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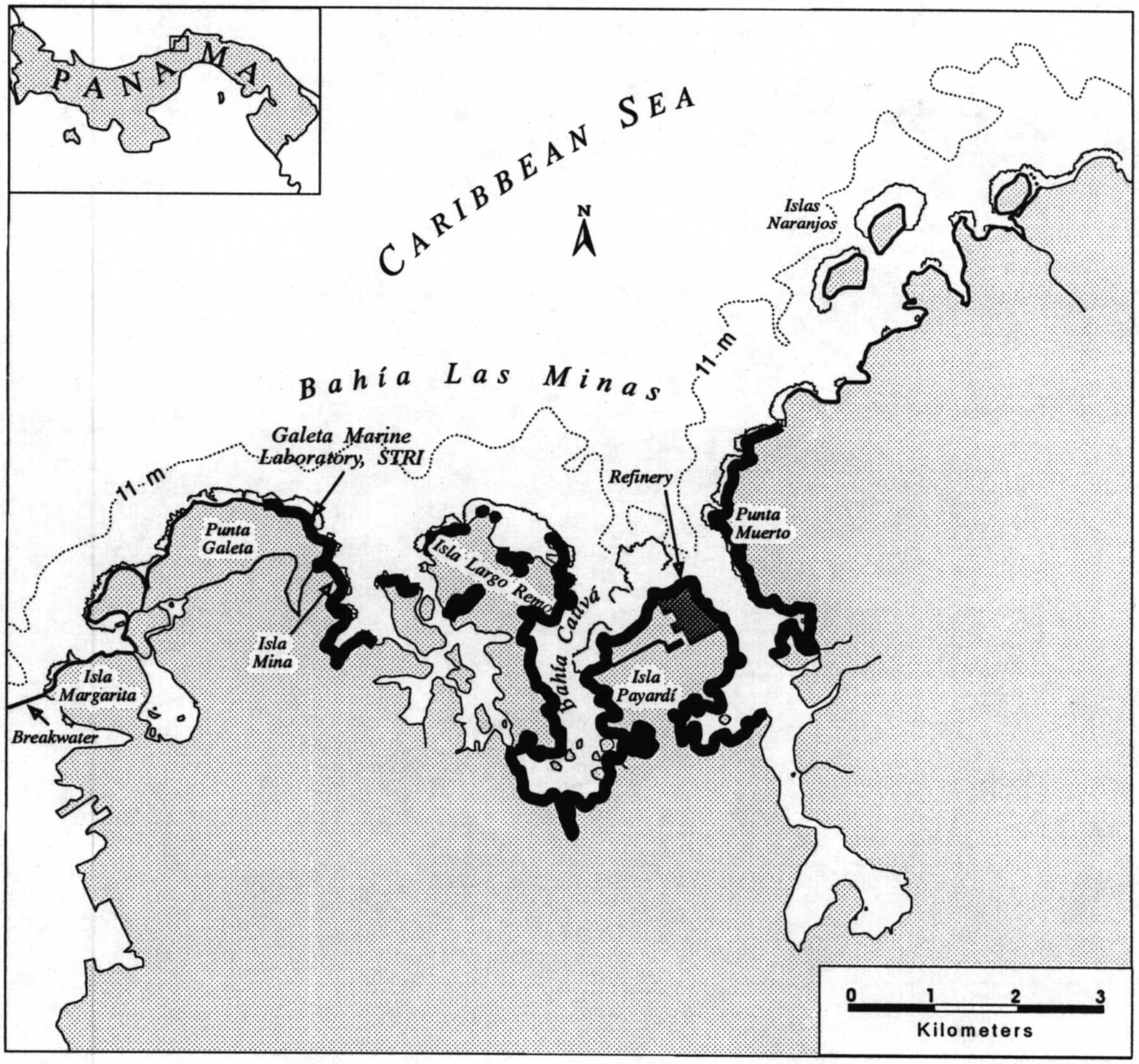
Long-term Assessment of the Oil Spill at Bahía Las Minas, Panama Synthesis Report

Volume II: Technical Report, Part 1



U.S. Department of the Interior
Minerals Management Service
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Long-term Assessment of the Oil Spill at Bahía Las Minas, Panama Synthesis Report

Volume II: Technical Report, Part 1

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Abbreviations and Acronyms

Refer to Keller et al. (Chap. 1) for a listing of site acronyms (Table 1.4; Fig. 1.5).

ANCOVA	analysis of covariance
ANOVA	analysis of variance
API	American Petroleum Institute
BBSR	Bermuda Biological Station for Research, Inc.
BCI	Barro Colorado Island, Panama
BLM	Bahía Las Minas
BMDP	BMDP Statistical Software, Inc.
CID	collection identification
CONSURV	name of a database (Cubit and Connor, Chap. 4)
CPI	carbon preference index
EC_{50}	effective concentration for 50% of the test population
EOM	extractable organic matter
ESP	Environmental Sciences Program, Smithsonian Institution
ex/em	excitation/emission
FID	flame-ionization detector
GC	gas chromatography
HPLC	high-performance liquid chromatography
HWL	high-water line
HWM	high-water mark
IAEA	International Atomic Energy Agency
IOC	Intergovernmental Oceanographic Commission
IRHE	Instituto de Recursos Hidraúlicos y Electrificación
IS	internal standard
IUCN	International Union for Conservation of Nature and Natural Resources
LAI	leaf area index
LMW	low molecular weight
LSD	least-significant difference
MANOVA	multivariate analysis of variance
MHW	mean high water
MLW	mean low water
MMS	Minerals Management Service
MS	mass spectrometry
m/z	mass/charge
NRC	National Research Council
NSL	non-saponifiable lipid
OCS	outer continental shelf
PAH	polynuclear aromatic hydrocarbon
PCC	Panama Canal Commission
ppm	parts per million

ppt	parts per thousand (o/oo)
PVC	polyvinyl chloride
REDGE	name of a database (Cubit and Connor, Chap. 4)
RF	response factor
RRI	relative retention index
SAS	SAS Institute, Inc.
SCUBA	self-contained underwater breathing apparatus
SD	standard deviation
SE	standard error
SI	Smithsonian Institution
SIM	selected-ion monitoring
SL	saponifiable lipid
SPM	suspended particulate matter
SPSS	SPSS, Inc.
SRB	Scientific Review Board
STRI	Smithsonian Tropical Research Institute
UNEP	United Nations Environmental Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
URE	unresolved
UVF	ultraviolet fluorescence
VMIC	Venezuelan/Mexican Isthmian Crude, the type of oil spilled at Bahía Las Minas

1
Introduction

**Brian D. Keller, Jeremy B. C. Jackson, John D. Cubit,
Stephen D. Garrity, and Héctor M. Guzmán**

1.1 Unplanned Environmental Assessments

Environmental assessments ideally consist of monitoring natural variation or of studying *planned* environmental modifications, such as the release of heated effluents from a power plant, and their effects. In both cases the kinds of ecological data to collect, degree of replication, frequency of sampling, and so on can be carefully designed (Green 1979; Stewart-Oaten et al. 1986; Underwood and Peterson 1988; NRC 1990; Keough and Quinn 1991; Underwood 1991). In contrast, unplanned environmental modifications, such as those caused by major oil spills, generally cannot be studied using optimal designs because pre-existing monitoring data are not available as balanced sampling at both affected and unaffected areas. In such cases all that can be done in designing an assessment is to make the best use of available data.

Even optimally designed environmental assessments lack the rigor of controlled experiments, and conclusions about cause and effect must consider factors other than the presumed one. For example, in comparing areas affected and unaffected by an oil spill, there may be additional area-specific differences not related to the spill that explain some of the observations. Because effects of major oil spills on benthic communities can be severe, studies employing suboptimal designs may detect postspill changes, as was the case after a major oil spill at Bahía Las Minas, Panama (Jackson et al. 1989; Keller and Jackson 1991).

1.2 Objectives of the Study and Organization of the Report

The study has two main objectives:

1. to monitor the long-term changes that may occur in the distribution and abundance of marine organisms as a result of the 1986 oil spill at Bahía Las Minas, and

2. to understand the ecological processes causing any observed changes.

This report is organized into chapters about the spread and stranding of oil under different sets of environmental conditions, the concentration and characterization of the spilled oil in environmental and organismal samples, and effects of the spill on particular populations and communities:

Chap. #	Chapter Title
1.	Introduction
2.	Weather, sea conditions, and topography affecting oil deposition during the spill
3.	Hydrocarbon chemistry
4.	Reef flat sessile biota, algal-turf infauna, and sea urchins
5.	Reef flat gastropods
6.	Reef flat stomatopods
7.	Subtidal reef corals
8.	Mangrove forests
9.	Mangrove fringe and epibiota of mangrove roots
10.	Subtidal seagrass communities
11.	Conclusions
	Literature cited
	Appendixes

Chapter subsections are numbered to three levels; further headings are indicated by bold typeface, italics, and italics at the start of a paragraph. References cited are given at the end of the report. In addition, there are appendix sections providing additional data.

Here, following a brief review of oil spills in tropical seas (Sect. 1.3), we describe the Bahía Las Minas ecosystem (Sect. 1.4) and the 1986 oil spill (Sect. 1.5), and relate its size to that of other major oil spills. The region of the spill has a history of development and industrialization, and was polluted by a major tanker spill of diesel oil and Bunker C in 1968 (Rützler and Sterrer 1970). After reviewing this background (Sect. 1.6), we briefly describe relevant biological studies that were conducted prior to the 1986 spill, review how existing information was incorporated into the design of each element of the study, and provide a listing and map of all study sites (Sects. 1.7-1.9).

1.3 Oil Spills in Tropical Seas

Most oil spills are unplanned events involving tankers, production platforms, storage tanks, pipelines, barges, and other vessels (NRC 1985). Between 1978 and 1990, the largest oil spills worldwide were from tankers (during 8 of the 13 yr) and production platforms or storage tanks (during 5 of the 13 yr; Welch et al. 1991). This section provides a brief review of tropical oil spills; particulars are presented in subsequent chapters.

At least 157 major oil spills, defined as more than 1,000 bbl, originated from ships and barges in the tropics between 1974 and 15 June 1990 (MMS Tanker Spill Database, App. Table A.1). Of these 157 spills, 99 occurred in coastal or restricted waters (51 of these involved groundings), 26 on the high seas, 20 at dock, and 12 in harbors. A conservative estimate, made by adding the 51 spills involving groundings in coastal or restricted waters with the 32 spills at dock and in harbors, shows that more than half of the 157 major tropical oil spills caused by shipping in recent years occurred near potentially vulnerable ecosystems such as coral reefs, seagrass beds, reef flats, sand beaches, and mangrove forests.

Between 1974 and 15 June 1990, there were 24 tanker or barge spills near coastlines in the Caribbean, four along peninsular Florida, two in the Bahamas, and two off the east coast of Mexico (Fig. 1.1; App. Table A.1; source: MMS Tanker Spill Database). There were also at least two spills in the open Caribbean Sea, one in the Atlantic Ocean, three each in the Orinoco and Amazon Rivers, and two at unspecified locations in the region (App. Table A.1).

Other past or possible oil spills in the Caribbean stem from the presence of at least 19 refineries (Ward 1990), a pipeline for transshipment of crude oil across Panama (Gundlach et al. 1985), and facilities in Bonaire, Curaçao (closed in 1985), the Bahamas, and Grand Cayman for transfer of crude oil from supertankers to smaller tankers (Fig. 1.1; Cintrón et al. 1981). Spills occurred recently at the Caribbean end of the pipeline across Panama (N. Duke and H. Guzmán, pers. obs.) and at two of the refineries (Cubit et al. 1987; Jackson et al. 1989; Bills and Whiting 1991).

Despite the frequency of spills in nearshore, tropical waters, very little work has been done on the effects of oiling on shoreline communities at low latitudes (NRC 1985). There is general agreement on the biological and economic value of mangroves forests and on the vulnerability of mangroves and their biota to oiling (Odum and Johannes 1975; Gundlach and Hayes 1978; Cairns and Buikema 1984; Linden and Jernelov 1980; Cintrón et al. 1981; Saenger et al. 1983; Teas 1983; Getter et al. 1984; Vandermeulen and Gilfillan 1984; Hatcher et al. 1989; NRC 1985; Jacobi and Schaffer-Novelli 1990). Similarly, reef flats are known to be highly productive (Hatcher 1988) and serve as nursery and foraging areas for spiny lobsters (e.g., Herrnkind and Butler 1986; Butler and Herrnkind 1991). However, knowledge of the effects of oil on mangroves and associated species is limited (Gundlach and Hayes 1978; Lewis 1983; NRC 1985), and reef flats have been "totally neglected" (NRC 1985). Of necessity, most studies of mangroves have been one-time efforts (Baker et al. 1980) following "spills of opportunity" (NRC 1985). They thus lack baseline, prespill data and seldom have long-term postspill monitoring (Baker et al. 1980).

In contrast to intertidal habitats, the vulnerability of coral reefs to oil spills has been much more controversial (see reviews by Loya and Rinkevich 1980, and Brown and Howard 1985; see Guzmán et al., Chap. 7). Although subtidal seagrass beds are vulnerable to human activities (Thayer et al. 1975), effects of oil spills on seagrass beds have received very little attention (Zieman et al. 1984).

1.4 The Bahía Las Minas Ecosystem

Bahía Las Minas is a topographically complex, shallow-water, tropical embayment whose margins were dominated at the time of the 1986 oil spill by

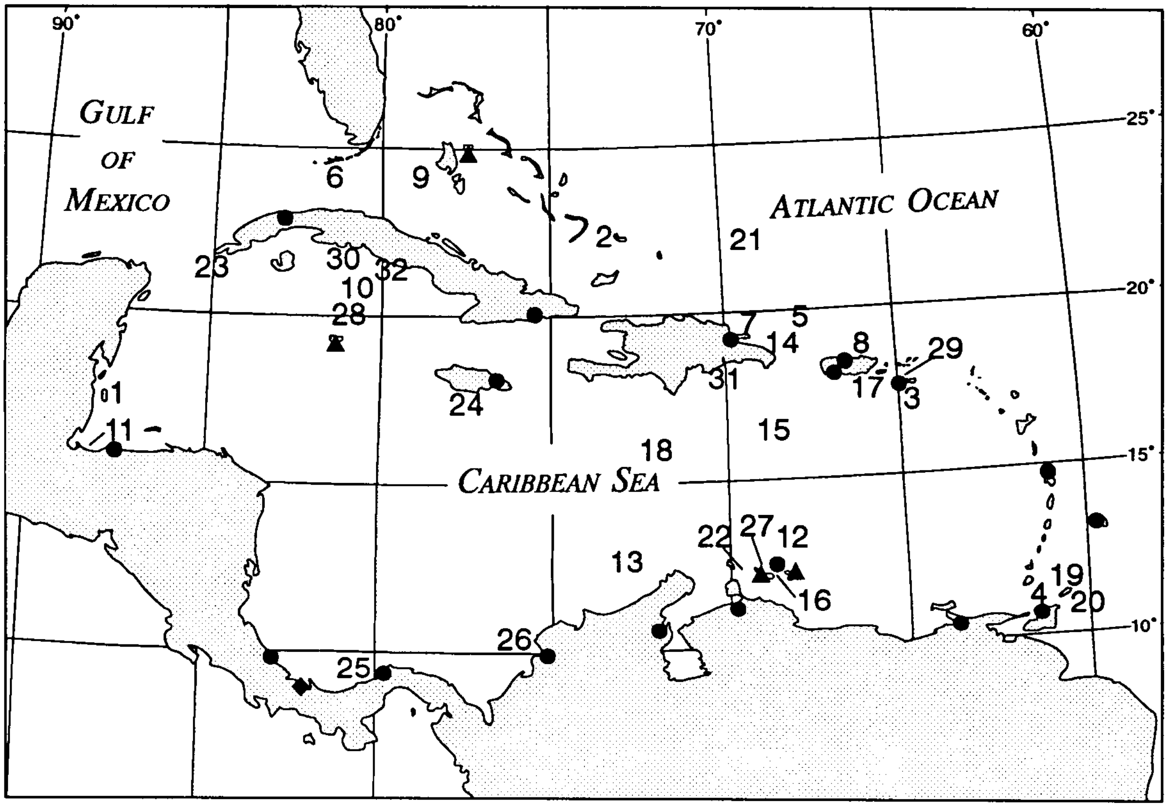

Fig. 1.1 Past oil spills and potential sources of oiling in the Caribbean. Numbers mark approximate locations of tanker and barge spills >1,000 bbl, 1978-June 1990 (source: MMS tanker oil spill database; see App. Table A.1 for data on each spill). *Circles* mark coastal oil refineries (source: NGS cartographic division; in Ward 1990). *Triangles* mark approximate locations of oil transshipment facilities (source: PNUMA/CEPAL 1979). *Diamond* marks the Caribbean terminus of the oil pipeline across Panama (Petroterminal de Panamá; source: Gundlach et al. 1985).

extensive mangrove forests, seagrass beds, and coral reefs (Cubit et al. 1985). These habitats characterize Caribbean shores and many other regions of the tropics worldwide (Phillips and McRoy 1980; Ogden and Gladfelter 1983; Dubinsky 1990; Robertson and Alongi 1992). In almost every case, the physical structure of these environments is built, stabilized, and maintained by a few species of relatively large, long-lived, photosynthetic organisms that together buffer the coastal zone from freshwater runoff and erosion from the land, and wave energy from the open sea. Mangroves, seagrasses, corals, and coralline algae produce enormous quantities of biogenic structural materials (wood, rhizomes, and limestone) whose very presence baffles water movements and promotes the deposition of sediments.

The red mangrove *Rhizophora mangle* forms dense, anastomosing thickets of prop roots and trunks that extend outward from land. Mangroves protect the shore from the impacts of debris such as floating logs and waves, reduce water circulation, increase sedimentation, and provide deep shade and shelter at all but the most open coastal margins. Seagrasses, especially turtlegrass (*Thalassia testudinum*), form dense beds that may extend for hectares seaward of the mangrove fringe, depending on the local bottom profile, size of the embayment, and degree of protection by coral reefs. Seagrass beds are supported by dense root and rhizome mats up to half-a-meter thick that stabilize sediments against erosion. In addition, long seagrass leaves slow water movements and increase sedimentation. Coral reefs are built primarily by a few species of corals and crustose coralline algae that produce the limestone framework and cement that is filled by skeletal debris of associated organisms. The physical complexity of reefs depends on the growth form of dominant coral species; branching corals form dense thickets whereas massive species form a more open framework. Regardless, reefs are the outer defense of the land against the sea, with calm-water, sediment-trapping lagoons and reef flats behind.

Mangroves, seagrass beds, and coral reefs provide habitat for a great diversity of species (Chapman 1977; Zieman 1982; Cubit and Williams 1983; Endean and Cameron 1990) that depend largely or entirely on biogenic characteristics of the habitat, much like the animals and herbaceous vegetation of a deep forest. Moreover, many of these organisms consume and thereby strongly influence the species composition and abundance of the habitat-structuring organisms on which they depend. The best-known cases are grapsid crabs feeding on mangrove seeds (Smith 1987; Smith et al. 1989), and sea urchins, schooling fishes, territorial damselfish, and snails feeding on seagrasses, reef corals, and fleshy macroalgae (Kaufman 1977; Ogden 1980; Lawrence and Sammarco 1982; Lessios 1988; Knowlton et al. 1990; Sale 1991). The principal groups of associated consumers and other organisms studied in this report are listed in Table 1.1.

Nearly all the oiled study sites and many of the unoiled mangrove sites listed later in this chapter (Sect. 1.9) were in Bahía Las Minas. However, many unoiled sites were northeast of this embayment in a region referred to here as the *Costa Arriba*, from María Chiquita to Isla Grande (Fig. 1.2). These included unoiled open-coast mangroves, reef flats (plus one site west of the bay), seagrass beds, and coral reefs. Environmental conditions and other factors differ between the two areas (Table 1.2). Implications of these differences to findings of the study are discussed in later chapters.

1.5 The 1986 Oil Spill at Bahía Las Minas, Panama

In 1986 a major oil spill polluted Caribbean coastal environments of Panama, including a biological preserve at a marine laboratory of the Smithsonian Tropical Research Institute (STRI). For the reef flat at this site, baseline biological and environmental data for some parameters had been collected for more than 15 yr (Cubit and Connor, Chap. 4). There had also been surveys or short-term studies of reef flat gastropods (Garrity et al., Chap. 5), reef flat stomatopod crustaceans (Steger and Caldwell, Chap. 6), coral reefs (Guzmán et al., Chap. 7), mangroves (Duke and Pinzón, Chap. 8), the epibiota of fringing mangrove roots (Garrity and Levings, Chap. 9), and seagrass communities (Marshall et al., Chap. 10). These prespill studies

Table 1.1 Principal habitat-structuring organisms and associated consumers and other organisms studied, in order of their presentation in the report.

Habitat-structuring Organisms	Associated Consumers and Other Organisms
Crustose coralline and fleshy macroalgae on reef flats	Sea urchins, stomatopods, snails, and infauna of *Laurencia* turf
Reef corals and associated sessile biota	Fishes and sea urchins
Red mangroves	Epibionts on prop roots, grapsid crabs
Seagrasses	Infaunal and epifaunal invertebrates, fish, and algae

provided a relatively comprehensive background for assessing biological effects of the spill. Furthermore, observations of effects of the spill began as oil was washing ashore. Such promptness is important because many ecological changes start immediately after such acute pollution (e.g., Sanders et al. 1980), and direct observations of immediate postspill die offs may be important.

The oil spill occurred on 27 April 1986 at a petroleum refinery at Bahía Las Minas, Panama (Cubit et al. 1987; Jackson et al. 1989; Fig. 1.2). Approximately 38.3 million L (240,000 bbl) of medium-weight crude oil drained from a ruptured storage tank. The oil was 70% Venezuelan and 30% Mexican Isthmian, with a specific gravity of 27° at 15.6°C (American Petroleum Institute) or about 0.89 g/cc. Approximately 22.3 million L (140,000 bbl) flooded through the containment dike around the storage tank and overwhelmed separators and a retaining lagoon. In May 1986 a refinery official reported recovery of 9.6 million L (60,000 bbl) of oil from the sea. We do not know, however, how much oil was *not* recovered and can only surmise that the volume of oil that spilled into the sea from the grounds of the refinery was at least 9.6-16.0 million L (60,000-100,000 bbl).

The volume of this spill was greater than that of any other oil spill reported near coral reefs and mangroves in the tropical Americas (Loya and Rinkevich 1980; Getter et al. 1981). Compared with recent major spills from oil tankers in other tropical areas, however, the Bahía Las Minas spill was moderate in size (Fig. 1.3; among the top 20% by volume). Considering some well-studied oil spills, the Bahía Las Minas spill was much larger than the spill from the barge *Florida* near Woods Hole, Massachusetts (Sanders et al. 1980), similar in size to the 1969 Santa Barbara spill (see Foster and Holmes 1977), and much smaller than the spills from the tankers *Torrey Canyon* (Smith 1968), *Amoco Cadiz* (see Teal and Howarth 1984), and *Exxon Valdez* (Houghton et al. 1991; Fig. 1.3).

During the first six days after the spill onshore winds held the spilled oil in Bahía Cativá, adjacent to the refinery (Fig. 1.4). Shifting winds and runoff from rains

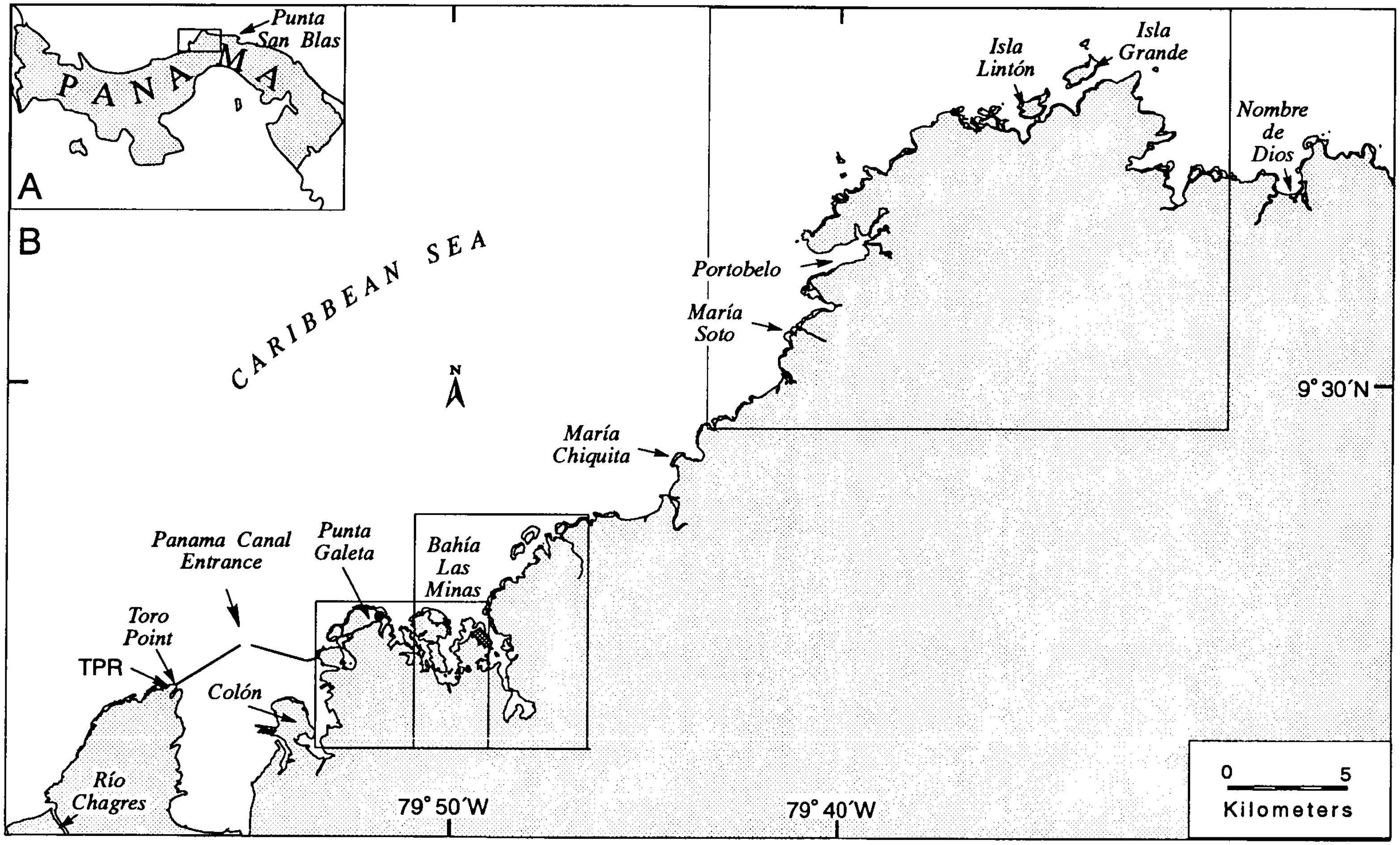

Fig. 1.2 Region of Panama affected by the 1986 oil spill. *A*. Location of the study region (*boxed area*) on the Caribbean coast of Panama. *B*. The study region; the *lower left boxed areas* include the most heavily oiled coastal habitats; lightly oiled and unoiled study sites are at Toro Point and northeast of Bahía Las Minas as far as Isla Grande (*upper right boxed area*), as well as certain areas within the heavily oiled region where oil did not reach. Enlargements of the boxed areas and the location of study sites within them are shown in Fig. 1.5.

Table 1.2 Comparison of the environments of Bahía Las Minas and the Costa Arriba.

Oiled and Unoiled Sites (Bahía Las Minas)	Unoiled Sites (Costa Arriba)
Industrial and other chronic pollution present	No sources of industrial pollution; less pollution from other sources
Water circulation blocked around islands by construction projects; severe floods of muddy freshwater	No major construction projects; muddy outflow from many rivers
Oiled open coastline faces tradewinds and tradewind-generated waves	Most open coastline obliquely oriented to tradewinds and tradewind-generated waves
Greater sediment load in water column	Less sediment load in water column

then pushed a large quantity of oil out to sea past a boom placed across the mouth of this embayment. Starting 6 May 1986, aircraft sprayed approximately 21,000 L of the dispersant Corexit 9527 (Exxon Chemicals) over oil slicks. A C-130 aircraft was observed spraying dispersant from a very low altitude (at or under tree level) at the mouth of Bahía Cativá, including areas near the coastlines of western Isla Largo Remo, northwestern Isla Payardí, and Punta Muerto. It was reported that more than 11,000 L of dispersant were used in this spraying alone. In addition, a small crop-duster aircraft was observed spraying dispersant on slicks between Islas Naranjos and the mainland. Other such applications of dispersant were observed off the breakwater of the Panama Canal, offshore of Bahía Las Minas, and offshore of Portobelo (Figs. 1.2 and 1.4). Additional back-pack spraying of dispersant was used in some areas of mangroves.

No dispersant was sprayed near Punta Galeta. Application of the dispersant nine days after the spill instead of within the first 24 hours, as well as calm sea conditions, probably rendered chemical dispersion ineffective. Although some coastal areas were exposed to dispersant, particularly Bahía Cativá and areas near Islas Naranjos, many oiled areas, including Punta Galeta, were not directly exposed to this compound. The overall dosage of dispersant was low based on a 1:20 dispersant:oil ratio from laboratory studies (NRC 1989). Such a ratio would have required a total of 480,000-800,000 L of dispersant, an order of magnitude greater than the estimated total used. Localized effects of the dispersant may have occurred, particularly at sites between Isla Largo Remo and Punta Muerto, and in Bahía Cativá (see Cubit and Connor, Chap. 4). However, the limited use of dispersant cannot explain the widespread subtidal biological effects reported later in this report (see Burns, Chap. 3).

By 15 May oil had spread along the coast and washed across fringing reefs into mangroves, small estuaries, and sand beaches within 10 km of the refinery.

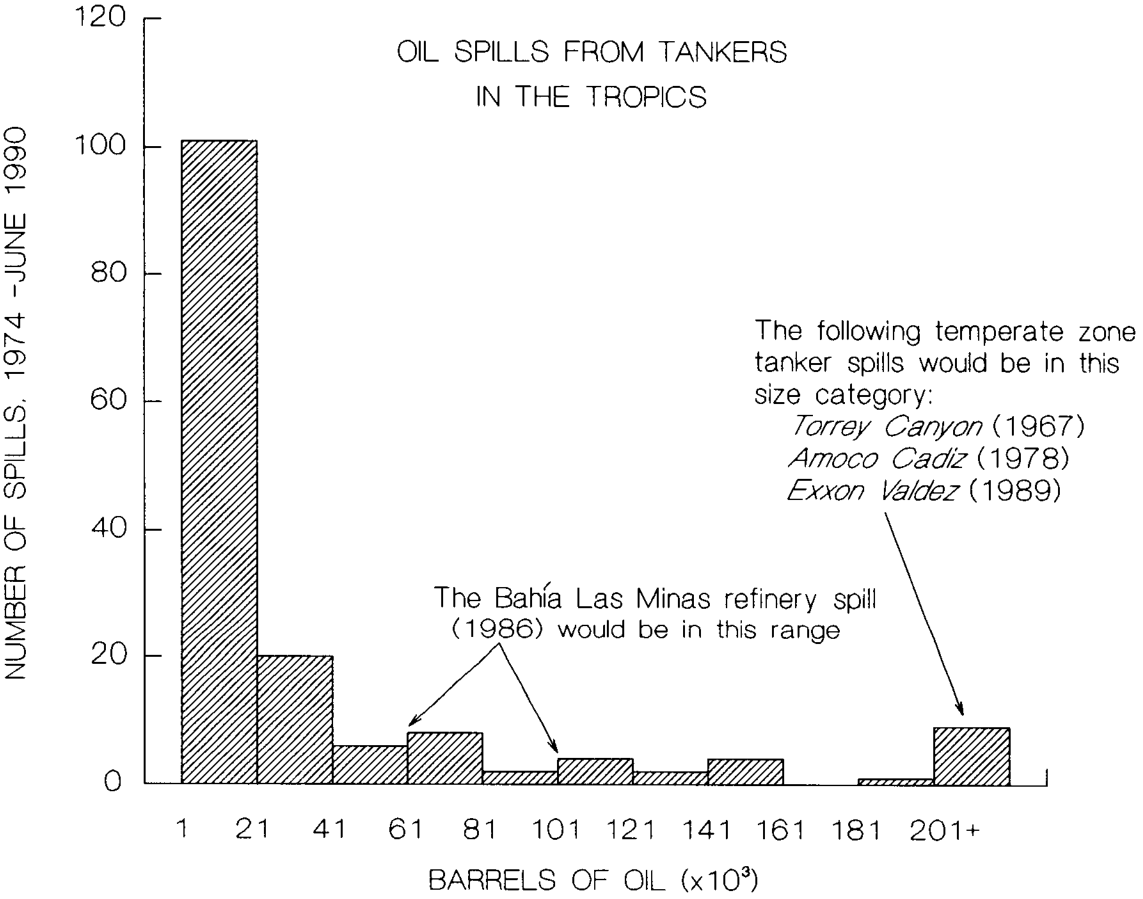

Fig. 1.3 Size frequency of recent oil spills from tankers in the tropics. The Bahía Las Minas refinery spill and some well-known temperate zone tanker spills are noted for comparison. Source: MMS Tanker Spill Database; see App. Table A.1.

During the first two months after the spill, the distribution of oil was surveyed from low-flying aircraft between Río Chagres, 27 km west of the refinery, and Punta San Blas, 98 km to the east (Fig. 1.2). Surveys by helicopter, airplane, foot, and boat were conducted from Río Chagres to Nombre de Dios. During these surveys visual assessments were made of the degree of oiling (heavy, moderate, light, or absent) and of the habitats and types of organisms obviously oiled or affected by oiling.

The shoreline deposition of extensive, black oil slicks was limited to the coast between Isla Margarita and María Chiquita with the exception of two partially isolated lagoons in Bahía Las Minas (Fig. 1.4; the lagoons east of Isla Margarita and southwest of Isla Largo Remo). The length of heavily oiled coastline was approximately 82 km (straight-line distance = 11 km) and included more than 1,000 ha of mangroves (Duke and Pinzón, Chap. 8) and extensive intertidal reef flats and

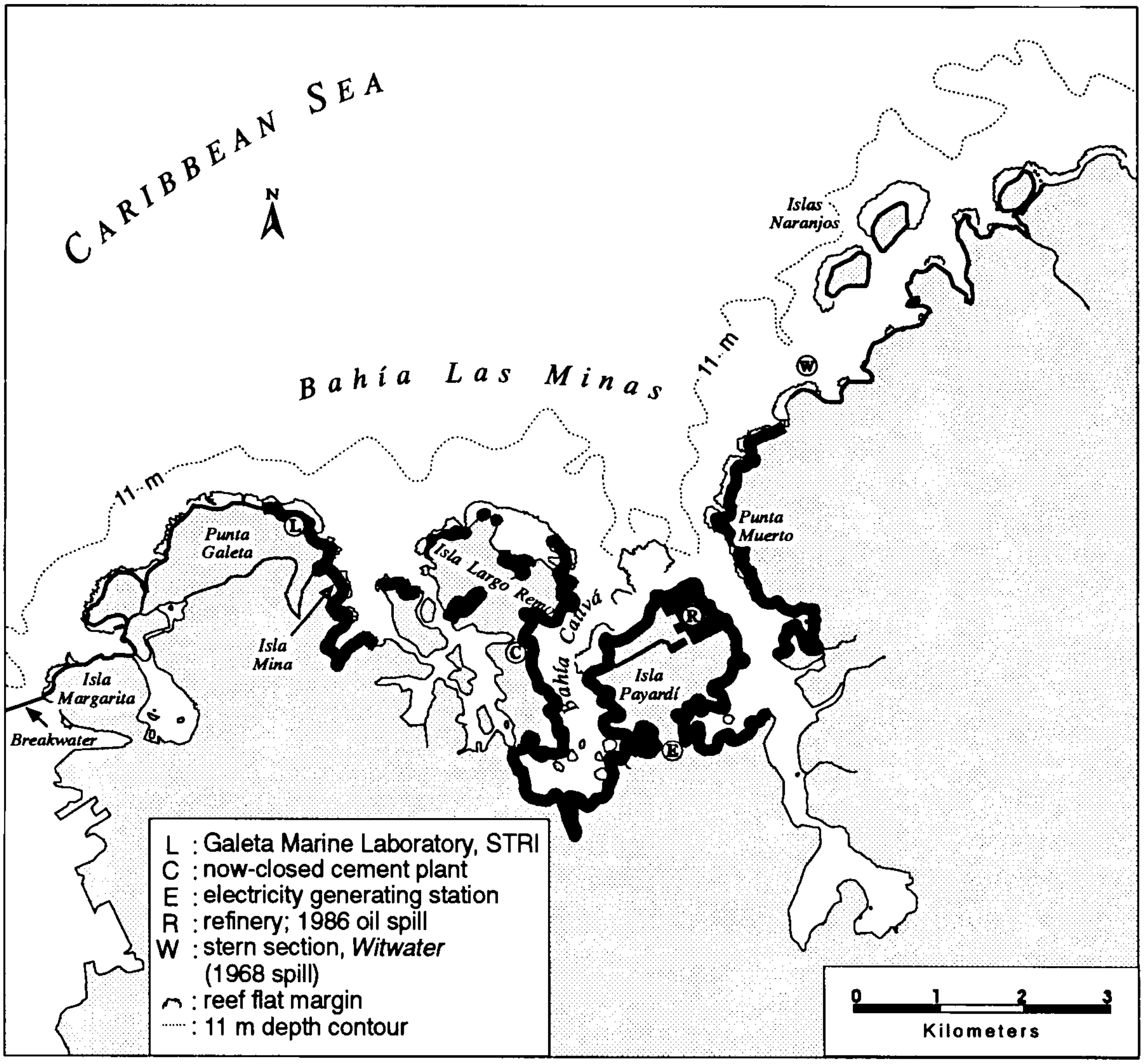

Fig. 1.4 Map of Bahía Las Minas depicting the approximate distribution of heaviest oiling (*heavy line*) and moderate oiling (*medium line*). Oiling was not observed in the embayments east of Isla Margarita, southwest of Isla Largo Remo, and southeast of Isla Payardí.

subtidal reefs. Only a few patches of oil were observed to strand east of María Chiquita and west of the entrance to the Panama Canal (Fig. 1.2). However, oily sheens were observed offshore from Isla Margarita to Nombre de Dios, the eastern limit of aerial surveys (Cubit et al. 1987). Approximately one month after the spill, oily sheen was observed offshore of Punta San Blas (J. Karr, pers. comm.). The offshore slicks appeared to be transported by the easterly coastal current, aided by an unusual period of southerly winds (J. Cubit, pers. obs.). Instances of strandings of black slicks occurred northeast of Portobelo (H. Caffey and A. Velarde, pers.

comm.) where generally prevailing northeasterly winds were likely to deposit slicks coming from far offshore.

In similar habitats within the heavily polluted area apparent degrees of oiling were highly variable. Probable causes of this heterogeneity included distance from the refinery, directions of movement of the spilled oil, and water depth. The greatest amounts of oil in mangroves, reef flats, and seagrass beds occurred within a few kilometers of the refinery. There was obviously less oil in these habitats at Islas Naranjos and Isla Margarita (Fig. 1.4). Large differences in visible oiling also occurred on a much smaller scale of a few hundred meters, depending on coastal orientation. Much of the oil escaping from Bahía Cativá spread to the west. Accordingly, coasts that faced north to northeast were much more heavily oiled than coasts that faced west or south. Furthermore, seasonal low tides (Cubit et al. 1986, 1988*a*, 1989) occurred between 10 and 19 May 1986, causing oil to accumulate along the seaward margins of reef flats (Cubit and Levings, Chap. 2; Cubit and Connor, Chap. 4). As a result, visible oiling was heaviest in intertidal habitats just above mean low water, such as mangrove roots and associated sediments, reef flat seagrass beds, coral rock, and beaches.

Chemical analyses of petroleum hydrocarbons in surface sediments generally verified these visual assessments of variability in degrees of oiling (Burns, Chap. 3). Samples collected five months after the spill contained concentrations of oil as high as 372,856 μg oil/g sediment and as low as 1,830 μg/g in heavily oiled mangrove surface sediments. Subtidally, surface sediments from heavily oiled seagrass beds ranged from 97 to 24,555 μg/g, and 19 to 715 μg/g at heavily oiled coral reefs. There was considerable variability in concentrations of oil among samples at a given site, confirming observations of small-scale patchiness as well.

Several different procedures were used to clean up the spilled oil. Some oil was removed from the sea using "skimmers" and shore-based pump trucks. As noted above, approximately 9.6 million L (60,000 bbl) of oil were recovered. Channels were dug through mangroves, apparently to drain oil from these areas. However, these channels appeared to increase the movement of oil beyond the seaward mangrove fringe to inner areas, as well (J. Cubit, pers. obs.). Disturbance from workers crushed windrows and may have increased subsequent erosion. In other areas oiled rocks, rubble, and debris were physically removed and seawater was sprayed onto sandy areas (Garrity et al., Chap. 5). Skimming and pumping floating oil appeared to be effective ways to recover oil from this kind of shallow-water spill. Shallow water and mangroves impeded many of the kinds of cleanup operations deployed after major oil spills, perhaps for the better, because some of these procedures can be environmentally or biologically destructive (NRC 1985; Houghton et al. 1991).

During the five years since the spill oil slicks have been regularly observed above coral reefs at Bahía Las Minas (Guzmán et al., Chap. 7) and along the mangrove fringe (Garrity and Levings, Chap. 9). The appearance of these slicks ranged from metallic sheens to brown patches. Slicks appeared to originate mainly from fringing mangroves, where much of the spilled oil washed ashore. As dead mangrove trees (*Rhizophora mangle*) decayed, the wooden physical structure disappeared, followed by erosion of oiled sediments. *Rhizophora* seedlings (survivors, recruits, and planted individuals) apparently have not prevented this erosion. Some slicks also appeared to come from oiled landfill beneath the refinery.

1.6 Other Human Disturbances in the Study Area

1.6.1 Coastal Development

Much of the study area has a long history of exposure to various kinds and degrees of human disturbance and pollution. More than a century ago construction of the Panama Canal and the city of Colón started, followed by decades of excavation, dredging, land filling, and erosion (McCullough 1977). Swamps were drained and sprayed for control of mosquitoes starting early this century (Curry 1925).

On the mainland there has been extensive deforestation for more than 30 years (Heckadon 1985), resulting in increased erosion, increasing siltation stress over the last 15 years (S. Garrity, pers. obs.), and heavy deposition of sediments on coral reefs (H. Guzmán, pers. obs.; Guzmán et al., Chap. 7).

1.6.2 Petroleum Refinery and Other Industrialization

During construction of a petroleum refinery at Bahía Las Minas (started in 1956), more than 4 million m^3 of fossil and living coral reef were excavated for landfill from areas totaling more than 15 ha (estimated from aerial photographs; see Duke and Pinzón, Chap. 8). The refinery started operating in 1961. Other industrialization at Bahía Las Minas includes an electricity generating station and a now-closed cement plant (Fig. 1.4). The cement plant operated from 1967 until 1975, and its small port facility is still in use.

1.6.3 1968 Tanker *Witwater* Spill

In 1968 the breakup of the tanker *Witwater* caused a spill of approximately 3.2 million L (20,000 bbl) of diesel oil and Bunker C at Bahía Las Minas (Rützler and Sterrer 1970; Birkeland et al. 1976; see Duke and Pinzón, Chap. 8). Observations of ecological effects of the spill were mostly qualitative or *ex post facto* because the Galeta Marine Laboratory was just being established.

1.6.4 Oil Spills Since the 1986 Refinery Spill

Since the 1986 refinery spill a few small oil spills have occurred in the study area. These included a small diesel spill near the Toro Point control site of the reef flat gastropod study in May 1988 (Garrity et al., Chap. 5), and fuel-oil spills in December 1988 and June 1990 from the electricity generating station at the mainland connection of Isla Payardí (Fig. 1.4). The June 1990 spill was followed by the death of some mangrove seedlings and an increased frequency of coral injuries (Guzmán et al., Chap. 7).

Also, occasional small spills occur at the port facility of the refinery, and some oil slicks and tar balls have washed ashore from offshore shipping. Chemical analyses enable most of these sources of oil contamination to be differentiated (Burns, Chap. 3). We have no information about the frequency or volume of such small spills before this study began.

1.7 Biological Studies Prior to the 1986 Oil Spill

1.7.1 Punta Galeta Reef Flat

Measurements of percent cover of plants and sessile invertebrates and counts of sea urchins constituted the longest-term biological baseline data available in the region. Monitoring of some parameters started as early as 1970, 16 yr prior to the spill. These data documented natural variability of populations in this habitat and allowed analyses of postspill changes through time and across space at Punta Galeta (Cubit and Connor, Chap. 4). No other sites were monitored prior to the spill.

1.7.2 1985 Coral Reef Survey

During 1985, the year before the spill, coral reefs were surveyed along the central Caribbean coast of Panama. Two of these reefs were in the region of the oil spill and provided the basis for a prespill-postspill comparison of percent cover for one reef that was heavily oiled and one reef that was moderately oiled. In addition, four similar reefs east of Bahía Las Minas were unoiled, thereby providing a form of control in evaluating effects of the spill (Jackson et al. 1989; Guzmán et al. 1991; Guzmán and Jackson 1991; Guzmán et al., Chap. 7 and App. Table C.1). These data and those used for retrospective analyses approximated an "optimal sampling design" for assessing effects of the spill, with data soon before and after the spill and at oiled and unoiled areas (Green 1979).

1.7.3 1979-1983 Reef Flat Stomatopod Studies

The population biology, ecology, and behavior of stomatopod crustaceans had been investigated for 5 yr at a number of reef flat sites near Punta Galeta (Steger and Caldwell, Chap. 6). These studies provided background data for examining populations after the oil spill, using two previously studied sites that were heavily oiled by the spill and two previously studied sites that were unoiled or lightly oiled. For some parameters and sites, prespill-postspill comparisons could be made.

1.7.4 Surveys of Epibiota of Mangrove Roots and Reef Flat Gastropods

In 1981 and 1982 surveys of epibiota attached to mangrove prop roots were conducted in three habitats: open coast, channel and lagoon, and drainage stream (Garrity and Levings, Chap. 9). Also, the gastropods at a reef flat near Punta Galeta were surveyed during 1982-1983 (Garrity et al., Chap. 5). These surveys provided data about community composition and population density prior to the spill that could be used in prespill-postspill comparisons.

1.7.5 Other Studies

Since 1968, ecological and biological investigations in the region of the 1986 oil spill have resulted in more than 130 publications (see bibliography in Cubit et al. 1988*b*). Detailed long-term data on hydrographic and meteorological conditions at Punta Galeta were also collected (Cubit et al. 1988*a*, 1989). Such extensive prespill information is rarely available in studies of the effects of oil spills.

1.7.6 Retrospective Analyses

Two additional approaches were employed to obtain prespill data: analyses of growth bands in coral skeletons (Guzmán et al., Chap. 7) and of aerial photographs of mangrove forests (Duke and Pinzón, Chap. 8). Both approaches add to our understanding of the history of the region and any biotic changes prior to the spill, and allow an examination of effects of the 1968 *Witwater* spill, which was never studied extensively (see Rützler and Sterrer 1970; Birkeland et al. 1976).

1.8 Incorporation of Available Studies in Sampling Designs

1.8.1 Some Principles of Sampling Design

An optimal design for assessing effects of a planned environmental change, such as initiation of the release of heated effluents into a body of water, consists of baseline data from both an affected and unaffected area (*main sequence 1* of Green 1979; Table 1.3; but see Hurlbert 1984; Stewart-Oaten et al. 1986; Underwood 1991). This design provides data both on variation through time and across space. Although this kind of design can be employed for a planned environmental alteration, assessments of unplanned events, such as the oil spill at Bahía Las Minas, generally must be made using suboptimal sampling designs, in which either variation through time or across space is analyzed. Both of these suboptimal designs result in data with possible confounding effects of variation that was not caused by the spill. Because some biological effects of major oil spills are far from subtle (see review by Teal and Howarth 1984), it may be possible to determine whether the spill was the cause of observed changes despite the possible confounding. Other biological effects may be too subtle to assess using suboptimal designs.

Table 1.3 Categories of environmental studies (from Green 1979).

Criteria			
1. Has the impact already occurred?	2. Is "when and where" known?	3. Is there a control area?	Main Sequence and Description
No	Yes	Yes	1. Permits an optimal impact study design.
No	Yes	No	2. Impact must be inferred from temporal change alone.
No	No	–	3. Baseline or monitoring study.
Yes	Yes	–	4. Impact must be inferred from spatial pattern alone.
Yes	No	–	5. "When and where" is the question.

If only one area was monitored prior to a spill and this area became oiled, long-term data may allow observed postspill changes to be ascribed to the spill (*main sequence 2* of Green 1979; Table 1.3). In this kind of temporal-variation design, determining whether postspill data are extraordinary depends on the history of variation at the site. Parallel patterns in the distributions of oiling and of biological changes, if present, greatly strengthen assigning cause and effect to the spill (Cubit and Connor, Chap. 4). Some spills may be associated with extreme environmental conditions, such as a storm causing the breakup of an oil tanker. In such cases, it may not be possible to distinguish effects of oiling from effects of the extreme conditions. Baseline data on the physical environment are necessary for the interpretation of biological variation through time.

The establishment of oiled and unoiled study sites after an oil spill can also provide useful, though confounded, data regarding biological effects, particularly if data collection starts immediately after the spill (*main sequence 4* of Green 1979; Table 1.3). In this kind of spatial-variation design it is not known whether preexisting differences between oiled and unoiled areas may confound ascribing biological variation between areas to the spill. Convergence through time of measurements at oiled and unoiled sites may indicate that the areas were similar before the spill and increase confidence that, if differences were observed soon after the spill, they were caused by oiling.

In a "hybrid" design, some sites were investigated prior to a spill (*main sequence 1* of Green 1979; Table 1.3), but the distribution of oiling left very different numbers of oiled and unoiled sites or all sites in one category of oiling. In this case, sites may be added to balance postspill designs and analyses; separate prespill-postspill analyses can also be conducted using the available data.

Issues of sampling units, replication, and pseudoreplication in ecological assessments are far from simple (e.g., Hurlbert 1984; Stewart-Oaten et al. 1986; Carney 1987; Underwood 1991). Primary considerations are appropriate spatial and temporal scales, which depend on many characteristics of the environment and organisms being investigated (e.g., NRC 1990; Goldsmith 1991). Sites within study areas, samples within sites, subsamples, and so on should be distributed spatially in such a way that assumptions of inferential statistics are met. Similarly, frequency of sampling should satisfy the criteria of repeated-measures analyses.

1.8.2 Statistical Analyses and Sampling Designs: General Considerations

There are two main features of sampling designs and analyses of data generated by long-term assessments of oil spills. First is the use of repeated-measures analyses, which are appropriate for monitoring the same sites through time. Sampling designs should employ fixed sampling units to eliminate confounding spatial and temporal variation, rather than randomly positioning sampling units at each survey (but see Hellawell 1991). Also, monitoring individual plants and sessile animals in fixed sampling units can produce data on survival and growth, which may contribute to a better understanding of ecological processes than data that did not come from monitoring individuals.

Second is the use of nested replication. Because there are only two or three kinds of areas (oiled, unoiled, and, in some cases, an intermediate category), all sampling units (sites, transects within sites, quadrats within transects, and so on) are nested. A key determination in such analyses is the level of nesting to test the significance of possible oiling effects.

Confidence in assigning "cause-and-effect" to oil contamination may be greatly strengthened if significant correlations exist between the concentration of oil in the environment or tissues of organisms and a response variable such as abundance, growth, injury, or reproduction. Also, a significant oiling x time interaction in repeated-measures analysis of variance (ANOVA) is statistical evidence for an effect related to the oil spill.

1.8.3 Subtidal Reef Corals

A survey of six reefs the year prior to the spill enabled an analysis of coral cover before and after the spill at oiled and unoiled reefs (Jackson et al. 1989; Guzmán et al. 1991; Guzmán and Jackson 1991; Guzmán et al., Chap. 7). Because only one reef was heavily oiled and only one moderately oiled, five additional heavily oiled reefs and one additional moderately oiled reef were included in postspill surveys. Because of this imbalance, some statistical analyses and the design of some postspill coral studies employed a balanced subset of sites, i.e., four oiled and four unoiled. As a result of this "hybrid" design certain statistical analyses employed the six reefs surveyed both before and after the spill (*main sequence 1* of Green 1979; Table 1.3), others employed the 12 reefs surveyed postspill (which included the six reefs in the prespill survey; *main sequence 4* of Green 1979), and a final type of analyses used a balanced subset of the 12 reefs. *Main sequence 1* also applied to analyses of coral growth using sclerochronology.

1.8.4 Reef Flat Sessile Biota, Algal-turf Infauna, and Sea Urchins

Monitoring of reef flat sessile biota and sea urchins started as long ago as 1970 at Punta Galeta (Cubit and Connor, Chap. 4). After the 1986 spill three additional sites were established, one oiled and two unoiled, and monitoring of sessile biota and sea urchins focused on the reef-edge zone, where effects of oiling had been most severe. Monitoring of infauna of algal turf was initiated postspill at all four sites. This study also was a "hybrid" design, with long-term, prespill-postspill analyses of sessile biota and sea urchins conducted for Punta Galeta (*main sequence 2* of Green 1979; Table 1.3) and postspill analyses of all three data sets for all four sites (*main sequence 4* of Green 1979). Because of the long-term, detailed biological and physical data across zones of the Punta Galeta reef flat and the spatial pattern of oiling of this habitat, the primary kind of data analysis for effects of the spill compared zones at this site at particular surveys (Cubit and Connor, Chap. 4).

1.8.5 Mangrove Forests

Monitoring of mangrove forests (Duke and Pinzón, Chap. 8) did not start until 3 yr after the oil spill, and the design of this study followed the design of the ongoing study of the mangrove fringe and epibiota of mangrove roots (Sect. 1.8.7; *main sequence 4* of Green 1979). Despite the lack of prespill monitoring, aerial photography of the region enabled an analysis of general patterns of vegetation before and after both the 1968 *Witwater* spill and the 1986 refinery spill (*main sequence 1* of Green 1979).

1.8.6 Reef Flat Stomatopods

Four of the reef flats studied from 1979-1983 were used in postspill monitoring; two were heavily oiled and two were lightly oiled to unoiled (Steger and Caldwell, Chap. 6). Because of a widespread recruitment failure not previously observed, two unoiled sites outside of Bahía Las Minas were added to the study a few years after the spill to document the spatial scale of the stomatopod recruitment failure. Data analyses also were "hybrid" (*main sequences 1*, *2*, and *4* of Green 1979; Table 1.3) because not all types of data had been collected at all sites prior to the spill and two sites were added postspill.

1.8.7 Mangrove Fringe and Epibiota of Mangrove Roots

Five sites surveyed in 1981 and 1982 were initially retained in postspill sampling; one of these sites was unoiled soon after the spill but became oiled 2 yr afterward, and monitoring of another site ended a year after the spill (Garrity and Levings, Chap. 9). To achieve adequate replication of oiled and unoiled conditions in the three habitats, additional sites were incorporated in the design of postspill monitoring. A year after the spill some new sites were selected to increase distance between them, sample the oiled area more representatively, and avoid pseudoreplication within areas. Data on long-term community composition were not compared statistically prespill and postspill due to a 4-yr gap in sampling and slightly different sampling methods. Collection of additional kinds of data was initiated postspill, and statistical analyses of all data sets compared oiled and unoiled sites postspill (*main sequence 4* of Green 1979; Table 1.3).

1.8.8 Reef Flat Gastropods

Reef flat gastropods were surveyed at one site during 1982-1983 that was oiled in 1986. Postspill monitoring was conducted at this site and two unoiled sites (certain intertidal zones at each one) that were selected after the spill. Statistical analyses were not conducted because of the 4-yr gap in pre- and postspill sampling at the oiled site (which invalidated the assumption of approximately equal sampling intervals in repeated-measures ANOVA) and oiling of one of the unoiled sites 2 yr after the spill (Sect. 1.6.4).

1.8.9 Subtidal Seagrass Communities

All monitoring of seagrass beds was initiated after the spill and statistical analyses were based on spatial variation (*main sequence 4* of Green 1979; Table 1.3).

1.8.10 Hydrocarbon Chemistry

The first postspill collection of samples for chemical analysis was made at some of the sites that had been established for biological studies. Additional sites were sampled to obtain a broader coverage (Burns, Chap. 3). Subsequent collections were all made at biological study sites.

1.9 Distribution of Study Sites

Study sites were distributed from Toro Point, just west of the entrance to the Panama Canal, to Isla Grande, with a concentration of sites in and near Bahía Las Minas (Figs. 1.2 and 1.5). A list of all study sites, degree of oiling, and type of study conducted is given in Table 1.4.

1.10 Acknowledgments

Xenia Guerra, Gabriel Jácome, and Karl Kaufmann prepared the figures.

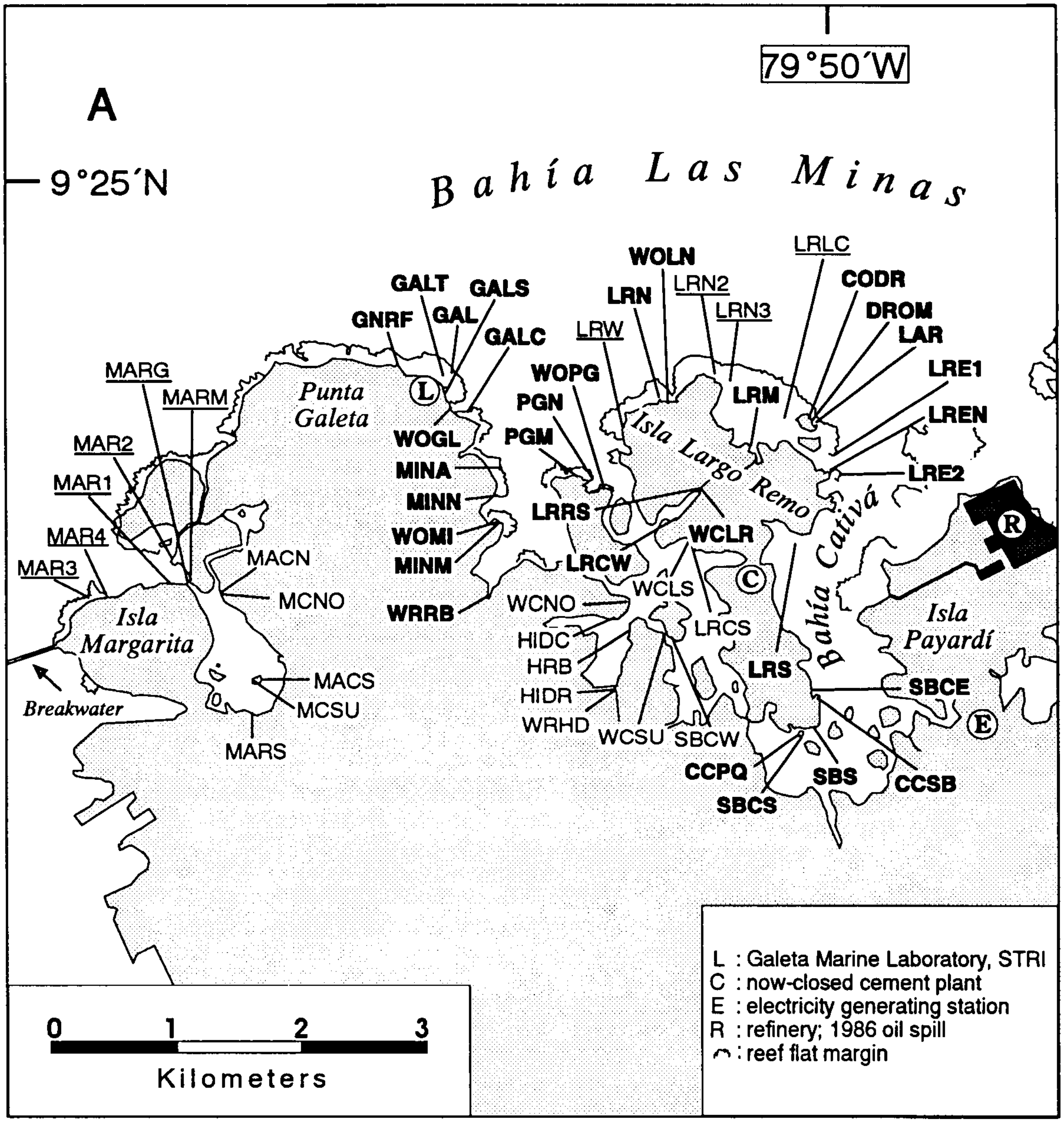

Fig. 1.5 Distribution of study sites. Bold: heavily oiled or oiled; underlined: moderately or lightly oiled; regular font: unoiled. *A*. Detail of Isla Margarita, Punta Galeta, and Isla Largo Remo. See Table 1.4 for a full list of study sites and Figure 1.2 for location in Panama.

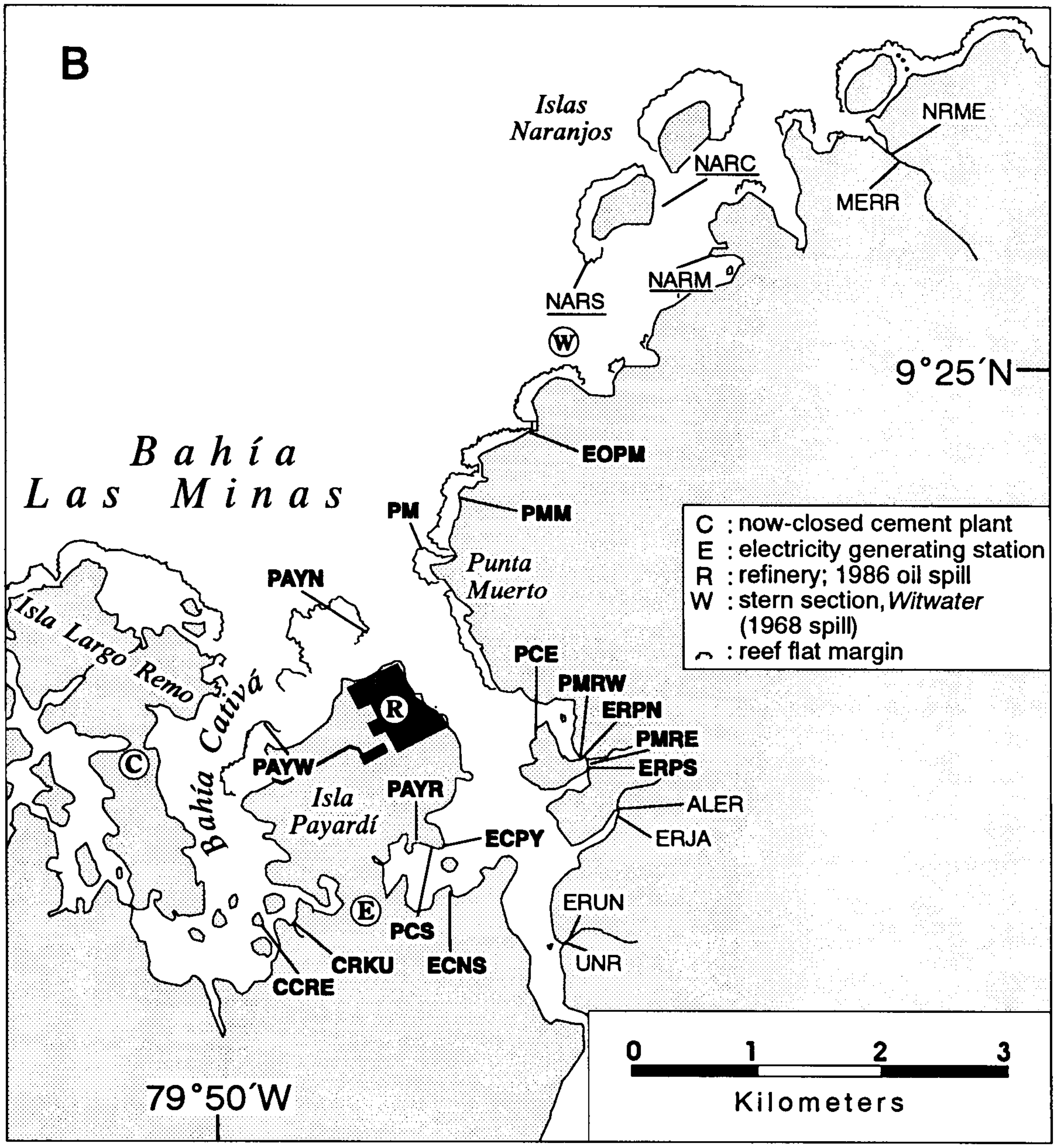

Fig. 1.5 Distribution of study sites (continued). *B*. Detail of Isla Payardí, Punta Muerto, and Islas Naranjos. Refer to caption of Figure 1.5*A* for further details.

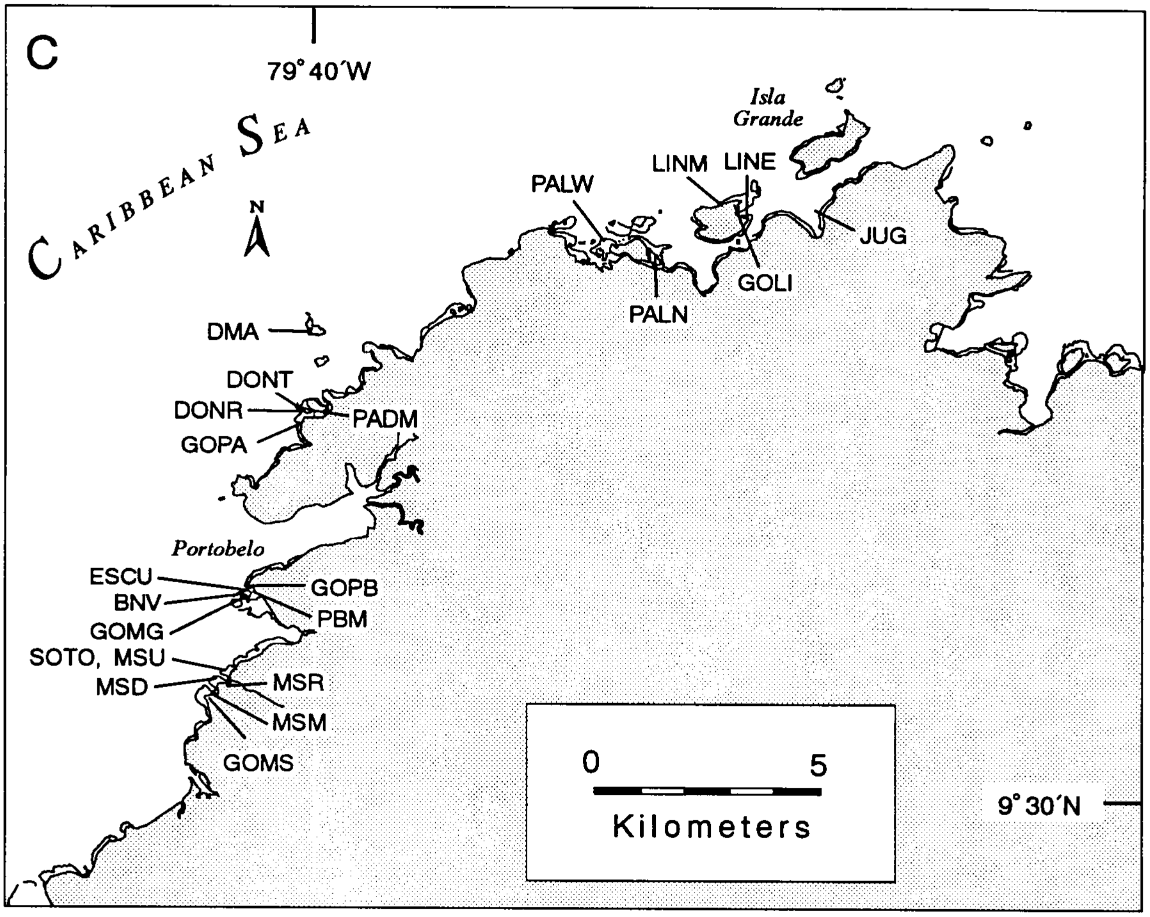

Fig. 1.5 Distribution of study sites (continued). *C.* Detail of unoiled sites northeast of Bahía Las Minas. Refer to caption of Figure 1.5*A* for further details.

Table 1.4 List of study sites (alphabetical by acronym), degree of oiling, and type of study conducted. Refer to Figures 1.2 and 1.5 for site locations.

	Acronym	Site Name	Degree of Oiling[1]	Type of Study[2]
1	ALER	Río Alejandro	U	MFE
2	BNV	Buenaventura	U	SSC
3	CCPQ	(Central, Channel) Pequeña	O	MF
4	CCRE	(Central, Channel) Reina	O	MF
5	CCSB	(Central, Channel) Samba Bonita	O	MF
6	CO3	Largo Remo Channel Site CO3	O	MFE (until May 1987)
7	CODR	(Central, Open) Droque	O	MF
8	CRKU	(Central, River) Kuna	O	MF
9	CSR	Río Coco Solo	U	MFE (until May 1987)
10	DMA	Dos Marias	U	SRC
11	DONR	Doncella Reef	U	SRC, HC86
12	DONT	Doncella *Thalassia*	U	SSC, HC86
13	DROM	Isla Droque Mangrove	O	MFE
14	ECNS	(Eastern, Channel) No Sé	O	MF
15	ECPY	(Eastern, Channel) Payardí	O	MF
16	EOPM	(Eastern, Open) Punta Muerto	O	MF
17	ERJA	(Eastern, River) Alejandro	U	MF
18	ERPN	(Eastern, River) Puerto Norte	O	MF
19	ERPS	(Eastern, River) Puerto Sur	O	MF
20	ERUN	(Eastern, River) Unnamed	U	MF
21	ESCU	Punta Escucha	U	RFS
22	GAL	Punta Galeta	O	RFB
23	GALC	Galeta Channel	H	SRC, HC86
24	GALM	Punta Galeta Mangrove	O	MFE (until August 1987)
25	GALS	Galeta Sand	O	HC86 (seawater)
26	GALT	Galeta *Thalassia*	H	HC86 (reef flat sediments)
27	GNRF	Galeta Navy Reef	O	RFG
28	GOLI	(Isla Grande, Open) Lintón	U	MF
29	GOMG	(Isla Grande, Open) Magoté Sur	U	MF
30	GOMS	(Isla Grande, Open) María Soto	U	MF
31	GOPA	(Isla Grande, Open) Padre	U	MF
32	GOPB	(Isla Grande, Open) Portobelo	U	MF
33	HIDC	Hidden Channel	U-O	MFE (oiled May/Aug. 1988)
34	HIDR	Hidden River	U	MFE
35	HRB	Hidden River Bay	U	HC86 (mangrove sediments)
36	JUG	Juan Gallego	U	SRC
37	LAR	Isla Largo Remo	O	RFB
38	LINE	Lintón East	U	SSC
39	LINM	Isla Lintón Mangrove	U	MFE
40	LRCS	Largo Remo Channel South	U	MFE
41	LRCW	Largo Remo Channel West	O	MFE
42	LRE1	Largo Remo East 1	H	SRC
43	LRE2	Largo Remo East 2	H	SRC, HC86
44	LREN	Largo Remo Entrance	H	SSC, HC86
45	LRLC	Largo Remo Lagoon Center	L	HC86 (seagrass sediments)

Table 1.4 List of study sites (alphabetical by acronym), degree of oiling, and type of study conducted (continued).

	Acronym	Site Name	Degree of Oiling[1]	Type of Study[2]
46	LRM	Largo Remo Mangrove	H	HC86 (mangrove sediments)
47	LRN	Isla Largo Remo North	H	RFS
48	LRN2	Largo Remo North 2	M	HC86 (seagrass sediments)
49	LRN3	Largo Remo North 3	L	HC86 (seagrass sediments)
50	LRRN	Largo Remo River North	O	MFE (until May 1987)
51	LRRS	Largo Remo River South	O	MFE
52	LRS	Largo Remo Sur	H	SSC, HC86
53	LRW	Isla Largo Remo West	L/U	RFS
54	MAR1	Margarita 1	M	HC86 (seagrass sediments)
55	MAR2	Margarita 2	M	HC86 (seagrass sediments)
56	MAR3	Margarita 3	M	SRC
57	MAR4	Isla Margarita	L/U	RFS
58	MACN	Margarita Channel North	U	MFE
59	MACS	Margarita Channel South	U	MFE
60	MARG	Margarita Grassbed	L	HC86 (seagrass sediments)
61	MARM	Margarita Mangrove	–	HC86 (mangrove sediments)
62	MARS	Margarita South	U	HC86 (mangrove sediments)
63	MCNO	Margarita Channel Norte	U	MF
64	MCSU	Margarita Channel Sur	U	MF
65	MERR	Quebrada Las Mercedes	U	MFE
66	MINA	Isla Mina	H	RFS
67	MINM	Isla Mina Mangrove	O	MFE
68	MINN	Mina North	H	SSC
69	MSD	María Soto Abajo	U	RFB
70	MSM	María Soto Mangrove	U	MFE
71	MSR	María Soto	U	RFG
72	MSU, SOTO	María Soto Arriba	U	RFB, RFS
73	NARC	Naranjos Channel	M	HC86 (seagrass sediments)
74	NARM	Naranjos Mangrove	L	HC86 (mangrove sediments)
75	NARS	Naranjos South	M	SRC, HC86
76	NRME	Naranjos (River) Las Mercedes	U	MF
77	PADM	Isla del Padre Mangrove	U	MFE
78	PALN	Palina North	U	SSC, HC86
79	PALW	Palina West	U	SRC, HC86
80	PAYN	Payardí North	H	SRC, HC86
81	PAYR	Payardí River	O	MFE
82	PAYW	Payardí West	H	SRC
83	PBM	Portobelo Mangrove	U	MFE
84	PCE	Payardí Channel East	O	MFE
85	PCS	Payardí Channel South	O	MFE
86	PGC	Peña Guapa Channel	O	MFE (until May 1987)
87	PGM	Peña Guapa Mangrove	O	MFE
88	PGN	Peña Guapa North	H	SSC
89	PM	Punta Muerto	H	SRC
90	PMM	Punta Muerto Mangrove	O	MFE

Table 1.4 List of study sites (alphabetical by acronym), degree of oiling, and type of study conducted (continued).

	Acronym	Site Name	Degree of Oiling[1]	Type of Study[2]
91	PMRE	Punta Muerto River East	O	MFE
92	PMRW	Punta Muerto River West	O	MFE
93	SBCE	Samba Bonita Channel East	O	MFE
94	SBCS	Samba Bonita Channel South	O	MFE
95	SBCW	Samba Bonita Channel West	U	MFE
96	SBS	Samba Bonita South	H	HC86 (mangrove sediments)
97	TPR	Toro Point	U	RFG
98	UNR	Unnamed River	U	MFE
99	WCLR	(Western, Channel) Largo Remo	O	MF
100	WCLS	(Western, Channel) Largo Remo Sur	U	MF
101	WCNO	(Western, Channel) Norte	U	MF
102	WCSU	(Western, Channel) Sur	U	MF
103	WOGL	(Western, Open) Galeta	O	MF
104	WOLN	(Western, Open) Largo Remo Norte	O	MF
105	WOMI	(Western, Open) Mina	O	MF
106	WOPG	(Western, Open) Peña Guapa	O	MF
107	WRHD	(Western, River) Hidden	U	MF
108	WRRB	(Western, River) Rabinowitz	O	MF

[1] Degree of oiling, by visual assessment. *H*: heavily oiled; *O*: oiled; *M*: moderately oiled; *L*: lightly oiled; *L/U*: lightly oiled to unoiled; *U*: unoiled.

[2] *HC86*: Hydrocarbon chemistry, 1986 sample collection, Chapter 3 (refer to Chap. 3 for sites sampled in 1988/89 and 1990).
MF: Mangrove forests, Chapter 8.
MFE: Mangrove fringe and the epibiota of mangrove roots, Chapter 9.
RFB: Reef flat sessile biota, algal-turf infauna, and sea urchins, Chapter 4.
RFG: Reef flat gastropods, Chapter 5.
RFS: Reef flat stomatopods, Chapter 6.
SRC: Subtidal reef corals, Chapter 7.
SSC: Subtidal seagrass communities, Chapter 10.

2

Weather, Sea Conditions, and Topography Affecting Oil Deposition During the 1986 Bahía Las Minas Oil Spill

John D. Cubit and Sally C. Levings

2.1 Abstract

In April 1986 a ruptured refinery tank spilled at least 60,000-100,000 bbl of medium-weight crude oil into the largest complex of reefs, mangroves, and seagrass beds on the central Caribbean coast of Panama. The Smithsonian Tropical Research Institute has maintained an environmental monitoring program in this area since the early 1970s. Movement and deposition of oil slicks were examined using aerial and ground observations, hydrographic and meteorological data from the monitoring program, and freshwater runoff modeling. Results indicated that the spatial pattern of oiling (and therefore the habitats and biota affected) were dependent on weather conditions at the time of the spill and would have been predictably different in other seasons. Patterns of oil deposition during the 1968 tanker *Witwater* spill were hindcast and found to be consistent with evidence for damage to reef flat biota and mangroves.

2.2 Introduction

When the Bahía Las Minas oil spill washed into the long-term ecological research site of the Smithsonian's Galeta Marine Laboratory, it became the most scrutinized oil spill in the tropics. From daily observations at this site it was apparent that spatial patterns of oil deposition were largely governed by weather and sea conditions, which in turn determined spatial patterns of biological damage in mangrove forests, reef flats, and other intertidal and subtidal habitats (Cubit et al. 1987; Jackson et al. 1989).

In constructing environmental impact studies and planning responses to oil spills, case studies are regularly used to estimate possible effects. As a measure of potential effects of other spills of similar oil, the results of the Bahía Las Minas spill must be qualified with the range of weather and sea conditions that could be expected during the whole year. To put the biological studies of the 1986 Bahía Las Minas oil spill in a context broader than the particular physical conditions during the spill, physical processes determining the pattern of oiling must be taken into account.

Weather and sea conditions on this coast have received considerable study and are known to be highly seasonal and variable from year to year (for recent summaries see Cubit et al. 1986, 1989). To examine possible effects of variations in weather and sea conditions on oil deposition in two kinds of coastal habitats in Bahía

Las Minas, we examine two factors transporting oil into them: (1) winds and waves moving oil onto the outer coast of broad, shallow fringing reef platforms, sand forests, and mangroves, and (2) freshwater runoff opposing the movement of oil into the stream channels of mangrove forests. We describe these conditions during the 1986 Bahía Las Minas oil spill and compare them with those in other months of the year and in other years, including the year of the 1968 tanker *Witwater* spill of approximately 20,000 bbl of diesel oil and Bunker C oil on this coast. On the basis of these comparisons, we predict the differences in oil deposition that would be produced by other combinations of weather and sea conditions.

2.2.1 Biological Communities and Physical Structure of the Coastline

The oil spill occurred on a coastline consisting of coral reefs, seagrass meadows, sand beaches, sand forests, small estuaries, and mangrove forests. This is the largest complex of mangroves and reefs on the central Caribbean coast of Panama. The outer coast in the region of the oil spill consists primarily of fringing reefs with extensive, periodically drying (intertidal) reef flats (Macintyre and Glynn 1976; Fig. 2.1). Going from seaward to landward, the reefs begin with a forereef slope of hard substrata covered predominantly by algae, and secondarily by corals and other sessile invertebrates (environment *a*; see Guzmán et al., Chap. 7). Where the forereef slope rises into the intertidal zone, it becomes part of the reef flat. Most reef flats on this coast have a raised outer crest of hard carbonate pavement deposited by crustose coralline algae, shown by the dark shaded structure in Figure 2.1 (environment *b*; Macintyre and Glynn 1976; see also Cubit and Connor, Chap. 4). In the study areas this reef crest is approximately at the level of mean low tide and is covered with a mixture of calcareous and fleshy algae. Landward of the reef crest is a mixture of coral bench, loose coral rubble, and loose sediments (environment *c*). Sediments usually become progressively finer toward the back reef (environment *d*). Seagrasses (mostly *Thalassia testudinum*) and rhizophytic green algae (mostly *Penicillus capitatus* and *Halimeda* spp.) grow in loose sediments of this interior portion of the reef flat, and various other algae grow epiphytically on seagrasses and attached to hard substrata of the exposed coral bench and coral rubble. Most of these sediments are produced by calcareous algae and invertebrates, and sediments are accumulated and held together by seagrasses, rhizophytic algae, and mats of attached algae.

Berms or beaches of sand often form on back-reef flats (environment *e*). Mangroves, mostly the red mangrove *Rhizophora mangle*, colonize intertidal levels of these sand deposits forming the seaward fringe of mangrove forests (environment *f*). On these elevated areas of sand, forests of other woody and herbaceous plants, including coconut palms (*Cocos nucifera*) and sea grape (*Cocolobo* sp.), grow just above the level of mean high tide. In intertidal areas behind beaches, mangroves continue landward and cover intertidal substrata (Fig. 2.1). Mangroves line the margins of freshwater streams where these streams enter the range of the tides.

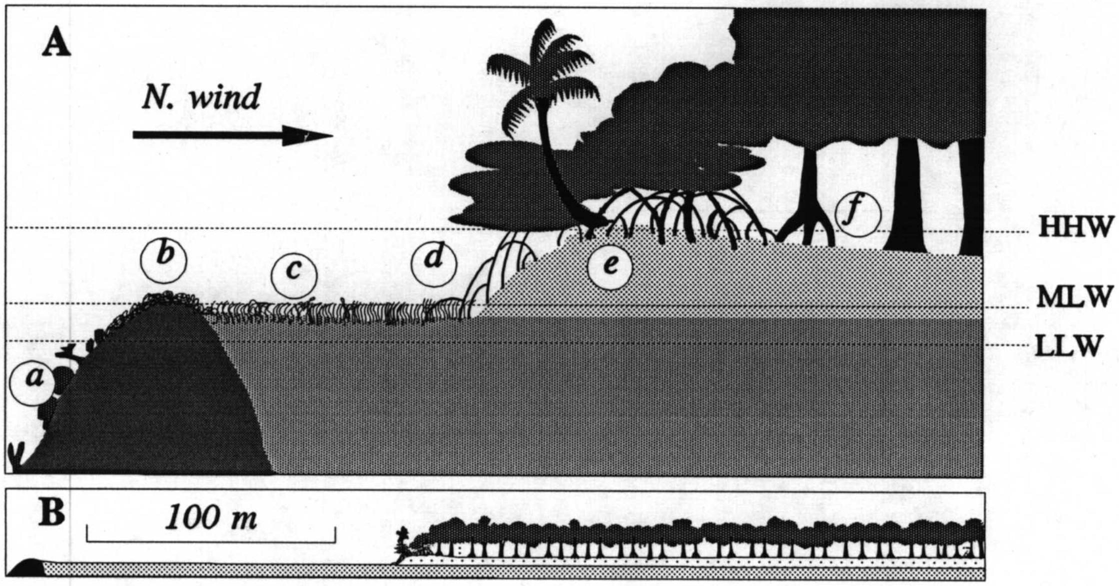

Fig. 2.1 Schematic cross sections of the wave-exposed outer coast of the central Caribbean coast of Panama. The prevailing wind is from the north. This shoreline consists of extensive, nearly flat habitats on a coral reef foundation (Macintyre and Glynn 1976). *A.* Cross section is compressed horizontally to show the principal habitats and benthic communities proceeding from seaward to landward: *a.* forereef slope of hard substrata covered by algae, corals, and other sessile invertebrates; *b.* elevated reef crest of hard substrata covered by algal turfs, crustose coralline algae, and sessile invertebrates; *c.* interior reef flat of coral bench, loose coral rubble, and sand covered by seagrasses, rhizophytic (root-forming) algae, and attached algae; *d.* back-reef flat of fine sediments covered by seagrasses and rhizophytic algae; *e.* beaches and berms of loose sediment supporting the seaward fringe of red mangroves (*Rhizophora mangle*) and sand forests covered by coconut palms, sea-grape, and other woody and herbaceous plants; and *f.* interior mangrove forests with coarse to fine substrata supporting primarily red, black, and white mangroves (*R. mangle, Avicennia germinans*, and *Laguncularia racemosa*). In this diagram the reef flat habitat corresponds to the area between the lowest low water level on the seaward side of the reef and beach or mangroves on the landward side. The *dotted lines* show approximate water levels: (*HHW*) the highest high water level produced by combinations of factors such as storm surges, waves, and seasonally high water levels; (*MLW*) mean low water level (i.e., the average level of low tides); (*LLW*) the lowest low water level produced by combinations of factors, including extreme low tides and seasonally low water levels. *B.* Cross section is shown to the approximately correct horizontal scale. The extensive reef flat is backed by beach, sand forest, and mangroves. Most of the mineral substrata in these habitats are produced by marine organisms. The hard substrata are constructed in place, mostly by crustose coralline algae and corals; the soft substrata consist of fragments of calcareous green algae (e.g., *Halimeda opuntia* and *Penicillus capitatus*), coralline (red) algae, corals, and other calcareous organisms.

2.2.2 Patterns of Weather and Sea Conditions in the Region Affected by the 1986 Oil Spill

Weather and sea conditions on this coast vary according to dry- and wet-season alternations (Figs. 2.2, 2.3), which are common in low-latitude tropics (Glynn 1972; Cubit et al. 1986, 1989). The dry season (roughly December to April) is

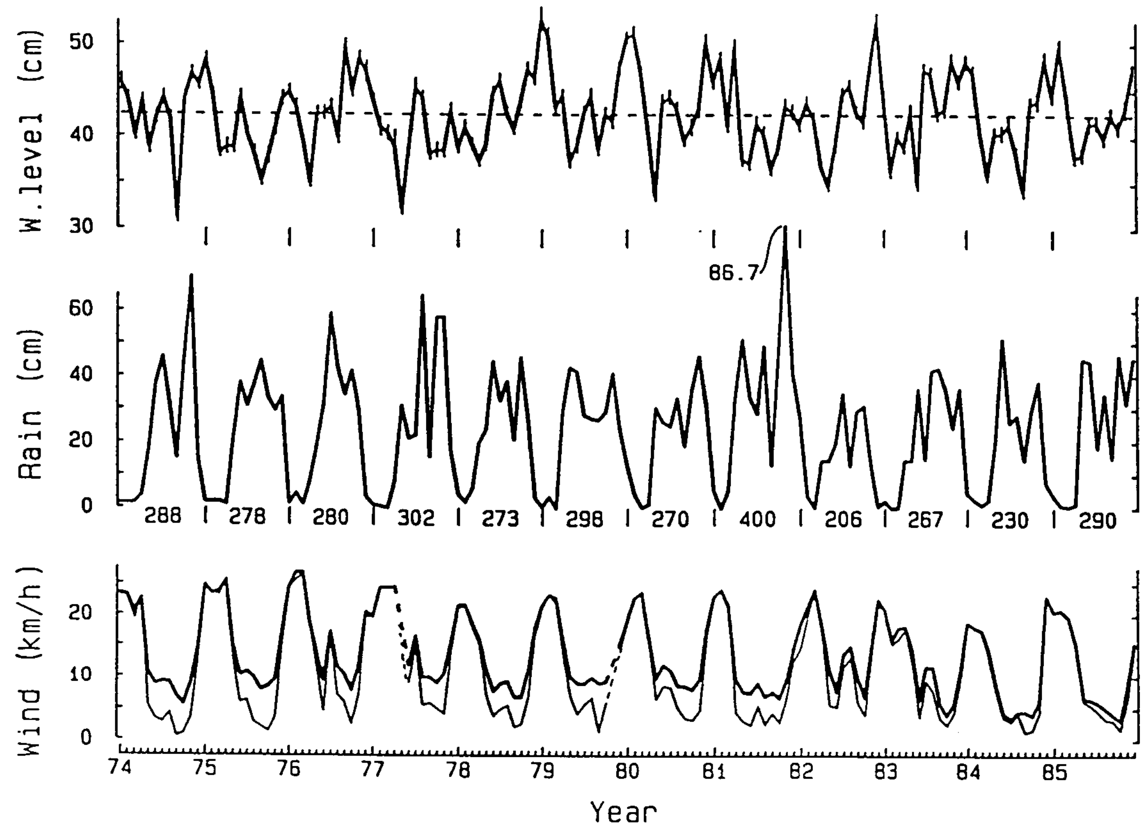

Fig. 2.2 Monthly water level, rainfall, and wind speed above the Punta Galeta reef flat. Water levels are monthly means with 95% confidence intervals; the *dashed line* is the mean water level for the 12-yr period. Heights are above the Punta Galeta datum. Rainfall values are monthly totals, with the yearly totals printed below. Wind speeds are monthly means; 95% confidence intervals (not shown) are within 1 km/h of the means. The *heavy line* connects the means of wind speed contributed by northerly winds (compass direction ≥292° and ≤68°). (From Cubit et al. 1989, with permission)

characterized by low rainfall, strong onshore (northerly) trade winds, and high mean water levels over reef flats (Glynn 1972; Cubit et al. 1986, 1989). During the wet season (roughly May to November) winds are variable (but predominantly onshore), mean water levels are lower, and rainfall ranges from 2,000 to 4,000 mm. In most years, a "little dry season," with strong onshore winds and little rain interrupts the wet season between July and October.

This coast has a microtidal regime; the mean diurnal range of the tides is approximately 30 cm. Actual water levels on these exposed windward coasts are determined by a combination of factors in addition to tidal fluctuations, including seasonal variations in mean sea level, wind stress, and wave buildup over reef flats (Cubit et al. 1986, 1989). Strong onshore trade winds of the dry season increase the amplitude of waves breaking onto fringing reef flats. The breaking waves raise water

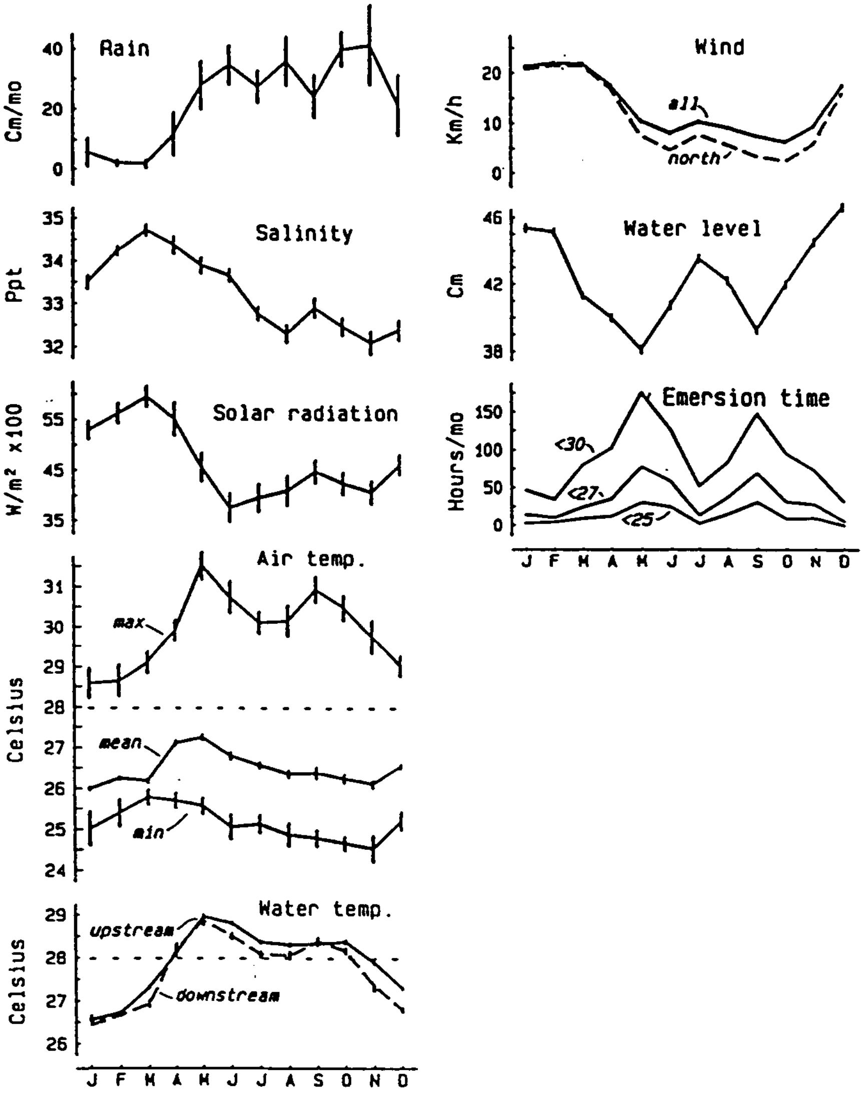

Fig. 2.3 Seasonal patterns of hydrographic and meteorological conditions at Punta Galeta. In all graphs the vertical bars are 95% confidence intervals around monthly means calculated from the following types of data: wind speed, water level, emersion time, mean air temperature, and water temperature are hourly means; rainfall values are monthly totals; salinity and maximum-minimum air temperatures are daily readings; solar radiation is W/m^2/d. See Cubit et al. (1989) for periods of data collection, which varied for different parameters between January 1974 and December 1985, and further details. Water levels are heights above the Punta Galeta datum. Exposure times are mean monthly totals for substrata at three elevations (cm) above the datum level. *Dashed lines* in the temperature graphs show the long-term mean of upstream water temperature. The dry season usually occurs December-April, and the wet season May-November. (From Cubit et al. 1989, with permission)

levels over reef flats and drive strong currents across them (Cubit 1985; Cubit et al. 1986, 1989). This water flow exits through channels in reef flats, through mangrove forests behind reef flats, or both.

The watershed for the area affected by the oil spill is a relatively narrow strip extending less than 10 km inland. Numerous small streams, but no large rivers, drain into the region of Bahía Las Minas (Instituto Geográfico Nacional "Tommy Guardia" 1988).

2.2.3 Observations of Oil Movement and Deposition

The 1986 Bahía Las Minas oil spill occurred on 27 April 1986 when a storage tank ruptured at the oil refinery on Isla Payardí (Fig. 1.4). Judging from the amount of oil recovered and the probable losses to evaporation, dissipation, and deposition in sediments, at least 60,000 to 100,000 bbl of medium-weight crude oil spilled into the sea. For 6 d most of the oil was contained within Bahía Cativá, which is bounded by Largo Remo Island, Samba Bonita Island, the town of Cativá, and Payardí Island. On 4 May 1986 refinery personnel reported that rainfall and shifting winds moved a large quantity of oil out to sea, where it could not be controlled (R. Morales, pers. comm.). The oil type was 70% Venezuelan Crude and 30% Mexican Isthmus Crude. The specific gravity of the blend was 27° API at 15.6°C (= about 0.89 gm/cm^3; R. Morales, pers. comm.).

Daily observations of oil movement onto the outer coast in Bahía Las Minas were made by J. Cubit beginning 9 May 1986, when the first large oil slicks arrived at Punta Galeta. Onshore winds drove accumulations of oil onto beaches and reef flats, concentrating the oil in small embayments, inlets, and lagoons. In subsequent surveys on foot, by car, and from low-flying aircraft, the most heavily oiled shoreline was between Isla Margarita and Punta Muerto (Cubit et al. 1987; Jackson et al. 1989; J. Cubit, pers. obs.; H. Caffey and A. Velarde, pers. comm.). In surveys between Isla Margarita and María Chiquita, the only areas that did not appear oiled were in upper reaches of very sheltered embayments lined by mangroves.

During the spill, oil accumulated in the following zones of the outer coast: (1) seaward edges of reef flats (between *a* and *b* in Fig. 2.1*A*), (2) seaward sides of berms (if they were present) on back-reef flats (near *d* in Fig. 2.1*A*), and (3) mid-to-low portions of sand beaches and outer-coast fringing mangroves (between *d* and *e* in Fig. 2.1*A*). In mangrove channels and streams, oil accumulated along berms at the outer edges of mangroves and in thickets of red mangrove roots lining channels and streams.

Meteorological and hydrographic conditions during the month of the spill and 2 mo thereafter are shown in Figure 2.4. The spill occurred during the late dry season, which is also a period of low tides and low mean water levels. These factors produced the patterns of oil deposition described above. During low tides, seaward borders of reef flats were exposed above water level and onshore winds concentrated oil slicks there. During high tides back-reef berms, beaches, and mangrove berms acted as barriers that also accumulated oil. Relatively little oil was able to move

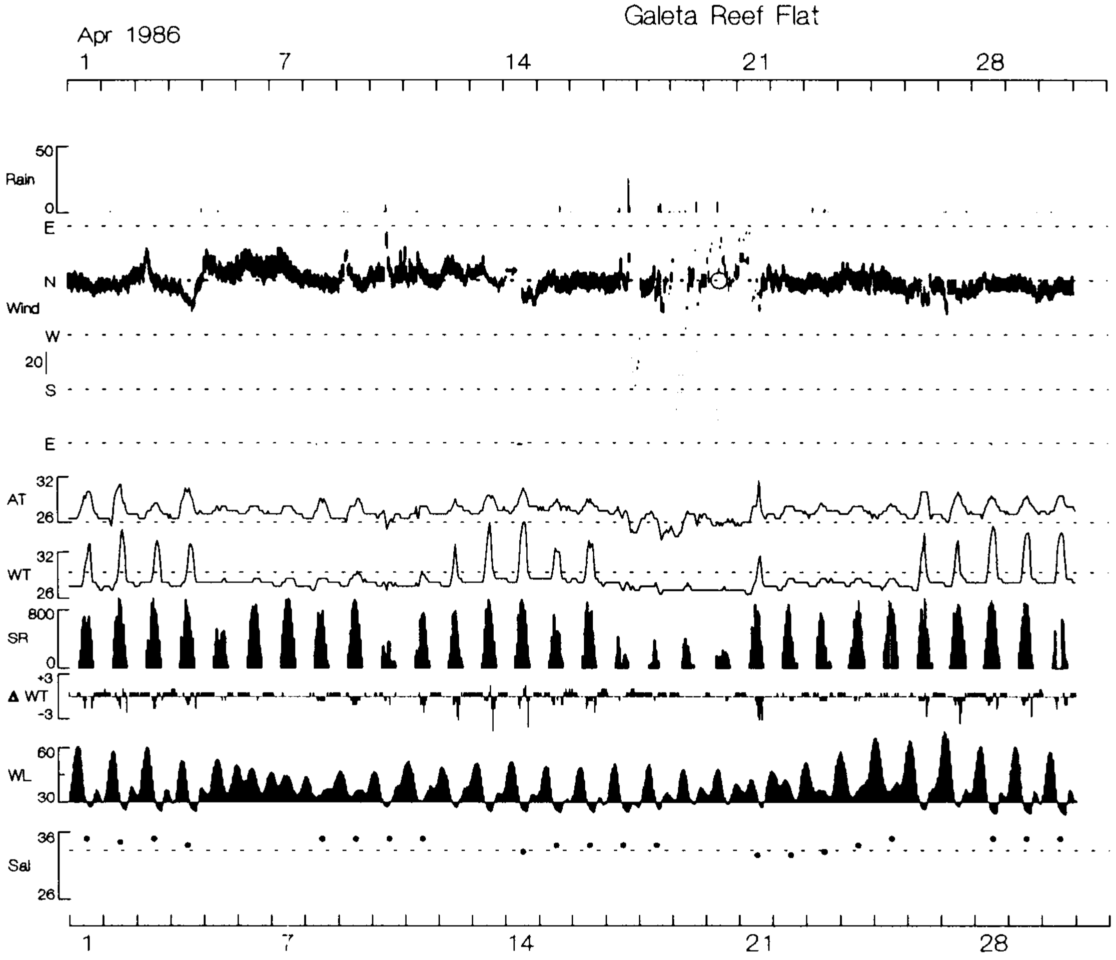

Fig. 2.4 Physical environmental data at Punta Galeta during the 1986 oil spill. *Rain* = rainfall (mm); *Wind* = both the average hourly wind speed and direction, the length of the vertical bar indicates wind velocity, the vertical bar between *W* and *S* is 20 km/h, the position of the vertical bars is the average hourly wind direction by compass heading, *open circles* show gusts >20 km/h more than hourly average; *AT* = air temperature (°C); *WT* = upstream water temperature (°C); *SR* = solar radiation (W/m^2); *Delta WT* = upstream minus downstream water temperature (°C); *WL* = water level relative to Punta Galeta datum (cm), extensive emersion of the reef flat occurs when water level is below 30 cm; *Sal* = salinity (o/oo). *A*. April 1986; the spill occurred on the 27th.

over these raised barriers and enter sand forests and interior mangrove forests. Because of the lack of rain, stream flow was slow during the oil spill and oil penetrated into stream channels. As stream flow increased through May, June, and July, stream discharge flushed out portions of the accumulated oil in mangroves growing along streams (Fig. 2.5).

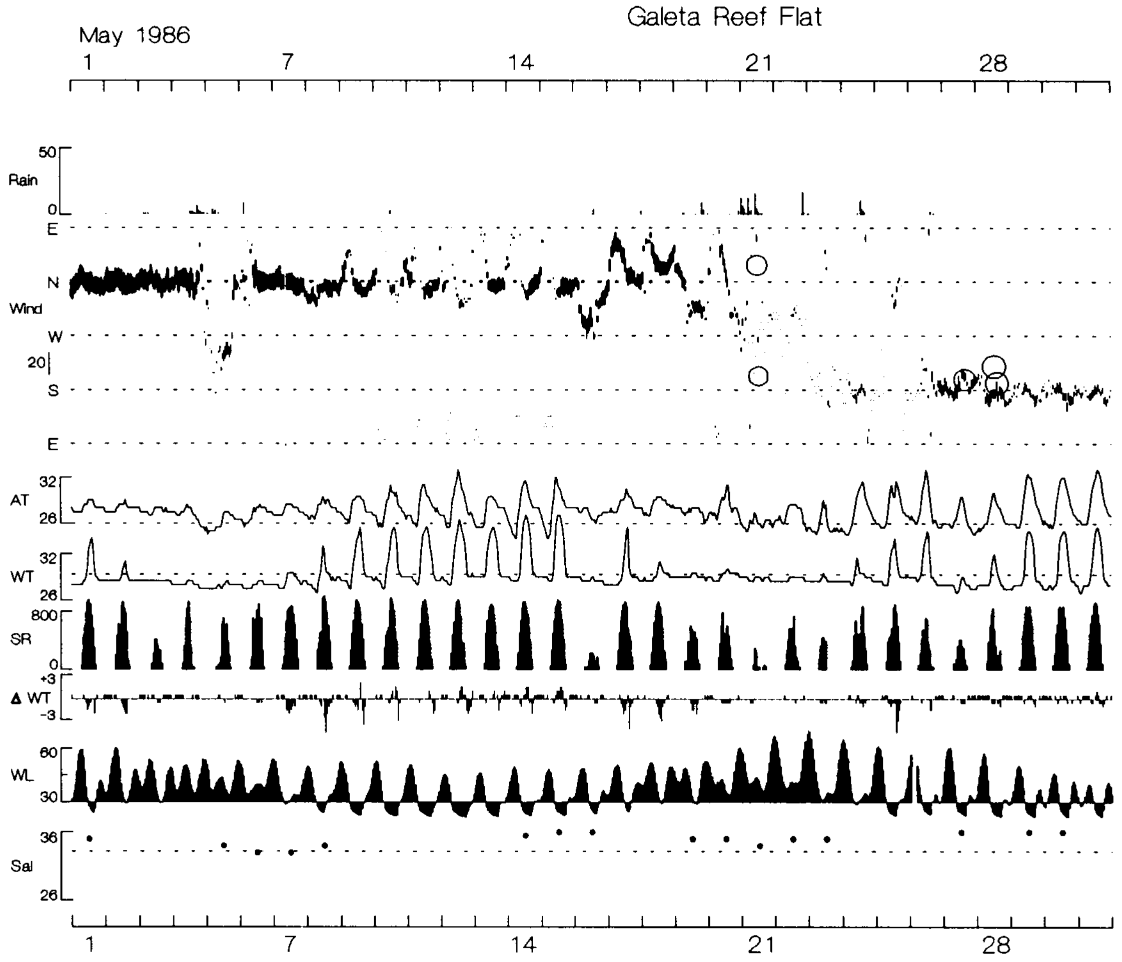

Fig. 2.4 **Physical environmental data at Punta Galeta during the 1986 oil spill (continued).** ***B.*** **May 1986.**

2.3 Objectives, Rationale, and Design

2.3.1 Objectives

For the purpose of generalizing results from the 1986 Bahía Las Minas oil spill to other oil spills, we examine possible patterns of oil accumulation produced by different combinations of weather and sea conditions.

Our goals in these analyses were to determine:

1. the extent to which winds, stream discharge, and water levels during the 1986 Bahía Las Minas spill represented conditions in other seasons and years, and

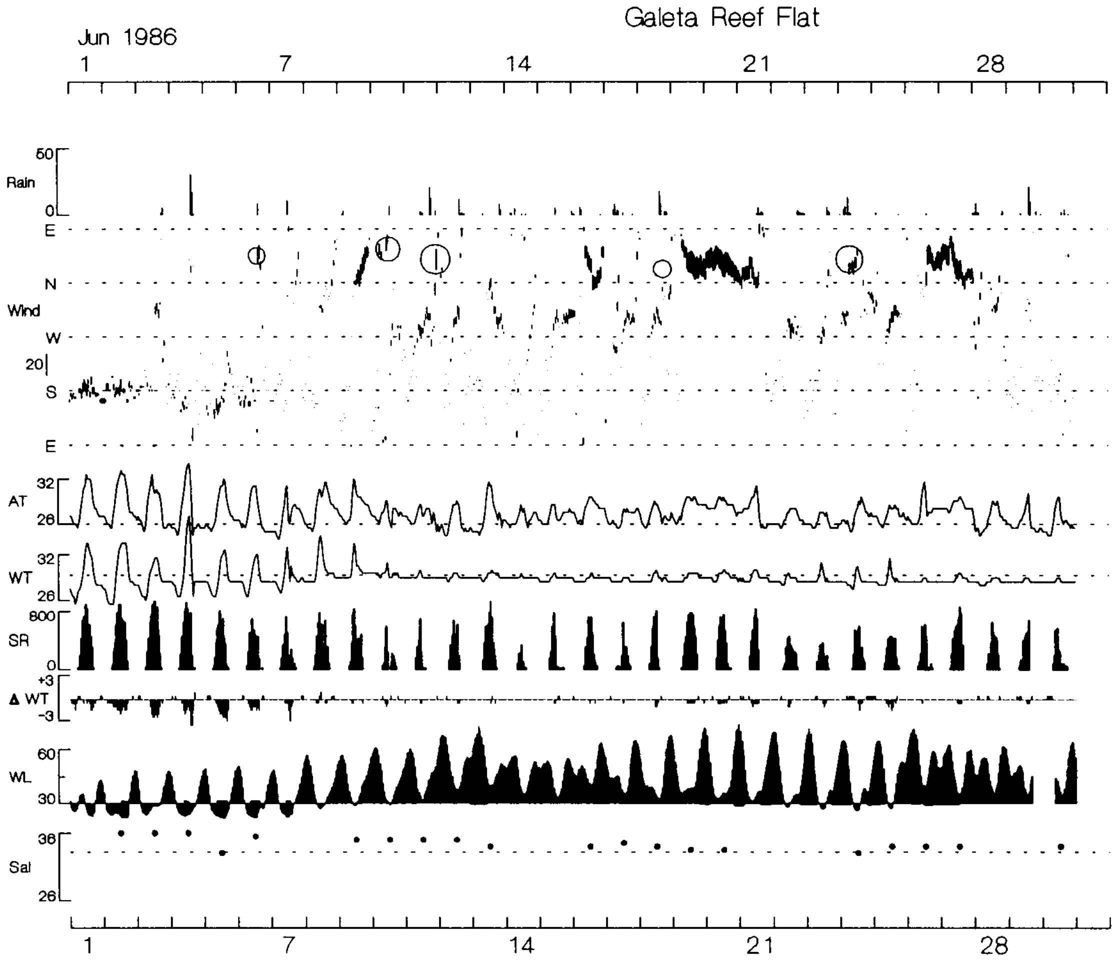

Fig. 2.4 Physical environmental data at Punta Galeta during the 1986 oil spill (continued). *C.* June 1986.

2. the frequencies of combinations of water levels, onshore winds, and stream discharge rates that would have produced other patterns of oil deposition and, therefore, different spatial distributions and severity of biological damage.

2.3.2 Rationale and Design

Daily field observations during the 1986 spill indicated that water levels, winds, waves, and rainfall were important factors in determining (1) the extent to which outer-coast habitats were exposed directly to oil slicks, and (2) the penetration of oil into freshwater streams.

Fig. 2.5 Oil being flushed out of a stream channel in Bahía Las Minas, July 1986, about 2.5 mo after the oil spill. Oil was deposited in the mangroves along this stream in May 1986 (early in the 1986 wet season), when rates of freshwater discharge were lower.

Using the Environmental Sciences Program database from Punta Galeta, we were able to (1) directly calculate the seasonal frequencies of various combinations of winds and water levels along the outer coast of Bahía Las Minas, and (2) combine rainfall data from Punta Galeta with the relationships between rainfall, soil moisture, and runoff developed for Barro Colorado Island, Panama (Windsor 1990), to estimate seasonal patterns of freshwater discharge from coastal streams.

2.3.2.1 The Outer Coast: Winds, Waves, and Water Levels

Oil damage to most outer-coast habitats would have differed under combinations of weather and sea conditions different from those during the 1986 spill (Cubit et al. 1987; Cubit and Connor, Chap. 4). Water levels over the Punta Galeta reef flat vary seasonally, and in some seasons lower elevations of the reef flat are rarely exposed above water level (Cubit et al. 1986, 1989).

The 1986 oil spill occurred during the season of low mean water levels and low tides when reef flat biota were frequently exposed above water level (Cubit et al., 1986, 1989). The low water levels exposed reef flat biota in some habitats directly to oil slicks, but restricted the movement of oil into sand forests and mangroves, which are at higher elevations behind reef flats (Fig. 2.1). If the 1986 oil spill had occurred in a period of higher water levels, the biota in most reef flat habitats would have escaped this prolonged direct immersion in oil, though more of the oil could have breached beaches and berms to enter sand forests and mangroves. These conditions are compared in the following analyses.

2.3.2.2 Mangrove Streams: Periods of Freshwater Discharge

The amount of oil that would penetrate into mangrove streams depends on winds, tidal fluctuations, absolute water levels, and discharge velocities at the surface of streams. In general, onshore winds and rising tides would tend to push oil into streams, while offshore winds and stream discharge would do the opposite. Because freshwater is less dense than saltwater, it determines the surface flow tending to transport oil out of the mouths of streams.

On this coast, reduced rainfall (and therefore runoff) coincides with strong onshore winds during the dry season; therefore, the dry season is a critical period when an oil spill could penetrate into mangrove streams. Two major oil spills have occurred on this coast near the dry-season period. The Bahía Las Minas oil spill occurred at the end of the dry season (April 1986), and the *Witwater* oil spill occurred at the beginning of the dry season (December 1968).

There are no direct measurements of stream discharge available for the area affected by the oil spill; however, stream discharge can be estimated from relationships between rainfall and runoff developed for Barro Colorado Island (BCI), Panama (Windsor 1990) in combination with rainfall data for Punta Galeta. BCI is approximately 25 km south of Punta Galeta, and relationships between rainfall and runoff are based on approximately 16 yr of daily observations (Windsor 1990).

2.3.2.3 Methods

Monitoring Data for Weather and Sea Conditions

Physical environmental data for Punta Galeta (Cubit et al. 1988*a*, 1989) were calculated from the long-term Punta Galeta database (since 1974) for weather and sea conditions. The database comprises hourly records of air and sea conditions, and provides a quantitative basis for defining physical conditions during the 1986 oil spill in comparison to seasonal and year-to-year variations in conditions recorded from this site. For movement of oil onto the outer coast, we used a corrected version of the 5 August 1991 update of the database. Methods of monitoring and variability of conditions are described in Cubit et al. (1986, 1988*a*, 1989). Weekly running averages of rainfall were used to calculate freshwater discharge rates.

2.3.2.4 Definitions and Methods of Calculation

Definitions

Onshore Winds

For the reef flat at Punta Galeta onshore wind direction was approximated using winds from the three northerly octants of northwest, north, and northeast, the equivalent of compass directions >292° and <68° (due North = 0°).

Water Levels

Water levels in the database were recorded from a tide gauge on the reef flat at Punta Galeta, and are given in relation to the Punta Galeta datum (the "0" level), which is approximately 30 cm below mean low water (Cubit et al. 1987, 1988*a*, 1989).

Winds and Water Levels on the Outer Coast

These analyses describe the actual monthly frequencies of two combinations of wind speeds, wind directions, and water levels that affected the deposition oil in outer-coast habitats near Punta Galeta. Both cases used onshore wind directions (i.e., directions that would transport oil toward the shore). The first (*Conditions A*) represents water levels low enough to expose any elevation of the reef flat above water level, and therefore to floating oil slicks (see Sect. 2.4.1). In the second case (*Conditions B*) water levels would be deep enough to allow floating oil slicks to pass at least 30 cm above the reef flat, but strong onshore winds would create wave turbulence and mix oil into the water column. Under these conditions high water levels would allow more oil to be deposited in the extensive habitats with loose sediment, such as mangroves, high beaches, and sand forests.

Specifications for Conditions A and B

Conditions A (direct exposure of benthic biota on the reef flat to oil slicks)

Conditions A include any onshore winds and any water levels below the reef crest (<30 cm above datum). The reef crest is the highest part of the reef flat, excluding loose pieces of coral rubble.

Conditions B (maximum transport of oil slicks into the interior portions of sand forests and mangrove forests)

Conditions B include water levels at least 30 cm above the reef crest (i.e., >30 cm above datum) with strong onshore winds and probably strong wave action. Here, onshore winds >20 km/h have been used to hindcast wave action; this underestimates wave action coming from distant sources. Under these conditions no biota of the reef flat would be directly immersed in floating oil slicks, though biota would be exposed to oil mixed into the water column by waves breaking onto reefs. Strong onshore winds and probably strong wave action would create the maximum force to drive oil into high-intertidal and supratidal habitats behind reef flats.

Calculation of Frequencies of Wind- and Water-level Combinations

Frequencies of hr/mo for Conditions A and B were calculated from the database described above. The combination of synchronous wind speed, wind direction, and water level data spanned January 1975 through December 1990. To reduce biases caused by missing data, these calculations used only months with at least 90% of the hourly records complete for simultaneous measurements of wind speed, wind direction, and water level. A total of 134 mo met these criteria. Within this group, corrections were made for missing data.

Explanation of Tukey Box Graphs

The frequencies of hr/mo for each set of conditions are presented as Tukey box graphs (also called Tukey box plots). The top and bottom of each box represent the upper and lower quartiles of the data. The horizontal line dividing the box represents the median. Thus, the length of the entire box is the interquartile range. The uppermost vertical line spans the distance from the top of the box to the maximum data point lying within 1.5 times the interquartile distance from the top of the box. All data lying beyond this point are plotted as individual points: the upper asterisks are less than three times the interquartile distance from the top of the box, the circles are more than three times that distance. The lower values are calculated by the same procedure. Unlike means and standard errors (or standard deviations) these plots more fully represent the actual distribution of the full set of data, including skewness, outlying values, and other irregularities.

Discharge from Freshwater Streams

To estimate the discharge of freshwater from coastal streams, we used the relationships established by Windsor (1990), which are based on a geometrically lagged moving average (one whose weighting decreases geometrically back through time) of weekly rainfall. When this average fell below 20 mm/wk, soils began to lose moisture. After a 4-wk run of these values, runoff almost ceased. When this value rose above 20 mm, soil moisture began to increase. Runoff began after a lag period when (1) the soil saturated or (2) when rainstorms exceeded the infiltration capacity of soils and overland flow occurs (see also Dietrich et al. 1982). Once soils were saturated, monthly rainfall and runoff were positively correlated.

We applied Windsor's (1990) procedures for BCI to determine periods of low freshwater runoff in the area of Bahía Las Minas. This stepwise procedure began by defining the wet and dry seasons, and then estimating dates of reduced runoff. To define the wet and dry seasons, Windsor (1990) created a running average over periods of 6 wk. This average was geometrically lagged so that rainfall for the ending week was weighted much more heavily than the beginning week 6 wk earlier. To do this he summed the following values: one-half of the rainfall for the ending week (time t), plus one-fourth the rainfall for the previous week ($t - 1$), plus one-eighth the rainfall for wk $t - 2$, and so on to wk $t - 6$. For Bahía Las Minas we used the Punta Galeta database to calculate the geometrically lagged moving average of rainfall (hereafter called *lagged average rainfall*) over the period January 1974 to June 1991. Gaps in the hourly records of this database were corrected using data from a rain collector mounted on the laboratory dock to give a continuous daily record (K. W. Kaufmann, pers. comm.).

The beginning of the dry season was defined as the first day of three consecutive weeks when the value for lagged average rainfall fell below 20 mm. The end of the dry season (i.e., the beginning of the wet season) was defined as the first day of three consecutive weeks when the lagged average rainfall exceeded 20 mm.

As a more conservative procedure, we then estimated the lengths of the periods of low stream discharge in two ways (*Cases A* and *B*), using different assumptions to estimate the ending dates (and therefore the lengths) of the periods of low discharge in the dry season. The procedure to estimate the reduction of stream discharge at the beginning of the dry season was based on the relationship between the onset of the dry season and the reduction of runoff at BCI, where discharge was greatly reduced approximately 4 wk after the dry season began (as defined above). In Case A we assumed that discharge began when the wet season began. In Case B we assumed a delay in the increase of stream discharge that was based on observations at BCI, where discharge rates lagged behind early wet season rainfall by up to 2 mo, depending on the timing and amount of early rains (Windsor 1990). For Case B stream discharge was estimated to resume after two conditions occurred: (1) the wet season began as defined above, and (2) rainfall increased to a level with at least three consecutive weeks of more than 35 mm of rain per week. Based on the BCI records, the Case B procedure appeared to be the more realistic method for estimating the length and ending date of the dry season periods of low stream discharge.

To estimate variations of runoff from successively larger basins, we added a series of constants to the lagged average rainfall and then calculated the period of low discharge as done for Case A. The added constants were 2.5, 5, 7.5, and 10.

Conditions During the 1968 Witwater Oil Spill

There are no equivalent meteorological records from Punta Galeta for 1968 when the tanker S.S. *Witwater* oil on this coast. Consequently, we used data from meteorological stations of the Panama Canal Commission (PCC) near Punta Galeta. Data for rainfall were obtained from three PCC stations: Cristóbal, Aguas Claras, and El Ciento. Cristóbal is located on Bahía Limón about 8 km southwest of Punta

Galeta (see Fig. 1.2). Aguas Claras and El Ciento are located at higher elevations near, but outside of, the highest part of the watershed of Bahía Las Minas. These two sites are approximately 18 and 20 km, respectively, southeast of Punta Galeta. Data for wind speed and direction were only available from Cristóbal. For the rainfall data, we applied the relationships developed above to hindcast conditions during the 1968 *Witwater* spill. All three rainfall records were used because of the east-west gradient in this area (Windsor 1990), which could affect estimates of seasonal changes in stream discharge.

2.4 Results and Implications

2.4.1 Winds and Water Levels on the Outer Coast

2.4.1.1 Conditions A

Reef flat habitats could be directly exposed to oil slicks at any time of year, but most often between February and August. The seasonal peak in exposure times was in April and May; thus, the 1986 oil spill occurred at the time of year when the lowest levels of reef flats were most vulnerable to direct contact with oil slicks. May 1986 was "average" because the total hours of exposure in May 1986 were close to the median value for the month of May in all years (Fig. 2.6*A*). The 1968 *Witwater* oil spill occurred near the end of December when the probability of exposure of reef flats was low.

2.4.1.2 Conditions B

The 1986 oil spill occurred at the beginning of the April-October period when combinations of high water levels and strong onshore winds were rare (Fig. 2.6*B*). In May 1986 these conditions were near zero; consequently, the spilled oil did not penetrate deep into sand forests and mangroves. Instead, low water levels caused most of the oil to be deposited in a narrow band along the seaward fringe of beaches and mangroves. If the oil spill had occurred between November and March, as did the December 1968 *Witwater* spill, the probability would have been much higher that strong onshore winds, high waves, and high water levels would have carried more oil into the extensive areas of sand forests and interiors of mangrove forests at Bahía Las Minas.

In summary for the outer coast, May 1986 was average for the conditions of winds and water levels compared with the month of May in other years. This was a time when strong onshore winds and high water levels were relatively infrequent, but low water levels were at their peak frequency. Thus, oil deposition was concentrated low on the shore instead of at higher tidal elevations.

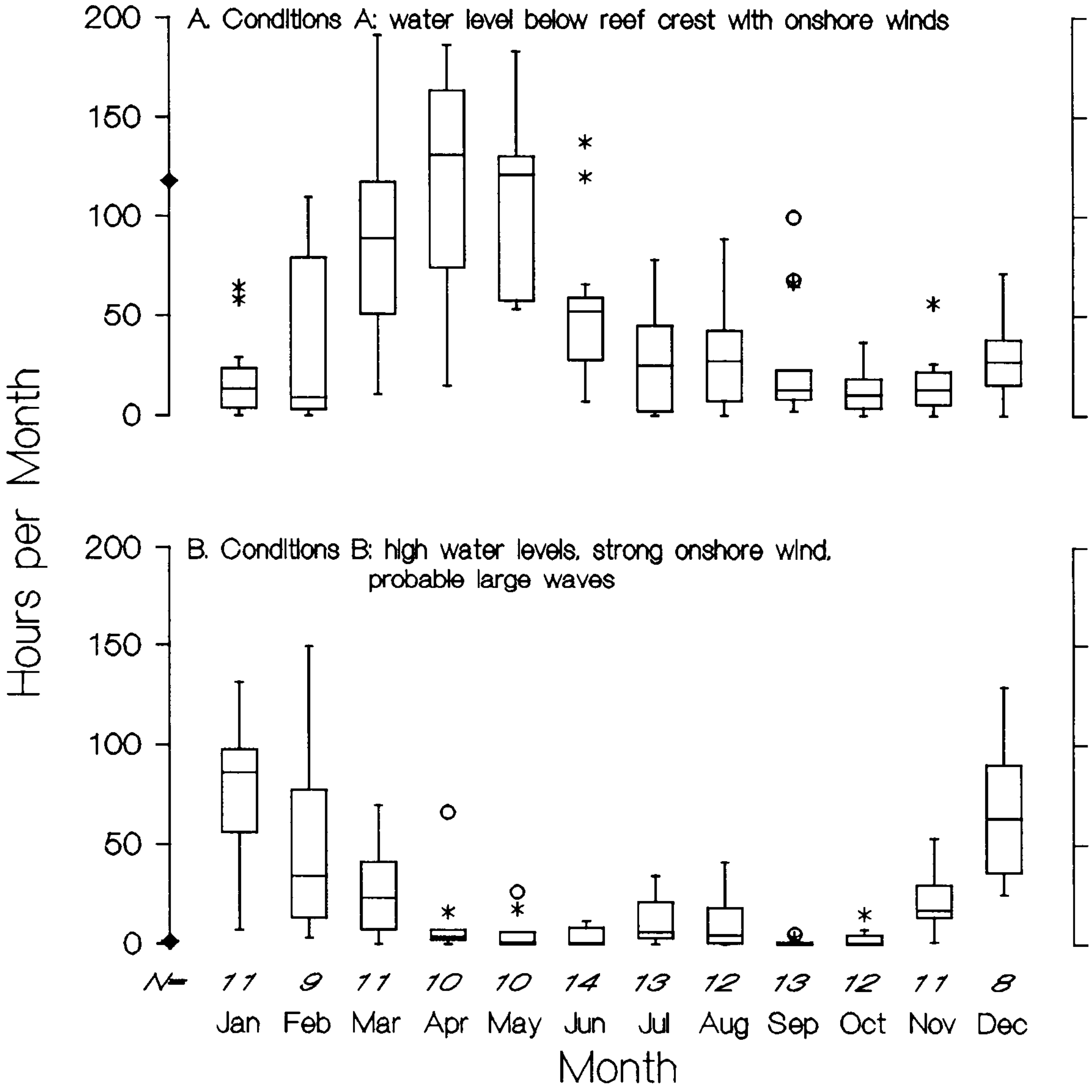

Fig. 2.6 Hours per month that outer coast habitats at Punta Galeta were exposed to the combinations of onshore winds and water levels specified for Conditions A and B in the text. Tukey box plots are explained in the text. The number of months included in the computations is given as *N* at the bottom of each set of graphs. Values for May 1986, the first month of the oil spill at Punta Galeta, are shown as *black diamonds* on the *y*-axis. *A*. Hours per month that the outer coast has been exposed to any onshore winds combined with water levels at, or below, the level of the reef crest. *B*. Hours per month that the outer coast has been exposed to strong onshore winds combined with water levels at least 30 cm above the reef crest.

2.4.2 Estimated Periodicity of Stream Discharge

2.4.2.1 Predicted Seasonal Patterns of Stream Discharge in Bahía Las Minas

Rainfall is highly seasonal at Punta Galeta (Fig. 2.7). The dry season varied in length between 10 and 23 wk in the 17 yr of rainfall data available (median = 18 wk of dry season, Table 2.1). The median start of the dry season occurred in the last week of December, with 53% of dry seasons starting between the third week of December and the first week of January. The last week of November was the earliest and the second week of February the latest start on record (a range of 11 wk). The wet season began as early as the last week of March and as late as the middle of May (a range of 7 wk). However, 59% of the wet seasons started in the last 2 wk of April. Every year there was a period when stream discharge was predicted to be low (Fig. 2.7, Table 2.1). By the more conservative measures of Case A, the median period of low flow was 14 wk, but ranged from 6 to 19 wk (Table 2.1). In these calculations low stream flow could begin as early as mid-December or as late as early March. In more than half the years reduced discharge could have started by the end of January. Flow was estimated to resume between March and May; in most years discharge would start in April.

According to the Case B calculations, stream discharge begins less predictably than the start of early wet season rains. In 5 yr, sustained heavy rains began as soon as lagged average rainfall went above 20 mm (1976, 1980, 1981, 1984, and 1987) and discharge probably began at approximately the same time (Table 2.1). However, in the other 11 yr with complete records for rainfall (1991 excluded), heavy rains began 2-13 wk after the defined start of the wet season (median = 7 wk, N = 11). In these more realistic estimates, which incorporated delays in heavy rains, the period of reduced flow was longer, up to 31 wk (*Case B* in Table 2.1). Sustained discharge could begin as early as April or as late as August. The procedure of adding a constant amount of soil moisture to lagged average rainfall tended to decrease the estimated length of the dry season and the estimated period when discharge was low (Table 2.1). Adding the maximum factor of 10 to lagged average rainfall only decreased the dry season by an average of 2 wk; however, in 1978 adding 10 eliminated the dry season entirely. This suggests that stream discharge could be continuous year round in larger streams from watersheds that had the same seasonal patterns of rainfall. Such streams would be unlikely to be oiled.

Adding soil moisture to lagged average rainfall changed the length of the dry season by delaying its start, not by ending it early. In other words, after the rains stopped, larger streams had positive downstream flow longer than smaller ones. Flow was reduced in all streams late in the dry season and for variable periods in the early wet season. Thus, the period of maximum vulnerability to oiling was late in the dry season and early in the wet season under all variations of the flow regime examined.

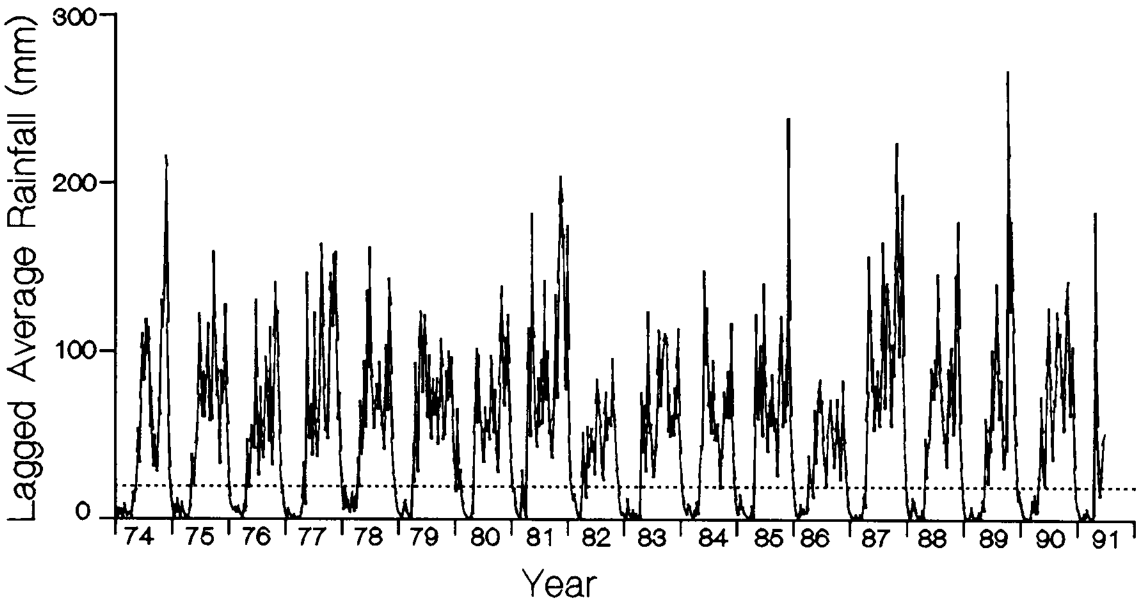

Fig. 2.7 Lagged average rainfall at Punta Galeta, January 1974-June 1991. The *dotted line* at 20 mm indicates the level below which stream discharge drops to levels near zero, and mangrove streams are most vulnerable to penetration by oil slicks. See text for definition of lagged average rainfall.

2.4.2.2 Stream Discharge Conditions During the 1986 Oil Spill

The Bahía Las Minas oil spill occurred at the end of April 1986, in the period of the dry-to-wet season transition. After being trapped in Bahía Cativá for about a week, oil escaped and began to move along the coast on 4 May (Cubit et al. 1987).

In 1986 the dry season ended the week of 14 April, based on lagged average rainfall (Fig. 2.8*A*). However, rainfall was sporadic or light until the first week of June, ranging from 3 to 118 mm per wk (total for 7 wk = 248 mm). Seventy-six percent of the total fell in 2 wk, 70 mm the week of 14 April and 118 mm the week of 19 May (Fig. 2.8*B*).

Case A estimates of freshwater discharge suggest that some discharge could have started during the early part of May 1986, but lagged average rainfall was barely above the 20-mm cutoff until the third week of May (Fig. 2.8*A*). Under Case B conditions, discharge probably did not begin until approximately the second week of June. Because the wet season started slowly in 1986 (Fig. 2.8*B*), we judge that Case B conditions better reflect conditions during the spill. Thus, stream discharge probably did not provide much force to oppose the entry of oil into stream channels while fresh oil slicks were coming ashore during the 1986 Bahía Las Minas spill.

Table 2.1 Estimates of lengths of periods (wk) when streams in the vicinity of Bahía Las Minas would have low rates of freshwater discharge. Cases are defined in text. Letters in columns with constants added denote effects on seasonal pattern: *E* = wet season starts early; *L* = dry season starts late. *ND* = no data.

Year	Length of dry season (wk)	Case A	Case B	Case A + 2.5	Case A + 5.0	Case A + 7.5	Case A + 10.0
1975	19	15	19	15	15	14 L	14 L
1976	19	15	15	12 E	12 E	11 E, L	11 E, L
1977	23	19	31	19	17 L	17 L	13 E, L
1978	16	12	14	7 E, L	7 E, L	7 E, L	0
1979	16	12	18	12	11 L	11 L	11 L
1980	12	8	8	8	7 L	7 L	7 L
1981	13	9	9	7	7 L	7 L	7 L
1982	10	6	19	5 L	5 L	2 L	2 L
1983	21	17	28	17	16 L	16 L	16 L
1984	16	12	12	11 L	11 L	11 L	11 L
1985	18	14	17	14	14	13 L	13 L
1986	14	10	17	10	9 L	9 L	9 L
1987	18	14	14	13 L	13 L	13 L	13 L
1988	17	13	15	13	13	13	12 L
1989	21	17	25	17	16 L	16 L	16 L
1990	19	15	22	15	13 L	13 L	13 L
1991	18	14	ND	14	13 L	13 L	13 L
Median	18	14	17	13	13	13	12
Range	10-23	6-19	8-31	5-19	5-17	2-17	0-16
N (yr)	17	17	16	17	17	17	17

2.5 Discussion

Coastal habitats in Bahía Las Minas consist of extensive areas of reef flats, mangroves, and beaches within the relatively narrow range of the tides. Weather and sea conditions during the spill determined the movement and deposition of oil into these habitats, and, therefore, spatial patterns and biota affected by oil. For any particular spill on this coast, the pattern and severity of damage would depend on when the oil spill arrived in the cycles between dry and wet seasons.

In actuality, if the same spill from the refinery had occurred during the strong and sustained onshore winds at the peak of most dry seasons, oil may have remained in Bahía Cativá until most of it was recovered. Onshore winds did, in fact, keep the oil trapped in this bay for the first week of the spill in April-May 1986 (Cubit et al. 1987). However, for the sake of generalization, the following discussion will assume the movement of oil slicks along the shore. This occurred during the 1986 spill and would also occur after a spill from a tanker or other source offshore.

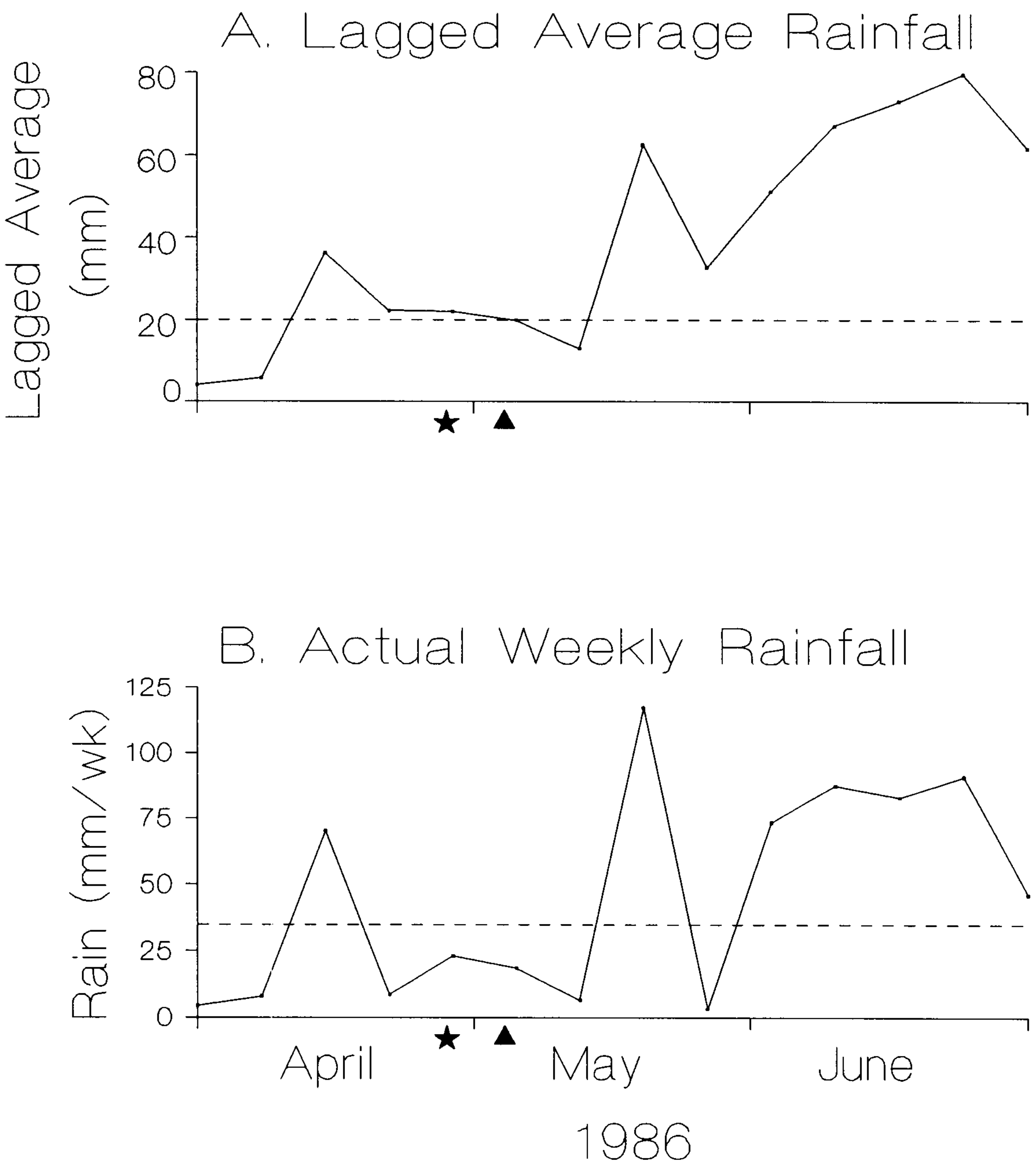

Fig. 2.8 Rainfall conditions affecting stream discharge during the 1986 Bahía Las Minas oil spill. The *star* marks the date of the spill into Bahía Cativá on 27 April 1986, and the *triangle* marks the date the first large masses of oil escaped from Bahía Cativá and began moving along the coast. *A.* Lagged average rainfall, as defined in the text. The *dashed line* at 20 mm indicates the level below which stream discharge was estimated to drop to near-zero levels. *B.* Actual weekly total rainfall for the same period. The *dashed line* at 35 mm per week represents the estimated increased rainfall required to initiate stream discharge after the start of the wet season.

2.5.1 Movements and Deposition of Oil in Habitats of the Outer Coast

Winds, waves, and water levels determined the extent of oil deposition in the outer-coast habitats illustrated in Figures 2.1*A* and *B*. The Bahía Las Minas oil spill occurred during the transition from the dry to wet season. Most of the oil spread along the coast and came ashore in May 1986. Frequency analyses (Fig. 2.6) indicate that conditions of winds and water levels during the 1986 oil spill were well within the normal range for the month of May. Due to the seasonally weak onshore winds and low mean water levels (Fig. 2.6), oil slicks accumulated along seaward edges of reef flats, beaches, and mangrove forests (habitats *b*, *c*, and *d* in Fig. 2.1). Biological damage was high in these zones of oil deposition (Cubit and Connor, Chap. 4; Garrity et al., Chap. 5; Duke and Pinzón, Chap. 8; Garrity and Levings, Chap. 9).

May 1986 was also a period of relatively calm seas with little turbulence to mix oil into the water column. However, during several days of strong wave action, oil was detected in the water column as it entered the laboratory seawater system (J. Cubit, pers. obs.). The seawater intake was about 1.5 m below water level in the relatively sheltered lagoon, and oil appeared as globules floating to the surface of seawater tanks in the laboratory. If this period of wave action had been longer, oil probably would have injured or killed more filter feeders in subtidal zones (habitat *a* in Fig. 2.1). Despite the infrequent conditions necessary to mix the oil with water, visible oil was present within live polyps of subtidal corals, and the mortality of subtidal corals was high (Guzmán et al., Chap. 7).

If the oil spill had occurred during the dry season, stronger onshore winds, higher water levels, and increased wave action would have driven more oil over natural barriers and into higher-elevation habitats of the outer coast, including high beaches, sand forests, and interior mangrove forests (habitats *e* and *f* in Fig. 2.1). Greater wave action would also have mixed more oil into the water column, and high water levels would have reduced the exposure of reef flats and other habitats to direct contact with oil slicks. In particular, biota at the seaward edge of reef flats would have escaped the prolonged direct immersion in oil that occurred during the 1986 oil spill. As discussed below, the 1968 tanker *Witwater* spill occurred during dry season conditions.

2.5.2 Movement of Oil into Streams Flowing through Mangrove Forests

Based on rainfall data from Punta Galeta, we estimate that there is a 3.5- to 4.5-mo "window" each year when stream discharge is weak and has little force to oppose the entry of oil. The size of this window decreases with increasing stream discharge, and increasing discharge is a function of amount of rainfall and area of watershed. Streams entering the region of Bahía Las Minas drain a relatively narrow watershed, and stream discharge was likely to be low during the low-rainfall period between early February and late April. Runoff could be more continuous from watersheds with other hydrological characteristics, such as larger size, more total rainfall, shorter dry season, or larger component of groundwater flow, upstream storage processes, or both. In coastal areas with such characteristics, there is a lower probability that the inner reaches of bays or stream mouths will be oiled.

Marine water levels interact with stream discharge in affecting the penetration of oil into freshwater streams. When water levels are high, water velocity is reduced because the same volume of water flows through a larger cross section. When water levels are low, water velocity increases as streams discharge through smaller cross sections.

In the region of Bahía Las Minas, stream habitats in mangroves are at greatest risk of oil contamination during the dry season, when onshore winds are stronger, water levels higher, and stream discharge rates lower. Thus, during the dry season the forces transporting oil into mangrove streams are at a maximum, and discharge velocities at a minimum. Stream habitats of mangroves are at least risk in the mid-to-late wet season, when onshore winds are weaker, water levels lower, and stream discharge rates higher.

2.5.3 Predicted Patterns of Oil Deposition and Possible Effects of the December 1968 *Witwater* Spill

On 13 December 1968, the 3,400-ton tanker S.S. *Witwater* ruptured about 4 km offshore from Punta Galeta, spilling approximately 20,000 bbl of diesel oil and Bunker C oil into the ocean (Rützler and Sterrer 1970). Based on the observed biological effects of the 1986 spill and local meteorological records for 1968, it is possible to predict the probable spatial patterns of oil distribution and the resulting biological effects of the *Witwater* spill.

Panama Canal Commission data for wind direction and speed at Cristóbal indicate that between 13 and 31 December 1968 wind directions were only from the north and northeast, and mean daily wind speeds ranged from 14 to 29 km/h. In the seasonal weather patterns analyzed for Punta Galeta (Cubit et al. 1986, 1989; Figs. 2.2, 2.3, and 2.6*A*, *B*), these conditions of onshore winds produce high mean water levels, few hours of reef flat exposures, and strong wave action. These conditions correspond to Rützler and Sterrer's (1970) reports of weather and sea conditions during the *Witwater* spill and to the sea conditions seen in aerial and ground-level photographs of the *Witwater* oil spill on file at the Galeta Marine Laboratory.

Wave action at the time of the *Witwater* spill probably churned oil into the water column. The lower levels of the reef flat were probably underwater through most of the spill and thus only exposed to the suspended and soluble portions of the oil. According to Rützler and Sterrer (1970), oil came ashore rapidly, passed over the reefs, concentrated in certain areas, and was quickly cleaned up. In this period of a few days or weeks in December, the highest elevations of reef flats were probably not exposed directly to accumulations of oil for more than a few hours total (Figs. 2.6 *A*, *B*).

Based on estimates from the rainfall data for the three stations examined, freshwater streams were discharging during the *Witwater* spill. At Cristóbal, the dry season began the second week of December, with runoff predicted to drop beginning approximately the second week of January 1969. At Aguas Claras and El Ciento, we calculated that the dry season began the third week of December, with the drop in runoff expected approximately the third week of January 1969. Depending upon the small-scale local conditions in 1968, it may have been possible for the strong onshore winds to push oil into some streams from small drainages, but it was unlikely that larger streams were oiled.

At present we have few data to test these hindcasts of biological damage caused by the *Witwater* spill. However, the existing data agree with our predictions. Patterns in the long-term data for sessile biota on the reef flat at Punta Galeta (started in 1970) are consistent with the hypothesis that some species on high substrata, but not low substrata, were recuperating from damage by the *Witwater* oil spill (Cubit and Connor, Chap. 4). The following information from the studies of Duke and Pinzón (Chap. 8 and App. D) provide more extensive corroboration for patterns of damage to mangroves caused by the *Witwater* oil spill. Patches of barren areas and young mangrove trees in 1973 aerial photographs of the Bahía Las Minas region indicate probable mortality of mangroves during the 1968 *Witwater* spill. Compared to patterns of mortality caused by the 1986 oil spill, the 1973 photographs showed areas of mortality that extended farther into mangrove forests and in some cases left a wider surviving fringe. In addition, oil from the 1968 *Witwater* spill apparently killed areas in the central interior of the mangrove forest, which had not recovered as of 1990. However, no such damage occurred in the 1986 oil spill. Consistent with the oil being driven by strong onshore winds, the mangrove mortality also extended deeper into the bays within Bahía Las Minas. However, despite the strong onshore winds, there were no signs of the oil penetrating into the channels of freshwater streams (Duke and Pinzón, Chap. 8 and App. D).

2.5.4 Summary of Overall Effects of Weather and Sea Conditions on Oil Deposition

In general, an oil spill in the dry season (Sect. 2.2.2) would cause greater immediate damage to biological communities along freshwater streams in mangroves and higher tidal habitats, such as sand forests and interior mangrove forests. Additionally, wave action would mix more oil into the water column. A spill in the wet season would cause greater immediate damage to biological communities in lower intertidal habitats, such as reef flats and fringing mangroves. Intermediate patterns of damage could occur in the transition periods between seasons.

An oil spill in the dry season would pose a much greater risk of long-term, chronic contamination of oil seeping from sediments. If oil slicks came ashore in January, February, or March, the probability is much higher that the combination of strong onshore winds, waves, high water levels, and low stream discharges (see Fig. 2.3) would result in a much greater deposition of oil in the extensive areas of sand forests and interior mangrove forests (habitats *e* and *f* in Fig. 2.1). All these areas have porous substrata of loose sediment. There appears to be no known way to clean oil from these sediments without destroying the forests, releasing masses of oily sediment into the surrounding environment, or both. Oil deposited in some mangrove sediments during the 1986 spill underwent little weathering over a 5-yr period (Burns et al. 1991; Burns, Chap. 3). Larger-scale deposition of oil in these environments would not only cause more extensive destruction of sand forests and mangroves than observed in the 1986 spill, but also deposits of oil in these habitats would constitute a larger reservoir of oil to feed the chronic oiling of all coastal habitats. Such oiling occurred on a smaller scale after the 1986 spill and caused chronic contamination of shoreline environments during the five subsequent years of our study (Burns et al. 1991; Guzmán et al. 1991; Burns, Chap. 3; Guzmán et al., Chap. 7; Garrity and Levings, Chap. 9). On a more massive scale, such oiling could have resulted in delayed, but repeated and more severe damage to biota in adjacent habitats, particularly biota growing on mangrove roots and in back-reef flats. Such damage would be aggravated by large-scale erosion of oily sediments and dead vegetation from sand forests and mangroves (Duke and Pinzón, Chap. 8).

2.5.5 Generalization to Other Spills

Case studies are often used to determine policies regarding oil spills, particularly to evaluate potential risks to marine resources and to plan responses for control and cleanup. Case studies are often cited as representative of conditions expected of other spills in similar habitats. If this typical approach were used for the Bahía Las Minas spill, it might begin like this:

> Damage was mostly confined to certain species of corals in subtidal habitats, biota near seaward edges of reef flats, and mangrove communities along the fringes of mangrove forests. Damage to biota of back-reef flats only occurred at some sites, and relatively little oil penetrated and damaged interior portions of mangrove forests.

Because the 1986 Bahía Las Minas oil spill is the most comprehensively studied oil spill in a tropical environment, it is likely to be used extensively in formulating oil spill policies. As shown by the potential wide-ranging effects of weather and sea conditions on oil deposition, generalizations from this case study require taking into account possible patterns of oil accumulation that would have been produced by other combinations of conditions. Without such considerations, potential risks could be considerably underestimated or overestimated, and oil spill plans could be inadequate or otherwise inappropriate.

2.6 Acknowledgments

This chapter could not have been completed without the assistance of D. M. Windsor, who also provided access to the long-term database of the Barro Colorado Island Environmental Sciences Project. K. W. Kaufmann kindly provided the database for summed daily rainfall from Punta Galeta and assisted with programming problems. The Punta Galeta database for weather and sea conditions was developed by C. Birkeland, D. Meyer, G. Hendler, R. Thompson, J. Cubit, H. Caffey, and K.

Kaufmann with support from the Smithsonian Environmental Sciences Program to C. Birkeland, D. Meyer, G. Hendler, and J. Cubit. The continuity and accuracy of the data are due to R. Thompson. We thank Joseph Brady and the Panama Canal Commission for providing meteorological data.

3
Hydrocarbon Chemistry

Kathryn A. Burns

3.1 Abstract

The hydrocarbon chemistry program interfaced directly with studies of biological effects and recovery in coral reef, seagrass, and mangrove ecosystems. The aims of the program were to provide dose-response information and to study biogeochemical processes acting to modify the spilled oil over time in tropical, coastal environments. Analysis of sediment samples collected over a wide area 6 mo after the spill generally confirmed visual rankings of severity of oiling and the large- and small-scale patchiness of the distribution of oil. Concentrations of oil were highest in organic-rich sediments of mangroves, reaching levels of more than 20% of sediment dry weight. Analysis by gas chromatography (GC) showed that the initial rate of degradation of alkane hydrocarbons in mangrove sediments was rapid compared to that in temperate salt marshes. The size distribution of coral reef sediments showed erosion already was the cause of deposition of increasing loads of fine particles onto reefs in heavily oiled areas of Bahía Las Minas within 6 mo of the spill. It was therefore anticipated that coastal ecosystems would continue to receive oil-contaminated sediments from increasing erosion of mangroves and seagrass beds.

Subsequent sampling was conducted at biological study sites, with chemical analyses providing the basis for interpreting biological effects of the spill. Weathering rates varied from site to site, and the chemistry data were essential for understanding trends in repopulation and recovery of community structure in the various habitats. Within two years of the spill, most hydrocarbon residues had been removed from sediments on high-energy coral reef platforms. By 4 yr postspill, although the residues were nearly nondetectable in this habitat, our techniques were sensitive enough that we could still differentiate oiled and unoiled reefs. High levels of oil persisted in seagrass beds for at least 2.5 yr. Pools of oil trapped in mangrove sediments were surprisingly consistent in composition for more than 5 yr, as seen by ultraviolet fluorescence, GC, and GC/mass spectrometry analyses. The relatively fast rate of degradation of alkanes was not maintained for aromatic hydrocarbons. Buried oil leached into coastal waters and was bioaccumulated by encrusting bivalve molluscs during the 5 yr of sampling. Major events of reoiling associated with erosion of heavily oiled sediments created chronic pollution of coastal waters. The most persistent aromatic hydrocarbons were the dibenzothiophene, phenanthrene, and higher ring-number series. Continued high concentrations of these toxic hydrocarbons, even in samples that appeared by GC to be highly weathered, indicates that the life span of these compounds in mangrove sediments is much longer than 5 yr. Effects of the 1986 oil spill were clearly discernible despite the history of repeated oil pollution in the region. For example, the proportion of dead mangrove

roots in mangrove sediment cores provided a record of long-term damage by major oil spills. Changes in the composition of residues bioaccumulated by bivalve molluscs 5 yr after the spill indicate the next 5 yr will be critical for the transformation of initial lethal effects into later sublethal effects in coastal populations and communities. To our knowledge this is the first set of long-term chemistry data from an oil spill in tropical, coastal environments.

3.2 Introduction

In April 1986 at least 9.6-16.0 million L (60,000-100,000 bbl) of medium-weight crude oil spilled from a ruptured storage tank into a complex region of mangroves, seagrasses, and coral reefs on the Caribbean coast of Panama just east of the entrance to the Panama Canal (Keller et al., Chap. 1). This spill, the largest recorded into coastal habitats in the tropical Americas, occurred close to the Galeta Marine Laboratory of the Smithsonian Tropical Research Institute. Because populations of plants and animals in both oiled and unoiled areas had been documented previously, the study of this spill afforded a unique opportunity to assess the effects of oil on tropical coastal ecosystems. Investigations began immediately and initial ecological effects were summarized by Cubit et al. (1987) and Jackson et al. (1989). Intertidal mangroves, seagrasses, algae, and associated invertebrates were covered by oil and died soon thereafter. There also was extensive mortality of subtidal reef corals and infauna of seagrass beds. These dramatic effects stand in sharp contrast to conclusions based on laboratory dosing experiments and small-scale field experiments. These were designed to assess short-term effects of oil spills and of dispersed oil, and indicated that corals under these circumstances suffer only transient physiological effects and should not be expected to exhibit mortality from oil spills unless oil is dispersed into subsurface waters (Knap 1987; Ballou et al. 1987). Small amounts of dispersant were sprayed from aircraft over offshore areas and channels (Sect. 1.5). Refinery officials estimated that less than 21,000 L of Corexit 9527 were used (Cubit et al. 1987). The amount of dispersant relative to oil, the limited areas over which it was sprayed, and weathering of the oil before dispersant was applied would result in insignificant dispersion of the large amount of spilled oil. Thus chemical dispersion alone would not account for the mortality of subtidal corals over the extended area documented in this study (Guzmán et al., Chap. 7).

The hydrocarbon chemistry program was designed to interface with studies of biological effects and recovery in coral reef, seagrass, and mangrove ecosystems. Sampling and analytical plans were designed to provide dose-response information and to study biogeochemical processes acting to modify the spilled oil over time in tropical coastal environments: the reactions, rates, routes, and reservoirs.

This chapter is divided into sections on methods (Sect. 3.3) and results of the initial assessment of effects in 1986 (Sect. 3.4), followed by the results of long-term studies of coral reefs (Sect. 3.5), seagrass beds (Sect. 3.6), and mangroves (Sect. 3.7). Section 3.8 presents summary conclusions of the hydrocarbon chemistry program.

Section 3.9 is a description of efforts to distinguish alternative sources of contamination in the system, and Section 3.10 describes analytical quality-assurance procedures. The entire data set for individual polynuclear aromatic hydrocarbons in sample extracts is contained in Appendix Tables B.1 and B.2.

3.3 Methods

3.3.1 Sampling Design and Methods

Where the spill occurred, the coastline is convoluted, with strips of beach bordering wave-exposed areas and mangroves lining sheltered areas. Subtidal seagrass beds occur just offshore, with coral reefs seaward of them. Most of the reefs in this study were within a few hundred meters of the shore. Photographs of the affected coastline are in Jackson et al. (1989).

To answer dose-response questions, replicate sites were established in each habitat. Sampling was designed to characterize hydrocarbon loads at each replicate site. To maximize spatial coverage but minimize the number of samples for analysis, samples were composited (combined together) from zones of interest within each site. The basic sampling design in each type of habitat is given in Table 3.1; see Appendix Table B.3 for a complete listing of samples analyzed. The timing of sampling efforts was planned for complete sediment and organism collections 2.5 or 3, and 4 yr postspill, with quarterly collections of bivalve molluscs starting 2.5 yr after the spill. The first major sampling effort during the long-term study was conducted in December 1988 and May 1989, and a second major sampling effort was completed in July 1990. A partial sampling of sites was conducted in 1986 (see Sect. 3.4).

Samples of coral tissues and associated sediments were collected from reefs where observations of coral growth and mortality were being conducted. Samples were taken from the same reefs, in areas close to, but just outside the areas marked for assessment studies to avoid possible interference. Sediments in small depressions at most reefs consisted of coarse coral-algal rubble, indicating high-energy sorting despite the proximity of seagrass beds landward. Galeta Channel (GALC), a site about 4 km west of the site of the spill (Fig. 1.5) and Payardí North (PAYN), within Bahía Las Minas, were heavily oiled. Sediments from these sites contained a relatively large proportion of small particles, indicating that more of the fine sediments eroded from seagrass and mangrove areas landward were retained. Oil slicks did not cover sampling sites the days corals were collected for hydrocarbon analyses, but on other occasions slicks were observed emanating from highly polluted mangrove areas due to runoff during heavy rains (Guzmán et al., Chap. 7).

Samples of scleractinian corals were collected from reefs at 3 to 6 m depth by a diver using a hammer and chisel. Each piece was brought to the surface, using care not to touch the living portions, and handed to assistants in a boat. The corals were sealed in solvent-washed aluminum foil. Each piece was numbered, and replicate pieces of each species were packed together in labeled polyethylene bags and packed on ice. Either immediately at the laboratory or, in most cases, after brief

Table 3.1 Sampling design and plan to answer dose-response questions using replicate oiled (*O*), moderately oiled (*M*), and unoiled (*U*) sites. Habitats: *S* = drainage stream; *C* = channel and lagoon; *OC* = open coast. Samples by this design were collected 2.5 or 3, and 4 yr postspill; partial sampling was conducted in 1986. Quarterly samples of indicator bivalves were collected 2.5-5 yr postspill. See Appendix Table B.3 for sites and dates of all samples analyzed.

A. Mangroves: 2 treatments (O and U) x 3 habitats (S, C, and OC) x 4 replicate sites = 24 stations. At each station:

- 3 surface sediment samples, each a composite of five 2-cm cores from across the site
- 3 samples from different depths of sediment cores, each a composite of 0-2, 8-10, and 18-20 cm sections from 3 replicate cores
- 1 composite sample of bivalves (false mussels or oysters) from across the site

B. Coral reefs: 3 treatments (O, M, and U) x 6, 2, or 4 replicate sites = 12 stations. At each station:

- 3 surface sediment samples, each a composite of 5 scoops of 0-5 cm of sediments
- 3 tissue samples of each major coral species, each a composite of 3 colonies

C. Seagrass beds: 2 treatments (O and U) x 4 replicate sites = 8 stations. At each station:

- 3 surface sediment samples, each a composite of 5 scoops of 0-5 cm of sediments

At selected oiled stations:

- 3 samples from different depths of sediment cores, each a composite of 3 cores

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storage in a freezer, samples were partially defrosted and tissues scraped into labeled glass jars. For analysis, replicate samples consisted of tissue scraped and composited from three pieces of coral into each replicate. Surface sediments were collected from small depressions at reef sites using solvent-cleaned glass jars as scoops. Composite samples were sealed with screw-cap lids lined with foil.

Sediments were collected at the subtidal seagrass beds selected for biological assessment studies and, during the initial survey in 1986 only, from a number of oiled intertidal seagrass beds. Surface sediments were collected using a glass jar as a scoop; several scoops were composited into replicate samples. A few cores were taken using a polyethylene tube approximately 8 cm in diameter. Cores were capped and then sectioned in the laboratory.

Mangrove sediments were collected in 1989 and 1990 at sites selected for studies of the mangrove fringe and epibiota of mangrove roots. Unfortunately, long-term biological study sites had not been selected in 1986 and the first collection covered a wide spatial range but included none of the long-term sites. Also, the 1989 and 1990 collections did not include all the sites for studies of mangrove forests (see Duke and Pinzón, Chap. 8 and Garrity and Levings, Chap. 9). Surface cores were taken with a 2-cm diameter cork borer and composited across study sites. Depth cores were taken at low tide using an aluminum tube, approximately 8 cm in diameter, hammered into the dense mat of roots and sediments in intertidal fringing mangroves. A long knife was then used to cut the sediment around the core for its removal. The ends of the tube were plugged and each core was wrapped in foil, placed on ice, and transported to the laboratory for sectioning. To prevent cross contamination of samples, the sampling gear was cleaned after each use with detergents followed by rinses with analytical grade acetone and hexane. Additionally, unoiled sites were sampled first, followed by lightly and, finally, heavily oiled sites based on visual assessment. The sediments at all mangrove sites consisted of densely compacted mangrove root material interspersed with fine sediments. At the laboratory, cores were sectioned after partial freezing. Replicate cores were extruded onto separate pieces of washed foil, and the layers at 0-2, 8-10, 18-20 cm depth, and any other interesting layers were cut with solvent-cleaned knives and placed on separate pieces of washed foil. Core edges were trimmed off and the replicate sections composited into labeled jars. Samples were frozen and the remaining core was split vertically for observations of visible oil content and live and dead roots.

Tissues of bivalve molluscs collected from mangrove roots were scraped into labeled glass jars, generally after the bivalves had been stored briefly in a freezer.

High-volume seawater samples were collected only in 1986 in mangroves (Largo Remo Mangrove [LRM]) and at Galeta Sand (GALS; Fig. 1.5) by passing water through a glass column packed with Amberlite XAD-2 resin. Columns were attached to mangrove roots with intakes positioned below the estimated low-tide mark. Sampling took approximately 5 hr at flow rates of 150 to 400 mL/min over bed volumes of 100 mL of resin. Upon retrieval, columns were sealed with glass caps, wrapped in foil, and refrigerated until analysis.

Samples were frozen or kept chilled and flown to Bermuda for analysis. At the Bermuda Biological Station for Research (BBSR), sediment and organism samples were stored in a locked freezer.

3.3.2 Analysis Plan

A combination of chemical methods was used, ranging from ultraviolet fluorescence spectrometry (UVF), which is selectively sensitive to aromatic hydrocarbons and their derivatives, to the more quantitative but less specific method of flame-ionization gas chromatography (GC), to the extremely sensitive and selective technique of selected-ion-monitoring gas chromatography/mass spectrometry (SIM GC/MS). Details of the extraction and analysis procedures followed the guidelines published by UNEP (1991).

Samples of organisms were extracted in a Soxhlet apparatus using a hydrolysis procedure. Sediments were extracted with $MeCl_2$ using sonication. Surrogate standards ($C_{18:1}$, $C_{22:1}$, and OTP) were added to extracts to track recovery through subsequent cleanup steps. Lipid weights, defined as total extractable organic matter (EOM), were determined by evaporating 10-μl aliquots of the $MeCl_2$-extracts onto the pan of an electronic microbalance. For organisms extracted by the hydrolysis procedure, separate subsamples of the homogenized tissues were sonicated with $MeCl_2$ for the EOM determinations.

All extracts were screened by UVF analysis. Oil units were computed by preparing a calibration standard from oil extracted in 1986 from heavily oiled mangrove sediments. This procedure provided the data needed for replication relevant to dose-response studies. Correlations were then established between UVF, GC, and GC/MS estimates of oil content.

The GC method analyzed the concentration of hydrocarbons eluting in the C_{14} to C_{34} *n*-alkane range. The columns were 30 m capillaries bonded with DB5 nonpolar phenyl, methyl silicon phase, with hydrogen as the carrier gas flowing at approximately 1 mL/min. Oil content was estimated as the total resolved plus unresolved (URE) hydrocarbon signal. Obvious biogenic hydrocarbons were excluded from the quantification of oil content. Samples with severe biogenic interference had oil content conservatively estimated as only the URE signal. See Section 3.10 for a description of analytical quality assurance procedures.

Tissue extracts always required precleaning with an adsorption chromatography step before UVF analysis; sediments were fractionated only before GC and GC/MS analysis. Most sample extracts were cleaned by passing them through a column of alumina and sequential elution of total hydrocarbon fractions with hexane and $MeCl_2$. A few extracts were separated into individual hydrocarbon classes using normal-phase high-performance liquid chromatography (HPLC).

A third measure of oil content was based on the sum of the individual polynuclear aromatic hydrocarbons and alkylated congeners in the naphthalene/fluorene, phenanthrene/anthracene, dibenzothiophene, pyrene/fluoranthene, chrysene/dibenzanthracene, and benzopyrene/benzoperylene series as determined by SIM GC/MS. In some cases replicate extracts were pooled before continuing with more detailed chemical characterization steps.

For the 1986 sediment collection, subsamples were dried at 90°C and sieved to determine the size distribution of particles. Sieves were stainless steel of standard mesh sizes, 125 μm and 1,000 μm. For mangrove cores, part of the 0-5 cm sections of the cores were dried, lightly ground with a mortar and pestle to break up peat and root material, and then sieved.

For the 1986 and the 1990 coral tissue collections, subsamples of coral tissue were analyzed for total protein content. Because fragments of coral skeleton were included when scraping the tissue, it was not possible to determine an accurate wet weight of tissues. Tissues were ground with precombusted Na_2SO_4 into a homogeneous paste. Protein determinations were made on aliquots of the sample paste as a measure of tissue mass, using the Folin-phenol procedure (Lowry et al. 1951). The aliquots were sufficiently diluted to preclude interference of the Na_2SO_4 in the protein assays, as determined by including the same amount of salt in the standards and blanks.

3.3.3 Methods Correlations

In Figure 3.1, the highly significant regression of GC and UVF estimates of oil content in mangrove sediments can be seen. The data span six orders of magnitude and 4 yr of sampling. The close regression between UVF and total content of polynuclear aromatic hydrocarbons (PAHs) determined by GC/MS is seen in Figure 3.2. In Figures 3.3 and 3.4, the same plots for false mussels and oysters collected as bioindicators are shown. The scatter is greater in the bivalves due, in part, to the phenomenon of selective bioaccumulation, in which bivalves preferentially accumulate the more soluble components of oil in water mixtures. Water-soluble components generally have weaker fluorescence intensity at the wavelengths used for estimating the content of crude oil. As shown below, part of the variation in the regression of the total data set for bivalves, collected between December 1988 and June 1991, is due to the changing composition of oil residues accumulated in the fifth year after the spill. The relationship was still highly significant for the larger data set. The dashed line in Figure 3.3 shows the distribution of points from sites visually assessed as oiled and unoiled.

These plots provide a new perspective on the concept of "legally defensible methods," a term used in recent damage assessments to include only those methods that measure the content of aromatic hydrocarbons by GC/MS. Aromatic hydrocarbons are, however, only one fraction of the toxic components of crude oils. No single method yielding quantitative data on the content of all petroleum components in environmental samples exists. Rather, each method measures a subset of the total petroleum-derived contamination. The use of a range of complementary methods, tailored to provide answers to specific environmental questions, allows for the most efficient use of resources, a significant lowering of analytical detection limits, and a more accurate understanding of the biogeochemical processes affecting changes in oil residues over time. This approach will be illustrated in the data and interpretations presented in this report.

3.4 Initial Sediment Survey, 1986

In September 1986 approximately 100 small-volume samples of surface sediments were collected from intertidal areas to estimate oil levels in sediments. The purpose of this collection was to support planning biological assessment studies, to assess spatial heterogeneity and temporal variation of oil concentration at study sites, and to document apparent topographical features associated with the distribution of oil.

UVF analyses showed the relative distribution of oil in surface sediments and confirmed the relative severity of oiling at the various sites. However, concentrations estimated by UVF were greater than actual amounts of hydrocarbons in sediments as measured gravimetrically or by GC. This difference occurs because UVF analysis is sensitive only to fluorescent aromatic hydrocarbons and derivatives. Light aromatics are water soluble and subject to rapid removal from environmental

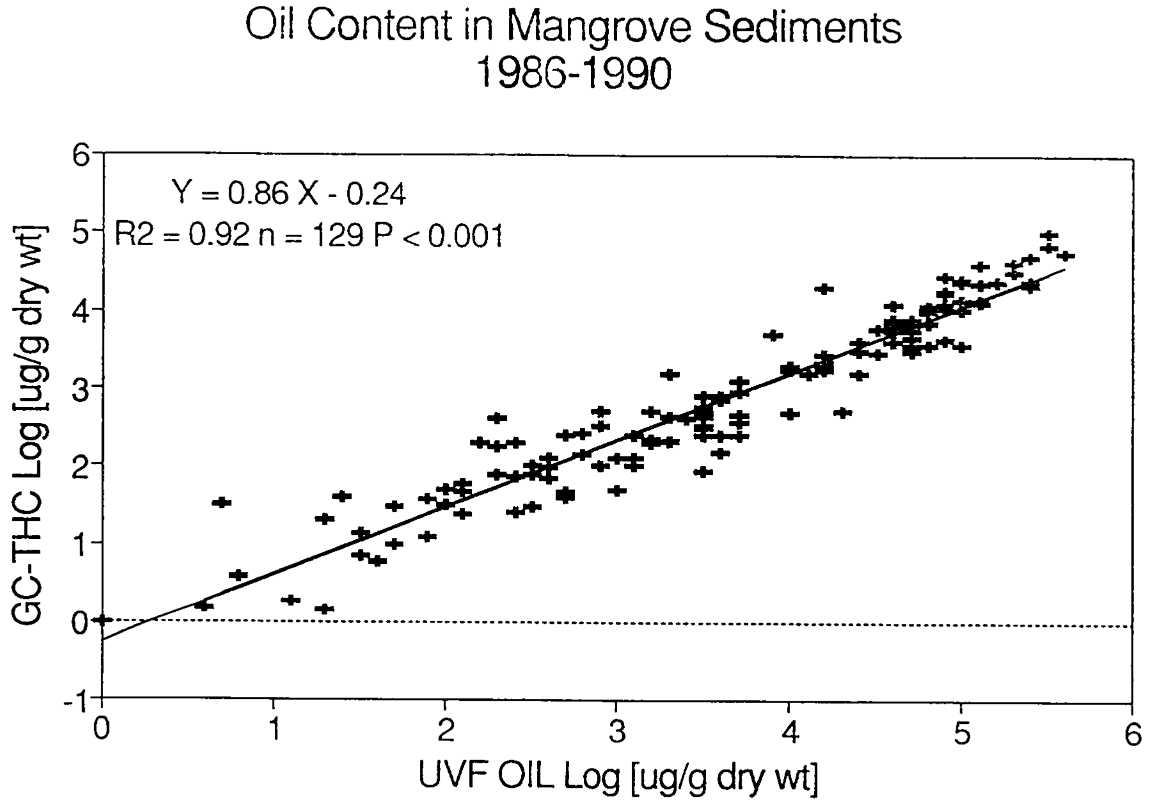


Fig. 3.1 Regression of the log of the concentration of oil in mangrove sediments (μg oil/g sediment dry weight) determined by UVF and GC methods. The data are from all cores and depths for samples collected in 1986, 1989, and 1990.

samples by dissolution and evaporation. Residual high-molecular-weight hydrocarbons generally have higher fluorescent intensities than low-molecular-weight hydrocarbons. Also, substituted derivatives of PAHs would be included in UVF measurements. Thus, as oil weathers it becomes more fluorescent.

To improve the quantitative accuracy of UVF analyses, weathered oil extracted from heavily contaminated mangrove sediments at station SBS (Fig. 3.5) was used to generate the response graph for UVF analyses, yielding estimates of the concentration of oil in units of degraded Venezuelan/Mexican Isthmian crude (D.VMIC), the type of oil spilled. Table 3.2 contains the summary UVF and GC estimates of oil content expressed as μg oil/g sediment (dry weight), oil composition determined by an assessment of the UVF and GC patterns, and the size composition of sediments sampled in 1986, all tabulated according to habitat type. Table 3.2 also shows the original classification of oiling severity based on visual assessment. Sampling sites are shown in Figure 3.5.

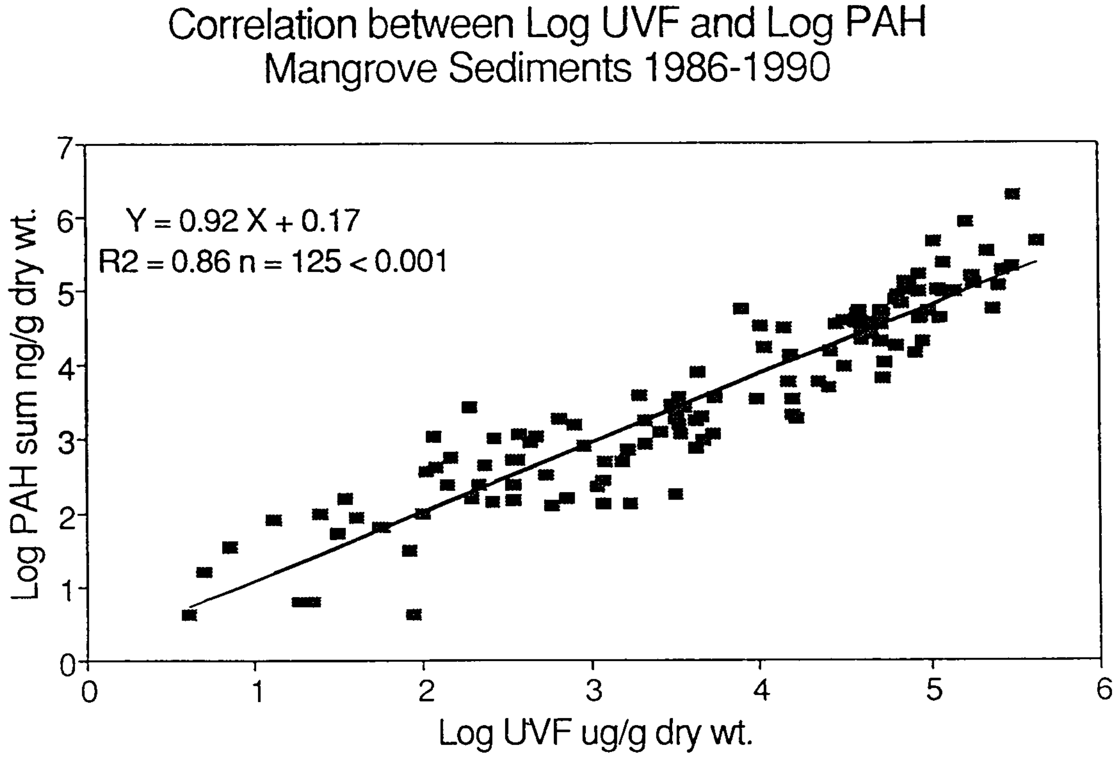


Fig. 3.2 Regression of the log of the concentration of oil in mangrove sediments determined by UVF and the concentration of total PAHs determined by GC/MS; log(μg oil/g sediment dry weight) for UVF and log(ng oil/g sediment dry weight) for PAHs. The data are from all cores and depths for samples collected in 1986, 1989, and 1990.

3.4.1 Mangrove Sediments

The unoiled mangrove site HRB (Fig. 3.5) contained trace residues of oil (<200 μg/g; Table 3.2), perhaps from earlier spills. MARS and NARM were lightly oiled with VMIC. In July 1986 MARS had high levels of fuel oil, determined by UVF spectra, in surface sediments. Fuel oil was detected in deeper core sediments at MARS in September 1986, but the surface of the core also contained trace VMIC. This site was classified as unoiled, but chemical analyses detected some contamination by VMIC. SBS was heavily oiled, with highest surface concentrations in the mid-forest, and LRM was heavily oiled from the front forest through the mid- and back forests. It was obvious by visual inspection that the distribution of oil was extremely patchy. This small-scale variation was reflected in replicate surface sediment samples. The coefficient of variation (standard deviation divided by the mean times 100) ranged from 14% to 56% within stations. To illustrate the extreme patchiness, three samples from the back forest of LRM were taken from patches of

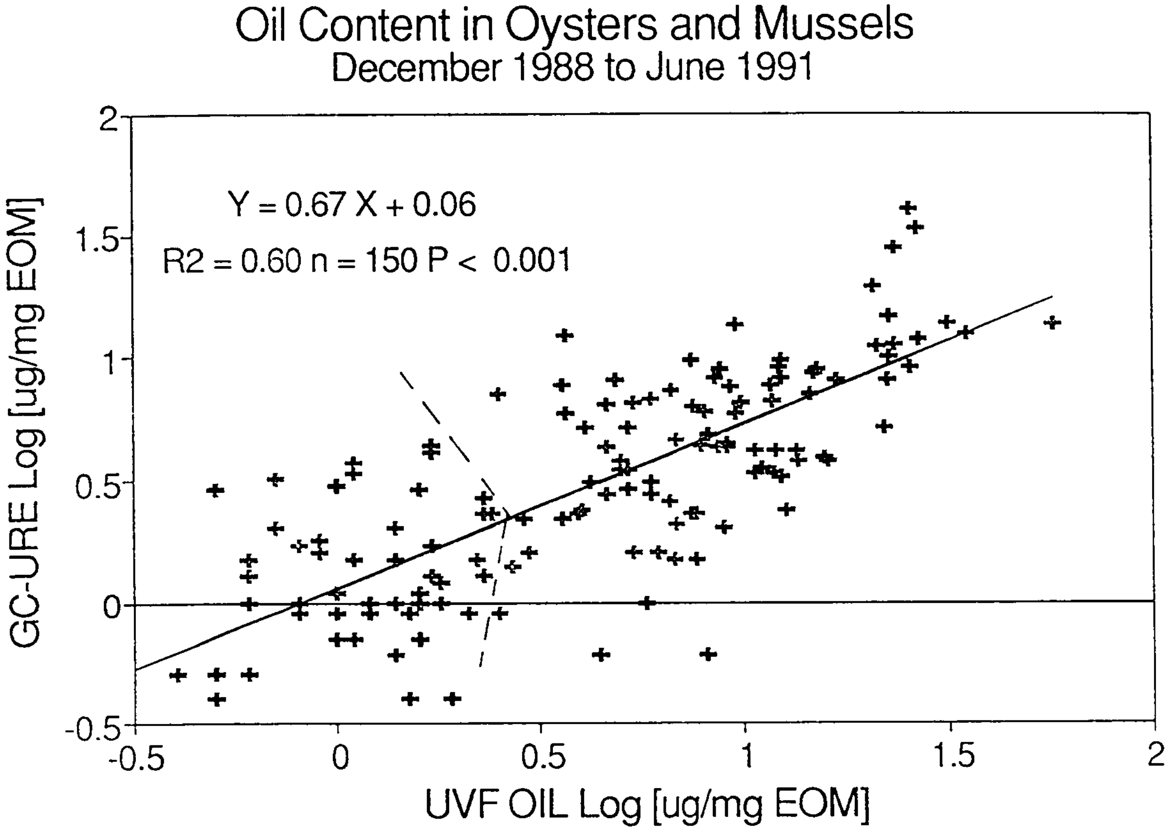


Fig. 3.3 Regression of the log of the concentration of oil in mangrove bivalves (μg oil/mg EOM) determined by UVF and GC. All samples were collected from December 1988 through June 1991. *Dashed line* indicates the break between samples from sites visually assessed as oiled (right of dashed line) and unoiled.

heavily oiled surface sediments. The coefficient of variation in these three samples was 122%, with oil levels ranging from 2 to 66% of sediment dry weight. Despite this patchiness over scales of meters, sites could be ranked in terms of relative degree of oiling. There was some disparity between visual assessments of oiling and amounts of oil in sediments determined by chemical analysis in areas initially classified as unoiled to lightly oiled. Mangrove sediment samples collected 5 mo after the spill demonstrated that VMIC had, in some cases, been transported into previously unoiled areas, such as the lagoon adjacent to Isla Margarita (Fig. 3.5). This finding agrees with visual observations of slicks emanating from heavily oiled mangroves and redistribution of oil along the coast. These observations were to be a repeated phenomenon, as shown in the results of the long-term study (Sects. 3.5-3.7).

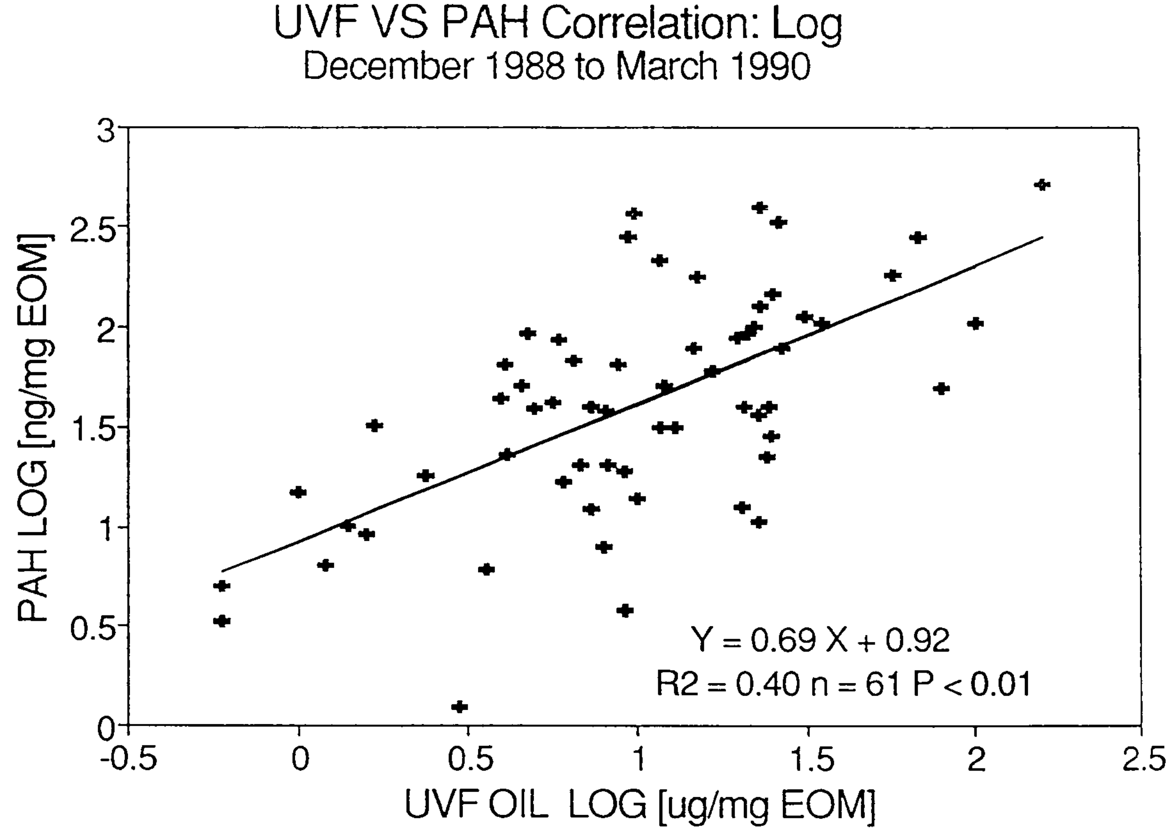


Fig. 3.4 Regression of the log of the concentration of oil in mangrove bivalves determined by UVF and the concentration of total PAHs determined by GC/MS; log(μg oil/mg EOM) for UVF and log(ng oil/mg EOM) for PAHs. All were samples collected from December 1988 through March 1990.

3.4.2 Shallow Sediments

Sediment samples were collected along the shore of Isla Largo Remo (LRN2) in approximately 0.5 m of water at about 10-m intervals. We observed that in some areas where currents were eroding the surface sand, layers of oiled sand were visible underneath. Thus, even in areas where surface sands appeared to be unoiled, layers of oil persisted at varying depths depending on erosion and burial rates. It was not possible to take compacted cores for depth distribution in these dynamic environments. Erosion and redistribution account for some of the variability in chemical measurements and for some chronic reoiling events, which were to persist in later years (Sects. 3.5-3.7).

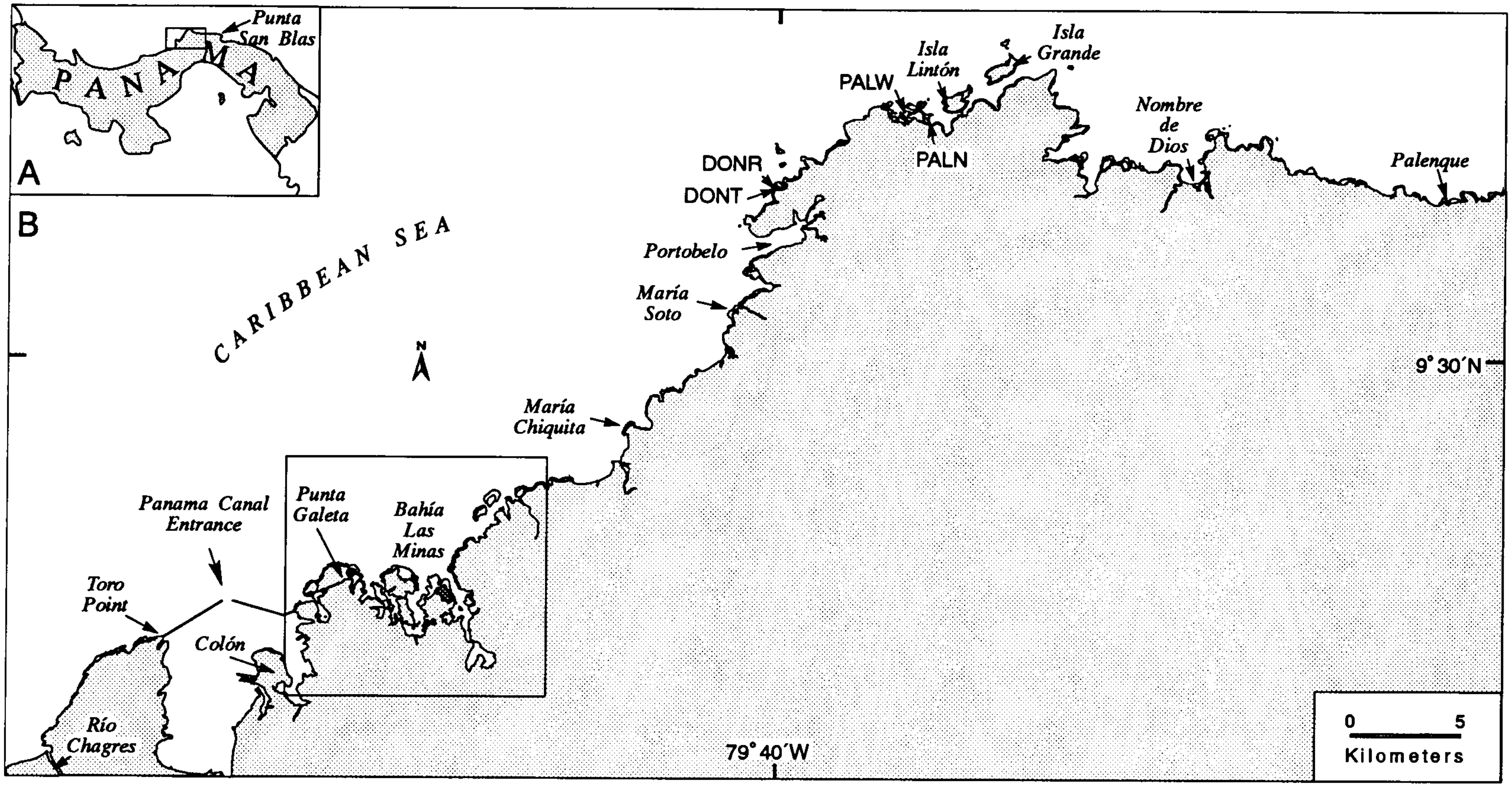


Fig. 3.5 Map of sampling sites, September 1986. Refer to Figures 1.2 and 1.5 for details.

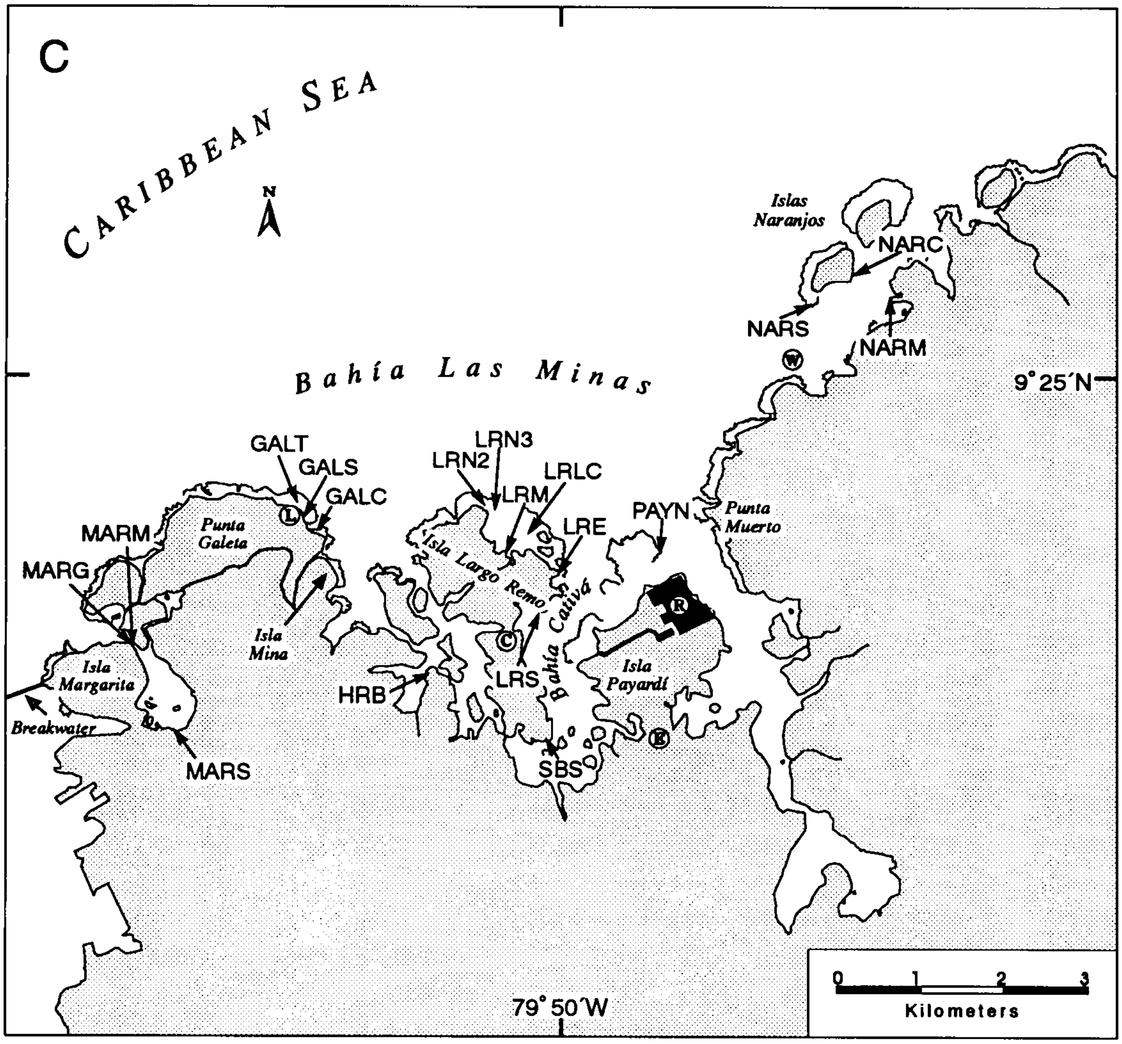


Fig. 3.5 Map of sampling sites, September 1986 (continued).

3.4.3 Seagrass and Coral Reef Sediments

Since some of the long-term study sites in seagrass beds and coral reefs were sampled in 1986, the hydrocarbon data for these sediment samples will be repeated in the time-series data (Sects. 3.5 and 3.6). Sediment size data (Table 3.2) indicated that reefs in Bahía Las Minas were receiving a larger percentage of fine particles from contiguous mangroves and seagrass beds than unoiled sites.

Table 3.2 Oil content of surface sediments (0-2 cm) and core sections (0-5 cm) collected in 1986 (September, unless noted otherwise) by UVF and GC analyses, sediment size composition, type of oil based on UVF spectrum, and visual assessment of degree of oiling (*H* = heavily; *M* = moderately; *L* = lightly; *U* = unoiled; *ND* = no data; *Nondet.* = nondetectable). Refer to Figure 3.5 for site locations.

	µg oil/g sediment		% Size Composition		
Site	UVF	GC	(> 1,000µm\ > 125µm\< 125µm)	UVF Spectrum[1]	Visual Assessment
Mangrove Sediments					
LRM (front[2])					H
Surface	37,881				
	17,037				
	50,283			VMIC	
Core	1,830	469	62\38\0	VMIC	
LRM (mid[2])					H
Surface	63,581			VMIC	
	49,275				
	123,421				
Core	35,195	16,715	16\67\17	VMIC	
LRM (back[2])					H
Surface	87,735			VMIC	
	8,840			VMIC	
	372,856			VMIC	
SBS (front[2])					H
Surface	66,848			VMIC	
	72,463			VMIC	
	12,439			VMIC	
Core	256,000	180,340	11\88\1	VMIC	
SBS (mid[2])					H
Surface	189,962				
	92,228			VMIC	
	159,513			VMIC	
Core	120,254	69,645	42\50\8	VMIC	
SBS (back[2])					H
Surface	42,967			VMIC	
	15,198			VMIC	
	21,824			VMIC	
Core	6,929	3,083	8\75\16	VMIC	

Table 3.2 Oil content of surface sediments (0-2 cm) and core sections (0-5 cm) collected in 1986 (September, unless noted otherwise) by UVF and GC analyses, sediment size composition, type of oil based on UVF spectrum, and visual assessment of degree of oiling (continued).

	µg oil/g sediment		% Size Composition		
Site	UVF	GC	(> 1,000µm\ > 125µm\< 125µm)	UVF Spectrum[1]	Visual Assessment
Mangrove Sediments (continued)					
MARM (seaward of boom)					L
Surface	2			Biogenics	
	4			Biogenics	
	6			Trace	
Core	650	665	13\73\14	VMIC	
NARM					ND
Core	182	100	17\77\6	VMIC	
MARS (July 1986)					U
Surface	1,091	381	42\50\7	L. Fuel	
	1,976			L. Fuel	
MARS (inside of boom)					U
Surface	5			Biogenics	
	5			Biogenics	
	3			Biogenics	
Core	250	135	19\70\10	Trace VMIC	
HRB (front[2])					U
Surface	151				
	181			VMIC	
	138				
Core	85	44	23\67\10	H. Fuel	
HRB (mid[2])					U
Surface	46				
	30			W. Crude	
	38				
Core	70	52	32\60\9	L. Fuel	
HRB (back[2])					U
Surface	53			W. Crude	
	67				
	47			W. Crude	
Core	131	46	17\74\9	W. Crude	

Table 3.2 Oil content of surface sediments (0-2 cm) and core sections (0-5 cm) collected in 1986 (September, unless noted otherwise) by UVF and GC analyses, sediment size composition, type of oil based on UVF spectrum, and visual assessment of degree of oiling (continued).

	µg oil/g sediment		% Size Composition		
Site	UVF	GC	(> 1,000µm\ > 125µm\< 125µm)	UVF Spectrum[1]	Visual Assessment
Seagrass Sediments					
LREN					H
Surface	7,274		5\59\36	VMIC	
	11,070		7\54\40		
	7,132		5\76\19	VMIC	
	24,555	5,561	4\68\28	VMIC	
LRS					H
Surface	335		30\67\2	VMIC	
	97		29\69\2	VMIC	
	331	88	33\65\2	VMIC	
NARC					M
Surface	150		4\54\42	VMIC	
	163		2\61\36	VMIC	
	48	58	7\57\36	VMIC	
LRN2					M
Surface	4		33\57\10		
	7		30\64\6	Biogenics	
	5		21\68\11	Biogenics	
	350		28\68\5	VMIC	
	35		33\62\5	VMIC	
	8		50\46\4		
	2		44\50\6		
	20		32\64\4	VMIC	
	14		25\72\3		
	7		14\82\4	Biogenics	
	500		21\73\6	VMIC	
MARG (June 1986)					L
Surface	144		100\0\0		
	104		3\69\28		
	59		9\70\21	VMIC	
MARG (June 1986, separate sample)					L
Surface	63		4\63\33	VMIC	
	28		2\65\33		
	81		3\66\31	H. Fuel	
	60	26	2\69\29	H. Fuel	

Table 3.2 Oil content of surface sediments (0-2 cm) and core sections (0-5 cm) collected in 1986 (September, unless noted otherwise) by UVF and GC analyses, sediment size composition, type of oil based on UVF spectrum, and visual assessment of degree of oiling (continued).

	µg oil/g sediment		% Size Composition		
Site	UVF	GC	(> 1,000µm\ > 125µm\< 125µm)	UVF Spectrum[1]	Visual Assessment
Seagrass Sediments (continued)					
LRLC					L
Surface	7		20\74\7		
	13		21\72\7		
	4		20\74\6		
	4	14	22\74\5	VMIC	
LRN3					L
Surface	3		33\65\3		
	5		53\46\1	Biogenics	
	8		35\59\7		
PALN					U
Surface	0		45\52\3		
	0		48\49\3		
	0		44\53\3		
	216	0	43\56\1	H. Fuel	
DONT					U
Surface	0		21\71\8		
	0		37\59\4		
	0		32\65\4		
	0	0	31\64\5	Nondet.	
Coral Reef Sediments					
PAYN					H
Surface	343		49\44\6		
	197		60\36\3	VMIC	
	217		48\47\5	VMIC	
	79	110	51\47\2	VMIC	
GALC					H
Surface	393		13\70\17		
	364		21\64\15	VMIC	
	136		16\69\15		
	715	85	16\78\6	VMIC	

Table 3.2 Oil content of surface sediments (0-2 cm) and core sections (0-5 cm) collected in 1986 (September, unless noted otherwise) by UVF and GC analyses, sediment size composition, type of oil based on UVF spectrum, and visual assessment of degree of oiling (continued).

	μg oil/g sediment		% Size Composition		
Site	UVF	GC	(> 1,000μm\ > 125μm\< 125μm)	UVF Spectrum[1]	Visual Assessment
Coral Reef Sediments (continued)					
LRE					H
Surface	41		21\79\0	VMIC	
	54		29\71\1		
	44		27\72\1		
	19	10	27\72\0	VMIC	
NARS					M
Surface	2		52\47\0		
	15		64\36\0		
	28		60\39\1	H. Fuel	
	25	6	66\34\1	H. Fuel	
DONR					U
Surface	0		90\10\0	Trace H. Fuel	
	0		94\5\1	Nondet.	
	0		94\6\0	Nondet.	
	41	0	94\6\0	H. Fuel	
PALW					U
Surface	0		77\23\0	Trace Fuel	
	0		86\14\0	Trace Fuel	
	0		82\18\0	Nondet.	
	0	1	89\11\0	Trace Fuel	
Intertidal Reef Flat Sediments					
GALT					M
	18		10\86\4		
	2		55\45\1	H. Fuel	
	116		51\47\2	VMIC	
	52		57\43\0	VMIC	
	94		19\80\1	VMIC	
	60		66\34\0	VMIC	

[1] The compositional evaluation of oils was based on UVF spectra. *VMIC* = Venezuelan/Mexican Isthmian Crude, the type of oil spilled; *L.* or *H. Fuel* refers to the spectra of predominantly 1-3 and 1-4 ringed aromatics with none of the higher wavelength emission seen in spectra from crude oils; *W. Crude* = weathered crude oil.

[2] *Front*, *mid*, and *back* are zones of fringing mangroves, moving landward from the seaward-most edge.

3.4.4 Initial Weathering Patterns

Gas chromatographic analyses showed that the oil in all sediment samples collected in September 1986, including many mangrove cores, was substantially altered within 6 mo of the spill. Most alkanes were missing, leaving primarily an unresolved signal that was quantified using an average response factor over the appropriate elution range on the GC. In Figure 3.6 are examples of GC traces of hydrocarbons ranging from the relatively least degraded oil residue in surface sediments at a protected channel mangrove site (SBS) to very degraded patterns at an open-coast mangrove site (LRM) and a seagrass bed (LREN). It was thus determined that peak ratios, such as *n*-heptadecane/pristane or *n*-octadecane/phytane, which are often used to estimate weathering rates of oil residues in marine sediments in temperate zones, would not be useful in this data set from a tropical area. Evaporation and dissolution processes removed most of the low-molecular-weight and acutely toxic hydrocarbons in the benzene through *n*-decyl benzene series. GC/MS analysis failed to detect these highly volatile compounds in any of the sediment extracts.

The relative ranking of site contamination is compared with residual oil levels in each type of habitat in Table 3.3. The mangrove sites LRM and SBS were classified as heavily oiled, MARM as lightly oiled, and HRB and MARS as unoiled, by visual observation (Table 3.2). Chemical analyses generally confirmed this assessment and quantified it for this spill. The data from depth cores (Sect. 3.7) showed that by September 1986 some oil had penetrated at least 20 cm into heavily oiled mangrove sediments.

Seagrass sites classified as heavily oiled had concentrations of oil in surface sediments >300 μg/g. Moderately oiled sites were in the 20 to 500 μg/g range (samples in which VMIC was identified), and lightly oiled sites were in the 4-150 μg/g range. VMIC was not detected at unoiled seagrass sites (PALN and DONT; Table 3.2). One sample from unoiled sites showed traces of tar balls (heavy fuel oil) even at relatively unpolluted areas. Such contamination is expected along the coast of Panama from ship traffic and previous incidents of contamination. The UVF spectra for residues of the crude oil spilled in 1986 at Bahía Las Minas were distinguishable from highly weathered fuel residues in tar balls (Fig. 3.7). LREN sediments, composed of fine silty sand, had much higher levels of oil than areas with coarser sands, such as LRS, despite the extremely high levels of oil in the mangroves fronting both of these seagrass beds. Oil levels dropped with distance from shore (LRN2 [onshore] compared to LRN3 [offshore] and LREN [onshore] compared to LRS [offshore]; Table 3.2).

Coral reefs at PAYN, GALC, and LRE were visually ranked as heavily oiled, and reef sediments contained 19-715 μg oil/g (Table 3.2). NARS was classified as moderately oiled, and associated sediments contained <30 μg oil/g of a heavy fuel oil; VMIC was not identified in the samples collected, although slicks were observed soon after the spill and years afterward (Guzmán et al., Chap. 7; Table 7.6).

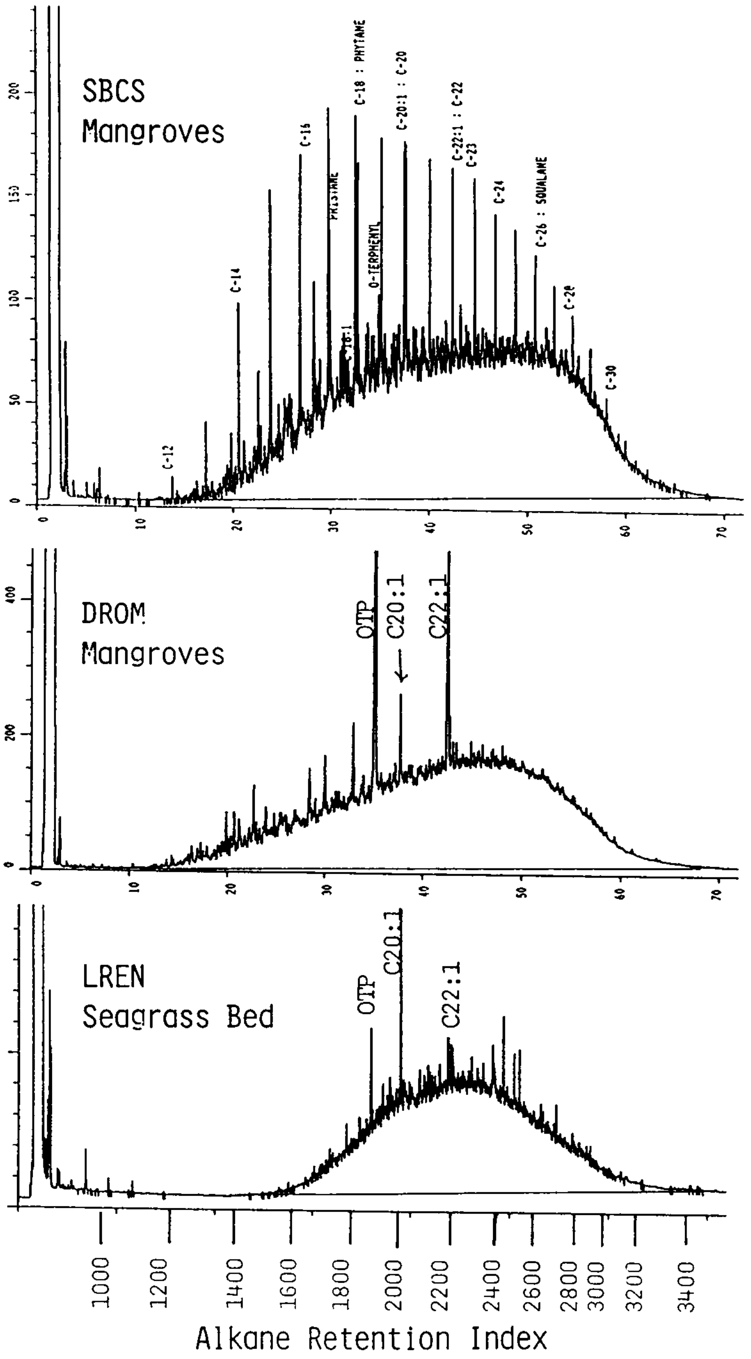


Fig. 3.6 Gas chromatograms of sediments collected in 1986, 6 mo after the oil spill. Except for sheltered channel mangrove sediments (*top*, SBS), patterns were very degraded. Note the examples of an open coast mangrove site (*middle*, LRM) and a seagrass site (*bottom*, LREN) where the residual hydrocarbon pattern was missing most volatile components ($<C_{20:1}$) and most resolved components.

Table 3.3 Visual degree of oiling related to UVF oil equivalents in surface sediments collected 6 mo after the Bahía Las Minas oil spill (see Table 3.2). Values are μg/g dry weight. *ND* = no data; *Nondet.* = nondetectable.

	Visual Degree of Oiling			
Habitat	Heavy	Moderate	Light	Unoiled
Mangrove	>1,800	ND	<700	<200
Seagrass Bed	>300	<200[1]	<150	Nondet.
Coral Reef	>40[2]	<30	ND	Nondet.

[1] Except two samples at LRN2.
[2] Except one sample at LRE.

Sediments at unoiled reefs contained only traces of fuel oil visible by UVF spectra. One of the samples contained 41 μg oil/g; the rest contained 0.

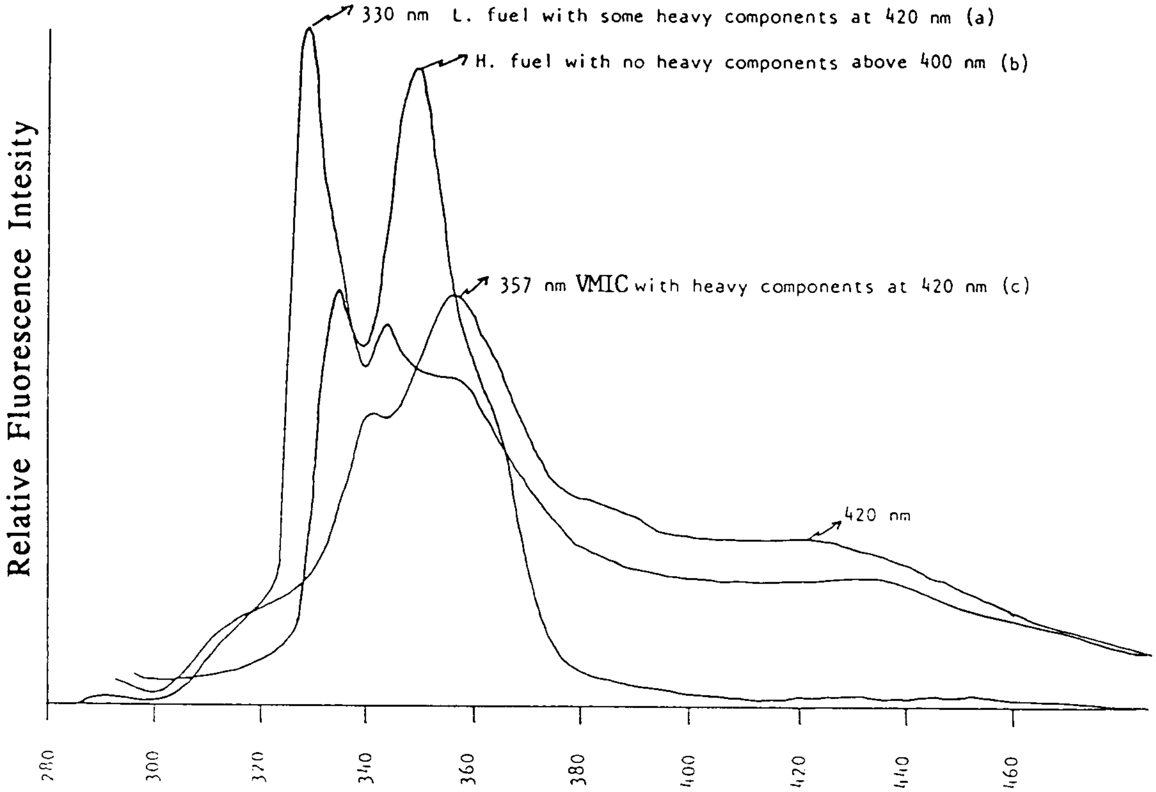
Chemical data generally corroborated visual estimates of the severity of oiling, as expected. Because some areas are flushed more than others by currents and waves, residual levels of oil at lightly and moderately oiled areas may change from initial levels.

3.4.5 Oil in the Water Column

In Table 3.4, the concentrations of oil suspended in the water column of heavily oiled mangrove areas based on GC and UVF analysis are listed. The chromatogram of the water sample from GALS compared to that of unweathered VMIC is shown in Figure 3.8. VMIC was 70% Venezuelan Crude and 30% Mexican Isthmian Crude, with a specific gravity of 27° API at 15.6°C and a reported density of 0.89 g/cc. Characterization of this oil using adsorption chromatography showed that it was 47.4% saturates, 6.4% light aromatics, 4.8% heavy aromatics, and 18.9% more polar aromatic hydrocarbons, which required $MeCl_2$ for elution; 22.5% remained unrecovered on the column. The carbon preference index (CPI), defined as the sum of odd-numbered *n*-alkanes divided by the sum of even-numbered *n*-alkanes over the C_{10} to C_{36} elution range, was close to 1. Residual oil suspended in the water column was devoid of most resolved components and was missing most of the low-molecular-weight components. This is the residual oil that would be available for washout to contiguous seagrass beds and reefs.

3.4.6 Conclusions

The 1986 survey of sediments generally confirmed the visually assessed relative ranking of severity of oiling of coastal habitats. Oil concentrations were highest in organic-rich sediments of mangroves; concentrations reached over 20% of



(a). Sample No. 83. Mangrove sediment. MARS.
(b). Sample No. 87. Reef sediment. DONR.
(c). Sample No. 85. Seagrass Sediment. MARG.

Fig. 3.7 Examples of synchronous excitation/emission UVF spectra. *L.* = light; *H.* = heavy.

sediment dry weight. It was anticipated that oil buried in mangrove sediments would act as a long-term reservoir, causing reoiling of coastal habitats over time.

GC analysis showed that initial rates of degradation of the spilled oil were high compared to oil trapped in temperate salt marshes, where reaching the stage of degradation observed in Panama would take close to 2 yr (Burns and Teal 1979). In assessing the GC and UVF patterns of 1986 samples, strategies for interpreting geochemical degradation patterns and integration of chemical data with biological studies had to be further developed to incorporate the rapid rate of degradation, as seen in this initial data set.

The size distribution of coral reef sediments showed that erosion was causing the deposit of increasing loads of fine particles on reefs in the heavily oiled areas of

Table 3.4 Concentration of petroleum hydrocarbons in water samples from two oiled mangrove sites. Determinations were by GC analysis of saturated fractions (*URE*) and UVF analysis of aromatic fractions.

Site	Volume (L)	URE (μg/L)	UVF (μg/L)
LRM	53	4.5	15.1
	45	6.7	29.1
GALS	115	1.9	4.8

Samples were collected by pumping seawater through glass columns filled with Amberlite XAD 2 resin. Columns were tied to mangrove prop roots with intakes approximately 0.5 m below the water surface. The water was saturated with crude oil emanating from sediments, as evidenced by surface sheens.

Bahía Las Minas compared to unoiled areas. Thus, it was anticipated that reefs would continue to receive oil-contaminated sediments from the increasing erosion occurring in contiguous mangroves and seagrass beds (Jackson et al. 1989; Guzmán et al. 1991).

3.5 Long-term Studies of Coral Reefs

3.5.1 Oil in Reef Sediments

Summary data for the content of oil in reef sediments over time, determined by GC and UVF analysis, are shown in Table 3.5. The same data are plotted in Figure 3.9 with UVF data on the x-axis and GC data on the y-axis. Using these two independent analytical methods, trace amounts of oil in reef sediments were clearly visible 4 yr postspill. The data in Figure 3.9 clearly show, however, that concentrations at oiled sites decreased overall through time and by 1990 heavily and moderately oiled sites contained only traces of oil (less than 7 μg/g dry weight by GC analysis). One unoiled reef (DMA) was contaminated by traces of heavy fuel oil visible in the sediments in 1988. As shown in Section 3.9, this oiling appeared to be recent heavy fuel oil and not VMIC. The other three unoiled reefs had just-detectable oil levels at all three samplings. Based on the content of oil in reef sediments, two sites visually classified as heavily oiled would have been chemically classified as moderately or lightly oiled (PM and LRE2).

The change through time in slope of the regressions (Fig. 3.9) indicated the composition of oil in sediments changed in such a manner that the most residual fractions were more fluorescent over time than the oil used for instrument calibration.

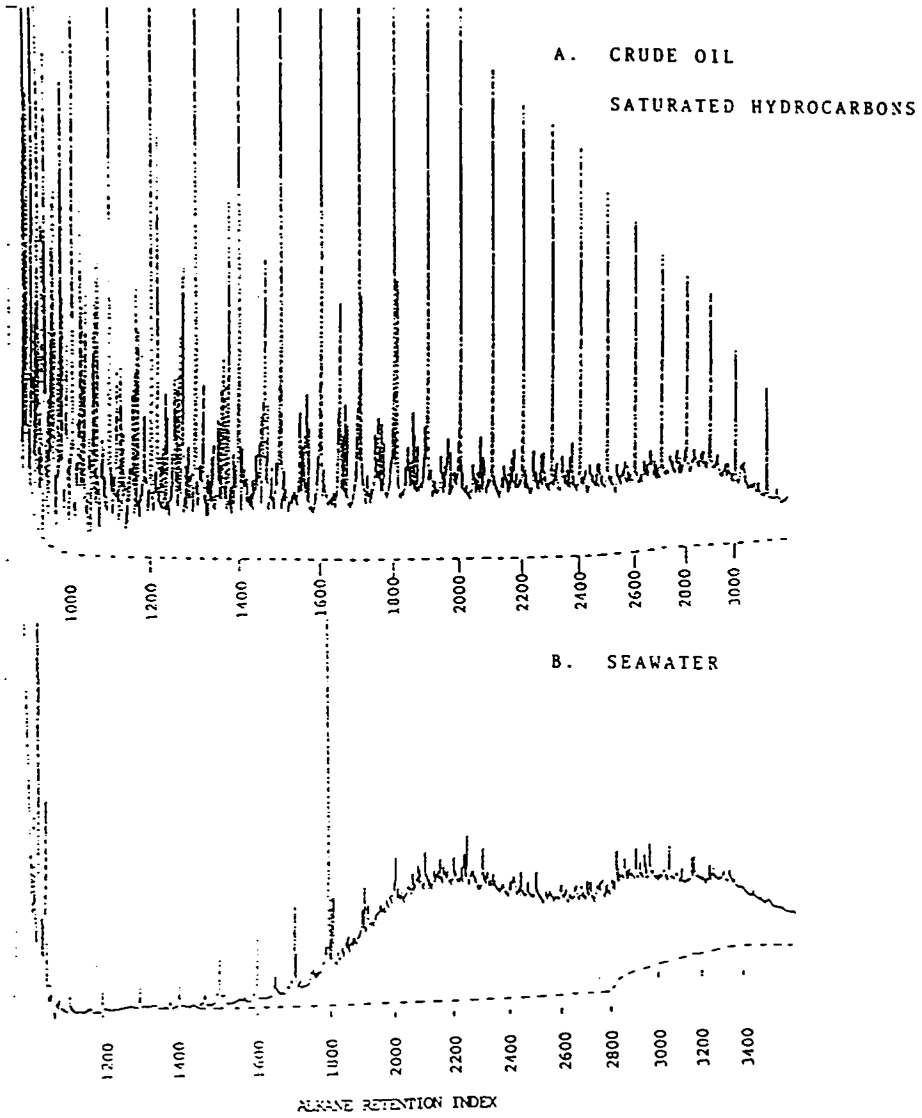


Fig. 3.8 Gas chromatograms of saturated hydrocarbons. *A*. VMIC oil; *B*. oil suspended in seawater (2 μg/L) at Galeta Sand (GALS, Fig. 3.5), September 1986.

3.5.2 Uptake of Oil by Corals in 1986

3.5.2.1 Quantitative Results

Corals collected in 1986 showed uptake of oil into tissues. The results of analyses of composited homogenized tissue from five pieces of *Siderastrea siderea*

Table 3.5 Summary of oil content in coral reef sediments by year, showing the visual classification of oiling of sites. The units are μg oil/g dry weight of sediments by UVF and GC-URE analyses. Samples were done in triplicate except for 1986 and 1988 extracts, which were pooled for GC analysis. The values shown are means (SD), except for the pooled samples.

Classif. and Site	1986			1988			1990		
	UVF	GC	Spectrum[1]	UVF	GC	Spectrum[1]	UVF	GC	Spectrum[1]
Unoiled Reefs									
JUG				1.7 (1.8)	0.2	Tr.H.F./~Ndt.	9.5 (8.8)	0.9 (0.1)	Ndt.
PALW	0 (0)	1	Tr.F./~Ndt.	1.7 (0.3)	0.0	Tr.H.F./~Ndt.	5.3 (3.4)	1.6 (0.7)	Ndt.
DONR	10 (18)	0.1	Tr.H.F./Ndt.	0.3 (0.1)	0.2	Ndt.	1.0 (0.7)	0.9 (0.6)	Ndt.
DMA				43 (12)	8.1	Cr./D.Cr. + alkanes	127 (21)	6.1 (0.7)	Cr./D.Cr. + alkanes
Moderately Oiled Reefs									
MAR3				54 (67)	7.0	Cr./V.D.Cr.	79 (30)	6.0 (2.0)	Tr.Cr./V.D.Cr.
NARS	18 (10)	6	Cr./D.Cr. or H.F.	14 (2)	0.4	Cr./~Ndt.	50 (11)	2.5 (1.1)	Tr.Cr./V.D.Cr.
Heavily Oiled Reefs									
LRE1				101 (33)	6.3	Cr./V.D.Cr.	37 (10)	5.6 (2.3)	Cr./V.D.Cr.
LRE2	40 (13)	9	Cr./D.Cr.	43 (14)	4.1	Cr./V.D.Cr.	70 (5)	6.2 (1.3)	Cr./V.D.Cr.
PM				15 (2)	1.7	Cr./V.D.Cr.	33 (12)	2.8 (1.0)	Cr./~Ndt.
GALC	406 (206)	85	Cr./D.Cr.	126 (62)	21.7	Cr./V.D.Cr.	38 (10)	3.0 (0.7)	Cr./V.D.Cr.
PAYN	209 (93)	110	Cr./D.Cr.	244 (137)	19.4	Cr./V.D.Cr.	34 (6)	3.0 (0.2)	Tr.Cr./V.D.Cr.
PAYW				264 (123)	21.6	Cr./V.D.Cr.	105 (36)	4.5 (0.5)	Cr. + F./D.Oil + alkanes

[1] Oil type by UVF spectrum: *Tr.F.* = trace fuel; *Ndt.* = nondetectable; *H.F.* = heavy fuel; *Cr.* = crude oil; *D.* = degraded; *V.D.* = very degraded.

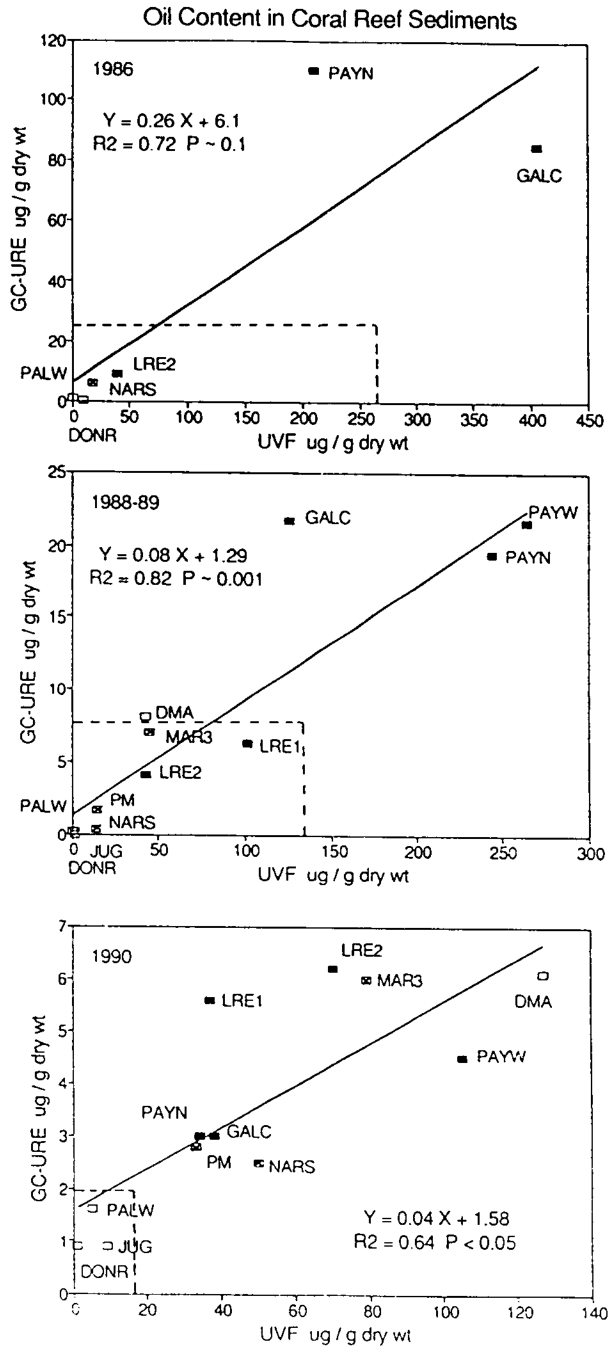


Fig. 3.9 Regressions of oil content in coral reef sediments estimated by UVF and GC-URE for each year of sampling. *Dotted boxes* in 1986 and 1988-89 show the range of values of the next sampling year. The *dotted box* for 1990 shows that the range of the three uncontaminated unoiled reefs was clearly distinguishable from the oiled reefs and the contaminated unoiled reef (DMA).

from each reef are shown in Table 3.6. These triplicate analyses established the means and standard deviations for samples collected over an area of several tens of square meters at depths ranging from 3 to 6 m. The horizontal lines break the data into statistically different groups of hydrocarbon concentrations based on 1-way analysis of variance (ANOVA) using group means and 95% confidence limits. The data include two estimates of the content of petroleum hydrocarbons, the amount of unresolved compounds (URE) determined by GC and UVF oil equivalents of the aromatic fractions. GC estimates, expressed as mg URE/g protein, split the data into two groups: low- and high-pollution areas. The same data expressed as mg URE/g lipid split the data into low-, medium-, and high-contamination areas. UVF oil equivalents per gram lipid split the data into unoiled, low-, medium-, and high-contamination areas. In all cases the relative ranking of contamination was from the unoiled site at PALW, through light to moderate levels at NARS and moderate to high levels at GALC, to very high levels at Payardí North (see Fig. 3.5).

This pattern was repeated in the quantitative results for *Agaricia tenuifolia*. The results of composite analyses of this species along with the means of the data for *Siderastrea siderea* and the visual evaluation of oiling severity are given in Table 3.7. Also listed in this table are the protein-to-lipid ratios of both species at each site. This ratio appeared to increase in heavily oiled samples.

3.5.2.2 Descriptive Results

Representative chromatograms of the saturated hydrocarbon fractions of corals at unoiled and oiled reefs are shown in Figure 3.10. These traces may be compared with the chromatograms of the original oil and the seawater extracts shown in Figure 3.8. Hydrocarbons in the spilled oil spanned the C_{10} to C_{36} elution range, with *n*-alkane peaks predominant over an unresolved mixture (URE). Chromatograms of extracts of corals from unoiled reefs showed clusters of discrete peaks at relative retention indices (RRIs) typical of the patterns of biogenic hydrocarbons synthesized by marine algae (RRIs of approximately 1500, 1700, and 1900 [Blumer et al. 1971]). These hydrocarbons were likely to have been synthesized by the dinoflagellate symbionts in coral tissues. Some of the other discrete peaks may also be biogenics. There was negligible contribution from plant waxes in the C_{21}-C_{31} elution range, which would be expected if corals accumulated biogenic hydrocarbons from surrounding seagrass beds and mangroves. Corals at oiled sites showed similar patterns of biogenics, but also an accumulation of hydrocarbons representative of a highly degraded oil. The oil pattern was similar to that seen in water samples collected at oiled mangrove sites (Fig. 3.8). Compared with the original oil, the volatile and low-boiling components were missing, as were most *n*-alkanes and other resolved hydrocarbons. This finding emphasizes the residual unresolved pattern of hydrocarbons typical of highly degraded oil residues.

These data documented the uptake of dissolved or dispersed hydrocarbons by subtidal corals within 1 yr of the oil spill. Corals appeared to take up hydrocarbons primarily from the water column, as indicated by the GC patterns. There were

Table 3.6 Concentration of hydrocarbons in *Siderastrea siderea* in 1986, expressed as mg/g lipid or protein. The horizontal lines mark significantly different sites (ANOVA, $P < .05$).

	Saturates			Unsaturates	
Site	Biogenics[1] (mg/g lipid)	URE[2] (mg/g lipid)	URE (mg/g protein)	UVF[3] oil (mg/g lipid)	URE (mg/g lipid)
PALW	1.4	1.8	0.40	0.2	2.8
	0.3	0.6	0.11	>0.1	1.8
	0.2	0.6	0.15	>0.1	0.2
NARS	2.4	1.6	0.30	1.8	2.5
	0.6	1.2	0.29	0.9	0.5
	0.6	1.3	0.40	1.0	0.6
GALC	3.8	2.6	0.82	5.8	2.5
	2.1	2.5	0.58	4.4	2.6
	1.5	2.5	0.71	2.7	1.9
PAYN	2.8	4.6	0.72	23.6	2.8
	3.8	3.9	0.93	21.3	4.0
	3.3	6.8	0.63	41.4	4.3

[1] Biogenics are the sum of multiple peaks with relative retention indices (RRI) near 1500, 1700, and 1900, which are common biogenic hydrocarbons produced by marine algae.

[2] URE is the GC signal generated by the complex mixture of hydrocarbon residues that cannot be resolved into individual peaks. This is a conservative estimate of petroleum hydrocarbons in samples.

[3] UVF oil units were determined using the spilled oil as the calibration standard (Sect. 3.3.2) and measuring fluorescence at 360 nm emission with 310 nm excitation.

similar levels of oil in sediments but a significantly lower concentration of hydrocarbons in coral tissues (when expressed per gram lipid) between GALC and PAYN. Thus, it would appear that oil in sediments was not necessarily available for uptake by corals (compare Tables 3.5 and 3.8). This conclusion is suggested by the lack of biogenic hydrocarbons typical of seagrasses or mangroves in coral tissues, despite the location of reefs where receiving significant input of detritus from these contiguous ecosystems is expected. High levels of oil hydrocarbons were suspended in seawater near mangroves (Table 3.4), indicating that highly oiled mangrove sediments would be a source of contaminated water for an extended period after the spill. Coral mortality, shown by decreases in coral cover, was consistent with the chemical ranking of the severity of oiling, determined by analyzing the hydrocarbon composition of surviving corals. The coral data in Jackson et al. (1989) showed that percent cover of corals decreased by 76% at 3 m depth and 45% at 9 to 12 m depth at GALC, which was visually assessed as heavily oiled. Reductions in cover were significantly less at moderately oiled reefs and were not observed at unoiled reefs in 1986.

Table 3.7 Concentration of petroleum hydrocarbons in coral tissues, 1986. Hydrocarbons are expressed as mg URE/g lipid or protein, or mg/g lipid by UVF. Also shown are protein-to-lipid ratios and visual site classifications.

Site	Coral	URE (mg/g lipid)	URE (mg/g protein)	UVF (mg/g lipid)	Protein/ Lipid	Degree of Oiling
PALW						unoiled
	Siderastrea	*1.0*	*0.2*	*0.1*	*4.6*	
	Agaricia	0.3	0.1	>0.1	2.5	
NARS						moderate
	Siderastrea	1.4	0.3	1.2	4.3	
	Agaricia	0.4	0.1	0.4	2.6	
GALC						heavy
	Siderastrea	2.5	0.7	4.3	4.2	
	Agaricia	2.8	1.0	3.1	4.5	
PAYN						heavy
	Siderastrea	5.1	0.8	28.8	6.1	
	Agaricia	8.3	1.2	49.7	6.4	

3.5.2.3 Discussion

Corals in experimental systems have been shown to incorporate petroleum hydrocarbons in their tissues as a result of dosing (Peters et al. 1981; Knap et al. 1982). In addition to documenting the incorporation of petroleum hydrocarbons into coral tissue and demonstrating a positive correlation between tissue burden and mortality, the data from the first year of this study suggested a possible modification in the protein-to-lipid ratios of corals heavily stressed by oil. Although the sample size was too small to show a statistically significant change in this ratio, the possibility is consistent with laboratory studies in which corals were stressed by addition of dispersed oil.

Cook and Knap (1983) reported a temporary reduction in photosynthetic rate and a decrease in lipid biosynthesis with wax ester and triglyceride synthesis reduced 97% and 95%, respectively, relative to undosed control corals. Fatty acid, sterol and polar lipid synthesis rates were reduced 83% to 21% compared to controls. While these effects were only visible for a few hours in laboratory tank experiments, data from samples collected 5 mo after the Bahía Las Minas oil spill indicate that such physiological effects may be chronic in highly contaminated reef systems.

Rinekevich and Loya (1979) reported that corals on reefs subjected to chronic oil pollution showed decreased reproductive success; the same is true for *Siderastrea siderea* from two heavily oiled reefs in Bahía Las Minas (Guzmán et al., Chap. 7). Peters et al. (1981) observed impaired development of reproductive tissues,

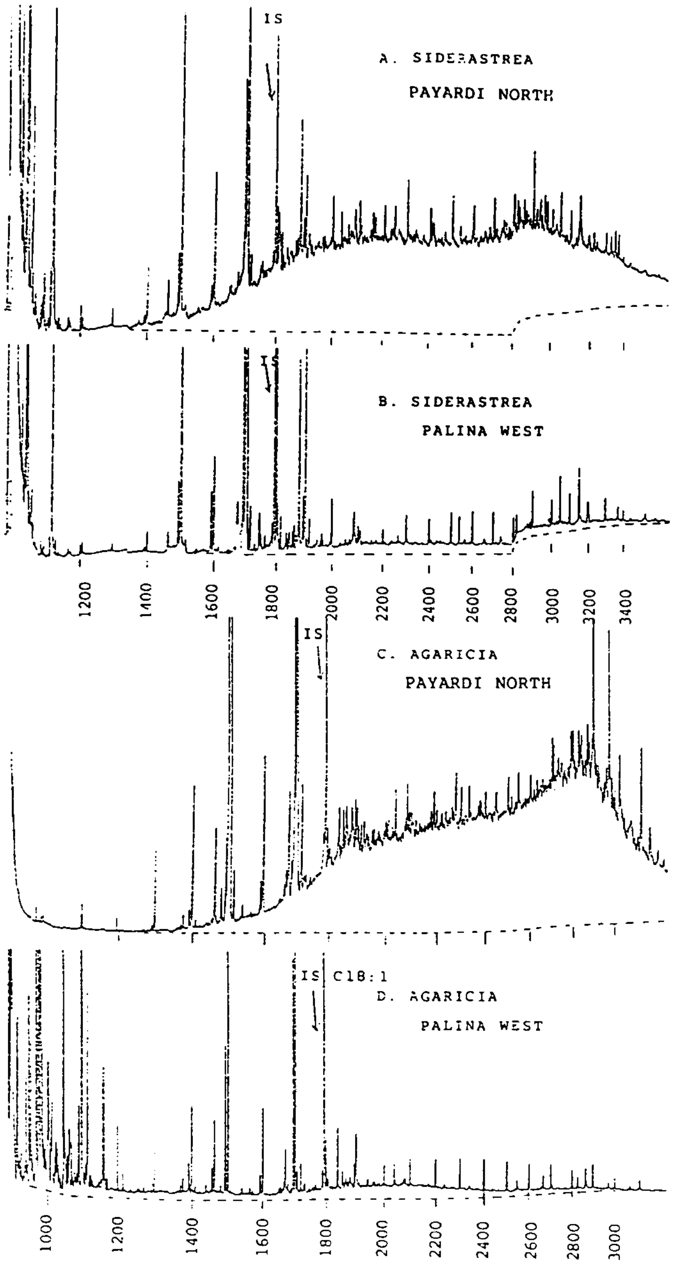


Fig. 3.10 Gas chromatograms (with FID) of saturated hydrocarbons in coral tissues in 1986. *Siderastrea* = *S. siderea*; *Agaricia* = *A. tenuifolia*. Payardí North (PAYN) and Palina West (PALW) are oiled and unoiled reefs, respectively. Shown also are the elution positions of *n*-alkane standards used to compute relative retention indices of resolved peaks; *IS* = *n*-octadecene added as an internal standard. The *dashed line* is the column bleed signal. See Table 3.2 for concentrations of petroleum hydrocarbons in these samples.

Table 3.8 Summary of oil content in *Siderastrea siderea* tissues by year. Units are mg oil/g EOM (Sect. 3.3.2) by UVF and GC-URE analyses. Samples were done in triplicate, except for some samples, which were pooled for GC analysis. The values shown are means (SD), except for the pooled samples.

	1986		1988/89		1990	
Site	UVF	GC-URE	UVF	GC-URE	UVF	GC-URE
Unoiled Reefs						
JUG			0.0 (0.0)	1.0 (1.0)		
PALW	0.1 (0.0)	1.0 (0.6)	0.0 (0.0)	0.8	0.7 (0.2)	0.0
DONR	0.1 (0.1)	0.0 (0.0)	0.3 (0.1)	1.6	0.1 (0.0)	0.0 (0.0)
DMA			0.5 (0.4)	0.8		
Moderately Oiled Reefs						
MAR3	0.1 (0.0)	0.0 (0.0)	0.4 (0.1)	1.2	0.1 (0.0)	0.0 (0.0)
NARS	1.2 (0.4)	1.4 (0.2)	0.3 (0.1)	0.7	0.1 (0.0)	0.0 (0.0)
Heavily Oiled Reefs						
LRE1			0.6 (0.5)	0.0		
LRE2	2.9 (0.3)	0.0 (0.0)	0.4 (0.2)	1.9	0.4 (0.0)	0.0 (0.0)
PM	0.1 (0.7)	0.0 (0.0)	0.1 (0.1)	0.4	0.1 (0.0)	0.0 (0.0)
GALC	4.3 (1.3)	2.5 (0.0)	0.2 (0.1)	1.7 (0.0)	0.1 (0.0)	0.0 (0.0)
PAYN	28.8 (9.0)	5.1 (1.2)	0.4 (0.2)	2.0 (2.6)	0.4 (0.1)	0.0
PAYW			0.4 (0.2)	1.4		

Other species analyzed but not shown here were *Porites asteroides*, *Agaricia tenuifolia*, *Acropora palmata*, and *Diploria clivosa* or *strigosa*.

degeneration and loss of symbiotic zooxanthellae, and atrophy of mucus secretory cells and muscle bundles in corals exposed to oil in long-term tank experiments.

In a study of effects of petroleum hydrocarbons on lipid metabolism of larval lobsters, Capuzzo et al. (1984) demonstrated that exposure to oil lowered levels of triglycerides and raised levels of sterols compared to controls. These authors also noted fatty acid stores were altered, indicating decreased storage in energy reserves and decreased mobilization of essential fatty acids into phospholipid pools. These alterations in lipid metabolism were related to alterations in larval development, metamorphosis, and growth rates (Capuzzo et al. 1984). Alterations in lipid metabolism would also be expected to have profound effects on the physiology and reproductive success of corals (see Guzmân et al., Chap. 7).

A large portion of the energy fixed in algal-coral photosynthesis goes into mucus production (Crossland et al. 1980). This mucus, which is rich in wax esters, triglycerides, and other lipids, is an energy-rich link in the coral reef food chain (Benson and Muscatine 1974). Thus, if the lipid synthesis system is disrupted in corals stressed by petroleum hydrocarbons, not only would coral physiology be adversely affected, but such an effect would cascade to other components of the ecosystem dependent on corals for food and space.

3.5.3 Corals in Subsequent Years

Initial findings about the uptake of oil into coral tissues in 1986 were published by Burns and Knap (1989). Samples collected in subsequent years did not show significant levels of oil in tissues of surviving corals at oiled reefs. Table 3.8 gives summary data for *Siderastrea siderea* with sites ranked according to the visual assessment of oiling. Concentrations of oil in tissues were consistent with the results of the sediment analyses shown in Table 3.5. In 1988 levels of oil in coral tissues at heavily oiled reefs had dropped to the same level as moderately oiled reefs. Also in agreement with the sediment data, corals at the unoiled reef, DMA, had trace oil contamination in 1988. Levels of oil at all reefs had dropped to just detectable by 1990, 4 yr after the oil spill. Tissues of four other scleractinian coral species were screened for oil in 1988. Table 3.9 shows summary data for the 1988-1989 collections of these other species. The most heavily oiled reef, PAYN, showed no significant difference in tissue oil levels compared to unoiled reefs (PALW and JUG) for all five species; only *S. siderea* was analyzed for hydrocarbons in 1990.

Protein-to-lipid ratios of the five species collected in 1990 showed that *S. siderea* and *Agaricia tenuifolia* at heavily oiled reefs had returned to levels at unoiled reefs (Table 3.10). None of the other species differed in this ratio between the heavily oiled and unoiled reef in 1990.

3.5.4 Conclusions about Long-term Effects of the Spill on Coral Reefs

Hydrocarbon chemistry data showed a clear association with toxic effects on coral populations soon after the oil spill (Guzmán et al., Chap. 7). Within 2 yr postspill, oil levels in sediments and coral tissues dropped to near-background levels; after 4 yr levels of oil were barely detectable. This pattern suggests the short-term damage to reefs noted by Guzmán et al. (1991) resulted from the initial dramatic alteration in habitat and species composition, aggravated by periodic inputs of contaminated fine sediments washing out of contiguous mangroves. It also appears that long-term, chronic damage to reefs resulted from increased sedimentation rather than from the persistence of toxic hydrocarbons in the reef ecosystem.

3.6 Oil in Seagrass Beds

Time-series data for sediments at long-term study sites in subtidal seagrass beds are shown in Table 3.11. Note that the 1986 values in this table are from analyses of separate samples collected in October and November 1986 to take advantage of a sediment core sample, and not those reported in Table 3.2. These data show the incorporation of high levels of oil at least 20 cm deep in seagrass bed sediments. Sites with thousands of μg of oil per g of sediments in 1986 dropped to

Table 3.9 Summary lipid, concentration of oil by UVF, and GC-URE determinations of the 1988/89 coral collection. Samples were scraped immediately after collection and maintained frozen until analysis in 1989 and 1990.

Site	No.	Species[1]/ Inv. no.	Wet wt. (g)	EOM (mg)	GC-URE (mg/g[2])	Rec. (%I.S.[3])	UVF crude (mg/g[2])	Spectra/GC Evaluation[4]
Unoiled Reefs								
PALW	P3 C65	PA 1,7,9	39.1	215	0.3	68	0.0	
	P3 C66	PA 2,4,5	40.4	211	0.3	67	0.2	Tr. Fuel/
	P3 C67	PA 3,6,8	40.0	232	0.1	82	0.1	~Nondet.
					0.2 (0.1)		0.1 (0.1)	
	P3 C71	DS 1,4,6	30.5	261	0.0	74	0.1	Tr. H.Fuel/
	P3 C72	DS 2,3,5	32.6	250	0.2	67	0.1	~Nondet.
	P3 C73	DS 7,8,9	30.8	354	0.0	76	0.0	
					0.1 (0.1)		0.1 (0.0)	
	P3 C77	AT 1,6,9	18.7	130	0.0	87	0.0	
	P3 C78	AT 2,3,5	47.6	481	0.0	112	0.0	~Nondet./
	P3 C79	AT 4,7,9	36.6	220	0.0	70	0.0	~Nondet.
					0.0 (0.0)		0.0 (0.0)	
	P3 C86	AP 1,2,5	40.7	288			0.0	
	P3 C87	AP 3,7,9	41.6	290			0.0	Tr.Fuel/
	P3 C88	AP 4,6,8	41.8	202	0.1		0.1	~Nondet.
					(combined)	52	0.0 (0.0)	
JUG	P3 C81	PA 1,2,4	20.2	112			0.2	
	P3 C82	PA 5,8	35.4	221			0.1	Tr. Fuel/
	P3 C89	PA 6,7,9	42.0	288	0.6		0.1	~Nondet.
					(combined)	44	0.1 (0.0)	
Moderately Oiled Reef								
MAR3	P3 C43	PA 1,7,9	34.7	28	1.7	70	0.5	
	P3 C44	PA 2,4,6	33.1	29	1.8	65	1.1	Tr. Crude
	P3 C45	PA 3,5	30.6	130	0.4	59	0.2	
					1.3 (0.6)		0.6 (0.4)	

Table 3.9 Summary lipid, concentration of oil by UVF, and GC-URE determinations of the 1988/89 coral collection (continued).

Site	No.	Species[1]/ Inv. no.	Wet wt. (g)	EOM (mg)	GC-URE (mg/g[2])	Rec. (%I.S.[3])	UVF crude (mg/g[2])	Spectra/GC Evaluation[4]
Heavily Oiled Reefs								
PAYN	P3 C60	PA 1,2,5	29.8	233	0.7	73	0.2	
	P3 C61	PA 3,4,8	30.2	lost			lost	
	P3 C62	PA 6,7,9	25.1	203	0.7	62	0.7	Tr. Crude
					0.7 (0.0)		0.5 (0.3)	
	P3 C68	DS 1,4,8	32.3	194	0.8	62	0.3	Tr.H.Fuel or Crude
	P3 C69/70	DS 2,5,6, 7,9	53.2	484	0.3	62	0.1	
					0.5 (0.2)		0.2 (0.1)	
PAYN	P3 C74	AT 1,3,7	52.3	374	0.2	68	0.2	
	P3 C75	AT 2,6,8	51.8	387	0.1	72	0.3	Crude/ ~Nondet.
	P3 C76	AT 4,5,9	48.0	402	0.2	55	0.1	
					0.2 (0.0)		0.2 (0.1)	
	P3 C83	AP 1,2,5	42.8	376			0.2	
	P3 C84	AP 4,6	47.1	279			0.2	Tr.H.F./Cr.[3]/ ~Nondet.
	P3 C85	AP 7,8,9	42.4	342	0.1		0.2	
					(combined)	68	0.2 (0.0)	

---

[1] *PA* = *Porites astreoides*; *DS* = *Diploria strigosa*; *AT* = *Agaricia tenuifolia*; *AP* = *Acropora palmata*.

[2] mg/g EOM.

[3] *Rec.* = recovery; *I.S.* = internal standard.

[4] *Tr.* = trace; *H.* = heavy; *F.* = Fuel; *Cr.* = Crude; *~Nondet.* = nearly nondetectable.

hundreds by 1988. This decrease in concentration suggests that the time span for possible major toxic effects on seagrass communities is on the order of 2.5 yr. Less toxic and sublethal effects probably persist for a longer period.

Oil residues in seagrass sediments were highly degraded within 6 mo of the spill. Figure 3.11 shows a time series of chromatograms for one heavily oiled site; oil residues were highly degraded at every sampling. PAH data for these samples are tabulated in Appendix Tables B.1 and B.2; no further analyses of seagrass sediments were undertaken. However, because of the close proximity of seagrass beds to heavily oiled mangroves, this habitat is expected to experience continued reoiling events during storms.

Table 3.10 1990 coral collection: lipid and protein determinations of fresh tissue samples.

Site	Sp.[1]	Sample no.	Lipid (mg/g wet wt.)	Protein (mg/g wet wt) $\bar{x}$	Protein (mg/g wet wt) SD	Ratio P/L[2]	Site	Sp.[1]	Sample no.	Lipid (mg/g wet wt.)	Protein (mg/g wet wt) $\bar{x}$	Protein (mg/g wet wt) SD	Ratio P/L[2]
PAYN (Oiled)							**PALW** (Unoiled)						
	SS	433	2.86	12.2	2.6	4.27		SS	424	2.37	9.7	0.2	4.11
		434	2.86	12.9	3.3	4.51			425	2.98	10.2	0.2	3.43
		435	3.65	12.4	1.5	3.39			426	2.80	12.7	0.4	4.52
						4.05 (0.48)							4.02 (0.45)
	PA	436	3.39	15.0	0.2	4.41		PA	427	2.43	14.5	0.1	5.94
		437	2.60	10.6	1.0	4.08			428	3.16	12.4	0.2	3.92
		438	3.77	12.8	0.4	3.40			429	3.55	15.6	0.3	4.40
						3.96 (0.42)							4.76 (0.86)
	AP	439	3.33	14.1	1.9	4.24		AP	430	3.59	11.9	0.2	3.30
		440	4.39	13.9	1.7	3.16			431	3.36	16.0	0.5	4.75
		441	4.25	13.1	1.0	3.07			432	4.47	11.4	0.1	2.54
						3.49 (0.53)							3.53 (0.92)
	AT	487	4.32	13.9	0.8	3.22		AT	484	4.41	8.7	0.7	1.98
		488	2.66	12.4	1.2	4.64			485	2.82	13.6	0.9	4.82
		489	2.39	8.7	0.5	3.64			486	1.60	10.2	0.6	6.37
						3.83 (0.60)							4.39 (1.82)
	DS	481	6.41	15.75	1.36	2.46		DS	478	5.67	17.4	1.3	3.07
		482	7.82	17.85	1.33	2.28			479	7.16	15.3	1.5	2.14
		483	5.84	17.75	1.94	3.04			480	10.20	16.13	1.1	1.58
						2.59 (0.32)							2.26 (0.61)

[1] *SS = Siderastrea siderea*; *PA = Porites astreoides*; *AP = Acropora palmata*; *AT = Agaricia tenuifolia*; *DS = Diploria strigosa.*

[2] *P* = protein; *L* = lipid.

Table 3.11 Hydrocarbons in seagrass sediments determined by UVF and GC analyses. Units are μg/g dry weight.

	Nov. 1986		Apr. 1987		Dec. 1988	
Site	UVF	GC-URE	UVF	GC-URE	UVF	GC-URE
Unoiled Beds						
DONT	0	0	6	0	0	0
PALN	0	0	1	0	0	0
LINE			102	14	4	0
BNV					6	0
Moderately Oiled Bed						
NARC	596	82			98	7
Heavily Oiled Beds						
PGN			211	19	188	11
LREN	4,020	2,021	6,683	203	658	60
LRS	792	111	1,198	137	501	51
MINN	1,499	151	957	75	607	52
LREN Core						
0-2 cm					391	19
8-10 cm					665	64
18-20 cm					553	39

Regression of log(μg/g) by UVF (X) vs. log(μg/g) by GC-URE (Y): $Y = 0.72X - 0.22$, $R^2 = 0.91$, $N = 25$, $P < .001$.

Even in the most heavily oiled subtidal seagrass beds, the root and substratum structure remained intact after the spill. Although some toxicity to seagrasses and associated animals was noted, the basic structure of the environment, the root and rhizome mat, remained intact except along shoreward margins of beds. As a result, the area of oiled beds decreased (Marshall et al., Chap. 10). This observation was in sharp contrast to the sediment movement associated with the erosion of shallower and intertidal seagrass beds. Massive erosion of the heavily oiled intertidal seagrass bed at LRN occurred in February 1988 (Steger and Caldwell, Chap. 6). As will be discussed again in Section 3.7, this was probably the mechanism for oil transport into previously unoiled mangrove sites, such as HIDC (see Garrity and Levings, Chap. 9).

Based on hydrocarbon chemistry for seagrass beds, it seems organic-rich sediments accumulated enough oil to exhibit acutely toxic effects on sensitive fauna for a period of at least 2.5 yr. During this time, major erosional events would be expected to redistribute oil loads in shallow subtidal and intertidal ecosystems.

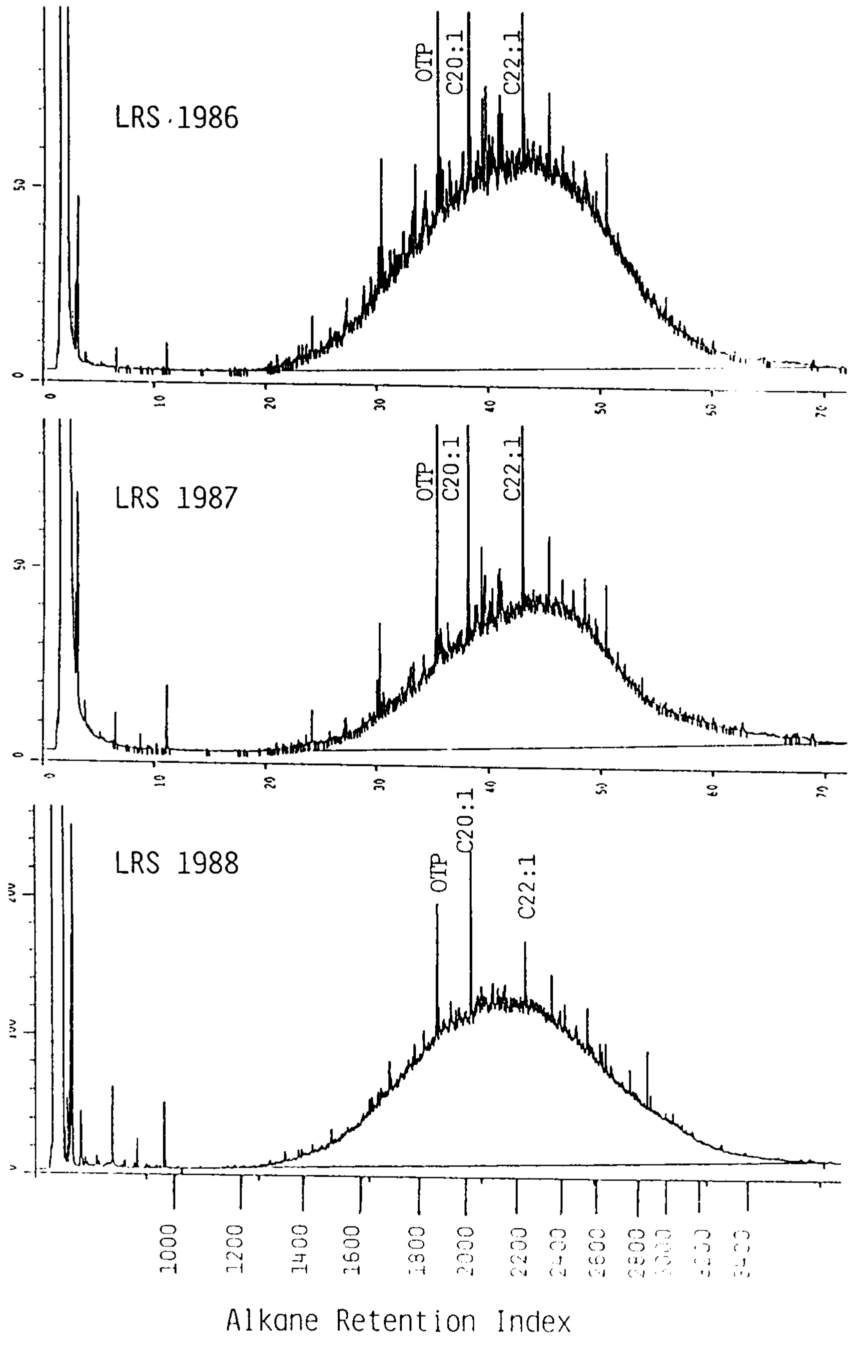


Fig. 3.11 Gas chromatograms through time of seagrass sediments at LRS (Fig. 3.5). Samples were highly degraded in all 3 yr sampled. The labeled peaks are surrogate and internal standards.

3.7 Persistence of Oil in Mangroves

This section focuses on the persistence of high levels of the spilled oil in mangrove sediments and the continued reoiling of coastal habitats for over 5 yr postspill.

3.7.1 Sediment Oil Loads and Weathering Patterns of the Spilled Oil

Sediment cores collected in mangroves at oiled areas in 1986 during the initial survey showed oil had penetrated at least 20 cm into sediments. The oil presumably moved by tidal action, diffusion, down animal burrows, and (in later years) down channels created by decaying roots of dead mangrove trees. Only a few samples were collected near long-term sites in the initial survey (App. Table B.3). After biologists selected study sites for the long-term assessment, triplicate sets of cores were collected at each site in 1989 and 1990 (3 and 4 yr postspill).

The time-series data show oil concentrations remained high in mangrove surface sediments for at least 4 yr and that more oil was transported to depth in later years. In Table 3.12, the oil determinations by UVF and GC and the determinations of the sum of individual polynuclear aromatic hydrocarbons (PAHs) by GC/MS are presented. There was some year-to-year variability in the data, probably due to patchy distribution of oil residues in sediments and across sites. An attempt was made to minimize sampling variability by combining the 0-2 cm, 8-10 cm, and 18-20 cm sections from three cores at each station. Archived drawings and photographs documented the patchy distribution of oil in cores. Tables 3.13, 3.14, and 3.15 present the untransformed data for UVF and GC determinations and the sum of individual PAHs. Concentrations of individual PAHs are tabulated for each sample in Appendix Tables B.1 and B.2.

Weathering processes of evaporation, dissolution, microbial degradation, and photochemical decomposition dramatically altered the composition of oil residues in surface sediments just 6 mo after the spill. Only in heavily oiled sediments could the straight-chain or branched-chain alkanes from the oil be quantified. In most sediment samples the hydrocarbon patterns determined by GC contained up to 99% unresolved components, indicating that microbial degradation had removed the resolvable components. In contrast, reaching this stage of degradation in temperate saltmarshes requires approximately 2 yr (Burns and Teal 1979). Very few mangrove sediment samples 3 and 4 yr postspill contained quantifiable levels of marker alkanes. However, residual pools of oil in mangrove sediments remained fluid enough to flow out when cores were extracted. Oil slicks have been observed repeatedly during the 5 yr since the spill to leach out of oiled mangroves and move along the coast, sometimes redistributing oil into previously unoiled sites.

Figure 3.12 shows again the gas chromatograms of the hydrocarbon fractions of the Venezuelan/Mexican Isthmian Crude (VMIC) spilled,oil oozing from depth in a core hole at a heavily oiled channel site (PMRE) 3.5 yr postspill, and oil

Table 3.12 Summary UVF and GC-THC oil content as log(μg/g dry weight) and total PAHs as log(ng/g dry weight) in mangrove sediments.

	Oiled										Unoiled								
	Sept. 1986			May 1989			July 1990				Sept. 1986			May 1989			July 1990		
	UVF	GC	PAH	UVF	GC	PAH	UVF	GC	PAH		UVF	GC	PAH	UVF	GC	PAH	UVF	GC	PAH
A. Stream Sites																			
LRRS (RO1)										HIDR (RU1)									
0-2 cm				4.2	3.3	3.4	5.0	4.1	4.7	0-2 cm				2.4	2.3	2.7	2.2	1.9	2.6
8-10 cm				3.7	2.6	3.2	4.9	4.1	4.7										
18-20 cm				2.5	2.0	2.2	4.2	3.3	3.8	15-20 cm									
PAYR (RO2)										UNR (RU2)									
0-2 cm				4.7	3.8	4.3	4.6	3.8	4.7	0-2 cm				2.1	1.7	2.6	2.3	2.6	2.4
8-10 cm				5.1	4.4	5.1	4.6	3.8	4.6										
18-20 cm				3.2	2.7	2.7	3.5	2.8	3.5										
PMRE (RO3)										ALER (RU3)									
0-2 cm				4.7	3.9	4.5	4.5	3.5	4.0	0-2 cm				2.1	1.8	3.0	2.7	1.7	3.0
8-10 cm				4.9	4.2	5.0	3.7	3.0	3.5										
18-20 cm				3.2	2.9	4.0	2.3	2.0	4.2										
PMRW (RO4)										MERR (RU4)									
0-2 cm				4.8	4.0	4.9	5.1	4.1	5.0	0-2 cm				1.7	1.5	1.8	2.3	1.9	2.2
8-10 cm				3.3	2.6	3.6	4.6	3.9	4.7										
18-20 cm				2.0	1.5	2.6	4.0	3.3	4.5										

Table 3.12 Summary UVF and GC-THC oil content as log(μg/g dry weight) and total PAHs as log(ng/g dry weight) in mangrove sediments (continued).

	Oiled										Unoiled								
	Sept. 1986			May 1989			July 1990				Sept. 1986			May 1989			July 1990		
	UVF	GC	PAH	UVF	GC	PAH	UVF	GC	PAH		UVF	GC	PAH	UVF	GC	PAH	UVF	GC	PAH
B. Channel Sites																			
SBCE (CO1)										MACS² (CU1)									
0-2 cm				5.5	4.8	6.3	4.7	3.5	3.8	0-2 cm	2.4	2.1		2.1	1.4	2.4	2.5	1.5	2.4
8-10 cm				5.3	4.6	5.2	5.4	4.6	4.8										
18-20 cm				4.0	2.7	3.6	5.4	4.7	5.3	18-20 cm	1.3	0.7							
SBCS¹ (CO2)										MACN³ (CU2)									
0-2 cm	5.0	5.1	6.3	5.3	4.6	5.2	5.0	4.4	4.7	0-2 cm	2.8	2.7		2.4	2.3	3.0	2.8	2.4	3.2
8-10 cm				4.6	3.9	4.6	4.6	4.2	4.5	8-10 cm				2.9	2.5		3.1	2.4	
18-20 cm	3.5	3.7	4.8	3.6	2.9	3.3	2.6	2.1	3.0	18-20 cm	3.2	4.0		2.8	2.2		2.8	2.7	3.2
PCE (CO3)										HIDC⁴ (CU3)									
0-2 cm				5.2	4.0	5.0	5.0	4.0	4.3	0-2 cm	1.6	1.5		1.9	1.6	1.5	3.3	2.3	2.9
8-10 cm				3.5	2.6	2.2	4.6	3.8	4.3	8-10 cm				1.4	1.6	2.0	3.2	2.9	2.9
18-20 cm				3.2	2.3	2.1	3.4	2.6	3.1	18-20 cm	1.6	1.6		1.3	1.3	0.8	2.6	1.9	2.7
PCS (CO4)										SBCW CU4)									
0-2 cm				4.9	4.1	5.1	5.1	4.6	5.4	0-2 cm				3.0	2.0	2.6	4.5	3.5	3.7
8-10 cm				5.3	4.5	5.5	5.0	4.4	5.7	8-10 cm				2.6	1.9	2.7	3.1	2.1	2.8
18-20 cm				4.6	3.6	4.6	3.5	2.5	3.4	18-20 cm				0.9	1.5	1.2	1.7	1.0	1.8
LRCW (CO5)										LRCS (CU5)									
0-2 cm				4.1	4.3	3.3	4.4	3.6	4.6	0-2 cm				3.5	2.9	3.0	3.8	3.1	3.1
8-10 cm				3.5	2.4	3.2	3.0	1.7	2.9	8-10 cm				3.4	3.2	3.6	3.0	2.4	2.8
18-20 cm				3.5	1.9	3.3	4.8	3.8	4.9	18-20 cm				2.4	1.4	2.1	1.8	1.7	2.0

Table 3.12 Summary UVF and GC-THC oil content as log(μg/g dry weight) and total PAHs as log(ng/g dry weight) in mangrove sediments (continued).

	Oiled										Unoiled								
	Sept. 1986			May 1989			July 1990				Sept. 1986			May 1989			July 1990		
	UVF	GC	PAH	UVF	GC	PAH	UVF	GC	PAH		UVF	GC	PAH	UVF	GC	PAH	UVF	GC	PAH
C. Open Coast Sites																			
MINM (OO1)										MSM (OU1)									
0-2 cm				4.2	3.3	3.6	4.7	3.7	4.0	0-2 cm				0.0	0.0	0.0	0.8	0.6	1.6
8-10 cm				3.1	2.0	2.2	3.5	2.7	3.3										
18-20 cm				1.9	1.1	0.6	3.7	2.7	3.3										
PGM (OO2)										PBM (OU2)									
0-2 cm				4.7	3.9	4.7	5.1	4.1	4.6	0-2 cm				0.6	0.2	0.6	1.6	0.8	1.9
8-10 cm				4.4	3.2	4.2	4.9	3.6	4.1										
18-20 cm				4.1	3.2	4.5	4.3	2.7	3.8										
DROM[5] (OO3)										PADM (OU3)									
0-2 cm	4.2	3.2		4.7	3.6	4.7	4.8	3.6	4.3	0-2 cm				1.3	0.2	0.8	1.1	0.3	1.9
8-10 cm	5.0	3.6	5.0	4.7	3.8	4.4													
18-20 cm	3.0	2.1		3.5	2.5	3.4													
PMM (OO4)										LINM (OU4)									
0-2 cm				4.0	3.2	2.3	5.4	4.3	5.1	0-2 cm				1.5	1.1	1.7	1.5	0.9	2.2
8-10 cm				3.6	2.2	2.9	4.8	4.1	4.8										
18-20 cm				2.8	1.6	2.1	4.2	3.4	4.1										

[1] 1986 site was SBS, approximately 200 m across the channel from SBCS (see Fig. 1.5).

[2] 1986 site was MARS, approximately 300 m south of MACS (see Fig. 1.5).

[3] 1986 site was MARM, outside an oil-containment boom; MACN was inside the boom on the opposite shore of the lagoon (see Fig. 1.5).

[4] HIDC initially was unoiled and became oiled before the 1990 collection (see Chap. 9). The 1986 site was HRB; approximately 250 m across the channel (see Fig. 1.5).

[5] 1986 site was LRM, approximately 500 m southwest of DROM (see Fig. 1.5).

Table 3.13 Oil content of mangrove sediments as measured by UVF. Units are mg/g dry weight. For sites separated by a "/," the second site was sampled only in 1986; refer to Figure 1.5. *Hab./Oil.* = habitat and oiling; *S* = stream; *C* = channel; *OC* = open coast; *O* = oiled, *U* = unoiled; ~ *Nondet.* = nearly nondetectable.

		Sept. 1986		May 1989			July 1990			1991 Ooze Oil (mg)
Hab./ Oil.	Site	0-5 cm	15-20 cm	0-2 cm	8-10 cm	18-20 cm	0-2 cm	8-10 cm	18-20 cm	
S/O	LRRS			16.33	5.40	0.35	89.56	85.68	14.86	19
	PAYR			51.45	123.66	1.54	37.87	30.85	3.37	11
	PMRE			50.29	72.07	2.46	30.73	5.56	0.24	120
	PMRW			64.33	1.97	0.10	116.85	36.72	10.34	650
S/U	HIDR			0.15			0.24			~ Nondet.
	UNR			0.12			0.21			~ Nondet.
	ALER			0.12			0.48			~ Nondet.
	MERR			0.06			0.20			~ Nondet.
C/O	SBCE			304.70	179.80	9.79	52.52	239.10	272.00	306
	SBCS/SBS	391.60	13.00	195.80	42.70	4.31	96.40	38.50	0.44	182
	PCE			85.87	3.22	1.74	90.98	39.00	2.69	2272
	PCS			73.82	222.22	36.59	121.86	105.01	3.00	242
	LRCW			12.49	3.36	3.19	28.14	0.91	63.02	22
C/U	MACS/MARS	0.25	0.02	0.14			0.35			~ Nondet.
	MACN/MARM	0.65	1.59	0.27	0.88	0.64	0.65	1.22	0.80	~ Nondet.
	HIDC/HRB	0.07	0.04	0.08	0.02	0.02	2.09	1.67	0.37	~ Nondet.
	SBCW			0.99	0.42	<0.01	25.26	1.17	0.06	~ Nondet.
	LRCS			3.14	2.68	0.26	5.68	1.19	0.23	~ Nondet.
OC/O	MINM			15.72	1.19	0.09	53.32	3.26	4.61	78
	PGM			52.20	25.68	14.12	113.69	80.56	22.00	201
	DROM/LRM	10.85	1.09	50.10	110.57	3.63	46.39	44.72	3.32	105
	PMM			3.21	4.22	0.57	254.93	69.08	15.31	11
OC/U	MSM			<0.01			<0.01			~ Nondet.
	PBM			<0.01			0.04			~ Nondet.
	PADM			0.02			0.01			~ Nondet.
	LINM			0.03			0.02			~ Nondet.

Table 3.14 Oil content of mangrove sediments as measured by gas chromatography. Units are mg/g dry weight. Refer to Table 3.13 heading for details.

Hab./ Oil.	Site	Sept. 1986		May 1989			July 1990			1991 Ooze Oil (mg)
		0-5 cm	15-20 cm	0-2 cm	8-10 cm	18-20 cm	0-2 cm	8-10 cm	18-20 cm	
S/O	LRRS			2.21	0.36	0.10	13.55	11.68	1.79	2
	PAYR			5.64	22.56	0.53	5.79	5.81	0.57	1
	PMRE			7.61	17.15	0.87	2.91	0.92	0.10	5
	PMRW			10.04	0.43	0.03	12.97	7.66	2.04	130
S/U	HIDR			0.21			0.08			~ Nondet.
	UNR			0.05			0.40			~ Nondet.
	ALER			0.06			0.05			~ Nondet.
	MERR			0.03			0.08			~ Nondet.
C/O	SBCE			67.77	41.87	0.48	3.16	24.35	47.70	43
	SBCS/SBS	127.99	4.88	42.09	7.26	0.74	23.77	14.12	0.13	20
	PCE			10.59	0.43	0.20	10.49	5.69	0.28	152
	PCS			11.29	34.01	4.38	35.59	25.77	0.32	35
	LRCW			19.10	0.23	0.09	4.11	0.05	6.86	2
C/U	MACS/MARS	0.10	<0.01	0.02			0.03			~ Nondet.
	MACN/MARM	0.50	11.15	0.21	0.32	0.15	0.27	0.26	0.53	~ Nondet.
	HIDC/HRB	0.02	0.04	0.04	0.04	0.02	0.22	0.22	0.07	~ Nondet.
	SBCW			0.10	0.08	0.03	3.35	0.13	0.01	~ Nondet.
	LRCS			0.80	1.61	0.03	1.21	0.24	0.05	~ Nondet.
OC/O	MINM			1.95	0.10	0.01	4.51	0.48	0.46	12
	PGM			7.39	1.53	1.57	13.23	4.16	0.52	33
	DROM/LRM	1.70	0.13	3.56	3.58	0.24	3.69	6.20	0.33	6
	PMM			0.60	0.16	0.04	22.00	11.70	2.68	1
OC/U	MSM			0.00			<0.01			~ Nondet.
	PBM			<0.01			<0.01			~ Nondet.
	PADM			<0.01			<0.01			~ Nondet.
	LINM			0.01			<0.01			~ Nondet.

Table 3.15 Sum of individual PAHs in mangrove sediments. Units are µg/g dry weight except values for ooze oil (µg). Refer to Table 3.13 heading for details.

Hab./ Oil.	Site	Sept. 1986		May 1989			July 1990			1991 Ooze Oil (µg)
		0-5 cm	15-20 cm	0-2 cm	8-10 cm	18-20 cm	0-2 cm	8-10 cm	18-20 cm	
S/O	LRRS			2.29	1.43	0.17	50.31	44.91	6.01	9
	PAYR			20.52	120.03	0.49	53.20	39.08	3.47	10
	PMRE			34.54	98.31	10.21	9.43	3.44	1.55	41
	PMRW			82.15	3.75	0.36	92.62	47.70	32.87	2,722
S/U	HIDR			0.56			0.43			
	UNR			0.41			0.25			
	ALER			1.06			1.11			
	MERR			0.07			0.16			
C/O	SBCE			208.29	156.88	3.65	6.93	61.96	188.62	407
	SBCS/SBS	1,920.76	65.60	156.95	39.03	1.79	53.71	28.87	0.92	221
	PCE			97.89	0.17	0.13	20.04	21.24	1.23	2,597
	PCS			112.23	336.11	37.25	237.64	448.68	2.76	464
	LRCW			2.10	1.53	1.82	35.61	0.79	74.57	8
C/U	MACS			0.23			0.24			
	MACN			1.01			1.64	0.00	1.51	
	HIDC			0.03	0.10	0.01	0.87	0.74	0.51	
	SBCW			0.37	0.52	0.02	4.90	0.70	0.07	
	LRCS			10.9	3.65	0.14	1.24	0.59	0.10	0.1
OC/O	MINM			3.65	0.16	0.00	11.13	2.13	1.99	121
	PGM			47.18	15.40	31.11	42.92	13.96	5.86	349
	DROM/LRM	16.67	0.37	52.18	104.51	2.71	17.79	24.78	2.72	96
	PMM			0.19	0.75	0.14	113.31	66.91	13.12	9
OC/U	MSM						0.04			
	PBM			<0.01			0.09			
	PADM			0.01			0.08			
	LINM			0.05			0.16			

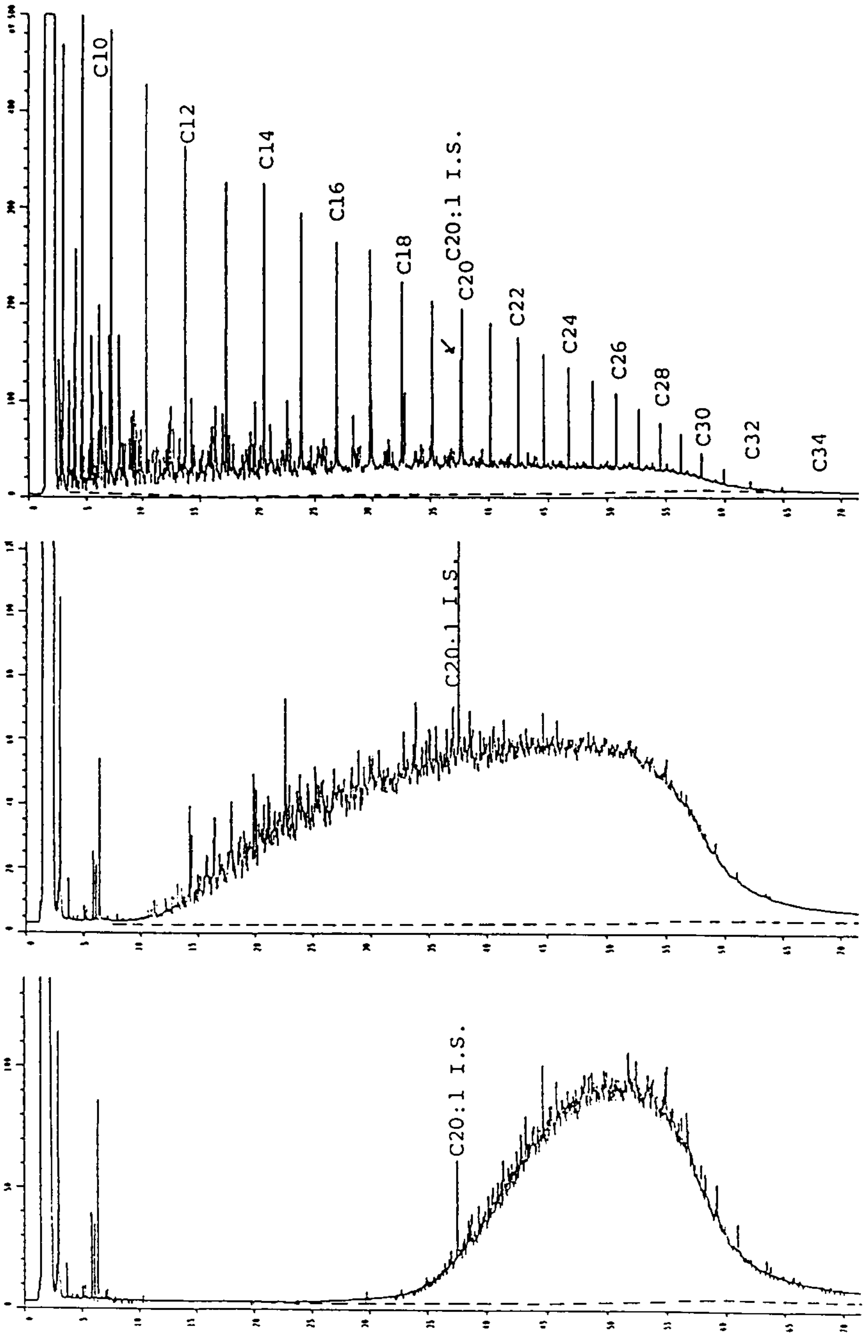


Fig. 3.12 Gas chromatograms of the spilled oil (*top*), oil oozing from mangrove sediments 3.5 yr postspill (*middle*), and the extensive alteration of patterns due to weathering of oil recoating mangrove roots 2.5 yr postspill (*bottom*). $C_{20:1}$ is an internal standard (*I.S.*) added for GC quantification.

recoating mangrove roots at a previously unoiled site (HIDC) 2.5 yr after the spill. These chromatograms show that the reservoirs of trapped oil still contained a significant fraction of low-boiling hydrocarbons, which could presumably still be toxic when leached into coastal waters. Note that a significant proportion of hydrocarbons eluted before the $C_{20:1}$ internal standard in the ooze oil sample. Residues redeposited on solid surfaces were so highly modified that source identification would be impossible by GC without additional supporting chemical evidence.

UVF spectra were conserved even in the highly modified oil residues (Fig. 3.13). Subtle changes in the spectra due to the disappearance of low-molecular-weight aromatics were evident in some samples. The majority of the fluorescence signal of this crude oil is contained in the higher-boiling residual aromatic hydrocarbons of the phenanthrene/anthracene, pyrene/fluoranthene, and chrysene/benzanthracene series.

The pattern of triterpane biomarkers seen by GC/MS selected-ion-monitoring of mass-to-charge ratio (m/z) 191 and related ions also was conserved (Fig 3.14). Triterpanes elute in the C_{26} to C_{30} boiling range. Using these selective techniques, the identity of even highly weathered residues can be verified.

In 1991 mangrove sediments were cored again. Oil oozing out of core holes during a 3-min period was collected at each site. For cores with no sign of oozing oil (unoiled sites), 20 mL of the water refilling the hole were collected. Figure 3.15 shows the logarithm of the amounts of oil determined by GC and UVF. From these data it is clear that significant amounts of oil oozed out of sediments 5 yr postspill.

Figure 3.16 shows the gas chromatograms of the oozing oil from stream, channel, and open-coast sites. In most cases the oil was highly degraded and similar to the residues seen in sediment extracts. However, PMRW, an oiled stream site, contained a surprisingly fresh oil residue with the full suite of *n*-alkanes preserved. The oil in surface sediments was more degraded. There was no evidence that this oil at depth was from a new source; reoiling would have been recorded by biologists studying mangrove root communities. S. Garrity and S. Levings (pers. comm.) reported no significant reoiling event that would have delivered enough oil to penetrate to deep sediments. The oil could have been preserved relatively intact since the 1986 spill in an anoxic zone of sediment, perhaps inside a decayed mangrove root.

Appreciation of the relatively intact composition of the oil oozing at PMRW in 1991 comes from comparing its GC pattern with oil from surface sediments in 1986 and with the ooze oil from PMRE in 1990 (Fig. 3.17).

Chemical evidence that oil oozing from deep sediments could be residual from the spilled VMIC comes from examination of individual PAH and triterpane biomarker patterns obtained by selected-ion-monitoring GC/MS. Figure 3.18 shows the distribution of parent and alkyl-substituted PAHs in the naphthalene (N), fluorene (F), dibenzothiophene (D), phenanthrene/anthracene (P), and chrysene/benzanthracene (C) series for VMIC, the ooze oil from PMRE in 1990, and the ooze oil from PMRW in 1991. Allowing for the loss of the most volatile naphthalenes, the substitution patterns are similar in the F-, D-, P-, and C-series.

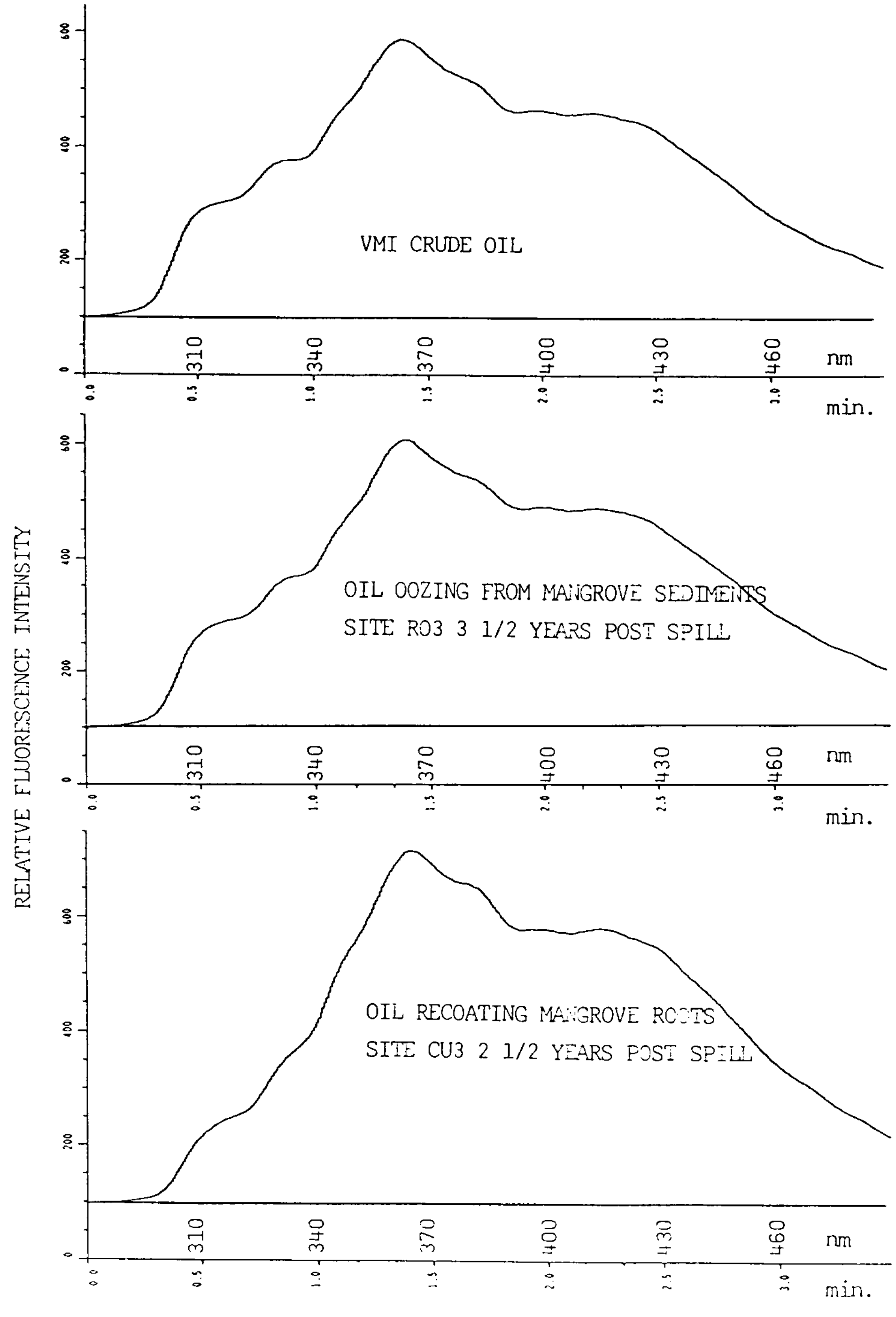


Fig. 3.13 Synchronous excitation/emission UVF spectra of the spilled oil in mangrove sediments showing pattern retention despite the extensive weathering apparent by GC analysis (see Fig. 3.12). *RO3* = PMRE; *CU3* = HIDC (Fig. 1.5).

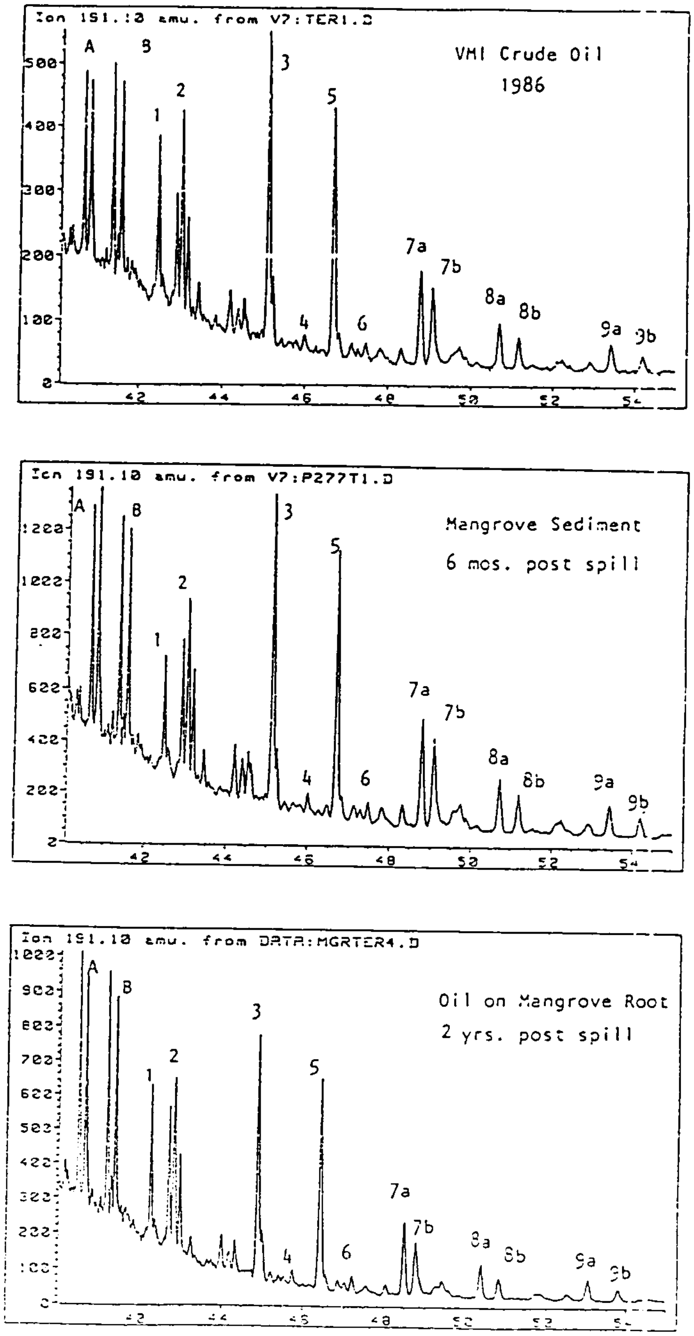


Fig. 3.14 GC/MS selected-ion-monitoring traces of ion 191 used for triterpane biomarkers of fresh and weathered VMIC. Note the retention of pattern even after the extensive weathering of oil residues seen by GC (see Fig. 3.12). Individual triterpanes (as labeled) are listed in Appendix Table B.4.

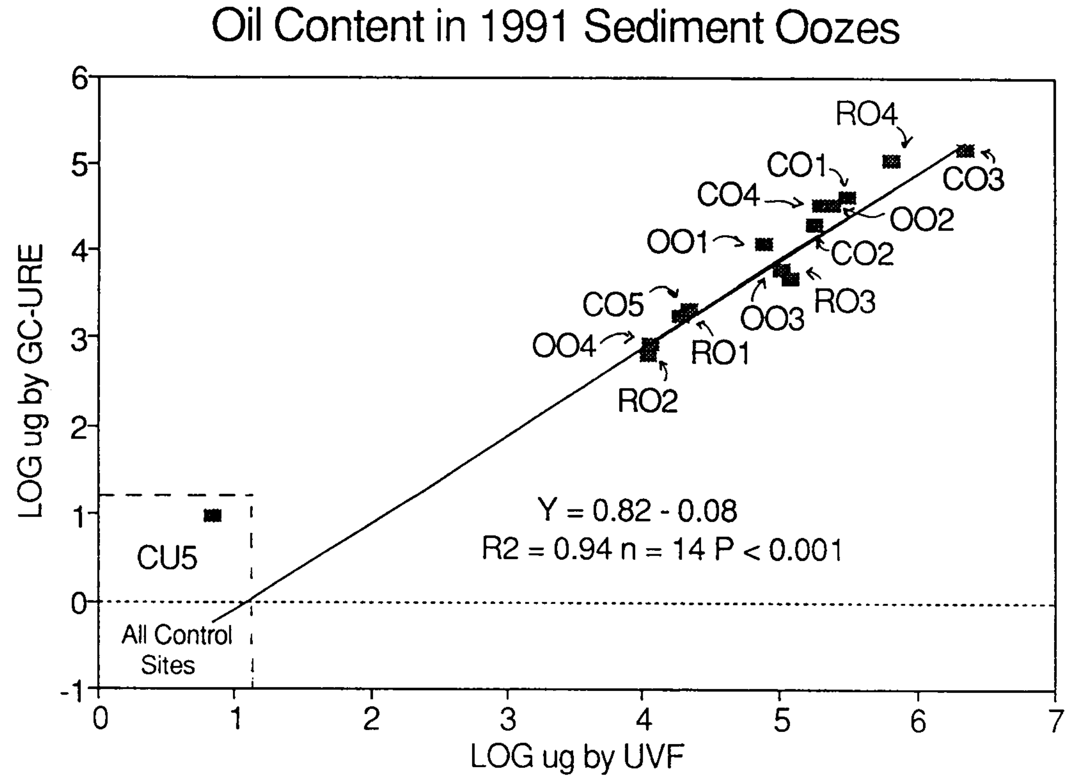


Fig. 3.15 Regression of the amount of oil oozing from mangrove sediments in 1991 determined by UVF and GC. Values are log(μg oil) collected after 3 min. All unoiled sites were analyzed by UVF; only CU5 was also analyzed by GC. Values for all unoiled sites were within the boxed area, *lower left*. Refer to Table 3.12 for site acronyms.

The entire data set of PAHs in the 1991 ooze oil samples is given in Table 3.16. It is apparent that significant levels of low-molecular-weight aromatics were still leaching from disturbed sediments 5 yr after the spill.

The data normalized to total hydrocarbon content determined by GC also showed a relative enrichment of the dibenzothiophene series within the total hydrocarbon load. Are the sulfur derivatives simply more residual than the parent hydrocarbons or are they being formed by substitution reactions within the anoxic, sulfur-rich mangrove sediments? Figure 3.19 shows the distribution of triterpane biomarkers monitored as m/z 191. The ratios of highly stable markers in the hopane series are used by petroleum geochemists to distinguish very closely related crude oils (e.g., Shen 1984; Siefert and Moldowan 1978, 1979). The criterion for mismatch is that the ratios of diagnostic compounds, such as the C_{27} pentacyclic triterpanes (T_m/T_s), vary by more than 20%. Weathering reduces the content of T_s before T_m, but peaks 3 and 5 are known to weather at the same rate as T_m (Shen 1984). As

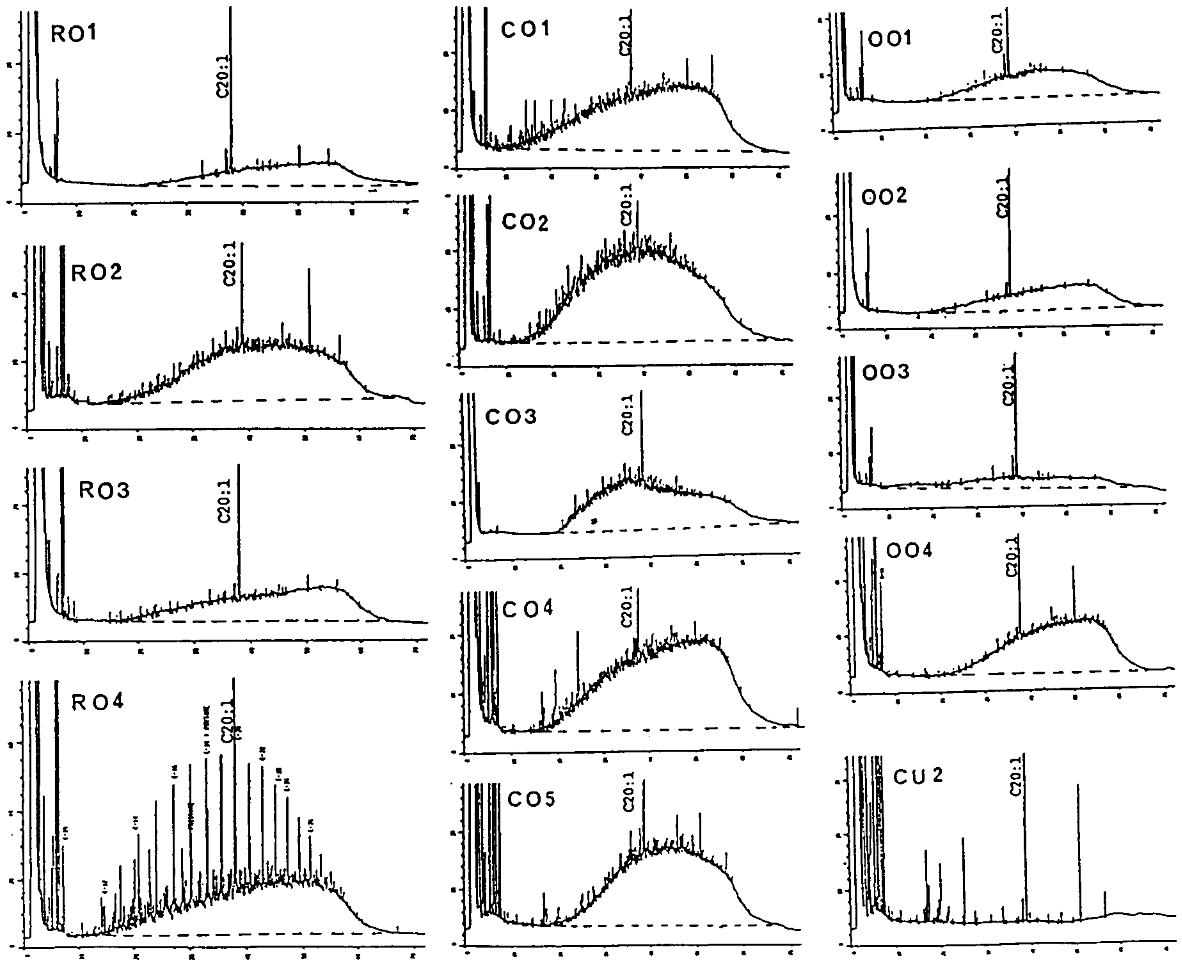


Fig. 3.16 Gas chromatograms of oil oozing from mangrove core holes in June 1991. $C_{20:1}$ is an internal standard added for GC quantification. *RO* = oiled stream sites; *CO* = oiled channel sites; *OO* = oiled open coast sites; *CU2* = unoiled channel site shown for comparison (see Table 3.12 and Fig. 1.5 for sites).

seen in Figure 3.19, the ratios of the T_m/T_s peaks in the same three samples shown in Figure 3.17 varied by no more than 2.3% and all agree with VMIC (not shown) within 20%. These ratios thus confirm that the relatively fresh oil seen at PMRW in 1991 is related to the original crude oil mixture spilled in 1986.

Note that the peak patterns in Figure 3.19, produced in 1991, varied slightly from those in Figure 3.14, which were produced in 1987. This difference occurred because the analyses were conducted on different GC columns with different carrier-gas-flow and oven-programming rates. Resolution increased in the 1991 analyses, and both sets of patterns are internally consistent.

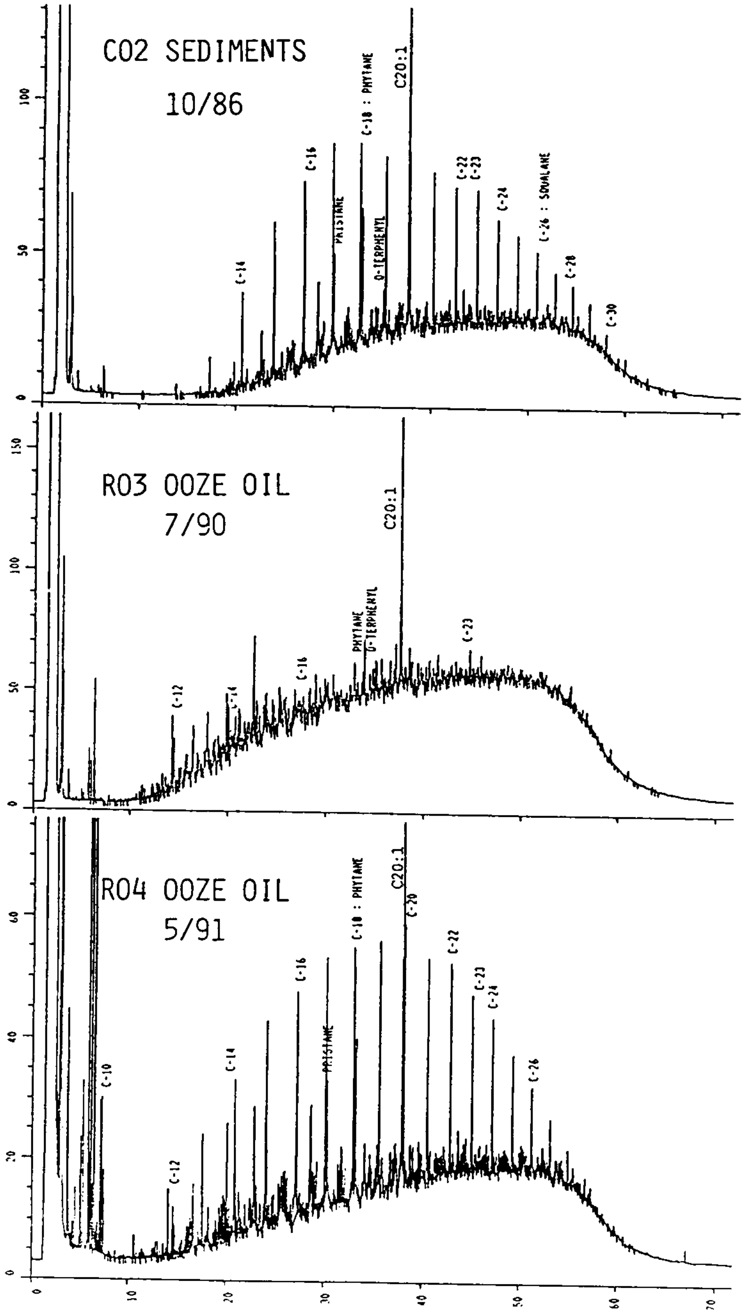


Fig. 3.17 Gas chromatograms of oil in mangrove sediments. *Top to bottom*: oil extracted from surface sediments at CO2 (= SBS; Fig. 1.5), October 1986; oozing from core holes at RO3 (= PMRE) in July 1990 and RO4 (= PMRW) in May 1991. Note the full suite of preserved alkanes in 1991 (*bottom*).

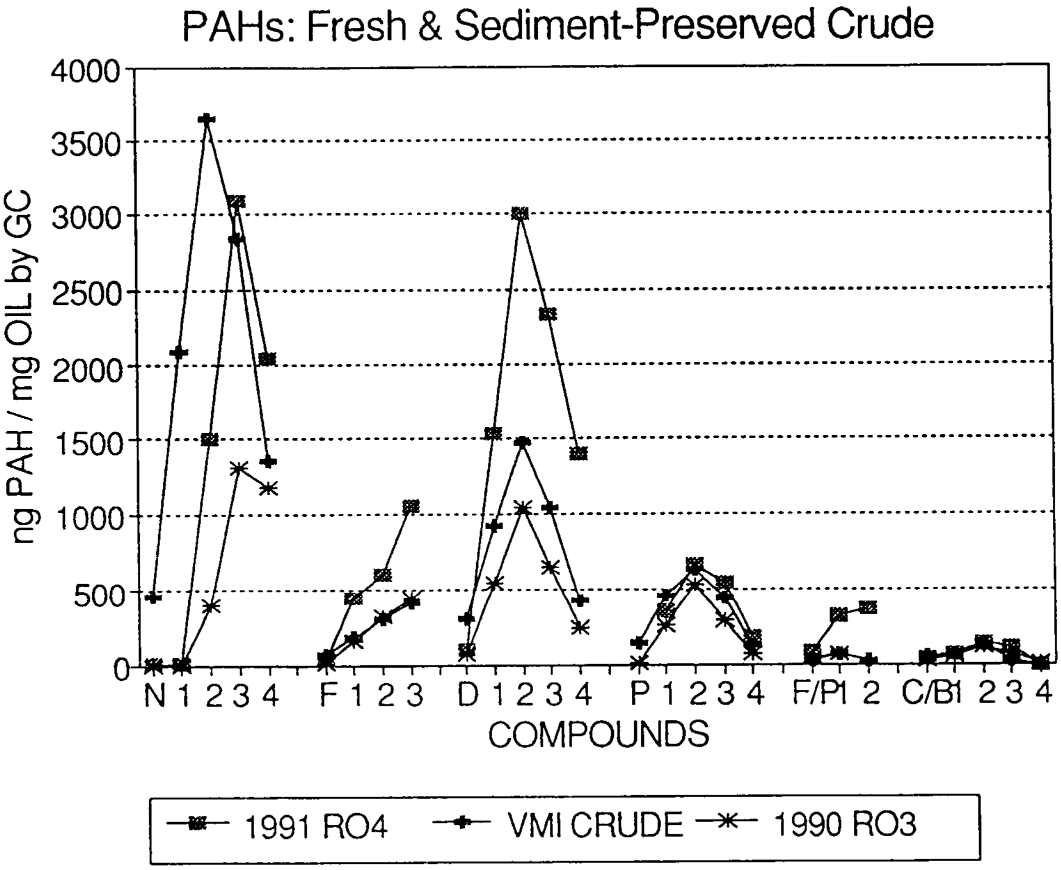


Fig. 3.18 Content of individual parent and alkyl-substituted PAHs in fresh VMIC and ooze oils from mangrove sediments in 1990 (RO3 = PMRE) and 1991 (RO4 = PMRW; Fig. 1.5). Units are ng PAH compound per mg oil determined by GC. Component data are in Table 3.16.

3.7.2 Long-term Toxicity

Evidence of effects of oil in sediments on mangrove trees comes not only from measurements of leaf production, seedling growth, and other indicators (Duke and Pinzón, Chap. 8), but also from observations of live and dead roots in sediment cores. Table 3.17 presents counts of roots greater than 5 mm in diameter, living and dead, for cores collected in 1989 and 1990. In Figure 3.20 are the scatter plot and regression, computed as follows. Oil content was patchy both horizontally and vertically. In some cores the highest concentrations of oil were at the surface, while in others the 8-10 cm section contained higher concentrations. Only a few cores had high concentrations in the 18-20 cm sections. To construct the correlation, the sediment content of oil in UVF units in the 0-2 and 8-10 cm core sections at oiled

Table 3.16 Individual PAHs in oil that oozed into mangrove core holes at each oiled site in May 1991. Units are ng PAH/mg total hydrocarbons determined by GC. LRCS, an unoiled channel site, shows ng subtracted from samples as a blank.

	Open Coast				Stream				Channel					
Compound	MINM	PGM	DROM	PMM	LRRS	PAYR	PMRE	PMRW	SBCE	SBCS	PCE	PCS	LRCW	LRCS
naphthalene	0	0	0	18	0	0	0	4	0	0	0	2	0	11
C_1-naphthalenes	0	0	0	28	0	0	0	10	0	0	0	1	0	13
C_2-naphthalenes	0	0	0	57	10	15	0	1,500	103	0	0	21	6	10
C_3-naphthalenes	164	18	87	68	64	72	0	3,092	472	295	73	109	88	0
C_4-naphthalenes	650	172	227	20	122	177	101	2,041	500	839	651	470	11	0
fluorene	0	0	0	0	0	5	0	35	21	4	4	6	3	0
C_1-fluorenes	26	24	37	23	19	24	0	441	109	66	29	55	14	0
C_2-fluorenes	157	141	71	34	58	67	55	608	175	339	147	347	17	0
C_3-fluorenes	692	789	737	338	326	668	598	1,047	908	920	1,233	1,160	135	0
dibenzothiophene	26	7	13	4	7	14	4	90	18	24	19	17	2	0
C_1-dibenzothiophenes	30	19	52	11	50	21	13	1,537	285	167	65	273	12	0
C_2-dibenzothiophenes	1,330	743	659	294	522	897	873	2,997	1,093	1,736	2,022	2,375	56	0
C_3-dibenzothiophenes	2,290	2,909	2,438	1,235	987	2,188	1,920	2,327	1,418	2,170	3,774	2,730	433	0
C_4-dibenzothiophenes	1,958	2,468	3,758	2,003	1,112	2,243	1,837	1,399	1,225	1,578	3,000	1,865	936	0
phenanthrene/anthracene[1]														
C_1-phenanthrenes	5	0	48	25	13	27	1	346	20	4	6	20	13	1
C_2-phenanthrenes	147	80	268	66	57	109	53	670	119	214	201	166	26	0
C_3-phenanthrenes	427	278	820	260	144	372	254	536	122	394	591	398	65	0
C_4-phenanthrenes	199	205	920	250	138	287	246	194	104	176	309	200	90	0
fluoranthene	18	0	50	40	15	25	18	31	15	26	46	28	19	1
pyrene[1]	17	0	76	118	17	69	73	56	75	65	134	73	49	2
C_1-pyrene/fluoranth	366	550	465	600	109	435	328	325	414	338	793	406	246	0
C_2-pyrene/fluoranth	507	734	674	882	174	627	556	359	527	351	961	461	360	0
benzanthracene/chrys	114	148	229	135	62	154	164	118	172	130	334	148	75	0
C_1-chrysene/benzanth	86	104	439	203	139	266	255	167	235	179	434	243	137	0
C_2-chrysene/benzanth	302	377	1,332	725	119	723	395	387	556	428	921	600	352	0
C_3-chrysene/benzanth	284	326	1,277	784	42	539	295	312	413	352	716	525	294	0
C_4-chrysene/benzanth	117	192	557	273	15	121	19	151	132	52	201	263	141	0
benzfluoranth(k)(b)	50	73	140	93	39	74	80	30	58	54	117	75	42	0
benzopyrenes	142	168	419	241	105	208	114	106	140	109	236	174	113	0

Table 3.16 Individual PAHs in oil that oozed into mangrove core holes at each oiled site in May 1991 (continued).

	Open Coast				Stream				Channel					
Compound	MINM	PGM	DROM	PMM	LRRS	PAYR	PMRE	PMRW	SBCE	SBCS	PCE	PCS	LRCW	LRCS
perylene	20	24	65	43	17	23	0	17	17	17	43	22	10	0
dibenzanthracene	0	0	18	0	0	0	0	0	0	0	0	5	0	0
benzoperylene	17	17	43	28	13	24	0	11	11	13	27	16	12	0
Total PAHs	**10,139**	**10,567**	**15,920**	**8,899**	**4,494**	**10,473**	**8,252**	**20,943**	**9,456**	**11,040**	**17,085**	**13,253**	**3,757**	**38**

[1] Phenanthrene was not quantified in this series due to a problem with the SIM acquisition window.

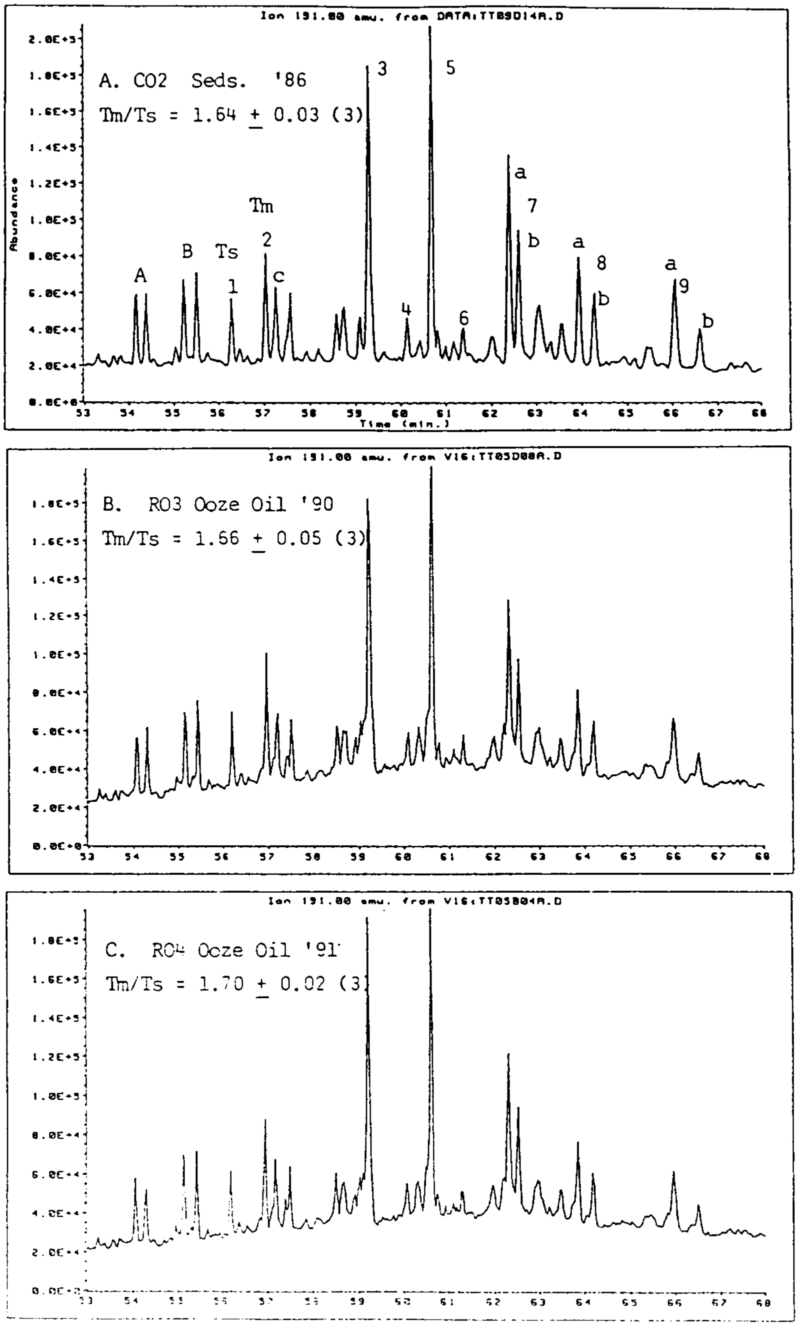


Fig. 3.19 GC/MS selected-ion-monitoring traces of ion 191 used for triterpane biomarkers of VMIC in mangrove sediments. The peaks are identified in Appendix Table B.4. Diagnostic ratios of T_m/T_s agree within 2.3% of each other and within 20% of VMIC (not shown). CO2 = SBS; RO3 = PMRE; RO4 = PMRW (Fig. 1.5).

Table 3.17 Number of live and dead mangrove roots in sediment cores. *A*. May 1989; *B*. July 1990.

Oiled Sites				Unoiled Sites			
Site	Live	Dead	Proportion Dead	Site	Live	Dead	Proportion Dead
A. May 1989							
MINM	4,8,3	3,0,0	0.17	MSM	4,2,5	1,3,0	0.27
PGM	3,6,4	4,4,10	0.58	PBM	13,3,6	0,0,0	0.00
DROM	2,2,8	4,6,6	0.57	PADM	7,9,8	0,1,1	0.08
PMM	12,10,5	2,0,1	0.10	LINM	5,10,5	0,0,0	0.00
Total	67	40	0.37		77	6	0.07
SBCE	3,3,4	8,4,3	0.60	MACS	10,14,8	0,0,1	0.03
SBCS	7,2,10	3,8,3	0.42	MACN	3,8,8	5,2,0	0.27
PCE	4,4,5	0,4,2	0.31	HIDC	5,8,6	3,2,1	0.24
PCS	4,4,3	2,4,6	0.52	SBCW	5,5,8	4,0,2	0.25
LRCW	10,12,7	3,0,0	0.09	LRCS	13,5,3	1,4,3	0.28
Total	82	42	0.34		108	27	0.20
LRRS	6,7,4	9,3,4	0.48	HIDR	3,7,2	0,0,2	0.14
PAYR	1,5,3	2,2,5	0.50	UNR	2,2,6	3,6,2	0.52
PMRE	0,1,2	4,5,10	0.86	ALER	5,6,6	4,3,1	0.32
PMRW	13,6,6	6,3,3	0.32	MERR	3,3,6	5,1,3	0.43
Total	54	56	0.51		51	30	0.37
B. July 1990							
MINM	5,2,2	2,1,2	0.36	MSM	6,4,5	1,1,3	0.25
PGM	13,2,3	8,6,6	0.53	PBM	6,5	1,2	0.21
DROM	2,2,4	5,4,1	0.56	PADM	8,9,8	1,0,0	0.04
PMM	7,2	5,1	0.40	LINM	9,11,9	0,0,2	0.06
Total	44	41	0.48		80	11	0.12
SBCE	6,2,1	0,4,4	0.47	MACS	4,4,6	0,0,0	0.00
SBCS	4,2,4	12,10,6	0.74	MACN	2,3,2	3,2,4	0.56
PCE	3,1,1	7,8,7	0.82	HIDC	1,7,5	6,3,5	0.52
PCS	3,4,4	6,8,2	0.59	SBCW	8,9,5	5,4,3	0.35
LRCW	4,10,2	6,7,12	0.61	LRCS	13,9,11	3,1,0	0.11
Total	50	99	0.66	Total	89	39	0.30
LRRS	6,2,4	8,6,6	0.63	HIDR	0,1	1,6	0.88
PAYR	5,4,4	5,8,4	0.57	UNR	9,9,8	1,1,2	0.13
PMRE	2,2,0	5,5,4	0.78	ALER	7,4,8	3,1,0	0.17
PMRW	2,1,1	5,8,9	0.85	MERR	3,3,2	2,1,0	0.27
Total	29	73	0.72		54	18	0.25

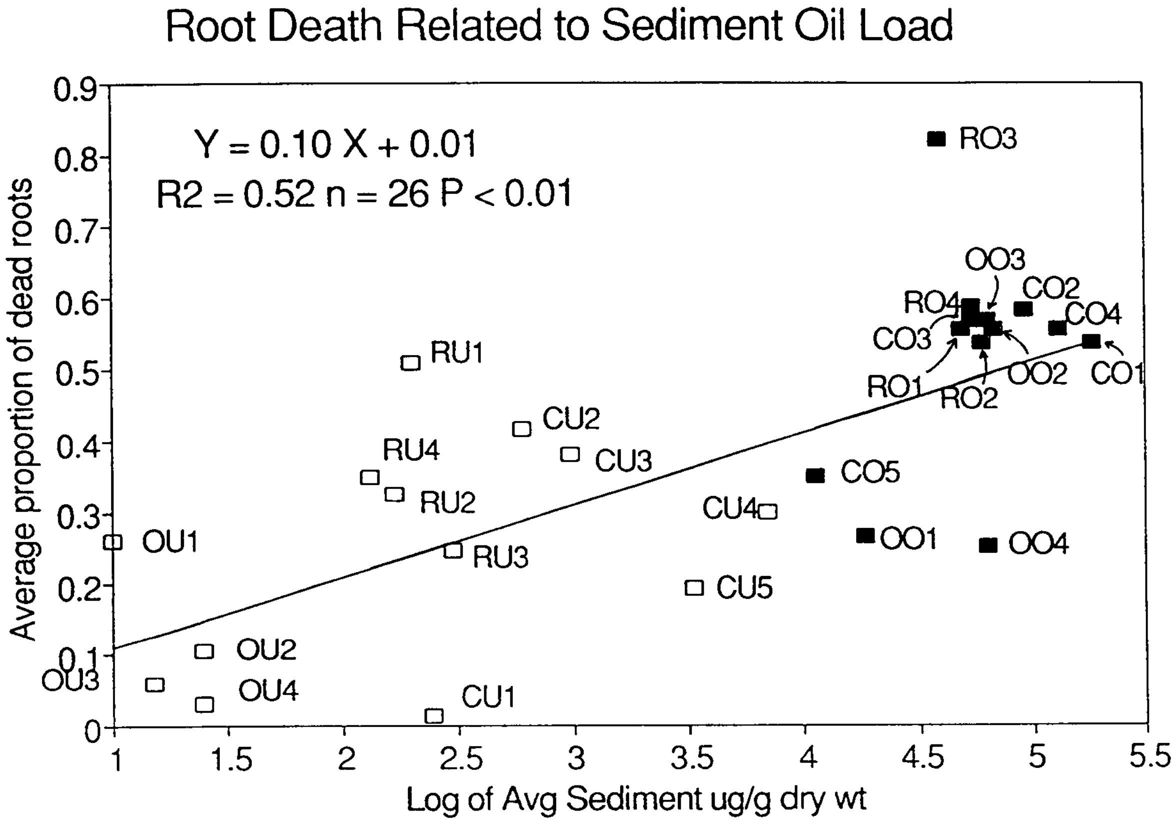


Fig. 3.20 Average proportion of dead mangrove roots in cores related to sediment oil load. Sites visually classified as heavily oiled are shown as *solid black squares*; *open squares* were classified as unoiled by the 1986 spill. See Table 3.12 and Figure 1.5 for sites.

sites were averaged over 1989 and 1990. This average is plotted as a log transformation against the average proportion of dead roots counted at each site in these 2 yr.

The relationship is significant and shows the distinction between heavily oiled and unoiled sites. However, there also appear to be intermediate sites showing effects of oil on roots. The unoiled stream and channel sites were mostly in Bahía Las Minas (Fig. 1.5). Not only was this area contaminated by the 1986 spill of crude oil, but also extensive areas of mangroves were killed in the same general area nearly 20 yr earlier following the 1968 wreck of the tanker *Witwater*, which was carrying fuel oil and Bunker C (Duke and Pinzón, Chap. 8). Traces of background oil were detected in deep layers of mangrove sediment cores taken at HRB in 1986. Also, MARS, located at Isla Margarita outside of Bahía Las Minas, showed relatively high levels of light fuel oil in 1986 that must have come from a separate spill. One result of the history of repeated oil pollution incidents along this coast appears to be a detectable effect on the condition of mangrove roots related to the degree of oil contamination.

Preliminary evidence of the toxicity of mangrove sediments came from adapting the Microtox bioluminescent bacterial assay to solid-phase testing (Ross et al. 1990; Buhlich et al. 1981). The data in Figure 3.21 show the technique has promise for demonstrating toxicity using frozen sediments.

3.7.3 Bivalve Bioindicators of Water Quality

The case for using bivalves as indicator organisms for petroleum contamination in coastal waters was published by Burns and Smith (1981). Two species are generally common in mangrove habitats near Bahía Las Minas. The oyster *Crassostrea virginica* and the false mussel *Mytilopsis sallei* are dominant encrusting bivalves on mangrove roots at sheltered channel and stream sites, respectively (Garrity and Levings, Chap. 9). Bivalves preferentially accumulate the more soluble, lower-molecular-weight hydrocarbons in an oil-contaminated ecosystem. As these compounds are also the most acutely toxic fractions of oil, the tissue content of bivalves should provide evidence of continued release of toxic, bioavailable petroleum residues into coastal waters. Doses estimated from bivalves may not correspond exactly with those estimated from sediments. Rather, they provide a time-integrated estimate of the degree of contamination in the surrounding water column.

Care must be used in selecting an oil for constructing the calibration curve for UVF analysis because the lower-molecular-weight, soluble aromatics have different emission maxima and lower fluorescent intensities than more-condensed-ring aromatics. GC analysis is quantitative despite different elution ranges of aromatic hydrocarbons associated with bioaccumulated oil residues. However, biogenic hydrocarbons filtered from food particles can interfere with the accurate quantification of degraded oil residues. These complicating factors account for some of the variation shown in Figures 3.3 and 3.4.

Uptake is related to the lipid content of the organisms in addition to the amount of oil in surrounding waters. Expression of results as oil content per mg lipid thus provides an effective comparison between sites. The maximum limit of accumulation in bivalves is generally up to approximately 30 μg oil/mg lipid based on GC analysis (Burns and Smith 1982).

Oysters were collected from only three sites in 1986. No living oysters or other obvious bivalves could be found in heavily oiled mangrove areas. Oysters were collected from mangrove roots at HRB, MARM, and MARS. Quarterly collections of false mussels at stream sites and oysters at channel sites began in December 1988, 2.5 yr after the spill.

Water-soluble fractions of crude oil were accumulated by bivalves during the entire study. Table 3.18 gives UVF estimates of oil content per mg of extractable lipid (EOM) for each sample. Table 3.19 gives the GC-URE estimates of oil content

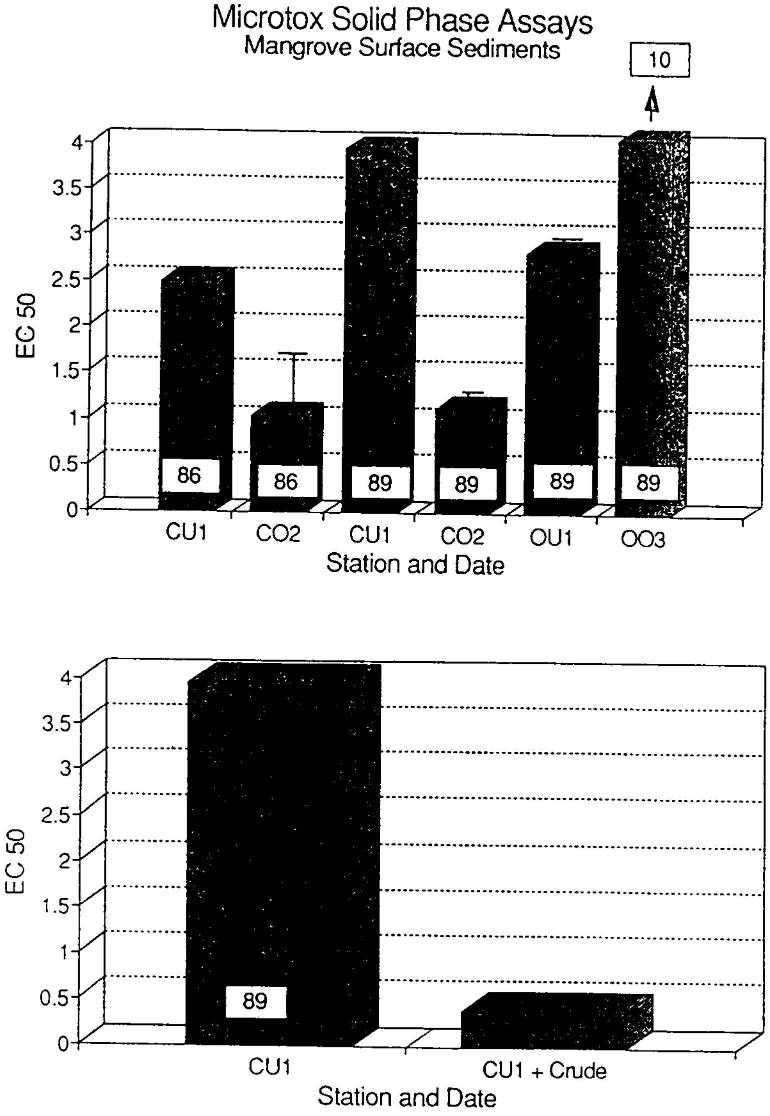


Fig. 3.21 Microtox EC_{50} values from solid-phase assay of mangrove sediments. The scale is percent dilution of seawater extract of 200 mg of sediment. Literature values indicate that unoiled sediments read 2.5 and above; lower values are considered toxic. The *top* graph shows that for this limited test, the bioassay shows promise for determining the relative toxicities of oiled sediments. The error bars show 1 SD for duplicate (CO2 = SBCS [Fig. 1.5], 1989; OU1 = MSM, 1989) or triplicate (CO2 = SBS, 1986) determinations; all others were single determinations. CU1 = HRB in 1986, MACS in 1989; OO3 = DROM.

Table 3.18 Mangrove organisms: oil content over time expressed as µg oil/mg EOM based on UVF analysis. *m* = false mussels; *o* = oysters; *b* = barnacles.

Site	Dec 88	Feb 89	May 89	Aug 89	Nov/Dec 89	Mar 90	May 90	Aug 90	Nov/Dec 90	May/Jun 91
Oiled Stream										
LRRS m	20.8	35.3	26.8		25.5	22.8	51.3	10.7	15.7	12.4
PAYR m	(9.6o), 23.5		16.9	22.8	11.8	6.1	3.7	8.5	14.6	12.1
PMRE m	(12.3o), 22.5				31.6	57.6	9.3	10.6	25.5	13.7
PMRW m	(4.6o), 8.8	23.4		21.2	12.2	14.8	9.6	11.1	3.7	22.0
Unoiled Stream										
HIDR m	5.0		2.3		2.2	1.4				1.4
UNR m	0.6	1.7			2.7					1.5
ALER m	0.9			1.1	0.8	1.8				1.2
MERR m	0.4	0.6	1.5	0.6	1.2	0.8				0.5
Oiled Channel										
SBCE o	4.0, 4.1	15.2			11.7	5.0	2.9	7.5	6.7	5.9
SBCS o	5.9, 6.6			9.1	5.2	9.3	5.3	5.2	12.4	4.6
PCE o	4.2, 2.4			8.0	8.1	5.7	2.3	4.0	7.9	2.5
PCS o	4.8, 26.6	9.9	9.2			8.2	3.6	4.6	5.9	5.2
LRCW o	3.6		6.8		3.0	8.7	3.0	2.7	3.9	6.8
Unoiled Channel										
MACS o	2.5					0.6				0.6
MACN o	1.7	1.4	1.7		1.9, 1.7	0.8				0.7
HIDC o	1.0			1.6	1.6	1.2	0.2	0.5	0.5	1.1
SBCW o	2.3	0.5	1.8	0.7	2.1	0.2	0.5	0.6	1.0	1.0
LRCS o	0.6				0.6	1.4	1.6	0.8	1.1	1.6
Oiled Open Coast										
MINM b	163.5	12.9			19.9				1.9, 13.6	
PGM b	67.8		7.4	24.6	25.2				43.6, 19.5	
DROM b	100.7		24.3						40.8, 4.5	
PMM b	10.1, 35.5	79.6		20.6					1.6, 0.8	
Unoiled Open Coast										
MSM b	0.2	0.3	0.2		0.6					
PBM b	0.3	0.4		1.3						
PADM b	0.1		1.2	0.7						
LINM b	0.0				1.0					

Table 3.19 Mangrove organisms: oil content over time expressed as μg oil/mg EOM based on GC-URE analysis. *m* = false mussels; *o* = oysters; *b* = barnacles.

Site	Dec 88	Feb 89	May 89	Aug 89	Nov/Dec 89	Mar 90	May 90	Aug 90	Nov/Dec 90	May/Jun 91
Oiled Stream										
LRRS m	19.7	12.5	12.0		40.9	14.7	114.3	3.4	3.9	3.3
PAYR m	(13.7o), 28.3		8.1	10.1	6.6	1.6	12.2	8.3	7.1	4.2
PMRE m	(8.3o), 8.1				13.8	13.7	7.6	4.2	9.1	3.8
PMRW m	(4.3o), 9.0	11.4		11.1	9.2	8.7	5.8	3.6	5.9	5.2
Unoiled Stream										
HIDR m	3.5		1.3		1.5	0.0				0.6
UNR m	1.3	1.3			2.9					0.4
ALER m	1.8			3.4	1.7	0.0				1.0
MERR m	0.5	1.5	0.9	0.5	0.0	0.0				0.5
Oiled Channel										
SBCE o	2.4, 5.2	8.9			7.8	3.8	2.2	6.4	1.5	2.8
SBCS o	7.3, 3.1			9.8	5.2	4.3	6.5	3.5	9.8	2.8
PCE o	3.1, 2.3			6.0	0.6	1.0	2.3	2.4	4.4	0.9
PCS o	8.2, 34.0	6.5	4.5		13.7	4.9	7.8	6.5	6.8	2.9
LRCW o	2.2		2.1		1.6	4.3	1.6	1.4	2.3	4.6
Unoiled Channel										
MACS o	7.1					Int				1.0
MACN o	4.1	2.0	1.7		0.4	1.6				3.2
HIDC o	0.7			0.0	1.1	0.9	0.0	0.4	2.9	0.7
SBCW o	2.7	lost	1.2	2.0	0.9	Int	0.3	0.5	3.0	1.1
LRCS o	1.0				0.0	1.5	0.3	0.9	1.5	0.7
Oiled Open Coast										
MINM b	44.0	4.1			5.5				1.9, 3.1	
PGM b	87.9		1.1	1.3	4.5				4.4, 2.9	
DROM b	18.2		6.4						4.8, 6.2	
PMM b	3.9	14.6		0.8					1.7, 0.7	
Unoiled Open Coast										
MSM b	2.0	0.0	0.0		0.0					
PBM b	0.5	3.3		0.0						
PADM b	1.5		3.0	0.0						
LINM b	1.8				0.5					

in μg/mg EOM and Table 3.20 gives the concentration of the sum of individual aromatic hydrocarbons as ng/mg EOM. Concentrations of individual PAHs can be found in Appendix Table B.2.

Figure 3.22 shows the concentrations of oil in oysters and false mussels as averages of the samples from replicate sites at each time. The tables show the substantial scatter in the results from replicate sites, but the distinction between oiled and unoiled remains. No samples were excluded from the averages despite a few anomalously high values, which would likely result from ingestion of suspended, rather than dissolved, oil. The immediate impression from this simple summary plot is that, at oiled sites, oysters had only about one-half the amount of oil in their tissues as false mussels. At three of the oiled stream sites in 1988, both species were collected from the same sites. False mussels contained on average about twice the levels of oysters (Table 3.18). Levels of oil in both species at all oiled sites and at a few unoiled sites subsequently oiled would be expected to cause at least sublethal stress responses in the bivalves (Widdows et al. 1990). Seasonality in levels of oil is suggested because the highest levels of oil in bivalve tissue occurred in the samples collected in February-March of 1989 and 1990. This season is the time of heaviest wind and wave action. This peak in concentration in bivalve tissue corresponds with increased oil mobilization visible as root and dowel coatings (Garrity and Levings, Chap. 9).

The disparity in levels of oil concentrated by these two species led us to conduct an uptake experiment. In May 1991 oysters and mussels were collected from unoiled sites and packed in open-mesh nylon bags, which were tied to subtidal mangrove roots at an oiled site, LRRS. Samples of each species were collected 0, 3, 6, 9, 12, 15, and 21 d later. Figure 3.23 shows the GC and UVF results from these samples. The time course of uptake and discharge of petroleum contaminants, as documented for similar species, indicates a half-life in tissues of approximately 3 d for the majority of the tissue burden (Burns and Smith 1981). Oysters showed only half the initial rate of uptake (R_i), half the average concentration (C_{avg}), and half the maximum concentration (C_{max}) of false mussels. This experiment shows the difference in accumulation characteristics between these two species.

The field data (Tables 3.18-3.20) can thus be interpreted to mean that similar amounts of soluble oil fractions were available for bioaccumulation at oiled stream and channel sites. This observation agrees with the sediment data and shows that potentially toxic hydrocarbons were available for uptake by bivalves 5 yr after the oil spill.

Barnacles, *Chthamalus* sp., from open-coast sites were also tested for their usefulness as bioindicators. However, this species was so small that it was not possible to dissect soft tissues from the shell, as was possible for bivalves. Barnacle shells therefore were included in extractions. Levels of oil in these samples were extremely variable and the residues appeared to be high-boiling, residual tar-type fractions, not the water-soluble fractions accumulated by bivalves. Examples of the GCs of oil residues from the three species are given in Figure 3.24. It must be concluded that this species of barnacle was not a useful bioindicator species.

Table 3.20 Mangrove organisms: PAH content over time expressed as ng PAH/mg EOM based on GC/MS analysis. *m* = false mussels; *o* = oysters; *b* = barnacles. No data May 1990-May/June 1991.

Site	Dec 88	Feb 89	May 89	Aug 89	Nov/Dec 89	Mar 90
Oiled Stream						
LRRS m	39.4	105.9	78.2		147.0	36.0
PAYR m	(281o), 393		59.7	10.6	31.1	16.7
PMRE m	100.6				112.1	185.1
PMRW m	(51o), 65	127.1		93.7	50.9	79.2
Unoiled Stream						
HIDR m						
UNR m						
ALER m						
MERR m						
Oiled Channel						
SBCE o	44.0, 64.4	177.8			214.3	38.9
SBCS o	66.7, 86.0			37.0		3.8
PCE o	22.7, 17.9			7.9	38.1	42.3
PCS o	93.9, 338.2	370.4	19.0		26.4	20.1
LRCW o	6.0		20.2		1.2	
Unoiled Channel						
MACS o						
MACN o					31.9	
HIDC o	14.7			9.2		6.5
SBCW o						
LRCS o	5.1				3.3	
Oiled Open Coast						
MINM b	518.8	31.3			87.5	
PGM b	281.7		12.3	39.9	28.3	
DROM b	105.2		22.2			
PMM b	13.8	49.7		12.6		
Unoiled Open Coast						
MSM b						
PBM b						
PADM b						
LINM b						

That the water-soluble fraction of the spilled crude oil was accumulated in bivalves was further demonstrated by the UVF spectra shown in Figure 3.25. Bivalves contained the water-soluble fraction in the 2- to 4-ringed series, while the oozing oil was more enriched in the heavier and more fluorescent 4-, 5-, and higher-ring series. The third trace in Figure 3.25 is of a fuel-oil residue in oysters from an unoiled site, which is easily distinguished both in quantity and quality from the crude-oil residues.

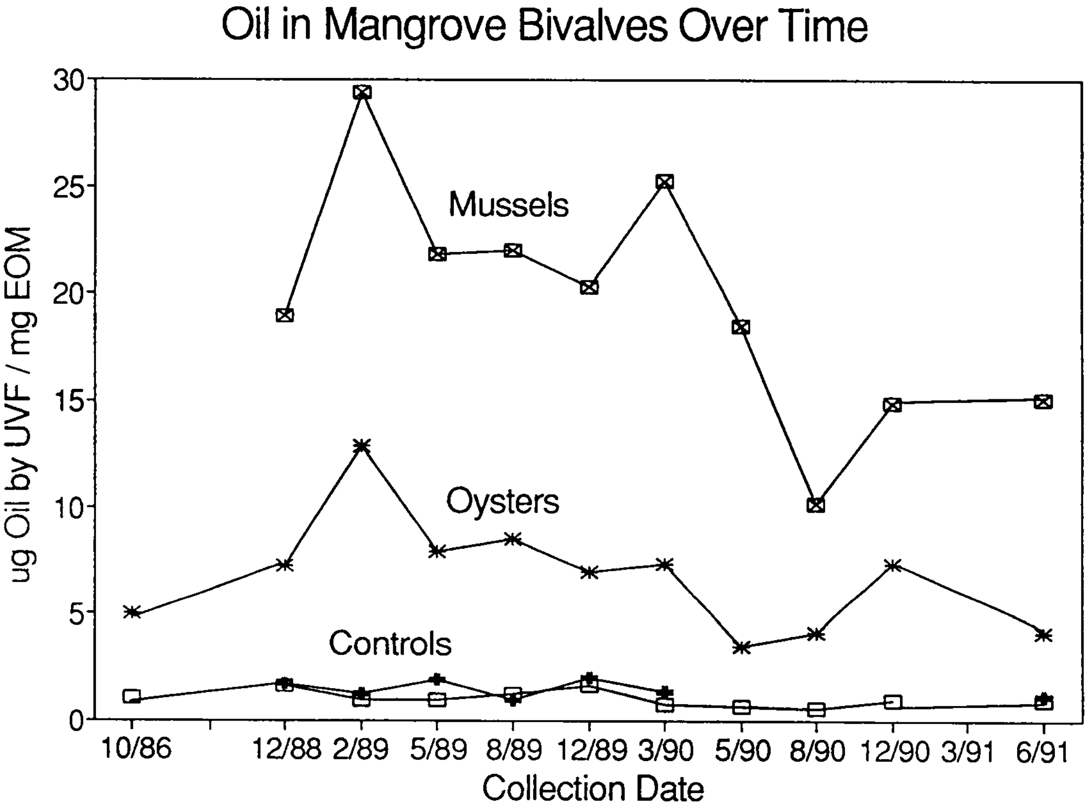


Fig. 3.22 Concentration of oil in false mussels and oysters as averages of replicate sites. Units are μg oil/mg EOM by UVF analysis. The data are from Table 3.18, which shows the range of values. *Controls* = values at unoiled sites.

3.7.4 Further Discussion of Changes in Oil Composition Over Time

Gas chromatograms of oil in sediments showed that, in most instances, the residues followed predictable weathering patterns. Most low-molecular-weight components disappeared by evaporation and dissolution. Alkanes and other resolved marker compounds disappeared within 6 mo of the spill, except for oil sheltered from degradation in anoxic pools in mangrove sediments. The persistence of the UVF pattern over time was unexpected. PAH analysis showed that, even in weathered samples depleted of naphthalene and lower molecular weight aromatics, this residual UVF signal was due primarily to the 3- to 4-ringed aromatics. As shown in Figure 3.18, the dibenzothiophene series also was persistent, and that the proportion of DBTs to total hydrocarbons increased with time.

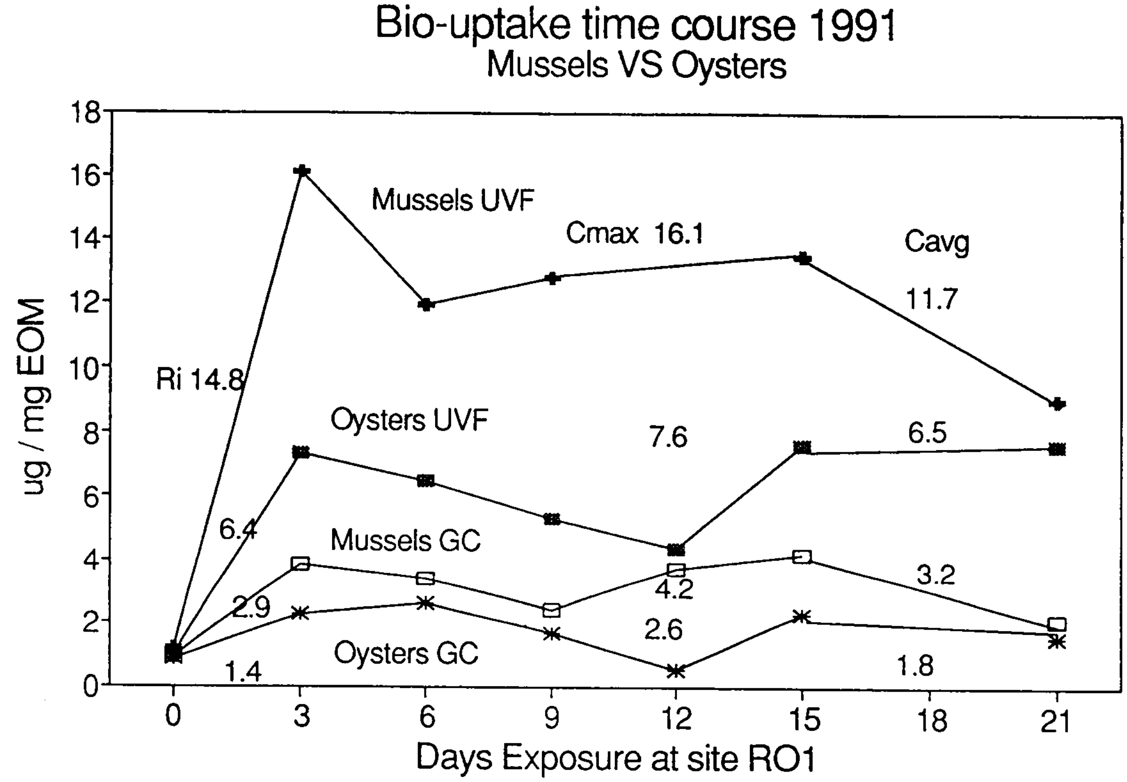


Fig. 3.23 Uptake of oil into bivalves transplanted from an unoiled site to an oiled site in May 1991. *Mytilopsis* accumulated twice as much oil as *Crassostrea*, calculated as R_i (initial rate of uptake), C_{max} (maximum concentration), or C_{avg} (average concentration) based on both UVF and GC analyses. RO1 = LRRS (Fig. 1.5).

Figure 3.26 shows examples of the distribution of individual PAHs in oiled sediments averaged over the 0-2 and 8-10 cm sections and during the 1989 and 1990 sampling periods compared to the average concentration in bivalve tissues over time. In both cases the PAHs were normalized to the total hydrocarbon content determined by GC. As for Figure 3.18, this presentation shows the relative change in composition of oil residues.

Total amounts of oil in tissues or sediments are given for each sample in Tables 3.5 and 3.20, as well as in Appendix Tables B.1 and B.2. The patterns show, in general, that the PAHs accumulated by bivalves were similar to the PAHs in the oil leaching or resuspended from sediments. Bivalves accumulated the whole suite of parent and substituted PAHs in the naphthalene through chrysene elution range. The most abundant PAHs in bivalve tissues were generally the dibenzothiophene series, followed by the chrysenes and phenanthrenes. This pattern agrees well with the UVF spectra of bivalve tissues. Oysters at SBCS contained a larger percentage

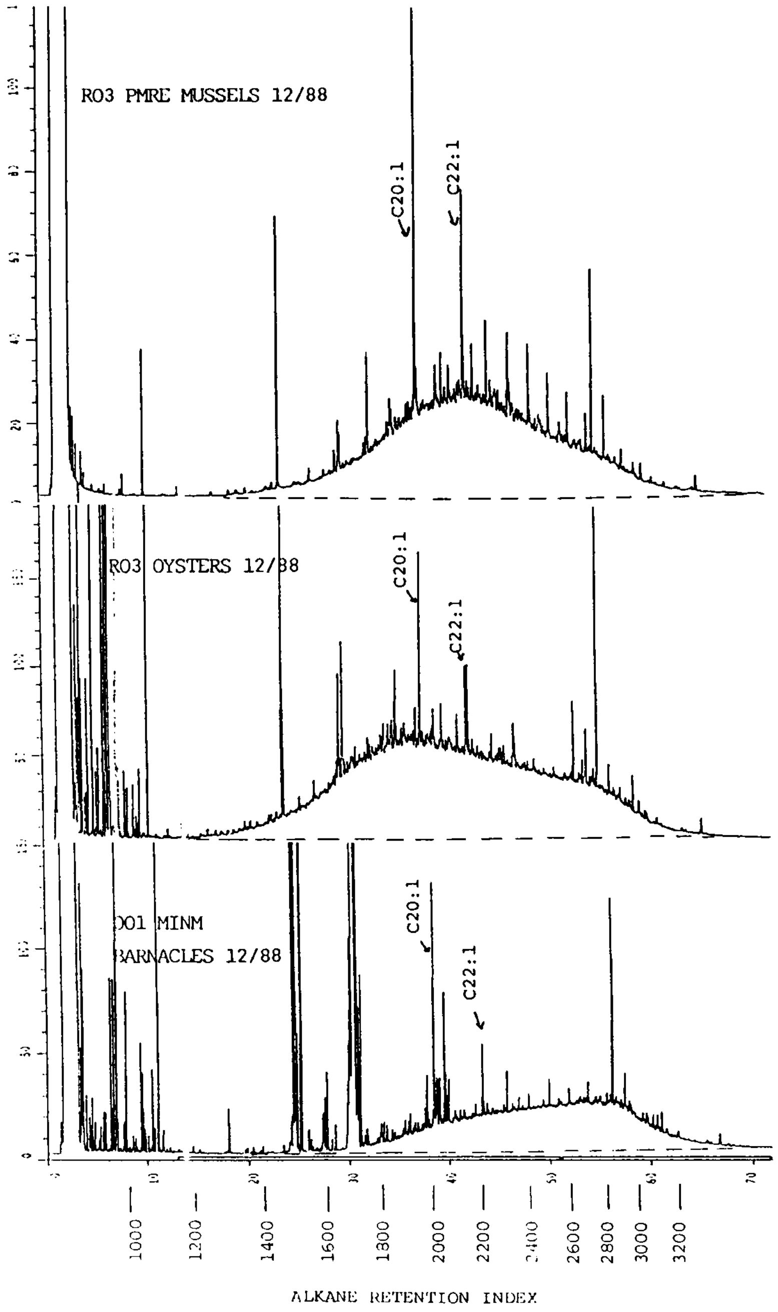


Fig. 3.24 Gas chromatograms of saturated hydrocarbons in false mussels, oysters, and barnacles collected in December 1988. Bivalves accumulated the relatively soluble fraction of oil from the water column, while residues on barnacles appeared to be more weathered, tar-like fractions. The labeled peaks are surrogate and internal standards. RO3 = PMRE; OO1 = MINM (Fig. 1.5).

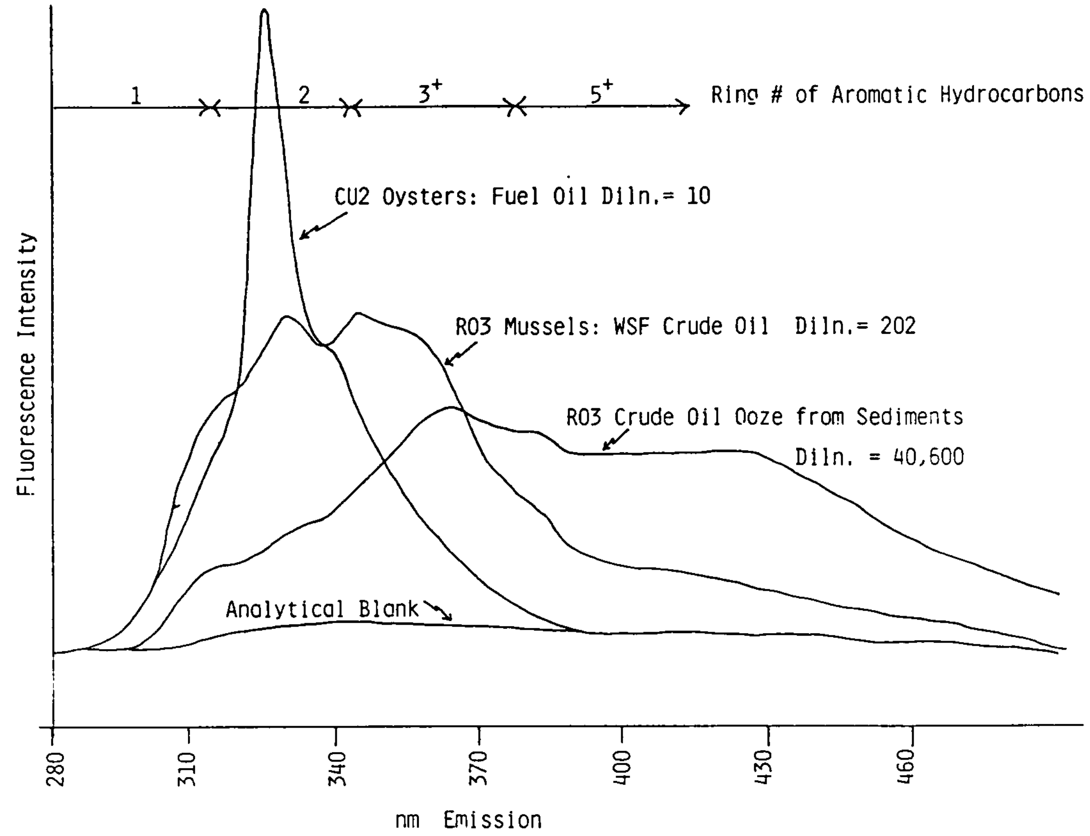


Fig. 3.25 Synchronous excitation/emission UVF spectra of aromatic hydrocarbons in false mussels compared to those in sediments at RO3 (= PMRE [Fig. 1.5], oiled with VMIC) and to trace fuel-oil residues at an unoiled site (CU2 = MACN). Note the lack of signal due to higher-ring structures in fuel residues. *Diln.* = dilution; *WSF* = water-soluble fraction.

of C_1- and C_2-phenanthrenes than sediments. Comparison of these patterns with VMIC, shown in Figure 3.18, suggests that the more soluble C_1- and C_2-compounds are being depleted in sediments and accumulated by bivalves. This process is shown most clearly at SBCE; sediments were relatively depleted in DBTs and phenanthrenes, which were predominant components in bivalves. As seen in Table 3.16, the most residual PAHs in sediments were the DBT, phenanthrene/anthracene, and pyrene/fluoranthene series.

A second way of displaying the individual PAH data is shown in Figure 3.27. Here, the relative concentrations of individual compounds in sediments are plotted against those in bivalves. The compounds falling below the regression line should be the most residual ones in sediments; those falling above the line should be depleted fastest.

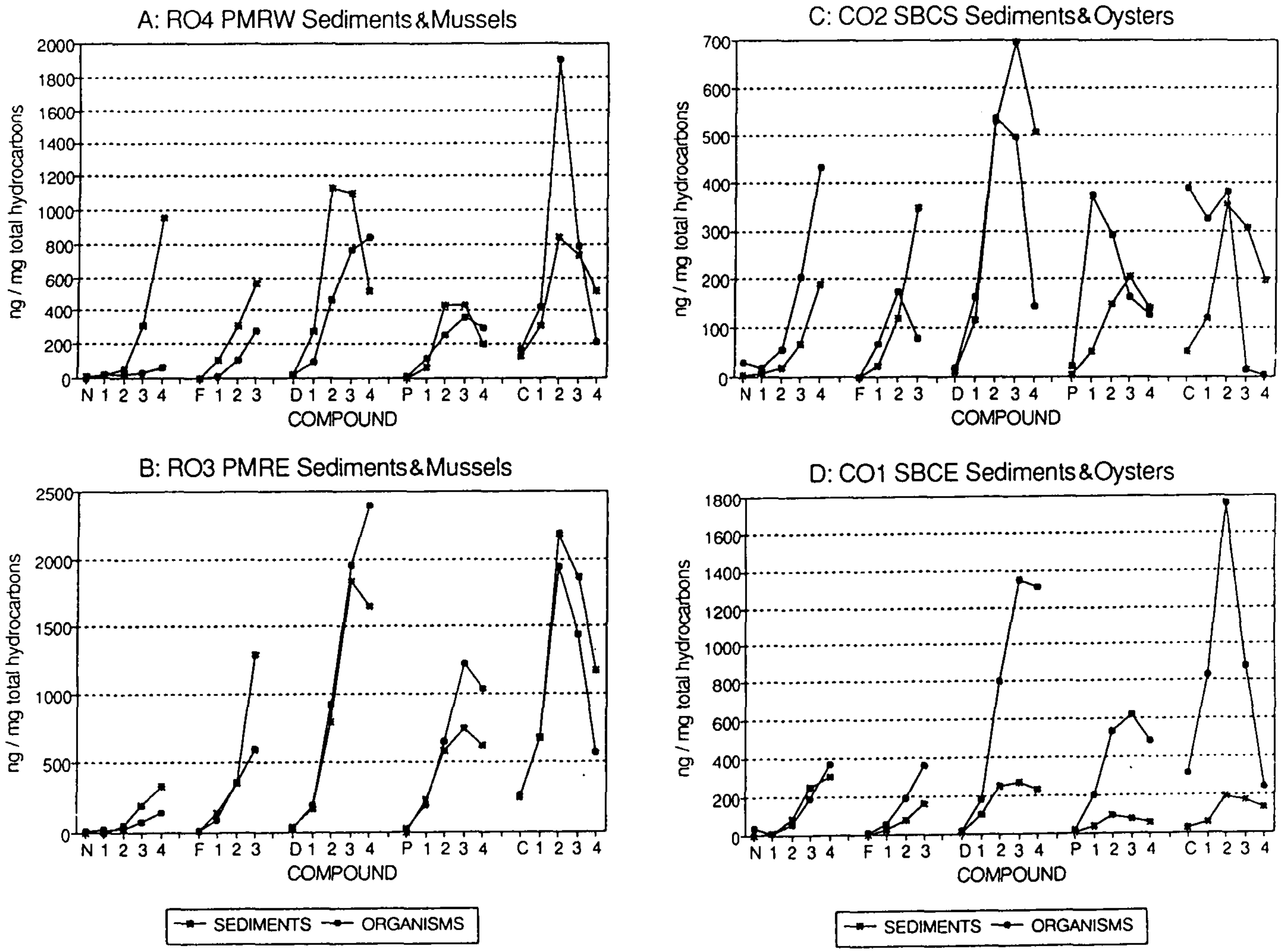


Fig. 3.26 Composition of individual PAHs in 0-2 and 8-10 cm core sections, 1989 and 1990 averaged, and average composition of PAHs in bivalves at the stream and channel sites indicated (see Fig. 1.5). Plotted as ng/mg total hydrocarbons determined by GC analysis.

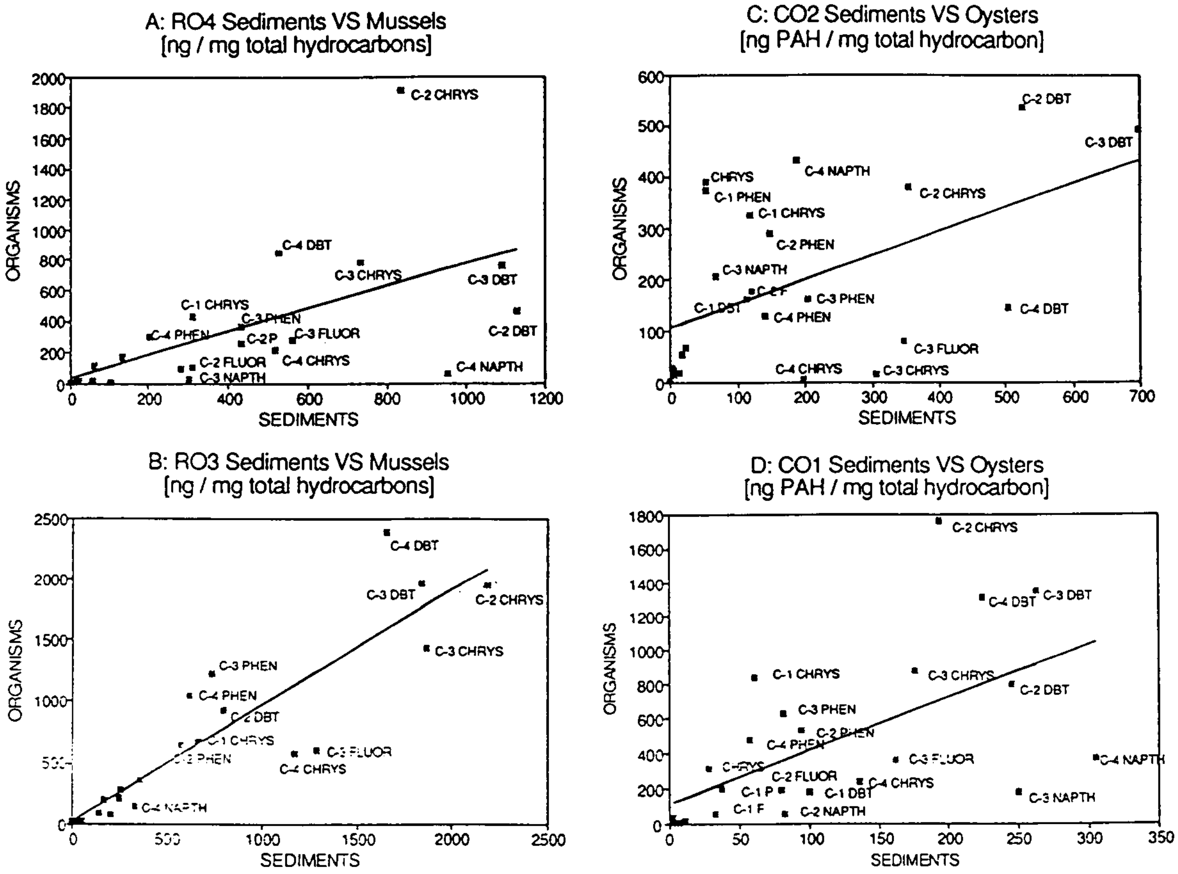


Fig. 3.27 Data in Figure 3.26 plotted as regressions of sediment content (x-axis) and organism content (y-axis). *RO4* = PMRW; *RO3* = PMRE; *CO2* = SBCS; *CO1* = SBCE (Fig. 1.5).

Evidence that the composition of residues leaching from sediments into the water column began to change detectably 5 yr after the spill comes by dissection of the correlation of UVF and GC analyses of bivalves. Figures 3.3 and 3.4 showed highly significant correlations of UVF and GC analyses of the entire data set, spanning samples collected in December 1988 through June 1991, and for UVF and total PAHs for samples collected December 1988 through March 1990 (PAH data are not yet available for bivalves collected later than March 1990). Some of the scatter in these plots can be accounted for if the data are grouped by time.

Table 3.21 shows that the slopes of the regressions between the UVF and GC determinations decreased after August 1990. Samples collected from November 1990 to June 1991 showed a detectable change in slope in the regression, indicating a change in the composition of the residues accumulated by bivalves. This change may be from a difference in the composition of PAHs accumulated or from a larger

Table 3.21 Regression equations for UVF (X) and GC (Y) determinations of oil residues in bivalve tissues. Concentrations were transformed to log(μg oil/mg EOM). N is the number of samples; R^2 is the correlation coefficient squared; P is the significance of the slope. Note that the final regression encompasses the entire sampling interval, 1988-1991.

Sampling Interval	Regression Equation	N	R^2	P
Dec. 88-Feb. 89	$Y = 0.74X + 0.16$	32	0.82	< .001
May 89-Dec. 89	$Y = 0.73X + 0.02$	33	0.63	< .001
Mar. 90-Aug. 90	$Y = 0.74X + 0.02$	41	0.68	< .001
Nov. 90-Jun. 91	$Y = 0.45X + 0.07$	44	0.45	< .01
Dec. 88-Jun. 91	$Y = 0.67X + 0.06$	150	0.60	< .001

percentage of the UVF signal due to fluorescent derivatives, rather than the parent hydrocarbons.

3.8 Summary Conclusions

The hydrocarbon-chemistry data confirm the long-term persistence of crude-oil residues deep in mangrove sediments. The initial fast rate of degradation of alkane fractions observed in 1986 was not maintained in later degradation rates of the more residual aromatic hydrocarbons. Pools of buried oil maintained surprising consistency in composition as seen by UVF, GC, and GC/MS analyses.

This buried oil was continually leached into coastal waters and accumulated by encrusting bivalves during the first 5 yr after the spill. Major reoiling events associated with erosion of heavily oiled coastal sediments created chronic oil pollution in coastal waters, with detectable, continuing biological effects in fringing mangroves (Garrity and Levings, Chap. 9), mangrove forests (Duke and Pinzón, Chap. 8), and reef corals (Chap. 7) 5 yr after the spill.

The most residual aromatic fractions appeared to be the dibenzothiophene, phenanthrene, and higher series. The continued high concentrations of these relatively toxic fractions, even in sediment residues appearing highly weathered by GC, indicates their life span in mangroves is much longer than 5 yr.

The effect of this oil spill was clearly discernible despite the history of oil pollution along this coast. The proportion of dead roots in mangrove-sediment cores provided a record of long-term damage by major oil pollution incidents.

Detectable changes in the composition of residues available for bioaccumulation 5 yr after the spill indicate that the next 5 yr will be critical for studying the gradation of lethal into sublethal biological effects in coastal systems. Justification for further study with emphasis on the functional ecology of mangroves is clear. Physiological stress measurements on key species would provide a sensitive means of determining sublethal effects. Chemistry should be expanded to attempt quantification of the residual derivatives of aromatic hydrocarbons because of the observed changes in the composition of oil residues over the first 5 yr postspill. The persistence for more than 20 yr of medium-weight aromatic hydrocarbons in heavily oiled sediments in a temperate saltmarsh was reported by Teal et al. (1992).

We hope that the potential for the study of the Bahía Las Minas spill site to provide further answers to remaining questions on the time scales and processes involved in environmental recovery will continue to be realized.

3.9 Alternative Sources of Contamination

The source of contamination was unquestionable in areas heavily contaminated by VMIC. GC and UVF patterns in environmental samples differed from the spilled oil in ways explained by weathering processes. Particular scrutiny was given to samples taken near the site of the 1968 *Witwater* wreck. Nearby mangroves, seagrass sediments, and coral reef sediments were contaminated primarily with degraded VMIC, although traces of degraded fuel oil were seen in mangrove and coral reef sediments. The only unoiled mangrove site with significant contamination by another oil was MARS. This contamination appeared to originate from a spill of light fuel oil; the concentration of oil was higher at depth than at the surface by September 1986. The surface assemblage of animals and plants seemed to be similar to that at MARM based on cursory observation. Thus the surface organisms appeared to have recovered from obvious effects by the time the core was taken in September 1986. One heavily oiled river site (PMRW) leached relatively fresh oil when cored in 1991. This oil was preserved from the 1986 spill, as shown by GC/MS analysis of triterpane biomarkers.

At unoiled reef and seagrass sites, residual contamination by tar balls and fuel residues likely originating from ships was visible by the highly sensitive UVF method. One unoiled reef (DMA) was contaminated by a relatively fresh oiling with heavy fuel oil. The chromatogram for this residue is shown in Figure 3.28. Judging by the rapid rate of degradation of oil residues in coral reef sediments, an estimate of less than 2 mo seems reasonable for timing this event. Because mangrove sediments at the closest site (MSM) did not show any detectable contamination in May 1989, this must have been an isolated incident that did not oil large areas of coastline. However, a reduction of benthic infauna, which may relate to this incident, occurred in algal beds directly downwind of the DMA reef (Cubit and Connor, Chap. 4).

During the 1990 collection, reef sites inside of Bahía Las Minas were subjected to a recent spill of fuel oil from the electricity plant. Fuel oil, however, was not detected in reef sediments or coral tissues. Similar events were evident in the UVF spectrum of oil residues in bivalves at mangrove site PAYR beginning in August 1989. This mixture of crude and fuel oils was visible in samples collected though May 1990; by August 1990 the spectrum was again dominated by crude-oil residues. These transient events were distinguished by fluorescence analysis.

Work was also carried out to characterize Sunniland crude oil from Florida in a comparison with the Venezuelan/Mexican Isthmian Crude to predict generally

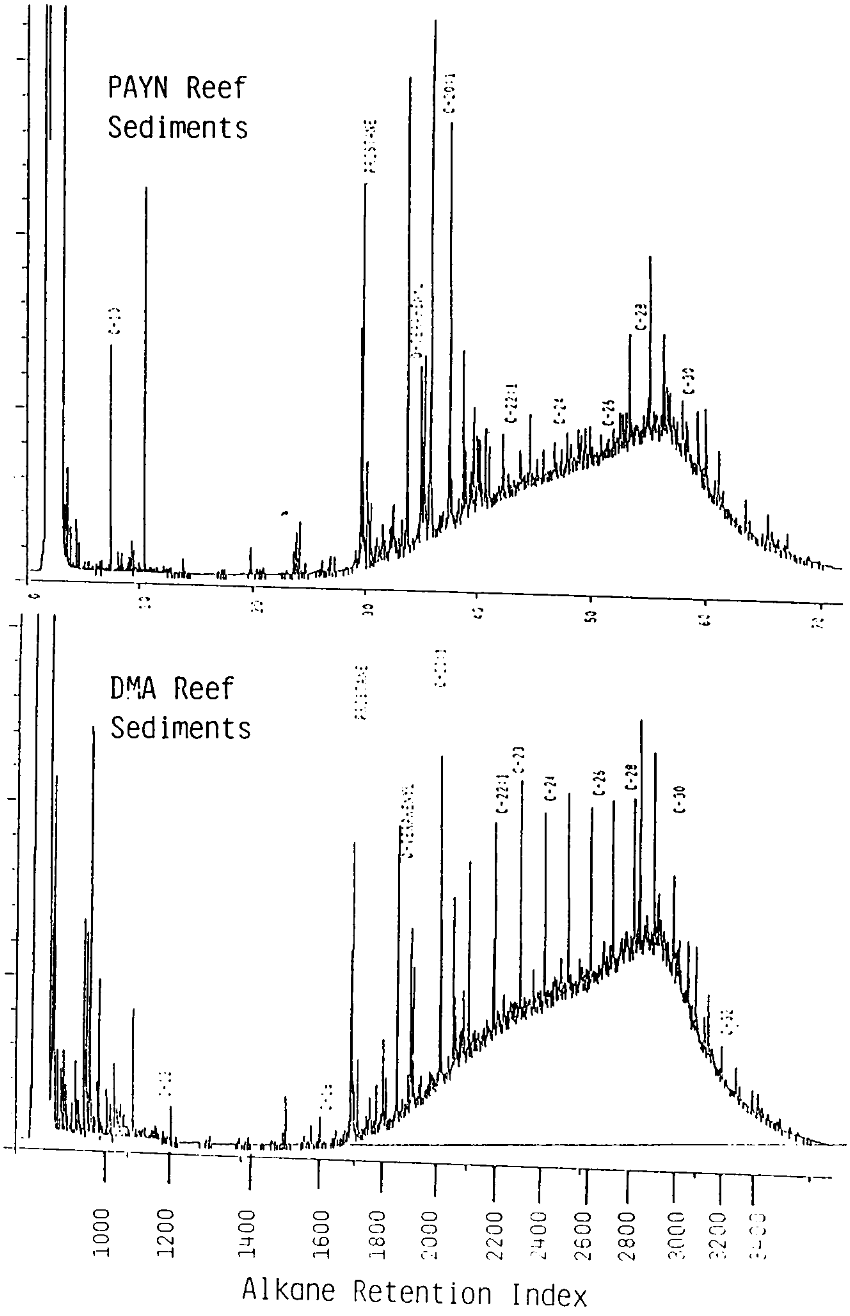


Fig. 3.28 Gas chromatograms of hydrocarbons in coral reef sediments, 1990. Note the content of a relatively fresh heavy fuel oil at DMA as seen in the distribution of the *n*-alkane peaks.

the relative toxicity of the two crude oils. This comparison was made to help assess the comparability of effects of the Bahía Las Minas spill with a possible spill of Sunniland crude oil on the coast of southern Florida. The densities of the two oils were: 0.846 g/cc (SD = 0.005, N = 9) for VMIC and 0.878 g/cc (SD = 0.007, N = 9) for Sunniland. Gas chromatography showed that the two crude oils have nearly identical elution ranges. Table 3.22 gives the results of GC/MS analysis of volatile benzene derivatives. There appeared to be no significant difference between the two crude oils.

Figure 3.29 shows the relative composition of individual PAHs in three reference crude oils: VMIC, Sunniland, and Prudhoe Bay crude oils. Sunniland appeared to be slightly more dense than VMIC and contained slightly lesser amounts of the more volatile PAHs. However, the composition followed the same pattern as the other medium-weight crude oils and would, in general, be expected to exhibit the same order-of-magnitude effects if spilled into coastal ecosystems. It is expected that weathering patterns would follow similar trends for all three crude oils. Sunniland Crude has a very different triterpane pattern, as shown in Figure 3.30. Thus VMIC and Sunniland could be distinguished by GC/MS analysis.

3.10 Analytical Quality Control and Quality Assurance Practices

The Bermuda Biological Station for Research (BBSR) organic chemistry laboratory follows quality and assurance procedures tailored to the needs of individual projects. Procedures relevant to petroleum-hydrocarbon, PAH, and oxidation-product analyses for the Bahía Las Minas oil spill study are described here.

3.10.1 Routine Instrument Calibrations

The basis of any chemical analysis is the analytical balance. BBSR has a complete set of calibration weights and the organic-chemistry staff routinely verify the accuracy of the balances. All calibration solutions of hydrocarbons are diluted using volumetric glassware. Solution weights are written on each vial and checked before use as calibration standards. Standards are the best-available purity and are tailored to meet specific analytical objectives. A reference alkane mixture was made in 1984 and aliquots of the concentrated stock solution were heat-sealed in glass ampoules. Stock solutions are made in isooctane or hexane, and dilutions for instrument calibrations are made fresh as use warrants. In addition to mixtures of pure compounds made in the lab, some commercial solutions are purchased to act as certified checks on laboratory accuracy. These solutions include a commercially available reference mixture of polynuclear aromatic hydrocarbons.

An initial calibration curve is generated over the linear range of the detector. A standard mixture within this linear range is run daily to ensure maintenance of instrument sensitivity and chromatographic integrity. The daily standard is compared with the initial calibration curve and must fall within 20% of known values. Daily response factors are used for sample quantification. The accuracy of a sample

Table 3.22 Content of volatile aromatics in the ethyl-benzene through decyl-benzene elution range in the Venezuelan/Mexican Isthmian Crude Oil and Sunniland Formation Crude Oil, for comparison. None of these volatile benzene derivatives could be detected in sediment core samples containing VMIC collected in September 1986. *Nondet.* = nondetectable.

Compound	VMIC Oil (μg/mg oil)	Sunniland Oil (μg/mg oil)
benzene[1]		
toluene[1]		
ethyl benzene	0.9	1.2
m,p-xylene	2.2	1.5
o-xylene	1.1	1.1
isopropyl benzene	0.1	0.2
n-propyl benzene	0.2	0.4
mesitylene	0.4	0.2
p-cymene	0.9	1.2
n-butyl benzene	0.4	0.2
n-hexyl benzene	Nondet.	Nondet.
n-octyl benzene	Nondet.	Nondet.
n-decyl benzene	Nondet.	Nondet.

[1] Benzene and toluene could not be quantified because of coelution with hexane used as the solvent for injection.

analysis thus is ensured not only by calculating sample data from response factors generated by standards run the same day, but also with the knowledge that the daily response factors are within the normal and well-characterized range.

If the daily standard falls outside of the satisfactory range, then the instrument undergoes trouble-shooting procedures to return it to optimum sensitivity. If this is not possible, the system is completely recalibrated and samples are only run when system performance is satisfactory. This applies to the gas chromatographs (GCs), the gas chromatograph/mass spectrometry system (GC/MS), the high-performance liquid chromatograph (HPLC), and the fluorometer (UVF).

3.10.1.1 GC-FID Calibrations

The linearity of the flame-ionization detector (FID) response is checked by injection of a series of dilutions of hydrocarbons, one of which is used as an internal standard. Calibration mixtures are made in the range of 5 to 100 ng/μL. The FID is linear over this range. A calibration mixture of saturated and unsaturated hydrocarbons is run daily when sample extracts are being analyzed. Samples are concentrated or diluted to yield accurately quantifiable results within the established linear range of the detector.

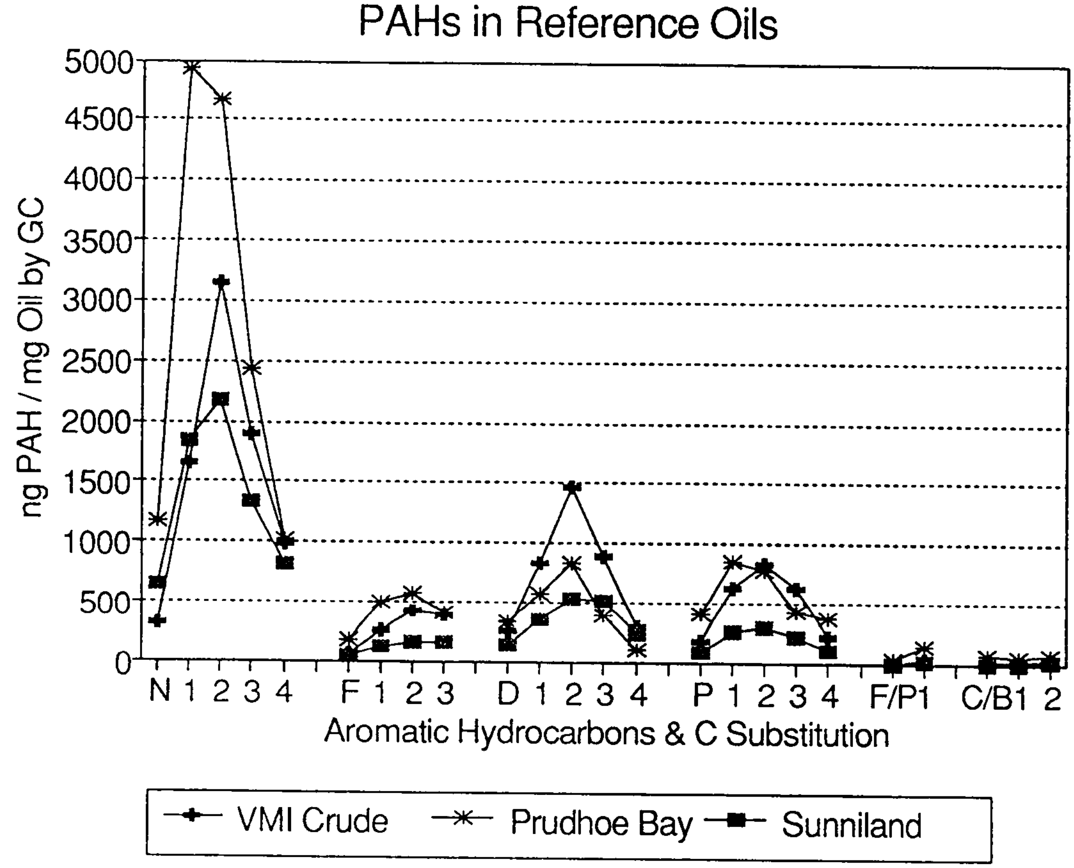


Fig. 3.29 Relative distribution of individual PAHs in three crude oils.

3.10.1.2 GC/MS

Internal auto-tune procedures conducted on a daily basis establish mass-accuracy and ion-abundance requirements. A daily calibration mixture is run in selected-ion-monitoring (SIM) mode. Relative response factors of peaks with mass/charge (m/z) ratios of compounds of interest are tabulated as per the GC-FID. When these deviate by more than 20%, evaluation and trouble-shooting procedures are initiated. Five-point calibration plots were produced for 20 PAHs and alkyl-substituted PAHs spanning the naphthalene through benzoperylene elution range. The m/z ratios for parent and confirming ions of the target PAHs are given in Table 3.23. When possible, response factors are based on pure PAH standards. Response factors for 21 other alkyl PAHs, for which no pure standards were available, were interpolated from the relative abundance of specific mass fragments

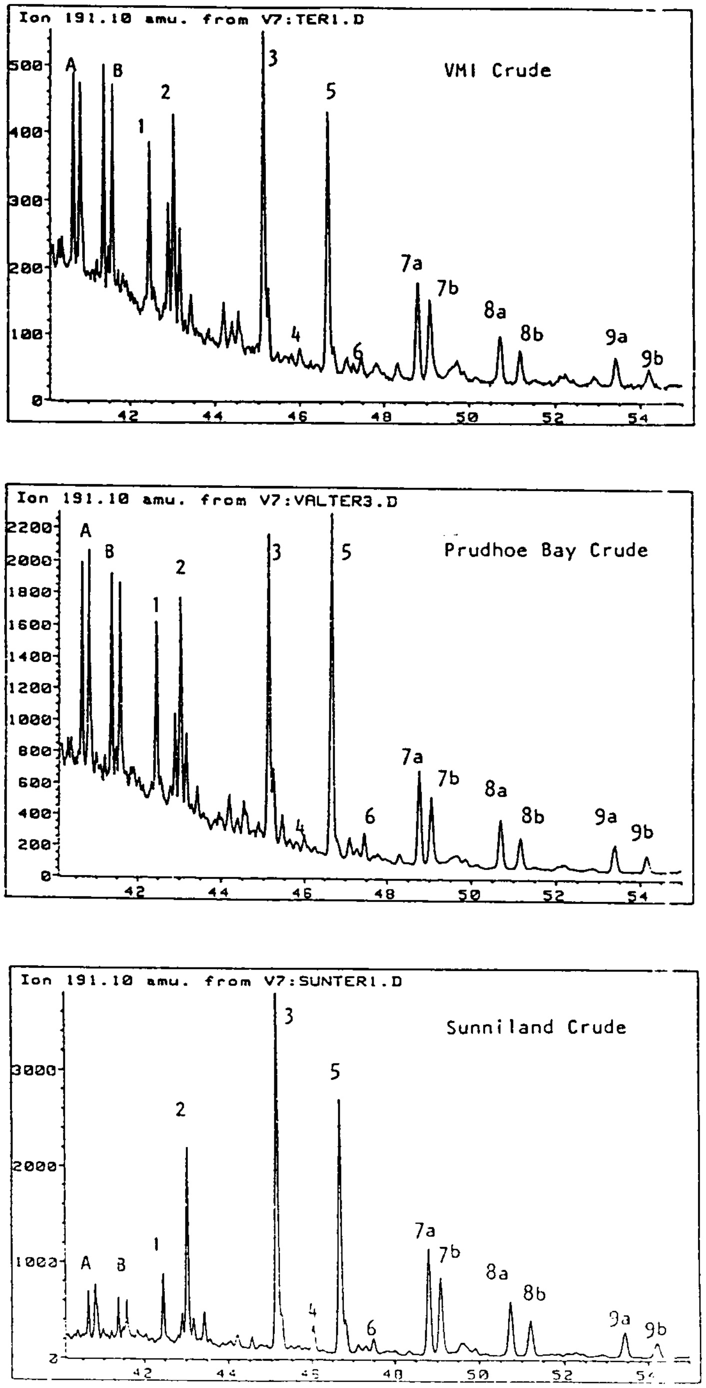


Fig. 3.30 GC/MS selected-ion-monitoring traces of ion 191 used for triterpane biomarkers of different crude oils. Ratios of specific peaks can be used for source oil differentiation; the peaks are identified in Appendix Table B.4.

Table 3.23 Selected-ion-monitoring program (m/z ratios) used for quantitative analyses of polynuclear aromatic hydrocarbons. *IS* = internal standard.

	Target Ion	Confirmation Ion
naphthalene	128	127
C_1 (2-methyl) naphthalene	142	141
C_1 (1-methyl) naphthalene	142	141
C_2-naphthalenes	156	141
d10-biphenyl (IS)	164	162
C_3-benzothiophenes	176	161
C_4-benzothiophenes	190	175
C_3-naphthalenes	170	155
C_4-naphthalenes	184	169
fluorene	166	165
C_1-fluorenes	180	165
dibenzothiophene	184	185
d10-phenanthrene (IS)	188	187
phenanthrene/anthracene	178	179
C_2-fluorenes	194	179
C_3-fluorenes	208	193
C_1-dibenzothiophenes	198	197
C_2-dibenzothiophenes	212	211
C_1-phenanthrenes/anthracenes	192	191
C_3-dibenzothiophenes	226	211
C_4-dibenzothiophenes	240	211
C_2-phenanthrenes/anthracenes	206	191
C_3-phenanthrenes/anthracenes	220	205
C_4-phenanthrenes/anthracenes	234	219/191
fluoranthene	202	200
pyrene	202	200
C_1-fluoranthenes/pyrenes	216	215
C_2-fluoranthenes/pyrenes	230	215
benzathracene	228	226
chrysene	228	226
C_1-benzanthracenes/chrysenes	242	243
C_2-benzanthracenes/chrysenes	256	241
C_3-benzanthracenes/chrysenes	270	
C_4-benzanthracenes/chrysenes	284	
benzofluoranthenes	252	241
benzopyrenes	252	253
d12-perylene (IS)	264	260
perylene	252	253
indenopyrene	276	138
dibenzanthracene	278	279
benzoperylene	276	138

as determined from scan data of the Venezuelan/ Mexican Isthmian crude oil and from published mass spectra. The deuterated internal standards used were: d10-biphenyl, d10-phenanthrene, and d12-perylene.

3.10.1.3 UVF

The signal-to-noise ratio of the fluorometer is checked routinely using the Raman emission of distilled water. If it drops below the specified limit, the lamp is replaced. Standard response graphs are made from fresh dilutions of crude oil or other relevant calibration mixtures. A new calibration graph is prepared for use with every batch of samples. The analyst initiates trouble-shooting procedures if standard graphs vary by more than 20% on a daily basis. Because UVF is only a semi-quantitative technique, calibration standards must be tailored to the application. For fractionated samples, D.VMIC was a quantitative standard for the aromatic fractions of the sediment and organism samples in this study.

3.10.2 Routine Blank and Recovery Experiments

Every batch of six to nine samples had a blank extraction spiked with a mixture of analytical standards. This blank underwent complete analysis as a sample, following all cleanup and evaporation stages. The procedures typically recover 80-100% of the C_{14} to C_{34} elution range. Recovery drops to 65-70% at C_{10} due to the evaporation steps.

Ideally, surrogate standards are added to every sample to compute recovery through the procedures and the results can then be corrected for recovery. This level of precise sample handling was used for samples that yielded similar levels of contaminants, such as coral tissue and reef sediments. In the seagrass and mangrove sediment samples, concentrations varied over many orders of magnitude making it difficult to estimate how much of the standards to add. Also, biogenic hydrocarbons in bivalve extracts often interfered with the accurate quantification of surrogate standards. Recovery was based on the standards in the spiked blanks and on the recovery of the spiked surrogate standards as determined by GC analysis, when possible. When samples were reduced in volume for GC analysis, a GC-quantification internal standard was added just before analysis to facilitate accurate determination of analytes. The surrogate standards used were $C_{18:1}$, $C_{22:1}$, and O-terphenyl and the internal standard was $C_{20:1}$. O-terphenyl is a particularly useful standard for samples undergoing UVF analysis as it elutes with the bulk of the aromatic hydrocarbons during isolation procedures, but has negligible fluorescence. For GC/MS analysis of PAHs, d10-biphenyl, d10-phenanthrene, and d10-perylene were added just prior to analysis. Each analysis report was then scrutinized for recovery of the internal standards. If recovery was unsatisfactory, the sample was reinjected.

3.10.3 Coding of Samples for Analysis

As samples were removed from the freezer for analysis they were assigned a sequential number on individual data sheets. The inventory number was listed against the sample analytical number in the laboratory notebook. From this stage onward, the analyst did not know the history of the samples. It was not until the data collation stage that the samples were grouped into sites and analytical replicates. In this way, analytical bias was minimized.

3.10.4 Analysis of Standard Reference Materials

The methods used in the BBSR organic chemistry laboratory for the analysis of petroleum hydrocarbons in marine samples conform to the UNEP Manual and Guides No. 20 (1991). A Quality Control Chart is kept by the laboratory using the Ma-K-1 oyster sample reference material for petroleum hydrocarbons. This material from the IAEA, Monaco, was analyzed yearly during the laboratory's participation in the Bahía Las Minas oil spill study. Results are shown in Figure 3.31. In 1990 the laboratory also participated in an international intercomparison exercise for analysis of petroleum hydrocarbons in a reference sediment by UVF and GC and individual PAHs by GC/MS. The BBSR laboratory results were excellent.

3.11 Acknowledgments

The hydrocarbon chemistry program was carried out under subcontract to the Smithsonian Tropical Research Institute with funding provided by the U.S. Minerals Management Service contract no. 14-12-001-30393. This chemistry work was done by Dr. K. Burns and the collaborating team of scientists and technical experts at the BBSR, D. Jorissen, J. MacPherson, M. Stoelting, J. Tierney, and L. Yelle. Dr. G. Kananen, Mrs. C. Hackett, and numerous Work Study students at BBSR contributed to the preparation and analysis of samples. Mrs. N. O'Leary, an instructor at the Bermuda College, did the protein analyses of coral tissues as a volunteer effort. Special collaboration with biologists Mr. S. Garrity and Dr. S. Levings in field work in the mangroves was essential. Additional help in field sampling was provided in 1986 by Ms. S. Wyers of BBSR and Drs. H. Caffey, M. Marshall, and J. Cubit of STRI. During the 1988/89/90 collections, additional assistance was provided by Dr. G. Kananen and Mrs. C. Hackett of BBSR, Messrs. S. Garrity and H. Guzmán, as well as several technical assistants at STRI. Mr. R. Burns of BBSR constructed the coring devices. Team work made this study successful. We thank these people and all the other collaborating biologists and staff at STRI.

This is contribution No. 1309 of the Bermuda Biological Station for Research.

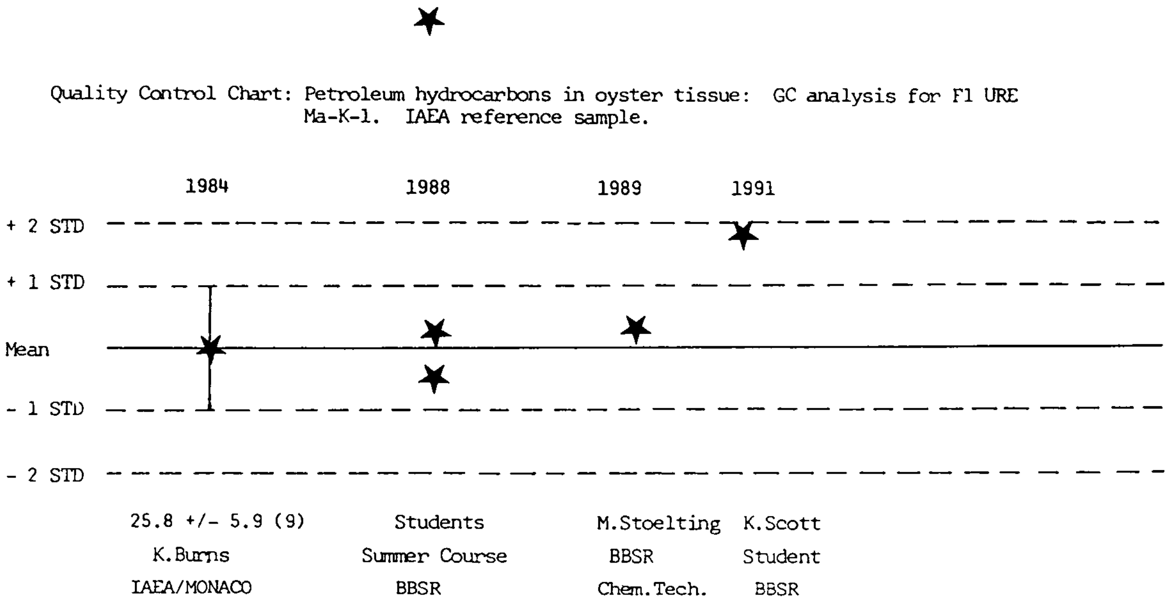


Fig. 3.31 Analytical quality control chart showing results of GC analysis of saturated petroleum hydrocarbons in reference oyster sample Ma-K-1. All analyses were done by or under the direction of Dr. K. Burns.

4

Effects of the 1986 Bahía Las Minas Oil Spill on Reef Flat Sessile Biota, Algal-turf Infauna, and Sea Urchins

John D. Cubit and Judith L. Connor

4.1 Abstract

On 27 April 1986 at least 60,000-100,000 bbl of medium-weight crude oil spilled from a refinery on the Caribbean coast of Panama. After weathering for 6 d in a small bay, the oil escaped and began to wash onto the fringing reef flats of Bahía Las Minas. The areas affected included the reef flat and biological reserve at Punta Galeta, which had been under study since 1970. The prespill database for Punta Galeta consisted of hourly hydrographic and meteorological records and detailed, time-series monitoring of the spatial distributions and abundances of algae, seagrasses, sessile invertebrates, and sea urchins on reef flats. Using these data, the populations of reef flat biota were compared before and after the oil spill. Postspill comparisons were also made among additional sites. The prespill baseline data established relationships between variations in the physical environment and changes in the reef flat biota, which were used to distinguish effects of the oil from natural physical stresses.

Continuous observations of oil movement and accumulation were made during the spill. Deposition of oil was not uniform over reef flats, which allowed population changes to be compared over gradients of oiling. Onshore winds and waves concentrated oil slicks against raised barriers, including seaward margins of reef flats, which were exposed above water level by seasonally low sea levels and low tides. At high tide oil floated over reef flats and exited through natural drainage channels or collected along beaches and natural berms on the landward sides of reef flats.

Mortality of plants and invertebrates was greatest in the zones where oil had accumulated. Where oil floated over the reef flat without prolonged direct contact with reef flat biota, no mortality of sea urchins, algae, or seagrasses was detected. Rates of regeneration varied among species and appeared to be dependent on a number of factors, including the severity of initial damage, the mode of recruitment of new individuals, the proximity of sources of propagules, and the pre-emption of space by competitors.

Populations of some sessile biota, such as fleshy macroalgae and crustose coralline algae, regenerated within a year. However, populations of some other sessile biota, such as stony corals and the zoanthids *Palythoa* spp. had not fully recovered as of the last surveys in June 1990. With the possible exception of one group of crustaceans, the tanaids, populations of infauna in the algal turfs (formed primarily by the fleshy red alga *Laurencia papillosa)* were re-established less than 15

mo after the oil spill. Patterns of recruitment of sea urchins suggested some persistent factor on the substratum diminished recruitment, possibly by inhibiting settlement of larvae. Apparently the regeneration of all macroalgae and sessile invertebrates depended on regrowth of fragments surviving *in situ* or washing in from nearby habitats. For these attached biota, no evidence of regeneration from spores or larvae was observed, and the species with the fastest regeneration times were those regrowing from fragments that survived *in situ*. Populations of biota slow to recover were those that were nearly eliminated from the reef flat (e.g., stony corals and *Palythoa* spp.); the regeneration of these biota depended on fragments washing in from deeper, subtidal habitats. These results indicate the recovery of other sessile biota would have also been delayed if longer exposure to oil, or exposure to more toxic oil, had reduced the *in situ* survival below a critical minimum.

4.2 Introduction

Reef flats are broad, shallow platforms of coralline rock and calcareous sediments formed by coral-algal reefs when the reefs reach sea level. Biological processes produce nearly all the structure and substrata of reef flats. Because sea level has been relatively stable for the past several thousand years, reef flats have formed on many actively growing reefs. These extensive, highly productive habitats are common throughout the tropical and subtropical areas of the world and account for much of the surface area of many warm-water reefs.

Reef flats are important components of coastal environments. They have been ranked among the most productive ecosystems on earth (Lewis 1977; Kinsey 1985; Hatcher 1988). Coastal ecosystems in the tropics and subtropics generally depend on benthic, rather than planktonic, productivity (Hatcher 1988), and an estimated 10 to 60 percent of the benthic productivity from reef flats is exported to other marine ecosystems. This material is thought to be a major food source for animals in deeper water, including corals (Kinsey 1985). Reef flats are important foraging grounds for fish, shore birds, lobsters, and other animals. Beds of the foliose red algae *Laurencia* spp. on tidal and shallow subtidal flats in Florida are essential as nursery and foraging sites for spiny lobsters (e.g., Herrnkind and Butler 1986; Butler and Herrnkind 1991). Consequently, the Florida Coastal Oil Spill Handbook lists these algae among the biota of concern for oil spill damage (State of Florida 1986). Reef flats act as natural breakwaters and shoreline revetments (Fig. 4.1). Hydrodynamically, the shallowest shore structures are the most important in protecting shorelines from wave action (U.S. Army Corps of Engineers 1984); therefore, along many tropical and subtropical coasts, reef flats, as well as other tidal flats, are the primary protective structures for developed property and coastal ecosystems, such as mangrove forests and coastal wetlands.

Reef flats tend to have certain features in common. As illustrated by the zonation map of Punta Galeta (Fig. 4.2), the seaward, or windward, sides of most reef flats are protected by concrete-like pavements, or revetments, built by coralline algae and corals. These masses of coralline rock are usually the highest parts of reef

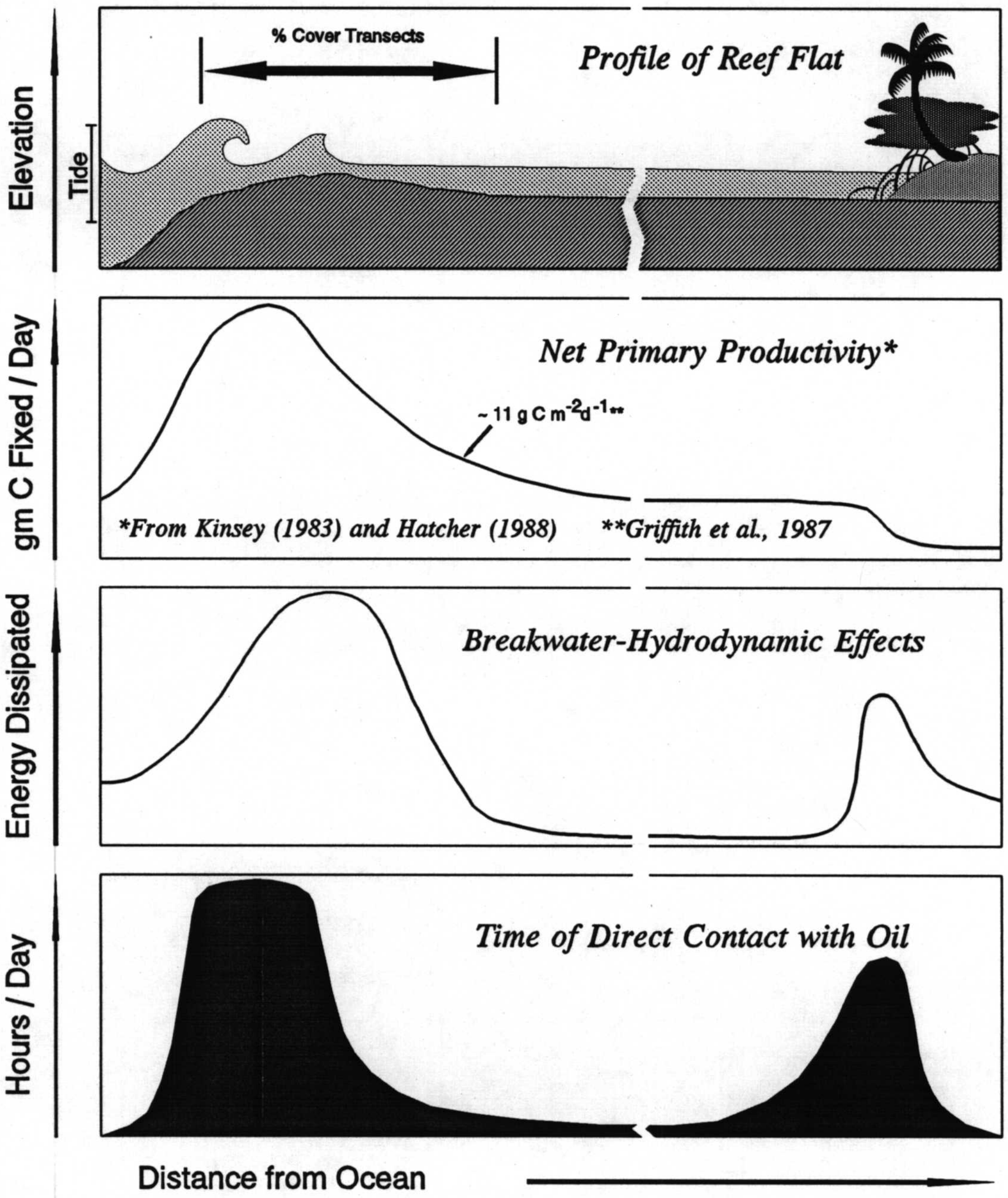


Fig. 4.1 Schematic profile of a fringing reef flat in Bahía Las Minas (*top*). The graphs show relative rates of primary productivity, hydrodynamic effects, and time of direct contact with oil during the 1986 Bahía Las Minas spill at positions corresponding to the reef flat profile. The *vertical bar* to the left of the reef flat profile represents the approximate range of water levels, including the extremes caused by storm surges. The span labeled *% Cover Transects* is the zone of the REDGE transects. As shown by the gap in the figures, the horizontal scale has been greatly shortened.

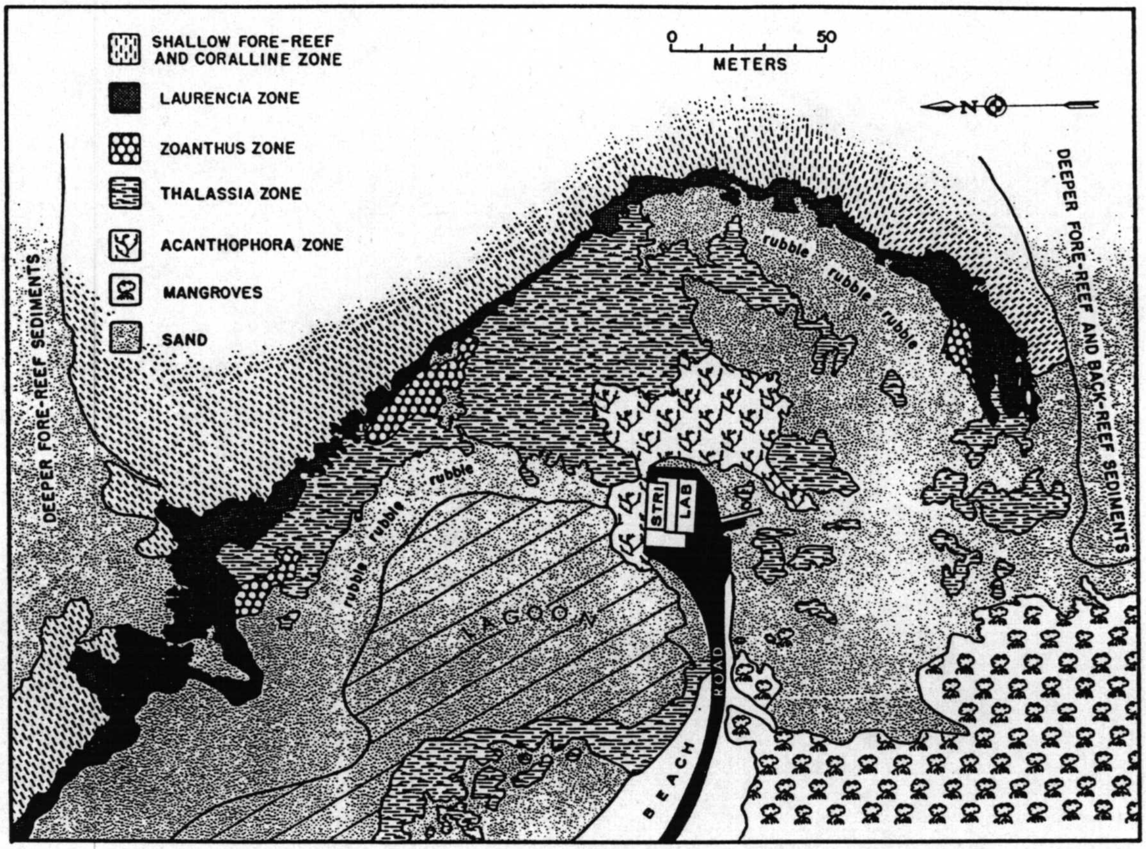


Fig. 4.2 Zonation map of the reef flat at Punta Galeta showing the spatial distribution of the principal biota. The zones are not mutually exclusive, i.e., the distributions overlap, and some species are found outside the zones shown on the map. This map was produced for the ZONE surveys (from Meyer and Birkeland 1974; see text for more explanation).

flats and are the principal structures determining the dissipation of wave energy, rates of water flow, and many other hydrographic factors on reef flats as a whole. Behind the protection of these revetments, coralline rubble and sediments accumulate. Much of this material is generated *in situ* by coralline red algae, calcareous green algae (especially *Halimeda* species), and various invertebrates with calcareous shells and skeletons. These sediments are stabilized primarily by plants. The root-like growths of rhizophytic (root-forming) green algae and seagrasses trap and bind the sediments. Canopies of algal mats further promote deposition and retention of sediments.

Although hundreds of species are found on reef flats (e.g., Cubit and Williams 1983), a few species tend to predominate. As shown in Figure 4.3, less than 10 species account for nearly all the biotic cover on the reef flat at Punta Galeta. Furthermore, these 10 species, or similar species in the same genera, are not found

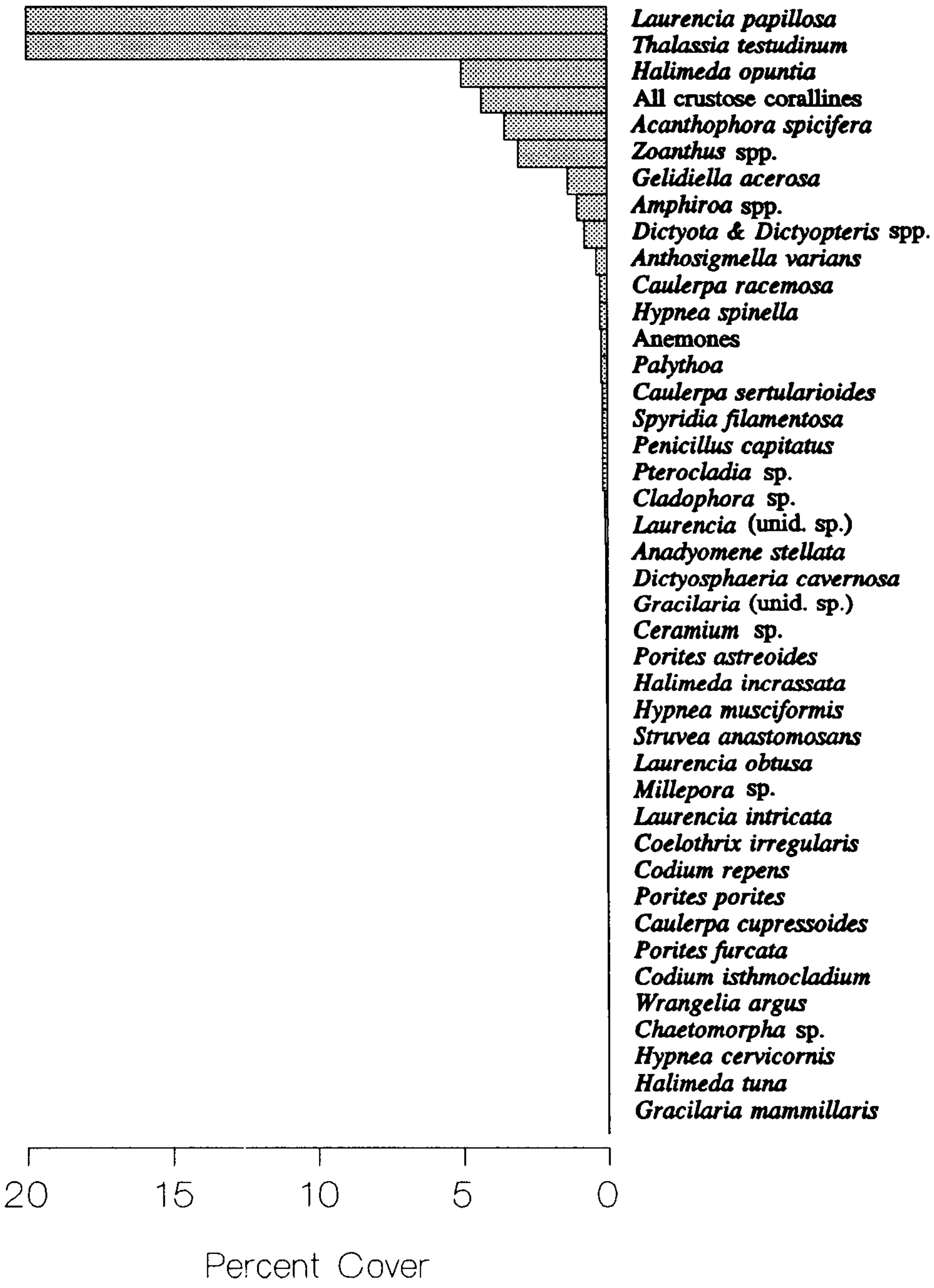


Fig. 4.3 Principal species and groups of species of sessile biota on the reef flat at Punta Galeta, as determined by the CONSURV surveys of the whole reef flat (1981-1982). The biota have been ordered by relative percent cover. The graph includes only the species or groups with >0.01 percent cover; bare space is not included.

just on the reef flats of Bahía Las Minas, but on various kinds of warm-water tidal flats throughout the tropics and subtropics. (Warm-water tidal flats include essentially any level, relatively stable substrata in the intertidal and shallow subtidal zones of the tropics and subtropics, such as reef platforms, limestone shelves, shell deposits, and accumulations of carbonate loose rock and sediment.) For example, crustose coralline algae, turfs of *Laurencia papillosa*, and the sea urchin *Echinometra lucunter* are the principal biota on hard substrata of reef flats in Bahía Las Minas (Birkeland et al. 1976) and on intertidal limestone flats along the Florida Keys (Stephenson and Stephenson 1950; Croley and Dawes 1970; Chan 1977). The predominant biota found in areas of sand and mud of back-reef flats in Bahía Las Minas are the seagrass *Thalassia testudinum*, rhizophytic (root-forming) green algae (e.g., *Halimeda* spp. and *Penicillus* spp.), and mats of fleshy red algae (e.g., *Laurencia* spp.). These are the same principal species or genera found on the sedimentary tidal flats of South Florida (e.g., Hoffmeister 1974; Zieman 1982; Shinn et al. 1989). However, factors that affect oil movement and deposition such as sea conditions, weather, and tidal flat topography must be carefully considered when generalizing results from the Bahía Las Minas oil spill to other tidal flat environments (Cubit and Connor 1993; Cubit and Levings, Chap. 2). As explained further in the Discussion and Conclusions (Sect. 4.5), topography and sea conditions would make the tidal flats in South Florida more susceptible to damage from oil spills.

Algae, seagrasses, and sessile invertebrates with photosynthetic symbionts cover most of the surface of reef flats with a highly productive photosynthetic lawn. The lawn provides both food and cover for high densities and diversities of mobile invertebrates (e.g., Cubit and Williams 1983). Mobile invertebrates (e.g., echinoderms, small crustaceans, polychaetes, and gastropods) provide the trophic link between primary producers and carnivorous predators, such as larger invertebrates, fishes, and shore birds.

The shallowness of reef flats and other tidal flat ecosystems makes them highly vulnerable to oil spills (Gundlach and Hayes 1978; NRC 1985; State of Florida 1986). Because reef flats are built by the plants and animals that grow on them, the lethal effects of an oil spill have the potential to damage the actual physical structure of reef flats, not just the veneer of biota on the surface. The solid, hard portions of reef flats are built by coralline algae and corals. The sedimentary portions are built primarily from the skeletons of calcareous algae and animals growing on the reef flat. These sediments are trapped and held together by the roots of root-forming algae and seagrasses. Both the solid and sedimentary structures of reef flats are subject to erosion through physical and biological processes (Steneck and Adey 1976; Lewis 1977; Trudgill 1983; Hubbard 1986). Without the construction and maintenance activity of the reef flat biota, the solid and sedimentary components of these structures would erode away.

Despite the vulnerability of reef flats to oil spills and the ecological and economic importance of this habitat, there is very little information regarding the effects of oil spills in these ecosystems. The *Oil in the Sea* report (NRC 1985, p. 412) describes the state of information as follows:

> **"One area that has been totally neglected is that of spill impact on tropical or warm-water microphytes and macrophytes. Of particular concern in this respect are the giant algal flats that constitute important components of tropical trophic systems and barrier reef systems. These are highly vulnerable to oiling because of their shallowness. Except for a single follow-up study (Lopez 1978), nothing is known of their recovery potential following oiling."**

The Minerals Management Service (MMS) of the U.S. Department of the Interior funded this study of the 1986 Bahía Las Minas oil spill primarily to evaluate the vulnerability of marine environments in South Florida to oil spills. The National Research Council's description of "totally neglected" is an accurate assessment for all tidal flats, including reef flats, in South Florida. Extensive and diverse tidal flat habitats exist in Florida Bay and along the Florida Keys to the Dry Tortugas (Mayer 1914; Stephenson and Stephenson 1950; Phillips 1959, Croley and Dawes 1970; Hoffmeister 1974; Chan 1977; U.S. National Ocean Service 1986; Jaap and Hallock 1990; J. Ogden, pers. comm.; M. Robblee, pers. comm.; J. Cubit, pers. obs.). The MMS funded and published a series of surveys of the marine habitats of South Florida to determine the environments at risk from oil spills. These surveys systematically covered the entire geographic area of shallow-water environments of Florida Bay and the Florida Keys to the Dry Tortugas. However, the surveys concentrated on particular, mostly subtidal, species and did not include the hundreds of hectares of biotic communities on reef flats and other tidal flats in this region (Jaap 1984; Environmental Science and Engineering, Inc. et al. 1987; Phillips and Larson 1990). In another study, the NRC (1989) evaluated these surveys of South Florida for their adequacy to assess potential effects of oil and gas activities and concluded "the shallow-water benthic communities, other than corals, have been inadequately studied and characterized."

The earlier published results of this study of the 1986 Bahía Las Minas oil spill have already been extrapolated to the extensive, seagrass-covered tidal flats of South Florida. Citing the mortality of reef flat vegetation caused by direct contact with oil (documented in Cubit et al. 1987 and Jackson et al. 1989), Zieman (1990) predicted an oil spill could destroy "the priceless nursery meadows" of shallow seagrasses in Everglades National Park and the western portions of the Lower Keys. The applicability of the results of this Bahía Las Minas study to other tidal flat environments are treated in more detail in the Discussion of this chapter (Sect 4.5).

In May 1986 the Bahía Las Minas oil spill washed onto the reef flat at Punta Galeta that had been under study since 1970. The prespill database consisted of extensive, detailed monitoring of the abundances and spatial distributions of plants and animals on the reef flat in relation to concurrent changes in weather and sea conditions. These data demonstrate that the abundances of reef flat species are highly dynamic, changing mainly in response to fluctuations of the physical environment (e.g., Cubit 1985; Cubit et al. 1986). These baseline data allowed us to compare abundances of the flora and fauna on the reef flat before and after the oil spill, using physical monitoring data to distinguish effects of the oil from effects of variations in sea and weather conditions. In addition, the distinct pattern of oil deposition on the reef flat provided the basis to compare changes in the biota in zones that differed in direct exposure to oil. After the oil spill we also began monitoring additional oiled and unoiled sites to measure the extent to which postspill events at Punta Galeta Reef represented general phenomena. The surveys of biotic cover and echinoid populations on the reef flat included more than 90% of the living biomass, sessile organisms, primary producers, structuring species, and reef builders at this site.

4.3 Objectives, Design, and Confounding Factors

4.3.1 Objectives

The purpose of this element of the study was to examine the types of ecological changes caused by the 1986 Bahía Las Minas oil spill and to elucidate, as well as possible, the processes of cause-and-effect that produced these changes and that can be applied to the effects of oil spills in similar shallow-water ecosystems. Unlike many studies of oil spills, this one was not performed to levy fines or charge for losses. Such studies are primarily concerned with assessing total damage and time to recovery after a particular oil spill. As discussed in more detail at the end of this chapter, these measures, *per se*, have limited value in understanding how oil spills affect marine biota. Consequently, they also have limited application to evaluating the potential effect of other oil spills. More important for generalization are the processes that determined the amount of damage, including the effects of factors such as sea conditions, weather conditions, shoreline configuration, biotic zonation, substratum types, and topography of the tidal flats of concern.

4.3.2 Design and Consideration of Confounding Factors

4.3.2.1 Problems in Designing a Post-hoc Study

To design this study, it was necessary to confront the serious dilemma that affects all unforeseen, after-the-fact studies of a single event. The 1986 oil spill was not a controlled experiment. The study was a post-hoc investigation of a single oil spill, and choosing treatments corresponding to "experimentals" and "controls" involved serious, unavoidable biases that could invalidate the results if not properly recognized and treated. Most of the information regarding effects of the oil was derived from surveys that compared the biota of sites or habitats differing in exposure to oil. Unlike a correctly designed experiment, there was only one, unreplicated oil spill, and the oil remained in one geographic area. Therefore, dividing this area into various "oiled" sites to obtain sample sizes greater than one is what Hurlbert (1984) called "pseudoreplication" in space. The various factors that moved and deposited oil slicks definitely did not produce a random distribution of oil. To compare "oiled" and "unoiled" sites meant comparing two different regions of the coastline (Sect. 1.4). All oiled sites were in the region of Bahía Las Minas, and all the unoiled sites were in the region of Costa Arriba (between María Soto and Isla Grande; Fig. 4.4); therefore, it was neither possible to randomly choose nor intersperse control and affected sites within the same area of coastline. Hurlbert (1984) suggested that lack of interspersion is a more severe problem than nonrandom selection. As described below, the environments of these two regions have many differences, and the resulting bias violates both the basic principles of experimental design and the fundamental requirements of ANOVAs and other statistics needed to analyze the data. These are not vague, hypothetical problems of concern only to statistical purists: if one compares two locations not only different in oil exposure, but also different in sea conditions, weather conditions, geology, and human modification, then there is a high risk of finding differences in the biota caused by differences in the basic environment, regardless of oil. In the science of investigating cause-and-effect and testing hypotheses, two principles are fundamental: (1) only the factor(s) of interest should vary among treatments, and (2) if other factors cannot be controlled, then consideration of the strongest possible alternative hypotheses is obligatory. If these procedures are not followed, the results are not valid. Consequently, the validity of such a post-hoc study depends on a very critical examination of assumptions and biases that could affect the findings of the study.

When studying impacts of particular events, Green (1979) and Stewart-Oaten et al. (1986) have discussed study designs to cope with the problems discussed above. These designs compare changes (1) at impact and control sites before and after an event, (2) at impact and control sites only after an event, or (3) at impact sites only (with no control sites) before and after an event. In this study we use a different design. To reduce dependence on comparisons between the regions of Bahía Las Minas and Costa Arriba, the primary source of information in this study is derived from *within-site* and *within-survey method* comparisons of zones of reef flats with different degrees of oiling. Within-site comparisons eliminated biases caused by regional differences, but were subject to smaller-scale potential biases resulting from comparisons of different zones. To compare biota under different exposures to oil, it was necessary to compare biota in close proximity, but in somewhat different physical environments. Consequently, an essential part of the reef flat study was the examination of the alternative hypotheses that mortalities during the oil spill were caused by differences existing among zones, including exposure to desiccation, insolation, high temperatures, and other physical stresses, the severity of which was at a seasonal peak during the oil spill. The detail of the long-term baseline data for changes in the biota at Punta Galeta relative to the monitoring of the physical environment allowed these hypotheses to be tested. The principal site was Punta Galeta, where detailed, long-term monitoring of the physical environment and reef flat biota formed a set of baseline data that could be used to make before-and-after comparisons in relation to physical conditions and to the gradient of oil deposition on the reef flat. In addition, the laboratory is located on this reef flat, which allowed direct, continuous observations of the processes of oil movement, oil deposition, and the immediate effects of oil on the reef flat biota. As described below, postspill comparisons were also made among additional sites.

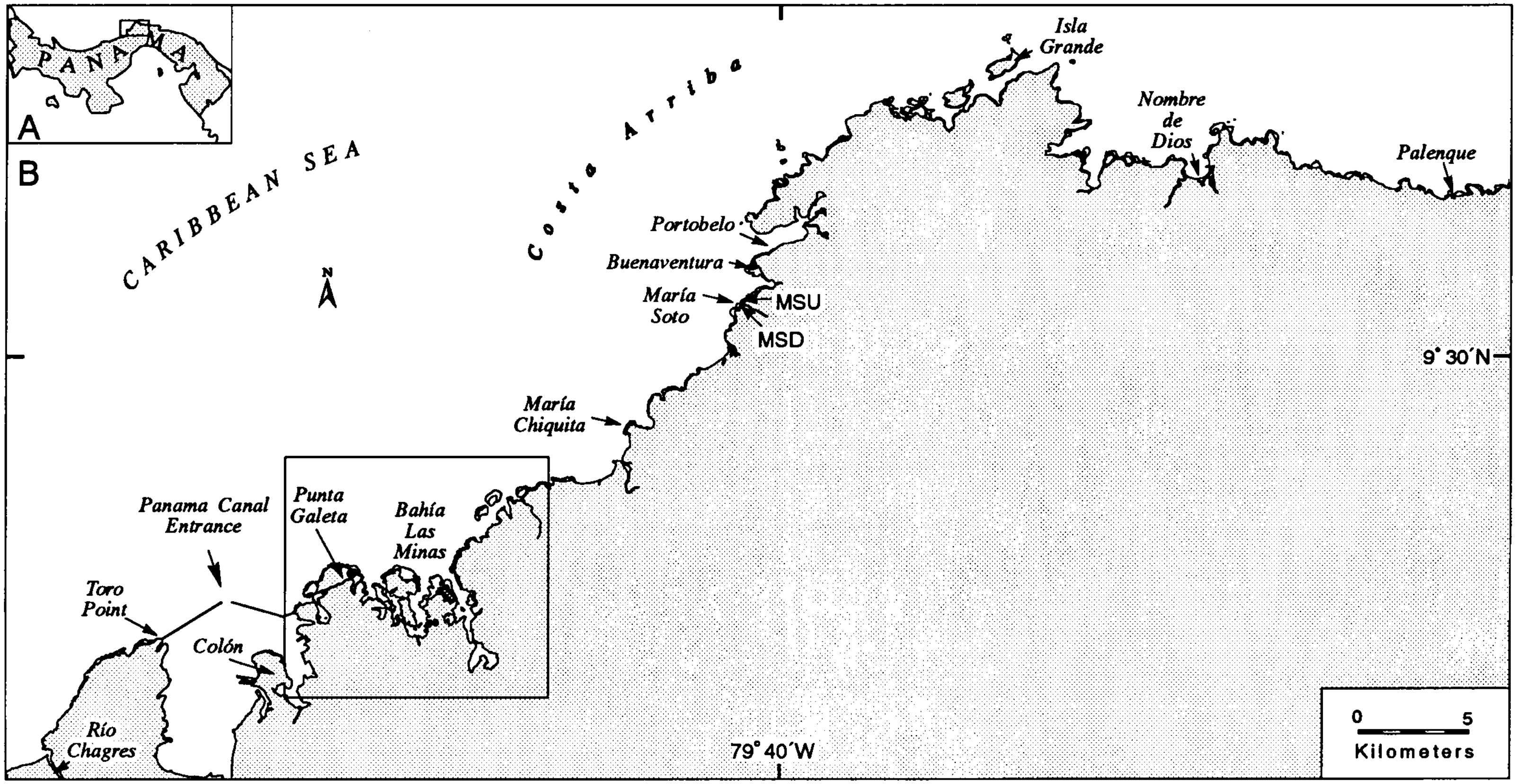


Fig. 4.4 Map of the central Caribbean coast of the Republic of Panama, shown as increasing enlargements. *A*. The Republic of Panama; the *box* designates the area of map *B*. *B*. The central Caribbean coast of Panama between the Río Chagres and the town of Palenque, showing areas surveyed for oil contamination and potential study sites. The unoiled sites for biological monitoring were María Soto Arriba (*MSU*) and María Soto Abajo (*MSD*).

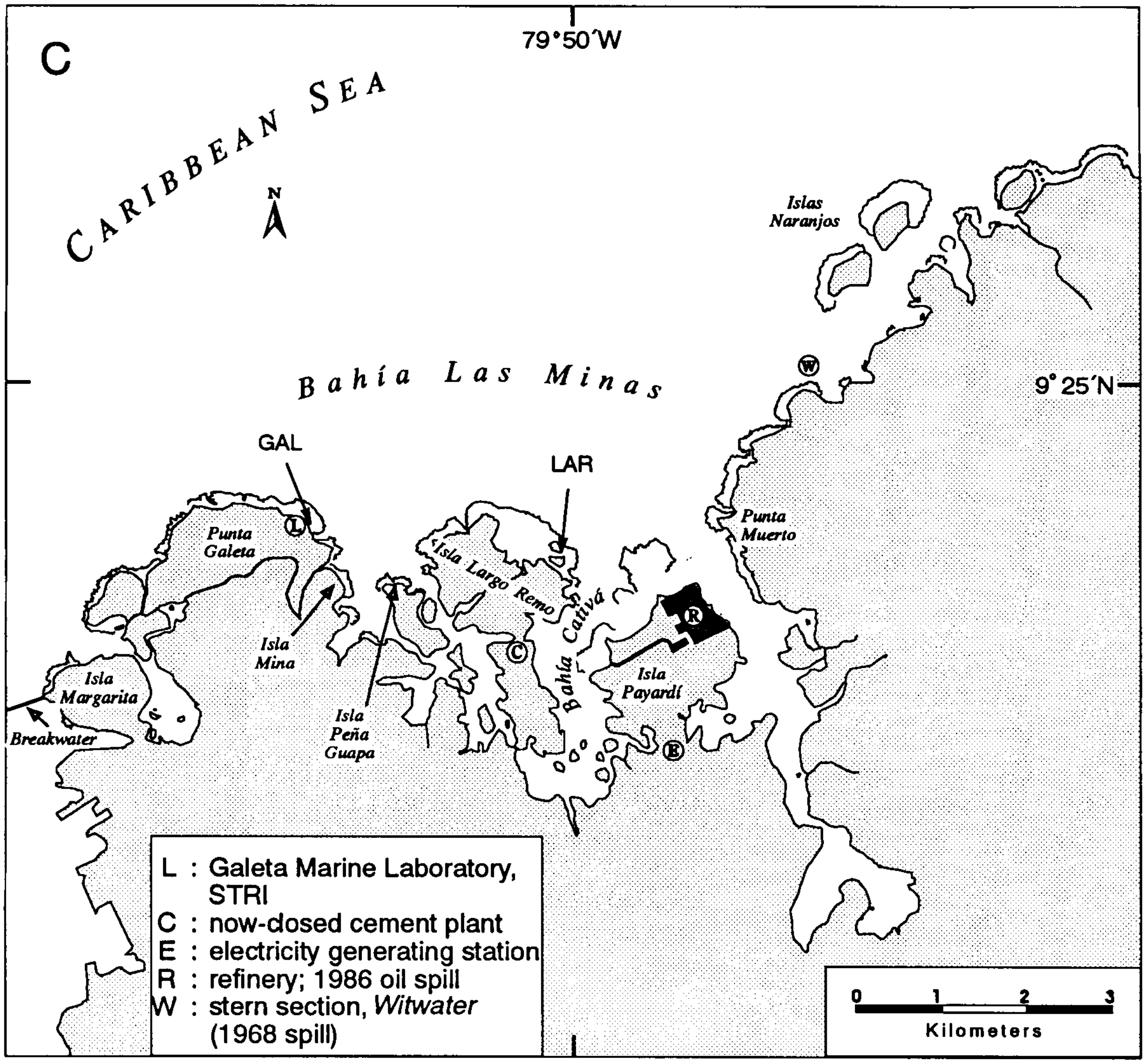


Fig. 4.4 Map of the central Caribbean coast of the Republic of Panama, shown as increasing enlargements (continued). *C.* Map of Bahía Las Minas and vicinity. The oiled sites for biological monitoring were Punta Galeta (*GAL*) and Isla Largo Remo (*LAR*).

4.3.2.2 Weather and Sea Conditions as Potential Confounding Factors in Examining Effects of the 1986 Oil Spill on Reef Flat Communities

Seasonal and year-to-year variations of weather and sea conditions strongly affect the abundances of plants and animals on reef flats in Bahía Las Minas (Hendler 1977; Connor 1984; Schneider 1985; Cubit 1985; Cubit et al. 1986, 1988*a*, 1989) and therefore must be taken into account in evaluating effects of the oil spill.

The single most important factor in this regard is the time the reef flat is exposed above water level during the warmer, sunnier parts of the day (Hendler 1977; Cubit 1985; Cubit et al. 1986, 1988*b*, 1989). These daytime exposures of the reef flat above water level (also termed "emersions" of the reef flat) are seasonal and most frequent between March and June (Cubit et al. 1986, 1989). Because the exposures naturally cause mass mortalities of many reef flat plants and animals, their effects must be taken into account in evaluating the 1986 Bahía Las Minas oil spill, which started at the end of April.

Descriptions of the physical environment in this chapter were calculated from the long-term database for weather and sea conditions at Punta Galeta. Implemented in 1974, this monitoring program provides a strong quantitative basis for defining the physical conditions during the 1986 oil spill in comparison to the seasonal and year-to-year variations in conditions recorded from this site. This constantly evolving and expanding database (see Cubit et al. 1989 for a list of published sources) consists of hourly records of air and sea conditions from the Smithsonian Tropical Research Institute Environmental Sciences Program. A corrected version of the 5 August 1991 update of the database was used, and includes corrections to earlier data added by K. Kaufmann, R. Thompson, and J. Cubit. The methods of monitoring and variability of conditions are described in Cubit et al. (1986, 1988*a*, and 1989). This section will summarize conditions of daytime water level during the 1986 oil spill. Cubit and Levings (Chap. 2) compare water levels and wind conditions during the 1986 oil spill with those conditions in other months and years.

The total time reef flats were exposed above water level was a critical factor in interpreting changes in the zonation and abundance of plants and animals on reef flats following the oil spill (Cubit and Levings, Chap. 2). To calculate average monthly water levels, biases caused by missing data were reduced by using data only from those months with at least 90% of the hourly records existent for daytime water level. The months of each year meeting these criteria are shown in Table 4.1. Within this group, proportional corrections were estimated for the missing data, standardizing each of these months to a full month of data. In the description of yearly total times of exposure above water level (*emersion*), only the actual data were used, without adjustments. The missing data in some months of frequent exposure may have produced substantial underestimates of total exposure times for some years, particularly 1975 and 1977 (Table 4.1). The exposure criteria used were hours with water levels below 26 cm above Galeta datum, which corresponds to exposures of most of the reef flat surface above water level (Meyer and Birkeland 1974; Meyer et al. 1975; Cubit et al. 1986, 1989). (Galeta datum is the fixed zero level from which all water level measurements at Punta Galeta are made. The datum level is approximately 30 cm below mean low water, and is defined in relation to regional tidal levels in Cubit et al. 1986). The daytime hours used were 0800 to 1800, inclusive, which is the portion of the day most exposed to the stresses of desiccation, insolation, and high temperatures.

Table 4.1 Percent of hourly records having valid data for the combination of water level, wind direction, and wind speed between 0800 and 1800, inclusive.

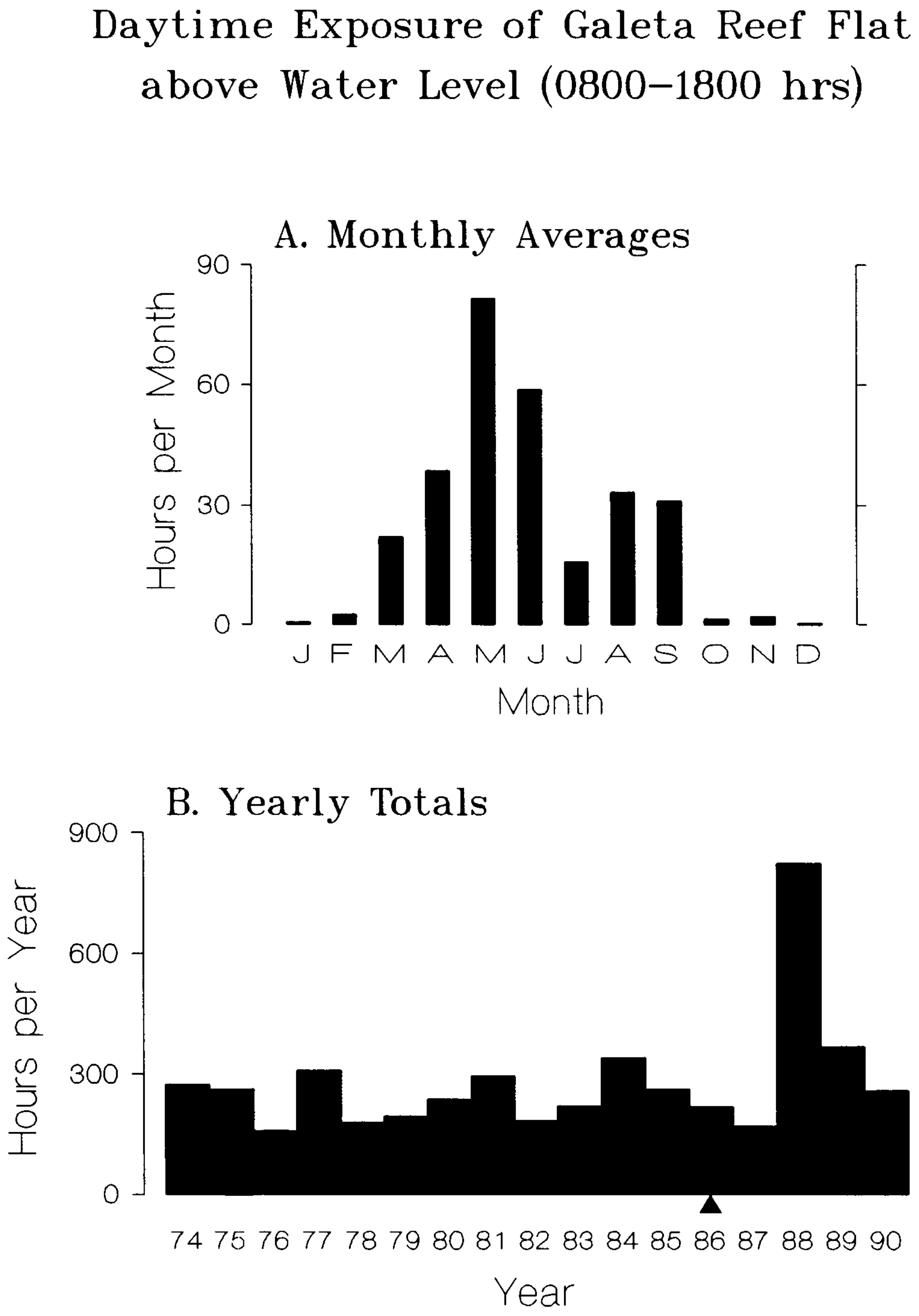
	Month												
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year Mean
1974	98	100	100	96	99	100	100	100	100	95	100	100	99
1975	100	100	100	73	88	100	75	58	100	66	69	100	86
1976	100	67	63	100	84	97	83	100	87	100	95	67	87
1977	91	35	66	1	76	59	51	92	100	76	100	96	70
1978	100	100	94	92	97	97	100	100	100	100	99	84	97
1979	20	43	100	96	76	100	100	100	100	92	98	83	84
1980	100	100	96	90	95	100	96	91	100	96	86	72	93
1981	96	88	82	100	96	99	100	100	100	95	100	100	96
1982	99	94	87	100	100	100	100	100	100	91	90	95	96
1983	89	100	93	100	100	100	100	100	100	100	100	100	98
1984	100	100	100	100	100	100	100	99	100	100	100	100	99
1985	96	100	99	100	100	100	100	100	100	100	100	90	98
1986	100	100	98	100	100	99	96	77	98	100	100	100	97
1987	100	100	100	100	100	100	100	100	100	95	100	100	99
1988	100	78	97	87	100	100	100	99	100	96	100	100	96
1989	99	81	98	100	100	100	96	100	100	100	99	80	96
1990	92	100	100	100	92	97	98	91	87	54	100	99	92

Figure 4.5 shows the average monthly patterns and yearly total of daytime exposures of the full reef flat. Daytime exposure frequencies were highest in the months of April, May, and June, the time of year when oil slicks reached most of the reef flats in Bahía Las Minas during the 1986 spill. The total daytime exposure times for 1986 were moderate compared with other years. In 1988, however, the total exposure times were much higher than any year on record.

4.3.2.3 Sites Added After the 1986 Oil Spill

Site Selection Process: Rationale and Procedure

More sites were added mainly for two reasons: (1) to determine the extent to which species composition, zonation patterns, and population dynamics allowed the results from Punta Galeta to be extrapolated to other locations, and (2) to detect major, widespread phenomena occurring after the oil spill, but not caused by the oil. Biota on the Caribbean coast of Panama can change dramatically for reasons other than the effects of oil spills. For example, changes in the abundances of sea urchins and the zonation of algae are associated with year-to-year variations in mean sea levels (Cubit 1985; Cubit et al. 1986). In the 1980s alone we have seen mass



Daytime Exposure of Galeta Reef Flat above Water Level (0800–1800 hrs)

A. Monthly Averages

B. Yearly Totals

Fig. 4.5 Monthly averages (*A*) and yearly totals (*B*) in the number of hours the reef flat at Punta Galeta was exposed above water level during the daytime (0800-1800). The monthly averages were calculated from the 1974-1990 data, excluding months with <90% valid data. The *solid triangle* denotes the occurrence of the 1986 oil spill. See text for further explanation.

mortalities of the sea fan *Gorgonia ventalina* (Guzmán and Cortés 1984; J. Cubit, pers. obs.) and the sea urchin *Diadema antillarum* (Lessios et al. 1984*a*, *b*). In addition, bleaching and other signs of stress have been observed for corals and other cnidarians (Glynn 1984). Such phenomena occurred over wide areas of the Caribbean Sea. In interpreting the long-term survey data from Punta Galeta, it would be easy to attribute such sudden changes occurring after the oil spill to effects of the oil. Thus, a primary purpose for adding the unoiled sites was to detect these large-scale phenomena that could appear in the before-and-after studies at Punta Galeta, but were not caused by the 1986 oil spill.

In addition, studies on this coast have demonstrated that the abundances of plants and animals on reef flats can show strong seasonal variations, year-to-year fluctuations, and multi-year trends (e.g., Hendler 1977; Connor 1984; Cubit 1985; Cubit et al. 1986; studies reviewed in Cubit et al. 1988*b*, 1989). Thus, a major challenge in this project was to distinguish natural changes in reef flat biota from changes caused by the oil spill.

Because precise abundances of the biota vary naturally from reef flat to reef flat, the added sites served mainly as comparisons for change of state, rather than absolute state. The principal habitats of interest were those where we made pre- and postspill comparisons at Punta Galeta. To reduce confounding effects of physical factors other than oil, the additional sites were selected to match the topography of the reef flat at Punta Galeta. Thus, sites were selected that had a forereef slope, emergent reef flat, and inner reef flat habitats with coral rubble and seagrasses. Efforts to match sites could not produce a set of sites equal in all respects except in their exposure to oil. The unavoidably wide geographical range between the oiled and unoiled sites also included environmental disparities. Bahía Las Minas is a shallow, turbid bay; Costa Arriba is on a section of more open coast with less turbid water. One of the oiled sites was at Isla Largo Remo, at the mouth of Bahía Cativá, which was exposed to additional pollutants besides oil from the 1986 spill. This narrow, shallow bay receives effluent from the refinery, discharges from the slaughter house of the city of Colón, raw sewage and garbage from the town of Cativá, and chronic small spills of diesel and other fuel oils from an electricity generating plant. Because of these environmental differences between the regions of the oiled and unoiled sites, differences in the biota between the regions must be interpreted with caution and cannot be regarded as sensitive measures of the 1986 oil spill. To fit time and logistical constraints, we selected one additional oiled site and two unoiled sites.

To find such sites, we surveyed the coastline from the Río Chagres to Palenque, a distance of approximately 110 km (Fig. 4.4*B*). Initial surveys were made by boat, on foot, and from low altitude (< 150 m) overflights. During these flights we also took photographs for later reference. Final selection of sites was made on foot. Our selection proceeded as follows.

1. The coastline between the Río Chagres and the city of Colón was eliminated because the reef flats did not comprise the same complement of habitats

found at Punta Galeta. Most of these were more elevated than the Punta Galeta reef flat (based on aerial surveys, followed up by surveys on foot).

2. The reef flat at Isla Margarita was rejected because dredging projects had changed the reef flat structure (based on surveys on foot).

3. The coastline between Isla Grande and Palenque was eliminated because extensive deforestation had resulted in heavy depositions of terrigenous sediments on reef flats (based on overflights).

4. The coastline between Portobelo and Isla Grande was eliminated because the reef flats were much more protected from wave action and lacked a forereef slope (determined from overflights).

5. The reef flats between Buenaventura and Portobelo were eliminated because of the presence of terrigenous sediments or lack of suitable reef flat structure (surveyed on foot).

6. The unoiled sites were thus narrowed to the coastline between Buenaventura and María Chiquita, and the oiled sites between Islas Naranjos and Punta Galeta (Fig. 4.4*B*, *C*). This coastline was surveyed on foot to make the following selections.

    a. Unoiled sites: both sites were in the María Soto region, where the reef flats most closely matched those at Punta Galeta. These reef flats are approximately 23 km east of the site of the oil spill.

    b. Additional oiled site: the seaward (northern) reef flat of Isla Largo Remo most closely matched Punta Galeta. This reef flat borders Bahía Cativá to the west, where the oil spill initially was concentrated. It probably received much more oil than Punta Galeta during the spill. In addition, after the spill it continued to receive more light oiling from chronic seepage at the refinery and adjacent mangroves. Thus, any effects of oil should be more pronounced at this site than at Punta Galeta.

Exposure to Oil at Oiled and Unoiled Sites

The terms "oiled" and "unoiled" are used here in a relative sense. In surveys by helicopter, small plane, small boat, and on foot, the deposition of extensive black oil slicks appeared to be limited to the shoreline between María Chiquita and Isla Margarita. However, during these surveys, oil sheen was seen offshore along the entire coast from Isla Margarita to beyond Nombre de Dios (Cubit et al. 1987). (Nombre de Dios was the eastern limit of overflights.) On 26 May 1986 oil sheen was during a flight extending as far east as Punta San Blas, about 50 km beyond Nombre de Dios (J. Karr, pers. comm.). The sheen appeared to be transported by the coastal current, which flows roughly west to east (i.e., along the coast from Bahía Las Minas toward Colombia), and was aided by an unusual period of south winds during the oil spill (J. Cubit, pers. obs.). H. Caffey and A. Velarde (pers. comm.) reported instances of black slicks arriving on the shoreline northeast of Portobelo, where resumption of the prevailing northeast winds probably deposited oil slicks that had been offshore. They also surveyed the shoreline between Portobelo and María Chiquita, but no oil was found or reported.

The term "oil" refers to the mixture of Venezuelan and Mexican Isthmus crude oils spilled, plus an unknown quantity of the dispersant Corexit 9527. On 27 April 1986 crude oil spilled from a ruptured storage tank on land at the refinery on Isla Payardí (Fig. 4.4). Because part of the oil soaked into the coral landfill beneath the refinery, the portion entering the sea was difficult to estimate. Texaco officials initially estimated that 35,000 to 50,000 bbl spilled into Bahía Cativá, (Cubit et al. 1987), which borders the Isla Largo Remo study site. However, Texaco officials later announced recoveries of 48,000 bbl (Cubit et al. 1987) and 60,000 bbl (A. Dodge, pers. comm.) of cleaned oil from the sea. Considering the amount of oil trapped in sediments and the probable losses of oil to the open sea and evaporation, the actual amount of oil spilled into Bahía Cativá was probably at least 60,000-100,000 bbl.

Northerly (onshore) winds held the oil in Bahía Cativá until 5 May 1986, when rain and slack onshore winds allowed oil to flow out of the bay. On 6 May 1986 a Hercules C-130 four-engined aircraft equipped with a Biegart Aviation, Inc., ADDS Pack sprayed Corexit 9527 on oil slicks at the mouth of Bahía Cativá and in front of the refinery (Cubit et al. 1987; J. Cubit, pers. obs.). This is the narrow channel between Isla Largo Remo and Isla Payardí, located adjacent to the study site at Isla Largo Remo (Fig. 4.4). Additional Corexit 9527 was sprayed offshore by a single-engine crop-duster airplane. On at least two days (11 and 12 May 1986), the crop duster sprayed dispersant on slicks in the open ocean offshore from the town of Portobelo. On 12 May the crop duster was also observed spraying dispersant in front of the refinery (again near the Largo Remo site) and in the open ocean approximately 2-3 km northeast of Punta Galeta (J. Cubit, pers. obs.). Refinery officials estimated the total Corexit 9527 sprayed was <21,000 L (Cubit et al. 1987). Possible effects of the dispersant will be addressed in the Discussion and Conclusions (Sect. 4.5).

4.4 Components of Research

This report is based on the following types of data:

1. field observations of oil movement and oil deposition in relation to water levels, winds, and waves,

2. initial effects of the oil on the biota of reef flats,

3. surveys of the sessile biota (seagrasses, algae, and sessile invertebrates of reef flats),

4. censuses of sea urchin populations,

5. censuses of the infauna living in turfs of the fleshy red alga *Laurencia papillosa*, and

6. the physical regime of the Punta Galeta reef flat affecting reef flat biota and oil deposition during the oil spill, compared with these factors in other seasons and years.

4.4.1 Field Observations of Oil Movement, Oil Deposition, and Initial Direct Effects of the Oil on Reef Flat Biota

4.4.1.1 Methods of Qualitative Sampling of Microalgal Mats Immediately After the Oil Spill at Punta Galeta

As part of the field observations during the oil spill, mats of microalgae growing on the reef flat were sampled as follows for examination in the laboratory:

7 June 1986: To examine the developing flora and fauna at Punta Galeta after the oil spill, samples of the hard carbonate substratum were collected from the most seaward portion of the reef flat surveyed in the transects for measuring spatial cover. Six pieces of substratum, each approximately 2 to 3 cm^2 in surface area, were wrapped in porous waxed paper and placed in paper envelopes to permit gas exchange while retaining moisture. Samples were microscopically examined while fresh and then preserved by desiccation at room temperature.

29 June 1986: Eighteen samples of algae were taken adjacent to nine of the 10 transect lines. The samples were taken 10 cm away from the line. Nine samples were taken 10 cm landward of the zero marker for the transects. The other nine samples were taken 2 m landward of this marker. The samples were processed the same way as before.

4.4.1.2 Field Observations of Oil Movements, Deposition, and Effects

During the first month of the oil spill, daily observations were made of oil movement, oil deposition, and apparent response of the flora and fauna of reef flats. Most of these observations were made from the laboratory at Punta Galeta and other sites accessible by land. Aerial observations were also made from helicopters and fixed-wing aircraft. The first large masses of oil arrived at Punta Galeta on 9 May 1986, 12 d after the spill at the refinery. Although the oil had weathered during this time, it was still liquid (not tarry) and had the appearance of thick, used crankcase oil. Dispersant was not sprayed on slicks at Punta Galeta, and any dispersant that may have been in these slicks would have been residual dispersant from spraying elsewhere.

Between 10 and 19 May 1986 the reef flat was exposed above water level during low tides (Fig. 4.6), creating a barrier for the oil being moved ashore by northerly winds. This caused oil to accumulate along the seaward side of reef flats for the duration of low tides (Figs. 4.1, 4.7). Rising tides lifted unadhered oil from the seaward edge of the Punta Galeta reef flat and floated the oil over the peak of the reef crest. Under these conditions the back-reef flat was flooded by the rising tide before the accumulations of oil moved over the reef crest, as illustrated in Figure 4.8. Consequently, as winds and water movement moved oil slicks across the back-reef flat, oil floated above the substratum and benthic biota until it exited through drainage channels or became deposited along beaches behind the reef flat. In contrast to the other reef flats in Bahía Las Minas described below, this combination of reef flat topography and direction of oil movement reduced the time the back-reef flat at Punta Galeta was in direct contact with oil slicks.

Organisms along the seaward edge of the Punta Galeta reef flat (approximately meters 0-6 in Fig. 4.9) were directly immersed in oil, and the surface of the substratum was obscured by oil. By 17 May 1986 the smell of rotting invertebrates and appearance of numerous sea urchin tests indicated massive mortality beneath this layer of oil. Thalli of the fleshy red algae *Laurencia papillosa* and *Gelidiella acerosa* dried at low tide, which allowed oil to adhere to these turf-forming algae. On the reef flat, the calcareous green alga *Halimeda opuntia* formed dense, sediment-filled mats, which absorbed oil like a sponge. By 24 May 1986, enough oil had worn away to reveal a 1-3 m wide band of white carbonate substratum, which included bleached corals and coralline algae. The band paralleled the shore near the lowest low water line, marking a zone previously occupied by a mixture of zoanthids, corals, and a mixture of calcareous and fleshy algae. By systematic visual estimation, less than 10% of the original sessile community still remained. A translucent fuzz of fine filamentous algae had started growing over the white carbonate substratum.

By search, we could find none of the crabs (e.g., species of *Microphrys*, *Pachygrapsus*, and *Grapsus*) normally present in this zone. Live *Grapsus grapsus*, however, were present on nearby emergent habitats (logs and coral rubble). These usually fast-running crabs were easily caught by hand, and appeared blind. Before the oil spill, the littorinid snail *Littorina nebulosa* had been abundant on drift logs on the back-reef flat (J. Cubit and R. Thompson, unpublished data). After the spill we searched this habitat for *L. nebulosa*, but found none of this normally common snail. Because of the small size of the shells (<2 cm maximum length) and the thick layer of accumulated oil and oily debris around the drift logs, any snails that had fallen from logs into the oil could not be found.

On 1 June 1986 patches of oil still adhered to the reef flat at Punta Galeta. The fleshy, turf-forming algae *Laurencia papillosa* and *Gelidiella acerosa* showed no

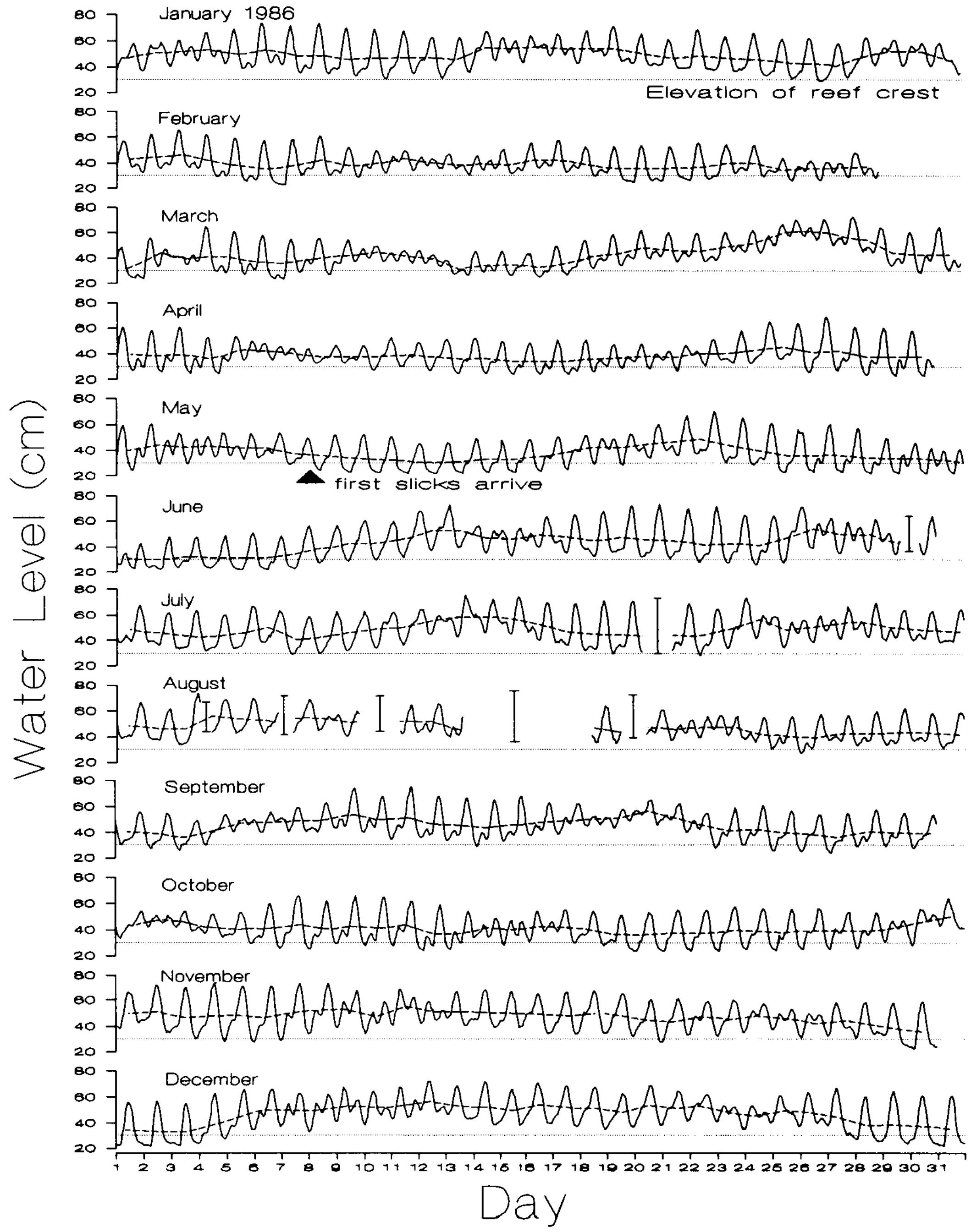


Fig. 4.6 Hourly water levels over the reef flat at Punta Galeta in 1986 (*solid line*). Heights are above Galeta datum. The daily mean water level is shown as a *dashed line*. The *horizontal line* at 30 cm is the height of the reef crest. The *solid triangle* marks the arrival of the first oil slicks at Punta Galeta in May. Gaps in the data resulted from chart stoppages; the *vertical bars* show the maximum and minimum water levels during the stoppages.

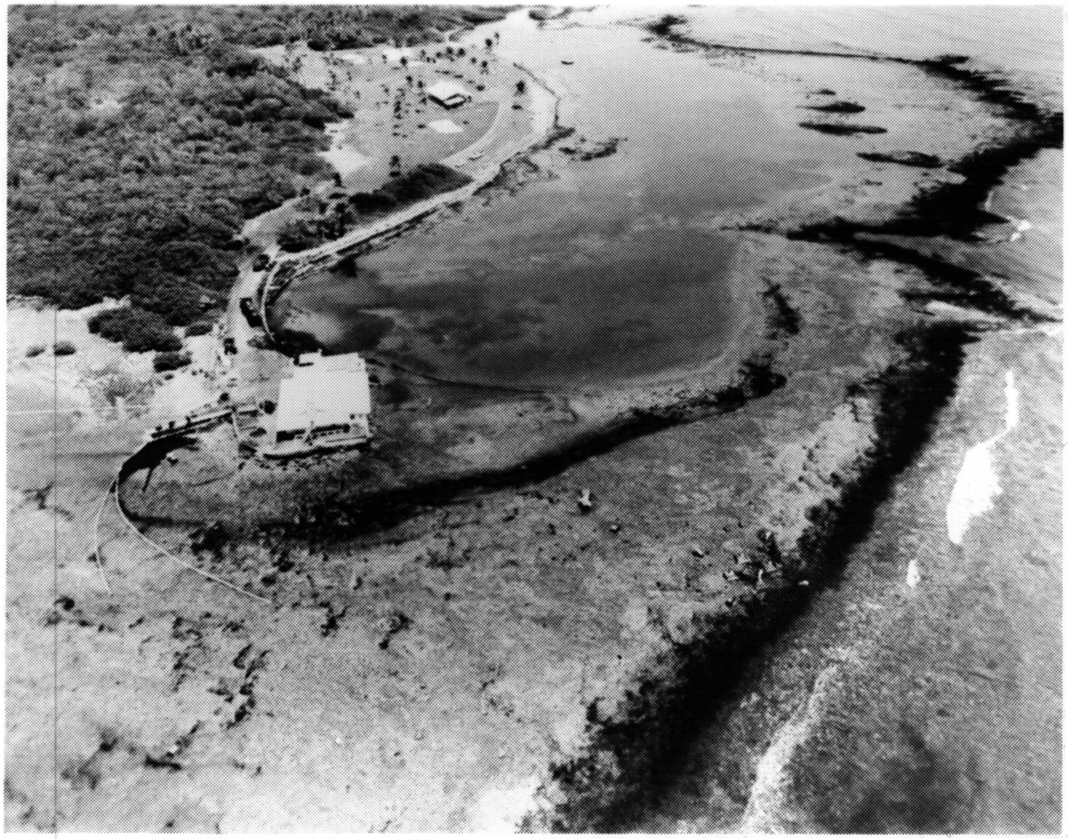


Fig. 4.7 Aerial photograph of Punta Galeta during the 1986 Bahía Las Minas oil spill, showing the accumulation of oil along the seaward edge of the reef flat.

visible damage in deeper tide pools and shallow subtidal areas, but were much less abundant than usual on emergent substrata of the reef flat where oil had adhered to their fronds. The algae gradually died back to basal tissue.

By 7 June 1986 the translucent, golden-green fuzz of microalgae was approximately 0.1-0.5 cm thick and covered an estimated 75% of the seaward edge of the reef flat. This is the zone where the spatial cover of sessile biota and the "reef-edge" populations of sea urchins are censused. The mat of microalgae was absent in the following areas: (1) pits where sand had accumulated, (2) pits where coralline algae were still present, and (3) patches ~2-10 cm in diameter around holes (~1 cm diameter) occupied by small crabs (*Pachygrapsus* sp., ~0.5 cm carapace width), which had recently colonized these holes. Similar holes without crabs were not surrounded by cleared patches. In microscopic examination of samples taken on this date, the algal fuzz consisted of the following (in order of abundance): the filamentous green alga *Cladophora* sp., the cylindrical green alga *Enteromorpha* sp.,

Fig. 4.8 Aerial photograph of oil streaming over the crest of the reef flat at Largo Remo during a rising tide. (The photograph shows approximately 200 m of reef crest extending from *upper left* to *lower right*.) During the preceding low tide, winds and waves deposited an accumulation of oil against the seaward side of the reef flat (*right*). The oil moved over the reef crest (from right to left) when the tidal level was high enough to protect the back-reef flat from direct contact with oil slicks.

and the filamentous red alga *Centroceras* sp. At least seven species of pennate and centric diatoms overgrew the substratum and other algae. In addition, ciliated protozoans, nematodes, and harpactacoid copepods were abundant among algal filaments. Small globules of oil were also found in the algal samples. Although blue-green algae were abundant in spot samples <50 m away on the back-reef flat, few blue-green algae were found in the samples from the reef-edge zone.

In contrast to the reef flat at Punta Galeta, raised berms and lack of drainage channels on the back-reef flats at some other sites in Bahía Las Minas caused oil to accumulate in beds of the seagrass *Thalassia testudinum*. This occurred on portions of the two reef flats southeast of Isla Mina, the reef flat on the north side of Isla Peña Guapa, and the reef flat on the north side of Isla Largo Remo (see Fig. 4.4). Seagrass leaves died and sloughed away within a month, but the mat of tough, fibrous rhizomes and roots remained in the soft substrata. This mortality did not occur in portions of the same *Thalassia* beds closer to the seaward edges of reef flats. In this

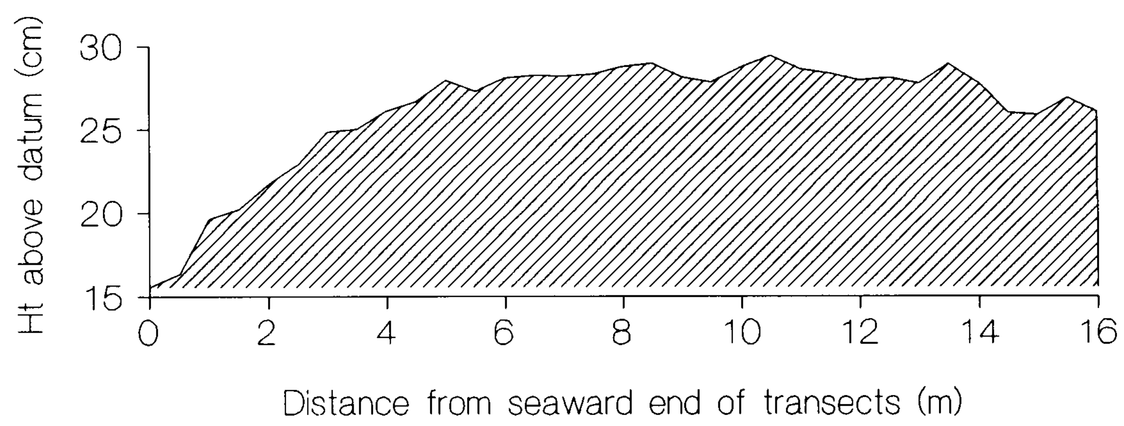


Fig. 4.9 Topographic profile of the seaward edge of the reef flat at Punta Galeta. Elevations are above Galeta datum, approximately 30 cm below mean low water (Cubit et al. 1986, 1988*a*, 1989). The profile is the average of elevations measured along the 10 transects used for the REDGE surveys, and the meter intervals correspond to those given for the REDGE data. The highest elevation of the reef flat is the reef crest, located approximately between meters 5 and 15 of this profile.

area, oil did not form pools, nor did it occur on reef flats, such as the one at Punta Galeta where the reef-crest barrier, tidal flow, and back-reef channels protected the back reef and allowed oil to drain away from the flat. In observations of sites at Peña Guapa and near Isla Mina, mats of rhizomes and roots of the dead *Thalassia* slowly decomposed, and the sand and silt of the *Thalassia* beds eroded down to hard coralline rock between 6 March 1987 and 16 June 1988. At the same time heavy erosion of the beach at Peña Guapa was also observed (4 August 1987). No recolonization of these seagrass beds was observed. Much more detailed and quantitative observations of the disappearance of the *Thalassia* bed at Largo Remo North are given by Steger and Caldwell (Chap. 6).

4.4.1.3 Summary and Discussion of Weather and Sea Conditions Affecting Oil Deposition and Biological Changes on the Punta Galeta Reef Flat

Daily field observations during the 1986 spill indicated water levels, winds, and waves were important factors in determining (1) the total surface area and specific zones of the reef flat directly exposed to oil slicks, (2) the duration that various zones were directly immersed in oil, and (3) the resulting mortality of biota on the reef flat. When the first oil slicks arrived at Punta Galeta in May 1986, low water levels, low wind speeds, and onshore wind direction caused them to accumulate along the seaward edge of the reef flat. Low water levels (Fig. 4.6), coinciding with the arrival of oil slicks, allowed the accumulations of oil to be in direct contact with the biota at the low elevations at the seaward edge of the reef flat. The effects of oil on the biota of the reef flat were measured in the quantitative surveys described below. The physical conditions determining the direct exposure to oil in May 1986 are compared with other seasons and years in Cubit and Levings (Chap. 2).

4.4.2 Spatial Cover of the Sessile Biota of the Reef Flat: Surveys of Algae, Seagrasses, and Sessile Invertebrates

4.4.2.1 Types of Surveys

The reef flat study includes a long-term database of the spatial cover of the sessile biota on the reef flat at Punta Galeta. The database was derived from three consecutive sets of surveys conducted between 1970 and 1990, as follows (the names of the databases are in capital letters).

1. *ZONE surveys* (1971-1977): percent cover of sessile flora and fauna in various zones on the reef flat. These surveys were conducted by C. Birkeland, D. Meyer, A. Reimer, and G. Hendler.

2. *CONSURV surveys* (1981-1982, plus two postspill surveys in 1986): percent cover of sessile flora and fauna in random samples over the entire reef flat. These surveys were conducted by J. Connor.

3. *REDGE surveys* (1983-1984, 1986-1990): percent cover of sessile organisms and volume of *Laurencia papillosa* bed in transects at the seaward section of the reef flat (Fig. 4.10). These surveys were conducted by J. Cubit and J. Burgett.

The surveys of biotic cover included more than 90% of the total reef flat fauna and flora by the following criteria: biomass, biotic cover, primary producers, structural components, and builders of the reef flat. The census information includes location on the reef flat, which allows data to be related among studies and to patterns of oiling. For the following analyses the databases were restructured to allow detailed comparisons among surveys, particularly to compare prespill and postspill data. As described in the following sections, the postspill CONSURV surveys of the whole reef flat (June and September 1986) indicated oil caused the heaviest detectable damage along the seaward side of the reef flat, in the zone sampled most intensively by the REDGE surveys. Thus, the REDGE surveys were chosen as the primary source of data for the oil spill studies and were duplicated at additional sites after the spill. The REDGE data were from transects at the seaward zone of the reef flat, the habitat that received the most direct exposure to oil during the spill.

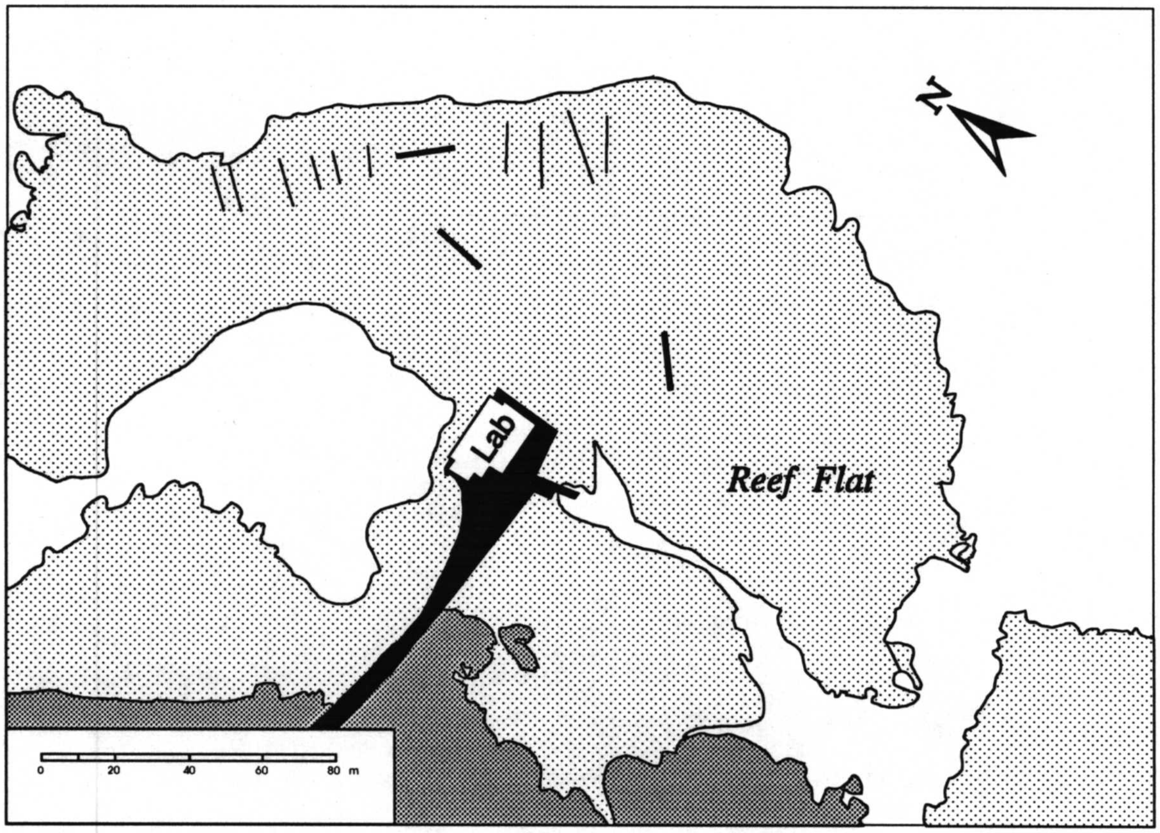


Fig. 4.10 Map of the reef flat (*shaded area*) at Punta Galeta showing the positions of the 10 REDGE and urchin-monitoring transects. The REDGE transects are the 10 *thinner, nearly vertical lines* near the upper edge of the reef flat. The urchin transects are the *thicker lines*.

4.4.2.2 Field Methods

Quantitative Surveys of All Sessile Biota Before and After the 1986 Oil Spill

ZONE Surveys

The percent cover of sessile organisms was measured using a point-intercept method in 1-m^2 quadrats, with 25 points in a uniform array per quadrat. There were 20 quadrats per survey in each zone, placed using random x and y coordinates measured from permanent transect axes in each zone. New random coordinates were used for each survey. The data used here represent the "coralline zone" and the "*Laurencia* zone" (Birkeland et al. 1976).

CONSURV Surveys

The percent cover of sessile organisms was measured using a point-intercept method in 0.5 x 0.5-m quadrats, with 100 points in a stratified-random array in each quadrat. There were 100 quadrats per survey, placed according to random *x* and *y* coordinates measured from permanent stakes in a grid over the whole reef flat. New random coordinates were used for each survey.

REDGE surveys

The percent cover of sessile organisms was measured using a point-intercept method on permanent line transects (criteria for this method are given below). For permanency, all transects were marked with steel stakes driven deep into the reef flat. Additional back-up stakes were set at fixed distances from the primary stakes. The positions of the stakes were mapped by measuring triangulation distances among stakes and by establishing lines-of-sight to local landmarks. At Punta Galeta there were 10 transects, with 10 random points per meter (stratified within 0.5 m intervals). A new arrangement of random points was used for each survey. The axes of the transects were perpendicular to the shoreline and established at random positions within 20 m intervals along the shore. The transects began in the "coralline zone" and spanned the *Laurencia papillosa* zone. The transects ranged in length from 9 to 21 m (mean = 15.6 m). After the oil spill, five matching transects were established at each of the three additional sites. These new transects were spaced roughly equidistantly along the portion of the shoreline that most closely matched the seaward edge of the Punta Galeta reef flat. The sections of shoreline surveyed at all four study sites were approximately 100 to 300 m between the first and last transects. The REDGE surveys were conducted by crawling along the transects on hands and knees and examining in minute detail the biota beneath each point on the transect line.

Criteria Used to Measure Percent Cover With the Point-intercept Method

With the point-intercept method, the species present under exact points (not areas) were recorded as the cover species. In the CONSURV and REDGE surveys, organisms attached directly to the substratum (i.e., carbonate rock or sand) were distinguished from those attached to other organisms. Following standard usage, the former were recorded as "primary cover" and the latter as "secondary cover." The values and validity of this field method are well established (e.g., reviews by Grieg-Smith 1983; Foster et al. 1991).

Methods of Comparing Data Among the ZONE, CONSURV, and REDGE Surveys (1970-1990)

The ZONE, CONSURV, and REDGE surveys were originally designed to monitor different aspects of the reef flat biota and not to be connected into a continuous time series. The data are presented here for only one purpose: to compare population changes *within* surveys, but *not* to compare absolute abundances among surveys. As explained below, the differences in sampling design among the surveys cause abundances to be averaged over different areas within zones, which could account for some of the between-survey differences in the absolute abundances of some species.

To compare the amount of fluctuation recorded *within* surveys, a database was constructed that matched the habitats and species groupings on the reef flat as closely as possible. To construct this database that spans 1970 to 1990, the species in all the surveys were assigned uniform identification codes and hierarchical grouping codes. Because the spatial organization of each survey type was different, subsets of data were extracted that corresponded to the sections of the reef flat sampled by all surveys in common. The sampling of the ZONE surveys was confined to transects placed within discrete zones; in contrast, the sampling of the CONSURV and REDGE surveys was conducted over a continuum spanning various zones. The organization of the ZONE surveys thus determined the spatial division of the comparison data extracted from the CONSURV and REDGE surveys. Two of the zones in the ZONE surveys corresponded to the areas of the reef flat also sampled by the CONSURV and REDGE surveys. These were the "coralline zone" and the "*Laurencia* zone" shown in Figure 4.2. Of these two zones, the *Laurencia* zone was the best represented in all the surveys. Although the CONSURV surveys used 100 quadrats per survey date, the quadrat positions were randomly dispersed over the entire reef flat; consequently, relatively few fell in the narrow seaward zones, particularly in the coralline zone. In this 1970 to 1990 comparison, the bias of small sample sizes was reduced by averaging the CONSURV data by year for the *Laurencia* zone and not using the CONSURV data for the coralline zone.

All data were checked against the original data sheets and reports, and any errors were corrected. A few errors were found in the published data for the ZONE surveys, but only the corrected data were used for this report.

4.4.2.3 Results: Spatial Cover of Sessile Organisms

Results for the cover of sessile biota are organized into five parts.

1. Seasonal patterns calculated from the REDGE data.
2. Prespill vs. postspill comparisons using the CONSURV data.

3. Broad-scale spatial and temporal changes seen during the 1986 oil spill at Punta Galeta compared with variations recorded in the ZONE, CONSURV, and REDGE surveys from 1970 to 1990. Variations recorded before the 1986 spill include possible residual effects of the tanker *Witwater* spill in December 1968.

4. Fine-scale spatial gradients of change at the seaward section of the reef flat recorded before and after the oil spill in the REDGE surveys at Punta Galeta (1983 to 1990).

5. Comparisons of the postspill surveys of the additional oiled and unoiled sites (1987 to 1990).

Seasonal Variations of the Sessile Biota

Categories of Space Occupancy

Patterns of seasonality for the sessile biota at Punta Galeta were calculated from the data of the REDGE surveys. These were the longest running surveys that transected the zone of heaviest oiling and biological damage at the seaward edge of the reef flat. Four categories of space occupancy accounted for approximately 98% of the spatial cover in the REDGE data: microalgae, macroalgae, sessile invertebrates, and bare substratum. Microalgae represents blue-green algae, diatoms, and other algae too small for identification by naked eye in the field. The macroalgae consist of all other fleshy and calcareous algae, including the crustose forms. The sessile invertebrates are those that adhere onto the substratum and have little or no mobility. Most of these in the study areas were cnidarians (e.g., anemones, zoanthids, and stony corals).

Little sandy substratum occurred within the REDGE transects; therefore, seagrasses only accounted for about 2% of the spatial cover data. To some extent the categories of bare substratum and microalgae represent a continuum. Truly bare substratum was a rare and temporary condition, existing for a few days before being overgrown by microalgae (usually blue-green algae, diatoms, and filamentous green algae). During the transition, assigning cover to one category or the other depended on lighting conditions and visual acuity, which were difficult to standardize. To avoid this ambiguity the two categories have been combined in Figure 4.11.

Seasonal Variation

The spatial covers of the macroalgae and microalgae-bare substratum categories were highly seasonal (Fig. 4.11). The cover of macroalgae peaked between November and March, when the reef flat was rarely exposed above water level in the daytime hours (Cubit et al. 1986, 1989; Fig. 4.5). The cover of macroalgae progressively decreased with the onset of daytime exposures of the reef

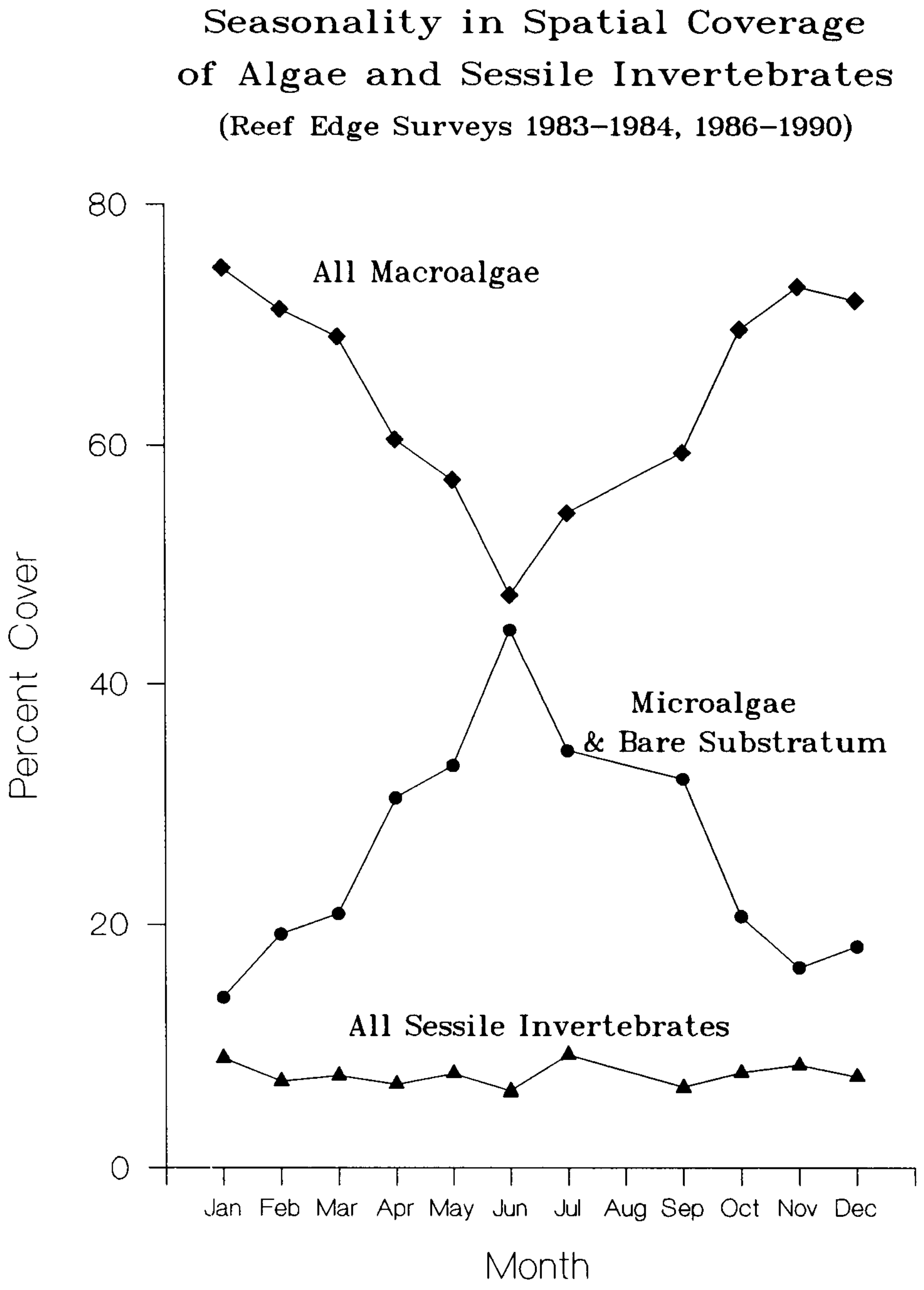


Fig. 4.11 Seasonality in spatial cover of algae and sessile invertebrates on the seaward portion of the reef flat at Punta Galeta. The data are monthly means from the REDGE surveys (1983-1990).

flat during Panama's wet season (usually beginning around March of each year). Cover of the microalgae-bare substratum category varied inversely with macroalgal cover and peaked in mid-year. The cover of sessile invertebrates was much more stable.

Nearly all the species of macroalgae and sessile invertebrates on the reef flat were perennials. Seasonal and other reductions in cover of these macrobiota resulted from mortality caused by exogenous factors, usually physical stresses.

CONSURV Prespill-Postspill Surveys of Biotic Cover on the Full Reef Flat

Two CONSURV surveys of biotic cover were conducted of the whole reef flat at Punta Galeta in June and September of 1986 for comparison with surveys using the same sampling design and methods used in the months of June and September of 1981 and 1982. The data were analyzed in two ways.

1. *Overall comparisons.* To reduce seasonal bias, the prespill data were lumped by calendar month (i.e., June and September) and compared by month with the postspill (1986) data for the same calendar month.

2. *Survey-by-survey comparisons.* To critically examine differences between the prespill and postspill surveys, the surveys for each prespill year (1981 and 1982) were compared one-by-one with the 1986 (postspill) data. This exposes the cases where the weight of one particular survey causes the ANOVAs of the lumped data ("1981 + 1982") to produce a statistically significant but less trustworthy effect of the oil.

For these comparisons the reef flat was divided into two primary sections:

1. the seaward edge of the reef flat, which received the heaviest oiling during the 1986 oil spill; this section corresponds to the seaward 50% of the REDGE transects, and

2. the inside (i.e., interior) section of the reef flat, which received less oil.

Before analyzing the data, a small portion (approximately 15%) of the inside section of the reef flat adjacent to the channel was excluded for the following reasons:

1. the amount of oiling was uncertain, and

2. this portion of the reef flat was subjected to other perturbations, including discharge of sewage and chemicals from the laboratory, disturbances resulting from the deployment of oil booms, and exposure to solvents and phenolic chemicals used to clean the booms and other oil spill control equipment.

Flow patterns through the channel (verified with dye markers) prevented these chemicals from reaching the other portions of the reef flat.

Seaward Edge of the Reef Flat in June

In the overall comparisons, abundances of the following species or groups of species were significantly lower after the oil spill in June 1986: crustose coralline algae, *Halimeda opuntia*, all macroalgae combined, and all sessile invertebrates and macroalgae combined (Table 4.2*A*). In the survey-by-survey comparisons, the June 1986 abundances for all categories except *Halimeda opuntia* were significantly lower than the abundances in prespill surveys for June. In all June comparisons, no statistically significant increases of any macrospecies were recorded at the seaward edge of the reef flat (Table 4.2*A*).

Inside Section of the Reef Flat in June

In the overall comparisons, only the abundance of *Halimeda opuntia* was significantly lower after the oil spill (Table 4.2*B*). However, in these overall comparisons abundances of the following species or groups of species were significantly higher after the oil spill: *Acanthophora spicifera*, all erect fleshy algae combined, all erect algae combined, and *Zoanthus sociatus* (Table 4.2*B*). In the survey-by-survey comparisons, none of these differences was statistically significant in every survey-by-survey comparison (Table 4.2*B*). Unlike the reef flats at Largo Remo North (see Steger and Caldwell, Chap. 6), Peña Guapa, and Mina South 1 and 2, beds of the seagrass *Thalassia testudinum* on the Punta Galeta reef flat did not markedly decrease after the 1986 oil spill.

Seaward Edge of the Reef Flat in September

In the overall comparisons, abundances of the following species or groups of species were significantly lower after the spill: crustose coralline algae, *Halimeda opuntia*, all macroalgae combined, and all sessile invertebrates, and macroalgae combined (Table 4.2*C*). The red alga *Acanthophora spicifera* showed a statistically significant increase. All of the above differences were also significant in the survey-by-survey comparisons, except for the category of "all macroalgae" (Table 4.2*C*).

Inside Section of the Reef Flat in September

In this category, all changes that are statistically significant are probably not biologically significant. In all cases of statistically significant increases or decreases, the 1986 values fell between the values for 1981 and 1982, and the prespill-postspill differences were only significant because the values for September 1981 were very different from the values for September 1986 (Table 4.2*D*). Therefore, the statistical

Table 4.2 Comparisons of percent cover of sessile biota at the Punta Galeta reef flat, as measured in the CONSURV surveys before and after the 1986 oil spill. The set of data labeled *1981 + 1982* was produced by pooling all quadrats surveyed in the months of June for those years. Differences significant at $P < .05$ are **bold**; "----" signifies insufficient data for test; *Crust. cor.* = crustose coralline; *Sess. invert./macro.* = sessile invertebrates and macroalgae.

	Percent Cover				Probability		
Biotic Category	1981	1982	1981 + 1982	1986	1981 vs. 1986	1982 vs. 1986	81+82 vs. 1986
A. Seaward Edge, June							
Acanthophora spicifera	2.5	0.7	1.6	1.3	.8	.2	.6
Erect fleshy algae	41.3	38.4	39.8	36.6	.9	.9	.9
All erect algae	42.4	43.8	43.1	36.9	.7	.5	.5
Stony corals	0.2	0.2	0.2	0.0	---	---	---
Crust. cor. algae	19.6	18.2	18.9	5.3	**.001**	**.001**	**.000**
Halimeda opuntia	10.1	6.6	8.3	3.2	**.041**	.226	**.046**
Laurencia papillosa	29.9	31.4	30.7	28.1	.9	.7	.9
All macroalgae	72.1	68.6	70.3	45.4	**.003**	**.013**	**.003**
Sess. invert./macro.	78.2	72.1	75.0	47.8	**.000**	**.006**	**.001**
Palythoa spp.	0.6	0.0	0.3	0.0	---	---	---
Zoanthus sociatus	5.2	3.1	4.1	2.2	.6	.8	.6
N (quadrats)	17	19	36	16			
B. Inside Section, June							
Thalassia testudinum	18.2	14	16.1	13	.2	.8	.3
Acanthophora spicifera	0.8	0.4	0.6	2.6	.08	**.026**	**.036**
Erect fleshy algae	11.1	11.6	11.4	17.5	**.025**	.07	**.021**
All erect algae	11.6	11.9	11.7	17.8	**.031**	.08	**.026**
Stony corals	0.1	0.1	0.1	0.0	.5	.3	.3
Crust. cor. algae	6.4	9.3	7.8	7.7	.3	.7	.7
Halimeda opuntia	8.3	7.6	8.0	4.4	**.018**	.052	**.009**
Laurencia papillosa	9.5	10.1	9.8	14.0	.2	.4	.2
All macroalgae	26.4	29.1	27.7	31.1	.2	.7	.4
Sess. invert./macro.	48.1	45.2	46.7	53.4	.2	.06	.057
Palythoa spp.	0.0	0.0	0.0	0.1	---	---	---
Zoanthus sociatus	2.7	1.5	2.1	8.3	.057	**.010**	**.019**
N (quadrats)	67	65	132	68			

Table 4.2 Comparisons of percent cover of sessile biota at the Punta Galeta reef flat, as measured in the CONSURV surveys before and after the 1986 oil spill (continued).

Biotic Category	Percent Cover				Probability		
	1981	1982	1981 + 1982	1986	1981 vs. 1986	1982 vs. 1986	81+82 vs. 1986
C. Seaward Edge, September							
Acanthophora spicifera	2.0	2.2	2.1	13.0	**.014**	**.018**	**.015**
Erect fleshy algae	55.9	74.7	66.3	65.5	.3	.3	.98
All erect algae	56.8	77.8	68.4	65.6	.3	.2	.8
Stony corals	0.0	0.5	0.3	0.3	---	.5	.9
Crust. cor. algae	16.2	7.3	11.3	0.8	**.000**	**.005**	**.000**
Halimeda opuntia	6.5	5.6	6.0	0.3	**.023**	**.001**	**.000**
Laurencia papillosa	45.1	63.7	55.4	50.3	.5	.2	.7
All macroalgae	79.5	90.7	85.7	66.7	.2	**.004**	**.017**
Sess. invert./macro.	87.7	95.1	91.8	68.9	**.04**	**.002**	**.007**
Palythoa spp.	2.1	0.3	1.1	0.0	---	---	---
Zoanthus sociatus	4.1	3.4	3.7	1.3	.4	.6	.4
N (quadrats)	17	21	38	12			
D. Inside Section, September							
Thalassia testudinum	26.2	20.1	23.2	20.8	.3	.9	.6
Acanthophora spicifera	2.2	9.3	5.8	9.0	**.001**	.842	.08
Erect fleshy algae	10.5	38.6	24.5	32.2	**.000**	.5	**.03**
All erect algae	11.3	39.4	25.3	34.6	**.000**	.6	**.01**
Stony corals	0.0	0.2	0.1	0.2	---	.7	.6
Crust. cor. algae	11.7	2.5	7.1	2.6	**.000**	.5	**.006**
Halimeda opuntia	5.6	4.7	5.1	3.4	.2	.6	.3
Laurencia papillosa	7.8	28.1	17.9	21.0	**.001**	.3	.4
All macroalgae	28.6	47.8	38.2	41.3	**.005**	.2	.4
Sess. invert./macro.	59.1	73.1	66.1	71.4	**.000**	.4	.1
Palythoa spp.	0.0	0.0	0.0	0.1	---	.6	.3
Zoanthus sociatus	3.7	3.9	3.8	7.2	.1	.2	.09
N (quadrats)	67	67	134	72			

significance of these comparisons is rejected as showing a biologically significant effect of the 1986 oil spill.

In summary, the CONSURV surveys showed that most of the decreases in biotic cover following the 1986 oil spill occurred at the seaward edge of the reef flat. *Halimeda opuntia*, the only exception, decreased over the whole reef flat. At the seaward edge of the reef flat, the only statistically significant postspill increase was the proliferation of the fast-growing red alga *Acanthophora spicifera*. In the interior of the reef flat, all statistically significant changes were postspill increases of cover, but none of these was statistically significant in each survey-by-survey comparison. More detailed, and longer-term comparisons of the biota at the seaward portion of the reef flat were made with the data from the REDGE surveys (see below).

ZONE, CONSURV, and REDGE Surveys at Punta Galeta: Changes During the 1986 Oil Spill in Relation to Variations Recorded in the Coralline Zone and Laurencia Zone Between 1970 and 1990

The primary value of the 1970-1990 data set is to compare changes in abundances *within* each type of survey, rather than to compare absolute abundances *among* surveys. As will be described in the following sections, some species, such as corals, coralline algae, and *Halimeda opuntia* were not uniformly distributed across the width of the coralline and *Laurencia* zones. Therefore, the ZONE surveys, which did not uniformly transect the zones, tended to produce different absolute abundances than the CONSURV or REDGE methods. However, methods were consistent within surveys, and the data from each survey type show the relative fluctuations of the biota for the survey period.

The spatial variation in oil deposition on the reef flat at Punta Galeta provided a form of "control" for making prespill and postspill comparisons at this site. Most of the coralline zone was covered by oil slicks at low tide during the first wk of the oil spill. The seaward portion (approximately 15%) of the *Laurencia* zone was also under oil at low tide, but the rest of this zone extended landward and upward onto the reef crest (Fig. 4.9), above and beyond the heavy accumulations of oil.

In these two zones nearly all the macrobiota (i.e., the predominant species of macroalgae and sessile invertebrates) were perennials. Seasonal variations in the abundances of these groups resulted from mortality caused by external physical factors, rather than from the endogenous programming of obligate annuals (Fig. 4.11).

In the coralline zone, macroalgae and sessile invertebrates suffered high mortality: both groups were nearly eliminated from this habitat (Figs. 4.12, 4.13). This was the greatest reduction in the abundance of macroalgae and sessile invertebrates recorded in this zone during any preceding or subsequent survey (i.e., from 1970 to 1990), including the catastrophic period of low water levels in 1988.

In the *Laurencia* zone the reduction in the abundance of macroalgae during the oil spill was marginally the greatest up to that date, but was exceeded during the stressful period of 1988 (Fig. 4.12). Sessile invertebrates were normally rare in the *Laurencia* zone, and the sparse populations showed little effect of either the oil spill of 1986 or the low water levels of 1988 (Fig. 4.13).

As a group, macroalgae regenerated rapidly in both zones after the oil spill, regaining prespill abundances within 6 mo after the spill. Macroalgae also exhibited a similar rapid rate of regeneration after the severe mortalities during the low water levels of 1988. In both situations macroalgae regenerated from surviving remnants, with no sign of regeneration from sporelings.

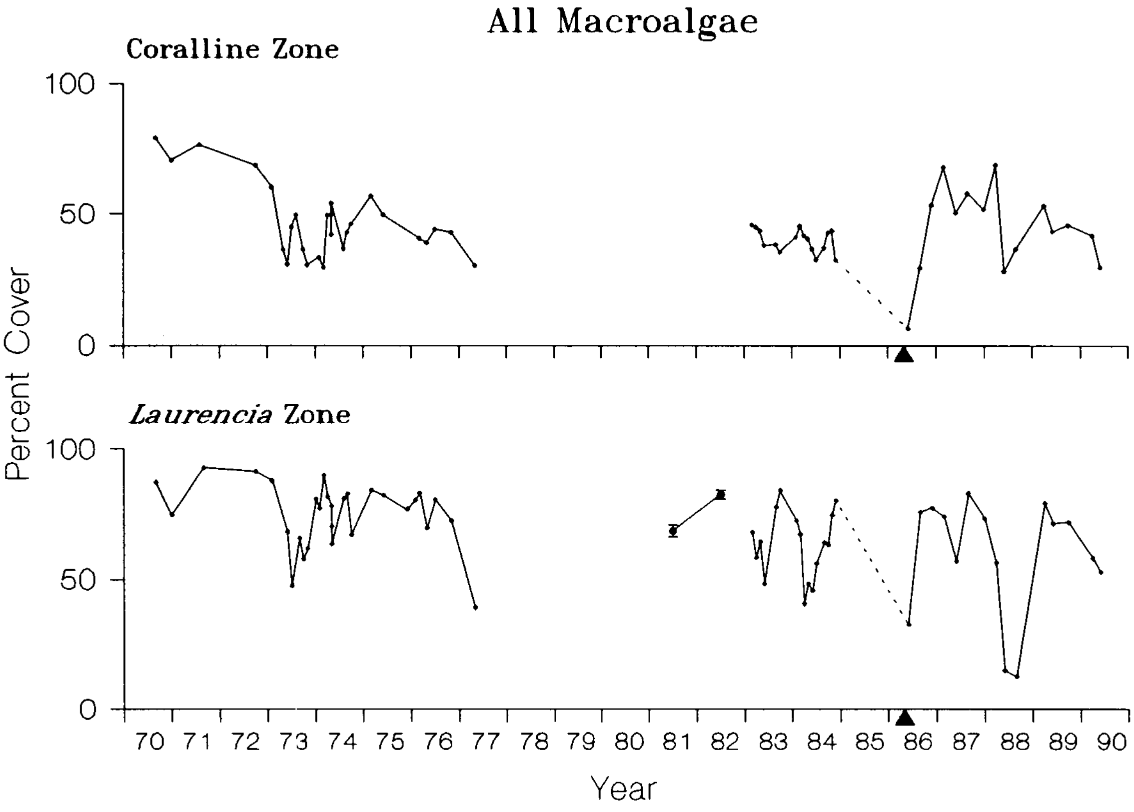


Fig. 4.12 Percent cover of macroalgae in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

As a group, populations of sessile invertebrates in the coralline zone were much slower to recover from the oil spill and appeared to be in the process of recovery through the last surveys of 1990 (Fig. 4.13). Like macroalgae, sessile invertebrates also regenerated from remnants surviving *in situ*, or fragments washing in from adjacent zones of greater survivorship. No evidence was seen of regeneration by means of larval settlement.

The calcareous green alga *Halimeda opuntia* is one of the main producers of loose sediment on the reef flat, and was abundant in both the coralline and *Laurencia* zones (Fig. 4.14). In the *Laurencia* zone the abundance of *Halimeda opuntia* was near zero in 1970, but increased steadily to about 25% cover by 1974. Much less variation and no trends were recorded for *Halimeda opuntia* in the coralline zone during this same period. As will be discussed later, the *Laurencia* zone was the portion of the reef flat most likely exposed to oil during the *Witwater* spill of December 1968. Following the 1986 oil spill, populations of *Halimeda* were

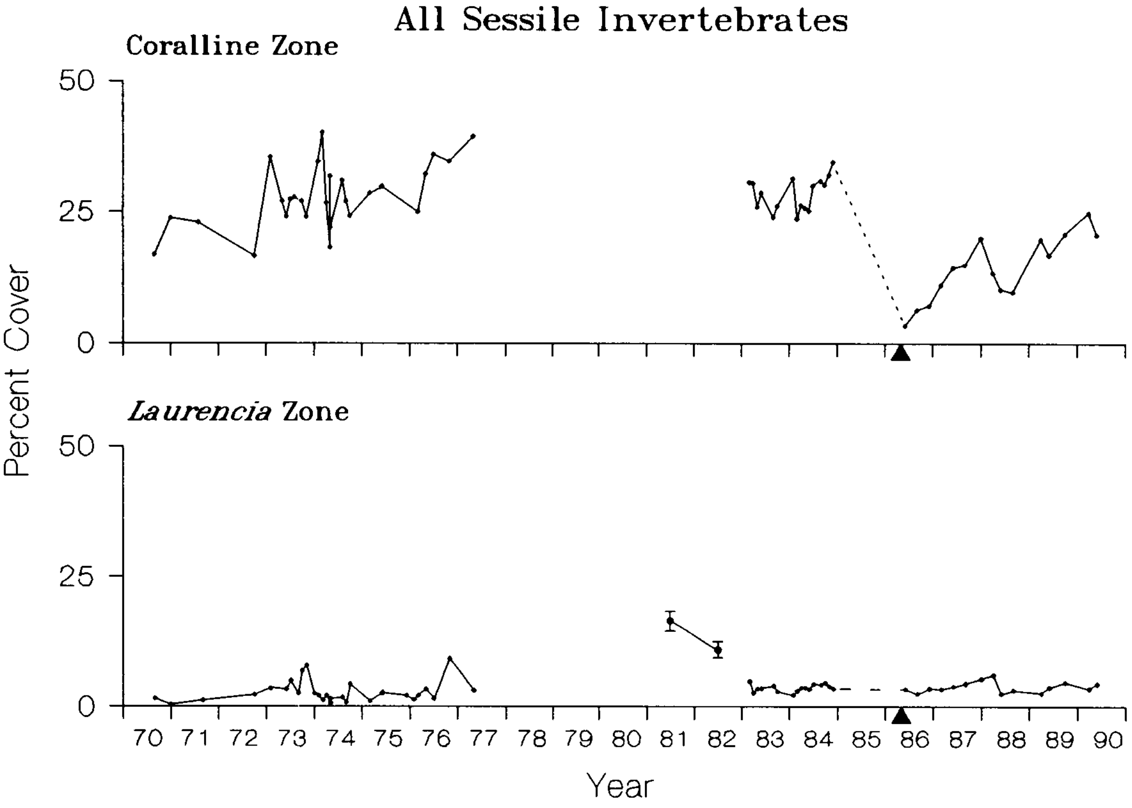


Fig. 4.13 Percent cover of all sessile invertebrates in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

reduced much more in the coralline zone than in the *Laurencia* zone. Recovery in the coralline zone was slow, and was set back by the stressful conditions of 1988. However, by 1990 the abundances of *Halimeda* in both zones equaled or exceeded prespill abundances in 1983-1984 (Fig. 4.14).

The red macroalga *Laurencia papillosa* was rare in the coralline zone during the 1970-1984 period before the 1986 oil spill (Fig. 4.15). After the 1986 spill *Laurencia papillosa* became more abundant in the coralline zone, forming a seasonally fluctuating population there. The dense cover of *Laurencia papillosa* in the *Laurencia* zone decreased between 1970 and 1977. In this zone the reduction in cover of *Laurencia papillosa* during the 1986 oil spill was well within the range of fluctuations seen before the spill, and was less than the reductions during the extreme low water levels of 1988.

Crustose coralline algae (i.e., Rhodophyta: Corallinaceae) were one of the main groups of organisms continuously producing the solid carbonate rock of the reef

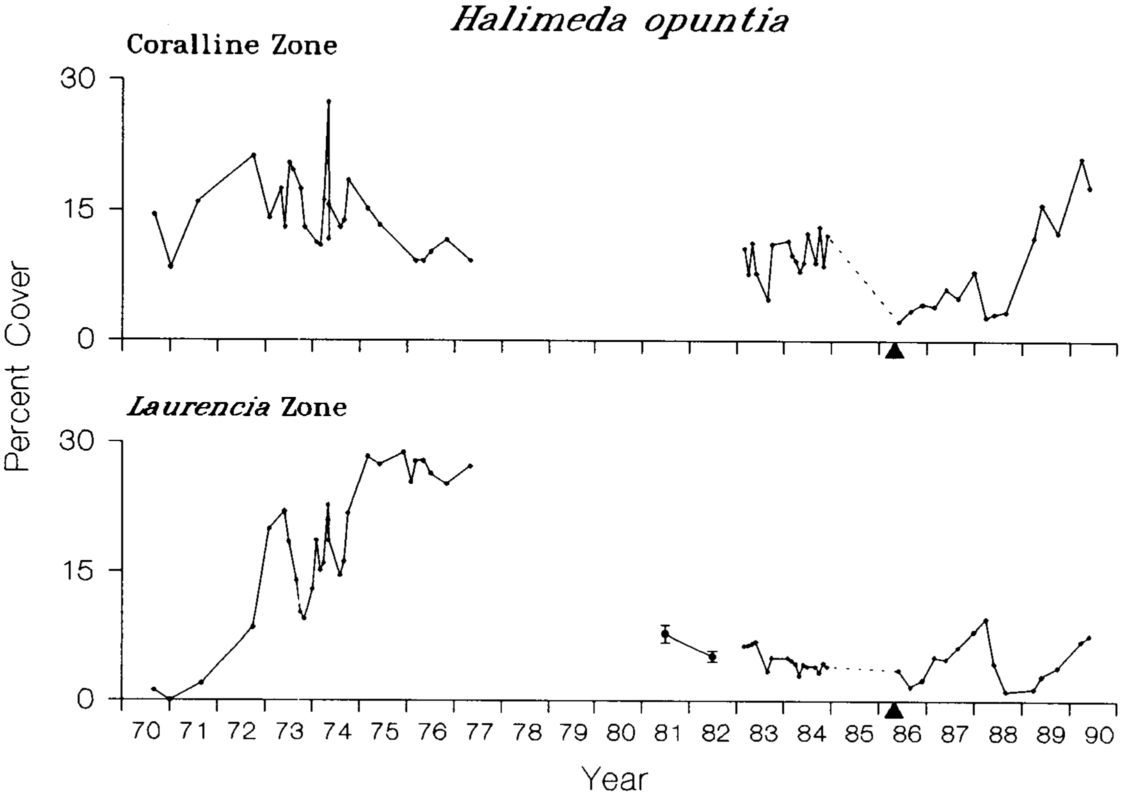


Fig. 4.14 Percent cover of calcareous green alga *Halimeda opuntia* in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

flat. In 1970 these algae formed more than 60% cover in the area then designated as the coralline zone (Fig. 4.16). Between 1970 and 1973 cover declined to about 30% in the coralline zone. During the 1986 oil spill crustose coralline algae were nearly eliminated from the coralline zone, but were re-established within a year, and persisted through the extreme low water levels of 1988 (Fig. 4.16).

In the *Laurencia* zone, crustose coralline algae were less abundant and more fluctuating in abundance. The 1986 oil spill appeared to have no effect on the crustose coralline algae in the *Laurencia* zone (Fig. 4.16).

On the reef flat, *Palythoa* (mostly *P. caribaeorum*) was restricted almost entirely to the coralline zone, where its abundance increased from near zero in 1970 to about 12% cover in 1984 (Fig. 4.17). During the 1986 oil spill *Palythoa* was nearly eliminated. After the 1986 spill populations of *Palythoa* regenerated primarily from one source: fragments of colonies washing in from deeper water. No evidence of growth from small larval stages was observed. The regeneration of *Palythoa*

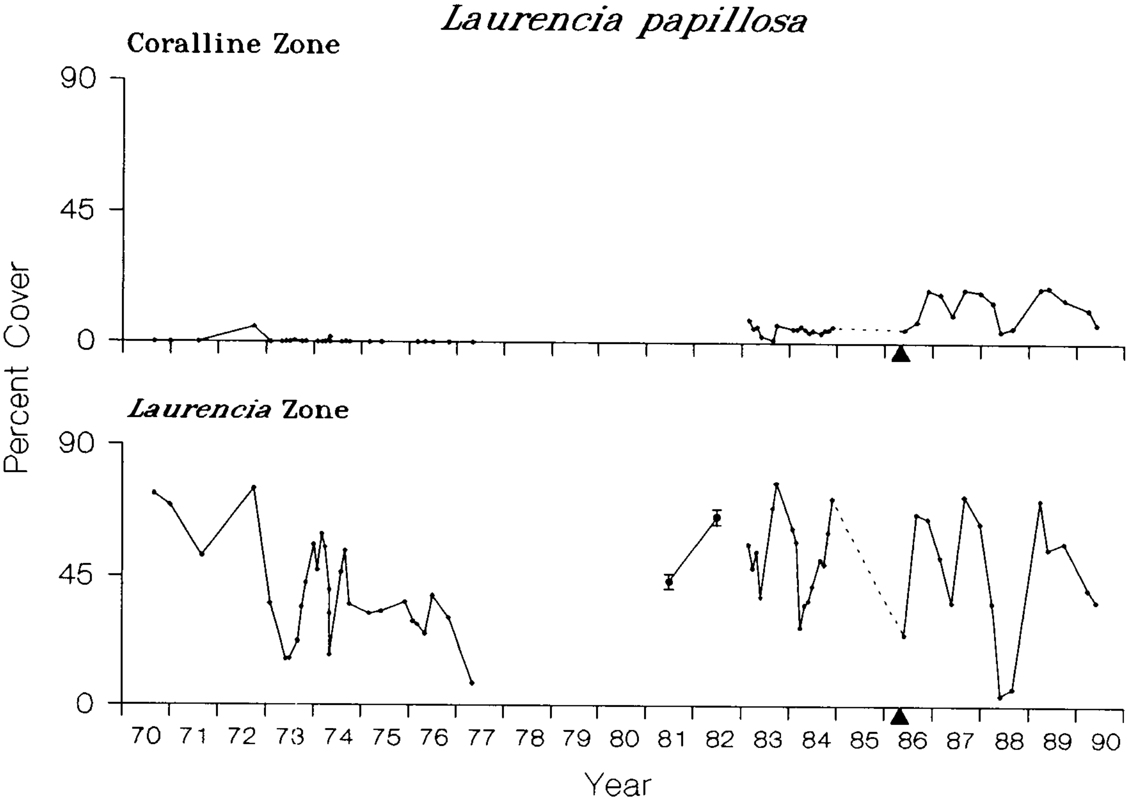


Fig. 4.15 Percent cover of the fleshy red alga *Laurencia papillosa* in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

populations was still in progress as of the last surveys in 1990 and was probably slowed by mortality during the seasons of low water levels in 1987 and 1988 (Fig. 4.17).

Populations of *Zoanthus sociatus* in the coralline zone suffered high mortality in the oil spill of 1986. These populations required about a year to regain abundances comparable to those present before the spill (Fig. 4.18).

Populations of *Zoanthus* in the coralline zone had three sources of material to contribute to regeneration: polyps that survived *in situ*, fragments that washed in from deeper water, and fragments that washed in from the *Laurencia* zone.

From 1970 to 1972, *Zoanthus sociatus* had been rare in the *Laurencia* zone, but afterward formed fluctuating populations, which showed little, if any, effect of the 1986 oil spill (Fig. 4.18).

The second major group constructing carbonate pavement on the reef flat was the stony corals (mostly *Porites* spp. and *Millepora* spp.). On the reef flat, stony

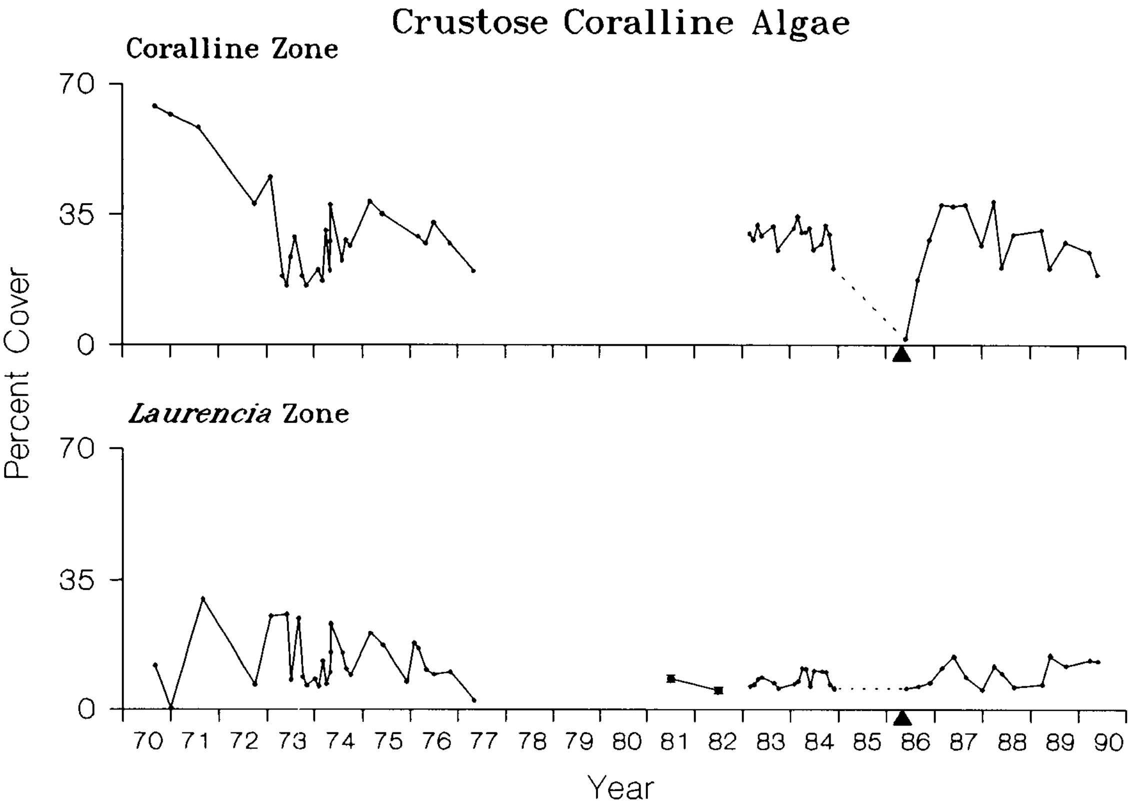


Fig. 4.16 Percent cover of crustose coralline algae in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

corals were restricted almost entirely to the coralline zone, where they were eliminated by the 1986 oil spill (Fig. 4.19). After the oil spill fragments of coral from surviving colonies in deeper water began washing onto the reef flat, re-establishing coral populations in the coralline zone. However, the immigrant corals did not survive the extreme low water levels of 1988, and as of the last surveys of 1990, coral abundances on the reef flat remained low (Fig. 4.19).

The REDGE Surveys at Punta Galeta: Changes of the Sessile Biota in Relation to Distance from the Oil Accumulation at the Seaward Edge of the Reef Flat

The transects of the REDGE surveys were started in 1983 to monitor variations in zonation on the seaward side of the reef flat. The transects began in the coralline zone (which was heavily oiled during the 1986 spill), crossed the *Laurencia* zone and the reef crest, and ended in the area of sediment deposition on

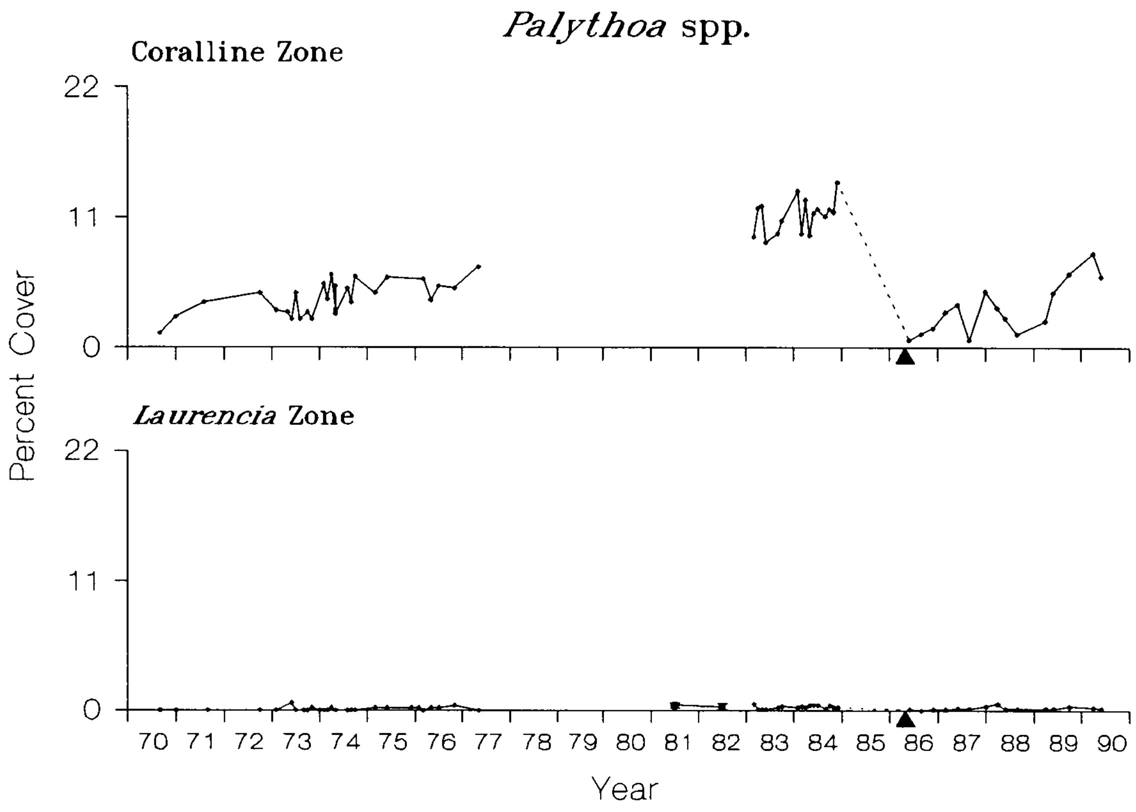


Fig. 4.17 Percent cover of the zoanthids *Palythoa* spp. in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

the landward side of the *Laurencia* zone (Figs. 4.1, 4.2, 4.7, 4.9). After the oil spill, these surveys were organized so that changes in the sessile biota could be examined meter-by-meter over the gradient of oiling at the seaward section of the reef flat. This procedure measured effects of oil at much higher resolution than the other survey methods on the reef flat. The REDGE surveys were replicated at additional sites after the oil spill (see next section) and were continued into 1990.

To show the spatial gradient of oil effects at Punta Galeta, the percent cover data from the REDGE surveys were graphed by 1 m intervals going from seaward (0 m) to landward. For statistical comparisons each transect was divided into two zones: (1) the seaward 30%, which was exposed to more direct contact with oil slicks, and (2) the landward 70%, which received less oil.

As noted in the field observations (Sect. 4.4.1), the mortality of sessile invertebrates and macroalgae at Punta Galeta (the macrospecies in Fig. 4.20) was concentrated at the seaward edge of the reef flat, and diminished in the landward

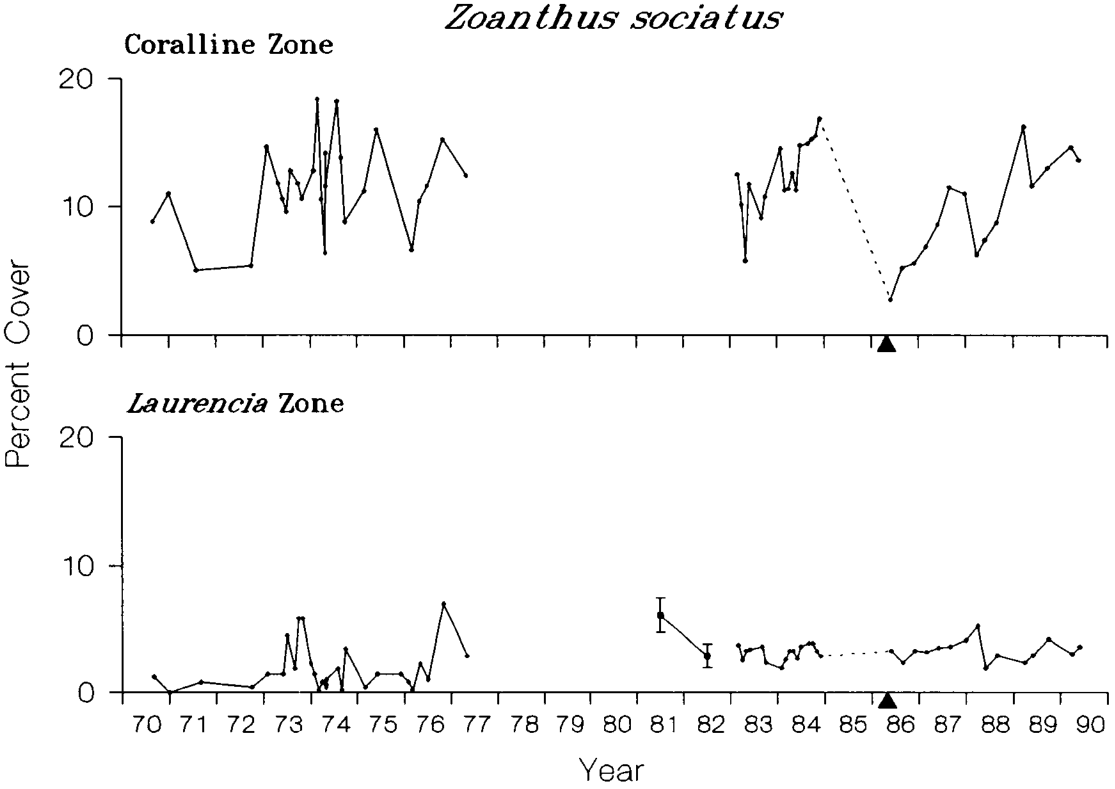


Fig. 4.18 Percent cover of the zoanthid *Zoanthus sociatus* in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

direction. In June 1986 the percent cover of macrospecies at the seaward 30% of the transects was lower than in any prespill survey (Fig. 4.20), including the two previous surveys for the months of June in 1983 and 1984 (Table 4.3*A*). These differences persisted through September 1986 (Table 4.3*D*). In the landward 70% of the transects these June differences were not significant for both prespill surveys (Table 4.3*B*). However, the rapid increase of macrospecies by September 1986 produced biotic covers that were significantly higher than in previous Septembers (Table 4.3*C*).

As described in the field observations, microalgae immediately colonized much of the substratum vacated by the mortality of macroalgae and invertebrates (the macrospecies in Fig. 4.20); consequently little open substratum existed after the oil spill. By the time of the first postspill census, the proliferation of microalgae at the edge of the reef flat had developed into an opaque, dark, golden-brown felt that covered nearly all hard substrata (Fig. 4.20); the cover was significantly higher than any prespill survey (Table 4.3*A*). This mat was composed of the same species found

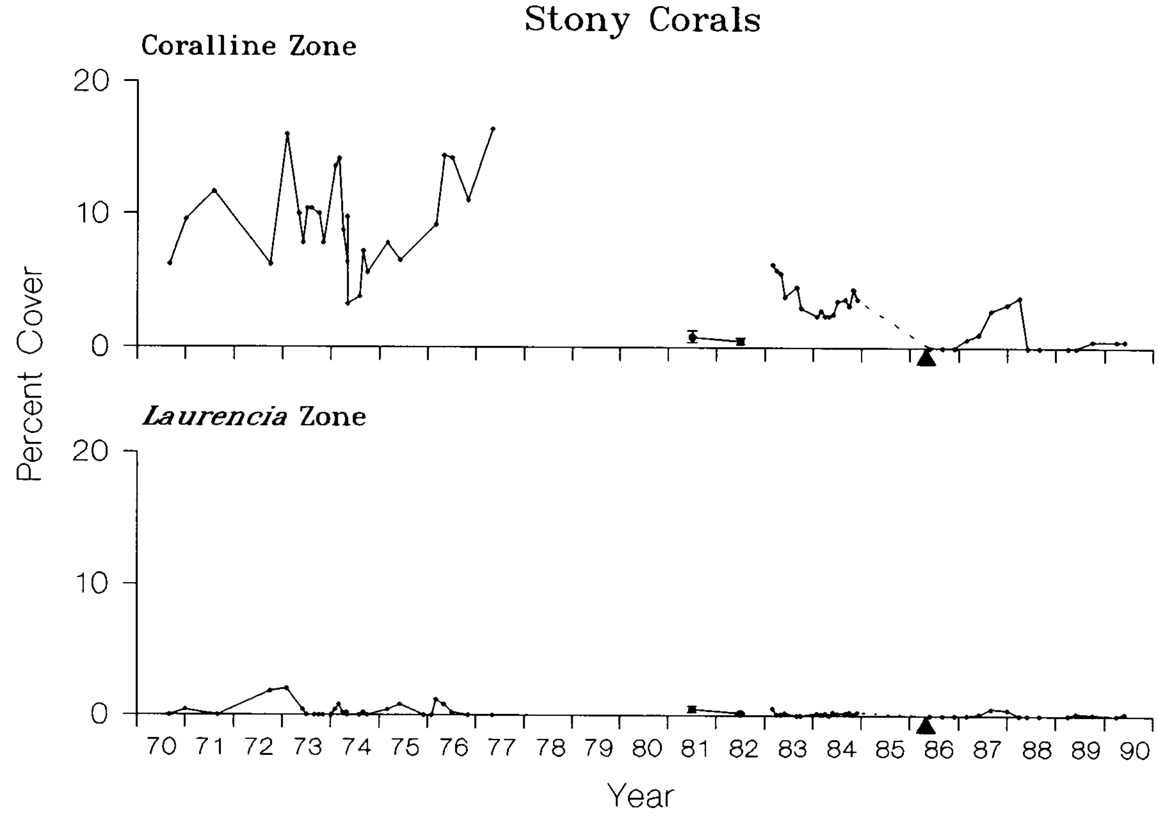


Fig. 4.19 Percent cover of all stony corals in the coralline and *Laurencia* zones at Punta Galeta, 1970-1990. Data for 1981 and 1982 are yearly means ± 1 SE from the CONSURV surveys. The *solid triangle* marks the time of 1986 Bahía Las Minas oil spill.

in the 7 June samples. Diatoms were a principal component, overgrowing both the substratum and other types of algae. In more sheltered areas blue-green algae, such as *Calothrix*, *Lyngbya*, and *Oscillatoria*, also formed thick mats, but were absent from the edge of the reef flat.

In the first postspill survey (June 1986) of the REDGE transects, the proliferation of microalgae was greatest at the seaward ends of the REDGE transects and diminished going landward (Fig. 4.20). This was the reverse of the pattern of zonation for microalgae before the oil spill, and at the seaward ends of the transects, microalgal cover was much greater than recorded in any of the 16 prespill surveys of these same transects.

By September 1986 the cover of microalgae had decreased to within the range of covers measured in the REDGE surveys before the oil spill. In 1988 the extensive exposures of the reef flat resulted in another bloom of microalgae having greater total cover than the postspill bloom (Fig. 4.20). The two blooms, however, exhibited

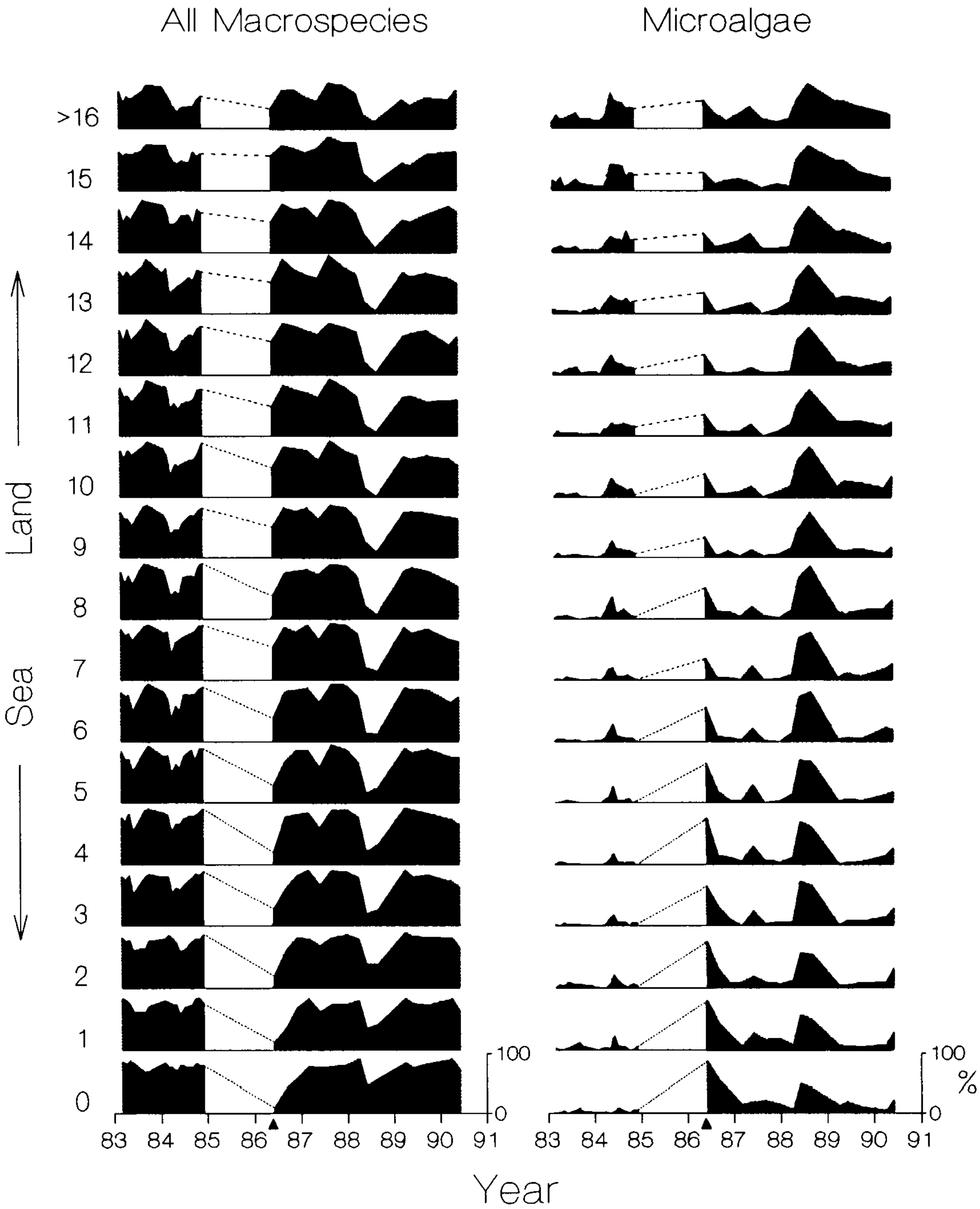


Fig. 4.20 Prespill-postspill changes in the percent cover and zonation of all macrospecies and microalgae on the reef flat at Punta Galeta, plotted by meter interval from the seaward edge of the reef flat. The numbers 0-16 at the *left* are meter intervals going away from the ocean (0 is closest to the ocean). The *solid triangle* on the baseline marks the time of the oil spill. The *dashed lines* span 1985, when no surveys were made.

Table 4.3 Fisher LSD contrasts for biotic cover in the reef edge (REDGE) transects at Punta Galeta (1983-1990). The contrasts were calculated for species or species groupings showing significant differences among times in repeated-measures ANOVAs. * = $P < .05$; *NS* = not significant. See text for further explanation.

	Contrasts				
Biotic Category	1983 vs. 1986	1984 vs. 1986	83+84 vs. 1986	83+84 vs. 87,88, 89,90	83+84 vs. 87,89, 90
A. Seaward 30%, June Surveys					
Acanthophora spicifera	NS	NS	NS	*	*
Erect fleshy algae	*	*	*	NS	*
Stony corals	*	*	*	*	NS
Crustose coralline algae	*	*	*	NS	*
Halimeda opuntia	*	*	*	NS	NS
Laurencia papillosa	*	*	*	NS	*
All macroalgae	*	*	*	NS	*
All erect algae	*	*	*	NS	*
Sessile invertebrates	*	*	*	NS	NS
All macrospecies	*	*	*	NS	*
Microalgae	*	*	*	*	*
Palythoa spp.	*	*	*	*	*
Zoanthus sociatus	NS	*	*	NS	NS
B. Landward 70%, June Surveys					
Acanthophora spicifera	*	*	*	*	*
Erect fleshy algae	*	*	*	*	*
Stony corals	NS	NS	NS	NS	NS
Crustose coralline algae	NS	*	NS	*	*
Halimeda opuntia	*	NS	NS	NS	*
Laurencia papillosa	*	*	*	*	*
All macroalgae	*	NS	*	NS	*
All erect algae	*	*	*	*	*
Sessile invertebrates	NS	NS	NS	NS	NS
All macrospecies	*	NS	*	NS	*
Microalgae	*	*	*	*	*
Palythoa spp.	NS	NS	NS	NS	NS
Zoanthus sociatus	NS	NS	NS	*	NS

Table 4.3 Fisher LSD contrasts for biotic cover in the reef edge (REDGE) transects at Punta Galeta (1983-1990; continued).

	Contrasts				
Biotic Category	1983 vs. 1986	1984 vs. 1986	83+84 vs. 1986	83+84 vs. 87,88, 89	83+84 vs. 87,89
C. Seaward 30%, September Surveys					
Acanthophora spicifera	NS	NS	NS	NS	NS
Erect fleshy algae	NS	NS	NS	*	NS
Stony corals	NS	*	*	NS	NS
Crustose coralline algae	NS	*	*	NS	NS
Halimeda opuntia	*	*	*	*	NS
Laurencia papillosa	NS	NS	NS	*	NS
All macroalgae	NS	NS	*	NS	*
All erect algae	NS	NS	NS	*	NS
Sessile invertebrates	*	*	*	NS	NS
All macrospecies	*	*	*	*	NS
Microalgae	*	*	*	*	*
Palythoa spp.	*	*	*	*	*
Zoanthus sociatus	NS	NS	NS	*	*
D. Landward 70%, September Surveys					
Acanthophora spicifera	NS	*	*	NS	NS
Erect fleshy algae	NS	*	*	*	*
Stony corals	NS	NS	NS	NS	NS
Crustose coralline algae	NS	*	NS	NS	NS
Halimeda opuntia	NS	NS	NS	NS	*
Laurencia papillosa	NS	*	*	*	*
All macroalgae	NS	*	*	*	*
All erect algae	NS	*	*	*	*
Sessile invertebrates	NS	NS	NS	*	NS
All macrospecies	NS	*	*	*	*
Microalgae	NS	*	NS	*	NS
Palythoa spp.	NS	NS	NS	NS	NS
Zoanthus sociatus	NS	NS	NS	*	*

almost the reverse patterns of zonation. In the 1988 bloom microalgae were least abundant at the seaward edge of the reef flat and increased going landward (Fig. 4.20).

Microalgal abundance was greatly altered by the postspill bloom of 1986 and by the postexposure bloom of 1988. As a result, all combinations of statistical comparisons for the month of June were significant regardless of zone (Table 4.3*A*, *B*). For the month of September all statistical comparisons were significant for the seaward 30% of the transects, but not the landward 70% (Table 4.3*C*, *D*).

In June 1986 the abundance of the fleshy (i.e., noncalcareous) macroalga *Laurencia papillosa* was significantly reduced over the width of the REDGE transects (Fig. 4.21; Table 4.3*A*, *B*). However, by September 1986 the abundances of this alga had greatly increased. As microalgae declined after the oil spill, *Laurencia papillosa* became more abundant at the seaward edge of the reef flat than it had been in the 16 prespill surveys (as shown by the increased cover of this alga in meters 0 and 1 of the transects in Fig. 4.21).

Before the oil spill the zoanthid *Palythoa caribaeorum* was found mainly at the seaward edge of the reef flat, where it was nearly eliminated by the oil spill (Fig. 4.22). As of the last REDGE survey in June 1990, *Palythoa* had not yet fully recovered, and postspill abundances in the seaward 30% of the transects were significantly lower than prespill abundances for the whole survey period (Table 4.3*A*, *C*). In the landward 70% of the transects, *Palythoa* was too rare for meaningful comparisons of oil spill effects.

While we were conducting these surveys, the zoanthid *Palythoa caribaeorum* was observed to recolonize the reef flat via fragments of colonies washing in from deeper, subtidal habitats. As shown in Figure 4.22, this produced a process of recolonization that was most rapid in the meter-interval nearest the ocean. This same process also occurred in the recolonization of the zoanthid *Zoanthus sociatus*. In addition, small fragments of *Zoanthus* colonies survived the oil spill *in situ*, and much of the subsequent recolonization of *Zoanthus* was produced by vegetative spreading from these fragments. *Zoanthus* regenerated more rapidly than *Palythoa* (Fig. 4.22).

As mentioned previously, stony corals and crustose coralline algae differed in total mortality during the oil spill and in mode and rate of recolonization after the spill. Like *Palythoa*, coral populations of the reef flat were present only in the seaward zone of heaviest oiling and were eliminated from the reef flat (Fig. 4.23). Also like *Palythoa*, corals recolonized from fragments washing in from subtidal habitats. This produced a slow, sporadic recolonization that did not survive the extreme low water levels of 1988 (Fig. 4.23). In contrast, crustose coralline algae were present over the full span of the transects. Significant reductions in the cover of coralline algae occurred in the seaward 30% of the transects (Fig. 4.23; Table 4.3*A*), but these reductions did not remain significant through September 1986 (Table 4.3*D*). Crustose coralline algae probably regenerated from tissue that survived below the layers of dead thalli at the surface. The regeneration of crustose coralline algae required less than a year and occurred uniformly over the entire width of the damaged area (Fig. 4.23).

Postspill Comparisons of Sessile Biota Among Sites

The lengths of the transects in the REDGE surveys varied according to the distance required to completely span the *Laurencia* zone at each transect location.

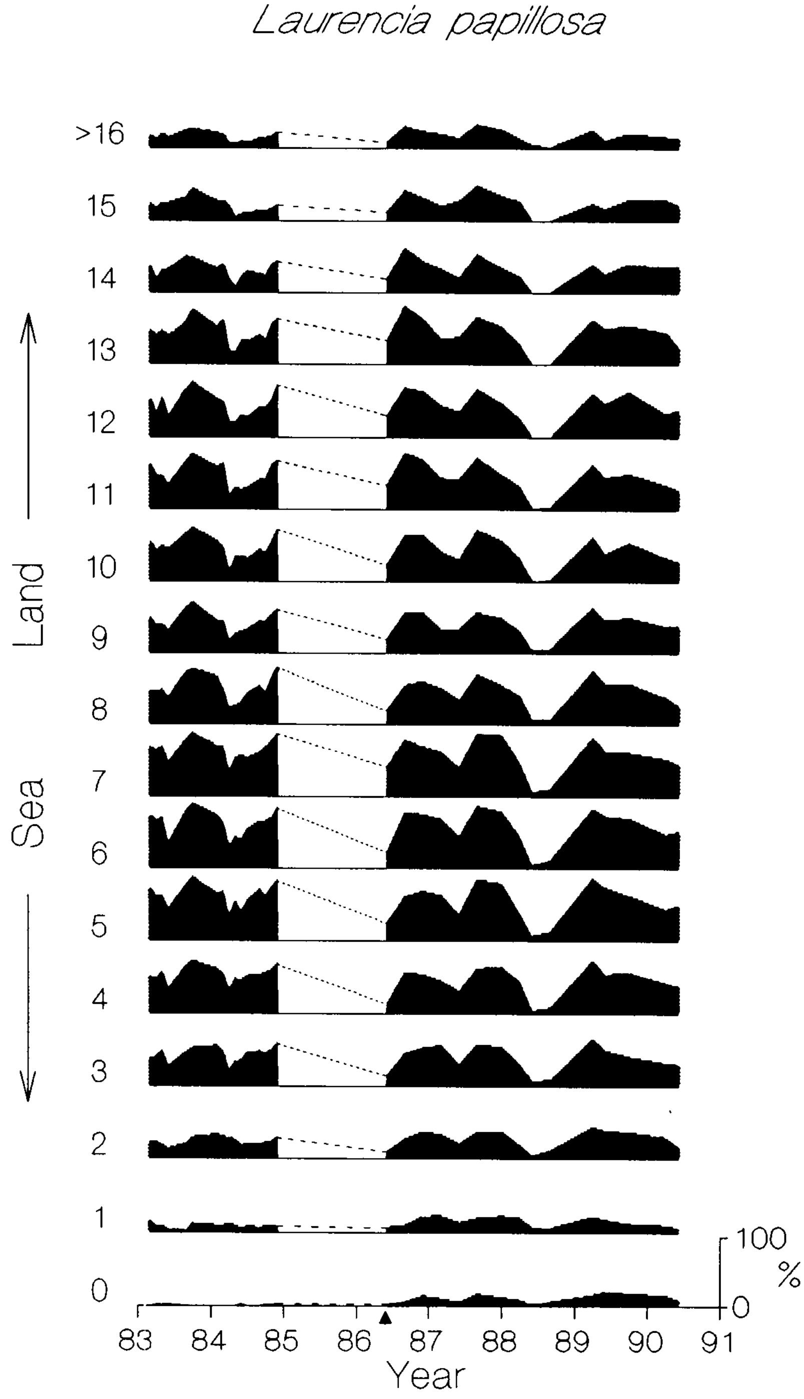


Fig. 4.21 Prespill-postspill changes in the percent cover and zonation of *Laurencia papillosa* on the reef flat at Punta Galeta, plotted by meter interval from the seaward edge of the reef flat. The numbers 0-16 at the *left* are meter intervals going away from the ocean (0 is closest to the ocean). The *solid triangle* on the baseline marks the time of the oil spill. The *dashed lines* span 1985, when no surveys were made.

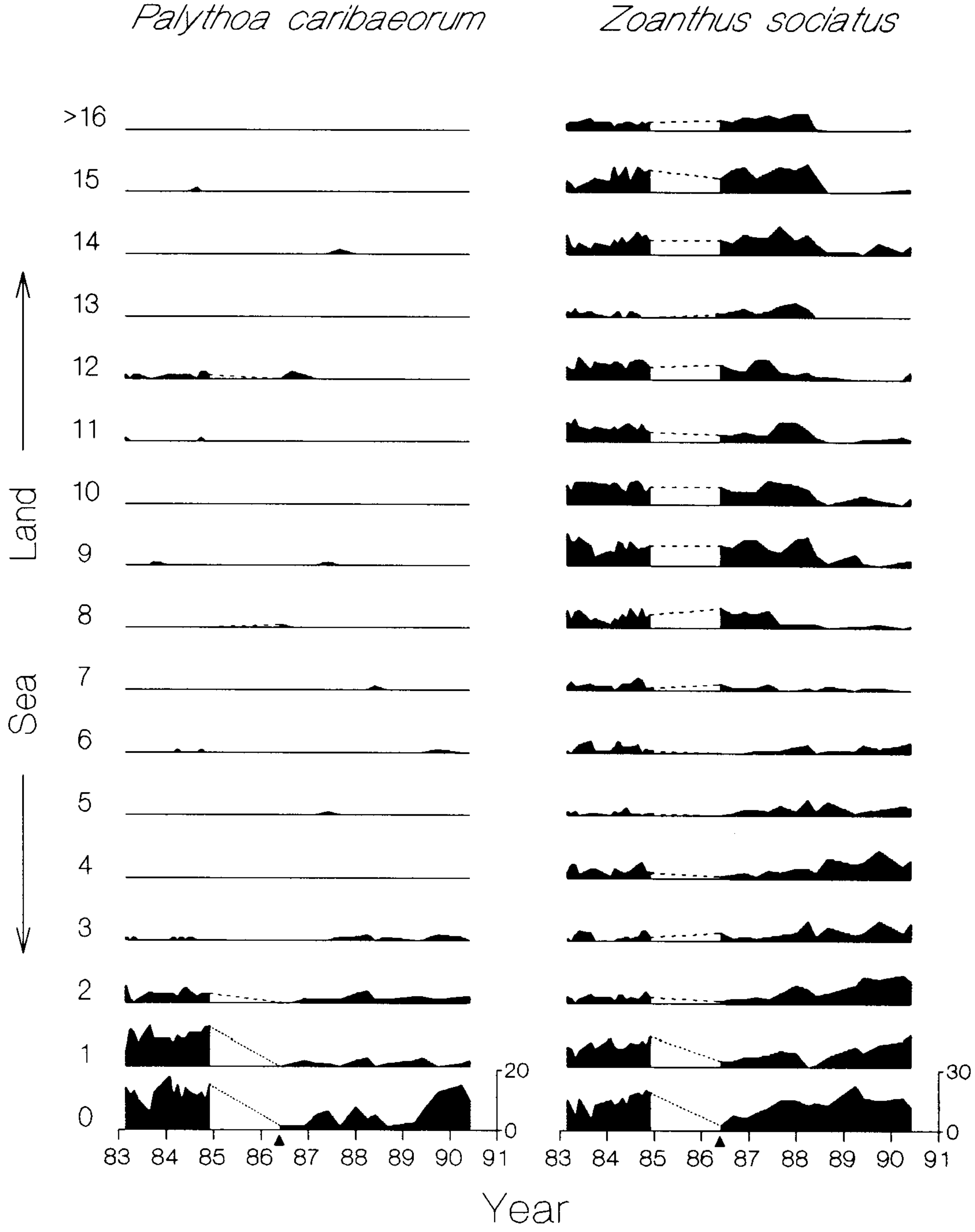


Fig. 4.22 Prespill-postspill changes in the percent cover and zonation of *Palythoa caribaeorum* and *Zoanthus sociatus* on the reef flat at Punta Galeta, plotted by meter interval from the seaward edge of the reef flat. Note that the vertical scales differ between the two columns of graphs to better show temporal variations in cover for each group. The numbers 0-16 at the *left* are meter intervals going away from the ocean (0 is closest to the ocean). The *solid triangle* on the baseline marks the time of the oil spill. The *dashed lines* span 1985, when no surveys were made.

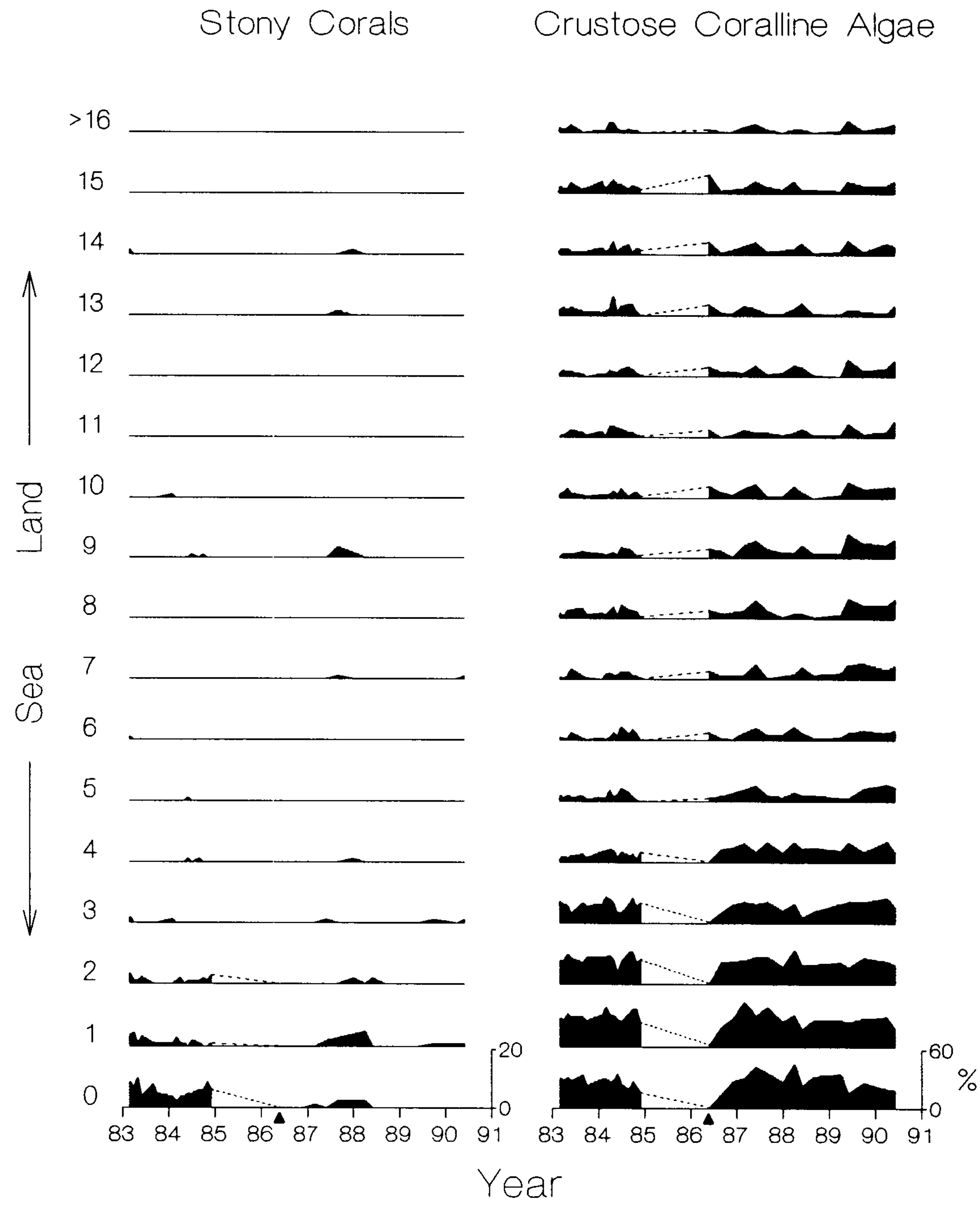


Fig. 4.23 Prespill-postspill changes in the percent cover and zonation of stony corals and crustose coralline algae on the reef flat at Punta Galeta, plotted by meter interval from the seaward edge of the reef flat. Note that the vertical scales differ between the two columns of graphs to better show temporal variations in cover for each group. The numbers 0-16 at the *left* are meter intervals going away from the ocean (0 is closest to the ocean). The *solid triangle* on the baseline marks the time of the oil spill. The *dashed lines* span 1985, when no surveys were made.

The width of this zone varied according to the wave exposure and topography of the reef flat. To compare the REDGE surveys among sites, transect lengths were standardized, and abundances of sessile organisms were plotted against the percent of transect length from the seaward end of the transects.

Figure 4.24 compares postspill patterns of microalgal abundance and zonation over time at all sites with reference to the patterns at Punta Galeta before and immediately after the oil spill (June 1986). At all sites after the spill, the abundance and zonation of microalgae in most years was similar to the pattern for Punta Galeta before the spill. However, the extreme low water levels of 1988 produced a bloom of microalgae synchronously at all sites (Fig. 4.24). At all sites the zonation of the natural blooms was not skewed seaward as was the postspill bloom at Punta Galeta (Fig. 4.24).

Intersite comparisons indicate that fleshy macroalgae in general, including the predominant species, *Laurencia papillosa*, had also regenerated at the other oiled site, Largo Remo, by the time of the first survey in September 1987 (Figs. 4.25, 4.26). However, during the extreme low water levels of 1988, *L. papillosa* and other fleshy macroalgae declined much more at the oiled sites than the unoiled sites, suggesting the re-establishing populations may have been more vulnerable to natural disturbances. After 1988, algae regrew again.

Intersite comparisons also indicate that the increased abundance of *Laurencia papillosa* and other fleshy macroalgae at the seaward edge of the reef flat at Punta Galeta (Figs. 4.12, 4.15, 4.21, 4.25, 4.26) may have been part of phenomena not caused by the oil spill. As of the 1990 censuses *Laurencia papillosa* and the other fleshy macroalgae had become more abundant in the seaward portions of the transects at all sites, both oiled and unoiled (Figs. 4.25, 4.26).

Although corals had been recruiting at the oiled sites via fragments washing in from deeper habitats, in 1987 and early 1988 corals were still less abundant at the oiled sites than the unoiled sites (Fig. 4.27). During the extreme low water levels of 1988 coral abundance was reduced at all sites, with near elimination of reef flat corals at Punta Galeta (Fig. 4.27). In early 1989 recruitment of corals occurred at all sites except at Punta Galeta, but afterward the decline resumed. As of the last survey (June 1990), corals had become rare at all sites, oiled and unoiled (Fig. 4.27). As previously described for Punta Galeta, the other group of reef builders, the crustose coralline algae, survived much better. As of June 1990 crustose coralline algae were the principal builders of hard substrata on the reef flats at all sites, regardless of exposure to oiling (Fig. 4.28).

The major producer of carbonate sediment, *Halimeda opuntia*, also persisted at the oiled and unoiled sites, including the period of extreme low water levels of 1988 (Fig. 4.29). Because zoanthids (*Zoanthus* and *Palythoa* spp.) were rare at the unoiled sites, oiled-unoiled comparisons were not appropriate.

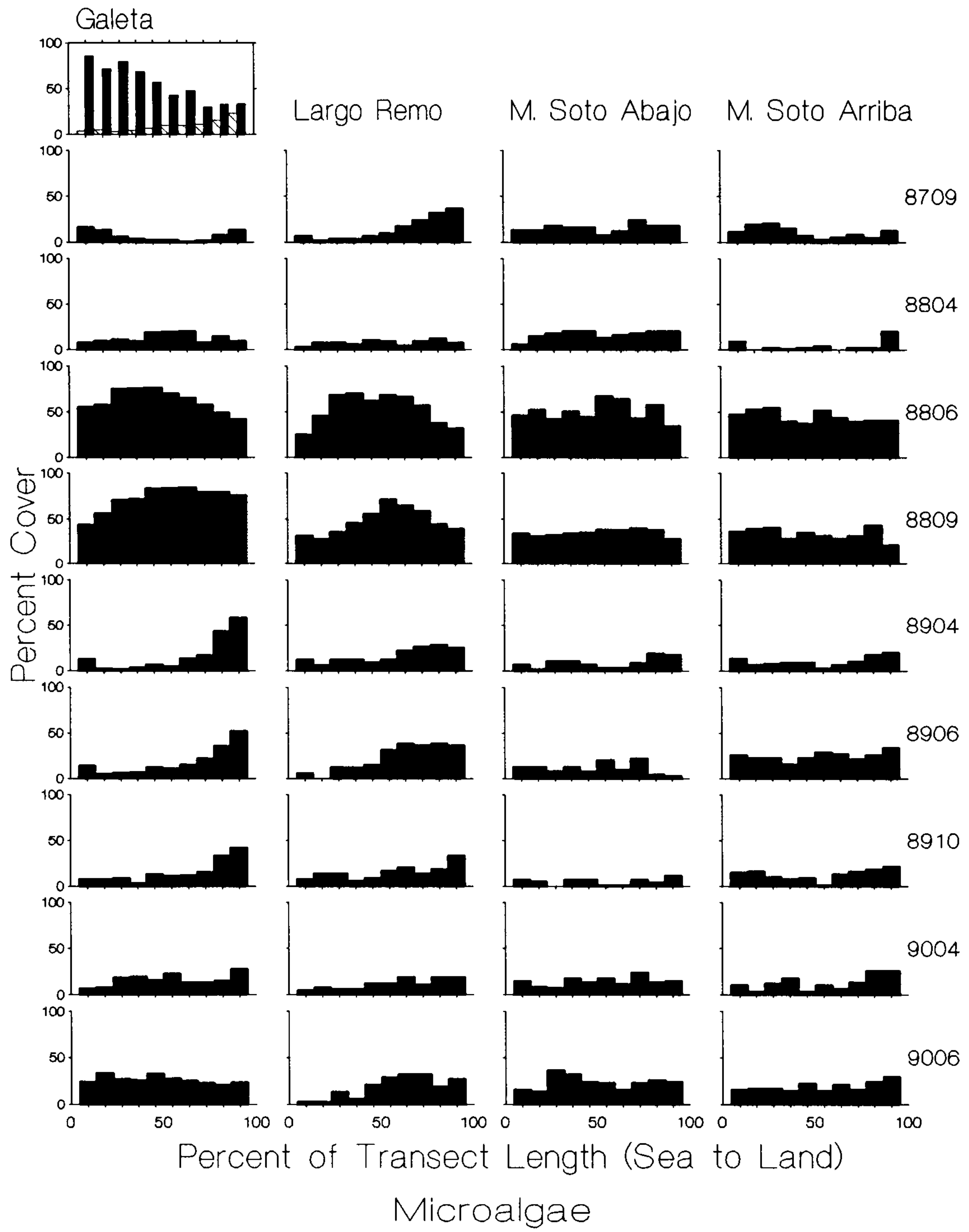


Fig. 4.24 Zonation of microalgae in the REDGE transects compared by sites. Transect lengths were standardized and are shown as percent distance from sea to land. For Punta Galeta, the *boxed graph* in the *upper left* compares the average cover in all prespill surveys (1983-1985: *hatched*) with cover in the first survey after the oil spill (June 1986: *solid*). The remaining graphs compare the four sites surveyed postspill. The two oiled sites are Punta Galeta and Largo Remo. The unoiled sites are María Soto (*M. Soto*) Abajo and Arriba. The survey dates are shown at right as four-digit year-months.

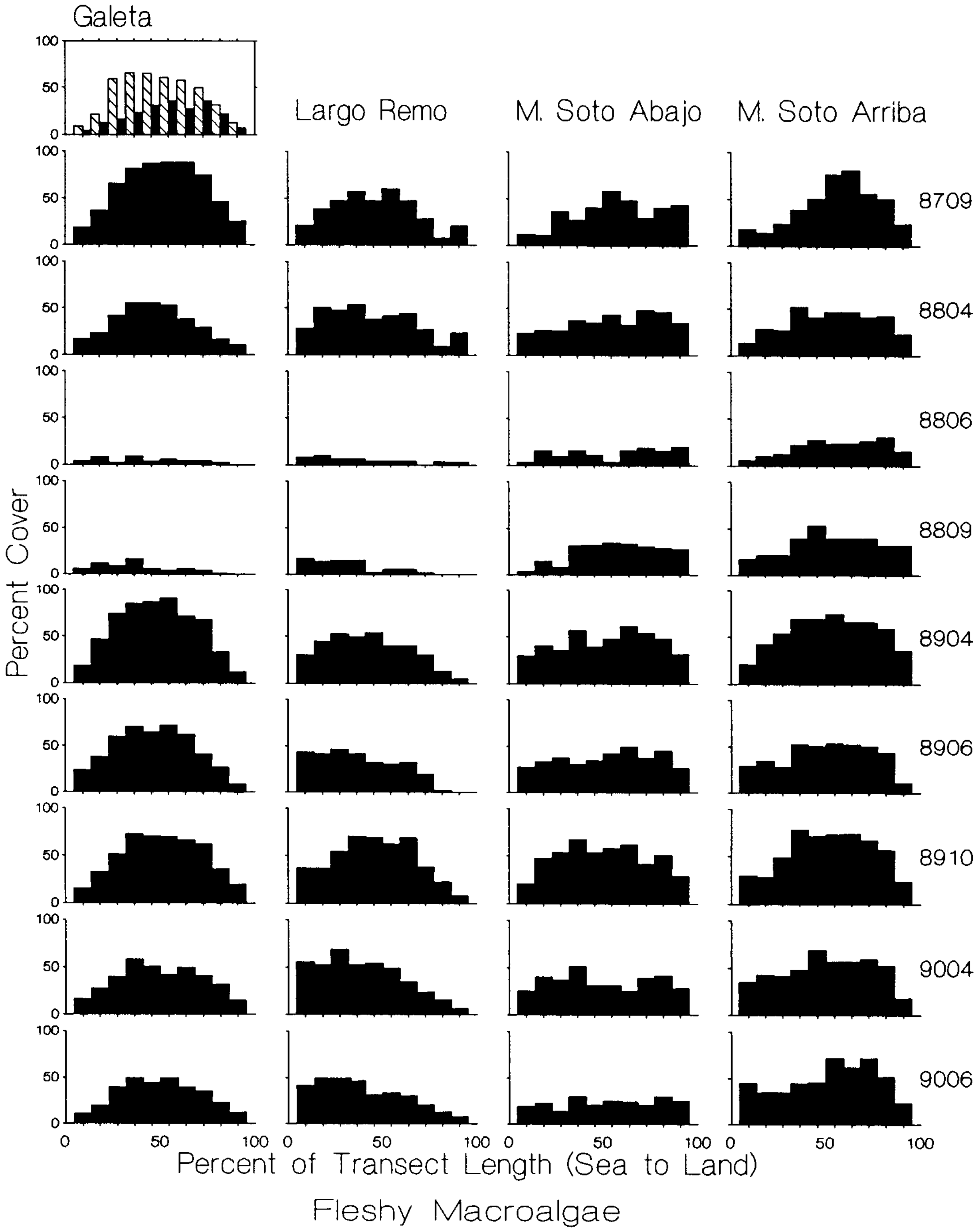


Fig. 4.25 Zonation of percent cover of fleshy macroalgae in the REDGE transects compared by sites. See Figure 4.24 for explanation of graph format.

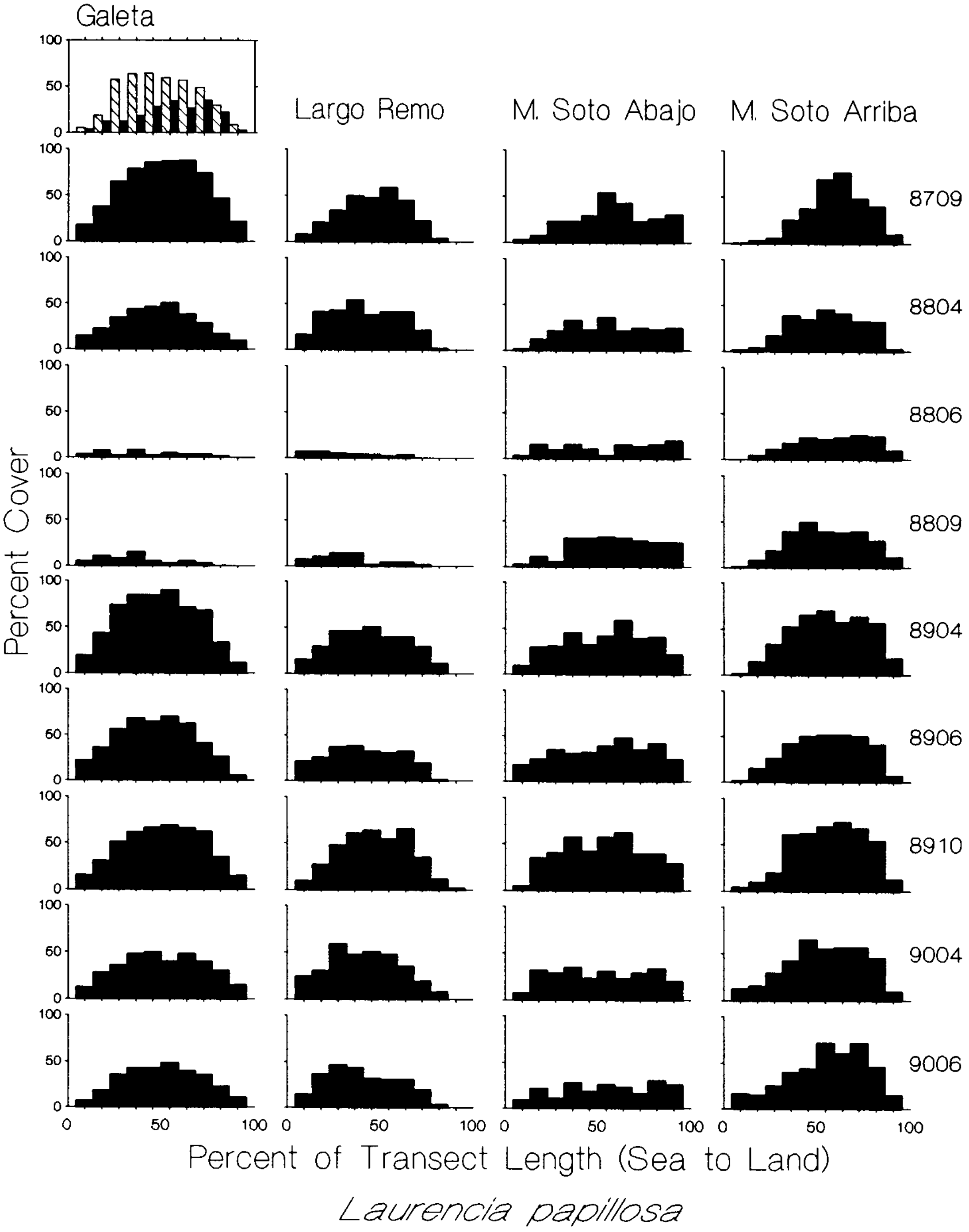


Fig. 4.26 Zonation of percent cover of *Laurencia papillosa* in the REDGE transects compared by sites. See Figure 4.24 for explanation of graph format.

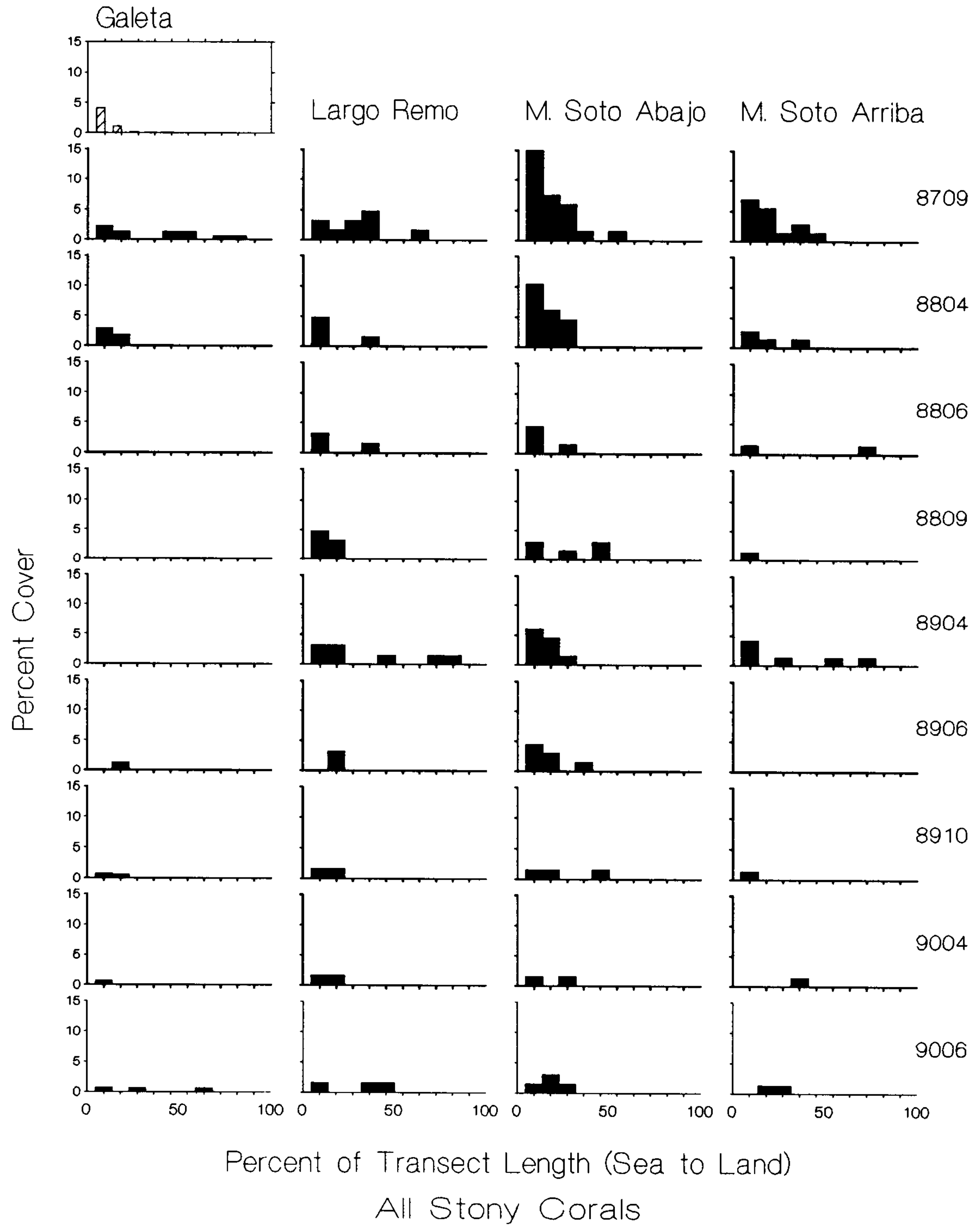


Fig. 4.27 Zonation of percent cover of all stony corals in the REDGE transects compared by sites. See Figure 4.24 for explanation of graph format.

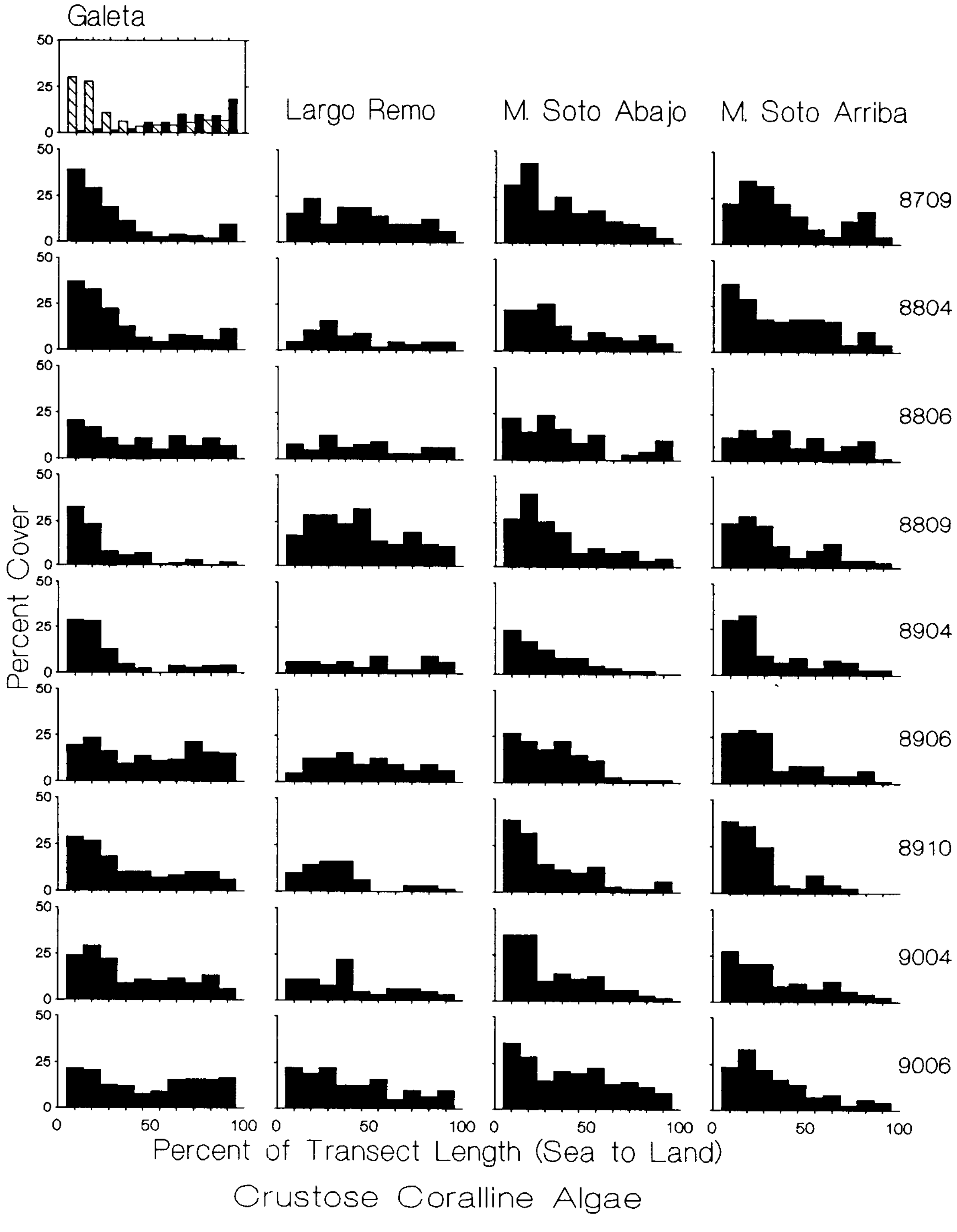


Fig. 4.28 Zonation of percent cover of crustose coralline algae in the REDGE transects compared by sites. See Figure 4.24 for explanation of graph format.

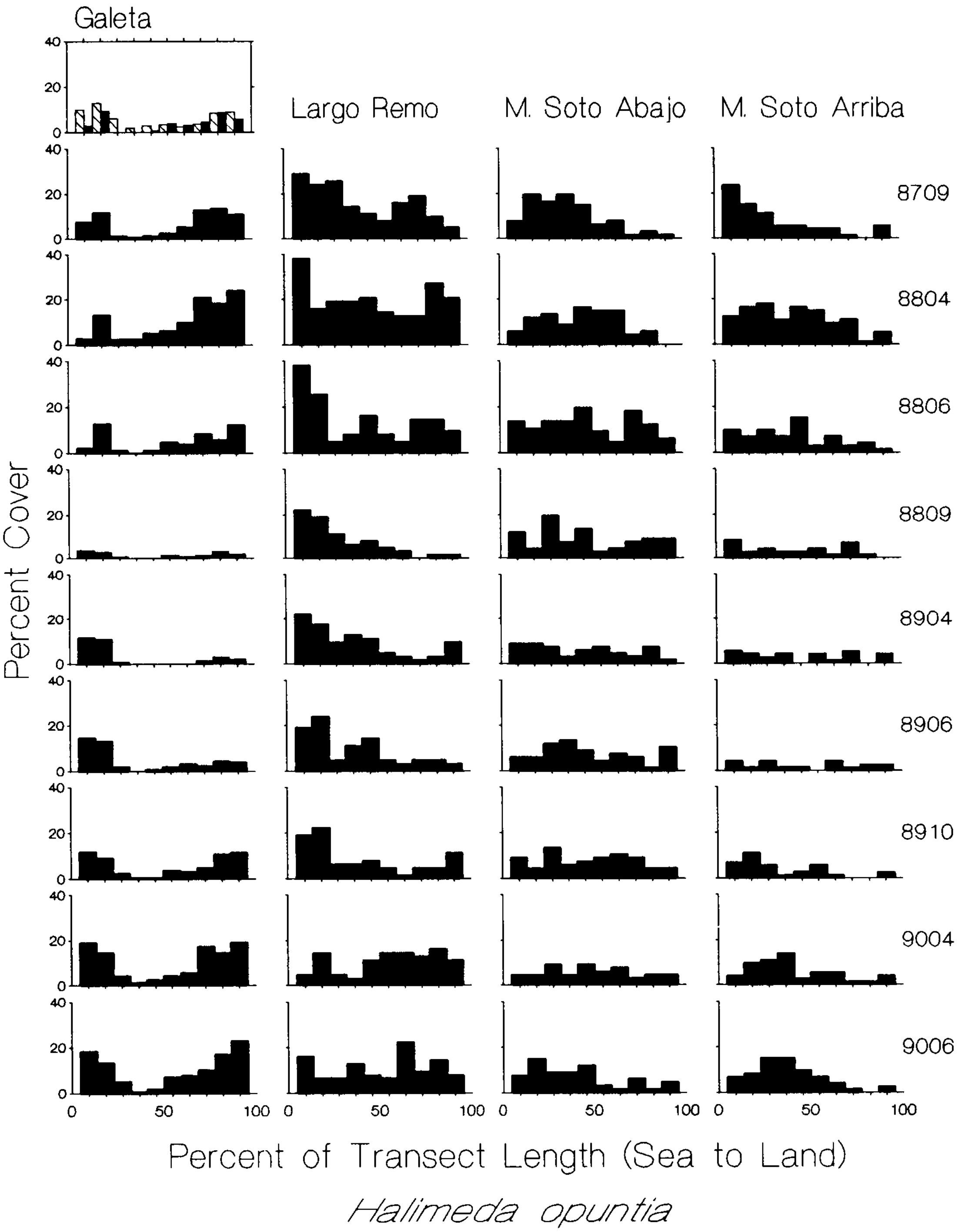


Fig. 4.29 Zonation of percent cover of *Halimeda opuntia* in the REDGE transects compared by sites. See Figure 4.24 for explanation of graph format.

4.4.2.4 Summary and Discussion of Effects on Sessile Biota

The seasonal patterns derived from the detailed, long-term baseline data show the oil spill occurred during the time of year when the predominant sessile biota, macroalgae, were at a natural low point in abundance. Therefore, if abundances during the oil spill had been compared with the overall averages for all prespill surveys (i.e., the abundances averaged for all months of the year), the results would have produced the appearance of a strong, detrimental effect of the oil spill, which would have been false. By making comparisons only within seasons, the seasonal component is reduced, allowing more reliable comparison of the spatial component corresponding to the gradient of oiling.

The CONSURV comparisons show that most of the mortality during the oil spill occurred near the seaward edge of the reef flat, in the area of the REDGE transects. The higher resolution data of the REDGE surveys demonstrated the mortality of sessile biota during the oil spill was graded according to the gradient of oil deposition on the reef flat. In general, algae regenerated faster after the oil spill than sessile invertebrates. The calcareous green alga *Halimeda opuntia*, however, was slow to recover.

The oil reduced the abundance of stony corals on the reef flat at Punta Galeta, and, in the first all-site census in 1987, stony corals were less abundant at the oiled sites than the unoiled sites. By June 1990 the abundances of stony corals at oiled and unoiled sites were approximately equal. The detailed monitoring data for the biota and physical environment of the reef flats demonstrate that this convergence in abundance can be clearly attributed to the mass mortality of organisms at all sites during a very unusual set of low water levels during 1988. The convergence neither indicates the corals recovered, nor that such mortality is "normal."

Spatial patterns of damage and processes of recovery are treated in the Discussion of this chapter (Sect. 4.4).

4.4.3 Censuses of Sea Urchin Populations

4.4.3.1 Methods

Censuses of sea urchin populations have been part of the long-term monitoring program at Punta Galeta. Urchins were counted in three permanent transects in three habitats on the reef flat (Fig. 4.10): at the seaward section of the reef flat (the "reef-edge transect"), in the intertidal *Thalassia* bed (the "*Thalassia* transect"), and in the back-reef area of coral rubble and loose sediment (the "rubble transect"). Each transect was 1 m wide by 20 m long (Fig. 4.30). At Punta Galeta the rubble transect was established in 1971, the *Thalassia* transect in 1972, and the reef-edge transect in 1977. In the expanded monitoring program after the spill, matching transects were established at the three additional sites and first censused

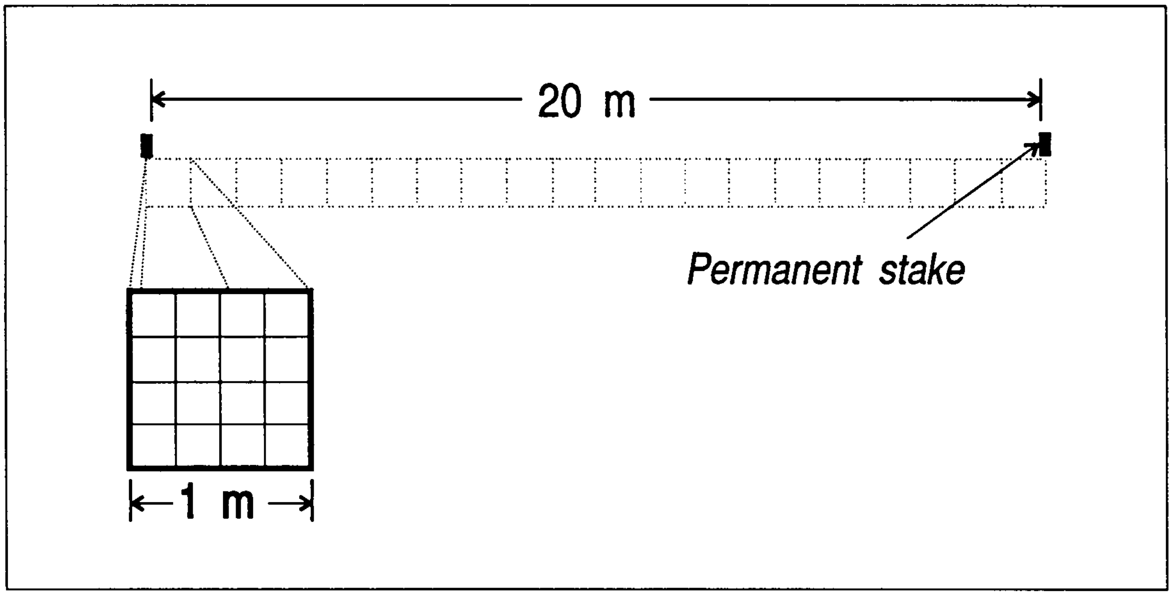


Fig. 4.30 Diagram of method of transect sampling used to monitor sea urchin populations. The permanent transects were 20 m long and 1 m wide. The sea urchins were counted in a 1 m by 1 m quadrat frame, which was divided into 16 subsections for easier counting.

in September 1987. All transects were permanently marked with steel stakes driven deep into the reef framework. The stakes, including back-up stakes, were mapped using direct linear measurements and triangulation to local landmarks.

To maintain continuity with the prespill surveys, censuses were made once per month at Punta Galeta, depending on water conditions. The other sites were monitored every 3 mo, water conditions permitting, with a minimum of three surveys per year. Counts were made in a square-meter quadrat placed at each meter interval of the transect (Fig. 4.30), and data were recorded individually for each quadrat; consequently, the data include the spatial distribution of sea urchins along the transect.

The substrata in each square meter were carefully inspected, including sorting through the overlying vegetation and lifting all pieces of rubble for examination. Glass-bottomed viewers were used when the transects were covered by water. However, counts were not made on days with strong wave action and currents over the transects because manipulation of the vegetation and rubble would have caused the sea urchins to become dislodged and wash away from the permanent transects. In addition, reliable counts could not be made when more than 15 cm of turbid water covered the transects. Vagaries of weather forced repeated rescheduling of surveys, resulting in much wasted time to complete the surveys of all sites within the same time period. Inspection of the data from the first two surveys of all sites indicated that use of 10 instead of 20 quadrats per transect would reliably census sea urchin populations; therefore, at the new sites, counts were made at every other meter interval. At Punta Galeta, consistency with the prespill data was maintained by censusing all 20 m of each transect on all dates.

At Punta Galeta, the sampling schedule was modified slightly during the oil spill. The first large slicks arrived in the afternoon of 9 May. Forewarned, we immediately censused the sea urchin populations. The first postspill censuses of the transects were made on 31 May and 3 June 1986, 23-26 d after the first oil slicks arrived at Punta Galeta. The censuses were repeated on 19-20 June, 43 d after the first slicks arrived at this site. The censuses made between 31 May and 3 June at Punta Galeta were treated as June data in the analyses.

4.4.3.2 Results of Sea Urchin Censuses

The format of the census data allowed changes to be analyzed at various spatial scales, including fixed 1-m^2 quadrats within the permanent transects, positions along the transects, distances of the transects away from the edges of the reef flats, and the locations of the reef flats sites relative to the oil spill. Changes observed in the long-term data before the oil spill were used to interpret the changes noted after the spill in 1986.

Prespill-Postspill Comparisons of Changes in Populations of Sea Urchins at Punta Galeta

The long-term monitoring of sea urchin populations on the reef flat at Punta Galeta demonstrated that the abundances of sea urchins varied seasonally, fluctuated from year to year, and exhibited long-term trends. Figure 4.31*A-F* provides monthly average abundances for each species of sea urchin in each transect. These averages were calculated from the data shown in Figures 4.32-4.34; the figures show the time span of, and gaps in, the data. *Echinometra lucunter*, *Echinometra viridis*, and *Echinoneus cyclostomus* normally reached maximum abundances during the middle of the year (Panama's wet season), while most of the other species reached maximum abundances around the beginning of the year (Panama's dry season) and completely disappeared in the middle of the year (Fig. 4.31*A-F*). Thus, the absence of *Lytechinus variegatus* and *Tripneustes ventricosus* on the reef flat in the months immediately following the oil spill was most likely a result of this natural seasonal variation, and not a result of the oil. Populations of *Diadema antillarum* and *Eucidaris tribuloides*, normally rare during the months of the oil spill, had declined in the years before the oil spill, thus precluding conclusions regarding effects of the 1986 oil spill on these species (Figs. 4.32-4.34).

When oil slicks arrived at Punta Galeta in May 1986, *Echinometra lucunter* was the most abundant sea urchin on the reef flat, followed by *Echinometra viridis* and *Echinoneus cyclostomus*. In addition, recruitment was underway for all three

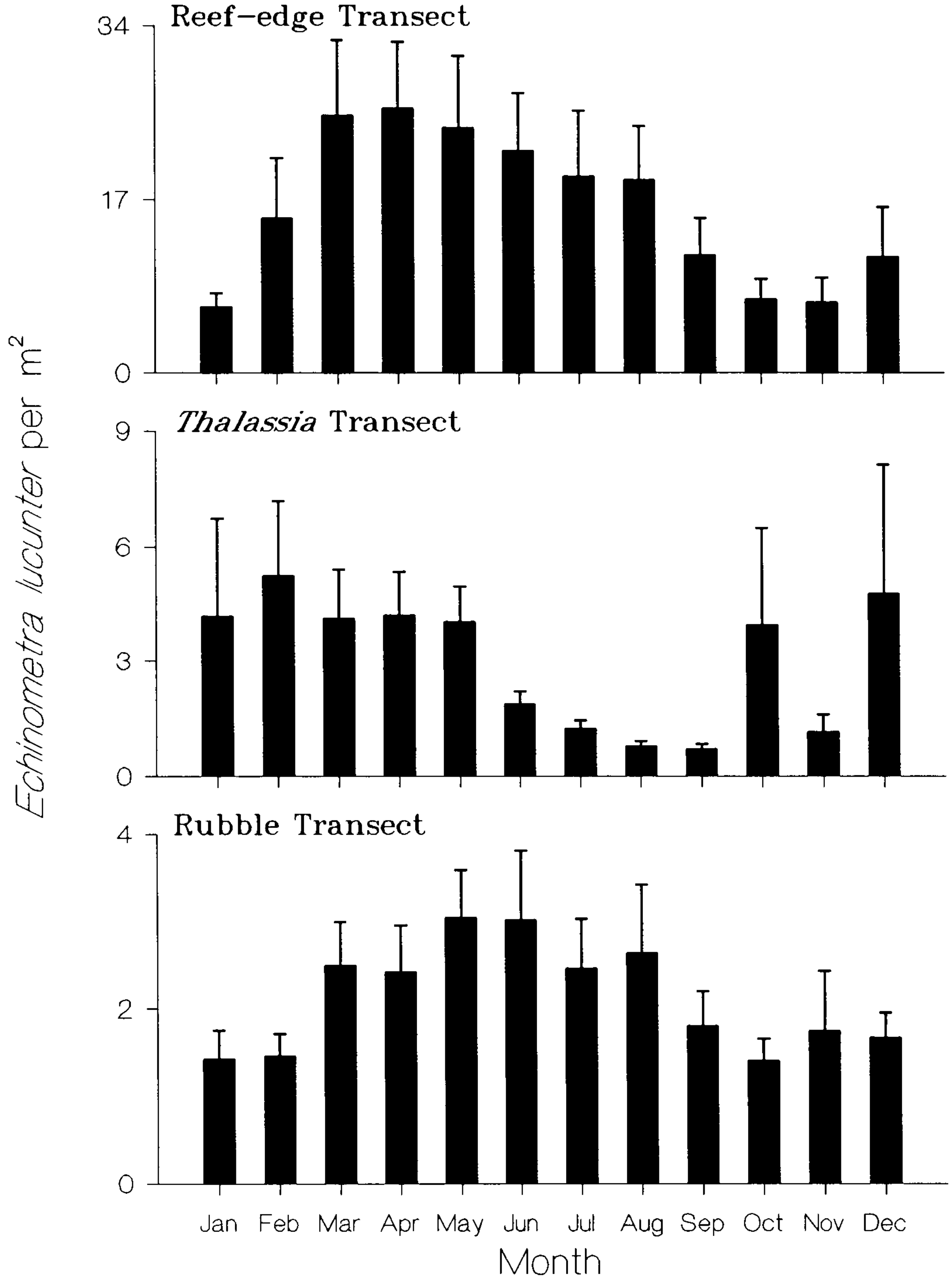


Fig. 4.31 Seasonal patterns of variation of sea urchin populations in the three permanent transects on the reef flat at Punta Galeta. *A. Echinometra lucunter.* Each *filled bar* is the monthly mean; the error bar is ± 1 SE. These patterns have been calculated from data collected over the following periods: reef-edge transect (1977-1991); *Thalassia* transect (1972-1991); rubble transect/mid-reef (1971-1991). Within these periods, data are missing for some species in some years. The actual dates of sampling for each species in each transect are shown as the data points in Figures 4.32-4.34. Note that the scales of the vertical axes differ to better show changes over time.

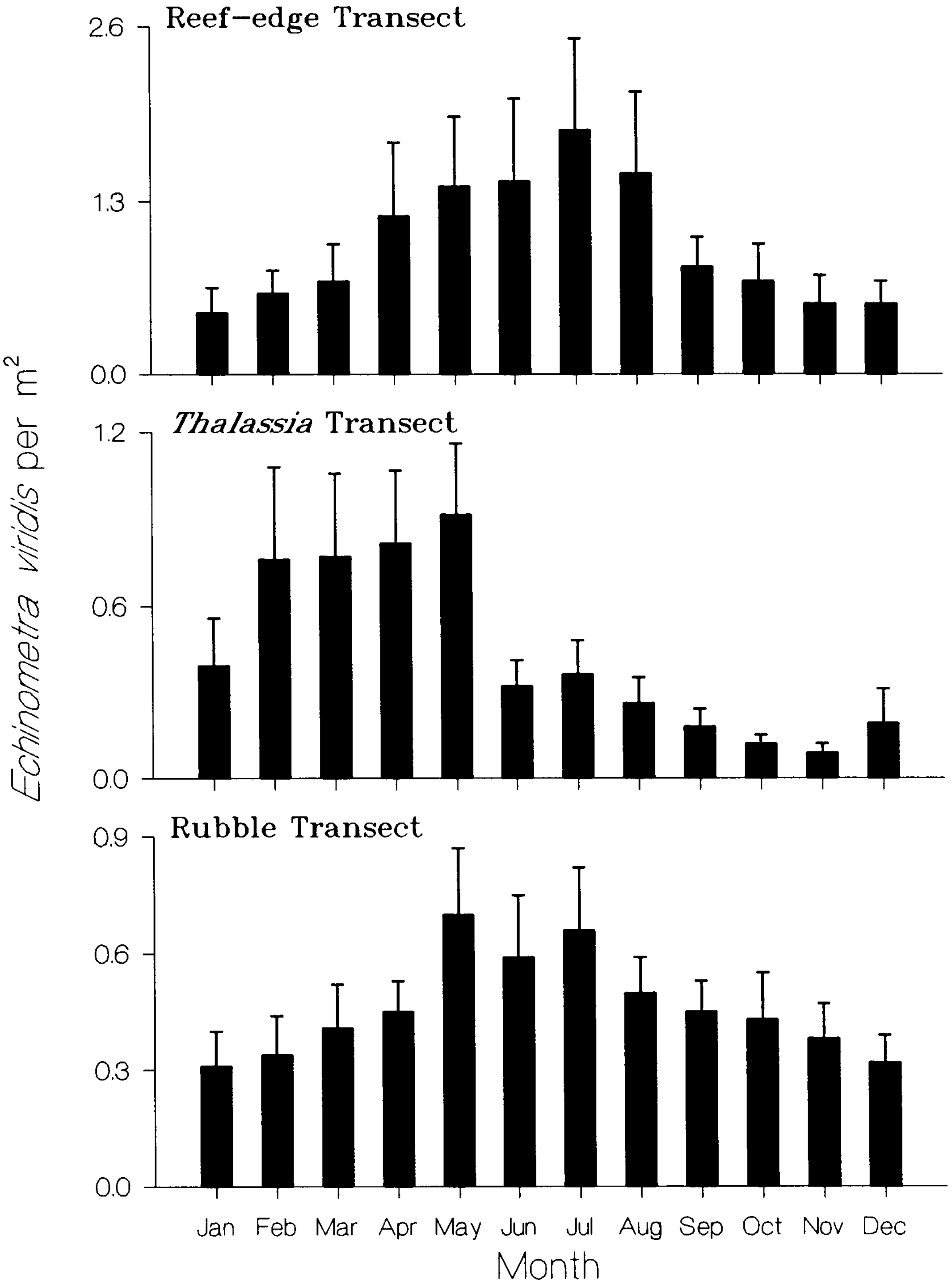


Fig. 4.31 **Seasonal patterns of variation of sea urchin populations in the three permanent transects on the reef flat at Punta Galeta (continued). *B. Echinometra viridis.* See Figure 4.31*A* for explanation of graph format.**

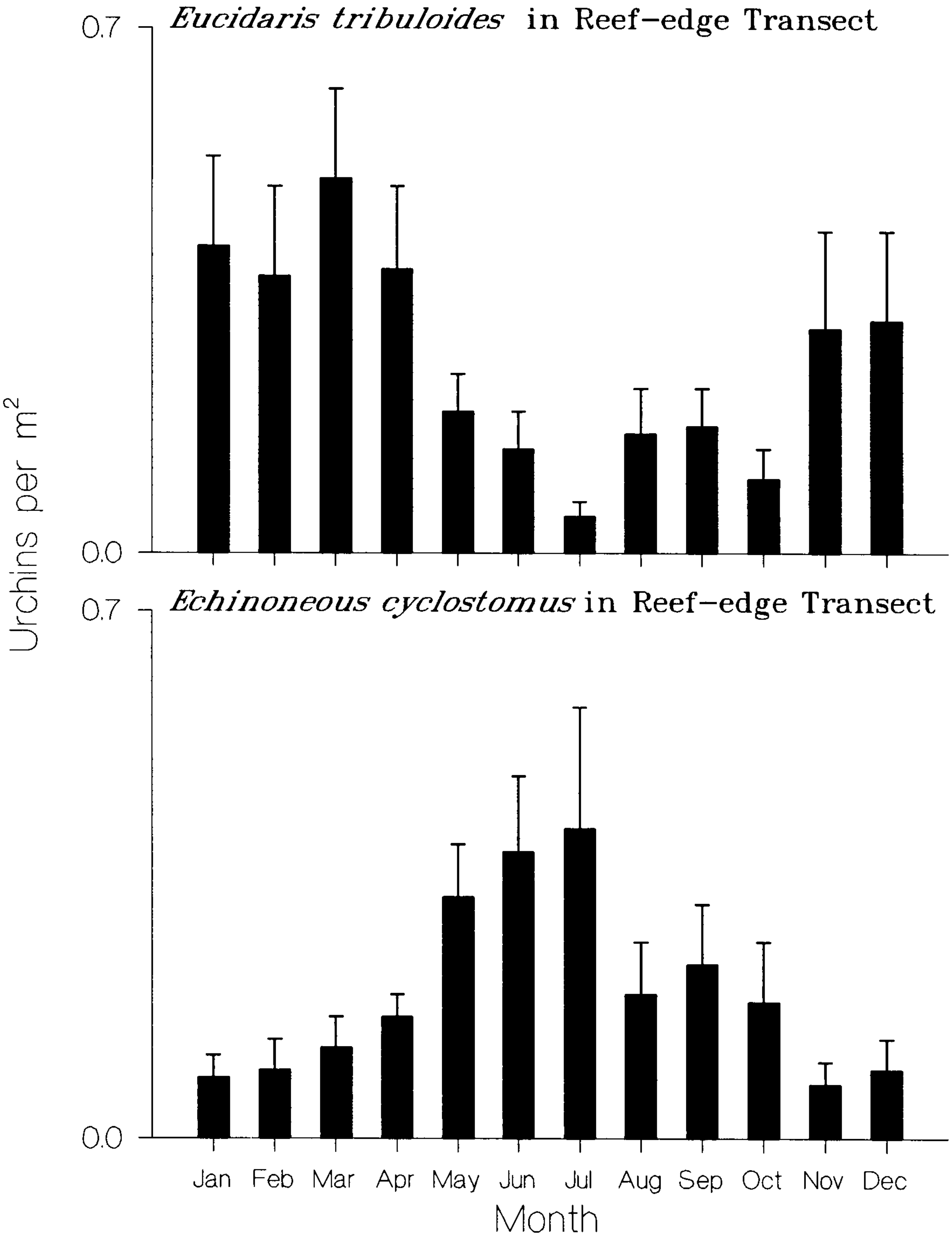


Fig. 4.31 Seasonal patterns of variation of sea urchin populations in the three permanent transects on the reef flat at Punta Galeta (continued). *C. Eucidaris tribuloides* and *Echinoneus cyclostomus*. The only significant populations of these two species occurred in the permanent reef-edge transect. See Figure 4.31*A* for explanation of graph format.

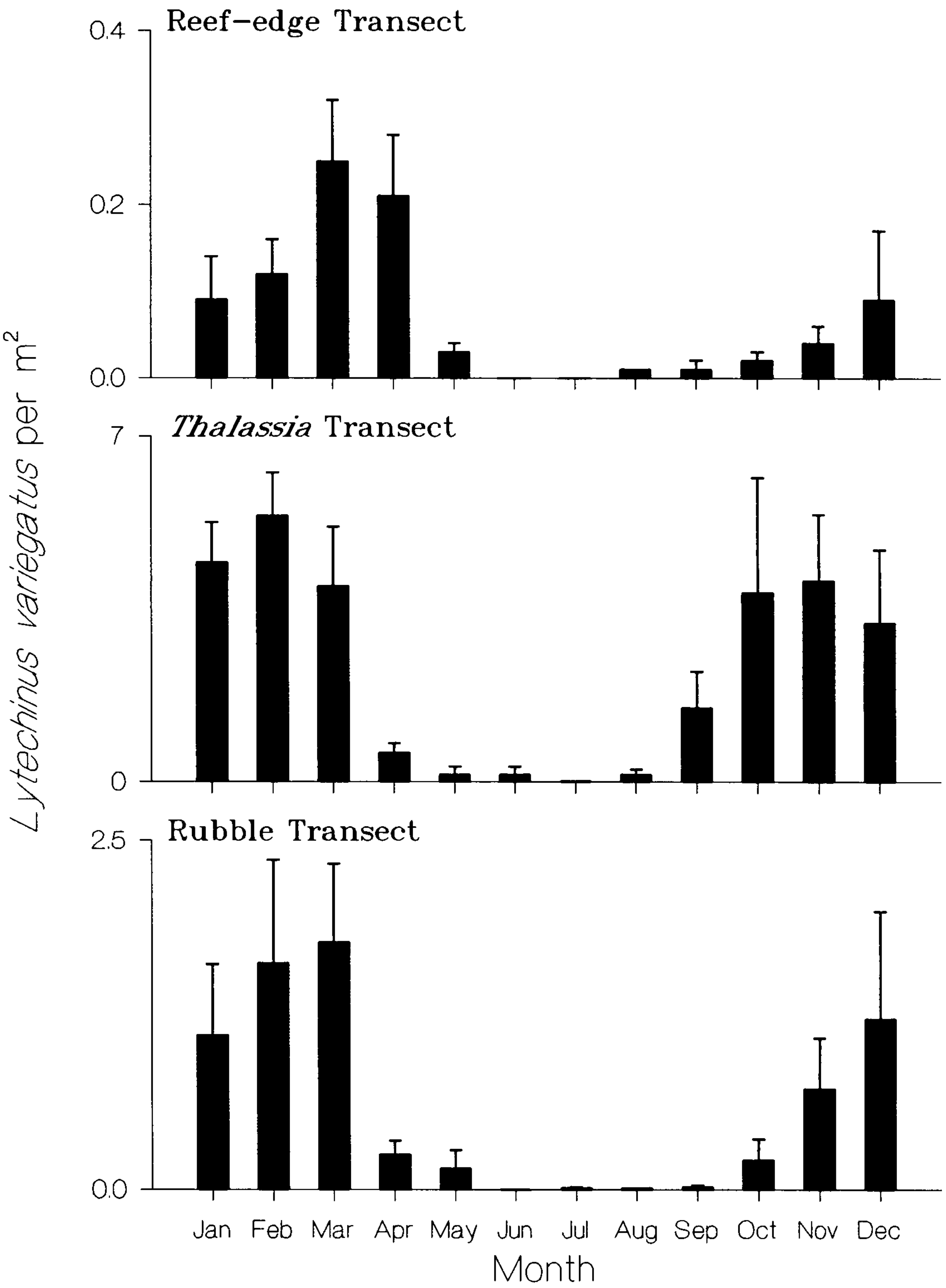


Fig. 4.31 Seasonal patterns of variation of sea urchin populations in the three permanent transects on the reef flat at Punta Galeta (continued). *D. Lytechinus variegatus.* See Figure 4.31*A* for explanation of graph format.

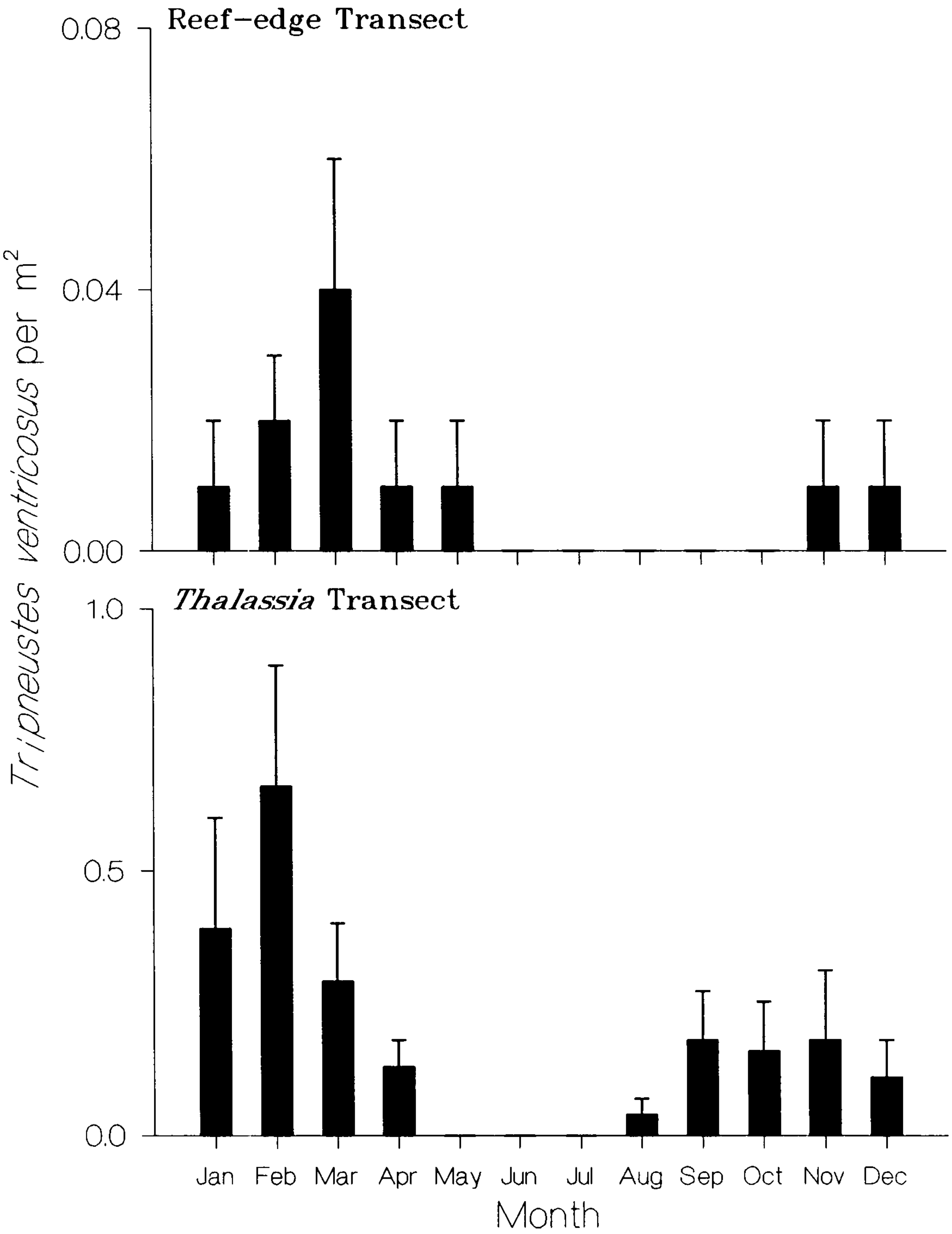


Fig. 4.31 Seasonal patterns of variation of sea urchin populations in the three permanent transects on the reef flat at Punta Galeta (continued). *E. Tripneustes ventricosus.* See Figure 4.31*A* for explanation of graph format.

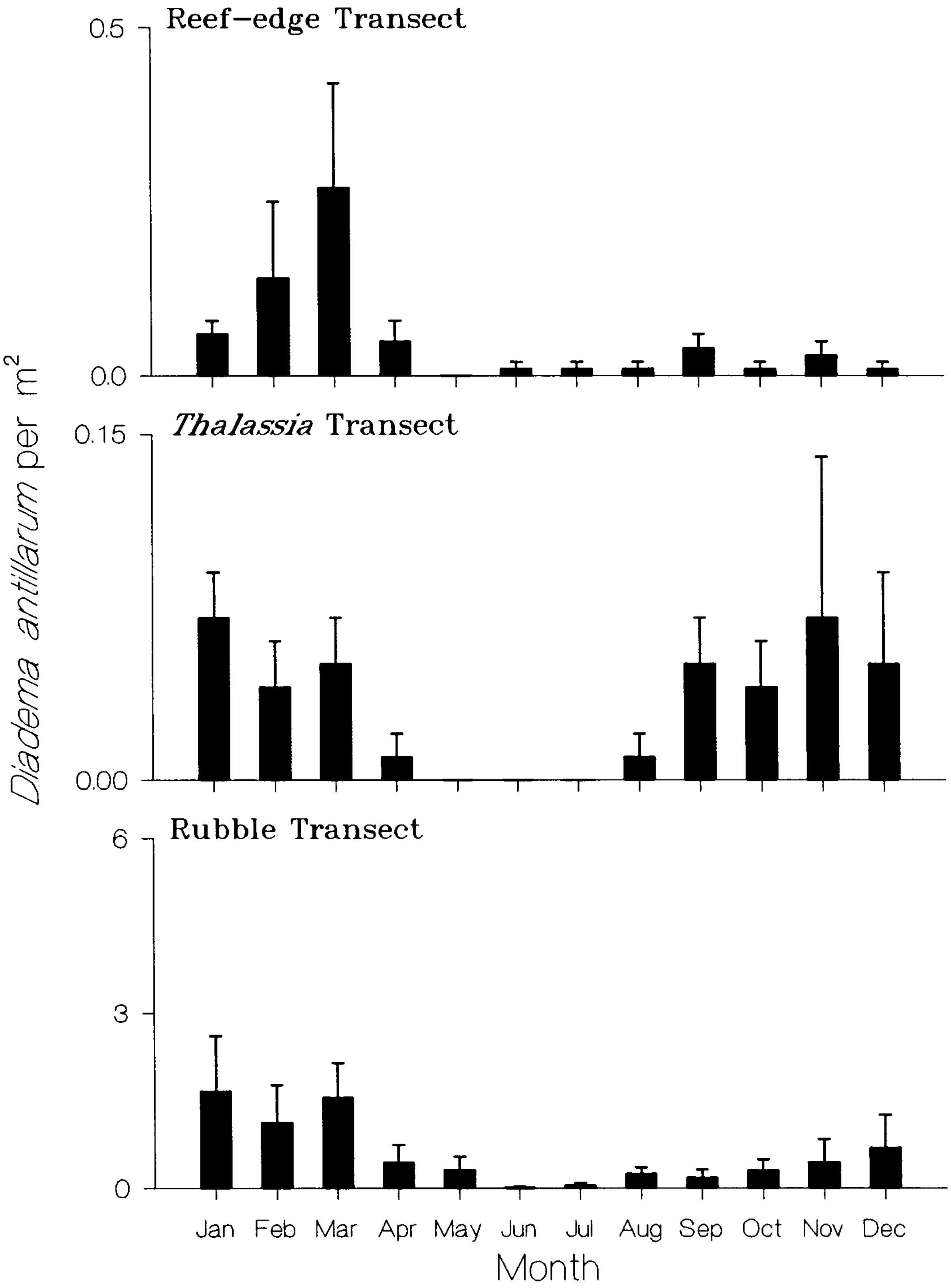


Fig. 4.31 Seasonal patterns of variation of sea urchin populations in the three permanent transects on the reef flat at Punta Galeta (continued). *F. Diadema antillarum.* See Figure 4.31*A* for explanation of graph format.

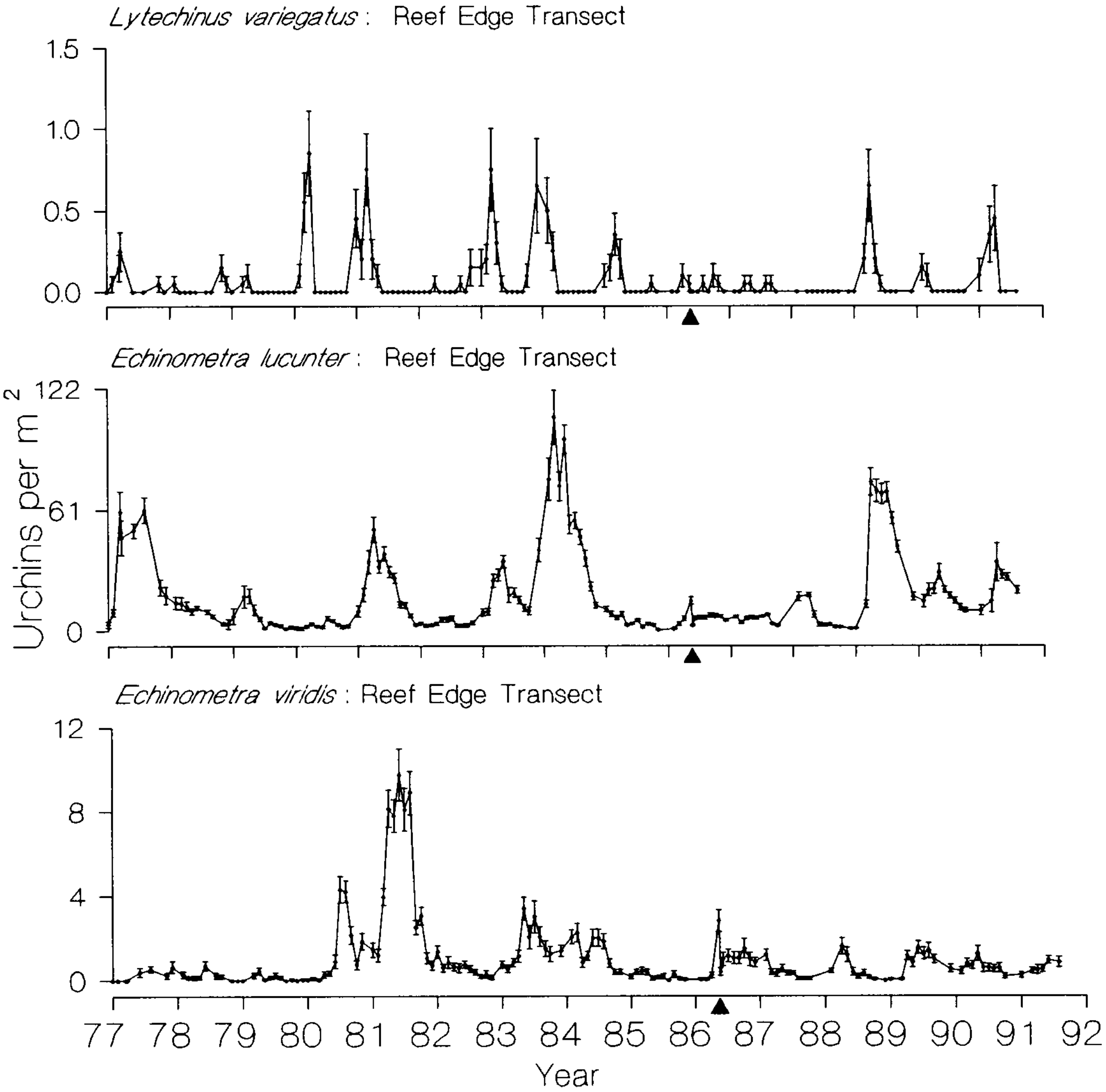


Fig. 4.32 Monthly population densities of sea urchin populations in the reef edge transect at Punta Galeta, 1977-1991. *A. Lytechinus variegatus, Echinometra lucunter,* and *E. viridis.* The data are means ± 1 SE of counts in the 20 square-meter quadrats for each survey date. The *solid triangle* marks the arrival of the first oil slicks at Punta Galeta in May 1986. Note that the scales of the vertical axes differ to better show changes over time.

species, and their populations had been increasing for several months before the spill (Figs. 4.32-4.34). (Recruitment here refers to the arrival of small urchins, approximately 0.5 cm test diameter, which had recently settled from the plankton.)

Changes in the abundances of these sea urchins during the spill corresponded to their position on the reef flat relative to the accumulation of oil along the seaward border of the flat. In the time-series graphs the largest apparent decrease in the

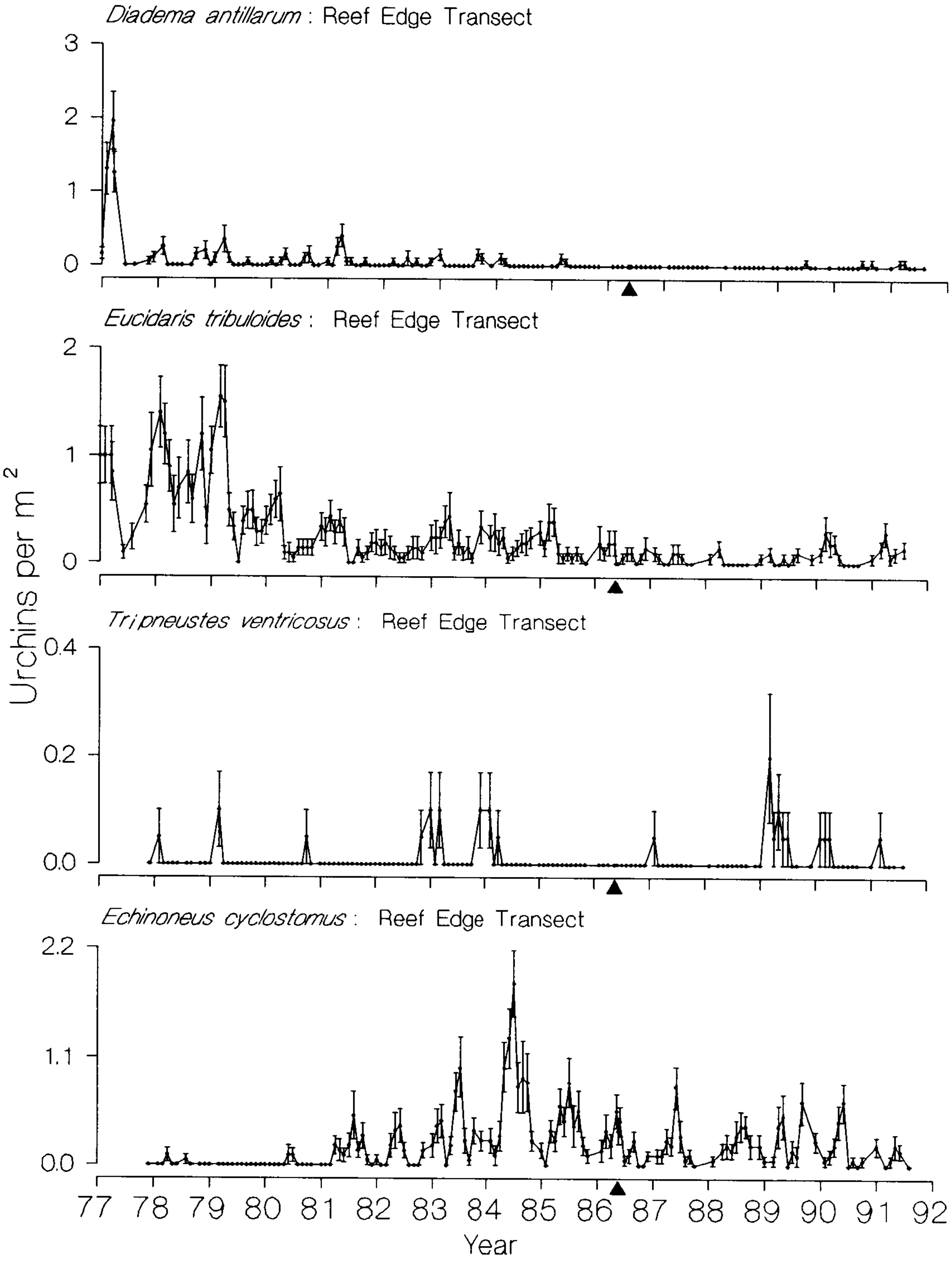


Fig. 4.32 Monthly population densities of sea urchin populations in the reef edge transect at Punta Galeta, 1977-1991 (continued). *B. Diadema antillarum, Eucidaris tribuloides, Tripneustes ventricosus,* and *Echinoneus cyclostomus.* See Figure 4.32A for explanation of graph format.

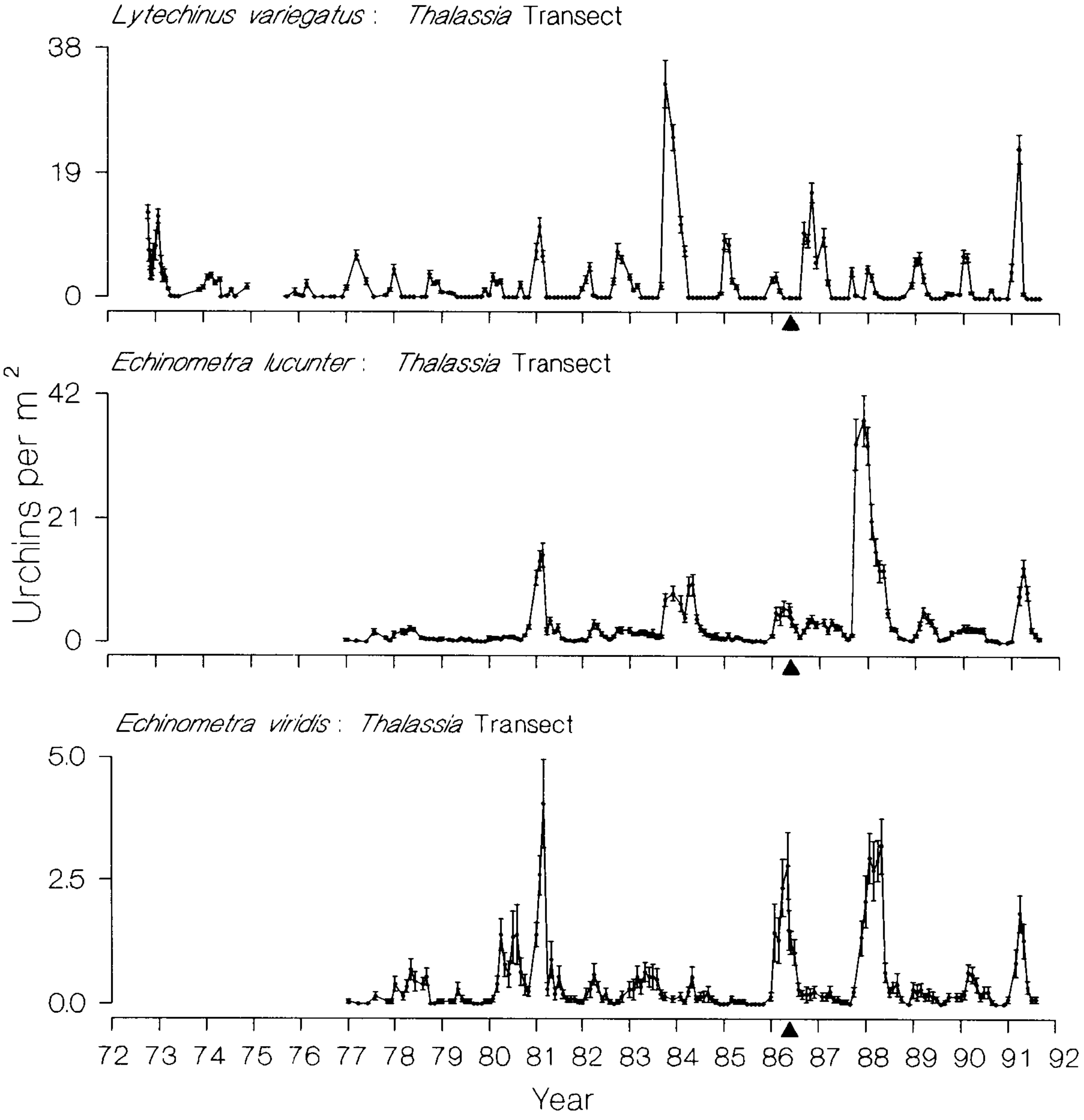


Fig. 4.33 Monthly population densities of sea urchin populations in the *Thalassia* transect at Punta Galeta, 1972-1991. *A. Lytechinus variegatus*, *Echinometra lucunter*, and *E. viridis*. The data are means ± 1 SE of counts in the 20 square-meter quadrats for each survey date. For standardization, the data for all species have been plotted on axes for 1972-1991; *blank spaces* in the graphs indicate periods of no records for that species, including species other than *L. variegatus* during 1972-1977. The *solid triangle* marks the arrival of the first oil slicks at Punta Galeta in May 1986. Note that the scales of the vertical axes differ to better show changes over time.

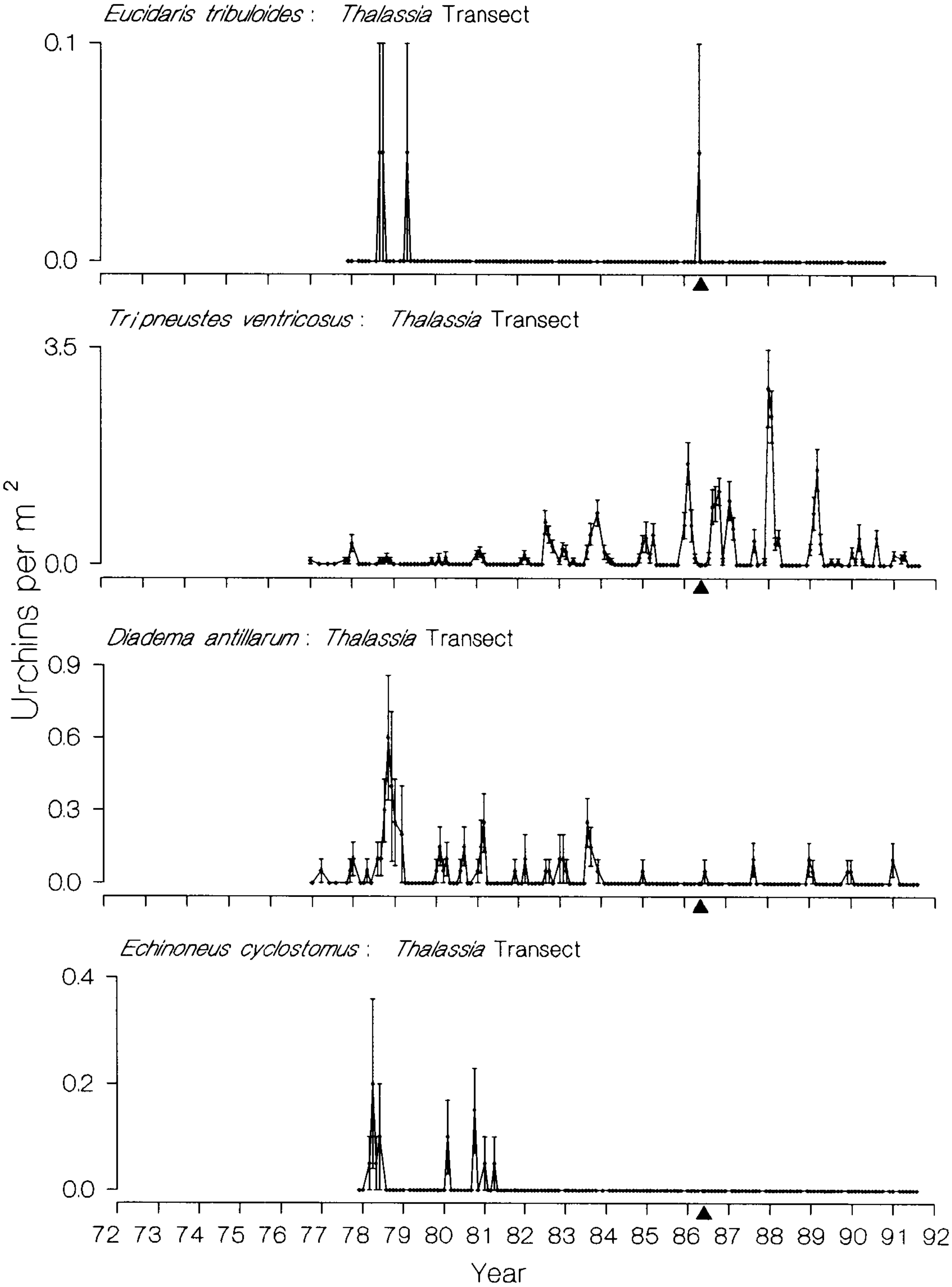


Fig. 4.33 Monthly population densities of sea urchin populations in the *Thalassia* transect at Punta Galeta, 1972-1991 (continued). *B. Eucidaris tribuloides, Tripneustes ventricosus, Diadema antillarum*, and *Echinoneus cyclostomus*. See Figure 4.33*A* for explanation of graph format.

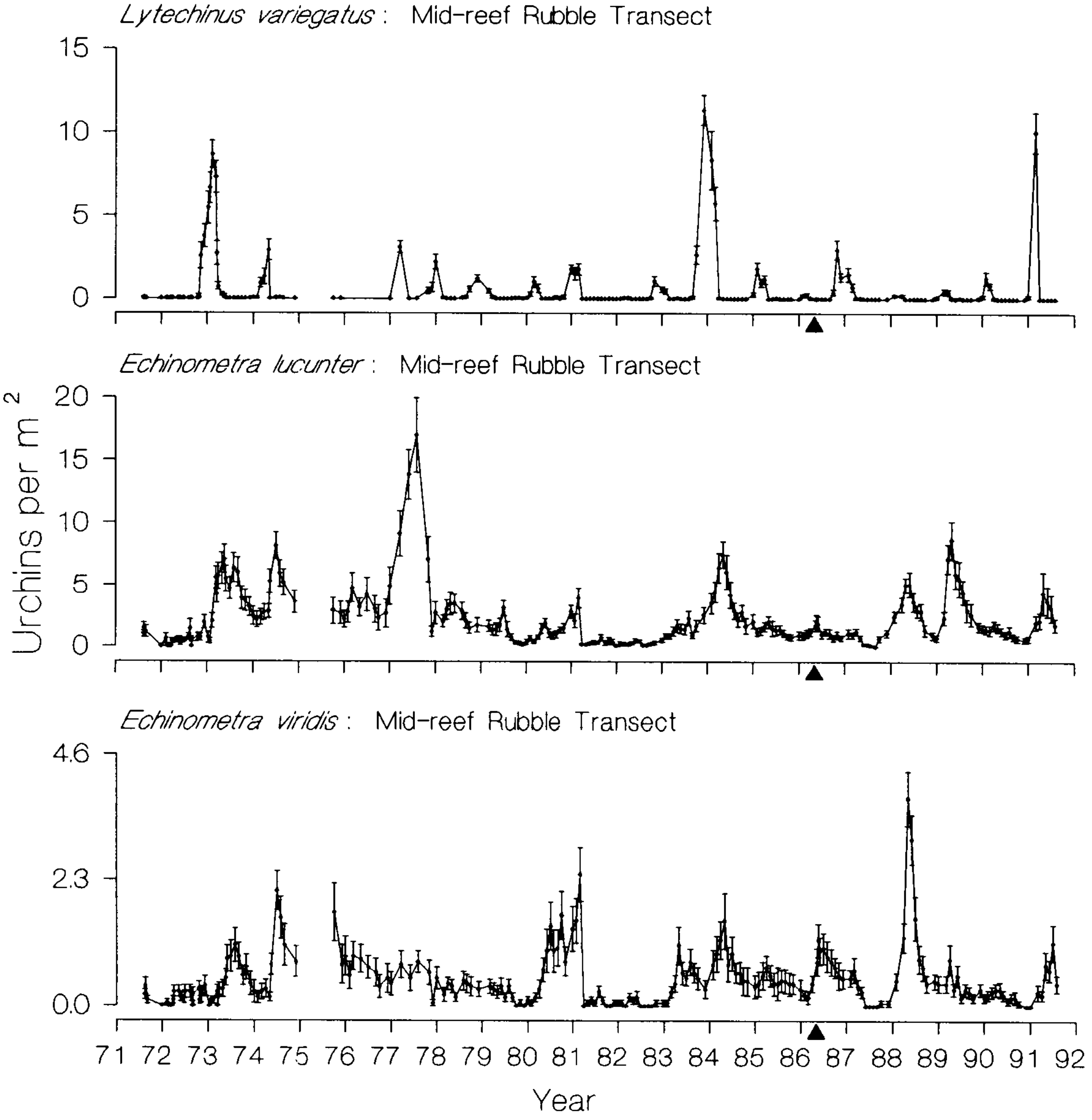


Fig. 4.34 Monthly population densities of sea urchin populations in the rubble transect at Punta Galeta, 1971-1991. *A. Lytechinus variegatus*, *Echinometra lucunter*, and *E. viridis*. The data are means ± 1 SE of counts in the 20 square meter quadrats for each survey date. For standardization, the data for all species have been plotted on axes for 1971-1991; *blank spaces* in the graphs indicate periods of no records for that species. The *solid triangle* marks the arrival of the first oil slicks at Punta Galeta in May 1986. Note that the scales of the vertical axes differ to better show changes over time.

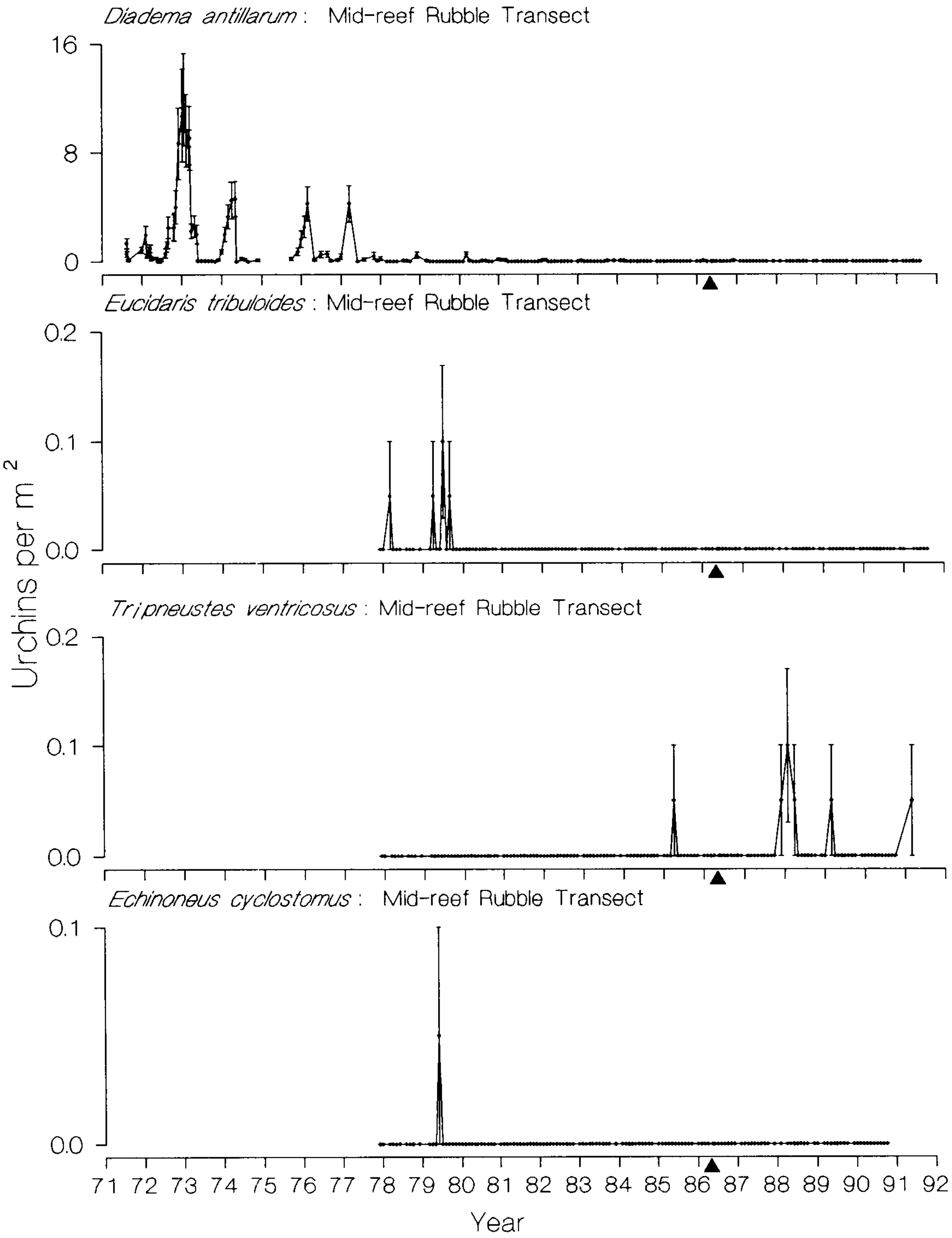


Fig. 4.34 Monthly population densities of sea urchin populations in the rubble transect at Punta Galeta, 1971-1991 (continued). *B. Diadema antillarum*, *Eucidaris tribuloides*, *Tripneustes ventricosus*, and *Echinoneus cyclostomus*. See Figure 4.34A for explanation of graph format.

Echinometra populations was in the reef-edge transect (Fig. 4.32). Lesser decreases occurred in the *Thalassia* transect, 26 m directly landward of the reef-edge transect. The abundances of the two *Echinometra* species in the rubble transect increased. The only significant populations of *Echinoneus cyclostomus* were in the reef-edge transect, where populations were reduced to near zero during the spill (Figs. 4.32-4.34).

To compare May-to-June 1986 changes in *Echinometra* populations with changes seen in the same period of other years, June populations were regressed on May populations of the same year. In any year, June populations are necessarily dependent on the population of the immediately preceding May, and changes due to recruitment and mortality occurring over the intervening month. Thus, the regression establishes an expected population in June relative to the population existing a month earlier in the same location. As a conservative test of the hypothesis that oil caused changes in *Echinometra* populations, the data for May and June 1986 were included in the regressions. In the reef-edge transect, which was nearest the heavy deposition of oil at the seaward edge of the reef flat, the decreases in populations of both *Echinometra* species from May to June 1986 appeared as points falling below the 95% confidence intervals (Fig. 4.35). Although the two *Echinometra* species also decreased in the *Thalassia* transects between May and June 1986, the decreases were less than the established norm for other years, as shown by the regression line. In the rubble transect, populations of both species increased from May to June 1986, even though oil was still coming ashore.

While weathered oil slicks were still present over the reef flat in the months following the spill, *Echinometra lucunter* resumed recruitment in the reef-edge and rubble transects, *Echinometra viridis* resumed recruitment in the *Thalassia* and rubble transects, and *Echinoneus cyclostomus* resumed recruitment in the reef-edge transect (Figs. 4.32-4.34).

Spatial differences in recruitment suggested a residual effect of the oil, as illustrated by the most abundant species, *Echinometra lucunter*. In the reef-edge transect after the oil spill, recruitment of this species was much reduced relative to previous years in the square-meter quadrats nearest the zone where oil had accumulated. Before the oil spill, timing of recruitment of *E. lucunter* was generally synchronized among the 20 quadrats of the reef-edge transect, producing the periodic, simultaneous increases of urchin populations shown in the 20 stacked graphs of Figure 4.36. In addition, during these events recruitment was relatively high in the quadrat nearest the ocean (Fig. 4.36). However, in the months following the spill, recruitment failed in this quadrat, but resumed in the quadrats farther from the seaward border of the reef flat (Fig. 4.36).

The distribution of *Echinometra lucunter* along the transects at Punta Galeta was also examined for indications that immigration and emigration of adult urchins, rather than recruitment of larvae and mortality of adults, could have explained the periodic changes in abundance, including the changes following the 1986 oil spill. Periods of change similar in magnitude to the change during the 1986 oil spill were chosen for comparison with the change in mid-1986. The monthly changes in spatial

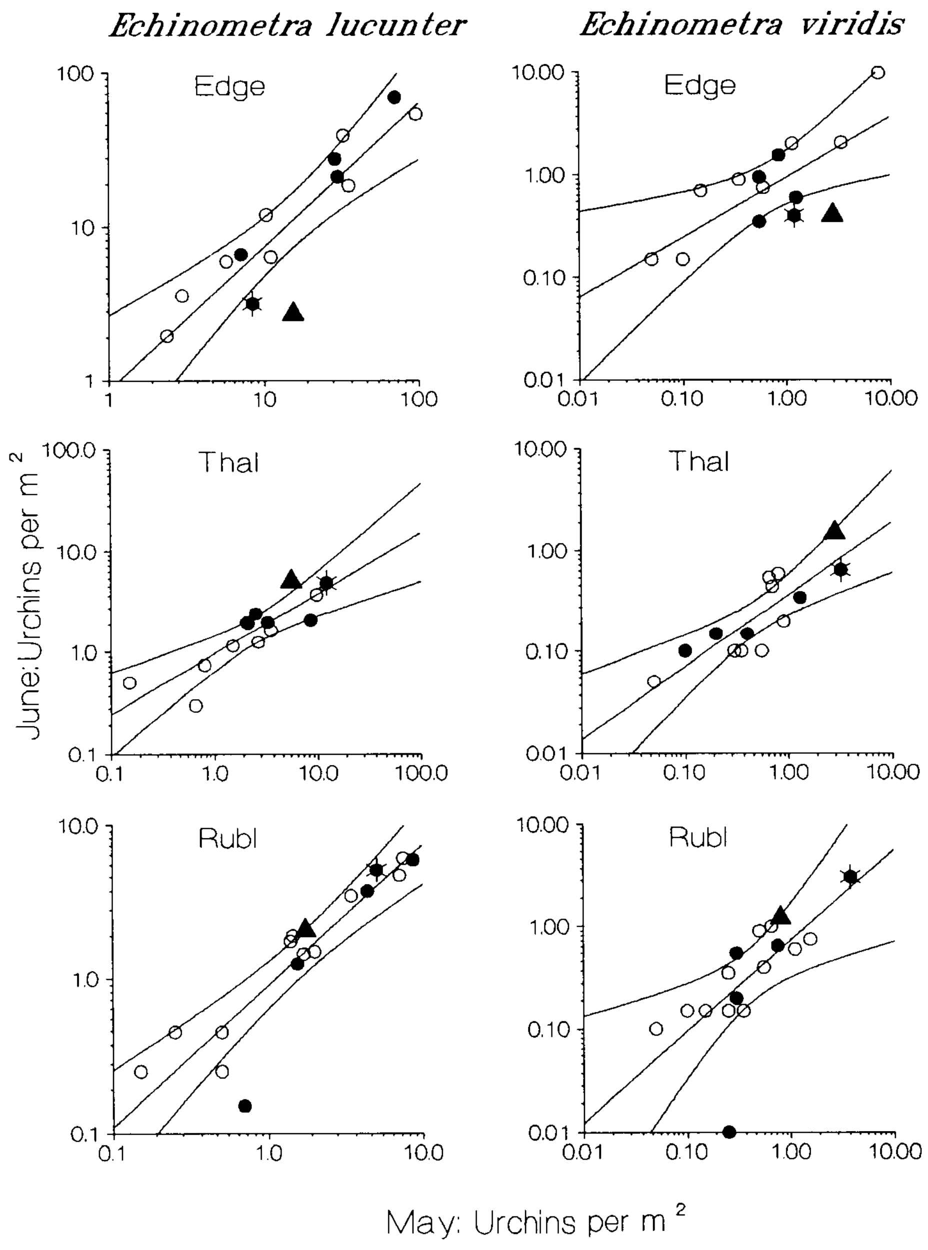


Fig. 4.35 The relationships between May and June populations of *Echinometra* spp. during the 1986 oil spill compared with the relationships between May and June populations of other years. Each data point represents a May-June combination for the same year. The data points for the prespill years are shown as *open circles*, for 1986 as *solid triangles*, and for the postspill years as *solid circles*. The *6-pointed stars* mark the solid circles for 1988, the year of severe exposures of the reef flat above water level. The *curved lines* are 95% confidence intervals on each side of the regression line for each year's June population on the preceding May population. The years of data for each species and transect are shown by the data points in Figures 4.32-4.34. Each transect is shown separately (*Edge* = reef-edge transect; *Thal* = *Thalassia* transect; *Rubl* = rubble transect).

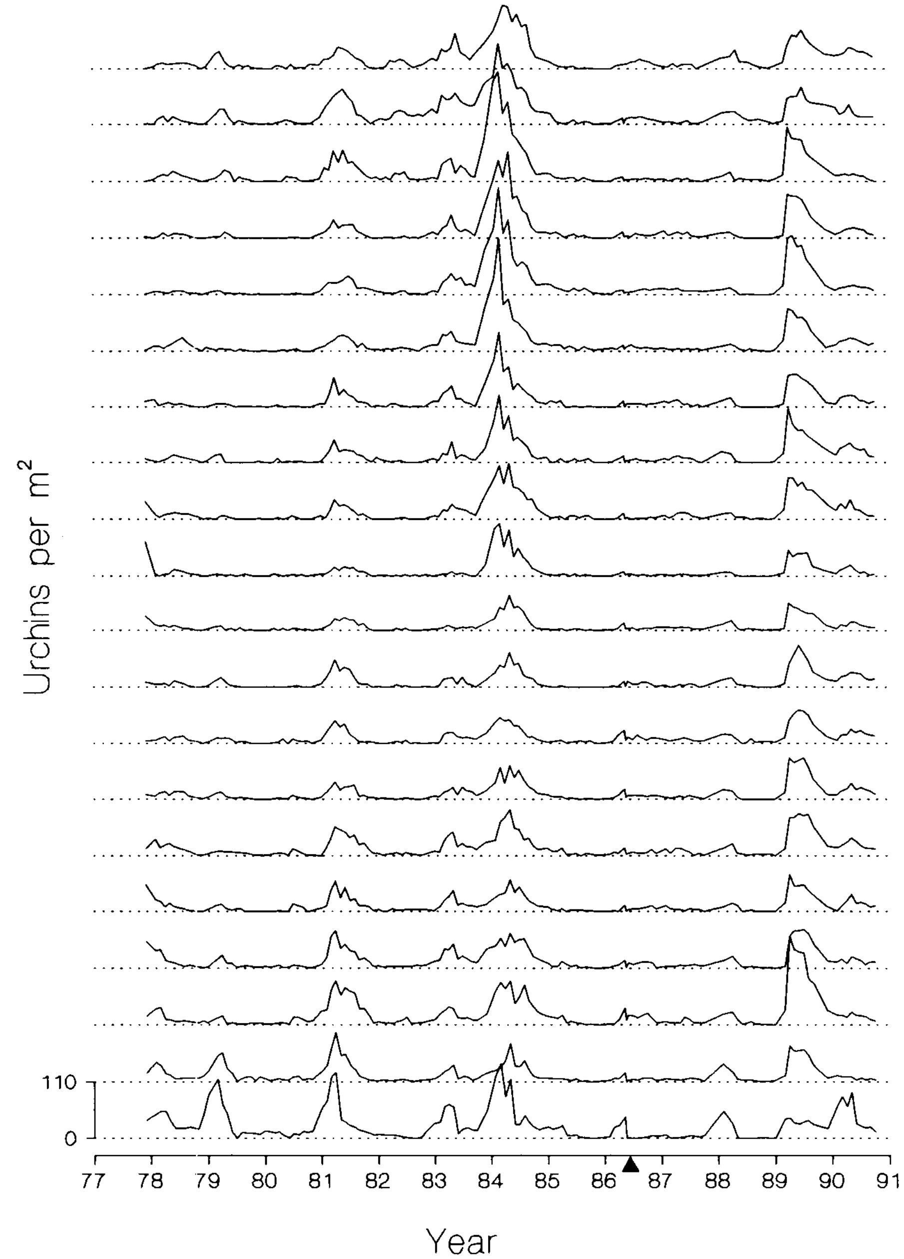


Fig. 4.36 Time series of changes in the populations of the sea urchin *Echinometra lucunter* in each fixed 1-m^2 quadrat of the reef-edge transect at Punta Galeta from 1977 through 1990. The transect was oblique to the shoreline, and the quadrat at the bottom of the graph was nearest the seaward edge of the reef flat. The *solid triangle* marks the arrival of the first oil slicks at Punta Galeta in May 1986. Note that recruitment events were generally synchronized among quadrats and that recruitment in the seaward quadrat accompanied overall recruitment events, except immediately after the spill.

distributions of urchins along the transects in 1986 and other years did not show shifts that would correspond to population migrations in and out of the transects (Fig. 4.37).

Postspill Comparisons of Sea Urchin Populations Among Sites

The graph comparing the total populations of all species of sea urchins among the four sites summarizes the most apparent difference among them: all species of sea urchins were consistently rare at Largo Remo, the oiled site nearest the refinery (Fig. 4.38). During the urchin censuses at this site, no recruitment of juvenile urchins was observed. This absence of sea urchins did not exist at the other oiled site (Punta Galeta), nor at the two unoiled sites (María Soto Arriba and María Soto Abajo).

With the possible exception of *Lytechinus variegatus*, the multi-site surveys showed no apparent additional effects of oil on sea urchin populations (Fig. 4.39*A*-*G*). In the reef-edge transects, *Lytechinus variegatus* remained near zero at Punta Galeta for about 3 yr after the oil spill, but this species was seasonally abundant at the unoiled sites (Fig. 4.39*A*). However, multi-year declines of this species have also occurred before in the reef-edge transect at Punta Galeta (Fig. 4.32*A*). In the *Thalassia* and rubble habitats, no exceptional differences existed in the abundance of *Lytechinus variegatus* between Punta Galeta and the two unoiled sites (Fig. 4.39*A*).

4.4.3.3 Summary and Discussion of Results of Sea Urchin Censuses

The detailed baseline data for sea urchin populations at Punta Galeta showed that after the oil spill the abundances of *Lytechinus variegatus*, *Eucidaris tribuloides*, *Tripneustes ventricosus*, and *Diadema antillarum* were much lower than the overall prespill mean. However, for each of these species, the low postspill abundances could be explained by (1) normal seasonal reductions that occur each year at the time when the spill also occurred (Fig. 4.31*A*-*F*) or by long-term trends that were in progress before the 1986 spill. Abundances of the two *Echinometra* species, *E. lucunter* and *E. viridis,* did show detectable decreases in the reef-edge transect (Fig. 4.35), the transect nearest the heavy deposition of oil at the seaward edge of the reef flat. Anomalous reductions of recruitment after the spill also occurred in the seaward portion of the transect. However, such changes in recruitment did not occur after mortality caused by the extremely low water levels of 1988 (Fig. 4.36), which suggests some persistent factor on the substratum, not heavy mortality *per se*, could have affected recruitment after the oil spill. Such a factor (or factors) could also have been responsible for the very low abundances of sea urchins in all surveys at Largo Remo. This possibility is covered in more detail in the general discussion below (Sect. 4.5).

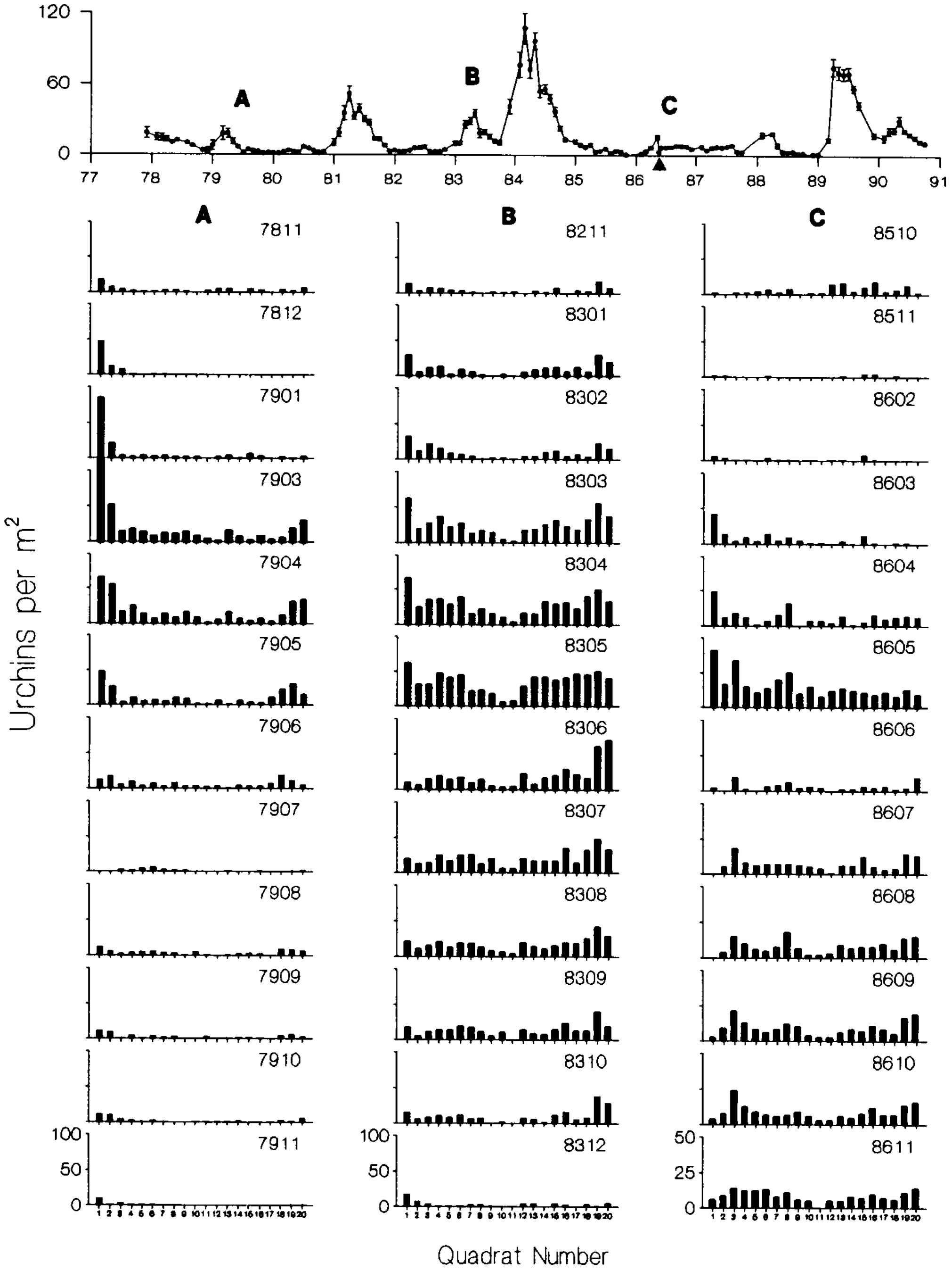


Fig. 4.37 Examination of spatial distributions of *Echinometra lucunter* populations for evidence of migrations. Data are from the reef-edge transect at Punta Galeta (*quadrat 1* is nearest the ocean). The periods of change chosen for comparison are marked as *A*, *B*, and *C* on the time-series graph (*top*). The spatial distributions for each survey date during these changes are shown as the histograms below each letter. The date of each survey is shown as a four-digit year-month. The *solid triangle* marks the arrival of the first oil slicks at Punta Galeta in May 1986.

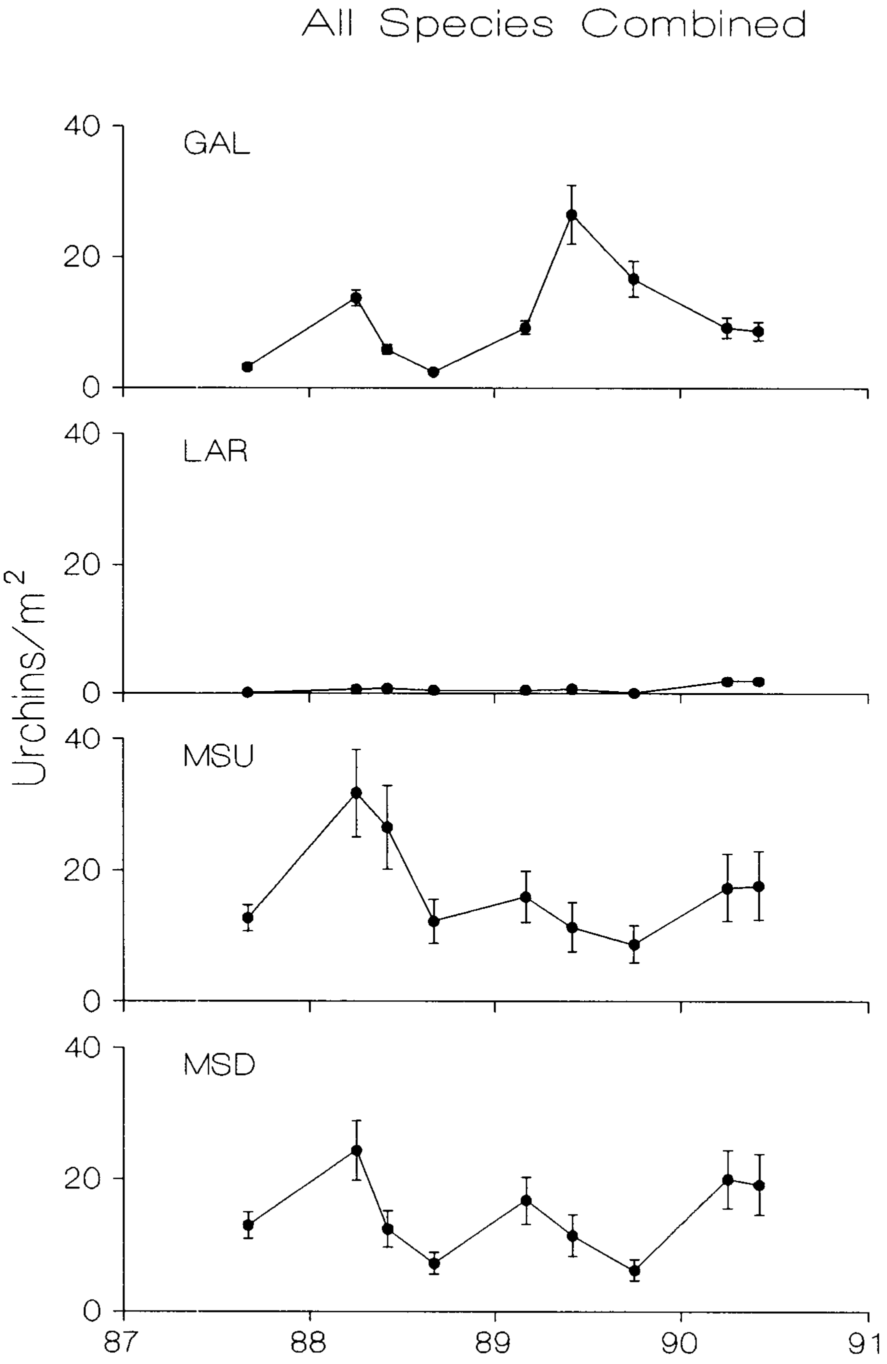


Fig. 4.38 Intersite comparison of total populations of all species of sea urchins in all transects. The data are means and standard errors. *GAL* = Punta Galeta, *LAR* = Largo Remo, *MSU* = María Soto Arriba, *MSD* = María Soto Abajo.

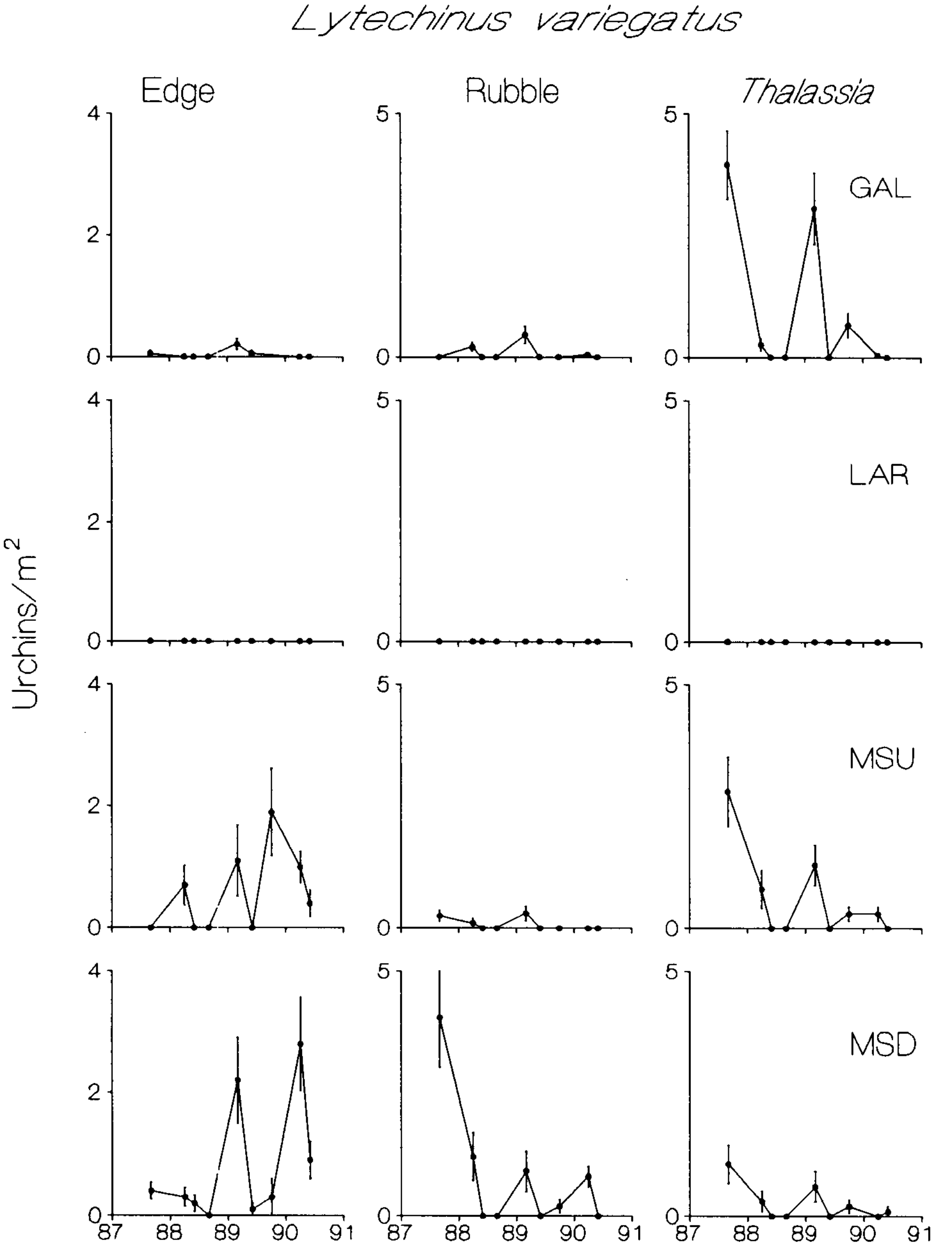


Fig. 4.39 Intersite comparison of populations for each species of sea urchin in each transect. *A. Lytechinus variegatus.* The data are means and standard errors. Transect names are shown at *top* (*Edge* = reef edge); *GAL* = Punta Galeta, *LAR* = Largo Remo, *MSU* = María Soto Arriba, *MSD* = María Soto Abajo.

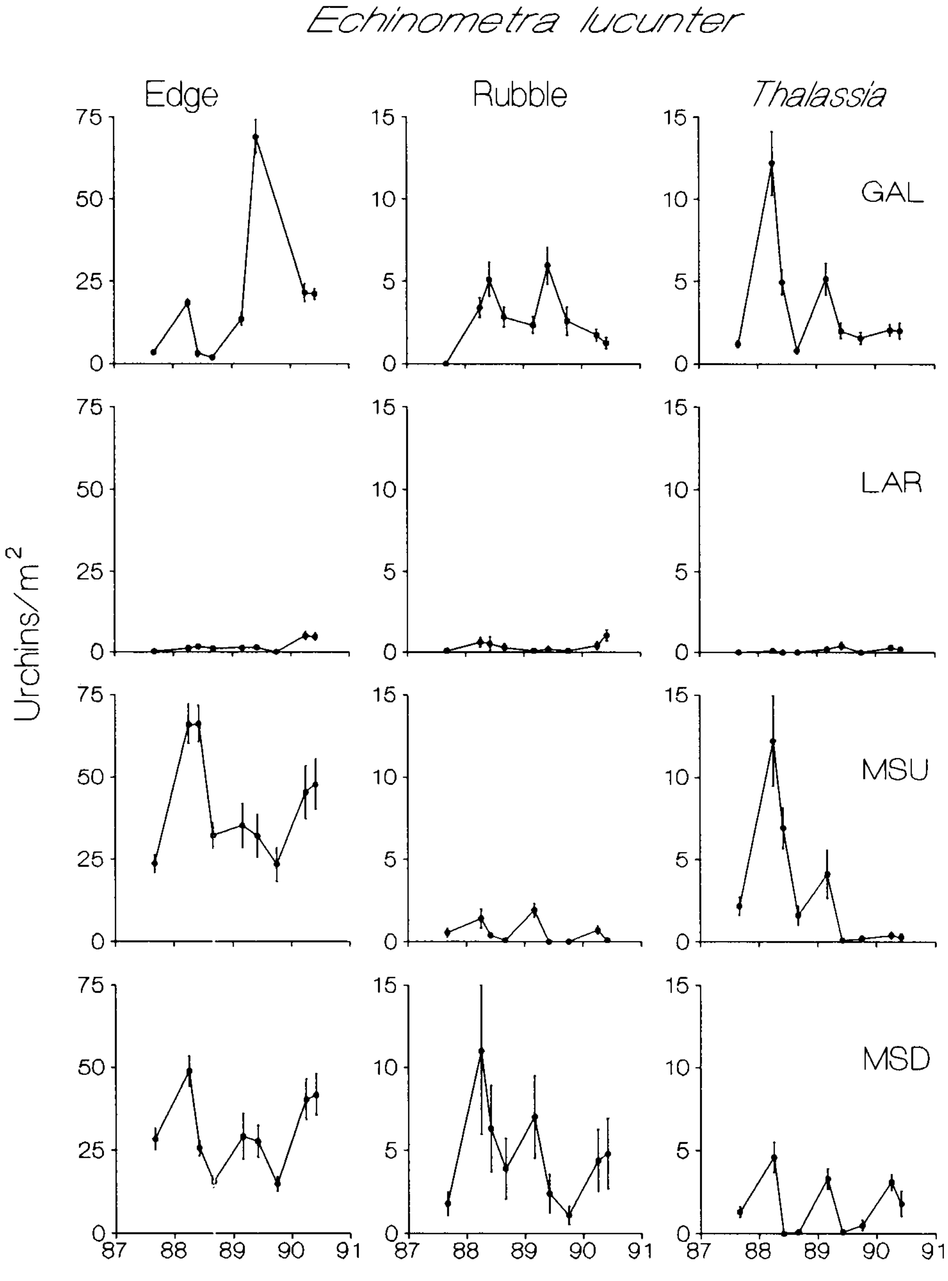


Fig. 4.39 **Intersite comparison of populations for each species of sea urchin in each transect (continued).** ***B. Echinometra lucunter.*** **See Figure 4.39*A* for explanation of graph format.**

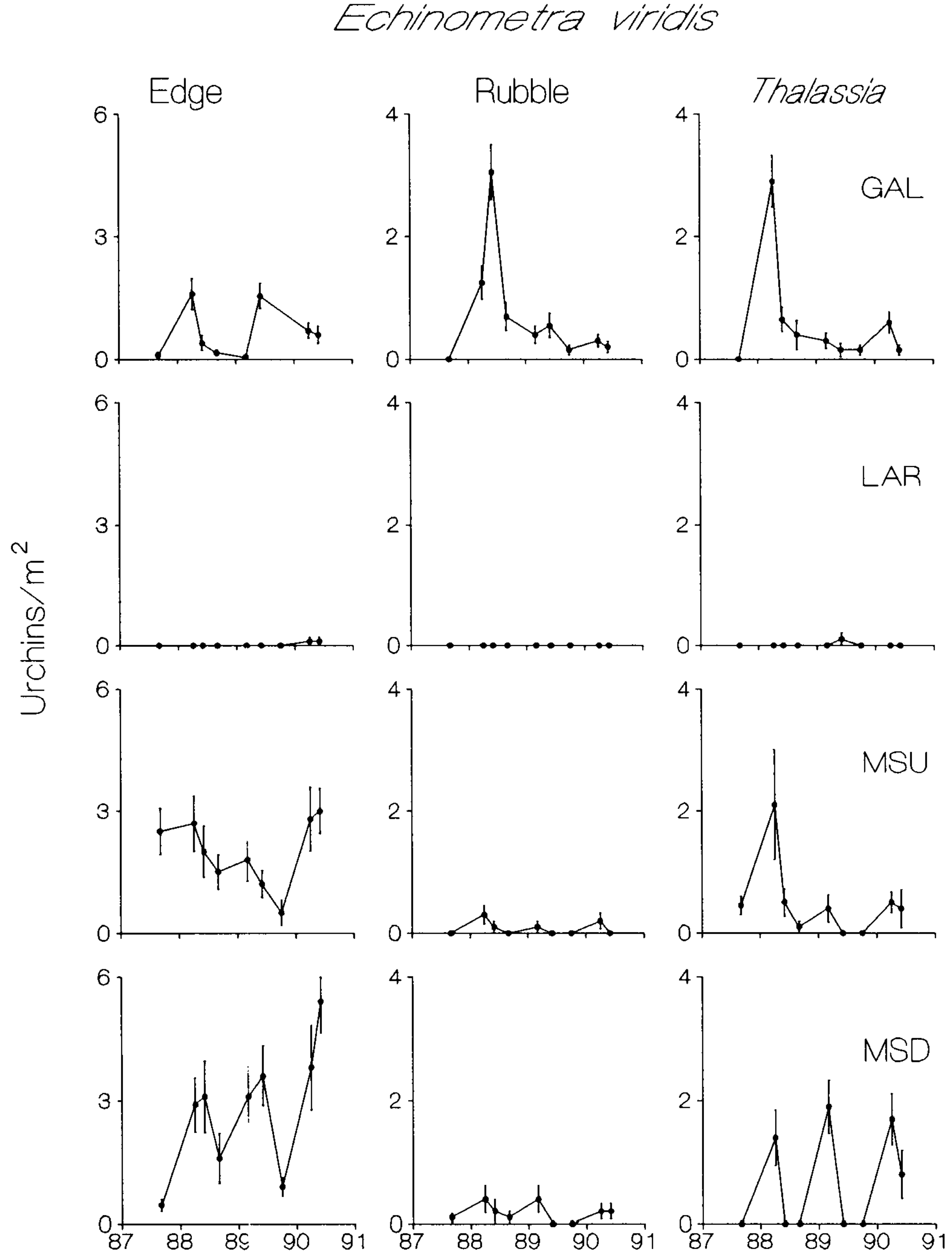


Fig. 4.39 Intersite comparison of populations for each species of sea urchin in each transect (continued). *C. Echinometra viridis.* See Figure 4.39*A* for explanation of graph format.

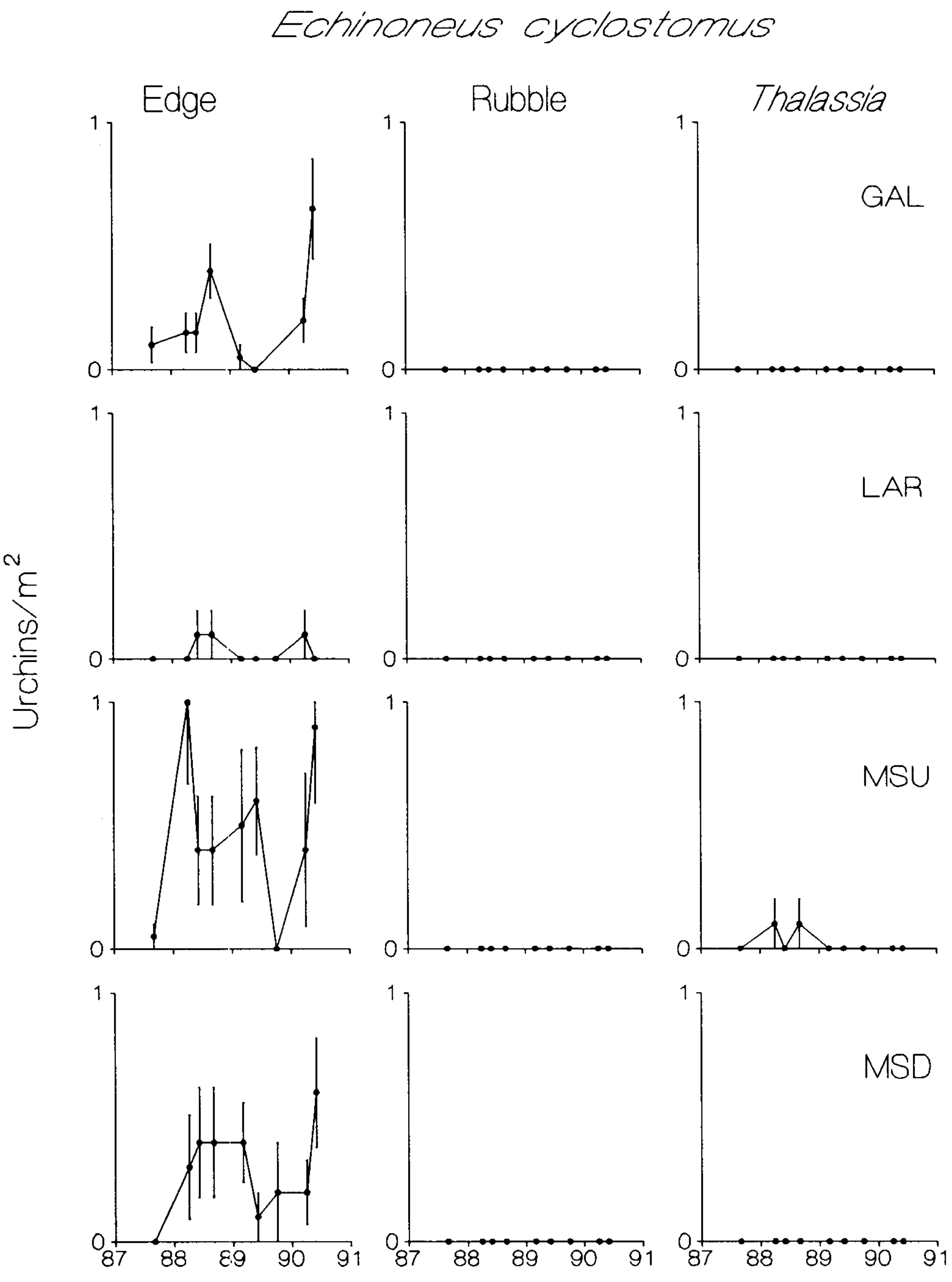


Fig. 4.39 **Intersite comparison of populations for each species of sea urchin in each transect (continued).** ***D. Echinoneus cyclostomus.*** **See Figure 4.39*A* for explanation of graph format.**

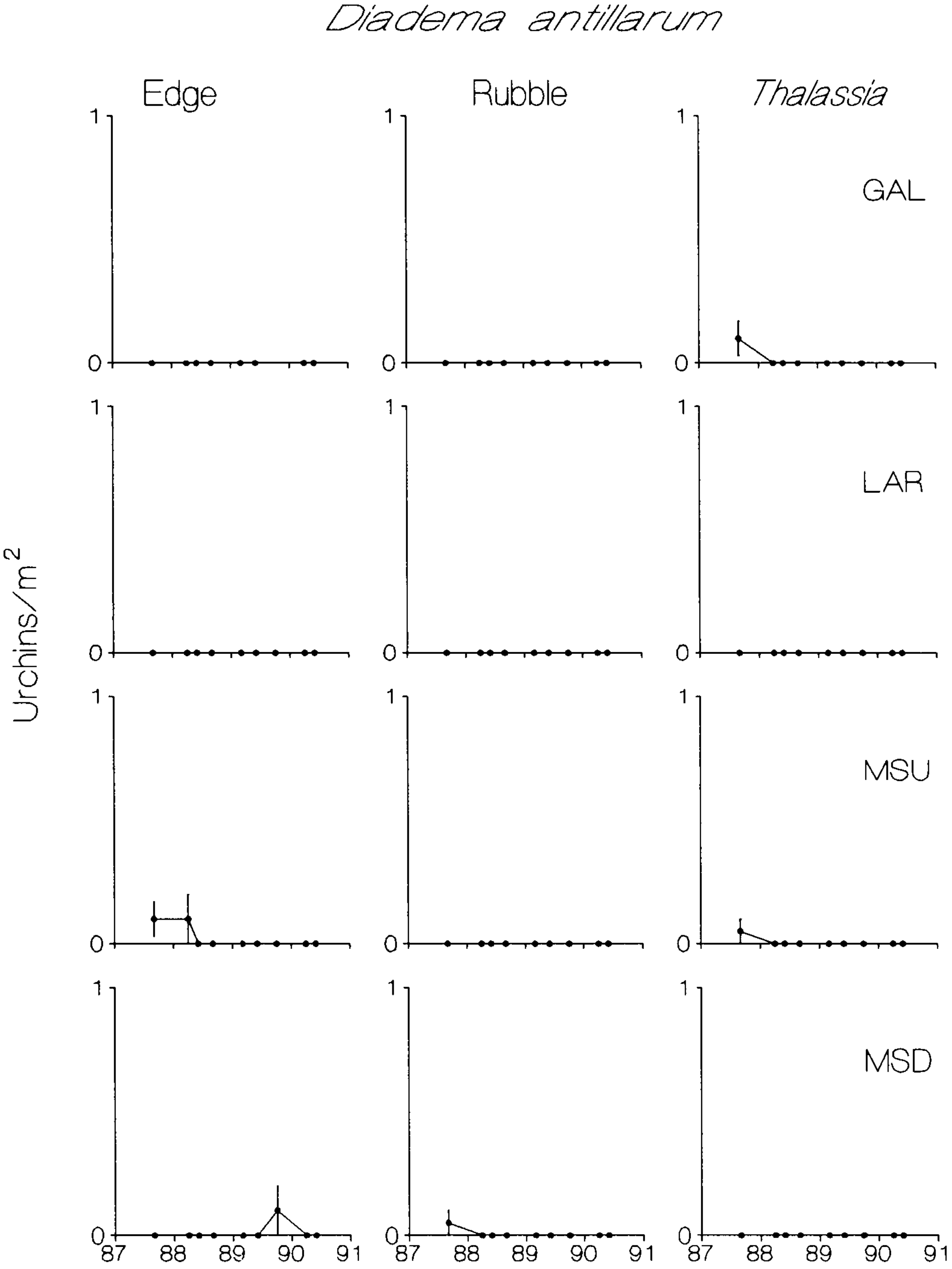


Fig. 4.39 **Intersite comparison of populations for each species of sea urchin in each transect (continued).** ***E. Diadema antillarum.*** **See Figure 4.39*A* for explanation of graph format.**

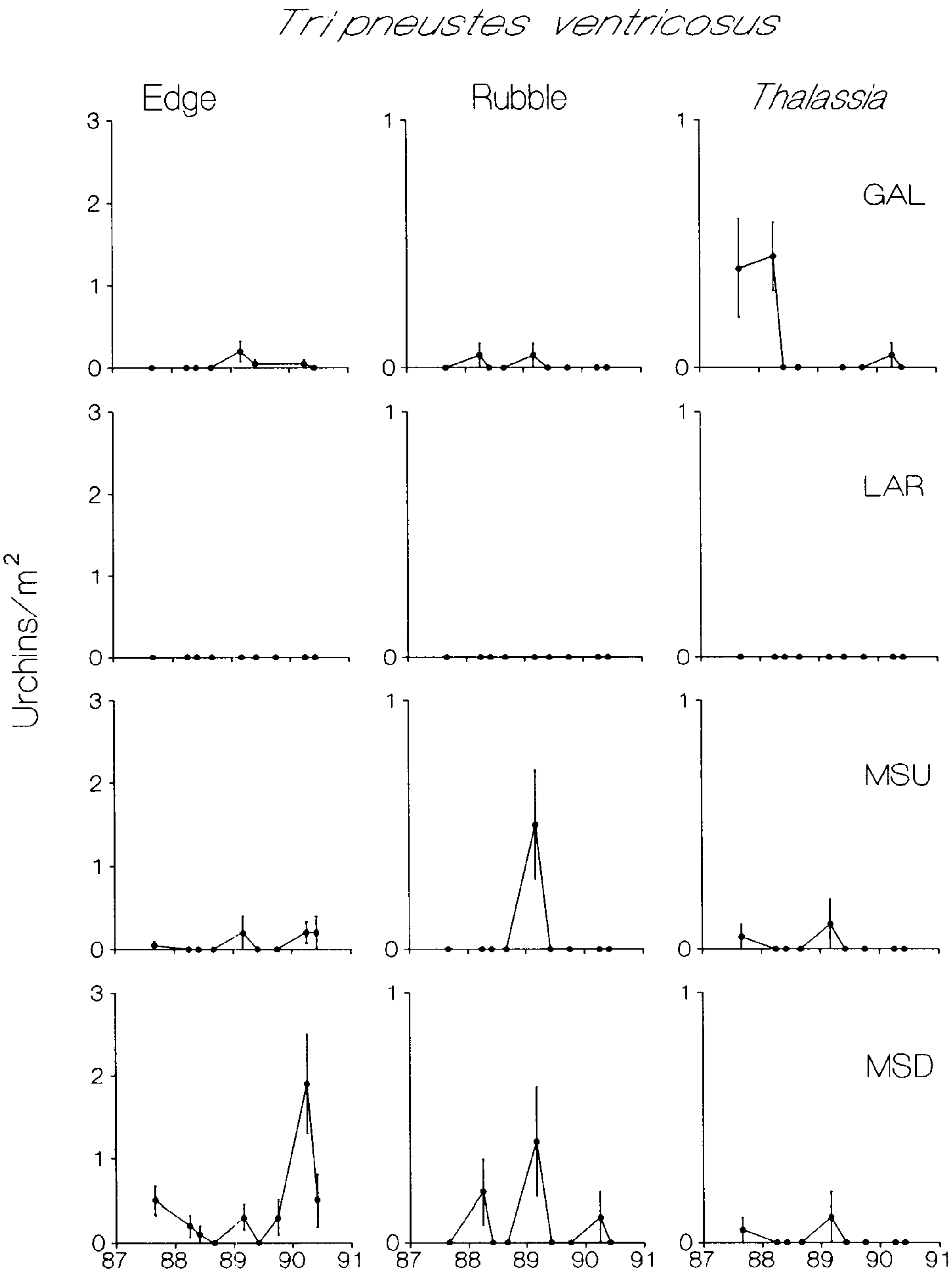


Fig. 4.39 Intersite comparison of populations for each species of sea urchin in each transect (continued). *F. Tripneustes ventricosus.* See Figure 4.39*A* for explanation of graph format.

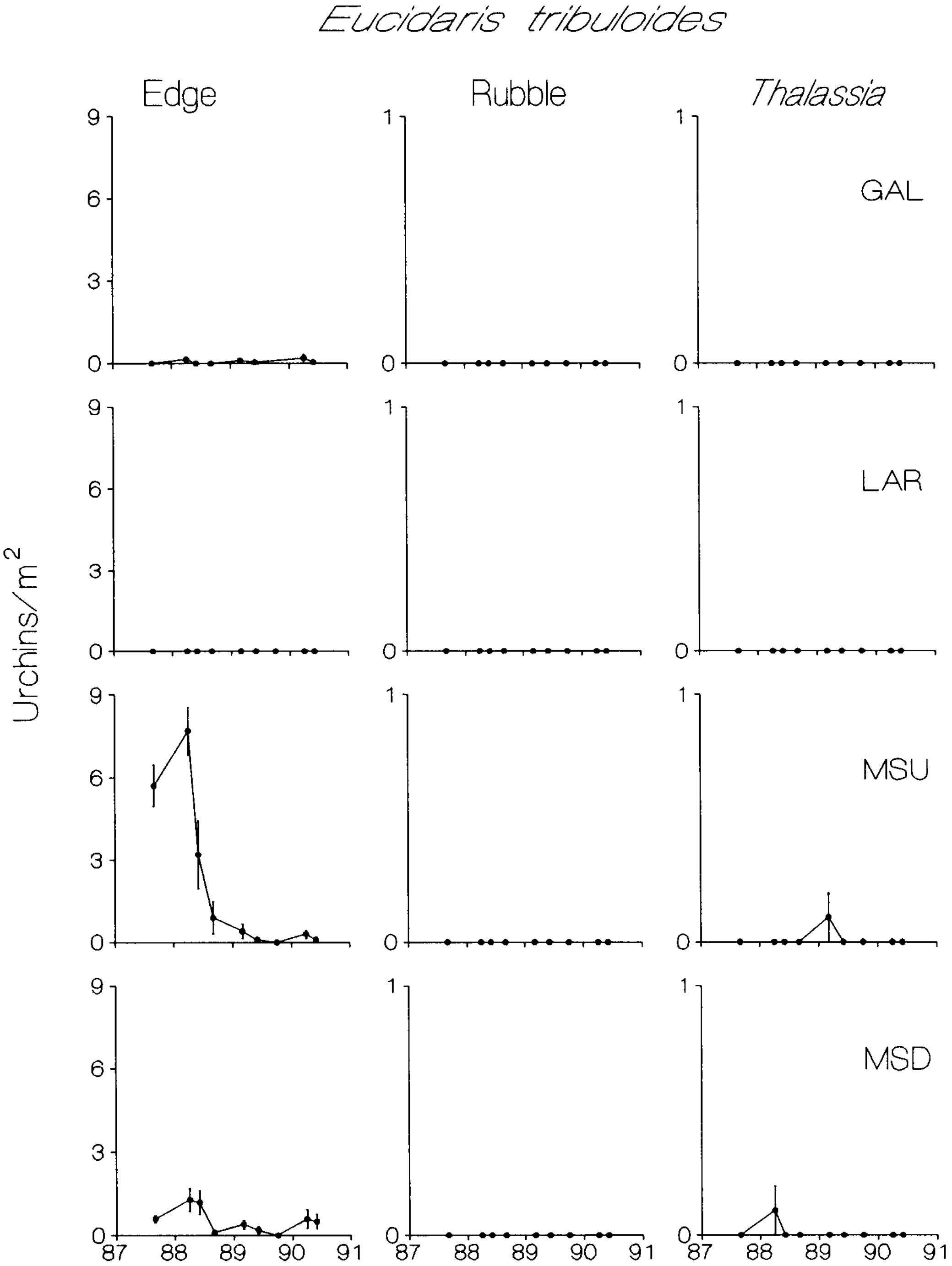


Fig. 4.39 Intersite comparison of populations for each species of sea urchin in each transect (continued). *G. Eucidaris tribuloides.* See Figure 4.39*A* for explanation of graph format.

4.4.4 Infaunal Populations of the *Laurencia papillosa* Turf: Postspill Comparisons Among Sites

4.4.4.1 Methods

To detect loss of infauna caused by the 1986 oil spill, infaunal populations were sampled at the oiled and unoiled sites after the spill. This is the only component study of the reef flat studies with no matched prespill data.

Infaunal sampling was designed to compare the following aspects of the infauna of *Laurencia* turf between the oiled and unoiled sites: (1) the types, abundances, and zonal distribution of the infauna, and (2) changes in these aspects of infaunal populations with time after the oil spill.

Sampling was performed approximately every 6 mo in the same zones of *Laurencia papillosa* surveyed as part of the monitoring of the sessile biota. Core samples were taken using a hand sledge to drive a stainless-steel pipe 5.2 cm in diameter (21.2 cm^2 area) through the algal turf into the hard substratum of the reef flat. To assure a complete core of the algal layer, cores were rejected if a plug of hard substratum was not present at the base of the core. The cores were located along five transect lines perpendicular to the shoreline (Fig. 4.40). The transects were randomly placed for each sampling, and the positions of the transects were recorded to avoid repeated sampling at the same location later. Samples were taken in triplicate clusters, repeated at four positions on each transect. The first position was at the seaward edge of the *L. papillosa* zone. The remaining positions were 2, 4, and 6 m from the seaward edge of the *L. papillosa* zone going in a landward direction (Fig. 4.40). Each core sample was taken in the densest portion of the *L. papillosa* turf within the same 50 x 50 cm quadrat, maximizing the distance among samples. If the algal turf did not exist within the designated location, the position was moved laterally (parallel to the shoreline) to the nearest area of algal turf. Where the algal zone was less than 6 m wide, a landward set of samples was not taken. To avoid interference, no samples were taken near the permanent transects used to monitor sessile biota and sea urchin populations. The samples were sealed in plastic bags with labels showing the position, site, and date of collection. The samples were preserved on the day of collection; some samples were initially frozen. All samples were fixed in 5% formalin buffered with sodium borate. After sorting and counting, the infauna were archived in 75% ethanol.

Two samples from each triplicate were processed per site for each sampling date, making a total of 40 samples processed for each site on each date. In the following presentation of results, all population densities are expressed as animals per unit area of algal turf. As will be discussed later, the total population densities per unit area of reef flat is a product of both the densities in the algal turf and the total cover of algal turf.

The samples were stained with rose bengal to assist distinguishing animals from debris. In the laboratory algae, substratum, and infauna were separated. A

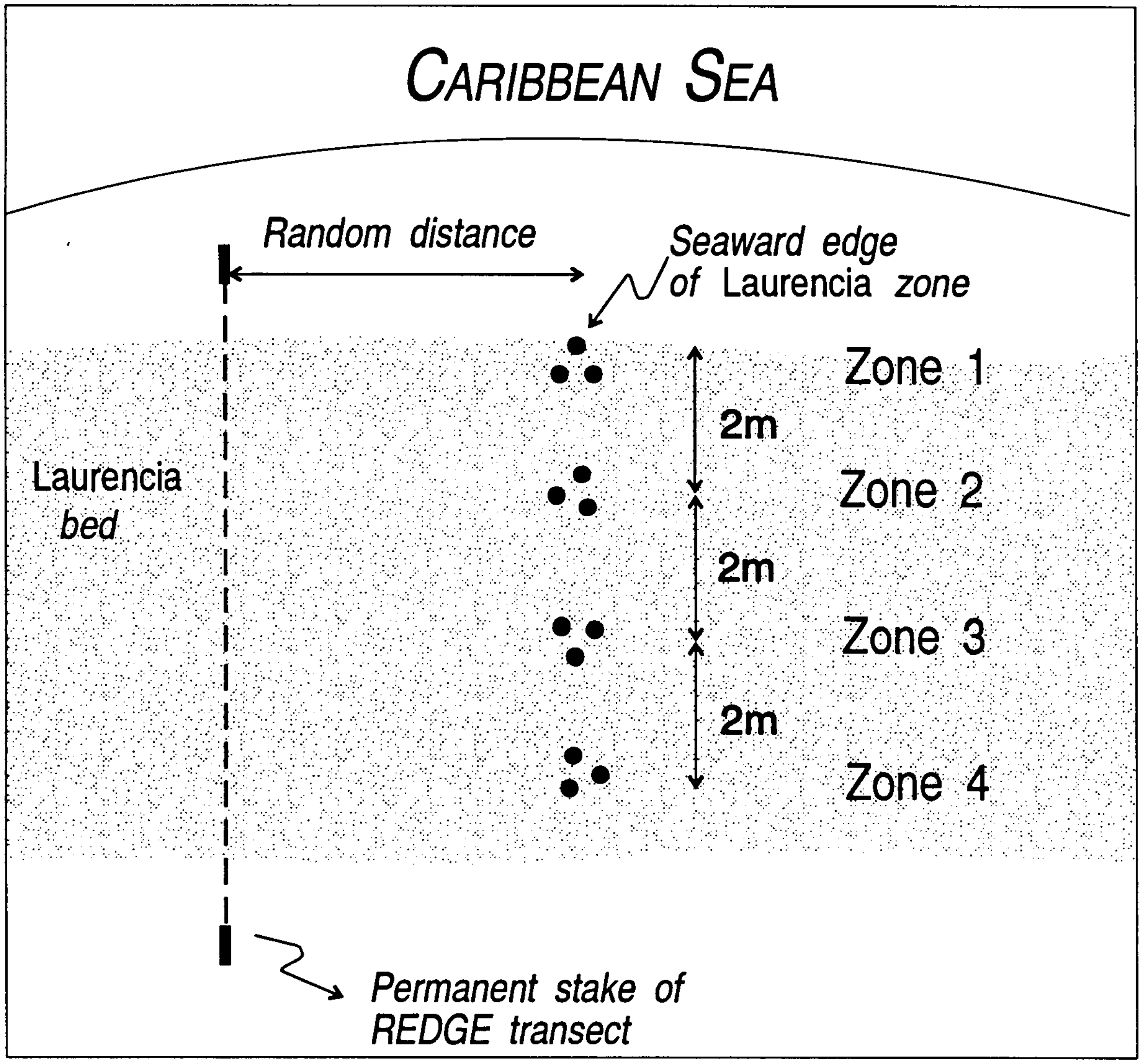


Fig. 4.40 Sampling scheme for the infauna of *Laurencia papillosa* beds. Samples were taken along a transect placed at a random distance from the permanent REDGE transects. The samples were taken in clusters of three; each cluster was 2 m apart, with the first cluster at the seaward edge of the *Laurencia* bed. In the data, the positions of the clusters are referred to as *zones 1* to *4*, as shown in the diagram. See text for further explanation.

stream of running water was used to wash the infauna from the sample, and any remaining animals were picked from the algal turf during examination under a dissecting microscope. The infauna processed were large enough to be retained by a sieve of 0.5-mm plankton netting.

The type of substratum and species of predominant macroalgae in the turf were recorded, and these materials were dried and weighed. At this first level of processing, infauna were separated into major taxonomic groups (usually Order). Except for polychaetes, all invertebrates were counted and their length measured. Because polychaetes tended to be in fragments and were elastic, no linear measurement proved satisfactory to quantify their abundance. Instead, only specimens with heads were counted and the width of the first segment behind the head was used as a measure of size. Although records were kept of all infauna, the data reported here are for the mobile infauna only. Anemones, zoanthids, sponges, and other sessile invertebrates are not included in the data from the infauna core samples, but are included in the more comprehensive biotic cover data of the ZONE, CONSURV, and REDGE surveys, described above.

To reduce possible systematic biases that could be contributed by the processors of samples, all processors shared the effort of processing the samples for all sites and dates. In addition, samples were processed in two rounds: one sample of every triplicate was processed for all dates and sites available before proceeding to the second sample of the triplicate.

Counts and length measurements were rechecked by repeated processing of random samples and by statistical comparison of data according to processor. In this screening, one of the three primary processors was found to have measured polychaetes and crustaceans in a manner that produced a slight, but statistically significant, difference in her set of samples. The assortment of samples among processors, as described above, made it unlikely that this difference would bias the results of the oiled-unoiled comparisons; nonetheless, to have maximum confidence in the data, all polychaetes and crustaceans measured by this processor were remeasured and recounted. The data were then reanalyzed for biases and no subsequent indications of bias were found.

Populations of infauna in beds of the alga *Laurencia papillosa* were sampled on six dates after the oil spill, from 1987 to 1990. The sampling underway in late 1989 was interrupted by warfare, with only one oiled and one unoiled site completed; therefore, a complete set of samples for all sites was taken again in April 1990. To maintain equal sample sizes for all sites, the following analyses were limited to the five complete sampling periods and did not include the partial sampling from late 1989.

4.4.4.2 Results and Discussion of Infauna Surveys

The total count of infauna averaged approximately 11 animals per cm^2 of algal turf for all 800 samples combined (all sites and dates). Isopods, amphipods, tanaids, gastropods, and polychaetes comprised most of the infauna. In all these major groups the highest abundances of animals were in the smallest size category (0.5 to 0.99 mm; Fig. 4.41*A-F*). Isopods and amphipods tended to be more abundant in zone 1, the zone nearest the sea; the other groups showed little or no patterns of zonation (Fig. 4.41*A-F*). Polychaetes and crustaceans as a group (including tanaids,

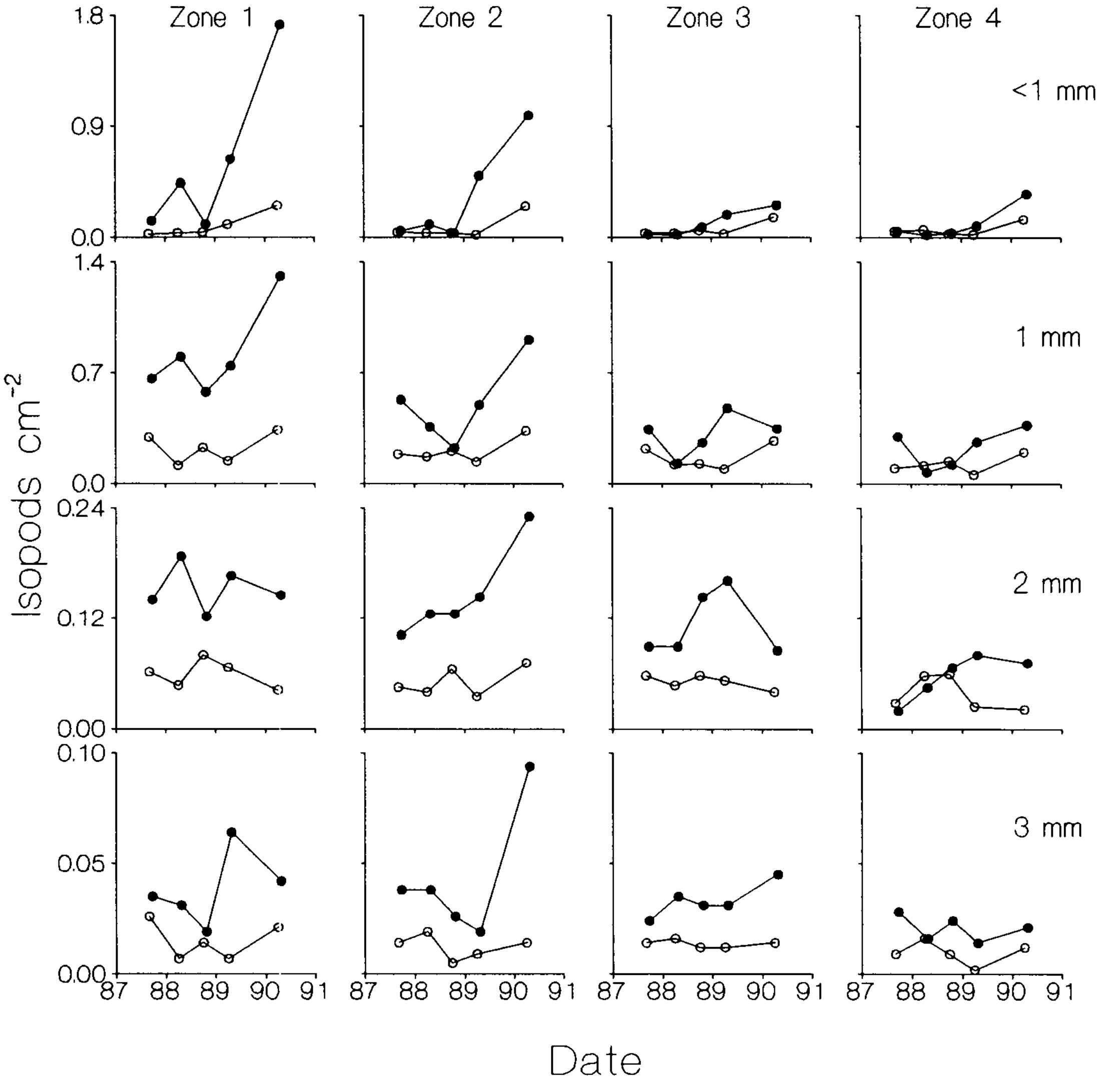


Fig. 4.41 Populations of infauna of *Laurencia papillosa* turf compared by oil condition of site, size class, and zone on the reef flats. *A*. Isopods. The *solid circles* are means for the two oiled sites (Largo Remo and Punta Galeta); the *open circles* are means for the two unoiled sites (María Soto Arriba and María Soto Abajo). Zones are listed at *top*, size classes are shown at *right*. Note that scales of abundance (vertical axes) differ among size classes to better show changes over time.

isopods, and amphipods) tended to increase with time at all sites, peaking in the 1989 or 1990 samples, as summarized in Figure 4.42. Gastropods only showed this pattern at the oiled sites (Fig. 4.42).

When the sampling began approximately 15 mo after the oil spill, tanaids were the only major group to show clearly lower populations at the oiled sites. In

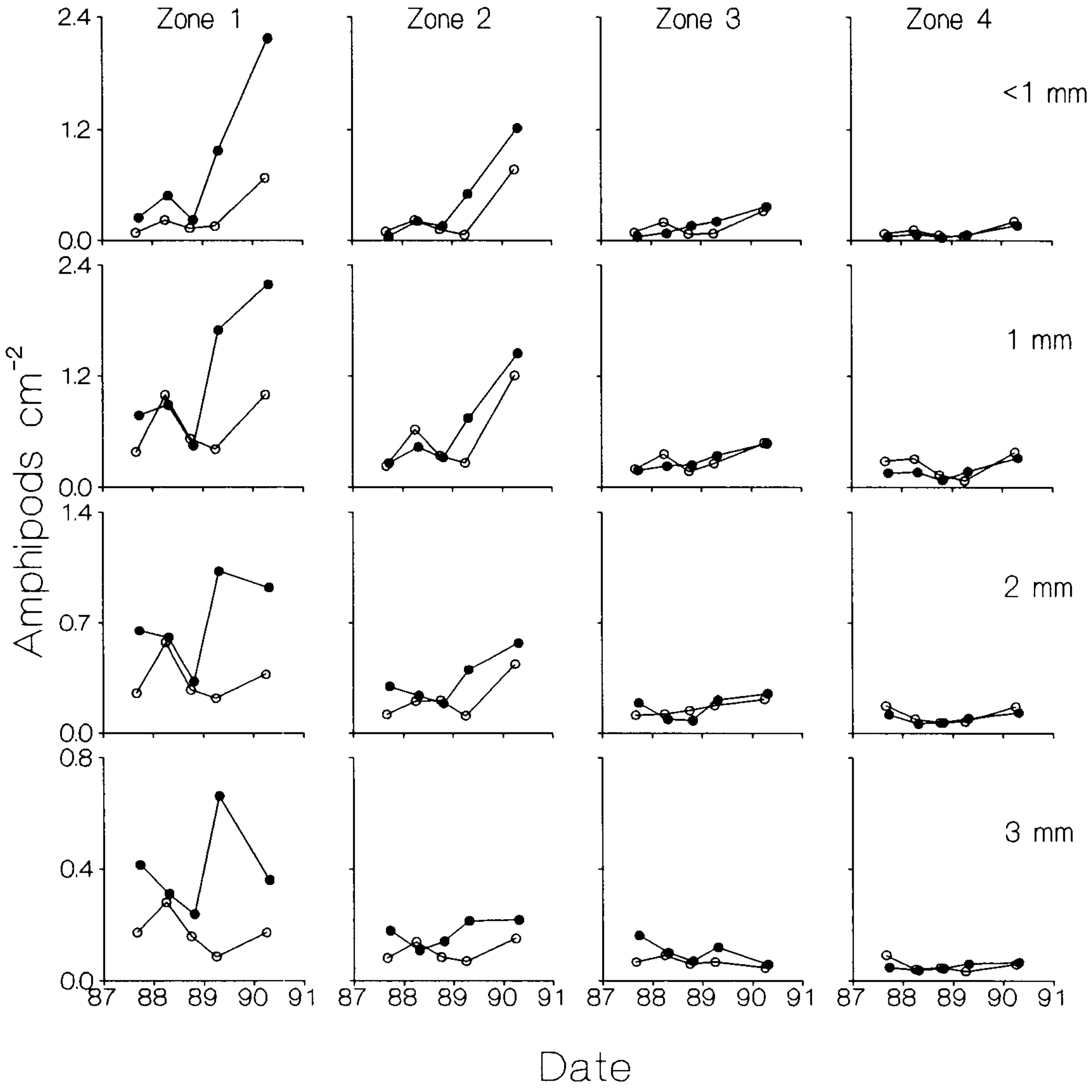


Fig. 4.41 **Populations of infauna of *Laurencia papillosa* turf compared by oil condition of site, size class, and zone on the reef flats (continued).** ***B.*** **Amphipods. See Figure 4.41*A* for explanation of graph format.**

September 1987 populations of tanaids in most size classes and zones were near zero at the two oiled sites (Figs. 4.41*C*, 4.42). Regeneration of tanaid populations was first recorded at the Largo Remo site in late 1988, but was not recorded at Punta Galeta until the last set of samples (April 1990). In the April 1990 sampling, tanaids were much less abundant at the oiled sites than the unoiled sites, even though tanaids also exhibited an extraordinary drop in abundance at the "unoiled" sites

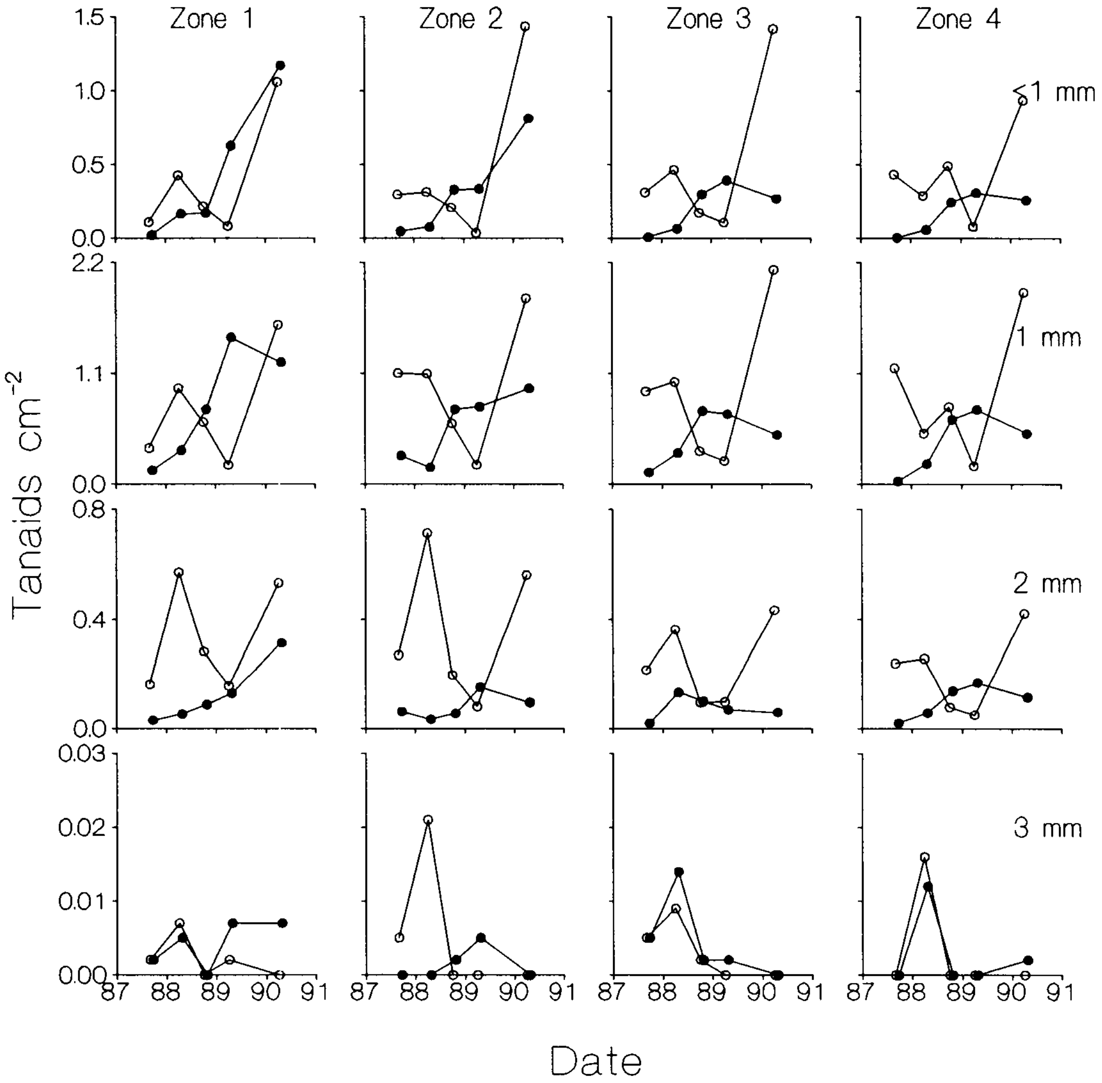


Fig. 4.41 Populations of infauna of *Laurencia papillosa* turf compared by oil condition of site, size class, and zone on the reef flats (continued). *C.* Tanaids. See Figure 4.41*A* for explanation of graph format.

between October 1988 and April 1989. This drop corresponded to the appearance of fresh heavy fuel oil in sediment samples taken at Dos Marias reef, approximately 7 km upwind from the "unoiled" sites (K. Burns, pers. comm., Sect. 3.5.1).

The other two major groups of infaunal crustaceans (isopods and amphipods) did not show these patterns over time, and by 1990 they were more abundant at the oiled sites than the unoiled sites. As a group, gastropods tended to be more abundant at the oiled sites than the unoiled sites at all sampling dates (Fig. 4.42).

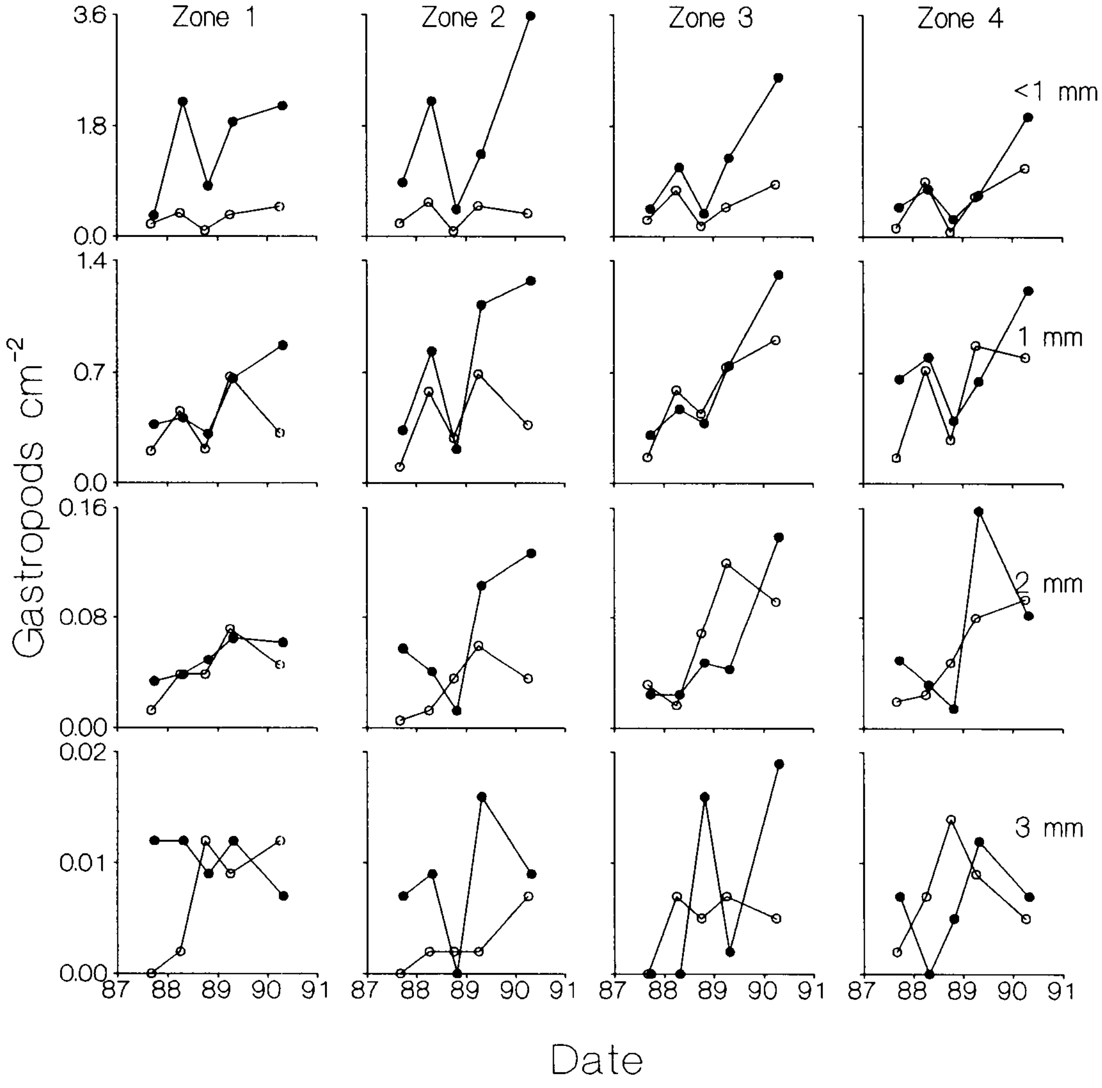


Fig. 4.41 Populations of infauna of *Laurencia papillosa* turf compared by oil condition of site, size class, and zone on the reef flats (continued). *D.* Gastropods. See Figure 4.41*A* for explanation of graph format.

Population densities of infauna were compared with the percent cover of algal turf to test the hypothesis that infauna concentrated in the surviving algal turf when turf covers were low. In Figure 4.43 infaunal counts per cm^2 of algal turf were plotted against the cover of algal turf. In these plots each point represents the mean density of the infaunal type in a given zone, at a given site, on a given date versus the mean percent cover of *Laurencia papillosa* in the same zone, site, and nearest

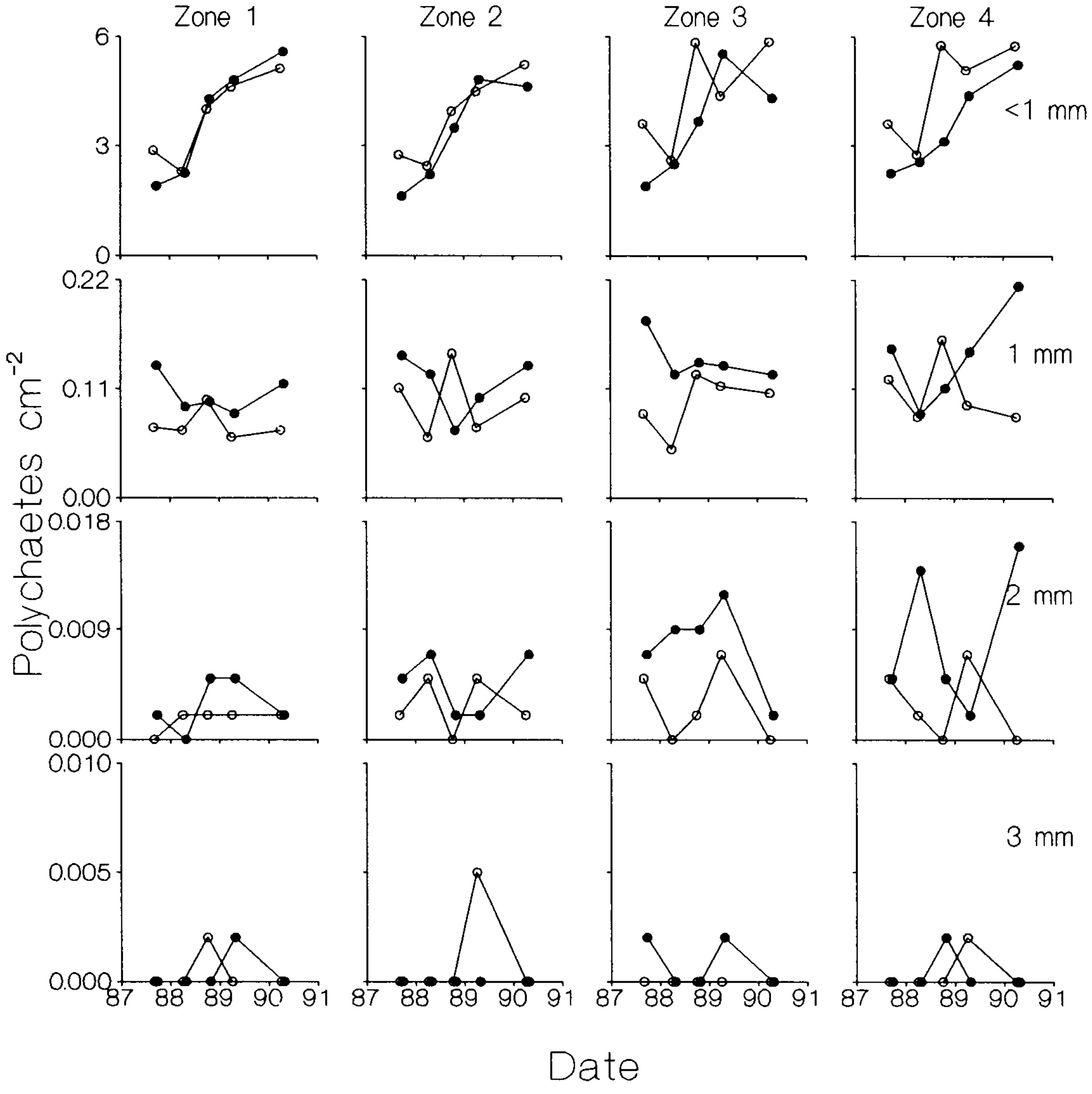


Fig. 4.41 Populations of infauna of *Laurencia papillosa* turf compared by oil condition of site, size class, and zone on the reef flats (continued). *E.* Polychaetes. See Figure 4.41*A* for explanation of graph format.

survey date. (The nearest survey date for cover of sessile biota was usually within a month of the sampling for infaunal populations.) As summarized for the three major groups of infauna, there was no apparent relationship between densities of infauna and percent cover of algal turf; in particular, no sign of infaunal densities increasing at low algal covers existed (Fig. 4.43).

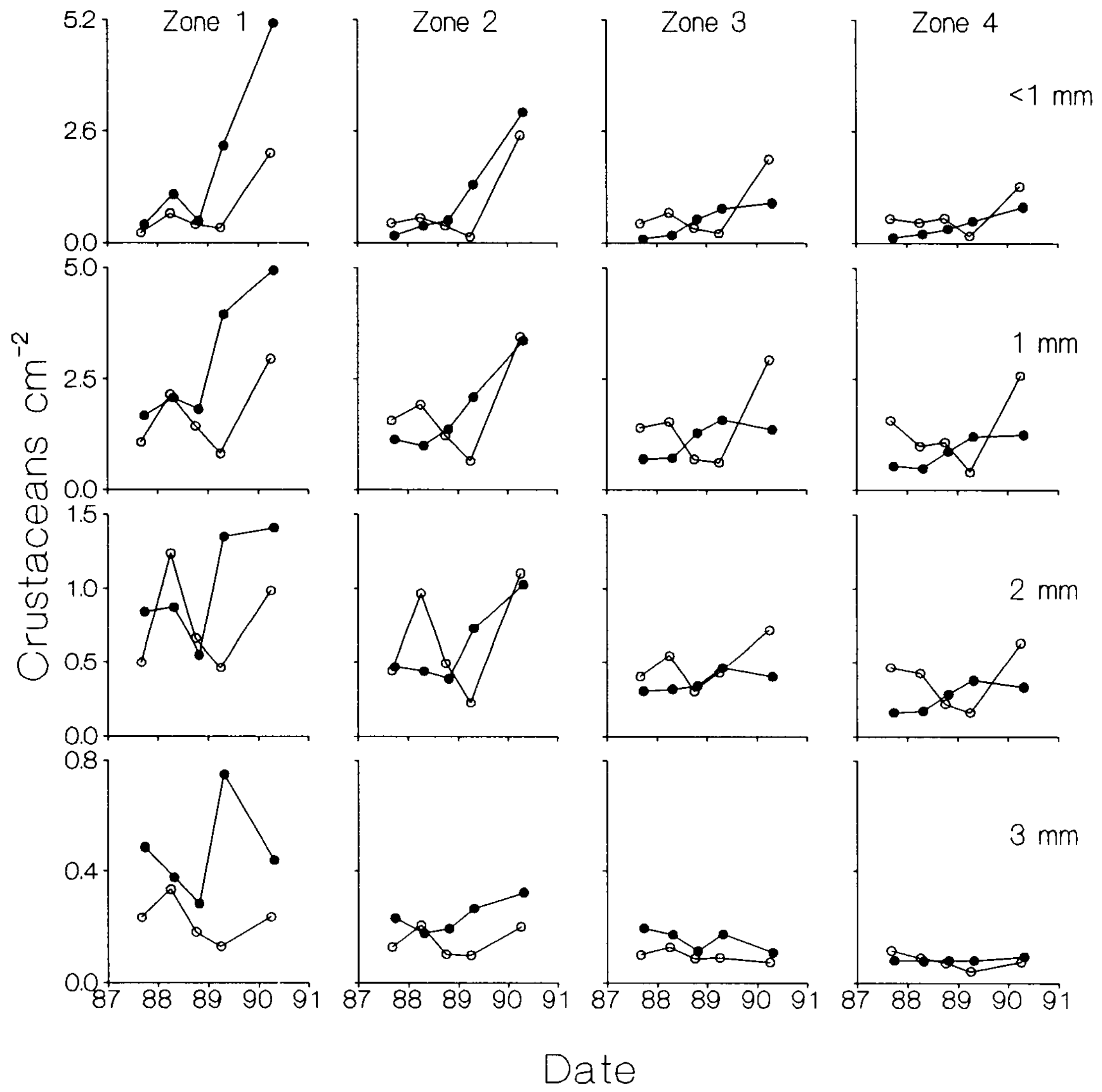


Fig. 4.41 Populations of infauna of *Laurencia papillosa* turf compared by oil condition of site, size class, and zone on the reef flats (continued). *F.* Total crustaceans (isopods, amphipods, tanaids, crabs, and stomatopods). See Figure 4.41*A* for explanation of graph format.

For most groups of infauna, the maximum populations were in the zone closest to the ocean. This zone was awash in oil during the low tides of May 1986 when oil slicks came ashore. Most of the turf of *Laurencia papillosa* in this zone was killed back to remnants of basal tissue, eliminating the normal habitat for infauna. The June 1986 samples of microalgal mats that quickly colonized this zone did not contain any of the amphipods, isopods, tanaids, gastropods, or polychaetes that

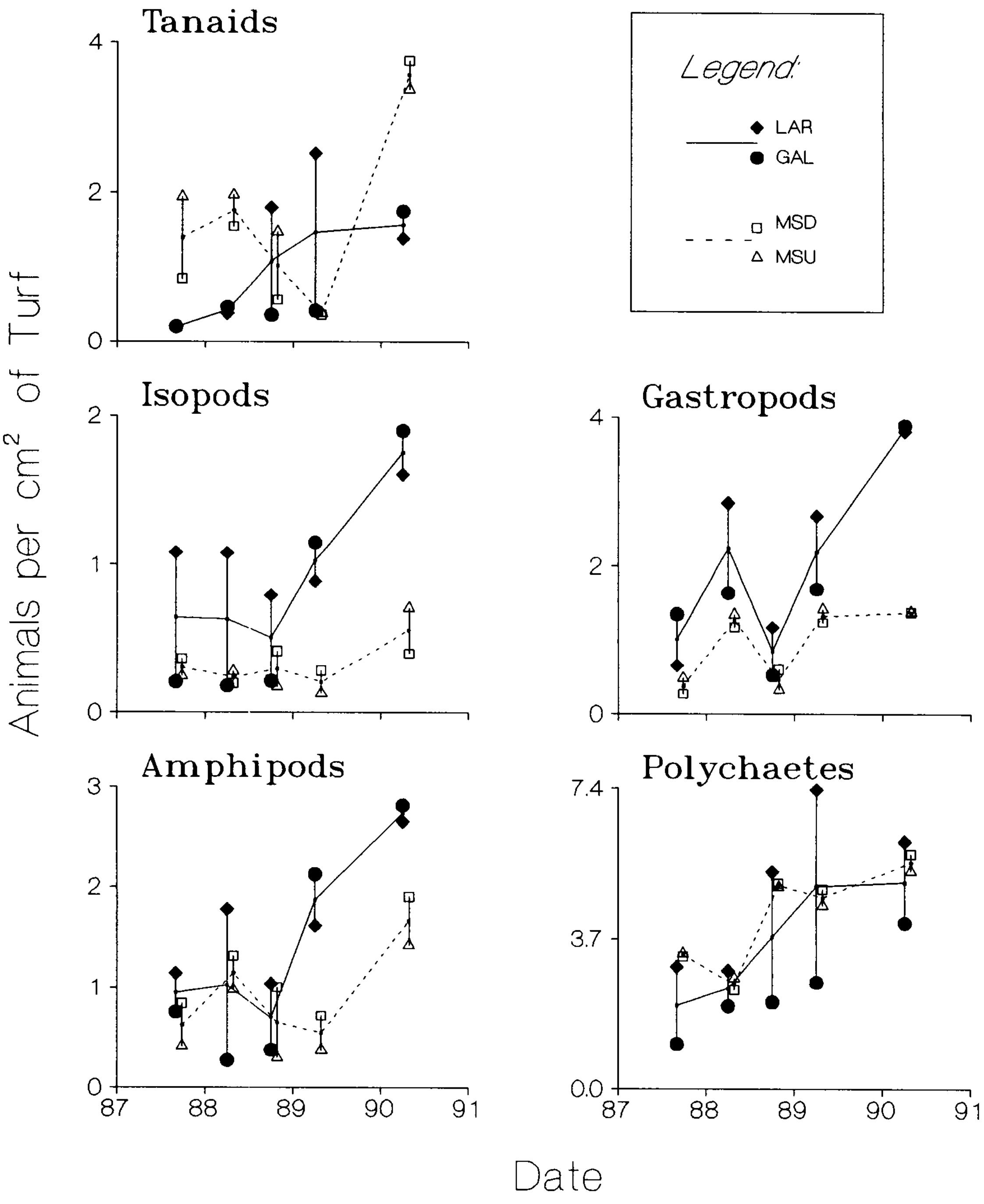


Fig 4.42 Summary comparisons of infaunal populations at oiled and unoiled sites. As shown in the legend at the *upper right*, each symbol represents a site; *solid symbols* are oiled sites, *open symbols* are unoiled sites. *Solid lines* connect mean values for the pair of oiled sites, *dashed lines* connect mean values for the pair of unoiled sites.

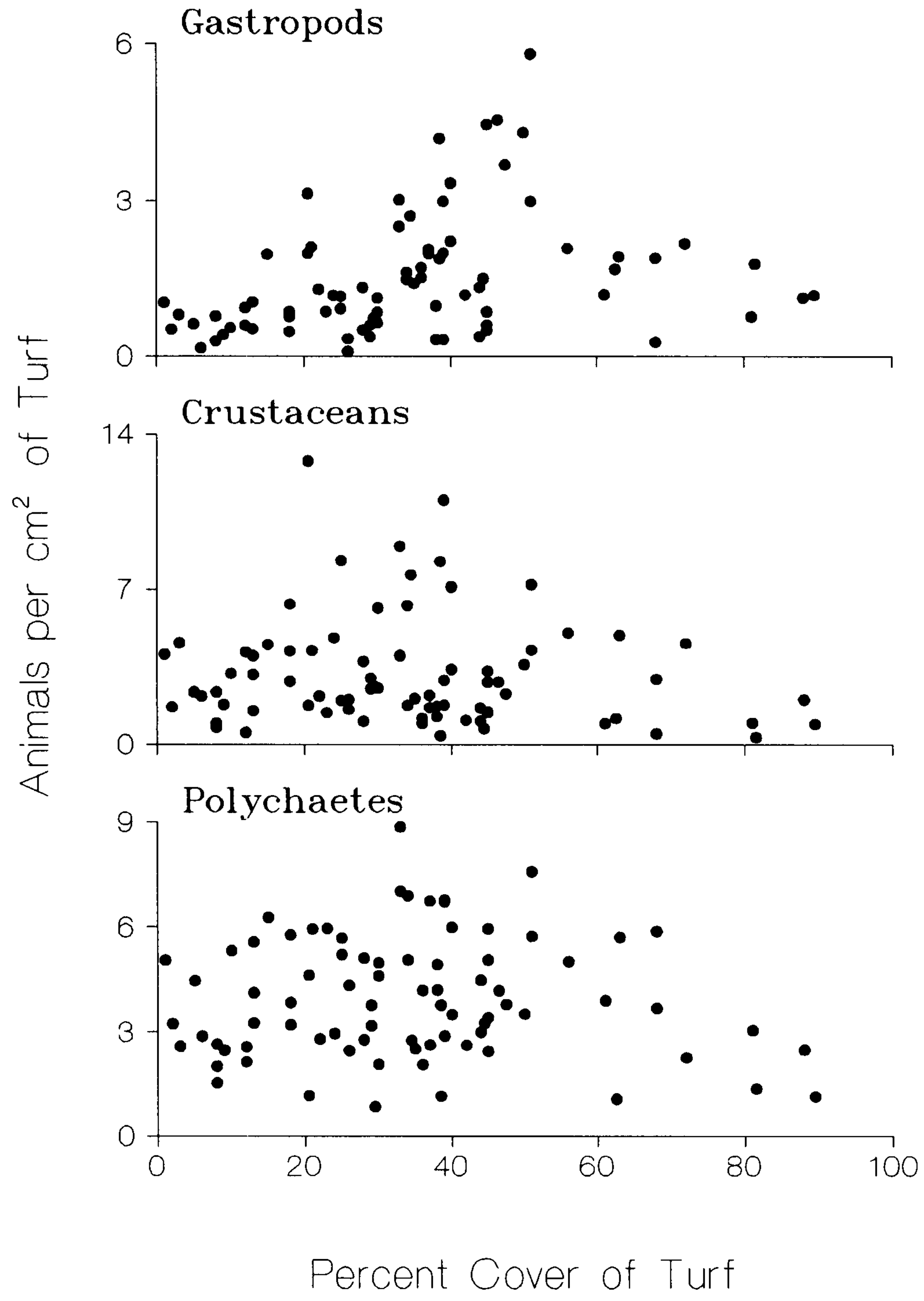


Fig 4.43 Population densities of infauna compared with percent cover of algal turf. Each point represents the mean density of the infaunal group from a given site, zone and date plotted against the percent cover of *Laurencia papillosa* at the same site and zone, and the nearest survey date for sessile biota.

postspill surveys indicated were probably abundant in this zone before the oil spill. If any of the original infauna from this zone survived the oil spill, they would have had to (1) avoid prolonged immersion in the oil that replaced seawater in this habitat and (2) found refuge in another habitat until the algal turf regrew. However, despite the severe loss of primary habitat and any mortality caused by the oil, the first infaunal samples (15 mo after the spill) showed abundances of most infaunal groups at the oiled sites were equal to or greater than abundances at the unoiled sites.

As a group, tanaids were the only group of infauna to show consistent indications of a deleterious effect of oil. In the samples (1987 and 1988), tanaids were much less abundant at the original oiled sites than original unoiled sites (Figs. 4.41*C*, 4.42). In 1989 tanaid populations declined at the original unoiled sites, corresponding to another oiling event. This was probably not caused by the severe low tides of mid-1988 because (1) tanaid populations persisted through this stress at Largo Remo and María Soto Arriba (LAR and MSU, respectively, in Fig. 4.42. Tanaids are probably more vulnerable to oil effects because of the following factors.

1. The exoskeletons of tanaids are probably susceptible to fouling by oil. In laboratory observations, tanaids were found to have strongly hydrophobic (water-repellant) exoskeletons. When sorted from algal mats under water, the living tanaids appeared to have dry surfaces and became trapped on the surface tension of the water, as though they were small shavings of paraffin (J. Cubit, pers. obs.). Oil does not readily adhere to wet surfaces, but readily adheres to waxy, hydrophobic surfaces. Regardless of any toxic effects, this probably put these small animals at the strong disadvantage of being fouled by oil in their environment. Although some other crustaceans may have waxy components in their cuticles, the exoskeletons of most crustaceans are hydrophilic (wettable), and the layer of surface water probably provided some protection against being coated with oil.

2. Tanaids appear to be mainly predators and filter feeders (Schram 1986). Recovery of predatory tanaids would necessarily lag the re-establishment of prey species, and the water-pumping activities of filter-feeding tanaid species would have increased their exposure to oil in the environment.

Consistent with the postulated greater sensitivity of tanaids to oil, populations decreased at the "unoiled" sites between October 1988 and April 1989, while oil was detected in sediment samples from Dos Marias reef, a location upwind from these sites. These sediment samples were collected in mid-December 1988, and GC analyses showed the presence of volatile fractions, indicating the oil was relatively fresh (K. Burns, pers. comm., see Sect. 3.9). In addition, surveys of invertebrates in subtidal seagrass beds indicated that tanaids were one of the groups most negatively affected by oil (Marshall et al., Chap. 10).

In the period of the infaunal surveys of the reef flat (September 1987 to April 1990), the results for other groups of infauna were also similar to the surveys of subtidal seagrass beds: populations of isopods, amphipods, and gastropods in the core samples at oiled sites were approximately equal to, or greater than, populations at unoiled sites. The relationship of repopulation of infauna to regeneration of algal beds is discussed in more detail in the general discussion at the end of this chapter (Sect. 4.5).

4.5 Discussion and Conclusions

4.5.1 General Discussion

Applying the results of this oil spill to other potential spills should be done on the basis of (1) the cause-and-effect processes that produced the spatial patterns and severity of damage, and (2) the factors that determined rates of recovery. Effects of this oil spill depended on a combination of many factors, including oil type, weather, sea conditions, and topography of the shoreline, all of which can vary from spill to spill. Therefore, absolute measures from this oil spill such as total damage or recovery rate have little valid application by themselves to other spills. As explained below, results of this study indicate effects of the 1986 Bahía Las Minas oil spill would have been predicatably different under other conditions of weather, sea state, and shoreline topography. Depending on conditions at the time of the spill, another spill at the refinery in Bahía Las Minas with an identical quantity of the same crude oil could cause biological damage very different in spatial pattern, severity, and recovery time. As explained below, a spill of the same type and quantity of oil under certain other conditions could spare the reef flat biota that suffered high mortality in the 1986 spill, but cause long-lasting damage to organisms that were little affected.

The dependence of damage on environmental conditions at the time of an oil spill is particularly important in using case studies to formulate policies regarding oil spills. This dependence, however, is often overlooked. Regarding the long-term implications of this spill, one of the observations made during this study is that it is common for research scientists, government officials, and oil industry representatives assume without reservation that (1) the absolute measures of damage and recovery recorded as effects of one spill are useful by themselves to estimate the effects of other oil spills in equivalent habitats, and (2) these estimates can be used without adjustment to determine plans and policies regarding oil spill risk and oil spill control. As discussed below, application of a particular case study to predicting oil spill effects and response planning can be made more comprehensive by including the predictable variations in oil movement and deposition that would occur under the full ranges of physical variables expected for the sites of application.

In the first surveys after the 1986 oil spill, the magnitude of changes in the reef flat biota at Punta Galeta coincided with the spatial pattern of direct deposition of oil on the reef flat: the greatest reductions in the abundances of invertebrates and macroalgae were at the lowest intertidal levels at the seaward edge of the reef flat, where oil accumulated during the extreme low tides of May. Much less damage occurred at higher tidal levels (on the reef crest) and in back-reef flat habitats.

These patterns of mortality coinciding with deposition of oil indicate that the primary cause of the initial mortality was direct contact with oil slicks and not a widespread, toxic effect of oil mixed in the water.

The exposure of the biota of reef flats to direct contact with oil depended on factors such as wind direction, wind speed, water level, wave action, topography of the reef flat, and zonation of the biota. The heavy deposition of oil along the seaward edge of reef flats was determined by the combination of onshore winds, seasonally low mean water levels, seasonally low tides, and relatively little wave action. During low tides the raised barrier formed by the reef crest protected the back-reef flat from the accumulating oil. On the rising tide at Punta Galeta, oil did not float over the reef crest until the back reef was underwater. This caused oil to float over the substrata and biota of the back reef and then exit through drainage channels at the back of the reef flat.

Differences in zonation patterns can account for many of the differences in proportional mortality among the various groups of organisms after the 1986 oil spill. Most of the reef flat populations of stony corals and the zoanthid *Palythoa* spp. were located at the seaward edge of the reef flat, which was the zone of highest mortality for all species. The populations of stony corals and *Palythoa* spp. on the reef flat were therefore almost eliminated by the oil spill. Another zoanthid, *Zoanthus sociatus*, was also common in this seaward zone, but survival of the *Zoanthus* population in the higher intertidal areas of the REDGE transects maintained a substantial population during the 1986 oil spill. At Punta Galeta, all the seagrasses of the reef flat (mostly *Thalassia testudinum*) were located on the back reef, where they were protected from the oil. These seagrasses showed no statistically significant reduction after the oil spill. However, oil did accumulate on the back-reef zones of other, nearby reef flats, causing extensive loss of *Thalassia* beds there.

Effects of the oil were concentrated on the part of the reef flat that probably supported the highest rates of productivity (Kinsey 1983; Hatcher 1988; Fig. 4.1). Algae, which account for much of the productivity, regenerated rapidly. However, the severity of effects would have depended on the consequences of (1) the lost period of full primary productivity, and (2) the lost period of full secondary productivity by the infauna and other fauna, which depend on algal beds for both forage and shelter. The ecological information available for reef flats is too meager to determine the effects of these losses. Without this information, assessment of total damage is not possible.

Processes of oil deposition and resulting mortality of reef flat biota were used to predict (i.e., hindcast) the widely different patterns of oil deposition and biological damage during the December 1968 spill of diesel oil and Bunker C oil from the *Witwater* (Rützler and Sterrer 1970; Cubit and Levings, Chap. 2). These predictions were tested against monitoring data that could show whether damage persisted after the *Witwater* spill. The apparent effects of this spill are consistent with the hypothesis that oil primarily causes mortality by direct contact. The steady increase of the calcareous green alga *Halimeda opuntia* in the *Laurencia* zone from the first surveys in 1970 (Fig. 4.14) may reflect regeneration from oil damage. Unlike the meandering oil slicks of the 1986 spill, oil slicks of the 1968 spill apparently came ashore during a short period of time and were quickly removed by cleanup crews (Rützler and Sterrer 1970). The expected durations of combinations of water levels and onshore winds for the month of December shown in Table 4.4 agree with the summary description of weather conditions during the *Witwater* spill (Rützler and Sterrer 1970; Cubit and Levings, Chap. 2): December is a month of high mean water levels, few hours of exposures, and strong onshore winds (the seasonal tradewinds), and strong wave action. Data from the Panama Canal Commission weather station at Cristóbal (approximately 8 km southwest of Punta Galeta) indicate the season of onshore tradewinds had started by the time of the *Witwater* oil spill (Cubit and Levings, Chap. 2). The expected total time of exposure of the coralline zone for the whole month of December was about 0 to 8 h (Table 4.4); therefore, the coralline zone was probably not exposed directly to accumulations of oil slicks for more than a few hours total in December 1968.

Oil slicks from the *Witwater* spill probably only came in contact with the highest parts of the reef flat, i.e., the reef crest in the *Laurencia* zone. Here the expected time of exposure was about 10 to 40 h for the whole month. As noted in the field observations (Sect. 4.4.1), clumps of *Halimeda opuntia* absorbed and held crude oil like a sponge. If this had occurred during the 1968 *Witwater* spill, the combination of the more toxic diesel and bunker fuel would have probably caused severe mortality of *Halimeda opuntia*. Progressive recuperation of *Halimeda opuntia* from heavy mortality in December 1968 could explain the steady increase of this alga in the *Laurencia* zone from 1970 to 1975. The rate of increase during this period was similar to the rate of increase of *Halimeda opuntia* in the coralline zone after its heavy mortality during the 1986 oil spill.

Based on the studies of the 1986 oil spill, *Halimeda opuntia* is probably the only species in these surveys that could have shown a detectable effect of the 1968 *Witwater* spill in the available (i.e., post-1970) survey data. Populations of fleshy algae would have regenerated by the time of the first surveys in 1970. Populations of sessile invertebrates did show slow recovery after the 1986 oil spill, but the high water levels during the *Witwater* oil spill would have protected most of the sessile invertebrates, which are found mainly in the deeper coralline zone. One exception is *Zoanthus sociatus*, which is found on higher substrata. Consistent with expected damage from the 1968 *Witwater* spill, *Zoanthus* was rare in the *Laurencia* zone in the first surveys of 1970 to 1972 and increased thereafter. However, populations of *Zoanthus* in the *Laurencia* zone are sparse and fluctuating, which reduces confidence in any conclusion that the rarity of *Zoanthus* from 1970 to 1972 was an effect of the December 1968 oil spill.

In the formulation of alternative hypotheses in this post-hoc study of the oil spill, the strongest alternative hypothesis was that seasonally low water levels, rather than oil, caused the postspill reductions in the biota of the reef flat. The stresses during low water levels include desiccation, insolation, and exposure to predatory shore birds. The deleterious effects of these factors are well documented for the reef flat at Punta Galeta, producing natural declines in the abundance of the sessile biota

Table 4.4 Expected conditions of wind and water level for the month of December. Values of expected hours per month are based on 8 yr of environmental data at Punta Galeta (see Cubit and Levings, Chap. 2).

Conditions		
Wind	Water Level	Expected h/mo[1]
Onshore, any speed	Extreme low (coralline zone emersed)	0 to 8
Onshore, any speed	Any part of reef flat emersed	10 to 40
Onshore, any speed	At reef crest	10 to 30
Onshore, >20 km/h [2]	Any level above reef crest	275 to 450
Onshore, >20 km/h [2]	At least 30 cm above reef crest	40 to 90

[1] Expected value for 50% of months (interquartile interval).

[2] Probable strong waves.

and certain species of sea urchins in the period between April and November (e.g., Birkeland et al. 1976; Hendler 1977; Schneider 1985; Cubit 1985; Cubit et al. 1986, 1988*b*, 1989; Figs. 4.11, 4.31*A*-*F*).

The detailed baseline data from Punta Galeta combined with the among-site comparisons ruled out the stress-based hypotheses in two ways: (1) compared with stresses and mortality recorded in other years, the stresses during the 1986 oil spill were not severe enough to account for the observed mortality in the oiled zones, and (2) even in the worst of years, these stresses produced spatial patterns of damage that were the reverse of the damage seen during the oil spill. In regards to the second point, the frequent and extensive exposures of the reef flat above water level in 1988 constituted a "natural experiment" that provided a strong test of the "stress hypothesis." The spatial patterns of reduced abundances were much different between the postspill censuses in June 1986 and the postexposure censuses in June 1988. Moreover, in each situation the reductions in abundances coincided with the exposure to each stress factor. After the oil spill, mortality was greater nearer the seaward edge of the reef flat. After the low water levels in 1988, mortality was greater on the reef crest. This difference occurred even though the exposures of the reef flat were the worst in 15 yr of records and could have swamped the effect of the oil spill. If natural physical stresses had produced the postspill reductions of biota near the seaward zone of the reef flat in 1986, then the reductions in this zone would have been even higher in 1988. They were not, indicating that it is highly probable that reductions in the cover of sessile biota after the oil spill were caused by oil and not by natural stresses of the physical environment.

The detailed time-series data from the biological surveys also demonstrated that some mortality associated with the oil spill and failure to recover afterward was probably *not* caused by oil. For example, the data showed the population declines of some biota (e.g., *Eucidaris tribuloides*) after the 1986 oil spill were probably continuations of declines in progress long before the 1986 oil spill (Figs. 4.32*B*, 4.44).

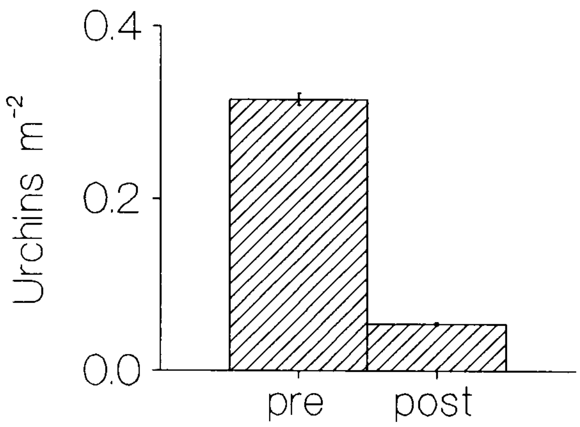


Fig. 4.44 Prespill-postspill comparison of the average population densities of the sea urchin *Eucidaris tribuloides* in the reef edge transect at Punta Galeta. The data are means and standard errors of monthly mean densities for 1977-1990 (see Fig. 4.32).

Because of population fluctuations, these trends would have been difficult to detect in less-detailed data, and the trends were lagged, or did not occur, at the unoiled sites. In a less-detailed study based only on intersite comparisons, these declines would have been falsely attributed to oil damage. Such declines have been noted for corals, sea urchins, seagrasses, and other marine organisms throughout the Caribbean (Williams and Bunkley-Williams 1990). Because these declines are not necessarily synchronous or of equal magnitude among sites, they have the potential to appear as oil damage in studies relying only on statistical comparisons of sites.

In addition, the combination of among-site comparisons and detailed physical monitoring data demonstrated that the abundance of corals on the reef flat did not continue to recover because the *coup de grace* was delivered by a period of the lowest water levels on record. Comparisons of oiled and unoiled sites showed that these low water levels killed corals regardless of persistent effects of oil.

Site comparisons by themselves, however, have significant limitations in demonstrating the effects of oil (Sect. 4.3.2). Without proper safeguards for between-region biases, differences in plant and animal populations between sites cannot be used to demonstrate effects of oil without making certain assumptions, including: (1) the regions were equivalent before the spill, and (2) differences after the spill were caused by oil. If this study had relied entirely on between-region comparisons, it would have been virtually guaranteed to find "oil effects" that were statistically significant, but false.

4.5.2 Processes Affecting Regeneration and Recovery

The mortality of sessile species during the 1986 oil spill and the exposures of 1988 resulted in little bare substratum. In both years, open space was immediately colonized by a bloom of microalgae, and in both years microalgae were most abundant in the zones of highest mortality of perennial species. The postspill bloom was concentrated at the seaward edge of the reef flat and diminished landward. The postexposure bloom was concentrated near the crest of the reef flat, and diminished seaward.

Blooms of benthic algae have been reported after oil spills elsewhere in the tropics (Russell and Carlson 1978) and in the temperate zones (Bellamy et al. 1967; Southward and Southward 1978); they appear to be a universal phenomenon. The cause of these blooms is not certain. The consensus explanation is that they are caused by mortality of herbivores (e.g., O'Brien and Dixon 1976; Southward and Southward 1978). This is consistent with our observations of the initial reductions in numbers of sea urchins, grazing crabs, and infauna, followed by the disappearance of microalgae as herbivores recolonized. However, release from competition and stimulation by increased nutrients cannot be ruled out as contributing to algal blooms (Russell and Carlson 1978). To our knowledge, the information from this study is the only before-and-after quantitative description of such a bloom and its demise following an oil spill.

The sea urchin data suggest that some persistent factor also may have negatively affected sea urchin populations in the oiled areas. After the May-June 1986 decreases in the reef-edge and *Thalassia* transects at Punta Galeta, *Echinometra* and *Echinoneus* species resumed recruitment even though oil slicks were still present on the water. Contrary to the prespill spatial patterns for the reef-edge transect, however, recruitment did not resume in the quadrat nearest the zone of heavy oiling at the seaward border of the reef flat. This interruption of recruitment may have been caused by some component of the oil absorbed into substrata or by a change in the biotic properties of substrata influencing settlement. Many marine organisms use the presence of various "cues" on the substratum to initiate settlement; these cues include those produced by coralline algae and other benthic organisms (Morse 1988*a*, *b*).

The same factors producing the small-scale failure of recruitment at Punta Galeta may have produced the absence of sea urchins on a much larger scale at Largo Remo. At this site, near the mouth of Bahía Cativá, sea urchin recruitment in all 4 yr of surveys was considered almost a total failure. Observations of higher densities of *E. lucunter* in subtidal areas adjacent to the reef flat at Largo Remo (J. Cubit, pers. obs.; Guzmán et al., Chap. 7) suggests that the near absence of urchins on this reef flat was caused by floating oil or some other factor near the surface of the water. The 1986 oil spill initially was concentrated in this bay, and mangrove sediments and coral landfill beneath the refinery continued to seep oil throughout the study period. In addition, several small spills of diesel and other fuel oils occurred at the electricity generating plant located at the head of this bay. This site also borders the path of heaviest aerial spraying of the oil dispersant Corexit 9527 during the oil spill in May 1986 (Cubit et al. 1987). The dispersant alone, emulsified oil, or some combination of these factors could have resulted in the absence of urchins. Punta Galeta was farther than Largo Remo from all such effects, and postspill recruitment was at times heavy (Figs. 4.32-4.34, 4.39). In field experiments in Panama, crude oil mixed with Corexit 9527 killed all the sea urchins (*Lytechinus variegatus* and *Echinometra lucunter*) in the treated area (Ballou et al. 1987). Chan (1977) also suggested surfactants as a possible cause of mortality of *Echinometra lucunter* after an oil spill in the Florida Keys. In addition, as described earlier, Bahía Cativá receives other pollutants and freshwater runoff. Intertidal habitats would have received the greatest exposure to these contaminants. The petroleum products and freshwater would be concentrated at the surface of the water. According to refinery officials (pers. comm.), the Corexit 9527 apparently did not disperse much oil into the water column because it was sprayed onto thick, weathered oil slicks during calm sea conditions. Thus, the combination of Corexit and oil probably remained floating at the surface of the water.

After the oil spill, *Laurencia papillosa* and other species of fleshy algae also increased their space occupancy in the zone previously occupied by *Palythoa* and corals. Because this phenomenon occurred at both the oiled and unoiled sites, it may have been unrelated to the oil spill. The increase of fleshy algae at the seaward edge of the reef flat may have been caused by factors similar to those causing shifts in the landward edge of the *L. papillosa* zone. The latter have been related to year-to-year changes in mean sea level (Cubit 1985). This project was discontinued before it was possible to determine if the zones of *L. papillosa* and other fleshy algae were restabilizing at different shore positions in the oiled and unoiled sites. As reported from other studies, the increase of fleshy algae at the seaward edge of the reef flat may have excluded corals (e.g. Loya and Rinkevich 1980), which declined at all sites.

The infauna data suggest that these small invertebrates probably recolonized the *Laurencia papillosa* turfs rapidly after the oil spill. Combined with the recolonization of sea urchins, this probably contributed to the disappearance of the microalgal bloom. The dense populations of infauna in *Laurencia papillosa* turf and sea urchins account for most of the animals on the reef flat. They probably constitute the predominant pathway for converting primary productivity of the reef flat algae into a form that is consumed by octopus, lobsters, fish, and other carnivorous predators that forage in and around this shallow reef environment.

Studies in Florida have demonstrated that the infauna inhabiting *Laurencia* beds constitute an essential food resource for spiny lobsters, particularly the juvenile stages (Marx and Herrnkind 1985*a*, *b*; Herrnkind and Butler 1986; Butler and Herrnkind 1991). Studies at Punta Galeta have also shown that spiny lobsters forage in *Laurencia* turfs of the reef flat at night (A. Richards, pers. comm.; J. Cubit pers. obs.).

The rapid recovery of populations of amphipods, isopods, small gastropods, and polychaetes was probably based in part on the fact that most of these animals are herbivores or omnivores (e.g., Barnes 1986; Schram 1986), and probably benefitted from the blooms of microalgae following the oil spill and the extreme low tides of 1988.

The time to recovery of infaunal abundances per unit area of turf is only one component assessing the severity of depopulation of infauna in the tidal flat ecosystem. A second component is the total area of the algal turf itself. Densities of infauna per unit area of turf did not indicate that infauna crowded into surviving algal turf when total turf areas were reduced. Therefore, the reduction of *Laurencia papillosa* turfs during the oil spill in 1986, and the disproportionate reductions of the turf at oiled sites during the extreme low water levels in 1988, both constituted severe, oil-related losses of infauna from tidal flat ecosystems. These results indicate that oil-caused reductions of habitat should be considered as deleterious for populations of small invertebrates as the direct lethal effects of oil.

In addition to lethal effects, chronic, low-level exposure to oil has been shown to decrease fecundity of crustaceans such as amphipods and copepods (studies reviewed in Neff and Anderson 1981). Sublethal exposure to petroleum hydrocarbons can also reduce the growth or survival, or both, of larvae of various invertebrates, including molluscs and crustaceans (Neff and Anderson 1981; Capuzzo et al. 1984). Despite these negative effects, these infauna were usually more abundant at Largo Remo than Punta Galeta, even though the Largo Remo site was more exposed to chronic reoiling from the adjacent Bahía Cativá. These results suggest that favorable factors (e.g., increased food supply or decreased predation rates) were overriding deleterious effects of chronic oiling or that oil affects these small invertebrates differently in closed systems of the laboratory than open systems of the field.

Populations of most major categories of infauna were much higher in the last sampling period (April 1990) than in the first sampling period (September 1987). This pattern included both the oiled and unoiled sites. Does this mean the initial abundances were depressed, or the final abundances were unusually high? Similar patterns were found after this oil spill for infauna of subtidal seagrass beds (Marshall et al., Chap. 10) and for stomatopods on reef flats (Steger and Caldwell, Chap. 6). In the first surveys of seagrass beds (September-November 1986), population densities of all invertebrate categories except burrowing shrimp were low at both the oiled and unoiled sites, but increased thereafter (Marshall et al., Chap. 10). Steger and Caldwell (Chap. 6) have the only prespill data in this regard. Their prespill-postspill comparisons indicate an anomalous regional reduction of larval recruitment following the 1986 oil spill that lasted until 1988-1989. In addition, fishermen in Col6n have reported a recent, dramatic decline in abundance of the heavily harvested blue mangrove crab *Cardisoma guanhumi* in the region from the city of Col6n to Portobelo, but not in locations farther down-current nor in areas up-current from Col6n (Borbua 1991). Although the decline may have been related to fishing pressure, this crab had been heavily harvested starting before 1974 (G. Hendler, pers. comm.; J. Cubit, pers. obs.) without the decline reported in 1991.

This phenomenon included a wide range of crustaceans, gastropods, and polychaetes. However, it did not occur among sea urchins. It also included both animals with planktonic larvae (e.g., stomatopods and polychaetes) and animals without planktonic larvae (e.g., most isopods and amphipods). This may have been a multi-year perturbation with natural causes. Unlike other multi-year perturbations, however, this one affected not just one taxonomic group, but a diversity of taxa. If this were an effect of the oil spill, it included the "unoiled" areas that were not directly inundated by black oil slicks. However, all "unoiled" sites for all projects were within the Bahía Las Minas area or downstream from Bahía Las Minas in the coastal current, where some oil was transported, as described in Section 2.2.3. Oil concentrations were low in most hydrocarbon samples taken beyond María Chiquita (Burns, Chap. 3), and an effect of the oil spill in these areas would indicate that small invertebrates are very sensitive to oil, the dispersants sprayed on the oil, or a combination of both.

This possibility could be directly tested with demographic analyses of the species of greatest tangible value: the long-lived and economically important groups (e.g., lobsters, crabs, shrimp, and conchs), which are found both down-current (toward Colombia) and up-current (toward Costa Rica) from Bahía Las Minas. If the oil spill had a widespread effect, the size-frequency distributions of only the down-current populations should show gaps in size classes corresponding to a loss of juveniles from mid-1986 to approximately 1988. Fishery statistics are not recorded for the Caribbean coast of Panama, but the economic importance of answering this question is illustrated by statistics from Florida (Table 4.5): crustaceans alone accounted for more than 50% of the dollar value of the commercial fishery, and a 2- or 3-yr gap in recruitment could have a severe economic impact.

Laurencia papillosa and other fleshy macroalgae of reef flats quickly regenerated after the oil spill; within 5 mo they had regained abundances within the range measured before the spill. This regeneration time was much faster than the 10-yr recovery time reported for some populations of fleshy intertidal algae in the temperate zone (NRC 1985). Populations of the calcareous green alga *Halimeda opuntia* were slower to regenerate, probably a direct result of a slower inherent growth rate.

After the 1986 oil spill rates of recovery of populations of plants and animals on reef flats depended on the following factors.

1. The severity of damage to the population.

2. Mode of recruitment and recolonization, e.g., recruitment of planktonic larvae and spores, immigration of mobile animals, regeneration from fragments surviving *in situ*, and vegetative spreading from fragments washed in by wave action (Table 4.6). Recolonization resulting from immigration of mobile animals or wave-driven transport of fragments of sessile organisms depended on the existence of source populations in refuges only a few meters from the damaged zones.

3. The inherent growth rate of the species.

Table 4.5 The commercial value of crustaceans from the Gulf Coast of Florida for 1987 and 1988. These are values of the landed commercial catch only and do not include the values of the marketed catch, support services, or the recreational fishery. (Source: U.S. Department of Commerce 1987, 1988)

	Year	
Group	1987	1988
Crabs	$10,379,000	$15,335,000
Spiny Lobsters	16,100,000	14,471,000
Shrimps	39,313,000	32,575,000
Total crustaceans	65,792,000	62,381,000
Total fishery (all species)	111,397,000	114,381,000
% of total from crustaceans	59%	55%

Table 4.6 Modes of recruitment and recolonization contributing to the recovery of benthic biota on reef flats after the Bahía Las Minas oil spill.

Mode	Examples
Arrival of planktonic larvae from open ocean	Sea urchins, some infauna
Immigration of mobile animals from adjacent, but less damaged, habitats	Infauna of algal beds, especially those without planktonic larvae, such as amphipods and isopods
Wave transport of fragments of sessile species from adjacent, but less damaged, habitats	Colonial animals (e.g., *Palythoa*, *Millepora*); possibly some algae
Regrowth from remnants surviving *in situ*	Most algae; some colonial animals (e.g., *Zoanthus* sp.)

4. Possible competition with other species.
5. Long-term destruction of the habitat.
6. The persistence of oil at the site.

For example, the fleshy red alga *Laurencia papillosa*, the most abundant species on the reef flat, was regularly killed back to basal tissue in seasons of physical stress, but regrew from this tissue during more favorable conditions. Oil produced similar damage to this alga, and it again regenerated from surviving basal portions. Unlike most algae, which have a single holdfast for attachment, plants of *Laurencia papillosa* grow in a tangled carpet of fronds with many points of attachment. This provides a single plant with many points for possible survival and regeneration. However, if the oil had been more toxic or the exposure to oil more prolonged, higher mortality of basal tissues could have slowed or prevented recovery, as seen in colonies of the zoanthid *Palythoa caribaeorum* and the hydrozoan coral *Millepora complanata*. Both of these sessile invertebrates can also spread vegetatively from surviving fragments. However, these species suffered nearly 100% mortality on the reef flat, and their slow, partial recovery was largely dependent on the slow growth of colony fragments washing in from refuges in deeper water.

The re-establishment of *Palythoa* and *Millepora* also may have been slowed by competitive interference from populations of fleshy algae (Loya and Rinkevich 1980), which became more abundant in the habitat vacated by the two sessile invertebrates. The loss of corals from the reef flat left coralline algae as the primary reef builders in these habitats. The rapid regeneration of coralline algae suggests they regrew from basal tissues protected deep within the ceramic-like thalli of these algae. If this is correct, recolonization succeeded because the weathered crude oil of the 1986 spill was not toxic or soluble enough to kill entire thalli. If a more toxic oil or more prolonged exposure to oil had killed entire thalli of the corallines, the most critical portion of the reef would have stopped accreting. The seaward portion of the reef flat is critically important for the hydrodynamics of the reef as a whole (Fig. 4.1; Cubit 1985; Cubit et al. 1989), and reduced accretion combined with presently rising sea level could cause rapid changes to the physical environment and biota of the whole reef (Cubit 1985, 1990; Cubit et al., 1986, 1989).

In the case of *Thalassia testudinum*, after the seagrass beds died, rhizomes and roots gradually rotted away over a period of several years. Without replenishment by sediment trapping and reinforcement by roots and rhizomes, sandy substrata eroded down to coral rock (see Steger and Caldwell, Chap. 6 for a well-documented case). Loss of substrata required by this seagrass may delay regeneration considerably.

The series of surveys comparing *Laurencia papillosa* populations at oiled and unoiled sites after the oil spill showed that populations that had regenerated after oil damage were more vulnerable to the stresses of low tides in 1988 than populations not damaged by oil. This result indicates claims of recovery based on measures of abundance may not be accurate indications of recovery in the sense that population dynamics of the species have returned to normal.

Ecological models for colonization and population dynamics of intertidal zones are usually based on the arrival of planktonic spores and larvae (e.g., Roughgarden 1989). These planktonic stages can be supplied from adult populations on distant, undamaged shores or from a pool of planktonic stages in the ocean offshore from damaged areas. This was not the primary mode of recolonization of reef flats (see also Guzmán et al. 1991, Chap. 7). Except for sea urchins and some infauna (those having planktonic larvae), recovery of most reef flat biota depended on perrenation (spreading growth) from fragments that survived *in situ* or arrival of fragments of sessile species and immigrants of mobile animals from immediately adjacent habitats that suffered less damage (Table 4.6).

These localized mechanisms of recovery imply that postspill recovery of biological communities on warm-water tidal flats is dependent on the survival of a critical minimum population for each species. Below this minimum, re-establishment of a species may not occur at all or not for a very long period. Delayed recruitment of the original set of species could allow the establishment and pre-emption of space by another assemblage of benthic organisms, or "alternate stable state," at the damaged site (Hatcher 1984). This could further impede re-establishment of the original community. Such changes have been seen following severe mechanical destruction of benthic communities by a shipwreck on the Great Barrier Reef (Hatcher 1984).

Differences in modes of regeneration and lack of severe damage from cleanup activities may explain why algal populations recolonized faster after the oil spill on the reef flats of Bahía Las Minas than reported after oil spills on intertidal shores in the temperate zone. Within a year after the oil spill, the predominant species of fleshy algae on the reef flat at Punta Galeta had regenerated to abundances approximately equal to both of those recorded on this same flat before the spill, and measured at unoiled reef flats after the spill. This regeneration time is much faster than the 10-yr recovery time reported for some populations of fleshy intertidal algae in the temperate zone (NRC 1985). This difference could be the result of two factors.

1. The common algae of warm-water flats regularly respond to seasonal stresses by dying back to basal portions and regrowing from the bases during more favorable conditions. This adaptation also served well when oil coated and killed the upper portions of algae on the reef flats in Bahía Las Minas. When subjected to natural physical stresses, algae of the intertidal zone on temperate shores are more "hardy," and their usual response is to persist, rather than to die back and regrow. The lack of selection for regrowth ability after severe tissue loss is one possible explanation for the slow recovery of temperate algae after oil spills, and is easily testable by field experiments.

2. Oil spills in the temperate zone have been subject to more intense cleanup activity, including the use of detergents for earlier spills, such as the *Torrey Canyon* spill. Detergents used during the *Torrey Canyon* spill were toxic to intertidal algae (Boney 1968). As discussed above, cleanup activities may have slowed recovery by removing the remnants of algae that survived the oil *per se*.

A construction project on the reef flat at Punta Galeta can be viewed as an experiment testing the hypothesis of the dependence of recovery on regeneration from localized survivors. In this project, a temporary causeway of rock and gravel over plastic sheeting was laid down on the back-reef flat. The causeway was approximately 1 m thick, 4 m wide, and 30 m long. The causeway stayed in place for approximately 3 mo in 1991 (22 April to 23 July). In the affected area, the biological damage caused by the construction project was much more severe than the damage caused by the 1986 oil spill. Virtually all the seagrasses, macroalgae, and other biota under the fill were killed. As of January 1992, approximately 6 mo after the causeway was removed, the former location of the causeway stood in sharp visual contrast to the rest of the back-reef flat. Though most of the area of the former causeway was covered with a fine layer of microalgae, it was essentially barren of macrospecies. The few clumps of macroalgae present in the area of damage grew on loose rocks that appeared to have washed into the zone of damage. In contrast to this lack of regeneration, in the seaward zone of the reef flat where oil caused the highest mortality, macroalgae had mostly regrown within 6 mo.

The dependence of postspill recovery on fragments that survive *in situ* or arrive from nearby sources implies that simple, but immediate, planting of fragments could speed recovery of the sessile flora and fauna, thereby reducing the risk of permanent change of tidal flat ecosystems suffering severe damage from oil.

4.5.3 Applicability of the Results of This Study to Oil Spill Risk Analyses and Planning for Oil Spill Response

This section will briefly address some general implications of this study for policies such as evaluating the risk of oil spills, selecting priorities for oil-spill control, and determining the methods for cleanup.

The results of this study indicate that the severity of damage to tidal flat communities depends on the following factors, in addition to the toxicity of the oil itself.

1. Winds, waves, currents, and other sea and weather conditions affecting the surface movement and vertical mixing of oil.

2. Water levels, tidal range, tidal flat topography, and other factors determining the amount of tidal flat habitat at or near water level at the time of the oil spill.

3. Shoreline configurations that concentrate or block oil slicks.

4. The zonation and along-shore distribution of biota in relation to the vertical and horizontal topography of the tidal flat.

5. The propensity of the substratum to absorb oil.

Such information is often overlooked in risk analyses. For example, MMS-sponsored analyses regarding the potential effects of oil spills in South Florida have been reviewed in the NRC (1989) report titled *The Adequacy of Environmental Information for Outer Continental Shelf Oil and Gas Decisions: Florida and California.* In this report, the NRC notes that most of the MMS information pertains to deeper-water communities and concludes: "The shallow-water benthic communities [in South Florida], other than corals, have been inadequately studied and characterized." In addressing the deficiencies of the MMS reports, the NRC (1989) specifies corals, mangroves, and seagrasses as the benthic groups of special concern that need further study. However, the remedial studies recommended by the NRC do not include the need for information about tidal elevation, zonation, substratum type, or other factors that would determine exposure of the biota to direct contact with oil. In addition, the NRC recommendations specify particular groups of organisms (e.g., seagrasses) as a way to define habitats. However, this approach omits some major, but less well-known, taxonomic groups such as most of the major groups of algae and mobile invertebrates. These deficiencies could be overcome by defining the needs for further studies in the same terms the NRC used to criticize the MMS studies, i.e., to directly study and characterize the distribution and abundance of the biota in the shallow habitats of South Florida, rather than presuppose that studies focused on particular taxa would serve this purpose.

The lack of attention to algae in these MMS and NRC studies is a major omission that can be demonstrated by just two examples: (1) most of the sand and finer sediments of Florida Bay and the Florida Keys are produced by calcareous green algae such as *Halimeda* spp. and *Penicillus* spp. (Hoffmeister 1974; Shinn et al. 1989), and (2) the successful settlement and survival of lobster larvae in Florida Bay depends on the masses of fleshy red algae *Laurencia* spp. (Marx and Herrnkind 1985*a*, *b*; Herrnkind and Butler 1986; Butler and Herrnkind 1991).

Zieman (1990) has extrapolated the results of this Bahía Las Minas study to the shallow seagrass beds on the mud banks of Florida Bay. Citing Chan (1977), Cubit et al. (1987), and Jackson et al. (1989), Zieman (1990) makes the following predictions for South Florida:

> "Where oil has contacted seagrasses that are just intertidal or just subtidal, damage has been extensive, while deeper subtidal beds are generally unharmed by direct effects of the oil (Chan 1977; Cubit et al. 1987; Jackson et al. 1989). These instances point out how vulnerable much of South Florida's seagrass meadows would be to an oil spill... Many of the deeper seagrass beds ... would probably survive [an oil spill], but the priceless nursery meadows of Florida Bay in Everglades National Park and much of the western portions of the Lower Keys could be destroyed. This is because so much of this area consists of shallow mudbanks that are emergent on very low tides... The average standing crop of [*Thalassia*] leaves for the entire [Florida] bay was 63.2 g dry wt/m^2, whereas the density in the upper meter of the western section of the bay, which would be by far the most vulnerable to oil spills, is 125 g dry wt/m^2 (Zieman et al. 1989)."

Although Zieman's (1990) predictions are based on the early results of the Bahía Las Minas study, they are entirely consistent with the final results. To elaborate further, it should be noted that damage to seagrasses in Bahía Las Minas was probably caused by oil penetrating the sediments around the roots and rhizomes of the seagrasses. Few seagrass flats in South Florida have the configuration that protected some of the seagrass beds on the fringing reef flats in Bahía Las Minas. Because reef flats in Bahía Las Minas are backed by land, oil slicks could only arrive over the raised reef crest, which protected back-reef flats through the processes described earlier. In South Florida many seagrass flats are on offshore formations, which would be exposed to oil slicks from all sides.

These exposed structures include the reef flats of South Florida, which are on bank reefs, rather than fringing reefs (e.g., Jaap 1984). In these offshore locations, the reef flats of South Florida are vulnerable to oiling on all sides of the reef crests, not just on the seaward sides, as in the case of Bahía Las Minas. In addition, the tides in South Florida have about twice the amplitude of the tides in Bahía Las Minas (U.S. National Ocean Service 1988). The combination of offshore reef locations and greater tidal range would allow oil slicks to be in direct contact with a much greater area of South Florida reef flats than Bahía Las Minas reef flats.

Oil on all tidal flats, including reef flats, creates a severe dilemma for cleanup decisions: on one hand, cleanup activities increase damage, but on the other hand, no cleanup of tidal flats with porous substrata could prolong a situation of chronic oiling. For all tidal flats, regardless of substratum, cleanup activities should be carefully controlled to maintain surviving populations above the critical minimum level required for regeneration and recovery, as discussed above. After an oil spill, most residual populations on tidal flats would suffer severe additional destruction from the usual methods used to clean up oil spills, such as hot water, steam, sandblasting, scrubbing with absorbent materials, or methods involving traffic of machinery or trampling by cleanup crews. Little is known about the effects of dispersants and other chemical agents on the biota of tidal flats.

In sandy or muddy areas on tidal flats, microbial decomposition of oil is slow because of anaerobic conditions. In addition, the natural mechanical washing of the biologically stabilized sediments is slow. Oil remaining in sediments would create a potential source to supply long-term chronic oiling, as seen in Bahía Las Minas after the 1986 oil spill (Burns, Chap. 3). Clearly, development of highly specialized cleanup methods, those removing residual oil but not residual biota, is needed for these environments. By removing the small surviving portions of tidal flat biota, cleanup activities could prevent or greatly delay the re-establishment of most plants and animals on tidal flats. Because of the multiple, long-term problems resulting from oil contamination, the best solution is to provide these sedimentary areas of tidal flats with high priority, maximally effective protection that would prevent oil from reaching environments located at or near water level.

The 1986 Bahía Las Minas oil spill was not the worst example of how an oil spill could affect reef flats. The oil on most of the reef flats in 1986 was weathered crude oil of relatively low toxicity. In addition, weather and sea conditions restricted the oil deposition to narrow portions of reef flats. As a result, biological damage resulting from this spill was relatively moderate, and populations of most of the surveyed species remained above the threshold of the "critical minimum." This allowed many, but not all, populations to regenerate rapidly. Populations of reef flat biota could have been reduced below the "critical minimum" required for *in situ* regeneration if conditions during the oil spill had prolonged exposure to oil, the oil had been more toxic, or standard cleanup measures had been used. Such damage could have lasted for years.

Simple enumeration of certain biota in a geographic area is the usual method of biological analysis used for studying the potential effects of oil spills and devising contingency plans for oil spill control. However, this type of information is not adequate for examining the potential effects of oil on a biological community. To evaluate risks to the biota, it is essential to include the range of expected weather and sea conditions, tidal elevations of the biota, and other factors that will determine the exposure of the biota to the damaging effects of the oil.

4.6 Acknowledgments

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5

Effects of an Oil Spill on the Gastropods of a Tropical Intertidal Reef Flat

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and Carlos González M.**

5.1 Abstract

The severity of effects of a major oil spill on reef flat gastropods on the Caribbean coast of Panama varied with the amount of oil deposited in each intertidal zone. Snails died immediately after the spill in zones with heavy accumulations of oil, and numbers decreased further in some zones after cleanup activities. Overall snail density and number of species decreased through time. Recruitment appeared reduced in some zones more than 2 yr after the spill. Such patterns were not observed at an unoiled site until a small spill of diesel fuel was followed by a similar reduction in snail populations. This small spill, however, did not have long-term effects. Effects of oil spills on these types of reef flats depend on emersion patterns because water levels determine where oil is distributed.

5.2 Introduction

On 27 April 1986 an oil storage tank at the refinery on Isla Payardí in the Republic of Panama ruptured, spilling at least 60,000-100,000 bbl of medium-weight crude oil into the Caribbean Sea (Cubit et al. 1987; Jackson et al. 1989). The Galeta Marine Laboratory of the Smithsonian Tropical Research Institute, located on a nearby reef flat, has been the focus of detailed investigations since 1968 (e.g., Birkeland et al. 1976; Cubit and Williams 1983). Oil began washing ashore at Punta Galeta in early May, as the area between the Caribbean entrance to the Panama Canal and the town of María Chiquita was extensively oiled (Fig. 5.1).

5.2.1 Physical Structure

Six discrete habitats (*zones*) on the reef flat were sampled (Fig. 5.2). Running roughly from the shore toward the seaward edge of the reef flat, they included:

1. high rubble,
2. low rubble,
3. sand,
4. reef rock,
5. beds of seagrasses, primarily *Thalassia testudinum*, and
6. beds of algae, primarily *Laurencia papillosa*.

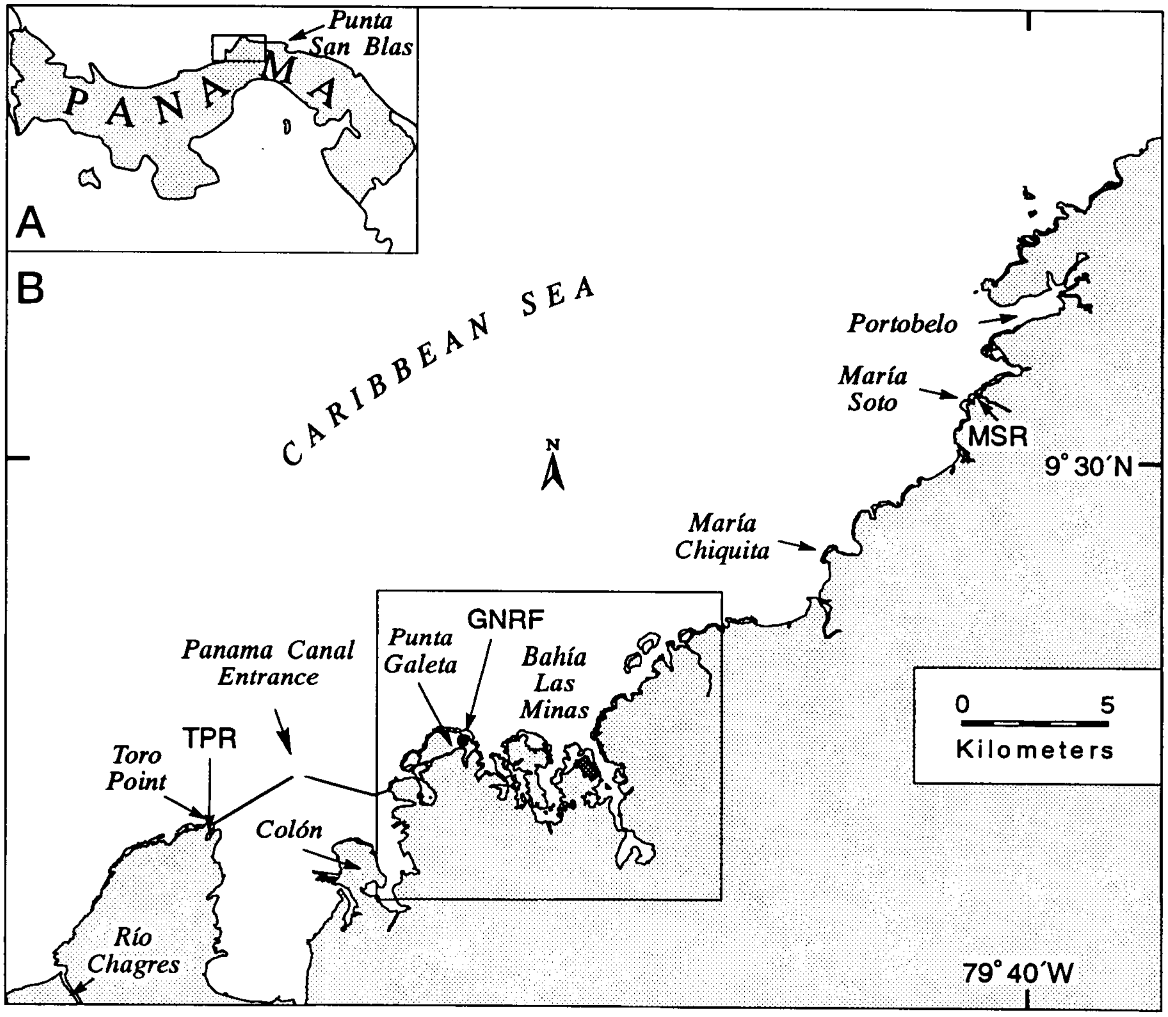

Fig. 5.1 Map of study sites. *A*. Location of study area in Panama. *B*. *GNRF* = Galeta Navy Reef (oiled), *TPR* = Toro Point (unoiled), *MSR* = María Soto (unoiled). See Figures 1.2 and 1.5 for further details.

The high rubble habitat was on the shoreward margin of the reef and consisted of an elevated, consolidated matrix of dead coral, shell fragments, and sand, with some loose rubble and shell (Fig. 5.3). It was never submerged, although waves might splash or surge onto the high rubble habitat during extremely rough weather (S. D. Garrity, pers. obs.).

The low rubble habitat occurred just below and seaward; it was less consolidated and consisted mainly of large fragments of coral rubble embedded in sand, and other relatively loose debris (Fig. 5.4). This habitat lay partly within the intertidal zone; it was regularly to intermittently submerged and emersed, depending

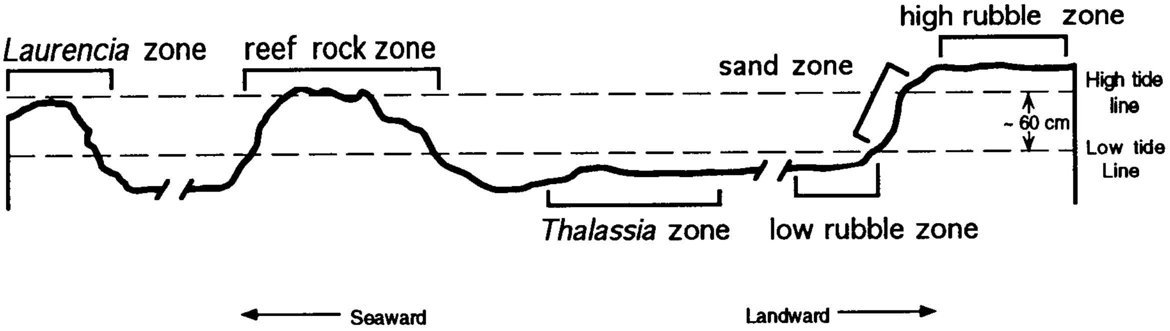

Fig. 5.2 Composite diagram of the six reef flat zones. Tide limits are approximate, and actual water levels may exceed upper and lower limits because of weather conditions; see text. *Thalassia* zone is shown as subtidal, but seagrass leaves may be exposed to air during low tide.

upon tidal cycle and wave action. Movement of snails between the high and low rubble zones was possible because the zones were contiguous sections of shore.

The sand habitat (Fig. 5.5) also occurred at the shoreward margin of reef flats, partly in and partly below the intertidal zone (Fig. 5.2). We monitored snails in the intertidal portion of this habitat.

The reef rock habitat, a relatively horizontal area of jagged and pocked rock, occurred seaward of the above habitats (Fig. 5.6). Well within the intertidal zone, it underwent fairly regular cycles of submergence and emersion. This habitat was physically isolated from the shoreward zones by deeper water.

Thalassia beds (Fig. 5.7) have been described by Heck (1977). Located within the lagoon and in deeper areas of the reef flat, they were partly emersed during low tides. *Laurencia* beds (Fig. 5.8; see also Connor 1984) occurred near the seaward fringe of the reef flat and were exposed above water level only when calm seas occurred with minus tides.

Cubit et al. (1986, 1989) discuss thoroughly the details of intertidal exposures at Punta Galeta. Tidal fluctuations are small and often overridden by winds and currents, leading to an irregular pattern of submersion and emersion of the reef flat. In general, water levels and wave action are high in the late wet (December) and especially dry seasons (January-March). In the early rainy season (April-May) onshore winds cease and water levels drop, often leading to long periods of exposures of the reef flat above water level. Exposures of the reef flat occur most often in this part of the year, but may happen at any time when winds and tides are both low.

5.2.2 Biota and Literature Review

Gastropod molluscs are abundant and diverse consumers on many tropical shores (Vermeij 1978), but relatively little is known about molluscs on coral reef flats

Fig. 5.3 High rubble zone, May 1989. *A.* General view, facing seaward, TPR. The quadrat is 0.5 x 0.5 m. Note transect tape and storm debris. *B.* Close-up of 0.25-m^2 quadrat, TPR. Note consolidated coral rubble substratum. Visible snails include *Tectarius*, *Nodilittorina*, and several species of *Littorina*.

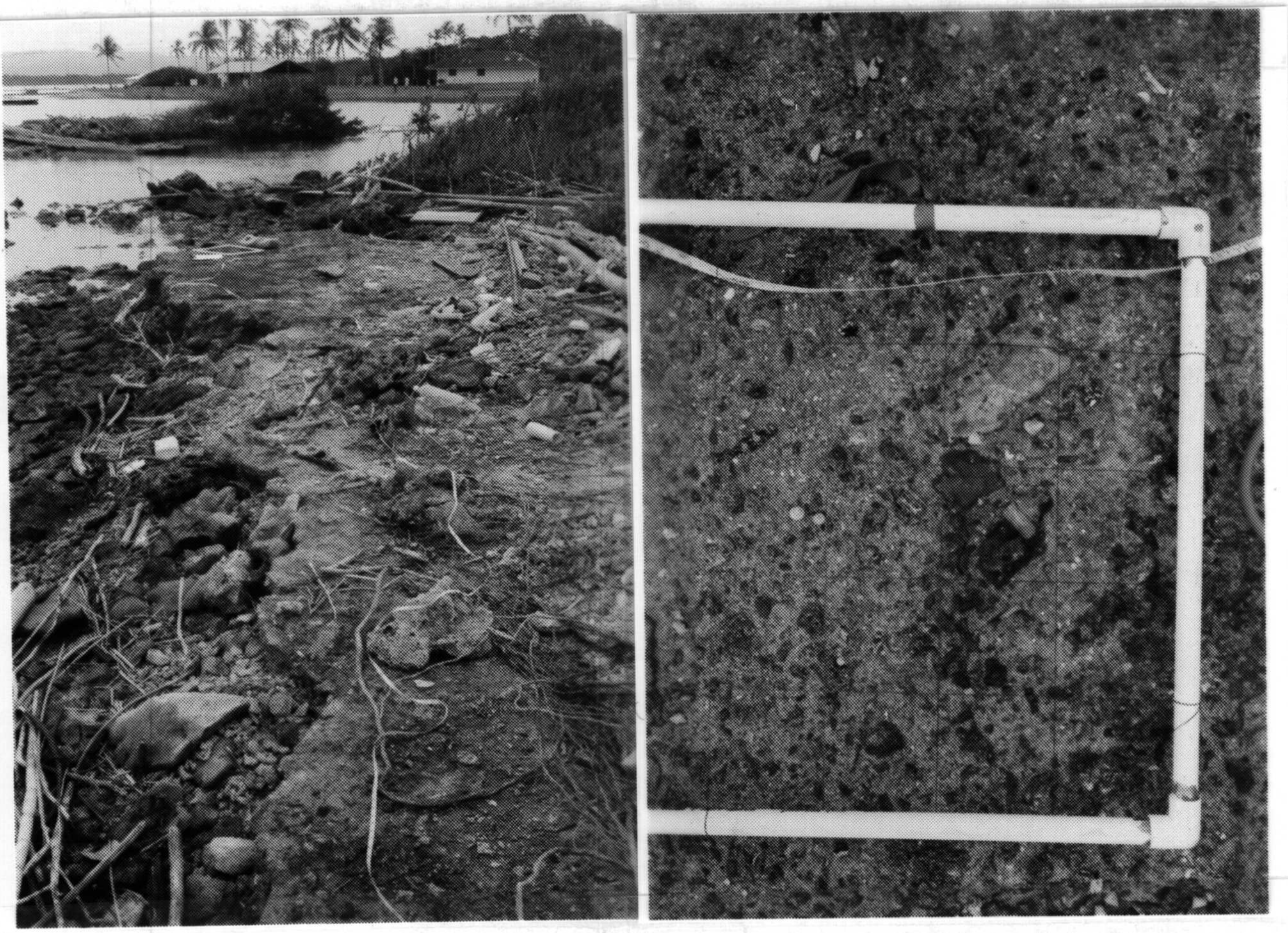

Fig. 5.3 High rubble zone, May 1989 (continued). *C.* General view, GNRF. Note storm debris and erosion of zone. *D.* Close-up of 0.25-m^2 quadrat, GNRF. Visible snails include *Tectarius* and *Littorina lineolata*.

(Kohn and Leviten 1976; Leviten and Kohn 1980). The relative abundance and size structure of molluscs in different habitats on the Galeta Navy Reef (GNRF), located approximately 0.5 km west of the reef flat at Punta Galeta (GAL, Figs. 1.5, 4.4; see Cubit and Connor, Chap. 4), were sampled quantitatively in 1982-1983 (Garrity and Guttierez, unpublished; Garrity and Levings 1990; Table 5.1). These are the most comprehensive data on gastropod ecology at GNRF before the spill (but see also Radwin 1969; Birkeland et al. 1976; Brattstrom 1985).

Little information exists on even short-term effects of oiling on the molluscs of reef flats (Birkeland et al. 1976; Eisler 1973, 1975*a*, *b*; Chan 1976, 1977; Maynard et al. 1977; Maynard 1984).

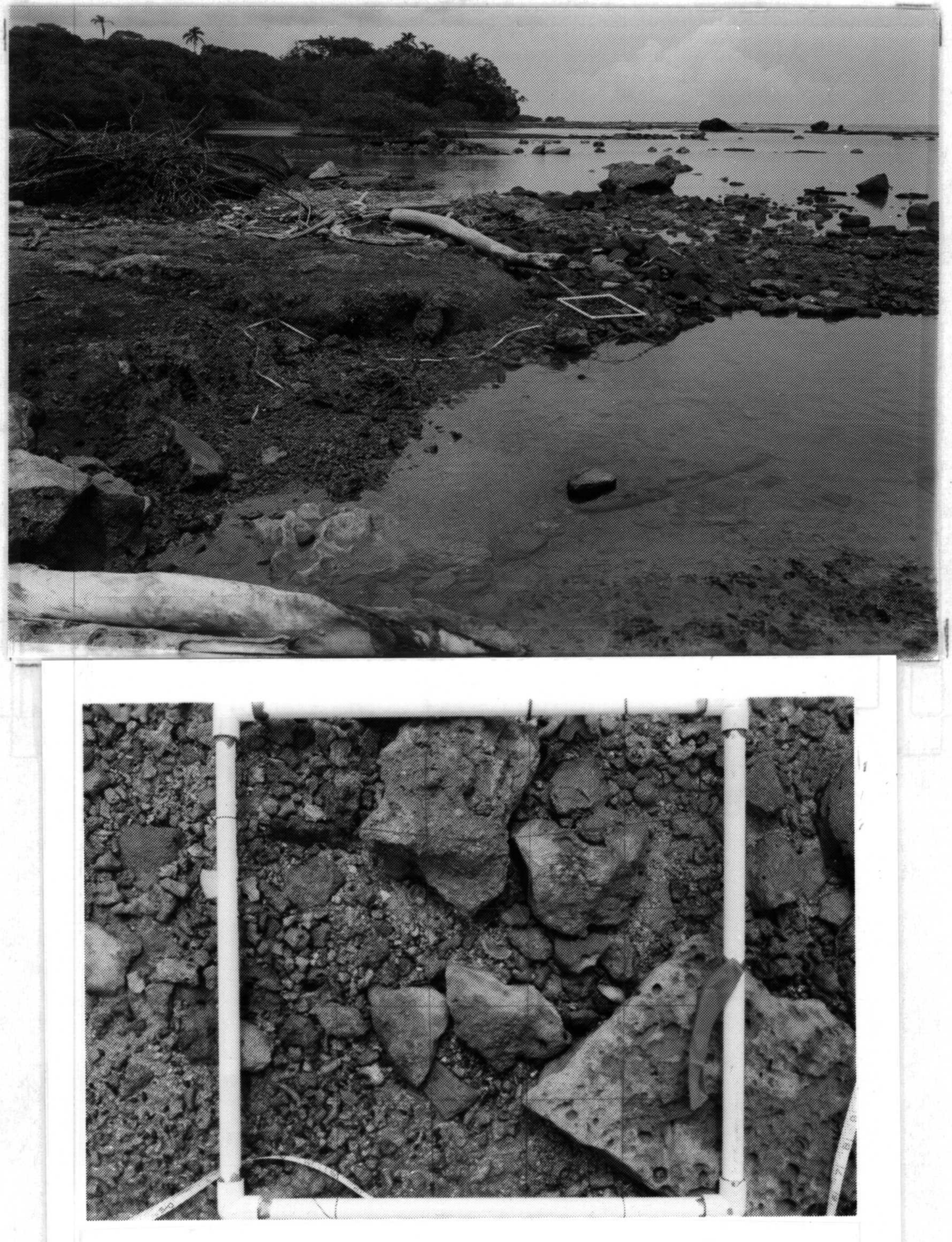

Fig. 5.4 Low rubble zone, May 1989. *A*. General view, TPR. Quadrat and line are in the low rubble zone; high rubble zone is visible *above* and to the *left*. Reef rock zone is visible in the *background* at the extreme *upper right*. *B*. Close-up of 0.25-m^2 quadrat, TPR. Note unconsolidated nature of the substratum relative to the high rubble zone. Visible snails are *Planaxis nucleus* in the *lower left* of quadrat.

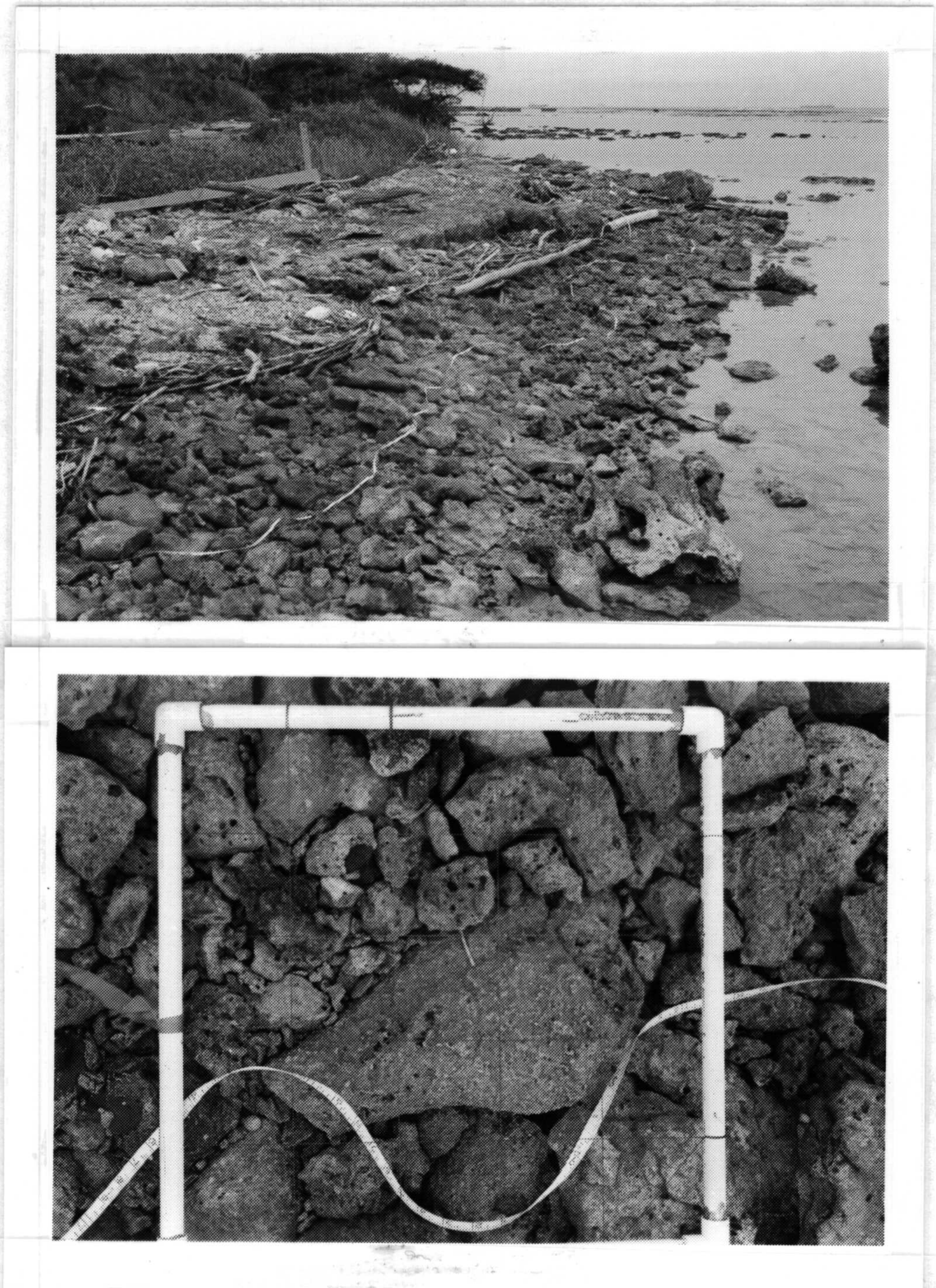

Fig. 5.4 Low rubble zone, May 1989 (continued). *C.* General view, GNRF. Note litter at high tide mark. *D.* Close-up of 0.25-m^2 quadrat, GNRF. No snails are visible; note tar balls in the *lower right* quadrat and outside the quadrat to the *upper right*.

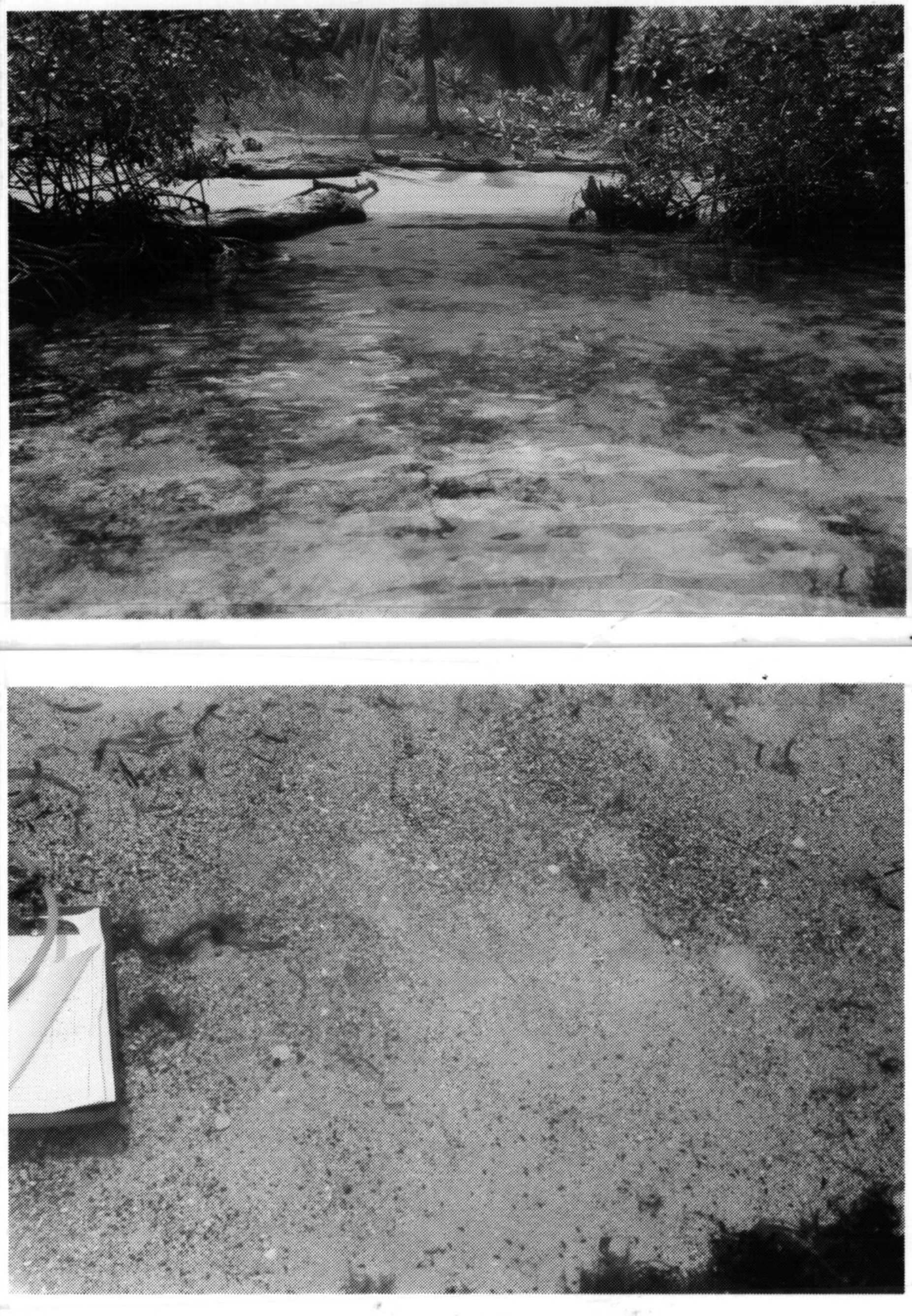

Fig. 5.5 Sand zone, May 1989. *A.* General view, MSR. Note lack of fringing hard substratum. The trees are red mangrove, *Rhizophora mangle*. *B.* Close-up of 0.25-m^2 quadrat, MSR. Clipboard is visible to the *left* for scale. Small black snails in the *foreground* are *Batillaria minima*.

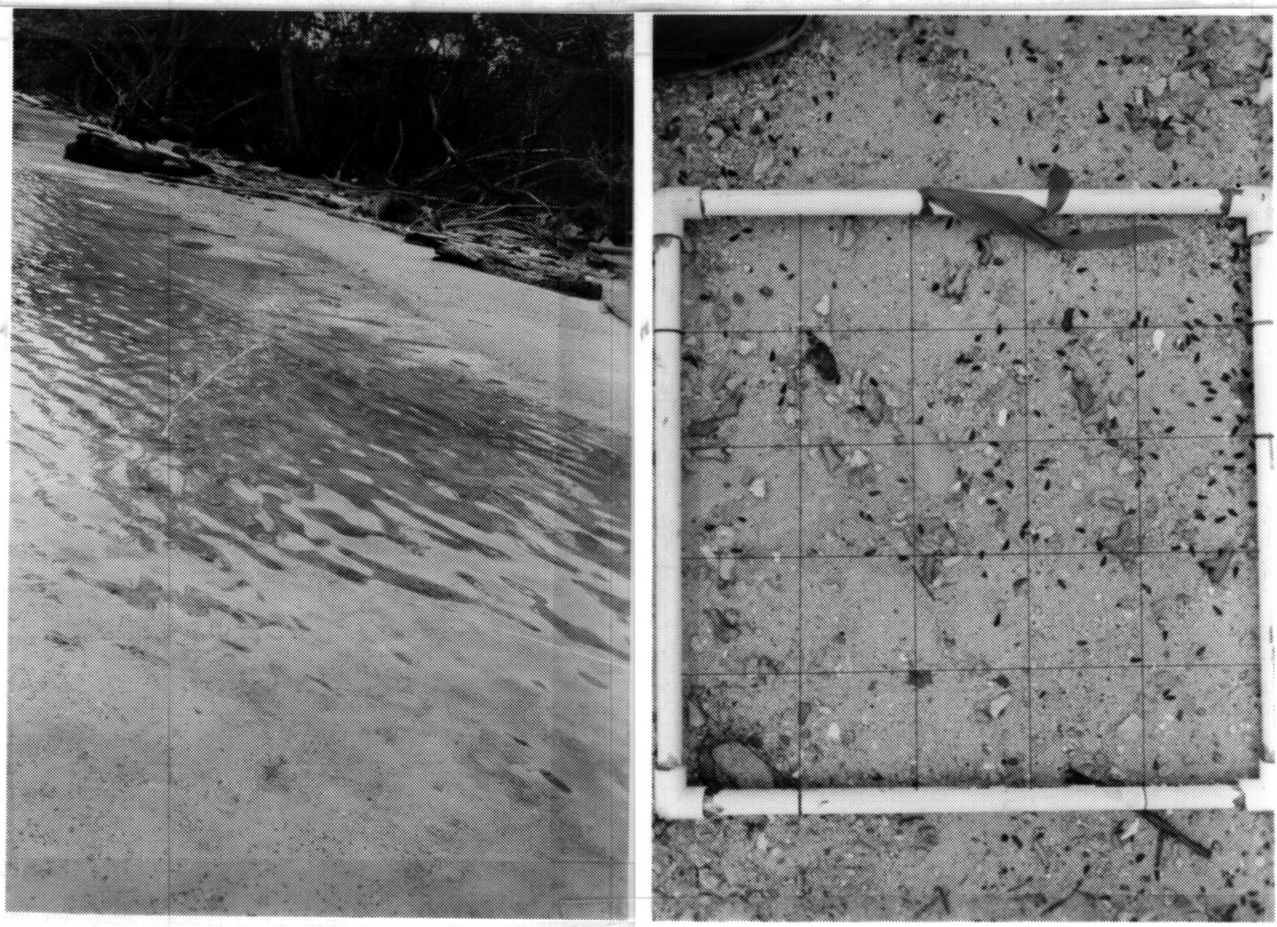

Fig. 5.5 Sand zone, May 1989 (continued). *C.* General view, GNRF. *D.* Close-up of 0.25-m^2 quadrat, GNRF. *Batillaria minima* is abundant within this quadrat.

5.3 Objectives, Rationale, and Design

5.3.1 Overview

In August 1986, roughly 3 mo after oil began coming ashore on the Punta Galeta reef flat, we initiated quarterly monitoring of snail abundance and size structure both at GNRF and at unoiled sites using methods identical to those in 1982-1983. These data provide an opportunity to examine effects over time of a major oil spill on the gastropods of a tropical reef flat. We present (1) data taken at GNRF before the oil spill, (2) data from 12 quarterly, postspill monitorings of the GNRF site (oiled), and (3) comparative data from 12 quarterly monitorings of control (unoiled) reef flats. A control site was oiled by a small diesel spill in May 1988; we discuss its effects and recovery of this site relative to overall results through May 1989, when the study ended.

Fig. 5.6 Reef rock zone, May 1989. *A.* General view, TPR. *B.* Close-up of 0.25-m^2 quadrat, TPR. Note pocked and creviced nature of rock. The chiton *Acanthopleura granulata* is in the *upper left*.

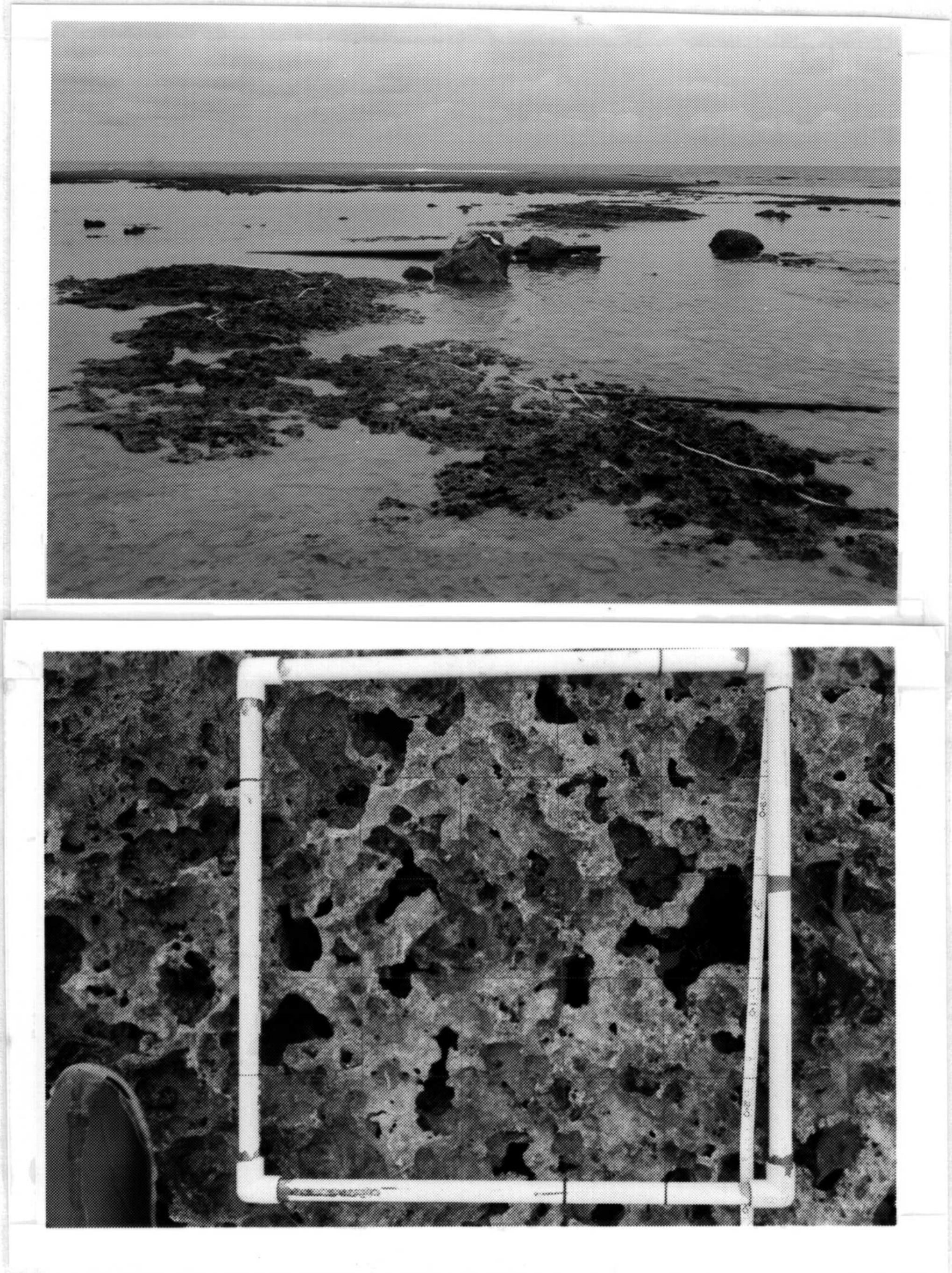

Fig. 5.6 Reef rock zone, May 1989 (continued). *C.* General view, GNRF. Note how deeper water isolates this zone. *D.* Close-up of 0.25-m^2 quadrat, GNRF. Substratum is pocked and creviced, as at TPR.

Fig. 5.7 *Thalassia* zone, May 1989. *A*. General view, MSR, high tide. Object in the *foreground* is a clipboard for scale. *B*. Close-up of 0.25-m^2 quadrat, MSR. Clipboard is visible to *right* for scale.

Time considerations limited monitoring to one replicate of each of the six oiled and unoiled habitats (henceforth referred to as *zones*). This lack of replication of zones within treatments prevents direct statistical comparison between oiled and unoiled treatments. Our data are thus best used to indicate the magnitude of reductions in mollusc populations at GNRF from 1983 levels and to follow population events at this site over time (*main sequence 2* of Green 1979). Population differences for common species between unoiled (Toro Point and María Soto) and oiled sites (GNRF) over time are also useful; we suggest some of these are related to oiling.

5.3.2 Sites

Due to its location in front of the U.S. Navy antenna site at Punta Galeta (Fig. 5.1), the section of reef flat sampled was protected from human interference. Beginning in August 1986, unoiled reef flats were sampled similarly for gastropod

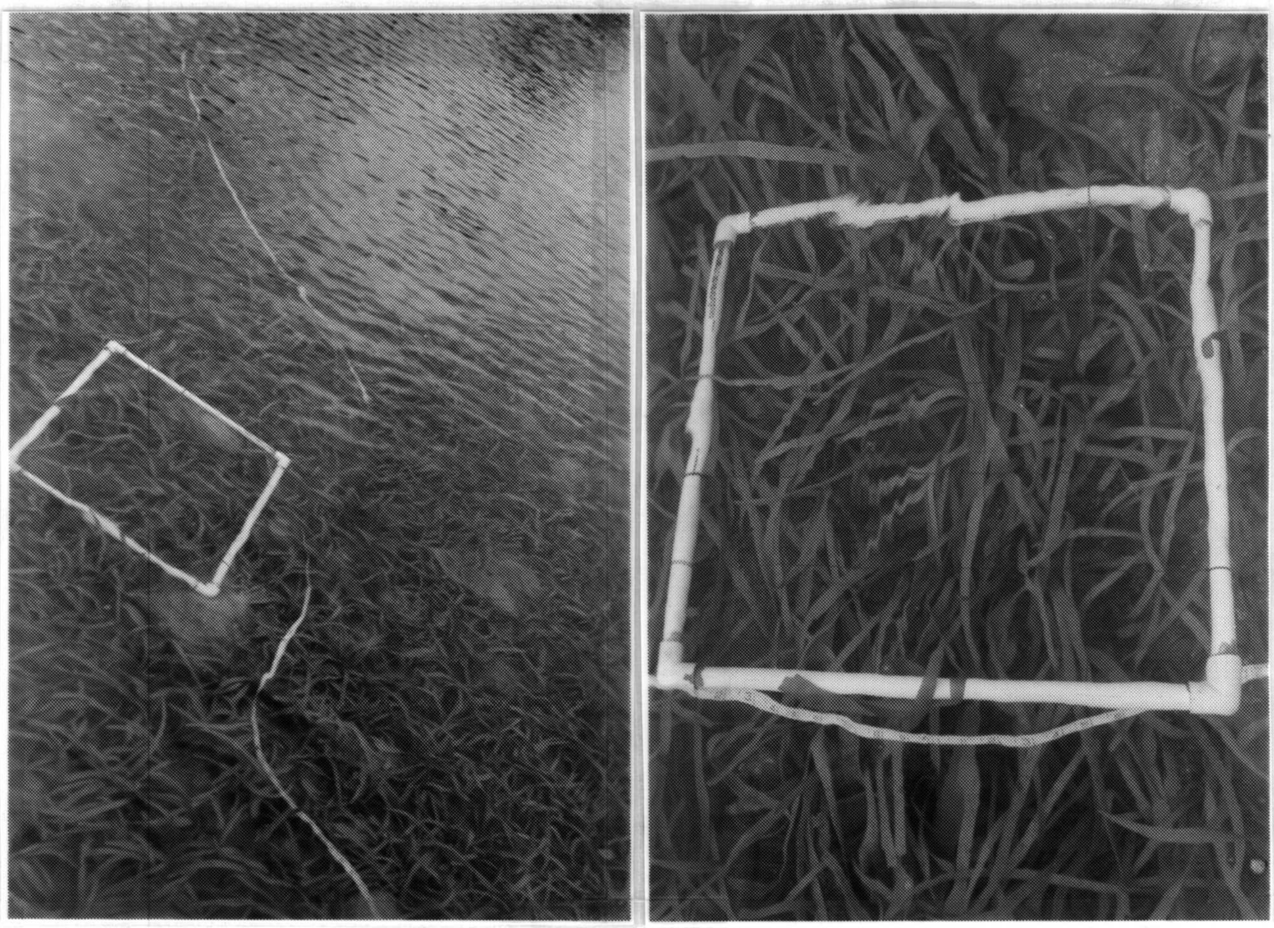

Fig. 5.7 *Thalassia* zone, May 1989 (continued). *C.* General view, GNRF, high tide. *D.* Close-up of 0.25-m^2 quadrat, GNRF. No snails are visible.

distribution, abundance, and size structure. Two unoiled sites were used because no one area had all the habitats found at GNRF. The unoiled flats were located (1) west of Toro Point (TPR) where high rubble, low rubble, reef rock, and *Laurencia* zones were monitored, and (2) seaward of María Soto (MSR) where we monitored gastropods found on sand and in the *Thalassia* bed.

At both TPR and GNRF some erosion of the surface was observed in the high rubble habitat during the dry season (December-March) of 1987-1988. This erosion followed periods of heavy wave action. Using the same quadrats used to measure density in May 1988, we visually estimated the extent of erosion as percent cover of newly eroded substratum. Further, on 14 May 1988, a week prior to quarterly monitoring, at least part of TPR was contaminated by a small diesel spill from a grounded sailboat. The monitoring immediately after this event is marked by an arrow on all figures, and observations are presented in subsequent sections. We again estimated percent cover of oil in the high rubble, low rubble, and reef rock zones at both TPR and GNRF in November 1988.

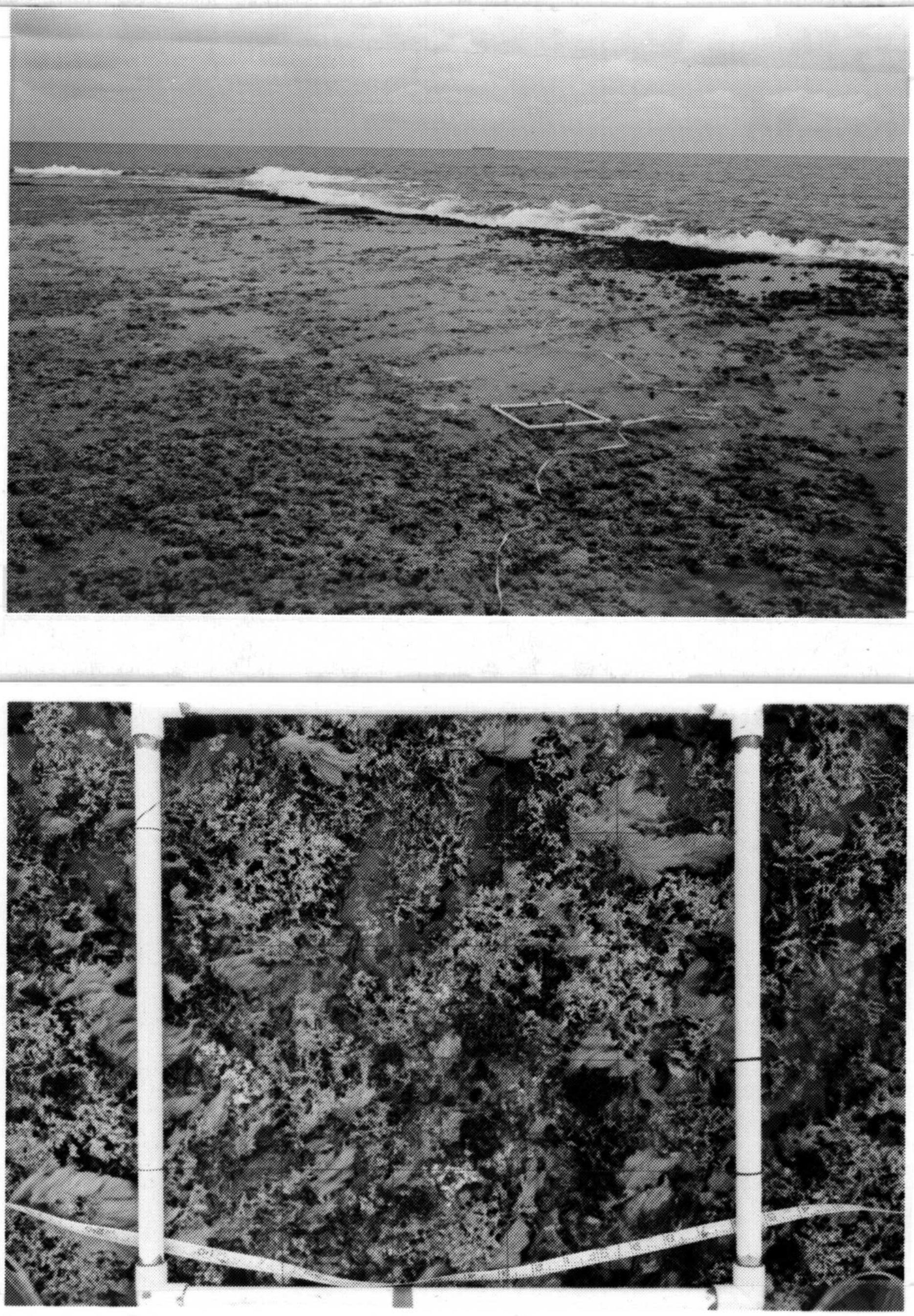

Fig. 5.8 *Laurencia* zone, May 1989. *A.* General view, TPR. Ships anchored in the *background* are awaiting transit through the Panama Canal. *B.* Close-up of 0.25-m^2 quadrat, TPR. Note bleaching of some algae.

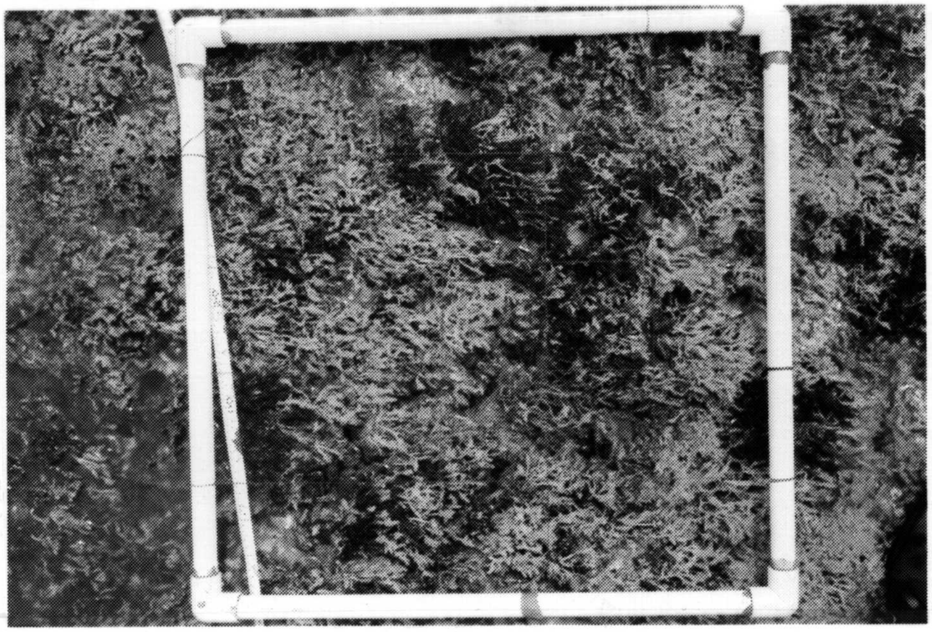

Fig. 5.8 *Laurencia* zone, May 1989 (continued). *C.* General view, GNRF. Most algae are bleached, except those on the outer fringes of the reef. *D.* Close-up of 0.25-m^2 quadrat, GNRF. No snails are visible. Most algae are bleached.

Table 5.1 Snail abundance by zone at Galeta Navy Reef, 1982-1983. Data are mean number (1 SD) per 0.25 m^2. $N = 20$ quadrats except in the *Thalassia* and *Laurencia* zones, where $N = 30$. - = absent.

Species	High Rubble	Low Rubble	Reef Rock	*Thalassia*	Sand	*Laurencia*
Tectarius muricatus	6.1 (8.6)	-	-	-	-	-
Nodilittorina tuberculata	2.8 (3.7)	0.9 (1.7)	0.1 (0.2)	-	-	-
Littorina angustior	10.8 (14.2)	8.8 (9.0)	1.0 (4.0)	-	-	-
L. lineolata	3.6 (5.2)	3.3 (4.4)	-	-	-	-
L. ziczac	0.3 (0.7)	1.3 (2.7)	0.2 (0.9)	-	-	-
Melampus coffeus	0.3 (1.1)	-	-	-	-	-
Nerita versicolor	0.7 (1.6)	11.4 (16.6)	-	-	-	-
N. fulgurans	-	0.8 (1.5)	0.3 (0.6)	-	-	-
N. peloronta	-	0.3 (0.7)	-	-	-	-
N. tessellata	-	12.1 (22.7)	10.8 (9.5)	-	-	-
Planaxis nucleus	-	0.6 (2.2)	-	-	-	-
L. meleagris	-	-	1.0 (2.8)	-	-	-
Gastropod sp. 1	-	-	0.1 (0.2)	-	-	-
Cerithium ebemeum	-	-	-	3.0 (5.8)	-	-
C. literatum	-	-	-	12.1 (14.7)	-	-
Thais rustica	-	-	-	0.03 (0.2)	-	-
Batillaria minima	-	-	-	-	96.4 (97.3)	-
Neritina virginea	-	-	-	-	0.3 (0.9)	-
Gastropod sp. 2	-	-	-	-	0.1 (0.3)	-
Astrea ?phoebia	-	-	-	-	-	0.2 (0.5)
Cyprea zebra	-	-	-	-	-	1.4 (2.6)
Diodora dysoni	-	-	-	-	-	0.1 (0.2)
Leucozonia nassa	-	-	-	-	-	0.03 (0.2)
Smaragdia sp.	-	-	-	-	-	0.2 (0.4)
Thais deltoidea	-	-	-	-	-	0.3 (0.9)
Nudibranch sp.	-	-	-	-	-	0.1 (0.4)
Gastropod sp. 6	-	-	-	-	-	0.03 (0.2)

5.4 Oiling and Reoiling

The following additional data were collected soon after the spill at GNRF. In August 1986 the amount of oil adhering to the substratum in each habitat was assessed. In the high rubble, low rubble, and reef rock habitats, percent cover of oil was visually estimated within 20 randomly placed 0.25-m^2 quadrats. Extensive field notes were taken of oiling in the intertidal sand habitat and in *Laurencia* and *Thalassia* beds, where the nature of the substratum precluded percent cover measurements.

Oil from the 1986 spill was not deposited uniformly across GNRF. The high rubble zone, which is wetted only by spray and splash, had a percent cover of 2.3 ± 2.9% oil in August 1986 (mean ± 1 SD, $N = 20$ quadrats). In contrast, the low rubble and reef rock zones were heavily oiled. In the low rubble zone oil covered 69.0 ± 17.6% and diatoms growing on hardened oil averaged 15.5 ± 14.0% cover. On reef rock, hardened oil or oil overgrown by diatoms accounted for 91.7 ± 9.6% cover. Oil was present in the sand, *Thalassia*, and *Laurencia* zones, but the amount could not be quantified, as on the rocky substratum of the three zones higher in the intertidal. However, slicks and oil globules were seen at high tide in August 1986 (S. D. Garrity, pers. obs.), and oil seeped visibly from sand disturbed at low tide. In *Thalassia* beds plants were heavily covered with epiphytes, especially the red alga *Acanthophora spicifera*, blue-green algae, and diatoms. The *Laurencia* bed was heavily silted and had high cover of turf-like microalgae and bare space (17.5 ± 17.9%).

Control areas showed no oil cover attributable to the spill. No sign of oiling was found at MSR through the 3 yr of the study. However, at TPR, located adjacent to the Atlantic entrance to the Panama Canal, a few tar balls were noted initially and were found with increasing frequency in the low rubble and reef rock zones at each visit (Fig. 5.9). This low-level, chronic oiling appears related to ship traffic. Additionally, in May 1988 a 75-foot sailboat ran aground on the reef at TPR, releasing an undetermined amount of diesel fuel. An oily sheen was seen across the flat, and dead crabs and snails were found in several habitats (C. González, pers. obs.). The volatility of diesel fuel and the small size of the spill suggest this spill may have caused short-term effects, but was unlikely to have persistent effects relative to the 1986 Bahía Las Minas spill.

Percent cover of oil was monitored again at both GNRF and TPR in November 1988. In the high rubble zone at GNRF, 2.6 ± 4.8% (mean ± 1 SD, $N = 20$ quadrats) of the substratum was covered with oil, most of which was highly weathered mats of tar. This is approximately equal to the cover of oil in the high rubble zone immediately after the spill. More oil was recorded at TPR (5.3 ± 17.1%) because a large tar ball covered 76% of one quadrat. In the low rubble zone, oil cover had dropped to 3.6 ± 3.7% in November 1988, compared with ~75% cover in August 1986. Only traces of oil were recorded at TPR. Oil only covered 14.4 ± 21.6% of the reef rock zone at GNRF (vs. ~90% in August 1986); this remaining oil was heavily overgrown by diatoms. Only traces of oil were recorded at TPR in an equivalent area. Because of the continuing presence of tar balls and the May 1988 diesel spill, TPR should be considered lightly oiled compared to GNRF (heavily oiled) or MSR (unoiled).

5.5 Components of Research

5.5.1 Gastropod Abundances Prior to Oiling

5.5.1.1 Methods

Monitoring was done during daylight, and generally when water levels and wave action were low to moderate. Within each zone gastropod abundance was estimated by placing 0.25-m^2 quadrats at 20 to 30 randomly selected points along a

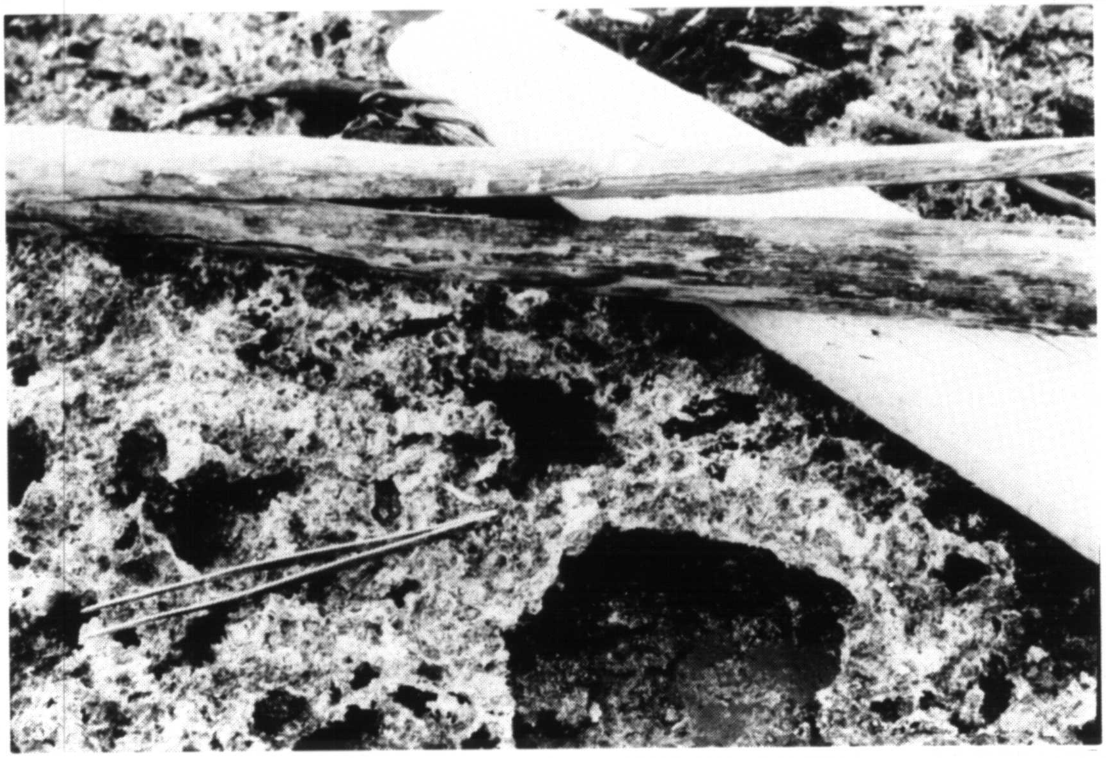

Fig. 5.9 Tar ball in the reef rock zone, TPR, May 1988. Forceps (*lower left*) are 25.4 cm long.

50-100 m transect line; all gastropods visible within each quadrat were identified and counted. We did not sample cryptic or burrowing gastropods because it would have involved considerable disturbance to most habitats. Prespill monitoring at GNRF was done between November 1982 and January 1983.

5.5.1.2 Results

Table 5.1 lists mean densities of gastropod species found in six reef flat habitats at GNRF in 1982-1983. The high rubble zone was characterized by the large-bodied, herbivorous snail *Tectarius muricatus* (mean density = 6.1/0.25 m^2) and the smaller *Littorina angustior* (10.8/0.25 m^2). *Nodilittorina tuberculata* (2.8/0.25 m^2) and *L. lineolata* (3.6/0.25 m^2) were also relatively common, while *L. ziczac*, *Nerita versicolor*, and *Melampus coffeus* were rare (<1 snail/0.25 m^2).

Nine species occurred in the low rubble habitat at GNRF prior to the spill. *Nerita tessellata* and *N. versicolor* were the most abundant (~12/0.25 m^2), and *N. fulgurans* and *N. peloronta* were rare (<1 snail/0.25 m^2). *Littorina lineolata*, *L. angustior*, and *L. ziczac* occurred in roughly the same abundance as in the high rubble zone, while *Nodilittorina* (we use generic names for genera with only one species present) was rarer (<1 snail/0.25 m^2) than higher on the shore. *Planaxis nucleus*, which forages under rocks, was present in low abundance (<1 snail/0.25 m^2).

Nerita tessellata was the most abundant snail (10.8/0.25 m^2) on reef rock at GNRF, while *Nerita fulgurans*, *Littorina ziczac*, *L. angustior*, *L. meleagris*, and *Nodilittorina* occurred in low density (≤1 snail/0.25 m^2).

Two species, *Batillaria minima* and *Neritina virginea*, occurred on intertidal sand at GNRF in 1982; *Batillaria* was patchily abundant (~100/0.25 m^2), while *Neritina* was rare (<1/0.25 m^2). *Thalassia* beds were characterized by two species of cerithids, *Cerithium eburneum* and *C. literatum* at a total density of ~15 individuals/0.25 m^2. These snails were found on and around *Thalassia* blades.

Laurencia beds had relatively high gastropod diversity, but low abundances in 1982 (total density <3 individuals/0.25 m^2). Both predaceous and herbivorous gastropods occurred within this habitat; the most common were *Cypraea zebra*, *Smaragdia* sp., and *Thais deltoidea*.

5.5.2 Initial Effects on Gastropod Populations: Oiling and Mortality of Snails

5.5.2.1 Methods

See Section 5.5.1.1; GNRF was first remonitored during July and August 1986, 3 mo after oiling.

To examine the size structure of common species of snails, individuals (usually ≥200) of each species were collected and shell lengths measured to the nearest mm. All snails were collected by hand from a separate series of contiguous 0.25-m^2 quadrats running from the upper to lower limits of each zone. Forceps were used to collect tiny individuals and those in crevices. Snails were measured with vernier calipers and returned immediately to the appropriate habitat. Snail species were identified and vouchers retained for verification. Size data were used to test for size-specific mortality following the spill.

During August 1986 sampling, snails within quadrats were recorded as having oiled or unoiled shells. In both August and November 1986 dead snails that were either (1) cemented by oil to the rock in the low rubble habitat or (2) found in the high rubble habitat (where movement of shells by wave action did not occur) were separately counted, along with live individuals in abundance transects. Dead snails were collected in these two habitats for size measurements. Size-selective mortality was examined by comparing the size-frequency distributions of live and dead snails collected at the same time and in the same habitat.

5.5.2.2 Results

Although the amount of oil deposited on the high rubble zone at GNRF was small (Sect. 5.4), a greater proportion of snails than of substratum was oiled in August 1986. Forty-eight percent of *Tectarius* counted in quadrats, 25% of

Nodilittorina, 28% of *L. angustior*, 17% of *L. ziczac*, and 6% of *L. lineolata* had oiled shells in August 1986. Dead individuals of 10 species were collected ($N = 283$ snails) within the high rubble zone outside of density quadrats (Table 5.2*A*). Included were *Nodilittorina*, *L. angustior*, *L. lineolata*, and *L. ziczac*, normally resident in the high rubble zone; *N. versicolor*, *N. tessellata*, *N. peloronta*, and *Planaxis nucleus*, usually found lower on the shore; and *Melampus* and *L. angulifera*, primarily inhabitants of mangroves (*N. versicolor* and *Melampus* were both rare in the high rubble zone before the spill). Live *L. lineolata* did not differ in size from empty shells (Table 5.3; $P > .1$, *G*-test with Williams' correction; Sokal and Rohlf 1981). For all other dead snails collected, too few live individuals were present for comparison.

Dead snails of 10 species were also collected from the high rubble zone in November 1986, about 7 mo after the spill ($N = 262$ snails); nine species appeared in density quadrats (Table 5.2*A*). Because all empty shells had been removed when found in August, these data represent mortality of snails between the August and November monitorings. They also suggest the higher proportion of oiled snails relative to oiled substratum in the high rubble zone (observed in August, above) was due to upward movement of oiled individuals following the spill. The absence of live individuals of species found dead in this zone supports this hypothesis (Table 5.2 vs. Table 5.4), but prevents a test for size-selective mortality.

Of the snails found in the low rubble zone in August 1986, 100% of *P. nucleus*, *L. angustior*, and *L. lineolata* had oiled shells, as did 68% of *N. versicolor* and 14% of *N. tessellata*. The single *L. ziczac* found was unoiled. Much of the low rubble zone was coated with a thick, viscous layer of oil (Sect. 5.4). We counted the number of dead snails stuck to or covered with oil (Table 5.2*B*) along with counts of live individuals, then removed the dead snails. At least five species were found (there were individuals of both *Littorina* and *Nerita* spp. too oiled to identify). Live and dead individuals of *N. versicolor* and *N. tessellata* were abundant enough to test for size-selective mortality; in both cases, mortality of small snails was greater than large ones ($P < .005$, *G*-test with Williams' correction; Table 5.3).

Individuals of four species were found dead in November 1986 (Table 5.2*B*). For *N. versicolor* and *P. nucleus*, small snails died more frequently than large ones (Table 5.3; $P < .005$, *G*-test with Williams' correction). The opposite was true of *N. tessellata* ($P < .005$, *G*-test with Williams' correction), but only 12 live *N. tessellata* were found. Seven of these were less than 8 mm long, while the smallest live snail found in August was 9 mm long.

5.5.2.3 Summary

Effects of the spill appeared minor in the high rubble zone. Wetted only by wave splash, this zone received only small amounts of oil. Many snails found in the first 6 mo after the spill had oil on their shells, and some newly dead individuals were recovered in August and November 1986. However, it is likely that these individuals had moved upwards from the low rubble zone in response to oiling below

Table 5.2 Abundance of dead snails in the high and low rubble zones at Galeta Navy Reef. Data are mean number (1 SD) per 0.25 m^2. $N = 20$ quadrats; + = present, but not found in quadrats; - = absent. *Nerita* spp. and *Littorina* spp. are individuals that were too oiled to identify to species.

Species	August 1986	November 1986
A. High Rubble Zone		
Tectarius muricatus	-	+
Nodilittorina tuberculata	+	0.1 (0.31)
Littorina angustior	+	0.3 (0.55)
L. ziczac	+	0.2 (0.67)
L. lineolata	+	0.1 (0.22)
L. angulifera	+	0.1 (0.22)
Nerita tessellata	+	1.0 (1.50)
N. versicolor	+	0.3 (0.73)
N. peloronta	+	-
Melampus coffeus	+	0.9 (1.50)
Planaxis nucleus	+	0.3 (0.57)
B. Low Rubble Zone		
Nerita fulgurans	0.6 (1.15)	-
N. versicolor	0.9 (1.40)	+
N. tessellata	0.3 (0.92)	+
Nerita spp.	1.6 (1.88)	-
Littorina ziczac	0.1 (0.22)	-
L. angustior	-	+
Littorina spp.	0.8 (1.27)	-
Planaxis nucleus	0.4 (0.67)	+

(*Littorina angulifera*, the mangrove littorinid, was observed to move up to 10 m in trees following oiling in the mangroves; S. D. Garrity, pers. obs.).

In contrast, data suggest that snails were killed initially by oil in the low rubble zone at GNRF. Species number and snail abundance were sharply reduced in the reef rock zone, and initial effects were suggested by data in the sand zone.

The spill had no detectable effects in *Thalassia* beds, given the variation in populations over time. In *Laurencia* beds, data from GNRF suggest a reduction in snail populations after the oil spill.

5.5.3 Initial Effects on Gastropod Populations: Effects of Reef Cleanup Crew, August-September 1986

5.5.3.1 Methods

See Section 5.5.1.1; less than 1 wk after the first postspill monitoring, a cleanup crew manually removed oil and oiled debris from the high and low rubble

Table 5.3 Size-selective mortality in the high and low rubble zones at Galeta Navy Reef. Data are number of snails in size classes, living or dead, after the oil spill (August or November 1986).

		Size (mm)																	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	P^1
High Rubble Zone																			
Littorina lineolata																			
8/86	Live				2	4	2	4	2	1									>.1
	Dead				16	17	17	23	48	7									
Low Rubble Zone																			
Nerita versicolor																			
8/86	Live				1	2	2	1	10	10	8	22	25	26	12	12	4		<<.005
	Dead		2	1		9	5	14	19	9	22	28	25	9	7	1	1		
11/86	Live					1	4	3	23	24	33	37	33	17	10	2	1	1	<<.005
	Dead				3	3	3	2	5	4	7	5	6	1	2	1			
N. tessellata																			
8/86	Live						2	7	14	27	34	22	22	15	5	1			<<.005
	Dead		1	4	9	11	19	21	24	42	54	36	37	16	6	1			
11/86	Live	2	2	2	1						1	2	1	1					<<.005
	Dead			2	4	2	6	3	7	10	17	19	9	5	1				
Planaxis nucleus																			
11/86	Live						1	2	5	10	21	7	4						<<.005
	Dead				2	1	2	4	9	6									

[1] Calculated using the *G*-test with Williams' correction (Sokal and Rohlf 1981).

habitats and used water hoses to blow oil from the sand habitat. These three habitats were remonitored within 2 wk to assess changes brought about by the cleanup.

5.5.3.2 Results

Gastropod abundances at GNRF were monitored twice in August-September 1986, before and immediately after the activities of a cleanup crew (Table 5.4). Cleanup occurred in the high and low rubble zones, where oiled rocks, rubble, and other debris were collected by hand and removed by wheelbarrow, and in the sand zone, where cleaning was attempted using saltwater pumps and hoses. Here, oil, snails, and small detritus were washed seaward several meters into deeper water (S. D. Garrity, pers. obs.).

In the high rubble zone 6 of 7 species found before cleanup were less abundant immediately afterward, with the abundance of *Tectarius* dropping 50%. One rare species increased slightly in abundance, and two new species appeared. In

Table 5.4 Density of gastropods before and after cleanup activities in the high rubble, low rubble, and sand zones at Galeta Navy Reef, 1986. Data are mean number (1 SD) per 0.25 m^2. $N = 20$ quadrats, each monitoring.

	August Postspill	August-September Post-cleanup	November
A. High Rubble Zone			
Littorina angulifera	0.1 (0.2)	0	0
L. angustior	14.8 (12.8)	13.0 (18.9)	10.4 (13.5)
L. lineolata	0.9 (1.4)	0	0.4 (0.8)
L. ziczac	0.6 (1.6)	0	0.8 (0.3)
Nodilittorina tuberculata	2.5 (3.5)	1.4 (2.7)	1.1 (1.2)
Tectarius muricatus	14.8 (10.2)	7.2 (13.3)	12.1 (11.3)
Nerita versicolor	0.1 (0.2)	0.7 (2.7)	0
L. nebulosa	0	0.1 (0.2)	0
N. peloronta	0	0.1 (0.3)	0
N. tessellata	0	0	0.6 (2.5)
Melampus coffeus	0	0	0.1 (0.5)
B. Low Rubble Zone			
Littorina angustior	0.2 (0.7)	1.4 (5.8)	1.0 (1.3)
L. ziczac	0.1 (0.2)	0	0.5 (1.1)
Littorina spp.[1]	0.1 (0.2)	0	0
Nerita tessellata	1.1 (2.6)	0.1 (0.2)	0.1 (0.3)
N. versicolor	1.3 (2.2)	0.3 (0.9)	4.3 (4.0)
Planaxis nucleus	0.1 (0.2)	0	1.2 (2.2)
P. lineatus	0	0.1 (0.5)	0
Tectarius muricatus	0	0.1 (0.5)	0
Melampus coffeus	0	0	0.1 (0.5)
C. Sand Zone			
Batillaria minima	11.1 (41.2)	0.1 (0.2)	0
Cerithium eberneum	24.3 (69.3)	0	0
Neritina virginea	0	0	415.0 (497.4)

[1] Too oiled to identify to species.

the low rubble zone 4 of 5 species decreased in abundance or disappeared, one increased, and two new species were found after cleanup. In the sand zone the most abundant snail disappeared, and another common species decreased in density by a factor of 10 following cleanup.

Changes in the high and low rubble zones appeared ephemeral. Table 5.4 also shows snail abundances for the following quarterly monitoring (November 1986). Four species which had appeared in the two rubble zones after cleanup did not persist. However, reductions in density of *Batillaria* and *C. eburneum* in the sand zone continued. These two species disappeared, and a third species, *Neritina virginea*, not previously found, appeared in high density.

5.5.3.3 Summary

Activities of a cleanup crew resulted in disturbance to some species in the high rubble zone, but this appeared temporary. In the low rubble and sand zones, some species showed a further reduction in abundance immediately after these activities.

5.5.4 Long-term Effects on Gastropod Abundance

5.5.4.1 Methods

See Section 5.5.1.1. Reef flat habitats were monitored quarterly after the spill until May 1989.

5.5.4.2 Results

High Rubble Zone

Overall Abundance

After the spill total snail abundance (all species combined) was similar (~30 snails/0.25 m^2) at TPR and GNRF in August and November 1986 (Fig. 5.10). From February 1987 through May 1989, however, density was greater at TPR than GNRF in all 10 monitorings. When we compared the number of species found at each monitoring, more species occurred at TPR in 8 of 12 instances, with one tie. More species were found at GNRF in the first two monitorings after the spill (when two "nonresident" species, *L. angulifera* and *Melampus*, were found) and in May 1988 (after a diesel spill at TPR).

Individual Species Abundances

The data in Figure 5.11 show the relative contribution of common species to overall snail abundance at each site. Between November 1986 and February 1987 a single species, *Littorina lineolata*, increased markedly in abundance at TPR only. This caused most of the difference in abundance between sites. Another contributor, *Littorina angustior*, was initially abundant only at GNRF, then disappeared during the same period. Two species, *Nodilittorina tuberculata* and *Tectarius muricatus*, showed roughly similar changes in abundance at both sites.

Figures 5.12 and 5.13 show long-term abundance data for individual species in this zone. Despite some gastropod mortality immediately after the spill (Sect. 5.5.2), abundances of most species at GNRF in August 1986 were roughly similar to those in 1982. *Tectarius* was the exception, having more than doubled in density (to ~15/0.25 m^2). The unoiled site at TPR, first monitored in August 1986,

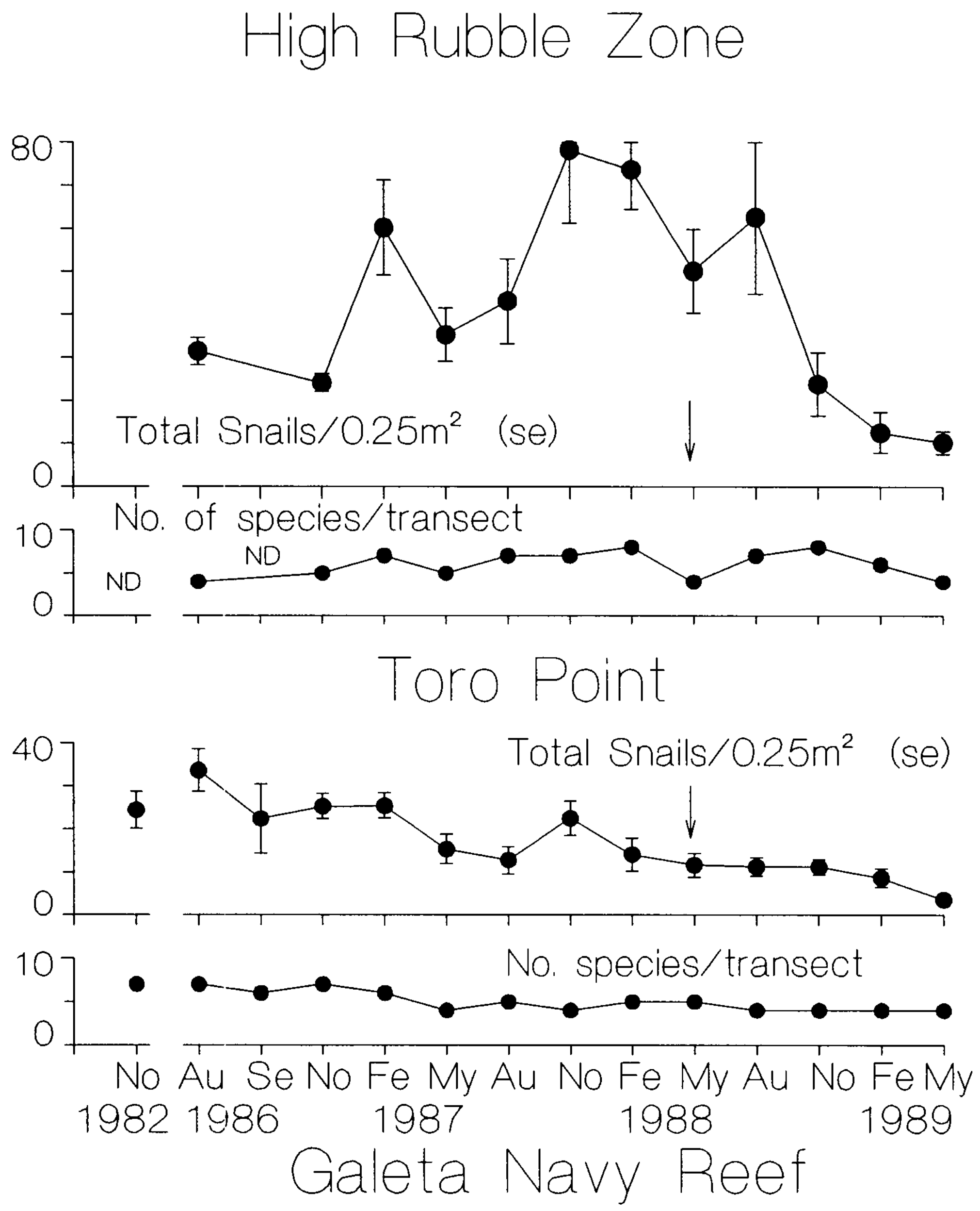

Fig. 5.10 Total gastropod abundance and number of species in the high rubble zone. Abundance data are mean densities (SE) of all gastropod species combined for each site at each sampling date. *Vertical arrow* indicates the diesel spill at TPR in May 1988. Number of species is the total recorded in 20 0.25-m^2 quadrats at each sampling date. *ND* = no data.

had three main differences from GNRF: *L. angustior* was absent, *L. ziczac* was far more common than at GNRF, and only four species were recorded.

Density at both oiled and unoiled sites remained about the same in November 1986, except for a decrease in *L. ziczac* at TPR and an increase in *Tectarius* at

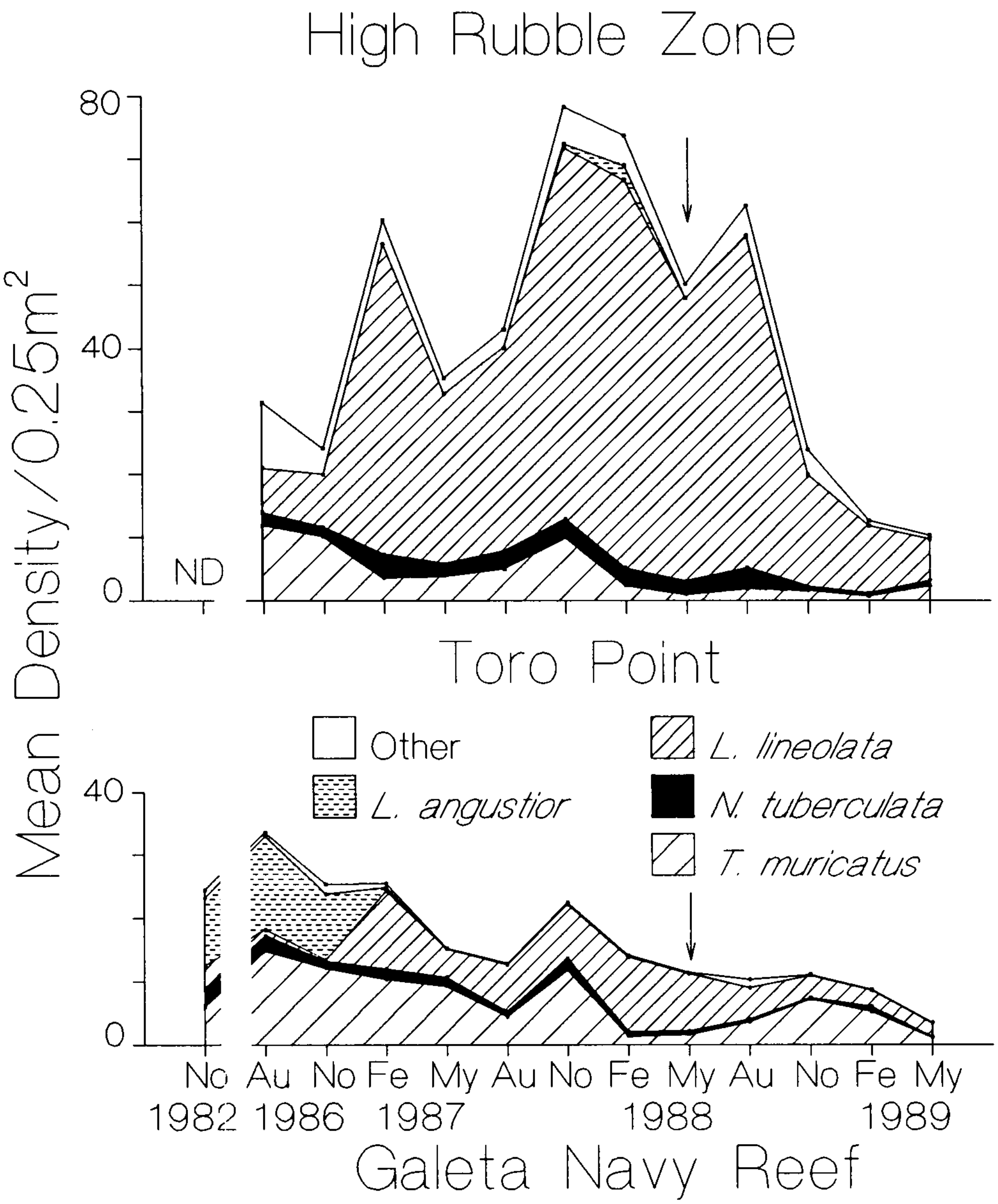

Fig. 5.11 Abundance of ***Littorina lineolata*, *L. angustior*, *Nodilittorina tuberculata*, *Tectarius muricatus*, and other gastropods combined in the high rubble zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.**

GNRF. Both oiled and unoiled sites had markedly increased numbers of *L. lineolata* in February 1987. *L. angustior*, abundant in all previous monitorings at the oiled site, essentially disappeared in February 1987. *L. ziczac* became rare at the unoiled site at the same time, and *Tectarius* also dropped sharply in abundance. *L. ziczac*

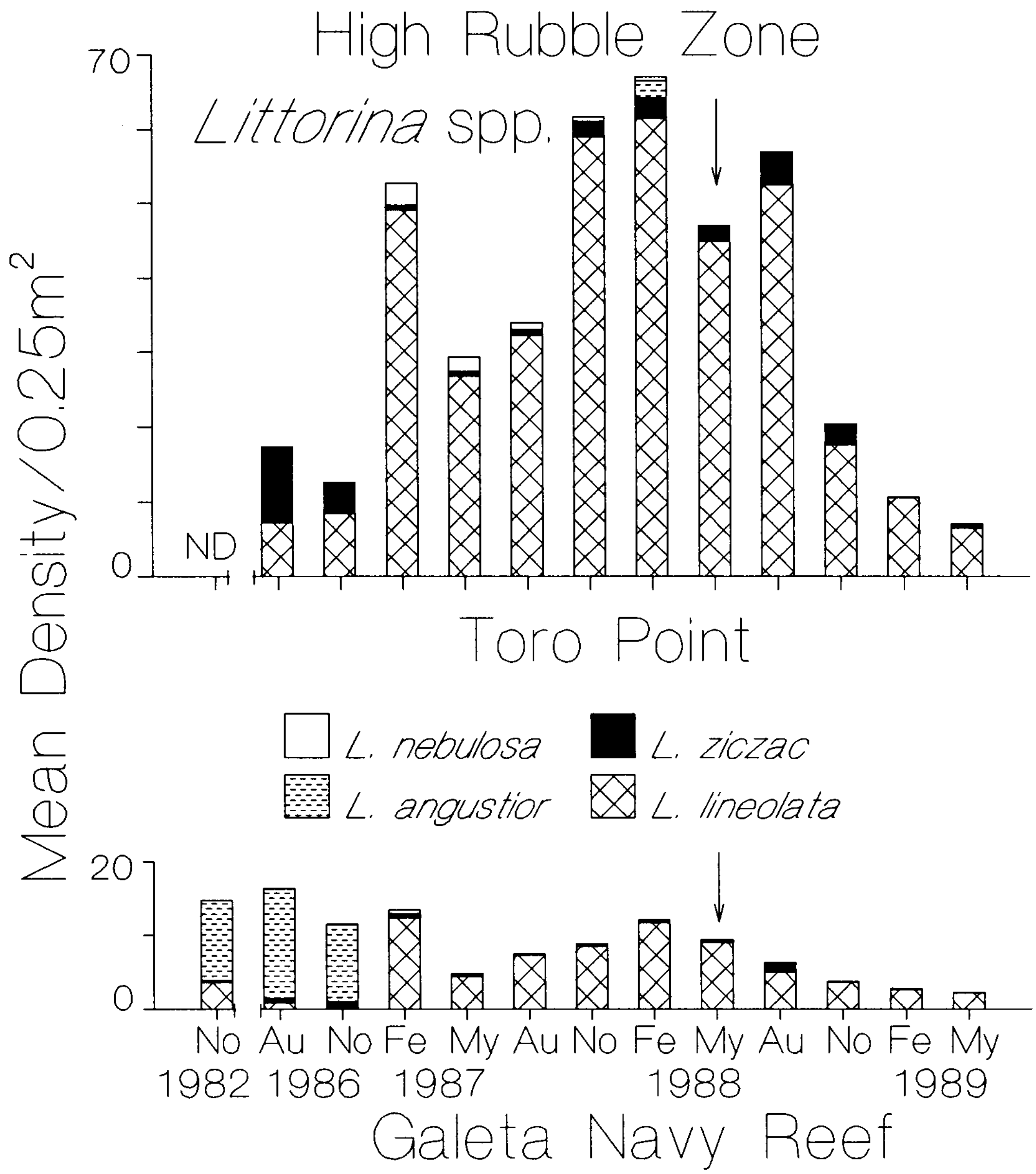

Fig. 5.12 Abundance of species of *Littorina* in the high rubble zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.

remained rare at both sites (< 1 snail/0.25 m^2) through the next several monitorings (rainy season), then increased at TPR during late rainy/early dry season 1987-1988 (2-4/0.25 m^2). *L. lineolata*, after a decrease in May 1987, increased at both sites through dry season 1988, then decreased again in May 1988.

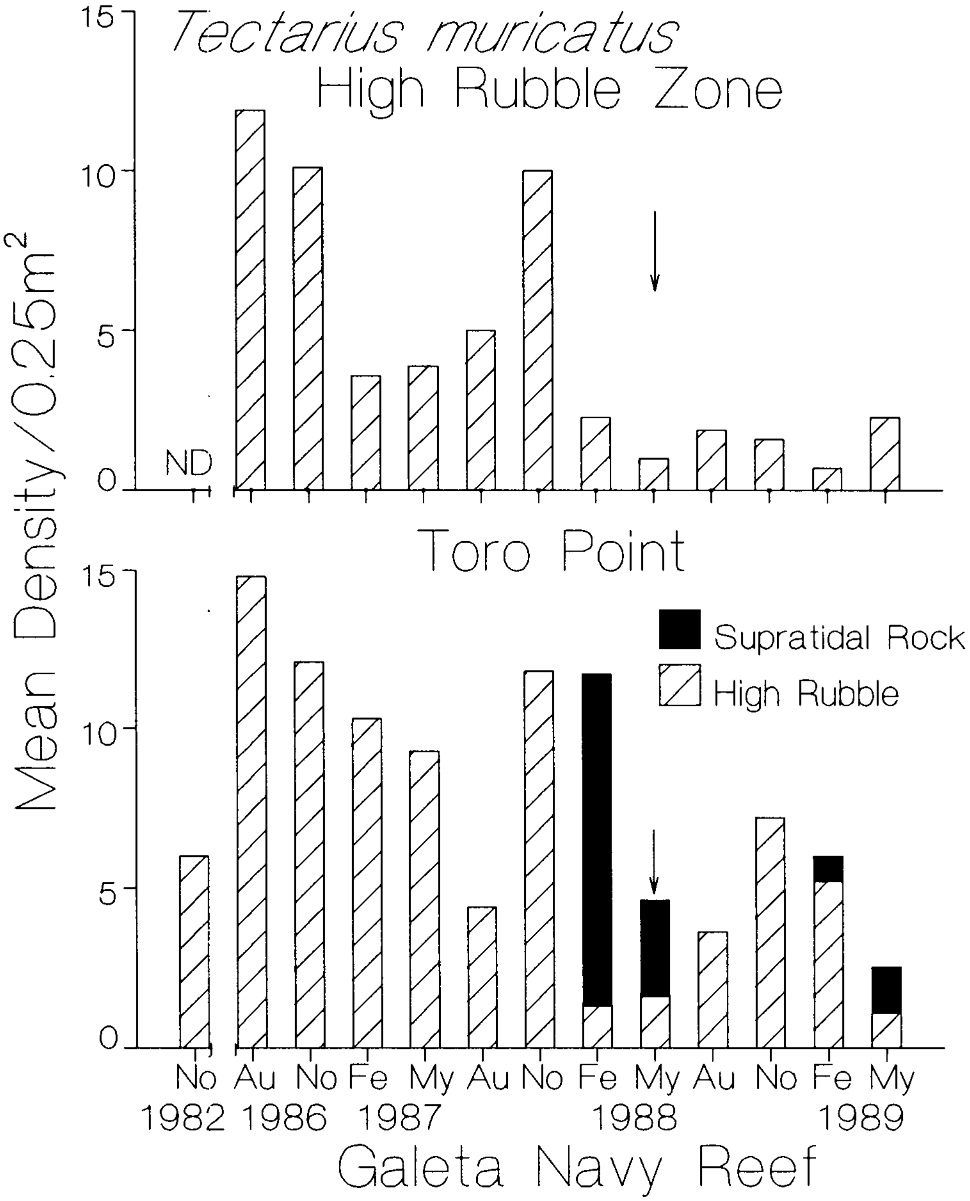

Fig. 5.13 Abundance of *Tectarius muricatus* in the high rubble zone. Data are means of 20 quadrats at each sampling date. Solid portions of bars for GNRF are means of 20 quadrats run along a transect line above the high rubble zone when snails were observed in the supratidal. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.

The 1988 dry season was windy, with extremely high wave action (R. C. Thompson, pers. comm.; S. D. Garrity, pers. obs.). By May up to 75% of the substratum in the high rubble zone at GNRF had been recently eroded (mean = 24.8%, range = 0-75%, N = 20). There was somewhat less erosion at TPR (mean = 10.5%, range = 0-50%, *N* = 20). In February 1988 *Tectarius* decreased greatly in abundance at both GNRF and TPR. At GNRF this reduction in number was partly caused by an upward migration of snails into supratidal debris (mean density ± 1 SD of *Tectarius* above its normal range was 10.5 ± 8.1/0.25 m^2, *N* = 20 quadrats; Fig. 5.13). In May 1988 *Tectarius* was rare at GNRF in both the high rubble zone and above its normal range (2.9 ± 3.3/0.25 m^2). This suggests delayed mortality. Similar reductions in density were observed at TPR, where less supratidal rock was available. The absence of a supratidal refuge equivalent to that at GNRF apparently led to greater immediate *Tectarius* mortality during storm conditions, and probably caused the reduction in numbers of *Tectarius* in February 1987 as well.

In May 1988 density of all species dropped at both GNRF and TPR (Figs. 5.10, 5.11); this also occurred in February to May 1987. It may reflect a regular migration of snails to lower levels of the shore at the end of the dry season, when water levels predictably drop and the reef flat is exposed for long periods (Cubit et al. 1986). However, at TPR there was a small spill of diesel fuel just prior to the May monitoring. Species number dropped from 8 to 4 (the four rarest species disappeared). *N. versicolor* was one; it had been present in 4 of 7 previous monitorings. This species died in large numbers in the lower intertidal after the diesel spill and may have been affected indirectly in the high rubble zone. Members of the genus *Nerita* typically exhibit rhythmic feeding migrations (e.g., Levings and Garrity 1983; Peckol et al. 1989), making them likely to be affected by conditions in a broad range of the intertidal. Overall, the data suggest that the diesel spill at TPR in May 1988 had effects in the high rubble zone only on *N. versicolor* and perhaps on three rare species of *Littorina*. Density returned to previous levels in August 1988.

Over the next four monitorings (August 1988-May 1989), *L. lineolata* slowly continued to decline at GNRF; at TPR, after a small increase in August 1988, it also declined through the remainder of the study. Rare species, including *L. ziczac* and *L. nebulosa*, also became rarer or disappeared at both sites. This general trend was correlated with further physical degradation of the habitat at both sites through continued erosion by wave action (S. D. Garrity, pers. obs.).

Low Rubble Zone

Overall Abundance

Total gastropod abundance in the low rubble zone was generally greater, but more variable at TPR than at GNRF (Fig. 5.14). For 3 yr following the spill, snail density was higher at TPR in 10 of 12 monitorings. In 2 of the 3 yr, snail density was highest in mid-rainy season (August) at the unoiled site, while there was little seasonality evident at GNRF (Fig. 5.14). At GNRF overall snail density remained 50-95% lower than it had been in 1982-1983 until August 1988 (over nine quarterly monitorings).

The number of species found per transect was higher at GNRF than TPR in 7 of 12 monitorings; the unoiled site had more or the same number of snail species

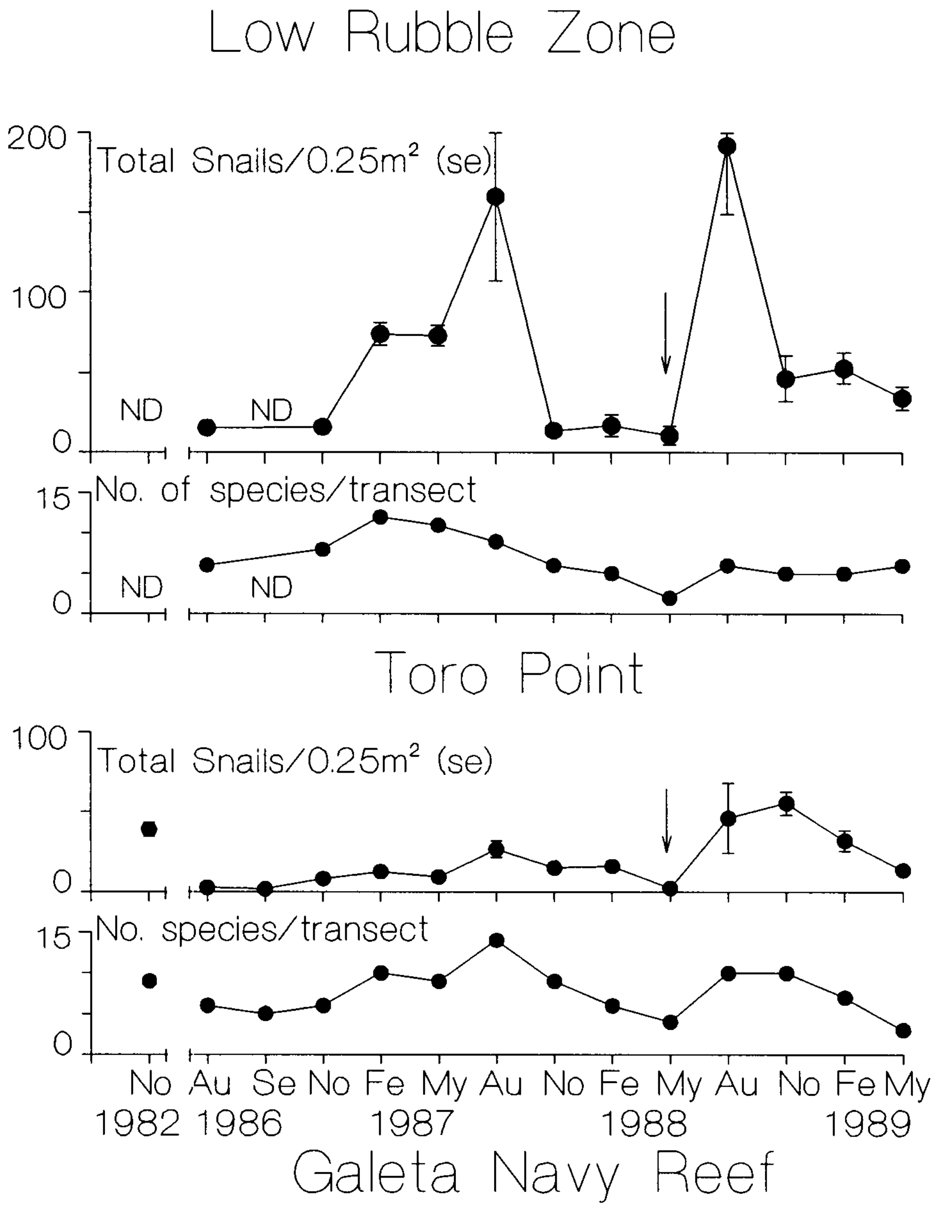

Fig. 5.14 Total gastropod abundance and number of species in the low rubble zone. Abundance data are mean densities (SE) of all gastropod species combined for each site at each sampling date. *Vertical arrow* indicates the diesel spill at TPR in May 1988. Number of species is the total recorded in 20 0.25-m^2 quadrats at each sampling date. *ND* = no data.

only for the first four monitorings following the spill and in the final (May 1989) monitoring (Fig. 5.14).

Individual Species Abundances

The relative contributions of various species or groups to overall snail abundance are shown in Figure 5.15. Littorinids and *Planaxis* showed the strongest differences between oiled and unoiled sites, while neritids did not differ as much. At GNRF only, neritids appeared to have a seasonal cycle of abundance, with lowest densities in May (early rainy season) in all 3 yr examined. At TPR littorinid abundance decreased greatly through rainy season in 1987 and never recovered. *Planaxis*, the major contributor to overall snail density, also showed highly seasonal patterns of abundance, with peaks in mid- to late rainy seasons 1987 and 1988 at TPR (and to a lesser degree at GNRF).

Figures 5.16 and 5.17 show individual species densities for littorinid and neritid snails. In the first monitoring after the spill, only five species were found at GNRF (Fig. 5.14, August 1986). *Nerita fulgurans*, *N. peloronta*, *L. lineolata*, and *Nodilittorina* disappeared there between 1982 and August 1986; abundances of remaining species dropped roughly by a factor of 10 (Figs. 5.16, 5.17). Immediately following activities of the cleanup crew, *L. lineolata*, *L. ziczac*, and *P. nucleus* disappeared while a few *P. lineatus* appeared (Table 5.4).

In November 1986 four species present in 1982 were absent, and one new species appeared (Tables 5.1 and 5.4). The latter, *Melampus coffeus*, usually is found in mangrove detritus. Of the two previously most abundant species, *N. tessellata* dropped further in density between August and November 1986, while *N. versicolor* increased. There were no major changes in density for any species of gastropod at the unoiled site (TPR) during the same period (Figs. 5.16, 5.17).

In February 1987 there were increases in density for 10 species (most notably *L. lineolata* and *N. versicolor*) at TPR (Figs. 5.16, 5.17). Three of these species also increased at GNRF (*N. tessellata*, *L. nebulosa*, and *Nodilittorina*), but abundances remained comparatively low (Figs. 5.16, 5.17). In contrast to events in the high rubble zone, most species in the low rubble zone did not decrease markedly in density in May 1987 (early rainy season); only *N. tessellata* and several rare species (combined as "other" on Fig. 5.16) became less abundant at GNRF. At TPR most species declined slightly, and *N. tessellata* increased (Figs. 5.16, 5.17).

In August 1987 *P. nucleus* (Fig. 5.15) and *N. fulgurans* (Fig. 5.17) markedly increased in abundance at the unoiled site, and *N. versicolor*, *N. tessellata*, and *P. nucleus* increased at GNRF (Figs. 5.15, 5.17). Most other species declined in abundance at both the oiled and unoiled site, notably *L. lineolata* and *L. angustior* (Fig. 5.16), or at TPR only (*N. versicolor* and *N. tessellata*, Fig. 5.17).

In November 1987 snail density at TPR dropped markedly. The two species whose densities had increased greatly in August were relatively rare in November. Only *N. tessellata* increased in density, and three previously rare species were not found. At GNRF the three species that had increased in abundance in August, as well as *L. ziczac*, markedly decreased. Three species that had been rare in August were not found in November samples, but *L. lineolata* increased by a factor of 20 (Figs. 5.15, 5.16, 5.17).

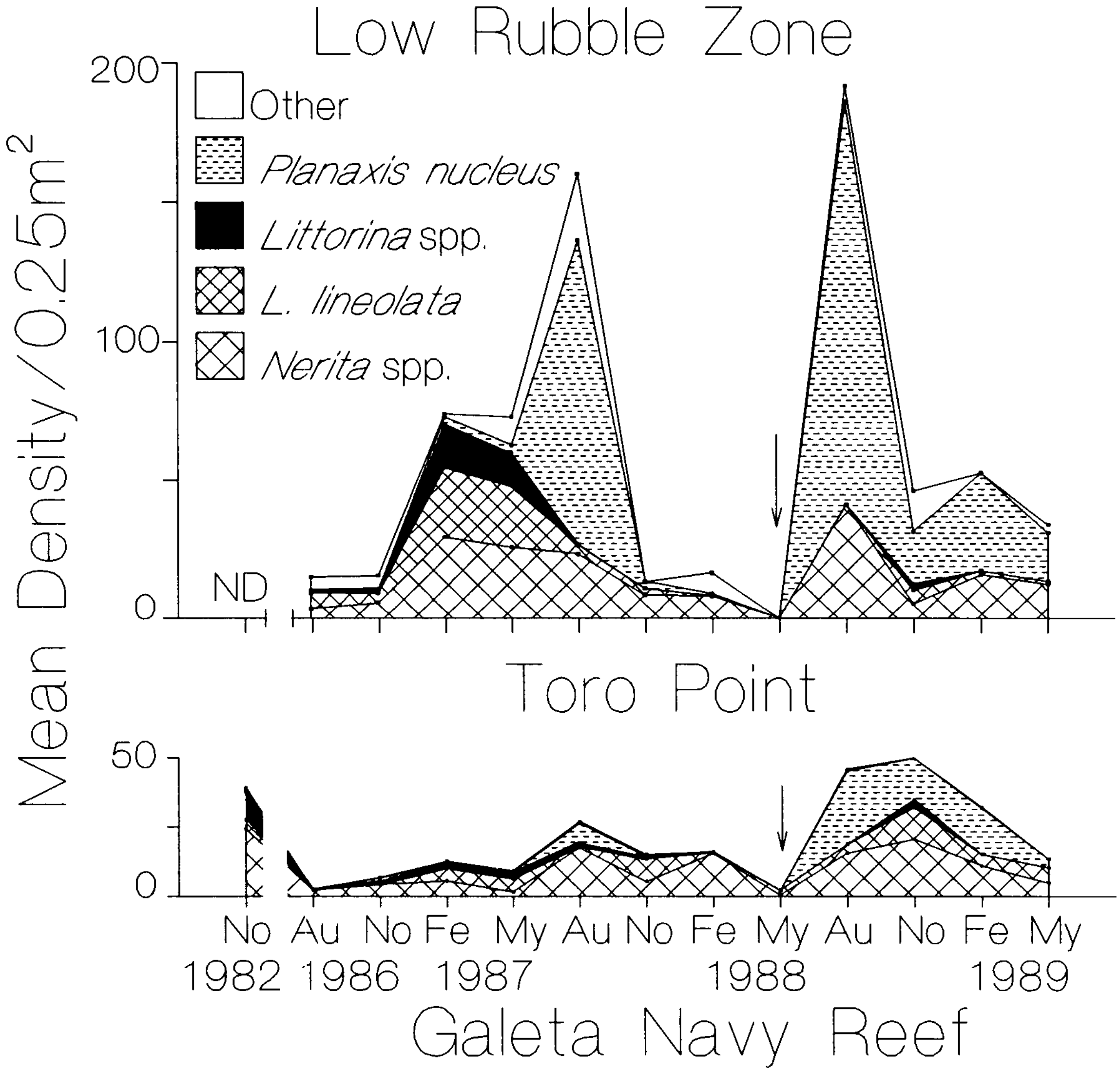

Fig. 5.15 Abundance of *Planaxis nucleus*, *Littorina* spp., *L. lineolata*, *Nerita* spp., and other gastropods combined in the low rubble zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.

Densities of snails in February 1988 were generally lower than in November 1987 at both sites. A notable exception was *Nerita tessellata* at GNRF; it increased by a factor of 10 (Fig. 5.17). The number of species decreased at both sites as well (from 9 to 6 at GNRF, and from 6 to 5 at TPR). This contrasted sharply to events seen the previous February. Field notes document storm damage and erosion of the habitat between November 1987 and February 1988.

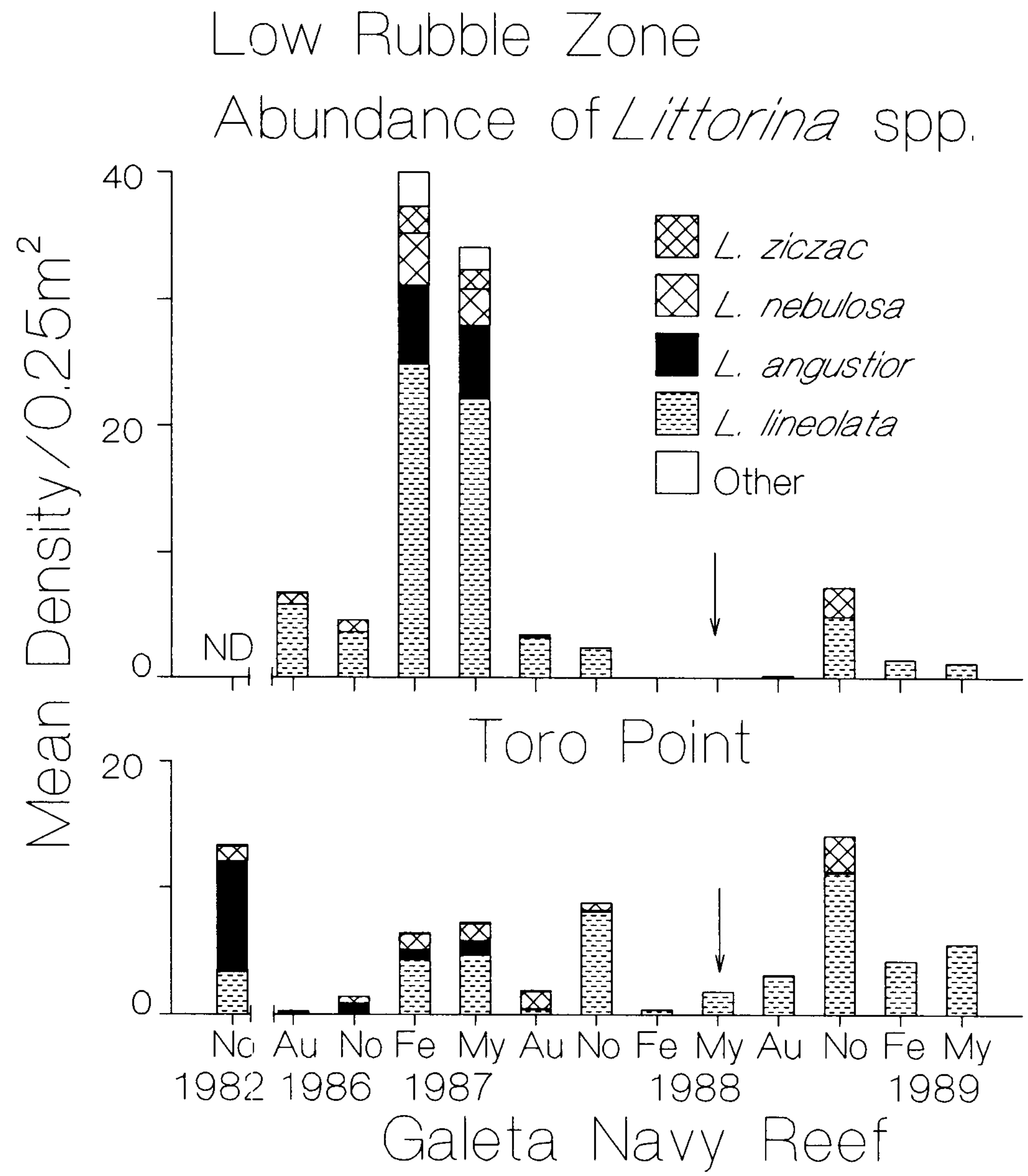

Fig. 5.16 Abundance of species of *Littorina* in the low rubble zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.

For most species, density at both GNRF and TPR dropped considerably between February and May 1988 (Figs. 5.15-5.17); only *L. lineolata* at TPR showed a small increase. The overall decreased densities, perhaps due to continuing effects of rough dry season weather, complicate interpretation of the effects of the diesel spill that occurred only at TPR. However, there is some evidence for mortality due

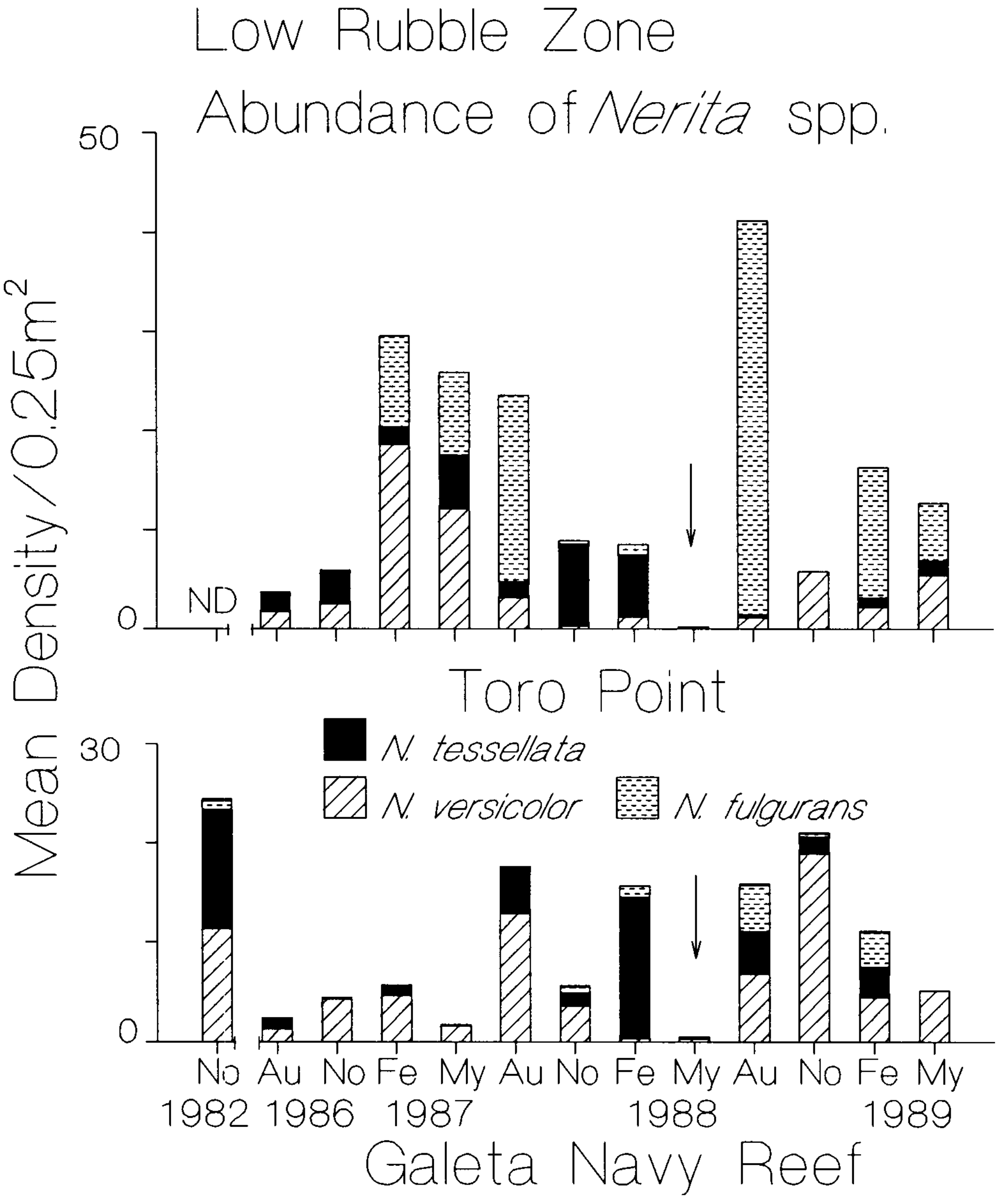

Fig. 5.17 Abundance of species of *Nerita* in the low rubble zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.

to the spill. Only two species were found at TPR in May, both at greatly reduced density; five species were recorded in February. All *Batillaria minima* in density quadrats were dead (mean density of dead snails ± 1 SD = 10.3 ± 25.1, $N = 20$). At GNRF total snail density also dropped, largely as a result of changes in numbers of *N. tessellata*. Three rare species disappeared (of six present at the previous monitoring), while one was added (*Nodilittorina*).

At the next monitoring (August 1988), three neritid species, particularly *Nerita fulgurans* (Fig. 5.17), as well as *Planaxis nucleus* (Fig. 5.15), had increased in abundance both at TPR and GNRF. Littorinid abundances remained low, although *L. lineolata* continued to increase slowly at GNRF only (Fig. 5.16). In November 1988 *N. fulgurans* disappeared from both sites, and *N. versicolor* increased (Fig. 5.17). *L. lineolata* continued to increase at GNRF and reappeared, along with *L. ziczac* (both sites) at TPR (Fig. 5.16).

After November 1988 littorinid abundance declined overall, with disappearances of *L. ziczac* at both sites, and a decrease in *L. lineolata* as well (with a slight increase in the latter at GNRF in May 1989; Fig. 5.16). In February 1989 *N. versicolor* declined in abundance at both sites, while *N. tessellata* and *N. fulgurans* reappeared at both sites. Neither species occurred at GNRF in May 1989; *N. versicolor* was the only neritid found. At TPR *N. versicolor* and *N. tessellata* were more abundant than in February, but *N. fulgurans* was rarer.

Reef Rock Zone

Overall Gastropod Abundance

The effects of oiling were severe in the reef rock zone. Total gastropod abundance was higher at TPR than at GNRF in 11 of 12 monitorings; only in May 1988, following the localized diesel spill, was snail abundance as low at TPR as at GNRF (Fig. 5.18). Similarly, the number of gastropod species found per transect was higher at TPR at all monitorings except May 1989. Like overall snail abundance, species number at TPR was lowest immediately after the May 1988 diesel spill, although there were still more species of molluscs found (three) than at GNRF (one).

The relative contribution of species or groups to overall patterns of abundance in the reef rock zone is shown in Figure 5.19. Both littorinids and neritids, present before the spill, were almost entirely absent from the reef rock zone at GNRF for 2 yr following the spill. Even after 3 yr, density at GNRF was <7.5 snails/0.25 m^2 (all species combined). At TPR there were 4-10 neritids/0.25 m^2, except after the diesel spill (May 1988), when they were rare (0.2 snails/0.25 m^2). At TPR littorinids were both more abundant and variable than neritids, with no apparent pattern of seasonality and lowest density immediately after the diesel spill. Several rare species, including *Purpura patula* and *Acanthopleura granulata*, were found at TPR in 9 of 12 monitorings, but only one individual was found at GNRF.

Individual Species Abundances

At GNRF in August 1986, of the species found prior to the spill only two species of neritids remained, and both were less abundant than in 1982 (Fig. 5.20; the

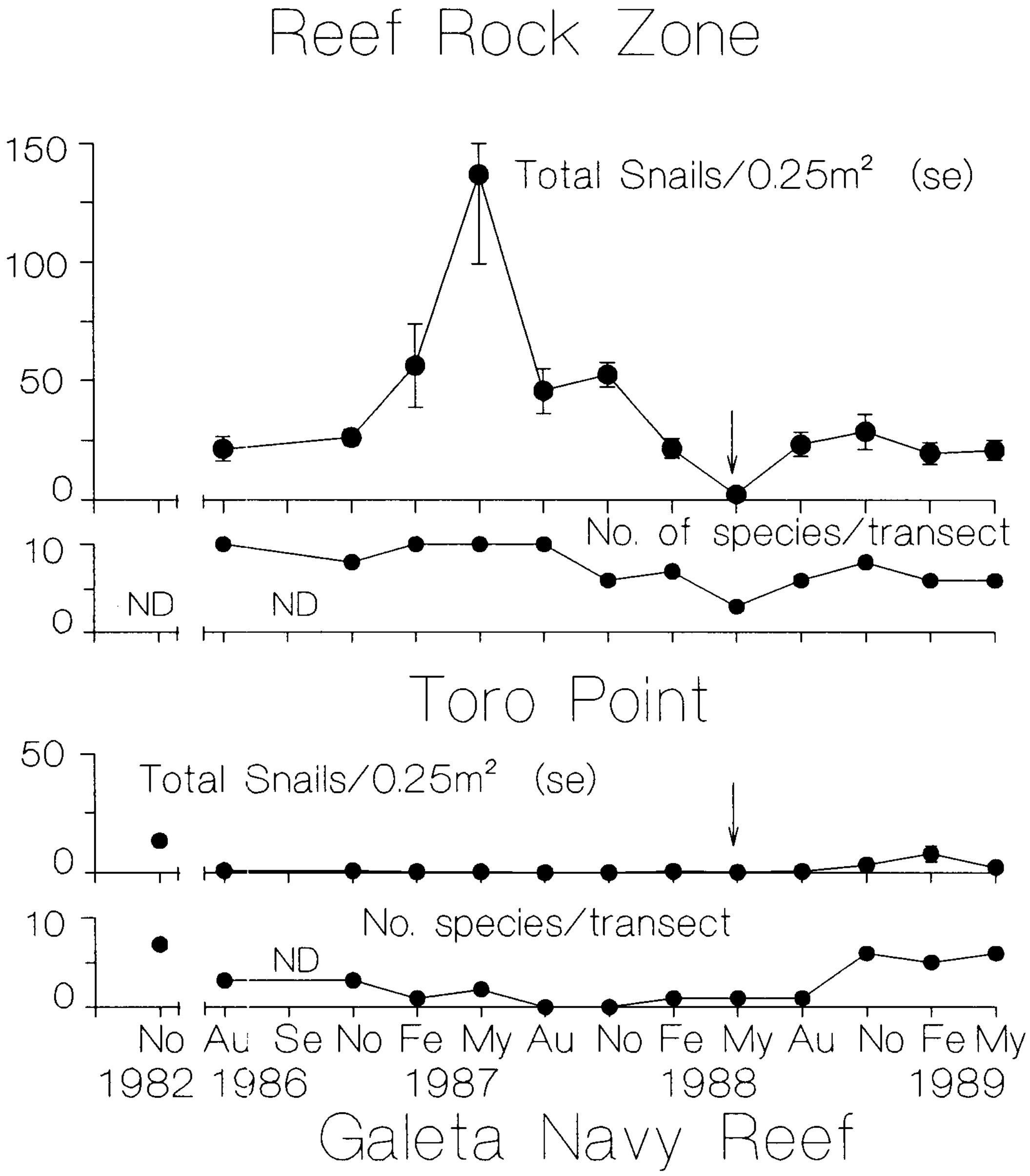

Fig. 5.18 Total gastropod abundance and number of species in the reef rock zone. Abundance data are mean densities (SE) of all gastropod species combined for each site at each sampling date. *Vertical arrow* indicates the diesel spill at TPR in May 1988. Number of species is the total recorded in 20 0.25-m^2 quadrats at each sampling date. *ND* = no data.

mean density of *N. fulgurans* in August 1986 was 0.1/0.25 m^2 and cannot be seen in the figure). *N. fulgurans* was not found between August 1986 and August 1988. *N. versicolor*, absent in 1982, was present in August 1986 (mean density = 0.1/0.25 m^2, not visible in Fig. 5.20), but was not recorded from May 1987 through February 1989.

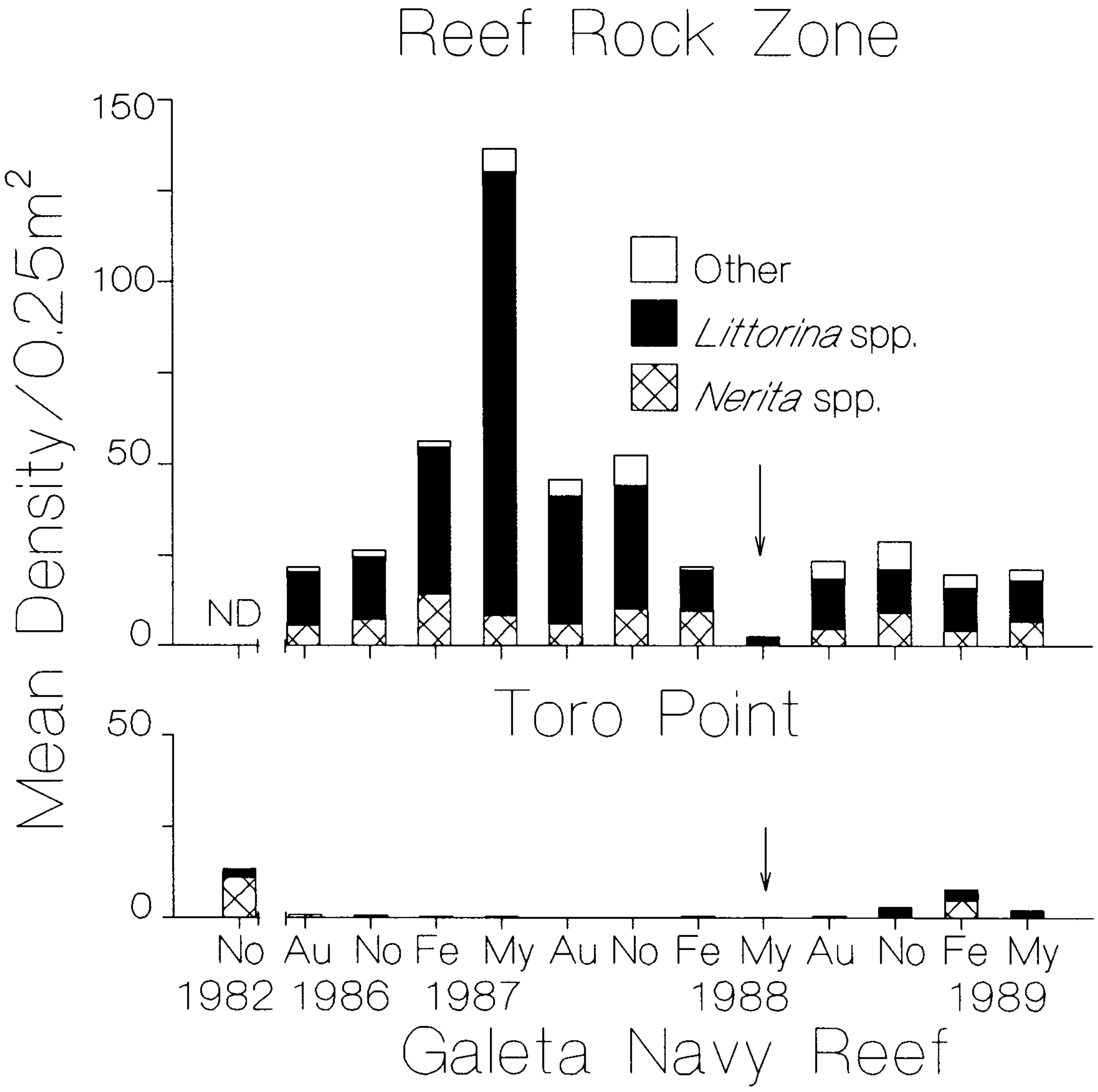

Fig. 5.19 Abundance of *Littorina* spp., *Nerita* spp., and other gastropods combined in the reef rock zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.

N. tessellata remained rare, never reaching a density of 1 snail/0.25 m^2. No littorinids reappeared through August 1988. A single chiton, *Acanthopleura granulata*, was found in November 1986. Recovery appeared to begin in earnest only in 1989 and was not complete in May when the study ended.

At the unoiled site the reef rock zone had more vertical relief and was more emergent than at GNRF. Three or four species of neritids were counted during all monitorings except May 1988, when only one species was found.

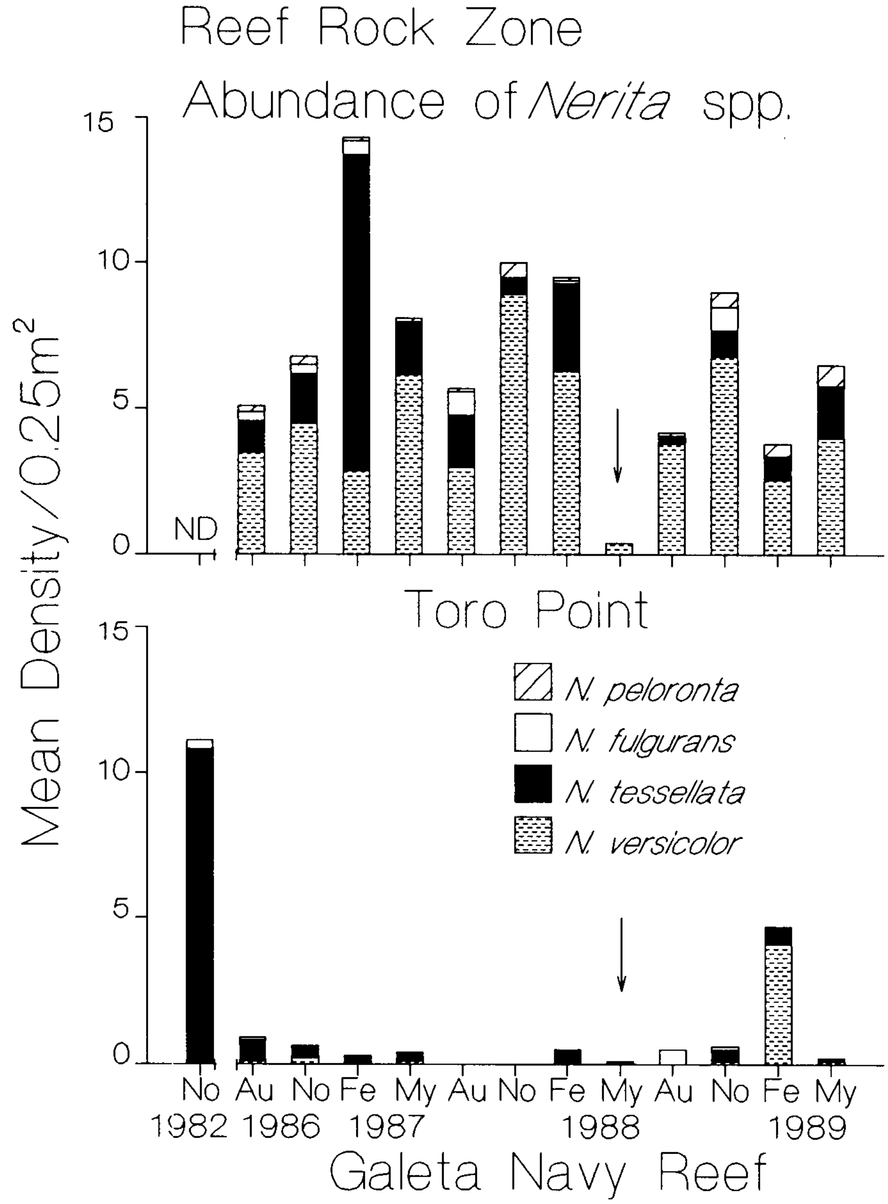

Fig. 5.20 **Abundance of species of *Nerita* in the reef rock zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. *ND* = no data.**

Four species of littorinids occurred in the reef rock zone (Fig. 5.21). Numbers of *L. lineolata* fluctuated, but remained high through February 1988, dropping in May 1988. *L. ziczac* remained rare until August 1988. *L. angustior* varied from 5 to 28 snails/0.25 m^2 between August 1986 and August 1987; none was recorded after August 1987. *L. meleagris* was ephemeral; it was found only in 1987.

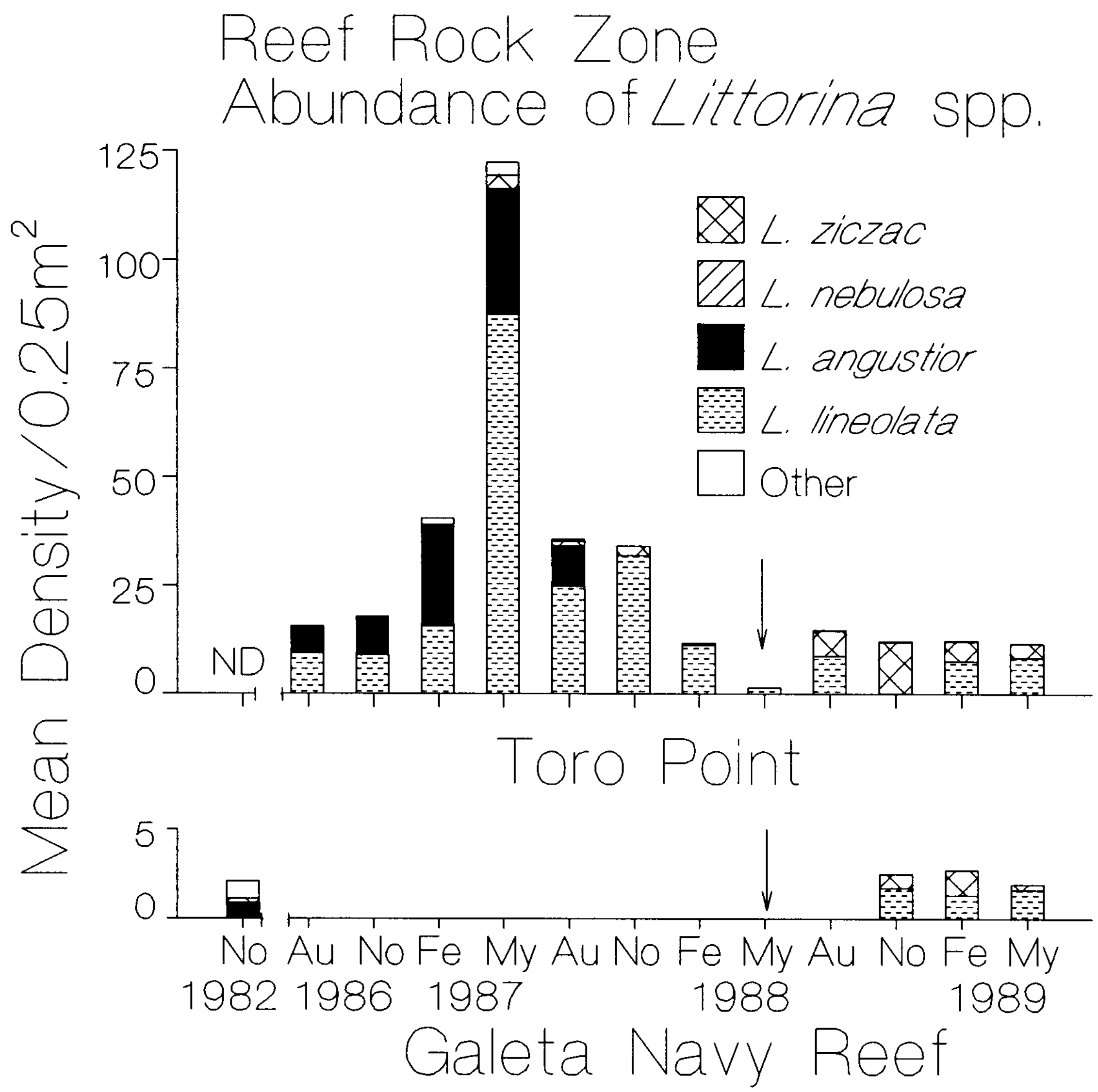

Fig. 5.21 Abundance of species of *Littorina* in the reef rock zone. *Vertical arrow* indicates the diesel spill at TPR in May 1988. Note difference in scales between TPR and GNRF. *ND* = no data.

Seven species were recorded at TPR in the reef rock zone in February 1988, at a total density of 21.8/0.25 m^2. In May only three species were found, with a total density of 2.4/0.25 m^2 (an order of magnitude fewer snails than in the seven previous monitorings). On 23 May more than 120 dead *N. versicolor* and numerous dead crabs were counted in a 5-m^2 area. Dead individuals of five species (*N. tessellata*, *N. versicolor*, *N. peloronta*, *N. fulgurans*, and *Tectarius*, *N* = 81 total, most *N. tessellata*) were found on 1 June. These data strongly suggest an immediate effect of diesel at

TPR in this zone. Density recovered in August 1988 and remained similar to that in February 1988 for the duration of the study.

Sand Zone

At GNRF *Neritina* was not found and *Batillaria* abundance was far lower in August 1986 than in 1982 (Tables 5.1, 5.5). No live *Batillaria* were found in November 1986. Only a few *Batillaria* were found at each monitoring until August 1987, and they were extremely rare again in November 1987. Since then, numbers fluctuated from <1 to >50 snails/0.25 m^2. *Cerithium eburneum*, absent in 1982, was common in August 1986. It was also washed seaward by cleaning, and only two individuals, found in August 1987, were counted afterward. The small-bodied *Neritina*, absent in August and September 1986, appeared in high density in November 1986. Its numbers gradually declined through 1987, and none was recorded after August 1987.

At the unoiled site (MSR), *C. eburneum* was absent in August and rare in November 1986, after which none was found. *Batillaria* was patchily abundant in all monitorings. *Neritina* was abundant in November 1986. None was found in February 1987 and only a few were counted in May 1987. None was recorded after the May 1987 monitoring.

Thalassia testudinum Zone

At GNRF in August 1986, *C. eburneum* was not found, and *C. literatum* was rare; in November 1986, neither species was found (Table 5.6). On the unoiled reef at MSR, both species occurred in low abundance during the August and November 1986 monitorings. Since then, snails were rare at both oiled and unoiled sites. No snails were recorded at either site after November 1987, except for a few *Batillaria* at MSR in August 1988 and February 1989.

Laurencia papillosa Zone

Gastropods were rare in this zone (Table 5.7). No gastropods were found in August and November 1986 at GNRF, but *T. deltoidea* reappeared in low abundance in February through August 1987. Two even rarer species were found then, *Olivella* in May 1987 and *Engina* in August 1987. No snails were found in sampling from November 1987 through May 1989.

At TPR 1 to 4 species were found in low abundance at each monitoring through August 1987. Since then, like at GNRF, no snails were recorded. No snails were found at TPR in May 1988 after the diesel spill and early wet season exposures. In July 1988 an unrelated search at this site for *Morum oniscus*, a cryptic species, yielded only dead individuals (C. González, pers. obs.).

Table 5.5 Density of gastropods in the sand zone. Data are mean number (1 SD) per 0.25 m^2. N = 20 quadrats.

		Galeta Navy Reef			María Soto		
		Cerithium ebemeum	*Batillaria minima*	*Neritina virginea*	*Cerithium ebemeum*	*Batillaria minima*	*Neritina virginea*
1986	August	24.3 (69.3)	11.1 (41.2)	0	0	901.3 (1137)	1.5 (6.5)
	November	0	0	415.0 (497.4)	0.3 (1.1)	172.8 (176)	91.5 (146.3)
1987	February	0	5.1 (13.9)	116.5 (12.6)	0	418.8 (527)	0
	May	0	0	71.9 (94.4)	0	26.6 (35)	10.7 (18.6)
	August	0.2 (0.4)	77.4 (163.5)	20.7 (30.3)	0	164.4 (552)	0
	November	0	0.3 (0.8)	0	0	96.7 (189)	0
1988	February	0	0.4 (0.8)	0	0	147.1 (233.4)	0
	May	0	42.3 (121.6)	0	0	57.2 (68.6)	0
	August	0	52.4 (137.6)	0	0	128.0 (182.7)	0
	November	0	7.8 (12.7)	0	0	8.2 (8.4)	0
1989	February	0	14.9 (27.1)	0	0	69.3 (69.1)	0
	May	0	19.1 (47.3)	0	0	24.8 (27.2)	0

Table 5.6 Density of gastropods in the *Thalassia testudinum* zone. Data are mean number (1 SD) per 0.25 m^2. N = 20 quadrats.

		Galeta Navy Reef		María Soto		
		Cerithium ebemeum	*Cerithium literatum*	*Cerithium ebemeum*	*Cerithium literatum*	*Batillaria minima*
1986	August	0	0.05 (0.2)	0.2 (0.5)	0.3 (0.6)	0
	November	0	0	0.9 (1.1)	0.6 (1.5)	0
1987	February	0.1 (0.3)	0	0	0	0
	May	0	0	0	0.1 (0.3)	0
	August	0.1 (0.3)	0	0.7 (1.5)	0	0
	November	0	0	0	0	0
1988	February	0	0	0	0	0
	May	0	0	0	0	0
	August	0	0	0	0	1.7 (2.6)
	November	0	0	0	0	0
1989	February	0	0	0	0	0.5 (1.3)
	May	0	0	0	0	0

Table 5.7 Density of gastropods in the *Laurencia papillosa* zone. Data are mean number per 0.25 m^2. N = 20 quadrats; - = no data. No molluscs were recorded at either site starting November 1987.

		1986		1987			
Species	1982-1983	August	November	February	May	August	November
A. Galeta Navy Reef							
Thais deltoidea	0.3	0	0	0.1	0.1	0.1	0
Engina turbinella	0	0	0	0	0	0.1	0
Leucozonia nassa	0.03	0	0	0	0	0	0
Astraea phoebia	0.2	0	0	0	0	0	0
Opisthobranch sp. 1	0.1	0	0	0	0	0	0
Diodora dysoni	0.1	0	0	0	0	0	0
Smaragdia sp.	0.3	0	0	0	0	0	0
Gastropod sp. 6	0.03	0	0	0	0	0	0
Cypraea zebra	1.4	0	0	0	0	0	0
Olivella pusilla	0	0	0	0	0.1	0	0
Total species	8	0	0	1	2	2	0
B. Toro Point							
Thais deltoidea	-	0.2	0.3	0.2	0.5	0.3	0
Olivella pusilla	-	0.4	0.2	0.1	0	0	0
Mitra sp.	-	0	0.1	0	0	0	0
Aplysia sp. 1	-	0	0	0.2	0	0	0
Conus sp. 1	-	0.1	0.1	0	0	0	0
Heliacus ?cylindricus	-	0	0	0	0	0.1	0
Engina turbinella	-	0	0	0	0	0.1	0
Total species	-	3	4	3	1	3	0

5.5.4.3 Summary

Species number and abundance in the high rubble zone fluctuated at both sites after the spill, but showed a downward trend, at least partly due to habitat loss unrelated to the spill. If recruitment continues to be below normal at GNRF relative to TPR, there will be a gradual reduction in gastropod density, an increase in larger size classes of snails, and eventually a further decrease in species number.

After the shoreline cleanup, despite the complicating factors of habitat loss and snail mortality (or movement) correlated with dry season storms, data indicate persistent effects of oiling in the low rubble zone through May 1988. By August 1988, snails were as abundant as they had been in 1982. The diesel spill at TPR had strong immediate effects, but was followed by the recruitment of several species and a return to prior patterns of abundance. This suggests few long-term detrimental effects of this small spill in the low rubble zone.

Our data show strong, persistent effects of oiling at GNRF in the reef rock zone, where species number as well as snail abundance was reduced sharply, relative to both conditions prior to the spill and to the pattern of population change at the unoiled site. There was a strong reduction in density at TPR after a relatively small diesel spill, but populations quickly recovered to previous levels.

After the spill and cleanup, short-lived pulses of recruitment caused large variations in gastropod density in the sand zone at both oiled and unoiled sites. The spill also had no detectable effects on snails in *Thalassia*, given the variation in populations over time. We have no explanation for the paucity of gastropods in this habitat since 1986 relative to 1982.

In *Laurencia* beds, the postspill reduction in snail populations was followed by gradual recovery. It is possible that snails moved up from crevices deep in the reef flat from subtidal populations. The small diesel spill at TPR had no detectable effects because snails were absent in previous monitorings.

5.5.5 Patterns of Recruitment: 1986-1989

5.5.5.1 Methods

See Sections 5.5.1.1 and 5.5.2.1. Size data were used to examine patterns of recruitment over time. A species was considered to have recruited if a pulse of small, previously absent individuals appeared. Because monitoring was quarterly, pulses of rapidly growing species could have been missed; estimates of recruitment should be considered conservative.

5.5.5.2 Results

Recruitment differed among species, as well as between oiled and unoiled sites. Size-frequency data on shell lengths are shown for three species with different patterns of recruitment in Figure 5.22. *Batillaria minima*, here shown from the sand zone at MSR, showed a relatively static population size structure from August 1986 through May 1987. A sharp pulse of recruitment in August 1987 was followed by increases in shell size between November 1987 and July 1988. A lesser event of recruitment occurred in December 1988, followed by rapid growth over the dry season. The relative increase in some smaller size classes in May 1989 may not have been due to recruitment because no size classes smaller than those in the previous monitoring were found. Rather, this probably represented movement or mortality of some larger snails, and was not counted as a recruitment event.

In contrast, *Tectarius muricatus*, shown from the high rubble zone at GNRF, remained relatively static throughout the study, with little or no change in shell size (Fig. 5.22). There were no large pulses of recruitment and only occasional appearances of small numbers of small individuals. A third species, *Littorina lineolata*, from the high rubble zone at TPR (Fig. 5.22) recruited in moderate

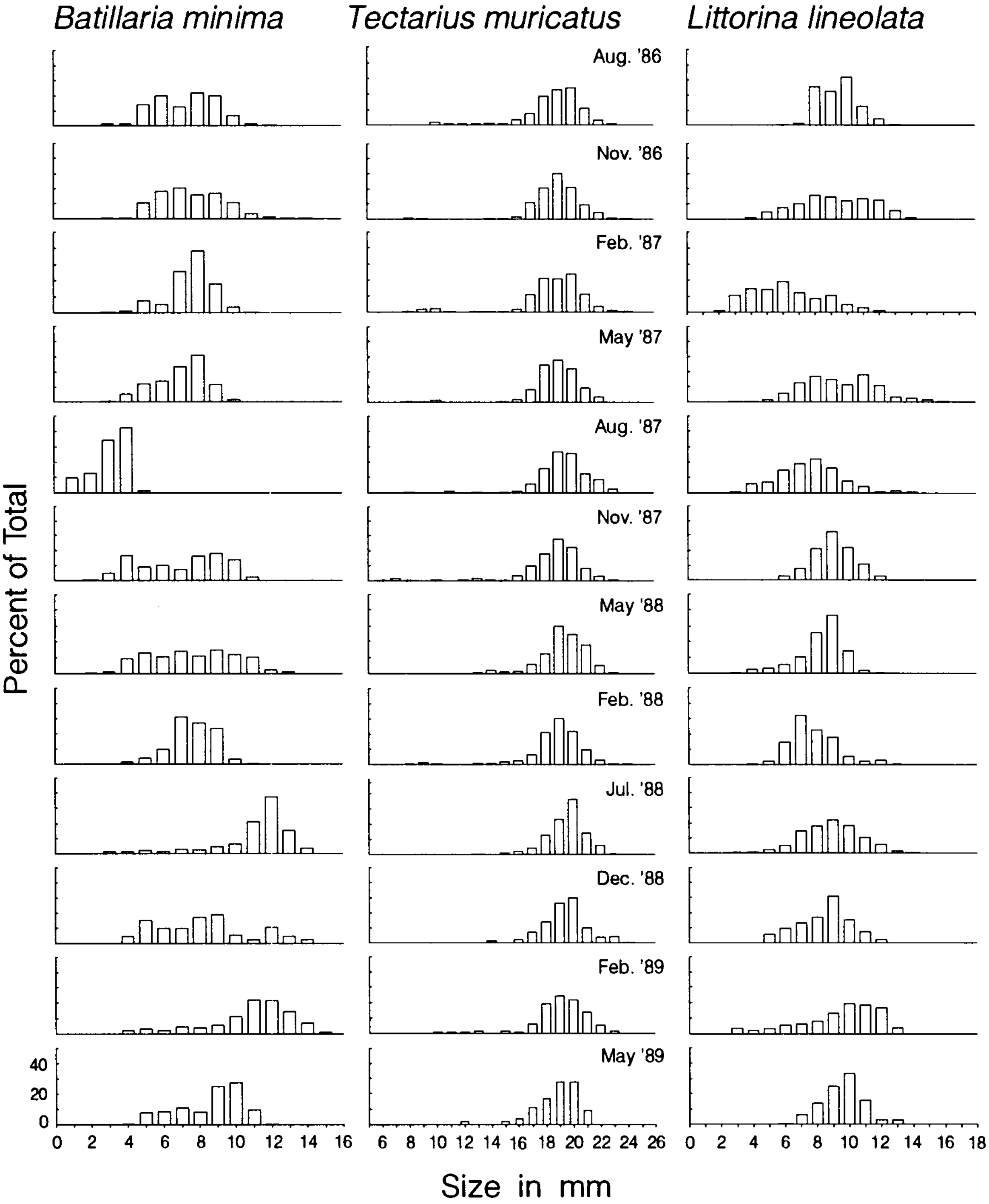

Fig. 5.22 Temporal changes in gastropod size frequency. Examples of recruitment in 3 species: *Batillaria minima*, *Tectarius muricatus*, and *Littorina lineolata*.

numbers in November 1986, February 1987, August 1987, February 1988, and February 1989.

Episodes of recruitment are listed for all species abundant enough to follow in Table 5.8. Most species have planktonic larvae (Vermeij 1978), with the exception of members of the genus *Cerithium*. Dispersal and settlement should thus be independent of snail population size at any given site.

In the high rubble zone, for the first year following the spill (August 1986-May 1987), only *Littorina lineolata* recruited at GNRF; this species, another littorinid, and two neritids recruited at TPR. Over the second year (August 1987-May 1988), *L. lineolata* again was the only species to recruit in the high rubble zone at GNRF, compared to three littorinids and two neritids at TPR. In the final year of the study *L. ziczac* and *Nodilittorina tuberculata* recruited at GNRF, and these two species, plus *L. lineolata* and *Tectarius*, recruited at the unoiled site. Overall, between August 1986 and May 1989, four pulses of recruitment were recorded in the high rubble zone at GNRF, while 13 were observed at TPR; three species recruited only at TPR (Table 5.8).

In the low rubble zone 10 species recruited at TPR between August 1986 and May 1987, compared to five at GNRF (Table 5.8). Only 1 of these 5, *L. lineolata*, did not also recruit at the unoiled site in this zone. However, it did recruit in the high rubble zone. In both the second and third years following the spill, about the same number of species recruited in the low rubble zone, although individual species varied between sites (Table 5.8). Over the 3 yr, 15 episodes of recruitment occurred at GNRF, compared to 19 at TPR. One species, *Planaxis lineatus*, recruited only at GNRF, while three species recruited only at TPR (Table 5.8).

On reef rock there was no recruitment at GNRF in either yr 1 or 2 following the spill. At TPR eight species recruited during yr 1, but only two in the second year. Recruitment was equivalent between sites in the third year, with four species at GNRF and five at TPR. Overall, there were four recruitment events in the 3 yr after the spill at GNRF, compared to 15 in the reef rock zone of the unoiled site, and four species that recruited at TPR during this period did not recruit at GNRF (Table 5.8).

On intertidal sand there were no differences in the number of recruitment events between sites. Overall, four episodes of recruitment occurred at GNRF between August 1986 and May 1989, and four at MSR (Table 5.8). No unique species recruited to either site.

Recruitment was impossible to follow in the *Thalassia* and *Laurencia* zones, due to the low abundance of gastropods in these zones from August 1986 through May 1989. In the former zone, two species of cerithids had been abundant in 1982-1983, indicating either a general disappearance of the genus in this region or a very patchy population structure. In the *Laurencia* zone, mobile molluscs are generally rare, and recruitment may be low, variable, or both.

Table 5.8 Episodes of recruitment, 1986-1989.

	1986	1987				1988				1989		
	No	Fe	My	Au	No	Fe	My	Au	No	Fe	My	Total
High Rubble												
Galeta Navy Reef		LL				LL		LZ	NT			4
Toro Point	LZ LL	LN NT		LL NV		LL	LZ LN		LZ	LL NT	 TM	13
Low Rubble												
Galeta Navy Reef		LL NS NV NT	PN	LZ PL			PN	NV NS NF	LL NT	NF	PL	15
Toro Point	LZ LL	LA LM LN NF NV NS NT PN		PN NF			PN	BM	LZ	LL NS	PN BM	19
Reef Rock												
Galeta Navy Reef									LL	NV LZ	 NS	4
Toro Point		LA LL NT NS NF	LM LZ NV	NS		NV		LZ NV	NF NS	LL		15
Sand												
Gal. Navy Reef	NE	BM		BM						BM		4
María Soto	NE	NE	BM						BM			4

BM = Batillaria minima, LA = Littorina angustior, LL = L.lineolata, LM = L. meleagris, LN = L. nebulosa, LZ = L. ziczac, NF = Nerita fulgurans, NS = N. tessellata, NV = N. versicolor, NE = Neritina virginea, NT = Nodilittorina tuberculata, PL = Planaxis lineatus, PN = P. nucleus.

5.5.5.3 Summary

Decreased recruitment at GNRF relative to that at TPR appeared to be the major long-term effect of the spill in the high rubble zone. In the low rubble zone there may have been decreased or failed recruitment during the first year after the spill. Recruitment in the second and third year after the spill appeared to be equivalent at GNRF and TPR.

Recruitment in the reef rock zone failed completely for 2 yr after the spill, resuming only in November 1988. There may also have been a reduction in recruitment at TPR in May 1988 after a relatively small diesel spill.

After the shoreline cleanup, short-lived pulses of recruitment occurred in the sand zone at both oiled and unoiled sites. The low abundance of gastropods made it impossible to follow recruitment in *Thalassia* and *Laurencia* beds.

5.6 Discussion

The effects of the oil spill at Bahía Las Minas on gastropod molluscs inhabiting the nearby reef flat at GNRF must be evaluated cautiously. Conclusions are speculative, given:

1. the 4-yr period between pre- and postspill monitoring,
2. the natural scarcity of gastropods in some habitats,
3. the patchy distribution of many species,
4. the unknown extent of natural variation, and
5. tack of data before the spill from TPR and MSR.

Despite this, there is evidence that the oil spill had strong effects on the molluscs of some zones at GNRF. Especially important are:

1. observations of dead snails glued to the rock by oil,
2. recovery of large numbers of dead snails at GNRF when none was recorded at TPR, and
3. failure of recruitment in the most heavily oiled zones.

Of the habitats sampled, oil was most heavily concentrated in the low rubble and the emergent portions of the reef rock zones, where wave action and the rise and fall of tides added layer upon layer of oil to rocky surfaces following the spill (J. Cubit, pers. comm.). Relatively little oil splashed up onto the high rubble, above the level of the tides. The intertidal sand, *Thalassia*, and *Laurencia* zones were also heavily oiled, but the physical evidence quickly disappeared as sand shifted, oiled plants rotted and drifted away, and ephemeral algae bloomed.

The observed pattern of oiling was due to the complicated pattern of tidal emersion at Punta Galeta (Cubit et al. 1986, 1989). By April and May, onshore winds, which usually keep water levels high and mask tidal fluctuations on the reef flat, have dropped. This results in the periodic emersion of much of the surface of the reef flat. In contrast, an oil spill during the dry season, when water levels and onshore winds are consistently high, would have concentrated oil on or at the edge of the high rubble zone (see Cubit and Levings, Chap. 2). Lower zones of the reef flat probably would have had little direct contact with surface slicks of oil, and were affected only by oil mixed into the water column. The latter scenario appears to have occurred when the Punta Galeta reef flat was oiled during dry season by the tanker *Witwater* spill in 1968 (Rützler and Sterrer 1970). Thus, effects of the oil spill were intimately dependent upon the pattern of wind and tides, combined with small differences in elevation across the reef flat (e.g., Connor 1984).

A small diesel spill at TPR occurred following the grounding of a 75-foot sailboat on 14 May 1988. Weather conditions were similar to those during the Bahía Las Minas spill. Intertidal reef gastropods were experiencing extended periods of daytime emersion. Extreme daytime exposures (J. Cubit, pers. comm.) occurred for five or more hours each day both before and during the May 1988 monitoring period (19-22 April, 2-5 May, 16-22 May, and 29 May-7 June). Although low water levels occur every year at this time, 1988 had an unusually high number of exposures (J. Cubit, pers. comm.; Cubit and Connor, Chap. 4). Mobile molluscs behaviorally reduce physical stress from heat and desiccation while emersed by moving lower on the shore, moving to vertical surfaces or crevices, or becoming inactive under cobble, coral rubble, sand, or debris (Garrity 1984). Some of these behaviors affected exposure of snails to diesel fuel at TPR. Fuel collected in depressions and on sand in the reef rock and low rubble zones (C. González, pers. obs.).

Physical stress alone does not explain the observed mortality at TPR. First, several hundred dead, freshly oiled individuals of six snail species were collected during the May 1988 monitoring at TPR; no such dead snails were found either at GNRF or MSR. Of the 206 *Batillaria minima* counted in quadrats in the low rubble zone at TPR, all were dead, while of the 237 individuals counted in quadrats from the sand zone at MSR, all were alive. Second, snail abundance generally increased at TPR from May to August 1988, despite continued daytime reef exposures in June, July, and early August. A number of species showed recruitment during these extended exposures, further suggesting adaptation of snail population to such exposures.

As a result of the pattern of oil deposition from the 1986 refinery spill, both reef rock and low rubble zones showed major and persistent changes in gastropod abundance at GNRF. These zones at TPR appeared briefly affected by a small diesel spill in May 1988, the same time of year as the spill at GNRF. At GNRF there was little evidence of recovery in the reef rock zone until 3 yr after the spill and its resultant mortality; recruitment was strongly affected until late 1988.

The high rubble zone received relatively little oil, and populations of snails varied after the spill at both oiled and unoiled sites. The presence of large numbers of oiled snails in this zone soon after the spill suggests vertical movement of snails from lower, more heavily oiled zones. The extent and timing of normal snail movement and migration may help explain differential mortality among species and may be a factor in some of the changes in abundance seen at both sites since the spill (e.g., Peckol et al. 1989). Activity patterns of gastropods elsewhere have been shown to influence susceptibility to pollutants (Dicks 1976). This aspect remains to be investigated.

In the *Laurencia* zone several species of gastropods are normally rare. Since the oil spill, few to no molluscs were found at GNRF. However, the same pattern has been observed at the unoiled site, indicating that oiling alone is not responsible for the reductions. More detailed examination of mollusc populations, perhaps focusing on micromolluscs, might have provided more conclusive results.

In the sand and *Thalassia* zones at GNRF, there were strong differences in snail abundance between 1982 and postspill monitorings. The oil spill or subsequent cleanup operations (or both) eliminated *Batillaria* from intertidal sand at GNRF. Numbers since have fluctuated, probably depending upon pulses of opportunistic settlement, and suggesting few persistent effects of oiling. In the *Thalassia* bed, two species of *Cerithium* were present at GNRF before the spill and were absent or rare after the spill. However, at the unoiled site in 1986 both species were also rare. They have been commonly reported in *Thalassia* beds (e.g., Heck 1977), but were rare along the entire coast during this study (S. D. Garrity, pers. obs.). We have no explanation for this phenomenon. Although oil may have affected the gastropods of the *Thalassia* bed at GNRF, results could also be explained by unrelated population fluctuations along this coast.

To confound our findings, results from sand and *Thalassia* beds highlight the potential for variation in recruitment among sites (e.g., Caffey 1983). Because only two sites could be monitored for each zone, we cannot separate random variation at each site from effects of oiling. Persistence of effects over time may indicate that recruitment is reduced at oiled sites, or merely that an individual site tends to have low recruitment.

This general problem strongly affects studies of pollution (e.g., Jones 1982; Lewis 1982; Underwood and Peterson 1988). Oil spills are unplanned events that occur rarely in areas where long-term monitoring programs have been in progress for many years or where the life histories of resident organisms are well known. As with many ecological problems, data from tropical habitats are even rarer than data from the temperate zone (but see Birkeland et al. 1976; Eisler 1973, 1975*a*, *b*; Chan 1976, 1977; Maynard et al. 1977; Maynard 1984; Cubit et al. 1986). Despite its imperfections, the present study represents the only long-term data available for tropical molluscs (Garrity and Levings 1990). Interpreted cautiously, the data show thus far that (1) mortality was extensive in some sections of the reef flat and (2) recovery had not been completed 3 yr after the spill. The separation of natural population fluctuations from those caused by oil, and any evaluation of time to final recovery must await further study.

5.7 Acknowledgments

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6
Reef Flat Stomatopods

Rick Steger and Roy Caldwell

6.1 Abstract

Stomatopod crustaceans, commonly known as mantis shrimp, are important components of reef flat and seagrass bed communities. In particular, gonodactylid stomatopods are abundant predators that live in cavities in hard substrata, such as coral rubble, specializing on gastropod and hermit crab prey. Prior to the 1986 Bahía Las Minas oil spill near the Galeta Marine Laboratory, gonodactylid densities of 7-8 individuals per m^2 were recorded from low-intertidal *Thalassia* beds in this area.

In September 1986, 5 mo after the oil spill, four study sites were established and a sampling regimen prescribed to determine immediate and long-term effects of oiling on gonodactylid populations. Two sites, Isla Mina and Isla Largo Remo North, had been heavily oiled. The other two, Isla Margarita and Isla Largo Remo West, received little or no direct oiling and served as reference sites. Three types of sampling were conducted. First, to determine densities, a reserve was established at each site for quadrat sampling. During each sampling period 0.5-m^2 quadrats were cleared of all hard substrata, which were then measured and broken apart to reveal resident stomatopods. Second, to provide greater numbers of animals, large pieces of rubble were haphazardly selected from areas near the reserves, measured, and broken apart. Finally, to determine the relationship between stomatopod size and the volume of the cavity it occupied, pieces of rubble known to contain at least one gonodactylid were taken from selected sites, the animals removed, and the cavity volumes measured. All gonodactylids collected were characterized according to species, sex, size, reproductive condition, injuries, and molt and growth status. Sampling was conducted at the four sites during the wet (September) and dry (February) seasons from September 1986 through February 1988. Dry-season-only sampling was continued from February 1989 through February 1991. Two control sites that had not been oiled (María Soto and Punta Escucha) were added in February 1988. Only the haphazard rubble samples were collected at these sites.

Results from the initial postspill samples in September 1986 showed there were significantly fewer large gonodactylids, particularly females, at the two heavily oiled sites. Also, individuals at the heavily oiled sites grew more per molt and had fewer wounds than individuals at the reference sites and at the same sites prior to the spill. At the one heavily oiled site sampled for gonodactylid-size cavity-volume relationships, individuals occupied larger cavities than did animals at reference sites both before and after the spill. These results suggest that loss of large gonodactylids at heavily oiled sites through mortality, emigration, or both resulted in reduced competition for cavities. This change allowed animals to occupy preferred-size cavities. Relaxed competition also resulted in less fighting and injuries, and increased

growth. Growth may also have been promoted at oiled sites due to an increase in available prey. Numbers of hermit crabs increased rapidly postspill, presumably due to snail mortality and a resulting increase in shell availability.

Long-term effects of oiling were measured in habitat changes at Isla Largo Remo North, the most heavily oiled site. Oiling killed *Thalassia* and a wide band of mangroves at this site. Subsequent erosion resulted in the loss of up to 14 cm of soft substratum and transformed the habitat into an area dominated by large pieces of unconsolidated coral rubble. Isla Largo Remo North no longer has the preferred habitat of gonodactylids, and stomatopod density remained low nearly 5 yr after the spill. *Thalassia* beds at the other heavily oiled site recovered within a year of the spill, and again provided suitable habitat for gonodactylids.

Oiling had a long-term effect on gonodactylid recruitment. Recruitment was reduced for 1 to 2 yr following the spill at heavily oiled sites relative to reference sites. This reduction resulted in a decline in densities of young and medium-aged gonodactylids at these locations. The long-term effect of reduced recruitment on gonodactylid populations was confounded, however, by a general depression of recruitment at all sites from 1987 through 1989. The cause of this phenomenon and its relationship to the oil spill are unknown.

No direct effects of the oil spill on gonodactylids were measured after 1988. Immigration of larger stomatopods into heavily oiled sites and a return to normal levels of recruitment eliminated or masked any direct effects of oiling that persisted.

Indirect effects of oiling brought about through reduced competition for cavities, including the gonodactylid-size to cavity-volume relationship and wounding, could not be discerned at any site other than Isla Largo Remo North after 1988. At this heavily oiled site, after significant erosion continued, low gonodactylid density and abundant available cavities resulted in high stomatopod-size to cavity-volume ratios and reduced wounding.

One other stomatopod, the large burrowing species *Lysiosquilla grabiuscula*, was common, particularly at Isla Mina, in intertidal *Thalassia* beds prior to the oil spill. Monogamous pairs apparently remain in one burrow for life and individuals are quite long lived. Prior to the spill adults were followed for up to 4 yr. Since the oil spill searches for *Lysiosquilla* have failed to reveal a single burrow at any of the oiled or reference sites. It appears this species is locally extinct, although why it has not been found even at the reference sites remains unclear.

6.2 Introduction

Stomatopods (mantis shrimp) represent an ancient lineage of predatory marine crustaceans found in most tropical and semitropical seas (Fig. 6.1). Most species occur in shallow coastal habitats burrowing in sandy or muddy sediments or occupying cavities in coral, rock, and other hard substrata. Mantis shrimp are characterized by a pair of enlarged thoracopods that form powerful raptorial appendages used to capture and process prey. The raptorial appendages are also potent offensive and defensive weapons used in intra- and interspecific combat.

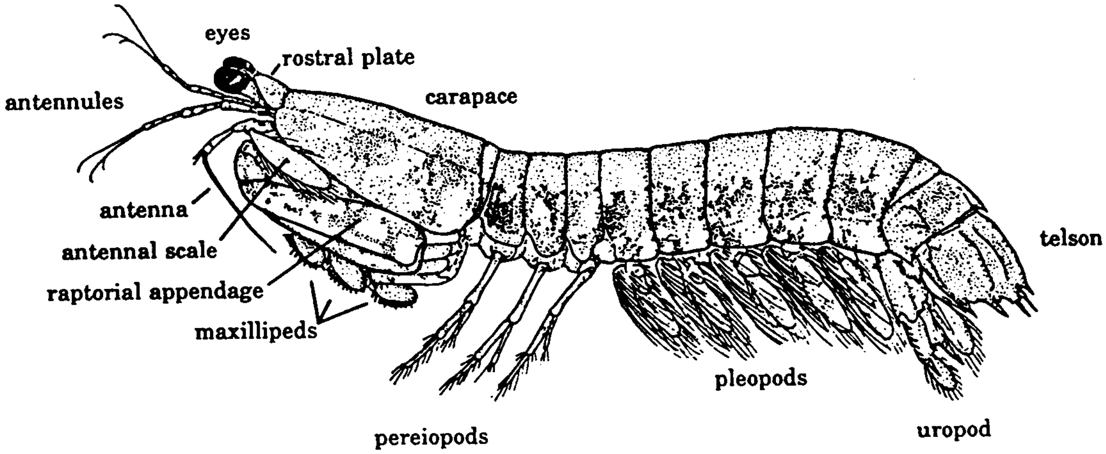

Fig. 6.1 Typical stomatopod crustacean with major body parts and appendages labeled.

Caldwell and Dingle (1976) divided stomatopods into two functional groups based on the morphology of the raptorial appendages, *spearers* and *smashers*. Raptorial appendages of spearers possess a slender, terminal dactyl armed with numerous spines. They are used to impale soft-bodied prey, such as shrimp and fish. Smashers typically have unarmed dactyls with greatly enlarged and heavily calcified heels that are used like hammers to smash armored prey, such as snails and crabs.

Gonodactylid stomatopods are smashers that occupy and defend cavities in hard substrata as refuges from predators. Gonodactylids are principal components of many intertidal communities due to their position as both important predators and prey. As prey, they serve as an abundant source of food for herons, egrets, fishes, and octopuses (Reaka 1985; Caldwell 1987). As predators, they are capable of structuring local populations of hermit crabs and gastropods, their primary prey (Caldwell et al. 1989). In addition, gonodactylids are used often in research on the function and expression of aggressive behavior because of their combativeness and highly developed systems of communication (for reviews see Caldwell and Dingle 1976; Reaka and Manning 1981; Steger 1985; Caldwell 1987).

The population biology, ecology, and behavior of gonodactylids have been studied in great detail around the Galeta Marine Laboratory of the Smithsonian Tropical Research Institute (STRI). These studies were carried out over a 5-yr period from 1979 to 1983 by R. Caldwell and R. Steger. During that period, we monitored gonodactylid populations on reef flats and seagrass beds within a 4-km radius of the Galeta Marine Laboratory. We collected approximately 150 stomatopods per week throughout most of this period to determine demographic structure and reproductive, growth, and recruitment patterns of populations. Sampling methods included quadrat- and transect-density studies, in which all animals were removed and recorded from prescribed areas (0.5 m^2, 1 m^2, and 49 m^2);

breaking selected pieces of rubble likely to contain at least one stomatopod; and collecting at prescribed intervals all gonodactylids that colonized grids of artificial concrete cavities set out in the field. In all, over 50,000 gonodactylids were collected and catalogued with respect to species, sex, size, reproductive condition, injuries, molt status, and growth. In addition, we undertook several sampling programs to determine local stomatopod densities, estimated stomatopod-size to cavity-size relationships, locally manipulated stomatopod densities to determine their effect on local prey populations, and conducted numerous field and laboratory observations to document various agonistic and reproductive behaviors. Much of the current information available on the ecology and behavior of gonodactylid stomatopods comes from this research program (e.g., Caldwell and Lamp 1981; Caldwell 1982; Berzins and Caldwell 1983; Steger and Caldwell 1983; Montgomery and Caldwell 1984; Steger 1985; Caldwell 1986*a*; Caldwell 1986*b*; Caldwell 1987; Steger 1987; Caldwell 1988; Caldwell et al. 1989; Shuster and Caldwell 1989; Adams and Caldwell 1990; Caldwell 1991). The following is a brief summary of the biology of Panamanian gonodactylids relevant to this study.

6.2.1 Physical Structure

The primary habitat where gonodactylid stomatopods are found on the central Caribbean coast of Panama is low intertidal and shallow subtidal *Thalassia testudinum* (seagrass) beds. These beds often form on wave-exposed coasts between the shore and the crustose coralline algal and coral reef crest. Frequently these beds are interspersed among coral-rubble-strewn reef flats or outcrops of reef platform, and they are often found directly in front of stands of red mangrove, *Rhizophora mangle*. Seagrass beds often form up to a few centimeters above mean low water, but just below the level of the reef crest, and are frequently exposed during low tides. During most of the year *Thalassia* (turtlegrass) beds are lush. Blades are several centimeters long and provide a thick, green canopy generally covering the substratum. However, during extreme diurnal low tides of May and June, if prevailing winds are light or offshore, beds are exposed for several hours during the heat of the day and *Thalassia* blades die back to a short brown stubble.

Rhizomes and root systems of *Thalassia* stabilize organically rich calcareous sediments to form a firm substratum. On wave-sheltered shores the reef crest is often lacking and seagrass beds typically grade subtidally into deeper water. In these areas sediments contain more silt, are softer, and are less favored by gonodactylids. Gonodactylids can also be found living in other intertidal and shallow subtidal habitats, particularly on reef flats containing consolidated carbonate rock, coral rubble, or heavy algal turfs. However, *Gonodactylus* are most numerous in intertidal seagrass beds, provided there are pieces of coral rubble, crustose coralline algae, or other hard substrata that provide cavities to serve as homes.

Our studies of gonodactylid populations took place at five primary locations within 4 km of the Galeta Marine Laboratory. Each location had extensive seagrass beds interspersed with pieces of coral rubble and coralline algae that served as homes for stomatopods. All selected seagrass beds were exposed at spring low tides.

6.2.1.1 Isla Margarita

Isla Margarita was the westernmost site (Fig. 6.2). We worked on a northwest-facing reef flat and *Thalassia* bed. The turtlegrass bed extended approximately 200 m along the shore. The distance between the reef crest and a concrete sea wall was 40-80 m. To the east the seagrass bed sloped subtidally into a shallow bay. To the west *Thalassia* gave way to a rubble-strewn reef flat interspersed with exposed reef platform, rubble and sand bars, deeper pools and channels, and occasional patches of seagrasses. During onshore winds waves broke over the crest and washed directly over the flat to the sea wall.

6.2.1.2 Punta Galeta

The second site was on Punta Galeta directly in front of the U.S. Navy antenna facility 1 km west of the Galeta Marine Laboratory (Fig. 6.2). This north-northwest facing location consisted primarily of rubble-strewn reef flat and exposed reef platform with occasional patches of seagrasses, and extended 100-200 m back from the reef crest. The shore consisted primarily of elevated reef platform and coral rubble. We sampled selectively over a 500-m section of this reef flat. During onshore winds waves swept across this flat from the north and the general drainage was to the southwest with return through various deep channels.

6.2.1.3 Isla Mina

The third site was on Isla Mina (MINA; Fig. 6.2). This location had a reef crest that faced north and curved around to the east. The reef flat behind the crest consisted of a large *Thalassia* bed with occasional rubble and sand bars and small patches of exposed reef platform. To the south the seagrass bed sloped subtidally into a quiet, protected bay. The shore consisted of a mix of sand beach with coconut palms and patches of red mangrove. The exposed seagrass bed was approximately 300 m long and 40-70 m wide. During onshore winds waves washed over the north- and east-facing reef crest. Water flowed the length of the reef flat and exited to the south onto subtidal seagrass beds in the back bay.

6.2.1.4 Isla Largo Remo West

The fourth site was on the northwest corner of Isla Largo Remo (LRW; Fig. 6.2). An expansive *Thalassia* bed (200 x 300 m) was situated behind the north- and west-facing reef crest. There were occasional outcrops of reef platform and large pieces of coral rubble. The reef flat continued to the east. The shore consisted entirely of red mangrove with a shallow channel running between the mangroves and

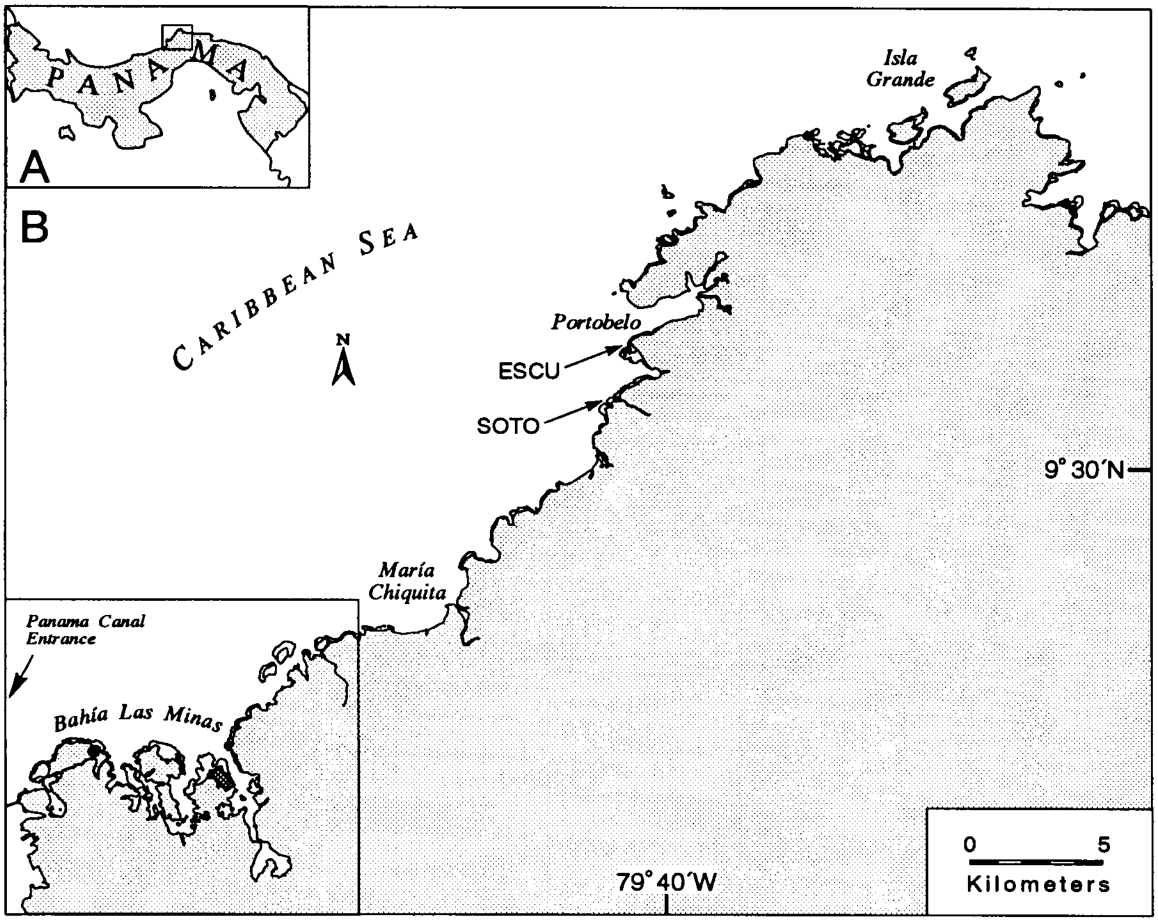

Fig. 6.2 Map of study sites used to survey effects of oiling on gonodactylid stomatopods. *A*. Location of the study region in Panama. *B*. Location of unoiled control sites; *SOTO* = María Soto; *ESCU* = Punta Escucha.

the reef flat. During onshore winds water came over the reef crest, traveled southwest across the flat, and exited through a shallow channel into the bay to the west.

6.2.1.5 Isla Largo Remo North

The fifth and most easterly site was on the north-northwest-facing shore of Isla Largo Remo (LRN; Fig. 6.2), just west of a deep pass that cuts through the reef crest and opens onto a shallow lagoon extending to mangroves. We sampled an 80 by 40 m exposed bed of *Thalassia* directly in front of an extensive stand of red mangrove forest. Toward the north the reef flat extended 150 m to the reef crest. This flat consisted of exposed platform, rubble, and patches of seagrasses. During onshore

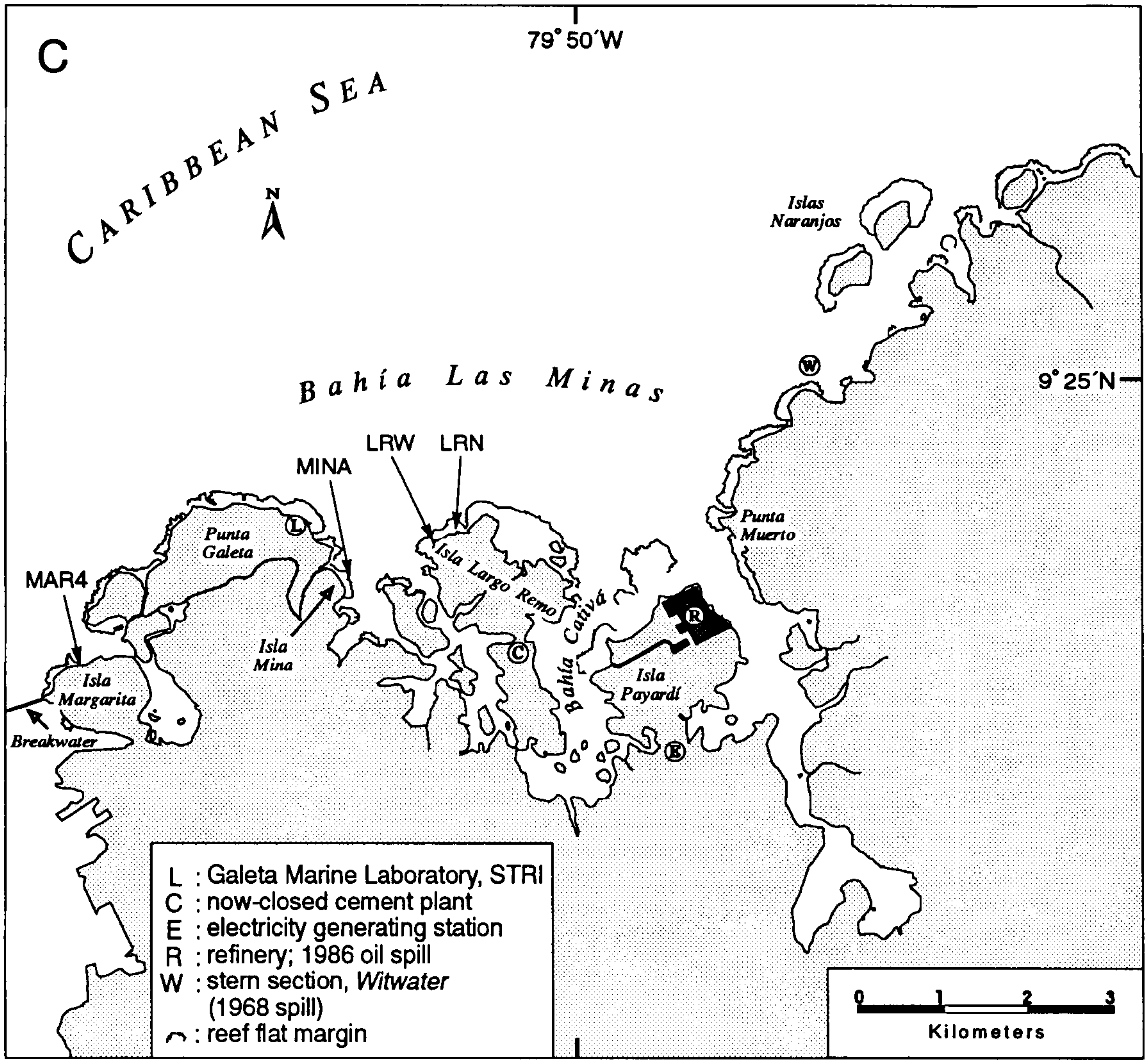

Fig. 6.2 Map of study sites used to survey effects of oiling on gonodactylid stomatopods (continued). *C.* Location of oiled sites (*LRN* = Isla Largo Remo North; *MINA* = Isla Mina) and lightly oiled to unoiled reference sites (*LRW* = Isla Largo Remo West; *MAR4* = Isla Margarita).

winds waves broke over the reef crest, entered through the pass, and moved across the seagrass bed into mangroves. Return flow was to the east back into the lagoon and to the west along the reef flat and mangrove edge.

6.2.2 Gonodactylid Stomatopods

Six species of *Gonodactylus* are found living in cavities in hard substrata on the Punta Galeta coast. However, two of these species have been recorded only subtidally. *G. curacaoensis* occurs on faces of fringing reefs at depths below 2 m and *G. torus* is a rare dwarf species occasionally taken at depths exceeding 10 m. Of the four remaining *Gonodactylus* found on intertidal reef flats and seagrass beds surrounding Punta Galeta, three species are common. *G. bredini* is the most abundant, accounting for approximately 80% of intertidal gonodactylids. *G. oerstedii* is the second-most-common species, making up about 15% of gonodactylids, and *G. austrinus* comprises about 5% of intertidal gonodactylids (Steger 1987). *G. spinulosis* is exceedingly rare; only two small males were recorded during prespill studies. It is unlikely that a breeding population of *G. spinulosis* exists on the Panamanian coast.

G. bredini and *G. oerstedii* are most abundant at Punta Galeta from the low intertidal to a subtidal depth of approximately 0.5 m. While both species occur considerably deeper, they become increasingly rare with depth. There is a tendency for small, nonbreeding *G. oerstedii* (7-40 mm total length) to be more common below mean low water and for large breeding adults (>45 mm) to be found in the intertidal, but any-sized individual can be found in rubble from the intertidal down. Both adult (>25 mm) and juvenile (7-25 mm) *G. bredini* are more common in the lower intertidal, but they too are found subtidally (Caldwell 1988).

G. austrinus tends to be a habitat specialist, occurring most frequently on reef crests where there is a swift current, wave action, or both, and complete exposure at low tide is rare. *G. austrinus* individuals are frequently associated with the calcareous alga *Halimeda opuntia*, which grows in dense mats over surfaces of coral rubble and coralline algae. Juveniles are often found living between algal mats and the coral surface, but adults seek cavities in rubble. Adult *G. austrinus* are occasionally collected inshore in the low intertidal, but we suspect many of these animals were transported there by wave action. For example, in March 1987, 3 wk after several days of high wind and heavy surf, we found several large pieces (20-40 cm diameter) of *Halimeda*-covered crustose coralline algae and coral rubble that had washed inshore onto exposed *Thalassia* flats. After several days of exposure *Halimeda* was dying, but inside these pieces of rubble were several *G. austrinus*. One large block contained nine *G. austrinus*, including a female with eggs. The closest source for such rubble was over 50 m away on the reef crest where *G. austrinus* were common. The rubble, along with its resident stomatopods, obviously had been transported inshore by the storm. Postlarval *G. austrinus*, however, frequently recruit onto intertidal *Thalassia* flats and juveniles are relatively common in this area.

The abundance of gonodactylids on reef flats around Punta Galeta is quite high. Our best estimate of prespill densities comes from data taken at Isla Margarita in August 1979. Ten 1 x 10 m transects were sampled. All rubble was removed from the transects in 1-m^2 quadrats and broken to extract all gonodactylids. The area sampled was predominantly *Thalassia* bed with some coral rubble outcrops. We estimated there were 9.6 ± 0.59 (mean ± 1 SE) *Gonodactylus* per m^2 at this site.

Density estimates taken from seagrass beds at other locations near Punta Galeta and at other times of the year generally were quite similar, particularly when excluding juveniles, which fluctuate markedly due to sporadic recruitment events.

6.2.2.1 *Gonodactylus* Life History

The life histories of all three species of *Gonodactylus* discussed in this report are similar. Where differences exist, they will be specifically noted. Individuals settle out of the plankton and onto reef flats as postlarvae. At this stage they are typically 6-9 mm total length (*G. oerstedii* are typically smaller than *G. bredini* and *G. austrinus*, but there is considerable overlap) and resemble miniature adults except that they lack external reproductive organs and are transparent. They begin to develop body pigment in 3 or 4 d and molt within 1 to 2 wk. Individuals continue to molt approximately every 2 wk and grow 15-20% per molt for the next 1 to 2 mo. When males reach 11 mm total length the gonopods become visible. Gradually, the rate of molting slows to once every 1 to 2 mo and growth drops to near 10% per molt. When females reach a total length of approximately 18 mm, gonopores become visible. One year after settling out of the plankton as postlarvae, these stomatopods are 25-33 mm long. Female *G. bredini* and *G. austrinus* may begin breeding when they are around 25 mm total length. *G. oerstedii* females delay first reproduction until they are approximately 40 mm long. Males of all three species appear to begin breeding at about the same size as is typical for females of their species. Once breeding is initiated, molting intervals decrease further to once every 2 to 4 mo, and growth per molt decreases to around 5% or less. By the end of the second year these gonodactylids are approximately 40 mm long. At 3 yr they are in the 45-50 mm range. Because they delay reproduction, *G. oerstedii* may grow somewhat more rapidly than the other two species, but they also decelerate their rate of growth once reproduction is initiated. A few *G. bredini* and *G. austrinus* achieve a maximum body size of 55-60 mm, and we estimate these individuals are no more than 5 to 6 yr old. *G. oerstedii* may achieve a slightly larger size (65 mm), but due to initial rapid growth, they are probably about the same age (Caldwell and Steger, unpubl. data).

Reproduction in these gonodactylids is on a lunar cycle, with most females mating and laying eggs near the time of the full moon (Fig. 6.3). They then brood their eggs and larvae for 1 mo, and larvae leave female cavities and enter the plankton on the next full moon. On the Atlantic coast of Panama full moons are associated with the most extreme spring tides, and therefore the greatest risk of tidal exposure, but they also coincide with the greatest tidal currents, when larvae can be transported off reefs and into the open ocean. Small females initially reproduce only every 3 to 6 mo, but older females typically breed every other month. Molting and reproduction are incompatible in gonodactylids. For a female to molt she must forego reproducing for at least 1 mo (Caldwell 1986*b*). At least some females breed in the population each lunar cycle; typically, 25 to 40% of sexually mature females are reproductive at any given time. However, for reasons we do not fully understand,

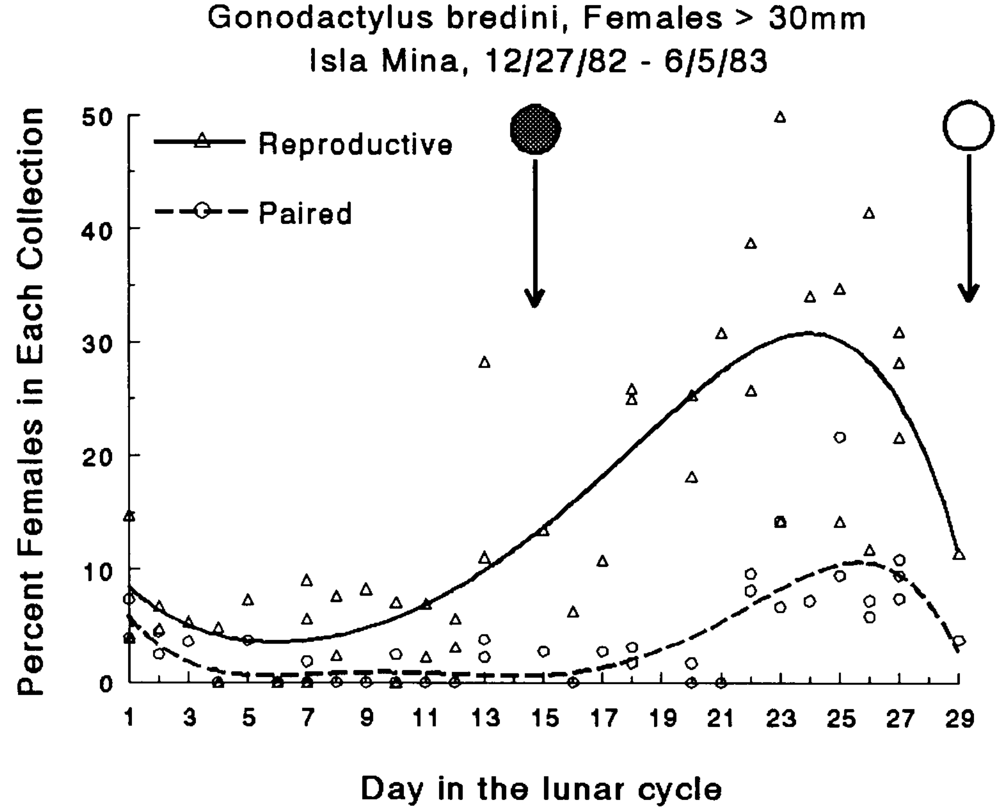

Fig. 6.3 Percent of female *Gonodactylus* >30 mm in total length reproductive (as indicated by well-developed cement glands) or captured paired with males. Animals were collected in 1983 at MINA. Data are derived from over 4,000 stomatopods, with at least 100 individuals captured every 5 d. *Open circle* represents the full moon; *solid circle* represents the new moon.

in most years during June, July, and August many small females (<35 mm long) do not reproduce.

Mating systems of these species of *Gonodactylus* appear to be similar. Males and females pair in either the male's or the female's cavity for a few days prior to oviposition (Fig. 6.3). During this time they mate repeatedly and the male guards the female from other males, thereby protecting his paternity (Shuster and Caldwell 1989). As soon as the female spawns the male leaves the cavity to the female and searches for another home (Fig. 6.4). This is a time of increased mortality for males, and with each breeding episode the number of surviving males declines. At the age of first reproduction the sex ratio in these gonodactylids is approximately 1:1. However, among older individuals the ratio may be as high as three females for every male.

We are uncertain how long larvae remain at sea in the plankton before they return to reef flats and settle out as postlarvae. Based on data from other populations and species, our best estimate is that larvae are planktonic for 2 to 3 mo

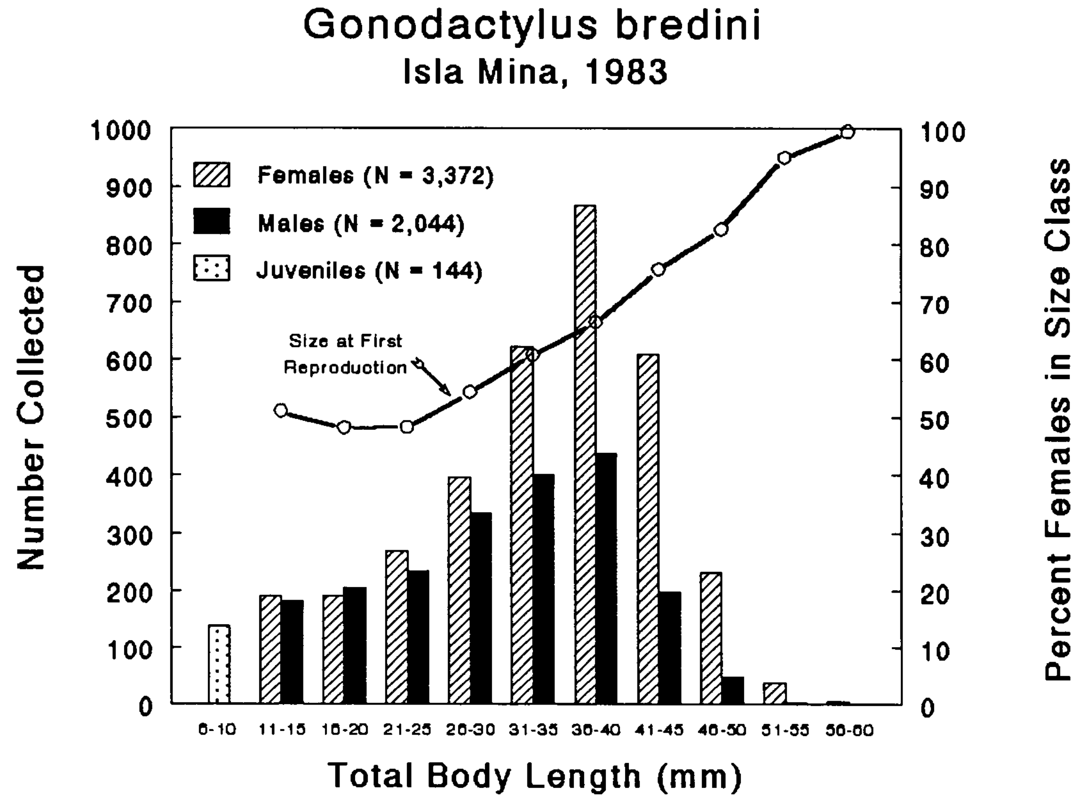

Fig. 6.4 Size frequency of female, male, and juvenile *Gonodactylus bredini* captured at MINA in 1983. Also plotted (*right axis*) is the percent of females in each size class.

and that this period is somewhat variable depending on local conditions (Caldwell, unpubl. data).

Recruitment typically occurs during the new moon, but does not occur every lunar cycle despite the fact that a considerable proportion of females in the population reproduce each month. Recruitment is most likely during the dry and early wet season (February-May), with a second small peak in October or November (Fig. 6.5). Factors controlling recruitment and the source of postlarvae that settle on reefs around Punta Galeta are poorly known (Steger 1987).

6.2.2.2 Physical Tolerances

The upper distributions of *G. bredini*, *G. oerstedii*, and *G. austrinus* at Punta Galeta are determined by physical limits. All three species have similar tolerances to the high temperatures, desiccation, and low salinities that may be encountered during tidal exposures. For example, adults of all three species can survive water temperatures up to 39°C for 30 min., but suffer greater than 50% mortality at 40°C

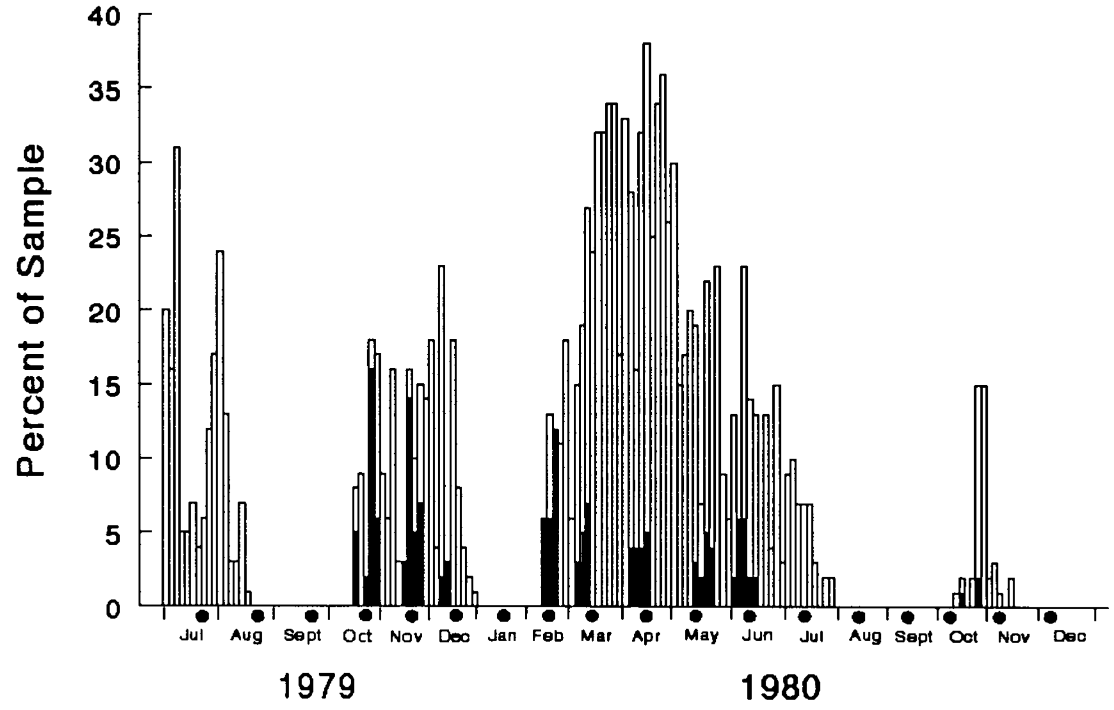

Fig. 6.5 Postlarval recruitment of *Gonodactylus* at Isla Margarita (MAR4), July 1979-December 1980. The bars show the percent of individuals in each sample whose total length was < 11 mm. The *darkened portions* indicate newly settled (transparent) postlarvae in each sample. Postlarvae were not specifically scored before October 1979. *Circles* below the *x*-axes represent new (*solid*) and full (*open*) moons (redrawn from Steger 1987, with permission).

(Caldwell 1988). Tide pool temperatures near 40°C are not uncommon when spring low tides occur during periods of bright sun and offshore winds.

Females of all three species brood eggs in cavities in the lower intertidal. Females care for their eggs for about 3 wk. After hatching, larvae remain in cavities for an additional week prior to entering the plankton during spring tides associated with the full moon. Temperature and salinity tolerances of eggs and larvae remaining in female cavities are high and match those of adults. The tolerance of eggs and early larvae to high temperatures and low salinities probably reflects the fact that if a severe exposure occurs while a female is brooding her young, she cannot easily transport them to deeper water to avoid extreme physical conditions (Caldwell 1988).

Even though eggs and larvae of gonodactylids are tolerant of extreme heat, we have observed mass mortality during exposures. For example, in May 1983 a series of unusually severe reef flat exposures occurred, coupled with light winds and bright sun. When the first exposure occurred, approximately 40% of the large gonodactylid females in the intertidal were brooding eggs and larvae. After five consecutive exposures, not a single female brooding eggs or larvae could be found. Most females survived by abandoning their cavities and crawling into shallow pools of water under pieces of rubble that they had occupied or moved to deeper water. However, most left their eggs or larvae in the cavity where they perished, or eggs were lost when they were removed from the protection of the cavity (Caldwell 1988).

Once larvae complete the fourth stadial molt and leave female cavities to enter the plankton, they are much more susceptible to thermal and osmotic stress (Caldwell 1988). However, planktonic stomatopod larvae occur in the open ocean where thermal and salinity fluctuations are much less extreme and rarely would approach lethal levels.

6.2.2.3 Competition for Cavities

Gonodactylids require cavities in hard substrata. These cavities provide a refuge from predators, a place to process prey, and a safe shelter in which to mate and brood eggs and larvae. On reef flats and seagrass beds around Punta Galeta, gonodactylids typically live in cavities in coral rubble or coralline algae, although they also can be found living in cavities in a variety of objects, including shells, sea urchin tests, basal attachments of detached sea fans, and even seeds and nuts. Without a cavity, the life expectancy of a gonodactylid is probably very short.

Suitable cavities, particularly for large individuals, are often in short supply (Steger 1985, 1987). While gonodactylids can modify existing cavities to some extent, they usually cannot create them. Competition, therefore, is often intense for existing cavities and much of the complex aggressive repertoire documented for these species involvs acquiring and defending homes (Caldwell 1987). Given the potent weapons these animals possess and the value of a cavity, it is not surprising that contests for cavities are fierce and frequently result in injuries. The ability of an animal to defend a cavity is related to how well it fits into it (Steger 1985). If the cavity is too large, bigger, stronger stomatopods are likely to enter and displace the resident. A cavity smaller than that preferred is easier to defend because often a larger intruder cannot fit through the entrance. However, if a cavity is too small to provide space for feeding and reproducing, a resident is unlikely to vigorously defend it when challenged. Competition for homes will tend to force animals to accept cavities smaller than preferred. The more intense the competition, the smaller the cavity-volume to stomatopod-size ratio. This relationship of cavity to stomatopod size is a good indicator of cavity limitation and competition in a population (Fig. 6.6; Steger 1985, 1987).

6.2.2.4 Other Stomatopods

Another stomatopod, a large burrowing spearer, *Lysiosquilla glabriuscula*, was common on reef flats surrounding Punta Galeta prior to the spill. These animals obtain a maximum body length of up to 22 cm, form lifelong pair bonds, and live for several years. Burrows of mature adults are *U*-shaped with two entrances 2-3 m

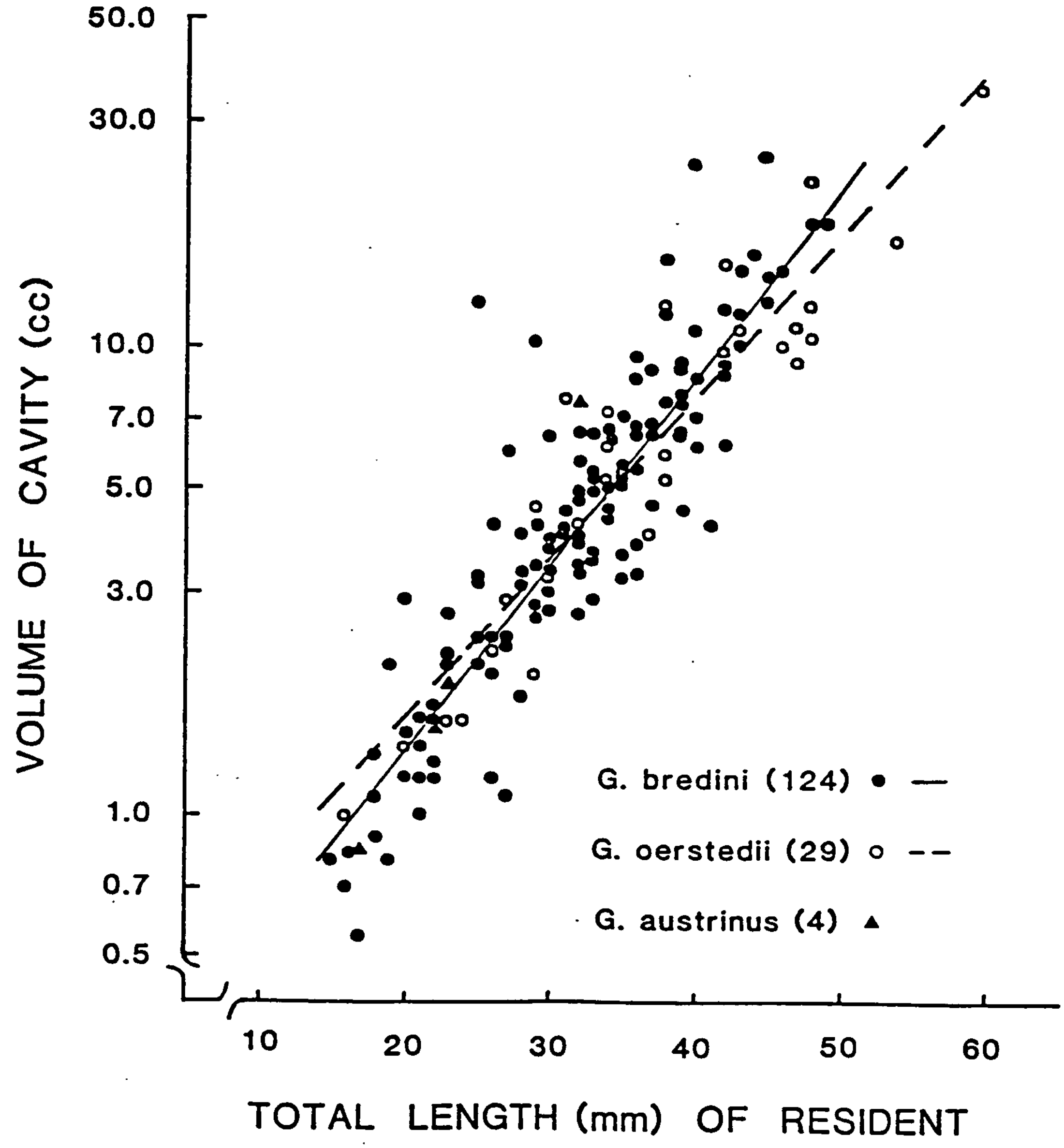

Fig. 6.6 Volume of cavities occupied by *Gonodactylus* in coral rubble. Regression lines represent the best fit of the data with an exponential relationship ($Y = ae^{bx}$). Cavities were collected at Isla Margarita in 1980 (from Steger 1985, 1987, with permission).

apart. The burrow connecting the two entrances runs only 20-40 cm under the surface. Each entrance has a diameter of 5-7 cm, but during the day these animals typically cap the entrances with a sand-mucus plug leaving only a small hole in the center for their eyes and antennules. When the burrow is covered by water the male sits in one camouflaged entrance waiting for passing prey, which are seized by a rapid strike of the barbed raptorial appendages. Females rarely hunt for themselves and are provisioned by their mates. At night males are more likely to hunt from an open burrow entrance, but they still rely exclusively on ambush to obtain prey. Except to locate another mate, should their own die, individuals rarely leave their burrows and persist in one location for several years, possibly their entire lives (Caldwell 1991).

By systematically walking across exposed seagrass beds at low tide, we located *Lysiosquilla* burrow entrances. Burrows were also discovered by searching at night when entrances are more frequently open. Once a burrow was found it was permanently marked by driving a stake near the entrance. Eventually, because males hunt from both openings to the burrow, the two entrances were identified and marked. Even when animals completely sealed the entrances for 2 or 3 wk to molt or brood eggs, the burrow could still be found by digging down 15-20 cm through the plug.

Between 1979 and 1983 we located and marked *Lysiosquilla* burrows on low intertidal seagrass beds and reef flats surrounding Punta Galeta, including Isla Mina, Isla Largo Remo, and Isla Margarita. Some burrows were observed for up to 4 yr and very few burrows "disappeared." In August 1983, when we last surveyed these sites prior to the 1986 oil spill, there were 17 marked and active *Lysiosquilla* burrows at MINA, two at LRW, one at LRN, and two near the Galeta Marine Laboratory dock.

6.2.3 Bahía Las Minas Oil Spill

On 27 April 1986 at least 60,000-100,000 bbl of medium-weight crude oil spilled from a ruptured storage tank into Bahía Las Minas near the Galeta Marine Laboratory. By 15 May oil had spread along the coast and washed across fringing reefs into mangroves, small estuaries, and onto beaches within 10 km of the refinery. By June heavy oiling was observed along much of the coast between Isla Margarita and Islas Naranjos, although the degree of oiling at specific sites was highly variable. Much of the coast hardest hit by the spill included sites where, from 1979 to 1983, we had studied the behavior and ecology of gonodactylid stomatopods. Because of the considerable knowledge we had of these populations and habitats combined with the importance of gonodactylids as predators in the fringing reef community, they seemed to be ideal candidates for study to determine the initial effects of the spill on inhabitants of these habitats and to follow any long-term consequences.

6.3 Objectives, Rationale, and Design of the Long-term Study

6.3.1 Overview

The initial September 1986 survey of reef flats around the Galeta Marine Laboratory was designed to document any effects of the oil spill on stomatopod populations. While we concentrated on population consequences, particularly demographic structure at oiled and unoiled sites, we also discovered physiological and behavioral effects associated with oiling that pointed to some of the mechanisms involved in producing these population changes.

An assessment of long-term effects of the oil spill on gonodactylid stomatopods from February 1987 to February 1991 was designed to examine the persistence and consequences of effects of the spill on gonodactylid stomatopods. We specifically examined several parameters that were, according to the initial postspill survey, affected by oiling.

6.3.1.1 Population Structure

In 1986 we documented selective mortality or emigration of large adults from oiled sites (Jackson et al. 1989). We wanted to determine whether large *Gonodactylus* would immigrate back into these oiled areas and whether young adults would be able to mature and continue to survive.

6.3.1.2 Recruitment

During the initial assessment of effects of the oil spill, we noted a lack of small stomatopods at both oiled and unoiled sites, with the greatest decrease at oiled sites. These juveniles should have recruited into the population during the 3 mo following the spill. However, recruitment is seasonal and sporadic on this coast and it was not possible to determine whether we were observing normal variation in recruitment or an effect specifically related to the spill. We planned to monitor recruitment over the next 5 yr to determine whether it was re-established at these sites and whether there would be a difference in the level of recruitment at oiled and unoiled sites. We also hoped to gain some understanding of natural variation in recruitment to help us interpret our findings. In addition, if recruitment continued to fail, we intended to follow subsequent changes in population structure, and ultimately the effects this might have on reproduction and survival of local populations.

6.3.1.3 Growth

The September 1986 study showed that adult gonodactylids at oiled sites were growing 50% more per molt than animals at unoiled sites. We postulated this increase was due to competitive release resulting from the loss of large individuals from the population, possibly because of an increase in the number of hermit crabs (potential prey) resulting from the sudden availability of empty gastropod shells (see Garrity et al., Chap. 5). We planned to monitor growth at oiled and unoiled sites to determine whether this effect persisted when and if competition and food limitation were restored.

6.3.1.4 Injuries

Large *Gonodactylus* had fewer wounds at oiled than at unoiled sites. We postulated this was due to the reduced density of large individuals in oiled populations and therefore less fighting for cavities. We planned to follow wounding rates to determine whether they increased in oiled populations if and when large individuals returned and competition for cavities was re-established.

6.3.1.5 Stomatopod-size to Cavity-volume Relationships

After the spill, gonodactylids at oiled sites occupied larger cavities than the same-sized individuals had prior to the spill. We hypothesized that the loss of large individuals from oiled sites reduced competition for cavities, and allowed animals to acquire cavities closer to the size they preferred. We planned to follow this stomatopod-size to cavity-volume relationship to see whether it returned to prespill levels if and when competition was restored as large individuals reappeared in the population.

6.3.1.6 Habitat Change

There was considerable loss of *Thalassia*-blade cover on lower intertidal seagrass beds at oiled sites following the oil spill. These seagrass beds are prime habitat for gonodactylids, and recovery of stomatopod populations in these affected habitats relied on the presence of healthy *Thalassia* beds. We planned to monitor intertidal seagrass beds to determine the extent of damage and the course of their recovery.

6.3.1.7 Effects on Other Stomatopods

In 1983 we recorded a number of *Lysiosquilla glabriuscula* living on reef flats around Punta Galeta, however, following the spill we failed to locate a single *Lysiosquilla* burrow. We planned to continue monitoring for the reappearance of this species.

6.3.2 Confounding Effects

Three factors conspire to make the interpretation of our results difficult with respect to the direct effect of oiling on stomatopods: (1) natural variation in recruitment, (2) changes in other animals that might affect stomatopods, and (3) habitat alteration.

6.3.2.1 Natural Variation in Gonodactylid Recruitment

Our prespill studies from 1979 to 1983 demonstrated that recruitment of juvenile *Gonodactylus* into the population varies from month to month, with season, and from year to year. While *Gonodactylus* breed and release larvae every month, recruitment is sporadic. A few recruits may appear each month, but generally there is a peak in recruitment during late dry season and early wet season (February through May). A second minor increase may also occur in October and November. However, recruitment totally fails during some months, and overall levels are higher in some years than in others (Steger 1985, 1987). This variation can have a marked effect on the number of small gonodactylids in the population. Pulses of or failures in recruitment can be followed in the age structure of a population for at least a year until these animals reach 25-35 mm total length. Because growth markedly slows in larger individuals, and because to some extent the number of larger gonodactylids is determined by cavity availability, large size classes remain relative constant unless recruitment declines markedly over two or more consecutive years. *Gonodactylus* populations have not been studied for a sufficient period to understand the extent of fluctuations in recruitment, whether these changes occur over a sufficiently long period to affect demographic structure, particularly in larger size classes, and the geographical extent of such changes. Nor do we understand the causes of these natural perturbations. It is against this background of natural variation in recruitment that we must then try to interpret possible consequences of the oil spill on stomatopod recruitment and population structure.

6.3.2.2 Effects of Other Animal Species on Stomatopods

We only surveyed stomatopods present at the study sites. We have no quantitative data on changes in abundance or behavior of other predators, prey, or competitors with stomatopods that lived in these habitats. Effects that we might attribute directly to oiling could be secondarily mediated through other species. For example, one of the major differences measured in the first sampling period after the spill was that large gonodactylids at oiled sites grew more than large gonodactylids at unoiled sites. It was our impression that many potential prey species, particularly gastropods, had been eliminated, or their numbers greatly reduced, at oiled sites. On the other hand the death of many gastropods may have provided an abundance of empty shells that could be used by hermit crabs. It appeared to us that hermit crabs were extremely numerous in the months following the spill, possibly because their numbers were no longer limited by shell availability. This dramatic increase in abundance of a prey species could account, at least in part, for the increased growth that we observed in stomatopods. However, without quantitative data on other biotic constituents of these habitats, such interpretations must remain tentative.

6.3.2.3 Habitat Change

One of the most dramatic effects recorded in our study was the destruction of the *Thalassia* bed at Isla Largo Remo North, the subsequent loss of soft sediment, and its replacement with an unconsolidated jumble of coral rubble. To further complicate matters, the red mangrove forest directly behind this site was also deteriorating. Many trees died, roots rotted, and substrata began to erode. This deterioration resulted in the release of some oil and silt back onto the former site of the *Thalassia* bed. These confounding factors make it difficult to attribute decreases in stomatopod recruitment and abundance to a particular cause. The decline in stomatopods could be due to a lack of suitable substrata, a lack of prey, reoiling of the site, or some combination of these or other unidentified factors. With only one study site having undergone such severe and confounded degradation, it is difficult, if not impossible, to tease apart cause and effect.

6.3.3 Site Selection and Design

6.3.3.1 Site Selection

To assess effects of the 1986 Bahía Las Minas oil spill on local gonodactylid populations, we planned to sample several sites that received significant oiling and compare them to other locations with little or no oiling. We also wanted to select sites with prespill data on stomatopod populations. That limited us to Isla Margarita, Punta Galeta, Isla Mina, and two sites on Isla Largo Remo (Fig. 6.2). Because gonodactylids are most numerous in low intertidal *Thalassia testudinum* beds (Steger 1987), it seemed appropriate to concentrate on this type of habitat.

A review of prespill data suggested that detecting differences in population structure among sites would require collections of at least 300 gonodactylids per site per sampling period. With only 3 wk per sampling period in the field and knowing that we could collect and process no more than 100 stomatopods per day, as well as the added time required to collect data for density and cavity-volume estimates, only two oiled and two unoiled sites could be studied.

In 9 September 1986 we inspected a number of seagrass beds for evidence of oiling and discussed with STRI personnel present during the spill which of the potential sites had been oiled and which had not. From a number of possible beds, we selected four for study that met our criteria.

Isla Largo Remo North (LRN) was closest to the source of the spill. There was considerable evidence of oiling; no live *Thalassia* blades could be found, much of the coral rubble was spotted with weathered oil, adjacent mangrove roots were caked with weathered oil, and an oil sheen was still being released from the substratum when we walked across the site. We visually classified this site as heavily oiled and established it as an oiled station.

Isla Mina (MINA) also showed signs of considerable oiling. The shore and mangrove roots were caked with weathered oil, there was considerable loss of

Thalassia cover, and an oil sheen appeared when the substratum was disturbed. We visually classified this site as moderately to heavily oiled.

Isla Largo Remo West (LRW), while only 400 m from LRN, showed little indication of oileding. *Thalassia* beds were lush and no oil was detected on rubble. There was no indication of oil being released when sediments were disturbed. STRI personnel reported that, during the spill, oil built up on the front of the reef crest at low tides, but floated over seagrass beds during high tides and was not deposited directly on the reef flat. We visually classified this site as having received light to no oiling and established it as a reference station.

Isla Margarita (MAR4) was the farthest of the original study sites from the source of the spill. There was no evidence of recent oiling at the site; seagrass beds were healthy and no oily sheen was released from disturbed sediments. We visually classified this site as having received light to no oiling and established it as the second reference station.

By February 1989 there was a general decline in gonodactylid recruitment at both the oiled and reference sites. To help determine the scale of this phenomenon, we established two control sampling sites up the coast that had received no oiling during the spill. These sites were María Soto, 23 km northeast of the source of the spill, and Punta Escucha, 26 km northeast of the site of the spill (Fig. 6.2). Both sites had lush *Thalassia* beds strewn with rubble.

Maria Soto (SOTO) was quite similar to sites at and near Punta Galeta. The seagrass bed was situated on a reef platform directly behind a northwest-facing reef crest. During onshore winds waves came over the crest, washed across the seagrass bed, and flowed along the shore to the southwest. One major difference between SOTO and three of the primary sites (LRN, LRW, and MINA) was the absence of mangroves along the shore. In this respect, SOTO was most like MAR4.

Punta Escucha (ESCU), situated in a somewhat protected bay, was shielded from large swells arriving from the north. The reef crest was less developed and the *Thalassia* bed was in slightly deeper water than the other sites. The seagrass bed selected for sampling was 50 to 150 m offshore, and there were few mangroves directly adjacent to the site. The seagrass bed had a softer, more silty substratum than the other sites.

6.3.3.2 Site Classification According to Oiling

None of the seagrass beds that we studied was sampled in 1986 for hydrocarbons, so they cannot be directly classified according to the system used by Burns (Chap. 3). Burns (Chap. 3) did sample coral reef sediments at LRE2, approximately 300 m east of LRN, and classified the site as moderately oiled by hydrocarbon analysis. However, inspection of LRN indicated that it was more severely affected than LRE2 and fits better with the heavily oiled category.

A subtidal seagrass bed was sampled on the northern end of Isla Mina (MINN). It was visually classified as heavily oiled and hydrocarbon analysis confirmed this assessment. This site was approximately 200 m north of MINA. From our inspection of MINA, we feel this site more closely corresponds to Burns's moderately oiled category.

No hydrocarbon sampling was conducted that closely corresponds with the reference sites, LRW and MAR4. Since oil was known to flow over LRW at high tide, it is reasonable to classify this site as having been lightly oiled. Due to the proximity of oil to MAR4, the same classification of lightly oiled is also appropriate for this site.

Hydrocarbon sampling at a subtidal reef in the vicinity of SOTO and ESCU (DONR) confirms that these sites were probably unaffected by this spill (Burns, Chap. 3).

6.3.3.3 Design

The original design consisted of two treatment levels, light to no oiling (reference) and moderate to heavy oiling (oiled), with two sites in each treatment. In 1989 a third treatment level, no oiling (control), was added, with two sites. We had hoped to analyze data using repeated-measures analysis of variance. However, over the course of the study, the two oiled sites physically and biologically diverged. The habitat at MINA recovered to its prespill state while LRN deteriorated and changed into an unconsolidated rubble bed. Later in the study, it did not seem valid to consider these two sites as part of the same treatment. To a lesser extent, the same was true of the two reference sites. Because of its proximity to LRN and the prevailing direction of currents, LRW experienced significant siltation as sediments at LRN were washed away. We saw no evidence of similar siltation at MAR4. Even the two control sites proved to be more dissimilar than expected. The silty and protected nature of ESCU made it physically and biologically different from the other five sites. For these reasons, and due to lack of normality and unequal variances in much of the data, we abandoned the repeated-measures design and relied, when necessary, on within-site variance estimates to compare sites with one another and across time. When it seemed biologically sound, particularly early in the study, sites were combined within treatment for analysis. We also frequently present data arranged by treatment, but in these cases mean them to be only broad qualitative categories.

6.4 Oiling and Reoiling

We recorded any evidence of oil observed at all sites during visits between 1986 and 1991. At LRN an oil sheen appeared on the surface of the water whenever someone walked through the area during each visit from September 1986 until February 1988. Oil sheens continued to appear sporadically at LRN through February 1991. Oil was also found in some coral rubble at LRN during every visit. At MINA we observed oil released from sediments during September 1986-September 1987. Oil was found in rubble at MINA in September 1986 and February

1987, but not in September 1987 or thereafter. We did not see oil released from sediments and did not find oil in coral rubble at LRW, MAR4, SOTO, or ESCU.

6.5 Components of Research

6.5.1 General Sampling Methods

Two types of sampling programs, *area quadrats* and *rubble samples*, were established in the initial September 1986 postspill survey. These sampling methods were continued at the two oiled and two reference sites throughout the study. Each site was sampled in September (wet season) and February (dry season) through February 1988. This provided a sufficient set of data to track any changes in stomatopod density at these locations and to detect any differences in seasonal effects between oiled and reference sites. Sampling in the wet season was discontinued after September 1988. The two unoiled control sites (SOTO and ESCU) were added in February 1989; only rubble sampling was undertaken.

6.5.1.1 Area-quadrat Samples

In September 1986 we established 400-m^2 reserves, which were used exclusively for quadrat sampling, at each of the four primary sites. Each reserve was selected as typical of seagrass beds in the area. The reserves were also matched across sites to be as similar as possible prior to the spill with respect to depth, percent *Thalassia* cover, and the amount and kinds of rubble and coralline algae they contained. To accomplish this matching we had to rely on field notes and our memory of the nature of these locations in 1983 when last surveyed. However, having spent 5 yr sampling these areas, we are confident that the characterization of the state of these reserves prior to the spill was accurate.

During each sampling period we sampled at each reserve 30 0.5-m^2 quadrats. The position of each quadrat was determined by laying down *x-y* coordinate axes and using pairs of random numbers to locate its placement. A 0.5-m^2 square frame was placed on the substratum, water depth and characteristics of substrata in the quadrat recorded, and all hard substrata removed, placed in buckets, and transported ashore. All pieces of hard substrata larger than 2 cc were measured and then broken to collect the resident stomatopods. These samples were also used to identify changes in the habitat and to estimate densities of rubble, as well as to determine gonodactylid density.

The removal and breaking apart of coral rubble is a destructive process that conceivably could alter habitat availability and, thus, stomatopod numbers across the study. Furthermore, because we processed 30 0.5-m^2 quadrats from the same 400-m^2 reserves each sampling period, it is likely that some areas were sampled more than once, although lack of precision in laying out *x-y* coordinates makes it unlikely that the exact same location was sampled repeatedly.

These habitats are dynamic, and most rubble in the areas sampled is not firmly embedded in the substratum. Through wave action, normal currents, storms, logs rolling over reef flats, and biological agents, rubble is constantly being created on the forereef and reef crest and then is transported across reef flats. Furthermore, tagging studies show considerable turnover in pieces of rubble typical of those containing stomatopods and, frequently, these pieces are moved or buried and re-exposed in this habitat (Caldwell 1988). We noted little effect of repeated sampling on rubble quality or abundance. Given the amount of suitable gonodactylid habitat in the area, gonodactylid density, and the frequency that local gonodactylids move (Caldwell et al. 1989), we feel the sampling program had little effect on local populations.

6.5.1.2 Rubble Samples

Pieces of coral rubble were sampled during every sampling period at all sites. Rubble was collected from areas within 100 m of the quadrat reserves at the oiled and reference sites, and from an approximately 2,000-m^2 area at the two control sites. Each piece of rubble was selected haphazardly, examined for cavities, placed in a plastic bag, and returned to shore. The criteria for selecting a piece were that it contain at least one hole that a stomatopod could use as a refuge and was representative of rubble sizes at the site. These generally ranged from 10,000 cc to 100 cc. Each piece was then measured and broken, and all stomatopods removed. Eight to 10 samples, each sample consisting of 10 pieces of rubble, were collected from every site each sampling period. Rubble samples were used to calculate densities of *Gonodactylus* per unit volume of rubble. Again, due to the generation and movement of rubble and the high density and movement of stomatopods in these areas, we feel the sampling effort had little effect.

6.5.1.3 Stomatopod Identification and Cataloguing

All gonodactylids collected in the field were returned to the laboratory and examined under a dissecting microscope to determine species, sex, size, reproductive condition, and injuries. They were then held individually in cups of seawater outside under ambient conditions to identify and measure any animal that molted. After 3 d the animals were returned to another reef flat at least 1 km from any of the study sites.

Four species of *Gonodactylus*, *G. bredini*, *G. oerstedii*, *G. austrinus*, and *G. spinulosis*, were collected during this study. However, only a single 16-mm male *G. spinulosis* was captured after the spill (September 1986 at MAR4). This species is so rare at the sites that it was eliminated from all analyses. The other three species, *G. bredini*, *G. oerstedii*, and *G. austrinus*, were collected in a ratio of approximately 7:2:1. Because they are all approximately the same size (maximum total length of 60-70 mm), occupy the same types of cavities in coral rubble, and generally can be found in the same types of habitat, they were combined for the sake of analysis.

6.5.2 Effects of Oiling on Habitat Characteristics

6.5.2.1 Methods

Using area-quadrat samples, the amount of rubble per unit area was estimated at oiled and reference sites for all sampling periods from September 1986 through February 1991. This determination was accomplished by measuring the volume of rubble broken in each 0.5-m^2 quadrat.

Physical characteristics of the substrata were also recorded from quadrat samples. We classified the physical characteristics found in quadrats as one of the following five categories.

1. ***Thalassia* Blades.** Quadrats were located in lush stands of *Thalassia* where blades of turtlegrass were abundant throughout the quadrat.

2. ***Thalassia* Rhizomes.** Quadrats contained no turtlegrass blades above the sand-silt bottom, but the substratum contained turtlegrass roots and rhizomes.

3. **Coral Bench.** Hard substratum in the form of dead coral bench covered at least 10% of the quadrat.

4. **Soft Sediment.** The substratum consisted of a sand-silt bottom with no evidence of coral bench or seagrasses.

5. ***Halodule* Blades.** Quadrats were dominated by the seagrass *Halodule*.

6.5.2.2 Results

The mean volume of rubble per 0.5 m^2 varied significantly through time at LRN and LRW but not at MINA and MAR4 (Table 6.1). The largest change occurred at LRN, where erosion exposed large amounts of rubble in 1990 and 1991 (see below). The mean number of pieces of coral rubble per 0.5 m^2 varied significantly at all sites (Table 6.2).

The habitat at LRN, the most heavily oiled site, changed completely during the course of monitoring (Table 6.3). Although this area was part of a large turtlegrass bed prior to the oil spill, not one of the 240 quadrat samples taken from the 400-m^2 sampling reserve at LRN after the oil spill contained live blades of *Thalassia*. All samples taken between September 1986 and February 1988 contained roots and rhizomes in the sand, evidence of the previous *Thalassia* bed that had existed at the site, but all of these roots and rhizomes were dead. By February 1988 the matrix of dead roots and rhizomes had begun to disintegrate and the soft substratum had started to erode. This exposed underlying hard substrata in 30% of the quadrats by February 1988. By February 1990 erosion had proceeded to a state where it was evident that hard substrata exposed by erosion was not coral bench, but

Table 6.1 Mean volume (cc) of coral rubble per 0.5 m^2. Means are the average volume of rubble from 30 quadrats taken at each site in September (*S*) and February (*F*) of the years indicated. Standard errors are in parentheses. Reference sites were lightly oiled to unoiled.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
S86	766 (486)	1,017 (373)	1,213 (674)	1,507 (579)
F87	280 (106)	1,949 (901)	1,192 (394)	924 (375)
S87	369 (148)	513 (157)	872 (306)	996 (347)
F88	399 (226)	636 (173)	721 (354)	700 (234)
S88	330 (198)	446 (99)	446 (117)	1,216 (358)
F89	556 (208)	485 (133)	410 (95)	767 (266)
F90	4,493 (667)	512 (115)	645 (112)	1,089 (303)
F91	7,075 (1,313)	564 (106)	1,161 (292)	928 (213)

Kruskal-Wallis ANOVA, all collections: LRN, $P < .001$; MINA, $P = .5$; LRW, $P = .04$; MAR4, $P = .4$.
Kruskal-Wallis ANOVA, February collections: LRN, $P < .001$; MINA, $P = .4$; LRW, $P = .03$; MAR4, $P = .1$.

Table 6.2 Mean densities of coral rubble per 0.5 m^2. Means are the average number of pieces of rubble from 30 quadrats taken at each site in September (*S*) and February (*F*) of the years indicated. Standard errors are in parentheses. Reference sites were lightly oiled to unoiled.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
S86	1.6 (0.29)	7.8 (1.65)	1.8 (0.30)	5.4 (0.92)
F87	0.9 (0.20)	5.4 (1.14)	2.6 (0.42)	3.6 (0.53)
S87	0.7 (0.21)	2.8 (0.53)	1.0 (0.19)	2.5 (0.41)
F88	1.0 (0.29)	4.6 (0.59)	1.5 (0.32)	4.0 (0.82)
S88	0.7 (0.16)	3.2 (0.40)	1.3 (0.26)	1.4 (0.20)
F89	1.5 (0.21)	3.2 (0.62)	1.4 (0.30)	1.3 (0.28)
F90	4.2 (0.33)	2.6 (0.42)	2.0 (0.25)	3.0 (0.40)
F91	4.7 (0.62)	4.7 (0.76)	2.4 (0.37)	4.1 (0.60)

Kruskal-Wallis ANOVA, all collections: LRN, $P < .001$; MINA, $P = .01$; LRW, $P = .003$; MAR4, $P < .001$.

Kruskal-Wallis ANOVA, February collections: LRN, $P < .001$; MINA, $P = .07$; LRW, $P = .02$; MAR4, $P < .001$.

large pieces of unconsolidated coral rubble. At that point these pieces were counted as pieces of rubble if they could be collected without disturbing the sand-silt bottom or coral bench, if they were still buried in soft sediments. Erosion therefore caused a large increase in the amount of rubble sampled from quadrats in 1990 and 1991 at LRN (Table 6.1).

Table 6.3 Physical characteristics of moderately to heavily oiled habitats. Counts were recorded qualitatively as the number of quadrats within each category at the site during the time indicated.

	Thalassia Blades	*Thalassia* Rhizomes	Coral Bench	Soft Sediment	*Halodule* Blades
LRN					
September 1986	0	29	1	0	0
February 1987	0	29	1	0	0
September 1987	0	30	0	0	0
February 1988	0	21	9	0	0
September 1988	0	0	6	22	2
February 1989	0	0	12	17	1
February 1990	0	0	6	22	2
February 1991	0	0	3	27	0
MINA					
September 1986	30	0	0	0	0
February 1987	28	2	0	0	0
September 1987	27	3	0	0	0
February 1988	29	1	0	0	0
September 1988	5	24	0	1	0
February 1989	29	0	0	1	0
February 1990	26	0	0	3	1
February 1991	21	5	0	1	3

Four small patches of *Thalassia* (all less than 0.75 m^2) survived oiling at LRN in an area next to the quadrat area. We estimated the amount of erosion by the difference in height between the center of a patch and the substratum immediately adjacent to the patch where turtlegrass had died and the substratum eroded. Between 10 and 12 cm of sediment had eroded by 1990, and between 11 and 14 cm of sediment had eroded by 1991. Survival of these patches is doubtful because they were growing smaller as edges continued to erode.

MINA, a heavily oiled site (Table 6.3), and both reference sites, LRW and MAR4 (Table 6.4), continued as thriving beds of turtlegrass with scattered patches of bench, sand-silt, and *Halodule* from September 1986 through February 1991. The only differences in the samples from these sites occurred in September 1988 and February 1990. Quadrat samples in September 1988 were taken after a long tidal exposure, which caused *Thalassia* blades to die, leaving the root and rhizome system intact. These areas quickly recovered after the event. In February 1990 a significant number of quadrats at LRW were classified as sand-silt. (The decision to classify the quadrats as sand-silt or *Thalassia* rhizomes was arbitrary.) Erosion at LRN caused large amounts of sediment to be carried by prevailing currents to the southwest. Much of this sediment was deposited at LRW as currents slowed over the *Thalassia* beds. The root and rhizome system was not killed by sedimentation and the area recovered by 1991.

Table 6.4 Physical characteristics of lightly oiled to unoiled reference habitats. Counts were recorded qualitatively as the number of quadrats within each category at the site during the time indicated.

	Thalassia Blades	*Thalassia* Rhizomes	Coral Bench	Soft Sediment	*Halodule* Blades
LRW					
September 1986	30	0	0	0	0
February 1987	30	0	0	0	0
September 1987	30	0	0	0	0
February 1988	30	0	0	0	0
September 1988	2	28	0	0	0
February 1989	29	0	1	0	0
February 1990	19	11	0	0	0
February 1991	27	0	1	2	0
MAR4					
September 1986	30	0	0	0	0
February 1987	29	0	1	0	0
September 1987	30	0	0	0	0
February 1988	30	0	0	0	0
September 1988	0	30	0	0	0
February 1989	29	0	0	1	0
February 1990	29	0	0	1	0
February 1991	26	0	2	2	0

6.5.2.3 Discussion of Habitat Change

The most dramatic long-term effect of the oil spill was the transformation of the habitat at LRN. Prior to the oil spill the site at LRN was an intertidal bed of *Thalassia testudinum* with scattered pieces of small coral rubble, bordered by an almost impenetrable stand of *Rhizophora mangle*. The area now is dominated by large pieces of unconsolidated coral rubble resting on a fine, sand-silt substratum, bordered by a 15 m wide mud flat. The oiling at LRN killed the *Thalassia* and a wide band of mangroves adjacent to the seagrass bed. There was a slow but continuous erosion of sediments at the site from 1987 through 1988 as the root systems of the turtlegrass and mangroves deteriorated, and dead mangrove trees were broken down by seasonal high tides and driftwood. During the strong wave action accompanying the dry season of 1989, the process accelerated as the vegetative structure holding the sediments completely broke down. The transformation of the area was essentially complete by 1990, although erosion continued through 1991. By 1991, 11-14 cm of substratum had eroded from the site. Considering the loss of sediments, turtlegrass, and mangroves, there is little chance in the near future for this habitat to "recover" to the state that existed prior to the oil spill.

MINA also was heavily oiled. However, it was not as heavily oiled or, at least, not as heavily affected by oiling as LRN. The *Thalassia* bed at MINA was killed back to the roots, but rhizomes were not killed by the oiling and there has been no overall deterioration of the habitat. Except for a few widely dispersed patches of dead mangroves along the shore, MINA appears today much as it did before the oil spill.

Why seagrass beds at LRN, but not MINA, were destroyed remains a puzzle (see Cubit and Levings, Chap. 2). Both sites received significant oiling, although we have no means to determine whether one site received more oil than the other. Possible explanations for the recovery of MINA may have to do with current patterns and the lack of a heavy stand of mangroves directly adjacent to the site. At LRN onshore winds push water directly against the shore and water flow is not strongly directional. At MINA water arrives on the seagrass bed over the reef crest and there is usually a strong flow parallel to the shore that exits into the bay south of the site. This flow may effectively flush hydrocarbons from the *Thalassia* bed. Perhaps more importantly, the shore immediately adjacent to MINA is primarily sand beach with a scattering of red mangroves. While thick stands of mangroves occur to the west and south of the site, there are relatively few trees directly adjacent to it. In contrast, LRN was directly in front of a dense red mangrove forest. At Isla Largo Remo, oil that was deposited in mangroves continued to flush back out across the seagrass bed for several months, and we recorded occasional reoiling even years later. At MINA there were few mangroves to hold oil, and prevailing current patterns led to little or no reoiling.

LRW was not affected directly by oiling, but it was affected by the erosion at LRN. Water comes across the north-facing reef flat at Isla Largo Remo driven by waves, washes around the island at the mangrove fringe, and exits through a break in the fringing reef after flowing over the west-facing reef flat. There was heavy sedimentation at LRW caused by the erosion at LRN. Almost half of the quadrat area was covered by 1-2 cm of sand-silt at LRW in February 1990. However, there seemed to be no long-term effects of sedimentation on the *Thalassia* bed at LRW.

MAR4 was lightly oiled to unoiled, and the habitat was not significantly affected by any oiling that may have occurred.

6.5.3 Densities of *Gonodactylus*

6.5.3.1 Methods

Densities of *Gonodactylus* were established per unit area from quadrat samples and per unit volume of rubble from coral rubble samples. Densities from quadrat samples were analyzed for four size categories: (1) *all sizes combined*; (2) *small* (6-16 mm total length); (3) *medium* (16-40 mm total length); and (4) *large* (>40 mm total length). Rubble samples generated greater numbers of individuals, which allowed analysis of densities in six size categories: (1) all sizes combined; (2) 6-10 mm total length; (3) 11-20 mm total length; (4) 21-30 mm total length; (5) 31-40 mm total length; and (6) >40 mm total length. Due to extreme deviations from normality and unequal variances, statistical analyses were carried out by Kruskal-

Wallis 1-way analysis of variance (ANOVA; Wilkinson 1986). Significance levels were set at $P < .05$.

6.5.3.2 Results

Table 6.5 provides the size distribution and sex, as well as an estimate of density per m^2, of gonodactylids collected in 0.5-m^2 quadrat samples for each site in September 1986. The only comparable data available for this time of year prior to the spill come from Isla Margarita, where Steger (1985) estimated a mean density and SE of 9.6 ± 0.59 *Gonodactylus* per m^2. This density is very close to the value of 9.9 ± 1.51 that we obtained for the same site in September 1986. Stomatopod densities at the other three sites were lower, but without prespill data we could not say with certainty whether population numbers had been affected by the spill.

One aspect of these data that appeared unusual was the lack of large gonodactylids over 40 mm in total length at the oiled sites (LRN and MINA). Animals this size would be at least 2 to 3 yr old, and their numbers typically do not fluctuate widely, as does the abundance of smaller animals, which reflects sporadic recruitment events (Steger 1987). The structure of populations at LRW and MAR4 was more typical of populations sampled prior to the spill. Individuals in excess of 45 mm total length were common and usually there were many more large females than males (see above). Of the 101 gonodactylids collected at MINA and LRN in September 1986, only three (all males) were 40 mm or longer. This finding contrasted with MAR4 and LRW, where 31 (10 males and 21 females) of the 240 animals captured were at least 40 mm in length (G-test, $G = 12.2$, $P < .001$).

Further evidence for a loss of large gonodactylids at oiled sites came from comparing the size-frequency distribution of gonodactylids collected from both Isla Largo Remo sites in 1986 with animals captured at the same locations and at the same time of year in 1981 (Fig. 6.7). In 1986 LRW had considerably more large animals (>44 mm long) than did LRN (G-test, $G = 25.4$, $P < .001$). In 1981 just the opposite was true (G-test, $G = 14.3$, $P < .001$).

Densities of *Gonodactylus* of all sizes varied significantly in quadrat samples at LRN, LRW, and MAR4 between September 1986 and February 1991 (Table 6.6). Densities of *Gonodactylus* of all sizes did not vary significantly at MINA. Mean densities of *Gonodactylus* of all sizes varied significantly in rubble samples at all sites, including the two unoiled sites added in 1989 (Table 6.7).

Densities of large *Gonodactylus* (individuals greater than 40 mm in total length) did not vary significantly in quadrat samples at both reference sites, LRW and MAR4, and at one heavily oiled site, LRN (Table 6.8). The density of large *Gonodactylus* increased significantly at MINA, a heavily oiled site (Table 6.8). The increase in density of large *Gonodactylus* at MINA took place between September 1986, when no gonodactylids longer than 40 mm were found in quadrat samples, and February 1987, when the density of large gonodactylids was 0.5 individuals per 0.5 m^2. The density of large *Gonodactylus* did not vary significantly at MINA between February 1987 and February 1991 after the initial increase (Kruskal-Wallis 1-way

Table 6.5 Numbers and sex of *Gonodactylus* found in 0.5-m^2 quadrats. Values are for 30 quadrats taken at each site in September 1986 4 mo following the Bahía Las Minas oil spill. Reference sites were lightly oiled to unoiled.

	Oiled				Reference			
	LRN		MINA		LRW		MAR4	
Size (mm)	M	F	M	F	M	F	M	F
<12[1]	0		2		1		5	
12-14	0	0	0	2	4	3	2	5
15-19	2	4	12	15	9	7	9	12
20-24	4	4	6	7	6	4	15	13
25-29	2	3	3	3	6	11	13	16
30-34	1	4	4	4	8	5	7	13
35-39	2	0	6	8	4	5	6	16
40-44	1	0	2	0	1	6	4	3
45+	0	0	0	0	4	7	1	5
Total[2]	12	15	33	39	42	48	57	83

[1] Sex cannot be determined in *Gonodactylus* juveniles less than 12 mm total body length.
[2] Juveniles are not included in totals.

ANOVA performed on MINA data in Table 6.8 excluding the September 1986 sample, P = .58).

Densities of large gonodactylids did not vary significantly overall at LRN due to the large variation among quadrats within a sampling period (Table 6.8). However, there was evidence of immigration of large gonodactylids into the site. The density of large *Gonodactylus* immediately after the oil spill in September 1986 (0.03 individuals per 0.5 m^2) was significantly lower than the density of large individuals at the end of monitoring in February 1991 (0.2 individuals per 0.5 m^2; Kruskal-Wallis ANOVA, P = .02).

Densities of large *Gonodactylus* in rubble samples varied significantly at all sites except MAR4 (Table 6.9). There were two patterns of change in densities of larger individuals. First, densities increased significantly at heavily oiled sites between September 1986 and September 1987. There were no significant changes in densities at reference sites during this period. Second, densities decreased significantly between 1989 and 1991 at sites where densities were greater than 0.3 individuals per 1,000 cc in 1989 (MINA, LRW, SOTO, and ESCU). Densities did not vary significantly between 1989 and 1991 at sites where densities in 1989 were less than 0.4 individuals per 1,000 cc (LRN and MAR4).

Densities of medium-sized *Gonodactylus* (16-40 mm in total length) varied significantly at LRN, a heavily oiled site, and at MAR4, a reference site, but not at MINA, a heavily oiled site, and LRW, a reference site, during this period (Table

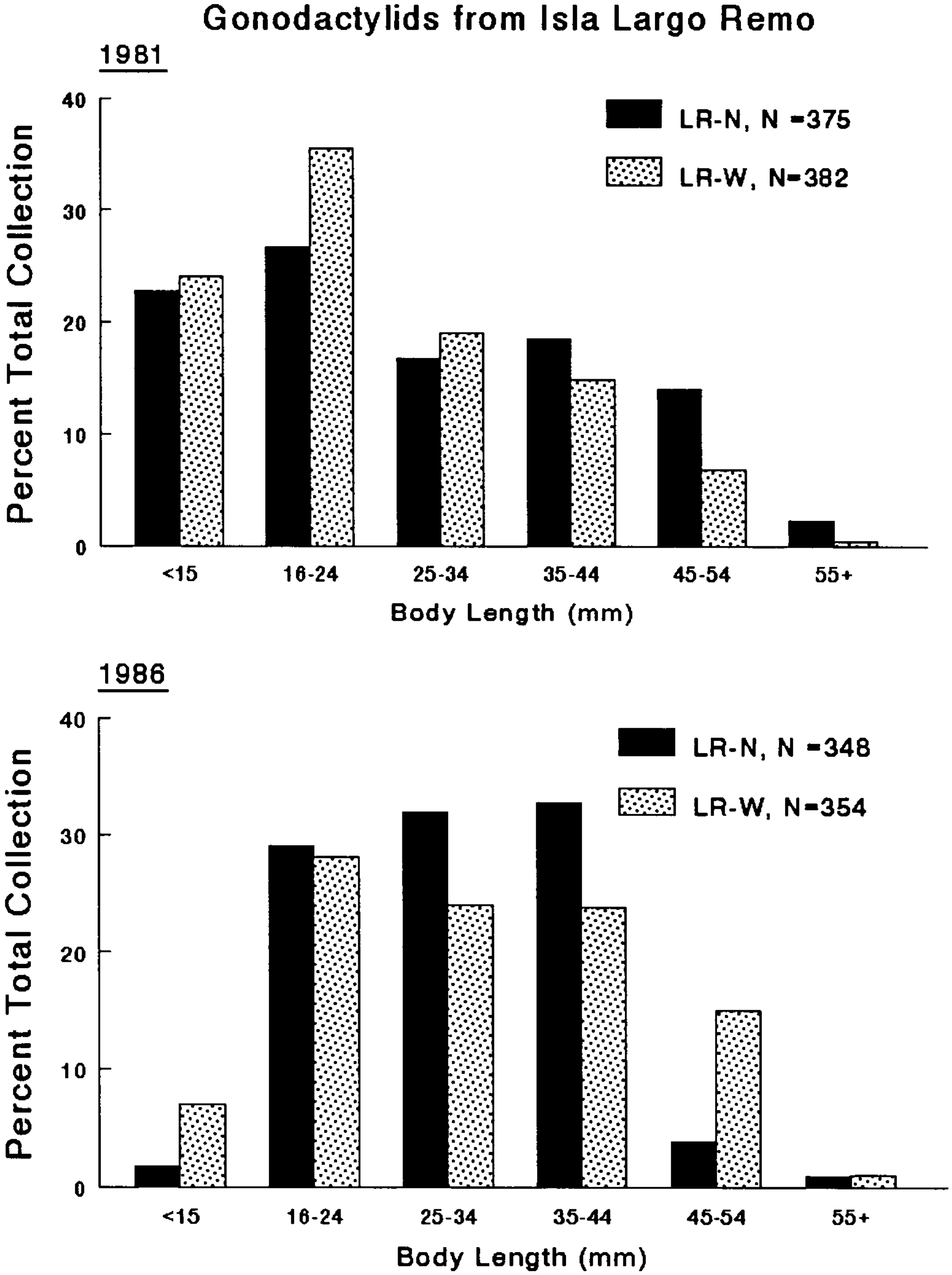

Fig. 6.7 Percent of individuals in each size class of *Gonodactylus* collected from Isla Largo Remo prespill in 1981 and postspill in 1986. All collections were made in August and September. *NORTH* = LRN; *WEST* = LRW.

Table 6.6 Mean densities of *Gonodactylus* per 0.5 m^2. Means are for 30 quadrats taken at each site in September (*S*) and February (*F*) of the years indicated. Standard errors are in parentheses. Reference sites were lightly oiled to unoiled.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
S86	0.9 (0.20)	2.5 (0.42)	3.0 (0.75)	5.0 (0.73)
F87	0.6 (0.31)	2.8 (0.44)	2.2 (0.51)	3.6 (0.66)
S87	0.7 (0.21)	2.2 (0.43)	1.4 (0.37)	3.0 (0.66)
F88	0.2 (0.10)	2.8 (0.45)	2.4 (0.86)	2.8 (0.56)
S88	0.3 (0.18)	2.3 (0.29)	1.3 (0.32)	1.7 (0.32)
F89	0.7 (0.15)	2.3 (0.55)	2.0 (0.48)	2.5 (0.59)
F90	2.4 (0.29)	3.9 (0.64)	3.7 (0.61)	5.3 (0.94)
F91	1.3 (0.20)	3.5 (0.53)	3.6 (0.57)	5.1 (0.73)

Kruskal-Wallis ANOVA: LRN, *P* <.001; MINA, *P* = .2; LRW, *P* = .002; MAR4, *P* <.001.

Table 6.7 Mean densities of *Gonodactylus* per 1,000 cc of coral rubble. Means are for 8-10 samples of 10 pieces of rubble taken at each site in September (*S*) and February (*F*) of the years indicated. Reference sites received light to no oiling. Control sites were unoiled. Standard errors are in parentheses. No data (*ND*) were taken at unoiled sites prior to 1989.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
S86	0.8 (0.05)	1.3 (0.11)	1.2 (0.12)	1.7 (0.23)	ND	ND
F87	0.6 (0.06)	2.2 (0.13)	1.7 (0.10)	1.7 (0.12)	ND	ND
S87	1.2 (0.08)	2.4 (0.13)	1.6 (0.05)	1.7 (0.01)	ND	ND
F88	0.5 (0.04)	2.2 (0.15)	1.3 (0.08)	1.4 (0.09)	ND	ND
S88	0.5 (0.06)	1.7 (0.13)	1.6 (0.07)	1.7 (0.16)	ND	ND
F89	0.7 (0.08)	1.7 (0.10)	1.5 (0.07)	2.1 (0.13)	1.6 (0.15)	0.9 (0.06)
F90	0.7 (0.06)	2.1 (0.19)	1.8 (0.13)	2.0 (0.06)	1.6 (0.13)	0.8 (0.08)
F91	0.4 (0.04)	1.1 (0.07)	1.2 (0.08)	1.5 (0.07)	1.2 (0.10)	0.6 (0.05)
P[1]	<.001	<.001	<.001	.007	.01	.03

[1] Kruskal-Wallis ANOVA.

6.10). The pattern of variation in densities, however, was the same at all sites: densities decreased, then increased.

Medium-sized *Gonodactylus* were analyzed in three size categories in rubble samples: (1) 11-20 mm total length; (2) 21-30 mm total length; and (3) 31-40 mm total length. Comparisons were made within sites by Kruskal-Wallis ANOVA among

Table 6.8 Mean densities of large *Gonodactylus* per 0.5 m^2. Means are for gonodactylids greater than 40 mm total length from 30 quadrats taken at each site in September (*S*) and February (*F*) of the years indicated. Standard errors are in parentheses. Reference sites were lightly oiled to unoiled.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
S86	0.03 (0.03)	0	0.5 (0.26)	0.4 (0.12)
F87	0.2 (0.11)	0.5 (0.17)	0.6 (0.18)	0.1 (0.06)
S87	0.07 (0.05)	0.4 (0.12)	0.5 (0.17)	0.3 (0.10)
F88	0.03 (0.03)	0.3 (0.11)	0.6 (0.25)	0.2 (0.11)
S88	0.1 (0.07)	0.2 (0.07)	0.3 (0.10)	0.4 (0.11)
F89	0.1 (0.08)	0.3 (0.14)	0.5 (0.18)	0.2 (0.10)
F90	0.2 (0.10)	0.5 (0.12)	0.6 (0.15)	0.2 (0.10)
F91	0.2 (0.08)	0.4 (0.15)	0.5 (0.13)	0.4 (0.11)

Kruskal-Wallis ANOVA: LRN, $P = .2$; MINA, $P = .02$; Isla LRW, $P = .8$; MAR4, $P = .05$.

Table 6.9 Mean densities of large *Gonodactylus* per 1,000 cc of coral rubble. Means are for 8-10 samples of 10 pieces of rubble taken at each site in September (*S*) and February (*F*) of the years indicated. Reference sites received light to no oiling. Standard errors are in parentheses. No data (*ND*) were taken at unoiled sites prior to 1989.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
S86	0.1 (0.01)	0.2 (0.03)	0.4 (0.10)	0.3 (0.07)	ND	ND
F87	0.2 (0.03)	0.6 (0.05)	0.4 (0.04)	0.2 (0.04)	ND	ND
S87	0.3 (0.04)	0.8 (0.05)	0.5 (0.02)	0.3 (0.07)	ND	ND
F88	0.1 (0.02)	0.5 (0.07)	0.3 (0.04)	0.2 (0.04)	ND	ND
S88	0.2 (0.04)	0.4 (0.06)	0.4 (0.04)	0.3 (0.05)	ND	ND
F89	0.2 (0.02)	0.4 (0.05)	0.4 (0.05)	0.3 (0.05)	0.7 (0.07)	0.5 (0.05)
F90	0.2 (0.03)	0.2 (0.04)	0.2 (0.08)	0.3 (0.05)	0.3 (0.06)	0.3 (0.04)
F91	0.1 (0.02)	0.2 (0.03)	0.2 (0.03)	0.3 (0.05)	0.3 (0.02)	0.3 (0.03)
P[1]	<.001	<.001	.005	.6	.005	.02

[1] Kruskal-Wallis ANOVA.

all samples. Comparisons were also made among only February samples (dry season) and only September samples (wet season) for the 11-20 mm and 21-30 mm size classes due to possible effects of seasonal recruitment on smaller sizes. Densities of 11-20 mm gonodactylids varied significantly among all samples combined and among dry season samples (February) at each site (Table 6.11). Among wet season samples

Table 6.10 Mean densities of medium-sized *Gonodactylus* per 0.5 m^2. Means are for gonodactylids 16-40 mm in total length from 30 quadrats taken at each site in September (*S*) and February (*F*) of the years indicated. Standard errors are in parentheses. Reference sites were lightly oiled to unoiled.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
S86	0.9 (0.20)	2.2 (0.37)	2.1 (0.47)	4.0 (0.57)
F87	0.4 (0.19)	2.0 (0.28)	1.2 (0.33)	2.3 (0.46)
S87	0.6 (0.19)	1.7 (0.36)	1.0 (0.25)	2.5 (0.46)
F88	0.1 (0.074)	1.5 (0.30)	1.2 (0.48)	1.3 (0.33)
S88	0.2 (0.12)	2.0 (0.28)	1.0 (0.23)	1.1 (0.24)
F89	0.2 (0.088)	1.4 (0.38)	0.7 (0.20)	1.3 (0.38)
F90	0.8 (0.16)	1.3 (0.29)	1.4 (0.27)	2.2 (0.33)
F91	0.7 (0.15)	1.7 (0.27)	1.5 (0.29)	1.8 (0.38)

Kruskal-Wallis ANOVA: LRN, $P < .001$; MINA, $P = .09$; LRW, $P = .06$; MAR4, $P < .001$.

Table 6.11 Mean densities of 11-20 mm *Gonodactylus* per 1,000 cc of coral rubble. Means are for 8-10 samples of 10 pieces of rubble taken at each site in September (*S*) and February (*F*) of the years indicated. Reference sites received light to no oiling. Standard errors are in parentheses. No data (*ND*) were taken at unoiled sites prior to 1989.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
S86	0.1 (0.01)	0.3 (0.02)	0.4 (0.09)	0.4 (0.04)	ND	ND
F87	0.07 (0.02)	0.5 (0.04)	0.4 (0.05)	0.3 (0.05)	ND	ND
S87	0.01 (0.01)	0.4 (0.07)	0.3 (0.04)	0.2 (0.03)	ND	ND
F88	0.2 (0.02)	0.4 (0.05)	0.3 (0.04)	0.3 (0.05)	ND	ND
S88	0.03 (0.01)	0.3 (0.04)	0.2 (0.06)	0.2 (0.04)	ND	ND
F89	0.2 (0.05)	0.7 (0.09)	0.5 (0.03)	0.9 (0.07)	0.2 (0.04)	0.2 (0.04)
F90	0.2 (0.04)	0.7 (0.10)	0.6 (0.05)	0.7 (0.06)	0.5 (0.05)	0.2 (0.03)
F91	0.06 (0.01)	0.2 (0.04)	0.2 (0.03)	0.2 (0.03)	0.3 (0.05)	0.05 (0.02)
P All[1]	<.001	<.001	<.001	<.001		
P Feb[2]	<.001	<.001	<.001	<.001	.02	.007
P Sep[3]	<.001	.09	.05	.01		

Kruskal-Wallis ANOVA: [1]all samples combined; [2]February samples only; [3]September samples only.

(September) densities varied significantly at LRN and MAR4, but not at LRW and MINA (Table 6.11). Densities of 21-30 mm gonodactylids also showed a seasonal pattern of density variation. Densities varied significantly among dry season samples at heavily oiled sites, but not at lightly oiled to unoiled reference sites and unoiled control sites (Table 6.12). Among wet season samples, mean densities of 21-30 mm gonodactylids decreased significantly at LRN and increased significantly at LRW, but did not vary significantly at MINA and MAR4 (Table 6.12). Densities of 31-40 mm gonodactylids varied significantly at all sites except SOTO (Table 6.13).

Densities of small *Gonodactylus* (6-15 mm in total length in quadrat samples, 6-10 mm total length in rubble samples) varied significantly at all sites for both quadrat samples (Table 6.14) and rubble samples (Table 6.15).

6.5.3.3 Discussion

The most apparent initial effect of the oil spill was the loss of large *Gonodactylus*, particularly females, from the two oiled sites. This was most likely a direct effect of oiling, which either produced the death of these individuals or caused them to leave the area. Because we did not survey this area until 4 mo after the spill, we cannot be certain whether mortality or emigration was more important in reducing the numbers of large stomatopods. Large gonodactylids seasonally move to deeper water during prolonged exposure of intertidal seagrass beds at the time of year of the spill. However, such movements typically take place over several days or weeks and usually do not result in mass mortality. By July and August large gonodactylids return to higher intertidal seagrass beds to resume breeding in these areas. The fact that they had not returned, but that many smaller individuals were breeding in these areas, strongly suggests they were killed either when oil was deposited on these beds or as they tried to flee the area.

Consequences of this loss of large individuals to the population, and subsequent perturbations in population size and structure detected throughout the remainder of the study are discussed in detail below.

6.5.4 Postlarval Recruitment

6.5.4.1 Methods

Densities of small *Gonodactylus* were analyzed in detail because these sizes represent recent postlarval recruitment. We paid particular attention to February data because it is the season when significant recruitment is most likely to occur. *Gonodactylus* postlarvae settle out of the plankton and recruit onto reef flats when they are 6-10 mm in length. It takes 2-4 mo for them to grow to 15 mm total length. To study recruitment at the various sites, we examined quadrat and rubble sample data for the occurrence of recruits. For data derived from 0.5-m^2 quadrats, the number of small animals was low and we therefore included all gonodactylids 15 mm or smaller. These animals had recruited within the past 4 mo. Rubble samples generated larger sample sizes, so we included only animals 10 mm or smaller. These animals had recruited within the past 4 wk, given their small size.

Table 6.12 Mean densities of 21-30 mm *Gonodactylus* per 1,000 cc of coral rubble. Means are for 8-10 samples of 10 pieces of rubble taken at each site in September (*S*) and February (*F*) of the years indicated. Reference site received light to no oiling. Standard errors are in parentheses. No data (*ND*) were taken at unoiled sites prior to 1989.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
S86	0.3 (0.03)	0.3 (0.05)	0.2 (0.03)	0.4 (0.07)	ND	ND
F87	0.08 (0.02)	0.2 (0.03)	0.2 (0.04)	0.2 (0.05)	ND	ND
S87	0.4 (0.05)	0.5 (0.10)	0.4 (0.02)	0.5 (0.07)	ND	ND
F88	0.02 (0.01)	0.4 (0.06)	0.3 (0.04)	0.2 (0.02)	ND	ND
S88	0.1 (0.03)	0.6 (0.10)	0.5 (0.07)	0.5 (0.08)	ND	ND
F89	0.05 (0.03)	0.3 (0.06)	0.2 (0.03)	0.2 (0.05)	0.1 (0.05)	0.03 (0.02)
F90	0.1 (0.01)	0.4 (0.08)	0.3 (0.03)	0.2 (0.04)	0.1 (0.03)	0.04 (0.02)
F91	0.09 (0.02)	0.2 (0.01)	0.2 (0.02)	0.2 (0.03)	0.1 (0.02)	0.03 (0.01)
P All[1]	<.001	.001	.001	.001		
P Feb[2]	.002	.02	.4	.99	.6	.08
P Sep[3]	<.001	.1	.04	.4		

Kruskal-Wallis ANOVA: [1]all samples combined; [2]February samples only; [3]September samples only.

Table 6.13 Mean densities of 31-40 mm *Gonodactylus* per 1,000 cc of coral rubble. Means are for 8-10 samples of 10 pieces of rubble taken at each site in September (*S*) and February (*F*) of the years indicated. Reference sites received light to no oiling. Standard errors are in parentheses. No data (*ND*) were taken at unoiled sites prior to 1989.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
S86	0.3 (0.03)	0.5 (0.08)	0.2 (0.02)	0.5 (0.09)	ND	ND
F87	0.2 (0.03)	0.7 (0.08)	0.5 (0.03)	0.5 (0.04)	ND	ND
S87	0.5 (0.04)	0.6 (0.09)	0.3 (0.03)	0.6 (0.06)	ND	ND
F88	0.2 (0.03)	0.6 (0.05)	0.5 (0.05)	0.4 (0.07)	ND	ND
S88	0.1 (0.04)	0.5 (0.10)	0.6 (0.04)	0.6 (0.10)	ND	ND
F89	0.2 (0.05)	0.4 (0.04)	0.4 (0.04)	0.4 (0.05)	0.5 (0.16)	0.1 (0.04)
F90	0.08 (0.03)	0.3 (0.06)	0.3 (0.05)	0.3 (0.04)	0.1 (0.03)	0.05 (0.01)
F91	0.1 (0.02)	0.09 (0.02)	0.3 (0.05)	0.2 (0.02)	0.2 (0.02)	0.1 (0.02)
P[1]	<.001	<.001	<.001	<.001	.1	.02

[1]Kruskal-Wallis ANOVA.

Table 6.14 Mean densities of small *Gonodactylus* per 0.5 m^2. Means are for gonodactylids 6-15 mm in total length from 30 quadrats taken at each site in September (*S*) and February (*F*) of the years indicated. Standard errors are in parentheses. Reference sites were lightly oiled to unoiled.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
S86	0	0.2 (0.09)	0.4 (0.16)	0.6 (0.16)
F87	0.03 (0.03)	0.3 (0.10)	0.3 (0.15)	1.2 (0.33)
S87	0	0.1 (0.06)	0	0.2 (0.09)
F88	0.07 (0.05)	1.0 (0.22)	0.6 (0.22)	1.3 (0.24)
S88	0	0.07 (0.05)	0.03 (0.03)	0.2 (0.32)
F89	0.3 (0.10)	0.5 (0.18)	0.7 (0.19)	1.0 (0.20)
F90	1.5 (0.20)	2.1 (0.36)	1.7 (0.31)	2.9 (0.60)
F91	0.4 (0.10)	1.4 (0.24)	1.6 (0.38)	2.8 (0.43)

Kruskal-Wallis ANOVA: LRN, $P < .001$; MINA, $P < .001$; LRW, $P < .001$; MAR4, $P < .001$.

Table 6.15 Mean densities of 6-10 mm *Gonodactylus* per 1,000 cm^3 of coral rubble. Means are for 8-10 samples of 10 pieces of rubble taken at each site in September (*S*) and February (*F*) of the years indicated. Reference sites received light to no oiling. Standard errors are in parentheses. No data (*ND*) were taken at unoiled sites prior to 1989.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
S86	0.02 (0.01)	0.02 (0.02)	0.03 (0.00)	0.02 (0.01)	ND	ND
F87	0.03 (0.02)	0.2 (0.04)	0.2 (0.03)	0.4 (0.04)	ND	ND
S87	0.01 (0.01)	0.02 (0.02)	0.1 (0.02)	0.1 (0.04)	ND	ND
F88	0.03 (0.01)	0.4 (0.07)	0.09 (0.02)	0.2 (0.05)	ND	ND
S88	0	0	0.02 (0.01)	0.02 (0.01)	ND	ND
F89	0.01 (0.01)	0.03 (0.02)	0.03 (0.02)	0.1 (0.05)	0	0.02 (0.02)
F90	0.07 (0.01)	0.3 (0.06)	0.3 (0.06)	0.5 (0.05)	0.5 (0.07)	0.2 (0.04)
F91	0.03 (0.01)	0.4 (0.04)	0.3 (0.04)	0.6 (0.06)	0.2 (0.03)	0.09 (0.01)
P^1	.009	<.001	<.001	<.001	.001	<.001

^{1}Kruskal-Wallis ANOVA.

6.5.4.2 Results

From Table 6.5 we see that in September 1986 the proportion of *Gonodactylus* under 15 mm total length taken in quadrat samples was quite low, particular at oiled sites. Combining oiled (LRN and MINA) and reference (LRW and MAR4) samples, significantly fewer *Gonodactylus* recruited at oiled sites (*G*-test, $G = 5.65, P < .02$).

Figure 6.7 presents data from the two Isla Largo Remo sites for September 1981 and 1986 using all types of samples. Prior to the spill, in 1981, no difference existed in the proportion of animals under 15 mm at LRN and LRW (*G*-test, $G = 0.8, P > .1$). However, after the spill, in 1986, there was a significantly smaller proportion of gonodactylids under 15 mm found at LRN than at LRW (*G*-test, $G = 11.3, P < .001$). There was also a significantly smaller proportion of animals under 15 mm at both sites after the spill in 1986 than in 1981 (*G*-tests; LRN, $G = 70.2, P < .001$; LRW, $G = 37.4, P < .001$).

In quadrat samples, the heavily oiled sites at LRN and MINA showed a significant increase in recruitment between 1987 and 1989, while recruitment did not change significantly at lightly oiled to unoiled sites (Table 6.16). Recruitment increased dramatically at all sites in 1990. Recruitment decreased significantly at LRN in 1991, but did not vary significantly from 1990 at the other sites (Table 6.16).

In rubble samples, recruitment decreased significantly at LRW and MINA between 1987 and 1989, but did not vary significantly at LRN and MAR4 (Table 6.17). Recruitment increased significantly at all sites in 1990. In 1991 recruitment decreased significantly at LRN, the most heavily oiled site, and at SOTO and ESCU, the unoiled control sites (Table 6.17).

Paired comparisons between sites of recruitment in quadrat samples indicated three levels of recruitment in 1987. Densities of 6-15 mm gonodactylids were significantly higher at MAR4, intermediate at MINA and LRW, and lower at LRN (Table 6.18). By 1990 recruitment had increased significantly at all sites and there were no significant differences between any of the sites. Recruitment decreased at LRN in 1991, but continued not to differ significantly among MINA, LRW, and MAR4. There were no significant differences in densities of 6-15 mm gonodactylids between MINA and LRW during each year.

Quadrat samples were analyzed for the effect of prior oiling by comparing recruitment at heavily oiled sites with recruitment at lightly oiled to unoiled reference sites (Table 6.18). An effect on recruitment of the amount of oiling was detected in 1987 and 1988; recruitment was significantly lower at heavily oiled sites. The amount of oiling in 1986 had no effect on recruitment after 1988 in areal samples.

Paired comparisons between sites of recruitment in rubble samples indicated two levels of recruitment in 1987. Densities of 6-10 mm gonodactylids were significantly lower at LRN (Table 6.19). By 1989 recruitment was extremely low at all sites and there were no significant differences between any sites, including the unoiled sites. Although recruitment increased significantly at all sites in 1990, recruitment did not increase equally at all sites and three different levels of

Table 6.16 Recruitment differences within sites from quadrat samples. The probability values are listed for pairwise comparisons of the number of 6-15 mm gonodactylids taken in quadrat samples at each site in February of the years compared. Reference sites received light to no oiling.

	Oiled		Reference	
	LRN	MINA	LRW	MAR4
1987 vs. 1988	.6	.02	.1	.2
1988 vs. 1989	.02	.1	.5	.3
1989 vs. 1990	<.001	<.001	.01	.001
1990 vs. 1991	<.001	.2	.5	.6
All Samples	<.001	<.001	<.001	<.001

Kruskal-Wallis ANOVA.

Table 6.17 Recruitment differences within sites from rubble samples. The probability values are listed for pairwise comparisons of the density of 6-10 mm gonodactylids per 1,000 cc of coral rubble taken in rubble samples from each site in February of the years compared. Reference sites received light to no oiling.

	Oiled		Reference		Unoiled	
	LRN	MINA	LRW	MAR4	SOTO	ESCU
87 vs. 88	.9	.1	.007	.07		
88 vs. 89	.4	.001	.02	.07		
89 vs. 90	.008	.001	.001	.001	.006	.002
90 vs. 91	.03	.3	.6	.8	.005	.002
All dry season	<.001	<.001	<.001	<.001	<.001	<.001

Kruskal-Wallis ANOVA.

recruitment were established among sites, with only LRN significantly lower than all other sites. In 1991 five levels of recruitment were established after recruitment decreased at LRN, SOTO, and ESCU. There were no significant differences in the densities of 6-10 mm gonodactylids between MINA and LRW during each year.

The effect of the amount of oiling on later recruitment was also analyzed for rubble samples (Table 6.19). Recruitment was significantly lower in 1987 at sites heavily oiled in 1986. There were no significant differences in recruitment among sites that could be attributed to oiling after 1987.

Table 6.18 Recruitment differences among sites from quadrat samples. Paired comparisons were made by Kruskal-Wallis ANOVA for the number of 6-15 mm gonodactylids taken in quadrat samples at each site in February of the years indicated. Sites are listed in ascending order of mean densities per 0.5 m^2. *Underlined sites* indicate no significant differences among the sites ($P > .01$). P -values are listed for comparisons between heavily oiled (LRN and MINA) and reference sites (LRW and MAR4) pooled.

					Oiled vs. Reference
1987	LRN	LRW	MINA	MAR4	$P = .005$
	—	—			
		—	—		
1988	LRN	LRW	MINA	MAR4	$P = .006$
		—	—		
			—	—	
1989	LRN	MINA	LRW	MAR4	$P = .02$
	—	—	—		
			—	—	
1990	LRN	LRW	MINA	MAR4	$P = .5$
	—	—	—	—	
1991	LRN	MINA	LRW	MAR4	$P = .8$
		—	—	—	

Table 6.19 Recruitment differences among sites from rubble samples. Paired comparisons were made by Kruskal-Wallis ANOVA for the number of 6-10 mm gonodactylids taken in rubble samples at each site in February of the years indicated. Sites are listed in ascending order of mean densities per 1,000 cc of rubble. Underlined sites indicate no significant differences among the sites ($P > .01$). Paired comparisons were also made among oiled sites (*OL*: LRN and MINA), reference sites (*RF*: LRW and MAR4), and unoiled sites (*CN*: SOTO and ESCU).

1987	LRN	LRW	MINA	MAR4			RF	OL	
		—	—	—					
1988	LRN	LRW	MINA	MAR4			RF	OL	
		—	—	—			—	—	
1989	SOTO	LRN	ESCU	LRW	MINA	MAR4	CN	OL	RF
	—	—	—	—	—	—	—	—	—
1990	LRN	ESCU	LRW	MINA	SOTO	MAR4	OL	CN	RF
		—	—	—				—	—
			—	—	—	—			
1991	LRN	ESCU	SOTO	LRW	MINA	MAR4	CN	OL	RF
			—	—			—	—	
				—	—			—	—
					—	—			

6.5.4.3 Discussion

Recruitment appeared to be depressed in the months immediately following the oil spill, and the decline seemed strongest at those sites receiving the most oil. However, June through September is not a time when high levels of recruitment typically occur, and the intensity of recruitment varies considerably from year to year (Steger 1985, 1987). Therefore, while these data suggest that oil was interfering with the settlement of new recruits into the general area and onto oiled sites in particular, we feel that more reliable indications of the effect of oiling on recruitment comes from February data, when recruitment levels are generally more constant and often several times higher than during early to mid-wet season. Our data suggest recruitment was curtailed for as long as 2 yr following the spill, and that the effect was most severe where oiling was heaviest. However, the effect was confounded by a general suppression of recruitment that seems to have taken place along this portion of the Panamanian coast. Because we lack data from sites throughout the western Caribbean, we cannot determine whether this decline was localized and related to the oil spill or was a more widespread phenomenon that affected the entire region. The implications of changes in recruitment, with respect to population structure, are discussed in detail below.

6.5.5 Cavity Volume

6.5.5.1 Methods

As we had done prior to the spill at Isla Margarita (Steger 1985, 1987), we monitored the pattern of cavity utilization by collecting intact pieces of coral rubble containing gonodactylids and bringing them into the laboratory. These pieces were haphazardly selected, contained at least one occupied cavity, and were typical of rubble at the site. Each resident was harassed into leaving its cavity by a combination of immersing rubble in freshwater for several seconds and prodding the gonodactylid with a blunt probe. After the animal vacated its cavity, it was measured and catalogued. The volume of the cavity was determined by the volume of lead shot required to fill it. Stomatopod size was measured as total length.

The relationship between size of a gonodactylid and the volume of its cavity was measured at LRN, an oiled site, during the initial study in September 1986. Due to limited time, we were not able to collect these data at the other sites. However, as these data turned out to be critical to our interpretation of nonlethal effects of the oil spill on gonodactylids, we measured the relationship between gonodactylids and their cavities at both reference sites (MAR4 and LRW) and one oiled site (LRN) beginning in February 1987 and continuing to the end of the study. The relationship between size of the resident and the volume of its cavity is used to assess the intensity of competition.

6.5.5.2 Results

The relationship between gonodactylid size and the volume of its cavity was measured at LRN (Fig. 6.8), LRW (Fig. 6.9), and MAR4 (Fig. 6.10). The same data were taken prior to the oil spill during 1980 at MAR4, and are included in each figure for comparison. An exponential model ($y = ae^{bx}$) best fit the data, where logarithmic transformations of cavity volumes (cc) were regressed on total length of *Gonodactylus* residents. There was a significant, linear relationship between the logarithm of cavity volume and total length of the cavity resident during all sampling periods at all sites (Table 6.20).

Cavity-volume regressions were analyzed in three groups of comparisons by analysis of covariance (ANCOVA; Wilkinson 1986). The three types of comparisons were: (1) paired comparisons of postspill regression statistics between samples at each site (Table 6.21), (2) paired comparisons of postspill regression statistics between sites during the same sampling period (Table 6.22), and (3) paired comparisons of prespill regression statistics from MAR4 (1980) with postspill statistics from each site (Table 6.23). ANCOVAs were made by first testing for the homogeneity of slopes by analysis of variance (ANOVA). If there was no significant difference between slopes, the regressions were compared by testing for differences between intercepts by ANOVA. Considering the number of comparisons made, a conservative approach was adopted by setting the significance level at $P = .01$ for each test.

Starting with the first set of comparisons (within site, between postspill samples), there were no significant differences between any of the cavity-volume regressions measured at LRW, a reference site (Table 6.21).

At the other reference site, MAR4, three of the paired comparisons were significant (Table 6.21). The slope of the cavity-volume regression for February 1987 was significantly different from September 1987 and February 1988, and the slope of the regression for September 1987 was significantly different from February 1991. All significant differences involved comparisons with 1987 measurements. For 1987 samples most cavities measured at MAR4 contained residents that fell into the 30 to 40 mm size range, with relatively few large or small animals included. We therefore question whether the calculated slope of the cavity-volume regression fairly represents the actual relationship existing in that population at that time.

At LRN, the most heavily oiled site, the cavity-volume regression for September 1986 was not significantly different from February 1987 (Table 6.21). The cavity-volume regression showed numerous significant changes between February 1987 through February 1989 as the slope and intercept of the regression varied. There were no significant differences between cavity-volume regressions measured at LRN after February 1989.

Switching to the next group of comparisons (between sites, within sampling periods), there were no significant differences between cavity-volume regressions measured at LRW and MAR4, the two reference sites (Table 6.20).

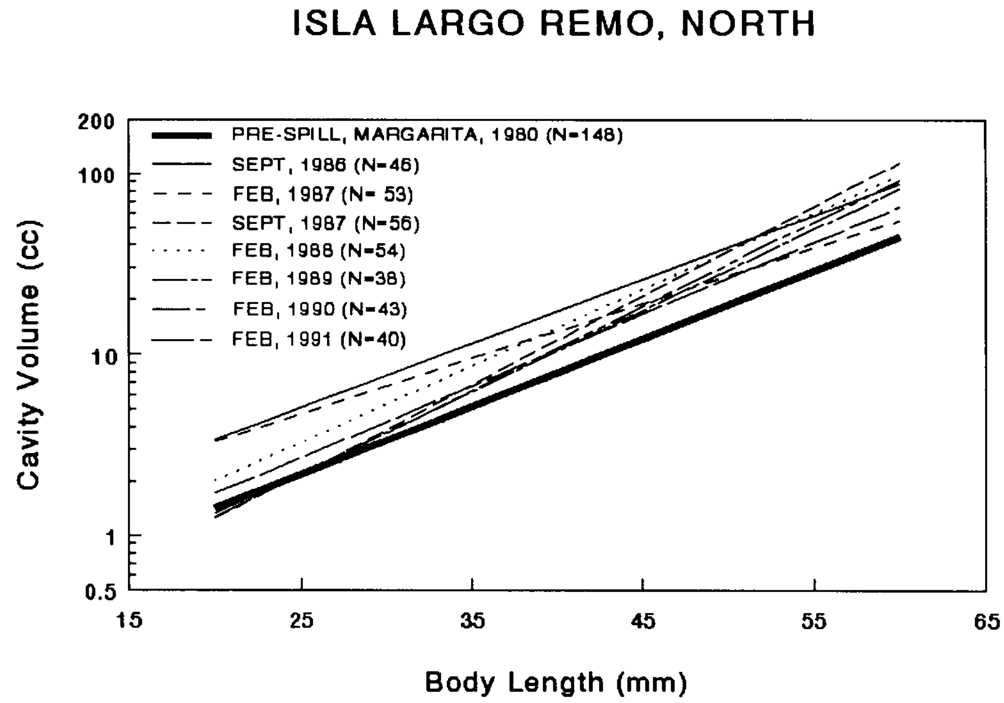

Fig. 6.8 Relationship at LRN between the size of resident *Gonodactylus* and the volume of the cavity occupied. All data are postspill for rubble taken from LRN except for prespill data taken at Isla Margarita in 1980 (*heavy solid line*). Sample sizes are in parentheses.

Comparing LRN, a heavily oiled site, and LRW, a reference site, there were no significant differences between the cavity-volume regressions in September 1987 and February 1989 (Table 6.22). The remainder of the paired comparisons were significantly different. Comparing LRN to the second reference site, MAR4, there were no significant differences between the cavity-volume regressions in February 1989 (Table 6.22). All other paired comparisons were significantly different.

Switching to the last group of comparisons (pre- vs. postspill), there were no significant differences between the cavity-volume regression measured at MAR4 in 1980, before the oil spill, and the cavity-volume regressions measured at MAR4 and LRW during all sampling periods after the oil spill (Table 6.23).

In contrast, all cavity-volume regressions measured at LRN, a heavily oiled site, were significantly different from the regression measured at MAR4 before the oil spill (Table 6.23). In September 1987 the slopes were significantly different; intercepts were significantly different for the other sampling periods.

The association between density and cavity volume of large gonodactylids was tested directly by regression analysis. The *x-y* pairs for the regression were the mean density of large gonodactylids per 0.5 m^2 (Table 6.8) and the cavity volume from the

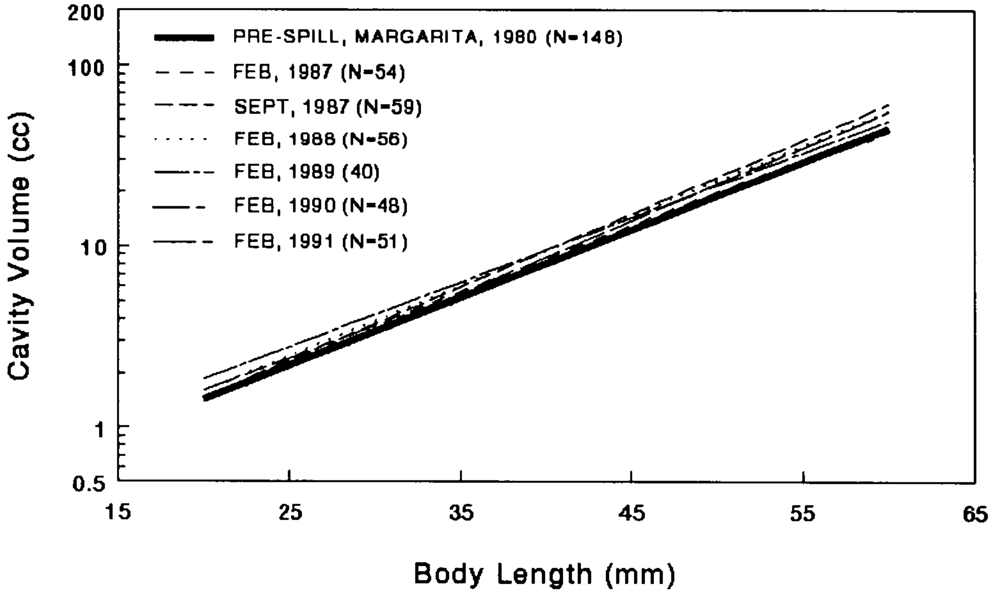

Fig. 6.9 Relationship at LRW between the size of resident *Gonodactylus* and the volume of the cavity occupied. All data are postspill for rubble taken from LRW except for prespill data taken at Isla Margarita in 1980 (*heavy solid line*). Sample sizes are in parentheses.

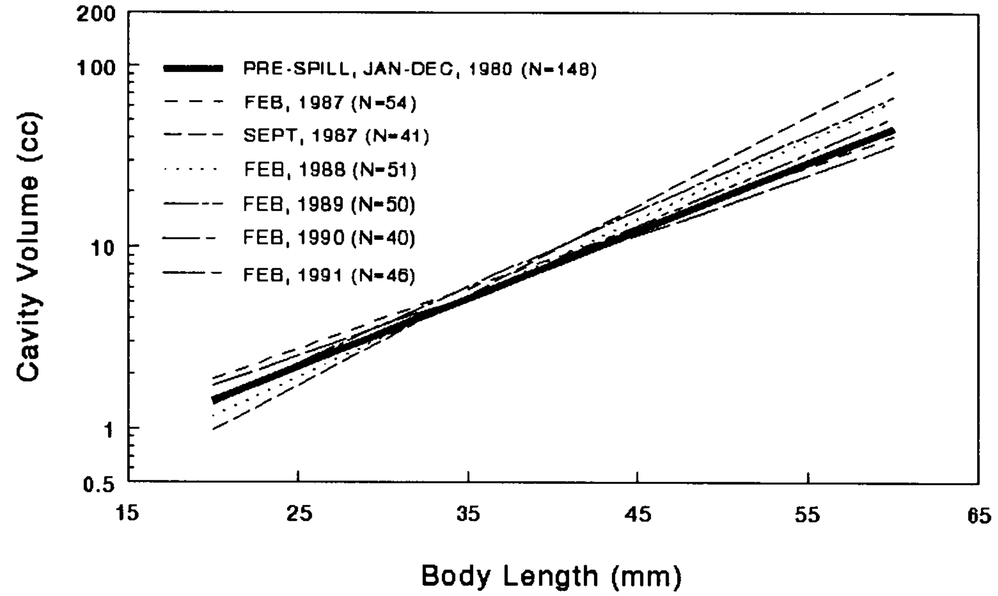

Fig. 6.10 Relationship at MAR4 between the size of resident *Gonodactylus* and the volume of the cavity occupied. All data are postspill for rubble taken from MAR4 except for prespill data taken at Isla Margarita (same location as MAR4) in 1980 (*heavy solid line*). Sample sizes are in parentheses.

Table 6.20 Summary of Model I regression parameters. The log of cavity volume (cc) was regressed on the total length (mm) of *Gonodactylus* residents. An exponential model ($y = ae^{bx}$) best fit the data. Cavity volumes obtained from the regression for 45 mm individuals are listed.

	N	b[1]	a	Pearson's Correlation	Predicted Cav. Vol.(cc) 45 mm
LRN					
September 1986 (S86)	46	0.081	0.68	.70	26.0
February 1987 (F87)	53	0.070	0.82	.79	19.1
September 1987 (S87)	56	0.113	0.13	.87	21.0
February 1988 (F88)	54	0.097	0.29	.80	22.8
February 1989 (F89)	38	0.103	0.17	.88	17.5
February 1990 (F90)	43	0.107	0.15	.82	18.5
February 1991 (F91)	40	0.091	0.28	.87	16.8
LRW					
February 1987 (F87)	54	0.084	0.30	.90	13.1
September 1987 (S87)	59	0.093	0.23	.94	15.1
February 1988 (F88)	56	0.089	0.27	.90	14.8
February 1989 (F89)	40	0.082	0.36	.91	14.4
February 1990 (F90)	48	0.082	0.31	.85	12.4
February 1991 (F91)	51	0.092	0.22	.85	13.8
MAR4					
Jan - Dec 1980[2] (1980)	148	0.086	0.26	.89	12.5
February 1987 (F87)	54	0.077	0.40	.89	12.8
September 1987 (S87)	41	0.114	0.10	.86	16.9
February 1988 (F88)	51	0.100	0.16	.92	14.4
February 1989 (F89)	50	0.096	0.21	.90	15.8
February 1990 (F90)	40	0.091	0.22	.88	13.2
February 1991 (F91)	46	0.076	0.38	.86	11.6

[1] Analysis of variance, H_0: b = 0, all $P < .001$.
[2] Prespill data.

regression for 45 mm individuals (Table 6.20; e.g., LRN, September 1986, *x*, *y* = 0.03 individuals per 0.5 m^2, 26.0 cc cavity volume). With all sites pooled, the regression was significant, and the degree of association between cavity volume and density was strong (Pearson correlation coefficient = .7; slope b = -0.71, ANOVA $P = .002$).

For individual sites there was no association between density and cavity volume at LRW and MAR4 ($P = .9$ and .3, respectively). Variation in cavity volume represented random variation when there were no significant differences in the density of large gonodactylids. There was, however, an association between cavity volume and density for large gonodactylids at the heavily oiled site, LRN ($P = .03$). Cavity volume was associated with density if density varied significantly.

There was no significant regression between cavity volume for 25 or 30 mm individuals and the density of medium-sized gonodactylids for any site or with sites

Table 6.21 Probabilities obtained from analysis of covariance (ANCOVA) for each site, between collections. Paired comparisons of postspill regression statistics were made between samples for each site. Regression statistics were obtained from the regression of the cavity volumes (cc) on the total length (mm) of *Gonodactylus* residents during September (*S*) and February (*F*) of the years indicated.

		Slopes Homogeneous	Intercepts Equal
LRW	F87 vs. S87	.2	.1
	S87 vs. F88	.6	.9
	F88 vs. F89	.4	.9
	F89 vs. F90	.9	.03
	F90 vs. F91	.4	.4
MAR4	F87 vs. S87	.001	
	S87 vs. F88	.2	.5
	F88 vs. F89	.6	.2
	F89 vs. F90	.6	.03
	F90 vs. F91	.2	.9
	F87 vs. F88	.005	
	S87 vs. F91	.004	
LRN	S86 vs. F87	.5	.1
	F87 vs. S87	<.001	
	S87 vs. F88	.2	.03
	F88 vs. F89	.6	.009
	F89 vs. F90	.8	.7
	F90 vs. F91	.3	.6
	S86 vs. S87	.04	<.001
	S86 vs. F89	.2	<.001
	S86 vs. F90	.2	<.001
	S86 vs. F91	.5	<.001
	F87 vs. F89	.01	.001
	F87 vs. F90	.01	.001
	F87 vs. F91	.07	.003
	F88 vs. F90	.5	.002

pooled (all $P > .05$). There was significant variation in densities of medium-sized individuals, but cavity volume was not associated with differences in density.

6.5.5.3 Discussion

Steger (1985, 1987) measured the ratio of cavity volume to stomatopod size preferred by *Gonodactylus* in populations around Punta Galeta and demonstrated that typically this ratio is not realized. Competition for homes forces gonodactylids, particularly those requiring large cavities, to fight for them. Usually an animal must settle for a cavity smaller than it would prefer. This effect cascades down through

Table 6.22 Probabilities obtained from ANCOVA for each collection, between sites. Paired comparisons of postspill regression statistics were made between sites for each sampling period. Regression statistics were obtained from the regression of the cavity volumes (cc) on the total length (mm) of *Gonodactylus* residents during September (*S*) and February (*F*) of the years indicated.

		Slopes Homogeneous	Intercepts Equal
LRW vs. MAR4	F87	.4	.8
	S87	.05	.05
	F88	.2	.1
	F89	.1	.8
	F90	.4	.9
	F91	.1	.3
LRN vs. LRW	F87	.2	<.001
	S87	.04	.05
	F88	.5	<.001
	F89	.05	.3
	F90	.08	.008
	F91	.9	.007
LRN vs. MAR4	F87	.5	<.001
	S87	.9	.004
	F88	.8	<.001
	F89	.5	.2
	F90	.2	.003
	F91	.2	<.001

the size distribution of stomatopods. The elimination of large gonodactylids from the population at oiled sites immediately following the spill opened up a large number of suitable cavities. As a result, surviving animals were able to occupy cavities close to the preferred size. However, as populations at oiled sites recovered and more large animals reappeared, competition was re-established, and animals again had to settle for suboptimal homes. At LRN, however, where habitat deterioration has produced an abundance of large cavities, animals can still find a home of the preferred size. The utility of the cavity-volume to stomatopod-size relationship and how it relates to competition is discussed in greater detail below.

6.5.6 Wounds and Injuries

6.5.6.1 Methods

One consequence of competition for cavities is that *Gonodactylus* are more frequently wounded as homes become limiting. Either they are more likely to be injured during more frequent and intense aggressive contests for homes or, if an

Table 6.23 Probabilities obtained from ANCOVA for each collection, between sites and the prespill regression for Isla Margarita. Paired comparisons were made between postspill regression statistics from each site and prespill regression statistics from Isla Margarita during 1980. Regression statistics were obtained from the regression of the cavity volumes (cc) on the total length (mm) of *Gonodactylus* residents during September (*S*) and February (*F*) of the years indicated. Prespill data from 1980 were taken throughout that year.

		Slopes Homogeneous	Intercepts Equal
LRW vs. ISLA MARGARITA 1980			
	F87	.7	.6
	S87	.3	.06
	F88	.7	.05
	F89	.6	.04
	F90	.6	.8
	F91	.5	.7
MAR4 vs. ISLA MARGARITA 1980			
	F87	.2	.2
	S87	.01	.2
	F88	.07	.8
	F89	.2	.1
	F90	.7	.6
	F91	.2	.7
LRN vs. ISLA MARGARITA 1980			
	S86	.6	<.001
	F87	.04	<.001
	S87	.004	
	F88	.3	<.001
	F89	.09	.004
	F90	.09	.006
	F91	.6	.001

animal is evicted from a cavity, it is more subject to injury from predators while searching for a new home. This effect is most pronounced in larger individuals because it is in these animals that competition for cavities is most intense (Berzins and Caldwell 1983; Steger 1985).

Using a dissecting microscope, we inspected all individuals collected during this study for injuries. Animals were examined the same day they were collected. Damage to stomatopod cuticle darkens after several hours, so it is possible to distinguish recent from old injuries. Also, we can usually identify wounds inflicted by other stomatopods due to their characteristic shape and location. Any injury that was judged to have taken place the day of collection and that did not appear to have been caused by another stomatopod was excluded from analysis.

We analyzed postspill wounding data for gonodactylids greater than 35 mm total length collected at all study sites. We also included in the analysis wounding data for animals greater than 35 mm collected at the same sites and seasons in years prior to the oil spill. Prespill data were not available for SOTO and ESCU.

6.5.6.2 Results

Data for injuries among individuals greater than 35 mm total length were collected at the study sites from all postspill samples and at the same sites in years prior to the oil spill (Table 6.24; Figs. 6.11, 6.12). We also examined the amount of injuries among larger individuals at unoiled control sites added in 1989 (Table 6.25). Injuries were analyzed by first examining the number of individuals with injuries at each site independently (Table 6.26), and then by examining the level of injuries across sites, factoring in the amount of oiling at the sites for wet season samples (Table 6.27) and dry season samples (Table 6.28).

Injuries were analyzed at sites by tests of independence in contingency tables of numbers of individuals injured and not injured (Tables 6.24, 6.25). Comparisons were made to determine differences among prespill samples, among postspill samples, and between pre- and postspill samples. Postspill samples were also examined for seasonal effects.

At MAR4 the number of gonodactylids injured and not injured was independent of date sampled among prespill samples, among postspill samples overall, and between pre- and postspill samples (Table 6.26). The number injured varied significantly only among postspill wet season samples. Variation in the number of injured gonodactylids at MAR4, a lightly oiled to unoiled reference site, was not associated with the oil spill.

At LRW the number injured was not independent of date sampled for pre- and postspill samples (Table 6.26). In postspill samples injuries were independent of season, but were associated with date sampled during each season. However, injuries were independent of date sampled when comparing pre- and postspill samples. Thus, the number of injured gonodactylids at LRW, a lightly oiled to unoiled reference site, varied significantly among samples, but differences in the number injured was not associated with the oil spill.

At MINA the number injured was independent of date sampled for prespill samples (Table 6.26). For postspill samples the number injured was not independent of date sampled and was not independent of season. The number injured was independent of date sampled in dry season samples, but was not independent in wet season samples. Also, the number injured was not independent of date sampled comparing pre- and postspill samples at MINA. The association between the number injured and date sampled in postspill samples and between pre- and postspill samples was caused by the low level of injuries found in a single sample, September 1986. The oil spill was followed by a decreased number of injured gonodactylids at MINA, a heavily oiled site, in the initial sample following the spill, but the level of injuries returned to prespill levels by February 1987, less than a year after the spill.

At LRN the number injured was not associated with date sampled in postspill samples (Table 6.26). In comparing a single wet season, prespill sample with the wet

Table 6.24 Number of injured *Gonodactylus* greater than 35 mm total length at oiled and reference sites, pre- and postspill. Gonodactylids were collected during September (*S*) and February (*F*) of the years indicated. Samples are listed by site, when collected relative to the oil spill (*Prespill* or *Postspill*), and season.

		Season	Date	Number Injured	No. Not Injured	Percent Injured
LRN	Prespill	Wet	S81	24	94	20
	Postspill	Wet	S86	12	113	10
			S87	10	62	14
			S88	7	53	12
		Dry	F87	21	112	16
			F88	11	77	13
			F89	10	62	14
			F90	15	62	19
			F91	9	64	12
MINA	Prespill	Wet	S81	22	41	35
			S83	47	155	23
	Postspill	Wet	S86	18	142	11
			S87	29	100	22
			S88	15	40	27
		Dry	F87	50	141	26
			F88	26	71	27
			F89	20	42	32
			F90	15	46	25
			F91	13	47	22
LRW	Prespill	Wet	S81	21	37	36
			S82	27	101	21
	Postspill	Wet	S86	49	79	38
			S87	21	78	21
			S88	16	95	15
		Dry	F87	42	140	23
			F88	47	49	49
			F89	38	86	31
			F90	42	110	28
			F91	42	107	28
MAR4	Prespill	Wet	S80	17	53	25
			S81	16	35	31
	Postspill	Wet	S86	42	70	38
			S87	21	61	26
			S88	22	82	21
		Dry	F87	27	54	33
			F88	33	49	40
			F89	32	69	32
			F90	25	74	25
			F91	26	93	22

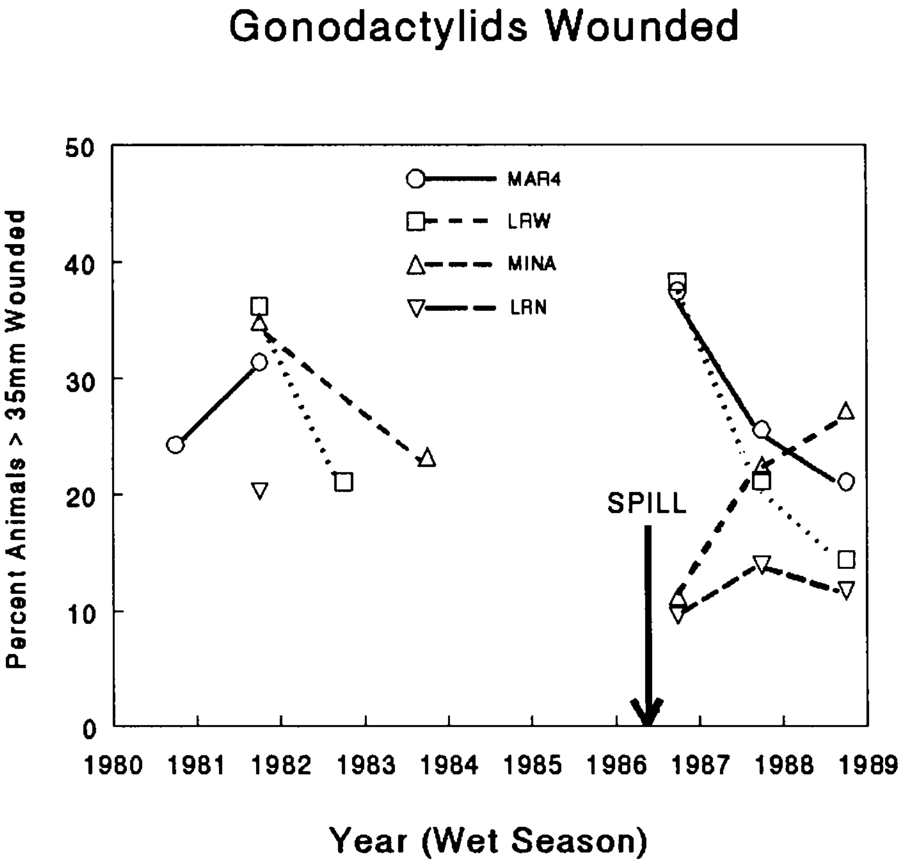

Fig. 6.11 *Gonodactylus* larger than 35 mm that were collected with injuries in September (wet season), pre- and postspill. See Figure 6.2 for an explanation of the site abbreviations.

season, postspill samples, the number injured was not independent of date sampled. The oil spill was also followed by a decrease in the number injured at LRN. The availability of only one prespill sample, however, makes this conclusion tentative.

At both unoiled control sites, SOTO and ESCU, injuries were not associated with date sampled for the three postspill, dry season samples taken at each location (Table 6.26).

Injuries were also analyzed across sites to factor in the amount of oiling at the sites. The analysis was carried out by a test of independence with a log-linear model in a 3-way contingency table (Wilkinson 1986). The main effects entered into the model were site, date of sampling relative to the oil spill (pre- and postspill), and injury state of gonodactylids (injured and not injured). Sites were not pooled into heavily oiled versus reference sites because differences between the two heavily oiled sites and the two reference sites would be ignored. The test was run only on wet season data because of the availability of prespill data.

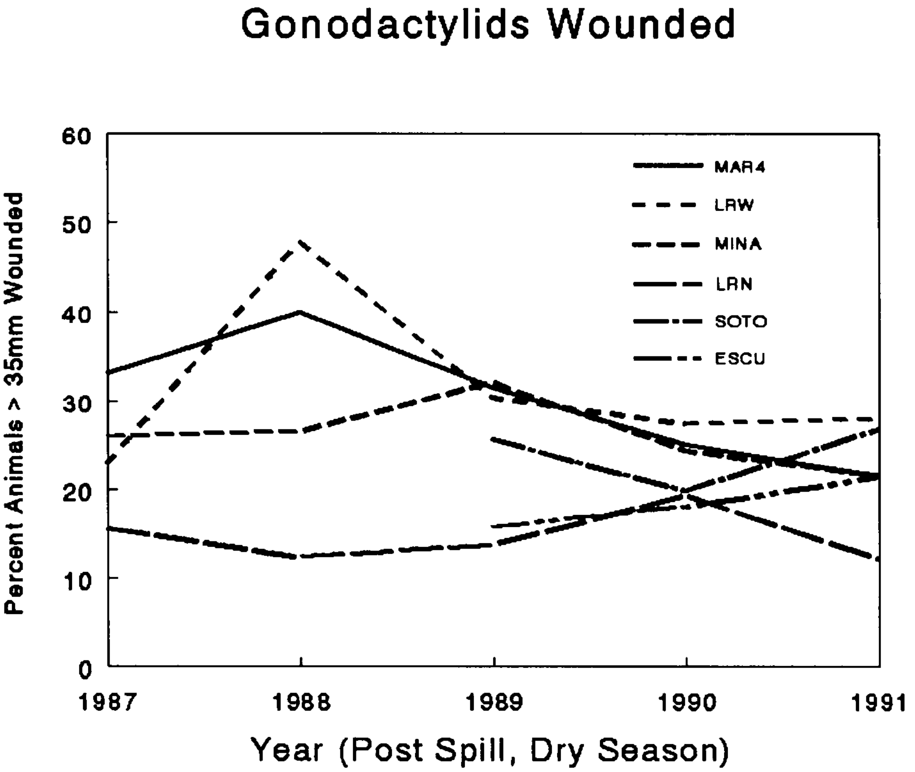

Fig. 6.12 *Gonodactylus* larger than 35 mm that were collected with injuries after the oil spill in February (dry season). See Figure 6.2 for an explanation of the site abbreviations.

The test for a 3-factor interaction was not significant (log-likelihood ratio X^2 = 6.55, df = 3, P = .08). Thus, the degree of association between pre- and postspill levels of injuries did not appear to depend on site. Still, given the known differences in site and the near-significance of the test for a 3-factor interaction, we decided to explore possible interactions further following the recommended procedure to make separate, 2-way tests of independence within each level of the main factors (Sokal and Rohlf 1981). This involved 2-way tests at each level of site for pre- and postspill injuries, at the prespill level of injury examining paired comparisons of sites, and at the postspill level of injury examining paired comparisons of sites.

The number injured was not associated with pre- vs. postspill sampling at the reference sites, MAR4 and LRW. However, the number injured was associated with pre- vs. postspill sampling at both heavily oiled sites. This is the same analysis of pre- vs. postspill injured at sites presented above (Table 6.26). A reduction of injuries followed the oil spill at heavily oiled sites.

Table 6.25 Number of injured *Gonodactylus* greater than 35 mm total length at two unoiled sites. Gonodactylids were collected during September (*S*) and February (*F*) of the years indicated. Samples are listed by site, when collected relative to the oil spill (*Prespill* or *Postspill*), and season.

		Season	Date	Number Injured	Number Not Injured	Percent Injured
SOTO	Postspill	Dry	F89	14	40	26
			F90	14	56	20
			F91	24	65	27
ESCU	Postspill	Dry	F89	7	37	16
			F90	10	45	18
			F91	16	58	22

Table 6.26 Probabilities obtained from log-likelihood ratio chi-square tests for injuries at each site. *P*-values were calculated from contingency tables using the number of gonodactylids, injured and not injured, for the samples indicated. Reference sites received light or no oiling.

	Reference		Oiled		Unoiled	
	MAR4	LRW	LRN	MINA	SOTO	ESCU
Prespill samples (Wet season only)	.4	.03	--	.07	ND	ND
Postspill samples						
Among all samples	.5	.01	.5	.001		
Wet vs. dry season	.1	.07	.5	.003		
Wet season	.02	<.001	.7	.01	ND	ND
Dry season	.05	<.001	.7	.8	.6	.7
Prespill vs. Postspill (Wet season only)	.8	.9	.02	.02		

-- = only one sample; *ND* = no data.

At the level of prespill samples, the number injured was not associated with site for any paired comparisons of sites (Table 6.27). That is, the amount of injury was independent of site in prespill samples.

At the level of postspill samples, the number injured was not associated with site for the comparison of reference sites (MAR4 vs. LRW) and the comparison of

Table 6.27 Probabilities obtained from log-likelihood ratio chi-square tests comparing injuries between sites. *P*-values were calculated from contingency tables using the number of gonodactylids, injured and not injured, for the samples indicated. Reference sites were lightly oiled to unoiled. All samples were collected during September (wet season).

	P
Level of analysis: between sites, prespill vs. prespill number injured.	
Oiled	
LRN vs. MINA	.3
Reference	
LRW vs. MAR4	.8
Oiled vs. Reference	
LRN vs. LRW	.3
LRN vs. MAR4	.2
MINA vs. LRW	.96
MINA vs. MAR4	.8
Level of analysis: between sites, postspill vs. postspill number injured.	
Oiled	
LRN vs. MINA	.02
Reference	
LRW vs. MAR4	.4
Oiled vs. Reference	
LRN vs. LRW	<.001
LRN vs. MAR4	<.001
MINA vs. LRW	.02
MINA vs. MAR4	.002

heavily oiled sites (MINA vs. LRN; Table 6.27). The number injured was associated with site for all paired comparisons of reference vs. heavily oiled sites. These results indicate the number of injured gonodactylids was associated with the amount of oiling at the sites. Heavily oiled sites had fewer injured gonodactylids after the oil spill than reference sites.

A similar analysis was also carried out on postspill dry season samples (Table 6.28; Fig. 6.12). Injuries were not associated with site at unoiled sites and lightly oiled to unoiled sites. Injuries were associated with site at heavily oiled sites. Gonodactylids at MINA carried more wounds and injuries than at LRN in dry season samples after the oil spill.

Injuries were associated with site for comparisons between LRN, a heavily oiled site, and LRW and MAR4, the lightly oiled to unoiled reference sites. Injuries were not associated with site for comparisons between MINA, a heavily oiled site,

Table 6.28 Probabilities obtained from log-likelihood ratio chi-square tests between sites postspill. *P*-values were calculated from contingency tables using the number of gonodactylids, injured and not injured, for the samples indicated. Reference sites were lightly oiled to unoiled. All samples were collected during February, the dry season of the years indicated.

		P
Level of analysis: between sites, postspill vs. postspill number injured.		
Unoiled (1989-91)		
	SOTO vs. ESCU	.2
Reference (1987-91)		
	LRW vs. MAR4	.9
Oiled (1987-91)		
	LRN vs. MINA	.001
Oiled vs. Reference (1987-91)		
	LRN vs. LRW	<.001
	LRN vs. MAR4	<.001
	MINA vs. LRW	.2
	MINA vs. MAR4	.3
Oiled vs. Unoiled (1989-91)		
	LRN vs. SOTO	.02
	LRN vs. ESCU	.3
	MINA vs. SOTO	.7
	MINA vs. ESCU	.1
Reference vs. Unoiled (1989-91)		
	LRW vs. SOTO	.3
	LRW vs. ESCU	.01
	MAR4 vs. SOTO	.7
	MAR4 vs. ESCU	.08

and the reference sites. Thus, of the heavily oiled sites, only LRN had fewer injuries than reference sites in dry season samples after the oil spill.

Injuries were also associated with site for comparisons between LRN and SOTO, but were not associated with site between LRN and ESCU. LRN had fewer injured gonodactylids than SOTO, but not ESCU. Injuries were not associated with site for comparisons between MINA and both unoiled sites. ESCU had low levels of injuries for an unoiled site when compared with heavily oiled sites.

Injuries were not associated with site for comparisons between lightly oiled to unoiled sites and unoiled sites, with the exception of ESCU and LRW. ESCU also had low levels of injuries with respect to comparisons between unoiled sites and lightly oiled to unoiled sites.

6.5.6.3 Discussion

Another measure of competition in gonodactylid populations for limiting resources is the proportion of wounding in the population (Berzins and Caldwell 1983; Steger 1985). The drop in wounding that we witnessed at oiled sites in 1986 immediately following the spill probably resulted from the reduced numbers of large gonodactylids and weakened competition for cavities. These findings are discussed in that context below.

6.5.7 Growth

6.5.7.1 Methods

Growth per molt in gonodactylids can be measured by comparing the length of the carapace before and after a molt. When recently molted gonodactylids are collected in the field with the old carapace intact, reliable growth data can be obtained. However, because the old carapace is frequently eaten within a few hours after molting, such data are difficult to obtain. By maintaining animals in the laboratory following capture, we can identify animals likely to molt, measure the carapace, and then measure the new carapace following a molt. However, smaller *Gonodactylus*, even when held in the laboratory for 1 or 2 d, show reduced growth per molt and molt less frequently than expected. Therefore, growth data obtained for small individuals held in captivity cannot be used for such an analysis. As animals become larger, the time that they can be maintained in the laboratory before growth rate is affected increases. Growth per molt and the frequency of molting for animals larger than 35 mm total length declines only after 3 or 4 d. We therefore can use laboratory-held *Gonodactylus* longer than 35 mm to estimate growth provided that they molt within 3 d. Also, growth per molt varies across season, so only data obtained from the same time of year can be compared (Steger and Caldwell, unpublished data).

The rate at which stomatopods grow depends both on how often they molt as well as how much they grow per molt. We can estimate the frequency of molting by calculating the percentage of animals that molt over a given period of time. However, one complication that affects estimations of intermolt intervals is that molting follows a lunar cycle, with most animals molting during, or just after a full moon. Therefore, data obtained from animals collected during the same phase in the lunar cycle must be compared.

All *Gonodactylus* collected during each sampling period and at each site were used to estimate percent growth by molting stomatopods. Carapaces were measured under a dissecting scope with the aid of an ocular micrometer. If recently molted animals were collected in the field with an intact old carapace that could be measured, it was retrieved along with the gonodactylid and both were measured when returned to the laboratory. All animals collected during this study were maintained individually in containers outside the laboratory under ambient conditions for 3 d.

If animals molted during this period, the exuviae were used to calculate growth per molt by measuring the change in carapace length. Only data obtained from animals greater than 35 mm total length were used in this analysis. Prespill data were obtained using the same procedures. For SOTO and ESCU, because of the lack of prespill data and small sample sizes, postspill data were not included in this analysis. We tested for differences among the various sites and years using analysis of covariance, following procedures outlined in Sokal and Rohlf (1981).

6.5.7.2 Results

Table 6.29 presents September 1986 data for percent growth recorded for individuals greater than 35 mm in total length collected at the two oiled and two reference sites following the spill. These data are compared to growth values obtained from animals collected prior to the spill at the same locations during the same season. Because of small sample sizes, we pooled data from MINA and LRN (significant oiling) and from MAR4 and LRW (little or no oiling). As discussed above, the relationship between body length and percent growth per molt has a negative slope. We tested for differences among the four groups using analysis of covariance following procedures outlined in Sokal and Rohlf (1981). The test for homogeneity of slopes was not significant (F = 1.82, df = 3, 45, P = .16). However, as expected, there was a significant negative common slope (-0.18%/mm; F = 7.78, df = 1, 48, P <.01). And, most interestingly, there was also a significant difference in adjusted mean growth (F = 9.24, df = 3, 48, P <.001); animals collected from oiled sites grew approximately 50% more per molt than those collected at the same locations prior to the spill. There were no differences between the adjusted mean growth values for the sites that received little or no oiling before and after the spill or between affected and unaffected areas prior to the spill. No differences in molting rate were found between any of the sites sampled in September 1986 or between pre- and postspill samples at the same site.

There were no significant differences in percent growth or rate of molting from prespill levels at any of the sites from February 1987 through the end of sampling in February 1991.

6.5.7.3 Discussion

The increase in growth rates that we observed at heavily oiled sites immediately following the oil spill did not persist after the first postspill sampling period. This may reflect the rapid return to normal levels of competition at all sites except LRN. The fact that growth levels fell to prespill levels at LRN, even though competition for cavities remained low at this location, may be explained by a concomitant drop in food availability at this site. While we did not quantitatively sample snail and hermit crab availability, our impression is that these prey items became relatively scarce as the habitat at LRN degraded into large pieces of unconsolidated coral rubble.

Table 6.29 Percent growth of *Gonodactylus* greater than 35 mm total length collected from the two reference sites (MAR4 and LRW) and the two oiled sites (LRW and MINA). Postspill data were from September 1986 and are compared to prespill data collected in the same season from the same locations, 1979-1983. Growth was measured as the percent change in the length of the carapace for animals captured in the field with intact molt skins or for animals that molted within 3 d of capture.

	Adjusted Mean Growth (%)	95% Confidence Interval	*N*
Prespill			
Reference	6.34	1.23	12
Oiled	6.09	1.14	14
Postspill			
Reference	6.28	1.21	13
Oiled	9.44[1]	1.15	14

[1] ANCOVA revealed no significant difference in the slope of growth vs. body length among the four regressions, but there was a significant difference in adjusted mean growth ($F = 9.24$; df = 3, 48; $P < .001$).

6.5.8 The Disappearance of *Lysiosquilla glabriuscula*

During the first sampling after the oil spill in September 1986, searches failed to locate any of the 22 *Lysiosquilla glabriuscula* burrows that we had marked in 1983, although the marking stakes still existed at several locations. Since the oil spill not a single *Lysiosquilla* has been recorded from this area despite repeated searches, and we must conclude that this species is now locally extinct. Given that these animals exist for long periods (up to 3 wk at a time) in sealed burrows containing stagnant water, it is somewhat surprising that animals were killed immediately after the spill in oiled areas, although we cannot explain why they disappeared from reference site LRW. Why no recruitment has occurred over the past 5 yr remains a mystery, although such events in these exceptionally long-lived animals may be rare.

6.6 Discussion and Conclusion

6.6.1 Effects of Oiling on Gonodactylid Population Structure and Density

In September 1986 we documented a significant loss of large *Gonodactylus* from oiled sites. The effect was particularly severe with respect to females. Why did these animals sustain greater mortality at the oiled sites? For reasons that we do not understand, large *Gonodactylus* typically move into the lower intertidal to mate and brood eggs. During severe, early wet season exposures, they are gradually driven to deeper water, but then move back up into these areas to breed later in the year. The spill occurred during the first extreme low tides of the wet season. Many gonodactylids in the lower intertidal would have been large breeding adults. A greater proportion of animals in deeper water would have been smaller individuals. When oil came ashore, it appears that effects on *Thalassia* beds were greatest in the intertidal; subtidal beds were not as severely affected (Cubit and Connor, Chap. 4; Garrity et al., Chap. 5; Marshall et al., Chap. 10). If direct contact with oil killed stomatopods, it is most likely that animals in the intertidal would have suffered most, including a greater proportion of large animals. Also, brooding females are very reluctant to leave their cavities (Montgomery and Caldwell 1984). They may have remained in oiled areas longer than males and, therefore, been even more at risk. Because we were not at Galeta during the spill, this is only speculation, but it is consistent with what has been reported about how oil came ashore and what we know of the biology of gonodactylids.

Monitoring of gonodactylid populations has shown that densities of *Gonodactylus* have varied significantly after the initial effects of the oil spill in 1986 (Table 6.30). Low levels of postlarval recruitment and migration of larger gonodactylids were two processes identified to affect densities significantly.

Large numbers of larvae settle on reefs around Punta Galeta from January through June every year (Steger 1987). Larvae metamorphose into postlarvae between 6 and 10 mm total length. It takes 2 to 4 mo for these juveniles to grow to 15 mm total length. Densities of small *Gonodactylus*, which include postlarvae and individuals that have recruited within the past few months, indicate that recruitment was extremely low from at least February 1987 through February 1989. The lack of recruitment is especially noteworthy in February samples because recruitment during September is normally low and highly variable from year to year (Steger 1987).

The effect of low recruitment was first detected in the decline in densities of medium-sized gonodactylids (20-40 mm long, 8 mo to 2 yr old). The magnitude of the decline at a particular site depended on the relative densities of the various size classes compared to the amount of recruitment at the site. The declines were greatest at LRN, the most heavily oiled site, where recruitment was extremely low, and MAR4, where recruitment was moderate but the initial density of medium-sized gonodactylids was high. The general pattern, however, was the same at all sites: densities declined initially following low recruitment and increased later as recruitment increased (Table 6.30).

Densities of larger *Gonodactylus* in rubble samples began to decline due to low recruitment in 1989-1990. The timing of the decline was consistent with the age of these individuals, which are all over 2 yr old (greater than 40 mm total length). The effect of low recruitment on the abundance of large *Gonodactylus* also depended on existing densities at a particular site. The amount of recruitment was sufficient to maintain moderate densities of larger individuals (LRN and MAR4), but the level of recruitment in 1987-1989 was not sufficient to maintain higher densities (LRW, MINA, SOTO, and ESCU). However, in quadrat samples there was no evidence of a decline in large gonodactylids due to low recruitment.

The second factor that affected densities of *Gonodactylus* was immigration of larger individuals into heavily oiled sites. This movement was most evident at MINA, but was also detected at LRN. Net immigration into sites ended by February 1987,

Table 6.30 Total number of *Gonodactylus* collected at each study site in September (*S*) and February (*F*) during the years indicated. Oiled sites received moderate to heavy oiling and reference sites received light to no oiling. These data include quadrat, rubble and cavity volume collections. No data (*ND*) were taken at control sites prior to 1989.

	Oiled		Reference		Unoiled		
	LRN	MINA	LRW	MAR4	SOTO	ESCU	Total
S86	230	238	212	402	ND	ND	1,082
F87	203	448	428	407	ND	ND	1,486
S87	326	309	316	307	ND	ND	1,258
F88	163	316	331	357	ND	ND	1.167
S88	76	210	206	253	ND	ND	745
F89	134	217	262	340	66	66	1,085
F90	220	302	414	473	222	126	1,757
F91	153	288	411	453	276	130	1,711
Total	1,505	2,328	2,580	2,992	564	322	10,291

when moderate to high densities of larger gonodactylids were established. There is some evidence that the initial densities reached by immigration were not stable, as densities at the heavily oiled sites dropped slightly after immigration before leveling out (Table 6.30).

Overall, the amount of postlarval recruitment between 1986 and 1989 was not sufficient to maintain *Gonodactylus* populations at 1986 levels at any of the sites. This pattern was consistent in areal samples (see below for discussion of possible spurious effects in rubble samples). Total densities of gonodactylids declined and increased with levels of recruitment. MINA superficially appeared to be an exception because the total density of gonodactylids did not vary throughout the monitoring (Table 6.30). Under close scrutiny, however, MINA actually illustrated best the complexity of factors affecting gonodactylid densities. Total densities were balanced early in the monitoring between a decline in medium-sized individuals due to low recruitment and an increase in larger gonodactylids due to immigration. Total densities were balanced later between a decline in larger individuals due to earlier low levels of recruitment and an increase in smaller gonodactylids due to increased recruitment. The net effect was that total densities of all sizes of gonodactylids did not vary significantly at MINA even though densities of most size classes varied significantly.

The long-term effect of the oil spill on densities of gonodactylid stomatopods focuses on one major question: did oiling cause the low levels of recruitment measured between 1986 and 1989, or was the reduced recruitment an effect of a large-scale natural, albeit unusual, phenomenon triggered by events unrelated to the oil spill? The evidence implies that oiling *and* a large-scale, natural phenomenon both contributed to low levels of recruitment.

The evidence that oiling reduced recruitment comes from two points. First, there were three levels of recruitment in 1987, the first period following the oil spill when high recruitment was expected. These levels corresponded to the amount of oiling or proximity to heavily oiled areas. LRN was the most heavily oiled site and showed the lowest level of recruitment. MINA was heavily oiled, but less than LRN, and LRW was lightly oiled to unoiled, but surrounded by heavily oiled areas. Both of these sites showed low, intermediate levels of recruitment. MAR4 was lightly oiled to unoiled and the most distant of the initial sites from Bahía Las Minas. MAR4 showed the highest level of recruitment.

Second, recruitment levels were significantly different between heavily oiled sites and reference sites when sites were pooled according to amount of oiling (heavily oiled vs. lightly oiled to unoiled). The effect of oiling was highly significant in February 1987. In February 1988 oiling may have had an effect on recruitment, but the evidence was mixed, as oiling had a significant effect in quadrat samples but not in rubble samples. Oiling had no significant effect on recruitment in 1989.

The evidence that an unexplained phenomenon not related to oiling was also affecting recruitment comes from data taken in 1989-1991 and the addition of two unoiled control sites in 1989. Both unoiled sites were outside the zone of effects of the oil spill and showed low postlarval recruitment in 1989. Recruitment was low at all sites and there was no significant effect of oiling among sites (heavily oiled vs. lightly oiled to unoiled vs. unoiled). Recruitment increased significantly at all sites in 1990 regardless of the amount of oiling after the 1986 spill.

Our conclusion is that recruitment on a large geographic scale was low as early as 1987 through 1989 due to an unexplained phenomenon and that oiling reduced the level of recruitment further at heavily oiled sites in 1987 and possibly 1988. The levels of recruitment measured in 1990 indicated that both the effects of oiling and the general depression of recruitment were finished at that time.

The strength of this conclusion must be tempered by two points. First, the number of sites involved in the monitoring was minimal. In retrospect, more effort should have been placed on quadrat samples at a larger number of sites and less effort on rubble samples. Second, the effect of oiling was mainly due to the difference between two of the sites, LRN and MAR4. Pooling blurred the fact that recruitment was not significantly different in any samples taken from MINA, a heavily oiled site, and LRW, a lightly oiled to unoiled site. Hindsight indicates that a more effective analysis could have been accomplished if the number of sites were increased to allow discrimination among the most heavily oiled sites (e.g., LRN), heavily oiled sites with no long-term habitat damage (e.g., MINA), lightly oiled to unoiled sites surrounded by heavily oiled sites (e.g., LRW), and unoiled sites (e.g., SOTO, ESCU, and possibly MAR4). However, it must be understood that the addition of 3 to 5 sites to this monitoring project would have involved a substantial increase in effort and cost. It is not clear that the additional effort and cost could be justified simply to analyze more effectively what already may have been examined adequately.

The mechanism behind the effect of prior oiling on recruitment remains open to speculation. While surviving females at all sites continued to produce eggs and release larvae, the scale of the oil spill may have been sufficient to reduce the larval pool through the mortality of larger individuals, especially females, that produce most of the larvae. Emigration of large individuals from primary habitats where most reproduction normally occurs could also have affected the larval pool. Oil at sea could have killed planktonic larvae. Chronic oiling, especially at LRN, could inhibit larval recruitment. Most simply, the dynamics of larval dispersal and recruitment are not understood sufficiently to pinpoint the mechanism behind the effect of oiling or behind the general depression in recruitment also measured.

There were some potential complications of our sampling methods that need to be discussed. Rubble sampling may have produced some spurious effects when used to calculate gonodactylid densities. Even though densities were calculated per unit volume of rubble to correct for differences in the amount of rubble among samples, stomatopod densities remained correlated significantly with the volume of rubble in some samples. We do not know what effect, if any, this may have had on our interpretation of intra- and intersite variation. Also, densities of gonodactylids in rubble samples may have been sensitive to the distribution of larger pieces of rubble (for a detailed discussion of effects of cavity distribution as opposed to cavity abundance, see Steger 1985, 1987). In general, our analysis has relied more heavily on quadrat-areal samples due to these potential problems, especially when there was a contradiction between results from quadrat and rubble samples.

Also, the timing of recruitment may have given a false impression about the amount of recruitment in 1991. Postlarval recruitment in gonodactylid stomatopods has both a seasonal cycle and a lunar cycle (Steger 1987). The sampling in February was scheduled to measure recruitment during the second or third lunar cycle of recruitment in the dry season. Recruitment normally occurs at peak intensity for the season by this time. Based on our analysis of size structure of populations at the time of sampling (it is possible to follow the monthly pulses of recruits for at least 2 to 3 mo), this timing held every year except 1991. Recruitment started late in 1991 and our sampling picked up only the first cycle of recruitment for the season. It is possible that the reduced recruitment seen at some sites in 1991 was a spurious effect of timing and that subsequent cycles were of typical intensity. We are therefore cautious in interpreting the recruitment event in 1991 due to this possibility.

6.6.2 Effect of Density on Competition

The intensity of competition for cavities is expected to be a function of the abundance of gonodactylids relative to the abundance of cavities. A good indicator of the intensity of competition is the relationship between the size of a gonodactylid and the size of its cavity. When the availability of cavities limits populations, the intensity of competition for cavities is high and individuals are forced to occupy cavities smaller than they prefer (Steger 1985). This leads to the prediction that as densities of gonodactylids decrease or increase, the cavity-volume relationship should change also, and individuals should occupy larger or smaller cavities.

The cavity-volume relationship will be discussed below in terms of gonodactylid densities. We will rely on densities from quadrat samples in the comparisons because areal samples are more representative of true densities than rubble samples. We focus our discussion on large and medium-sized gonodactylids because cavity volumes were not measured (for logistical reasons) for individuals smaller than 20 mm total length.

At LRW there were no significant differences among the cavity-volume regressions for any samples taken during the monitoring. This similarity is consistent with the densities of gonodactylids, as there were no significant differences in the densities of large and medium-sized individuals at LRW.

At MAR4 the cavity-volume regression showed significant differences in slope in 1987. There were no significant changes in densities of large gonodactylids at MAR4, but densities of medium-sized individuals varied significantly as densities dropped and rose with recruitment. It appears that changes in the slope of the cavity-volume relationship are associated with changes in the density of smaller individuals, but not larger individuals.

At LRN the cavity-volume regression adjusted almost continuously from 1987 until 1989. Changes in slopes of the regression were followed by changes in intercepts. The density of large gonodactylids increased significantly during the monitoring due to immigration. The density of medium-sized gonodactylids varied significantly with the level of recruitment in previous years. Changes in densities of both large and medium-sized individuals resulted in both slope and intercept changes in the cavity-volume regression.

Comparisons between sites showed no significant differences between the cavity-volume regressions measured at reference sites. The cavity-volume relationship was the same for all samples from MAR4 and LRW.

Intercepts of cavity-volume regressions were significantly different between LRN and the reference sites for every year except 1989. It is interesting that there were no differences in slopes among any of the sites. This finding indicates that the changes in slopes measured at sites were taking place at all sites during the same sample period. This is consistent with the overall pattern of decline in medium-sized individuals driven by low recruitment, as the pattern was the same at all sites.

The intercepts of the regressions indicate that the cavity-volume regressions at LRN were converging with the reference sites through 1989 as the density of large individuals increased at LRN. The regressions moved apart in 1990 and 1991 although densities did not vary. It appears that the differences in 1990 and 1991 were due to the huge increases in rubble (i.e., abundance of cavities) at LRN.

Comparisons between sites and prespill data from MAR4 in 1980 indicate there were no significant differences between reference sites and prespill data. It should be noted that the prespill cavity-volume regression is an average relationship for an entire year. The changes in densities and cavity-volume regressions measured at MAR4 after the spill were not large enough to cause cavity-volume relationships to be significantly different than an average prespill year.

The cavity-volume relationship at LRN never reached prespill conditions. Although densities changed and caused changes in the cavity-volume regression over the course of monitoring, the density increases were not sufficient to drive the cavity-volume relationship all the way to prespill conditions.

The results of this project have given us a good understanding of how densities of various sizes of gonodactylids affect competition and establish the cavity-volume relationship. The density of large gonodactylids drives competition. If the density of large individuals decreases, the intercept of the cavity-volume regression shifts up as all sizes of gonodactylids move up into larger, more preferred cavities. If the density of large individuals increases toward the carrying capacity of cavities, the intercept of the cavity-volume regression shifts down as all sizes of individuals are forced into smaller cavities.

Densities of smaller gonodactylids do not drive competition overall, but they do affect competition among smaller individuals. The density of smaller gonodactylids can affect the slope of the regression. That is, the density of larger individuals affects the cavities occupied by large and small gonodactylids through a cascading effect, while the density of smaller individuals only partially affects the sizes of cavities available to smaller gonodactylids.

The cavity-volume relationship can be pictured as a line anchored by the density of large individuals. If the density of large animals changes, the entire position of the line changes. If the density of smaller animals changes without a change in the density of larger animals, the line is still anchored at the high end, but the tail of the line shifts. If all densities are changing, the line moves in complex patterns, with both position and slope of the line changing.

This model of gonodactylid competition was supported by the direct test of association between densities and cavity volumes. The degree of association between density and cavity volume was strong for larger gonodactylids. The lack of association between density and cavity volume for smaller sizes indicates the cavities occupied by smaller individuals are affected strongly by something other than density. This is consistent with the theory that competition and the cavity-volume relationship is directed most strongly by the density of larger individuals.

Oiling decreased the density of large gonodactylids and depressed recruitment at heavily oiled sites. The nonlethal, indirect effects of these density changes as described above should be viewed as a long-term effect of the oil spill.

6.6.3 Injuries, Densities, and Cavity Competition

Often we find that a significant number of larger *Gonodactylus* are injured. These injuries include missing raptorial appendages, regenerating appendages, and tears and fractures in the exoskeleton. Most injuries are clearly wounds inflicted by other gonodactylids such as damage to the exoskeleton caused by stabs and smashes from the raptorial appendages. The wounds are received during fights for cavities.

Presumably, as the density of gonodactylids increases or decreases, competition for cavity refuges changes, and the level of injuries in the population varies in a predictable manner. This project has provided solid evidence about the association among injuries, densities, and competition.

There were two objectives in our analysis of the amount of injuries. First, we wished to determine whether oiling had an effect on the number injured. Second, we wished to determine whether differences in the number of injured gonodactylids were caused by changes in densities.

Amount of oiling had a significant effect on the number of injured gonodactylids at the study sites. The first evidence came from the analysis of pre- and postspill number injured at the sites. The number injured was associated with the time of the oil spill at heavily oiled sites, but not at lightly oiled to unoiled sites. Between-site comparisons were also consistent with the amount of oiling. Before the oil spill, number injured was not associated with site of sampling. There were no differences in the number of injured gonodactylids caused by inherent differences among LRN, LRW, MINA, and MAR4. After the oil spill injuries were associated with site and based on the amount of oiling at the site. Heavily oiled sites had lower numbers of injured gonodactylids.

Further evidence came from heavily oiled sites. There was significant immigration of large gonodactylids into MINA between September 1986 and February 1987. Oiling had an effect on the density of large gonodactylids at MINA only in the September 1986 sample. Similarly, oiling had an effect on the number injured at MINA only in September 1986. When density was low, the number injured was low. When density increased, injuries increased. LRN showed some evidence of immigration by larger *Gonodactylus* into the area, but densities remained low at LRN during all postspill sampling. The number injured also remained lower at LRN, and this site differed from MINA (after September 1986, but not before), both lightly oiled to unoiled sites, and the unoiled site at SOTO. The exception was ESCU, an unoiled site, which will be discussed below.

At some sites, however, changes in the number of injured gonodactylids were not always tightly associated with changes in densities. For example, LRW showed no significant variation in the density of large gonodactylids in quadrat samples and perhaps only a slight decrease in rubble samples. LRW also showed the least variation in the cavity-volume regression. Injuries, on the other hand, varied significantly at LRW, ranging from 15% to 49%. Why this site was so variable with respect to wounding remains a mystery, although its proximity to heavily oiled sites and changes in substrata due to erosion at LRN could be involved.

ESCU is another example of a site where injuries and gonodactylid density were poorly correlated. This location had a relatively low number of injured gonodactylids although the density of large gonodactylids per unit volume of rubble was relatively high. The number of injured gonodactylids at ESCU was almost as low as at LRN. The total density of gonodactylids per unit volume of rubble was low at ESCU, as at LRN, but it is difficult to explain the low number of injured large gonodactylids in terms of total density because most combat for cavities is between similar-sized individuals. One possibility is that gonodactylids were concentrated in relatively few, large pieces of rubble at ESCU, and that there were fewer interactions among animals due to overall low densities or lack of movement among pieces. ESCU was slightly deeper than the other sites, and gonodactylids are less likely to move in deeper water (Caldwell 1988). The lack of quadrat samples at ESCU precludes density-per-unit-area estimates, but our impression is that animals were relatively scarce at this location.

In summary, oiling had a measurable effect on the number of injured gonodactylids. The reduced number of injured individuals was an indirect effect brought about by the reduction in densities of large gonodactylids caused by oiling. Except when there was a very large effect of oiling on densities, the association between density and number injured was not clear and other factors besides density *per se* were affecting the number injured at sites independent of the amount of oiling.

6.7 Summary

Oiling had an initial effect on gonodactylid stomatopods by reducing the density of larger gonodactylids, especially females, at heavily oiled sites.

Long-term effects of oiling were measured in changes in habitat at the most heavily oiled site. Oiling killed *Thalassia* and a wide band of mangroves. Erosion then caused a loss of up to 14 cm of substratum and transformed the habitat into an area dominated by large pieces of unconsolidated coral rubble. This site no longer has the preferred habitat of gonodactylids and the density of gonodactylids remains low.

Oiling also had a long-term effect on recruitment of gonodactylids. Recruitment was reduced for at least one and possibly as long as 2 yr following the oil spill. This reduced recruitment caused a decline in densities of gonodactylids in the years following the oil spill. The long-term effect of reduced recruitment on gonodactylid populations was not completely clear, however, because of a general depression of recruitment that occurred from 1987 through 1989.

Indirect effects of oiling were also measured in the level of competition for cavities and the number of injured gonodactylids. The indirect effects were brought about by changes in density.

No direct effects of the oil spill were measured on gonodactylid stomatopods after 1988. Immigration of larger gonodactylids into heavily oiled sites and a return to normal levels of recruitment eliminated or masked any direct effects of oiling that persisted. The indirect effect of the transformation of the habitat at the most heavily oiled site, however, will persist for many years.

In addition to fulfilling the primary goal of studying the effects of the oil spill on gonodactylid stomatopods, long-term monitoring has also increased our understanding of basic dynamics in gonodactylid populations.

The disappearance of *Lysiosquilla* from the area around Punta Galeta following the oil spill and the failure of these stomatopods to recruit since then provides a clear example of a species eliminated from these coastal habitats by oil, although the mechanisms responsible remain unclear.

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7
Changes and Recovery of Subtidal Reef Corals

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7.1 Abstract

In 1986 a major crude oil spill on the Caribbean coast of Panama polluted extensive areas of coral reefs, seagrass beds, and mangroves. Oil slicks originating from mangroves are still observed passing over reefs after 5 yr. We studied long-term effects of the spill on shallow (0.5-6 m) subtidal reef corals for 6 yr at the individual, population, and community level. Results demonstrate by several methods deleterious effects of oil on reef corals. These effects include: (1) a striking reduction of cover, abundance, and diversity of corals immediately after the spill (the elkhorn coral *Acropora palmata* suffered much more than other common species, most of which are massive), (2) a strong positive correlation between injured corals and the concentration of hydrocarbons in reef sediments, (3) a decrease in the growth rate of four massive coral species on oiled reefs during the year of the spill and 3 yr thereafter (growth of two coral species was negatively correlated with hydrocarbon concentrations in reef sediments), (4) a decline in fecundity of surviving colonies of the important reef-building coral *Siderastrea siderea* on oiled reefs more than 3 yr after the spill (furthermore, colonies with recent injuries showed impaired reproductive activity), and (5) little recovery, as indicated by very low coral recruitment rates on oiled reefs. Effects of the spill on reef corals in Bahía Las Minas were severe, and populations have not begun to recover. Increased erosion, reoiling, and sedimentation from degraded mangroves and seagrass beds suggest that populations will be reduced even further.

7.2 Introduction

Coral reefs are shallow-water communities formed by growth of corals, crustose algae, and other calcareous organisms whose skeletons form a stable, wave-resistant structure. Reefs are a major habitat of tropical waters, where they may form a continuous band fringing the coast or offshore barriers seaward of mangroves and seagrass beds. Coral reefs are highly resistant to storms and erosion, and are important in stabilizing coastlines, especially in low-lying areas subject to tropical storms and rising sea levels (Smith and Buddemeier 1992). Reefs are also primary areas of subsistence fisheries throughout the tropical Pacific Ocean and the Caribbean Sea, and in general have high cultural, economic, and recreational value (UNEP/IUCN 1988).

Coral reefs support a high diversity of plants and animals (Huston 1985; Jackson 1991). Reef community structure reflects the influence of both physical (sedimentation, light, and currents) and biological (competition, disease, and predation) factors. Patterns of larval recruitment, juvenile and adult mortality, regeneration, sexual and asexual reproduction, and grazing pressure are among the most important ecological processes that affect the dynamics of coral populations and their distribution and abundance (Connell 1973, 1978; Bak and Engel 1979; Birkeland et al. 1981; Rogers et al. 1984; Huston 1985; Jackson 1991). Predictability of the distribution, abundance, and diversity of reef communities increases with increasing spatial scale (Done 1983; Geister 1977; Wilkinson and Cheshire 1988; Jackson 1991). Studies of several reef habitats and larger reef areas show predictable reef zonation as a result of interspecific interactions in association with resource use, individual life histories, and the effect of disturbances (Huston 1985; Jackson 1991).

Coral reefs are ranked among the most biologically productive and diverse of all natural ecosystems (Hatcher 1988, 1990). Approximately 14% (84,000 km^2) of the areal extent of coral reefs worldwide occur in the Caribbean Sea (Smith 1978). The Caribbean region also is potentially one of the largest oil-producing areas in the world (Rodriguez 1981), and contamination by petroleum hydrocarbons is considered to be the most serious marine pollution problem of the region (IMCO 1979). Nevertheless, there has been no long-term study of oil pollution in coral reef ecosystems of the Caribbean. Of particular concern are oil terminals, tanker traffic, refineries, and offshore oil reserves adjacent to reefs, all of which are potential sources of contamination (UNEP/IUCN 1988; Keller et al., Chap. 1). Designation of the new Florida Keys National Marine Sanctuary heightens these concerns. Thus, studies of the long-term effects of oil pollution on Caribbean reef corals is a regional research priority.

The extent of the effects of oil on reef corals in their natural environment is controversial and poorly understood (Loya and Rinkevich 1980; Brown and Howard 1985). Some studies suggest harmful effects and lasting damage in oiled populations, as measured by abundance, mortality, reproduction, and recruitment (Lewis 1971; Johannes et al. 1972; Loya 1975, 1976; Reimer 1975; Birkeland et al. 1976; Rinkevich and Loya 1977, 1979; Loya and Rinkevich 1980; Peters et al. 1981; Bak 1987; Jackson et al. 1989; Guzmán et al. 1991). Field studies supporting this view are generally for intertidal corals or lack observations before pollution began. In contrast, results of experiments on the effects of oil and dispersants in the laboratory or field are contradictory. Some studies suggest little or no mortality or persistent sublethal effects of oil on corals (Reimer 1975; Elgershuizen and Kruijf 1976; Knap et al. 1985; Cook and Knap 1983; Dodge et al. 1984; Wyers et al. 1986; Knap 1987; Shinn 1989), while others suggest more serious or lasting damage (Lewis 1971; Reimer 1975; Birkeland et al. 1976; Rinkevich and Loya 1977; Peters et al. 1981). In either case, extrapolation of such experimental results to the field is hampered by the necessarily unnatural spatial scale and durations of the manipulations (Loya and Rinkevich 1980; Knap et al. 1985; Brown and Howard 1985; Capuzzo 1987), which were not designed to mimic large-scale oil spills with chrinic contamination and other possible real-world factors.

7.2.1 Coral Reefs of the Study Region

Subtidal reefs along the Caribbean coast of Panama are mostly fringing reefs that give way to sediment plains at depths of 10 to 25 m (Guzmán et al. 1991). Despite heavy sedimentation and runoff, live coral cover averaged 27% before the oil spill (Guzmán et al. 1991).

The study reefs include eight within or near Bahía Las Minas that were varyingly affected by the oil spill, and four "control" reefs between Portobelo and Isla Grande (Fig. 7.1). Bahía Las Minas has been subjected to intense human disturbance since long before the 1986 oil spill, beginning in the 1870s with excavation, dredging, land filling, and erosion due to construction of the Panama Canal and the city of Colón (McCullough 1977). Chemical pollution began with drainage and intensive oiling and spraying of mangroves for mosquito control early in this century (Curry 1925). Construction of a refinery and a large cement plant were completed in the 1950s, and erosion and sediment discharge, due to increased deforestation and housing construction over the past 30 yr, is greater. There was also a major spill of 3.2 million L (20,000 bbl) of diesel and Bunker C fuel oil following the wreck of the tanker *Witwater* in December 1968 (Rützler and Sterrer 1970; Birkeland et al. 1976). During that time of year low tides are higher than normal, so oil from the *Witwater* spill may have passed over reefs without noticeable effect (Rützler and Sterrer 1970).

The region of the control reefs has also suffered from human disturbance. Deforestation and consequent heavy discharge of sediments have been extensive since the 1970s, and are still increasing, as evidenced by dark, bauxite-laden river discharge after heavy rains.

7.2.2 Physical Environment

Monthly observations of salinity and temperature began in May 1987; observations of suspended and resuspended sediments began in October 1987 and continued until December 1991, weather permitting. All parameters were measured on all reefs but Largo Remo 1 (Table 7.1). Values of salinity (± 1 o/oo) were determined using a hand-held refractometer (American Optical Corporation). Temperature was measured using a mercury thermometer with 0.5°C divisions. Suspended sediments were determined by filtration (Cortés and Risk 1985; Tomascik and Sander 1985; Guzmán 1986). Six replicate 1-L samples of seawater were collected at each reef. Samples were filtered using preweighed Millipore filters (0.45 μm). A few drops of sodium azide (NaN_3) were used to poison the samples to prevent decomposition of organic matter. Filters were oven-dried at 45°C for 24 h, cooled, and reweighed to the nearest 0.01 mg. Resuspended sediments were sampled using six sediment traps per reef (Cortés and Risk 1985; Guzmán 1986). The traps were constructed of PVC pipe 8 cm by 24 cm (height/width ratio of 3), and were set on iron stakes 20 cm above the bottom. The traps were collected 1-5 d after placement on reefs, and the sediments trapped were recovered by filtering the water

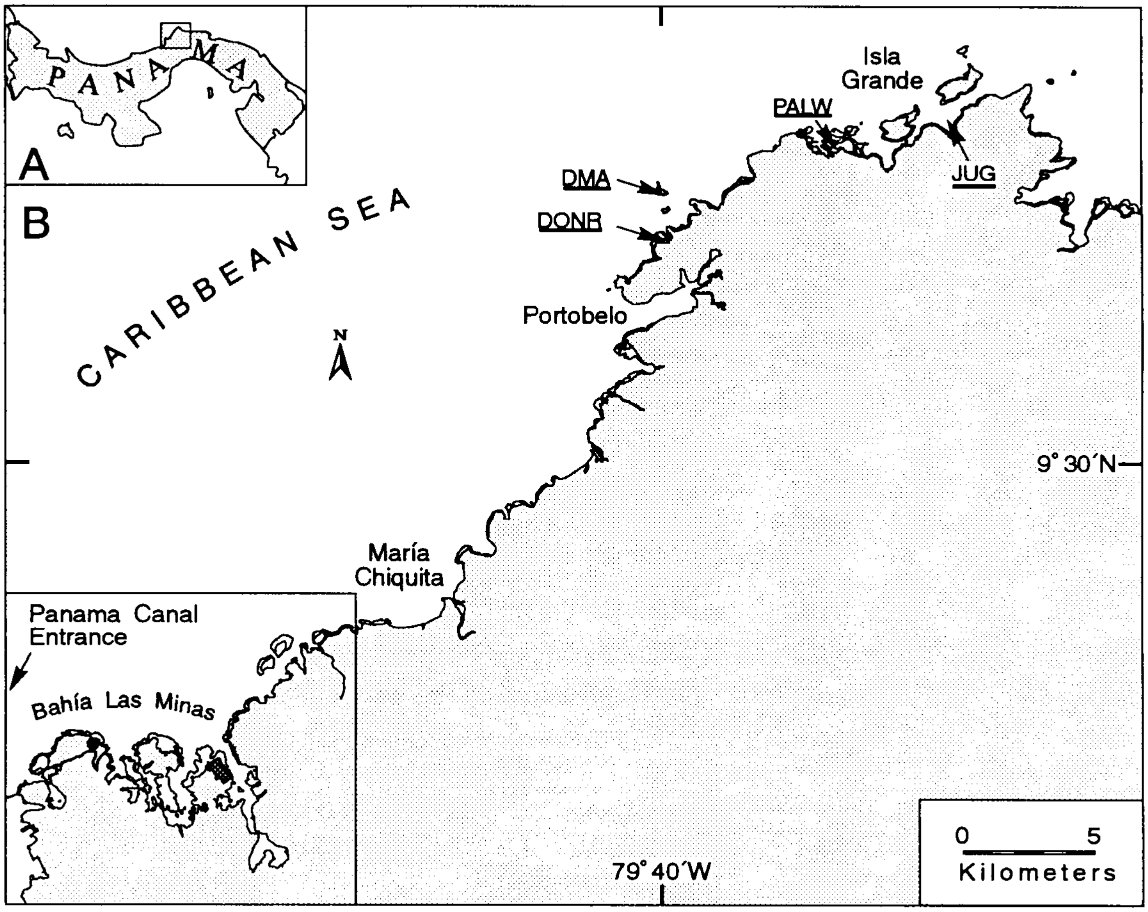


Fig. 7.1 Region affected by the 1986 oil spill shown as increasing enlargements, (*A-C*). *A*. Location within Panama, just east of the Caribbean entrance to the Panama Canal. *B*. The *boxed area* includes the most heavily oiled coastal habitats. Punta Galeta is at 9°24'N, 79°52'W. Lightly oiled and unoiled reefs are east of Bahía Las Minas near Portobelo and Isla Grande. Reef names are listed in Table 7.1. Reefs surveyed in 1985 (before the oil spill) are *underlined*.

on preweighed filter papers (Whatman #1, 12.5 cm). Samples were then processed following the same protocol described for suspended sediments.

Figure 7.2 shows monthly average values of four physical parameters for reefs grouped according to exposure to oil during the spill. Sea-surface salinities were generally lower and temperatures somewhat higher at Bahía Las Minas than at unoiled reefs. Sediment loads showed considerable variation throughout the year, but higher values were always observed at reefs in Bahía Las Minas, where values reached 1,550 mg/cm^2/yr. In general, both suspended and resuspended sediments were high during the dry season (December-April). Although only one survey was made during January due to generally rough sea conditions, an elevated sediment load probably occurs this month as well as the rest of the dry season.

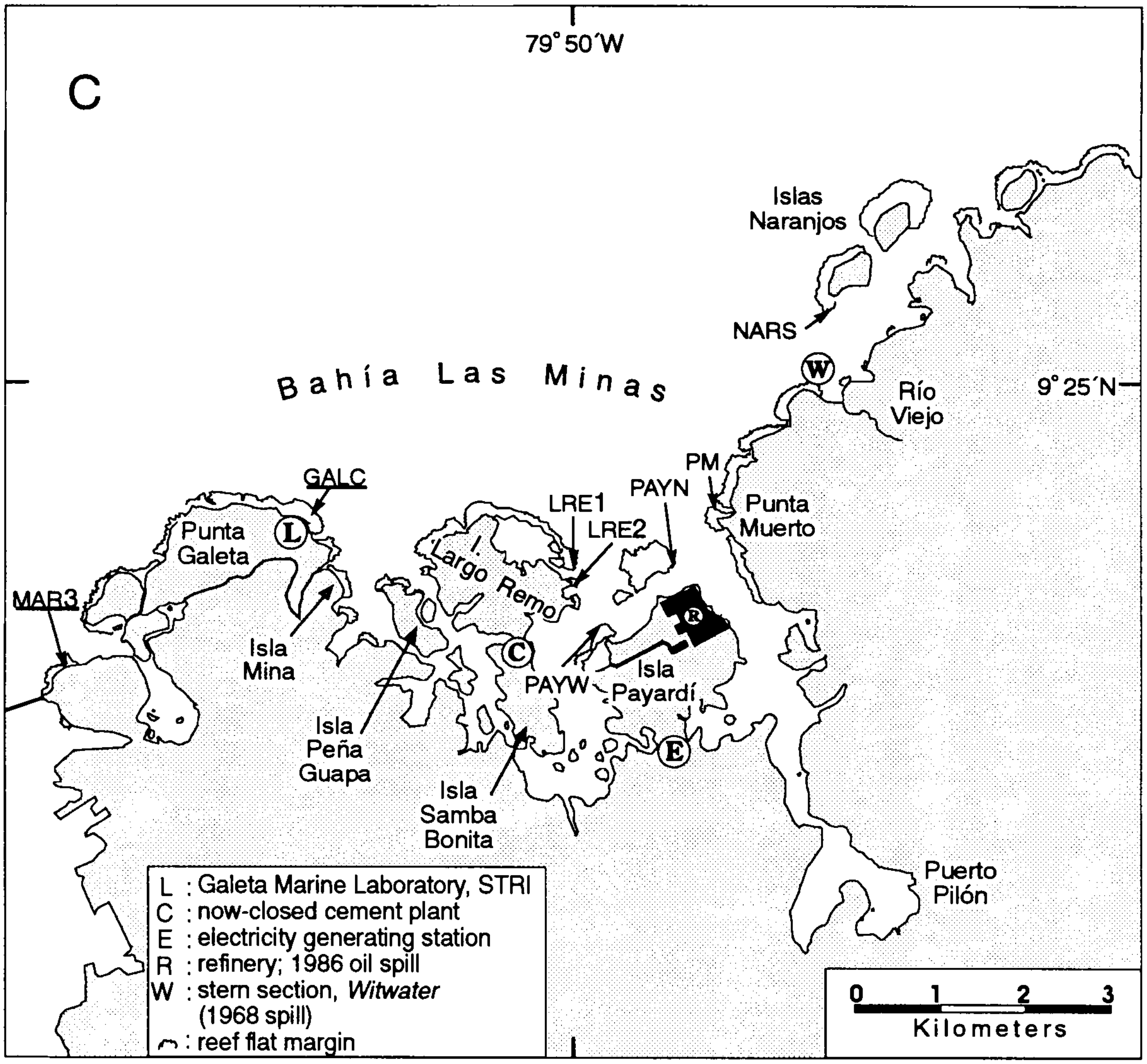


Fig. 7.1 Region affected by the 1986 oil spill shown as increasing enlargements (continued). *C.* Detail of the most heavily oiled area and location of study reefs.

Yearly averages for the same four environmental parameters are shown in Figure 7.3 and results of repeated-measures analysis of variance (ANOVA) for these data in Table 7.2. The oil x time interaction was not significant for any of the variables. Salinity varied significantly during the study, and was significantly higher at unoiled reefs, whereas temperature was higher at oiled reefs and did not change during the 4 yr of observations. Sediment load varied considerably between years. Resuspended sediments were significantly higher at heavily oiled reefs and over time. The approximately linear increase in resuspended sediments was significant during

Table 7.1 Subtidal reefs studied for the different tasks. *Cov* = cover of sessile organisms; *Inj* = coral injury; *Reg* = regeneration experiments; *Scl* = sclerochronology; *Mon* = physical monitoring (salinity, temperature, suspended and resuspended sediments); *R/R* = coral recruitment and reproduction; *Hyd1* = hydrocarbon samples in 1986; *Hyd2* = hydrocarbon samples in 1988-89 and 1990; *H* = heavy; *M* = moderate; *U* = unoiled.

Reef	Acronym	Oiling	Cov	Inj	Reg	Scl[1]	Mon	R/R	Hyd1[2]	Hyd2[3]
Galeta	GALC	H	X	X		X	X	X	X	X
Largo Remo 1	LRE1	H	X	X		X		X		X
Largo Remo 2	LRE2	H	X	X	X	X	X	X/X		X
Payardí West	PAYW	H	X	X	X	X	X	X		X
Payardí North	PAYN	H	X	X		X	X	X/X	X	X
Punta Muerto	PM	H	X	X		X	X	X		X
Naranjos South	NARS	M	X	X		X	X		X	X
Margarita	MAR3	M	X	X		X	X			X
Doncella	DONR	U	X	X		X	X	X		X
Dos Marias	DMA	U	X	X		X	X	X		X
Palina West	PALW	U	X	X	X	X	X	X/X	X	X
Juan Gallegos	JUG	U	X	X	X	X	X	X/X		X

[1] Coral colonies were not collected at LRE1 (heavily oiled) during 1987.

[2] Reefs sampled by BBSR for hydrocarbons in 1986.

[3] *Siderastrea siderea*, *Porites astreoides*, and reef sediments were collected at all reefs. *Acropora palmata*, *Agaricia tenuifolia*, and *Diploria strigosa* were collected only at PAYN and PALW.

the 4-yr period (repeated-measures ANOVA by contrast; $F = 7.87$; df = 1, 8; $P = .023$). Suspended sediments decreased over time at unoiled sites, but were more variable at oiled reefs.

Rainfall data were obtained from Instituto de Recursos Hidraúlicos y Electrificacíon, Departamento de Hidrometeorología, Panama. There are two meteorological stations within the study region: San Pedro (9°23'N, 79°49'W; 9 m altitude) is close to the refinery and Portobelo (9°33'N, 79°40'W; 2 m altitude) is in the region of the control reefs. Rainfall is higher and the dry season shorter at Portobelo than Bahía Las Minas throughout the year (Fig. 7.4). Figure 7.5 shows annual rainfall at both sites from 1985 to 1991. The consistently higher rainfall at Portobelo suggests that increasing sediment loads at Bahía Las Minas are not due to differences in runoff, and may therefore be related to the oil spill.

7.2.3 Sessile Biota

The sessile biota of subtidal coral reefs along the central Caribbean coast of Panama is composed mainly of scleractinian corals, hydrocorals, gorgonians, crustose algae, macroalgae (including fleshy and calcareous species), zoanthids, anemones, and sponges. The most abundant reef organisms before the oil spill were macroalgae (45% cover), scleractinian corals (27%), sponges (5%), and crustose

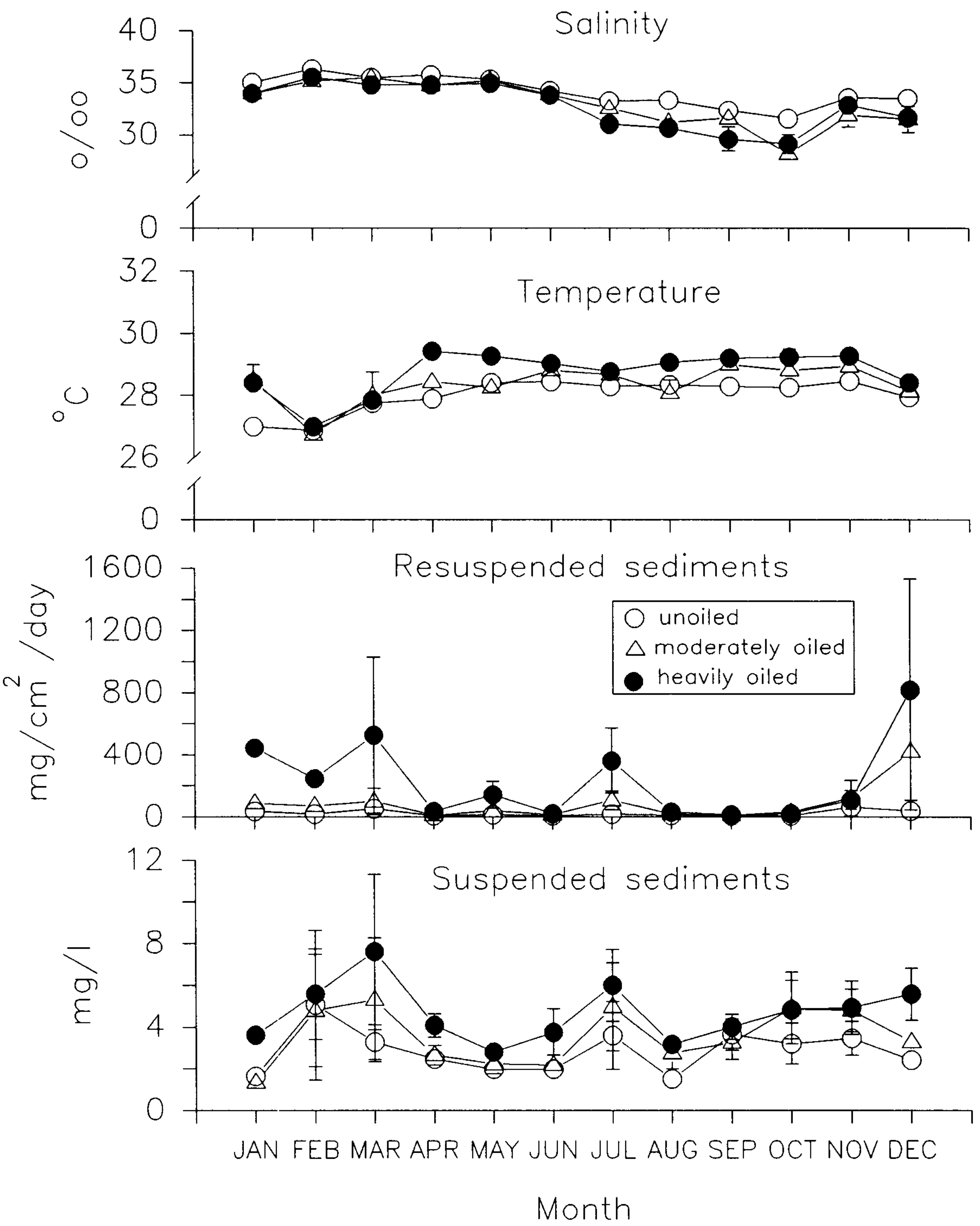


Fig. 7.2 Monthly average of sea surface salinity, temperature, resuspended sediments, and suspended particulate matter recorded from October 1987 to December 1991 in relation to the degree of oiling (unoiled, moderate, and heavy). Error bars are 1 SE.

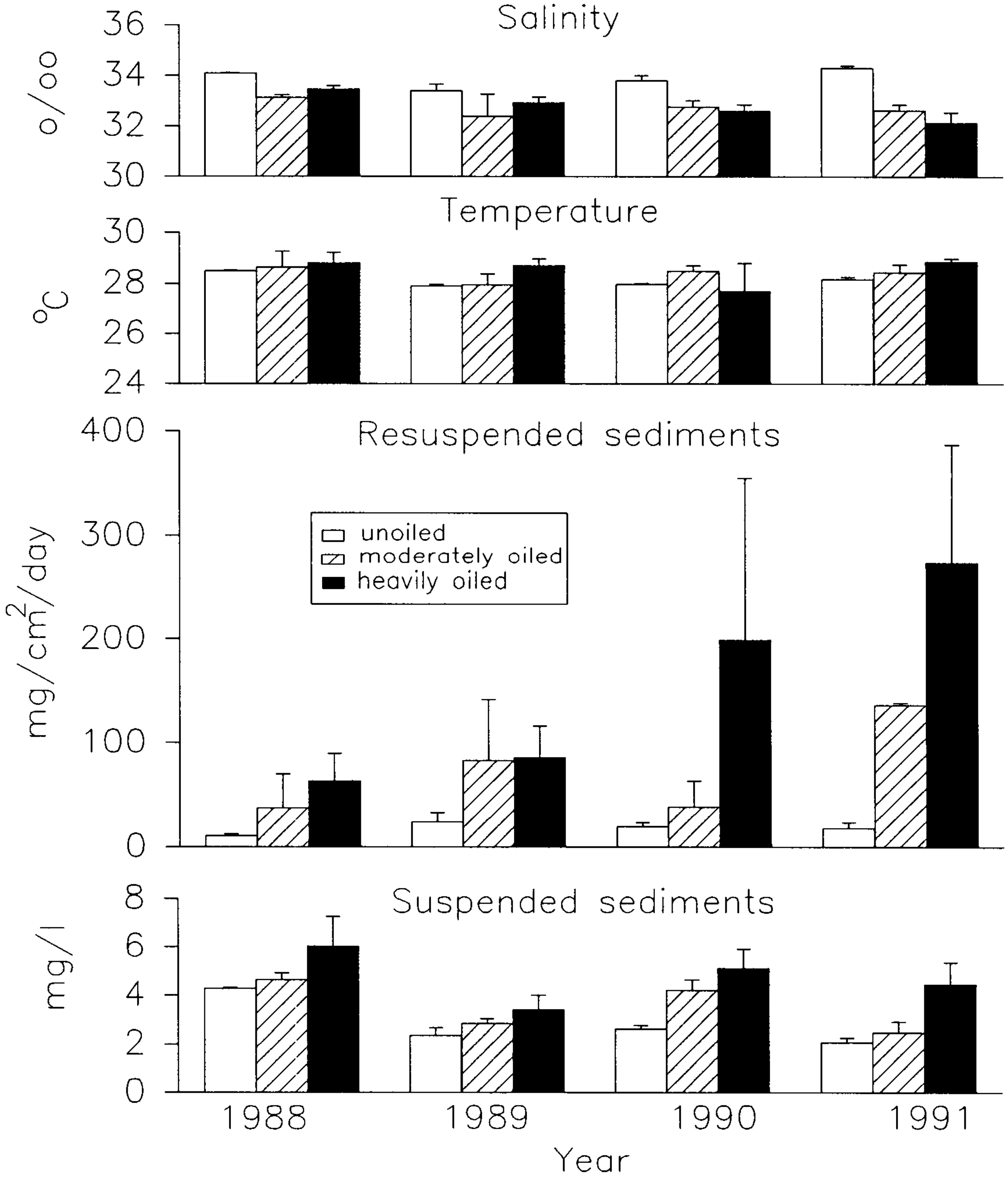


Fig. 7.3 Yearly average of sea surface salinity, temperature, resuspended sediments, and suspended particulate matter in relation to the amount of oiling (unoiled and oiled). Based on data recorded from October 1987 to December 1991. Error bars are 1 SE.

Table 7.2 Repeated-measures analysis of variance for four physical parameters on reefs grouped according to exposure to oil in 1986 (heavily oiled, moderately oiled, unoiled; factor = *Oil*). Data are yearly averages from 1988 to 1991. See Figure 7.3.

Parameter	Factor	*F*	df	*P*
Salinity	Oil	16.23	2, 8	.001
	Time	4.35	3, 24	.01
	Oil x Time	2.00	6, 24	.1
Temperature	Oil	9.18	2, 8	.008
	Time	1.49	3, 24	.2
	Oil x Time	0.37	6, 24	.9
Resuspended Sediments	Oil	6.17	2, 8	.02
	Time	5.83	3, 24	.003
	Oil x Time	1.30	6, 24	.3
Suspended Sediments	Oil	2.25	2, 8	.2
	Time	21.88	3, 24	.000
	Oil x Time	1.67	6, 24	.2

algae (3%; see Sect. 7.5.1); the main reef-building scleractinian and hydrocoral species are the major focus of this study. The Caribbean region has about 70 hermatypic scleractinian species (UNEP/IUCN 1988); Jaap and Hallock (1990) reported 63 scleractinian species, subspecies, and formae from the Florida Keys. Porter (1972) reported 49 hermatypic coral species for Panama, however, the list recently has been increased to 58 hermatypic species (Holst and Guzmán *in press*), and will increase further with detailed taxonomic study (Knowlton et al. 1992; E. Weil, unpublished data). Of these, a total of 52 scleractinian and four hydrocoral species have been recognized in the area between Isla Margarita and Isla Grande (Table 7.3; Fig. 7.1). The most abundant coral species on the study reefs were the scleractinians *Siderastrea siderea*, *Porites astreoides*, *Diploria clivosa*, *Agaricia agaricites*, and *D. strigosa*, and the hydrocoral *Millepora complanata* (UNEP/IUCN 1988; Guzmán et al. 1991). Other, more patchily abundant species included *Agaricia tenuifolia*, *Montastrea annularis* (now three species; see Knowlton et al. 1992; they were not distinguished during our surveys), *Acropora palmata*, and *Porites* spp. All these species are widely distributed throughout the Caribbean at shallow depths. One coral species, *Porites colonensis*, however, is endemic to this region. Because of the depth distribution of corals and the likely greater effects of the spill in shallow water, all work was conducted in depths less than 10 m.

Crustose algae and macroalgae (fleshy and calcareous) are the most abundant sessile organisms other than corals (Table 7.4). Both groups flourished after coral mortality throughout the region at all depths (Sect. 7.5.1; Guzmán et al. 1991).

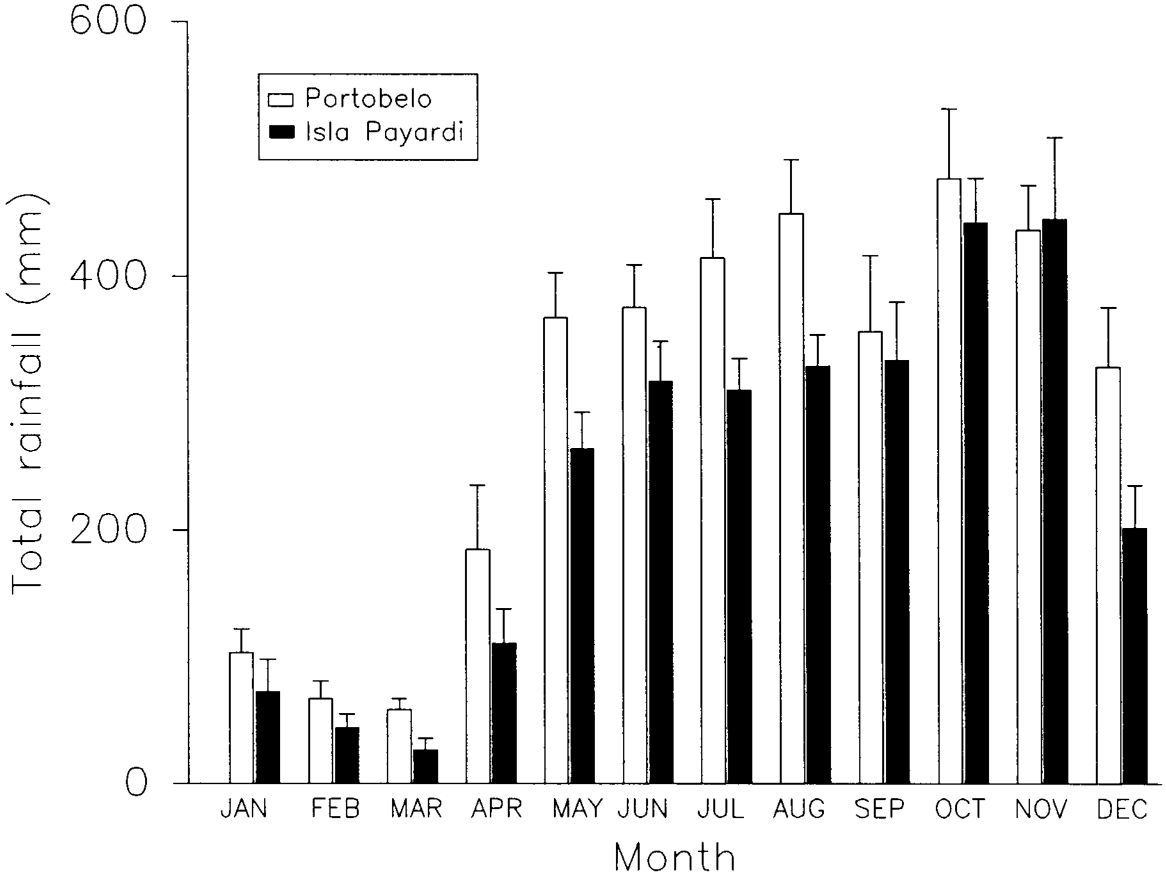


Fig. 7.4 Monthly average of total rainfall at Isla Payardí (oiled area) and Portobelo (unoiled area) based on records from 1979 to 1991. Error bars are 1 SE.

Halimeda spp., *Sporolithon episporum*, *Hydrolithon boergesenii*, *Caulerpa* spp., and *Dictyota* spp. are the most abundant species from 0.5 to 12 m depth.

Other groups, such as sponges, gorgonians, and zoanthids, may compete aggressively for reef space, and overgrow living corals and colonize large areas of dead reef framework. The gorgonian *Erythropodium caribaeorum* and the zoanthids *Palythoa* spp. commonly overgrow surviving corals on disturbed reefs. Other common gorgonians include *Muriceopsis flavida* and *Gorgonia flabellum*. The sea anemone *Stoichactis helianthus* occurs in large numbers in shallow water, growing in dense patches over large dead coral heads. Encrusting sponges are diverse and abundant on the study reefs. Boring sponges such as *Cliona* spp. are quite destructive by weakening the support of corals (Hein and Risk 1975). Other sponges may help to bind coral skeletons to the reef substratum (Wulff 1984).

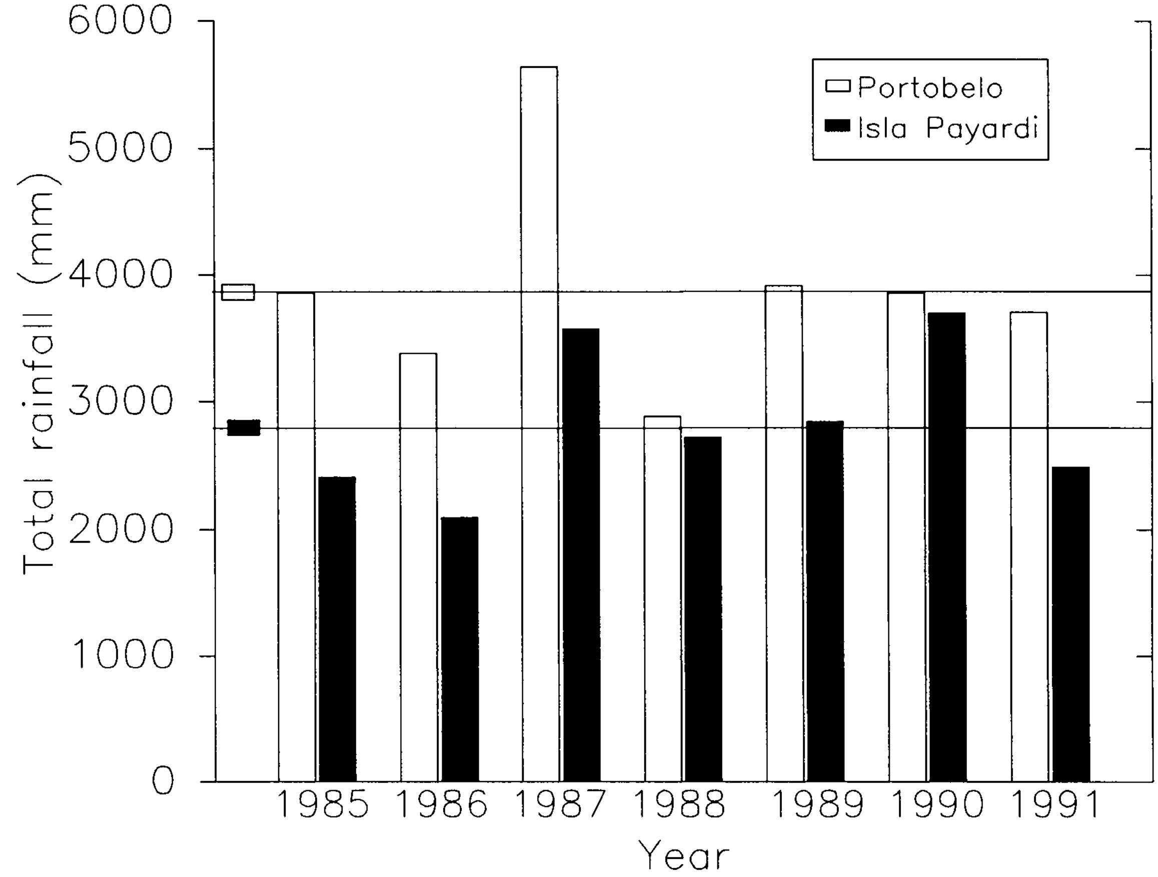


Fig. 7.5 Total rainfall each year of the study period (1985-1991) at Isla Payardí (oiled area) and Portobelo (unoiled area). *Horizontal lines* represent total annual average for the last 12 yr (1979-1991) at Isla Payardí (*bottom line*) and Portobelo (*top line*).

7.3 Objectives, Rationale, and Design

Here we describe responses of the reef community and some reef coral populations during 6 yr of continuous biological and physical monitoring (Table 7.1). The study included annual surveys of the cover of sessile organisms (1985-1991, except 1987) and different assays to evaluate sublethal effects of oil on common massive corals. The latter included quarterly monitoring of four species that showed considerable recent injury just after the oil spill (1986-1991), regeneration of experimentally induced injuries in two species, rates of growth of four species, and sexual reproduction of one species. Temperature, salinity, presence of oil slicks overlying reefs, suspended sediments, and resuspended sediments were also measured (1987-1991). Different assessments were, of necessity, performed on different subsets of reefs for reasons of availability of corals and logistics.

Table 7.3 List of scleractinian and hydrocoral species observed in the study area. A total of 52 scleractinians and four hydrocorals are reported.

Scleractinians (Hermatypic)
- *Stephanocoenia intercepta*
- *Madracis decactis*
- *Madracis mirabilis*
- *Madracis senaria*
- *Acropora cervicornis*
- *Acropora palmata*
- *Acropora prolifera*
- *Agaricia agaricites*
- *Agaricia agaricites danai*
- *Agaricia agaricites purpurea*
- *Agaricia agaricites humilis*
- *Agaricia agaricites carinata*
- *Agaricia tenuifolia*
- *Agaricia fragilis*
- *Agaricia* sp.
- *Leptoseris cucullata*
- *Siderastrea siderea*
- *Siderastrea radians*
- *Porites porites*
- *Porites furcata*
- *Porites divaricata*
- *Porites astreoides*
- *Porites colonensis*
- *Colpophyllia natans*
- *Colpophyllia breviserialis*
- *Diploria strigosa*
- *Diploria clivosa*
- *Diploria labyrinthiformis*
- *Favia fragum*
- *Manicina areolata*
- *Cladocora arbuscula*
- *Montastrea "annularis"* sp. 1
- *Montastrea "annularis"* sp. 2
- *Montastrea "annularis"* sp. 3
- *Montastrea cavernosa*
- *Solenastrea hyades*
- *Solenastrea bournoni*
- *Oculina diffusa*
- *Meandrina meandrites*
- *Dichocoenia stockesii*
- *Dichocoenia stellaris*
- *Dendrogyra cylindrus*
- *Isophyllia sinuosa*
- *Isophyllastrea rigida*
- *Mussa angulosa*
- *Scolymia lacera*
- *Scolymia cubensis*
- *Mycetophyllia aliciae*
- *Mycetophyllia ferox*
- *Mycetophyllia danaana*
- *Mycetophyllia lamarckiana*
- *Eusmilia fastigiata*

Hydrocorals
- *Millepora alcicornis*
- *Millepora complanata*
- *Millepora squarrosa*
- *Stylaster roseus*

Table 7.4 List of the most abundant species of algae (crustose, calcareous, fleshy) observed at reefs in the study area (based on Hillis-Colinvaux 1980; Littler et al. 1989).

Crustose Coralline Algae
- *Amphiroa* spp.
- *Lithophyllum* sp.
- *Titanoderma* sp.
- *Porolithon pachydermum*
- *Sporolithon* sp.
- *Hydrolithon boergesenii*

Fleshy and Calcareous Macroalgae
- *Dictyota* spp.
- *Dictyopteris* spp.
- *Hypnea* spp.
- *Sargassum* spp.
- *Lobophora variegata*
- *Acetabularia calyculus*
- *Caulerpa racemosa*
- *Caulerpa sertularioides*
- *Caulerpa verticillata*
- *Halimeda opuntia*
- *Halimeda tuna*
- *Halimeda discoidea*
- *Halimeda incrasata*
- *Halimeda gigas*
- *Halimeda copiosa*

The goal of our study was to determine lethal and sublethal effects of the oil spill on subtidal reef corals and other sessile organisms, and prospects for recovery. We used three different statistical designs based on the type of data available before the spill and observations made soon afterward that helped to define the spatial scale of effects (Table 7.5).

A survey of six reefs in the study area less than 1 yr before the spill provided baseline data to assess immediate effects on distribution and abundance of sessile taxa (Guzmán et al. 1991). The reefs surveyed were not balanced relative to the subsequent oiling (one heavily oiled, one moderately oiled, and four unoiled; Table 7.5). Nevertheless, pre- and postspill comparisons (*main sequences 1* and *2* of Green 1979; Table 1.3) revealed striking decreases in coral abundance on oiled reefs.

Observations of coral condition in August 1986, less than 4 mo after the spill, revealed extensive injuries to corals on oiled reefs (Jackson et al. 1989). To document this finding, six oiled reefs were surveyed for the first time in 1986-1988 (five heavily oiled and one moderately oiled) in addition to the six original reefs. This addition gave 12 reefs (six heavily oiled, two moderately oiled, and four unoiled by the Bahía Las Minas spill) to be compared postspill. These data were analyzed using Green's *main sequence 4*, wherein effects must be defined from the spatial pattern of change alone. With the exception of coral growth measurements using sclerochronology (*main sequence 1*), other data collected postspill fit a *main sequence 4* model (Table 7.5).

The statistical procedure for all three designs is repeated-measures analysis of variance (ANOVA) with individual reefs as replicates for each treatment (amount of oiling). Repeated-measures procedures are necessary because all observations to test for biological effects involve repeated measurements of the same corals or reefs (Underwood 1981; Hand and Taylor 1987).

Because the oil spill was not a controlled experiment, unoiled reefs are not controls in the sense of "no treatment" in a planned experiment. Nevertheless, because there were prespill data on community composition and only some of the surveyed reefs were oiled by the spill, the unoiled reefs are the closest approximation to controls under these circumstances.

The use of control reefs places the oiled reefs within a wider context, with sampling methods standardized across all study sites (*sensu* Lincoln 1991). Many ecological studies compare an altered site with a single control site, assuming that they were not different before the alteration. This is not the case for coral reef communities, where reef habitats and populations vary greatly over all spatial and temporal scales (Jackson 1991). This range of natural variation can be measured comparing several control sites (Lincoln 1991; Underwood 1991). When only a single control site is used, interpretation of the effects of an anthropogenic disturbance is confounded with any natural change (Underwood 1991).

Two moderately oiled reefs (Table 7.1) were included in our design for the assessment of oil effects on reefs (e.g., cover, recent injury, and growth). However, the inherent ambiguity of this category and large differences in the structure and species composition between the two reefs complicated interpretation of the results.

Table 7.5 Summary of experimental design (after Green 1979) for the study of the effects of the oil spill on subtidal reef corals. See Table 1.3.

Task	Green's Main Sequence	Experimental Design and Time-Spatial Replication
Cover of Sessile Organisms	1	4 unoiled sites and 2 oiled sites before the spill. Annual survey.
	4	4 unoiled and 8 oiled sites after the spill. Annual survey.
	2	1 oiled reef surveyed before and after the spill. Annual survey.
Coral Recent Injury	4	4 unoiled sites and 8 oiled sites after the spill. Quarterly survey.
Coral Regeneration	4	2 unoiled sites and 2 oiled sites after the spill. Annual survey.
Sclerochronology (Growth)	1	4 unoiled sites and 8 oiled sites before and after the spill. Provides several annual records for before and after the spill.
Coral Reproduction	4	2 unoiled sites and 2 oiled sites after the spill. Monthly survey 3 yr after the spill for 15 mo.
Coral Recruitment	4	4 unoiled sites and 4 oiled sites after the spill. Annual survey (5th yr).
Herbivores	4	4 unoiled sites and 4 oiled sites after the spill. Annual survey (5th yr).

All statistical analyses (cover, injury, growth, reproduction, recruitment, and physical monitoring) were done using BMDP (1990 version for microcomputers). Percent cover data were arcsine transformed, and analyses were run group by group because of interdependence of this type of data. Variation in these parameters was evaluated by ANOVA with amount of oiling and depth as between-factor variables and time of census as a within-factor variable. Whenever significant interactions were found (oil x time, oil x depth, and depth x time) differences were examined among levels of one factor at each level of the other factor, and vice versa. In these cases, interpretations cannot be based on differences among means of particular levels of oiling (or differences among individual times) without taking into consideration the other factor because of nonindependence of factors (Underwood 1981). Only when no interactions exist can differences in means among levels of one factor can be examined over all levels of the other factor (i.e., looking at main effects in the resulting ANOVA).

The same is true for second-order interactions, which measure the extent to which the interaction between factors *A* and *B* varies among levels of factor *C* (e.g., whether the interaction of oil and depth varies over time or the interaction of oil and time varies between depths or the interaction of depth and time varies among different levels of oiling). A significant oil x time x depth interaction suggests no factor is independent of the others, and comparisons should be made at different depths for each of the combinations of oil and time, at different times for each combination of oil and depth, and at each level of oiling for each combination of depth and time. Graphs provide an invaluable analytical tool in the interpretation of such complex results. Again, if there are significant second-order interactions, it is misleading to consider only the first-order interactions or main effects.

BMDP 5V was used when unbalanced repeated-measures ANOVAs and contrast analyses were required because of missing observations in the repeated-measures factor. The 5V program uses maximum-likelihood criteria and provides a greater choice of covariance structure. The type of structure (compound symmetry or unstructured, fully parameterized covariance matrix) was chosen using the maximum Akaike's Information Criterion (AIC) value (Schluchter 1990). Differences among individual years and level of oiling were compared by contrast procedures (using BMDP 5V). To maintain the overall significance level (error rate) at 5%, the significance of contrasts was adjusted using Bonferroni's methods (α/p, where p = the number of contrasts).

7.4 Oiling and Reoiling

The amount of oil floating above reefs was visually assessed during the 3 mo following the spill (May-July 1986) by air, boat, and underwater observations (Cubit et al. 1987; Jackson et al. 1989). Oiling was ranked on the basis of the amount of oil visible at the surface as heavy, moderate, or none (see Table 7.1). Results of subsequent analyses of petroleum hydrocarbons in tissues of the corals *Siderastrea siderea* (Ellis and Solander) and *Agaricia tenuifolia* (Dana), and of reef sediments, generally corroborated the classification of reefs based on visual inspection (Burns, Chap. 3; Jackson et al. 1989; Burns and Knap 1989; Burns et al. 1991). Also, colonies of the coral *Siderastrea siderea* with recently dead areas covered with filamentous algae were carefully removed, placed inside clean plastic bags, and transported from oiled and control reefs to the Galeta Marine Laboratory, where they were placed in clean tanks with running seawater. After several hours the surface of colonies was squeezed and only colonies from Bahía Las Minas released oil, forming an oily film on the water surface (E. Weil, unpublished data).

Reef sediments and coral tissues were collected for hydrocarbon analyses during August-September 1986, December 1988-March 1989, and July 1990 at oiled and unoiled reefs where we recorded coral cover, injury, growth, and reproduction (Table 7.1). No oil slicks were observed over the reefs when samples were collected in 1988-1989 and 1990, but were always present over oiled reefs during the 1986 collection. Corals were collected by divers, sealed aboard a boat with solvent-washed aluminum foil, labeled, and packed in polyethylene bags, using care not to touch the living portion of the colonies. Reef surface sediments were collected by divers using solvent-cleaned glass jars sealed with screw-cap lids lined with foil. A complete description of sampling and analytical methods and results is given in Burns and Knap (1989) and in Burns (Chap. 3). In this chapter we correlate those results with data on recent injuries and growth of corals.

We also recorded the presence or absence of oil slicks over all reefs from August 1987 to December 1991 (Table 7.6). Heavy oil slicks continued to emerge from mangroves (Fig. 7.6) and landfill beneath the refinery, most frequently following heavy rains, but also after very high tides. The pattern of this chronic oiling was consistent with observations immediately after the oil spill (Table 7.6). Reefs heavily oiled by the spill still had oil over them 43-84% of the days we collected observations. In contrast, we observed oil at the moderately oiled reefs only 4-31% of the days visited and found no oil at unoiled reefs except for a small gasoline spill at one control site (JUG). Nevertheless, one control reef (DMA) was contaminated after the 1986 spill by considerable amounts of fuel oil (Burns, Chap. 3) not obvious to divers.

Some of the oil from these postspill slicks is almost certainly reaching the bottom adsorbed to suspended and resuspended sediments, which averaged 5 mg/L and 155 mg/cm^2/day, respectively, over reefs in Bahía Las Minas (based on measurements from 1988 to 1991). Given the large amounts of oil apparently still trapped in mangrove sediments, this chronic pollution *due to the original oil spill* is likely to last many more years. The chemical composition and toxicity of the oil has changed considerably, so chronic effects may be less than observed soon after the spill. Nevertheless, "the reservoirs of trapped oil still contained a significant fraction of low-boiling hydrocarbons, which could presumably still be toxic when leached into coastal waters" (Burns, Chap. 3; see Tables 3.5, 3.8; Fig. 3.9).

According to the Instituto de Recursos Hidráulicos y Electrificación (IRHE) and refinery personnel, during December 1988 and June 1990 an unestimated amount of diesel fuel spilled into the Bahía Las Minas area from a storage tank at the IRHE electricity generating station (Fig. 7.1, *E*), about 1 km from the refinery. Three of our study sites (LRE2, PAYW, and PAYN) may have been affected by these additional spills.

After the 1986 spill, much oil sank and became incorporated in sediments and coral tissues (Jackson et al. 1989; Burns and Knap 1989; Burns et al. 1991; Burns, Chap. 3). Initial collections for chemical analyses of hydrocarbons at heavily oiled reefs were made 4 mo after the spill, and still showed average concentrations per reef of 40 (LRE2) to 402 (GALC) μg oil/g dry wt. of sediment (range = 19-715; Table 7.7). These values are higher than reported for sediments near oil depots in the Persian Gulf (291 μg oil/g dry wt.), although lower than "hot spots" in the Gulf of Paria, Trinidad (17,148 μg oil/g dry wt.; Agard et al. 1988). Oil concentrations in reef sediments decreased by 1990, but traces of oil were still detected 4 yr after the spill (Table 7.7; Burns, Chap. 3). Oil concentrations in coral tissues in 1986 averaged

Table 7.6 Percent days when oil slicks were observed above the study reefs from August 1987 to December 1992. Numbers of observations are in parentheses. Reefs are listed from east to west within each category of oiling. *U* = unoiled; *M* = moderate; *H* = heavy.

	Percent Days With Oil Slicks[1]				
Reef	1987	1988	1989	1990	1991
Unoiled					
JUG	17 (6)	0 (18)	0 (31)	0 (41)	0 (24)
PALW	0 (7)	0 (18)	0 (30)	0 (37)	4 (26)
DMA	0 (6)	0 (16)	0 (26)	0 (28)	0 (27)
DONR	0 (6)	0 (14)	0 (26)	0 (30)	0 (26)
Moderately Oiled					
NARS	8 (12)	36 (28)	24 (38)	52 (42)	29 (31)
MAR3	0 (2)	0 (7)	0 (25)	0 (27)	4 (23)
Heavily Oiled					
PM	30 (10)	68 (19)	59 (39)	65 (63)	35 (40)
PAYN[2]	67 (12)	92 (40)	69 (45)	79 (72)	77 (44)
PAYW[2]	33 (3)	82 (11)	78 (46)	85 (65)	79 (43)
LRE1	25 (4)	100 (7)	65 (34)	71 (55)	80 (35)
LRE2[2]	22 (9)	82 (39)	55 (54)	63 (75)	67 (46)
GALC	80 (10)	81 (21)	23 (47)	21 (78)	11 (46)

[1] Oil is commonly observed every day throughout the year in different areas of Bahía Las Minas.
[2] May have been affected by diesel fuel spills in December 1988 and June 1990.

0.1 to 28.8 μg oil/mg EOM by UVF analysis, and subsequently declined to almost nondetectable levels during 1988-1989 and 1990 (Table 7.8).

The dispersant Corexit 9527 was both observed and reported to have been applied mostly offshore in May 1986, and always >2-3 km away from the heavily oiled reef at Punta Galeta. Thus, its use could not have been a major factor in the effects of oil at Punta Galeta, but may have contributed to effects observed at other oiled reefs in Bahía Las Minas (LRE1 to NARS; Fig. 7.1). The dispersant was used too late and at concentrations too low, relative to the volume of spilled oil, to be effective (Cormack 1983), and may have mixed directly into the water column soon after spraying, or accumulated on top of the floating oil (see Keller et al., Chap. 1; Cubit and Connor, Chap. 4). Corexit 9527 has been described as toxic to reef corals (Ballou et al. 1989; Thorhaug et al. 1989), or not toxic at concentrations up to 50 ppm (Knap et al. 1985), but there are no data on dispersant concentrations at any of the reefs.

While concentrations of oil in reef sediments (Table 7.7) and coral tissues (Table 7.8) generally declined between 1986 and 1990 at oiled sites, rates of settlement of resuspended sediments increased from 1988 to 1991 (Table 7.3). Oiling and sedimentation are confounded as possible factors causing deleterious effects on reef corals at oiled sites years after the spill. Some combination of oiling and

Fig. 7.6 Aerial photograph of oil slicks moving out of a partially deforested mangrove area, and coastline erosion 3 yr after the oil spill (by C. Hansen).

sedimentation, are clearly implicated, but we cannot separate them nor estimate their relative contributions, which may have paralelled their levels in reef environments.

7.5 Components of Research

Biological studies of possible effects of the oil spill included measurements of the cover of sessile organisms, injury, regeneration of lesions, growth, reproduction, and recruitment of corals.

7.5.1 Cover of Sessile Organisms

Annual surveys were made to measure the percent cover of different dominant groups of organisms (macroalgae, sponges, corals, etc.) and the cover, abundance, size, and diversity of scleractinian coral species at different depths on 12 reefs (Tables 7.1, 7.3, 7.4).

Table 7.7 Mean oil content in coral reef sediments by year. Units are μg oil/g dry wt. by UVF analysis; SD in parentheses. Samples were done in triplicate. Detailed information is in Burns (Chap. 3).

Reef	1986	1988	1990
Unoiled			
JUG		1.7 (1.8)	9.5 (8.8)
PALW	0 (0)	1.7 (0.3)	5.3 (3.4)
DONR	10 (18)	0.3 (0.1)	1.0 (0.7)
DMA[1]		43 (12)	127 (21)
Moderately Oiled			
MAR3		54 (67)	79 (30)
NARS	18 (10)	14 (2)	50 (11)
Heavily Oiled			
GALC	402 (206)	126 (62)	38 (10)
LRE1		101 (33)	37 (10)
LRE2	40 (13)	43 (14)	70 (5)
PAYW		264 (123)	105 (36)
PAYN	209 (93)	244 (137)	34 (6)
PM		15 (2)	33 (12)

[1] Contaminated by heavy fuel oil of unknown origin.

Table 7.8 Mean oil content in *Siderastrea siderea* tissues by year. Units are μg oil/mg EOM by UVF analysis. For triplicate samples (SD in parentheses) as described in Burns (Chap. 3).

Reef	1986	1988	1990
Unoiled			
JUG		0.0 (0.0)	
PALW	0.1 (0.0)	0.0 (0.0)	0.7 (0.2)
DONR	0.1 (0.1)	0.3 (0.1)	0.1 (0.0)
DMA[1]		0.5 (0.4)	
Moderately Oiled			
MAR3	0.1 (0.0)	0.4 (0.1)	0.1 (0.0)
NARS	1.2 (0.4)	0.3 (0.1)	0.1 (0.0)
Heavily Oiled			
GALC	4.3 (1.3)	0.2 (0.1)	0.1 (0.0)
LRE1		0.6 (0.5)	
LRE2	2.9 (0.3)	0.4 (0.2)	0.4 (0.0)
PAYW		0.4 (0.2)	
PAYN	28.8 (9.0)	0.4 (0.2)	0.4 (0.1)
PM	0.1 (0.7)	0.1 (0.1)	0.1 (0.0)

[1] Contaminated by heavy fuel oil of unknown origin.

7.5.1.1 Methods

Surveys of reefs for coral cover, abundance, size, and diversity were done using 1-m^2 quadrats (Guzmán et al. 1991; Weil and Jackson, unpubl. data). Six reefs within the study area were surveyed between July and October 1985, including four that were subsequently unoiled (DONR, DMA, PALW, and JUG), one that was lightly to moderately oiled (MAR3), and one that was heavily oiled (GALC; Table 7.1). These reefs were surveyed again after the spill during July and August 1986 together with three additional oiled reefs (LRE2, PAYW, and NARS). In 1988 three more heavily oiled reefs were included (PAYN, LRE1, and PM). A total of 12 reefs (four unoiled, two moderately oiled, and six heavily oiled) were surveyed from 1988 to 1991.

At each reef four or five line transects were extended perpendicularly to the shore from haphazardly chosen points at the shoreward edge to the bottom of the reef. Where a reef flat occurred the transects were extended from the seaward edge of the reef flat. Along each line 1-m^2 quadrats were placed contiguously or spaced at regular intervals up to 3 m apart, depending on the length of the transect, which varied with profile of the reef. All quadrats were placed subtidally and were not exposed, even during seasonal periods of extreme low water. The quadrats were divided by strings into one-hundred 100-cm^2 cells. Percent cover of all sessile organisms was estimated visually using these cells. Only scleractinian corals were identified to species. Quadrats from all transects on each reef were pooled into two depth intervals: 0.5-3 m (shallow) and >3-6 m (deep). The typical total number of quadrats per reef was 120; the range was 40 to 150.

Data analysis for coral cover, numbers and sizes of colonies, numbers of species, and Shannon-Wiener diversity H' (based on percent cover of corals and on number of coral colonies) per m^2 was done using single mean values for each depth interval on each reef. All percentage data were transformed using the arcsine function, which generally causes data to follow more closely assumptions of ANOVA such as normality and homogeneity of variances (e.g., Sokal and Rohlf 1981). Differences in these variables on six reefs (four unoiled, one moderately oiled, and one heavily oiled) between the 1985 and 1986 censuses were compared in order to assess immediate effects of the oil spill. These analyses were done by calculating the natural logarithm of the value for 1985 divided by the value for 1986 (ln[mean cover 1985/mean cover 1986]) and testing for the effect of oil by ANOVA with one heavily oiled, one moderately oiled, and four unoiled reefs. This is an appropriate model for examining changes over a short period (Green 1979). For species common at both depth intervals we used instead 2-way ANOVA with depth and degree of oiling as factors.

Variation in the same variables for scleractinians (cover, number and sizes of colonies, and diversity) and all other sessile organisms over all censuses (1986, 1988, 1989, 1990, and 1991) after the oil spill, and including all reefs (four unoiled, two moderately oiled, and six heavily oiled) were evaluated by repeated-measures

ANOVA with amount of oil and depth as between-factor variables and time (year of census) a within-factor variable (see summary in Table 7.5). A guide for interpreting interactions obtained by repeated-measures ANOVA and contrast analysis is given in Section 7.3.

7.5.1.2 Results

Differences between 1985 and 1986, 4 mo after the oil spill, revealed extensive effects of oil (Guzmán et al. 1991; Table 7.9; App. Table C.1). Total coral cover decreased by 76% at 0.5-3 m and by 56% between >3 and 6 m at the heavily oiled Punta Galeta reef (Fig. 7.7), and this was highly significant (2-way ANOVA; $F = 11.69$; df = 2, 8; $P = .004$; Table 7.10). The decrease was less at the moderately oiled reef, and coral cover generally increased or stayed the same at the four unoiled reefs (Fig. 7.8; Table 7.9). *Acropora palmata* was practically eliminated from the heavily oiled Punta Galeta reef (Fig. 7.9), whereas its abundance increased from 7% to 9% cover at the four unoiled reefs during the same year (1-way ANOVA; $F = 21.0$; df = 2, 3; $P = .017$). No other species showed a statistically significant reduction at the oiled reefs, despite the significant overall decline, although reduction in *Porites astreoides* was nearly significant (Table 7.10). Death of *Acropora palmata* represented about 29% of total coral mortality in shallow water (0.5-3 m). However, cover of all five other coral species with >1% cover before the spill decreased at the heavily oiled reef by 40 to 86% at both depths between 1985 and 1986, but generally increased at the control reefs over the same interval. These results clearly demonstrate a detrimental effect of oil on reef corals as indicated by the general decrease in cover of almost all coral species, and major effects on some species.

The average size of coral colonies (all species) between 1985 and 1986 also decreased significantly in relation to oiling and depth, whereas the number of colonies did not (Fig. 7.8; Tables 7.9, 7.10). This suggests that more corals suffered partial mortality, and thus decreased in size and total cover, than died outright. Similarly, diversity based on coral cover decreased on oiled reefs, whereas diversity based on number of colonies was unchanged as a function of oiling (Fig. 7.10; Table 7.10). No major reduction in the number of species was observed.

Comparable data for all 12 reefs after the spill are shown in Figures 7.11 and 7.12, and were used for repeated-measures analysis of effects of the spill from 1986 to 1991 (Tables 7.11, 7.12). The principal change in pattern by the third (1988) census was a striking decrease in coral cover, size, and diversity (cover) on unoiled reefs. Average total coral cover decreased from 28 to 13% on the four unoiled reefs at 0-3 m, and from 23 to 18% at >3-6 m, with more than half of the decrease due to marked declines of *Acropora palmata* and *Montastrea annularis* (Table 7.9; App. Table C.1). The result of the repeated-measures analysis showed amount of oiling, year, and the oil x year interaction were significant for all measured variables, whereas only number of species was significantly different between the two depths. Because cover was similar between depths, this variable was not used for contrast analyses. Contrast analyses of scleractinian cover for all possible comparisons

Table 7.9 Percent cover of common coral species by depth, level of oiling, and date of census. Common species were defined arbitrarily as those with >2% cover in at least 2 of 72 possible occurrences (12 reefs x 2 depths x first 3 censuses). Data are means (SE in parentheses). Total coral cover (scleractinians) and number of species per m^2 are shown in Figures 7.11 and 7.12, respectively. There were no replicates for moderately and heavily oiled reefs during 1985. In 1986 four unoiled, two moderately oiled, and three heavily oiled reefs were surveyed. After 1986 there were four unoiled, two moderately oiled, and six heavily oiled reefs.

Species	Depth (m)	Year	Unoiled (*N* = 4)	Moderately Oiled (*N* = 1 or 2)	Heavily Oiled (*N* = 1,3, or 6)
Acropora cervicornis	0.5-3	85	0.32 (0.32)	0.00	0.00
		86	0.47 (0.47)	0.00 (0.00)	0.00 (0.00)
		88	0.07 (0.07)	0.00 (0.00)	0.00 (0.00)
		89	0.11 (0.11)	0.00 (0.00)	0.00 (0.00)
		90	0.05 (0.05)	0.00 (0.00)	0.00 (0.00)
		91	0.31 (0.31)	0.00 (0.00)	0.00 (0.00)
	>3-6	85	0.81 (0.81)	0.00	0.00
		86	0.80 (0.80)	0.00 (0.00)	0.00 (0.00)
		88	0.18 (0.17)	0.00 (0.00)	0.00 (0.00)
		89	0.15 (0.15)	0.00 (0.00)	0.00 (0.00)
		90	0.23 (0.23)	0.00 (0.00)	0.00 (0.00)
		91	0.29 (0.29)	0.00 (0.00)	0.00 (0.00)
Acropora palmata	0.5-3	85	6.81 (1.34)	0.59	6.56
		86	9.04 (2.22)	1.02 (1.01)	0.09 (0.09)
		88	2.27 (1.24)	0.51 (0.48)	0.01 (0.01)
		89	4.55 (2.25)	0.91 (0.91)	0.27 (0.13)
		90	2.46 (1.07)	0.07 (0.02)	0.08 (0.06)
		91	1.31 (0.93)	0.04 (0.06)	0.17 (0.12)
	>3-6	85	0.97 (0.56)	0.00	0.00
		86	2.79 (0.85)	0.00 (0.00)	0.00 (0.00)
		88	0.27 (0.16)	0.00 (0.00)	0.00 (0.00)
		89	0.43 (0.32)	0.00 (0.00)	0.00 (0.00)
		90	1.43 (1.29)	0.00 (0.00)	0.00 (0.00)
		91	0.36 (0.71)	0.00 (0.00)	0.06 (0.14)
Agaricia agaricites	0.5-3	85	3.07 (0.79)	2.50	1.28
		86	3.00 (0.82)	2.22 (0.78)	0.22 (0.08)
		88	0.83 (0.31)	1.25 (0.13)	0.55 (0.15)
		89	0.78 (0.26)	1.32 (0.43)	0.86 (0.24)
		90	0.11 (0.04)	0.44 (0.28)	0.28 (0.08)
		91	0.36 (0.39)	0.40 (0.51)	0.07 (0.07)
	>3-6	85	5.79 (1.63)	7.56	5.52
		86	4.10 (1.33)	2.59 (0.19)	1.78 (0.60)
		88	2.44 (1.18)	1.59 (0.60)	1.38 (0.53)
		89	1.94 (0.30)	2.53 (0.48)	3.58 (1.11)
		90	0.23 (0.08)	1.06 (0.83)	0.36 (0.07)
		91	0.38 (0.26)	0.97 (0.06)	0.28 (0.23)

Table 7.9 Percent cover of common coral species by depth, level of oiling, and date of census (continued).

Species	Depth (m)	Year	Unoiled	Moderately Oiled	Heavily Oiled
Agaricia tenuifolia	0.5-3	85	0.11 (0.11)	0.00	0.00
		86	0.55 (0.42)	0.21 (0.17)	0.21 (0.10)
		88	0.05 (0.05)	0.32 (0.18)	0.27 (0.17)
		89	0.46 (0.19)	0.13 (0.13)	0.36 (0.12)
		90	0.85 (0.21)	1.07 (0.07)	0.57 (0.15)
		91	1.15 (1.03)	1.24 (0.07)	0.46 (0.12)
	>3-6	85	0.90 (0.85)	0.00	0.02
		86	2.12 (1.53)	1.26 (0.24)	2.67 (1.06)
		88	1.98 (1.86)	1.04 (0.78)	0.93 (0.52)
		89	1.71 (0.75)	0.52 (0.52)	0.78 (0.26)
		90	2.82 (0.93)	2.71 (2.07)	1.76 (0.40)
		91	2.03 (0.61)	2.79 (1.66)	1.71 (0.63)
Colpophylia natans	0.5-3	85	0.05 (0.05)	0.00	0.04
		86	0.11 (0.11)	1.14 (1.14)	0.02 (0.02)
		88	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)
		89	0.03 (0.03)	0.00 (0.00)	0.27 (0.21)
		90	0.00 (0.00)	0.00 (0.00)	0.01 (0.01)
		91	0.04 (0.04)	0.05 (0.05)	0.03 (0.03)
	>3-6	85	0.51 (0.26)	0.00	0.65
		86	1.01 (0.47)	2.31 (2.05)	0.30 (0.13)
		88	0.45 (0.30)	0.12 (0.12)	0.19 (0.16)
		89	1.36 (1.09)	0.15 (0.15)	0.00 (0.00)
		90	0.66 (0.31)	0.03 (0.03)	0.52 (0.28)
		91	0.13 (0.08)	0.00 (0.00)	0.49 (0.63)
Diploria clivosa	0.5-3	85	1.59 (0.72)	0.72	4.96
		86	2.30 (0.79)	2.12 (0.57)	1.03 (0.04)
		88	0.97 (0.64)	1.02 (0.99)	0.73 (0.21)
		89	0.75 (0.27)	0.29 (0.01)	0.99 (0.36)
		90	0.80 (0.47)	0.36 (0.25)	0.66 (0.33)
		91	0.53 (0.46)	0.17 (0.17)	0.17 (0.06)
	>3-6	85	0.06 (0.03)	0.00	0.00
		86	0.15 (0.04)	0.00 (0.00)	0.22 (0.22)
		88	0.12 (0.11)	0.06 (0.06)	0.02 (0.02)
		89	0.05 (0.04)	0.00 (0.00)	0.03 (0.03)
		90	0.05 (0.03)	0.02 (0.02)	0.02 (0.02)
		91	0.00 (0.00)	0.00 (0.00)	0.03 (0.02)

Table 7.9 Percent cover of common coral species by depth, level of oiling, and date of census (continued).

Species	Depth (m)	Year	Unoiled	Moderately Oiled	Heavily Oiled
Diploria strigosa	0.5-3	85	1.79 (0.64)	0.04	3.39
		86	1.08 (0.40)	0.53 (0.49)	0.27 (0.10)
		88	1.50 (0.34)	0.69 (0.69)	0.24 (0.07)
		89	1.49 (0.64)	1.20 (1.20)	0.39 (0.15)
		90	1.27 (0.25)	1.14 (1.14)	0.30 (0.11)
		91	0.47 (0.18)	0.22 (0.32)	0.24 (0.07)
	>3-6	85	0.92 (0.63)	0.00	0.50
		86	0.63 (0.30)	1.29 (0.94)	0.19 (0.11)
		88	1.09 (0.42)	0.35 (0.35)	0.31 (0.28)
		89	0.39 (0.11)	0.66 (0.66)	0.34 (0.26)
		90	0.24 (0.10)	0.06 (0.06)	0.08 (0.07)
		91	0.19 (0.10)	0.00 (0.00)	0.19 (0.10)
Montastrea annularis	0.5-3	85	2.44 (1.77)	0.00	0.00
		86	3.33 (2.43)	0.41 (0.41)	0.01 (0.01)
		88	0.93 (0.80)	0.16 (0.16)	0.02 (0.01)
		89	1.12 (0.89)	0.13 (0.13)	0.38 (0.30)
		90	1.44 (1.29)	0.00 (0.00)	0.36 (0.24)
		91	0.41 (0.25)	0.00 (0.00)	0.24 (0.19)
	>3-6	85	4.24 (2.94)	0.00	0.01
		86	3.54 (1.62)	1.37 (1.37)	0.00 (0.00)
		88	0.71 (0.41)	0.13 (0.13)	1.52 (1.23)
		89	2.46 (2.26)	0.19 (0.19)	0.45 (0.26)
		90	1.50 (1.24)	0.07 (0.07)	0.32 (0.32)
		91	2.68 (1.62)	0.00 (0.00)	0.60 (0.40)
Porites astreoides	0.5-3	85	2.30 (0.82)	3.45	3.96
		86	2.49 (0.37)	2.02 (0.69)	1.39 (0.30)
		88	1.68 (0.26)	1.40 (0.39)	1.39 (0.16)
		89	1.61 (0.13)	1.64 (0.11)	1.69 (0.12)
		90	1.00 (0.21)	1.49 (0.30)	1.27 (0.10)
		91	1.19 (0.15)	1.79 (0.36)	1.16 (0.17)
	>3-6	85	1.49 (0.34)	1.35	3.09
		86	1.47 (0.17)	0.79 (0.02)	1.75 (1.00)
		88	1.25 (0.59)	0.23 (0.19)	0.52 (0.11)
		89	1.22 (0.31)	0.64 (0.37)	0.78 (0.16)
		90	0.98 (0.24)	0.74 (0.40)	0.75 (0.19)
		91	0.74 (0.15)	1.04 (0.07)	0.68 (0.21)

Table 7.9 Percent cover of common coral species by depth, level of oiling, and date of census (continued).

Species	Depth (m)	Year	Unoiled	Moderately Oiled	Heavily Oiled
Porites furcata	0.5-3	85	0.11 (0.06)	2.27	0.11
		86	0.18 (0.08)	1.32 (0.28)	0.80 (0.66)
		88	0.10 (0.04)	0.79 (0.13)	0.38 (0.08)
		89	0.05 (0.03)	0.54 (0.43)	0.20 (0.06)
		90	0.04 (0.02)	1.06 (0.72)	0.14 (0.04)
		91	0.07 (0.03)	0.45 (0.38)	0.10 (0.03)
	>3-6	85	0.13 (0.05)	7.28	4.30
		86	0.24 (0.14)	1.54 (0.82)	1.27 (0.49)
		88	0.23 (0.05)	1.28 (1.08)	0.33 (0.17)
		89	0.08 (0.04)	0.70 (0.52)	0.39 (0.11)
		90	0.04 (0.03)	0.77 (0.45)	0.19 (0.06)
		91	0.10 (0.05)	0.34 (0.33)	0.23 (0.07)
Porites porites	0.5-3	85	0.24 (0.11)	2.67	0.27
		86	0.80 (0.33)	1.05 (0.11)	0.22 (0.15)
		88	0.02 (0.02)	0.00 (0.00)	0.00 (0.00)
		89	0.05 (0.02)	0.02 (0.02)	0.04 (0.02)
		90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		91	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	>3-6	85	0.66 (0.60)	0.10	0.13
		86	0.42 (0.30)	0.00 (0.00)	0.07 (0.07)
		88	0.02 (0.02)	0.03 (0.03)	0.00 (0.00)
		89	0.13 (0.07)	0.01 (0.01)	0.01 (0.01)
		90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		91	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Siderastrea siderea	0.5-3	85	1.96 (0.51)	4.68	1.62
		86	2.96 (0.92)	5.65 (1.03)	4.11 (2.28)
		88	3.08 (0.41)	5.01 (3.43)	3.91 (1.16)
		89	1.17 (0.49)	7.81 (4.40)	4.65 (0.96)
		90	2.68 (0.87)	4.77 (4.34)	3.39 (0.77)
		91	2.00 (0.87)	4.79 (2.54)	3.07 (0.63)
	>3-6	85	4.64 (1.20)	6.81	7.04
		86	4.06 (1.08)	9.57 (0.84)	5.00 (1.80)
		88	4.32 (1.57)	4.30 (0.26)	2.28 (0.83)
		89	2.49 (0.59)	3.76 (1.07)	2.94 (0.55)
		90	1.97 (0.90)	5.37 (0.98)	2.78 (0.60)
		91	1.17 (0.53)	3.69 (0.50)	2.10 (0.53)

Table 7.9 Percent cover of common coral species by depth, level of oiling, and date of census (continued).

Species	Depth (m)	Year	Unoiled	Moderately Oiled	Heavily Oiled
Total Coral Cover	0.5-3	85	21.96 (3.24)	18.46	22.78
		86	27.79 (4.70)	18.91 (4.37)	8.83 (1.99)
		88	12.18 (0.92)	11.86 (4.66)	7.94 (1.10)
		89	12.82 (2.98)	14.28 (6.15)	10.36 (1.43)
		90	11.42 (1.05)	10.52 (4.86)	7.44 (1.31)
		91	8.21 (1.60)	9.37 (2.62)	5.77 (0.73)
	>3-6	85	23.12 (4.92)	26.26	28.00
		86	23.95 (1.83)	23.52 (1.40)	13.68 (3.26)
		88	15.28 (2.26)	11.26 (3.15)	8.54 (2.13)
		89	14.12 (2.44)	11.53 (2.50)	10.00 (1.01)
		90	10.88 (2.84)	11.67 (0.39)	7.36 (1.04)
		91	8.84 (2.34)	9.31 (1.68)	6.65 (1.30)
Number of coral species per m^2	0.5-3	85	3.77 (0.35)	3.79	2.61
		86	4.05 (0.11)	4.05 (0.30)	2.56 (0.43)
		88	2.54 (0.09)	3.03 (0.12)	2.66 (0.16)
		89	3.02 (0.08)	3.79 (0.34)	3.16 (0.10)
		90	2.16 (0.14)	2.66 (0.05)	2.34 (0.19)
		91	1.96 (0.13)	2.58 (0.18)	1.42 (0.15)
	>3-6	85	3.63 (0.23)	3.92	3.91
		86	3.99 (0.13)	4.55 (0.31)	3.29 (0.17)
		88	2.89 (0.36)	2.81 (0.53)	2.57 (0.35)
		89	3.26 (0.21)	3.71 (0.15)	3.18 (0.20)
		90	2.07 (0.21)	2.80 (0.38)	2.02 (0.15)
		91	1.60 (0.13)	2.20 (0.29)	1.47 (0.16)

(1986-1988, 1986-1989, 1986-1990, 1986-1991, 1988-1989, 1988-1990, 1988-1991, 1989-1990, 1989-1991, and 1990-1991) were significant for level of oiling. Year and the oil x year interaction for coral cover were only significant for the first four comparisons, 1986 versus all other censuses, indicating that 1986 was different from the other years. These results indicate that despite decreases in coral cover throughout the entire region, the differences between oiled and unoiled reefs still persist, and coral cover has not changed significantly after the 1988 regional mortality.

Analysis of numbers of coral colonies (Fig. 7.11; Tables 7.11, 7.12) showed a near doubling and subsequent decline on oiled reefs, in contrast to a smaller decrease on unoiled reefs. The observed increase in 1989 followed by a decrease at oiled reefs was significant, as indicated by the oiling x year interaction. Contrast analyses for all comparisons were significant for level of oiling, whereas the oil x year

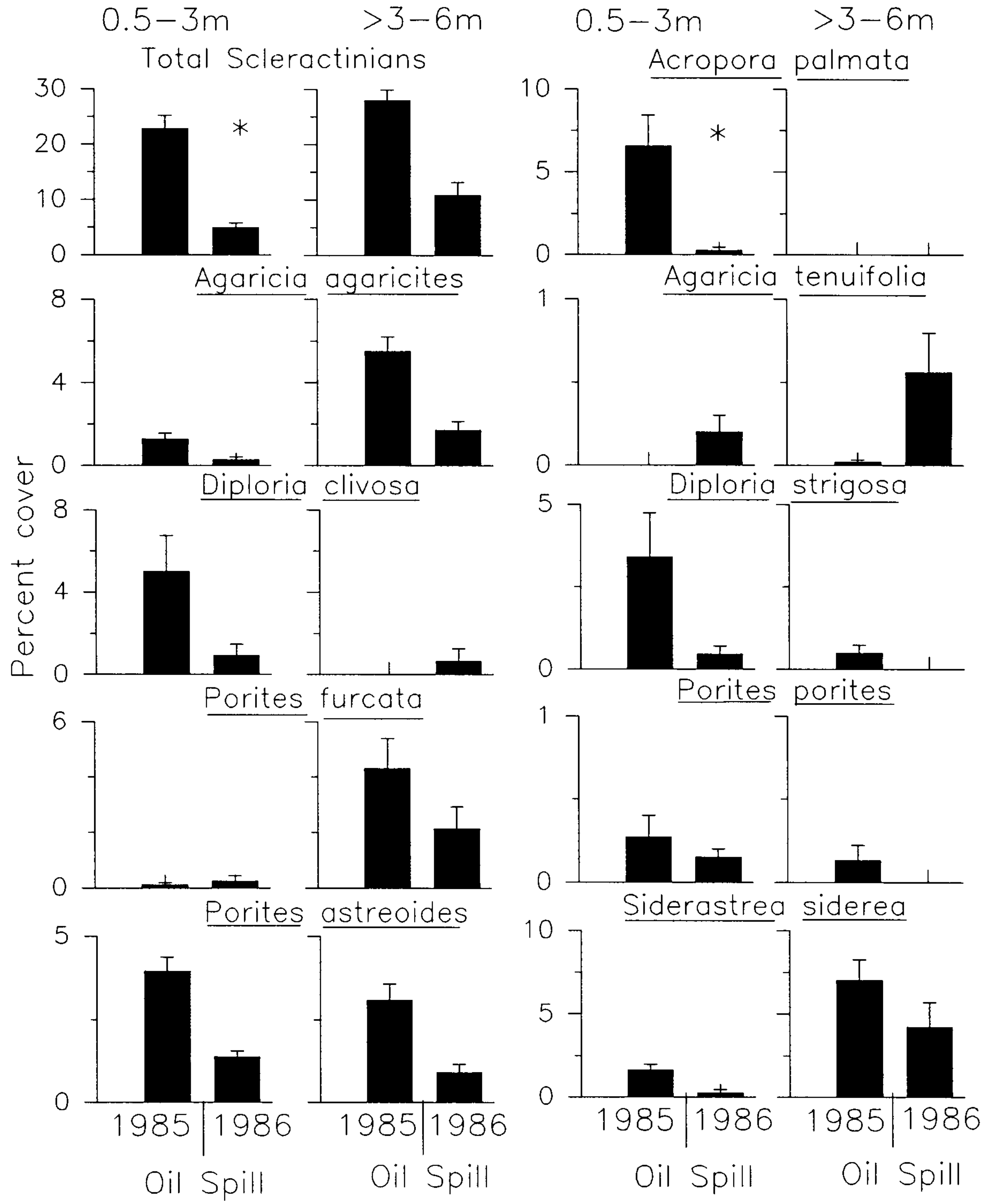


Fig. 7.7 Before (1985) and after (1986) comparison of mean coral cover (per m^2) of total scleractinians and the most important species at the Galeta Marine Laboratory (GALC), a heavily oiled reef. Error bars are 1 SE. Significant changes are marked with an asterisk; the change in *P. astreoides* was nearly significant (Table 7.10).

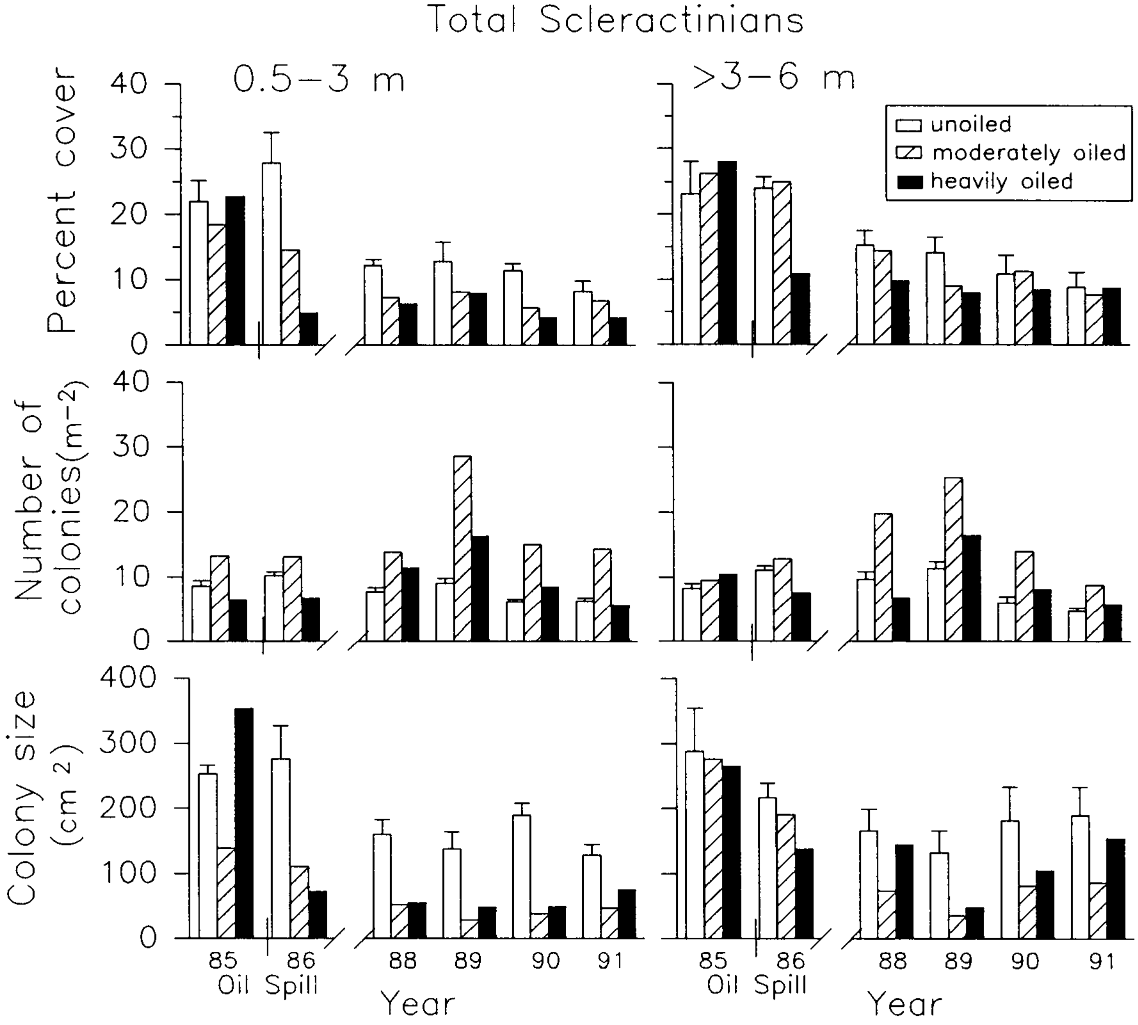


Fig. 7.8 Changes through time in mean number and size of coral colonies and percent cover of all coral species combined in relation to the degree of oiling from the 1986 spill and depth for four unoiled, one moderately oiled, and one heavily oiled reef for all years. The time of the oil spill is indicated by the *vertical line*. SE is shown for the four unoiled reefs, except when smaller than the symbols used.

interaction indicated 1988 and 1989 were different from the other years. Colony size showed the opposite pattern (decline, then increase) on oiled reefs, with the factors *oil* and *year* and the oil x year interaction all significant (Table 7.11). The increase between 1989 and 1990 was the only significant contrast (Table 7.12). These two patterns were due to partial mortality and survival of small parts of colonies on oiled reefs, followed by mortality of smaller survivors in subsequent years.

The number of coral species varied somewhat on oiled reefs, and unoiled reefs showed a significant reduction in species/m^2 after 1986 (Fig. 7.12; Tables 7.11, 7.12). The oil x year interactions were not significant for any comparison except marginally

Fig. 7.9 Elkhorn coral (*Acropora palmata*) after the 1986 oil spill at Galeta reef (*top*) and in 1974, 12 yr before the spill (*bottom*, courtesy of D. Meyer).

Table 7.10 Two-way ANOVAs for before-after comparison using ln(mean cover 1985/mean cover 1986), with depth and level of oiling as factors, for total scleractinians (only species common at both depth intervals) and *Porites astreoides*.

Variable	Factor	*F*	df	*P*
Total Coral Cover	Oil	11.69	2, 8	.004
	Depth	0.02	1, 8	.9
Number of Colonies	Oil	1.34	2, 8	.3
	Depth	0.19	1, 8	.7
Size of Colonies	Oil	5.91	2, 8	.03
	Depth	0.03	1, 8	.9
Number of Species	Oil	1.75	2, 8	.3
	Depth	0.45	1, 8	.5
Diversity H' (cover)	Oil	19.71	2, 8	.001
	Depth	0.23	1, 8	.6
Diversity H' (colonies)	Oil	2.46	2, 8	.1
	Depth	0.15	1, 8	.7
Porites astreoides	Oil	4.14	2, 8	.058
	Depth	0.04	1, 8	.8

for 1986-1988; oiling was significant for all comparisons except 1988-1991 and 1989-1991, and years were not different for four of the 10 comparisons (1986-1989, 1986-1990, 1988-1989, and 1989-1990). Differences in coral diversity (based on cover and number of colonies) persisted between oiled and unoiled reefs for the 10 comparisons. The factor *year* and the year x oiling interaction showed that 1986 differed from all other years. In short, diversity was higher at unoiled reefs than at oiled reefs during the study, and decreased and leveled for all reefs after 1986.

Differences between 1985 and 1986 did not reveal extensive effects of oil in other groups of sessile organisms (Fig. 7.13; Table 7.13; App. Table C.2). Macroalgal cover did not change immediately in response to the oil spill, although an increase in crustose coralline algae was nearly significant on the oiled reefs at both depths. Other sessile animals behaved differently in response to the oil spill; cover of gorgonians and sponges decreased significantly, whereas those of the hydrocoral *Millepora* spp. and zoanthids were unchanged. The increase of crustose coralline algae from 1986 to 1990 on the heavily oiled reef, and from 1988 to 1990 on the unoiled reefs, were correlated with decreases in coral cover, whereas the general reduction in crustose coralline algae during 1991 on all reefs was correlated with a general increase in cover of fleshy macroalgae. The most likely explanation for this

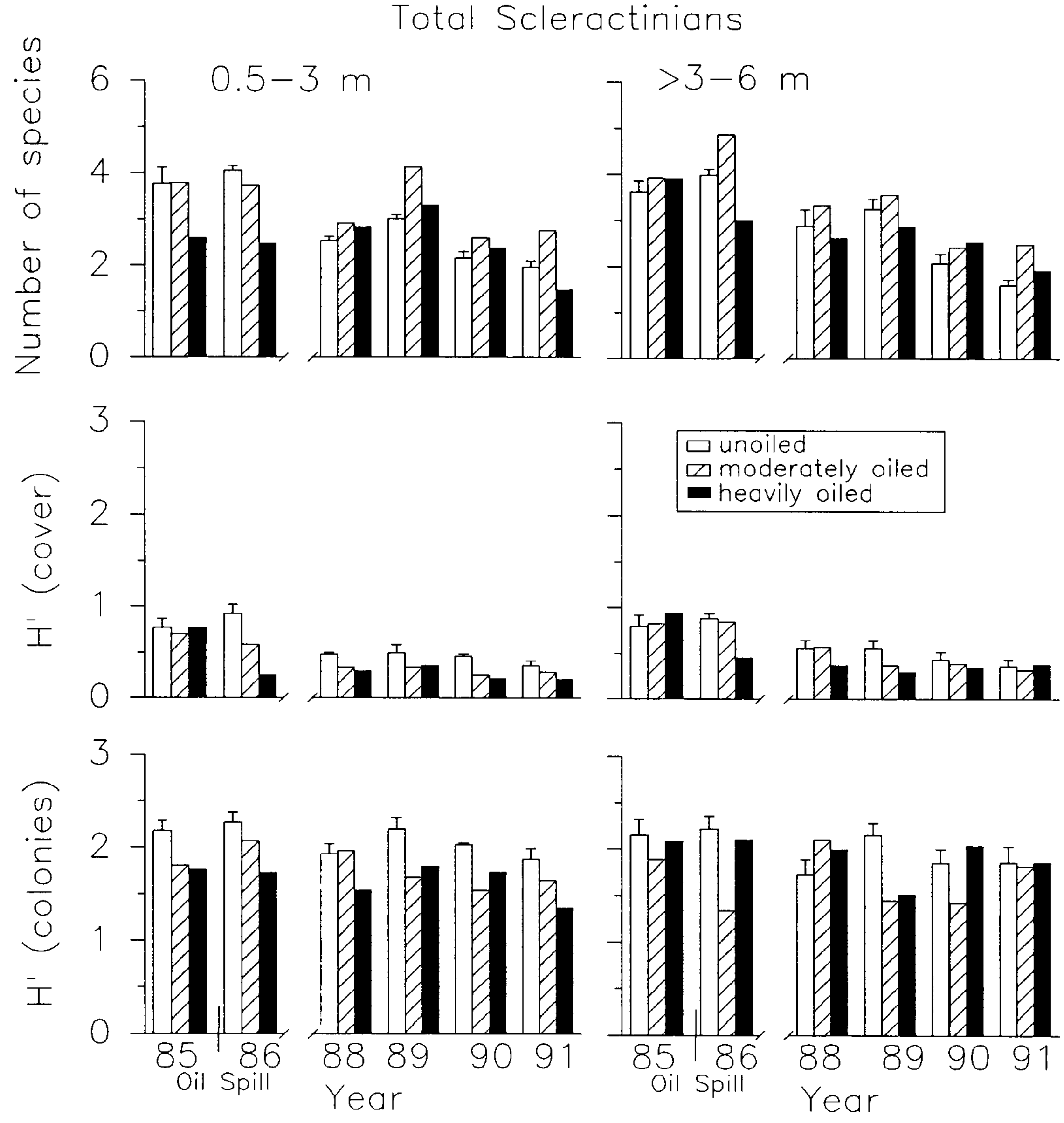


Fig. 7.10 Changes through time in average numbers of coral species per m^2 and Shannon-Wiener diversity per m^2 in relation to the degree of oiling and depth for four unoiled, one moderately oiled, and one heavily oiled reef for all years. The time of the oil spill is indicated by the *vertical line*. SE is shown for the four unoiled reefs, except when smaller than the symbols used.

switch in algal dominance is the destruction on reef framework by bioeroders, which has created new bare space for fast-growing fleshy algae. At the heavily oiled Punta Galeta reef, sponges were reduced by half their cover after the spill in 1986, but soon recovered.

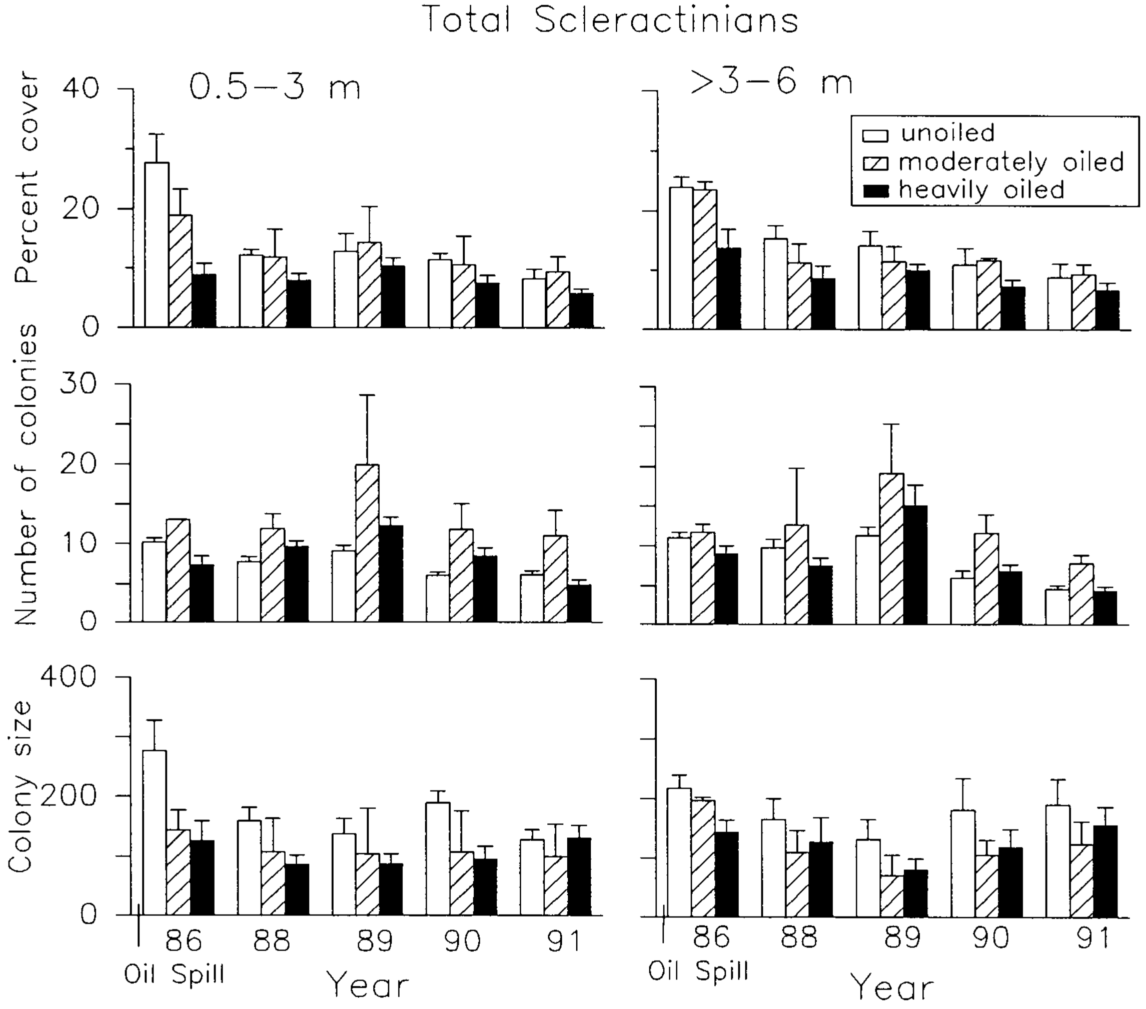


Fig. 7.11 Changes through time in mean number and size of coral colonies and the percent cover of all coral species combined in relation to the degree of oiling from the 1986 spill and depth. The time of the oil spill is indicated by the *vertical line*. SE is shown for the four unoiled reefs, except when smaller than the symbols used. In 1986 four unoiled, two moderately oiled, and three heavily oiled reefs were surveyed; after 1986 the number of heavily oiled reefs was increased to six.

Similar patterns were observed for all 12 reefs (Fig. 7.14; App. Table C.2). Cover of macroalgae varied greatly over time. Repeated-measures ANOVA showed that the factor *year* and the oil x year interaction were significant for all sessile organisms, whereas level of oiling and depth were not (Tables 7.14, 7.15). In general, macroalgal cover increased after 1986 at all reefs and both depths. This effect was greater but not significantly different at moderately and heavily oiled reefs. Crustose coralline algae showed an opposite pattern of increase, then decrease. Contrast analysis indicated that cover of crustose coralline algae for all subsequent years was

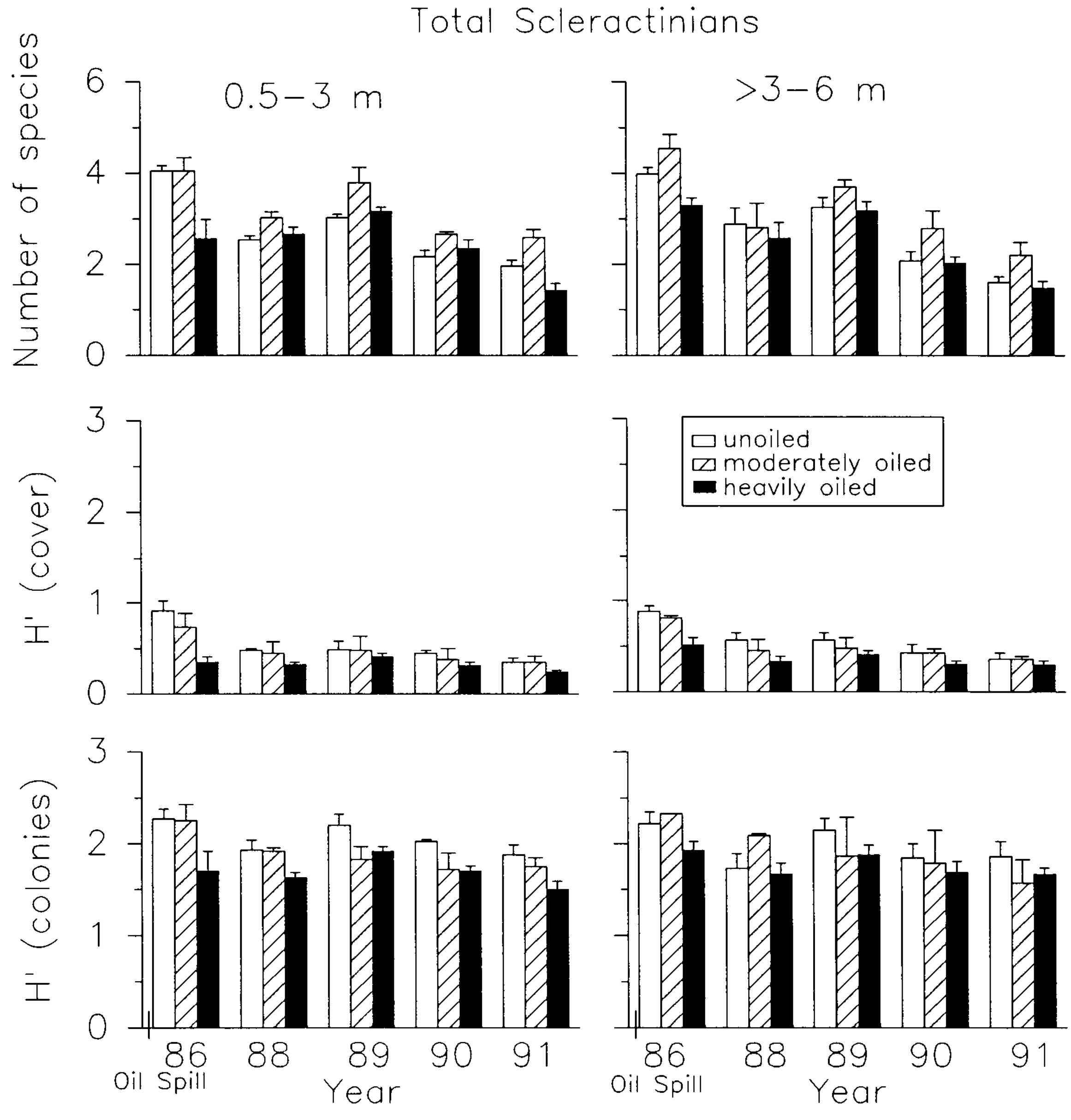


Fig. 7.12 Changes through time in average numbers of coral species per m^2 and Shannon-Wiener diversity per m^2 in relation to the degree of oiling and depth. The time of the oil spill is indicated by the *vertical line*. SE is shown for the four unoiled reefs, except when smaller than the symbols used. In 1986 four unoiled, two moderately oiled, and three heavily oiled reefs were surveyed; after 1986 the number of heavily oiled reefs was increased to six.

Table 7.11 Repeated-measures analysis of variance of percent cover per m^2 for scleractinian corals, and for Shannon-Wiener diversity (H'), number of coral species, number of coral colonies, and size of colonies per m^2. See Methods (Sect. 7.5.1) for explanation.

Variable	Factor	X^2	df	P
Cover	Oil	17.17	2	.000
	Depth	0.19	1	.7
	Year	206.32	4	.000
	Oil x Year	32.94	8	.000
	Oil x Depth	0.10	2	.9
	Depth x Year	2.03	4	.7
	Oil x Depth x Year	12.62	8	.1
Number of Colonies	Oil	16.65	2	.000
	Depth	0.19	1	.7
	Year	427.88	4	.000
	Oil x Year	52.38	8	.000
	Oil x Depth	0.44	2	.8
	Depth x Year	32.57	4	.000
	Oil x Depth x Year	18.77	8	.02
Size of Colonies	Oil	8.57	2	.01
	Depth	0.23	1	.6
	Year	149.62	4	.000
	Oil x Year	25.79	8	.001
	Oil x Depth	0.31	2	.9
	Depth x Year	8.63	4	.07
	Oil x Depth x Year	19.22	8	.01
Number of Species	Oil	7.41	2	.03
	Depth	44.04	1	.000
	Year	90.90	4	.000
	Oil x Year	21.68	8	.006
	Oil x Depth	8.07	2	.02
	Depth x Year	28.05	4	.000
	Oil x Depth x Year	26.79	8	.001
Diversity (cover)	Oil	23.45	2	.000
	Depth	0.75	1	.4
	Year	284.37	4	.000
	Oil x Year	62.22	8	.000
	Oil x Depth	0.08	2	.9
	Depth x Year	1.18	4	.9
	Oil x Depth x Year	9.04	8	.3
Diversity (colonies)	Oil	18.15	2	.000
	Depth	0.00	1	.9
	Year	74.31	4	.000
	Oil x Year	20.43	8	.009
	Oil x Depth	1.40	2	.5
	Depth x Year	1.78	4	.8
	Oil x Depth x Year	6.04	8	.6

Table 7.12 Contrast analysis (10 possible comparisons) from 1986 to 1991 of percent cover for scleractinian corals, and for Shannon-Wiener diversity (H'), number of coral species, number of coral colonies, and size of colonies. See Methods (Sect. 7.5.1) for explanation. Significance level is $P < .005$ based on Bonferroni's methods (e.g., Milliken and Johnson 1984).

Variable	Factor	86-88	86-89	86-90	86-91	88-89	88-90	88-91	89-90	89-91	90-91
Cover	Oil	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	Year	.000	.000	.000	.000	.5	.4	.03	.1	.003	.2
	Oil x Year	.05	.001	.007	.000	.7	.8	.5	.9	.9	.9
Number of Colonies	Oil	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	Year	.001	.010	.02	.000	.000	.9	.000	.000	.000	.000
	Oil x Year	.004	.000	.000	.5	.8	.000	.02	.006	.004	.000
Size of Colonies	Oil	.3	.05	.2	.1	.04	.1	.6	.001	.1	.06
	Year	.000	.000	.7	.004	.000	.01	.03	.000	.000	.3
	Oil x Year	.3	.02	.08	.003	.04	.04	.5	.001	.4	.008
Number of Species	Oil	.000	.000	.000	.001	.000	.000	.2	.000	.011	.005
	Year	.000	.6	.7	.000	.02	.001	.000	.4	.000	.000
	Oil x Year	.03	.7	.1	.07	.6	.04	.2	.1	.4	.06
Diversity (cover)	Oil	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	Year	.000	.000	.000	.000	.5	.4	.07	.1	.01	.4
	Oil x Year	.010	.000	.000	.000	.7	.8	.5	.9	.9	.9
Diversity (colonies)	Oil	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	Year	.000	.2	.000	.000	.04	.7	.1	.02	.000	.3
	Oil x Year	.6	.009	.07	.1	.05	.3	.2	.8	.8	.9

different from 1986. Cover of sessile animals varied greatly over time. Repeated-measures ANOVA showed that the factor *year* and the oil x year interaction were significant for all taxa, whereas level of oiling was only marginally significant for gorgonians and zoanthids.

7.5.2 Recent Injury of Corals (Partial Mortality)

Patches of tissue of colonial animals such as corals are frequently injured or harmed by natural enemies or physical processes (Fig. 7.15; Loya 1976; Bak et al. 1977; Jackson and Palumbi 1979; Bak and Steward-Van Es 1980; Palumbi and Jackson 1982, 1983; Jackson 1983; Wahle 1983; Guzmán 1986; Guzmán and Cortés 1989*a*). Such injuries expose the white skeleton of coral colonies, which, after a few weeks, is overgrown by algae and other organisms (see references cited above). Eventually the coral may regenerate the injury by overgrowing these invaders, or the lesion may persist or expand.

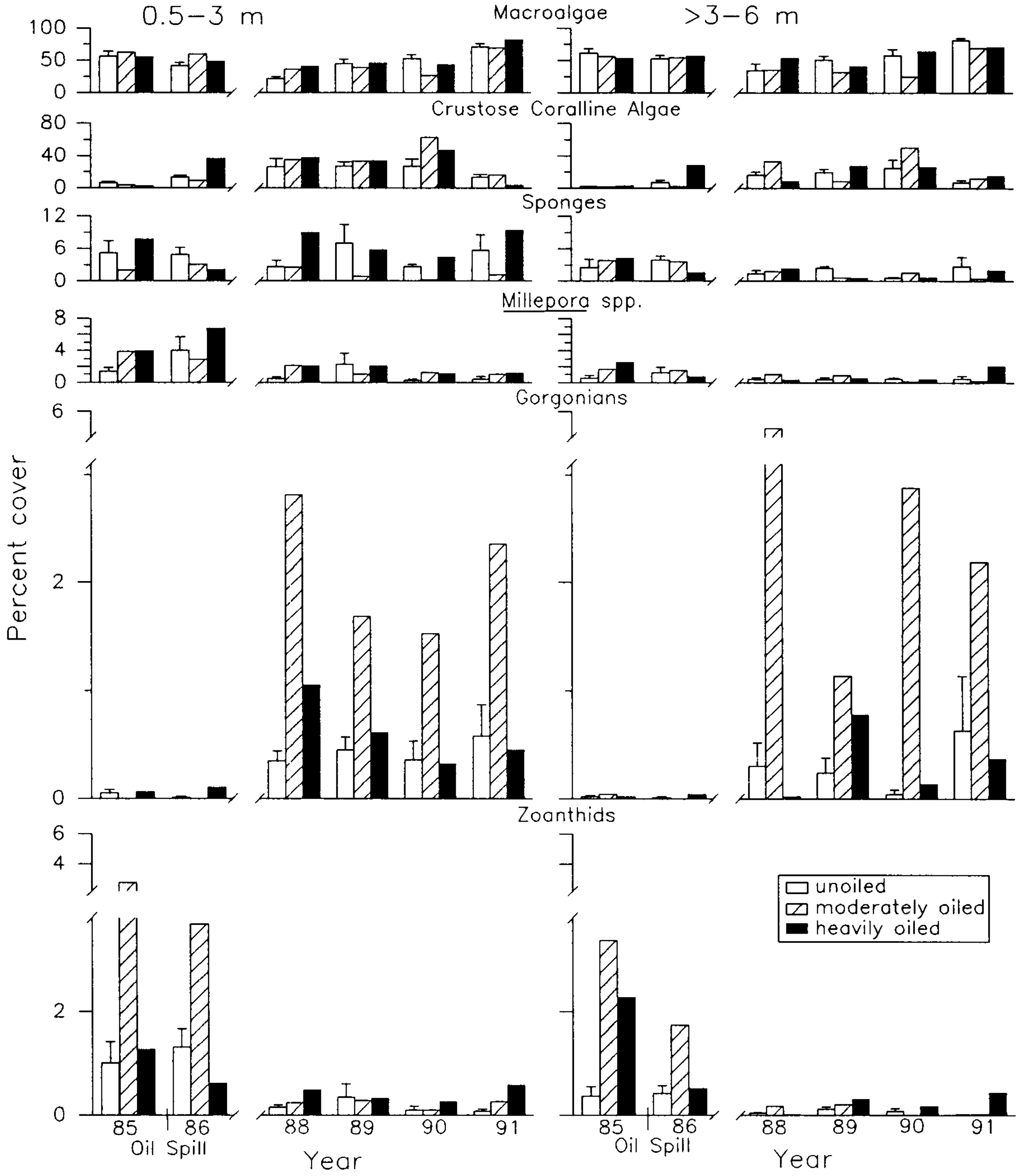


Fig. 7.13 Changes through time in mean percent cover per m^2 of all major sessile organisms in relation to the degree of oiling from the 1986 spill and depth for four unoiled, one moderately oiled, and one heavily oiled reef for all years. The time of the oil spill is indicated by the *vertical line*. SE is shown for the four unoiled reefs except when smaller than the symbols used.

Table 7.13 Two-way ANOVAs for before-after comparisons using ln(mean cover 1985/mean cover 1986), with depth and level of oiling as factors, for sessile organisms.

Taxon	Factor	F	df	P
Macroalgae	Oil	0.43	2, 8	.7
	Depth	0.45	1, 8	.5
Crustose	Oil	3.25	2, 8	.08
Coralline Algae	Depth	4.86	1, 8	.04
Sponges	Oil	5.99	2, 6	.04
	Depth	0.88	1, 6	.4
Millepora spp.	Oil	1.90	2, 8	.2
	Depth	1.22	1, 8	.3
Gorgonians	Oil	273.28	1, 1	.04
	Depth	1.62	1, 1	.4
Zoanthids	Oil	1.13	2, 7	.4
	Depth	0.75	1, 7	.4

7.5.2.1 Methods

Quarterly surveys were made on 12 reefs (Table 7.1) for *Siderastrea siderea*, *Diploria strigosa*, *D. clivosa*, and *Porites astreoides* from August 1986 to March 1991. The proportion of corals showing recent injury was estimated from the percentage of recently bare (white) colony surface (Fig. 7.15), and the apparent causes of mortality (physical or biological) recorded when possible. It is important to note, however, that the validity of censusing injuries is not dependent on knowing the causes of injuries observed.

At each census two haphazardly placed 50-m transects were run on each reef parallel to the reef crest, one at 0.5-1 m water depth and the other between >1 and 2 m. The two transects were a minimum of 10 m apart, depending on the horizontal profile of the reef. All colonies of the four species in a 1-m band to either side of the transect were recorded for a total area of 100 m^2 per transect. The sizes of corals were estimated in five size classes (1-100 cm^2, 100-200 cm^2, 200-400 cm^2, 400-800 cm^2, and >800 cm^2) and the proportion of recent injury estimated visually (none, <10% injury, and >10%).

Temporal variations in injury were analyzed by repeated-measures ANOVA with amount of oil and depth as between-factor variables and time of census as the within-factor variable. The variable time was composed of five 1-yr periods, every 3-4 censuses (i.e., I = Aug. 1986 to Apr. 1987, II = Aug. 1987 to May 1988,

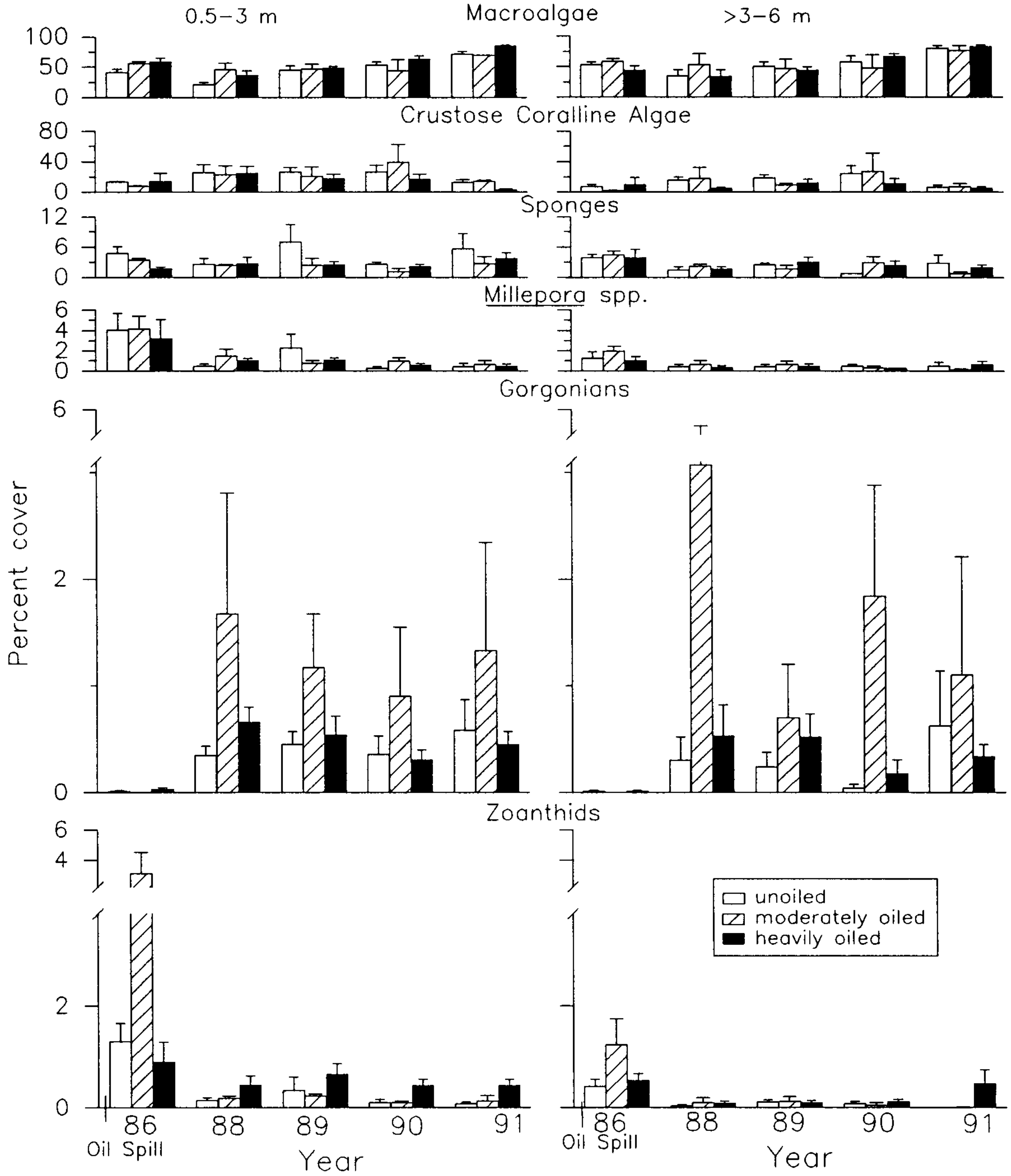


Fig. 7.14 Changes through time in mean percent cover per m^2 of all major sessile organisms in relation to the degree of oiling from the 1986 spill and depth. The time of the oil spill is indicated by the *vertical line*. SE is shown for the four unoiled reefs except when smaller than the symbols used. In 1986 four unoiled, two moderately oiled, and three heavily oiled reefs were surveyed; after 1986 the number of heavily oiled reefs was increased to six.

Table 7.14 Repeated-measures analysis of variance of percent cover for all major reef sessile organisms. *Macroalgae* includes fleshy and calcareous forms. See Methods (Sect. 7.5.1) for explanation.

Taxon	Factor	X^2	df	P
Macroalgae	Oil	2.30	2	.3
	Depth	0.5	1	.5
	Year	196.62	4	.000
	Oil x Year	23.86	8	.002
	Oil x Depth	5.33	2	.07
	Depth x Year	2.76	4	.6
	Oil x Depth x Year	7.14	8	.5
Crustose Coralline Algae	Oil	3.59	2	.2
	Depth	4.33	1	.04
	Year	41.47	4	.000
	Oil x Year	24.53	8	.002
	Oil x Depth	0.09	2	.9
	Depth x Year	2.45	4	.7
	Oil x Depth x Year	7.46	8	.5
Sponges	Oil	1.14	2	.6
	Depth	1.14	1	.3
	Year	52.11	4	.000
	Oil x Year	37.84	8	.000
	Oil x Depth	2.50	2	.3
	Depth x Year	21.24	4	.000
	Oil x Depth x Year	10.61	8	.2
Millepora spp.	Oil	1.18	2	.6
	Depth	7.89	1	.005
	Year	40.46	4	.000
	Oil x Year	18.62	8	.02
	Oil x Depth	0.09	2	.9
	Depth x Year	6.44	4	.2
	Oil x Depth x Year	20.21	8	.01
Gorgonians	Oil	8.27	2	.02
	Depth	0.61	1	.4
	Year	99.22	4	.000
	Oil x Year	38.40	8	.000
	Oil x Depth	0.47	2	.8
	Depth x Year	4.99	4	.3
	Oil x Depth x Year	22.49	8	.004
Zoanthids	Oil	7.87	2	.02
	Depth	23.51	1	.000
	Year	224.91	4	.000
	Oil x Year	81.41	8	.000
	Oil x Depth	1.49	2	.5
	Depth x Year	24.75	4	.000
	Oil x Depth x Year	39.93	8	.000

Table 7.15 Contrast analysis (10 possible comparisons) from 1986 to 1991 of percent cover for sessile organisms. See Methods (Sect. 7.5.1) for explanation. Significance level is $P < .005$ based on Bonferroni's methods.

Variable	Factor	86-88	86-89	86-90	86-91	88-89	88-90	88-91	89-90	89-91	90-91
Macroalgae	Oil	.05	.02	.01	.6	.01	.04	.3	.006	.1	.08
	Year	.9	.1	.3	.000	.03	.02	.000	.002	.000	.000
	Oil x Year	.9	.05	.2	.02	.05	.2	.1	.09	.1	.8
Crustose Coralline Algae	Oil	.02	.01	.001	.5	.000	.000	.000	.000	.000	.04
	Year	.000	.000	.000	.2	.2	.6	.000	.07	.000	.006
	Oil x Year	.001	.02	.000	.000	.2	.5	.8	.3	.2	.3
Sponges	Oil	.07	.7	.1	.8	.3	.01	.3	.03	.04	.3
	Year	.000	.000	.000	.000	.03	.09	.8	.003	.2	.06
	Oil x Year	.003	.001	.000	.000	.02	.03	.01	.04	.2	.08
Millepora spp.	Oil	.2	.05	.3	.1	.2	.02	.4	.9	.04	.4
	Year	.2	.2	.004	.000	.9	.6	.05	.6	.006	.2
	Oil x Year	.05	.003	.9	.001	.005	.04	.002	.06	.2	.08
Gorgonians	Oil	.003	.7	.03	.7	.8	.4	.6	.2	.5	.4
	Year	.000	.000	.000	.000	.2	.9	.1	.4	.002	.1
	Oil x Year	.000	.4	.000	.3	.2	.08	.05	.002	.5	.000
Zoanthids	Oil	.02	.02	.000	.000	.03	.001	.002	.01	.008	.000
	Year	.000	.000	.000	.000	.04	.004	.000	.06	.000	.000
	Oil x Year	.000	.000	.000	.000	.000	.001	.001	.8	.000	.000

III = Aug. 1988 to Jun. 1989, IV = Sep. 1989 to Jun. 1990, and V = Sep. 1990 to Mar. 1991).

7.5.2.2 Results

Both the frequency and size of recent injuries increased substantially during 1986 with the amount of oiling, particularly at the shallower depth (Fig. 7.16). *Siderastrea siderea* was affected more than *Diploria clivosa* and *Porites astreoides*. There were too few data for *D. strigosa* to merit analysis, but the trend suggested an oil effect. Some decrease in recent injuries was observed for *S. siderea* during November 1987, November 1988, and March 1989; November 1987, August 1988, and March 1989 for *P. astreoides*; and during August 1986, November 1988, June 1989, June-December 1990, and March 1991 for *D. clivosa* (Fig. 7.16).

Variation through time in total recent injury indicates colonies were most affected at heavily and moderately oiled sites during the first year after the spill, and then again beginning in June 1989 until the final census in March 1991 (Figs. 7.17,

Fig. 7.15 **A *Siderastrea siderea* colony showing recent injury at Punta Muerto reef (*PM*; Fig. 7.1).**

7.18). This second period of high levels of injuries corresponded to spills of diesel fuel in Bahía Las Minas (Sect. 7.4). Repeated-measures ANOVAs incorporating oil and depth as factors showed significant effects of the factors *oil* and *year* and the oil x year interaction (groups of years as indicated in Figs. 7.17, 7.18) for each of the three coral species (Table 7.16). Injuries were similar at both depths for all species. Contrast analyses between defined time periods (I to V in Figs. 7.17, 7.18) showed a significant oil effect for all eight possible comparisons for the three coral species. Year and the oil x year interaction were significant only for I-II, I-V, II-IV, and III-IV for *P. astreoides*, and I-IV, I-V, II-IV, II-V, and III-V for *S. siderea*. These contrasts clearly indicate that the percentage of injured colonies (*Siderastrea* and *Porites*) became stabilized between August 1987 and June 1989, although injuries were higher at oiled reefs than unoiled reefs. However, injuries for *D. clivosa* were significantly greater on oiled reefs at all times, and the oil x year interaction was significant in all cases except II-V. Thus, the numbers of injured *Diploria* varied throughout the study period, and only the periods August 1987-May 1988 and September 1990-March 1991 were similar.

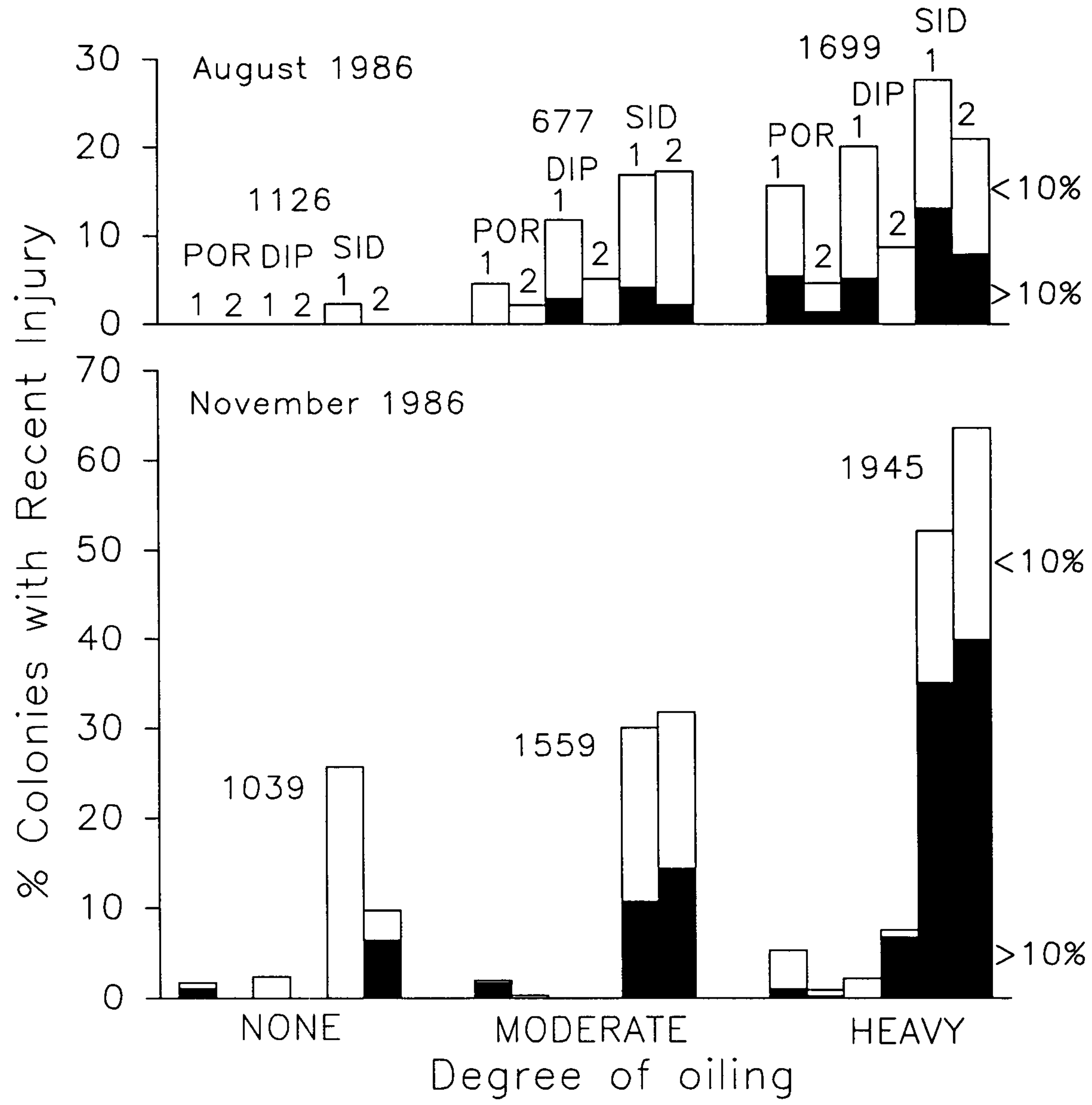


Fig. 7.16 Frequency of recent injury for three species of massive corals by depth, and in relation to the degree of oiling at 12 reefs (four unoiled, two moderately oiled, and six heavily oiled). *Open bars* represent coral colonies with injuries that did not exceed 10% of the surface area of the coral; *solid bars* represent corals with injuries >10% (maximum observed = 100% injury = colony death). Coral species and depths are indicated by: *POR* = *Porites astreoides*, *DIP* = *Diploria clivosa*, *SID* = *Siderastrea siderea*, *1* = 0.5-1 m, *2* = >1-2 m depth. Total numbers of corals surveyed at each census for each level of oiling (all species combined) are indicated by the numbers above the histograms. August and November 1986.

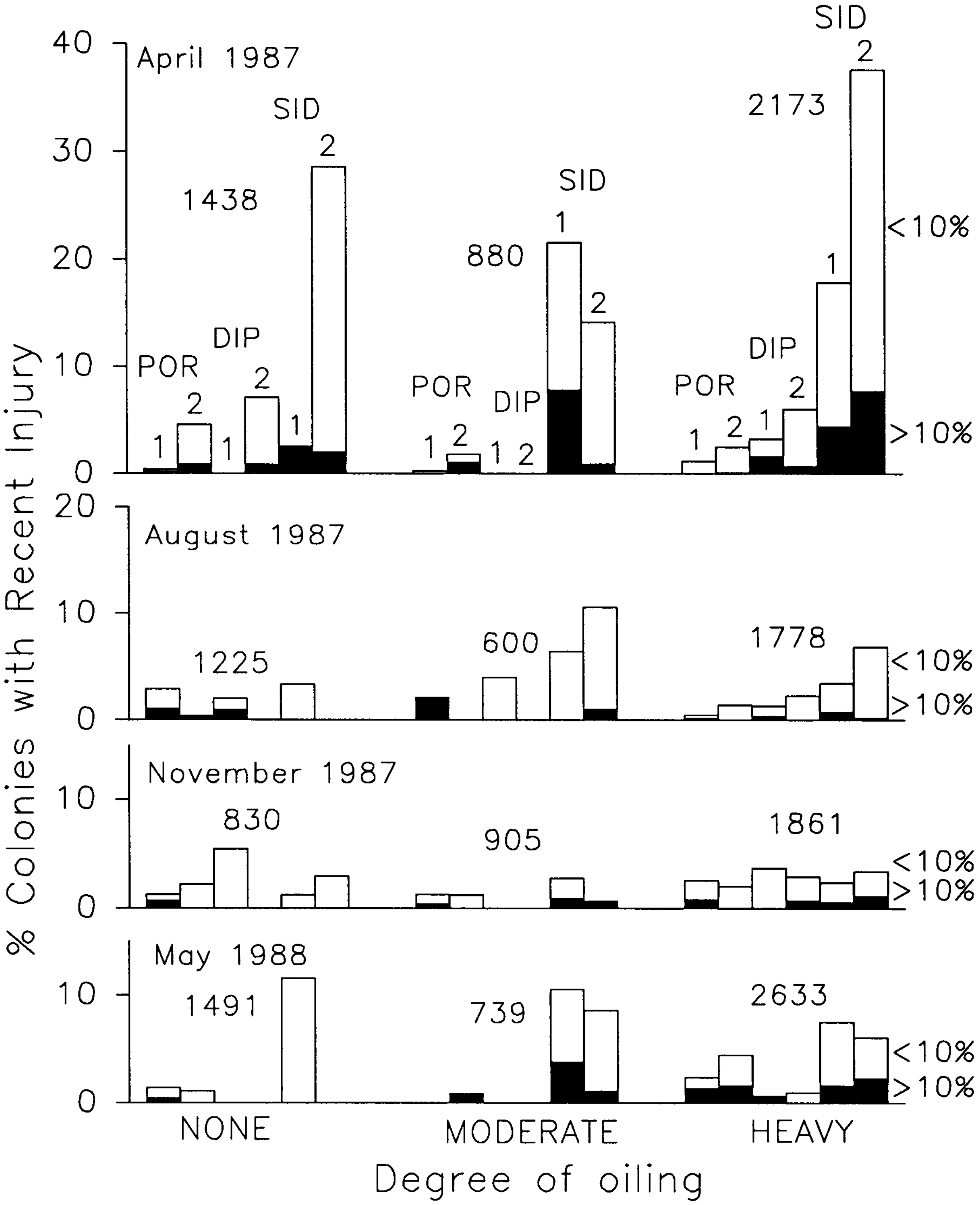


Fig. 7.16 Frequency of recent injury for three species of massive corals by depth and in relation to the degree of oiling at 12 reefs (continued). April 1987-May 1988.

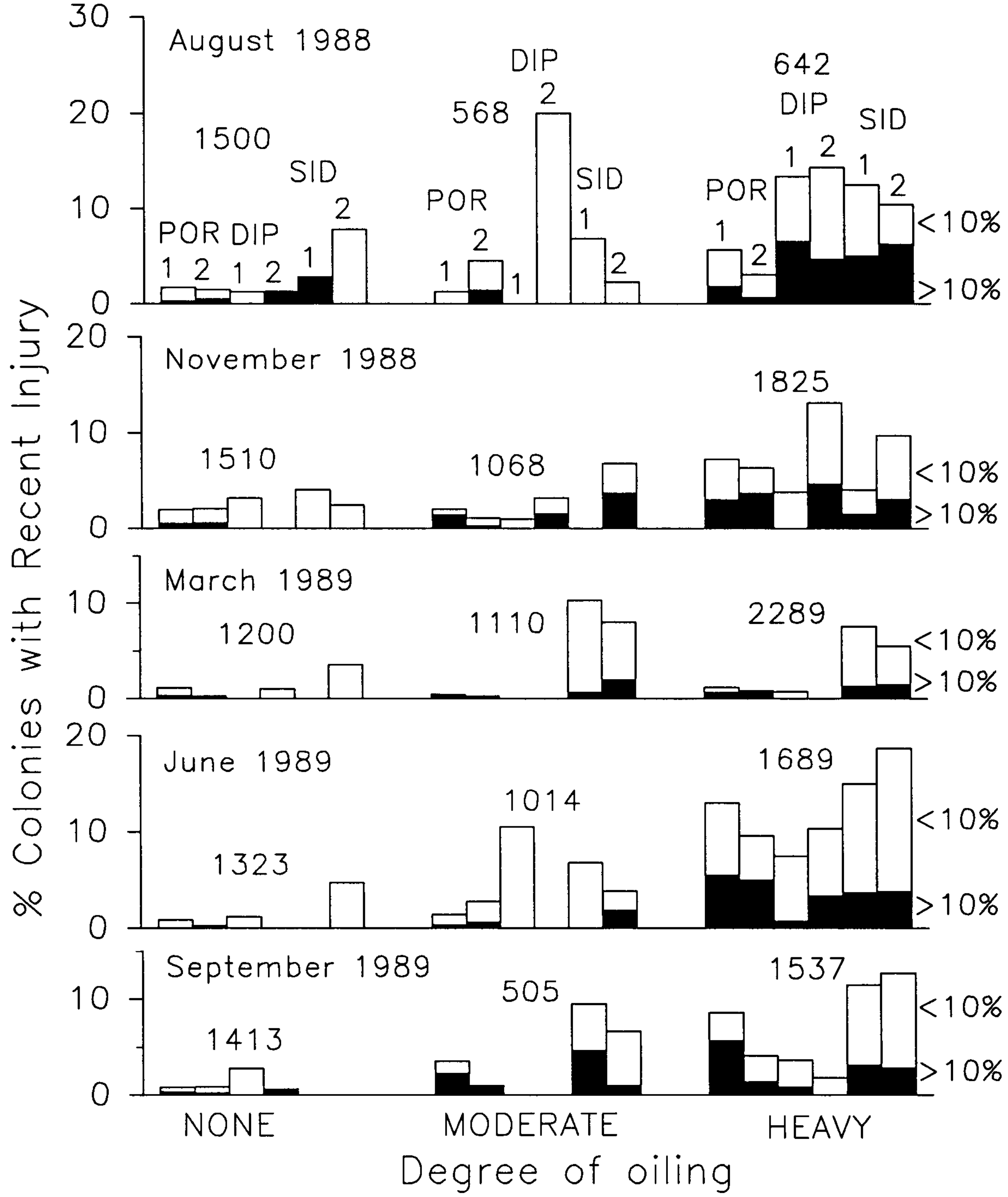


Fig. 7.16 Frequency of recent injury for three species of massive corals by depth and in relation to the degree of oiling at 12 reefs (continued). August 1988-September 1989. The first diesel spill occurred at the end of December 1988.

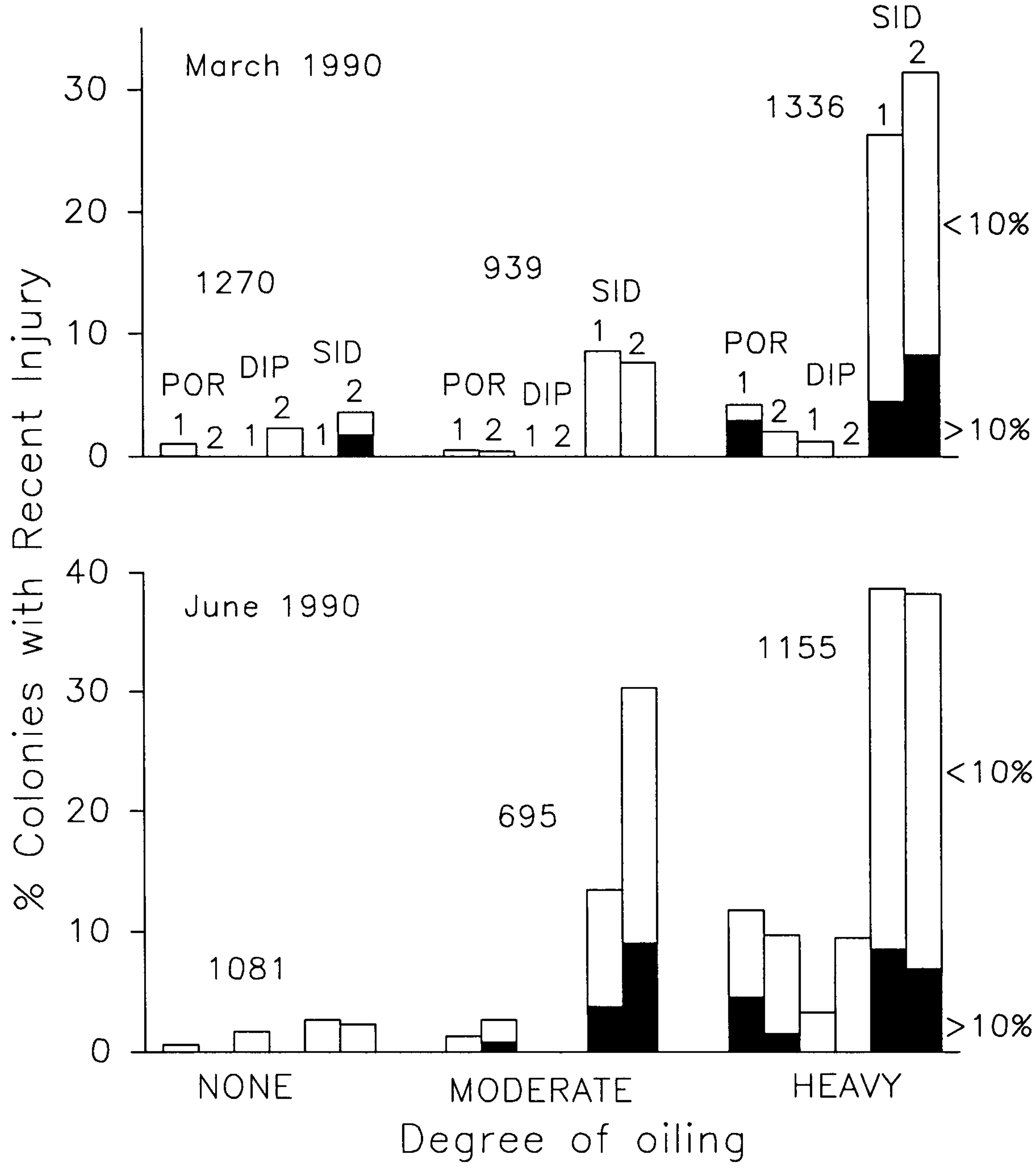


Fig. 7.16 Frequency of recent injury for three species of massive corals by depth and in relation to the degree of oiling at 12 reefs (continued). March and June 1990. The second diesel spill ocurred during June.

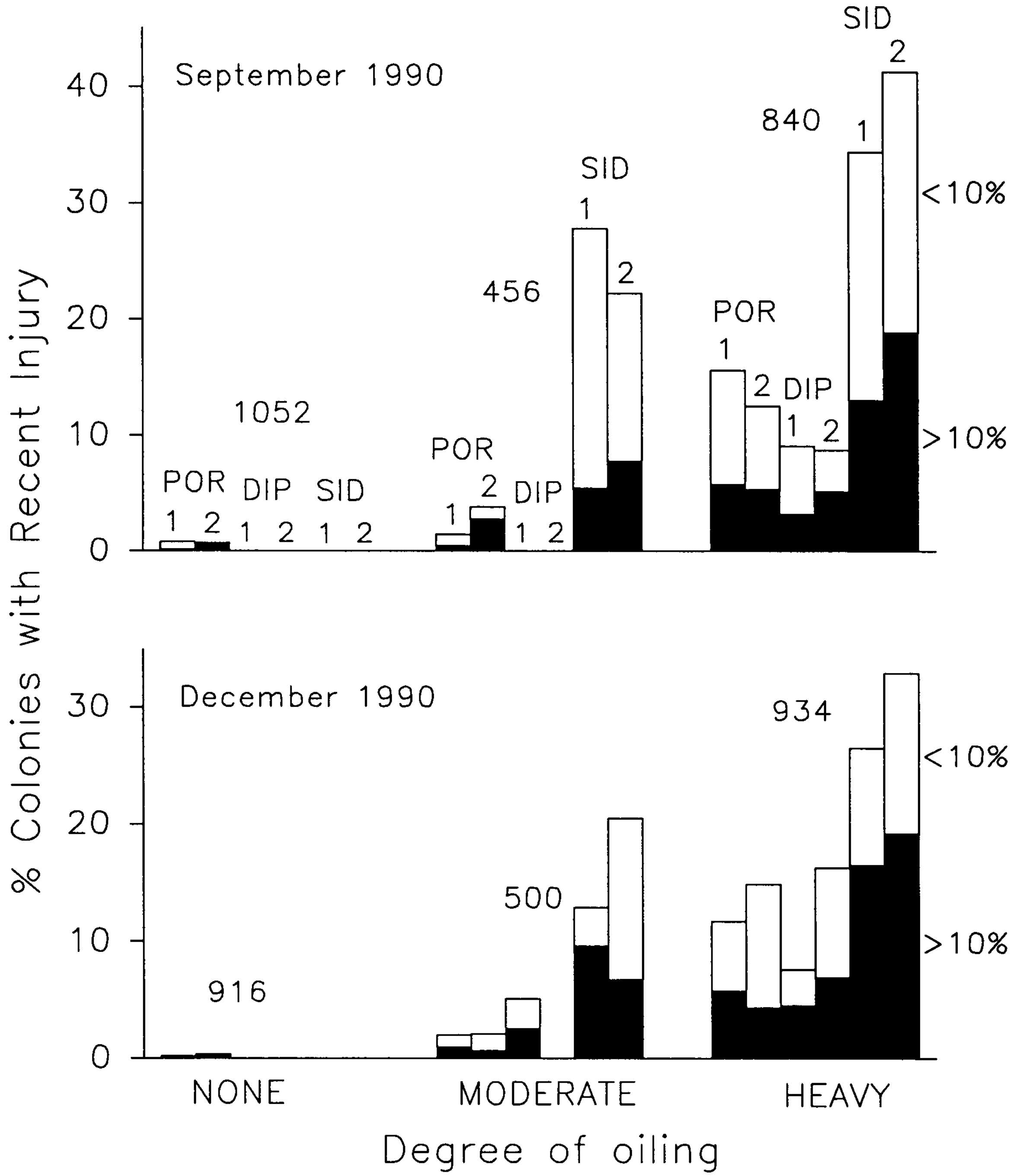


Fig. 7.16 Frequency of recent injury for three species of massive corals by depth and in relation to the degree of oiling at 12 reefs (continued). September and December 1990.

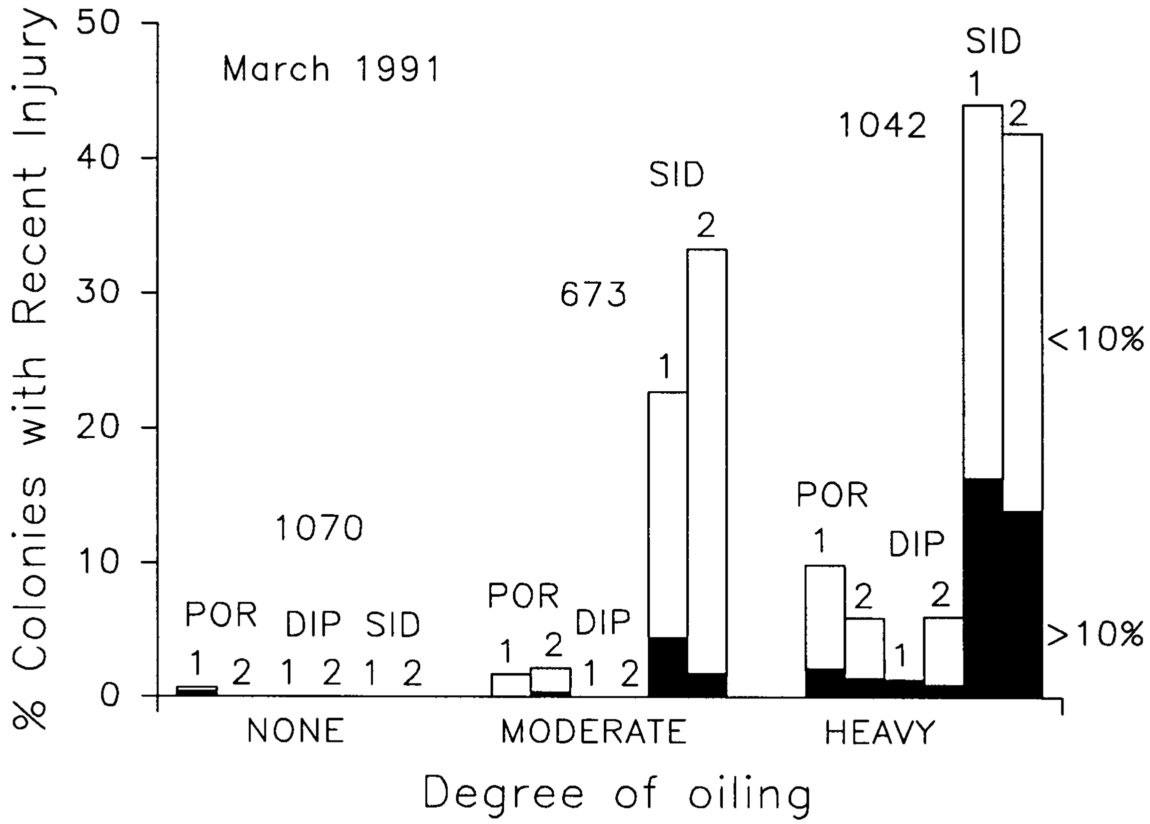


Fig. 7.16 Frequency of recent injury for three species of massive corals by depth and in relation to the degree of oiling at 12 reefs (continued). March 1991.

Numbers of colonies in these transects (all species combined) showed no major changes from August 1986 to March 1991 at unoiled reefs, but increased soon after the oil spill and did not decline until 38 mo later on oiled reefs (Fig. 7.19). This trend matches that of the numbers of colonies in the coral cover survey (Figs. 7.9, 7.11), further supporting the idea that most corals suffered partial rather than entire mortality of colonies after the spill. Size-frequency distributions of colonies in the transects were stable over time, with populations dominated by small individuals on all reefs (Fig. 7.20).

The amount of hydrocarbons in reef sediments (log-transformed data) was significantly positively correlated with the percentage of injured colonies of *Siderastrea siderea* (arcsine-transformed data) during 1986 and 1990, but not during 1988 (Fig. 7.21). Injury of *Porites astreoides* was not significantly correlated with amounts of hydrocarbons in 1986, but increased in relation to hydrocarbons in sediments during 1988 and 1990, whereas *Diploria clivosa* showed no clear relationship with amounts of hydrocarbons in reef sediments. Percent injury for *S. siderea* during 1986 was marginally correlated with the concentration of hydrocarbons

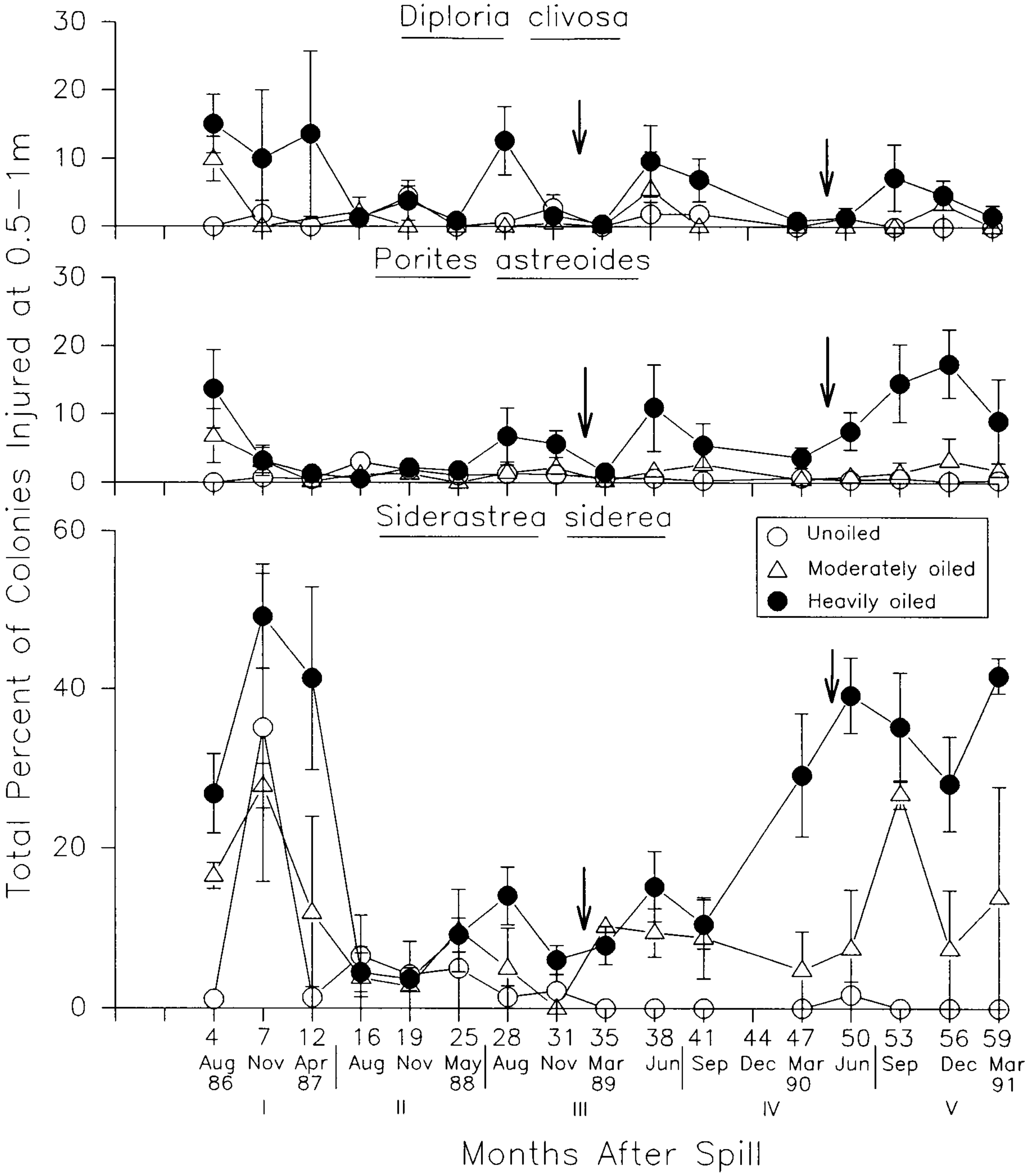


Fig. 7.17 Changes through time in the percentage of recently injured corals in relation to the degree of oiling and depth (0.5-1 m) from August 1986 to March 1991. Error bars are 1 SE. *Vertical arrows* indicate the 1988 and 1990 diesel spills. The *vertical lines* at the bottom indicate the pooled surveys used for statistical analyses.

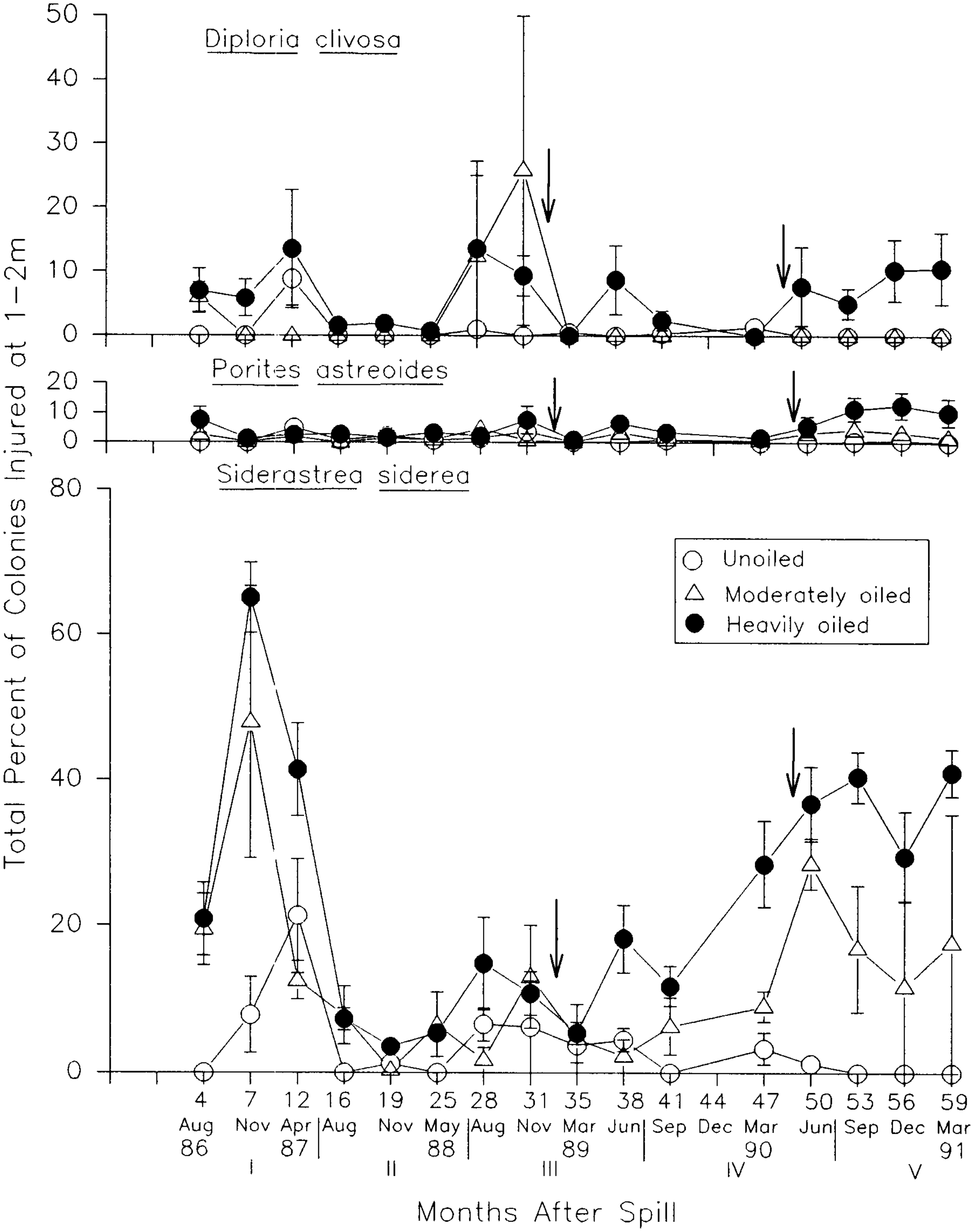


Fig. 7.18 Changes through time in the percentage of recently injured corals in relation to the degree of oiling and depth (1-2 m) from August 1986 to March 1991. Error bars are 1 SE. *Vertical arrows* indicate the 1988 and 1990 diesel spills. The *vertical lines* at the bottom indicate the pooled surveys used for statistical analyses.

Table 7.16 Repeated-measures ANOVA of percent of total injury for three coral species. See Methods (Sect. 7.5.2) for explanation.

Species	Factor	X^2	df	P
Siderastrea siderea	Oil	248.64	2	.000
	Depth	1.55	1	.2
	Year	110.83	4	.000
	Oil x Year	157.00	8	.000
	Oil x Depth	0.21	2	.9
	Depth x Year	15.39	4	.004
	Oil x Depth x Year	32.45	8	.000
Porites astreoides	Oil	33.57	2	.000
	Depth	0.02	1	.9
	Year	33.20	4	.000
	Oil x Year	141.65	8	.000
	Oil x Depth	0.03	2	.9
	Depth x Year	1.81	4	.8
	Oil x Depth x Year	11.17	8	.2
Diploria clivosa	Oil	33.51	2	.000
	Depth	0.17	1	.7
	Year	129.31	4	.000
	Oil x Year	52.92	8	.000
	Oil x Depth	0.49	2	.8
	Depth x Year	25.57	4	.000
	Oil x Depth x Year	28.75	8	.000

in tissues ($r = .66, P = .07$). Hydrocarbon levels in coral tissues in subsequent years (1988 and 1990) were near nondetectable levels, and showed no relationship with injury (Table 7.17).

7.5.3 Resistance of Corals to Stress

Organisms may be weakened by exposure to oil in their natural environment in a complex, time-dependent fashion (Loya and Rinkevich 1980; Brown and Howard 1985). This is particularly likely for corals in very shallow water that suffered high levels of injury in oiled areas (Guzmân et al. 1991). Without moving corals to the laboratory we were able to study their responses to stress on oiled reefs and to compare their performance with that of corals on unoiled reefs.

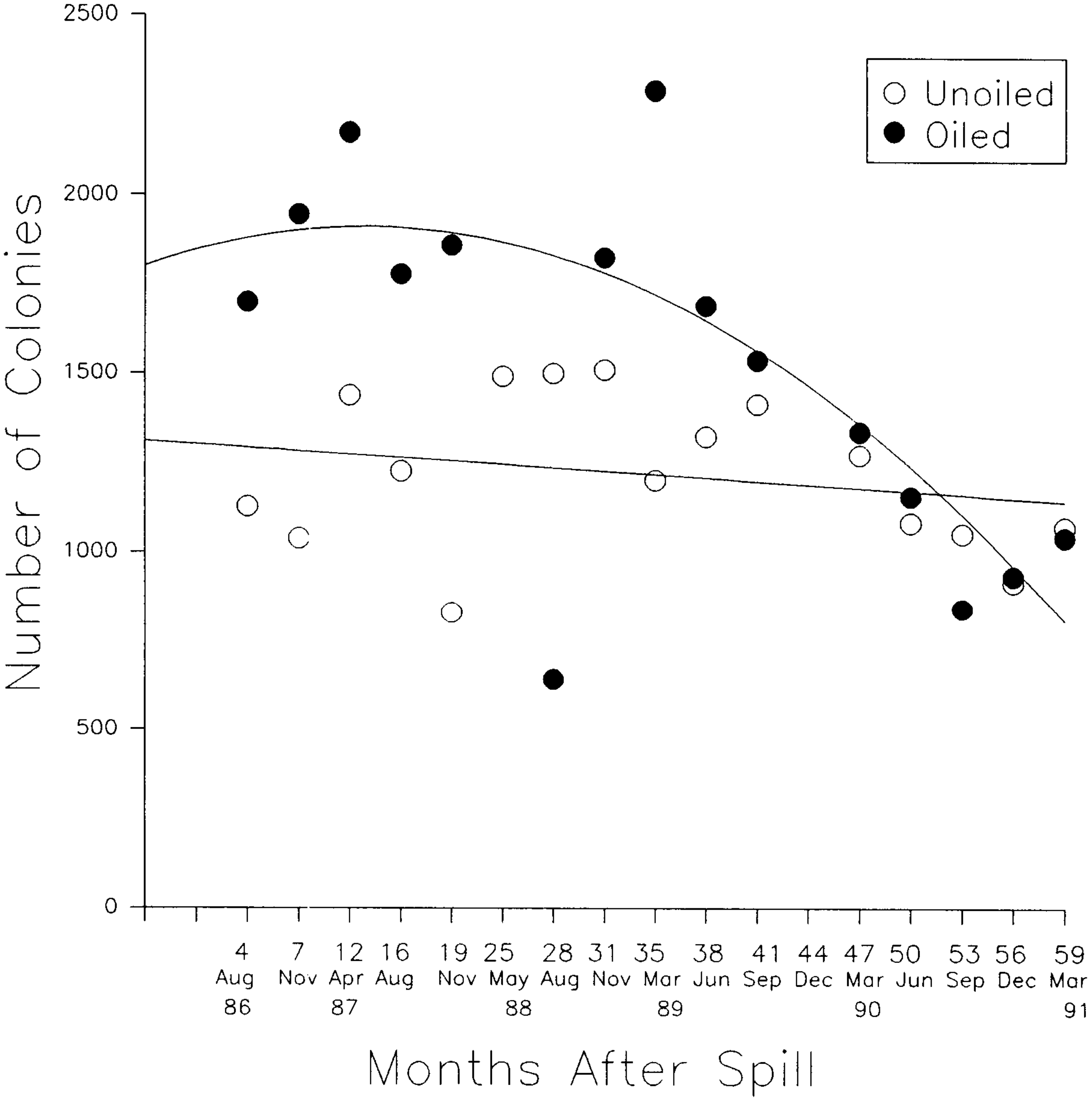


Fig. 7.19 Changes through time (August 1986 to March 1991) in the total number of colonies of all three species combined in relation to degree of oiling (only unoiled and heavily oiled reefs).

7.5.3.1 Methods

We developed a technique for measuring regeneration of injuries and tested the time intervals necessary to follow the recovery of injured corals using close-up photography. The procedures were based on those used in Curaçao for different species (Bak et al. 1977). Two different types of injuries, each 5.7 cm^2, were inflicted

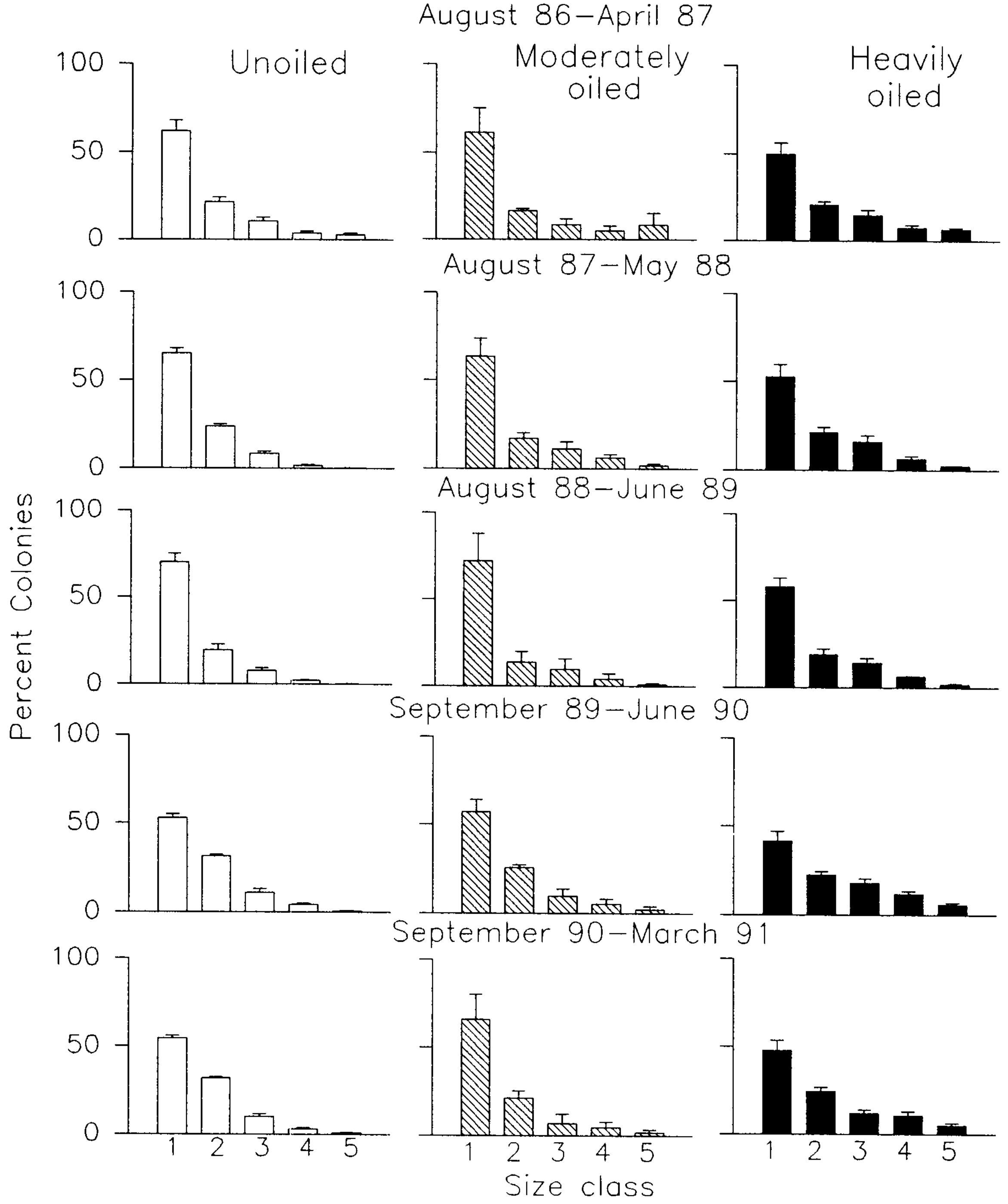


Fig. 7.20 Colony size-class distribution of the total number of colonies of all three species combined over time (five survey periods; see Sect. 7.5.2) for each degree of oiling. Size classes in cm^2 are: *1* = <100; *2* = 100-200; *3* = 200-400; *4* = 400-800; *5* = >800. Error bars are 1 SE.

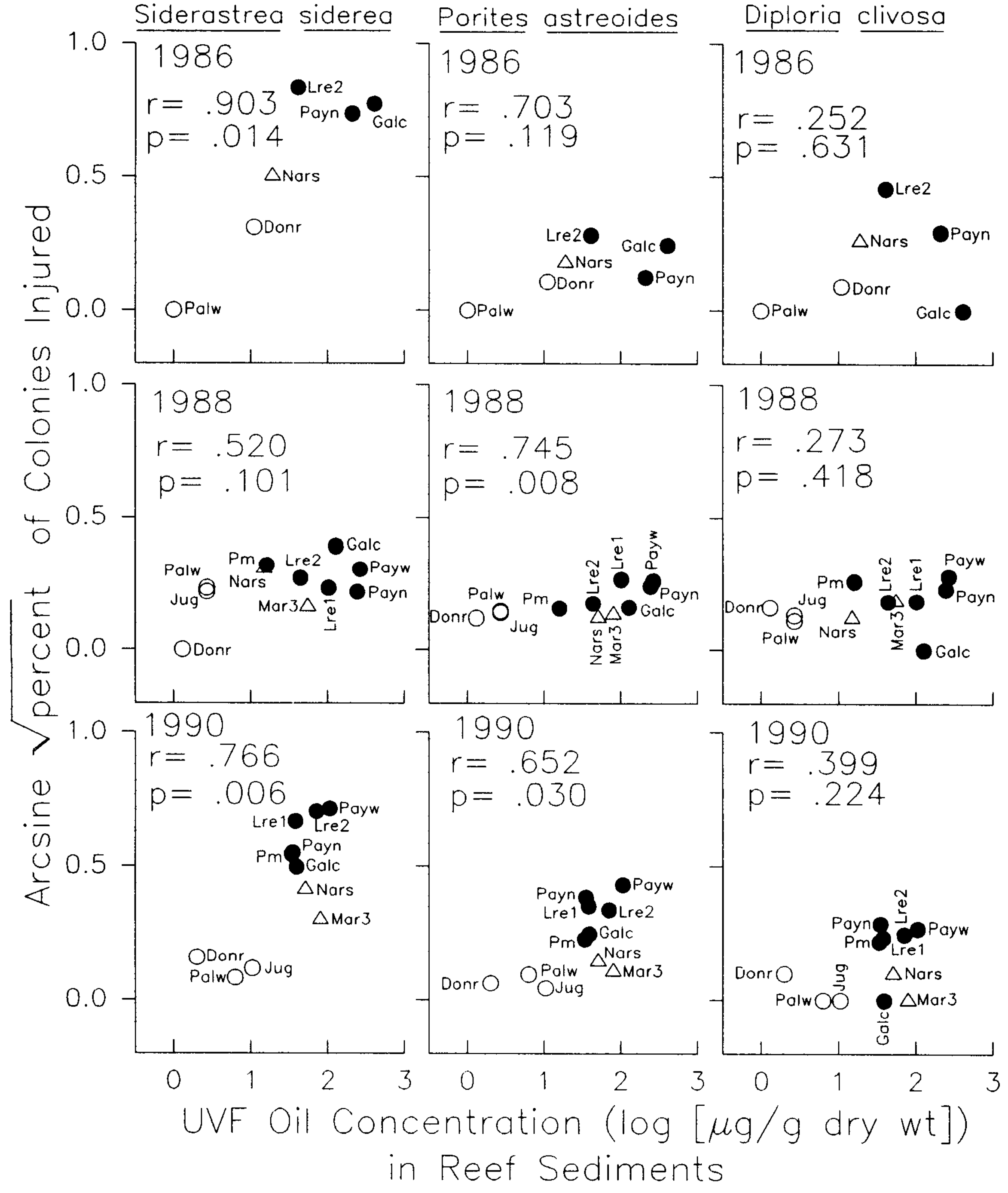


Fig. 7.21 Relationship between percent of colonies injured and oil concentration in reef sediments during 1986, 1988, and 1990 for *Siderastrea siderea*, *Porites astreoides*, and *Diploria clivosa*. Notice that the correlation for *S. siderea* was not significant during 1988. *Solid circles*: heavily oiled; *open triangles*: moderately oiled; *open circles*: unoiled.

Table 7.17 Correlation coeficient between percent of colonies injured (arcsine transformed) and oil in coral tissue (log μg oil/mg EOM by UVF analysis) during 1986, 1988, and 1990 for *Siderastrea siderea*. Values for hydrocarbon concentrations are given in Table 7.6.

Year	*N*	*r*	*P*
1986	8	.663	.07
1988	11	-.127	.7
1990	8	-.137	.7

on a single colony. One type was made by rasping the skeleton with a rotating tool (skeleton and tissue lesions that resemble predation), the other by blasting coral tissue with air (tissue lesion that resembles diseases and bleaching). Photographs were taken 1 yr after the injuries (Guzmán and Jackson 1991, Fig. 5.6). The experiments were carried out on two heavily oiled reefs (LRE2 and PAYN) and two unoiled reefs (PALW and JUG) at 1 m depth, using five replicate colonies per species per reef. Colony size (diameter) was 20-25 cm and 60-70 cm for *P. astreoides* and *S. siderea*, respectively. Experiments were run from September 1988 to October 1989, and from November 1990 to November 1991; the latter was not completed due to bad weather.

7.5.3.2 Results

Figure 7.22 shows the percent regeneration after 1 yr for *S. siderea* and *P. astreoides* subjected to the two kinds of experimental injuries (blasting and rasping). *Porites* recovered faster than *Siderastrea* on all reefs. Regeneration of skeletal injuries (rasping) was similar for both species (Mann-Whitney *U*-test, $P > .05$). Regeneration was significantly faster at oiled reefs than at unoiled reefs for tissue lesions on *S. siderea*, but not for *Porites* (Mann-Whitney *U*-test, $P = .007$ and .8, respectively). Necrosis of colony tissue around the experimental injuries was observed mainly in *S. siderea*, where 60% and 20% of the colonies at unoiled and oiled reefs, respectively, were affected (Table 7.18). Increase of injury size by necrosis may explain the much slower regeneration by *S. siderea*. At the oiled sites one of the two necrotic *Siderastrea* colonies died; no mortality occurred at unoiled reefs (Fig. 7.23). In contrast, *Porites* showed similar rates of necrosis at unoiled and oiled reefs (30% of the colonies), but all three of the necrotic colonies at oiled reefs died and two out of three died at unoiled reefs. Nevertheless, these differences in mortality of both species by necrosis of tissues were not significant between oiled and unoiled sites ($X^2 = 0.61$, 2 x 2 contingency table, $P > .05$). Figure 7.23 suggests *Porites* is less tolerant of injuries than *Siderastrea*, but such differences are not significant for the limited data available. All of these results are complicated by secondary biological interactions. Grazers and predators were observed to affect injured coral colonies, although these appeared to be different at oiled and unoiled

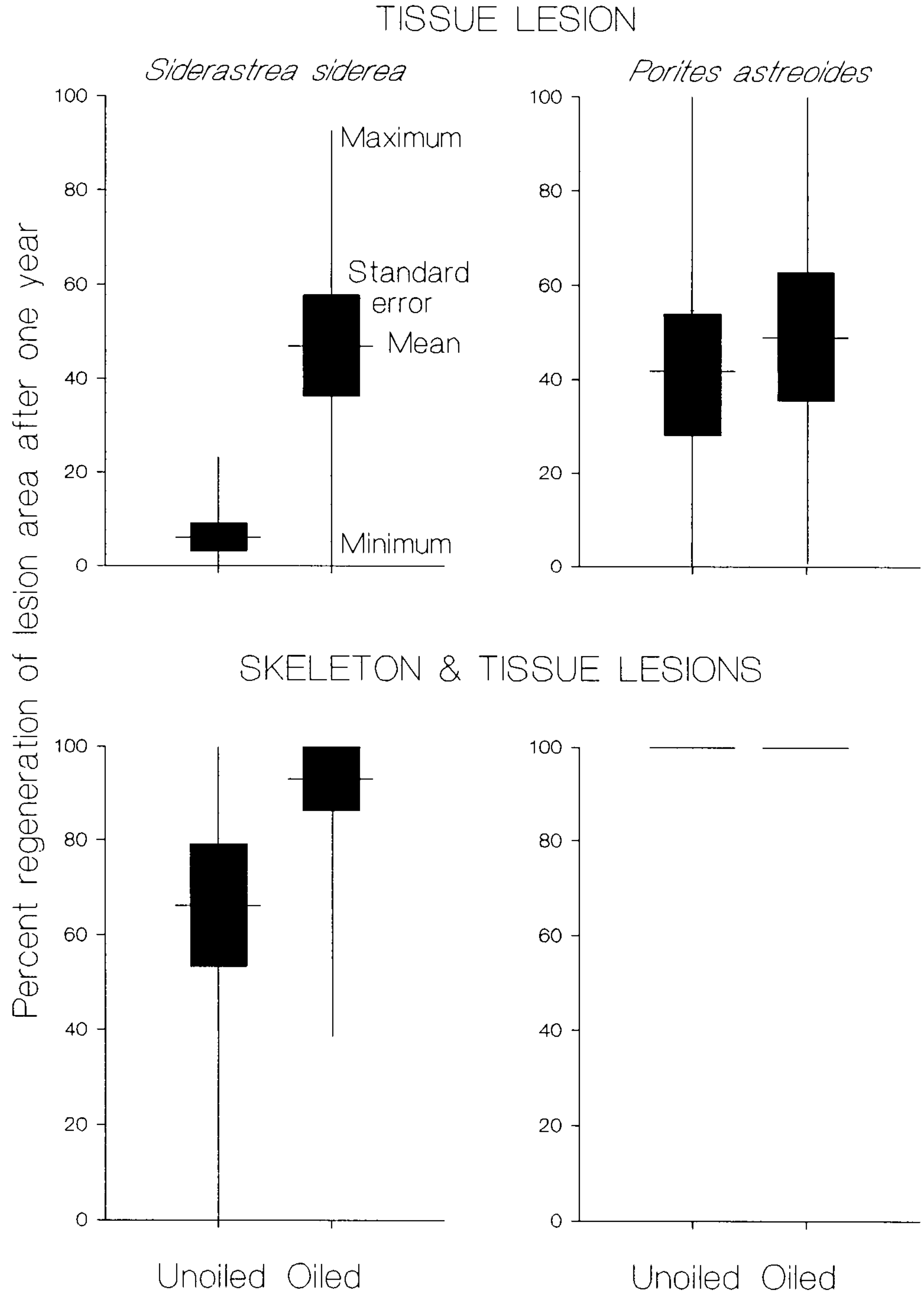


Fig. 7.22 Percent regeneration after 1 yr of *S. siderea* and *P. astreoides*. Colonies were subjected to two kinds of experimental injury (blasting and rasping) at two unoiled and two oiled reefs. The experiment was initiated in September 1988.

Table 7.18 Percent of colonies that suffered necrosis (increase in size of injury) after artificial lesions (only blasting), and percent of necrotic colonies that died. Colonies within treatments were pooled. Number of colonies in parentheses.

		Percent Necrotic Colonies		Percent Necrotic Colonies That Died	
Species	*N*	Unoiled	Oiled	Unoiled	Oiled
Siderastrea siderea	10	60 (6)	20 (2)	0 (0)	50 (1)
Porites astreoides	10	30 (3)	30 (3)	66 (2)	100 (3)

reefs. Additional experiments with larger sample size coupled with observations of differences in grazer and predator abundance would be required for a better understanding of consequences of injuries and their recovery.

7.5.4 Coral Growth (Sclerochronology)

Coral sections contain a wealth of information concerning growth rates, past and present incidents of stress, and, to some extent, the history of the environment in which colonies live (Knutson et al. 1972; Dodge et al. 1974; MacIntyre and Smith 1974; Hudson et al. 1976; Dodge and Vaisnys 1980; Dodge and Lang 1983; Jackson 1983; Brown and Howard 1985). Growth rate (i.e., skeletal extension) can be determined from the width of annual growth bands using X-ray techniques (see references cited above). *Montastrea annularis* and *Diploria strigosa* are not as abundant as *Siderastrea siderea* and *Porites astreoides*, but could be found on most of the reefs. Little previous work had been done on the latter two species (Hubbard and Scaturo 1985; Huston 1985), and it seemed prudent to compare these results to published standards for better-studied taxa. Growth of *Montastrea annularis* has been studied more than any other Caribbean coral (Aller and Dodge 1974; Dodge et al. 1977; Gladfelter et al. 1978; Dodge and Brass 1984; Hubbard and Scaturo 1985; Huston 1985), although the value of these studies has now been compromised by the discovery of cryptic species that differ significantly in growth rates (Knowlton et al. 1992). *Diploria strigosa* was chosen because of the detailed and extensive experimental work done at the Bermuda Biological Station for Research (BBSR) on the effects of oil on this species (Dodge et al. 1984, 1985; Knap 1987).

7.5.4.1 Methods

Four coral species were studied (*Montastrea annularis*, *Diploria strigosa*, *Porites astreoides*, and *Siderastrea siderea*) using standard techniques (Dodge 1980; Guzmán and Cortés 1989*b*; Guzmán et al. 1991). Colonies or cores were collected from shallow water (1-3 m) and rinsed with freshwater (Table 7.1). In the laboratory, slabs

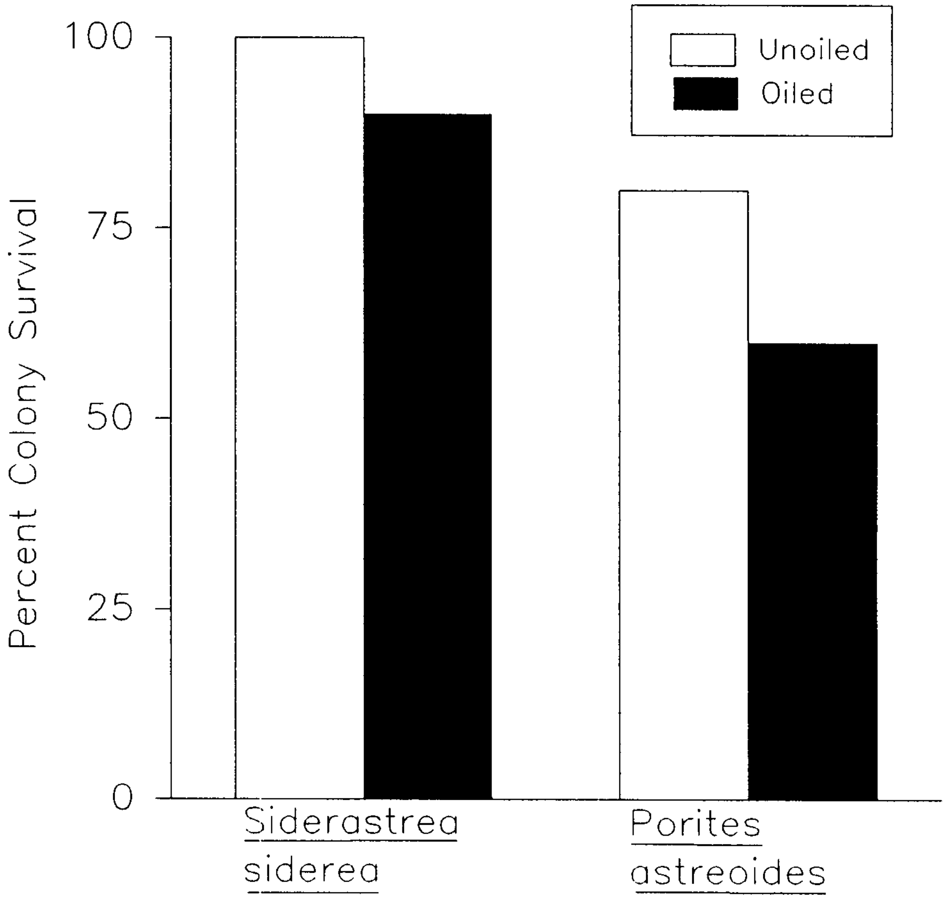


Fig. 7.23 Percent survival after 1 yr of artificially injured colonies of *S. siderea* and *P. astreoides* at unoiled and heavily oiled reefs.

5-7 mm thick were cut using a rock saw (see Guzmán and Jackson 1991) parallel to the axis of growth. The coral slabs were x-rayed on a Universal X-ray unit (model Little Giant 30) using Kodak Industrex AA film (see photograph in Guzmán and Jackson 1991). Exposures were made at 60 KVp, 30 ma for 15 sec with a source-to-film distance of 1 m. Contact X-radiograph prints were made on high-contrast paper for analyses of growth rate. Growth bands were measured (Fig. 7.24) using a fine ruler (resolution 0.5 mm). One transect along the axis of maximum growth was measured for each coral. Growth bands were measured inward along the transect starting at the outer (distal) surface of the most distal high-density band to the distal surface of the next-oldest high-density band. Alizarine staining (Lamberts 1978) of 10 corals (five *S. siderea* and five *P. astreoides*) was done in November 1988 at Punta Galeta reef to confirm annual banding. We had, however, demonstrated that growth bands were annual by collecting several specimens at different times of

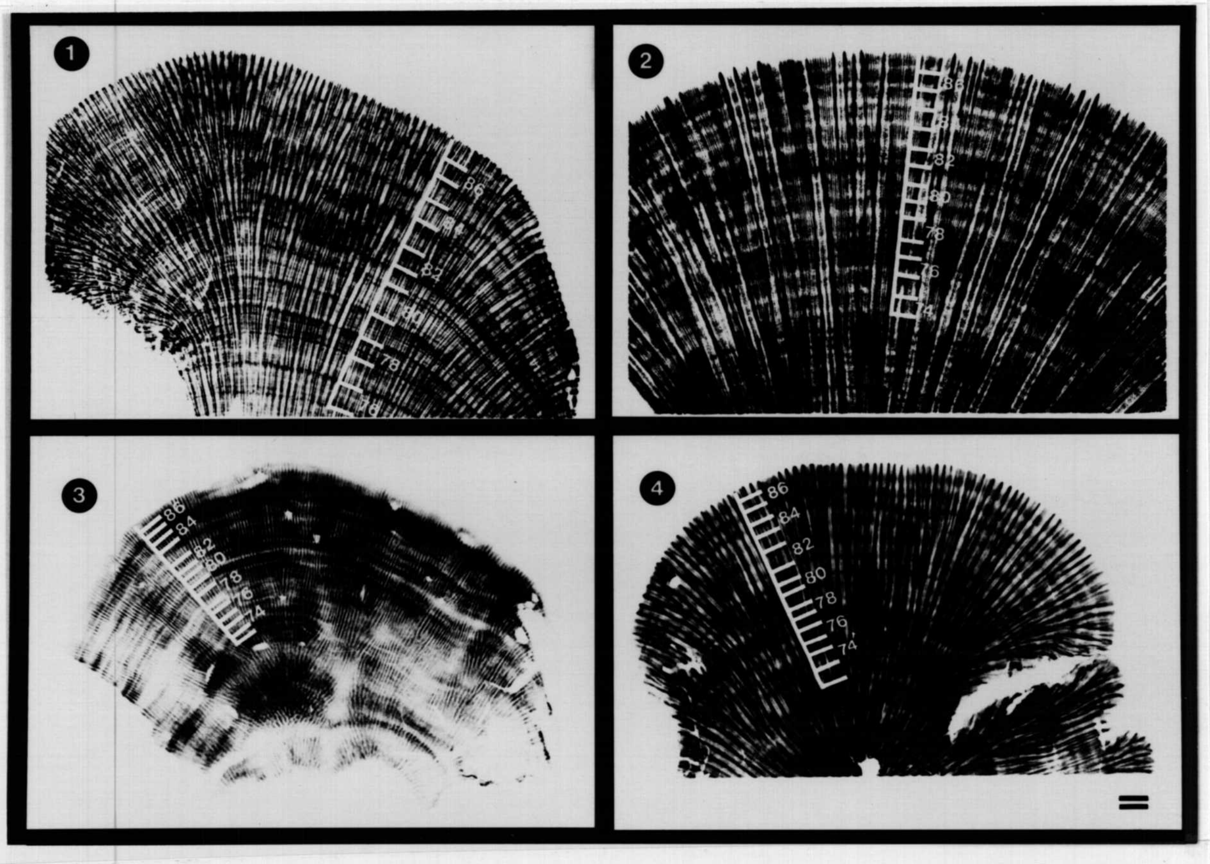


Fig. 7.24 **X-ray photograph of corals showing growth bands in all four species used:** ***1*** **=** ***Montastrea annularis*****;** ***2*** **=** ***Diploria strigosa*****;** ***3*** **=** ***Porites astreoides*****; and** ***4*** **=** ***Siderastrea siderea.*** ***Scale bar*** **= 1 cm** ***(bottom right corner)*****.**

the year (i.e., beginning and ending of dry and rainy seasons) and comparing their band structure. Comparison of corals, collected during mid- to late 1987 and early 1988, documented that the beginning of the low-density band coincides with the onset of the dry season in late December or early January, so that each primary couplet of light and dense bands coincides approximately with the calendar year.

Three sets of corals were collected for analyses of growth. First, a total of 190 colonies (four coral species) were collected at 11 reef sites in 1987: four unoiled reefs, two moderately oiled, and five heavily oiled. Colonies were of similar size (30-40 cm diameter). Ten specimens did not show clear bands and were therefore excluded from the analyses. Annual variation in growth was examined for each species for the 9 yr previous to the spill (1977-1985) by 2-way repeated-measures ANOVA, using the three groups of reefs (unoiled, moderately oiled, or heavily oiled in 1986) and time (years) as factors. In this case repeated-measures analysis is required because successive growth bands are measured in the same corals. We then compared growth in the year of the oil spill to the nine previous years by calculating the natural logarithm of the ratio of the mean width in 1986 and the mean width for 1977-1985, and by comparing these values in a 2-way ANOVA with species and amount of oil as factors (*main sequence 1* of Green 1979; Table 1.3). Second, a collection was made to assess possible sublethal effects on growth by comparing growth 3 yr before and 3 yr after the oil spill. Nine colonies each of *S. siderea* and *P. astreoides* were collected at similar depths from each of the 12 reefs at the end of 1988 and during 1989. All of these corals were smaller than 30 cm in diameter. A total of 108 colonies were collected for each species, but only 104 and 76 for *S. siderea* and *P. astreoides*, respectively, showed clear bands. Finally, in order to develop a long-term chronology of environmental changes and degradation in Bahía Las Minas for at least 100 yr, we drilled large heads of *Siderastrea siderea* at a depth range of 1-3.5 m using an underwater hydraulic drill. A total of 231 colonies were drilled and 1-m long cores obtained from most of the colonies. The cores provide a unique historical record of coral growth before, during, and after two major oil spills and the construction of the refinery. However, it was not possible to analyze these cores during this study.

7.5.4.2 Results

1987 Collection

Yearly mean growth rates were measured for each of the four species from 1977 to 1986 (Fig. 7.25; App. Tables C.3-C.6). Results showed no significant 9-yr variations in growth among reefs grouped by their subsequent exposure to oil in 1986 for any of the four coral species (repeated-measures ANOVAs; all $P > .05$).

The lowest mean annual growth rates for all four species (marginally for *P. astreoides*) on oiled reefs occurred in 1986, the year of the spill (Fig. 7.25). This change in growth in 1986, relative to the previous 9 yr and degree of oiling, is shown in Figure 7.26 using data only for corals with bands for all 10 yr. There was a substantial reduction in growth on moderately and heavily oiled reefs relative to unoiled reefs for all species except *Siderastrea siderea*. Two-way ANOVA, using the data from Figure 7.25 and level of oiling (reef groups) and species as factors, gave a nearly significant result for oiling ($F = 2.93$; df = x, y; $P = .057$), but not for species or the interaction of species and oil. Subsequent 1-way ANOVA for oiling alone, to test all species combined (general coral growth), was significant ($F = 3.35$, df = 2, 154, $P = .038$).

1988/1990 Collection

When growth rates for the 3 yr before and after the oil spill were compared, both *Siderastrea* and *Porites* showed a general reduction in growth rate after the oil spill at all reefs, regardless of the amount of oiling (Fig. 7.27). However, growth after the spill declined significantly only for *S. siderea* and not for *P. astreoides* at

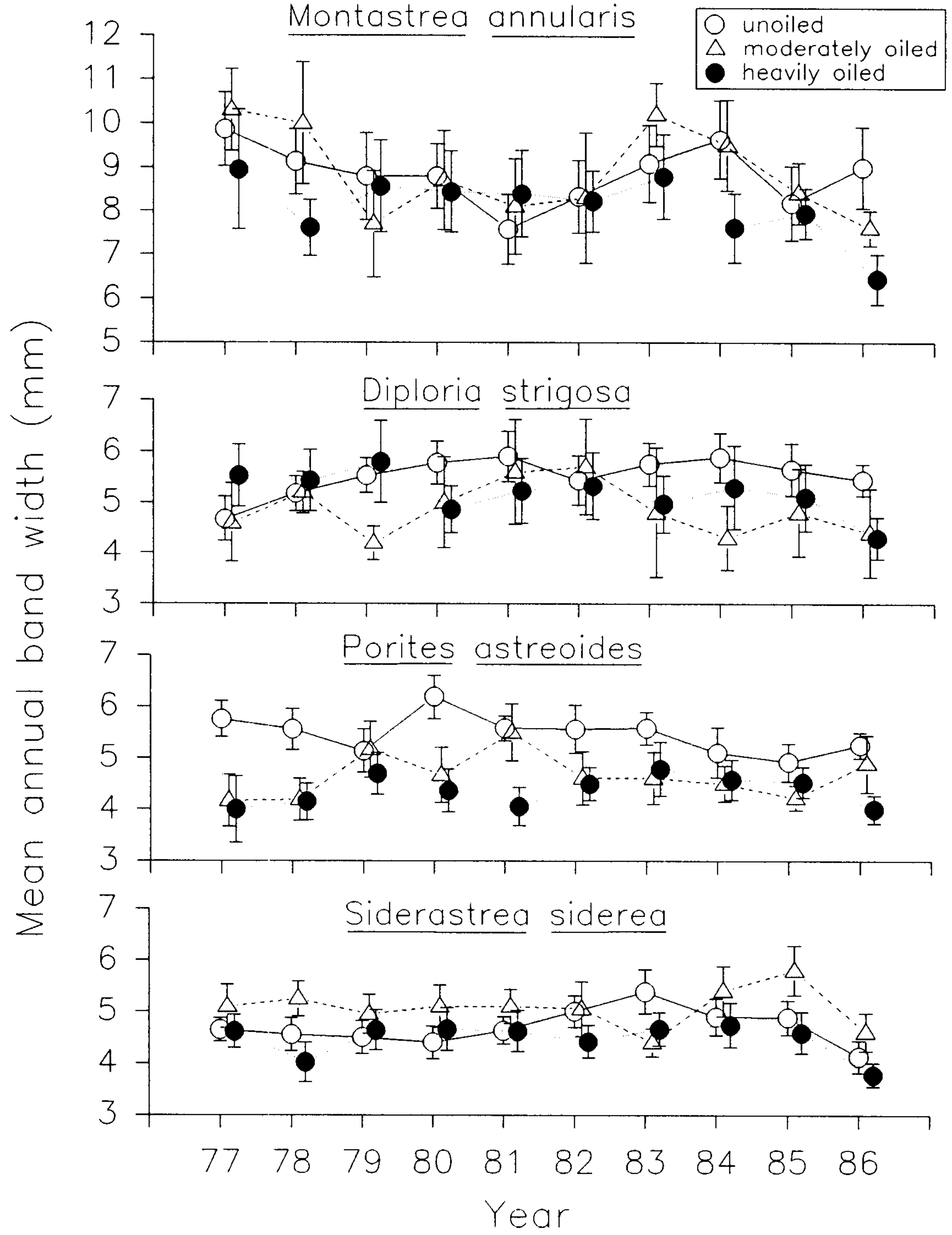


Fig. 7.25 Changes in average mean annual growth rates over 10 yr for four species of corals at 11 reefs grouped by exposure to oil during the 1986 spill. *Bars* mark 1 SE. Numbers of corals analyzed for each reef are given in Appendix Tables C.3-C.6.

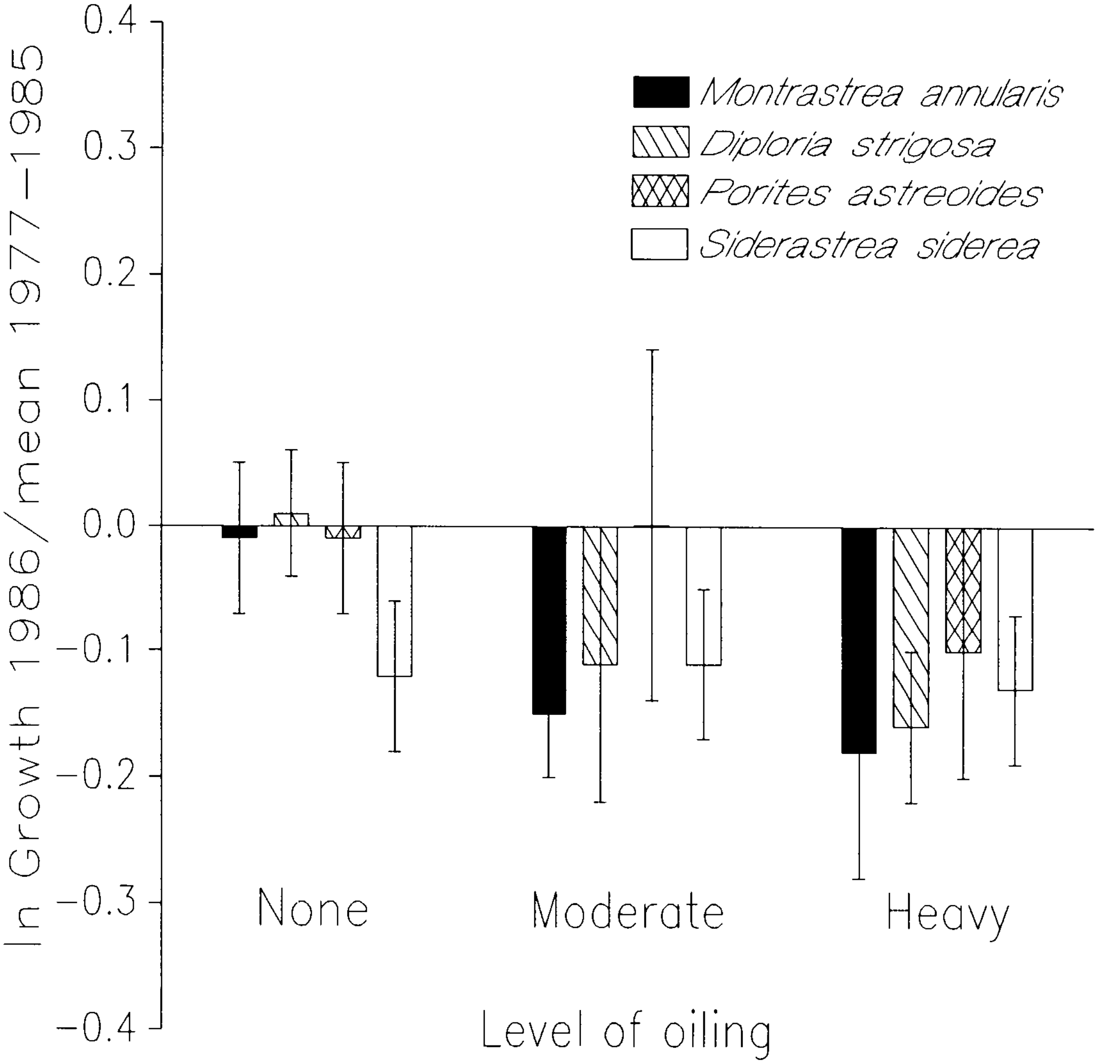


Fig. 7.26 Mean growth and SE of four species of corals in 1986 relative to their mean annual growth during the 9 previous yr and exposure to oil during the 1986 spill. Data include only those corals with bands for the most recent 10 yr. Values below the *0.0-line* show growth in 1986 less than the average for the previous 9 yr.

heavily oiled reefs (paired *t*-tests; $t = 3.63$, df = 5, $P = .015$; $t = 0.52$, df = 5, $P = .627$, respectively). Repeated-measures ANOVAs were only significant for oil and year for *S. siderea*, but not for *P. astreoides*; the oil x year interactions were not significant for either species (Table 7.19). Thus, growth of *S. siderea* was affected only in the medium term (3-yr average, 1986-1988) because no difference in growth was observed in 1986 (see Fig. 7.26), whereas *Porites* showed an initial reduction in 1986 but no effect 2 yr later.

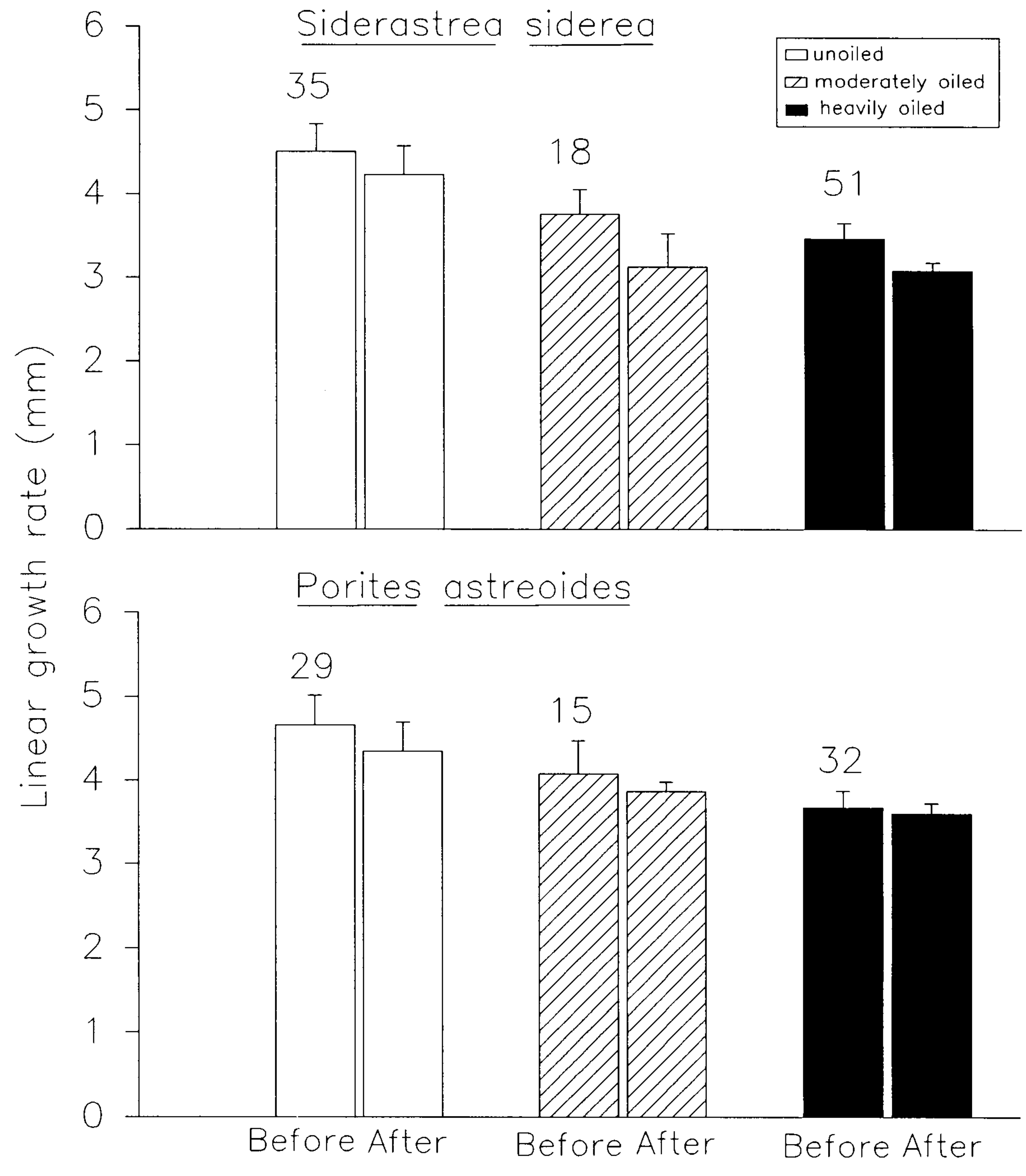


Fig. 7.27 Growth average and 1 SE of two species of corals 3 yr before the oil spill (1983-1985) compared to 3 yr afterward (1986-1988) in relation to exposure to oil during the 1986 oil spill. Numbers of colonies are indicated by the numbers above the histograms.

Table 7.19 Repeated-measures analysis of variance of growth rates before (1983-1985) and after (1986-1988) the oil spill for two coral species.

Species	Factor	*F*	df	*P*
Siderastrea siderea	Oil	6.43	2, 9	.02
	Time	31.87	1, 9	.000
	Oil x Time	1.55	2, 9	.3
Porites astreoides	Oil	3.63	2, 9	.07
	Time	2.74	1, 9	.1
	Oil x Time	0.49	2, 9	.6

A significant negative correlation between levels of oil in reef sediments and linear growth rates in corals from both collections was observed for *S. siderea* and *P. astreoides* during 1986 and 1988 (Fig. 7.28). *Montastrea annularis* and *Diploria strigosa* showed a nonsignificant negative relationship in 1986 (no growth data for other years). Growth rates were not measured for any coral species during 1990. Furthermore, growth of *S. siderea* was not correlated with hydrocarbon levels in coral tissues during 1986, but it was in 1988 (Fig. 7.29). However, because values were so low by 1988, this precluded any biological significance.

7.5.5 Coral Reproduction

Previous studies suggest that oil pollution may significantly decrease survival of coral larvae (Cohen et al. 1977; Loya and Rinkevich 1979; Rinkevich and Loya 1977) and development of coral reproductive tissues (Peters et al. 1981). Fertilization may be depressed in coral species with external fertilization, such as *Siderastrea siderea*, if expulsion of gametes occurs in response to oil pollution (Loya and Rinkevich 1979), as has been demonstrated in other marine invertebrates (Nicol et al. 1977).

7.5.5.1 Methods

Siderastrea siderea was chosen to evaluate sublethal effects of oil pollution on coral reproduction. *S. siderea* is gonochoric (separate sexes), and broadcasts gametes into the water column where fertilization and embryogenesis occur. In Panama the species has one reproductive cycle per year, and the spawning season is between August and September, most likely after the September full moon (Soong 1990, 1991; Guzmán and Holst 1993). Four reefs were selected, two unoiled (PALW and JUG) and two heavily oiled (LRE2 and PAYN). To reduce possible between-reef (or treatment) and within-reef variation in fecundity, all colonies were sampled the same day (less than 5 h between unoiled and oiled reefs) at a depth of 1.5-3 m. Fragments

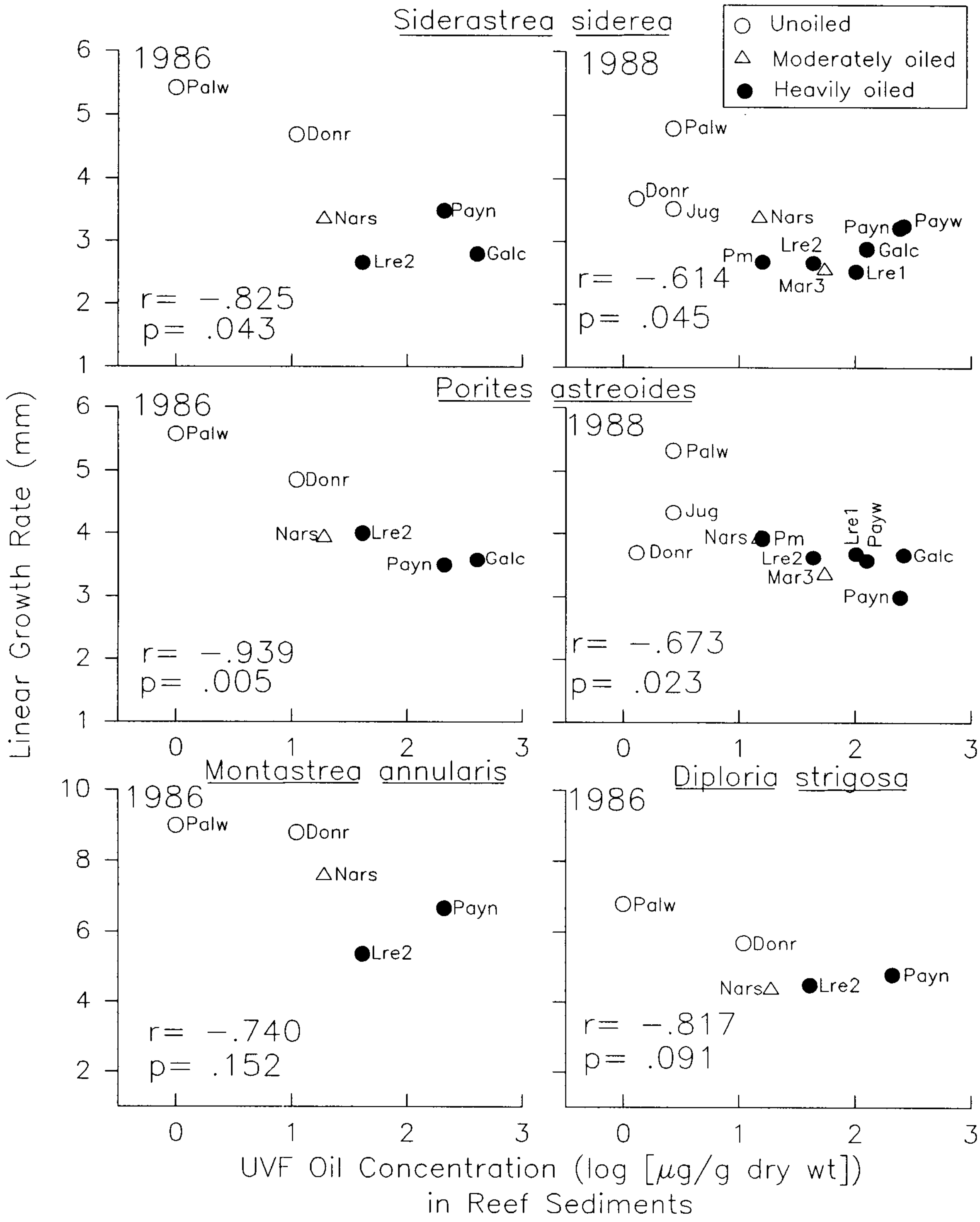


Fig. 7.28 Linear growth rates of *Siderastrea siderea*, *Porites astreoides*, *Montastrea annularis*, and *Diploria strigosa* vs. oil concentration in reef sediments during the year of the spill, and 1988 (only the two former species). Notice that the concentration of oil in sediments was always higher at the heavily oiled sites PAYN and GALC.

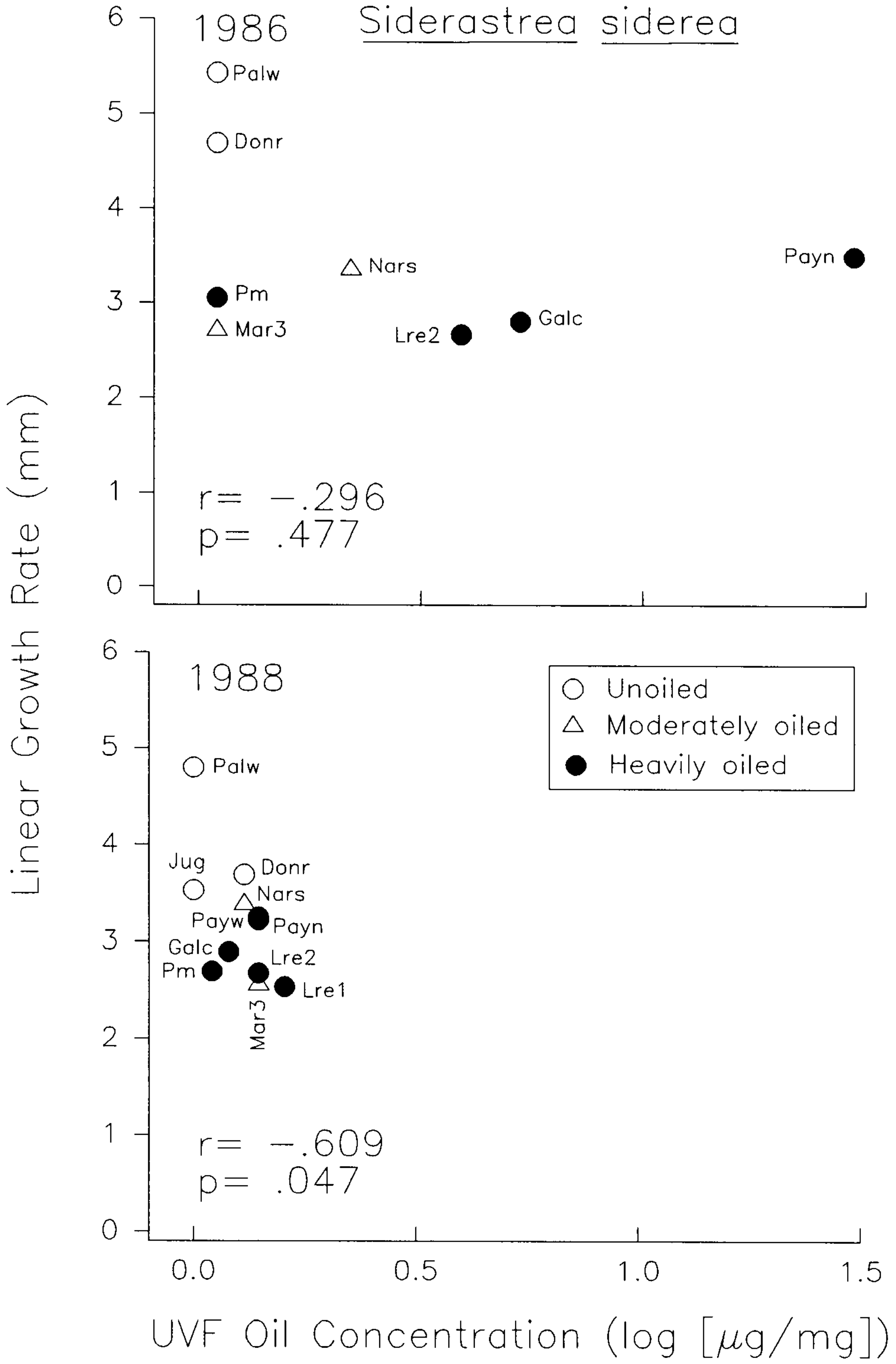


Fig. 7.29 Linear growth rate of *Siderastrea siderea* vs. oil concentration in coral tissues during the year of the spill (1986) and 1988.

of 10 different, large (>60 cm diameter), healthy-looking colonies were haphazardly collected from each reef at each census using a hammer and chisel. This study began 39 mo after the 1986 oil spill. Samples were taken during full moon from August 1989 to November 1990 (except January 1990; 15 censuses x 4 reefs x 10 replicates). Colonies at one oiled reef (LRE2) were tagged and sampled from August 1989 to February 1990, but this procedure was discontinued to prevent excessive damage to colonies due to increasing partial mortality at oiled reefs (see Sect. 7.5.2). Samples were only collected on horizontal surfaces, avoiding marginal infertile areas and vertical surfaces of the colonies (*sensu* Soong 1990). Within a few minutes after collection coral fragments were fixed in a seawater Zenker's solution with 5% formalin (Yevich and Barszcz 1981) for 16-20 h, washed in running tap water for 24 h, and stored in 70% ethanol.

For histological studies coral samples were decalcified in a solution of 5% nitric acid. The solution was replaced twice daily until no skeletal material (calcium carbonate) was detected using sodium oxalate. Samples were then placed in 4% sodium sulfate for 12 h to neutralize the tissue, washed in running tap water for 24 h, and stored in 70% ethanol. Tissue samples were dehydrated in a series of alcohol and xylene treatments, embedded in Paraplast using standard techniques, and oriented so that both longitudinal and cross sections were obtained (Szmant-Froelich et al. 1980). Tissues were serially sectioned at 10 μm; only sections taken every 200-250 μm along the vertical axis (from distal to basal portion; an average of 10-13 slides per tissue) were stained with Heidenhain's azocarmine-aniline blue method (Luna 1968).

The average number of polyps per histological section was 12 (N = 155, range = 4-29) or an average of 22 polyps per cm^2 (N = 108, range = 14-34). For each of the tissue samples the slide with polyps containing the most reproductive material was used to estimate coral fecundity (gonad number and size). In order to standardize results 10 randomly chosen reproductive polyps were scored for total number of female and male gonads, gonad size, and developmental stage according to the modified criteria of Szmant-Froelich et al. (1985). Gonads were counted with a Wild M5A stereomicroscope and an Olympus BH-2 light microscope. The size (area) of five gonads per reproductive polyp (N = 10 per colony) in cross section was digitized with a Java video system (Jandel Video Analysis Software) adapted to the optical equipment. There was no significant difference between mean gonad area using five or 10 replicate gonads per polyp (t = 0.188, df = 88, P = .851 for females; t = 0.297, df = 49, P = .768 for males). Gonad area was measured only during the reproductive peaks (August, September, and October 1989 and 1990).

These data were used to assess temporal patterns of reproduction at oiled and unoiled sites by calculating: (1) the percentage of colonies containing female or male gonads at any stage of development (all polyps per colony), (2) numbers of gonads per polyp using 10 reproductive polyps, and (3) the size (area) of gonads per reproductive polyp.

An assessment of reproduction in injured colonies was conducted during October 1991 (10 d after September full moon), 53 mo after the oil spill. Sixteen colonies (0.6-1.3 m diameter) of *Siderastrea siderea* were sampled at one previously studied heavily oiled reef (PAYN). All colonies had a least 1/3 of their surface showing ongoing symptoms of stress (e.g., bleaching, swollen tissue) or recent injury. Two fragments of similar size (2.5 cm diameter) were collected from each colony using a small pneumatic drill, one fragment from the healthy portion and one fragment from the bleached portion of the colony. All samples were fixed, preserved, and decalcified following the above procedures. Ten polyps were randomly selected from each of the colony fragments, longitudinally dissected under a stereomicroscope, and stained directly with azocarmine-aniline blue. We measured size (length and diameter) of gonads (female and male), number of oocytes/gonad, and size (length and diameter) of oocytes.

7.5.5.2 Results

The assessment of oil effects on reproduction was found to be difficult for gonochoric coral species. We used mainly female gonads to measure colony fecundity (as indicated in Connell 1973; Giese and Pearse 1974). Numbers of colonies with gonads and numbers of gonads per colony were not sensitive to level of oiling, but gonad size varied significantly between oiled and unoiled reefs. The percentage of reproductive colonies was similar for all reefs (Fig. 7.30), indicating that gametogenesis occurred in almost all sampled colonies. Figure 7.31 shows the temporal pattern of gametogenesis for the species. No gametocytes of either sex were observed during February. Primary oocytes and spermatocytes were first found during March, and developed further during the rest of the year. Timing of spawning was similar in both years, with peaks in August and September. Although mature female gonads were observed after this period (October-December), spawning might not have occurred and gonads may have been reabsorbed. This was confirmed by measuring the size (area) of gonads during October (see below).

Numbers of female gonads did not differ significantly between oiled and unoiled reefs during the spawning peak (August-September; Fig. 7.31). Nested ANOVAs using oil and sites (*site* as nested factor under *oil*) as factors for each month showed no effect of oil on number of gonads/polyp, whereas the two oiled reefs were significantly different in September 1989 ($F = 10.31$; df = 2, 15; $P = .001$) and nearly so in September 1990 ($F = 3.29$; df = 2, 11; $P = .075$). It is not clear why male gonads/polyp at oiled reefs dipped below values at unoiled reefs in October-November 1989. Spawning is not completely synchronous, and it is noteworthy that the reverse pattern occurred in November 1990. Figure 7.32 shows results during the spawning period separated for each reef. Corals on one oiled reef, LRE2, had fewer female gonads than all the others (Fig. 7.32), including the heavily oiled reef PAYN. LRE2 is located at the mouth of Bahía Cativá and the channel of Isla Largo Remo lagoon (see Fig. 7.1). Both areas were heavily oiled during 1986, and continued to have (by 1988-1990) oil concentrations as high as 650 μg/g (in 1988) and 195,000 μg/g (in 1990) trapped in seagrass and mangrove sediments, respectively (see Burns, Chap. 3).

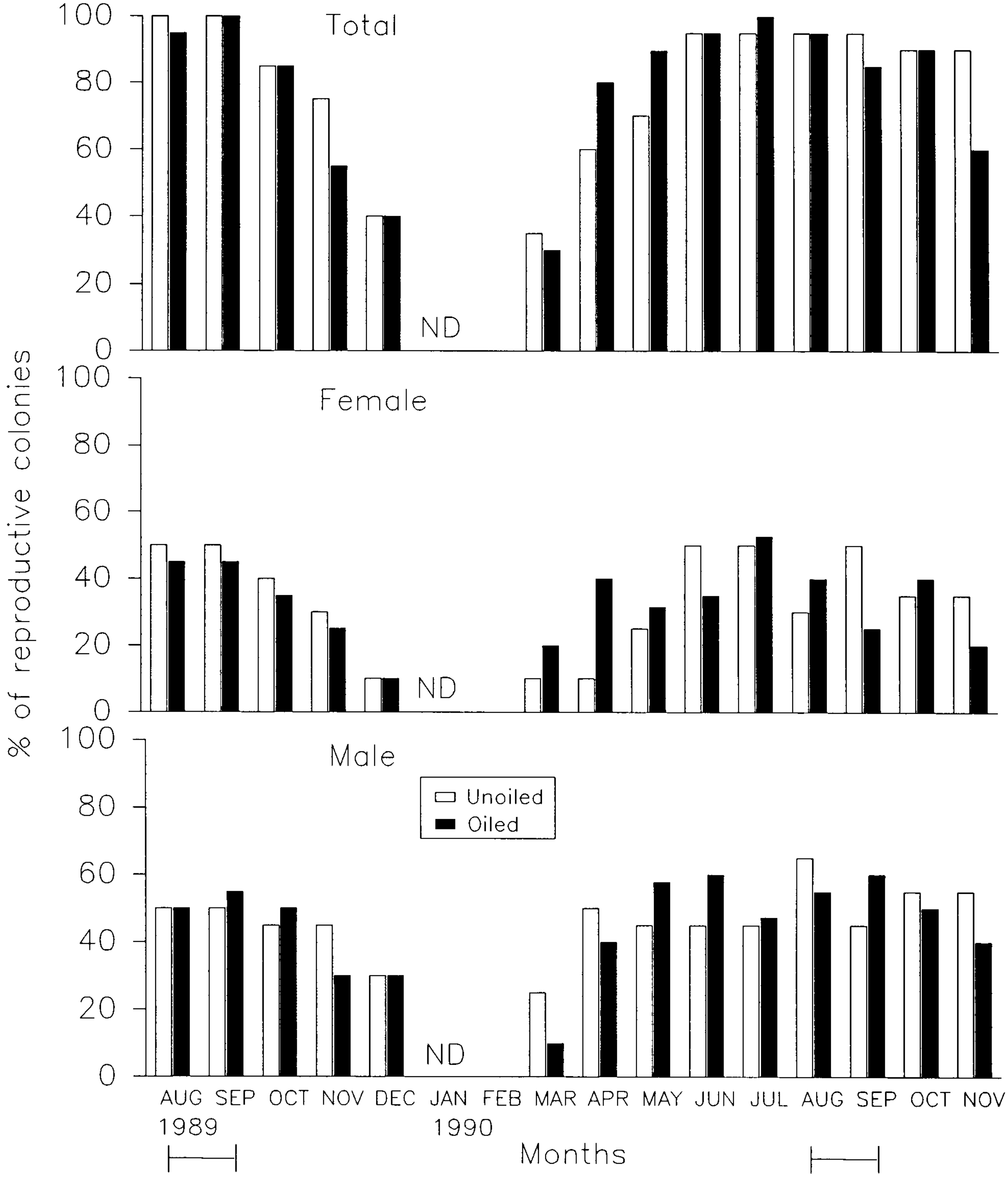


Fig. 7.30 Percentage of reproductive colonies of *Siderastrea siderea* from August 1989 to November 1990 in relation to the degree of oiling. *Total* indicates that all colonies were combined for both sexes. The *horizontal bars* indicate the August-September spawning peak.

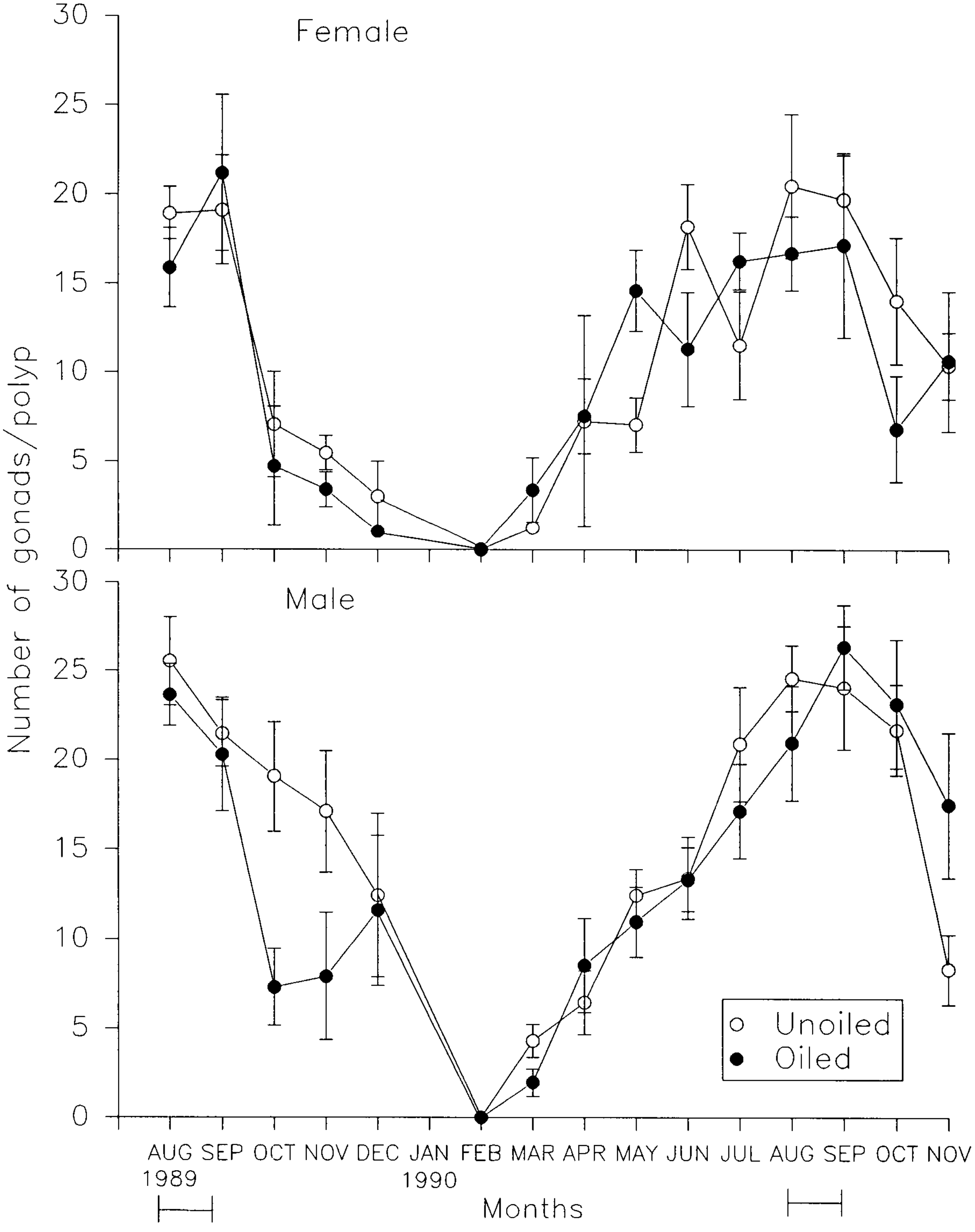


Fig. 7.31 Reproductive cycle based on mean number of gonads per polyp (10 polyps/colony; 10 colonies per reef; two reefs per oil category) of *Siderastrea siderea* from August 1989 to November 1990 in relation to the amount of oiling. SE is given for each mean.

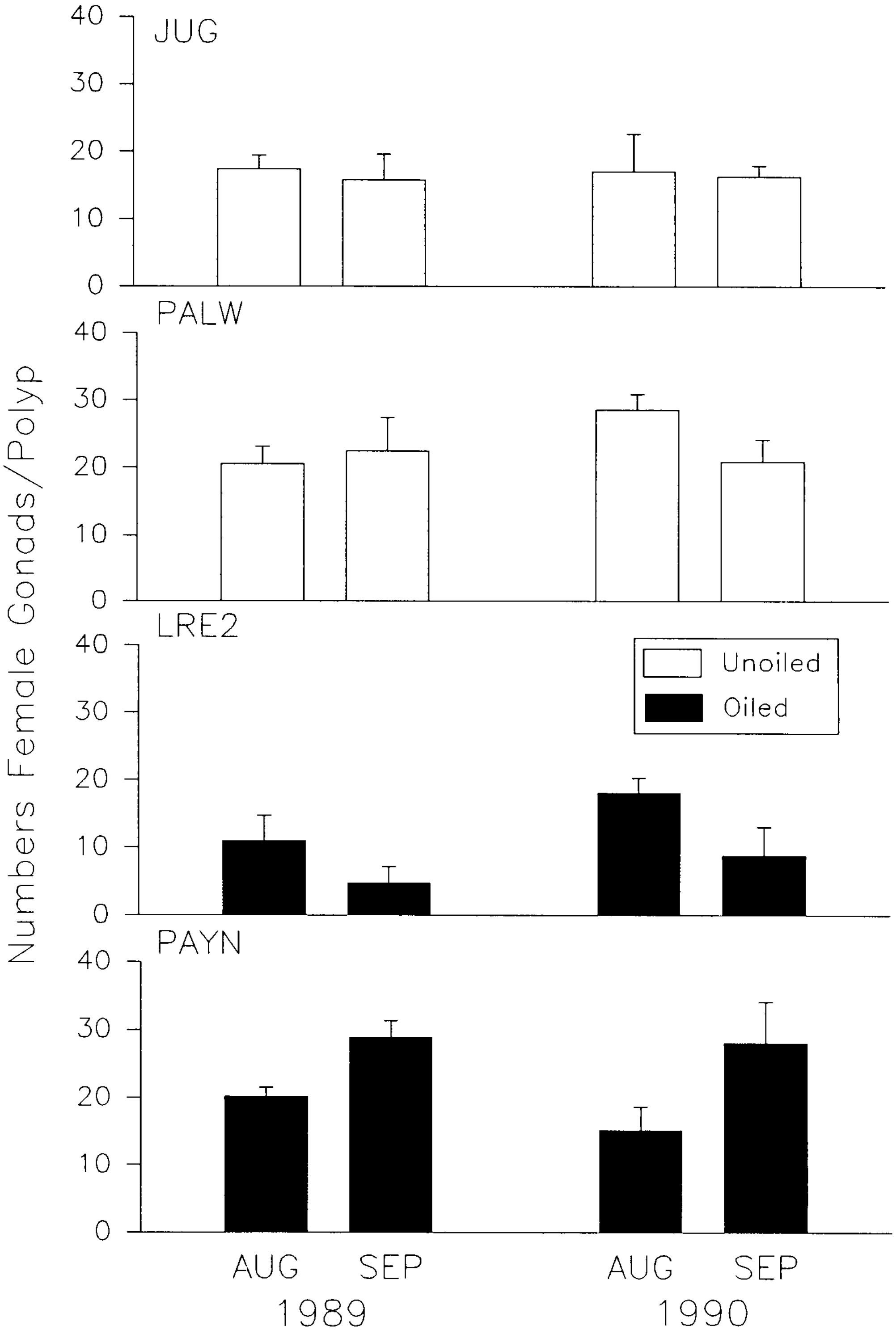


Fig. 7.32 Number of female gonads per polyp for *Siderastrea siderea* at individual reefs during the months of major reproductive activity in 1989 and 1990. SE is given for each mean.

In contrast to the above results, gonad size (area) was larger at unoiled than oiled reefs for most sampling periods (Figs. 7.33, 7.34). Female gonad size was significantly different for both years during September and in October 1989, and nearly significant for August 1989, again indicating an oil effect (Table 7.20). Male gonads were significantly larger at unoiled reefs during September and October 1989 (Table 7.20). It is important to note that almost all sampled female gonads were ripe (stage IV of development; Fig. 7.33). Thus, the decline in size of gonads for both years during October supports the hypothesis that spawning does not occur after September.

Colonies with recent injuries demonstrated a consistent decrease in colony fecundity relative to uninjured colonies. All parameters measured indicated a significant effect of injuries or bleaching on size of gonads, and the size and number of oocytes per gonad (Table 7.21). This reduction in gonad size (area) in association with recent injuries mirrors the small gonad size observed in healthy colonies at heavily oiled reefs (Fig. 7.34). Thus, the size of gonads is a more sensitive measure of long-term (more than 3 yr in this study) sublethal effects of oil on reproduction than is the number of gonads. In addition, small mature eggs may be less viable than larger ones, but this possible difference has to be tested.

Soong (1990) found that average size at puberty of *S. siderea* was 156 cm^2, and the smallest reproductive colony was 144 cm^2. Surveys conducted for recent injuries of corals (see Sect. 7.5.2) showed long-lasting effects of oil on oiled reefs. The increased number of injuries (Figs. 7.16-7.18) and associated reduction in colony size on oiled reefs may reduce the number of reproductively viable colonies (egg viability) in coral populations at Bahía Las Minas (as shown in Table 7.21), and ultimately reduce population survival.

7.5.6 Coral Recruitment

Loya (1975, 1976) suggested that recovery of coral communities affected by chronic oil pollution may be reduced by altering the viability and behavior of coral larvae, inhibition of larval settlement, or increased larval mortality. Also, changes in the distribution and abundance of other reef taxa after severe perturbations may affect patterns of coral recruitment, coral composition, and recovery (Sammarco 1980). High rates of coral recruitment tend to be associated with low algal biomass and high grazing pressure (Rylaarsdam 1983; Rogers et al. 1984).

7.5.6.1 Methods

We surveyed the four unoiled and six heavily oiled reefs (Table 7.1) for recent recruitment in September 1991. The total number of scleractinian recruits (new individuals apparently derived from sexually produced larvae and less than 4 cm diameter) at each reef were counted along 30-m^2 transects at two depths (30 quadrats each at 1.5-3 and 4.5-6 m) parallel to the shore.

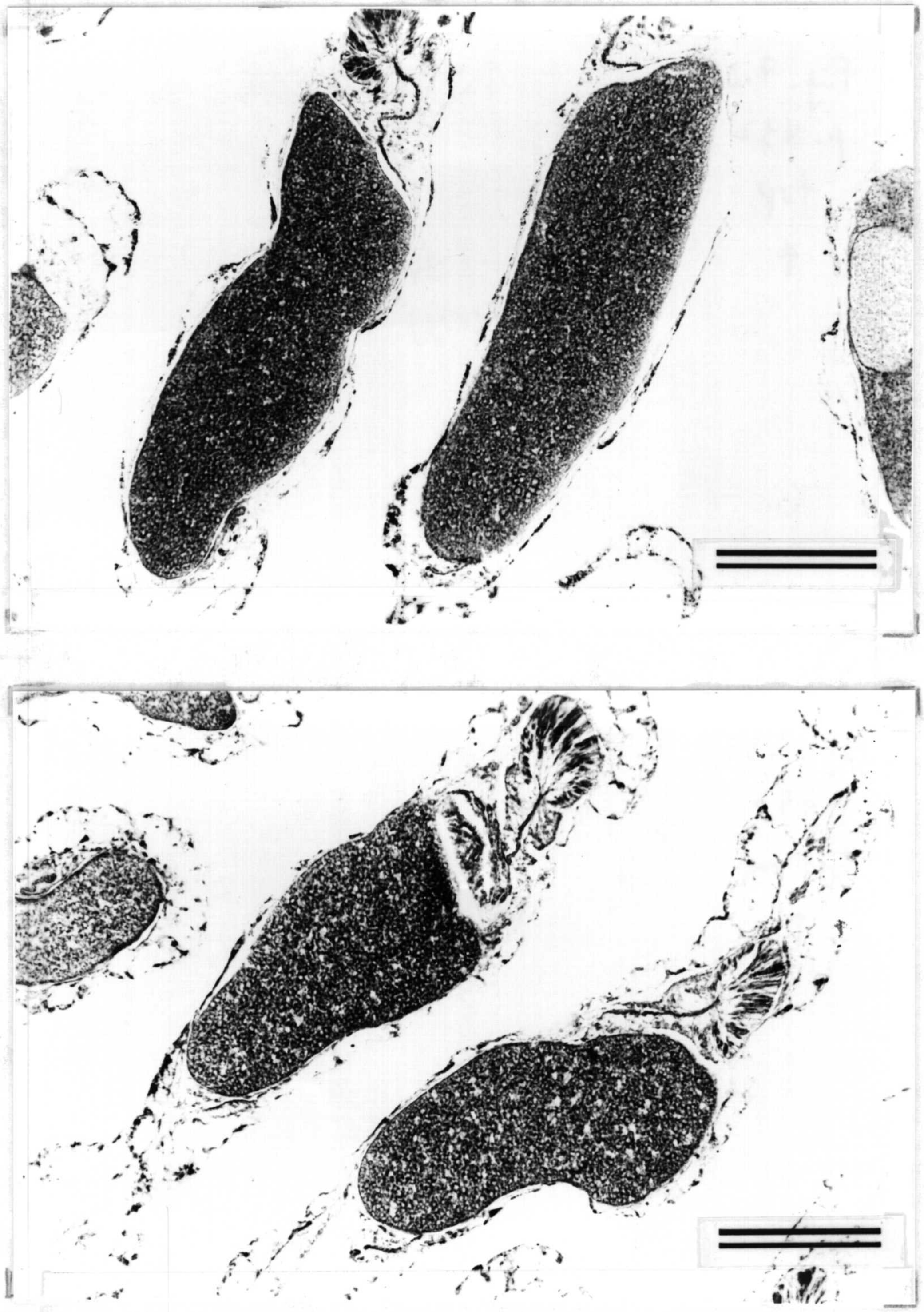


Fig. 7.33 Photomicrographs of histological preparations of *Siderastrea siderea* tissue showing cross sections of female gonads. Eggs are at stage IV of development at an unoiled reef (*top*) and a heavily oiled reef (*bottom*). Notice that egg area is greater at the unoiled reef. Specimens were collected at the same time. Scale bar = 100 µm.

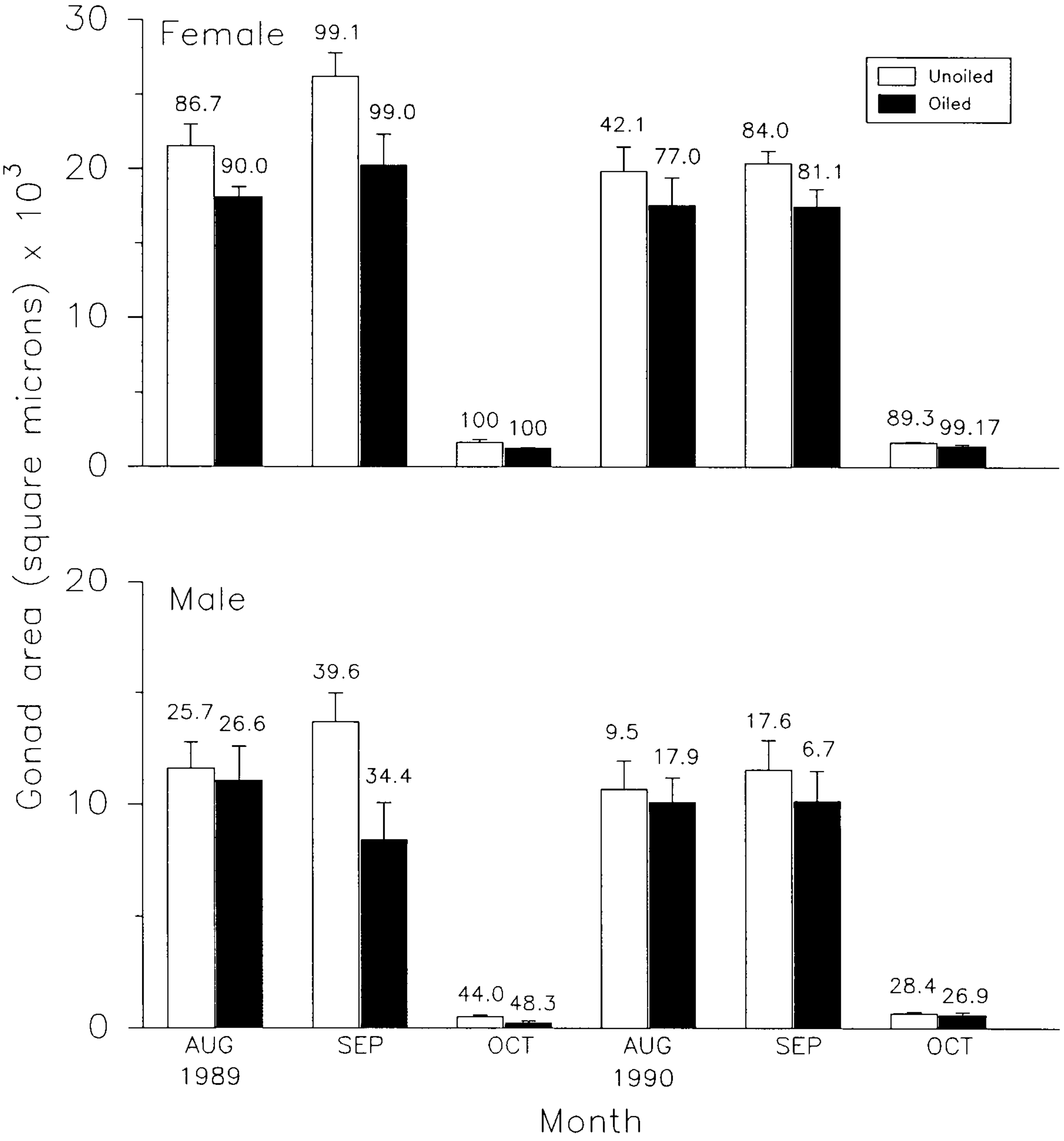


Fig. 7.34 Mean gonad area (and 1 SE) of *Siderastrea siderea* during the 3 mo of maximum reproductive activity in 1989 and 1990 in relation to the degree of oiling. The numbers at the top of the histograms indicate the percent of polyps per colony at stage IV of development.

Table 7.20 Analysis of variance for gonad size (area).

Sex		1989 Aug.	1989 Sep.	1989 Oct.	1990 Aug.	1990 Sep.	1990 Oct.
Female	*F*	4.18	7.31	7.25	1.04	7.48	3.49
Gonads	df	1, 15	1, 15	1, 7	1, 10	1, 11	1, 10
	P	.06	.02	.03	.3	.02	.09
Male	*F*	0.09	11.36	6.54	0.14	0.57	0.42
Gonads	df	1, 16	1, 17	1, 16	1, 19	1, 17	1, 17
	P	.8	.003	.02	.7	.5	.5

Table 7.21 Comparison of reproductive condition among healthy and bleached or injured portions of the same colonies ($N = 16$) of *Siderastrea siderea* at a heavily oiled reef (PAYN). Values are means (SE) for size of gonads (mm), number and size of oocytes per gonad, and matched *t*-test probability. Sex ratio was 1:1. Notice that all comparisons were significant.

Reproductive Parameter	Healthy	Bleached	*P*
Size of male gonads (length)	2.13 (0.18)	1.14 (0.23)	.02
Size of male gonads (diameter)	0.21 (0.01)	0.14 (0.03)	.03
Size of female gonads (length)	1.35 (0.17)	0.30 (0.12)	.000
Size of oocytes per gonad (diameter)	0.28 (0.02)	0.11 (0.04)	.001
Number of ova per gonad	2.01 (0.32)	0.39 (0.11)	.000
Size of ova per gonad (length)	0.67 (0.04)	0.29 (0.11)	.02

7.5.6.2 Results

Five-and-a-half years after the oil spill the number of recruits/m^2 (all coral species combined) was significantly higher at unoiled reefs than at oiled reefs ($t = 5.62$, df = 11.5, $P < .001$; Fig. 7.35). Eighteen species of recruits were identified, most of which are brooders (Fig. 7.36). Recruits of seven of the 18 species were not observed at heavily oiled reefs, including *Acropora palmata*. A similar pattern of recruitment was observed along a eutrophication gradient on the west coast of Barbados (Tomascik 1991).

7.5.7 Abundance of Herbivores

Herbivores have a major influence on recruitment, growth, and maintenance of coral populations (Bak and Van Eys 1975; Ogden and Lobel 1978; Sammarco 1980; Hay 1981; Bakus 1983; Hatcher 1983; Hay and Taylor 1985; Lewis 1986).

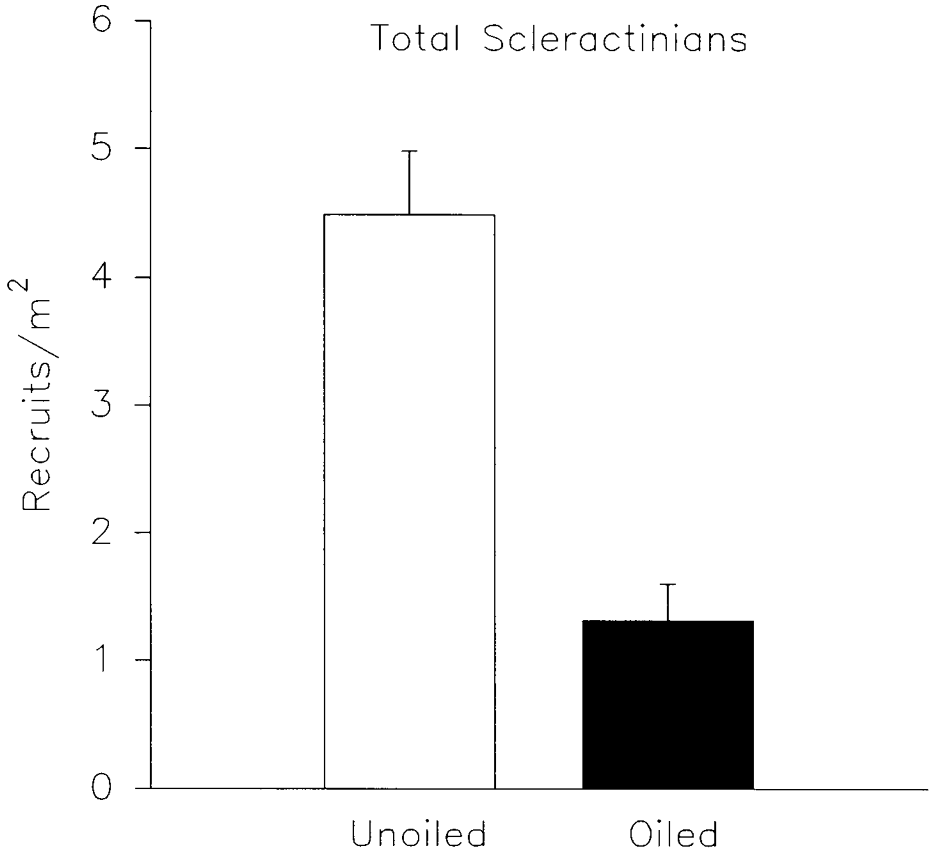


Fig. 7.35 Mean number of recruits (all scleractinians combined) per m^2 at unoiled and oiled sites. Total reef area surveyed was 480 m^2 and 720 m^2 at unoiled and oiled reefs, respectively. SE is given for each mean.

Under natural conditions, herbivores influence reef community structure by altering the balance between the abundance of corals and fleshy macroalgae. Grazing by echinoids can erode attachment sites of small and large coral colonies, resulting in dislodgment and mortality (Ogden 1977; Glynn et al. 1979), and may affect coral settlement and juvenile survival (Sammarco 1980). Abundant macroalgae can overgrow relatively slower-growing crustose coralline algae and corals (Hatcher 1983; Lewis 1986; Hughes et al. 1987), and may also reduce coral recruitment. Damselfishes modify effects of herbivores and prevent the monopolization of reef surfaces by competitively dominant macroalgae (Williams 1980). Damselfishes establish and maintain algal territories by excluding other herbivores and by nipping and killing live corals, so that space is prepared for the settlement and growth of filamentous algae (Kaufman 1977; Wellington 1982). Kaufman (1979) estimated that territorial damselfishes can garden more than 6% of reef surfaces every year. Hay

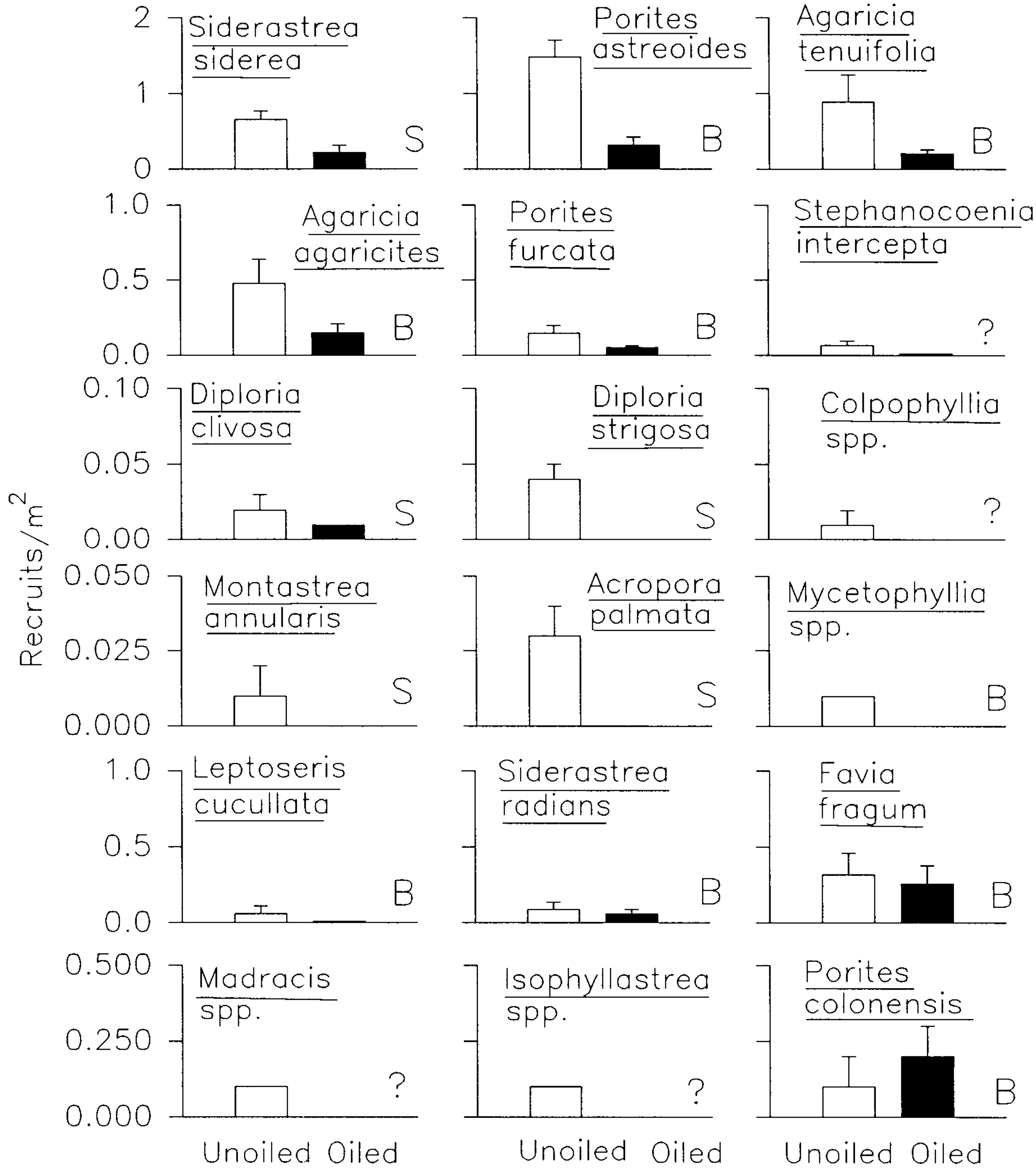


Fig. 7.36 Mean number of recruits per m^2 for all coral species observed at unoiled and heavily oiled reefs. *B* = broods larvae; *S* = spawns gametes, for all cases known (*?* = not known; from Szmant 1986; Soong 1991). SE is given for each mean.

et al. (1983) reported herbivory to be intense in areas of *Acropora palmata* and moderate in habitats of massive and *Porites* corals at Punta Galeta.

7.5.7.1 Methods

We considered four groups of herbivores during this study: sea urchins, parrotfishes (Scaridae), damselfishes (Pomacentridae), and surgeonfishes (Acanthuridae). Surveys were conducted once at the end of the 5-yr study to obtain a general idea of herbivore abundance and levels of herbivory.

At each of the unoiled and heavily oiled reefs the abundance of sea urchins was estimated along four different 1 x 30 m transects at the following depths: 0.5-1 m, 2-4 m, 5-7 m, and lower slope). Four species were counted: *Diadema antillarum*, *Eucidaris tribuloides*, *Echinometra lucunter*, and *Echinometra viridis*.

Abundance of grazing reef fishes was estimated by swimming along depth contours at 1-2 and 5-7 m, and recording the total number of fishes observed within a 6 x 100 m transect. Damselfish densities were estimated along 2 x 100 m transects. Fishes were recorded only at the level of families. Three censuses per transect were made in a single day, normally in the mornings (0900-1200) at each of the unoiled and heavily oiled reefs.

Log-transformed data were used to run 2-way ANOVAs, using oil and depth as factors.

7.5.7.2 Results

Sea urchins were more abundant on heavily oiled reefs, but the differences were not significant (Fig. 7.37; Table 7.22). Large, mixed schools of surgeonfishes and parrotfishes were commonly observed at unoiled reefs. Although fishes were not identified to species, the limited surveys suggested that diversity was similar at unoiled and heavily oiled reefs. However, the relative abundance of nonterritorial surgeonfishes and parrotfishes was significantly higher at unoiled reefs than oiled reefs, whereas damselfishes were more abundant at oiled reefs (Fig. 7.38; Table 7.23). Amesbury (1981) documented a similar pattern in relation to sedimentation stress.

7.6 Discussion and Conclusions

7.6.1 Does Oil Affect Reef Corals?

Results of this study demonstrate by several methods deleterious effects of oil pollution on Caribbean reef corals. These effects include the following.

1. A great reduction of live coral cover, abundance, and diversity down to 6 m depth immediately after the spill (Figs. 7.7-7.12).

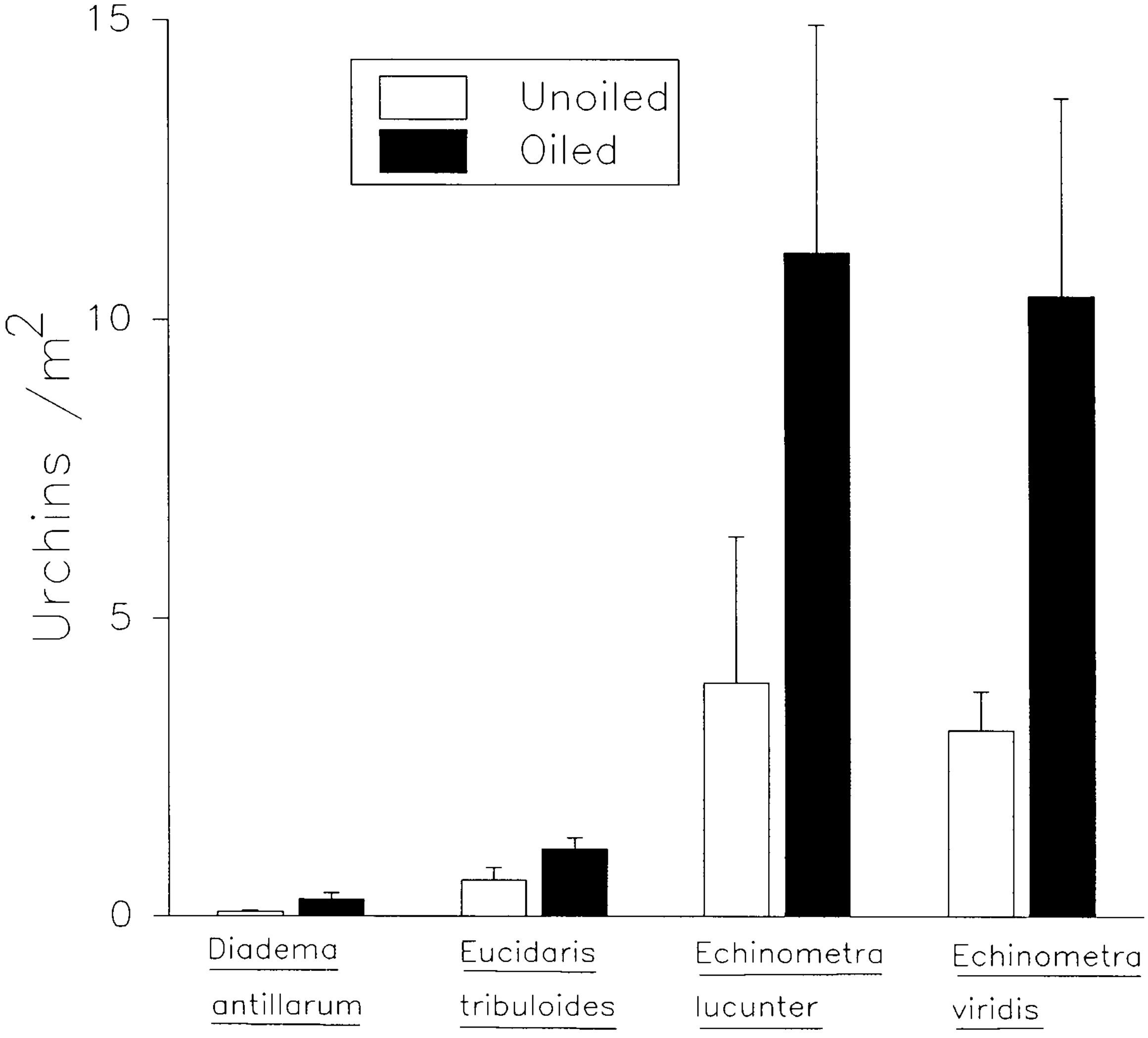


Fig. 7.37 Relative abundance of four species of sea urchins in relation to the degree of oiling. Censuses were done in 1990. Mean densities are shown with 1 SE.

Table 7.22 Paired *t*-test for abundances of four species of sea urchins between oiled and unoiled reefs. Data were log(*x* + 1)-transformed.

Species	*t*	df	*P*
Diadema antillarum	-2.37	7	.05
Eucidaris tribuloides	-1.13	7	.3
Echinometra lucunter	-1.88	7	.1
Echinometra viridis	-2.00	7	.09

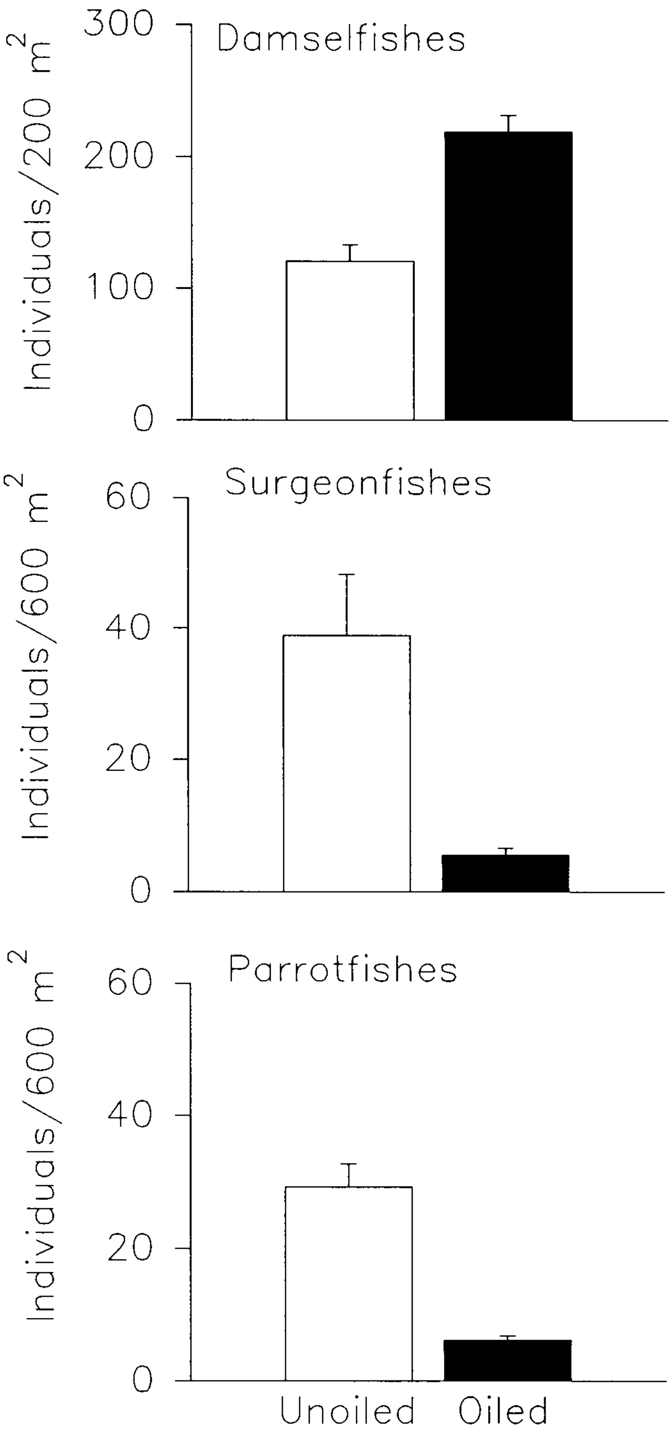


Fig. 7.38 Relative abundance of three families of reef fishes in relation to the degree of oiling. There was one census during 1991. Mean densities are shown with 1 SE.

Table 7.23 Paired *t*-test for abundances of three families of reef fishes between oiled and unoiled reefs. Data were log(x + 1)-transformed.

Group	*t*	df	*P*
Damselfishes	-4.05	8	.003
Surgeonfishes	5.71	8	.000
Parrotfishes	8.28	8	.000

2. A strong, positive correlation between the frequency and number of injured corals in relation to oiling and, more directly, to the concentration of hydrocarbons in the environment for some species (Figs. 7.16, 7.17, 7.18, 7.19, 7.21). This high percentage of injured colonies reduced individual and population fecundity on oiled reefs (Fig. 7.34; Table 7.21; see below).

3. A decrease in coral growth rates on oiled reefs during the year of the spill and 3 yr thereafter. There was a negative correlation between growth of two coral species and the concentration of oil in reef sediments during the year of the spill (1986) and 2 yr afterward (Figs. 7.26-7.28).

4. A decline in fecundity of the important reef-building coral *Siderastrea siderea* on oiled reefs, as measured by size of gonads in surviving colonies 5 yr after the oil spill (Figs. 7.33, 7.34). Furthermore, corals with recent injuries showed impaired reproductive activity (Table 7.21).

5. Little prospect for rapid reef recovery, as indicated by very low coral recruitment rates on heavily oiled reefs (Figs. 7.35, 7.36).

7.6.2 Effectiveness of Different Assays of Environmental Effects on Coral Reef Communities

Different techniques vary greatly in utility for assessment of environmental effects on coral reefs (Brown and Howard 1985; Brown 1988). Community measurements of cover or diversity may be insensitive and slow to respond to all but extreme disturbances, whereas results of experiments may fail to scale up to realistic conditions, and assays of sublethal effects require basic knowledge of coral biology that is generally unavailable.

The present study confirmed the danger of inference based on laboratory or small-scale field experimental studies of effects of oil on corals (reviewed in Brown and Howard 1985). One important reason for this is the problem of temporal scale (Spies 1987). In experiments, oil is typically removed after a short period (Loya and Rinkevich 1980; Knap et al. 1985; Knap 1987; Capuzzo 1987; Spies 1987). In contrast, oil may persist in protected coastal environments long after a major spill

(Vandermeulen 1982; Teal et al. 1992; Burns, Chap. 3). This persistent pollution reflects the time required to flush oil from the fine sediments of mangrove forests and seagrass beds, and from porous reef framework, habitats which occur along >80 km of convoluted coast within Bahía Las Minas (Figs. 7.1, 7.6). The oil trapped in mangrove sediments (average in cores was 191,000 μg/g and 13,400 μg/g [UVF] at 0-2 cm and 18-20 cm, respectively) still contained high concentrations of relatively toxic fractions (dibenzothiophene, phenanthrene, and higher series) 4 yr after the oil spill (Burns, Chap. 3).

Experiments to test effects of such chronic oiling must apply oil with seawater over many months or years (Bayne et al. 1982; Capuzzo 1987). For example, *Stylophora pistillata*, subjected to such conditions, suffered considerable depression of reproduction comparable to that on a chronically oiled reef in the Gulf of Eilat (Rinkevich and Loya 1979). Moreover, no mortality or injury occurred during the first 2 mo of that experiment, but 80% of the corals chronically exposed to oil died after 2 to 6 mo of oiling compared to only 10% of the controls (Rinkevich and Loya 1979). This pattern matches the delayed and extended patterns of injury and reduced sexual reproduction we have observed at heavily oiled reefs in Panama. Similar depression of growth and physiological deterioration resulting from chronic low levels of oiling have been shown for several bivalves (Anderson et al. 1983).

The approaches used in this study differ in their difficulty and effectiveness for detection of responses of corals to oil, particularly with regard to possible differences among species. Most difficult and least effective are surveys of distribution and abundance to measure changes in populations and communities. These surveys require extensive prespill data for oiled and unoiled reefs; otherwise it is necessary to wait several years to detect changes in abundance patterns over time (Green 1979). The natural patchiness of coral distributions on most reefs (Hughes and Jackson 1985; Guzmán et al. 1991) is also a major problem. High within-group variance in almost any population characteristic requires large numbers of replicate reefs, as well as transects or quadrats on each reef, for each treatment. This can require prohibitively large amounts of time underwater. These problems are further confounded by the temporal instability of most reef communities owing to almost certain covariation among predators, disease, competitors, physical disturbances, and pollution (reviews in Hutchings 1986; Brown 1987; Rogers 1990; Jackson 1991). These uncertainties are universal in studies of natural populations (Clark 1982; Carney 1987). Nevertheless, measurement of population and community parameters is essential as the only assay of the consequences of oil pollution for an ecosystem (Loya 1976; Bak 1987).

Monitoring community composition would have been far more effective and useful if combined with repeated observations of marked corals or fixed quadrats (Hughes and Jackson 1985; Guzmán 1986; Knowlton et al. 1990). Such methods are necessary to obtain data on changes in processes affecting coral populations (spatial competition, grazing, disease, sedimentation, etc.) that cannot be obtained from randomized transect methods.

The simplest and most sensitive technique was the survey of recent injuries. Healing of wounds by corals is known to occur relatively quickly on unoiled reefs (Bak et al. 1977; Bak and Steward-Van Es 1980; present study). Thus, changes in the numbers and sizes of recent injuries are more likely to reflect conditions of the moment, including levels of oil pollution, than abundance and retrospective analyses of growth. Moreover, although natural variance is high due to the myriad causes of injuries, observations can be made easily and quickly, so that replication is not demanding of field time underwater.

Reproductive condition during the spawning season (by the dissection method) and survey of recruitment onto natural substrata also provided simple, yet highly sensitive measures of effects of the oil spill. These data are of particular importance for projection of possible scenarios of reef community recovery (Sect. 7.6.4).

Problems of high variance and adequate replication also complicate measurement of the effects of oil on growth rates of corals in the field using sclerochronology (Hudson et al. 1976), but the potential for powerful retrospective analysis is worth the effort (Dodge and Vaisnys 1980). Growth of corals is notoriously variable, both between environments and over time (Dodge and Lang 1983; Dodge and Brass 1984; Hubbard and Scaturo 1985). Medium- to long-term variations in growth between reefs and years require large numbers of replicate corals for each species and treatment (i.e., habitat type or form of pollution) over many years. A better approach to measuring growth effects would be the construction of master-index chronologies of growth for populations of large corals using several cores that span at least 50 yr (Dodge and Vaisnys 1980). This procedure can help to factor out local variations in growth from coral to coral, due to considerable differences we have seen among individuals on the same or nearby reefs (Fig. 7.25), while preserving variance due to pervasive changes in environment (climate change, dredging, oil spills, etc.).

A final statistical issue is that virtually all observations to test for biological effects of oil involve repeated measures on the same corals or reefs, and thus require special statistics for that purpose (Underwood 1981; Hand and Taylor 1987). This is different from procedures appropriate for most other types of environmental studies, such as remote sampling of level bottoms on continental shelves, where the same spot is not repeatedly sampled except by chance (Carney 1987).

7.6.3 General and Specific Oil Effects

The oil spill affected various coral species very differently. The elkhorn coral *Acropora palmata* suffered much more (Figs. 7.7, 7.8) at the two oiled reefs than other common species, most of which are massive. Elkhorn corals also have not started recruiting onto oiled reefs. These results lend further support to the claim that branching corals are most sensitive to human disturbance (Brown and Howard 1985). There were no statistically significant differences related to oiling in abundance, size, or cover among the common massive species, except for the reduction in cover of *Porites astreoides*. However, means for most variables are low and variances high due to the natural patchiness of coral distributions. Thus, the survey data cannot be used to distinguish any but the most striking contrasts.

Recent injuries were most frequent for *S. siderea* and least for *Porites astreoides*. This was surprising in light of earlier experimental evidence that the presence of oil did not affect the ability of *S. siderea* to rapidly remove sand from the colony surface, whereas *P. astreoides* rejects sand, with or without oil, very slowly (Bak and Elgershuizen 1976), and was reported to suffer extensive mortality in relation to dredging in Curaçao (Bak 1978). A possible explanation lies in the differing abilities of the two species to reject silt; *S. siderea* rejects silt at only 35% of the rate for sand, whereas *P. astreoides* cleanses itself of silt more than 10 times faster than sand (Bak and Elgershuizen 1976). The critical missing data, therefore, are for effects of oil on rejection rates of silt, because siltation rates are high in Bahía Las Minas (Sect. 7.2.2). Differences may also be due to delayed effects of oil or the presence of chronic oil slicks or both.

The association of oil with decreased growth was different from that for increased injury. *Porites astreoides*, *Montastrea annularis*, and *Diploria strigosa* showed sharp reductions in growth in the year of the oil spill, whereas *Siderastrea siderea* showed no effect of oil on growth during 1986 (Fig. 7.26). However, when growth 3 yr before and after the spill was compared (Fig. 7.27), *S. siderea* showed a significant decrease in linear growth, suggesting a possible delayed effect of oil for this species. Decrease in *D. strigosa* growth (Fig. 7.26) is different from results of short, one-time exposure to oil in field experiments (Dodge et al. 1984; Knap 1987). However, experiments in Curaçao showed that, unlike other species tested, *D. strigosa* was 2.7 times slower in clearing oiled sand than similar unoiled particles (Bak and Elgershuizen 1976).

7.6.4 Reef Recovery

Recovery from a disturbance requires the unlikely assumption that the community will not have changed for other reasons during the interval (Sutherland 1974; May 1977; Connell and Sousa 1983). Indeed, most coral populations exhibit considerable temporal instability due to effects of predators, disease, competitors, and physical disturbance (Connell 1978; Bak and Luckhurst 1980; Davis 1982; Knowlton et al. 1990; Jackson 1991). The general decline in coral populations at the four control reefs between 1986 and 1988 is a case in point. It may be related to the demise of the sea urchin *Diadema antillarum* in 1983-1984 (Lessios 1988), associated increases in abundance of macroalgae that overgrow corals (Hughes et al. 1987), epidemic disease of acroporid corals (Gladfelter 1982), and coral bleaching (Williams and Bunkley-Williams 1988). All of these events have been observed on Caribbean reefs of Panama during the last 5 to 10 yr, but the extent of their effects and interaction is poorly understood (D'Elia and Taylor 1988).

Processes that may affect the response of corals to a major disturbance include recruitment of new individuals from unaffected areas, the abundance and condition of survivors, and the nature and intensity of biological interactions and physical disturbance affecting their growth, survival, and reproduction. We can, therefore, estimate minimum times of recovery based on what is known about rates of larval recruitment and population growth, and our results.

Recruitment of juvenile corals onto Caribbean reefs is slow (Bak and Engel 1979; Rogers et al. 1984; Rylaarsdam 1983; Hughes 1985; Tomascik 1991), even without possible reductions due to chronic oil pollution as exists now throughout Bahía Las Minas. Half to 90% of these recruits are typically agariciids and poritids. Reproduction and larval biology of these groups have been studied, and both brood planula larvae, which are released, often on a lunar cycle, throughout much of the year (Fadlallah 1983; Szmant 1986; Soong 1990, 1991). In contrast, recruits of the other common coral species, *Acropora palmata*, *Siderastrea siderea*, *Diploria* spp., and the *Montastrea annularis* complex, are usually very rare. All these spawn gametes that are fertilized externally and undergo an undetermined period of development in the plankton (Fadlallah 1983; Szmant 1986; Soong 1990, 1991). Recruitment at Bahía Las Minas is low, as observed during our surveys, 5.5 yr after the oil spill. Only 10 coral species were recorded, of which eight were brooding species (Fig. 7.36). Spawning is seasonal, roughly July-November in Panama (Soong 1991), which includes most of the rainy season (Fig. 7.4).

Oil in coral tissues inhibits gonad development and reproduction. This has been demonstrated for brooding species (Rinkevich and Loya 1977, 1979; Loya and Rinkevich 1979; Peters et al. 1981) and broadcasting species even 3 yr after the oil spill (Sect. 7.5.6; Guzmán and Holst 1993). To the extent that reproduction does occur within Bahía Las Minas or that larvae are transported in from elsewhere, the common occurrence of oil slicks may affect differentially the reproductive success and recruitment of brooding and broadcasting corals. Gametes of spawning species are released during the rainy season (Figs. 7.4, 7.31) when oil slicks are common, whereas brooding species release planulae throughout the year, including times when oil slicks are absent or infrequent (Soong 1991). Moreover, gametes of most spawning species tend to rise to the surface just after spawning (Harrison et al. 1984; Szmant 1986), where they are more likely to encounter oil, and their larvae spend one to several weeks in the plankton before attaining competence to settle (Fadlallah 1983; Jackson 1986; Richmond and Hunter 1990). Brooded planulae may also rise to the surface or settle directly within a few hours or 1-2 d of release (Fadlallah 1983; Richmond and Hunter 1990), which should decrease chances of encountering oil slicks. Oil induces premature expulsion of planulae (Loya and Rinkevich 1979) and probably gametes. Although *S. siderea* showed a high percentage of reproductive colonies (Fig. 7.30), gonads were small during the spawning peak (September) at oiled reefs (Fig. 7.34), suggesting lower fertility due to stress.

Gonad size may be a sensitive indicator of coral viability. Lipids are the main storage product in coral eggs, and spawned eggs have a calorific value similar to pure lipids (B. Willis, unpubl. data in Harrison and Wallace 1990). Lipids can be reduced rapidly (less than 15 d) in corals during periods of stress (Clayton and Lasker 1982; Glynn et al. 1985). In Bahía Las Minas corals are under chronic stress (e.g., oil and siltation), and we can expect surviving corals to divert resources away from sexual reproduction to other functions, particularly regeneration of injuries and sediment removal (Harrison and Wallace 1990).

The small size of surviving colonies (Fig. 7.20) and the high percentage of colonies showing recent injuries (Figs. 7.17, 7.18) affect the viability of coral populations. Fecundity increases with colony size (Szmant-Froelich 1986; Babcock 1986), and polyps close to recent injuries or peripheral tissues of the colony have lower fecundity (Wallace 1985; Chornesky and Peters 1987). Soong (1990, 1991) described this pattern for several Panamanian corals, and showed that decreased reproduction near colony margins was due to wounds or competition rather than differences in the age of the polyps. Likewise, we observed that both size and numbers of eggs in *Siderastrea siderea* were reduced within injured colonies compared to healthier areas (Table 7.21).

In summary, assuming larvae are available at least some of the time, and that sexual reproduction is the primary mode of repopulation by corals in Bahía Las Minas, we would expect a slow shift toward greater relative abundance of brooding species (Fig. 7.36), including agariciids, poritids, and perhaps small species like *Favia fragum* (Esper) and *Siderastrea radians* (Pallas). This suggests that several decades might be required to develop populations similar to those before the oil spill (based on data in Hughes 1985; Rogers et al. 1984; this study). Alternatively, repopulation may occur chiefly by growth and asexual proliferation of surviving portions of colonies (Highsmith 1982*b*; Rogers et al. 1984; Hughes 1985), which are rare but do occur on oiled reefs. Assuming these remnants continue to survive and grow at normal rates, which is less likely for smaller colonies than for larger colonies even in the absence of oil (Figs. 7.17-7.19; Hughes and Jackson 1985), equivalent populations might become established in 10-20 yr. These estimates are minimal, however, based on the unlikely premise that chronic oil pollution and other events will not further depress coral populations, as occurred at unoiled reefs between 1986 and 1988.

7.6.5 Conclusions

Effects of the spill on corals in Bahía Las Minas were severe, and populations have not begun to recover. Levels of injury are still as high as those immediately postspill, sexual reproduction and larval recruitment are reduced, and there has been virtually no recruitment of most formerly dominant coral species. Moreover, increased erosion and sedimentation from degraded mangroves (Teas et al. 1989; Duke and Pinzón 1991; Duke and Pinzón, Chap. 8) and seagrass beds (Marshall 1991; Marshall et al., Chap. 10; Steger et al. 1991; Steger and Caldwell, Chap. 6) suggest that populations will be reduced even further. Likewise, reduced populations of surgeonfishes and parrotfishes imply that macroalgal populations will increase and overgrow the few surviving corals.

Beyond this generally grim picture, it will be impossible to make more precise projections without data on the rates and magnitude of processes affecting coral recruitment, growth, survival, and reproduction comparable to that being employed to reveal the dynamics and consequences of reef community change in response to outbreaks of the crown-of-thorns starfish *Acanthaster planci* (Done 1985, 1988, 1992). This means repeated and frequent monitoring in fixed quadrats of corals, algae, predator and grazer abundances and interactions, and incidence of new physical disturbances, coupled with experiments to test effects of specific processes (D'Elia and Taylor 1988). This monitoring should be conducted over a large enough area to discriminate localized phenomena such as the oil spill from changes on a regional scale.

8
Mangrove Forests

Norman C. Duke and Zuleika S. Pinzón M.

8.1 Abstract

The mangrove forests of Bahía Las Minas, Panama, have been studied closely starting approximately 3 yr after the 1986 oil spill to gauge both its effects and the status of habitat recovery. This assessment is based primarily on trees, the basic structural component of the habitat, with studies focused on their structure, recruitment, and growth. The main effect of the spill was obvious because large areas of trees died within 2 mo of oiling. However, more subtle changes extended into surrounding forests, as well as affecting seedling growth in 6-yr-old gaps in the canopy caused by the spill. Apparently, these more subtle effects were caused by residual oil, which lies in pockets in anaerobic sediments, protected from weathering until it resurfaces.

The primary effect of the oil spill was clearly shown by the extent of deforestation, which totaled 64 ha, 7% of the mangroves in the bay. This deforestation chiefly involved just one species, *Rhizophora mangle*. Areas of deforestation were determined from vegetation maps traced from aerial photographs taken before and after the spill. They revealed not only the extent and location of gaps in the canopy after the oil spill, but also particular patterns that appear to characterize deforestation caused by large oil spills. Recognition of these patterns in older photographs showed, for the first time, that a major oil spill in 1968 also caused extensive deforestation. In this case 46 ha, 5% of the area of mangroves, were destroyed. The 1968 spill resulted from the sinking of the tanker *Witwater*, and although there was a substantial biological effect reported for the supralittoral fauna, there were no reports of die back in mangrove forests. The recognition of these much older canopy gaps provided a set of comparable sites, which formed the basis of a recovery model covering two decades.

Mature trees surviving the initial oiling in 1986 suffered canopy deterioration, showing lower levels of mean annual leaf biomass (down 23-33%) and leaf numbers per shoot (down 12-17%); oiling appeared to have reduced their ability to support canopies. This reduction possibly reflects an equivalent loss of feeding roots because of the remaining oil in sediments. In this scenario, trees may shed leaves to compensate for loss of roots, leading to loss of shoots in the upper canopy, as measured in shoot studies and as additional wood litter fall. If this is the case, these factors had not stabilized at oiled sites, and numbers of leaves per shoot continued to decline in the second year of the study, 1990-1991, 4 yr after the spill. Trees were apparently still adjusting their growth to postspill conditions, budgeting total leaf area and carbon-fixation processes with prespill growth structures. Surviving forests

adjacent to deforested areas therefore appeared to be unstable and vulnerable, and had not recovered 6 yr after the spill.

In oiled canopy gaps, seedling recruitment and growth 5 to 6 yr postspill was extremely variable, ranging from prolific to nonexistent at particular sites. Sheltered sites had apparently normal recruitment, and seedling growth also appeared normal. However, detailed comparative measurements indicated significant growth suppression in sediments with high concentrations of residual oil. In contrast, survivorship was extremely low at exposed sites, and the presence of oil was less important because erosion had removed 1-2 cm of surface sediments, and huge drifting logs scoured the surface. The fate of these sites appears inevitable, and in another year damaged fringes are expected to retreat permanently as many of the remaining survivors succumb. Collectively, mature trees usually form a strong barrier at exposed sites, forming a complex tangle of strong and looping prop roots. However, there was insufficient time for new recruits to develop such protection before the once-protective dead trees finally rotted away.

Recovery of oiled gaps was reportedly assisted by a massive planting effort, in which most gaps around the bay were planted with *R. mangle* seedlings in sods of unoiled upland soil. These seedlings often grew well, but general site recovery was not improved; planted sites had significantly fewer seedlings than sites of no planting. Despite the further damage of the planting effort, all sheltered oil-spill gaps are expected to recover, while most exposed sites are not. The latter require a different kind of assistance, such as protecting seedlings and young trees from physical damage while they become self supporting. Otherwise, prospects for recovery of exposed sites are not good, and areas are expected to be lost in the same way as comparable gaps created by the tanker *Witwater* spill two decades earlier.

This study provides a detailed assessment of effects on and long-term recovery of mangrove forests following the 1986 spill in Panama, as well as a preliminary assessment of the 1968 spill. Our findings identify serious primary and long-term effects, suggesting that responses to tropical oil spills must concentrate on keeping mangroves free of oil. Because oiling of mangroves is often unavoidable, these findings suggest strategies for minimizing damage and long-term effects. In particular, mangrove fringes damaged at exposed coastal sites need special protection, especially considering the trade-offs faced by spill-response decision-makers in directing limited resources for protecting particular sections of coastline. If this approach fails, it may be necessary to clean individual trees, concentrating first on exposed stands. If trees die, a possible solution is to install protective barriers while seedlings recolonize the site. Where natural recruitment fails, planting could be done carefully, but only to supplement natural processes because it is clear that misdirected assistance can retard recovery and promote further deterioration.

8.2 Introduction

8.2.1 Mangroves and Large Oil Spills

Mangrove forests comprise a diverse group of specialized rain-forest species from 20 plant families throughout the world (Duke 1992). These species are mostly trees that grow along subtropical and tropical seashores within a very restricted vertical range between mean sea level and highest spring tides. In the Caribbean Sea, this range is often less than 0.5 m, but this belies the potential areal extent of mangroves, which sometimes extends many kilometers in width, especially near large estuaries and rivers. In this environment, mangroves have an influential role interacting with, and buffering between, terrestrial and marine habitats. Hence mangroves might be alternately viewed as sinks for land runoff and protection for coastal margins from erosion to sites of high primary production and nursery grounds for local and offshore fisheries (see, e.g., Lugo and Snedaker 1974; Cintrón and Schaeffer-Novelli 1983).

Because sea level is constantly fluctuating, this zone is dynamic and progressively changing on both the short and long term. This variability implies that mangrove species are able to accommodate to changes, and, not surprisingly, mangroves display a unique combination of adaptations compared with upland counterparts (e.g., Tomlinson 1986). These include the ability to grow in salt water, specialized root structures for both physical support and for breathing air, buoyant propagules for dispersal by sea currents, and numerous other physiological adaptations. The latter are largely designed to cope with high concentrations of salt in the sap; for example, several species exude salty, perspiration-like droplets through tiny pores on leaf surfaces.

Also important are specialized attributes promoting establishment success. These features are most apparent in the important mangrove genus, *Rhizophora*, and entail highly developed large, buoyant propagules. *Rhizophora* is viviparous in that seeds germinate and continue to grow while the fruits remain attached to the parent tree. This growth is rapid, utilizing the resources of the parent tree (Pannier and Pannier 1975), so that when the propagule drops off, it has a well-advanced primary root structure and large amounts of food reserves. Some of these attributes enable propagules to drift at sea for long periods, but they are perhaps more importantly designed to promote rapid development as soon as conditions are suitable. Propagules do this by quickly putting down holding roots when they become stranded during low tide (Fig. 8.1). But at certain sites, repopulation may be delayed when older trees die or when sites are disturbed and damaged.

Large oil spills in tropical coastal areas may disrupt these processes and cause serious problems for mangrove forests. For example, when exposed roots, lower trunk, and surrounding substratum become oiled, many trees die from either suffocation or toxicity, depending on the type and condition of oil (see Getter et al. 1984; Evans 1985). This may occur chiefly when oil, perhaps spilled offshore, floats in on a rising tide driven by wind and waves, and remains after the tide ebbs. Many

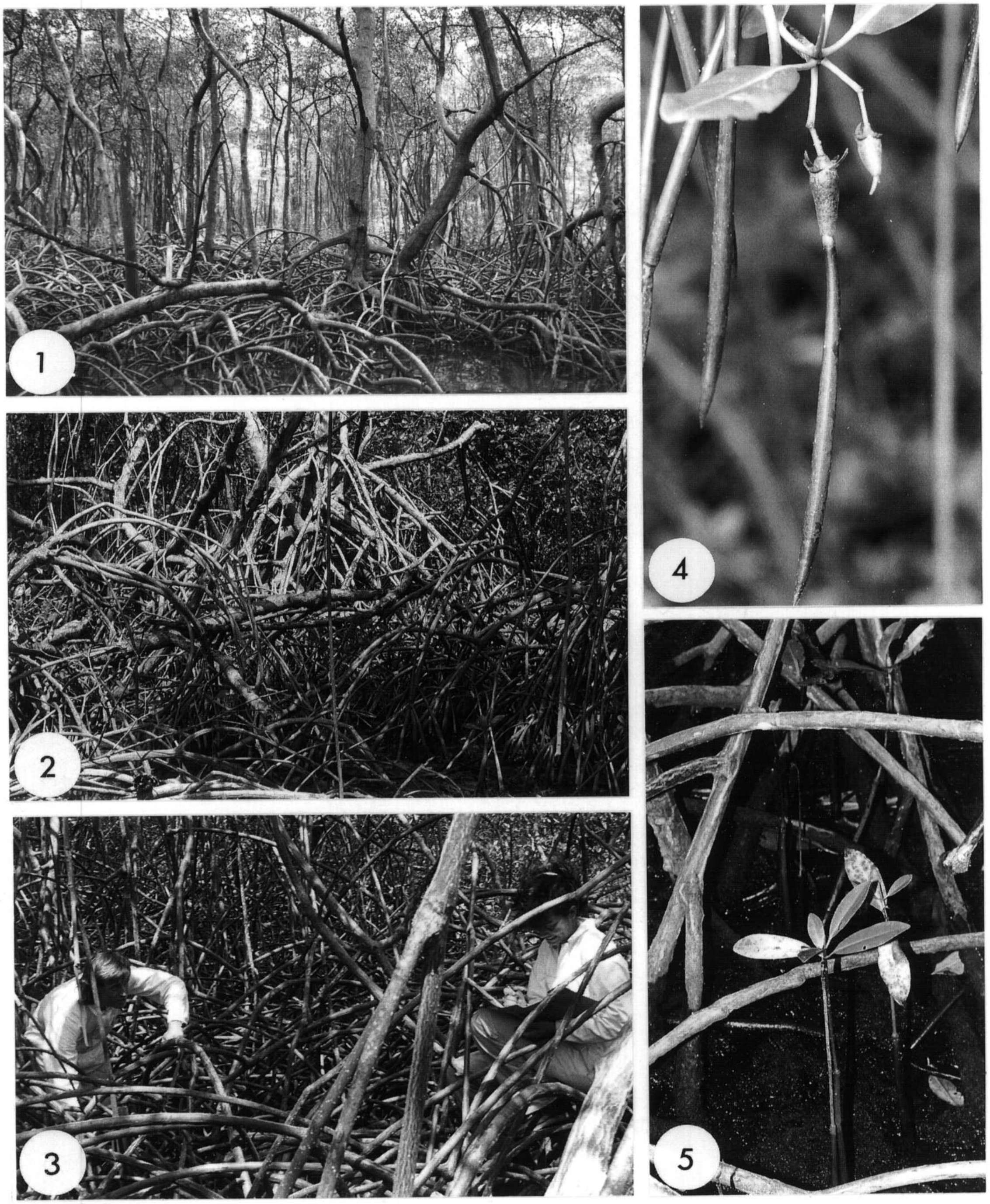


Fig. 8.1 Photographs taken in 1991 show major structural aspects of fringing mangrove forests, dominated by *Rhizophora mangle*, at Bahía Las Minas. *1.* The canopy is often uniform and low in density, and there is no undercanopy. A tangle of looping prop roots arises chiefly from the lower trunk. *2.* Where the canopy is lower, approximately 5 m, the tangle of roots is increased with both higher trunk roots and other aerial roots, i.e., roots emerging from foliage branches. *3.* Studies of recruitment and

Fig. 8.1 (continued) growth involved quantification and monitoring of seedlings among these roots. *4.* Seedlings emanate from advanced, viviparously grown young plants known as hypocotyls, or *Rhizophora* propagules, often mistakenly noted as seeds or fruits. The fruit, in fact, is the inverted pear-shaped body with four pointed lobes, from which the hypocotyl emerged. *5.* When the hypocotyl is mature, it falls from the fruit, which remains on the tree a little longer, and hypocotyls often take root among the roots of established stands. Here they are subject to low light where canopies remain closed, and they are often grazed on by herbivorous crabs. Our studies demonstrated that a seedling bank forms among these roots, presumably in readiness for an opening in the canopy.

trees die subsequently in patches of various sizes and shapes where oil beached along higher tidal contours within forests. Characteristics of these areas are variable, depending largely on the amount and type of oil, the mangrove site, and prevailing climatic and tidal conditions. In such cases, and often before the trees actually die, animals that depend on them will also perish, leaving desolate oily, deforested gaps, here called *oil gaps*, among surrounding bands of surviving trees.

The occurrence of forest gaps is not unique to oil spills or mangroves (Mabberley 1983). Oil gaps are different, however, because oil often remains in the environment long after a spill (Lee 1980; Cintrón et al. 1981; pers. obs.; Burns, Chap. 3). In these cases, recovery may be retarded, but this phenomenon has not been critically evaluated over a long period following a large spill. Habitat recovery depends chiefly on the establishment and growth of new trees, and this may take several decades under normal conditions. Assessment of these processes also needs to be based on recognized forest parameters (e.g., Cintrón and Schaeffer-Novelli 1984) for comparison with measures of health and condition of equivalent forests in other regions. Evaluation of these factors for making long-term projections for recovery, however, is hampered by a lack of any method to age mangrove trees retrospectively (Tomlinson 1986), a problem shared with most other tropical trees around the world (Bormann and Berlyn 1981). Nevertheless, for mangroves, a partial solution was offered during these studies with the discovery of a method to age *Rhizophora* seedlings and small trees using leaf-scar nodes (Duke and Pinzón 1992). More data on this method are presented in this report, and they provide insights into some recovery processes and the influence of residual oil. Because tree growth is relatively slow, the discovery of such aging techniques and the establishment of long-term study plots are basic to our immediate needs of understanding and preserving these threatened mangrove habitats.

8.2.2 The 1986 Spill at Bahía Las Minas, Caribbean Coast of Panama

On 26 April 1986 at least 60,000-100,000 bbl of oil was lost from a ruptured tank at the refinery at Bahía Las Minas, on the Caribbean coast of Panama (Fig. 8.2; Jackson et al. 1989; Keller et al., Chap. 1). The subsequent spill took place in three main phases. First, most oil overflowed retaining facilities to gather temporarily in the central mangrove-lined embayment. At this time, it was fresh and concentrated, forming an estimated average 5-mm-thick layer throughout the estuary

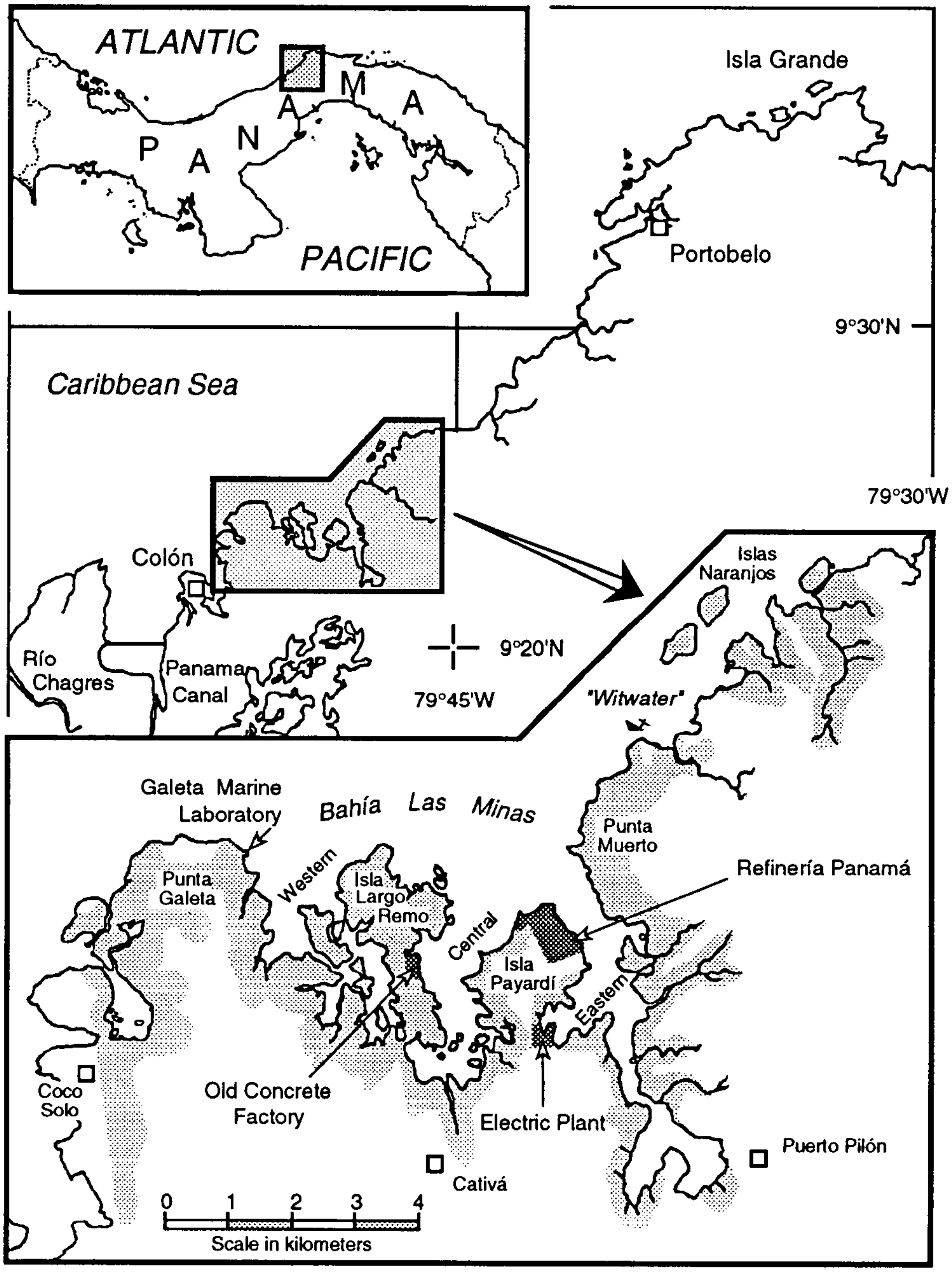


Fig. 8.2 Map of the study area. *Stippled areas* (inset map) denote mangrove forests around Bahía Las Minas. *Open squares* denote major population centers. Note the position of the Galeta Marine Laboratory, the refinery, and the exposed stern section of the tanker *Witwater* wreck north of Punta Muerto. The bow section sank just north of Isla Largo Remo. The nearest possible unoiled sites for exposed habitats were located in the vicinity of Portobelo and Isla Grande.

(approximately 2 km^2), although the layer was presumably much thicker on windward margins. As expected, effects of the spill on mangroves were most severe in this embayment, and approximately half the surrounding forested area of mangroves was killed within 2 mo. Trees probably died from a combination of toxic and suffocating effects, and few trees remained alive from the water's edge to half-way up the intertidal zone. The second phase of the spill occurred a few days later when estuarine outflow, increased by rainfall coupled with a temporary wind shift, washed floating oil over retaining booms and out to sea. When the wind returned to onshore shortly afterward (Cubit and Levings, Chap. 2), oil was then redeposited on other mangroves, extending widely across the bay from Punta Galeta to Punta Muerto, and, to a lesser extent, farther up the coast (Jackson et al. 1989; Keller et al., Chap. 1; Fig. 1.4). On this occasion, damage was patchy, and many oiled sites faced the open sea. With few exceptions, these sites were all characterized by bands of deforestation behind rows of one or two trees bordering the water's edge (see Fig. 3*E* in Jackson et al. 1989). Other instances of oiling formed characteristic crescent shapes, at sites on exposed faces of small islands and headlands, or funnel shapes at sites with smaller drainage channels. In each case, the pattern of deforestation was apparently related to topographic contours because oil tended to accumulate at upper levels of tidal variation at the time. This pattern of accumulation might have also contributed to the curious survival of trees lower in the topographic zone. Because these trees were also awash with oil, it is possible that the deposition of oil higher on trunks somehow missed critical breathing surfaces, presumably located lower on trunks and exposed roots. Oiling was obviously also catastrophic for intertidal fauna, as marked by an overwhelming smell of rotting flesh in and around Bahía Las Minas for several weeks following the spill (J. Cubit, pers. comm.). Finally, some of the spilled oil penetrated into sediments, particularly in mangroves (Burns, Chap. 3), where it degraded further, was released as chronic slicks (Guzmán et al., Chap. 7; Garrity and Levings, Chap. 9), and redistributed along the shore.

Effects of the spill were obvious soon after organisms came in contact with oil, although response times varied for particular species. Intertidal animals with thick coatings of oil died after 2 or 3 d, whereas mangrove trees with oiled roots took approximately 1 or 2 mo before losing their leaves (Jackson et al. 1989). For *Rhizophora*, total defoliation is indicative of imminent death; in Bahía Las Minas this defoliation resulted in extensive areas of deforestation that are still clearly visible 6 yr later. Delay in tree death appears to be normal because it has been observed at other oil spill sites (Cintrón et al. 1981). Other mangrove species, notably *Laguncularia*, apparently were less affected by the spill because individuals often were observed in oil gaps previously dominated by *Rhizophora*. This variation identifies a major difference in the response of different mangrove species, and the ability to recover from total defoliation may account for the greater effects presently observed in *Rhizophora* forests. In this context, it would be interesting to compare areas of defoliation with those of subsequent tree death. This is no longer possible for the Bahía Las Minas spill, however, but it would be informative to follow the relationship in future instances. Furthermore, this effect is expected to extend beyond biota coated with oil because there is evidence of oil adversely affecting subtidal sessile biota (Guzmán et al. 1991, Chap. 7).

Oil remained in mangrove areas long after the spill. This oil is mostly trapped in anaerobic sediments where it apparently entered during initial contact, assisted by tidal fluctuations, and presumably penetrating crab burrows, which later became blocked. There now appears to be a myriad of air-tight reservoirs, or pockets, of oil in the substratum, comparable to a giant oil-soaked sponge. Walking on the surface still causes oil to ooze out. In addition, heavy rains disturb and flush out these pockets, causing new slicks. Biological effects of this spill therefore are expected to continue for at least two reasons. First, sites of deforestation must regenerate. Second, all sites in the vicinity must exist in the presence of residual oil.

These points are summarized schematically in Figure 8.3. This diagram illustrates effects of large oil spills on mangrove forests, initially oiling some parts and not others. In the primary effect, some oiled trees die while others survive. The latter are expected to return to normal relatively quickly because the structure was left intact. Deforested areas, however, require much more time to recover because the establishment and growth of new recruits are required to replace dead trees. If recruitment fails, then mangrove habitat is lost. Such loss is likely to reflect changed environmental conditions, considering that mangroves are long lived. However, habitat loss may also result from short-term factors affecting success of recruitment and survival, e.g., erosion of substratum. All stages are also likely to be influenced by secondary effects, chiefly from residual oil. In each case these secondary effects might become more detrimental, notably those toward the left side of the diagram.

Furthermore, because sites of deforestation lack both trees and canopy, productivity and physical structure may be strongly affected. Where trees are missing, the nature of the substratum changes considerably due to a wider range of temperatures, desiccation, and erosion from rainfall, currents, and tides. These different conditions may not support the same flora or fauna present earlier and, therefore, those species able to utilize gaps will have the best opportunities for occupying the site. During this time habitats are vulnerable to invasion, and the inclusion of new species may alter them permanently. This effect is compounded for sites of oil-spill deforestation by both the areal extent and presence of oil. Different species may become established and others excluded because of the influence of oil. An example involves crabs that eat selected species of mangrove propagules (Smith et al. 1989). At sites where these crabs were removed, albeit temporarily, the usually eaten tree species had an opportunity to become established. Because trees are long lived, so is the potential effect of such a change.

The 1986 spill killed approximately 64 ha of mangrove forests dominated by one tree species, *Rhizophora mangle*. Nevertheless, the total effect is expected to extend much further, possibly affecting up to 500 ha. Studies were divided into three major parts: describing the primary effect of deforestation, recovery of deforested areas, and the status of surviving forests surrounding oil gaps. Considering the extent of a planting conducted by the Refinería Panamá in 1986 and 1987 (Teas et al. 1989), we evaluated its possible benefit.

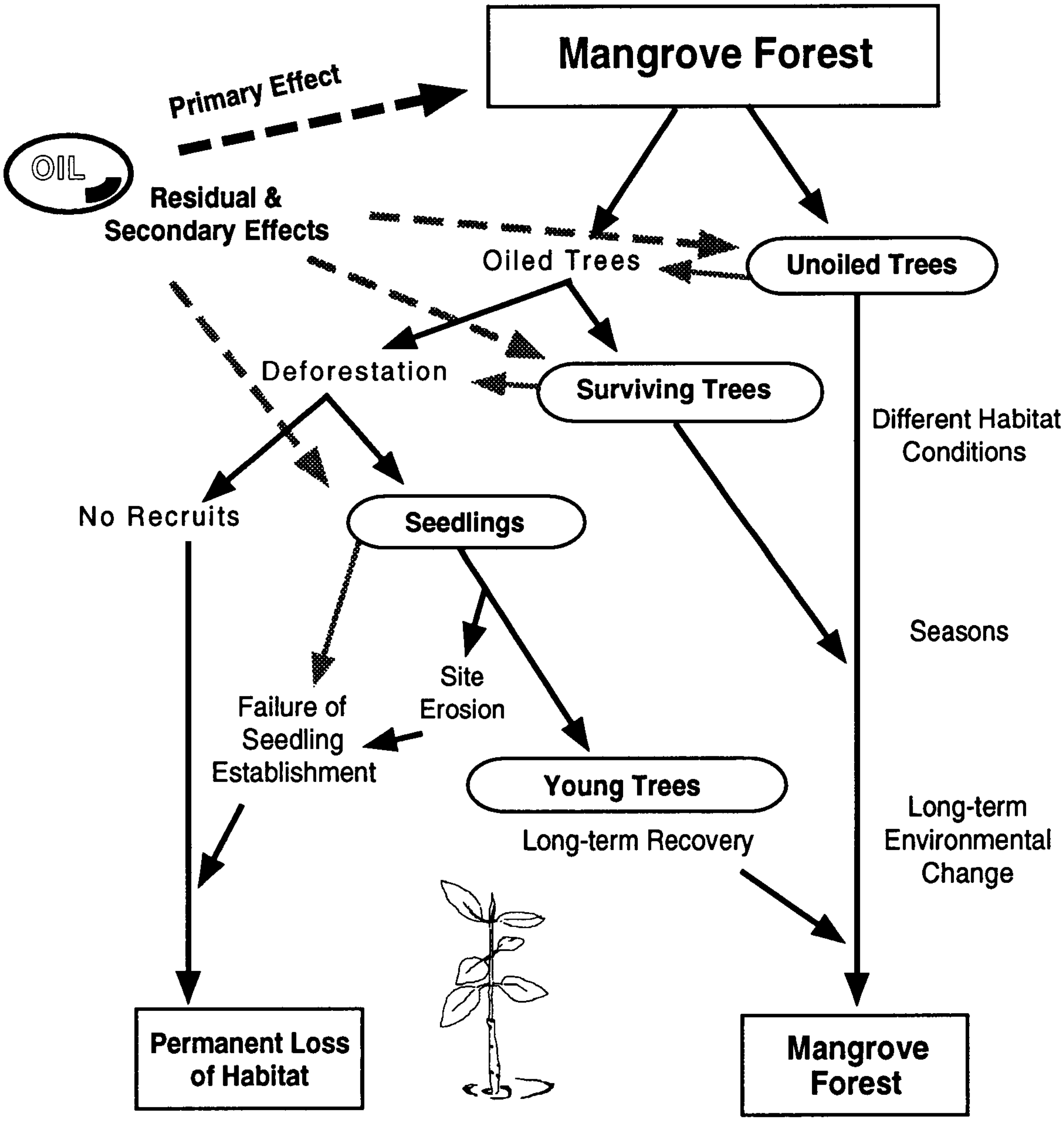


Fig. 8.3 Schematic diagram depicting the primary and secondary effects of a large oil spill on mangrove forests, and the major paths to either recovery (*solid arrows*) or permanent loss of habitat (*narrow dashed arrows*). This study paid particular attention to both seedlings in oil gaps and mature trees that survived oiling. During the recovery process, which may take several decades, mangrove forests are also subject to natural fluctuations in environmental conditions, further influencing their recovery.

8.2.3 Another Large Spill in Bahía Las Minas in 1968

In December 1968 the tanker *Witwater* was making its way from Bahía Las Minas toward Port Limón, near Colón (Fig. 8.2), when its hull seams ruptured in heavy seas just west of Punta Galeta. After drifting inshore for some time, the stricken vessel was taken in tow back toward the refinery, but it broke in half just north of Isla Largo Remo, in the middle of Bahía Las Minas. The bow section sank, and the stern section, still afloat, beached just north of Punta Muerto on the far eastern side of the bay. Apparently, no oil was recovered and 15,000-20,000 bbl of diesel and Bunker C were released along 10-20 km of coastline from Isla Margarita and the breakwater of Port Limón to shores east of Bahía Las Minas.

At that time of year sea levels were relatively high, and oil washed well inshore by strong onshore winds and large waves (Cubit and Levings, Chap. 2). The effect on mangrove trees was not initially noted, but the supralittoral fauna were reported to be extensively damaged soon after oil reached shore (Rützler and Sterrer 1970; Birkeland et al. 1976). Damage to mangrove forests was observed during the present study when examination of aerial photographs taken in 1973 revealed large areas of deforestation similar to those seen in 1990 aerial photographs of the 1986 spill. These observations were later verified in ground surveys, where previously deforested sites had smaller trees growing at much higher densities than older, surrounding mature forests (Fig. 8.4). Although retreating fringes were observed at several exposed sites, deforestation from this spill was farther back. This pattern often left broader bands of surviving trees along the water's edge, and even extended into at least one basin forest. In this instance, the effect was long-lasting because these latter sites still remain as deforested gaps.

Because of the extent of this spill's effects, its similarities with the present spill, its occurrence in the same bay, and the fact that mangrove trees are long lived, it was essential for the present study to include an appraisal of deforestation and forest recovery after the *Witwater* spill.

8.2.4 Major Objectives and Focus of This Element of the Study

This study began as an assessment of effects of the 1986 oil spill and the potential for long-term recovery of mangrove forests in Bahía Las Minas, Panama. From the beginning of studies in 1989, as outlined in Duke and Pinzón (1991), great emphasis was placed on processes in the ecosystem to better assess habitat recovery, the time this might take, and whether rehabilitation was beneficial. Two major discoveries, the technique for aging *Rhizophora* seedlings (Duke and Pinzón 1992) and the *Witwater* deforestation, have provided unanticipated assistance with the major objectives. This development allowed the focus of research to be directed particularly toward forest structure, recruitment, and growth.

The major objectives of this study were: (1) to delineate the extent of mangrove forests and of deforestation in and near the area of the oil spill and (2) to

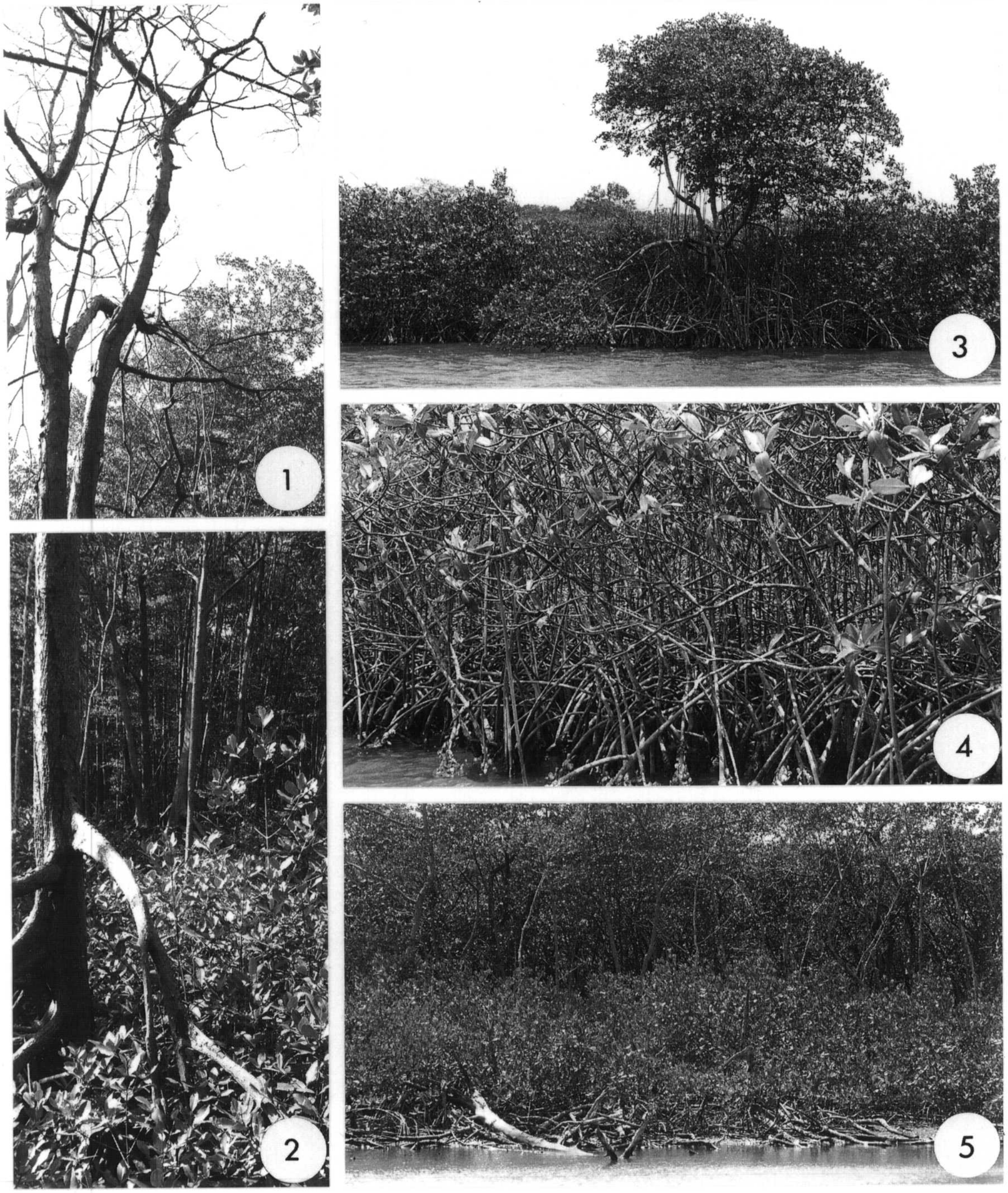


Fig. 8.4 Photographs taken in 1991 show recovery following light-gap creation in *Rhizophora mangle* forests at Bahía Las Minas. *1.* Natural gaps are created by trees that die standing in groups of one to 10. *2.* In the gap below, seedlings grow rapidly and in strong competition because taller plants shade shorter ones. *3.* Oil gaps were often much larger areas of deforestation than natural gaps and, after the 1968 *Witwater* spill, seedlings far outnumbered the few fringe survivors, which still remain isolated in

Fig. 8.4 (continued) 1991. This pattern may reflect the incomplete recovery of these forests. *4.* The structure of 23-yr-old post-*Witwater* forests is variable, but trees are always much shorter, have smaller girths, and are very dense compared with mature forests. *5.* By further comparison, seedlings recovering from the 1986 refinery spill appear to have similar growth in height; residual oil in sediments may be a major influence on seedling growth. Consequently, oil-gap recovery to prespill conditions is likely to take much longer than recovery in the absence of oil.

study forest structure, recruitment, and growth in both deforested and surviving oiled forests.

8.3 Methods and Techniques

8.3.1 Study Area and Vegetation Maps

The study was conducted chiefly in the vicinity of Bahía Las Minas (9°25' N, 79°50' W), on the Caribbean coast of Panama, but also extended north of Portobelo to Isla Grande (Fig. 8.2). Bahía Las Minas is a northward-facing bay, approximately 6 km across the mouth, situated immediately east of the Atlantic entrance to the Panama Canal and the city of Colón. Its location and physical characteristics are described further by Cubit et al. (1987, 1989) and Jackson et al. (1989). The bay is densely fringed with mangrove forests growing in a variety of habitats, from exposed coastal sites behind reef flats to those bordering tidal channels and those farther upstream along freshwater-dominated estuarine reaches. Parts of these forests have been altered and removed for port and industrial development but, apart from this, their chief use includes fishing, scavenging, and cutting of mangrove saplings at a subsistence level.

Study sites were chosen mainly in coordination with ongoing studies of mangrove roots (Garrity and Levings, Chap. 9) and hydrocarbons in mangrove sediments (Burns, Chap. 3). These sites included at least three replicates from both oiled and unoiled areas for mangroves in two major habitat types affected by the spill in Bahía Las Minas, exposed, open-coast fringing forests backing shallow reef flats and sheltered, channel-fringing forests bordering deeper embayments. A third habitat, unaffected by oiling, was sheltered, river-fringing forest bordering streams and influenced by frequent runoff. Sites were included in river habitats for further comparison, especially because of the influence of salinity on growth of mangrove trees.

The aerial photography gathered, including that flown specifically for this study, is listed in Table 8.1. Using these photographs, routinely enlarged to a standard working scale of 1:5,000, outlines of mangrove forests and other features were drawn on overlays of transparent acetate film. Traced areas were categorized as mangrove areas, oil gaps, other deforestation, intertidal bare areas, natural upland areas, roads, major buildings, and port facilities. Determinations of categories on the maps were based on detailed ground-truth observations and familiarity with mangrove plants.

Table 8.1 Aerial photography used in the study of mangrove forests. "*" denotes those providing the greatest information for the present study.

Date	Coverage	Scale
January 1955	Western-Central Bahía Las Minas	1:20,000
*August 1966	Northern Bahía Las Minas. Portobelo vicinity	1:20,000
*February 1973	Chagres-Toro Point-Colon-Bahía Las Minas	1:25,000
*August 1976	Isla Margarita-Punta Galeta	1:20,000
June 1979	Isla Margarita-Bahía Las Minas-Maria Chiquita	1:40,000
August 1981	Portobelo-Isla Grande	1:40,000
January 1987	Isla Margarita-Bahía Las Minas. Portobelo-Isla Grande	1:30,000
September 1987	Isla Grande	1:30,000
*December 1990	Isla Margarita-Bahía Las Minas	1:12,000

8.3.2 Mangrove Species, Forest Structure, and Allometric Relationships

Most studies discussed in this chapter relate to *Rhizophora mangle*, the red mangrove. This species dominated the areas of extensive deforestation of lower intertidal forests, although a smaller number of four other species were also affected or were in close proximity: *Avicennia germinans* (L.) L. (black mangrove), *Laguncularia racemosa* (L.) Gaertn.f. (white mangrove), *Pelliciera rhizophorae* Triana and Planchon (piñuelo), and *Conocarpus erectus* L (buttonwood). These species and their distributions are described in detail by Tomlinson (1986).

Forest structure was assessed in two ways, for seedlings and for trees. Seedlings were studied chiefly in fixed 3 x 4 m plots, established in a selection of sites throughout the study area. In each plot all seedlings were tagged for future reference and scored for hypocotyl height, total height, diameter just above the hypocotyl, node count, and species. Tree structure was characterized by measurements of tree height, girth, basal area, and stem density based on the angle-gauge technique (Cintrón and Schaeffer-Novelli 1984).

Because controls were not established when deforestation occurred in 1986, there was a problem in comparing oiled and unoiled sites for seedling growth. Instead, the present work relied on finding reforested sites resulting from either cutting by local fishermen or natural light gaps. Their ages were estimated from both aerial photographs and *in situ* measurements of node counts and internodal sequences.

Derived parameters of leaf and total biomass are a common way of describing forest structure (see Clough 1992). Estimates of these for *R. mangle* were calculated from the allometric relationships described by Cintrón and Schaeffer-Novelli (1984), which established relationships of total above-ground biomass and leaf biomass (g dry weight) with tree height (m) and girth of the trunk (cm). The relationships are a power function:

$$\text{Leaf Biomass} = 125.9571\,[(\text{Diameter})^2\,(\text{Height})]^{0.8557} \qquad r^2 = .99;\ N = 26 \quad (1)$$

$$\text{Total Biomass} = 23.6398\,[(\text{Diameter})^2\,(\text{Height})]^{0.5902} \qquad r^2 = .99;\ N = 26 \quad (2)$$

Allometric relationships for *R. mangle* leaves were developed during this study, establishing a relationship between green leaf area and dry weight. The overall relationship was developed in two steps. First, 56 mature green leaves were collected from several localities with the intention of encompassing the full range of variability in size and shape. These leaves were photocopied half an hour later, taking care to note that dimensional characteristics were faithfully recorded. Based on these photocopies, blade lengths and widths were compared with respective areas, determined using a digitizing tablet and computer. The relationship is a simple linear one within the range of leaf dimensions collected in Bahía Las Minas. It relates green leaf area (cm^2) to the multiple of green leaf blade length and width (cm):

$$\text{Leaf Area} = 1.1183 + 0.6995\,(\text{Length x Width}) \qquad r^2 = .998;\ N = 56 \quad (3)$$

The second step, to establish site-specific leaf character, involved collections from each of the 25 litter-fall sites of 20 mature green leaves from shoots ranging from upper to lower canopy positions. Leaves were harvested in October 1991, blade lengths and widths were measured within an hour of collecting, and then leaves were oven dried at 80°C for 4-5 d while loosely pressed to maintain their flatness. These leaves were then weighed. Simple linear regressions were used to equate green leaf area (cm^2) with average dry weight per leaf (g) for each site (App. Table D.1) and each habitat-treatment combination: exposed unoiled (*OU*), exposed oiled (*OO*), channel unoiled (*CU*), channel oiled (*CO*), and river unoiled (*RU*):

$$\text{OU: Leaf Area} = 3.3175 + 40.6686 \text{ x Leaf Weight} \qquad r^2 = .931;\ N = 88 \quad (4)$$

$$\text{OO: Leaf Area} = 4.3170 + 41.7281 \text{ x Leaf Weight} \qquad r^2 = .855;\ N = 78 \quad (5)$$

$$\text{CU: Leaf Area} = 4.6666 + 42.3758 \text{ x Leaf Weight} \qquad r^2 = .912;\ N = 99 \quad (6)$$

$$\text{CO: Leaf Area} = 9.4630 + 42.3450 \text{ x Leaf Weight} \qquad r^2 = .897;\ N = 156 \quad (7)$$

$$\text{RU: Leaf Area} = 10.3184 + 44.3558 \text{ x Leaf Weight} \qquad r^2 = .846;\ N = 79 \quad (8)$$

Equations 4 to 8 provide treatment/habitat-specific relationships between green leaf area and dry leaf weight, enabling total leaf area for specific mangrove forests to be determined from litter-fall data. Total leaf area is a measure of the photosynthetic ability of plant communities, and in the present context represents a measure of forest community well-being.

8.3.3 Seedling Growth and Demography

Seedlings were monitored chiefly in two ways, using marked seedlings to follow individual growth and fixed plots to describe seedling demography. The manner in which these two studies were conducted are described separately.

In late 1989, 252 *Rhizophora mangle* seedlings were selected from 12 light-gap sites, including both exposed and sheltered habitats, and oiled and unoiled areas (Table 8.2). At each site 21 seedlings (roughly divided into three groups of seven) were chosen to represent the full range of age classes, determined by counting leaf-scar nodes (Duke and Pinzón 1992; Fig. 8.5). Each seedling was scored for various physical parameters, including height of each node along the main stem from the substratum to the topmost (apical) node, stem diameter just above the "zero" hypocotyl node, total number of leaves, and total number of leafy shoots. All seedlings were tagged with a Dymo label on a stainless steel wire stuck through the stem, identifying both the seedling and the position of an upper node for future reference. This procedure was used at all sites, so it was not likely to influence among-site differences. This possibility was tested in a small study of shoot growth for 20 shoots on three trees over 1 yr. The results showed no significant effect of tagging on leaf production rate (Table 8.3). All sites were revisited, starting in late 1990, and the same parameters were scored again, except for node-height measurements, which could this time be more simply measured above the reference wire. This point is important because lower nodal scars become harder to discriminate in older seedlings as bark develops, and branches and roots form.

The demographic study of seedlings commenced in April 1990, and involved establishment of 42 plots, each 12 m^2. This total included three replicates at each of 14 sites throughout the bay, including both exposed and sheltered habitats, and oiled and unoiled light gaps (Fig. 8.6). All seedlings in these plots were tagged, numbered, and scored for species, total height to the topmost leaf node, hypocotyl height (for *Rhizophora* and *Laguncularia*), girth just above the hypocotyl or established prop roots, and total number of nodes along the main stem. Sites were revisited in June-July 1991 and the seedlings were retagged and rescored in the same way.

Growth was also studied using some small trees growing in an area that was previously a channel between two islands in Bahía Las Minas, Isla Largo Remo and Samba Bonita. This channel was filled during construction of a causeway and port for a cement factory, providing a new area that was rapidly colonized by mangroves. This change was evident from aerial photographs (see below, Figs. 8.12, 8.13). Tree growth was rapid, and after approximately 10 yr the trees were 12 m tall. It was possible to count leaf scar nodes along the main stem of these trees because of a lack of bark development, presumably because the trees grew exceptionally fast. Thus, there was a complete sequence of leaf scar nodes along main stems of each of the four specimens collected. Total above-ground biomass of these trees was estimated using equation 1, and it is apparent that growth of *R. mangle* in Panama is comparable to *R. apiculata* in Thailand (Aksornkoae 1975; Christensen 1978).

Table 8.2 Sites used for studies of seedling growth in unoiled natural light gaps and refinery oil gaps in exposed and sheltered habitats with the two dates of observation. *OO* = open coast oiled; *OU* = open coast unoiled; *CO* = channel oiled; *RU* = riverine unoiled.

No.	Code	Description	Site Code	CID 1	CID 2
2	OO	Peña Guapa - West BLM	WOPG	27 Sep 89	30 Oct-1 Nov 90
3	OO	Droque - Central BLM	CODR	28 Sep 89	1 Nov 90
4	OO	Punta Muerto - East BLM	EOPM	28 Sep 89	30 Oct-1 Nov 90
6	OU	Portobelo - I. Grande	GOPB	22 Nov 89	15 Feb 91
7	OU	Padre - I. Grande	GOPA	26 Oct 89	14 Feb 91
8	OU	Lintón - I. Grande	GOLI	22 Nov 89	14 Feb 91
19	CO	Puerto Sur - East BLM	ERPS	24 Jan 90	15 Feb 91
20	CO	Puerto Norte - East BLM	ERPN	21 Nov 89	13 Dec 90
21	CO	Rabinowitz -West BLM	WRRB	27 Sep 89	1 Nov 90
24	RU	Unnamed - East BLM	ERUN	24 Jan 90	13 Dec 90
25	RU	Alejandro - East BLM	ERJA	24 Jan 90	13 Dec 90
26	RU	Las Mercedes - Naranjo	NRME	30 Oct-21 Nov 89	7 Feb 91

A further study focused on the influence of light and how this factor affects seedling growth. Eight unoiled closed-canopy sites were selected in the western bay of Bahía Las Minas. At each site two 8-m crossing transects were marked, one E-W and the other N-S. Within 1 m of these transects all undercanopy plants (seedlings) were tagged, measuring position, height, girth, and nodes. To test whether seedlings normally survive light-gap creation, 10 seedlings were tagged in each of the eight sites mentioned above and monitored each month for node production, height increase, and survival. Dead seedlings were replaced with others nearby. After about 4 mo the canopies at half of the sites were opened by cutting away all smaller foliage branches, forming light gaps approximately 6 m in diameter. Monthly monitoring continues to determine if and when a change in seedling growth will occur, and whether this will be reflected in the sequence of nodal scars.

The demographic study of seedlings was extended to provide a balanced set of planted and unplanted areas in both exposed and sheltered habitats. These areas included an array of three replicate plots for each of 12 oil gaps in Bahía Las Minas (Fig. 8.6). Seedlings in the plots were tagged and scored for several parameters, including height, girth, and number of nodes on the main stem. Also, the seedlings were identified as being planted or not, based on several factors, including disturbance of sediment, different soil, and presence of plastic bags.

Plots were also set up at six sites deforested by the 1968 *Witwater* oil spill. The size of these plots varied from that of the seedling plot (3 x 4 m) to plots four times larger (6 x 8 m) depending on stem density, which was targeted at approximately 200 per plot for logistical reasons. Node counts were scored for

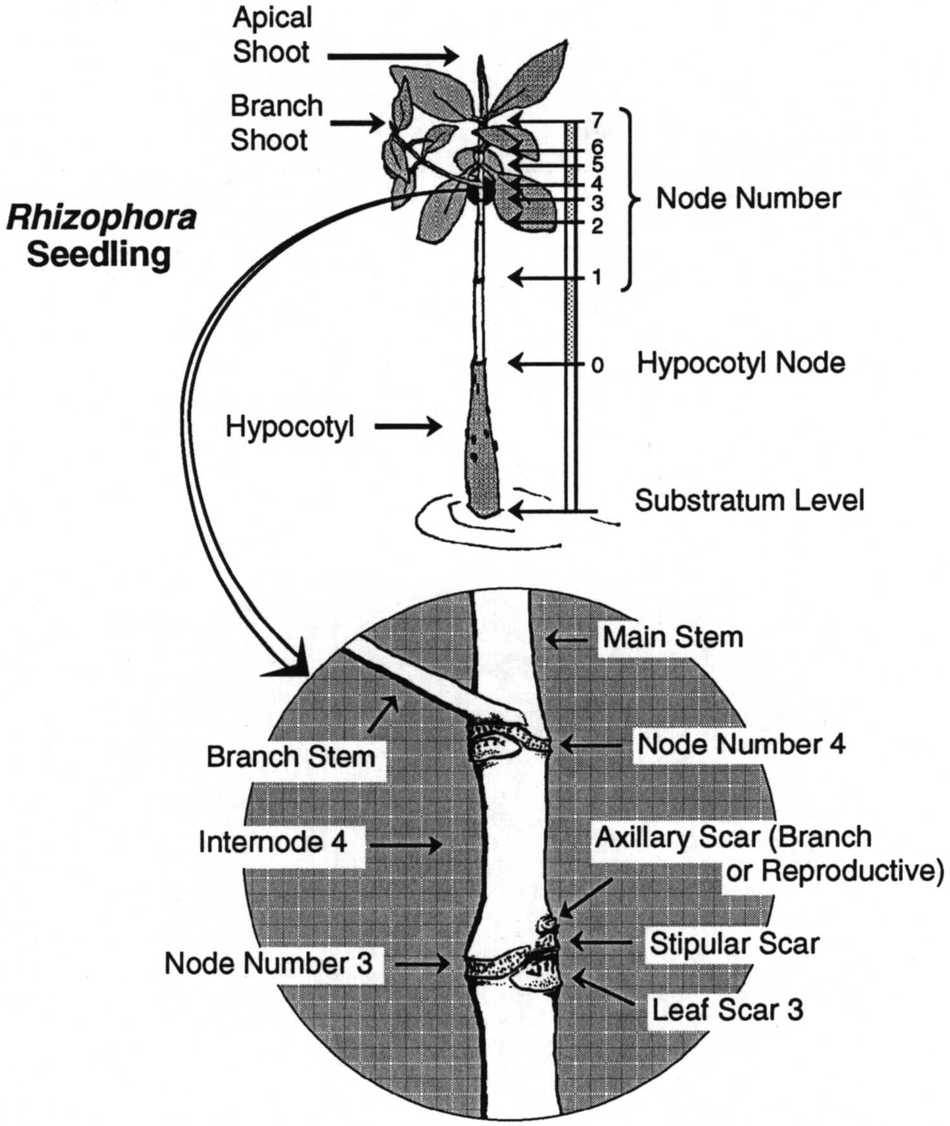


Fig. 8.5 Diagram of a *Rhizophora* seedling with a portion of the stem magnified to show the nodes and the hypocotyl. Note the node structure, each comprising three parts, of two leaf-petiole scars, two encircling stipular scars, and some axillary structures or their scars. Also included is the convention used for numbering leaf nodes and internodes from above the hypocotyl or "zero" node, up to the one positioned immediately below the apical shoot. In this example there are seven nodes along the main stem. Note also that approximately half the hypocotyl is beneath the substratum. The aging method using nodes is described in detail in Appendix Abstract and Description D.1 (Duke and Pinzón 1992).

seedlings, but this was not possible for trees. Otherwise, data were scored for the same parameters described for the study of seedling demography.

Table 8.3 Comparison of the effect of wire tags used to mark node position on shoot growth of mature *Rhizophora mangle* trees near the Galeta Marine Laboratory, Bahía Las Minas. Leaves per shoot and leaf production of 30 shoots were scored monthly on three trees for an entire year, June 1989 to July 1990. Data are presented as annual means with estimates of SE among trees. Half of the shoots were tagged with short pieces of stainless steel wire gently pushed through the stem just below the leaves, and between two nodes. In all shoots, nodal positions were marked with an arbitrary numbering sequence written on the leaves using a black felt pen.

	Wire	No Wire
Leaves/Shoot	9.21 (0.41)	9.13 (0.74)
Leaf Production	3.68 (0.07)	3.95 (0.27)

8.3.4 Condition and Growth of Tree Canopies

Canopy growth and condition of mature *Rhizophora mangle* were based on studies of litter fall and observations of upper-canopy shoots. These studies were conducted concurrently at approximately monthly intervals at the same 25 sites plus one other, ranging from Isla Margarita to Isla Grande (Fig. 8.7). An additional shoot site was established at María Soto for further comparative purposes. In general, there were at least three sites in each of the oiled and unoiled habitats. Theft of traps was common at many sites, but by selective replacement litter collections continued at all sites. In contrast, shoot observations were never disturbed by humans, although some tags were removed by insects.

In June 1989, 72 litter-fall traps, each 1 m^2, were suspended above the highest tides under mature *Rhizophora mangle* trees at 25 sites. They were constructed of mosquito mesh, cut and sewed funnel-shaped, approximately 50 cm deep, and fitted with a perimeter seam enclosing a square frame of PVC electrical conduit, forming the trap mouth. Litter was collected monthly until October 1990. After each collection, litter samples were returned to the laboratory and sorted, counted, dried, and weighed prior the next collection. Sorting of plant material was based on prior experience (e.g., Duke et al. 1984), and categories for respective species included leaves, interpetiolar stipules, wood, immature buds, mature buds, flowers, immature fruit, mature fruit, immature hypocotyls, mature hypocotyls, expended fruit, and miscellaneous debris.

Also, in June 1989, 21 leafy-shoot replicates of *Rhizophora mangle* were tagged in the uppermost canopy of individual trees at all 26 sites. The choice of shoot position was based on the highest reached at each site, then ranging down 1-2 m. Their positions from the top of the canopy were measured, and showed comparable representation of position classes for the habitat/treatment site groupings (App. Table D.2). In general, shoot position was measured from the apical shoot tip to the upper surface of the canopy, arbitrarily defined as the nearest (usually vertically above) uniform upper boundary. All shoots were monitored monthly until August

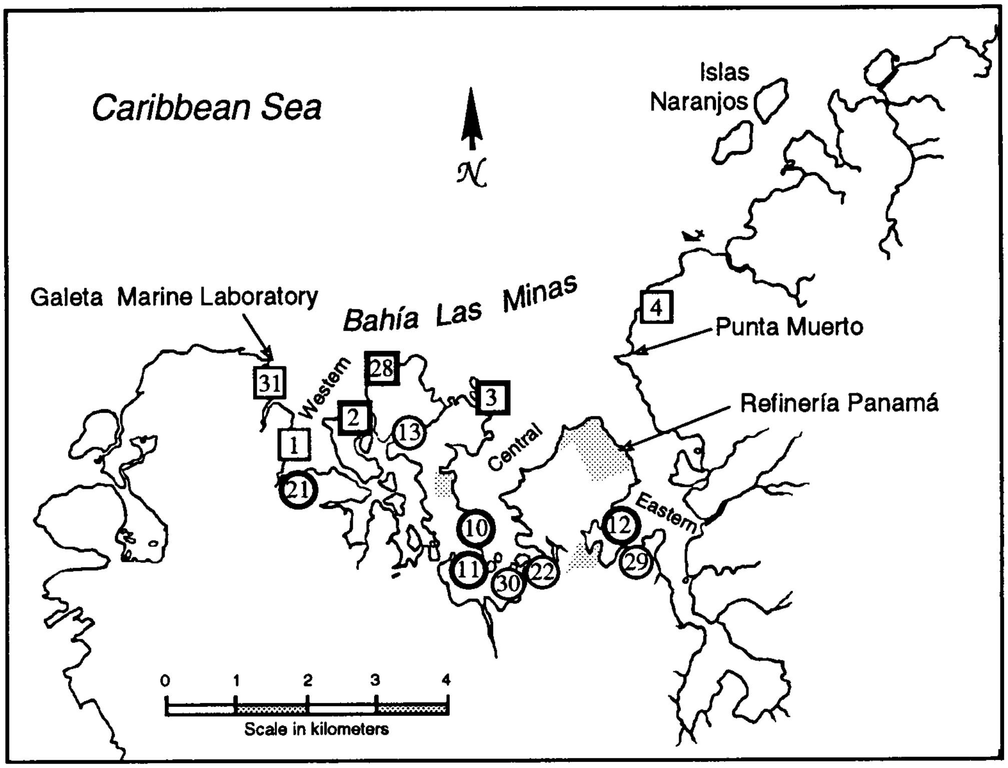


Fig. 8.6 Map of sites used for demographic studies of *Rhizophora mangle* seedling assemblages growing in 1986 oil gaps. Deforestation was confined mostly to Bahía Las Minas, extending from Punta Galeta (#31) to Punta Muerto (#4). *Square symbols* denote exposed habitats, *circles* denote sheltered habitats, and **bold** indicates sites planted with seedlings.

1991. A small number of shoots was lost during the study, and these were replaced with new ones in similar positions. Monthly shoot observations entailed scores of entire shoot status, noting node positions for particular leaves, branch shoots, and reproductive parts in various stages of development (listed for litter-fall sorting, above). From these data various parameters were calculated, including leaves per shoot, leaf production, and leaf fall.

Leaf size and weight were quantified in various combined studies. Mean leaf weights were best estimated from litter fall because these averaged possible seasonal variation and differences in canopy position. Leaf size was determined during the allometric study, noted earlier, and was clearly a function of weight for respective sites.

Another selection of forest-canopy parameters was derived using the combination of specific measures of canopy condition from several studies, including concurrent litter and shoot studies, and the allometric study of leaf area and weight. Source measurements included annual leaf production rates, standing crop (mean

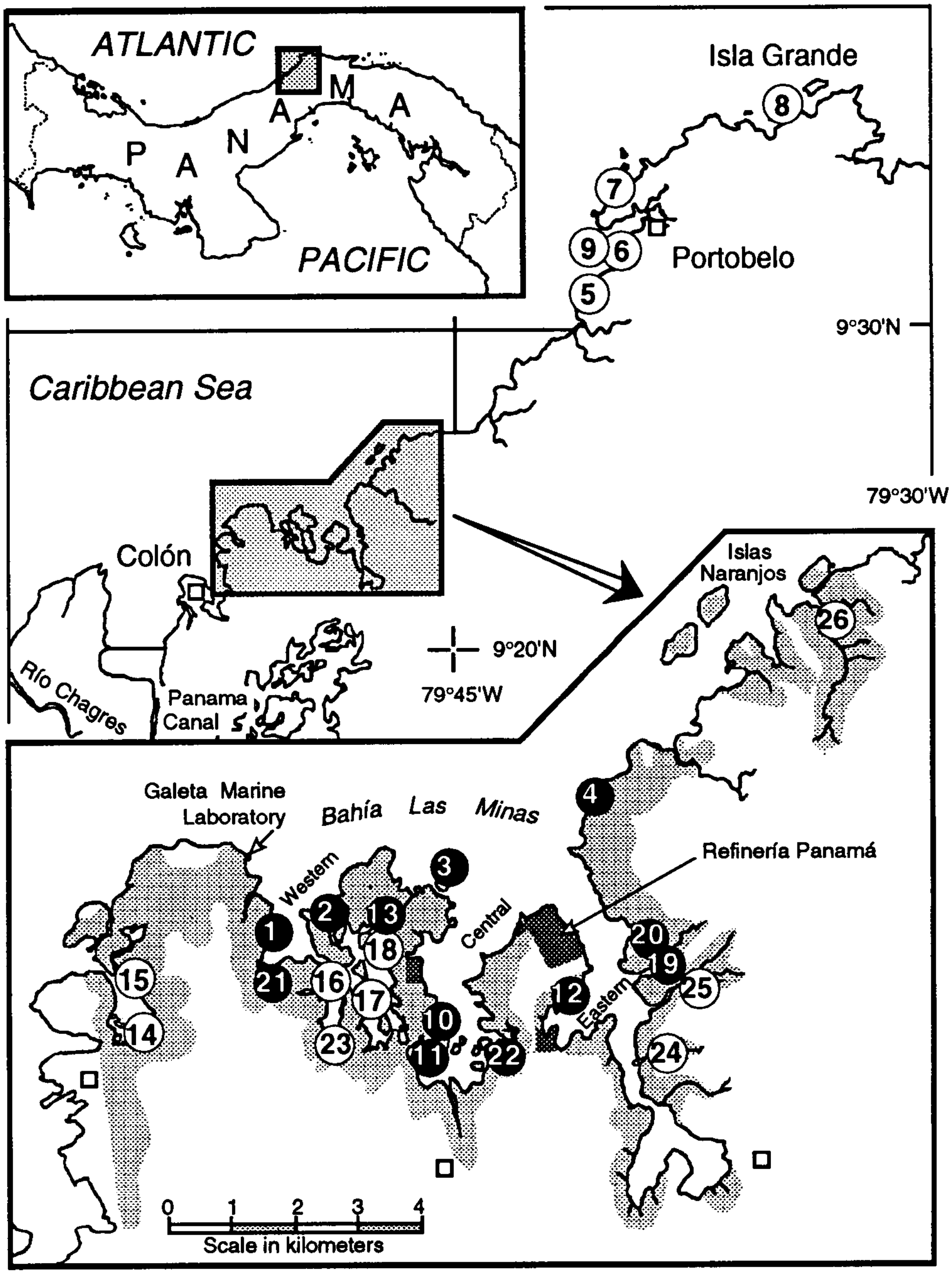


Fig. 8.7 Map of the 26 sites used chiefly for litter fall and shoot studies. *Stippled areas* (lower map) denote mangrove forests in and around Bahía Las Minas. Site numbers also refer to descriptions in Table 8.8 (below). *Open circles* denote unoiled sites and *filled circles* denote oiled ones. Sites 14 and 15 were located in Margarita Lagoon. Note also the locations of the Galeta Marine Laboratory and the refinery. *Open squares* denote major population centers.

annual number) of leaves per shoot, and mean average leaf weight. Derived parameters included density of shoots, density of leaves, canopy leaf biomass, and canopy leaf area. Their derivation from the source measurements is shown in the schematic diagram in Figure 8.8, which also notes the units concerned.

8.3.5 Trials to Estimate Canopy Herbivory

Canopy herbivory is known to have a significant effect on canopy production (Robertson and Duke 1987), and this factor is missing from productivity estimates derived from litter fall. In Australia Robertson and Duke reported that the herbivores were chiefly insects. In Panama the amount of herbivory appeared to be similar, but the herbivores included insects, snails, and arboreal crabs. An exploratory study was set up to test a method that might exclude at least the crabs, and thereby provide a means to estimate separate contributions to overall herbivory.

Virtually all green-leaf consumption by crabs appears to be done by one species, *Aratus pisoni*, the mangrove tree crab (Warner 1967). It is commonly observed in New World mangroves climbing along branches and roots from forest-floor roots and the water's edge to the uppermost canopy shoots. It also appears to prefer *Rhizophora mangle* over other tree species.

Leaf herbivory in Panama is rarely locally destructive though it mostly appears to be evenly distributed over large areas of canopy. Loss of leaf area is approximately 10%, and concentrated on older leaves. Tree crabs normally crawl along roots and branches, and over crossovers, jumping between branches only when threatened. This behavior was tested in a preliminary trial by placing excluders on branches and inspecting the damage to leaves on the isolated leafy shoots. Excluders consisted of plastic dinner plates, cut to the center, and placed centrally on 1-2-cm wide stems. Cut plates were repaired with staples and glue, and provided 12-cm smooth, plastic barriers, which were effective in casual observations of crabs that confronted them. The excluded stems supported approximately 10 to 20 leafy shoots, or about 40 to 100 leaves. Observations were not rigorous, due to time constraints, but after a full year damage to new leaves on excluded branches was noticeably less than on neighboring unmanipulated branches. This method is therefore considered to be effective and, as such, offers future studies with the means to accurately assess the importance of different herbivores.

8.3.6 Environmental Factors and the Amount of Residual Oil in Sediments

Salinity and water temperature fronting each of the 26 sites were measured at approximately 20 cm depth at each monthly collection from June 1989 to August 1991. Annual means of salinity and water temperature are summarized in Table 8.4 and Figure 8.9, with a full listing of data by site in Appendix Table D.3.

Monthly values of solar radiation and rainfall were obtained from the Galeta Marine Laboratory, situated at the western side of Bahía Las Minas (Fig. 8.7), through the Environmental Sciences Program of the Smithsonian Tropical Research

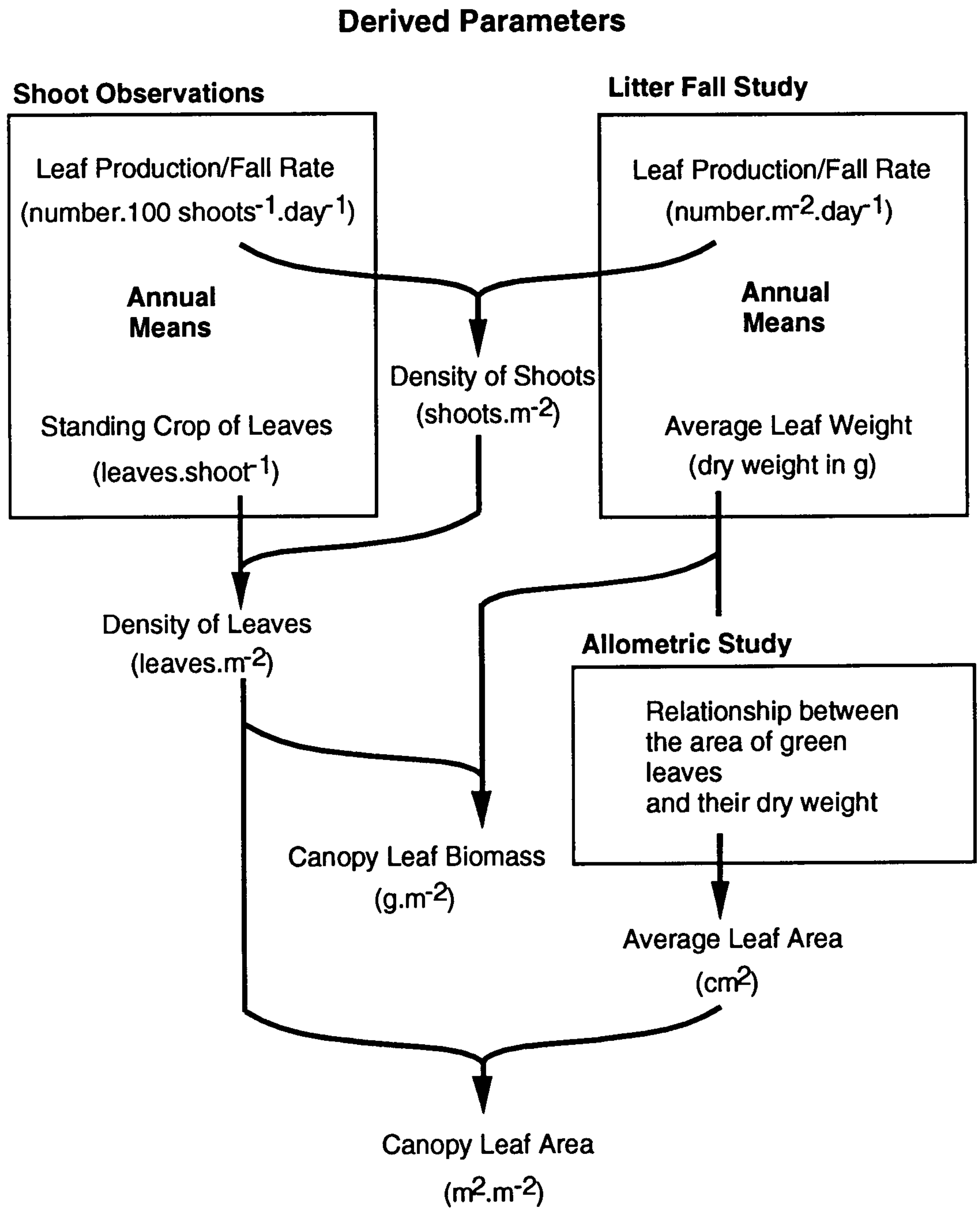


Fig. 8.8 Schematic diagram showing derived parameters, using shoot observations, litter-fall data, and the allometric relationship for leaf dry weight and green leaf area. These parameters include densities of shoots and leaves, canopy leaf biomass, and canopy leaf area (or Leaf Area Index, LAI).

Table 8.4 Mean annual salinity and temperature of water fronting oiled and unoiled locations in three fringe habitats, 1990-1991. SE of among-site variation is in parentheses. Site means are in Appendix Table D.3.

	Exposed		Sheltered		
	Open		Channel		River
Year	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Salinity (ppt)					
1989-1990	34.0 (0.2)	33.0 (0.4)	27.1 (0.9)	28.3 (0.7)	12.7 (2.1)
1990-1991	33.3 (0.5)	33.1 (0.3)	25.5 (1.4)	27.8 (0.7)	15.4 (1.5)
Average	33.7 (0.3)	33.0 (0.3)	26.3 (1.1)	28.0 (0.6)	13.7 (1.7)
Temperature (°C)					
1989-1990	29.4 (0.2)	29.3 (0.2)	28.7 (0.5)	29.4 (0.2)	27.5(0.3)
1990-1991	29.6 (0.2)	29.5 (0.1)	29.5 (0.3)	30.0 (0.2)	28.2 (0.2)
Average	29.6 (0.2)	29.4 (0.2)	29.1 (0.4)	29.7 (0.2)	27.8 (0.3)

Institute. Other climatic data and mean annual seasonal patterns (Fig. 8.10) referred to in the text were derived from the same station (Cubit et al. 1989).

Residual oil in sediments was analyzed by Burns (Chap. 3). Data used in this chapter were chiefly those from the UVF method of determining oil concentration in mangrove sediments. Data were reduced by averaging estimates for respective levels in depth cores (0-20 cm), summarized by habitat and condition in Table 8.5. Site means were compared with a selection of biological parameters.

8.4 Deforestation of Mangrove Forests

8.4.1 Extent of Deforestation Caused by the Refinery Spill in 1986

Maps of Bahía Las Minas were drawn from aerial photographs, showing all oil gaps from the 1986 spill, the surrounding area and mangrove forests, and sites of major human development (Fig. 8.11). The nine full-page plates are presented in Appendix Figures D.1 to D.19, and one, Plate IV (Fig. 8.12), is shown as an example because it displays the major patterns observed. These maps were based on 1990 aerial photographs. In the upper center is the large mangrove island, Isla Largo Remo, dominating the central part of Bahía Las Minas. This island was joined to the mainland by a causeway and port facilities at its southern margin. Notice that dredging had removed a large portion that was used to fill the older channel separating it from Isla Samba Bonita. The latter island has been drastically altered, but Isla Largo Remo still remains a mangrove-dominated island. The 1986 spill

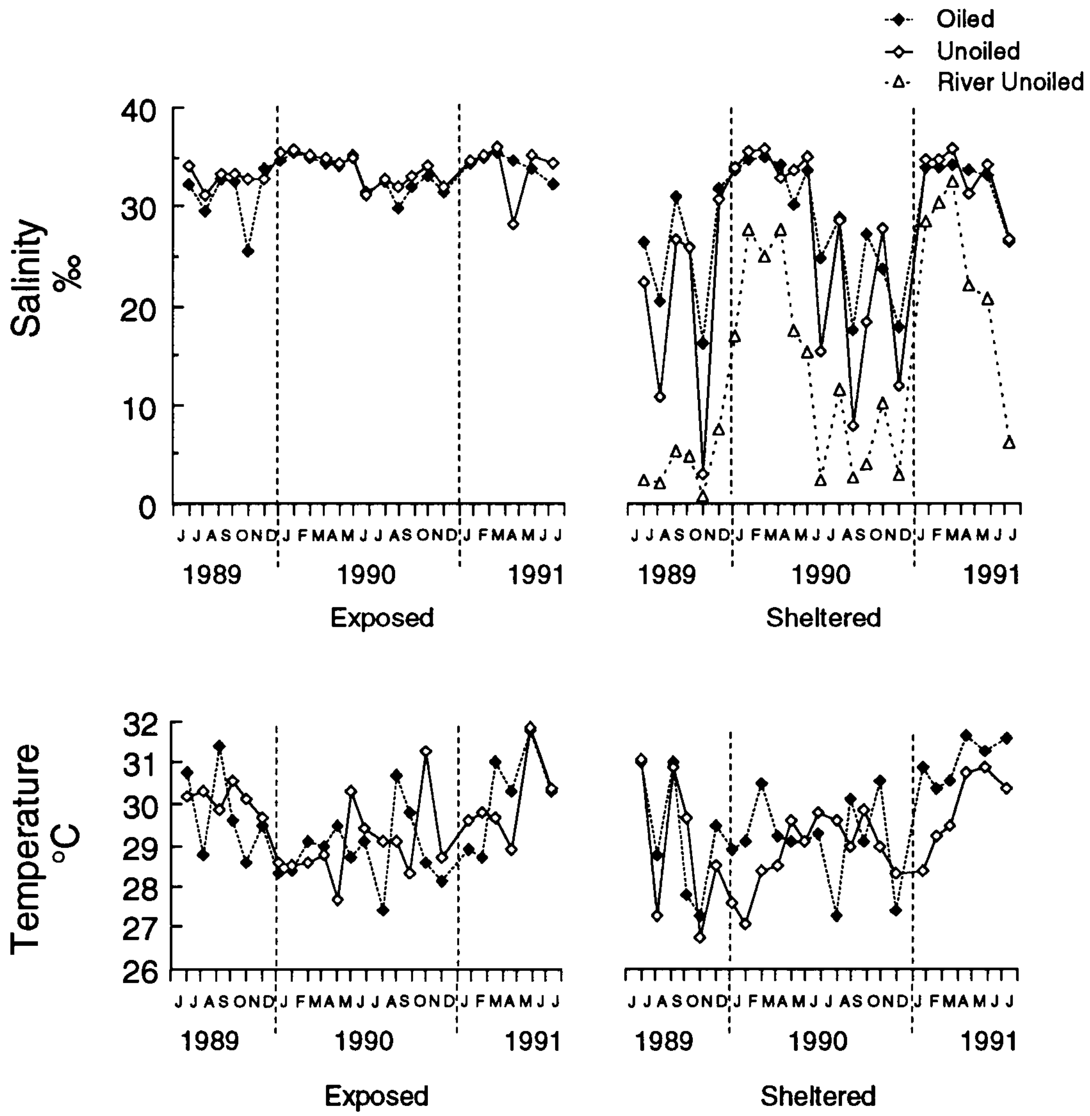


Fig. 8.9 Seasonal variation of water salinity and temperature for oiled and unoiled sites in exposed, sheltered, and river-fringe habitats, July 1989 to July 1991. Values are means for each collection at the 26 sites (Fig. 8.7).

caused extensive areas of deforestation, particularly in the northeastern-facing, shallow embayment. Along its margin there was a long fringe of deforestation, approximately 50 m wide. Oil apparently killed trees inside the forest, while leaving a thin, seaward veneer of one to two trees that survived the spill. Notice, also, the older coastal outline and missing mangrove clumps, shown with dotted lines beyond

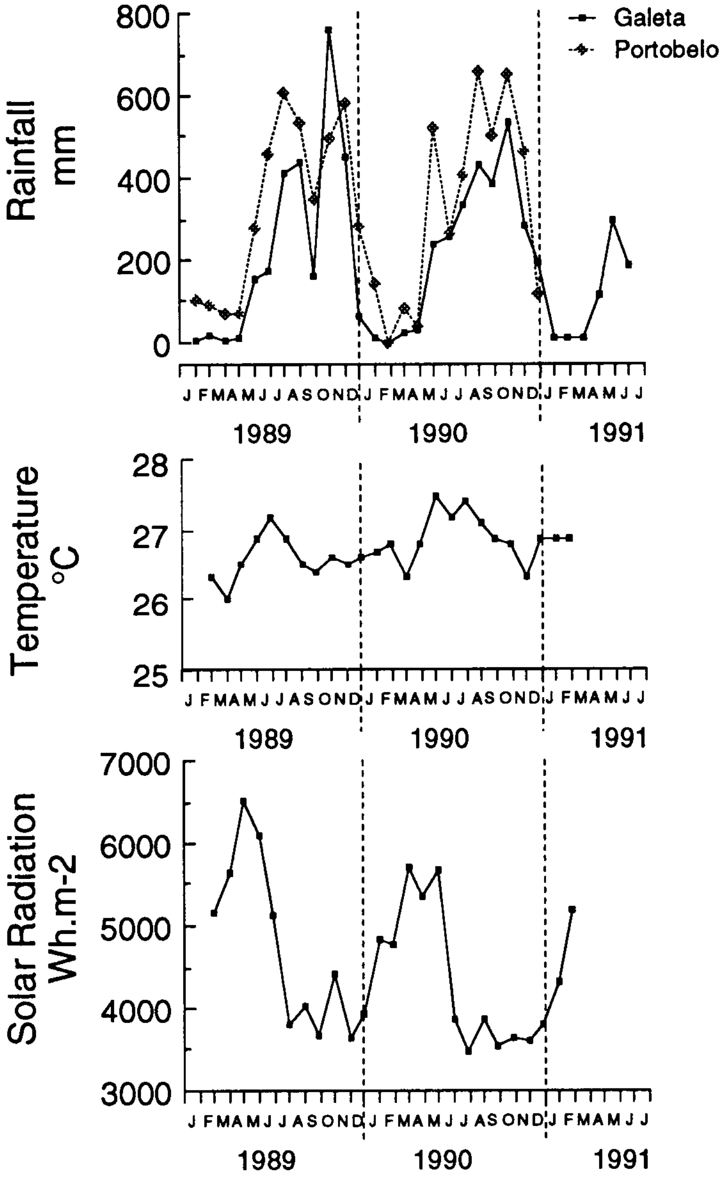


Fig. 8.10 Seasonal variation of rainfall, air temperature, and solar radiation at the Galeta Marine Laboratory, January 1989 to July 1991. These data are based on calendar months. Rainfall data from Portobelo (supplied by Instituto de Recursos Hidráulicos y Electrificación, Panama) are included for comparison because this station is closer to the unoiled exposed sites.

the island margin. These stands were alive just prior to oiling in 1986, as determined by comparing older photography from 1973 to 1986, particularly 1973.

Table 8.5 Summary of oil concentrations in mangrove sediments (mg/g), determined using the UVF method described in Burns (Chap. 3) from oiled and unoiled sites in three fringe habitats in 1986, 1989, and 1990. Values used for comparative analyses in this treatment were averaged from samples taken through the top 20 cm of mangrove sediments. SE of among-site variation is in parentheses. Site means are in Appendix Table D.4.

	Exposed		Sheltered		
	Open		Channel		River
Year	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Sept 1986	-	5.97	0.44 (0.34)	202.30	0.80
May 1989	0.01 (0.00)	23.44 (12.18)	0.65 (0.36)	71.11 (24.44)	0.11 (0.02)
July 1990	0.02 (0.02)	59.27 (21.11)	2.76 (1.55)	67.85 (25.60)	0.28 (0.07)
Average 89/90	0.01 (0.00)	41.35 (9.91)	1.71 (0.81)	69.48 (24.10)	0.20 (0.04)

8.4.2 Changes Prior to 1986 – the 1968 *Witwater* Spill

Another series of maps, using the same layout of plates (Fig. 8.11), was based on 1973 photographs and shows changes since 1966. These maps are also presented in Appendix Figures D.2 to D.10, and the same example, Plate IV (Fig. 8.13), shows the unexpected series of older deforested areas. These areas show the deforestation caused by the earlier spill, which resulted from the breakup and sinking of the bow section of the tanker *Witwater* in 1968, just north of Isla Largo Remo. Once again, notice the older coastal outline and missing mangroves, shown with dotted lines. Furthermore, the small stream dividing the island was not affected by this earlier spill. In contrast, an exposed small embayment on the eastern edge of the island was damaged severely, and it had not recovered when the next spill struck in 1986. The deforestation in the large northeastern embayment is also situated farther back from the water's edge, and there was a much wider surviving fringe. The reason for this difference is apparently related to higher tides and stronger onshore winds at the time. Furthermore, some inner, bare "wet desert" areas were apparently also created by the 1968 spill, and these have still not recovered, possibly because residual oil remains in the soil.

8.4.3 Comparing the Effects of Two Spills

These maps provide the quickest and most effective means of describing deforestation caused by large oil spills on mangrove forests, and for comparing effects of the different spills. They provide a rapid appraisal of differences in habitat damage and change, identifying not only the magnitude, but also patterns of deforestation and precise locations. They also provide the means to estimate actual areas, as was determined for the two large oil spills in Bahía Las Minas (Table 8.6).

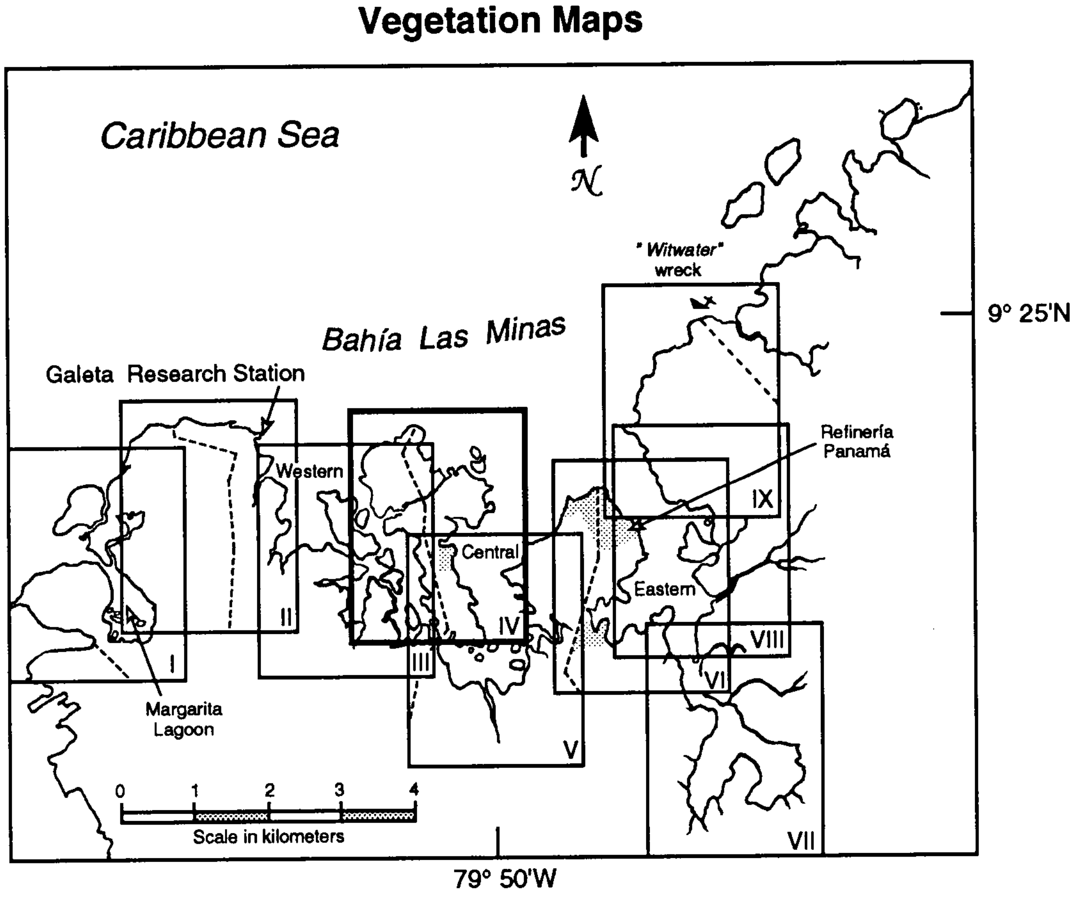


Fig. 8.11 Map of Bahía Las Minas showing outlines of the nine vegetation maps drawn from aerial photographs to show mangrove forests and the deforestation caused by oiling from the refinery spill in 1986 and the tanker *Witwater* in 1968. The two sets of maps are presented in Appendix Figures D.1 to D.19; Map IV is also presented as Figures 8.12 and 8.13 for the respective spills. Also, note the nominal partition of the study area into four sections (marked with *dashed-line* borders), referred to in Table 8.6. These sections include Laguna Margarita, and the three embayments of Bahía Las Minas: Western, Central (also called Bahía Cativá), and Eastern.

The 1986 oil spill killed 64 ha of mangrove forests, approximately 7% of intertidal forested areas in the bay. Most of this area (69%) was concentrated in the central embayment where oil was first contained, and 23% was killed in the eastern embayment. By comparison, 46 ha, or 5% of total forested areas, were killed after

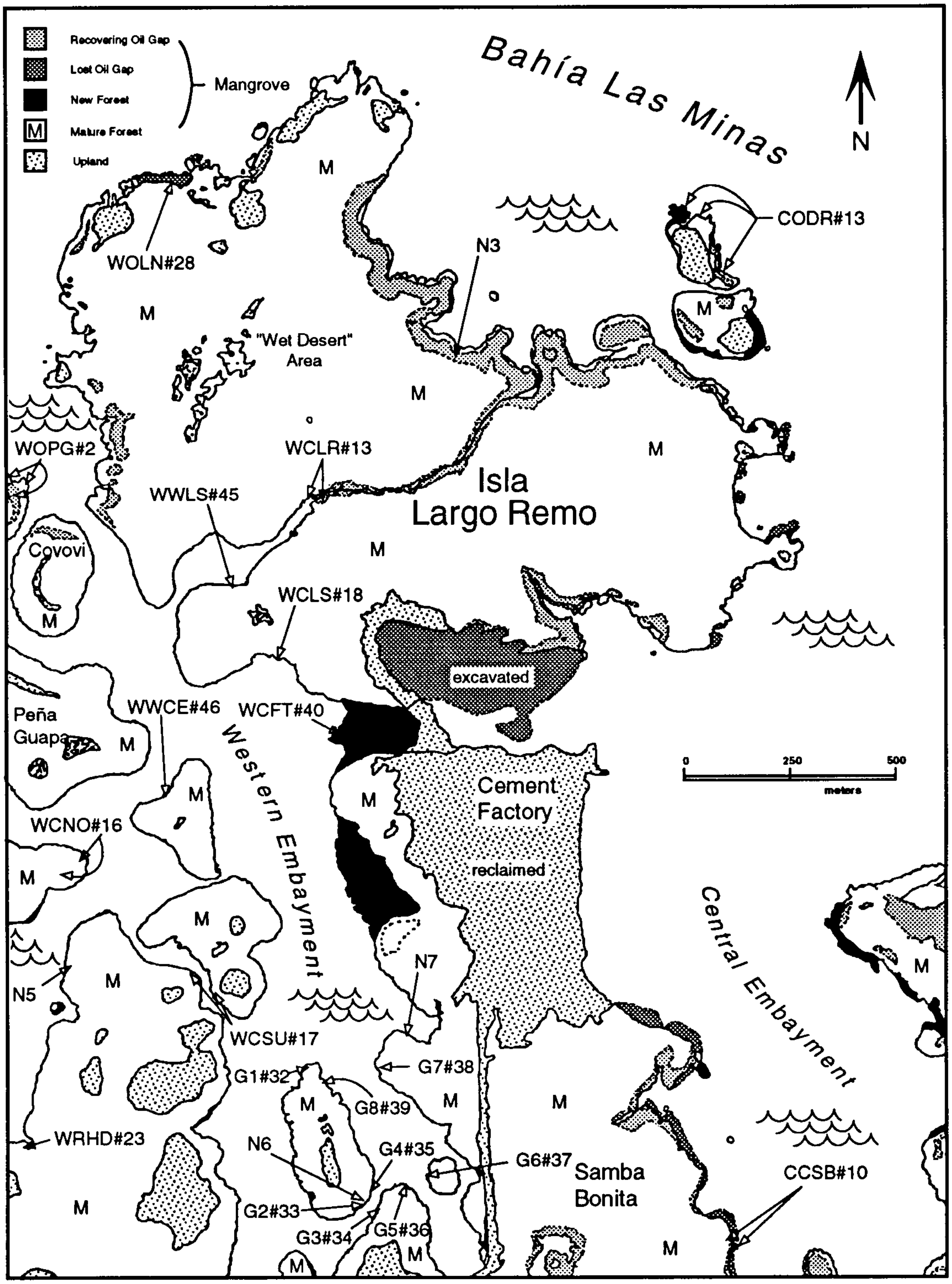

Recovering Oil Gap
Lost Oil Gap
New Forest
Mature Forest
Upland
Mangrove
Bahía Las Minas
N
CODR#13
N3
WOLN#28
M
"Wet Desert" Area
WOPG#2
WCLR#13
WWLS#45
Isla Largo Remo
Covovi
WCLS#18
excavated
Peña Guapa
WWCE#46
WCFT#40
Western Embayment
0
250
500
meters
Cement Factory
reclaimed
WCNO#16
Central Embayment
N5
N7
WCSU#17
G1#32
G7#38
G8#39
WRHD#23
N6
G4#35
G6#37
Samba Bonita
CCSB#10
G2#33
G3#34
G5#36

Fig. 8.12 (facing page) Map, based chiefly on 1990 aerial photographs, showing major oil-deforestation patterns in mangrove forests following the 1986 refinery spill. It centers on the islands of Largo Remo and Samba Bonita, located in the middle of Bahía Las Minas (note Area IV in Fig. 8.11). The legend includes: recovering and lost oil gaps, the latter being those with no recruitment and apparently changed into other habitats; new areas of mangrove forest created since 1973 (most of these are associated with the construction of the cement factory and port); mature, essentially intact forests; and upland areas above highest tidal levels. Notice that oil gaps are mostly narrow bands and patches, fringing seaward margins of forests, or along small inlets and tidal streams. No deforestation was observed in the western embayment, while it was extensive in the central area. Note the site locations used in the present study (see Appendix Table D.14 for all site codes and studies).

the 1968 *Witwater* spill, and this area was concentrated in the western and central embayments (52% and 30%, respectively). The proportion of exposed habitats affected was slightly greater after the *Witwater* spill (22% of total deforestation). The lower loss (17%) after the 1986 spill reflects the early entrapment of oil in the sheltered central embayment, compared with the immediate contact of the *Witwater* oil with exposed coastlines.

In summary, the two spills have two major differences in patterns of deforestation, the different topographic levels and occurrence in different parts of the bay. These differences were probably influenced by the different seasons and tidal regimes, as much as by the different sources and types of oil (Cubit and Levings, Chap. 2). Despite these differences, however, it is interesting to note the similarities because these were instrumental in the discovery of the earlier deforestation. Major similarities include coastal-fringing (contour-like) strips of deforestation approximately 50 m wide and often fronted by an isolated row of surviving trees along the original fringe, lens-shaped oil gaps on convex coastal margins, and funnel-like gaps at smaller stream entrances. Such observations have not been reported before, so it is not known whether these characteristics of oil gaps compare with those from other large oil spills in mangrove forests.

8.5 Condition of Mangroves Surrounding Deforested Areas

8.5.1 Forest Structure

From the aerial photographs, forest damage was clearly seen in large bands of total deforestation, creating an impression of specific and clearly delimited areas of effects of the oil spill on mangrove forests. However, two observations (Galeta Marine Laboratory: 1986 oil spill log) suggest that this picture was not complete, and that the full effect of the spill probably extended much farther. First, oiling was observed on more trees than eventually died. Second, intertidal fauna died throughout the wider oiled area. It therefore seems highly probable that trees have a wider response to oiling than dying, and that oiled survivors may eventually recover. For example, it is possible that the oiled forests surrounding gaps may have become less dense.

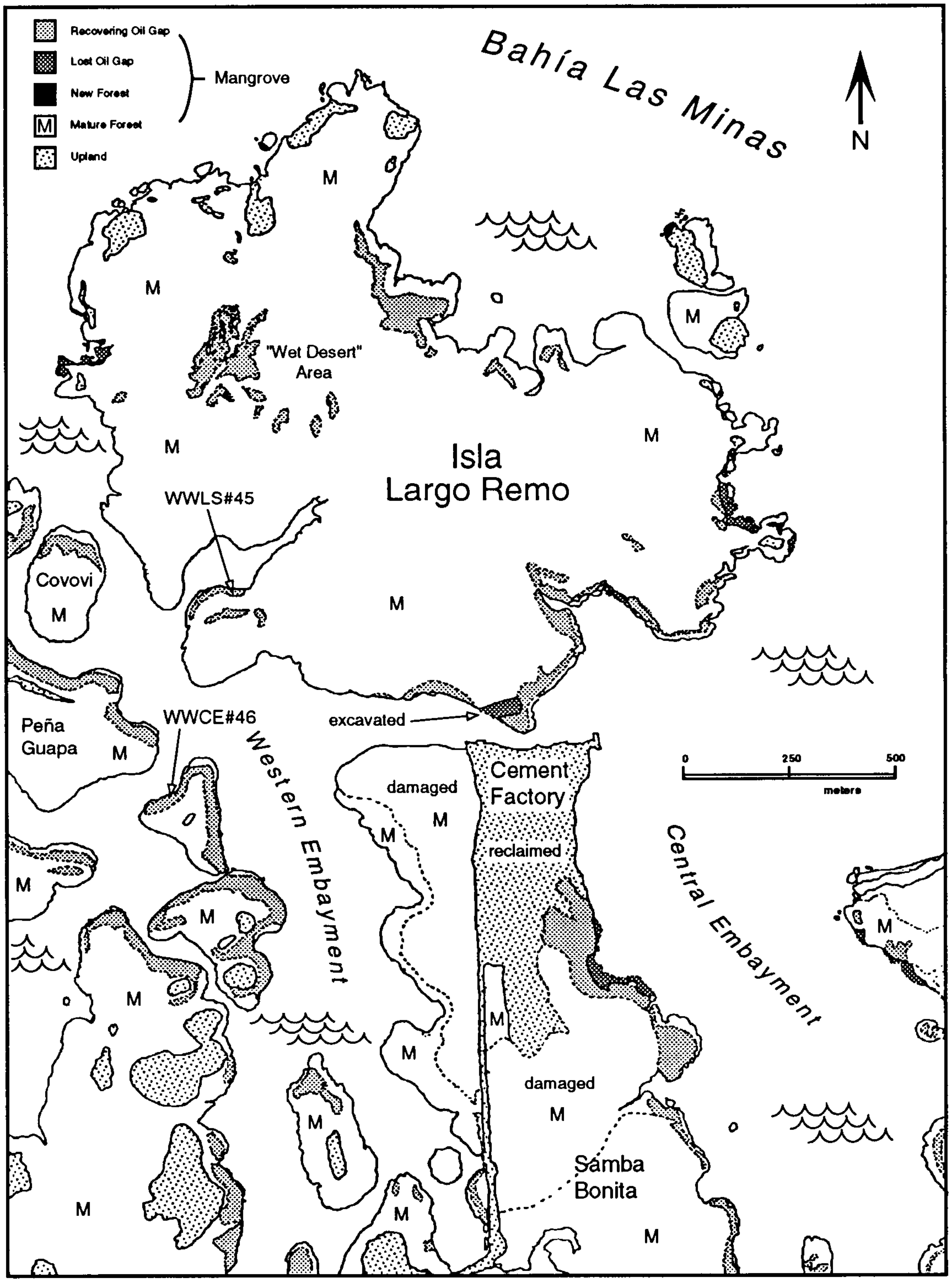

Recovering Oil Gap
Lost Oil Gap
New Forest
Mature Forest
Upland
Mangrove
Bahía Las Minas
N
M
"Wet Desert" Area
Isla Largo Remo
WWLS#45
Covovi
Peña Guapa
WWCE#46
excavated
Western Embayment
damaged
Cement Factory
reclaimed
Central Embayment
0
250
500
meters
Samba Bonita

Fig. 8.13 (facing page) Map, based chiefly on 1973 aerial photographs, showing major oil-deforestation patterns in mangrove forests following the 1968 *Witwater* spill. It centers on the islands of Largo Remo and Samba Bonita, located in the middle of Bahía Las Minas (note Area IV in Fig. 8.11). The legend includes: recovering and lost oil gaps, the latter being those with no recruitment and apparently changed into other habitats; new areas of mangrove forest created since 1966; mature, essentially intact forests; and upland areas above highest tidal levels. Notice that oil gaps were mostly narrow bands and patches, fringing seaward margins of forests, or along small inlets and tidal streams. However, others were also evident in the center of Isla Largo Remo, as a large "wet desert," or bare area, which was still present in 1991 (Fig. 8.12). In 1968 deforestation was extensive in both the western and central embayments. Furthermore, the channel between the two larger islands had not been filled, although dredging and excavation had commenced at Isla Largo Remo, and large areas of mangroves were noticably damaged by reclamation of the cement- factory site. Note the site positions of preliminary studies of forest structure, particularly regarding the older *Witwater* oil gaps.

These ideas were assessed in a study of the structural characteristics of these forests, comparing them with similar sites and habitats in places away from the 1986 oiling, including forests in exposed-coast, sheltered-channel, and river-fringing habitats. The data are summarized in Table 8.7 and listed for all 25 litter-fall sites in Appendix Table D.5. In Table 8.7 sites are also grouped by oiled and unoiled condition, showing the lack of significant differences. These measurements and derived estimates demonstrate that site selection for treatment comparisons were based on forests having obvious structural similarities. On the other hand, there are important differences between habitats, with exposed sites having significantly lower total biomass, approximately 80 t/ha, reflected in smaller trees with an average lean angle of approximately 45° and a relatively low canopy approximately 3.5 m tall. The total biomass of channel and river sites was equivalent, at approximately 190 t/ha, but there were obvious differences, with river sites having taller, more erect trees, (approximately 14 m) compared with channel sites (approximately 5.5 m). Densities, however, were significantly less at river sites. Comparison of structural parameters was also useful, showing that mean tree diameter for a site, for example, could be used to predict mean canopy height (Height [m] = -1.4397 + 0.7599[Diameter {cm}]; $r = .91$; $N = 25$; $P < .001$).

Species composition also changed between habitats, with a greater presence of species other than *Rhizophora*, particularly *Laguncularia racemosa*, at river sites. In all cases there were no undercanopy trees, but *Rhizophora* seedlings were abundant at channel sites, less abundant at river sites, and virtually absent at exposed sites.

Clearly, exposed sites were predominantly influenced by marine conditions, while those in channel and river habitats had respectively greater amounts of influence from river outflow. The designation of habitat type matched mean annual water salinity, shown in Figure 8.14, including major seasonal variation expressed as standard errors and range. These data along with water temperature, summarized in Table 8.4, demonstrated the comparable environments with respect to site groupings of habitat and the occurrence of oiling in 1986.

Table 8.6 Areas of oil-gap deforestation and mangrove vegetation in the three parts of Bahía Las Minas and neighboring Margarita Lagoon, Panama (Fig. 8.11). The areas include two major habitats (exposed and sheltered), and were measured from traced outlines of aerial photographs taken in 1973 and 1990.

Vicinity of Bahía Las Minas	Vegetation Area (ha)					
	1973			1990		
	Exposed	Sheltered	Total	Exposed	Sheltered	Total
Oil Gaps (Fringing)						
Margarita	0.00	3.43	3.43	0.05	0.05	0.10
Western	6.74	17.69	24.43	2.13	2.87	5.00
Central	3.71	10.60	14.31	7.26	37.09	44.35
Eastern	0.00	3.48	3.48	1.31	13.38	14.68
Total	10.45	35.20	**45.65**	10.75	53.38	**64.14**
Lost Habitat (Fringing)						
Margarita	0.00	0.13	0.13	0.00	0.05	0.05
Western	0.97	0.00	0.97	0.69	0.20	0.89
Central	1.05	0.14	1.19	0.50	3.08	3.57
Eastern	0.00	0.94	0.94	0.23	0.37	0.61
Total	2.02	1.21	**3.24**	1.42	3.69	**5.12**
All Mangrove Forests						
Margarita			309.22			275.38
Western			333.36			340.82
Central			258.66			230.00
Eastern			344.31			331.47
Total			**1,245.55**			**1,177.68**
Other Deforestation						
Margarita	0.00	0.00	0.00	0.22	0.15	0.37
Western	0.00	0.09	0.09	0.00	7.42	7.42
Central	0.00	10.71	10.71	0.00	11.01	11.01
Eastern	0.00	1.87	1.87	0.00	9.03	9.03
Total	0.00	12.66	**12.66**	0.22	27.61	**27.83**

In general, major structural parameters, including mean height and diameter, were inversely correlated with mean annual salinity (height: $r = -.65$; $P < .001$; $N = 25$). This finding shows that taller fringing forests characterize sites with lower mean salinities and greater river runoff. However, it does not follow that these upstream sites with larger trees have greater amounts of plant biomass per unit area. In fact, site estimates of total above-ground biomass were not correlated with salinity. This

Table 8.7 Characteristics of *Rhizophora mangle* forests in unoiled and surviving-oiled locations in three fringe habitats in 1991. The means are based on 26 litter fall and shoot study sites (Table 8.8). SE of among-site variation is in parentheses. Site means are in Appendix Table D.5.

	Exposed		Sheltered		
	Open		Channel		River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Stem Height (m)	3.47 (0.26)	3.64 (0.37)	5.53 (0.51)	5.90 (0.74)	14.30 (4.60)
Stem Diameter (cm)	5.8 (0.6)	6.4 (1.0)	9.5 (0.8)	10.9 (1.6)	19.7 (4.7)
Basal Area (m^2/ha)	20.11 (2.02)	14.63 (2.33)	16.09 (1.94)	16.46 (3.05)	17.37 (2.79)
Stem Density (stems/ha)	11,376 (3,150)	8,365 (2,493)	7,042 (3,829)	7,122 (3,199)	1,939 (1,350)
Stem Angle (degrees from horizontal)	40.5 (4.4)	46.1 (6.4)	51.6 (2.8)	54.7 (9.9)	73.1 (8.6)
Proportion of *Rhizophora* Stems to Total	1.00 (0.00)	0.97 (0.03)	0.96 (0.04)	0.89 (0.11)	0.72 (0.12)
Tree Density (trees/ha)	3,380 (618)	1,994 (373)	3,362 (1,741)	3,901 (2,465)	1,513 (951)
***Rhizophora* Seedling Density** (number/m^2)	0.02 (0.02)	0.03 (0.01)	1.71 (0.38)	1.76 (1.41)	0.33 (0.11)
***Rhizophora* Leaf Biomass** (t/ha)	4.10 (0.68)	3.51 (0.83)	5.97 (2.66)	5.53 (1.87)	3.35 (0.55)
***Rhizophora* Total Biomass** (t/ha)	80.5 (9.9)	80.7 (20.8)	194.0 (88.2)	199.8 (86.7)	182.3 (50.6)

was also the case for leaf biomass, which generally makes up 2-5% of the total above-ground biomass.

These structural characters and estimates of biomass are best viewed as prespill, therefore, because tree height does not appear to have been significantly reduced in surviving trees 3-5 yr postspill. Shedding of topmost branches was not evident in these forests. Nor was there any apparent change in densities, as noted earlier, where tree death appears to be almost exclusively in large deforestation patches, rather than selectively affecting certain individuals or species in a process of forest thinning. Field observations support this view. These major structural characters of surviving forests therefore remained essentially unchanged following oiling.

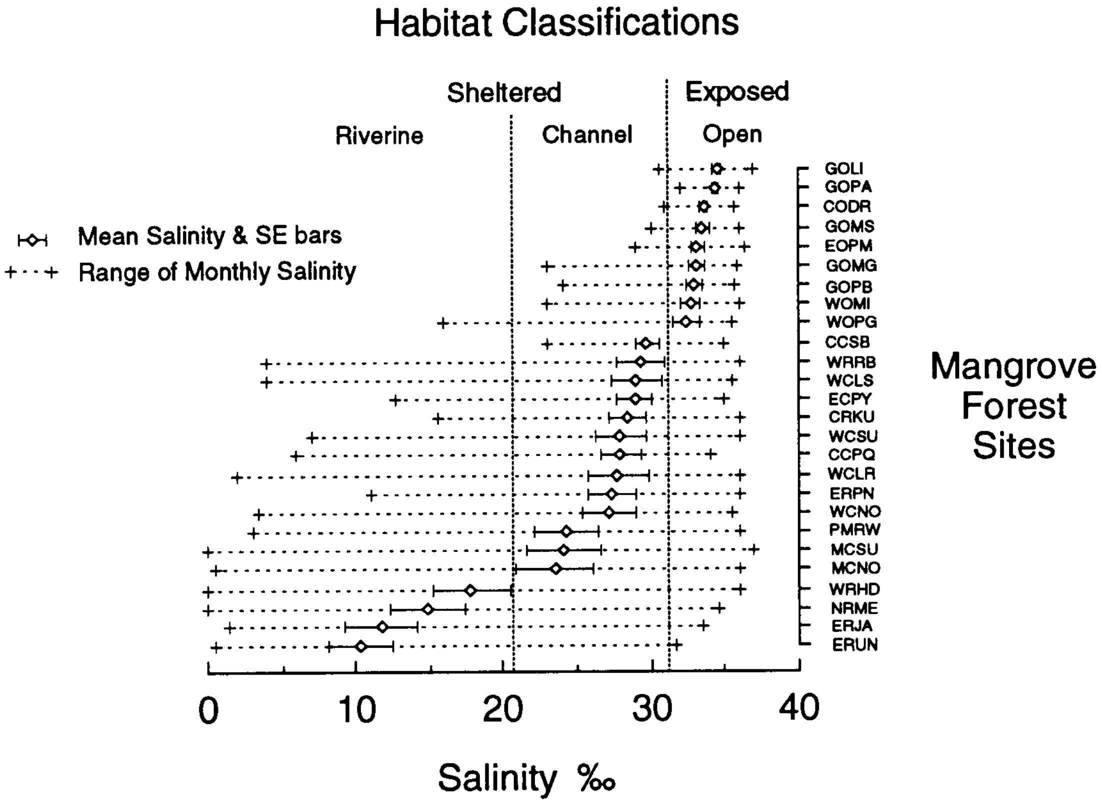


Fig. 8.14 Annual means and ranges of monthly records of salinity at all sites, which provided a basis for categorization of sites on exposed open coasts, sheltered channels, and riverine streams. Measurements were scored from water collected 5-10 cm below the surface of channels and streams immediately fronting fringing forests. Explanations for site codes are in Table 8.8. Seasonal variation in salinity is shown in Figure 8.9.

8.5.2 Seedling Assemblages in Unoiled Forests

Seedling assemblages were assessed at sheltered sites, and node counts provided a determination of age structure (Fig. 8.15). Note the higher frequencies at 1 node and 4-5 nodes, forming two apparent cohorts. These peaks were confirmed as annual because we measured node production rates of 3-4 nodes/yr for comparable seedlings (Duke and Pinzón 1992). Seedlings, therefore, apparently survive under closed canopies for up to 2 yr before dying while this part of the population, or "seedling bank," is replenished annually.

8.5.3 Canopy Condition and Productivity 3-5 Years Postspill

The canopy of mangrove forests is much more flexible than forest structure and clearly reflects seasonal changes corresponding to obviously different wet- and

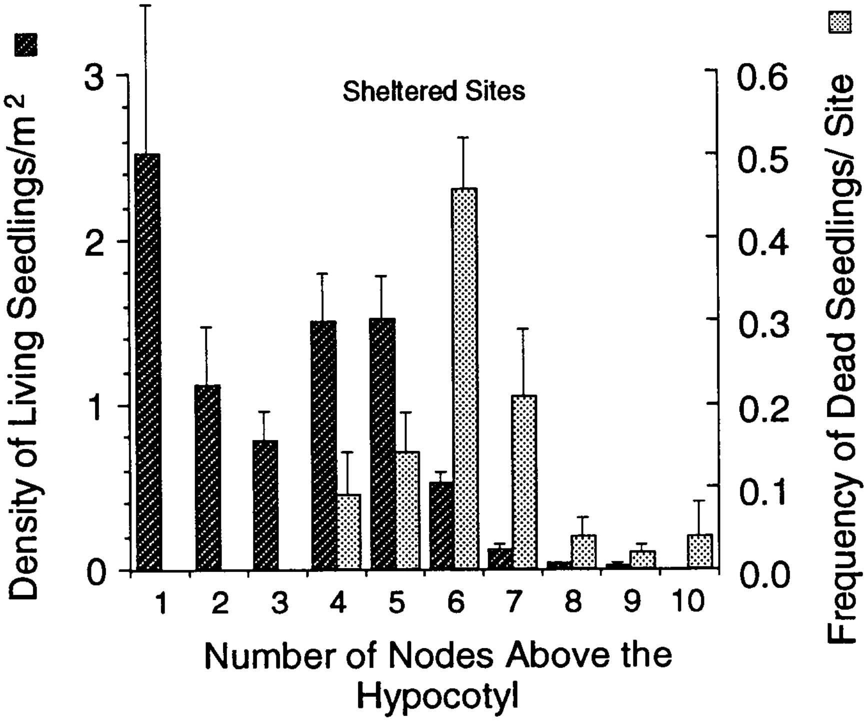


Fig. 8.15 Density of living seedlings and frequency of dead seedlings at sheltered sites. Mature hypocotyls supply a seedling bank of 1- to 3-yr-old recruits established under the closed canopy of *Rhizophora mangle* forests. This graph was based on sheltered sites; exposed sites had similar patterns, although they had much lower densities. The plot compares densities of living recruits with particular leaf node counts along the main stem (mean 3.3 ± 0.2 nodes/seedling), scored in July 1990, with others that died during the year (mean 6.2 ± 0.2 nodes/seedling). The number of nodes was taken as a measure of age because annual node-production rates measured in similarly shaded sites were constant (3.7 ± 0.3 nodes/year). Notice also that in July the 1990 cohort of established seedlings had 1 node and the 1989 cohort had 4 to 5 nodes. Based on these data, turnover time of the seedling bank was estimated to be approximately 2 to 3 yr.

dry-season climatic conditions (e.g., Duke et al. 1984). The canopy may therefore be considered as a pulse by which we may gauge the condition of the forest. The main functional component of the canopy are the leaves, and in *Rhizophora* trees these are clustered at the ends of shoots. The following studies focus on these leaves and shoots, noting their present character and the processes of production and longevity. The objective was to show any changes, either between oiled and unoiled sites, or during the 2 yr of the study.

8.5.3.1 Shape and Size of Leaves

Leaves were collected from 26 sites in the study area (Table 8.8). Data on mean lengths, widths, and weights are listed in Appendix Table D.1, and are summarized for habitat/treatment groupings in Table 8.9. A plot of leaf lengths and widths (Fig. 8.16) shows that all site means follow the same significant relationship ($P < .001$). Close examination of these data suggests there are effects of salinity and oil, not on shape, but on size. This effect was shown by nonsignificant regressions of the length-to-width ratio, for shape, and significant regressions for size, based on area estimates ($P < .05$). Mean leaf area for respective sites was estimated from allometric equation 3 (Sect. 8.3.2) for the multiples of length times width for each leaf. Plots of the relevant regressions are shown in Figure 8.17, with larger leaves found at lower salinities and higher concentrations of oil in the sediment.

Another characteristic of leaves is thickness, which is proportional to weight per unit area. This measure of thickness was also compared with salinity and oil concentration showing only a significant effect of salinity ($P < .001$). This effect, however, differed with respect to the presence of oil (Fig. 8.18), and leaves were noticeably thicker at higher salinities, but at oiled sites they tended to be thinner.

8.5.3.2 Seasonal Influences and Canopy Variation

Total canopy condition was monitored each month in the concurrent studies of litter fall and shoot observations for comparable oiled and unoiled sites in two habitats, exposed open coasts and sheltered channels. Unoiled river sites were also considered for comparison. Litter-fall and shoot observations showed comparable trends for leaf production and leaf fall (Figs. 8.19, 8.20), and reproductive development (Fig. 8.21). It needs to be emphasized that while the two methods are complementary, they differ because litter fall relates to total canopy production and shoot observations are based on productivity per leafy shoot. In either case, there are clear seasonal trends in vegetative and reproductive growth. For example, compare stipule fall in litter, a measure of new leaves produced on the tree (Duke et al. 1984), with the actual measure of new leaves produced on shoots. Generally, leaf production was lowest from December to February, the dry season, and peaked in May to July, during the wet season. Leaf fall tended to follow this trend at channel and river sites but not at open-coast sites, where the previous trend was reversed. This difference is probably related to the mean life span of leaves at particular sites, which will be estimated later in this assessment. It will also be noted in these plots, with regard to leaf production and fall, that oiled sites showed no consistent differences from the fluctuations at unoiled sites.

In contrast, there were consistent differences in the numbers of leaves per shoot from the shoot observations (Fig. 8.20). These values also fluctuated with season, and there was a positive relationship with rainfall and a negative one with solar radiation, but numbers were always lower by one or two leaves at oiled sites. Furthermore, these numbers declined over the 2 yr of observations, shown by

Table 8.8 Mangrove Forest Project site descriptions and codes, with corresponding Mangrove Root Project site codes (Garrity and Levings, Chap. 9). The first letter of the Forest Code refers to locations in Bahía Las Minas (i.e., in *BLM*, as western = *W*, central = *C*, and eastern = *E*), toward Isla Grande (= *G*), near Isla Margarita (= *M*), and near Islas Naranjos (= *N*). The second letter refers to habitats. The third and fourth letters of the Forest Code relate to site description names.

Site No.	Site Code	Forest Code	Root Code
Exposed (Open) Oiled			
1	Mina - West BLM	WOMI	MINM
2	Pena Guapa - West BLM	WOPG	PGM
3	Droque - Central BLM	CODR	DROM
4	Punta Muerto - East BLM	EOPM	PMM
Exposed (Open) Unoiled			
5	María Soto - I. Grande	GOMS	MSM
6	Portobelo - I. Grande	GOPB	PBM
7	Padre - I. Grande	GOPA	PADM
8	Lintón - I. Grande	GOLI	LINM
9	Magoté Sur - I. Grande	GOMG	-
Sheltered (Channel) Oiled			
10	Samba Bonita - Central BLM	CCSB	SBCE
11	Pequeña - Central BLM	CCPQ	SBCS
12	Payardi - East BLM	ECPY	PCS
13	Largo Remo - West BLM	WCLR	LRCW
Sheltered (Channel) Unoiled			
14	Sur - Margarita	MCSU	MACS
15	Norte - Margarita	MCNO	MACN
16	Norte - West BLM	WCNO	HIDC
17	Sur - West BLM	WCSU	SBCW
18	Largo Remo Sur - West BLM	WCLS	LRCS
Sheltered (Channel) Oiled			
19	Puerto Sur - East BLM	ERPS	PMRE
20	Puerto Norte - East BLM	ERPN	PMRW
21	Rabinowitz - West BLM	WRRB	-
22	Kuna - Central BLM	CRKU	-
Sheltered (River/Stream) Unoiled			
23	Hidden - West BLM	WRHD	HIDR
24	Unnamed - East BLM	ERUN	UNR
25	Alejandro - East BLM	ERJA	ALER
26	Las Mercedes - Naranjo	NRME	MERR

significant regressions ($P < .01$) through these monthly data only for oiled sites and not unoiled sites, and in the two mean annual estimates (Fig. 8.22).

Seasonal production of reproductive parts (Fig. 8.21) appeared to be more influenced by habitat differences than by differences caused by oiling. For example, notice that open sites had relatively low production of advanced reproductive stages.

Table 8.9 Parameters of leaves of *Rhizophora mangle* measured and weighed green (wet), and dry for mangrove forest sites in oiled and unoiled locations of three fringe habitats in September 1991. SE of among-site variation is in parentheses. Site means are in Appendix Table D.1.

	Exposed		Sheltered		
	Open		Channel		River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Wet Length (cm)	99.97 (2.23)	102.95 (1.63)	109.55 (4.18)	120.80 (2.24)	120.35 (2.59)
Wet Width (cm)	46.08 (0.82)	50.17 (1.03)	52.11 (1.97)	58.70 (1.76)	59.13 (1.38)
Wet Weight (g)	1.84 (0.09)	2.32 (0.15)	2.28 (0.19)	2.65 (0.15)	2.51 (0.05)
Dry Length (cm)	93.21 (2.06)	94.45 (1.51)	101.00 (3.50)	110.71 (1.81)	111.44 (1.92)
Dry Width (cm)	40.77 (1.31)	44.15 (1.5)	46.54 (1.53)	52.26 (1.40)	52.64 (1.45)
Dry Weight (g)	0.76 (0.04)	0.81 (0.02)	0.88 (0.07)	1.00 (0.06)	0.93 (0.03)
Wet Area (cm^2)	34.21 (1.44)	38.25 (0.98)	41.89 (3.01)	51.80 (2.37)	51.70 (1.37)

8.5.3.3 Annual Measures of Canopy Condition and Production

Canopy production is clearly seasonal, so the best way to reduce this effect and determine canopy condition and productivity is to estimate annual means of the various measures.

Data from 2 yr (1989-1990 and 1990-1991) of shoot observations are in Table 8.10 (site data are in Appendix Table D.6), and show mean annual estimates of leaf production, leaf fall, numbers of leaves per shoot, and derived parameters of net canopy production (subtracting leaf loss from leaf production) and turnover (dividing standing crop leaves per shoot by leaf production). There was no appreciable difference between leaf production and fall at oiled and unoiled sites, although there was a trend toward lower production, greater loss, and a canopy debit in lower- salinity habitats. Numbers of leaves per shoot were previously shown to be related to both salinity ($r = .51$; $N = 11$; $P < .05$) and oil ($r = -.63$; $N = 19$; $P < .005$), with lower numbers of leaves per shoot at low salinities and oiled sites. Turnover of leaves in the canopy was slowest at unoiled open sites, taking about 9-10 mo. At sheltered sites turnover was approximately 7 mo. The overall relationship with salinity, however, was not significant, but turnover was correlated with residual oil in sediments (Fig. 8.23). Oil concentration was also correlated with the standing-crop measure of the number of leaves per shoot (Fig. 8.24), and leaf longevity was reduced by 18% in comparable oiled habitats.

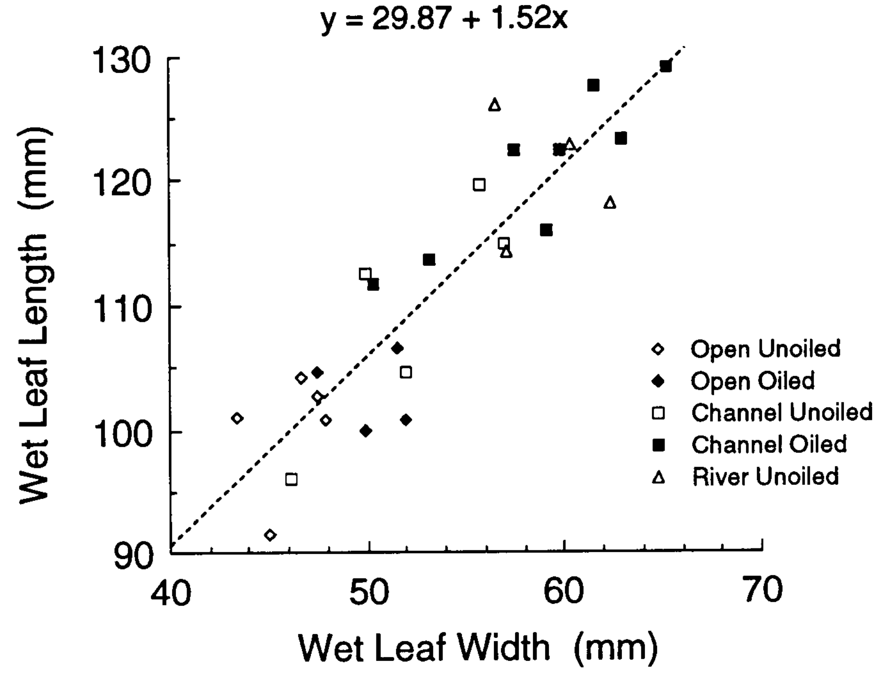


Fig. 8.16 Mean length and width of *Rhizophora mangle* green leaves from unoiled (*open symbols*) and oiled sites (*solid symbols*) in three fringe habitats of the study area. Notice that they form a single regression ($r = .89$; $N = 26$; $P < .001$), showing that leaf shape (length/width) did not change significantly at oiled sites. On the other hand, leaf size varied substantially, with the largest leaves generally found at sheltered sites.

The responses of shoots to oil also changed with respect to position in the canopy. Numbers of leaves per shoot and leaf production were both highest in the upper canopy of unoiled sites, but each parameter tended to be more even among canopy positions in oiled habitats (Fig. 8.25). They differed, however, and leaves per shoot were significantly higher in the upper canopy of unoiled habitats compared to oiled ones. Leaf production was significantly greater at a canopy position 1-1.5 m lower in oiled habitats than in unoiled habitats. This difference is possibly a result of more light reaching lower canopy shoots at the lower-density oiled sites.

Annual litter production by counts is summarized in Table 8.11, showing means for oiled and unoiled habitats of five major litter components: stipules, leaves, total reproductive parts, flowers, and mature hypocotyls. These counts match the values reported in shoot observations.

Annual litter production by weight is summarized in Table 8.12, showing means for oiled and unoiled habitats of four major litter components: leaves, stipules, wood, and reproductive parts, and the totals. The one trend in these data was a slightly higher amount of wood litter at oiled sites, and lower total litter and reproductive parts at open sites.

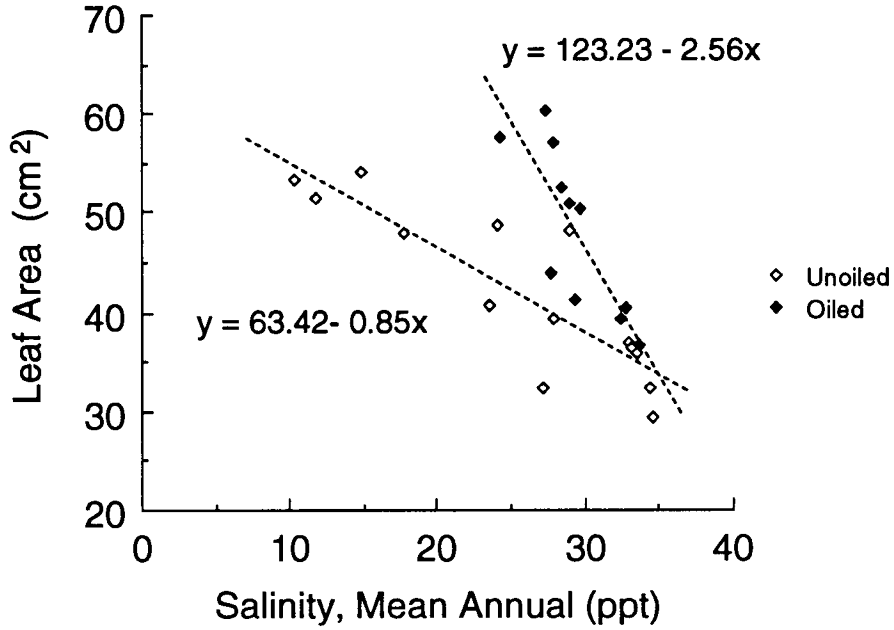


Fig. 8.17 Mean area of *Rhizophora mangle* green leaves and mean annual water salinity at unoiled (*open symbols*) and oiled sites (*solid symbols*) in three fringe habitats of the study area. Notice that unoiled and oiled sites form two different regressions (unoiled: r = -.86, N = 14, P <.001; oiled: r = -.84, N = 12, P <.001), showing that leaf size increased more at lower salinities.

Several parameters of canopy condition derived from litter and shoot data are presented in Table 8.13, showing respective means of grouped data and estimates of standing leaf biomass. Canopy leaf area, or leaf area index (LAI), is a measure of total photosynthetic area of the canopy. This parameter was correlated with salinity (Fig. 8.26), but not with oil. On the other hand, canopy leaf biomass was correlated with oil (Fig. 8.27), and not with salinity. Mean leaf area was correlated with both salinity and oil (P <.005).

These results clearly show a marked difference in all habitats, representing a reduction in all estimates of canopy density, notably those for shoot numbers, leaf numbers, and total leaf weights. Note that leaf biomass was similar in all unoiled habitats, but was substantially lower at oiled sites. Where oil was present there was a consistent 23-33% reduction in leaf biomass, as standing crop in the canopy. The comparison of standing-canopy biomass and annual turnover is important because their ratio provides a further measure of leaf turnover. Data are summarized in Table 8.14, showing estimates in t/ha for the respective oiled and unoiled habitats. Essentially, the turnover ratios reflect standing crop differences because there was no appreciable difference in annual leaf production. These changes in turnover indicate that leaf longevity was reduced by about 18% at oiled sites.

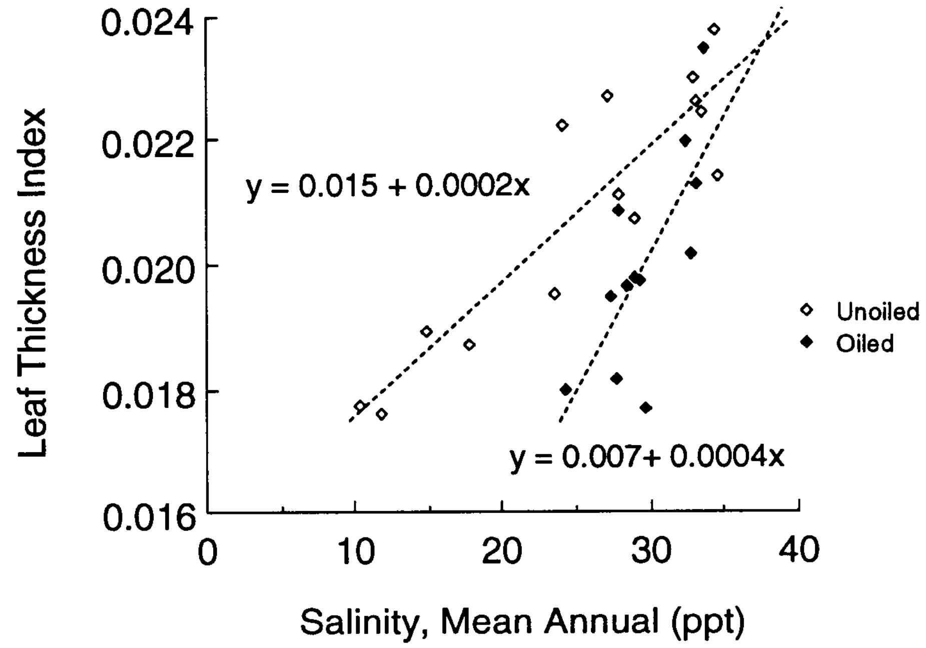


Fig. 8.18 Mean density (weight/size) of *Rhizophora mangle* green leaves and mean annual water salinity at unoiled (*open symbols*) and oiled sites (*solid symbols*) in three fringe habitats of the study area. Notice that unoiled and oiled sites form two different regressions (unoiled: $r = .90$, $N = 14$, $P < .001$; oiled: $r = .73$, $N = 12$, $P < .005$), showing that thicker leaves occurred at higher salinities, but that in oiled areas leaves were thinner at lower salinities.

8.6 Status of Deforested Areas

8.6.1 Recruitment and Growth of Seedlings in 1986 Oil Gaps

8.6.1.1 Individual Seedlings

Areas deforested by the 1986 spill were well defined spatially in aerial photographs, and often had wide-open patches of dead trees with few, scattered survivors. These areas could not be expected, therefore, to recover entirely through processes of stem sprawling and expansion of fringing survivors. Consequently, recovery processes in these oil gaps were restricted almost entirely to seedling recruitment and growth.

However, shortly after assessment of seedlings commenced 3 yr after the spill, it was found that some seedlings were apparently older than the expected range of postspill recruits. Based on the sequence and number of leaf scar nodes along the main stem (Duke and Pinzón 1992; Appendix Abstract and Description D.1), it was concluded that these seedlings survived the oiling that killed the surrounding trees.

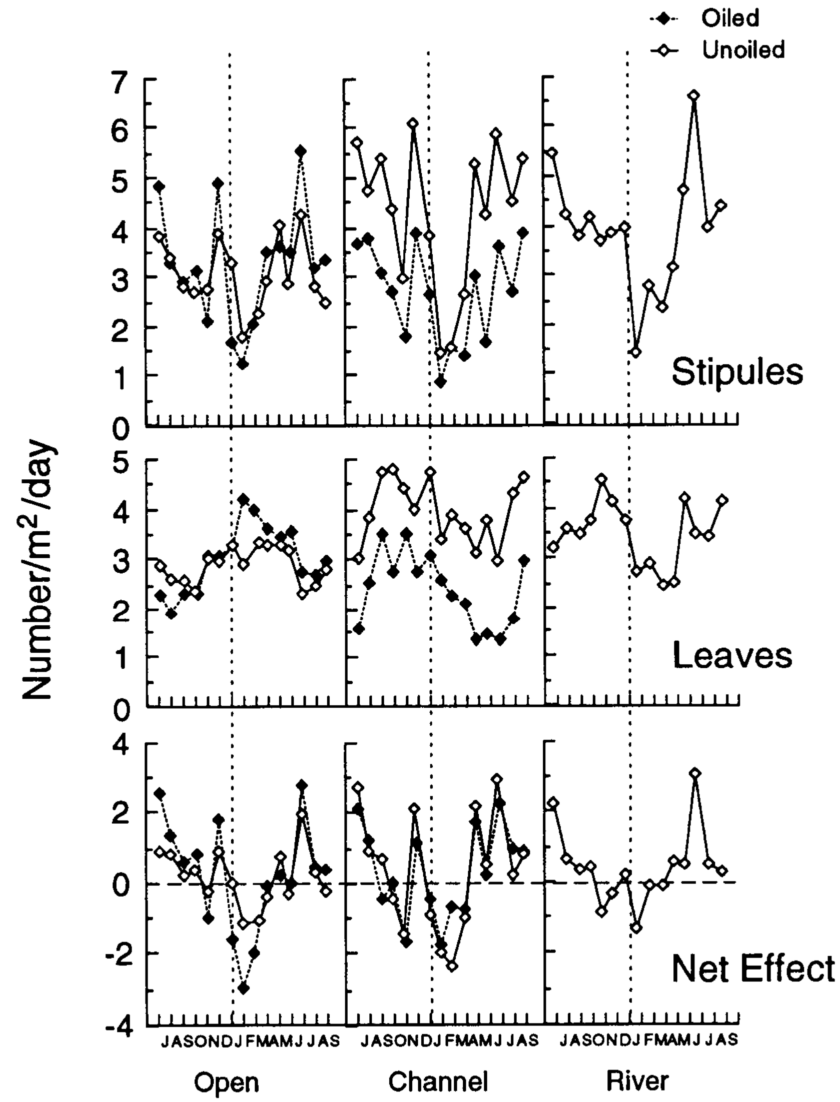


Fig. 8.19 Mean number of leaves and stipules in monthly litter fall of *Rhizophora mangle* trees at unoiled (*open symbols*) and oiled sites (*solid symbols*) in three fringe habitats of the study area, 1989 to 1990. Stipules in litter represent new leaves on the tree, so the difference in these parameters shows the net monthly canopy condition, which fluctuated around 0 during the year. Notice that the canopy was in debit in October and later, from January to March in all habitats, matching periods of low rainfall (Fig. 8.10). Sites at oiled areas had the same pattern.

Accordingly, seedlings in oil gaps appear to have grown first under a closed canopy, indicated by more than six nodes of limited internodal extension (up to 2-3 cm/node), a characteristic of shaded growth. This phase was followed by more rapid height extension in distinct seasonal pulses of approximately 9-10 cm/node,

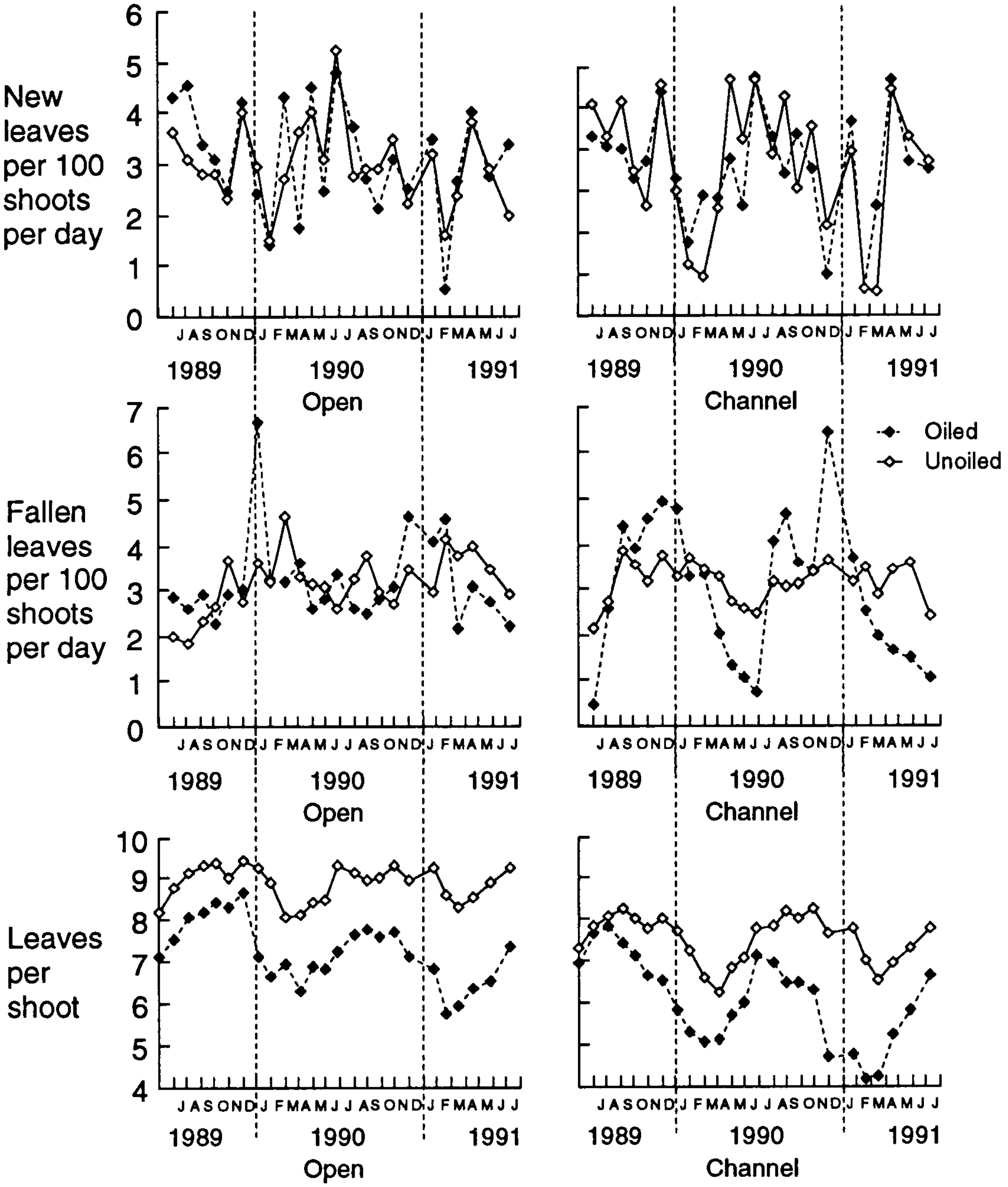


Fig. 8.20 Mean number of new leaves and fallen leaves in monthly shoot observations of *Rhizophora mangle* trees at unoiled (*open symbols*) and oiled sites (*solid symbols*) in two fringe habitats of the study area during 2 yr, 1989 to 1991. Notice that these rates followed the same pattern as equivalent litter-fall parameters (Fig. 8.19). In addition, a standing crop measure, leaves per shoot, showed distinct seasonal variation, and also was lowest in the dry season (Fig. 8.10). Furthermore, notice that this measure was always lower at oiled sites for open coast and channel sites.

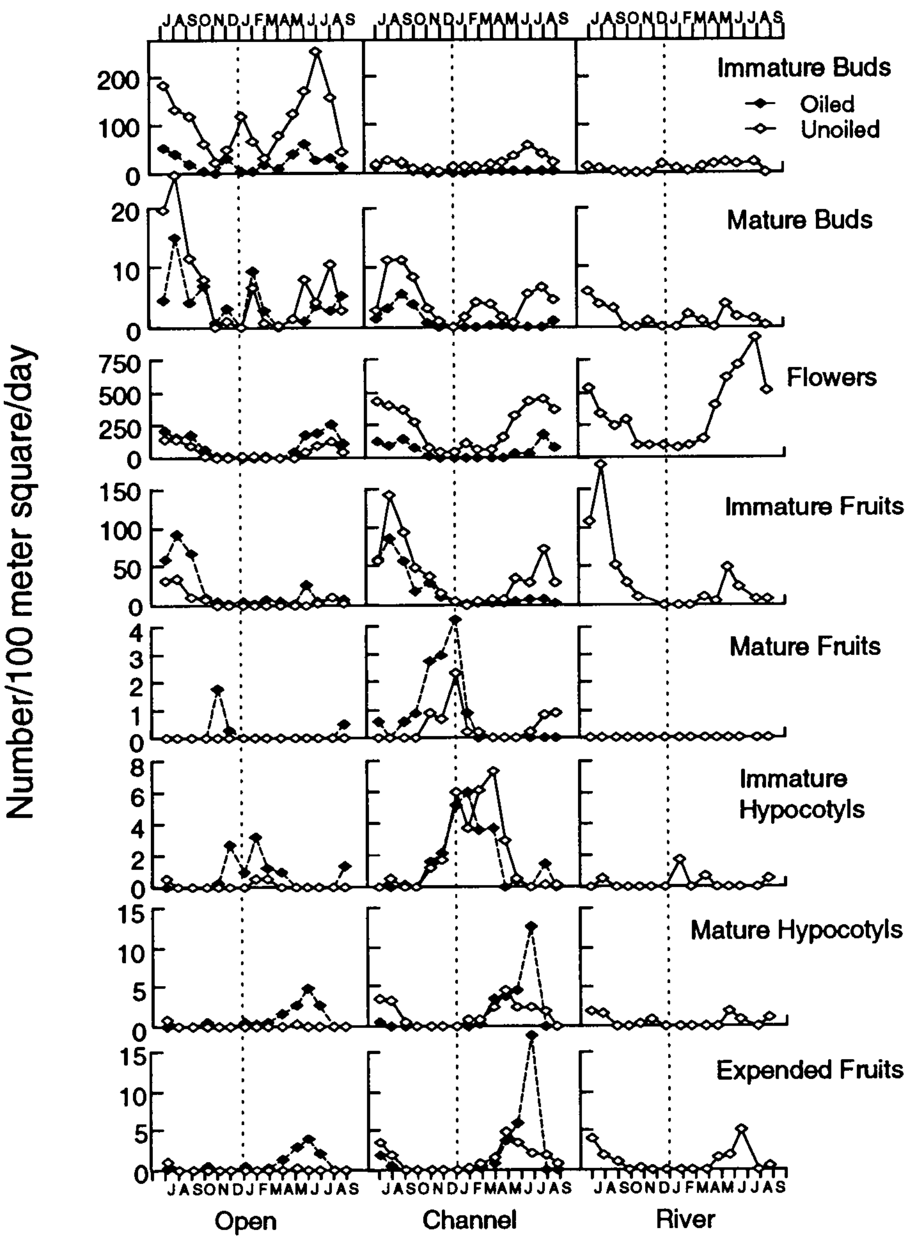


Fig. 8.21 Mean number of eight types of reproductive parts in monthly litter fall of *Rhizophora mangle* trees at unoiled (*open symbols*) and oiled sites (*solid symbols*) in three fringe habitats of the study area, 1989 to 1990. Notice the life-history phenology of these components, ordered in the plot from top to bottom by increasing maturity. The complete cycle took just over 1 yr, so that reproductive material was present in the canopy all through the year. Sites at unoiled, exposed areas had poor levels of hypocotyl maturation, but otherwise followed the same pattern as other sites. Notice that hypocotyls matured in May to July.

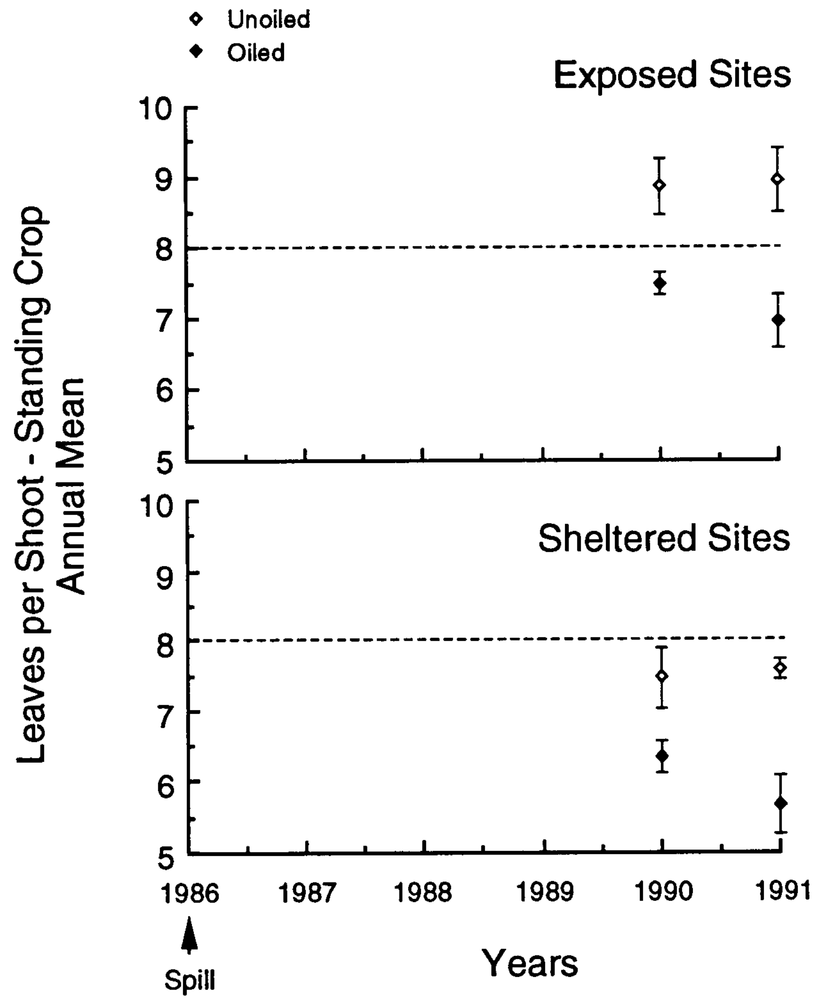


Fig. 8.22 Mean annual estimates of leaves per shoot from shoot observations of *Rhizophora mangle* trees at unoiled (*open symbols*) and oiled sites (*solid symbols*) in exposed open coast and sheltered channel habitats of the study area for 2 yr, 1989-1990 and 1990-1991. Notice that unoiled sites were always greater than oiled sites and that they were unchanged between the 2 yr. In contrast, oiled sites had much lower levels in the second year, representing an apparent decline since oiling in 1986.

characteristic of open-canopy growth. These seedlings apparently changed node production rates from under-canopy values of 3-4 nodes per yr, to about 7-8 per yr after the canopy disappeared.

Furthermore, peaks of internodal extension in these seedlings possibly also reflect a diminishing influence of residual oil on growth following the spill. This effect was, however, site dependent; consider the examples of four seedlings from four different sites in Bahía Las Minas (Fig. 8.28). Three were from different areas of oil-deforestation, and the other was from a natural light gap. All were apparently

Table 8.10 Annual estimates of new leaves, leaves lost, net production, leaves per shoot, and turnover ratio in *Rhizophora mangle* canopies. Data were derived from monthly shoot observations of mangrove forests in oiled and unoiled locations of 3 fringe habitats during 2 yr, 1989-1991. SE of among-site variation is in parentheses. Site means are in Appendix Table D.6.

	Exposed		Sheltered		
	Open		Channel		River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
New Leaves (number/100 shoots/d)					
1989-1990	3.04 (0.16)	3.23 (0.07)	3.00 (0.19)	2.99 (0.10)	2.61 (0.20)
1990-1991	3.04 (0.19)	2.97 (0.10)	2.94 (0.13)	2.89 (0.08)	2.56 (0.22)
Leaves Lost (number/100 shoots/d)					
1989-1990	3.03 (0.12)	3.22 (0.10)	3.09 (0.12)	3.16 (0.10)	3.05 (0.13)
1990-1991	3.35 (0.32)	3.23 (0.15)	3.22 (0.22)	3.09 (0.07)	2.93 (0.09)
Net Leaf Production (number/100 shoots/d)					
1989-1990	0.01 (0.13)	0.01 (0.07)	-0.09 (0.08)	-0.17 (0.08)	-0.44 (0.07)
1990-1991	-0.31 (0.23)	-0.26 (0.08)	-0.28 (0.21)	-0.20 (0.05)	-0.37 (0.15)
Standing Crop (leaves per shoot)					
1989-1990	8.86 (0.41)	7.49 (0.14)	7.48 (0.43)	6.38 (0.19)	6.61 (0.86)
1990-1991	8.94 (0.45)	6.96 (0.38)	7.58 (0.15)	5.92 (0.25)	6.32 (0.64)
Turnover Ratio (standing leaves per shoot/new leaves per shoot)					
1989-1990	0.80 (0.02)	0.64 (0.02)	0.69 (0.06)	0.59 (0.02)	0.69 (0.07)
1990-1991	0.81 (0.03)	0.65 (0.05)	0.71 (0.04)	0.56 (0.03)	0.69 (0.10)

older than the respective light gaps, and all show marked seasonal fluctuations of internodal increments. Notice the abrupt increase of peak values for the unoiled seedling (Fig. 8.28*a*), with a light gap appearing sometime prior to 1989. This timing was confirmed, in part, from aerial photographs. However, it is not known whether there is a significant delay, as presented, in the growth response under natural conditions. This delay is not expected to be longer than 1 yr because seedlings in oil gaps often showed significant increases 1 yr after the spill. This effect depended on the site, however, particularly regarding the presence of oil. In the first instance (Fig. 8.28*b*), the site was very exposed to waves and wind and there was virtually no protective barrier of lower-intertidal mature surviving trees. In this case, growth increased abruptly, as observed in the seedling from the unoiled gap site, while those from more sheltered locations exhibited greater delays in growth, with progressive increases (Fig. 8.28*c*, *d*). This pattern is suggestive of a negative influence of oil, which diminishes more rapidly at sites of greater exposure.

Individual seedlings therefore appear to survive oil spills and continue to grow afterward. These individuals are very important in habitat recovery because they

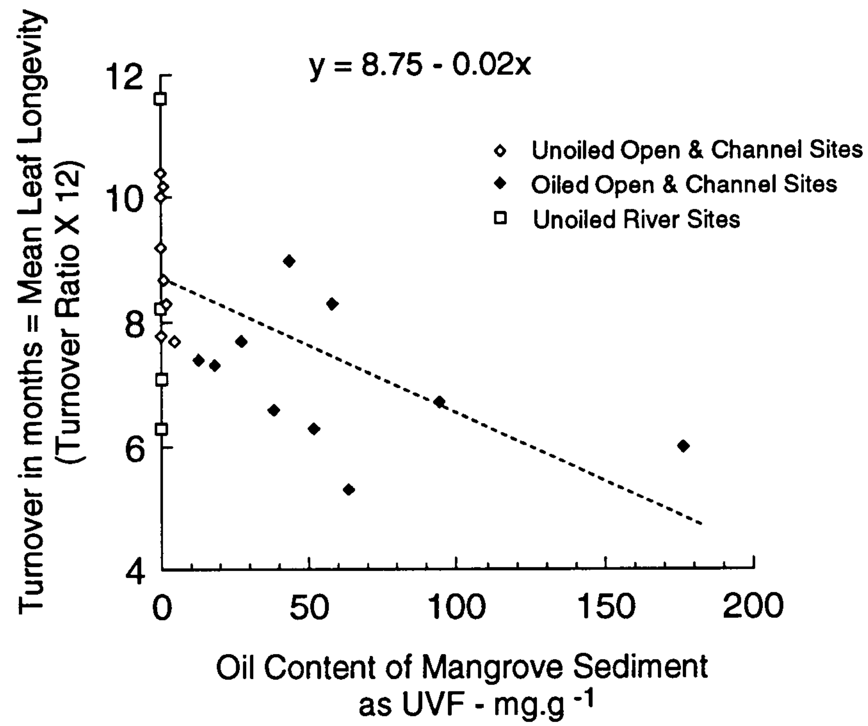


Fig. 8.23 Mean annual canopy turnover (standing crop/annual production) of *Rhizophora mangle* trees vs. the mean concentration of oil in sediments (UVF; see Burns, Chap. 3) during 1989-1990. Notice that turnover decreased on the open coast and in channels from 8 to 9 mo at unoiled sites to a minimum of approximately 6 mo at oiled sites (r = -.64; N = 19; P <.01; unoiled river sites not included).

represent the first cohort of creators of the new canopy. Because light is important for the growth of *Rhizophora* seedlings, all younger and shorter recruits are expected to grow appreciably less, and eventually die. So, how does this process affect seedling assemblages, and would gap recovery be influenced further by the presence of the remaining oil? It appears so, and several studies highlight the apparent success of natural recovery in oil gaps in Bahía Las Minas.

In one study, growth of seedlings over 1 yr (1989-1990) was monitored for 216 individuals, for all age groups from the time of the spill and throughout the range of oiled and unoiled habitats. These seedlings were grouped in sheltered and exposed sites. The results for three parameters of growth, node production, height increase, and mean internodal length, are presented in Table 8.15. These values show significantly greater node production rate at oiled sites, and a nonsignificant difference between habitats (2-way ANOVA for site means of annual node production normalized by an inverse transformation, grouped by habitat, P >.5, and the presence of oil, P <.001). This unexpected result was possibly due to the greater size of oil gaps, resulting in more light compared with natural ones. As noted earlier,

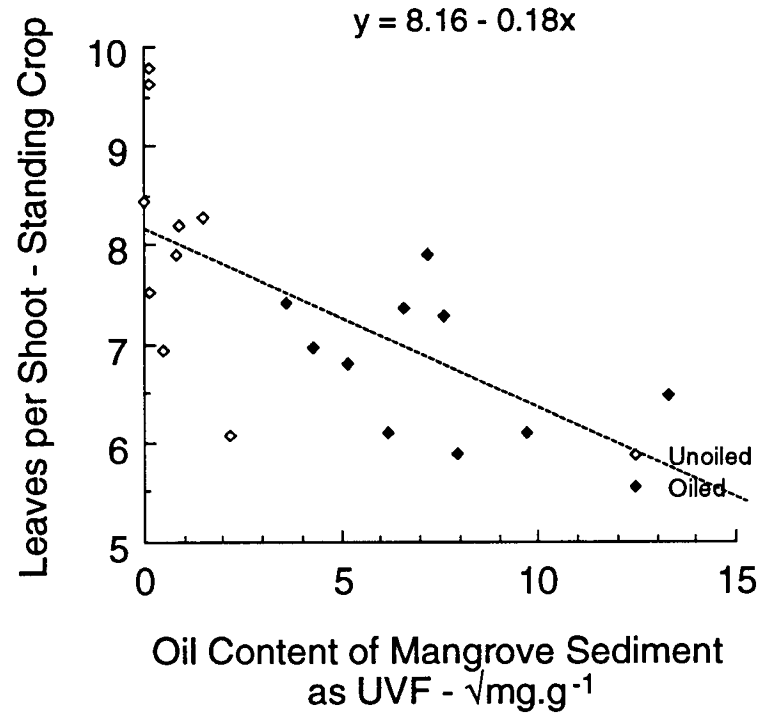


Fig. 8.24 Mean annual number of leaves per shoot of *Rhizophora mangle* trees vs. the mean concentration (square-root transformation) of oil in sediments (UVF; see Burns, Chap. 3) during 1989-1990. Notice that leaves per shoot decreased from approximately 8 at unoiled sites to a minimum of approximately 6 at oiled sites (r = -.63; N = 19; P <.005).

the greater amount of light would contribute to higher node production rates. In comparison, there were no significant differences for height estimates (2-way ANOVA for site means of annual height increase normalized as log transformation, grouped by habitat, P >.4, and the presence of oil, P >.1). There was, however, a relationship between these parameters, with node production and height (log transformation) positively correlated (r = .77, N = 214, P <.001). These data are presented in Figure 8.29, which displays the range of the respective parameters and the line of best-fit for the linear regression coefficients. Notice for the lower range of node production rates that extrapolated seedling heights increased by approximately 1 cm per yr, or 0.5 cm/node. This value is very close to the length of the leaf-scar node, and is indicative of growth in low-light conditions. Also notice that as node production and height increased, the range of annual height production also increased, indicating the independence of these parameters when node production rates were high, particularly above 8 nodes per yr. In general, these data indicate that seedlings in oil gaps and unoiled natural gaps grew at comparable rates 4 yr after the spill, and oil appeared to suppress growth chiefly in faster-growing individuals. It must be emphasized that these seedlings will shade smaller plants and

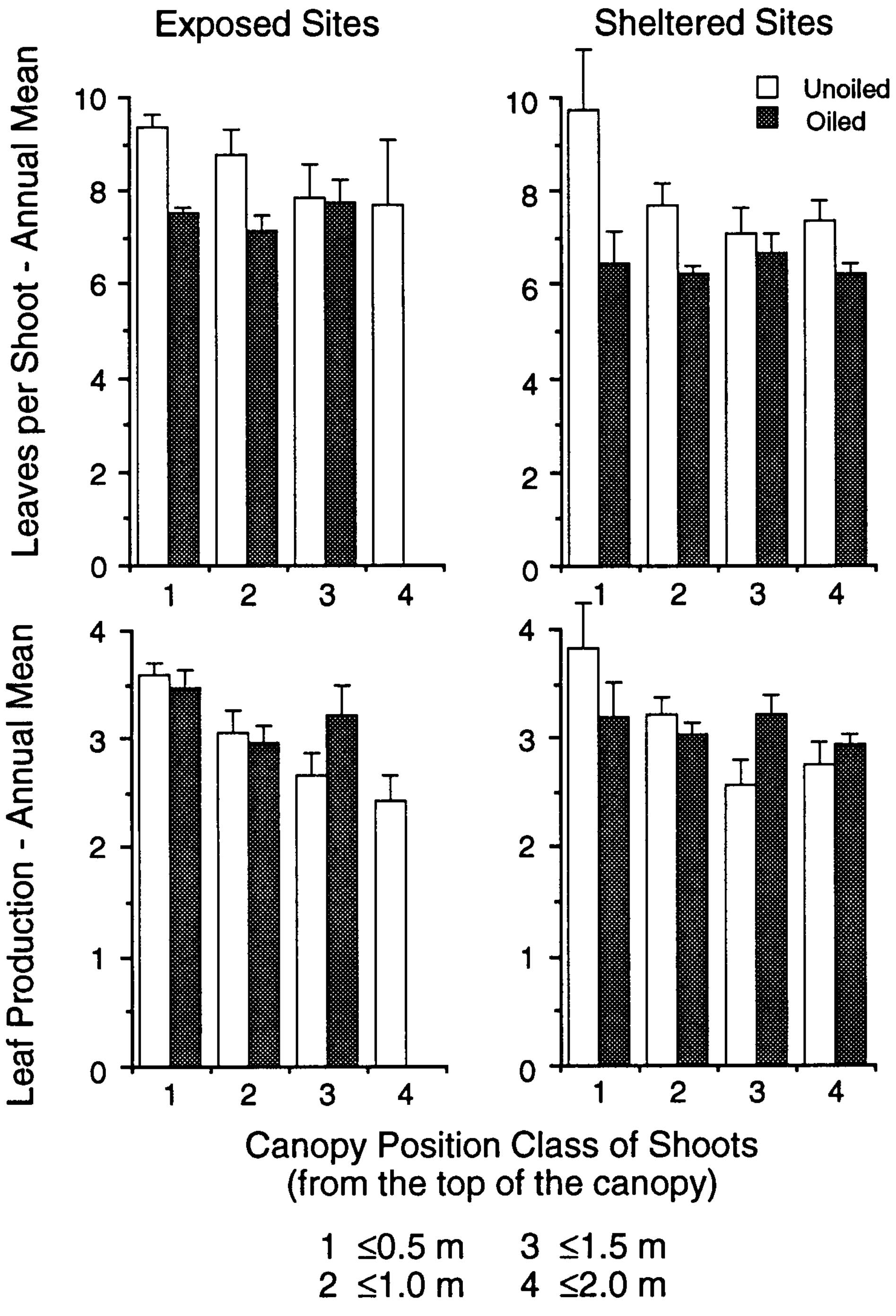


Fig. 8.25 Histograms of canopy-position classes of *Rhizophora mangle* leafy shoots with respect to annual means of leaves per shoot and leaf production as numbers of leaves per 100 shoots per d. Estimates were derived from monthly scores from mature canopies from July 1989 to June 1990. Position classes were measured down from the upper canopy edge. Error bars are 1 SE. Shoot positions for each site are summarized in Appendix Table D.2.

Table 8.11 Annual falls (number/m^2) of five components of *Rhizophora mangle* canopies found in monthly litter fall from oiled and unoiled locations of three fringe habitats during 1989-1990. SE of among-site variation is in parentheses. Site means are in Appendix Table D.7.

	Exposed Open		Sheltered Channel		Sheltered River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Total Stipules (= New Leaf Appearance)					
	1,103 (68)	1,112 (121)	1,424 (165)	1,064 (145)	1,357 (139)
Total Leaves					
	1,083 (50)	1,104 (100)	1,394 (198)	1,019 (132)	1,276 (71)
Total Reproductive Parts					
	544 (69)	475 (80)	913 (117)	456 (106)	1,080 (175)
Total Flowers					
	175 (37)	372 (66)	836 (110)	427 (102)	1,040 (172)
Mature Hypocotyls					
	0.37 (0.36)	3.29 (1.83)	5.84 (2.19)	5.48 (1.46)	2.19 (0.73)

Table 8.12 Annual fall rates as dry weight (g) per m^2 for five components of *Rhizophora mangle* canopies found in monthly litter fall from mangrove forests in oiled and unoiled locations of three fringe habitats during 1 yr, 1989-1990. Also included are mean leaf weights. SE of among-site variation is in parentheses. Site means are in Appendix Table D.8.

	Exposed Open		Sheltered Channel		Sheltered River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Stipules (New Leaf Appearance)					
	0.23 (0.01)	0.22 (0.02)	0.26 (0.03)	0.19 (0.02)	0.29 (0.03)
Leaves					
	1.49 (0.05)	1.55 (0.10)	1.99 (0.10)	1.54 (0.15)	1.93 (0.11)
Total Reproductive Parts					
	0.12 (0.02)	0.24 (0.06)	0.47 (0.07)	0.31 (0.11)	0.44 (0.10)
Wood					
	0.10 (0.02)	0.19 (0.07)	0.10 (0.02)	0.51 (0.25)	0.35 (0.10)
Total					
	1.93 (0.04)	2.20 (0.17)	2.82 (0.12)	2.56 (0.26)	3.00 (0.19)
Average Leaf Weight (g dry weight)					
	0.51 (0.02)	0.47 (0.02)	0.53 (0.05)	0.69 (0.07)	0.54 (0.02)

eventually overwhelm them, making it essential to consider total population growth rather than concentrating on selected individuals.

Table 8.13 Derived parameters of canopy condition, based on shoot observations (Table 8.10), litter fall studies (Table 8.11), and allometric relationships (Sect. 8.3.2). *Rhizophora mangle* canopies were sampled at monthly intervals over 1 yr, 1989-1990, in oiled and unoiled locations of three fringe habitats. SE of among-site variation is in parentheses. Site means are in Appendix Table D.9.

	Exposed		Sheltered		
	Open		Channel		River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Shoot Density (shoots/m^2)					
1989-1990	96.7 (0.8)	95.5 (7.1)	131.8 (21.9)	93.8 (13.4)	133.3 (19.3)
Leaf Density (leaves/m^2)					
1989-1990	884.3 (31.6)	715.8 (76.6)	1,007.5 (169.2)	619.6 (82.9)	935.1 (86.8)
Canopy Leaf Biomass (g dry weight/m^2)					
1989-1990	448.0 (16.7)	333.6 (41.2)	508.9 (62.2)	369.8 (46.2)	507.0 (46.0)
Average Leaf Area (green leaves, cm^2)					
1989-1990	23.8 (0.9)	23.6 (0.3)	26.0 (2.6)	35.8 (2.1)	34.2 (1.2)
Canopy Leaf Area - LAI (m^2/m^2)					
1989-1990	2.10 (0.02)	1.69 (0.19)	2.50 (0.34)	2.14 (0.24)	3.20 (0.29)

8.6.1.2 Seedling Assemblages – Demographic Structure

In order to study seedling assemblages, plots were established in a range of oil gaps and unoiled natural gaps. Summaries of the scored parameters are presented in three figures, which compare density of different height classes (Fig. 8.30), density of node classes (i.e., based on node counts along the vertical stem; Fig. 8.31), and estimated total above-ground biomass for node classes (Fig. 8.32). These plots display data collected from two census dates, a little over a year apart, and consequently show demographic changes in the structure of seedling assemblages.

In general, there were many smaller plants and progressively fewer taller ones at these sites; the situation changed in the second year of the study, apparently only with the tallest seedlings growing taller (Fig. 8.30). These patterns were equivalent at sheltered and exposed sites, but densities at exposed sites were five times less. A plot comparing seedling densities by respective node classes (Fig. 8.31) gives a completely different perspective by presenting a view based more on age structure, because node counts equate to age (Duke and Pinzón 1992; Appendix Abstract and Description D.1). At sheltered sites, an older peak of seedlings advanced by about 3-4 node classes in the second year, while the younger peak stayed fixed within 1-3 nodes. This younger peak represents the reformation of a seedling bank, and comprises new recruits that die after 2 to 3 yr if they have remained shaded by older, larger plants. Therefore, the canopy has reformed in these particular plots and is comparable to that seen in mature forests, albeit at a much lower height. The situation at exposed sites was quite different, however, because these areas had no

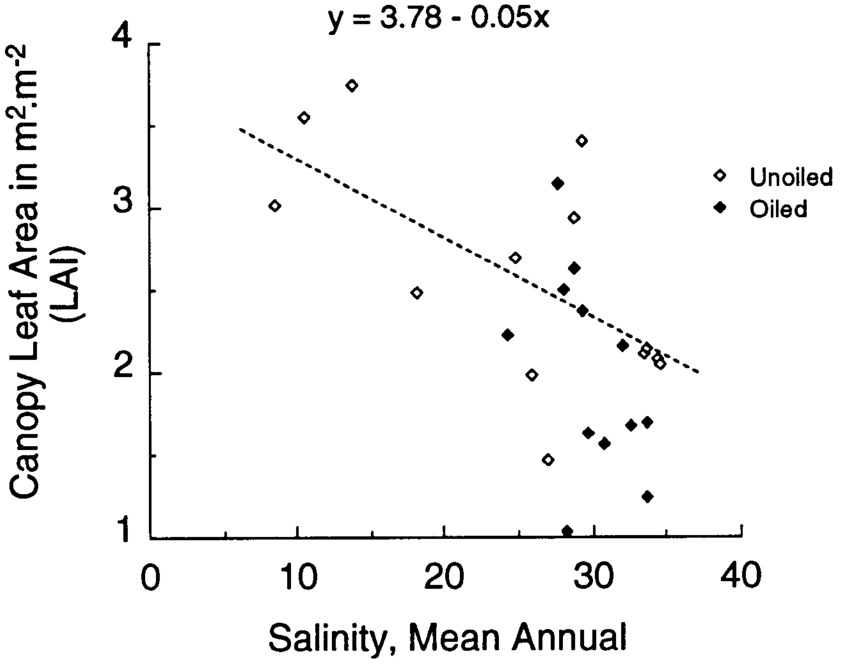


Fig. 8.26 Mean annual estimate of canopy leaf area (LAI) of *Rhizophora mangle* trees vs. mean annual water salinity during 1989-1990. Notice that total leaf area increased at lower salinities (r = -.63; N = 13; P <.05; for unoiled sites only), but was not correlated with oil concentration in oiled sites.

seedling-bank peak, indicating that there was no canopy closure. Canopy closure appears to represent the end of the first stage of forest recovery.

The third plot (Fig. 8.32) compares node classes with extrapolated amounts of total above-ground biomass, based on the allometric equation of height and girth, with biomass for trees in Puerto Rico (Cintr6n and Schaeffer-Novelli 1984; Sect. 8.3.2). Older seedlings had both the greater biomass and contribution toward the reforming or reformed canopy, depending on habitat. Clearly, seedlings at exposed sites were far less dense and more poorly developed than at sheltered sites, with only a fraction of the total biomass produced in the same period.

It is important to point out that recruitment of individuals that make up the new canopy, particularly at sheltered sites, appeared to peak during 1986, with fewer numbers before and after the spill. In understanding these differences between sheltered and exposed sites, it needs to be emphasized that these data only show survivors. Nevertheless, a similar pattern prevailed at all sites.

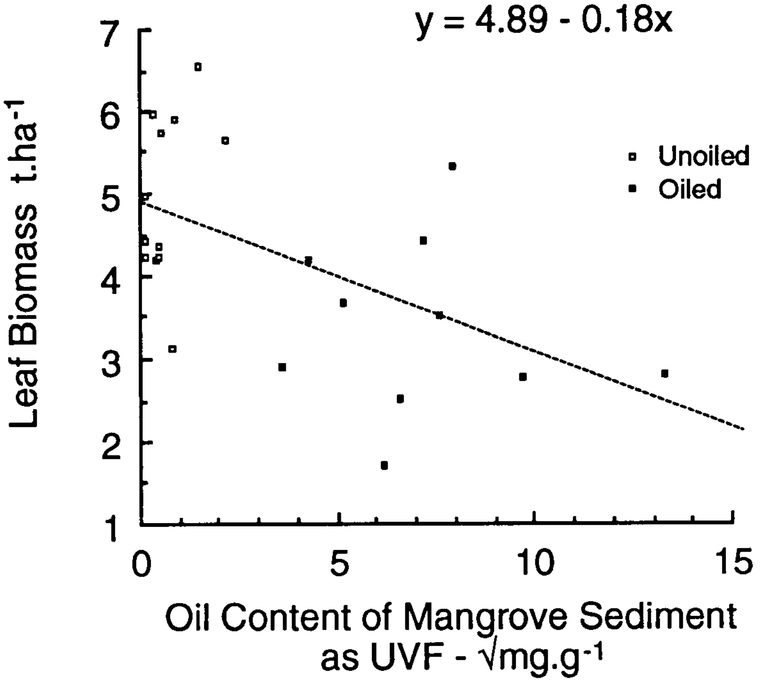


Fig. 8.27 Mean annual estimate of leaf biomass of *Rhizophora mangle* trees vs. the mean concentration (square-root transformation) of oil in sediments (UVF; see Burns, Chap. 3) during 1989-1990. Notice that leaf biomass was highest, around 5 t/ha, at low concentrations of oil (r = -.54; N = 22; P <.01), but was not correlated with salinity.

8.6.1.3 Seedling Assemblages – General Site Variation

Densities of recruits were relatively high in some oil gaps in Bahía Las Minas, but they were variable, as shown by mean estimates ranging from <0.1 to 23.4 seedlings/m^2. These data were collected approximately 4 yr after the spill, and represent both annual restocking and survivorship. Given that comparable densities were observed regularly in unoiled gaps of similar age, it is assumed that the major influencing factors of recruitment and establishment were less related to oil than to natural factors, although there may have been secondary consequences of oiling. In this sense, there are no natural phenomena in Panama known to cause such short-term destruction of mangrove habitat as seen resulting from the two large oil spills documented in this report.

Site means of major structural parameters of seedling assemblages in oil gaps are listed in Table 8.16 for April 1990, and in Table 8.17 for June 1991. These data are further divided into younger seedlings with ≤12 nodes, representing the seedling bank, and older seedlings (noted as saplings), planted and natural recruits, and planted and naturally recruited sites. Planted recruits and sites will be discussed in

Table 8.14 Canopy leaf biomass (t/ha) estimated from shoot observations with litter fall of *Rhizophora mangle* trees and annual leaf production (t/ha) determined from monthly litter fall from mangrove forests in oiled and unoiled locations of three fringe habitats during 1 yr, 1989-1990. The turnover ratio represents the proportion of the year taken to replace the standing canopy biomass. SE of among-site variation is in parentheses. Site means are in Appendix Table D.10.

	Exposed		Sheltered		
	Open		Channel		River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Standing Crop - Canopy Leaf Biomass					
1989-1990	4.48 (0.17)	3.34 (0.41)	5.09 (0.62)	3.70 (0.46)	5.07 (0.46)
Annual Leaf Production					
1989-1990	5.48 (0.17)	5.12 (0.47)	7.08 (0.63)	5.99 (0.58)	6.91 (0.33)
Turnover Ratio					
1989-1990	0.82 (0.01)	0.65 (0.03)	0.72 (0.07)	0.61 (0.04)	0.73 (0.06)

the next section (8.6.2) and, for the moment, attention is directed to natural sites. The parameters include density, height, girth, length of the hypocotyl above the sediment, and estimates of leaf biomass and total above-ground biomass. Density of seedlings 3-5 yr postspill was apparently not a function of oil, but rather a function of habitat. Recruitment was far more successful at sheltered sites than exposed ones. This appears to be a function of greater erosion at exposed sites, noting the change in the height of hypocotyls from 1990 to 1991. Hypocotyl height would only be expected to change where sediment levels change, because this attribute of seedlings is usually unaffected during subsequent growth (Duke and Pinzón 1992). Clearly, in this case, hypocotyls of saplings increased in length at exposed sites significantly more than they did at sheltered sites, indicating the loss of 1-2 cm of surface sediments at exposed places.

In contrast, growth in height in the two habitats appeared to be more a function of oil concentration. In Figure 8.33 mean seedling height is correlated with oil in 1990 and 1991 and, as seedlings grew, the effect of oil was clearly more exaggerated, shown by the higher level of significance for the 1991 regression and its increased slope. It appears the presence of residual oil had a compounding effect on oil-gap seedlings, and retarded forest recovery in the bay.

8.6.2 Assisted Recruitment in 1986 Oil Gaps

The recovery of mangrove forests in Bahía Las Minas following the 1986 spill was further influenced by a large-scale restoration project. Shortly after the spill the Refinería Panamá commenced extensive tests in anticipation of implementing a planting program. These tests established a procedural methodology by which, over the next year or so, at least 86,000 seedlings were planted (Teas et al. 1989). In fact,

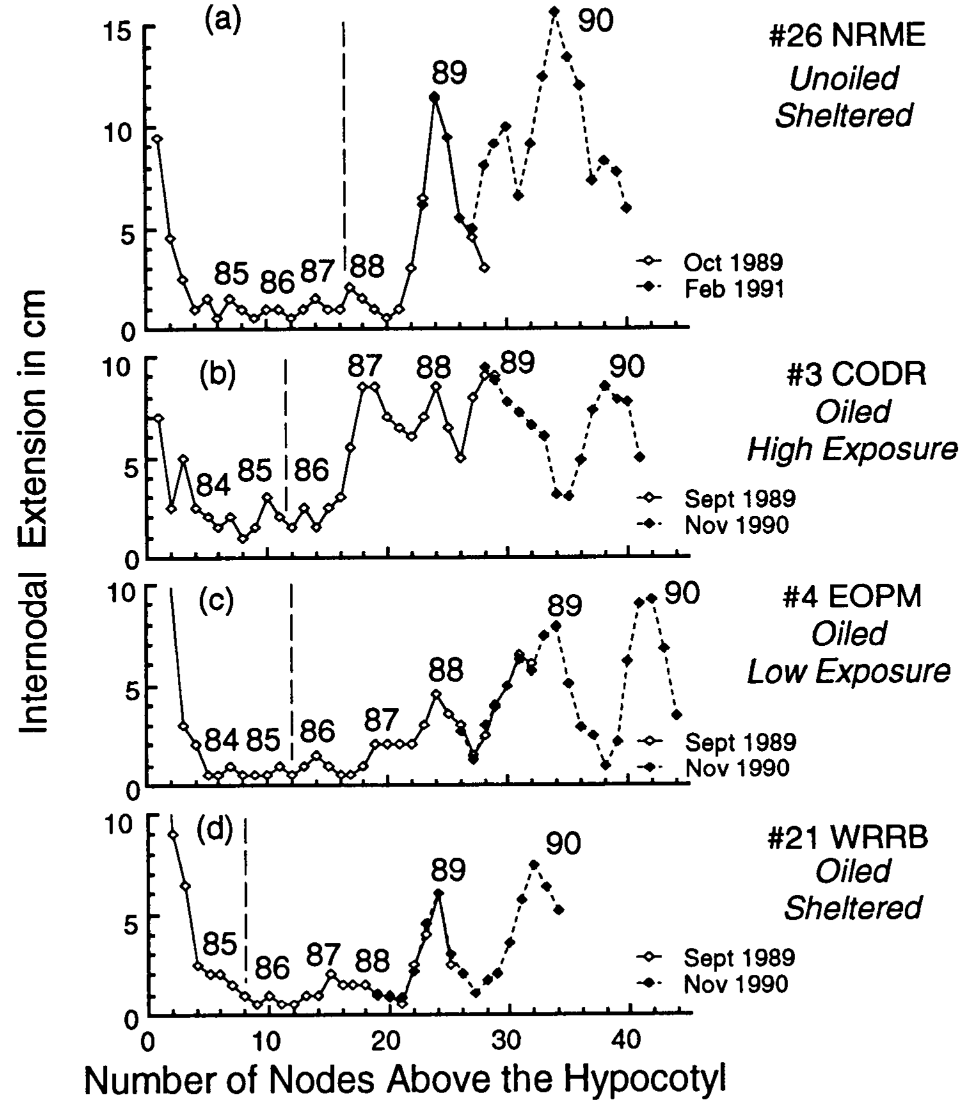


Fig. 8.28 Sequences of internodal extension by node position for four natural recruits of *Rhizophora mangle* in Bahía Las Minas. The first one (*a*) was growing in a natural light gap in a sheltered site, while the others (*b-d*) were growing in oil gaps where site conditions ranged from very exposed (*b*) and exposed (*c*) to sheltered (*d*). These recruits were scored on two occasions, noted in their respective legends, providing a measure of node production rate. Accordingly, nominal annual peaks were identified. Notice that each recruit apparently survived the estimated creation date of its respective light gap, indicated by a *vertical dashed line*. Also notice the abrupt increase in internodal extension and node production rate at the unoiled site and the very exposed oiled site, while the more sheltered oiled sites had depressed peaks of internodal extension that progressively increased following deforestation.

Table 8.15 Growth parameters of *Rhizophora mangle* seedlings in refinery spill oil gaps, compared with those in similar-aged natural gaps in two fringe habitats, scored between late 1989 and late 1990. SE of among-site variation is in parentheses. Site means are in Appendix Table D.11.

	Exposed		Sheltered	
	Unoiled	Oiled	Unoiled	Oiled
Node Production (nodes/yr)	7.4 (0.1)	8.9 (0.5)	7.8 (1.3)	8.4 (0.2)
Height Increase (cm/yr)	29.7 (3.4)	43.8 (14.4)	53.8 (24.2)	36.4 (7.0)
Height/Node (cm/node)	3.7 (0.4)	4.6 (1.2)	6.0 (2.5)	4.1 (0.7)

there were possibly many more planted because anecdotal accounts indicate twice this number. Seedlings were often planted with the addition of some broad-spectrum fertilizer and unoiled upland soil because the latter was shown to improve early growth and survival at oiled sites. These procedures were based on developing the most efficient and successful method for planting seedlings and promoting their survival. The need for planting was not first established by an assessment of natural recruitment and growth. Considering the area covered and the likely outcome of this effort on the recovery of mangrove forests affected by the 1986 spill, it was essential in the present study to quantify the status of planted and natural recruits. This study was designed to identify long-term growth and survival, and provide an independent evaluation of the possible benefit of planting to oil-gap recovery.

The expected density of planting was estimated by dividing the reported numbers of planted seedlings by the total area planted, approximately 80% of oil gaps. This calculation was not meant to be precise, but rather an approximation for comparison with the observed densities found in the randomly chosen plots of this study. The expected planting density ranged from 0.1 to 0.3 seedlings/m^2, in the lower range of the observed standing-stock densities of natural recruits summarized in Tables 8.16 and 8.17 for 1990 and 1991, respectively. The observed density of planted recruits corresponded with the expected estimate derived above. These data further showed two age classes of planted recruits, corresponding to two major planting efforts made approximately 1 yr apart, around mid-1986 and mid-1987. The second effort appeared to be much more extensive than the first, and added exposed sites and more recruits at many sheltered sites.

Data on densities in April 1990 show the contribution of the massive planting exercise was quite small compared with natural recruitment. At planted sites there were 5-11 times more natural recruits than planted ones, compared with sites of no planting, where we counted 11-39 times more natural recruits. Planted and natural cohorts were also compared using node counts, allowing a partial demographic analysis of the restoration and recovery of both seedling assemblages. This assessment is summarized in Table 8.18. Four cohort groups are presented, representing the two plantings, and older and younger plants. These estimates

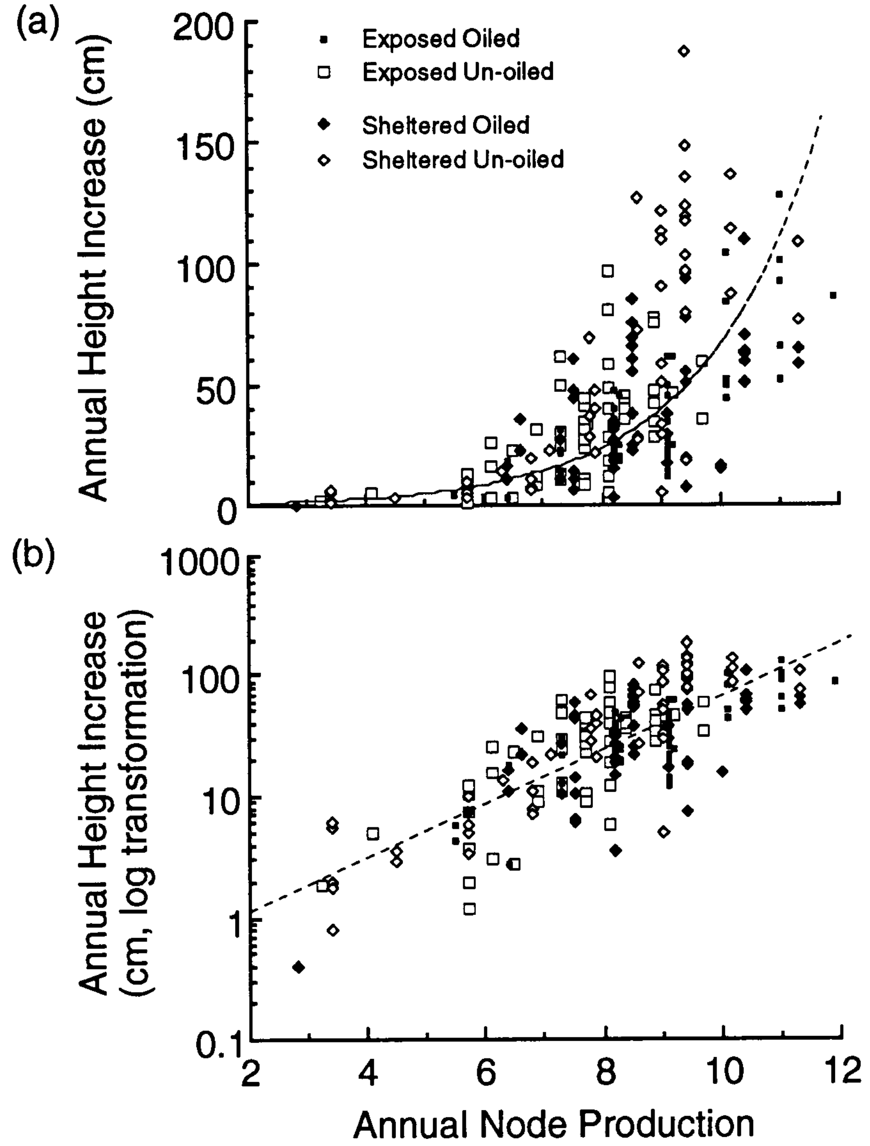
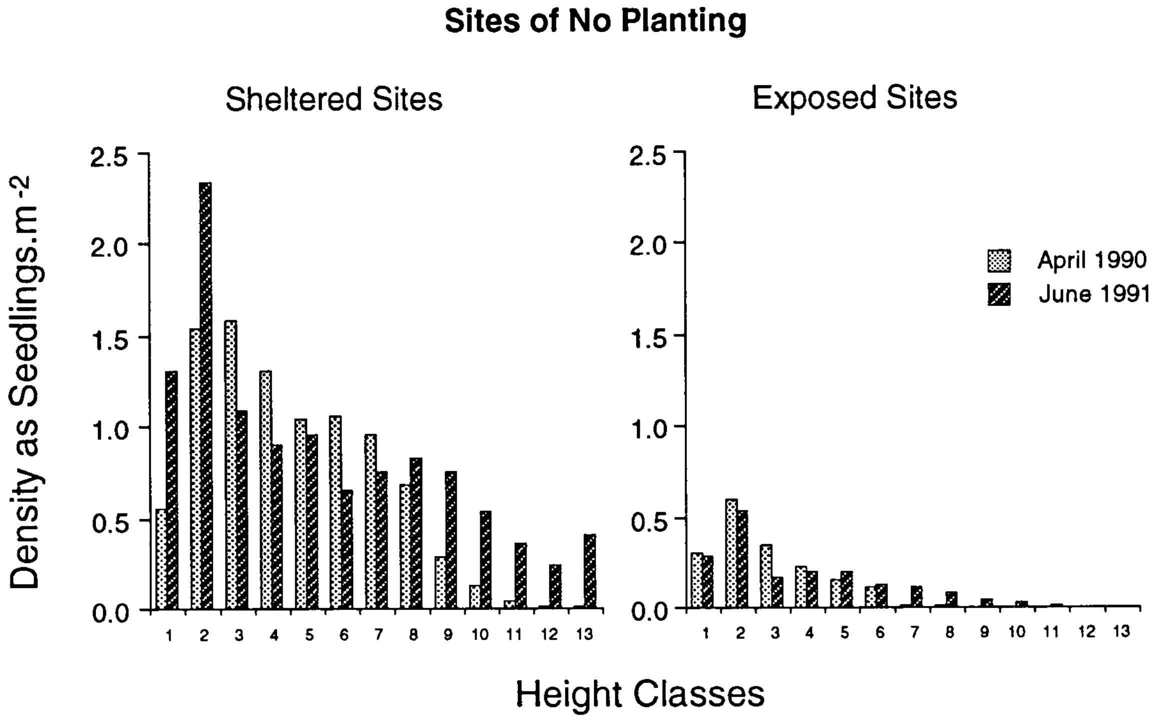


Fig. 8.29 Node production rate and height increase during 1 yr, 1989-1990, for 216 natural recruits of *Rhizophora mangle* growing in natural light gaps or oil gaps. Data were grouped according to habitat condition (exposed or sheltered) and whether sites were oiled in 1986. One plot (*a*) shows the data on untransformed scales; the second (*b*) shows the same data with a log transformation for height increase. The significant regression of best-fit was plotted for the latter case because it appeared that this adequately described the trends observed. The first plot shows the separation of oiled and unoiled seedlings, where node production rates and height increased the most. The second plot displays the regular trend across the range of node production rates, and a minimum that corresponded with rates noted earlier in shaded seedlings, approximately 0.5 cm/node.

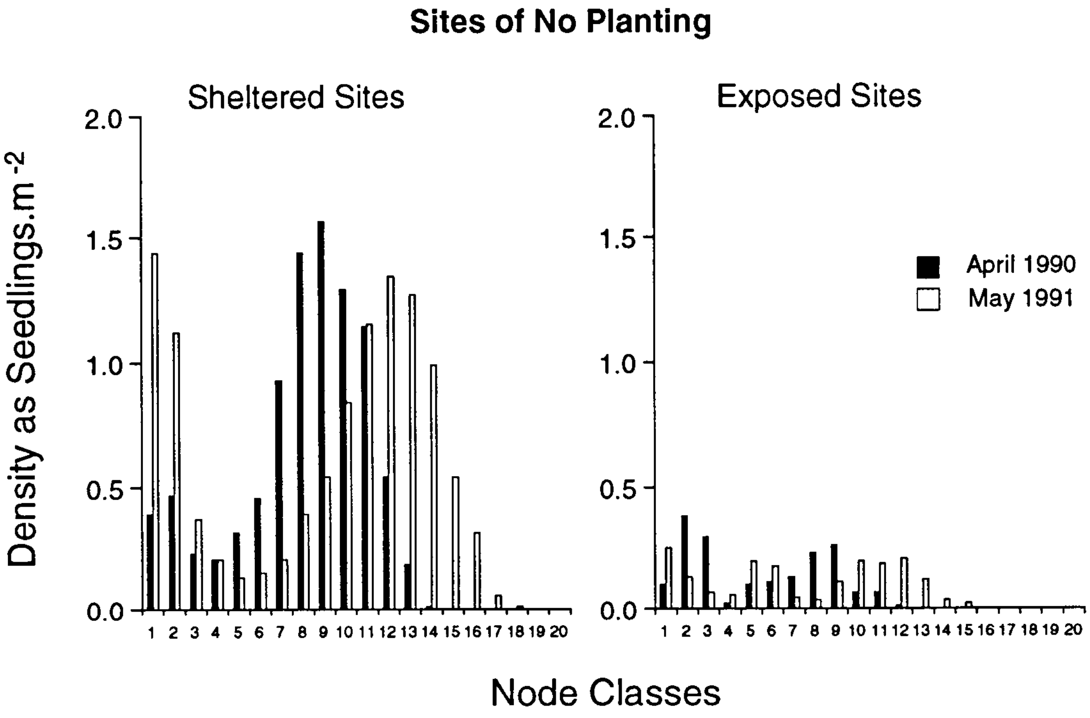


	cm		cm
1	0-30	8	151-170
2	31-50	9	171-190
3	51-70	10	191-210
4	71-90	11	211-230
5	91-110	12	231-250
6	111-130	13	>250
7	131-150		

Fig. 8.30 Height classes and mean densities of *Rhizophora mangle* seedlings (*cum* saplings) at exposed and sheltered sites scored in April 1990 and June 1991. Notice the higher densities at sheltered sites compared to exposed ones. Also note how height classes, unlike node classes (Fig. 8.31), are skewed left. This pattern indicates that while there were many older recruits, they were not equally tall, and growth in height was suppressed, presumably by lack of light. This is indicative that only those recruits in the greater height classes would have any chance of contributing to the mature canopy.

indicate that planted recruits were placed among existing natural recruits. At sheltered sites, it also appears that the number of surviving older recruits was reduced by planting. Furthermore, recruitment of natural recruits in undisturbed (unplanted) oil gaps was equal to or higher than equal-aged recruits at planted sites.

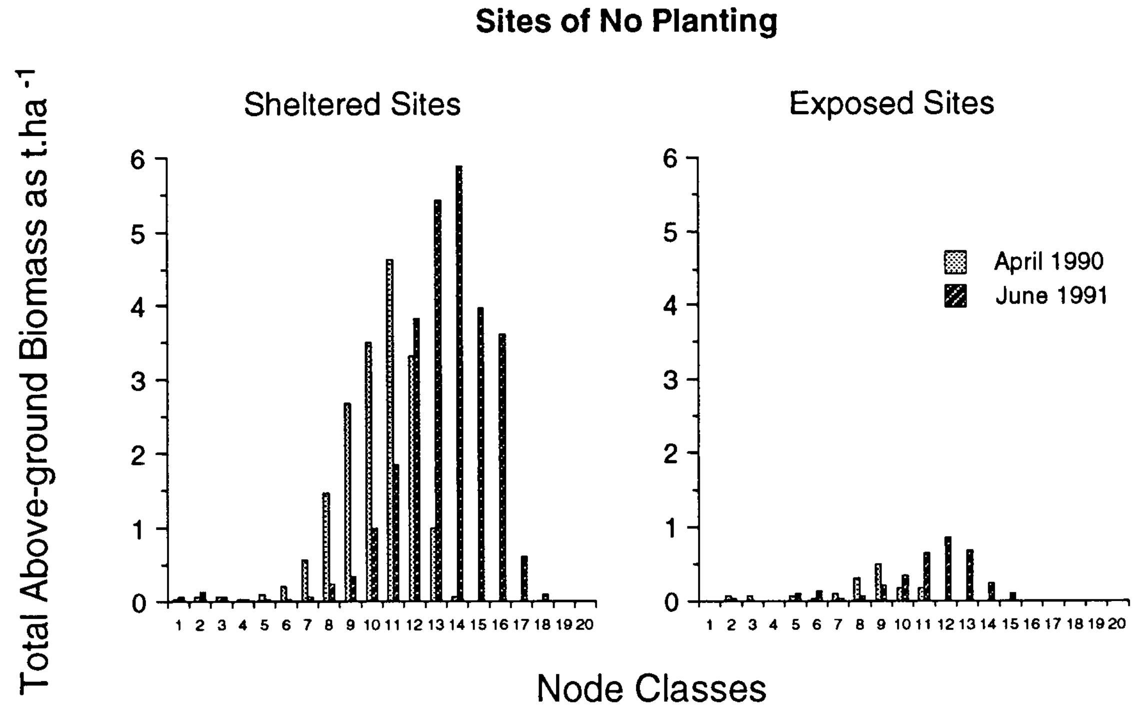
These data reveal that the high levels of natural recruitment, alone, question the justification for the planting program. In this case, natural recruitment was



	count		count
1	0-3	11	31-33
2	4-6	12	34-36
3	7-9	13	37-39
4	10-12	14	40-42
5	13-15	15	43-45
6	16-18	16	46-48
7	19-21	17	49-51
8	22-24	18	52-54
9	25-27	19	55-57
10	28-30	20	58-60

Fig. 8.31 Node classes and mean densities of *Rhizophora mangle* seedlings (*cum* saplings) at exposed and sheltered sites scored in April 1990 and June 1991. At sheltered sites notice the bimodal peaks, with the younger group (node classes 1-4 = 1-12 nodes) representing the presence of a seedling bank at these sites, and the older group, which noticably moved up 3 to 4 node classes by the second scoring date. This change matched the expected rate of node production (9-12 nodes/yr), showing that recruitment of this cohort was mostly during 1987, the year after the spill. In contrast, exposed sites showed no establishment of a seedling bank.

adequate for these sites in Bahía Las Minas, and this fact apparently was evident at the time of planting. This assessment also found other problems with the planting effort, displayed by the significant negative effect on natural recruitment where



	count		count
1	0-3	11	31-33
2	4-6	12	34-36
3	7-9	13	37-39
4	10-12	14	40-42
5	13-15	15	43-45
6	16-18	16	46-48
7	19-21	17	49-51
8	22-24	18	52-54
9	25-27	19	55-57
10	28-30	20	58-60

Fig. 8.32 Node classes and mean total above-ground biomass of *Rhizophora mangle* seedlings (*cum* saplings) at exposed and sheltered sites scored in April 1990 and June 1991. Notice that biomass was predominantly found in older plants, and these moved up 3 to 4 node classes by the second scoring date. This pattern matched the expected rate of node production (9-12 nodes/yr), again showing that recruitment of this cohort was mostly during 1987, the year after the spill.

planting took place. In Figure 8.34 the data already discussed are presented in four histograms with densities for each of the node classes, in sites grouped by habitat and whether or not they were planted. Notice the trend from greatest densities at sheltered, nonplanted sites to exposed, planted ones. Furthermore, both effects were significant (2-way ANOVA for site means of total seedling densities normalized with

Table 8.16 Six parameters of forest structure scored in refinery spill oil gaps during April 1990. Data are based on *Rhizophora mangle* seedlings and saplings, planted and natural recruits in two fringe habitats and in planted and naturally recovering sites. Seedlings were defined as plants with ≤12 nodes along the main stem. SE of among-site variation is in parentheses.

	Exposed		Sheltered	
	Natural Site	Planted Site	Natural Site	Planted Site
Density (number/m^2)				
Natural Seedlings	0.79 (0.39)	0.09 (0.05)	1.29 (1.09)	0.64 (0.17)
Planted Seedlings	-	0.00	-	0.04 (0.01)
Natural Saplings	0.95 (0.35)	0.69 (0.32)	7.88 (3.78)	2.17 (0.53)
Planted Saplings	-	0.16 (0.06)	-	0.20 (0.05)
Total Natural	1.74 (0.72)	0.79 (0.37)	9.16 (4.84)	2.81 (0.62)
Total Planted	-	0.16 (0.06)	-	0.24 (0.04)
Diameter (cm)				
Natural Seedlings	0.50 (0.02)	0.61 (0.01)	0.54 (0.04)	0.57 (0.03)
Planted Seedlings	-	-	-	0.74 (0.10)
Natural Saplings	1.10 (0.09)	1.43 (0.18)	1.46 (0.21)	1.46 (0.21)
Planted Saplings	-	1.35 (0.08)	-	1.29 (0.19)
Total Natural	0.85 (0.04)	1.37 (0.18)	1.40 (0.20)	1.27 (0.19)
Total Planted	-	1.35 (0.08)	-	1.24 (0.20)
Stem Height (m)				
Natural Seedlings	0.35 (0.01)	0.33 (0.01)	0.32 (0.01)	0.36 (0.02)
Planted Seedlings	-	-	-	0.51 (0.08)
Natural Saplings	0.65 (0.08)	0.87 (0.12)	1.19 (0.13)	1.00 (0.17)
Planted Saplings	-	0.65 (0.02)	-	0.81 (0.12)
Total Natural	0.52 (0.04)	0.83 (0.12)	1.12 (0.14)	0.86 (0.14)
Total Planted	-	0.65 (0.02)	-	0.77 (0.12)
Hypocotyl Height (m)				
Natural Seedlings	0.19 (0.01)	0.16 (0.01)	0.18 (0.02)	0.17 (0.00)
Planted Seedlings	-	-	-	0.15 (0.01)
Natural Saplings	0.20 (0.01)	0.18 (0.02)	0.20 (0.01)	0.19 (0.00)
Planted Saplings	-	0.16 (0.01)	-	0.16 (0.01)
Total Natural	0.20 (0.01)	0.18 (0.02)	0.20 (0.01)	0.19 (0.00)
Total Planted	-	0.16 (0.01)	-	0.16 (0.01)
Leaf Biomass (t/ha)				
Natural Seedlings	0.05 (0.02)	0.006 (0.003)	0.08 (0.06)	0.04 (0.01)
Planted Seedlings	-	-	-	0.004 (0.002)
Natural Saplings	0.23 (0.10)	0.26 (0.15)	2.52 (0.62)	0.72 (0.12)
Planted Saplings	-	0.04 (0.01)	-	0.08 (0.04)
Total Natural	0.28 (0.12)	0.26 (0.15)	2.59 (0.68)	0.76 (0.11)
Total Planted	-	0.04 (0.01)	-	0.08 (0.03)
Total Biomass (t/ha)				
Natural Seedlings	0.14 (0.07)	0.02 (0.01)	0.24 (0.20)	0.12 (0.02)
Planted Seedlings	-	-	-	0.02 (0.01)
Natural Saplings	1.36 (0.61)	2.05 (1.43)	17.67 (2.75)	5.46 (1.36)
Planted Saplings	-	0.25 (0.07)	-	0.63 (0.35)
Total Natural	1.49 (0.67)	2.07 (1.44)	17.90 (2.88)	5.59 (1.34)
Total Planted	-	0.25 (0.07)	-	0.64 (0.35)

Table 8.17 Six parameters of forest structure scored in refinery spill oil gaps during June 1991. Data are based on *Rhizophora mangle* seedlings and saplings, planted and natural recruits in two fringe habitats and in planted and naturally recovering sites. Seedlings were defined as plants with ≤12 nodes along the main stem. SE of among-site variation is in parentheses.

	Exposed		Sheltered	
	Natural Site	Planted Site	Natural Site	Planted Site
Density (number/m^2)				
Natural Seedlings	0.49 (0.27)	0.02 (0.01)	3.13 (2.70)	1.43 (0.81)
Planted Seedlings	-	0.00	-	0.00
Natural Saplings	1.29 (0.56)	0.54 (0.26)	7.92 (3.78)	2.46 (0.59)
Planted Saplings	-	0.15 (0.05)	-	0.23 (0.04)
Total Natural	1.83 (0.78)	0.57 (0.27)	11.06 (6.43)	3.89 (1.23)
Total Planted	-	0.15 (0.05)	-	0.23 (0.04)
Diameter (cm)				
Natural Seedlings	0.46 (0.03)	0.45 (0.05)	0.43 (0.03)	0.48 (0.02)
Planted Seedlings	-	-	-	-
Natural Saplings	1.40 (0.06)	2.14 (0.30)	1.60 (0.21)	1.79 (0.21)
Planted Saplings	-	2.05 (0.21)	-	1.73 (0.26)
Total Natural	1.18 (0.03)	2.12 (0.31)	1.40 (0.20)	1.44 (0.20)
Total Planted	-	2.05 (0.21)	-	1.73 (0.26)
Stem Height (m)				
Natural Seedlings	0.33 (0.04)	0.38 (0.10)	0.33 (0.01)	0.35 (0.01)
Planted Seedlings	-	-	-	-
Natural Saplings	0.83 (0.12)	1.37 (0.24)	1.61 (0.21)	1.51 (0.29)
Planted Saplings	-	1.16 (0.07)	-	1.35 (0.28)
Total Natural	0.70 (0.06)	1.35 (0.23)	1.38 (0.19)	1.22 (0.26)
Total Planted	-	1.16 (0.07)	-	1.35 (0.28)
Hypocotyl Height (m)				
Natural Seedlings	0.18 (0.01)	0.14 (0.06)	0.17 (0.01)	0.17 (0.00)
Planted Seedlings	-	-	-	-
Natural Saplings	0.21 (0.01)	0.20 (0.02)	0.20 (0.01)	0.19 (0.01)
Planted Saplings	-	0.18 (0.01)	-	0.15 (0.01)
Total Natural	0.20 (0.01)	0.20 (0.02)	0.19 (0.00)	0.19 (0.00)
Total Planted	-	0.18 (0.01)	-	0.15 (0.01)
Leaf Biomass (t/ha)				
Natural Seedlings	0.02 (0.01)	0.001 (0.001)	0.13 (0.10)	0.08 (0.04)
Planted Seedlings	-	-	-	-
Natural Saplings	0.48 (0.23)	0.32 (0.14)	3.37 (0.82)	1.30 (0.15)
Planted Saplings	-	0.10 (0.04)	-	0.17 (0.07)
Total Natural	0.51 (0.23)	0.34 (0.15)	3.50 (0.92)	1.38 (0.13)
Total Planted	-	0.10 (0.04)	-	0.17 (0.07)
Total Biomass (t/ha)				
Natural Seedlings	0.06 (0.03)	0.003 (0.002)	0.32 (0.26)	0.23 (0.12)
Planted Seedlings	-	-	-	-
Natural Saplings	3.46 (1.75)	2.95 (1.45)	27.15 (4.24)	12.43 (2.63)
Planted Saplings	-	0.90 (0.38)	-	1.72 (0.95)
Total Natural	3.57 (1.74)	3.13 (1.61)	27.49 (4.47)	12.66 (2.55)
Total Planted	-	0.90 (0.38)	-	1.72 (0.95)

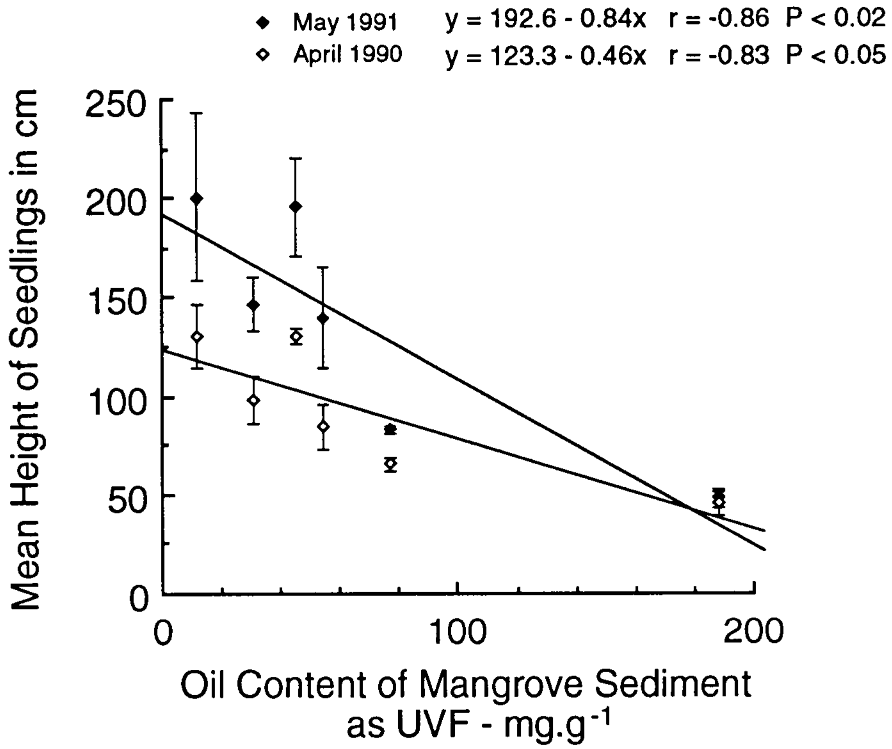


Fig. 8.33 Mean height of *Rhizophora mangle* plants at sheltered sites scored in April 1990 and June 1991 vs. the mean concentration of oil in sediments (UVF; see Burns, Chap. 3) in July 1990. Notice that plants were tallest at low concentrations of oil, approximately 1.25 m in 1990 (r = -.83; N = 6; P <.05) and 1.90 m in 1991 (r = -.86; N = 6; P <.02), and smallest (around 0.50 m) at high concentrations. Exposed sites showed a similar trend for surviving plants, but mean site patterns were affected by loss of plants from erosion.

a square-root transformation, grouped by habitat, P <.001, and the presence of planted seedlings, P <.026).

Nevertheless, despite a negative effect of planting on general site recovery, the average growth rate of planted seedlings was greater than natural seedlings. This difference was assessed by tallying all information on heights of both planted and natural recruits of the same node numbers at the same sites. As these individuals covered a range of node numbers, the estimates were reduced to mean internodal distances for heights above the hypocotyl, and the results are presented in Table 8.19. In all cases there were significantly greater height increases for planted recruits in both habitats and cohorts. The growth-enhancement measures utilized in this planting effort therefore had a positive influence on growth of planted recruits, although this difference could also have been a function of lower competition.

Table 8.18 Mean densities of natural and planted seedlings per m^2 for particular stem-node classes of *Rhizophora mangle* for 48 quadrats (12 m^2 each) in 16 oil-deforested sites in two major mangrove habitats in Bahía Las Minas (Fig. 8.6). These were scored in April 1990 and correspond with surviving cohorts before, during, and after those planted, around mid-1986 and mid-1987 (Teas et al. 1989). Sites are further categorized according to planting activities, as planted once, twice, or undisturbed. Virtually all recruits are natural, and where values include planted seedlings, a second figure for natural recruits only is bracketed. SE of among-site variation is in parentheses.

Habitat	Site Condition	> 33 cm "Older"	19-33 cm "First Planting"	10-18 cm "Second Planting"	< 10 cm "Younger"
Exposed	Planted Once	0.02 (0.01)	0.39 (0.20)	0.48 (0.22) [0.31 (0.19)]	0.06 (0.03)
	Undisturbed	0.01 (0.01)	0.74 (0.31)	0.22 (0.12)	0.76 (0.37)
Sheltered	Planted Once	0.03 (0.03)	3.00 (0.61)	1.01 (0.24) [0.92 (0.22)]	0.68 (0.24)
	Planted Twice	0.13 (0.07)	1.22 (0.07) [1.07 (0.09)]	0.42 (0.10) [0.25 (0.11)]	0.40 (0.15)
	Undisturbed	0.72 (0.27)	6.36 (3.27)	0.97 (0.84)	1.06 (0.87)

Any benefit of planting was far outweighed by the apparent disruption of natural recruitment and growth. Furthermore, despite their higher growth rate, most planted recruits face the prospect of losing essential light because only the tallest seedlings survive. In most cases the tallest seedlings were natural recruits (Fig. 8.35). In contrast, similar data for nodes (Fig. 8.34) shows that greater densities were skewed right and seedlings of greatest height usually had lowest densities. Notice in these histograms that few of the planted recruits are present in the upper height or age classes. Also, in reference to the proposed model of gap recovery, only a smaller number of older and larger individuals will ultimately contribute to the future mature canopy. Canopy density or total leaf biomass is expected to increase up to some maximal value, as observed in mature forests. This value would represent the end of the initial phase of gap recovery during which the substratum was only partially shaded and younger seedlings colonizing more open positions may still contribute to canopy closure. Subsequent development in the second phase would then involve direct competition between neighboring plants, as the requirement for greater canopy space for each individual increases with height and age. Furthermore, during this latter phase, the substratum is expected to be shaded, as in a mature forest, and the seedling bank would be re-established. In this phase, also, leaf biomass is expected to be relatively constant and independent of either tree density or height.

To assess the status of recruitment and gap recovery in oil gaps, seedling density and height were compared with estimates of standing leaf biomass. The latter were derived from the allometric relationship equating tree heights and girths to dry weight of leaves, recorded by Cintrón and Schaeffer-Novelli (1984). This value was calculated per tree in the plots, and total densities per m^2 were then

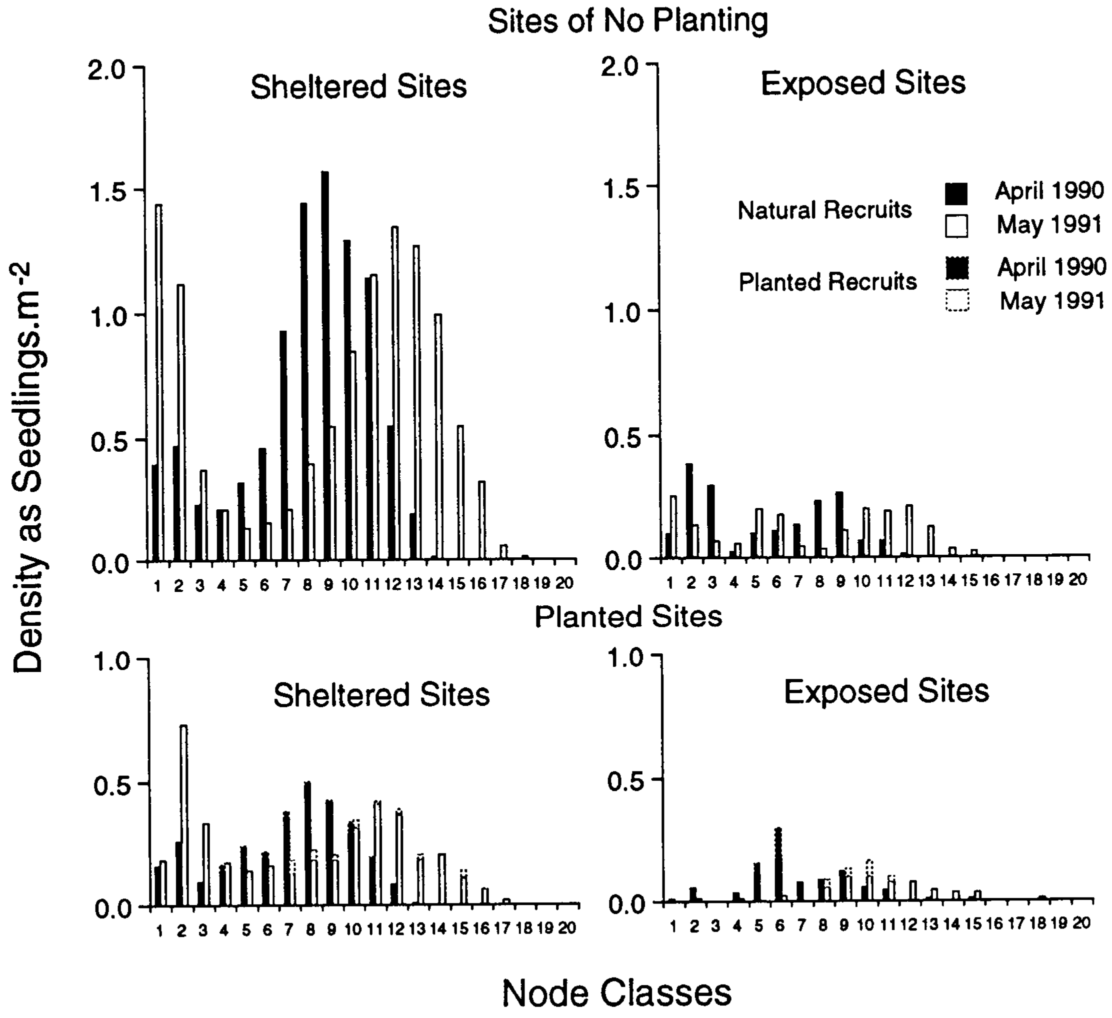


Fig. 8.34 Node classes and mean densities of *Rhizophora mangle* seedlings (*cum* saplings) at exposed and sheltered sites of no planting (see Fig. 8.31) and planted sites scored in April 1990 and June 1991. Notice the lower densities, the proportion of planted recruits, the bimodal peaks in sheltered sites, and the lack of a seedling bank at exposed sites. Planted exposed-coast sites were the least populated in the entire survey.

estimated because the area and number of trees were known. These values were then presented for all sites in two plots (Fig. 8.36) comparing leaf biomass with seedling height and density. These plots show leaf biomass as having a significantly closer relationship with density ($r = .87, N = 41, P < .001$) than with seedling height ($r = .45, N = 41, P < .005$), suggesting that height is less important in this process. Nevertheless, 4 yr after the spill standing leaf biomass in the range of sites increased, as suggested earlier, approaching the levels observed in mature forest canopies. These values were derived from litter fall and shoot data collected over 1 yr in the

Table 8.19 Mean internodal distance (cm) along the main stem, above the hypocotyl, for 130 planted and 208 natural recruits of *Rhizophora mangle* in Bahía Las Minas. These data were collected in April 1990 at each of eight sites in exposed and sheltered habitats. Seedlings were chosen to be approximately the same age, as determined by equivalent node classes, either 19-33 nodes for those first planted around mid-1986, and 10-18 nodes for the second planting around mid-1987. SE of among-site variation is in parentheses.

	Exposed Sites	Sheltered Sites	
Seedling Type	Second Planting	First Planting	Second Planting
Natural	2.11 (0.16)	2.35 (0.26)	3.69 (0.32)
Planted	3.03 (0.33)	3.67 (0.31)	4.14 (0.31)

same area, and the mean annual values were relatively constant between habitats, depending chiefly on whether sites were oiled (336-346 g/m^2) or not (452-484 g/m^2). Notice that these mean estimates closely match the maximum values computed for the more densely stocked plots (Fig. 8.36*a*), the sheltered, nonplanted sites. These sites are therefore the most advanced, having presumably achieved canopy closure, albeit at a relatively low height. It is also evident that exposed sites had greatly reduced seedling density and growth, and that the planting effort failed to significantly enhance recruitment in this obviously vulnerable habitat.

8.6.3 Status of Forest Recovery in 1968 *Witwater* Oil Gaps

Witwater oil gaps ranged over habitats similar to those studied for the assessment of the 1986 refinery spill. Ten comparative plots were established in three comparable exposed open sites and three sheltered channel areas, based on interpretations of 1973 aerial photographs (Sect. 8.4.2). Results, showing means of stem density, height, and girth, with estimates of both leaf and total above-ground biomass, are presented in Table 8.20. These plants were divided into seedlings (≤12 nodes, representing the seedling bank) and trees, and exposed and sheltered habitats. Densities at sheltered sites were more than double those at exposed sites, a feature reflected in slightly smaller trees. Despite these differences, however, estimates of total above-ground biomass were equivalent, at approximately 71-73 t/ha. There was a tendency for lower standing leaf biomass, from 3.5-4.4 t/ha, but this difference was not considered to be biologically significant. Leaf biomasses also compared closely with the range of mature forests (Table 8.7) and more advanced seedling stands (Tables 8.16, 8.17).

It was important in this assessment to consider the seedling bank separately, considering the decidedly bimodal distribution of individuals in these assemblages (Fig. 8.37), and the need for comparative estimates from studies of seedling plots and tree forests alike. In the latter case, seedling assemblages have rarely been quantified, and clearly their equivalent numbers greatly influence estimates of

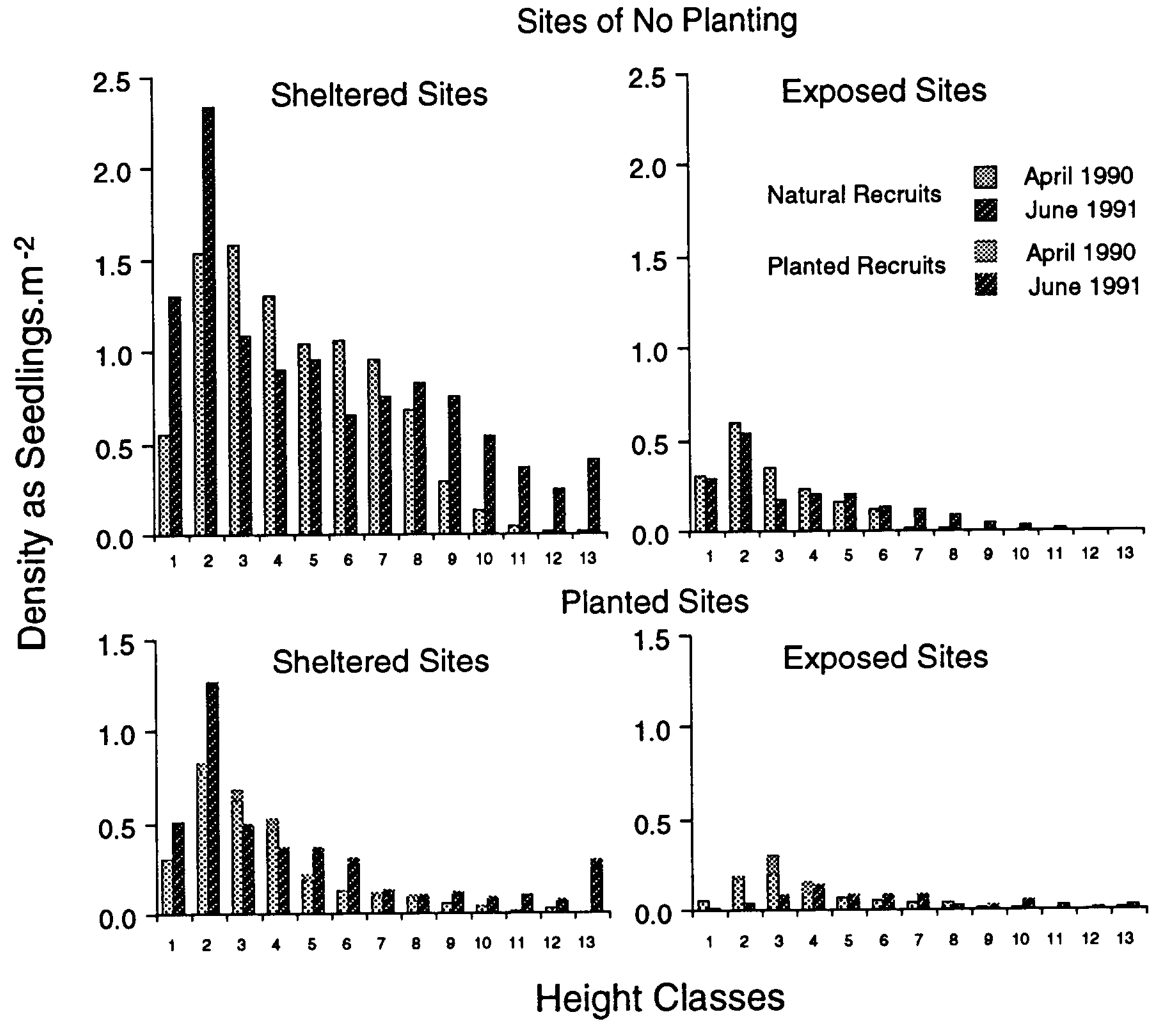


Fig. 8.35 Height classes and mean densities of *Rhizophora mangle* seedlings (*cum* saplings) at exposed and sheltered sites of no planting (see Fig. 8.30) and planted sites scored in April 1990 and June 1991. Notice the lower densities overall, and the proportion and displacement of planted recruits.

density, mean diameter, and mean height. Furthermore, sheltered sites had greater densities of plants in every height class, compared with exposed places. It was not possible to age larger individuals, but nodes were counted for seedlings and it is useful to describe the assemblage in these terms, where node counts are considered measures of age. In Figure 8.38 the demographic isolation of the seedling bank is clearly shown, isolated to the first three node classes, while the larger trees are around node-class 70, using an annual node production rate of 9 for 23 yr. The range of node classes in the latter group is expected to be similar to that shown for the older seedlings in sheltered-habitat seedling plots (Fig. 8.31).

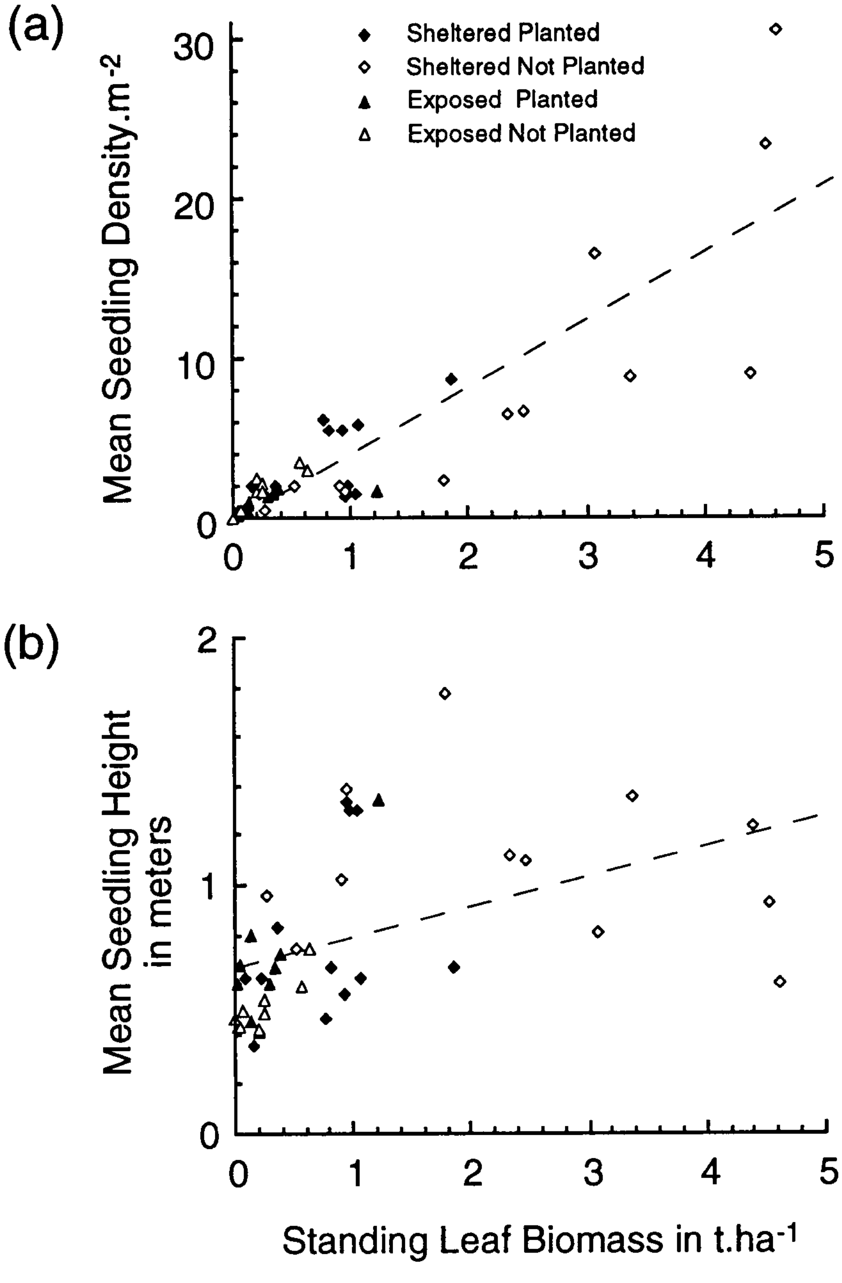


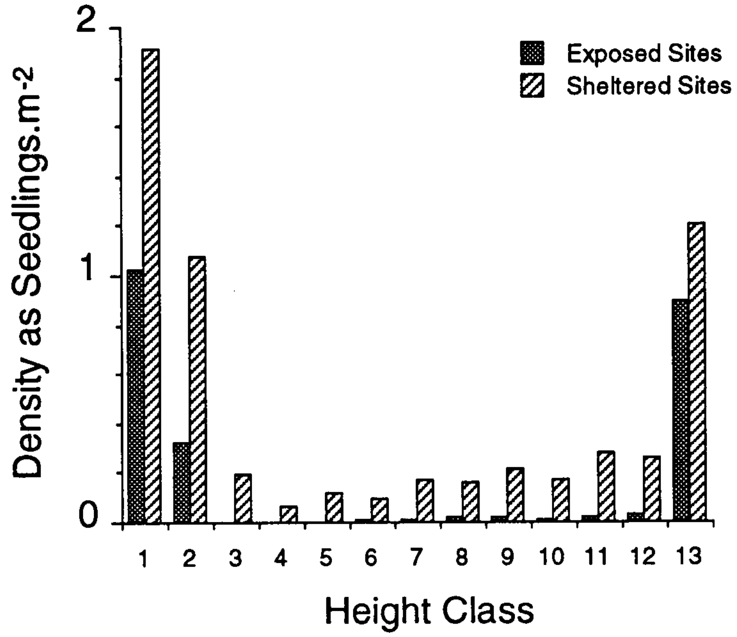
Fig. 8.36 Comparisons of estimated leaf biomass (t/ha) with density (*a*) and height (*b*) of *Rhizophora mangle* plants at planted and not-planted sites in exposed and sheltered oil gaps. Linear regressions show the overall trends. The study plots appeared to be in the first phase of gap recovery, since most estimates of leaf biomass fell below mean annual estimates for nearby mature forest canopies, approximately 4.0 ± 0.5 t/ha. Notice that plots with values approaching those in mature canopies were at sheltered sites where there was no planting.

Table 8.20 Five parameters of forest structure for *Witwater* spill oil gaps in October 1991, based on *Rhizophora mangle* seedlings and trees in two fringe habitats. Seedlings were defined as plants with ≤ 12 nodes along the main stem. SE of among-site variation is in parentheses. Site means are in Appendix Table D.12.

	Exposed	Sheltered
Density (number/m^2)		
Seedlings	1.33 (0.43)	2.98 (0.95)
Trees	1.03 (0.28)	2.94 (1.18)
Total	2.37 (0.38)	5.92 (2.12)
Diameter (cm)		
Seedlings	0.37 (0.01)	0.36 (0.01)
Trees	4.30 (0.53)	3.37 (1.18)
Total	1.99 (0.35)	1.59 (0.29)
Height (m)		
Seedlings	0.28 (0.02)	0.29 (0.02)
Trees	5.03 (0.59)	4.17 (1.87)
Total	2.27 (0.46)	1.84 (0.52)
Leaf Biomass (t/ha)		
Seedlings	0.04 (0.01)	0.09 (0.03)
Trees	3.47 (0.42)	4.39 (0.58)
Total	3.51 (0.42)	4.48 (0.59)
Total Biomass (t/ha)		
Seedlings	0.09 (0.03)	0.21 (0.08)
Trees	71.15 (6.56)	72.68 (24.00)
Total	71.24 (6.53)	72.89 (23.94)

8.6.4 Growth of Trees in an Unoiled 1979 Gap

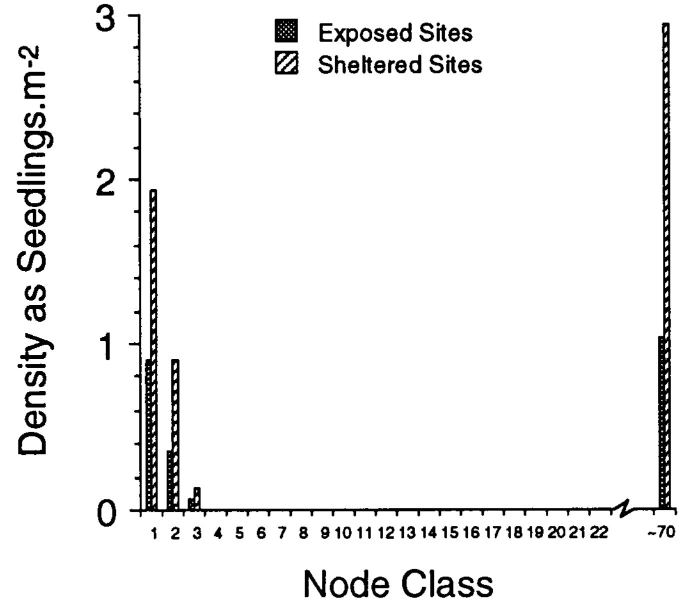
Growth of *R. mangle* at an unoiled site was assessed briefly, establishing that it can be quite rapid. A well-developed forest, visited in May 1990, was found to have been newly colonized on dredge spoil in 1979, based on aerial photographs (Table 8.1). This situation provided a measure of site age for this stand of trees of roughly equivalent ages, a character shared with the larger oil-gap stands. Subsequently, four trees, each approximately 12 m tall, were carefully removed and a range of parameters was scored, including height, girth, and internodal increments (Table 8.21). The latter were traced from the uppermost shoot down the main stem to the hypocotyl, or very close to it. For some reason, these trees had very little bark development obscuring the old leaf scars. The growth history of these trees could therefore be reconstructed based on node height, which extended at a rate of approximately 10 cm/node. Thus, a plot of tree height and node number (Fig. 8.39) formed a curve that can be compared with the sigmoidal curve of the logistic equation (Odum 1971), which is equivalent to this pattern of growth over time. These trees, therefore, grew slowly at first, with height later approaching a putative



	cm		cm
1	0-30	8	151-170
2	31-50	9	171-190
3	51-70	10	191-210
4	71-90	11	211-230
5	91-110	12	231-250
6	111-130	13	>250
7	131-150		

Fig. 8.37 Height classes and mean densities of *Rhizophora mangle* plants (seedlings and trees) at exposed and sheltered *Witwater* oil-gap sites scored in September 1991. Compare with the refinery oil-gap sites in Figure 8.30. Notice the similar densities of the seedling bank, the bimodal distribution with the taller-class trees forming a prominent group compared with the refinery gaps, and the higher overall densities of plants at sheltered sites.

climax at *K* of the logistic equation, of approximately 13 m. This model, although requiring the refinement of a better mathematical model, is in agreement with the type of development expected for trees that grow and attain specific, uniform stand heights, depending on location both within and among sites. In mangroves, this pattern is notable in transects across the topographic profile, but it is also influenced greatly by climate, salinity, tidal inundation frequency, and site exposure. Estimates of total biomass for these trees (Table 8.21) show that rates of growth equal those of *R. apiculata* in Thailand (Aksornkoae 1975; Christensen 1978).



	count		count		count
1	0-3	11	31-33	-70	>207
2	4-6	12	34-36		
3	7-9	13	37-39		
4	10-12	14	40-42		
5	13-15	15	43-45		
6	16-18	16	46-48		
7	19-21	17	49-51		
8	22-24	18	52-54		
9	25-27	19	55-57		
10	28-30	20	58-60		

Fig. 8.38 Node classes and mean densities of *Rhizophora mangle* plants at exposed and sheltered *Witwater* oil-gap sites scored in September 1991. Compare with refinery oil-gap sites in Figure 8.31. Notice the comparable densities of seedling-bank plants and the much greater age difference to older trees, based on node counts, estimated for trees to be approximately 210 (i.e., 9 nodes/yr for 23 yr), or node class 70. Although there were plants in node classes from 4 to 20 in the refinery oil gaps, none was found in *Witwater* oil gaps.

Table 8.21 Five structural parameters (means) of four *Rhizophora mangle* trees growing in an approximately 10-yr-old unoiled gap, sampled in May 1990. SE of among-tree variation is in parentheses. Values for each tree are in Appendix Table D.13.

Parameter	(units)	Mean	(SE)
Height	(m)	12.20	(0.38)
Diameter	(cm)	7.92	(0.68)
Node Count	(no.)	103	(3)
Leaf Biomass	(t/tree)	11.98	(1.38)
Total Biomass	(t/tree)	378.36	(60.37)

8.7 Discussion

8.7.1 Natural Processes of Forest Recovery

8.7.1.1 Turnover and Replacement of Trees

Deforestation and the creation of light gaps are natural and common phenomena in tropical forests (e.g., Mabberley 1983), and mangrove forests are no exception. In the latter case, however, gaps in mangroves differ chiefly because they result from trees that die standing, instead of from tree falls, which characterize tropical rain forests, particularly those in the Americas. Furthermore, light gaps in mangroves are rarely created by the death of only old trees. It is more common to see them comprised of approximately 10 dead trees of various age groups and often appearing as roughly circular holes in the continuous canopy because the dead trees rapidly rot and collapse in a few years. These "pot-hole" impressions form a broad mosaic of regeneration pockets of different depths, reflecting various ages and stages of regrowth. Below the canopy, this variation is also apparent for several reasons, depending on gap age. When a gap is young, there is considerable extra light coming through the gap, illuminating patches of prolific young plants immediately below and in an otherwise vacant undercanopy. Later, in older refilled gaps, groupings of younger trees are clearly apparent because of smaller girths and higher density. This process of gap creation and closure is believed to be the main process by which mangrove forests naturally regenerate.

The reason for tree death in these gaps is not known, but gap formation is first observed when a small group of trees wilt and drop their leaves in an otherwise green and continuous canopy. Death involves the entire tree, rather than limb shedding or partial die back. Potential causes depend on the site, but range from episodic events, such as violent storms, frost damage, plant pathogens, and lightning strikes, to slow and progressive changes in sea level, water courses, and seasonal flooding or drought. In any case, it is not surprising that plants growing in this

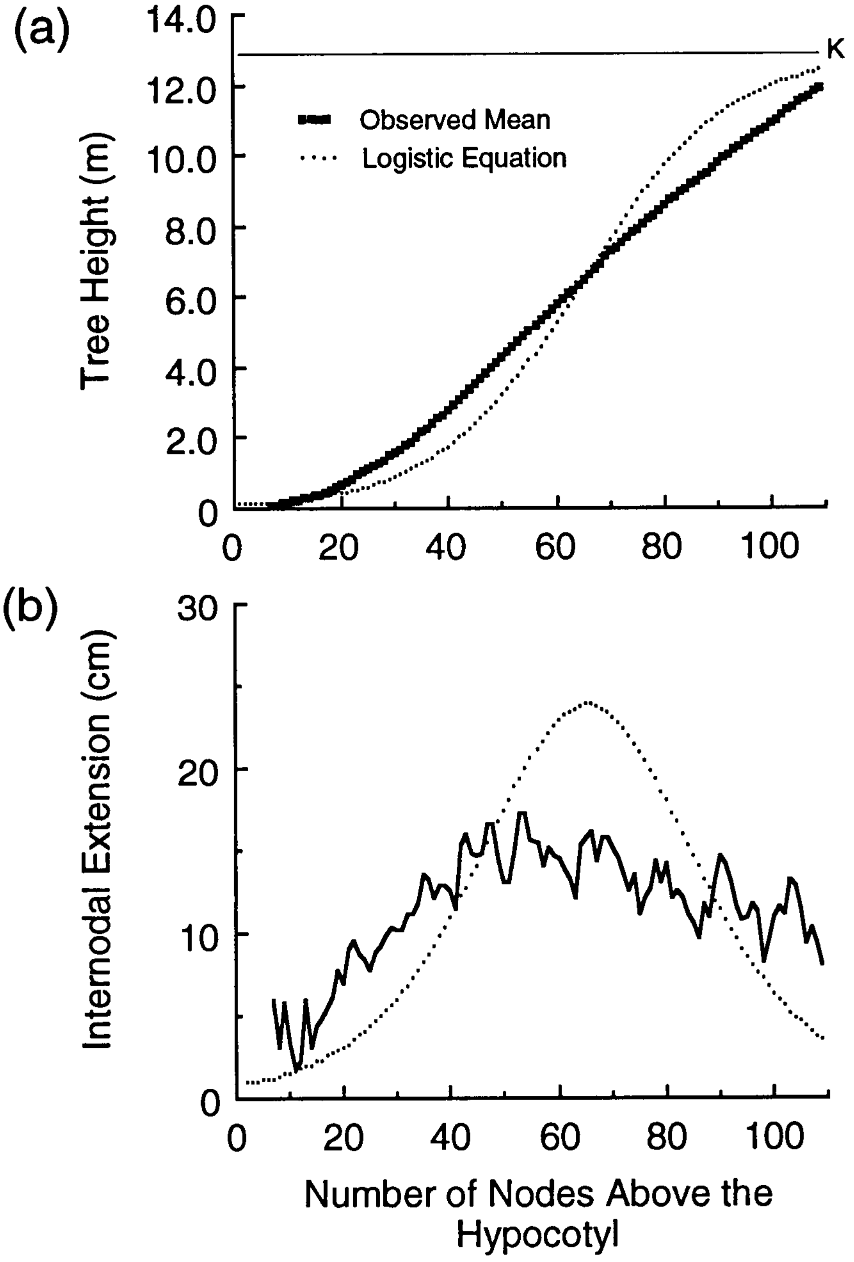


Fig. 8.39 Four trees of *Rhizophora mangle*, approximately 12 m tall, were sampled from an apparently single-aged stand approximately 10 yr old. The two plots present data of (*a*) mean heights and (*b*) mean internodal extension, derived from leaf scar nodes which were discernible along the main stems of each tree. In addition, and in conjunction with each plot, the best-fitting curve for the logistic equation is included for comparison. In this way, the idea of these trees approaching some maximal height, around 13 m (*K*), was considered in this assessment.

fluctuating intertidal habitat might reflect both subtle and episodic changes in local conditions, including those caused by man.

In Panama the most likely cause of natural light gaps is considered to be lightning strikes. It seems reasonable to assume that a surge of electricity striking a central tree would also reach those in the immediate vicinity, especially as root grafting, joining neighboring trees, is very common. This connectedness is observed even between different species of the same genera, notably for *Rhizophora*. But, apart from the common occurrence of electrical storms in mangrove areas, there are no other data supporting this notion, and few gaps show signs of being created by lightning.

While these gaps probably represent the most common form of tree death of mangroves in Panama, this is not necessarily the case at other locations. For example, in areas of frequent hurricanes, the influence of small light gaps is possibly irrelevant, considering the putative life span of these trees. During violent storms, trees are often stripped of leaves and, for *Rhizophora* species, this results in death. However, this is not the case for all mangrove species. Other species, notably *Avicennia germinans* and *Laguncularia racemosa*, apparently recover rapidly because lost leaves are quickly replaced. This difference is expected to profoundly influence species composition of mangrove forests in respective regions, e.g., comparing Panama with Florida.

Nevertheless, it appears that *Rhizophora* forests have several recuperative processes, ostensibly for colonizing new intertidal banks and refilling natural light gaps. In the latter case regeneration appears to begin long before trees die, and a "seedling bank," reminiscent of the seed bank of terrestrial forests, provides the means for these forests to recuperate more rapidly than expected. However, there is an important difference between terrestrial and mangrove forests because most mangroves do not have seed propagules and, for those that do, the seeds are not long-lived. Instead, many mangroves depend on some degree of viviparous development of seedlings. This attribute was considered to have developed especially for longevity and long-distance dispersal in the marine habitat, but it is possibly more important for re-establishment. Many nonmangrove plants have durable and buoyant seeds, capable of greater long-distance dispersal than mangroves. The importance of vivipary therefore appears to be related more to rapid establishment because vivipary is part of a process of continuous growth characteristic of certain mangroves. Our observations therefore suggest that the role of *Rhizophora* propagules is not simply to transport the species to new and distant sites, but more importantly to recolonize and restock existing populations each season. This notion, and that of long-distance dispersal, are each supported by the dominance of *Rhizophora* species in virtually all tropical mangrove forests worldwide. It appears therefore that the persistence of these forests is improved by the rapid establishment and recovery made possible by viviparous propagules in conjunction with the seedling bank, as individuals available to fill the next gap opening.

8.7.1.2 Seedling Bank and Herbivorous Crabs

For *Rhizophora* forests on the Caribbean coast of Panama, the undercanopy seedling bank comprises established young plants with 1 to 3 pairs of leaves on a single stem, between 0.5 and 1 m tall. However, these seedlings are not easily visible because they rarely reach higher than the common tangle of above-ground roots. They are also shade intolerant and usually die before reaching 1 m tall, when they are between 2 and 3 yr old. Their initial growth response is undoubtedly under endogenous control because they have a rapid burst of growth when first established. This period is evident as rapid main-stem extension for the first 1 to 4 leaf nodes. However, this growth declines from 5-10 cm in the first year to approximately 1.5 cm in the second. It is clear from this difference that early growth of *Rhizophora mangle* seedlings relies chiefly on internal resources derived originally from the parent (Pannier and Pannier 1975). As these resources become depleted in the growing seedling, height extension slows and the plant eventually dies under the closed canopy. During this time, leaf-node production rate remains relatively constant, at approximately 3.7 ± 0.3 nodes/yr (Duke and Pinzón 1992).

Furthermore, the density of seedlings is patchy, and depends on tides and topography. In addition, there appears to be another unexpected factor, as indicated from several observations of mangrove forests in Panama. First, propagules of established seedlings are invariably buried in the substratum by at least one-third of their length. This observation was unusual because the substratum in these mature forests is often not soft silt, but rather a tough mat of fibrous roots, apparently impenetrable to floating seedlings. Second, seedlings are often positioned away from established prop roots, where they were expected to be established if entrapment among roots was important. In fact, those near roots had severe leaf damage caused by root-crawling herbivorous crabs. Third, smaller crabs make propagule-sized burrows approximately 15 mm in diameter, slightly larger than the diameter of *Rhizophora* hypocotyls, through the root mat. Therefore, considering these observations and others, we suggest that *Rhizophora* propagules can be "crab-hole planted" by drift-placement into crab burrows. Their rapid subsequent development of holding roots ensures that they become established quickly. An alternate hypothesis suggests that falling propagules "spear" into the substratum, but this mechanism is considered less likely based on the observation of seedlings being equally common under prolific seedling-bearing trees and sterile ones, and that the substratum mat is tough and penetration resistant. Furthermore, crab-hole planting is favored over root entrapment because those crabs that eat and damage leaves, mentioned earlier, apparently prefer to stay on roots. Such observations need to be assessed further, but the notion of seedlings becoming planted commonly in crab holes has interesting implications in the evolution of both the forests (their propensity to vivipary) and the presence and behavior of crabs. For example, while there does not appear to be any immediate benefit to the small crabs, they do benefit indirectly by helping to maintain the seedling bank, which, in turn, maintains the forests they depend on.

8.7.2 The Influence of Oil on Forest Recovery

One of the chief questions asked in all of these studies is whether gaps deforested by oil have different rates of recovery than natural gaps. Before launching on this discussion, one qualification needs to be made: oil gaps are usually much larger than natural gaps, at least in Panama (Fig. 8.40). The importance of this point is found in the amplification of at least two very important factors, light and substratum stability. While larger deforested areas offer more light and, consequently, faster tree growth, they also provide less protection for new recruits. Clearly, there is a trade-off in these opposing factors for the recovery of mangrove forests. It is also clear that the influence of these factors changes considerably from one place to another. For example, consider the differences between sheltered and exposed sites, with the latter having much greater erosion and consequent loss of recruits. In fact, losses have been so great at some exposed sites it seems inevitable that these habitats will be lost (Fig. 8.41). In contrast, sheltered sites are recovering much better, although seedling growth is depressed in proportion to the amount of oil in the sediment.

This study quantified particular growth parameters with respect to the presence of both oil and a small number of environmental factors. The most important environmental factor appeared to be salinity of surrounding waters, which clearly relates to both rainfall and the location of each site with respect to riverine outflow. This factor was essentially removed, however, by comparing oiled and unoiled effects only for sites with similar annual salinity variation. These sites were grouped as the open (exposed) and channel (sheltered) sites, with unoiled river sites for comparison. Using these criteria, data gathered in this study are presented in several comparisons, identifying life-history development of mangrove forests and comparing oiled and unoiled stands, of known and unknown ages, growing in Bahía Las Minas and elsewhere.

Furthermore, recovery of surviving forests, i.e., those surrounding oil gaps, was not apparent either. Estimates of standing crop leaf biomass of forest canopies indicated a 23-33% loss in oiled places, matching the continued decline in 1990 and 1991 of numbers of leaves per shoot, lower numbers of shoots, and 18% lower leaf longevity. This reduction appears to represent a response by trees to lighten the upper canopy by self-pruning, notable as increased wood litter, presumably because of greater stress on roots from oil in sediments (Fig. 8.27). At the same time, average leaf area increased at oiled places (Fig. 8.17), indicating a possible countermeasure by trees to maximize falling photosynthetic capability. However, while our data offer some insights into this effect on surviving forests and its continued influence, it is impossible to predict how and when the positive aspect of recovery might begin. To answer these questions, it is critical to continue monitoring canopy condition longer than 3 to 5 yr postspill, especially in this instance.

This might also be said of oil-gap recovery, except that our projections can be greatly extended because of the discovery of the 23-yr-old recovering *Witwater* sites. Therefore, while there was insufficient time to assess old surviving forests, there was

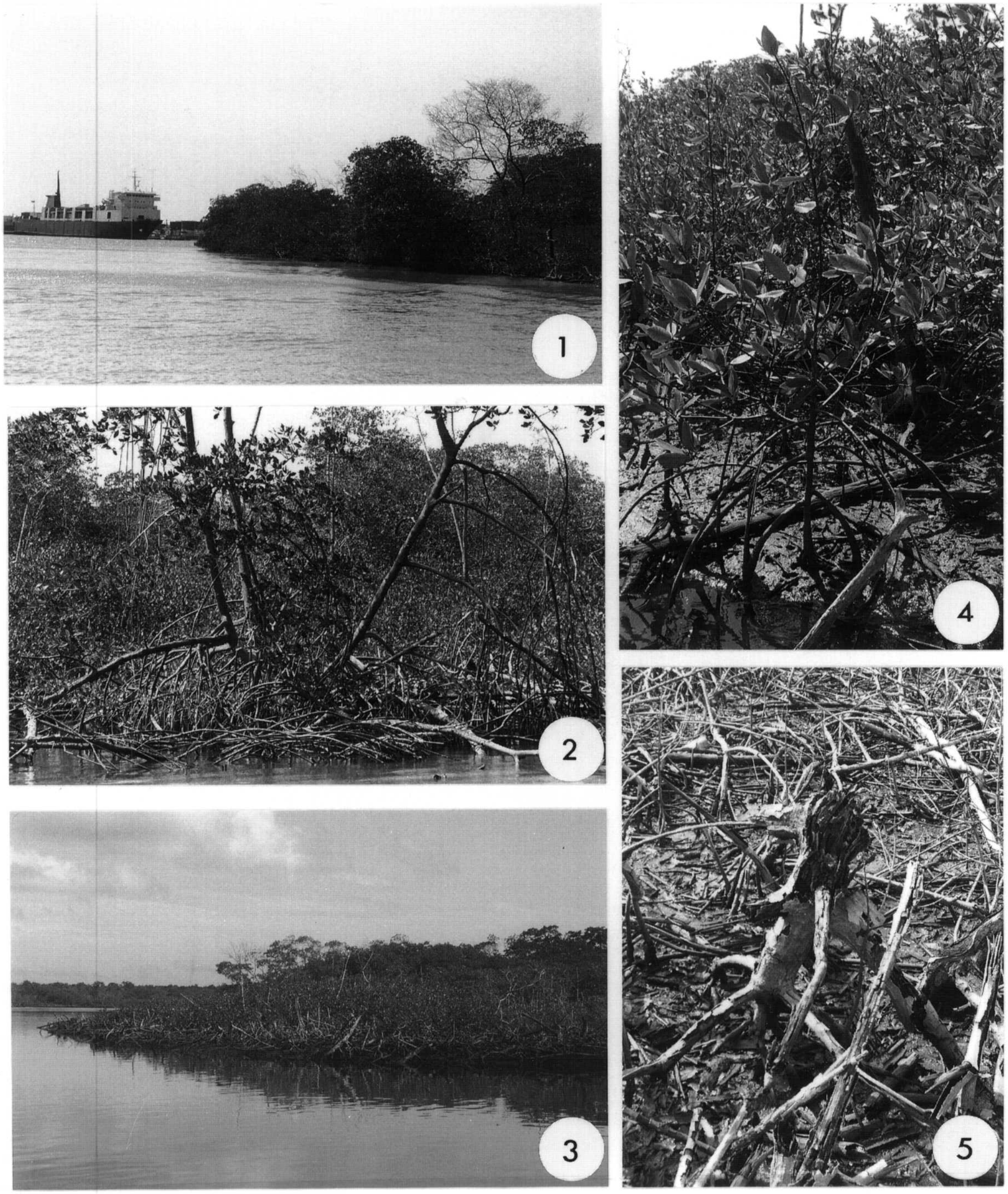


Fig. 8.40 Photographs taken in 1991 show oil-gap damage and recovery of sheltered mangrove forests in Bahía Las Minas following the 1986 refinery spill. *1.* The refinery and port activities result in numerous lesser spills, but their total effect has not been as obviously destructive as the single large spills, particularly the one in 1986. Trees often died behind surviving fringes, and the true magnitude of this disaster is shown more clearly in the maps derived from aerial photographs (Figs. 8.12, 8.13; App.

Fig. 8.40 (continued) Figs. D.2-D.19). *2.* In 1991 surviving fringe trees are clearly evident in front of seedling-filled oil gaps, backed by surviving upland mangroves. *3.* In some areas fringes did not survive, notably in the central embayment where oil was entrapped shortly after it was spilled. *4.* Seedlings were planted in many recovering gaps by the refinery, but natural recruitment appears to have been sufficient. *5.* A small number of sheltered areas had little or no recovery in 1991. This lack of recovery was more prevalent in the central embayment and along the lower intertidal fringe.

time to include a brief survey of six reforested sites, three each in open and channel habitats. The result is that data on forest structure now span 23 yr of postspill recovery for oil gaps.

Studies from Puerto Rico (Cintrón and Schaeffer-Novelli 1983) described some relationships that appear to be followed by natural forests of *R. mangle* wherever they grow. One of these relationships is between girth and density, and is shown in Figure 8.42 as the *dashed straight line*, along with data from the present studies in Bahía Las Minas. Notice that seedling assemblages approach the line from the left, *Witwater* sites are more advanced down the line, and oiled and unoiled mature forests occur at the lower right end of the line. Clearly, these forests follow the regression shown for trees both without oil and a long way from Panama, showing that this fundamental biological relationship remained unaffected by oil.

The question, therefore, does not appear to be one of status, but rather of time. In other words, how long does recovery take, and is this different for oil gaps? To explore this question, it was important to summarize parameters of biomass, notably for leaves and total above-ground matter.

Data on leaf biomass are listed in Table 8.22, showing estimates for the 2 yr of seedling growth from the 1986 spill, the *Witwater* plots, and the surviving mature forests. Estimates were generally lower in the seedling plots, 4 and 5 yr postspill, but those at *Witwater* sites were comparable to those in mature forests. The estimates for mature forests were based on two techniques, and consequently indicate different aspects of leaf biomass. The canopy estimate was derived from litter and shoot data and represents the postspill condition of surviving canopies. The structure estimate, on the other hand, was derived from an allometric equation based on tree height and girth, characteristics that do not appear to have changed significantly since the trees were oiled. The latter estimates are therefore believed to best represent prespill canopy condition. Unfortunately, the error terms on the latter estimates are too large for a before-and-after comparison (requiring more within-site replication, in the first instance); differences between oiled and unoiled habitats were not significant, while they were for the canopy estimates. Plotting these data with respect to gap age (Fig. 8.43), the increase in leaf biomass is seen to sharply increase in the first few years, after which it apparently levels off at the amounts observed in mature canopies.

This leveling off with age is not apparent in total above-ground biomass. These data are summarized in Table 8.23, showing the same study sites as before, noting the prespill data of forest structure showed no difference in total biomass between oiled and unoiled sites. In contrast, there was a significant difference

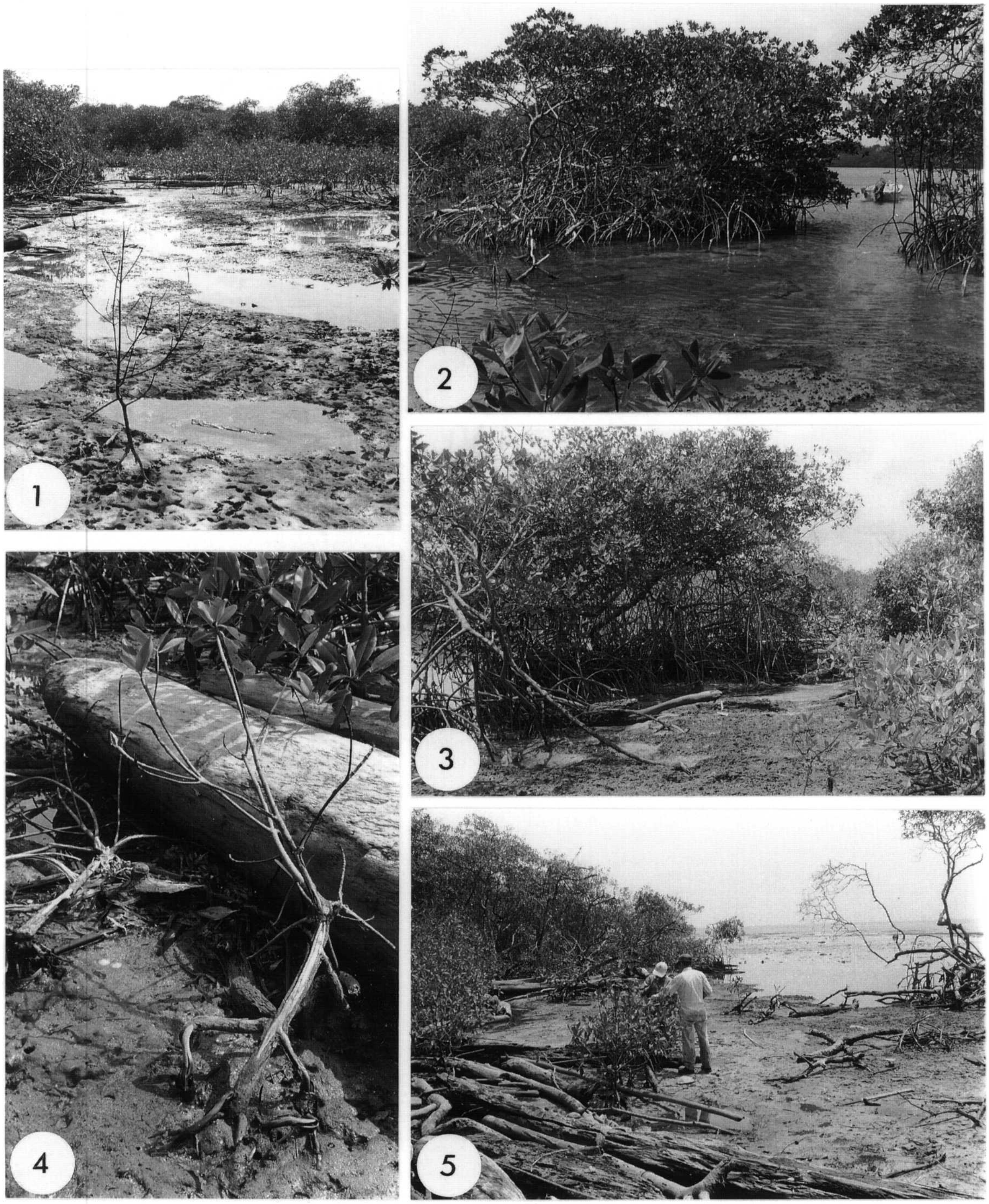


Fig. 8.41 Photographs taken in 1991-1992 show the oil-gap damage and condition of exposed mangrove forests at Bahía Las Minas following the 1986 refinery spill. *1.* Large gaps were often nearly desolate, with only scattered seedlings. *2.* Survivors were also found in narrow fringes facing the sea, with occasional openings. *3.* Other oil gaps were more enclosed and narrow, but recruitment was still minimal. *4.* Seedlings were clearly being killed by fallen trees and driftwood. *5.* In addition, some sites

Fig. 8.41 (continued) were without a surviving fringe, and piles of driftwood had scoured the surface where mature trees stood 5 to 6 yr before.

between habitats. There was also a marked difference between the apparent progress of *Witwater* gaps toward pre-oiled conditions. Therefore, while exposed sites showed almost a complete return to normal, sheltered sites appeared to be stunted by more than 50%. If these sites were to continue growing at this rate, then recovery might be expected to take another 30-40 yr (Fig. 8.44), an estimate that stands in stark contrast with the rate of growth scored for the four unoiled trees nearby (Table 8.21). In their case, normal stand biomass is expected to be achieved approximately 15 yr from recruitment. This latter rate is not unusual; similar values were reported for *Rhizophora* forests in Thailand (Aksornkoae 1975; Christensen 1978).

8.7.3 Concerns with Assisted Rehabilitation

Rehabilitation following large oil spills has traditionally focused on deforested oil gaps, where recovery would understandably take at least 25-30 yr for forests to attain something approaching prespill conditions. This period represents the time it takes for seedlings to grow into trees. The kinds of assistance measures considered include site drainage, removal of oil, planting, or some combination of these steps. However, in providing help we presume, suspect, or know that natural processes are unable to repair the damage or, at least, not as efficiently, because the supply of seedlings was reduced, their growth inhibited, or erosion too rapid. Unfortunately, our collective knowledge and experience with these matters is inadequate, and there is a serious risk that added interference may worsen this already fragile and vulnerable habitat. At the very least, human assistance may not improve recovery compared with situations where there is no further interference. Until we learn more about these matters, it seems essential to study both the natural processes involved and to follow the fate of earlier efforts that were implemented to attempt to accelerate recovery. By knowing their fates, we will further improve the prospects for future projects faced with the same questions.

Such considerations are spill- and site-specific, so the insights and lessons gained during these studies in Panama have their own value, both at a local level and in comparisons elsewhere. In general terms, however, mangrove forests appear to be more variable within single locations than between distant sites, a feature undoubtedly related to their genetic uniformity, their climatic range, and the intertidal habitat. Assessment of the rehabilitation effort in Panama suggests two important factors affecting longer-term recovery of oil gaps. First, although planting provided no apparent benefit toward the general recovery of mangroves in Bahía Las Minas, growth of some planted seedlings was improved with fresh soil. Second, standing dead timber and roots appear to provide shelter for new recruits, so these structures should not be cut or removed.

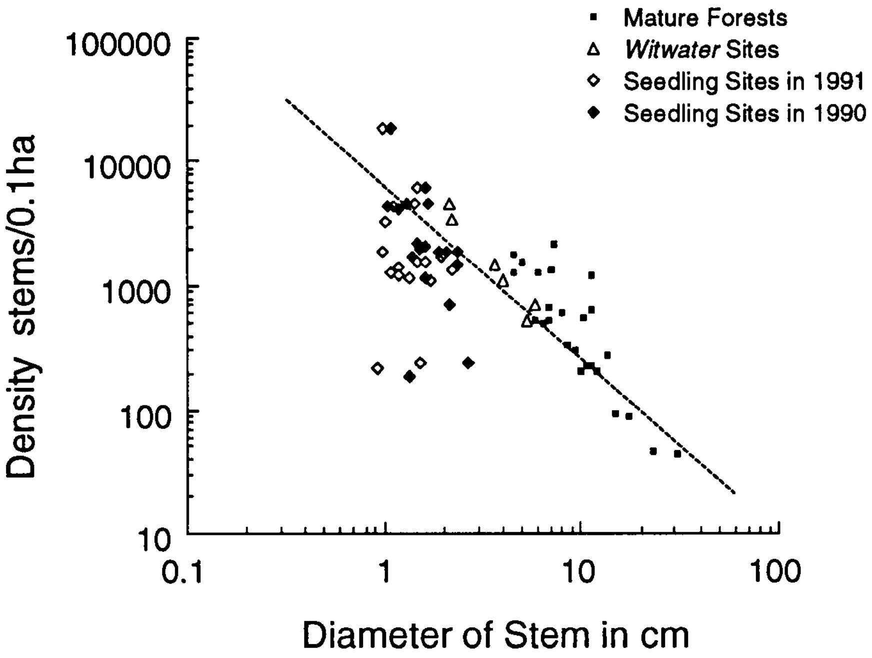


Fig. 8.42 Mean girth and density of *Rhizophora mangle* plants from refinery oil gaps, *Witwater* oil gaps, and mature trees, showing the correspondence of these plants in Panama with those in Puerto Rico (regression line; see text). Also included are the densities for mature-site seedling banks and hypocotyls in litter fall. These provide a pattern for the growth development of *R. mangle*, which followed the linear regression to lower densities and larger girths in older plants. Oil did not appear to alter this relationship, although it may have affected the growth rate and maximum tree size.

Table 8.22 Canopy leaf biomass (t/ha) estimated from height and girth of *Rhizophora mangle* plants in respective sites of mangrove forests in oiled and unoiled locations in three fringe habitats during 1990-1991. SE of among-site variation is in parentheses.

	Exposed Open		Sheltered Channel		Sheltered River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Refinery Spill 1986 - seedlings and saplings					
1990	-	0.27 (0.08)	-	1.48 (0.37)	0.76 (0.12)
1991	-	0.45 (0.11)	-	2.38 (0.44)	1.38 (0.03)
Refinery Spill 1986 - mature trees, prespill					
1991	4.10 (0.68)	3.51 (0.83)	5.97 (2.66)	5.53 (1.87)	3.35 (0.55)
Refinery Spill 1986 - mature trees, postspill					
1990	4.48 (0.17)	3.34 (0.41)	5.09 (0.62)	3.70 (0.46)	5.07 (0.46)
***Witwater* Spill 1968** - young trees					
1991	-	3.47 (0.42)	-	4.39 (0.58)	-

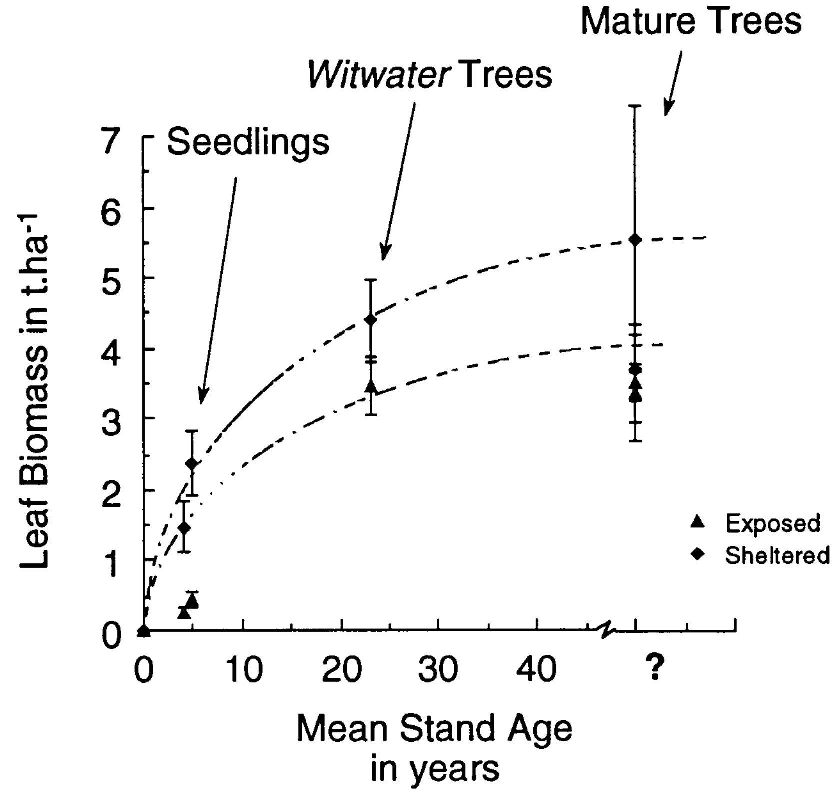


Fig. 8.43 Mean leaf biomass and age of *Rhizophora mangle* plants from refinery and *Witwater* oil gaps, showing a trend toward an upper level found in unoiled mature forests in exposed and sheltered habitats. The low levels in refinery oil gaps apparently were depressed because they included several places that continued to deteriorate, and were not expected to recover.

In Bahía Las Minas erosion and deterioration of protective standing dead trees at exposed sites was accelerated by large drift-logs buffeting the damaged fringe, causing permanent loss of habitat in some cases. In order to prevent windrow-scouring, it is suggested that simple wooden structures, such as posts, be positioned among planted or natural recruits to protect them until they are big enough to withstand scouring by larger drift fragments. In this instance, it may be necessary to plant seedlings because natural recruitment is apparently quite low at exposed sites (Table 8.7). These observations demonstrate where and how assistance might be better applied in the future to improve recovery of mangrove forests in Bahía Las Minas, and elsewhere.

The decision to act by initiating projects of habitat restoration when mangroves have been damaged is based on the assumption that natural processes are inadequate and that they may be beneficially accelerated. However, to have value, this need must first be demonstrated with a thorough assessment of ongoing recruitment and establishment success prior to taking further steps. It seems

Table 8.23 Total above-ground biomass (t/ha) estimated from height and girth of *Rhizophora mangle* plants in respective sites of mangrove forests in oiled and unoiled locations in three fringe habitats during 1990-1991. SE of among-site variation is in parentheses.

	Exposed		Sheltered		
	Open		Channel		River
	Unoiled	Oiled	Unoiled	Oiled	Unoiled
Refinery Spill 1986 - seedlings and saplings					
1990	-	1.83 (0.70)	-	10.72 (2.31)	5.75 (1.12)
1991	-	3.65 (0.92)	-	20.39 (3.01)	12.28 (0.04)
Refinery Spill 1986 - mature trees					
1991	80.48 (9.88)	80.65 (20.80)	193.99 (88.20)	199.81 (86.65)	182.28 (50.61)
***Witwater* Spill 1968** - young trees					
1991	-	71.15 (6.56)	-	72.68 (24.01)	-

important to be aware of natural rehabilitation processes, otherwise the outcome of good intentions, implicit in projects of habitat restoration (e.g., by attempting to remove oil or by planting) could result in further destruction of an already disrupted and fragile environment. Accordingly, it makes good sense to learn about the natural functioning of the ecosystem, as well as studying earlier restoration projects, in the hope of developing suitable process-based solutions that do not unwittingly promote further longer-term damage.

8.7.4 Loss of Habitat, Particularly Exposed Coastal Fringes

Erosion of oil gaps at exposed sites was more evident 3 or 4 yr after the spill. This process involves the mobilization of rotting and broken tree fragments. It was less important at sheltered sites, but at exposed locations, especially where frontal trees were killed, the substratum was noticeably scoured by the bulldozing movement of wind- and wave-driven windrows of broken timber. These were chiefly made up of the fragmented remains of trees killed during the initial effects of the spill. Furthermore, the bulldozing process removed most established seedlings. It is suggested that colonization and re-establishment of these sites is significantly retarded, and some oil gaps on exposed coasts may be altered permanently.

8.8 Conclusions

8.8.1 Listing of Major New Findings

This study made observations of mangrove forests, particularly regarding effects of a large oil spill, rehabilitation by restoration projects, and natural growth and recovery. The major results and findings include the following.

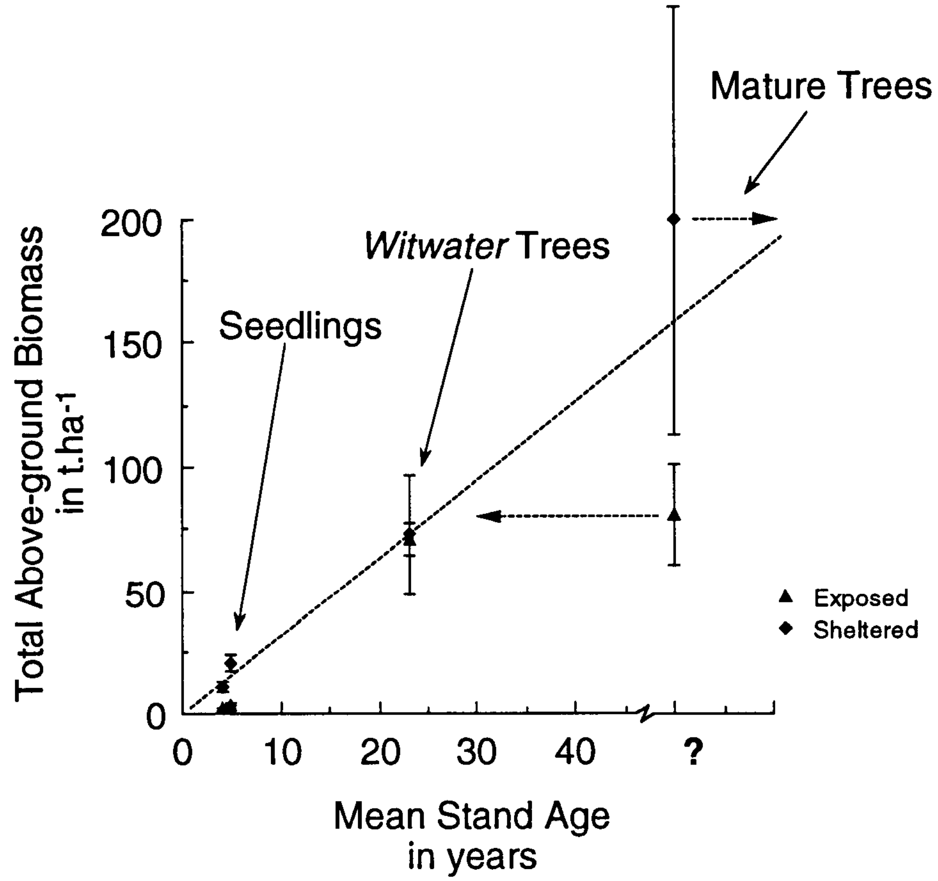


Fig. 8.44 Mean total above-ground biomass and age of *Rhizophora mangle* plants from refinery and *Witwater* oil gaps showing a linear trend toward higher amounts in progressively older stands. These values may be compared with mature forest estimates for exposed and sheltered habitats, but while exposed *Witwater* oil gaps had apparently returned to normal, the sheltered sites appeared to be severely suppressed compared with growth at the unoiled sheltered site. Estimates showed that this site was close to the normal mature-forest biomass after approximately 10 yr, and this compared well with growth of *R. apiculata* in southeast Asia (see text).

1. **Maps of the 1986 Refinería Panamá Spill.** Vegetation maps delineate areas of mangroves and oil-gap deforestation.
2. **The 1968 *Witwater* Spill.** Similar maps delineate deforestation caused by this spill, providing the first record of this effect.
3. **Canopy Deterioration of Surviving Forests.** Forest canopies were reduced by oil in sediments, and showed no signs of recovering 3-5 yr after the spill.

4. **Retardation of Seedling Growth.** Recruitment can be prolific, but growth was retarded by residual oil in sediments.

5. **Site Erosion.** Recruitment at exposed oil-gap sites was destroyed by drift-logs and removal of sediment.

6. **Protection and Planting.** Refinery planting provided no benefit to recovery, but site protection from drift-logs might help.

7. **Aging Seedlings.** Node counts and sequences provided a much-needed method to age *Rhizophora* seedlings.

8. **Seedling Bank.** Seedlings under closed canopies pre-empt gap creation, and possibly promote rapid closure.

8.8.2 Suggestions for Future Studies of Large Oil Spills

In this chapter we have presented a wide view of postspill recovery of mangrove forests by considering natural processes operating in the ecosystem. In this way, we have shown these forests to be well-adapted for small-scale deforestation, because it is apparently the major process of turnover, regeneration, and succession. Deforestation exposes an undercanopy seedling bank of established recruits, apparently able to grow rapidly when greater light conditions are presented by the creation of a gap in the canopy.

Clearly, there is more to learn, but it is hoped that future efforts to restore mangrove forests might include greater consideration of natural processes, some of which are discussed in this chapter. Accordingly, and based on our observations and others (e.g., Cintrón and Schaeffer-Novelli 1983), a tentative protocol for postspill efforts might include the following.

1. Protect mangrove forests from major oil spills. Once mangrove sediments are oiled, there are no known ways of removing the oil that do not damage surviving or recovering forests.

2. Carefully remove oil reaching mangrove trees, where practical. This cleaning would include wiping oil coated on sensitive trunk and root surfaces without using solvents or harsh emulsifiers. Each tree saved represents an enormous investment toward habitat restoration, particularly where they protect exposed and vulnerable coastal margins. Furthermore, during these efforts surviving seedlings of the seedling bank need to be identified and protected.

3. Cutting and removal of trees, dead or alive, should be discouraged because seedling recruits rely on these structures for shelter and support. This is particularly important for exposed coastal sites.

4. The extent of the affected area needs immediate quantification for future reference. This documentation entails mapping the coastal areas affected, identifying the areal extent of mangrove forests and oiled places, and mapping subsequent deforestation. For this task it is essential to have aerial photographs flown immediately, with supporting ground-truth data for areas of deforestation, including tree and animal species most affected by the spill.

5. The potential for natural recovery needs to be evaluated once the extent of obvious damage, such as deforestation, is known. For example, the extent and proximity of unoiled habitats, where these exist, need to be evaluated because they represent the source of both natural, and planting recruits, if necessary. The decision to plant would depend primarily on site character, the status of surviving recruits, propagule supplies, and the opportunities for dispersal to sites of deforestation. Planting should only be necessary if it can be shown that natural processes are failing or that they could be beneficially accelerated. At exposed sites there is the additional problem of erosion possibly causing permanent changes to site topography. This problem is apparently exacerbated by the destruction of recruits by the scouring action of windrow drift. In these cases it might be more beneficial to position degradable wooden structures in the substratum to protect young seedlings during early growth.

6. Sites of future long-term biological evaluation need to be established immediately. This step is necessary for collecting basic comparative data on forest structure and species composition in deforested areas. Other scientific sites could be set up with plots of marked recruits. In this way, future recruitment and growth of existing earlier plants could be accurately followed. This monitoring requires regular site visits over a long period. It is expected that constant re-evaluation might redefine precise goals and procedures.

7. In addition, there should be investigations to determine the mechanisms of how plant growth is suppressed in oiled sediments. In particular, it is not known if there is direct, sublethal toxicity of oil, an alteration of sediment nutrient dynamics, a change in below-ground root biomass and physiology (e.g., gas exchange, nutrients), an effect on mangrove infauna, or other factors. Another area to consider is the infuence of oiling on the herbivore/propagule-predator relationship noted in Section 8.7.1.2.

8.9 Acknowledgments

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