Effects of a Dispersed and Undispersed Crude Oil on Mangroves, Seagrasses and Corals

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FOREWORD

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EXECUTIVE SUMMARY

The primary objective of this study was to evaluate the application of dispersant to spilled oil as a means of reducing adverse environmental effects of oil spills in nearshore, tropical waters. The results of numerous laboratory and field studies have suggested that dispersants may play a useful role in reducing adverse impacts on sensitive and valued environments such as mangroves, seagrasses, and corals. However, the use of dispersants has not been allowed thus far in most situations because **of** a lack of direct experimental data on the various effects of dispersants and the environmental trade-offs presumed to occur as a result of their application to crude oils. To accomplish this objective, a 21/2-year field experiment was designed in which detailed, synoptic measurements and assessments were made of representative intertidal and nearshore subtidal habitats and organisms (man-groves, seagrass beds, and coral reefs) before, during, and after exposure to untreated crude oil and chemically dispersed oil. The results were in-tended to give guidance in minimizing the ecological impacts of oil spills through evaluation of trade-offs in the relative impacts of chemical dispersion to tropical marine intertidal and subtidal habitats.

METHODS

The experimental design was intended to simulate a severe, but realistic, worst-case scenario of two large spills of fresh crude oil in nearshore waters, one treated with chemical dispersant and the other left untreated. The experimental scenarios used in this project were developed on the basis of the collective experience of the API Task Force members and the project scientists, and the oil and dispersed oil volumes selected were uniformly acknowledged as being very strong tests of the potential impacts of each. This was particularly true for the dispersed oil scenario since almost all recommended dispersant use strategies call for treatment of oil slicks in deep water after a certain amount of natural weathering has occurred. This is al-most totally contrary to the experimental procedure used in this study, so it must be noted that the dispersed oil scenario represents an extreme case, such as might occur if a large (relative to the area of water), fresh oil slick were chemically dispersed in the shallow waters of a slowly flushed, semi-enclosed bay.

Three sites were chosen for intensive study: **one** site was treated with **953** liters (L) [about 1 liter/square meter (L/m^2) j Prudhoe Bay crude oil (Site 0); a second site (Site D) was **treated** with 715 L Prudhoe Bay crude and **a** commercial dispersant concentrate [to achieve a target concentration of 50 parts per million (ppm)]; and a third site was used as an untreated reference site (Site R). The study sites chosen were typical of nearshore, microtidal tropical marine habitats. The intertidal portion of each site consisted of well-developed red mangrove (Rhizophora mangle) forests. The sub-tidal portion consisted of turtle grass (Thalassia testudinum) beds and coral reefs composed primarily of Porites porites and Agaricia tennuifolia.

Each site was studied twice (8 months and 1 week) prior to treatment to determine baseline, prespill values of chemical, biological, and physical parameters. Each site was then completely enclosed within an oil spill containment boom. Oil **and** oil plus dispersant were released through Six poiyeinyiene¬ tubes located at various locations within each study site. A total of 715 L [4.5 barrels (bbl)] of dispersed oil was released over a 24-hour period at Site D. Real-time measurement of petroleum hydrocarbons in the water column was used as feedback to achieve a target concentration of 50 ppm. This scenario simulated a worst-case scenario in which a large oil spill is dispersed in shallow, nearshore waters, resulting in environmentally significant concentrations of dispersed oil for an extended period. This scenario is sharply different than a more likely scenario in which dispersion occurs far away from sensitive nearshore environments, resulting in reduced or no exposure. A total of **953** L (6 bbl) of untreated crude oil was released over several hours at Site 0 and was allowed to remain within the boomed-in area for two days. This resulted in exposure of the entire site to 1 L of oil per square meter. Site R was treated exactly the **same** as Sites D and 0 except no oil was released there.

During site treatment, and for 20 months afterward, detailed chemical and biological measurements were made at each site using the same methods used in the prespill studies. These analyses are summarized below.

Chemistry Studies

Petroleum hydrocarbon concentrations were measured in intertidal and subtidal sediments using gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS). The water column was monitored during site

treatment using UV fluorometry. Water was sampled from six locations, as duplicates from the subtidal portions of the mangrove, seagrass, and coral habitats. Discrete water samples were taken at regular intervals during site treatment for subsequent GC analyses of low-molecular-weight (LMW) hydrocarbons, and spectrofluorometric analyses of high-molecular-weight hydrocarbons. Large-volume water samples were taken using XAD filters for later GC analysis. Samples of mangrove leaves, seagrasses, and oysters were analyzed to determine uptake of oil.

Biological Studies

Intertidal Systems - Mangrove **Forests**

Survival, leaf canopy coverage and condition, leaf production rates, leaf length/width ratios, prop-root growth, and lenticel production of adult Rhizophora mangle were measured. Survival and colonization rates of juvenile R. mangle also were determined. Surveys of mangrove tree snails (Littorina angulifera) were conducted to determine changes in abundance and distribution, and the survival of mangrove tree oysters (mainly Isognomon alatus) was measured.

Subtidal Systems - Coral Reefs

Detailed transects were conducted to measure the relative abundance of epifauna and epiflora living on the reef surface. This included measurements of the percent coverage of four assessment categories (total organisms, total animals, corals, and total plants). Growth rates of four coral species (Porites porites, Agaricia tennuifolia, Montastrea annularis, and Acropora cervicornis) **also** were measured.

Subtidal Systems - Seagrass Beds

The growth rate, total leaf area, and density of Thalassia testudinum were measured. The relative abundance of the dominant epifauna (the sea urchin species Echinometra lacunter and Lytechinus variegatus) was deter-mined using transect and quadrat measurements. Density and diversity of infaur ▸ al communities were determined.

RESULTS Chemistry

Studies

Prior to site treatment, each site was found to be uncontaminated by petroleum hydrocarbons. During treatment, there was no cross-contamination between any of the sites.

During site treatment, concentrations of dispersed oil in the water column at Site D ranged from 3 to over 80 ppm oil equivalents, averaging close to the 50-ppm target concentration. Because of nearshore water currents, it was not possible to maintain exactly 50 ppm of dispersed oil during the 24-hour release. Figure A shows the measured concentration of dispersed oil at two sampling locations over the seagrass bed. Note that the concentrations exceeded 80 ppm for a number of hours at these locations (the fluorometer was unable to resolve concentrations above 80 ppm). Table A presents the exposure concentrations in ppm-hours for each sampling lo-cation. Table A shows that the dispersed oil target exposure of 1,200 ppm-hours (50 ppm x 24 hours) was exceeded at the mangrove and seagrass sampling locations, and the overall average exposure for the entire site was about 1,470 ppm-hours, or about 20 percent higher than the planned expo-sure. LMW hydrocarbon concentrations were also high at Site D, ranging between 293 and 684 parts per billion (ppb).

Site 0 was exposed to thick oil slicks, and total oil in the water column ranged from 1 to 4 ppm oil equivalents. LMW hydrocarbon concentrations ranged from 33 to 46 ppb. Total exposure to subtidal habitats ranged from 65 to 165 ppm-hours.

Three days after site treatment, 8.9 and 10.2 ppb total hydrocarbons (collected by large-volume sampler) were measured in the water at Sites D and 0, respectively, and these concentrations slowly decreased through the end of the study. Concentrations of hydrocarbons in the water column were very low and comparable at both sites through the 20-month postspill sur*vey.*

After discharge, oil was found in the mangrove sediments at both treatment sites. Not all of the chemically treated oil was completely dispersed in the water. There was always some surface slick which moved into the man-grove forest. The oil coverage was not uniform and was reflected in the very high variability between samples that were analyzed for hydrocarbon content. In general, more oil was found in the sediments at Site 0 than at

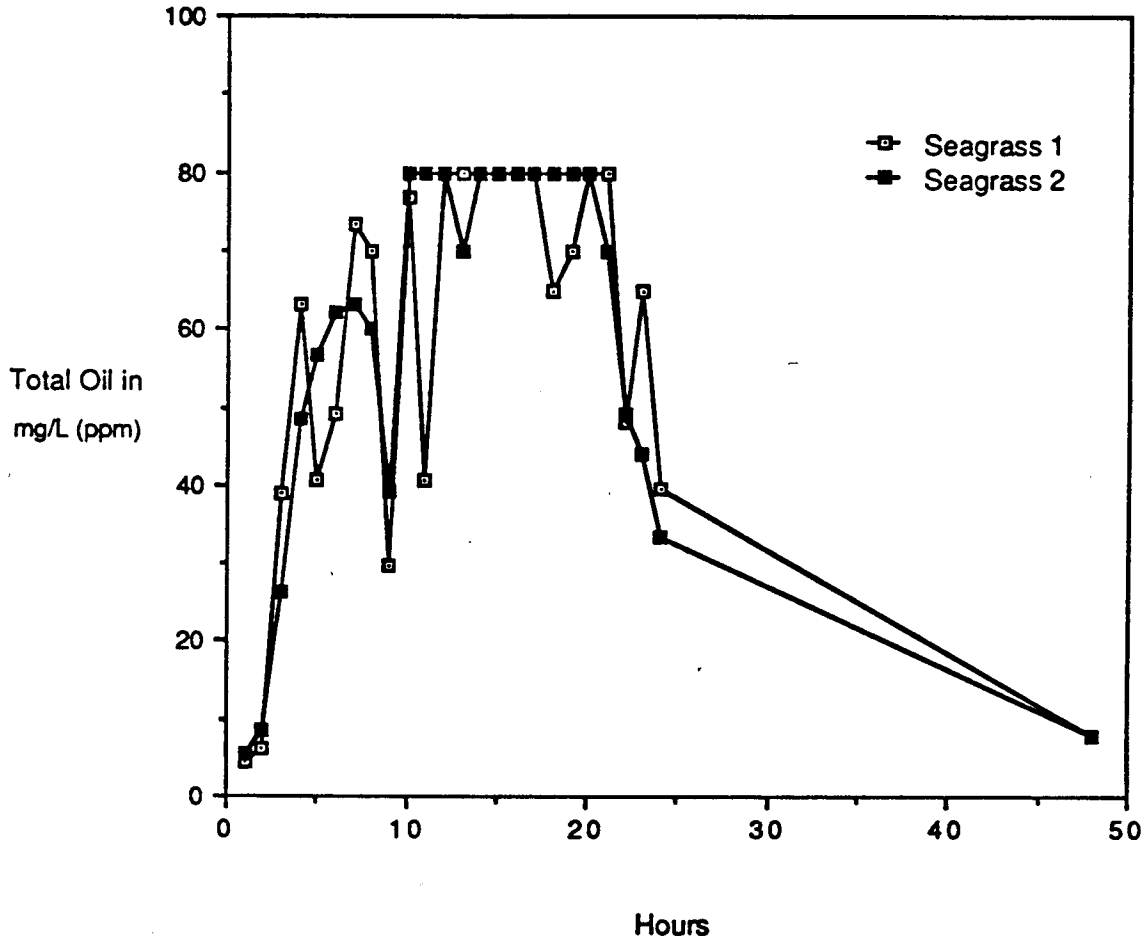

FIGURE A. Total oil in water over seagrasses at Site D during site treatment (0-24 hours) and 24 hours after termination of treatment.

TABLE A. Total hydrocarbon exposures in the water column expressed as ppm-hours (area under the curves for the dispersed and untreated oil releases).

	DISPERSED OIL SITE	UNTREATED OIL SITE
Mangrove sample location 1	1,515	150
Mangrove sample location 2	1,915	166
Seagrass sample location 1	1,930	103
Seagrass sample location 2	2,235	165
Coral sample location 1	475	65
Coral sample location 2	755	106

Site D (Fig. B). Three days postspill (December 1984), the oil content measured at Site D was 16 ppm. In June 1985, 180 ppm was measured at Site D. Thereafter, the sediment content appeared to remain constant. Postspill hydrocarbons at Site 0, initially about 95 ppm, likewise increased. Because more oil was not added to the sites, the apparent increases are due to redistribution of oil from areas that were not sampled initially. More uniform distribution in the sediments that were sampled later resulted in the apparent higher oil content. However, it is difficult to draw conclusions because of the high variance and limited number of samples.

Hydrocarbons in the seagrass sediments were very much lower than in the mangroves. At 3 postspill sample times (December 1984, March 1985, and December 1985), the concentrations ranged from 20 to 45 ppm at Site D and from 1 to 6 ppm at Site O. Because of the limited number of samples and large variance, visual observations will be included in the following discussions of biological results. Hydrocarbons measured in mangrove leaves, seagrass leaves, and mangrove oysters will be discussed when those biological habitats are reviewed.

Biological Studies

Intertidal Systems - Mangrove Studies

The release of whole oil at Site 0 resulted in heavy contamination of the entire intertidal portion of the site. Oil was deposited on exposed sediments during low tide and moved throughout the site during high tide. Westerly winds tended to push much of the oil toward the downwind (eastern) half of the site. Visible contamination of sediments and mangrove prop roots was evident throughout the immediately postspill monitoring period, and the water surface was covered with sheen. During later site visits, less oil was visible, but sheen and small black globules of oil were released from disturbed sediments during the remainder of the study (20 months postspill).

The effects of the untreated crude oil on adult and juvenile mangroves at Site 0 were severe. Mortality and defoliation of adult and juvenile man-groves were very evident four months after site treatment (Fig. C). Defoliation was especially pronounced in the eastern half of the site (that portion receiving the greatest quantity of oil). In this area, 18 trees were dead, and 34 trees were 50-100 percent defoliated. The number of dead trees increased to 25 by June 1985 (7 months postspill), after which no

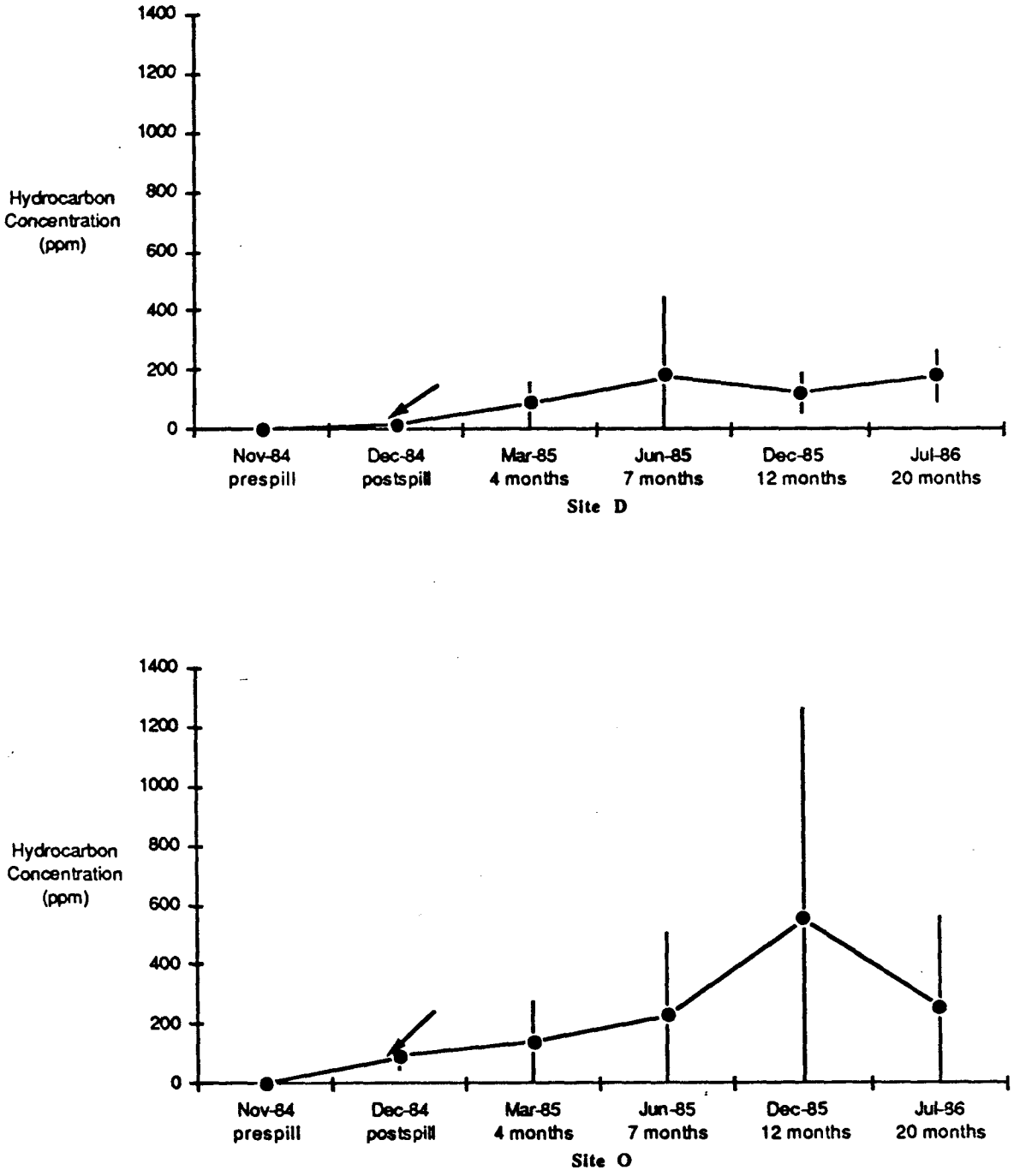

FIGURE B. Hydrocarbon concentrations in mangrove sediments at Site D (top) and Site O (bottom). Arrow indicates date of site treatment.

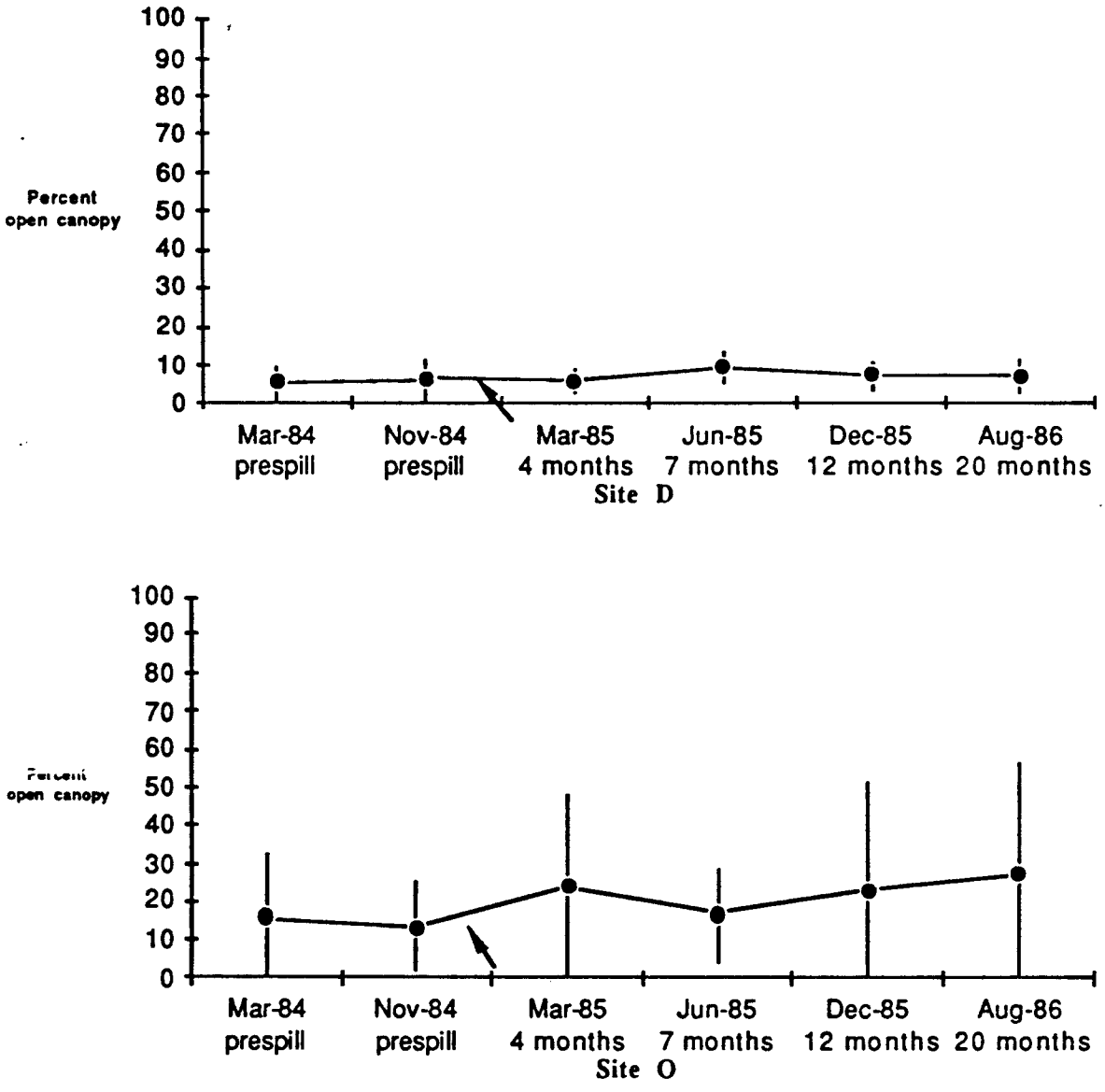

FIGURE C. Percent open canopy of the mangrove forest at Site D (top) and Site O (bottom). Arrow indicates date of spill.

additional mortality was detected. Partial defoliation of living trees was evident throughout the site and averaged about 45 percent. Trees located in the outer fringe next to the water showed no observable effects, possibly because they were rooted in sediments that were always underwater, thereby reducing exposure to oil.

The defoliated area with dead adult trees increased space and sunlight. A - number of propagules (seeds) entered but did not sprout successfully until after June 1985. The number of live juveniles increased from 18 in June 1985 to 175 in December 1985. This demonstrated the start of colonization of this severely damaged area, but it will require from 10 to 20 years for the seedlings to become adult trees.

The relative abundance of the snails at Site 0 was reduced by about 50 percent following site treatment. Snail numbers remained lower than prespill levels until about one year later. A significant shift in the vertical distribution of tree snails was also measured at Site 0, with more snails occur-ring in the upper levels of the forest than before site treatment.

Mangrove oysters at Site 0 were exposed to heavy concentrations of floating slick oil, but no measurable increase in mortality was observed. This occurred despite uptake of high levels of hydrocarbons (to 678 ppm) during the early postspill period. Tissue levels eventually decreased to low levels after one year.

The intertidal portion of Site D was exposed to dispersed oil and small patches of whole, floating oil. There was a light coating of sheen on exposed sediments and prop roots, but after a few days, the quantity of oil had decreased, and after 4 months, it was difficult to detect that the site had been oiled. Only one small area that had been exposed to a surface slick showed evidence of contamination after 4 months.

No measurable effects on adult mangroves trees occurred at Site D. Short-term survival and growth of juvenile mangroves were reduced compared to prespill levels, and long-term growth and survival were comparable to Site R.

The abundance of tree snails at Site D also was reduced by about 50 percent after site treatment, and recovery to pretreatment levels occurred after one year. No changes in the distribution of tree snails was measured at Site D.

Mangrove oysters at Site D also had high survival rates following treatment. Tissue levels of hydrocarbons were high (506 ppm) 3 days postspill and declined over the next 12 months to very low levels.

Subtidal Systems - Coral Studies

The percent coverage of coral reef substrate by epifauna and epiflora at Site D declined abruptly following site treatment. This decline continued through the 12-month postspill survey, after which the decline in coverage appeared to have leveled off. The percent coverage by all assessment categories (total organisms, total animals, corals, and total plants) declined during the study period (Fig. D).

There was a slight, but statistically significant, decrease in coral coverage over time at Site 0. The other three assessment categories did not exhibit a decline over time at this site.

No significant changes were measured in the growth rates of two coral species (Montastrea annularis and Acropora cervicornis) at Site D or Site 0. The effects of dispersed oil on Porites porites were not great, but a slight, significant reduction in 2 of 3 growth parameters was measured during the initial postspill survey period. No effect was seen at Site 0 on P. porites. The coral species Agaricia tennuifolia showed clear evidence of reduced growth rates at Site D. All three growth parameters were significantly reduced during the duration of the study. No effects were seen on growth rates of this species at Site 0.

Subtidal Systems - Seagrass Studies

No significant effects on seagrass growth rates or blade areas were measured at either Site D or 0 following treatment. Seagrass density declined following site treatment at Site D but returned to greater than prespill levels after 7 months. Density of seagrasses at Site 0 also declined after treatment but did not return to pretreatment levels.

The abundance of sea urchins was reduced drastically at Site D such that no live urchins were present four months after site treatment. Sea urchin populations reappeared 12 months after site treatment. At Site 0, there was a slight decrease in sea urchin abundance after site treatment, followed by relatively stable numbers until the 7-month postspill survey, at wh!ch` time large increases in abundance were recorded (Fig. E).

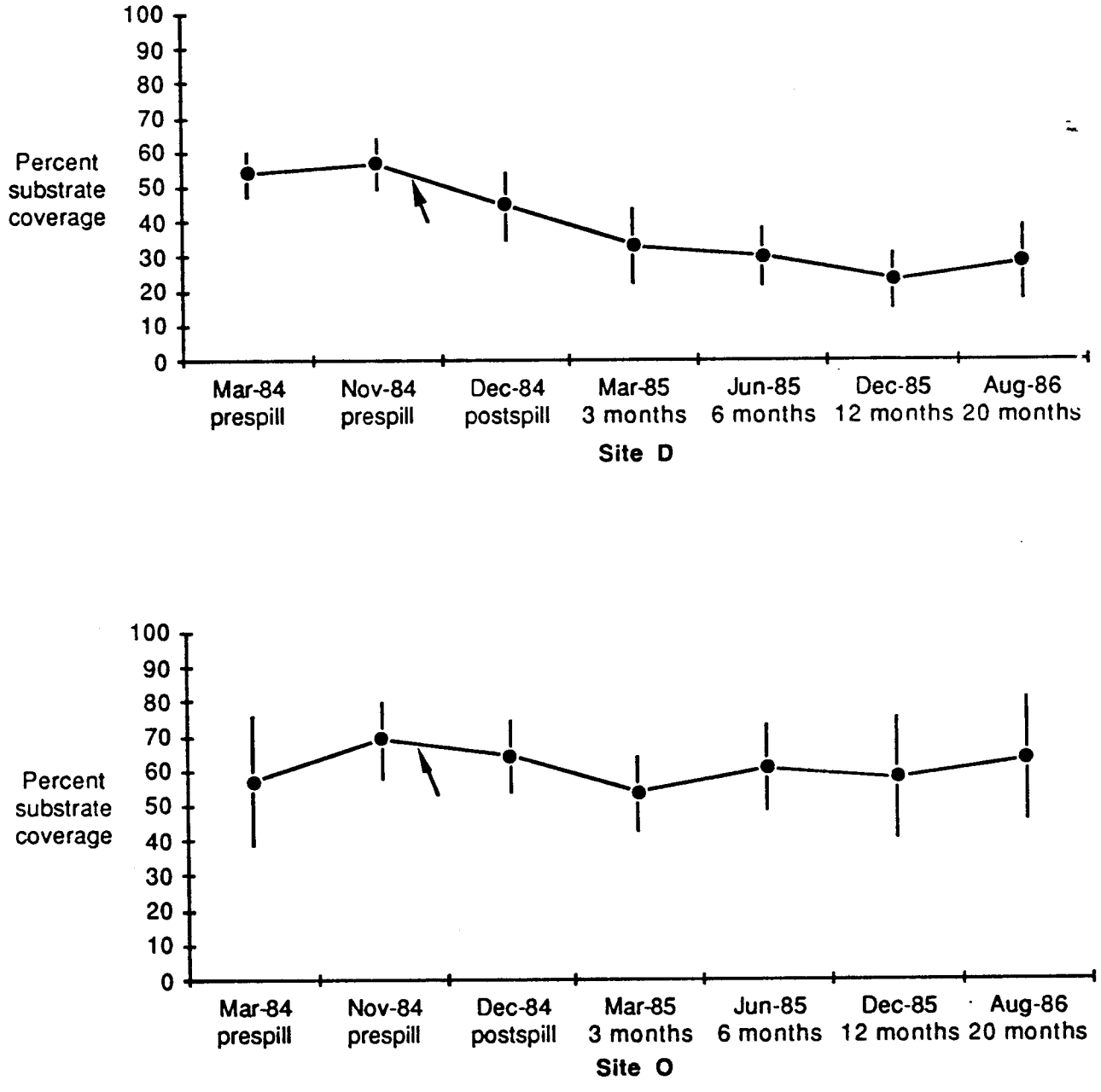

FIGURE D. Total organism coverage of reef substrate at Site D (top) and Site O (bottom). Arrow indicates date of site treatment.

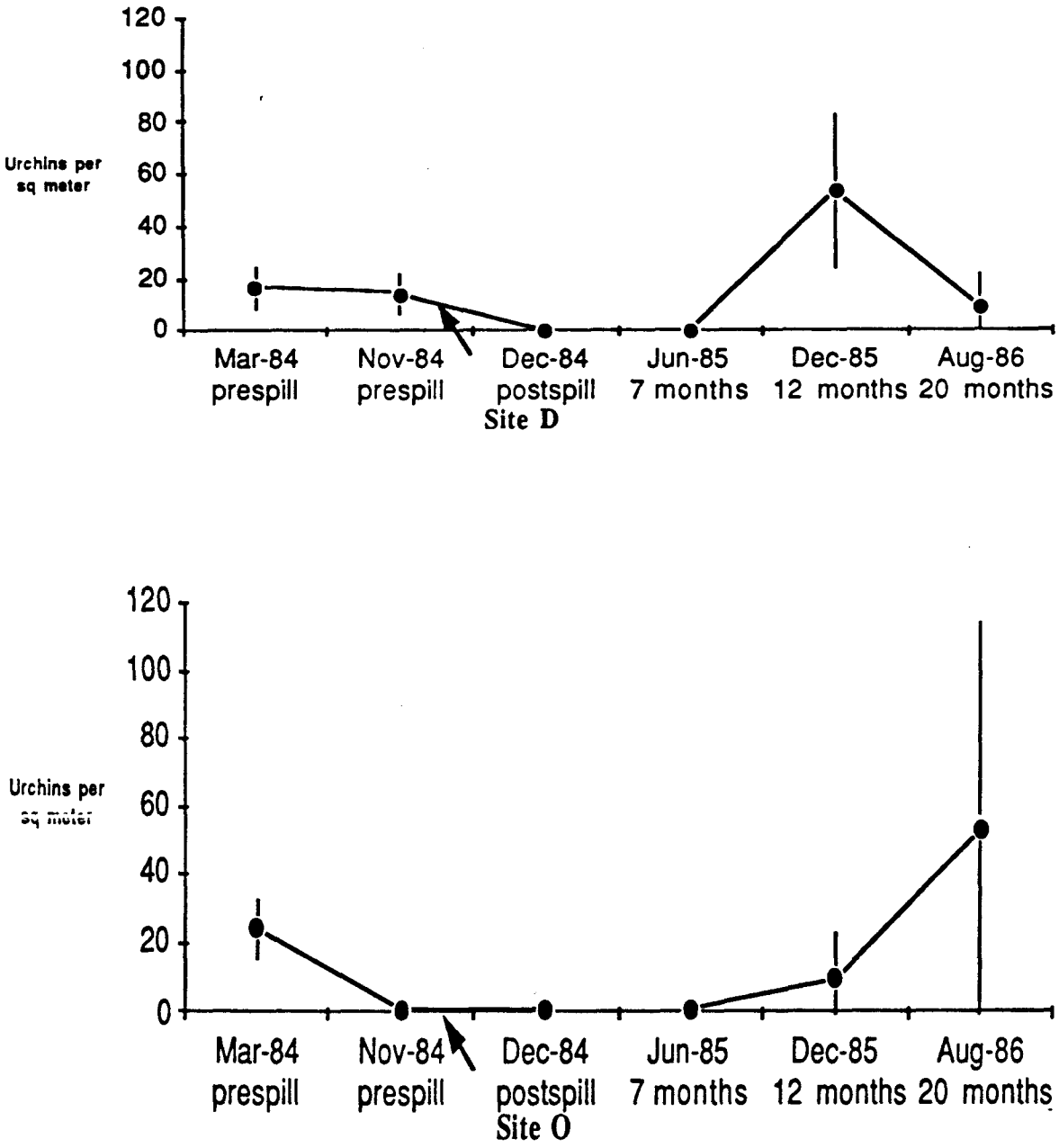

FIGURE E. Density of sea urchins (Echinometra lacunter) in the seagrass beds at Site D (top) and Site O (bottom). Arrow indicates date of site treatment.

The density and diversity of infauna were extremely variable at all sites at all sampling periods. No discernible patterns over time or between sites were seen.

CONCLUSIONS

The purpose of this study was to obtain experimental data to determine if the use of chemical dispersants will reduce or exacerbate adverse impacts of oil spills upon sensitive and valued tropical environments such as man-grove forests, seagrass beds, and coral reefs.

The question of possible trade-offs in effects between intertidal and subtidal habitats was explored to determine if there was a net benefit to be gained, such as reduction in impacts to one or both habitats, or increase in recovery rates of affected habitats. This would allow evaluation of various available response options based on different spill scenarios. These options are discussed below.

Option 1 - No Action

This option was simulated by the untreated oil scenario (Site 0). The experimental data for Site 0 clearly show that whole, untreated crude oil has severe, long-term effects on the intertidal components of the study site (mangroves and associated fauna) and relatively minor effects on subtidal environments (limited to a slight decline in coral abundance). These results and the results of numerous other studies of oil spills have shown consistently that intertidal habitats are exposed to much higher concentrations of oil than subtidal habitats when no action is taken to prevent stranding of oil or when mechanical collection or containment procedures are ineffective. In those cases where the intertidal environment is highly sensitive to oil pollution, the no-action response option has a relatively high probability of resulting in significant adverse environmental impacts, and therefore, the no-action option is not recommended in these cases. Some form of response is warranted, either chemical dispersion of the oil (within the framework out-lined below) or mechanical containment and recovery. In situations where intertidal environments have low inherent sensitivities, the no-action response option may be an acceptable approach.

Option 2 - Apply Dispersants in Shallow, Nearshore 'waters Directly Over or Adjacent to oral and Seagrass Habitats

An extreme case of this option was simulated by the dispersed oil scenario (Site D). The experimental data show that the use of dispersants under this scenario had a positive effect in reducing or preventing adverse impacts to the mangrove forest, but this was accompanied by relatively severe, long-term effects on the coral and seagrass environments. It must be noted that the implementation of this scenario at Site D resulted in an extreme, worst case of Option 2 because of the volume of oil used, the lack of weathering, and the duration of exposure.

Under more likely conditions in which the floating, untreated oil has weathered for several hours and is dispersed into the water column over a relatively short period of time, it is reasonable to assume that the magnitude of impacts to subtidal environments would be less than was measured in this study. Under less extreme conditions, one would expect the balance in environmental trade-offs to shift in favor of Option 2; for example, more physical weathering of the oil and shorter exposure periods to the dispersed oil (such as would occur in more realistic conditions) would probably result in fewer impacts to nearshore, shallow-water coral reefs and seagrass beds and, at the same time, reduce or prevent impacts to mangrove forests, even if dispersants were applied directly over coral/seagrass habitats. Therefore, the use of dispersants in shallow waters to protect highly sensitive intertidal habitats should be considered a viable option, with the realization that significant subtidal impacts may occur and that overall environmental damages may not necessarily be reduced. All efforts should be made to apply dispersants in water as deep as possible to promote dilution of dispersed oil.

Option 3 - Apply Dispersants in Deep Water, Offshore from Mangrove, Seagrass, and Coral Environments

This option was not directly tested during the study, but the experimental data presented here indicate that this option is likely to result in prevention or reduction of damages to mangroves without significant effects on seagrass or coral habitats. Any action taken to prevent or reduce stranding of oil in mangrove forests is likely to have a positive effect in reducing damages to mangroves. Chemical dispersion of oil in deep water, 2wcy from nearshore environments, is likely to allow dilution of dispersed oil

such that exposure of sensitive subtidal environments to toxic concentrations is not likely to occur. The amount of dilution required or the threshold levels of exposure concentration or duration are not readily identifiable from the experimental data presented here. However, it is reasonable to speculate that any reduction in exposure of nearshore corals and seagrass habitats to dispersed oil would tend to reduce damages to them. Therefore, it is recommended that the use of dispersants be considered whenever highly sensitive intertidal environments are threatened by spilled oil and that dispersant application is conducted in water as deep as possible.

Reduction in exposure of subtidal environments to dispersed oil is achieved through dilution into deep water or by high rates of mixing with water currents. It is possible to identify in advance areas where local physical processes would tend to promote rapid dilution of dispersed oil. Advance planning of this type would be similar to existing oil spiti Iliapp;ng methods based on sensitivity analyses and would provide spill response personnel with a practical guide in the decision-making process.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	analysis of variance
API	American Petroleum Institute
APN	Autoridad Portuaria Nacional
BIOS	Baffin Island Oil Spill
BBS	Bermuda Biological Station
cm	centimeter
DBH	diameter at breast height
CC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
km	kilometer
L	liter
LAI	leaf area index
LMW	low-molecular-weight hydrocarbons
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
nm	nanometer
P^i	3.14
ppb	parts per billion
ppm	parts per million
ppt	parts per thousand
psi	pounds per square inch
PTP	Petroterminal de Panama
RPI	Research Planning Institute, Inc.
sec	second
SNK	Student-Neuman-Keuls
TROPICS	Tropical Oil Pollution Investigations in Coastal Systems
ug	micrograms
UV	ultraviolet water-
WAF	accommodated fraction

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INTRODUCTION

This report describes a 21/2-year field experiment conducted by Research Planning Institute, Inc. (RPI) and Bermuda Biological Station, Inc. (BBS) to determine the relative effects of the undispersed and dispersed forms of a selected crude oil upon tropical intertidal and subtidal habitats dominated by mangroves, seagrasses, and corals.

In the tropics, mangrove forests, seagrass beds, and coral reefs con-tribute significantly to water quality, estuarine productivity, and coastal stabilization. These ecosystems also are considered to be among the most sensitive to the effects of oil spills. A recent publication by the American Petroleum Institute (API, 1985) indicated that there are presently no known effective mechanical means to clean them following impact. In fact, all known, mechanical cleanup procedures in these habitats have the potential to render more harm than the oil itself. It is important, therefore, that new means be examined to provide protection from oil spills which are threatening to impact these habitats.

A number of industry- and government-sponsored field experiments have been conducted in recent years to determine the effects of oil and dis[persant]s on arctic, temperate, and tropical ecosystems. A series of tests conducted by API off New Jersey and southern California in 1978 and 1979 focused on evaluation of dispersant effectiveness and measurement of chemical and natural dispersion of oil. McAuliffe et al. (1980, 1981) present a summary of these studies. The most significant findings were that chemical dispersion of oil greatly exceeded natural dispersion such that concentrations up to 40 milligrams per liter (mg/L) of crude oil were measured at 1-meter (m) depth under the best chemically-dispersed oil slicks, while naturally dispersed (untreated) oil concentrations were generally less than 1 mg/L.

In 1980, the Baffin Island Oil Spill (BIOS) project was begun to investigate arctic marine oil spill fate, effects, and countermeasures, with particular emphasis on the environmental consequences of dispersant use. Summaries of the results of this 4-year, multidisciplinary study are given in Blackall and Sergy (1981, 1983) and in Sergy (1985). Their findings indicate that untreated oil persisted for several years in intertidal sediments, which acted as a source of contamination to adjacent nearshore subtidal habitats. Chemical dispersion of oil in nearshore waters resulted in short-term exposure of subtidal habitats and organisms to high levels of dispersed oil,

which was rapidly associated with sediments and accumulated by benthic organisms. This was accompanied by severe, acute behavioral and physiological effects. However, during the two years after the oil and dispersed oil releases, no large-scale mortality or significant change in community structure was measured in benthic faunal communities. The researchers concluded that, as a result of their findings, there were no ecological reasons why dispersants should not be used on arctic nearshore oil spills, in light of the relatively minor acute effects seen and the long-term, heavy contamination caused by untreated oil.

The Tidal Area Dispersant Experiment, conducted in Searsport, Maine, starting in 1981, was similar to the BIOS project in that detailed quantitative measurements of the chemical fates and biological effects were made to compare the environmental consequences of chemically dispersed and untreated oil. Gilfillan et al. (1983, 1985) and Page et al. (1984) present detailed summaries of methods and results. In this study, untreated oil was Incorporated into surface sediments, while chemically dispersed oil was not. Two species of bivalve molluscs showed rapid uptake and depuration of untreated oil; no significant uptake was measured following exposure to dispersed oil. Uptake of untreated oil was correlated with transient alterations in enzyme activity. Untreated oil was found to reduce mollusc larval colonization rates compared to chemically dispersed oil, and the density of opportunistic oligochaete worms was found to increase after exposure to untreated oil. The more severe and long-lasting effects from the untreated oil were believed to be due to its greater persistence in intertidal sediments, as compared to the relatively minor, transient effects of the dispersed oil.

In 1983, a field experiment was conducted by RPI in Laguna de Chiriqui, Panama, to determine the relative effects of untreated and chemically dispersed oil on mangrove forests (Getter and Ballou, 1985). This project was part of an ongoing research program on the effects of oil pollution on tropical marine ecosystems, which included studies of several oil spill sites (Getter et al., 1981) and various laboratory studies (Getter et al., 1984; Ballou, 1986). The most significant finding was that chemically dispersed oil caused very much less adverse effects on mangrove forests than did untreated oil. There appeared to be potential for impacts to subtidal habitats from dispersed oil. These impacts were not quantified at that time, and this question of trade-offs in impacts formed part of the basis for the study described in the present report.

Field and laboratory studies have been conducted by *BBS* on the effects of oil and dispersants (Knap et al., 1983). These studies indicated that exposure to dispersed oil resulted in short-term, sublethal behavioral effects on corals. No changes in growth rate were detected.

The need for more realistic exposure conditions, more detailed analytical chemistry data, and long-term data on growth rates and other parameters led to the development of a multidisciplinary program to determine the sublethal effects of dispersed oil on corals. The present study was designed as a joint effort by RPI and BBS to examine in detail the environmental consequences of dispersant use on tropical marine environments.

PROJECT PLANNING

The first objective was to select an area where an oil/dispersant study would be permitted which contained mangrove, seagrass, and coral communities. A number of possible research areas were evaluated on the basis of suitability of coastal environments for the study, presence and availability of logistical support, and the support of local government regulatory agencies. Of all the areas considered, the Republic of Panama offered the best combination of these factors. It is located in the tropical region of this hemisphere and has large, undeveloped coastal areas with pristine mangrove forests, seagrass beds, and coral reefs. The climate is moderate, undergoes regular seasonal changes in rainfall and winds, and is not subject to hurricanes which could destroy a field study such as the one described here.

Petroterminal de Panama (PTP), a government- and industry-operated pipeline company with facilities in the provinces of Chiriqui and Bocas del Toro, Panama, had been instrumental in providing logistical support during previous environmental surveys of this region conducted by RPI for the start-up of PTP operations. RPI had also conducted an industry-sponsored field experiment on the effects of oil and dispersant on mangroves with the direct support of PTP (Getter and Ballou, 1985). Through this arrangement, we had access to air and water transportation including pilot boats, , launches, airplanes, and helicopters; oil spill response equipment such as a Marcos skimmer, curtain booms, sorbent pads, and dispersants; ground sup-port from PTP personnel to assist in logistical arrangements; supplies of Prudhoe Bay crude oil; housing, food, and communication facilities; some office facilities; diving equipment and compressors; and building materials such as cement, wood, nails, and tools.

The Autoridad . Portuaria Nacional (APN) is charged with the responsibility of controlling oil pollution in Panama and has the authority to allow the release of oil into the marine environment for the purposes of scientific investigation.

Within the framework of the law and their interest in reducing the environmental effects of oil pollution, APN and PTP were very helpful and cooperative throughout the course of this study. APN provided RPI the opportunity to present some of the findings of our study to interested groups in Panama City and also provided an observer during the actual spills in December 1984.

PTP provided unparalleled logistical support throughout the course of this study. Sr. Ricardo Brin, Captain Geoffrey Moss, Sr. David Jimenez, and the entire staff at PTP consistently provided the most complete support possible and took a personal interest in the successful completion of our studies.

REGIONAL CHARACTERIZATION

CLIMATOLOGY AND METEOROLOGY

General

The geographical location of Panama is between 7°N and 10°N latitude, and the entire country experiences tropical weather conditions. The region is humid, with relatively high average annual rainfall and frequency of thunderstorms.

Winds

The prevailing wind direction in northwestern Panama is from the northeast (Cornthwaite, 1919). While these tradewinds dominate, the area experiences diurnal wind changes related to the intense heating of -land areas daily. Wind squalls frequently accompany thunderstorms, and the wind may blow from any direction depending on the weather conditions. These squalls often have maximum sustained velocities from 40 to 75 kilometers (km) per hour.

Rainfall

The mean annual rainfall at Bocas del Toro is 287 centimeters (cm) (Gordon, 1982) and is somewhat higher on the southeastern side of Laguna de Chiriqui. No clear wet and dry seasons exist, but rather, there are at least 2 periods of reduced rainfall and 2 periods of heavy rainfall. Reduced rainfall occurs in March and again in September/October, when monthly rain-fall averages about 15 cm. Heavy rainfall occurs in July and again in December, when monthly rainfall averages 35 cm. Rainfall during the drier seasons usually consists of light local showers, but heavy rains associated with frontal systems extending southward from North America occur occasion-ally along the Caribbean coast. Rainfall in the Laguna de Chiriqui is fairly evenly distributed between daytime and nighttime.

Storms

Tropical storms and hurricanes rarely, if ever, affect Panama. However, thunderstorms are relatively numerous in Panama, averaging between 100 and 140 per year (Cornthwaite, 1919). Thunderstorms usually travel across the Isthmus from the Caribbean to the Pacific coasts, which is the general

direction of prevailing air circulation patterns. Inland thunderstorms are usually an afternoon phenomenon in response to convective processes, while coastal thunderstorms are often nighttime and morning phenomena in response to cooling of air masses.

HYDROGRAPHIC REGIME

The shoreline embayment of Bahia Almirante and Laguna de Chiriqui (Fig. 1) occurs adjacent to the Caribbean Sea, which in general is not characterized by strong, oceanic conditions. Wave action is strongest outside the embayment in open waters, and portions of northwestern Panama's coast-line are characterized by active marine erosion. At the mouth of the estuary, wave action is reduced by fringing coral reefs and islands which shelter the embayment. Waves within the Laguna de Chiriqui range from 2 to 15 cm, while currents show little directional order and are less than 40 centimeters/second (cm/sec).

The Laguna de Chiriqui is microtidal with a mean tidal range at Bocas del Toro of 24 cm (National Ocean Survey, 1984). The actual fluctuation of water levels within the lagoon is influenced primarily by winds and wind-driven currents. The prevailing tradewinds blow into the embayment, but frequent thunderstorms often are accompanied by strong winds which affect water levels.

The salinity at the estuary mouth is approximately 35 parts per thou-sand (ppt) but may be significantly less on the landward side of the lagoon because of freshwater input from rivers. The water temperature is approximately 30°C, and the pH of the water is 7.5.

GEOLOGIC SETTING

Regional Geology

Central America is characterized by complex terrain with rugged mountains, volcanic peaks, long serrate ridges, and narrow coastal plains. In Panama, the Central Cordilleran mountain range extends almost the length of the country, with their crests dividing the Isthmus into a series of Atlantic-and Pacific-facing slopes. The mountain ridges, which trend roughly east-west to southeast-northwest, are composed of both nonvolcanic crystalline rocks and extinct volcanic peaks. In western Panama, the mountains are

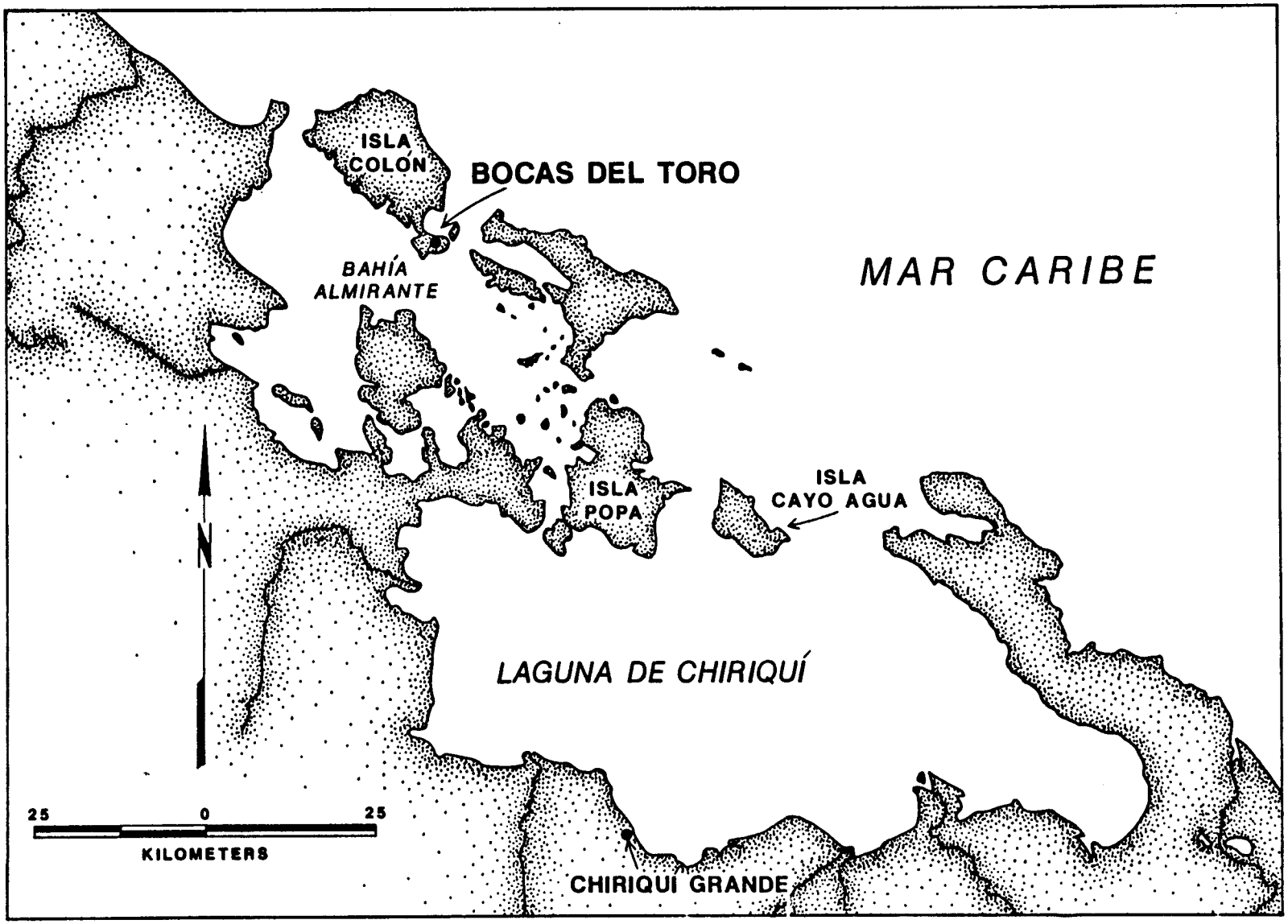


FIGURE 1. The Laguna de Chiriqui is located in northwestern Panama on the Caribbean Sea.

considerably larger than those to the east, reaching elevations in excess of 3,300 m in the vicinity of extinct volcanoes.

The youngest volcanoes of the mountain ranges of western Panama be-came extinct during the Pleistocene (Terry, 1956), while others became extinct considerably earlier. Volcanic ash deposits exist on Pacific-facing slopes where they have been weathered into fertile soils, while volcanic ash deposits on Atlantic-facing slopes have been largely eroded by extensive rainfall runoff in the area (Gordon, 1982).

The relatively regular coastline of northwestern Panama is interrupted by a large two-part embayment (Fig. 1), with Bahia Almirante to the west and Laguna de Chiriqui occupying the eastern portion of the embayment. This embayment is backed by forest-covered ridges to its landward side, while extensive coastal lowlands lie adjacent to the embayment along the coastline, and the Caribbean Sea is to the northeast. Laguna de Chiriqui and Bahia Almirante are separated by Isla Popa, Cayo de Agua, and an eastward-extending peninsula of the mainland (Fig. 1). Laguna de Chiriqui is generally deeper and less protected from the sea than Almirante Bay, as several large islands shelter the bay from open marine conditions. Numerous small islands exist within the embayment, while the seaward side is a relatively shallow shelf that is dotted with small patches of coral reefs.

The study area of this report consists of two small islands: Cayo Fresca and Cayo Ramirez (see Fig. 4). The substrate of these islands is an algae-covered, peaty marl with pockets of coral fragments.

Coastal Geomorphology

Extensive coastal lowlands occur to the northwest and southeast of Almirante Bay and Laguna de Chiriqui, respectively, while elevated peaks of the Central Cordilleran Range foothills surround the embayment to the south and southwest. Coastal environments within the embayment include fine-grained beaches, coarse-grained beaches, marshes, vertical bedrock, and mangrove habitats.

Almost no beaches exist in Almirante Bay as mangroves girdle much of the coastline and cover the lower elevations of the islands. Approximately half of the total shoreline of Laguna de Chiriqui is covered by mangrove habitats. The increased percentage of occurrence of mangroves in Almirante Bay is probably due to shelter from oceanic conditions.

Drainage Patterns

No large streams enter Almirante Bay, as the relatively large Rio Changuinola is deflected northwestward and reaches the coast west of the embayment. Five rivers drain into the Laguna de Chiriqui, with Rio Cricamola being the largest stream entering the embayment. Rio Guarumo is the next largest, although it and the other three rivers are considerably smaller. Freshwater/saltwater mixing occurs on the mainland side of the la-goon, as coral reefs near the estuary mouth are unaffected by any fresh-water influence.

Sedimentology

Continentally-derived clastic sediments are introduced by rivers into Laguna de Chiriqui, and sedimentation on the mainland side of the lagoon is dominantly terrigenous clastic material. Extensive sandbars have developed at the mouth of Rio Cricamola, and these sands have been reworked into broad beaches extending away from the river mouth in both directions. Transition from terrigenous clastic to carbonate sediments occurs witIll the lagoon in a seaward direction, with the outer portion of the lagoon dominated by carbonate sedimentation.

SITE SELECTION AND EVALUATION

The sites selected for the experiment were in the northwestern Laguna de Chiriqui, located on the Caribbean coast of Panama (Fig. 1). This is a tropical estuarine system which (at its greatest dimensions) measures 54 km long by 24 km wide. The northwestern Laguna de Chiriqui is dominated by mangrove shorelines with associated seagrass beds and nearshore coral reefs.

Since 1982, baseline physical, chemical, and biological data have been collected throughout the estuary in preparation for the environmental assessment of the coastal impact and a contingency plan for the tanker port associated with the cross-Panama oil pipeline (RPI, 1984). In 1983-1984, RPI con-ducted an oil and dispersant study on the mangroves in the region, which was sponsored by Exxon Production Research Company (Getter et al . , 1984). Among the data bases available from these studies for the Laguna de Chiriqui are:

1) Coastal maps showing the distribution of mangroves, seagrasses, and coral reefs with details on the shoreline and shallow-water distribution of these communities.
2) A large-scale trajectory model for the estuary with one year's data on winds, currents, and tides.
3) Baseline, spill, and follow-up biological and chemical data from our 1982 experimental spill in mangroves study.

In March 1984, a field survey was conducted to locate 3 sites for use in the present study. A large area in the northwestern Laguna de Chiriqui (Bahia de Almirante) was surveyed, and 12 candidate sites were selected. Each site was evaluated as to the suitability of the intertidal and subtidal habitats for use in the study.

Evaluation of intertidal habitats during site selection involved detailed structural analyses which allowed comparisons between mangrove forests to locate those which were comparable with regard to species composition, height, stand maturity, density, condition, and location respective to the seagrass and coral habitats. Forest characteristics of mangroves of the Laguna de Chiriqui vary according to substrate, slope, wave height, and proximity to freshwater runoff. Sites were selected which contained dense stands of healthy, nonstressed adult trees, saplings, and seedlings.

Evaluation of subtidal habitats during site selection involved comparisons between coral reefs and seagrass beds to locate those which had the

best combination of depth, density, condition, and location respective to the intertidal components of the research project. Three sites were selected which contained dense stands of healthy seagrass plants (greater than 300 plants/m^2) and well-developed coral communities. Healthy condition was determined by the measurable presence of abundant growth. Subtidal sites typically were dense seagrass beds between coral reefs and mangroves. These 2 types of subtidal habitats are most representative of those which occur in the tropics (Figs. 2 and 3). In addition, consideration was given to the physical layout of each site with respect to proximity to other sites and human inhabitants, fetch, longshore currents, and relative location of each habitat.

The subtidal and intertidal habitats at 12 candidate sites were ranked on a scale of 1 to 10, with 1 being the best score, and the best sites were reevaluated for final consideration. The final three sites chosen were judged to possess the best overall combination of comparable mangrove, seagrass, and coral habitats and the best available physical orientation and location.

After site selection, three sites were marked and measured. Each site was 30 m x 30 m and was approximately one-half covered by mangroves and one-half covered by coral and seagrasses. Water depths averaged 0.48 m over the seagrasses and 0.63 m over the corals. Additional corals were found on a steeply sloped drop-off extending from 3- to 10-m water depths outside the outer edge of each plot.

Two of the study sites were located on 1 island (Cayo Fresca), approximately 0.5 km apart, and the third site was located on a separate island (Cayo Ramirez) about 5 km to the east (Fig. 4). This latter site was designated as the reference site (Site R) since its physical layout was less suitable for containment of oil and was unlikely to receive even very small levels of contamination from the other sites. One of the sites on Cayo Fresca was found to have slightly lower current velocities than the other site and was therefore selected to be the dispersed oil site (Site D) because less dispersed oil would be required to maintain the target water-column concentration. The remaining site received the untreated oil (Site 0).

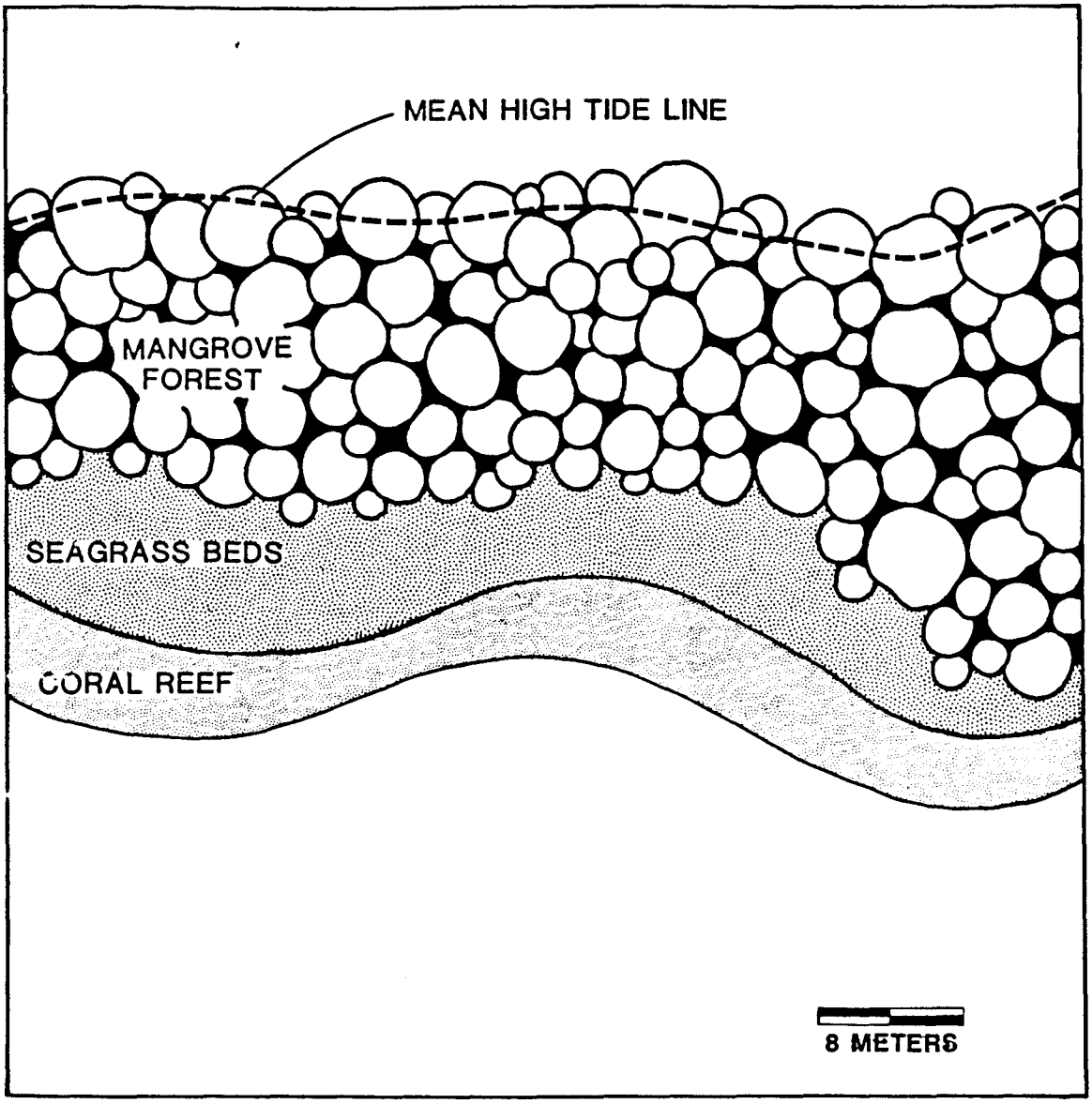


FIGURE 2. The mangrove, seagrass, and coral components of the intertidal and subtidal ecosystems at the study sites were located in close proximity to each other.

FIGURE 3. The mangrove forest, seagrass beds, and coral reefs typified the coastal ecosystems in many tropical areas. This photo demonstrates the development and physical appearance of these areas.

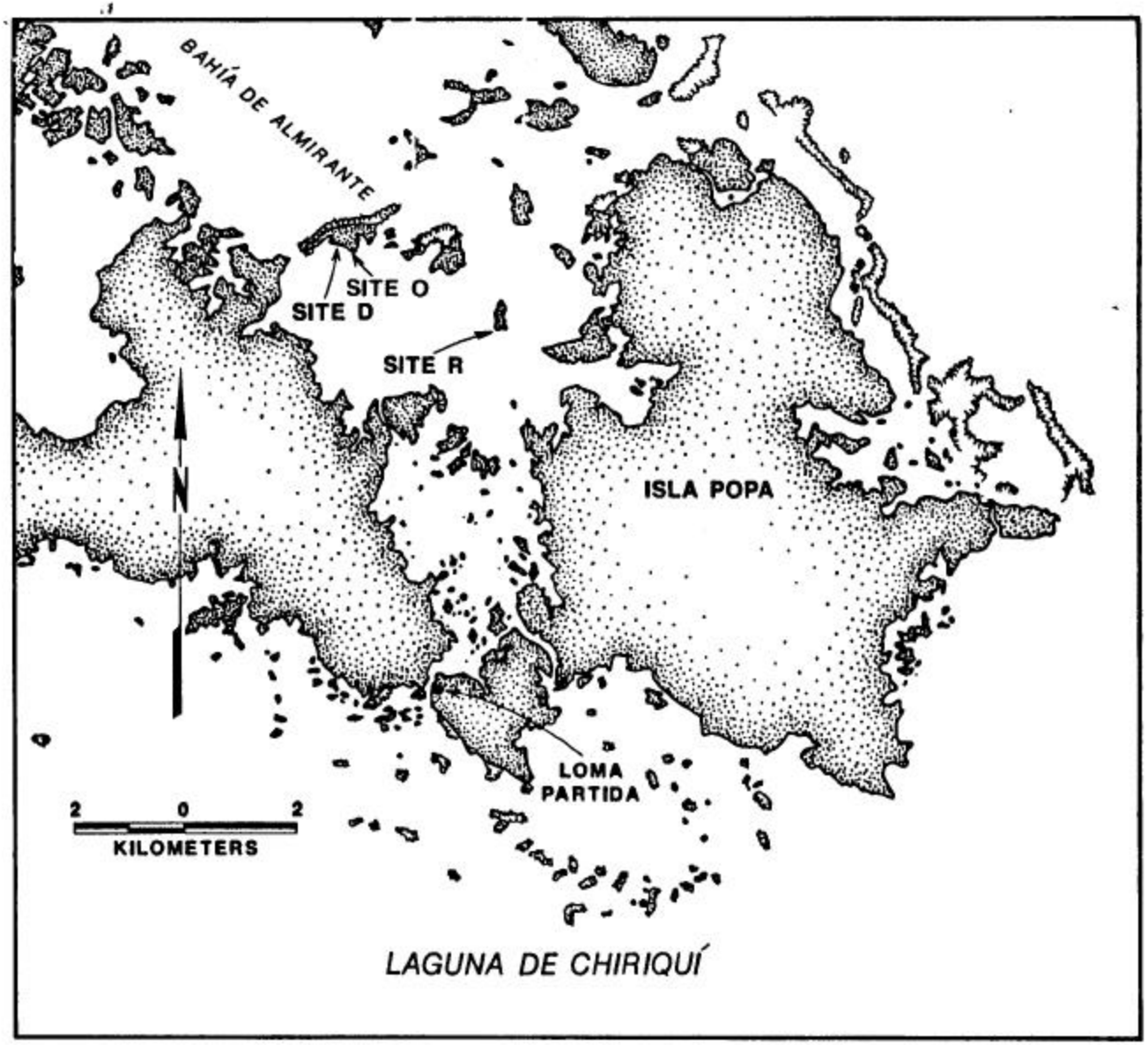


FIGURE 4. The study sites were located on a chain of islands known as the Cayos Viscanyos. Sites D and O were located on Cayo Fresca, and Site R was on Cayo Ramirez.

SITE PREPARATION AND COLLECTION OF BASELINE DATA FOR EACH EXPERIMENTAL SITE

After selecting the three experimental sites, a baseline, prespill study was conducted to determine the following:

1) Waves, tides, currents, and small-scale circulation patterns.
2) Geomorphic data, including grain size, sorting, organic matter content, carbonate content, compaction, and bathymetry tied to onshore profiles.
3) Chemical and water-quality baseline data, including background petroleum and biogenic hydrocarbon levels, pH, temperature, and salinities.
4) Biological community and ecosystem data, including species composition, abundance, and distribution of resident plant and animal communities.

Prespill biological, chemical, and physical parameters were collected in March 1984 and again in late November and early December 1984. At each time, the same measurements were repeated at each site. Immediately following the November 1984 survey, preparations were begun for the treatment of each site according to a preestablished protocol described below.

Prior to release of oil or dispersed oil, study Sites D and 0 were en-closed within a 45-cm-deep containment boom anchored at 6 points. The booms were drawn through a channel cut through the mangrove prop roots around the perimeter of the mangrove study area (Fig. 5), extended across the edge of the seagrass and coral habitats, and joined in the middle at a point just outside the seawardmost part of the œoral reef (Fig. 6). This boom deployment enclosed the area around the study site and helped restrict the movement of untreated oil and dispersed oil to allow a controlled expo-sure to the habitats and organisms present in the site (Fig. 7). A second containment boom was positioned between the two study sites to prevent cross-contamination. This boom was a large, open-water boom 1 m deep and approximately 100 m long. It was tied to shore at one end and anchored in deep water at the other end, oriented perpendicular to shore.

Immediately outside the enclosed area, a small, shallow-draft barge was anchored perpendicular to shore. This barge served as a work platform and as a storage area. All the oil, booms, and other spill control equipment

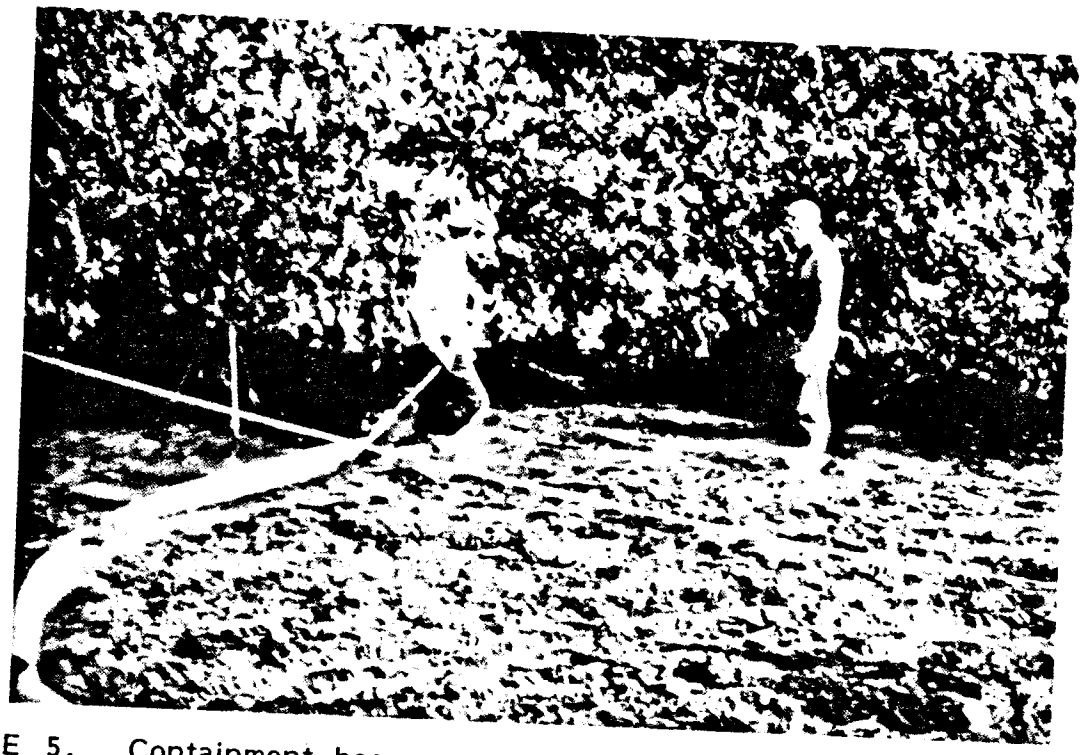


FIGURE 5. Containment booms were laid out to surround each study site. A channel was cut along the outside perimeter of the intertidal area of each site to allow the boom to be threaded through the prop roots.

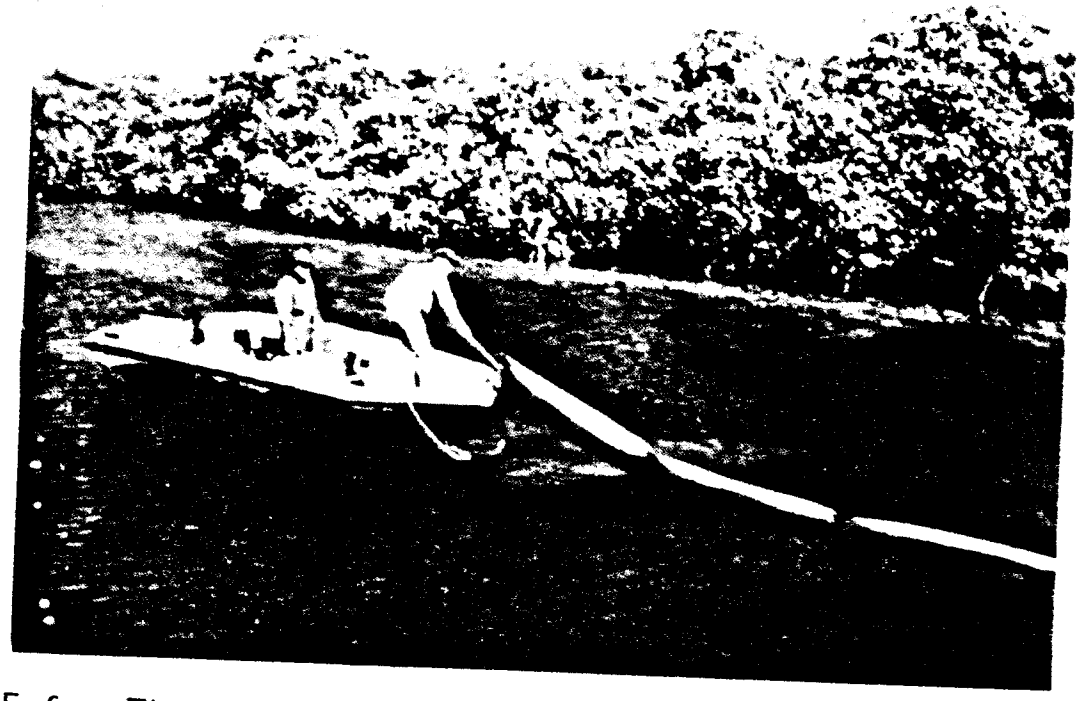


FIGURE 6. The booms extended out around the outer perimeter of the subtidal areas, creating an entirely enclosed area around the site.

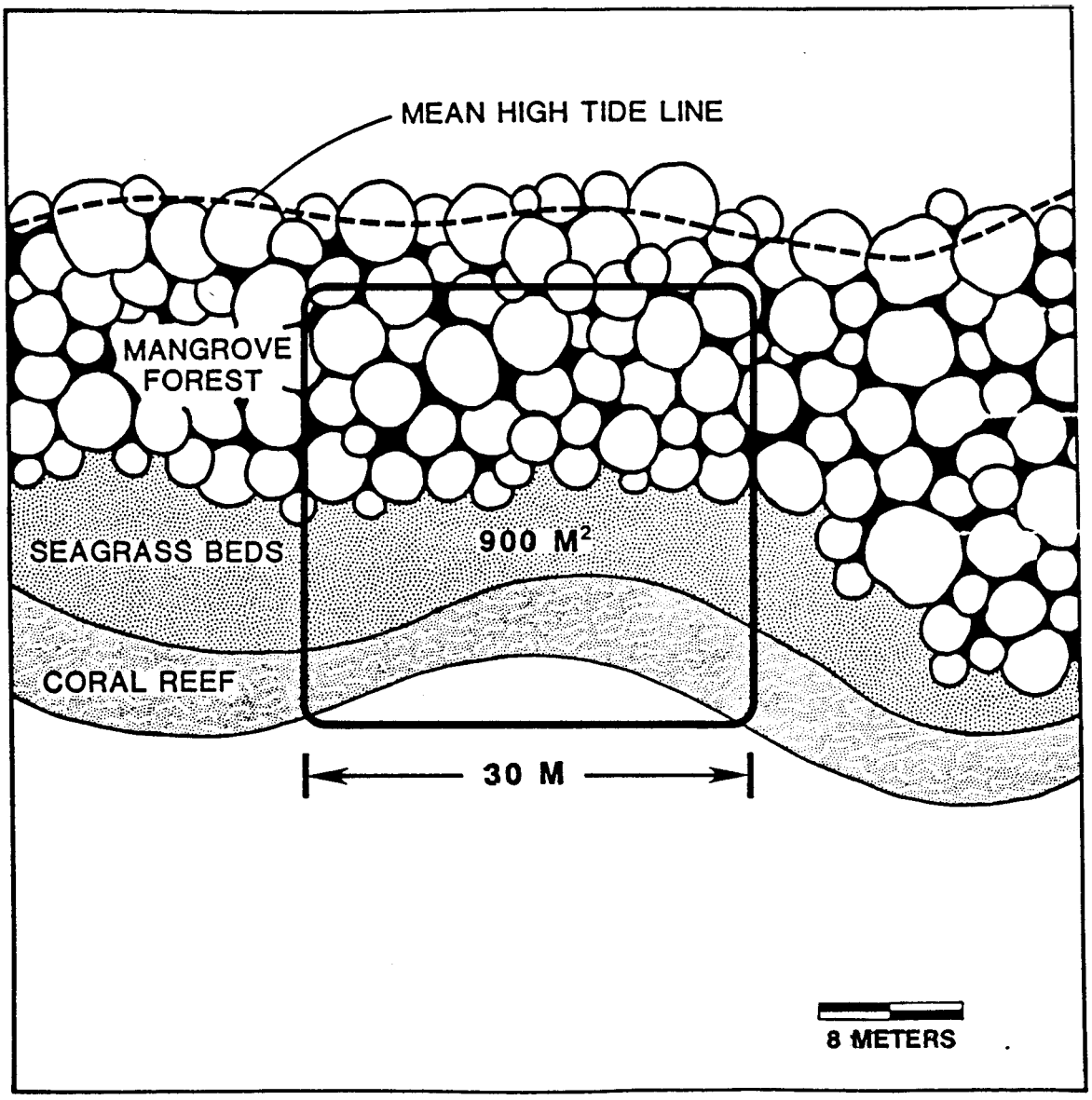


FIGURE 7. The sites were completely surrounded by booms that helped contain the oil and dispersed oil and allow a relatively con-trolled exposure of the site.

were transported to the site on this barge, and it functioned as the oil de-livery vessel during the spills.

A large workboat was positioned adjacent to the barge. This vessel served as the main work area and observation area during the spills. Fluorometric analyses and water sampling were conducted from this vessel, and it provided an area from which to observe, monitor, and document the progress of site treatment. The water sampling and hydrocarbon monitoring system was located on the stern of this vessel (Figs. 8 and 9).

Following placement of the booms, barge, and workboat, the oil delivery and monitoring systems were set up. The oil delivery system consisted of a battery-powered electric pump with six outlet valves. Each valve was connected to a length of 0.5-inch polyethylene tubing that was connected at the other end to a floating oil-release apparatus. This apparatus consisted of a 1.0-m section of 0.75-inch PVC pipe with a row of small holes drilled into it. This tube was fastened to a section of wooden board that was loosely anchored to the bottom with a short section of cord. This allowed the board to float at the surface. Two of these oil-release devices were located in the coral area, 2 in the seagrass area, and 2 in the outer fringe of the man-grove area (Figs. 10 and 11). The release rate was 10 liters/minute (L/min) (0.167 L/sec).

The oil monitoring system consisted of a Turner Designs field fluorometer, a battery-powered electric pump, a 6-way gang valve, and 6 lengths of 0.5-inch polyethylene tubing (Figs. 12 and 13). The ends of the tubing were located at 6 points in the study site and were anchored approximately 10 cm above the bottom. The oil monitoring points were located within approximately 3 to 5 m of the oil release points. These tubes were connected to a 6-way gang valve which then led to an electric pump and then through the field fluorometer. Between the pump and the fluorometer was a separate valve from which discrete water samples could be drawn. This apparatus al-lowed water to be sampled from six different locations within the study site for analysis by the fluorometer or for analysis of discrete water samples. These were subsequently analyzed for volatile hydrocarbons (C_1 to C_{10}) and for intermediate-range carbon number hydrocarbons by GC or GC/MS in the laboratory.

The oil used during the site treatments was Prudhoe Bay crude oil. It is of medium viscosity and density and is frequently transported through tropical coastal waters. Prudhoe Bay crude is one of the most important

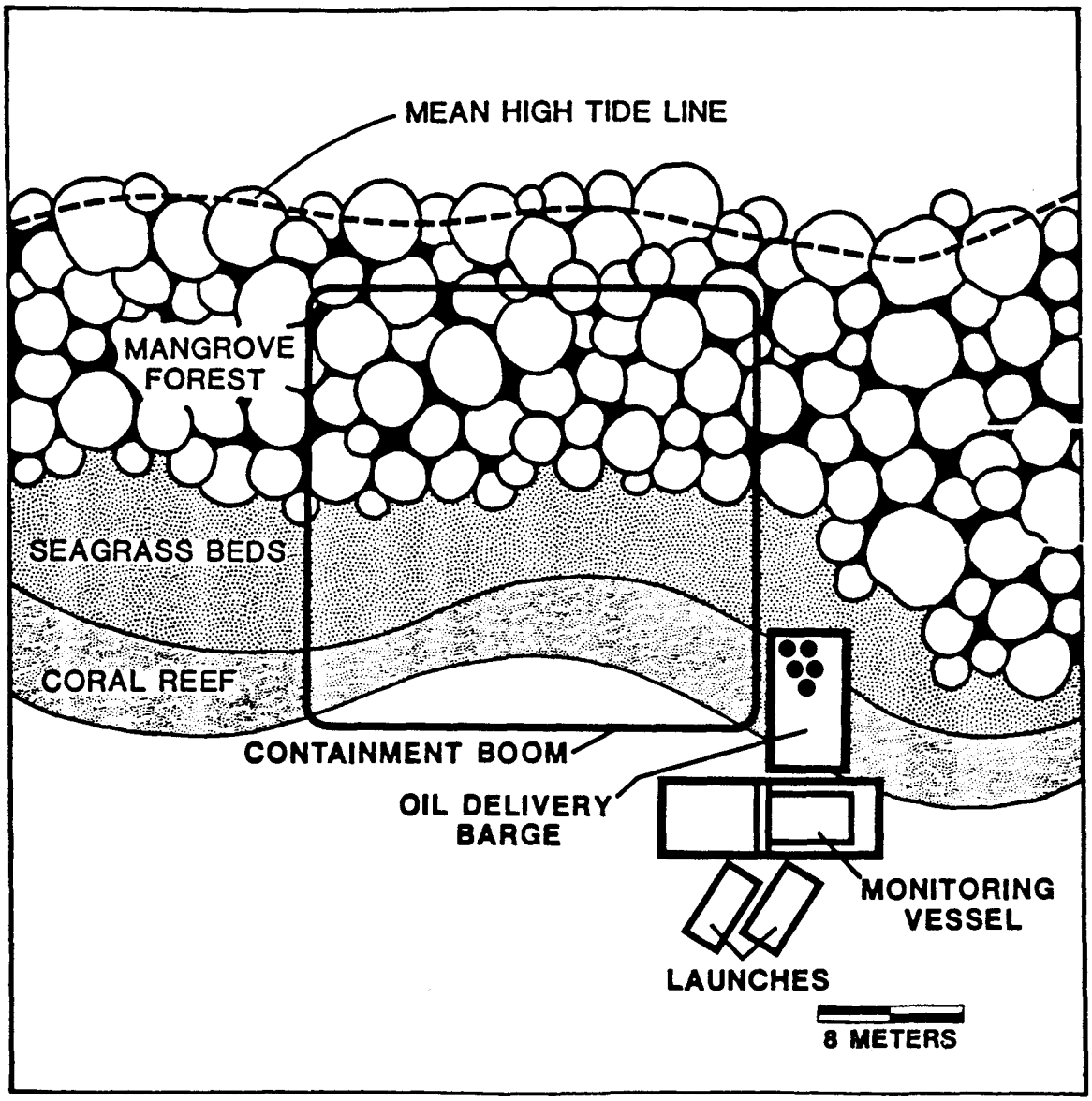


FIGURE 8. A small barge and workboat were stationed outside the boomed-in area. They functioned as work platforms and storage areas during the treatment periods.

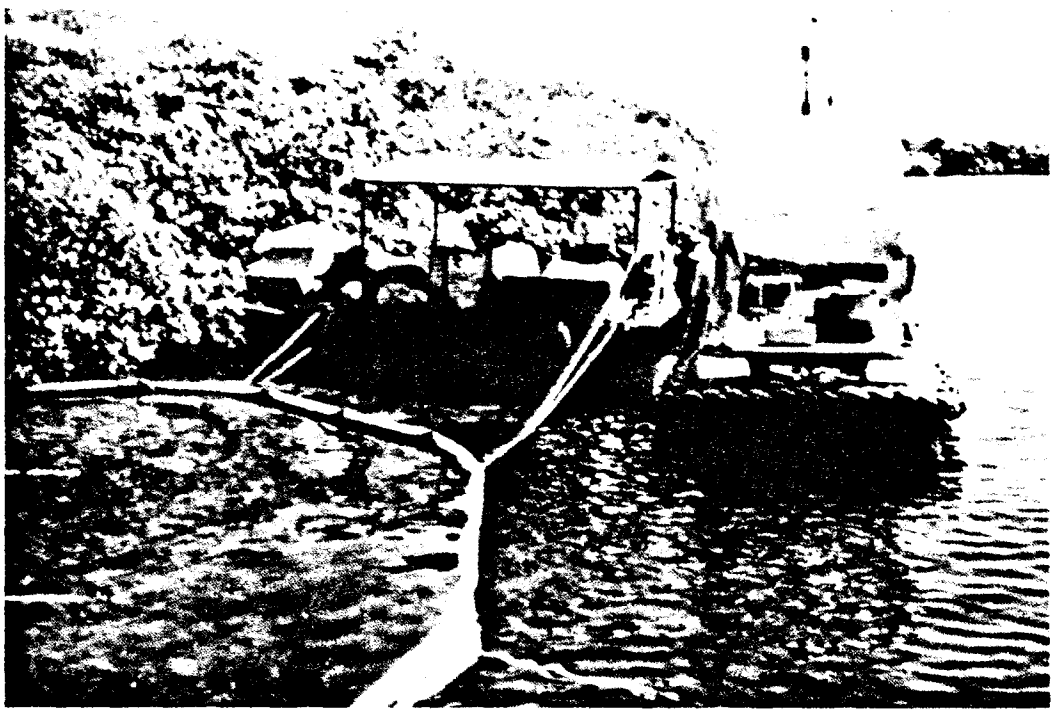


FIGURE 9. The oil delivery system was operated from the barge, and the oil monitoring system was operated from the stern of the workboat.

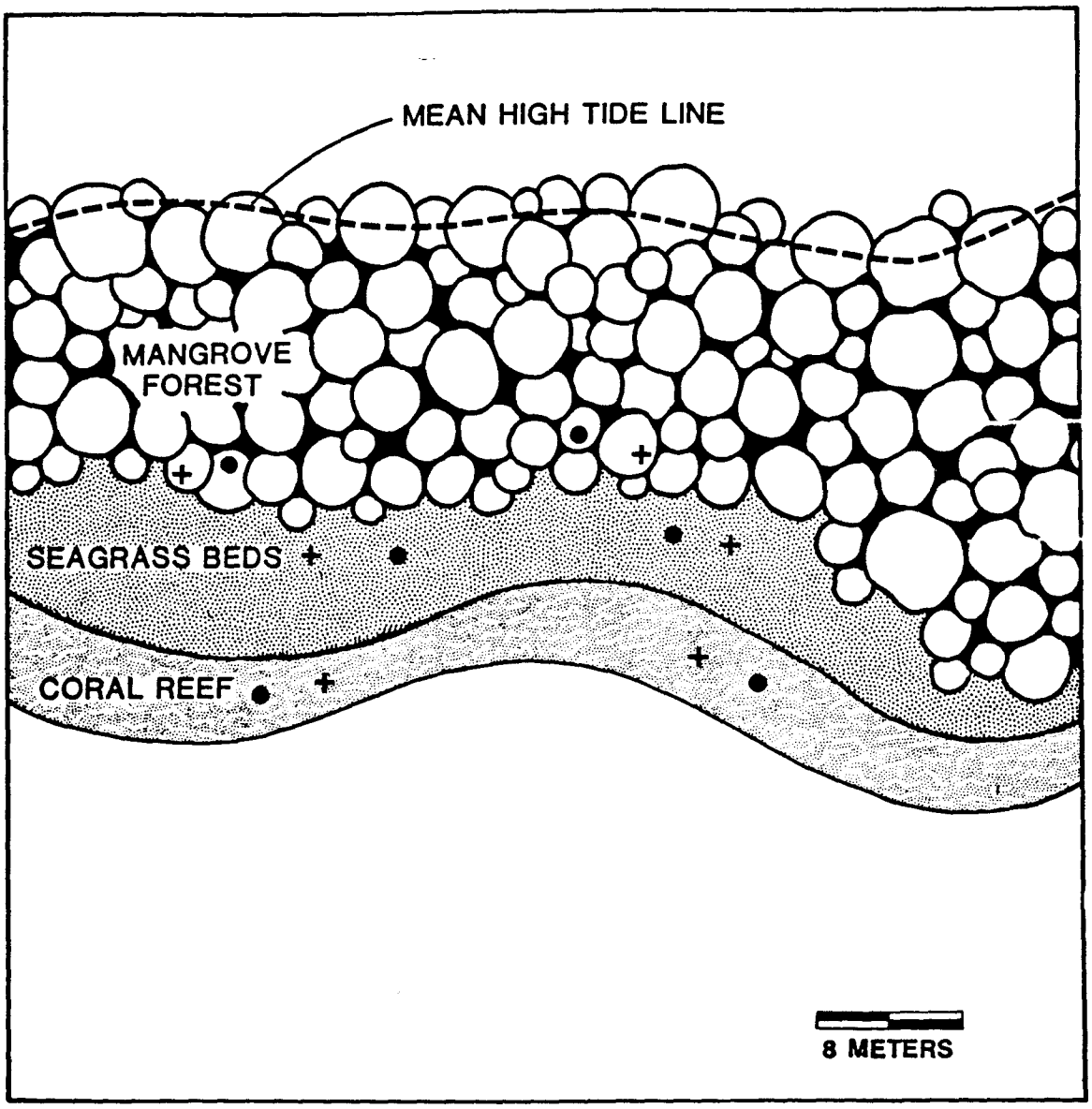


FIGURE 10. Approximate location of oil-release points (+) and water-intake points (●) at Site D.

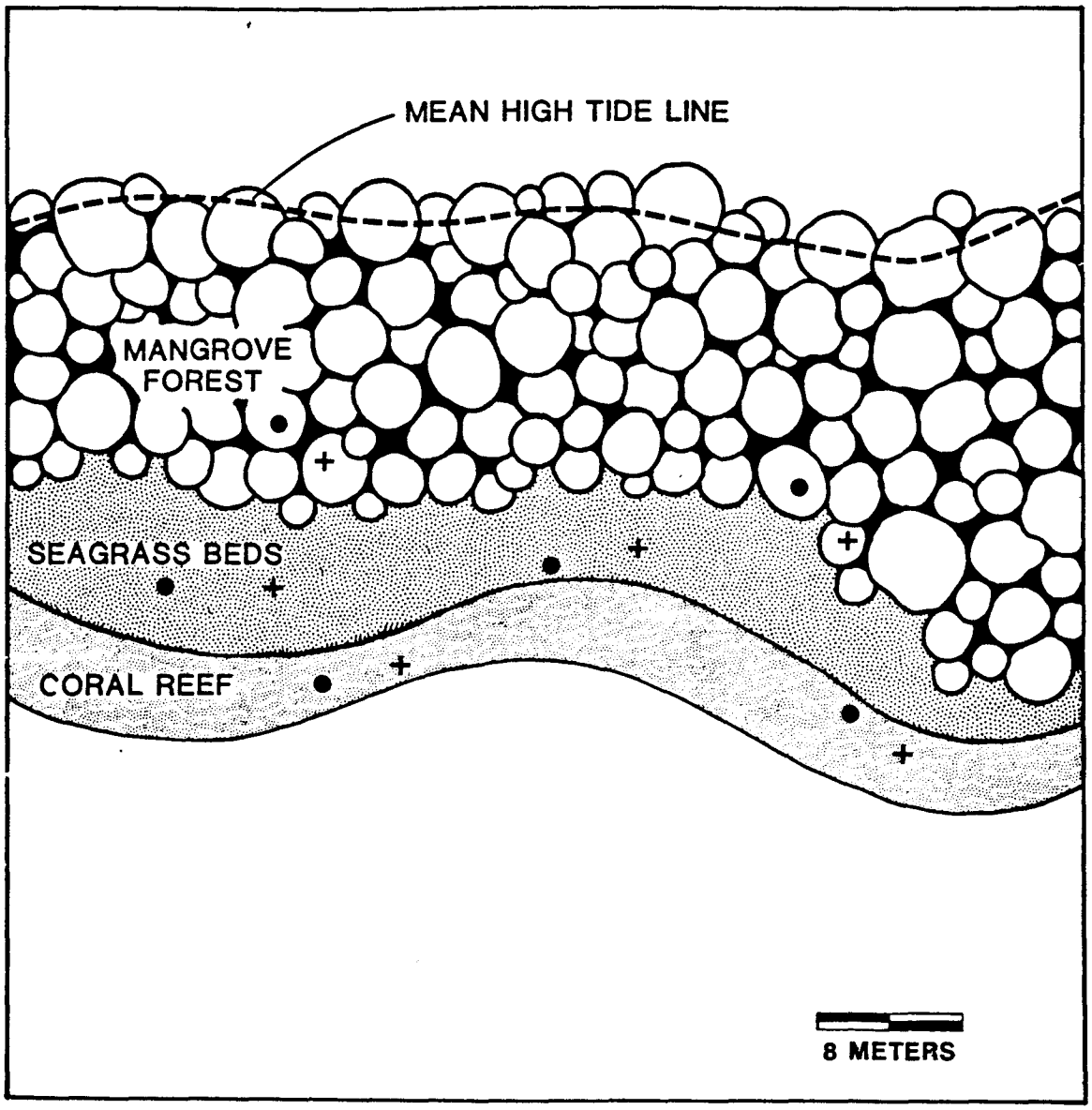


FIGURE 11. Approximate location of oil-release points (+) and water-intake points (●) at Site O.

FIGURE 12. A Turner Designs field fluorometer was used to monitor oil concentrations. It was used to sample water from six locations within the study site.

FIGURE 13. Polyethylene tubing was used to take water samples from six locations in each study site during treatment.

crude oils in terms of volume transported in U.S. coastal waters. Table 1 presents a summary of physical and chemical characteristics *of* this oil. The dispersant used in this study was a commercial nonionic glycol ether-based dispersant concentrate. It is stockpiled by PTP and is recommended for use on offshore oil spills. It is a highly effective dispersant concentrate.

All of the oil used in the study was obtained directly from the PTP pipeline and placed into clean, metal drums. Forty-two gallons of Prudhoe Bay crude oil was placed into each of 10 metal 55-gallon drums.

Dispersant was added directly to each barrel in a 20:1 oil-to-dispersant ratio. In addition, hexadecane was added as a chemical label to aid in the identification and quantification of the dispersed oil. Octadecane was added as a chemical label to the untreated oil. At each site, the chemical label and dispersant were added to each barrel and then thoroughly mixed by physical agitation for several minutes.

GENERAL SCENARIO DEVELOPMENT

The volume and discharge rates of crude oil used in this study were based on calculations derived from accidental oil spills and from the experimental oil spills previously described. This study was intended to simulate an unusually high exposure level *of* chemically dispersed oil and a moderate exposure level of untreated oil. This level of dispersed oil would be approached only if fresh oil were dispersed directly adjacent to coral and seagrass habitats in shallow, nearshore waters. The exposure level chosen for the untreated oil was based on field observations of estimated amounts that caused mangrove tree mortalities. Emphasis was placed on ensuring that the chemically-dispersed oil scenario was a severe test of dispersed oil's toxicological effects so that the subsequent analysis of data would be based on a worst-case scenario for dispersant use.

It has been shown that oil slicks rapidly spread to thicknesses of 0.1 to 0.01 millimeters (mm) (API, 1986; McAuliffe, 1986). For 0.1-mm-thick oil slicks, the maximum oil concentration in the top 1 m of water would be 100 ppm if the slick was completely dispersed and uniformly mixed. If uniformly mixed in 10 m, there would be 10 ppm. Under actual conditions, the concentration at 10 m may be 1 ppm or less (Mackey and Wells, 1983).

The target concentration *of* dispersed oil in this study was to simulate the chemical dispersion of an oil slick with an average thickness of 0.1 mm.

TABLE 1. Physical and chemical characteristics of Prudhoe Bay crude oil.

API Gravity (20°C)	27.8° API
Specific Gravity (15°C)	0.89 g/mL
Pour point	-10°C
Viscosity (38°C)	14.0 cst
Yield:	
Aromatics	25.3% volume
Paraffins	27.3% volume
Naphthenes	36.8% volume
Others	10.6% volume
Composition:	
Sulfur	0.94% weight
Nitrogen	0.23% weight
Vanadium	18 ppm
Nickel	10 ppm

Data from Thompson et al. (1971) and Coleman et al. (1973).

The slick was to be large relative to the surface area of the receiving waters, which in turn were to be slowly flushed. The slick was to be dispersed before stranding; therefore, the dispersed oil was subject to dilution by intervening water. Thus, the concentration that reached shore would be diluted to about 15 ppm or less, lasting for 3 days. However, because of manpower and budget limitations, it was not technically feasible to release dispersed oil at this concentration for such a long period. Thus, a target concentration of 50 ppm released over 24 hours was selected, producing an exposure of 1,200 ppm-hours. Under normal conditions, this would occur only in very shallow, slowly flushed waters and, therefore, is a very vigorous test for chemically dispersed oil (Fig. 14).

The dispersed oil was released over a 24-hour period starting at 1430 hours on 28 November 1984 and ending at 1445 hours on 29 November 1984. A total of 4.5 barrels (715 L) of oil was released. The release of oil was governed by continuous measurements from the fluorometer monitoring system. The actual, measured concentrations fluctuated considerably, primarily as a result of differential water flow rates within the enclosed area and the relative location of oil release points and water intake points.

The untreated oil was released into the study site at an application rate of 1 liter/square meter (L/m^2). This would represent the amount of oil that would strand from a 100- to 1,000-barrel spill, depending on wind and cur-rent conditions. This concentration was based on levels known to have caused adverse effects on mangroves (Getter et al . , 1981; Getter and Ballou, 1985).

Four barrels of untreated oil were released from 1250 hours to 1700 hours on 1 December 1984. On 2 December 1984, an additional 2 barrels were added between 1245 and 1500 hours, making a total of 953 L released into the 900-m^2 site.

The whole, untreated oil was allowed to remain within the area enclosed by booms until 1630 hours on 3 December 1984, at which time the free, floating oil was removed with sorbents. This allowed sufficient time for tides and winds to distribute the oil throughout the site.

FIGURE 14. The dispersed oil formed large clouds underwater and some small slicks of undispersed oil on the surface. Dispersed oil moved slowly through the site under the influence of nearshore currents, and the slick oil collected in the intertidal area and in downwind sections of the boomed area.

PHYSICAL CHARACTERIZATION

Detailed measurements of the hydrology, geology, and water quality were conducted at each site to establish the baseline characteristics of each site and to assist in the design and implementation of the site treatments. Parameters measured included current velocities, water depths, tidal regime, waves, sediment characteristics, and various water-quality parameters.

METHODS

The speeds and directions of nearshore water currents were measured by timing the movement of a neutrally buoyant float over a premeasured distance. Water depths were measured at various points with a 1.5-m profile rod. Tidal regime was determined from published tide tables for the nearest station (Bocas del Toro, Panama) and from on-site observations. Wave height and direction were determined from daily observations at each site.

Within each study site, sediment samples from mangrove, seagrass, and coral habitats were collected to determine grain-size distribution, percentages of peat (organics) and calcium carbonate, and compaction. A 3-inch diameter aluminum coring pipe was inserted into the sediments and withdrawn. The contents were then placed in a plastic bag and shipped to the RPI laboratory. Grain size was determined by sorting of sediments into three size classes (gravels, sands, and muds) and measuring the percentage of each size class by weight. Percentage of peat and other organics and calcium carbonates was determined by visual observation and by acidification of the samples with nitric acid to dissolve any carbonates present.

Water-quality parameters were measured using a Hydrolab to determine water temperature, pH, conductivity, and dissolved oxygen.

RESULTS

Wave and Tidal Regime

Surface water movement at each site was dominated by wind-driven longshore currents that ranged from 0.35 to 1.0 meters/minute (m/min). Close to shore at the edge of the mangrove prop roots, a very weak countercurrent of less than 0.05 m/min was measured.

Wave height depended on local weather conditions. Under typical conditions, wave height ranged from less than 0.5 cm to 4.0 cm. During thunderstorms, wave height reached a maximum of about 10.0 cm.

Water depth at each site changed with distance from shore. Maximum depth within the sites was less than 3 m and was usually less than 1 m throughout most of the site. At the crest of the coral habitat and in most of the seagrass habitat, water depths were as little as 10 cm, depending one the tide and local weather conditions.

The tidal regime in this area is microtidal diurnal with a mean range of 0.84 feet. The actual height and time of the tides is highly variable de-pending on local weather conditions.

Sediment Analyses

The sediment types of the study sites consisted of two categories; calcium carbonate sediments derived from coral and calcareous algae, and organic peats derived from mangrove root and leaf material. The study sites were located on small islands formed by mangrove trees growing on top of coral rubble fragments. A thick deposit of peat formed on top of the coral rubble, forming a distinct transition zone located at the edge of the man-grove prop roots. Seaward of this area, the sediments are almost exclusively coral fragments, and landward, the sediments are fibrous peat.

Coral Bed Sediments

The sediments collected at the 3 coral experiment sites were very similar in both composition (Table 2) and size distribution. The samples are composed of coral fragments (Porites porites) with a mean clast size of 50.4 mm. All of the sediments sampled fell into the gravel size class (greater than 2 mm). An occasional piece of Halimeda also was present in the samples. These sediments were very loosely compacted.

Seagrass Bed Sediments

The composition of the sediments at the seagrass experiment sites were predominantly calcium carbonate with only 1 to 2 percent of the samples being organic material (Table 2). Size distribution of the samples ranged from gravel to mud. Gravel-size coral fragments of Porites porites were most common, comprising an average of 57 percent for the 3 samples. The mean

TABLE 2. Size distribution and composition of sediments from coral, seagrass, and mangrove components of test sites.

	CORAL	SEAGRASS	MANGROVE
TEXTURAL SIZE CLASSES			
Gravel (greater than 2.0 mm)	100.0%	56.7%	0.0%
Sand (0.0625-2.0 mm)	0.0%	41.6%	0.0%
Mud (less than 0.0625 mm)	0.0%	1.7%	1.9%
COMPOSITION			
Peat/ organics	0.0%	1.3%	98.1%
Calcium Carbonate	100.0%	98.6%	1.9%

size for the coral fragments was 43.6 mm. An average of 42 percent of each sample was sand-size coral fragments with grain sizes ranging from 0.088 mm to 0.25 mm (very fine- to medium-grained sand). The remaining portion of the sediments was either organics or calcium carbonate mud. The sediments in the seagrass habitats were loosely compacted.

Mangrove Forest Samples

Sediments sampled from the mangrove experiment sites were predominantly peat (97 percent organic material; see Table 2). One to 2 percent of each sample was calcium carbonate mud. The peat was of a fibric grade, being spongy, elastic, and compact with plant residues well preserved (root-mat). These sediments were very dense and formed a coherent plug of root-mat when removed by the sediment corer.

Water Quality

Temperature, pH, and salinity were very uniform throughout the study area. Water temperature averaged 28.5°C, pH averaged 7.4, and salinity averaged 32.0 ppt.

CHEMICAL SAMPLING AND ANALYSES

An intensive analytical survey was conducted at each site prior to treatment, during treatment, and following treatment to determine the chemical characteristics with reference to petroleum hydrocarbons.

This analytical program had a number of specific objectives, the first of which was to evaluate the suitability of the sites selected for treatment. This was done to detect any evidence of previous exposure to petroleum hydrocarbons, to allow a comparison to be made between the sites based on organic chemistry, and to establish prespill baseline levels of naturally occur-ring hydrocarbons.

A second major objective was to determine real-time concentrations of petroleum hydrocarbons during the site treatment periods, both to determine the magnitude of the spills and to provide a feedback mechanism for control-ling the dose of oil at each site.

The third major objective was to conduct postspill analyses of the water, sediments, and biota to determine exposure levels and uptake that could be related later to any measured biological effects and to determine the long-term changes in hydrocarbon chemistry at the sites.

METHODS

Water Sampling Large-

Volume Samples

Hydrocarbons were adsorbed from water onto Amberlite XAD-2 resin in glass columns. Columns were placed in areas within the sites connected with Tygon tubing leading to Masterflex pumps placed in a small boat at the edge of the site. Water was pumped through each column for approximately 5 hours at a flow rate of 250 mL/min for a total sample of about 75 L. The columns were treated with saturated mercuric chloride solution to prevent biodegradation of hydrocarbons sealed and transported to Bermuda for analysis.

Spill Monitoring

At each of the 2 experimental sites (D and 0), sample tubes were led into 6 areas of the site (Figs. 10 and 11). Tube inlets were placed

approximately 10 cm off the bottom attached to galvanized reinforcing bars driven into the sediments at locations representing coral, seagrass, and mangrove areas within sites. The tubes led to a 6-port sampling manifold, through the flow-cell of a Turner portable fluorometer to a 12-volt impeller pump. By selectively opening a valve, water could be drawn from each sampling location through the fluorometer to obtain a real-time reading of fluorescence. The instrument had been calibrated to convert fluorescence intensity to the concentration of physically and chemically dispersed oil. Calibration produced a standard curve of fluorescence versus oil concentration, and all fluorescence readings taken in the field were compared to this standard curve. The oil concentrations derived from the standard curve are known as oil equivalents since they are estimated from the curve rather than gravimetrically or volumetrically quantified. The outlet tube was used to collect discrete water samples that were collected at time intervals tnrougnout the 24-hour dosing period as well as 24 hours after the addition of oil. In addition to measuring hydrocarbon concentrations, the readings from the fluorometer provided feedback to control the addition of oil.

Water samples were collected in 1-L separatory funnels and extracted twice with 50-mL dichloromethane. The extracts were sealed in glass ampoules that had been precleaned with chromic acid, washed with distilled water, and then solvent-rinsed. These were transported to BBS for total extractable organic matter analysis. Throughout the dosing period, samples also were collected and sealed in crown top glass bottles for the analysis of LMW hydrocarbons (C_1-C_{10}).

Sediment Sampling

Sediments were collected in the intertidal area using 10-cm-diameter x 30-cm-long aluminum corers. Once above water, cores taken in the seagrass area were transferred to glass jars. Three replicates were taken at each sampling point and stored separately. The large amounts of coral rubble made coring extremely difficult, so in many cases, coral rubble was collected by hand using plastic gloves and placed in glass jars. Mangrove and inter-tidal areas were sampled by coring in areas within the site at low tide. These samples were extruded from the corer into glass jars. Other samples were taken using a stainless-steel knife to cut the peaty material to a depth of 0.5 cm. These are called "surface scrapes." One sample is made up by

combining 5 scrapes from an area within a 1-m radius from a central sampling point. All samples were treated with saturated mercuric chloride solution to prevent sample biodegradation during transport.

Sampling of Biota

Mangrove leaves' and seagrass leaves were collected by hand, placed in plastic bags at the site, and then transferred to glass jars. Leaves were collected from each site in triplicate and then later pooled at the BBS laboratory to create fewer samples.

The coral tissue was sampled with an air-pik (Knap and Sleeter, 1984). The tissue was dissociated from the carbonate matrix using a stream of air, blown into a clean beaker, and then transferred to a glass jar. Mercuric chloride was added to prevent sample degradation.

Analytical Techniques

Samples of water, sediment, and biota were treated by similar techniques once they arrived at the BBS laboratories. All the solvents and other chemicals used were of high purity, confirmed in our laboratory by raniltary GC.

The dichloromethane extracts of the water samples extracted in the field were dried over anhydrous sodium sulfate, adjusted to an appropriate volume to prevent quenching, and analyzed by scanning and fixed wavelength UV fluorometry on a Perkin-Elmer 650 10S scanning spectrofluorometer. A synchronous scan of the excitation and emission wavelengths was carried out from 250 to 500 nanometers (nm) with an excitation wavelength 25 nm shorter than the emission wavelength. The spectra were compared to those generated using Prudhoe Bay crude oil. The extracts were calibrated with whole oil and whole oil plus 5 percent dispersant.

Selected samples were analyzed further by capillary GC and capillary GC/MS after further purification and separation. The extract was evaporated to approximately 100 microliters, and 1-3 microliters were injected into a Hewlett-Packard 5840 gas chromatograph equipped with a capillary injector and flame ionization detector. Aliphatic hydrocarbons were analyzed using a 30-m x 0.25-mm fused silica capillary column coated with SE-30 (J&W Scientific). The GC conditions were temperature programming from 55° to 250°C at 4 degreesC/min, and hydrogen at a head pressure of 10 pounds per square inch

(psi) which gave a column flow rate of 1 mL/min. The same conditions were used for aromatic hydrocarbons, but a more polar column (SE-52) column was used. Aliphatic fractions were recorded by integrator and interpreted ac-cording to their retention index. Peaks in the aromatic fraction were qualitatively identified using authentic standards as well as by using the system developed by Lee et al. (1979) which indexes the aromatic hydrocarbons on the basis of naphthalene, phenanthrene, chrysene, and picene. Quantitatioo was carried out using peak area of the unknown relative to authentic hydro-carbon standards and using an internal standard as a measurement of method efficiency. Full procedural and sample blanks were carried out with every sample set.

Water Sample Analysis

The XAD-2 resin was removed from the column and extracted [c]- hours with aqueous acetone (1 :1). This process was repeated for a further 24 hours with fresh solvent and the 2 extracts combined. The acetone was removed by rotary evaporation under vacuum, and the aqueous phase was extracted twice with n-hexane. The hexane extracts were evaporated to approximately 0.5 mL and applied to a 1-cm-diameter x 5-cm-long column of Florisil that had been deactivated with 2.5 percent water. Two fractions were eluted from the column using hexane alone for the first fraction containing the aliphatic hydrocarbons and a 10 percent diethyl ether in hexane solution for the second fraction which contained the aromatic hydrocarbons. These fractions were then analyzed by GC and GC/MS. The results are ex-pressed in micrograms per liter (ug/L) by dividing the hydrocarbon concentration by the amount of water that passed through the resin.

Sediment Analyses

Sediment samples were sieved to remove all debris over 1 mm in order to remove coarse material. Three replicates from each site were pooled to give 1 sample from each habitat (mangrove, seagrass, and coral). The sediments were alkaline-digested for 8 hours using 0.5-N potassium hydroxide in methanol. The nonsaponifiable lipids were partitioned into n-hexane (3 times with 20-mL aliquots of n-hexane), evaporated to 0.5 mL, subjected to Florisil cleanup, and separated and analyzed as described above. The results were

reported as hydrocarbon per unit wet weight of sediment. Internal and external standards were added to correct for method losses.

Tissue Analyses

Plant and animal samples were homogenized, subjected to alkaline digestion, and analyzed for hydrocarbons as described above for sediments.

Gas Chromatography/Mass Spectrometry

Verification of compound identification in sample extracts was carried out using a Hewlett-Packard 5970 quadrupole mass selective detector with a capillary direct interface. This was coupled with a 5790 Hewlett-Packard capillary gas chromatograph, and a Hewlett-Packard 59970A data system with the NBS Reference Library. Unknown peaks that did not correspond with known samples were identified by interpretation of their mass spectra as well as by library data searches.

RESULTS

Baseline Characterization

Analysis of the samples collected during the first sampling trip (March 1984) indicated that the sites were free of petroleum hydrocarbons and, therefore, were suitable for the experiment (Table 3). Hydrocarbons present in water samples taken from the 3 sites ranged from 0.1 to 0.2 ug/L. The GC pattern of hydrocarbons indicated a biogenic origin. There was no evident unresolved complex mixture in the chromatograms, thus indicating that the area was free of oil inputs. Sediment and tissue samples also were free of oil hydrocarbons, and there was little difference between sites. Site R, the reference site, had less variability between replicates, but we cannot determine the reason for this.

Monitoring During Site Treatments

Tables 4 and 5 summarize the water sampling data obtained during site treatment. The concentrations of oil were taken at arbitrary time intervals by switching sampling ports and reading the concentrations in terms of fluorescence intensity. These readings then were converted to ppm oil using a calibration graph. The concentrations achieved were time-averaged at

TABLE 3. Analysis *of* baseline samples taken in March *1984* (mean *of* samples) showed very low levels of biogenic hydrocarbons only.

	SITE D	SITE 0	SITE R
Water samples (ppb)	0.14 +/- *0.11*	*0.20 t 0.14*	*0.18* +/- 0.06-
Sediment samples (ppm wet weight)			
Mangrove	*2.04 ± 0.53*	*1.44 +/- 0.51*	*0.97* t 0.39
Seagrass	*1.85 t 1.14*	*1.02* +/- 0.16	0.69 ± 0.07
Plant tissue (ppm wet weight)			
Mangrove	*0.90**	*0.69**	*1.02**
Seagrass	*0.61**	*1.94**	1.93*

*Results of pooled samples consisting of at least 10 mangrove and 30 seagrass leaves.

TABLE 4. Fluorometry readings as oil equivalents (in ppm) during dosing period at the dispersed oil site (Site D). Concentrations of dispersed oil were somewhat variable over the site, with highest concentrations present over the seagrass areas.

Hours After Dosing	AREA 1 (Coral)	AREA 2 (Coral)	AREA 3 (Seagrass)	AREA 4 (Seagrass)	AREA 5 (Mangrove)	AREA 6 (Mangrove)
0	-	3.0	-	4	-	-
1	5.3	2.0	4.3	5.5	4.2	4.5
2	5.0	4.5	6.0	8.5	8.0	10.5
3	27.5	21.0	39.0	26.3	48.0	24.0
4	27.0	35.3	63.3	48.5	58.3	36.0
5	33.7	26.0	40.7	56.7	67.3	49.3
6	13.0	27.7	49.3	62.0	34.7	54.0
7	7.0	19.7	73.3	63.3	28.0	44.7
8	4.0	13.0	70.0	60.0	54.0	32.5
9	7.1	19.0	29.8	39.3	-	-
10	4.3	21.0	76.7	80.0+	20.0	55.0
11	11.3	33.0	40.5	80.0+	-	-
12	9.7	35.3	80.0+	80.0+	-	-
13	6.5	31.0	80.0+	70.0	-	-
	7.1	16.8	80.0+	80.0+	-	-
15	12.6	10.6	80.0+	80.0+	20.5	42.0
16	20.0	10.0	80.0+	80.0+	49.3	43.3
i7	10.8	11.2	80.0+	80.0+	23.5	34.0
18	18.2	5.0	65.0	80.0+	67.0	35.7
19	22.0	24.8	70.0	80.0+	27.5	50.0
20	6.3	21.3	80.0+	80.0+	15.0	53.3
21	5.0	19.3	80.0+	70.0	23.3	65.0
22	7.5	18.5	48.0	49.0	40.0	47.3
23	6.8	28.5	65.0	44.0	52.7	70.0
24	12.3	19.0	39.5	33.5	52.5	64.0
48	3.0	4.6	7.7	8.0	5.3	8.4
For 0-24 hr: Mean (SD)	12.1 (8.3)	19.1 (9.7)	59.0 (23.0)	57.6 (25.0)	36.5 (18.9)	42.9 (16.7)

TABLE 5. Fluorometry readings as oil equivalents (in ppm) during dosing period at the oil site (Site 0). Oil concentrations were much lower and more uniform at this site.

Hours After Dosing	AREA 1 (Coral)	AREA 2 (Coral)	AREA 3 (Seagrass)	AREA 4 (Seagrass)	AREA 5 (Mangrove)	AREA 6 (Mangrove)
0	1.6	-	-	-	-	-
1	1.9	3.0	2.2	1.8	2.5	2.5
2	1.8	2.9	2.7	2.0	3.0	3.5
3	1.5	4.0	3.1	2.7	3.6	3.3
4	1.5	4.0	3.1	2.7	3.6	3.3
5	-	2.0	2.2	2.5	3.5	3.4
23	1.3	1.4	1.9	-	2.3	2.5
24	1.3	1.8	-	-	-	-
26	1.0	2.1	1.8	1.3	2.0	2.6
27	1.2	2.1	1.9	1.8	2.3	3.1
28	-	-	-	-	2.3	3.4
148	1.1	2.1	1.9	1.3	2.1	2.8
49	-	1.9	1.7	1.2	2.0	2.8
Mean (SD)	1.4 (0.3)	2.5 (0.9)	2.3 (0.5)	1.9 (0.6)	2.6 (0.6)	3.0 (0.4)

one-hour intervals. The results in Table 4 show variability, and generally, the concentrations at Site D were lower over the coral area and higher over the seagrass area. The readings listed as 80 ppm are actually higher as the fluorometer quenched at this reading; thus, these readings are 80 ppm plus. Concentrations of oil in the mangrove area were a little lower than the sea-grass area, but generally, an oil dose exceeding that desired for seagrasses was achieved. Table 5 shows the hourly averaged concentrations under the oil slick at Site O. Concentrations are less than 3 ppm and are fairly uniform. These low concentrations were expected because the oil would be more in the dissolved phase than present as an oil dispersion as was the case for Site D.

Samples taken during the dosing period and analyzed by GC and GC/MS are expressed as oil equivalents in Table 6. These also show some variability but indicate that the measurements reached concentrations as high as 222 ppm. Qualitative analysis shows a full range of oil hydrocarbons present in the extracts. The dichloromethane extracts of the water samples taken during the introduction of chemically dispersed oil show the hexadecane (C_{16}) spike, as well as a clear definition between C_{17}/pristane and C_{18}/phytane. Discrete water samples taken during the oiling of Site 0 are shown in Table 7. Chromatograms of dichloromethane extracts from Site 0 show the larger octadecane peak from the addition of this hydrocarbon to the oil as an internal standard prior to oil discharge. The qualitative analysis indicated no cross-contamination between sites.

Water samples taken during the dispersed and untreated crude oil re-leases were analyzed for LMW hydrocarbons. The results are given in Table 8. The table is self-explanatory except for the term "octanes or cyclo-hexanes" which refers to hydrocarbon peaks of specific chromatographic retention time.

The results indicate that, as expected, the water column concentrations of petroleum hydrocarbons at Site D were much higher than at Site O. The coral area of Site D averaged 293 ppb during the period of dosing, while the corals at Site 0 averaged 33 ppb. The seagrass area had the highest concentrations with an average total LMW hydrocarbons of 684 ppb, while at Site 0, seagrasses received 44 ppb. The mangrove area of Site D was exposed to an average of 367 ppb, while Site 0 averaged 56 ppb. The aromatic hydrocarbons benzene, toluene, the xylenes, and the trimethyl benzenes had the highest concentrations at Site D. The cycloalkanes were next which fits

TABLE 6. Analysis of oil hydrocarbons as oil equivalents (in ppm) in discrete extracts of the dispersed oil site (Site D) show the higher variability and exposure level at this site.

Hours Dosing	AREA 1 (Coral)	AREA 2 (Coral)	AREA 3 (Seagrass)	AREA 4 (Seagrass)	AREA 5 (Mangrove)	AREA 6 (Mangrove)
5	-	5.8				
6	-	-	3.9	9	2.2	0
7	-	4.9		28.4	4.2	
8	-	1.5		8.9	-	189.4
9	0.22	-	32.2			
11	-	14.9				
12	3.1	-	70.1	10.9		
13	-	5.6				
14	2.0	-	31.5			
16	11.7	-		0.24	222.0	
20	-	-		11.0	-	20.0
21	-	5.1				
22	-	-	35.7	14.1		
24	-	-	7.5	-		

TABLE 7. Analysis of oil hydrocarbons as oil equivalents (in ppm) in discrete extracts at the oil site (Site 0) indicate lower, more uniform exposure.

Hours After Dosing	AREA 1 (Coral)	AREA 2 (Coral)	AREA 3 (Seagrass)	AREA 4 (Seagrass)	AREA 5 (Mangrove)	AREA 6 (Mangrove)
1	0.7	-	-	0.07	-	-
2	-	0.03	-	0.04	-	0.07
3	0.02	-	0.07	-	-	-
4	-	0.02	-	0.06	0.06	-
24	0.03	-	-	-	-	-
25	-	-	0.04	0.02	0.02	0.03
26	0.01	0.05	0.04	0.09	-	-
27	0.02	-	0.06	-	-	-

TABLE 8. Concentrations (in ppb) of low-molecular-weight hydrocarbons during oil dosing at both sites.

HYDROCARBON	SITE D (Coral)	SITE O (Coral)	SITE D (Seagrass)	SITE O (Seagrass)	SITE D (Mangrove)	SITE O (Mangrove)
methane	0.48	0.23	1.74	0.27	0.34	0.49
ethane	0.27	0.07	0.88	0.17	0.37	0.37
propane	1.81	0.12	3.98	0.31	2.59	0.48
isobutane	0.92	0.16	2.81	0.15	1.78	0.21
n-butane	5.42	0.29	11.53	0.30	7.22	0.74
isopentane	4.47	0.30	9.41	0.66	5.87	0.61
n-pentane	6.58	0.45	13.94	0.42	7.98	0.85
2,2-dimethylbutane	0.46	0.24	1.01	0.00	0.28	0.06
cyclo + 2-methylpentane	6.23	0.40	13.87	0.36	7.97	0.67
3-methylpentane	1.90	0.13	4.45	0.11	2.19	0.18
n-hexane	4.37	0.35	9.85	0.19	4.78	0.30
methylcyclopentane	8.91	0.67	18.96	0.51	10.63	1.03
benzene	14.69	0.66	39.13	9.79	23.92	15.89
cyclohexane	15.95	0.80	35.04	0.77	19.48	1.65
n-heptane	7.03	0.36	16.90	0.40	7.90	1.02
methylcyclohexane	12.60	0.73	29.34	0.64	14.42	1.09
toluene	48.39	8.36	106.45	4.64	63.37	5.29
octanes or cycloheptanes	3.83	1.89	6.11	2.52	3.16	1.85
octanes or cycloheptanes	5.96	2.17	12.20	3.68	6.42	2.15
octanes or cycloheptanes	4.38	0.23	6.55	0.30	5.22	0.65
octanes or cycloheptanes	4.58	0.33	7.01	1.38	2.19	0.45
octanes or cycloheptanes	2.49	0.09	5.78	0.27	3.31	0.22
ethylbenzene	7.55	0.00	23.95	0.12	10.42	1.10
m- & p-xylene	38.78	2.75	90.86	3.73	44.35	4.94
o-xylene	27.45	1.38	80.46	3.64	39.31	2.80
isopropylbenzene	3.37	0.48	8.06	0.28	4.16	0.87
C3-benzenes	14.49	2.87	32.49	1.99	16.52	2.85
o-methylbenzene	10.13	2.30	9.93	2.62	5.78	2.35
1,2,4-trimethylbenzene	22.69	2.61	62.81	2.23	35.41	2.95
1,2,3-trimethylbenzene	7.14	1.99	18.91	1.49	9.41	1.86
Total Average Concentration	293	33	684	44	367	56

well with expected solubility data. As with the higher-molecular-weight hydrocarbons, it is obvious that the chemical dispersant significantly increased the concentration of oil hydrocarbons for the whole molecular-weight range to which the organisms were exposed.

Sediment Analysis

The hydrocarbon concentration in the sediments of the 3 areas are low (less than 1 ppm) prior to the addition of oil (Table 9). A few days after site treatment, Site D showed higher concentrations in the seagrass sediments (45 ppm) than in the mangrove sediments (16 ppm) However, at Site 0, the mangrove sediments averaged 92 ppm, while the seagrass sediments were relatively uncontaminated and were slightly higher than the baseline hydrocarbon concentrations. At both sites, the petroleum hydrocarbon concentrations were highly variable, as indicated by the high standard deviation in these measurements (Table 9). This high variability was more evident at Site D than at Site O. At Site D, there were pockets of slick oil that re-formed on the water surface during treatment and resulted in a very patchy coating of oil, while at Site O, the slick oil formed a relatively even coating over the entire site.

Two days after site treatment, the highest concentrations of whole oil at Site 0 were measured in the high intertidal portion of the mangrove forest

(Fig. 15) . Lower oil concentrations were measured in the midintertidal and subtidal areas of the mangrove forest. The distribution of oil at Site D was almost reversed, with the highest concentrations measured in the subtidal sediments.

The concentration of petroleum hydrocarbons in the subtidal mangrove sediments at Sites D and 0 were about the same (Fig. 15), possibly because the water depth in both sites was about 10-20 cm, and with such a high concentration of oil, the hydrocarbons transferred quickly to these subtidal sediments. The sediments in the seagrass area of Site D were similarly affected. After a few days, the mean concentration of these subtidal sediments in Site D (20- to 40-cm water depth) was 44.7 ppm.

Four months after site treatment, there were still high concentrations of oil in the mangrove and seagrass sediments at Sites D and O. Figure 15 illustrates an increase in the concentrations of oil in the high intertidal and midintertidal mangrove sediments. This was more evident at Site 0 than at

TABLE 9. Petroleum hydrocarbon results for sediment samples (ppm wet weight).

	SITE D	SITE 0	SITE R
PRESPILLL (November 1984)			
Mangrove sediments	0.6 +/- 0.3	0.9 ± 0.6	0.6 ± 0.6
Seagrass sediments	0.3 ± 0.1	0.4 ± 0.3	0.3 ± 0.0
Coral sediments	-	-	0.9 ± 0.7
3 DAYS POSTSPILL (December 1984)			
Mangrove sediments	16 ± 27	93 ± 47	0.5 ± 0.2
Seagrass sediments	45 ± 33	1.2 ± 0.7	0.5 ± 0.2
4 MONTHS POSTSPILL (March 1985)			
Mangrove sediments	89 ± 77	140 ± 136	0.8 +/- 0.4
Seagrass sediments	21 ± 10	6 ± 4	0.4 ± 0.1
7 MONTHS POSTSPILL (June 1985)			
Mangrove sediments	179 ± 263	229 ± 281	-
12 MONTHS POSTSPILL (December 1985)			
Mangrove sediments	125 ± 66	552 ± 713	-
Seagrass sediments	27 ± 4	2 +/- 1	-
Coral sediments	7 +/- 2	0.4	-
20 MONTHS POSTSPILL (August 1986)			
Mangrove sediments	185 ± 88	254 ± 307	-

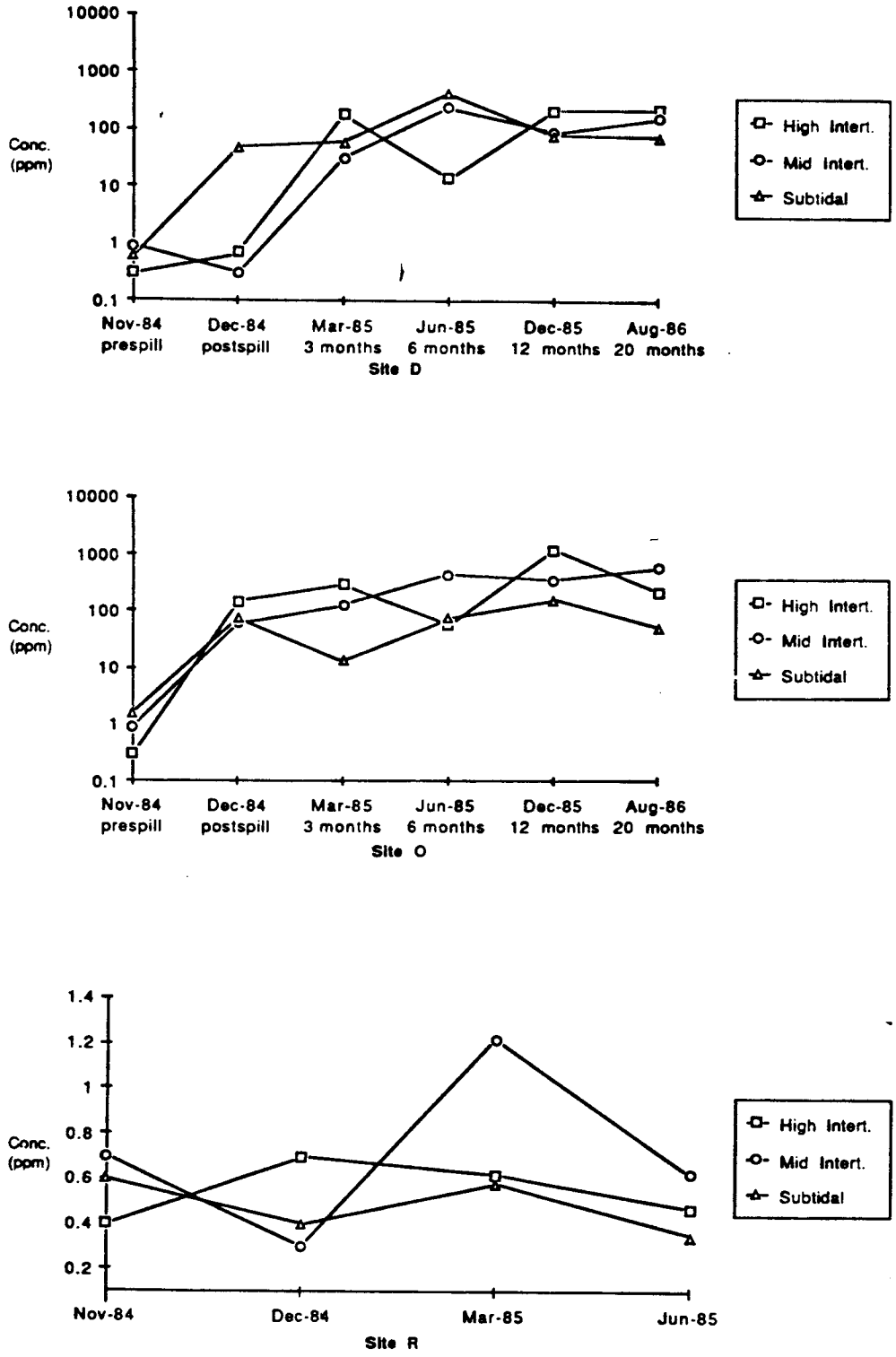


FIGURE 15. Hydrocarbon concentrations of mangrove sediments.

Site D and is presumably attributable to the redistribution of oil around the sites due to tidal and current action. The subtidal sediments were not affected to the same degree with mean concentrations of 31 ppm and 123 ppm, respectively. The hydrocarbon levels in the seagrass sediments were 21 ppm at Site D and also slightly elevated in Site 0 at 6 ppm (Fig. 16).

At 7 months after the spill (June 1985), there was evidence of even greater redistribution of oil. The means of all mangrove sediments at Sites D and 0 increased to 179 ± 263 ppm and 229 ± 282 ppm, respectively. Due to sampling bias in trying to gain more information on the intertidal area, we obtained only a few samples from this high intertidal area and, therefore, appeared to sample only patches that were not severely contaminated. How-ever, in the midintertidal area, 7 full sets of intertidal surface scrapes were sampled; the means were 232 ± 302 ppm and 420 ± 317 ppm for Sites D and 0, respectively. The subtidal sediments were higher at Site D than at Site 0, although this result was biased by one sample with a high hydrocarbon content at Site D. Unfortunately, the seagrass samples were damaged in transit so it was not possible to obtain data at this time period.

At the 12-month sampling period (December 1985), sampling was concentrated in Site 0 where there was severe adult mangrove damage. The mean of hydrocarbon concentrations in Site 0 was 552 ± 713 ppm, the high standard deviation indicating the high variability. At Site D, the levels were only 125 ± 66 ppm. The high intertidal sediments were again an area of high hydrocarbon concentration. Whole liquid oil was still present in areas of both sites even one year after the dosing indicating the long-term nature of a spill in these ecosystems. Also, the concentration of oil in the seagrass sediments of Site D was still elevated, which may indicate a long-term build-up of these compounds in these sediments because of the chemical dispersion of oil and subsequent incorporation into the coral-rubble substrate. Figure 16 illustrates the pattern of hydrocarbon buildup over the 12 months in the seagrass sediments. There is no data point for the 20-month visit since sampling was concentrated in the areas that showed biological damage (the mangroves).

After 20 months (August 1986), the oil in the surface mangrove sediments was still high and extremely variable, with the greatest variability in the sediments of Site O. Concentrations were 185 ± 88 ppm for Site D and 254 ± 307 ppm for Site O. There was still a great deal of whole oil locked up in the porous, peaty sediment material. GC analysis of the samples

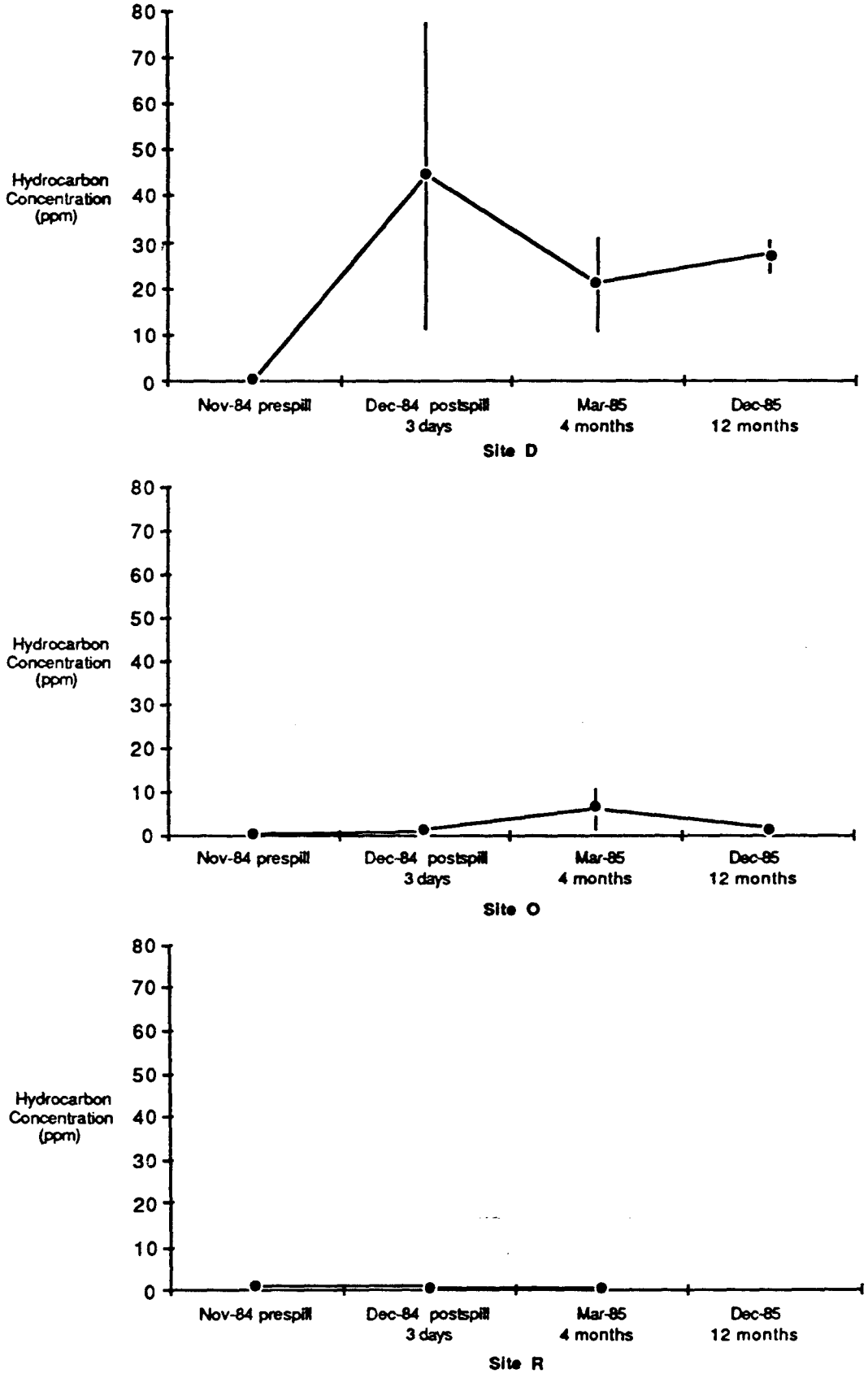


FIGURE 16. Hydrocarbon concentrations of seagrass sediments.

indicated that although some degradation occurs, it is very slow. The presence of whole oil after 20 months in some areas of both sites confirmed this.

Analysis of Sediment Cores

Sediment cores were taken to determine if oil penetrated further into the sediments from dispersant addition. The first cores were taken at the 7-month sampling period (June 1985). At 7 months, a core taken at the high-tide area of Site D indicated slight hydrocarbon contamination (16 ppm) of the surface sediments and no contamination at 8-10 cm depth (Table 10). The subtidal core had higher oil concentrations at the surface but no oil contamination at 8-10 cm. At the 12-month sampling period, there was significant oil penetration of the high intertidal and midintertidal sediments at Site D, but none in the subtidal area. At Site 0, there was no change of hydrocarbon concentration with depth in the high intertidal and subtidal area sediments. There was penetration in the midintertidal sediments. The oil penetration of the high and midintertidal sediments of Site D and midintertidal of Site 0 is presumably caused by the hydraulic motion of the tide as well as the presence of holes caused by burrowing organisms.

The subtidal core taken at Site D at the 20-month time period indicates low surface concentrations with higher concentrations at depth. This anomaly may be caused by the presence of a bore hole. One core was sampled 20 months after the spill in the high intertidal area of Site D, and no contamination was found at the 33-cm level.

A more detailed analysis of one core from the high-tide area of Site 0 where mangrove death was evident indicated a surface sediment oil content of 109 ppm at 0-2 cm depth. There was then a large increase in concentration to 902 ppm at 3-5 cm followed by a tailing off of 55 ppm at 6-10 cm depth. Background levels existed below 10 cm.

Unfortunately, there was high variability in petroleum hydrocarbon concentrations, and only a limited amount of samples could be taken. To draw conclusions on this small and highly variable data set is difficult. However, there did not appear to be much difference in sediment accumulation of petroleum hydrocarbons whether the oil was chemically dispersed or not.

TABLE 10. Analysis of sediment cores taken in the mangrove area at Sites D, 0, and R. All results reported as ppm wet-weight hydro-carbon. Depth of sample (in cm) given in parentheses.

	SITE D	SITE 0	SITE R
HIGH- INTERTIDAL AREA			
7 Months Postspill (June 1985)	16 (0- 2) 5 (8-10)		
12 Months Postspill (December 1985)	208 (0- 2) 33 (8-10)	357 (0- 2) 4 (8-10)	
20 Months Postspill (August 1986)	252 (0- 2) 5 (33-35)	109 (0- 2) 902 (3- 5) 55 (6-10) 8 (11-17) 6 (18-21) 5 (22-24)	
MIDINTERTIDAL AREA			
7 Months Postspill (June 1985)	210 (0- 2) 32 (8-10)		
Months Postspill (December 1985)	78 (0- 2) 87 (8-10)	133 (0- 2) 43 (8-10)	
20 Months Postspill (August 1986)	101 (0- 2) 77 (15-18)	173 (0- 2) 9 (10-12)	
SUBTIDAL AREA			
7 Months Postspill (June 1985)	421 (0- 2) 8 (8-10)		0.5 (0- 2) 0.5 (8-10)
12 Months Postspill (December 1985)	68 (0- 2) 5 (8-10)	1612 (0- 2) 6 (8-10)	
20 Months Postspill (August 1986)	2 (0- 2) 61 (8-10)		

Seawater Analysis

Water samples were passed through XAD-2 resin columns at each sampling period. The results are given in Table 11 and Figure 17. During the prespill sampling (November 1984), the blanks were somewhat higher than normal, and this is reflected in the higher concentrations. However, each sample could be compared to the value of Site R at each sampling period as the XAD-2 resin columns are prepared in batches and are handled similarly at each time period. Also, care was taken to ensure that the samples were taken prior to any other work being carried out at each site. This prevented oil release caused by human activity in the site; therefore, we believe that these data are a true reflection of the concentration of hydrocarbons emanating from the sites.

In the immediate postspill period, the levels were similar at Site D (8.9 ppb) and Site 0 (10.2 ppb). After 4 months, hydrocarbons were still being leached out of both sites with indications of higher concentrations present at Site D (5.4 ppb). As was the case in the sediments, there was a high degree of variability. At month 7, the levels had decreased to 0.6 and 0.7 ppb for Sites D and 0, respectively. At months 12 and 20, there were still traces of hydrocarbons emanating from Site D. As discussed in the previous section, there appeared to be such a large amount of residual hydrocarbons remaining in the sediments of the sites that even after month 20, there were detectable levels of hydrocarbons in water at the prop roots of the man-groves at Site D. The concentrations at Sites D and R were background, and the water was essentially clean.

Plant Tissue Analysis

The analyses of leaf samples from mangroves and seagrasses are shown in Table 12. Soon after site treatment, the seagrass leaves at Site D were coated with oil. This appeared not to be a tissue uptake mechanism as after the 4-month sampling period, the hydrocarbon concentration of samples had reduced to baseline levels. Unfortunately, the seagrass samples from Site 0 did not survive the transport to the BBS laboratory; therefore, there are no data on the seagrass concentrations at Site 0 soon after oiling. The elevated levels reported in the Site 0 seagrasses at the 12-month period are natural lipids and not petroleum hydrocarbons. No explanation was found for why this is three times higher than Site R.

TABLE 11.. Analysis of XAD-2 resin columns of water samples. All concentrations reported as ppb ±1 standard deviation.

	SITE D	SITE 0	SITE R
Prespill (November 1984)	0.7 ± 0.2	0.6 ± 0.0	0.6 ± 0.2
3 Days Postspill (December 1984)	8.9 t 2.1	10.2 ± 6.0	0.8 t 0.5
4 Months Postspill (March 1985)	5.4 ± 3.8	- 1.5 ± 0.3	0.9 ± 0.2
7 Months Postspill (June 1985)	0.6 ± 0.2	0.7 ± 0.3	0.1 ± 0.0
12 Months Postspill (December 1985)	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
20 Months Postspill (August 1986)	0.4 ± 0.5	0.1 ± 0.0	0.1 ± 0.0

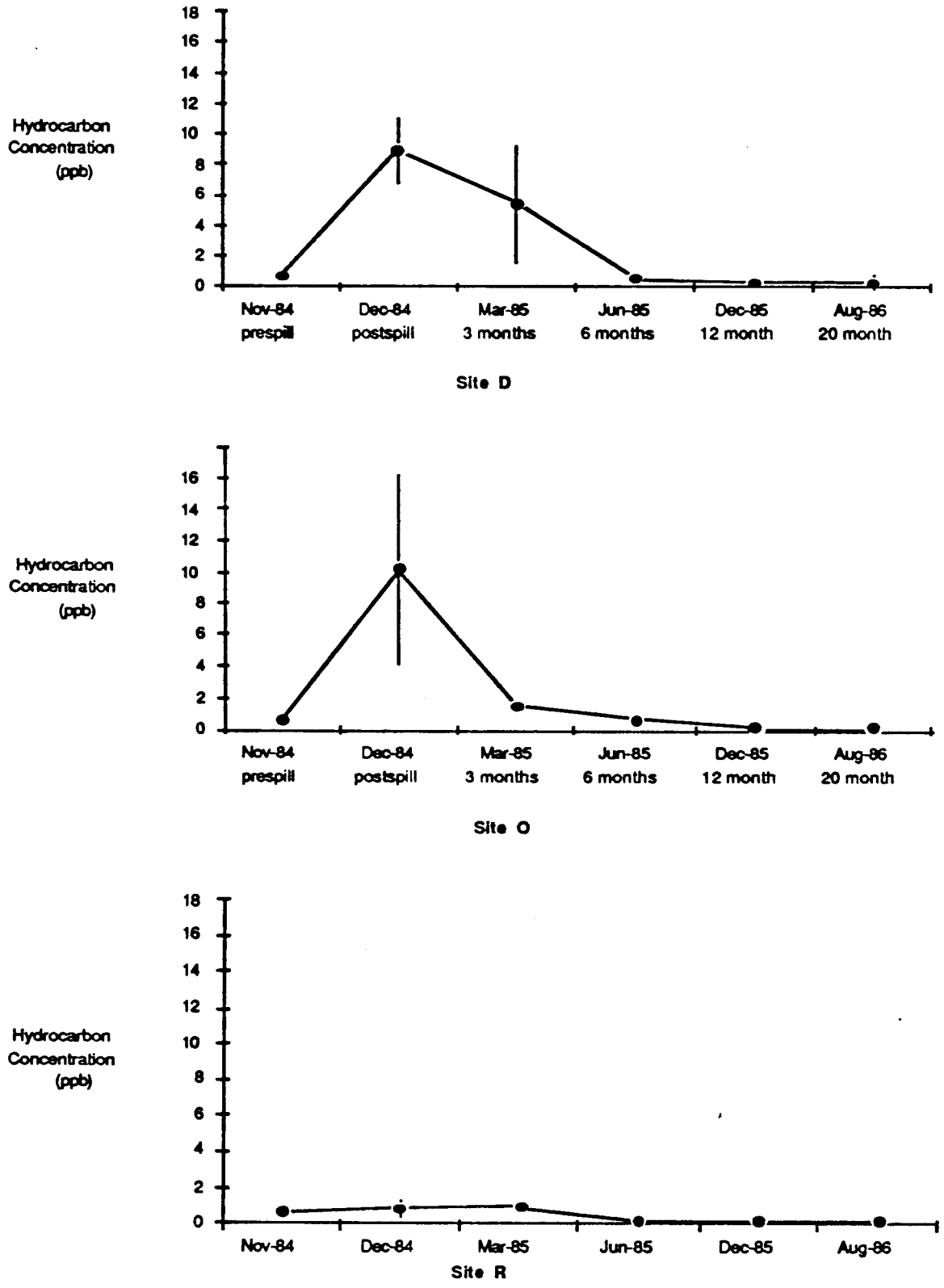


FIGURE 17. Hydrocarbon concentrations of XAD-2 water extracts.

TABLE 12. Analysis of plant tissue samples. All concentrations are reported as ppm wet-weight equivalents ±1 standard deviation. [* = Combined group of 30 seagrass leaves. ** = Combined group of 10 mangrove leaves.]

	SITE D	SITE 0	SITE R
PRESPILL (November 1984)			
Mangrove leaves	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
Seagrass leaves	0.7 +/- 0.1	0.1 ± 0.0	0.2 ± 0.2
3 DAYS POSTSPILL (December 1984)			
Mangrove leaves	0.6 ± 0.5	0.5 ± 0.5	0.4 ± 0.3
Seagrass leaves	6,465 ± 512	(5,851.4)	0.4*
4 MONTHS POSTSPILL (March 1985)			
Mangrove leaves	0.2 ±	3**	0.8 ± 0.1
Seagrass leaves	0.9 ± 0.	2 +/- 0.7	0.8 +/- 0.3
7 MONTHS POSTSPILL (June 1985)			
Mangrove leaves	3 +/-	0.5 ± 0.6	0.5 ± 0.3
Seagrass leaves	3 ± 1	2 +/- 3	0.3 ± 0.3
12 MONTHS POSTSPILL (December 1985)			
Mangrove leaves	2 ± 0.9	3 # 2	2**.
Seagrass leaves	5*	11 t 2	4*
20 MONTHS POSTSPILL (August 1986)			
Mangrove leaves	4**	3**	
Seagrass leaves	0.2*	2*	

The mangrove leaves (Table 12) did not take up oil, although 1 sample at Site 0 was extremely high a few days after the treatment (5,851 ppm). GC analysis indicated this was whole oil and was probably inadvertent contamination. The concentrations over time were not significantly higher than the control. It was concluded that the mangrove leaves did not significantly take up hydrocarbons from the seawater or sediments.

Oyster Tissue Analysis

Samples of Crassostrea rhizophorae were taken to determine uptake and depuration of hydrocarbons. Groups of 100 oysters were taken during each time period up to 12 months from each site. The results of the analysis are given in Table 13 and Figure 18. The results show a rapid tissue uptake of 506 and 679 ppm for oyster tissues in Sites D and 0, respectively. At the 4-month postspill period, there is a reduction to 161 and 134 ppm, respectively. However, after 12 months, most of the oil hydrocarbons were no longer present in the tissue of oysters from both sites.

The byssal threads exhibit a similar pattern, with a rapid uptake to 4,015 and 5,303 ppm, respectively (Table 13). This increase in concentration is most probably due to surface adsorption rather than tissue uptake. After 4 months, the levels were reduced to 422 and 1,182 ppm, respectively. It is interesting to note that at Site 0, the levels were significantly higher than at Site D. This may have been due to a sampling bias or to a greater exposure of oysters to floating oil from Site O. The small sample size could not resolve this difference. However, the loss of oil from the threads was greater than in the tissue which is due to the more rapid removal of the ad-sorbed hydrocarbons than tissue-accumulated hydrocarbons.

TABLE 13. Analysis of oyster tissue and byssal threads. All values re-ported as ppm hydrocarbon wet weight. Prespill concentrations are the mean from the reference Site R. All analyses were made from 100 individual oysters and the standard deviation of replicate analyses was ±18 percent.

	SITE D		SITE 0		SITE R	
	Tissue	Threads	Tissue	Threads	Tissue	Threads
Prespill (November 1984)					34	21
3 Days Postspill (December 1984	507	4,015	679	5,303		
4 Months Postspill (March 1985)	161	422	134	1,182		
7 Months Postspill (.June 1985)	120	122	114	66		
12 Months Postspill (December 1985)	31	246	17	281		

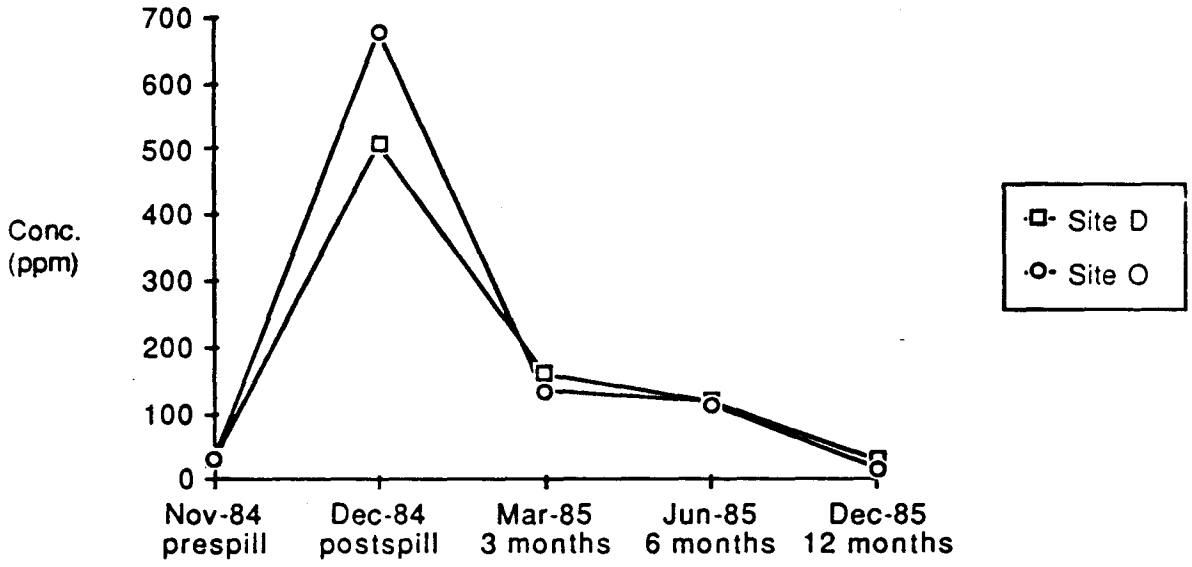


FIGURE 18. Hydrocarbon concentration of oyster tissue.

INTERTIDAL SYSTEMS

The intertidal ecosystem of the study sites was dominated by moderately developed mangrove forests typical of sheltered coastal areas throughout the Caribbean Sea. The mangrove communities were dominated by red mangroves (Rhizophora mangle). A few white mangroves (Laguncularia racemosa) also were present atone of the sites. The area within each site was intertidal and was completely inundated during all high tides and almost completely exposed during low tides, except for the outer fringe of the forest.

Molluscs, echinoderms, and crustaceans dominated the fauna of the man-grove forest. Prop roots of R. mangle were colonized by several species of oysters. Sea urchins, snails, brittle stars, and other mobile animals also were common. Within the forest, the mangrove tree snail (Littorina angulifera) was the dominant macrofaunal species. Tree crabs (Aratus pisonii) and land crabs (Cardisoma guanhumi and Gecarcinus lateralis) also were relatively common. Many species of fish were found among the man-grove prop roots, especially snapper, barracuda, and anchovies. Larval shrimp (Penaeus sp.) also were abundant.

METHODS AND MATERIALS - MANGROVE STUDIES

Determination of Macrostructural Characteristics

The parameters used to describe the structural characteristics of the mangrove communities were individual tree location, species composition, di-ameter at breast height (DB H) , tree height, leaf area index (LAI), and canopy density. In addition, a photographic record of each site was made showing canopy closure, forest density, and other points of interest.

The sites were measured with fiberglass measuring tapes and a Suunto siting compass. The four corners were permanently marked with stakes and surveyors' flagging.

Within the mangrove forest at each site, 3 transects were established running perpendicular to the water's edge, each incorporating 3 sample stations. The transects were used for measurements of canopy density, LAI, phenology, sediment sampling, soil water salinity, and other measurements.

All trees within the study sites were identified as to species and numbered; their location, trunk diameter, and total height were determined and re.:crded (Fig. 19). Location was *found* by measuring the X-Y coordinates

FIGURE 19. Each mangrove tree within the study sites was located, la-belled, and measured to determine the structural characteristics of the mangrove forest.

of each tree from a known datum. The distances were determined with a fiberglass tape and optical distance measurer (Opti-Meter, Ranging, Inc., Rochester, N.Y.). Tree height was determined with either the above-referenced optical distance measurer or a telescoping fiberglass measuring pole (Forestry Supplies, Inc., Jackson, Miss.). Height was measured in meters from the surface of the substrate to the highest point on the tree. The diameter of the tree bole (DBH) was measured in centimeters at 1.3 m above the substrate surface. DBH is measured with a special tape which is graduated in pi ($3.1^{1}4$) units. The tape is wrapped around the trunk (bole) of the tree and is read as tree diameter. Because of the unusual growth forms of some mangrove trees, the determination of bole diameter and number of boles is sometimes difficult. To standardize these measurements, the recommendations of Cintron and Schaeffer-Novelli (1984) were used. They are as follows:

1) If the tree has multiple boles and these boles fork or sprout from a common base below breast height (1.3 m), each is measured as a separate bole.
2) If the tree has multiple boles and they fork above or at breast height, determine the DBH at 1.3 m or just below the swelling caused by the fork (i.e., if the fork is at or above breast height, it is considered as only one bole).
3) If the bole has a fluted trunk or prop roots at breast height, the diameter is measured above them at a location where the fluting and/or prop roots do not bias the measurements.
4) If the bole has swellings, branches, or abnormalities at breast height, the DBH should be measured either above or below the irregularity where it stops affecting normal form.

LAI was determined using a modification of the plumb-bob method, in which a rod was inserted through the forest canopy and the number of leaves touching the rod was counted. The number of leaves touching the rod is the LAI. Forest canopy density measurements were determined with a spherical densiometer (Lemmon, 1956, 1957). This pocket-type instrument employs a spherical convex mirror for the reflection of the overhead forest canopy. The mirror is scribed with a grid system used to estimate percent-age of coverage and equipped with a bubble level so the unit can be held level during use. All readings were taken at 1.3 m above the forest floor.

LAI and canopy density were collected along three transects within each study site. Three locations along each study transect were permanently marked. At these stations, 3 canopy density and 5 LAI measurements were taken.

Basal area is the space occupied by a tree bole at the point where the DBH is measured. Basal area is usually reported as surface area of bole (m^2) per hectare. The equation to calculate basal area is as follows when DBH is in meters: basal area (m^2) = $(DBH/2)^2$ x pi. Density is the number of individuals per unit surface area (usually reported as individuals per hectare). Mean stand diameter is the diameter of the bole of mean basal area. This is not the same as the arithmetic mean of the DBH measurements for the forest stand. Bole volume is a function of DBH and height and is a measure of the area occupied by the tree bole. The equation for volume is as follows when DBH and height are measured in meters: bole volume (m^3) - 0.333 x pi x $(DBH/2)^2$ x h.

The structural characteristics of each site were determined during the first baseline survey in March 1984. These measurements included tree lo-cation, species composition, DBH, and tree height. All statistical comparisons of data were accomplished using analysis of variance (ANOVA) with p = 0.05 set as the level of significance.

Determination of Microstructural Characteristics

The functional and microstructural characteristics of individual trees were determined for each of the three study sites. Three trees along the center study transect were chosen for these determinations. The trees were located near the water's edge, in the center, and at the rear of the study site. Each tree was evaluated for leaf production rates, leaf length and width, the incidence of herbivory and deformities, and growth of respiratory organs.

Three branches from each tree were selected to measure new leaf pro-duction. Each branch was tagged with an identification number, and the leaves were marked in reference to their positions on the branch. The leaf marking was done by punching a series of small holes in the leaf, the number corresponding to its position on the branch (Fig. 20). The branches, leaf numbers, and positions were monitored for the duration of the study.

FIGURE 20. Nine branches on adult mangrove trees were tagged and monitored at each site to determine longevity of leaves and to measure new leaf production. Small holes were punched into each leaf, the number of holes corresponding to the location of the leaf on the branch.

Three branches from each tree were chosen to determine the structural characteristics of the leaves. The length, width, and incidence of herbivory and deformities were recorded. Herbivory (consumption of leaf material by herbivores, especially insects) and leaf deformities (abnormal shape) were determined by visual inspection.

Several aerial roots were tagged and measured in each site. These organs were monitored for growth rate (cm/day) and density of lenticels (gas exchange organs present on the roots). It has been proposed that when mangroves are exposed to respiratory stress (e.g., coating with oil), one response of the plant is to rapidly generate new lenticels.

Microstructural characteristics of the intensively monitored trees were measured during both baseline surveys and through the 20-month postspill survey. These measurements included leaf phenology, leaf structure, LAI, canopy density, seedling density, and seedling growth rates. Certain parameters (prop-root growth and lenticel growth) were monitored only through the 7-month revisit.

Propagules

At each site, 3 groups of 25 red mangrove propagules were planted during the March 1984 baseline survey and 1 week after the site treatments in December 1984. These propagules were monitored for sprouting success, height, leaf number, phenology, and leaf structure at each site survey peri-od.

Fauna

Detailed transect surveys of the density and distribution of L. angulifera were conducted during each survey period. Observations were made at 9 permanent stations established on the 3 transects previously de-scribed. At each station, the number of snails present in 6 vertical compartments from the sediment surface to the canopy was counted. All snails present within a 5-m radius of the station were counted. This allowed a de-termination of the density and horizontal and vertical distribution of tree snails within each site.

At each site, five separate prop roots were permanently marked, and the number of each species of mangrove oyster present was counted. The

survival of these groups of oysters was monitored throughout the study period.

Interstitial Water Quality

Measurements of interstitial water salinity and pH were conducted at each site. Three PVC pipes of different lengths were driven into the sediments to depths of 20 cm, 60 cm, and 150 cm. Water samples were obtained from each depth during each sampling period through the 7-month revisit.

RESULTS AND DISCUSSION

The three sites can be classified as overwash forests (Snedaker and Lugo, 1973; Lugo and Snedaker, 1974). The forests exhibited the typical structural, topographical, and hydrographical characteristics of this forest type. The study sites are inundated during each tidal cycle, thus resulting in the removal of most of the accumulated litter fall and organic material. The tree architectural characteristics, moderately sized trees dominated by red mangroves with extensive prop-root development, were similar to over-wash forests located on the west coast of southern Florida. Study Sites D and R contained only red mangroves. Site 0 contained red mangroves and one white mangrove.

Table 14 presents the structural characteristics of the 3 study sites. The most obvious difference among the sites was in regard to density. Site 0 had a higher density of individual trees than the two other sites. This difference in density was within the range of densities reported for other similar mangrove forests in the Caribbean (Table 15).

General Observations of the Intertidal Study Sites During and After Site Treatment

Dispersed Oil Site (Site D)

The release of the premixed oil and dispersant caused the formation of large clouds of dispersed oil and some small slicks of undispersed oil, both of which entered the mangrove forest with the rising tide. The intertidal surface of the mangrove prop roots and the substrate surface received a light coating of oil that appeared as a dull, greasy sheen. In a few areas, especially in depressions on the substrate, small quantities of undispersed,

TABLE 14. Structural characteristics of mangrove forests at each study site measured in March 1984.

	SITE D (Dispersed Oil)	SITE 0 (Oil)	SITE R (Reference)
Surface area (m^2)	462	479	533
Density (individuals)*			
Per site	72	149	108
Per hectare	1,555	3,109	2,028
Basal area (m^2)*			
Per site	0.67	0.65	0.72
Per hectare	14.45	13.48	13.58
Volume (m^3)*+			
Per site	0.98	1.05	1.08
Per hectare	21.10	21.95	20.36
Mean stand diameter (cm)*	10.88	7.43	9.24
Mean height (m)	4.9	4.2	4.3

*All trees.
+Volume calculated at 1.3 m above ground surface.

TABLE 15. Comparison of selected structural components for mangrove overwash forests. The structural characteristics of the mangrove forests used in this study are *very* similar to these other forests.

LOCATION	BASAL AREA (m^2/hectare)	STAND HEIGHT (m)	DENSITY (#/hectare) (>2.5 cm DBH)	COMPLEXITY INDEX
FLORIDA*				
Ten Thousand Islands	15.0	6.3	1,000	3.4
PUERTO RICO*				
Jobos Bay	15.5	4.8	11,650	26.0
MEXICO*				
Isla Roscell	28.5	8.0	1,480	10.1
PANAMA				
Site D	14.4	8.7	1,555	1.9
Site 0	13.5	8.9	3,109	3.6
Site R	13.6	9.5	2,028	2.6

* Data from Pool et al., 1977.

slick oil had collected. With each tidal cycle, the quantity of oil within the forest was reduced until by day 4 postspill, little or no slick oil remained. The surface of the prop roots retained a very light coating of oil that was detectable by touch and smell.

Observations taken 48 hours after dosing showed that the mangrove forest floor had been completely covered with a thin layer of oil. A layer of oil that was not dispersed had moved into the mangrove forest and covered the substrate and prop roots. The prop roots were coated with a light film of oil to the level of the high tide. Small quantities of oil were observed to a height of 2 m on some of the trees. This oil was most probably transported by animals (tree crabs and snails). At low tide, the substrate was covered with a thin film of oil, and in low areas, small pools of standing oil had accumulated. The air was pungent with the smell of the oil. Active lenticels which were covered with the oil film showed a black obtained center¬. All tree crabs and many tree snails observed were covered in oil. Crabs were occupying a lower-than-usual vertical position within the forest and exhibited minimum or no escape response. Several dead crabs were observed.

Three days after the spill, an oil sheen was observed throughout the site. Along the fringing edge of the mangroves, within their prop-root zone, an oil slick was observed. All tree crabs and snails observed were coated with oil. Some dead tree crabs were observed (total of 4). The air was pungent with the smell of oil, and the smell of dead animals was also noticeable. Sea anemones attached within the mangrove forest were coated with oil and fully extended, although they still responded when touched.

Six days after the spill, a light oil sheen was present on the mangrove forest substrate and surface waters. The amount of oil on the substrate appeared to have decreased. An oil coating was still observed on the prop roots, although the amount was less than previously observed. Sea anemones had returned to normal, and all tree crabs observed were still oiled and showed no escape or defense response. Oiled' snails were still observed.

During the March 1985 survey (4 months postspill) of Site D, it was difficult to detect it had been oiled at all. No oil was observed on the prop roots or sediment surface. Tree crabs behaved normally, and crabs and snails were evident throughout the site. One tree located in the outer fringe was in seed. The only oil detected was in the pocket area (described

above) in which oil had accumulated during site treatment. Walking on the peat substrate in this area resulted in the release of an oil sheen, and further compaction of the substrate would release small oil droplets. This was the only area in Site D where this was observed.

The condition of Site D remained this way through the final site survey in August 1986., The appearance of the adult and juvenile mangroves appeared unchanged compared to the prespill condition, and the abundance and behavior of resident fauna appeared unchanged as well. The sediments at Site D continued to release *very* small quantities of sheen when disturbed.

Oil Site (Site 0)

During site treatment, large quantities of black oil entered the man-grove forest and tended to collect in the upper portion of the site toward the downwind, eastern corner. Oil was very thick in this area and coated all intertidal surfaces. The oil was deposited directly on the substrate surface during low tides and moved throughout the site when the tide was high.

Following the 48-hour postrelease exposure period, most of the floating slick oil was removed using sorbent materials. The oil had partially weathered by this time and appeared to be slightly more viscous and had less of an odor than during the spill.

Seventy-two hours after the spill, prop roots were covered with oil to the height of high water, although the amount of oil adhering to the roots was much less than that observed at oil spills in which the oil has weathered more before coming ashore. The thickness of oil on the prop roots appeared to be much greater than in the dispersed oil site. The substrate was covered with oil, and heaviest oiling was observed in the eastern portion of the study area. In this area, the ground was completely covered with oil. Many of the snails and tree crabs were covered with oil, and the crabs showed no escape or defense response when approached. The pungent smell of oil was noticeable throughout the site.

Five days after the spill, the amount of oil present was reduced, al-though a heavy coating of oil was still on the prop roots, and oil droplets and small pools of oil were still present. The water surface was covered with an oil sheen. All tree crabs observed were coated with oil and exhibited no escape or defense response.

During the 4-month postspill survey, oil was visibly evident in most of the site. Small patches of oil could be found in depressions on the substrate, and the entire forest floor was blackened and had an oily texture. Sheen was released wherever the substrate was disturbed even slightly. No oil was found absorbed on the mangrove prop roots.

The adult mangroves had experienced severe effects by 4 months. Defoliation of adult trees was evident throughout much of the site and was especially pronounced in the area receiving the greatest quantity of oil. This defoliated area covered about 25 percent of the mangrove area. Eighteen trees were dead, and 34 trees were 50 to 100 percent defoliated. Many of the trees that had not lost all their leaves retained only the two leaves of the first whorl and the juvenile bud.

Adult trees continued to show severe effects from oiling during the 7-, 12-, and 20-month postspill surveys. The number of dead trees increased to 25 by June 1985, after which no additional mortality of adults was detected. Partial defoliation of living trees was evident throughout the site, and the defoliation of each tree averaged about 45 percent.

The trees located in the outer fringe of the forest showed no observable effects, possibly because they were rooted in sediments that were al-ways underwater, thereby reducing exposure to the oil. Only 18 trees (12 percent) showed no signs of defoliation by August 1986.

During this period of increasing impacts to the adult trees, there was a large increase in the number of mangrove propagules in the areas opened by the death of the adult trees. The appearance of large numbers of sprouting mangroves was delayed until after June 1985 when the numbers of live juveniles increased from 18 to 175 in December 1985.

The sediments at Site 0 also continued to release large amounts of sheen when disturbed; this effect was observable through the final visit. In a few places, black oil was released. The odor of oil was also noticeable.

Reference Site (Site R)

This site appeared completely normal and unchanged, and there were no signs of any lethal or sublethal effects resulting from the site preparation methods used. As previously mentioned, a path approximately 0.6 m wide was cut through the prop roots around the perimeter of the site to allow the

curtain booms to be placed. This procedure caused no observable effects on any of the trees in or near the cut area.

Effects on Adult Mangroves

Table 16 presents a summary of the LAI and canopy coverage data for the 3 sites, and Figure 21 presents the canopy coverage data. Canopy coverage proved to be a much more sensitive measure of defoliation than LAI measurements, and only this parameter will be discussed here.

Inspection of the data presented in Table 16 shows that the canopy coverage at Site D did not change appreciably during the entire study period. The means and standard deviations fluctuated slightly above and below prespill values, and these measurements supported on-site observations of the forest canopy, which remained dense, with no observable changes between prespill and postspill conditions (Fig. 22).

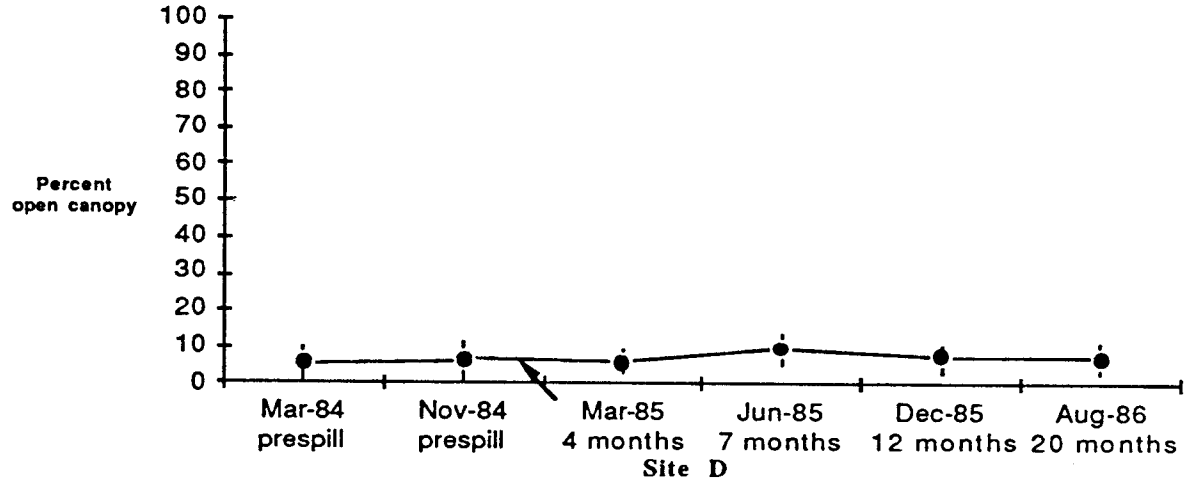
The canopy coverage measurements at Site 0 increased dramatically following site treatment, but the distribution of affected trees within the site was not uniform. This is reflected in the decrease in mean canopy coverage and the increase in standard deviation of the means. Visual inspection of Site 0 showed that most of the observable defoliation occurred in the eastern half of the site behind the outer fringe trees, where much of the oil accumulated during site treatment. All of the trees in this area were completely defoliated (Fig. 23). Individual trees scattered throughout the remainder of the site also were defoliated, but there were a substantial number of trees (especially in the outer fringe and the extreme western edge of the site) that were only slightly defoliated and still contributed to the overall canopy coverage. The level of defoliation and the distribution of affected trees resulted in an overall increase in the mean percentage of open canopy accompanied by an increase in the standard deviation since certain areas (especially the outer fringe trees) were relatively unaffected.

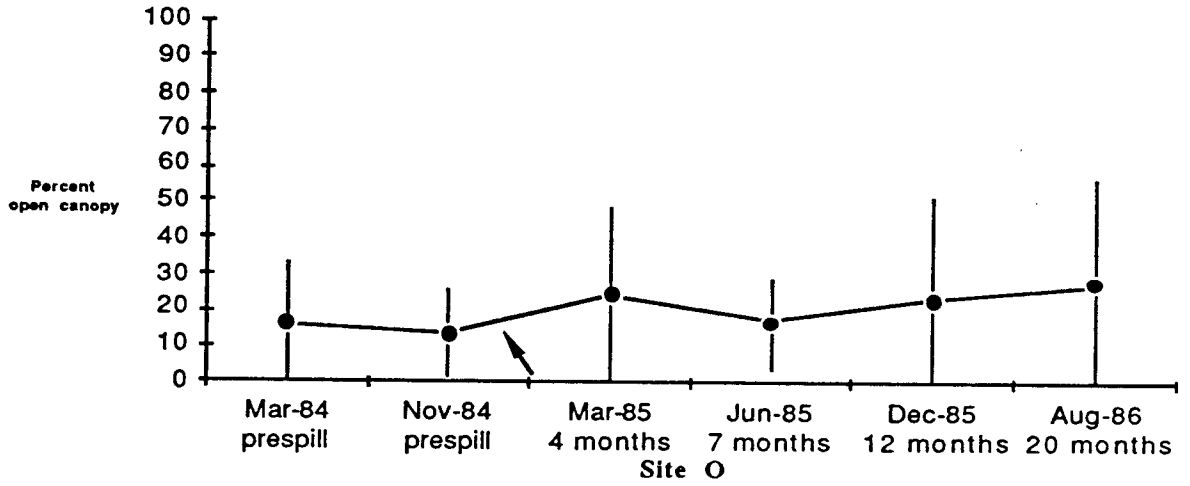
Estimates of the percentage of defoliation of each tree were made at Site 0 during each postspill survey. Most of the observed defoliation occurred during the 4-month period after oiling, during which time 18 trees were completely defoliated. The average defoliation for the entire site was 43.1 per-cent after 4 months. The number of dead trees increased slightly during the following year and a half, to 25, and the average defoliation increased to 47.5 percent by the end of the study at 20 months postspill. The canopy at

TABLE 16. Leaf area index and canopy coverage of study sites indicate a large increase in the open canopy at Site 0 following treatment.

	CANOPY COVERAGE*		LAI	
	Mean	(SD)	Mean	(SD)
SITE D				
March 1984 (8 months prespill)	5.6	(4.6)	3.3	(1.4)
November 1984 (1 week prespill)	6.4	(5.4)	2.8	(1.2)
March 1985 (4 months postspill)	5.7	(3.2)	2.3	(1.1)
June 1985 (7 months postspill)	9.5	(4.1)	2.7	(1.5)
December 1985 (12 months postspill)	7.5	(3.9)	3.5	(1.2)
August 1986 (20 months postspill)	7.3	(4.5)	3.0	(0.8)
SITE 0				
March 1984 (8 months prespill)	15.8	(17.2)	2.7	(1.8)
November 1984 (1 week prespill)	13.6	(11.6)	2.7	(1.5)
March 1985 (4 months postspill)	24.1	(24.2)	1.7	(1.5)
June 1985 (7 months postspill)	17.0	(12.5)	2.0	(1.8)
December 1985 (12 months postspill)	23.1	(28.6)	2.1	(1.8)
August 1986 (20 months postspill)	27.4	(29.3)	1.7	(1.4
SITE R				
March 1984 (8 months prespill)	10.5	(8.0)	2.2	(1.6)
November 1984 (1 week prespill)	13.7	(5.7)	2.5	(1.7)
March 1985 (4 months postspill)	8.7	(3.2)	2.0	(1.4)
June 1985 (7 months postspill)	13.1	(9.7)	2.5	(1.9)
December 1985 (12 months postspill)	12.3	(6.6)	2.4	(1.3)
August 1986 (20 months postspill)	10.6	(6.7)	2.1	(1.1)

* Percentage of open canopy.





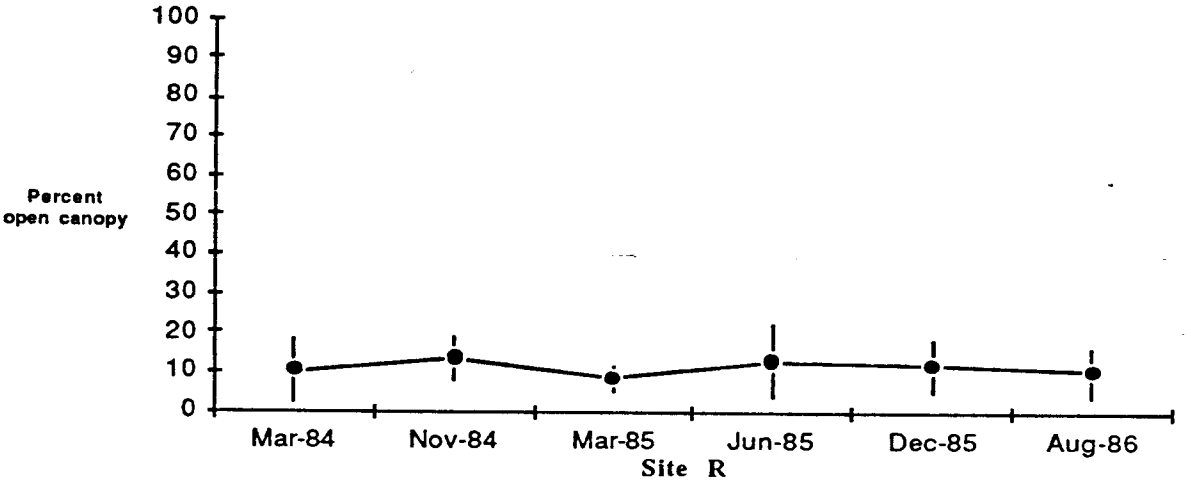


FIGURE 21. Percent open canopy coverage of mangrove forests at Site D, Site O, and Site R. Arrow indicates site treatment date.

FIGURE 22. The canopy of the mangrove forest at Site D showed no observable defoliation 4 months after treatment.

FIGURE 23. Defoliation was widespread throughout Site 0 4 months after treatment.

Site 0 became much more open, and many dead trees were evident through-out the site. The canopy in the eastern portion of the site was completely lost, and the overall canopy coverage for the whole site was greatly reduced.

The relationship between the analytical chemistry data on sediment hydrocarbon concentrations (presented in Table 9) and the effects seen on adult mangroves at Sites D and 0 were somewhat unusual. The sediment hydrocarbon concentrations at Site 0 were at all times higher compared to those' at Site D, but when different sampling periods are compared, this relationship was sometimes reversed (for example, 7-month postspill levels at Site D were higher on average than 4-month levels at Site 0). Also, the high standard deviations of the chemical data in Table 9 would suggest that there was considerable overlap in the hydrocarbon concentrations of individual samples from different sites. While Site 0 levels were consistently higher than at Site D, the difference in measured hydrocarbon concentrations is less than the measured differences in biological effects, especially in mortality and defoliation of adult mangroves. This would suggest that the dispersant may in some way reduce the toxicity of crude oil to mangroves.

Table 17 presents a summary of leaf production rates (new leaves/ month) of adult mangroves at each study site. No significant differences in leaf production rates over time were measured at any of the sites. This result may be biased in part by the uneven distribution of impacts within Site *0. The t*hree trees used to determine leaf production rates were not located in the area of greatest impact, in which little or no new leaf production occurred following defoliation of the trees. That partially affected trees continued to produce new leaves at a normal rate is an interesting and unusual finding.

The lengths, widths, and length/width ratios of leaves from selected adult trees were measured at each sampling period. Alterations in these parameters have been shown to occur in mangroves when subjected to stress from oil pollution, high salinity, and other factors. A summary of these data is presented in Table 18, and each site is discussed below.

At Site D, significant decreases in leaf length were measured in June and December 1985, compared to the baseline measurements of March and November 1984. No significant changes in leaf width occurred during the course of the study, and a significant decrease in the length/width ratio of adult lleaves was measured in August 1986.

TABLE 17. New leaf production rates (leaves/month) of adult mangroves at Sites D, 0, and R. (NM = not measured)

	SITE D		SITE 0		SITE R	
November 1984 (1 week prespill)	0.67	(0.17)	0.64	(0.053)	0.78	(0.36)
March 1985 (4 months postspill)	0.62	(0.23)	0.86	(0.49)	0.86	(0.26)
July 1985 (7 months postspill)	0.687	(0.35)	NM		0.75	(0.38)
December 1985 (12 months postspill)	0.50	(0.33)	0.57	(0.34)	0.67	(0.24)
August 1986 (20 months postspill)	0.44	(0.32)	0.48	(0.27)	0.63	(0.18)

TABLE 18. Lengths, widths, and length/width ratios of leaves from adult trees at Sites D, 0, and R. (NM = not measured)

	LENGTH	WIDTH	L/W
SITE D			
March 1984 (8 months prespill)	12.46	5.71	2.19
November 1984 (1 week prespill)	12.21	5.34	2.29
March 1985 (4 months postspill)	12.20	5.64	2.15
June 1985 (7 months postspill)	11.85*	5.35	2.19
December 1985 (12 months postspill)	11.31*	5.23	2.11
August 1986 (20 months postspill)	12.07	5.84	2.06*
SITE 0			
March 1984 (8 months prespill)	13.81	6.29	2.21
November 1984 (1 week prespill)	13.06	6.02	2.18
March 1985 (4 months postspill)	12.96	5.99	2.11
June 1985 (7 months postspill)	NM	NM	NM
December 1985 (12 months postspill)	9.49*	4.46*	1.97*
August 1986 (20 months postspill)			
SITE R	11.15*	5.29*	2.11
March 1984 (8 months prespill)	13.63	6.13	2.37
November 1984 (1 week prespill)	12.99	5.85	2.23
March 1985 (4 months postspill)	13.09	5.88	2.24
June 1985 (7 months postspill)	12.59	5.67	2.18
December 1985 (12 months postspill)	12.23*	5.49*	2.16
August 1986 (20 months postspill)	13.06	5.91	2.22

* Significantly different (p less than 0.05) from March 1984 (8 months pre-spill) or November 1984 (1 week prespill).

At Site 0, leaf length decreased in December 1985 and August 1986, as did leaf width. Significant changes in length/width ratios occurred only in December 1985. No measurements were taken in June 1985 of Site 0 since the leaves marked for this purpose were lost as a result of the almost complete defoliation of the adult tree. Leaf lengths and widths at Site R remained unchanged during all surveys except in December 1985, and the length/width ratios remained unchanged during the entire course of the study.

Previous studies (Getter et al., 1984) have measured significant reductions in leaf length and width in Rhizophora mangle juveniles exposed to whole and dispersed oils, but the net effect was to increase the length/width ratio. The opposite effect was measured in the present study.

The timing of the effects on leaf length and width at Sites D and 0 may be indicative of a delayed, sublethal response that does not appear i!n*[Hi 7] to 12 months after exposure to oil.

The rate of prop-root elongation was monitored at all sites during the prespill baseline period and through the 7-month postspill survey. The rate of elongation for the collection period is shown in Table 19. No significant difference in prop-root growth rates (cm/day) of adult trees was measured at any site. There were also no significant changes in the density of lenticels at any of the study sites (Table 20).

Table 21 lists the pH and salinity results for the interstitial and surface waters. The interstitial salinity values, in most cases, were less than or equal to that of the surface waters, indicating that the water exchange with-in the substrate was good and that buildup of high salt concentrations was not a problem. The pH values at Site D tracked those of the surface waters (low-high-low). At Site 0, the pH values followed an opposite pattern from that of the surface waters. The pH values dropped 5 days after the oiling and were still below their initial values 90 days after the oiling. This reduction in pH may indicate an alteration in the microbial activity and/or decomposition rates.

Effects on Juvenile Mangroves

The survival of propagules planted at each site was highly variable. Average sprouting success of propagules planted at the first baseline survey in March 1984 ranged from 0 to 77 percent. None of the propagules planted

TABLE 19. Prop-root growth rates (cm/day) of adult mangroves at Sites D. 0, and R. Means and standard deviations (in parentheses) are presented.

	SITE D	SITE 0	SITE R
November 1984 (8 months prespill)	0.24 (0.04)	0.26 (0.09)	0.29 (0.19)
December 1984 (1 month postspill)	0.24 (0.14)	0.27 (0.07)	NM
March 1985 (4 months postspill)	0.19 (0.11)	0.27 (0.18)	0.30 (0.13)
June 1985 (7 months postspill)	0.26 (0.14)	0.34 (0.31)	0.31 (0.21)

TABLE 20. Lenticels/cm^2 on prop roots of adult mangroves at Sites D, 0, and R. Means and standard deviations (in parentheses) are presented.

	SITE D	SITE 0	SITE R
November 1984 (1 week prespill)	0.37 (0.16)	0.44 (0.27)	0.42 (0.19)
March 1985 (4 months postspill)	0.43 (0.16)	0.46 (0.14)	0.38 (0.16)
June 1985 (7 months postspill	0.37 (0.11)	0.46 (0.19)	0.54 (0.17)

TABLE 21. Surface and interstitial water pH was reduced at Site 0 and remained unchanged at Sites D and R. Salinity was not affected at any of the sites.

SITE .	DEPTH	PRESPILL		POSTSPILL 5 days		POSTSPILL 112 days	
		pH	S°/oo	pH	S°/oo	pH	S°/oo
OIL AND DISPERSANT SITE							
1	surface	7.2	31	7.8	31	7.2	33
1	20 cm	6.7	30	7.0	27	6.8	32
1	60 cm	6.7	31	7.1	29	6.5	32
1	150 cm	6.8	32	6.9	32	6.5	32
OIL SITE							
5	surface	7.8	32	7.9	31	7.5	34
5	20 cm	7.4	29	7.1	31	6.7	32
5	60 cm	6.9	30	6.3	32	6.7	32
5	150 cm	7.1	28	6.7	30	6.5	32
REFERENCE SITE							
12	surface	7.2	23*	-	-	7.2	32
12	20 cm	6.8	32	-	-	6.7	32
12	60 cm	6.9	32	-	-	6.6	32
12	150 cm	6.8	32	-	-	6.6	32

* Measurement taken during heavy rainfall.

at Site 0 in the March 1984 baseline survey were found during the November 1984 baseline survey. Naturally colonizing propagules were present at Site 0, and the survival of planted propagules at the other sites was quite high (76 percent at Site D and 77 percent at Site R).

Following site treatments in December 1984, 3 additional groups of 25 propagules each were planted at each site. The sprouting success of these propagules after four months was also quite variable. Sprouting success at Site R was lower than during the baseline period. Sprouting success was also much lower at Site D, where only 32 percent of propagules planted 1 week after site treatment had sprouted within 4 months. At Site 0, survival of planted propagules also was very poor. Only 24 propagules were found, and none of these had sprouted 4 months after site treatment.

The long-term survival of the planted propagules was monitored through the remainder of the study. By August 1986 (20 months postspill), only 8 percent of the propagules planted at Site D were alive, 11 percent survived at Site 0, and 30 percent survived at Site R. These propagules were intended originally for measurements of leaf production, but the *very* low survival rates made this impossible.

Figures 24-26 show some of the seedlings planted at Sites R, D, and 0, respectively, 4 months after site treatment, showing the relative condition of some of the juvenile mangroves at each site. Figure 24 shows seedlings at Site R. They were healthy and producing a normal complement of leaves. Seedlings at Site D (Fig. 25) appeared to be stressed compared to those at Site R. Sprouting had occurred, but there were fewer live seedlings present, and they were shorter and had fewer leaves than those at Site R. Seedlings at Site 0 (Fig. 26) were highly stressed and were either dead or had not sprouted during the 4-month postspill period.

In addition to the planted propagules, there were many naturally colonizing propagules present at each site. These were counted during each site visit to determine the survival rates of those propagules that successfully sprouted within each site. Mangrove propagules typically have very high sprouting rates in intertidal habitats (in contrast to those planted at Site 0), but their long-term survival is dependent on their ability to compete with larger trees for light. In mature mangrove forests, propagules usually survive only in areas where adults have been removed by fire, cutting, or some other cause.

FIGURE 24. Red mangrove seedlings at Site R appeared healthy and were growing well during the Month 4 follow-up visit.

FIGURE 25. At Site D, seedlings had sprouted and were growing, but many had died shortly after sprouting; deformities were common.

FIGURE 26. No propagules at Site 0 had sprouted 4 months after site treatment.

The number of live and dead seedlings at each site is presented in Table 22. The total number of seedlings increased at each site during the period from November 1984 to August 1986, with most of the increase occurring after June 1985. The reason for this increase is unknown but may be a result of seasonal or yearly variations in propagule production in the study area. The most striking feature of the data presented in Table 22 was the very large increase in live seedlings at Site 0 seen in December 1985 and August 1986 (12 and 20 months postspill, respectively). This increase rep-resented an almost tenfold increase in the number of live seedlings at Site 0. Most of the seedlings were growing in the area of greatest impact to adult trees. The defoliated adults opened up a large area within Site 0 that became suitable for colonization by mangrove propagules. Significant reduction in canopy cover had occurred as early as March 1985 (4 months postspill), but contamination of the sediments by whole oil possibly prevented successful colonization until after June 1985 (7 months postspill). The *very* low percentage of dead seedlings present after June 1985 suggested that the conditions at Site 0 are suitable for seedling growth and the process of recovery has started there, despite the relatively high concentrations of hydrocarbons present in the sediments at Site O.

As mentioned above, increases in seedlings were measured at Sites D and R, but the magnitude of increase was much less than at Site 0, and the ratio of live to dead seedlings also was much lower. A low live/dead ratio indicated that long-term survival of seedlings was not very high. A ratio at or near 1.0 indicated that mortality of seedlings was about equal to successful sprouting of new propagules. Site D appeared to be near this equilibrium, with little or no net increase in new mangroves; Site R appeared to be increasing slightly; Site 0 was increasing very rapidly in terms of new man-groves filling in areas opened by the death of adults.

Effects on Mangrove Fauna

Tree snail (Littorina angulifera) surveys indicated that the site treatments had a significant effect on both the density of tree snails and their vertical distribution within each site. Treatment of Site D with oil and dispersant was followed by a 48 percent reduction in the snail population 14 days later (Table 23). When this site was resurveyed 4 months later, the snail population within the site had increased but was still 23 percent below

TABLE 22. Number of live and dead red mangrove seedlings at Sites D, 0, and R.

	TOTAL PROPAGULES		LIVE/DEAD RATIO
	LIVE	DEAD	
SITE D			
November 1984 (1 week prespill)	33	59	0.56
March 1985 (4 months postspill)	**15**	57	0.26
June **1985** (7 months postspill)	55	34	1.62
December 1985 (12 months postspill)	72	62	1.16
August 1986 (20 months postspill)	72	53	1.36
SITE 0			
November 1984 (1 week prespill)	13	38	0.34
March 1985 (4 months postspill)	11	46	0.24
June **1985** (7 months postspill)	18	44	0.41
December 1985 (12 months postspill)	175	15	11.70
August 1986 (20 months postspill)	212	15	14.10
SITE R			
March 1984 (8 months prespill)	26	16	1.63
March 1985 (4 months postspill)	24	21	1.14
June 1985 (7 months postspill)	26	12	2.16
December 1985 (12 months postspill)	92	25	3.68
August **1986** (20 months postspill)	115	**60**	1.91

TABLE 23. Relative abundance [N_1 (percentage of total)] of tree snails by elevation compartment at Site D. Site treatment caused a significant reduction in snail density.

ELEVATION (cm)	TIME					
	December 1984 (Prespill)	December 1984 (4 Days Postspill)	March 1985 (4 months postspill)	June 1985 (7 months postspill)	December 1985 (12 months postspill)	August 1986 (20 months postspill)
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1-24	22 (7.4)	9 (5.9)	27 (11.9)	21 (8.8)	32 (8.3)	48 (18.6)
25-60	64 (21.5)	41 (26.8)	50 (22.0)	55 (22.9)	91 (23.6)	39 (15.2)
61-100	113 (38.1)	54 (35.3)	52 (22.9)	75 (31.3)	122 (31.6)	46 (17.9)
101-170	58 (19.3)	27 (17.6)	41 (18.1)	49 (20.4)	87 (22.5)	41 (15.9)
greater than 170	40 (13.5)	22 (14.4)	57 (25.1)	40 (16.7)	54 (13.9)	83 (32.3)
TOTAL	297	153	227	240	386	257
Percentage of prespill abundance		51	76	81	130	87

the prespill level. For the duration of the study, the number of Littorina within Site D remained approximately equal to or greater than during the prespill period. An increase in Littorina abundance was seen during December 1985 (12 months postspill) at all study sites, followed by a decrease the following August. A significant change in the distribution of Littorina over time at Site D was measured only during the December 1985 survey (1 year postspill) (Table 24). This change was manifested by an increase in snail density at the middle level of the forest.

At Site 0, treatment with oil was followed by a significant reduction in the tree snail population (Table 25). Snail density dropped 51 percent during the 4 days after site treatment. Unlike Site D, however, snail density remained highly reduced after 4 months when snail density had increased by only 19 percent. By June 1985 and December 1985, tree snail abundance had increased substantially and was only slightly below prespill levels

A sharp drop in snail abundance was measured at Site 0 in August 1986. The reason for this drop is unknown, and it follows a similar de-crease in Littorina abundance that occurred at Sites D and R. In addition, the vertical distribution of tree snails changed at Site 0 following site treatment. The relative abundance of tree snails was significantly increased (p less than 0.05) in the upper levels of the mangrove forest and reduced in the lower levels (Table 26). This upward shift in the remaining population persisted through the 20-month postspill visit.

At Site R, no significant change in tree snail density was measured until December 1985 when snail abundance increased sharply (Tables 27 and 28), followed by an even greater decline the following August. The cause of this sudden rise and fall is unknown. A shift in tree snail distribution was noted also at Site R after 4 months, but it was a bidirectional shift with snail abundance increasing in the lower and upper levels of the forest, rather than only in the upper levels as at Site 0. In June 1985 and December 1985, other shifts in Littorina distribution were noted at Site R, when the abundance of snails increased at higher elevations and then at lower elevations, respectively.

Survival of the mangrove oysters was very high at all sites and at all sampling times (Table 29). Very high short-term survival rates were observed at Sites 0 and D 4 days after site treatment. Survival of Crassostrea rhizophorae at Site D was 96.9 percent, and 100 percent for Isognomon alatus and Pinctada imbricata for the period from the beginning of

TABLE $2^1$4. Two-way analysis of variance of tree snail density and distribution data at Site D shows that snail density was affected by site treatment over time, but there was no effect on the distribution of snails within the forest over time (interaction).

FACTOR	DEGREES OF FREEDOM	F	PROBABILITY
Time	5	9.23	p less than 0.05
Elevation	5	38.26	p less than 0.05
Interaction	25	2.63	p greater than 0.05
Error	72		

TABLE 25. Relative abundance [N_1 (percentage of total)] of tree snails by elevation compartment at Site O. Snail density was greatly reduced by site treatment, and this effect persisted through month 4.

ELEVATION (cm)	TIME					
	December 1984 (Prespill)	December 1984 (4 Days Postspill)	March 1985 (4 months postspill)	June 1985 (7 months postspill)	December 1985 (12 months postspill)	August 1986 (20 months postspill)
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1-24	172 (22.5)	45 (12.0)	31 (7.0)	50 (7.8)	102 (14.1)	63 (15.9)
25-60	202 (26.4)	61 (16.3)	42 (9.4)	80 (12.5)	161 (22.2)	79 (20.0)
61-100	225 (29.4)	90 (24.0)	92 (20.6)	162 (25.3)	158 (21.8)	55 (13.9)
101-170	119 (15.5)	90 (24.0)	129 (28.9)	165 (25.8)	151 (20.9)	90 (22.8)
greater than 170	48 (6.2)	89 (23.7)	152 (34.1)	183 (28.6)	150 (20.8)	107 (27.2)
TOTAL	766	375	446	640	722	394
Percentage of prespill abundance		50	58	84	94	51

TABLE 26. Two-way analysis of variance of tree snail density and distribution data at Site 0 indicates a significant reduction in snail density over time and a significant change in their distribution over time (interaction).

FACTOR	DEGREES OF FREEDOM	F	PROBABILITY
Time	5	7.81	p less than 0.05
Elevation	5	24.84	p less than 0.05
Interaction	25	3.24	p less than 0.05
Error	72		

TABLE 27. Relative abundance [N_1 (percentage of total)] of tree snails by elevation compartment at Site R did not change over time.

ELEVATION (cm)	TIME					
	December 1984	December 1984	March 1985	June 1985	December 1985	August 1986
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1-24	34 (7.6)	39 (9.6)	68 (19.7)	27 (6.3)	125 (20.3)	53 (18.2)
25-60	116 (26.1)	94 (23.1)	61 (17.6)	78 (18.2)	157 (25.6)	66 (22.6)
61-100	177 (39.8)	112 (27.6)	68 (19.7)	93 (21.7)	198 (32.2)	48 (16.5)
101-170	72 (16.2)	79 (19.5)	59 (17.0)	107 (25.0)	75 (12.2)	51 (17.5)
greater than 170	46 (10.3)	82 (20.2)	90 (26.0)	123 (29.2)	59 (9.6)	73 (25.1)
Total	445	406	346	428	614	291
Percentage of December 1984 abundance		91	78	96	138	65

TABLE 28. Two-way analysis of variance of tree snail density and distribution data at Site R indicate a significant alteration in distribution over time (interaction), but the change was toward the canopy and forest floor, rather than away from the forest floor, at Site O.

FACTOR	DEGREES OF FREEDOM	F	PROBABILITY
Time	5	6.49	p less than 0.05
Elevation	5	27.72	p less than 0.05
Interaction	25	3.67	p less than 0.05
Error	72		

TABLE 29. Survival of mangrove oysters was *very* high at each site, indicating no acute or chronic effects on these species. Presented below are the total numbers of live oysters of each species at each site present on five prop roots monitored during the study.

	C. rhizophorae	*I. alatus*	*P. imbricata*
SITE D			
December 1984			
(1 day prespill)	33	48	2
(4 days postspill)	32	48	2
March 1985			
(4 months postspill)	21	46	1
June 1985			
(7 months postspill)	21	41	2
December 1985			
(12 months postspill)	37	45	
August 1986			
(20 months postspill)	52	39	1
SITE 0			
December 1984			
(1 day prespill)	16	53	3
(4 days postspill)	14	53	3
March 1985			
(4 months postspill)	13	67	3
June 1985			
(7 months postspill)	20	72	2
December 1985			
(12 months postspill)	8	88	2
August 1986			
(20 months postspill)	11	111	3
SITE R			
December 1984			
(1 day prespill)	23	61	3
(4 days postspill)	23	61	3
March 1985			
(4 months postspill)	23	54	2
June 1985			
(7 months postspill)	17	48	3
December 1985			
(12 months postspill)	11	54	5
August 1986			
(20 months postspill)	41	34	1

site treatment to 4 days later. Survival of oysters at Site 0 for the same period also was very high. C. rhizophorae was the only species to have any reduction in survival (87.5 percent survival). All oysters at Site R had 100 percent survival for this period.

When these oysters were examined during the 4-month postspill revisit, some changes in population had occurred. Overall survival still was quite high. At Site D, survival of C. Rhizophorae was 60.9 percent; I. alatus, 95.8 percent; and P. imbricata, 50.0 percent. The survival rates of P. imbricata are based on a change from 2 individuals to 1 individual present on the marked roots. At Site 0, the numbers of I. alatus had increased 26.4 percent (from 53 to 67 individuals). C. rhizophorae had 81.2 percent survival and P. imbricata had 100.0 percent survival. At Site R, C. rhizophorae had 100.0 percent survival, I. alatus had 88.5 percent survival, and P. imbricata had 66.6 percent survival after 4 months. These data suggest that the mangrove oyster species are quite resistant to the short- and long-term toxic effects of exposure to fresh oil and dispersed oil.

The high survival rates of oysters at Sites D and 0 occurred in spite of the high levels of petroleum hydrocarbons present in the oyster tissues (see Table 13). This would indicate a relatively high resistance to the toxic effects of whole and dispersed oil. The addition of dispersant appeared to make no difference in the uptake of oil. Other researchers (Gilfillan et al., 1985) have found that uptake of dispersed oil was reduced in two bivalve species (Mya arenaria and Mytilus edulis) compared to untreated oil.

In addition to the tree snails and oysters, a survey was conducted of other macrofauna present at each site. A species of brown, intertidal anemone was present at Site D. Ten individual anemones were located within the study area during the November 1984 prespill survey. During site treatment, they were exposed to dispersed oil and some slick oil within the man-grove forest. Despite this direct exposure, none of the anemones had died 4 days or 4 months after site treatment. No anemones of this species were present at Site 0 or R.

SUBTIDAL SYSTEMS

The subtidal ecosystem of the study sites is composed of fringing sea-grass beds and coral reefs that parallel the outer mangrove fringe. The most common seagrass species is Thalassia testudinum (turtle grass) (Fig. 27). It is the dominant angiosperm in tropical subtidal zones and is considered the most important seagrass in the Caribbean Sea. It provides habitat to many economically and ecologically important fish and shellfish, and seagrass beds contribute significantly to the primary productivity to near-shore tropical areas.

Associated with the seagrass beds are fringing, nearshore coral reefs. Coral reefs are uniquely significant to the world's oceans. They are extremely productive ecosystems in oligotrophic seas and are regarded as the most diverse and complex of marine communities. The basis for the existence of the coral reef ecosystem is the hermatypic (reef-building) coral which, in addition to their structural role, provide shelter, substrate, and a source of nutrients for a wide variety of organisms.

Fringing reefs are commonly situated very close to mangrove stands and may be interspersed with individual seagrass plants or with well-developed seagrass beds. The seagrass beds are most dense within the small lagoonal area between the mangrove fringe and the reef crest.

The reef crest is typically in water of 1 m or less. At the crest is a predominately rubble zone composed primarily of Porites porites fragments. Living corals occur on the crest and landward reef portions, consisting predominantly of P. porites, Millepora alicicornis, and Siderastrea spp., with lesser abundance of Agaricia spp., Porites astreoides, and occasional Oculina spp. Within the lagoonal seagrass beds, P. porites is a common coral component. The extreme landward portion where mangrove prop roots enter the water contains few corals (Fig. 28). Some species encrust these roots (Millepora alicicornis, Agaricia), and Agaricia in flattened growth form may be found within the mangrove prop root as may be Siderastrea siderea, occasionally in large colonies up to 1 m in diameter.

At the seaward reef crest, the coral species Porites porites dominates with a coverage of approximately 30 percent. Seaward from the crest, the reef slopes abruptly to depths of 10-15 m. Typically, the reef slope is densely covered by hard corals. In the shallower portions, species composition is dominated by Porites porites and Agaricia tennuifolia. Below 2-3 m,

FIGURE 27. Turtle grass (Thallasia testudinum) was the dominant seagrass species in the study area. It formed dense beds in the sub-tidal zone and was also present scattered throughout much of the coral reefs.

FIGURE 28. Prop roots of the red mangrove (Rhizophora mangle) merged with turtle grass and various species of corals (Porites, Agaricia, Millepora, and others) in the subtidal zone of the study sites.

Agaricia is dominant. On some reefs in depths greater than approximately 2 m, patches of Acropora cervicornis occur. The branching species Madracis mirabilis also may be locally abundant. At the deeper and basal portions of the reef, more massive corals of the species Montastrea annularis, Montastrea cavernosa, and Colpophyllia natans may occur. Branching forms at depth include Oculina spp., Agaricia agaricites, Porites porites, and A. tennuifolia.

Due to the low current and wave energy at the crest, P. porites colonies are fragile, delicate, and poorly cemented. In addition, the upper 1.5 m of the water column appears to be subject to lenses of lowered salinity from the frequent rains and surface runoff from the mangrove forest. This effect possibly acts to exclude many species from the crest region and may contribute also to the fragility of specimens.

The seagrass- and coral-associated fauna and flora are very diverse and abundant. The most common organism is the colonial anthozoan, Zooanthus pulchellus. It forms dense colonies throughout the subtidal zone. Sponges, anemones, sabellid worms, starfish, urchins, and sea cucumbers are also common. These areas support large populations of fish, especially parrot fish, snapper, gobies, barracuda, and other species commonly associated with seagrass and coral habitats.

In addition to the large faunal assemblage, many species of algae are present in these areas. Halimeda, Dictyota, Caulerpa, Acanthophora, Penicillus, Udotea, and various blue-green algae are found throughout the nearshore zone from the mangrove prop roots to the outer slope of the coral reef.

There have been few studies of the effect of oil and dispersed oil on turtle grass and other seagrasses. Most field studies are the result of observations of accidental oil spills. Laboratory studies of turtle grass by RPI in the last 2 years have provided some information on the toxicity of oil and dispersed oil (Baca and Getter, 1984). It was determined that dispersants enhanced the uptake of hydrocarbons by turtle grass. More recent work has indicated that the water-accommodated fraction (WAF) was more toxic than dispersed oil when actual hydrocarbon concentrations were compared and that turtle grass was relatively resistant to dispersed oil in short-term doses.

Oil pollution from any of a variety of sources holds the potential for serious impact on corals. This is largely a perceived threat because only a few reports refer specifically to corals. Experiments have shown effects ranging from none at all to growth suppression and mortality. These studies have been accomplished in a wide variety of areas and have used a variety of different coral species and different assessment techniques, with widely varying oil concentrations and treatment procedures.

METHODS AND MATERIALS - CORAL STUDIES

Floral/Faunal Assessment - Transects

To evaluate ecological parameters of the coral reefs in the shallow zone (0-2 m) where impact was expected to be greatest from experimental treatment, the point plotless line transect method was chosen. Reef assessment methods, including plotless line methods, are described in Loya (1978). The point plotless line method is described in detail in Dodge et al. (1982).

At each site, 4 locations for semipermanent line transects were established by marking end points with metal rods 1.0-1.5 m in length driven into the reef substrate. Each transect line was 10 m in length. Two transect lines were laid parallel to the reef crest and were established in water depths of approximately 1 m. Two other lines were laid parallel to the first in depths of approximately 1.3 m. Data were collected from the transect lines by a diver swimming over the line and recording the identity of the substrate which lay beneath points established at 10-cm intervals. There-fore, for each transect, 100 data points were collected. For each site, major parameters were averaged over transects for a mean representation.

The substrate was identified as either bare substrate or as one of several categories of living organisms. In essentially all cases, bare substrate consisted of rubble formed by dead Porites porites or other coral species. Organisms were further subcategorized into epifaunal and epifloral groups. Stony corals were identified to species. Other animals were classified to phylum or order. The anthozoan Zooanthus pulchellus was identified to species. In terms of plants, fleshy and calcareous algae were distinguished. In addition, seagrass was identified when present.

Four assessment categories were created: corals, total animals (including corals), total plants, and total organisms. Changes in coverage

of reef substrate by each category were used as a measure of relative abundance. Reductions in coverage of reef substrate by sessile organisms such as coral is the result of mortality, unless an increase in the coverage of other organisms such as algae occurs.

During the initial survey in March 1984, 4 transects at each site were accomplished. In November 1985, the transect marker rods of most sites could not be relocated. Consequently, new markers were established and four transects were resurveyed on all subsequent assessments. Each transect line was assessed in duplicate during each site survey. Consequently, the data set of the initial survey (March 1984) is not directly comparable to that of later surveys in terms of precise transect locations. Statistical analyses were conducted appropriately to reflect fixed or variable transect lo-cations.

Coral Growth Assessment

General

The coral species Montastrea annularis, Agaricia tennuifolia, Porites porites, and Acropora cervicornis were chosen for growth assessment follow-treatment by oil or dispersed oil.

During the initial survey period (March 1984) at each of the 3 main sites (Sites D, 0, and R), approximately 5 specimens of each species were fixed to cement blocks with underwater quick-setting cement. Cemented specimens were placed at 1-2 m depth in the central portion of each site. The coral species Montastrea annularis and Acropora cervicornis did not occur naturally at this shallow depth and were transplanted at the same reef from approximately 4 and 3 m, respectively. The other two species (Porites porites and Agaricia tennuifolia) were collected from the same shallow depths in which they were cemented.

Immediately prior to the November 1984 treatment period, coral specimens were surveyed for survival and retention on the cement blocks. Approximately 60 percent were intact. The others had apparently become loosened from the cement and had toppled onto the reef substrate. Missing specimens were replaced (see following discussion) by fresh corals as in the above cementing procedure. No specimens of Montastrea annularis had to be ʳeplaced.

A second reference site (Site R-2) for growth assessment was located midway between Sites D and O. This was established for confidence in the reference nature of Site R. At this second reference site, corals of the species Porites porites, Agaricia tennuifolia, and Acropora cervicornis were stained and cemented onto cement blocks at 1-2 m depth using the procedures as above.

After specimen selection and at least 7 days prior to treatment with oil dispersed oil, specimens at each site were stained with alizarin red S dye (Lamberts, 1978) for approximately 5 hours. Staining was accomplished by securing a clear plastic bag around cemented corals, injecting a mixture of alizarin red S dye and distilled water at sufficient concentration to bring the alizarin concentration to between 10 and 15 ppm, and closing the bag opening securely around the coral base. For not-yet-cemented specimens, plastic zip-lock bags were used. Bags were removed after approximately 3 hhvui s. Several days after staining, any nonsecured specimens were cemented to blocks where appropriate.

In growth assessments made from November 1984 through December 1985, staining was accomplished exclusively in zip-lock bags. After an approximately one-day recovery period, specimens were cemented onto cement blocks. The corals were inspected to ensure proper staining and secure attachment to the cement base blocks.

At the start of the following survey, coral specimens were then collected, air-dried, labeled, and packaged for shipment to the laboratory at Nova University (Dania, Fla.). The period between staining and collection was variable, depending primarily on the length of time between site visits.

The growth periods for each species were as follows:

M. annularis	126 days	(November 1984 - March 1985)
A. cervicornis	122-128 days	(November 1984 - March 1985)
A. tennuifolia	122-128 days	(November 1984 - March 1985)
	162-164 days	(June 1985 - December 1985)
	234-235 days	(December 1985 - July 1986)
P. porites	122-127 days	(November 1984 - March 1985)
	89- 91 days	(March 1985 - June 1985)
	162-164 days	(June 1985 - December 1985)
	234-235 days	(December 1985 - July 1986)

In the laboratory, specimens were bleached in a dilute solution of Clorox to remove remaining coral tissue and to better expose the alizarin stain line. Specimens of Montastrea annularis were not bleached because remaining tissue was needed to locate certain skeletal structures.

Remarks on the Nature of Coral Colonies

There was no ambiguity about the diserete nature of coral colonies for the species Montastrea annularis. This is a hemispherical-type coral forming individual heads. The species Porites porites, however, is a branehed coral (Fig. 29). In small specimens, it is simple to trace individual branches to a common base and therefore identify several branches which are of the same genotype. In larger clumps, breakage from handling and/or prior breakage from bioerosion often obscures these relationships. Therefore, for this species, the operational unit was a well-defined branch. In general, each staining unit (i.e., each cluster of P. porites which was stained) was composed of several branches, some of which were probably of the same genotype.

Colony considerations for the species Agaricia tennuifolia were similar to that of Porites porites. In this case, however, A. tennuifolia forms blade-like growths (Fig. 29). The operational unit of measurement was a blade which w generally distinct from other colony parts. As with P. porites, each stained cluster was composed of several blades, most of which were probably a similar genotype.

For Acropora cervicornis, transplantation was conducted by breaking branch tips (greater than 10 cm in length) from in situ living colonies, staining, and cementing branches to blocks. Transplanted branches were, in general, from different genotypes and so each block contained several branches from a different original genotype.

Growth Measurement Procedures Montastrea annularis

Measurement procedures for this species are described in more detail in Dodge (1982). Each specimen was trimmed to obtain several slabs which were from 3 to 4 cm thick. These slabs were dried under low heat and subsequently embedded in clear casting polyester resin. Upon hardening, resin slabs were sectioned with a rock saw along surfaces parallel to corallite

FIGURE 29. The shallow subtidal zone was heavily colonized by Porites porites (1) and Agaricia tennuifolia (2). These species and others were analyzed to determine growth rates and percentage of coverage.

growth axes. Embedded cut surfaces were sequentially polished using lapidary sandpaper and grit to a clear surface suitable for viewing under low-power microscopy.

Specimens were surveyed for stain uptake. Septa increase was measured along well cross-sectioned septa which revealed the alizarin stain line and current septa top. In addition, fossa length was measured as the distance from the top of the current septa to the current colurnella. The parameter is an index of calical relief. Unfortunately, the stain was not well revealed in the columella of colonies. This precluded quantitative analysis of the fossa length in the predosed condition of the corals.

Agaricia tennuifolia

When possible, individual blades were identified on each stained cluster. Five measurements per blade were made with calipers from the stain lines to the blade distal surface. Observation of the stain line was made by holding the blade in front of a strong back light. The thickness of the blade at the stain line also was measured with calipers.

For analysis, blade length (extension) was converted to an annual basis (cm/yr). Because blade thickness (width) appeared relatively constant, this parameter was not normalized to yearly growth. Cross-sectional blade area (cm^2/yr) was calculated as the product of blade width and extension rate.

Porites porites

Where possible, at least five branch tips were identified from each staining unit. These were cut from the branch (below the stain line) using a dentist saw. Each tip was labeled and its basal thickness measured at the stain line using calipers.

Each tip was then cut or ground to produce a medial surface suitable for stain-line observation. The linear growth (extension) from the stain line top to the branch tip top was recorded as length. The width of the tip at the top of the stain line also was recorded. Length (normalized to yearly growth rate in cm/yr) and width (cm) measurements were used to calculate tip growth volume (cm^3/yr) by the formula of a cylinder.

Acropora cervicornis

Stained tips of individual branches were cut from the main branch body using a dentist saw. Each tip was labeled by site and staining unit. Tips were sectioned medially, and a length measurement was taken by caliper from the uppermost stain line to the new tip growth surface. Branch width also was measured at the stain line. This measurement is considered to be less precise because of the irregular growth surface of A. cervicornis.

RESULTS OF CORAL STUDIES

Floral/Faunal Assessment - Transects of Coral Reefs

Tables 30-32 summarize the results for all survey periods obtained from each of the 4 plotless line transects at Sites D, 0, and R, giving the means and standard deviations of each assessment category for each site. The following discussion will be concerned only with the initial survey period.

Initial Survey (March 1984)

Figure 30 is a bar graph presentation for total coral coverage, total animal coverage, total plant coverage, and total organism coverage at each of the 3 sites during the March 1984 survey.

Reference Site R exhibits slightly lower total coral coverage in comparison to Sites D and 0. This difference is compensated by a slight increase in zooanthid coverage at Site R which causes total animal coverage to be similar between the three sites.

Algal coverage is similar between the sites; however, Site R has much greater plant coverage because of its relatively high seagrass cover. For total organism coverage, Site R is higher than the other two sites.

To quantify site differences, a one-way ANOVA (fixed model) was con-ducted on the parameters total coral coverage, total animal coverage, total plant coverage, and total organism coverage.

There were no statistical differences between sites in total corals and total animals at the p-less-than-0.05 level. For total plants and total organisms, ANOVA indicated significant site differences at the p-less-than-0.0005 and p-less-than-0.01 levels, respectively. Testing with the Student-Neuman-Keuls (SNK) test revealed that Site R was significantly different than both Sites D and 0 in total plant coverage (at the p-less-than-0.01

TABLE 30. Percentage of coverage of the reef substrate by the listed organism categories at the dispersed oil site (Site D) at each of the assessment categories.

ASSESSMENT PERIOD	MARCH 1984 (8 months prespill)		NOVEMBER 1984 (3 days prespill)		DECEMBER 1984 (5 days postspill)		MARCH 1985 (4 months postspill)		JUNE 1985 (7 months postspill)		DECEMBER 1985 (12 months postspill)		AUGUST 1986 (20 months postspill)	
	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.
ASSESSMENT CATEGORY														
Porites porites	32.50	11.73	26.13	13.91	20.25	14.51	19.38	11.96	16.13	12.57	9.50	10.78	8.63	9.40
Agaricia tennuifolia	1.00		1.13	1.13	1.38	1.69								
Millepora alcicornis									0.13					
Total Corals	33.50	11.73	27.25	13.79	21.63	14.15	19.38	11.96	16.26	12.57	9.50	10.78	8.63	9.40
Zooanthids	7.25	2.63	14.63	5.15	13.25	2.92	6.50	2.78	6.75	3.24	6.50	3.38	12.25	5.73
Sponges	9.50	7.05	12.38	6.84	7.38	5.04	6.13	3.52	6.63	3.89	6.38	3.78	6.25	1.67
Anemonies			0.50	0.76	0.13	0.35			0.25	0.46	0.50	0.53	0.38	0.74
Total Other Animals	16.75	7.54	27.50	9.24	20.76	5.97	12.63	2.20	13.63	5.73	13.38	2.62	18.88	4.76
TOTAL ALL ANIMALS	50.25	8.30	54.75	7.55	42.39	9.68	32.00	10.43	29.88	8.51	22.88	8.27	27.51	10.45
Calcareous Algae	3.75	2.22	2.00	2.93	1.88	2.10	1.13	1.55					0.38	
Fleshy Algae					0.63	1.06			0.25	0.46	0.25	0.46	0.50	0.83
Total Algae	3.75	2.22	2.00	2.93	2.51	3.02	1.13	1.55	0.25	0.46	0.25	0.46	0.88	0.83
Seagrass													0.00	
TOTAL PLANTS	3.75	2.22	2.00	2.93	2.51	3.02	1.13	1.55	0.25	0.46	0.25	0.46	0.88	0.83
TOTAL ORGANISMS	54.00	6.48	56.75	7.38	44.90	9.70	33.13	10.63	30.13	8.34	23.13	8.17	28.39	10.57
Rubble/Bare Substrate	46.00	6.48	43.25	7.38	55.10	9.70	66.87	10.63	69.87	8.34	76.87	8.17	71.61	10.57
GRAND TOTAL	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00

TABLE 31. Percentage of coverage of the reef substrate by the listed organism categories at the oil site (Site O) at each of the assessment categories.

ASSESSMENT PERIOD	MARCH 1984 (8 months prespill)		NOVEMBER 1984 3 days prespill)		DECEMBER 1984 (5 days postspill)		MARCH 1985 (4 months postspill)		JUNE 1985 (7 months postspill)		DECEMBER 1985 (12 months postspill)		AUGUST 1986 (20 months postspill)	
	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.
ASSESSMENT CATEGORY														
Porites porites	28.25	17.73	25.00	11.44	21.75	7.81	21.38	11.11	24.88	7.32	19.50	8.67	15.63	10.72
Agaricia tennuifolia	0.75	0.50	1.38	1.19	1.38	1.30	1.13	1.55	0.13		0.79	0.71	1.38	1.06
Millepora alcicornis	1.50	3.00	1.25	1.39	0.88	1.25	0.88	1.64	0.63	1.19	1.13	1.64	1.63	2.77
Total Corals	30.50	18.72	27.63	13.21	24.01	9.35	23.39	12.08	25.64	7.80	21.38	9.04	18.64	11.87
Zooanthids	11.00	3.37	19.38	5.01	20.25	8.56	15.38	5.10	21.38	7.07	25.38	12.30	31.63	8.25
Sponges	8.75	5.56	8.63	1.77	7.38	1.51	9.38	3.46	10.00	2.56	7.63	2.69	10.25	4.13
Anemonies					0.13	0.35								
Total Other Animals	19.75	7.23	28.01	5.66	27.76	9.24	24.76	7.46	31.38	6.50	33.01	9.75	41.88	4.88
TOTAL ALL ANIMALS	50.25	19.09	55.64	13.27	51.77	10.57	48.15	11.83	57.02	11.10	54.39	16.89	60.52	15.35
Calcareous Algae	5.50	0.58	12.00	4.60	9.88	3.64	5.00	2.93	2.88	2.23	3.25	2.31	1.50	1.41
Fleshy Algae	1.50	1.91	1.50	1.93	2.75	2.31	0.50	0.76	0.88	1.64	0.38	0.74	1.50	1.31
Total Algae	7.00	1.41	13.50	4.50	12.63	2.50	5.50	3.30	3.76	2.43	3.63	2.00	3.00	2.56
Seagrass														
TOTAL PLANTS	7.00	1.41	13.50	4.50	12.63	2.50	5.50	3.30	3.76	2.43	3.63	2.00	3.00	2.56
TOTAL ORGANISMS	57.25	18.57	69.14	10.99	64.40	10.21	53.65	10.62	60.78	12.22	58.02	17.27	63.52	17.57
Rubble/BareSubstrate	42.75	18.57	30.86	10.99	35.60	10.21	46.35	10.62	39.22	12.22	41.98	17.27	36.48	17.57
GRAND TOTAL	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00

TABLE 32. Percentage of coverage of the reef substrate by the listed organisms at the reference site (Site R) at each of the assessment categories.

ASSESSMENT PERIOD	MARCH 1984 (8 months prespill)		NOVEMBER 1984 3 days prespill)		DECEMBER 1984 (5 days postspill)		MARCH 1985 (4 months postspill)		JUNE 1985 (7 months postspill)		DECEMBER 1985 (12 months postspill)		AUGUST 1986 (20 months postspill)	
	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.	Mean %	s.d.
ASSESSMENT CATEGORY														
Porites porites	21.25	2.50	15.25	4.74	15.75	5.44	8.38	4.81	13.88	5.03	8.63	6.55	7.25	6.54
Agaricia tennuifolia									0.25	0.46				
Millepora alcicornis														
Total Corals	21.25	2.50	15.25	4.74	15.75	5.44	8.38	4.81	14.13	5.44	8.63	6.55	7.25	6.54
Zooanthids	23.00	7.79	34.75	6.76	32.88	6.64	27.38	3.38	27.75	3.92	28.25	4.06	22.63	7.56
Sponges	5.00	2.94	3.63	2.00	5.00	2.51	6.75	4.40	5.00	2.07	6.88	2.80	7.13	1.25
Anemonies													0.38	0.74
Total Other Animals	28.00	6.32	38.38	5.37	37.88	4.70	34.13	4.45	32.75	5.44	35.13	5.46	30.14	7.32
TOTAL ALL ANIMALS	49.25	7.89	53.63	3.34	53.63	3.54	42.51	7.58	46.88	10.22	43.76	10.42	37.39	10.99
Calcareous Algae	4.00	0.82	4.75	4.13	4.13	3.14	2.88	1.73	0.88	0.83	4.25	2.87	1.50	0.93
Fleshy Algae	1.25	1.89	10.75	8.45	10.38	8.96	21.00	16.93	23.88	18.43	21.25	17.83	18.63	10.03
Total Algae	5.25	1.89	15.50	10.66	14.50	7.98	23.88	16.75	24.76	18.30	25.50	16.12	22.50	10.18
Seagrass	14.00	4.08	14.13	6.29	12.88	5.00	13.75	2.31	14.88	3.04	16.50	4.93	17.25	6.45
TOTAL PLANTS	19.25	4.11	29.63	7.50	27.38	4.96	37.63	16.12	39.64	16.22	42.00	11.89	39.75	8.56
TOTAL ORGANISMS	68.50	4.65	83.26	5.99	81.01	4.75	80.14	10.95	86.52	7.84	85.76	4.86	77.14	6.53
Rubble/Bare Substrate	31.50	4.65	16.74	5.99	18.99	4.75	19.86	10.95	13.48	7.84	14.24	4.86	22.86	6.53
GRAND TOTAL	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00

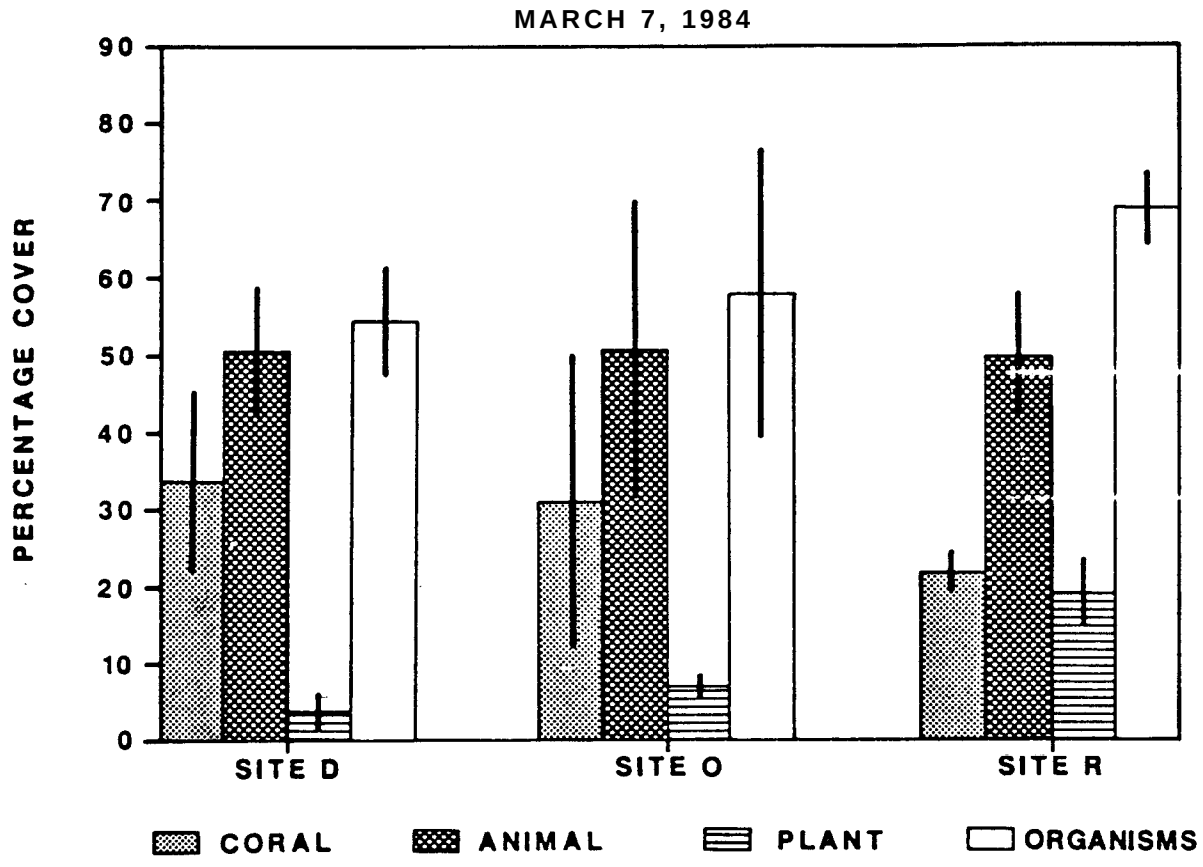


FIGURE 30. The relative relative abundance of epifauna and epiflora (measured as percentage of coverage of the reef substrate). Site R was statistically different from Sites O and D for both total plant and total organism coverage.

level). In addition, Site R was statistically different than Site 0 in total organism coverage (at the p-less-than-0.05 level).

Pretreatment Survey (November 1984)

Tables 30-32 include summaries of transect results for Sites D, 0, and R for each formal assessment period. The following discussion will be limited to between-site comparisons during the pretreatment survey assessment period.

Figure 31 presents a bar graph depicting total coral, total animal, total plant, and total organism coverage for each of the 3 sites in the November 1984 baseline survey.

Sites D and 0 had similar total coral coverage. This parameter at Site R was relatively lower. Total other animal coverage was greater at Site R because of relatively high coverage by zooanthids (Zooanthus sociatus) in comparison to Sites D and 0. Total animal coverage was similar between all three sites.

Algal coverage was similar between Sites 0 and R and relatively lower at Site D. Seagrass coverage was markedly higher at Site R. Total organism coverage was greatest at Site R (83 percent), moderate at Site 0 (69 percent), and lowest at Site D (56 percent).

To assess and quantify specific site differences, a one-way ANOVA (fixed model) was employed to test the parameters total coral, total animal, total plant, and total organism coverage between the three sites. ANOVA revealed no site differences (p less than 0.01) in the parameters total coral and total animal coverage. Total plant coverage was revealed to be different (p less than 0.0005) between the 3 sites. SNK testing indicated that all sites were different from each. other (p less than 0.05). Site differences were revealed also for total organism coverage (p less than 0.005). SNK testing revealed that all sites were significantly different from each other (p-less-than-0.05 level) for this parameter.

Initial Survey and Pretreatment Survey Differences Between Sites Before Treatment

For both the initial survey in March 1984 and the pretreatment survey in November 1984, significant preexisting differences in sites were

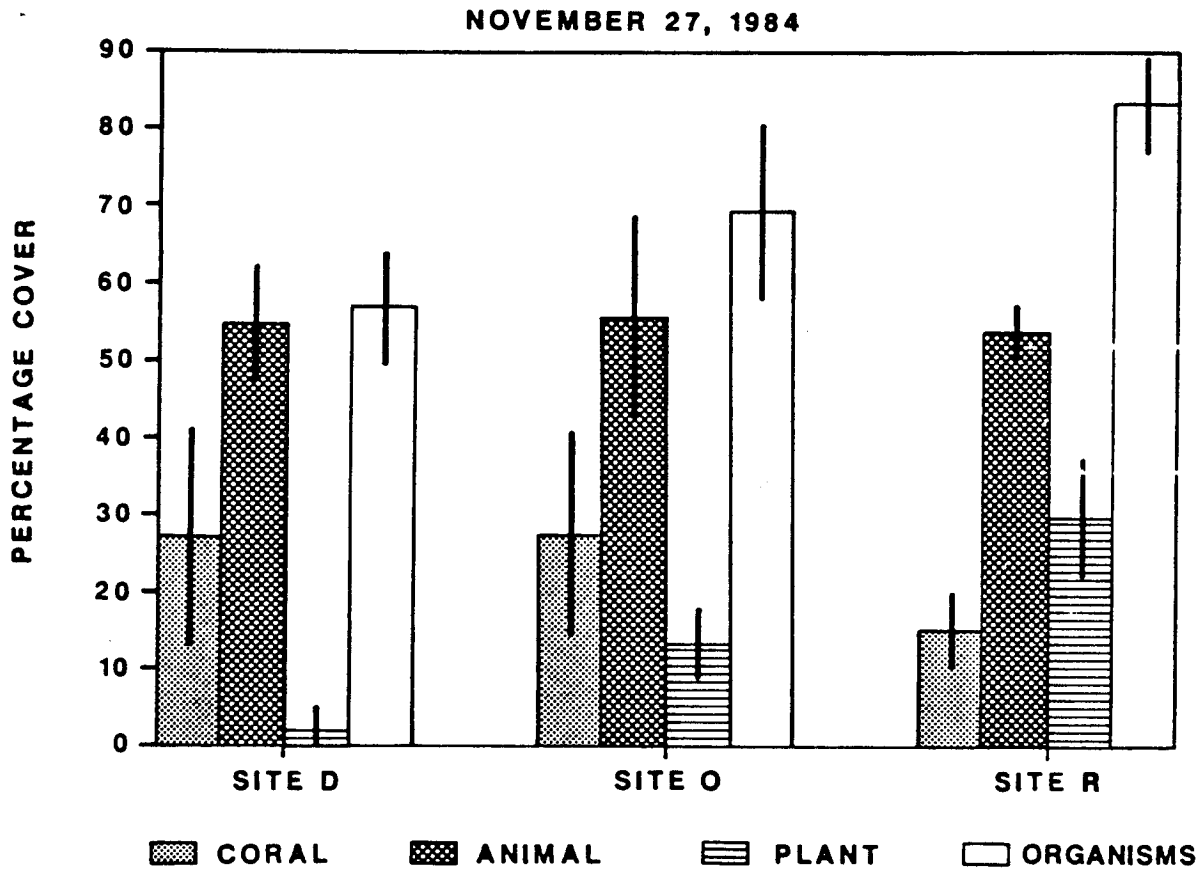


FIGURE 31. For the November 1984 pretreatment survey, coverage of total coral and total animals was similar between sites. Each site, however, was significantly different for total plants and total organisms.

discovered. Sites were most similar in total coral and total animal coverage. Sites were statistically different in total plant and total organism coverage. Site R was most different than other sites because of lower coral coverage and higher algal, plant, and organism coverage.

Comparisons Within Sites

The previous results of between-site comparisons for both the initial survey and the pretreatment assessment indicated significant site differences for most parameters. Therefore, it was apparent that each site had unique characteristics which would preclude using any one site as a "control" for changes in another caused by experimental perturbation. It was decided to evaluate changes caused by treatments in the following ways.

Two-way ANOVA was used on data from each site for each of the four parameters (total coral, total animal, total plant, and total organism cover-age). A fixed-model ANOVA was employed. This statistical analysis evaluates differences between the six assessment periods and accounts for the variance component of transects within each site and replication of each transect. Consequently, the November 1984 prespill period may be considered as an experimental control or reference period for comparison to the postspill periods. The March 1984 data are not included in this statistical analysis because transect locations had to be changed after that assessment
period

Unless otherwise noted, the significance level for the analyses is the p-less-than-0.01 level. This 99 percent probability level was chosen for higher statistical confidence in the results. Where appropriate, the p-lessthan-0.05 level of testing was used occasionally and is noted in the text. If the ANOVA test indicated a significant period difference was present, further testing was conducted using the SNK test to isolate specifically those periods which were significantly different from each other.

Site R has been designated as a reference site. Between-site comparisons in the pretreatment periods had indicated that Site R could not be used as a "control" site in the strict sense. This site was designated as the "reference" site to be used primarily for monitoring any seasonal or other changes in the floral and faunal composition. Site R data were subjected therefore to the same statistical analyses as described above.

Site D: __ Dispersed Oil Treatment

The mean values (+/-1 standard deviation) of each of the major assessment categories (total coral, total organism, total plant, total animal cover-age) for each assessment period are graphed in Figures 32 and 33. The data for the initial survey are graphed for reference.

Table 30 provides a summary of these data, including means and standard deviations for individual assessment categories and totals for corals, animals, plants, and organisms. Nearly all major assessment categories declined abruptly in the first posttreatment assessment period and continued to decline through December 1985 (12 months postspill).

Total coral coverage declined over the entire postspill study period. Two-way ANOVA for total coral coverage indicated a significant difference between assessment periods (p less than 0.05). SNK testing (Table 33) revealed that the November 1984 pretreatment period was significantly greater than the December 1985 and August 1986 periods (12 and 20 months post-spill, respectively). This decrease in total coral coverage was the result of a 67 percent decrease in P. porites coverage, and the complete elimination- of A. tennuifolia from the transect locations.

For total animals, ANOVA indicated significant period differences at the p-less-than-0.01 level. SNK testing revealed that the prespill period was significantly greater than all other periods. Total animal coverage was affected primarily by the loss of sponges during the immediate postspill period. Sponge coverage declined by 40 percent during this time. On-site observations indicated that sponges were severely affected by dispersed oil; sponges in the treatment area were covered by a white, fungus-like layer and were very friable. As mentioned above, P. porites coverage also declined, contributing to the overall decrease in total animal coverage.

For total plants, ANOVA did not reveal any significant period effects. For total organisms, ANOVA revealed significant period differences. SNK indicated the prespill period was significantly greater than all postspill periods. In addition, the December 1984 (5 days postspill) period was greater than all other postspill periods. Total organism coverage declined by almost 50 percent between November 1984 and August 1986 (3 days prespill and 20 months postspill, respectively), accompanied by a 40 percent increase in bare substrate.

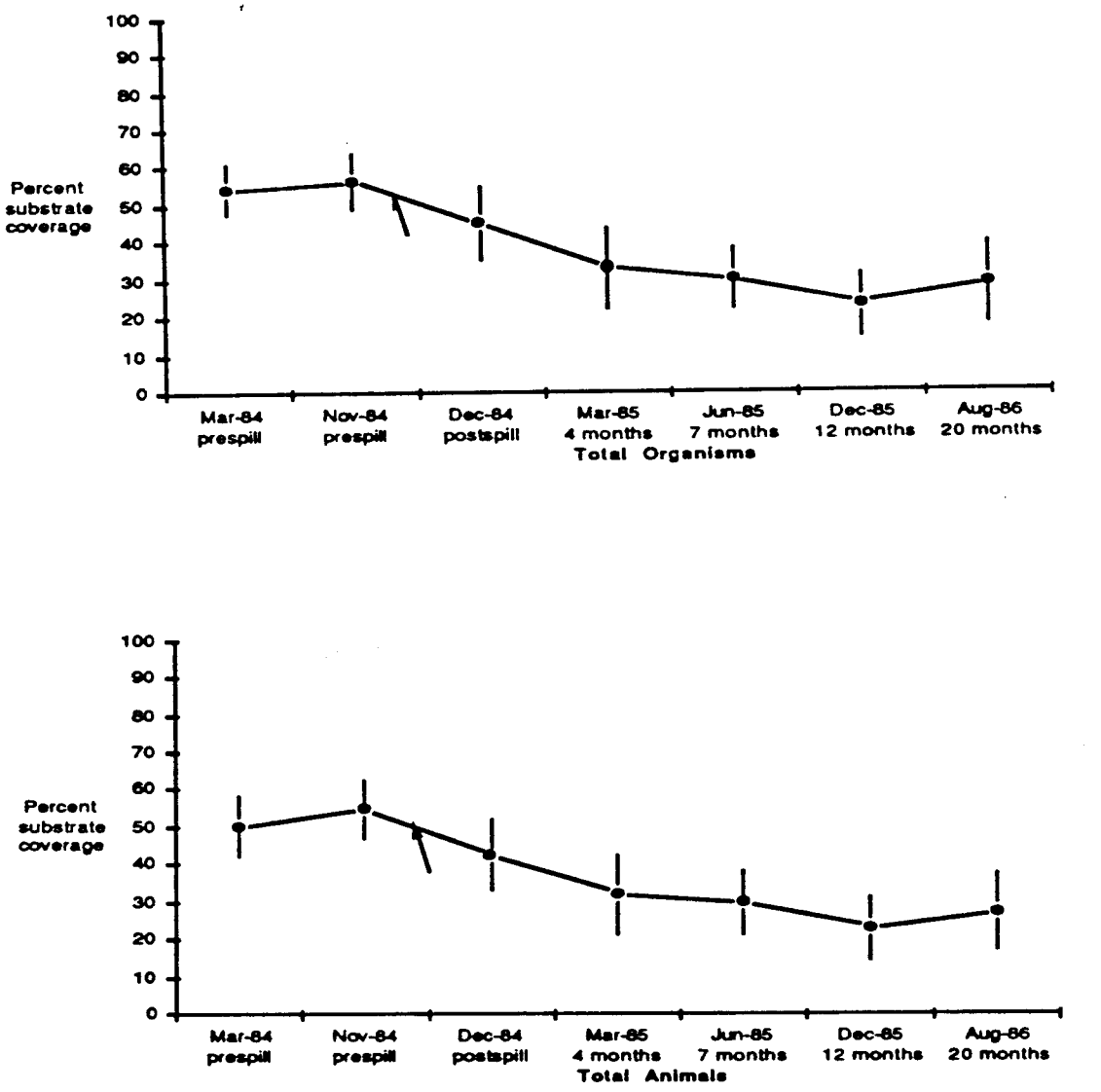
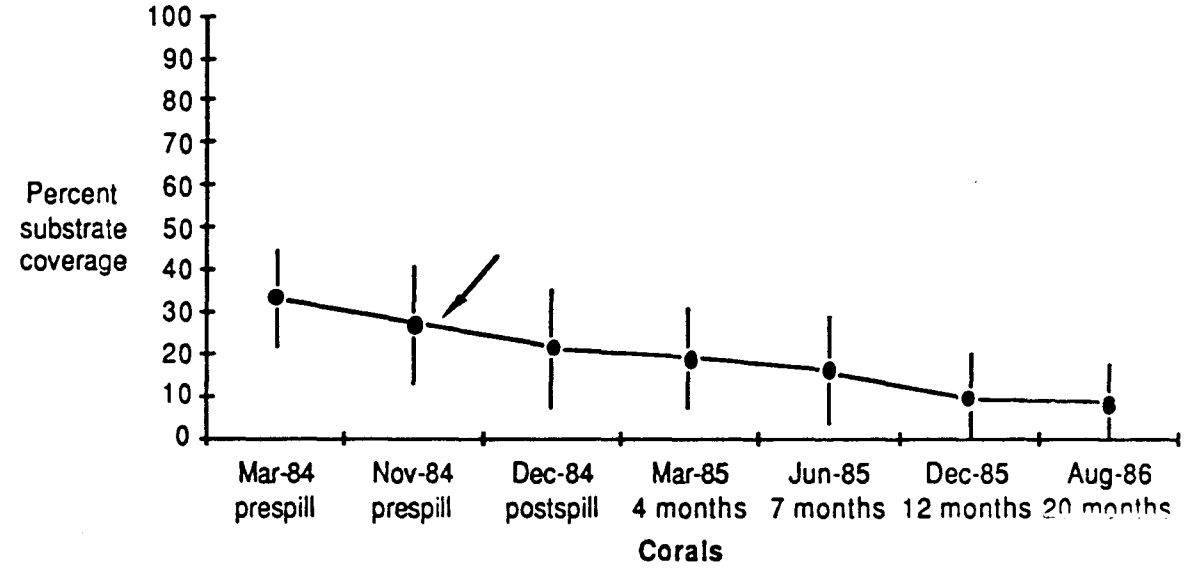


FIGURE 32. The percentage of reef substrate coverage of total organisms (top) and total animals (bottom) at Site D. Error bars are ±1 standard deviation.


Percent substrate coverage
100
90
80
70
60
50
40
30
20
10
0
Mar-84 prespill
Nov-84 prespill
Dec-84 postspill
Mar-85 4 months
Jun-85 7 months
Dec-85 12 months
Aug-86 20 months
Corals

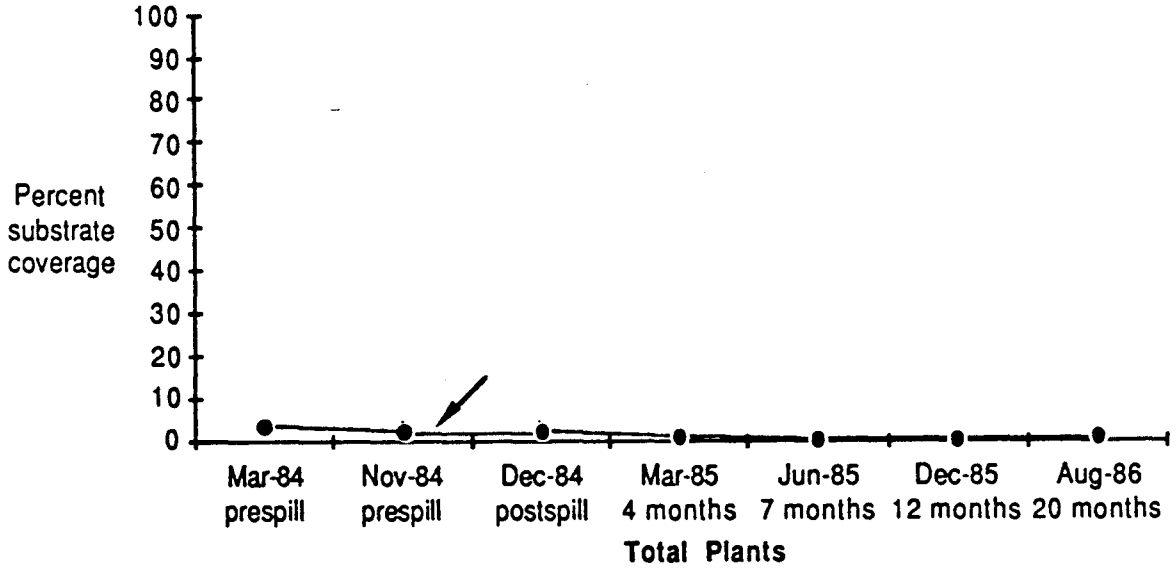

Percent substrate coverage
100
90
80
70
60
50
40
30
20
10
0
Mar-84 prespill
Nov-84 prespill
Dec-84 postspill
Mar-85 4 months
Jun-85 7 months
Dec-85 12 months
Aug-86 20 months
Total Plants

TABLE 33. Results of SNK testing for dispersed oil Site D for period differences for each of the parameters total coral, total animal, total plant, and total organism coverage. Equality or inequality of means determined at p less than 0.01, except for coral coverage where p is less than 0.05.

PERIOD COMPARISON	PARAMETERS			
	Coral Coverage	Animal Coverage	Plant Coverage	Total Organism Coverage
November 1984 -vs- December 1984	=	≠	=	≠
November 1984 -vs- March 1985	=	≠	=	≠
November 1984 -vs- June 1985	=	≠	=	≠
November 1984 -vs- December 1985	≠	≠	=	≠
November 1984 -vs- August 1986	≠	≠	=	≠
December 1984 -vs- March 1985	=	=	=	≠
December 1984 -vs- June 1985	=	=	=	≠
December 1984 -vs- December 1985	=	≠	=	≠
December 1984 -vs- August 1986	=	≠	=	≠
March 1985 -vs- June 1985	=	=	=	=
March 1985 -vs- December 1985	=	=	=	=
March 1985 -vs- August 1986	=	=	=	=
June 1985 -vs- December 1985	=	=	=	=
June 1985 -vs- August 1986	=	=	=	=
December 1985 -vs- August 1986	=	=	=	=

In each ANOVA, the factor "transects" was highly significant indicating that individual transects differed from each other. This was an expected result because of the spatial heterogeneity of the reefs assessed.

Site 0: __ Oil Only Treatment

The mean values (±1 standard deviation) of each of the major assessment categories (total coral, total organism, total plant, and total animal coverage) for each assessment period are graphed in Figures 34 and 35. The data for the initial survey are graphed for reference. All data are presented in Table 31, and SNK results appear in Table 34.

In general, while there appears to be a slight decline for some parameters following site treatment, the overall impression is one of fairly stable conditions over time. Total plant coverage appears to decline; however, this organism category was scarce and, consequently, is represented by relatively few data points.

No significant differences in total coral coverage between sampling periods were detected using ANOVA. Inspection of the data in Table 31 indicates that coral coverage appears to decline gradually over the entire study period, but none of the between-period means are significantly different. Therefore, within the range of measured variability at Site 0, it is not possible to detect (using ANOVA) any overall effect on corals.

Significant ANOVA differences in total animal coverage were detected at Site 0, but these differences involved increases in animal coverage during the March 1985 to August 1986 period (4-20 months postspill). No significant reduction was detected in animal coverage during the postspill period. The increase in total animal coverage appeared to be the result of a 63 per-cent increase in zooanthid coverage between November 1984 and August 1986 (3 days prespill and 20 months postspill, respectively).

For total plants, ANOVA revealed significant period effects. SNK testing revealed the prespill period was significantly greater than all the post-spill periods from March 1985 to August 1986 (4 to 20 months postspill). In addition, the December 1984 (4 days postspill) period was significantly greater than all other postspill periods.

For total organism coverage, ANOVA revealed significant period effects. SNK testing revealed that only the March 1985 period was significantly less than the prespill period. This appears to be the result of small,

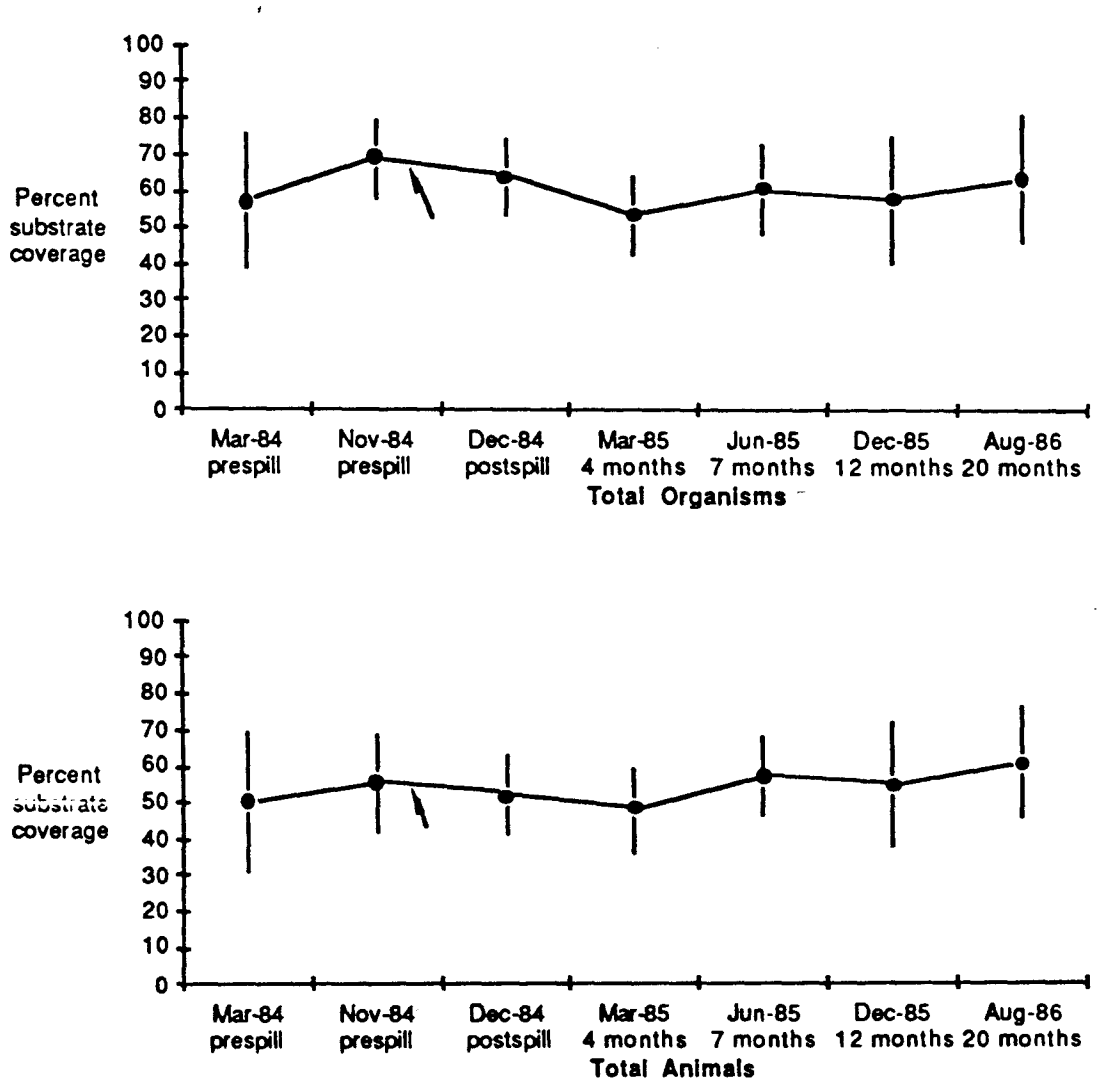


FIGURE 34. The percentage of reef substrate coverage of total organisms (top) and total animals (bottom) at Site O. Error bars are ±1 standard deviation. Arrow indicates date of site treatment.

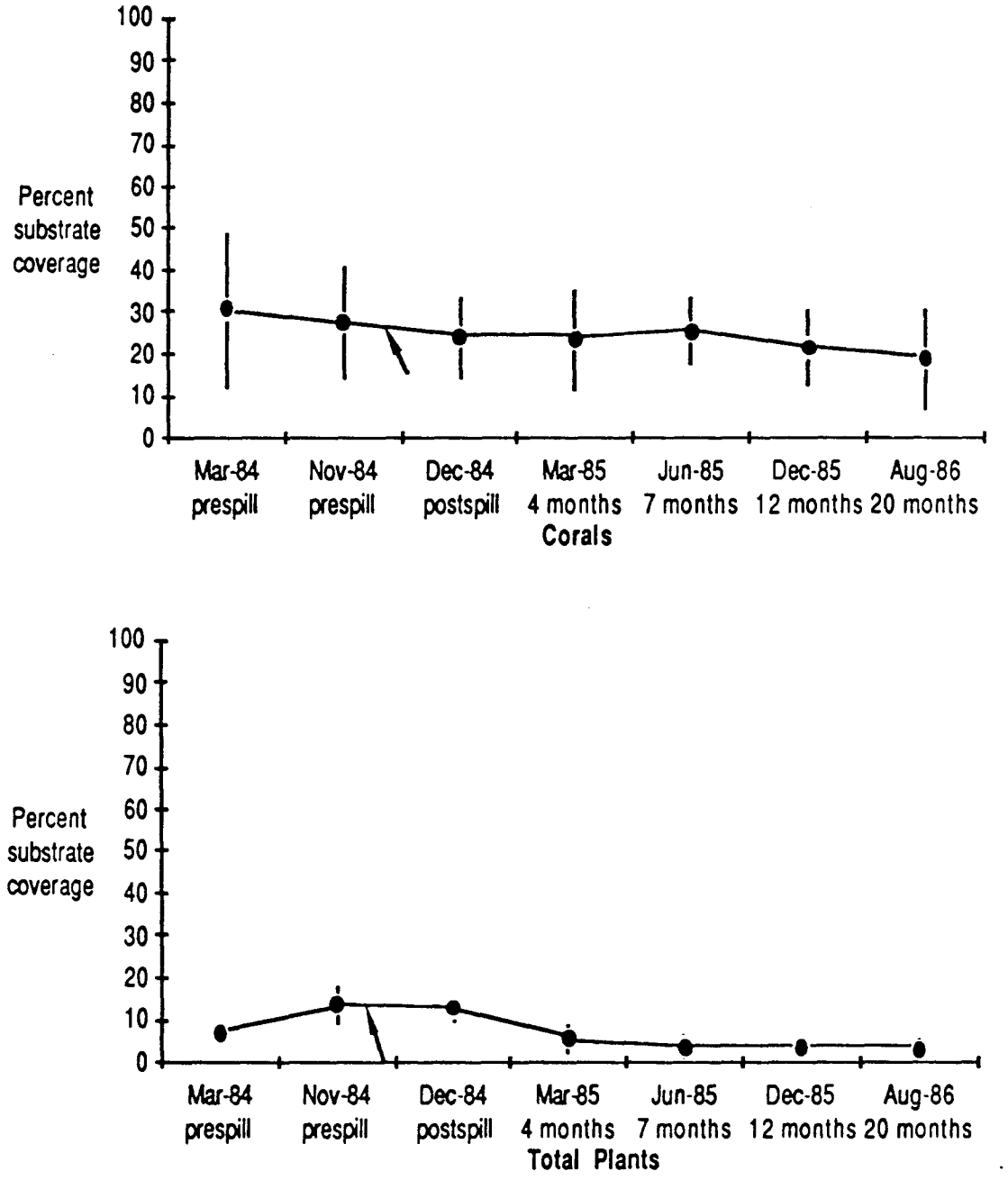


FIGURE 35. The percentage of reef substrate coverage of corals (top) and total plants (bottom) at Site O. Error bars are ± 1 standard deviation.

TABLE 34. Results of SNK testing for oil only Site 0 for period differences for each of the parameters total coral, total animal, total plant, and total organism coverage. Equality or inequality of means determined at p less than 0.01, except coral coverage where p is less than 0.05.

PERIOD COMPARISON	PARAMETERS			
	Coral Coverage	Animal Coverage	Plant Coverage	Total Organism Coverage
November 1984 -vs- December 1984	=	=	=	=
November 1984 -vs- March 1985	=	=	≠	≠
November 1984 -vs- June 1985	=	=	≠	=
November 1984 -vs- December 1985	=	=	≠	=
November 1984 -vs- August 1986	=	=	≠	=
December 1984 -vs- March 1985	=	=	≠	=
December 1984 -vs- June 1985	=	=	≠	=
December 1984 -vs- December 1985	=	=	≠	=
December 1984 -vs- August 1986	=	=	≠	=
March 1985 -vs- June 1985	=	=	=	=
March 1985 -vs- December 1985	=	=	=	=
March 1985 -vs- August 1986	=	≠	=	=
June 1985 -vs- December 1985	=	=	=	=
June 1985 -vs- August 1986	=	=	=	=
December 1985 -vs- August 1986	=	=	=	=

nonsignificant decreases in corals, zooanthids, and algae combining to cause an overall reduction in total organism coverage at that time. Four months later, however, total organism coverage had increased to prespill levels, indicating that the effects of site treatment on this parameter were relatively short-lived.

In each ANOVA except for total plants, the factor "transects" was highly significant indicating that individual transects differed from each other. This was an expected result because of the spatial heterogeneity of the reefs assessed. For total plants, transects were similar because of the very low numbers found on each.

Reference Site R

The mean values (±1 standard deviation) of each of the major assessment categories (total coral, total organism, total plant, and total all animal coverage) for each assessment period are graphed in Figures 36 and 37. The data for the initial survey are included for reference. All data are presented in Table 32.

In general, parameters for Site R show relatively uniform values over assessment period times. Variability is high, and there are some apparent period differences. As previously discussed, this site was the most different from the other two treatment sites. This was most evident for the parameters total corals and total plants which are relatively lower and higher, respectively. With the exception of the December 1985 (12 months postspill) data, there appears to be a gradual increase in total plant coverage with a corresponding decline in total coral coverage. This is probably explained by an overgrowth phenomenon whereby either fleshy algae or seagrass were covering corals during the assessment. These results do not necessarily imply coral death.

For total coral coverage, ANOVA indicated significant period differences. SNK testing, however, did not resolve any particular period as significantly different from another. For total animal coverage, ANOVA indicated significant period differences. SNK indicated that the November 1984 pretreatment period was significantly greater than August 1986 period.

For total plants, ANOVA indicated significant period differences. SNK testing revealed the December 1984 period to be significantly lower than the

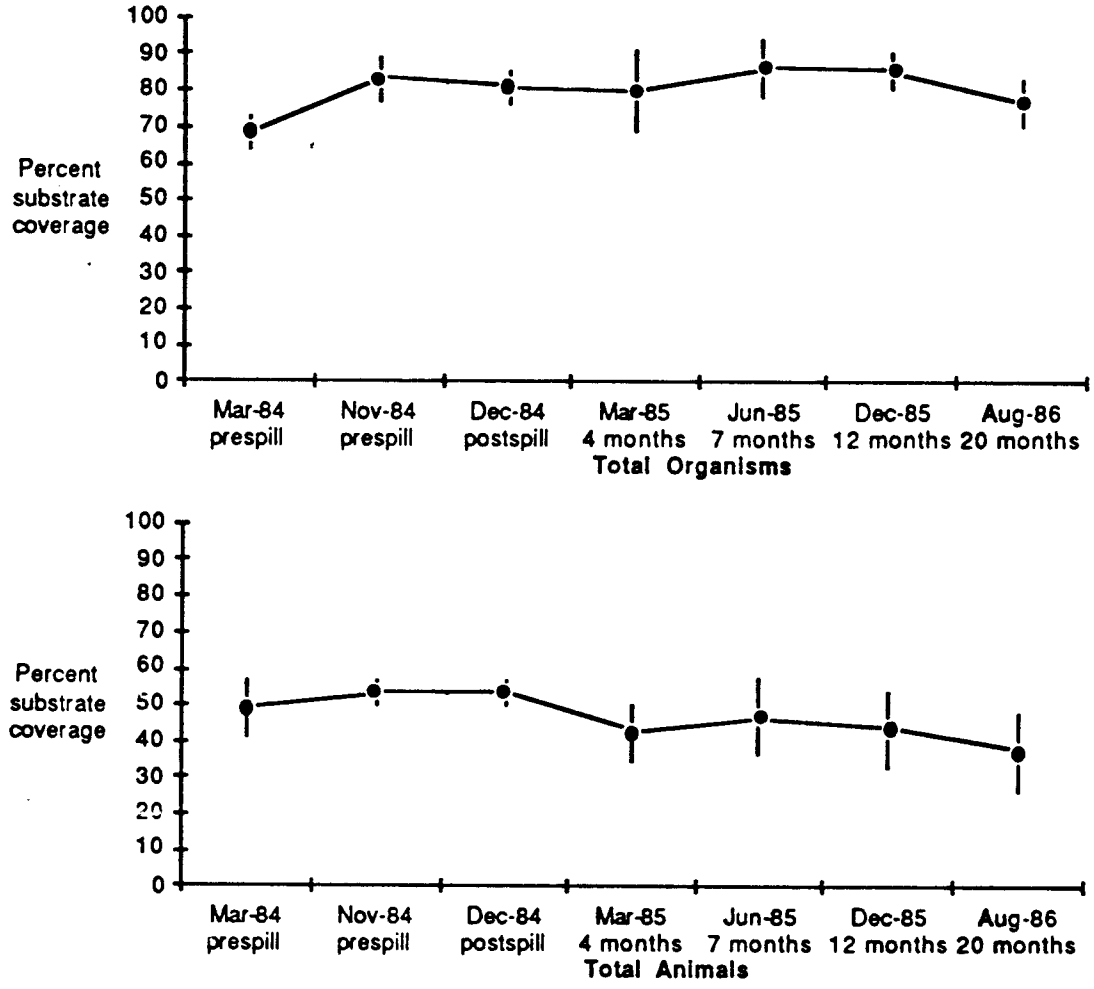
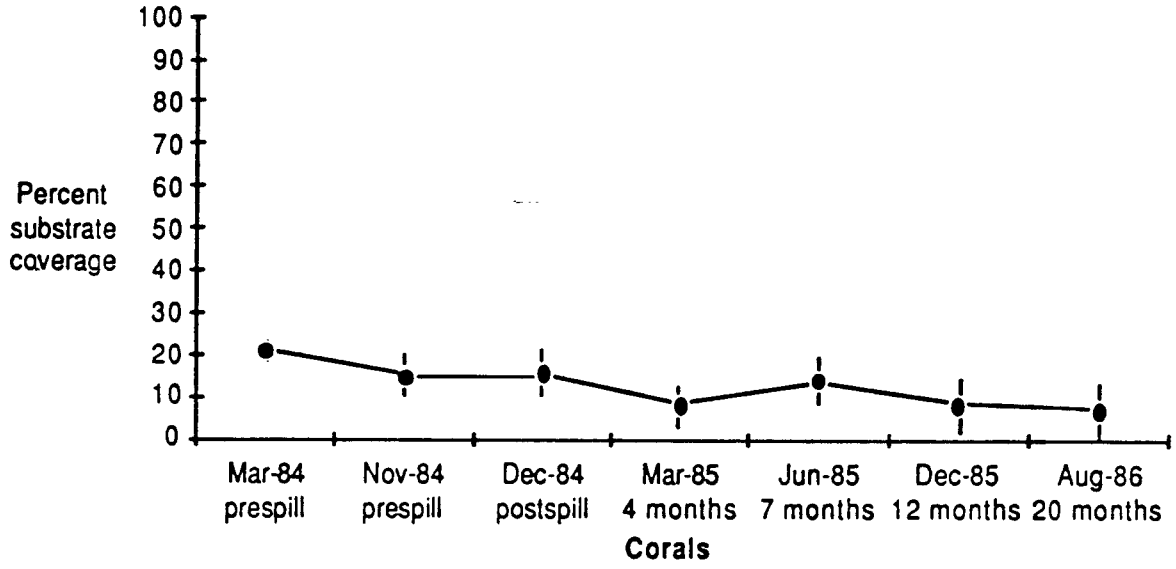


FIGURE 36. The percentage of reef substrate coverage of total organisms (top) and total animals (bottom) at Site R. Error bars are ±1 standard deviation.



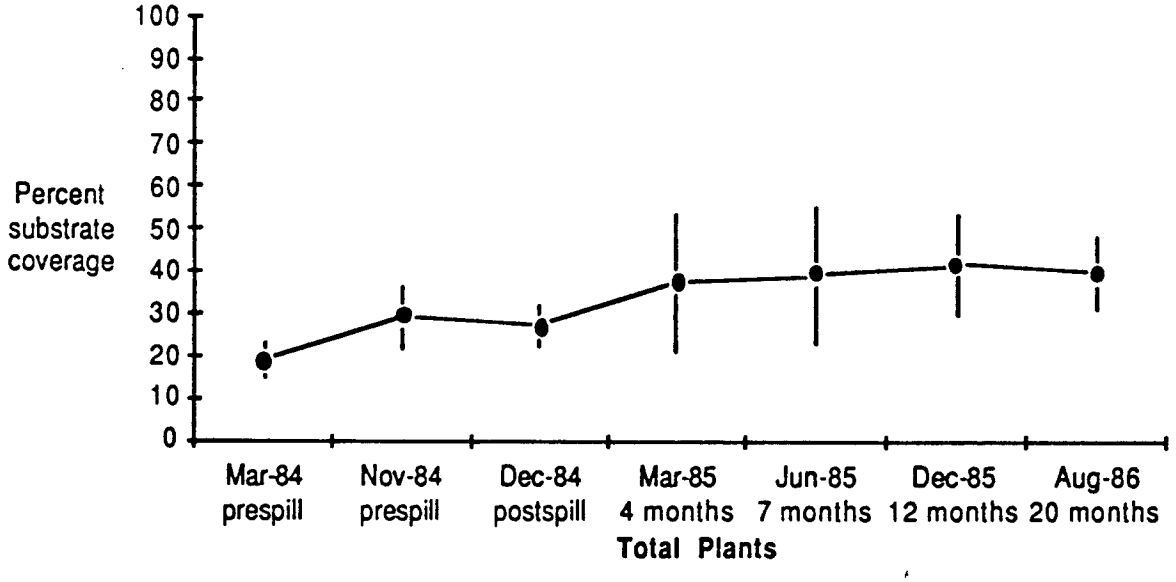


FIGURE 37. The percentage of reef substrate coverage of corals (top) and total plants (bottom) at Site R. Error bars are ±1 standard deviation.

June 1985-August 1986 period. For total organisms, ANOVA indicated no significant differences between periods.

Table 35 presents SNK results in detailed form.

In each ANOVA, the factor "transects" was highly significant indicating that individual transects differed from each other. As with the dispersed oil Site D and oil only Site 0, this was an expected result because of the spatial heterogeneity of the reefs assessed.

Regression Analysis of Floral/Faunal Assessment Data

A linear regression analysis method (Sokal and Rohlf, 1981) was used to determine if a regression line fitted to transect parametric data for each site would have a slope significantly different from zero. A regression line with a slope not significantly different from zero indicates that there was no change in the dependent variable (coverage by each assessment parameter) over time (i.e., within the range of variability present in the data, no statistically significant effect over time is indicated). The analysis also incorporated an ANOVA to further test inequality of parametric means.

The parameters tested for each site were total coverage of all organisms, animals, corals, and plants. The initial assessment period data (March 1984) were not included for the reasons previously stated (initial transect positions were different). The independent variable X consisted of the time prior to and after site treatment for each of 6 assessment periods (November 1984, December 1984, March 1985, June 1985, December 1985, and Au[9]ust 1986; or prespill and 4, 7, 12, and 20 months postspill). The de-pendent variable Y consisted of means of parametric data from each transect at each period.

Results are summarized in Table 36. Listed for each site are the coverage parameters, the F value from the ANOVA for comparisons among groups, its statistical significance, the F value from the ANOVA for the linear regression, its statistical significance, and the positive or negative character of the slope of the regression line.

Stated briefly:

> Both the ANOVA and the linear regression test the same null hypothesis--equality of means, the regression test is more powerful . . . against the alternative hypothesis that there is a linear relationship between the group means and the independent variable X. Thus, when the means increase or decrease slightly as X in-creases, they may not be different enough for the mean square

TABLE 35. Results of SNK testing for reference Site R for period differences for each of the parameters total coral, total animal, total plant, and total organism coverage. Equality or inequality of means determined at p less than 0.01, except coral coverage where p is less than 0.05.

PERIOD COMPARISON	PARAMETERS			
	Coral Coverage	Animal Coverage	Plant Coverage	Total Organism Coverage
November 1984 -vs- December 1984	=	=	=	=
November 1984 -vs- March 1985	=	=	=	=
November 1984 -vs- June 1985	=	=	=	=
November 1984 -vs- December 1985	=	=	=	=
November 1984 -vs- August 1986	=	≠	≠	=
December 1984 -vs- March 1985	=	=	=	=
December 1984 -vs- June 1985	=	=	≠	=
December 1984 -vs- December 1985	=	=	≠	=
December 1984 -vs- August 1986	=	≠	≠	=
March 1985 -vs- June 1985	=	=	=	=
March 1985 -vs- December 1985	=	=	=	=
March 1985 -vs- August 1986	=	=	=	=
June 1985 -vs- December 1985	=	=	=	=
June 1985 -vs- August 1986	=	=	=	=
December 1985 -vs- August 1986	=	=	=	=

TABLE 36. Summary of ANOVA and regression analysis. [NS = not significant]

PARAMETER	F (5,42)*	ANOVA SIGNIFICANCE OF GROUPS	F (1,4)*	SIGNIFICANCE OF LINEAR REGRESSION	SLOPE
SITE D					
Organisms	14.548	0.001	5.105	NS	minus
Animals	12.965	0.001	5.197	NS	minus
Corals	2.778	0.050	24.717	0.010	minus
Plants	1.915	NS	2.989	NS	minus
SITE O					
Organisms	1.278	NS	0.165	NS	minus
Animals	0.822	NS	2.850	NS	plus
Corals	0.695	NS	12.365	0.025	minus
Plants	20.324	0.001	7.248	NS	minus
SITE R					
Organisms	1.991	NS	0.394	NS	minus
Animals	4.818	0.005	13.201	0.025	minus
Corals	3.785	0.010	6.017	NS	minus
Plants	2.121	NS(.10)	5.697	NS(.10)	plus

* Degrees of freedom.

> among groups to be significant by ANOVA, yet a significant regression can be found. Where we find a marked regression of the means on X, . . . we usually will find a significant difference among the means by an ANOVA. However, we cannot turn this argument around and say that a significant difference among means as shown by an ANOVA necessarily indicates that a significant linear regression can be fitted to these data (Sokal and Rohlf, 1981).

For example, means can be significantly different from each other, yet the regression line may explain only little of the variation.

For Site D, only total coral coverage exhibits both statistically significant ANOVA and linear regression (negative slope). Clearly, there is a strong relationship of decreasing coral coverage over the entire study period. Total organism and total animal coverage exhibit significant group differences but not a significant regression.

In order to understand the ANOVA results, reference must be made to the SNK results of Table 33 where for animal coverage, the December 1985 and August 1986 data (12 and 20 months postspill) are significantly less than the December 1984 (1 week postspill) data and all postspill periods are less than the prespill period. Coverage of all organisms during the period from March 1985 to August 1986 (4 to 20 months postspill) are less than December 1984 data and the November 1984 prespill period. Although these differences are indicative of a change over time, the regression is not significant for the full duration of the study probably because there were slight (but not statistically significant by ANOVA) increases during the final assessment period (August 1986) in these parameters. These increases toward the end of the study were large enough to cause the regression line fitted to the data to have a slope equal to zero, indicating that no significant change occurred from the beginning to the end of the study. The ANOVA analysis shows that significant decreases occurred between individual assessment periods. The overall conclusion drawn from these analyses is that coral coverage was reduced and no recovery occurred, and total organism and total animal coverage decreased but there were indications that recovery of these parameters had begun during the final assessment period 20 months after site treatment. Results for plant coverage are not discussed further because of the low numbers of counts on the transects.

For Site 0, only coral coverage exhibited a significant regression (the accompanying ANOVA did not exhibit significance). This demonstrates the power of regression to indicate a difference not revealed by ANOVA alone.

There was a slight, but statistically significant, decrease in coral coverage over time. Coral coverage was the only parameter that showed a consistent downward trend over the entire study period, resulting in a regression line that was significantly different from zero. The incremental decreases between periods were too small to be significant by ANOVA, but the greater sensitivity of the regression detected the downward trend. As in the above discussion of Site D, plants are not discussed because of low coverage at this site.

For Site R, the only parameter that exhibited a significant regression is total animal coverage, which showed decreasing coverage over time. This must be tempered with on-site observations which indicated an increase in fleshy algae over time. Increased algal coverage made observation of animals more difficult. Although only significant at the 90 percent level, the table results for plants tend to confirm this with an increase in the regression line over time. No significant regression was found for coral coverage, indicating that there was no measurable decrease in coral coverage through the du-ration of the study at Site R.

In summary, regression analysis confirms and provides additional information to the ANOVA and SNK analyses. Although the data are variable, there is a significant decline in coral coverage over time at both sites D and 0. It is also important to note that if data from the last assessment period (August 1986) are omitted from the analyses of Site D, the regression would have been more apparent (and significant) for the parameters of total organisms and total animals. This is because total animal and organism coverage appeared to have reversed (or at least stabilized) their decline by the final assessment period. For Site R, the statistical result of a decline in total animal coverage over time is explained most likely by a corresponding increase in total plant (algae) coverage which made animal observation more difficult.

Summary of Floral/Faunal Assessment Results

Of the three sites, the dispersed oil Site D exhibited a relatively dramatic and consistent decline in parameters for most periods following treatment. In most cases, this also could be resolved into statistically significant differences. As observed in Figures 32 and 33, it is apparent that the de-cline has leveled or reversed from December 1985 to August 1986 (12 to 19 months postspill). This may mean that the reef is on the *way* to partial or

full recovery. Given the variability in the data and the small differences between successive assessment periods, it is impossible to make a firm pre-diction on the degree or duration of recovery at this time.

For the oil only Site 0, there are few conclusive indications of a negative effect. There are suggestions, supported in some cases by the statistical results, that a decline in some parameters may have occurred up to March 1985 (4 months postspill). The results for total plants may not be as reliable as for other categories because of low sample size, characteristic of this site.

The effects on percentage of coverage of epiflora and epifauna at Sites D and 0 appear to be strongly correlated with exposure to petroleum hydro-carbons (see Tables 4-9). Site D was exposed to much higher levels of waterborne hydrocarbons than Site 0 during site treatment, and the levels of contamination of the nearby seagrass sediments were also consistently higher over time at Site D than Site 0.

The data of reference Site R are marked by somewhat higher variability. There are some period differences for certain of the assessment parameters. The lack of a clear time trend, however, indicates that these random or seasonal occurrences probably are not responsible for the sequentially lower differences exhibited by the periods of the dispersed oil Site D.

Coral Growth

Statistical analyses consisted of nested ANOVA and SNK tests. The first test was employed to evaluate if significant site differences were present for the growth parameter under consideration. The second test was used to identify the specific site differences in those factors. The significance level used was 0.05 (95 percent) unless otherwise noted.

Montastrea annularis

Septa increase data (cm/yr) for the period from November 1984 to March 1985 (4 months postspill) were analyzed by one-way two-level nested ANOVA to evaluate if significant differences between site means were present. The F-test revealed no significant site differences between those three evaluated (Sites D, 0, and R). This particular species was apparently little affected by dosing with either dispersed oil or nondispersed oil only.

Acropora cervicornis

Branch tip length (cm/yr) and width (cm) were determined for specimens from each of the sites during the period from November 1984 to March 1985 (4 months postspill). One-way nested ANOVA testing of each parameter (length and width of tips) at each site revealed no significant differences.

Agaricia tennuifolia

A summary of growth data for A. tennuifolia is presented in Table 37 and Figures 38-43. A discussion of each growth parameter measured for this species follows.

Blade Length (Extension Rate)

Measurements of A. tennuifolia collected in March 1985 (4 months post-spill) indicated that the blade extension rate at Site D was clearly lower than that at Sites 0, R, or R-2. Data from the 4 sites were compared using one-way nested ANOVA and Satterwaith's approximation (Sokal and Rohlf, 1969) and there was a significant difference between site means. Comparison using the SNK test (at the p-less-than-0.01 level) revealed that Site D was significantly less than Sites 0, R, and R-2. No significant differences were measured between the other sites.

A[g]aricia tennuifolia specimens stained in June 1985 and collected in December 1985 also showed differences between sites. The mean blade extension rate at Site D was significantly lower than at Sites 0, R, or R-2, which were not different compared to each other.

This pattern persisted through the end of the study. Specimens of A. tennuifolia stained in December 1985 and collected in August 1986 showed significantly reduced blade extension rates at Site D compared to Sites 0, R, or R-2. No difference was measured between Sites R-2 and O.

Blade Thickness (Width)

Measurements of blade thickness of A. tennuifolia specimens stained in November 1984 and collected in March 1985 (4 months postspill) were found to show significant differences between sites. Mean values for Site D were clearly lower than the other three sites. SNK testing (at the p-less-than-

TABLE 37. *Agaricia tennuifolia* growth statistics. Asterisks (*) indicate significant differences (p = 0.05) compared to Site R or Site R-2.

	EXTENSION RATE (cm/yr)				MEAN WIDTH (cm)				BLADE AREA (cm^2/yr)			
SITE	D	O	R	R-2	D	O	R	R-2	D	O	R	R-2
November 1984 - March 1985												
MEAN	1.33*	2.81	2.84	2.32	0.24*	0.28	0.34	0.28	0.16*	0.40	0.48	0.33
SD	0.38	0.47	0.50	0.32	0.06	0.04	0.04	0.04	0.06	0.10	0.10	0.06
N	55	50	60	30	55	50	60	30	55	50	60	30
June 1985 - December 1985												
MEAN	1.01*	2.62	2.63	2.62	0.21*	0.32	0.28	0.35	0.11*	0.43	0.38	0.46
SD	0.31	0.65	0.77	0.57	0.07	0.04	0.04	0.05	0.06	0.13	0.14	0.13
N	25	20	20	20	25	20	20	20	25	20	20	20
December 1985 - August 1986												
MEAN	1.00*	2.80*	1.96	3.25	0.26	0.37	0.35	0.40	0.15*	0.53*	0.34	0.66
SD	0.47	0.57	0.23	0.42	0.10	0.08	0.04	0.06	0.13	0.23	0.28	0.16
N	15	25	10	15	15	25	10	15	15	25	10	15

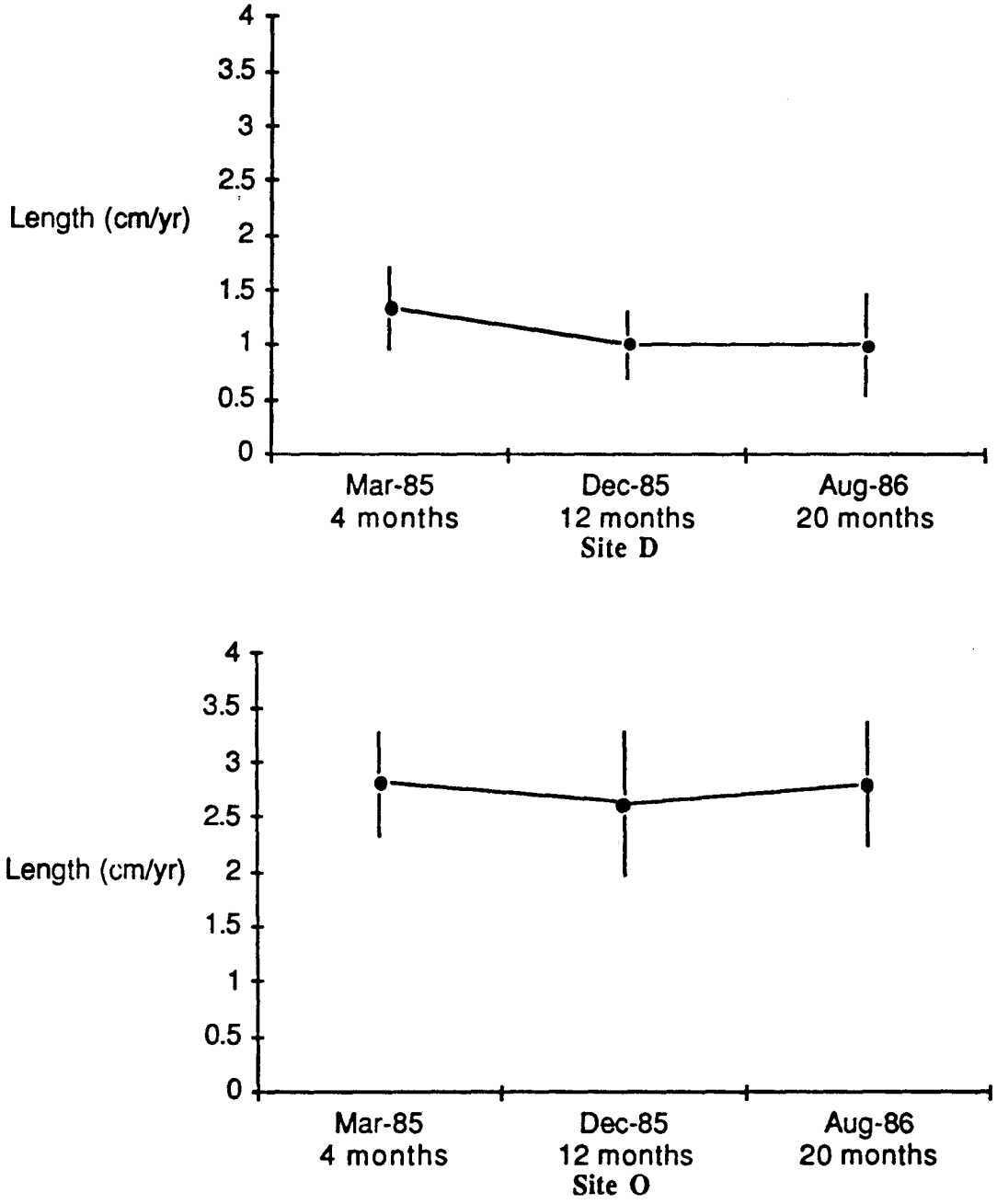


FIGURE 38. Blade length (extension rate) of A aricia tennuifolia specimens at Site D (top) and Site 0 (bottom

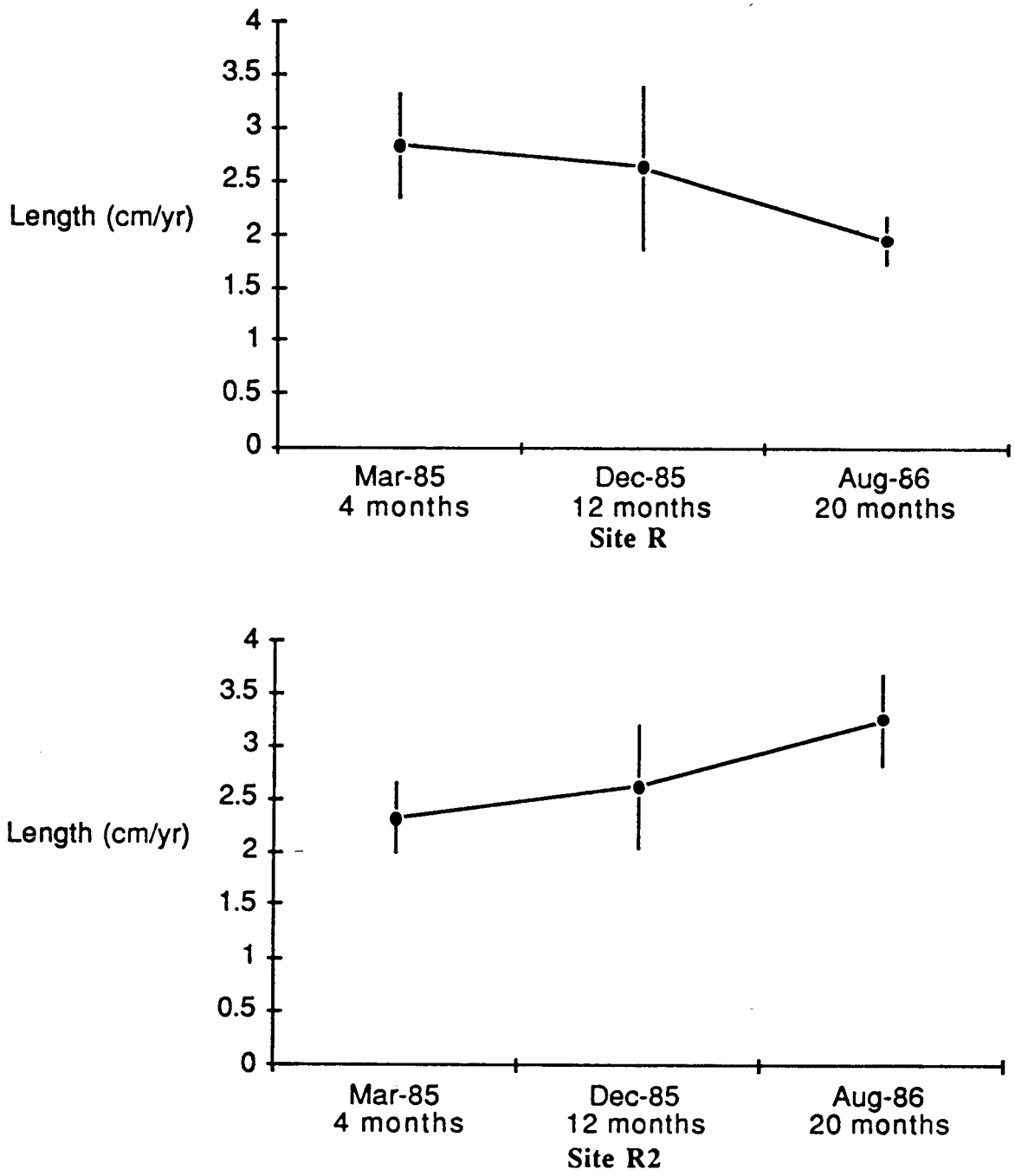


FIGURE 39. Blade length (extension rate) of Agaricia tennuifolia specimens at Site R (top) and Site R-2 (bottom).

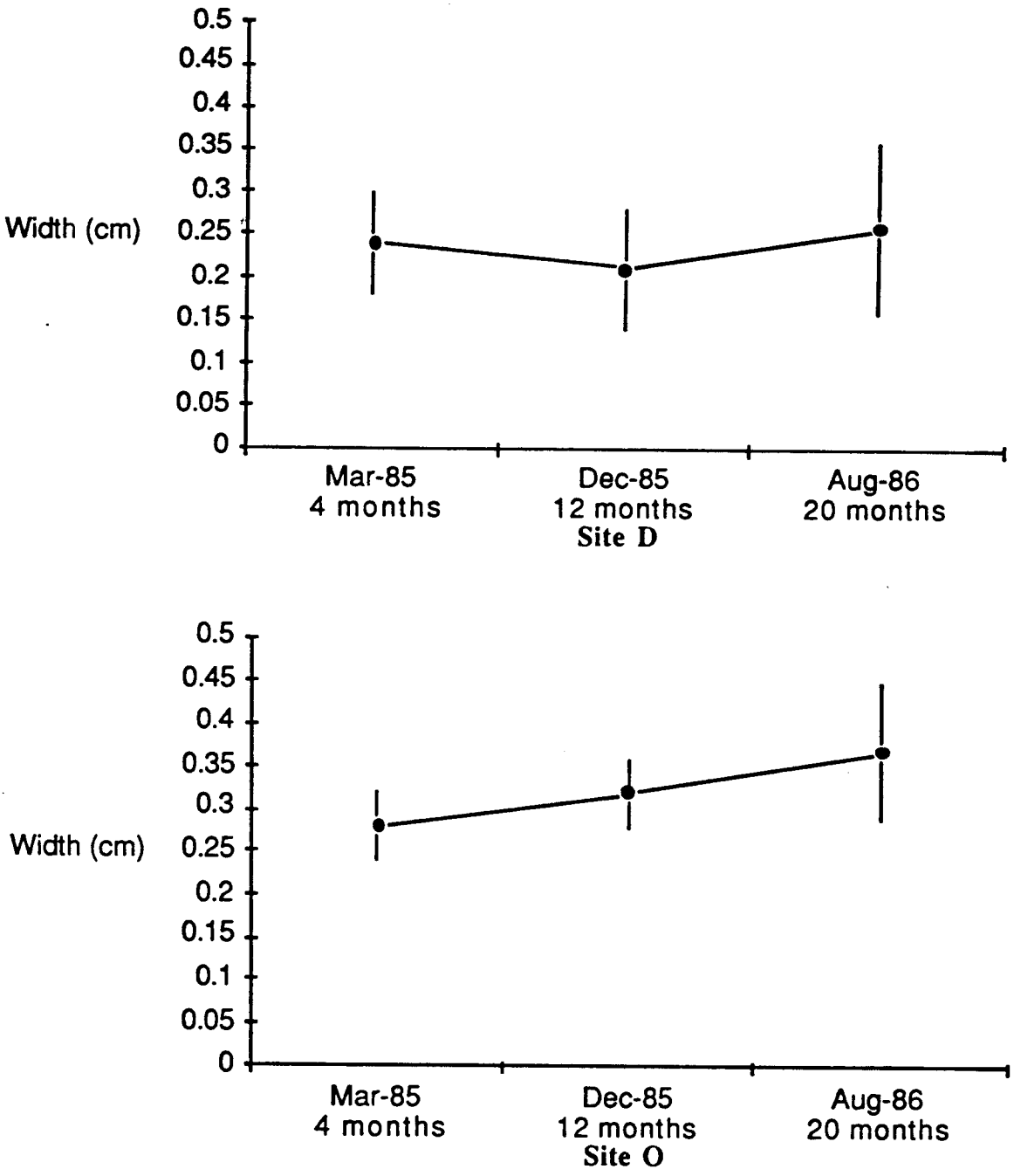


FIGURE 40. Blade thickness (width) of A. tennuifolia specimens at Site D (top) and Site 0 (bottom).

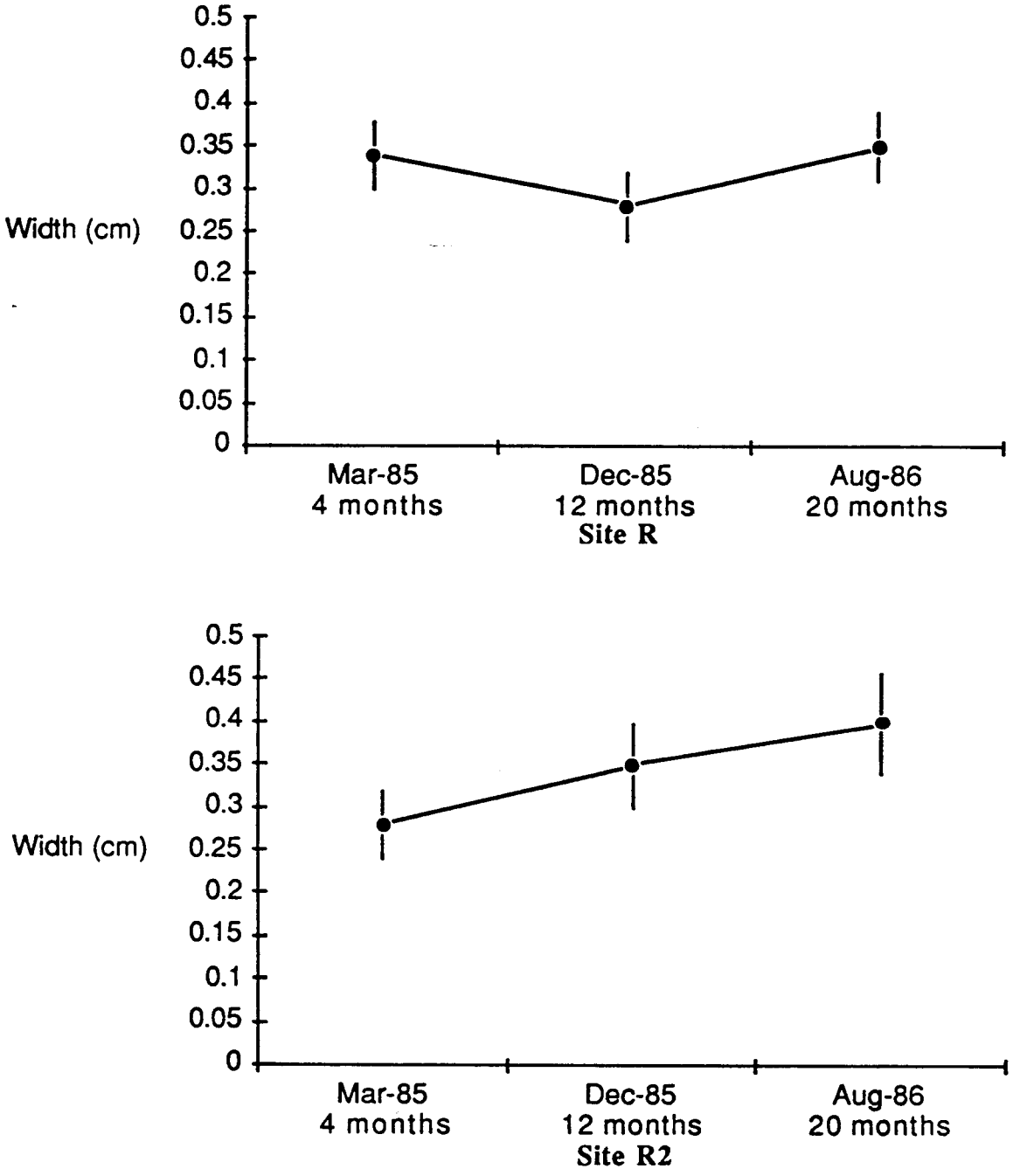


FIGURE 41. Blade thickness (width) of A. tennuifolia specimens at Site R (top) and Site R-2 (bottom)

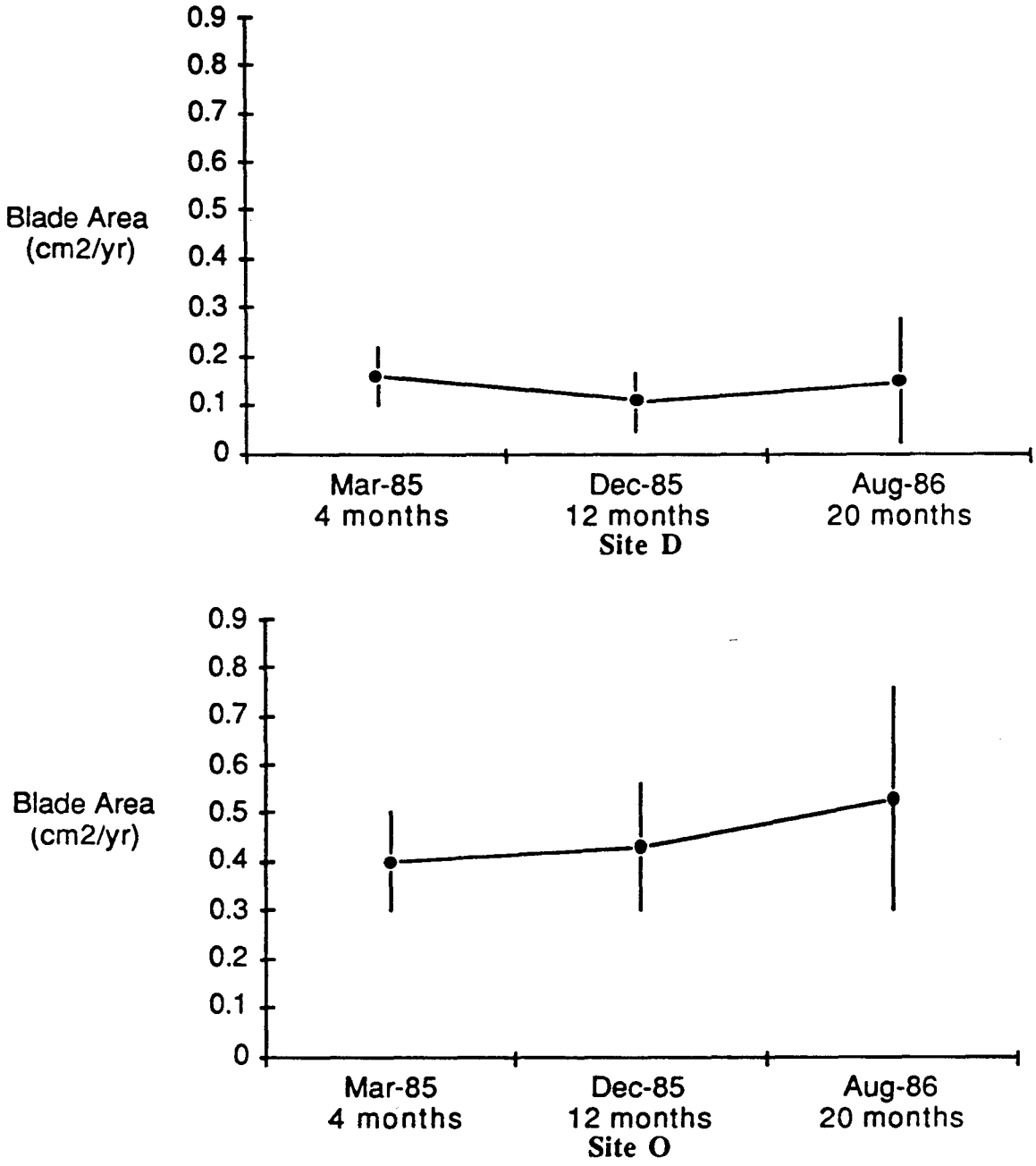


FIGURE 42. Cross-sectional blade area of A. tennuifolia specimens at Site D (top) and Site 0 (bottom).

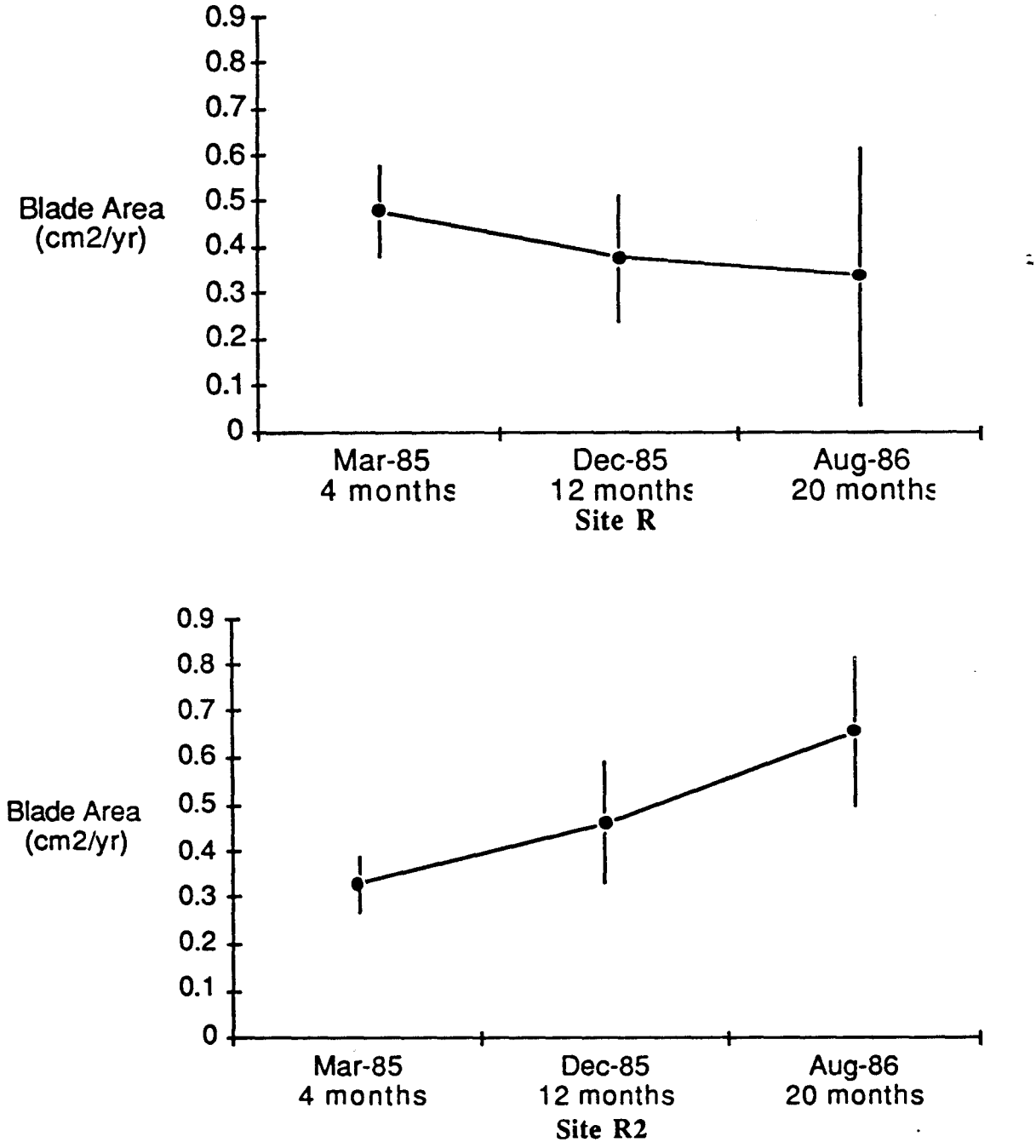


FIGURE 43. Cross-sectional blade area of A. tennuifolia specimens at Site R (top) and Site R-2 (bottom).

0.05 level) indicated that Site D was significantly less than Sites *0, R,* and R-2. All other sites were not statistically different.

Specimens from Site D stained in June 1985 and collected in December 1985 also showed significantly reduced blade thickness compared to Sites 0, R, and R-2. No difference was measured between Sites 0 and R-2 and Sites O and *R.* Sites R and R-2 were significantly different. During the final growth period (December 1985 to August 1986), the only significant difference in blade width was measured between Sites D and R-2.

Cross-Sectional Blade Area

An index of the mass growth of these corals is given by the cross-sectional blade area (product of extension rate and thickness divided by 2). The blade area of A. tennuifolia specimens stained in November 1984 and collected in March 1985 at Site D was significantly lower than at Sites 0, R, or R-2. SNK testing revealed that all sites were significantly different at the p-less-than-0.05 level, with the exception that Sites 0 and R-2 and Sites 0 and R were statistically indistinguishable.

Blade area was significantly lower at Site D compared to the other sites during all ensuing measurement periods (June 1985 to December 1985 and December 1985 to August 1986, 12 and 20 months postspill, respectively). In

1985, Site D was significantly lower than Sites 0, R, or R-2. There were no other site differences at that time. In August 1986, no difference was measured between Sites R-2 and 0, while all other sites were significantly different.

Figures 42 and 43 present a summary of data for growth parameters for A. tennuifolia measured at the end of each growth period at each site. While Site D values are much lower than for the other study sites, there is no readily apparent temporal trend of either decreasing or increasing grow'th rates for the treatment sites. The analysis of these data indicates that A. tennuifolia growth rates at Site D were significantly affected by treatment with dispersed oil and that recovery has not yet begun there.

Porites porites

Table 38 and Figures 44-49 present a summary of growth data for the coral species P. porites. The growth parameters tip extension rate (length), tip width, and tip growth volume were determined for P. porites specimens during each postspill period. Statistical results significant at the p-less-than-0.05 level are discussed below.

Tip Extension (Length)

Measurements at each site over time indicate that P. porites at Site D had depressed, but not significantly different, tip extension rates compared to all the other sites during the periods from November 1984 to March 1985 and from March 1985 to June 1985 (4 and 7 months postspill). During June 1985 to December 1985 (12 months postspill), tip extension at Site D was lower (but not significantly) than at all the other sites but was significantly greater than Site R. Site R-2 was significantly greater than Site R, and Site 0 was significantly less than Site R. All other sites were not signifi-
cantly different. No data for Sites D and R-2 are available for the period from December 1985 to August 1986 due to loss of the stained specimens at these sites during that period, possibly because of storms or vandalism.

Extension rates at Site 0 were greater (but not always significantly different) than those at Site D at all measurement periods (Table 38). Site 0 was statistically greater than Site R.

The reference sites' extension rates were quite variable. Clear trends over time or between reference sites were not apparent; extension rates were both less and greater than those measured at one or both of the treatment sites.

Tip Width

In March 1985, Site D had significantly lower tip width than Site 0, while all other sites were similar. No significant differences in tip width between any sites were measured during any of the following measurement periods. Tip widths at Site D were lower than at the other sites during the periods from November 1984 to March 1985 and from March 1985 to June 1985 (4 and 7 months postspill, respectively). At Site 0, tip width alternated between greater and less than the other sites over time, as did both the reference sites.

TABLE 38. *Porites porites* growth parameters. Asterisks (*) indicate significant differences (p = 0.05) compared to Site R or Site R-2.

	EXTENSION RATE (cm/yr)				MEAN WIDTH (cm)				BLADE AREA (cm^2/yr)			
SITE	D	O	R	R-2	D	O	R	R-2	D	O	R	R-2
November 1984 - March 1985												
MEAN	2.47	3.64	2.81	2.78	0.61	0.74	0.70	0.72	0.75	1.58	1.02	1.18
SD	1.00	1.08	1.36	1.23	0.15	0.11	0.08	0.21	0.50	0.63	0.32	0.86
N	34	16	9	22	34	16	9	22	34	16	9	22
March 1985 - June 1985												
MEAN	2.14	3.13	4.38	2.23	0.61	0.77	0.80	0.75	0.71*	1.62	2.22	1.11
SD	1.41	1.28	1.77	1.23	0.12	0.14	0.10	0.19	0.69	0.96	1.17	0.89
N	12	17	35	24	12	17	35	24	12	17	35	24
June 1985 - December 1985												
MEAN	2.47*	3.13	2.17	3.66	0.74	0.70	0.74	0.66	1.09	1.28	1.12	1.39
SD	0.59	1.12	0.81	0.88	0.16	0.11	0.27	0.18	0.52	0.73	1.31	0.94
N	24	9	20	13	24	9	20	13	24	9	20	13
December 1985 - August 1986												
MEAN		2.95*	2.81			0.73	0.85			1.36	1.17	
SD		1.12	0.53			0.22	0.34			0.99	1.78	
N		36	21			36	21			36	21	

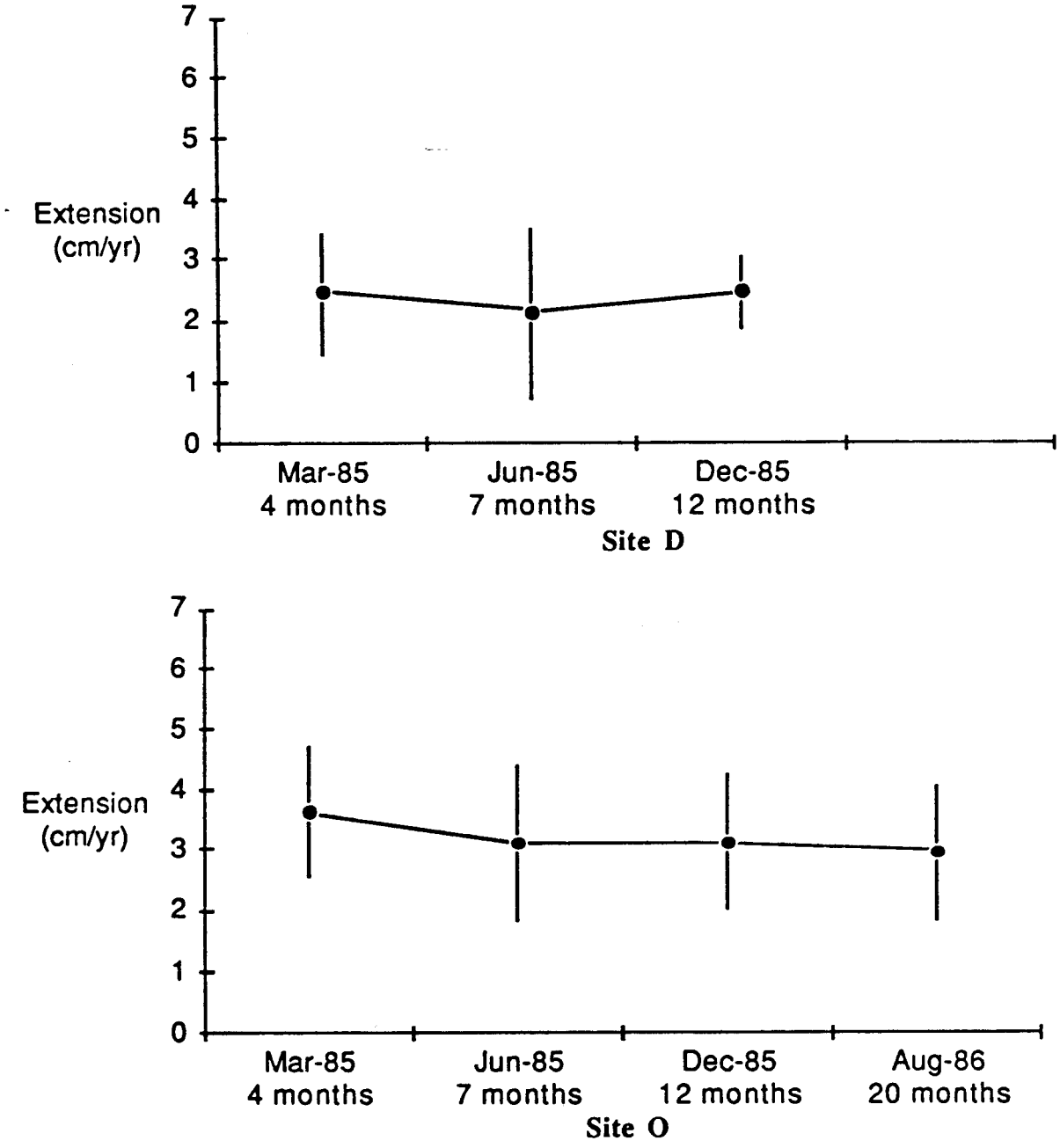


FIGURE 44. Tip extension rates of Porites porites specimen at Site D (top) and Site 0 (bottom).

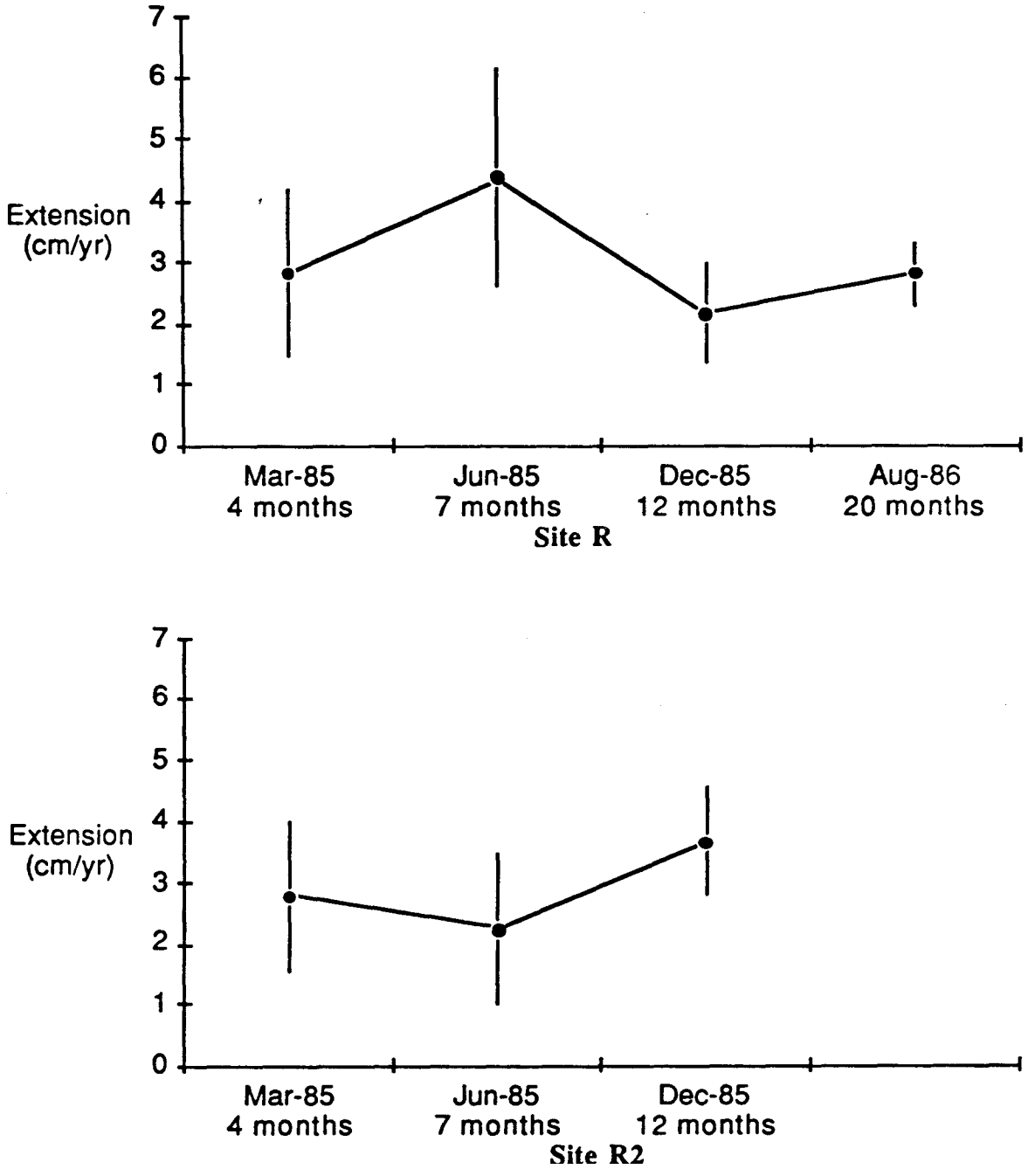


FIGURE 45. Tip extension rates of P. porites specimen at Site R (top) and Site R-2 (bottom).

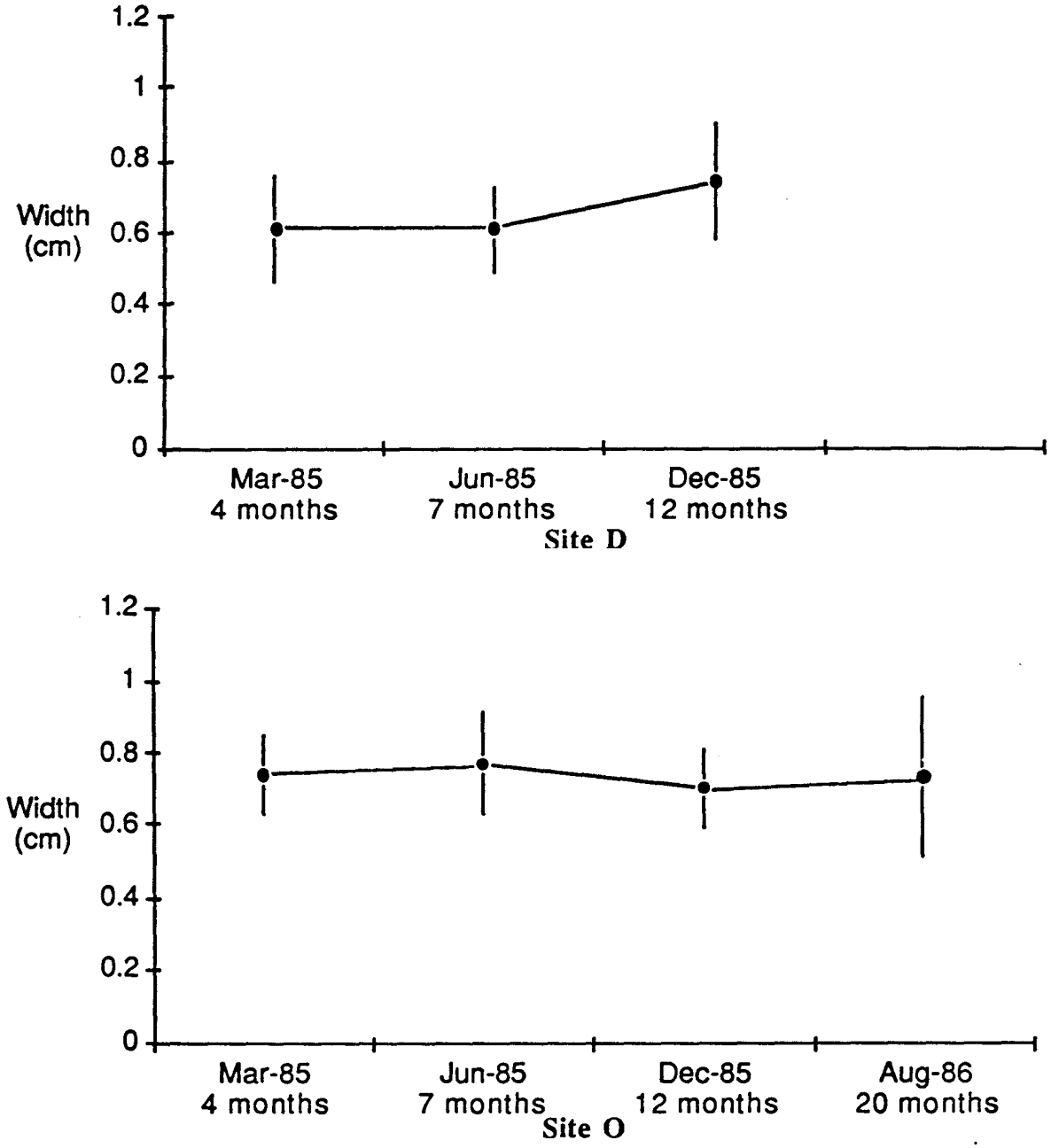


FIGURE 46. Tip widths of P. porites specimen at Site D (top) and Site 0 (bottom).

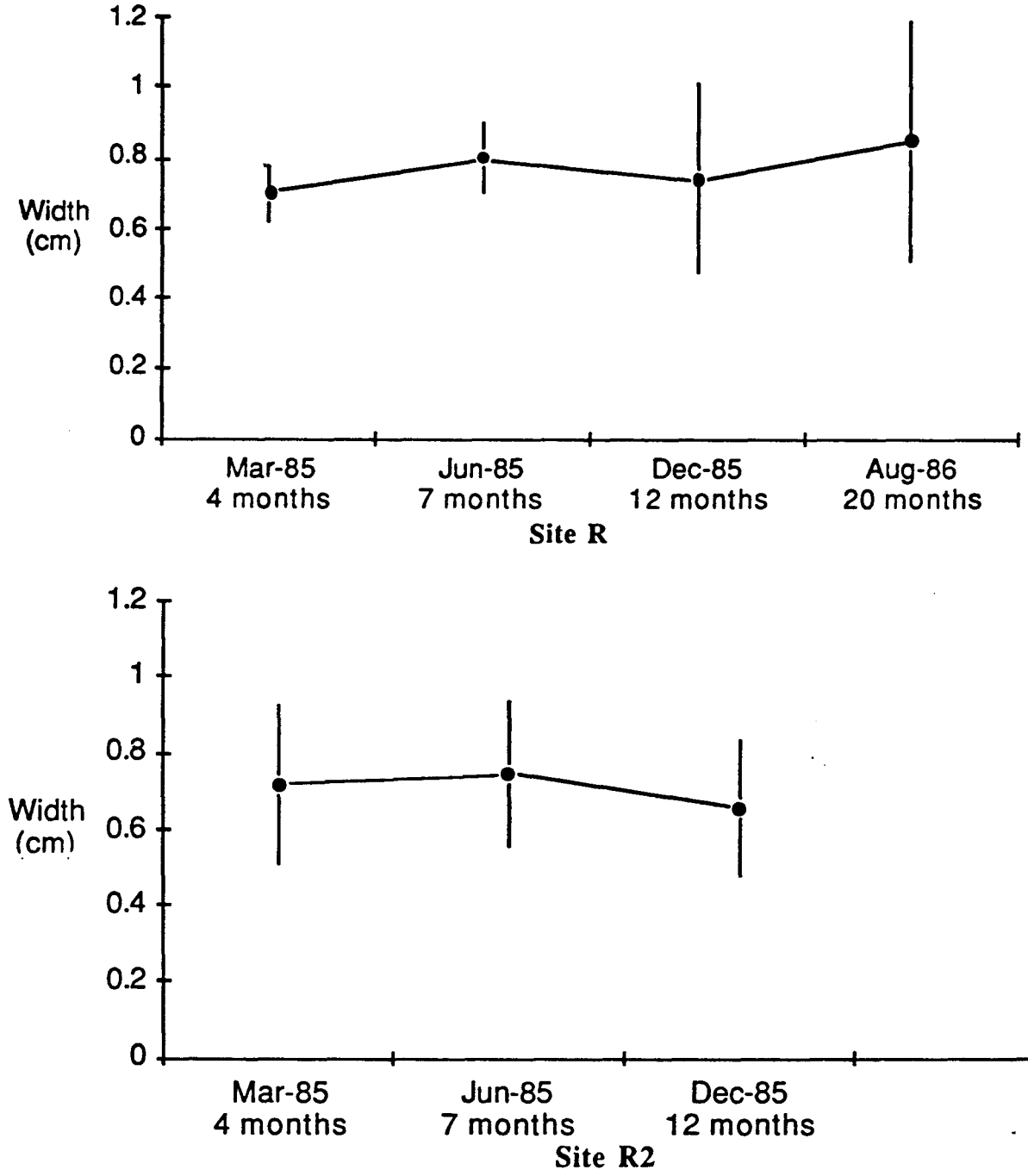
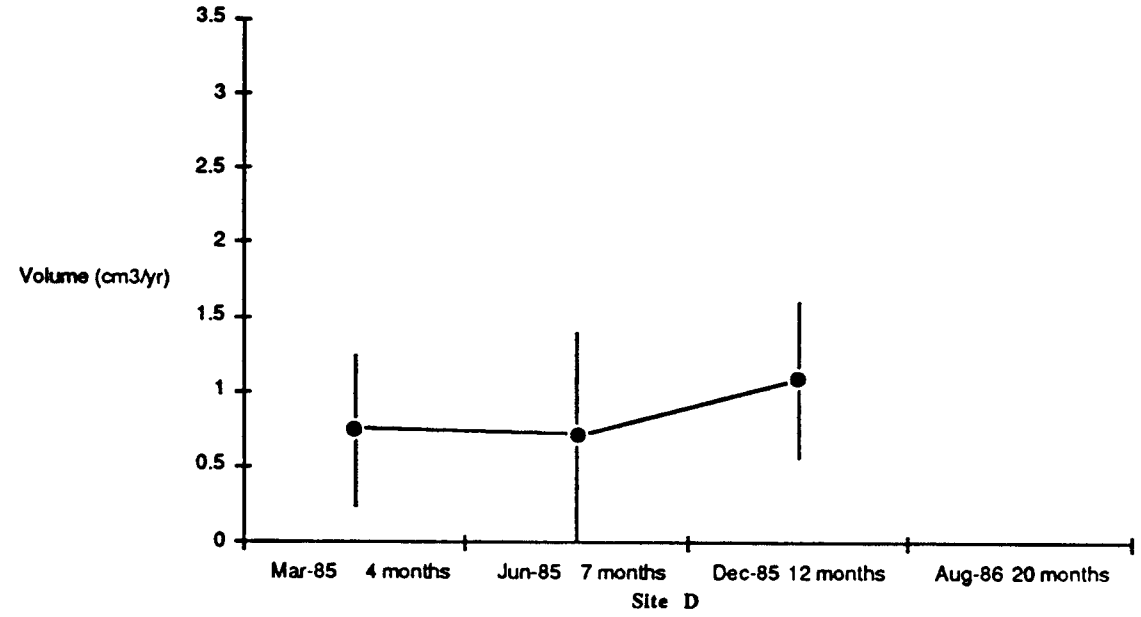


FIGURE 47. Tip widths of P. porites specimen at Site R (top) and Site R-2 (bottom).



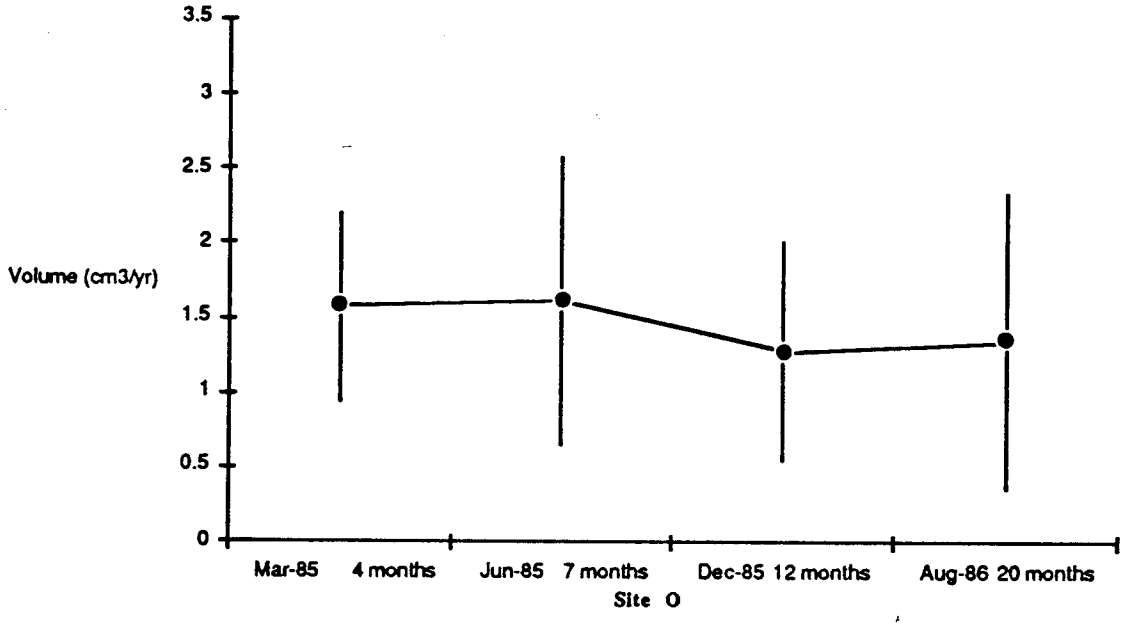


FIGURE 48. Tip volumes of P. porites specimen at Site D (top) and Site 0 (bottom).

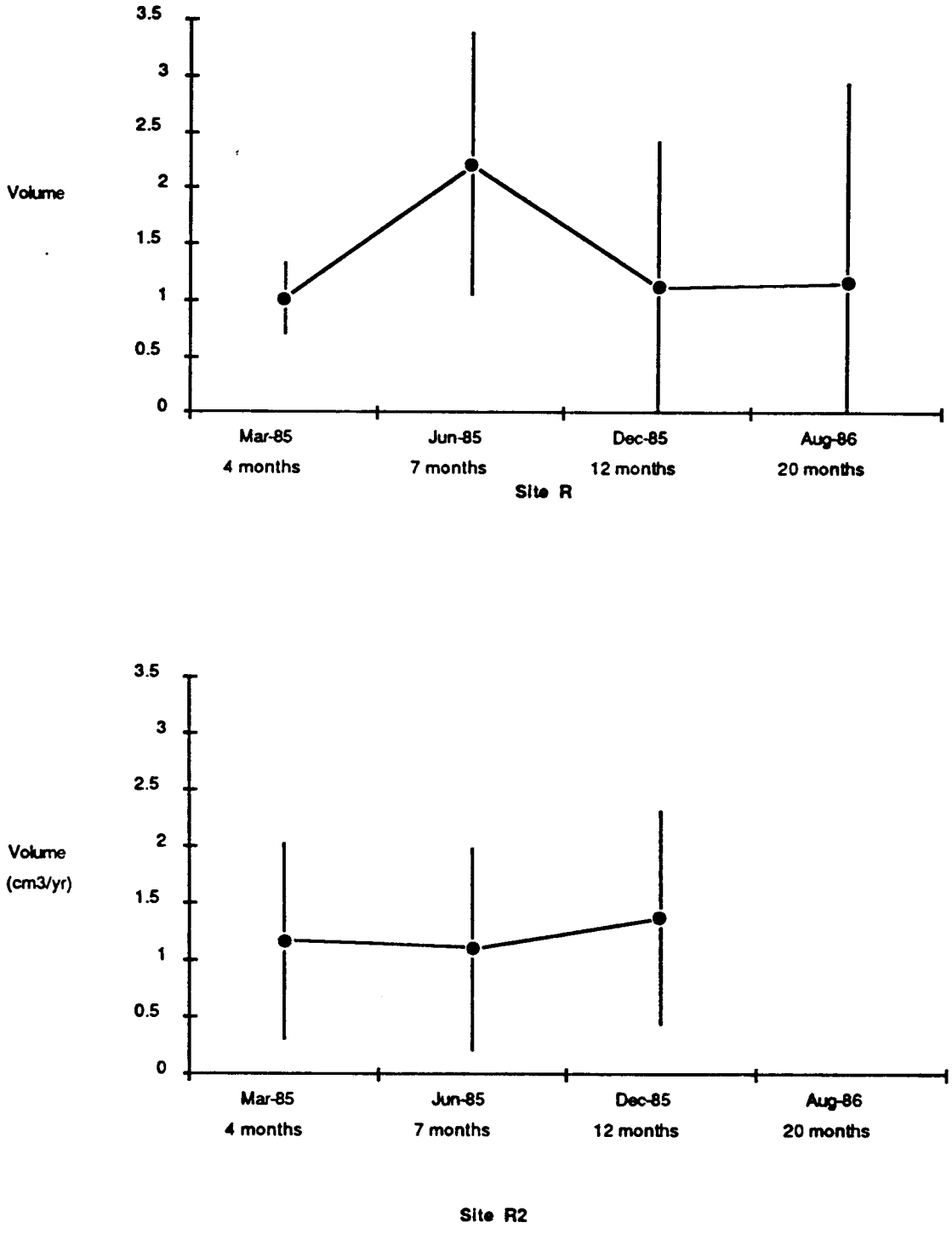


FIGURE 49. Tip volumes of P. porites specimen at Site R (top) and Site R-2 (bottom).

Tip Volume

Tip volume of P. porites specimens at Site D was significantly less than at Site 0 in March 1985, but there was no difference between Site D and either reference site. In June 1985, tip volume at Site D was significantly less than at Site R-2, but there_ was no difference between Site D and Site R or O. Site R-2 was significantly less than Site R. After June 1985, no significant differences between any sites were measured.

Summary of Coral Growth Results

The growth rates of suites of 4 coral species at each of the experimental sites and the reference sites have been evaluated for the first growth assessment period (November 1984 to March 1985). Growth rates of 2 coral species (A. tennuifolia and P. porites) were evaluated at 2 and 3 additional assessment periods for most sites.

Two coral species, Montastrea annularis and Acropora cervicornis, showed no effects from the treatment dosing. Growth rates (linear extension rate and tip widths) were similar between sites.

The coral species Agaricia tennuifolia showed significantly reduced blade extension rate (length), blade thickness (width), and blade area (mass index) growth at the dispersed oil site with respect to the oil site and the two reference sites at each growth assessment period. Growth parameters at all sites do not exhibit clear increasing or decreasing trends over the experimental assessment periods. This suggests that Site D recovery has not yet begun over approximately 20 months since treatment.

Statistical results for the coral species Porites porites are not as clear-cut as for the above A. tennuifolia, due primarily to the high degree of variability within and between sites. At the first and second postspill assessment periods, P. porites at Site D exhibited depressed values in the parameters of branch length (extension), width, and volume compared to the other sites. No statistically significant differences between Sites D and 0 were noted except in tip width and volume during the 4-month postspill period. The data suggest a possible small growth effect to this species at Site D from dispersed oil treatment for as much as 8 months following dosing.

METHODS AND MATERIALS - SEAGRASS STUDIES

To determine the impact of crude oil and dispersed crude oil on subtidal grass beds (Thalassia testudinum) and the associated infauna and epifauna, a variety of parameters were monitored in situ. These are listed below:

Floral Assessments	Faunal Assessments
o Growth rates	° Macroepifauna
o Blade area	° Macroinfauna
o Plant density	

Floral Assessments

For the purposes of Thalassia blade area and growth studies, 3 plots were established within each of the study sites (Fig. 50). Plots were 0.5 m x 0.5 m and were permanently marked with iron rods driven into the substrate at each of the 4 corners. Plots were situated along the approximate central axis of the Thalassia bed, in shallow water landward of the reef crest. Within each study plot, 10 plants were selected at random and marked with nylon tie-wraps (Fig. 51). These were secured at the base of the plant around the basal sheath that surrounds the emergent blades. Tie-wraps were tightened sufficiently to preclude loss through wave action or other disturbances, yet loosely enough to prevent damage or stress to the plant. These nylon markers also had the advantages of being durable, non-biodegradable, noncorrosive, and noncontaminating. Each of the 10 plants within a plot were numbered by punching the edges of the tie-wraps with a single-hole punch. This method proved highly successful, as plant relocation success was nearly 100 percent throughout the study. During later visits to the study sites, old tags were discarded and a suite of ten new plants marked.

Subsequent to marking each plant, the length and width of all blades for each plant were recorded. Measurements were made in situ using clear, plastic millimeter rules. Width measurements were taken near the midpoint of the length of each blade. Length measurements were taken from the inflexible margin of the basal sheath to the distal limit of the blade. Typically, Thalassia plants consist of 3 or 4 blades, the outermost 2 being the oldest. These are often necrotic for a considerable portion of the distal extreme. Accurate length measurement in these situations is frequently difficult, as

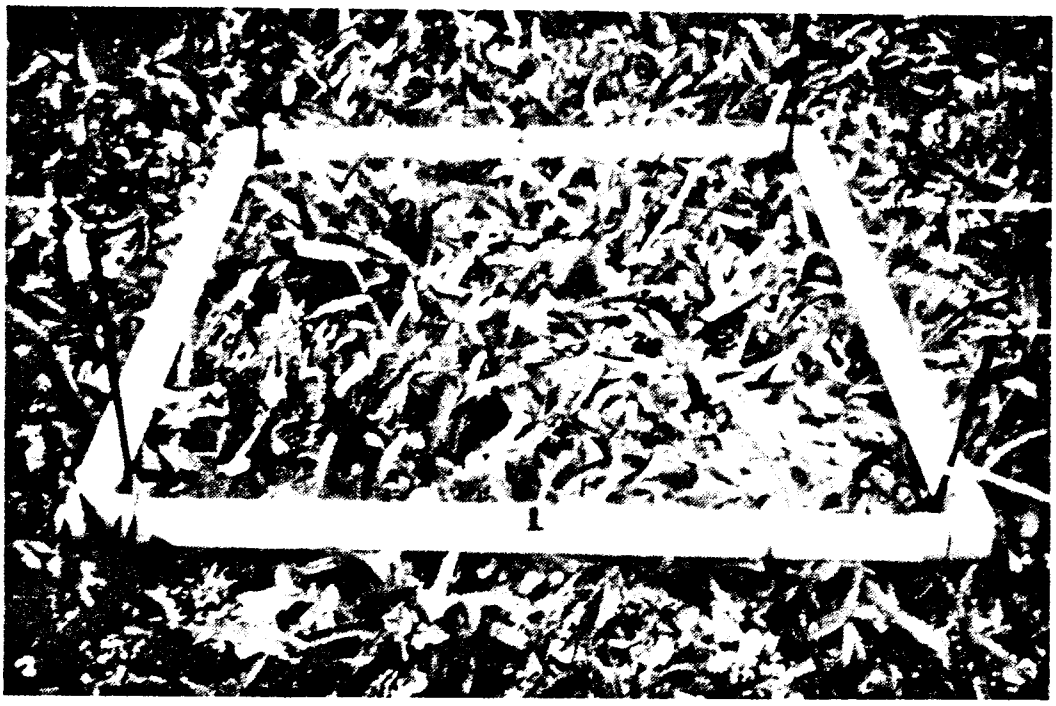


FIGURE 50. Replicate plots were established within the seagrass area of each site.

FIGURE 51. Within each plot, individual seagrasses were tagged and measured to determine growth rates.

the blade tips are fragile and often frayed. In these instances, the outer leaves were trimmed perpendicular to the length axis, so as to provide a standard-length reference point, a feature valuable to both initial measurements and the reidentification of specific blades during the second measurement. Trimming was limited to the necrotic area, well away from the healthy, photosynthetically-active portion of the blade. Because of the artifacts imposed by tip damage of outer blades, growth determinations were based upon the inner (newest), undamaged blade. Approximately 72 hours after the initial length measurements, the central blade of each numbered plant was remeasured. Actual growth rate data were calculated from the difference of these 2 values divided by the number of hours separating measurements and then multiplied by a factor of 24. This provided a per-day growth rate for each plant. Results were recorded in cm/day.

Blade areas were determined from the same data collected during the growth studies. Blade area for each plant was calculated as the product of blade length and blade width.

Plant density data were taken using a different methodology. A 0.5-m x 0.5-m PVC quadrat subdivided into 25 œlls (each cell = 100 square cm) was placed at random within the grass bed. Counts of the number of plants per cell were made for 16 cells within each quadrat. A total of four quadrat replicates were conducted within each site during each sampling period. Thus, calculations of plant densities within each site were based on 64 cells of data for each period. Plant densities are reported as the number of plants per square meter.

Total blade area within a study area also was determined. This value is actually the total surface area (one-side) of new blades per square meter. Values were determined by taking the product of the mean new blade area (10 plants) and plant density. This resulted in single values for each plot for each sampling period.

Statistical treatment of seagrass data involved three steps. First, descriptive statistics (means, standard deviations, ranges) were determined for growth rates, blade areas, and densities. Second, ANOVA statistics were applied to determine if there were statistically significant differences in mean seagrass variables. Third, if differences were detected, SNK multiple-range tests were used to identify which means differed statistically. These procedures permitted a statistically valid assessment of the effects, if any, of crude oil and dispersed oil of subtidal grass beds.

Faunal Assessments

A wide variety of organisms were present in the seagrass beds, including echinoderms, holothurians, polychaetes, poriferans, coelenterates, and calcareous and noncalcareous algae. Two species of sea urchin (Echinometra lacunter and, to a lesser degree, Lytechnius variegatus were by far the most abundant macroepifauna present at all study sites and, for this reason, were chosen for detailed evaluation before, during, and after site treatment.

Sea urchin abundance was assessed using two techniques: (1) linear transects, and (2) random-placed quadrats.

Three 30-m line transects were conducted within the seagrass community at each study site during each sampling period except March 1984. Transect lines consisted of 30-m fiberglass tapes. Data were recorded in 2-m increments over the length of the transect.

Areal densities of sea urchins also were assessed at each site during each sampling period. This was accomplished using the same 0.5-m x 0.5-m quadrat locations used to determine plant densities.

Macroinfaunal assessments were effected through core sampling. Nine aluminum push-core samples were collected at each site during each sampling period. Core tubes were 7.6-cm diameter and were inserted into the sediments approximately 15 cm. Cores were transferred to 0.5-mm mesh Nytex bags, fixed in 10 percent formalin, and stained with Rose Bengal. Samples were returned to the laboratory, where they were sorted and the organisms identified to the lowest possible taxonomic level. Species diversity and abundance were recorded for each sample. Two diversity indices were calculated for each sample. These were:

1) Shannon-Weaver diversity index $H' = E\ P_i/InP_i$, where H' = diversity index, P_i = proportion of occurrence of each species calculated from N_i/N, where N_i = number of specimens of species i, and N = total number of species in the sample.
2) Gleason diversity index $D' = (s-1)/InN$, where D' = diversity index, s = number of species, and N = total number of specimens.

Results of Floral Assessments

Tables 39-41 present means and standard deviations for all sites and sampling periods for seagrass growth, blade areas, and densities,

TABLE 39. Means, standard deviations (in parentheses), and sample sizes for seagrass growth rates (in cm/day) by site and sampling period.

SAMPLING PERIOD	SITE D	SITE 0	SITE R
PRETREATMENT			
March 1984 (8 months prespill)	0.16 (0.20) N=30	0.19 (0.21) N=28	0.25 (0.13) N=5
November 1984 (1 week prespill)	0.39 (0.20) N=29	0.49 (0.30) N=26	0.46 (0.13) N=27
DURING TREATMENT			
December 1984 (During spill)	0.38 (0.13) N=27	0.36 (0.10) N=27	NM*
POSTTREATMENT			
December 1984 (4 days postspill)	0.46 (0.34) N=25	0.48 (0.23) N=29	NM*
March 1985 (4 months postspill)	0.39 (0.19) N=30	0.46 (0.17) N=29	0.55 (0.29) N=10
June 1985 (7 months postspill)	0.53 (0.29) N=30	0.39 (0.20) N=30	0.51 (0.23) N=30
December 1985 (12 months postspill)	0.50 (0.22) N=30	0.46 (0.22) N=30,	0.53 (0.26) N=30
August 1986 (20 months postspill)	0.69 (0.42) N=30	0.43 (0.22) N=30	0.81 (0.42) N=30

* NM = no measurements

TABLE [1]40. Means, standard deviations (in parentheses), and sample sizes for seagrass blade areas (in cm^2) by site and sampling period.

SAMPLING PERIOD	SITE D	SITE 0	SITE R
PRETREATMENT.			
March 1984 (8 months prespill)	22.21 (5.17) N=30	23.81 (6.71) N=30	37.47 (8.08) N=25
November 19814 (1 week prespill)	24.35 (7.75) N=30	22.16 (7.44) N=30	27.61 (7.78) N=30
POSTTREATMENT			
March 1985 (4 months postspill)	25.35 (8.36) N=30	18.58 (5.44) N=30	33.21 (19.22) N=10
June 1985 (7 months postspill)	28.34 (11.92) N=30	18.83 (16.58) N=30	34.18 (13.85) N=30
December 1985 (12 months postspill)	22.90 (28.45) N=30	23.21 (35.14) N=30	24.33 (8.37) N=30
August 1986 (20 months postspill)	27.02 (17.55) N=30	14.88 (5.92) N=30	28.52 (23.96) N=30

TABLE 41. Mean plant densities (in number/m^2) by site and sampling period.

SAMPLING PERIOD	SITE D	SITE 0	SITE R
PRETREATMENT			
March 1984 (8 months prespill)	422.7	356.0	379.2
November 1984 (1 week prespill)	816.7	841.7	666.7
POSTTREATMENT			
March 1985 (4 months postspill)	673.3	682.2	NM*
June 1985 (7 months postspill)	922.0	603.0	488.0
December 1985 (12 months postspill)	911.0	598.0	692.0
August 1986 (20 months postspill)	862.5	579.7	720.3

* NM = no measurements

respectively. Mean growth rates, blade areas, and plant densities are depicted in Figures 52-54.

Seagrass growth rates showed considerable variation both within and among sites during sampling periods and between different periods. This variability is evidenced by the high standard deviations noted for growth rates at several sites. At Site D, growth ranged from 0.16 to 0.39 cm/day during the pretreatment periods. During posttreatment periods, growth at Site D ranged from 0.39 to 0.69 cm/day. Slowest growth was in March 1984, while fastest growth was recorded in August 1986. At Site 0, pretreatment growth ranged from 0.19 to 0.49 cm/day. Posttreatment growth at Site 0 was more stable, ranging from 0.39 to 0.46 cm/day. Again, slowest growth was in March 1984 and fastest in March and December of 1985.

At the reference site, Site R, growth was slowest in March 1984 (0.25 cm/day) but increased in November 1984 to 0.46 cm/day, 0.51 cm/day in June 1985, and 0.81 cm/day in August 1986. In general, absolute growth rate values increased at all three sites following treatment. Mean pretreatment growth rates were 0.28, 0.34, and 0.36 cm/day at Sites D, 0, and R, respectively. Posttreatment site means were 0.53, 0.43, and 0.60 cm/day, respectively. Statistical treatment of these trends is presented below.

Mean blade areas at Site D ranged from 22.2 to 24.4 cm^2 during pre-treatment sampling. Posttreatment mean blade areas were approximately equal ra^{n}ging from 22.9 to 28.3 cm^2. At the oiled site, Site 0, pretreatment blade areas were 23.8 cm^2 (March 1984) and 22.2 cm^2 (November 1984). After treatment, mean blade areas were lower in 3 of the 4 sampling periods. These values ranged from 14.9 to 18.8 cm^2. In December 1985, however, mean blade areas were intermediate to the 2 pretreatment levels (23.2 cm^2). Seagrasses at Site R generally had the largest blade areas. Pretreatment areas were 37.5 and 27.6 cm^2. Posttreatment values ranged from 24.3 to 34.2 cm^2. In general, absolute values for blade areas at Site D indicate an increase in blade area. Mean pretreatment blade area was 23.3 cm^2, while the grand mean for posttreatment periods was 25.9 cm^2. Site 0 showed an opposite trend. Mean blade area declined from 23.0 cm^2 during pretreatment to 18.9 cm^2 during posttreatment. Site R also showed a decline in blade area following the treatment date, but the absolute value of the reduction in blade area was only slightly more than half that recorded at Site O.

Plant density values demonstrated considerable variability within each site throughout the study. This was, in part, a result of the patchy

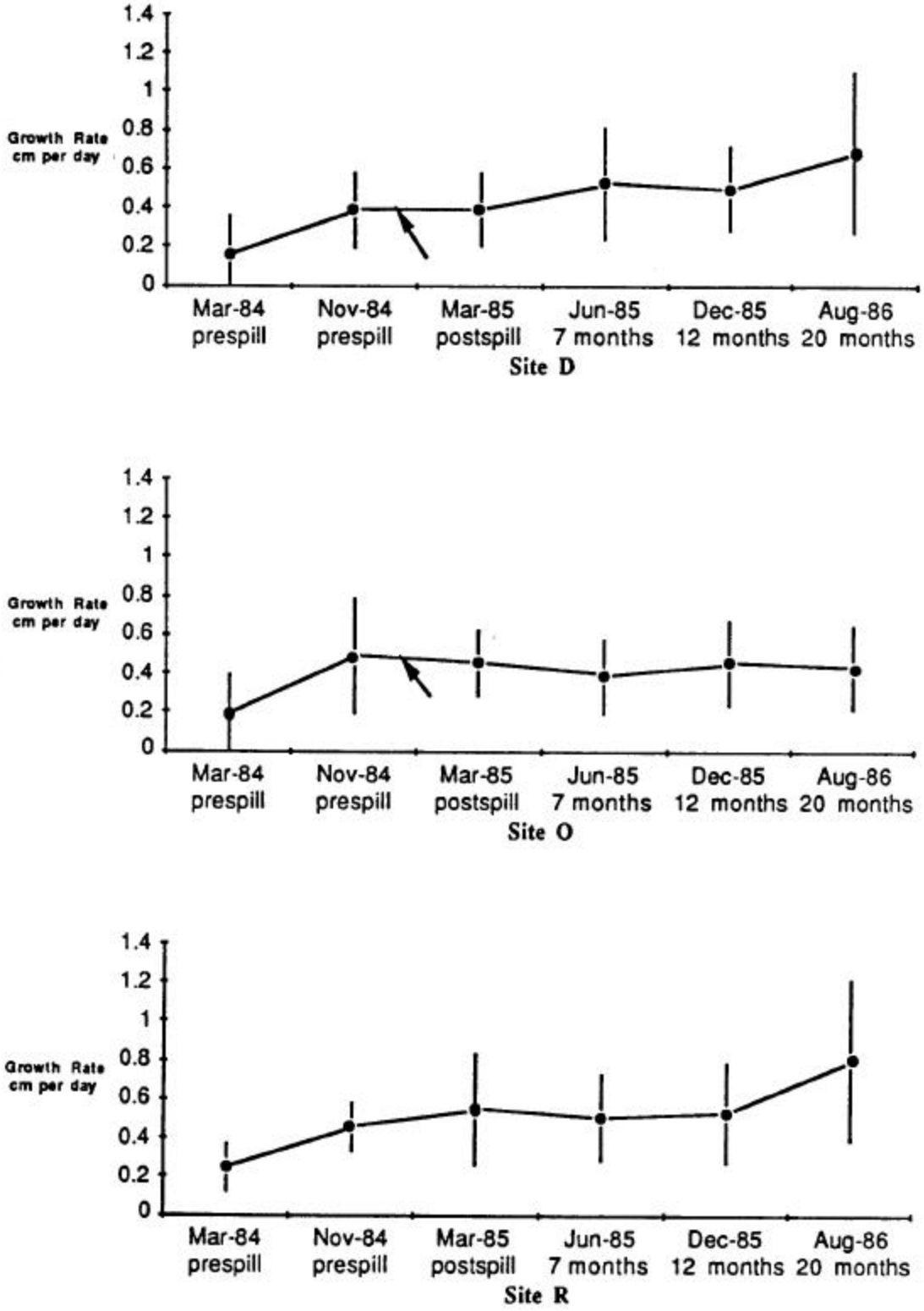


FIGURE 52. Growth rates (cm/day) of Thalassia testudinum at Site D (top), Site 0 (middle), and Site R (bottom). Arrow indicates date of site treatment.

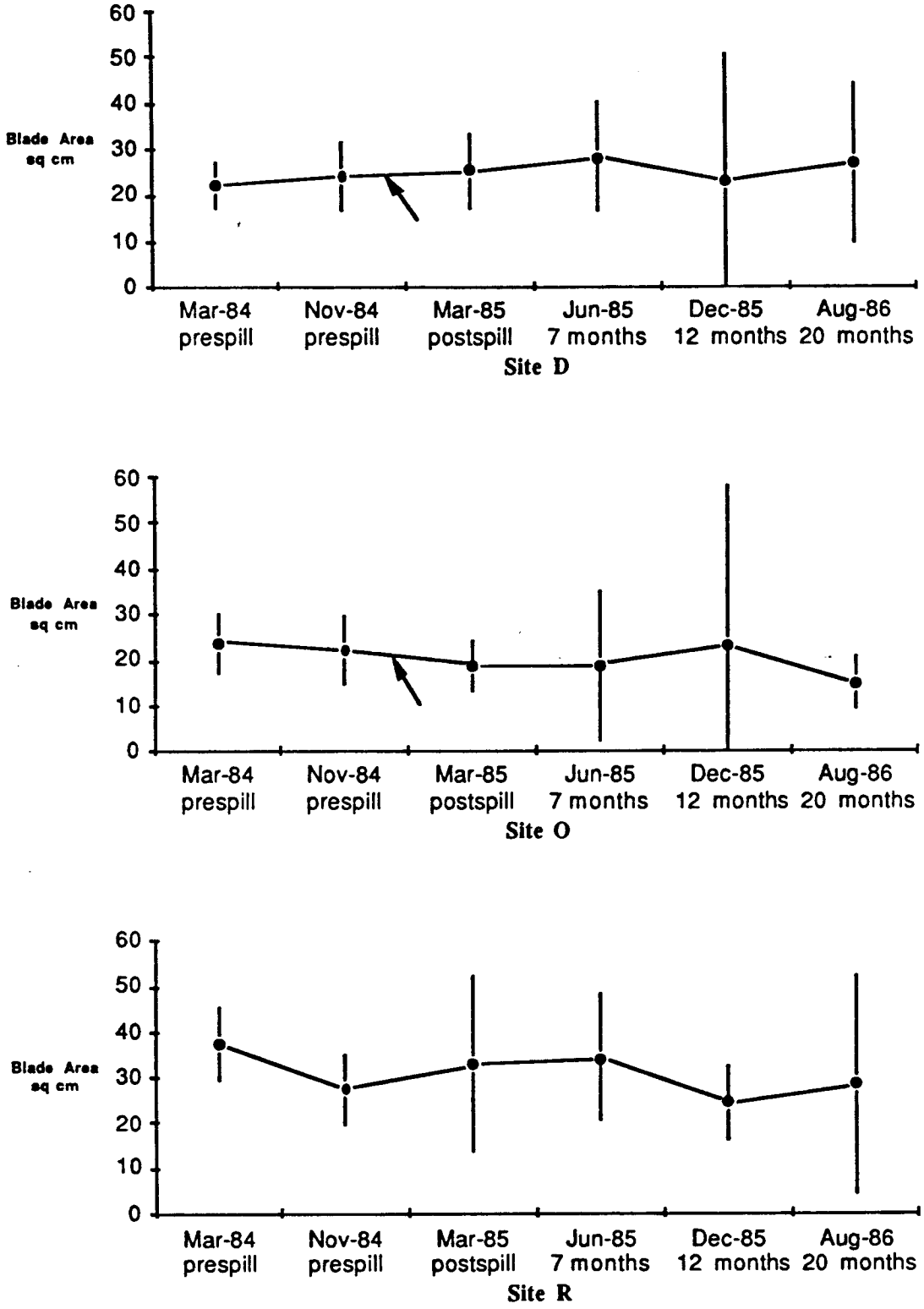


FIGURE 53. Blade areas (cm^2) of T. testudinum at Site D (top), Site 0 (middle), and Site R (bottom). Arrow indicates date of site treatment.

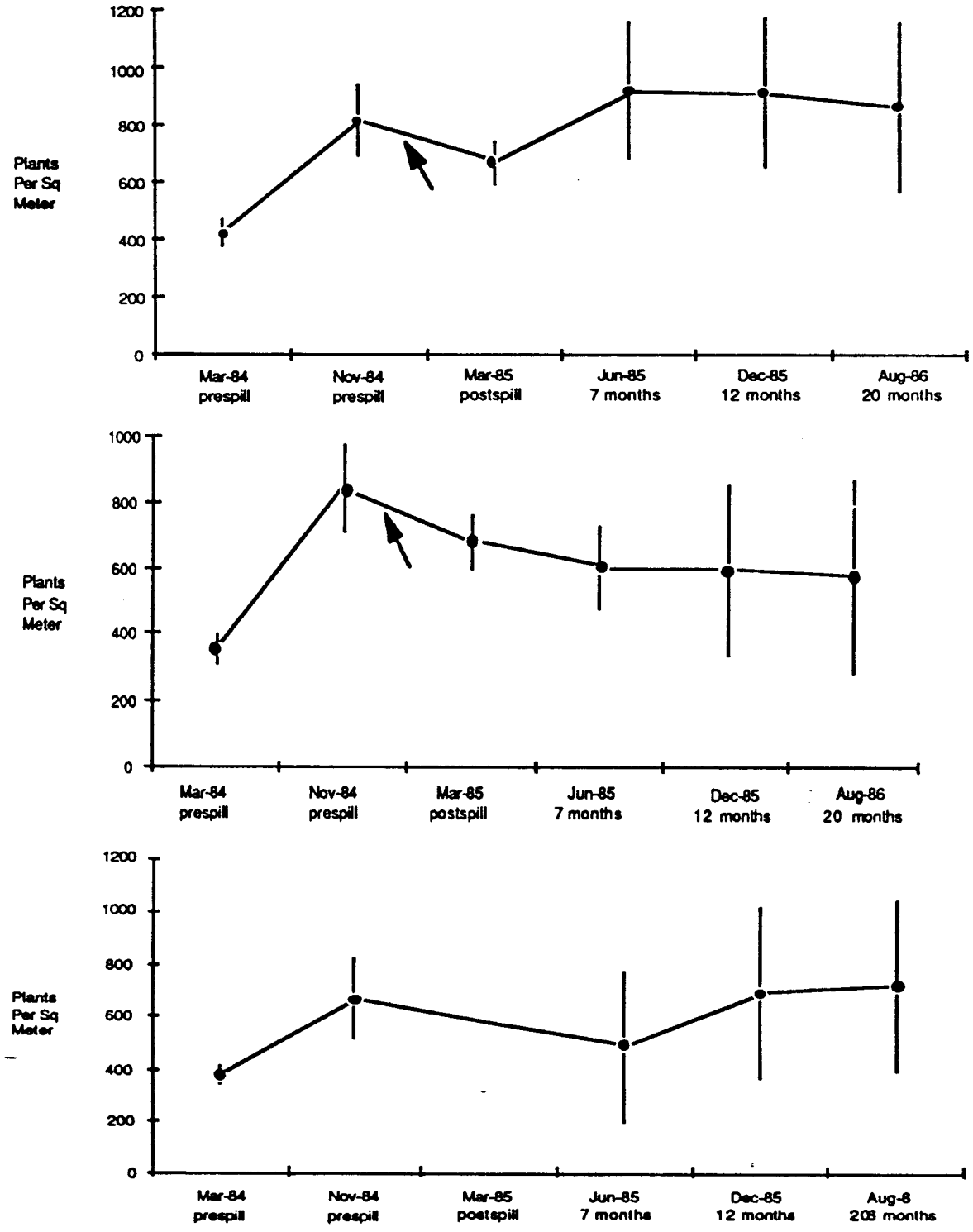


FIGURE 54. Seagrass plant densities (#/m') at Site D (top), Site 0 (middle), and Site R (bottom). Arrow indicates date of site treatment.

distribution of plants which reflected the patchiness of substrate suitability. That is, the substrate within the seagrass zone of each site was not homogeneous. Varying amounts of sediment and coral rubble occurred throughout each site. The ratio of sediment to coral rubble appeared to play an important role, if not the dominant role, in governing plant density. This factor should be considered in interpreting the density data presented below.

Site D was characterized by pretreatment densities of 422.7 plants/m^2 in March 1984 and 816.7 plants/m^2 in November 1984. Posttreatment densities ranged from 673.3 to 922.0 plants/m^2. Densities at Sites 0 and R were, in general, lower than those seen at Site D. Site 0 pretreatment densities ranged from 356.0 (March 1984) to 841.7 (November 1984) plants/m^2. After treatment, densities ranged from a high of 682.2 in March 1985 to a low of 579.7 plants/m^2 in August 1986. Site R pretreatment densities were 379.2 in March and 666.7 plants/m^2 in November. Posttreatment plant densities ranged from 488.0 in June to 720.3 plants/m^2 in August 1986. Density measurements were not taken at Site R in March 1985. Interestingly, all 3 sites demonstrated uniformly low plant densities in the March 1984 sampling. By November 1984, plant densities had increased dramatically. After oiling, Site 0 showed a gradual decline in plant density from the November 1984 high. Site D, on the other hand, showed an initial decline after November 1984, followed by densities in excess of the pretreatment maximum. Site R showed no clear trends. Instead, densities fluctuated at levels near those recorded during pretreatment periods. Because of the high variability in plant density and the substantial disparity in the two pretreatment density samples for each site, comparisons of pre- and posttreatment means were considered unadvisable.

Statistical Treatment of Results

To interpret and determine the statistical significance of the trends expressed in the raw data, two basic comparative approaches were undertaken. First, the differences in growth rates, blade areas, and areal densities were compared between sites for each sampling period. Second, changes in these 3 parameters were compared within sites over time (among the 6 sampling periods). Two statistical procedures, one-way ANOVA and SNK tests, were employed. The former serves to identify if any differences exist between sample means. The latter test (SNK) identifies which means are equivalent

TABLE 42. Summary of ANOVA statistics for seagrass parameters, testing for differences among sites within sampling periods.

PERIOD	SITES	NUMBER OF OBSER-VATIONS	PARAMETER	D.F.	F-VALUE	PROBABILITY	UNEQUAL MEANS?
March 1984	All(3)	63	Growth	2	0.46	0.6312	No
(8 months prespill)		85	Area	2	41.89	0.0001	Yes
		75	Density	2	23.44	0.0001	Yes
November 1984	All(3)	82	Growth	2	1.56	0.2175	No
(1 week prespill)		90	Area	2	3.85	0.0250	Yes
		90	Density	2	>99.00	0.0001	Yes
March 1985	All(3)	69	Growth	2	2.58	0.0836	No
(4 months postspill)		79	Area	2	9.56	0.0002	Yes
		60	Density	1	>99.00	0.0001	Yes
June 1985	All(3)	90	Growth	2	2.87	0.0623	No
(7 months postspill)		90	Area	2	8.88	0.0003	Yes
		90	Density	2	>99.00	0.0001	Yes
December 1985	All(3)	90	Growth	2	0.64	0.5296	No
(12 months postspill)		90	Area	2	0.02	0.9760	No
		90	Density	2	>99.00	0.0001	Yes
August 1986	All(3)	90	Growth	2	8.35	0.0005	Yes
(20 months postspill)		90	Area	2	5.49	0.0057	Yes
		90	Density	2	>99.00	0.0001	Yes

or different on a basis of statistical significance. Results of ANOVA analyses are presented in Tables 42 and 43. Results of SNK tests are shown in Tables 44-47.

Comparison of Sites Within Sampling Periods

Table 42 summarizes the results of ANOVA testing for seagrass parameters among sites within sampling periods. Degrees of freedom, F-values, probabilities, and the equality/inequality of means are reported for each parameter -

Table 44 summarizes the results of the SNK tests applied to each sampling period. This table indicates whether mean values of parameters for specific comparisons are statistically equal or different at the 0.05 level.

Growth Rates

Mean growth rates of seagrasses at each site during the March 1984 pretreatment survey were not statistically different ($p = 0.6312$). Similar results were obtained in the November 1984 pretreatment survey. No significant differences in growth rates were found among all 3 sites ($p = 0.2175$).

Following the application of oil and dispersant, no significant differences in growth rates were found among the 3 sites for the 3 sampling periods March, June, and December 1985 ($p = 0.0836$, 0.0623, and 0.5296, respectively). In August 1986, mean growth rates were found to be different among sites. At that time, the mean seagrass growth rates at Sites D and R were found to be equal, but both were significantly greater than the growth recorded at Site 0. Site R showed the highest mean growth, but this was not significantly greater than that at Site D. Thus, growth rates at all three sites were the same within each of the pretreatment periods. Also, growth rates at all 3 sites were statistically equivalent within each of the first 3 posttreatment surveys.

Blade Area

Mean blade areas during the March 1984 survey were not statistically different for Sites 0 and D, but both were significantly different than Site R ($p = 0.0001$). ANOVA results for the November 1984 period were the same, although SNK results for November were ambiguous.

TABLE 43. Summary of ANOVA statistics for seagrass parameters, testing for differences within sites over time (among sampling periods).

SITE	PERIOD	NUMBER OF OBSER-VATIONS	PARAMETER	D.F.	F-VALUE	PROBABILITY	UNEQUAL MEANS?
D	All(6)	179	Growth	5	13.29	0.0001	Yes
		180	Area	5	0.71	0.6151	No
		180	Density	5	>99.00	0.0001	Yes
O	All(6)	173	Growth	5	6.92	0.0001	Yes
		180	Area	5	1.26	0.2830	No
		170	Density	5	>99.00	0.0001	Yes
R	All(6)	132	Growth	5	6.93	0.0001	Yes
		155	Area	5	3.09	0.0111	Yes
		145	Density	4	>99.00	0.0001	Yes

TABLE 44. Summary of SNK results for seagrass parameters, testing among sites within sampling periods.

SAMPLING PERIOD	SITE COMPARISON	Means are . . .		
		GROWTH	AREA	DENSITY
March 1984	D -vs- O	=	=	≠
(8 months prespill)	D -vs- R	=	≠	≠
	O -vs- R	=	≠	≠
November 1984	D -vs- O	=	=	≠
(1 week prespill)	D -vs- R	=	=*	≠
	O -vs- R	=	=*	≠
March 1985	D -vs- O	=	≠	≠
(4 months postspill)	D -vs- R	=	≠	nd
	O -vs- R	=	≠	nd
June 1985	D -vs- O	=	≠	≠
(7 months postspill)	D -vs- R	=	=	≠
	O -vs- R	=	≠	≠
December 1985	D -vs- O	=	=	≠
(12 months postspill)	D -vs- R	=	=	≠
	O -vs- R	=	=	≠
August 1986	D -vs- O	≠	≠	≠
(20 months postspill)	D -vs- R	=	=	≠
	O -vs- R	≠	≠	≠

* Results obtained were ambiguous. Two conflicting rankings for Site R. More data required to resolve using SNK.

nd = no data

TABLE 45. Summary of SNK results for seagrass parameters at Site D, testing over time (among sampling periods).

PERIOD COMPARISON	Means are . . .		
	GROWTH	AREA	DENSITY
March 1984 -vs- November 1984	≠	=	≠
March 1984 -vs- March 1985	≠	=	≠
March 1984 -vs- June 1985	≠	=	≠
March 1984 -vs- December 1985	≠	=	≠
March 1984 -vs- August 1986	≠	=	≠
November 1984 -vs- March 1985	=	=	≠
November 1984 -vs- June 1985	=	=	≠
November 1984 -vs- December 1985	=	=	≠
November 1984 -vs- August 1986	≠	=	≠
March 1985 -vs- June 1985	=	=	≠
March 1985 -vs- December 1985	=	=	≠
March 1985 -vs- August 1986	≠	=	≠
June 1985 -vs- December 1985	=	=	≠
June 1985 -vs- August 1986	≠	=	≠
December 1985 -vs- August 1986	≠	=	≠

TABLE 46. Summary of SNK results for seagrass parameters at Site O, testing over time (among sampling periods).

PERIOD COMPARISON	Means are . . .		
	GROWTH	AREA	DENSITY
March 1984 -vs- November 1984	≠	=	≠
March 1984 -vs- March 1985	≠	=	≠
March 1984 -vs- June 1985	≠	=	≠
March 1984 -vs- December 1985	≠	=	≠
March 1984 -vs- August 1986	≠	=	≠
November 1984 -vs- March 1985	=	=	≠
November 1984 -vs- June 1985	=	=	≠
November 1984 -vs- December 1985	=	=	≠
November 1984 -vs- August 1986	=	=	≠
March 1985 -vs- June 1985	=	=	≠
March 1985 -vs- December 1985	=	=	≠
March 1985 -vs- August 1986	=	=	≠
June 1985 -vs- December 1985	=	=	=
June 1985 -vs- August 1986	=	=	≠
December 1985 -vs- August 1986	=	=	≠

TABLE 47. Summary of SNK results for seagrass parameters at Site R, testing over time (among sampling periods).

PERIOD COMPARISON	Means are . . .		
	GROWTH	AREA	DENSITY
March 1984 -vs- November 1984	=*	=	≠
March 1984 -vs- March 1985	=*	=**	nd
March 1984 -vs- June 1985	=*	=	≠
March 1984 -vs- December 1985	=*	=**	≠
March 1984 -vs- August 1986	≠	=	≠
November 1984 -vs- March 1985	=	=	nd
November 1984 -vs- June 1985	=	=	≠
November 1984 -vs- December 1985	=	=**	≠
November 1984 -vs- August 1986	≠	=	≠
March 1985 -vs- June 1985	=	=	nd
March 1985 -vs- December 1985	=	=**	nd
March 1985 -vs- August 1986	≠	=	nd
June 1985 -vs- December 1985	=	=**	≠
June 1985 -vs- August 1986	≠	=	≠
December 1985 -vs- August 1986	≠	=**	≠

* March 1984 growth means yielded ambiguous SNK results. One ranking showed March 1984 different from all other means, while an alternative ranking showed March 1984 equal to December 1985, June 1985, and November 1984. More data required for SNK.

** December 1985 area comparisons yielded ambiguous results. SNK showed December 1985 mean equal to all other means except March 1984 in one ranking and unequal to all other means in an alternative ranking. More data required to resolve this ambiguity by SNK.

nd = no data

After treatment, mean blade areas for sites within sampling periods showed varying results. In March 1985, blade areas were shown to be significantly different at each site ($p = 0.0002$). SNK procedures for these data showed that Site R had the greatest mean blade area, followed by Sites D and O. This pattern of higher mean areas at Site R was similar to that seen in both pretreatment periods. In June 1985, the mean blade area for Site 0 was significantly lower than the blade areas measured at Sites D and R, while no significant difference was measured between Sites D and R. In December 1985, no statistical differences in blade areas were measured at all 3 sites ($p = 0.9700$). In the last sampling period, ANOVA tests showed that mean blade areas were different ($p = 0.0057$). Sites D and R had statistically equal levels and were both significantly greater than the mean area noted at Site O.

In summary, blade areas for Sites 0 and D were equal within each pre-treatment survey, but different with reference to Site R. Following treatment, Sites 0 and D were found to have statistically different mean blade areas within all but one (December 1985) of the posttreatment surveys. In each of these cases, Site D had greater blade areas than Site O.

Plant Density

Within both pretreatment periods (March 1984 and November 1984), plant densities were found to be significantly different among sites ($p = 0.0001$, both periods). SNK techniques demonstrated that mean plant densities were statistically different for each site within each of the two surveys. Similar results were derived for each of the 4 posttreatment periods (p less than or equal to 0.0001 for all 4 periods). According to SNK results, each site was characterized by a significantly different mean density within each of the four surveys. This pattern might be expected following treatment but is difficult to interpret, since pretreatment densities also were site-specific. Changes in plant densities within sites are discussed further in the following section.

Comparison of Sites Between Sampling Periods

Table 43 summarizes the results of the site-specific ANOVA testing of mean seagrass parameters over the 6 sampling periods. Means are compared to determine how each site responded to treatment over time.

Tables 45-47 summarize the results of SNK testing for Sites D, 0, and R, respectively. These tables document the statistical differences for specific comparisons of means at each site. Fifteen comparisons were made for each parameter at each site.

Growth Rates

At Site 0, a statistically significant difference (p less than 0.05) was noted between the 2 pretreatment periods, the March 1984 levels being much lower. The same situation was evident at Site D. Results for Site R were ambiguous, but one analysis showed significant differences between March and November 1984 levels. The exceptionally low growth rates at all 3 sites in March 1984 was perplexing. A careful review of data gathering, recording, and analysis did not pinpoint any source of error. A comparison of growth rates for March 1984 and March 1985 did not indicate any natural, annual cycle of reduced growth during that time of the year. This leads to the conclusion that some unusual event had occurred or was active that pro
duced the March 1984 levels, although a complete review of field observations defined no discernible event. As a result, the depressed March 1984 growth rates remain enigmatic. It seems advisable, then, that greater reliance should be placed on the November 1984 levels. These rates are closer to those recorded during and well after oiling and probably represent more typical growth.

Growth rates also were recorded during site treatment, during the first 4 days following treatment, and at 4-6 days following treatment. At Site D, absolute growth rates showed a slight increase during the spill, but these levels were not statistically significant. Site D growth rates at 0-4 days and 4-6 days postspill also were slightly elevated but did not prove statistically significant.

Comparison of growth rate means at Site D between the November 1984 pretreatment levels and the next 3 sampling periods (March, June, and December 1985) revealed no increase in March and slight increases in June and December. None of these, however, were found statistically significant through ANOVA and SNK tests. Growth rates at Site D in August 1986 were significantly greater than any of the levels noted before or after treatment. August levels at Site D were approximately 77 percent higher than the highest pretreatment growth and 30 percent higher than the highest

posttreatment value. Whether this faster growth was a result of treatment remains unclear. Additional sampling would be necessary to clarify this possibility. As shown in Table 45, statistical comparison of growth rates at Site D showed no significant differences among the November pretreatment level and all subsequent periods except the August 1986 (20 months postspill) level.

At Site 0, growth rates during treatment decreased slightly from pre-treatment levels (0.49 to 0.41 cm/day). During the first 4 days postspill, Site 0 growth was higher than pretreatment values. By the 4- to 6-day sampling, growth had returned to the prespill levels of November. ANOVA testing of these data showed no significant differences in growth rates from prespill values.

As shown in Table 46, statistical comparison of growth rates at Site 0 among the November pretreatment level and all subsequent periods demonstrated no statistically significant changes in growth. Therefore, it would appear that undispersed crude oil has no measurable effect on Thalassia growth within the time period and under the conditions of this study.

Thalassia growth was not measured during treatment or in the six days following treatment at Site R. ANOVA and SNK comparisons of the November 1984 levels with all subsequent sampling periods showed that Site R behaved in the same manner as Site D. Growth rates in March, June, and December 1985 were not significantly different from prespill levels (November 1984). In addition, a significant increase in growth was recorded in August 1986. August 1986 levels at Site R were 76 percent higher than the highest pre-spill value and 47 percent higher than the highest posttreatment growth rate. Although the reference site should not be regarded as a control, the distinct August growth increase suggests that the August increase at Site D was a natural fluctuation and not one resulting from treatment.

Blade Area

While absolute blade areas generally increased at Site D following treatment, ANOVA tests showed there were no significant differences between either pretreatment surveys or between prespill and postspill periods (p = C.6151), as shown in Tables 43 and 45. This indicates that dispersed oil had no significant impact on Thalassia blade areas, when contrasted with prespi!I values.

In contrast, Site 0 blade areas were characterized by a reduction in area during most periods. However, like Site D, ANOVA statistics (Tables 43 and 46) revealed that these changes were not statistically significant ($p = 0.2830$). Thus, crude oil appears not to affect Thalassia blade area significantly, when comparing pre- and postspill levels of the periods studied.

ANOVA testing of blade areas for Site R between periods indicated that means were different ($p = 0.0111$). However, SNK testing yielded ambiguous results. Blade areas during December 1985 were higher, but their statistical significance remains in doubt. More data are required to resolve this ambiguity, but it appears that blade areas were not significantly different at Site R throughout the project.

Plant Density

ANOVA comparison of plant densities between the 2 prespill periods showed significant differences at all 3 sites. Like the situation seen with the growth data, plant densities showed a greater difference between the 2 prespill surveys than between any other 2 periods. Similar to the depressed growth levels noted in March 1984, plant densities also seem to have been affected by some irregular environmental factor. ANOVA comparison of plant densities showed significant differences for all sites (p less than or equal to 0.0001, Table 43) over the complete study period. SNK testing revealed that plant densities were significantly different between each period at Site D. The initial postspill period at Site D had significantly lower densities, while the three later periods displayed significantly higher densities. At Site 0, density values also were significantly lower in March 1985 as compared to November 1984. The two following periods (June and December) were characterized by statistically equal levels; however, both were significantly different (lower) from November 1985. August 1986 densities were significantly different from all preceding periods and still lower. At Site R, plant densities were irregular (Table 47). The density in each sampling period was significantly different from all other periods, but no clear trend was evident.

In summary, postspill plant densities at Site D were initially reduced but recovered and were found at significantly higher levels in all other post-spill periods. At Site 0, postspill densities declined gradually but significantly. Levels were not reduced, however, to the low densities found in the

first prespill survey. At Site R, significant fluctuations were identified, but these followed no clear trend.

Results of Faunal Assessments

Tables 48 and 49 and Figures 55-58 present a summary of means and standard deviations for all sites and sampling periods for sea urchin density. Of the two urchin species present, Echinometra lacunter was by far the most abundant at all sites. The data presented in Tables 48 and 49 indicate that there was very high variability in the density measurements during the pre-treatment period, both between sites and between the 2 sampling periods. Given this high variability and the lack of data for certain sampling periods, the following discussion is restricted to within-site comparisons only and focuses on changes measured from the November 1984 prespill survey onward.

Site D

At Site D, total prespill urchin density was relatively high. In November 1984, E. lacunter density averaged 14.22/m^2 and L. variegatus density averaged 4.44/m^2. Both species were evident throughout the site and in all nearby areas. During treatment, these organisms experienced a drastic de-cline in abundance such that a few days after the release of dispersed oil, no live urchins of either species were present anywhere in the seagrass beds at Site D. Both areal (#/m^2) and linear (#/m) densities were 0.0 at this time. Numerous dead urchins were evident throughout the site.

Four months after site treatment (March 1985), no live urchins were recorded in any of the linear transects. Inspection of the site revealed no urchins anywhere (a few urchins were found in the deeper water outside of and adjacent to the site). Quadrat density measurements were not taken during this survey period.

By the 7-month postspill survey (June 1985), sea urchins had begun to recolonize Site D. The linear and areal densities of both urchin species were well below the prespill levels of November 1984. Although no size measurements were taken, observations of the urchins present indicated that most were relatively small, suggesting that those present represented newly-recruited juveniles.

In December 1985 (12 months postspill), the density of both urchin species at Site D had increased dramatically to levels 3 times those of the

TABLE 48. Mean sea urchin density (#/m²) for ¼-m quadrats for all sites by sample period (standard deviation in parentheses).

	SITE D		SITE O		SITE R	
	Number	N	Number	N	Number	N
MARCH 1984 (8 months prespill)						
E. lacunter	16.80 (8.19)	5	24.80 (8.67)	5	31.20 (11.79)	5
L. variegatus	3.20 (3.34)	5	2.40 (3.57)	5	0.80 (1.78)	5
NOVEMBER 1984 (1 week prespill)						
E. lacunter	14.22 (7.51)	9	1.10 (1.95)	7	NM	-
L. variegatus	4.44 (5.45)	9	0.60 (1.51)	7	NM	-
DECEMBER 1984 (1 week postspill)						
E. lacunter	0.00 (0.00)	4	0.80 (1.78)	5	NM	-
L. variegatus	0.00 (0.00)	4	0.80 (1.79)	5	NM	-
MARCH 1985 (4 months postspill)						
E. lacunter	NM	-	NM	-	NM	-
L. variegatus	NM	-	NM	-	NM	-
JUNE 1985 (7 months postspill)						
E. lacunter	0.33 (0.58)	4	1.00 (1.00)	4	6.00 (5.20)	4
L. variegatus	0.00 (0.00)	4	2.00 (1.70)	4	2.00 (1.70)	4
DECEMBER 1985 (12 months postspill)						
E. lacunter	53.00 (29.10)	4	10.00 (13.27)	4	13.00 (10.52)	4
L. variegatus	15.00 (12.38)	4	5.00 (3.83)	4	3.00 (6.00)	4
AUGUST 1986 (20 months postspill)						
E. lacunter	9.00 (12.81)	4	54.00 (60.00)	4	35.00 (35.38)	4
L. variegatus	8.00 (7.30)	4	3.00 (3.83)	4	6.00 (4.00)	4

TABLE 49. Mean sea urchin density (#/m) for linear transects for all sites by sample period (standard deviation in parentheses).

	SITE D			SITE O			SITE R		
	Number		N	Number		N	Number		N
MARCH 1984 (8 months prespill)									
E. lacunter	NM		-	NM		-	NM		-
L. variegatus	NM		-	NM		-	NM		-
NOVEMBER 1984 (1 week prespill)									
E. lacunter	1.53	(0.11)	2	1.93	(1.02)	3	0.77	(0.25)	2
L. variegatus	0.13	(0.04)	2	0.43	(0.58)	3	0.00	(0.00)	2
DECEMBER 1984 (1 week postspill)									
E. lacunter	0.10	(0.07)	4	0.75	(0.29)	4	NM		-
L. variegatus	0.00	(0.00)	4	0.21	(0.10)	4	NM		-
MARCH 1985 (4 months postspill)									
E. lacunter	0.00	(0.00)	3	0.90	(0.18)	3	NM		-
L. variegatus	0.00	(0.00)	3	0.05	(0.05)	3	NM		-
JUNE 1985 (7 months postspill)									
E. lacunter	0.44	(0.32)	3	1.87	(0.43)	3	1.49	(0.31)	3
L. variegatus	0.02	(0.04)	3	0.07	(0.00)	3	0.19	(0.11)	3
DECEMBER 1985 (12 months postspill)									
E. lacunter	3.58	(1.26)	3	0.71	(0.45)	3	0.89	(0.33)	3
L. variegatus	0.01	(0.02)	3	0.00	(0.00)	3	0.00	(0.00)	3
AUGUST 1986 (20 months postspill)									
E. lacunter	4.05	(1.21)	3	2.11	(0.64)	3	1.42	(0.10)	3
L. variegatus	0.01	(0.02)	3	0.08	(0.04)	3	0.18	(0.11)	3

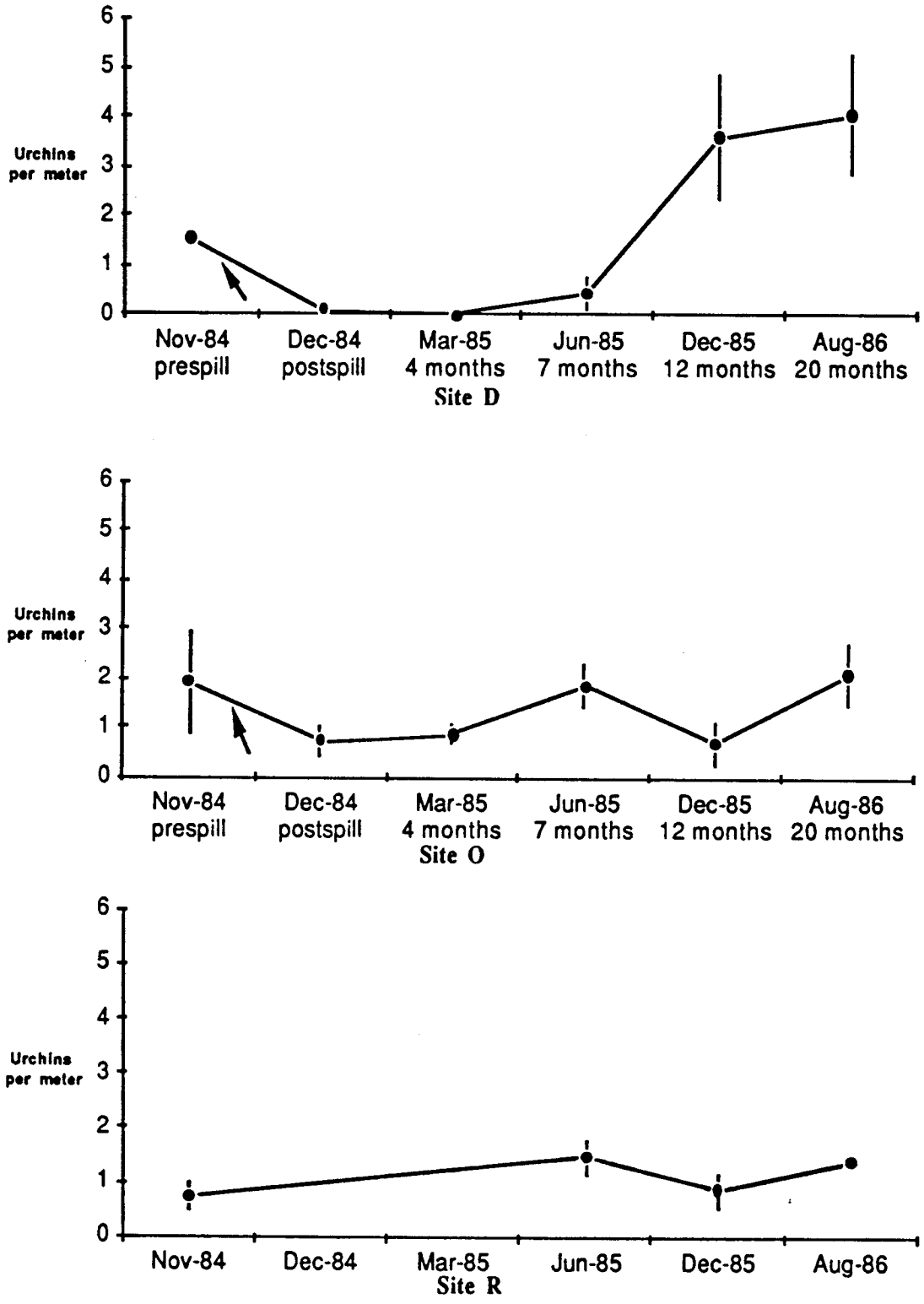


FIGURE 55. Linear densities (#/m) of Echinometra lacunter (black sea urchins) at Site D (top), She 0 (middle), and Site R (bottom). Arrow indicates date of site treatment.

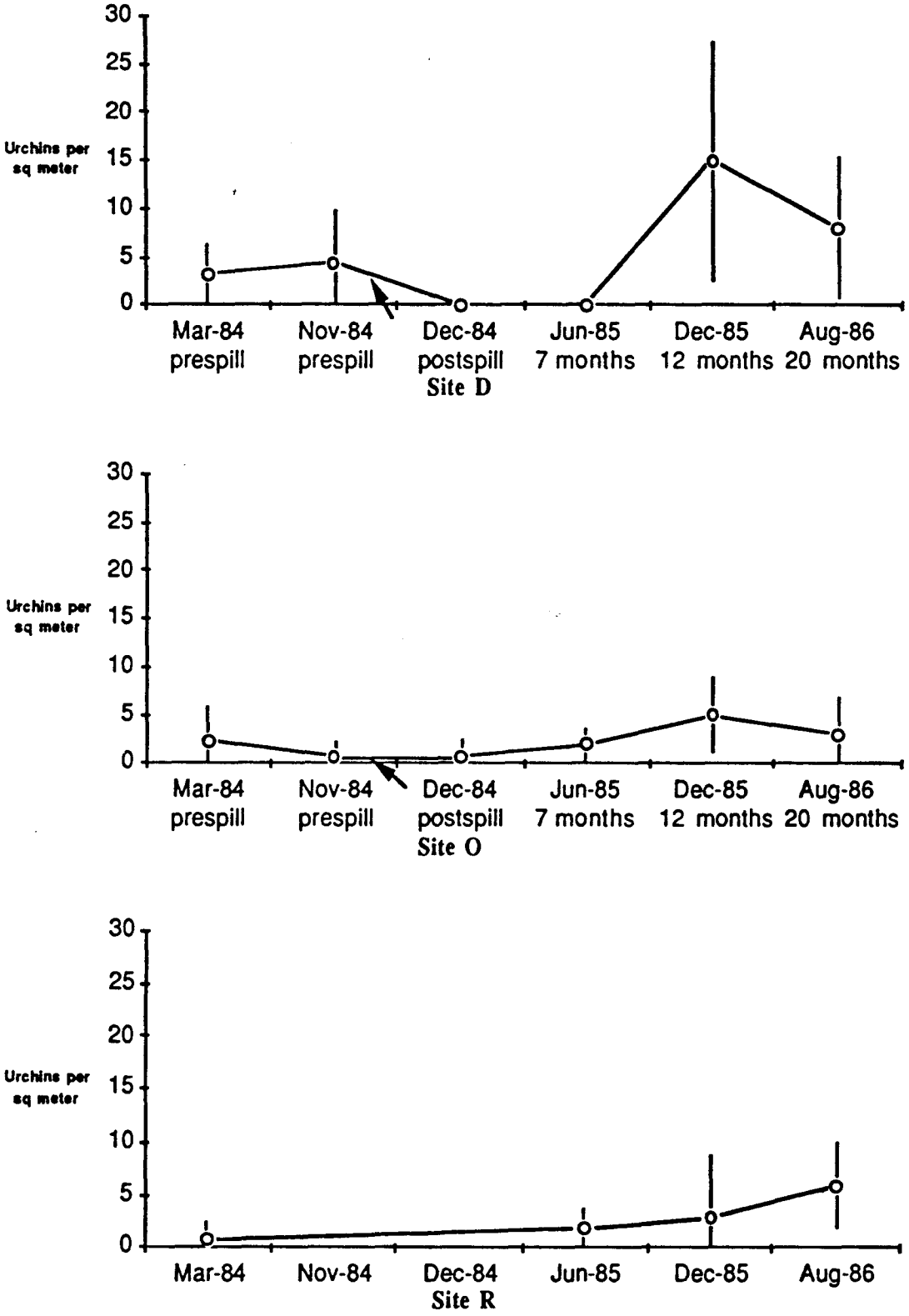


FIGURE 56. Areal densities (#/m^2) *of* E. lacunter at Site D (top), Site 0 (middle), and Site R (bottom). Arrow indicates date *of* site treatment.

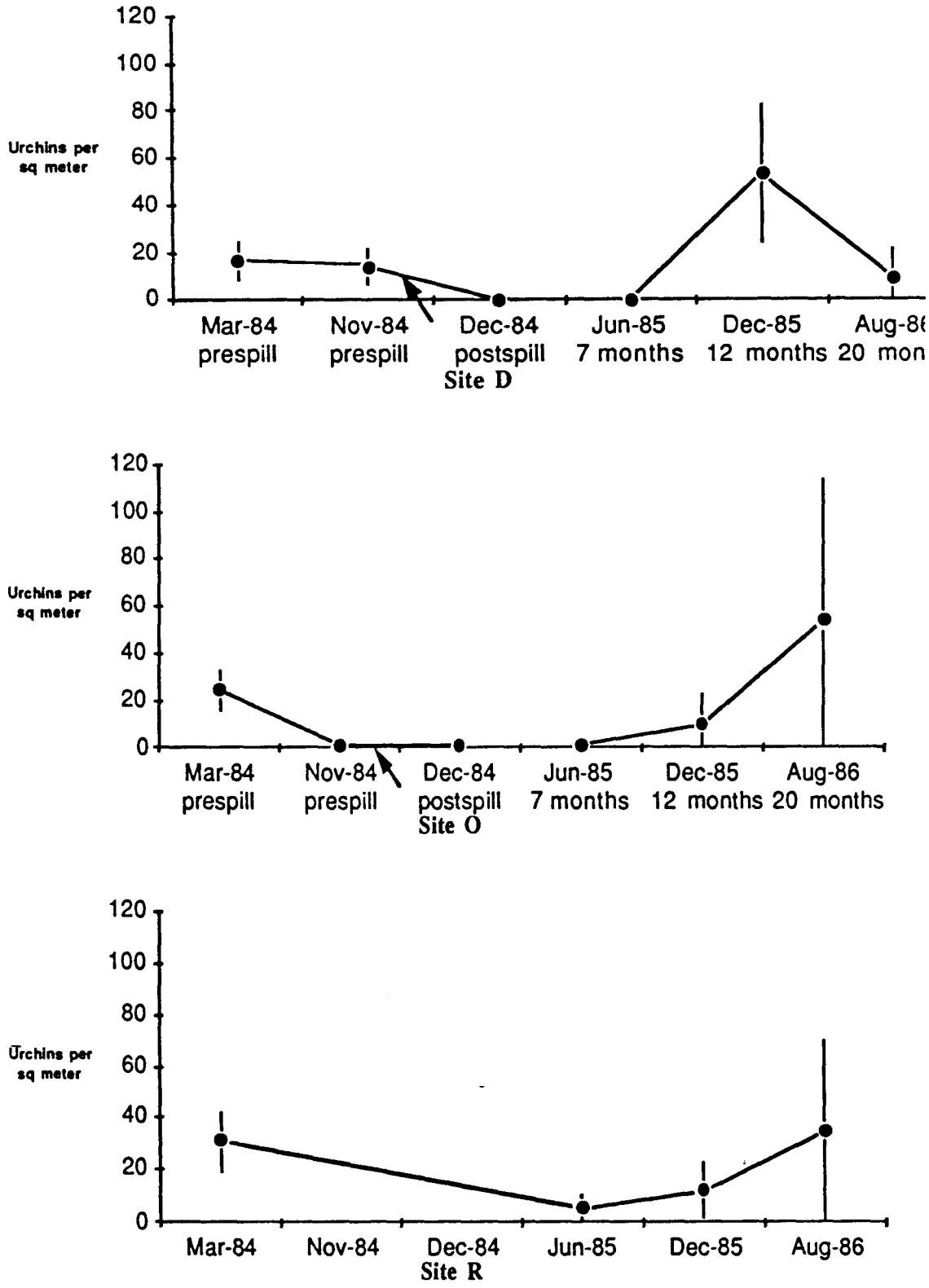


FIGURE 57. Linear densities (#/m) of Lytechinus variegatus (white sea urchins) at Site D (top), Site 0 (middle), and Site R (bottom). Arrow indicates date of site treatment.

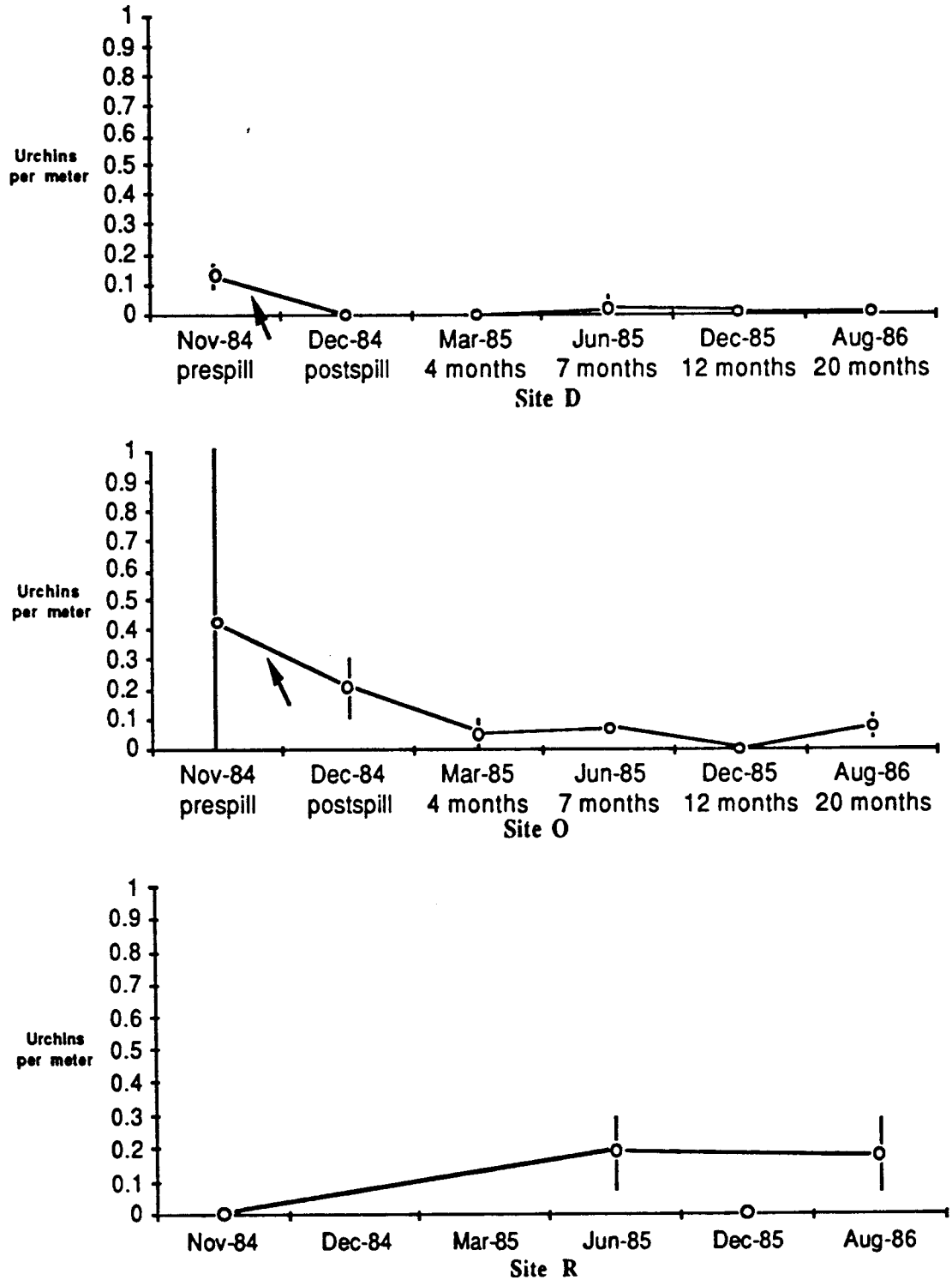


FIGURE 58. Areal densities (#/m^2) of L. variegatus at Site D (top), Site 0 (middle), and Site R (bottom). Arrow indicates date of site treatment.

prespill period (November 1984), followed 8 months later by another decline in density. The reason for this decline is unknown but may simply reflect the natural variation in density such as that measured at Sites 0 and R.

Site 0

Urchin density at Site 0 was highly variable during the prespill period. Comparison of the first prespill and first postspill data (November 1984 and December 1984) showed a slight decrease in density, followed by relatively similar densities in the 7-month postspill period (June 1985). In December 1984, and especially in August 1986, large increases in density of both species were measured at Site O.

Site R

Density of the undisturbed urchin populations at Site R demonstrated high variability. The wide range in mean density and the high standard deviations presented in Tables 48 and 49 give an indication of what is presumably natural variability.

Infauna

Tables 50 and 51 present summaries of density and diversity data for infauna sampled in the seagrass habitats at each site. The infaunal communities at each site were dominated by polychaete worms and bivalve molluscs. The most common polychaetes were representatives of the following genera:

Nereidae
Syllidae
Eunicidae
Maldanidae
Terrebellidae
Flabelligeridae

A total of nine species of polychaetes from these genera were present. Two species of the bivalve genera Codakia (C. orbicularis and C. orbiculata) also were relatively abundant.

In addition to the polychaetes and bivalves, amphipods, sea urchins, brittle stars, sponges, crabs, shrimps, and isopods appeared in some of the samples. These organisms generally are not considered to be true infauna

TABLE 50. Infaunal density (numbers/m^2) for the seagrass bed habitats at each site. Means and standard deviations are presented.

	SITE D	SITE 0	SITE R
March 1984 (8 months prespill)	1,072.1 (643.5)	1,218.3 (719.9)	755.4 (835.8)
November 1984 (1 week prespill)	0.0 (0.0)	292.4 (245.2)	36.5 (89.5)
March 1985 (4 months postspill)	93.9 (172.5)	407.3 (20.2)	250.6 (151.3)
June 1985 (7 months postspill)	584.8 (379.8)	548.2 (438.6)	274.1 (304.5)
December 1985 (12 months postspill)	511.7 (525.8)	268.0 (239.7)	877.2 (863.4)
August 1986 (20 months postspill)	657.9 (580.2)	536.1 (549.5)	1,047.7 (868.7)

TABLE 51. Diversity indices for infauna sampled in the seagrass habitat at each site. H' = Shannon-Weaver index; D' = Gleason diversity index.

	SITE D	SITE 0	SITE R
March 1984			
H'	1.086	0.730	0.545
D'	1.303	0.912	0.813
November 1984			
H'	0*	0.223	0*
D'	0*	0.421	0*
March 1985			
H'	0.098	0.197	0.197
D'	0.206	0.309	0.412
June 1985			
H'	0.670	0.587	0.156
D'	1.060	0.986	0.271
December 1985			
H'	0.481	0.301	0.330
D'	0.816	0.582	0.461
August 1986			
H'	0.575	0.340	0.695
D'	0.799	0.474	0.883

* Of nine samples taken, one or zero specimens were present.

and were not quantified in our analyses, primarily because their populations were assessed in other components of the site investigations and because they occur infrequently in the sediment cores used to sample infauna. The results of infaunal sampling were extremely variable at all sites. Density measurements ($1/m^2$) at various sampling periods varied at least one order of magnitude at each site. In addition, there was no readily discernible pat-tern in the abundance or diversity of infauna.

This high variability may be due to the relatively heterogeneous substrate present in the seagrass beds. The seagrass substrate was composed almost entirely of P. porites coral rubble, which formed a relatively coarse, poorly sorted layer throughout each site. Scattered throughout the seagrass beds were small areas with high levels of organic material, areas devoid of organic materials, and areas covered by Zooanthus and other epifauna. This wide variety of microhabitats within the seagrass bed almost undoubtedly played a role in the highly variable density measurements obtained. The highly variable infaunal density among sites and sampling periods precludes a scientifically sound assessment of the effects of crude oil or dispersed oil on the infauna of this study area. Future assessments directed toward this question will necessitate more extensive sampling.

SUMMARY

The methods used in this study were successful in implementing the experimental scenarios and in assessing biological and chemical effects and trends over the course of the study. The site treatments resulted in expo-sure levels to whole or dispersed oil that were close to the target exposure levels set for each site; the oil site (Site 0) was exposed to a moderate-to-high dose of fresh crude oil, and the dispersed oil site (Site D) was exposed to a high dose of dispersed, fresh oil such as would occur if a large spill was dispersed in or adjacent to coral and seagrass habitats in shallow, near-shore waters.

Short-term and long-term monitoring of each site was carried out over a 20-month period following site treatment, during which time a number of biological and chemical parameters were measured. A summary of the major findings at each site is presented below.

SITE 0

- The intertidal sediments of Site 0 were heavily contaminated by crude oil; hydrocarbon concentrations ranging from 92 to 552 ppm were measured during the postspill period.
- The subtidal habitats were exposed to relatively low concentrations of hydrocarbons; exposure levels in the water ranged from 1.0 to 3.5 ppm during site treatment, and 0.1 to 10.2 ppb afterward. Hydrocarbon concentrations in subtidal sediments ranged from 7 to 44 ppm during the posttreatment period.
- Adult mangroves (Rhizophora mangle) experienced a high level of defoliation and death; these effects stabilized 7 months after site treatment, at which time the average amount of defoliation was 45 percent and 17 percent of the adult trees were _dead. Death and defoliation of adult mangroves resulted in a doubling of the amount of open forest canopy by the end of the study.
- No significant changes in leaf production of surviving mangroves were measured; changes in leaf length were detected one year after site treatment. Lenticel density and growth rates of prop roots of surviving trees were not affected.
- None of the juvenile mangroves planted 1 week after site treatment were alive 4 months later. Defoliation of adult trees allowed successful

natural colonization of substrate below defoliated areas by juvenile mangroves; one year after site treatment, the number of juvenile mangroves had increased tenfold over pretreatment numbers.

- The abundance of tree snails (Littorina angulifera) decreased more than 50 percent 4 days after site treatment; the population recovered to pretreatment levels after 1 year. No mortality of mangrove tree oysters (Crassostrea rhizophorae, Isognomon alatus, and Pinctada imbricata) occurred as a result of site treatment, but the C. rhizophorae accumulated over 500 ppm hydrocarbons. Tissue levels decreased to pretreatment levels after one year.
- Regression analysis indicated that the coverage of reef substrate by corals decreased significantly over the 20-month posttreatment period, although ANOVA indicated no significant differences between assessment periods. No significant changes in coverage by total animals and total organisms were detected by ANOVA or regression. Plant coverage was found to decrease by ANOVA, but no change was detected by regression.
- No significant effects on growth rates of the four coral species studied (Porites porites, Montastrea annularis, Agaricia tennuifolia, and Acropora cervicornis) were measured.
- No significant effects on seagrass (Thalassia testudinum) growth rates leaf blade areas were detected. Gradual but significant reductions in seagrass density were measured.
- No significant changes in sea urchin (Lytechinus variegatus and Echinometra lacunter) abundance were detected, and infaunal density and diversity were too variable to allow statistical analysis.

SITE D

- The subtidal habitats were exposed to high concentrations of dispersed oil in the water, ranging from about 5 to over 80 ppm during site treatment.
- Subtidal sediments were contaminated with hydrocarbons, but at a lower level than at Site 0. Concentrations ranging from 7 to 44 ppm were measured during the posttreatment surveys (3 days to 20 months after treatment).

- Intertidal sediments also were contaminated, but at a lower level than at Site O. Concentrations of hydrocarbons in the intertidal sediments at Site D ranging from 16 to 185 ppm were measured during the posttreatment period.
- No significant effects on adult mangroves were measured during the en-tire posttreatment period; no mortality occurred, and no significant increase in defoliation was measured. No significant changes in leaf production, root growth, or lenticel density were measured; significant decreases in leaf length were measured 7 and 12 months after site treatment.
- Short- and long-term survival rates of juvenile mangroves were not affected. No large-scale increases in colonization by juveniles occurred at Site D.
- Abundance of tree snails decreased by about 50 percent 4 days after site treatment; tree snail numbers recovered to pretreatment levels 7 months later. No mortality of tree oysters occurred as a result of site treatment. C. rhizophorae accumulated hydrocarbons to 506 ppm 4 days after site treatment, and after 12 months, concentrations in tissues dropped to pretreatment levels.
- Coverage of reef substrate by corals was found to decrease significantly by ANOVA and regression. Significant decreases in total animal and organism coverage were detected by ANOVA, but not by regression, suggesting that total animal and organism coverage stabilized or re-versed at 20 months postspill. No significant changes in plant coverage were detected.
- Growth rates of A. tennuifolia were significantly reduced at Site D, and P. porites had marginal reductions in growth. No effects on growth of A. cervicornis or M. annularis were detected.
- No significant reductions in seagrass growth rates or leaf blade areas were measured. Density of seagrass declined at Site D but later recovered to greater than pretreatment levels.
- The abundance of sea urchins was greatly reduced; no urchins at Site D survived site treatment. Urchin populations recovered within 12 months. No changes in infaunal density or diversity were detectable because of extremely high variations in their data.

SITE R

- oNo contamination of sediments or water occurred at Site R as a result of site treatment at Sites D or 0, or from other unknown sources.
- oNo significant changes in adult or juvenile mangrove survival, foliation, leaf production, prop-root growth, or lenticel density were measured. Only adult leaf length and width showed any significant change at any time in the study.
- oSignificant period differences in coral coverage were detected by ANOVA, but SNK testing was unable to resolve any particular period as different from any other. Coral coverage was found to increase and decrease throughout the study period with no apparent overall pattern. Regression analysis indicated no significant decline in coral coverage.
- oTotal animal coverage was found to significantly decrease by ANOVA and regression. SNK testing showed only the final assessment period (20-month postspill) to be significantly different compared to the pre-spill period. Animal coverage was affected most by decline in coral coverage associated with marginally significant increases in plant cover-age. No significant changes in total organism coverage were detected by ANOVA or regression.
- oNo significant changes in coral growth were measured at Site R or the supplementary reference site (R-2).
- oSeagrass parameters were highly variable at Site R, but no clear in-creasing or decreasing trends in growth rate, blade areas, or densities were detected.
- o No significant changes in the abundance of sea urchins were measured.

DISCUSSION

The TROPICS project was the most comprehensive and detailed evaluation of the effects of oil and dispersed oil on tropical nearshore environments ever conducted. The findings of this study provide environmental managers with experimental data that will allow informed, intelligent decisions regarding dispersant use in tropical areas dominated by the three habitat types studied.

Two important design features must be considered in the interpretation of the results of this study. First, the experimental scenario called for the use of fresh, unweathered crude oil. The lack of any weathering undoubtedly contributed to the toxic effects measured at both study sites. The use of unweathered oil, while somewhat unrealistic, provided an extreme case study from which the effects of less extreme situations might be extrapolated. The second factor is the duration of exposure at Site D. Under normal circumstances, it would be unlikely that such high concentrations of dispersed oil would be maintained for a 24-hour period.

The results of sediment analyses indicated that both sites had relatively high and roughly equivalent levels of contamination of intertidal sediments. This may seem unusual in light of the wide disparity in biological effects on the mangroves at each site. This conflict in results probably is related to the sampling protocol used. Inspection of the data in Table 9 shows that the lowest oil concentrations were measured 3 days after site treatment, when visual observations (and common sense) dearly indicated that the highest levels occurred then. The low levels measured at this time undoubtedly reflect a sampling bias away from the most heavily contaminated portions at each site. Later measurements probably reflect a combination of similar sampling effects, natural variability, and redistribution of oil within each site over time. Sediment sampling is particularly sensitive to small-scale spatial variations in the distribution of oil because of the relatively small volume of material obtained in each sample. This effect would tend to be greatest during the early posttreatment period before waves and tides have redistributed oil within the site.

The sediment chemistry data, therefore, should be viewed in terms of the general levels of contamination indicated by the mean values, tempered by acknowledgment of the difficulty in obtaining a truly representative assessment of a highly variable parameter. Within this context, it is clear that

Site D had lower levels of contamination in the intertidal sediments and higher levels in subtidal sediments than Site O.

One ramification of these data is that dispersants somehow may act to reduce the toxicity of oil to mangrove trees. It is evident that the man-groves at Site D were exposed to what appears to be a fairly high level of contamination without any apparent biological effects. It is not known if this is merely an artifact of highly variable chemical data (as discussed above), if dispersant actually reduces oil toxicity through some unknown mechanism, or if the measured levels of contamination at Site D represent some threshold value above which toxic effects occur.

The water chemistry data clearly show that subtidal areas of Site D were exposed to very high dispersed oil concentrations during treatment. Site D also showed the most immediate and prolonged effects both in terms of decreasing coverage of reef substrate by living organisms, decreasing growth rates of at least one coral species, and abundance of sea urchins. The decline in substrate coverage and coral growth persisted for at least one year, suggesting that the high initial concentrations of dispersed oil had residual effects on these parameters.

The long-term effects of the whole and dispersed oil on the intertidal and subtidal habitats persisted through the 20-month postspill survey, but there were indications that recovery was beginning at this time. Colonization by juvenile mangroves of substrate below dead and defoliated adult man-groves was very vigorous at Site 0 after 12 and 20 months, and it appeared that these new plants would eventually replace those killed by the whole oil. Obviously, this process will take many years, and recovery will not be complete until the juveniles have reached adult size (probably 10 to 20 years).

Recovery of the only significantly affected component of the seagrass habitat at Site D (sea urchins) was already complete after one year. Recovery of corals and other encrusting organisms in the coral reef at Site D will be much slower, but there are indications that the decline in abundance of corals was beginning at 20 months postspill. The time to full recovery is unknown but is probably on the order of several years.

The physical environment in the study region may play an important role in interpretation and application of the experimental results. The sheltered, microtidal conditions in Bahia Almirante are typical of many mangrove-, seagrass-, and coral-dominated nearshore environments through-out the Caribbean basin and other tropical regions. In these areas,

seagrass and coral habitats are able to exist in very shallow waters close to shore. These factors will tend to reduce the potential for dilution of dispersed oil in many tropical areas, thereby aggravating the adverse impacts of dispersant use in shallow waters. On the other hand, many of the shallow nearshore environments of the Caribbean and elsewhere are adjacent to deep-water areas that would be very conducive of rapid dilution of dispersed oil. Identification of deep- and shallow-water habitats and resident, sensitive biological communities would greatly aid advance planning efforts in areas where oil spills may occur.

The data presented in this report and on-site observations made over the 21/2 years of the project clearly indicate that the intertidal and subtidal habitats studied are highly sensitive to the effects of whole and dispersed oil, respectively. The effects on the Rhizophora mangle forest from both whole and dispersed oil were not unexpected and are very similar to those measured at a previous study conducted in Laguna de Chiriqui in 1983 (Getter and Ballou, 1985), as well as numerous accidental oil spills (Getter et al., 1981). The effects of oil and dispersed oil on seagrass and coral habitats are much more poorly known. This study provides one of the first detailed analyses of these effects in a controlled field setting. The only previous studies of seagrasses were conducted by Baca and Getter (1984), Thorhaug et al. (1986), and Thorhaug and Marcus (1987) ; these studies showed that Thalassia testudinum is relatively resistant to dispersed and whole oil when exposed to 5 to 20 ppm for short periods. The results of the present study confirm these findings and provide the only field experiment data to date on the short- and long-term effects of whole and dispersed oil on seagrasses.

The results of this study also expand upon the presently small data base on the effects of oil and dispersants on corals. The Coroil project (Knap et al., 1985) and similar studies conducted at BBS have found that one coral reef species (Diploria strigosa) is relatively tolerant to dispersed oil in terms of long-term effects on growth and survival. The present study increases the available data for four additional coral species and supports casual observations of the sensitivity of other reef-inhabiting invertebrates. These noncoral invertebrates of the reef community (and the invertebrates of the seagrass beds) have not been studied in much detail, and as the TROPICS results have shown, they may be one of the most sensitive components of these habitats.

Comparison of the TROPICS experiment to other, similar studies con-ducted in temperate and arctic environments (Tidal Area Dispersant Experiment and BIOS) indicates several major differences. The results of the TROPICS study were very different from the Tidal Area Dispersant Experiment, which found no evidence of contamination of subtidal sediments by dispersed oil or uptake of dispersed oil by benthic molluscs. In the BIOS project, dispersed oil resulted in contamination of subtidal sediments and a variety of acute, temporary, and sublethal effects on macrobenthos. Both studies concluded that there were no compelling environmental reasons for not using dispersants in arctic and temperate environments.

The primary reasons for these major differences in experimental results are differences in the physical environment, experimental methods, and biological communities. The TROPICS study focused on sheltered, shallow-water communities and used a rather complex oil release mechanism that resulted in relatively high exposure levels to the biological communities being studied. The *very* shallow, sheltered waters, microtidal conditions, and *very* high dose of dispersed oil resulted in much higher short-term exposures to the dispersed oil cloud, and higher long-term exposures to the residual dispersed oil retained by the porous peat and coral rubble sediments. Mangrove, seagrass, and coral habitats also are typically much more complex than temperate or arctic intertidal and subtidal habitats. Tropical habitats are not subject to many of the physical forces present in temperate or arctic areas, such as ice scour and freezing, and consequently tend to have much higher densities of plants and animals present in very shallow waters. These factors resulted in relatively more severe effects than in the other studies mentioned above.

CONCLUSIONS

The purpose of this study was to obtain experimental data to determine if the use of chemical dispersants will reduce or exacerbate adverse impacts of oil spills upon sensitive and valued tropical environments such as man-grove forests, seagrass beds, and coral reefs.

The experimental design was intended to simulate a severe, but realistic, worst-case scenario of two large spills of fresh crude oil in nearshore waters, one treated with chemical dispersant and the other left untreated. It must be noted that the dispersed oil scenario represents an extreme case, such as might occur if a large (relative to the area of water), fresh oil slick were chemically dispersed in the shallow waters of a slowly flushed, semi-enclosed bay.

The question of possible trade-offs in effects between intertidal and subtidal habitats was explored to determine if there was a net benefit to be gained, such as reduction in impacts to one or both habitats, or increase in recovery rates of affected habitats. This would allow evaluation of various available response options based on different spill scenarios. These options are discussed below.

OPTION 1 - NO ACTION

This option was simulated by the untreated oil scenario (Site 0). The experimental data for Site 0 clearly show that whole, untreated crude oil has severe, long-term effects on the intertidal components of the study site (mangroves and associated fauna) and relatively minor effects on subtidal environments (limited to a slight decline in coral abundance). These results and the results of numerous other studies of oil spills have shown consistently that intertidal habitats are exposed to much higher concentrations of oil than subtidal habitats when no action is taken to prevent stranding of oil or when mechanical collection or containment procedures are ineffective. In those cases where the intertidal environment is highly sensitive to oil pollution, the no-action response option has a relatively high probability of resulting in significant adverse environmental impacts, and therefore, the no-action option is not recommended in these cases. Some form of response is warranted, either chemical dispersion of the oil (within the framework out-lined below) or mechanical containment and recovery. In situations where

intertidal environments have low inherent sensitivities, the no-action response option may be an acceptable approach.

OPTION 2 - APPLY DISPERSANTS IN SHALLOW, NEARSHORE WATERS DIRECTLY OVER OR ADJACENT TO CORAL AND SEAGRASS HABITATS

An extreme case of this option was simulated by the dispersed oil scenario (Site D). The experimental data show that the use of dispersants under this scenario had a positive effect in reducing or preventing adverse impacts to the mangrove forest, but this was accompanied by relatively severe, long-term effects on the coral and seagrass environments. It must be noted that the implementation of this scenario at Site D resulted in an extreme, worst case of Option 2 because of the volume of oil used, the lack of weatnering, and the duration of exposure.

Under more likely conditions in which the floating, untreated oil has weathered for several hours and is dispersed into the water column over a relatively short period of time, it is reasonable to assume that the magnitude of impacts to subtidal environments would be less than was measured in this study. Under less extreme conditions, one would expect the balance in environmental trade-offs to shift in favor of Option 2; for example, more physical weathering of the oil and shorter exposure periods to the dispersed oil (such as would occur in more realistic conditions) would probably result in fewer impacts to nearshore, shallow-water coral reefs and seagrass beds and, at the same time, reduce or prevent impacts to mangrove forests, even if dispersants were applied directly over coral/seagrass habitats. Therefore, the use of dispersants in shallow waters to protect highly sensitive intertidal habitats should be considered a viable option, with the realization that significant subtidal impacts may occur and that overall environmental damages may not necessarily be reduced. All efforts should be made to apply dispersants in water as deep as possible to promote dilution of dispersed oil.

OPTION 3 - APPLY DISPERSANTS IN DEEP WATER, OFFSHORE FROM MANGROVE, SEAGRASS, AND CORAL ENVIRONMENTS

This option was not directly tested during the study, but the experimental data presented here indicate that this option is likely to result in prevention or reduction of damages to mangroves without significant effects on seagrass or coral habitats. Any action taken to prevent or reduce

stranding of oil in mangrove forests is likely to have a positive effect in reducing damages to mangroves. Chemical dispersion of oil in deep water, away from nearshore environments, is likely to allow dilution of dispersed oil such that exposure of sensitive subtidal environments to toxic concentrations is not likely to occur. The amount of dilution required or the threshold levels of exposure concentration or duration are not readily identifiable from the experimental data presented here. However, it is reasonable to speculate that any reduction in exposure of nearshore corals and seagrass habitats to dispersed oil would tend to reduce damages to them. Therefore, it is recommended that the use of dispersants be considered whenever highly sensitive intertidal environments are threatened by spilled oil and that dispersant application is conducted in water as deep as possible.

Reduction in exposure of subtidal environments to dispersed oil is achieved through dilution into deep water or by high rates of mixing with water currents. It is possible to identify in advance areas where local physical processes would tend to promote rapid dilution of dispersed oil. Advance planning of this type would be similar to existing oil spill mapping methods based on sensitivity analyses and would provide spill response personnel with a practical guide in the decision-making process.

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