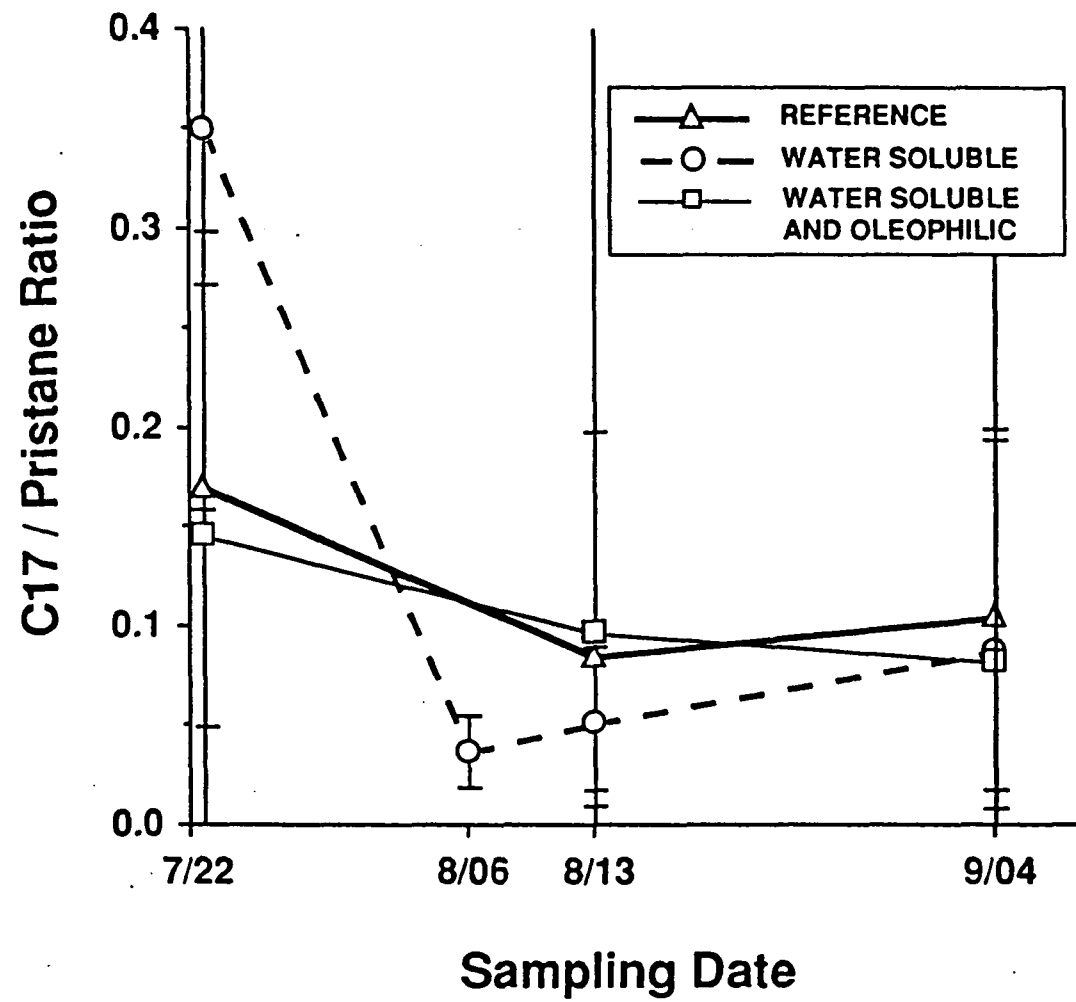


Figure 8.2 Mean C17 / Pristane Ratio at Passage Cove Cobble Plots, Top, All Zones.

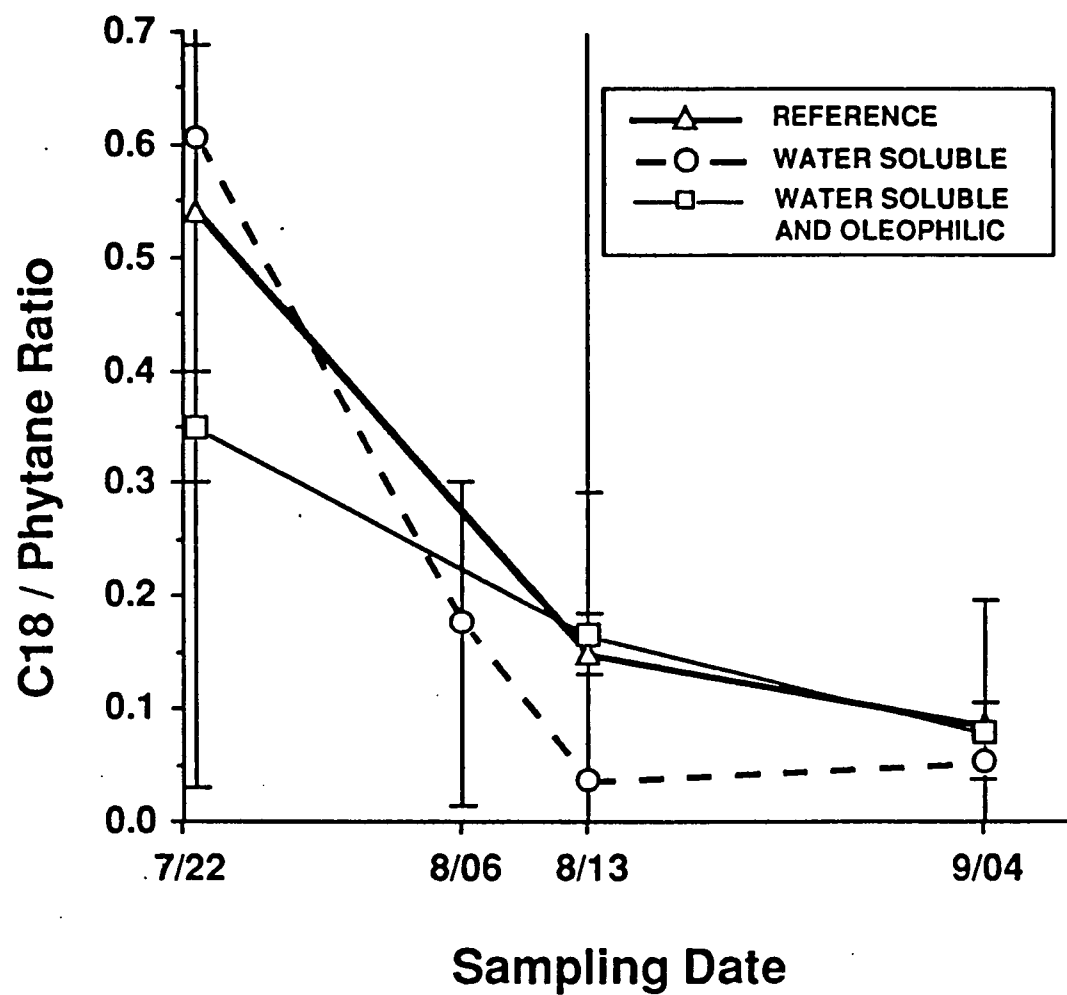


Figure 8.3 Mean C18 / Phytane Ratio at Passage Cove Cobble Plots, Top, All Zones.

As with much of the Passage Cove data, this information is still being analyzed. It will require more time and probably special statistical analyses, to verify the indicated trends.

#### Gas Chromatographic Profiles

Unfortunately only a few computer recreated gas chromatographic profiles are available. Examples using floating concentrations on the plots are shown in Figures 8.4 and 8.5. Comparing profiles in the fertilizer solution-treated beach (sprinkler system) prior to, 2 and 3 weeks following application, it is apparent that both the amount of oil analyzed and the relative concentrations of the alkanes in the profile changed quickly and dramatically through time. Thus, under conditions where microbial communities experience repeated and controlled exposure to nutrients, degradation of oil on the rock surfaces occurred within 1 week. The extensive degradation apparent at 3 weeks, the point in time when oil was visually disappearing from the rocks, suggests that degradation of other fractions (aromatics, waxes, asphaltenes, polars) of the oil may be occurring. Additional chemical and mass spectral analysis of the oil will provide insight into this supposition.

Profiles from two sampling times from the reference plot are compared (Figure 8.5). Degradation does not appear as extensive, yet it occurred to a significant extent.

#### Total Alkane Concentrations

Examination of changes in the total (summed) aliphatic hydrocarbons (normalized to oil residue weight) as determined from the gas chromatographic profiles (Figure 8.6) show how rapidly degradation proceeded in Passage Cove. Again, the absence of complete statistical analyses makes definitive statements difficult. The visual impression is that the reference beach may have had the slowest degradation and the oleophilic/slow release granule fertilizer combination (in terms of total aliphatic hydrocarbons degraded) may have been the most rapid. The initial rise in total hydrocarbons in this latter plot remains unexplained. However, if the worst case is considered and the assumption is made that the sampling on the week following application (week 1) is a fluke, the overall decrease in total hydrocarbons is still as fast as that seen in the fertilizer solution-treated plot. Thus, as more data are analyzed, evidence for nutrient enhanced bioremediation may become stronger.

# Passage Cove - Cobble Surface - Water Soluble Fertilizer - Before Treatment

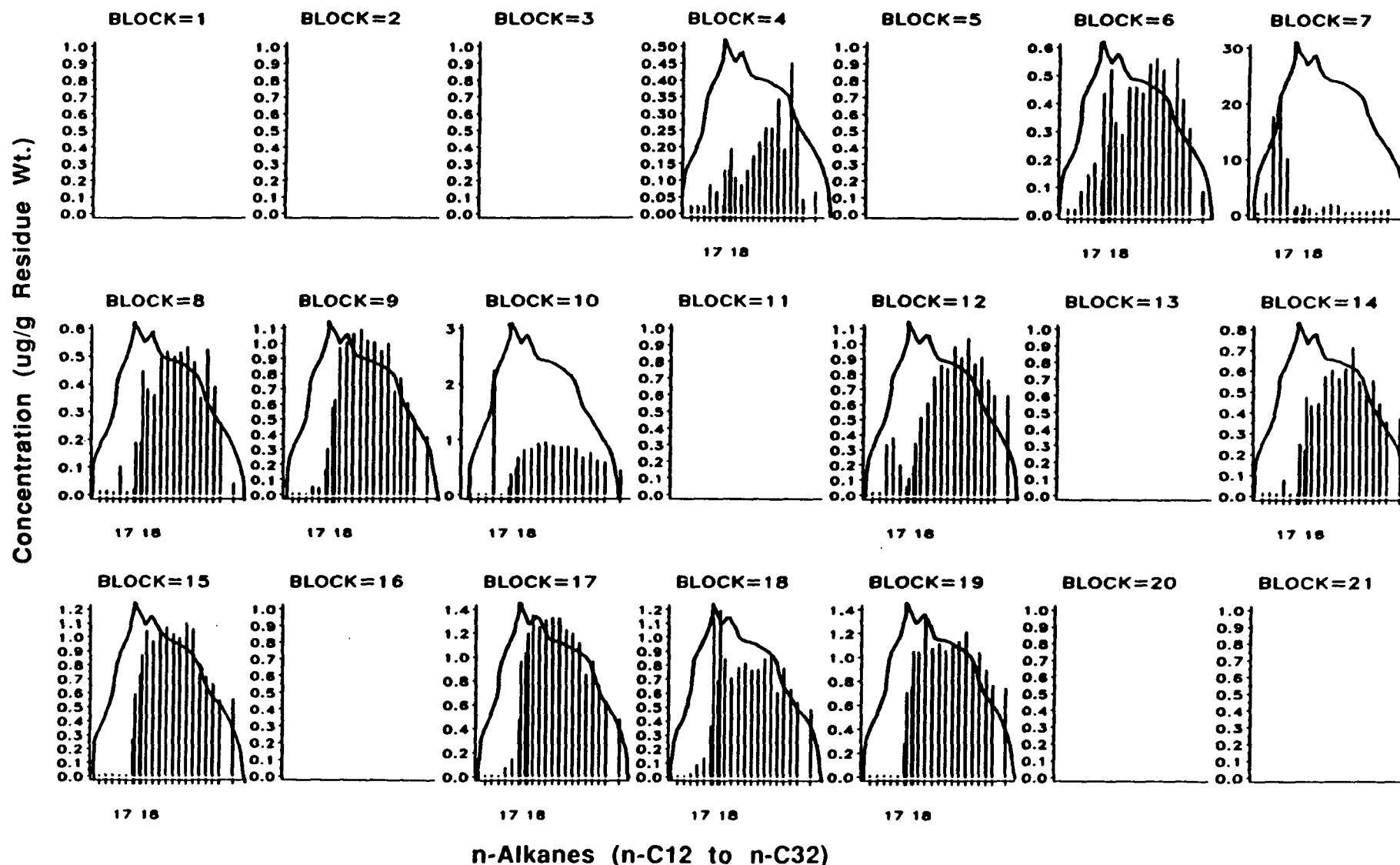


Figure 8.4a. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble before application of water soluble fertilizer (sprinkler system) on Kittiwake Beach in Passage Cove. Blanks indicate data not available. Solid line profile estimates peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.

# Passage Cove - Cobble Surface - Water Soluble Fertilizer - 2 Weeks After Application

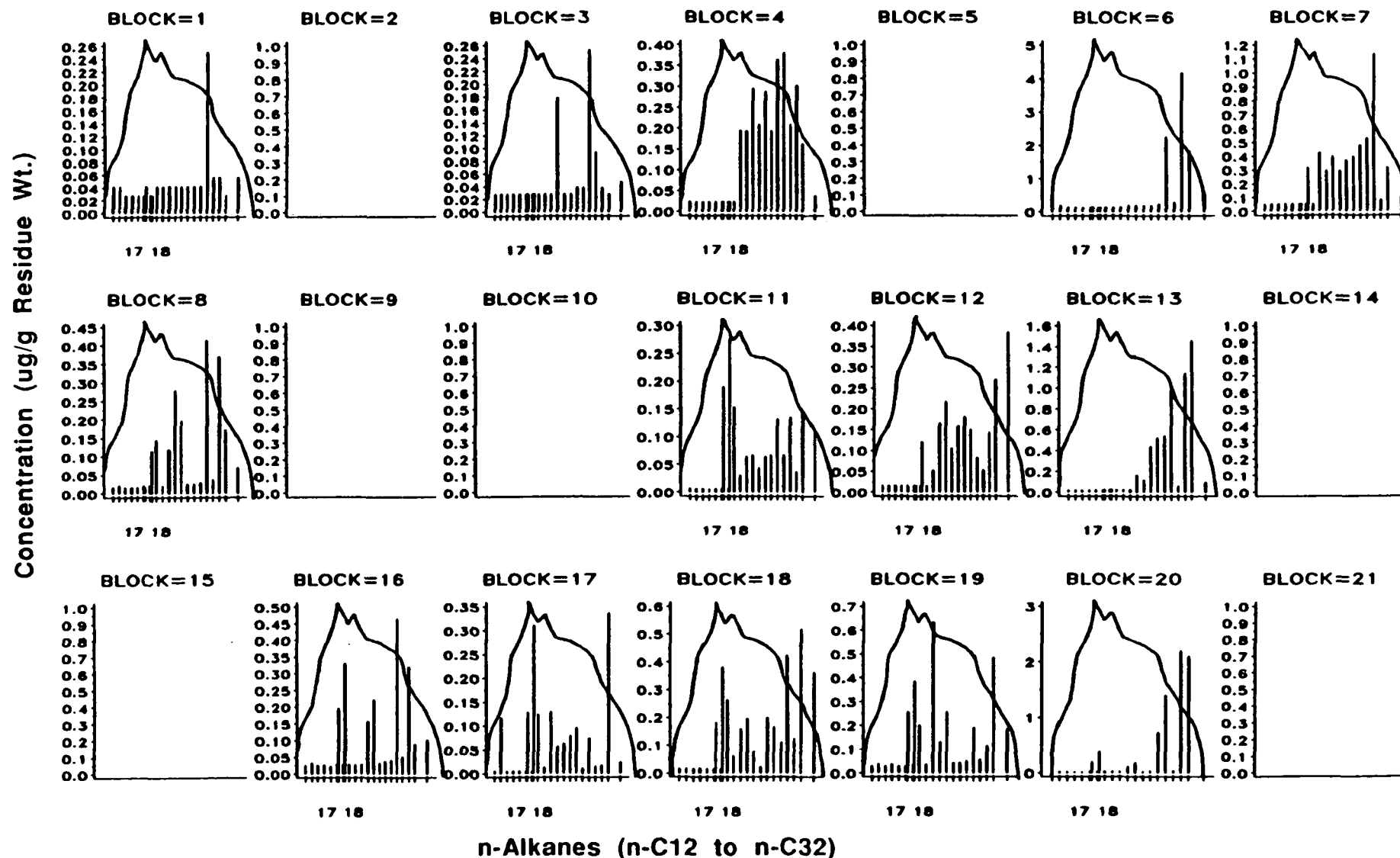


Figure 8.4b. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble two weeks after application of water soluble fertilizer (sprinkler system) on Kittiwake Beach in Passage Cove. Blanks indicate data not available. Solid line profile estimates peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.

# Passage Cove - Cobble Surface - Water Soluble Fertilizer - 3 Weeks After Application

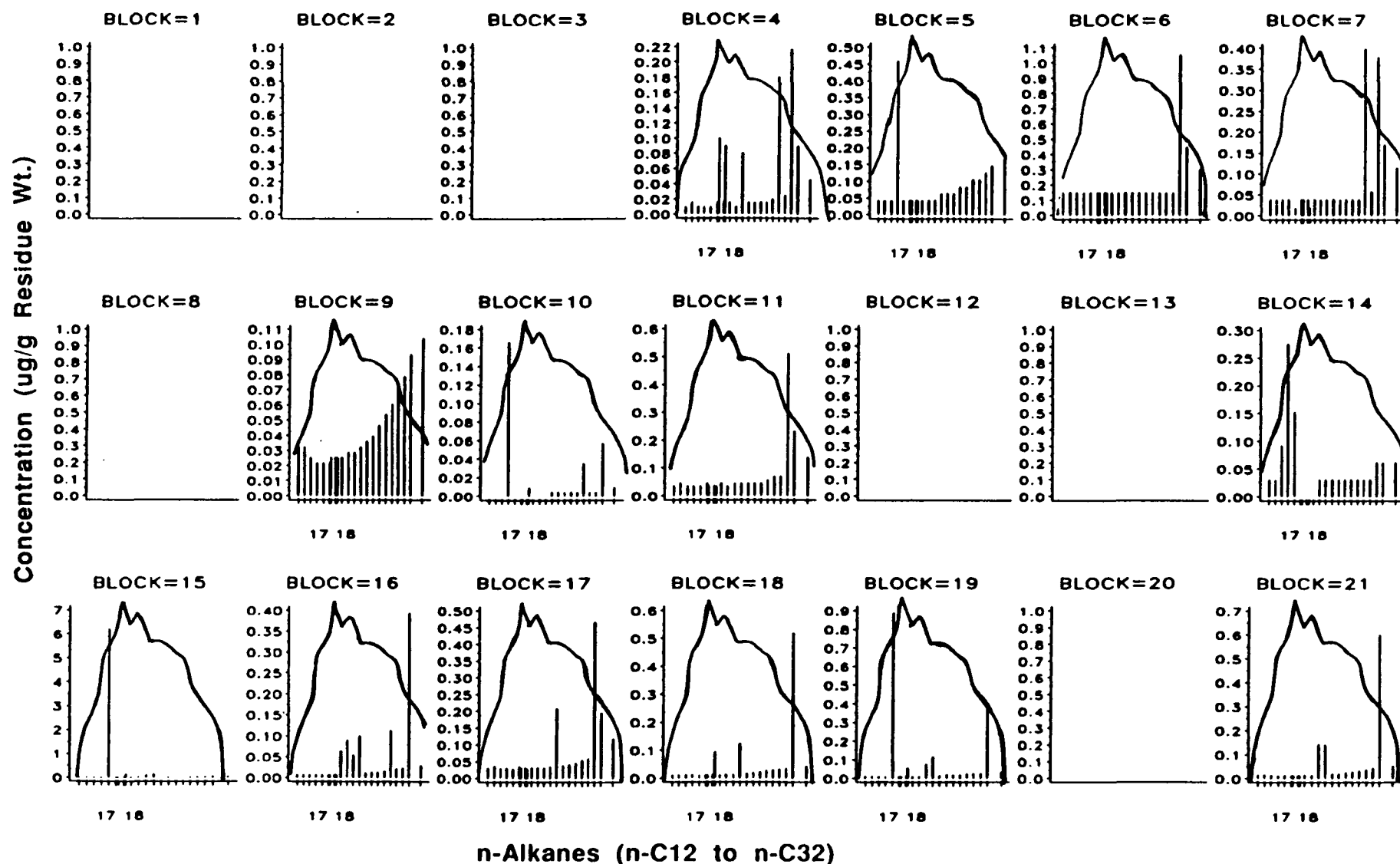
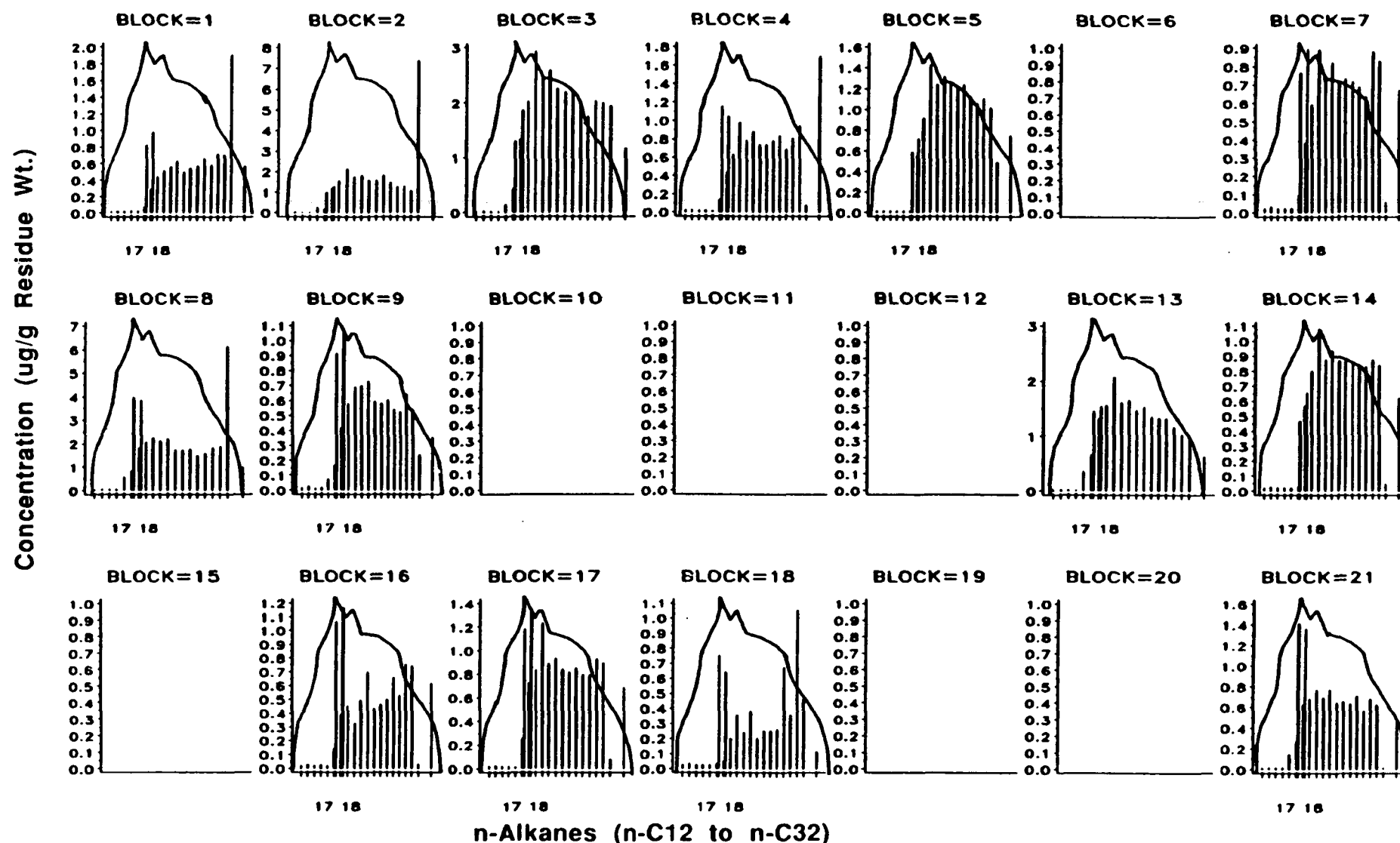


Figure 8.4c. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble three weeks after application of water soluble fertilizer (sprinkler system) on Kittiwake Beach in Passage Cove. Blanks indicate data not available. Solid line profile estimates peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.

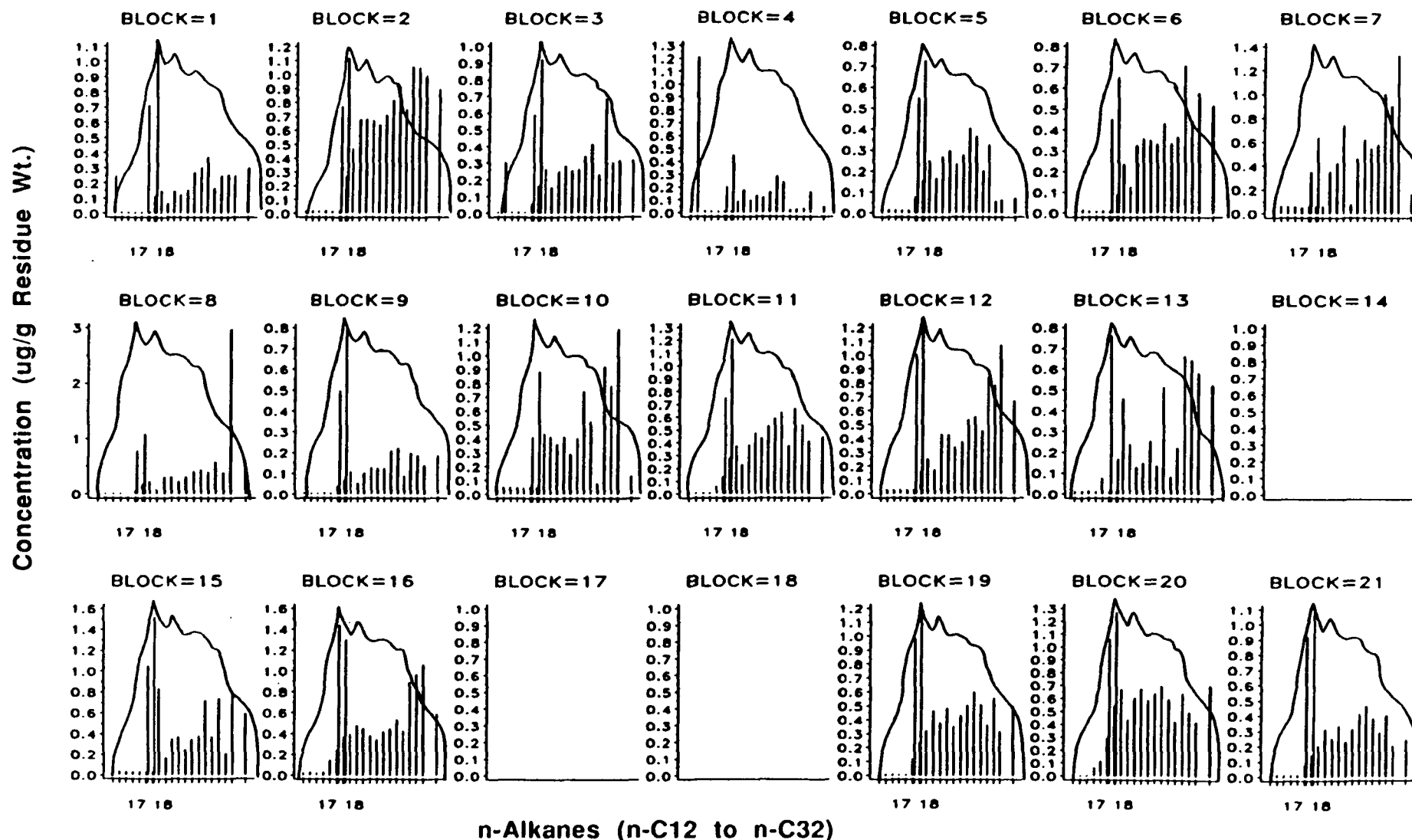
**Passage Cove - Cobble Surface - Untreated - Before  
Fertilizer Application to Nearby (Treated) Beaches**



**Figure 8.5a. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble at the control beach (Raven Beach) before application of water soluble fertilizer at Passage Cove. Blanks indicate data not available. Solid line profile is an estimated line connecting peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.**



**Passage Cove - Cobble Surface - Untreated - 2 Weeks  
After Fertilizer Application to Nearby (Treated) Beaches**



**Figure 8.5b. Recreated gas chromatographic profiles from samples of oil extracted from the surface of cobble at the control beach (Raven Beach) two weeks after application of water soluble fertilizer (sprinkler system) to nearby beaches at Passage Cove. Blanks indicate data not available. Solid line profile estimates peak heights of alkanes in oil that has undergone minimal biodegradation. Note floating concentration scale.**

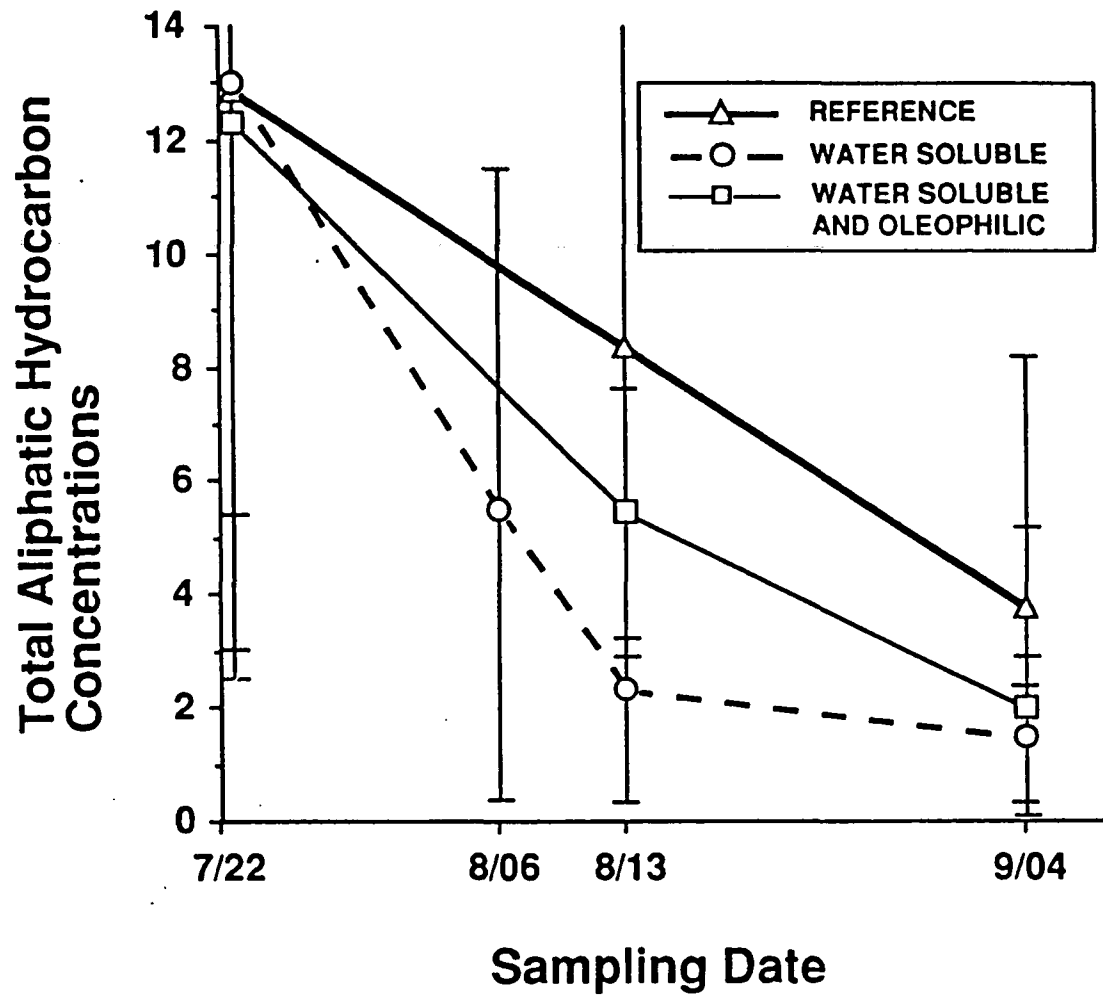


Figure 8.6 Median of Total Aliphatic Hydrocarbon Concentrations (Normalized to Oil Weight) on Treated and Untreated Passage Cove Cobble Plots, Top, All Zones.

## MICROBIOLOGY

The number of oil-degrading bacteria present on beach materials has also been 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 a modification of the dilution to extinction method used for Snug

Five replicate dilution series were prepared from the \*p244XHa initial 1:10 dilution. The relative numbers of bacteria in each sample was an average of the five replicate dilution series.

Results from these studies are shown in Table 8.1. 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 that 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, no increase in oil-degrading microorganisms occurred. This could be the result of a relatively constant sloughing of microbial biomass from the surfaces of the beach material, perhaps as caused by tidal flushing action. Grazing by protozoans could also keep the microbial numbers at a specific density. The presence of high numbers of oil-degrading bacteria in the reference beaches made differences in the numbers of degrading organisms between treatments subtle and difficult to detect.

In early August, several beaches that had not been impacted by the oil spill were sampled to determine realtive levels of oil-degrading microorganisms. Samples were collected from the high, mid, and low tide areas at each beach. The bacterial densities are shown in Table 8.2. The range in concentration of oil degrading organisms was much greater than that observed for oil impacted beaches. It is clear that the number of oil degraders in uncontaminated areas was 1000-100,000 times lower than in contaminated areas. Thus the presence of oil causes a significant enrichment of oil degrading microorganisms.

The rate of mineralization of  $C^{14}$ labeled substrates is being used to determine the physiological competence of microbial populations to degrade specific crude oil components. The ability to utilize these components will be compared to the treatments at test sites.

Three  $C^{14}$ labeled constituents of crude oil were used: naphthalene, phenanthrene, and hexadecane. The rate of conversion of each of these compounds to  $^{14}CO_2$  will establish the activity of the microorganisms as opposed to the number of microorganisms. Preliminary mineralization data from the Passage Cove sampling site indicated that the initial sampling times for  $^{14}CO_2$  were early; no  $^{14}CO_2$  was produced. Due to the number of samples

Table 8.1. Relative Concentration ( $\text{Log}_{10}$  of the cell number/g of beach material) of Oil-Degrading Microorganisms in Passage Cove.

Sampling Date	Plots		
		Fertilizer-Treated	
<u>Before Application</u>	<u>Reference</u>	<u>Water Soluble</u>	<u>Oleophilic &amp; Water soluble</u>
07/22/89	6.44 $\pm 1.44$	6.31 $\pm 1.36$	6.44 $\pm 1.33$
<u>After Application</u>			
08/06/89	5.32 $\pm 1.12$	5.78 $\pm 1.45$	5.71 $\pm 0.67$
08/19/89	6.60 $\pm 1.83$	5.47 $\pm 1.34$	5.66 $\pm 0.35$

Table 8.2. Relative concentratrion ( $\text{Log}_{10}$  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.

<u>Site</u>	<u>High Tide</u>	<u>Mid Tide</u>	<u>Low Tide</u>
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

being processed and the current capability to analyze them, only four sampling times were possible. The first studies indicated that after 48 hours of incubation, significant amounts of  $^{14}\text{CO}_2$  were produced. Therefore, further studies will use an extended incubation period of 3 to 5 days.

## **ECOLOGICAL MONITORING**

The same environmental parameters were monitored at the Passage Cove study site as were monitored at the Snug Harbor study site, using a somewhat modified strategy for sample site location. Sample stations were located along the central axis of the embayment and along 3 nearshore areas where fertilizers were applied (see Sections 5 and 6). Reference sites for the Passage Cove study were established outside of 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, whereas 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**

Only limited data are available from analyses of water samples from Passage Cove for ammonia, nitrite, nitrate, and phosphorus. Assessment of eutrophication resulting from fertilizer additions must await additional sample analyses.

### **Chlorophyll Analysis**

Phytoplankton chlorophyll data showed little change over the course of the study period (Figure 8.7). No trends consistent with nutrient effects were observed. An increase observed on 8/27 was seen in the 0.5 m sample from all mid-channel stations and the reference site.

### **Phytoplankton Primary Productivity**

Results from the pre-treatment sample (7/21), Day 3 (7/28), and Weeks 1 (7/31) and 2 (8/2) are shown in Figure 8.8. These data showed no trends toward greater primary productivity for Passage Cove stations as a result of nutrient additions, except on 7/31. Primary productivity estimates on this date showed greater values for stations 5, 6, and 7, the nearshore stations along the treated shoreline. This increase was not observed 1 week later, and it was not borne out in the chlorophyll data. If primary productivity was enhanced along the shoreline due to nutrient input, the effect on plankton growth was not sufficient to overcome dilution and transport due to tidal exchange, i.e.,

## PASSAGE COVE

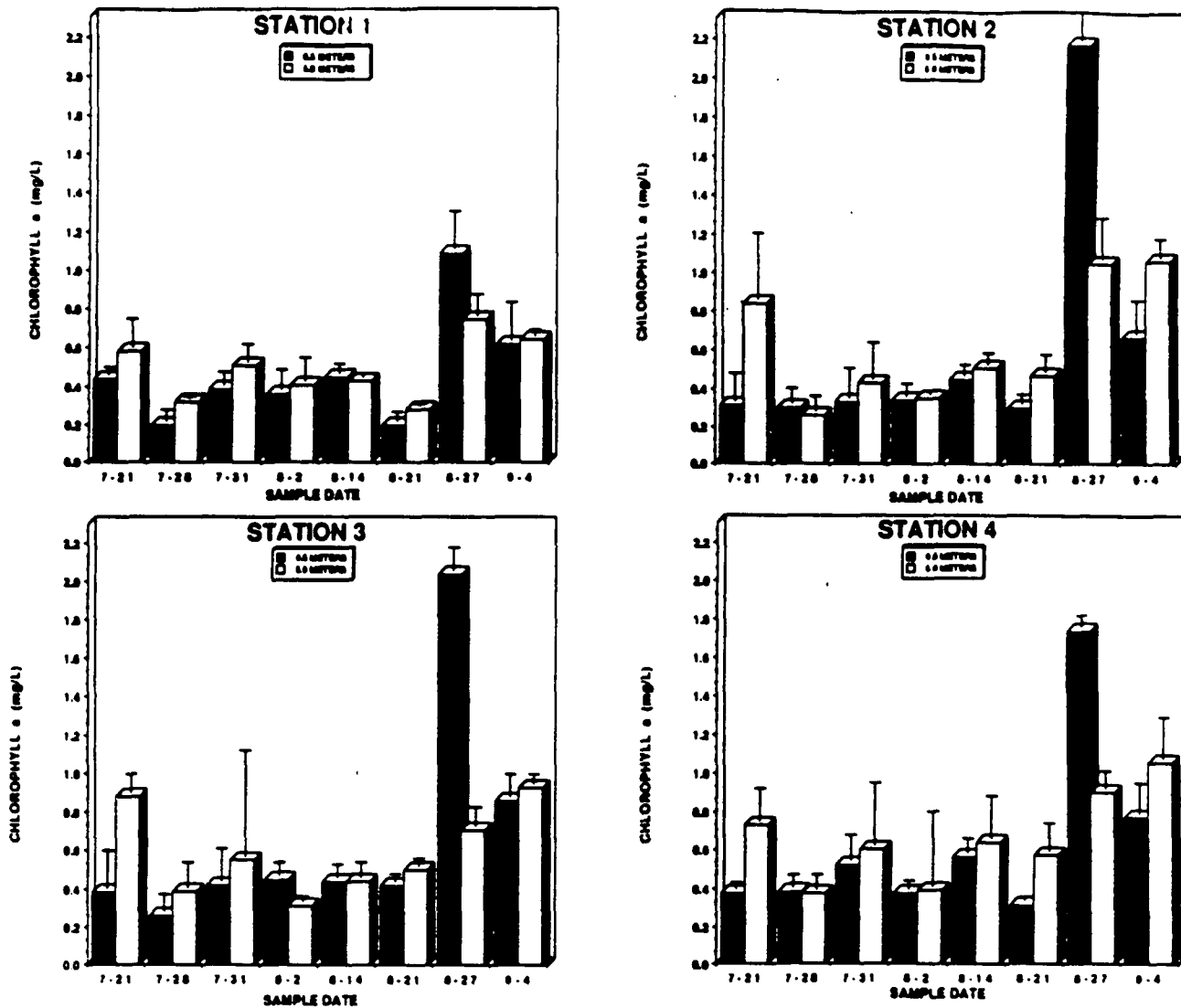


Figure 8.7 Mean Chlorophyll Measurements (+ SD) From 4 Replicate Plankton Samples Taken at Passage Cove Study Sites Before and After July 25, 1989, Fertilizer Applications to Shorelines

## PASSAGE COVE

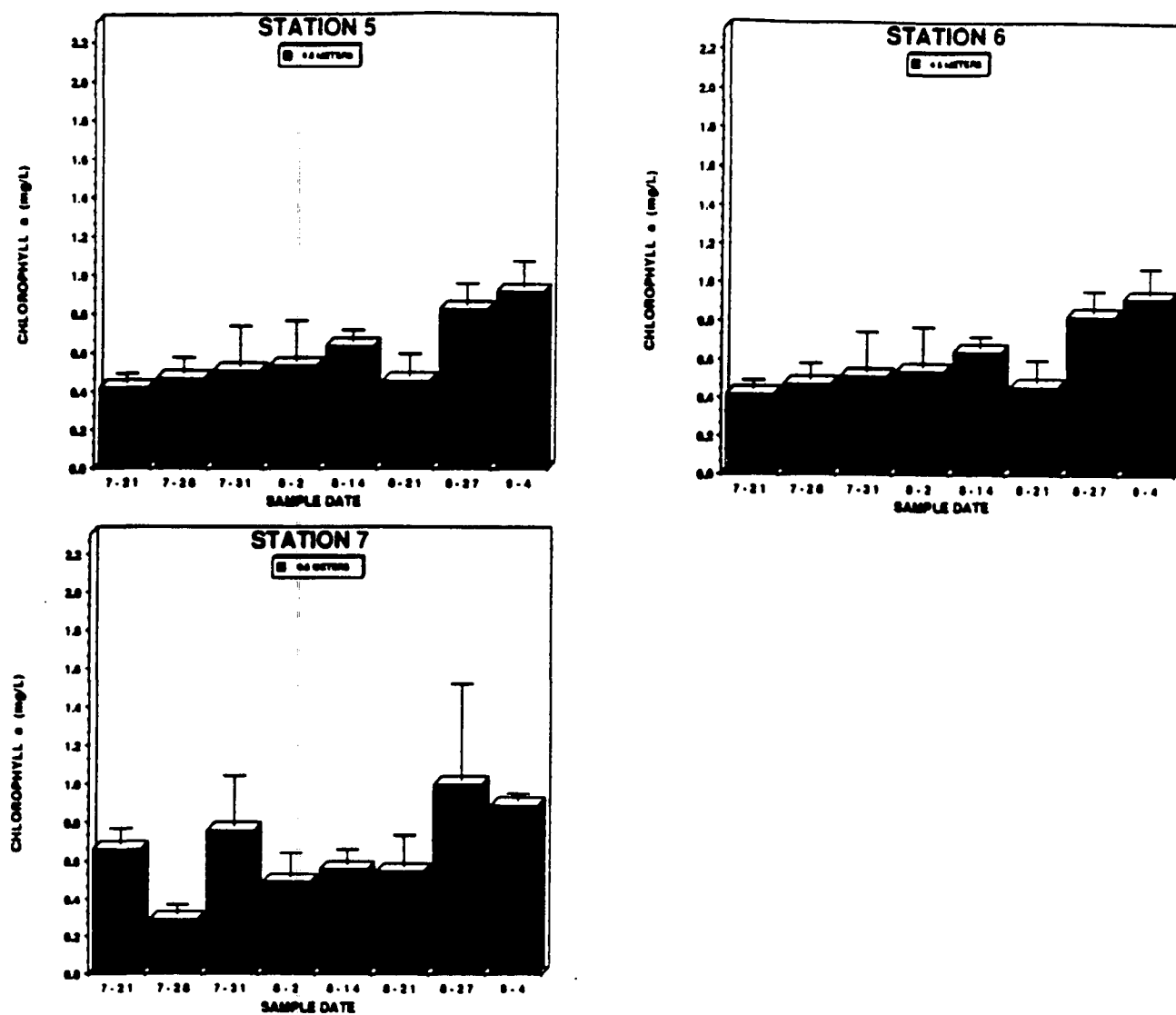


Figure 8.7 (Continued)



## PASSAGE COVE

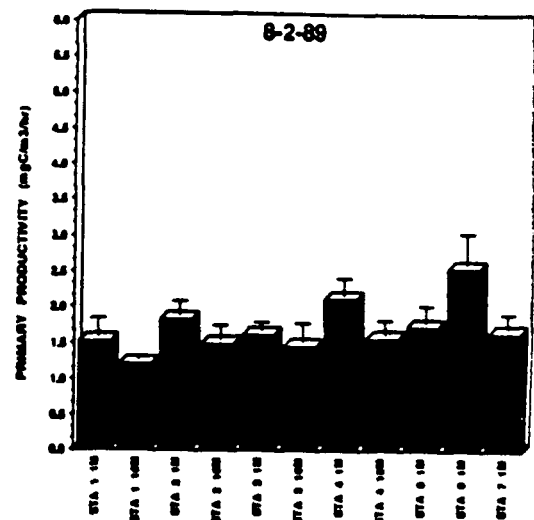
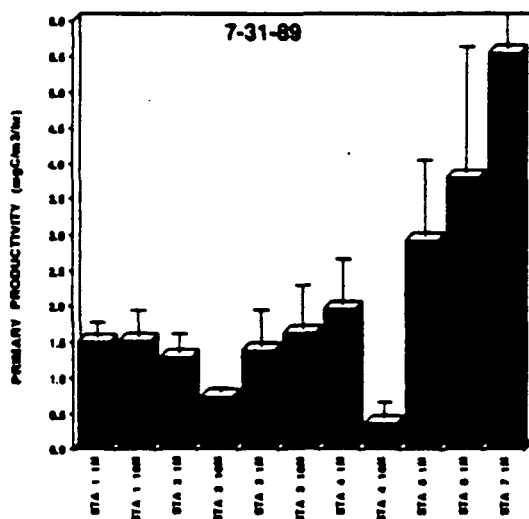
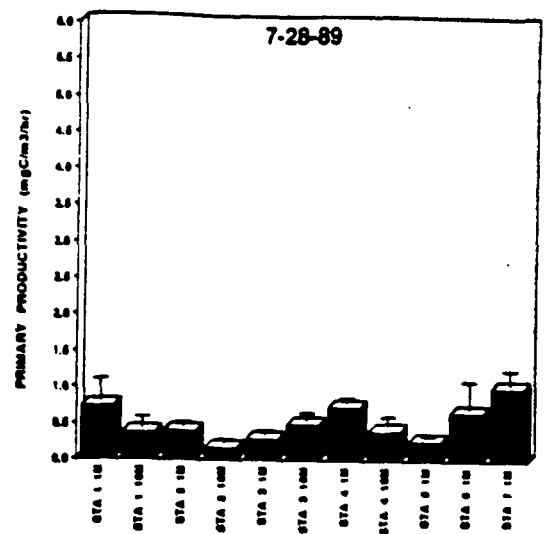
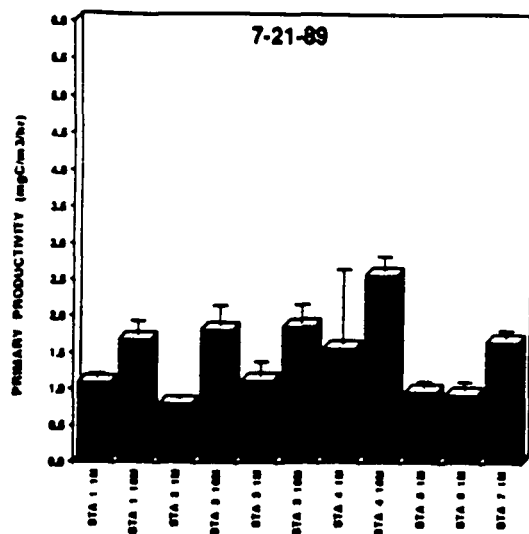


Figure 8.8 Mean Primary Productivity Measurements (+ SD), as <sup>14</sup>C-Uptake From 4 Replicate Plankton Samples Taken at Passage Cove Study Sites Before and After July 25, 1989, Fertilizer Applications to Shorelines

there was no persistent increase in plankton chlorophyll. These conclusions will be reevaluated once all data are available.

### Bacterial Abundance

The mean number of bacterial cells per liter of water at Passage Cove sample sites ranged from approximately  $0.4$  to  $1.2 \times 10^9$  over the 7-week sample period (Figure 8.9). All stations followed the same general pattern of greater numbers on the first three sample dates with lesser abundances thereafter. No trends were observed for nearshore and offshore comparisons, treated versus control comparisons, or 0.5 m to 5.0 m sample comparisons. Fertilizer additions had no stimulatory effect on bacterial numbers.

### Bacterial Productivity

Bacterial productivity, measured by bacterial uptake of tritiated thymidine, demonstrated considerable variability between sample dates with no consistent trends through time or with fertilizer treatments (Figure 8.10). The samples with prominently 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

Analyses of mussel tissues is still proceeding, only 20% of the samples have been analyzed to date. These samples represent a cross-section of the stations and times sampled at the Passage Cove study site. An inspection of available results to date indicate that slightly more than half of the samples analyzed had no detectable polycyclic aromatic hydrocarbons (PAH) residues ( $<0.05 \mu\text{g/g}$ ) and, when present, total PAH concentrations were always less than  $1 \mu\text{g/g}$  (ppm). The predominant PAH present in samples with residues was benzo(a)pyrene. This compound is not prominent in Prudhoe Bay crude oil or its degradation products, but is more likely an indicator of the presence of diesel combustion products from the myriad of vessels working in Prince William Sound on oil spill clean-up efforts. None of the mussel tissue data to date indicates any enhanced residues from oil degradation resulting from bioremediation activities. A definitive assessment must await completion of analytical work on a greater number of samples.

### Field Toxicity Tests of Oleophilic Fertilizer at Passage Cove

Water samples were collected at specified intervals before and after the July 25, 1989, application of Inipol and slow release granules to the treated shoreline. These samples were sent to a consulting laboratory for 48-hr toxicity tests with

## PASSAGE COVE

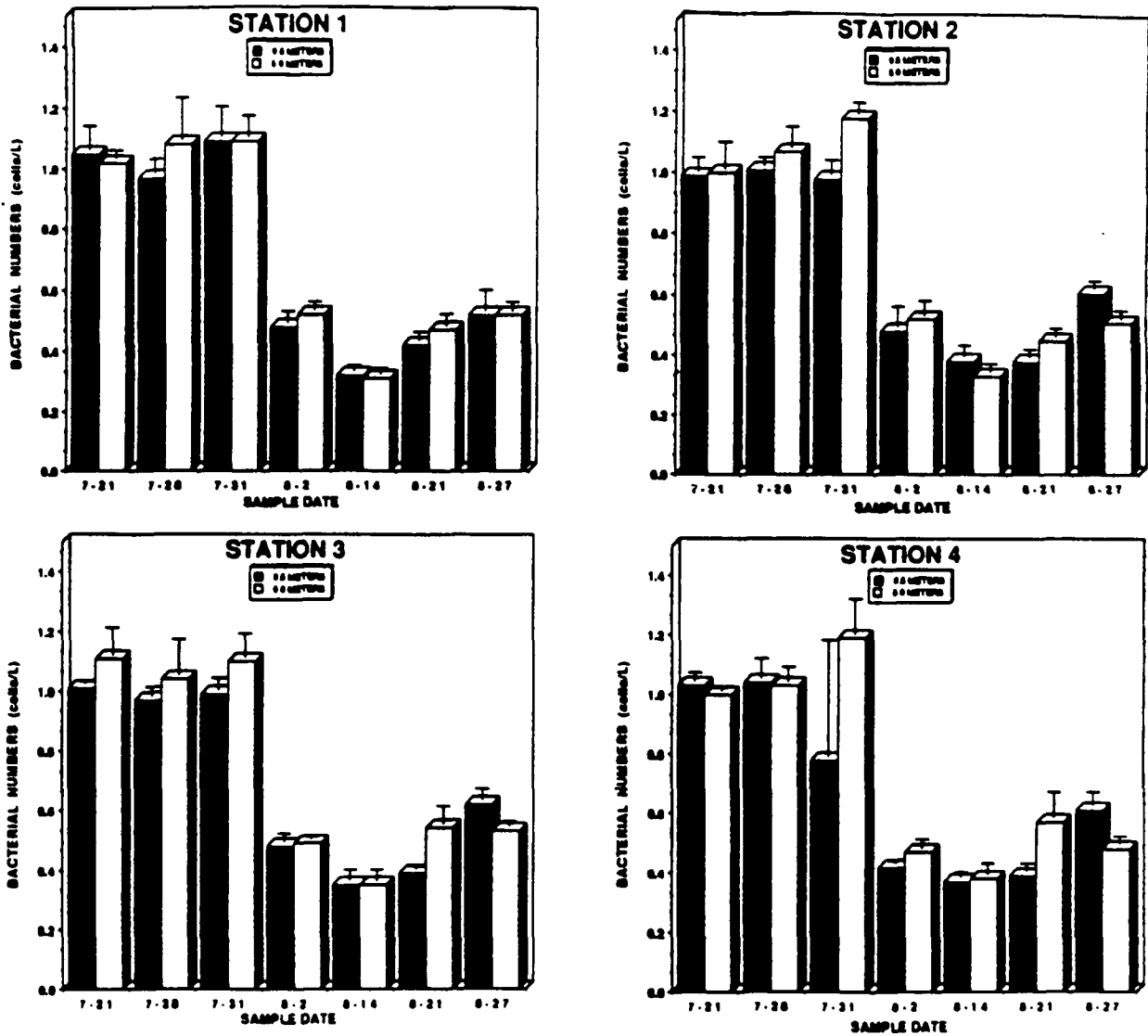


Figure 8.9 Abundance of Bacterial Cells ( $\times 10^9$ ) (Means + SD) From Water Samples Taken at Passage Cove Study Sites Before and After July 25, 1989, Fertilizer Applications to Shorelines

## PASSAGE COVE

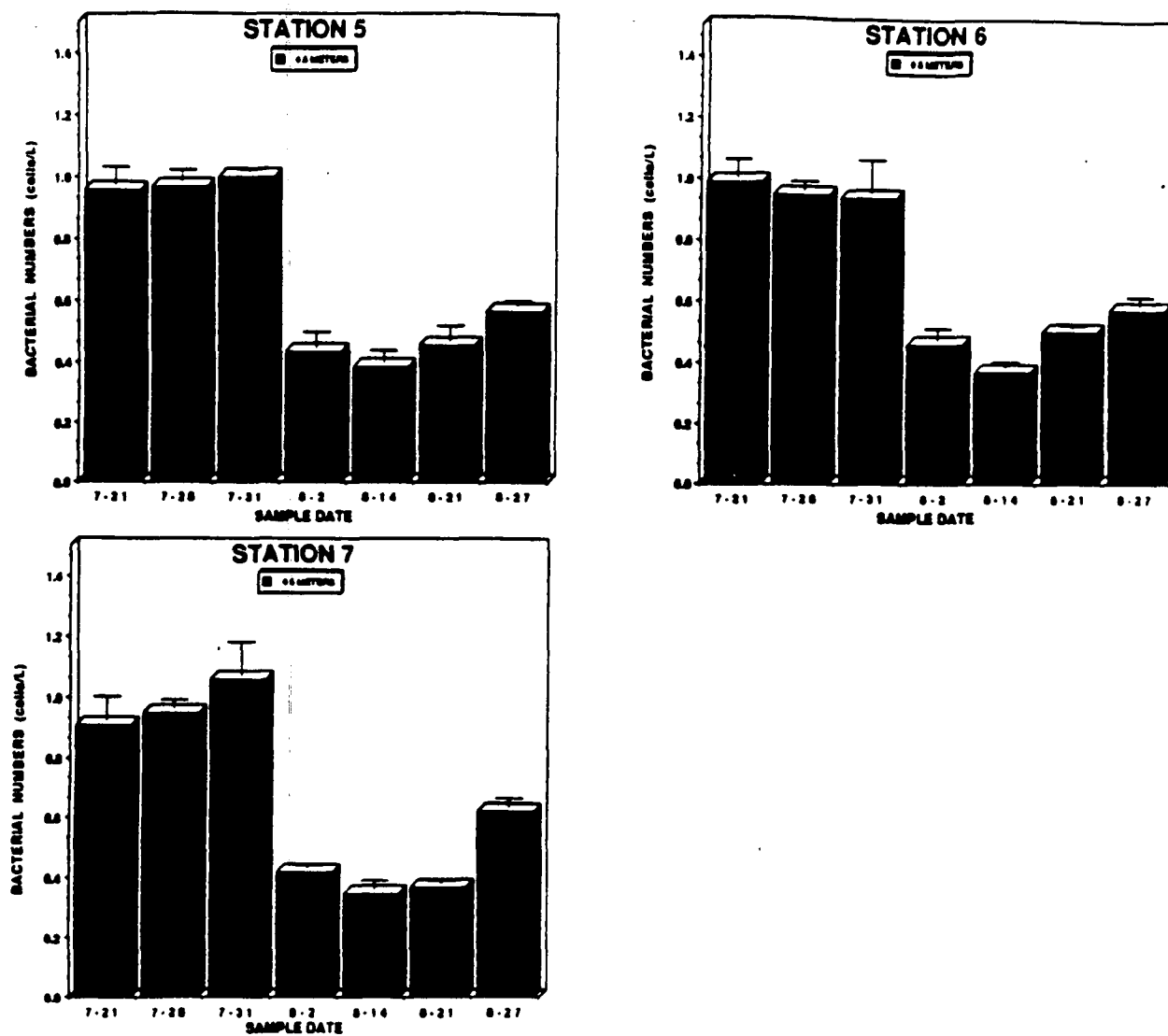


Figure 8.9 (Continued)

## PASSAGE COVE

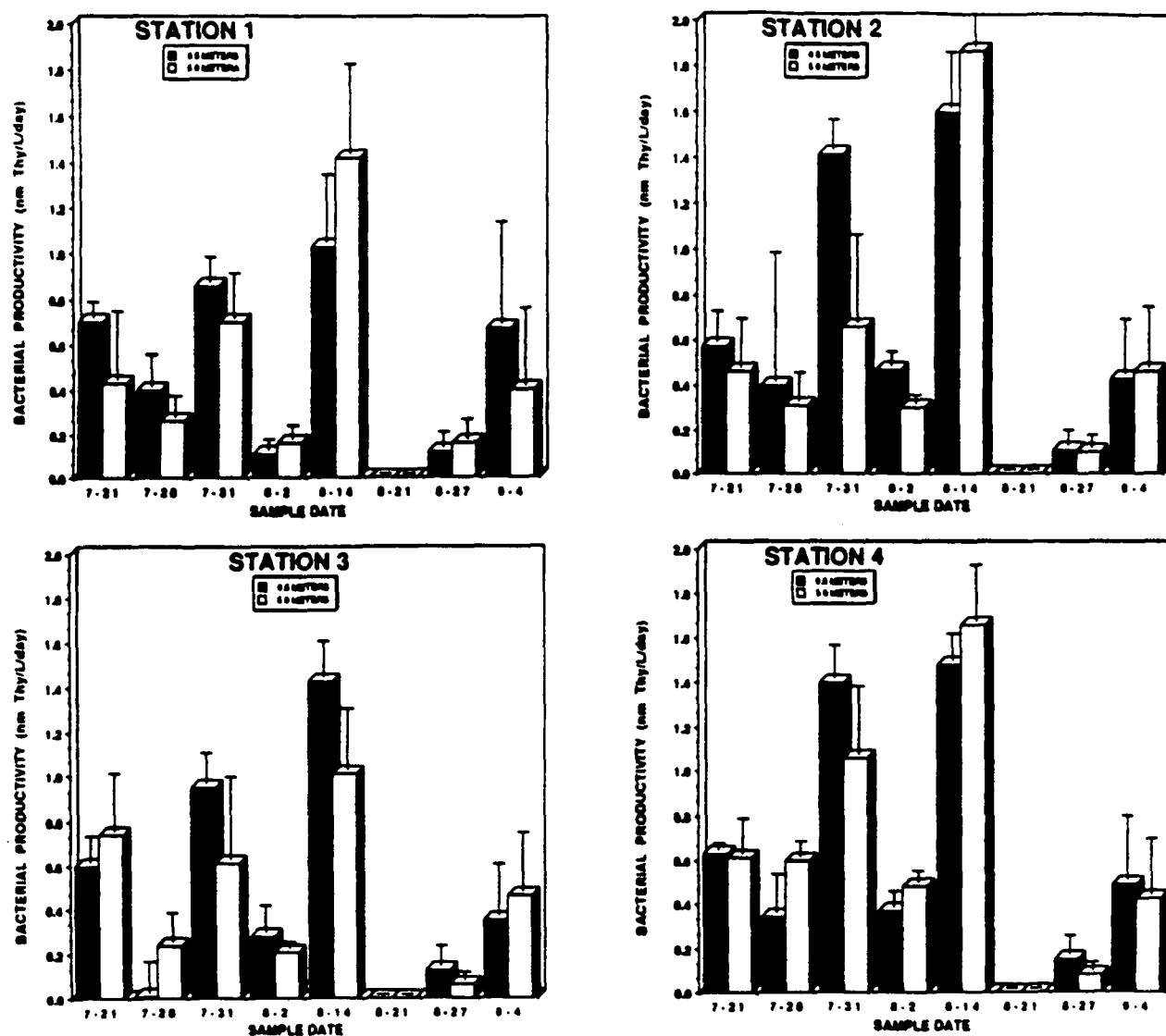


Figure 8.10 Bacterial Productivity Measurements (Means + SD), From Tritiated Thymidine Uptake by Water Samples Taken at Passage Cove Study Sites Before and After July 25, 1989, Fertilizer Applications to Shorelines

## PASSAGE COVE

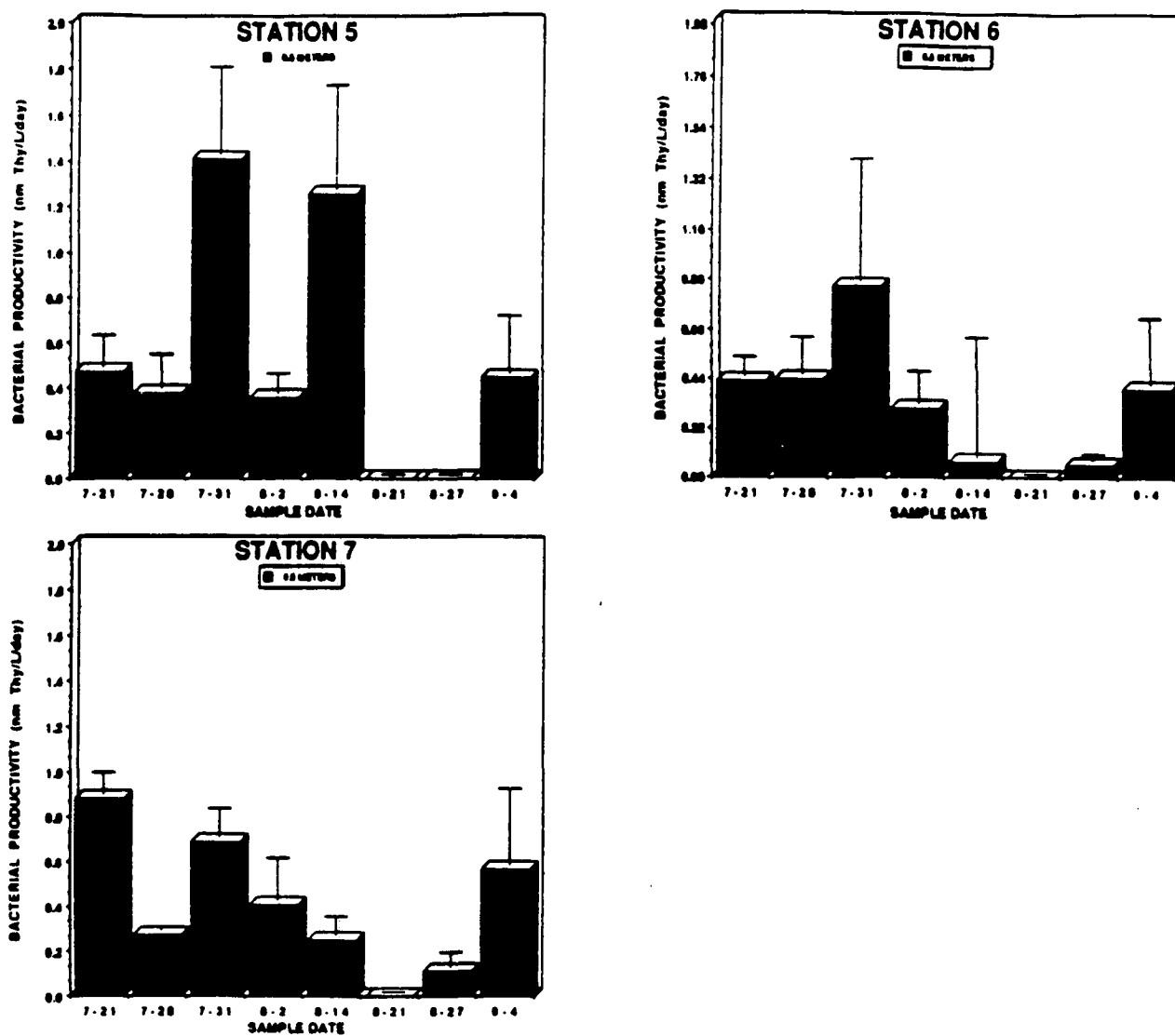


Figure 8.10 (Continued)

oyster larvae, Crassostrea gigas. Endpoints monitored for these tests were larvae survival to test termination and percentage of larvae that exhibited abnormal development. The data are given in Table 8.3.

Test acceptability criteria dictated that for each series tested, control survival must be greater than 70% with abnormality 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 in 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 indicated survival values 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 hr sample, an LC50 could be computed for the 18 hr sample only. 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 hr test samples, were significantly different from the field control survival and pre-application survival of 70% and 74%, respectively. In addition, none of the percentages of abnormally developed larvae for these samples was significantly different from those of the field control and pre-application samples, 8.4% and 10.4%, respectively. Comparison with laboratory seawater controls showed that significant effects occurred for several samples, but these comparisons combine Inipol toxicity with residual toxicity in site water at Passage Cove prior to fertilizer additions.

The water sample taken 18 hours post treatment killed 61% of the oyster larvae during the toxicity test. A 48 hr LC50 of 58% of full-strength water was calculated using the dilution series. Thus, when full-strength site water was diluted to 58% of its original concentration, it would kill 50% of the oyster larvae during a 48 hr 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 controls and laboratory controls. Dilution to 56% of the full strength concentration would produce abnormal larvae at a rate not significantly different than the field control and pre-application samples.

Table 8.3. Larval Survival and Development After 48 hours in Salinity-Adjusted Prince William Sound Water.

<u>Sample Designation</u>	<u>Survival</u>	<u>Abnormal</u>	<u>Tidal Stage</u>
Lab Seawater Control	92%	9.5%	
Hypersaline Control (28 ppt)	75%	7.8%	
Field Control	70%	8.4%	2-hr pre-low
Pre-Application 74%	10.4%	2-hr pre-low	
Inipol Application 10 AM - 2 PM			
1-hr Post Application	62%	14.2%	3-hr post-low
3-hr Post Application	87%	16.1%	near high tide
6-hr Post Application	77%	10.5%	mid-tide, outgoing
12-hr Post	58%	10.1%	mid-tide, incoming
18-hr Post Application	39%	31.4%	mid-tide, outgoing



## Discussion and Conclusions

Toxic effects from misapplication of Inipol or immediate release from the shoreline during initial tidal flooding were not seen. Test results indicated 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 hours after application. The 48 hr 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 the initial flooding. This was unexpected. No unusual weather or oil movements that could have caused this effect 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<sup>2</sup>, concentrations of 4,500 mg/l would be expected if 100% of the applied Inipol was immediately released into water 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, ie, 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 gives 50% mortality should have 50 mg Inipol/l. Thus, a 58% dilution of 18 hr water would get concentrations down to 50 mg/l.
- e) Thus, the initial concentration in the 18 hr sample may have been 90 mg/l Inipol (dilution to 58% yielded 50 mg/l). This concentration was 2% (90 mg/l divided by 4,500 mg/l) of the "no-dilution and 100% release" assumption.
- f) This crude estimate of the release rate (2%) is within the range of expectations for initial releases of Inipol following application.

## DISCUSSION AND CONCLUSIONS

Much of the data from the Passage Cove study is still being processed. However, several points can be discussed at this time.

The biological cleaning effect of oleophilic fertilizer observed in Snug Harbor also occurred in Passage Cove. However, the effect was perhaps more dramatic in that oil from all areas of the treated plots disappeared. It is possible that the homogeneous distribution of oil over a large extensive surface area by physical washing promoted the biological degradation of the oil in the presence of the fertilizer.

Application of nutrients from the sprinkler system proved to be the most efficient system for exposing oil-degrading bacteria to nutrients in a controlled and reproducible manner. As a result, oil degradation was extensive enough to cause removal of the oil from the surfaces of the beach materials. Since there were no chemicals involved in this treatment except inorganic nutrients, it would appear that biodegradation activities were responsible for the oil removal and these activities were enhanced by the nutrient addition.

The action of oleophilic fertilizer probably involved a stimulation of microbial degradation activities through sustained and controlled nutrient addition. The shorter time for this stimulation may have resulted from the softening of the oil caused by the mild surfactants in the fertilizer increasing bioavailability.

No occurrence of eutrophication was revealed by extensive monitoring studies, nor was oil released from nutrient addition.

## SECTION 9

### SUPPORTING STUDIES

#### MICROCOSMS

##### Background

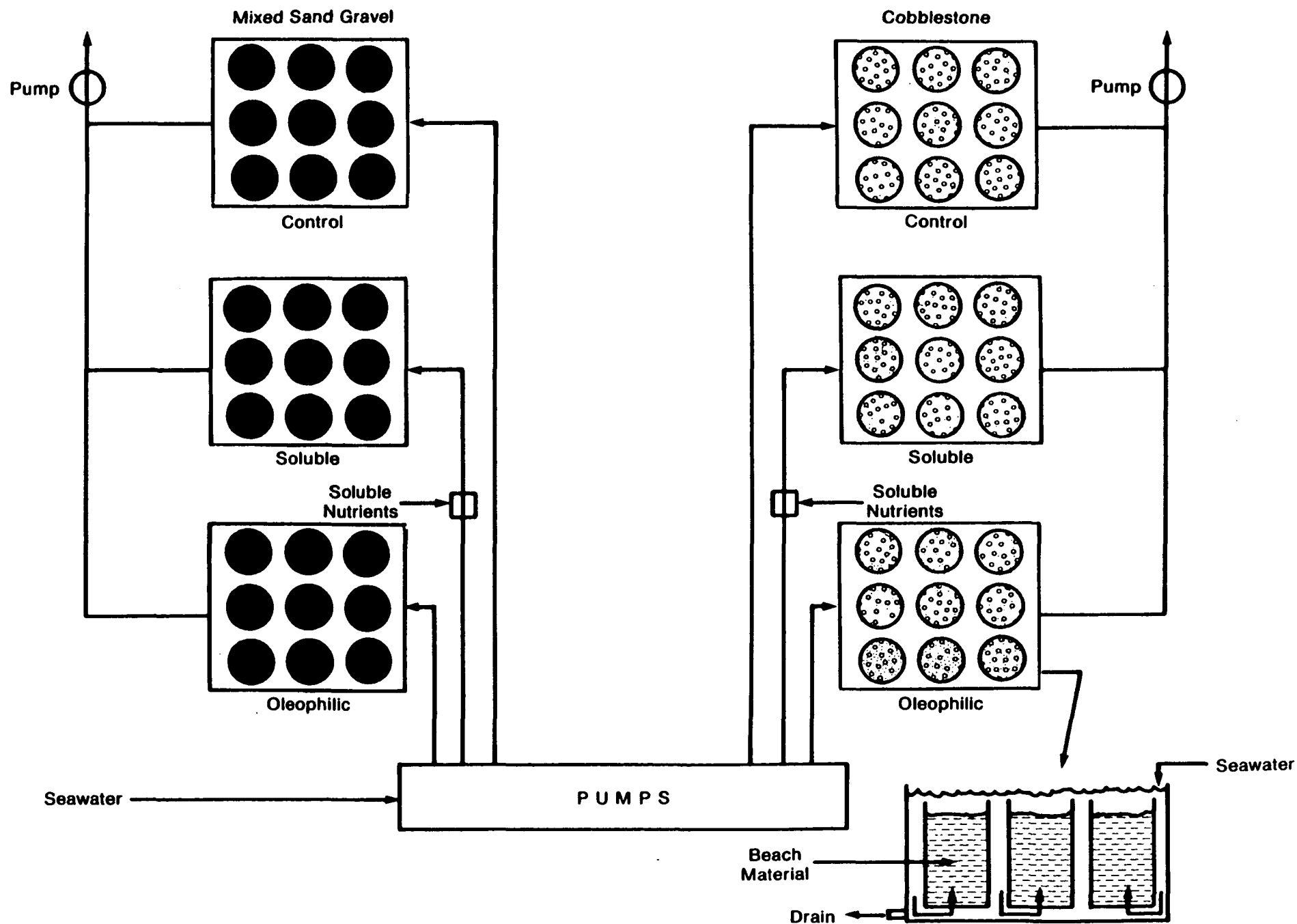
The purpose of these studies was to provide supplemental information to the field demonstration project. In the event of a major storm event, or some other unforeseen complication, significant amounts of data from the field demonstration project could be lost. Microcosm studies that were designed to simulate the field demonstration project could, therefore, provide a basis from which scale-up decisions could be based.

In addition, microcosm studies allow the testing of bioremediation concepts under idealized conditions to provide complementary data and information to the field demonstration projects. For example, if biodegradation of oil occurs in microcosms operated at constant and slightly elevated temperatures, the study could act as a prelude to what would happen in the field where conditions are less constant. It is also desirable to demonstrate that changes in the composition of the oil caused by biodegradation correspond with significant decreases in the weight of the oil. In the field, high spatial variability of oil concentrations may prevent this observation. However, in microcosms, oil concentrations can be standardized and thus indications of weight loss can be readily obtained.

Finally, the field verification of microcosm results lend weight to results from other microcosm tests which cannot be coupled with a field demonstration component. Such related, but non-field verified, microcosm tests can be used with confidence in making decisions about other approaches to the bioremediation of oil-contaminated beaches.

##### Methods

Microcosms were constructed on board the F.V. AUGUSTINE to simulate treatment and control plots in the field demonstration. Six tanks (representing the six plots) were used to hold 9 two-gallon polyethylene containers per tank. A schematic of the microcosm system is shown in Figure 9.1. Twenty-seven of the containers were filled with homogenized sand and gravel obtained from the same area as the sand and gravel used for in situ containers, and mixed in the same manner. The remaining 27 containers were first filled about one-fourth full with homogenized sand and gravel, and then filled with oiled cobble. The microcosm containers had four one-inch holes in the bottom to



**Figure 9.1. Schematic Diagram of the Microcosms**

allow percolation of the water through the beach material as the tanks filled. Seawater from the harbor was pumped into the tanks, held for 6 hours (high tide) and withdrawn to simulate tidal cycles. The tanks, therefore, remained dry for 6 hours. This cycle was then repeated over the next 12 hours, simulating 2 tidal cycles. Within each tank with nine containers, three replicate containers were sacrificed at three intervals. These were analyzed to characterize the remaining oil. Intermittent samples were taken for nutrient analyses.

Fertilizer was added to the microcosms on June 16th. The oleophilic fertilizer was applied by portable backpack sprayers. Enough fertilizer was applied to coat the exposed surface of the beach material in the microcosms. For the water-soluble fertilizer, 80 IBDU briquettes were placed in a container such that water entering the microcosm flushed over the briquettes. However, since ammonia concentrations in the microcosms were never above background during the first week of operation, the briquettes were replaced with small bags filled with commercial granular fertilizer (N:P:K, 16:5:5 not slow-release), to ensure adequate levels of nutrients were maintained. This approach continuously produced ammonia concentrations around 400-700 mg/l at each filling of the microcosms.

## Results

The first set of mixed sand and gravel microcosms was sampled on July 7 (22 days post application) and a set of cobble microcosms was sampled on July 11 (26 days post application) and July 26 (41 days post application). Visual observations at the time of sampling indicated that the oleophilic fertilizer-treated cobble microcosms appeared to have the least oil on the surface, but the difference from other treated and control microcosms was not dramatic. The cobble microcosms treated with the oleophilic fertilizer had a mottled appearance, suggesting that the oil on the surface had been partially removed or degraded. Oil was apparent under the rocks, but it was very black and viscid. This consistency appeared to be due to the oleophilic fertilizer dissolving into the oil.

Amounts of surface oil in the control and water-soluble fertilizer-treated microcosms appeared the same. Cobble systems showed some rocks with clean surfaces, but generally fewer than in the oleophilic fertilizer-treated systems. Oil on the rock surfaces appeared gray and dried. Oil under the rocks was drier and less fluid than oil observed in the oleophilic fertilizer treated microcosms.

After sampling the microcosms, it was noted that the inside walls of the water-soluble fertilizer microcosms and the reference microcosms were spotted with oil smudges. This was not the case in the oleophilic fertilizer-treated set, where the

walls generally appeared free of oil. Small particles of white waxy material were also observed throughout the sand and gravel in the oleophilic fertilizer-treated set of microcosms, even with the daily influx of fresh seawater. This material may have been residual oleophilic fertilizer.

### Discussion and Conclusions

Results from chemical analyses sampling dates are available. In a sand and gravel microcosm sampled on July 7 (22 days post fertilizer application), the C17/pristane and C18/phytane ratios in the oleophilic fertilizer-treated microcosms were the same as those in the untreated reference microcosms (Table 9.1). Ratios in the oil from microcosms treated with the water soluble fertilizers were almost half of those for the other microcosms. There was also approximately 20% less oil residue by weight. These data suggest that the more rapid degradation of oil was occurring in the water-soluble fertilizer treatments, assuming oil concentration and composition were approximately the same in all microcosms at the start of the experiment (data not yet available). Because of the large amount of readily degradable carbon added with the oleophilic fertilizer, enhanced degradation of the oil may not occur until after much of the carbon is degraded. In the cobble microcosms sampled on July 11, similar results were observed (Tables 9.2 and 9.3). The most active degradation, in terms of loss of weight and change in composition, appeared to be in the water-soluble fertilizer-treated systems. Degradation of oil in the oleophilic fertilizer-treated systems was about the same as in the untreated systems. Oil residue weights in the former gravel systems were, on the average, 6 times those in the reference microcosms. This indicates that a component of the oleophilic fertilizer may contribute to the residue weight.

Analysis of the data from the July 26 sampling of a cobble microcosms is only partially complete. For those samples available, mass spectral analysis was performed. A summary of the results is given in (Tables 9.4 and 9.5). In contrast to the July 11 data, C17/pristane and C18/phytane ratios for July 26 samplings indicated that the control microcosms were degrading oil faster than the water-soluble fertilizer-treated microcosms (Table 9.4). This is in contrast to the initial sampling in which the results showed greater degradation in the water-soluble fertilizer-treated microcosms. However, the hydrocarbon ratios may yield false indications of limited degradation for samples in which marked degradation of the oil has occurred if pristane is degraded along with straight chain hydrocarbons. Additionally, more degradation may be occurring in the oleophilic fertilizer-treated microcosms than the pristane or phytane ratios suggest.

Table 9.1. Chemical Analysis of Mixed Sand and Gravel Microcosms  
Sampled 17 Days After Initiation of Fertilizer Application.

<u>Treatment</u>	<u>Residue Weight (mg/kg)</u>	<u>C17/Pristane</u>	<u>C18/Phytane</u>
Control 1	1570	0.5	0.8
Control 2	913	0.4	0.6
Control 3	<u>790</u>	<u>0.4</u>	<u>0.6</u>
Average	1091	0.4	0.7
Oleophilic 1	1490	0.4	0.7
Oleophilic 2	1360	0.4	0.8
Oleophilic 3	795	<u>0.4</u>	<u>0.7</u>
Average	1215	0.4	0.7
Soluble 1	913	0.3	0.4
Soluble 2	916	0.1	0.3
Soluble 3	<u>845</u>	<u>0.3</u>	<u>0.3</u>
Average	891	0.2	0.3

Table 9.2. Residue Weight of Oil in Cobble Microcosms Analyzed  
26 Days After Fertilizer Application

Treatment	<u>Residue Weights (mg/kg)</u>		
	<u>Top Cobble</u>	<u>Bottom Cobble</u>	<u>Gravel</u>
Control 1	1420	1120	889
Control 2	889	1090	1090
Control 3	<u>1040</u>	<u>722</u>	<u>1030</u>
Average	1116	977	1993
Oleophilic 1	1770	1910	6350
Oleophilic 2	1260	2460	5580
Oleophilic 3	2340	<u>3550</u>	<u>6960</u>
Average	1790	1640	6297
Soluble 1	161	1310	1020
Soluble 2	1240	725	714
Soluble 3	<u>383</u>	<u>664</u>	<u>814</u>
Average	595	900	849



Table 9.3. Ratios of Hydrocarbons in Oil From Cobble Microcosms Analyzed 26 days After Fertilizer Application

<u>Treatment</u>	<u>C17/Pristane</u>			<u>C18/Pristane</u>		
	<u>Top Cobble</u>	<u>Bottom Cobble</u>	<u>Gravel</u>	<u>Top Cobble</u>	<u>Bottom Cobble</u>	<u>Grave</u>
Control 1	0.8	0.7	0.3	1.3	1.1	0.5
Control 2	0.9	0.6	0.4	1.3	1.0	0.5
Control 3	<u>0.7</u>	<u>0.7</u>	<u>0.4</u>	<u>1.3</u>	<u>1.1</u>	<u>0.5</u>
Average	0.8	0.7	0.4	1.3	1.0	0.5
Oleophilic 1	0.9	0.8	1.0	1.3	1.4	1.5
Oleophilic 2	1.0	0.9	0.9	1.2	1.5	1.4
Oleophilic 3	<u>1.0</u>	<u>0.9</u>	<u>0.8</u>	<u>1.2</u>	<u>1.4</u>	<u>1.3</u>
Average	1.0	0.9	0.9	1.2	1.4	1.4
Soluble 1	0.2	0.3	0.5	0.4	0.4	0.5
Soluble 2	0.6	0.1	0.6	1.1	0.3	0.7
Soluble 3	<u>0.6</u>	<u>0.2</u>	<u>0.5</u>	<u>0.9</u>	<u>0.3</u>	<u>0.5</u>
Average	0.5	0.2	0.5	0.8	0.3	0.5

Table 9.4. Comparison of C17/Pristane Ratios and C17/Norhopane Ratios as Measures of Oil Degradation in Samples Taken From Cobble Microcosm 42 Days After Initiation Of Fertilizer Application.

<u>Microcosm</u>	<u>C17/ Pristane</u>	<u>C17/ Norhopane</u>	<u>Pristane/ Norhopane</u>	<u>Norhopane/ Hopane</u>
Control	.19	1.03	5.44	.78
Water Soluble	.49	.22	.44	.75
Standard	1.7	17.50	10.68	.78

Table 9.5. Use of Dibenzothiophene Peaks/Norhopane Ratios as Relative Measures of the Degradation of Aromatic Components in Oil Sampled From Cobble Microcosms 42 Days After Initiation of Fertilizer Application.

---



---

Dibenzothiophene Peaks <sup>a</sup> /Norhopane Ratios			
<u>Microcosms<sup>b</sup></u>	<u>Peak 1</u>	<u>Peak 2</u>	<u>Peak 3</u>
Control 1	.40	.54	.60
Control 2	.49	.66	.71
Control 3	.46	.70	.71
Water Soluble 1	.08	.13	.13
Water Soluble 2	.10	.12	.19
Water Soluble 3	.11	.13	.17
Oleophilic 1	.82	1.21	1.06
Oleophilic 2	.81	1.15	1.01
Oleophilic 3	.85	1.17	.99
Standard	1.06	1.84	1.54

---

<sup>a</sup> In the mass spectral analysis of oil, C-2 dibenzothiophenes and their homologs show a series of peaks at mass ion 212. Three prominent peaks (labeled here 1, 2, and 3) were selected for comparison.

<sup>b</sup> Average of three replicates

Further gas chromatography/mass spectrometry data provided sufficient data to evaluate this possibility. By extracting and analyzing all microcosm samples in the same manner, compounds that did not change in concentration in any of the treatments were identified. Two compounds, norhopane and hopane were identified. Their concentrations did not change, and the ratio of norhopane to hopane remained constant at 0.76 (Table 9.4.). Constructing C17/norhopane and pristane/norhopane ratios indicated that C17 was degraded 5 times more effectively in the water-soluble fertilizer-treated than in the control microcosms (Table 9.4). Surprisingly, pristane was also degraded in both the control and water-soluble, microcosms, thereby supporting the suggestion that C17/pristane ratios were inadequate indicators of biodegradation. It was concluded that norhopane may be a better choice of a very slowly degraded oil component to be used as an internal marker for the undegraded oil components.

The ratios of the three major dibenzothiophene peaks to the very poorly degraded hydrocarbon, norhopane, were also examined using mass spectral analysis (Table 9.5). Further differences between the treatments were observed. Water-soluble fertilizer-treated microcosm samples showed the greatest degree of degradation of the dibenzothiophene isomers. Interestingly, the ratios for the oleophilic treatment indicated little change in the dibenzothiophene isomers, compared with the ratios observed in a Prudhoe Bay crude oil standard. These observations are consistent with the C17/pristane data from previous samplings, which also indicated that oil degradation in the oleophilic treatment was less active than the degradation in both the water-soluble and control treatments. The dibenzothiophene to hopane ratio may be useful to estimate degradation of the sulfur-heterocyclics in oil. Ratios with other aromatic compounds (phenanthrene, fluorene, etc.) may also provide a similar tool to evaluate the homocyclic aromatic fraction.

From these initial microcosm results, it can be concluded that if sufficient nutrients are supplied to the microorganisms, then enhanced biodegradation of the oil will occur. Because the microcosms represent the test systems that best reflect field conditions, a similar response could be expected in the field, if nutrient concentrations can be maintained at adequately high levels. The microcosm studies also showed that pristane and phytane are readily biodegraded and as such they are not good markers for assessing changes in oil composition. Mass spectral analysis may provide other markers to use in this regard.

## LABORATORY BIODEGRADATION SCREENING EVALUATIONS<sup>a</sup>

### Background

Studies have shown that oil biodegradation can be enhanced by the addition of inorganic nutrients under controlled laboratory conditions. While it could be assumed that similar enhancement would occur in the field for oil spilled in Prince William Sound if degradation was limited by nutrient availability, laboratory studies using samples of weathered oil and beach material from the Prince William Sound were needed to verify this assumption. Laboratory flask studies were designed to investigate the validity of this assumption, using various nutrient sources, inocula, and temperatures. The results of these studies will be used to help interpret the results of field observations.

### Methods

Flask studies used samples of Prince William Sound water and/or oiled beach material. All flasks were incubated with slow shaking at constant temperature. At each sampling, flask contents were sacrificed and extracted with methylene chloride. Extracts were dried and then analyzed by flame ionization detection gas chromatography (GC/FID). Experiments were conducted as follows:

- Effects of different inocula: Samples of artificially weathered Prudhoe Bay crude oil (30% weight loss induced by distillation) (1% by weight) were placed in sterile Bushnell-Haas medium, a defined nutrient medium containing 0.03% nitrogen and 0.04% phosphorous. This mixture was added at a rate sufficient to provide nitrogen and phosphorus equal to 3.5% and 4.1% by weight of oil, respectively. This mixture was used uninoculated (control) or inoculated with either a 10% inoculum of water from the Alyeska ballast treatment facility or seawater from Prince William Sound. All flasks were incubated at 15°C for 16 days before the oil composition was analyzed.
- Effect of incubation temperature: Artificially weathered Prudhoe Bay crude oil was added to sterile Bushnell-Haas medium and inoculated with 10% Prince William Sound water. Flasks were incubated for 38 days at 15° and 5°C before the oil composition was analyzed.

---

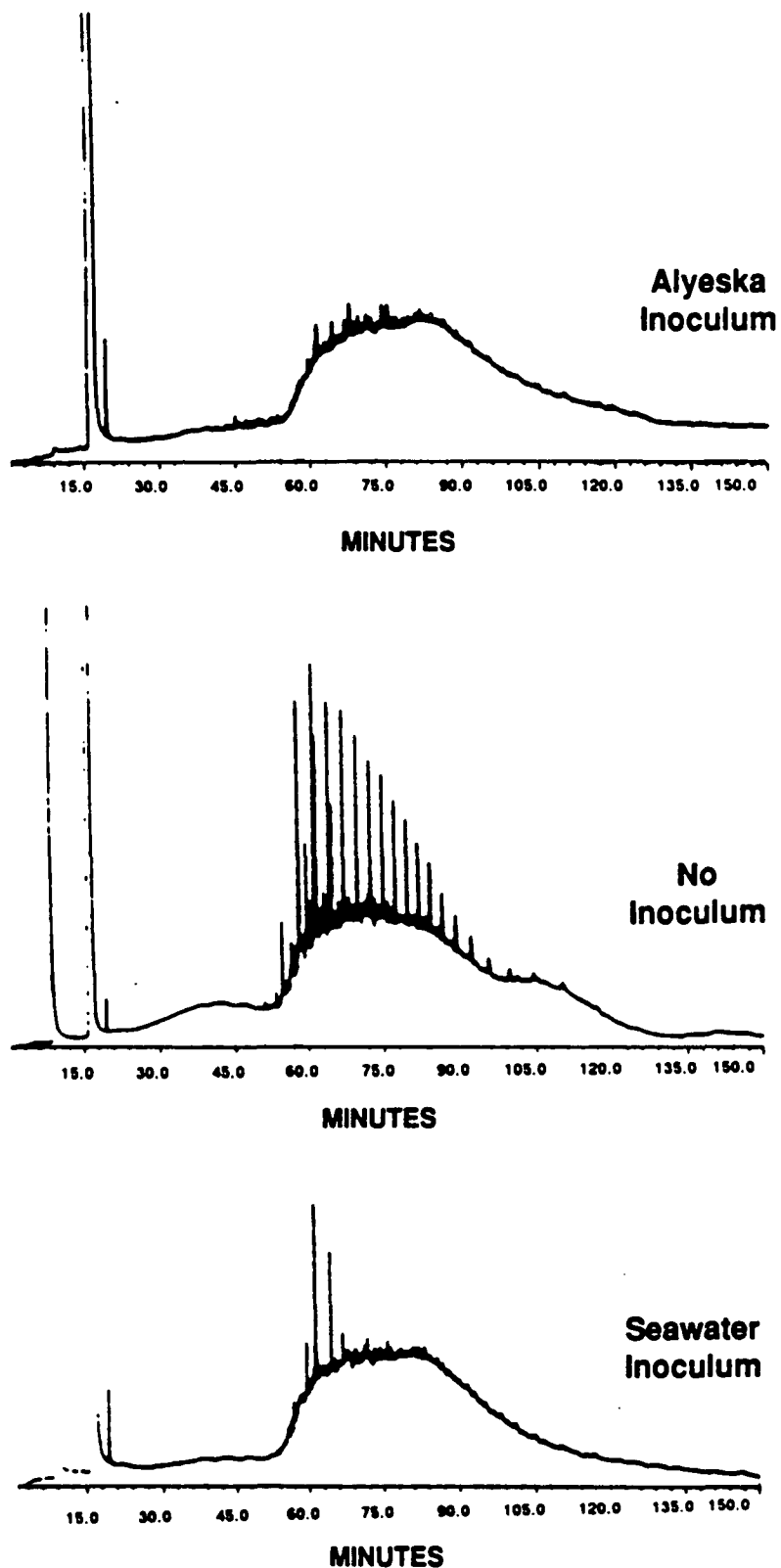
<sup>a</sup> Experiments conducted by Exxon Researchers at Research Laboratories in New Jersey and Texas.

- **Relative effectiveness of Inipol:** Two sets of flasks containing artificial seawater and 1% by weight of artificially weathered Prudhoe Bay crude oil were made; one set was then poisoned with 50 mg/l  $\text{HgCl}_2$ . Inipol, at 10% of the oil concentration, was added to a poisoned and a nonsterile flask. Water-soluble fertilizer (Woodace; N:P:K = 14:3:3) was added to a nonsterile flask at a rate sufficient to produce a mixture of fertilizer and oil that had 0.4% added N and 0.09% added P, Inipol (10%) and fertilizer were added to a second nonsterile flask. All flasks were inoculated with 10% Prince William Sound water, and incubated for 16 days before the oil composition was analyzed.
- **Optimal Inipol concentration:** Flasks for this study contained artificial seawater and 1% of artificially weathered crude oil. Inipol, at concentrations of 3, 10, 20 and 50% of the oil concentration was added to the flasks. Flasks were inoculated with 10% and water from the Alyeska ballast water treatment facility, and incubated for 16 days at 15°C before the oil composition was analyzed.
- **Effect of temperature on Inipol enhancement:** Flasks for this study contained artificial seawater and artificially weathered crude oil. One flask received 10% Inipol and another received fertilizer. Both flasks were incubated at 5°, 15°, and 20°C for 38 days before the oil composition was analyzed.
- **Inipol enhanced oil degradation on rock surfaces:** Oiled beach material from Prince William Sound was placed in flasks and covered with Inipol at concentrations approximating 10% of the oil concentration. Untreated oiled beach material was used as a control. A poisoned control of oiled rocks and Inipol was established using 50 mg/l  $\text{HgCl}_2$ . All beach materials were covered with artificial seawater. Flasks were incubated at 15°C for 16 days before the oil composition was analyzed.

## Results

Indigenous organisms have an ability to degrade weathered crude oil if provided with adequate nutrients. Initial experiments showed that organisms in both the Prince William Sound seawater and in the 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 9.2). There was a substantial decrease in the amount of dichloromethane extractable material, and substantial degradation of both the

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



**Figure 9.2 Gas Chromatographic Profiles Showing the Effect of Different Inocula on Degradation of Artificially Weathered Prudhoe Bay Crude Oil.**

resolvable fractions and the unresolvable fractions on GC analysis. Very little organic carbon remained in the aqueous phase after dichloromethane extraction once the precipitated 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 9.3).

Inipol EAP 22 stimulated the biodegradation of crude oil. Flask experiments revealed that the extent of biodegradation increased with the concentration of Inipol (Figure 9.4).

Water soluble fertilizers and Inipol had at least an additive, and perhaps a synergistic effect on biodegradation (Figure 9.5).

Inipol EAP 22 shows a sharper temperature dependency than water soluble fertilizers, and at 2°C there was very little biodegradation when Inipol EAP 22 was used alone (Figure 9.6).

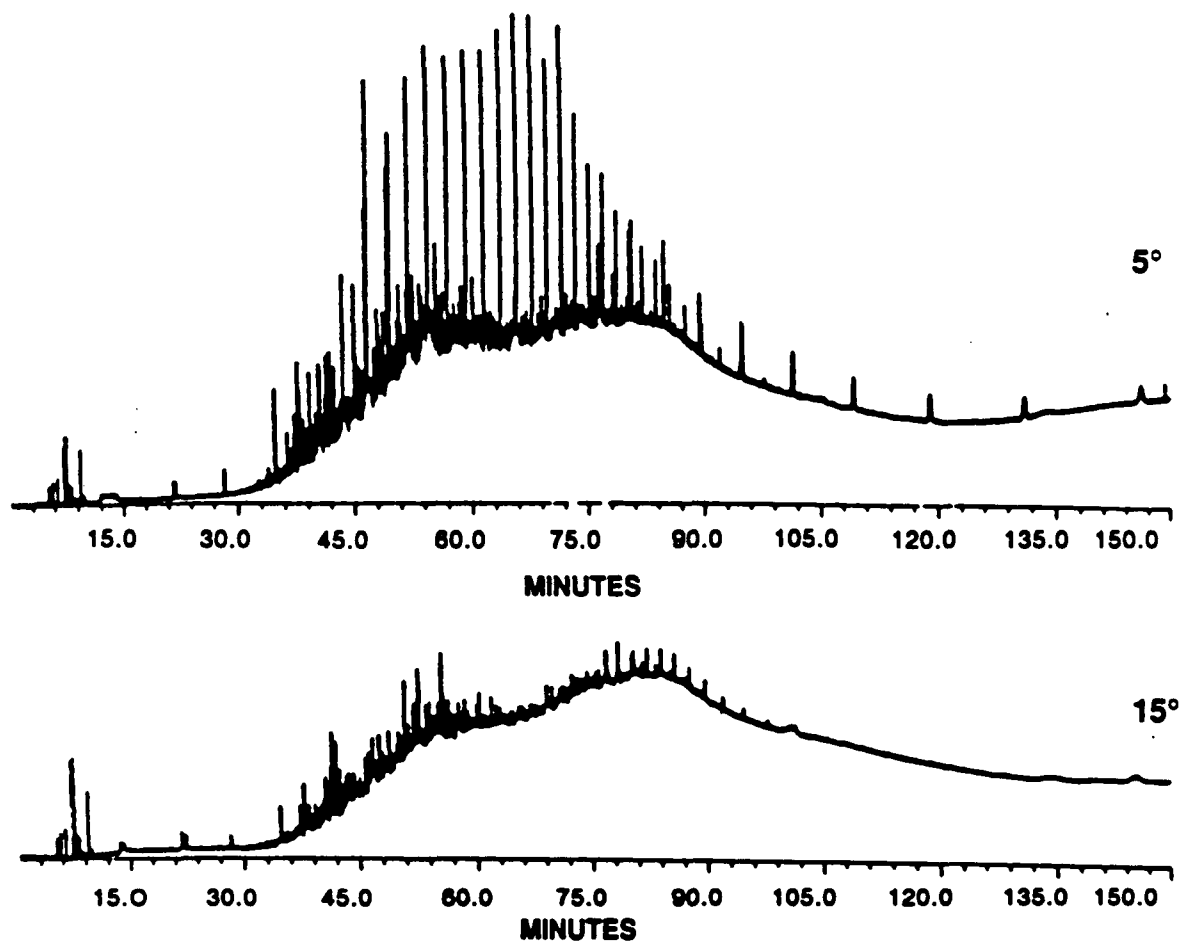
Inipol EAP 22 stimulated the biodegradation of oil on Prince William Sound beach material (Figure 9.7). Rocks treated with Inipol and then incubated at 15°C became significantly cleaner after 14 days; all the resolvable peaks had disappeared in the GC analysis, and 50% of the total dichloromethane-extractable material had disappeared; furthermore, the rocks were 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.

### Discussion and Conclusions

These studies showed that weathered oil can be degraded by organisms indigenous to the Prince William Sound, and that addition of either oleophilic or water-soluble fertilizer accelerated the degradation of weathered oil. Oil degradation from the flasks was also temperature dependent, and was a function of the concentration of added nutrient, as long as an adequate inoculum was present. These data indicate that either oleophilic or water-soluble fertilizers can be used to enhance biodegradation of weathered oil; they also suggest that a further enhancement may be possible by using the two types of fertilizer together.

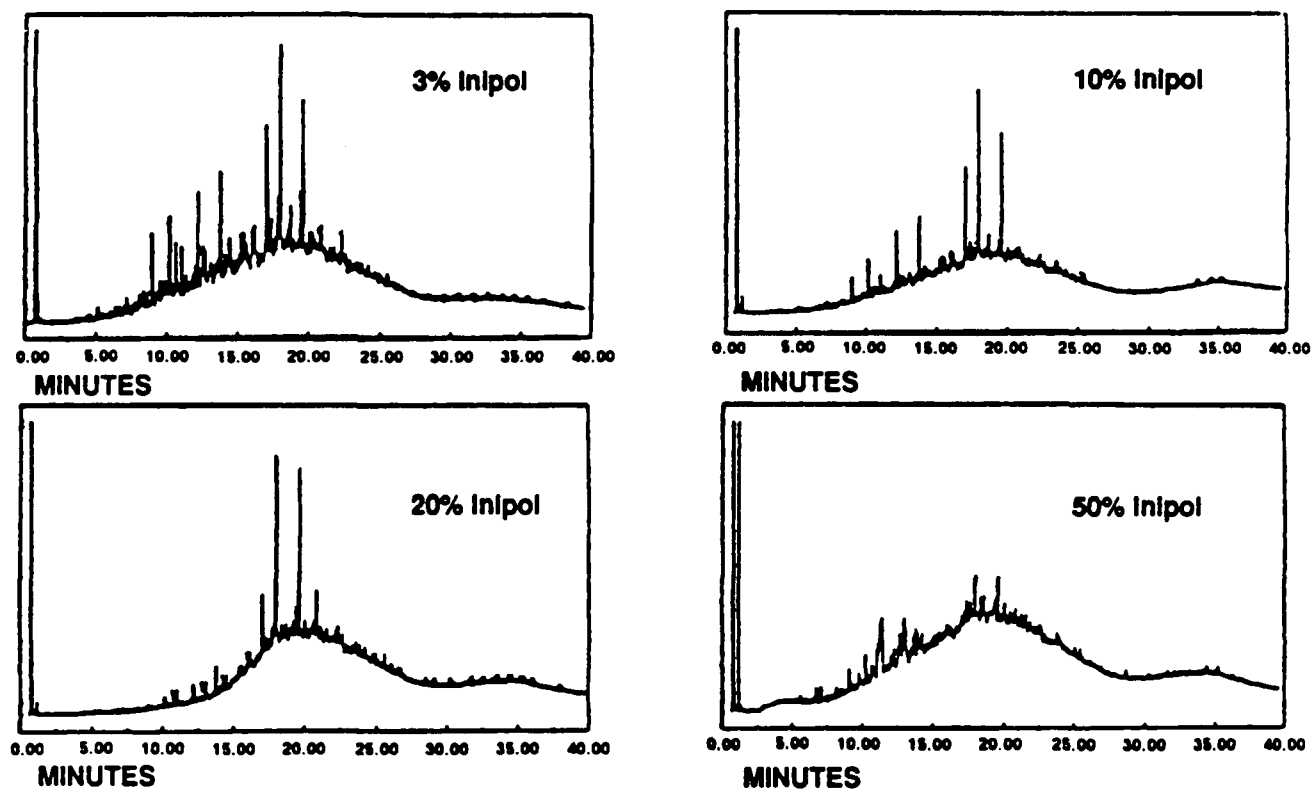


**Bushnell-Hass Broth  
(3.5% N, 4.1% P)  
38 days**

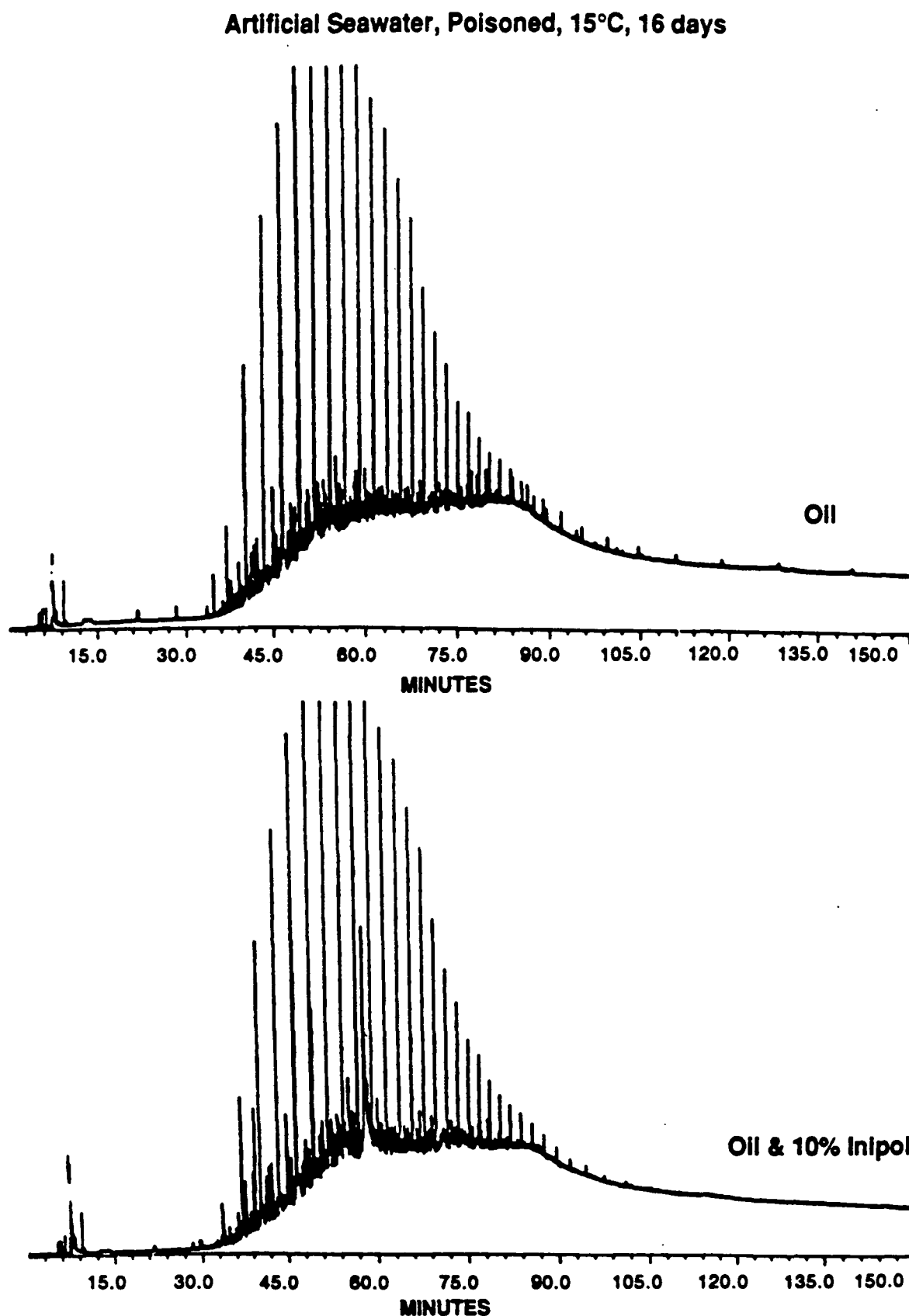


**Figure 9.3. Gas Chromatographic Profiles Showing the Effect of Temperature on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil.**

**Artificial Seawater, 15°C, 16 Days**

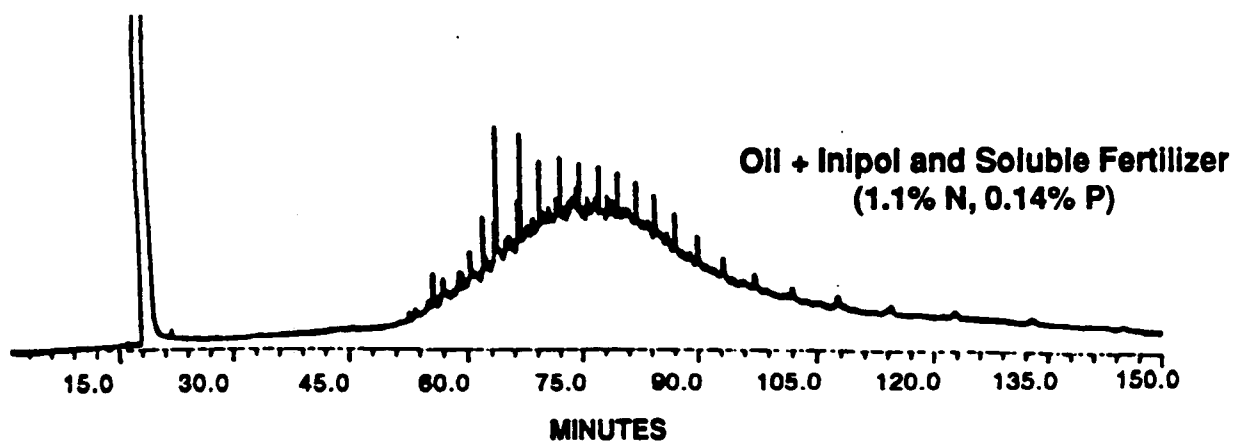
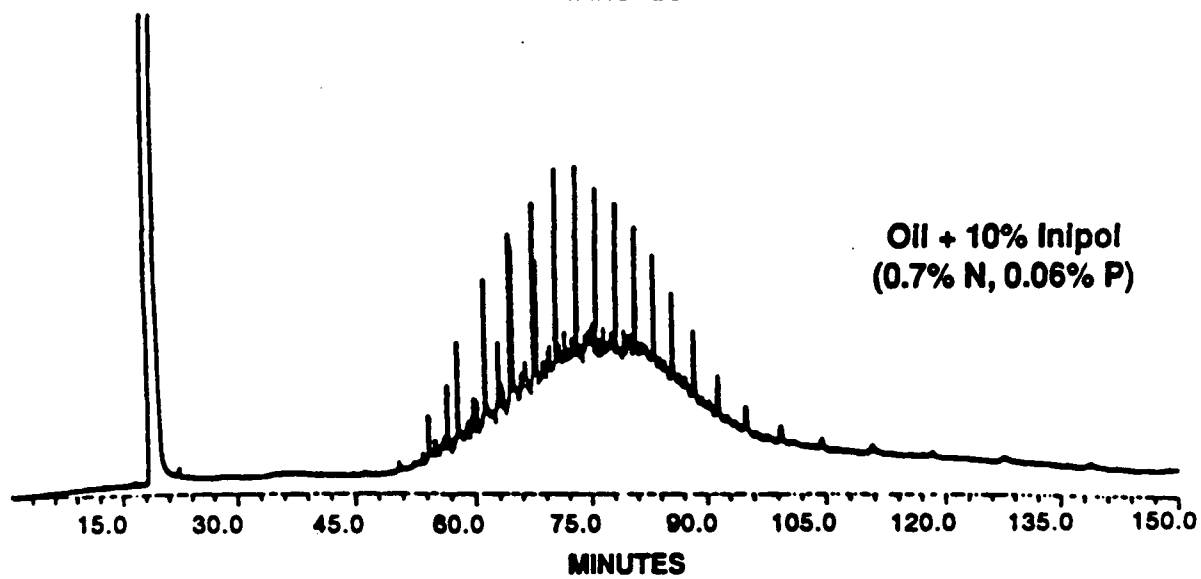
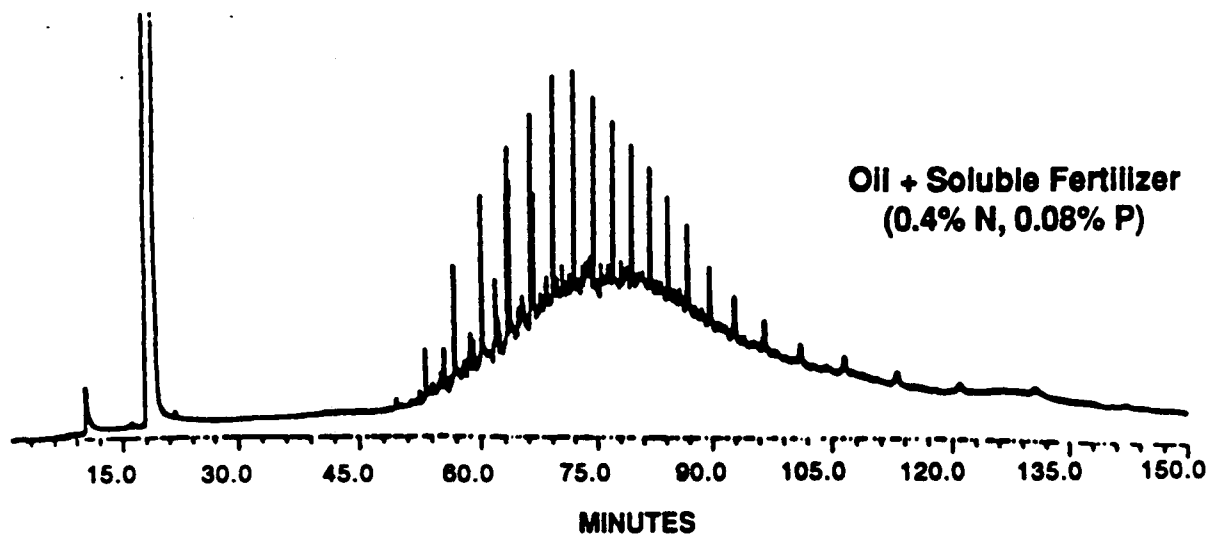


**Figure 9.4. Gas Chromatographic Profiles Showing the Effect of Different Concentrations of Inipol (% of Oil Concentration) on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil.**



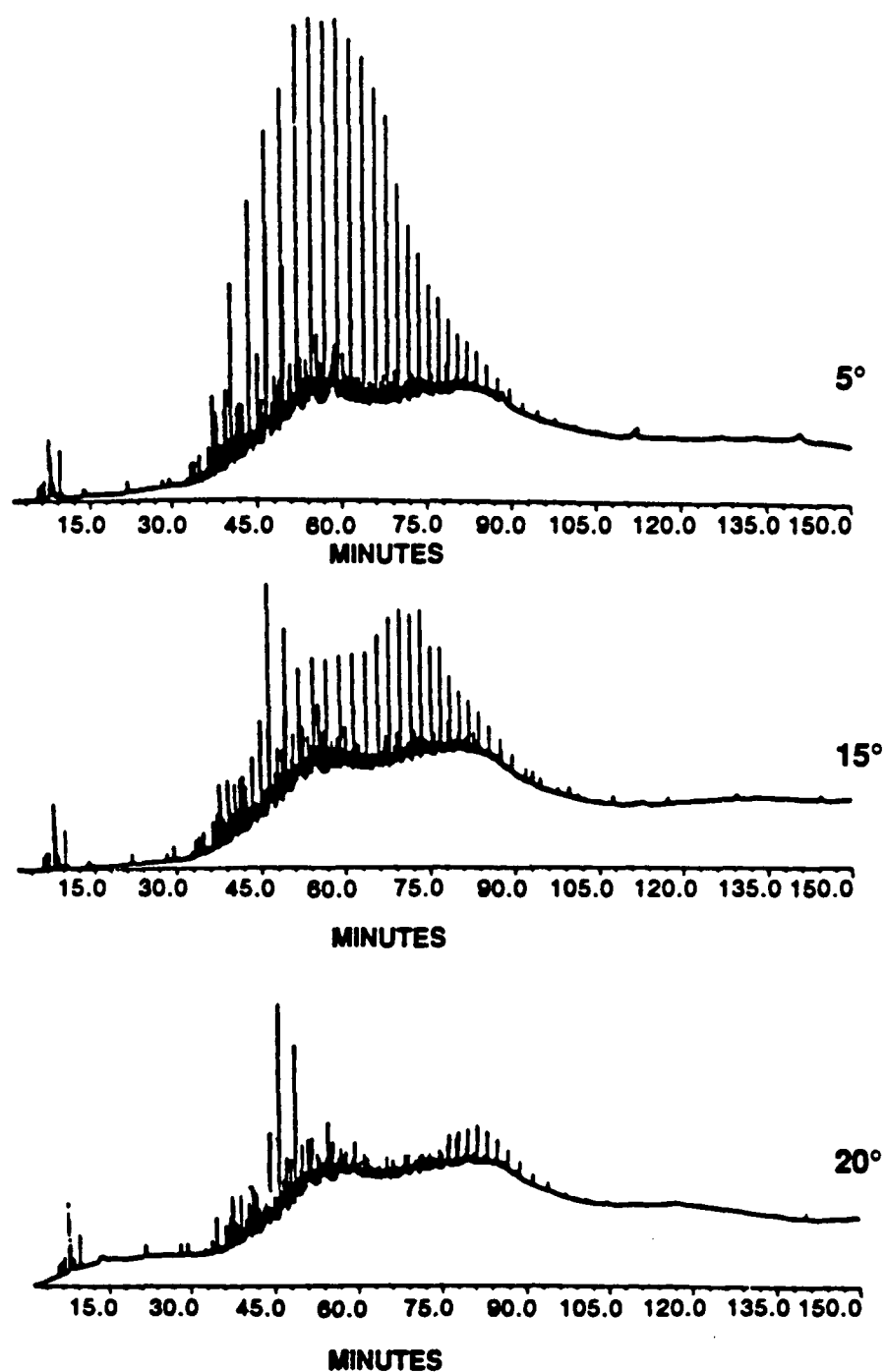
**Figure 9.5. Gas Chromatographic Profile Showing the Effect of Different Fertilizers, Under Poisoned and Unpoisoned Conditions, on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil.**

**Artificial Seawater, Active, 15°C, 16 days**

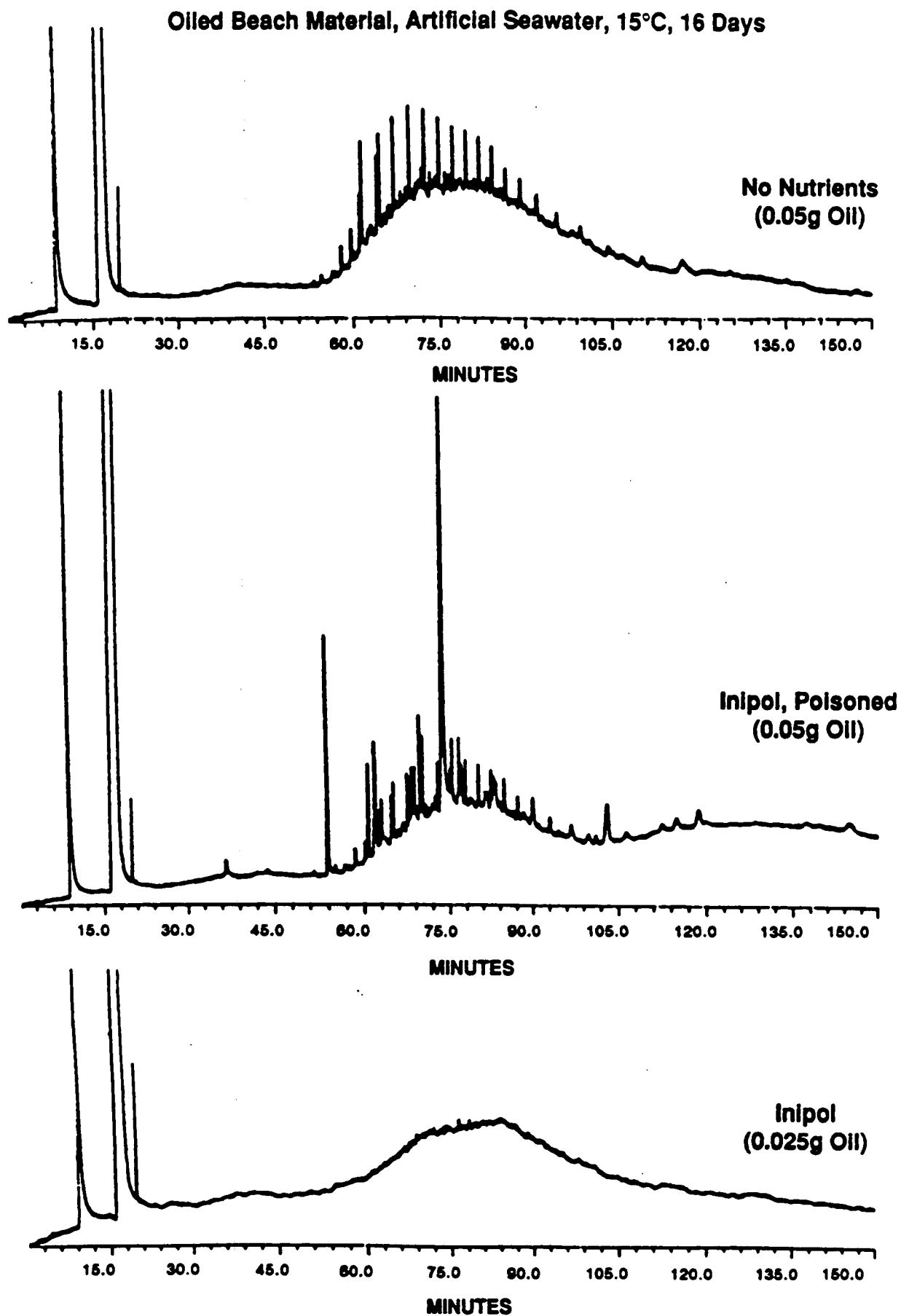


**Figure 9.5. (Cont.)**

**Artificial Seawater, 10% Inipol, 38 days**



**Figure 9.6. Gas Chromatographic Profiles Showing the Effect of Temperature on the Degradation of Artificially Weathered Prudhoe Bay Crude Oil Treated with Inipol.**



**Figure 9.7. 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.**

## RESPIROMETRIC ANALYSIS OF BIODEGRADATION

### Background

To obtain additional information on the effect of Inipol for enhancing, under very controlled conditions, the degradation of different concentrations of artificially weathered oil, laboratory flask studies were conducted. These studies were designed to evaluate the inherent ability of the indigenous Prince William Sound microflora to degrade weathered Prudhoe Bay crude oil. Water from the Alyeska ballast water treatment plant was also evaluated as a source of oil-degrading microorganisms to enhance the natural microbiota for biodegrading the oil. Analytical respirometry was used as the primary tool for studying rates of biodegradation of the oil. To corroborate the oxygen uptake measurements collected in the respirometric reactors, GC/FID chromatography scans of aliphatic and aromatic hydrocarbons were performed at various times on samples taken from batch flasks. This was done to determine which oil constituents were being biodegraded in the closed systems.

### Methods

#### Nutrient Media--

Two nutrient formulations, Inipol and a defined minimal-salts medium (OECD), were compared for their ability to support the growth of hydrocarbon degraders on weathered Prudhoe Bay crude oil.

#### Microbial Inocula--

The microbial inocula consisted of seawater from Snug Harbor, beach material collected from an uncontaminated beach in Valdez, weathered crude oil from the spill, and indigenous biota from the Alyeska ballast water treatment plant.

#### Chemical Analyses--

The oil was fractionated into the aliphatic, aromatic, and polar fractions using standard silica gel column chromatography. Composition of the aliphatic fraction was measured by gas chromatography using flame ionization detection (GC/FID). Composition of the aromatic fraction was characterized by gas chromatography/mass spectrometry (GC/MS). Samples were collected at 0 weeks, 6 weeks, and 26 weeks.

#### Analytical Respirometry--

Respirometry experiments were carried out in a Voith Sapromat B-12 respirometer. This instrument consisted of a temperature controlled water bath containing 12 measuring units, a recorder for direct plotting of the decomposition velocity curves, and a cooling unit for conditioning and continuous recirculation of water bath volume. Each measuring unit comprised a reaction vessel with a CO<sub>2</sub> adsorber, an oxygen

generator, and a pressure indicator. Microbial activity created a vacuum in the reaction vessel, which was recorded by the pressure indicator. Pressure was balanced by electrolytic oxygen generation from the dissociation of copper sulfate and sulfuric acid. The recorder/plotter constructed an oxygen uptake graph automatically.

Design of the respirometry experiments is summarized in (Table 9.6). All vessels contained 2 grams of uncontaminated beach sand from Valdez and 1000 ml of seawater collected offshore at Snug Harbor. The vessels containing beach material, oil, and Inipol were charged by first adding the beach material, pouring a measured amount of oil onto the sand, adding the Inipol to the oiled rocks, and finally filling the vessel with the Snug Harbor seawater. All reaction vessels were mixed with stirring turbines and incubated at 15°C in the dark.

#### Flask Studies--

Flask microcosm experiments were conducted to provide further support for the respirometric studies. Each flask contained 20 gm of uncontaminated beach material and 1000 ml of Snug Harbor seawater. The flasks were charged with the various additives in the same fashion and order as above. The experimental design for these experiments is summarized in (Table 9.7). Flasks were incubated on a shaker at 15°C.

### Results

#### Analytical Respirometry--

Results of the analytical respirometry experiments are summarized in Figure 9.8. The figure displays cumulative oxygen uptake as a function of time in the respirometric vessel containing 1000 mg/l oil and Inipol (5% by weight of oil) and in the respirometric vessel containing only Inipol. Oxygen uptake began in both vessels after only 1.5 days lag period. Maximum uptake of Inipol occurred by the 9th day, then leveled off at approximately 150 mg/l. The oxygen uptake rate on weathered oil with Inipol 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 with no Inipol or oil) was always close to background (data not shown).

The vessel containing oil, Inipol, and the Alyeska ballast water biomass exhibited an oxygen uptake curve that was almost superimposable on the curve for oil plus Inipol (data not shown). Thus, in the closed environment of the respirometric vessel, no



Table 9.6. Experimental Design for Respirometric Studies

Reaction Vessel <sup>b</sup>	Oil Concentration (mg/l) _	Inipol Concentration (mg/l)	Alyeska Ballast Water (ml) _
V1,V1R	1000	50	-
V2,V2R	300	15	-
V3,V3R	100	5	-
V4,V4R	1000	50	10
C5	-	50	-
C6	-	-	-
C7	1000	50	-
C8	-	50	-

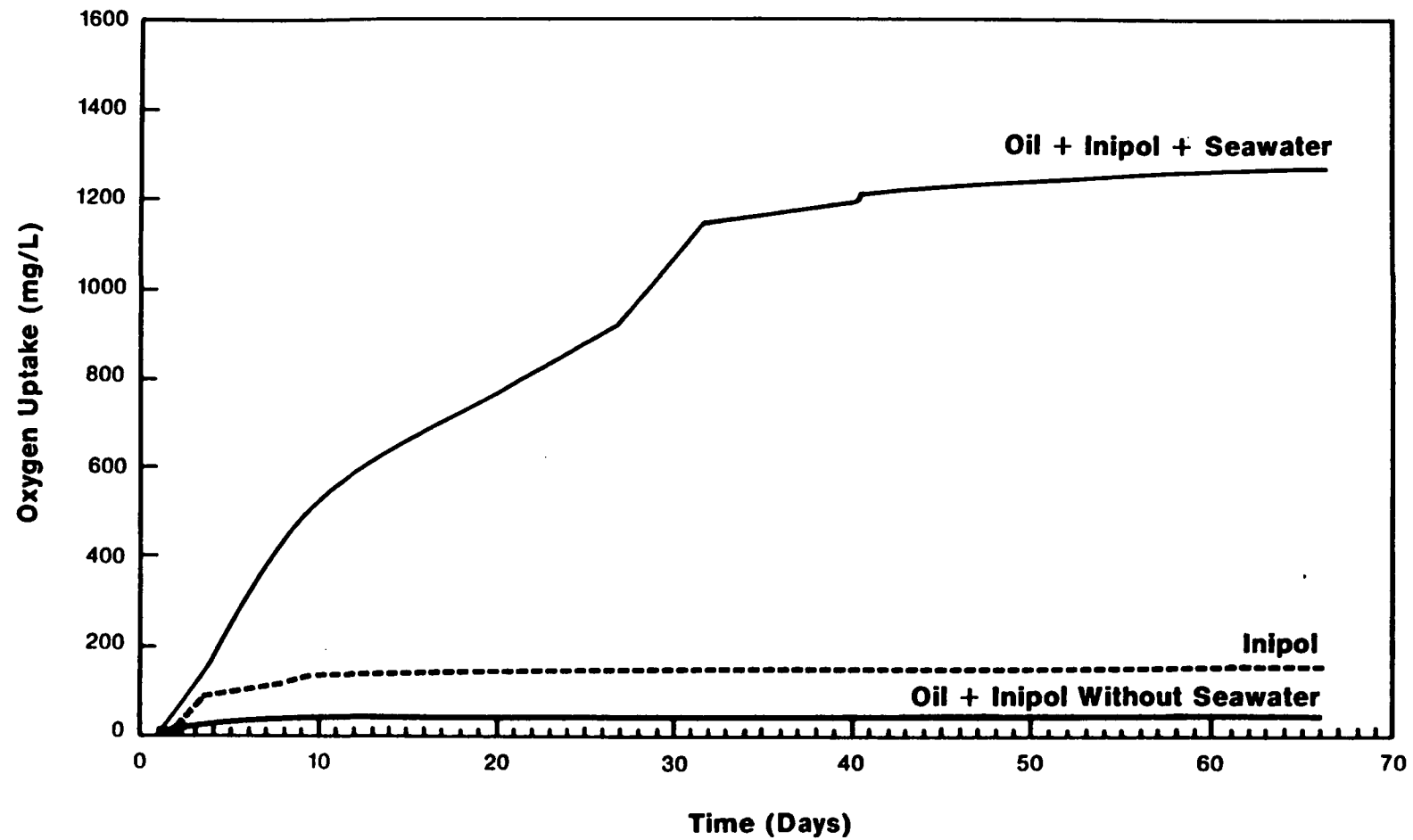
<sup>b</sup> V = Vessel  
R = Replicate  
C = Control

Table 9.7. Experimental Design of Flask Studies.

Flask <sup>c</sup>	Oil Concentration (mg/l)	Inipol Concentration (mg/l)	OECD <sup>d</sup>	Alyeska Ballast (ml)
F1, F1R	10,000	500	-	-
F2, F2R	10,000	500	-	10
F3, F3R	10,000	-	+	-
F4, F4R	10,000	-	+	10
C1	10,000	-	-	-
C2	10,000	-	-	10

<sup>c</sup> F = Flask  
R = Replicate  
C = Control

<sup>d</sup> OECD, a defined minimal-salts medium was composed of the following constituents added to provide the specified final concentration (mg/l) in the test solution:  $\text{KH}_2\text{PO}_4$  (170),  $\text{K}_2\text{HPO}_4$  (435),  $\text{Na}_2\text{HPO}_4$  (668),  $\text{NH}_4\text{Cl}$  (50),  $\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$  (45),  $\text{CaCl}_2$  (55),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (2.5). It included the following trace elements added to provide final concentrations ( $\mu\text{g/l}$ ) in the test solution:  $\text{MnSO}_4$  (60.4),  $\text{H}_3\text{BO}_3$  (114.4),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (85.6),  $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}$  (69.4), and  $\text{FeCl}_3$ , EDTA (200). To prevent trace nutrient limitation, either 1 ml/l of a stock yeast extract solution (15 mg/100 ml), or the following vitamins, biotin (0.4), nicotinic acid (4.0), thiamine (4.0), p-aminobenzoic acid (2.0), pantothenic acid (2.0), pyridoxamine (10.0), cyanocobalamine (4.0), and folic acid (10.0).



**Figure 9.8. Cumulative Oxygen Uptake on Weathered Prudhoe Bay Crude Oil**

enhancement of oil degradation by an external source enriched with oil-degrading organisms was detected.

#### Flask Studies--

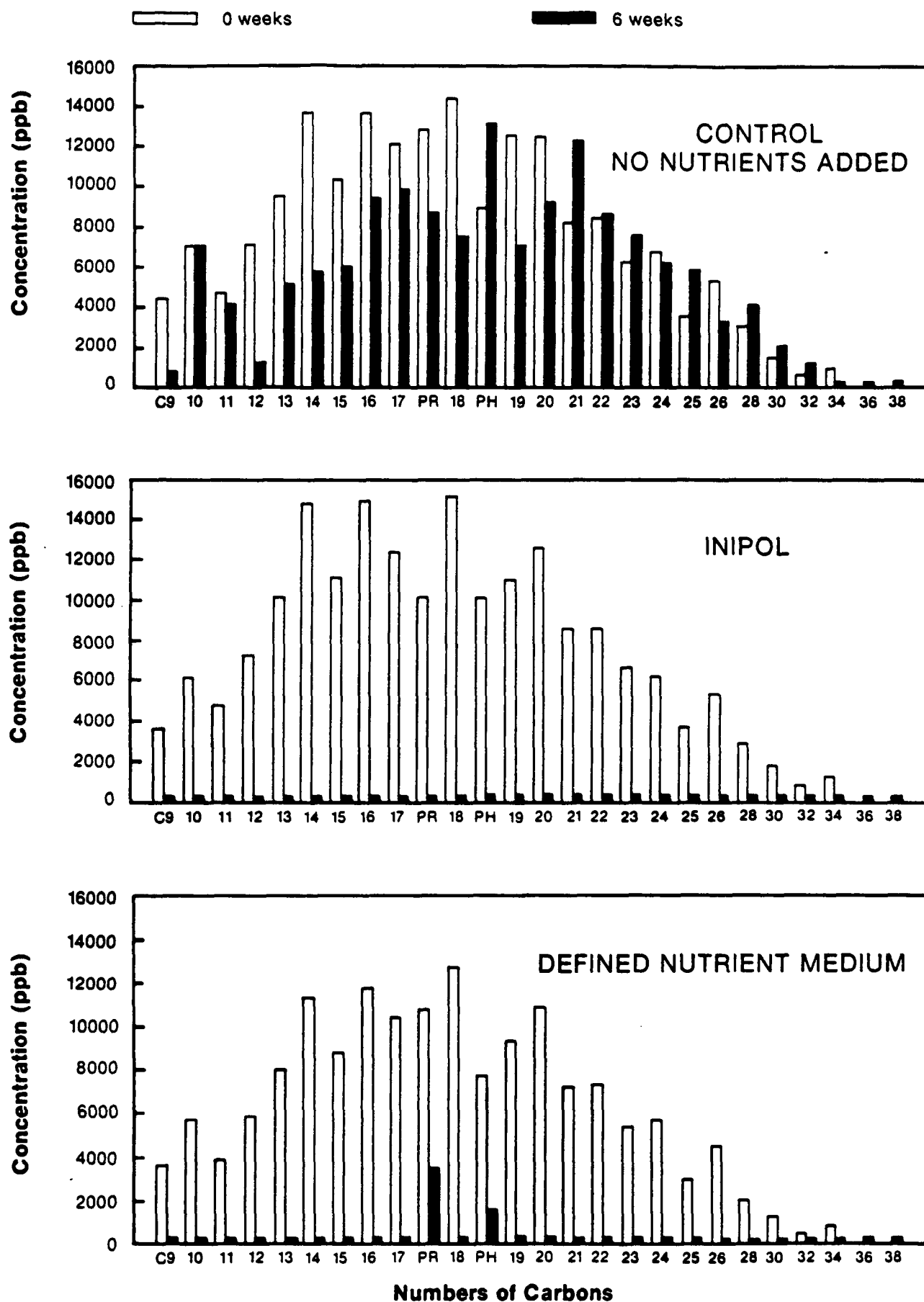
Figure 9.9 summarizes the results of GC/FID scans of the alkane hydrocarbons from three sets of flasks: control (containing 10,000 mg/l weathered crude oil and no nutrients) Inipol-treated (containing (10,000, mg/l) oil and (500 mg/l) Inipol, and defined minimal salts-treated (containing 10,000 mg/l oil and OECD). In the control, some minor changes in the alkane fractions are 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 was the cause, the magnitude of the changes was relatively insignificant.

Flasks containing oil plus Inipol 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 at six weeks, although the levels were significantly reduced from the controls. These results suggest that Inipol may have enriched a different type of microbial population than that enriched by the minimal-salts solution. The Inipol-enriched organisms were able to break down not only straight chain components at a very rapid rate but branched-chain components as well. The organisms enriched by the minimal-salt solution were also able to degrade the branched chain aliphatics, but at a reduced rate or after a longer lag period.

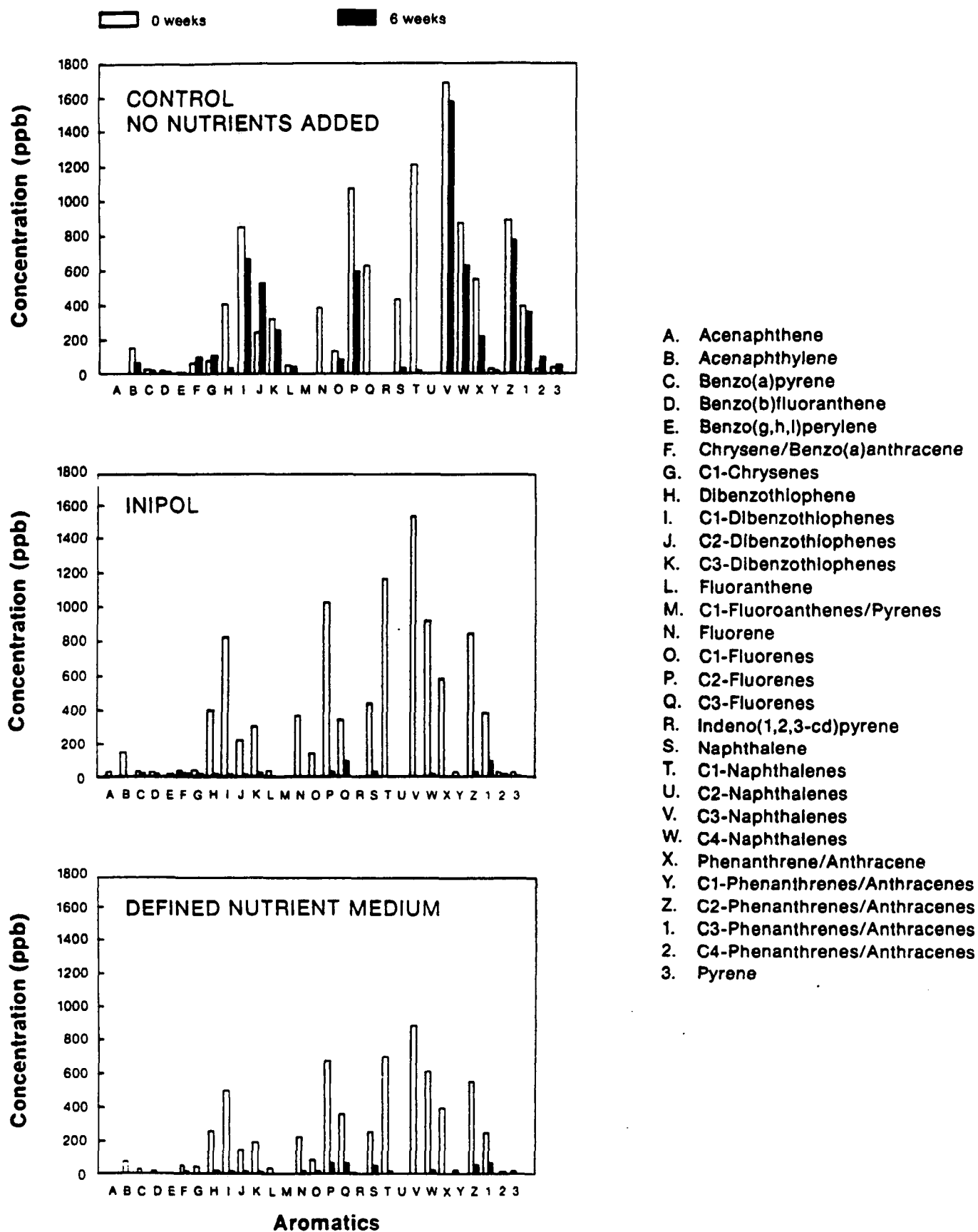
The GC/MS traces of the aromatic fractions are presented in Figure 9.10. In the control, several of the components were reduced to undetectable levels after six weeks (note fractions H, Q, S, and T, corresponding respectively to dibenzothiophene, C3-fluorenes, naphthylene, and C1-naphthylene). The traces from the Inipol and minimal-salt solution flasks exhibited virtually complete removal of all aromatic fractions after six weeks incubation.

#### Discussion and Conclusions

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 (Inipol and a minimal-salt solution). This



**Figure 9.9. Gas Chromatographic Profiles of Alkanes at 0 and 6 Weeks After Initiation of Flask Studies.**



**Figure 9.10. Gas Chromatographic Profiles of Aromatics at 0 and 6 Weeks After Initiation of Flask Studies.**

suggests that perhaps a combination of Inipol and a water-soluble source of nutrients may ultimately be the appropriate manner of stimulating 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.

## **MECHANISM OF ACTION OF INIPOL-ENHANCED OIL DEGRADATION**

### **Background**

A laboratory study was conducted to investigate the mechanism by which the Inipol fertilizer enhanced oil degradation. Numbers of oil-degrading microorganisms and oleic acid-degrading microorganisms were specifically examined along with changes in oil composition. The study was performed in a manner which would, to some extent, simulate environmental conditions; i.e., no shaking and daily water change to simulate tidal flushing. Results are currently available for oil-degrading and oleic acid-degrading microbial populations.

### **Methods**

The experimental design is shown in Table 9.8. Studies were conducted in chemically clean (I-Chem) jars, each containing approximately 200 g of oiled rocks and either seawater, defined nutrient medium, or sodium chloride solution (20%). The defined nutrient medium (DNM) used in these tests contained (per liter of distilled water): NaCl (24g)  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (1.0 g) KCL (0.7 g)  $\text{KH}_2\text{PO}_4$  (2.0 g).  $\text{Na}_2\text{HPO}_4$  (3.0 g), and  $\text{NH}_4\text{NO}_3$  (1.0 g). The pH of the medium was adjusted to 7.4 with 1.0 N NaOH following autoclaving. For sterile systems, the oil-contaminated rocks were autoclaved in I-Chem jars. This removed the water from the oil, but did not remove the oil from the rocks. Inipol application consisted of dripping 3 ml of Inipol (sterile) over the rock surface and allowing the treated rocks to incubate for 3 hours before filling the jars with the appropriate aqueous phase (about 100 ml). Except for the jar containing unautoclaved seawater, sterile medium (seawater, defined nutrient medium, or NaCl solution) was used in each microcosm. Subsamples of 1.0 ml for bacterial enumeration were collected from all jars at 24-hour intervals. Oleic acid-degrading bacteria were enumerated on oleic acid-containing agar plates supplemented with nitrogen and phosphorous. Oil-degrading bacteria were enumerated by the dilution to extinction technique described in Section 7. After collecting bacterial enumeration samples, the aqueous phase from one set of jars was decanted into a sterile I-Chem jar and

Table 9.8. Experimental Design for Laboratory Microcosm Study

Flask

Seawater

Artificial SeawaterSterile

NaCl

Inipol added  
Seawater

Artificial SeawaterNonsterile

NaCl

Seawater

Artificial SeawaterSterile

NaCl

No Inipol added  
Seawater

Artificial SeawaterNonsterile

NaCl



replaced with fresh sterile medium (fresh seawater was added to the nonsterile seawater jar). The decanted solution was frozen for analysis of residual oil components.

### Results

The results from these studies indicated that the addition of Inipol led to a substantial increase in the number of organisms capable of growth on oleic acid-agar plates (Figure 9.11). High background concentrations of oleic acid-degrading bacteria were observed in the water even before Inipol treatment.

Since the aqueous phase at each water change was sterilized, the number of oleic acid degraders possibly reflected those that sloughed off the oiled rocks during a 24 hour period. However, no obvious differences were observed for the different aqueous phases. Similar results were observed in systems that did not have daily water changes.

Results from the enumeration of oil-degrading organisms (Figure 9.12.) indicated that in all cases the populations increased to a high value by day 3 and then decreased to an intermediate but variable level for the following 6 days. Similar results were seen in those jars that did not have a daily water change. Although all the samples showed a peak after 3 days of incubation, jars containing only seawater appeared to have the fewest microorganisms in the 6 days following the 3-day peak. Chemical analysis of the water samples is being preformed. Information on how effectively the enriched oleic acid degraders can degrade the oil also is forthcoming.

### Discussion and Conclusions

Inipol increased the number of oleic acid-degrading bacteria in flask studies designed to approximate field conditions. This situation would theoretically result in competition for available nutrients between oleic acid-degrading and oil-degrading bacteria. This competition could explain the decrease in oil-degrading bacteria following their initial rise after initiation of the experiment. Tests of oleic acid-degrading bacteria are currently being conducted to determine the percentage which are also hydrocarbon degraders. Supplying dissolved nutrients in addition to those nutrients in Inipol did not seem to affect the oleic acid- and oil-degrading bacterial populations.

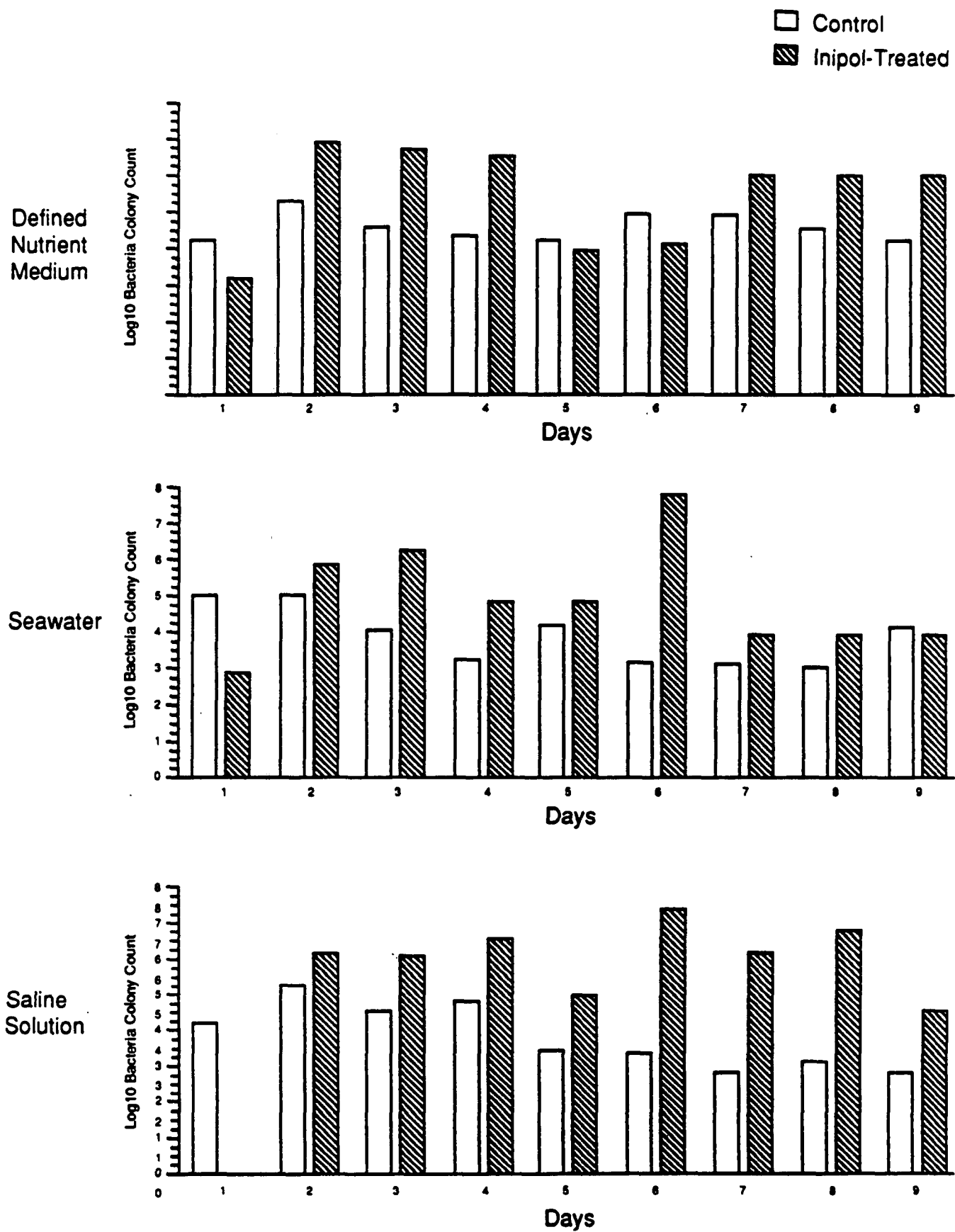


Figure 9.11. Effect of Inipol on the relative numbers of oleic acid-degrading bacteria in jars containing oiled rocks and seawater, defined nutrient medium, or saline solution. Incubated with daily change of water.

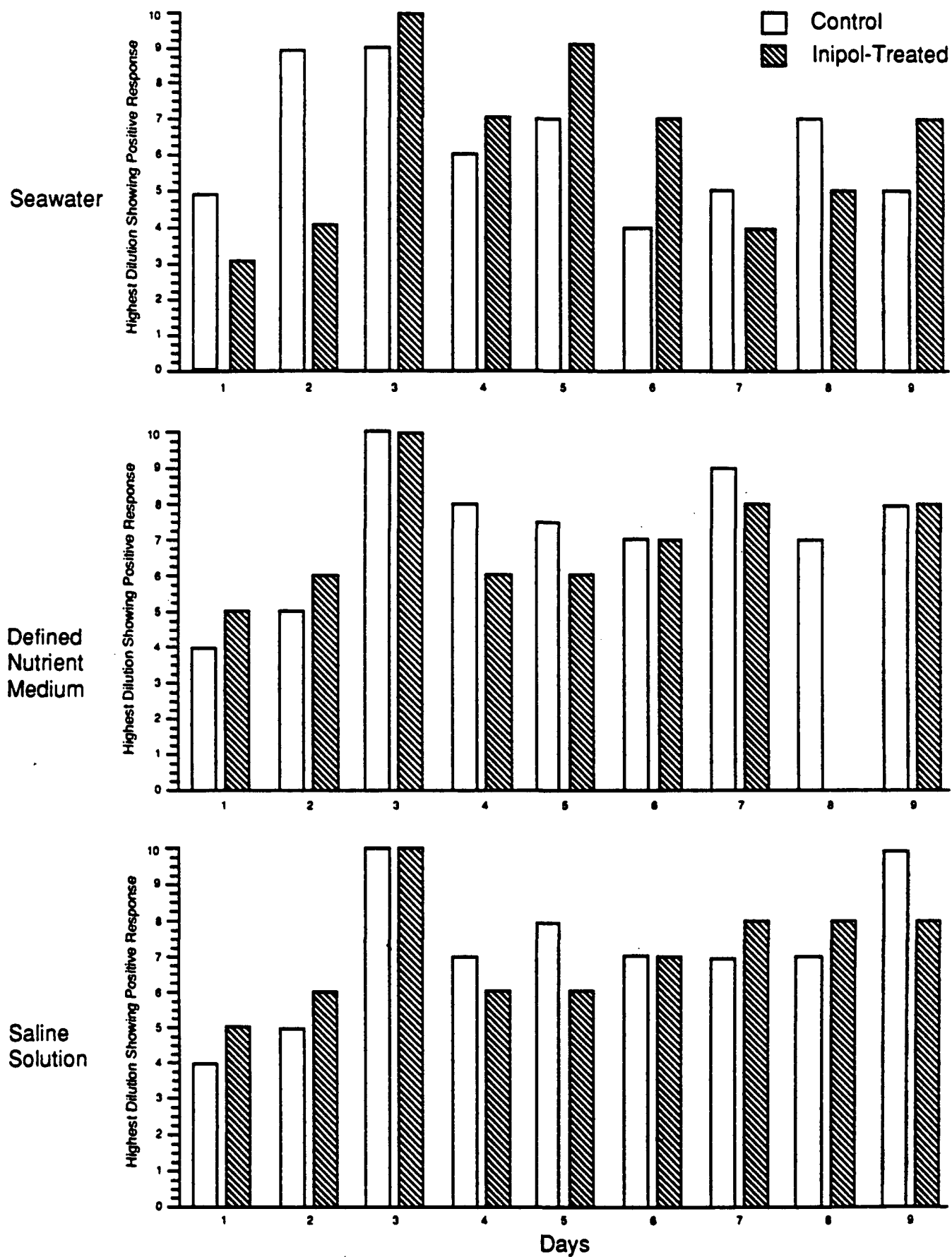


Figure 9.12. Effect of Inipol on the relative numbers of oil-degrading microorganisms in jars containing oiled rocks and seawater, defined nutrient medium, or saline solution. Incubated with daily change of water.

## CHEMICAL EFFECT OF OLEOPHILIC FERTILIZER\*

### Background

The mechanism by which oil is removed from substrates is important for interpreting the results of biodegradation studies. Since several substances in the Inipol fertilizer formulation are known to act as surfactants or to otherwise change the consistency of oil on rock surfaces, the question arose as to whether or not Inipol acted to alter the physical characteristics of the oil such that removal of oil from rock surfaces could occur in the absence of biodegradation. This study was designed to evaluate the rock-washing characteristics of Inipol under conditions that precluded biological activity.

### Methods

The test system was designed to address the efficacy of various chemicals as potential "rock-washers". Each chemical was applied to fully oiled gravel, and the gravel was then refrigerated at 5°C for 1 hour. Artificial sea water at 5°C was then added to cover the gravel, and the gravel refrigerated at 5°C for 6 hours. The gravel was then drained, and the amount of oil in the water was estimated. A typical test used four washing cycles.

### Results

Inipol EAP 22 removed only 0.6% of the oil in a first test at a normal application rate of 5% by weight of oil, and 0.84% of the oil in a test where the Inipol was added to ensure complete surface coverage. Both tests indicated an insignificant amount of oil was removed (more than 30% of the oil was removed by some preparations sold specifically for this purpose). Approximately 45% of the Inipol, by weight, remained with the oiled rock in the regime used in these, which were designed to remove as much of the rock-washing material as possible. Similarly, about 50% of the available nitrogen, (in the Inipol) was released in the first 2 wash cycles, with the remainder being released more slowly.

### Discussion and Conclusion

Inipol EAP 22 does not wash oil off rocks at typical Prince William Sound water temperatures. Based on the results of this study, it is reasonable to expect that oil removal associated with Inipol EAP 22 applications is the result of something other than a physical process. In addition, associated tests by Exxon

---

\* These tests were conducted by Exxon Researchers at laboratories in Houston and New Jersey.

demonstrated that Inipol enhanced biodegradation was not accompanied by a detectable lowering of interfacial tension between oil and brine. This suggests that the microorganisms did not produce significant amounts of surfactants under the conditions tested.

## **TOXICITY OF OLEOPHILIC FERTILIZER**

### **Background**

Little information is available in the literature regarding the toxic effects of Inipol on sensitive marine biota. Studies were designed in response to requests for information regarding the possible toxic effects of oleophilic fertilizer on indigenous biota of the Prince William Sound. However, several marine species commonly used in toxicity testing are known to be more sensitive than species indigenous to the Prince William Sound. Therefore, the toxicity testing conducted included both species that are commonly used in toxicity testing and species from the Prince William Sound.

### **Methods**

Toxicity of Inipol EAP 22 and weathered oil were tested in 3 ways:

- 1) To account for worst-case conditions, the Inipol EAP 22 was tested in a mixture with seawater.
- 2) Because Inipol EAP 22 is very likely to become bound to oil after application to an oil-contaminated shoreline, and because data generated by the manufacturer show that toxicity of the fertilizer is appreciably decreased in the presence of oil, a second treatment involved spraying fertilizer on a layer of oil on seawater.
- 3) Finally, the oil was tested alone to provide data for comparisons.

Toxicity tests were conducted by Battelle and E.V.S. Consultants under contract to the US EPA for the development of definitive, acute LC50 values for fishes, invertebrates, and algae (Table 9.9). Organisms tested by Battelle included silver salmon smolts, herring fry, and mussel larvae. The oleophilic fertilizer was tested both alone and in seawater plus weathered Prudhoe Bay crude oil. E.V.S. Consultants conducted similar tests with an alga, oyster larvae, mysids, grass shrimp, and sticklebacks, and a sperm cell fertilization test with sand dollars. Final test results are not yet available at this time for all test species.

Table 9.9. Results of Laboratory Toxicity Tests with Oleophilic Fertilizer, Inipol EAP 22, and Various Marine Species. (Values are 96-hour LC50 estimates unless otherwise noted.)

<u>Organism</u>	<u>Inipol</u>	<u>Inipol Plus Oil</u>
<b>Fish</b>		
Salmon smolts	2,500 ppm <sup>a</sup>	6,700 ppm
Herring	200 ppm	800 ppm <sup>a</sup>
Sticklebacks	100 ppm <sup>a</sup>	range-finder underway
<b>Invertebrates</b>		
Mussel larvae	35 ppm (48hr)	70 ppm (48hr)
Oyster larvae	>10ppm <100ppm (48hr)	range-finder underway
Mysids	range-finder <100ppm	range-finder underway
Pandalid shrimp	400 ppm <sup>a</sup>	range-finder underway
<b>Algae</b>		
Skeletonema	range-finder underway	range-finder underway

---

<sup>a</sup> Best estimate from non definitive test.

## Results

General trends show that larvae of mussels, oysters, and juvenile mysids are two orders of magnitude more sensitive than salmon and approximately one order of magnitude more sensitive than herring and sticklebacks. When mixed with oil, the toxicity of Inipol is reduced two- to four-fold.

## Discussion and Conclusions

These data were provided to the Shoreline Committee and to advisory groups in Valdez, Seward, and Homer to assist in the evaluation of potential toxic effects associated with large-scale application of Inipol as a clean-up technique. In addition, a risk assessment procedure was suggested as a means to establish a benchmark that identifies the concentration where no acute effects are observed. Such a benchmark would be useful for comparison with possible environmental concentrations following shoreline treatment. This method was modified by the Shoreline Committee in Valdez and Homer and used to assist in approval of shoreline segments for fertilizer application.

Toxicity of Inipol to marine biota was thought to be possible as a result of unintentional over-spraying of marine waters during application, or release of Inipol from the shoreline into the bay immediately after application. Analysis of a worst-case example considered the effects of elevated levels of Inipol in protected embayments with minimal tidal exchange and maximum shoreline-to-water volume ratios (long, narrow bays with constricted openings). The standard application rate for Inipol applied to oiled shorelines was  $293 \text{ g/m}^2$  ( $0.06 \text{ lb/sq ft}$ ) in the bioremediation program. Assuming this rate was applied to 100 m of shoreline on 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 Inipol was washed in a pulse into completely mixed nearshore water that was 100 m long, 10 m wide, and had an average depth of 1 m ( $1000 \text{ m}^3$ ), the "worst-case" expected environmental concentration would be 293 ppm. This value is considerably less than the 96 hr LC50 value for salmon and is comparable to the LC50 for herring. Toxicity to marine invertebrates residing in the area next to shore is possible at these concentrations, should unrealistic application conditions exist. Any marine invertebrate exposure that resulted from shoreline applications would be mitigated by tidal mixing, dilution, and transport out of the system into the Prince William Sound. Initial concentrations should decrease by orders of magnitude within one to two days, to levels considerably less than acutely toxic concentration measured in laboratory tests. Thus, it is predicted that the prospect of sustained lethal concentrations for any biota is very unlikely.

There are no proven analytical methods to quantify Inipol in seawater, so environmental concentrations of Inipol could not be measured to compare with worst-case predictions. However, daily input into nearshore waters was estimated to be in the range of 1% to 10% of the applied material, based on 1) visual observations of a colored film present after spraying, 2) sustained nutrient enriched pore water observed in the intertidal zone following nutrient additions, and 3) the lack of measured nutrient increases in the nearshore zone. If this estimated input (10%) was 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 would be between 3 and 30 ppm. These values indicate that peak environmental concentrations would be less than the laboratory LC50 values of Inipol and oil mixtures for invertebrates. Peak values would develop immediately after application, and would be subjected to subsequent tidal mixing, dilution, and transport. When considered in this light, the potential for toxic effects of Inipol applied to oiled beaches at recommended rates appears to be minimal.

Inipol 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. Enhanced microbial biomass and available nutrients associated with mixtures of Inipol and oil should result in their rapid degradation. In their mixed form, oil and Inipol have less toxicity than does Inipol by itself for marine biota, as demonstrated by the reduced toxicity of Inipol in laboratory tests where it was mixed with oil.

## **BEACH HYDRAULICS**

### **Background**

Numerous hydrological factors could influence the redistribution of oil on contaminated beaches or affect the release and distribution of nutrients applied as fertilizers. Prior to the initiation of these studies, no knowledge of the flow of water in the highly porous beaches of the Prince William Sound was available. Beach hydrological studies were conducted to identify the primary factors that influence the distribution of fresh and salt water and the dynamics of aqueous flow in these beaches.

### **Methods**

Hydrological evaluation of Kittiwake Beach at Passage Cove, Knight Island was implemented through installation of sample wells, instrument packages, a tide gauge and a weather station. Concurrently, Kittiwake Beach was used to test the efficacy of nutrient application via a sprayer using water-soluble



fertilizer. The orientation of wells installed on the beach and a diagram of the instrument packages installed in the wells are shown in Figures 9.13 and 9.14, respectively.

Nutrient samples were collected every two weeks between August 6 and September 12, 1989. Samples were collected using peristaltic pumps to withdraw water from each of three small tubes placed alongside the major well casing. At each sample location these sample tubes extended to specific depths: two feet below the beach surface, one foot above the bottom of the well, and the bottom of the well, respectively. Clean 250 ml polyethylene bottles were filled with water and frozen as soon as possible after collection. For each sampling period, samples were collected every three hours over two tidal cycles. It was not feasible to collect samples over 24 hour cycles due to weather conditions and the hours of darkness. Salinity and temperature data were collected in the field concurrently with nutrient sample collection. Samples were analyzed for ammonium, nitrite, nitrate, and phosphorous.

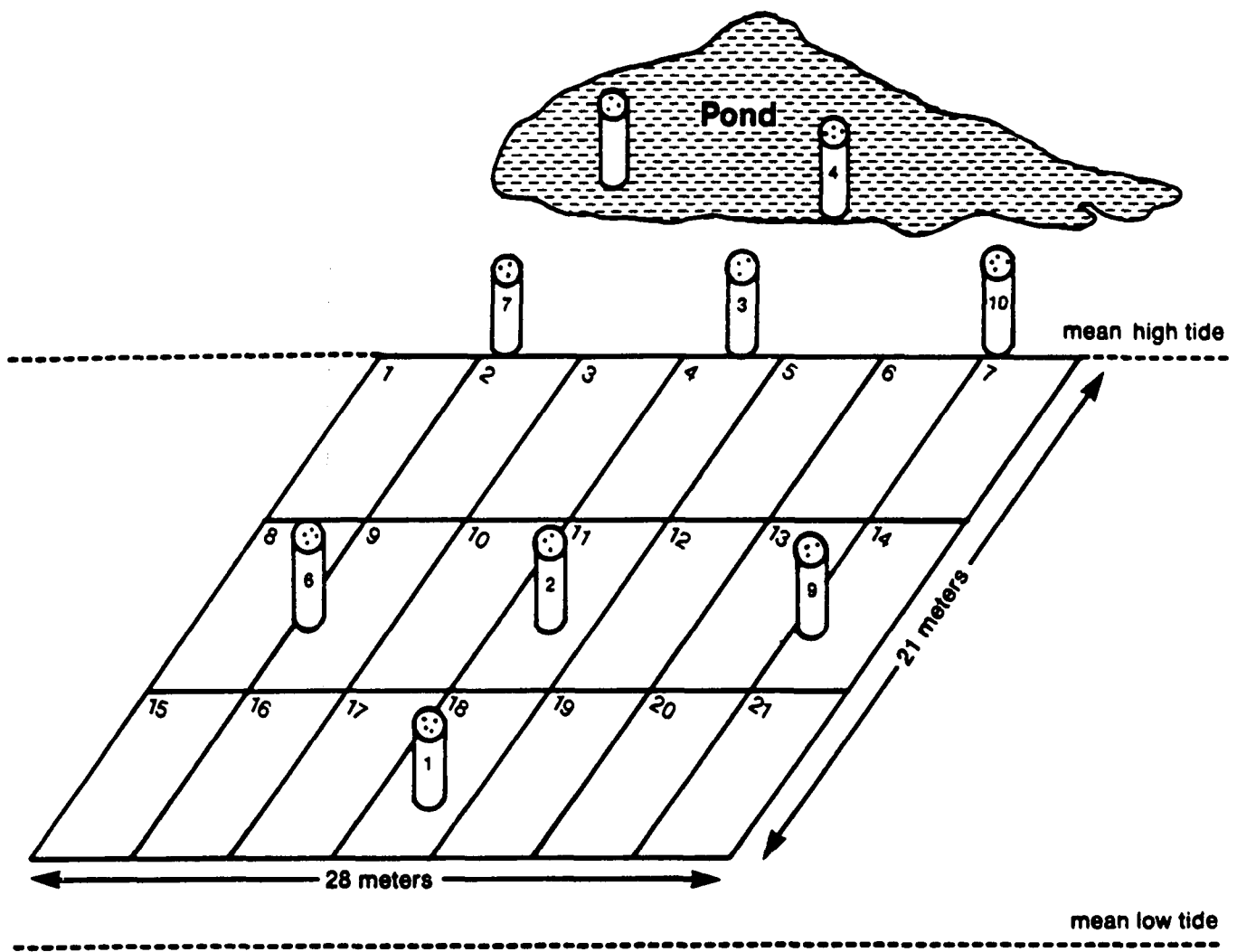
Due to the vertical changes in sea level over a tidal cycle, often a complete series of nutrient samples could not be obtained. Survey samples were collected in groups. Groups 1 through 7 were collected September 10 and 11, 1989. Sample sets were collected at about three hour intervals, unless otherwise indicated.

## Results

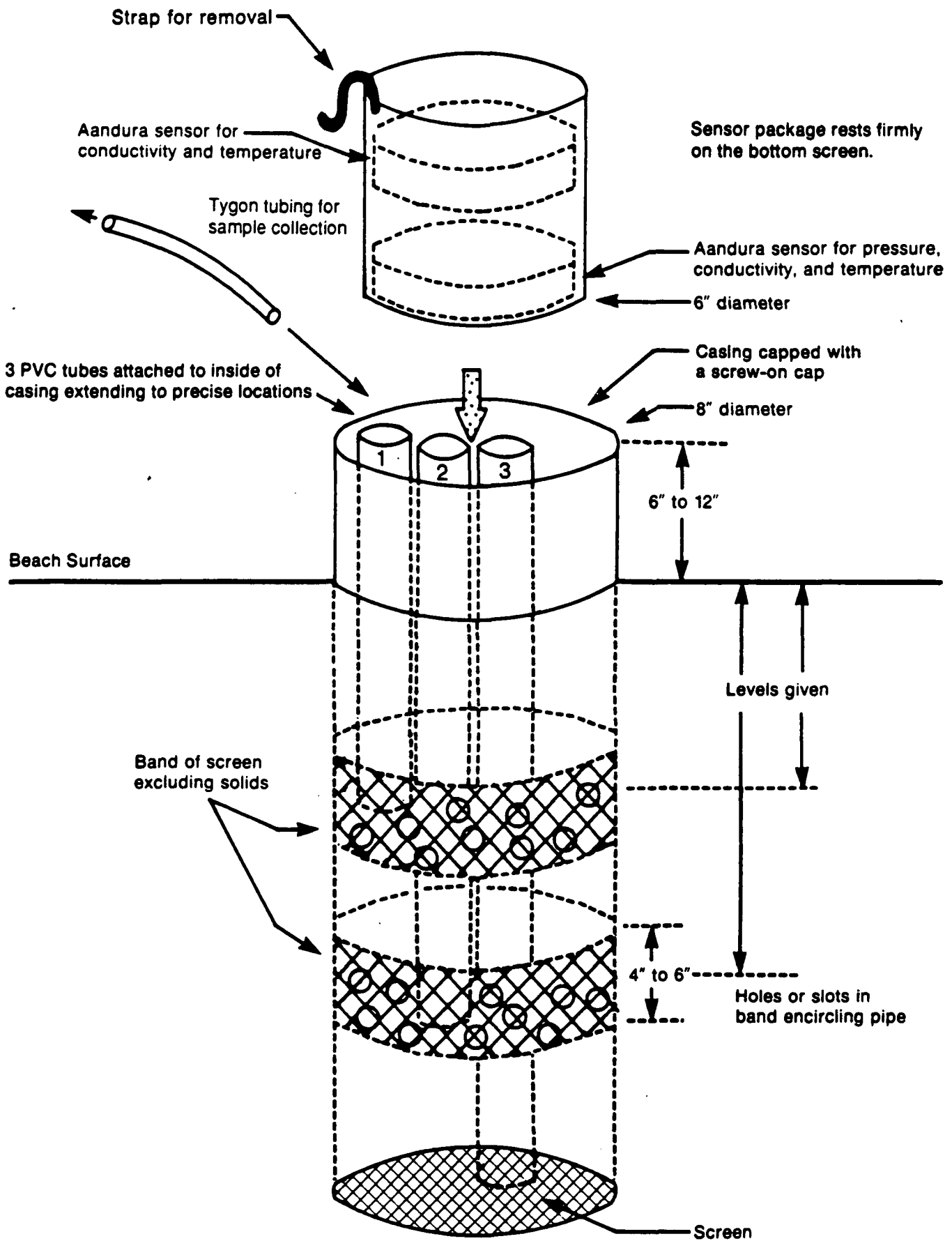
Tables 9.10 through 9.34 show the salinity, temperature, ammonium, nitrate, and phosphorous data from sample groups 1 through 25 respectively. The data indicate rapid changes in salinity and nutrient content in each series of samples over tidal cycles. The presence of nitrate in many samples indicates that anaerobiosis was not particularly evident during the sample period.

Samples taken August 6 and 7 (Tables 9.10 through 9.16) were collected two days after fertilization had begun. The ammonium data indicated that fertilizer had penetrated from the surface of the beach to the bottom of the wells. The ammonium concentration in interstitial water reached a maximum of 179  $\mu\text{M}$  in the center of the plot. Salinities were also at a minimum in the same samples series. The salinity data indicated that the subsurface water flow was very complex and not easily described. No nitrate data were available for this series at the time of writing.

Samples taken August 20 and 21 (Tables 9.17 through 9.26) were collected after a two day hiatus in fertilizer application. The data showed that ammonium and nitrate persisted in the body of the beach even after cessation of fertilizer application. The ammonium data usually showed less than 100  $\mu\text{M}$  concentration.



**Figure 9.13. Location of Wells for Beach Hydraulics Experiment at Passage Cove**



**Figure 9.14. Casing Configuration**

Table 9.10. Passage Cove Beach Hydraulics: August 6, 1989;  
4:30 a.m.; High Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	400
Temperature ( $^{\circ}\text{C}$ )	13
$\text{NH}_4$ ( $\mu\text{M}$ )	1.1
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	0.2

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	15	15	-	11	12	-	16	16
Temperature ( $^{\circ}\text{C}$ )	-	14	14	-	14	13	-	13	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	108	110	-	45	41	-	54	97
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	0.9	1.7	-	0.6	0.9	-	0.5	0.9

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	0	0	0	11	11	13	0	0	0
Temperature ( $^{\circ}\text{C}$ )	14	14	14	15	15	14	14	14	15
$\text{NH}_4$ ( $\mu\text{M}$ )	111	114	90	81	71	179	70	64	53
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	3.1	3.7	4.0	2.2	1.3	6.1	0.9	0.7	2.3

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	-	-
Temperature ( $^{\circ}\text{C}$ )	-	-	-
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )
Temperature ( $^{\circ}\text{C}$ )
$\text{NH}_4$ ( $\mu\text{M}$ )
$\text{NO}_3$ ( $\mu\text{M}$ )
$\text{PO}_4$ ( $\mu\text{M}$ )

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.11. Passage Cove Beach Hydraulics: August 6, 1989;  
7:30 a.m.; Falling Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	340
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	0.8
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	0.4

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	-	9	-	-	10	-	-	-
Temperature ( $^{\circ}\text{C}$ )	-	-	15	-	-	15	-	-	-
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	43	-	-	29	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	1.1	-	-	0.3	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	17	16	16	-	10	17	17	17	16
Temperature ( $^{\circ}\text{C}$ )	15	15	15	-	15	15	14	14	15
$\text{NH}_4$ ( $\mu\text{M}$ )	157	130	151	-	57	33	89	93	109
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	4.3	3.7	5.1	-	2.3	1.8	3.7	2.5	4.1

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	16	16	14
Temperature ( $^{\circ}\text{C}$ )	15	15	15
$\text{NH}_4$ ( $\mu\text{M}$ )	33	47	44
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	0.9	0.8	1.1

Offshore

Salinity ( $\mu\text{Mho}$ )
Temperature ( $^{\circ}\text{C}$ )
$\text{NH}_4$ ( $\mu\text{M}$ )
$\text{NO}_3$ ( $\mu\text{M}$ )
$\text{PO}_4$ ( $\mu\text{M}$ )

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.12. Passage Cove Beach Hydraulics: August 6, 1989;  
10:00 a.m.; Low Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	0
Temperature ( $^{\circ}\text{C}$ )	16
$\text{NH}_4$ ( $\mu\text{M}$ )	1.7
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	0.2

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	15	14
Temperature ( $^{\circ}\text{C}$ )	-	16	16
$\text{NH}_4$ ( $\mu\text{M}$ )	-	46	46
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	0.9	0.6

Offshore

Salinity ( $\mu\text{Mho}$ )	13
Temperature ( $^{\circ}\text{C}$ )	17
$\text{NH}_4$ ( $\mu\text{M}$ )	1.2
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	0.3

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.13. Passage Cove Beach Hydraulics: August 6, 1989;  
1:00 p.m.; Rising Tide

Pond

Station Number	4
Salinity ( $\mu$ Mho)	350
Temperature ( $^{\circ}$ C)	16
NH <sub>4</sub> ( $\mu$ M)	35
NO <sub>3</sub> ( $\mu$ M)	-
PO <sub>4</sub> ( $\mu$ M)	0.3

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	-	16	15	-	12	12	-	15	15
Temperature ( $^{\circ}$ C)	-	15	15	-	16	16	-	16	16
NH <sub>4</sub> ( $\mu$ M)	-	48	52	-	22	27	-	7.6	7.1
NO <sub>3</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
PO <sub>4</sub> ( $\mu$ M)	-	1.2	1.6	-	0.7	0.9	-	0.9	1.0

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	19	15	15	18	14	16	12	12	12
Temperature ( $^{\circ}$ C)	16	16	18	17	17	17	18	17	18
NH <sub>4</sub> ( $\mu$ M)	83	73	21	5.3	34	38	52	34	38
NO <sub>3</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
PO <sub>4</sub> ( $\mu$ M)	8.5	2.9	4.0	1.8	1.3	1.3	2.2	1.2	1.6

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu$ Mho)	-	15	15
Temperature ( $^{\circ}$ C)	-	16	17
NH <sub>4</sub> ( $\mu$ M)	-	36	32
NO <sub>3</sub> ( $\mu$ M)	-	-	-
PO <sub>4</sub> ( $\mu$ M)	-	1.1	0.9

Offshore

Salinity ( $\mu$ Mho)
Temperature ( $^{\circ}$ C)
NH <sub>4</sub> ( $\mu$ M)
NO <sub>3</sub> ( $\mu$ M)
PO <sub>4</sub> ( $\mu$ M)

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.14. Passage Cove Beach Hydraulics: August 6, 1989;  
5:10-7:30 p.m.; High-Falling Tide

Pond

Station Number	<u>4</u>
Salinity ( $\mu\text{Mho}$ )	200
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	13
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	3.5

High Tide Wells

Station Number	<u>7</u>			<u>3</u>			<u>10</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	17	17	-	12	13	-	17	17
Temperature ( $^{\circ}\text{C}$ )	-	15	15	-	16	15	-	15	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-	40	38	-	19	21	-	17	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	1.4	1.7	-	0.7	0.8	-	1.0	1.0

Mid Tide Wells

Station Number	<u>6</u>			<u>2</u>			<u>9</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	16	17	17	17	16	16	16	16	17
Temperature ( $^{\circ}\text{C}$ )	15	15	15	14	14	15	14	14	14
$\text{NH}_4$ ( $\mu\text{M}$ )	45	40	51	35	32	39	31	29	30
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	4.1	3.9	3.0	2.6	2.1	3.4	2.1	1.9	2.0

Low Tide Wells

Station Number	<u>1</u>		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Time	5:10	7:30
Salinity ( $\mu\text{Mho}$ )	20	-
Temperature ( $^{\circ}\text{C}$ )	15	14
$\text{NH}_4$ ( $\mu\text{M}$ )	0.06	13
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	0.6	3.5

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well



Table 9.15. Passage Cove Beach Hydraulics: August 6, 1989;  
9:00 p.m.; Low Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	0
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	1.2
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	18	17	-	17	18	-	18	18
Temperature ( $^{\circ}\text{C}$ )	-	15	15	-	15	14	-	14	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	64	66	-	35	32	-	43	43
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	18	17	17
Temperature ( $^{\circ}\text{C}$ )	-	-	-
$\text{NH}_4$ ( $\mu\text{M}$ )	15	23	23
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )
Temperature ( $^{\circ}\text{C}$ )
$\text{NH}_4$ ( $\mu\text{M}$ )
$\text{NO}_3$ ( $\mu\text{M}$ )
$\text{PO}_4$ ( $\mu\text{M}$ )

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.16. Passage Cove Beach Hydraulics: August 7, 1989;  
6:00 a.m.; High Tide

Pond

Station Number	<u>4</u>
Salinity ( $\mu\text{Mho}$ )	
Temperature ( $^{\circ}\text{C}$ )	
$\text{NH}_4$ ( $\mu\text{M}$ )	
$\text{NO}_3$ ( $\mu\text{M}$ )	
$\text{PO}_4$ ( $\mu\text{M}$ )	

High Tide Wells

Station Number	<u>7</u>			<u>3</u>			<u>10</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	15	15	-	12	13	-	15	16
Temperature ( $^{\circ}\text{C}$ )	-	13	13	-	13	13	-	13	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	28	51	-	13	10	-	3.8	6.9
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	<u>6</u>			<u>2</u>			<u>9</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Low Tide Wells

Station Number	<u>1</u>		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Salinity ( $\mu\text{Mho}$ )	-
Temperature ( $^{\circ}\text{C}$ )	-
$\text{NH}_4$ ( $\mu\text{M}$ )	8.5
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.17. Passage Cove Beach Hydraulics: August 20, 1989;  
7:20 a.m.; Falling Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	320
Temperature ( $^{\circ}\text{C}$ )	13
$\text{NH}_4$ ( $\mu\text{M}$ )	<0.13
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	-	11	-	-	6	-	-	2
Temperature ( $^{\circ}\text{C}$ )	-	-	14	-	-	14	-	-	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	24	-	-	19	-	-	0.4
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	214	-	-	210	-	-	16
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	12	12	12	-	10	12	9	8	9
Temperature ( $^{\circ}\text{C}$ )	14	14	14	-	14	14	14	14	14
$\text{NH}_4$ ( $\mu\text{M}$ )	4	43	46	-	38	33	24	24	25
$\text{NO}_3$ ( $\mu\text{M}$ )	216	219	224	-	200	230	115	105	73
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	13	13	13
Temperature ( $^{\circ}\text{C}$ )	14	14	14
$\text{NH}_4$ ( $\mu\text{M}$ )	104	42	40
$\text{NO}_3$ ( $\mu\text{M}$ )	299	260	221
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	17
Temperature ( $^{\circ}\text{C}$ )	13
$\text{NH}_4$ ( $\mu\text{M}$ )	1.4
$\text{NO}_3$ ( $\mu\text{M}$ )	10.8
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.18. Passage Cove Beach Hydraulics: August 20, 1989;  
10:00 a.m.; Low Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	200
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	<0.1
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )				-	-	11			
Temperature ( $^{\circ}\text{C}$ )				-	-	15			
$\text{NH}_4$ ( $\mu\text{M}$ )				-	-	243			
$\text{NO}_3$ ( $\mu\text{M}$ )				-	-	227			
$\text{PO}_4$ ( $\mu\text{M}$ )				-	-	-			

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	-	12
Temperature ( $^{\circ}\text{C}$ )	-	-	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	64
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	282
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	15
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	<0.1
$\text{NO}_3$ ( $\mu\text{M}$ )	0.4
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.19. Passage Cove Beach Hydraulics: August 20, 1989;  
12:50 p.m.; Rising Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	500
Temperature ( $^{\circ}\text{C}$ )	18
$\text{NH}_4$ ( $\mu\text{M}$ )	<0.1
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	11	13	-	-	8	-	7	9
Temperature ( $^{\circ}\text{C}$ )	-	16	17	-	-	16	-	16	18
$\text{NH}_4$ ( $\mu\text{M}$ )	-	40	50	-	-	37	-	396	48
$\text{NO}_3$ ( $\mu\text{M}$ )	-	212	291	-	-	226	-	159	145
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	16	12	12
Temperature ( $^{\circ}\text{C}$ )	15	16	17
$\text{NH}_4$ ( $\mu\text{M}$ )	24	41	43
$\text{NO}_3$ ( $\mu\text{M}$ )	82	240	256
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	21
Temperature ( $^{\circ}\text{C}$ )	18
$\text{NH}_4$ ( $\mu\text{M}$ )	<0.1
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.20. Passage Cove Beach Hydraulics: August 20, 1989;  
4:15 p.m.; High Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	210
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	<0.1
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	21	19	20	12	8	9	19	19	19
Temperature ( $^{\circ}\text{C}$ )	17	18	20	15	15	15	16	16	17
$\text{NH}_4$ ( $\mu\text{M}$ )	-	2	3	25	28	19	<0.1	<0.1	<0.1
$\text{NO}_3$ ( $\mu\text{M}$ )	8	9	7	74	166	54	25	13	9
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Salinity ( $\mu\text{Mho}$ )	21
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	4.8
$\text{NO}_3$ ( $\mu\text{M}$ )	4.9
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.21. Passage Cove Beach Hydraulics: August 20, 1989;  
9:15 p.m.; Falling Tide

Pond

Station Number	<u>4</u>
Salinity ( $\mu\text{Mho}$ )	5
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	<u>7</u>			<u>3</u>			<u>10</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	6	8	-	5	5	-	-	4
Temperature ( $^{\circ}\text{C}$ )	-	14	14	-	14	14	-	14	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	119	156	-	253	286	-	27	32
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	<u>6</u>			<u>2</u>			<u>9</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	16	16	16	13	14	18	13	14	14
Temperature ( $^{\circ}\text{C}$ )	15	15	15	15	14	14	14	14	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	176	211	216	172	169	151	144	161	161
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	<u>1</u>		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Salinity ( $\mu\text{Mho}$ )	21
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	20
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.22. Passage Cove Beach Hydraulics: August 21, 1989;  
6:30 a.m.; Falling Tide

Pond

Station Number	4
Salinity ( $\mu$ Mho)	140
Temperature ( $^{\circ}$ C)	13
NH <sub>4</sub> ( $\mu$ M)	-
NO <sub>3</sub> ( $\mu$ M)	0.05
PO <sub>4</sub> ( $\mu$ M)	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	-	8	10	-	5	5	-	5	8
Temperature ( $^{\circ}$ C)	-	13	13	-	13	13	-	13	13
NH <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
NO <sub>3</sub> ( $\mu$ M)	-	65	84	-	160	112	-	21	5
PO <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	17	17	18	14	15	17	15	15	15
Temperature ( $^{\circ}$ C)	14	14	14	13	13	14	14	14	14
NH <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
NO <sub>3</sub> ( $\mu$ M)	176	189	172	115	97	131	119	115	70
PO <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu$ Mho)			
Temperature ( $^{\circ}$ C)			
NH <sub>4</sub> ( $\mu$ M)			
NO <sub>3</sub> ( $\mu$ M)			
PO <sub>4</sub> ( $\mu$ M)			

Offshore

Salinity ( $\mu$ Mho)	21
Temperature ( $^{\circ}$ C)	13
NH <sub>4</sub> ( $\mu$ M)	-
NO <sub>3</sub> ( $\mu$ M)	12
PO <sub>4</sub> ( $\mu$ M)	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well



Table 9.23. Passage Cove Beach Hydraulics: August 21, 1989;  
10:00 a.m.; Low Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	300
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )				-	-	15			
Temperature ( $^{\circ}\text{C}$ )				-	-	13			
$\text{NH}_4$ ( $\mu\text{M}$ )				-	-	-			
$\text{NO}_3$ ( $\mu\text{M}$ )				-	-	172			
$\text{PO}_4$ ( $\mu\text{M}$ )				-	-	-			

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	16	15
Temperature ( $^{\circ}\text{C}$ )	-	15	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	237	245
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	22
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	1.6
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.24. Passage Cove Beach Hydraulics: August 21, 1989;  
12:00 noon; Rising Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	280
Temperature ( $^{\circ}\text{C}$ )	16
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.06
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	15	14	-	-	12	-	9	11
Temperature ( $^{\circ}\text{C}$ )	-	17	17	-	-	16	-	15	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	220	99	-	-	-	-	144	148
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	15	15	15
Temperature ( $^{\circ}\text{C}$ )	16	16	16
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	140	92	226
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	20
Temperature ( $^{\circ}\text{C}$ )	17
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	<0.06

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.25. Passage Cove Beach Hydraulics: August 21, 1989;  
3:45 p.m.; Rising Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	500
Temperature ( $^{\circ}\text{C}$ )	17
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	0.2
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	19	19	21	19	11	11	19	19	20
Temperature ( $^{\circ}\text{C}$ )	18	18	17	16	17	17	17	17	17
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	0.7	3.2	3.1	48	151	173	1.7	0.4	5.2
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Salinity ( $\mu\text{Mho}$ )	21
Temperature ( $^{\circ}\text{C}$ )	17
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	0.5
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.26. Passage Cove Beach Hydraulics: August 21, 1989;  
5:00 p.m.; Falling Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	0
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	<0.04
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	13	17	-	14	14	-	13	16
Temperature ( $^{\circ}\text{C}$ )	-	15	16	-	15	15	-	14	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	167	-	-	176	77	-	40	26
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	-	-
Temperature ( $^{\circ}\text{C}$ )	-	-	-
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	75
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	21
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	6.9
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.27. Passage Cove Beach Hydraulics: September 10, 1989;  
12:20 noon; Falling Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	350
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	-	3	-1000	1	-	-	-	800
Temperature ( $^{\circ}\text{C}$ )	-	-	14	-	14	14	-	-	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	7	14	14	4	7	12	7	9	15
Temperature ( $^{\circ}\text{C}$ )	14	14	13	13	14	13	14	13	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Salinity ( $\mu\text{Mho}$ )	17
Temperature ( $^{\circ}\text{C}$ )	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.28. Passage Cove Beach Hydraulics: September 10, 1989;  
3:10 p.m.; Low Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	500
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	5	6	9	-	6	13	3	3	4
Temperature ( $^{\circ}\text{C}$ )	14	14	14	-	15	14	15	15	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	7	5	4
Temperature ( $^{\circ}\text{C}$ )	14	14	18
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	15
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.29. Passage Cove Beach Hydraulics: September 10, 1989;  
6:15 p.m.; Rising Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	400
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
	T	M	B	T	M	B	T	M	B
Sample Position*	-	-	2	-	-	3	-	-	1
Salinity ( $\mu\text{Mho}$ )	-	-	2	-	-	3	-	-	1
Temperature ( $^{\circ}\text{C}$ )	-	-	13	-	-	14	-	-	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
	T	M	B	T	M	B	T	M	B
Sample Position*	9	7	9	6	6	10	16	9	10
Salinity ( $\mu\text{Mho}$ )	9	7	9	6	6	10	16	9	10
Temperature ( $^{\circ}\text{C}$ )	15	12	13	14	14	14	14	13	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
	T	M	B
Sample Position*	-	-	-
Salinity ( $\mu\text{Mho}$ )	-	-	-
Temperature ( $^{\circ}\text{C}$ )	-	-	-
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	19
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.30. Passage Cove Beach Hydraulics: September 10, 1989;  
8:45 p.m.; Rising Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	400
Temperature ( $^{\circ}\text{C}$ )	12
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	4	5	-	2	2	-	5	5
Temperature ( $^{\circ}\text{C}$ )	-	14	13	-	12	13	-	13	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )			
Temperature ( $^{\circ}\text{C}$ )			
$\text{NH}_4$ ( $\mu\text{M}$ )			
$\text{NO}_3$ ( $\mu\text{M}$ )			
$\text{PO}_4$ ( $\mu\text{M}$ )			

Offshore

Salinity ( $\mu\text{Mho}$ )	16
Temperature ( $^{\circ}\text{C}$ )	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well



Table 9.31. Passage Cove Beach Hydraulics: September 11, 1989;  
8:10 a.m.; Rising Tide

Pond

Station Number	4
Salinity ( $\mu$ Mho)	300
Temperature ( $^{\circ}$ C)	13
NH <sub>4</sub> ( $\mu$ M)	-
NO <sub>3</sub> ( $\mu$ M)	-
PO <sub>4</sub> ( $\mu$ M)	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)									
Temperature ( $^{\circ}$ C)									
NH <sub>4</sub> ( $\mu$ M)									
NO <sub>3</sub> ( $\mu$ M)									
PO <sub>4</sub> ( $\mu$ M)									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	-	-	-	-	-	8	-	-	6
Temperature ( $^{\circ}$ C)	-	-	-	-	-	15	-	-	15
NH <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
NO <sub>3</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
PO <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu$ Mho)	7	7	7
Temperature ( $^{\circ}$ C)	-	-	-
NH <sub>4</sub> ( $\mu$ M)	-	-	-
NO <sub>3</sub> ( $\mu$ M)	-	-	-
PO <sub>4</sub> ( $\mu$ M)	-	-	-

Offshore

Salinity ( $\mu$ Mho)	15
Temperature ( $^{\circ}$ C)	14
NH <sub>4</sub> ( $\mu$ M)	-
NO <sub>3</sub> ( $\mu$ M)	-
PO <sub>4</sub> ( $\mu$ M)	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.32. Passage Cove Beach Hydraulics: September 11, 1989;  
11:45 a.m.; High Tide

Pond

Station Number	<u>4</u>
Salinity ( $\mu$ Mho)	350
Temperature ( $^{\circ}$ C)	13
NH <sub>4</sub> ( $\mu$ M)	-
NO <sub>3</sub> ( $\mu$ M)	-
PO <sub>4</sub> ( $\mu$ M)	-

High Tide Wells

Station Number	<u>7</u>			<u>3</u>			<u>10</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	-	4	5	-	2	2	-	3	3
Temperature ( $^{\circ}$ C)	-	14	13	-	13	13	-	14	13
NH <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
NO <sub>3</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
PO <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-

Mid Tide Wells

Station Number	<u>6</u>			<u>2</u>			<u>9</u>		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu$ Mho)	-	-	-	14	14	14	-	-	-
Temperature ( $^{\circ}$ C)	-	-	-	14	13	13	-	-	-
NH <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
NO <sub>3</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-
PO <sub>4</sub> ( $\mu$ M)	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	<u>1</u>		
Sample Position*	T	M	B
Salinity ( $\mu$ Mho)			
Temperature ( $^{\circ}$ C)			
NH <sub>4</sub> ( $\mu$ M)			
NO <sub>3</sub> ( $\mu$ M)			
PO <sub>4</sub> ( $\mu$ M)			

Offshore

Salinity ( $\mu$ Mho)	14
Temperature ( $^{\circ}$ C)	13
NH <sub>4</sub> ( $\mu$ M)	-
NO <sub>3</sub> ( $\mu$ M)	-
PO <sub>4</sub> ( $\mu$ M)	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.33. Passage Cove Beach Hydraulics: September 11, 1989;  
3:00 p.m.; Falling Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	350
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	9	9	9	-	8	10	7	8	7
Temperature ( $^{\circ}\text{C}$ )	14	14	14	-	14	9	14	14	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	11	9	10
Temperature ( $^{\circ}\text{C}$ )	14	14	13
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	13
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Table 9.34. Passage Cove Beach Hydraulics: September 11, 1989;  
6:30 p.m.; Low Tide

Pond

Station Number	4
Salinity ( $\mu\text{Mho}$ )	300
Temperature ( $^{\circ}\text{C}$ )	12
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

High Tide Wells

Station Number	7			3			10		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )									
Temperature ( $^{\circ}\text{C}$ )									
$\text{NH}_4$ ( $\mu\text{M}$ )									
$\text{NO}_3$ ( $\mu\text{M}$ )									
$\text{PO}_4$ ( $\mu\text{M}$ )									

Mid Tide Wells

Station Number	6			2			9		
Sample Position*	T	M	B	T	M	B	T	M	B
Salinity ( $\mu\text{Mho}$ )	-	11	13	-	8	9	-	8	10
Temperature ( $^{\circ}\text{C}$ )	-	12	12	-	14	14	-	14	12
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-	-	-	-	-	-	-

Low Tide Wells

Station Number	1		
Sample Position*	T	M	B
Salinity ( $\mu\text{Mho}$ )	11	11	12
Temperature ( $^{\circ}\text{C}$ )	14	14	15
$\text{NH}_4$ ( $\mu\text{M}$ )	-	-	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-	-	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-	-	-

Offshore

Salinity ( $\mu\text{Mho}$ )	16
Temperature ( $^{\circ}\text{C}$ )	14
$\text{NH}_4$ ( $\mu\text{M}$ )	-
$\text{NO}_3$ ( $\mu\text{M}$ )	-
$\text{PO}_4$ ( $\mu\text{M}$ )	-

\* T = Top - 2 feet below beach surface  
M = Middle - 1 foot above bottom of well  
B = Bottom - bottom of well

Samples from groups 9 and 10 (Tables 9.18 and 9.19) had  $\text{NH}_4$  concentrations greater than 100  $\mu\text{M}$ . The nitrate data showed definite high concentrations of  $\text{NO}_3$  at virtually every depth sample. The lowest concentrations were observed in the well samples on the right side of the plot. This indicates that wither fertilizer application was uneven or that the freshwater flow was greater on the right side of the plot. The salinity tended to support the conclusion that freshwater flow was greater on the right side of the plot. The salinity data tended to support the conclusion that freshwater was more prevalent under the right side of the plot. Similarly, the lowest nutrient concentrations were observed when salinities were closest to seawater. Surface samples collected offshore in water about 0.4 meters deep had very low nutrient concentrations in each case. Nitrate never exceeded 20  $\mu\text{M}$  immediately outside the plot area, indicating very little loss of nutrients to the sea, or that nutrient loss was thoroughly diluted by the time it reached the sample location. The highest nutrient concentrations were associated with salinities intermediate between seawater and freshwater, indicating that the nutrients were partially confined to the zone where mixing of fresh and salt water occurred. One possible explanation is that the incoming tide pushes water into the face of the beach, rather than flowing under and pushing water up in the body of the beach.

Nutrient data for samples collected September 10 and 11 (Tables 9.27 through 9.34) were not available at the time of writing. Final conclusions regarding the beach hydraulics experiments will have to be made after reception and consideration of the data. Tentative conclusions based on evaluation of data from tables 9.20 through 9.26 include: 1) the use of water soluble fertilizers seems to be effective in distributing nutrients on beaches to support biodegradation of crude oil, 2) nutrients persist in the body of the beach at least two days after the last fertilizer application, and 3) fresh and salt water flow dynamics in beaches are sufficiently complex to require additional study. In addition, hydrologic studies of other types of beaches may be required to describe the hydrology of oil coated beaches in the Prince William Sound in order to improve the results of bioremediation efforts. However additional evidence argues that nutrient loss from the plots was minimal.

### Discussion and Conclusions

The data provided by nutrient analysis, salinity measurements, well instruments (salinity, temperature, water depth), the tide gauge, and the weather station will be incorporated into a mathematical model of hydrology of a beach in Prince William Sound. The model is likely to be very complex due to the number of variables affecting water movement in this area.

Data analysis, model development, and incorporation of data into the model are still in progress. Detailed nutrient analysis and the hydraulic model will be presented in the final report of this project.

## MUTAGENICITY TESTS

### Background

The types of health hazards for which monitoring is most difficult are those that have chronic, delayed effects such as carcinogenicity, neurotoxicity, and mutagenicity. Fortunately, for mutagenicity there are short-term in vitro tests that demonstrate whether or not a pollutant interacts in a detrimental manner with DNA. Due to the mechanistic research with oncogenes, available evidence shows that oncogene activity can be initiated by mutation. Mutation assays, although not definitive, can be used as screening tests for the presence of potential carcinogens. When performed in a quantitative, dose-responsive fashion, one can use these bioassays to detect alterations in the quantity of mutagens present within complex mixture samples. One of the methods used to assess potential health effects associated with this and similar spills, therefore, is the examination of mutagenicity associated with the oil spill, the weathered oil, and the products associated with bioremediation. The most commonly used mutation assay is the Salmonella typhimurium / mammalian microsome assay developed by Ames.

Experiments were initiated to determine the potential mutagenic activity associated with biodegradation of oil. An early pilot study had demonstrated that extracts of spilled oil are mutagenic in the Salmonella typhimurium bioassay for mutagenicity. This meant that the removal of genotoxic components from the oil by biodegradation could be monitored with this assay.

### Methods

The bioassay chosen for the monitoring of these samples is the spiral Salmonella assay as described by Houk, et. al. This bacterial assay is a modification of the standard Salmonella plate incorporation assay. It requires less total material, accommodates more samples per unit time, and samples do not have to be solvent-exchanged into dimethylsulfoxide.

Preparation for samples for this assay was accomplished by extracting the samples using a sonication procedure and dichloromethane (DCM). The samples are filtered through silanized glass wool are concentrated to <100 ml using roto-evaporation. After drying with anhydrous NaSO<sub>4</sub>, all samples are then concentrated or diluted to a concentration of 10 mg/ml (a

reference point derived from preliminary testing) and stored in a freezer at -30°C until taken for bioassay.

## Results

Due to the characteristics of some complex mixture samples (e.g., insolubility), the standard assay can sometimes be impractical. The Alaskan oil samples are examples of mixtures that are difficult to test in the standard assay. Due to the physical properties of the samples, therefore, tests were done using the spiral Salmonella assay. Both the Prudhoe Bay crude oil and the weathered oils tested were weakly mutagenic using TA100. The commercial nutrient formulations were negative. Organic samples collected from the beaches showed varying results depending upon the type and timing of the treatments. Although the data is in final analysis, we do know that the mutagenicity of the organic extracts from both treated and untreated beaches decreases over time when based upon the amount of extracted organic material applied to the test. This result means that the mutagenicity is being lost at a rate greater than the rate at which the organic material is depleted. Calculations showing the mutagenic response per area or volume of beach treated have not yet been done; therefore, we cannot yet compare treated with untreated beaches.

## Discussion and Conclusion

In final analysis, these mutagenicity studies show that mutagenic toxins associated with spills of Prudhoe Bay crude oil are lost over time. In conjunction with chemical analysis, these studies will also help to demonstrate whether or not these decreases in toxicity are due to bioremediation efforts, or other natural processes, or to some combination of effects. These studies will assist in the selecting of appropriate bioremediation procedures for environmental oil spills.

## REFERENCES

- Caron, D.A., 1983. Technique for Enumeration of Heterotrophic and Phototrophic Nanoplankton, using Epifluorescence Microscopy and Comparison with Other Products. *Applied and Environmental Microbiology* 46:491-498.
- Fuhrman, J.A. and F. Azam. 1982. Thymidine Incorporation as a Measure of Heterotrophic Bacterioplankton Production in Marine Surface Waters: Evaluation and Field Results. *Marine Biology* 66:109.
- Hildebrand, Robert, 1989. Draft Fucus Protocols. United States Coast Guard Research and Development, Mobile Laboratory.
- Hobbie, J.E., R.J. Daley, and S. Jasper. 1977. Use of Nucleopore Filters for Counting Bacteria by Fluorescence Microscopy. *Applied Environmental Microbiology* 33:1225-1228.
- Houk, V.S., S. Schalkowsky, and L.D. Claxton, 1989. Development and Validation of the spiral salmonella assay; an automated approach to bacterial mutagenicity testing. *Mutation Res.* 223:49-64.
- Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemical and Biological methods for Seawater Analysis. Pergamon Press, Inc., Maxwell House, Elmsford, N.Y.
- Sveum, P., 1987. Accidentally Spilled Gas-Oil in a Shoreline Sediment on Spitzbergen: Natural Fate and Enhancement of Biodegradation. Sintef, Applied Chemistry Division, N-7034 Trondheim, Norway. 16 pp.).
- Whitledge, Malloy, Patton, and Wirick (1981).