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Symposium Proceedings: Gulf of Mexico and Caribbean Oil Spills in Coastal Ecosystems: Assessing Effects, Natural Recovery, and Progress in Remediation Research

New Orleans, July 14-15, 1994

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

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PREFACE

This volume is the proceedings of a symposium entitled *Gulf of Mexico and Cairbbean Oil Spills in Coastal Ecosystems: Assessing Effects, Natural Recovery, and Progress in Remediation Research* held in New Orleans on July 14-15, 1994. Both the symposium and these proceedings were supported under a cooperative agreement between MMS and McNeese State University. The papers in this report were provided by the invited symposium speakers. They have been reviewed editorially, but have not been subjected to peer review. Included at the end of the report are outline summaries of discussion sessions held near the end of the symposium.

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NOTE TO READERS

There are many instances in which oil spills have adversely affected ecosystems and wildlife. Many scientists and environmental managers have approached various aspects of the problem for decades. Despite advances in science and the technology of cleanup and remediation, our understanding of the effects of oil on ecosystems, the rates of natural degradation of oil once in these systems, and effective techniques for remediation of adverse environmental effects is marginal, at best.

For this reason, the Minerals Management Service (MMS) and the Louisiana Environmental Research Center at McNeese State University (LERC) organized and hosted this symposium of invited papers. This proceedings includes a number of papers discussing on-going research on the ecological and genetic effects of oil on biota, bioremediation, and in-situ marsh burns. Other papers provide summaries and syntheses of states-of-knowledge on oil effects and various remediation techniques.

To further address the problems, MMS and LERC are holding follow-up workshop-style meetings to focus on specific problems identified during the symposium. From these workshops a series of technical papers on specific topics will be authored by participants and published by MMS. These technical papers will contain reviews of specific topics and opinions by the invited experts on research needs and best management practices. The workshop on Mangroves and Oil Spills has already been held (August 1995, at McNeese State University) and the technical paper is in preparation. An additional workshop will be held during 1996.

We hope that these efforts will result in significant advances in our understanding of oil spill effects and how to respond to them in ways that minimizes effects on coastal, estuarine, and marine wildlife and ecosystems.

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Experimental Analyses of the Effects of Oil on Mangrove Seedlings and Saplings and the Mangrove Gastropod *Melampus coffeus* L.

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INTRODUCTION

Mangroves dominate about 75% of the world's low energy coastline between 25° N and 25° S latitude. Mangroves also occur well outside this range along eastern North America and other coasts where cold temperatures are buffered by warm water currents. Many trade routes, ports, petroleum storage and refining facilities, and naturally occuring oil fields are located in these same regions, making mangrove communities epecially susceptable to contamination from oiling.

There are numerous documentations of death, defoliation, genetic, and other damage to mangroves and their associated communities after exposure to oil (Baker *et al.* 1981; Baker 1982; Dicks 1986; Duke 1991; Garrity *et al.* 1994; Getter *et al.* 1981; Gilfillan *et al.* 1981; Jackson *et al.* 1989; Kahn 1992; Klekowski *et al.* 1994; Jacobi and Schaeffer-Novelli 1990; Lewis 1983; Proffitt *et al.* 1993, 1995; Snowden and Ekweozor 1987).

Once contamination has occured, characterization of the effects of oils on living organisms is difficult. Oils vary in composition, toxicity and viscosity. These factors are secondarily affected by the environment as the oils breakdown or "weather" (Proffitt *et al.* 1995).

Assessing the effects of crude or refined petroleum in mangrove forests is particularly challenging. The intertidal location and complex structure of mangrove forests provides habitat for diverse faunal assemblages. The canopy supports large suites of birds, arthropods and other invertebrates. Submerged roots provide surfaces for colonization by encrusting organisms including oysters, barnacles, sponges, tunicates, algae and other species; as well as refuge and foraging habitat for numerous fish and crustaceans. Burrowing and epibenthic crustaceans, molluscs and other invertebrates live the soft substratum and litter on the forest floor. Algae and salt tolerant herbaceous plants are also common.

Damage to mangrove forests varies with the amount and toxicity of the spilled oil product(s); tidal height and range; the oil residence time, the season of the oiling event, and other factors. Spilled oil may coat both plant and animal life and/or be absorbed by

substratum. The rate of subsequent degradation of oil in the sediment is influenced by the sediment type, oxygen content and bacterial component of the sediment; availability and level of nutrients in the sediment and at the oil/soil interface; and the depth to which the oil has penetrated.

A primary cause of death in oiled mangroves is reported to be the disruption of gas exchange when aerial roots are coated with oil and can no longer supply oxygen to root tissues below ground in hypoxic soils (Teas *et al.* 1993). Getter *et al.* (1981, 1985) found that hydrocarbons can enter mangroves through the root system, be translocated to the leaves and accumulate in the stomata. They speculate that the hydrocarbons may interrupt transpiration or poison biochemical pathways. Page *et al.* (1985) demonstrated that petroleum can disrupt root membranes and allow lethal concentrations of salt to accumulate in mangrove tissues.

Toxicity varies with the composition of the oils and in combination dispersants. Getter *et al.* (1985) report that light crudes and No. 2 fuel oil decreased foliage production by mangroves more than the heavier Bunker C oil, while dispersants decreased toxicity of the bunker C oil but increased the toxicity of light oils.

Effects also differ with life history stage, and may effect the growth forms of young trees. Duke (1991) found that 1-2 year old *Rhizophora mangle* L. survived oiling by medium weight crude while surrounding mature trees died. Getter (1982) found that seedlings took up oil more rapidly than did larger trees. Seedlings growing in oiled sediments sometimes have a modified growth form. There is also some evidence suggesting that stands in areas near the physiological tolerance limits for the species or in other stressful environments, may be more vulnerable to oil damage than forests in less stressful environments (Proffitt *et al.* 1995).

Lewis (1983) suggests that natural recolonization of an mangrove habitat oiled during the *Zoe Colocotroni* spill "appears to be successful." However, he also states that other mangrove experts (A. Lugo and G. Cintron) have testified that the plants colonizing this site will likely remain stunted or die. Getter *et al.* (1985) opined that the tall, spindly seedlings noted growing in at the *Zoe Colocotroni* spill site may be less able to withstand storm winds.

Studying the effects of spills in mangrove systems is often difficult. Mangals around the world have divergent suites of species and different levels of biological diversity. Mangals are protected by law in some areas which often precludes manipulative field experiments on oil effects. Especially poorly understood are the effects of oil contamination on mangrove fauna and food webs; and, on ecological functions such as detrital decomposition and transport.

Here we summarize experiments, some of which are on-going at time of this symposium, of the effects of oil on growth and survival of *Rhizophora mangle* seedlings and saplings. Complete results of this study will be presented in a paper that is in preparation at time of publication of this document. We also present limited experimental data on survival of the mangrove-dwelling gastropod, *Melampus coffeus* L. when exposed to oiled seagrass. Two oils, No. 6 fuel oil (both fresh and weathered) and Louisiana crude, were used in different studies.

METHODS

EXPERIMENT 1 (fresh No. 6 fuel oil): *Rhizophora mangle* propagules were collected at War Veteran's Park on Boca Ciega Bay, a part of the Tampa Bay estuary system. Two-hundred eighty propagules varying from 15 to 25 cm in length were selected for use. Two treatments 50% oiled and 100% oiled and a control were established. For the oil treatments, propagules were either dipped halfway (50%) or completely immersed (100%) in fresh No. 6 fuel oil. Small amounts of the oil flowed from the propagules into the sediment for several hours after planting. A control group received no oil.

EXPERIMENT 2 (spilled and weathered No. 6 fuel oil): *R. mangle* propagules and blades of the seagrass *Thallasia testudinum* coated with No. fuel oil were collected from a beach in Tampa Bay (south Pinellas County) in July of 1991 when an oil slick came ahore. The fuel oil had leaked from a pipe at Port Manatee several days previously. *R. mangle* propagules were scored as lightly oiled ($<$ 50% cover) or heavily oiled ($\geq$ 50% cover). Unoiled control propagules were collected from the driftline of a nearby cove not affected by the spill. Data from a second type of "control" group (a "cohort control") consisting of propagules all from one parent tree are not presented here since these seedlings had very different growth patterns than any (oiled or unoiled) taken from the driftline.

All propagules from the two fuel oil experiments were planted in commercial potting media and watered with fresh water when necessary. Water levels in external containers were maintained at approximately 1 - 2 cm below the surface of the sediment. Plants were kept at ambient temperatures in a shaded site. Experiments 1 and 2 were not run concurrently.

EXPERIMENT 3 (Effects of weathered No. 6 fuel oil on *M. coffeus*): Oiled *T. testudinium* blades were placed on the floor of an uncovered aquaria in which 20 *M. coffeus* had been kept for several months for feeding observations. In a second aquarium, 20 *M. coffeus* were presented with unoiled seagrass blades. There was approximately 1-2 mm of water on the floor of both aquaria. An unoiled food supply of mangrove leaves was available in Petri dishes placed on the floor of both aquaria. This was a simple, unreplicated experiment-of-convenience resulting from our having collected a small amount of oiled seagrass during the spill discussed in Experiment 2 and also having a number of *M. coffeus* available and acclimated to aquaria.

EXPERIMENT 4 (Effects of Louisiana crude oil on 34 month old *R. mangle* saplings: In this study, plants surviving Experiment 2 were re-potted and allowed to acclimate for two months in a greenhouse water table at McNeese State University in Lake Charles, Louisiana. Three experimental groups with 16 replicates per group were established: an unoiled control, a low crude oil treatment (1.6 L/m^2) and a high crude oil treatment (16 L/m^2). Oil was applied to the sediment. At the date of the symposium this on-going study has been in progress for about 4 months and preliminary growth data for 92 days are presented. Survival data include the entire year-long study period as these data were available prior to finalization of the paper for the proceedings. At time of publication of this proceedings, a

paper analyzing data from exeriments 2 and 4 together is being submitted for publication (Proffitt and Devlin in prep).

MEASUREMENTS: In each mangrove experiment, survival, primary stem growth and lateral branch growth of all treatment and control plants were measured to the nearest mm with vernier calipers. Numbers of leaves, leaf scars and attached leaves by degree of senesence were recorded. Primary stem growth was measured from the tip of the hypocotyl to the base of the apical leaf primordium. Lateral branch growth was measured from the point of formation of the axillary branch to the base of the leaf primordium at the tip of the branch. Total leaf production was calculated as follows:

$$(L_{t2} + LS_{t2}) - (L_{t1} + LS_{t1})$$

Where L and LS are numbers of leaves and numbers of leaf scars at times 1 and 2.

For the Louisiana crude oil study, primary stem lignification was also measured and plants were ranked as to general condition: good, fair or poor. Condition was determined by acessing the following indicators of plant stress: leaf yellowing, browning, and wilting; production of deformed leaves; growing tip necrosis; lateral branch death, primary stem death; and plant death. Data on lignification and condition are preliminary and not presented here. One goal of this on-going experiment is to assess effects of oiling on first reproduction since red mangroves can flower and produce propagules as early as 3 years of age. As yet, no plant has reproduced so data on this is not presented.

RESULTS

EXPERIMENT 1 (Fresh No. 6 Fuel Oil):

Approximately 80% of unoiled *R. mangle* propagules produced their first leaves between 50-80 days. The majority of propagules in the 50% and 100% oil treatments did not produce their first leaves until 80-120 days. By the end of the study (12 months), 90-100% of seedlings in all groups had survived and produced leaves (Fig. 1).

At 12 months the control group had significantly greater stem growth (Fig. 2) and leaf production (Fig. 3) than both oil treatments (ANOVA and Tukey multiple comparison tests [$p < 0.05$]). There were no significant differences between the 50% oil treatment and the 100% oil treatments.

EXPERIMENT 2 (Spilled and Weathered No. 6 Fuel Oil):

Survival of treatment and control plants was $\geq$ 80% at 10 months, with a slight trend of reduced survival with greater surface area oiled (Fig. 4). At 32 months, survival remained at 83 % for the control, but declined to 71% for the low oil treatment, and 60% for the high oil treatment.

There were no significant differences in total stem growth among control and treatments throughout the study (Fig. 5). Lateral branch growth in the high oil treatment plants was greater at 32 months (One Way ANOVA and Tukey Multiple Comparisons Test, $P<0.05$) than the low oil group, but not the control group (Fig. 5).

Total leaf production was lowest in the low oil treatment (One Way ANOVA and Tukey Multiple Comparisons Test, $P<0.05$) (Fig. 5). Lateral branch leaf production was also greater ($P<0.05$) in the high oil treatment (Fig. 5). Leaf production on the primary stem was virtually identical for treatment and control groups.

EXPERIMENT 3 (Effects of Weathered No. 6 Fuel Oil on *Melampus coffeus*):

Changes in *Melampus coffeus* behavior occurred nearly immediately upon exposure to the oiled *Thallasia testudinum* blades. Most (85%) quickly moved as far away from the oil as possible by climbing the aquaria sides. Three individuals were overtaken by the spreading oil leachate and upon contact, fell limp and hung out of their shells. Mortality of all 20 gastropods in the oil treatment occurred within 17 days (Fig. 6). There was no mortality in the unoiled control group during the same 17 day time frame or over the ensuing 8 weeks.

EXPERIMENT 4 (Effects of Louisiana Crude Oil on *R. mangle* Saplings):

At experiment initiation there were no significant differences among control and treatments in height, primary stem length, lateral branch length, numbers of leaves on the plants or numbers of leaf scars.

Survival

There was a lag time of about 30 days before any detrimental effects of oil were noted. At 30 days, wilting and necrosis was evident in some leaves of the high oil treatment. At 92 days reduced survival (78%) was recorded in the high oil treatment (Fig. 7). At 379 days, survival was 31% in the high oil treatment and 100% in both the control and low oil treatment (Fig. 7).

The trend in mortality is best described by an exponential model. Since this is an on-going study, it is unclear if mortality in the high oil treatment will continue or level-off. Thus, we present two preliminary alternative models that encompass these two possibilities: a linear regression model of percent survival on days and an exponential regression model of ln(proportion surviving) on days.

These provided the following models of survival.

Linear Regression Model:

$$\text{Percent Survival} = -0.176 \times \text{Days} + 89.436$$
$$R^2 = 0.861,\ n=17$$

Exponential Regression Model:

Proportion Surviving = $e^{(-0.003 \times days) + 4.562}$
$R^2 = 0.943$, n=17

It should be emphasized that these are preliminary emperical fits of linear and exponential models to the data and should not be used to exptrapolate mortality outside the 379 days of data.

Growth

At 62 and 92 days, there was significantly less total stem growth, primary stem growth and lateral branch growth in the high oil treatment relative to the control and low oil treatment (Fig. 8). Growth data analyses for time periods >92 days and analyses assessing prior oiling history of the plants is provided in Proffitt and Devlin (in prep.).

There is a trend toward fewer leaves on the high oil plants at 62 and 92 days (Fig. 9). Statistically significant differences occur only for leaf number on primary stems. There is extensive within-group variability in numbers of leaves among replicates on lateral branches.

There are significant differences between the high oil treatment and other groups for numbers of leaves produced, leaves dropped and leaves on saplings at both 62 and 92 days (Table 1). The high oil treatment produced fewer new leaves (2.5x less); dropped more leaves (at least 3 x more) and had fewer leaves (net) at 62 days. Net leaf production was very low in the high oil treatment (9-10 x less than control and the low oil treatment) and by 92 days, net leaf production in the high oil treatment was negative (i.e., more leaves were being dropped than produced).

DISCUSSION

Effects of No. 6 Fuel Oil

The similar mortality over the first year in the weathered oil and fresh oil experiments was unanticipated since fresh oil is generally considered to be more toxic because of a greater proportion of low molecular weight fractions Baker (1970). The differences in suvivorship between control and heavily oiled propagules in the weathered oil study first occurred at 10 months (12% lower survival in the heavily oiled plants) and increased to a 23% difference between treatments by 32 months. The fresh oil experiment showed 10% lower survival in the highly oil cover group at 12 months but was not followed longer. Results of the experiment with weathered oil suggest that some effects may be long term and that experimental studies on oil effects should be lengthened in order to accurately determine differences among treatments and controls.

Effects of Louisiana Crude Oil

Mortality was greater in the high oil (16 l/m^2) treatment and the surviving saplings exhibited characteristics which are indicative of stress, including lower growth rates, decreased numbers of leaves, and lower net leaf production (total leaf production-leaf drop). The means of application in this experiment was designed to mimic a fresh oil slickcoming ashore in a *Rhizophora mangle* stand, with oil remaining in the sediment and ponding in low areas. These results are consistent with those reported by Getter *et al.* (1985) that mortality and stress in *R. mangle* probably represent responses to uptake of oil and subsequent poisoning of transpiration or blockage of metabolic activity.

Survival of all saplings in the low oil treatment indicates that there is some threshold amount of oiling below which mortality of this age class does not occur. However, further studies are required to support this contention and quantify the threshold since our experiment did not utilize natural environmental conditions such as a salt water medium or winter cold. Environmental growing conditions have been shown to modify outcomes of oil effects in mangroves (Proffitt *et al.* 1995).

The manner of leaf necrosis in this experiment was interesting. Leaf browning usually began at the distil tip of the leaf and progressed toward the point of attachment at the proximal end of the leaf. A few pairs of leaves browned along one side. Generally a pair, or two pairs of leaves located close together, browned simultaneously and at similar rates. Observations continue in this on-going study.

Potential Mechanisms of Oil Stress in Red Mangroves

Snowden and Ekweozor (1987) found that after a spill of Nigerian crude oil, mangrove seedlings oiled over an average of 32% of their surface area survived less than 3 months. Getter *et al.* (1985) suggest that the toxic effects of oil on mangroves are short term, occurring within 4 months. The results of our weathered oil experiment and the Louisiana crude experiment are not consistent with the hypothesis that effects of oiling are short term. In these experiments, new mortalities in the High Oil treatment occurred nearly a year after application of crude oil; and, in the weathered No. 6 fuel oil study differences in leaf production were not noted at 10 months but were found at 32 months.

Getter *et al.* (1985) found that application of oil to the waxy stem of the *R mangle* seedlings resulted in less toxicity than when oil was applied to the roots or leaves. They suggest that the thick waxy covering of the stem may protect the plant from the oil. Other authors have reported that a coating of oil blocks gas exchange in red mangrove prop roots and can cause mortality (Teas *et al.* 1993). Jimenez *et al.* (1985) cite several instances where mass mangrove mortalities occurred when aerial roots are covered for too long a period with water, interrupting gas exchange. In our experiment with weathered oil, the medium was thicker and more sticky than the fresh oil and may have acted to block oxygen intake through the lenticels. However, we observed that at some time after the No. 6 fuel oil coatings in both fresh and weathered experiments had hardened, a brown slightly raised lip formed around the lenticels of surviving plants and the lenticels appeared to reopen. This phenomenon requires further study.

Proffitt *et al.* (1995) suggest that mangrove forests in stressful environments such as areas near the physiological tolerance limits for the species, may be more vulnerable to oil damage than those in less stressful environments. They also show in experiments using lubricating oil that significant interaction terms between oil treatments and environmental growing conditions can confound the prediction of effects of oil on red mangrove seedlings.

Several researchers have noted that in some cases oil seems to act as a growth enhancer, resulting in increased stem growth or elongation of mangroves in some oiled sites. It has been suggested that the mechanism of growth enhancement may be the release of nutrients as oil degrades or increased metabolic rates resulting from oiled (dark) propagules being warmer than the unoiled area. We suggest that it is more likely that the increased growth resulting in abnormal tree architecture may occur as a result of stress. The increased lateral stem growth and associated leaf production may occur as a result of damage to apical meristems followed by induced growth of lateral stems.

Melampus coffeus and Spilled No. 6 Fuel Oil

The leachate from weathered oil was lethal to adult *Melampus coffeus*. In the field, *M. coffeus* larger than *ca.* 4 mm shell length avoid tidal waters by climbing trees to a height above the high tide level or onto floating debris. They tend to stay in a relatively small area, often climbing up the same or nearby tree for several consecutive days or weeks (unpublished data). Since *M. coffeus* in an aquarium avoided the No. 6 fuel oil which was weathered by natural processes, it is probably reasonable to assume that the larger snails would also avoid patches of oil in the forest. Juvenile *M. coffeus*, less than about 4 mm in length seldom climb to escape flooding tides, but rather remain in the debris on the forest floor. Eggs are enclosed in small gelatinous masses attached to leaves or woody debris and are also covered by incoming tides (pers. obs.). Larvae are released into the water column (Apley 1968). Eggs, small individuals on the forest floor and larger individuals on floating debris would be the most likely be killed at the time of the initial oiling. Individuals on trees may be able to temporarily avoid contact with the oil. It seems clear that unless > 4mm *M. coffeus* are adaptable enough to change their habitat, and are physiologically able to remain out of the water for long periods of time, that they would not survive a spill event that oiled extensive portions of the mangrove forest floor. If this is the case, all life stages within the affected area would be exterminated if a No. 6 oil spill contaminated much of their mangrove forest habitat. Recolonization would have to occur through larval settlement after oil had degraded to the point where it would no longer be toxic to the juvenile snails. The time period required for oil degradation to levels not toxic to *M. coffeus* is unknown.

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Table 1

Sapling *Rhizophora mangle* numbers of new leaves produced, dropped, and net (Production-Drop) at 62 and 92 days following experimental exposure to Louisiana sweet crude oil applied to the sediment and base of plant. Values are means (1 standard deviation). Letters indicate no significant differences among treatments (one-way ANOVA and Tukey multiple comparisons tests).

	Numbers of New Leaves Produced	Numbers of Leaves Dropped	Net Leaf Production
62 DAYS			
Control	3.9(3.3) A	0.1(0.3) A	3.9(3.3) A
Low Oil	4.5(3.8) A	0.1(0.3) A	3.8(3.7) A
High Oil	1.4(2.0) B	0.9(1.3) B	0.4(2.3) B
92 DAYS			
Control	6.1(4.7) A	0.3(0.8) A	5.8(4.2) A
Low Oil	6.3(4.4) A	0.8(1.2) A	5.5(4.4) A
High Oil	2.4(2.8) B	2.9(1.8) B	-0.5(1.9) B

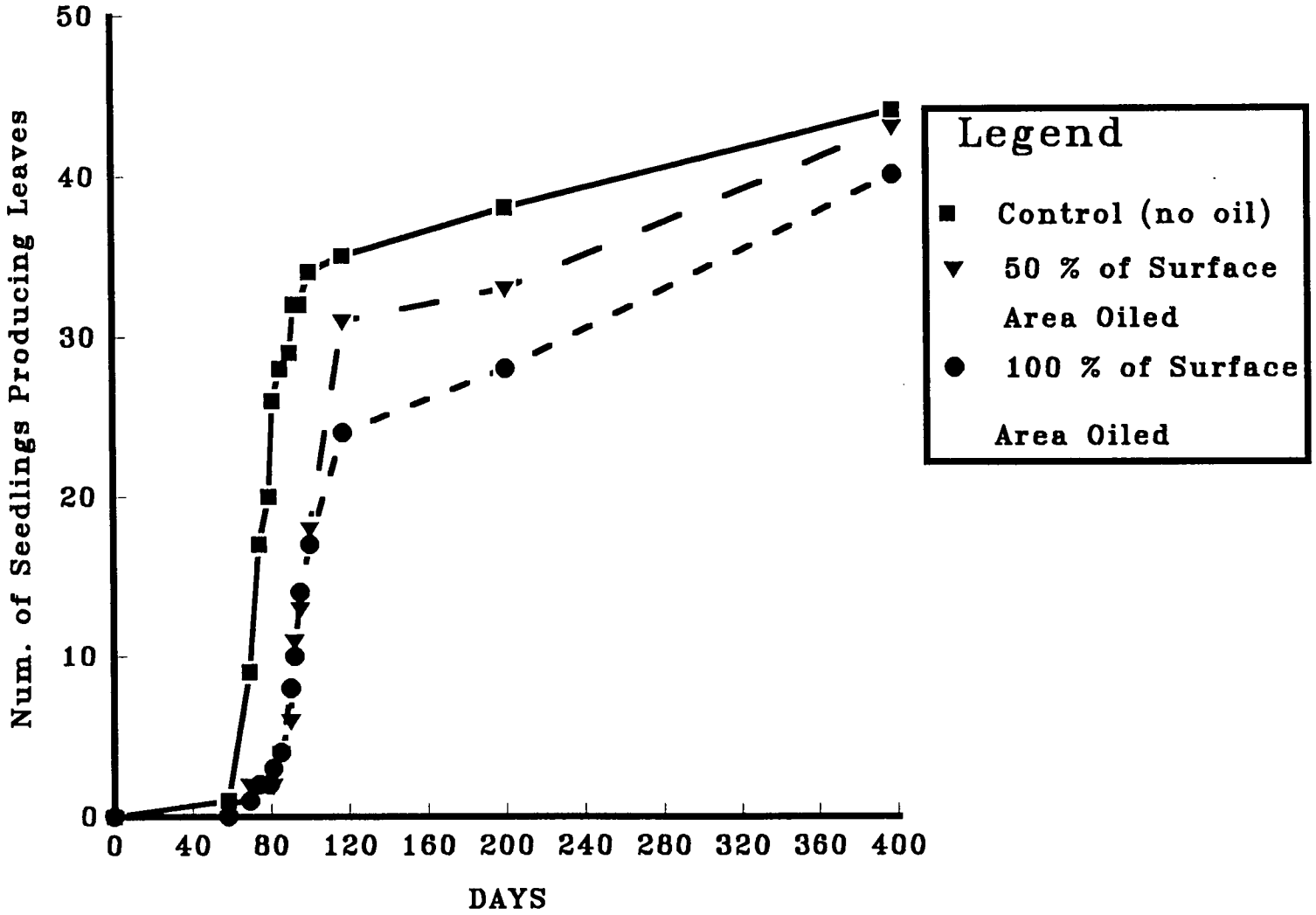

Figure 1. *Rhizophora mangle* cumulative numbers of seedlings producing leaves after experimental oiling of propagules with unweathered No. 6 fuel oil.

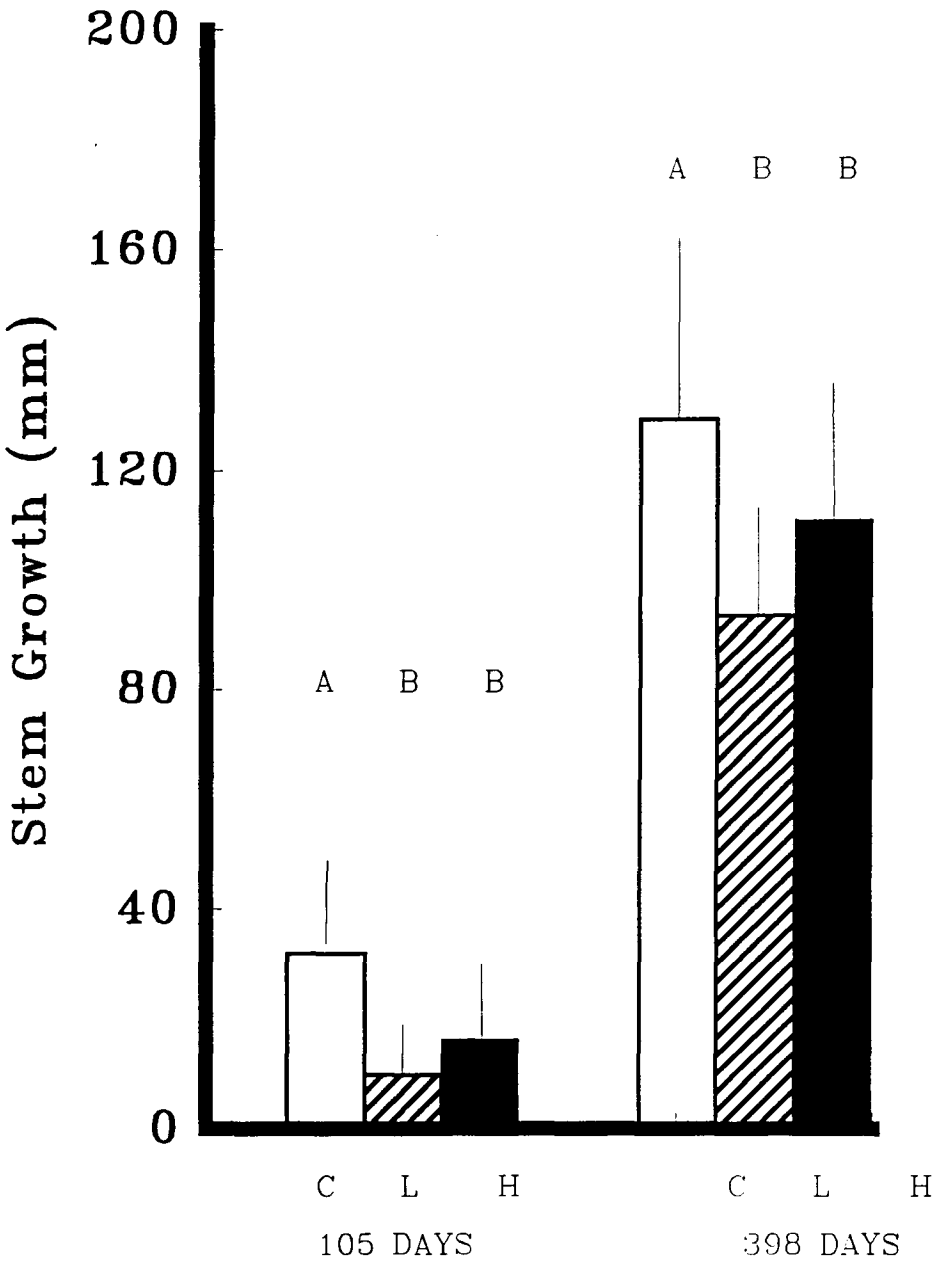

Figure 2. *Rhizophora mangle* Seedling propagule stem growth under different experimental treatments of unweathered No. 6 fuel oil applied to the surface of the propagules. Values are means and 1 S.D. Letters indicate significant differences (one-way ANOVA and Tukey multiple comparisons tests, $p<0.05$.

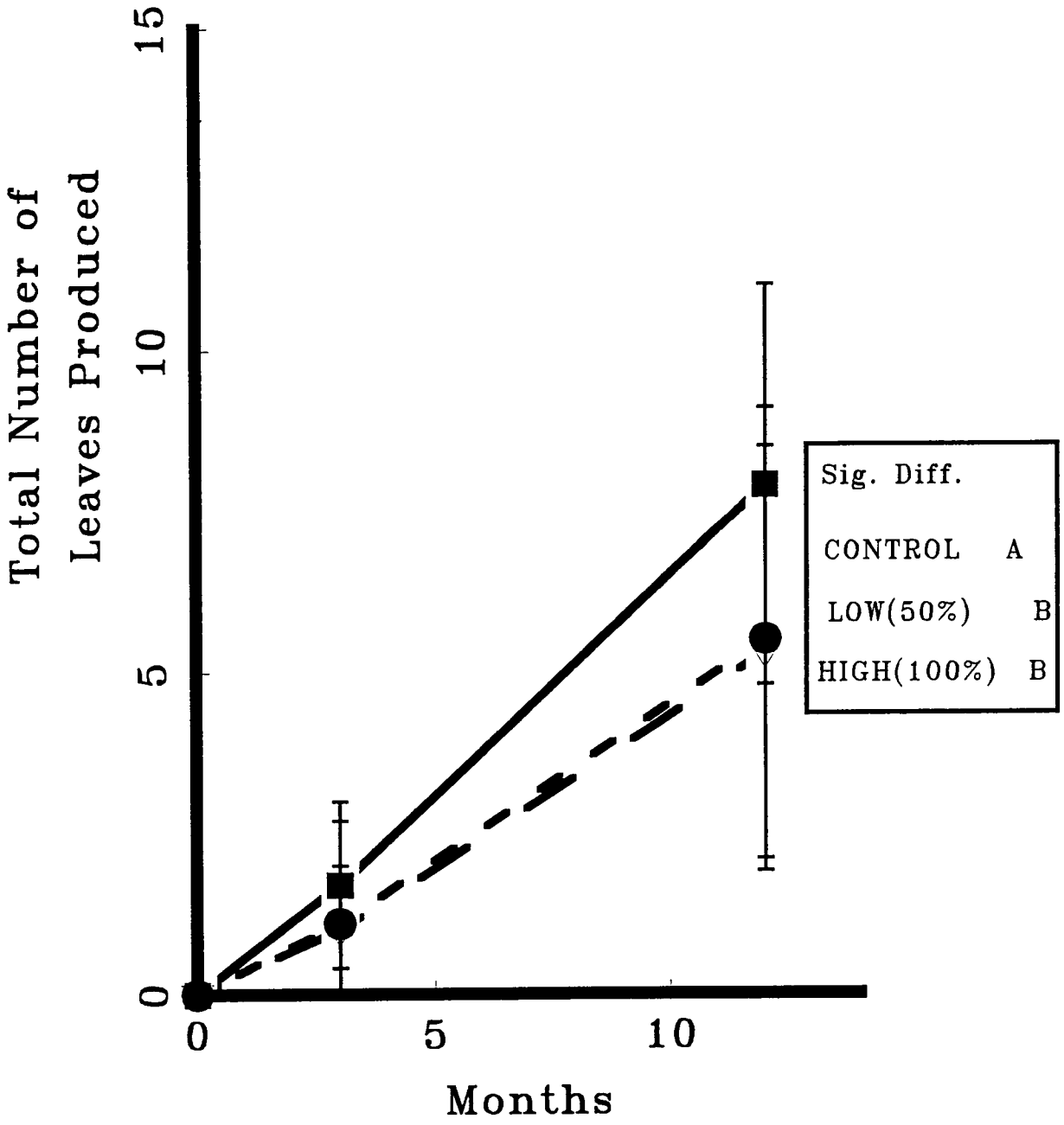

Figure 3. *R. mangle* seedling propagule leaf production under different experimental treatments of unweathered No. 6 fuel oil applied to the surface of the propagules. Values are means and 1 S.D. Letters indicate significant differences (one-way ANOVA and Tukey multiple comparisons test, $p<0.05$).

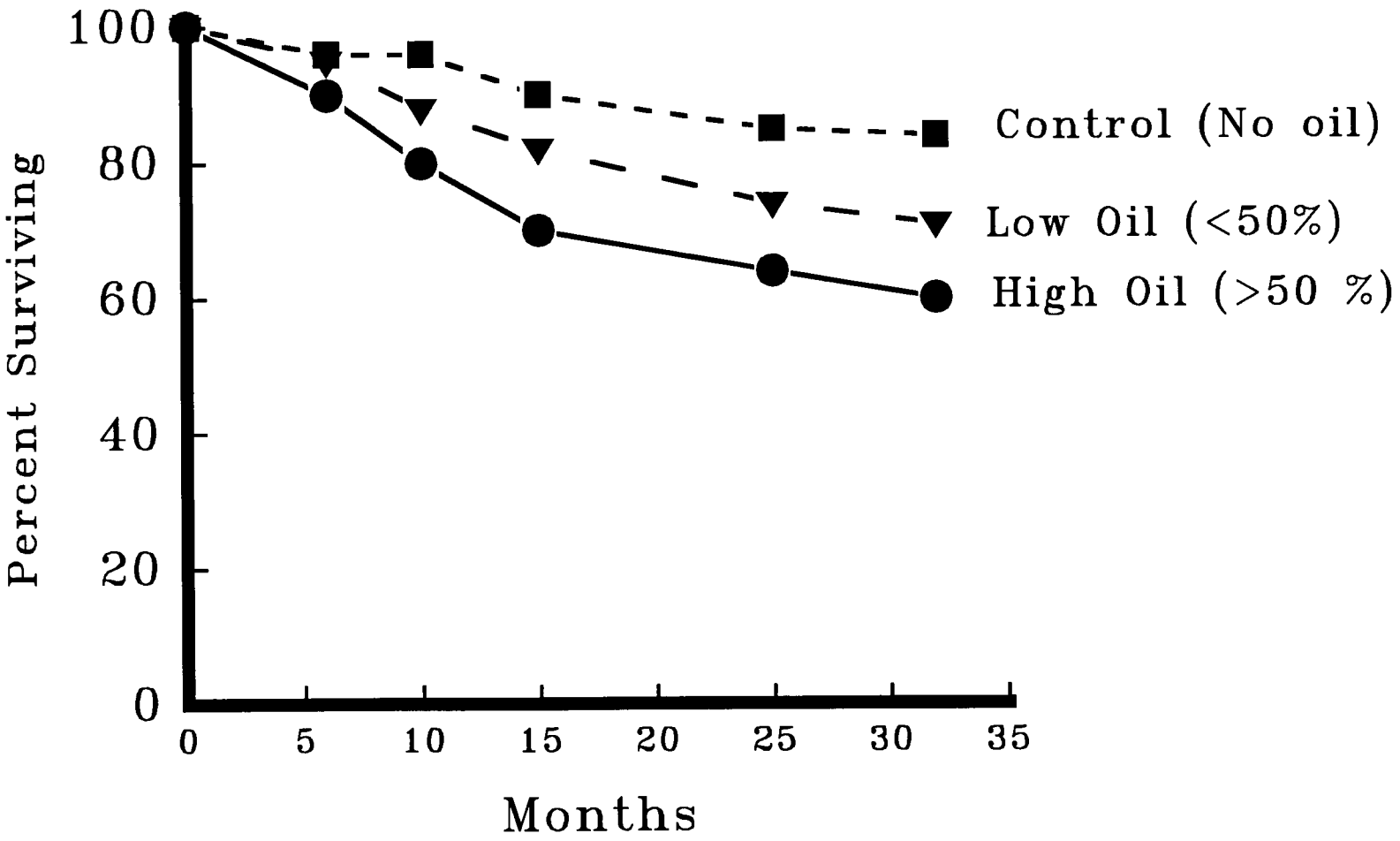

Figure 4. Percent of *R. mangle* seedling propagules surviving being oiled during a spill of No. 6 fuel oil (weathered several days prior to coming ashore). Controls had no oil cover, Low Oil had <50 % of surface area covered with oil, and High Oil had >50 % surface area cover by oil.

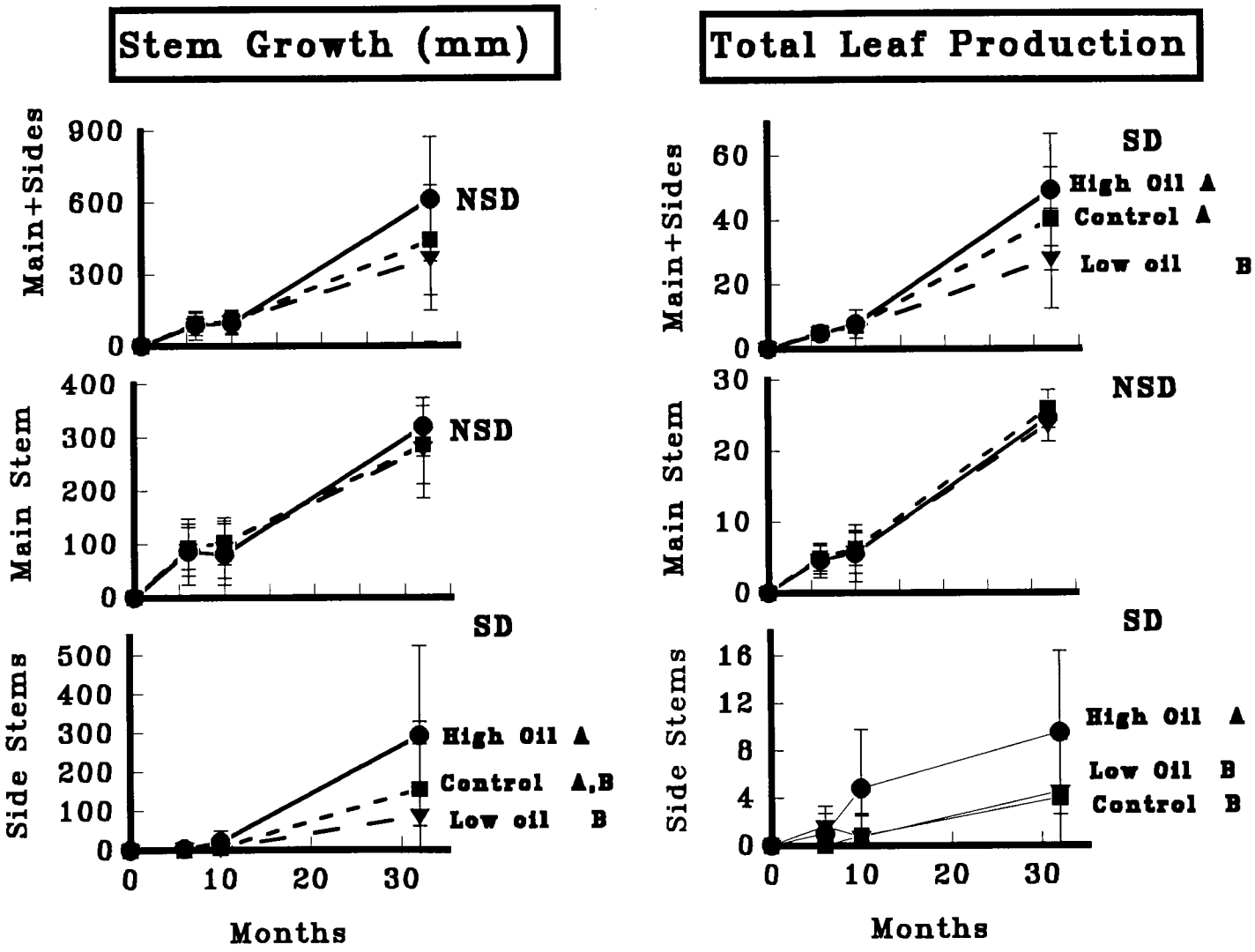

Figure 5. Stem growth and leaf production of *R. mangle* seedlings following oiling by a spill of No. 6 fuel oil. Controls had no oil cover, Low Oil had <50% surface area cover, and High Oil had >50 % cover with oil. NSD = No significant difference among groups. SD = significant difference at $p<0.05$ and letters indicate differences among means (one-way ANOVA and Tukey multiple comparisons. Main+Sides=total growth.

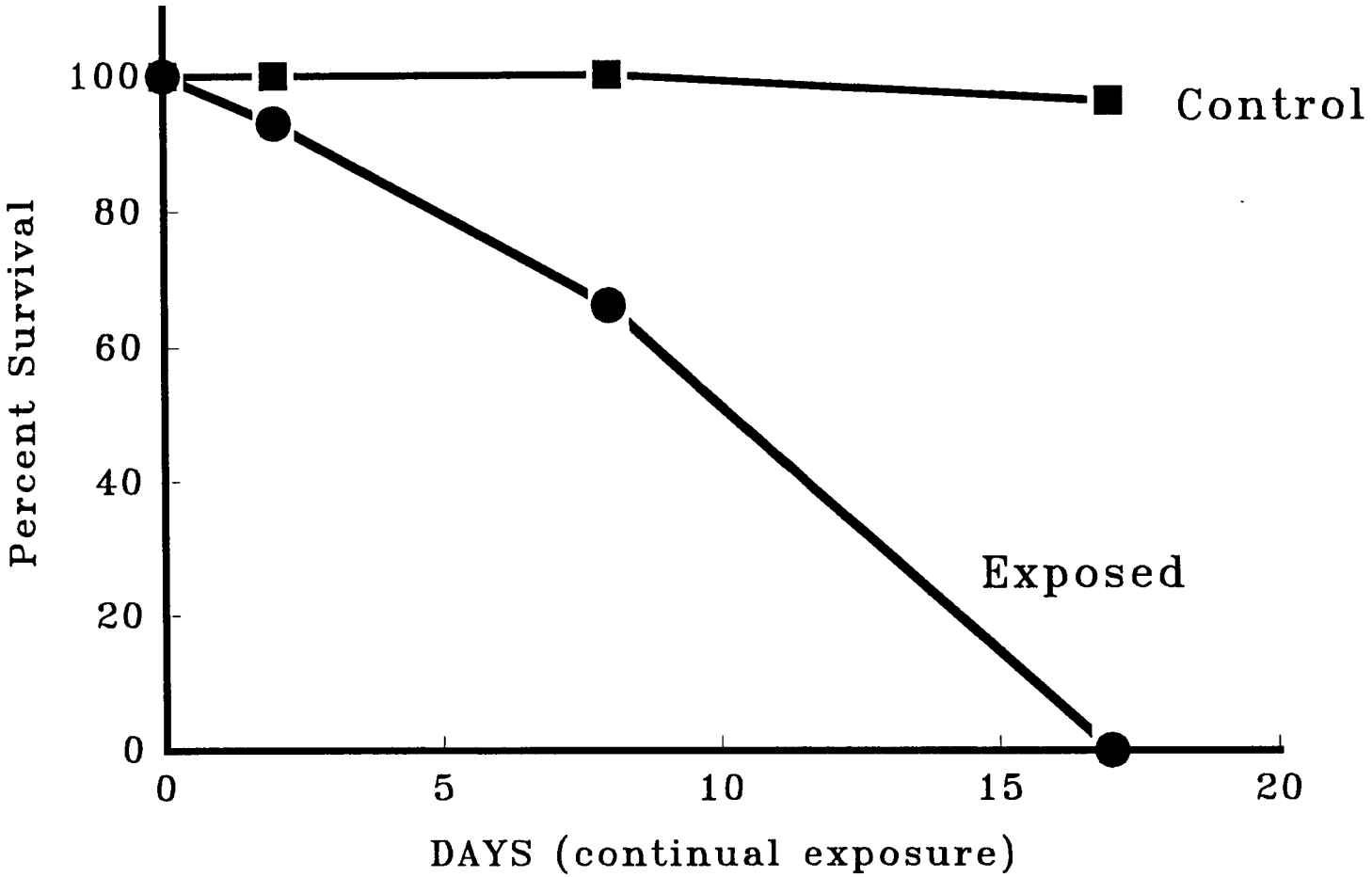

Figure 6. Percent survival of the mangrove forest gastropod *Melampus coffeus* L. following exposure in aquaria to seagrass oiled by a No. 6 fuel oil spill in Tampa Bay, FL.

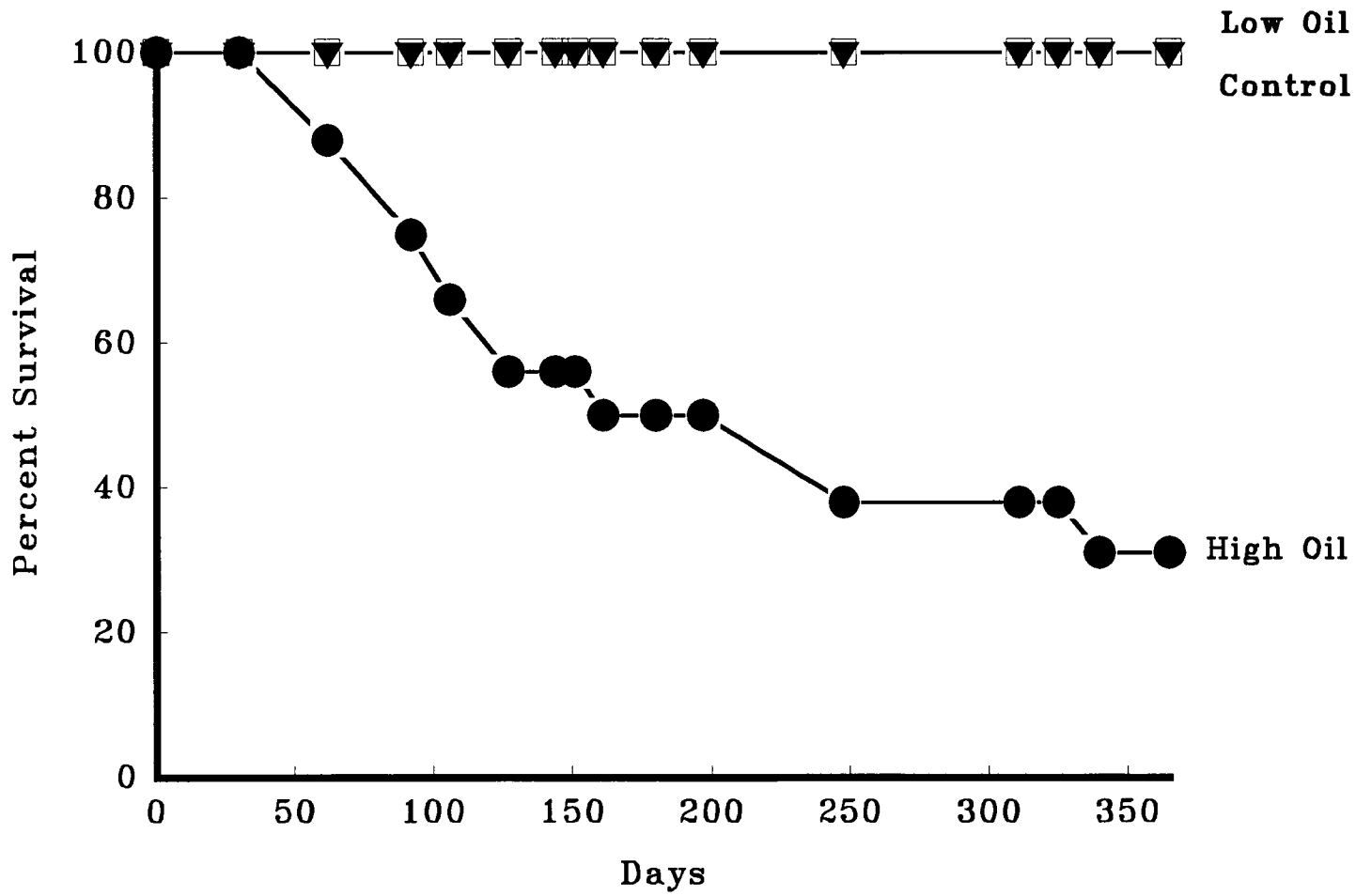

Figure 7. Percent survival of *R. mangle* saplings exposed to no oil (Control), Low Oil (1.6 L/m^2), or High Oil (16.0 L/m^2). The oil used is south Louisiana sweet crude.

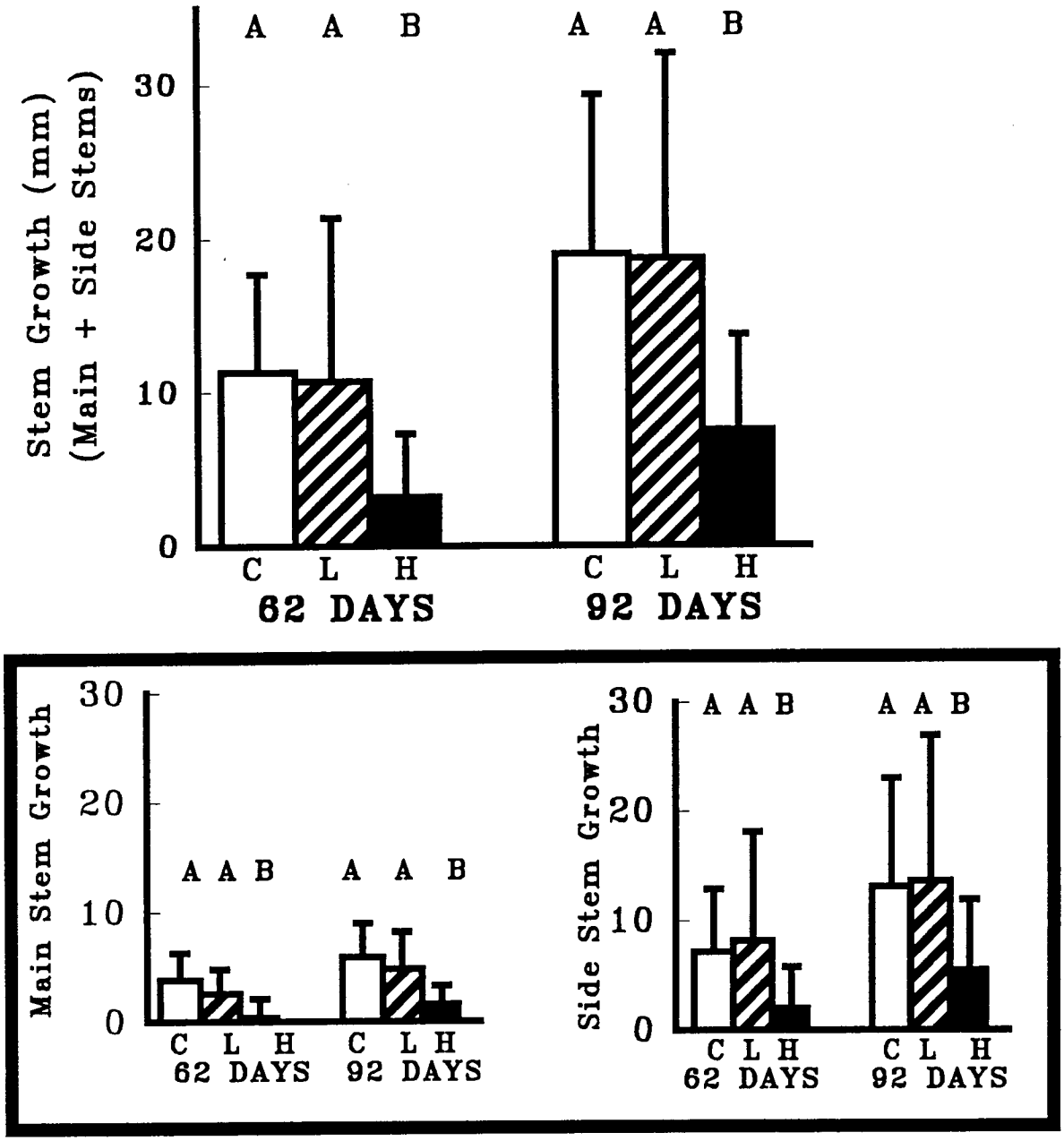

Figure 8. R. mangle sapling stem growth following experimental exposure to Louisiana sweet crude oil. C=control (no oil), L=low oil (1.6 L/m^2), H = high oil (16 L/m^2). Top graph is total stem growth. The bottom two graphs give the breakdown of total growth into main stem growth and lateral branch growth. Values are means and 1 S.D. Letters indicate signficant differences (one-way ANOVA and Tukey multiple comparisons tests).

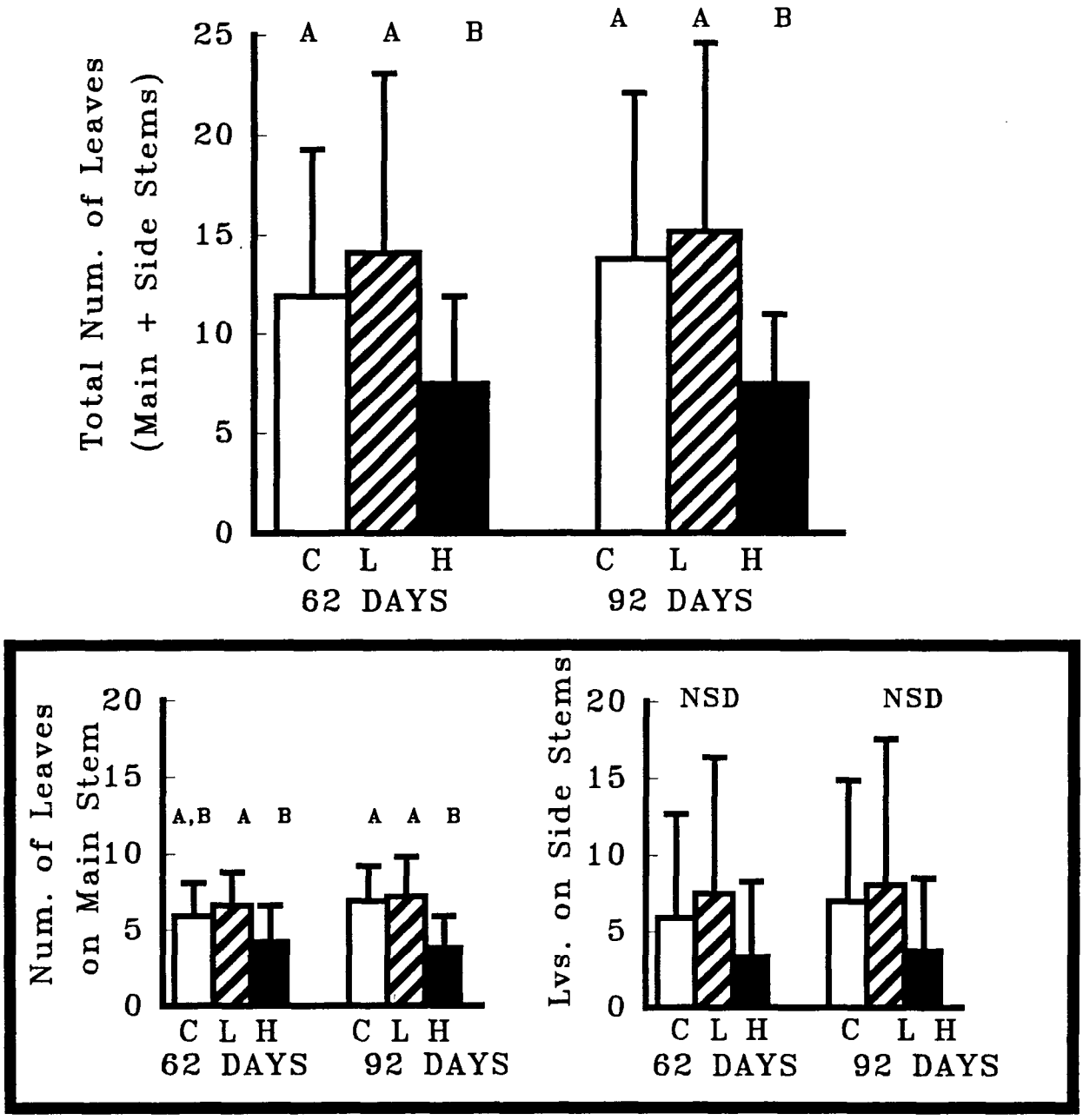

Figure 9. *R. mangle* sapling numbers of leaves at 62 and 92 days follfowing experimental exposure to Louisiana sweet crude oil. C_2=control (no oil), L=low (1.6 L/m^2). H=high (16.0 L/m^2). The top graph is total numbers. The of leaves per plant. The bottom two graphs give the breakdown of the total into leaves on main stems and leaves on lateral stems. Values are means and 1 S.D. Letters indicate significant differences (one-way ANOVA and Tukey multiple comparisons tests).

The 1993 Tampa Bay Spill: Preliminary Assessment of Natural Resources

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INTRODUCTION

At 5:50 am on August 10, 1993, two tank barges and a phosphate freighter collided at the mouth of Tampa Bay, between Egmont Key and Fort DeSoto Park. The collision caused an explosion igniting a fire on the Maritrans Barge Ocean 255, which continued to burn for 18 hours. Before they abandoned ship, the crew on the burning barge grounded the vessel at Ft Desoto Park, thereby saving the vessel and most of the nine million gallons of petroleum onboard. Approximately 300,000 gallons of mixed light product (diesel, jet A and gasoline) was missing from the Maritrans barge when it was lightered off. An estimated 30,000 gallons of this was discharged into Tampa Bay. The remainder allegedly consumed by the 18 hour fire onboard. The Barge B-155, owned by Bouchard Transportation, was struck by the Balsa 37 in the #1 port tank sustaining a rupture the size of a three story building, causing an instantaneous release of an estimated 328,440 gallons of the 5 million gallons of No. 6 fuel oil. The phosphate freighter Balsa 37, owned by the Tsacaba Shipping company of the Philippines sustained major damages to its starboard side. It was grounded off Egmont Key to prevent its sinking.

Initially, oil came ashore at Fort Desoto Park and Egmont Key impacting beaches, seagrass beds and mangroves. But the vast majority of the oil was carried up to 13 miles offshore and remained off Pinellas County for several days, before west winds carried the oil onshore the 14th of August. The oil was deposited above the mean high water line along 14 miles of beaches. It was also carried through several tidal passes up the Pinellas County coastline impacting commercial, residential and environmentally sensitive areas.

Immediate impacts included the closure of the Port of Tampa, portions of the Intra-Coastal Waterway, Fort DeSoto Park, Egmont Key State Park, and 14 miles of recreational beaches. Natural resource damages included the impacts to the water column, fisheries resources, mangroves, salt marshes, seagrass beds, birds, and sea turtles.

The most visible portion of the cleanup effort was focused on the beaches. Over 1,900 cleanup workers and hundreds of graders, bulldozers and dump trucks were on the beach at one time. The gross contamination was removed by hand with subsequent reworking by heavy equipment. The beaches were officially declared clean by Sept 6th for the Labor Day Weekend. Since that time 12 reoilings have occurred in response to storm fronts from the west. Subsequent cleanup efforts have taken from 2 to 7 days.

The back beach cleanup continued long past the Sept 6th date. Cleanup crews hot washed approximately 21 miles of seawall, innumerable docks and cleaned hundreds of vessels. Experienced crews were detailed to work the environmentally sensitive areas. Cleanup crews worked the John's Pass area until December. Areas of submerged oil have continued to be discovered, with the latest oil recovery operations occurring in June.

The Oil Pollution Act of 1990 (OPA 90) provides for the designation of natural resource trustees to act on behalf of the public to recover damages to natural resources. The Department of Environmental Protection as trustee for the State of Florida, the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Department of the Interior (DOI) as Federal trustees entered into a Memorandum Of Understanding to jointly perform a Natural Resource Damage Assessment (NRDA) in accordance with OPA 90. Under applicable laws the trustees are allowed to develop their own assessment strategies for each event. Florida's Pollution Discharge and Prevention Control Act provides a compensation schedule to calculate the natural resource damages for discharges less than 30,000 gallons. For spills greater than 30,000 gallons the responsible parties (RPs) have the option to request a formal damage assessment. Each vessel owner involved has opted for the formal scientific damage assessment.

The assessment approaches discussed were developed by the trustees, with participation and comment by the Potential Responsible Parties (PRPs) technical and legal consultants. Nothing in this document is meant to reflect a final determination of the facts of this incident or the final assessment, as the assessment activities are ongoing.

METHODS AND MATERIALS

Injury categories were identified as: mangroves, water column (finfish, plankton), birds, sea turtles, seagrasses, salt marshes, shellfish beds (biological and recreational use closures), bottom sediment injury, beach physical injury, shoreline lost use value, and surface water access.

Extensive amounts of baseline data on the natural resources of Tampa Bay exist. State and Federal environmental programs have collected and synopsized data concerning floral and faunal distribution, abundance, and population trends in Tampa Bay. Current land use, wetlands mapping, aerial photography, population and recreational use data were available from a variety of sources. At the time of the spill State and Federal studies pertaining to surf zone fishes, invertebrates, seagrass beds, turtle nesting activity, zooplankton and larval fish were ongoing. These studies were identified and the principal investigators contacted so that these studies could be expanded to include assessing the impacts of the oil spill. The National Pollution Fund Center made available video of the spill and associated response.

Most components of the damage assessment will be quantified using site specific information and analysis. However, for some injury components, conducting detailed site specific studies was determined not to be cost effective. These assessments will be determined through the use of applicable models.

Impacted habitats and their pre-spill condition was initially documented using historic aerial photographs. Since the spill two series of aerial real color and infrared photographs have been taken to document initial contact and post spill condition of mangroves, saltmarsh, seagrass, and shellfish systems.

A professional survey was performed to precisely measure the extent of impact. DEP coordinators flagged the extent of oiling for the survey crews. Lightly oiled areas were not included in the professional survey to minimize the impacts of survey activities.

SHORELINE RECREATION, SHELLFISH RECREATIONAL HARVESTING, SURFACE WATER AND PORT CLOSURES

The trustees have contracted for studies to assess the injuries resulting from the lost public use and/or nonuse of recreational beaches, recreational shellfish harvesting, surface water access and port closure. Preliminary studies will conclude in August, with the full study and final report scheduled to be completed in December 1995.

BIRDS

Injuries from oil included direct injury as a result of oiling, ingestion, and stress resulting from capture and cleaning, and indirect injury through habitat loss and disruption of nesting and foraging activities. Bird rescue efforts accounted for the recovery of 371 oiled birds of which 53 individuals were recorded dead on arrival, euthanized or died in treatment. 318 birds were released after rehabilitation. The trustees estimate that at least twice that number have been affected to date.

TURTLES

Four (4) loggerhead sea turtle (*Caretta caretta*) (federally threatened) hatchlings were killed. Twelve loggerhead sea turtles and one juvenile green sea turtle (*Chelonia mydas*, 25 cm carapace length) (federally endangered) were recovered oiled, cleaned, rehabilitated and released.

At the time of the spill, there were 115 known loggerhead nests on the Pinellas County beaches. Of these 96 nests were at risk. Fourteen nests had to be protected from oil by trenching and booming. Subsequently two nests were inundated by oil. Evaluation of the two oiled nests revealed that they contained a total of 176 unhatched and 9 hatched eggs. A third nest was partially destroyed by heavy cleanup equipment, resulting in the destruction of five loggerhead sea turtle eggs.

Twenty-nine nests hatched during the spill response. Hatchlings from 23 of these nests (about 1530 turtles) were restrained after nest emergence and released at oil free sites. About 413 hatchlings from 6 nests were not restrained and entered the water at sites where surface waters may have contained oil.

Although the number of sea turtles directly observed to be affected was small, these species are listed as Federal and State endangered or threatened species. Restoration options include funding of sea turtle management activities or stranding network activities.

MANGROVE

Mangrove impacts occurred in two areas; Bonne Fortune Key at Ft Desoto Park and on four keys inside John's Pass in Boca Ciega Bay. The mangroves impacted were within mature forests which included black mangroves (*Avicennia germinans*), red mangroves (*Rhizophora mangle*), white mangroves (*Laguncularia racemosa*) and buttonwood *(Conocarpus erectus*).

The trustees propose using a habitat replacement analysis to determine the amount of necessary restoration. The analysis provides an estimate of the amount of new mangrove acreage that will have to be created in order to compensate the public for both direct injury and the ecological services lost prior to the restored mangrove areas reaching baseline (pre-discharge) productivity. The biological parameters needed for the habitat replacement analysis include: extent of oiling; percent of ecological services lost initially; number of years to full natural recovery; the functional form (shape) of the recovery curve; years to full ecological service flow after creation; functional form (shape) of the "maturity" curve for the created habitat; relative productivity of created vs. natural mangrove habitat; and costs to create mangrove habitat. The inputs for these parameters will involve a combination of field measurements, literature values and professional judgment. Specific data needs were identified and contracts have been entered into to meet these requirements.

Prior to the commencement of the professional survey, DEP coordinators, Florida Game Fresh Water Fish Commission biologists, the survey project manager and the RP's biological representatives met onsite to discuss the methods and constraints on the survey due to nesting and roosting colonial waterbirds. DEP coordinators flagged the extent of oiling for the survey crews. Lightly oiled areas were not included in the professional survey to minimize the impacts of survey activities on the rookery. At least 5.5 acres of mangroves were either moderately or heavily oiled.

NOAA contracted with Coastal Zone Analysis (pers. com.) for a study to estimate the amount of injury to the mangrove islands directly or indirectly caused by the oil. Further to document the extent and nature of the injury, predict the long-term impacts and evaluate possible restoration alternatives. Since August field work has included:

1) Documenting percent of cover of oil and epibiota on submerged red mangrove prop roots, root shape and size and root density. Black mangrove pneumatophores were collected and length, percent cover of oil and epibiota recorded for each. Leaves were collected from the canopy for each species of mangrove and pressed.

2) Extent of oil penetration from the fringe inward was recorded.

3) Forest structure was recorded for both Eleanor and Rookery Key.

4) Seedling and sapling mortality was examined by marking juvenile red, black and white mangroves (n=650 plants tagged).

5) Toxicity of oiled sediments was examined by using common garden plot experiments (n=29 paired replicates, 522 propagules planted).

The University of South Florida and Mote Marine Laboratory (pers. com.) under contract with DEP will commence a study to assay the toxicity of the sediments to further address the ability to proceed with restoration activities. Five cores will be collected from oiled areas, as well as three cores from unoiled areas, and will be divided into two equal sections for analysis. Hydrocarbons will be extracted and separated into aliphatic and aromatic fractions using methods currently in use by USF and Mote.

WATER COLUMN

Over the course of its trajectory, the discharged oil cumulatively contacted approximately 300 square miles of open Gulf waters and 27 square miles of embayment waters. Elevated Total Petroleum Hydrocarbon (TPH) levels were recorded during the days following the discharge in water samples from several of the bay areas (Mote Marine Lab, pers. com.).

Due to the extremely difficult and expensive task of directly measuring injury to water column organisms, the trustees propose that models, including the Type A Model, be considered to assess water column injuries (e.g., finfish, plankton, etc.). DEP has collected a large amount of pre- and post-discharge data applicable to this injury. To the extent possible, site specific information will be used to validate the predictions of the model selected.

SALTMARSH

The discharge resulted in 0.85 acres of salt marsh vegetation being exposed to heavy oiling, including marsh vegetation within Boca Ciega Bay from mouth of John's Pass to Gulfport. Field surveys in April indicated that 0.75 acres were damaged.

SEAGRASS

The discharge resulted in the loss of approximately 2.5 acres of seagrasses from exposure to oil mats and response activities. Most of the heavy to moderate oiling of seagrasses occurred in Boca Ciega Bay in the area near John's Pass and southward. Additionally, 255 acres near Mullet Key, Egmont Key, and in Boca Ciega Bay near John's Pass, are known to have been exposed to the floating oil slick from the discharge.

DEP's professional survey of impacted seagrass beds included a number of different elements. Those areas which had been manually cleaned (vacuumed) prior to the survey. Areas

where oil was still present as patchy mousse patties within the seagrass beds (often covered by silt) and areas mechanically denuded by the boat\barges and other cleanup equipment and activities. All three of these area types were included within the seagrass survey.

The Technical Working Group attempted to evaluate the extent of remaining submerged oil within seagrasses in early January 1994 by transect survey methods. While trace amounts of oil were noted during the survey, no areas of gross contamination were detected at that time. Subsequent to that survey submerged patties have been documented in the area and four different cleanup efforts initiated by the RPs. This month the USCG has contracted with Oceans Systems for a diving survey of Blind Pass to document and map the submerged patties in the channel. Submerged oil remains a persistent problem.

Aerial photography, historical and ongoing research activities are being used to further document seagrass bed loss. Comparison of historical and post-discharge studies of seagrass community structure may be used to evaluate the significance of the floating oil exposure for the additional 255 acres.

Prior to the spill, Hall and Bell (pers. com.) had an ongoing research project with sites within the spill trajectory. At the trustees request these investigators revised their sampling dates and sampled sites within the spill area on August 13th. Observations made suggested that Egmont Key was the site most directly affected by oil although an oil sheen was present at Ft. Desoto (east beach site). *Thalassia testudinum* was the seagrass present at all sites except for Bunces Pass and Ft. Desoto where *Halodule wrightii* was dominant. All sites were sampled August 13th and midday December 14-January 27 1994. At each site samples were collected for fish, macroepifauna and blade dwelling meiofauna. Three throw traps were haphazardly tossed into each site. From within each trap, three samples of seagrass blades were collected according to the methods of Hall (1988). Fish and macroepifauna were collected by dipnetting and seining both with a 3.2 mm mesh net. Repetitive dipnetting was conducted until five dipnets in a sequence failed to capture any fish. Approximately 95% of all shrimp are also collected by this procedure (Bell and Fonseca, unpubl.). After dipnetting the traps were seined until three successive passes failed to capture any fish. This technique efficiently captures both the macroepifauna and the small (15-60 mm standard length) size class of fish in seagrass beds. In addition to these samples, three benthic cores were collected from each trap and archived.

SHELLFISH BEDS

Preliminary shellfish studies focused on public health concerns involving shellfish consumption. Three areas within the projected spill trajectory were closed to shellfishing on August 10. Lower Tampa Bay (Manatee Co.) remained closed until September 23, 44 days after the spill. At that time clams in the Mullet Key area were found to still have continued elevated levels of hydrocarbon contamination so that the south Boca Ciega Bay shellfishing area remained closed until November 30, 112 days after the spill. The oyster sample collected in John's Pass was found to be grossly contaminated by oil (Sherblom and Pierce 1993). This organism had significant tissue burdens of not only the n-alkanes, but also polycyclic aromatic hydrocarbons

and other cyclic compounds. The body burden of these organisms was thought to be of sufficient concentration that detrimental effects were likely to be observed in the impacted population. The oil related components found in this sample were not only potentially toxic to the oysters themselves, but would also be detrimental to any organisms preying on the stressed oysters.

The University of South Florida and Mote Marine Laboratory under contract with DEP will commence a study to assay the residual oil within the oyster bed to help address the ability to proceed with restoration activities. Live oysters and shell hash will be collected at three places on each of two oyster beds. Live oyster tissue will be analyzed for contamination and shell hash will be analyzed for residual oil within the oyster bed.

BEACH PHYSICAL INJURY

Beaches from Indian Shores in central Pinellas Co. to Egmont Key on the southern tip of Pinellas Co. were oiled. As a result of cleanup activities associated with this oiling at least 39,827 cubic yards of sand were removed from public beaches. Beach impacts were documented via the State survey, aerial real color photos and video. Under a State permit issued during the spill response, the cost of sand replacement must be paid by the responsible party. Natural resources associated with beach oiling impacts are assumed to be short term in nature and the replacement of the sand on the beaches will fully compensate for these impacts. Other impacts are being assessed indirectly by a surf zone fish study which was in progress at the time of the spill and is still in progress.

SEDIMENTS

Several areas of submerged oil in contact with the subtital sandy sediments just off Pinellas County beaches, as well as in mudflats and in deeper areas of Boca Ciega Bay were documented. Surveys conducted during spill response indicated that at least 58,540 square feet (1.3 acres) of subtidal sediments had been covered by submerged oil patties or mats. Subsequent reoilings on the beach and within Boca Ciega Bay indicate that submerged oil continues to spread and move, exposing and re-exposing additional areas. This oil is well weathered and relatively insoluble, thus minimizing toxicity from external exposure, but physical smothering in areas of these deposits would result in injury to sediment associated biota. Ingestion of oil particles or oil contaminated sediments would also result in injury to the sediment associated biota.

The trustees have determined that it is not technically feasible to determine by direct survey the extent of the remaining submerged oil in the Gulf of Mexico and Boca Ciega Bay. Alternative assessment approaches include: 1) calculation of the potential amount of oil remaining unaccounted for, using a mass balance approach, or 2) applying specific oil fate models which can be used to estimate the amount of injury to the biota, as well as the value of the injury if appropriate.

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Environmental Distribution of Oil-Related Hydrocarbons Following a Spill of Number 6 Fuel Oil in Tampa Bay

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INTRODUCTION

In the early morning on August 10, 1993, approximately 1.2 million liters (320,000 gal) of Bunker C (No. 6) fuel oil spilled from the fuel tanker *Bouchard 155* after it collided with the jet fuel barge *Ocean 255*, and the phosphate freighter *Balsa 37* in a shipping channel at the entrance to Tampa Bay, Florida. The position of the leaking tanker was N 27°36.41', W 82°40.30' at 0600 hours on that morning. The collision caused explosions, fire and a 27 km long oil spill.

The *Balsa 37*, carrying 6000 tons of phosphate, was outbound in a shipping channel leading from Tampa Bay, Florida, the nation's seventh busiest port. It cut left across the bow of the *Ocean 255*, carrying 30 million liters (7.9 million gallons) of jet fuel, which was passing to the left of the *Bouchard 155*. Both vessels were headed inbound when the collision occurred with all three vessels. The *Ocean 255* exploded and burned sending flames over 100 feet in the air. The highly volatile jet fuel quickly burned or vaporized apparently leaving no lingering fuel. The *Balsa 37* sustained no serious damages and no phosphate cargo discharge. However, the *Bouchard 155* suffered severe injury, and spilled 7600 barrels of highly viscous, high molecular weight Bunker C fuel oil. The barge began leaking at the point of impact, but came to rest near the Sunshine Skyway Bridge which spans the mouth of Tampa Bay, Florida. Although winds and currents carried the majority of the spilled oil out of Tampa Bay and into the Gulf of Mexico, significant quantities were deposited on nearby beaches and in mangrove and seagrass habitats. Subsequent changes in wind direction brought the oil back to Gulf beaches and estuarine habitats north of the mouth of Tampa Bay.

The distribution and fate of oil entering the marine environment depends on properties of the oil and the environmental conditions. The complex mixture of petroleum components is altered (weathered) through processes such as evaporation, dissolution, vertical dispersion, emulsification, sedimentation, photo- and microbial degradation (Farrington 1980, NRC 1985, Volkman *et al.* 1992). Weathering of the entire mixture of compounds occurs simultaneously, however, the hydrocarbon profile changes over time due to differential degradation/dissolution of the various chemical compounds. Petroleum hydrocarbon alteration from weathering can be summarized as follows: loss of low boiling ($< nC_{22}$) hydrocarbons through evaporation and dissolution, biodegradation of the *n*-alkanes with a proportional increase in the percent of the

unresolved complex mixture (UCM) and polycyclic aromatic hydrocarbons (PAH's), followed by a subsequent shift in the relative abundance within the (UCM) towards higher molecular weight compounds (Farrington 1980, Van Vleet *et al.* 1984, Pierce *et al.* 1986, Burns *et al.* 1993).

On August 11, 1993 (one day following the Bunker C fuel oil spill from the *Bouchard 155*), a cooperative University of South Florida (USF), Florida Institute of Oceanography (FIO), United States Geological Survey (USGS) cruise was carried out in the area to collect sediments from the area of the spill. At the same time, Mote Marine Laboratory of Sarasota, Florida, was carrying out a similar study in the area on water and shellfish. USF has continued a year long monitoring of sediments in some of the affected areas, particularly Eleanor Island, a protected mangrove island inside Boca Ciega Bay. In addition, Mote Marine and USF have entered into a joint post-spill assessment of this island.

METHODS AND MATERIALS

Water Sampling Sites and Collection

Distribution and transport of the oil in the water column was investigated by collecting near-surface (0.3 m depth) water samples from 20 sites throughout the mouth of Tampa Bay and the near-shore Gulf of Mexico on August 12, 1993 (Figure 1). Samples were also collected from 2 m depth at three of the sites, to assess the potential for vertical mixing of the oil. Near surface water samples were collected from an additional 12 sites through out Bunces Pass and Johns Pass on August 17, 1993, to observe redistribution of the oil by wind and surface currents. A patch of the spilled oil with associated seawater was collected on each of the above dates to assist in identification of the source material. A sample of the barge oil was obtained from The National Oceanic and Atmospheric Administration, Office of Damage Assessment, for comparison with environmental samples.

Water samples were collected by siphoning through precleaned silicone (silastic) tubing into clean, 4-liter amber glass bottles. Surface tar ball and oil slick samples were collected by submersing the mouth of the bottle half-way into the slick/water to skim the oil and associated water from the surface.

Water Sample Extraction and Analyses

Samples were stored over ice for transport to the laboratory, spiked with recovery surrogates (octadecene, $C_{18:1}$ for aliphatics, and deuterated *p*-terphenyl for aromatics), and stored under refrigeration until extraction. Storage time was less than 24 hours for all water samples except the two oil-water mixed samples, which were refrigerated for several days before extraction. For the oil-water samples, the aqueous phase was separated from the overlying oil either by passing it through a pre-cleaned glass wool plug (August 12), or by carefully pipetting the water into a separatory funnel, avoiding the transfer of any visible oil droplets (August 17). Oil solutions were prepared for chromatographic analysis by diluting a weighed amount of oil (4-6 mg) with hexane in a 10 ml volumetric flask.

All water samples were extracted three times with dichloromethane (DCM). Extracts were reduced in volume using rotary evaporation; samples which still contained water were placed in small separatory funnels and the aqueous phase was rinsed (3x) with DCM, which was collected and further reduced in volume. The concentrated extracts were passed over anhydrous

sodium sulfate and exchanged into hexane. The hydrocarbon fraction was isolated using combined silica gel/alumina columns (12 g/6 g) slurry packed into chromatography columns. The extract was applied to the column in 0.5 ml hexane and the hydrocarbons were eluted using 20% DCM in hexane.

The hydrocarbon fraction was reduced to near dryness with N_2 and taken up in a solution containing a quantitation standard (docosene, $C_{22:1}$ and *o*-terphenyl) and analyzed by capillary gas chromatography with flame ionization (GC/FID). Chromatographic analyses were performed with a Varian 6000 gas chromatograph with a flame-ionization detector. The column was a 30 m J&W DB5, with He carrier gas and a temperature program of 45°C to 280°C at 6°C/min, holding at 280°C for 30 min, interfaced with a Perkin-Elmer/Nelson 2600 chromatography data system and a 486 computer for data compilation, analysis and presentation.

Efficiency of hydrocarbon extraction and recovery from the water samples was determined by comparison to the known amounts of reference surrogates (RS) added to each water sample prior to extraction. Quantitation of the extractable hydrocarbons was provided by normalization to the known amounts of quantitation standards (QS) added after extraction. Selected confirmatory analyses were performed with a Varian Saturn II ion trap GCMS using a J&W DB5-MS capillary column.

Sediment Sampling Sites and Collection

Surface sediments (approximately top 5 cm) were collected near the spill site in lower Tampa Bay on August 11, 1993 (1 day following the spill) and on September 1, 1993 (approximately three weeks after the spill). Twelve different sampling sites were chosen (six each day) for sediment collection in order to investigate the transport of oil to sediments near the spill (Figure 2B). Four days after the spill (August 14, 1993), oil began to wash ashore along Pinellas County beaches located to the north. Oil was also carried through John's Pass into Boca Ciega Bay where it fouled several mangrove islands. The island that was oiled most was Eleanor Island, located just inside John's Pass (Figure 2A). Oil globs were first collected along this island on August 25, 1993 (approximately two weeks after the spill). Oil globs were then collected from this same site approximately every 2 weeks through June 10, 1994. The purpose of this portion of the study was to investigate the chemical weathering of the Bunker C fuel oil over time in this subtropical environment. As of the June 10 collection date, intact oil patches (1-3 cm thick) were still widely distributed along this portion (and along other portions) of the island.

Sediment Extractions and Analyses

Approximately 100 g (wet weight) of each sediment sample collected near Egmont Key were extracted by refluxing with 2:1 (v/v) dichloromethane:methanol. Internal standards (5α-androstane and *o*-terphenyl) were added to each sample prior to extraction for quantitative hydrocarbon analysis. The extracts were separated into F1 (aliphatics/saturated) and F2 (aromatics/unsaturated) fractions by silica gel-alumina column chromatography, and eluted with hexane for the F1 fraction and 1:1 hexane:toluene for the F2 fraction. Each oil (tar) sample collected from Eleanor Island was dissolved in hexane, in the presence of an internal standard, and separated by column chromatography as above.

All hydrocarbon fractions were analyzed by high resolution gas chromatography (GC) using a Shimadzu GC-14A equipped with a 25 m DB-5 fused silica column and flame ionization

detector, using hydrogen as the carrier gas. Oven temperature was programmed from 80°C to 280°C at 4°C/min and held at 280°C for 10 minutes. The peaks in the F1 fraction were identified by comparing peak retention times with a known alkane standard or by co-injection with authentic standards. A sample of the Bunker C fuel oil taken from the *Bouchard 155* by the Coast Guard, was analyzed by GC for matching the alkane characteristics of the Bunker C fuel oil with those found in the samples. Total F2 hydrocarbon fractions were analyzed by GC and quantitated by comparison of the total F2 area with the area of the internal standard. Selected F2 fractions were analyzed by combined gas chromatography-mass spectrometry (GCMS) for qualitative and quantitative identification of individual PAH's. Samples were analyzed by electron impact ionization (70 eV) using a Finnigan INCOS-50 GCMS system interfaced with a Hewlett Packard 5890 gas chromatograph. Gas chromatographic conditions were the same as those used above, however helium was used as the carrier gas. The mass spectrometer was scanned from mass 40-500 in 0.5 sec. All mass spectral data was compared to spectra produced by authentic standards and by comparison to previously published spectra.

RESULTS AND DISCUSSION

Water Samples

Characterization of the oil is provided by a gas chromatographic (GC) hydrocarbon profile of the No. 6 fuel oil collected from the barge, *Bouchard 155*. The results of the total hydrocarbon (THC) profile are shown in Figure 3, along with the profile of a tarball collected from the mouth of Tampa Bay 8/12/93, and a sample of a larger oil patch collected in the Gulf 8/17/93. The original oil sample exhibited a *n*-alkane distribution from nC_{10} to nC_{32}, with the most abundant *n*-alkanes in the range of nC_{16} to nC_{22}. The UCM, consisting of branched and cyclic alkanes and aromatic/olefinic hydrocarbons was most pronounced throughout the nC_{24} to nC_{32} *n*-alkane range. These chromatograms show that the tarball and oil patch samples had lost some of the lower molecular weight hydrocarbons ($< nC_{14}$) observed in the original No. 6 fuel oil sample, while maintaining the higher molecular weight components, characteristic of the original oil.

Although the tar ball and oil patch samples retained most characteristics of the source oil, changes in hydrocarbon composition in water, due to rapid dilution and complex weathering processes, hinder the ability to definitively characterize the oil. This is exemplified in the hydrocarbon composition of the two aqueous phases collected with the above oil samples (Figure 4). The aqueous water samples show completely different hydrocarbon profiles from the overlying oils and from each other, representing different mechanisms for oil dispersion into the water column. The sample collected on the 12th of August contained primarily the lower molecular weight fractions (nC_{12}-nC_{22}), with a predominance of the UCM and no definitive *n*-alkane homologous series. This sample is representative of the water-soluble fraction of the oil with the majority of the *n*-alkane homologous series degraded. The August 17th sample shows a higher molecular weight distribution of the alkanes and the UCM than that exhibited in the August 12 sample. Comparison of the aqueous phase hydrocarbons with the overlying oil reveals loss of the lower molecular weight *n*-alkanes as well as the lower boiling components of the UCM. The presence of predominantly higher molecular weight compounds in the water would suggest the presence of weathered oil particles dispersed in the aqueous phase (possibly

colloidal). The peak labels in these chromatograms indicate the following compounds: RS 1: 1-Octadecene; QS 1: *o*-terphenyl; QS 2: 1-Docosene; RS 2: *p*-terphenyl-D14.

The distribution and concentrations of total extractable hydrocarbons from water samples are given in Figure 5 for samples collected August 12, and in Figure 6 for samples collected August 17. These results show that the spatial distribution of oil-related hydrocarbons was patchy, ranging from purely biogenic hydrocarbons to a predominance of petrogenic hydrocarbons. The total hydrocarbon concentration is shown for each sample. Those representative of primarily biogenic hydrocarbons are indicated with a B. Biogenic hydrocarbons are distinguished by discrete sets of alkanes such as pristane, pentadecane and heptadecane. Petroleum hydrocarbons are identified by a homologous series of *n*-alkanes, with no predominance of odd or even carbon numbers, approximately equal ratios of pristane and phytane and numerous aromatic and olefinic hydrocarbons exemplified by an unresolved complex mixture (UCM) on the chromatographic trace (Farrington 1980, Van Vleet *et al.* 1984, NRC 1985, Pierce *et al.* 1986).

Examples of hydrocarbon profiles in water samples are illustrated in Figure 7. The hydrocarbon profiles in Figure 7 are indicative of marine biogenic hydrocarbons with no petroleum impact, dissolved-weathered oil with a predominance of the UCM, and a high molecular weight distribution of *n*-alkanes, indicative of weathered oil particles as opposed to dissolved hydrocarbons, respectively, top to bottom.

Of the 23 samples collected from twenty sites on the 12th of August, three contained below detectable limits of hydrocarbons (<0.5 μg/L), six had predominantly biogenic (B) hydrocarbons with concentrations <5 μg/L. The remaining sixteen samples contained primarily petroleum hydrocarbons exhibiting dissolved/dispersed hydrocarbons and hydrocarbon profiles representing suspended particles of weathered oil in concentrations up to 46 μg/L.

Vertical distribution of the oil was observed at three stations by collecting samples from both 0.3 m and 2 m water depths. Stations 13 and 17 exhibited higher hydrocarbon concentrations at depth, demonstrating that the deeper waters were also impacted by oil. Station 15 showed petroleum hydrocarbons in the near-surface water while biogenic hydrocarbons were found at the 2 m depth. The profiles observed at these stations (oil and weathered oil) indicated that oil was well-dispersed into the water column at certain sites.

Subsurface currents flowing in different direction from surface movement could have led to separation between the surface oil slick and hydrocarbons in the lower water column, resulting in a wider distribution of hydrocarbon contamination than could be observed by following the movement of the surface slick. The contrary is also true, water movement under the surface slick could bring water with low hydrocarbon content into areas visibly impacted by the surface slick. Thus, an area bathed in low hydrocarbon content water when sampled would not necessarily avoid exposure during a different tidal cycle.

The water samples collected on August 17th gave a wide range (from <0.5 to 39 μg/L), similar to that observed for the August 12 samples. The John's Pass area was visibly oiled and high concentrations of weathered oil were observed in water samples from the west side of Eleanor Island, with lower concentrations found in the water at other locations (Figure 6). Bunces Pass had no visible oiling, however, there were stations with hydrocarbon concentrations and profiles indicating contamination by suspended oil particles (Figure 6). Patchy contamination of the benthic environment was evident from globules floating just above the bottom among the seagrass beds in Johns Pass, however, these oil globules were not collected for this study.

The presence of petroleum hydrocarbons in water samples throughout the mouth of Tampa Bay and the near-shore Gulf of Mexico following the oil spill provide an indication of the oil transport and potential for short term ecological impact in the water column. Clams (*Mercenaria merceneria*) were collected from the Bunces Pass area on September 29, 1993, to assess shellfish contamination from oil distributed in the water as opposed to direct oiling. The high molecular weight distribution of *n*-alkanes was observed in several of the clams, indicating uptake of oil-containing particulates (Sherblom and Pierce, unpublished data).

Sediment Samples

Petroleum hydrocarbons were present in both fractions (F1 and F2) in all sediments collected near Egmont Key following the spill. Alkanes in the range from nC_{15} to nC_{32} were quantified separately in order to estimate the contribution of Bunker C fuel oil to the sediments. These alkane concentrations characteristic of Bunker C ranged from 8 μg/g to 41 μg/g (dry weight sediment; Figure 2B). *n*-Alkanes derived from the Bunker C fuel oil comprised approximately 30-90% of the total F1 hydrocarbons in these samples. The contribution of Bunker C fuel oil *n*-alkanes to sediments taken on August 11 (1 day after the spill) averaged 28.8 $\pm$ 13.2 μg/g while those collected on September 1 (three weeks post-spill) averaged 14.5 $\pm$ 4.1 μg/g. While the hydrocarbon concentrations found three weeks after the spill were lower than those found the day after the spill, this does not necessarily represent a temporal decrease in the concentrations of hydrocarbons in the sediments resulting from the *Bouchard 155* spill. The differences in hydrocarbon concentrations may have resulted from collecting the samples in slightly different areas.

Previous studies on sediments from the mouth of Tampa Bay have indicated that background levels of hydrocarbons in sediments from this area average less than 10 μg/g and normally show a hydrocarbon pattern characteristic of biogenic contributions with little petrogenic input to sediments in this area (Van Vleet and Reinhardt, 1983; Van Vleet *et al.* 1986). A gas chromatogram of the hydrocarbons isolated from sediments collected on August 11 is shown in Figure 8. Although the alkane distribution is clearly petrogenic, this chromatogram shows that *n*-alkanes below nC_{20} were not incorporated into the sediments. Visible oil was not observed in these sediments (*i.e.* no large tar particles or globs). Transport of hydrocarbons to these sediments appears to have resulted from adsorption to particles in the water column with subsequent transport to the bottom. Lower carbon-numbered *n*-alkanes are more soluble and less readily adsorbed to particles than the longer chain alkanes. Hence it is not unusual that the lower carbon-numbered alkanes are preferentially removed prior to deposition, and the *n*-alkane maximum has shifted from nC_{21} to nC_{26} (Farrington 1980, Burns *et al.* 1993, Volkman *et al.* 1992). Due to the lack of any significant petrogenic signal in sediments reported by other studies from this area, and also due to the sheer mass of oil present at the time of the spill, the petrogenic hydrocarbons observed in these sediments can be attributed to release from the *Bouchard 155*.

Approximately four days after the initial spill (August 14, 1993), oil washed ashore and coated the mangroves of Eleanor Island just inside John's Pass (Figure 2A). The distribution of oil visible in surface sediments along the mangrove fringe decreased through December, 1993, but then appeared to remain fairly constant through March (R.A. Davis, unpublished data). Samples were collected approximately biweekly since the spill at one of the most heavily

oiled sites along the mangrove fringe (northern face of island's northeast quadrant) to investigate chemical changes in the oil due to weathering in a subtropical sediment.

Gas chromatograms of the F1 fraction of oil collected from Eleanor Island in December, 1993, and June, 1994, are shown in Figure 9 along with a chromatogram of the F1 fraction of the *Bouchard 155's* Bunker C fuel oil prior to spillage. From these chromatograms, it can be seen that much of the lower carbon-numbered *n*-alkanes have been weathered from the oil, although the *n*-alkane maximum remains at nC_{21} throughout the 10 months following the spill. The reason for the difference in the *n*-alkane maximum in the bulk oil versus the oil found in the sediments taken near Egmont Key is likely due to the mode of transport and preservation. The deposition of oil in the sediments near Egmont Key appears to have been by adsorption onto particles followed by deposition, a process which will preferentially enrich the sedimentary hydrocarbons in higher carbon-numbered alkanes. The oil present on Eleanor Island, however, is simply the remains of the original oil following dissolution, evaporation, and microbial degradation. There was a direct transport of the intact oil to these intertidal sediments; hence there was no preferential enrichment due to adsorptive transport processes. Clearly, the bulk portion of the oil has retained a large portion of its *n*-alkanes even though it has been exposed to tidal flushing and water temperatures as high as 31 °C. Although higher temperatures might be expected to increase the solubility and volatilization of the hydrocarbons, as well as to increase microbial degradation rates, it is clear that the bulk of the oil, which is unexposed, is protected against these enhanced rates and the chemical nature of the oil is largely retained.

Figure 10 shows the gas chromatogram of a sample taken from the outer surface of an oil glob collected on April 5, 1994. This suggests that the *n*-alkanes in the outer surface of the tar have been preferentially microbially degraded relative to the isoprenoids pristane and phytane. This preferential degradation of *n*-alkanes has been shown to occur in several other studies of petroleum degradation (NRC 1985). The percentage that nC_{28} comprises of the total alkanes in the nC_{15}-nC_{32} range is shown in Figure 11. As can be seen from this figure, the nC_{28} percentage falls into two general ranges, those making up approximately 14-19% and those making up approximately 3-7%. Samples with nC_{28} percentages falling into the higher range (14-19%) were all found in samples where the bulk oil was dissolved and analyzed. Samples with nC_{28} percentages falling into the lower range 3-7%) were all found in samples taken from the outer surface of an oil glob. These percentages result from the fact that the nC_{28} and other *n*-alkanes are being degraded relative to the isoprenoid and branched alkanes on the outer portion of the tar. This further illustrates that the outer portion of the oil is preferentially degraded while the inner, bulk portion of the oil retains more of its original chemical signature.

Combined gas chromatography-mass spectrometry was used to investigate the distribution of polycyclic aromatic hydrocarbons in the oil obtained from Eleanor Island following the *Bouchard 155* spill. Although several other PAH's were investigated, the most abundant PAH's observed in the oil were the C_0-C_4 homologs of naphthalene, fluorene, phenanthrene, pyrene, and chrysene. The C_0-C_4 homolog distribution of each of these were very similar in the bulk oil obtained from Eleanor Island following the spill to the Bunker C fuel oil obtained directly from the *Bouchard 155*. Even the most volatile naphthalene series was still present with approximately the same relative homolog distribution throughout the study (Figure 12). However, relative to the higher four-ring PAH series (pyrene and chrysene), the two and three ring aromatic hydrocarbon series (napthalene, phenanthrene, and fluorene) decreased in relative concentration throughout the study (Figure 13). Nonetheless, significant concentrations of all PAH's were still present in the oil for at least 10 months following the spill.

Several other spills of Bunker C fuel oil have occurred in the marine environment, however most of these spills took place in temperate or cold water climates. These include the *Arrow* spill (1.3 million liters) in Chedabucto Bay, Nova Scotia (Michalik 1971, Scarratt 1972, Vandermeulen 1977, Vandermeulen *et al.* 1977, Keizer *et al.* 1978), the *Metula* spill (2.1 million liters of Bunker C spilled with 53 million liters of crude oil) in the Strait of Magellan (Hann 1977), the San Francisco Bay spill (3.2 million liters; Chan 1977), the *Sansinena* spill (5.1 million liters) in outer Los Angeles Harbor (Soule *et al.* 1978), the *Blue Magpie* spill (0.3 million liters) in Yaquina Bay, Oregon (Kemp *et al.* 1986), and the *Nestucca* spill (0.9 million liters) off the Washington coast (Strand *et al.* 1992). No reports have been found of major spills of Bunker C fuel oil in subtropical or tropical environments prior to the *Bouchard 155* spill investigated in this study.

In studies following several of the spills in cold water environments, acute toxicity and lethal effects of Bunker C fuel oil were observed. Effects on the softshell clam, *Mya arenaria*, following the *Arrow* spill, included lower total numbers, fewer mature adults, lower tissue and shell growth rates, lower carbon assimilation rates, and poor recovery (Gilfillan and Vandermeulen 1978). Following the *Sansinena* spill, Soule and Oguri (1978) observed reduced phytoplankton productivity and reduced benthic populations. Kemp *et al.* (1986) observed reduced populations and lowered recruitment of the amphipod, *Rhepoxynius abronius* following the *Blue Magpie* spill. In addition, in a laboratory study on the toxicity of Bunker C fuel oil to cod eggs and larvae, Kuhnhold (1978) observed reduced survival, retarded growth, loss of buoyancy, abnormal development and lower heartbeat rates.

Based upon the persistance of the aromatic hydrocarbons in the Bunker C fuel oil recovered 10 months after the *Bouchard 155* spill in the present study, it is clear that the toxicity of the oil can persist in the Tampa Bay environment for at least several months. Since there was no indication of a significant reduction of these PAH's in the bulk oil found in the intertidal sediments, it appears that this oil may remain toxic in the environment for substantially longer than the 10 months observed in this study. The higher annual temperatures found in this subtropical environment do not seem to mediate the degradation, dissolution, or volatilization of the oil when deposited in large masses. Studies are continuing to assess the longer term fate of this oil.

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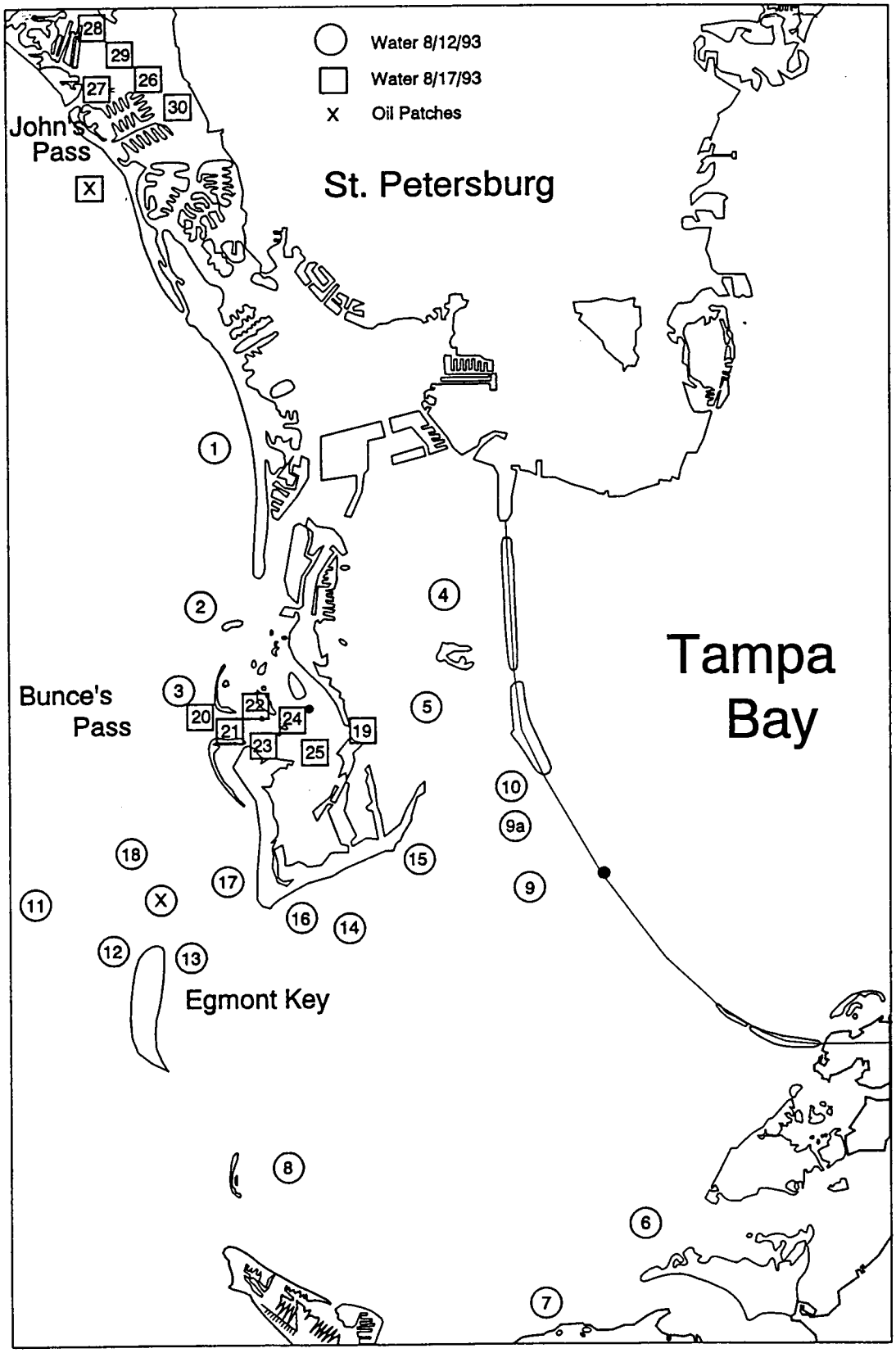

Figure 1. Location of water sample collection sites in the vicinity of the 8/10/93 Tampa Bay oil spill.

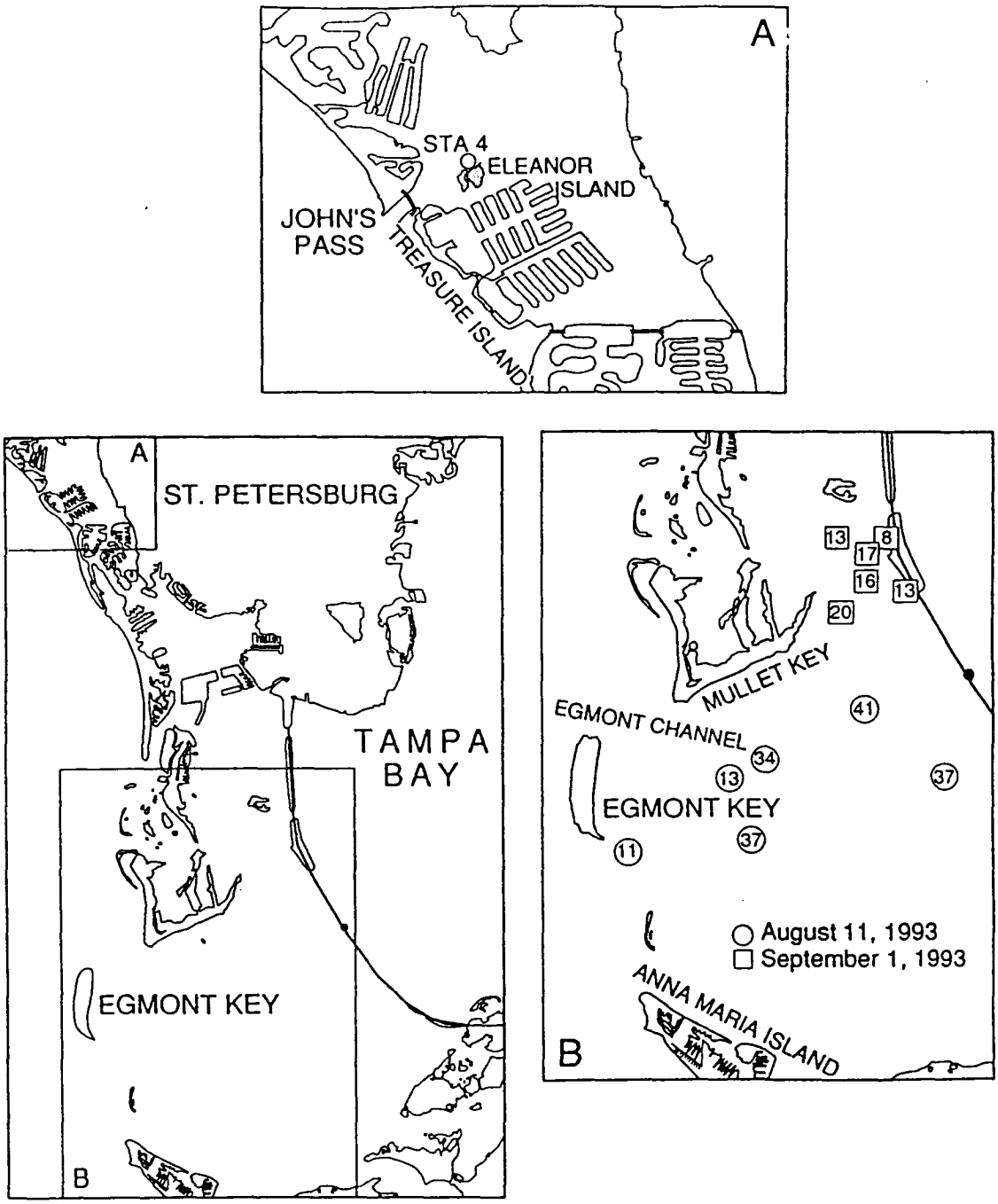

Figure 2. Locations of sampling sites for sediments collected (A) near the spill, and (B) in the mangrove island intertidal sediments. Concentrations are shown for each site in terms of μg Bunker C fuel oil/g dry weight sediment.

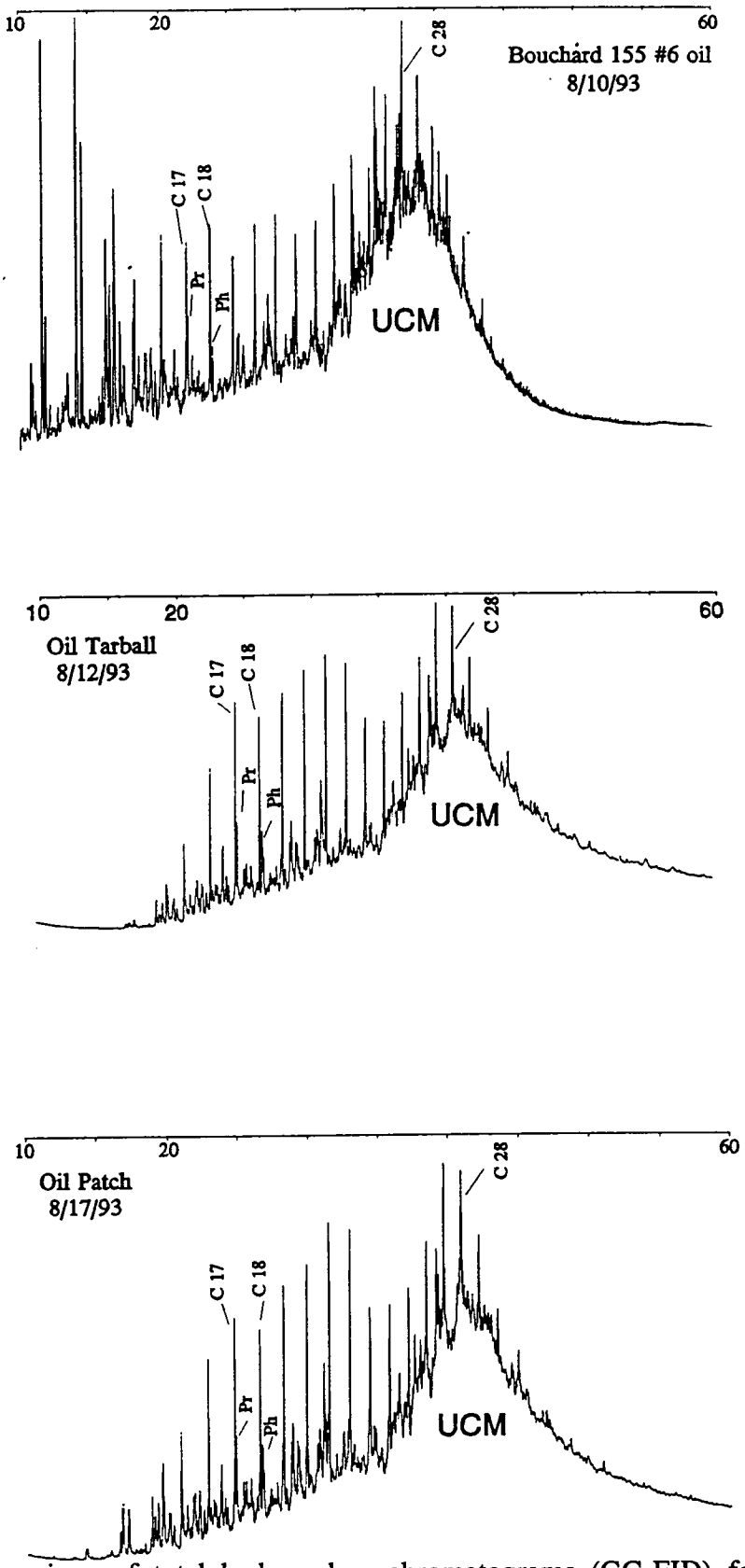

Figure 3. Comparison of total hydrocarbon chromatograms (GC-FID) for oil from the barge, *Brouchard 155*, oil patches collected from the mouth of Tampa Bay (8/12/93), and from near-shore Gulf of Mexico (8/17/93).

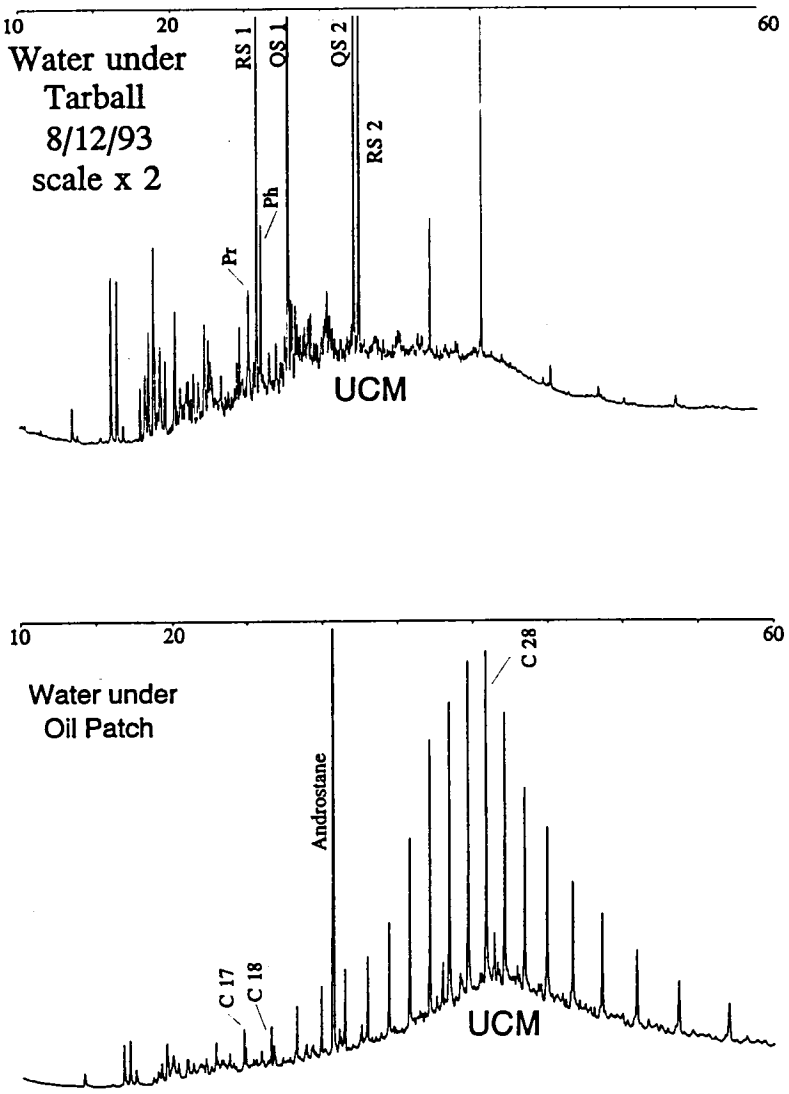

Figure 4. Comparison of total hydrocarbon chromatograms (GC-FID) for water samples collected along with a tarball from the mouth of Tampa Bay (8/12/93) and with an oil patch from near-shore Gulf of Mexico (8/17/93).

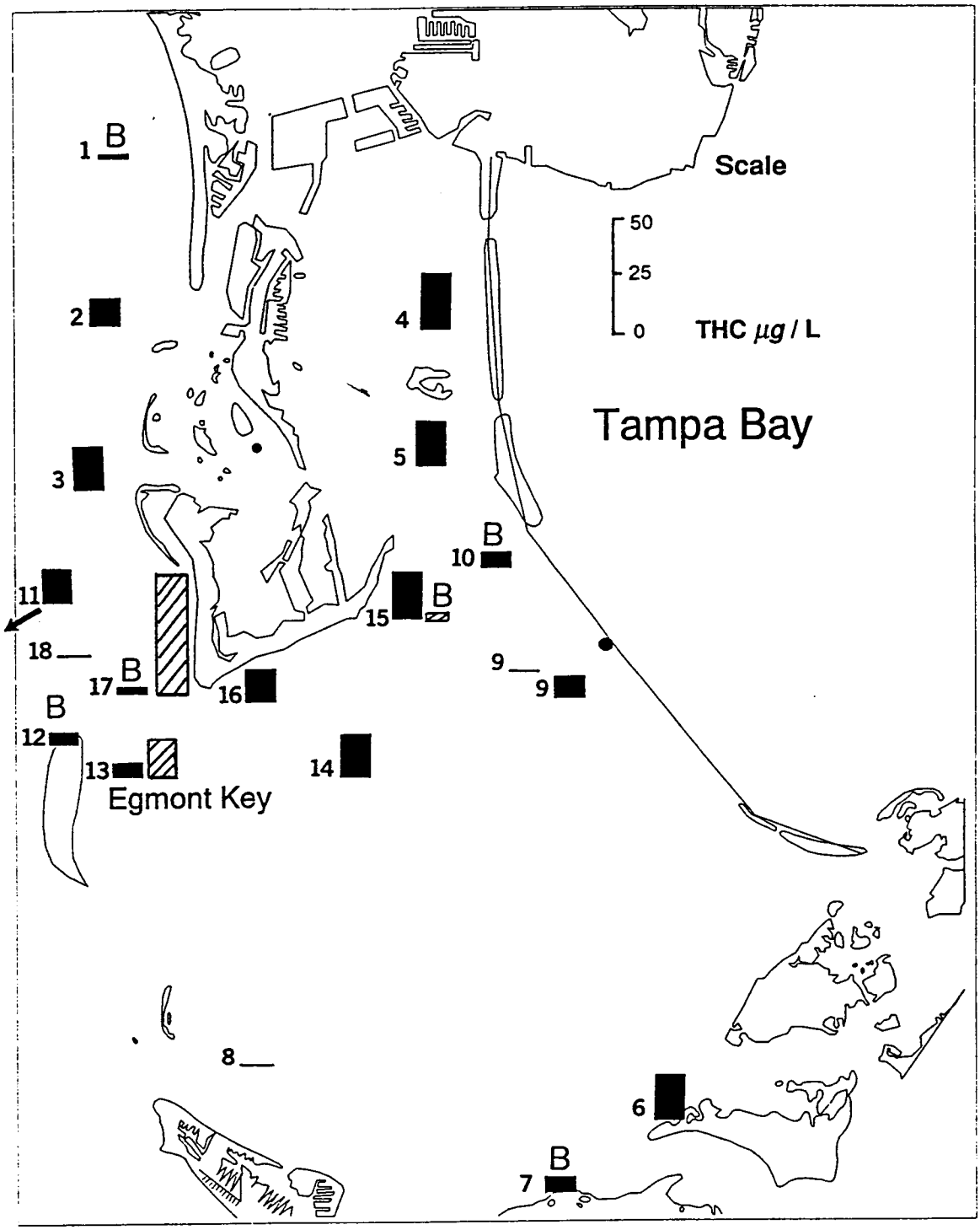

Figure 5. Total hydrocarbon (THC) concentrations in water samples collected 8/12/93, reported as µg/L.

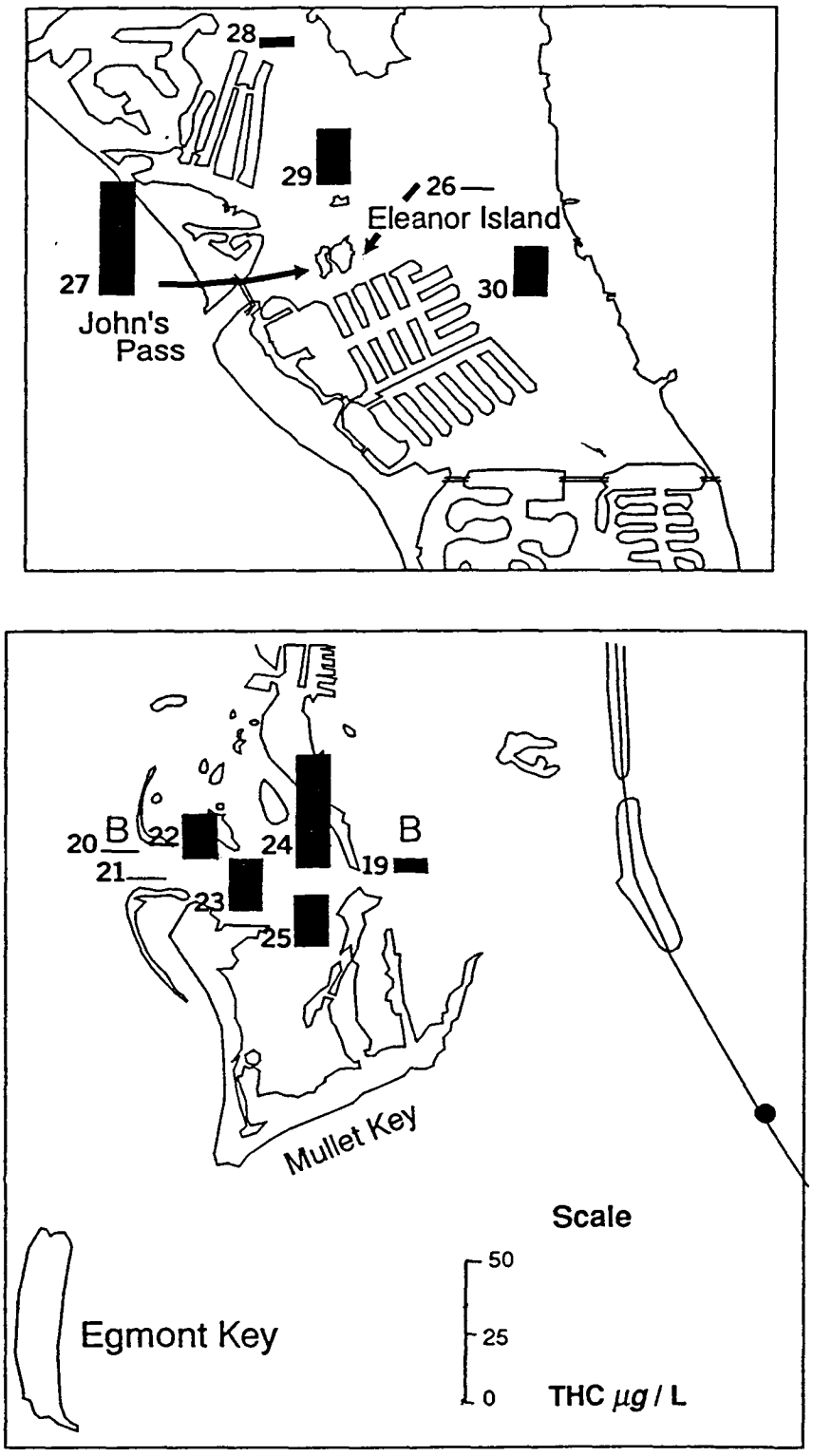

Figure 6. Total hydrocarbon (THC) concentrations in water samples collected 8/17/93, reported as μg/L.

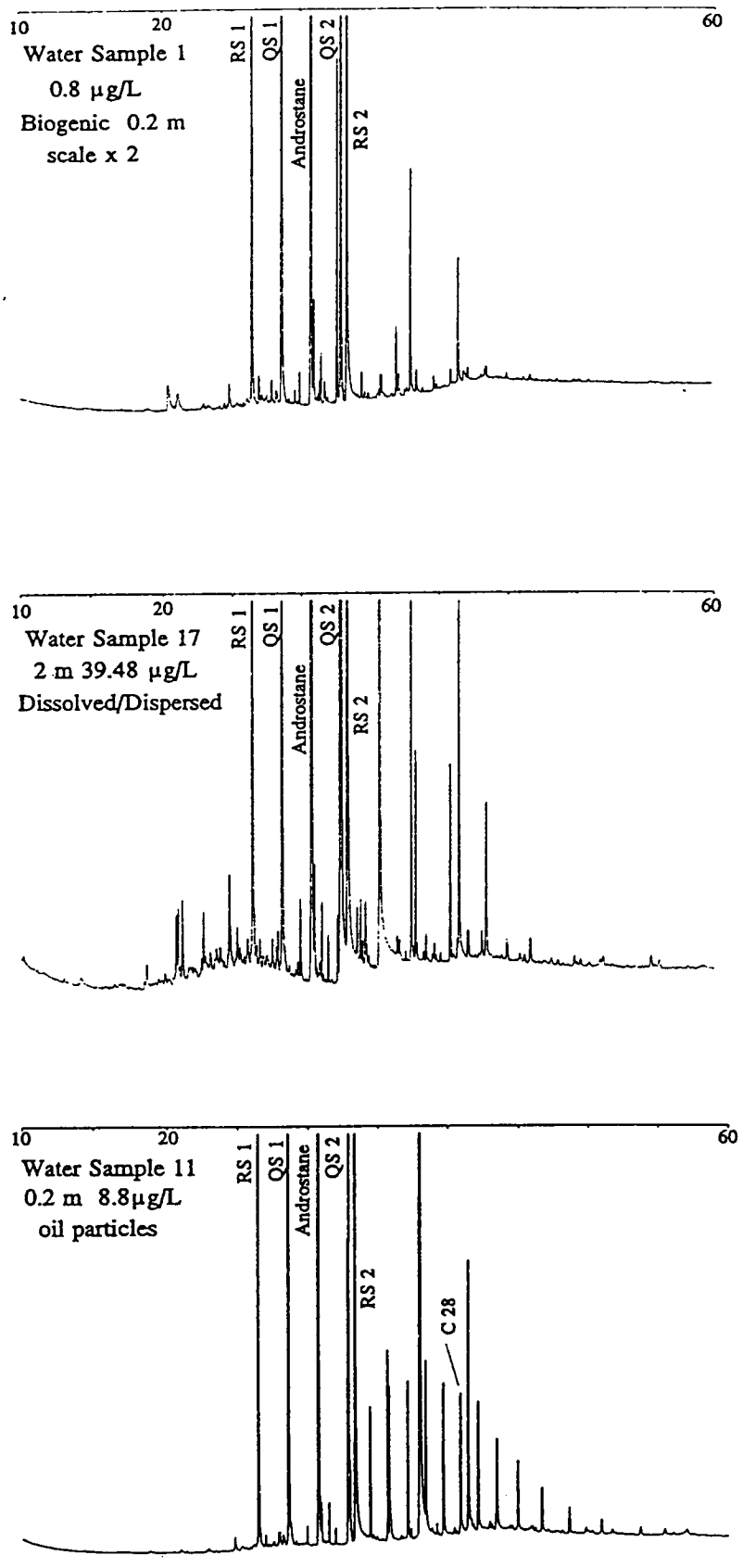

Figure 7. Comparison of total hydrocarbon chromatograms (GC-FID) representative of water samples collected throughout the Tampa Bay oil spill area.

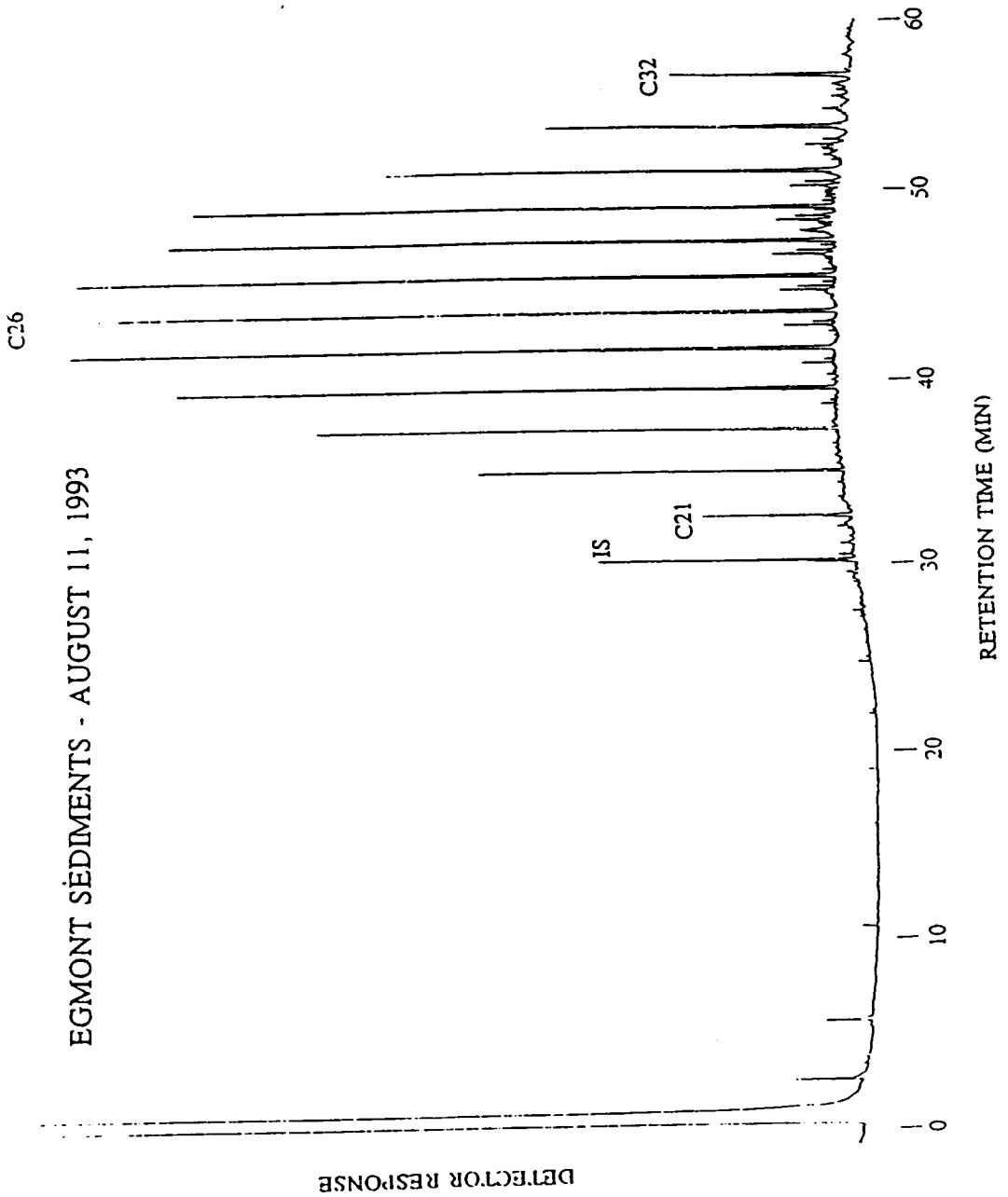

Figure 8. Gas chromatogram of F1 (alkane) fraction of hydrocarbons isolated from sediments near Egmont Key one day after the spill. IS = Internal Standard.

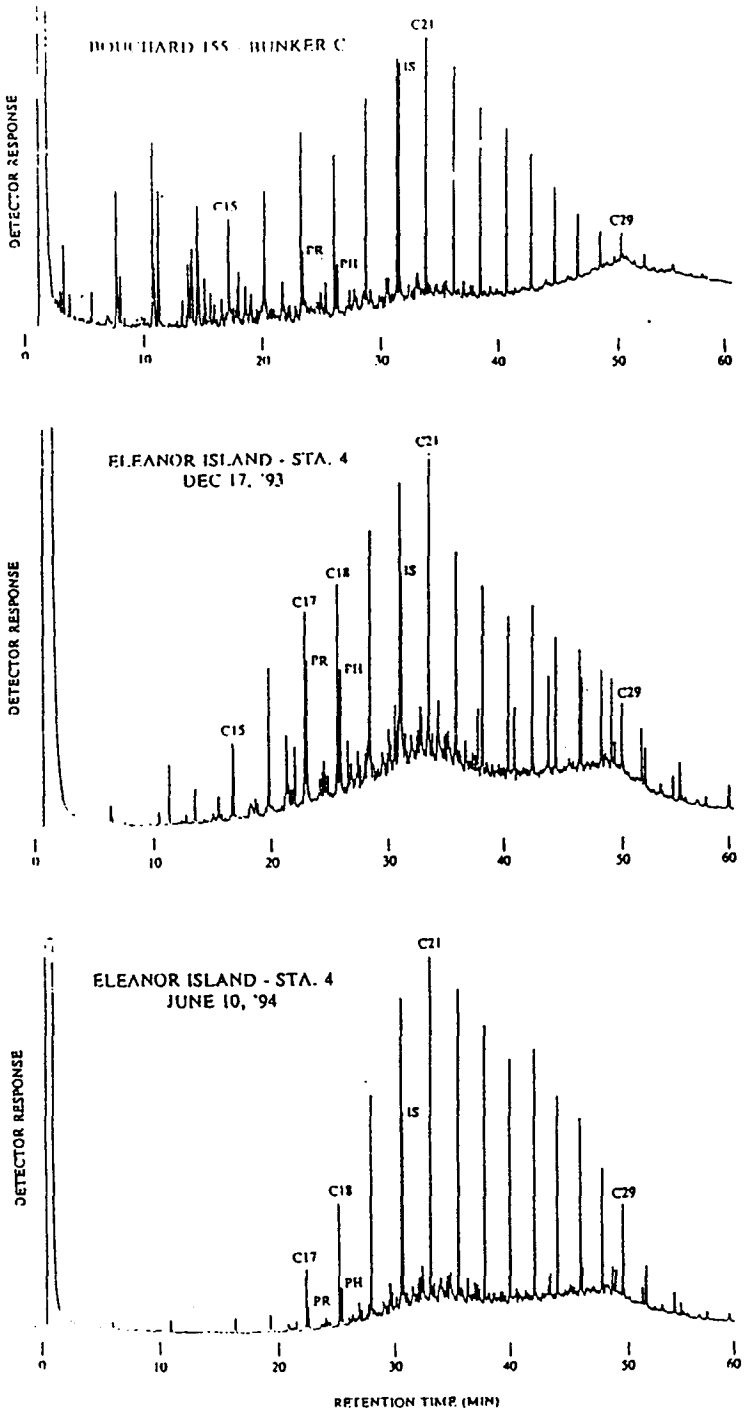

Figure 9. Gas chromatograms of F1 (alkane) fraction of hydrocarbons isolated from (A) Bunker C fuel oil carried by the *Bouchard 155*, (B) tar taken from Eleanor Island on December 17, 1993, and (C) tar taken from Eleanor Island on June 10, 1994. IS = Internal Standard. PR = Pristane. PH = Phytane.

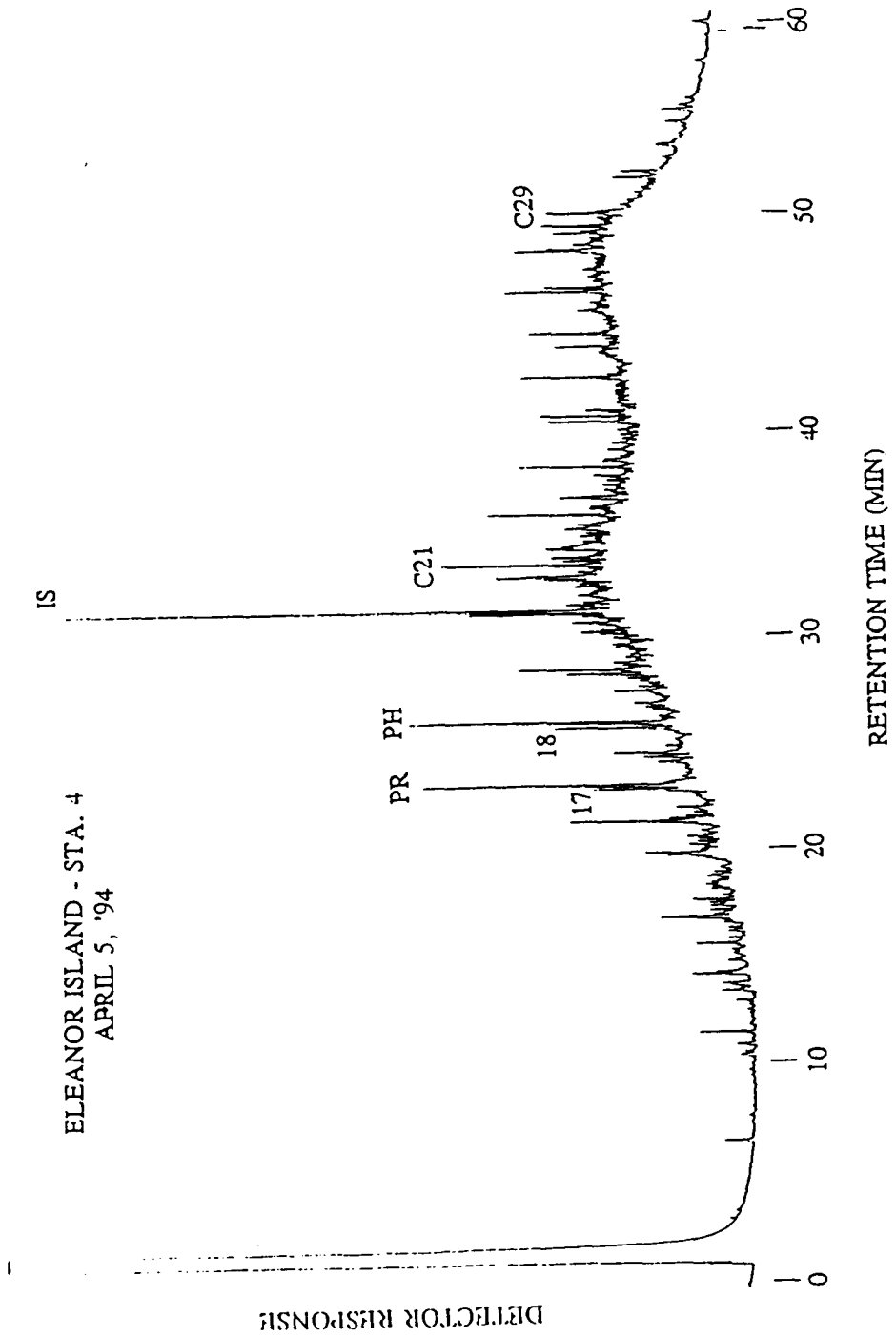

Figure 10. Gas chromatogram of F1 (alkane) fraction of hydrocarbons isolated from the surface of tar taken from Eleanor Island on April 5, 1994. IS = Internal Standard. PR = Pristane. PH = Phytane.

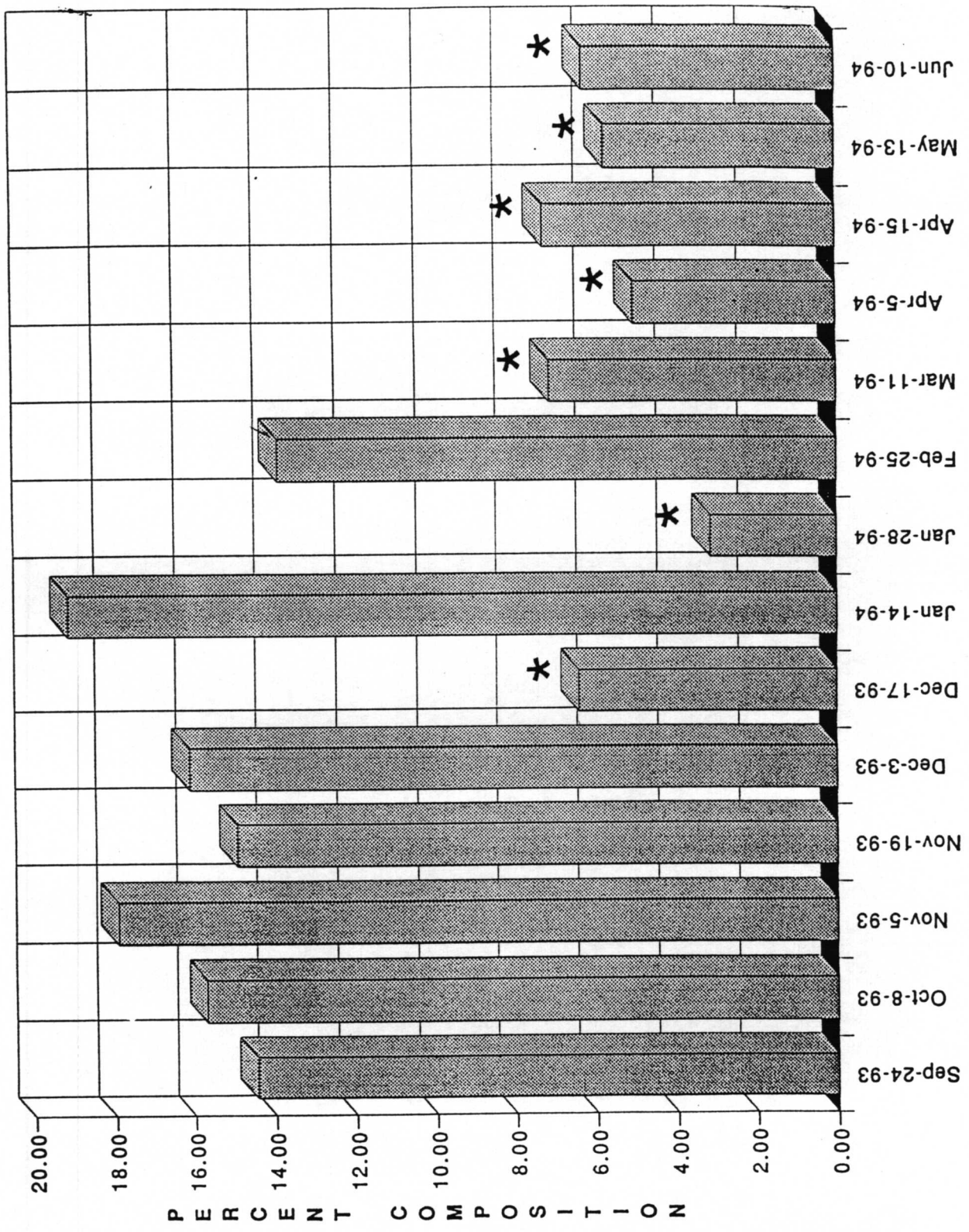

Figure 11. Percentage of nC_{28} in the nC_{15}-nC_{32} *n*-alkane range isolated from tar taken from Eleanor Island on different sampling dates. Asterisks indicate samples taken from surface of tar. All other samples were of the composite bulk tar.

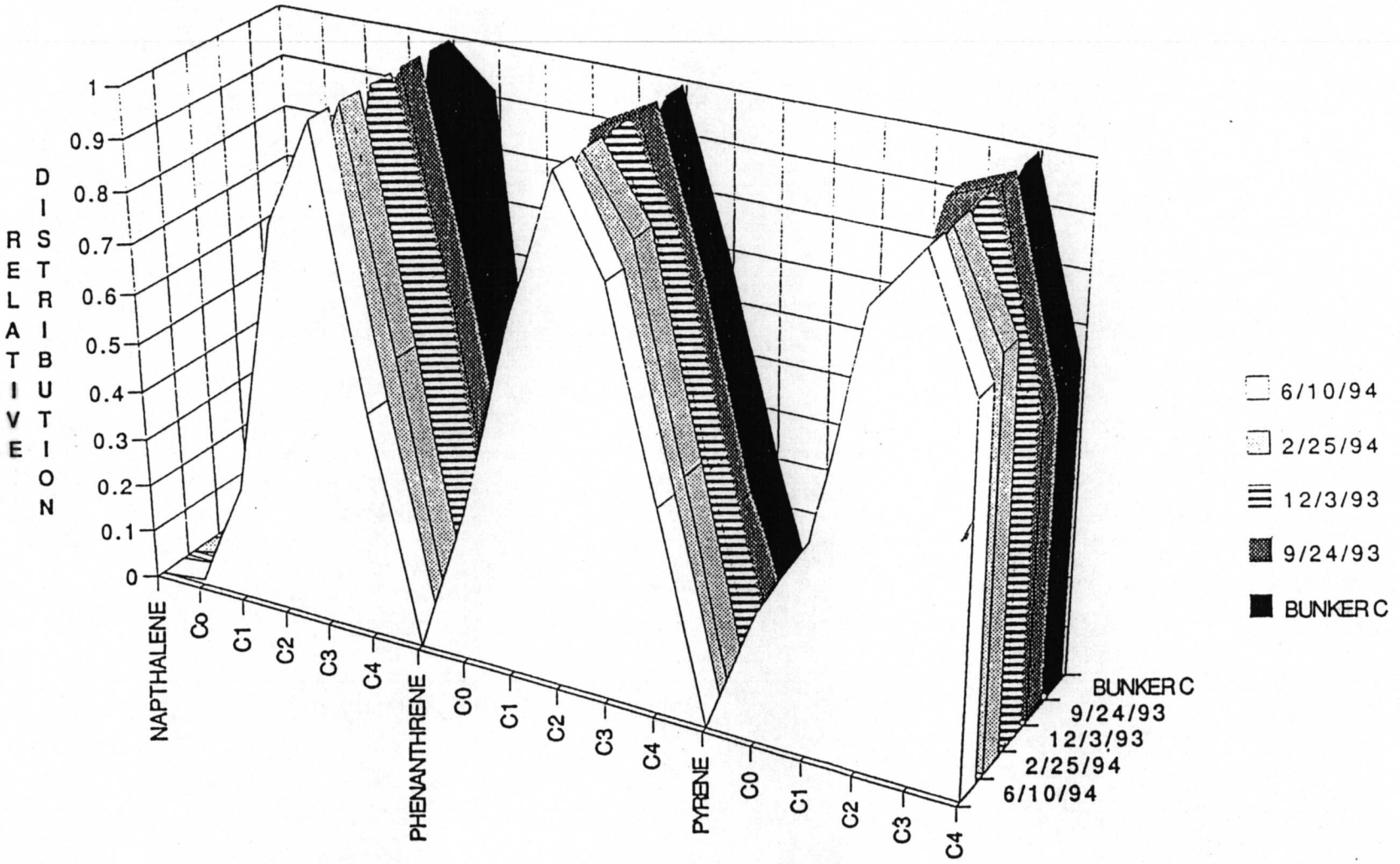

Figure 12. C_0-C_4 homolog distributions of naphthalenes, phenanthrenes, and pyrenes in F2 fractions of tar collected on 4 sampling dates throughout the study (see legend). The homolog distributions are also shown for Bunker C fuel oil for comparison.

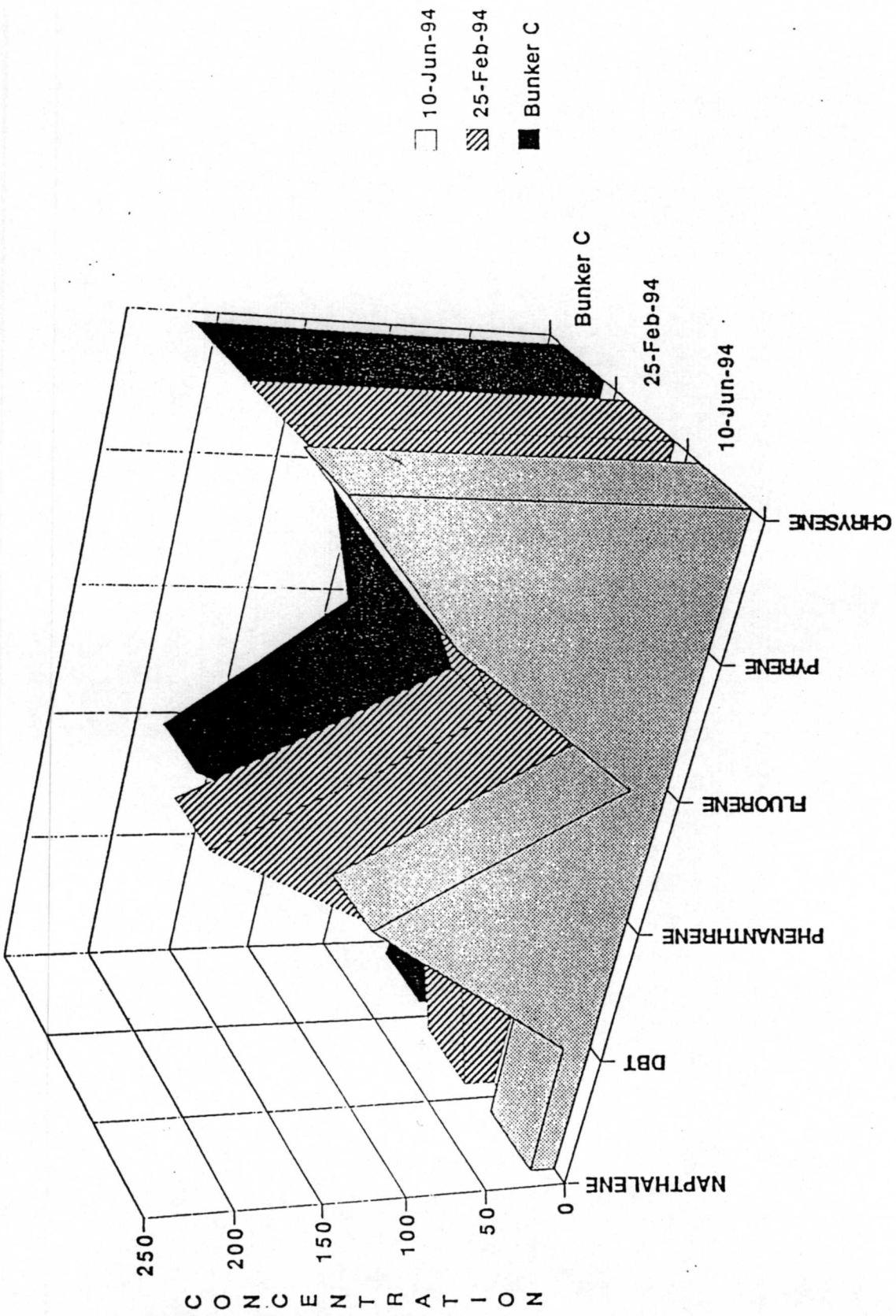

Figure 13. Concentrations (in μg PAH/g oil) in samples taken on February 25, 1994 and June 10, 1994. Concentrations in the intact Bunker C fuel oil carried by the *Bouchard 155* are also shown for comparison.

Effects of Oil on Salt Marshes

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INTRODUCTION

Coastal salt marshes are valuable resources. Salt marshes are composed of various plant communities and associated bay water, bayous and tidal creeks with many animal inhabitants. The lower intertidal zone dominated by smooth cordgrass (*Spartina alterniflora*) is one of the most important plant and animal communities of a tidal marsh because of its importance to estuarine processes. Because of daily tidal flooding it is a habitat that is often impacted by oil spills originating from tankers, pipelines, or storage tanks. Thus, an understanding of the effects oil spills may have on the habitat and functions of marshes is important.

It is valuable as a nursery habitat because young and juvenile stages of fish and shellfish utilize this plant community zone for protection and to derive nutrition (McHugh 1968; Lindall and Saloman 1977; Peters *et al.* 1978; Herke 1971; Zimmerman and Minello 1984). Some scientists estimate that from 63 to 99 percent of the commercially important fish and shellfish of the southern Atlantic and Gulf Coasts utilize estuaries and their associated wetlands during their life cycle (McHugh 1968). Some species such as brown shrimp (*Penaeus aztecus*) strongly select for the smooth cordgrass habitat (Zimmerman and Minello 1984; Zimmerman *et al.* 1984).

The smooth cordgrass habitat is important in protection of shorelines from wave erosion (Webb 1977; Woodhouse *et al.* 1972). This species grows in the lower intertidal zone where wave action can erode shorelines. The plant biomass produced ranks as one of the highest in the world. The biomass produced appears to provide significant amounts of detritus to the estuarine ecosystem (Odum *et al.* 1972). The detritus provides the base to an energy pathway that is important in many estuarine systems. It also enhances water quality through immobilization of nutrients and filtering of heavy metals and toxic materials from the water column (Getter *et al.* 1984).

Despite the high priority for protection in oil spill response plans (Lindstedt-Siva 1977; Gundlach and Hayes 1978), salt marshes are frequently impacted by oil spills (Crow 1974; Hershner and Moore 1977; Hampson and Moul 1978; Webb *et al.* 1981). The reason for the frequent oil spill impacts is that the Gulf Coast of the U.S. is a highly utilized tanker transportation route, a high exploration and producing zone, an area with many refineries, and an area with many oil storage tanks.

Since the marshes have high value and the U.S. Gulf Coast has high oil exploration and production activities an understanding of the effects of oil on salt marshes is important in planning for spill events. Plants form the habitat in which animals live, therefore, an understanding of the response of plants to oil is imperative in any planning. This paper

reviews the past work by the author and others in development of an understanding of plant response to oil spills.

METHODS

Our first evaluations of the effects of oil spills occurred in 1977 as crude oil flowed into a Galveston Bay smooth cordgrass marsh following a barge collision at the junction of the GIWW and the Houston Ship Channel (Webb *et al.* 1981). Experimental studies in smooth cordgrass marshes were conducted from 1981 through 1986 by spraying oil on 1 m^2 plots. Effects of type of oil, seasonal effects, and height of coverage of plants were evaluated (Alexander and Webb 1985; Webb *et al.* 1985). Specific effects of No. 2 fuel oil and residual toxicity effects on smooth cordgrass were evaluated by recording initial response and regrowth into center and edge portions of large plots in 1983 (Webb and Alexander 1991). Clean-up techniques were evaluated in a separate study from 1985 to 1986 (Kiesling *et al.* 1988). The effects of various amounts of crude oil on sections of a salt marsh along Dickinson Bayou were evaluated in 1986 (Alexander and Webb 1987). In 1991 the effects of the Apex oil spill, clean-up, and oil-remediation were evaluated (Webb 1993).

RESULTS

Types of Oil

Oil types that can affect salt marshes vary from crude oil to light refined oils. Four general types are recognized. Three of the four types were evaluated in experimental studies.

Crude oils and heavy fuel oils can vary in specific gravity, amount of sulfur, and other properties. However, they are generally the same in overall effects on plants (Figure 1). Oils that have been evaluated include Arabian Crude, Libyan Crude, Isthmus (Mexico) crude, and No. 6 fuel. Little toxic effects to plants appear to occur unless oil penetrates into the soil and chronic toxicity to the plants occurs as roots are continuously exposed to oil. Oil spill studies at Dickinson Bayou indicated that in actual spills the oil may accumulate in the soil causing long-term effects on growth.

Lighter and more refined oils such as No. 2 fuel oil are extremely toxic to smooth cordgrass. This type oil acts as a contact herbicide with some translocation capabilities to the rootstock.

Amount of Plant Coverage

Tidal conditions can greatly affect the oil coverage on plants and the amount of oil dispersion throughout a marsh system. The amount of plant coverage is very important in determination of effects on plants and animals.

With crude oils smooth cordgrass is affected primarily by coverage of the surface of plants. Respiration and photosynthesis are blocked when oil coats plants. The aboveground portion of smooth cordgrass is generally killed only when oil covers all plant surfaces.

Regrowth from rootstock generally will occur soon after death of aboveground portions of the plants (Figure 2). Plant recovery can become a problem if new shoots must penetrate a continuous mass of oil or sediment contamination by oil occurs in significant amounts.

In experimental studies No. 2 fuel oil was applied to 4-m^2 plots of smooth cordgrass as four treatments (control, sediment only, sediment plus lower plant surfaces, and sediment plus entire plant surfaces). Damage to plants was much greater with complete coverage of plants (Figure 3). With complete coverage of foliage by oil, above-ground plant material died rapidly and regrowth from rootstock did not occur. With partial coverage of stems, some discoloration of plants occurred but little difference in stem density occurred between controls and treated plots. When oil was applied to the sediment alone, stem density was only slightly reduced. The reduction in stem density apparently resulted from oil penetrating the soil to the roots. No accumulation of hydrocarbons in the sediments occurred.

Regrowth of smooth cordgrass did not occur from the rootstock when plants were killed by complete foliar coverage of the No. 2 fuel oil. Regrowth did occur with lower stem coverage and sediment only coverage by oil. The conclusions were that No. 2 fuel acts as a contact herbicide. Some oil accumulation in roots and rhizomes after spraying of complete foliage indicates that some translocation of the oil from foliage to roots and rhizomes can occur, preventing regrowth from rootstock in many instances.

Seasonal Effects

Seasons play a role in determination of damage to salt marshes. Damage to smooth cordgrass was more detrimental during the spring than fall. During the spring plants are actively growing and have presumably used available stored nutrients. During the fall plants are beginning their dormancy and many flowering stems are dying. Regrowth the following spring does not appear to be greatly reduced by the oil spill.

Oil Amounts

Most of our studies were designed to test initial effects of an oil spill. Sufficient oil was applied to completely cover sediments and/or plants, generally 1 to 2 liters per m^2, depending on whether coverage was intended for sediments or plants and sediments. Oil generally dispersed with tides, leaving little to penetrate sediments and cause long-term damage. In actual spills the oil often accumulates on shorelines in large quantities. Complete death of large areas of smooth cordgrass generally occurs only when high levels of crude and heavy fuel oils accumulate in the sediments or remain within the marsh for long periods of time (Holt *et al.* 1978; Alexander and Webb 1987; Krebs and Tanner 1981). As indicated in our studies of plant coverage, the constant presence of oil can coat new stems and cause chronic effects on plants. Refined oils such as No. 2 fuel oil spilled in large amounts can destroy large salt marsh plant communities (Hershner and Lake 1980; Hampson and Moul 1978).

Oil Spill Cleanup

Following a spill that impacts a salt marsh, the logical question is what must be done to remove the oil and return the marsh to its full functioning capabilities. Studies in 1985 and 1986 on marsh plots attempted to provide necessary information.

Cleanup techniques implemented 18-24 hours after the application were not effective in removing oil that had penetrated the surface. When oil remained on the sediment surface, flushing techniques were most effective at removal, reducing levels of oil by 73 to 83 percent. When dispersants were added to the water during flushing, oil removal was only slightly enhanced. Clipping of vegetation followed by sorbent pad application to sediments was moderately effective, reducing added oil by 36 to 44 percent. Burning had a negative effect on oil removal; oil increased in sediments of burned plots compared to controls.

Consideration should be given to natural flushing, evaporation, and natural microbial breakdown of the oil. When large amounts of oil are present in the marsh, clean-up measures may be necessary to remove oil and return the marsh to its natural state. Damage from trampling can be severe, causing damage to plants and forcing oil into sediments (Webb 1993). Where possible trampling and destructive damage to the marsh should be avoided.

CONCLUSIONS

Type of oil is important in determination of effects. Light refined oils kill on contact. Crude oils block photosynthesis and respiration. Amount of oil and amount of plant coverage are important. Coating of entire plant causes death of aboveground material. Plants can regenerate from rootstock with crude oils. Roots are killed by light refined oils, including number 2 fuel oil. Oil remaining in the sediments can cause long-term effects on plants. Season can be important. Plants are least affected by oils during late fall and winter when plants are dormant or approaching dormancy.

Clean-up and restoration techniques must be based on oil type, plant and marsh coverage, and amount of oil. Cleanup decisions following a spill must be based on judgements of the long-term damage projected for the impacted marsh. The type and amount of oil must be carefully judged. The do-nothing alternative should be carefully considered. Flushing is effective when narrow marshes are impacted. Clipping and use of sorbents are moderately successful, but trampling can cause damage and force oil into the sediment. Burning may be ineffective.

ACKNOWLEDGMENTS

Experimental research performed between 1981 and 1986 was done in conjunction with Dr. Steve Alexander, who is co-author on many of the papers cited in this summary publication. Experimental work testing clean-up techniques cited in this paper was done in conjunction with Russell Kiesling, who is a former graduate student. Funding for various aspects of the studies were provided by the Center for Energy and Mineral Resources at Texas A&M University, Harris and Eliza Kempner Fund, National Wildlife Federation,

American Petroleum Institute, and the Texas Water Commission. Oils and dispersants were supplied by Exxon USA. Sorbent pads were supplied by 3M Company.

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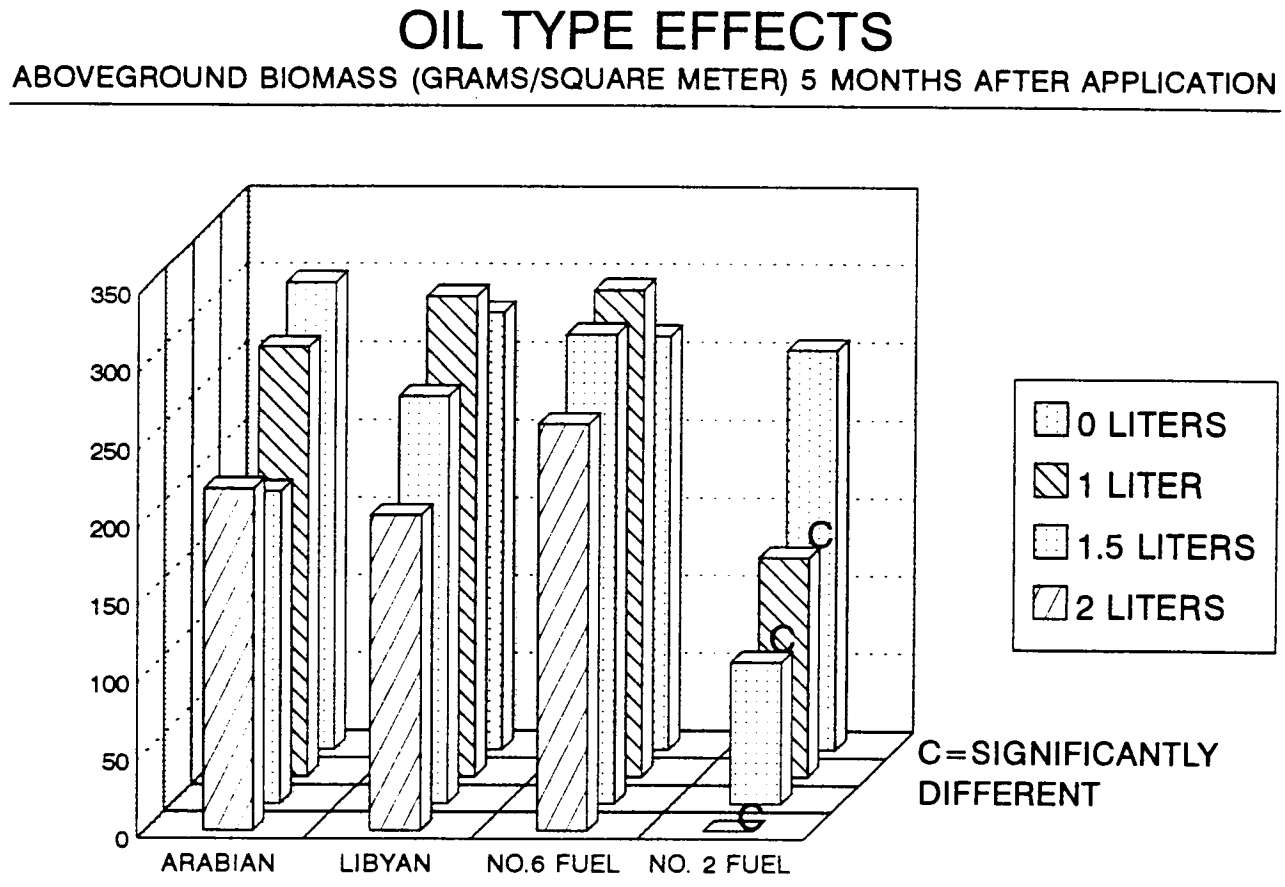

Figure 1. Oil type effects on smooth cordgrass aboveground biomass (g/m^2) five months after application of crude, heavy fuel, and light fuel oils to a salt marsh.

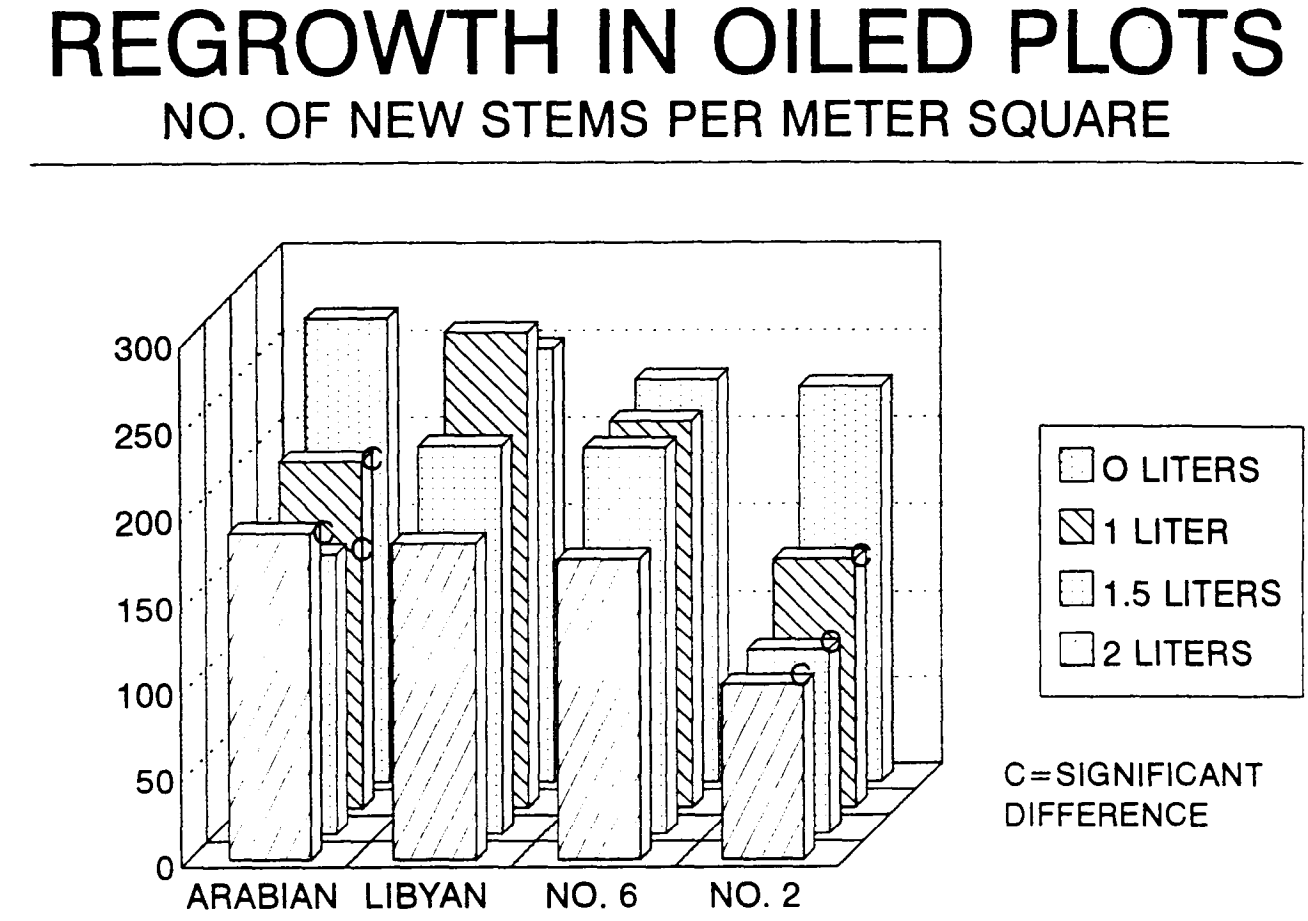

Figure 2. Regrowth (no. of new stems/$m^{2)}$ in smooth cordgrass plots sprayed with crude, heavy fuel, and light fuel oils.

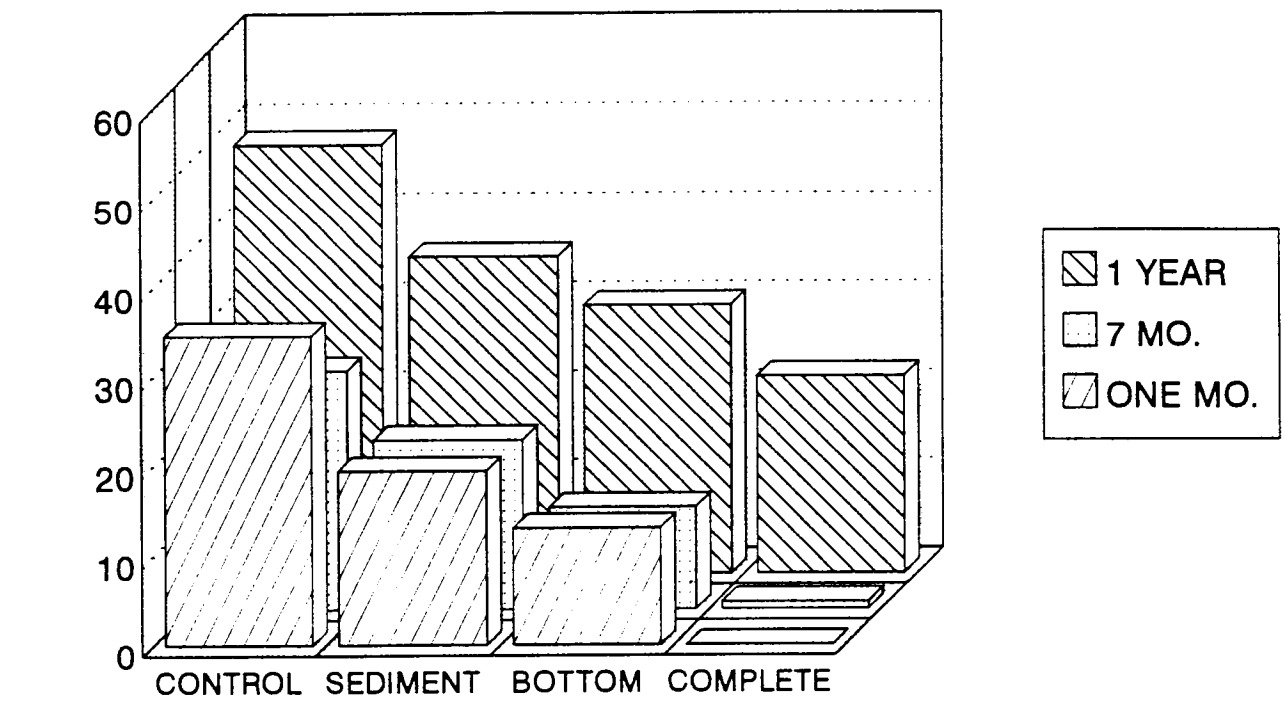

Figure 3. Initial and residual effects of No. 2 fuel oil on live stem density of smooth cordgrass plots at 1, 7, and 12 months.

The Applicability of Predictions Made from Other Oil Spills to the 1986 Bahia Las Minas, Panama, Crude Oil Spill: Seagrass Communities

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INTRODUCTION

The general ecological role of seagrasses throughout the world has been documented by numerous authors. Seagrass beds are important nursery grounds for many species of fish and crustaceans, they stabilize sediments, they act as sediment traps, they provide substrata for many species of epiphytes and epifauna, and they provide food as both green leaves and detritus for many small invertebrates and juvenile fish species. Damage to or the loss of a seagrass meadow may have ecological effects which extend well beyond the boundaries of that particular meadow. Seagrass communities can also be strongly perturbed by factors that do not kill or otherwise affect seagrass standing crop.

Seagrasses and their associated animal communities, in Panama (as described in Heck 1977; Marshall *et al.* 1990) and in other areas, are susceptible to many types of man-induced (Jacobs 1980; Phillips 1980; Williams 1988; Thayer *et al.* 1975; Zieman *et al.* 1986) perturbations. The response of seagrass communities to oiling (Zieman *et al.* 1986; Marshall *et al.* 1990) seems to be strongly dependent on the elevation of individual seagrass beds within the littoral zone. Plants within deep, subtidal seagrass beds are less likely to be killed than are seagrasses in intertidal areas. Local climatic and oceanographic conditions at the time of oil exposure and many other factors also undoubtably play a role in the degree of oiling. Sediment texture, degree of exposure to waves and currents, tidal flushing, seasonal timing of recruitment for affected species, suspended sediment loads, initial seagrass density, etc. may all have a role in determining the severity of the initial impact and the rate of the recovery process. Certain heavy fractions of crude oil sink when they are emulsified and mixed with sediment particles through turbulence under rough seas or coastal breakers. Emulsified oil droplets were seen on numerous occasions, at the deep edges of reef-protected seagrass beds near the Bahia Las Minas refinery, during the early months of the Panama oil spill (Marshall *et al.* 1990).

Seagrass meadows elsewhere in the tropics, subtropics, and temperate zone have been contaminated with oil from tanker wrecks and intentional discharges from tankers (Marshall *et al.* 1993). Despite an omnipresent oil spill threat to seagrass meadows and despite a currently perceived world-wide decrease in seagrass acreage little is known about their recovery potential from oiling (Zieman *et al.* 1986; Thorhaug and Marcus 1987).

The *Amoco Cadiz* spill off the coast of France oiled temperate *Zostera* meadows (denHartog and Jacobs, 1980; Jacobs 1980). Panama's tropical seagrass beds and the French *Zostera marina* meadows contain many groups in common at higher taxonomic levels.

Species compositions are probably completely different but the initial spill effects on common groups, such as the Amphipoda, Tanaidacea, and Ophiuroidea, proved to be similar in the two areas (denHartog and Jacobs 1980; Jacobs 1980; Marshall *et al.* 1993). The French studies continued for one year after the spill event; they were based on prespill studies undertaken at the oiled seagrass beds approximately one year before the *Amoco Cadiz* occurred. Several major groups virtually disappeared after the spill but quickly recovered. Twenty six species of Amphipoda disappeared from the oiled *Zostera* beds and after one year only one species had returned.

The Bahia Las Minas refinery spill in Panama presented an opportunity to observe both the amount of damage done by various degrees of oiling to numerous, spatially separated seagrass meadow communities and to observe their resilience to this type of damage under a range of environmental conditions. Comparisons between the effects of the *Amoco Cadiz* spill and the Panama spill are instructive for the shared faunal groups but there are many plant and animal taxa in the tropical seagrass beds of Panama that are not present in the French *Zostera* beds.

At present there is a continuing debate about the advisability of allowing oil and gas exploration and development on the continental shelf off southwestern Florida (Marshall *et al.*, 1990). Tankers passing through the Florida Straits provide a present and continuous threat of oil spills to shallow marine habitats if they run aground, rupture a tank, sink, or pump their bilges in this area. Several predictions (Table 1), about the potential effects of oil on Panama's Bahia Las Minas seagrass meadows, were made from a survey of the reported effects of oil on seagrass meadows oiled by other spills (Marshall *et al.* 1990). This summary of the effects of the Bahia Las Minas spill's effect on seagrass communities is aimed at deciding whether or not these predictions applied to the Panama spill.

RESULTS AND DISCUSSION

Certain Organisms Are More Oil Sensitive than Others

Epifauna were sorted to the major taxa listed in Table 2. Responses to initial oiling and recovery patterns varied widely between taxa. Gastropods (Fig. 1) existed in similar densities at oiled and unoiled sites throughout most of the 36 months covered by this study. Ophiuroidea (Fig. 1) were never abundant in oiled seagrass beds. Fish (Fig. 1) densities were initially widely different but trends at oiled and unoiled sites eventually converged.

Amphipod (Fig. 1) densities were initially widely different between sites types but the long-term population trends quickly converged. Amphipoda oiling x time interactions were significant and amphipod counts were consistently higher at unoiled sites until the last 9 months of our three year long study. Mysids (Fig. 1) were consistently most abundant at oiled sites while isopods were nearly equally abundant in oiled and unoiled sites. Tanaidacea (Fig. 1) were consistently absent or rare at oiled sites but showed seasonal spikes of abundance in unoiled seagrass beds.

Amphipod temporal trends in oiled Panamanian seagrass meadows fit the pattern displayed by amphipods in French *Zostera* meadows following the *Amoco Cadiz* oil spill (denHartog and Jacobs, 1980). In both locations amphipods proved to be highly oil sensitive and slow to recover from spill effects.

Ophiuroids were quickly killed and had not recovered after almost three years (infaunal data) in Panamanian seagrass beds. In France they were reduced in numbers but

were not totally eradicated by the *Amoco Cadiz* oil spill. Surface dwelling species of *Ophiothrix* and *Ophiocoma*, those species most common in pushnet samples, may have been killed by direct contact with oil. The oil sensitivity of ophiuroids and other echinoderms suggests that they may serve as excellent indicator organisms of oil effects on faunal communities in tropical seagrass meadows. Our pushnet and core samples were extremely expensive to sort. Large, easy to count echinoderm species may provide a means of rapidly determining where oil toxicity has had an effect on seagrass communities.

Oil Sensitivity Depends on Feeding Type and Filter Feeders Are the Most Oil Sensitive

Density trends of numerous polychaete families and epifaunal taxa suggested a wide-spread sensitivity to oil. Populations of other taxa appeared to be insensitive. Some of the "insensitive" taxa may have been able to recover before this study began (at five months post spill). Hydrocarbon analyses of seagrass bed sediments (Burns 1993; this report) demonstrated that the oil present in those sediments was highly weathered by five months after the spill (September 1986). Oil washing out from the mangrove forests of Bahia Las Minas, considering its relatively unweathered condition (Burns 1993) could have an effect on sensitive surface dwelling species for years after the spill.

Density changes of polychaete families through time suggested that oil effects were much the same for all feeding types (Marshall *et al.* 1993). Feeding type categories are based on a polychaete family-level, guild classification scheme developed by Fauchald and Jumars (1979). Initial densities were lower at oiled than at unoiled sites for 12 of the 13 most abundant families. Recovery, defined by convergence of densities at oiled and unoiled sites, was seen in 9 of the 13 families. No consistent patterns of significant oiling x time interactions were apparent within or between feeding strategies.

Seagrass Plants Shelter Infauna from Oil Exposure by Preventing Oil from Mixing into the Sediments

Early reports of *Thalassia* intertidal bed die-offs (Jackson *et al.* 1989) after the 1986 Panama spill suggested that oil might harm or kill very shallow seagrass beds if they are oiled during low tide exposures. Not all of the oiled, intertidal seagrass beds died after contact with oil (J. Cubit STRI, personal communication and personal observation). Subtidal *Thalassia*, which had been exposed to crude oil (Jackson *et at.* 1989; Marshall *et al.* 1993) was not killed. This supports laboratory results (Thorhaug and Marcus 1987) which suggest a high level of oil tolerance for this species. *Syringodium* biomass appeared to decrease in oiled areas while undergoing seasonal fluctuations in unoiled seagrass meadows. This finding, and toxicity tests (Thorhaug and Marcus 1987), suggest that *Syringodium* is sensitive to oiling and dispersants.

Chronic oiling after a spill, the situation in Panama, has never been considered in laboratory or field experiments) with seagrasses. Oil seeping from the rocks beneath the refinery and washing out from oil-saturated sediments in the shoreline mangrove forests (Garrity and Levings 1993), after the initial exposure to "fresh" crude oil, apparently did not kill *Thalassia*, away from the shoreline in deeper water, but it may have damaged *Syringodium* populations. Seagrass meadow edges slowly receded from oiled mangrove shorelines suggesting that the oil concentrations reached during chronic re-oiling events were toxic to seagrasses. It is also possible that high concentrations of weathered oil in sediments

near the shoreline slowly killed *Thalassia* plants. A general thinning of subtidal *Thalassia* at oiled and unoiled sites, as observed in our study, was not attributable to oil exposure.

Ultraviolet fluorescence (UVF) and GC/MS analyses of a set of deep sediment samples (Table 4) demonstrated that, unlike *Zostera marina* in French grassbeds, the root and rhizome mats of Panama's seagrasses did not prevent oil penetration into deep sediment layers. Oil concentrations were higher in deep sediments, at 8-10cm and at 18-20 cm, than at the sediment water interface (0-2 cm). UVF analysis of sediment surface scrapes showed that oil concentrations in some areas (especially LREN) remained high through 1987. After that date surface sediment oil concentrations decreased by an order of magnitude. Chromatograms in Burns (1993) and in Marshall and Sherblom (1994) show a continued weathering of oil throughout the study for surface scrape sediment samples collected from seagrass beds in Bahia Las Minas. Seagrass did not protect infauna from oil.

Opportunistic Species Become More Abundant in Oil Exposed Sediments

Capitellid polychaetes, usually considered to be a family of mostly opportunistic species and which have both been seen to increase in numbers following other oil spills, did follow this pattern at oiled sites in Panama. Capitellids were far more abundant at oiled sites (Table 4) but they were not the most abundant polychaete family. They were outnumbered by the serpulidae, which live attached to seagrass blades above the sediment surface. The serpulids were more abundant at oiled sites than at unoiled sites. Perhaps this was a response to lowered predation intensities by other animal taxa (fish, crabs, snails, etc.) that were affected by the crude oil. Cirratulids were about equally abundant at oiled and unoiled sites.

Densities of Sensitive Organisms Should Be Inversely Correlated to the Oil Content of the Sediment

Weathered oil remained at high concentrations in seagrass bed sediments throughout this study (Fig. 2). Thus the recovery patterns seen for many organisms happened despite the presence of crude oil. The major changes in oil constituents, and presumed lowering of oil toxicity, were perhaps more important than the total oil content of the sediment.

Reproductive and Recruitment Processes Are Often Affected by Exposure to Oil

Caribbean caridean shrimp species typically show continuous reproduction coupled with high temporal and spatial variation in recruitment patterns (Bauer, 1987). The apparent lack of an effect on the development, reproductive output, and recruitment of *Hippolyte zostericola* and *Latreutes fucorum* (Fig. 3) beginning from the first post-spill collection, suggested that the biological activity of the weathered oil was low for these shrimp if they survived or recruited to the grassbeds after the initial exposure to crude oil. It is assumed that the density responses to oiling shown by species at oiled and unoiled sites were mostly due to the initial toxic effects of the oil concentrations experienced in oiled grassbeds soon after the spill began. Oil effects on reproduction in other taxa were not observed and no generalizations can be made.

SUMMARY

Oil appeared to have a transitory effect on Panamanian subtidal seagrasses and a lasting effect on some animal taxa. The faunal component of oiled seagrass meadows remained highly altered over the time span covered by the infaunal and epifaunal studies. It was apparent, that the oil effect was quickly overcome by species with high reproductive potentials and/or by those with highly mobile planktonic stages. A more exact statement on oil sensitivity cannot be made until more hydrocarbon analyses are fully completed. It is important to realize that, despite the existence of strong faunal and floral affinities between Caribbean Panama's and other shallow seagrass meadows in the Caribbean and the Gulf of Mexico, there are numerous differences between these areas that could drastically affect the outcome of a major oil spill. Since the oil spill project was funded, by the U.S. Minerals Management Service and Marine Spill Response Corporation, because of its relevance to shallow tropical ecosystems on Florida's southern coast, it seems appropriate to direct attention to the differences between these two areas. Vast expanses of shallow seagrass meadows exist on mud banks within Florida Bay. The mud banks support over 1/2 of the standing crop of seagrasses in Florida Bay (Powell *et al.* 1989). Fleshy algae are also much more important in Florida Bay (Lapointe 1989) than in Panama. The bank tops are nearly exposed, with seagrass blades protruding through the water surface, on extreme tides. Flushing rates, a factor that can alter oil removal rates (Burns *et al.* 1991), are slow in Florida Bay because tidal flow is restricted by the numerous mud banks (Holmquist *et al.* 1989).

The Panamanian seagrass beds included in this study are all in lagoons that are bordered by subtidal coral reefs and which lie adjacent to mangrove lined shores. They are tiny in comparison to south Florida's seagrass meadows, they are rarely exposed on low tides, and they are flushed by relatively strong tidal and/or wind-generated currents. It would be a mistake assume that the effects of the Panama spill on seagrass beds would be identical to what might happen in Florida Bay. Mud bank flow restriction and seagrass location and other differences in hydrodynamics, topography, and ecology between our study sites in Panama and in Florida Bay could lead to a much different result.

The fauna of seagrass meadows can also be highly variable from site to site. In seagrass beds amphipod abundance tends to decrease with latitude and caridean species composition varies greatly between seagrass beds within the tropics and subtropics (Bauer 1987). These studies and the present report each identify different numerically dominant species of caridean shrimp in *Thalassia* meadows. *Latreutes fucorum*, an oil-sensitive species in our study, accounted for 57% of the total numbers of carideans in Puerto Rican *Thalassia* beds. It was much less abundant in Bahia Las Minas and at the unoiled control sites in Panama. The total impact of an oil spill can only be predicted from a thorough knowledge of by the assemblage of oil sensitive and tolerant species within individual seagrass meadows. It is not safe to assume that the effects of a spill, of a similar oil type, over shallow seagrass beds of the same plant species would be the same in any other area.

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Table 1.

Observations of oil spill effects on seagrass beds from other studies from around the world.

1)	Certain organisms are more oil sensitive than others.
2)	Oil sensitivity depends on feeding type with filter feeders being the most oil sensitive.
3)	Seagrass plants shelter infauna from oil exposure by preventing oil from mixing into the sediment.
4)	Opportunistic species become more abundant in oil exposed sediments.
5)	Densities of sensitive organisms should be inversely correlated with the oil content of the sediment.
6)	Reproduction and recruitment are often affected by oil exposure.

Table 2.

Total counts of epifaunal taxa from November 1986 through April 1989. Percents are based on the overall total number of organisms collected by pushnetting as described in the text.

Taxonomic Group	UNOILED SITES Count	UNOILED SITES Percent	OILED SITES Count	OILED SITES Percent
Shrimp (Caridea and Penaeidea)	89,248	58.1	59,573	59.4
Tanaidacea	18,055	11.8	714	0.7
Amphipoda	12,561	8.2	9,647	9.6
Gastropoda	7,793	5.1	3,788	3.8
Paguroidea	6,342	4.1	12,136	12.1
Bracyhura	5,613	3.7	4,767	4.8
Fish	4,061	2.7	1,455	1.5
Isopoda	2,483	1.6	1,527	1.5
Ophiuroidea	2,188	1.4	106	0.1
Cumacea	2,104	1.4	987	1.0
Mysidacea	1,105	0.7	3,566	3.6
Pycnogonida	558	0.4	804	0.8
Stomatopoda	484	0.3	893	0.9
Holothuroidea	251	0.2	7	<0.1
Echinoidea	80	<0.1	9	<0.1
Totals				
Site Type	**153,533**		**100,337**	
Overall Total		**253,870**		

Table 3.

Hydrocarbons in seagrass sediment surface scrapes (4a) and at depths (4b) as determined by ultraviolet fluorescence (UVF) and by gas chromatograph (GC-URE) analysis. Units are ug/g dry wt. Site categories, heavy, moderate and unoiled are based on oiling levels based on knowledge of the spill path and on oil observed at each site.

Station	1986		1987		1988		1989	
	UVF	GC-URE	UVF	GC-URE	UVF	GC-URE	UVF	GC-URE
(4a.) Surface Scrape Samples								
Heavily Oiled Sites:								
LREN	4020	2021	6683	203	658	60	277	18
LRS	792	111	1198	137	501	51	263	50
MINS/N	1499	151	957	75	607	52	747	128
PGN	127	50	211	19	188	11	143	71
Moderately Oiled Site:								
NARC	596	82			98	7		
Unoiled Sites:								
DONT	0	0	6	0	0	0	0	2
PALN	0	0	1	0	0	0	0	9
LINE	5	0	102	14	4	0	0	11
BNV	9	0			6	0	0	4
(4b.) Subsurface Oil								
LREN								
0-2cm	4020	2021	6683	203	391	19		
8-10cm					665	64		
18-20cm					553	39		

Table 4.

Total counts of polychaete families from September 1986 through April 1989.

Family	Unoiled Sites		Oiled Sites	
	Count	Rank	Count	Rank
Sabellidae	22,547	1	18,150	3
Syllidae	19,855	2	11,210	4
Paraonidae	15,161	3	4,423	6
Capitellidae	9,103	4	16,103	2
Spionidae	4,857	5	7,894	5
Eunicidae	3,576	6	1,547	9
Dorvilleidae	3,541	7	1,697	8
Cirratulidae	3,190	8	2,855	7
Serpulidae	2,380	9	25,765	1
Onuphidae	2,208	10	1,303	11
Orbiniidae	2,106	11	674	12
Nereidae	2,092	12	1,428	10
Amphinomidae	724	13	16	31
Terebellidae	617	14	128	18
Ampharetidae	589	15	745	13
Glyceridae	537	16	107	19
Maldanidae	466	17	27	28
Opheliidae	346	18	433	14
Polynoidae	202	19	53	21
Lumbrinereidae	179	20	150	17
Arabellidae	156	21	192	16
Chaetopteridae	143	22	34	26
Hesionidae	107	23	254	15
Chrysopetalidae	106	24	12	33
Magelonidae	76	25	28	29
Trichobranchidae	62	26	1	36
Oweniidae	59	27	50	22
Phyllodocidae	41	28	37	25
Sigalionidae	26	29	22	30
Poechilochaetidae	22	30	68	20
Pilargidae	17	31	45	23
Pectinariidae	16	32	-	-
Arenicolidae	11	33	16	31
Bogueidae	9	34	-	-
Polyodontidae	8	35	-	-
Goniadidae	4	36	3	34
Flabelligeridae	6	37	42	24
Aphroditidae	1	38	-	-
Total	**95,150**		**95,567**	

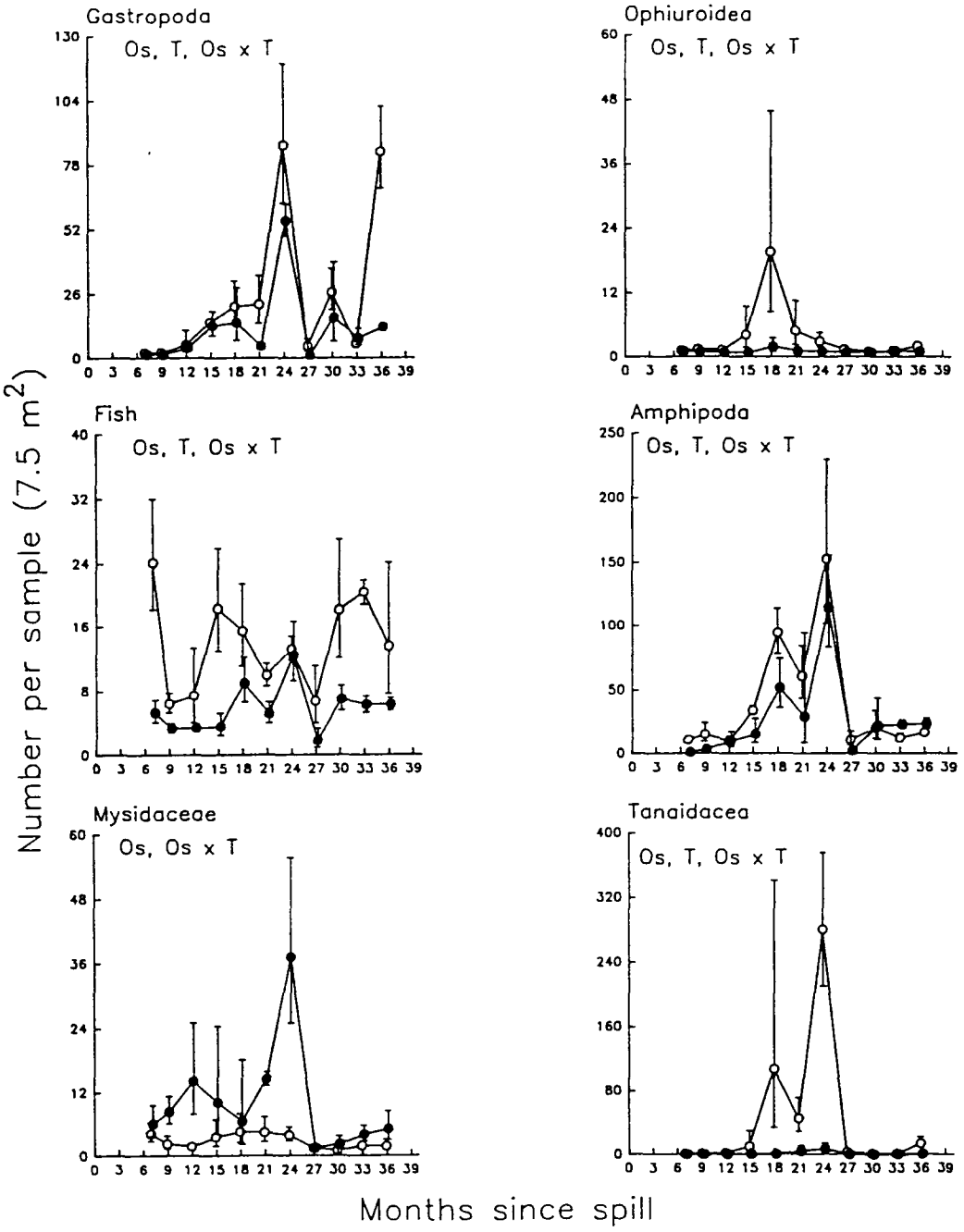

Figure 1. Total counts of six epifaunal taxa collected by pushnet at oiled and unoiled sites, September 1986 through April 1989. Standard error bars were back-transformed from ln(x+1). Results of repeated measures ANOVAs are: Os = significant difference ($P<.05$) between oiled and unoiled sites, T = significant difference through time, Os x T = significant interaction between oiling and time, and all NS = no significant differences.

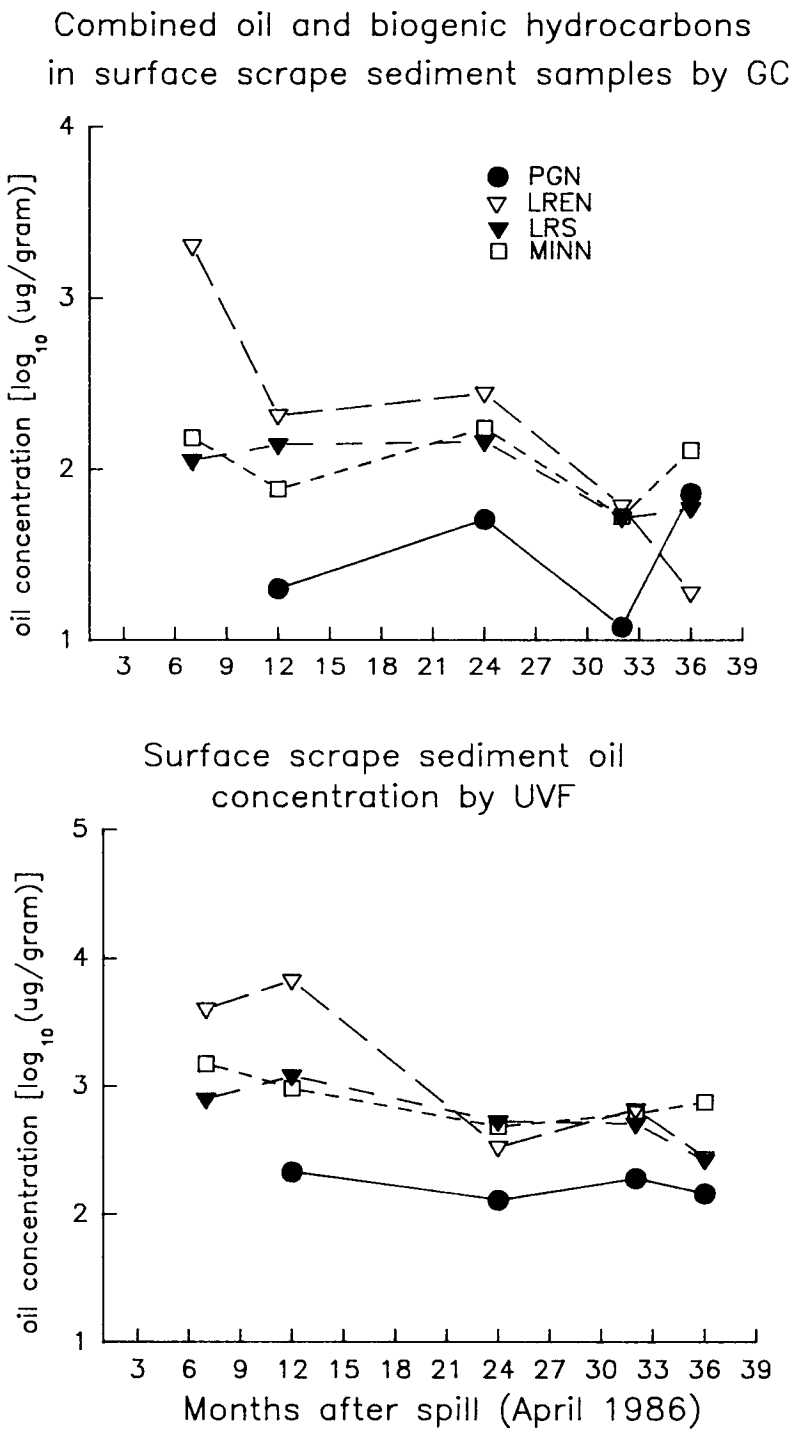

Figure 2. Oil concentrations at oiled sites from September 1986 through April 1989. Methods used to measure oil concentrations were gas chromatography (GC) and ultraviolet fluorescence (UVF).

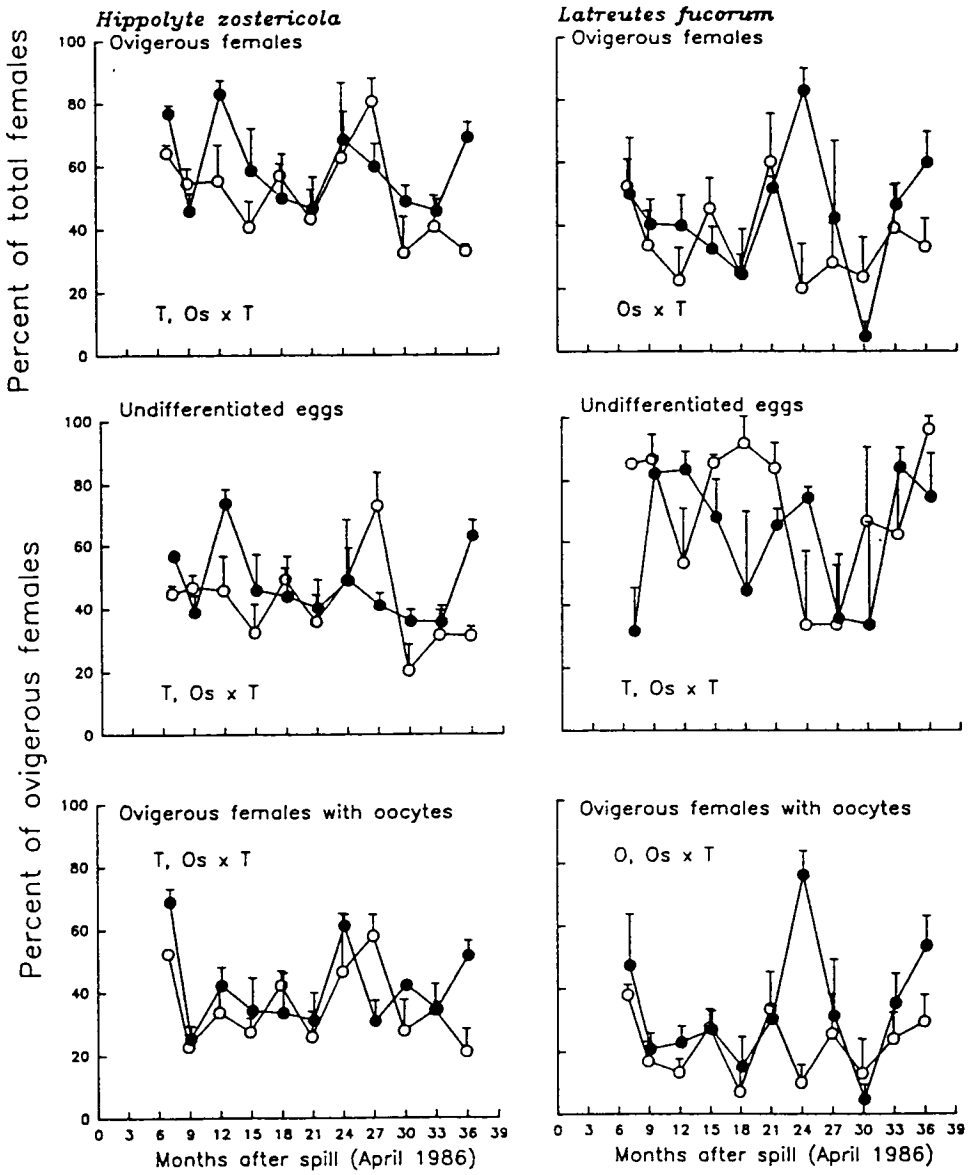

Figure 3. Reproductive patterns for two hippolytid shrimp (*Hippolyte zostericola* and *Latreutes fucorum*) based on egg and ovarian developmental stages as described in Marshall and Sherblom (1994).

The 1986 Bahía Las Minas Oil Spill: Summary Results from the Red Mangrove (*Rhizophora mangle*) Fringe

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INTRODUCTION

In the Caribbean and portions of the Gulf of Mexico, red mangroves (*Rhizophora mangle*) fringe most mangrove shorelines (West 1977). Red mangroves are characterized by the presence of numerous prop roots which grow from the lower part of the stem, anchoring the tree at multiple points. At the edge of the shore, submerged prop roots grow into the water, hanging in the water column until they grow into the substrate. This shoreline edge is known as the red mangrove fringe. The red mangrove fringe is an ecologically important habitat. Submerged prop roots are often the only hard surfaces in areas of soft sediments where mangroves grow. The thickets of submerged prop roots serve as substrate for a complex assemblage of attached plants and animals (the epibiota) and are critical habitat for many mobile, associated species (Odum *et al.* 1982, Rützler and Feller 1987, Odum and McIvor 1990). Species such as spiny lobsters, larval fishes, crabs and shrimp spend some or all of their life cycle among the submerged prop roots of the red mangrove fringe; these in turn are eaten by higher order predators like birds and large fish (Odum *et al.* 1982, Hatcher *et al.* 1989). Some species dependent upon the mangrove fringe are commercially or recreationally important (i.e. shrimp, spiny lobsters, snook, Lewis *et al.* 1985). The red mangrove fringe and the complex of associated species are a major component of the mangrove nursery grounds. The productivity of these nursery areas is directly related to the abundance and persistence of submerged prop roots. Damage to this biogenic habitat after an oil spill could have prolonged negative ecological effects.

In 1986, the rupture of an oil storage tank spilled an estimated 75,000 - 100,000 barrels of medium weight crude oil into Bahía las Minas on the Caribbean coast of Panamá (Cubit *et al.* 1987, Jackson *et al.* 1989). For five years, we studied the effects of this major

oil spill on fringing red mangroves (*Rhizophora mangle*) and the plants and animals that live attached to submerged mangrove roots. Results of this study have now been published in technical reports, in summary overviews and in a series of technical articles (Appendix 1, in References). We here briefly summarize our major findings, both chemical and biological, from the point of view of natural recovery patterns and the biological processes which control recovery. We examine the direct negative effects of the oil spill and how long we estimate they will last. We then discuss some indirect effects of oiling, estimating habitat losses for associated species. Physical factors that affected oil deposition patterns, and their general importance in interpreting the results of case studies, are presented. Finally, we suggest areas where further information is needed.

SUMMARY RESULTS

Initial Effects

Immediately after the spill, there were massive die-offs of plants and animals attached to submerged mangrove roots (Cubit *et al.* 1987, Jackson *et al.* 1989, Garrity and Levings 1993a). Oil coated roots repeatedly as tides rose and fell and slicks circulated throughout the bay. Thus the mechanical effect of oil (smothering) extended through the intertidal to shallow subtidal portions of roots, while species attached deeper on submerged roots were exposed only to dissolved petroleum hydrocarbons (toxicity). Most plants and animals directly coated with oil died and subsequently sloughed off submerged prop roots. Some plants and animals growing deep on roots survived, at least until defoliation resulted in roots lifting from the water; survivors then died from heat and desiccation stress. This process continued for almost a year, as oil moved through the bay, entering previously unoiled areas and reoiling sites previously oiled. It would not have been possible to estimate the full extent of losses for attached plants and animals for 6 months to one year after the spill.

The Pattern of Mangrove Mortality

After oil coated roots and soaked into sediments where mangroves were rooted, die-offs of red mangrove trees began (Table 1). The time course of defoliation, death and collapse of dead trees varied, depending upon location in the bay (Garrity *et al.* 1994). Damage was heaviest in sheltered, interior sections of the bay and lighter at the edges of the bay where oceanic waves washed the fringe. In general, where leaf loss began first, damage to the fringe was most severe five years later. Within the bay, sheltered drainage streams drained uplands and mixed species stands of mangroves. Here, overall damage was highest, most trees in the mangrove fringe were defoliated within 6-9 months, and collapse following death began in less than 2 years. An average of 66% of fringing mangroves died. In channels and lagoons, which comprised most of the shoreline of the bay, about 23% of the fringe died. At the mouth of the bay and on the open coast, where mangroves grew behind reef flats, approximately 13% of the fringe died. Defoliation took longer to occur in channels and lagoons and on the open coast than in streams, with new defoliation up to 2 years after the spill. Most collapse of dead trees occurred between 2.5 and 4 years after oiling. Based on

our observations, it would not have been possible to estimate the full extent of damage to the mangrove fringe throughout the bay for approximately 3 years.

Five years after the spill and after considerable natural recruitment and planting of propagules behind the original fringe (Teas *et al.* 1989), some new trees were beginning to grow into the fringe (Duke and Pinzon 1993). However, in channels and lagoons and on the open coast, $<1\%$ of the fringe had regrown; reestablishment was so low on the open coast that sections of the fringe were expected to be permanently lost (Duke and Pinzon 1993). In sheltered drainage streams, trees had recruited along $\sim 5\%$ of the original fringe. For all areas, the estimated time for the fringe to regrow to its pre-spill state was on the order of decades rather than years.

Long-term Structural Changes in the Mangrove Fringe

Even in surviving portions of oiled mangrove fringe, both the physical structure of the trees and environmental conditions in and around submerged prop roots changed (Table 1) (Garrity *et al.* 1994). First, there were fewer submerged prop roots at oiled than unoiled sites within areas of surviving fringe. Second, submerged prop roots of fringing mangroves were shorter at oiled than unoiled sites on the open coast and in drainage streams, but not in channels. Third, throughout the bay, more submerged prop roots were dead at oiled sites. Fourth, surviving oiled trees were partially defoliated up to 5 years post-spill when our observations ended, resulting in increased light reaching root level.

These finer scale alterations to the habitat in turn further affected associated species, already negatively affected by the absolute loss of major sections of the fringe. Lowered density of roots per unit shoreline and decreased root length per root reduced the total area of submerged root surfaces, thus quantitatively reducing the amount of available habitat for both sessile and mobile species. Qualitatively, at oiled sites, increased levels of light reaching root level, increases in the proportion of dead vs. live submerged prop roots and decreases in the depth of submergence (depth of submergence is dependent on root length) created a different habitat for the plants and animals that live attached to submerged prop roots and for the species that live or feed among submerged prop roots. These changes will likely persist until the mangrove fringe is fully re-established; this process will probably take decades.

Long-term Reductions in Plants and Animals Encrusting Submerged Prop Roots

In an attempt to factor out the effects of habitat loss from those of oiling itself, we compared the cover of plants and animals on remaining submerged prop roots at oiled sites to that on similar roots at unoiled sites. These analyses showed that the cover of plants and animals on submerged prop roots decreased significantly after oiling (Garrity and Levings 1993b, Levings *et al.* 1994). Restricting our discussion to major groups of sessile invertebrates, oiling reduced cover for at least 4-5 years (Figure 1).

On the open coast, sessile invertebrates on submerged prop roots included sponges, corals, anemones, tunicates, bryozoans, vermetids and hydroids. Cover was significantly reduced for four years after oiling, but did not differ significantly between oiled and unoiled sites in the fifth year after the spill. However, some groups (e.g. corals, anemones and

tunicates) remained rarer at oiled than unoiled sites in year 5 post-spill. In channels and lagoons, roots were typically encrusted with oysters (*Crassostrea virginica*). Oyster abundance dropped by approximately an order of magnitude after oiling. Although cover increased over time at oiled sites, differences between oiled and unoiled sites remained significant through five years post-spill, when observations ceased. In drainage streams, the false mussel *Mytilopsis sallei* typically covered more than 25% of the space on submerged prop roots. Within three months after oiling, false mussels had almost disappeared from oiled drainage streams. False mussels were significantly less abundant at oiled than unoiled streams for five years post-spill. Unlike the partial recovery of oysters in channels, there was little evidence of recovery in drainage streams by 1991.

Reductions in the cover of abundant sessile invertebrates on remaining prop roots thus persisted through at least 4-5 years after oiling. The full extent of the damage was evident within approximately six months to one year after the spill. Full recovery remains to be documented in any habitat.

Estimated Reductions in Standing Crop

We estimated reductions in standing crop by combining data on the reduction in surface area on submerged prop roots with the reduction in percent cover of groups of sessile invertebrates at oiled as compared to unoiled sites (Garrity *et al.* 1994, Levings *et al.* 1994). On the open coast, area of submerged root surfaces was reduced 33% five years post-spill, but there was no statistically significant difference in percent cover of sessile invertebrates at oiled as compared to unoiled sites. Thus we estimate that standing crop was reduced by ~33%, caused by a reduction in the amount of habitat available. In channels and lagoons, surface area on submerged prop roots was reduced ~38% and cover of oysters was reduced 44% at oiled as compared to unoiled sites. Combined, we estimate a reduction of 65% in standing crop of oysters five years post-spill. In drainage streams, surface area on submerged prop roots was reduced by 74% and false mussels covered 96% less space on roots. Combined, the net reduction in standing crop was estimated to be ~99% in drainage streams. The severity of estimated losses in standing crop five years post-spill illustrates the strength and persistence of negative effects from the 1986 Bahía las Minas oil spill.

Chronic Oiling and the Continued Presence of Toxic Hydrocarbons in the Environment

Initial weathering processes removed most volatile hydrocarbons and all marker alkanes in oil adsorbed to surface sediments within six months after the spill (Burns *et al.* 1994). This initially fast rate of biodegradation was not maintained in the rate of disappearance of aromatic hydrocarbons over time. Anoxic mangrove muds served as long-term reservoirs for toxic hydrocarbons, causing chronic contamination of contiguous coastal communities for over five years.

We documented chronic oiling by recording the incidence and thickness of oil slicks and the amount of oil deposited on artificial prop roots (dowels) at quarterly intervals (Levings *et al.* 1994). On the open coast, relatively little oil was deposited on dowels, except during occasional wet season washouts. The frequency and thickness of oil slicks also

declined at oiled sites, with most slicks recorded as iridescence or silvery sheen by five years post-spill; slicks were seen at five of nine visits in May 1991. In channels and lagoons, pulses of oil were frequently recorded, with the greatest washouts occurring from February - August 1989 (approximately 2.5 years post-spill). The frequency of slicks did not decline over time and some black oil was observed in May 1991. In drainage streams between 1987 and 1991, oil slicks were seen on 128/129 visits and ranged from black oil to iridescent patches.

Oil leaching out of heavily contaminated sediments was bioaccumulated by oysters in channels and false mussels in drainage streams for at least five years after the spill. Both species accumulated the whole range of alkylated polynuclear aromatic hydrocarbons in the naphthalene through benzoperylene elution range, seemingly in proportion to what was leached from sediments (Burns and Yelle-Simmons 1994). Levels of oil in bivalve tissues were high enough to reduce significantly the growth and reproductive rate of both species examined (Bayne *et al.* 1982). Continuing contamination of oysters and false mussels attached to submerged mangrove prop roots suggests the possibility that oil may contaminate near-shore food chains and/or local fisheries. More data are needed to examine this possibility.

There was a change in the composition of aromatic hydrocarbons in the fifth year post-spill; this indicated the depletion of the most soluble and acutely toxic hydrocarbons in readily leachable reservoirs. We anticipate that over the next 5 - 10 years, impacts will probably grade from acutely lethal to sublethal levels. However, some sediment cores retained significant levels of the more volatile and toxic aromatic hydrocarbons through year six post-spill (Burns and Yelle-Simmons 1992), indicating the persistence of some sediment reservoirs of highly toxic hydrocarbons. These results are in contrast to expectations that oil residues will degrade quickly under tropical conditions.

Indirect Effects of Oiling: Estimated Long-term Effects on Associated Species

We estimated the effects of the 1986 Bahía las Minas oil spill using (1) direct measures of the abundance of wood-boring isopods and (2) losses of epibiota used as shelter, settlement and juvenile habitat by spiny lobsters (*Panulirus argus*, Levings and Garrity 1994). For boring isopods in sheltered channels and lagoons, there was no significant difference in isopod abundance on suitable (live) roots at oiled and unoiled sites. However, suitable (live) submerged prop roots were less abundant at oiled sites, leading to a reduction of over 60% in the number of submerged prop roots containing isopods at oiled as compared to unoiled sites 5 years post-spill. We estimated effects on non-boring species using two groups of epibiota (foliose red algae and arborescent hydroids and bryozoans) used as juvenile habitat by spiny lobsters. We estimated the amount of habitat for juvenile spiny lobsters in the mangrove fringe was reduced ~40-50% five years after the oil spill on the open coast and in sheltered channels and lagoons. Such reductions may have effects on local populations of mobile species associated with the mangrove fringe.

Factors Important in Generalizing from our Results

Case histories are used routinely to estimate possible damages from future spills. However, the generality of results depends in part upon the probability that patterns of oil deposition would be similar during other spills. For maximum usefulness for planning, results from any spill must be put into a broader context than the particular physical conditions that prevailed during that spill. In particular, variability in pertinent physical factors must be examined.

Movement and deposition of oil slicks were studied using aerial and ground observations of the oil spill, hydrographic and meteorological data from environmental monitoring, and freshwater runoff modeling (Cubit and Levings 1993). The spatial pattern of oiling, and therefore the habitats and biota affected, was dependent on weather conditions at the time of the spill, and would have been predictably different in other seasons. Conditions at the time of the 1968 Witwater spill were used to predict patterns of oil deposition; data on damage to reef flat biota and mangroves were consistent with our predictions of the spatial pattern of damage. Use of this, and other case studies, requires the consideration of other possible patterns of oil accumulation that would have been produced by other combinations of conditions. Such considerations must be included in predictions of potential risks of future oil spills. If they are not, oil spill contingency plans might be inadequate.

DISCUSSION

Patterns of Mangrove Mortality

Sediment oil concentrations were of the same order of magnitude (10^4-10^5 ug/g dry weight) at all oiled sites. However, there were order of magnitude differences in the percent of the mangrove fringe that died among sites. The amount of damage to a site was probably related to the pattern of oil deposition, the persistence of the oil both on prop roots and in sediments, and the particular physical characteristics of the site. Depth and type of substrate appeared to be of importance. In streams and channels, most trees rooted entirely in the intertidal zone died, while trees with some or most prop roots anchored in subtidal sediments often survived. On the open coast, trees rooted in coarse sediments like sand and coral rubble survived better than trees rooted in mud or peat; depth was also important. Death of mangroves after oil coating of gas exchange surfaces has been reported (e.g. Cintron and Schaeffer-Novelli 1983, Odum and Johannes 1975); survival of the outer fringe in spills that killed interior mangroves has also been noted (Baker *et al.* 1980, Cintron *et al.* 1981, Nadeau and Berquist 1977). Getter *et al.* (1981, 1984) presented a model of oil impact on fringing mangroves based upon topography and forest type, suggesting that maximum damage occurs in shallow waters near the berm (unless oil is carried over the berm during high tides)(see also Lewis 1983, Jacobi and Schaeffer-Novelli 1990).

In our study, more mangroves died where (1) prop roots were heavily coated with oil for at least 18 months and (2) sediments were soaked with oil. Survival was variable (and often high) unless both sediments and prop roots were oiled. However, knowledge of the

effects of oil on mangroves is far from complete; we suggest further study of the biological mechanisms controlling mangrove mortality.

Natural Recovery

Oil spills are distinct from natural disasters such as hurricanes in that chemical contamination may be long-lasting. Recovery from a hurricane may take many years (Smith *et al.* 1994), but will not be slowed by the presence of toxic compounds. Hydrocarbons deposited in soft sediments may persist for decades and have negative effects when leached from sediment reservoirs (Bayne *et al.* 1982, Corredor *et al.* 1990, Teal *et al.* 1992). We know of no way to remove oil from soft sediments without harming or destroying the habitat itself.

Understanding the biological basis of recovery processes, both with and without toxicants, is the key to effective contingency planning and mitigation. Biogenic habitats, that is those created by their constituent plants or animals, are especially vulnerable to negative impacts of oil spills. Examples of biogenic habitats include salt marshes, seagrass beds, coral reefs and mangrove forests. Damage to the structuring organism can cause loss of the habitat (i.e. erosion of the salt marsh), with cascading effects on the suite of associated species (loss of salt marsh crab populations and the birds that eat them). Biogenic habitats thus by their nature are uniquely susceptible to stressors that affect the major structuring organisms. In contrast, communities occurring in non-biogenic habitats (such as on rocky shores) may be devastated by oiling, but the habitat remains, ready to be repopulated. Recovery processes may be prolonged and ecologically complex (e.g. Southward and Southward 1978), but the potential habitat itself (rock surfaces) has not been destroyed. Recovery in biogenic habitats is linked to repopulation of the structuring organism(s) and associated species.

Five years after the 1986 Bahía las Minas oil spill, trees were beginning to regrow into the mangrove fringe, but occupied $<5\%$ of the fringe at any site. Regrowth was lowest on the exposed open coast, where drift logs crushed recruits. Duke and Pinzon (1993) predicted that sections of the fringe on the open coast would probably be lost due to this natural source of disturbance. Thus prospects for regrowth may actually be lowest where damage from the oil spill was least overall. Until we understand the factors controlling regrowth of mangroves after natural or anthropogenic damage (e.g. Smith *et al.* 1994), it will be difficult or impossible to predict the time course required for repopulation.

With respect to species associated with the mangrove fringe, structural changes had reduced the amount of habitat (submerged root surfaces) at all oiled sites. Only on the open coast did sessile invertebrates cover as much space on a per root basis at oiled and unoiled sites. There were additional reductions in percent cover of oysters and false mussels in channels and streams that may have been related to release of toxic hydrocarbons from sediments. Reductions in standing crop of between 33-99% were estimated 5 years post-spill. However, epibiota were returning in all habitats, especially growing on prop roots just entering the water. In this case, regrowth of the fringe and return of associated species were occurring at the same time, even though there were significant differences in the percent cover of major groups between oiled and unoiled sites in channels and streams.

Mitigation and Natural Recovery

Many methods of clean-up or mitigation may actually slow recovery when considered in the light of what is known about the biology of plants and animals affected by oil. For plants or animals that recolonize by regrowth from surviving fragments (i.e. some algae and corals), at least partial protection of the existing population may be crucial to the recovery process. This was the major mechanism for repopulation on reef flats after the Bahía las Minas oil spill (Cubit and Connor 1993a, b). Cleanup methods such as dispersants or hot water may kill surviving fragments and slow repopulation. Even for species that recolonize by settlement and growth of spores or larvae, prospects for recovery may be better if local, parent populations persist. In the case of the mangrove fringe, we recommend protection of at least some sections (1) to serve as a potential source for mangrove seedlings that can recolonize bare areas after oiling, and (2) to slow erosion, limiting deposition of oily sediments in adjacent habitats, like subtidal grass beds or coral reefs. Such protected sections of fringe could also (3) serve as sources for larvae and spores of species that live attached to submerged prop roots, speeding the recovery of associated species and returning food sources for higher order predators. Thus, even if it is not possible to keep oil completely out of mangroves, protection of at least sections of the fringe will probably assist natural recovery. Other factors, such as gregarious settlement (Levings *et al.* 1994) or the absence of settlement cues, may also substantially affect the dynamics of the recovery process. On one hand, we know too little of the dynamics of dispersal and settlement to assume that recolonization from distant sources will be sufficient to repopulate an area after a major spill. On the other, well-meaning attempts to assist recovery may actually slow the process; clearing and replanting of dead areas appears to have slowed repopulation of mangroves after the Bahía las Minas spill (Duke and Pinzon 1993). The need for much additional research is clear. Prior planning and approval of clean up and mitigation procedures after detailed consideration of possible direct and indirect effects is highly recommended.

Factors Important in Generalizing from our Results

Case studies are routinely used to estimate possible damage from future oil spills. However, results from studies of the 1986 Bahía las Minas oil spill cannot be accurately used unless the biological basis of injury and recovery patterns are understood. In addition, physical factors control the spread and persistence of oil and must be taken into account. As the best studied tropical oil spill, results of the Bahía las Minas oil spill are likely to be widely cited. However, damage would have been predictably different under different physical conditions because oil would have been deposited differently along the shore. For example, if more oil had been deposited deep in mangrove forests, there might have been less damage to corals and sea-grass beds, and more damage to interior mangrove forests. Repopulation would then have followed a different (and probably slower) course in interior mangroves, with more oil sequestered in sediments with little water exchange. Because seagrass beds died only where sediments were oil-soaked, it is unlikely that any seagrass beds would be killed if oil came ashore at higher water levels. As is shown by the potentially wide-ranging effects of weather and sea conditions on oil deposition, the use of

this case study requires taking into account the possible patterns of oil deposition that would have been produced by other combinations of conditions.

SUMMARY

In April 1986, an estimated 75,000 -100,000 barrels of medium-weight crude oil spilled into Bahía las Minas on the Caribbean coast of Panamá. As part of a five year study of this spill, we examined the habitat formed by the outer shoreline fringe of red mangroves (*Rhizophora mangle*) and the plants and animals encrusting the submerged prop roots of these mangroves. Submerged prop roots serve as hard substrata for algae and sessile invertebrates, a limited resource where mangroves grow. Thickets of submerged prop roots and plants and animals living on the roots are nursery grounds for many ecologically and economically important species. The productivity of these nursery areas is directly related to the abundance and persistence of submerged prop roots.

Evidence from our studies shows that damage from an oil spill was much longer lasting than the initial rapid and massive die-offs of oil-coated plants and animals. Five years after the Bahía las Minas spill, there were significant reductions in the total length of shoreline fringed by red mangroves. In areas where red mangroves survived or regenerated, submerged prop roots were fewer and extended less deeply into the water. Oysters and false mussels collected from submerged prop roots between 1986 and 1991 had continued high tissue levels of oil residues, which were associated with reduced populations of oysters and false mussels during the five year study. Release of oil from sediments caused chronic reoiling; virtually undegraded oil residues were found in some heavily oiled sediments 6 years post-spill. These data also indicated possible oil contamination of the near-shore food chain. Additionally, we estimated that this spill reduced the number of roots bored by isopods by over 60% and the amount of habitat for post-larval spiny lobsters (*Panulirus argus*) by 40-50%.

Workers attempting to generalize our results must carefully consider short-term, seasonal and annual variation in physical conditions (e.g. weather, sea, and runoff). During this spill, the spatial pattern of oiling, and therefore the habitats and biota affected, was dependent upon conditions at the time of the spill, and would have been predictably different in other seasons.

Considering that more than half of the tropical and subtropical shorelines of the world are fringed with mangroves, oil spills have the potential to cause severe and persistent damage to the ecologically and economically important species dependent upon these critical coastal habitats. This potential must be carefully studied as part of the contingency planning process. It is critical that we understand the biological processes underlaying the type of damage observed. In the case of mangroves, data are not presently available to determine fully the biological causes of oil-related mortality. Until we understand the processes causing mortality after oiling, we cannot develop better risk and damage assessments or more effective cleanup and mitigation strategies.

CONCLUSIONS

1. It can take months to years to estimate how much habitat will be lost, even as a direct effect of oiling. Less obvious direct and indirect negative effects may be subtle and/or difficult to identify.

2. Recovery of the trees (biogenetic substrate) and reestablishment of the structure and environment of the mangrove fringe will probably take decades or longer.

3. Toxic hydrocarbon compounds will probably persist for at least 20 years, and continue to affect the plants and animals of the mangrove fringe. Transmission of toxic compounds up the food chain or to surrounding habitats is possible, but has not been documented.

4. Contingency planning should combine knowledge about patterns of damage from oiling with estimates of where oil will penetrate. These estimates should be based on seasonal and annual variation in weather and sea conditions that affect oil deposition.

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Appendix 1

Publications: Studies of effects of the 1986 Bahía las Minas oil spill on the mangrove fringe

Journal Articles

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Table 1

Changes in the mangrove fringe five years after the Bahía las Minas oil spill: Comparisons of oiled vs. unoiled sites

	Open Coast	Channels and Lagoons	Drainage Streams
Quantitative changes:			
Percent of fringe lost	- 13%	- 23%	- 66%
Percent reduction in density of submerged prop roots	- 24%	- 20%	- 16%
Percent change in root length	- 5%	no change	- 32%
Total area of submerged root surfaces	- 34%	- 38%	- 74%
Qualitative changes:			
Percent increase in the proportion of dead roots	+ 63%	+ 950%	+ 750%
Percent increase in light transmission to the water surface	+ 46%	+ 46%	+ 164%

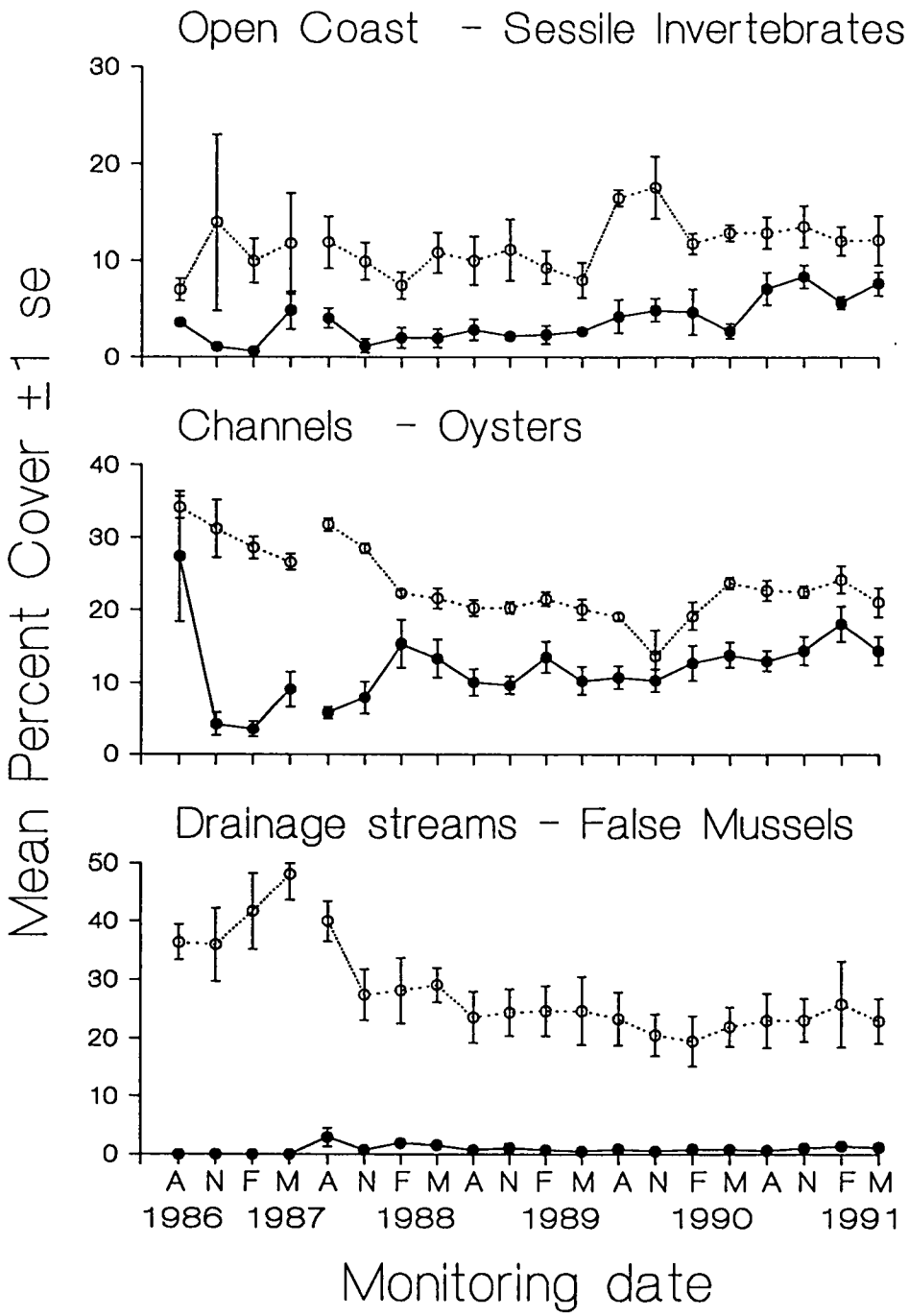

Figure 1. Mean percent cover of major sessile animals. Among site means ± one standard error (n = 3 - 5 sites/point). Open symbols = unoiled sites, filled symbols = oiled sites. Quarterly monitoring began in August 1986, three months after the oil spill. Lines are broken where site locations were changed one year after monitoring began. Note that scales differ among panels.

Is Genetic Degradation of Mangroves a Consequence of Petroleum?

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INTRODUCTION TO THE PROBLEM

The detection of dispersed mutagens in ecosystems involves overcoming a number of obstacles: generally the chemical nature of the mutagen or mutagens is unknown; these unknowns are quite often in low concentration and may be episodic with reference to their presence in the environment; and finally, the mutagenic material may be restricted to, or more concentrated in, a specific portion of the environment (for example, in a marine environment the mutagens may be in the water or bound to organic material or clay micelles). Because of these complexities the employment of a single assay protocol to screen ecosystems for dispersed mutagens runs a high risk of generating "false negatives," *i.e.* not detecting mutagenic activity when mutagens are present. Thus satisfactory screening of an ecosystem must be based upon diversity of assay protocols.

Four general approaches appear feasible for mutagens. These four assays either concentrate mutagens from an ecosystem by physical or biological means or detect mutational damage in test organisms by the dispersed mutagens. The following list will illustrate these assays:

(1) Sampling and concentrating a fraction of the environment (atmosphere, water, and soil) and assaying formutagenicity with a laboratory based assay (Pelon *et al.* 1977);

(2) Introducing the mutagen assay organism into the environment for a period of time and monitoring changes in mutation rate (Schairer *et al.* 1978);

(3) Using indigenous bioconcentrators to accumulate material from the ecosystem and then testing extracts of these organisms with laboratory based mutagen assays (Parry *et al.* 1976; Barnes and Klekowski 1978);

(4) Measuring genetic damage in selected populations of organisms naturally occurring in the ecosystem, an *in situ* bioassay (Klekowski 1978). This communication will consider red mangrove (*Rhizophora mangle*) as an *in situ* bioassay organism.

Mangrove-fringed coasts are a common sight along many tropical shores. In the Caribbean and the Gulf of Mexico the red mangrove, *Rhizophora mangle*, often forms large forests between the land and the sea. These arborescent flowering plants are one of the few tree species that has the necessary adaptations to grow in sea water. In addition, red mangroves possess reproductive traits that permit the easy detection of nuclear and cytoplasmic mutations in the field. Because of these reproductive characteristics, coastal mangrove forests may be assayed for genetic damage in the same way as colonies of bacteria or yeasts are screened for mutation (Lowenfeld and Klekowski 1992). The difference between mangroves and microbes is one of scale; mangroves are larger, have longer life cycles, and are exposed to environmental mutagens for greater periods of time (*i.e.* years rather than hours) (Klekowski *et al.* 1994 a, b, c).

GENETIC ENDPOINTS

An *in situ* mutagen bioassay is based upon the detection of genetic damage (mutation) in selected components of the genetic system of an indigenous bioassay species. Measurements of mutation may be based on a variety of genetic endpoints. In red mangrove two genetic endpoints are convenient to measure in the field:

(1) A suite of approximately 300 *nuclear* gene loci that in some way control the presence of chlorophyll in the chloroplasts (Corredor *et al.* 1995; Klekowski 1992).

(2) A variety of mutations of the *plastid* genome that result in chloroplasts that lack chlorophyll (Klekowski *et al.* 1994 a, b, c).

Nuclear Mutations

The key features of red mangroves that allow for genetic screening are vivipary and frequent self-fertilization. Red mangrove is one of those rare flowering plant species with a sexual viviparous life cycle. In the majority of flowering plants, pollination and fertilization are followed by the development of an embryo that remains dormant within the seed while the seed matures within the ovary of the flower. Upon ovary (fruit) maturity the seeds are dispersed, germinate and the next generation seedling begins to grow. In viviparous plants a number of these post-fertilization stages are either omitted or modified.

In red mangrove the embryo that develops after fertilization does not enter a period of dormancy, but rather continues to grow and develop within the ovary. A seed never develops. The ovary remains attached to the parental plant throughout the growth of the embryo. Eventually the embryo ruptures through the stigma end of the ovary and continues

growth, sometimes reaching a length of approximately 20 cm. During this period of viviparous growth the embryo obtains almost all of its nourishment and important metabolites from the maternal parent. Thus vivipary permits the survival and growth of metabolically defective mutant offspring genotypes.

In addition to enhancing the survival of mutant offspring, vivipary results in the long-term ($\sim$6 months) retention of families of sibs attached to their maternal trees; thus families of embryos may be scored for mutant traits while they are still attached to their maternal trees. Segregation ratios for mutant and wildtype embryo phenotypes can be readily determined; 3:1 ratios for wildtype to mutant phenotypes indicate that the tree is a monohybrid and that self-fertilization occurred.

In red mangroves the most prominent portion of the viviparous embryo is the green hypocotyl. Mutations which result in embryos unable to produce chlorophyll are easily detectable. These mutant embryos are albino or chlorophyll-deficient in appearance. If a tree is heterozygous for a chlorophyll-deficient allele (mutation), self-fertilization will result in a random pattern of green and albino viviparous embryos hanging from the tree. Consequently vivipary and frequent self-fertilization allow for the easy recognition of trees heterozygous for individual chlorophyll-deficient alleles (Klekowski and Godfrey 1989; Klekowski *et al.* 1994 a, b, c).

The nuclear-inherited chlorophyll-deficient genotype is due to a mutant allele at any one of a suite of nuclear genes that controls the synthesis or stabilization of chlorophyll or the development of a chloroplast. In flowering plants this suite contains approximately 300 different nuclear genes (Klekowski 1992); chlorophyll-deficient mutations represent, therefore, a mutational endpoint that monitors genetic damage at 300 genes. Because of the number of genes screened, chlorophyll deficiency is a mutational endpoint that can be used successfully to screen relatively small populations (*ca.* 1,000 trees) (Klekowski *et al.* 1994b).

In natural populations two different manifestations of heterozygosity for chlorophyll-deficiency may occur:

(1) The mutant allele may have arisen in a previous generation and have been passed on to the zygote. In this case all of the cells of the tree are heterozygous for the genetic defect, and, consequently, recessive homozygous viviparous embryos together with wildtype viviparous embryos occur randomly on all branches of the tree;

(2) Mutations may also occur within the somatic cells of a growing mangrove tree; such trees are chimeras. If the mutant cells give rise to apical or axillary buds, there is some chance that these cell lineages may give rise to reproductive cells (pollen in the anthers and/or eggs in the ovary of the flower). Trees with such post-zygotic mutations have a distinctive phenotype. One branch system will segregate for green and albino viviparous embryos while the rest of the tree forms only green viviparous embryos. Such chimeras are the hallmark of recent mutational events, *i.e.* events that have occurred after the tree was established.

Cytoplasmic Mutations

All plant cells have their genetic material partitioned into three subcellular compartments: the nucleus, the mitochondria, and the plastids. In red mangrove it is comparatively easy to detect mutations of the genetic material of the plastid (plastid genome). Although all plant cells have plastids, the visual recognition of plastid genome mutations is easiest in those tissues in which chloroplasts develop. Chloroplasts with defective genomes are not green but are white or yellow, depending upon the mutational lesion. During mitotic cell divisions the chloroplasts divide and are randomly sorted into the two daughter cells. Cells that contain mutant and wildtype chloroplasts are termed heteroplasmic; cells with either all mutant or all wildtype chloroplasts are homoplasmic.

A homoplasmic cell containing only mutant chloroplasts has a white or yellow color in contrast to the surrounding green cells. Homoplasmic cells in the apical meristems (stem tips and axillary buds) may give rise to cell lineages in the leaves that have conspicuous and relatively predictable patterns (Klekowski 1988). Plastid genome mutations occur as chimeras on trees, *i.e.* one branch system will have variegated leaves and the other branches of the tree will have nonvariegated green leaves. Plastid genome mutations are not usually transmitted sexually since the resulting offspring are inviable. A branch system of variegated leaves typically represents a single post-zygotic mutational event of the plastid genome.

Population Analysis

Screening red mangrove populations for an increased incidence of nuclear-based chlorophyll-deficient mutations is conceptually straight forward. One must simply screen 500 to 1,000 trees that have each formed 10 or more viviparous embryos and determine the frequency of trees with green and albino embryos. The trees with green and albino embryos are usually heterozygous for a chlorophyll-deficient mutation. Of course actually screening a mangrove forest often requires some ecological ingenuity. One method is to survey the forest edge from a slow-moving, small boat and score and count trees.

In situations where the incidence of somatic mutation has increased during the lifetimes of the mangroves, *i.e.* the trees are chimeras for either nuclear or plastid genome mutations, the frequency of mutational events is simply the frequency of branch systems exhibiting mutational changes (chlorophyll-deficient embryos or variegated leaves). Each mutant branch system ultimately originates from a single mutational event. For comparative purposes between populations, one useful metric is simply the number of single-origin mutant branch systems per tree.

CASE STUDY

Petroleum Pollution in Puerto Rico

The relationship between sediment hydrocarbon loads and mangrove albinism at sample sites along the southwestern coast of Puerto Rico was studied (Klekowski *et al.* 1994 b,c). For each site sediment samples were analyzed for biogenic and petrogenic hydrocarbons and

the frequency of red mangrove trees heterozygous for chlorophyll-deficient mutations was determined. A positive correlation was found between the concentration of polycyclic aromatic hydrocarbons (PAH) and the frequency of nuclear mutations in the mangroves growing in the contaminated sediments (Klekowski *et al.* 1994 c). Although controversy still exists regarding the occurrence of biogenic PAHs, consensus is that biosynthesis of such compounds is rare. On the other hand, petrogenic hydrocarbons exhibit a characteristically high PAH content.

LIMITATIONS AND ADVANTAGES

Limitations

As in all bioassays, the key problems are false positives, *i.e.* increased mutation for reasons not related to environmental pollution, and false negatives, *i.e.* failure to respond to an environmental mutagen. An example of a false positive is already in the literature; the spontaneous mutation rate for San Salvador Island and Puerto Rico differs by a factor of two (Klekowski *et al.* 1994 b, c). This problem of geographic differences in spontaneous mutation rate is easily resolved by using control populations and establishing a dose response relationship between mutagen and mutation (*e.g.* Klekowski *et al.* 1994 b, c).

The false negative problem is more significant in most bioassays. In red mangroves one can speculate on a number of scenarios for false negatives. The sensitivity of mangroves to mutagens is dependent upon the ability of foreign chemicals (xenobiotics) to enter the xylem stream and be translocated to the apical meristems. It is only when the apical initials are mutated that mutant phenotypes will be manifested either in the embryos or in clear patterns in the leaves.

Many xenobiotics must be modified by cellular metabolism before they are active mutagens, *i.e.* promutagen activation. The extent to which plant metabolisms can activate promutagens and to what degree this process mimics similar metabolism in mammals is an area of active research (Sandermann 1988; 1992; Higashi 1988; Plewa *et al.* 1988). In this regard, the capacity of mangrove metabolism to activate different promutagens is unknown.

Advantages

The majority of human carcinogens have mutagenic activity. Probably the most significant attribute of the mangrove bioassay is that it screens for mutagens and thus may document the occurrence of pollutants that negatively impact on human health. Related to this point are the immediate socio/economic implications; the bioassay documents the genetic effects of environmental mutagens on the indigenous biota of the contaminated environment at environmental concentrations and exposures. Thus the results cannot be dismissed as laboratory artifacts that only occur at artificially high doses or exposures.

Finally, members of the genus *Rhizophora* are pan tropical. Thus although we have developed this bioassay for Caribbean populations of red mangrove, *R. mangle*, other species of the genus *Rhizophora* may also be potential bioassay organisms. The red mangrove

bioassay is inexpensive, is very low tech, and can be taught to technicians with only a rudimentary knowledge of plant biology. If other species of *Rhizophora* share similar genetic characteristics with the red mangrove (*R. mangle*) then the mangrove *in situ* environmental mutagen bioassay have broad utility in tropical coastal environments.

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Session II. Remediation Techniques: Reviews and Current Research

Chair: Dr. Pasquale Roscigno

Presentation	Author / Affiliation
Bioremediation: Statistical and Analytical Needs	Dr. W. James Catallo Louisiana State University
Toxicology Research: An Update on EPA Methods for the Evaluation of Oil Spill Dispersants	Dr. Carol B. Daniels Environmental Research Laboratory U.S. Environmental Protection Agency
Remediation Techniques: an Overview	Dr. William A. Kucharski Louisiana Department of Environmental Quality Dr. Paul Kostecki University of Massachusetts
Responding to Oil Spills in Marshes: The Fine Line Between Help and Hindrance	Ms. Rebecca Z. Hoff NOAA Hazardous Materials Response and Assessment Division

Bioremediation: Statistical and Analytical Needs

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INTRODUCTION

The techniques employed for biodeterioration and "bioremediation" studies typically are diverse and technically complicated (Blumer 1975; Catallo and Gambrell 1995; Grady 1989; Smith *et al.* 1978; US Environmental Protection Agency 1979; US Environmental Protection Agency 1993a; Wolfe 1977). Laboratory and field work addressing the effects of biological processes and human manipulations on chemical fates in environmental systems rely on procedures from microbiology, analytical chemistry, soil science, aquatic chemistry, and multivariate statistics. While there is no doubt that naturally occurring micro- and macro- organisms can degrade or transform organic chemicals under a range of conditions (Catallo 1991; Grady 1989; US Environmental Protection Agency 1979; Wolfe 1977), the principle appeal of bioremediation techniques is the claim that pollutant degradation rates are significantly enhanced by biotreatment approaches, and that the toxicity of chemical mixtures is reduced concomitantly (Catallo 1991). This claim has been applied not only to simple mixtures of labile hydrocarbons (e.g., n-alkanes in jet fuels), but to virtually every kind of pollutant chemical class and mixture of concern including chlorinated pesticides, petroleum and petrochemicals, chlorinated solvents, halogenated biphenyls and their congeners, heterocycles, and polynuclear aromatic hydrocarbons in soils, sediments, surface water, and ground water (Grady 1989; Hazardous Materials Control Research Institute 1989; US Environmental Protection Agency 1993 a).

Critical evaluation of the analytical and statistical methods of many bioremediation studies suggest otherwise: as a result of a) inherent complexity and variability of environmental systems, b) the extreme complexity of many pollutant mixtures of concern and, c) important (and sometimes fatal) experimental method limitations, the effects of biotreatment on pollutant degradation rates and toxicity frequently cannot be decided with confidence, even in cases where large differences are reported (Bragg *et al.* 1994; Catallo 1991; US Environmental Protection Agency 1993 b). In general, the method limitations of primary concern are those relating to a) experimental design and statistical analysis of laboratory and field data, b) the analytical chemistry of environmental mixtures, c) recognition of method limitations, propagation of errors, and sources of bias and, d) rules of deduction for developing conclusions from frequently incomplete experimental data (Bain and Engelhardt 1992; Davis 1986; Poole and Poole 1991; Snedecor and Cochran 1980).

Recent reviews of the use of basic statistical models and techniques by environmental scientists have provided noteworthy results. For instance, a critical analysis of 151 papers in seven prestigious journals showed that only 12% of the papers were free from basic errors in

design, selection of tests, execution of analysis, and interpretation of results (Underwood 1981). Similarly, there has been consistent criticism of the ways in which the handling, extraction, and analysis of chemical and microbiological samples have been performed as well as increasing dissatisfaction with interpretational problems caused by inappropriate sampling designs (Harmsen 1986; Kirchmer 1983; Taylor 1986; Thomas 1994). Some workers have concluded that estuarine research has stopped progressing, largely as a result of these problems (Schoer and Duwe 1986). Related developments have included "re-evaluation" of long-term biological data sets in light of new data on the effects of extreme environmental variability which was insufficiently addressed by sampling designs (Cullinane and Whelan 1983; Steimle and Caracciolo-Ward 1989).

Although it would be easy to find a large complement of statistical and analytical flaws in recent bioremediation papers (most of which are found in venues not subject to rigorous peer review, e.g., Catallo 1991), it is doubtful that this would have any positive effect on the way these studies are performed and evaluated. Rather, it seems more productive simply to indicate several of the major challenges that are involved in the design and implementation of a statistically robust and analytically sound bioremediation study. This will include a brief overview of experimental design and general systems theory from the chemometric standpoint, a discussion of the assumptions and limitations of prominent analytical techniques used widely in bioremediation experiments, and consideration of the effects of error propagation, bias, poor replication, and insufficient sample size on the conclusions of bioremediation studies. Although these topics will be segregated where possible, statistical principles apply not only to design and analysis of experimental systems, but also to the routine operation and data handling associated with extraction and instrumental analysis of chemicals from soils, water, or tissues. As a result, statistical and analytical procedures are integral parts of the deductive process in bioremediation experiments, and the effects of their cumulative assumptions and errors can have significant impacts on what may be concluded, even when things appear obvious or straightforward. What follows is a series of concepts that hopefully will serve as heuristic tools for the design and evaluation of future work. In keeping with the format of these proceedings, statistical and analytical postulates given below are stated rather than proved, with rigorous handling to be found in the references (Aldrich and Nelson 1984; Bain and Engelhardt 1992; Blackburn 1989; Davis 1986; Deming and Morgan 1987; Edwards and Hamson 1989; Harmsen 1986; Kirchmer 1983; Lande and Arnold 1983; Perko 1991; Ruhla 1992; Snedecor and Cochran 1980; Taylor 1986; Thomas 1994; Underwood 1981).

EXPERIMENTAL DESIGN

A fundamental difference between science and "common sense" is that scientific conclusions rely on controlled experimentation, replication of results, and statistical methods of inference. Statistical inference is governed by mathematically consistent rules and procedures for tracking the sources and flows of error in experimental systems and for determining differences (with specified levels of uncertainty) between groups of observations being compared (Bain and Engelhardt 1992; Davis 1986; Perko 1991). In general, these

mathematical rules apply to specific experimental configurations and data types (Aldrich and Nelson 1984) and are invalid when used outside their domain. The most widely used system is the general linear models family of techniques, including ordinary least squares regression, probit, and logit (Aldrich and Nelson 1984). These models are invalid if used to compare classes of data not conforming to the so-called Gauss-Markov Assumptions (Aldrich and Nelson 1984; Bain and Engelhardt 1992). Briefly, these assumptions require that a) independent variables not be collinear, b) no superfluous independent variables are incorporated in the analysis, but all relevant variables are included (see below) and c) random error is not correlated with any independent variables and has a mean of zero. It is no surprise that experimental biology generally, and bioremediation specifically, deals with processes that are influenced by a large number of factors (many of which are nonlinear, highly correlated and/or unknown) and variability of important properties tends to be large and uneven (Aldrich and Nelson 1984; Davis 1986; Lande and Arnold 1983; Ruhla 1992, Thomas 1994). Application of linear statistical models to these studies can be problematic, particularly when input and output variables (below) can be complex and numerous.

An essential first step to valid statistical inference is to develop a model of the system under consideration (Deming and Morgan 1987; Edwards and Hamson 1989). As shown in Fig 1., the experimental system is a group of interacting elements behaving as a unit. Among other things, it is necessary to characterize the system at least conceptually, and estimate the character and likely extent of variability within the experimental units (study sites, analytical configurations). A design can then be made that allows for robust and powerful statistical treatment of data.

Although there are inherent or self-evident characteristics that aid system identification and delimitation (e.g., open water vs. wetlands vs. uplands), system boundaries tend to be chosen more or less at will. Ideally, the experimental system should be approximately homogenous in its properties, and its dimensions should be large enough so that important processes are not altered by the boundaries (e.g., as when surface area to volume ratios of microorganisms decrease to such an extent that adsorption phenomena at the surface of the system are significantly relative to adsorption on sediments) (Blackburn 1989) or significantly perturbed by experimental conditions (e.g., field workers stomping around marsh plots, see Bender *et al.* 1981). Input variables impinging on the system are called "factors" if they exert a real effect on the system. Typical input factors in bioremediation might include experimental treatments such as use of nutrients, bacteria, and tilling as well as natural physicochemical processes affecting the recovery of analyte chemicals being treated (e.g., volatilization, plant root processes, sediment properties, photochemical oxidation, leaching, complexation or acid-base reactions followed by phase transfer) (Parris 1980; Ponnamperumma 1972; Rapaport and Eisenreich 1984; Smith *et al.* 1977). In each setting, these input factors can be known , unknown, controlled (i.e., limited experimentally by one value or a predetermined range) or uncontrolled. It is necessary to know all factors of importance to processes under study, and account for as many as possible in the experimental design.

Similarly, the system outputs (measurable responses to inputs) can be known, unknown, important or unimportant. The importance of individual outputs frequently depends on the kind of problem being addressed. For example, flavinoid derivatives

generated in the brewing of beer may be significant in a chemical sense, but not of concern to brew masters who assay for taste and color. This output would be considered known but unimportant. If, however, these compounds caused allergic reactions in beer drinkers, this output becomes known and important. Clearly, it is important to fully realize the set of outputs from experimental systems so that they can be prioritized with respect to importance, and appropriate measurement techniques can be deployed. Another important type of output is called a feedback: a system output that influences the system as an output (e.g., accumulation of enzymatic products that inhibit further enzyme activity). Feedbacks are widespread control elements in environmental and biological systems, and tend to give rise to threshold and nonlinear behavior in the system.

Experimental design of both laboratory and field bioremediation experiments should account for important input and output variables through controls and replication. As shown in Fig 2, mistakes, omissions, or oversimplifications can have a range of consequences, from virtually benign to disastrous. For example, if important input variables are accurately identified, and insignificant variables are discounted, then the desirable results in blocks A and D can be achieved (Fig. 2). If an input variable is incorrectly identified as true and important (block C), the experimental data set can become unnecessarily large and labor intensive, but this mistake can wash out of a correctly executed study. The failure to identify a true and important input variable (block B) can have negative consequences, ranging from loss of statistical power to virtually complete vitiation of the worth of the results. Extremes of this problem occur readily in field work, but also can be found in laboratory experiments, e.g., when adequate controls are not established.

Under-specification of output variables can have a range of consequences (Fig. 3). As with input factors, accurate and complete specifications of responses can give rise to results that are powerful and elegant (i.e., shaved by Occam's Razor, blocks A and D). An erroneous claim that a response is true and important (block C) may cause no problems, because analysis of output data frequently will show no significant relationships between an unimportant response and the input factors. But, underspecification of true responses can have very unfortunate consequences, as when drugs designed for specific purposes have unforeseen, toxic side effects. Another example would be remediation techniques that give rise to toxic materials of high mobility that escape the treatment system unmonitored (more on this below, see Catallo and Gambrell 1995; Catallo 1993; Corke *et al.* 1979; Grady 1989; Pieper *et al.* 1992).

A CASE STUDY

Consideration of the general material above suggests some elements that should be looked for when designing a bioremediation study or evaluating the results and conclusions of completed work. Primary issues include whether the appropriate input and output variables have been identified, measured, and controlled and whether sources of bias have been rigorously prosecuted in the design. For example, consider an experiment recently conducted in my laboratory on the effects of biological treatment on benzo(f)quinoline (I., below) in estuarine water.

I. Estuarine water was spiked with 20 ug/g of benzo(f)quinoline in sealed glass flasks, followed by perturbation with three controlled inputs (microbial inocula, inorganic trace nutrients, oxygenation). The output variable monitored was the concentration of benzo(f)quinoline, as determined using solvent extraction followed by GC and GC-MS. Several identical treatment chambers (replicates) and an equal number of controls (sterile estuarine water receiving benzo(f)quinoline, oxygen and nutrients) were established. During the experiment, precautions were taken against competing processes (volatilization, photochemical modification) and the analytical approach was "tight " (good recovery, acceptably low systemic error). After some weeks it was found that the magnitude of the output variable (benzo(f)quinoline concentration) of the treatments decreased in time relative to little decrease in the controls (Fig. 4A). It was concluded that the addition of the microbes promoted removal of the benzo(f)quinoline relative to sterile controls. Fortunately, data from other experiments suggested that this system was under-controlled in an important sense: passive adsorption of benzo(f)quinoline to microbial cells without degradation could have occurred thereby limited recoveries so as to look like removal from the system. Unchecked, this case of unconscious bias could have resulted in wasted time and effort in a pilot or field experiment. In order to determine causality in this system, it was necessary to re-design the experiment: the input variable combinations were permuted vs. appropriate controls with replicates, and "killed" biomass controls were added. Hence, every nontrivial treatment combination was tested vs. controls, including a set of controls obtaining dead biomass to examine the effect of non-metabolic biosorption on time course recovery of the analyte. As shown in Fig. 4B, time-dependent biosorption was in fact very significant to benzo(f)quinoline recovery: as the least squares regression lines and 95% confidence intervals for control, killed control, and estuarine microbes show, passive biosorption and systematic analytical error had the effect of looking like degradation in the killed controls. In fact, the confidence intervals showed that only the last few time points in the live microbe treatments were clearly distinguishable from the killed control function. Luckily, the expanded design contained the adequate controls, and this indicated that product analysis also should be incorporated at each time point. This allowed for a sound conclusion to be reached: benzo(f)quinoline is degraded slowly by estuarine microflora to an epoxide product (II., below) that is stable for extended periods, mobile, and probably mutagenic (Catallo and Gambrell 1995). This was confirmed using data from pathway studies using deuterated benzo(f)quinoline synthesized in this laboratory. A competing process is quasi-reversible or irreversible sorption of the analyte to organic matrices (colloids, micelles, cells). Further degradation of the epoxide can occur via microbial and chemical action (Catallo and Gambrell 1995). These processes are very slow, and residual benzo(f)quinoline and its transformation products can be detected in water and sediments for months to many years, depending on conditions. One wonders how often processes such as sorption to surfaces and biogenic matter (e.g., large amounts of microbial biomass that are dumped into field sites to be bioremediated) limit recoveries in this way and pass for biodegradation of the analyte.

It can be seen that demonstrating causality and generating accurate interpretations even in this simple system involved a large amount of multidisciplinary work (microbiology, synthetic organic chemistry, analytical chemistry, statistics). The addition of other variables (e.g., sediments, redox potentials, plants) and compounds (complex mixtures such as

petroleum) to these studies adds to the complexity and labor needed to provide defensible results. It is important to note that the error of each procedure and analytical technique in these complex experimental systems is roughly multiplicitive. Hence, a five step sampling, extraction, analytical, data reduction hierarchy involving 5% experimental error at each step (an almost unbelievably low level of error in real settings) has a total experimental error as high as $(0.95)^5$ or about 23%. The random errors at each step also introduce further increases in absolute error, especially if there is inadequate replication. This is another major reason why extensive replication and adequate controls are needed in experimental biology, and why results from extremely precise and accurate analyses (e.g., quantitative chemical analysis) can frequently provide very little help in resolving subtle effects between treatments: total error running through complex experimental systems can be so large as to "swamp" the deductive power of very precise analyses. Replication adds power to procedures that may have large experimental errors, and can reduce significantly the random error. When field experiments are considered, the ability to control or even anticipate important input variables becomes limited. The disappearance of target chemicals frequently results from factors not related to microbial metabolism, and large variabilities within experimental units can make things look conclusive (Bragg *et al.* 1994; Catallo 1991; Cullinane and Whelan 1983; Schoer and Duwe 1986; Smith *et al.* 1977; Steimle and Caracciolo-Ward 1989; Taylor 1986; US Environmental Protection Agency 1993 b). The notes on chemical analyses given below suggest that total analytical error alone can limit interpretations to order-of-magnitude changes. The best way to compensate for this is to have a large enough set of random samples and replicates to overcome the effect of natural variances, and to measure explicitly all known significant input variables. The addition of exhaustive controls also is critical to the success of field approaches. The entire enterprise usually requires multivariate statistical designs and analyses (e.g., structural equation modeling) (Davis 1986; Lande and Arnold 1983).

Consideration of the laboratory example above also illustrates some major differences between laboratory and field studies. In conclusive laboratory work, the system is well defined (i.e., true replication is possible), the data have homogenous variances or nearly so, and the input factors are known and are largely controllable. Under these conditions, a well implemented experimental approach (i.e., with adequate sample size, no bias, requisite true controls, and true replication) can determine causality, usually after a great deal of work. Conversely, field systems frequently are not well delimited, there are high degrees of internal variability, true replication frequently is problematic, and input-output factor sets are complex, not well known and/or "lurking", and largely uncontrollable. Hence, results from field work generally end up being correlative. The confidence that can be ascribed to correlations in field data is greatly influenced by experimental design and analytical protocols as well as the definitiveness of the corresponding laboratory work. Also involved is the assumption (hardly ever examined) that the results of well constructed laboratory studies (microcosms) bare some straightforward relationships to analogous experiments in closely related field settings (macrocosm);

$$P(A)_m \ \alpha \ k\, P(A')_M$$

where the probability P of some complex event(s) A measured in the microcosm m is proportional to the probability of the corresponding event(s) A' in the macrocosm M multiplied by some weighting *k*. We are far from measuring these weighting terms systematically, or testing this mapping with respect to complex sets of variables. In general, we hope that the true impact of *k* is minimal or at least predictable.

Without any abstraction, it seems clear that determining the efficiency of bioremediation in field settings (hazardous waste sites, oil spills) is a task requiring adequate statistical design, multidisciplinary expertise, and analytical savvy. The experimental system must be known, the winnowing of input and output variable sets to a manageable monitoring scheme must be accurate and unbiased, and the field experiments must be well controlled, highly replicated, randomly sampled, and analyzed with accuracy. It follows that the results of field evaluation projects with a small number of experimental units (e.g., 1 - 3 plots), few or no true replicates, multiple uncontrolled inputs and unmonitored outputs will be difficult to assess with any power. In fact, a large proportion of bioremediation pilot projects under the EPA Superfund Innovative Technologies Evaluation (SITE) program suffer from these kinds of limitations [11]. Other "showcases" are similarly limited, with adequate controls lacking or altered during the experiments, sparse replication, and QA on site selection, analyses, and sampling difficult to evaluate (Bragg *et al.* 1994).

EXTRACTION AND INSTRUMENTAL ALAYSIS OF CHEMICALS

An area of continuing difficulty in the interpretation of environmental data is analytical chemistry in general, and the sciences of chromatography and mass spectrometry in particular (Harmsen 1986; Kirchmer 1983; Poole and Poole 1991; Thomas 1994). In the last two decades, a revolution in separation technologies and microelectronics has made sensitive analytical instrumentation and reasonably friendly data handling available to most laboratories. Of particular interest here is the identification and "quantification" of semivolatile organic chemicals using some form of internally standardized chromatography followed by mass spectrometry. The industry standard is capillary column gas chromatography (GC) followed by mass spectrometry (usually quadrupole "mass selective detection") or GC-MS. Chromatography with other detection systems such as the flame ionization or electron capture detection are two dimensional techniques: for each time point in the separation (x), there is a detector response value (y) that is proportional to compound concentration. Compounds in environmental matrices can be tentatively identified using these 2-D techniques, but further characterization is needed for conclusive work because of problems such as co-elution (particularly in complex environmental or biological mixtures). Mass spectrometry adds a third dimension to 2-D GC: for each x,y pair, a mass spectrum (z) is acquired. The mass spectrum can provide definitive information on identity of the analyte even in cases of co-elution. Hence, conclusive demonstration of the presence of a chemical can result without further analysis. Also,integration and comparison of mass spectral intensities from analytes and known standard curves can allow for semiquantitative or quantitative analyses to be performed (Table 1).

A popular, and frequently mandatory, approach to documenting the presence and concentrations of organic chemicals is EPA Method 625 CAP and a modifications/extensions thereof. In bioremediation studies, these methods frequently are used to document the "removal" of target analytes (e.g., PAHs given in the EPA Toxic Substances List) using selective ion monitoring (SIM). SIM approaches are appropriate for monitoring low levels of selected analytes and providing macro-driven data analysis (e.g., so-called "fingerprinting" approaches), but information falling outside SIM windows is irrevocably lost (Fig. 5). The power of GC-MS for qualitative and semi-quantitative identification of unknown compounds in mixtures has promoted a tendency among bioremediation workers to spuriously regard these methods as conclusive and accurate for detecting differences of a few percent in complex samples. In addition to many fallacies promoted by this widespread "black box" attitude, there are many systemic limitations to GC-MS techniques as commonly deployed that are germane to the valid interpretation of bioremediation data:

1) The EPA 625 CAP and related methods are semiquantitative at best with respect to many selected ions of interest. Quantitative analysis (Table 1) would require a) a broad series of internal and surrogate standards many of which are not commercially available and must be synthesized, b) isotopic dilution approaches for each analyte to be quantitated, c) double blind intercalibration, and d) extremely well-maintained and monitored instrumental conditions. In cases where moderate (> six/day) or large sample loads are processed, this level of effort and quality assurance simply is not sustainable in most commercial and academic settings. Indeed, the EPA Method 625 CLP document states: "*These extraction and preparation procedures were developed for rapid and safe handling...of hazardous wastes. The design...does not stress efficient recoveries or low levels of quantitation. Rather, procedures were designed to screen, at moderate recovery and sufficient sensitivity, a broad spectrum of organic chemicals.*" In other words, this disclaimer suggests that semi-quantitation may be the best that can be expected for real-world analysis of selected compounds on the EPA Toxic Substances List (for which these methods were developed to detect) with virtually everything else missed. It is useful to consider what "order-of-magnitude" means with respect to propagation of errors (above) and the facile conclusion that biodegradation has occurred in samples showing small differences. Although trained chemists with adequate leisure, support, and facilities can quantitate organic chemicals in defined systems, the effort required is, quite simply, enormous and unlikely to be sustained in most settings. In this regard, it is noteworthy that numerous lawsuits have been filed against EPA certified contract labs for corner-cutting on the execution of Method 625 CLP.

2) The selection of target analytes to be monitored at bioremediation sites usually is driven by regulatory requirements, not by data on the presence, environmental significance, and toxicity compounds. In other words, these methods "see what they look for, and look for what they can see." For

instance, in bioremediation of creosote and heavy asphaltic waste oils, the concentrations of PAHs e.g., naphthalene, anthracene/phenanthrene, and pyrene (on the List) are monitored routinely and changes in their measured concentrations are used as evidence of the effectiveness of the remediation effort. These compounds and their known degradation products are not mutagenic or carcinogenic in bacterial or mammalian assays [6]. Heterocyclic congeners in these mixtures (i.e., quinoline, acridine/ benzo(c)quinoline, and azapyrene) are mutagens/carcinogens and are transformed to mobile oxygenated products that probably are biologically deleterious (Catallo and Gambrell 1995; Rostad *et al.* 1984). But these hardly ever are included in analytical monitoring during remediation of creosote because they are not on the List. (Another reason is that these heterocycles are notoriously difficult to biotreat. More than one site manager has told the author "we don't need any more problems." This meant that unexamined outputs, such as the escape of undegraded, unregulated toxic chemicals from the site had no legal significance and therefore could be regarded as nonexistent *ipso facto*. The equation of unexamined outputs with unimportant ones on this basis is a fallacy that impedes true R&D as aggressively as it does mechanistic understanding, cf. ref. Grady 1989).

3) Potentially very significant transformation products of the target analytes in bioremediation studies are not examined. Hence, the modification of benzo(a)pyrene (BaP) to a phenol or diol would be interpreted as "removal" of BaP because the oxygenated products have different chromatographic and mass spectrometric properties (i.e., they fall outside the mandated SIM window). But the removal of BaP coincided with the generation of a mobile and available mutagen. As above with the undegraded heterocyclyes, this unidentified, uncontrolled output from the system could have both regulatory and toxicological significance.

While these limitations clearly need to be addressed (i.e., new protocols need to be developed for analysis of compounds not on the Toxic Substances List, transformation pathways need to be documented and evaluated, and sites to be delisted should be detoxified as well as "cleaned up" from a regulatory standpoint) they do not in themselves preclude a good field bioremediation study from being performed. With attention to the experimental design features and interpretational limitations discussed above, semi-quantitative analyses of complex environmental mixtures can provide very compelling evidence in support of remediation. The addition of full scan analyses of each extract subjected to SIM analysis can add valuable insight to processes of concern, as can multi-tier toxicity evaluation over the course of the treatment. What is needed beforehand is adequate, unbiased design and explicit recognition of method strengths and limitations.

CONCLUSIONS

As suggested above, the importance of several design and analytical elements of experiments cannot be minimized. The work of Huff (Huff) provides an accessible set of touchstones for evaluating scientific claims that are applicable to the subjects of concern here. When confronted with research designs or conclusions in bioremediation work, the following grid of questioning is called for:

1. "Who says so?" When this is asked (even by editors), a typical response is to produce a list of publications. The point, however, is not to judge data by reputations, but the other way around. It is always appropriate to look for conscious bias (e.g., the intentional citing of controls and treatments that are most likely to give anticipated results, the selection of chemical or statistical analyses that miss or obscure important limitations of the approach, attempts to over interpret marginal results, or under value the limitations of techniques). Conflicting interests (business affiliations), institutional pressures (agency deadlines and funding requirements), and other concerns (i.e., rushing work in order to pass tenure review, gain a promotion, or limit a fine or damage settlement) also can cause bias, and should be examined where possible. It is equally important to account for unconscious bias (e.g., under specification of the input and output variables; under controlling experiments, other conceptual gaps). Accountability (rather than deniability) should be stressed. This means that system models, site selections, experimental and analytical configurations, raw data, assumptions, error estimates, and statistical approaches should be available and made part of the deliverables provided to the funding organization.

2. "How does he know?" Is the sample large enough to permit a conclusion, is the system specified adequately and correctly, are there adequate true replications, what real bearing does previous work have on current claims (e.g., are past mistakes or shortcomings recognized and rectified in current work)? Does the design and implementation of the study clearly support claims, or is there unwarranted subjectivity in the conclusions?

3. "What's missing?" Are controls adequate and appropriate, is there true replication, are raw numbers presented, or only "crunched" values, do we have access to information on the original work, the sites, and detailed descriptions of operating conditions, has error been addressed specifically and can it be found stated in plain form?

4. "Did somebody change the subject?" Confusing correlation with causality, or changing the statement "x changes with y" to "x changes because of y"; under valuing the importance of variability, competing processes, and systemic error.

After this gauntlet has been run, detailed evaluation of the effectiveness of a bioremediation technology may begin. As rude and invasive as this approach may seem, it is an integral part of the critical scientific method and should be welcomed rather than shunned. In all critical analysis, arguments *ad hominem* (to the man), *ad verecundiam* (from authority), *ad ignorantium* (from ignorance) and *ad baculum* (from threat) are discounted immediately as fallacious and flawed in form. Only the data may speak through an argument *ad judicium*: a grid of unbiased statistics and logical rules of deduction and inference. To identify scientific flaws, or huge bodies of flaws (Underwood 1981) is no attack (i.e., *ad hominem*), it is among the most constructive things critical people can do for one another and their institutions. Even in the emerging age of forced "tech transfer" and mandatory "industry partnerships", the only truly lethal mistake for researchers is international fraud. Objectivity is safeguarded when scientists open their approaches to dispassionate, disinterested critique. Once done, even experiments that are highly flawed methodologically can be found to contain useful information. While it is best to submit experimental designs to statisticians for critique before initiating work, statisticians also can perform triage on finished studies in order to extract valid, if not very powerful, interpretations. Other insights can be gained on appropriate ways to compare past results, or those from other studies, to issues under investigations. When the mainstream of environmental scientists, managers, and regulators (Particularly those interested in bioremediation) remember this, much productive work is likely to result.

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Table 1.
Working Definitions of Selected Terms

Bias: Conscious or unconscious deviations from randomness in design (selection of experimental groups, locations, sampling patterns, etc.) so that certain outcomes are favored or enhanced in the data set. Introduction of a "tilt" to data through unsound design or execution.

Calibration Curves: Curves of compound concentration vs. detector response constructed using known standards prior to sample analyses. The slopes of these curves correspond to a number of factors including detector response factor, type of integration, instrument tune. Integrals of analytes in samples are referenced to these curves, and concentrations are determined accordingly.

Internal Standard: A compound or series of compounds added in known amounts to finished extracts prior to analysis by chromatography or GC-MS. These standards are used to correct for sample loss in the analytical system (in the injection port, on the column, or at the detector).

Locality: A property of biased data in which values collected and statistical results derived from them are valid only for the limited area sampled.

n: Number of observations in the experimental population.

Nonlocality: Property of unbiased design in which sufficient random data have been collected so that statistics generated are good estimators of population values for the whole system.

Power: Refers to the ability of a statistical procedure to correctly reject false hypotheses, such as spurious difference between experimental treatments. In subjects of concern here, power can be increased by decreasing system variability and/or increasing sample sizes.

Psuedoreplicate: Multiple samples taken from an experimental system, each of which are treated (erroneously) as separate observations from different populations. e.g., two tanks used for biotreatment of compound R. One receives fertilizer, the other does not (control). At intervals, three samples are drawn from each tank, and are analyzed. The three values from each tank are then averaged and compared. These are pseudo-replicates (see "replicate") if used in statistical comparisons as "n" values. In order to be true replicates, three tanks would be necessary each for control and treatments (six total).

Table 1 (continued)

Quantitative: Refers to analytical procedures having the ability to discriminate between chemical concentrations differing by $<25\%$. These analyses typically feature calibration curves of five points or higher, use of large numbers (e.g., >12) of internal and surrogate standards, and auxillary procedures and measurements including intercalibration, determination of time-dependence of surrogate recoveries, and double-blind quality assurance.

Replicate: Single samples taken from multiple experimental systems under tha same conditions (i.e., treatments). In a sense, replicates are separate observations from different populations exposed to the same input variable set that are sampled and analyzed without bias.

Robustness: Refers to techniques that are powerful for examining a wide range of population types, or cases where large measurement errors occasionally are encountered.

Sample: A randomly selected element of an experimental population.

Semiquantitative: Refers to environmental chemical analyses with the ability to discriminate between chemical concentrations differing by an order of magnitude or more (see "quantitative").

Surrogate Standard: A compound or series of compounds added to the sample matrix (soil, water , tissue) prior to extraction and used to monitor recovery. They also can be used to correct for loss of analytes during sample preparation (volume reduction, chemical drying, fractionation). Sometimes called "recovery standard".

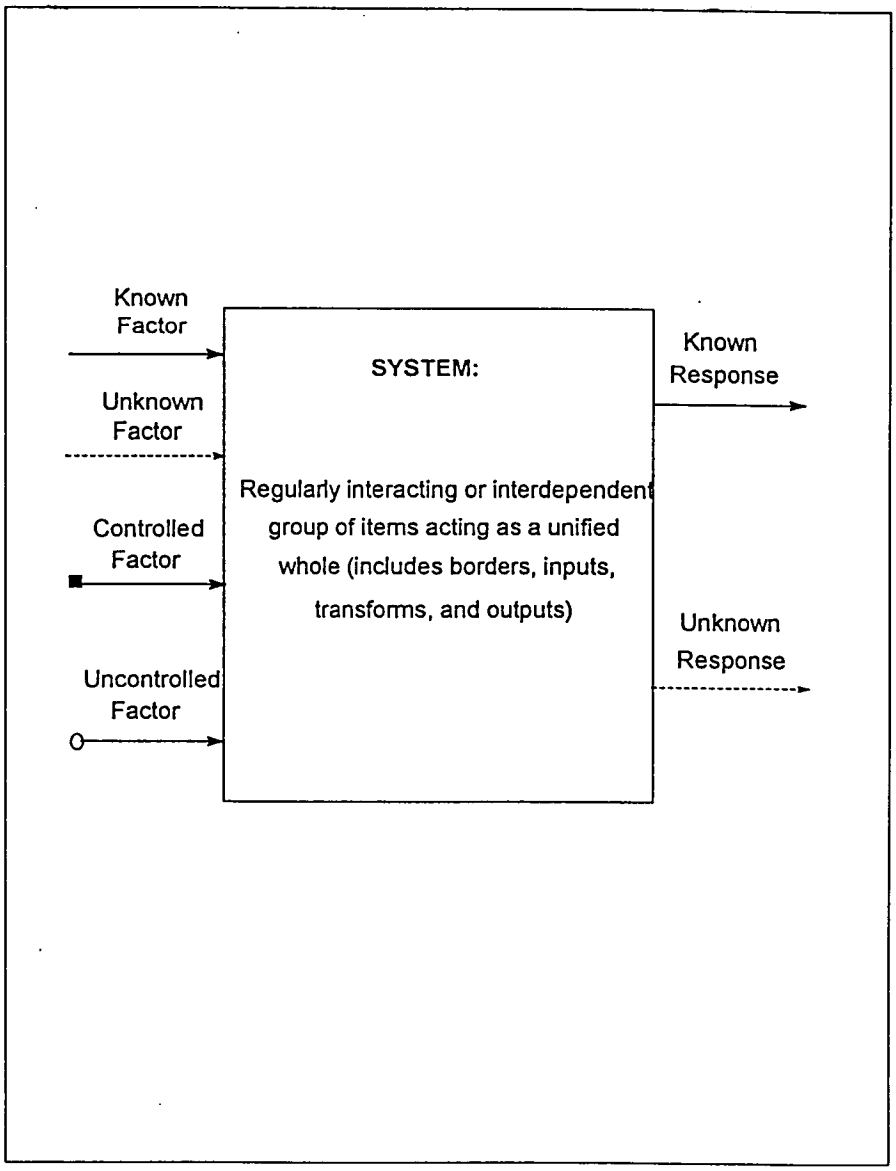

Figure 1. Schematic representation of an experimental system with selected input and output factors.

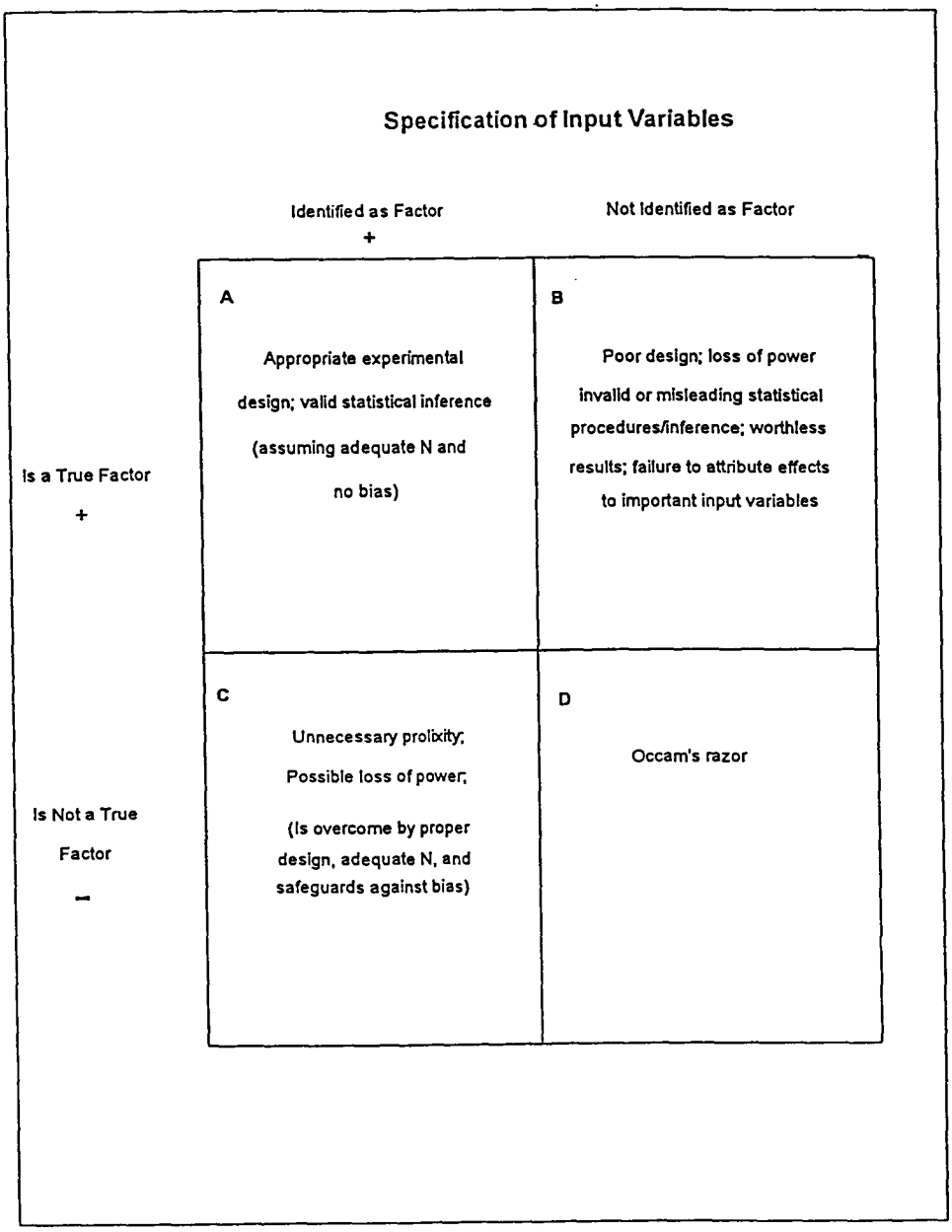

Figure 2. Probable results of adequate and inadequate specification of input variables in experimental systems.

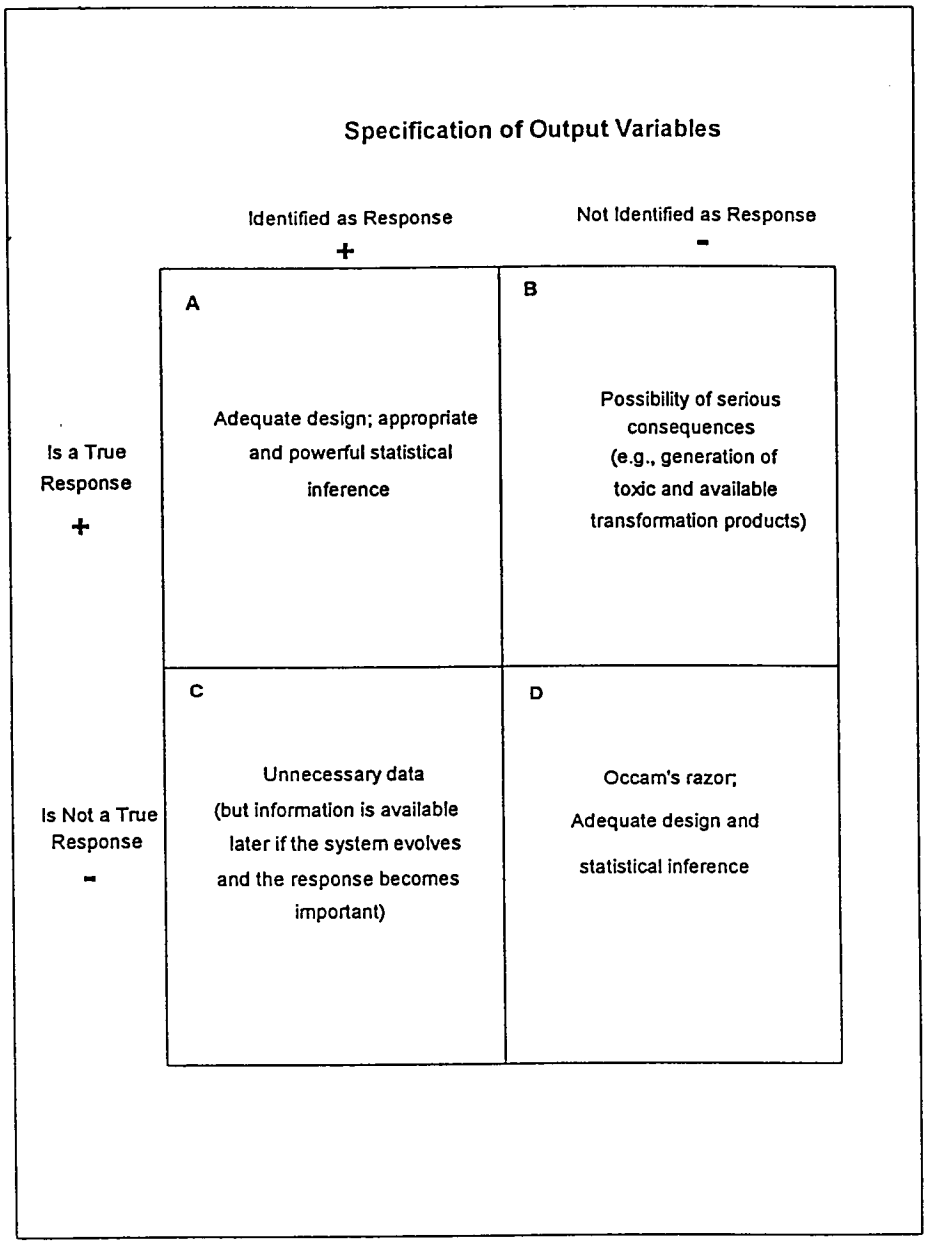

Figure 3. Probable results of adequate and inadequate specification of output variables in experimental systems.

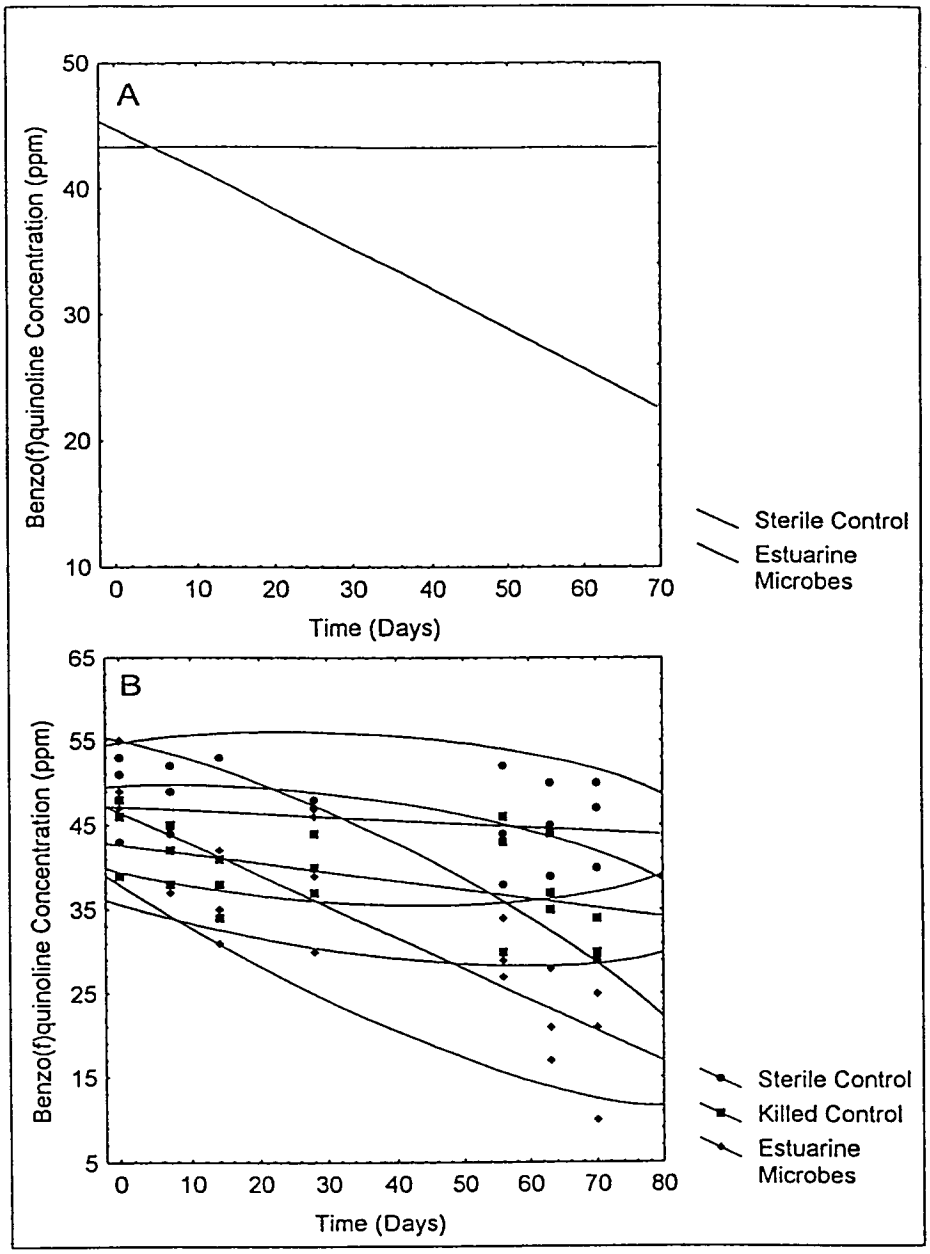

Figure 4. Effects of under-controlling a laboratory biodegradation study. A.) Apparent degradation of benzo(f)quinoline by estuarie microbes vs. sterile conditions. B.) Effect of killed biomass control and system variability on the apparent degradation of benzo(f)quinoline (ellipses are 95% confidence bands on the least squares linear regression lines, n=3).

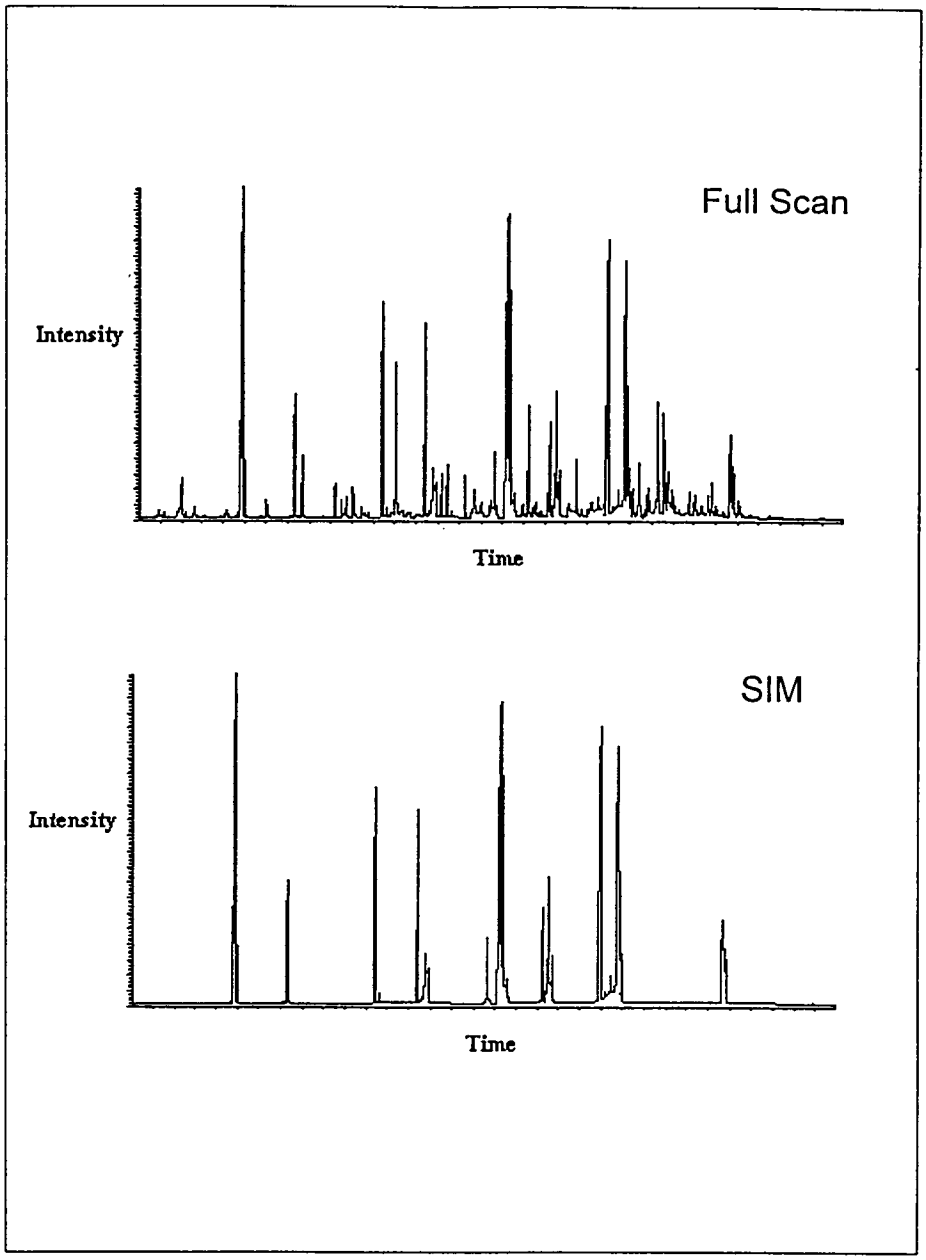

Figure 5. Effect of SIM approaches on GC-MS data sets. Full scan shows approximately 60 resolved compounds and unresolved envelopes while SIM data provides only selected ions (c. 20) within preselected elution ranges.

Toxicology Research: An Update on EPA Methods for the Evaluation of Oil Spill Dispersants[1]

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INTRODUCTION

Change is the hallmark of progress. In science, progress, i.e. change, is typically denoted by the advance of technology. The evolution of each new technology, encourages the stabilization and, in some instances, the demise of other related technologies. Development of bioremediation as a technology for oil spill abatement, is a clear example of waning interest in one technology, chemical dispersants, and the increasing interest in another.

Oil spill research at the Environmental Protection Agency (EPA) has followed a similar trend of expansion and stabilization, with funding of dispersant research declining in recent years. The nutrient enrichment study conducted in Alaska subsequent to the grounding of the EXXON *Valdez* was a significant event which spurred the advancement of other spill response technologies including bioaugmentaion. Since that time, research on bioremediation processes has moved forward, while research on oil spill dispersants has plateaued within the federal, state and private sectors.

Although bioremediation remains as the primary focus of EPA's research program in Oil Spill Pollution, studies have been conducted to enhance our knowledge of dispersants and to minimize potential hazards associated with their use. Within the Agency (EPA), the current effort on dispersants is focused primarily in three areas: (1) spill response and contingency planning; (2) re-evaluation of regulatory test methods for efficacy and toxicity; and (3) risk estimation. This discussion will highlight toxicological research on dispersants as it relates to the latter two categories, regulatory test methods and risk estimation.

Review of Regulatory Test Methods

The bioassays currently described in Subpart J of the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) and used to evaluate the toxicity of dispersants

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[1] Contribution No. 910 from the Environmental Research Laboratory, Gulf Breeze, FL. The research described in this article has been subjected to Agency Review. The views expressed in the article are that of the author, and no official endorsement should be inferred. Use of trade names in this publication does not constitute endorsement by the U.S. Environmental Protection Agency.

and other oil cleaning agents have remained relatively unchanged since written (49 FR 29192; July 18, 1984). The last changes were promulgated March 18, 1990 (55 FR 8666). The standard dispersant toxicity test (SDTT) protocol requires use of a saltwater fish, *Fundulus heteroclitus*, and an invertebrate species, *Artemia salina*, in acute bioassays designed to screen dispersants and other chemical agents for potential effects on the survival of aquatic species. Lethality data from the SDTT is required for the inclusion of a dispersant on the NCP product schedule. These organisms are exposed to a dispersant in a graded-series of dispersant concentrations for a period of 96h under static-flow conditions. The relevance of the toxicity tests described in the SDTT protocol has come into question since *Artemia salina* and *Fundulus heteroclitus* are used as test species and have been reported in some cases to lack sensitivity to crude oils. Use of *Fundulus* poses some limitations on the quality of the test methods employed relative to reproducibility and variability in the data since these organisms are generally not reared in the laboratory.

Concern over the lack of sensitivity of *Artemia* to oil and related by-products has prompted research on determining the sensitivity of other marine test species to oil contaminants. Data derived from routine use of cultured organisms such as the inland silverside *(Menidia beryllina)* and mysid shrimp *(Mysidopsis bahia)* for other forms of regulatory testing (e.g., National Pollution Discharge Elimination System (NPDES) testing in the case of mysids) coupled with toxicological data on pure chemicals (e.g., pesticides, industrial agents, etc.) has lead to extensive toxicological databases describing the relative sensitivity of these organisms to various contaminants (Hemmer *et al.* 1992; Cripe 1990; and Cripe and Cripe 1990). Despite limited information on the effects of oil on these two species (Daniels and Whiting 1993; Daniels and Whiting 1992) collection of comparative data on the sensitivity of *M. beryllina* and *M. bahia* to fuel oils and chemical dispersants has been encouraged by regulatory agencies and industry groups relative to other test organisms *(Artemia* and *Fundulus)*.

To address concerns about the sensitivity of the test species and to accommodate State requests for incorporation of regional test species into the SDTT protocol: EPA scientists conducted more than forty acute toxicity tests to evaluate the comparative sensitivities of five species, the sheepshead minnow (*Cyprinodon variegatus)*, inland silverside (*Menidia beryllina),* mysid shrimp *(Mysidopsis bahia)*, brine shrimp *(Artemia salina)* and killifish *(Fundulus heteroclitus)* using API#2 fuel oil and the dispersants, Corexit 9527 and 7664, in the SDTT protocol for dispersant toxicity. The results of these tests are the focus of this presentation.

96h static acute tests were performed according to the SDTT protocol (48 FR 29192) using the five species noted above (Brizeno *et al.* 1992), The data indicate that the two Gulf of Mexico species, *M. beryllina* and *M. bahia*, were more sensitive to these agents than were *Fundulus*, *Artemia or Cyprinodon*. Seven-day (7d) chronic-estimator tests were performed subsequent to the 96h tests, to assess the benefits of performing longer duration tests with the more sensitive test organisms, *M. beryllina* and *M. bahia* (Whiting *et al.* 1992). Protocols for chronic-estimator tests were also modified to incorporate use of oil-water dispersions (OWD) and water-soluble fractions (WSF) of the API#2 fuel oil. Comparative data on the toxicity of oil, dispersants and oil-dispersant mixtures were also derived.

Chronic toxicity test data indicated that mean 7d LC50 values derived from the chronic-estimator tests were not significantly ($p < 0.05$) different from mean 96h LC50 values from acute tests for either *M. bahia* or *M. beryllina*. Sensitivity of organisms in the 7d tests was dependent on the toxicant, and the rank order for toxicity indicates oil-dispersant mixtures were more lethal than were either API#2 fuel oil or dispersant alone. Growth and reproductive effects, where observed, typically occurred at a concentration just below that noted for a significant change in mortality.

These data provided the Agency's regulatory staff (EPA, Office of Solid Waste and Emergency Response) with the information necessary to recommend use of *Menidia* and *Mysidopsis* as alternatives to the less sensitive *Fundulus* and *Artemia* in the NCP dispersant toxicity test. The revisions to the proposed changes (revised standard dispersant toxicity test; RSDTT) were published in the Federal Register in October, 1993 (58 FR 54702). These changes, coupled with the proposed 45 percent cut-off for efficacy are expected to provide an effective screening mechanism for On-Scene Coordinators and other decision makers seeking safer and more effective agents.

Risk Estimation

Although regulatory test methods play a significant role in the Agency's risk assessment paradigm, supplementary data on the sublethal effects of dispersants to marine species help to define the hazards associated with use of oil spill dispersants in coastal and marine habitats. Toxicological data on the growth, reproduction and developmental success of a species provide key information in the evaluation of ecological hazards for these agents. Shrimp represent a significant economic resource in the Gulf of Mexico region. Despite this, limited toxicity data are available on the effects of dispersants on the development of shrimp (Anderson *et al.* 1987; Anderson *et al.* 1981; Tatem *et al.* 1978 and Shuba and Heikamp 1989); a fact often related to the unavailability of laboratory reared organisms. Use of organisms of similar genetic stock reduces variability within a bioassay making for a more toxicologically-sound test.

Shrimp Embryo Toxicity Test

A toxicity test was developed by EPA researchers, using grass shrimp (*Palaemonetes pugio)* embryos, with the intent of minimizing genetic variation within the test (Fisher and Foss, 1993). Developing eggs from a single ovigerous female were exposed to different concentrations of two dispersants (Corexit 9527 and Corexit 7664), a water-soluble fraction of API #2 fuel oil, and combinations of oil plus dispersant, and monitored for survival and hatch. Although several females were used for a series of experiments, eggs from a single brood were employed for each independent event which was found to greatly enhance the reproducibility of a test.

Data from these developmental tests indicated that oil-dispersant mixtures were generally one order of magnitude more toxic to developing shrimp embryos than was the dispersant alone (Fisher and Foss 1993). Toxicity of the oil-dispersant mixtures was found to be temperature- and salinity- dependent, with elevation of temperature causing a shift in

onset of mortality to an earlier age. In cases where hatching was observed (i.e., control, 10% water soluble fraction of oil (WSF) and 10% WSF plus Corexit 9527), it was relatively synchronous with the onset of hatch which occurred between days 11 and 14.

Wetland Plants

Like the shellfish industry, coastal wetlands are integral to the vitality of the Gulf of Mexico. Their importance as a nursery for coastal and marine fishes has long been recognized. However in ecological risk assessments, the significance of plants associated with these wetland communities, is frequently overshadowed by concern for vertebrate and invertebrate species inhabiting these ecosystems. Preliminary studies were initiated to determine the effect of impact of dispersant use on wetland vegetation. Measures of effect (survival and growth) were assessed for wetland species *Sesbania macrocarpa* (river hemp) and *Spartina alterniflora* (smooth cordgrass) following exposure to a Louisiana crude oil and oil-dispersant mixtures (Weber *et al.* unpublished). Studies to assess the potential impact of a highly refined Alaskan oil on the assimilative capacity of microorganisms associated with the rhizomes of *Spartina alterniflora* were also initiated (Frederick *et al.*, 1993). Because of the preliminary nature of these studies, the data will not be presented here. However, the potential significance of these data and that of others (Mendelssohn and Hester 1990; Webb and Alexander 1991) derives from the fact that these studies may be used to assess the impact of clean-up technologies on wetland vegetation. This type of research may assist in spill response activities by providing a greater understanding of the effectiveness and consequences of clean-up strategies in wetlands. These studies may also emphasize the need for preservation of wetland habitats.

This presentation provides a brief synopsis of toxicological studies currently underway at EPA's Environmental Research Laboratory, Gulf Breeze to enhance EPA oil spill response programs. Toxicity data on mysids, *M. bahia*, and silversides, *M. beryllina*, reviewed here, provided in part, the basis for changes to the dispersant toxicity test described as part of the National Oil and Hazardous Substances Pollution Contingency Plan, Final Rule (FRL-5028-6; 59 FR 47384). Information from studies with oil spill dispersants and aquatic species, *P. pugio, S. alterniflora* and *S. macrocarpa*, can help to broaden our understanding of the effectiveness and ecological consequences of this clean-up technology.

In the current risk assessment paradigm, constant-exposure studies such as those with the mysid and silverside serve as effective tools for hazard identification in a regulatory framework. Despite their current usefulness, research should be expanded to include studies of discontinuous/episodic exposures to enhance the realism of the exposure regimes used in dispersant testing protocols. Expansion of the protocol to include freshwater species has been suggested by some. Performance of field studies is encouraged to judge the true impact of dispersants on marine organisms and to assess the predictive nature of these short-term tests.

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Remediation Techniques: An Overview

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INTRODUCTION

Remediation is a word widely used in conversation and technical journals but you will not find it in an American dictionary because it is actually a newly created word. The root of the word, remedy, can be found however. A *remedy* is defined as "A means of counteracting or removing an error, to make right, correct." Even if American dictionaries do not contain a definition of remediation, the term is used to mean *an applied* remedy. Since this section covers an overview of remedial technologies, the starting point for the section is a definition of the topic. For the purposes of this paper, the term Remediation will be defined more precisely as follows:

> The application of a process or technology to a mixture of materials such that the problem or hazard associated with the mixture is counteracted or removed.

There are three physical principles that form the basis of all remedial technologies and designs. These principles are *physical separation*, *physio-chemical reactions* and *chemical/biochemical reactions*. The second law of thermodynamics states that all natural processes take place so as to increase entropy, that is, the universe tends toward a state of equilibrium, randomness or sameness. This principle is illustrated by this simple question: which requires more energy and effort, spilling oil on the ground or removing the oil from the soil? This law of nature that it is easier to make a problem than it to fix one, defines the basic challenge of any remedial technology: how do we engineer the separation of two types of materials once they have become mixed? In the alternate, if we do not separate the materials, how can the mixture be stabilized, contained, modified or reused? When we include in this discussion the nature of a petroleum product, which after all is a mixture of hundreds of specific chemical compounds, the potential solutions become both more numerous and simultaneously, more difficult.

The remainder of the paper will describe several different remedial technologies that have been applied to petroleum contaminated soil and ground water. As the various types of remedial technologies are described, the basic principal that drives the technology will be discussed. The technologies will be grouped and discussed by these principles.

Physical separation

The process of physical separation can be thought of as a separation based on physical differences between the mixture components and equilibrium differences of these components in liquid and vapor phases. More precisely, *a separation process takes a combination of chemical species and by applying physical or chemical principles, creates two or more separate products which differ in composition*. Most, if not all of these separation processes are based on an equilibrium principle. The refining of crude oil into different products is a direct application of basic chemical engineering separation processes. When petroleum products have been spilled or deposited on the ground or in a water source, we utilize the identical principles that were applied in the original refining process to separate that petroleum from either the earth or the water. This we call remediation.

There are several examples of physical separation processes that will be discussed in this section, but two brief examples follow. Vapor extraction works by utilizing vapor pressure differences between petroleum and water or petroleum and soil. Soil washing (solvent extraction) on the other hand, is a process in which the difference in the solubility of petroleum and the soil in water (usually enhanced by the addition of a surfactant), is the equilibrium principle that drives the process. Other examples of this principle would be air stripping, thermal desorption, microwave enhanced vapor extraction or air sparging. Each of these remedial technologies uses equilibrium driving forces as the primary separation mechanism.

Physio-Chemical Reactions

Physio-chemical processes are remedial technologies that do not result in the separation of materials. The process principles consist of a change in the chemical bonding or structure of the material required to be treated. This type of process may include the immobilization of the material, such as in a solidification/fixation process. Other physical reaction methods would be adsorption or the oxidation of the material during incineration. Another such process is asphalt incorporation of petroleum contaminated soils. Each of these mechanisms depends on a bonding phenomenon or structural decomposition through non-biological oxidation.

Chemical / Biochemical Reactions

The third major remediation technique used for petroleum contaminated media is biological activity as a means of oxidation/reduction of petroleum. Selected microorganisms ingest various hydrocarbon groups and the resultant decomposition by-products are then ingested by other groups (and types) of organisms. Under ideal aerobic conditions, carbon dioxide and

water are the ultimate residuals from this microbial process. The molecular material ingested by these microorganisms provides the energy necessary to maintain the life of these organisms.

Here is the listing of the technologies to be reviewed and discussed:

Physical separation

SOILS

Thermal Desorption

Thermal desorption consists of heating the contaminated soils with an external heat source such as heated air flow, or with an internal heat source such as an oil heated screw feeder. The major difference between thermal desorption and incineration is that in thermal desorption, the volatilized materials are either captured or combusted away from the soil substrate. The limitations on soil size and moisture content relate to the amount of energy that must be added to the desorber to volatilize the hydrocarbons in the soils. While many systems usually operate at 400°to 600° F, thermal desorbers are operated at temperatures as high as 1200° F. In order to determine the temperature at which a thermal desorber is to be operated, the boiling point range of the treated petroleum mixtures should be considered.

Gasoline volatilizes at 105° - 440° F (40°-225° C); Kerosene at 350°-640° F (180°-300° C); Diesel at 390°-640° F (200°-340° C); Lubricating oil and crude oil has constituents that volatilize at high temperatures, i.e., greater than 800° F. The volatilized gases can be either released to the atmosphere untreated or be combusted, condensed or adsorbed in an activated carbon system.

Thermal desorbers have been designed and operated using several different designs. Rotary drum dryers utilize either co-current or counter current heated air flow, while thermal screw designs use hollow screw feeders that usually contain hot oil as the heat source. The thermal screw design has one inherent advantage; it can maintain a low oxygen atmosphere in the chamber. Conveyor furnaces move the soil through a heated chamber on a metal belt. It is similar to the rotary dryer in principle.

Factors for Consideration (US EPA 1994):

Soil Particle size: <4.4 cm dia.
Moisture Content: 10%-25%
Heating Value of Soil/Petroleum Fraction: <2000 BTU/lb
Composition of Fraction: Boiling Point <1200° F

Types of systems:

Rotary Dryer, Thermal Screw, Conveyor Furnace

Vacuum Extraction

Vacuum extraction is a process that utilizes the volatility of the petroleum constituents and equilibrium concentrations between the liquid and vapor phase as the primary method of remediation. Normal slotted wells are placed in the vadose zone of the soil, in or around the contaminated soil. The well is sealed at the surface and connected to a vacuum pump. The resultant vacuum in the extraction wells causes a subsurface air flow to pass over the adsorbed soil/petroleum matrix. Utilizing vapor phase equilibrium as the driving force, volatile components are moved from the liquid phase into the vapor phase and removed via the vacuum extraction system. Low soil permeability and high soil moisture both inhibit air flow and therefore petroleum constituent recovery. In a wet soil, the soil is usually desiccated before there is significant recovery of the petroleum constituents. This process can be enhanced by injecting heated air or by applying microwaves to increase the temperature, and therefore the volatilization of the petroleum constituents.

The process is generally utilized to remediate the more volatile petroleum products such as gasoline and aviation fuel but it can be used to reduce the mobility and hazard of diesel, kerosene and some heating oils by removing the more volatile fractions from the soil.

Soil vapor extraction is usually limited to material with a boiling point less than 480° F or 250° C and high vapor pressure (greater than 0.5 mm Hg). Henry's Law constant is a measure of the volatility of compounds dissolved in water. Compounds with a Henry's law constant greater than 100 atmospheres tend to be removable from water using vapor extraction.

The efficacy of this technology is enhanced whenever the temperature of the constituents/soil can be increased. Techniques that provide this increased temperature include microwaves, heated and injected air and steam injection.

Vacuum pressures can be as high as 200 inches of H_2O in very tight soils. Most systems operate between 30 and 60 inches of water and 20-cfm air flow from each well. In shallow aquifers, those less than ten feet from the surface, horizontal extraction wells can be installed.

Factors for Consideration (US EPA 1994):

Intrinsic Soil Permeability: k (cm2) $> 10^{-8}$
Depth to Groundwater: >10 ft
Vapor Pressure of Petroleum Fraction: >0.5 mm's Hg
Henry's Law Constant: >100 atm
Composition of Fraction: Boiling Point: $<250°$ C, 480° F

Types of Systems

Microwave Enhanced, Heated Air Injected, Steam Injected, High Vacuum

Soil Washing (Solvent Extraction)

Solvent extraction - This process operates on the principle of differential solubilities of materials. There are processes that utilize extracting solvents such as triethylamine, carbon dioxide or propane. For general heavy oil recovery however, water with an added surfactant, is the most common processing solvent. In this process, contaminated soils are usually excavated (although in situ soil flushing systems do exist) and the soil screened to remove large rocks and materials. If there are large amounts of clay or other very fine soil particles, soil washing can be limited by the ability of the soil particle to settle after the washing/extraction has been accomplished. Early surfactants were simple laundry detergents. These detergents work by attaching a hydrophilic molecule (a molecule that "likes" water, therefore dissolves in it), to and insoluble particles (dirt/grease) thereby making that particle soluble. The detergent allowed the dirt particle to remain in solution, producing what we know as dirty water. When this principle is applied to soil washing, the problem with the detergent type surfactant is that the oil/water emulsion must be treated and the soil must be separated from the solution. Newer surfactants have been developed that do not increase the solubility of the petroleum constituent in water, but form a separate phase that is easily separated from the soil/water phase. If there are many fine (e.g., clay) particles in the soil, mechanical separation of the soil from the liquid phase may be instituted in order to remove the water from the soil. Some new surfactants also contain many of the base nutrients necessary to support microbiological activity. A major impediment to using soil washing as a remediation technique has been the inability of the process to remediate fine particle substrates and to treat the wash water in a cost-effective way.

Factors for Consideration (EPRI *et al.* 1988):

Soil Characteristics: <30% Clays
Depth of Contaminated Soil: <25 ft
Characteristic of Solvent: Treatability, separation characteristics

Types of Systems

Excavated with mechanical washing/extraction, in situ soil flushing

WATER

Air Stripping (Sparging)

Air Stripping/Air Sparging - This process is based on the principle of enhancing the volatilization of dissolved petroleum constituents in the water phase. A stripper utilizes air, which is added to the base of a packed tower; contaminated water is added to the top. As the air moves upward and the contaminated water phase moves down, the air/water interface increases the mass transport potential and drives the vapor phase equilibrium to favor the transfer of the dissolved constituent into the vapor phase. The contaminant is then captured in the vapor phase and either recovered or combusted. Air sparging utilizes the same principles.

A well is constructed into contaminated groundwater and air is pumped into the well. An air sparging unit creates fine bubbles so that the air is distributed into the soil/groundwater matrix. This enhances the volatilization of dissolved constituents. Air sparging is often used in conjunction with a vapor extraction system or an in situ bioremediation process. The probable efficacy of air sparging is evaluated in a similar manner to the evaluation of a vapor extraction process. High levels of iron in the ground water may pose a problem. If the Fe^{+2} is contacted by air, the iron may oxidize and precipitate, thereby reducing the permeability of the effected area. When this sparging technique is used in conjunction with bioremediation, the process is termed Bioventing.

Factors for Consideration (US EPA 1994):

Intrinsic Soil Permeability: k (cm2) $> 10^{-9}$
Iron concentration in Groundwater: $Fe^{+2} < 10$ mg/l
Volatility of Petroleum Fraction: Boiling Point $<250°$ C, 480° F
Henry's Law Constant: >100 atm

Types of Systems

Packed tower, in situ air sparging

Physical Reaction / Incorporation

Solidification

Solidification - This process combines a solidification material such as Portland cement or a calcium rich pozzolanic material such as coal fly ash with a soil contaminated with a petroleum product. The process simply renders the soil and the petroleum constituents immobile. Construction materials such as bricks may be manufactured from contaminated soils, the volatile constituents being removed during the baking/curing process. Solidification is usually chosen when it is necessary to decrease the leachability (i.e., mobility) of the contaminants on the soil. Very fine soils may inhibit the solidification process.

Factors for Consideration (Western States Petroleum Association 1989):

Soil Particle size: >200 mesh
Petroleum Constituents: Semi-volatiles <10,000 ppm, cyanides <3,000 ppm
Metals in soil or petroleum: Mn, Sn, Zn, Cu, Pb and sodium salts
Future Land use

Types of Systems

Cement based, pozzolanic (fly ash), Chemical fixation

Asphalt Incorporation

Asphalt Incorporation - This process has been used for many years as a means of using petroleum contaminated soils in a productive manner. Hot batch asphalt plants remove volatile components from contaminated soils in the initial combustion chamber and utilize the heavy ends in the asphalt itself. The lighter ends are volatilized in the asphalt dryer (400°-500° F) and combusted in the process. The soil bound to the heavy ends make very good aggregate replacement if the soil particle size is large enough. A recent process design has been developed that can utilize large quantities of very fine soil particles with heavy petroleum residues (weathered lubricating oils were used in the study). This process has great promise for the utilization of such contaminated soils. If the soil has even a small organic/humic content, asphalt incorporation becomes very difficult. Both hot rolled asphalt and cold batched asphalt processes are available.

Factors for Consideration (EPRI *et al.* 1988):

Soil Particle Size: <200 mesh <10%
Soil Organic content: Limited
Addition to normal aggregate: Limited to 5%
Petroleum Constituents: Volatiles limited

Types of Systems

Hot batch, cold batch

Incineration

Incineration - This process utilizes an external source of energy (natural gas or fuel oil) to combust the petroleum residues and the soil substrate to which these constituents are adsorbed. The more volatile the petroleum product and the dryer the soil, the less expensive the process. Soils containing large sized particles are a problem because of the greater heat capacities of the solids, thereby requiring more energy in the process. Incinerators use various designs to combust contaminated soils. Rotary kilns, fixed hearths and fluidized beds are typical. Wastes with an intrinsic thermal value greater than 5000 BTU/lb are considered incinerable. Incinerators generally operate at combustion temperatures of 600°-1000°C (1100°-1800°F). The volume of material able to be processed is dependent upon the resultant residence time of the waste in the combustion chamber at a given chamber temperature. Liquids and sludge wastes can also be incinerated but these forms usually contain large amounts of water and therefore require greater amounts of external energy to be added to the system. Air emissions are generally required to be treated if the soil contains significant quantities of chlorine or volatile metals such as mercury.

Factors for Consideration (EPRI *et al.* 1988):

Soil Particle size: (<2 inch's dia.)
Moisture Content:
Metals content of Soil: (As, Hg, Pb, Sn)
Heating Value of Soil/Petroleum Fraction
Composition of Petroleum Fraction

Types of systems:

Rotary Kiln, Fluidized Bed

Vitrification

Vitrification - This process uses large quantities of electrical energy to physically melt the contaminated soil. This also provides for the oxidation of any organic associated with the soil. The process cannot be used in situ if there are high percentage of volatile organics in the soil as this type of material tends to advance before the melt zone, that is, the rate of volatilization and resultant movement is more rapid than the advancement of the melt itself. This phenomenon can therefore spread the contamination. The melt vitrifies, or makes the soil into a glass like substance. This will encapsulate any metals contained in the melt for many hundreds of years. The in situ trials have produced problems, but there are facilities that can vitrify soils above ground in engineered containers.

Factors for Consideration (EPRI *et al.* 1988):

Petroleum Constituents: <15%, volatiles
Soil Permeability: $>10^{-9}$ cm/sec
Availability of Electric Power: Average 3,200 kW
Depth of Contamination: 30 foot maximum
Moisture Content: <25%

Types of Systems

In situ, excavated/engineered

Chemical / Biochemical Reactions

Bioremediation

Bioremediation - This process is a natural phenomenon that has been used since time in memorial to recycle materials on the earth. Simply, aerobic microorganisms ingest petroleum

hydrocarbon molecules and excrete carbon dioxide and water. Different species utilize different portions of the petroleum mix. Bonds are cleaved and new compounds are thereby formed. To be successful, every aerobic bioremediation process requires several basic elements, oxygen, phosphorus, nitrogen and sulfur. If any of these things are missing, the process will not go on.

There are also anaerobic organisms that will ingest petroleum in the absence of oxygen. The end products for these organisms are usually methane, water and hydrochloric acid. Most commercial processes are aerobic however. By monitoring the level of dissolved oxygen in ground water, or the levels of carbon dioxide in soil, one can determine if microbiological activity is occurring. Generally, the addition of oxygen, water and base nutrients to a food supply, such as petroleum, will encourage the growth of a microbiological colony. The positive thing about this process is that when the food is gone (the petroleum), the organisms also die. Microbiological systems can be designed to treat soils or ground water in situ or after excavation. The excavated soil can be land farmed or placed in a Bioreactor for treatment. Every successfully engineered aerobic bioremediation process must create an environment where there are sufficient oxygen and nutrients and where the buildup of waste products such as carbon dioxide, water and dead cells can be controlled. All of the techniques described in this section contain the same elements.

There are two basic methods of ensuring an adequate microbial colony. One is to encourage the growth of indigenous colonies and the second is to separate and select a colony of microorganisms that are then nurtured in the laboratory to preferentially ingest the waste to be remediated. After the desired colonies have been selected and grown, the cells are reintroduced into the natural environment. Both techniques have been used successfully.

Factors for Consideration (US EPA 1994):

Microbial Population: >1,000 CFU/GM
Soil pH: Between 6 and 8
Moisture Content of soil: >40%
Petroleum fraction composition: TPH<50,000 ppm
Rainfall: <30 in/yr

Types of systems:

In Situ - Land farming, Bioreactor, Bioventing, Biopiles

GOALS

Before any cleanup process can be selected, there must have been consideration as to why the cleanup is occurring and what the cleanup is designed to effect. In other words, if an area is to be cleaned up because it poses a threat to human health, how much and therefore, how you clean up will be very different from if the cleanup is occurring to return a contaminated area to how it was before the contaminant was released. If the cost of the remediation is not the primary

issue, the determination of the ultimate goal of the remediation is usually agreed upon before any remediation process is selected. On the other hand, if a total cost for the cleanup has been set, then alternative technologies can be evaluated to determine what can be done for the amount of money allocated for the cleanup. It is important to have this decision made before choosing a specific remediation path.

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Responding to Oil Spills in Marshes: The Fine Line Between Help and Hindrance

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INTRODUCTION

There is general understanding that marsh environments are highly sensitive to oiling and thus receive high priority for protection. Lessons from past spills have also documented that response activities have the potential to cause additional harm to oil-impacted marshes. Less clear is how to determine when cleanup is desirable, when it should stop, and which methods should be employed.

The basic lessons about impacts of oil and subsequent response activities in marshes have been known for years (Mattson *et al.* 1977; Westree 1977; McCauley and Harrel 1981). The *Amoco Cadiz* spill in France taught us the complications from sediment removal at the Ile Grande marsh, when such activities greatly increased erosion of the marsh and substantially delayed vegetative recovery (Baca *et al.* 1987; Vandermeulen *et al.* 1981). However, in cold temperate environments, it has also been clearly documented that heavily oiled marshes where oil is not removed may be impacted for decades (Table 1). The *Metula* spill in Chile is an extreme example of slow recovery after 20 years, little change has occurred (Baker *et al.* 1993; Teal *et al.* 1992; Vandermeulen and Jotcham 1986). At some spills occurring in warmer regions less severely impacted by crude oils, very limited clean-up has constituted a successful "response" (e.g., Neches River Unocal spill; NOAA 1993).

Additional monitoring studies conducted in oil-impacted marshes, as well as experimental research during the past two decades have documented the complexity of marsh ecology and the numerous parameters that affect the severity of impact to these systems (DeLaune *et al.* 1984, de la Cruz *et al.* 1981, Alexander and Webb 1985). This knowledge complicates decisions regarding cleanup in marshes, because we now know that such parameters as substrate type, plant species, season of impact, oil type and climate may all affect the eventual recovery of an oil-impacted marsh (Table 3).

A myriad of questions await responders faced with an oiled marsh. Under what circumstances is cleanup appropriate not only in removing oil, but in speeding recovery of the marsh? If the decision is made to respond in a marsh, what methods should be employed and how should these be chosen? Where is the line where cleanup should cease lest it cause more harm than good? How can seemingly conflicting resource needs be balanced in cleanup decision making (marsh cleanup is often suggested as a way to prevent oiling of birds or other animals, or to prevent oil from moving to other sensitive environments)? To develop some general guidelines for answering these questions, I have perused numerous follow-up studies after oil spills, and reviewed the scientific literature on this subject.

CLEANUP DECISION MAKING

A simple starting point for determining whether cleanup is appropriate for an oiled marsh is to assess the severity of the impact, and attempt to estimate the time-frame for recovery. Predictions of future recovery times will, of course, be approximations, but can provide a general sense of whether impacts are likely to be short term (less than 1 year), medium-term (1-3 years), or long-term (greater than 3 years). Having an idea of the likely time frame for recovery without cleanup allow us to assess whether undertaking some response is likely to speed the natural recovery process or impede it.

Defining recovery

Recovery is an easily misunderstood and difficult term to define, but the concept is a necessary endpoint for environmental monitoring studies. Generally, recovery is used to denote a return to some un-impacted state of the environment in question. Ecological communities are not static and undergo changes based on environmental perturbations both naturally occurring and human caused. It is common to measure recovery by comparing oil impacted sites with nearby 'control' (un-impacted) sites. When these sites resemble each other in important ecological parameters (such as percent cover of vegetation for marshes) they can be considered to be recovered.

For marsh studies, the most commonly measured parameter is the percent cover of vegetation, sometimes accompanied by diversity indices or height and weight of plants. However, each follow-up study is different, and may measure additional indices. For the purposes of this paper, recovery will refer to vegetative cover, largely because percent cover is measured by a number of different studies. Commonly, some measurable concentration of petroleum hydrocarbon remains in sediments of marshes that appear to be "recovered" from looking only at the vegetation. It is important to remember that vegetative recovery may not include measurements of subtle differences in species diversity or use of the habitat by other organisms.

Timelines for "recovery"

Documented recovery times for oiled marshes range from a few weeks to decades (Tables 1-3). As might be expected, the cases on the extreme ends of this spectrum are the easiest to delineate. On the worst case side, are several well-studied marsh sites where recovery times ranged from 5 years to greater than 20 years. These include two sites in Buzzard's Bay Massachusetts, the *Miguasha* spill in Quebec, the *Metula* in Chile and the *Amoco Cadiz* in France (Table 1). These examples share the following characteristics:

north-temperate (cold) environments,
sheltered location
heavy oiling
spills of fuel oils (bunker C or No. 2 fuel)
in some cases intensive cleanup methods delayed recovery

In contrast, recovery times of two years or less have been documented for sites at the following spills (examples are taken from Gulf of Mexico): Neches River, Texas (*Esso Bayway*), Harbor Island, Texas pipeline, and the Shell pipeline in SE Louisiana (Table 2). These marshes showing quicker recovery share the following characteristics:

warm climate
light to moderate oiling
spills of light to medium crude oil
variety of cleanup methods used
(often no cleanup resulted in fastest recovery time)

Based on these examples, it is easy to conclude that marshes lightly oiled in warm climates have a good potential for natural recovery in a comparatively short period of time, and that cleanup may be more of a hindrance rather than a help. However, decisions to implement a "no response" option may be complicated by other concerns, such as potential oiling of birds or other animals or concerns about leaching of oil to nearby environments.

In contrast, heavily oiled marshes in colder climates have the potential to be impacted for many years, depending on the type of oil spilled and the degree of penetration of the oil into the marsh substrate, among other parameters (Vandermeulen and Singh 1994). Cleanup activities in these situations have a higher potential for accelerating the recovery of the marsh, if only because of the expected long residence time of oil if left to weather naturally.

Between these two extremes, however, lies the vast middle ground of spills with less clear-cut solutions to the cleanup question. These include moderate to heavy impacts in marshes in warm climates, spills of medium crude oils or refined oils, and issues involving conflicting resource uses. Deciding when cleanup activities are appropriate and what cleanup methods to use are challenging issues that usually must be made on a site-specific basis. Since all cleanup techniques have some detrimental impact associated with them, choosing any particular techniques requires a balancing of the expected gains versus the costs. Many techniques can be applied in a very judicious manner, thus minimizing their detrimental impacts.

MARSH CLEANUP TECHNIQUES

Historically, most techniques available for oil spill cleanup have been tried at one time or another in marshes. Table 5 summarizes the advantages and disadvantages of several techniques that are currently either commonly used, or are of strong research interest.

Natural Degradation/No Response

No response at all is an ideal approach when natural weathering and biodegradation are expected to occur quickly (see Table 4 for more details). Choosing natural degradation/no response is the only way to completely eliminate physical impacts resulting from workers or mobilization of equipment. Natural degradation is often used as the last stage of a response where physical removal has already been implemented, since most physical removal methods

will reach a point where oil can no longer be effectively removed, leaving some level of residual oiling. The no response option has a cost when oiling is heavy and/or degradation is expected to be very slow (greater than 1-2 years). Asphalt pavements may form from heavy layers of oil left undisturbed (in very sheltered areas or when oil strands in the upper or supratidal zone). Such pavements were found at the *Metula* spill, in experimental "set-aside" plots at the *Exxon Valdez*, and were observed from historic spills in the Persian Gulf. In these cases, initial efforts to remove thick layers of oil (or manually remove asphalt after hardening) are warranted.

To ensure that areas left to degrade naturally do not contaminate adjacent sites, sorbents may be used to collect sheen or other mobile oil.

Vacuum/Pumping

Physical removal of pooled oil on marsh sediment or water surfaces using vacuum or pumping apparatus have been quite successful at a number of marsh spills. Large quantities of oil can be removed, though at some point, residual oiling will remain after most of the heavy oil is collected. Use of vacuum removal in conjunction with low pressure flushing can also be very successful.

Two main environmental impacts from using this technique are the physical impact of deploying the equipment and the workers to operate it, and the potential to inadvertently remove plants along with oil. Careful monitoring of the use of this technique in the field is important to ensure that impacts are minimized. Access to remote sites may also be difficult, although vacuums can be deployed from barges as was done at the Tampa Bay spill to clean an area of oiled mangroves.

Low Pressure Flush

Low pressure flushing is usually used to assist in moving oil towards collection points where other removal equipment, such as vacuums or boom/skimmer collection operate. Flushing can also be helpful in lifting oil off the sediment surface during times when the marsh is not flooded.

Flushing may be difficult to apply correctly, since slight changes in water pressure can turn a low-impact technique into a high impact one. Thus, workers must be carefully supervised and it is a good idea to undertake trials to work out the details of application. Access by workers will physically impact the marsh, and this should be minimized, either by working from boats during high tide, or by using board walkways.

Vegetation Cutting

Cutting of oiled vegetation has been tried in numerous spills, many times with quite drastic consequences, i.e. death of plants, increased erosion, and permanent loss of marsh. When oil covers sediment surfaces, cutting near the base of the plant can increase penetration of oil into the sediment, damaging plant roots. Studies that monitor oiled marshes where cutting has been used show that non-cut areas may recover as fast or faster (e.g., *Esso*

Bayway spill and the American Petrofina pipeline spill). However, cutting impacts in many of these studies were confounded with impacts from physical trampling by workers (Hershner and Moore 1977; Mattson *et al.* 1977; Holt *et al.* 1978; McCauley and Harrel 1981).

In general, cutting should be avoided except in special circumstances, since the likelihood of killing the plants and causing permanent damage to the marsh is high. Special circumstances include concerns about potential oiling of birds from residual oiling on plant fronds, or aesthetic considerations in high public use areas. At the *Candian Liberty* spill on the Delaware River, careful cutting of *Phragmites* and *Scirpus* was used to minimize risk to birds using the marsh. Follow-up monitoring 3 months after cutting found that plants from cut sites appeared similar to control sites, and no obvious adverse impacts were observed (NOAA 1993).

Burning

Burning of marsh grasses has been practiced as a vegetation management technique for many years, but burning of oiled marshes is relatively new. Two recent incidents where burning was used to remove oil in freshwater marshes in Texas and Maine has sparked increased interest in this response option. Burning of oil in marshes has the potential to remove large quantities of oil quickly while potentially minimizing physical impacts.

However, the technique has not yet been well documented and many questions remain about the specific conditions (different from the two recent examples) under which burning can be successfully used in marshes. Both of the recent burns in Texas and Maine were conducted while the marshes were inundated, and the Maine burn was conducted under ice and snow conditions. What degree of inundation of the marsh is necessary to minimize impacts to plant roots and rhizomes? Information on the effects of burns on a variety of plant species as well as particulars about recovery of marshes after burning are topics for further research.

Bioremediation

Bioremediation is similar to burning in its stage of development with respect to application in marshes. There is great interest in using the technique, and positive data from laboratory studies, but little information on its successful use in oiled marshes. From experimental data we can infer that bioremediation would be a potential low-impact cleanup technique for residual oiling of marsh sediments. Questions remain about the potential for eutrophication of marsh environments, and potential limitations to degradation from low-oxygen conditions in marsh sediments.

Sediment Removal/Replanting

Sediment removal followed by replanting can be considered more of a remediation technique than an actual response, since it is in many ways, a technique of last resort. It may be the only option for some cases where sediments are very heavily oiled. However, this is indeed an example of destroying the marsh to save it, since existing vegetation and

roots would be removed along with sediment. There is great potential for increased erosion and a danger that if sediments are not replaced, changes in elevation will prevent regrowth of plants. Several of the long term impact case studies (e.g. *Miguasha* and *Amoco Cadiz*) provide examples of unsuccessful use of this technique (Vandermeulen and Jotcham 1986; Baca *et al.* 1987). Therefore, it should be considered a last resort option, and proceeded with very cautiously.

A less intrusive related technique is tilling of contaminated sediments to try and break up heavily oiled sediments, providing aeration and possible channels for seeds to reach cleaner soil. Again, there is little data on successful use of this technique at field sites.

SUMMARY/CONCLUSIONS

Deciding how to respond in an oiled marsh is clearly a complex issue for which there cannot be a single answer. Decisions will need to be made on a case by case basis, and usually with a degree of uncertainty. However, the lessons of the past give us a good deal of guidance at least about what techniques to avoid and where to tread cautiously. The following guidelines give a simple outline for making these decisions.

Begin by evaluating the impact, oil residence time and general situation:

(1) Assess the impact of oil by conducting a field survey. Estimate what percentage of the marsh is oiled, how heavily, whether the species impacted are known to be sensitive or more tolerant.

(2) Estimate the likely oil residence time by considering the potential for natural weathering and biodegradation, along with the characteristics of the marsh, such as the deposition rate, the type of vegetation, etc. (Table 4).

(3) Consider other cleanup concerns, such wildlife that may be at risk of being oiled, whether the area is used by the public or has other special concerns associated with it.

Review whether cleanup is necessary or desirable. Clean-up in a marsh appears to be justified when oil can be removed with minimal impact, when other resources are at high risk of being oiled (such as migrating birds) and when unassisted recovery is likely to be very slow (greater than 2-3 years).

Natural (unassisted) recovery may be the best option in cases where oiling is light and natural recovery is likely to occur in a short time frame (such as one year or less), where cleanup activities would detrimentally impact the marsh and where wildlife are at low risk of being oiled.

If choosing to proceed with cleanup options, review the site limitations, consider the options that seem appropriate, keeping in mind the need to minimize the physical impacts on the marsh. In many cases, trial applications of cleanup techniques will help determine if the technique is appropriate at a given site. Trials can also refine techniques such as low pressure flushing to make sure they are having the desired effect. Most responses rely on a

combination of cleanup techniques. And always keep the ultimate objectives of the response in mind: to minimize adverse impacts of oil on the marsh itself, on the organisms that use it for habitat, and to speed its ecological recovery.

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Table 1.
Examples of oil-impacted marshes with recovery times of five years or more, documented by follow-up studies.

Location	Vegetation	Oil type	Date of oiling	Cleanup	'Recovery time'
Chile *Metula* [1]	*Salicornia ambigua* *Suaeda argentinensis*	Arabian crude Bunker C	Aug, 1974	none	>20 yr
Quebec *Miguasha* [2]	*Spartina alterniflora,* *Spartina patens*	Bunker C	Sept. 1974	sediment removal manual burning digging	> 11 yr <11 yr
Brittany, France *Amoco Cadiz* [3]	*Salicornia* *Suaeda* *Halimione*	Arabian light, Iranian light crude Bunker C	March, 1978	sediment removal	5 - >8 yr
West Falmouth, MA , *Florida* [4]	*Spartina alterniflora,* *Salicornia europaea,* *Spartina patens*	No. 2 fuel	Sept ,1969	?	> 8 yr
Buzzard's Bay, MA, *Bouchard 65* [5]	*Spartina alterniflora,* *Salicornia virginica*	No. 2 fuel	Oct, 1974	?	> 3 yr

1) Baker *et al.* 1993
2) Vandermeulen and Jotcham 1986
3) Baca *et al.* 1987
4) Burns and Teal 1979; Teal *et al.* 1992
5) Hampson and Moul 1978

Table 2.
Examples of oil-impacted marshes with recovery times of 3 years or less, documented by follow-up studies.

Location	Vegetation	Oil type	Date of oiling	Cleanup	'Recovery time'
Hackensack estuary, NJ Wellen tank farm[1]	*Spartina alterniflora*	No. 6 fuel	May, 1976	none cutting	?
Galveston Bay, TX, Dickinson Bayou pipeline [2]	*Spartina alterniflora* *Juncus roemerianus*	light crude	Jan, 1984	none sorbents flushing	8 mos - > 2 .5 yrs
Harbor Is., TX *Am Petrofina pipeline* [3]	*Spartina alterniflora* *Avicennia germinans*	crude oil	Oct, 1976	none sorbents burning * clipping	6 mos- > 6 mos
Aransas River Chilitpin Creek [4]	*Spartina alterniflora*	S. Texas light crude	Jan, 1992	burning	>2 yr
Neches River, TX *Esso Bayway* [5]	*Spartina patens*	Arabian crude	Jan, 1979	none sorbents flushing burning cutting	7 mos > 7 mos
Neches River, TX *Unocal* [6]	*Spartina alterniflora*	light crude	April, 1993	none sorbents flushing	?
Nairn, LA *Shell pipeline* [7]	*Spartina patens* *Spartina alterniflora* *Distichlis spicata*	Louisiana crude	April, 1985	flushing trampling	< 1.5 yr

1) Mattson *et al.* 1977
2) Alexander and Webb 1987
3) Holt *et al.* 1978
4) Tunnell and Hicks 1994, Gonzalez and Lugo 1994
5) McCauley and Harrel 1981, Meyers 1981, Neff *et al.* 1981
6) NOAA 1993
7) Mendelssohn *et al.* 1990, Fischel *et al.* 1989

Table 3.
Data from field studies on impacts to marsh vegetation from experimental, single-dose oiling.

Location	Vegetation	Oil type	Time of oiling	Cleanup	'Recovery time'
Galveston Bay, TX (1)	*Spartina alterniflora*	Arabian crude Libyan crude No. 6 fuel No. 2 fuel	Nov.	none	1 yr 1 yr 1 yr 2 yr
Louisiana (2)	*Spartina alterniflora*	S. La . crude	June	none flushing cutting	3 mos 3 mos 2.5 yr
York River, VA (3)	*Spartina alterniflora*	S. La. crude: fresh /weathered	Sept.	none	>1 yr
St. Louis Bay, MS (4)	*Juncus roemerianus*	Empire Mix crude Saudi crude	March	none	1-3 yr

1) Webb *et al.* 1985
2) DeLaune *et al.* 1984
3) Bender *et al.* 1977
4) de la Cruz *et al.* 1981

Table 4.
Factors to consider when evaluating whether impacts to marshes from spilled oil are likely to be short-term (1-2 years) or longer.

Evaluation factors	Examples
Severity of impact	
oil persistence & toxicity	oil type surface area covered, thickness % of plant oiled
oil penetration in sediment	substrate type, coarseness oil viscosity
type of marsh vegetation	sensitivity of plants (annuals, perennials)
season of impact	growing season vs. dormant season
Oil residence time without cleanup	
weathering	exposure, tidal flushing precipitation
biodegradation "potential"	oil type temperature/climate previous exposure to oiling
Ability of marsh to self-recover	
sedimentation over oiled layer	deposition rate
recolonization	intact adult plants nearby reproductive strategy
elevation	ideal for species or marginal?
environmental stresses	heavy rainfall, cold temperatures, drought

Table 4. con't

Other cleanup concerns

use of site (over short & long term)	human users ecological users
species of special concern	migrants, endangered species
impacts to adjacent areas	mobility of remaining oil sensitivity of adjacent habitats

Table 5.
Cleanup techniques used in marshes and their advantages and disadvantages.

Advantages	Disadvantages
No response	
minimal impact (if oil degrades quickly) no physical impact	potential oiling of birds or wildlife oil may impact adjacent areas heavy oils may degrade slowly or form asphalt
Vacuum/pumping	
can remove large quantities of oil	access /deployment of equipment physical impacts
Low pressure flushing	
assists in removal by herding oil lifts oil off sediment surface	requires careful monitoring pressure must be controlled physical impacts
Burning	
recent successes: Maine, Texas potential to remove oil quickly minimizes physical impacts	little known about specific conditions (season, inundation of marsh, plant species) air pollution regulatory concerns
Sediment removal	
may be only remediation for heavily oiled sediments	"destroy marsh to save it" increased erosion potential elevation changes may impede regrowth of plants replanting necessary
Vegetation cutting	
leaves most of plant intact prevents oiling of birds	may kill plant potential for increased erosion must be monitored
Bioremediation	
great theoretical potential low impact	few case studies available potential for nutrient enrichment oxygen may be limiting

Session III. An Overview of Remediation Studies in the Gulf of Mexico

Chair: Dr. Irving A. Mendelsshon

Presentation	Author / Affiliation
Evaluation of Commercial Bioremediation Products for Oil Biodegradation in Salt Marshes	Dr. R.W. Weaver[1] Mr. B. Crites[1] Mr. S. Neralla[1] Mr. A. Wright[1] Dr. James Webb[2] 1 Texas A&M University College Station 2 Texas A&M University Galveston
The Development of Bioremediation for Oil Spill Cleanup in Coastal Wetlands: Product Impacts and Bioremediation Potential	Dr. Irving A. Mendelssohn[1] Ms. Qianxin Lin[1] Ms. Karolien Debusschere[2] Mr. Charles B. Henry, Jr.[1] Dr. Edward B. Overton[1] Dr. Shea Penland[1] Dr. Ralph J. Portier[1] Dr. Nancy N. Rabalais[3] Ms. Maude M. Walsh[1] 1 Louisiana State University 2 Coastal Environments, Inc. 3 Louisiana Universities Marine Consortium
Bioremediation Studies: Effects on the Marsh Infaunal Community	Dr. Nancy N. Rabalais[1] Ms. Nazan Atilla[2] 1 Louisiana Universities Marine Consortium 2 Louisiana State University
In-Situ Burn as an Oil Spill Response Technique	Mr. Gus Stacey Marine Spill Response Corp.

Evaluation of Burning as an Oil Spill Cleanup Technique in a High Marsh Community Along the South Texas Coast	**Mr. Beau Hardegree Mr. David W. Hicks Dr. John W. Tunnell, Jr. Center for Coastal Studies Texas A&M University Corpus Christi**
Fates of Oil in Salt Marsh Sediments	**Edward B. Overton, Charles B. Henry and Paulene Roberts Louisiana State University**

Evaluation of Commercial Bioremediation Products for Oil Biodegradation in Salt Marshes

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INTRODUCTION

Bioremediation requires large populations of microorganisms to be effective in rapidly degrading high concentrations of organics. Often soil contains the necessary microorganisms for degrading compounds formed in nature (Alexander 1994). Sometimes the microorganisms may not be present or are present but at population levels that are too low. In such cases it may be useful to bioaugment the populations to enhance the rate of bioremediation. Products that provide microorganisms or nutrients are commercially available to stimulate oil bioremediation.

Studies on the role of bioaugmentation in the enhancement of biodegradation indicate some promise (Leahy and Colwell 1990). Microbial degradation of oil appears to be the major process through which petroleum hydrocarbons are removed from the sediment environment (Zobell 1946). Some predominant hydrocarbon degrading bacteria found in soils are *Pseudomonas, Achromobacter, Bacillus, Flavobacterium*, and *Nocardia* (Leahy and Colwell 1990). Inoculating a known oil-degrading strain of *Pseudomonas* resulted in increased biodegradation of oil (Atlas and Busdosh 1976). Antai (1990) reported that *Bacillus* sp. and *Pseudomonas* sp. were suitable for application in oil spill cleanup of the Nigerian terrestrial ecosystem. However, Venosa *et al.* (1992) found that indigenous Alaskan microorganisms were primarily responsible for the biodegradation of the weathered Alaska north slope crude oil. Dott *et al.* (1989) reported varying abilities of commercial cultures to degrade fuel oil. Introduction of new microorganisms into a polluted site does not always yield successful results. The potential success of inoculation in bioremediation depends on factors such as inoculant formulation, adaptation, inoculant concentration, and the availability of ecological niches (Pritchard 1992).

Considerable oil transportation occurs along the Gulf Coast of Texas because of oil production and processing facilities (Geyer 1980). Oil spills that occur in coastal marsh environments are difficult to remediate because they are fragile ecosystems which are susceptible to physical disturbances. The question arises as to whether commercial inoculants enhance the rate of bioremediation in coastal marsh environments. Such a question cannot be easily answered because relatively little data are available regarding the kinetics of bioremediation in marsh environments and the need for additional microorganisms. Each product must be evaluated on its own merit and under conditions that allow it to perform. Conditions must be suitable for microbial survival and development of biomass. Complete evaluation of any product is beyond the means of most laboratories and only preliminary investigations can be undertaken to see if there seems to be potential for using the products.

Because of environmental regulations it was not possible to intentionally spill oil in a marsh environment to test products, and it became necessary to approximate field conditions under laboratory and glasshouse conditions. The results of testing only pertain directly to the conditions under which the tests were performed and extrapolation to field conditions is limited. It is generally thought, however, that field conditions would be more rigorous and affect product performance.

MATERIALS AND METHODS

Soil

Soil was collected from a salt marsh near Galveston, TX and is classified as a Follet series. The soil is a Hyperthermic Typic Haplaquent. For the laboratory experiment, soil was collected and screened wet to remove large debris. After screening, the soil was thoroughly mixed so that it would be homogeneous. For glasshouse experiments, soil was collected and placed directly into 7.5 L pots.

Oil

Arabian lubes crude oil was used for experimentation since it is commonly transported along the Gulf Coast. Oil was artificially weathered before experimentation so that it would more accurately mimic weathered oil that would be remaining for bioremediation following an actual spill. Oil was heated at 150°C for 8 h. Heating reduced the volume considerably and further loss by volatilization during experimentation was negligible.

Bioremediation Products

Ten commercial products from the list of products prepared by the US EPA as part of the National Contingency Plan Product Schedule were chosen for the investigation. The products used were Oppenheimer Formula I (Oppenheimer Environmental Corp., Austin, Texas); Munox-712 (Osprey Biotechnics, Sarasota, Florida); Micro-Blaze Out (Verde Environmental Inc., Houston, Texas); BioGEE HC Concentrate (BioGEE International, Inc., Houston, Texas); WMI 2000 (Waste Microbes Inc., Houston, Texas); Marine-D (Environmental Remediation, Inc., Baton Rouge, Louisiana); Alpha Biosea (Alpha Environmental, Inc., Austin, Texas); Hydrobac (Polybac Corp., Bethlehem, Pennsylvania); Oil Spill Eater-II Concentrate (OSEI Corp., Dallas, Texas); and Mycobac-Tx 20 (Mycobac Inc., Houston, Texas). The products were numbered P1 through P10 in the order mentioned. The products were prepared and applied according to the literature provided by the manufacturers. Larger quantities of products were prepared for application than were actually used so that a more representative sample of the product would be applied.

Enumeration of Hydrocarbon-Degrading Microorganisms

Populations of hydrocarbon-degrading microorganisms were determined using a modified Sheen Screen Method (Brown and Braddock 1990). Basically it is a most probable number procedure (MPN). The diluent was a solution of 8.5 g NaCl L^{-1}. Serial dilutions of 10 with three tubes for each dilution were employed. Bushnell Haas medium (Bushnell and Haas 1941) was used in the tubes and the same weathered crude oil used in the experiments provided the carbon source. Tubes were considered positive if there was obvious turbidity due to microbial growth or if the oil layer was clearly dispersed or reduced. Tubes were incubated for approximately 1 month before final readings were made.

Microcosm Experiment in the Laboratory

The objective of this experiment was to determine the ability of commercial products to enhance oil bioremediation in flooded soil. Microcosms consisted of 0.47 L glass canning jars containing 60 g of soil and were kept flooded with approximately 1 cm of water above the soil surface. Oil was added to the surface of the water column to provide a layer approximately 0.5 mm thick (1.0 g oil). A completely randomized experimental design was used with a factorial arrangement of treatments. Incubation temperatures were 10 and 30°C . Two control treatments were included and consisted of not applying fertilizer and applying 110 mg ammonium nitrate and 45 mg potassium phosphate to the water column. Duplicate microcosms of each treatment were processed at 21 d to determine the population of hydrocarbon-degrading microorganisms. The contents of the entire container were mixed with 200 ml of saline (8.5 g NaCl L^{-1}) and placed on a reciprocating shaker for 30 minutes. Dilutions were made and populations enumerated as already described.

At 90 d, oil and grease (O&G) and total petroleum hydrocarbons (TPH) were determined on duplicate microcosms of all treatments. Total oil and grease was extracted from the samples using dichloromethane. Oil in the water column and on the soil surface was extracted by adding 20 ml of dichloromethane and rinsing the surface of soil and decanting. Oil in the soil was extracted by adding 20 ml of dichloromethane to the microcosm and thoroughly mixing into the soil and decanting. A second extraction was made and all extractions were combined. A portion of the final mixture (10 ml) was evaporated to dryness to gravimetrically determine oil and grease content. Total petroleum hydrocarbons were determined by dissolving oil and grease (obtained by evaporating 1 ml of the final mixture) in freon and passing it through a silica gel column and analyzing by infrared spectrophotometry.

Mesocosm Experiments in the Glasshouse

The objective of this experiment was to determine if addition of bioremediation products would aid in the bioremediation of oil in mesocosms. Mesocosms consisted of *Spartina alterniflora* growing in 7.5 L pots that had been acclimated in a marsh for two months for the plants to become established under natural conditions before they were transported to the glasshouse. Pots containing the plants were placed inside 19 L containers and flooded with enough water for a 3.5 cm layer over the soil surface. Water was added as needed to keep the

pots continuously flooded. Two experiments were conducted, one beginning in December and the other beginning in May so that a cool season and a warm season would be represented. The same bioremediation products were used in both experiments.

Oil was added at a rate of 28 ml to each pot and formed a layer approximately 0.5 mm thick. A completely randomized experimental design was used with two replications. Treatments consisted of five commercial bioremediation products being applied singly and fertilized and non-fertilized controls. In the cool season, only ammonium sulfate was added for the fertilizer control treatment and it was added to the water column at a rate of 1.12 g of N pot^{-1}. Nitrogen was applied in four equal split applications beginning the day after oil application. For the experiment conducted during the warm season, the fertilizer source for the control treatment was Max Bac® CB24-89, a granular slow release commercial nutrient source for bioremediation, with an analysis of 25-5-0 (N-P-K). It was obtained from Grace-Sierra Horticultural Products Co., Milpitas, CA. The product is a mixture of ammonium nitrate, calcium phosphate, ammonium phosphate, and urea. It was added at a rate of 2.24 g pot^{-1} (providing 0.56 g N and 0.112 g P) to the surface of the water and it settled to the bottom of the water column onto the soil surface. Supplemental P was added as sodium phosphate to the water column to increase the amount of P by 20 kg ha^{-1} (0.112 g P pot^{-1}).

Populations of hydrocarbon-degrading microorganisms were determined at the time of harvest for the cool season experiment and at two and four weeks after the start of the warm season experiment. The determinations were made on water samples collected from various locations in the water column the day after water additions were made to maintain the water level.

Mesocosms were destructively harvested on March 15 for the cool season experiment and on June 19 for the warm season experiment to collect residual oil. Plants were removed at the water level by clipping. Oil on the plant tissue was collected by rinsing tissue in dichloromethane and oil floating on the water surface was skimmed off. Some oil adhered to the sides of pots and was scraped off and combined with oil skimmed from the water surface and collected from plant material. The quantity of oil recovered was determined gravimetrically and percent TPH was determined by infrared spectrophotometry as described for the microcosm experiment.

RESULTS AND DISCUSSION

The fertilized control treatment incubated at 30°C contained the lowest quantity of TPH at 90 d (Fig. 1). Only 17% of the TPH remained for this treatment while 74% remained for the unfertilized control treatment. Similar increases in oil biodegradation by fertilization were also reported by other workers (Dibble and Bartha 1979; Jobson *et al.* 1972). Fertilization was not as beneficial for the microcosms incubated at 10°C since 60% of the TPH remained in the fertilized control treatment and 79% in the unfertilized treatment at 90 d.

Only Product 4 significantly reduced the TPH below that of the unfertilized control when microcosms were incubated at 10°C but did not significantly reduce the TPH below that of the fertilized control (Fig. 1). All products except Products 2 and 3 reduced the TPH below that of the unfertilized control when incubated at 30°C but no product was as effective as the fertilized control treatment in reducing TPH. Considerably more bioremediation occurred when microcosms were incubated at 30°C rather than 10°C.

The number of hydrocarbon degrading microorgansisms in treatments receiving products was not significantly larger at 21 d of incubation than the no product treatment at either 10 or 30°C (data not shown). The population size was not significantly different for the two temperatures. Addition of oil increased the populations from approximately 3 x 10^4 to 1 x 10^5 cells g^{-1} soil.

Neither product nor fertilizer application reduced the quantity of oil that remained in mesocosms at the end of the glasshouse experiment conducted during the cool season (Fig. 2). On average, 24% of the added oil remained at the end of the experiment. The second mesocosm experiment was conducted during the warm season and visual inspection indicated that the oil began disappearing more rapidly than in the cool season experiment. Therefore, the incubation time was reduced from 98 d of the previous experiment to 33 d to better reveal which products increased bioremediation most rapidly. No product reduced the quantity of oil remaining at 33 d significantly more than that in the fertilized control treatment (Fig. 3). The percentage of oil remaining for the fertilized control treatment was 33 and the average percentage of oil remaining for the other treatments was 51. In this experiment both N and P were provided as fertilizer because in the cool season experiment no response was obtained to nitrogen fertilization alone. Perhaps P was needed more than N. Products contained both N and P, but the quantity provided was much lower than that provided for the fertilized control treatment. Populations of hydrocarbon-degrading microorganisms were not influenced by treatments except that addition of oil to mesocosms increased populations of hydrocarbon-degrading microorganisms by approximately 10 times.

SUMMARY

Experiments were conducted under laboratory and glasshouse conditions to evaluate effectiveness of commercial products in enhancing bioremediation of oil spilled onto water columns over soil. One product of ten increased oil bioremediation in the laboratory in comparison to the unfertilized control but no product was as effective as the fertilizer in the fertilized control treatment. The population size of hydrocarbon-degrading microorganisms was not influenced by bioremediation products. The experiment conducted during both cool and warm seasons in the glasshouse indicated that the products did not significantly enhance bioremediation. Disappearance of oil during the warm season was more rapid than during the cool season. Soil from the Gulf Coast apparently contained adequate populations of hydrocarbon-degrading microorganisms necessary for bioremediation.

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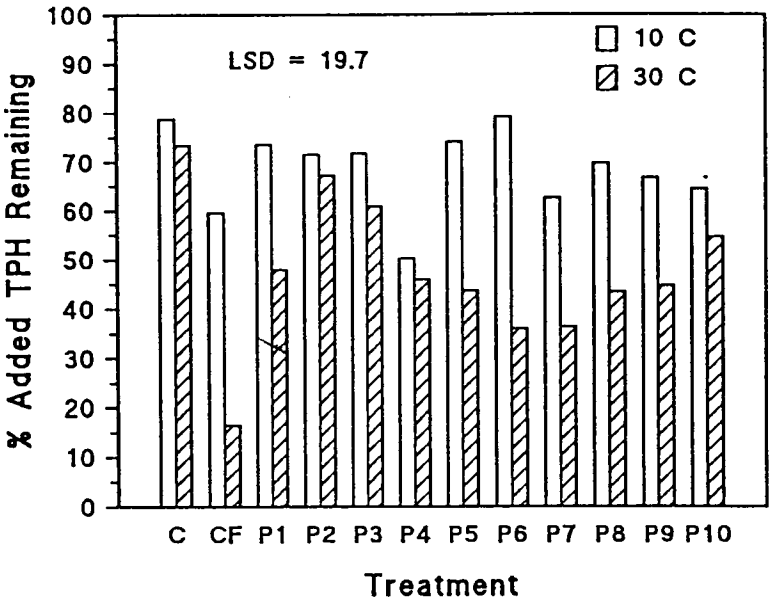

Figure 1. Percentage of added total petroleum hydrocarbons (TPH) remaining in microcosms that were unfertilized (C), fertilized (CF), and those treated with 10 bioremediation products (P1-P10) 90 d following incubation at 10 and 30^{0} C.

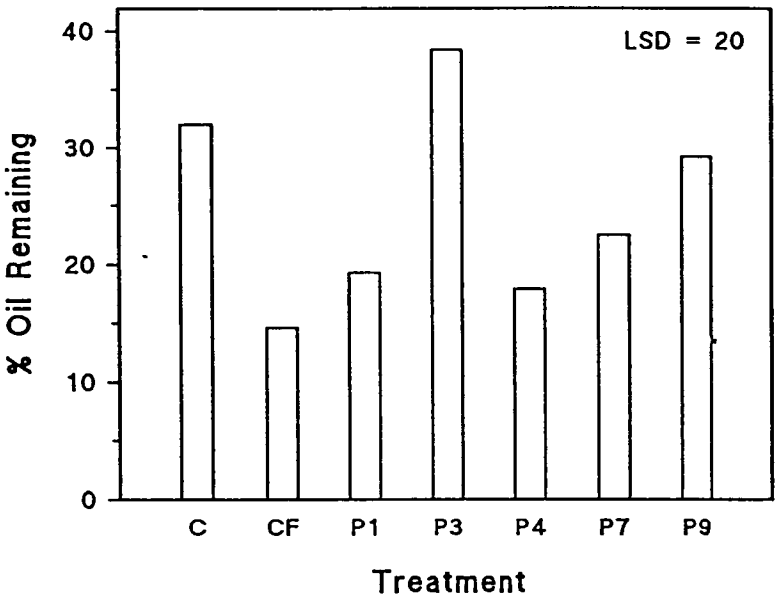

Figure 2. Percentage of added oil remaining at 98 d in mesocosms that were unfertilized (C), fertilized (CF), and those treated with bioremediation products (P1, P3, P4, P7 and P9) and maintained in a glasshouse during the cool season.

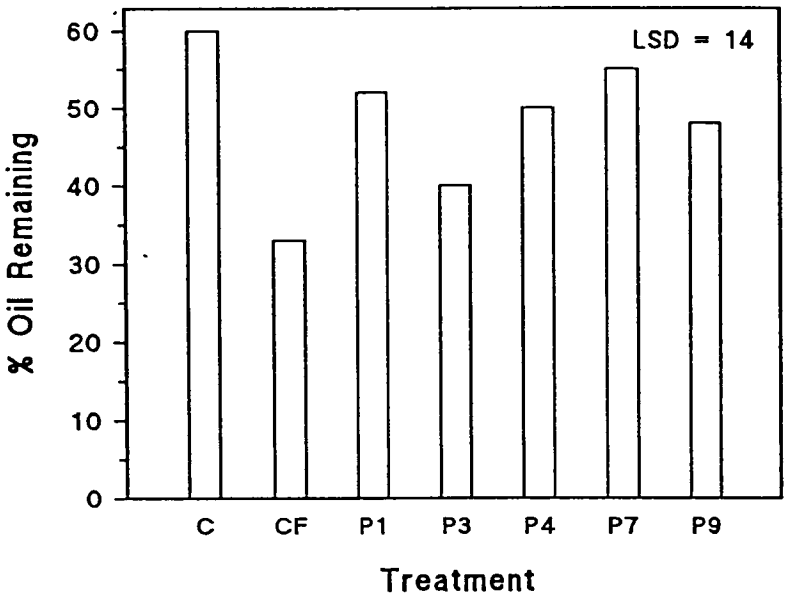

Figure 3. Percentage of added oil remaining at 33 d in mesocosms that were unfertilized (C), fertilized (CF), and those treated with bioremediation products (P1, P3, P4, P7 and P9) and maintained in a glasshouse during the warm season.

The Development of Bioremediation for Oil Spill Cleanup in Coastal Wetlands: Product Impacts and Bioremediation Potential

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INTRODUCTION

The northern Gulf Coast of the United States is a region of intense oil exploration, production and transmission. Consequently, coastal states, such as Louisiana, are subject to oil spills resulting from shipping accidents, production-related incidents, and pipeline ruptures. Since these incidents often occur in the nearshore environment, coastal salt marshes are frequently the first wetland habitats to be subjected to the oil. As a result, a large number of investigations have documented the effect of petroleum hydrocarbon spills on the dominant salt marsh plant species, *Spartina alterniflora* (for example, Hershner and Lake 1977; Lee *et al.* 1981; Alexander and Webb 1983; Ferrell *et al.* 1984; Mendelssohn *et al.* 1990). In addition, some investigations (e.g., DeLaune *et al.* 1984) have evaluated the

impact of oil cleanup procedures in salt marshes. Not only can petroleum hydrocarbons have detrimental impacts on coastal marshes, but additionally, the cleanup of the oil from these highly sensitive environments is often more damaging that the oil itself. Hence, it is more important to develop less intrusive oil spill cleanup procedures that exert little or no impact on wetland ecosystems.

Bioremediation, the act of adding materials to contaminated environments, such as oil spill sites, to cause an acceleration of the natural biodegradation process (U.S. Congress 1991) is a promising means by which oil released into salt marshes, as well as other wetland types, can be removed with little or no impact to the habitat. Bacteria, cultured and selected for high rates of oil degradation, and fertilizers, which enhance native microbial activity, are two types of bioremediation products that can be added to oil contaminated wetlands. Although data in the scientific literature demonstrating the relative effectiveness of bioremediation as an oil cleanup procedure in wetlands is lacking, a number of studies have demonstrated, in general, the potential for enhanced oil degradation as a result of bioremediation, especially through nutrient additions (for example, Tabak *et al.* 1991; Safferman 1991; Lee and Levy 1987; Lee and Levy 1991; Bragg *et al.* 1993; Lee *et al.* 1993). Specifically for wetlands, Scherrer and Mille (1990) confirmed enhanced degradation of oil in a West Indies mangrove swamp after the addition of an oleophilic fertilizer. Similarly, Lee and Levy (1991) found enhanced degradation of oil, this time in salt marsh sediments, treated with inorganic nutrients. However, critical evaluations of oil bioremediation potential, based on oil chemical analyses that can unequivocally identify enhanced biodegradation, in wetland environments are lacking in the published literature.

Microbial seeding as a means of enhancing oil biodegradation, has even greater uncertainties associated with it, especially in systems such as wetlands where hydrocarbon degrading bacteria are naturally prevalent. For example, microbial seeding was used in an experimental mode to test its effectiveness in cleaning up an oil spill in a marsh (Marrow Marsh) in Galveston Bay. The reported results did not indicate that the microbial seeding significantly degraded oil at this marsh site (Mearns 1991). In a recent investigation (Venosa *et al.* 1992) two microbial products, which exhibited enhanced biodegradation of Alaska North Slope crude oil in shaker flask tests, did not accelerate biodegradation in a field experiment conducted on an oiled beach in Prince William Sound, although the high variability in the data, the highly weathered nature of the oil, and a lack of sufficient time for biodegradation were cited as possible reasons for the lack of response. Regardless of these equivocal results, many microbial products have been commercialized. If added microbes, *per se*, are not effective in increasing oil degradation, the high costs of microbial amendments may not be warranted. Oil response agencies, both public and private, require a critical evaluation of microbial seeding in enhancing oil biodegradation. Finally, the ecological impacts of these amendments, microbial as well as fertilizer, must be identified.

A multi-disciplinary, multi-investigator research program has been initiated to address the question: Is bioremediation, via fertilizer or microbial seeding, an effective and ecologically safe means of oil spill cleanup in coastal wetlands? The specific objective of this paper is to summarize the overall scope of the study and to present some preliminary findings concerning marsh plant response to the bioremediation agents.

PROJECT GOAL AND APPROACH

The overall goal of the proposed project is to determine the potential for the use of bioremediation as a oil-spill cleanup technique in wetlands. Specifically, we shall determine the effects of both fertilization and microbial seeding on (1) petroleum hydrocarbon degradation and (2) the impacts, if any, of these spill cleanup methods on the wetland. To accomplish the preceding goal, we have divide the project into three phases (Figure 1):

Phase 1. Project Design - This component, whose objective was to design the Phase 2 efforts, was funded by Exxon and has been completed; this paper is the result of that effort.

Phase 2. Greenhouse Bioremediation Trials - The present paper describes the first experiment within the Phase 2 effort to determine the potential for oil spill bioremediation in wetland mesocosms. This phase of the research consists of four tasks described below and is being funded jointly by Exxon USA and the Minerals Management Service's Coastal Marine Institute at Louisiana State University.

Phase 3. Field Demonstration Project - If the greenhouse experiments indicate a potential for bioremediation of oil spills in wetlands, a field demonstration will be conducted.

PROJECT DESCRIPTION

Controlled greenhouse experiments, as well as field trials, are planned to test the efficacy and ecological safety of microbial seeding and fertilization as enhanced biodegradation methodologies. This three year study is designed to test the following aspects of bioremediation in coastal marshes:

(1) Product Toxicity: Determine if the maximum allowable loading rate (as defined by the product manufacturer) of the selected bioremediation products generates adverse impacts on the wetland plants, infaunal animals and microbial communities. This experiment is required to ensure that the product loading rate suggested by the manufacturer is not toxic to wetland plants and estuarine animals. Only products on the National Contingency Plan (NCP) list, with defined maximum loading rates, will be used in this study.

(2) Biodegradation Potential: Determine the effect of fertilizer and microbial seeding on oil biodegradation in salt marsh soil mesocosms. This experiment is essential to determine the potential for enhanced oil degradation via bioremediation in salt marsh substrates and is the first step before large scale field trials.

(3) Marsh Soil Type: Determine to what extent product-enhanced oil biodegradation is modified by marsh soil type. Salt marsh soils, depending on their texture and specific microbial communities, may exhibit different capacities for bioremediation which must be quantified in order to access the variability in bioremediation potential of salt marshes.

(4) Product Reapplication: Determine (a) if product reapplication is required to maintain an enhanced rate of biodegradation and, thus, to maximize total hydrocarbon degradation and (b) whether reapplication rate is a function of the initial oil dosage. Reapplication of the fertilizer bioremediation product is likely during a real cleanup operation. Thus, the efficacy of reapplication as a means of maintaining maximum biodegradation rates at a different oil dosing levels will be evaluated.

(5) Field Bioremediation Trial: Determine, under real-world conditions, the degree to which the potential for bioremediation demonstrated in the greenhouse marsh mesocosms is realized in the field. This experiment will be designed to assess bioremediation in both streamside salt marshes, where subsurface hydrology is a relatively active, and immediately adjacent inland salt marshes, where subsurface hydrology is minimal. Both bioremediation efficacy and ecological safety will be evaluated.

The effectiveness of bioremediation and its ecological safety will be assessed in the above experiments by evaluating the following: (1) petroleum hydrocarbon chemistry to identify and quantify the degree of oil biodegradation (Overton and Henry), (2) oil morphology, which will be related to oil chemistry, as an inexpensive means of evaluating oil biodegradation (Debusschere), (3) soil microbial response to determine the effect of the bioremediation products on the microbial communities that are performing the oil biodegradation (Portier and Walsh), (4) soil chemistry to determine the effect of the bioremediation products on those factors that limit the growth of microbes and plants (e.g. nutrients, soil reducing conditions and soil toxins) (Mendelssohn), and (5) plant and infaunal response to evaluate the combined effects of the oil and products on plant and animal components of the marsh system (Mendelssohn and Rabalais).

IDENTIFICATION OF PRODUCT TOXICITY AT MAXIMUM LOADING RATE

Objective and Rationale

This experiment is designed to determine if the maximum allowable loading rate of the selected bioremediation products generate adverse impacts to wetland plants, infaunal animals and microbial communities. We specifically ask the question: Can bioremediation be used for oil spill cleanup without causing negative impacts to wetland structure and function? Selected plant and soil responses are presented.

Experimental Design and Methods

Sods of marsh (soil and vegetation intact), approximately 28 cm in diameter (0.06 m^2) and 30 cm deep, were collected from the inland zone (approximately 5 m from the creekbank natural levee) of a *Spartina alterniflora* dominated salt marsh located west of Cocodrie, Louisiana and used as the experimental units. Inland sods were chosen because the inland zone comprises the largest aerial extent of most salt marshes. We recognize that soil type will likely influence bioremediation, and, thus, this factor will be examined in future research. *Spartina alterniflora* is the dominant intertidal salt marsh grass along the Atlantic and Gulf Coasts of the United States and thus result from this study will be generally applicable to many other salt marshes.

In the greenhouse, the following treatments were randomly assigned to the collected sods: (1) fertilizer product, (2) seeding product, and (3) control (Table 1). The experimental design was a randomized block with a 3 x 2 factorial treatment arrangement (three bioremediation types [mentioned above] and two oil dosage levels [oiled with 1 l/m^2 (1mm of oil thickness) and control) (Table 1). Each treatment combination was replicated five times for a total of 30 sods of marsh. Analysis of variance was used to test for statistically significant differences ($P < 0.05$) among the treatments.

A reduced crude with nC-13 and below removed in order to stimulate oil spilled in open water and subsequently transported into a salt marsh by winds or tides was added to the surface water of the mesocosms. The water was then drained from the bottom of the pots to allow the oil to come in contact with the surface of the sods. The bioremediation products utilized were those proven to be most successful in enhancing oil biodegradation from marsh sediment-microcosm experiments performed by Ms. Sara McMillen of Exxon Production Research, Houston as part of Phase I of this project. This work employed both respirometry and oil chemistry (GC-FID) to identify enhanced oil biodegradation. The results indicated that Customblen, a fertilizer product used during the Valdez Spill and Petrobac, a microbial product, show promise as bioremediation agents. Thus, we used these two products in the present experiment.

The Costomblen used in this study contained 28% N and 8% P as ammonium nitrate, calcium phosphate and ammonium phosphate (Bragg *et al.* 1992). Petrobac contains microbes, without any fertilizer, selected for hydrocarbon degradation in a saline medium (R. Drake, Polybac Corp., personal communication). The products were applied to the soil surface in a manner similar to that during a field application and following the manufacturer's specifications (Customblen: 93 g m^{-2}; Petrobac:0.8331 m^{-2} of inoculum [46 g of Petrobac l^{-1} of deionized water]). The sod-mesocosms were kept moist, but drained by maintaining an average water level at 5 cm below the soil surface. Water levels were allowed to fluctuate due to evapo-transpiration, but they were reflooded daily with deionized water to 5 cm below the soil surface to maintain relatively constant salinities.

Plant responses were measured during a three month period (at 0, 1, 2, 4, 8, and 12 weeks after product addition) to determine the effect , if any, of product addition on plant growth and photosynthesis (a highly sensitive indicator of plant response to stress). Soil respiration was determined with an infra-red gas analyzer by measuring carbon dioxide production in a flow through respiration chamber placed over the soil surface. Microbial,

infaunal, and soil chemical responses were also evaluated and will be presented in future publications.

RESULTS

The effect of the bioremediation products and oil on the growth response of *Spartina alterniflora* was assessed by determining leaf elongation and photosynthetic (leaf CO_2 exchange) rates. Bioremediation had a significant effect on both leaf elongation (Figure 2) and photosynthesis (Figure 3). The bioremediation effect was due to the fertilizer product which significantly increased leaf elongation and net CO_2 exchange rates (photosynthesis) above the control, regardless of the presence of oil (Figures 2 and 3). The addition of the microbial product had no significant effect on the plant growth responses (Figures 2 and 3). These results demonstrate that the bioremediation agents tested were not toxic to the vegetation. In fact, the fertilizer product, Customblen, stimulated plant growth , a response that was not unexpected. The addition of a reduced crude oil to the marsh mesocosms had no significant impact on plant responses (Figures 2 and 3), and there was no significant interaction between bioremediation products and the oil.

In situ respiration was measured in order to identify if the bioremediation agents were affecting the metabolism of the soil community. The living soil community is composed of bacteria, fungi, invertebrates and roots of *Spartina alterniflora*. The fertilizer treatment had a significant positive effect on soil respiration compared to both the control and the microbial treatments (Figure 4). The soil respiration of the microbial treatment, however, was not significantly different from that of the control (Figure 4). Oil addition also had a significant effect on soil respiration, with the oiled mesocosms exhibiting significantly higher soil respiration than the unoiled mesocosms (Figure 5). The increase in soil respiration due to the fertilizer and oil treatments could be a response to either increased microbial activity or to greater root density within the soil. Although we presently cannot separate the two, soil cores have been collected from the sods and root density will be evaluated in an attempt to separate these factors.

SUMMARY AND CONCLUSIONS

1. The fertilizer product significantly increased the growth response of *Spartina alterniflora* and the rate of soil respiration, while the microbial product did not significantly affect either of these processes.

2. Oil significantly increased soil respiration, but had no influence on plant growth.

3. The bioremediation products tested did not negatively impact plant growth response, and , therefore they appear to have no toxic affect to the application rates used in this investigation.

4. The results of this investigation, in conjunction with our findings from ongoing

bioremediation experiments, will help to determine if bioremediation is a suitable oil spill cleanup technique in the wetland environment.

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Table 1.

Experimental design for Task 1. The three bioremediation treatments (fertilizer, microbial seeding, and control) were applied to both oiled and unoiled marsh sods.

	Fertilizer	Microbial Seeding	Control
No Oil	5 replicates	5 replicates	5 replicates
Oil	5 replicates	5 replicates	5 replicates

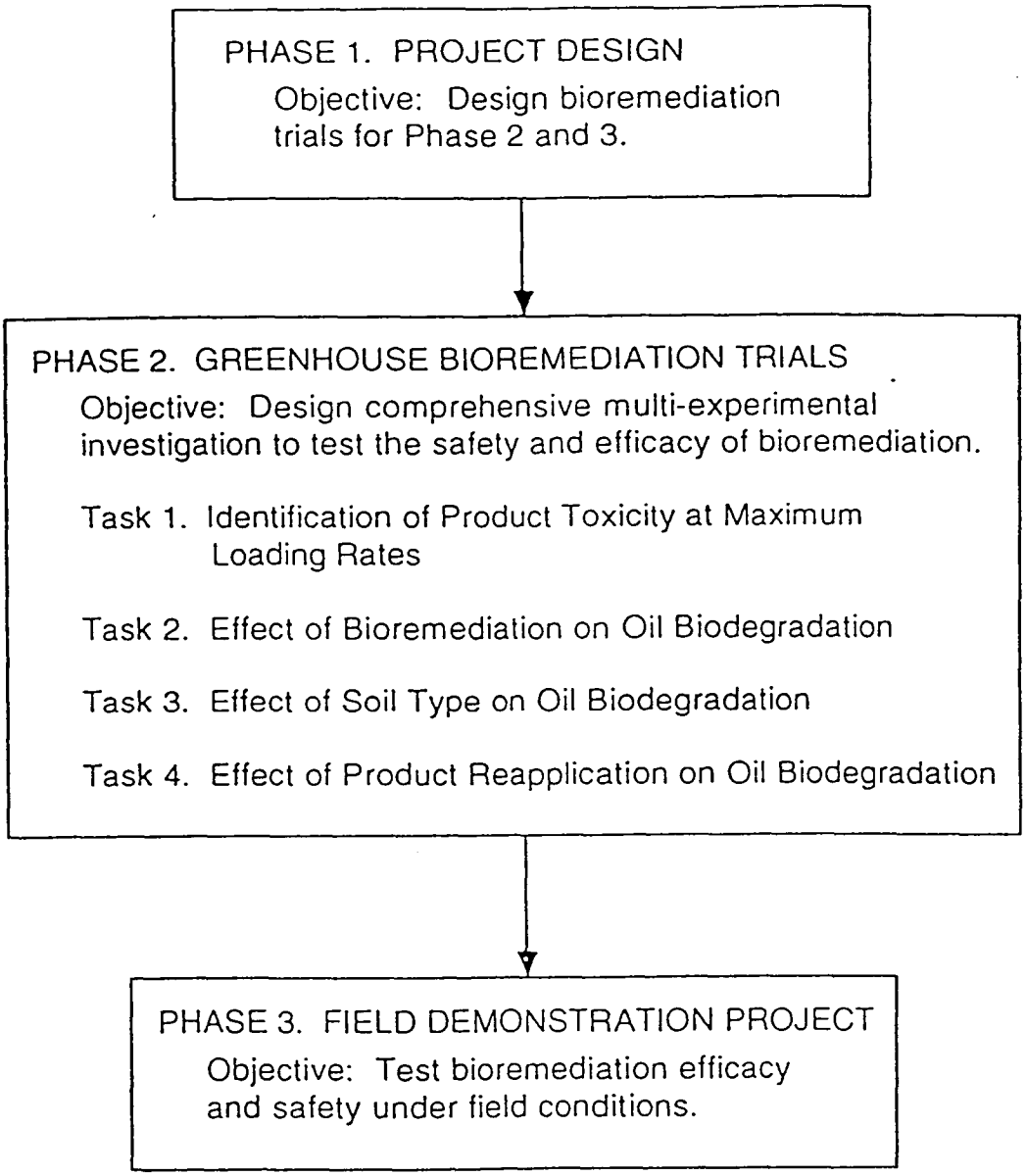

Figure 1. The overall project was designed in three phases: Phase I - Project Design has been completed; Phase 2 - Greenhouse Bioremediation Trials are ongoing; Phase 3 - Field Demonstration Project will be pursued if the greenhouse trials indicate a potential for bioremediation.

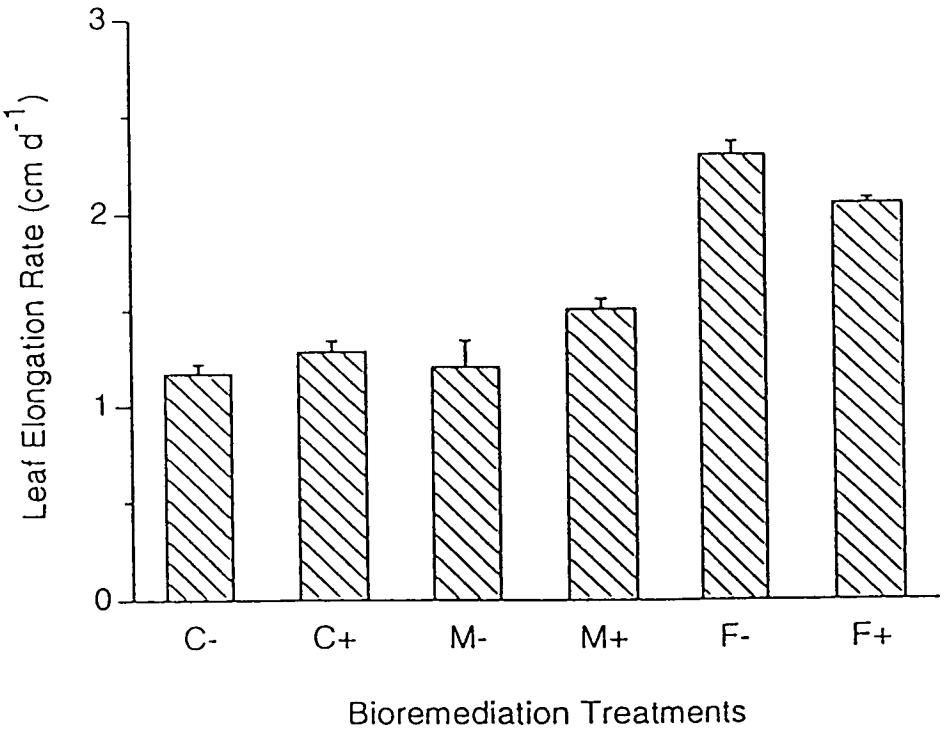

Figure 2. The response of leaf elongation rate to bioremediation and oil treatments (C=no bioremediation product, M=microbial product, F=fertilizer product; + indicates oil added, - indicates no oil). Means of 5 replicates and standard error bars are presented.

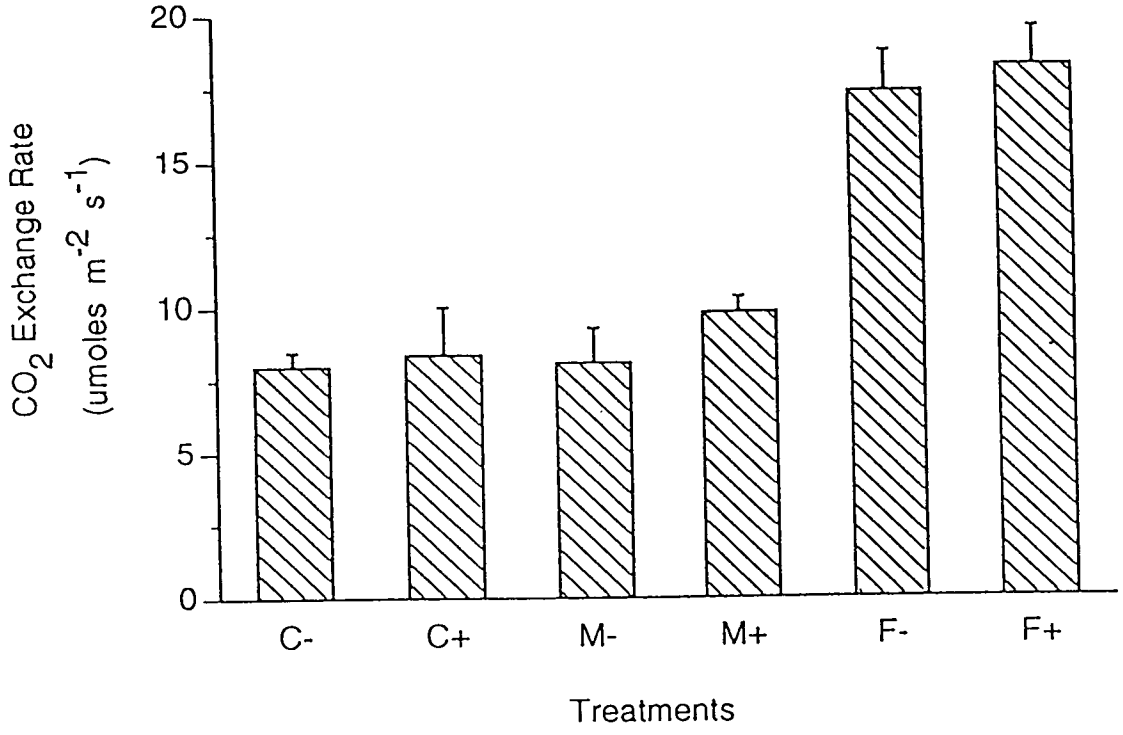

Figure 3. The effect of bioremediation and oil treatments on net leaf $C0_2$ exchange rate (C=no bioremediation product, M=microbial product, F=fertilizer product; + indicates oil added, - indicates no oil). Means of 5 replicates and standard error bars are presented.

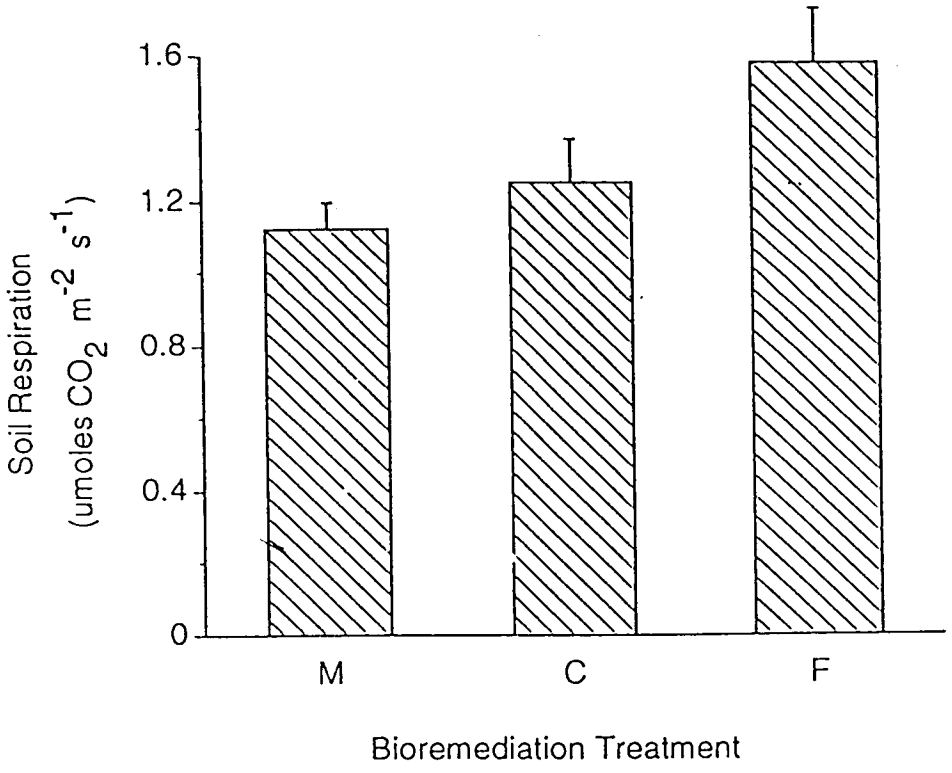

Figure 4. The effect of the bioremediation products, averaged over oil treatments, on soil respiration rate (C=no bioremediation product, M=microbial product, F=fertilizer product; + indicates oil added, - indicates no oil). Means of 10 replicates and standard error bars are presented.

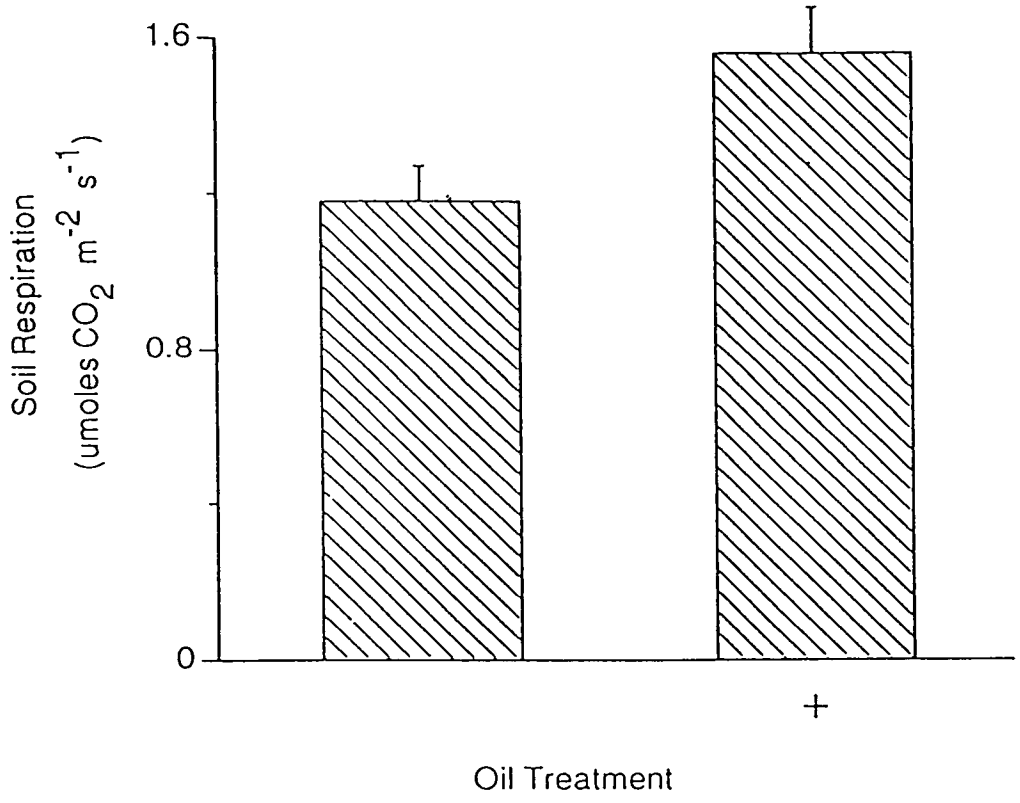

Figure 5. Soil respiration as a function of oil addition (+Oil, -Oil). Means of 15 replicates and standard error bars are presented.

Bioremediation Studies - Effects on the Marsh Infaunal Community

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INTRODUCTION

Wetland habiats, when subjected to oil spills, may be impacted either directly by the petroleum hydrocarbons or by physical or chemical removal of the oil through various cleanup attempts. Bioremediation, the act of adding materials to contaminated sediments, such as oil spill sites, to cause an acceleration of the oil biodegradation process is a promising methodology for restoring oil contaminated environments.

The studies of the effects of chemical discharges, oil spills and bioremediation treatments for oil spills have necessarily focused on the benthos, because (1) they are relatively sedentary, (2) their life spans, depending on the organism, may be long enough to integrate water/sediment quality conditions or short enough to respond to changes in the environment, (3) different species or taxonomic gropups exhibit different tolerances to stress, (4) they are an important component of the food web, and (5) they function in the cycling of nutrients and other chemicals between the sediment and water column.

A comprehensive, multi-disciplinary experimental program (I. Mendelssohn *et al.*) is underway as part of the Louisiana State University, Coastal Marine Institute, to examine microbial seeding and fertilizers as a means of oil spill cleanup in coastal salt marshes. During the first year's effort, currently in progress, controlled greenhouse experiments are being used to determine product toxicity on wetland plants, infaunal organisms, and microbial communities. The objectives of our research are to determine the effects of the bioremediation products, the oil, and/or the interaction of the bioremediation products with the oil on the indigenous salt marsh meio- and macroinfaunal communities.

STUDY DESIGN

Sods of marsh grass (*Spartina alterniflora*) were collected from an inland marsh west of the LUMCON Marine Center in Cocodrie, Louisiana and transported to a greenhouse at Louisiana State University. In the greenhouse, randomly assigned treatments were set up in a 3 x 2 factorial design to test for the effects of oil vs. no oil, and for the effects of fertilizer addition, microbial seeding, or no treatment. Five replicates of each treatment were established and are shown in the following chart.

	Fertilizer	Microbial Seeding	Control
No Oil	5 replicates	5 replicates	5 replicates
Oil	5 replicates	5 replicates	5 replicates

Macroinfauna will be collected in 3-in diameter core tubes to a depth of 10 cm. Five replicates from similarly treated plots were taken prior to the beginning of the experiment. One core from each replicate plot will be taken at the termination of the experiment.

A series of studies are being conducted on the meiofaunal component of the experiment. A comparison of meiofauna cores taken from the marsh will be compared to cores taken from acclimated plots in the greenhouse, to determine the effects of acclimation period on the meiofauna community. Fifteen replicates from the field will be compared to 15 of the 30 replicates in the greenhouse plots. These cores will also quantify the variability in meiofaunal communities.

A simple experiment was conducted on extra greenhouse plots to determine where the majority of the meiofauna were distributed vertically in a core. Splits of cores were made at 0-1, 1-2, 2-3, and 3-4 cm. We determined that most of the meiofauna were in the upper 3 cm of the cores; this protocol was used for the remainder of the experiment.

For the definitive experiment, meiofauna were collected with a bulk density coring device, approx. 2-cm diameter, to depth of 3 cm at day 0, 1 week, 2 weeks, 4 weeks and 12 weeks. When a meiofuana core is removed from a greenhouse plot, a core from a similarly treated plot is used to replace it. The area of the replaced core is not resampled on subseuqent dates.

Time Table for Experiment:

Sod collection from marsh - March 22, 1994
Vertical distribution of meiofauna, post-acclimation effects - May 2, 1994
Day 0 - post-acclimation effects, initial status - May 12, 1994
1 Week - May 20, 1994
2 Week - May 27, 1994
1 Month - June 10, 1994
3 Month - August 5, 1994

Standard meiofauna and macroinfauna processing techniques will be used to determine taxonomic composition and abundance of organisms. Benthos responses will be tested by determining changes in abundance, species composition, diversity, population structure (adult vs. juvenile, presence of eggs), taxonomic composition, and, possibly, size of nematodes.

RESULTS

As can be seen from the above schedule, the experiment is currently in progress. Preliminary results from a limited number of meiofauna cores indicates no dramatic toxicity of any treatment within one week of experiment initiation. Expected effects may be seen in an initial toxicity of oil to copepods, but possibly not nematodes. There may be an organic stimulation of the meiofaunal community from either oil, microbes, or microbes stimulated by fertilizer additions.

ACKNOWLEDGMENTS

Acknowledgments are due to the U. S. Minerals Management Service and Exxon Corporation funding to the Louisiana State University, Coastal Marine Institute, for project funding and to the Louisiana Universities Marine Consortium Foundation, Inc. for a Graduate Student Research Grant to Ms. Nazan Atilla. Dr. John Fleeger, LSU, has provided expertise and insight into the conduct of the meiofaunal sampling.

In-Situ Burn as an Oil Spill Response Technique

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The Marine Spill Response Corp. (MSRC) is a national, not-for-profit corporation formed by the major oil companies and the American Petroleum Institute following the Alaskan- *Exxon Valdez* oil spill in 1989. MSRC will respond to catestrophic oil spills in certain U.S. coastal waters up to 200 miles offshore.

In-situ burning is the deliberate ignition and controlled combustion of spilled oil "in place," or, under special circumstances, the allowing of an accidental burn to continue to burn. Prior to the early 1980s and the development of the fire resistent boom, the effectiveness of burning depended on other structures or forces such as docks, breakwaters, shorelines, winds, or ice to keep the oil thick enough to support combustion.

After the advent of the fire-resistant boom, it was possible to collect spilled oil on the water and burn large quantities of oil with great efficiency. Experimental burns including small scale, meso-scale, and a few large-scale burns were under taken to learn more about this response technique. Three large-scale field burns, two experimental and one actual spill makeup the bulk of our present day knowledge about in-situ burning in a real world environment. These were the Trondheim, Norway experimental burn of 500 gallons in 1988; the Newfoundland Offshore Burn Experiment experimental burn involving two burns totaling approximately 20,000 gallons in 1994; and the burning of 15-20,000 gallons in Prince William Sound, Alaska after the wreck of the *Exxon Valdez* in 1989.

In the 1967 grounding of the *Torry Canyon* off the English coast, 1,000-pound high explosive bombs, napalm bombs, rockets, and other tecniques were used in unsuccessful attempts to ignite the oil.

Since the development of fire-resistant boom, many small scale laboratory burns, a number of meso-scale field burns, and at least three large-scale burns have demonstrated the effectiveness of burning in eliminating large volumes of oil from the water. Oil removal efficiency rates in access of 95% are routinely reported. The small amount of residue remaining is a small fraction of the original oil volume; it is lighter, more toxic components have been removed; and it is easy to handle.These factors greatly reduce storage and disposal requirements.

Numerous experimental burns and the few field burns that have been done have reported elimination rates of 95-99%. Since oil spill responders work to prevent spilled oil

from impacting sensitive areas and resources concentrated on or near the shoreline, in-situ burning has the potential to mitigate environmental impacts from catastrophic oil spills

In-situ burning is relatively simple in terms of logistics: a basic unit consists of a fire boom towed in a "u" shaped configuration by two vessels of opportunity and an ignition source. The burn residue is normally easy to handle and might require a gill net, shrimp net, or other device for collection. Efficiency of the burn operation would be improved with aerial support from an airplane or helicopter for the observation of slick movements, smoke plumes, safety factors, response activities, and resources at risk. Another operational advantage is the storage and disposal requirements inherent in mechanical cleanup are minimized with in-situ burning.

Probably the major reason for the reluctance to accept in-situ burning as a major response option is the large amount of black smoke generated by the burn and the resultant air pollution. Combustion of oil produces a number of compounds, paticulates and gases that could constitute a health threat to humans or wildlife directly in the path of the smoke plume.

The tradeoffs involve the rapid removal of oil from the surface of the water by burning, preventing shoreline impacts, contact of wildlife with floating oil, and environmental impacts of the slow evaporation and weathering of spilled oil weighed against concerns for worker safety, and environmental impacts associated with air emissions, heating of the water, and residue from the burn.

Safety considerations are the number one priority in assessing the option of in-situ burning. Considerations include: worker safety training, safe boom handling, planning for the possibility of unwanted ignition and secondary fires, personnel exposure, and proximity issues.

The combustion of oil on water produces an abundance of thick, black smoke which contains emissions that are potentially harmful to the environment. Of primary concern are PM_{10}s (particulate matter $<$ 10 microns in size), PAHs (polycyclic aromatic hydrocarbons), VOCs (volatile organic compounds), gases (such as carbon monoxide, carbon dioxide,and sulphur dioxide), metals, and exotic organics.

The recent Newfoundland Offshore Burn Experiment (NOBE), the largest most comprehensive experimental burn conducted to date, indicated that emissions from the two burns were less than expected. At a distance of 200 meters from the fire, all measurements of emissions were below health concern levels. At 500 meters, very little was detected. Water under the burn was analyzed and observed and no compounds of concern were detected and no measurable increase in water temperature was found. The residue did not sink, was easy to recover, contained no detectable acutely toxic compounds, and comprised only a small percentage the original oil volume.

The major sponsors of the NOBE were: U.S. Minerals Management Service, Environment Canada, Canadian Coast Guard, U.S. Coast Guard, U.S. Environmental Protection Agency, Canadian Association of Petroleum Producers, MSRC, American Petroleum Institute, 3-M Corp., and the Canadian Petroleum Products Institute.

The experimental burn involved 234 people at sea, 22 ships and boats, 7 aircraft, and over 200 sensors and samplers. ignition source for the burns was a helitorch. A number of special sampling techniques including remote controlled boats, submersables, helicopters and other aircraft were developed to collect the desired data.

Due to the rapid emulsification of spilled oil on the open ocean, the window of opportunity for the successful use of burning is narrow, typically ranging from only a few hours to a day or two. The narrow window of opportunity makes preapproval by the Regional Response Teams (RRT) a necessity if the FOSCs and responders are to have the use of this valuable tool.

On January 7, 1994, Regional Response Team VI which covers the Gulf Coast, approved a plan that allows the FOSC to approve the use of burning three miles or farther off the coasts of Louisiana or Texas. This landmark action is the first preapproval for in-situ burning in the continental U.S. and was presented to the RRT by MSRC on behalf of the response industry and its clients. The approval will encourage response community members to acquire fire boom to begin to work out the operational details and conduct the training necessary to conduct safe burns.

In spite of all the current interest in in-situ burning and the promising results from the NOBE, much needs to be done before this technique becomes a widely accepted and utilized.

Due to the rapid emulsification of spilled oil on the open ocean, the window of opportunity for the successful use of burning is narrow, typically ranging from only a few hours to a day or two. The narrow window of opportunity makes preapproval by the RRTs a necessity if the FOSCs and responders are to have the use of this valuable tool.

The approval process involved encouraging comments from the numerous federal and state agencies involved in the RRT approval process and responding to these comments. Of most concern to the resource agencies commenting were: air emissions, burn residue issues, and monitoring.

Concerns about air emissions were answered by establishing a 3 mile limit to burning. Previously reported research results and the recently completed NOBE indicated that 3 miles at least tripled the safe distance for the measuring of levels of concern.

Residue concerns were addressed primarily by the establishment of exclusion zones to prevent Gulf of Mexico sensitive biological communities associated with "hard" bottom from being contacted by sinking residue.

Monitoring became a big issue. It was finally decided that a concurrent proposal by the U.S. Coast Guard to monitor for dispersants and in-situ burning be included as a part of the burn plan. The Special Response Operations Monitoring Program (SROMP) was developed by the Coast Guard and will be carried out by the Gulf Strike Team.

The Strike Team will be monitoring real time during a burn operation to report PM_{10} levels directly to the FOSC. When levels of cocern are read, the FOSC will be advised to terminate operations.

However, much needs to be done before in-situ burning becomes a widely accepted and utilized response technique. Operational techniques and methods need to be worked out and improved. Boom towing must be praticed before crews become proficient with the technique. Training of this type can be done in pre-planning courses and drills. however, there is still a need to practice in-situ burning with oil deliberated spilled and ignited for that specific purpose.

Research is needed on the ignition of weathered oil, on rates of weathering of various types of oil and the effects of oil chemistry on other aspects of burning. In a broader

scope, research needs to be done on burning in wetlands where mechanical cleanup would result in more damage to this fragile resource.

From the results summarized above, as well as other reseasrch on burning spilled oil, it is felt that in-situ burning can be used as a safe response technique. Its use in combination with the other response tools, dispersants and mechanical recovery, will allow responders to mitigate environmental damage from catestrophic oil spills.

Evaluation of Burning as an Oil Spill Cleanup Technique in a High Marsh Community along the South Texas Coast

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INTRODUCTION

A rupture in a 40cm diameter underground oil transfer pipeline on January 7, 1992, resulted in the spillage of approximately 2,950 barrels (469 m^3) of API gravity 37 south Texas light crude oil into 15.5 ha of a high marsh community near Chiltipin Creek, San Patricio County, Texas (Strand and Schultz 1992). Because this high marsh is part of a natural drainage system of upland farmland that flows towards the confluence of Chiltipin Creek and the Aransas River, approximately 0.8 km to the N.E., there was concern that oil would enter the river possibly affecting a greater area. This concern was elevated by the fact that the marsh was inundated with several centimeters of water from recent heavy rain falls and continuing precipitation was predicted. Therefore, the situation required rapid oil removal and on January 9, 1992, the Texas General Land Office (designated On-Scene-Coordinator), after consultation with other resource agency personnel, authorized the burning of the oil as the primary cleanup technique. Other agency personnel included representatives of the Texas Natural Resource Conservation Commission, Texas Railroad Commission, U.S. Fish and Wildlife Service, Texas Parks and Wildlife Department, and the U.S. Coast Guard. Their rationale was based on the conclusion that trampling of marsh plants during mechanical removal techniques might result in the total loss of the existing marsh, and it was believed that the below-ground root and rhizome systems of the marsh vegetation would be protected against burn injury by the layer of standing water inundating the marsh, therefore allowing regrowth in the spring. The burn was initiated at 1820 hrs on January 11, 1992, by using varisol as a propellent and within two hours flames were in excess of 46 m high (Strand and Schultz 1992). The primary burn persisted for more than 18 hours after which minor pockets of unburned oil were ignited. Post-burn inspection revealed that burned oil residue, consisting of a waxy paraffin, as well as a brown and black mousse-like material, remained in significant quantities throughout the marsh. It was decided that if plants were to recover in the spring this residue would have to be removed by manual cleanup efforts. To minimize trampling of plants, work crews were required to strategically place plywood walkways throughout the marsh and remain on these walkways during the cleanup. Manual cleanup techniques included low pressure, high volume flushing and pumping, sorbent booms, pads, and pom poms. Final estimates for recovered oil were 1,250 bbls (198.7 m^3) from the blow-out hole, 500 bbls (79.5 m^3) were pumped from the marsh, and 50 bbls (8 m^3) were extracted with sorbent booms, pads and pom poms. Of the 1,150 bbls (183 m^3) that were unaccounted for, it was estimated that 350 bbls (56 m^3) probably evaporated into the air, leaving 800 bbls (127 m^3) either burned or remaining in the marsh. Of the 15.5 ha

surrounded by sorbent booms roughly half, 6.6 ha, were burned to the ground, causing extensive bare area throughout the impacted site.

The great value of coastal tidal wetlands or salt marshes is well established, including characteristic functions such as habitat, feeding, and nursery grounds for a variety of species, high productivity, shoreline stabilization, reduction of coastal flooding, and enhancement of water quality through immobilization of nutrients and filtering of heavy metals and toxic materials from the water column (for example, Lindall and Saloman 1977; Mitsch and Gosselink 1993; Rabalais 1980; Getter et al. 1984). Because of these important ecologic and economic values, as well as their high environmental sensitivity, salt marshes receive the highest priority for protection in oil spill contingency and response plans for coastal areas (Lindstedt-Siva 1979; Gundlach and Hayes 1978). However, despite their environmental sensitivity and priority for protection, salt marshes are still occasionally impacted by oil via accidental discharges from barge traffic (Hershner and Moore 1977; Ayers 1978; Hampson and Moul 1978; Sanders *et al.* 1980; Webb *et al.* 1981), oil transfer pipelines (Crow 1974; Holt *et al.* 1978; Kiesling *et al.* 1988), and oil storage tanks (Mattson *et al.* 1977).

After oil enters a marsh, the method(s) of cleanup, as well as assessment of environmental impacts, must be determined. Kiesling *et al.* (1988) and Alexander and Webb (1985, 1987) have thoroughly reviewed oil spill cleanup techniques and their impacts on salt marshes along the upper Texas coast. Their field work on actual oil spills and experimental test plots, as well as a careful review of the literature reveal multiple clean-up technologies available and varied results depending upon the ecological setting and the type and volume of oil spilled. Of the clean-up methods, including absorption, flushing, clipping, burning, or doing nothing, any could be chosen as appropriate, depending on the spill scenario. Generally, the most accepted ideas are to: 1) do nothing in the case of light to moderate oiling and adequate tidal flushing, because trampling of the marsh can cause more damage in the long-term than the oiling itself; 2) use flushing and absorption with heavier oiling or deeper penetration into the marsh; 3) use clipping when heavy oil cannot be effectively removed from vegetation by flushing; 4) do not use burning because it enhances oil penetration into the sediment and causes substantial initial plant damage. Additionally, it was noted that lighter fuel oils were more damaging to the salt marsh vegetation than heavier crude oils.

Alexander and Webb (1985) identified three spill situations where impact was long-term: 1) spillage of No. 2 fuel oil; 2) penetration of large quantities of oil into the sediment; and 3) complete oil coverage of plant surfaces during active growth periods. When any of these three situations are identified, the above-mentioned clean-up responses are recommended. In contrast to Alexander and Webb (1985, 1987), Holt *et al.* (1978) did find burned and clipped areas to have slightly better recovery than non-cleaned areas in a 1976 Harbor Island, Redfish Bay pipeline spill.

All of the above mentioned studies and reviews dealt with low salt marshes dominated by *Spartina alterniflora* distributed in the intertidal zone of estuarine shorelines. None of them were concerned within the supratidal, high marsh zone and none considered burning as the primary clean-up strategy.

In situ burning is viewed with growing interest as a response tool and has distinct advantages over other techniques. It offers the potential to rapidly convert large quantities of oil into its primary combustion products, carbon dioxide and water, with small percentages

of other unburned and residue by-products (Evans *et al.* 1992). The removal of oil from environmentally sensitive areas, such as high marshes, by burning is very rapid and may prove less damaging (long term) to vegetation than physical removal techniques, where trampling of plants is inevitable. This rapid removal of the oil also reduces the likelihood of it spreading into other sensitive areas. In-situ burning also requires minimal equipment and less labor than other removal techniques, and because the oil is converted to gaseous products of combustion by burning, the need for physical collection, storage, and transport of recovered fluids is reduced to a few percent of the original spill volume (Evans *et al.* 1992). Obvious disadvantages of burning oil are that it produces a visible smoke plume giving rise to public health concerns related to the chemical content of the smoke plume and the downwind deposition of particulates (Evans *et al.* 1992) and in vegetated areas, causes substantial initial plant damage.

The purpose of this study is to evaluate the effectiveness of burning oil as a clean-up method in a high marsh habitat by measuring changes in the marsh plant community and soil Total Petroleum Hydrocarbon (TPH) content over time (Tunnell *et al.* 1994).

STUDY AREA

The impacted high marsh area is located in San Patricio County, Texas, at approximate coordinates 28°04'09" N, 97°16'01" W (Fig. 1).

The study area is characterized by typical south Texas middle and high marsh plant species, small unvegetated areas usually covered by blue-green algal mats, and several ephemeral brackish-water ponds. Two sampling areas for study are located in the marsh, one impacted by the oil and burn and the other unimpacted. The adjacent unimpacted site was used as the control site for this study (Fig. 2).

The climate in the area is considered to be semi-arid, receiving approximately 89 cm of precipitation annually. Average temperatures range from 8.3 °C in the winter to 33.3 °C in the summer (Brown *et al.* 1976). Persistent southeast winds occur from March through September, while northeasterly winds occur with frontal passages from October through February (Behrens and Watson 1973.)

As a high marsh this area is rarely covered with water but because at the time of the spill this location was in a period of above normal rainfall there was 10 to 15 cm of water inundating the marsh. Throughout the spill and clean up efforts, rainy conditions persisted.

METHODS

Methodology for evaluating the effectiveness of burning oil as a cleanup method included a marsh plant frequency and biomass study as well as a study of soil TPH. Frequencies for marsh plants was obtained utilizing a line-point intercept transect technique. Twenty 30 m transects were systematically located in both the impacted and control areas of the marsh with points examined every 0.5 m. The plant species touching or immediately below the point being examined was recorded. The frequency of plant species was determined by totaling the number of times a given species was recorded for each transect, yielding a maximum mean frequency of 60. The 20 transects were used as replicates for

analyses in comparisons between impacted and control areas. Marsh plant frequency sampling was initiated in October 1992, and transects were repeated once in July 1993, during the first year of the study, after which sampling was conducted quarterly (October 1993, January 1994, and April 1994).

Marsh plant biomass was obtained by clipping all above-ground tissue without regards to species composition from 100 randomly located 25 cm^2 quadrats in each area. All plant tissue removed from the quadrats with electric hedge trimmers was placed in marked brown paper bags and returned to the lab. The aggregate plant samples were dried for 72 hours in a drying oven before being weighed to the nearest mg. Plant biomass samples were taken seasonally for the first year of the study (March, April, and May for cool season plants and June, July, September, and October for warm season plants). Beginning in October 1993, biomass was taken quarterly along with plant frequency sampling. Mean dry weight biomass was compared by independent t-test analysis after each sampling date.

Because little is known about high marsh vegetation species and their tolerance to fires, a separate investigation was begun to test the initial premise that marsh plants would continue to grow after having all of their above-ground tissue destroyed. In February 1994, two 1 m square quadrats were established in monotypic stands of *Borrichia frutescens*, *Salicornia virginica*, *Monanthochloe littoralis*, and *Distichlis spicata*. All above-ground tissue was removed by clipping and the remaining stubble was burned to ground level utilizing a pear burner. Prior to burning, the plants within the quadrats were isolated from clones outside the quadrat by cutting around the edges to a depth of 40 cm with a shovel. A 15.2 cm wide plastic garden edging was placed around each quadrat to prevent roots and rhizomes from regrowing into the isolated quadrats. Regrowth was qualitatively observed and photographed.

Soil for TPH analysis was collected in December 1992 and repeated one year later in December 1993. Initially, seventy core samples (8 cm length X 5 cm diameter), fifty from the impacted area and twenty from the control, were randomly collected using lexan cores and sent to the Texas Parks and Wildlife laboratory in San Marcos, Texas, for analysis. Areas found to have TPH values higher than 100 ppt were resampled in the subsequent sampling period. Because spiked recovery rates were different (91.6% in 1992 compared to 81.0 % in 1993) results were normalized to 100% spiked recoveries and then analyzed using a paired samples t-test.

RESULTS AND DISCUSSION

Thirteen species of plants were identified inhabiting the marsh area (Table 1). Of these thirteen, *Distichlis spicata*, *Batis maritima*, *Salicornia virginica*, *Borrichia frutescens*, *Monanthochloe littoralis*, and *Scirpus maritima*, along with bare area accounted for over 90 % of the observed frequencies along the line-point intercept transects. Although species richness was similar in the impacted and control areas, the relative abundance of each species was found to be different.

Approximately 16.4 acres were burned to the ground in the cleanup effort. This newly created bare area was for the most part rapidly recolonized by invasive pioneering species (*Distichlis spicata* and *Scirpus maritima*). *Distichlis spicata*, which accounts for

approximately 80% of the frequencies of the impacted area, is regarded as a disturbance-dependent species whose success is a product of its ability to tolerate and recover from disturbance in high salt marsh habitats (Bertness and Ellison 1987; Bertness *et al.* 1992; Hartman 1988). *Scirpus maritimus* thrives in standing water, the condition that was predominant throughout the first growing season. The bare areas resulting from the burn, made ideal habitat for *Scirpus maritimus*, whose only competition was from *Distichlis spicata*. The combined frequencies of *Distichlis spicata*, *Scirpus maritima*, and bare area account for 85.5% of the total observed frequencies of the impacted area between October 1992 and April 1994, compared to 50.3% in the control area (Figs. 3 and 4). The other half of the control area is for the most part comprised of climax perennial vegetation with mean combined frequencies averaging 27.68 compared to the impacted area where these perennial species made up 12.7% of the total combined frequencies (Figs. 5 and 6). There is no clear observable trend in the combined mean frequencies for vegetation patterns in the control area, unlike that of the impacted site. In the impacted site combined perennial climax vegetation frequencies are steadily increasing at approximately the same rate that combined pioneering species and bare area are decreasing. Regressions based on these trends yield estimates of between 10 and 12 years from initiation of the study in October 1992, until vegetation patterns resemble those observed in the control site. Although the changes in the impacted area are not expected to remain linear, it is believed that the predicted recovery times does illustrate how slow recovery of climax perennial vegetation lost due to the burn is likely to be.

These trends are also reflected in species diversity. In the control area, species diversity values have remained fairly constant over time. In the impacted area, as *Distichlis* colonized bare patches, the overall dominance was shifted to one species, and the diversity index value decreased. This decrease in diversity values continued until increases in frequency of *Borrichia frutescens* and *Scirpus maritimus*, at the expense of *Distichlis spicata*, between January and April 1994, caused the diversity index value to rise (Fig. 7).

Plant dry weight biomass was significantly higher ($P < 0.05$) in the control area in all months sampled. Seasonal trends apparent in the control area were masked by steadily increasing biomass in the impacted area until June 1993 when the two areas began tracking fairly closely (Fig. 8).

Results from the qualitative burning experiment show that all plots had new growth and by June 1994 *Distichlis spicata* plots were nearly indistinguishable from surrounding areas. Only *Salicornia virginica* plots were severely lagging behind others in regrowth. Although the *Borichia frutescens* plants were obviously shorter in burned plots, the number of plants within the plots is similar to that observed outside. The isolated plots of *Monanthocloe littoralis* also showed considerable regrowth and other than the obvious loss of the understory detrital buildup, would be indistinguishable from the surrounding area.

TPH values for the 20 samples collected from the control area in December 1992, ranged from 16.4 to 72.0 ppm ($\bar{x} = 50.5$, SE = 2.9). Of the 50 TPH samples randomly collected in the impacted area, 20 were found to have TPH values greater than 100 ppm. These twenty ranged from 108.0 to 4538.51 ppm ($\bar{x} = 1064.3$, SE = 256.7) The twenty impacted site samples were repeated in December 1993. TPH values for the repeated samples ranged from 29.38 to 4440.4 ppm ($\bar{x} = 897.1$, SE = 258.1) The overall decrease,

in mean TPH values, was not significant by paired sample t-test analysis. Although the TPH values have remained much higher than those of the control area ($\bar{x}$ = 50.5), it is not clear if they are at a level that would be detrimental to plant recovery. Levels of TPH < 5 ppt were found to have little effect on *Spartina alterniflora* marshes in Texas (Alexander and Webb 1987).

SUMMARY

The most significant disadvantages of burning as a clean-up method appears to be the substantial initial plant damage, especially to climax perennial vegetation. Residual hydrocarbon levels in the sediment remain elevated even after two years. Kiesling *et al.* (1988), during studies evaluating oil spill cleanup techniques, demonstrated that burning actually increased oil content in sediment by 27-72%.

Results of the qualitative burning experiment lead to concluding that the initial premise was not flawed, and that most high marsh plants could regenerate above-ground parts from below-ground tissue. The loss of the climax perennial vegetation in the impacted area was either a result of intense heat from the fire caused by the oil, or hydrocarbons were forced into the sediment from the heat, killing the roots and rhizomes. Because of the rapid rate at which the area was recolonized by *Distichlis spicata* it is not believed that toxins are preventing the regrowth of perennial climax species, rather the roots of perennials were probably killed by the intense heat transferred by the water covering the plants. Heat from a fire producing flames > 46 m high and persisting for > than 18 hours would certainly penetrate into the shallow root and rhizome layer of the marsh plants.

The combined mean frequencies of perennial climax species are slowly increasing over time at about the same rate as the combined mean frequencies of *Distichlis spicata*, *Scirpus maritima* and bare area are decreasing. Estimates of recovery are from 10 to 12 years based on regressions of trends in combined mean frequencies of perennial climax and pioneering vegetation in the impacted area.

It is obvious that burning oil as a clean-up method has merit but it should be used with caution. In this situation there was almost no other choice, the spill was to large to be left to degrade naturally, and manual cleanup efforts would have only served to trample the plants as well as push crude oil into the sediment. Fear of the oil washing into the nearby Aransas River also played an important role in the decision to burn the oil. From this study it should be realized that the damage to plants is severe and with respect to perennial climax vegetation, long term. In the future this long-term plant damage should be considered in burn decision making.

ACKNOWLEDGMENTS

The authors would like to thank the Texas General Land Office for funding this important three year study of injury evaluation in high marsh community due to an oil spill and ensuing burn. With the support of the GLO it is our goal to expand scientific knowledge of high salt marsh communities and assess their ability to recover from oil spills and to evaluate the use of burning as a cleanup procedure. We would like to acknowledge and

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Table 1.

A taxonomic list of plants inhabiting Chiltipin Creek marsh.

Division Spermatophyta
- Class Angiospermae
  - Subclass Monocotyledoneae
    - Order Najadales
      - Family Ruppiaceae
        - *Ruppia maritima* L.
          - Wigeongrass
      - Family Cyperaceae
        - *Scirpus maritimus* L.
          - Bulrush
      - Family Gramineae
        - *Distichlis spicata* L.
          - Saltgrass
        - *Monanthochloe littoralis* Engelm.
          - Key grass
        - *Spartina spartinae* (Trin.) Hitche.
          - Gulf Cordgrass
        - *Sporobolus virginicus* (L.) Kunth.
          - Seashore Dropseed
    - Order Caryophyllales
      - Family Chenopodiaceae
        - *Salicornia bigelovii* Torr.
          - Annual Glasswort
        - *S. virginica* L.
          - Perennial Glasswort
        - *Suaeda linearis* (Ell.) Moq.
          - Seablite
    - Order Bataceae
      - Family Bataceae
        - *Batis maritima* L.
          - Saltwort
    - Order Primulales
      - Family Plumbaginaceae
        - *Limonium nashii* Small
          - Sea Lavender
    - Order Polemoniales
      - Family Solanaceae
        - *Lycium carolinianum* Walt.
          - Wolfberry
    - Order Asterales
      - Family Asteraceae
        - *Borrichia frutescens* (L.) DC.
          - Sea Ox-eye Daisy

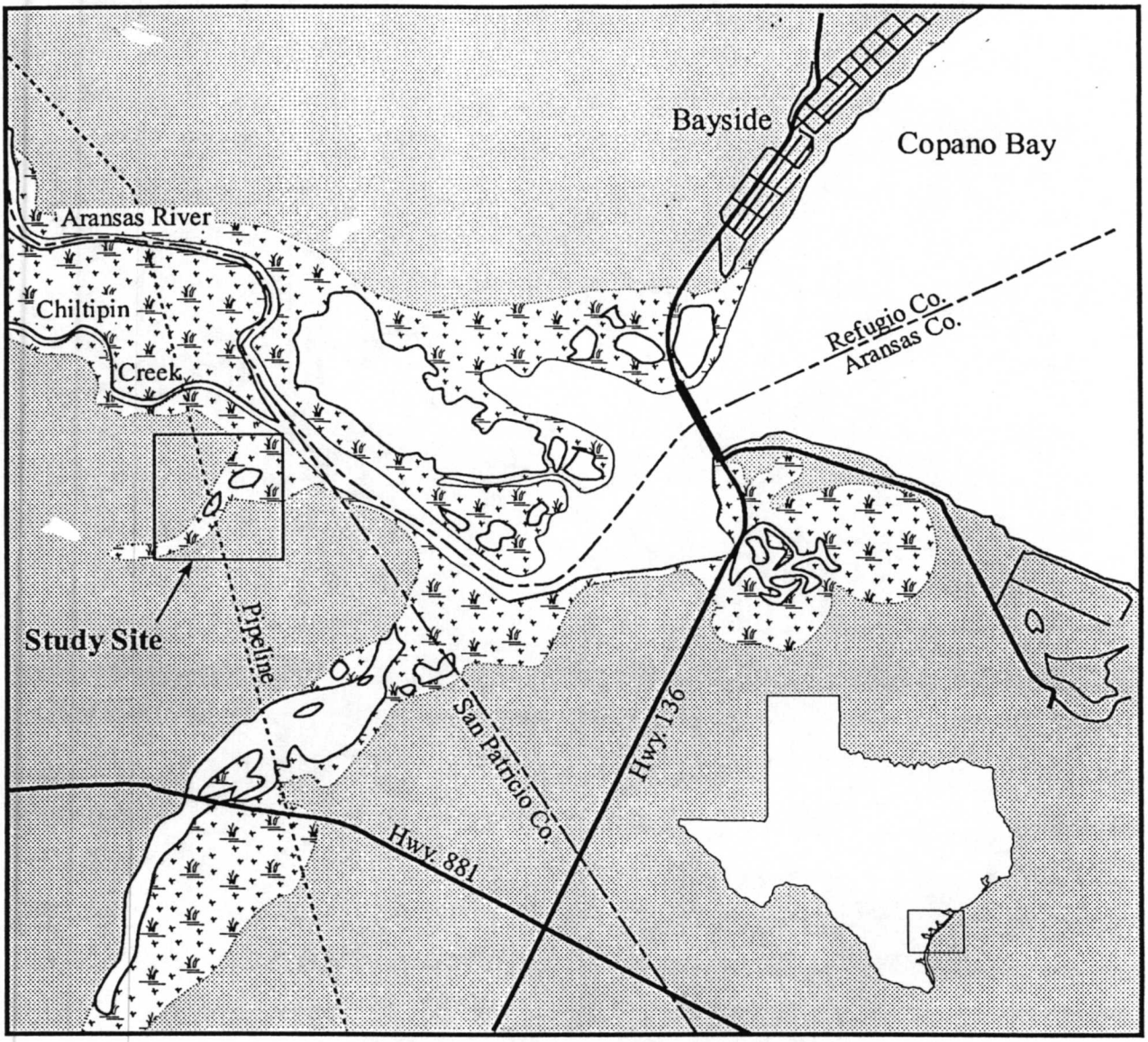

Figure 1. Location of the Chiltipin Creek marsh study site on the Texas coast.

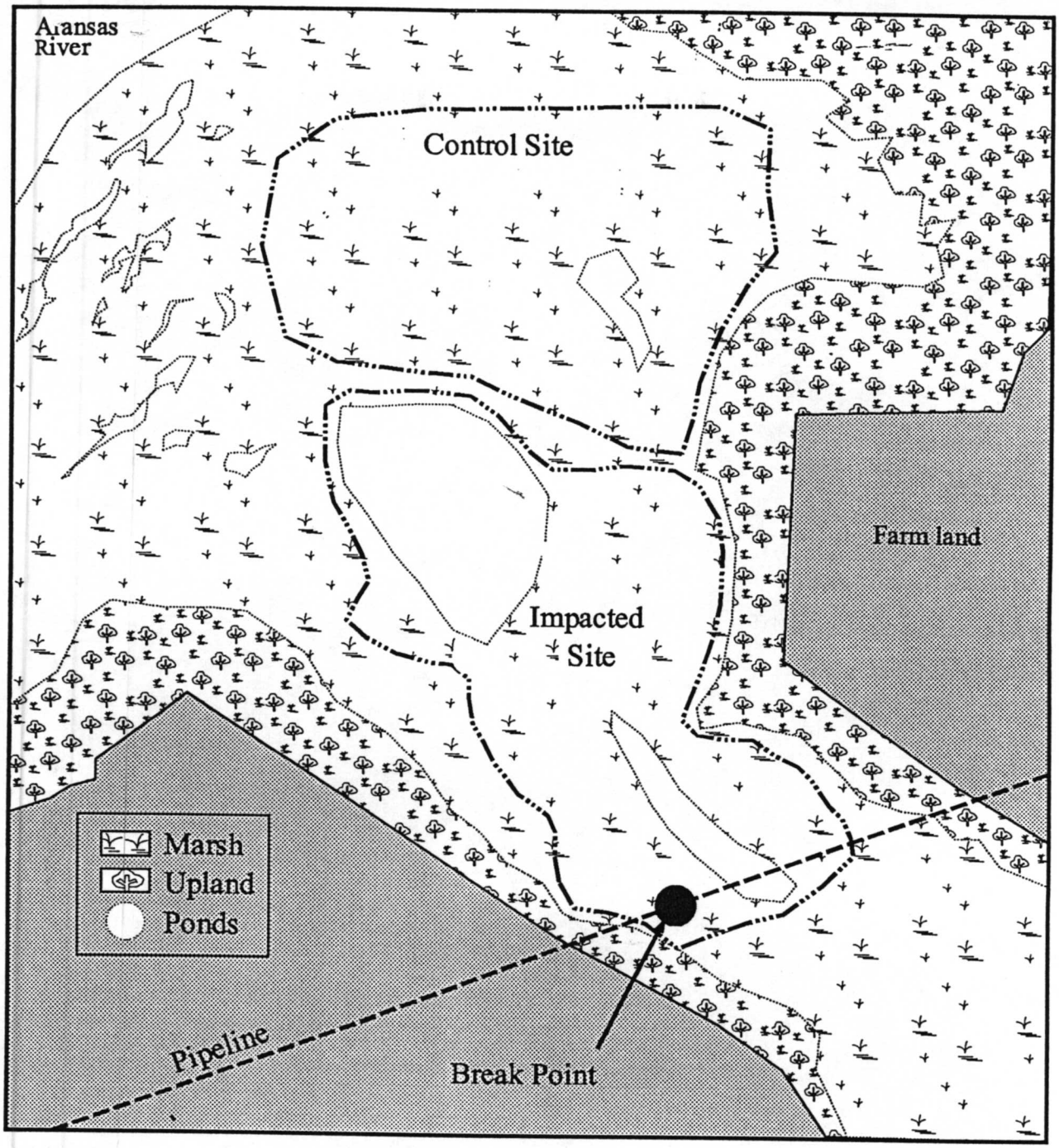

Figure 2. Spill site in Chiltipin Creek showing the impacted and control areas.

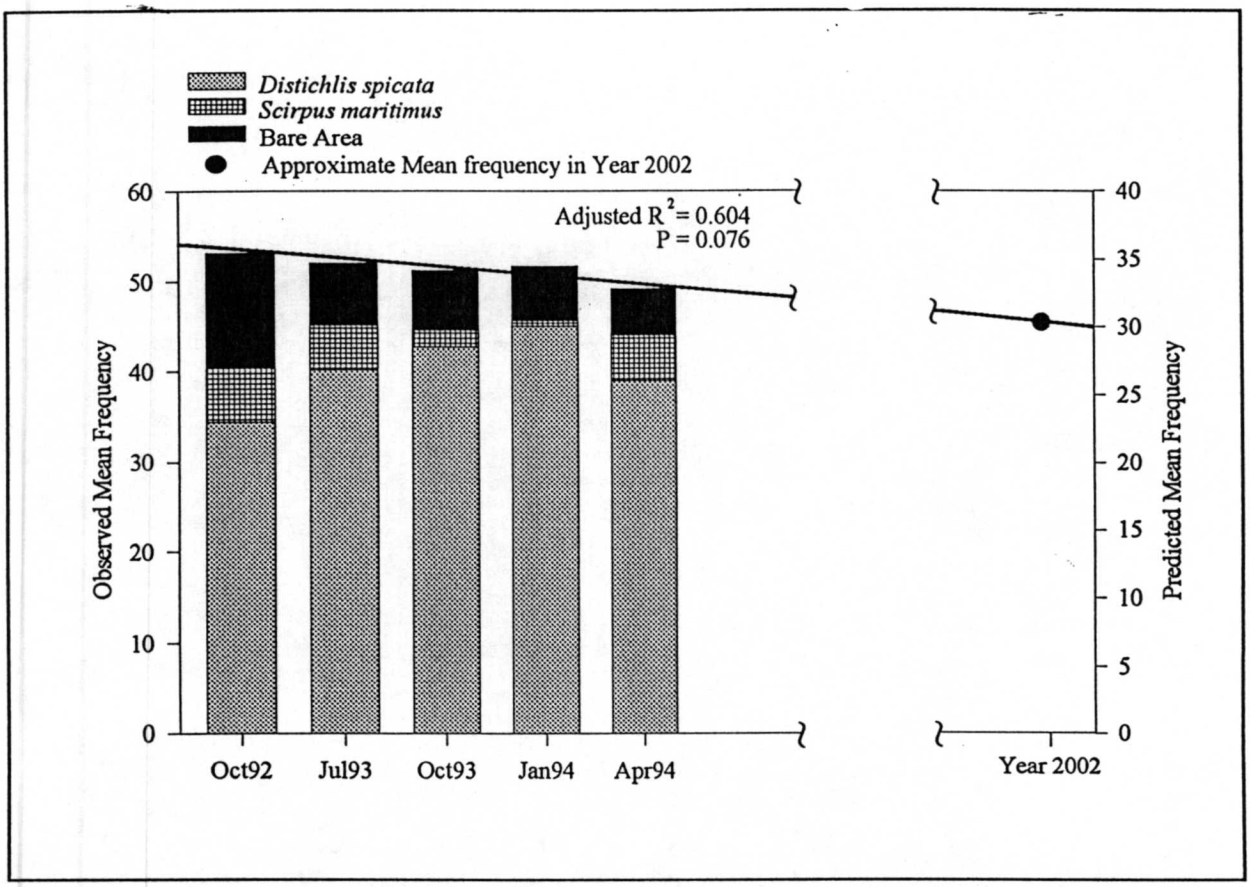

Figure 3. Combined mean frequencies of pioneering species and bare area observed in the impacted Chiltipin Creek marsh site, along with estimated time for levels to decrease to those observed in the control site

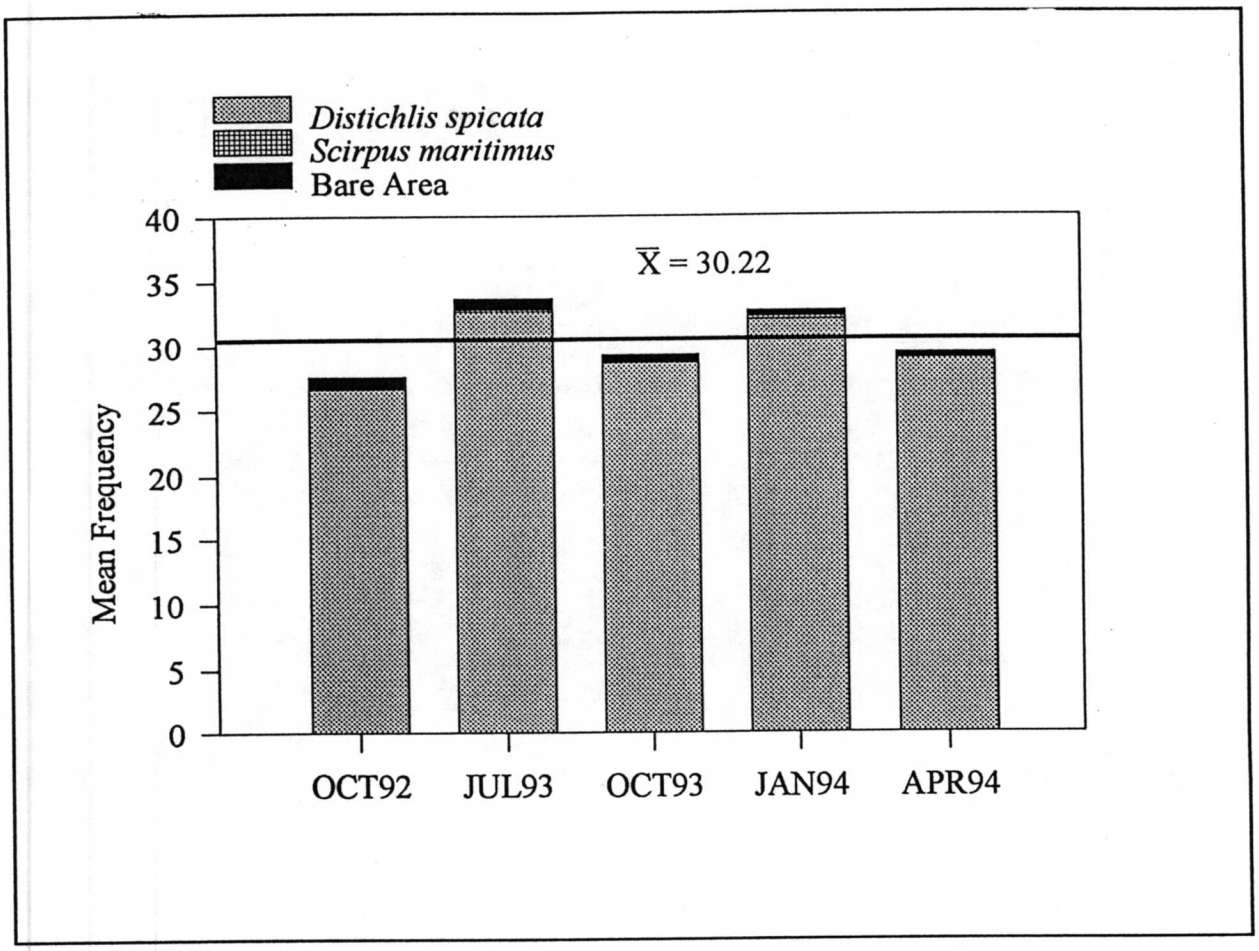

Figure 4. Combined mean frequencies of pioneering species and bare area observed in the control site of the Chiltipin Creek marsh.

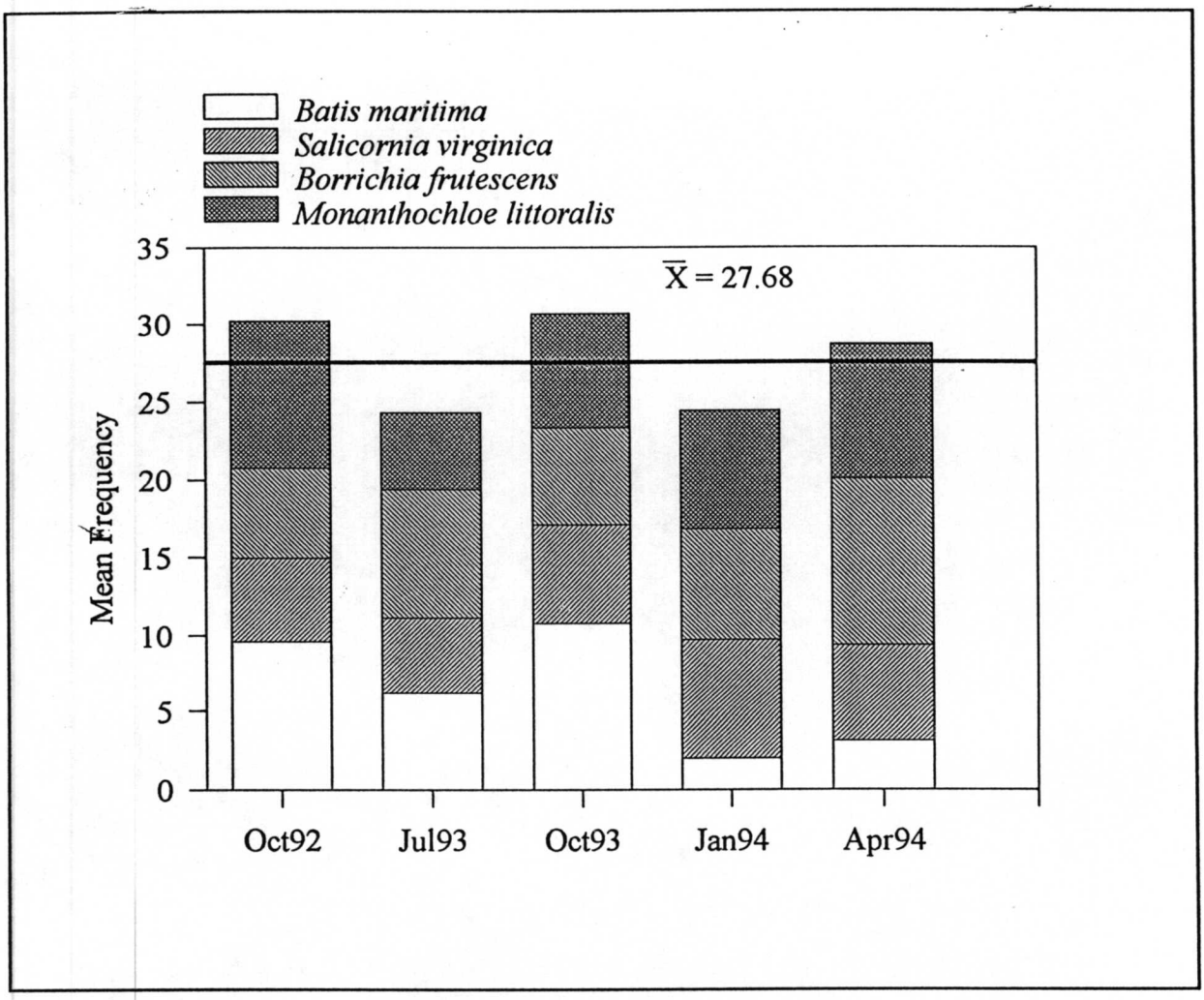

Figure 5. Combined mean frequencies of climax vegetation species observed in the control site of the Chiltipin Creek marsh.

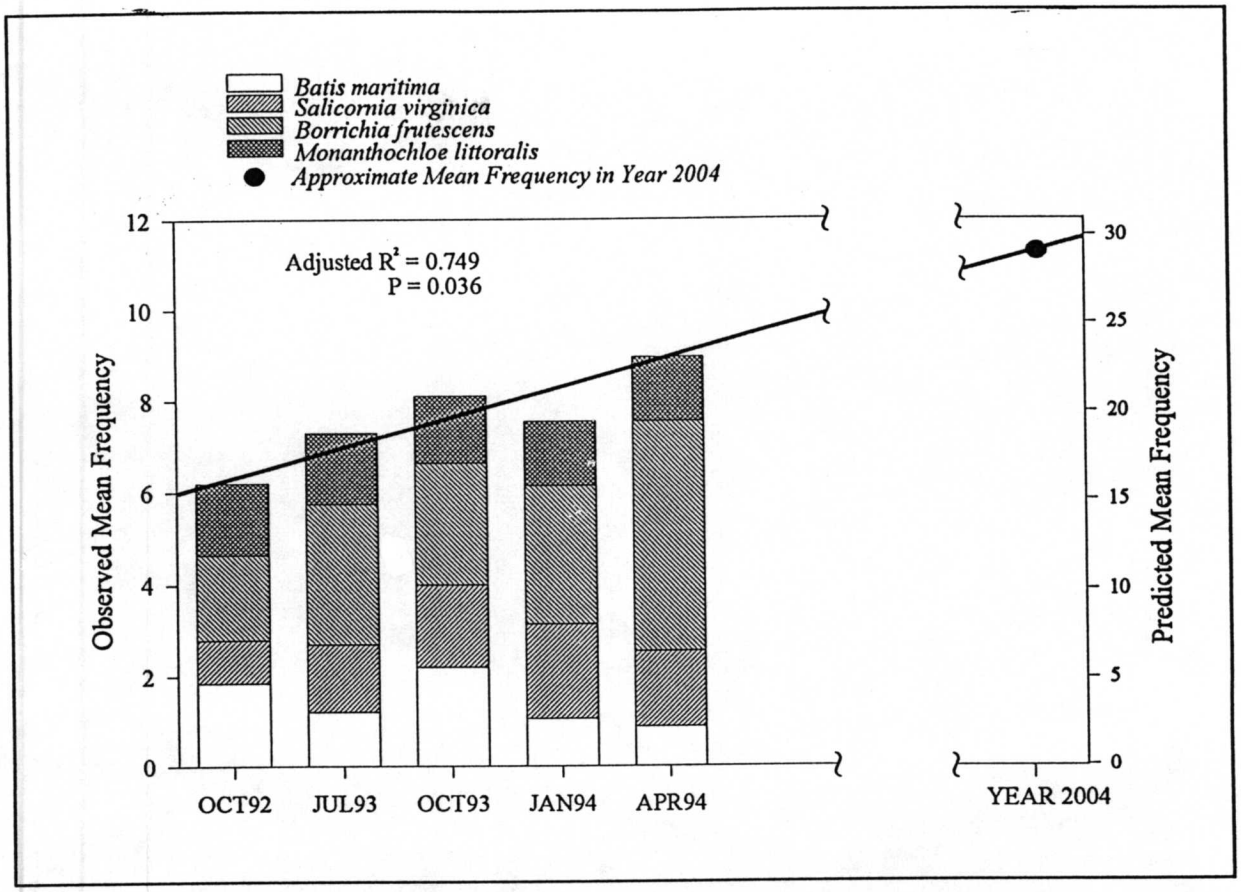

Figure 6. Combined mean frequencies of climax vegetation species observed in the impacted area of the Chiltipin Creek marsh, along with prediction of estimated time for levels to increase to those observed in the control site.

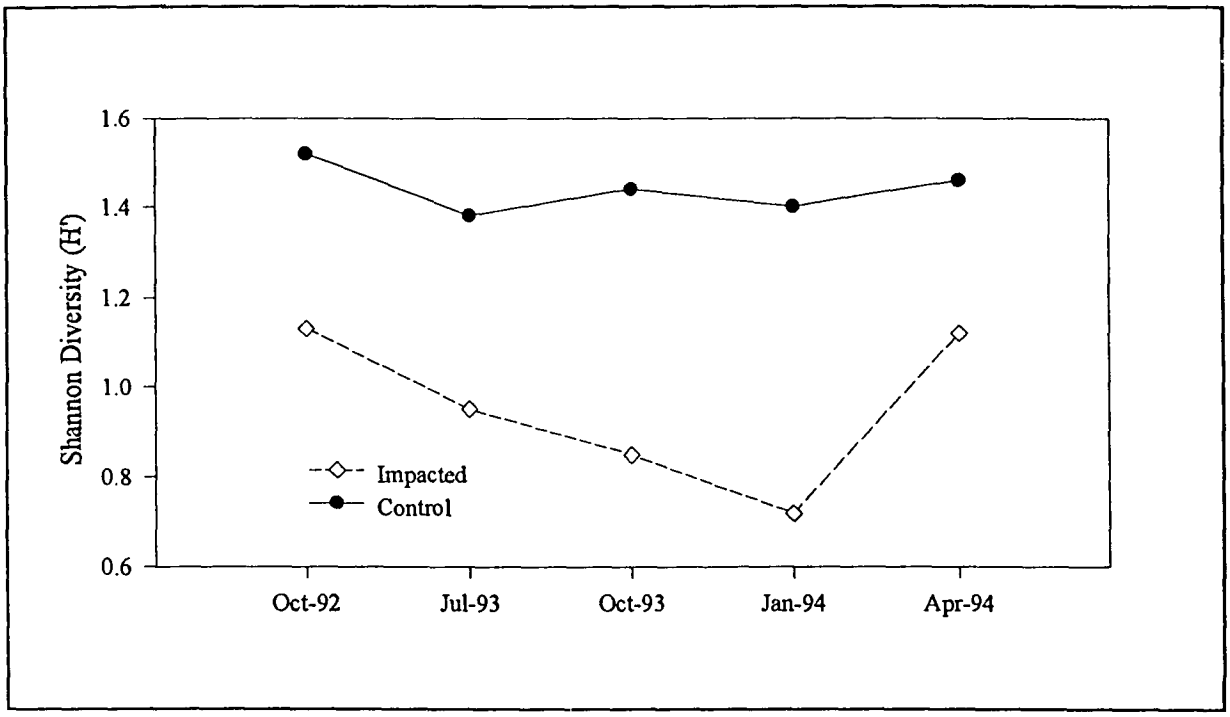

Figure 7. Shannon Diversity (H') values for the impacted and control areas of the Chiltipin Creek marsh.

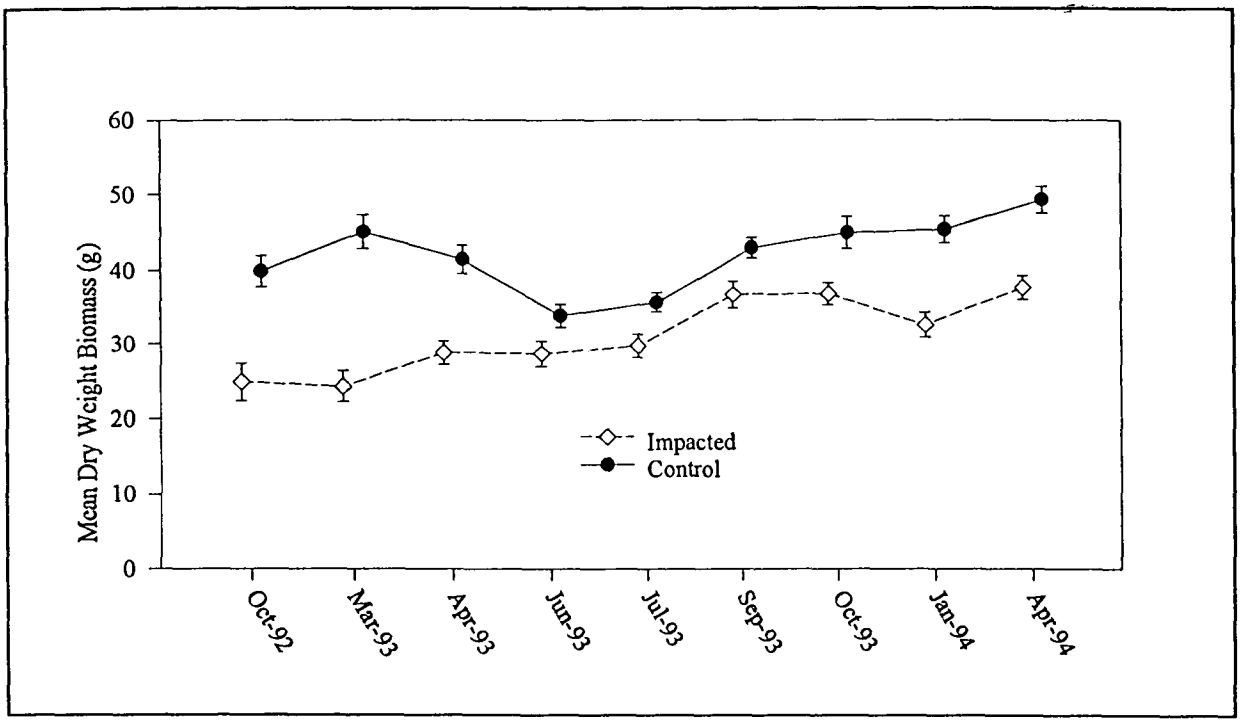

Figure 8. Mean plant dry weight biomass for the impacted and control areas of the Chiltipin Creek marsh.

Fates of Oil in Salt Marsh Sediments and Stabilization of Oil Residue

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The natural composition of crude oils is very complex and, to some extent, varies between petroleum reservoirs. In general, crude oil is a complex mixture of hydrocarbons (molecules made up of only carbon and hydrogen) and nonhydrocarbons (molecules made up of carbon, hydrogen, and other elements such as sulfur, nitrogen, and oxygen) produced from the partial decomposition of biogenic material. The slow breakdown process deep within the earth, known as diagenesis, produces a range of molecular structures significantly altered from the structures found in the original biomass. Understanding petroleum chemistry is important in understanding the fate of spilled oil in the marine environment. The ultimate fate of most spilled oil is microbial degradation in which the indigenous decomposing community attack the oil in much the same manner as any other detridal carbon source. Oil chemistry and oil degradation are the focus of this paper.

Composition of Crude Oils

Each petroleum reserve is a unique combination of biomass breakdown products. Hence, each crude oil has a unique compositional complexity with variations occurring within the individual petroleum reservoir (Speight 1991). Libraries of these compositional variations, known as molecular fingerprint patterns, have been developed using analytical methods for selective petroleum compound analysis. A gas chromatograph/mass spectrometer (GC/MS) is one such analytical system commonly used for analysis of complex petroleum hydrocarbon mixtures. A GC/MS system is capable of isolating individual compounds within the complex crude mixture. Separated compounds are then identified by electron impact mass spectra and ion fragment pattern recognition. Analytical data are stored in a computer and displayed in various formats that allow researchers to identify and quantify specific molecular components of crude oils. These data displays are frequently in the form of gas chromatograms of components with the same molecular weight (that is, mass chromatograms). Detailed analyses of specific components and their ion patterns provides insight into the quantitative differences in the makeup of individual components in these oils. As a general rule, all crude oils are complex mixtures composed of the same compounds however the quantities of the individual components differ in crude oils from different locations. This rule of thumb implies that the quantities of some compounds can be zero (extremely limited or reduced) in a given mixture of components that comprise a crude oil from a specific location, yet abundant in oil from a different location.

The nomenclature for petroleum-based hydrocarbons focuses primarily on the carbon bond and several of its structural formations. The following classification scheme breaks petroleum components into five major groups. The subcategories are based on the majority of the compounds in that subcategory, but many more classifications could exist.

Petroleum Composition Classification

Saturates	normal paraffins (straight-chain hydrocarbons), isoparaffins (branched-chain hydrocarbons), cycloparaffins or naphthenes (cyclic saturated and partially saturated hydrocarbons)
Aromatics	aromatic hydrocarbons (AH), polynuclear aromatic hydrocarbons (PAH) and their C_1 to C_4 alkyl homologs
Polar	sulfur-containing aromatic compounds, nitrogen-containing aromatic compounds, oxygen-containing aromatic compounds
Porphyrins	Complex large cyclic carbon structures derived from chlorophyll and characterized by the ability to contain a central metal atom. Trace metals are commonly found within these compounds.
Asphaltenes	Composition is dependent upon source. Asphaltenes and resins have the highest individual molecular weight of all crude oil components and are basically colloidal aggregates.

The saturated carbon bond, or carbon bound to four other atoms, in hydrocarbon molecules can be arranged as either straight-chained, branched, or cyclic hydrocarbon structures. The straight-chain compounds are found as a homologous series (same molecular structure separated by only CH_2 groups) in most crude oils, with carbon numbers ranging from 5 up to 30 and often more. Saturated hydrocarbon compounds are generally the major components of crude oil. Branched-chain compounds, or isoparaffins, are primarily methylated straight-chain hydrocarbons. From nine carbons and higher, many isoparaffins are isoprenoids originating from a side chain of the chlorophyll molecule with methyl group attached to every third carbon in the chain. Branched and isoprenoids hydrocarbons are generally slower to degrade by microorganisms than the normal paraffins. Two common paraffins found in most petroleum are heptadecane, a straight 17-carbon chain molecular structure, and pristane, a branched 19-carbon chain isoprenoid structure. Both components have similar boiling points and gas chromatographic retention times. Their microbial degradation rates in the environment are very different. Heptadecane rapidly degrades in the environment, while pristane remains and is often used as a biomarker for weathering and degradation studies.

Another abundant structure in most fresh crude oils is cyclic hydrocarbons. The two basic cyclic structures are cyclohexane and cyclopentane, with five and six carbon rings, respectively. Through combinations of these two ring structures assembled with up to five rings, many complex molecules are formed during petroleum diagenesis. Two of the larger structures commonly used in characterizing and fingerprinting oil are the triterpanes and the steranes, with a subclassification of hopanes. Hopane is not degraded by microorganisms to any appreciable extent and is often used as a degradation biomarker (Prince *et al.* 1994).

Carbon atoms that are bound to three or fewer other atoms in a hydrocarbon molecule are referred to as unsaturated and their physical structure may be a straight, branched, or aromatic form. As with the saturate petroleum hydrocarbons, aromatic ring structures in crude oils commonly range from one to five ring combinations. Two or more five- or six-member carbon rings are fused together to form polycyclic aromatic compounds (PACs). Petroleum aromatic hydrocarbons have abundant alkyl group substitution on their ring structures. The alkyl groups generally have from one to four saturated carbon atoms and thus can produce many different structural isomers and homologs for each aromatic hydrocarbon family. The most abundant aromatic hydrocarbon families generally have two and three fused rings with one to four carbon atom alkyl group substituents. Oil toxicity is primarily associated with exposure to these aromatic structures, many of which are known mammalian carcinogens. The most acutely toxic compounds are the single aromatic ring variations, including benzene, toluene, and the xylenes. All are volatile compounds with relatively high water solubility for insoluble compounds. The dibenzene ring structures, or naphthalene and its homologous series, are less acutely toxic than benzene but are more prevalent for a longer period of time during oil spills (Overton *et al.* 1994).

The polar compounds in oil are classified as nonhydrocarbons. For these compounds, the primary functional group is associated with nitrogen, sulfur, or oxygen atoms. The classification applies to sulfur compounds such as cyclic sulfides and thiophenes, basic and neutral nitrogen compounds, and oxygen compounds, primarily naphthenic acids. The porphyrins, asphaltene, and resin compounds in petroleum are considered the residual oil, or residuum. During weathering processes, this fraction is the last to degrade, and its persistence in the environment over years has been noted (Leahy and Colwell 1991).

Weathering of Crude Oils

"Weathering" encompasses the physical and chemical changes that crude oils undergo as a result of interaction with the environment. The fate of oil in an environment, as determined by weathering, is governed by the cumulative physical and chemical properties of the individual constituents in the bulk oil (Payne and McNabb 1984). The factors affecting the fate and transport of oil spilled or released in a water environment include: evaporation, dissolution, emulsification, absorption and photochemical and microbial degradation.

Figure 1 outlines the affects of weathering on spilled crude oils. The ultimate fate of petroleum spilled in the marine environment is degradation, primarily biological and, to a lesser extent, photolytic degradation. The rate at which these processes occur is controlled by many factors, such as the physical properties and chemical composition of the oil, the exposure to physical processes, abiotic environmental factors, and the existence or absence of hydrocarbon-degrading microorganisms (bacteria, mold, yeast, and fungus) and essential nutrients. Most chemical breakdown processes affecting whole oil occur at the oil/water interface. Chemical actions in aquatic systems include biodegradation and photolytic degradation, which are surface-active processes. Therefore, the amount of surface area affects the rate of degradation. Certain factors hinder degradation; one factor is emulsification, which slows biodegradation because the stable "mousse" formed has a smaller surface area compared to very thin oil sheens. Another factor, cooler weather, often

results in slower rates of microbial activity, which results in less consumption or degradation of the oil (Malins 1987). Microbes utilize the oil as a carbon source which is converted to energy, biomass, carbon dioxide, and water (Fig. 1). Physical processes, such as high energy storms, increase the dispersion of oil, thus increasing surface areas and generally reducing the persistence of oil spilled in the environment.

Weathered crude oil can be distinguished from fresh or unweathered crude oil by the loss of the low-molecular-weight constituents such as the normal hydrocarbons less than n-C_{12}, the alkylbenzenes and, to some extent, naphthalene and its alkyl homologs. These initial changes are primarily due to evaporative losses and, to a lesser degree, dissolution (less than 5% of benzene is generally lost to dissolution, while more than 95% is lost to evaporation). The low-molecular-weight constituents are more volatile and more water soluble than the constituents with high molecular weight. The rate and extent at which the components evaporate and undergo dissolution, sedimentation, and degradation are factors which affect persistence and environmental toxicity of crude oils.

Figures 2 and 3 compare the composition, from capillary GC/MS analysis, of a fresh sample and a sample of seven day evaporatively weathered crude oil, south Louisiana crude (SLC). Oil was placed in a beaker of water (100 ml oil and 900 ml water). Samples were taken for analysis a day 0 and 7. The oil residue was dissolved in hexane and analyzed by GC/MS. As seen from examining these data, the fresh oil contains normal hydrocarbons with carbon numbers from 9 (retention time = 6 min.) to 30 (RT = 51). Eluting between the normal hydrocarbons are the branched and cyclic hydrocarbons. Pristane and n-C17 are the doublet that elutes near 28 min. while phytane and n-C18 elutes near 31 min. Evaporative weathering has changed the composition in a very predictable fashion causing loss of the volatile components below n-C12. The relative composition of the crude oil above n-C15 remains unchanged. Examination of the data in Figure 3 shows similar results for the composition of the aromatic hydrocarbons, from naphthalene (MW =128) and its alkyl homologs bibenzo (a,h) anthracene (MW=278). (Note that the data on the one ring aromatic hydrocarbons in crude oils is not shown in this figure.) Evaporation for seven days causes loss of the volatile naphthalene and C1-naphthalenes while the less volatile aromatics are retained in the weathered residue essentially unchanged in composition.

Figures 4, 5, 6, and 7 show SLC GC/MS data analysis after 7 day laboratory evaporative weathering and after 28 day microbial weathering. The microbial weathering involves placing 0.5 g of oil in a shaker flask with 100 ml sea water and agitating the mixture for 28 days. The samples were analyzed by GCMS in a similar fashion to the samples shown previously. Examining the data in Figure 4, it is evident that microbial degradation causes loss by molecular composition rather than by component volatility. For example, virtually all of the normal hydrocarbons, out to n-C30, have been microbially degraded and, consequently, lost, while the less degradable isoprenoid hydrocarbon, with carbon numbers from 14 (RT=20 min) to 20 (RT=31 min.) have not been degraded and remain in the oil residue. Note the ratio of pristane (RT=29 min.) to phytane (RT=31 min.) remains virtually unchanged when compared to this ratio in fresh oil. Clearly, microbial degradation is selective in the process of hydrocarbon metabolism.

A similar conclusion can be drawn from examining the aromatic hydrocarbon data in Figure 5. As a general rule, the parent aromatic hydrocarbon structure and its C1 alkyl

homolog isomers are more readily degraded than the C2 and C3 homologs. For example, the heavily degraded residue stilled contained C3 (MW=170) and C4 naphthalenes (MW=184) but none of the larger phenthrene (MW=178), dibenzothiophene MW=184, and their respective C1 homologs (MW=192 and 196). Even some pyrene (MW=202) degraded by microbial attack. Clearly, microbial degradation is selective and dependent on molecular structure. Figure 6 shows the compositional profile of compounds that are not subjected to microbial degradation, the hopanes and triterpanes. These compounds, commonly called biomarkers, are saturate hydrocarbons with fused multiple rings. Data clearly shows the complete lack of degradation for these compounds in a sample that was heavily weathered by microbial processes. This lack of microbial degradation of these cyclic structures is the justification for the use of the so called "biomarker normalization" data treatment method when studying the progress of microbial weathering of oils in the environment (Prince *et al.* 1994).

It is important to remember the factors that affect microbial degradation and their impact on the fate of oil in the environment. These factors include the existence of microbial colonies adapted to the degradation of petroleum hydrocarbons, nutrients, oxygen, and a mechanism for exposing the microbes to the oil. Exposing the microbes to oil implies significant surface contact between the oil and microbe bearing aerated waters. In salt marshes, significant colonies of microbes exist that have adapted to degrade the hydrocarbons associated with the vegetative matter in the marsh. These hydrocarbons are predominately the long chained saturated compounds. Consequently, the colonies in salt marshes readily degrades the normal hydrocarbons over other substituents in oils.

Figure 7 shows a comparison of the saturate hydrocarbons in the *Exxon Valdez* oil to the oil extracted from an intertidal salt marsh near surface sediment sample collected five years after the spill. The saturates are heavily degraded with only the isoprenoid hydrocarbons remaining in the sample extract. Figure 8, on the other hand, shows data on the aromatic hydrocarbon composition from the same two oil extracts. Microbial attack has removed the parent and C1 aromatic hydrocarbons but significant quantities of the higher homologs remain. Even the relatively volatile naphthalenes and fluorenes are still found in the near surface sediment five years after the spill. This is very surprising considering the fact that near surface sediments are generally considered to be aerated, not anoxic.

We believe colonies adapted to degrade the naturally occurring hydrocarbons in marsh grasses readily degrade the saturate hydrocarbon from the spilled oil but are not suitable for degradation of the aromatics. With plenty of biomass containing saturated hydrocarbons available from biogenic sources, microbial colonies never adapted to significantly degrade the aromatic components of the spilled oil. This same pattern of preferential degradation of the saturate verses aromatic hydrocarbons is common for oil spilled into environments rich in naturally occurring biogenic saturated hydrocarbons.

Figures 9, 10, 11, and 12 show data from samples collected 3 and 5 years respectively after the *Exxon Valdez* spill. However, these samples were collected from a rocky shoreline which contained little or sporadic levels of background biogenic hydrocarbons. The near surface sample (Figures 9 and 10), collected 3 years after the spill, contained an oily residue that was heavily degraded in terms of both its saturate and aromatic hydrocarbon content. High energy beaches, which provide abundant mixing energy for oil and aerated water

contact, are environments where all fractions of the exposed oil are readily degraded. This degradation pattern is in sharp contrast to the selective degradation pattern seen in the salt marsh samples as shown in Figures 7 and 8.

Figures 11 and 12 show an oil extracted from the same general area as the sample used in Figures 9 and 10; however, this sample was collected 60-65 cm subsurface five years after the spill. The sample was only moderately weathered in terms of both its saturate and aromatic fractions. Altered ratios of the normal to isoprenoid content indicates some microbial degradation but evaporative/dissolution processes clearly have contributed significantly to the weathering of this sample. We believe this example represents a case where the oil was trapped in an anoxic zone are removed from vigorous aerobic microbial degradation. Clearly, the location contained colonies capable of degrading oil as demonstrated in the surface sample. However, an essential ingredient of microbial attack, oxygen, may have been lacking. Subsurface oil removed from the active microbial degradation communities will remain essentially undegraded for many years after a spill.

In conclusion, careful study is needed to determine the fates of oil spilled into various environments. In addition to the normal weathering processes, the composition of microbial communities often are determined by substrate or detritus available at the scene of the spill and is an important factor in determining the speed and extent of natural degradation for spilled oils. Mixing energy also has a large influence on the speed and selectivity of environmental degradations. The combination of these variables in various environments lead to the persistence or rapid elimination of hydrocarbons.

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IMPLICATIONS OF WEATHERING

Weathering Process

FRESH	---------->	**RESIDUE**
Non viscous liquid	---------->	Solid
Smelly	---------->	No odor
Toxic	---------->	Non toxic
Flammable	---------->	Non flammable
Degradable	---------->	Non degradable
Recoverable	---------->	Non recoverable

Figure 1. Weathering process diagram.

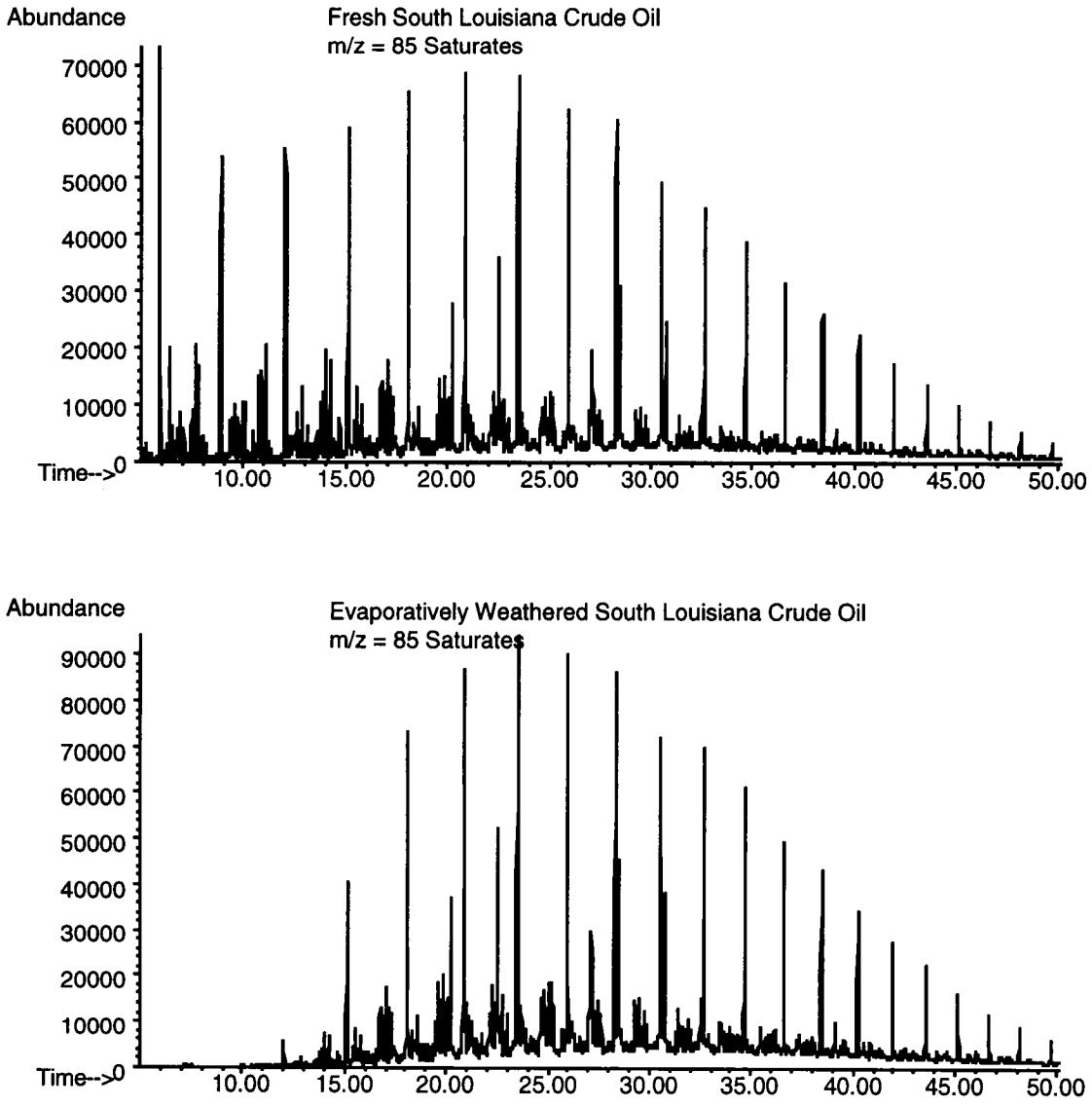

Figure 2. Chromatographic comparison of m/e 85 for unweathered South Louisiana crude oil (top) and the oil which has undergone initial weathereing (bottom).

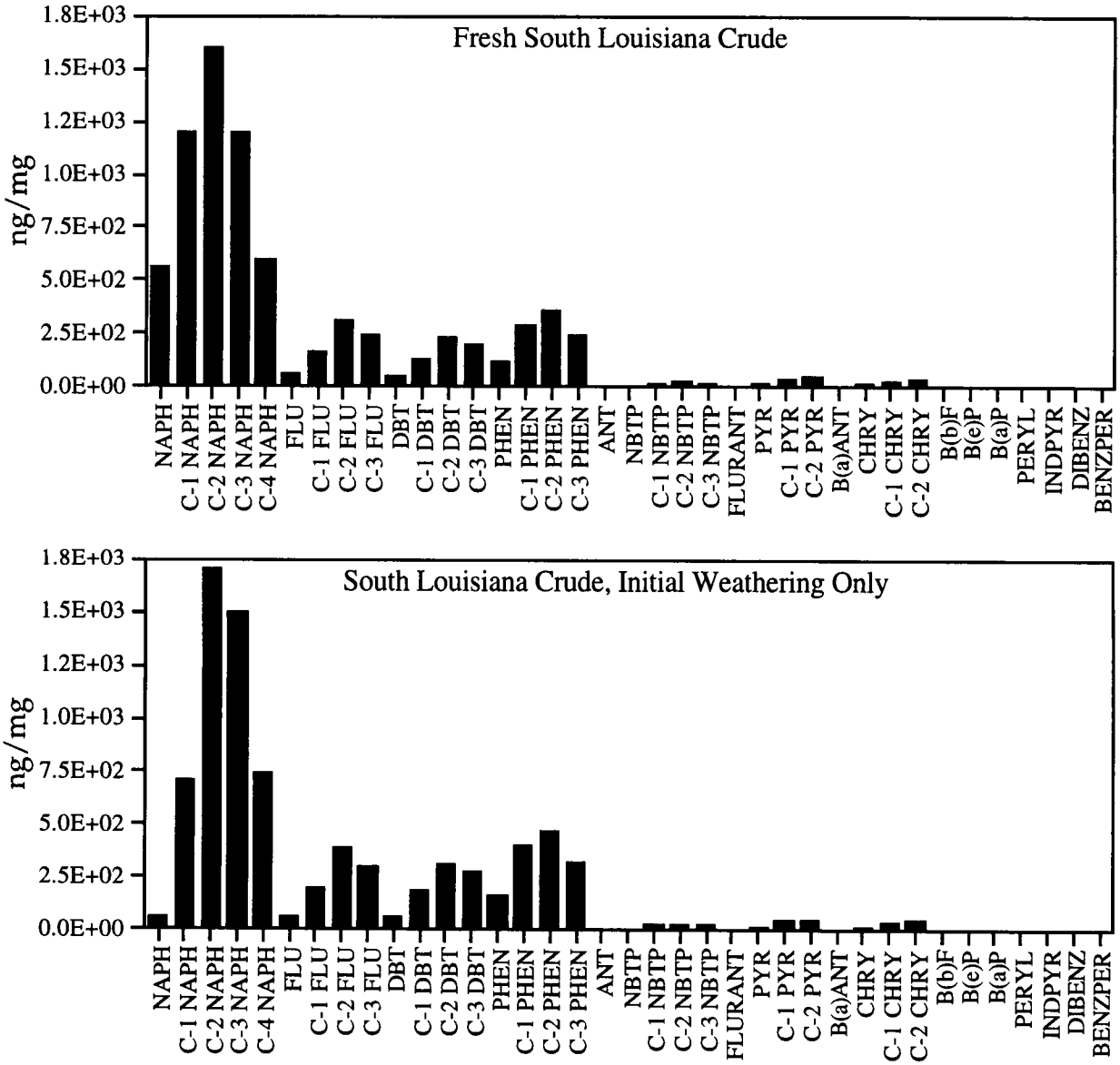

Figure 3. Aromatic hydrocarbon profile of unweathered South Louisiana Crude oil (top) and the same oil after being initially weathered on water (bottom). The oil has undergone an enrichment of semivolatile aromatic hydrocarbons caused by the evaporation of the volatile "gasoline" fraction and a dilution of the aromatic hydrocarbons shown due to the formation of a water-in-oil emulsification; the two effects nearly cancel each other relative to the net concentration of the constituents shown.

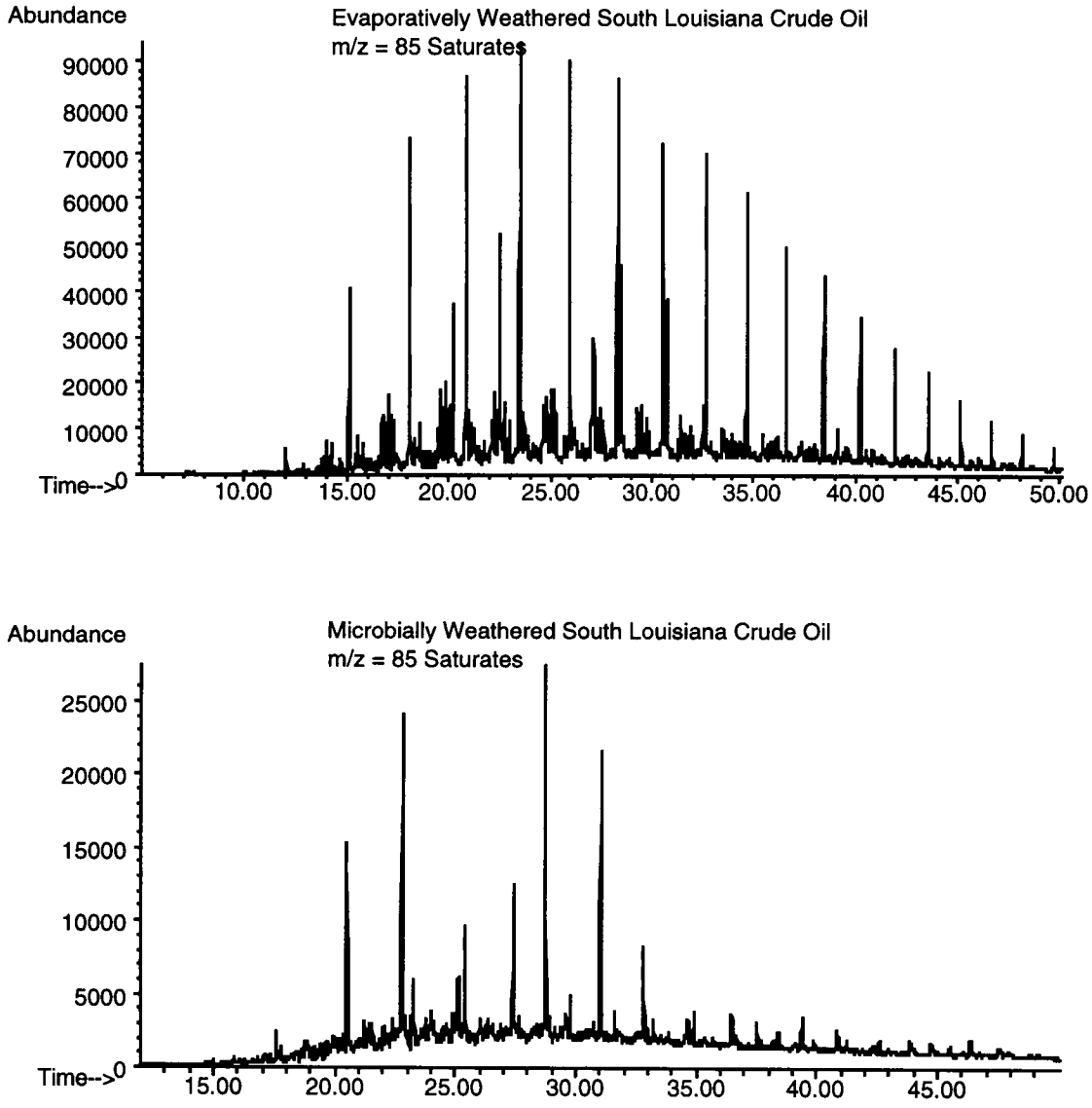

Figure 4. Chromatographic comparison of m/e 85 for slightly weathered South Louisiana crude oil (top) and the same oil after extensive microbial degradation (bottom).

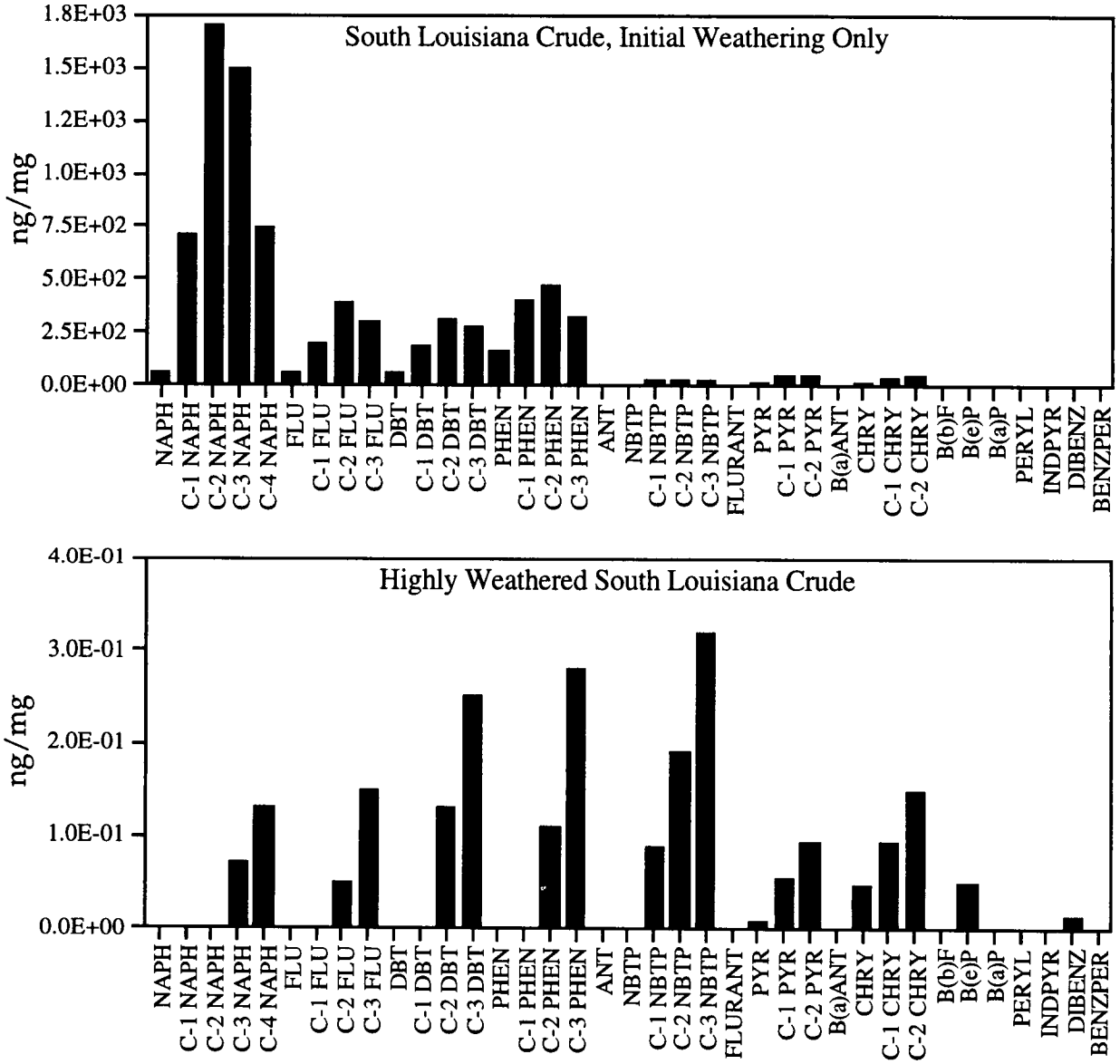

Figure 5. Aromatic hydrocarbon profile of a slightly weathered South Louisiana Crude oil (top) and the same oil after being heavily biodegraded (bottom).

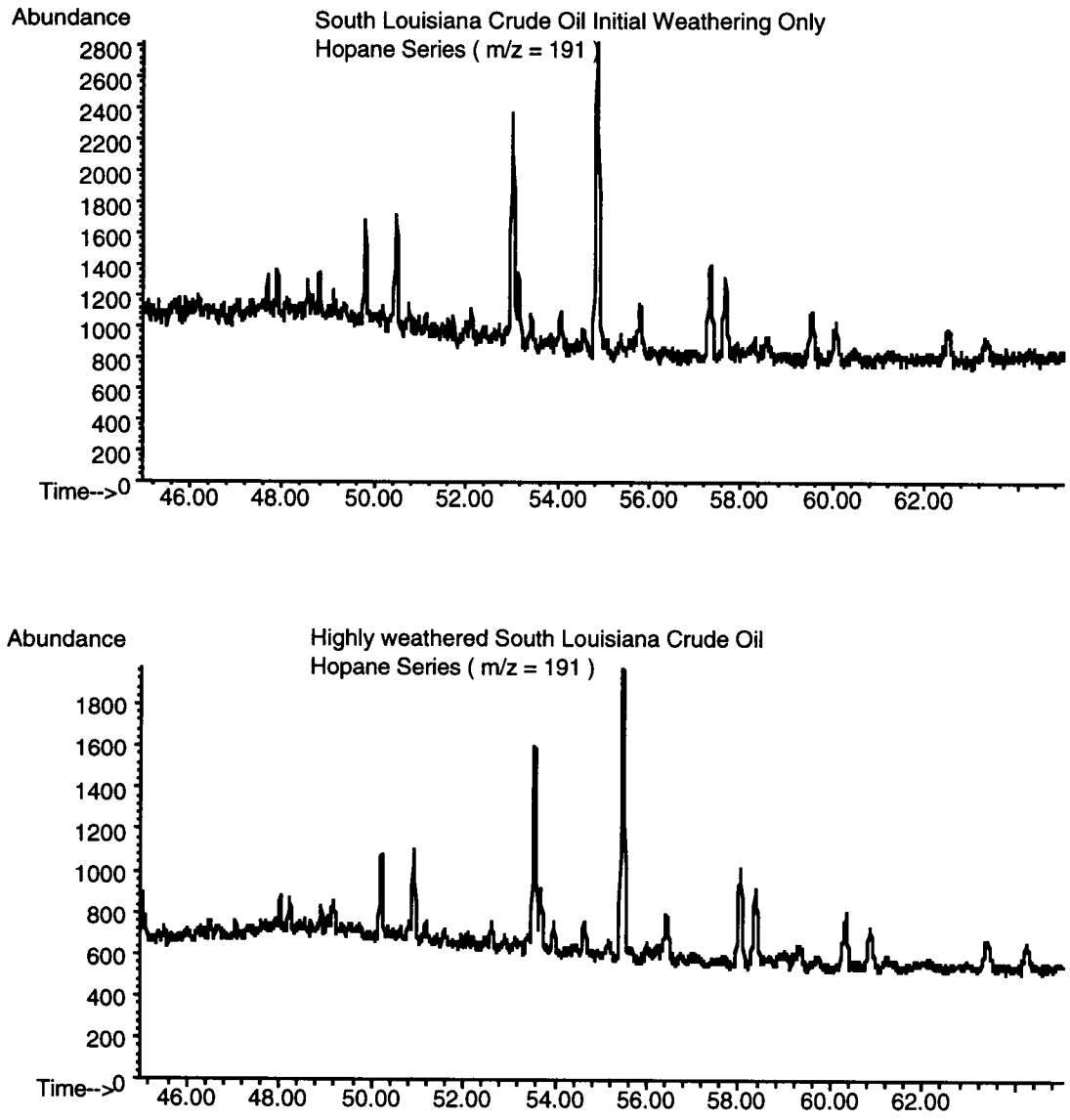

Figure 6. Chromatographic comparison of hopane series for slightly weathered South Louisiana crude oil (top) and the same oil after extensive microbial degradation (bottom).

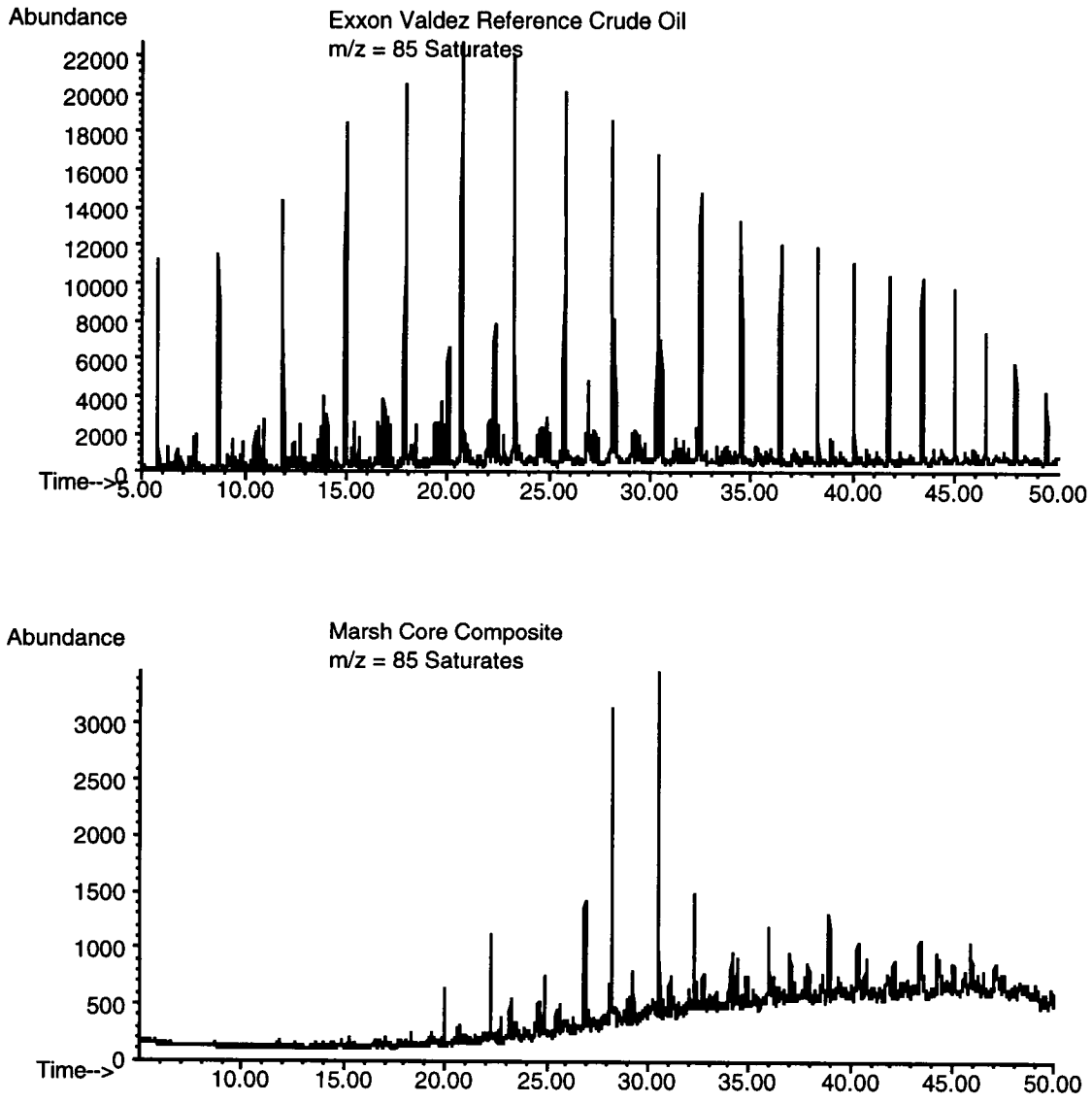

Figure 7. Chromatographic comparison of the alkane profile for the *Exxon Valdez* reference oil (top) and oil extracted from surface marsh sediments 5 years after being impacted by the *Exxon Valdez* spill (bottom).

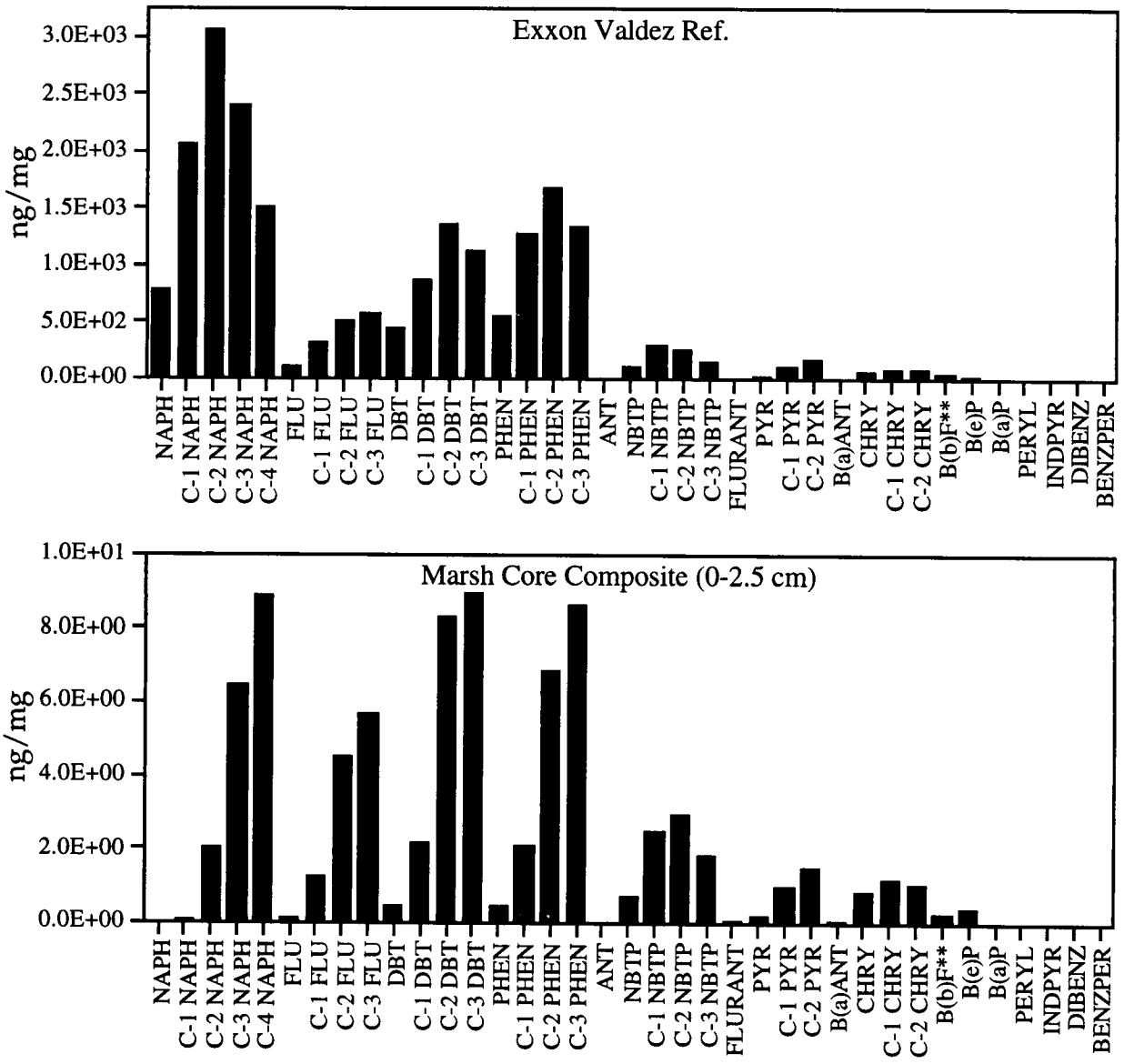

Figure 8. Aromatic hydrocarbon profile *Exxon Valdez* Reference Oil (top) and slight to moderately weathered oil extracted from an intertidal marsh sample collected 5 years after the spill (bottom). The marsh sample is a composite of the upper 2.5 cm from 5 different cores.

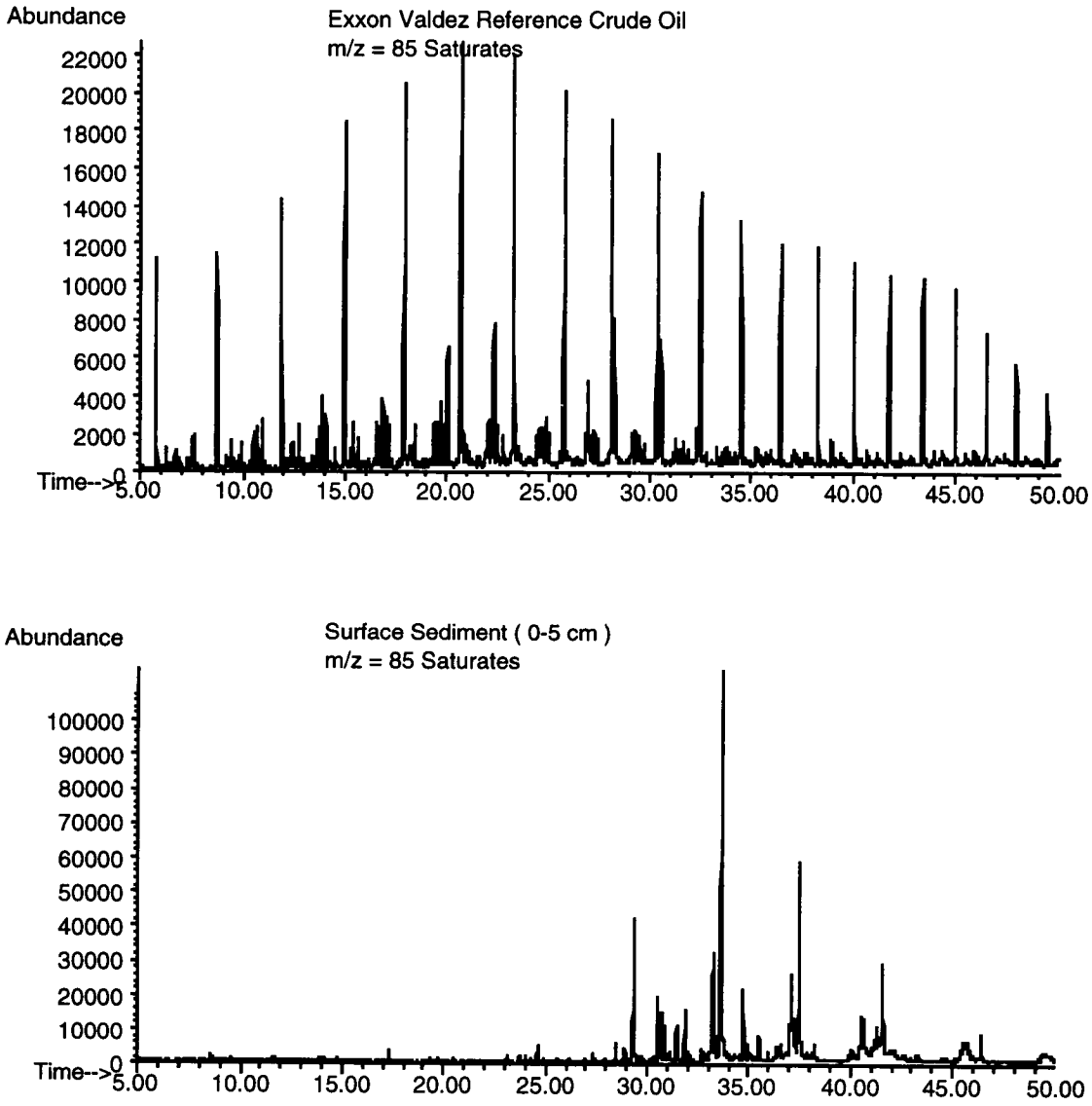

Figure 9. Chromatographic comparison of the alkane profile for the *Exxon Valdez* reference oil (top) and oil extracted from surface beach sediments 3 years after being impacted by the *Exxon Valdez* spill (bottom).

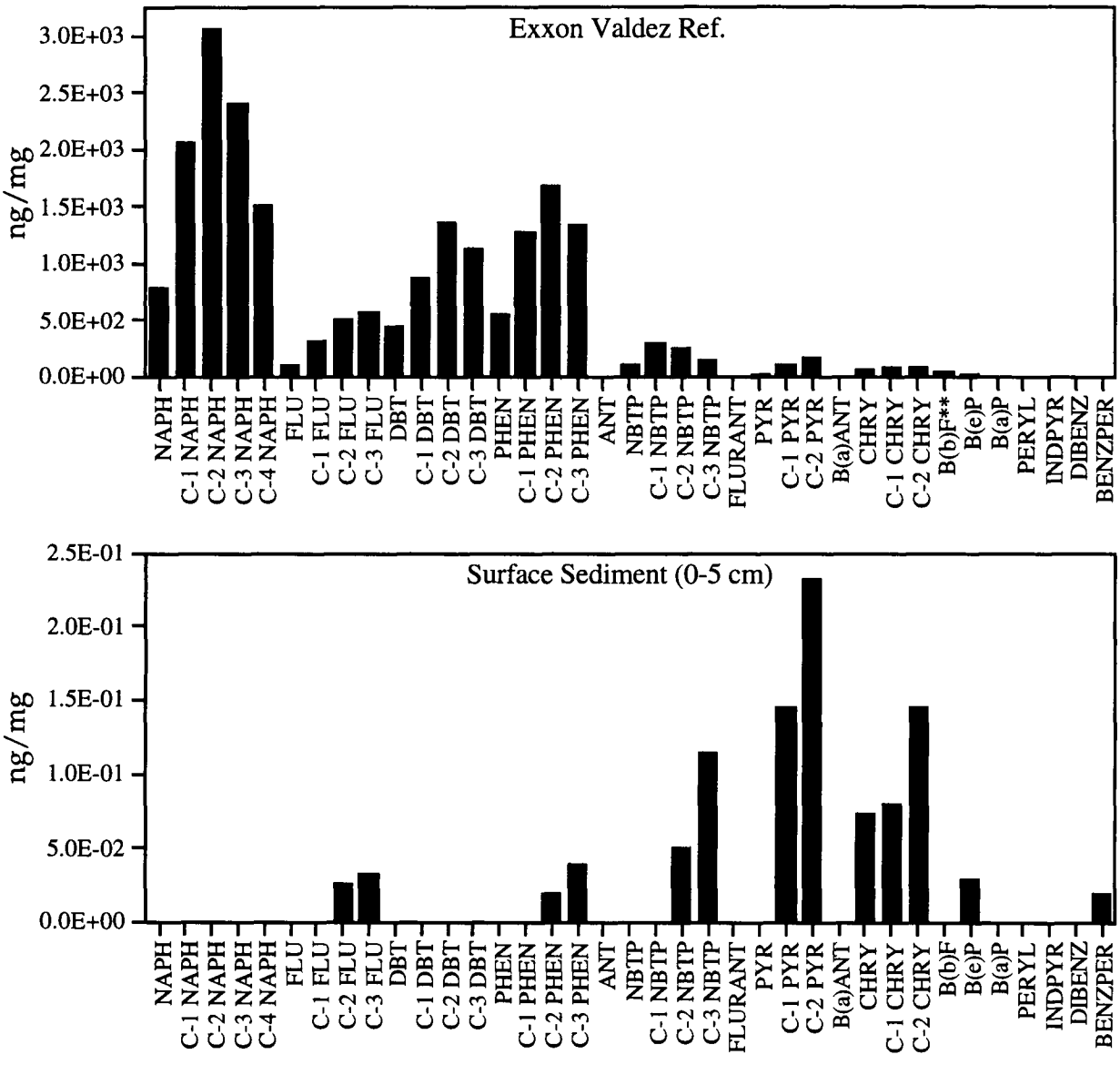

Figure 10. Aromatic hydrocarbon profile *Exxon Valdez* Reference Oil (top) and heavily weathered oil extracted from intertidal surface sediment, or gravel, collected 3 years after the spill (bottom).

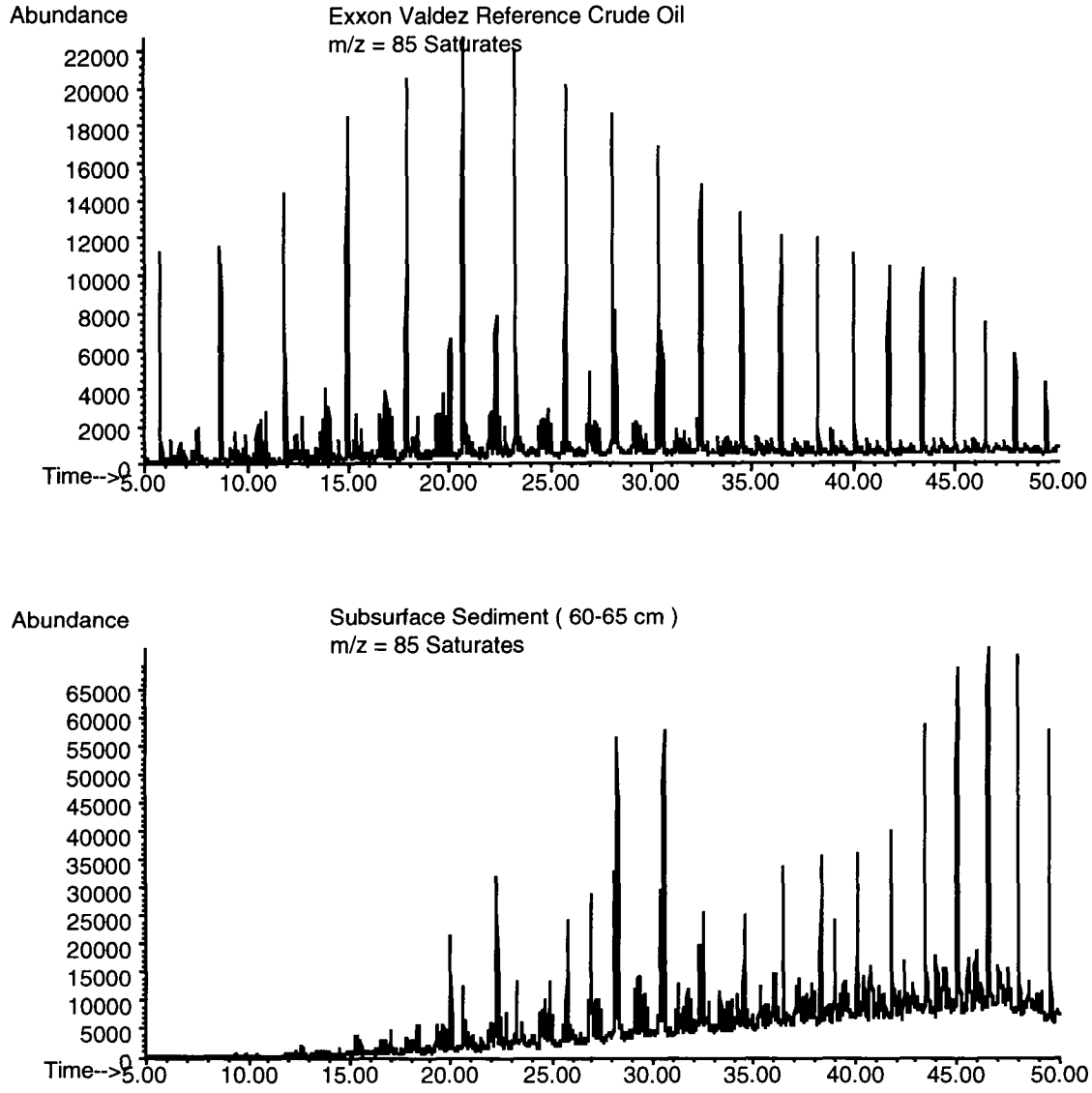

Figure 11. Chromatographic comparison of m/e 85 for the Exxon Valdez reference oil (top) and the oil extracted from a subsurface sediment sample collect at a depth of 60-65 cm (bottom).

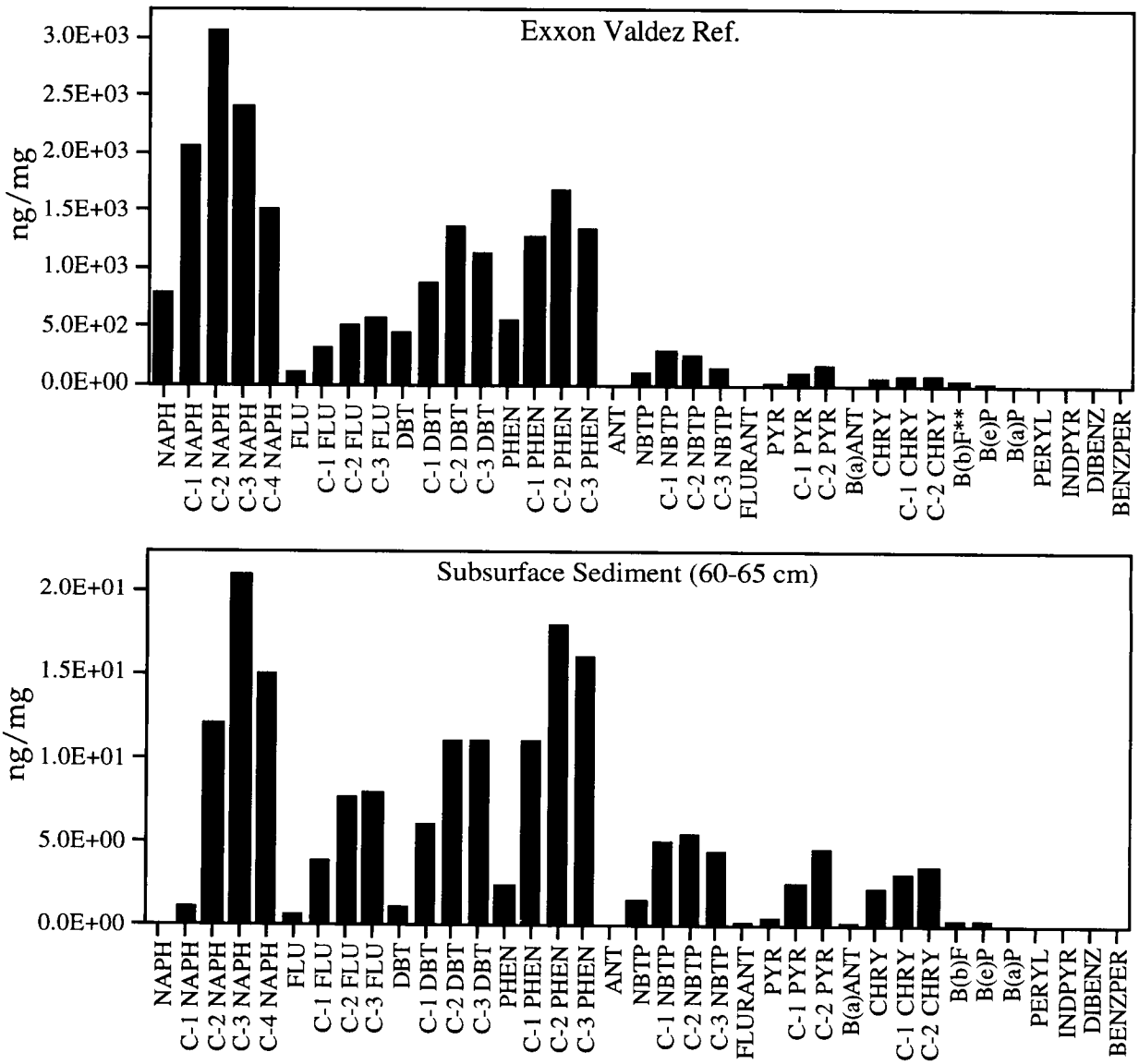

Figure 12. Aromatic hydrocarbon profile *Exxon Valdez* Reference Oil (top) and slight to moderately weathered oil extracted from intertidal subsurface gravel sediments collected 5 years after the spill (bottom).

Session IV. Group Discussions

Chair: **Dr. C. Edward Proffitt**

Discussion Sections	Leaders
Oil Spills and Mangroves	Ms. Donna J. Devlin Department of Biology University of Southwestern Louisiana Dr. Sally Levings Coastal Zone Analysis, Inc.
Oil Spills and Marshes	Dr. Irving A. Mendelsshon Wetland Biogeochemistry Institute Louisiana State University Dr. James Webb Marine Science Department Texas A&M at Galveston
Oil Spills and Seagrasses	Dr. Michael Marshall Mote Marine Laboratory
Oil and Dispersants	Dr. Carol B. Daniels U.S. Environmental Protection Agency

The Following Are Outline Summaries of Notes from the Discussion Sessions.

I. Mangrove Discussion Group: Group Leaders - Ms. Donna J. Devlin and Dr. Sally Levings

The mangrove workshop determined that several research priorities exist.

1. Not enough basic biology of the mangroves is known to allow a proper understanding of oil effects and remediation. Basic research needs include:

    A. Physiological ecology

        1. root zone aeration
        2. subsurface roots
        3. adventitious buds
        4. other areas

    B. Tree growth

    C. Genetics

    D. Anatomy of plant uptake mechanisms. This is of special concern when dispersants are used since the mangroves inhabit impact areas.

2. The oil death studies done thus far have been superficial ones for two reasons:

    A. Measuring effects based on leaf deaths are not accurate because in some cases the leaves die from the tip back and in other situations, there is a general wilting of the green leaves before they turn brown and die.

    B. Mode of death is dependent on the type of exposure or oil type.

3. Thus, a test is needed across the oil types that plots the causes of death. The following exposure cases should be considered:

    A. Oil floating on the tide so that the mangroves and the sediment are slightly coated and some die.

    B. Oil settles in the sediment and coats the plants and all die.

4. There is a window of time in any clean-up situation after which cleanup efforts are ineffective or even harmful. How long is that time window and how is it influenced by other parameters such as fisheries and wildlife? Baseline data need to be collected before a clean-up.

5. How effective are chemical oxidants in degrading oil in sediment or standing oil in a forest? Since oxygen diffuses slowly, oxidants may not be effective in large areas.

6. How are resident organisms such as crabs and snails impacted by oil in the mangroves?

7. How beneficial are fertilizers in breaking down oil in mangrove systems?

8. What are the sublethal versus the lethal effects of oil and dispersants?

9. How does oil affect stressed versus healthy plants?

10. Fundamental experiments need to be conducted to provide a baseline before extrapolation to field areas can be accurate.

11. In making on site decisions, certain pieces of information would be helpful:

    A. When should the situation be left alone?

    B. When is our interference a help?

    C. When is our interference a hindrance?

12. As a result of the mangrove discussions, some recommendations were made:

    A. The study of root physiology in all mangroves needs to be a research priority.

    B. The mode of injury of dead mangroves needs to be determined.

    C. The basic biology and genetics of mangroves needs to be studied.

    D. The effects on the community need to be studied and recorded so that in a spill event the effects can be predicted based on the type of oil and mode of oiling. The window of time must also be established.

    E. The effects of artificial additives, i.e. fertilizers and oxidants, needs to be studied.

II. Marsh Discussion Group: Group Leaders - Dr. Irving A. Mendelsshon and Dr. James W. Webb

The workshop on marshes resulted in the following recommendations being made:

1. Experimental spills should be conducted for scientific research.

2. Oil spill remediation methodologies in wetlands need to be investigated thoroughly; included in these studies should be:

   A. The efficiency and effects of physical removal

      1. both natural and man-made sorbents

      2. vacuum removal

      3. flushing

   B. Burning

      1. the effects of the residue

      2. factors controlling success

   C. Bioremediation

      1. microbes vs nutrients

      2. the effects of varying
         a. oil types
         b. marsh and sediment types
         c. tidal energy

      3. spills of opportunity should be used to test these techniques.

3. Mechanisms that enable the response agencies to incorporate scientific evaluations of the effects of the chosen response should be in place *a prior*.

   State and Federal agencies should be responsible for directing the funds needed for these evaluations.

4. Research to understand the processes (biological, physical, chemical etc.) that are altered by oil should be conducted.

5. Strong statistical approaches need to be developed so that all levels of oil pollution (i.e the spill, field research,and lab research) can be evaluated.

6. Sensitive environments need to be identified and the use of GIS needs to be expanded.

III. Seagrasses Discussion Group: Group Leader - Dr. Michael Marshall

1. The seagrass discussions resulted in the identification of several areas of research that were lacking. Some of the questions that the group would like to see scientifically answered are:

   A. What are the effects on the seagrass at the ecosystem level when it is directly impacted by oil?

   B. What are the impacts of physical disturbances?

   C. How do location and other physical characteristics influence the impacted seagrass?

   D. What happens in the long run (> 3 years) if the oil is not removed?

   E. What are the differences in spill situations? How does exposure affect the seagrass i.e. oil trapped in nearby mangroves may affect the seagrass differently than an oilslick that floats over and by the seagrass.

2. The recommendations that were made are as follows:

   A. Due to the lack of controlled studies of impacts and long term studies at the habitat level more microcosm studies are called for.

   B. Dispersant toxicity to seagrasses and other fauna need to be studied.

   C. It should be a priority in response situations to keep the oil away from shallow habitats by:
      1. burning to prevent movement to shallow water
      2. booming to prevent contact

   D. A clean up manual needs to be developed that accounts for specific oil types, seagrass species and the physical characteristics of the habitat.

   E. Protection priorities need to be defined.

IV. Toxicity of Dispersants Discussion Group: Group Leader - Dr. Carol B. Daniels

1. Problems with current knowledge:

   A. The research is always done in a laboratory with constant concentrations over a 96-hour period. Are these 96 hour toxicity tests going overboard? In a real life situation, the oil concentrations would not remain constant for 96 hours.

   B. Several questions remain unanswered.
      1. Where to use which dispersant?
      2. Are the water soluble components of the dispersants staying behind when the oil is gone?
      3. How does the depth of the water affect the toxicity of the dispersants?

2. What actions are needed:

   A. More realistic protocols that could reflect dilution in the marine environment.

   B. Tests that are capable of mimicking currents (for example, flow through versus static renewal).

   C. To determine the length of time the concentration remains high. This could be accomplished by monitoring the concentrations of dispersants where the oil is, not where it is going.

   D. When monitoring an oil spill, two issues need to be determined
      1. What was the damage done by the oil
      2. Would the application of dispersants be worse than the alternatives?

3. Areas of active research:

   A. When oil is dispersed, do the water soluble particles increase or is the oil being broken down into microscopic particles so that suspended oil concentrations increase? This would indicate whether the toxicity is persistent or not.

   B. The life history regional species. At what stage are the organisms found in the top of the water column?

   C. The chemical properties of dispersed oil
      1. When the oil is chemically dispersed can the suspended oil reach the bottom of a 30 foot water column?
      2. What is the buoyancy of this suspended oil?
      3. How is this buoyancy effected by
         a. adsorption to particulate matter in the water

b. water currents
c. microbial activity

D. How persistent is the toxicity of dispersants in shallow water? Which is more toxic, dispersants or the alternatives?

4. Future research needs

A. Field research for a better understanding of the ecological effects.

B. Develop probes to identify the hazards *in situ* and to monitor the recovery of survivors

C. Develop toxicity tests which incorporate more realistic exposure regimes

D. Standardization of testing methods for after the spill and standardized terminology.

E. Better studies of mode of action of the dispersant relative to the toxicity.

5. Recommendations

A. Prepare experimental design for follow-up studies prior to a spill

1. groups to conduct early studies need to be identified

2. funding needs to be set aside for R&D outside of clean-up

3. research data need to be shared
   a. spill of opportunity research should be circulated
   b. data from European countries that have been using dispersants in shallow water should be obtained

4. Test spills need to be conducted to see what actually happens in the environment so that lab tests can be developed to accurately model real life

6. There were a few final questions:

A. How do we go about getting test spills? It was stated that a test spill has not been allowed thus far because an "adequate plan" has not been submitted to the EPA. The bounds of what an "adequate plan" need to be determined. It was decided that the test spill would be a good subject for the MMS information transfer meeting.

B. What if we try to disperse oil and it is not effective? How will that effect mechanical clean ups? i.e. How effective are other clan up techniques subsequent to the use of an ineffective dispersant?

C. What about biosurfacants? Should they be promoted? Will the organisms produce the material rapidly enough to be effective?

Gulf of Mexico and Caribbean Oil Spills in Coastal Ecosystems: Assessing Effects, Natural Recovery, and Progress in Remediation Research

July 14-15, 1994
Hotel Inter-Continental, New Orleans, LA

Agenda

Thursday July 14, 1994

8:15-8:30 Welcome and Introduction
Dr. Edward Proffitt
Louisiana Environmental Research Center
McNeese State University

Session I. Experiments And Case Studies on the Effects of Oil Spills on Seagrass, Tidal Marsh, and Mangrove Ecosystems

Chair, Dr. Edward Proffitt, McNeese State University

8:30-9:00 Experimental Analysis of the Effects of Oil on Mangrove Seedlings and Saplings
Edward Proffitt[1] and Donna J. Devlin[2]
[1]McNeese State University and [2]University of Southwestern Louisiana

9:00-9:30 The 1993 Tampa Bay Spill: Preliminary Assessment of Natural Resources
Jane Urqhart-Donnelly and George Henderson
Florida Marine Research Institute

9:30-10:00 The 1993 Tampa Bay Spill: Tracking the Fate of Oil Richard P. Pierce[1], E.S. Van Vleet[2], D.L. Wetzel[2], P.M. Sherblom[1], and M. Henry[2]
[1]Mote Marine Laboratory and [2]Dept. of Marine Science, University of South Florida

10:15-10:45 Effects of Oil on Salt Marshes
James Webb
Texas A&M University at Galveston

10:45-11:15 The Panama Spill: Effects of Oil and Natural Recovery: Seagrass Communities
Michael Marshall
Mote Marine Laboratory

11:15-11:45 The Panama Spill: Effects and Natural Recovery: Mangroves
Sally Levings
Coastal Zone Analysis, Inc.

Session II. Remediation techniques: Reviews and Current Research
Chair, Dr. Pasquale F. Roscigno, Minerals Management Service

1:30-2:00 Evaluating Remediation Performance: Statistical and Analytical Needs
James Catallo
Louisiana State University

2:00-2:30 Dispersants: Current Toxicology Research
Carol Daniels
U.S. Environmental Protection Agency

2:30-3:00 Critique of Upland Soil Remediation Techniques and their Applicaiton to Marine Spill Situations
Paul Kostecki
University of Massachusetts, Amherst

3:00-3:30 Cleanup Techniques in Marshes: The Fine Line Between Help and Hindrance
Rebecca Hoff
NOAA

Session III. An Overview of Bioremediation Studies in the Gulf of Mexico
Chair, Dr. Irving A. Mendelsshon, Louisiana State University

3:45-4:15 Fate of Oil in Salt Marsh Sediments and Stabilization of Oil Residue
Edward Overton
Louisiana State University

4:15-4:45 Microbial Inoculants for Oil Bioremediation Evaluated Using Salt Marsh Mesocosms
Richard Weaver[1], B Crites[1], S. Neralla[1], A. Wright[1], and J. Webb[2]
[1]Texas A & M University (College Station) and [2]Texas A & M Univ. at Galveston

4:45-5:15 Mitigating and Oil Spill in Timbalier Bay, LA: NOAA's Damage Assessment and Restoration program in Action
Thomas Osborn, Erik Zobrist, Richard D. Hartman, B. Julius, and M. Newell
NOAA

Friday July 15, 1994

Session IV. (Continued) Overview of Bioremediation Studies in the Gulf of Mexico
Chair, Dr. C. Edward Proffitt, McNeese State University

8:30-9:00 Enhancement of Growth of the Marsh Natural Microbial Community
Ralph Portier
Louisiana State University

9:00-9:30 A Preliminary Assessment of the Toxicity of Bioremediation Agents on Salt Marsh Mesocosms: Vegetation
Irving A. Mendelsshon
Louisiana State University

9:30-9:45 Effects on the Marsh Infaunal Community
Nancy Rabalais
Louisiana Universities Marine Consortium

9:45-10:15 Use of In Situ Burning as an Oil Spill Remediation Technique
Gus Stacey, III
Marine Spill Response Corp.

10:15-10:45 Evaluation of Burning as an Oil Spill Clean-up Technique in a High Marsh Community Along the South Texas Coast
Beau Hardegree, D.W. Hicks, and J. W. Tunnell, Jr.
Center for Coastal Studies
Texas A&M University, Corpus Christi

Session V. Discussion and Planning: Recommending the Best Approaches for the Study of the Effects and Remediation of Oil Spills in Coastal Ecosystem
Chair, Dr. Edward Proffitt, McNeese State University

11:00-1:00	Discussion Groups	Facilitators
	Marshes	Mendelsshon and Webb
	Mangroves	Devlin and Levings
	Seagrass	Marshall
	Toxicology of Dispersants	Daniels

1:30-2:00 Group Summaries by Facilitators - General Discussion
2:00 Closing Remarks and Adjournment
Dr. C. Edward Proffitt
McNeese State University

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.