FINAL REPORT
STUDY OF THE EFFECTS OF OIL
ON MARINE TURTLES

OCS STUDY
MMS 86-0070

Prepared for the U.S. Department of the Interior
Minerals Management Service
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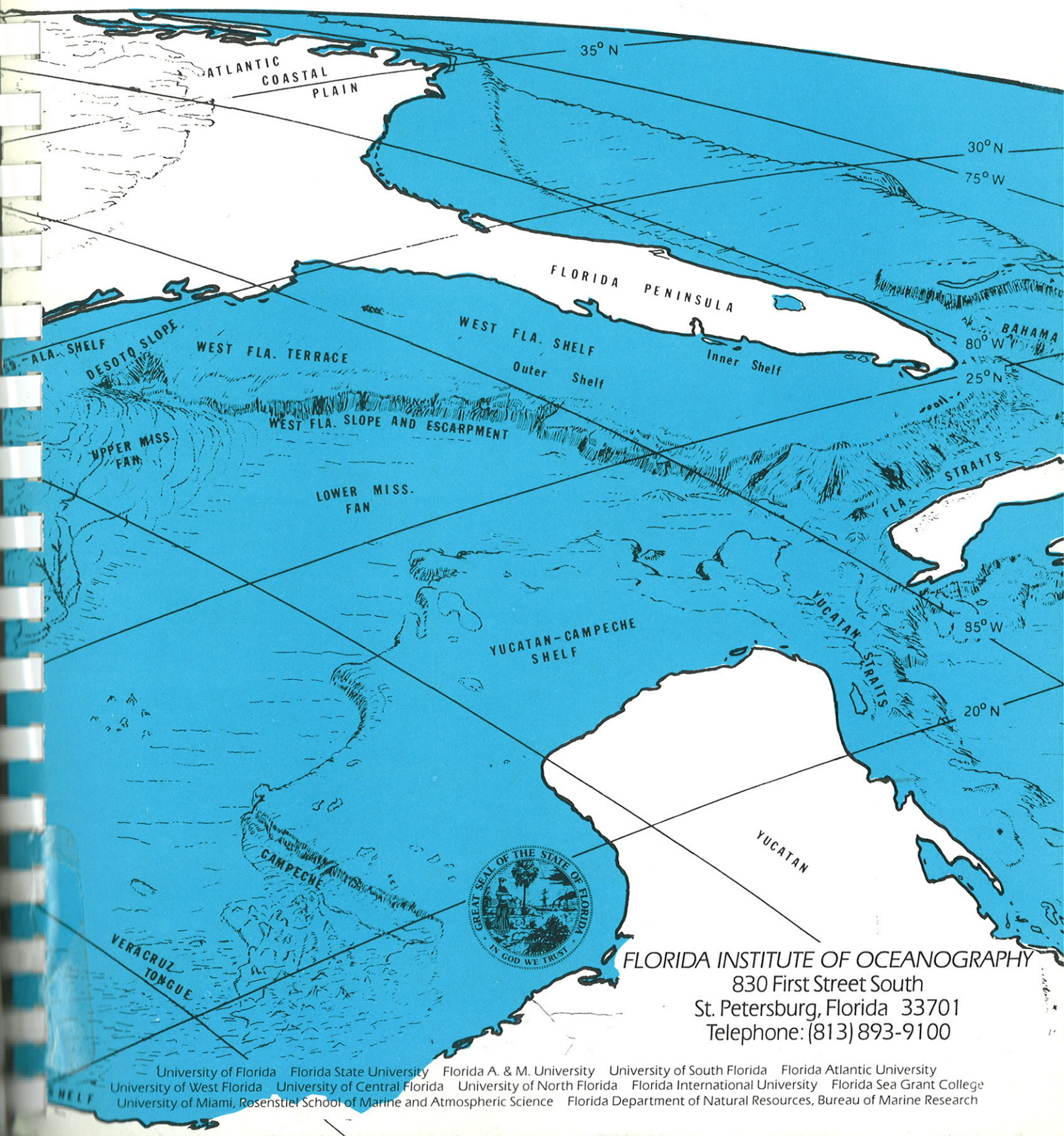
Volume 2 - Technical Report





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16. Abstract (Limit: 200 words)

The objective of this study was to determine the effects of oil on marine turtles. An experimental program was carried out on 3-20 month old loggerhead and 3-16 month old green turtles to determine behavioral and physiological effects of oil using South Louisiana Crude Oil (SLCO) preweathered for 48 hrs. The behavioral experiments indicated that both species of marine turtles had a limited ability to avoid oil slicks, but experiments to determine avoidance/attraction to floating tar balls were inconclusive. Controls (wooden balls) were struck at a greater rate than tar balls, but overall strike rates were low and interpretation complicated by feeding schedule and interspecific differences. The physiological experiments showed that the respiration, skin, some aspects of blood chemistry and composition, and salt gland function of 15-18 month old loggerhead sea turtles were significantly affected. Oil was observed clinging to the nares and eyes and in the upper portion of the esophagus and was found in the feces of all turtles in the physiological experiments. Some similar effects were found in stranded oil fouled turtles. Based on these experimental results, it is our opinion that given the proper circumstances, sea turtles will be at risk in the event of an oil spill. Spills in the vicinity of nesting beaches are of special concern. Mitigating strategies for such an event are discussed. Dispersants with microbial nutrients are of interest, but they should only be used if they are found harmless to sea turtles.

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## 1.0 PROGRAM BACKGROUND AND OBJECTIVES

The Department of the Interior (DOI) bears the responsibility for managing the petroleum and other mineral resources of the submerged lands of the continental shelf seaward of state territorial waters. The administration of leasing and supervision of production have been centralized under the Minerals Management Service (MMS). Under statutory authority from the OCS Lands Act Amendments of 1978 the Secretary is authorized to conduct studies in areas or regions of lease sales to obtain the information to predict, assess, and mitigate the potential impacts on the coastal and offshore areas that may be affected by oil and gas development.

In environmental impact assessments for numerous lease sales, the effects of oil and gas activities on marine turtles have frequently been listed as a matter for concern. Of marine species, marine turtles are particularly vulnerable to oil spills or pelagic tar because they must surface to breathe, increasing the possibility of repeated contact with the oil or tar which floats at the surface.

All marine turtle species are classified as either threatened or endangered under the Convention of International Trade in Endangered Species (CITES) of wild fauna and flora, Appendix 1. The final two populations of marine turtles accorded this protective status were the Australian populations of Chelonia mydas (green turtle) and Chelonia depressa (Australian flatback turtle) which were added to Appendix 1 at the 1981 CITES Meeting (Pritchard et al., 1982). In 1978, subsequent to the enactment of the U.S. Endangered Species Act in 1973, sea turtles were placed on the Federal List of Endangered and Threatened Species. Their status is reviewed every five years by the National Marine Fisheries Service (NMFS) and the Fish and Wildlife Service (FWS). Under the U.S. Endangered Species Act of 1973 federal actions must not jeopardize the existence of endangered or threatened species nor destroy or modify their habitats.

Recognizing the need for additional information, the MMS initiated a competitive request for proposals (RFP No. 3063) to address relevant questions regarding the effects of oil on marine turtles. In September, 1983 Minerals Management Service awarded MMS Contract No. 14-12-0001-30063 entitled "Study of the Effects of Oil on Marine Turtles" to the Florida Institute of Oceanography/University of South Florida acting as a consortium of University of South Florida, University of Miami, Florida Department of

Natural Resources, University of Central Florida, and Wildlife Veterinary Center.

The MMS goal for this research was to determine the possible effects of oil on hatchling, juvenile, and adult sea turtles by gathering existing information and generating new experimental information on two selected species, Chelonia mydas (green sea turtle) and Caretta caretta (loggerhead sea turtle). Sub-objectives of these overall goals were as follows:

a. To determine the ability of marine turtles to detect and avoid, or be attracted to tar balls and/or oil slicks.

b. To determine the effects of oil on the integument and various physiological systems of marine turtles.

c. To evaluate the long term impact of a. and b. on marine turtle survival and behavior, such as feeding, breeding, nesting, and migration and to analyze the potential for bioaccumulation of petroleum hydrocarbons and metabolites in marine turtles.

d. To predict, considering the known life history of sea turtles, records of past oil spills, and known climate or natural conditions, the possible impacts of oil on turtle nesting sites and on hatchlings, juveniles, and adults in an oil contaminated nearshore and open ocean environment.

e. To identify a range of mitigating measures which would eliminate or minimize the effects of oil contamination on marine turtles.

Objective b) was largely accomplished in this study while a), c) and d) were partially accomplished. Mitigating strategies (e) were identified from techniques reported in the literature taking into consideration the results of a) and b).

## 2.0 OVERALL SCIENTIFIC PLAN

The overall scientific plan for this program was designed to meet the research objectives stated in Section 1.0. It was divided into five main components:

1. Literature Survey
2. Analytical Studies (Petroleum Hydrocarbon Types and Concentrations)
3. Behavioral Response to Oil
4. Effects of Exposure to Oil
5. Field Studies (Analysis of Stranding Data)

Several general considerations were common to all experiments performed. The selected animals were tested under as realistic conditions as possible given experimental requirements. All experiments were designed to be sublethal. Experimental animals were healthy and the minimum number of animals was used that would still yield statistically valid results given the design of each experiment. Exposure levels for behavioral and physiological studies were selected to facilitate comparison of results, taking into account the differences in goals and experimental protocol. Stranded animals were not considered suitable for exposure experiments as they were already stressed by unknown factors. Clinical examinations of stranded animals, however, were conducted to aid in the interpretation of experimental results.

Two other general considerations are relevant to all the experimental studies, the stocks of experimental animals and oil.

### Turtle Stock

As marine turtles have a limited hatching season, from approximately mid-August to the end of September, it was essential to obtain animals as soon as possible after the commencement of the program in October, 1983. Hatchling to 18 month old turtles were required for use in the behavior experiments. Due to the program starting date, only limited numbers of hatchling green and loggerhead turtles were available for behavior experiments in Year 1. Hatchling turtles were obtained through the Florida Department of Natural Resources (FDNR) headstart programs at Clearwater Marine Science Center (Clearwater, Florida), House of Refuge (Jensen Beach, Florida), and Marineland, Inc. (St. Augustine, Florida).

The hatchling turtles were transferred to the Sea World Marine Science and Conservation Center (MSCC) on Long Key, Florida (formerly the Shark Institute) to acclimate them to the holding facilities prior to the beginning of the experiments. The hatchlings were first held in large outdoor fiberglass circular tanks approximately 8 feet (231 cm.) in diameter with a water depth of 20 inches (50 cm.) and then, as they grew larger, moved into a shallow concrete walled shallow pond system measuring about 20 feet (577 cm) by 40 feet (1155 cm) ranging in depth from 75 to 105 cm. The sea water supplied to these ponds was filtered and aerated. The turtles were fed daily on a mixed diet consisting of Purina turtle chow, shrimp, and squid. They were weighed and measured at least monthly to insure that they were growing normally and to detect disease problems before they became serious. Despite these precautions there was considerable mortality during the first three months in year 1 due primarily to bacterial infections. Such infections are not uncommon, even under good culture conditions, in captive marine turtles. The guts of marine turtles contain high concentrations of gram negative bacteria, many of which are pathogens. Thus, in captive conditions any small cut or abrasion offers an easy route for infection (G. Bossart, personal communication).

In Year 2 (August - September, 1984) a larger stock of hatchling green and loggerhead turtles was obtained, again through the FDNR headstart programs. A stock of about 50 loggerheads and 50 greens was maintained at the MSCC. Due to the mortalities experienced with hatchlings in the first three months in Year 1 of the program, a backup stock was maintained in year 2 at the Clearwater Marine Science Center for replenishing the MSCC stock. The mortality due to bacterial infections was much less in year 2. This may have been due to the fact that the turtles were moved to the more controlled rearing conditions at MSCC immediately after hatching rather than being held for 2-3 months at other locations.

A separate stock of 15-18 month old loggerhead turtles, Caretta caretta, weighing 10-20 pounds was used for the physiological effects experiments. These turtles were obtained from Seaquarium Inc. in Miami, Florida. They had been reared at this facility and were fed Purina turtle chow exclusively.

Oil Stock

The oil type designated by MMS to be tested in this program was South Louisiana Crude Oil (SLCO). A uniform supply of SLCO was required as SLCO from different oil fields, and even wells within the same field, can differ in composition (Hallett et. al., 1983). Texaco, Inc. provided a supply of 250 gallons of SLCO. The oil was provided at no cost from the Houma, Louisiana field, and was a South Delta Crude subtype.

## 3.0 ANALYTICAL STUDIES (E. S. Van Vleet)

During Years 1 and 2 of this project, hydrocarbon analyses were carried out at the Department of Marine Science, University of South Florida. The goals of this portion of the research were:

(1) To determine the nature and concentration of petroleum hydrocarbons in water to which turtles were exposed.

(2) To determine changes in concentration and nature of the water, oil, and tar balls over the course of the exposure experiments.

(3) To analyze tissue samples from exposed turtles as well as stranded or beached turtles.

(4) To conduct ancillary analyses on tar and oil collected in the environment that may have had an impact on natural turtle populations.

### 3.1 Analytical Methods

Extraction and Isolation.

Internal standards (5α-androstane and O-terphenyl in acetone) were added to the turtle tissues, tar balls, water samples or crude oils (as described in subsequent sections), prior to analysis, to provide reference compounds for later quantification of the hydrocarbon fractions. Water samples were extracted with hexane. Tar and tissue samples were saponified-extracted for 2 hours with a five-fold excess of 2:1 0.5N KOH-MeOH:Toluene. After filtration through pre-combusted glass fiber filters, the organic phases were partitioned with $H_2O$ and the aqueous-methanol phases extracted twice more with hexane. Organic phases were combined and evaporated to near dryness. Extracts were diluted to 1 ml with hexane and applied to a 10 cm x 1 cm i.d. glass column containing 2.5 g of 5% deactivated alumina over 5.0 g activated silica-gel to separate the aliphatic and aromatic hydrocarbon fractions. Aliphatic ($F_1$) hydrocarbons were eluted with two bed volumes of hexane, after which the aromatic ($F_2$) hydrocarbons were eluted with two bed volumes of toluene.

Gas Chromatography.

Each $F_1$ and $F_2$ hydrocarbon fraction was evaporated to near dryness and analyzed by high resolution glass capillary gas-liquid chromatography (GC) using a Hewlett-Packard Model 5880A gas chromatograph. Samples were analyzed on a 30 m x 0.25 mm i.d., 0.25 μm film thickness DB-5 (bonded/cross linked dimethyl (95%)-diphenyl (5%)-polysiloxane) fused silica capillary column (J. W. Scientific, Rancho Cordova, California). Splitless injection mode (injection port temperature = 240°C) was used with a helium carrier gas flow rate of 2 ml $min^{-1}$. Oven temperature was programmed from 100-280°C at 4° $min^{-1}$. Hydrocarbons were detected using a flame ionization detector (@300°C) and integrated using an integrating data processor equipped with BASIC programming capability. This data processor allowed storage and retrieval of raw chromatographic data as well as multiple programmed calculations based upon reassigning baseline conditions. All integrated hydrocarbon data was automatically corrected for individual response factors and compared with the areas of internal standards for compound quantitation.

Combined Gas Chromatography-Mass Spectrometry.

Selected aromatic hydrocarbon samples were analyzed by combined high resolution gas chromatography-mass spectrometry using a Hewlett-Packard Model 5992B computerized GCMS system. Samples were analyzed on a 30 m DB-5 fused silica column. Running conditions were as follows: splitless injection mode; carrier gas = helium; column flow rate = 2 ml $min^{-1}$; injection port temperature = 240°C; oven temperature programmed from 90°-255°C at 40°C $min^{-1}$; electron multiplier voltage = 1200-1800 eV; scan mode @690 amu $sec^{-1}$; selected ion monitoring mode 100 msec dwell time per ion. Selected aliphatic ($F_1$) hydrocarbon fractions were also analyzed by GCMS for structural confirmation.

Intercalibration Studies.

In order to confirm that all hydrocarbon analyses to be performed at USF would be precise and accurate, several intercalibration exercises were initially carried out in cooperation with a private research foundation (Mote Marine Laboratory, Sarasota, Florida) and an independent consulting firm (Interscience, Inc., Tampa, Florida). Originally the intercalibration exercise called for intercalibrating with Mote Marine Laboratory, University

of Miami, and Interscience, Inc. Six to ten samples were envisioned to be sent to each laboratory. One of the comments on our initial proposal was that we had just finished an extensive intercalibration study with these labs, so why was it necessary to do another? This comment was taken into consideration when the budget was renegotiated (downward), and the intercalibration exercise was modified. Since the principal investigators from the University of Miami were not involved with any GC work and since we had established a closer working relationship with Mote Marine Laboratory (for GC analyses), it was determined to include only Mote (GC) and Interscience (GCMS) in the intercalibration. The following four samples were distributed for analysis during the intercalibration exercise: (1) South Louisiana Crude Oil; (2) Tar ball collected from Mustang Island Beach, Texas; (3) Kuwait Crude Oil, and (4) Hillsborough River sediment extract (Sta. 12). No spiked tissue samples were proposed or planned for this intercalibration exercise.

Saturated/aliphatic (F1) hydrocarbons were analyzed by USF and Mote Marine Laboratory using high resolution gas chromatography (GC). Key aliphatic ratios as well as n-alkane range, n-alkane maximum, carbon preference index (CPI) and resolved versus unresolved components were measured for comparison of GC results. Unsaturated/aromatic (F2) hydrocarbons were analyzed by USF and Interscience, Inc. using combined high resolution gas chromatography-mass spectrometry (GCMS). Key aromatic components and their $C_1$-$C_3$ alkylated homologs were measured for comparison of GCMS results.

GC intercalibration results (Table 3-1) indicate good agreement between USF and Mote Marine Laboratory for South Louisiana Crude Oil, Kuwait Crude Oil, and Tar ball NNQ-507. Agreement in aliphatic components for these samples was generally within ±10%. Results for the Hillsborough River sediment extract were not as good. The main discrepancies for this sample are believed to result from the uncertainty in the measured pristane values. Concentrations of n-alkanes, as well as pristane and phytane, were very low in this sample and difficult to quantitate. Any errors measured in individual concentrations would be compounded in the component ratios. Despite disagreement in some of the ratios measured for the Hillsborough River sediment extract, the intercalibration results between the two laboratories were considered quite acceptable.

Table 3-1. GC intercalibration results between the University of South Florida Marine Science Department and Mote Marine Laboratory for F1 (saturated) hydrocarbon fractions. (n= number of replicates; 17= $nC_{17}$; 18= $nC_{18}$, Pr= pristane; Phy= phytane, CPI= carbon preference index; res= area of resolved peaks; unres= area of unresolved peaks).

| | | $F_1$ Parameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | Lab | 17/Pr | 18/Phy | 17/18 | Pr/Phy | n-alkane range | n-alkane maximum | CPI | res/unres |
| South Louisiana Crude Oil | USF (n=9) | 1.2 ± 0.1 | 1.9 ± 0.1 | 1.1 ± 0.1 | 1.8 ± 0.2 | $nC_{13}$- $nC_{35}$ | $nC_{15}$ | 1.0 ± 0.1 | 0.3 ± 0.0 |
| | Mote (n=4) | 1.3 ± 0.0 | 2.0 ± 0.0 | 1.0 ± 0.0 | 1.6 ± 0.0 | $nC_{13}$- $nC_{30}$ | $nC_{14}$ | 1.0 ± 0.0 | 0.3 ± 0.0 |
| Tarball NNQ-507 | USF (n=1)[1] | 1.9 ± 0.1 | 1.6 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.2 | $nC_{12}$- $nC_{36}$ | $nC_{18}$, $nC_{32}$ | 0.8 ± 0.1 | 0.9 ± 0.0 |
| | Mote (n=4) | 2.4 ± 0.0 | 1.6 ± 0.0 | 0.9 ± 0.0 | 0.6 ± 0.1 | $nC_{12}$- $nC_{34}$ | $nC_{18}$, $nC_{32}$ | 0.9 ± 0.0 | 0.4 ± 0.0 |
| Kuwait Crude Oil | USF (n=3) | 4.9 ± 0.2 | 2.7 ± 0.0 | 1.1. ± 0.1 | 0.6 ± 0.0 | $nC_{13}$- $nC_{35}$ | $nC_{17}$ | 1.1 ± 0.1 | 0.4 ± 0.0 |
| | Mote (n=3) | 6.6 ± 0.1 | 2.5 ± 0.0 | 1.0 ± 0.1 | 0.4 ± 0.0 | $nC_{13}$- $nC_{34}$ | $nC_{16}$ | 1.0 ± 0.0 | 0.4 ± 0.0 |
| Hillsborough River Sediment Extract | USF (n=2)[1] | 4.5 ± 0.4 | 9.3 ± 0.5 | 0.1 ± 0.0 | 0.2 ± 0.1 | $nC_{13}nC_{32}$ | $nC_{16}$ | 0.1 ± 0.0 | 0.2 ± 0.0 |
| | Mote (n=3) | 0.5 ± 0.0 | 5.9 ± 0.1 | 0.1 ± 0.1 | 1.3 ± 0.1 | n.d.[2] | n.d. | n.d. | 0.1 ± 0.0 |

[1] Standard deviations estimated from South Louisiana Crude Oil runs (n=9)
[2] n.d. = not determined

GCMS results (Table 3-2) indicate good agreement between USF and Interscience, Inc. for selected aromatic components in most samples. Agreement in aromatic components was generally <20-30%, a value within our analytical precision and acceptable for quantitative GCMS results. Plotting the alkylated homolog series for these samples (Figures 3-1 to 3-4) confirms the good agreement between the two laboratories. Aromatic components were not detected by Interscience, Inc. in the Kuwait Crude Oil sample, although the internal standard (O-terphenyl) was detected. It is believed that the sample provided to Interscience was too dilute for reasonable quantitation. Since agreement for the other samples was quite good, it was not deemed necessary to re-run this sample at additional project cost.

Based upon results of the intercalibration exercise, it was concluded that hydrocarbon results obtained during this project would be accurate and acceptable.

## 3.2 Results and Discussion

### Background Hydrocarbon Studies

Prior to the turtle exposure studies, we investigated background hydrocarbon levels in waters found in the MSCC holding tanks and experimental tanks as well as in the food pellets to be fed to the turtles. Total hydrocarbon levels in all tanks were below 8 $\mu g\ l^{-1}$ and in most tanks were <2 $\mu g\ l^{-1}$. In all cases hydrocarbon distributions suggested biogenic origins of these compounds rather than petroleum contamination. These very low background levels presented little possibility of significant contamination during the chronic, low-level exposure studies. Food pellets also indicated a composite of biogenic hydrocarbons ($\cong$45 $\mu g\ g^{-1}$). These food pellets were later used to feed the turtles being held for exposure studies, however the alkane distributions of the food were not reflected in the turtle tissues or fecal samples analyzed in later experiments.

### Characterization of South Louisiana Crude Oil

In order to insure comparability of all oil exposure experiments, a uniform and standardized crude oil was used throughout the study. South Louisiana Crude Oil (SLCO) was designated by MMS for this program. All South Louisiana Crude oils appear to have several features in common based upon

Table 3-2. GCMS intercalibration results between the University of South Florida Marine Science Department and Interscience, Inc. for F2 (unsaturated/aromatic) hydrocarbon fractions.

| Compound | SLCO[1] | | KCO[2] | | Tarball[3] | | Sediment[4] | |
|---|---|---|---|---|---|---|---|---|
| | USF | INT | USF | INT | USF | INT | USF | INT |
| N | 0.03 | 0.04 | 0.00 | * | 0.00 | 0.00 | 0.0 | 0.0 |
| $C_1N$ | 0.12 | 0.12 | 0.04 | * | 0.00 | 0.00 | 0.0 | 0.0 |
| $C_2N$ | 1.00 | 0.80 | 0.36 | * | 0.21 | 0.00 | 0.0 | 0.0 |
| $C_3N$ | 1.00 | 1.00 | 1.00 | * | 1.00 | 1.00 | 0.2 | 0.0 |
| P | 0.14 | 0.04 | 0.00 | * | 0.05 | 0.02 | 0.5 | 0.5 |
| $C_1P$ | 0.38 | 0.25 | 0.49 | * | 0.20 | 0.10 | 1.4 | 0.6 |
| $C_2P$ | 1.00 | 1.00 | 1.00 | * | 0.92 | 0.87 | 1.3 | 1.7 |
| $C_3P$ | 0.47 | 0.89 | 0.72 | * | 1.00 | 1.00 | 1.5 | 1.0 |
| Py | 0 | 0 | 0 | * | 0.10 | 0.01 | 0.6 | 1.7 |
| $C_1Py$ | 0 | 0 | 0 | * | 0.15 | 0.03 | 0.5 | 0.6 |
| $C_2Py$ | 0 | 0 | 0 | * | 0.96 | 0.50 | 0 | 0 |
| $C_3Py$ | 0 | 0 | 0 | * | 1.00 | 1.00 | 0 | 0 |
| BaA | 0 | 0 | 0 | * | 0.02 | 0.15 | 0 | 0 |
| $C_1BaA$ | 0 | 0 | 0 | * | 0.59 | 1.00 | 0 | 0 |
| $C_2BaA$ | 0 | 0 | 0 | * | 1.00 | 0.81 | 0 | 0 |
| $C_3BaA$ | 0 | 0 | 0 | * | 0.96 | 0.80 | 0 | 0 |
| | | | | | | TOTAL ( g/g) = | 6.1 | 6.0 |

[1]SLCO = South Louisiana Crude Oil. Concentrations relative to most abundant homolog in series.
[2]KCO = Kuwait Crude Oil. Concentrations relative to most abundant homolog in series.
[3]Tarball NNQ-507 from Mustang Island Beach, Texas. Concentrations relative to most abundant.
[4]Sediment from Hillsborough River (Station 12). Absolute concentrations in $g\ g^{-1}$.
USF = University of South Florida, Department of Marine Science.
INT = Interscience, Inc., Tampa, Florida 33607.
N = Naphthalene
P = Phenanthrene
Py = Pyrene
BaA = Benz(a)anthracene
$C_1$, $C_2$, $C_3$ = alkylated homolog series.
*No aromatic hydrocarbons were detected in this sample.

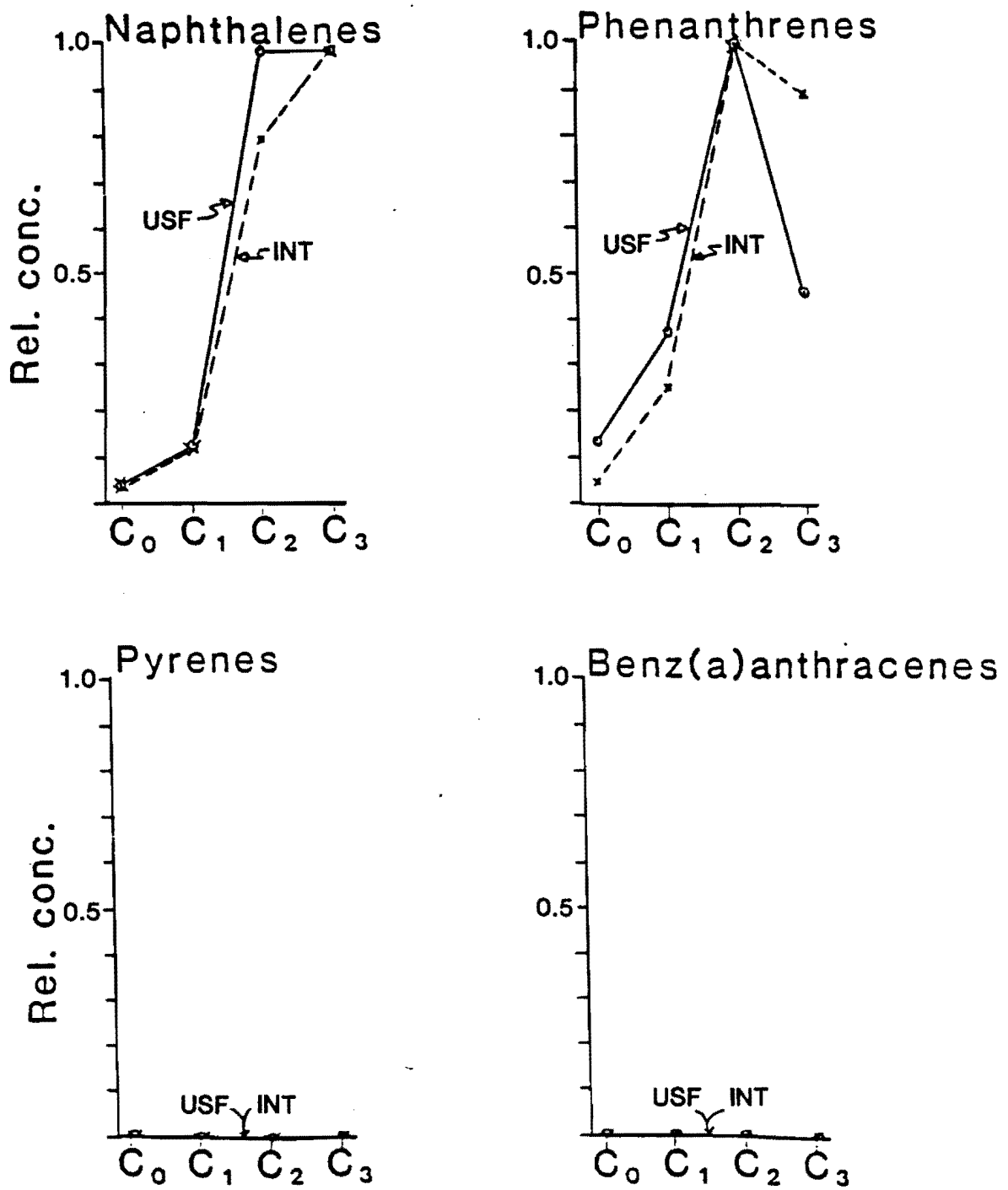



FIGURE 3-1. PAH alkyl distributions in South Louisiana Crude Oil analyzed as part of intercalibration study. Concentration is reported relative to most abundant peak. For pyrenes and benz(a)anthracenes, no peaks were detected. $C_0$ = parent compound, $C_1$ = methyl homolog, $C_2$ = dimethyl + ethyl homolog, $C_3$ = trimethyl + (methyl-ethyl) + propyl homologs. USF = University of South Florida. INT = Interscience, Inc.

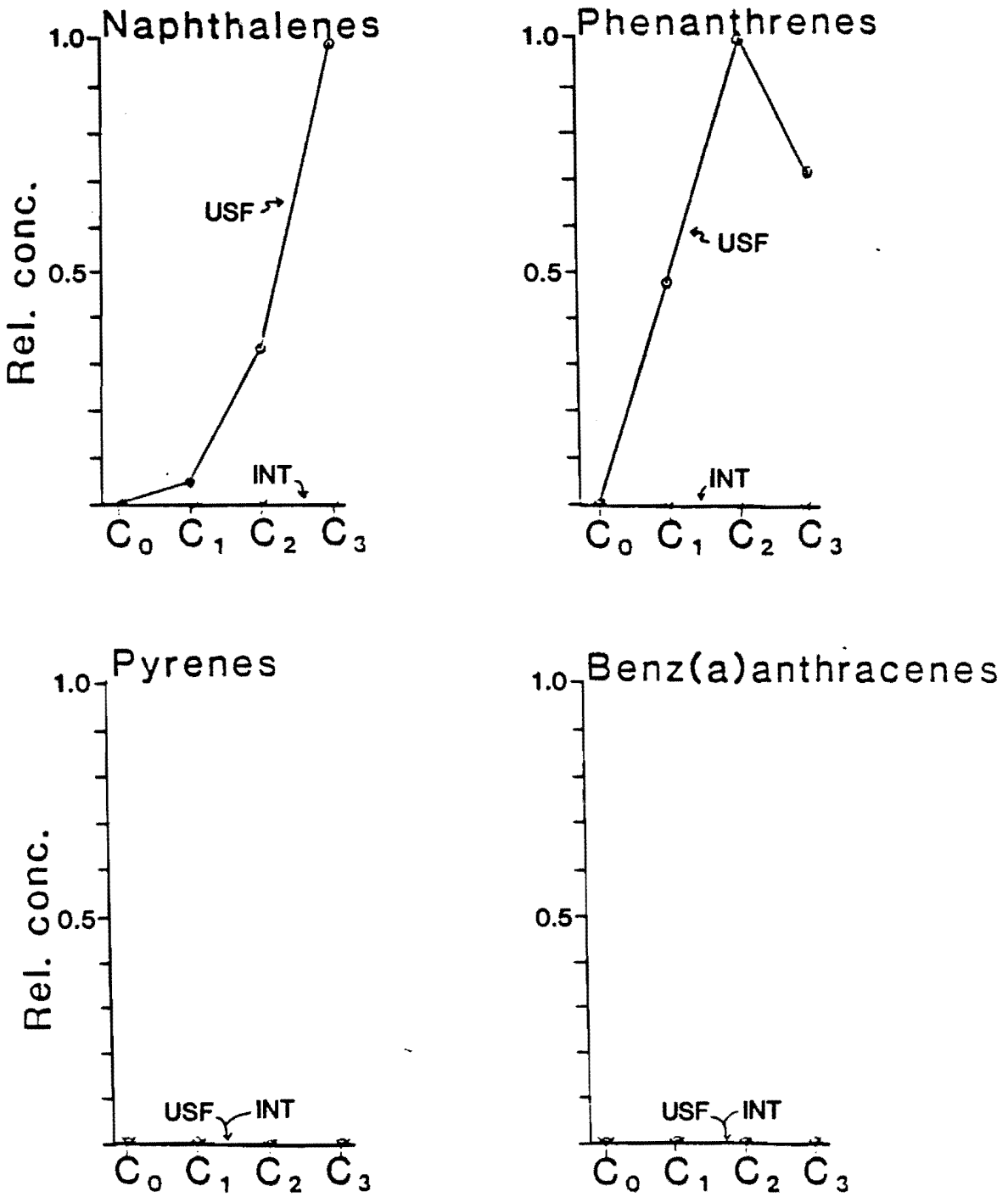



FIGURE 3-2. PAH alkyl homolog distributions in Kuwait Crude Oil analyzed as part of intercalibration study. Concentration is reported relative to most abundant peak. For pyrenes and benz(a)anthracenes, no peaks were detected. $C_0$ = parent compound, $C_1$ = methyl homolog, $C_2$ = dimethyl + ethyl homolog, $C_3$ = trimethyl + (methyl-ethyl) + propyl homologs. USF = University of South Florida. INT = Interscience, Inc.

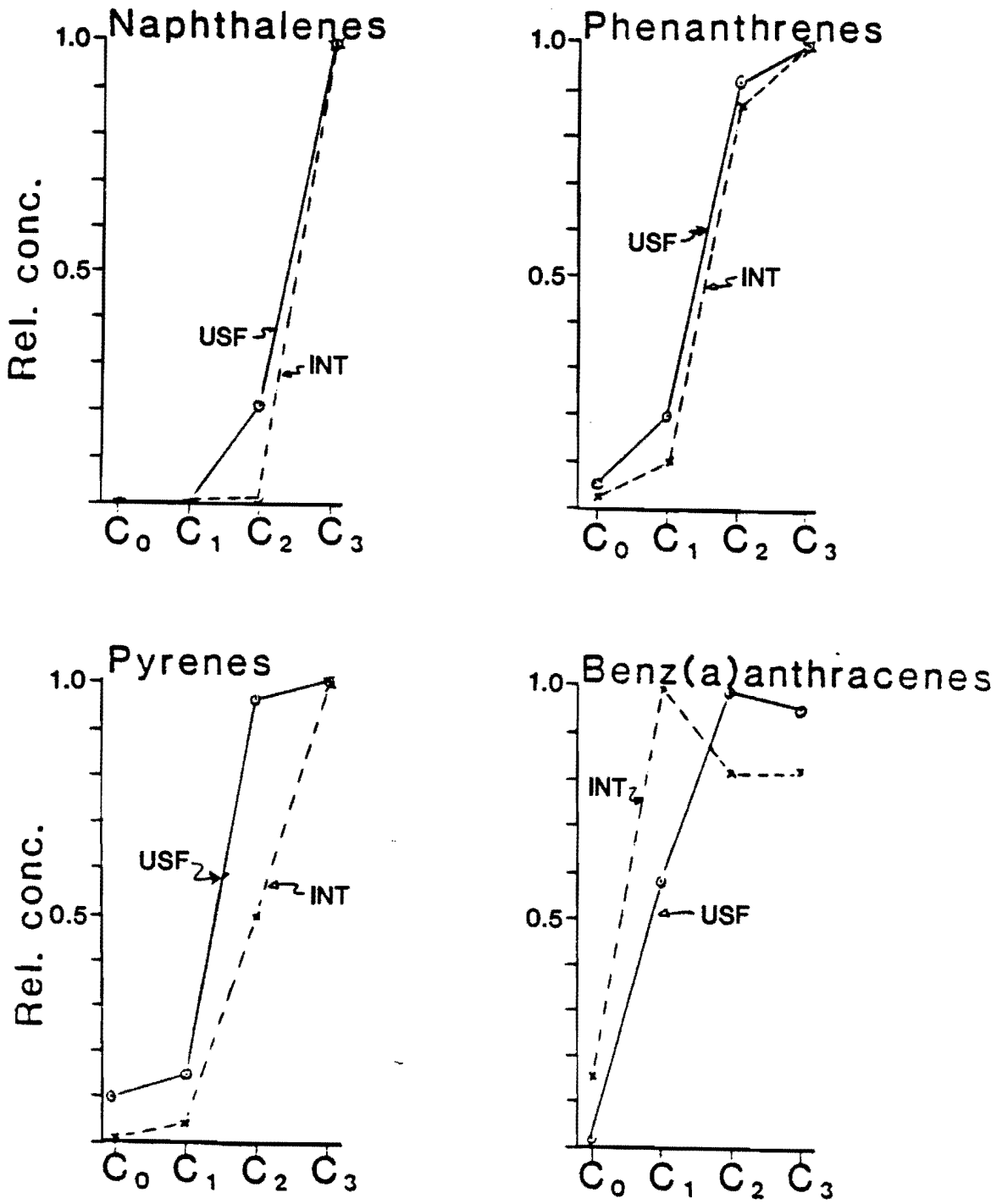



FIGURE 3-3. PAH alkyl homolog distributions in Tarball NNQ-507 analyzed as part of intercalibration study. Concentration is reported relative to most abundant peak. $C_0$ = parent compound, $C_1$ = methyl homolog, $C_2$ = dimethyl + ethyl homolog, $C_3$ = trimethyl + (methyl-ethyl) + propyl homologs. USF = University of South Florida. INT = Interscience, Inc.

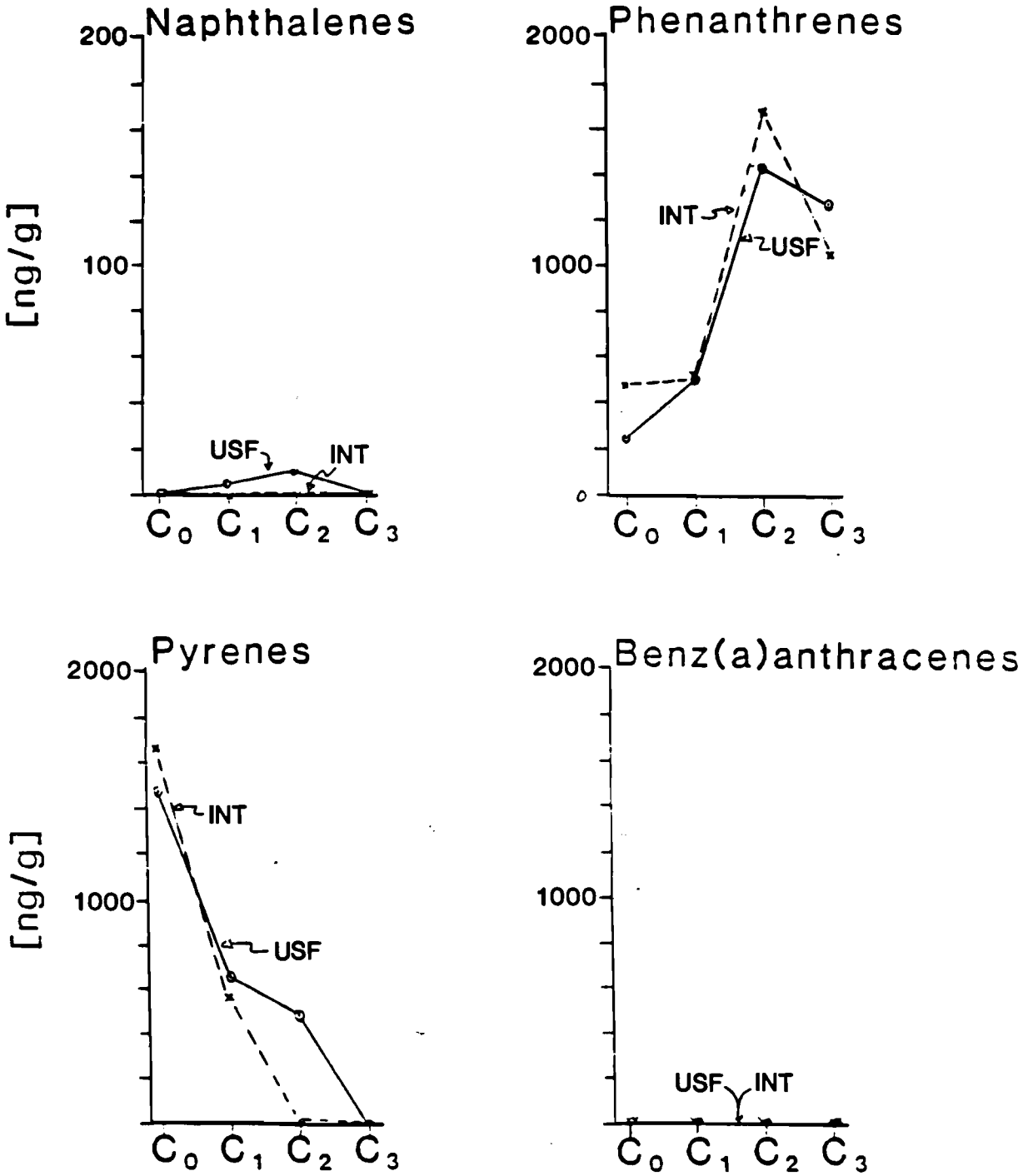



FIGURE 3-4. PAH alkyl homolog distributions in Hillsborough River sediment analyzed as part of intercalibration study. Concentrations of each compound are reported in ng/g dry weight sediments. $C_0$ = parent compound, $C_1$ = methyl homolog, $C_2$ = dimethyl + ethyl homolog, $C_3$ = trimethyl + (methyl-ethyl) + propyl homologs. For benz(a)anthracenes, no peaks were detected. No napthalenes were detected by Interscience. No benz(a)anthracenes were detected by either laboratory.
USF = University of South Florida. INT = Interscience, Inc.

similarities reported in the literature. South Louisiana Crudes are very light, highly paraffinic oils with low density, low viscosity, and low sulfur contents. These properties generally enable SLCO to spread rapidly on a water surface. Aliphatic hydrocarbons comprise approximately 73-81% of SLCO while aromatic hydrocarbons make up 16-19% and polar compounds make up 3-8% (Table 3-3; Lefcourt, 1973; Clark and Brown, 1977).

According to Hallett et al. (1983), however, all standard South Louisiana Crude Oils are not chemically alike. Studies in our laboratory confirmed this observation. Two South Louisiana Crude Oils provided by different companies (Gulf and Texaco) were analyzed by glass capillary gas chromatography (Figs. 3-5 and 3-6). The $nC_{17}$/pristane, $nC_{18}$/phytane, and pristane/phytane ratios were significantly different for the aliphatic fractions of the two crude oils. Similar molecular differences could be distinguished between the oils for other aliphatic and aromatic components.

Four drums of South Louisiana Crude Oil (SLCO) were received from Texaco for use during the present project. Nine replicate samples from these four drums were analyzed by GC and five replicates by GCMS. Results were compared with previously analyzed South Louisiana Crude Oil from Texaco (Table 3-4) and indicated that there were no significant aliphatic hydrocarbon differences between the old and new Texaco oils or between aliphatic and aromatic hydrocarbon components from different drums of Texaco oil to be used in the present study. Hence all new drums of South Louisiana Crude Oil on hand were considered uniform for use during this project.

## Solubility and Weathering of South Louisiana Crude Oil

Molecular variations in SLCO do not appear large enough to substantially alter the total solubility of South Louisiana Crude Oil. Anderson et al. (1974) determined the characteristics of water soluble extracts of SLCO (Table 3-5). According to Anderson et al. (1974), the solubility of total SLCO hydrocarbons in $20^{o}$/oo salinity ocean water at 20°C is 19.8 mg $l^{-1}$(ppm) measured by infra-red spectroscopy and 23.8 mg $l^{-1}$ measured by gas chromatography. Thus there is ≅14% variation in the reported solubility based on the two methods of analysis. These authors have shown that there is a preferential enrichment of aromatic hydrocarbons over n-paraffins in the water soluble fraction due to the higher solubilities of the aromatics.

Table 3-3. Chemical and physical characteristics of unweathered South Louisiana Crude Oil (from Clark and Brown, 1977).

| Characteristic or component | Crude oil: South Louisiana |
|---|---|
| API gravity (20°C) (°API) | 34.5 |
| Sulfur (wt%) | 0.25 |
| Nitrogen (wt%) | 0.69 |
| Nickel (ppm) | 2.2 |
| Vanadium (ppm) | 1.9 |
| Naphtha fraction (wt%) | 18.6 |
| Paraffins | 8.8 |
| Naphthenes | 7.7 |
| Aromatics | 2.1 |
| Benzenes | 0.2 |
| Toluene | 0.4 |
| $C_8$ aromatics | 0.7 |
| $C_9$ aromatics | 0.5 |
| $C_{10}$ aromatics | 0.2 |
| $C_{11}$ aromatics | 0.1 |
| Indans | - |
| High-boiling fraction (wt%) | 81.4 |
| Saturates | 56.3 |
| n-paraffins | 5.2 |
| $C_{11}$ | 0.06 |
| $C_{12}$ | 0.24 |
| $C_{13}$ | 0.41 |
| $C_{14}$ | 0.56 |
| $C_{15}$ | 0.54 |
| $C_{16}$ | 0.58 |
| $C_{17}$ | 0.59 |
| $C_{18}$ | 0.40 |
| $C_{19}$ | 0.38 |
| $C_{20}$ | 0.28 |
| $C_{21}$ | 0.20 |
| $C_{22}$ | 0.15 |
| $C_{23}$ | 0.16 |
| $C_{24}$ | 0.13 |
| $C_{25}$ | 0.12 |
| $C_{26}$ | 0.09 |
| $C_{27}$ | 0.06 |
| $C_{28}$ | 0.05 |
| $C_{29}$ | 0.05 |
| $C_{30}$ | 0.04 |
| $C_{31}$ | 0.04 |
| $C_{32}$ plus | 0 |
| Iso-paraffins | 14.0 |
| 1-ring cycloparaffins | 12.4 |
| 2-ring cycloparaffins | 9.4 |
| 3-ring cycloparaffins | 6.8 |
| 4-ring cycloparaffins | 4.8 |
| 5-ring cycloparaffins | 3.2 |
| 6-ring cycloparaffins | 1.1 |
| Aromatics (wt%) | 25.0 |
| Benzenes | 7.0 |
| Indans and tetralins | - |
| Dinaphthene benzenes | - |
| Naphthalenes | 9.9 |
| Acenaphthenes | - |
| Phenanthenes | 3.1 |
| Acenaphthalenes | - |
| Pyrenes | 1.5 |
| Chrysenes | - |
| Benzothiophenes | 1.7 |
| Dibenzothiophenes | 1.3 |
| Indanothiophenes | - |
| Polar materials (wt%) | 2.9 |
| Insolubles (wt%) | 1.2 |

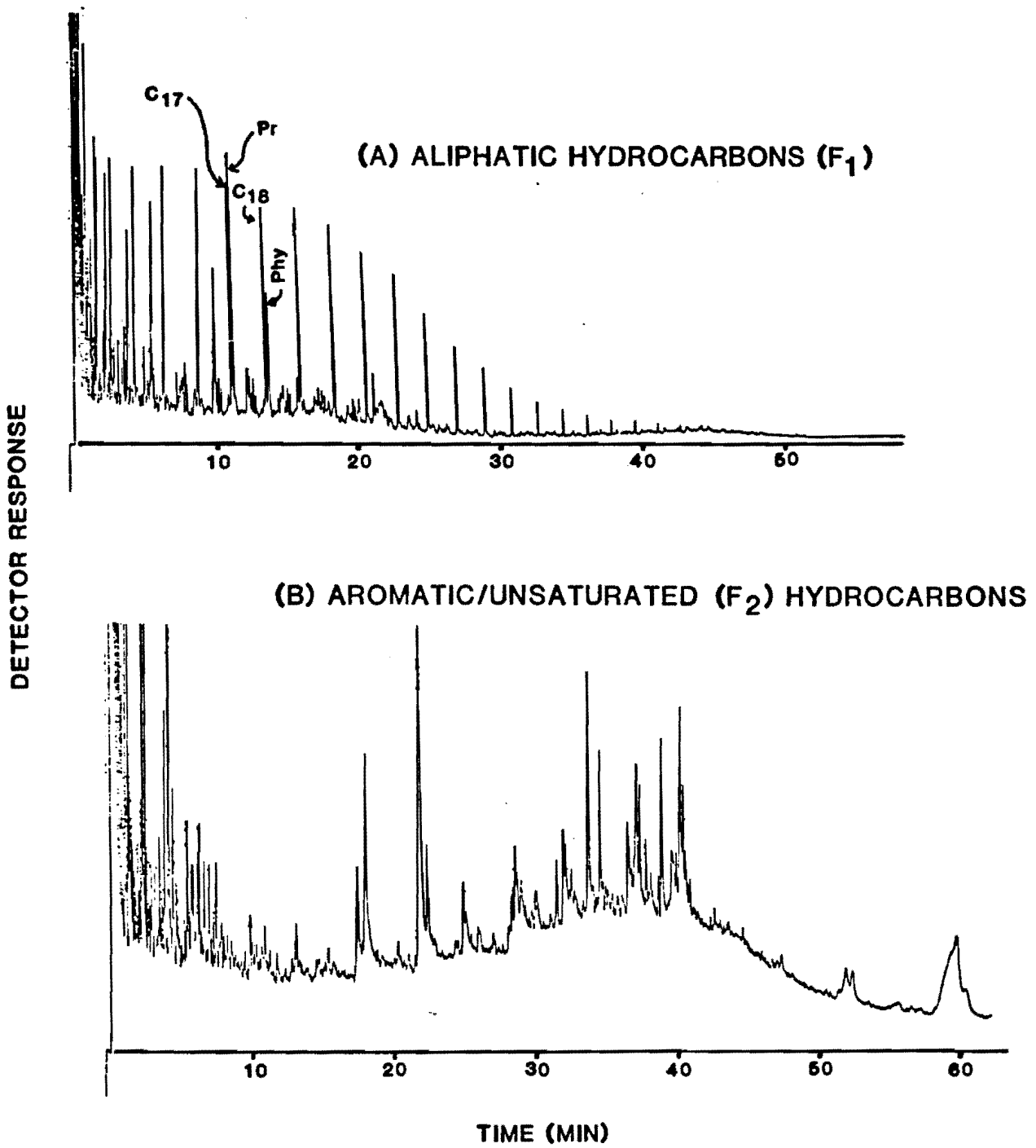



FIGURE 3-5. High resolution gas chromatograms of South Louisiana Crude Oil obtained from Gulf Oil Corporation. Pr = pristane, Phy = phytane

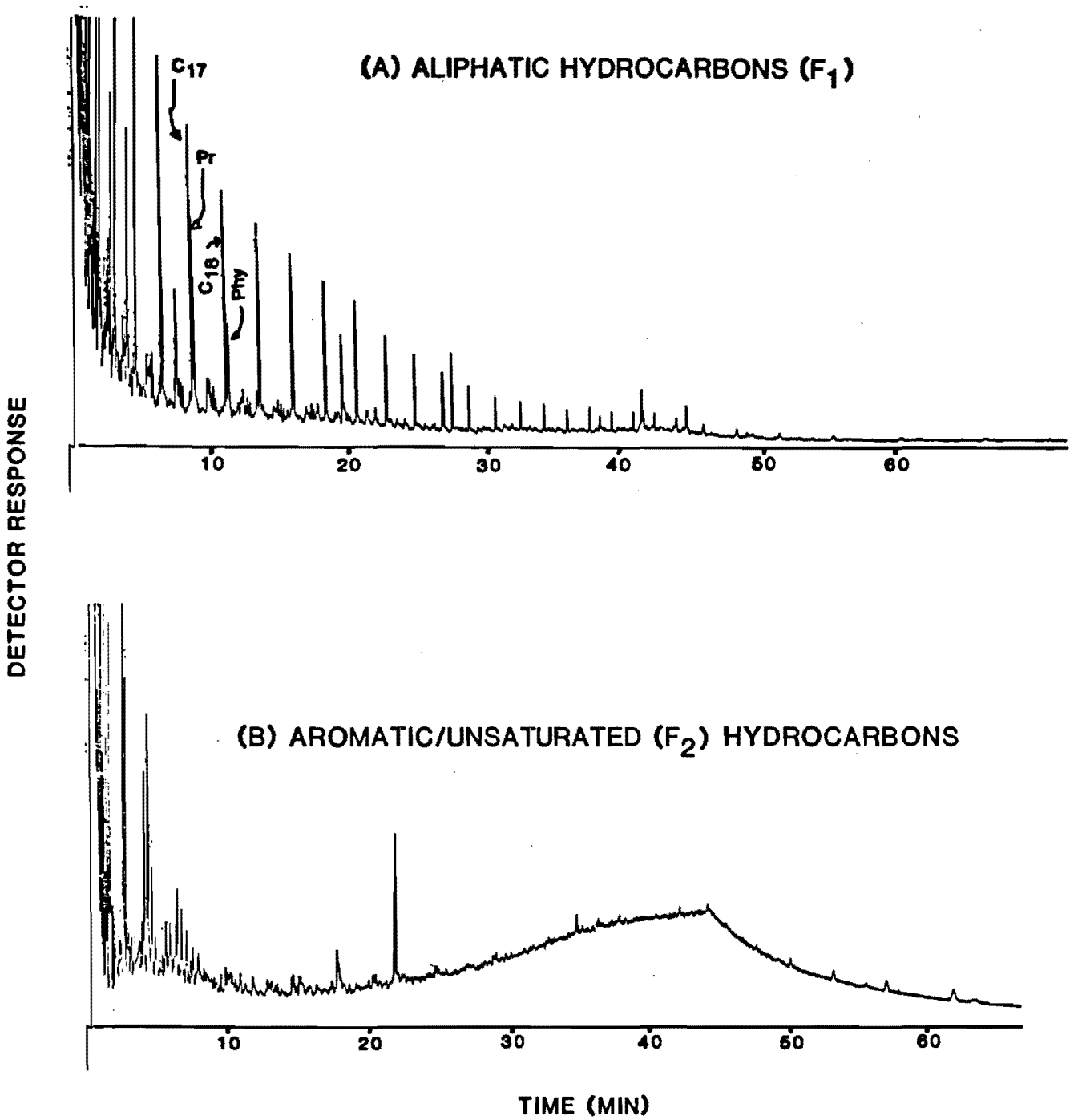



FIGURE 3-6. High resolution gas chromatograms of South Louisiana Crude Oil obtained from Exxon Corporation. Pr = pristane, Phy = phytane

Table 3-4. Characterization of previously analyzed (old) South Louisiana Crude Oil and newly acquired South Louisiana Crude Oil (Drums 1-4). SLCO= South Louisiana Crude Oil; 17= $nC_{17}$; 18= $nC_{18}$; Pr= pristane; Phy= phytane; n= number of replicates.

Aliphatic (F1) Fraction

| OIL | 17/Pr | 18/Phy | 17/18 | Pr/Phy | n-alkane range | n-alkane maximum |
|---|---|---|---|---|---|---|
| SLCO (old; n=1) | 1.1 | 1.9 | 1.2 | 2.0 | $nC_{13}$ - $nC_{35}$ | $nC_{15}$ |
| SLCO (new; n=9)[a] | 1.2±0.1 | 1.9±0.1 | 1.1±0.1 | 1.8±0.2 | $nC_{13}$ - $nC_{35}$ | $nC_{15}$ |
| Weathered Crude (n=6)[b] | 1.2±0.1 | 2.0±0.5 | 1.1±0.1 | 1.9±0.3 | $nC_{13}$ - $nC_{35}$ | $nC_{15}$ |

[a]No significant difference was observed between Drums 1, 2, 3, and 4.
[b]No significant difference was observed between pre-test and post-test.

Aromatic (F2) Fraction (Relative Concentrations)[c]

| | Naphthalenes | | | | Phenanthrenes | | | |
|---|---|---|---|---|---|---|---|---|
| OIL | $C_0$ | $C_1$ | $C_2$ | $C_3$ | $C_0$ | $C_1$ | $C_2$ | $C_3$ |
| SLCO (old; n=1) | 0.18 | 0.23 | 1.00 | 0.69 | 0.14 | 0.53 | 1.00 | 0.39 |
| SLCO (new; n=5) | 0.04±0.02 | 0.11±0.03 | 0.89±0.15[d] | 0.99±0.02 | 0.07±0.04 | 0.37±0.04 | 1.00 | 0.44±0.03 |

[c]No pyrenes or benz(a)anthracenes were observed.
[d]Represents only significant variation between Drums 1, 2, 3 and the weathered crude oils. (Drum 4 not yet analyzed).

Table 3-5. Chemical characteristics of water- soluble fraction of South Louisiana Crude Oil (from Anderson et al., 1974). Solubilities expressed in terms of mg/l.

| Compound | Solubility |
|---|---|
| Alkanes | |
| Ethane | 0.54 |
| Propane | 3.01 |
| Butane | 2.36 |
| Isobutane | 1.69 |
| Pentane | 0.49 |
| Isopentane | 0.70 |
| Cyclopentane + 2-methylpentane | 0.38 |
| Methylcyclopentane | 0.23 |
| Hexane | 0.09 |
| Methylcyclohexane | 0.22 |
| Heptane | 0.06 |
| $C_{16}$ n-paraffin | 0.012 |
| $C_{17}$ n-paraffin | 0.009 |
| Total $C_{12}$-$C_{24}$ n-paraffins | 0.089 |
| Aromatics | |
| Benzene | 6.75 |
| Toluene | 4.13 |
| Ethylbenzene + m-, p-xylenes | 1.56 |
| o-xylene | 0.40 |
| Trimethylbenzenes | 0.76 |
| Naphthalene | 0.12 |
| 1-Methylnaphthalene | 0.06 |
| 2-Methylnaphthalene | 0.05 |
| Dimethylnaphthalenes | 0.06 |
| Trimethylnaphthalenes | 0.008 |
| Biphenyl | 0.001 |
| Methylbiphenyls | 0.001 |
| Dimethylbiphenyls | 0.001 |
| Fluorene | 0.001 |
| Methylfluorenes | 0.001 |
| Dimethylfluorenes | 0.001 |
| Dibenzothiophene | 0.001 |
| Phenanthrene | 0.001 |
| Methylphenanthrenes | 0.002 |
| Dimethylphenanthrenes | 0.001 |
| Total saturates | 9.86 |
| Total aromatics | 13.90 |
| Total dissolved hydrocarbons measured | 23.76 |

Lefcourt (1973) determined the total solubility of SLCO in distilled water to be approximately 2 to 8 mg total hydrocarbons per liter (assuming 85% of the hydrocarbons in the crude oil is carbon). Lefcourt's calculations, however, were based solely on total organic carbon analyses of the subsurface waters and did not measure hydrocarbons directly. An additional difference between the two sets of data is that Lefcourt used organic-free distilled water as a substrate, while Anderson et al. (1974) used synthetic seawater. Synthetic seawater (i.e., Instant Ocean) is known to contain soluble organic matter. Boehm and Quinn (1973) reported that solubility of No. 2 fuel oil in seawater can increase by 2.5 times due to the presence of naturally occurring marine organic matter. Thus it is possible that Anderson et al.'s (1974) higher solubility levels reflect enhanced hydrocarbon dissolution due to the presence of dissolved organic matter. If Lefcourt's (1973) SLCO solubility values are multiplied by 2.5, the corrected solubility of SLCO ranges from 5 to 20 mg $l^{-1}$. Higher or lower values of dissolved organic matter in seawater will alter these values.

Lefcourt (1973) conducted an extensive study on the early weathering of South Louisiana Crude Oil. He examined evaporation, dissolution, and chemical alteration of the oil as a function of film thickness, exposure time, turbulence, water temperature and sunlight. Although all of Lefcourt's studies used distilled water as the substrate (in order to avoid any interfering effects from dissolved organic matter), the trends he observed would be expected to hold independent of salinity. Lefcourt's observations can be summarized as follows. The percent loss of selected n-alkanes in SLCO due to evaporation increases as the film thickness decreases. Most differential weathering effects take place below 1-2 mm film thickness. (Greater film thicknesses were therefore used throughout the present study.) As turbulence increases, the percent alkanes remaining in the oil decreases rapidly (i.e. dissolution and evaporation increase). As the oil-water exposure time increases, the percent hydrocarbons remaining rapidly decreases. Losses are accelerated by the presence of light and increased temperature. The most rapid dissolution effects occur within the first five hours of exposure.

Several authors (Boehm and Quinn, 1973; Sutton and Calder, 1975; Eganhouse and Calder, 1976) have shown that hydrocarbon solubility decreases with increasing salinity. Thus, if salinity effects dominated solubility

experiments conducted in seawater, Anderson et al.'s (1974) solubilities would have been expected to be lower than Lefcourt's (1973) values. Since Anderson et al.'s values were higher, the increased solubilities were probably promoted by the presence of dissolved organic matter. Hence, Anderson et al.'s data is expected to be more representative of Florida's coastal waters than Lefcourt's values. Thus the total solubility of South Louisiana crude oil is expected to be approximately 20 mg $l^{-1}$. However, this is not the value of naturally occurring hydrocarbons expected in Florida's coastal waters. Illiffe and Calder (1974) measured hydrocarbon values in the Gulf of Mexico Loop Current of up to 75 μg $l^{-1}$ (average = 47 μg $l^{-1}$) and in the Mid-Gulf and Yucatan Strait of up to 24 μg $l^{-1}$ (average 12 μg $l^{-1}$). These values are well below the solubility limits of South Louisiana Crude Oil. Based upon the above results, we suggested that behavioral studies on sea turtles exposed to the water soluble fraction (WSF) of South Louisiana Crude Oil be conducted in the concentration range of 10 μg $l^{-1}$ (the averaged observed in the mid-Gulf and Yucatan Strait) to ≅20,000 μg $l^{-1}$ (i.e. 20 mg $l^{-1}$; the upper limit expected in the proximity of a major spill).

Additional weathering experiments on SLCO received from Texaco Corp. were conducted at the University of South Florida. Oil was placed in two 2 gallon glass aquaria and was exposed to the environment for a period of six weeks. In one test, oil was spread on water collected from Bayboro Harbor ($S^{o}/oo \cong 26^{o}/oo$). A second test was conducted where the oil was placed in an aquarium with no initial subsurface water. Both aquaria were open at the top and exposed to sunlight on the roof of the Marine Science building at The University of South Florida. Despite efforts to cover each aquarium manually in the event of rain, this was not always successful due to unexpected sudden rainfalls which resulted in rainwater mixing with the two test solutions. By the end of the experiments approximately one inch of rain water accumulated in each aquarium. Gravimetric analysis indicated that in Experiment 1 (i.e., unfiltered Bayboro estuarine water), approximately 49.9% of the SLCO was lost due to dissolution or evaporation. In Experiment 2 (exposed to rainwater only), approximately 32.5% of the SLCO was lost due to these processes. Chromatograms of these weathered fractions are shown in Figures 3-7 and 3-8. The SLCO remaining after six weeks of weathering was an extremely viscous liquid. However, it never reached a solidified or semi-solidified state that could be used in the tar ball experiments. The reasons for this were

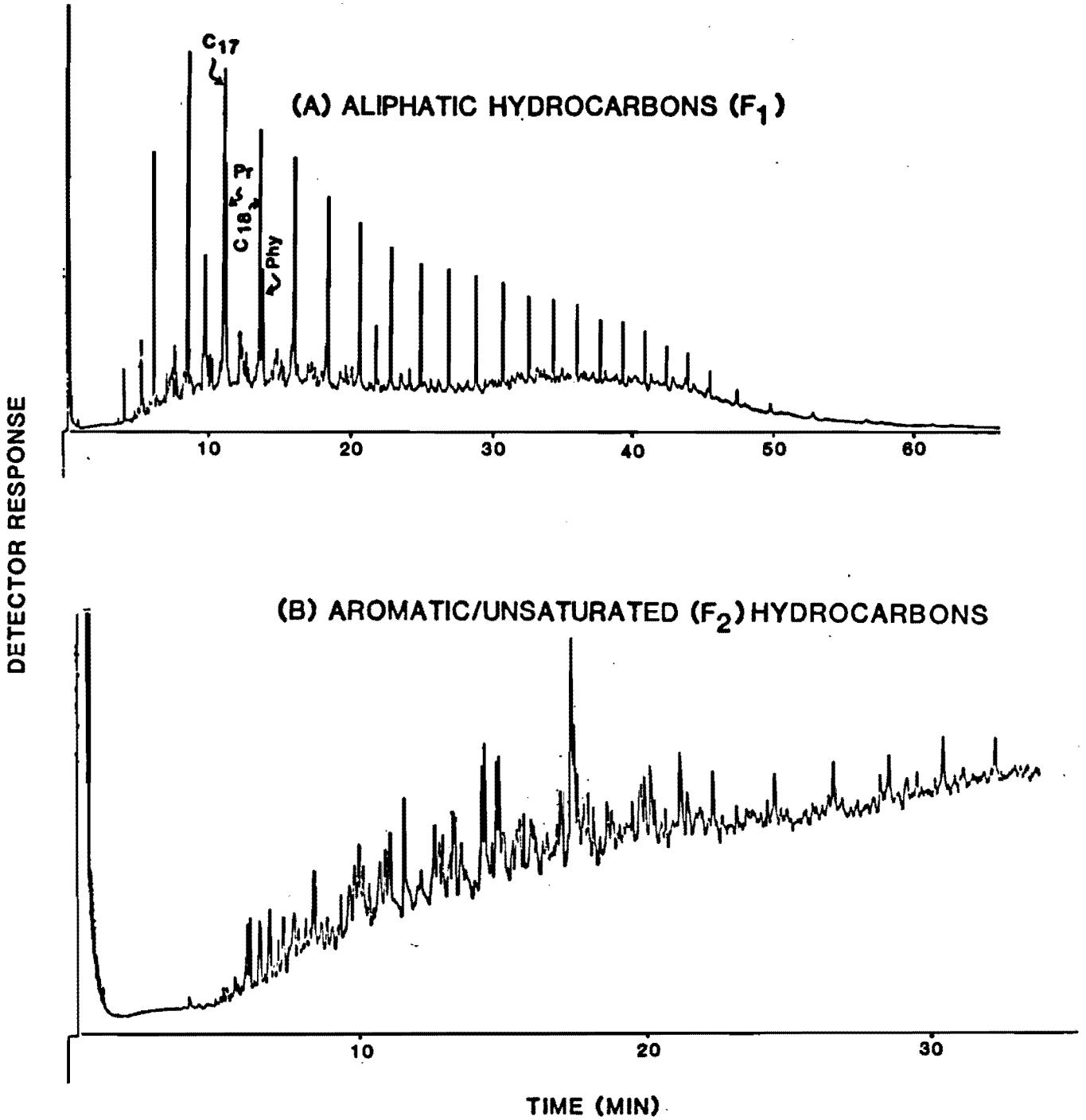



FIGURE 3-7. High resolution gas chromatograms of South Louisiana Crude Oil weathered over Bayboro Harbor estuarine water.
Pr = pristane, Phy = phytane

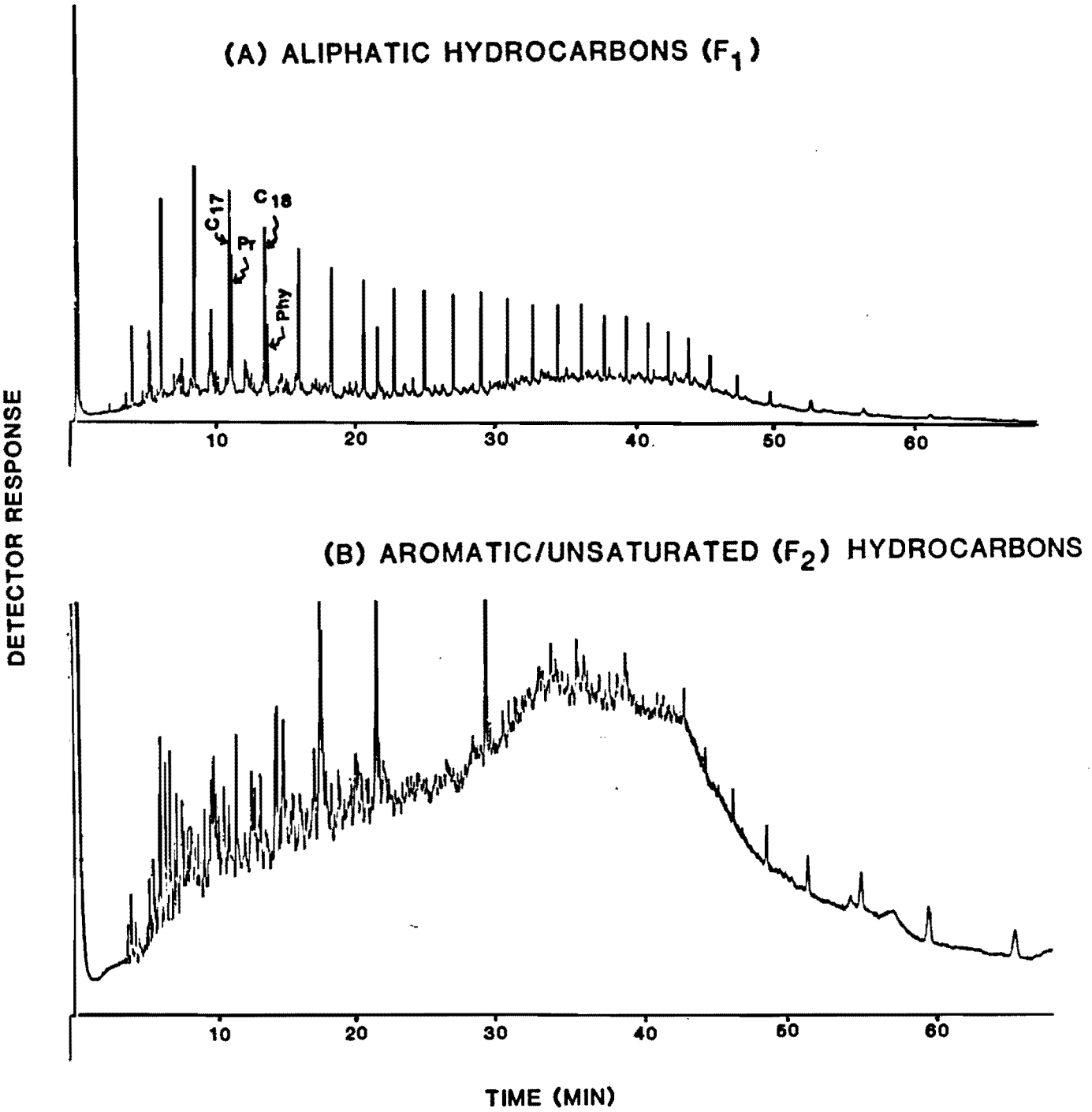



FIGURE 3-8. High resolution gas chromatograms of South Louisiana Crude Oil weathered over rainwater that accumulated during the experiment. Pr = pristane, Phy = phytane

twofold: (1) the inherent light nature of SLCO, and (2) the absence of agitation in the weathering experiments. Use of a submerged airstone to increase agitation may have enhanced the weathering process. Although we did not attempt this approach, we suspect that given the rather light nature of SLCO as well as the short weathering time scales available to us, we felt it was unlikely that tarballs would have been formed even with the use of airstones. It is more likely that a mousse would have formed. That would have been unusable for the behavioral and physiological studies in this project. Furthermore, later results showed that weathering SLCO for 48 hours over sea water with bubbling from an airstone providing mixing did not produce tarballs. We therefore suggested that it would be fruitless to try to generate tar balls by artificially weathering South Louisiana Crude Oil and recommended the use of tar balls collected in the field.

Weathering studies carried out on SLCO during a turtle behavior experiment indicated that although key aliphatic hydrocarbon ratios did not change during the course of an experiment (Table 3-6), these ratios did change upon longer weathering times (Figs. 3-7 and 3-8). Laboratory and tank weathering studies confirmed that key aliphatic and aromatic hydrocarbon ratios remained constant for the period of artificial weathering prior to and during exposure experiments.

Behavioral Studies

Based upon an extensive literature survey, we suggested that in behavioral studies, sea turtles should be exposed to an SLCO water accommodated fraction in the concentration range of 10 to ≅20,000 $\mu g\ l^{-1}$ (p. 23). It was also concluded that pre-weathering the oil would produce a more realistic exposure situation for the turtles as an oil spill would weather rapidly under natural conditions. The major changes in oil composition occur in the first 24-48 hrs. Therefore, the oil for behavior and exposure studies was weathered for 48 hrs over sea water with mixing by bubbling with an airstone.

Analyses of pre-test, mid-test, and post-test tank water used in behavioral studies at Sea World's MSCC (under the direction of Dr. Daniel K. Odell) indicated that initial tests were run at 70 to 2043 $\mu g\ l^{-1}$ while later tests were run at hydrocarbon levels of <1 to 116 $\mu g\ l^{-1}$ (Table 3-6). We do not know why the concentrations were higher in SLCO I than in the other

Table 3-6. Results of hydrocarbon analyses of tank water and weathered crude oil used in behavioral studies. (17= $nC_{17}$; 18= $nC_{18}$; Pr= pristane; Phy= phytane; SLCO= South Louisiana Crude Oil).

| Sample | Alkane Conc. (µg/l) | 17/Pr | 18/Phy | 17/18 | Pr/Phy | n-alkane Range[b] | n-alkane Maximum[c] |
|---|---|---|---|---|---|---|---|
| SLCO I - Pre-test water (n=1) | 70 | n.d.[a] | n.d. | n.d. | n.d. | $nC_{16}$-$nC_{22}$ | uniden. |
| SLCO I - Mid-test water (n=1) | 2043 | 0.5 | 0.8 | 0.7 | 1.3 | $nC_{14}$-$nC_{35}$ | pristane |
| SLCO I - Post-test water (n=1) | 1014 | 0.2 | 0.6 | 0.5 | 1.3 | $nC_{14}$-$nC_{25}$ | pristane |
| SLCO II - Pre-test water (n=1) | 0 | - | - | - | - | - | - |
| SLCO II - Mid-test water (n=1) | 1.35 | 0 | - | - | - | pr-C23 | pr |
| SLCO II - Post-test water (n=1) | 0.71 | 0 | - | - | - | C13-pr | pr |
| SLCO II - Pre-test weathered SLCO | - | 1.3 | 1.9 | 1.1 | 1.6 | C13-C27 | C13 |
| SLCO II - Post-test weathered SLCO | - | 1.3 | 2.3 | 1.4 | 2.4 | C13-C31 | C14 |
| SLCO III - Pre-test water (n=1) | 0 | - | - | - | - | - | - |
| SLCO III - Mid-test water (n=1) | 26.7 | 1.0 | 1.6 | 0.8 | 1.3 | C16-C28 | C19 |
| SLCO III - Post-test water (n=1) | 115.8 | 1.3 | 1.6 | 1.2 | 1.5 | C13-C32 | C17 |
| SLCO III - Pre-test weathered SLCO | - | 1.4 | 2.1 | 0.6 | 1.7 | C13-C31 | C13 |
| SLCO III - Post-test weathered SLCO | - | 1.5 | 2.2 | 1.3 | 1.8 | C13-C32 | C13 |
| SLCO IV - Pre-test water (n=1) | 26.4 | - | - | - | - | $nC_{23}$-$nC_{28}$ | $C_{23}$,$C_{28}$ |
| SLCO IV - Mid-test water (n=1) | 36.8 | - | - | - | - | $nC_{13}$-$nC_{28}$ | $nC_{20}$ |
| SLCO IV - Post-test water (n=1) | 141.3 | 0.84 | 2.63 | - | - | $nC_{13}$-$nC_{29}$ | $nC_{15}$ |
| SLCO IV - Pre-test weathered SLCO | - | 1.1 | 1.5 | - | - | $nC_{13}$-$nC_{29}$ | $nC_{13}$ |
| SLCO IV - Post-test weathered SLCO | - | 1.0 | 1.5 | - | - | $nC_{13}$-$nC_{30}$ | $nC_{13}$ |
| SLCO V - Pre-test water (n=1) | 1.5 | - | 0.3 | - | - | $nC_{18}$-$nC_{32}$ | $nC_{31}$ |
| SLCO V - Mid-test water (n=1) | 33.9 | 0.9 | 1.6 | - | - | $nC_{15}$-$nC_{29}$ | pr,$nC_{18}$ |
| SLCO V - Post-test water (n=1) | (Sample lost during analysis) | | | | | | |
| SLCO V - Pre-test weathered SLCO | - | 0.2 | 1.0 | - | - | $nC_{11}$-$nC_{33}$ | pr,$nC_{13}$ |
| SLCO V - Post-test weathered SLCO | - | 0.2 | 1.0 | - | - | $nC_{11}$-$nC_{32}$ | pr,$nC_{14}$ |
| Tar Ball I - Pre-test water | 37.8[d] | - | 3.1 | - | 1.56 | $C_{16}$-$C_{25}$ | $C_{20}$ |
| Tar Ball II - Post-test water | 0 | - | - | - | - | - | - |

a. none detected
b. series not homologous
c. unidentified biogenic peak
d. C17 is anomalously large or, most likely, masked by co-eluting peak.

experiments. Experimental conditions were the same. Although the <1 μg/l hydrocarbon experiments were conducted at lower than recommended values, these levels are obviously representative of hydrocarbon concentrations in certain parts of the Gulf of Mexico as witnessed by the very low background (i.e. pre-test) levels in several of the experiments (Table 3-6). In two of the five tests conducted, hydrocarbon concentrations increased in the mid-test samples and then decreased towards the end of the experiment. This decrease probably resulted from evaporation or physical mixing processes during the course of the experiments. In the SLCO III and IV, water accommodated hydrocarbons increased throughout the experiment. These results were probably due to differences in evaporation or mixing during the study due to the swimming of the turtles. No differences in behavioral response were noted despite the differences in water accommodated hydrocarbon concentrations (Section 4.0). It should be noted that the behavior experiments were designed to determine changes in behavior in the presence of an oil slick, not at a specific concentration of water accommodated oil. Analyses were done to ensure that we did not exceed reasonable levels which might be found in the natural environment or in the vicinity of a spill.

Physiology Studies

Periodic water samples were collected throughout the chronic exposure studies in Dr. Peter L. Lutz's laboratory. A complete series from Chronic Exposure Experiment #5 is shown in Table 3-7. Analyses of the water accommodated fraction, the particulate fraction, and turtle feces indicated that most of the oil resides in the "water accommodated" fraction (Table 3-7). However, it is inaccurate to designate these samples as the water accommodated fraction. Due to the movements of the turtles oil droplets were entrained continuously making analysis of the actual water-accommodated fraction impossible. These oil droplets are responsible for the extremely high values reported in Table 3-7. Only small amounts of hydrocarbons were observed in the particulate (filtered) fraction. Substantial amounts of hydrocarbons were observed in the turtle feces from Chronic Exposure Experiment V (Table 3-7). The chemical nature of these hydrocarbons, however, did not resemble the initial oil until several days after the turtles had been exposed to the oil. The turtles were exposed to 0.05 cm

(chronic) layer of oil for 96 hours. After 9 days, concentrations of oil in fecal material accounted for nearly 100% of the fecal hydrocarbons.

Small amounts (28-93 mg) of turtle neck and flipper tissues were received from the physiological experiments to investigate hydrocarbon contamination (Tables 3-7 and 3-8). These samples were near our lower detection limits for hydrocarbon analyses. Therefore, these results are probably only accurate to within a factor of two. Based upon this, there appear to be no significant differences between tissues from control and oiled turtles. There also appear to be no systematic differences between neck and flipper tissues, with hydrocarbon concentrations generally ranging from 0.2 to 3.9 mg $g^{-1}$. Evidence of oil contamination was observed in only one experiment (Expt. 4, Table 3-8). Both neck and flipper tissues from this experiment showed minor amounts of oil present, but these amounts were significantly overshadowed by naturally occurring biogenic peaks ($nC_{19}$ and $nC_{31}$), a recurring problem in tissue analyses. However, we cannot make the inference that there was no uptake of hydrocarbons by these tissues during the experiment. We can only state that the uptake was not significantly different from the control turtles based on our limits of detection.

## 3.3 Summary

The objectives of this portion of the program were to provide analysis of water, oil, tar balls, and turtle tissue for both the behavior and physiological experiments. Ancillary samples were also analyzed from field collected stranded turtles. Oil samples were analyzed to determine weathering characteristics of the SLCO to develop standardized dosing procedures and insure that the supply of SLCO was characteristic of the type. It was decided that preweathering the oil was more representative of oil that turtles would encounter in the natural environment. Therefore, the oil used for the behavior and physiology experiments was weathered outdoors with mixing over sea water for 48 hrs prior to use. Based on the literature survey (see discussion on p. 23) a concentration range of 10 to about 20,000 μg $l^{-1}$ was determined to be suitable for the behavior studies. Water analyses from the behavior experiments indicated that the concentrations ranged from 33.9 to 2043 μg $l^{-1}$ depending upon water temperature and mixing.

Table 3-7. Hydrocarbon analyses of samples from chronic oil exposure experiment (physiology experiment) #5 and acute exposure experiment #3.

| Sample | Day | Oiled | Control | n-alkane | n-alkane maximum |
|---|---|---|---|---|---|
| Water (mg $l^{-1}$) | 1 | 164.2 | 1.0 | $nC_{13}$-$nC_{32}$ | nC13 |
| | 4 | 62.4 | 0.1 | $nC_{13}$-$nC_{29}$ | $nC_{18}$ |
| Filter (mg $l^{-1}$) | 1 | 0.1 | 0.1 | $nC_{16}$-$nC_{32}$ | $nC_{18}$ |
| | 4 | 0.1 | 0.1 | $nC_{18}$-$nC_{25}$ | $nC_{21}$ |
| Feces (mg $l^{-1}$) | 1 | 80.9 | -- | none detected | -- |
| | 2 | -- | 33.5 | $nC_{15}$,$nC_{17}$ | $nC_{15}$ |
| | 9 | 22000 | 0.6 | $nC_{13}$-$nC_{29}$ | $nC_{17}$ |
| Flipper (mg $g^{-1}$) | 4 | 2.6 | 1.0 | none detected | -- |
| | 4 | 2.5 | -- | none detected | -- |
| Acute Exposure Experiment #3 | | | | | |
| Feces (mg $l^{-1}$) (water extract only) | 2 | 5.89 | (22% oil, 78% biogenic) | | |

Table 3-8. Turtle tissue samples (Received 11/5/84)[1] from chronic oil exposure experiments analyzed at the University of South Florida.

| Turtle Experiment | Tissue | Total Hydrocarbons (mg/g) Oiled | Control | Comments |
|---|---|---|---|---|
| #1 | Neck | 1.64 | 3.2 | No evidence of oil in sample or control. $C_{19}$ and $C_{31}$ alkanes dominant in both. |
| | Flipper | n.a.[2] | n.a. | Sample not provided. |
| #2 | Neck | 0.68 | 0.62 | Evidence of trace amounts of oil in sample and control. $C_{19}$ and $C_{31}$ dominant. |
| | Flipper | 0.67 | 1.05 | No evidence of oil in sample or control. $C_{19}$ and $C_{31}$ alkanes dominant in both. |
| #3 | Neck | 0.17 | 1.27 | No evidence of oil in sample or control. $C_{19}$ and $C_{31}$ alkanes dominant in both. |
| | Flipper | 3.88 | 1.34 | No evidence of oil in sample or control. $C_{19}$ and $C_{31}$ alkanes dominant in both. |
| #4 | Neck | 1.82 | n.a. | Evidence of minor amounts of oil present. Alkane range $C_{22}$-$C_{28}$. $C_{31}$ biogenic peak dominant. |
| | Flipper | 0.86 | n.a. | Evidence of trace amount of oil in sample. $C_{31}$ alkane dominant. |

[1] Sample amounts (28-93 mg) near lower detection limits for extraction.
[2] n.a. = not analyzed.

In the physiology experiments the concentration of the water accommodated fraction was open to question due to the entrainment of oil droplets due to the turtle's movements. Analysis of water, particulate, and fecal samples indicated that most of the oil remained in the water fraction. Substantial amounts of hydrocarbons were found in the feces which showed that the turtles did actually ingest the oil. Neck and flipper tissue samples from the oiled turtles did not show significant oil contamination. However, these samples were at the lower limits for hydrocarbon analyses and were overshadowed by naturally occurring biogenic peaks.

## 4.0 BEHAVIORAL RESPONSE TO OIL (D. K. Odell and C. MacMurray)

The possible effects of oil pollution on sea turtles were reviewed at a workshop held in Mississippi in 1982 (Keller and Adams, 1983; Witham, 1983). Possible routes of contamination are through direct contact with oil floating on the surface of the sea, direct ingestion of oil or tar, ingestion of food items contaminated with petroleum hydrocarbons, and inhalation of volatile hydrocarbons of turtles. Witham (1978, 1983) expressed particular concern with the occurrence of external fouling of turtles with tar and ingestion of tar balls by young sea turtles. Externally fouled loggerhead, green and hawksbill sea turtles have been found. The route of contamination is not clear. Is the contact accidental or do the turtles actively approach the tar? Do the turtles treat the tar balls as potential food items? Oil/tar fouled sea turtles occur relatively infrequently. From 1980-1984, 50 (3.2%) of the stranded turtles reported in Florida were categorized as petroleum related (Section 6.0). It should be noted that these 50 reports represent a relative, not absolute figure, for the number of turtles contaminated with oil. We do not know how many turtles never beach or go unreported.

The scientific literature contains only limited direct reference to the effects of oil on sea turtles (e.g. Witham, 1978). Kleerekoper and Bennett (1976) concluded that the water soluble fraction of Louisiana crude oil did have an effect on locomotor behavior of 'juvenile' green sea turtles. However, they were not in a position to state the biological significance of these observations.

Turtles may or may not respond to the presence of water soluble hydrocarbons (approach or avoid). However, to respond the turtles must first detect the presence of these compounds. Detection could be by either chemoreceptory, visual, or tactile means. Earlier studies (Manton, Karr, and Ehrenfeld, 1972) demonstrated the chemoreceptive capabilities of sea turtles. Ehrenfeld and Koch (1976) indicated that aerial vision in sea turtles was apparently poor. Vision underwater is presumably good, however. Incidental observations made during this study on captive sea turtles feeding on the bottom of their tanks or pools strongly support assumptions of good aquatic visual capabilities. Thus, sea turtles may be able to detect oil spills and tar balls either visually or with chemoreception, but their responses are unknown.

In addition to encountering oil or tar in the open ocean, turtles face another risk in that they must come to shore to lay their eggs. Beach areas suitable for turtle nesting with a high rate of hatching success have dwindled due to destruction of habitat and the activities of man. During the nesting season, turtles aggregate in large numbers making them particularly susceptible to an oil spill occurring during this time. Oil and large amounts of tar in the water could also present a barrier to hatchlings as they move down the beach into the sea. They would be coated with oil or tar before they could reach open water.

The objective of this portion of the study was to determine if loggerhead and green sea turtles of a variety of age classes showed a behavioral response (attraction/repulsion) to weathered South Louisiana Crude oil or to tar balls. A key factor in reaching the experimental goals and in designing the experimental protocol was that the studies be carried out under conditions (e.g. light, water temperature, salinity) that approximated the natural environment. This would allow a more realistic interpretation of the experimental results with respect to sea turtles in their natural environment.

## 4.1 Methods

The major experimental milestones of this study are summarized in Table 4-1. Hatchling loggerhead and green sea turtles were obtained from turtle headstart programs (= hatched under controlled conditions). Loggerhead hatchlings were from programs in Florida in both year 1 and 2. In year 1, green sea turtles were obtained from a program in Texas but in year 2 they were all from Florida. We encountered a shortage of sea turtles (particularly green sea turtles) in year 1 due to the October award of the contract. The nesting/hatching season ends in September and by October most headstart turtles had already been released.

Mortality, primarily due to bacterial infections, further reduced stocks of year 1 animals and, as a result, a full suite (20/species) of turtles was not available for the initial experiments. During year 1 an ample supply of hatchlings of both species was secured for the experiments in Year 2. Turtles were taken from several clutches to maximize genetic variability of the stock. A backup stock of turtles was maintained at the Clearwater Marine

Table 4-1. Summary of major activities and milestones for the study of the effect of weathered crude oil and tar balls on sea turtle behavior.
CC = Caretta caretta (loggerhead turtle); CM = Chelonia mydas (green turtle)

| ACTIVITY/MILESTONE | DATE | STUDY CC | YEAR I CM | TURTLE AGE | STUDY CC | YEAR II CM | TURTLE AGE |
|---|---|---|---|---|---|---|---|
| Start Program | 10/83 | | | | | | |
| Equipment Purchase & Apparatus Constr. | 11/83 | | | | | | |
| Obtain Turtles | 12/83 | X | X | 6 MO | | | |
| Normal Behavior OBS (w/o Oil Boom) | 01/84 | X | X | 7 | | | |
| Mineral Oil Experiment I | 03/84 | X | X | 9 | | | |
| Long Term Behavior OBS | 04/84 | X | | 10 | | | |
| Mineral Oil Experiment II | 04/84 | X | X | 10 | | | |
| Weathered Crude Oil Experiment I | 05/84 | X | X | 11 | | | |
| Normal Behavior, Additional Turtles | 06/84 | X | | 12 | | | |
| Preliminary Tar Ball Experiments | 06/84 | X | | 12 | | | |
| Normal Behavior, Additional Turtles | 07/84 | X | | 13 | | | |
| Obtain New Turtles | 08/84 | | | | X | X | 0 MO |
| Transport New Turtles to Sea World | 08/84 | | | | X | X | 1 |
| Weathered Crude Oil Experiment II | 09/84 | X | X | 15 | | | |
| Tar Ball Experiment I | 10/84 | X | X | 16 | | | |
| Normal Behavior OBS | 11/84 | | | | X | | 3 |
| Normal Behavior OBS | 12/84 | | | | | X | 4 |
| Weathered Crude Oil Experiment III | 12/84 | X | | 18 | X | X | 4 |
| Tar Ball Experiment II | 02/85 | X | | 20 | X | X | 6 |
| Weathered Crude Oil Experiment IV | 03/85 | | | | X | X | 7 |
| Qualitative Behavior OBS w/Sargassum | 03/85 | | | | X | X | 7 |
| Preliminary Straight Boom OBS | 03/85 | | | | X | X | 7 |
| Tar Ball Experiment III | 05/85 | | | | X | X | 9 |
| Weathered Crude Oil Experiment V | 06/85 | | | | X | X | 10 |
| Tar Ball Experiment IV | 07/85 | | | | X | X | 11 |
| End of Project | 09/85 | | | | | | |

Science Center in Clearwater, Florida. About 50 each of loggerhead and green sea turtles were maintained at the Sea World MSCC where all the experiments were conducted. Turtles were maintained in shallow ponds with flowing sea water. Each turtle was individually tagged for identification purposes with a 'spaghetti' tag and later with a metal cattle ear tag as they increased in size. Spaghetti tags were placed in the webbing of the hind flipper and the metal tags in the axilla of the front flipper. Turtles were fed a combination of cut shrimp and squid, and Purina turtle chow. The turtles consumed all three food items readily. They were weighed and measured (carapace length and width using calipers) at least once per month to document growth. Overall health of the turtles was monitored daily by the Sea World staff and monthly by the project veterinarian.

All experiments were conducted in fiberglass pools about 8 feet (231 cm) in diameter with a working depth of about 50 cm. Pool color was light blue (the only color available). During control and experimental observations the pool circulation and filtration was shut off. Pool water was filtered and exchanged overnight except when oil was on the surface. Pool water was not filtered or exchanged between observations on individual turtles. Water temperature was not regulated but approximated ambient values ($\pm$1-2°C) in Florida Bay. Light intensity was reduced by greenhouse shade cloth above the pools and provided an environment similar to that in which the turtles were maintained between experiments. Diurnal patterns of light intensity and angle of incident light were not otherwise altered.

Each experimental trial was recorded on videotape using a Panasonic PK-957 camera and a Quasar portable VHS video tape recorder. A separate voice track was added later in an attempt to obtain better data on respiration intervals. Videotapes were reviewed as many times as necessary in the laboratory for data extraction.

Normal behavior of the sea turtles in the experimental pool was documented after the animals had been at the Sea World facility for about one month. This documentation of normal behavior was used to determine test design for the experiments. Additionally, the behavior of a control group of the turtles was videotaped prior to each experimental series. This procedure corrected for changes in behavior as the turtles aged or ambient conditions changed seasonally. All turtles (experimental and control) were fasted a minimum of 24 hours prior to use. Turtles were fasted to provide a uniform

base for comparative purposes. Turtles used immediately after feeding may respond differently from those tested a longer time after feeding. Fasting was used to stimulate feeding activity in the tar ball tests as well as provide a uniform base for comparison. Data collected included horizontal position in the pool (center, middle, edge), position in the water column (surface, middle, bottom), activity (swimming vs. not swimming), and direction of swimming (clockwise, counter clockwise). The pool was also divided into 8 "quadrants" for further partitioning of turtle location (Figure 4-1). Initially (normal behavior, year 1 turtles), each turtle was observed once for 45 minutes and data collected at 1 minute intervals. In all trials only one turtle was in the pool. We subsequently determined that a 30 minute period was adequate to document behavior. Comparisons made with 45 minute trials of normal behavior with 30 minute trials showed no difference by the Chi-square test. Long-term (6-8 hour) observations on individual turtles in the experimental pool revealed no change in behavior from that seen during the first 30 minutes in the pool. We also determined that data collected at one minute intervals (i.e. stratified sampling) was statistically equivalent to 30 observations taken randomly over the 30 minute observation period. Data on light intensity at the surface and water temperature in the experimental pool were collected between individual trials.

The ages of the turtles used in this study ranged from hatchlings (3-4 months old) to about 20 months. Loggerheads older than about 20 months and greens older than about 16 months were too large and too active, respectively, for use in the experimental pool. Larger pools and the space to keep the minimum number of turtles (20 of each species) were not available. We did not determine the sex of our turtles as this requires biochemical or histological (lethal) tests. The sex of juvenile (hatchling to age of sexual maturity) sea turtles is not obvious from external morphology.

Oil Slick Experiments (Colored Mineral Oil and South Louisiana Crude Oil)

The details of the design of both the equipment and protocol for the study of the behavioral response of sea turtles to a slick of weathered crude oil was based on our initial observations of turtle behavior in the experimental tank and could not be predicted *a priori*. The turtles, both

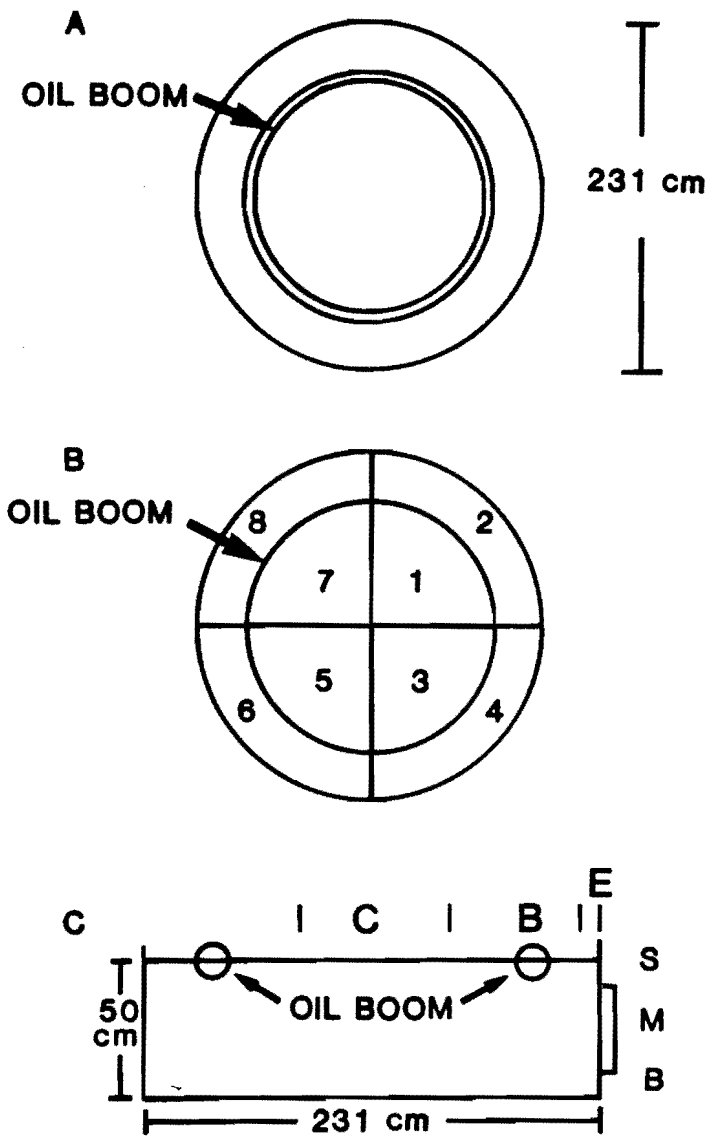



FIGURE 4-1. Diagram of experimental setup for oil slick experiments. A. Top view of experimental tank showing oil boom in place. Oil is placed in the outer annulus during an experiment. B. The surface of the tank divided into eight equal area quadrants to document the horizontal position of the turtle. Note that even numbered quadrants are edge quadrants and odd numbered quadrants are center quadrants. C. The vertical profile of the tank showing the three areas used to document the vertical position of the turtle, S = surface, M = mid-water, B = bottom, C = center, B = intermediate, E = edge.

loggerheads and greens, spent almost all of their time at the edge of the pool (Tables 4-2 to 4-5; Figures 4-2 to 4-7). To further complicate analysis of their behavior, it became apparent, when we analyzed their position by edge quadrant (2, 4, 6, and 8), that the turtle's presence in these edge quadrants was not random (Tables 4-6 to 4-9; Figures 4-8 to 4-12) for either loggerhead or green turtles. The turtles showed a distinct preference for specific edge quadrants, primarily quadrants 2 and 4 in the January 1984 normal behavior tests (Tables 4-6 and 4-7; Figures 4-8 and 4-10). This preference was not constant, however, in that in the December 1984 normal behavior tests for Year 2 animals, both species appeared to prefer edge quadrants 2 and 8 (Tables 4-8 and 4-9; Figures 4-11 and 4-12). At least one possible factor in this preference appeared to be differences in lighting as the sun angle changed. Although the shade cloth over the tanks reduced the light intensity, it did not change the sun angle. This hypothesis concerning the causes for quadrant preference was not tested as it was not directly related to the objectives of this study.

This preference for the edge and variable preference in edge quadrant made design of a test protocol challenging. The proposed circular design for the boom to separate the pool into equal areas was still the best option (Fig. 4-1). We selected the circular boom configuration (as opposed to a straight boom) because it allowed uniform light conditions as the sun angle changed over the course of the day. A straight boom configuration, under ambient light conditions, would have differing light conditions on the oiled and unoiled areas depending on the time of day. Therefore, considering the data on normal behavior, we submitted a revised experimental plan (Appendix B) suggesting that we place the oil (mineral and weathered crude) at the edge of the pool in order to maximize contact. Although we recognized that the avoidance response to oil by the turtles would have to be stronger than the preference for the edge, any avoidance response would indicate a definite behavioral change in the turtle.

We constructed a circular oil boom from 2 inch diameter swimming pool vacuum line. The boom floated at the surface and divided the pool into two parts with equal surface area (see Figure 4-1). It allowed the turtles to swim freely between quadrants. The boom was secured to the edge of the pool with monofilament line and duct tape.

Table 4-2. Summary of observations on year 1 (CC1) loggerhead sea turtles (*C. caretta*); horizontal and vertical distribution in pool (See Figure 4-1c)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | HORIZONTAL POSITION CENTER | MIDDLE | EDGE | TOTAL | VERTICAL POSITION SURFACE | MIDDLE | BOTTOM | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal behavior | 26 | 7 | 270 ±97a | 01/84 | 17b<br>3.0c | 66<br>13.0 | 408<br>83.0 | 491 | 76<br>15.0 | 127<br>26.0 | 288<br>59.0 | 491 |
| Mineral Oil I | | | | 03/84 | | | | | | | | |
| control | 6 | 9 | 320 ±29 | | 3<br>2.0 | 11<br>6.0 | 165<br>93.0 | 179 | 27<br>15.0 | 44<br>25.0 | 108<br>60.0 | 179 |
| experimental | 14 | 9 | 379 ±91 | | 13<br>3.0 | 53<br>13.0 | 354<br>84.0 | 420 | 63<br>15.0 | 46<br>11.0 | 311<br>74.0 | 420 |
| Mineral Oil II | | | | 04/84 | | | | | | | | |
| control | 6 | 10 | 580 ±39 | | 7<br>4.0 | 4<br>2.0 | 169<br>94.0 | 180 | 72<br>40.0 | 46<br>26.0 | 62<br>34.0 | 180 |
| experimental | 14 | 10 | 652 ±130 | | 9<br>2.0 | 40<br>10.0 | 371<br>88.0 | 420 | 100<br>24.0 | 80<br>19.0 | 240<br>57.0 | 420 |
| SLCO I | | | | 05/84 | | | | | | | | |
| control | 6 | 11 | 847 ±163 | | 3<br>1.0 | 13<br>7.0 | 164<br>91.0 | 180 | 100<br>56.0 | 40<br>22.0 | 40<br>22.0 | 180 |
| experimental | 8 | 11 | 827 ±180 | | 1<br>0.4 | 14<br>6.0 | 225<br>94.0 | 240 | 50<br>21.0 | 12<br>5.0 | 178<br>74.0 | 240 |
| normal behavior replacement turtles | 10 | 12 | 559 ±226 | 06/84 | 12<br>4.0 | 23<br>8.0 | 265<br>88.0 | 300 | 144<br>48.0 | 69<br>23.0 | 87<br>29.0 | 300 |
| SLCO II | | | | 09/84 | | | | | | | | |
| control | 6 | 15 | 2733 ±439 | | 1<br>1.0 | 6<br>3.0 | 173<br>96.0 | 180 | 75<br>42.0 | 37<br>21.0 | 68<br>38.0 | 180 |
| experimental | 12 | 15 | 2041 ±910 | | 8<br>2.0 | 23<br>6.0 | 329<br>91.0 | 360 | 106<br>29.0 | 18<br>5.0 | 236<br>66.0 | 360 |
| SLCO III | | | | 12/84 | | | | | | | | |
| control | 10 | 18 | 4105 ±1270 | | 1<br>0.3 | 6<br>2.0 | 293<br>98.0 | 300 | 75<br>25.0 | 53<br>18.0 | 172<br>57.0 | 300 |
| experimental | 12 | 18 | 3078 ±1565 | | 3<br>1.0 | 28<br>8.0 | 328<br>91.0 | 359 | 67<br>19.0 | 18<br>5.0 | 274<br>76.0 | 359 |

a. weight is mean ±sd
b. frequency count
c. % frequency

Table 4-3. Summary of observations on year 1 (CM1) green sea turtles; horizontal and vertical distribution in pool (See Figure 4-1c)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | HORIZONTAL POSITION CENTER | MIDDLE | EDGE | TOTAL | VERTICAL POSITION SURFACE | MIDDLE | BOTTOM | TTL # POSITIONS RECORDED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal | 9 | 7 | 686 ±136a | 01/84 | 2b | 11 | 111 | 124 | 120 | 2 | 2 | 124 |
| behavior | | | | | 1.6c | 8.9 | 89.5 | | 96.8 | 1.6 | 1.6 | |
| Mineral Oil II | | | | 04/84 | | | | | | | | |
| control | 4 | 10 | 1096 ±348 | | 1 | 2 | 117 | 120 | 113 | 7 | 0 | 120 |
| | | | | | 0.8 | 1.6 | 97.5 | | 94.2 | 5.8 | 0 | |
| experimental | 4 | 10 | 1150 ±272 | | 22 | 11 | 87 | 120 | 43 | 28 | 49 | 120 |
| | | | | | 18.3 | 9.2 | 72.5 | | 35.8 | 23.3 | 40.8 | |
| SLCO I | | | | 05/84 | | | | | | | | |
| control | 4 | 11 | 1403 ±488 | | 0 | 1 | 119 | 120 | 117 | 3 | 0 | 120 |
| | | | | | 0 | 0.8 | 99.2 | | 97.5 | 2.5 | 0 | |
| experimental | 5 | 11 | 1400 ±377 | | 10 | 12 | 128 | 150 | 79 | 8 | 63 | 150 |
| | | | | | 6.7 | 8.0 | 85.3 | | 52.7 | 5.3 | 42.0 | |
| SLCO II | | | | 09/84 | | | | | | | | |
| control | 4 | 15 | 3204 ±517 | | 0 | 3 | 117 | 120 | 95 | 19 | 6 | 120 |
| | | | | | 0 | 2.5 | 97.5 | | 79.2 | 15.8 | 5.0 | |
| experimental | 5 | 15 | 3506 ±458 | | 20 | 21 | 109 | 150 | 52 | 25 | 73 | 150 |
| | | | | | 13.3 | 14.0 | 72.7 | | 34.7 | 16.7 | 48.7 | |

a. weight is mean ±sd
b. frequency count
c. % frequency

Table 4-4. Summary of observations on year 2 (CC2) loggerhead sea turtles (*C. caretta*); horizontal and vertical distribution in pool (See Figure 4-1c)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | HORIZONTAL POSITION CENTER | MIDDLE | EDGE | TOTAL | VERTICAL POSITION SURFACE | MIDDLE | BOTTOM | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal | 20 | 3 | 98 ±25a | 11/84 | 15b | 31 | 554 | 600 | 373 | 101 | 126 | 600 |
| behavior | | | | | 2.5c | 5.2 | 92.3 | | 62.2 | 16.8 | 21.0 | |
| SLCO III | | | | 12/84 | | | | | | | | |
| control | 10 | 4 | 93 | | 2 | 15 | 282 | 299 | 240 | 30 | 29 | 299 |
| | | | | | 0.7 | 5.0 | 94.3 | | 80.3 | 10.0 | 9.7 | |
| experimental | 10 | 4 | 103.5 | | 3 | 28 | 269 | 300 | 239 | 22 | 39 | 300 |
| | | | | | 1.0 | 9.3 | 89.7 | | 79.7 | 7.3 | 13.0 | |
| SLCO IV | | | | 03/85 | | | | | | | | |
| control | 10 | 7 | 223 | | 0 | 0 | 300 | 300 | 141 | 42 | 117 | 300 |
| | | | | | 0 | 0 | 100 | | 47.0 | 14.0 | 39.0 | |
| experimental | 10 | 7 | 267.5 | | 0 | 0 | 300 | 300 | 193 | 19 | 88 | 300 |
| | | | | | 0 | 0 | 100 | | 64.3 | 6.3 | 29.3 | |
| SLCO V | | | | 06/85 | | | | | | | | |
| control | 10 | 10 | 552.5 | | 7 | 19 | 274 | 300 | 175 | 48 | 77 | 300 |
| | | | | | 2.3 | 6.3 | 91.3 | | 58.3 | 16.0 | 25.7 | |
| experimental | 10 | 10 | 560 | | 12 | 38 | 250 | 300 | 186 | 27 | 87 | 300 |
| | | | | | 4.0 | 12.7 | 83.3 | | 62.0 | 9.0 | 29.0 | |

a. weight is mean ±sd
b. frequency count
c. % frequency

Table 4-5. Summary of observations on year 2 green sea turtles (C. mydas); horizontal and vertical distribution in pool (See Figure 4-1c)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | HORIZONTAL POSITION CENTER | MIDDLE | EDGE | TOTAL | VERTICAL POSITION SURFACE | MIDDLE | BOTTOM | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal | 20 | 4 | 182 ±26a | 12/84 | 27b | 94 | 479 | 600 | 177 | 182 | 241 | 600 |
| behavior | | | | | 4.5c | 15.7 | 79.8 | | 29.5 | 30.3 | 40.2 | |
| SLCO III | | | | 12/84 | | | | | | | | |
| control | 10 | 4 | 254 | | 7 | 29 | 264 | 300 | 88 | 61 | 151 | 300 |
| | | | | | 2.3 | 9.7 | 88.0 | | 29.3 | 20.3 | 50.3 | |
| experimental | 10 | 4 | 233 | | 31 | 44 | 224 | 299 | 50 | 17 | 232 | 299 |
| | | | | | 10.4 | 14.7 | 74.9 | | 16.7 | 5.7 | 77.6 | |
| SLCO IV | | | | 03/85 | | | | | | | | |
| control | 10 | 7 | 585.5 | | 3 | 12 | 255 | 270 | 104 | 96 | 100 | 300 |
| | | | | | 1.1 | 4.4 | 94.4 | | 34.7 | 32.0 | 33.3 | |
| experimental | 10 | 7 | 560 | | 24 | 45 | 231 | 300 | 61 | 29 | 210 | 300 |
| | | | | | 8.0 | 15.0 | 77.0 | | 20.3 | 9.7 | 70.0 | |
| SLCO V | | | | 06/85 | | | | | | | | |
| control | 10 | 10 | 1024 | | 9 | 17 | 275 | 301 | 152 | 43 | 105 | 300 |
| | | | | | 0.3 | 5.6 | 91.4 | | 50.7 | 14.3 | 35.0 | |
| experimental | 10 | 10 | 1065.5 | | 27 | 60 | 213 | 300 | 102 | 39 | 160 | 301 |
| | | | | | 9.0 | 20.0 | 71.0 | | 33.9 | 13.0 | 53.2 | |

a. weight is mean ±sd
b. frequency count
c. % frequency

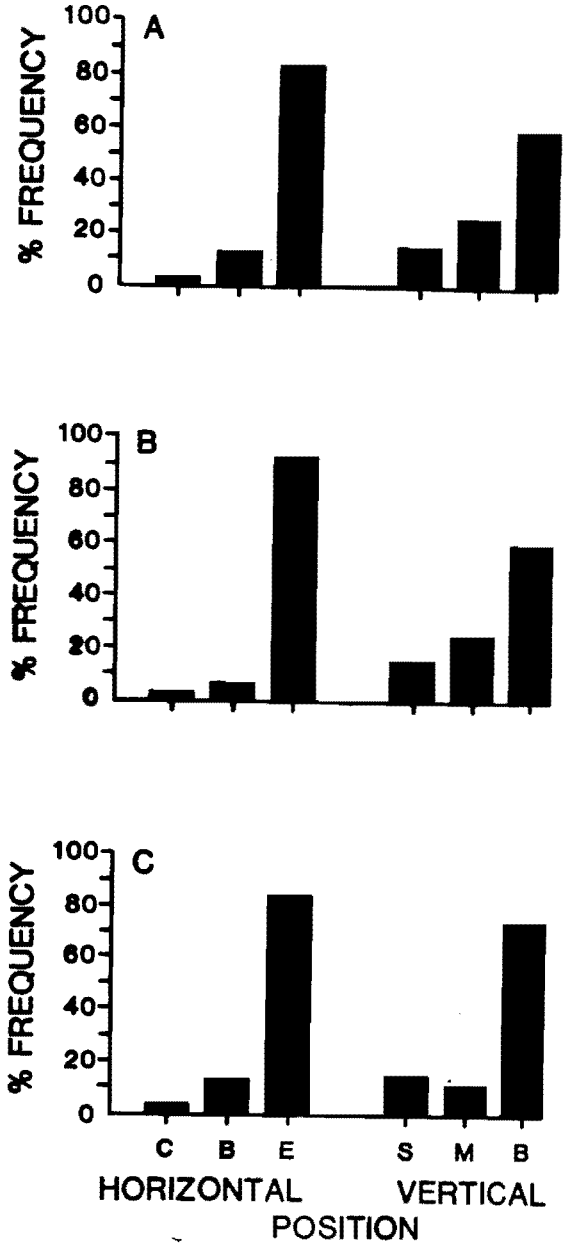



FIGURE 4-2. Horizontal and vertical position of year 1 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-2). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool.

A. Normal behavior, no oil boom in pool, turtles are 7 months of age
B. Control behavior - Mineral Oil I, turtles are 9 months of age
C. Experimental behavior - Mineral Oil I, turtles are 9 months of age

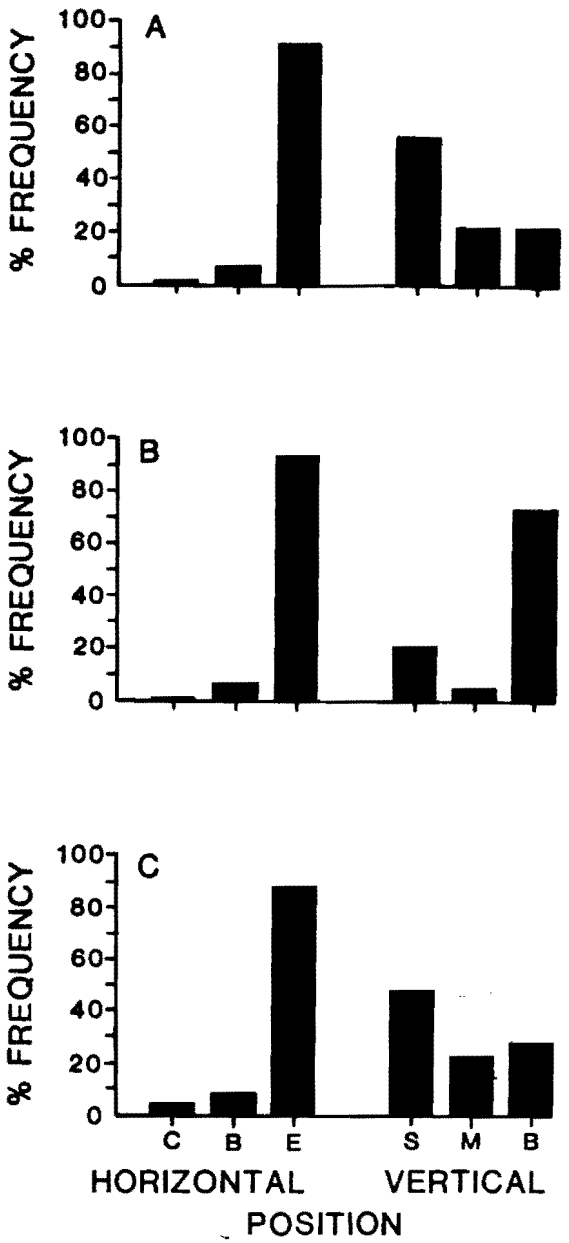



FIGURE 4-3. Horizontal and vertical position of year 1 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-2). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. These turtles are 11 months of age.

A. Control behavior - South Louisiana Crude Oil I (SLCOI)
B. Experimental behavior - South Louisiana Crude Oil I (SLCOI)
C. Normal behavior (no oil boom) - replacement turtles

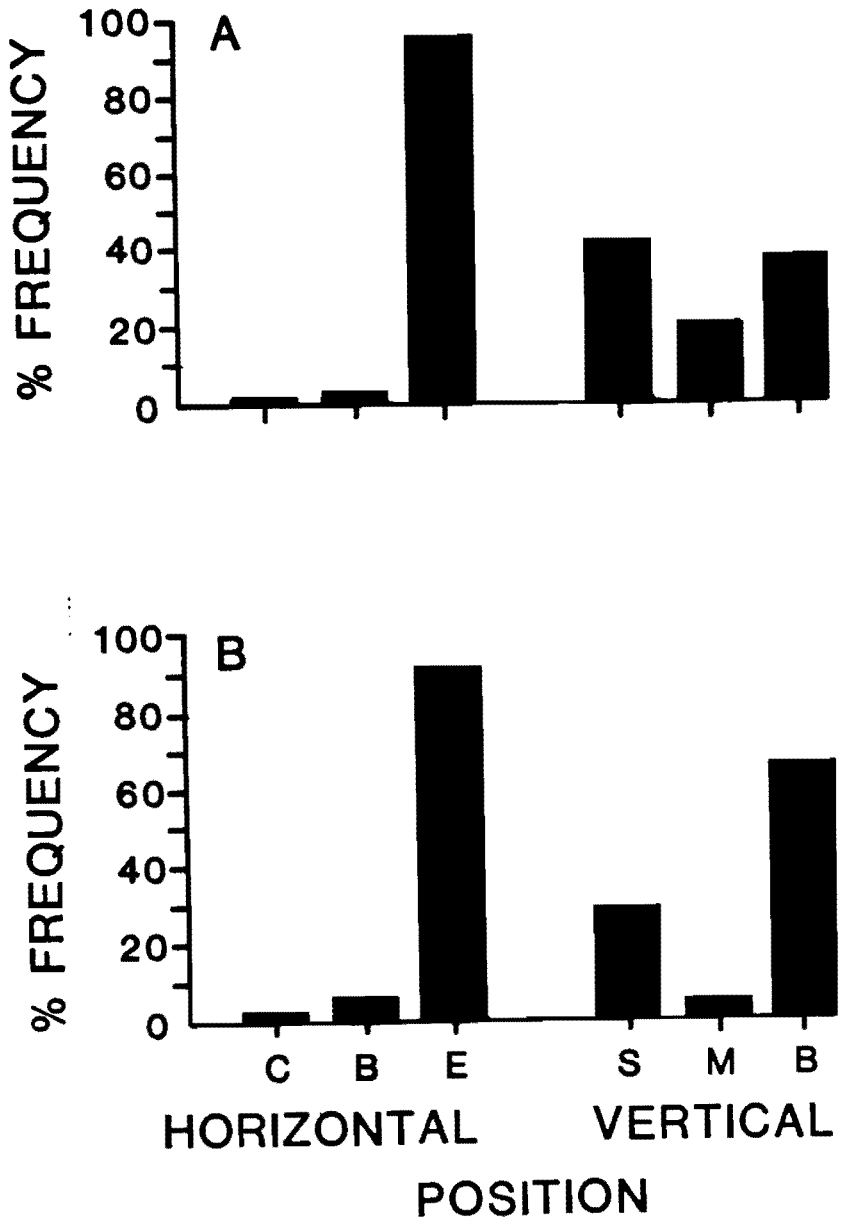



FIGURE 4-4. Horizontal and vertical position of year 1 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-2). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. These turtles are 15 months of age.

A. Control behavior - South Louisiana Crude Oil II (SLCOII)

B. Experimental behavior - South Louisiana Crude Oil II (SLCOII)

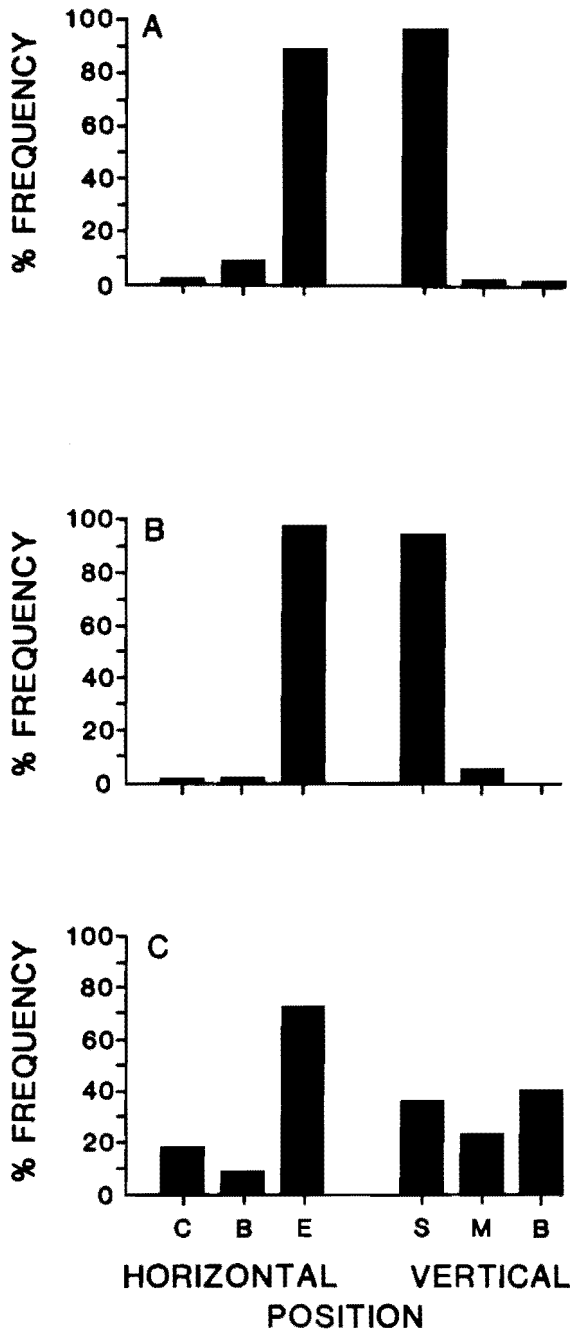



FIGURE 4-5. Horizontal and vertical position of year 1 green sea turtles (C. mydas) in the experimental pool (see Figure 4-1C). Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-3). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool.

A. Normal behavior - no oil boom, turtles are 7 months of age
B. Control behavior - Mineral Oil II, turtles are 10 months of age
C. Experimental behavior - Mineral Oil II, turtles are 10 months of age

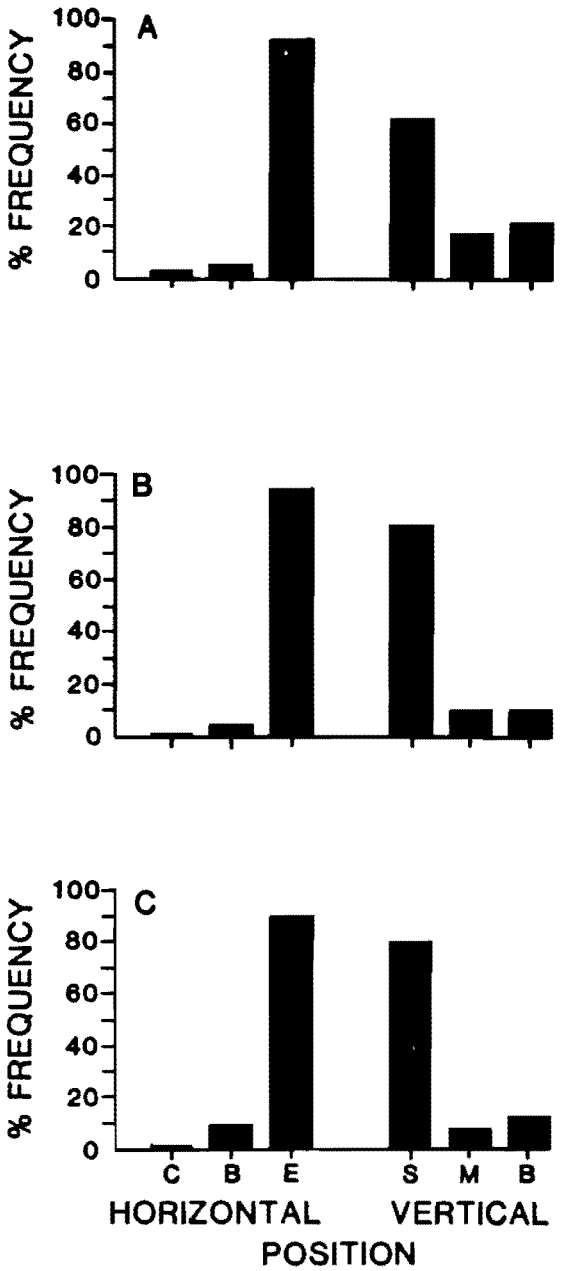



FIGURE 4-6. Horizontal and vertical position of year 2 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-4). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool.

A. Normal behavior - no oil boom, turtles are 3 months of age
B. Control behavior - South Louisiana Crude Oil III (SLCOIII), turtles are 4 months of age
C. Experimental behavior - South Louisiana Crude Oil III (SLCOIII), turtles are 4 months of age

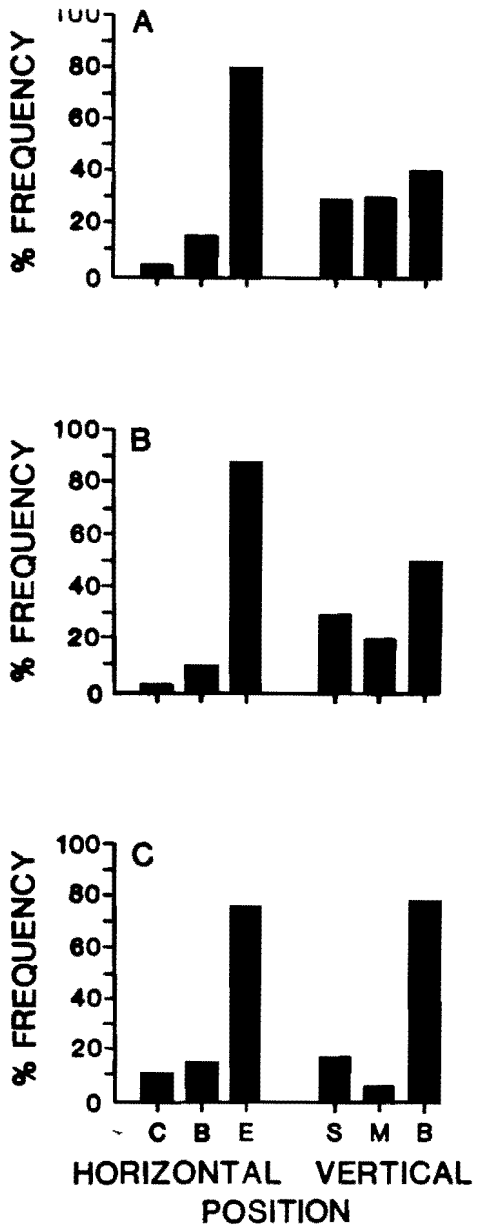



FIGURE 4-7. Horizontal and vertical position of year 2 green sea turtles (C. mydas) in the experimental pool (Figure 4-1C). Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-5). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. These turtles are 4 months of age.

A. Normal behavior - no oil boom
B. Control behavior - South Louisiana Crude Oil III (SLCOIII)
C. Experimental behavior - South Louisiana Crude Oil III (SLCOIII)

Table 4-6. Summary of observations on year 1 (CC1) loggerhead sea turtles (*C. caretta*) location by pool quadrant (see Figure 4-1B)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | FREQUENCY OF OCCURRENCE BY QUADRANT 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | TTL # OF POSITIONS RECORDED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal behavior | 26 | 7 | 270 ±97a | 01/84 | 6b<br>1.7%c | 154<br>31.4 | 7<br>1.4 | 128<br>26.1 | 7<br>1.4 | 94<br>19.1 | 9<br>2.1 | 86<br>17.5 | 491 |
| Mineral Oil I | | | | 03/84 | | | | | | | | | |
| control | 6 | 9 | 320 ±29 | | 2<br>1.1 | 34<br>19.0 | 0<br>0 | 36<br>20.1 | 1<br>0.6 | 52<br>29.0 | 1<br>0.6 | 53<br>29.6 | 179 |
| experimental | 14 | 9 | 379 ±91 | | 2<br>0.5 | 94<br>22.5 | 4<br>1.0 | 99<br>23.7 | 1<br>8.2 | 107<br>25.7 | 7<br>1.7 | 103<br>24.7 | 417 |
| Mineral Oil II | | | | 04/84 | | | | | | | | | |
| control | 6 | 10 | 580 ±39 | | 4<br>2.2 | 44<br>24.4 | 2<br>1.1 | 27<br>15.0 | 1<br>0.5 | 41<br>22.8 | 0<br>0 | 61<br>33.9 | 180 |
| experimental | 14 | 10 | 652 ±130 | | 5<br>1.2 | 111<br>26.5 | 3<br>0.7 | 95<br>22.7 | 2<br>0.5 | 89<br>21.2 | 5<br>1.2 | 110<br>26.3 | 419 |
| SLCO I | | | | 05/84 | | | | | | | | | |
| control | 6 | 11 | 847 ±163 | | 1<br>0.6 | 29<br>16.1 | 1<br>0.6 | 47<br>26.1 | 1<br>0.6 | 41<br>22.8 | 1<br>0.6 | 59<br>32.8 | 180 |
| experimental | 8 | 11 | 827 ±180 | | 2<br>1.4 | 40<br>27.2 | 2<br>1.4 | 40<br>27.2 | 1<br>0.7 | 30<br>20.4 | 1<br>0.7 | 31<br>21.1 | 147 |
| normal behavior replacement turtles | 10 | 12 | 559 ±226 | 06/84 | 1<br>0.3 | 61<br>20.3 | 4<br>1.3 | 49<br>16.3 | 5<br>1.7 | 92<br>30.7 | 6<br>2.0 | 82<br>27.3 | 300 |
| SLCO II | | | | 09/84 | | | | | | | | | |
| control | 6 | 15 | 2733 ±439 | | 0<br>0 | 36<br>20.0 | 1<br>0.5 | 50<br>27.8 | 1<br>0.5 | 50<br>27.8 | 1<br>0.5 | 41<br>22.8 | 180 |
| experimental | 12 | 15 | 2041 ±910 | | 3<br>1.1 | 69<br>26.2 | 5<br>1.9 | 46<br>17.5 | 4<br>1.5 | 57<br>21.7 | 6<br>2.3 | 73<br>27.8 | 263 |
| SLCO III | | | | 12/84 | | | | | | | | | |
| control | 10 | 18 | 4105 ±1270 | | 0<br>0 | 76<br>25.0 | 1<br>0.3 | 78<br>26.0 | 1<br>0.3 | 72<br>24.0 | 0<br>0 | 72<br>24.0 | 300 |
| experimental | 12 | 18 | 3078 ±1565 | | 4<br>1.3 | 70<br>23.0 | 2<br>0.7 | 69<br>22.5 | 1<br>0.3 | 74<br>24.1 | 3<br>1.0 | 84<br>27.4 | 307 |

a. weight is mean ±sd
b. frequency count
c. % frequency

Table 4-7. Summary of observations on year 1 green sea turtles (C. mydas) location by pool quadrant (see Figure 4-1B)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | FREQUENCY OF OCCURRENCE BY QUADRANT 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | TTL # OF POSITIONS RECORDED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal | 9 | 7 | 686 ±136a | 01/84 | 0 | 39b | 0 | 51 | 1 | 20 | 2 | 11 | 124 |
| behavior | | | | | 0 | 31.4c | 0 | 41.1 | 0.8 | 16.1 | 1.6 | 8.9 | |
| Mineral Oil II | | | | 04/84 | | | | | | | | | |
| control | 4 | 10 | 1096 ±348 | | 1 | 44 | 0 | 28 | 0 | 24 | 0 | 23 | 120 |
| | | | | | 0.8 | 36.7 | 0 | 23.3 | 0 | 20.0 | 0 | 19.2 | |
| experimental | 4 | 10 | 1158 ±272 | | 3 | 25 | 9 | 27 | 5 | 23 | 7 | 19 | 118 |
| | | | | | 2.5 | 21.2 | 7.6 | 22.9 | 4.2 | 19.5 | 5.9 | 16.1 | |
| SLCO I | | | | 05/84 | | | | | | | | | |
| control | 4 | 11 | 1403 ±488 | | 0 | 21 | 0 | 38 | 0 | 27 | 0 | 34 | 120 |
| | | | | | 0 | 17.5 | 0 | 31.7 | 0 | 22.5 | 0 | 28.3 | |
| experimental | 5 | 11 | 1400 ±377 | | 3 | 33 | 1 | 28 | 3 | 31 | 9 | 37 | 145 |
| | | | | | 2.1 | 22.8 | 0.7 | 19.3 | 2.1 | 21.4 | 6.2 | 25.5 | |
| SLCO II | | | | 09/84 | | | | | | | | | |
| control | 4 | 15 | 3204 ±517 | | 0 | 30 | 0 | 27 | 0 | 21 | 1 | 41 | 120 |
| | | | | | 0 | 25.0 | 0 | 22.5 | 0 | 17.5 | 0.8 | 34.2 | |
| experimental | 5 | 15 | 3506 ±458 | | 8 | 22 | 7 | 26 | 6 | 20 | 7 | 36 | 132 |
| | | | | | 6.1 | 16.7 | 5.3 | 19.2 | 4.6 | 15.2 | 5.3 | 27.3 | |

a. weight is mean ±sd
b. frequency count
c. % frequency

Table 4-8. Summary of observations on year 2 loggerhead sea turtles (*C. caretta*) location by pool quadrant (see Figure 4-1B)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | FREQUENCY OF OCCURRENCE BY QUADRANT 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | TTL # OF POSITIONS RECORDED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal | 20 | 3 | 98 ±25a | 11/84 | 5b | 183 | 4 | 150 | 9 | 86 | 7 | 156 | 600 |
| behavior | | | | | 0.8c | 30.5 | 0.7 | 25.0 | 1.5 | 14.3 | 1.2 | 26.0 | |
| SLCO III | | | | 12/84 | | | | | | | | | |
| control | 10 | 4 | 93 | | 0 | 102 | 1 | 72 | 0 | 39 | 2 | 83 | 299 |
| | | | | | 0 | 34.1 | 0.3 | 24.1 | 0 | 13.0 | 0.7 | 27.8 | |
| experimental | 10 | 4 | 123.5 | | 1 | 75 | 3 | 58 | 2 | 58 | 5 | 82 | 284 |
| | | | | | 0.4 | 26.4 | 1.1 | 20.4 | 0.7 | 20.4 | 1.8 | 28.9 | |
| SLCO IV | | | | 03/85 | | | | | | | | | |
| control | 10 | 7 | 223 | | 4 | 76 | 2 | 75 | 4 | 57 | 7 | 75 | 300 |
| | | | | | 1.3 | 25.3 | 0.7 | 25.0 | 1.3 | 19.0 | 2.3 | 25.0 | |
| experimental | 10 | 7 | 262.5 | | 24 | 51 | 20 | 43 | 13 | 43 | 23 | 41 | 258 |
| | | | | | 9.3 | 19.8 | 7.8 | 16.7 | 5.0 | 16.7 | 8.9 | 15.9 | |
| SLCO V | | | | | | | | | | | | | |
| control | 10 | 10 | 552.5 | 06/85 | 8 | 81 | 1 | 79 | 1 | 70 | 3 | 57 | 298 |
| | | | | | 2.7 | 27.2 | 0.3 | 26.5 | 0.3 | 23.5 | 1.0 | 19.1 | |
| experimental | 10 | 10 | 560 | | 6 | 56 | 3 | 70 | 5 | 56 | 8 | 62 | 266 |
| | | | | | 2.3 | 21.1 | 1.1 | 26.3 | 1.9 | 21.1 | 3.0 | 23.3 | |

a. weight is mean ±sd
b. frequency count
c. % frequency

Table 4-9. Summary of observations on year 2 green sea turtles (C. mydas); location by pool quadrant (see Figure 4-1B)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | TURTLE WEIGHT(g) | DATE | FREQUENCY OF OCCURRENCE BY QUADRANT 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | TTL # OF POSITIONS RECORDED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| normal | 20 | 4 | 182 ±26a | 12/84 | 12b | 187 | 10 | 127 | 11 | 91 | 14 | 148 | 600 |
| behavior | | | | | 2.0c | 31.2 | 1.7 | 21.2 | 1.8 | 15.2 | 2.3 | 24.7 | |
| SLCO III | | | | 12/84 | | | | | | | | | |
| control | 10 | 4 | 184 | | 5 | 107 | 2 | 82 | 1 | 51 | 3 | 49 | 300 |
| | | | | | 1.7 | 35.7 | 0.7 | 27.3 | 0.3 | 17.0 | 1.0 | 16.3 | |
| experimental | 10 | 4 | 233 | | 21 | 41 | 7 | 38 | 7 | 46 | 20 | 39 | 219 |
| | | | | | 9.6 | 18.7 | 3.22 | 17.4 | 3.2 | 21.0 | 9.1 | 17.8 | |
| SLCO IV | | | | 03/85 | | | | | | | | | |
| control | 10 | 7 | 585.5 | | 0 | 90 | 3 | 87 | 1 | 56 | 0 | 63 | 300 |
| | | | | | 0 | 30.0 | 1.0 | 29.0 | 0.3 | 18.7 | 0 | 21.0 | |
| experimental | 10 | 7 | 560 | | 15 | 58 | 16 | 34 | 7 | 47 | 16 | 54 | 247 |
| | | | | | 6.1 | 23.5 | 6.5 | 13.8 | 2.8 | 19.0 | 6.5 | 21.9 | |
| SLCO V | | | | 06/85 | | | | | | | | | |
| control | 10 | 10 | 1024 | | 8 | 34 | 2 | 84 | 6 | 100 | 3 | 63 | 294 |
| | | | | | 2.7 | 11.6 | 0.7 | 28.6 | 2.0 | 34.0 | 1.0 | 21.4 | |
| experimental | 10 | 10 | 1065.5 | | 18 | 54 | 7 | 47 | 16 | 54 | 14 | 72 | 282 |
| | | | | | 6.4 | 19.2 | 2.5 | 16.7 | 5.7 | 19.2 | 5.0 | 25.5 | |

a. weight is mean ±sd
b. frequency count
c. % frequency

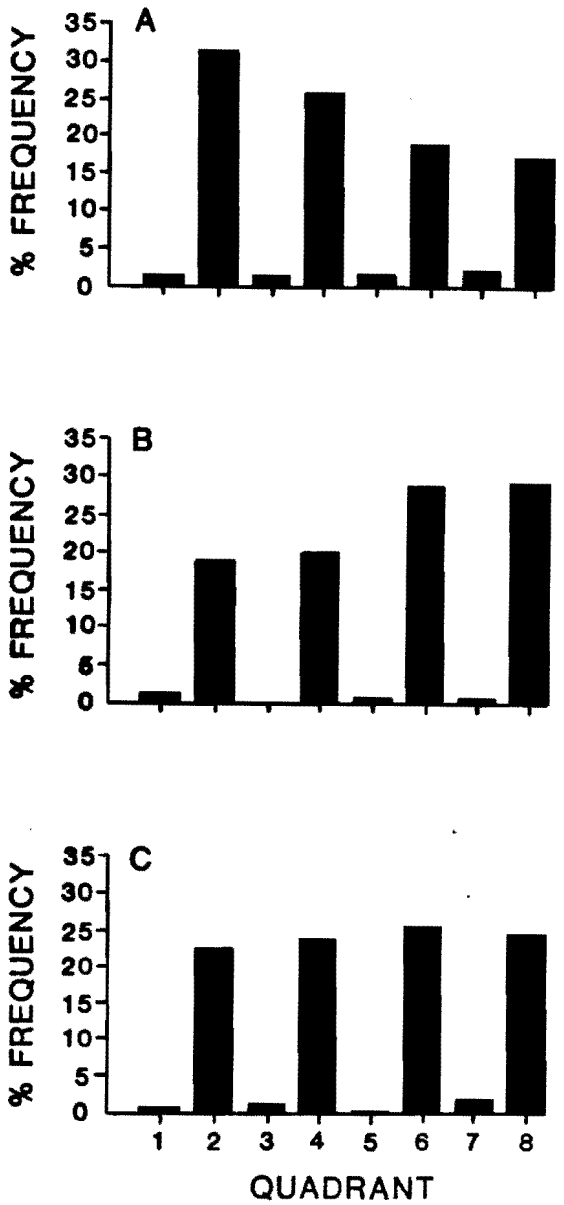



FIGURE 4-8. Distribution of year 1 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-6).
A. Normal behavior, no oil boom in pool, turtles are 7 months of age.
B. Control behavior - Mineral Oil I, turtles are 9 months of age.
C. Experimental behavior - Mineral Oil I, turtles are 9 months of age.

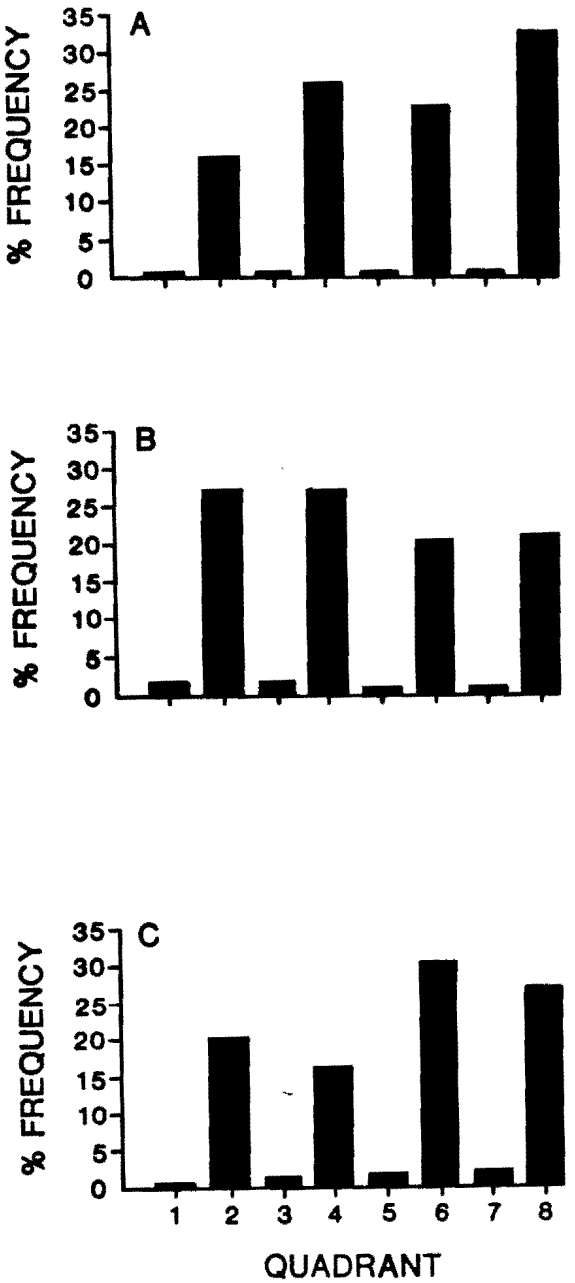



FIGURE 4-9. Distribution of year 1 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-6). These turtles are 11 months of age.

A. Control behavior - South Louisiana Crude Oil I (SLCOI)
B. Experimental behavior - South Louisiana Crude Oil I (SLCOI)
C. Normal behavior - replacement turtles, turtles are 12 months of age

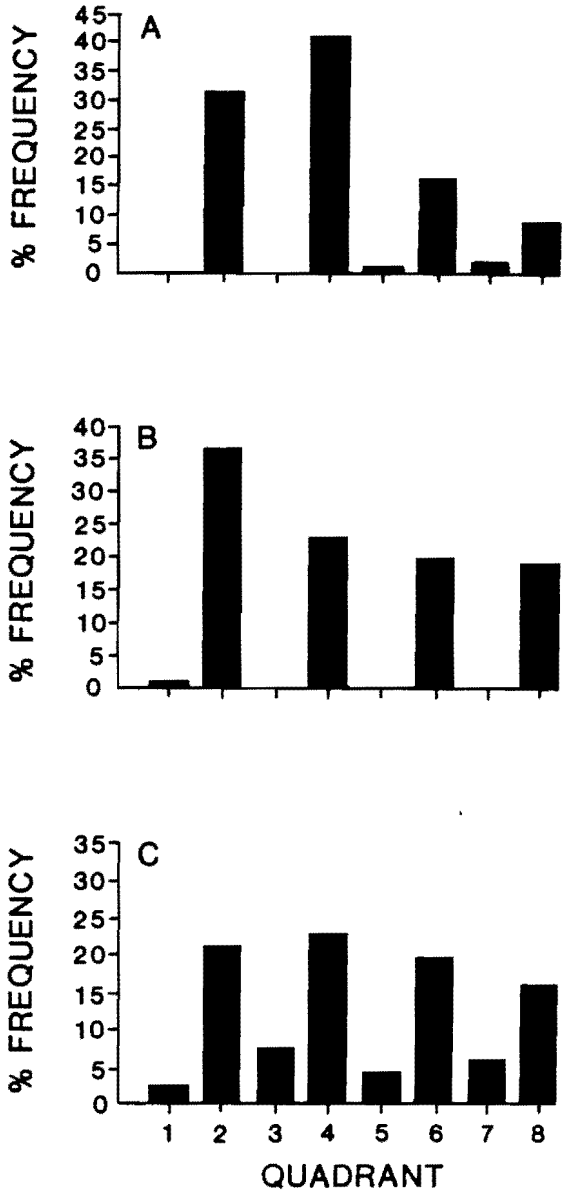



FIGURE 4-10. Distribution of year 1 green sea turtles (C. mydas) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-7).
A. Normal behavior - no oil boom, turtles are 7 months of age.
B. Control behavior - Mineral Oil II, turtles are 10 months of age
C. Experimental behavior - Mineral Oil II, turtles are 10 months of age

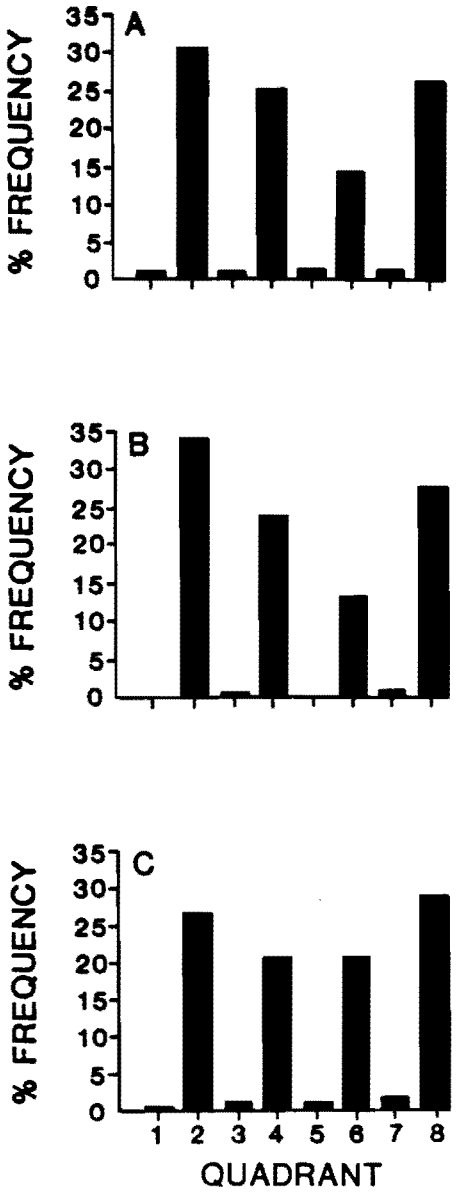



FIGURE 4-11. Distribution of year 2 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-8).

A. Normal behavior - no oil boom, turtles are 3 months of age.
B. Control behavior - South Louisiana Crude Oil III (SLCOIII), turtles are 4 months of age
C. Experimental behavior - South Louisiana Crude Oil III (SLCOIII), turtles are 4 months of age

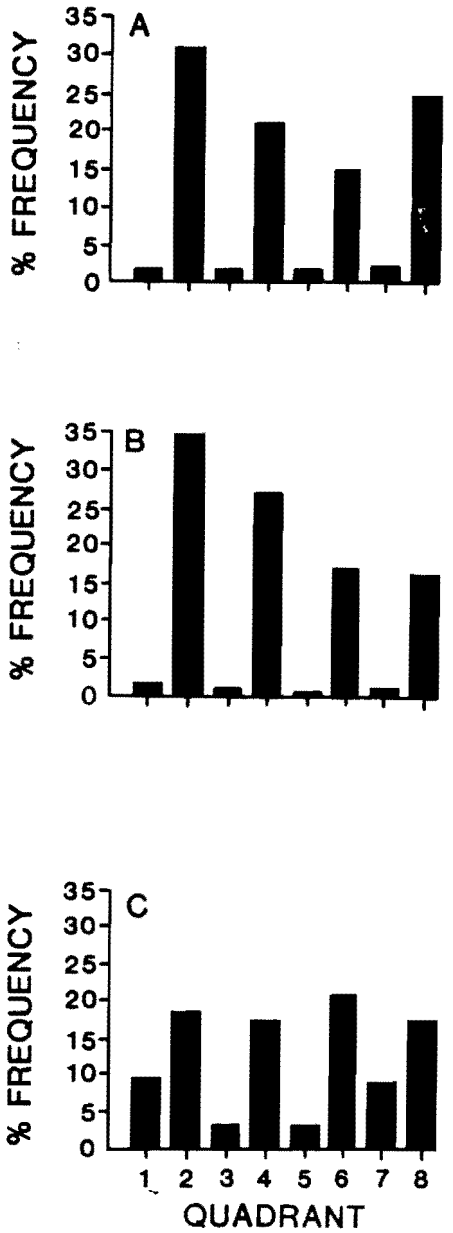



FIGURE 4-12. Distribution of year 2 green sea turtles (C. mydas) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-9). These turtles are 4 months of age.

A. Normal behavior - no oil boom
B. Control behavior - South Louisiana Crude Oil III (SLCOIII)
C. Experimental behavior - South Louisiana Crude Oil III (SLCOIII)

Under experimental conditions, a 0.5 cm layer of mineral oil (with coloring agent) or weathered crude oil was placed on the surface of the water outside the boom. Crude oil was weathered for 48 hours over sea water in a 10 gallon container with an aquarium air pump and airstone to ensure continual turnover of the oil. A sample of weathered oil was taken at the beginning of each set of experimental trials and a second sample from the surface of the water at the end of the trials. Water samples for dissolved hydrocarbon analyses were taken prior to the addition of weathered oil, midway through the given set of trials, and at the end of the trials before the oil was removed from the surface. Water samples were taken from the middle of the water column.

The sequence in which the individual turtles were used in each experimental series was randomly determined to compensate for any effects due to light conditions or time of day. During year 1 due to lack of availability and mortality, less than 10 turtles were taped. Under optimal conditions, a total of ten turtles were taped for the control tests (boom but no oil) and ten turtles for each oil slick exposure test. Ideally, each experiment used 20 turtles, 10 controls, and 10 experimentals. Each turtle was observed for one 30 minute period. If all the turtles were healthy 3 months later, the same turtles were used in the next experiment. Experimentals were never used as controls. If a turtle died, it was replaced with an animal from the reserve pool (year 2). The site of introduction of the turtle into the pool (center or edge) was alternated with each turtle (i.e. center, edge, center, edge ...) such that 5 turtles in each group (control and experimental) were introduced in the center and 5 at the edge. During the oil slick exposure, therefore, 5 turtles were introduced directly into the oil (edge) and 5 into the clear water (center). Between individual trials, any oil that had accumulated in the center of the pool was removed using 3M oil absorbent pads to maintain a clear visual difference between the two areas. Additional oil was added as necessary to keep the layer about 0.5 cm thick. Oil adhering to the turtles was removed as thoroughly as possible using the absorbent pads. At the end of a set of trials, the remaining oil was removed with absorbent materials and the pool scrubbed with detergent. Concentrations ranged from 1.35 - 2043 $ug\text{-}l^{-1}$ for the five oil slick experiments (Table 3-6).

Tar Ball Experiments

Tar balls (real and substitute) were presented to each turtle in a circular array designed to maximize discovery by the turtles (Figure 4-13). Tar balls about 2 cm diameter were molded from beach tar because the South Louisiana crude oil could not be weathered into tar (Section 3.0). Wooden spheres of a similar diameter were painted black and served as substitute tar balls for both control and discrimination studies. The painted wooden spheres were soaked in sea water for several days prior to use to remove any constituents that were easily soluble in water. Thus, the wooden balls would resemble the tar balls visually but not offer a chemical signal. To ensure that none of the experimental animals could actually ingest the tar, both real and substitute tar balls were individually wrapped in a coarse black plastic mesh pouch and suspended from the apparatus with monofilament line so that they floated on the surface. The control situation consisted of 12 wooden spheres equally spaced around the array. The experimental situation consisted of 6 tar balls and 6 wooden spheres. The positions occupied by the tar balls were determined randomly for each turtle. All turtles were started at the center of the pool. Each trial lasted for 30 minutes. One trial was run for each turtle, experimental or control. The order in which the individual turtles were used was determined randomly for both experimental and control situations for each set of experiments (i.e. 3 months, 6 months, 9 months ...). Pre- and post experiment water samples were collected during the first tar ball experiment for dissolved hydrocarbon analyses. Levels were extremely low (Table 3-6) and water sampling was not done in subsequent tar ball experiments.

## 4.2 Results

South Louisiana Crude Oil (SLCO) and Colored Mineral Oil

During our efforts to determine the effect of weathered crude oil on the behavior of loggerhead and green sea turtles we made and studied over 160 hours of video tape. The turtles studied ranged in age from about 2 to 18 months (Table 4-1). Older (larger) turtles were too big to be properly studied in our pools. All turtles, regardless of age or experimental protocol, showed a very strong tendency to stay at the edge of the pool (Tables 4-2 to 4-5, Figures 4-2 to 4-7). We found no significant difference

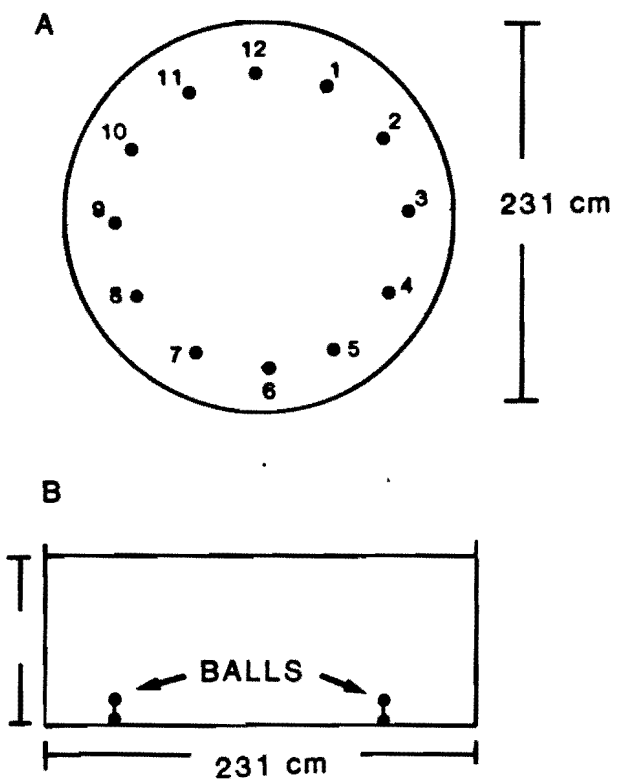



FIGURE 4-13. Diagram of the experimental setup for the tar ball experiments. A. Top view of experimental tank showing the horizontal arrangement of the tar balls at the surface. B. Side view of tank showing the position of the submerged tar balls 5 cm off the bottom in Tar Ball IV.

(Chi-square, $P<.01$) between control and experimental observations with respect to preference for the edge of the pool. The location (center vs. edge) where the turtle was placed in the pool did not alter the edge preference. However, the turtles were not always evenly distributed in the 4 edge quadrants as one would predict if all quadrants were equally attractive to the turtles. We feel it is probable that the varying angle and intensity of the ambient light accounted for this difference. While control and experimental observations were statistically identical with respect to preference for the edge, we observed a slight tendency for the turtles (particularly the green sea turtles, Table 4-5) to occur slightly more often in the center of the pool under experimental (= oil on surface of pool perimeter) conditions. The fact that this also occurred when dark colored mineral oil was used (Table 4-3, Figure 4-5) instead of weathered crude oil led us to conclude that this slight behavioral change was due to the dark color of the surface layer and not to chemical factors. Mineral oil is virtually insoluble in water and thus releases minimal chemical cues in comparison to crude oil. It is also possible that tactile factors were involved. We did not have the opportunity to test turtles with clear mineral oil to determine if tactile factors alone would cause the behavioral change.

The presence of a dark surface layer clearly affected the position of the turtles in the water column (Tables 4-2 to 4-5; Figures 4-2 to 4-7, 4-14 to 4-21) causing them to move away from the surface. This pattern was again particularly apparent in the green sea turtles (Tables 4-3 and 4-5; Figures 4-16, 4-17, 4-20, 4-21). We conclude that this behavior change under experimental conditions was in fact due to the dark color of the surface layer because it also occurred when we used the dark mineral oil (Figures 4-5 and 4-7). An analysis by pool quadrant showed a similar result (Tables 4-6 to 4-9; Figures 4-8 to 4-12; 4-22 to 4-30).

We also found that the mean dive times tended to increase under experimental conditions and in some cases the differences were significant (t-test, $p = 0.01$) (Tables 4-10 to 4-13). Here too, the green sea turtles showed the greatest differences (Tables 4-11 and 4-13). Here again, we conclude that, since the same pattern of behavior occurred when dark mineral oil was used, that the behavior change was probably due to the dark surface layer. Water temperature was relatively constant within a given experiment, but it varied between experiments. The total range was 21.5°C to 30.4°C.

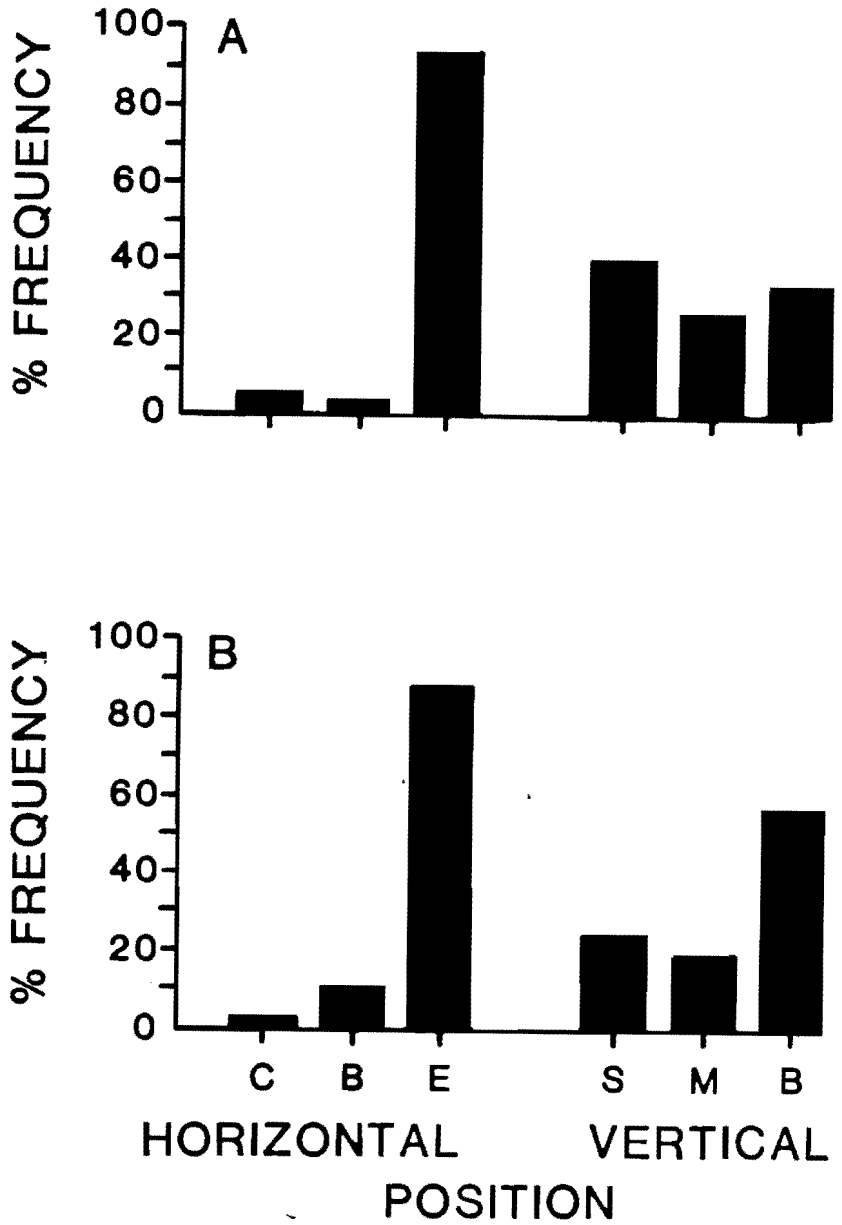



FIGURE 4-14. Horizontal and vertical position of year 1 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-2). These turtles are 10 months of age.

A. Control behavior - Mineral Oil II

B. Experimental behavior - Mineral Oil II

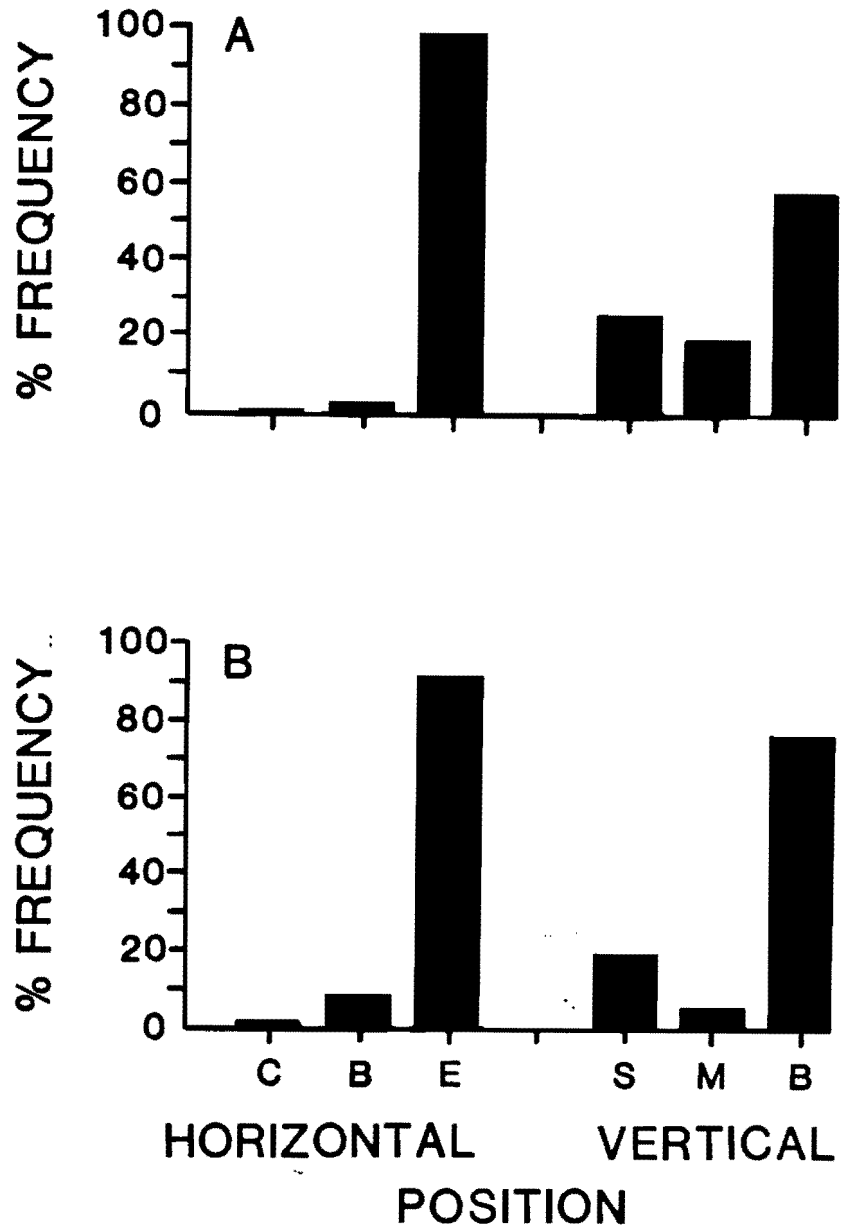



FIGURE 4-15. Horizontal and vertical position of year 1 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-2). These turtles are 18 months of age.

A. Control behavior - South Louisiana Crude Oil III (SLCOIII)

B. Experimental behavior - South Louisiana Crude Oil III (SLCOIII)

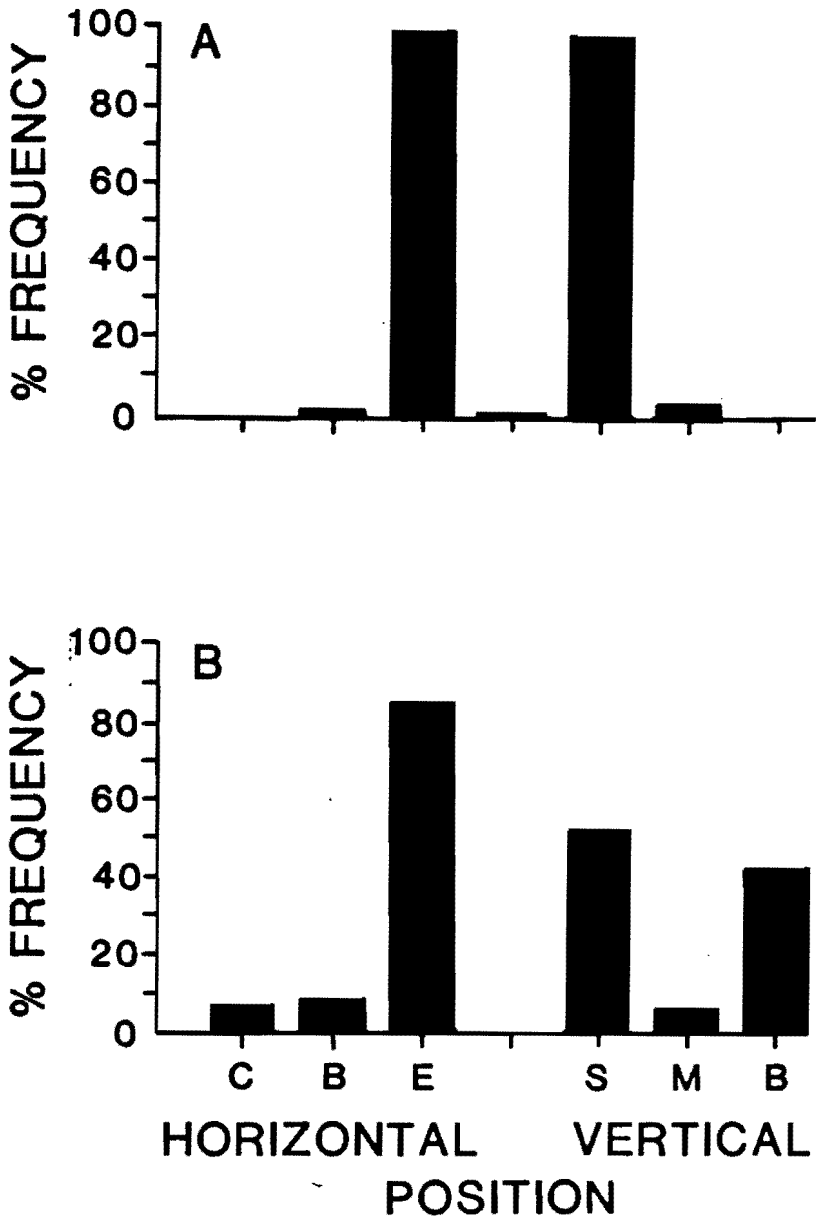



FIGURE 4-16. Horizontal and vertical position of year 1 green sea turtles (C. mydas) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-3). These turtles are 11 months of age.

A. Control behavior - South Louisiana Crude Oil I (SLCOI)

B. Experimental behavior - South Louisiana Crude Oil I (SLCOI)

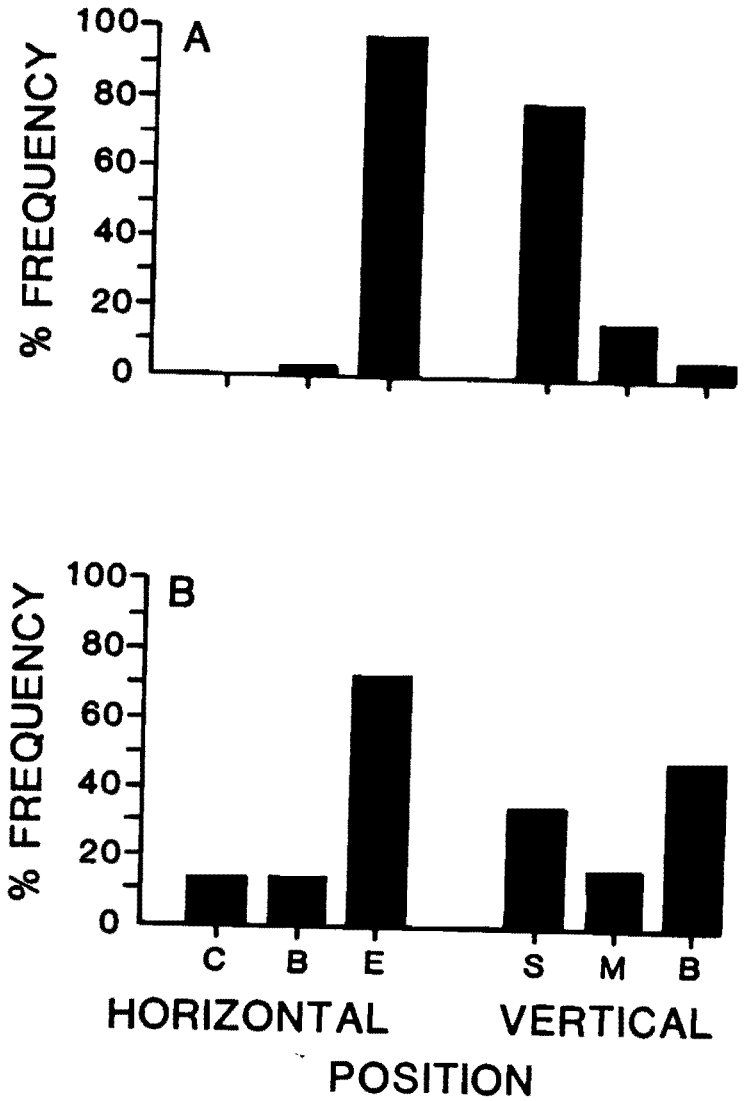



FIGURE 4-17. Horizontal and vertical position of year 1 green sea turtles (C. mydas) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-3). These turtles are 15 months of age.

A. Control behavior - South Louisiana Crude Oil II (SLCOII)

B. Experimental behavior - South Louisiana Crude Oil II (SLCOII)

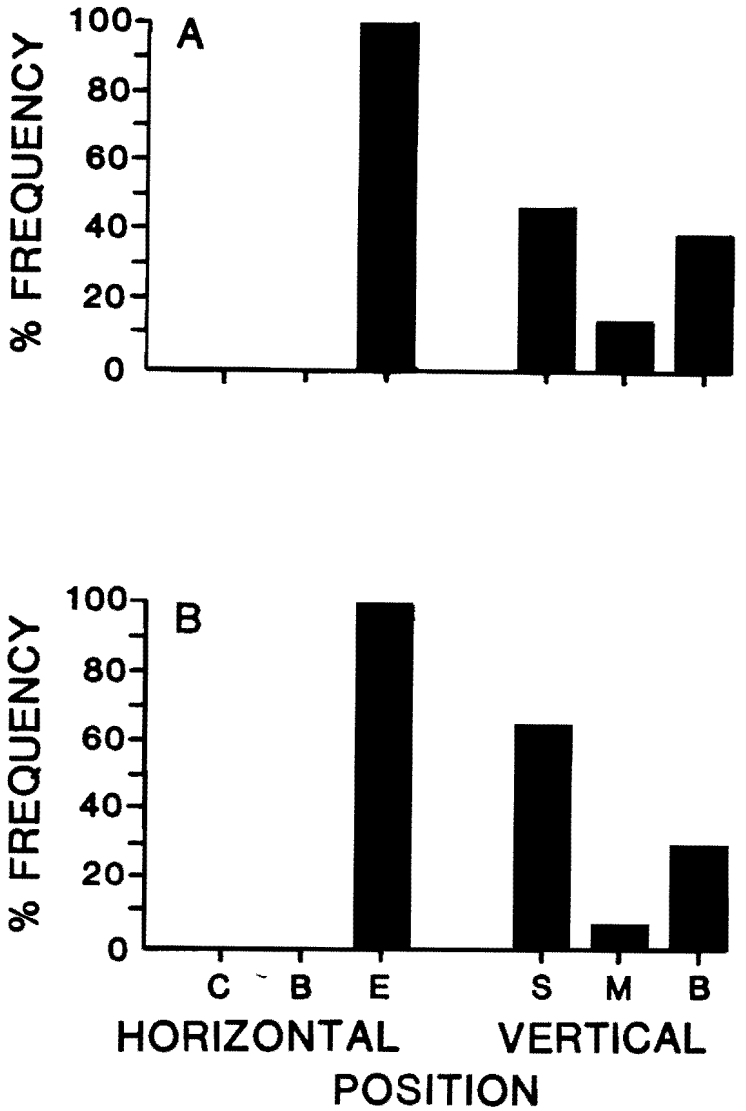



FIGURE 4-18. Horizontal and vertical position of year 2 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-4). These turtles are 7 months of age.

A. Control behavior - South Louisiana Crude Oil IV (SLCOIV)

B. Experimental behavior - South Louisiana Crude Oil IV (SLCOIV)

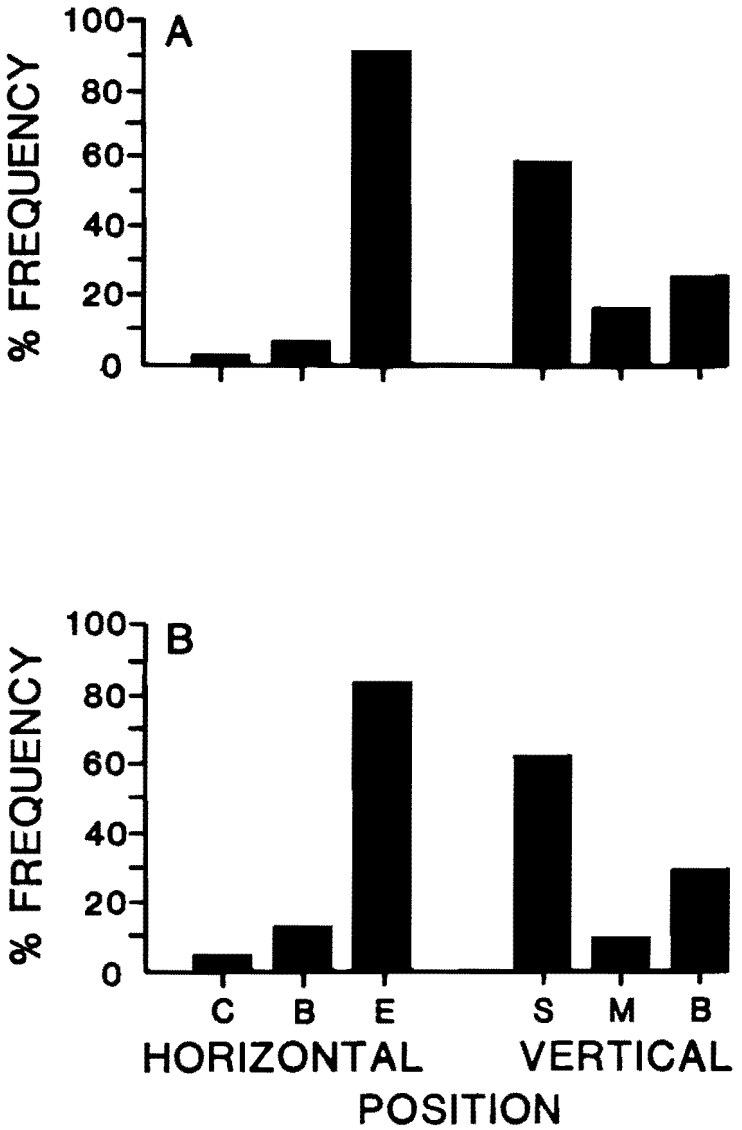



FIGURE 4-19. Horizontal and vertical position of year 2 loggerhead sea turtles (C. caretta) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-4). These turtles are 10 months of age.

A. Control behavior - South Louisiana Crude Oil V (SLCOV)

B. Experimental behavior - South Louisiana Crude Oil V (SLCOV)

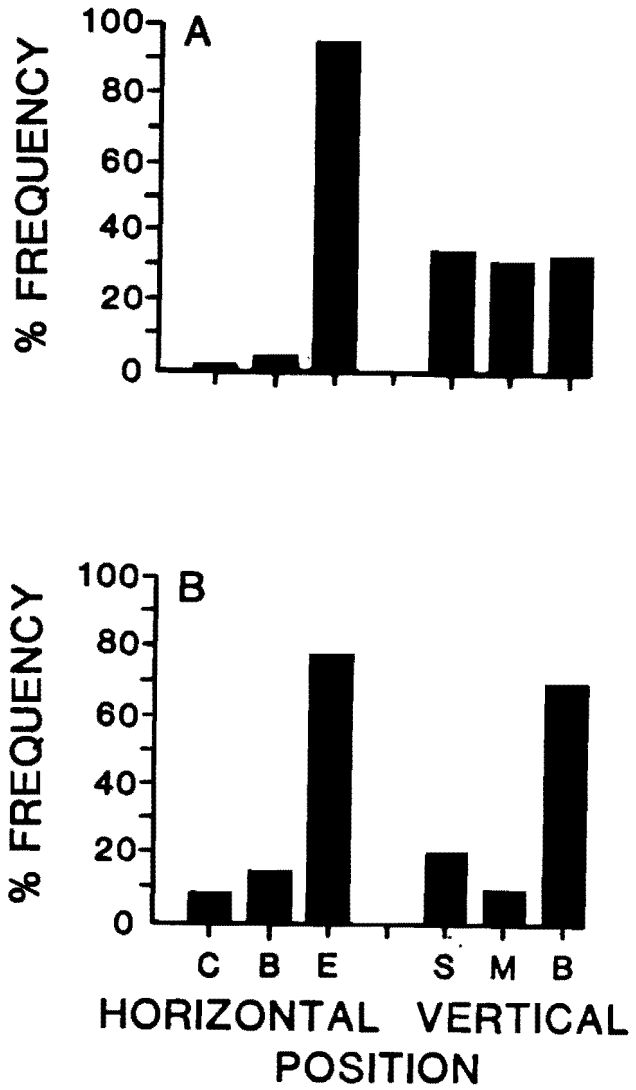



FIGURE 4-20. Horizontal and vertical position of year 2 green sea turtles (C. mydas) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-5). These turtles are 7 months of age.

A. Control behavior - South Louisiana Crude Oil IV (SLCOIV)

B. Experimental behavior - South Louisiana Crude Oil IV (SLCOIV)

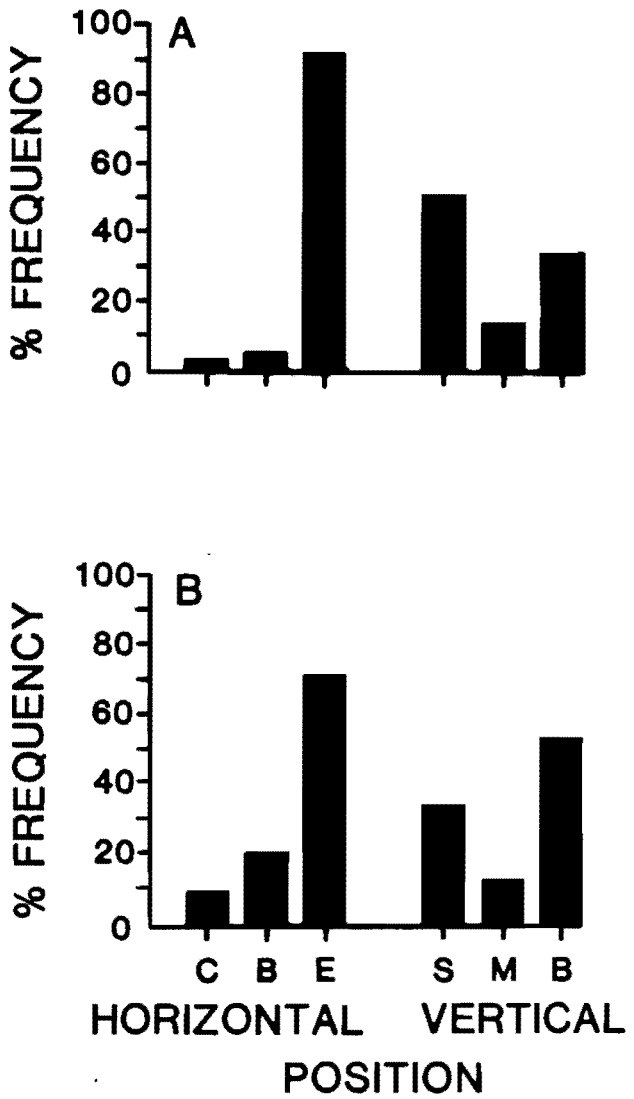



FIGURE 4-21. Horizontal and vertical position of year 2 green sea turtles (C. mydas) in the experimental pool (see Figure 4-1C). C=center; B=intermediate between center and edge of pool; E=edge of pool; S=surface; M=mid-water; B=bottom of pool. Percent frequency is the number of times the turtles were recorded in that location divided by the total number of positions recorded (Table 4-5). These turtles are 10 months of age.

A. Control behavior - South Louisiana Crude Oil V (SLCOV)

B. Experimental behavior - South Louisiana Crude Oil V (SLCOV)

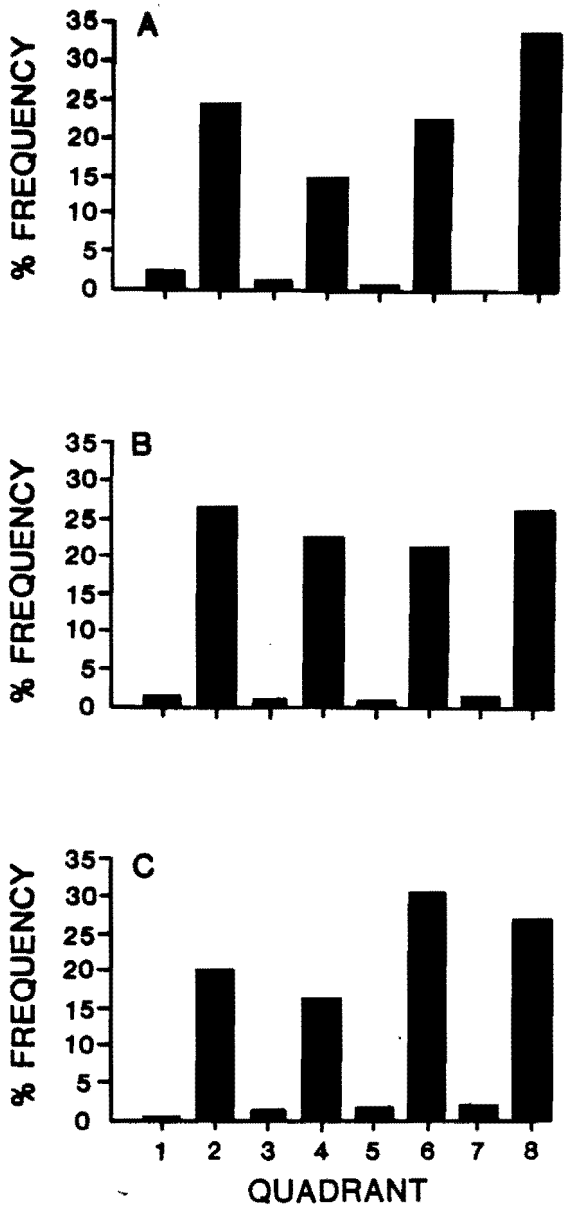



FIGURE 4-22. Distribution of year 1 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1C). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-6). These turtles are 10 months of age.

A. Control behavior - Mineral Oil II
B. Experimental behavior - Mineral Oil II
C. Normal behavior - Replacement Turtles

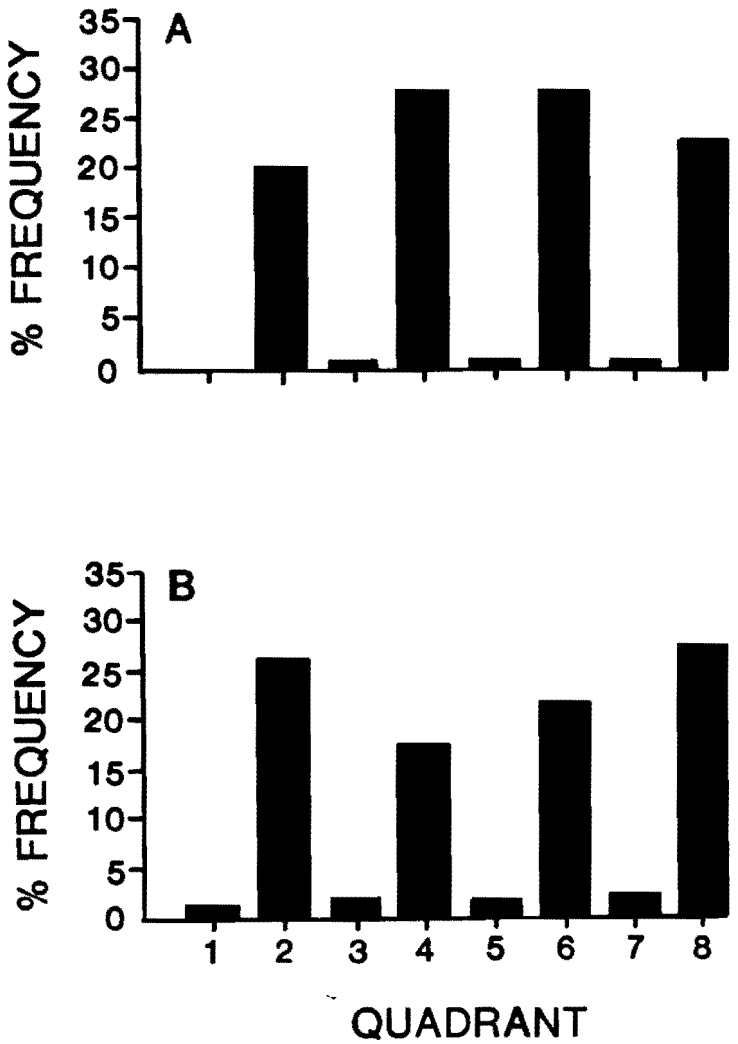



FIGURE 4-23. Distribution of year 1 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-6). These turtles are 15 months of age.

A. Control behavior - South Louisiana Crude Oil II (SLCOII)

B. Experimental behavior - South Louisiana Crude Oil II (SLCOII)

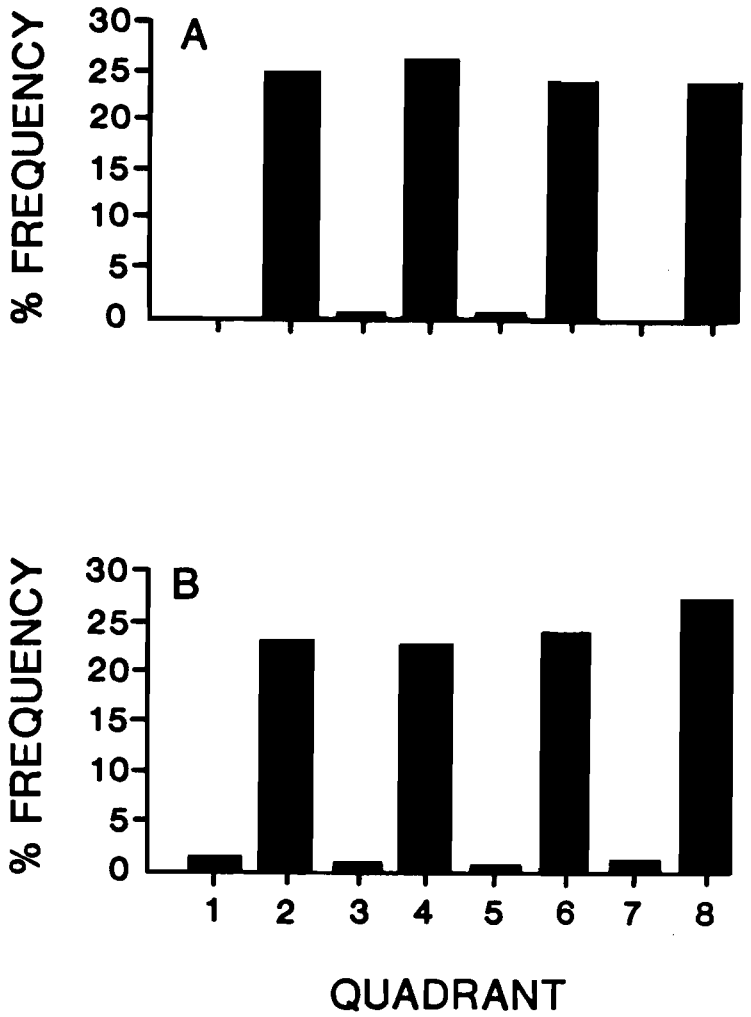



FIGURE 4-24. Distribution of year 1 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-6). These turtles are 18 months of age.

A. Control behavior - South Louisiana Crude Oil III (SLCOIII), group 1 turtles

B. Experimental behavior - South Louisiana Crude Oil III (SLCOIII), group 1 turtles

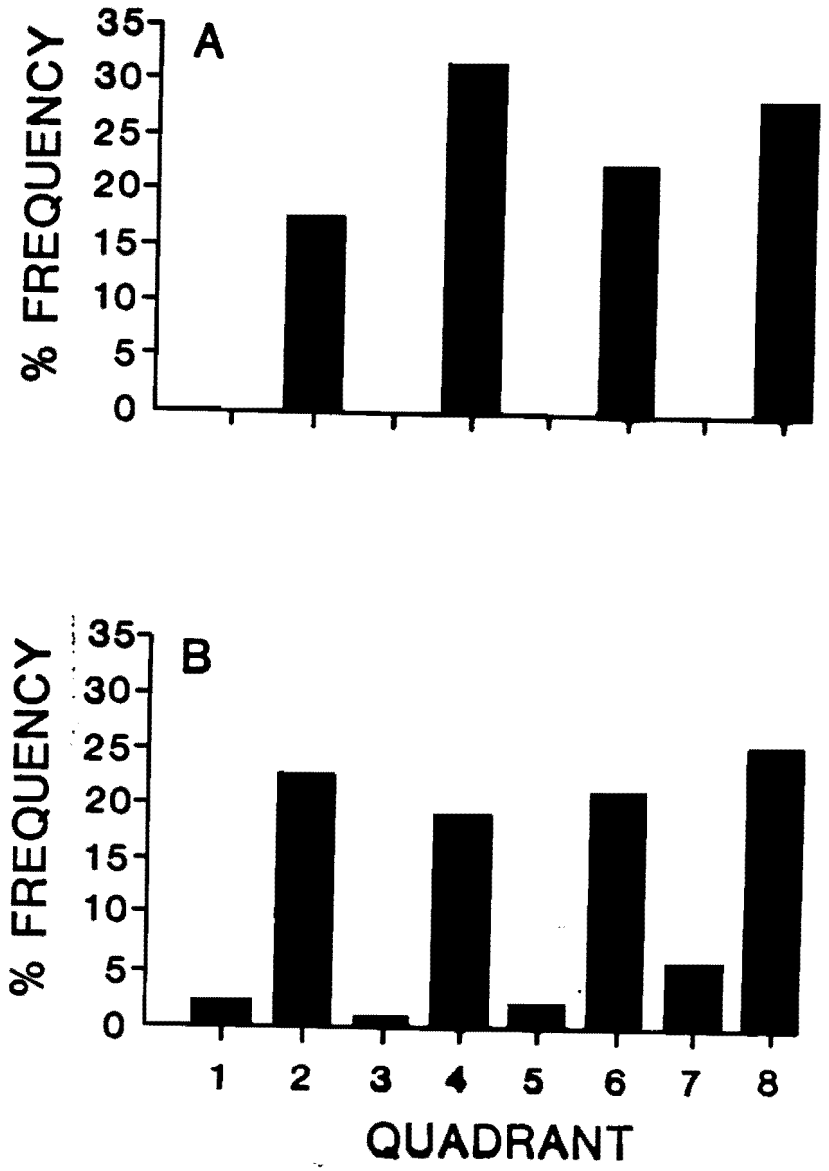



FIGURE 4-25. Distribution of year 1 green sea turtles (C. mydas) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-7). These turtles are 11 months of age.

A. Control behavior - South Louisiana Crude Oil I (SLCOI)

B. Experimental behavior - South Louisiana Crude Oil I (SLCOI)

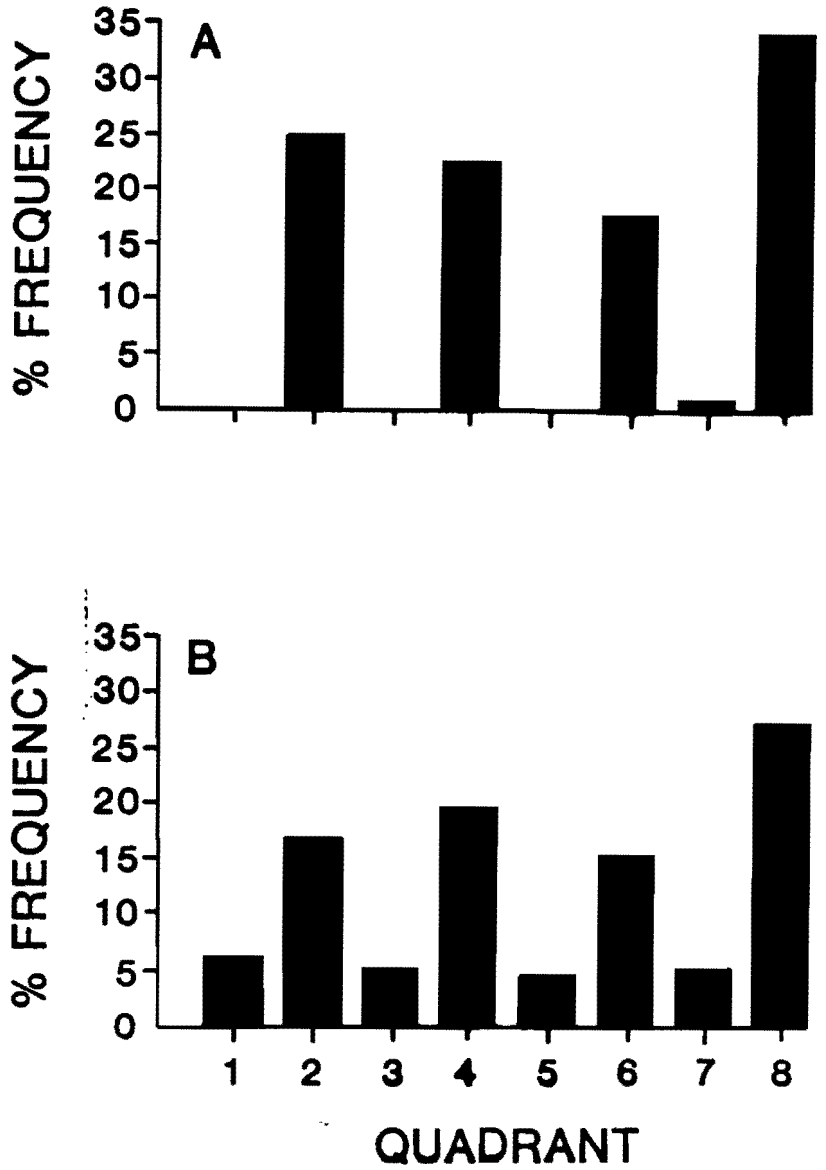



FIGURE 4-26. Distribution of year 1 green sea turtles (C. mydas) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-7). These turtles are 15 months of age.

A. Control behavior - South Louisiana Crude Oil II (SLCOII)

B. Experimental behavior - South Louisiana Crude Oil II (SLCOII)

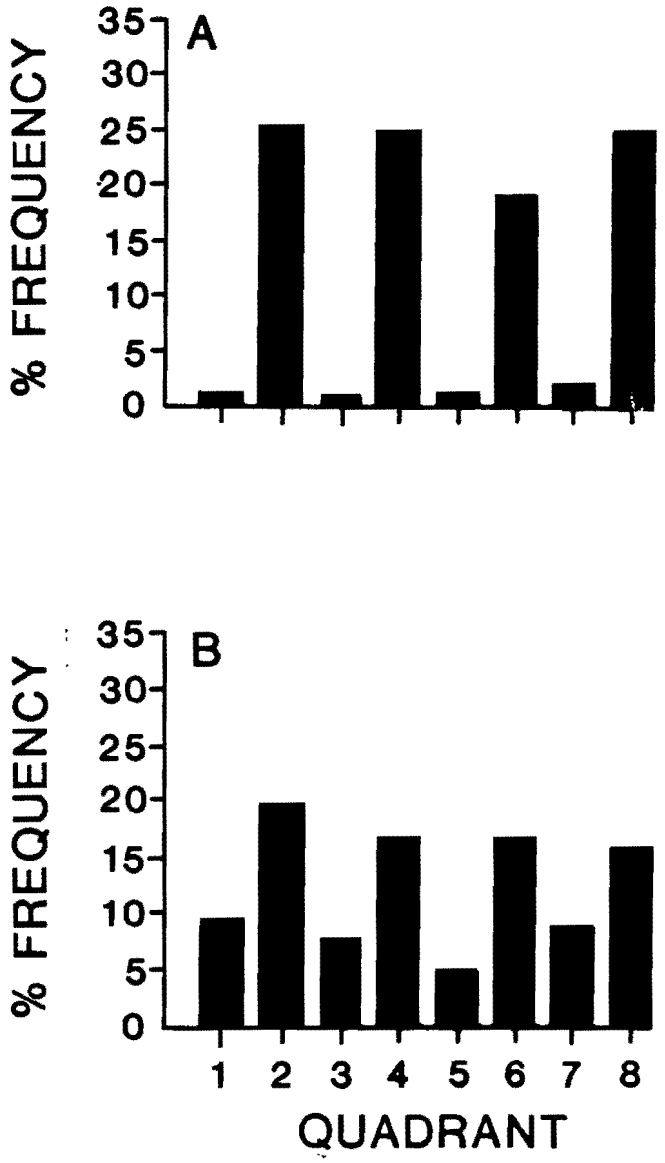



FIGURE 4-27. Distribution of year 2 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-8). These turtles are 7 months of age.

A. Control behavior - South Louisiana Crude Oil IV (SLCOIV)

B. Experimental behavior - South Louisiana Crude Oil IV (SLCOIV)

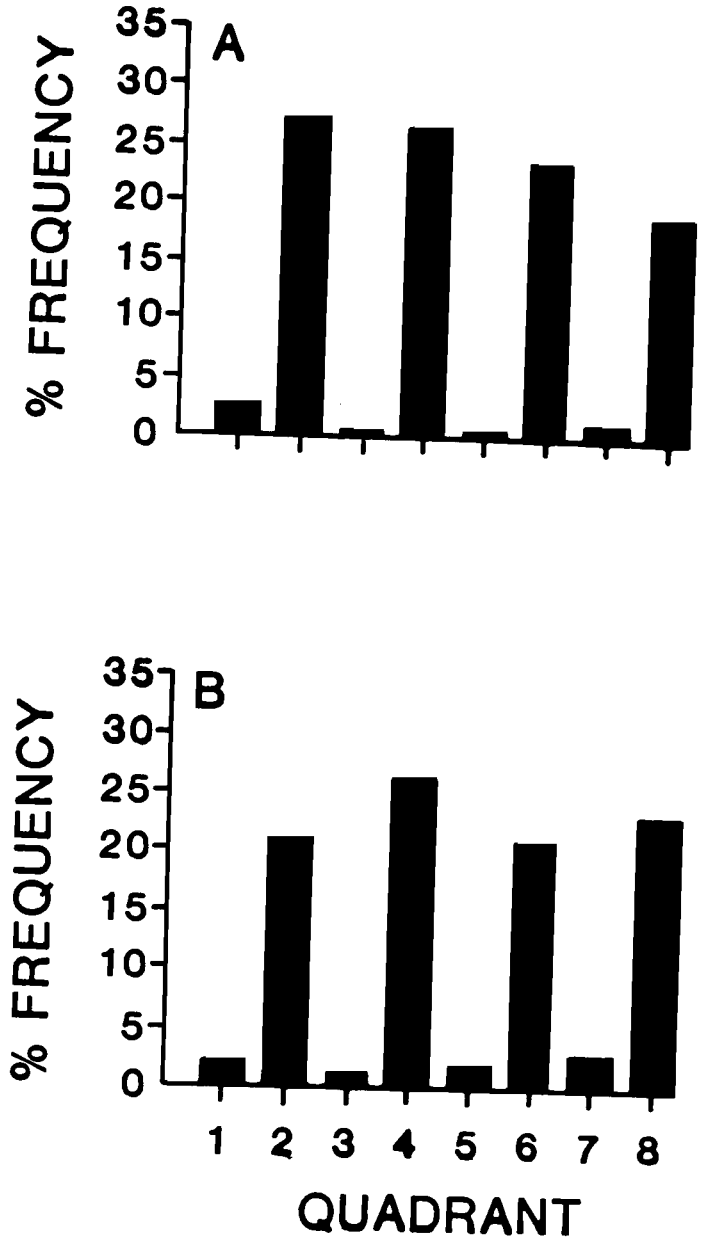



FIGURE 4-28. Distribution of year 2 loggerhead sea turtles (C. caretta) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-8). These turtles are 10 months of age.

A. Control behavior - South Louisiana Crude Oil V (SLCOV)

B. Experimental behavior - South Louisiana Crude Oil V (SLCOV)

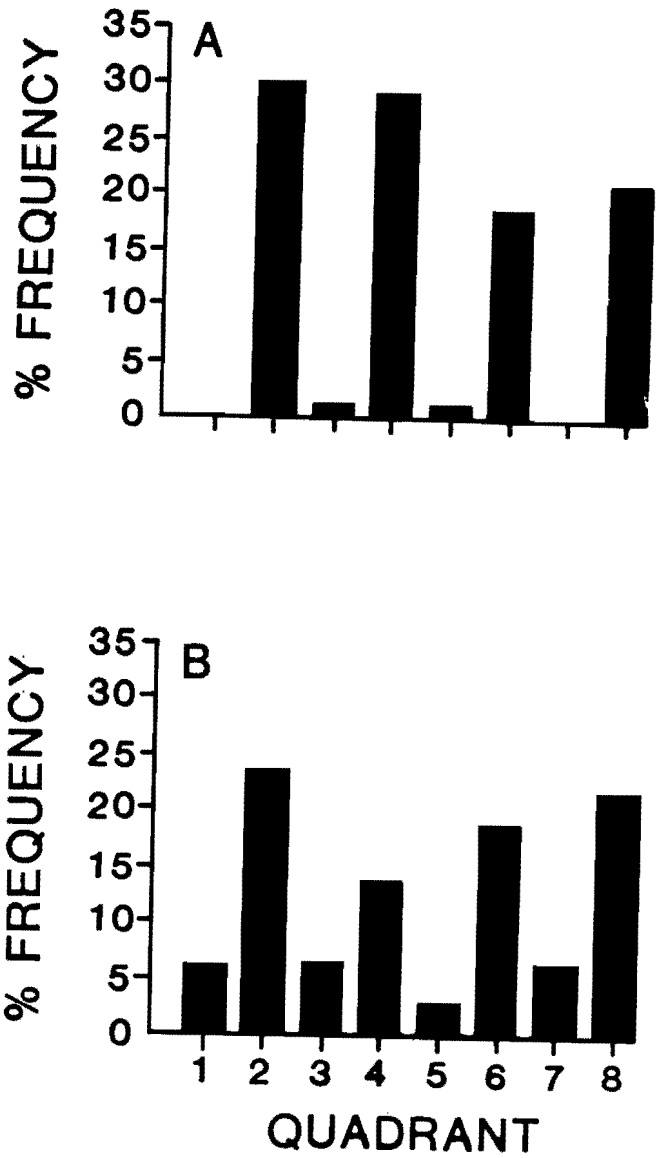



FIGURE 4-29. Distribution of year 2 green sea turtles (C. mydas) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-9). These turtles are 7 months of age.

A. Control behavior - South Louisiana Crude Oil IV (SLCOIV)

B. Experimental behavior - South Louisiana Crude Oil IV (SLCOIV)

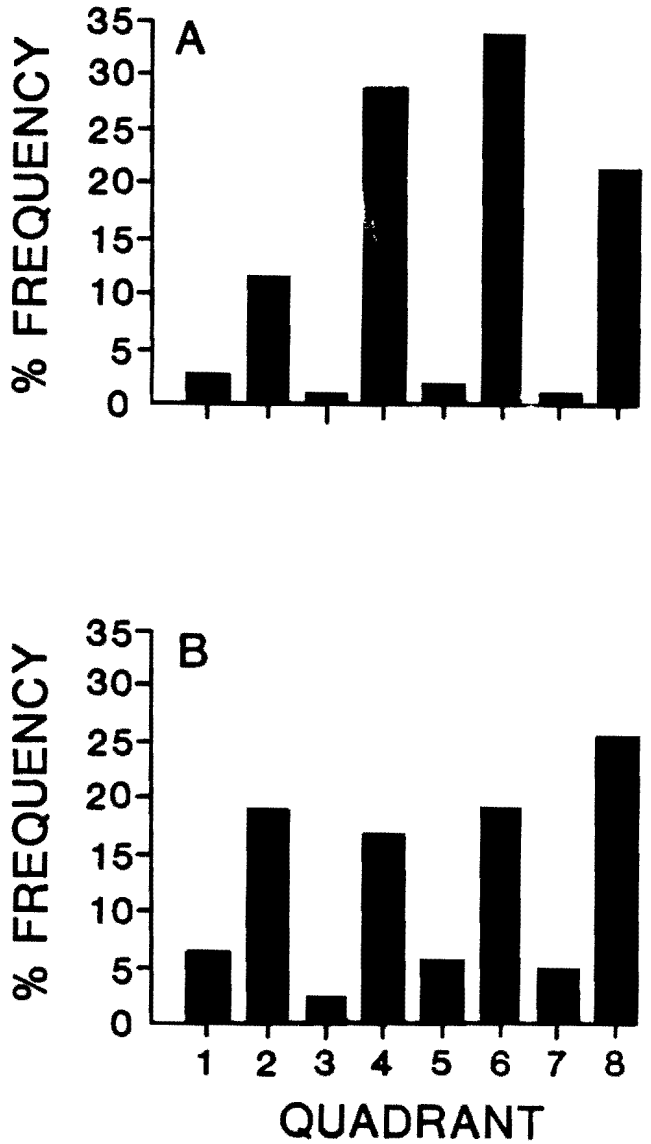



FIGURE 4-30. Distribution of year 2 green sea turtles (C. mydas) in the 8 quadrants of the experimental pool (see Figure 4-1B). Percent frequency is the number of times the turtles were recorded in a specific quadrant divided by the total number of positions recorded (Table 4-9). These turtles are 10 months of age.

A. Control behavior - South Louisiana Crude Oil V (SLCOV)

B. Experimental behavior - South Louisiana Crude Oil V (SLCOV)

Table 4-10. A comparison of means of initial and all dive times, breaths at center pool, and water temperature for year 1 (CC1) loggerhead sea turtles.

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | DATE | DIVE TIME (sec.) INITIAL DIVE (a) $\bar{x}$ S.D. | DIVE TIME (sec.) ALL DIVES (b) $\bar{x}$ S.D. | BREATHS AT CENTER POOL PER TURTLE | MEAN POOL WATER TEMPERATURE °C. |
|---|---|---|---|---|---|---|---|
| Mineral Oil I | | | 03/84 | | | | |
| control | 6 | 9 | | 106.6 ±78.8 | - | 0.5 | |
| experimental | 14 | 9 | | 113.6 ±118.6 | - | 2.4 | 23.6 |
| Mineral Oil II | | | 04/84 | | | | |
| control | 6 | 10 | | 81.7 ±44.3 | 28.2 ±30.4 | 0.7 | |
| experimental | 14 | 10 | | 78.0 ±58.6 | 31.4 ±35.7 | 5.2 ** | 26.6 |
| SLCO I | | | 05/84 | | | | |
| control | 6 | 11 | | 50.5 ±45.5 | 42.4 ±33.3 | 0.3 | |
| experimental | 8 | 11 | | 86.5 ±70.2 | 33.4 ±25.5 * | 5.9 ** | 28.3 |
| Normal Behavior replacement turtles | 10 | 12 | 06/84 | 61.8 ±37.2 | - | - | 30.0 |
| SLCO II | | | 09/84 | | | | |
| control | 6 | 15 | | 79.0 ±30.6 | 37.4 ±26.6 | 0.3 | |
| experimental | 12 | 15 | | 84.8 ±81.3 | 43.3 ±36.1 * | 5.3 ** | 27.7 |
| SLCO III | | | 12/84 | | | | |
| control | 10 | 18 | | 125.7 ±5.55 | 47.0 ±33.4 | 0.1 | |
| experimental | 12 | 18 | | 143.6 ±167.4 | 50.1 ±49.8 | 2.0 | 21.5 |

* = $p<.01$, t-test
** = $p<.01$, Chi-square, 1df
a. mean of first dive times for turtles in a series
b. mean of all dive times for turtles in a series

Table 4-11. A comparison of means of initial and all dive times, breaths at center pool, and water temperature for year 1 green sea turtles (*C. mydas*)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | DATE | DIVE TIME (sec.) INITIAL DIVE (a) $\bar{x}$ S.D. | DIVE TIME (sec.) ALL DIVES (b) $\bar{x}$ S.D. | BREATHS AT CENTER POOL PER TURTLE | MEAN POOL WATER TEMPERATURE °C. |
|---|---|---|---|---|---|---|---|
| Mineral Oil II | | | 04/84 | | | | |
| control | 4 | 10 | | 21.3 ±14.6 | 20.1 ±11.6 * | 0.3 ** | 26.6 |
| experimental | 4 | 10 | | 14.8 ±6.9 | 25.6 ±23.6 | 5.8 | |
| SLCO I | | | 05/84 | | | | |
| control | 4 | 11 | | 28.8 ±12.6 | 18.4 ±11.1 * | 0.8 ** | 28.3 |
| experimental | 5 | 11 | | 35.0 ±24.2 | 22.4 ±16.0 | 10.2 | |
| SLCO II | | | 09/84 | | | | |
| control | 4 | 15 | | 51.0 ±27.7 | 17.3 ±11.4 * | 0.5 ** | 27.7 |
| experimental | 5 | 15 | | 49.2 ±9.9 | 36.6 ±23.7 | 19.4 | |

* = $p<.01$, t-test
** = $p<.01$, Chi-square, ldf
a. mean of first dive times for turtles in a series
b. mean of all dive times for turtles in a series

Table 4-12. A comparison of means of initial and all dive times, breaths at center pool, and water temperature for year 2 loggerhead sea turtles (C. caretta)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | DATE | DIVE TIME (sec.) INITIAL DIVE (a) $\bar{x}$ S.D. | DIVE TIME (sec.) ALL DIVES (b) $\bar{x}$ S.D. | BREATHS AT CENTER POOL PER TURTLE | MEAN POOL WATER TEMPERATURE °C. |
|---|---|---|---|---|---|---|---|
| SLCO III | | | 12/84 | | | | |
| control | 4 | 4 | | 100.8 ±46.5 | 19.6 ±20.9 | 0.8 | |
| | | | | | | ** | 21.5 |
| experimental | 4 | 4 | | 106.8 ±51.2 | 18.4 ±19.2 | 7.0 | |
| SLCO IV | | | 03/85 | | | | |
| control | 5 | 7 | | 214.8 ±118.4 | 75.6 ±71.5 | 2.4 | |
| | | | | | * | ** | 20.2 |
| experimental | 6 | 7 | | 110.3 ±71.1 | 36.3 ±25.8 | 14.3 | |
| SLCO V | | | 06/85 | | | | |
| control | 10 | 10 | | 38.5 ±24.7 | 27.4 ±28.4 | 1.1 | |
| | | | | | | ** | 30.4 |
| experimental | 8 | 10 | | 30.1 ±38.9 | 30.2 ±25.6 | 7.9 | |

* = $p<.01$, t-test
** = $p<.01$, Chi-square, ldf
a. mean of first dive times for turtles in a series
b. mean of all dive times for turtles in a series

Table 4-13. A comparison of means of initial and all dive times, breaths at center pool, and water temperature for year 2 green sea turtles (C. mydas)

| EXPERIMENT | NUMBER OF TURTLES | TURTLE AGE (MONTHS) | DATE | DIVE TIME (sec.) INITIAL DIVE (a) $\bar{x}$ S.D. | DIVE TIME (sec.) ALL DIVES (b) $\bar{x}$ S.D. | BREATHS AT CENTER POOL PER TURTLE | MEAN POOL WATER TEMPERATURE °C. |
|---|---|---|---|---|---|---|---|
| SLCO III | | | 12/84 | | | | |
| control | 5 | 4 | | 86.2 ±23.6 | 35.4 ±20.4 | 1.8 | |
| | | | | | * | ** | 21.5 |
| experimental | 9 | 4 | | 87.0 ±29.1 | 42.6 ±28.9 | 13.7 | |
| SLCO IV | | | 03/85 | | | | |
| control | 9 | 7 | | 70.6 ±36.6 | 45.7 ±25.2 | 0.7 | |
| | | | | | | ** | 20.2 |
| experimental | 10 | 7 | | 83.6 ±45.6 | 44.6 ±36.0 | 14.4 | |
| SLCO V | | | 06/85 | | | | |
| control | 9 | 10 | | 18.3 ±15.3 | 29.3 ±20.1 | 2.3 | |
| | | | | * | | ** | 30.4 |
| experimental | 10 | 10 | | 48.0 ±31.8 | 27.5 ±17.7 | 23.2 | |

* = p<.01, t-test
** = p<.01, Chi-square, ldf
a. mean of first dive times for turtles in a series
b. mean of all dive times for turtles in a series

Since turtles are poikilotherms, one would expect longer dive times (= breath holding) when the water was relatively cold. In addition, one would also expect longer dive times as the turtles grew and weight-specific metabolic rate decreased. Because of this interaction it is not possible to accurately predict the effect of temperature on dive times.

Another factor that also differed between control and experimental (including mineral oil) situations was the location in the pool (edge vs. center) where the turtles surfaced to breathe (Tables 4-10 to 4-13). In all cases there was a strong tendency for turtles to breathe in the center of the pool under experimental conditions. Here too, we conclude that this behavior was due to the dark color of the edge surface layer.

Tar Ball Experiments

The results of the tar ball experiments are summarized in Table 4-14. The overall results are often inconsistent between and within experiments and, in some cases, the strike frequency was so low that results are inconclusive. In year 1 loggerheads (Table 4-14) the numbers of strikes between control and experimental groups were not equal ($p<.01$, Chi-square, 1df) and, in the experimental situation, wooden balls were struck more frequently than tar balls. In other experiments (year 2, greens) the overall number of strikes was low and not always equal between control and experimental situations. However, wooden and tar balls were struck with equal frequency.

We were unable to explain the overall low strike frequency as the turtles were fasted 24 hours before the experiments. It was assumed hungry turtles would strike at anything floating at the surface. A wide variation in the numbers of strikes by individual turtles within a given group was also noted. An analysis of the number of strikes by individual turtles (year 1 loggerheads, Tar Ball 1) as a function of time of day revealed an increased number of strikes/turtle in early and mid-afternoon (Fig. 4-31A) even though all turtles had been fasted for at least 24 hours prior to any observation. This response pattern may have been due to anticipation of regularly scheduled feedings in the afternoon. Subsequent feeding schedules (after Tar Ball I) were varied over the work day but had little apparent effect on the remaining tar ball experiments.

Table 4-14. Summary of results of tar ball experiments for loggerhead (C. caretta) and green sea turtles (C. mydas)

| EXPERIMENT | # T | TURTLE AGE (MONTHS) | DATE | YEAR 1 (c) LOGGERHEADS (CC1) # STRIKES | YEAR 1 (c) LOGGERHEADS (CC1) # BOUTS(a) | # T | AGE | YEAR 2 LOGGERHEADS (CC2) # STRIKES | YEAR 2 LOGGERHEADS (CC2) # BOUTS | # T | YEAR 2 GREENS (CM2) # STRIKES | YEAR 2 GREENS (CM2) # BOUTS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tar Ball I | | | 10/84 | | | | | | | | | |
| control | 16 | 16 | | 377 | 250 | | | - | - | | - | - |
| experimental | 15 | 16 | | tar(b) 82 * | 67 | | | - | - | | - | - |
| | | | | wood 169 * | 99 | | | - | - | | - | - |
| Tar Ball II | | | 02/85 | | | | | | | | | |
| control | 10 | 20 | | 53 * | 39 | 10 | 6 | 2 | 1 | 10 | 31 - | 25 |
| experimental | 10 | 20 | | tar 4 * | 4 | 10 | 6 | 0 | 0 | 10 | 24 | 13 |
| | | | | wood 9 = | 6 | | | 2 | 2 | | 17 = | 10 |
| Tar Ball III | | | 05/85 | | | | | | | | | |
| control | | | | - | - | 10 | 9 | 20 * | 11 | 10 | 53 * | 40 |
| experimental | | | | - | - | 10 | 9 | tar 0 | 0 | 10 | 2 | 2 |
| | | | | | | | | wood 2 | 2 | | 8 = | 5 |
| Tar Ball IV | | | 07/85 | | | | | | | | | |
| control | | | | - | - | 10 | 11 | 228 * | 55 | 10 | 17 * | 16 |
| experimental | | | | - | - | 10 | 11 | tar 29 | 24 | 10 | 11 | 10 |
| | | | | | | | | wood 129 * | 41 | | 25 = | 21 |

a) a bout is a group of 1 or more strikes grouped closely in time and separated from the next group of strike(s) by an arbitrarily variable time interval.
b) tar = strikes on tar balls; wood = strikes on wooden balls
c) data for group 1 loggerheads are a summation of 2 sets (replicates) of control observations and 2 sets (replicates) of experimental observations.
*) $p<.01$, Chi-square, df=1
=) $p>.01$, Chi-square, df=1
#T) number of turtles

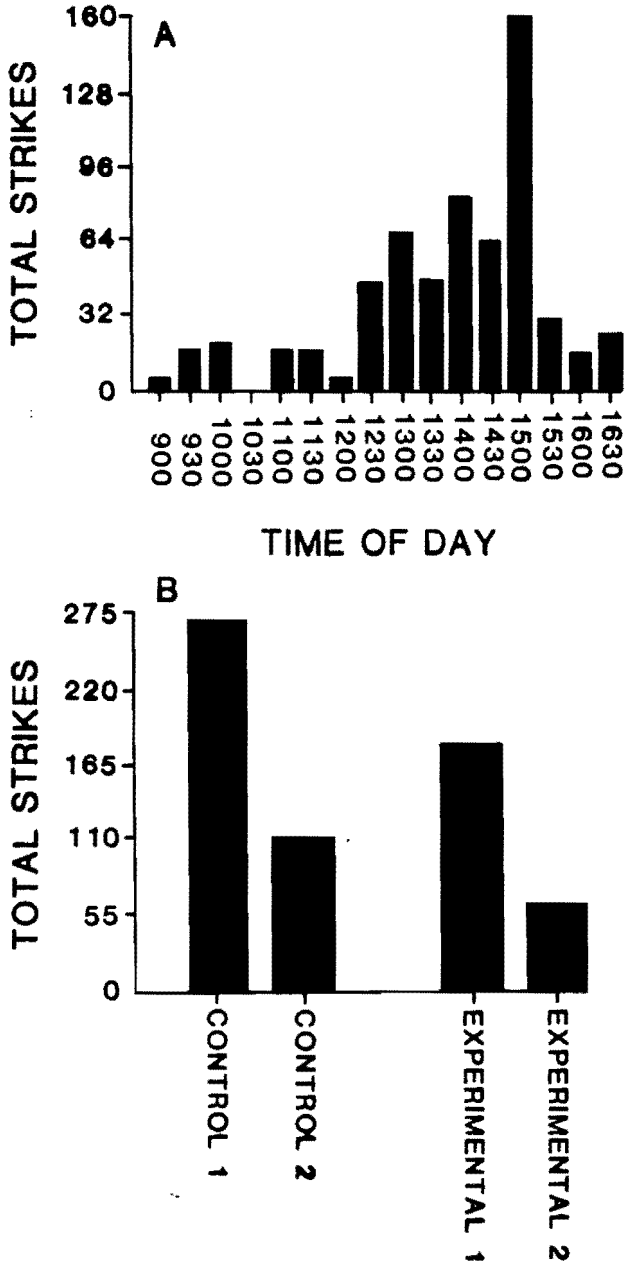



FIGURE 4-31. A. Temporal distribution of total strikes at tar and wooden balls by year 1 loggerhead sea turtles (C. caretta). B. Summary and comparison of total strikes at tar and wooden balls by year 1 loggerhead sea turtles (C. caretta). The data are a compilation of 2 sets of control and 2 sets of experimental observations from Tar Ball I experiment. These turtles are 16 months of age. The distribution of strikes between tar and wooden balls is as follows: Experimental 1 - 50 tar, 133 wood; Experimental 2 - 32 tar, 36 wood.

In the first tar ball experiment (year 1 loggerheads) we were able to repeat both control and experimental observations on the same turtles after a 48 hour interval. The results (Figure 4-31B) indicate a significant decrease in the total number of strikes by about 60% in both control and experimental situations. This suggested that the turtles may have learned that the objects were inedible. This opportunity to learn may not occur in the natural environment where the turtles could actually ingest the tar. While the trend was the same in both control and experimental trials (i.e. a decrease in strikes), the two groups were not equal in their response to the balls. The control group had a significantly higher ($p<.01$) strike total than the experimental group (Figure 4-31B). We can not explain this difference.

Analyzing the factors which might have accounted for the overall low strike frequency on the floating balls we hypothesize that it may have been related to the way in which the turtles were routinely fed. They were fed cut shrimp and squid and Purina turtle chow. The turtle chow floated for some time, but the shrimp and squid tended to sink to the bottom of the pool. It is possible that if the turtles preferred the food which sank they would be more responsive to items at the bottom of the pool than those at the surface.

To test this hypothesis, in experiment Tar Ball IV (Table 4-14), we sank the array with lead weights so that the balls floated about 5 cm. above the bottom of the pool (Figure 4-13B). This had an obvious effect on year 2 loggerheads. The control groups had significantly higher strike rates than the experimental groups and wooden balls were struck more frequently than tar balls. However, the change in experimental configuration had no apparent effect on year 2 greens (Table 4-14). This suggests interspecific differences in feeding behavior.

## Additional Studies

In addition to the long term studies with the circular oil boom, we experimented with a straight boom in March, 1985 (Table 4-1). The boom bisected the experimental pool (Figure 4-32). Our objective was to determine if this boom configuration and the resulting wider area that was covered with oil had an effect on turtle behavior that was different from the circular boom. We used two 6 month old loggerhead and two 5 month old green sea

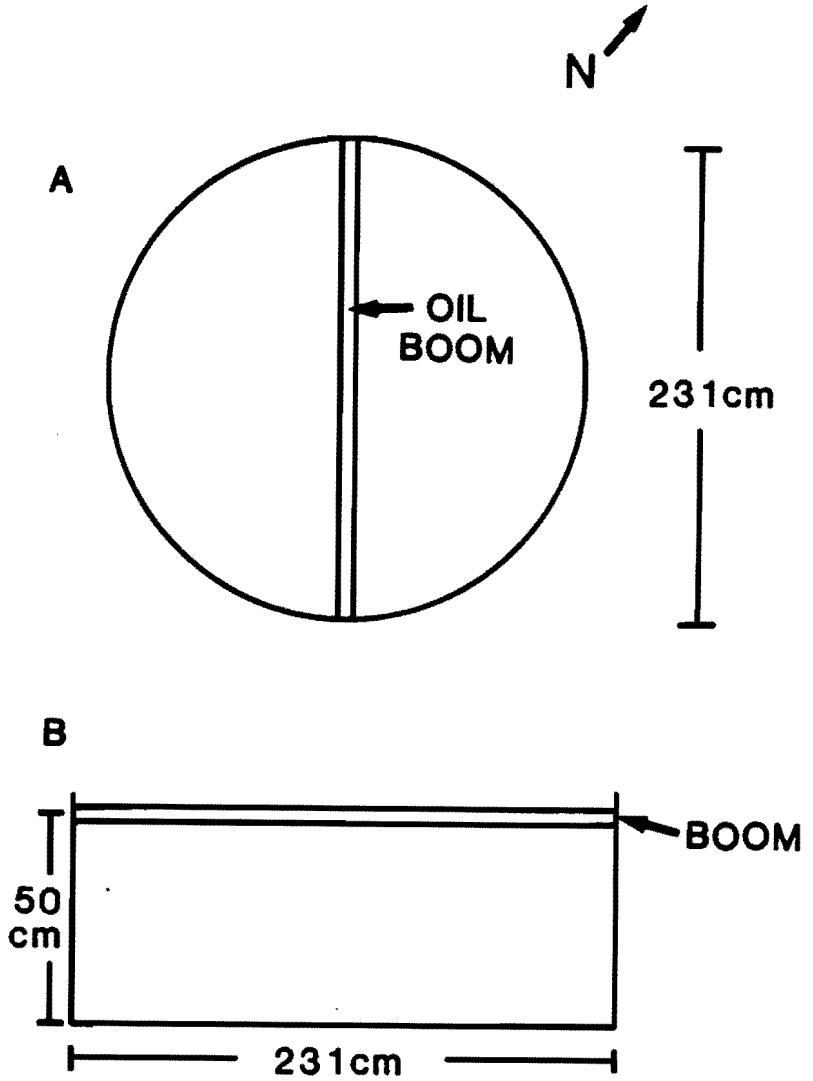



FIGURE 4-32. Diagram of the experimental setup for the straight boom oil slick trial. A. Top view of tank showing straight boom in place. B. Side view of tank showing vertical position of tank.

turtles. In the control situation with the boom in place, each turtle was placed at the center of the pool and observed once for 30 minutes. The group of 4 turtles spent 95% of their time at the edge of the pool with no preference for the left or right side of the boom. The group of control animals spent about twice as much time under water than at the surface. This summation is biased by species differences. The loggerheads spent twice as much time at the surface as did the greens. The overall results of the control observations and the species differences are similar to the results of control observations with the circular boom.

In the experimental situation a 0.5 cm layer of weathered crude oil was placed on the right side of the boom. Each turtle was introduced at the center of the pool on the unoiled side. Twenty-four hours later we repeated this study with the two loggerheads but entered them on the oiled side of the pool. As with the circular boom studies, the entry location (oil vs. clear water) had no apparent effect on subsequent behavior. The behavior of the turtles in the experimental situation was altered by the dark surface layer on half of the pool. Turtles spent 67% of their time in the clear water and 85% of their time at the edge of the pool on either the oiled or unoiled side. The latter figure is probably too low because we could not determine a turtle's horizontal position when it was on the oil side unless it was at the surface. When the turtles were on the unoiled side they (as a group) spent equal amounts of time at the surface and under the water. When they were on the oiled side they spent 3 times as much time under the water as they did at the surface. Given the small sample size and the summation of the results over two species, the behavior of the turtles in the straight boom pool configuration was quite similar to that in the circular boom configuration. Under experimental conditions, the turtles preferred unoiled water and, when on the oiled side of the pool, spent more time under water than at the surface. There was a clearer preference for unoiled water in the straight boom study than in the circular boom studies. This is probably due to the availability of 'edge' on the unoiled side but could also be related to the relatively wider patch of oil in the straight boom configuration. Turtles at sea encountering a oil slick of similar thickness to the ones used in these experiments may be likely to avoid surfacing through it, other factors being equal.

One of the factors that might not be equal under natural conditions is the presence of Sargassum. Carr (1986) has demonstrated that young sea turtles seek out Sargassum as a source of shelter and food (other animals that live in the Sargassum) after they leave the nesting beaches. We briefly examined the effect of Sargassum on the behavior of 6 month old loggerhead and green sea turtles by placing a mass of Sargassum in the experimental pool. The mass covered about 20% of the surface area. Turtles were placed in the pool individually and away from the Sargassum. When the loggerheads found the Sargassum they entered the mass and stopped swimming floating with the mass. This contrasts sharply with the control situation in which the turtles swam incessantly around the edge of the pool. The color of the carapace of young loggerheads is very similar to the color of Sargassum. The green sea turtles basically ignored the Sargassum and continued to swim around the pool for the 30 minute observation period. The color of young green sea turtles contrasts sharply with the Sargassum. At sea the Sargassum is concentrated in rows by the same winds and currents that might concentrate oil or tar. Young turtles could be in a compromised situation if they were attracted by the Sargassum but, at the same time, repelled by the dark surface layer of oil.

## 4.3 Summary

The objectives of this study were to determine the effects of weathered South Louisiana crude oil and tar balls on the behavior of loggerhead and green sea turtles under environmental conditions that approached the natural environment as closely as possible. The study also included the use of dark colored mineral oil to provide a dark layer on the surface of the water similar to weathered crude oil but without the potentially toxic components and chemical cues.

We recorded and analyzed over 215 hours (160 oil slick and 55 tar ball) of videotaped turtle behavior. Turtles ranged in age from about 3 to 20 months. All groups of turtles responded to the dark surface layer (mineral or crude oil) by spending significantly more time deeper in the water column and by coming to the center of the pool (unoiled water) to breathe more frequently than in the control situations. Turtles still surfaced to breathe through the oil layers and ingested some oil in the process. Most turtles

were contaminated externally with oil. None of our turtles died or became sick as a result of the brief (30 minute) contacts with oil. It is our conclusion that the turtles were responding visually to the dark surface layer and not necessarily to petroleum hydrocarbons. This conclusion was supported by the fact that the same behavior patterns were seen when non-toxic, non-soluble mineral oil was used. The mineral oil would not provide the same chemical cues as SLCO, but would resemble it visually. Tactile detection is also a possibility that was not tested.

Based on these experimental results, in the natural environment turtles may respond to an oil slick (depending on its thickness and surface area) by seeking open water, increasing dive times, and descending in the water column. In the worst case scenario, spills with a large surface area which remain cohesive may be, in practice, virtually impossible for turtles to avoid due to the absence of clear openings. The behavior of such a spill would be heavily dependent on weather conditions at the time it occurred. Similarly, spills in the vicinity of nesting beaches could be particularly detrimental, especially at hatching time if the oil came ashore or acted as a barrier as the hatchlings swam away from the shore. Since our studies were limited to relatively small turtles, we do not know how larger (= older) turtles would respond.

Additionally, behavior is determined by a complex set of cues. As a relatively simple example loggerhead sea turtles have a strong attraction to floating Sargassum. An oil slick that is mixed with Sargassum may provide a conflicting situation for the turtles, although this situation was not tested in this study. If the attraction to the Sargassum is greater than the 'repulsion' from the dark surface layer created by the oil, actual contact with the slick could be prolonged and result in metabolic and skin disorders (Section 5.0).

The tar ball portion of this study sought to determine if young sea turtles selectively struck at tar balls floating on the surface of the water. Young turtles have been found coated with tar on beaches and it could be disastrous if they were selectively taking tar balls that they considered to be food items. The results of this study were inconclusive and may have been complicated by a regimented feeding schedule and the physical characteristics of the food that the turtles were fed. However, interspecific differences in feeding behavior between loggerhead and green sea turtles may also play a

role. Overall strike frequencies were low and control and experimental groups were often not statistically equal with respect to the number of strikes at the floating balls. While total strikes were low for the green sea turtles, wooden and tar balls were struck with equal frequency. In two loggerhead sea turtle experiments, wooden balls were struck more frequently than tar balls. In one set of repeated experiments with loggerheads the turtles appeared to learn the floating balls were inedible.

From our results we can not conclude that turtles actively strike at tar balls they encounter at sea. The route of contamination of tar fouled stranded turtles remains unknown. We do know that tar accumulates in the windrows with the Sargassum, as do many other floating materials. One hypothesis, not tested in this study, would be that the turtles, at least loggerheads, may accidentally come into contact with tar because they have sought out the Sargassum as a refuge. During attempts to clean the tar from their flippers, the turtles may contaminate their mouths with tar, impeding feeding and, in the worst case, leading to starvation.

# 5.0 EFFECTS OF EXPOSURE TO OIL

## 5.1 Physiological Effects (P.L. Lutz, M. Lutcavage, and D. Hudson)

A large portion of the U.S. oil production and tanker traffic occurs in the Southeast and Gulf of Mexico - Caribbean areas (National Academy of Sciences, 1985) which coincides with the largest distribution of north Atlantic sea turtle populations. Relatively little data is available concerning effects of oil on sea turtles (Appendix A). The primary intent of this project was to determine if exposure to crude oil, such as might be encountered in an actual oil spill or blowout at sea, is detrimental to the health of sea turtles. No information was previously available regarding toxic effects of oil in sea turtles. Those regulatory systems that had been previously shown in marine birds and mammals to be impacted by oil contamination were chosen for initial study.

### 5.1.1 Methods

Two levels of oil exposure were used. In chronic exposure experiments we used the minimum practical oil layer thickness that would ensure that the surface of the experimental tank was completely covered by oil (0.05 cm). Exposure to oil lasted 96 hours. At this concentration a turtle would be exposed to oil in an amount that might reasonably prove deleterious if there were no means of escape. During the second year of research we selected an additional "acute" exposure level of 0.5 cm surface oil for 48 hours to provoke a "worst case" response. These layers may be much higher than those that a sea turtle might reasonably encounter in the wild, and it is obvious that under these experimental conditions the turtle has no means of avoiding the oil. Yet there is some evidence (Section 4.0) that some sea turtles may drift or selectively feed in surface wrack lines (Carr, 1986) which could result in prolonged contact with viscous weathered oil.

<u>Animals</u>

Subadult loggerhead turtles (<u>Caretta caretta</u>) ranging from 8 to 12 kg (approximately 15 - 18 months old) were obtained from mixed clutches that had been reared in headstart programs in Florida and the Bahamas. Turtles were kept outdoors in large 4' x 6' fiberglass tanks plumbed with a flow-through seawater system pumped from Biscayne Bay. Turtles were fed only pellet

Turtle Chow #45 (Purina) ad libitum 3 x per week. Minor bites and skin lesions were routinely treated with Betadine solution applied directly to the wounds as they occurred.

Exposure Studies

Sea turtles were brought indoors, weighed and held in a 30" x 30", 340 liter experimental tank filled with fresh unfiltered seawater (salinity 33 0/00 ±3 0/00) at room temperature (22-24° C) 24 hours before experiments were begun.

South Louisiana Crude Oil (SLCO) was selected for exposures studies (as stipulated by the study contract) and obtained from the Texaco batch used in behavioral experiments. Oil was weathered outdoors over sea water for 48 hours in a wide glass container using an aerator stone for mixing. Following baseline day the chronic (0.05 cm layer) or acute (0.5 cm layer) amount was then poured on top of seawater in the experimental tanks. Measurements on control turtles were obtained simultaneously with and following the same procedures of sea turtles exposed to oil. Each turtle was first a control turtle, then an experimental turtle.

Soon after experimental turtles were placed in the tank with oil their swimming movements tended to disperse the surface film and we observed a reduction in visible amounts of oil with time. Since we could not easily estimate the amount of oil lost in adhering to the equipment, turtle, etc. we did not compensate by adding more SLCO to the tank. We sampled seawater from the middle of the tank and collected oil from the surface slick on exposure days 1 and 3. Water and oil samples were submitted to Dr. Van Vleet for hydrocarbon analysis and determination of initial and final concentration of oil. These results are presented in Section 3.0, Table 3-7.

At least one hour before baseline (Day 0) measurements were begun a lid was placed over the seawater in the tank so that the turtle could only surface to breathe in a 600 ml glass funnel (Figure 5-1). Turtles usually learned to breathe in the funnel within one or two attempts at finding the surface. Our procedure was to record ventilation rate, tidal volume and oxygen uptake for 60 - 90 minutes once turtles appeared able to breathe in this manner with no obvious distress. Ventilation rate (f) and inspired tidal volume (Vt) were recorded continuously using a digital #1 pneumotachograph (Hewlett Packard) attached to the funnel with a low

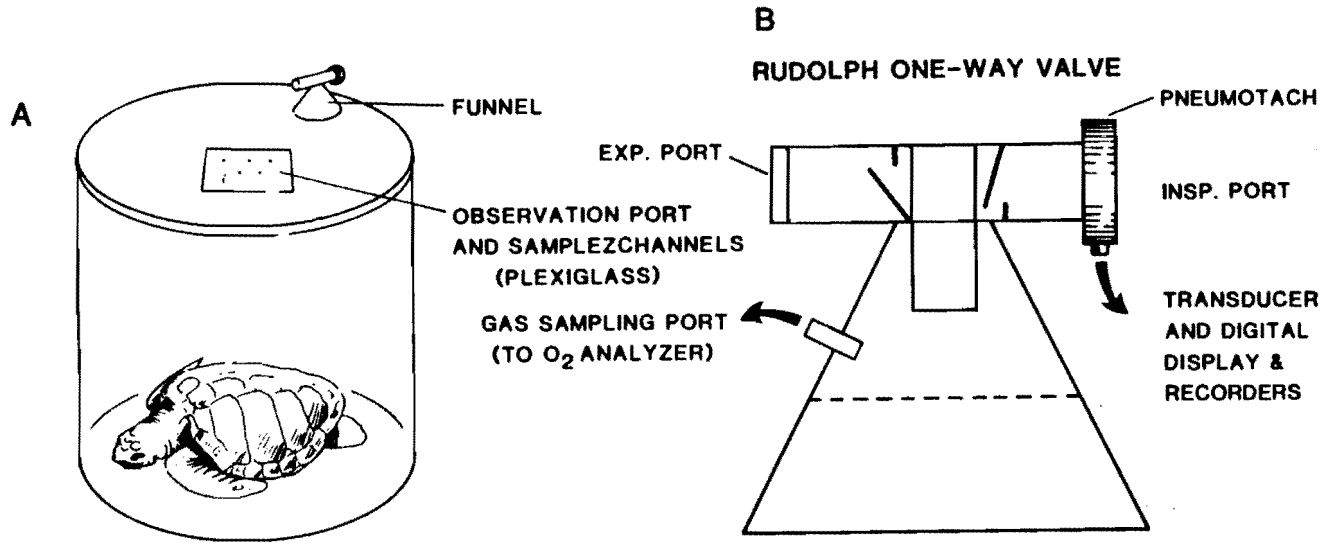



FIGURE 5-1. Diagram of experimental apparatus for oil exposure experiments. A. The turtle is placed in the tank, the oil added to the surface, and the lid put in place. Breathing and oxygen consumption is measured through the funnel and Rudolph one-way valve on the lid. B. Blowup of funnel showing detail of Rudolph one-way valve and hookups for pneumotachograph and oxygen analyzer.

volume one-way respiratory valve (Rudolph, Inc.). The pneumotach was calibrated with a 3-liter precision syringe and its response was linear from 100 - 3000 ml.

Whenever possible oxygen uptake per breath was recorded using the funnel system. A downstream pump drew room air into the funnel through the one-way valve. Excurrent air from the funnel was first drawn through a drierite column followed by Ascarite ($CO_2$ absorbant). Oxygen partial pressure was measured using an Applied Electrochemistry S 3-A $O_2$ analyzer. The analyzer was calibrated before each experiment with room air and oxygen partial pressure ($pO_2$) was recorded continuously from the excurrent funnel air. Oxygen uptake ($VO_2$) was calculated according to the respirometry equations of Withers (1977).

Respiration is intrinsically difficult to measure in sea turtles because they have an irregular breathing pattern and their ventilation rate not only depends on activity and temperature but may also be influenced by the stress involved in most experimental procedures (Glass and Wood, 1983). In our protocol turtles are not restrained and may surface at will to breathe. Nonetheless, their "baseline" diving behavior in the tank is in itself extremely variable and individuals may be influenced differently by the restrictions imposed by breathing in the funnel.

Sea turtles are episodic breathers and spend only about 2% of the time at the surface to breathe (Lutz and Bentley, 1985). In contrast to mammals that have a regular rhythm in breathing, in turtles a breathing bout may consist of one to several breaths of varying length. In consequence, the conventional parameters used to describe mammalian respiration such as frequency (breaths per minute) would not distinguish between a turtle that took a series of 6 breaths and submerged for 60 minutes ($f=0.1\ b \cdot min^{-1}$) from a turtle that took a breath every 10 minutes ($f=0.1\ b \cdot min^{-1}$).

We characterized turtle breathing patterns using both the mammalian analog of breathing frequency (f) and modified scheme of Glass and Wood (1983). They define the breathing ratio as the time spent breathing, the ventilatory period (VP), over total measurement time, i.e. VP + non-ventilatory period (NVP). An increase in breathing ratio means that the turtle is spending more of its time at the surface to breathe. Frequency (f) may be given both as breaths per minute and as the number of breaths in a ventilatory sequence ($f_{vP}$) times the breathing ratio i.e. f mean = ($f_{vP}$)[VP /

(VP + NVP)]. These respiratory indices were used in an attempt to determine whether sea turtle respiration and diving patterns are affected by exposure to crude oil.

Venous blood was sampled atraumatically from the dorsal cervical sinus and whole blood was used for determination of blood gases, hematocrit, cell counts, packed cell volume and hemoglobin analysis. Serum and plasma were separated from whole blood and used for determination of blood glucose and serum protein. Other subsamples were processed and submitted to Drs. Bossart and Van Vleet for clinicopathology and hydrocarbon analysis, respectively. Venous blood pH, oxygen ($pO_2$) and carbon dioxide partial pressure ($pCO_2$) were determined immediately using a digital Mk 2 Radiometer Acid-Base digital analyzer thermostatted to room temperature. Hematocrit was determined following conventional procedures and blood glucose and serum protein were determined photometrically using Sigma Chemical kits.

Each time oiled turtles were removed from the tank for collection of blood samples, etc. some of the oil adhering to their body was unavoidably removed by special oil sorbent towels (T151, 3M Corporation) used to hold the turtles. Other than this we made no attempt to remove oil until the recovery period. Following the 96th hour sampling point we used the 3M oil sorbent sheets to remove oil adhering to the skin of the turtle. The mouth cavity and eyes were also blotted with this material to remove oil but no detergents or chemicals were used to clean the turtle. Although some oil may have remained in the mouth and cloacal cavities most visible oil was effectively removed by the sorbent sheets.

After turtles were cleaned of visible oil they were removed to the large outdoor holding tanks until the next sampling period and at this time had access to turtle chow. Turtles were removed to indoor experimental tanks at least 24 hours before recovery measurements were begun.

## Analysis of Data

The data were expressed as means $\pm$ standard error and compared using one-way anova at each sampling point with mean separation performed using the Fisher's least significant difference t-test (SAS, Fifth Edition 1985). We also compared each experimental day with baseline day 0 (prior to dosing). In acute studies, experimental days were contrasted using baseline day 0 as a control. The confidence level was selected at the 95% level for all

experiments unless indicated otherwise (data tables are presented in Appendix C). In acute studies with very limited sample size, data were normalized as per cent change from baseline (day 0) for glucose, white blood cell and red blood cell counts.

### 5.1.2 Results and Discussion

Chronic Exposure (0.05 cm layer) - Ventilation and Diving

Compared with prior experience of the researchers with respiration measurements in sea turtles, oxygen uptake ($VO_2$) remained remarkably constant in both control and contaminated turtles and through the recovery period (Figure 5-2). No evidence of alteration of metabolic rate was produced by oil exposure. Oxygen consumption in other loggerheads of similar size is within the range we have recorded in earlier studies (Lutcavage and Lutz, 1983; Lutz and Bentley, 1985) but is lower than values we have recorded using rebreathing methods (Lutcavage et al., 1985).

Chronically oiled turtles did not spend significantly different amounts of time at the surface compared to controls. Both groups spent approximately one third of their total time at the surface (Figure 5-3). Loggerhead turtles in large outdoor tanks surface for only 2% of their time (Lutz and Bentley, 1985) so it is important to note that the breathing patterns recorded here are strongly influenced by experimental conditions. No significant differences were found in tidal volumes or breathing frequencies (Figure 5-4) although there is some suggestion of a reduced ventilation rate in contaminated turtles during recovery. The lowest f values in oiled turtles were 0.3 $b \cdot min^{-1}$ at this time, while in control turtles f values were about 0.7 $b \cdot min^{-1}$. Tidal volumes and breathing frequency of oiled turtles were within the range recorded previously in loggerheads of similar mass (Lutcavage and Lutz, 1983).

Chronic Exposure - Skin

In loggerheads exposed to chronic oil levels the skin began to slough off in layers especially around the neck and flipper area. The soft skin then exposed was reddened and lacked the leathery texture of the original epidermis. The skin continued to peel off for several weeks of recovery. Skin appeared superficially to return to its normal leathery texture and

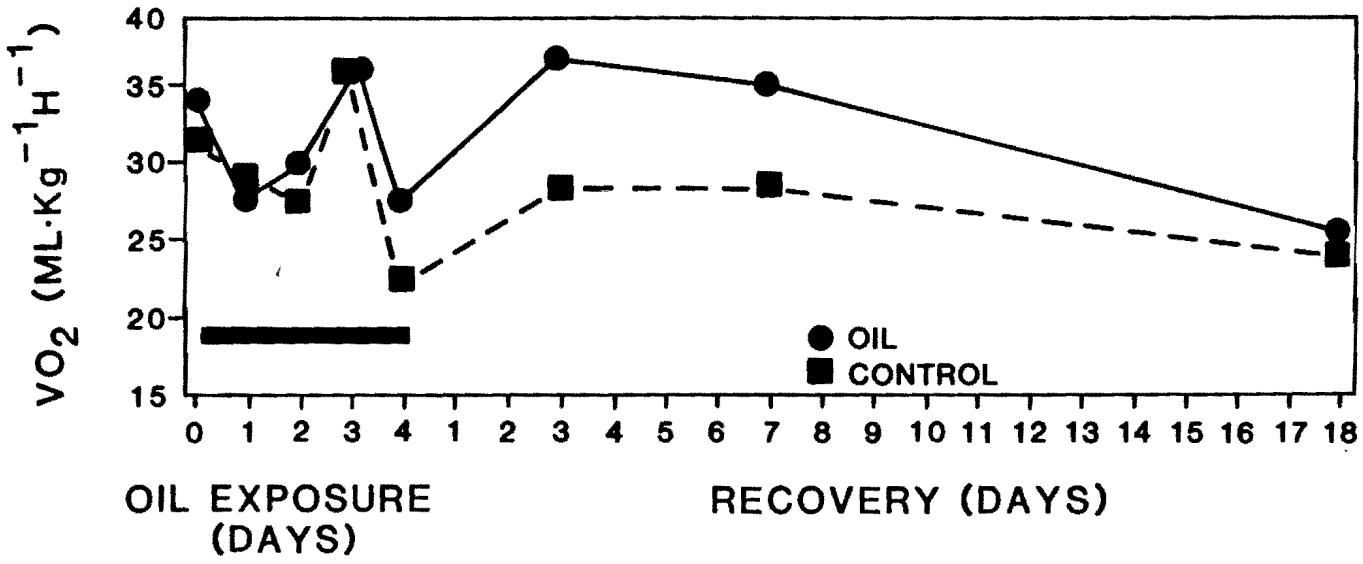



FIGURE 5-2. Mean oxygen uptake ($VO_2$) of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) and eighteen day recovery period.

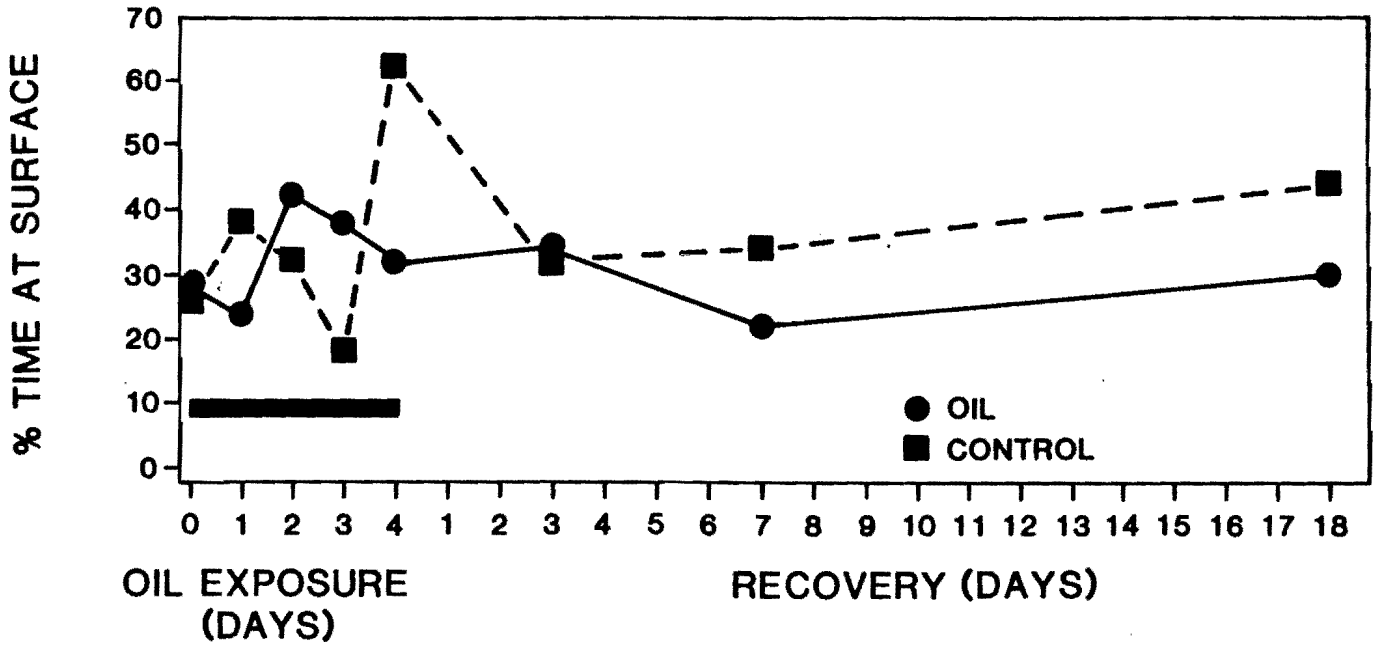



FIGURE 5-3. Mean percent time turtles spent at surface under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

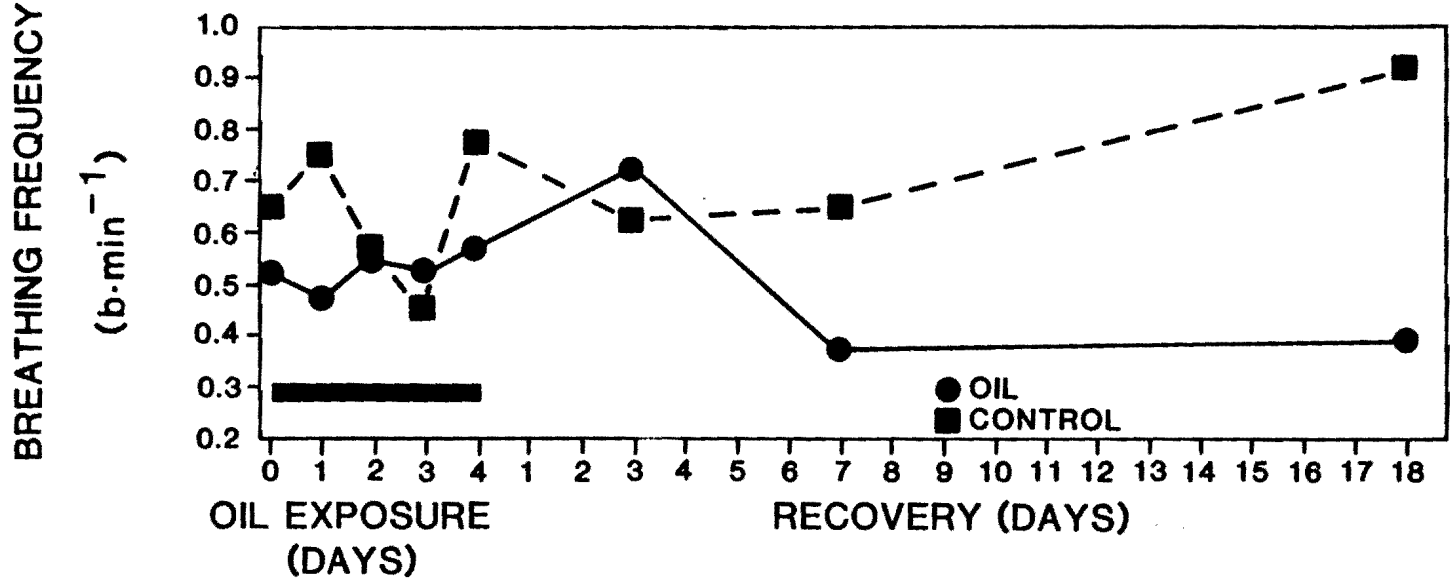



FIGURE 5-4. Mean breathing frequency of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

healing was evident within one month following oil exposure. The pathology of this condition is described in Section 5.2.

Chronic Exposure - Blood Chemistry

Again based on prior experience of the researchers, serum protein was remarkably constant in both oiled and control loggerheads (Figure 5-5). Albumin (used as an indicator of liver function) declined in the oil contaminated turtles (Figure 5-6) during the first six days of recovery but this difference was not statistically different from controls. No significant difference was found in the first three days of recovery in aspartate aminotransferase (serum AST) (Figure 5-7) between the groups. In mammals high levels of AST would indicate liver necrosis was occurring. Whether this is a valid interpretation for marine turtles is unknown.

Serum glucose declined significantly in both oiled and control groups (Figure 5-8). The general trend of declining values common to both groups is undoubtedly a function of starvation during confinement. The decline was reversed with feeding, but the rate of recovery in oiled turtles was slower than that of controls. At day 3 of recovery (the first point at which it was measured during recovery) contaminated turtles showed reduced levels relative to controls statistically significant at the $p<0.05$ level of significance. Therefore, it appears possible then that oil may interfere with uptake of energy substrates from the gut. An alternative possibility is that serum glucose falls as a result of a reduction in internal glucose metabolism. Levels were not statistically different on Day 7 and 15 of the recovery period.

Blood urea nitrogen (BUN) shown in Figure 5-9 decreased during the exposure period in both groups and this trend may also be related to starvation. In the control group BUN values showed an immediate increase with feeding (day 1, recovery) while in oiled turtles mean values continued to decline for ten days.

Hemoglobin concentration (an index of anemia) was not found to be significantly affected by oil contamination (Figure 5-10) and apparently increased during oil exposure, but during the recovery period experimental turtles appeared to suffer a decline in hemoglobin concentration though the difference was not statistically significant. In marine birds receiving large oral doses of Prudhoe Bay crude oil, hemolytic anemia (decreased

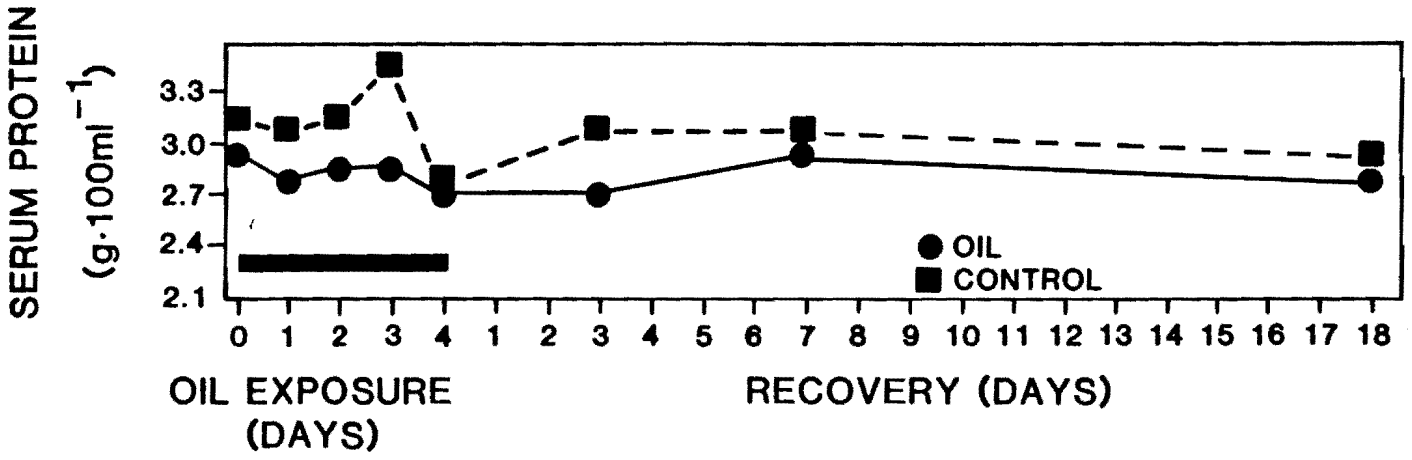



FIGURE 5-5. Mean serum protein concentrations in g·100 ml$^{-1}$ plasma of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

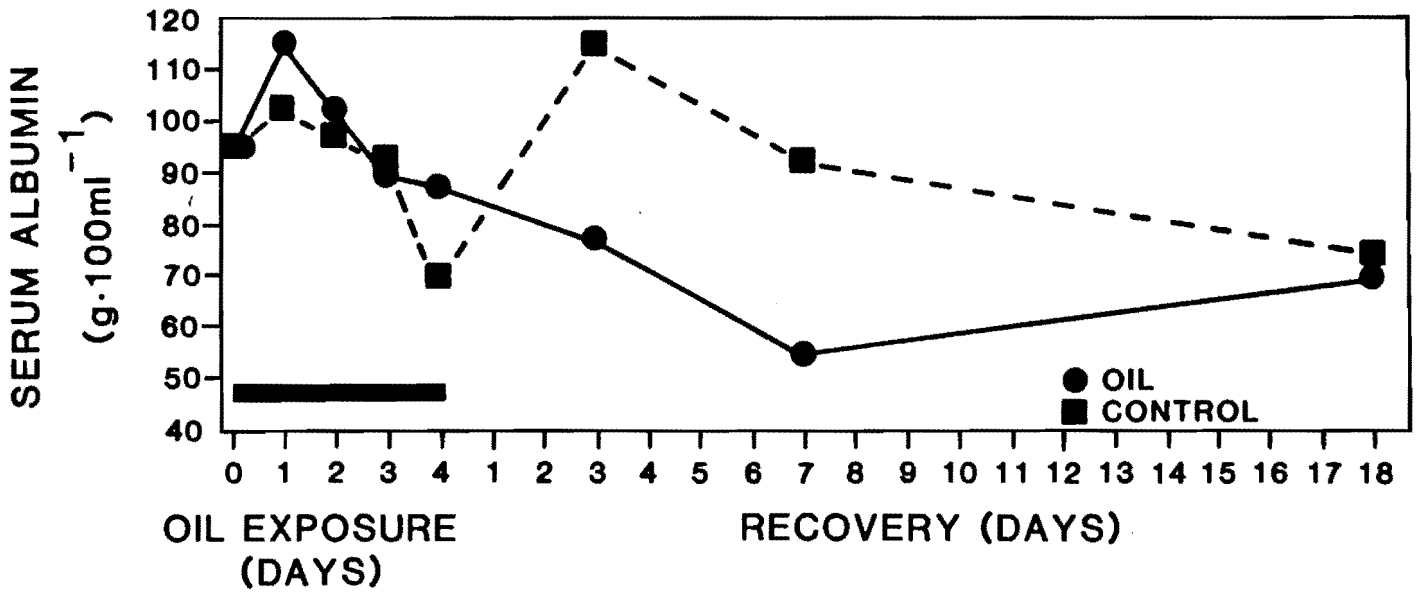



FIGURE 5-6. Mean serum albumin concentrations in g·100 ml$^{-1}$ plasma of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

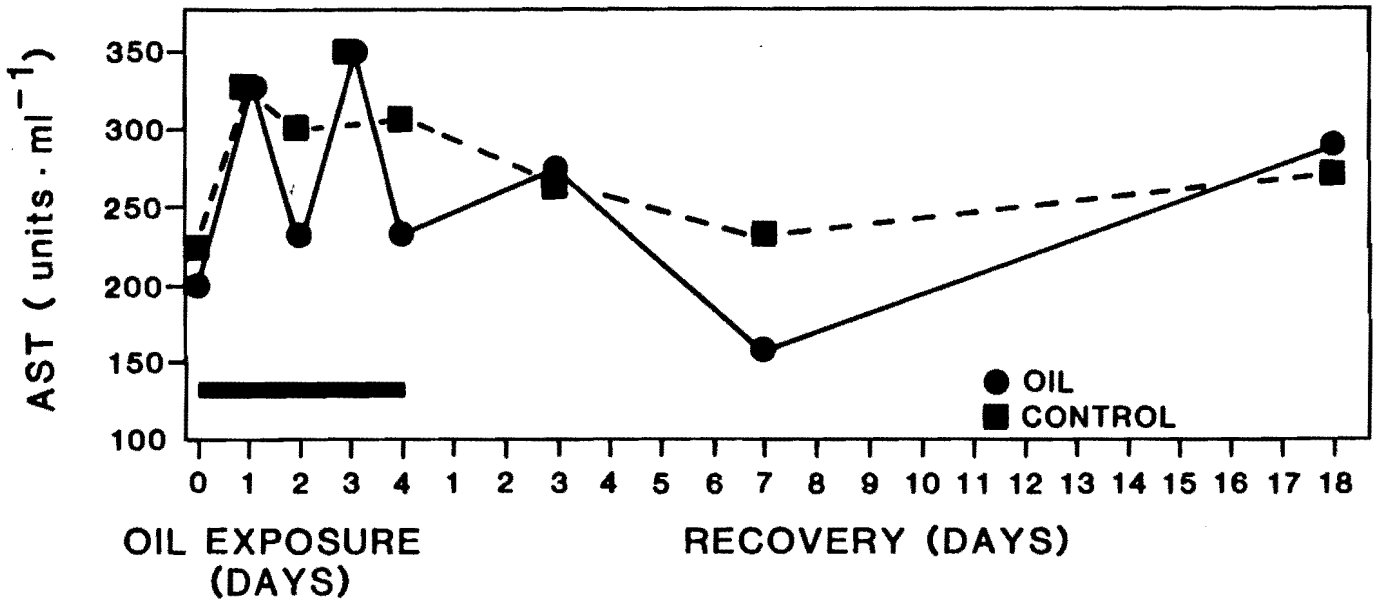



FIGURE 5-7. Mean serum aspartate aminotransferase (AST) concentrations in units·ml$^{-1}$ plasma of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) and eighteen day recovery period.

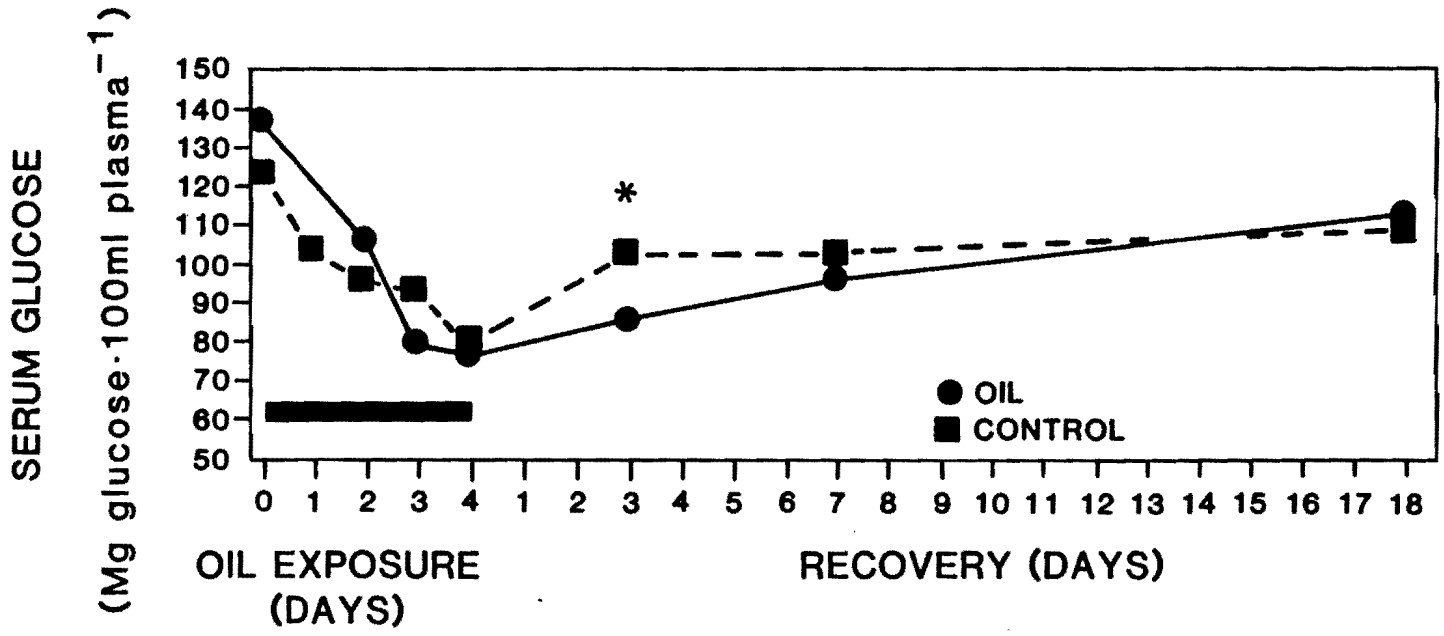



FIGURE 5-8. Mean serum glucose concentrations in mg glucose·100 ml$^{-1}$ plasma of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period. The asterisk indicates a statistically significant ($p<0.05$) decline on Day 3 of the recovery period.

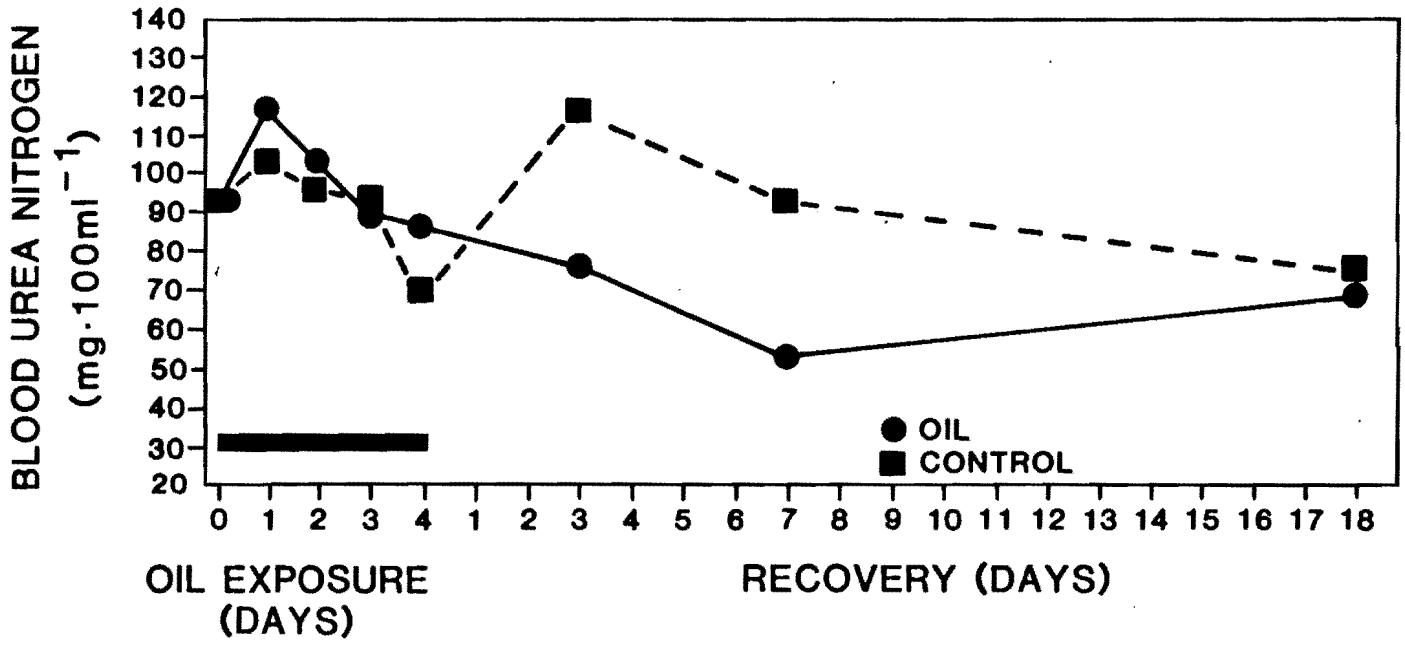



FIGURE 5-9. Mean blood urea nitrogen (BUN) concentrations in mg·100 ml$^{-1}$ plasma of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period. Note the increase in control values with feeding on Day 1 of the recovery period.

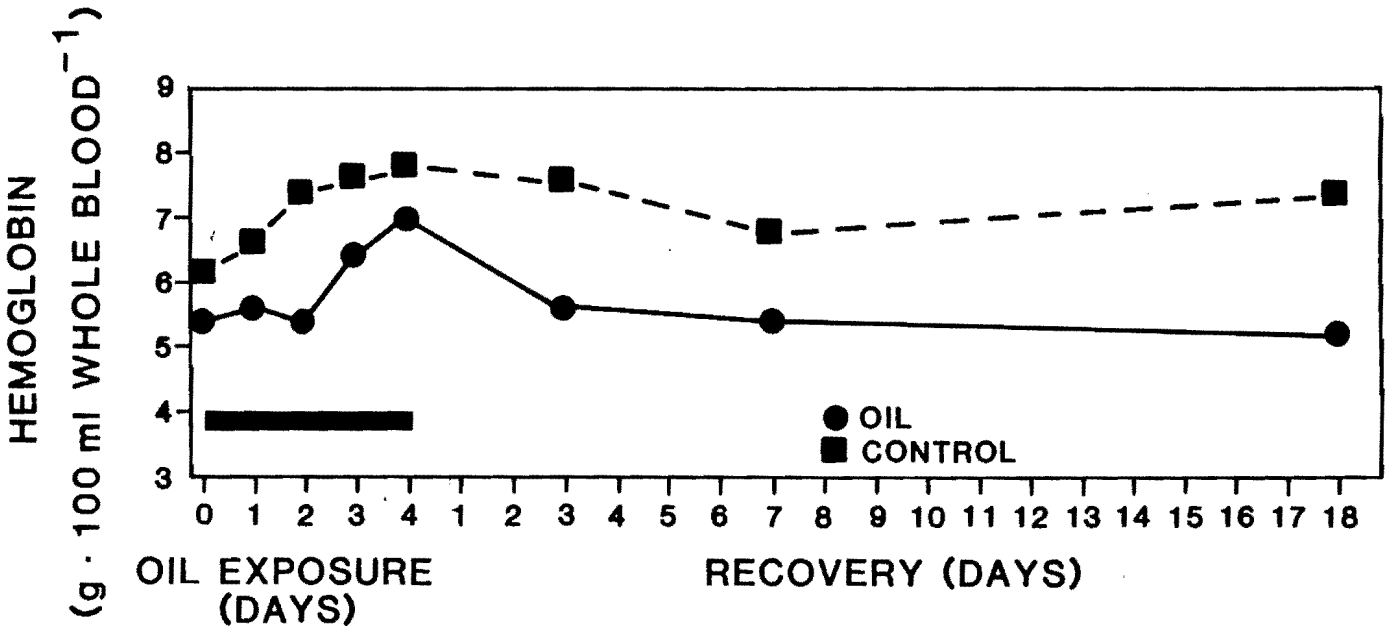



FIGURE 5-10. Mean hemoglobin concentrations in g·100 ml$^{-1}$ whole blood of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

hemoglobin), became pronounced following 5 days of exposure (Leighton et al., 1983). However, sea turtles do not appear to develop a severe hemolytic anemia at the exposure level we have tested.

The red blood cell count was not statistically significantly affected by oil exposure. However, counts in contaminated turtles soon after oiling were reduced almost by 50% and did not return to baseline values during the study period (Figure 5-11). This reduction was not statistically significant due to the large variation in red blood cell counts between individual turtles.

Hematocrit [packed cell volume (PCV)] was relatively constant throughout sampling and no statistically significant differences were found between groups (Figure 5-12). Mean values ranged from 20-25%, which is less than values we have obtained from wild loggerhead turtles (35%) sampled shipboard at Port Canaveral, Florida (Lutz and Dunbar Cooper, in press).

There was a rapid and significant rise in white blood cell count which in oiled turtles persisted to the last sampling point (Figure 5-13). By day 3 of oil exposure the experimental group white blood cell count had increased four-fold. This is evidence that the immune response of sea turtles was activated by oil exposure.

Chronic Exposure - Blood Gases

Venous $pO_2$ remained almost constant throughout the sampling period, showing no disturbance in tissue oxygen uptake (Figure 5-14). This finding is also supported by a relatively constant $VO_2$. Venous $pCO_2$ was not significantly affected by oiling (Figure 5-15). However, in both groups recorded $pCO_2$ values are very low, suggesting that hyperventilation occurred during the sampling period. This corresponds with the much greater time spent at the surface relative to turtles not subject to experimental stress.

Both groups showed alkalosis in terms of high venous pH values (Figure 5-16), although the oiled group had slightly but not significantly more acidic values. With condition of high pH concomitant with low venous $pCO_2$ it appears that respiratory alkalosis resulting from hyperventilation is the contributing factor.

It is possible then that the lower pH of the oiled group relative to controls, contrasted with their very similar $pCO_2$ values, is a result of metabolic acidosis. There are two types of physiological acidosis, metabolic and respiratory. Metabolic acidosis is almost invariably due to increases in

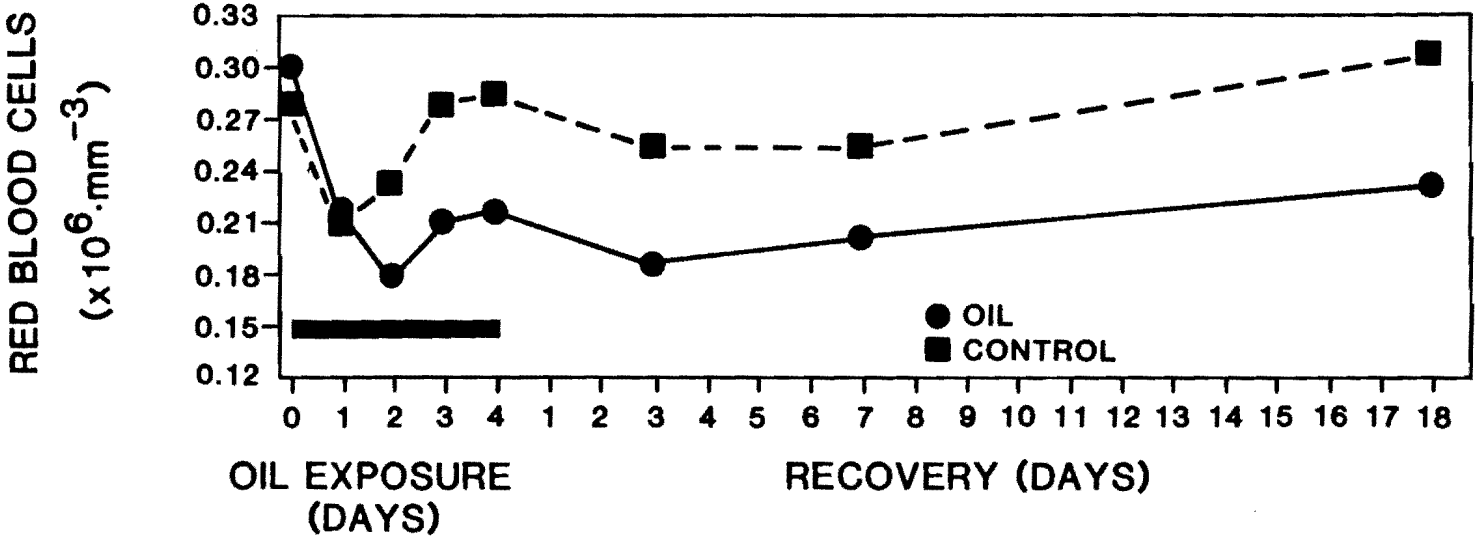



FIGURE 5-11. Mean red blood cell count per cubic millimeter of whole blood of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

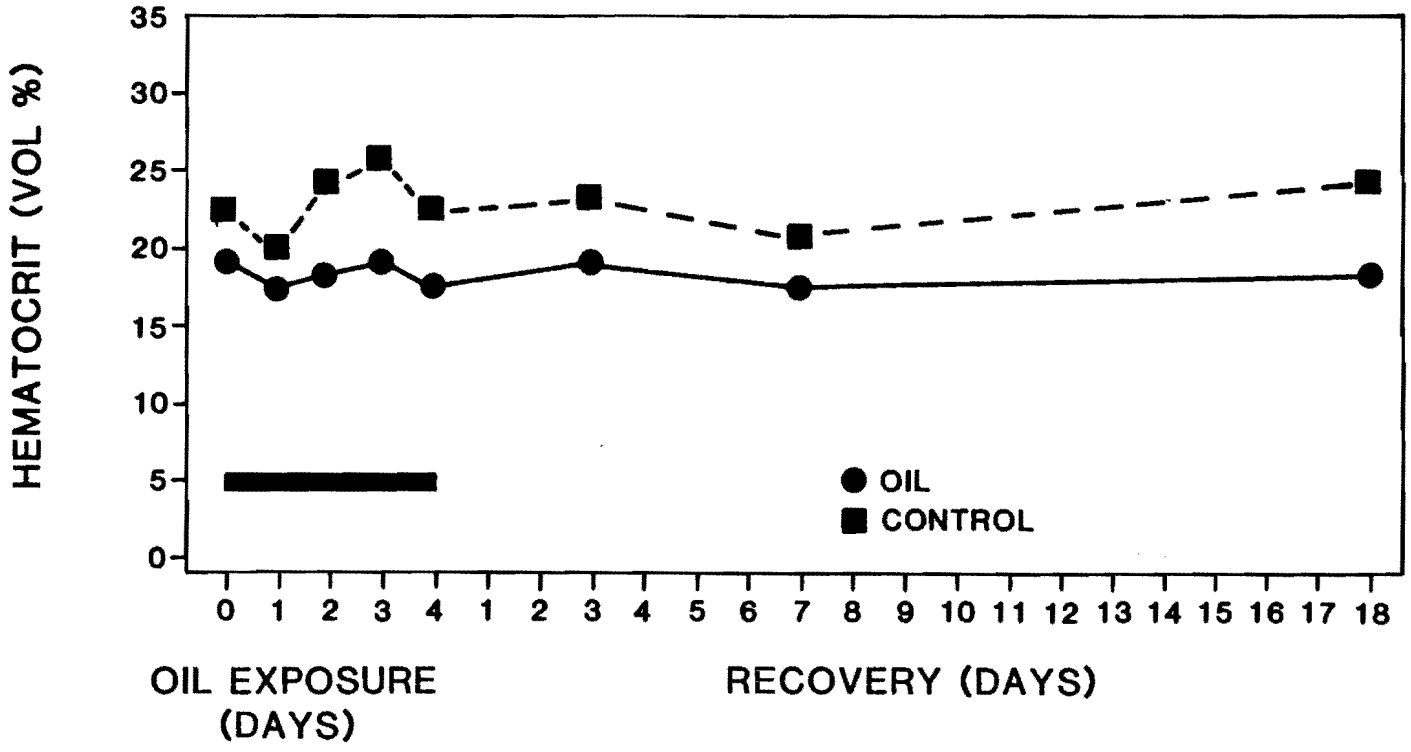



FIGURE 5-12. Mean hematocrit as percent volume of whole blood of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

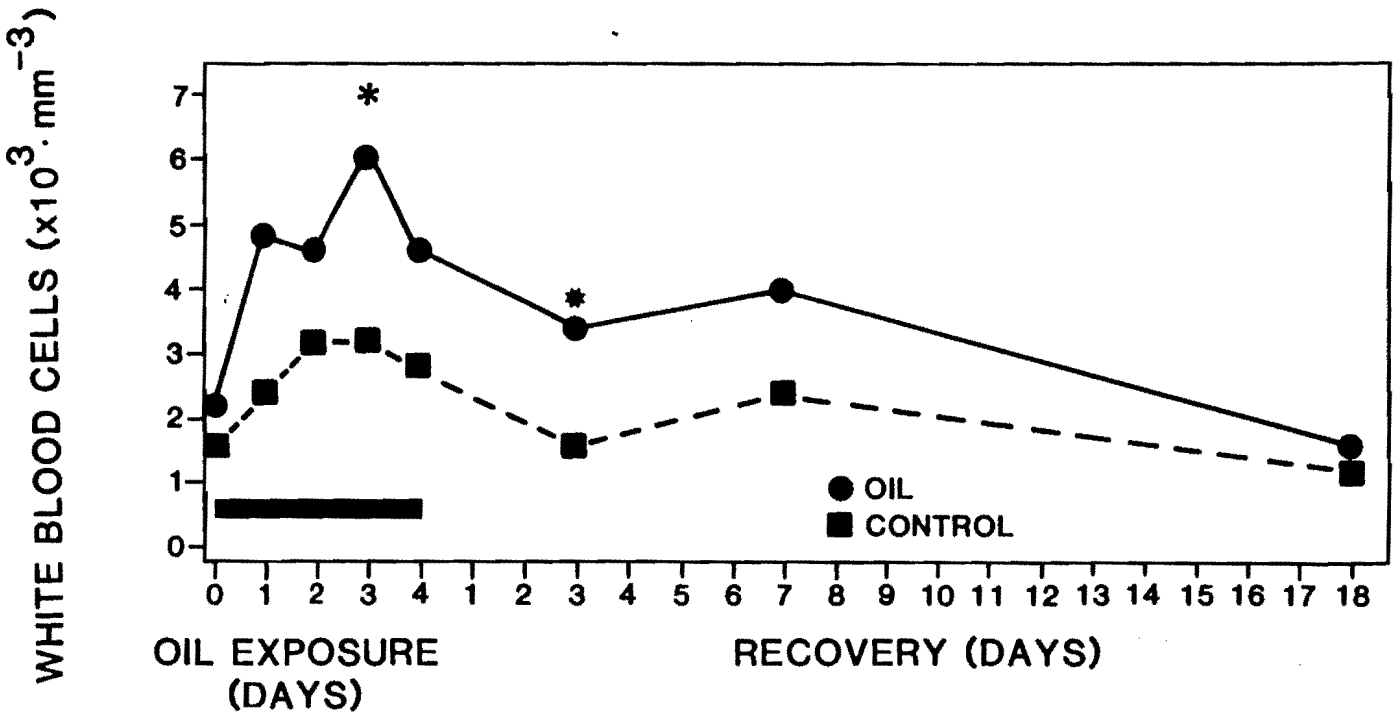



FIGURE 5-13. Mean white blood cell count per cubic millimeter of whole blood of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period. Asterisks indicate statistically significant differences.

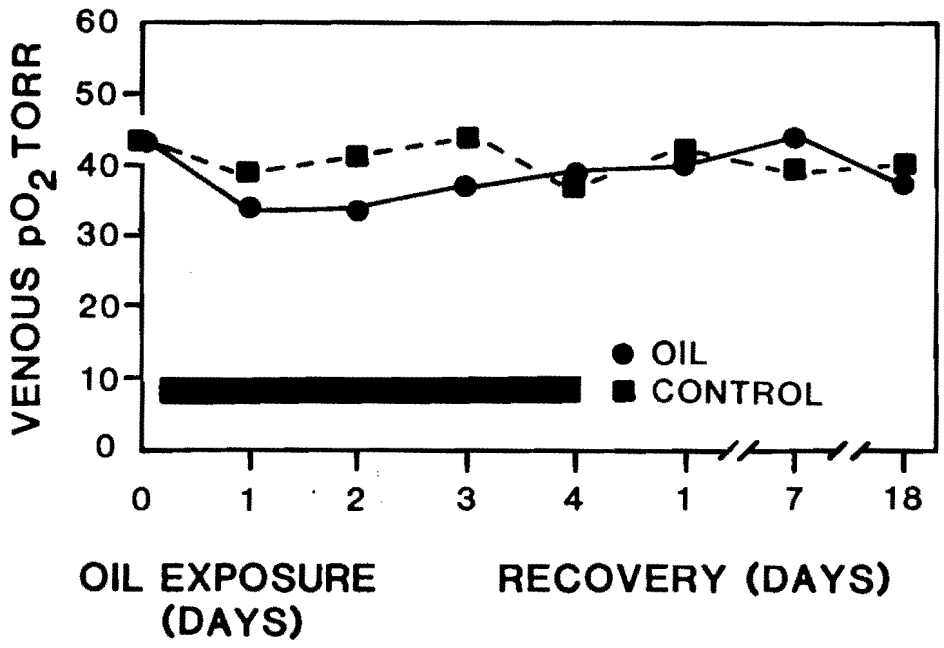



FIGURE 5-14. Mean venous oxygen partial pressure ($pO_2$) of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

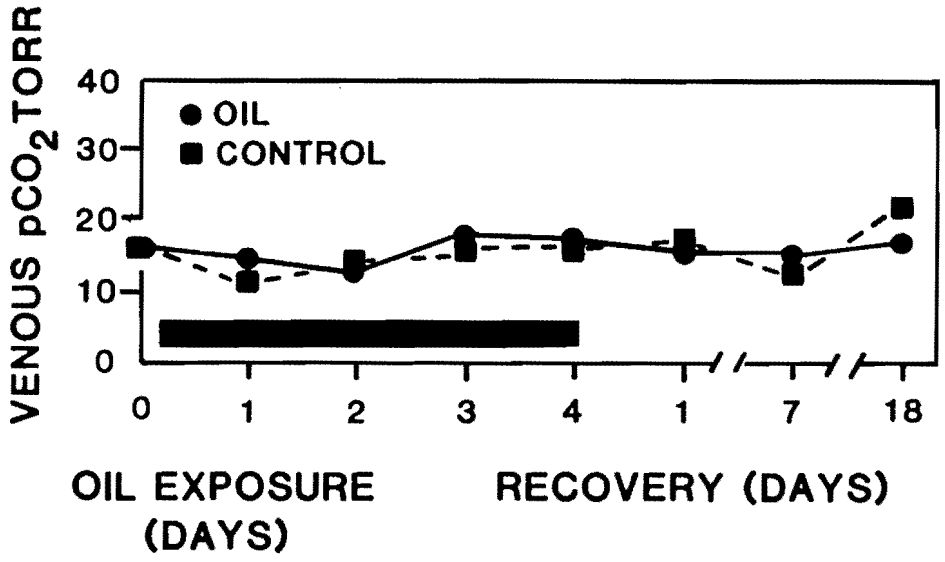



FIGURE 5-15. Mean venous carbon dioxide partial pressure ($pCO_2$) of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

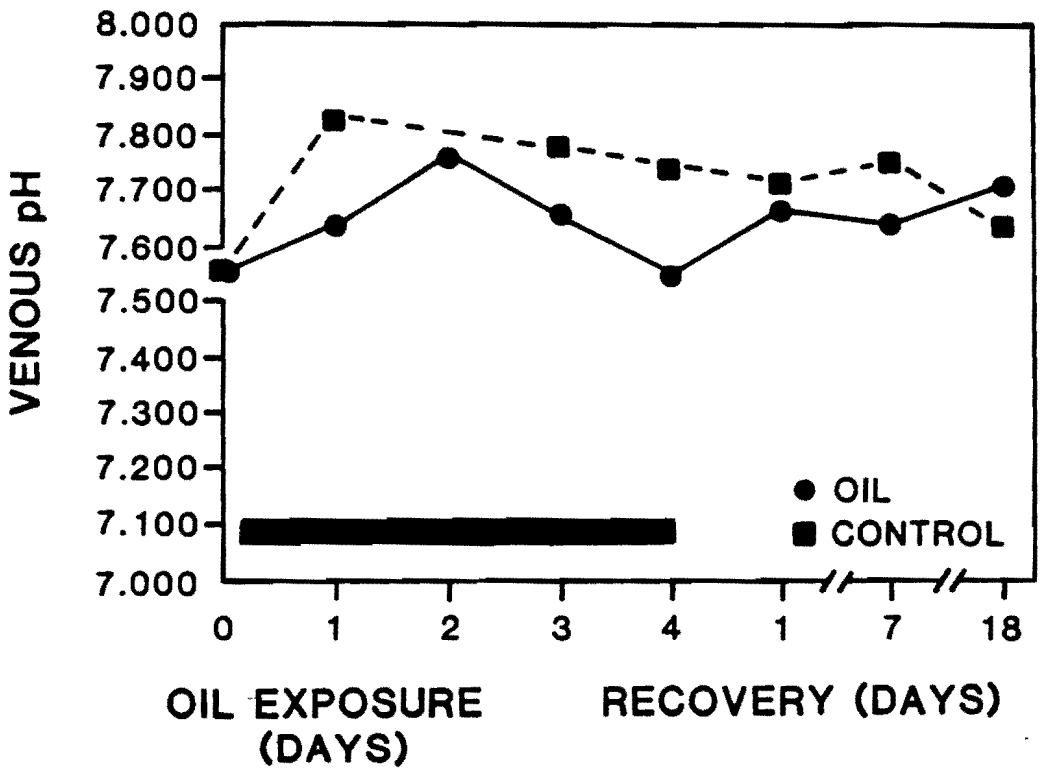



FIGURE 5-16. Mean venous hydrogen ion concentrations (pH) of turtles under control conditions, and during four day chronic oil exposure (0.05 cm oil layer) shown by the black bar and eighteen day recovery period.

blood lactate, respiratory acidosis is due to hypercapnia (high $CO_2$ concentrations in the blood). Hypercapnia was not seen in these turtles therefore it is probable that the lower pH was due to increased lactate. However, lactate was not measured during this study.

Acute Exposure - Ventilation and Diving

Loggerheads placed in tanks with a 0.5 cm layer of oil responded by first showing hyperactivity and vigorous swimming motions. This behavior continued in two out of four turtles, and they appeared stressed while surfacing to breathe. Each time the loggerhead surfaced to breathe oil residue was observed around its eyes and in its nasal and oral cavities, and its tongue was darkly stained with oil. Some oil was undoubtedly consumed incidentally by the loggerhead, but we were unable to calculate or estimate the amount ingested.

On several occasions we could not use the breathing funnel to record ventilation because turtles struggled if forced to do so. However, another turtle spent extremely long periods on the bottom of the tank with dives as long as 45 minutes, rarely surfacing to breathe. This is circumstantial evidence that the loggerhead was avoiding the oil.

Oxygen uptake ($VO_2$) and ventilation was measured in acute exposures where the turtle served as its own control on day 0 (prior to oiling). In response to a heavy surface slick turtles showed a pronounced change in $VO_2$ (n=4) relative to day 0 (Figure 5-17) yet it is difficult to classify a general response of the limited number of samples. After 24 hours there was almost a 2 fold increase in mean oxygen consumption (Appendix C). However, while three out of four turtles greatly increased $VO_2$ (up to 7-fold increase in Acute #2) and frequency (f), one loggerhead responded by making extremely long dives with limited oxygen consumption. Figure 5-18 is a reproduction of this turtle's dive pattern before and after one day of oil exposure.

The opposing respiration patterns seen in loggerheads exposed to the dense oil layer is probably both a reaction to the apparatus as well as to the presence of oil. It then seems reasonable that the increase in ventilation frequency we have recorded during oil exposure is necessary to maintain oxygen uptake. Respiratory changes were not distinguished in chronic exposures, but it is possible that the increase in ventilation was sufficiently great to mask any effect or graded response that chronic

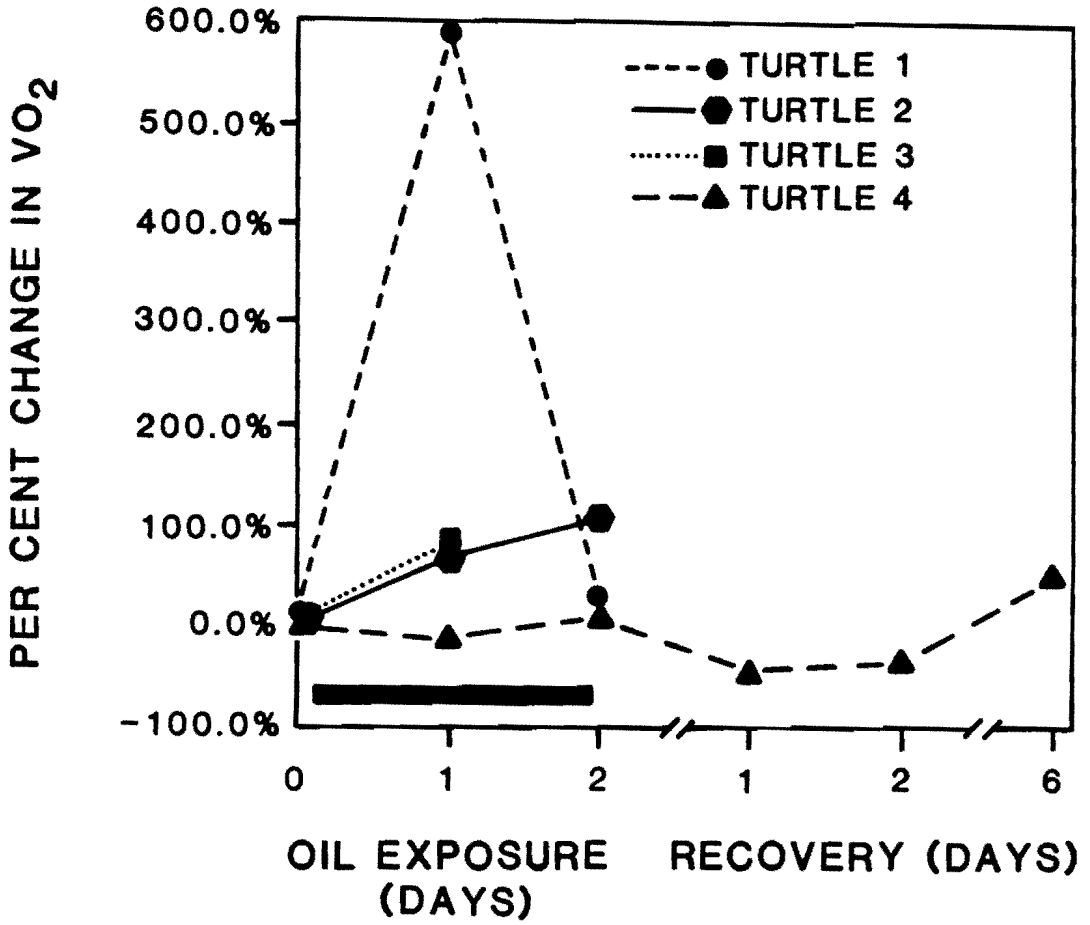



FIGURE 5-17. Percent change in oxygen uptake ($VO_2$) relative to Day 0 (prior to oiling) of individual turtles during two day acute oil exposure (0.5 cm oil layer) shown by the black bar and first six days of the recovery period.

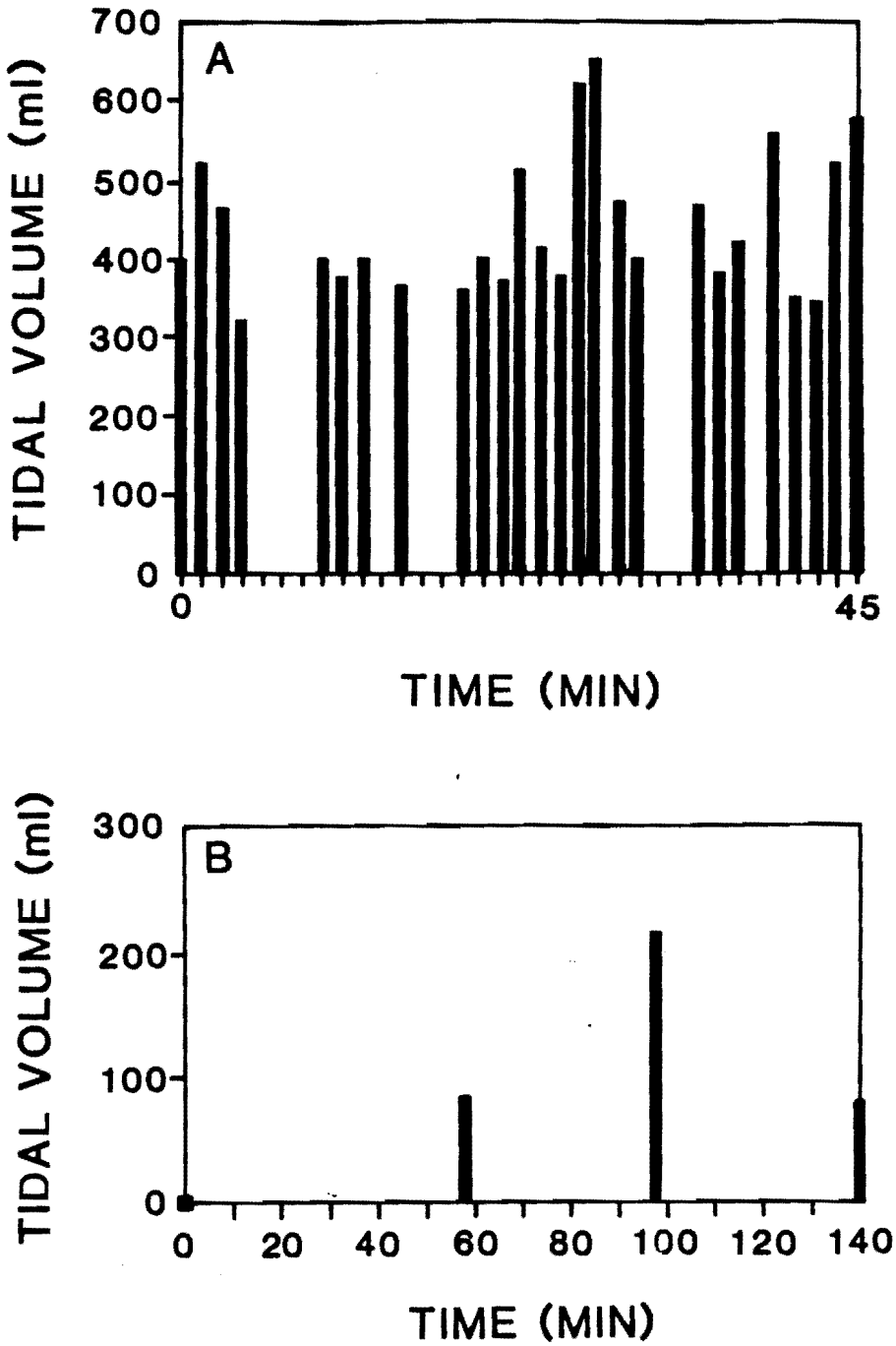



FIGURE 5-18. The dive pattern shown as tidal volume of an individual turtle on Day 0 before acute oil exposure (0.5 cm layer)(A) and after one day of acute oil exposure (B).

exposure might have on oxygen uptake. Acute exposures seemed to induce a more extreme disturbance that leads either to a marked increase in $VO_2$ or to a prolonged dive where the loggerhead reduced metabolism (as seen in its low $VO_2$) [Figure 5-19]).

Acute Exposure - Skin

In loggerheads exposed to acute oil levels the skin also began to slough off in layers especially around the neck and flipper area. The soft skin then exposed was reddened and lacked the leathery texture of the original epidermis. This response was more pronounced in acute oil exposures than in chronic oil exposures. In both groups skin continued to peel off for several weeks of recovery. Skin appeared superficially to return to its normal leathery texture and healing was evident within one month following oil exposure. The pathology of this condition is described in Section 5.2.

Acute Exposure - Blood Chemistry and Cells

Mean serum glucose concentration in acute turtles steadily fell to 35% of baseline values by day 3 of recovery and was 15% lower than baseline values on day 11 (Figure 5-20). This pattern was also seen in chronic exposures and may indicate a disruption of glucose metabolism.

Red blood cell concentration (Figure 5-21) following 48 hours of exposure to the acute oil slick declined slightly and did not return to baseline values during recovery. The large drop at recovery day 5 reflects an extremely low value in one individual. White blood cell count increased dramatically immediately after oiling (Figure 5-22) yet reflected baseline values by recovery day 11.

Acute Exposure - Digestion

Digestion efficiencies were measured on two control turtles. One had a digestion efficiency of 72.9% by the total ash method and 85.5% by the acid insoluble ash method. The second turtle had values of 75.9% and 74.8%, respectively. The total and insoluble ash estimates were in reasonable agreement with each other and the values are in the range reported by Bjorndal (1985) for the green sea turtle *Chelonia mydas*. After three days of recovery similar values were obtained from a turtle exposed to 0.05 cm oil. One experiment yielded 76.6% total ash and 80.1% acid insoluble ash.

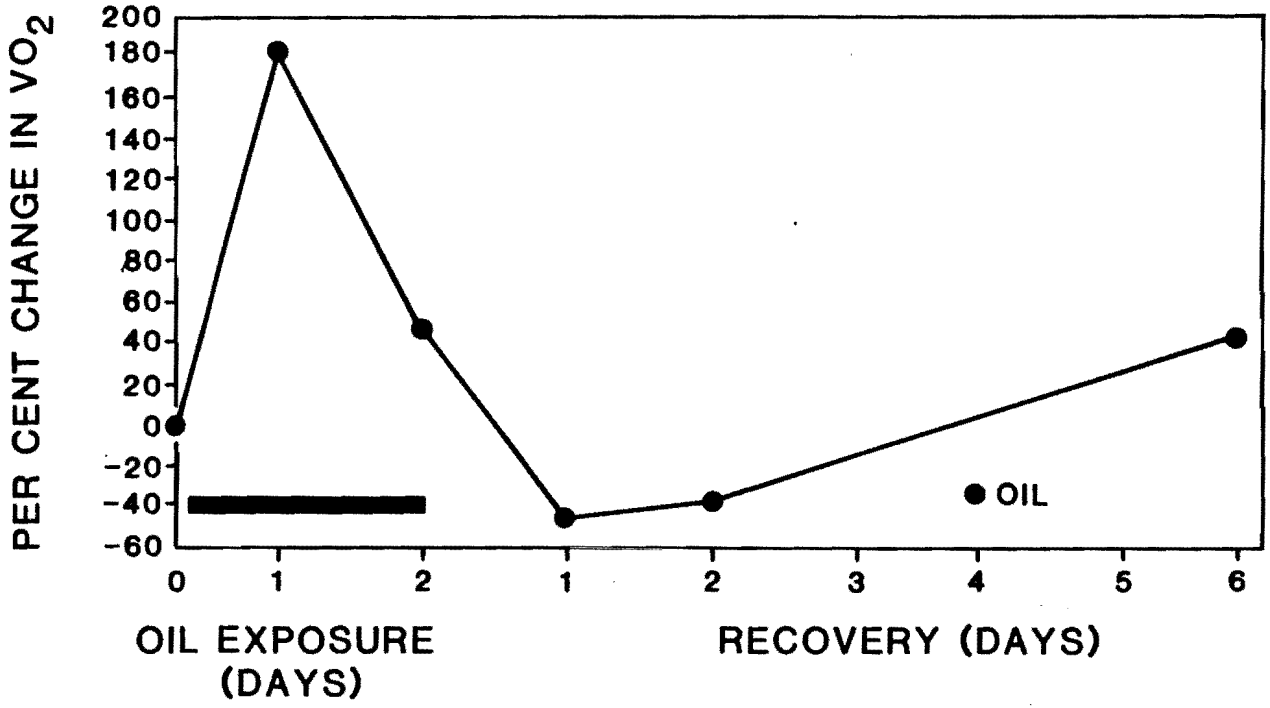



FIGURE 5-19. Mean percent change in oxygen uptake ($VO_2$) relative to Day 0 (prior to oiling) of individual turtles during two day acute oil exposure (0.5 cm oil layer) shown by the black bar and first six days of the recovery period.

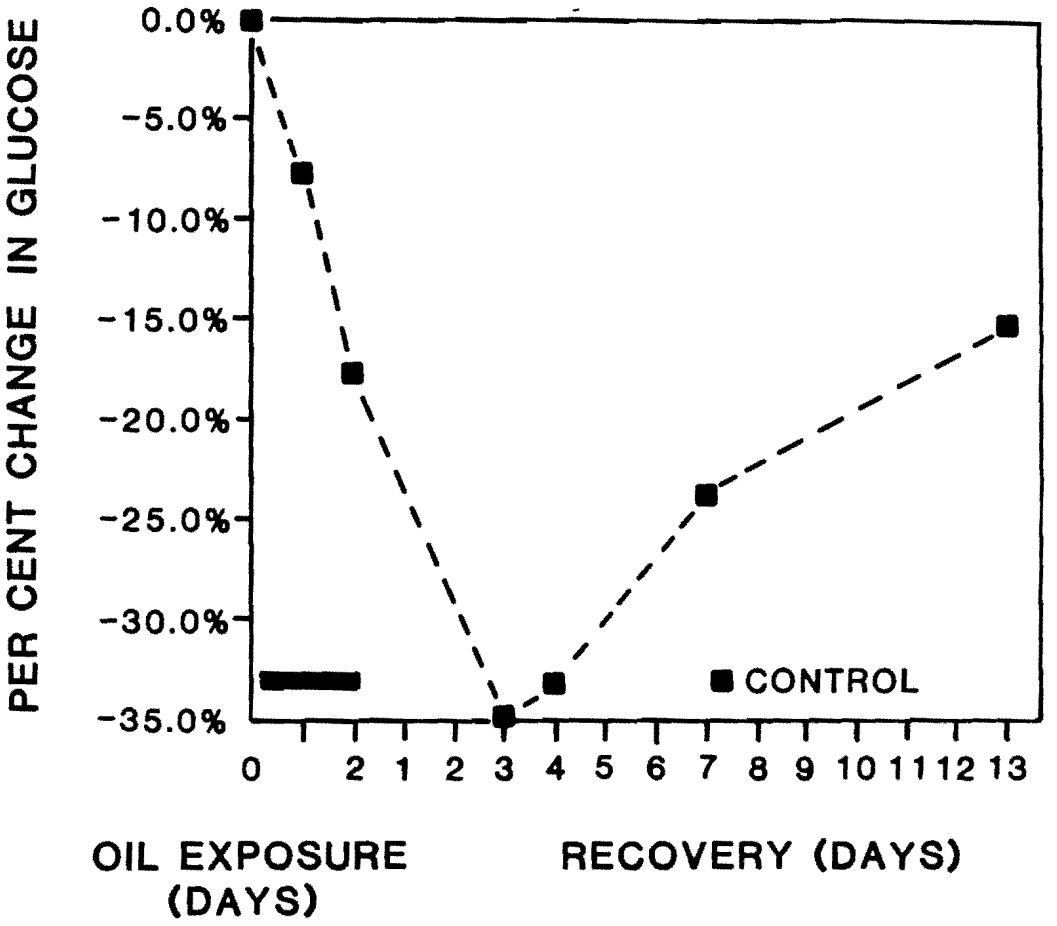



FIGURE 5-20. Mean percent change in glucose concentration relative to Day 0 (prior to oiling) of turtles during two day acute oil exposure (0.5 cm oil layer) shown by the black bar and first six days of the recovery period.

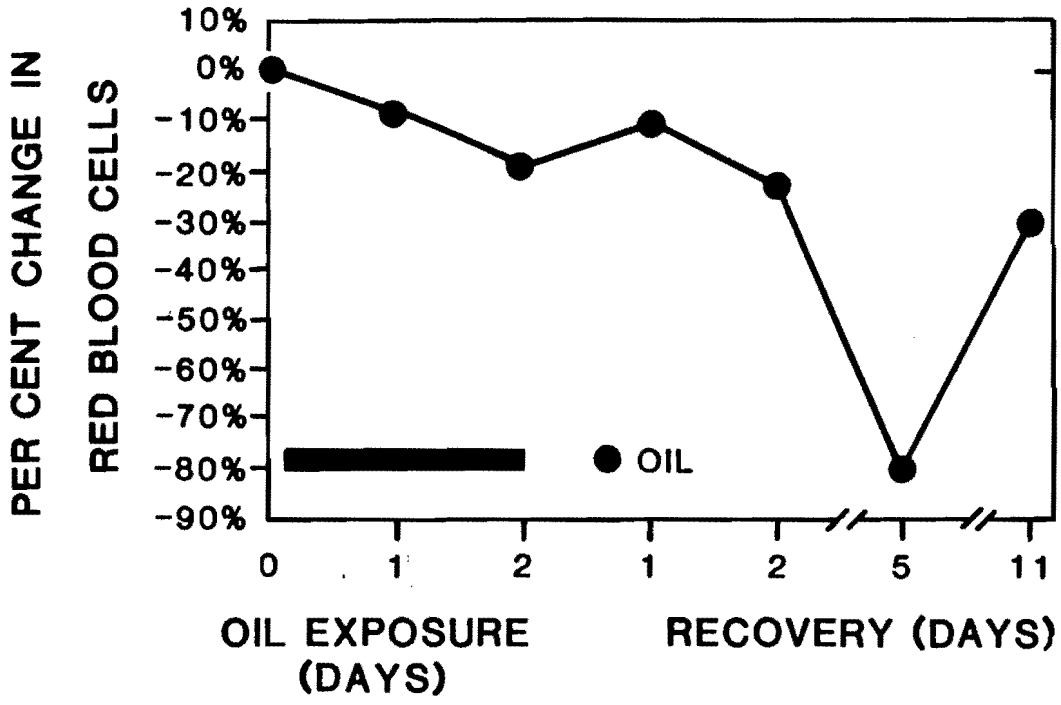



FIGURE 5-21. Mean percent change in red blood cell count relative to Day 0 (prior to oiling) of turtles during two day acute oil exposure (0.5 cm oil layer) shown by the black bar and first six days of the recovery period.

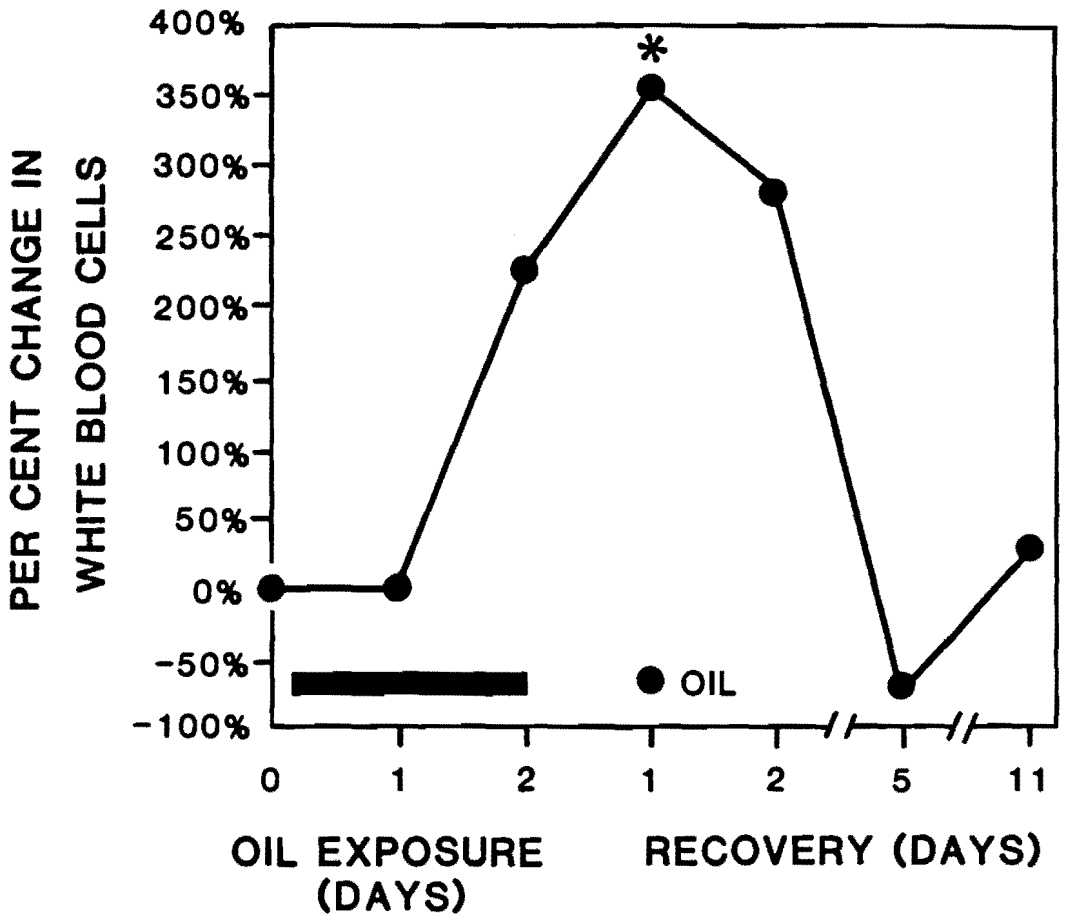



FIGURE 5-22. Mean percent change in white blood cell count relative to Day 0 (prior to oiling) of turtles during two day acute oil exposure (0.5 cm oil layer) shown by the black bar and first six days of the recovery period.

However, after 11 days recovery the single turtle measured showed a marked discrepancy between the total ash and the insoluble ash methods, i.e., 51.8.% total ash and 75.3% insoluble ash, respectively. One acutely oiled turtle showed a similar effect. Digestion efficiency six days after removal from oil was 42.1% for total ash and 86.3% for the acid insoluble method.

On the assumption that the acid insoluble method is the better measurement of absorption, these data indicate that exposure to oil has little effect on digestion efficiency, in all cases within 74 - 86%. The overall mean digestion efficiency was 80.4 $\pm$ 5.43%. But the large discrepancies between total ash and acid insoluble ash techniques that appeared some time after oil exposure indicated that the gut's role in mineral metabolism may have changed.

An erroneous underestimate of digestion efficiency would result from the total ash method if part of the total ash was reduced during passage through the gut. On the assumption that the acid insoluble component does not change as it passes through the gut then the change would have occurred in the acid soluble part such as the minerals sodium, chloride, calcium and magnesium. This could be due to an increase in salt uptake or a decrease in salt releasing mechanisms in the gut.

Acute Exposure - Salt Gland Function

It has been well established that the lachrymal salt gland of sea turtles is capable of producing fluid more concentrated than sea water (Prange and Greenwald, 1980) and that it has an important role in maintaining water balance against its environment (Hudson and Lutz, 1986; Bennett et al, 1986).

In order to measure flow rate and collect fluids for analysis the salt glands of experimental loggerhead sea turtles were cannulated with PE 50 tubing. Cannulation was achieved by simple insertion of the tubing into the external opening of a salt gland. The cannula was secured to the head by "super glue" (cyanoacrylate). Salt gland secretion was stimulated by injecting a predetermined amount of fluid (usually 30 ml of sterilized sea water) and salt gland secretion commenced within 1-1/2 hours. Osmotic pressure measurements were determined using a Wescor vapor pressure osmometer and salt gland flow rates were measured.

The results were most satisfactory as far as consistency was concerned. After a delay dependent on the volume and concentration of fluid injected, the osmotic pressure of salt gland fluid would quickly reach a plateau of 1500-2000 mOsm i.e. almost two times that of sea water or six times that of blood (Figure 5-23).

Since sucrose solutions produced the identical effects as sea water solution, salt gland flow was not strictly dependent on the composition of injected fluid. The rates of flow and the duration of flow appeared to be sensitive to the amount and concentration of the interperitoneal injection (Figure 5-24).

Chemical analysis showed that the fluid was not only rich in sodium (Na) and chloride (Cl), but also had very high relative concentrations of potassium (K), calcium (Ca), magnesium (Mg), and bromine (Br) (Table 5-1). The maximal salt gland/plasma ratios were strikingly high, and Br can be concentrated at least twenty-fold (Table 5-2). The data suggest that the gland possesses active pumps for these ions. There is no other report for an osmoregulatory gland producing Br. This indicates for the first time that the gland is not only important in water balance but has a role in ion regulation. It should be noted that this study is the first to deal with salt gland fluid in such detail.

Initially oiled loggerhead sea turtles showed the identical response as controls. But after three days the salt gland of the acutely exposed turtles showed no response (Figure 5-23), i.e. it appeared to have shut down. Two weeks later the turtles were retested and the salt glands were found to be fully recovered. Tests were not performed within this two week period so the time for recovery cannot be more accurately stated.

### 5.1.3 Summary

South Louisiana crude oil produced changes in ventilation and some blood serum chemistries, induced an immune response involving white blood cells, and appeared to have cytotoxic effects on regulatory systems including the epithelium, gut and salt gland. Most of these impacts attenuated during recovery, but we have no information regarding long-term effects of petroleum exposure on the contaminated loggerheads. It should be pointed out that under experimental conditions the turtles had no means of avoiding the oil and it is possible that some exposures might have been higher than turtles

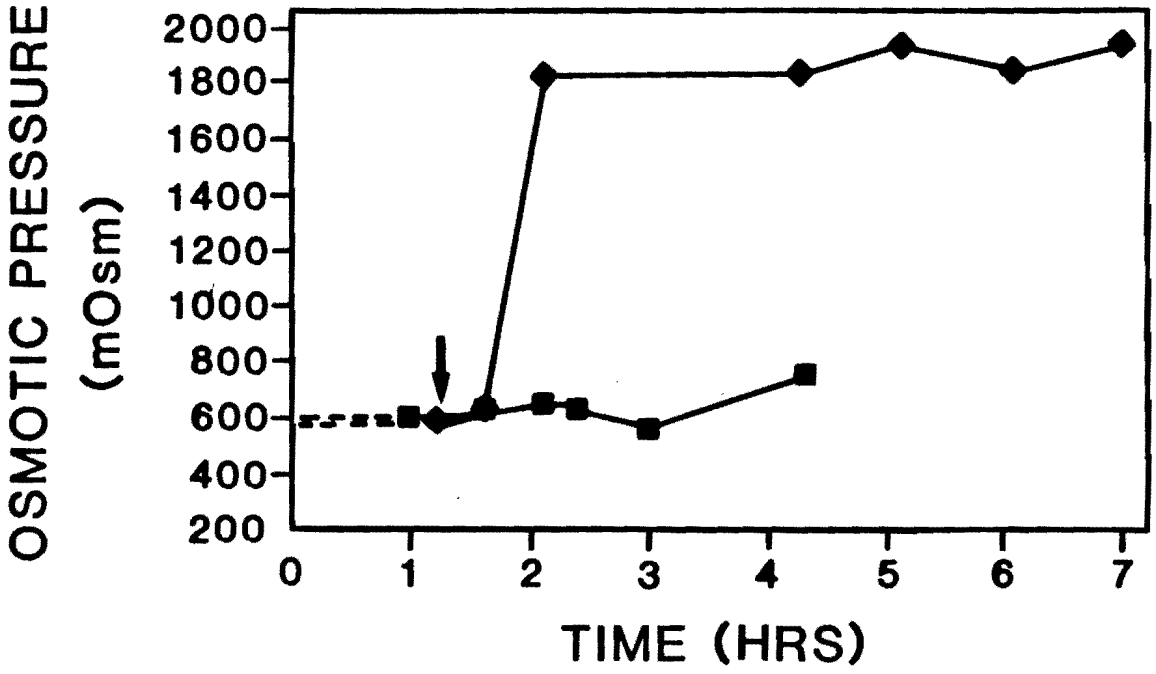



FIGURE 5-23. Osmotic pressure of salt gland fluid in control (unoiled) turtle (◆) and oil turtle three days after acute oil exposure (■). The arrow indicates the point at which sterilized sea water was injected.

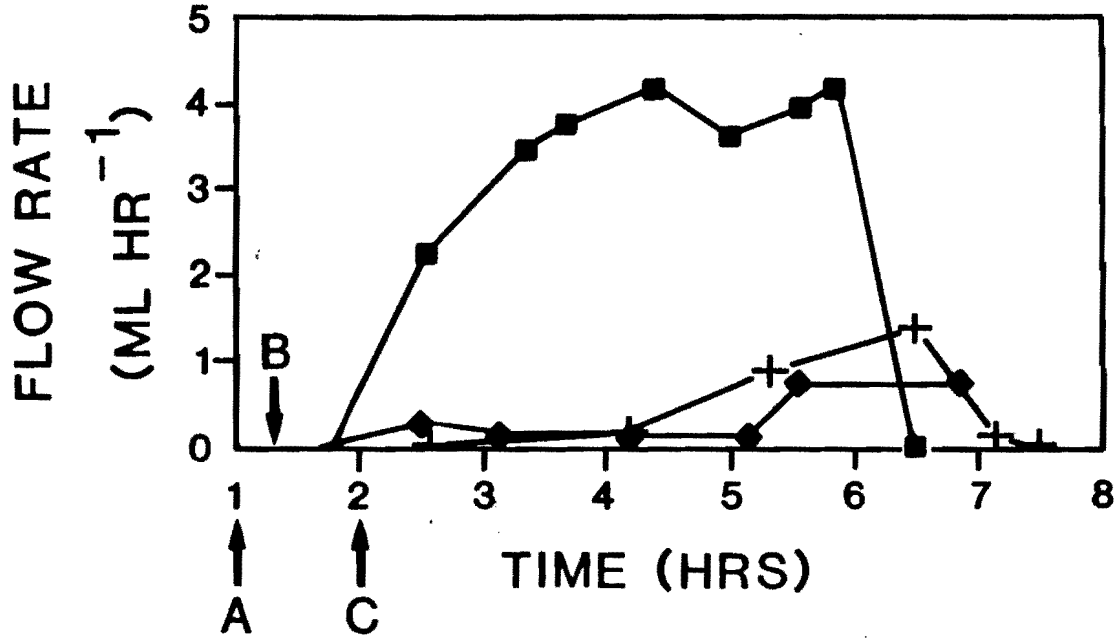



FIGURE 5-24. Effect of 30 ml isotonic saline load (A,◆), 100 ml sea water (B,■), and 30 ml isotonic sucrose (C, +) on the flow rate of the salt gland of the loggerhead sea turtle (C. caretta).

TABLE 5-1

AVERAGE COMPOSITION OF SALT GLAND SECRETION

FROM THE LOGGERHEAD SEA TURTLE

| | $Mm \cdot L^{-1}$ |
|---|---|
| K | 12.5 ±6.14 |
| Cl | 940.0 ±112.4 |
| Na | 786.9 ±93.9 |
| Ca | 20.4 ±3.86 |
| Mg | 44.3 ±5.23 |
| Br | 2.18 ±0.03 |

Values expressed as mean ± standard deviation, n = 8.

TABLE 5-2

MAXIMAL SALT GLAND/PLASMA RATIOS

FOR LOGGERHEAD SEA TURTLE

| | Salt Gland (mM) | Plasma (mM) | Ratio |
|---|---|---|---|
| K | 21.00 | 3.2 | 6.3 |
| Cl | 1057.00 | 110.0 | 9.6 |
| Na | 1167.00 | 140.0 | 8.3 |
| Ca | 36.00 | 1.3 | 27.7 |
| Mg | 54.00 | 1.2 | 45.0 |
| Br | 6.24 | 0.3 | 20.8 |
| mOsm | 1930.00 | 320.0 | 6.0 |

would encounter in an oil spill. On the other hand the oiled turtles were cared for carefully. After exposure they were cleaned of all oil, held in filtered sea water, given high quality food, and treated with antibiotics. It is possible that in the natural environment disease, predators and feeding would have posed problems for these animals. The physiological effects of SLCO on subadult loggerheads are summarized below.

Respiration

The experimental protocol that we used did not distinguish significant differences in respiration between control and oiled turtles; oxygen uptake ($VO_2$) remained constant, but there was a slight increase in time spent at surface and respiration rate in oiled turtles. Hyperventilation resulted in a very low venous $pCO_2$ in both controls and oiled turtles. However turtles exposed to acute level (0.5 cm) showed some signs of "distress" with at least two reactions. Two turtles responded with an increased ventilation rate and oxygen uptake, while another showed substantially decreased ventilation and $VO_2$ by making what appeared to be an emergency dive.

Skin

In loggerheads exposed to both chronic and acute oil levels the skin began to slough off in layers especially around the neck and flipper area. The soft skin then exposed was reddened and lacked the leathery texture of the original epidermis. This response was more pronounced in acutely oiled turtles, and in both groups skin continued to peel off for several weeks of recovery. Skin appeared to return to its normal leathery texture and healing was evident within one month following oil exposure. The pathology of this condition is described in Section 5.2. These effects on the skin were not observed in the behavioral experiments. This was probably due to the very short exposure time (30 minutes) compared to the 48 to 96 hour exposures used for the physiological studies.

Blood serum chemistry and cells

Most blood serum chemistries including total protein, albumin, and AST were not significantly affected by oiling, yet there was some evidence of interference with glucose metabolism. Hemoglobin concentration declined slightly during recovery in chronically oiled turtles but there was no

indication that hemolytic anemia had occurred. Red blood cell count and hematocrit remained the same in both groups but there was a significant increase in the number of white cells in oiled turtle groups. This reaction was more pronounced following acute exposures and suggests that in both exposure levels the immune system was responding to the presence of oil. White cell counts and hemoglobin concentrations (Leighton et al., 1983) are dramatically reduced and hematocrit increased in some birds dosed with oil Brown, 1982) so it appears that target organs and responses to oil by marine birds and turtles may be quite different.

Digestion

The presence of oil did not appear to result in significant changes in digestion efficiency, but gut processes involving mineral metabolism were possibly affected. We do not have sufficient data to draw conclusions as to whether the altered salt transport was due to an interference in absorption or secretion mechanism.

Salt gland function

The failure of the salt gland to produce fluids several days after exposure points to attenuated or delayed effects in salt transport. The delayed shutdown was unexpected and unfortunately we were not set up to study in detail this response to oiling. The nasal salt gland of sea birds orally dosed with crude oil also shows a loss of responsiveness which has been attributed to suppression of adrencortical activity (Holmes et al., 1980). The primary site of action in both sea birds and turtles may be the adrenal glands. The salt glands had recovered when function was tested after two weeks of recovery.

Prolonged interference with salt gland functioning could have serious consequences since it would not only interfere with water balance, but as this study has found, it would also upset internal ion regulation. Changes in body ions, particularly highly bioactive Mg, could be expected to affect many physiological and biochemical processes (Lutz, 1975). Interference with bromine metabolism has an unknown significance.

## 5.2 Clinicopathological Effects (G. Bossart)

Evidence of the harmful and often lethal effects of oil on some marine animals is well recognized, however few studies have been directed to the effects of oil on threatened or endangered sea turtles (Moore and Dwyer, 1974; Light and Lanier, 1978; Witham, 1983; Geraci and St. Aubin, 1982; Hartung and Hunt, 1966; Solangi and Overstreet, 1982). Direct effects of oil include direct contact with oil in an oil spill situation compromising optic, olfactory, integumentary or other sensory systems; inhalation of volatile hydrocarbons altering normal pulmonary function; changes in locomotion caused by oiling; and ingestion of oil or oil-contaminated food items resulting in gastro-intestinal compromise or malnutrition.

The direct effects of oil can be lethal or sublethal involving compromise to organ functions such as the reproductive, olfactory, or optic systems. Sublethal and lethal exposure effects of oil in other vertebrates, including numerous fish species and marine birds such as mallard ducks (*Anas platyrhynchos*), herring gulls (*Larrus argentanus*), and Atlantic puffins (*Fratercula artica*), have included aspiration lipid pneumonia, gastro-intestinal hemorrhage, degeneration of kidney tubular cells, tremors, anemia (including lymphoid depletion in primary lymphoid tissues as well as lipid depletion), and necrosis in the adrenal steriod producing cells (Rice, 1973; Couch, 1975; Eisler, 1975; Thomas, 1980; Martin, 1980; Coon, 1981; McKeown, 1982; Leighton, 1986). The indirect effects of oil on sea turtles through habitat alteration (including quantity and quality of food items) may also be far reaching.

The objective of this portion of the overall study was to determine selected clinicopathologic parameters from the captive behavioral and physiological experimental sea turtle population as well as stranded oil-fouled turtles salvaged along the Atlantic and Gulf coastlines. Since knowledge of the basic physiology of sea turtles was limited, this protocol permitted the establishment of "normal" baseline values for captive sea turtles and consequently a method to determine changes which occurred as a result of association with oil. This report will present evidence that exposure to oil does have certain deleterious clinicopathologic effects in experimental animals and in some oil-fouled stranded sea turtles.

### 5.2.1 Methods

Clinicopathologic data from control and experimental loggerhead and green sea turtles was obtained from behavioral and physiological studies described in appropriate sections. Parameters measured included blood hemograms, selected serum chemical compounds, oropharyngeal and fecal microbiologic flora, fecal blood, serum protein electrophoresis, and histologic evaluation of tissues. Similar data was collected from 6 oil-fouled stranded green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) sea turtles reported in Section 6.3.

#### Hematology and Serum Chemistries

Blood was collected aseptically from the dorsal cervical venous sinus. Hemograms were calculated from EDTA anticoagulated blood and included determinations of red blood cell indices and white blood cell total and differential counts. Red blood cell indices included determination of packed cell volume, plasma total solids, and hemoglobin concentration.

Red and white blood cell counts were calculated in a hemocytometer charged with a 1:100 dilution of blood to Shaw's stain and counted by conventional methodology (Todd, et al, 1953).

Packed cell volume was determined by the microhematocrit method (Cole, 1974) and plasma total solids by a commercial refractometer (Schuco clinical refractometer). Hemoglobin concentration was determined spectrophotometrically described below. Differential white blood cell counts were performed on peripheral blood smears stained with Wright-Giemsa. Two hundred cells were enumerated and percent cellular differential count was based on the resultant average.

Selected serum chemical compounds were determined with a multifilter photometer that spanned the visible spectrum from approximately 450-610 nm. Individual blood chemistry kits were available commercially (Mallinckrodt, New York). Serum compounds measured included lipase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), uric acid, albumin, alkaline phosphatase, glucose, lactate dehydrogenase, creatinine, calcium, and phosphorous. Electrolytes including sodium, potassium, and chloride were determined via a flame photometer.

Microbiology

Microbiologic samples were collected with sterile swabs in transport media available commercially (Culturette, American Scientific Products). Oropharyngeal cultures were obtained by opening the mouth with padded hemostats and then swabbing the oropharynx. Fecal cultures were obtained from fresh feces or from cloacal swabs collected with sterile K-Y® jelly. The culturettes were then returned to the laboratory, plated, and aerobically incubated at 37° C and room temperature. Fecal samples were processed on phenylethyl agar with sheep blood, Hektoen enteric agar, MacConkey agar, and in selenite-F broth. Oropharyngeal cultures were plated on trypticase soy broth and sheep blood agar. Identification of organisms followed standard microbiologic procedures (Buchanan and Gibbons, 1974; Cowan, 1975).

Fecal Blood

The presence of fecal blood was determined by a commercially prepared strip colorimetric test (Multistix, Ames).

Histopathology

Tissues for histologic evaluation were fixed immediately in 10% neutral buffered formalin and processed routinely. These specimens were cut at 5 μm and stained with hematoxylin and eosin.

Skin biopsies were obtained aseptically via an incisional or a 5 mm punch biopsy. The sites biopsied included the dorsal mid-zone surface of a foreflipper and the ventrolateral skin of the neck.

Various formalin-fixed tissues from oil-fouled stranded yearling Kemps ridley sea turtles (Lepidochelys kempi) were provided by Bob King from Padre Island National Seashore Park, Texas. The turtles washed ashore approximately 24 hours after their release from a head-start program. Eighteen were dead and 80% had tar in their mouths according to Mr. King. Tissues submitted from these sea turtles including liver, oropharynx, esophagus, pancreas, and small intestine.

Tissues from all organs were also examined grossly and histologically from a yearling green sea turtle which stranded covered with tar and later died at the Sea World Marine Science and Conservation Center in spite of several weeks of supportive medical treatment. Results from these stranded turtles are discussed in Section 6.3.

Protein Electrophoresis

Serum protein electrophoresis was performed on experimental and control sea turtles from an oil exposure physiological experiment by standard cellulose acetate methodology (Bauer, et.al., 1974).

No deaths occurred in any sea turtles as a direct result of experimental procedures.

Analysis of Data

The data were expressed as means ± standard error and compared using one-way anova at each sampling point with mean separation performed using the Fisher's least significant difference t-test (SAS, Fifth Edition 1985). We also compared each experimental day with baseline day 0 (prior to dosing). In acute studies, experimental days were contrasted using baseline day 0 as a control. The confidence level was selected at the 95% level for all experiments unless indicated otherwise (data tables are presented in Appendix C). In acute studies with very limited sample size, data were normalized as per cent change from baseline (day 0) for glucose, white blood cell and red blood cell counts.

5.2.2 Results and Discussion

Exposure to crude oil has direct effects on certain clinicopathologic parameters in sea turtles. These changes were observed in 15 - 18 month old loggerhead sea turtles exposed to oil in the physiology experiments (Section 5.1) and in 4 of 6 green and hawksbill sea turtles which were found on or near the shore line stranded oil-fouled (see Sect. 6.3, Field Study, Clinicopathologic Analysis Section).

No statistically significant differences in measured clinicopathologic parameters (including hematologic, serum chemistry, fecal blood, and bacteriologic factors) were present between control and experimental, hatchling to 18 month old sea turtles utilized in behavioral studies. These loggerhead and green sea turtles provided comparable baseline values for control animals in physiological experiments.

Hematology and Serum Chemistries

The most striking hematologic finding between sea turtles exposed to oil and control animals from the acute exposure studies (Section 5.1) was an

elevation of total white blood cell (WBC) count. These statistically higher white blood cell counts were present in sea turtles exposed to oil and were an average of 350% higher following oil exposure when compared to control sea turtles. Similarly, on day 3 of chronic oil exposure studies, sea turtles exposed to oil had significantly higher total WBC counts than control sea turtles not exposed to oil. In both acute and chronic oil exposure groups the total WBC count returned to within "normal" baseline levels by recovery day 18.

These elevated white blood cell counts may indicate a stress reaction related to oil exposure and/or toxicity. The response was oil duration - dependent, characteristically increasing during oiling periods and returning to pre-oiling counts after oil exposure ceased. A stress related leukocytosis similar to these sea turtles has been described in mammals (Cole, 1974).

There were no statistically significant differences in red blood cell parameters between oil-exposed and control sea turtles from acute and chronic exposure studies (Appendix C). However, the mean red blood cell (RBC) count from oil exposed turtles from acute studies fell an average of 80% from baseline "normal" values on recovery day 7 and similar lower mean RBC trends were present in sea turtles exposed to oil from chronic studies. The large variation and limited sample size from some acute exposure studies may have precluded the finding of statistically significant differences between oil-exposed and control sea turtles.

It is interesting to note that peripheral blood smears from these sea turtles, exposed to oil and having depressed RBC counts, exhibited red blood cell polychromasia. This diffuse red blood cell basophilia, in mammals, occurs in association with regenerative anemic conditions and represents an immature form of an erythrocyte. While these apparent regenerative anemic states were temporary, the pathogenesis of such conditions remains speculative. Similar regenerative anemic conditions which eventually resolve with intensive medical care and rehabilitation have been reported in marine birds exposed to oil from oil spill situations (McNair, 1980). Some parallels indicating the primary target of oil toxicity is the red blood cell with secondary stress related lesions have been found in marine birds that ingested crude oil (Leighton, 1986).

On recovery day 3, oil exposed sea turtles from chronic studies had statistically significant lower serum glucose levels than control turtles (Figure 5-8). The serum glucose levels were not statistically significantly lower on Day 7 and 18 of the recovery period. A decrease in blood glucose can result from normal hepatic glucose output with increased peripheral uptake; a decrease in hepatic gluconeogenesis (glucose production) when coupled with normal peripheral utilization of glucose; or a combination of these two mechanisms.

In other vertebrates hypoglycemic (low blood sugar concentrations) states have been reported following extreme exertion, in starvation, and in association with hormonal alterations accompanying hypothyroidism, adrenal cortical insufficiency, and hypopituitarism. The specific causative factor(s) in this situation could not be determined and require further investigation.

No significant differences, as indicated in the physiological studies (Section 5.1), were detected in total or daily means between chronically oiled or control turtles for serum total protein, albumin, LDH, lipase, AST, ALT, BUN, creatinine, alkaline phosphatase, calcium, phosphorus, uric acid, sodium, chloride, or potassium. If extrapolations can be made from mammals and birds to sea turtles, this data suggests that exposure to oil (within the framework of the study) has little effect on most liver, renal, pancreatic, or metabolic electrolyte-related processes. This interpretation is based on the assumption that these measured parameters in sea turtles are indicators of specific tissue or organ function as they are in mammals and some birds (Petrak, 1982; Cole, 1974).

Fecal Blood

Occasional traces of both hemolyzed and non-hemolyzed blood in feces were present in control and experimental animals from behavioral and physiological experiments. This trace amount probably reflects a dietary origin or is a result of minor trauma from obtaining cloacal fecal specimens.

Oil was found in the feces of oil exposure turtles from physiological studies and may have been absorbed from gastro-intestinal tract resulting in the observed hematologic abnormalities. If oil did pass from the gastro-intestinal tract into the turtles' system it was not of sufficient quantity or quality to cause intestinal bleeding and anemia as reported in aquatic birds (McNair, 1980; Hartung and Hunt, 1966).

Other pathologic or functional non-hemorrhagic gastro-intestinal oil related effects such as maldigestion or malabsorption were not examined and deserve further investigation.

Microbiology

Predominantly gram-negative bacterial flora representing a diverse population were isolated from the oropharynx and feces of both control and experimental sea turtles from behavioral and physiological studies. Oropharyngeal flora isolated in high numbers (from 30-50% of the total population) included *Pseudomonas putrefaciens*, *Citrobacter freundii*, *Acinetobacter calcoaceticus*, *Vibrio alginolyticus* and other species of the *Aeromonas-vibrio* group and *Pseudomonas* group. Species of bacteria in less than 30% of the oropharyngeal isolates included *E. coli*, *Proteus vulgaris*, *Salmonella* (groups D and E), and *Klebsiella pneumoniae*.

Gram-positive oropharyngeal bacterial isolates in widely diverse population numbers included alpha *Streptococcus* sp., *Corynebacterium* sp., *Bacillus* sp., and *Lactobacillus* sp.. These bacteria were also found in both experimental and control groups.

Fecal flora isolated was similarly diverse, predominately gram-negative, and found distributed in both experimental and control experimental animals. Species ranging from 30-50% of the total population included *Pseudomonas putrefaciens*, *Citrobacter freundii*, *Proteus vulgaris*, *Vibrio alginolyticus*, *E. coli*, and other species of the *Aeromonas-vibrio* group.

Gram-negative fecal flora in less than 30% of the isolates included *Acinetobacter calcoaceticus*, *Klebsiella pneumoniae* and *Salmonella* sp. (groups D and E). Gram-positive isolates were diverse in numbers and included *Streptococcus faecalis*, *Micrococcus* sp., *Staphylococcus epidermidis*, and *Bacillus* sp..

There were no statistically significant differences in bacterial populations from both the fecal and oropharyngeal bacterial cultures from control and experimental sea turtles. In the physiological oil exposure studies the overall oropharyngeal and fecal bacterial populations had a greater diversity of species in both control and experimental groups. This occurred at days 7-10 of the experiments and returned to a near base line population of bacteria at day 22.

The cause of the greater bacterial diversity could not be determined in the control and experimental groups. It may, however, simply represent the result of proliferating organisms in a stagnant media since tank water was not filtered or circulated during the oil exposure period.

Serum Protein Electrophoresis

Serum protein electrophoresis was performed on samples obtained at various times from a chronic, oil exposure physiological experiment during actual exposure and during recovery. Serum samples were analyzed prior to experimentation, day 2, day 4 (exposure), and day 11 (recovery) from both unexposed control and oil-exposed experimental loggerhead sea turtles.

In both control and experimental turtles, four protein fractions were consistently present in the electrophoretic studies. While rates of protein fraction separation were somewhat different, the overall pattern was typically mammalian corresponding to albumin, alpha, beta, and gamma globulin fractions.

Total protein and albumin concentration remained similar and unchanged in both turtles for each testing day (total protein 2.7 and 2.8 mg/dl; albumin 1.3 and 1.4 mg/dl), for control and oil-exposed experimental sea turtles respectively.

The fractions corresponding to gamma globulin were consistently higher in concentration and beta globulin lower in concentration in the oil-exposed turtle versus the control turtle (gamma globulin in oil-exposed sea turtle averaged 0.6 mg/dl and control turtle 0.3 mg/dl). This trend was present throughout the sampling period. This may represent a normal protein fraction distribution range for the loggerhead sea turtle. Alternatively, it may represent a state of immunological stimulation in the oil-exposed sea turtle reflected in an elevated gamma globulin fraction. An immunologic reactive state would be further supported by the elevated WBC counts and chronic-active inflammatory changes present in the integument of the oil-exposed sea turtles (see Histopathology). These inflammatory changes were associated with infiltrates of cells resembling plasma cells which in other species produce antibodies or gamma globlins (Outteridge, 1985).

Any further interpretation requires additional electrophoretic studies as well as a thorough understanding of the sea turtle immunologic system.

Histopathology

Marked consistent gross and histologic changes were present in the skin of loggerhead sea turtles exposed to oil from the physiological effects experiments. These changes were present in the chronically exposed sea turtles but were more severe in turtles from the acute oil exposure studies. No similar consistent dermatologic change were present in control sea turtles that were not exposed to oil (Figs. 5-25 and 5-26).

After exposure to oil had ceased (day 2, acute oil; day 5, chronic oil) marked microscopic changes were observed in the skin. In the epidermis, extensive numbers of acute inflammatory cells (heterophils) diffusely infiltrated the cellular layers beneath the outer epidermal zone, the stratum cornium (Fig. 5-27). Associated with the acute inflammatory cell epidermal infiltrates was moderate to extensive, multifocal epithelial cell death (necrosis) (Figure 5-28). These necrotizing changes were characterized by loss of intercellular bridges and cellular cohesion, with shrunken, thickened hyperchromatic nuclei. In addition, there was moderate abnormal cellular development (dysplasia) characterized by loss of individual cell uniformity, loss of cellular architectural orientation, and atypical proliferative changes (Figure 5-29). During this time period there was also marked diffuse epidermal thickening and proliferation of the horny or cornified layer.

The dermis of the skin of these same sea turtles was characterized by moderate diffuse superficial edema with mild diffuse and perivascular infiltrates of heterophils and occasional macrophages. There was also moderate multifocal, superficial dermal hemorrhage.

The changes during this time period were more extensive in the soft pliable skin of the neck and less severe in the hard, heavily keratinized skin of the flippers. Depending on the type of skin tissue these changes were still apparent seven to nine days after oil exposure ceased (Fig. 5-30).

Nine to 13 days after exposure to oil had ceased epidermal inflammatory cell infiltrates, cellular death, and dermal hemorrhage were no longer present in the skin biopsies. There was still moderate epidermal thickening and proliferation with marked sloughing of the cornified layer of the epidermis. Inflammatory cells had disappeared for the most part from the dermis although there was still mild superficial dermal edema.

At this time also and up to 21 days after exposure to oil had ceased marked grossly observable sloughing of skin and exposed mucous membranes

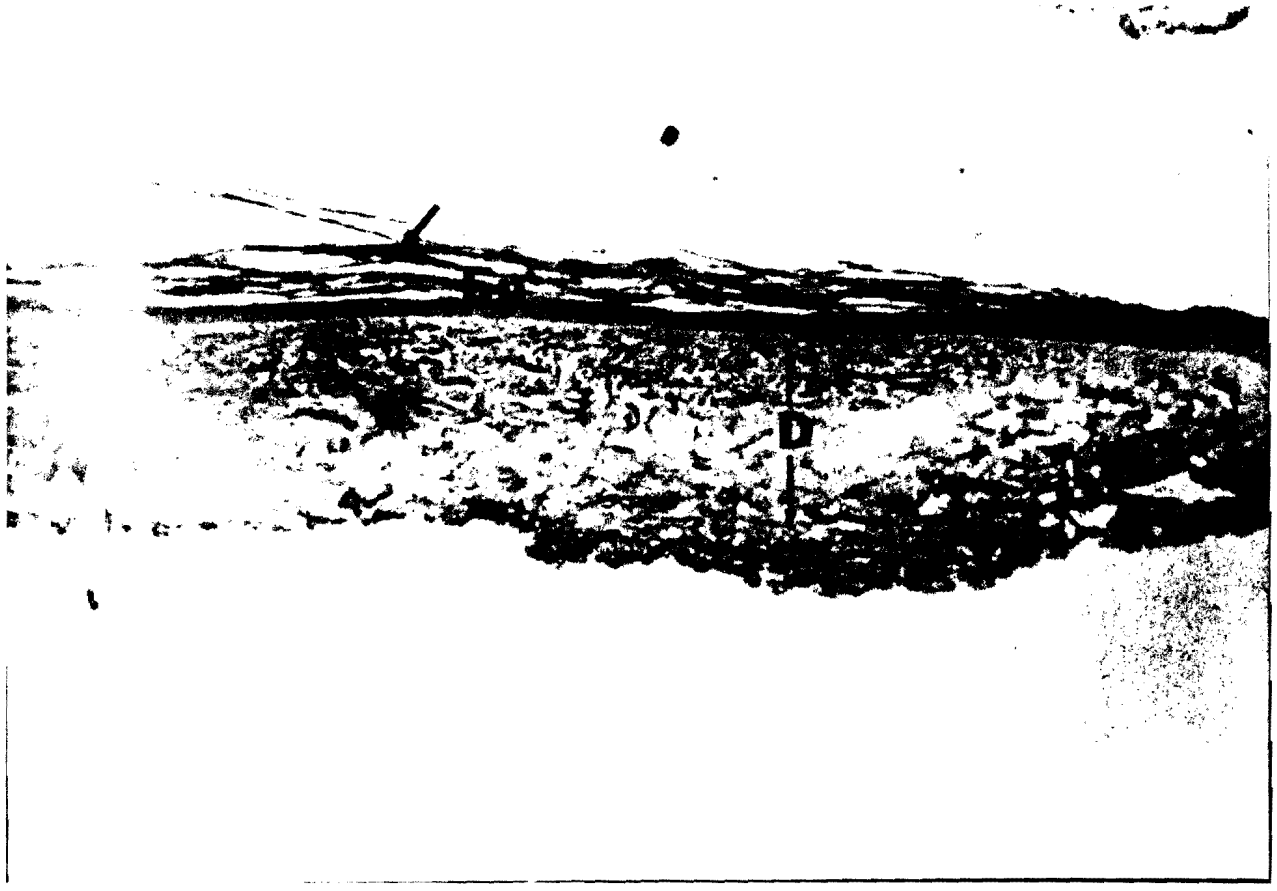



FIGURE 5-25. Low-power photomicrograph of a skin biopsy from the flipper of a loggerhead sea turtle that was not exposed to oil (control). The epidermis (Ep) is characterized by a moderate to heavily keratinized outer layer, the stratum corneum (arrow). The remaining epidermal cell layers are uniform in thickness. The dermis (D) is homogeneous and relatively acellular, characterized by dense connective tissue and occasional blood vessels. Hematoxylin and eosin stain, 20x.

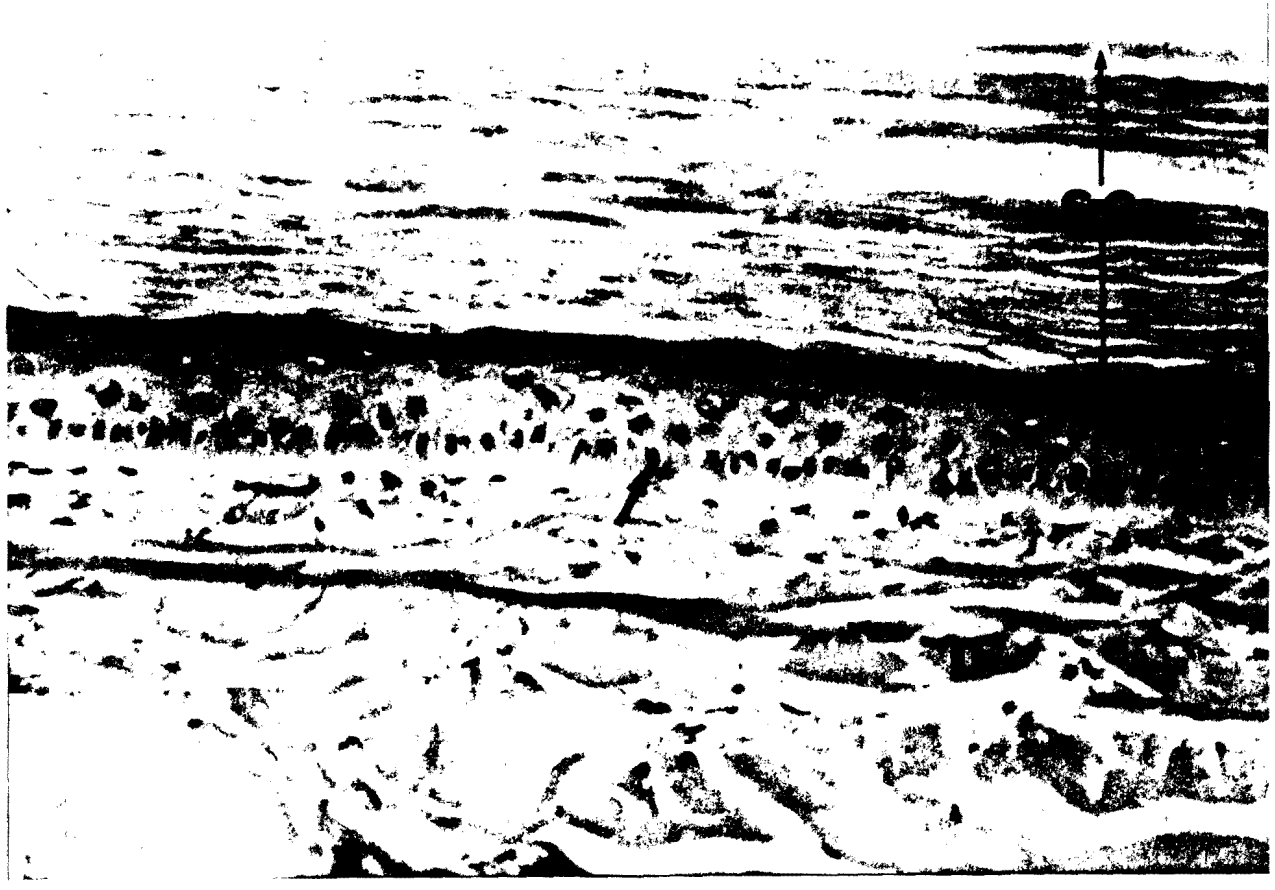


FIGURE 5-26. Medium-power photomicrograph of a skin biopsy from the neck of a loggerhead sea turtle that was not exposed to oil (control). Notice the wavy, laminated, uniform outer keratinized layer (SC) and evenly stratified, 4-5 cell layer thickness of the remaining epidermis. These epithelial cells exhibit orderly maturation from the round to oval cells of the deeper germinal layer (arrow) to the more polygonal then flattened and keratinized cells of the more superficial layers. The underlying dermis (D) is characterized by dense collagenous connective tissue and occasional spindle-shaped fibroblasts and cells containing dark brown to black cytoplasmic pigment granules. Hematoxylin and eosin stain, 100x.

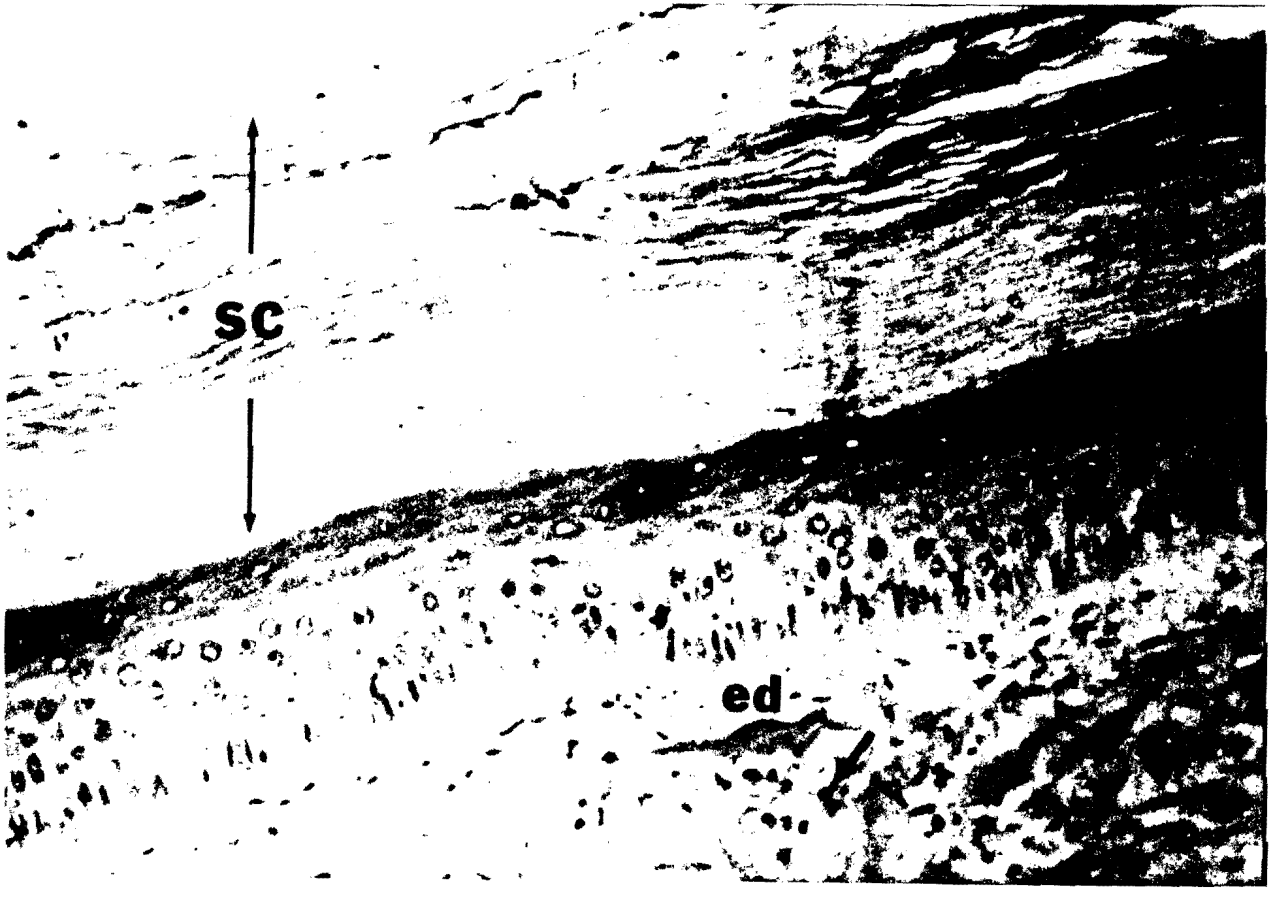



FIGURE 5-27. Medium-power photomicrograph of a skin biopsy from the neck of a loggerhead sea turtle that was exposed to oil for 2 days. The outer epidermal keratinized layer (SC) is markedly thickened and irregular with occasional inflammatory cells and focal necrosis. There is a concurrent thickening of the remaining epidermal strata (ES) characterized by an increased number of cell layers. The superficial dermis is characterized by mild to moderate, diffuse edema (ed) with associated, primarily perivascular, infiltrates of acute and chronic inflammatory cells (arrow). Hematoxylin and eosin stain, 100x.

FIGURE 5-28. Medium-power photomicrograph of a skin biopsy from the neck of a loggerhead sea turtle 3 days after oil exposure had ceased from an acute oil exposure physiological study. The base of the epidermal keratinized layer is characterized by multifocal necrosis with infiltrates of primarily heterophils (large arrows). As in Fig. 5-27 there is epidermal thickening with mild epidermal dysplasia. The superficial dermis is characterized by moderate to severe, diffuse edema with associated extensive infiltrates of acute and chronic inflammatory cells primarily heterophils with occasional plasma cells, histiocytic cells, and small lymphoid cells (small arrows). Hematoxylin and eosin stain, 100x.

FIGURE 5-29. A high-power photomicrograph of Figure 5-28. Notice focal necrosis and inflammatory cell infiltrates of the lower epidermal keratinized layer (N). Epidermal cells exhibit mild, dysplastic changes. Cell maturation is not as uniform with some cells containing large pleomorphic, round to oval nuclei and prominent, often multiple nucleoli in the superficial epidermal strata (arrows). The upper dermal edema with mixed inflammatory cells infiltrates is demonstrated. Hematoxylin and eosin stain, 400x.

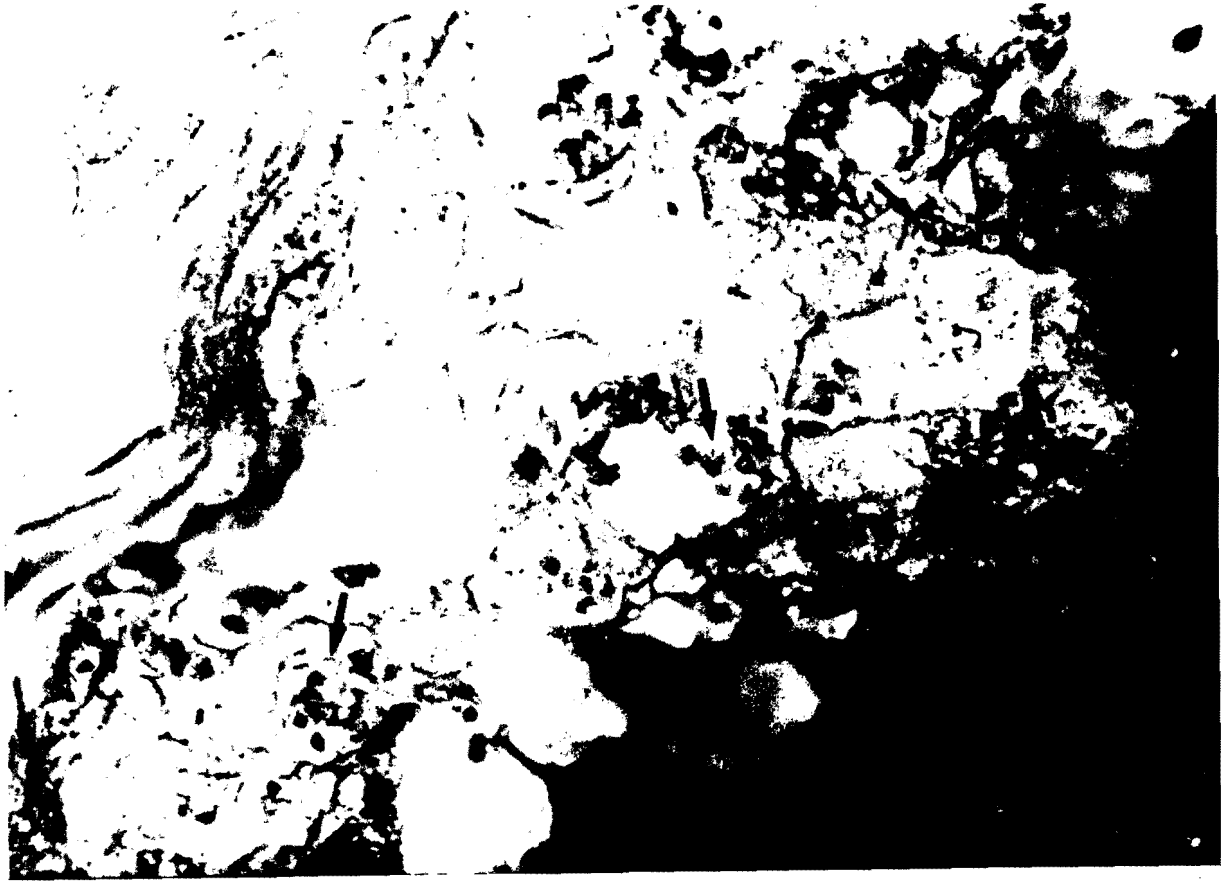


FIGURE 5-30. A high-power photomicrograph of periorbital tissue from a loggerhead sea turtle 7 days after exposure to oil had ceased (from an acute oil exposure physiological study). This tissue is characterized by extensive diffuse coagulative necrosis with focal infiltrates of primary heterophilic inflammatory cells (arrows). Hematoxylin and eosin stain, 400x.

occurred, involving primarily the skin of the neck, top and sides of the head, and inguinal and axillary regions. Sloughing mucous membranes involved the periorbital conjunctiva, nasal mucosa, and oral mucosa. Microscopic evaluation of this sloughing mucosal tissue revealed degenerating and dead epithelial cells admixed with extensive amounts of keratin.

Similar gross or microscopic lesions were not present on sea turtles from behavioral studies. This is probably a result of diminished oil exposure contact as well as the availability of a non-oiled environment.

The changes present in the skin biopsies at day 2 to day 4 are consistent with an acute, primary, contact or irritant dermatitis as seen in mammals (Muller and Kirk, 1976). During this period of time, dysplastic cellular changes in the epidermis are present and often marked. Dysplasia is generally a reversible condition. When the underlying inciting stimula is removed the dysplastic alterations generally revert back to a normal state. In some mammalian species, however, cancerous transformation supervenes (Robbins and Cotran, 1979). It should be noted that these changes in the skin of loggerhead sea turtles are in marked contrast to the lack of skin damage with oil exposure observed by Geraci and St. Aubin (1982) in porpoises.

Perhaps more worrisome than cancerous transformation is, that due to the nature of the acute dermatitis, a break could result in the integumentary barrier and act as a portal of entry for pathogenic organisms. This could occur as a result of the epidermal erosive changes and potential ulcerative nature of the observed dermatitis, either due to acute contact with oil or prolonged low level chronic oil exposure (i.e., if repair processes could not keep up with the irritant properties of the oil). Local bacterial infection and an ultimate septicemic condition could result especially in light of the predominant gram negative bacteria associated with the sea turtles of the study.

In the present acute oil exposure studies the epidermal dysplasia and most of the acute inflammatory changes had resolved by the ninth day following cessation of oil exposure. The dermatologic changes present at this time were consistent with a chronic resolving inflammatory process.

Chronic oil exposure studies demonstrated similar integumentary changes. However, these were not as extensive and repair processes following experimental cessation were more rapid.

### 5.2.3 Summary

Exposure to SLCO in the physiological experiments (chronic and acute) caused two statistically significant changes in the hematology and serum chemistries of loggerhead sea turtles. White blood cell counts increased greatly after exposure to oil (both chronic and acute levels) and did not return to baseline levels until Day 18 of the recovery period. Serum glucose concentrations declined significantly in oil exposed animals on Day 3 of the recovery period. No statistically significant differences were detected in serum protein, albumin, LDH, lipase, AST, ALT, BUN, creatinine, alkaline phosphatase, calcium, phosphorus, uric acid, sodium, chloride, or potassium.

The most severe pathological effect on the turtles due to oil exposure was the marked histopathological changes present in the skin. Changes were more extensive in the soft, pliable skin of the neck than the hard, keratinized skin of the flippers. These histopathological changes were consistent with acute, primary, contact or irritant dermatitis as seen in mammals. These cutaneous changes could ultimately lead to a break in the integumentary carrier; bacterial infection, and subsequent septicimia; and neoplastic transformation. Most of the acute changes were resolved by the ninth day after cessation of oil exposure.

# 6.0 FIELD STUDIES (R. Witham, L. Ehrhart, W. Conley, E. Van Vleet, and G. Bossart)

## 6.1 Analysis of Stranding Network Data

The Sea Turtle Stranding and Salvage Network was created in November, 1979 through the cooperation of sea turtle biologists from Gulf and Atlantic coastal states coordinated by National Marine Fisheries Service, Southeast Fisheries Center, Miami, Florida. The network's primary goal is to document strandings of marine turtles along U.S. Atlantic and Gulf coasts; it is composed of volunteer individuals and organizations, federal, state, and university personnel. All participants submit written reports, via a stranding/salvage form, of local strandings to their respective state coordinators. Mr. Ross Witham, Florida Department of Natural Resources (FDNR), has been the Florida state coordinator since the project's initiation. Copies of reports sent to the Florida state coordinator are forwarded to the Southeast Fisheries Center, Miami, and the originals are sent to the Bureau of Marine Research, St. Petersburg.

The objective of this data analysis for this program was to use this stranding network information to evaluate the mortality associated with oil versus mortality attributed to other causes. We recognize that the estimates of mortality generated from this data base were not absolute rates in that there was no way to estimate the numbers of turtles which were not found or sank before they stranded. However, as a percentage of all stranded turtles, the relative significance of petroleum related mortality should be accurate. It should be emphasized, however, that petroleum related does not definitely mean that petroleum toxicity was the cause of death, but that the turtle was fouled with oil or tar, some of which may have been ingested.

In September, 1983, the Florida Institute of Oceanography (FIO), in cooperation with FDNR, coded all stranding/salvage reports from FDNR's St. Petersburg laboratory for entry into FDNR's IBM 4341 Group II System computer. All written comments and recorded wounds were coded. Several categories were established to reflect possible causes for the strandings. Necropsies were not performed in most cases and comments consisted of observations made by investigating volunteers. These data were then analyzed via SAS utility programs (Statistical Analysis Systems, SAS Institute, Inc.,

SAS Circle, Cary, N.C.) to detect trends in sea turtle strandings. Since complete records were only available for the years 1980-1984, data analysis was limited to this five year period.

In Florida, 1,551 sea turtle stranding reports were received from January, 1980 to December, 1984. Most strandings (1,236) were by loggerhead turtles (Caretta caretta), followed in number by 192 green turtles (Chelonia mydas), 34 hawksbills (Eretomochelys imbricata), 21 Kemp's ridleys (Lepidochelys kempi), and 16 leatherbacks (Dermochelys coriacea). An additional 52 turtles were not identified (Table 6-1).

Necropsies were seldom performed, hence possible cause of stranding was inferred from the information reported. Stranding categories were determined from written comments and/or wounds as noted on the sea turtle stranding/salvage forms. Six categories were chosen to represent stranding type:

1) Drowning suspected: This category was used when a large number of unwounded carcasses washed ashore concurrent with intense local commercial fishing activity;
2) Environmentally related: This category included all turtles suffering thermal shock and those that stranded due to severe storm activity;
3) No comment: Utilized when the stranding report made no mention of wounds and included no written comment;
4) Petroleum related: Stranded turtles that were covered with petroleum, or had ingested its tar residue, were included in this category;
5) Illness induced: This category included turtles that were severely emaciated or had a severe case of papillomas;
6) Wounded: Strandings with a recent, severe wound were included in this category.

Stranding categories may not be mutually exclusive. For example, if a turtle drowned in a fishing net, and was subsequently wounded, that turtle would not be recorded as a suspected drowning. Further, a small thermally shocked individual could become more susceptible to attack by natural predators, such as sharks, and could, therefore, be recorded as a wounded turtle.

Some strandings (n = 232, 14.9%) involved turtles which were still alive when found. However, most died shortly thereafter. Approximately 37.8%

Table 6-1: Species composition of stranded sea turtles along the Gulf and Atlantic coasts of Florida (1980 - 1984).

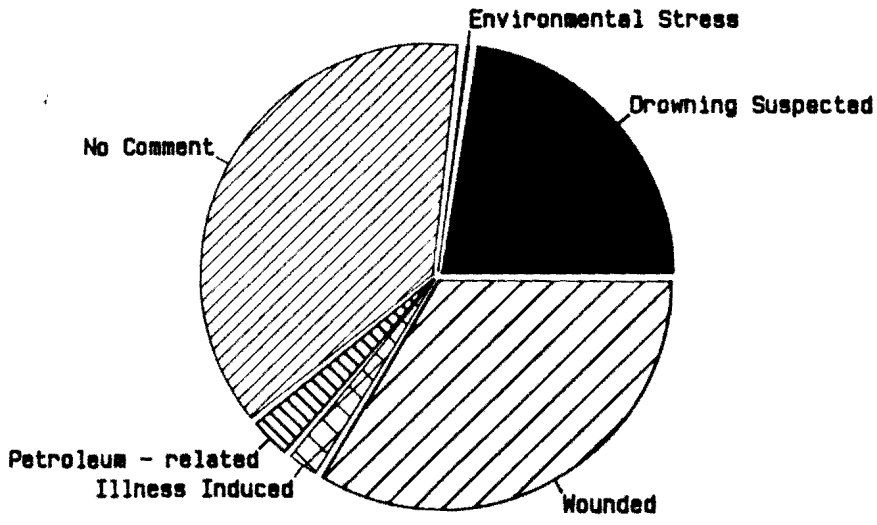
| SPECIES | NUMBER OF STRANDINGS | PERCENT OF TOTAL |
|---|---|---|
| *Caretta caretta* | 1,236 | 79.7 |
| *Chelonia mydas* | 192 | 12.4 |
| *Eretomochelys imbricata* | 34 | 2.2 |
| *Lepidochelys kempi* | 21 | 1.3 |
| *Dermochelys coriacea* | 16 | 1.0 |
| Unknown | 52 | 3.4 |
| Total | 1,551 | 100.0 |

(n = 584) of the recorded strandings received "no comment" (Figure 6-1, Table 6-2) and, therefore, no cause for the stranding could be inferred. A large portion (n = 521; 33.6%) of stranded turtles had been inflicted with a wound of some type (Figure 6-1, Table 6-2). 350 or 22.6% of the total, were suspected to have drowned as part of the incidental capture of commercial fishing vessels (Figure 6-1, Table 6-2). Several (n = 46; 2.9%) turtle strandings were attributed to natural causes (Figure 6-1). Illness, as evidenced by severe emaciation, accounted for 39 of these strandings while the remaining 7 were attributed to temperature stress or severe storm activity.

The first records of petroleum-related strandings in Florida involved two small green turtles (Witham, 1978). Petroleum-related strandings included turtles that were externally fouled or had ingested petroleum residue. Petroleum products accounted for 50 (3.2%) strandings during 1980-1984 (Table 6-2). These strandings tended to be localized, occurring more frequently in the southeastern section of Florida (Table 6-2, Figure 6-2). Southeastern Florida receives the heaviest concentration of pelagic tar from the Florida Current and the Loop Current (Van Vleet et al. 1983, 1984). In Dade County, 37.5% of strandings were petroleum related (Figure 6-2). Broward County observers reported 16 petroleum-related strandings, accounting for 21.9% of their total. However, Broward County observers did not submit individual stranding reports for all Broward strandings to FDNR in 1983. During that year, 18 petroleum-related deaths were recorded (Fletemeyer, 1983). These incompletely documented strandings bring Broward total to 34, and the state total to 68. Palm Beach and Martin counties reported 11.9% and 25.0% of their strandings as petroleum related, respectively.

Petroleum products affected the least abundant species more frequently (Table 6-3). Although 79.7% of Florida's sea turtle strandings were loggerheads (Table 6-1), this species accounted for only 22.0% of the total petroleum-related strandings (Table 6-3). Green turtles were the most affected species, comprising 46.0% of petroleum-related strandings. Hawksbill turtles, which were only 2.2% of the total strandings, comprised 28.0% of petroleum-related strandings. Only one Kemp's ridley, and no leatherback turtles were involved in petroleum-related strandings.

STRANDING TYPES FOR FLORIDA
SEATURTLES
(1980 - 1984)




All Species

FIGURE 6-1.

Table 6-2. Cross tabulation of stranding categories versus region.
Northeast - Nassau through Volusia counties.
Southeast - Brevard through Monroe counties.
Southwest - Collier through Pinellas counties.
Northwest - Pasco through Escambia counties.

| | NORTHEAST | | SOUTHEAST | | SOUTHWEST | | NORTHWEST | | NOT LOCATED | | TOTAL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number | % of Regional Total | Number | % of Regional Total | Number | % of Regional Total | Number | % of Regional Total | Number | % of Not Located Number | Number | % of Total |
| Drownings Suspected | 216 | 32.9 | 92 | 13.9 | 28 | 20.1 | 6 | 14.3 | 8 | 15.7 | 350 | 22.6 |
| Environmentally Related | 2 | 0.3 | 4 | 0.6 | 0 | 0.0 | 1 | 3.4 | 0 | 0.0 | 7 | 0.4 |
| No Comment | 266 | 40.5 | 216 | 32.6 | 44 | 31.7 | 25 | 59.5 | 33 | 64.7 | 584 | 37.7 |
| Petroleum Related | 1 | 0.1 | 49 | 7.4 | 0 | 0 | 0 | 0 | 0 | 0.0 | 50 | 3.2 |
| Illness Induced | 7 | 1.1 | 32 | 4.8 | 0 | 0 | 0 | 0 | 0 | 0.0 | 39 | 2.5 |
| Wounded Turtles | 165 | 25.1 | 269 | 40.7 | 67 | 48.2 | 10 | 23.8 | 10 | 19.6 | 521 | 33.6 |
| Total | 657 | | 662 | | 139 | | 42 | | 51 | | 1551 | |

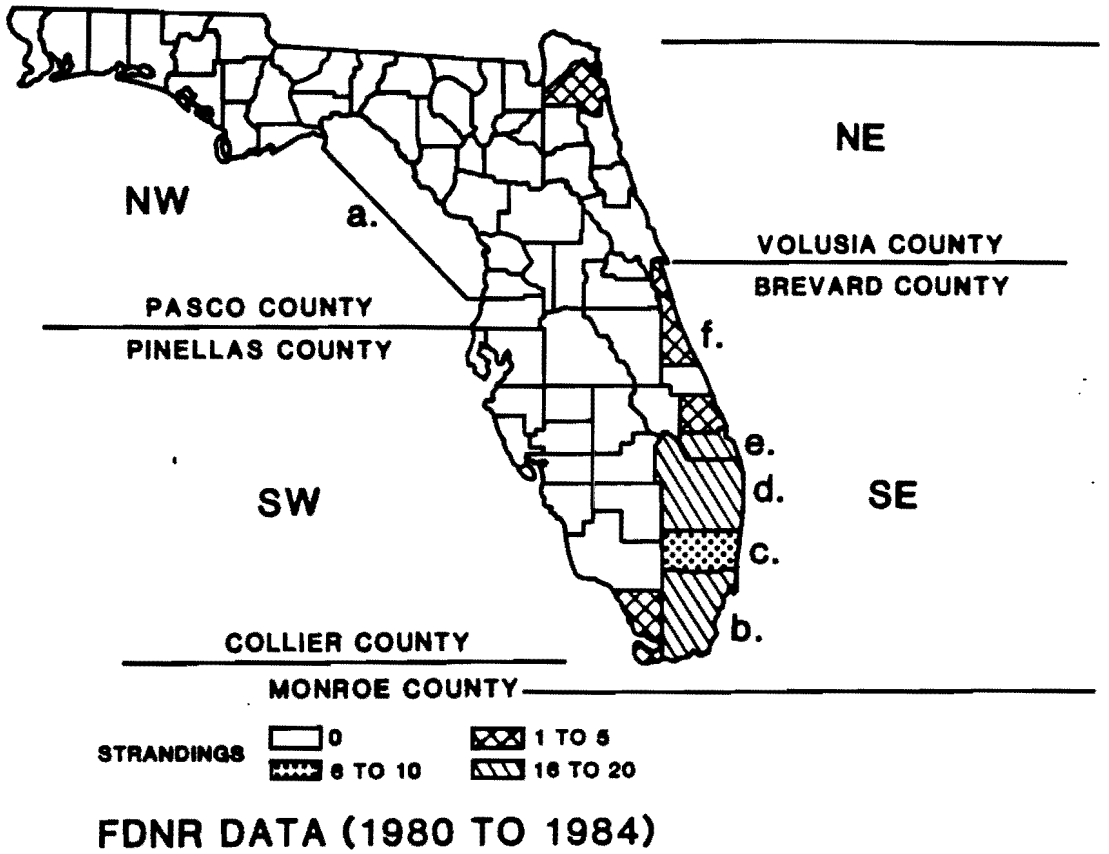



FIGURE 6-2. Areal trends in petroleum related strandings. a. Submergent coastline unsuitable for nesting. b. Dade County. c. Broward County. d. Palm Beach County. e. St. Lucie County. f. Brevard County.

Table 6-3: Species composition of petroleum related strandings in Florida.

| SPECIES | TOTAL | PERCENT OF TOTAL |
|---|---|---|
| *Caretta caretta* | 11 | 22 |
| *Chelonia mydas* | 23 | 46 |
| *Dermochelys coriacea* | 0 | 0 |
| *Eretomochelys imbricata* | 14 | 28 |
| *Lepidochelys kempi* | 1 | 2 |
| Unknown | 1 | 2 |
| Total | 50 | 100 |

Petroleum-related strandings also occurred more frequently among juvenile sea turtles. Approximately 92.0% of all petroleum affected turtles had a carapace length of 50 cm or less (Figure 6-3). Only 20.3% of the total strandings were in this size class. Witham (1983) noted that most petroleum-related strandings were encountered after episodes of sustained easterly winds. It is probable, therefore, that many petroleum-fouled young turtles were never observed because they were transported offshore during other wind conditions.

Many of the observed trends can be partially explained by habitat preferences. For example, 98.0% of petroleum-related strandings occurred in the southeastern section of the state, and approximately the same percentage of nesting turtles utilize southeastern beaches (Harris et al., 1984), thus the high frequency of petroleum-fouled juveniles. In addition, some species of sea turtles do not frequent habitats outside southeastern Florida. Green turtles rarely nest outside of the southeastern area (Hoffman et al.; M.S. in preparation), and hawksbills are commonly observed among the numerous coral reefs that line southeastern Florida.

In summary, petroleum related strandings made up a relatively small portion of the turtle strandings in Florida (3.2%). The two greatest causes of stranding were drowning (22.6%) and wounded (33.6%). An equal portion of the strandings (37.7%) could not be attributed to any specific factor. A more detailed analysis of the petroleum related strandings revealed that petroleum related stranding occurred more frequently in the southeastern section of the State. Analyzing for interspecific differences, green sea turtles were most affected and juveniles were more frequently involved than adults. Many of the trends can be explained by habitat preference and geographical location of nesting beaches.

## 6.2 Analysis of Stranded Turtles

### Hydrocarbon Analyses

As a part of this research project, we analyzed several samples of tar or oil from beached sea turtles as well as samples of the beached turtles' internal organs. The objectives were to determine the origin of the tar and if the petroleum compounds had been taken up by the turtles. Initially, four tar samples were provided by Dr. Tony Amos (University of Texas, Table 6-4). Three of these tar samples came from a beached Ridley turtle (two samples

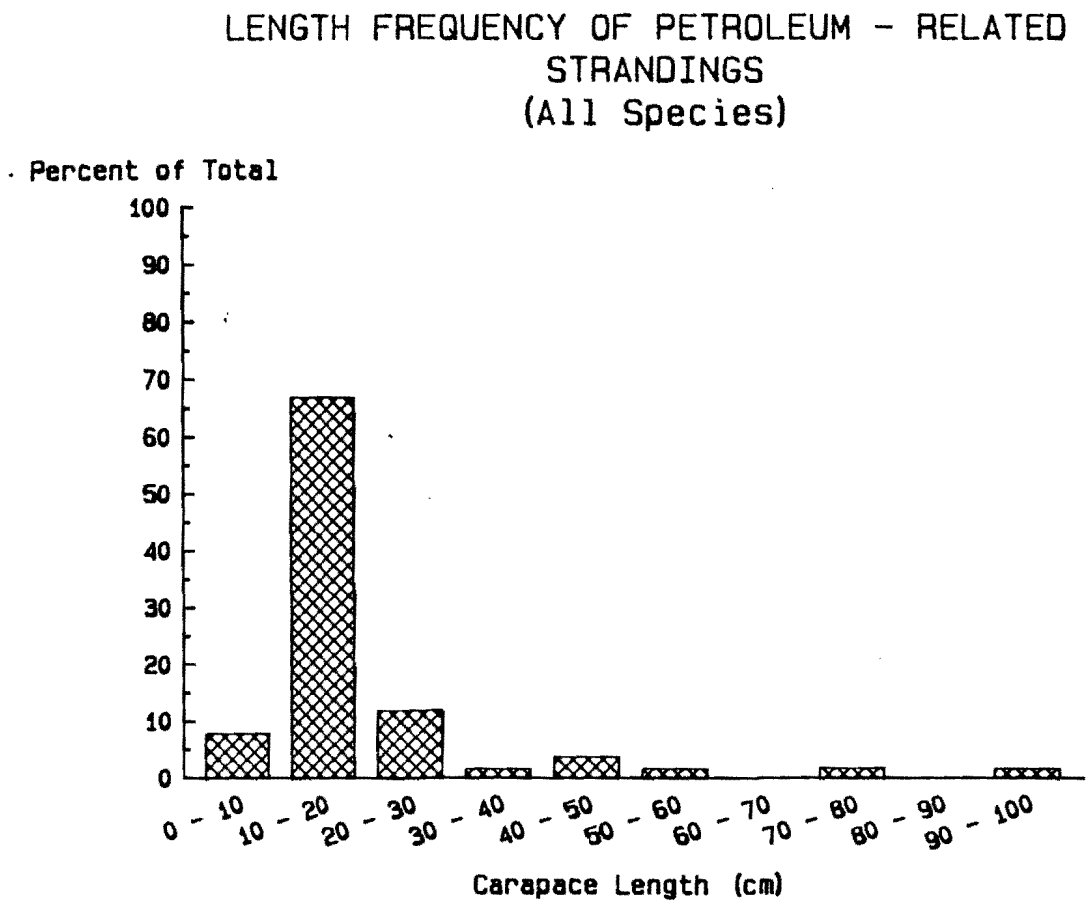



FIGURE 6-3. Size frequency distribution of stranded sea turtles.

Table 6-4. Characterization of tarballs from live Ridley turtles and Mustang Island Beach. All samples exhibited significantly different alkyl homolog distributions for $C_0$-$C_3$ naphthalenes, phenanthrenes, pyrenes, and benz(a)anthracenes. These differences were due to both different chemical compositions of the tars, and different weathering histories. Only tars NNQ-507 and NNQ-518 could have had similar sources based on their PAH distributions. (17= $nC_{17}$; 18= $nC_{18}$; Pr= pristane; Phy= phytane).

Aliphatic (Fl) Characteristics

| SAMPLE | 17/Pr | 18/Phy | 17/18 | Pr/Phy | n-alkane range | n-alkane maximum |
|---|---|---|---|---|---|---|
| NNQ-507 (Ridley Mouth) | 1.9 | 1.6 | 0.8 | 0.8 | $nC_{12}$-$nC_{36}$ | $nC_{18}$, $nC_{32}$ |
| NNQ-518 (Ridley Mouth) | 1.2 | 1.2 | 0.9 | 0.8 | $nC_{14}$-$nC_{36}$ | $nC_{18}$, $nC_{31}$ |
| NNQ-569 (Ridley-External) | 1.5 | 1.5 | 0.4 | 0.5 | $nC_{17}$-$nC_{32}$ | $nC_{19}$ |
| ABS-84839 (Beach tar) | 0.6 | 0.9 | 0.6 | 0.9 | $nC_{16}$-$nC_{38}$ | $nC_{27}$ |

from mouth, one from an external composite). The fourth sample was a beach tar collected on Mustang Island Beach (Texas) approximately two days after the turtle was collected (June 19-21, 1984). The two tar samples from the turtle's mouth appeared to come from the same source, although different degrees of weathering were observed. Both of these tars were judged to originate from crude oil tanker washings. The external composite sample was not from the same source, but appeared to be a refined oil based upon its narrow boiling range. None of the three samples matched the tar from Mustang Island Beach.

Later in June, 1984, oil from a tanker spill washed ashore in Galveston, Texas. High resolution GC and GC/MS analyses of this tar was also carried out (Table 6-5) to compare with possible future turtle impacts. No beached turtles were subsequently collected with tar that matched this source.

In September, 1984, a beached green turtle was provided by Dr. Amos for hydrocarbon analyses. Tar obtained from the mouth of this turtle also resembled that expected from most crude oil tanker discharges. Analyses of the stomach and esophagus gave evidence of similar hydrocarbon contamination (Table 6-6) and indicated that this turtle was indeed feeding upon the pelagic tar. Analyses of liver, intestines and muscle tissues from this turtle yielded only biogenic hydrocarbon peaks, indicating that the petroleum hydrocarbons had not been incorporated into other internal tissues at the time of death.

Tar isolated from two hawksbill turtles, two other green turtles and one loggerhead turtle also showed the bimodal n-alkane distribution characteristic of crude oil tanker discharge (Figure 6-4). In addition, analyses of internal organs of the loggerhead turtle indicated hydrocarbon contamination of the stomach and intestines. In summary, these results confirm essentially all of our other analyses of impacted turtles, and clearly show that crude oil tanker discharge is being ingested by marine turtles in the Gulf of Mexico.

## Clinicopathologic Analyses

To determine if experimental effects of oil exposure were indicative of exposure to oil in the natural environment clinicopathologic measurements identical to those performed in the experimental studies (Section 5.2) were performed on stranded, living, oil-fouled sea turtles during the 2 year contract period.

Table 6-5. Hydrocarbon characterization of oil washed ashore in Galveston, Texas, June 1984, from tanker spill. (17= $nC_{17}$; 18= $nC_{18}$; Pr= pristane; Phy= phytane).

**Aliphatic Characterization (F1)**

| 17/Pr | | 18/Phy | | n-alkane 17/18 | n-alkane Pr/Phy | | range | maximum | CPI | Res/Unres |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.97 | 9.96 | 0.14 | 1.38 | $nC_{16}$-$nC_{30}$ | $nC_{18}$ | 0.34 | 0.50 | | | |

**Aromatic Characterization (F2) (Relative Concentrations)**

Resolved/unresolved areas = 0.40

| Naphthalenes | | | | Phenanthrenes | | | | Pyrenes | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_0$ | $C_2$ | $C_2$ | $C_3$ | $C_0$ | $C_1$ | $C_2$ | $C_3$ | $C_0$ | $C_1$ | $C_2$ | $C_3$ |
| 0.01 | 0.21 | 0.30 | 1.00 | 0.28 | 0.22 | 1.00 | 0.79 | 0.01 | 0.08 | 0.34 | 1.00 |

Table 6-6. Analysis of Green turtle received from Dr. Tony Amos (10/16/84).

Data from Tony Amos:

Date Found: 14 September 1984
Location: 27°49.8'N 97°03.0'W (south of the jetty, Mustang Island Beach, Texas)

| Size: | length | width |
|---|---|---|
| straight | 5.60 cm | 4.84 cm |
| curved | 5.9 cm | 5.1 cm |

Weight: 20.32 grams

Description: Tar was in roof of mouth, scraped out with spatula and put into glass vial with Teflon-lined cap. Sample kept frozen until analyzed.

Chemical Data from USF:

| Subsample | Total Hydrocarbons ( g/g wet weight) | Qualitative Description |
|---|---|---|
| tar from mouth | - | $nC_{15}$-$nC_{32}$, OEP 1, no biogenic peaks |
| stomach and esophagus | 1025 | $nC_{21}$-$nC_{31}$, OEP 1, plus biogenic peaks |
| liver | 1399 | biogenic peaks only |
| intestines | 807 | biogenic peaks only |
| muscle | 563 | biogenic peaks only |

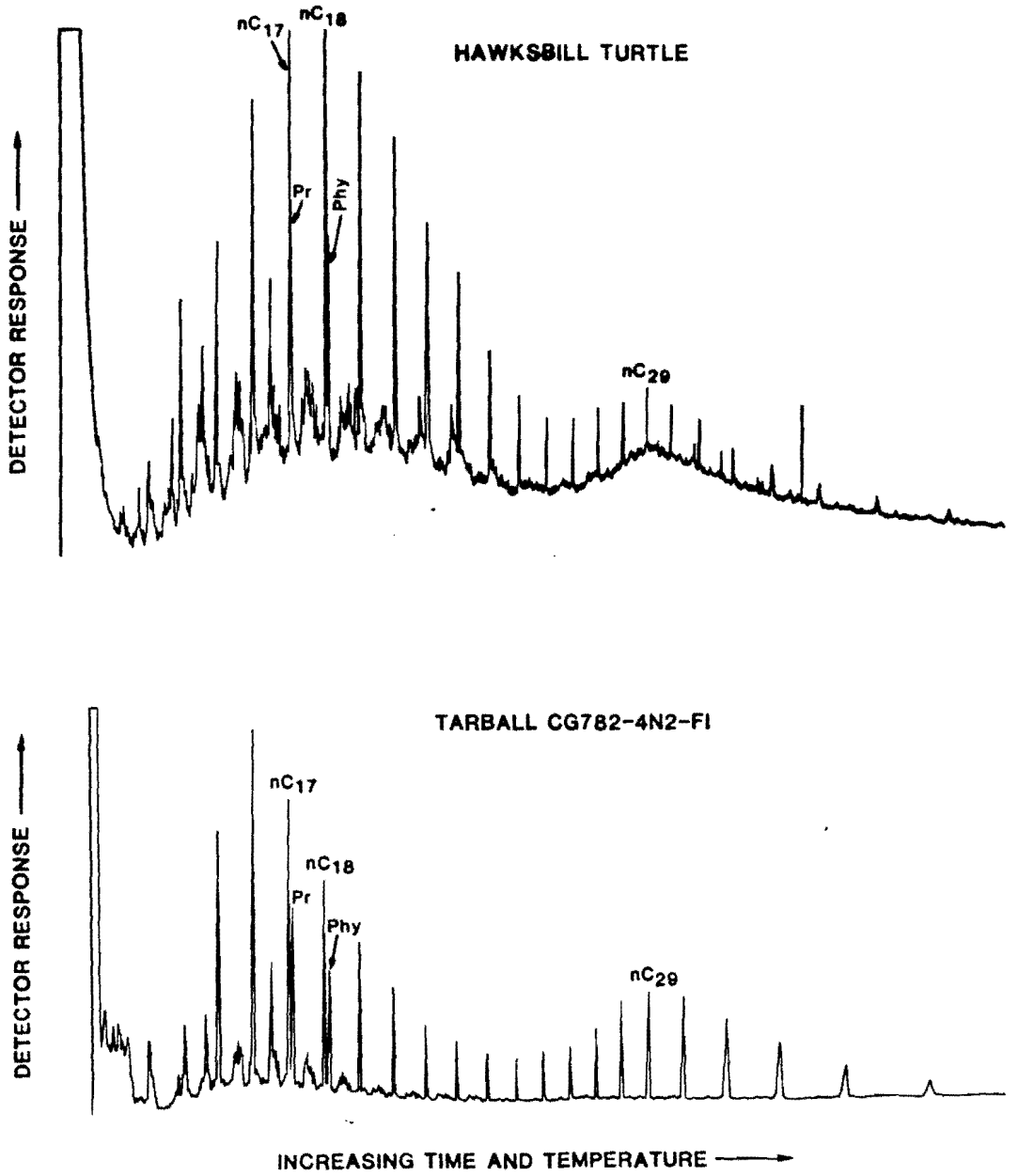



FIGURE 6-4. High resolution gas chromatograms of tar from a stranded hawksbill sea turtle (A) and pelagic tar ball (B).

During this period 3 hawksbill (Eretmochelys imbricata) and 3 green (Chelonia mydas) sea turtles were found beached and oil-fouled along the southeastern coastline of Florida. One hawksbill and 2 green sea turtles were found in the Ft. Lauderdale area and taken to Ocean World for rehabilitation and clinicopathologic sample collection. One hawksbill was found in the Miami area and taken to RSMAS. The remaining sea turtles were found in the central Florida Keys and taken to the Sea World MSCC. All these stranded turtles were living when found.

All of these sea turtles appeared to be subadult juveniles weighing between approximately 0.5 kg and 1 kg. Three of these turtles had depressed red blood cell indices including low red blood cell counts; depressed hemoglobin levels; and low hematocrits. This was similar to trends observed in experimental animals. However, differences between control and oil values were not statistically significant in the short-term laboratory experiments. One hawksbill sea turtle was hypoproteinemic (low blood protein). Four of these sea turtles were emaciated and had a low blood glucose at the time of stranding which was a similar trend present in chronic oil exposed turtles from physiological experiments. None had evidence of fecal blood. Several of the turtles had reddening or inflammation of the skin in areas where oil or tar had adhered. These lesions healed over a 2 - 4 week period. Skin biopsies were not taken due to the weakened condition of the turtles. Despite several weeks of supportive care, one of the stranded green sea turtles died. The only remarkable histopathologic change observed in this turtle involved the liver. The liver of this turtle was characterized by severe, diffuse hepatocellular fatty change and cellular degeneration. These are non-specific hepatic changes and in other species are associated with a wide variety of causes including nutritional, metabolic, and toxic factors (Jones and Hunt, 1983). The remaining turtles are still alive in captivity and their measured hematologic and blood chemistries have returned to within "normal" captive ranges from data established in the experimental studies.

The formalin fixed tissues from 12 oil-fouled, stranded Kemp's ridley (Lepidochelys kempi) sea turtles were evaluated grossly and microscopically. A large number of specimens were provided by Bob King from Padre Island National Seashore Park, Texas. These subadult turtles washed ashore approximately 24 hours after their release from a headstart program. Eighteen turtles were dead and 80% of those stranded (living and dead) had

tar in their mouths according to Mr. King. Tissues submitted from these sea turtles included liver, oropharynx, esophagus, pancreas, and small intestine.

Grossly, from the tissues submitted, the only remarkable finding was varying amounts of a black, tenacious, tar-like substance in association with the oropharynx and intestines. Histologic evaluation of these tissues revealed numerous consistent changes. The livers were characterized by mild to moderate chronic-active inflammation characterized by diffuse and subcapsular infiltrates of acute and chronic inflammatory cells, primarily heterophils, plasma cells, and macrophages.

In three cases, the livers had a multifocal type of inflammation that involved the formation of granulomas. These granulomas were characterized by central necrosis surrounded by multinucleated giant cells, heterophils, and macrophages. Certain fungi and bacteria in other species are associated with this type of inflammation. Special histochemical stains for these organisms in the livers, however, were negative. All livers also exhibited mild to severe hepatocellular degeneration, atrophy and fatty change.

Oropharyngeal changes associated with the tenacious tar-like deposits included mild to moderate acute inflammation characterized by submucosal infiltrates of primarily heterophils. These changes are consistent with a mild to moderate acute oropharyngitis. Implicating a causal relationship, however, requires further investigation.

The small intestinal specimens submitted were also characterized by granulomatous inflammation. Special histochemical staining demonstrated numerous acid fast bacteria not unlike those that cause tuberculosis. Definitive identification of such organisms would have required bacterial culture which is not possible with formalin fixed tissue or immunohistochemical staining which was not available at the time.

The remaining microscopic changes in the varied specimens submitted were either mild or non-specific related to terminal shock.

The majority of the inflammatory changes observed in these Kemp's ridley sea turtles were of a chronic-active nature. Therefore, they were probably present before their release 24 hours previous to the stranding and not a result of the tar.

The cause of this mass stranding and resultant death of many of these sea turtles was probably multifactorial. From the overall history for the Kemp's ridleys, the stress of release together with concurrent ongoing

chronic active disease states and resultant exposure to tar (varying degrees of airway obstruction) can not be ruled out as significant contributing factors.

In conclusion, from these field studies it is apparent that some clinicopathologic correlations can be made with the experimental data. New questions arise, however, on the potential effects of oil on the oropharyngeal mucosa and potential for functional compromise as an air or food conduit.

# 7.0 OVERALL PROGRAM SUMMARY AND SUGGESTED MITIGATING STRATEGIES

## 7.1 Program Summary and Synthesis

The program, as originally funded, was a two year program from September 30, 1983 to October 1, 1985. During this two year program we have gained a substantial data base of information relevant to the MMS stated program goal to determine the effects of oil on hatchling, juvenile, and adult sea turtles.

The experimental program was carried out using two species of marine turtles, loggerheads and greens. The potential effects of oil on the marine turtles is summarized as a conceptual model in Figure 7-1. These potential effects were drawn from documented effects on birds and marine mammals reported in the literature (Appendix A). It can be seen that the potential effects are both numerous and detrimental.

As specified by MMS the oil used in this program was a South Louisiana Crude Oil (SLCO). A sufficient supply was obtained so that the same oil could be used for all phases of the program. Analysis of the oil indicated it was consistent in overall characteristics with other SLCOs and a standardized dosing procedure was developed using preweathered oil. Direct analysis indicated that the petroleum hydrocarbon concentrations used in the behavioral, physiological, and clinicopathological studies were consistent with expected concentrations during an oil spill event at various locations within the spill.

The experiments on behavior addressed the ability of marine turtles to detect and avoid or be attracted to oil slicks and/or tar balls. Figure 7-2 illustrates potential effects of oil on behavior, again drawing on effects previously reported in the literature.

Loggerhead (*C. caretta*) and green sea (*C. mydas*) turtles were used in the behavior experiments (oil slick and tar ball) and ranged in age from 2 to 18 months. In the oil slick experiments both species responded to the oil slick by spending significantly more time deeper in the water column and coming to the unoiled center of the pool to breathe more frequently than in the control situation. However, because the turtles responded to dark colored mineral oil, which did not release chemical cues, in the same way as for SLCO, it was our conclusion that they were responding to the dark visual surface barrier not specifically to SLCO. In the natural environment,

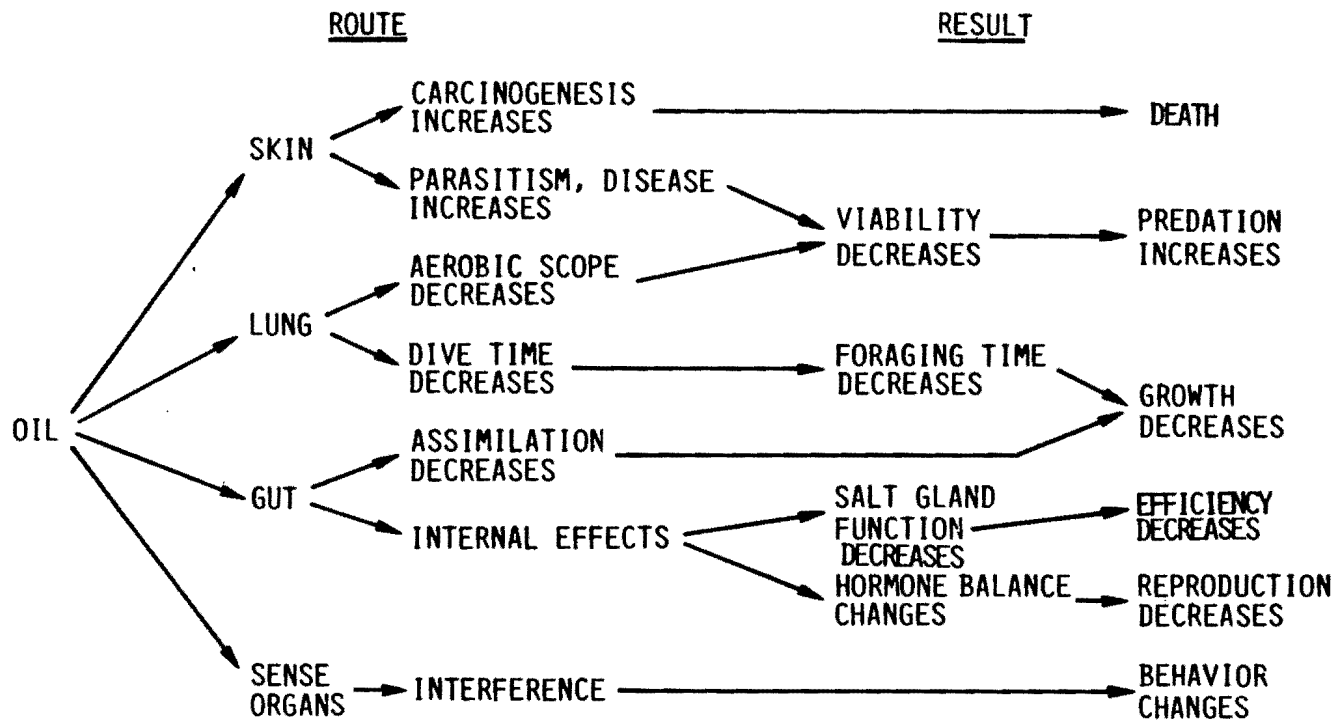



FIGURE 7-1. Conceptual model of potential effects of oil on marine turtles drawn from literature reports (Appendix A) of the effects of oil on turtles and other vertebrates.