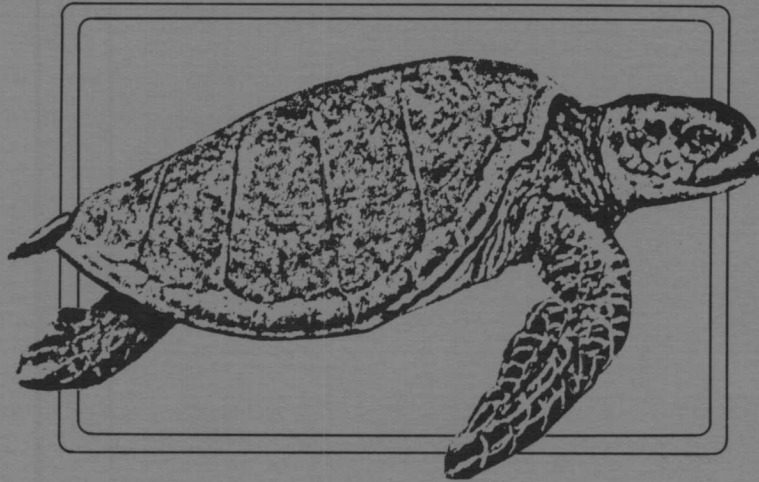




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*Effects of Petroleum on the
Development and Survival of
Marine Turtle Embryos*



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**EFFECTS OF PETROLEUM ON THE DEVELOPMENT AND SURVIVAL OF
MARINE TURTLE EMBRYOS**

by

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SUMMARY

An investigation of the effects of petroleum on the development and survival of marine turtle embryos was conducted to determine the vulnerability of marine turtle progeny to petroleum spills in adjacent and distant waters. Field studies of Lepidochelys kempi (Kemp's ridley turtle) involved the analysis of the concentration and distribution of hydrocarbons on the nesting beach in Tamaulipas, Mexico and the effects of petroleum on the development of embryos. Analysis of sands from the nesting beach indicated that the oil spilled in marine waters was deposited within the nesting zone by wave action. Field experiments in which paired samples of turtle eggs were incubated in clean and contaminated sands from the beach did not result in significant effects related to oil contamination.

In laboratory experiments, eggs of Caretta caretta (the loggerhead turtle) were incubated in sands treated with varying amounts of crude oil at different times during incubation. Experiments using varying quantities of oil mixed with the sand at the initiation of incubation resulted in differences in hatchling morphology but not in survival. Experiments in which oil was added on top of the sand containing the eggs after incubation was partially complete resulted in significant embryonic mortality and differences in hatchling morphology. The results suggested that aged petroleum is less toxic to turtle embryos than is fresh petroleum. The aged oil found on the beach studied in field experiments produced no detectable effects on turtle embryos, whereas fresh oil in the laboratory produced a variety of effects.

Our results suggest that a marine oil spill resulting in contamination of turtle nesting beaches before the nesting season may affect nesting success for only a short period, if at all. However, a spill resulting in the deposition of oil on eggs or on top of a nest already constructed is likely to increase mortality and affect hatchling morphology. The timing of the spill and age of oil may be critical in determining the overall effect on turtles.

The effects of oil contamination on marine turtles in ocean waters remain unknown. However, the present study provides a basis for evaluating potential effects of oil contamination and for developing oil spill contingency plans for turtle nesting beaches.

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INTRODUCTION

The effects of petroleum and petroleum products on the development and survival of marine turtle embryos during incubation are unknown (Cox 1977; Frazier 1980; Hall 1980). As a result of increased oil and gas exploration in marine coastal areas and environmental concern for declining populations of marine turtles, information is needed to define potential effects of oil on all aspects of marine turtle life history (Frazier 1980; Hall 1980).

Four species of marine turtles which occur in the waters off the southeastern United States are listed as endangered in all or part of their range. A fifth species is considered threatened. All of these turtles nest in areas where pollution resulting from oil and gas exploitation in the Gulf of Mexico and along the Atlantic coast of the United States could reach necessary beaches. Two species, Caretta caretta, the loggerhead, and Lepidochelys kempfi, Kemp's ridley, have major nesting beaches near Outer Continental Shelf (OCS) oil and gas lease areas in the southeastern United States. Dermochelys coriacea, the leatherback, and Chelonia mydas, the green turtle, also nest in limited numbers on the coast of the southeastern United States.

Except for the land basking of green turtles in Pacific waters (Bustard 1973; Balazs 1976; Fritts 1981), marine turtles occur on land only during nesting forays by the females and the subsequent development of embryos and hatchlings. The eggs generally are deposited about 20 cm below the surface of the beach at a level above the high tide line. Development usually takes 40 to 60 days. Upon hatching, the young must climb out of the nest by excavating the roof of the nest chamber and crawl down the beach into the ocean.

The developmental period spent in the nest is especially subject to environmental influences. The position of the nest on the beach determines exposure to environmental factors such as moisture gradients, sand particle size, and human activity. Nests are subject to predation by vertebrates and invertebrates. The nest may also be exposed to tidal inundation after storms and beach erosion, which can have lethal effects (McGehee 1979). Eggs, embryos, and hatchlings were judged to be more vulnerable to volatile and water soluble contaminants than adults (Frazier 1980).

The purpose of this investigation was to determine the effects of petroleum on the development and survival of embryos in the eggs of Lepidochelys kempfi and Caretta caretta. These studies were conducted during the 1980 reproductive season under a memorandum of understanding between the Bureau of Land Management (New Orleans OCS Office) and the U.S. Fish and Wildlife Service. The objectives were to determine effects of oil on eggs and hatchlings in relation to oil concentrations, aging of oil, and time of contamination in both field and laboratory environments. The IXTOC spill which occurred from 3 June 1979 to 24 March 1980 provided an opportunity to investigate possible effects under field conditions. The observations and results described herein are essential in anticipating appropriate responses to emergency situations.

MATERIALS AND METHODS

FIELD OBSERVATIONS

Observations of the effects of an oil spill were made at Rancho Nuevo, Tamaulipas, Mexico, on a beach monitored since 1966 by the Departamento de Pesca of Mexico (Figure 1). This beach is the only major nesting area of Lepidochelys kempi, an endangered species critically reduced in numbers due to a variety of factors. The beach is north of major oil fields in Mexico, and oil residues from natural seeps or oil spills have been present in variable quantities on the beach for several years and at least since 1966 when the Mexican project was initiated. The beaches in the vicinity of Rancho Nuevo were oiled during the IXTOC oil spill in 1979. Oil was first observed on the beach on 17 July 1979 after all nesting for 1979 was completed but before all hatchlings had completed development and entered the water (Pritchard 1980).

Nearly all known clutches had been collected and incubated in a protected beach enclosure as a part of the normal conservation program. Consequently, the oil arriving in July and August 1979 did not contact developing eggs. Mexican biologists evacuated the hatchlings from the oiled onshore waters to offshore areas presumed to be free from oil. The beach was not monitored after 11 August 1979, but significant residues of petroleum were present in April 1980 when Mexican and U.S. biologists returned in anticipation of the 1980 reproductive season (P. C. H. Pritchard, Florida Audubon Society, Maitland, Florida; pers. comm. in memo dated 28 April 1980).

Data on the nature and abundance of oil substances on the nesting beach were obtained by direct examination of the beach by Fritts on 28 to 29 May and 20 to 26 June 1980 and by McGehee on 3 to 13 August 1980. The distribution and abundance of petroleum residues were quantified along linear transects parallel to the beach. A 50-m string was extended at various levels along the beach, and the number of individual clumps of oil lying immediately under the string was counted. Any evidence of oil ranging from a 1-mm ball to masses of heavy tar 0.5 m in diameter was scored as one clump. Transects were conducted at three positions on the beach (Figure 2) where nests were likely to be deposited. Position A was approximately halfway between the high tide level and the base of the foredune. This area also contained the most beach litter (bottles, driftwood, and other debris). Position B was at the seaward base of the foredune. This position was only slightly higher in elevation than position A but was at the level where major sand deposition for dune formation had occurred. Position C was at the crest of the foredune and consequently varied in elevation with the height of the dune.

Transects were conducted at approximately 1 mi (1.6 km) intervals along the principal 6 mi (9.6 km) of beach from Barra Coma to San Vicente. Most of the transects were 50 m long but occasionally were shortened to 25 m due to disturbance of areas by turtles and humans. Most transects were performed 20 to 26 June 1980, but others were on 12 August 1980 after passage of Hurricane Allen. The means and standard deviations of the numbers of petroleum clumps per 50 m were computed for each position. The form and appearance of the petroleum residues also were noted. Oil was still evident in the water during June, July, and August 1980, and the hurricane had a major impact on

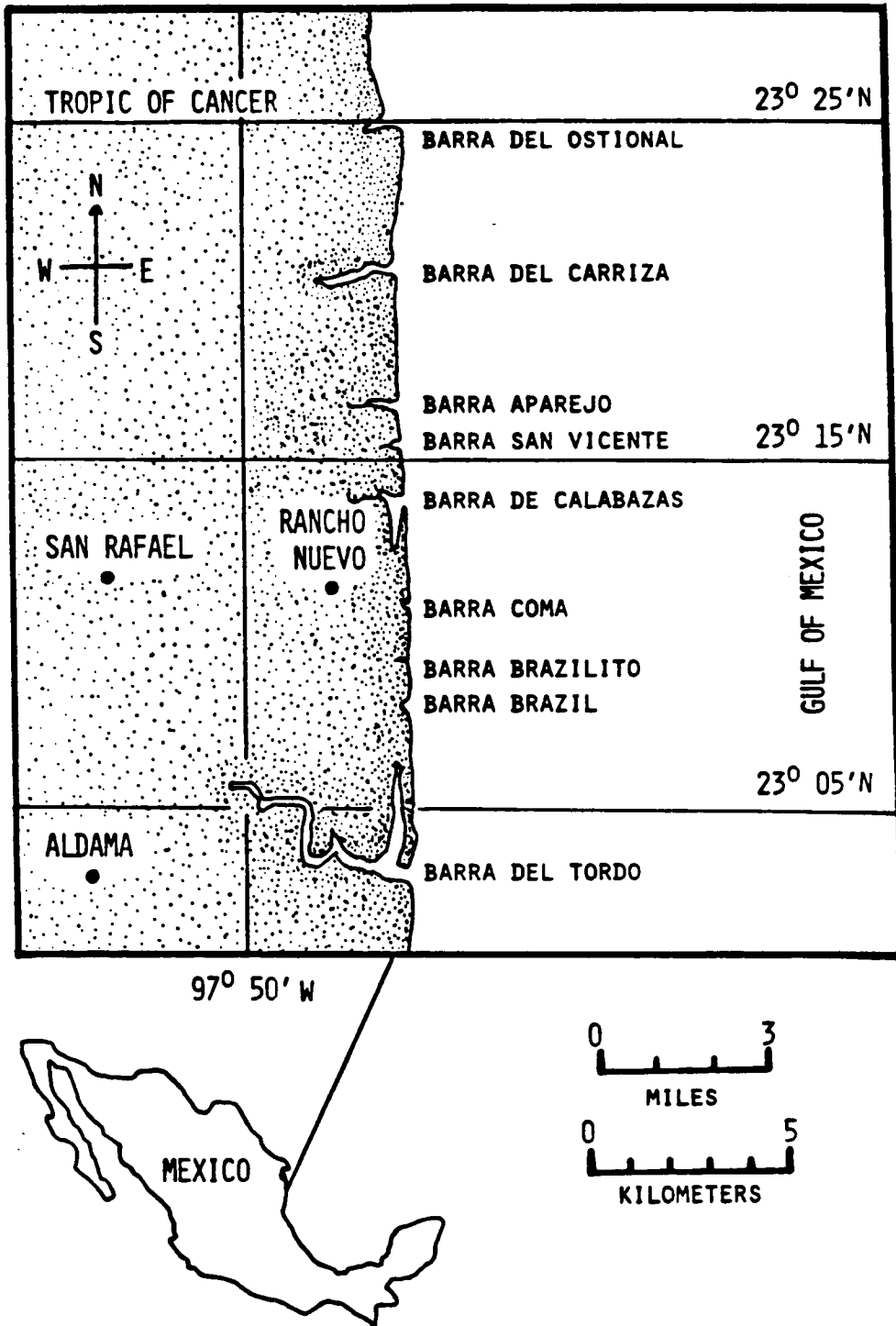


Figure 1. The location of the principal nesting beach of Kemp's ridley turtle in Tamaulipas, Mexico. The limits of the area studied are indicated by place names followed by mileages (in parentheses) from Barra Coma camp . Adapted from Casas-Andreu (1978).

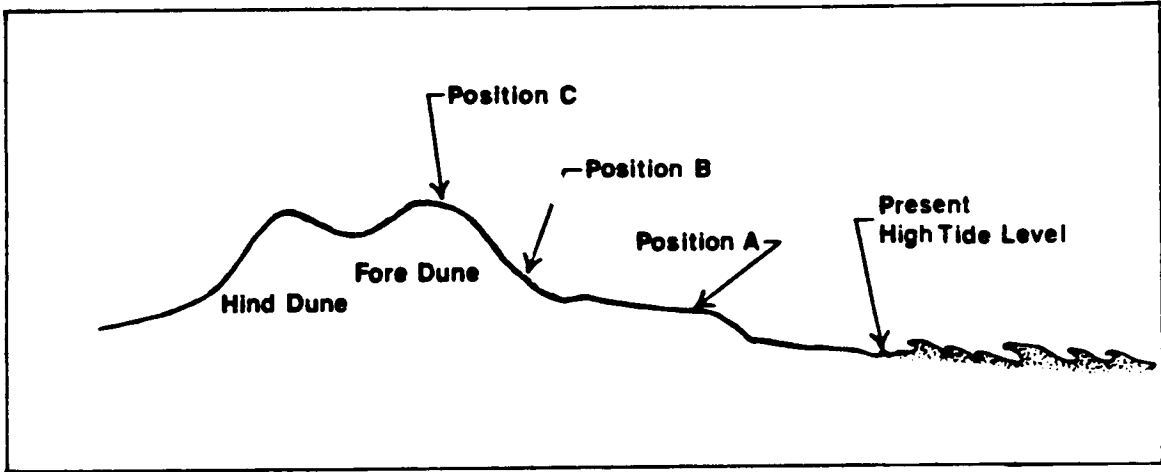


Figure 2. Rancho Nuevo beach showing the relative location of positions A, B, and C where transects were performed to measure the degree of oil contamination.

the beach form as well as the distribution of oil. Comparisons of the distribution of oil during the two periods were performed. Presumably the hurricane which hit the area on 10 August 1980 moved oil residues farther up the beach.

Samples of sand from various locations and positions were taken for analysis of hydrocarbon concentrations. Samples were collected systematically at depths 10 to 20 cm below the surface to determine oiling of subsurface sand. Samples were collected in 0.55 liter (1 pint) glass canning jars with metal lids. They were sealed and transported to New Orleans where they were stored in a freezer chest until analyzed. Hydrocarbon analyses were performed by Analysis Laboratories Inc. of Metairie, Louisiana. Samples were extracted with methyl chloride which subsequently was evaporated to recover the oil and grease. These residues were measured gravimetrically. Oil contamination was computed as milligrams of hydrocarbons per kilogram of sand. No attempt was made to identify the source of the petroleum encountered in the sand, but IXTOC oil was known to have reached this area.

FIELD EXPERIMENTS

The existence of an ongoing conservation project involving Kemp's ridley sea turtles at a site contaminated by IXTOC provided suitable conditions for studying possible effects of petroleum pollution. Since eggs were being laid and incubated in sands contaminated by oil, it was possible to design a series of experiments which compared clutches in contaminated and clean sands without risk of mortality above that expected as a result of normal beach operations. In brief, by placing 50% of a clutch in relatively clean sand it was possible to obtain comparative development data without further sacrificing an already endangered species.

In cooperation with the Departamento de Pesca, nine clutches of eggs were collected from nesting Lepidochelys kemp from 19 to 22 June 1980. Each clutch was divided into two approximately equal subgroups containing 42 to 56 eggs. One subgroup from each clutch was incubated in sand collected from the beach at the approximate location where natural nests were found. The other subgroup was incubated in relatively clean sand collected from the landward base of a large dune near Barra Coma. The beach sand was collected from 3.2 km (2.0 mi) north of Barra Coma and from Jarcias, 3.9 km (2.4 mi) north of Barra Coma. The sand from the former locality was moderately coarse, containing much broken shell. Clumps of oil 6 to 36 cm in diameter were on the surface of this sand (Figure 3). Oil was not visible below the surface. The sand collected at Jarcias had moderately abundant oil on the surface and some oil evident as far as to 10 cm below the surface. The sand from both locations was collected from 10 to 30 cm below the surface to eliminate the large clumps of oil on the beach surface. The sand collected from the landward side of the dune was considered free of significant oil contamination based on its location and the absence of visible oil on the surface. This sand was excavated from 10 to 30 cm below the surface to eliminate any surface contaminants.

An attempt was made to duplicate the incubation conditions as closely as possible in beach sand and dune sand subgroups of the same clutch. An equal amount of fresh water was added to the sand for each incubation container before adding eggs to insure that moisture conditions were comparable.



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Figure 3. A thin clump of oil partially covered by sand and shell fragments in position B on the beach at Rancho Nuevo, Tamaulipas, Mexico.

Three types of containers were used to incubate eggs, but the beach and dune subgroups from any one clutch were always housed in comparable containers. Four clutches (eight paired subgroups) were incubated in large plastic trash cans (approximately 65 cm in height and 50 cm in diameter) in which holes were cut in the bottoms to allow rain to drain and potentially to allow moisture from the water table to diffuse upward into the nest. The cans were buried flush with the sand on the beach at a level where nesting is common. Eggs were placed 20 to 40 cm below the surface of the sand in the cans and covered with about 20 cm of sand. Use of the cans permitted samples to be incubated on the beach while isolated from potentially contaminated sand adjacent to the container.

One clutch (two paired subgroups) was incubated in styrofoam boxes 36 x 27.5 x 22.5 cm. Five centimeters of sand were placed in the boxes, and the eggs were placed on the sand in two layers but not in contact with the walls of the box. Sand was packed against the walls and up to 5 cm deep on top of the eggs. Styrofoam lids were placed on the boxes to reduce moisture evaporation. Holes were punched in the bottoms and tops to allow drainage and air circulation. The boxes were stored in a thatched building constructed for incubating eggs. In previous years, large numbers of eggs were incubated in this manner as protection against predators and tidal inundation. Boxes were stored on wooden shelves 69 to 111 cm above the floor.

Four clutches (eight paired subgroups) were incubated in plastic buckets with a diameter of 30 cm and a height of 35 cm. Five centimeters of sand were placed in the buckets, and three centimeters of sand were packed around the sides before introducing the eggs. Five centimeters of sand were placed on top of the egg mass. The buckets were stored in a cement block building designed and used to incubate eggs in a protected environment. The buckets were not covered; consequently, water was added to them as necessary to keep the sand moist 2 cm below the surface. Buckets were stored on wooden shelves 69 to 105 cm above the floor.

Three types of containers were used to maximize the possibility that hatching would occur. The types of containers and the incubation sites used were ones employed in various turtle conservation projects in the United States and Mexico. The incubation of samples in cans on the beach, instead of an artificial environment, was considered important in duplicating natural incubating temperatures, rainfall, insolation, and air circulation. Due to the risk that such containers might be contaminated by petroleum in adjacent beach sand via air circulation, diffusion through drain holes, or by ground water transport, other containers were maintained away from the beach in protected buildings. Plastic buckets and styrofoam boxes on racks in the buildings were protected from extraneous oil and extreme weather but were exposed to a more artificial environment.

LABORATORY EXPERIMENTS

An additional series of experiments was conducted in laboratory conditions at University of Central Florida with eggs of loggerhead turtles, Caretta caretta. Louisiana crude oil (Vermilion Block 247, Shell Oil Company) was applied in varying quantities and at various times to subgroups of eggs.

Five clutches of loggerhead turtle eggs were collected on the same night they were laid, 20 August 1980, on Merritt Island, Brevard County, Florida. Ninety eggs were selected randomly from each clutch for incubation experiments. The remaining eggs were incubated at Merritt Island National Wildlife Refuge and were not included in the present study. A random sample of 20 eggs from each clutch was weighed and measured for diameter. The 90 eggs from each clutch were divided into six subsamples of 15 eggs each. The six subsamples from each clutch were labeled A through F and received the following treatments:

- A. Control - no oil added.
- B. $\frac{1}{2}$ -time - 30.0 ml of oil added for last $\frac{1}{2}$ of incubation period (poured on top of sand after 42 days).
- C. $\frac{1}{3}$ -time - 30.0 ml of oil added for last $\frac{1}{3}$ of incubation period (poured on top of sand after 28 days).
- D. Light dosage - 7.5 ml of oil (0.5 ml per egg) mixed with sand at initiation of incubation.
- E. Medium dosage - 15.0 ml of oil (1.0 ml per egg) mixed with sand at initiation of incubation.
- F. Heavy dosage/full-time - 30.0 ml of oil (2.0 ml per egg) mixed with sand at initiation of incubation.

The last subsample (F) served as an extreme in two analyses: effects related to quantity of oil and effects related to time of oiling. Methods of applying oil differed between samples focused on time of oiling, whereas the application methods were uniform in samples focused on quantity of oil.

Each subsample was placed in a new 4-liter cardboard bucket with 4 kg of sand collected from the nesting beach where the eggs were found. Eggs of each subsample were packed in three layers of five eggs each with a minimum of 2 to 3 cm of sand on all sides of the egg mass. All eggs were packed in buckets within 12 hours after deposition, and all subsamples were placed in incubators within 48 hours of deposition. The eggs were maintained in thermostatically controlled incubators at 29° to 30° C until hatching was complete. Temperature and moisture were monitored regularly to maintain comparable conditions in the four incubators used. An incubator consisted of a styrofoam chest equipped with an electric heat source controlled by a thermostat wafer. All subsample containers were supported about 4 cm above the incubator floor by a wire rack to allow air circulation.

The quantities of crude oil applied to the sand in which subsamples were incubated were 7.5 to 30.0 ml (1.9 to 7.5 ml of oil per kilogram of sand or 0.5 to 2.0 ml per egg). This oil had a specific gravity of about 0.83. Equivalent amounts were 1,577 to 6,225 mg/kg and 415 to 1,660 mg/egg. Since the oil was applied to the sand, it was assumed that more oil would be required to produce detectable effects than if it were applied directly to the egg. No previous studies had been done on the sensitivity of reptiles to crude oil; therefore, the quantities used were subjectively chosen.

DATA COLLECTED

In both field and laboratory experiments, all subsamples were checked daily to monitor hatching initiation and progress as the end of the incubation period approached. Minimum incubation time (number of days from oviposition to first sign of hatching) was recorded for each subsample. In the laboratory experiments, hatchling incubation time also was recorded for each hatchling as the number of days from oviposition to the initiation of active movement following emergence from the eggshell; thus, pipped young that remained in the shell and those that remained quiescent were not judged as hatched until activity increased.

Three to five days after hatching had occurred in a subsample, all unhatched eggs were opened and examined to evaluate embryological development. Eggs in subsamples with no signs of hatching were opened after all others had hatched. Eggs which contained no signs of embryos were recorded as undeveloped. For developed eggs, the size of the embryo was described.

In all experiments the following data were recorded for each subsample:

1. Hatchlings released - the number of hatchlings surviving to be released to the ocean.
2. Dead hatchlings - the number of turtles that pipped the eggshell but died before release.
3. Unhatched eggs - the total number of eggs that did not hatch.
4. Eggs with embryos - the number of unhatched eggs that contained signs of embryonic development.
5. Embryos near full term - the number of unhatched eggs with embryos greater than 60 mm in total length.
6. Embryos 21 to 60 mm in length - the number of unhatched eggs with embryos 21 to 60 mm in total length.
7. Embryos 1 to 20 mm in length - the number of unhatched eggs with embryos 1 to 20 mm in total length. In field experiments, categories 6 and 7 were grouped together and described as embryos less than or equal to half developed.

Hatchlings were weighed and measured about 24 hours after hatching. The measurements taken were as follows: carapace length, carapace width, plastron length, and body depth at the second vertebral. Weight was recorded in grams, and all linear measurements were recorded in millimeters with vernier calipers. For each measured hatchling the numbers of marginal scutes, costal scutes, and vertebral scutes were recorded. In the laboratory experiments, all hatchlings released were measured. In the field experiments, no more than 20 hatchlings from each subsample were measured. After measurements were taken, hatchlings were released to the ocean near the site of egg collection.

STATISTICAL ANALYSES

Means, standard deviations, and percentages of subsamples were computed on appropriate variables. For comparison of experimental and control treatment groups, analyses of variance were used to define significant differences. The F values and probabilities provided in the summary tables were from univariate analyses of variance comparing experimental and control treatment groups. Similar comparisons evaluating differences related to clutch and incubation containers were performed. These results are detailed in the text. Results where $p > 0.05$ were not considered to be significant. Due to the large number of variables being examined and the number of subsamples being compared, multivariate analyses of variance (MANOVA) were used as a precaution against Type I error. Rao's approximate F values were calculated in each MANOVA to evaluate the overall significance of the test. Newman-Keuls and Duncan's range tests were used for subsequent comparisons of more than two groups. Dunnett's tests were used for pair-wise comparisons of the control with each treatment group.

RESULTS

FIELD OBSERVATIONS

Notes

As an aid to the evaluation of crude oil contamination on the Mexican study site, a visual inspection of the principal nesting area was made in June 1980. The extent of visible petroleum was variable. In some sections, the clumps of oil were confined to a narrow zone above the high tide mark. In others, the petroleum was scattered throughout the forebeach and onto the face of the dune. Not all sections of the beach were equally suitable for nesting by turtles. The presence of rocks and dense beach litter interfered with nesting in some areas of the beach. Petroleum frequently adhered to such objects more than to the loose sand substrate. The petroleum was in several forms, which differed in size, texture, form, and degree of hardness. Thin disks and pellets were frequently found but were not conspicuous due to sand covering them.

The following observations were summarized from the field notes of T. H. Fritts.

Mile 0.2 had large disks of oil and a relatively rocky surface. The beach profile included a ledge 20 to 30 cm high, and most oil was between this ledge and the base of the foredune (Figure 4).

Mile 0.4 had some rocks on the surface and significant particles of oil in positions A and B. Some of the oil was in a dried tar-like state with embedded shells. Other disks of petroleum were soft and adherent.

Mile 0.6 had no ledge but had oil scattered in small pellets up onto the face of the foredune as well as down toward the lower beach below position A.

Mile 0.8 had a rocky surface above a low erosion ledge. Small pellets and chunks of oil were present in positions A and B, but these did not reach the top of the dune. Walking in a normal stride, it was impossible to avoid stepping on oiled sand.

Mile 1.0 had a significant erosion ledge below the dune. Above the ledge were broken shells and some rocks. The oil in this area was patchy, and overall it was sparse except immediately above the ledge on exposed rocks.

Mile 1.2 had heavy concentrations of oil in thin scabs of varying sizes and in dried chunks with embedded shells. Most oil contamination was in positions A and B above a slight ledge. A Kemp's ridley turtle had nested in position A in an area with soft oil scattered on the surface.

Mile 1.4 was similar to Mile 1.2 except the oil was more patchy in distribution.

Mile 1.6 had a narrow beach with rocks near the base of the dune. Oil disks were 2 to 20 cm in diameter and sparsely scattered.

Mile 1.8 had heavy oil contamination in position B and had signs of a turtle nesting in the area. The beach was approximately 20 m wide from the base of the foredune to the high tide line.

Mile 2.0 had sparse oil chunks up to 36 cm in diameter extending from the upper beach up onto the face of the foredune (positions B and C; Figure 5).

Mile 2.2 was moderately clean except for scattered large scabs of oil which remained soft and pliable.

Mile 2.4 had scattered oil in small to medium-sized chunks. Most oil was in positions A and B.

Mile 2.6 had a relatively clean lower beach with most oil in position B. Oil contamination was sparse to moderate.

Mile 2.8 had only scattered oil in position B and overall was relatively uncontaminated.

Mile 3.0 had oil on the face of the dune and in some places on top of the dune. Large chunks and scabs were widely scattered in position B.

Mile 5.0 was lightly oiled in relation to other areas. Many small chunks of oil were visible. Oil adhered to some rocks on the surface. The dune here was 1 to 1.5 m high and had vegetation on top. The oil did not reach the top of the dune except for wind-blown debris which had accumulated lower on the beach.

Mile 6.0 (near San Vicente) had a significant amount of oil concentrated at the base of the foredune (position B). The oil was relatively soft to the touch and adhered to anything it contacted. Most of the oil appeared to be on the surface of the sand.

Mile 7.0 (approximately 1 mile or 1.7 km north of San Vicente) was relatively free of oil. Some masses of oil were present which were 10 cm in diameter. Most of the flotsam and beach debris had some oil adhering to the surface. The top of the foredune had no apparent oil contamination.

Transects

The density of oil clumps on the beach surface varied along the length and breadth of the beach. In June 1980, most oil on the beach surface was concentrated in positions A and B. The mean number of oil clumps in 50-m transects was 48.0 in position A and 56.4 in position B. Oil was sparse in position C with a mean of 2.4 clumps in five transects (Table 1).



Figure 4. The middle beach (position A) showing disks of petroleum and dried plant material littering the beach surface at Rancho Nuevo, Tamaulipas, Mexico.



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Figure 5. The beach at Rancho Nuevo, Tamaulipas, Mexico showing chunks of petroleum and dried plant material. Chunks of oil similar to the one indicated in the photograph were distributed from the forebeach to the base of the dune shown in the background.

Table 1. Mean numbers of oil clumps along linear transects (50 m) of the beach at various positions before and after Hurricane Allen in 1980. See Figure 2 for relative location of positions A, B, and C.

Date	Position	Range	Mean	Standard deviation	No. of transects
22 to 26 June	A	15 to 117	48.0	43.0	8
	B	5 to 121	56.4	37.1	8
	C	0 to 4	2.4	3.3	5
12 August	A	36 to 94	66.2	32.1	4
	B	11 to 46	24.8	15.1	4
	C	4 to 13	7.2	4.0	4

The distribution of oil was altered by Hurricane Allen with subsequent means of 66.2, 24.8, and 7.2 for positions A, B, and C, respectively (Table 1). Apparently oil was deposited on top of dunes by the high waves of the storm, and other oil stirred up from submerged areas was deposited by subsequent tidal action after the storm.

Sand Analyses

The results of hydrocarbon analyses of beach sand suggested that oil was not uniformly distributed (Table 2). Four of the eleven samples had 4 to 6 mg of hydrocarbons/kg of sand, which is probably an ambient baseline level. In six samples from position A, hydrocarbons were 4 to 1282 mg/kg (\bar{x} = 306). In four samples from position B, hydrocarbons were 4 to 3192 mg/kg (\bar{x} = 847). A single sample from position C had 6 mg/kg.

FIELD EXPERIMENTS

No effect due to incubation in oiled sands was evident. The mean hatching success (defined by hatchlings released) for all subgroups with clean dune sand was 64.8%, whereas that for oiled beach sand was 67.7%. The observed difference was not significant (Table 3). Refer to Tables 3 and 4 for means and standard deviations; ranges are presented in the text.

The number of hatchlings released from each subgroup varied from 1.8 to 95.6% of the eggs incubated. The numbers from paired subgroups derived from single clutches were more closely matched, with deviations of 2.0 to 9.3%. This suggests that more variation is attributable to differences in clutches than to experimental treatment. The dune and beach subgroups of clutch 3 had 1.8 and 3.8% hatchlings, respectively, suggesting that this clutch was largely nonviable. Clutch 3 was the only clutch with subgroups producing fewer than 50% hatchlings released.

Hatching success was highest in buckets (72.7 to 95.6%; \bar{x} = 85.1%). Hatching success in cans was more variable (1.8 to 86.0%; \bar{x} = 50.1%) largely due to the low success of clutch 3 subgroups. The hatch from styrofoam boxes ranged from 62.5 to 67.3%.

The minimum incubation time (i.e., number of days until the first sign of hatch) for each subgroup varied more between types of incubation containers than between oiled and control groups (Table 3). Within similar containers containing both oiled and clean subgroups the maximal deviation in incubation time was 1 day. The mean incubation time for dune sand was 49.8 days, whereas that for beach sand was 50.0 days. These means did not differ significantly (Table 3). The minimal incubation time for subgroups in cans was 47 to 48 days; buckets, 51 to 52; and boxes, 52 to 53.

The number of dead hatchlings varied from 2.4 to 8.2% in cans, 1.9 to 3.8% in buckets, and was 0.0% in boxes. The numbers of dead hatchlings in dune and beach sand were not significantly different (Table 3).

The number of unhatched eggs varied from 9.3 to 98.2% in cans, 4.4 to 27.3% in buckets, and 32.7 to 37.5% in boxes. No significant difference was evident between dune and beach groups (Table 3).

Table 2. The concentration of hydrocarbons in sand samples collected on the nesting beach of Kemp's ridley turtle in Rancho Nuevo, Tamaulipas, Mexico.

Position	Sample no.	Mile	Hydrocarbon concentration mg/kg
A	1	2.5	500
A	4	7.0	23
A	5	4.6	4
A	7	2.5	23
A	9	4.0	1,282
A	11	2.4	4
B	2	7.0	120
B	3	6.0	70
B	8	4.6	3,192
B	10	2.4	4
C	6	7.0	6

Table 3. Subsample data from the nine clutches used in the field experiment. F values and probabilities are derived from analyses of variance of oil treatment group means.

Container and treatment	\bar{x} sub-sample size	\bar{x} minimum incubation time (days)	\bar{x} hatchlings released	\bar{x} dead hatchlings	\bar{x} unhatched eggs	\bar{x} eggs with embryos	\bar{x} embryos near full term	\bar{x} embryos half developed
Cans (4 clutches)								
Dune sand (s. d.)	50.2 (6.2)	47.5 (0.6)	23.8 (47.3%) (15.4)	1.2 (2.5%) (1.9)	25.2 (50.2%) (21.5)	2.0 (4.0%) (2.7)	1.2(2.5%) (1.3)	0.8 (1.5%) (1.5)
Beach sand (s. d.)	50.0 (5.1)	47.8 (0.5)	24.2 (48.5%) (15.3)	2.0 (4.0%) (1.4)	23.8 (47.5%) (19.2)	3.8 (7.5%) (4.3)	1.8(3.5%) (2.4)	2.0 (4.0%) (2.8)
Boxes (1 clutch)								
Dune sand	55.0	52.0	37.0 (67.3%)	0.0	18.0 (32.7%)	2.0 (3.6%)	2.0 (3.6%)	0.0
Beach sand	56.0	53.0	35.0 (62.5%)	0.0	21.0 (37.5%)	0.0	0.0	0.0
Buckets (4 clutches)								
Dune sand (s. d.)	50.5 (4.7)	51.5 (0.6)	41.2 (81.7%) (3.0)	0.5 (1.0%) (1.0)	8.8 (17.3%) (4.3)	2.5 (5.0%) (1.3)	1.2 (2.4%) (1.3)	1.3 (2.6%) (1.5)
Beach sand (s. d.)	51.2 (4.5)	51.5 (0.6)	45.0 (87.8%) (2.9)	0.5 (1.0%) (0.6)	5.7 (11.2%) (5.0)	0.8 (1.6%) (1.0)	0.5 (1.0%) (1.0)	0.3 (0.6%) (0.5)

Table 3. Concluded.

Container and treatment	\bar{x} sub-sample size	\bar{x} minimum incubation time (days)	\bar{x} hatchlings released	\bar{x} dead hatchlings	\bar{x} unhatched eggs	\bar{x} eggs with embryos	\bar{x} embryos near full term	\bar{x} embryos half developed
Grand means (9 clutches)								
19 Dune sand (s. d.)	50.9 (5.0)	49.8 (2.2)	33.0 (64.8%) (13.1)	0.8 (1.6%) (1.4)	17.1 (33.6%) (15.7)	2.2 (4.3%) (1.9)	1.3 (2.5%) (1.1)	0.9 (1.8%) (1.4)
Beach sand (s. d.)	51.2 (4.6)	50.0 (2.2)	34.7 (67.7%) (14.1)	1.1 (2.1%) (1.3)	15.4 (30.2%) (15.3)	2.0 (4.0%) (3.2)	1.0 (2.0%) (1.7)	1.0 (2.0%) (2.0)
$F_{1,16}$		0.04	0.07	0.28	0.05	0.03	0.24	0.02
p		0.84	0.80	0.60	0.82	0.86	0.63	0.89

The numbers of dead embryos near full term in dune and beach treatment groups were not significantly different (Table 3). The number of dead embryos near full term in cans varied from 1.8 to 9.0%, in buckets from 1.8 to 5.8%, and in boxes from 0.0 to 3.6%.

The number of eggs with dead embryos less than half developed varied from 0.0 to 11.5% in cans, 0.0 to 5.5% in buckets, and was 0.0% in boxes. Dune and beach treatment groups did not differ significantly (Table 3).

A MANOVA of the hatching characteristics presented in Table 3 indicated no significant differences between dune and beach treatment groups for all dependent measures (Rao's approximate $F_{8,9} = 1334.72$; $p > 0.05$).

In order to evaluate the interactive effects of clutches and treatment groups on size and scutellation of hatchlings, a two-way MANOVA was performed on subgroup data. Clutch 4 subgroups were eliminated from this analysis because hatchlings from the dune subgroup of this clutch were not available for study. Treatment effects were significant (Rao's approximate $F_{10,239} = 1.88$; $p < 0.05$) but only marginally so; therefore, caution is advised in this interpretation. Among the size and scutellation variables analyzed, only the number of left marginal scutes was significant in a univariate test ($F_{1,248} = 7.95$; $p < 0.05$; Table 4). The beach sand group had fewer marginals.

In contrast to treatment groups, differences related to clutches were much greater and more important in level of significance (Rao's approximate $F_{70,1400} = 6.88$; $p < 0.001$). This is probably a normal genetic influence. All size variables examined were significantly different ($p < 0.05$). Among the scutellation variables, the number of left costals and the number of vertebrae were significantly different at $p < 0.05$.

The effects of dune (clean sand) and beach (oiled sand) treatments do not appear to be significantly different. The possible effect on the number of left marginals is overshadowed by the lack of a similar effect on the right side of the body and the more conspicuous differences related to clutch (genetic and environmental) effects.

A two-way MANOVA was performed to identify possible effects of incubation containers in relation to oil treatment. Container effects were judged to be highly significant (Rao's approximate $F_{20,524} = 27.29$; $p < 0.0001$). All univariate size and scutellation variables, except the right marginals, were affected significantly ($p < 0.01$). Oil treatment effects were less conspicuous (Rao's approximate $F_{10,262} = 3.27$; $p < 0.001$). Only three univariate size and scutellation variables (carapace length, left marginals, and right costals) were significantly affected at $p < 0.05$, and none were affected significantly at $p < 0.01$. The interaction analysis suggested a significant interaction between treatment and container groups, with the two variables measuring costals significant in univariate analyses ($p < 0.05$). This suggests that the differences seen in costals in treatment and container groups are not interpretable. Overall container effects were more pronounced than any treatment effect. Treatment effects in this analysis potentially represent Type I error (i.e., different by chance alone).

Table 4. Hatchling data from the nine clutches used in the field experiment. F values and probabilities are derived from analyses of variance of oil treatment group means.

Container and treatment	No. of hatchlings measured	\bar{x} weight (g)	\bar{x} carapace length (mm)	\bar{x} carapace width (mm)	\bar{x} plastron length (mm)	\bar{x} depth (mm)	\bar{x} left marginal scutes	\bar{x} left costal scutes	\bar{x} vertebral scutes	\bar{x} right costal scutes	\bar{x} right marginal scutes
Cans (4 clutches)											
Dune sand (s. d.)	41	15.9 (1.2)	43.4 (1.0)	35.3 (0.7)	33.4 (1.1)	17.7 (0.8)	13.5 (0.3)	5.3 (0.2)	5.4 (0.3)	5.3 (0.2)	13.3 (0.2)
Beach sand (s. d.)	36	15.8 (1.1)	43.7 (1.3)	35.7 (0.8)	33.7 (1.0)	17.6 (0.7)	13.1 (0.1)	5.1 (0.6)	5.7 (0.5)	5.0 (0.0)	13.4 (0.3)
Boxes (1 clutch)											
Dune sand (s. d.)	20	14.4 (1.1)	41.4 (1.3)	35.0 (0.9)	31.2 (0.7)	16.3 (0.8)	13.0 (0.0)	5.0 (0.0)	5.0 (0.0)	5.0 (0.0)	13.2 (0.3)
Beach sand (s. d.)	20	14.7 (1.1)	41.1 (0.9)	35.2 (0.7)	31.2 (0.5)	16.1 (0.6)	13.0 (0.0)	5.0 (0.0)	5.1 (0.1)	5.0 (0.0)	13.2 (0.3)
Buckets (4 clutches)											
Dune sand (s. d.)	80	17.2 (1.6)	44.4 (1.1)	37.0 (1.3)	33.5 (1.0)	17.9 (0.8)	13.3 (0.2)	5.0 (0.0)	5.2 (0.2)	5.0 (0.0)	13.2 (0.2)
Beach sand (s. d.)	80	17.4 (1.9)	44.8 (1.2)	37.0 (0.9)	33.4 (0.5)	18.0 (0.8)	13.2 (0.1)	5.0 (0.0)	5.3 (0.1)	5.0 (0.0)	13.3 (0.2)
Grand means (9 clutches)											
Dune sand (s. d.)	141	16.4 (1.8)	43.7 (1.7)	36.2 (1.7)	33.2 (1.4)	17.6 (0.9)	13.3 (0.5)	5.1 (0.3)	5.2 (0.5)	5.1 (0.3)	13.2 (0.5)
Beach sand (s. d.)	136	16.6 (2.0)	44.1 (1.7)	36.4 (1.4)	33.2 (1.3)	17.6 (1.1)	13.2 (0.4)	5.1 (0.3)	5.3 (0.6)	5.0 (0.3)	13.4 (0.5)
$F_{1,248}$		0.51	0.83	0.00	1.00	0.00	7.95	1.66	1.72	1.39	1.06
p		0.47	0.36	1.00	0.32	0.95	0.01	0.20	0.19	0.24	0.30

LABORATORY EXPERIMENTS

Quantity of Oil

The minimum incubation time for subsamples was not affected by oil treatment (Table 5). However, a significant difference was evident among subsample means of hatchling incubation times ($p < .01$; Table 6). A Newman-Keuls range test of hatchling incubation time (Table 7) demonstrated that lightly oiled subsamples had shorter incubation times than controls (52.3 vs 53.3 days), and subsamples with a medium or heavy dosage of contamination required significantly longer time to incubate (53.9 days).

Other parameters of hatching were not affected significantly by oil treatments (Table 5). The number of hatchlings released from oiled subsamples did not differ from controls. Similarly, all treatments with oil at initiation of development did not significantly affect the numbers of dead hatchlings, unhatched eggs, eggs with embryos, or the size of embryos (Table 5).

Hatchling morphology was affected by the amount of oil added (Tables 6 and 7). Hatchling weight was significantly lower in the light dosage subsamples than in the heavy dosage treatment ($p < 0.01$). However, neither of these treatment groups were significantly different from controls or medium dosage subsamples. On the basis of hatchling length and width measurements, light dosage subsamples included significantly smaller hatchlings than other subsamples ($p < 0.01$). The heavy dosage subsamples also contained small hatchlings in relation to the control and medium dosage treatments, but this difference was significant only in carapace length. Turtles from controls and medium dosage subsamples did not exhibit significant differences in hatchling measurements. The univariate F test of hatchling depth measurements indicated a difference between treatment groups ($p < 0.01$), but other analyses indicated nothing significant about this variable ($p > 0.05$). Depth was found to involve more error than other measurements and was not analyzed further.

Differences in scutellation related to the amount of oil were evident (Tables 6 and 7). Eggs exposed to oil produced hatchlings with significantly more vertebral scutes than turtles from controls ($p < 0.01$). Vertebral counts did not appear to vary with the amount of oil to which the eggs were exposed. Medium and heavy dosage subsamples yielded hatchlings with significantly fewer left and right marginal scutes than young from control and light dosage subsamples ($p < 0.01$). The left marginal counts for hatchlings from the light dosage treatment were significantly lower than the controls, but the right marginal counts were not. Left costal scutes did not differ between treatments, but right costals did ($p < 0.05$). Hatchlings from control subsamples averaged more right costals than the other treatment groups; hatchlings from the heavy dosage treatment averaged the least.

Two-way MANOVA's were performed on the subsample data to evaluate the direct and interactive effects of clutches and oil treatments on hatchling morphology. Treatment effects were significant (Rao's approximate $F_{33,640} = 9.90$; $p < 0.001$) as previously described. Clutch effects also were significant (Rao's approximate $F_{44,832} = 24.36$; $p < 0.001$); all variables differed according to clutch, but this may be attributed to natural genetic variation between parent turtles and to environmental effects before oviposition. Clutch-treatment interaction was significant (Rao's approximate $F_{132,1793} = 1.83$; $p < 0.001$); hatchling incubation time, weight, length, and

width measurements were affected ($p < 0.05$ for all variables). Clutches responded somewhat differently to treatments, but the relationship is unclear. Caution is advised in interpreting treatment influences on the affected variables.

Time of Oiling

The time at which the marine turtle eggs were exposed to oil and possibly the method of applying oil had a significant effect on survival and on hatchling morphology (Tables 8 and 9). Although the time of first hatching (minimum incubation time) in any subsample did not vary in response to oil treatment (Table 8), hatchling incubation time was affected by oil contamination ($p < 0.01$; Table 9). Duncan's range test indicated that $\frac{1}{2}$ -time oil subsamples required a longer time to incubate than controls ($p < 0.05$).

The numbers of hatchlings surviving to be released (Tables 8 and 10) were significantly higher in control and full-time oil subsamples (88.0 and 82.7%, respectively) than in $\frac{1}{2}$ -time and $\frac{1}{4}$ -time oil subsamples (34.7 and 18.7%, respectively; $p < 0.01$). The difference in survival was not significant in the number of dead hatchlings but was in the number of unhatched eggs ($p < 0.01$). Control and full-time oil subsamples had 9.3 and 10.7% unhatched eggs, respectively whereas $\frac{1}{2}$ -time and $\frac{1}{4}$ -time treatments had 61.3 and 74.7% unhatched eggs, respectively.

A differential mortality related to oil treatment was further documented by the numbers of dead embryos in unhatched eggs (Tables 8 and 10). Unhatched eggs with embryos were fewest in controls (2.7%) and in full-time subsamples (8.0%). Unhatched eggs with embryos occurred in significantly greater numbers in $\frac{1}{2}$ -time and $\frac{1}{4}$ -time oil treatments (60.0 and 69.3%, respectively; $p < 0.01$). In all of these variables (hatchlings released, unhatched eggs, and eggs with embryos) control and full-time oil treatments were not different from each other, and $\frac{1}{2}$ -time and $\frac{1}{4}$ -time subsamples were not different from each other (Table 10).

Eggs with dead embryos near full term occurred in largest numbers in the $\frac{1}{2}$ -time subsamples (58.7%), whereas near full term embryos in other treatment groups (1.3 to 16.0%) were significantly fewer ($p < 0.01$; Tables 8 and 10).

The greatest number of eggs with dead embryos 21 to 60 mm in total length was produced by the $\frac{1}{2}$ -time oil subsamples (48.0%). Other treatment groups were not different from each other in the numbers of eggs with 21 to 60 mm embryos (0 to 1.3%) but were significantly different from the $\frac{1}{2}$ -time oil treatment ($p < 0.01$; Tables 8 and 10).

Hatchling morphological characteristics were affected by oil treatments (Tables 9 and 10). The $\frac{1}{2}$ -time oil subsamples produced hatchlings that were significantly smaller in weight and carapace length than those from control and full-time oil treatments ($p < 0.01$). Control, full-time, and $\frac{1}{4}$ -time subsamples did not differ significantly, nor did the $\frac{1}{2}$ -time subsamples differ from the $\frac{1}{4}$ -time treatment in weight and carapace length. The $\frac{1}{4}$ -time treatment produced hatchlings with the smallest mean carapace width. However, this difference was significant only when compared with hatchlings from the controls ($p < .01$) and may be due to chance. The $\frac{1}{2}$ -time treatment yielded turtles with the smallest average plastron length. This difference was significant only when compared with controls ($p < 0.01$) and may be due to chance. Hatchling depth did not vary between oil treatment groups.

The scutellation of the carapace appeared to be affected by the experimental treatments (Tables 9 and 10). On both right and left sides, marginals were fewer in hatchlings from full-time oil subsamples than in control and $\frac{1}{2}$ -time subsamples. Controls were not different from $\frac{1}{2}$ -time treatments, and full-time subsamples were not distinct from $\frac{1}{2}$ -time. Costal scute counts were not different between treatments. Analysis of variance of vertebral scutes did not differentiate treatment groups, but multiple range analysis indicated that hatchlings from full-time oil samples had more vertebral scutes than those from other treatments.

To determine direct and interactive effects of treatments and clutches on hatchlings, two-way MANOVA's were performed on the morphology data. Treatment effects differed significantly (Rao's approximate $F_{33,334} = 3.90$; $p < 0.001$) as discussed previously. Clutch effects also were different (Rao's approximate $F_{33,334} = 12.54$; $p < 0.001$); all variables except vertebral and costal scutes were affected ($p < 0.01$). Clutch effects may be due to normal genetic variation and environmental effects prior to oviposition. Clutch-treatment interaction was significant, but only marginally so (Rao's approximate $F_{99,808} = 1.28$; $p < 0.05$), and caution is advised in interpreting this relationship. Only weight, width, and left marginal scutes were affected ($p < 0.05$). The marginal significance of this interaction did not appear sufficient to influence our conclusions.

Table 5. Subsample data from the five clutches used in the quantity of oil experiment in the laboratory. F values and probabilities are derived from analyses of variance of oil treatment group means.

Treatment	\bar{x} minimum incubation time (days)	\bar{x} hatchlings released	\bar{x} dead hatchlings	\bar{x} unhatched eggs	\bar{x} eggs with embryos	\bar{x} near full term	\bar{x} embryos 21 to 60 mm in length	\bar{x} embryos 1 to 20 mm in length
Control (s. d.)	51.8 (0.4)	13.2 (88.0%) (1.1)	0.4 (2.7%) (0.5)	1.4 (9.3%) (0.9)	0.4 (2.7%) (0.9)	0.4 (2.7%) (0.9)	0.00	0.00
7.5 ml oil (s. d.)	51.0 (1.2)	11.6 (77.3%) (2.2)	0.6 (4.0%) (0.9)	2.8 (18.7%) (1.3)	1.8 (12.0%) (1.9)	1.6 (10.7%) (1.5)	0.00	0.20(1.3%) (0.4)
15.0 ml oil (s. d.)	52.0 (0.7)	12.2 (81.3%) (1.5)	0.8 (5.3%) (1.3)	2.0 (13.3%) (0.7)	1.2 (8.0%) (0.4)	0.8 (5.3%) (0.8)	0.00	0.40 (2.7%) (0.5)
30.0 ml oil (s. d.)	51.8 (0.8)	12.4 (82.7%) (1.7)	1.0 (6.7%) (1.2)	1.6 (10.7%) (1.8)	1.2 (8.0%) (1.3)	0.2 (1.3%) (0.4)	0.20 (1.3%) (0.4)	0.80 (5.3%) (0.8)
F_{3,16}	1.36	0.79	0.31	1.22	1.03	1.92	1.00	1.94
p	0.29	0.52	0.82	0.34	0.41	0.17	0.42	0.16

Table 6. Hatchling data from the five clutches used in the quantity of oil experiment in the laboratory. F values and probabilities are derived from analyses of variance of oil treatment group means.

Treatment	No. of hatchlings released	Hatchling incubation time (days)	\bar{x} weight (g)	\bar{x} carapace length (mm)	\bar{x} carapace width (mm)	\bar{x} plastron length (mm)	\bar{x} depth (mm)	\bar{x} left marginal scutes	\bar{x} left costal scutes	\bar{x} vertebral scutes	\bar{x} right costal scutes	\bar{x} right marginal scutes
Control (s. d.)	66	53.3 (0.7)	17.7 (1.4)	43.1 (0.7)	33.2 (0.6)	33.6 (1.2)	18.2 (0.7)	12.7 (0.3)	5.0 (0.0)	5.1 (0.1)	5.1 (0.1)	12.7 (0.3)
7.5 ml oil (s. d.)	58	52.3 (0.8)	17.4 (1.3)	41.3 (0.7)	32.2 (0.9)	32.7 (1.0)	18.5 (0.6)	12.4 (0.3)	5.0 (0.1)	5.3 (0.4)	5.0 (0.1)	12.6 (0.3)
15.0 ml oil (s. d.)	61	53.9 (1.4)	18.0 (1.7)	43.2 (1.3)	33.3 (0.7)	33.5 (1.3)	18.2 (0.8)	12.2 (0.3)	4.9 (0.2)	5.4 (0.3)	5.0 (0.1)	12.4 (0.4)
30.0 ml oil (s. d.)	62	53.9 (1.5)	18.2 (1.7)	42.7 (1.6)	32.9 (0.9)	33.3 (1.5)	18.4 (0.6)	12.2 (0.3)	4.9 (0.2)	5.4 (0.4)	4.9 (0.1)	12.3 (0.3)
$F_{3,227}$		35.17	7.92	46.16	13.43	10.97	4.22	19.08	1.81	5.27	2.91	10.17
p		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.15	0.01	0.04	0.01

Table 7. Results of Newman-Keuls range tests for selected variables from the quantity of oil experiment in the laboratory. Means with the same letter are not significantly different ($p > .05$).

Variable	Grouping	Treatment (ml oil)	Mean
Hatchling incubation time (days)	A	7.5	52.3
	B	Control	53.3
	C	15.0	53.9
	C	30.0	53.9
Hatchling weight (g)	A	7.5	17.4
	A B	Control	17.7
	A B	15.0	18.0
	B	30.0	18.2
Hatchling carapace length (mm)	A	7.5	41.3
	B	30.0	42.6
	C	Control	43.1
	C	15.0	43.2
Hatchling carapace width (mm)	A	7.5	32.2
	B	30.0	32.9
	B	Control	33.2
	B	15.0	33.3

Table 7. Concluded.

Variable	Grouping	Treatment (ml oil)	Mean
Hatchling plastron length (mm)	A	7.5	32.7
	B	30.0	33.3
	B	15.0	33.5
	B	Control	33.8
Hatchling left marginal scutes	A	15.0	12.2
	A	30.0	12.2
	B	7.5	12.4
	C	Control	12.7
Hatchling vertebral scutes	A	Control	5.1
	B	7.5	5.3
	B	15.0	5.4
	B	30.0	5.4
Hatchling right marginal scutes	A	30.0	12.3
	A	15.0	12.4
	B	7.5	12.6
	B	Control	12.7

Table 8. Subsample data from the five clutches used in the time of oil experiment in the laboratory. F values and probabilities are derived from analyses of variance of oil treatment group means.

Treatment	\bar{x} minimum incubation time (days)	\bar{x} hatchlings released	\bar{x} dead hatchlings	\bar{x} unhatched eggs	\bar{x} eggs with embryos	\bar{x} embryos near full term	\bar{x} embryos 21 to 60 mm in length	\bar{x} embryos 1 to 20 mm in length
Control (s. d.)	51.8 (0.4)	13.2 (88.0%) (1.1)	0.4 (2.7%) (0.5)	1.4 (9.3%) (0.9)	0.4 (2.7%) (0.9)	0.4 (2.7%) (0.9)	0.0	0.0
½-time oil (s. d.)	52.8 (0.4)	5.2 (34.7%) (2.8)	0.6 (4.0%) (0.5)	9.2 (61.3%) (2.9)	9.0 (60.0%) (3.4)	8.8 (58.7%) (3.3)	0.0	0.2 (1.3%) (0.4)
½-time oil (s. d.)	53.0 (1.4)	2.8 (18.7%) (3.1)	1.0 (6.7%) (1.4)	11.2 (74.7%) (3.2)	10.4 (69.3%) (3.3)	2.4 (16.0%) (2.3)	7.2 (48.0%) (4.7)	0.8 (5.3%) (1.8)
Full-time oil (s. d.)	51.8 (0.8)	12.4 (82.7%) (1.7)	1.0 (6.7%) (1.2)	1.6 (10.7%) (1.8)	1.2 (8.0%) (1.3)	0.2 (1.3%) (0.4)	0.2 (1.3%) (0.4)	0.8 (5.3%) (0.8)
$F_{3,15}^a$	2.74	22.34	0.66	21.53	20.37	19.19	15.66	1.01
p	0.08	0.01	0.59	0.01	0.01	0.01	0.01	0.42

^a One subsample in the ½-time oil category did not hatch and so was not included in the analyses.

Table 9. Hatchling data from the five clutches used in the time of oil experiment in the laboratory. F values and probabilities are derived from analyses of variance of oil treatment group means.

Treatment	No. of hatchlings released	\bar{x} hatchling incubation time (days)	\bar{x} weight (g)	\bar{x} carapace length (mm)	\bar{x} carapace width (mm)	\bar{x} plastron length (mm)	\bar{x} depth (mm)	\bar{x} left marginal scutes	\bar{x} costal scutes	\bar{x} vertebral scutes	\bar{x} right costal scutes	\bar{x} right marginal scutes
Control (s. d.)	66	53.3 (0.7)	17.7 (1.4)	43.1 (0.7)	33.2 (0.6)	33.8 (1.2)	18.2 (0.7)	12.7 (0.3)	5.0 (0.0)	5.1 (0.1)	5.1 (0.1)	12.7 (0.3)
½-time oil (s. d.)	26	54.2 (1.2)	17.3 (2.4)	42.2 (1.9)	32.6 (1.4)	33.1 (1.9)	18.5 (1.0)	12.8 (0.4)	5.0 (0.0)	5.0 (0.0)	5.0 (0.0)	12.7 (0.5)
¼-time oil (s. d.)	14	53.4 (1.0)	16.4 (1.1)	41.6 (1.5)	32.9 (1.1)	32.6 (1.3)	18.5 (0.8)	12.4 (0.5)	5.0 (0.0)	5.0 (0.0)	5.0 (0.0)	12.4 (0.5)
Full-time oil (s. d.)	62	53.9 (1.5)	18.2 (1.7)	42.7 (1.6)	32.9 (0.9)	33.3 (1.5)	18.4 (0.6)	12.2 (0.3)	4.9 (0.2)	5.4 (0.4)	4.9 (0.1)	12.3 (0.3)
$F_{3,123}^a$		4.05	14.63	11.81	6.79	5.36	2.28	8.42	1.64	1.48	1.61	5.90
p		0.01	0.01	0.01	0.01	0.01	0.08	0.01	0.18	0.22	0.19	0.01

^a One clutch which contained a subsample that did not hatch was excluded from the analyses.

Table 10. Results of Newman-Keuls range tests for selected variables from the time of oil experiment in the laboratory. Means with the same letter are not significantly different ($p > 0.05$).

Variable	Grouping	Treatment	Mean
Hatchlings released	A	1/2-time oil	2.8 (18.7%)
	A	1/2-time oil	5.2 (34.7%)
	B	Full-time oil	12.4 (82.7%)
	B	Control	13.2 (88.0)
Unhatched eggs	A	Control	1.4 (9.3%)
	A	Full-time oil	1.6 (10.7%)
	B	1/2-time oil	9.2 (61.3%)
	B	1/2-time oil	11.2 (74.7%)
Unhatched eggs with embryos	A	Control	0.4 (2.7%)
	A	Full-time oil	1.2 (8.0%)
	B	1/2-time oil	9.0 (60.0%)
	B	1/2-time oil	10.4 (69.3%)
Embryos near full term	A	Full-time oil	0.2 (1.3%)
	A	Control	0.4 (2.7%)
	A	1/2-time oil	2.4 (16.0%)
	B	1/2-time oil	8.8 (58.7%)

Table 10. Continued.

Variable	Grouping	Treatment	Mean
Embryos 21 to 60 mm in length	A	Control	0.0
	A	1/4-time oil	0.0
	A	Full-time oil	0.2 (1.3%)
	B	1/4-time oil	7.2 (48.0%)
Hatchling weight (g)	A	1/4-time oil	16.4
	A B	1/4-time oil	17.3
	B	Control	17.7
	B	Full-time oil	18.2
Hatchling carapace length (mm)	A	1/4-time oil	41.6
	A B	1/4-time oil	42.2
	B	Full-time oil	42.7
	B	Control	43.1
Hatchling carapace width (mm)	A	1/4-time oil	32.6
	A B	1/4-time oil	32.9
	A B	Full-time oil	32.9
	B	Control	33.2

Table 10. Concluded.

Variable	Grouping	Treatment	Mean
Hatchling plastron length (mm)	A	1/2-time oil	32.6
	A B	1/2-time oil	33.1
	A B	Full-time oil	33.3
	B	Control	33.8
Hatchling left marginal scutes	A	Full-time oil	12.2
	A B	1/2-time oil	12.4
	B C	Control	12.7
	C	1/2-time oil	12.8
Hatchling vertebral scutes	A	1/2-time oil	5.0
	A	1/2-time oil	5.0
	A	Control	5.1
	B	Full-time oil	5.4
Hatchling right marginal scutes	A	Full-time oil	12.3
	A B	1/2-time oil	12.4
	B	1/2-time oil	12.7
	B	Control	12.7

DISCUSSION

The most extreme effect of petroleum noted in the present study was the significant decrease in survival to hatching in laboratory samples where oil was poured on top during the last half and the last quarter of the incubation period. The $\frac{1}{2}$ -time oil group contained a large number of dead embryos of medium size, whereas the $\frac{1}{4}$ -time oil group, which received the oil later, contained numerous dead embryos of larger size. Thus, it appears that embryonic death in these two groups was related to the time of oil application. Biderman and Drury (1980) found this to be true in mallard eggs; embryonic death usually began within 3 days of treatment with oil. In subsamples to which oil was added during the last half or quarter of the incubation period, we noticed that only eggs near the bottoms of the buckets survived to hatch. Some eggs took on a characteristic gray coloration that was observed only in these subsamples. Exposure to oil appeared to cause this discoloration and probably was responsible for embryonic death.

This suggests two possibilities: (1) that embryos in early stages of development were not as sensitive to the toxic components of oil as later embryos, or (2) that mixing of the oil with the sand reduced the amount of oil that each egg received.

In full-time subsamples the oil was mixed with the sand, whereas $\frac{1}{2}$ -time and $\frac{1}{4}$ -time subsamples had oil poured on top of the sand. This difference may have affected the results. The small size of the containers in relation to the egg mass suggests that all eggs in full-time subsamples were exposed adequately to the oil. All eggs in $\frac{1}{2}$ -time and $\frac{1}{4}$ -time subsamples probably did not receive equal exposure to oil. The possibility exists that mixing diminished the effects of oil and resulted in a decreased effect in full-time subsamples. Only further experimentation can confirm this possibility.

The sample of crude oil used in the laboratory experiments appeared opaque dark brown in color, had a viscosity similar to water, no apparent sediments, and a distinctive odor similar to shoe polish. Volatile components in this sample began to evaporate immediately upon exposure to the atmosphere at room temperature. The odor was obvious in the sand when crude oil was mixed with it, regardless of experimental quantity, and permeated the atmosphere inside the incubators containing oiled subsamples (for this reason, nonoiled subgroups were kept in separate incubators). The volatile hydrocarbons (especially the aromatics) in crude oils contribute significantly to their toxicity. However, "the differences between aromatic toxicity and overall petroleum toxicity . . . are largely undefined" (NAS 1975).

Aromatic compounds in crude oil readily evaporate and enter into water solution, and solubility in distilled water is higher than in seawater (Jordan and Payne 1980). All subsamples in the laboratory experiments were kept moist as necessary by sprinkling the top of the sand with tapwater. This practice probably facilitated dispersion of oil through the sand and may have contributed to its degradation. Rainwater falling on nests in nature might produce similar results. Viable loggerhead eggs normally take up moisture from the surrounding substrate as they develop (McGehee 1979). It is possible that crude oil aromatics in water solution in the sand around incubating sea turtle clutches may be absorbed by the eggs and poison them.

In mallard eggs, embryonic mortality did not occur when the egg shells were coated with the alkane or paraffin components of crude oil, indicating that death was not caused by oxygen deprivation due to clogged shell pores; applications of artificially formulated mixtures of aromatic compounds found in crude oils caused greater embryonic mortality than controls or treatments with the alkane or paraffin mixtures (Stickel and Dieter 1979). These findings may also apply to marine turtle eggs. Tar residues from petroleum apparently are not extremely toxic to sea turtle eggs or hatchlings. Pieces of tar were present in the beach sand used in the field experiments, but this did not appear to influence embryonic survival.

Ackerman (1980) indicated that oxygen consumption by loggerhead turtle eggs increases sigmoidally throughout development, and "embryonic growth slows and mortality increases in environments in which gas exchange is reduced below naturally occurring levels." This may explain why loggerhead eggs appeared to become more sensitive to oil contamination toward the middle stages of development when the rate of oxygen consumption was approaching its peak. This also may be responsible for the smaller hatchling size seen in the $\frac{1}{2}$ -time oil subgroups. Crude oil aromatics in the nest atmosphere may displace or inhibit use of available oxygen; slower growth rates and greater mortality may occur due to oxygen depletion, aromatic poisoning, or both. Oxygen consumption by marine turtle eggs appears to be a function of growth rate and the egg or embryonic mass (Ackerman 1980), so sea turtle eggs may respond differently to oil contamination according to clutch size, degree of embryonic development, or species.

Minimum incubation times for the subsamples were not influenced by oil treatment, but hatchling incubation time may have been affected. Unlike minimum incubation time, hatchling incubation period includes the time required for hatchlings to emerge from the eggs. Since minimum incubation times were not affected, it appears that treatments with oil influenced hatchling emergence. It remains to be determined why application of oil during the last quarter of the incubation period might extend the time required for hatchlings to emerge.

The earlier hatching in the light dosage groups contrasts with the longer incubation period in medium and heavy dosage samples. The shorter incubation time of light dosage subsamples was accompanied by a lower body weight and smaller size relative to heavy dosage subsamples and controls. Whether light dosage contamination could act as an irritant which accelerates development or emergence while heavy dosage application could retard development is not clear from the present data.

Size parameters of hatchlings are subject to an error due to the curvature of the carapace resulting from the egg shape. Hatchlings normally assume a flatter, more typical body shape within 24 hours of leaving the eggshell. For this reason all measurements were taken after the hatchling had been out of the egg for 24 hours and after the yolk plug was nearly totally absorbed into the umbilicus. The smaller size of hatchlings from light dosage subsamples could result from a retardation of flattening rather than an absolute size difference, although this phenomenon should not affect weight.

The differences in question are slight and the sample size is small; therefore, it is sufficient to note variations in size and incubation times as possible effects of oil treatment awaiting confirmation by further investigations. It is relevant to note that

differences in mean incubation times between all groups in the laboratory are small in relation to differences attributable to standard incubation techniques in the field (beach vs. artificial incubation building). Among the laboratory samples incubated in varying amounts of oil but at the same temperature, mean incubation times differed by 1.6 days, whereas a difference of up to 5 days was produced in the field as a result of differing incubation methods and temperatures.

Hatchling scutellation in subsamples treated with oil at the beginning of incubation exhibited significant deviations from controls. Medium and heavy dosage contamination produced the most deviations. Hatchlings from these treatments averaged more vertebrals and fewer marginals than controls. How exposure to oil might induce differences in vertebral scutes and reduce variation in marginals remains undetermined. This may be correlated with the fact that vertebral scutes differentiate before marginals during embryonic development.

It is not surprising that scutellation differences were detectable only in subsamples which were exposed to oil at the beginning of the incubation period. Undoubtedly this is due to the fact that the basic body form, including the carapace morphology, is determined in the early stages of development. Presumably embryos influenced by oil only during the last half or quarter of development had already formed the scutes of the carapace. The lower number of marginal scutes seen in oiled subsamples in the laboratory were also weakly indicated in oiled subsamples (beach sand) in the field experiments.

The minimal quantity of oil required to produce measurable effects on the survival of a turtle embryo remains unknown, however, we do have a few points in the continuum. Thirty milliliters of fresh oil mixed with the sand at the initiation of incubation (7.5 ml oil/kilogram of sand) did not produce significant effects on survival, though some change in scutellation was observed. Extensive mortality occurred in samples where oil was poured on top of the clutch during the latter portion of incubation. When oil is poured on top there potentially is a gradient produced such that eggs near the top of the buckets receive a higher concentration of oil. The observation that 30 ml mixed with the sand at the start of incubation did not reduce survival whereas 30 ml poured on top of the sand later in the incubation period did lower survival may reflect the amount of oil any single egg received. Potentially, mixing resulted in a sublethal dose for embryos at the start of incubation. Further research is needed to determine the minimum quantity of oil which effects survival and the variables which can influence this.

The results of all experiments in the present study indicated significant clutch effects. These effects may be attributable to normal genetic variation. Such effects also may be related to environmental influence on the female turtle before and during ovulation and egg formation. Differences in egg weights and diameters were noted between clutches; such differences in egg characteristics may in turn be related to differences noted in progeny. It appears that clutch effects, whether genetically or environmentally caused, did not influence our conclusions. Subsamples from each clutch were distributed evenly among treatments; therefore, clutch effects were balanced throughout the experiments.

The quantities of crude oil used in laboratory experiments cannot be directly compared with the quantities of hydrocarbons found 10 cm below the surface of the

beach at Rancho Nuevo. Beach samples of sand had up to 1,282 mg/kg but were presumed to contain predominantly weathered components of petroleum (the least volatile compounds of high molecular weight). Thus, the concentration found on the beach represented only part of the oil originally entering the environment. The lowest concentration used in the laboratory experiments (1,577 mg/kg) was slightly higher than the highest concentration found in samples from the beach. The highest concentration in the laboratory was four times as high as that from the beach.

It is important to note that oiled sand taken from the beach in Mexico failed to produce measurable effects in survival and in morphology. Turtles from beach and dune treatment groups appeared to be equally vigorous when they were released on the beach and made their way into the surf. The oil present on the beach had accumulated over a period of 9 months and had been weathered by natural processes; therefore, the most volatile components were undoubtedly lower in concentration or absent in relation to fresh crude oil. The toxicity of oil is reduced significantly after 2 to 3 weeks of weathering (Stickel and Dieter 1979). The type of petroleum (e.g., crude oil from various origins or different kinds of refined products) also may have different toxic effects (NAS 1975) and may weather differently.

In the Mexican oil studies, weathering in the ocean and on the beach due to physical and biological factors was certainly more complete than weathering of crude oil during the laboratory experiment. The data suggest that oil contamination of turtle nesting sites would be most detrimental if the oil was not weathered and arrived during the nesting season. Oil that arrived as little as a few weeks before nesting by turtles might conceivably have no or minimal effect on embryonic development. This is even more likely because of the way oil is deposited on beaches. Oil is like other flotsam; not all areas of a beach receive equal amounts, and it generally is deposited just above high tide levels.

The concentration of hydrocarbons in the sand samples analyzed was largely consistent with visual observations made on the beach. Hydrocarbon concentrations were highest in positions A and B and lower in position C. The variation within any position suggested that contamination was not uniform. Thus, the degree of effects expected in any actual spill situation might also vary significantly along the beach length. Storms and abnormally high tides can move incoming oil higher up a beach than normal tides, and a concentrated band of petroleum similar to that recorded in positions A and B can form above the high tide line. Since most turtles nest significantly above the normal high tide level for the nesting season, little oil arriving during the nesting season would be deposited high enough on the beach to contact eggs or the normal nesting zone except during hurricanes or other extreme conditions. Petroleum was observed to be deposited in the nesting zone of Lepidochelys in Mexico, but this oil was undoubtedly deposited there during winter months when tides were higher and beaches were somewhat eroded by a hurricane the previous fall. Such oil would have been considerably weathered and potentially detoxified with respect to turtle eggs. The presumed origin of most of the petroleum on the beach at Rancho Nuevo was the IXTOC well; this oil may have been less toxic than oil from other sources. The toxicity of crude oil is highly variable depending on the actual chemical makeup of the oil.

If fresh oil contacts the nesting zone, the effects may be catastrophic to turtle reproduction. In experiments involving developing bird eggs, Stickel and Dieter (1979) applied very small amounts of petroleum (5 to 50 μ l per egg) directly to the shells early

in development. Such application increased mortality drastically. In our experiments with turtle eggs, oil was not applied directly to the eggs but was added to the sand in quantities of approximately 0.5 to 2.0 ml per egg. An alternative way of expressing this experimental condition is 1.9 to 7.5 ml oil per kg of sand. Eggs were not exposed to all oil in the sand because they lacked direct contact with all sand particles. If the toxic components of petroleum do not diffuse through sand, turtle eggs may be protected from most oil pollution.

The present data do not allow consideration of possible effects of petroleum ingested by a female turtle before or during ovulation and shelling of eggs. The experiments in the present study have addressed the two most likely possibilities for petroleum contamination of turtle eggs: oil on the beach before oviposition and oil arriving during incubation. The results of field and laboratory experiments suggest that weathering will reduce or eliminate toxic components if oil washes up on a beach before the nesting season. The exact time needed to weather the oil adequately is unknown. Oil mixed with beach sand and oil on top of the beach could be pushed into the nest by the female turtle during excavation of the egg chamber and during covering of the nest. Such oil, if weathered, is expected to have minimal effect on the development of the embryos. However, this conclusion must be mitigated by considering the quantity of oil and the rate of weathering under various conditions.

If oil washes up on a beach during the nesting season and reaches the level of nest sites, significant mortality could occur. Based on the laboratory experiments in which oil was added to the sand covering a subsample after half of the incubation period, oil could permeate the sand covering the eggs and result in embryonic death. However, for oil to reach the nesting zone, it must be transported there by extremely high tides. Since saltwater inundation is known to kill developing sea turtle embryos (McGehee 1979), such a perturbation, even in the absence of oil, might cause clutch mortality.

The results at hand do not indicate whether nests deposited high on a beach would be affected by oil contamination at a lower level. The $\frac{1}{2}$ -time and $\frac{1}{4}$ -time oil subsamples received only a small volume of oil in relation to the amount of sand in which the eggs were buried, and yet these subsamples had significant mortality. Actual inundation by petroleum may not be necessary to kill embryos; the mere presence of freshly oiled sand in the vicinity may suffice.

From a management viewpoint, turtle nesting beaches are not as vulnerable to petroleum damage as might be expected. Apparently, the most drastic effects of a one-time oil spill are relatively short-lived and would threaten at worst a single year's reproductive effort. Except under unusual conditions it seems impractical and detrimental to move the eggs to another location. If possible, clutches should be incubated on the beach where natural nest conditions are approximated. The concentration of eggs in transplant areas exposes all to any one potential risk (e.g., hurricane damage and saltwater inundation). An important and wide-reaching effect of artificial incubation of marine turtle eggs is the likelihood that sex ratios are altered by incubation temperatures (Yntema and Mrosovsky 1980). Another point to consider is the type of sand used for artificial incubation. In the field experiments, we noticed that dune sand taken from outside the usual nesting zone was of a very fine particle size; beach sand was much coarser. This difference in particle size could affect moisture and gas conditions around an incubating sea turtle clutch. In order to approximate natural conditions, transplanted nests should be incubated in sand from within the normal nesting zone if at all possible.

Petroleum arriving at a beach during the nesting season would be expected to be deposited at the normal high tide level and below the nesting area. In order to prevent this petroleum from being moved higher up the beach by subsequent high tides, mechanical removal of the petroleum may be possible. Transplanting the most threatened nests to a higher position at the same location seems preferable to concentrating all nests in a single transplant area. Since the oil is not uniformly deposited along the beach, some nests will survive at the same beach level where others are damaged.

As in any transplanting situation, care must be taken to minimize handling and prevent disruption of polarity in eggs older than 24 hours (McGehee 1979), to approximate optimum environmental conditions, to protect against vulnerability to predators, and to shelter nests from catastrophic inundation by oil or seawater. By these methods a maximally efficient conservation program may be maintained for marine turtles.

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The Department of the Interior Mission

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



The Minerals Management Service Mission

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

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

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