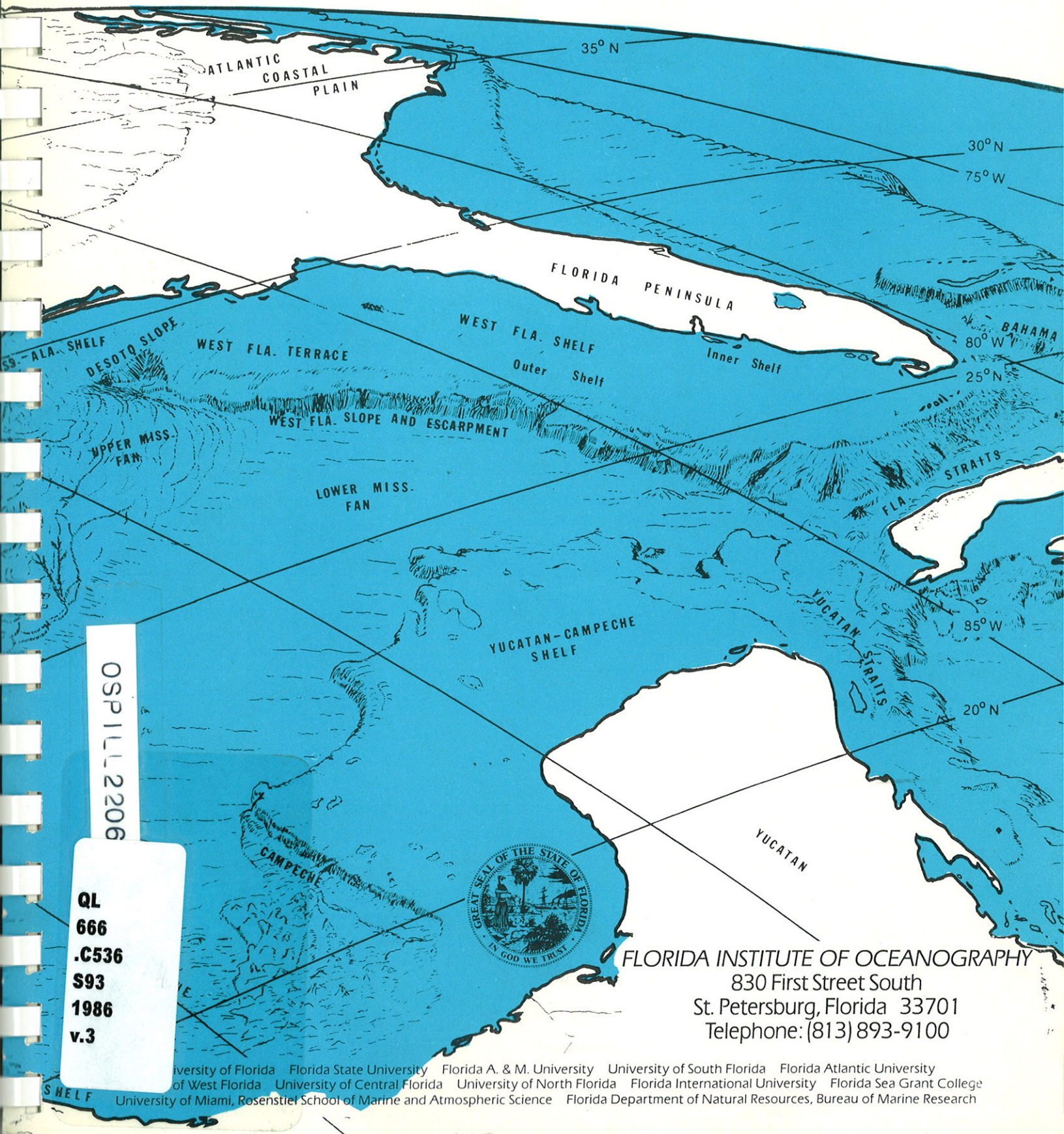
FINAL REPORT
STUDY OF THE EFFECTS OF OIL
ON MARINE TURTLES

OCS STUDY
MMS 86-0070

Prepared for the U.S. Department of the Interior
Minerals Management Service
MMS Contract Number 14-12-0001-30063

Volume 3 - Appendices



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FLORIDA INSTITUTE OF OCEANOGRAPHY
830 First Street South
St. Petersburg, Florida 33701
Telephone: (813) 893-9100

iversity of Florida Florida State University Florida A. & M. University University of South Florida Florida Atlantic University
of West Florida University of Central Florida University of North Florida Florida International University Florida Sea Grant College
University of Miami, Rosenstiel School of Marine and Atmospheric Science Florida Department of Natural Resources, Bureau of Marine Research

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Volume 3 - Appendices

Submitted September 15, 1986

Final Report for September 30, 1983 - October 1, 1985

Availability unlimited with approval of Minerals Management Service

Submitted by

Florida Institute of Oceanography
830 First Street South
St. Petersburg, Florida 33701

OCS STUDY
MMS 86-0070

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO. MMS 86-0070	2.	3. Recipient's Accession No.

4. Title and Subtitle

Effects of Oil on Marine Turtles

5. Report Date

6. September 15, 1986

7. Author(s) S. Vargo, P. Lutz(RSMAS), D. Odell(RSMAS), E. Van Vleet (USF) and G. Bossart (WVC)

8. Performing Organization Rept. No.

9. Performing Organization Name and Address

Florida Institute of Oceanography
830 First Street, South
St. Petersburg, Florida 33701

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.

(C)
(G) 14 12 0001 30063

12. Sponsoring Organization Name and Address

Atlantic and Gulf of Mexico OCS Regions
Minerals Management Service
U.S. Department of the Interior
1951 Kidwell Drive, Suite 601
Vienna, Virginia 22180

13. Type of Report & Period Covered

Final Report
September 30, 1983-
October 1, 1985

14.

15. Supplementary Notes

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.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement:	19. Security Class (This Report)	21. No. of Pages
Release unlimited with Minerals Management Service approval	Unclassified	
	20. Security Class (This Page)	22. Price

(See ANSI-Z39.18) See Instructions on Reverse OPTIONAL FORM 272 (4-77) (Formerly NTIS-35) Department of Commerce

PARTICIPANTS

PRINCIPAL INVESTIGATORS:

Dr. Peter L. Lutz
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Dr. Daniel K. Odell
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Dr. Llewellyn M. Ehrhart
Department of Biological Science
University of Central Florida

Dr. Edward S. Van Vleet
Department of Marine Science
University of South Florida

Mr. Ross Witham
Florida Department of Natural Resources

Dr. Gregory D. Bossart
Wildlife Veterinary Center

MANAGEMENT and FACILITIES:

Dr. Sandra L. Vargo
Florida Institute of Oceanography

Dr. Peter L. Lutz
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Mr. James S. Kepley
Sea World Shark Institute

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APPENDIX A

REVISED LITERATURE SURVEY AND SYNTHESIS

UNIVERSITY OF SOUTH FLORIDA

Host for

FLORIDA INSTITUTE OF OCEANOGRAPHY

REVISED LITERATURE SURVEY AND SYNTHESIS

Submitted in Partial Fulfillment of

MMS Contract #14-12-001-30063

entitled

"STUDY OF THE EFFECTS OF OIL ON MARINE TURTLES"

by

M. Lutcavage, P. L. Lutz, and D. K. Odell
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Date of Submission of Revision: May 25, 1984

Effective Date of Contract: September 30, 1983

Expiration Date of Contract: October 1, 1985

Amount of Contract: $552,135 with $501,199 currently allocated

Authorized to conduct Technical and Cost discussion: Dr. Sandra L. Vargo

Authorized to contractually bind for this agreement: Dr. A. Riley Macon

Florida Institute of Oceanography
830 First Street South
St. Petersburg, Florida 33701
Telephone: (813)893-9100

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1. General Background

The biological effects of petroleum on marine life including fish, marine mammals and birds have received substantial attention in recent years (Moore and Dwyer, 1974; Light and Lanier, 1978). Despite the endangered status of most sea turtle species virtually nothing is known regarding potential biohazards of oil on these animals (Witham, 1983).

Out of seven species of sea turtles living today, five are found in the Gulf of Mexico OCS development area. Oil pollution poses a potential threat to these animals and may even challenge the survival of the species most at risk, the Atlantic, or Kemp's ridley.

Evidence of the harmful effects of oil on sea turtles has been established in several published accounts. Oil covered hatchlings and juveniles have stranded on beaches in Panama (*Caretta caretta*, Rutzler and Sterrer, 1970), in Puerto Rico (Diaz-Piferrer, 1962) and in Florida (*Chelonia mydas*, Witham, 1978; Chan, 1977).

The IXTOC I oil blowout released crude oil near the Kemp's ridley's only known nesting beach (Delicat, 1980) and recent popular news accounts describe oiled turtles off the coast of Kuwait. Witham (1978) and Lutz (personal communication) rehabilitated several juvenile green turtles whose oral cavities were occluded by tar, while other oiled turtles apparently died as a result of oil ingestion.

There are very few studies of hydrocarbon effects on sea turtles. Studies of toxic substance effects on reptiles (reviewed by Hall, 1980) for the most part, are limited to investigation of pesticide and heavy metal residues. Frazier (1980) outlines potential impacts of pollutants for sea turtles in the coastal zone. Baseline levels of heavy metals and pesticides in feral sea turtles and their eggs were provided by Hillestad et al. (1974),

Schwartz and Flamenbaum (1976), Thompson et al. (1974), Stoneburner et al. (1980), and Clark and Krynitsky (1980).

Bennett and Kleerekooper (1978) examined changes in locomotion caused by oiling. Fritts and McGehee (1981) looked at the effects of oiled sands on incubating loggerhead (Caretta caretta) and Kemp's ridley (Lepidochelys kempi) eggs. Interestingly, they found that while loggerhead eggs incubated in artificially oiled sands appeared to suffer reduced survival and altered morphology, Kemp's ridley eggs incubated in oiled sand on their native beach showed no significant differences in embryo survival. These field study results indicate that oil effects on sea turtle eggs may be complex and determined by the interaction of several factors. These may include type of oil, concentration, degree of weathering, and the nest environment itself.

Although present accounts of oil effects on turtles describe and evaluate sources of oiling none critically assess how oil exposure disrupts behavior and physiological functions in these animals. However, it is only by understanding the cause-effect relationship that the full impact of oil exposure on sea turtle populations can be appreciated and realistic mitigating measures formulated.

In contrast to the situation for sea turtles, the potential threat of oil pollution has been well recognized with respect to marine mammals (LeBoeuf, 1971; Geraci and Smith, 1976a,b; Kooyman and Costa, 1979; Baker, 1981; Geraci and St. Aubin, 1982) and sea birds (Bourne, 1968; Beer, 1970; Bourne and Bogan, 1976; Dieter, 1976; King, 1979; Peakall et al., 1979; Falandysz and Szefer, 1982) and this awareness has resulted in a considerable amount of research and a solid ground of knowledge. Geraci and St. Aubin (eds., 1982) experimentally determined some effects of petroleum on cetaceans

and provided a comprehensive review and assessment of literature concerning oil exposure and ingestion in mammals.

The literature reviewed here concerns the effects of petroleum hydrocarbons (PHC) on marine vertebrate behavior and biology based on the assumption that similar effects will be seen in sea turtles. We have also included studies that involve the basic biology and physiology of sea turtles (see behavior section, also Bentley, 1981; Bentley and Lutz, 1983; Dunbar-Cooper and Lutz, 1983; Lutcavage and Lutz, 1983). However, knowledge of basic biology of sea turtles is limited. We do know that these species share biological characteristics of both homeothermic and poikilothermic vertebrates. Nonetheless, the effects of oil on the reptilian system may prove to be unique.

2. Oiling and Aquatic Vertebrate Behavior

A search of the scientific literature revealed only a few papers concerning sea turtle behavior with specific reference to feeding/food habits (e.g. Bjorndal, 1980; Grassman and Owens, 1982; Nuitja and Uchida, 1982; Hadjichristophorou and Grove, 1983). Among these, there are no data which document their behavioral response to tar balls or oil slicks. Tar balls are chronically present in the environment and show regional differences in concentration (Van Vleet and Sackett, 1981; Van Vleet et al., 1983 a, b, c).

Most of the recent literature on sea turtle behavior concerns reproduction (e.g. nesting, hatching) and migration. This reflects the fact that sea turtles are studied primarily when they return to land to nest.

Studies on sea turtle chemoreception have documented their ability to detect chemicals dissolved in the water (including the water soluble fraction of crude oil (Kleerekooper and Bennett, 1976 ms). These animals appear to

"smell" underwater by operating their nostrils and pumping water over the olfactory epithelium by raising and lowering the floor of the oral cavity (Walker, 1959; Manton et al., 1972a,b). With the release of chemicals the turtles showed increased flipper movements with a sometimes violent reaction.

These and other observations of turtle olfaction (Tucker, 1963; Tucker and Shibuya, 1965) raise the strong possibility that sea turtles could be attracted to tar balls and oil slicks by chemical cues. Some investigators think (no published accounts) that young sea turtles will strike at objects indiscriminantly while hunting for food. Apparent ingestion of tar balls could, therefore, be purely accidental.

Sea turtle vision out of the water is apparently poor (Ehrenfeld and Koch, 1967) but Ehrenfeld and Carr (1967) have found orientation of green turtles to be largely a visual process. Others support this finding and believe that light is the motivating force which guides the turtle from the nesting site on the beach toward the sea (Daniel and Smith, 1947; McFarlane, 1963).

Several theories have been advanced to explain sea turtle navigation and orientation. Van Rhijn (1979) suggested that in addition to optic components, non-optic components, i.e. the sloping of the beach surface towards the sea and the distribution of silhouette patterns, help to orient the sea turtle. Sound may be a contributing factor in orientation. Ridgeway et al. (1969) found the ear of the adult green turtle to be a low-frequency receptor, most usable in the range of 60-1000 HZ.

On return from sea to the natal beach for nesting, it has been proposed that the release of some chemical substance guides the turtle along a trail (Koch et al., 1969). Furthermore, chemical imprinting of neonates has been suggested (Owens et al., 1982). Pritchard (1969) suggested that Rathke's

glands located on the turtle plastron could provide a pheromone system used in navigation and homing. Ehrenfeld and Ehrenfeld (1973) have shown these to be typical exocrine glands with a glycoprotein secretion.

Fletemeyer (1978) and Carr and Meylan (1980) provided evidence that neonatal loggerhead sea turtles seek shelter in masses of Sargassum. Currents, especially in the Gulf Stream, concentrate floating material along boundaries with other water masses. One might hypothesize that turtles (especially neonates) would be concentrated in the same area as Sargassum and tar balls. This would increase the probability of the turtles encountering tar balls. The CETAP study (Winn, 1982) found most loggerhead sea turtles concentrated primarily shoreward of the 200 m depth curve with warming temperatures in the spring. This aerial survey along the northeast coast of the U.S. stopped at Cape Hatteras. In addition, Fritts et al. (1983) documented the distribution of sea turtles in the Gulf of Mexico OCS development area. It is likely, however, that small sea turtles are more at the mercy of the currents than the larger individuals and would tend to be hidden from view if associated with Sargassum or Sargassum wrack lines.

Studies of sea turtle feeding and activity patterns (Mendonca and Ehrhart, 1982; Ogden et al., 1983) reveal that juveniles are found in seagrass beds and lagoons and follow a diurnal cycle. From this, one might expect these turtles to be carried into an oil slick at night before they could take avoidance action. Witham (1980) supports the capacity of currents to move turtles. Galt (1983) has proposed a trajectory model for the dispersion of oil. Even if the turtle remains stationary, the oil may be carried into seagrass communities or onto nesting beaches. In field studies it was found that the Sargassum community was "measurably contaminated" by petroleum (Morris et al., 1976; Burns and Teal, 1973).

It remains that some sea turtles become contaminated with oil but the circumstances under which this occurs are unknown. Although there may be accumulation in part through feeding, magnification along the food web has not been identified (Morris et al., 1976). More likely, contamination is through the respiratory surface and via the gut by way of ingestion of tar balls (Morris et al., 1976). At any rate, oil could be a deterrent to nesting and migration, and its effect on sea turtle reproduction seems threatening.

3. Petroleum Hydrocarbon Toxicology

Some clues regarding the possible effects of oil on sea turtles may be obtained from studies of the toxic effects of petroleum fractions on other vertebrates, including marine fishes (Morrow, 1973; Eisler and Kissil, 1975; Meyerhoff, 1975; Martin, 1980), birds (King, 1979; Stickel and Dieter, 1979; Matko and King, 1980), and mammals (Baker, 1981). These demonstrate wide-ranging physiological effects caused by acute and chronic exposure to hydrocarbons (e.g. Payne, 1975; Stegeman and Sabo, 1976; Rice et al., 1976; Ernst et al., 1977; Engelhardt, 1978; Solangi and Overstreet, 1982; Weber, 1982). The sensitivity to oil type (crude vs. refined), concentration, degree of weathering and constituents (aliphatic vs. aromatic) varies tremendously among and within marine vertebrate groups (see reviews in Malins, ed., 1977; Tatem and Anderson, 1973). Several of these factors are likely to affect toxicity of particular oils to sea turtles.

There is evidence that the toxicity of oil is increased by the presence of polycyclic aromatic fractions which are more soluble than alkanes (Jordon and Payne, 1980; Neff and Anderson, 1981). The duration of exposure, life stage, sex, and whether and how the animal metabolizes petroleum also affect toxicity. In an experimental exposure study of a particular species these

factors must be evaluated against potential physiological differences that may occur between sexes and age classes.

Sea turtles are exempt from the obvious damage associated with oiling of structures such as feathers and gills (Hartung and Hunt, 1966; Solangi and Overstreet, 1982) and from ingestion of petroleum via preening (Miller et al., 1978). Nonetheless, there are several reasons why sea turtles are exceptionally vulnerable to contamination by petroleum. As air-breathers, sea turtles must come to the surface to breathe. Therefore, in an oil spill area they would continually come into contact with the oil slick. Surface film oil could be aspirated incidentally via the glottis during ventilation. Oil might also adhere to the keratinized pharyngeal papillae used in swallowing food and regurgitating sea water.

Aspiration of petroleum in other air breathers is known to induce pneumonia and associated symptoms (Stula and Kwon, 1978; Geraci and St. Aubin, 1982). If turtles occur in the immediate vicinity of newly released, unweathered crude oil, it is possible that inhaled or ingested fresh oil could results in pulmonary edema. Minor interference with ventilation could compromise diving capacity, hamper predator avoidance, and reduce the time available for feeding and underwater travel. Ventilation in the sea turtle is extremely dynamic (Tenney et al., 1974; Lutcavage and Lutz, 1983). A severe reduction in ventilation scope could ultimately lead to drowning.

Sea turtles face potential exposure to oil on land as well as in the sea. During the nesting season, petroleum contaminated beaches could adversely affect reproduction success by deterring egg laying, directly disturbing the developing egg, or changing the composition of the nest sand overlay.

Ackerman (1981a,b) studied gas exchange in sea turtle nests and found that embryonic development and hatching success is dependent on maintenance of effective oxygen and carbon dioxide tensions. These factors would be altered by oiled sands. Sex ratios of incubating eggs, known to be governed by temperature of incubation (Mrosovsky and Yntema, 1980; Morreale et al., 1982) might be skewed by increased mean incubation temperature induced by a low-albedo, oiled nest surface.

Studies of the acute or chronic toxicity of oil components have not been conducted on juvenile or subadult sea turtles. The toxicity of a substance is determined most often in standard marine test animals or indicator species (LaRoche et al., 1970). The nature of these studies requires that the test animal be both tolerant of captivity and also show reasonable sensitivity to oil components. A common experimental aim is to determine the LC-50 concentration for a given species. However, LC-50 studies are inappropriate for determining the ecological effects of a toxic substance. In addition, LC-50 studies are ill-advised for endangered or threatened species due to the numbers of organisms required for the tests.

It would appear that in general, relatively high concentrations of crude oils are required to cause mortality in most vertebrates examined. These levels would not be expected to occur often in a natural spill situation. However, sublethal exposure effects were evident following acute exposure to various fresh oil components. These include tremors, convulsions, degeneration of kidney cells, lipid pneumonia, gastrointestinal hemmorhage, and alteration of plasma cholinesterase activity (Morrow, 1973; Rice, 1973; McEwan et al., 1974; Couch, 1975; Morrow et al., 1975; Eisler and Kissil, 1975; Meyerhoff, 1975; Crocker, 1975, 1976; Rice et al., 1976; Craddock, 1977; DiMichele and Taylor, 1978; Holmes et al., 1979; Thomas et al., 1980,

Martin, 1980; Coon and Dieter, 1981; McEwan et al., 1982). Leighton et al. (1983) found that Heinz-body hemolytic anemia was a primary toxic effect from crude oil ingestion in herring gulls and Atlantic puffins. A similar disturbance could be envisioned in sea turtle red blood cell function with far reaching consequences for survival.

4. Physiological Effects of Petroleum

Knowledge of the physiological effects of petroleum hydrocarbons on marine animals is very limited and focused on invertebrates and fishes (Vernberg and Vernberg, 1974; Neff et al., 1976a; Yarbrough et al., 1976; Thurberg and Gould, 1978). In fishes, studies have been complicated by the fact that locomotion, activity and feeding behavior disruptions are sometimes induced by captive conditions alone (Craddock, 1977). Therefore, it has been difficult to distinguish physiological features that are solely due to oil exposure.

Our literature search for physiological effects of oil on marine mammals, birds, and fishes documented extremely variable reactions to crude oil exposure. These ranged from no visible effects to major disruptions such as eye irritation (Nelson-Smith, 1970; Geraci and Smith, 1975, 1976a,b), respiratory changes (Brocksen and Bailey, 1973; Thomas and Rice, 1975; Gentry and McAllister, 1976), elevated metabolic rate (Hartung, 1967; McEwan et al., 1974; Dieter, 1976; Korn et al., 1976; Wang and Nicol, 1977; Kovaleva, 1979; Patton and Dieter, 1980), bradycardia (Kiceniuk et al., 1978), rapid heat loss (Kooyman et al., 1977; Erasmus et al., 1981; Costa and Kooyman, 1982), and reduced reproduction (Hartung, 1965; Grau et al., 1977; Eastin and Hoffman, 1978; Vangilder and Peterie, 1980; Ainley et al., 1981; Erasmus et al., 1981; Harvey et al., 1981; Peakall et al., 1982).

In Tursiops exposure to oil produced depression of phospholipid synthesis in epidermal cells and transient changes in creatine kinase (Geraci and St. Aubin, 1982). Trace elements and lipids were lost in baleen fouled with various oils, although impairment of baleen function was only temporary (Geraci and St. Aubin, 1982).

Sea birds fed oral doses of crude oil had altered blood parameters including ionic composition and cortisol levels (Holmes, 1972). Oral doses of crude oil fed to test animals have been very wide, ranging from 0.2mg to 5.0mg/kg. In both birds and mammals PHC levels in tissue fall rapidly following exposure. Most investigators conclude that PHC are either rapidly metabolized by mixed-function oxidase systems or are excreted in urine and bile (Zobova et al., 1976; Engelhardt et al., 1977; Varanasi and Malins, 1977; Sakaguchi and Hamaguchi, 1979; Peakall et al., 1980).

Adrenal steroid hormone and electrolyte imbalances are well-documented in oil exposure studies of sea birds (Holmes et al., 1979; Gorsline, 1983). These findings are important to this study as they are directly applicable to sea turtles. In sea turtles excess salts are lost across salt glands through the functioning of comparable systems (Schmidt-Nielsen and Fange, 1958; Holmes and McBean, 1964; Holmes, 1972; Staub, 1975). Disruption of salt-pumping enzyme systems such as Na^+-K^+ - ATPase under adrenal steroid control could also result in dehydration of sea turtles. As their body fluids are much more dilute than that of the surrounding sea water, any failure of the salt glands would result in a rise in internal ions, which in turn would upset many physiological processes, with likely fatal results.

A wide variability of response is shown by the findings that, in some studies, sea birds show no obvious physiological damage after ingestion of as much as one liter of crude oil (Gorsline and Holmes, 1981, 1982). In

Tursiops, chronic ingestion of some oils resulted in no clinically detectable effects (Geraci and St. Aubin, 1982). In other studies, however, oiled birds that showed no obvious gross symptoms of physiological damage were found to be less resistant to cold and osmoregulatory stress (Hartung, 1967; Holmes et al., 1979). The authors concluded that ultimately, elevated metabolic rates would result in rapid starvation and mortality. In addition, treatments with crude oil is believed to impair growth in birds due to reduced nutrient transport in the intestine (Crocker, 1975, 1976; Miller et al., 1978).

5. Effects on Resistance to Disease and Histopathology

Although many studies have been conducted to determine specific toxicity of crude oil on aquatic animals, almost nothing is known about oil effects on resistance to disease and general health (see review by Hodgins et al., 1977; Sindermann, 1982). Recently, published accounts of aquatic animals from chronically polluted areas describe a high incidence of tumors and hyperplastic disease in fishes and invertebrates relative to frequencies in less polluted areas (Kimura et al., 1967; Brown et al., 1973; Mearns and Sherwood, 1976; Minchew and Yarbrough, 1977; Tomita et al., 1978; Rau et al., 1981; Stott et al., 1981). The etiology of cellular and morphological changes of the integument is unknown. No direct evidence exists which relates environmental pollutants to occurrence of skin tumors or epidermal pappillomas.

It appears that PHC exposure and other toxic substances may damage disease resistance barriers in systems such as integument and blood (Papermaster et al., 1964; Hodgins et al., 1977; Payne et al., 1978). In fishes exposed to an oil slick morphological changes in gill epidermal and mucous cells occurred (Blanton and Robinson, 1973). In fishes it is likely

that the primary site of uptake of toxic components occurs via the gills to the blood (Hodgins et al., 1977).

A key concern regarding the effects of PHC exposure is its potential for suppressing an organism's immune response and resistance to disease (Papermaster et al., 1964; Sniezko, 1974). Temperature changes and other stresses including confinement and starvation are known to suppress immunity to bacterial and other infectious agents (Hodgins et al., 1977) in aquatic vertebrates.

In our own studies we have observed a higher frequency of infectious diseases and epidermal ulcerative lesions in sea turtles exposed to extremes in environmental temperature. Bacteria and fungi responsible for these syndromes are typically present in sea water (e.g. Citrobacter). A reduction in cellular immunity could potentially result from chronic exposure to PHC and increase the incidence of infectious disease in sea turtles. We and others have observed epidermal fibrillomas in green sea turtles found in coastal Florida water. These turtles were anemic with hematocrits as much as 75% lower than unaffected turtles. In some cases the location and size of lesions covered eyes and limbs and disturbed swimming movements.

It is clear from published accounts that chemical and physical agents including petroleum products and toxic substances may inhibit immune response, alter functioning of metabolic organs such as liver, blood and kidneys and affect an animal's overall vitality (Thomas et al., 1980). No direct evidence exists for petroleum induced increase in susceptivity to disease or secondary stress in aquatic animals. However, it is likely that some exposure level would have considerable impact on fitness and survival in the sea turtle.

6. Uptake and Metabolism of Petroleum Compounds

The uptake and release of petroleum hydrocarbons by marine vertebrates has recently been studied using labelled alkanes and aromatic hydrocarbons (Diamond and Clark, 1970; Ahearn et al., 1971; Lee et al., 1972; Anderson et al., 1974; Lee et al., 1976; Roubal et al., 1977; Ball et al., 1979; Foureman, 1982). Substantial differences occur in metabolism, retention and depuration of oil among animals studied (Neff et al., 1976b; Philpot et al., 1976). Following both experimental (Gruger et al., 1976; 1977; Dixit and Anderson, 1977; Lawler et al., 1978; McKeown, 1982; Armant et al., 1980) and field exposures (Pohl et al., 1974, 1977; Nulton and Johnson, 1981; Spies et al., 1982; Falandysz and Szefer, 1982) labelled hydrocarbons or their metabolites are usually found in the liver, bile gallbladder, and excreta of challenged animals. In contrast, blood, plasma, muscle and lung tissue show very little concentration (Gaurino et al., 1972; Lee et al., 1972; Sabo et al., 1975; Roubal et al., 1977; Miller and Connell, 1980; Nava, 1980; Nava and Engelhardt, 1980).

Metabolism of PHC occurs through the action of mixed-function oxidases (MFO) or mono-oxidase enzyme systems located in microsomal fractions of lipogenic organs (e.g. liver) (Omura and Sato, 1964; Gelboin, 1969; James et al., 1974; Jerina and Daly, 1974; Payne, 1977; Payne and Fancey, 1982; Stegeman, 1978, 1980; Walton et al., 1978). These enzymes have been isolated in all vertebrates studied so far (see review by Lech et al., 1982; also Dewalde, 1970; Bend et al., 1976; Gruger et al., 1977; James and Bend, 1980; Lech and Bend, 1980; Malins et al., 1981; Law, 1982) it is likely that sea turtles possess MFO systems.

Mixed function oxidases allow for the uptake of foreign hydrocarbons

into body tissue. The oxidized products of these systems are more polar than the lipid-soluble petroleum substrates. Subsequently, metabolized products can be discharged from body tissues by diffusion across membranes, via serum components, and in excretion products (Wood et al., 1976; Pohl et al., 1977). However, it is important to note that some enzymes are substrate-specific and their presence alone does not guarantee that PHC can be metabolized to more soluble products. Alternatively, it is uncertain whether MFO enzyme transformations are responsible for cytotoxicity observed in animals exposed to PHC (Jerina and Daly, 1974; Kluwe and Hook, 1978; Law, 1982).

MFO activities may be related to metabolic rate as marine invertebrates and fish MFO activities studied so far are approximately one tenth of those measured in mammals. As yet there is no information regarding MFO enzyme systems in sea turtles or for most reptiles in general. Provided that sea turtles can metabolize PHC, the question remains as to how the resulting metabolites affect physiological processes as these can be highly toxic themselves.

It is clear from previous discussion that in aquatic and terrestrial vertebrates, petroleum products have been implicated in disruption of lipid metabolism, storage, and excretion as well as glucose metabolism. Alteration of hormonal levels and ATP-ase function may also occur (Rattner and Eastin, 1981; Peakall et al., 1981). Difficulties arise in attempting to predict in advance what significant alterations in key biochemical processes would occur following oil exposure in sea turtles.

If sea turtles are incapable of metabolizing PHC, the potential for bioaccumulation over a long life span exists. In this case, chronic exposure to low levels of PHC in the environment or from biomagnification via the food chain deserve consideration. Loggerhead turtles, for example, are

opportunistic feeders of benthic organisms such as crustaceans and molluscs as well as pelagic prey such as jellyfish (Hendrickson, 1980; Hughes, 1974). Some of these organisms demonstrate the largest enrichment of petroleum components following pollution exposure (Blackman and Mackie, 1974; Neff and Anderson, 1981) and could easily pose a source of oil contamination to turtles via accumulation in the food chain.

Whether sea turtles would consume petroleum contaminated food or sea grass remains to be determined, and as yet little information is available concerning biomagnification of oil components in marine vertebrates. It would be helpful to consider the extent to which invertebrate prey of sea turtles (e.g. crustaceans, jellyfish) accumulate petroleum hydrocarbons (Lee et al., 1972; Neff and Anderson, 1973; Petrocelli et al., 1975; Lee, 1975; Fong, 1976; Thurberg and Gould, 1978; Burns, 1976). This information would be useful in estimating a reasonable natural petroleum body load a sea turtle is capable of accumulating over time in a polluted environment.

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APPENDIX B

REVISED LOGISTICS AND EXPERIMENTAL PLAN

LOGISTICS AND EXPERIMENTAL PLAN

Submitted in Partial
Fulfillment of

MMS Contract #14-12-001-30063
entitled

"STUDY OF THE EFFECTS OF OIL ON MARINE TURTLES"

(Revised - September 10, 1984)

Florida Institute of Oceanography
830 First Street South
St. Petersburg, Florida 33712

PARTICIPANTS

PRINCIPAL INVESTIGATORS:

Dr. Peter L. Lutz
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Dr. Daniel K. Odell
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Dr. Llewellyn M. Ehrhart
Department of Biological Science
University of Central Florida

Dr. Edward S. Van Vleet
Department of Marine Science
University of South Florida

Mr. Ross Witham
Florida Department of Natural Resources

Dr. Gregory D. Bossart
Wildlife Veterinary Center

MANAGEMENT and FACILITIES:

Dr. Sandra L. Vargo
Florida Institute of Oceanography

Dr. Peter L. Lutz
Rosenstiel School of Marine and Atmospheric Science
University of Miami

Mr. James S. Kepley
Sea World Shark Institute

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1.0 INTRODUCTION

The Experimental and Logistics Plan submitted in December, 1983 was based on the research plan described in the Revised Technical Proposal submitted August 26, 1983. Sections were added to deal with such considerations as sources of crude oil, preliminary dosing procedures, licenses, acquisition of animals, and animal maintenance. The Literature Review and discussions between the Principal Investigators had confirmed early hypotheses and had not resulted in any alteration of the proposed experiments. Therefore, this plan reiterated previously submitted documents and expanded them in the stated areas. Based on preliminary experimental results, some revisions to this Logistics and Experimental Plan submitted in December, 1983, were desirable. These revisions in procedure are included in the following sections.

2.0 OVERALL SCIENTIFIC PLAN

The overall experimental scientific plan is designed to meet the following objectives:

1. To test the selected animals under as realistic conditions as possible to increase the validity of using experimental data to make predictions about turtles in the wild;

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

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

The experiments proposed are designed to meet the guidelines and limitations stated in the RFP, Section B, Item 3A. Experiments are designed to be sublethal in nature and to determine the dosage dependency of effects both for concentration and time of exposure. Experimental animals will be healthy and the minimum number of animals will be used that will still yield statistically valid results. Stranded animals are not considered suitable

for exposure experiments as they may already be stressed by unknown factors. Clinical examination of stranded animals, however, will be conducted to aid in the interpretation of experimental results. The experimental conditions will be as realistic as possible. The oil standardization experiments will be considered in designing the actual exposure systems. Exposure levels for behavioral and physiological studies will be selected to facilitate comparison of results. Any proposed changes in experimental protocol by an individual investigator will be communicated to the other Principal Investigators for comment and discussion.

3.0 ANALYTICAL STUDIES

3.1 Oil Standardization Studies (Item 3AB)

a. Background

To conduct the proposed oil exposure studies, it is necessary to know the exact characteristics and properties of the dosing agent. For repetitive or long term studies, this means preparing a standard oil or oil mixture for use. Two types of oil are envisioned for the proposed studies: crude oil and pelagic tarballs.

Crude oil studies will be conducted using South Louisiana Crude Oil (SLCO) as the basic dosing agent. The Organic Geochemistry laboratory at the Department of Marine Science, University of South Florida, has been using South Louisiana Crude Oil in previous studies for the past three years (Figure 3-1, Table 3-1). SLCO will be used directly to generate slicks for specific behavioral response studies. Weathered oil is more typical of the forms of oil the animals will encounter in the environment (than fresh SLCO). If, after preliminary weathering experiments, it proves to be feasible logistically, preweathered crude oil will be used for behavioral and exposure studies.

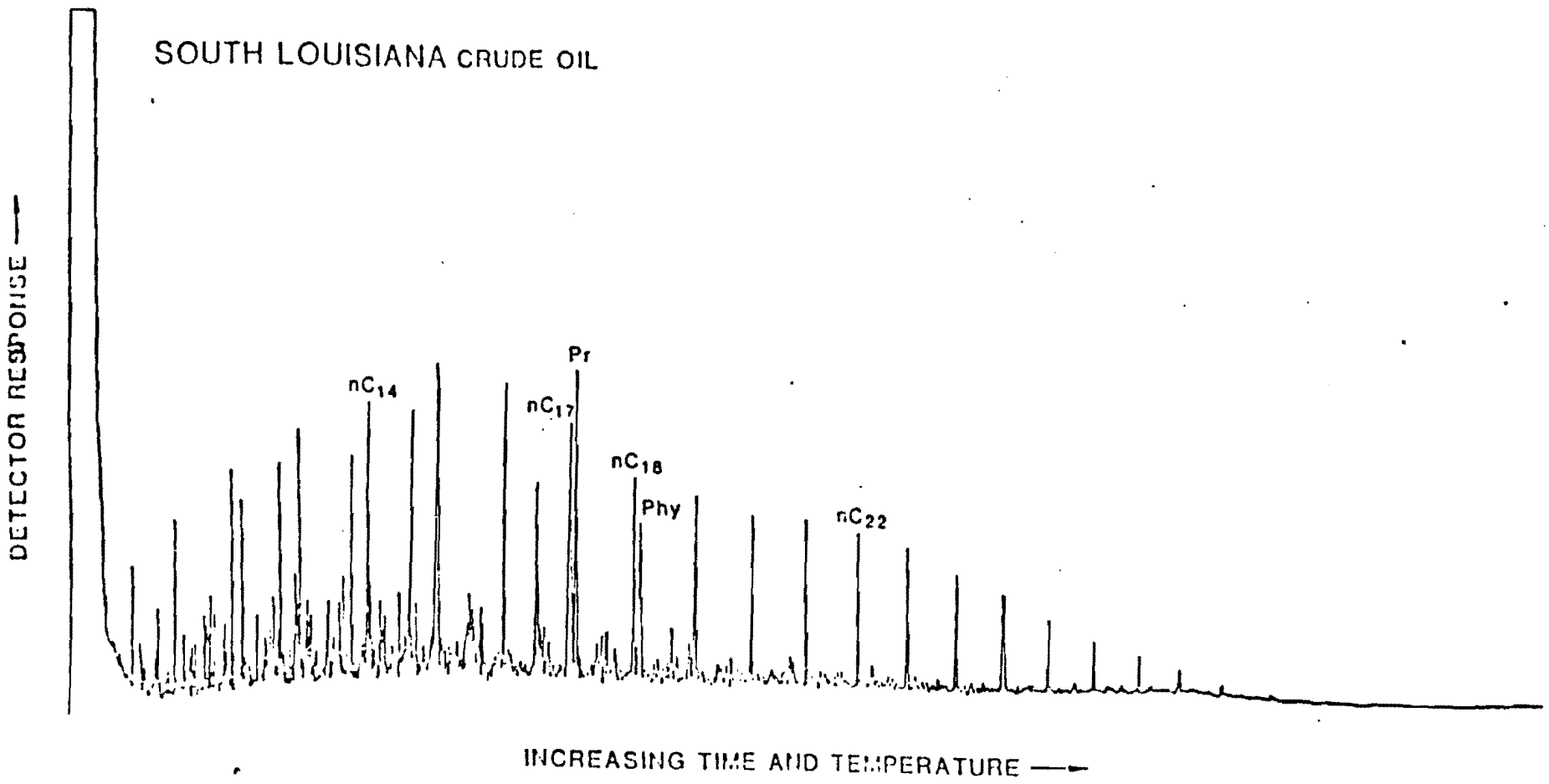

Figure 3-1. High resolution gas chromatogram of South Louisiana Crude Oil as measured during previous hydrocarbon studies at the University of South Florida.

Table 3-1. Isotopic and chromatographic data for South Louisiana Crude Oil as determined in previous hydrocarbon studies at the University of South Florida

PARAMETER	MEASURED VALUE
$\delta^{13}C_{PDB}$	
total oil	-27.0°/₀₀
aliphatic (f_1)	-27.5°/₀₀
aromatic (f_2)	-26.2°/₀₀
aliphatics/aromatics	2.90
nC_{17}/pristane	0.65
nC_{18}/phytane	1.08
pristane/phytane	2.10
n-alkane range	nC_{13}-nC_{31}
aromatic range	2R-5R

The solubility behavior of hydrocarbons in seawater has been examined in several studies (McAuliffe, 1968; Boehm and Quinn, 1973, 1974; Sutton and Calder, 1975; Eganhouse and Calder, 1976). Boehm and Quinn (1973) and Gordon et al. (1973) suggest that hydrocarbons in seawater primarily form colloidal micelles and are not truly dissolved in the water. What normally is referred to as the "water soluble fraction" (WSF) of crude oils is more accurately defined as the "water accommodated fraction" (WAF). Gordon et al. (1973) found that the concentration of water accommodated oil in seawater is related directly to the amount of oil added and the degree of turbulence, but inversely related to temperature. Consequently, in preparing a standardized dosing procedure for South Louisiana Crude oil, each of these factors must be considered.

Behavioral responses also will be determined as a function of tarball occurrence. Since most of the tar in the ocean is not freshly created, generating fresh tarballs from SLCO does not seem appropriate, and generating "standardized" or "representative" tarballs from SLCO is virtually impossible. For the past three years, the Florida Institute of Oceanography and University of South Florida Marine Science Department have been involved in tarball research in the eastern Gulf of Mexico (Van Vleet and Sackett, 1981; Van Vleet et al., 1983a, b, c). The results of this study have indicated the spatial and temporal variation of pelagic tar over a two year period (Figure 3-2). As a result, we have an extremely comprehensive data base (>400 net collections) on the concentrations and distribution of pelagic tar in the eastern Gulf of Mexico. We suggest, therefore, that the most appropriate way to determine behavioral responses to tarballs is to actually collect tar from the Gulf or beaches, analyze a portion, and use the remainder as a dosing agent. The past experience of the principal

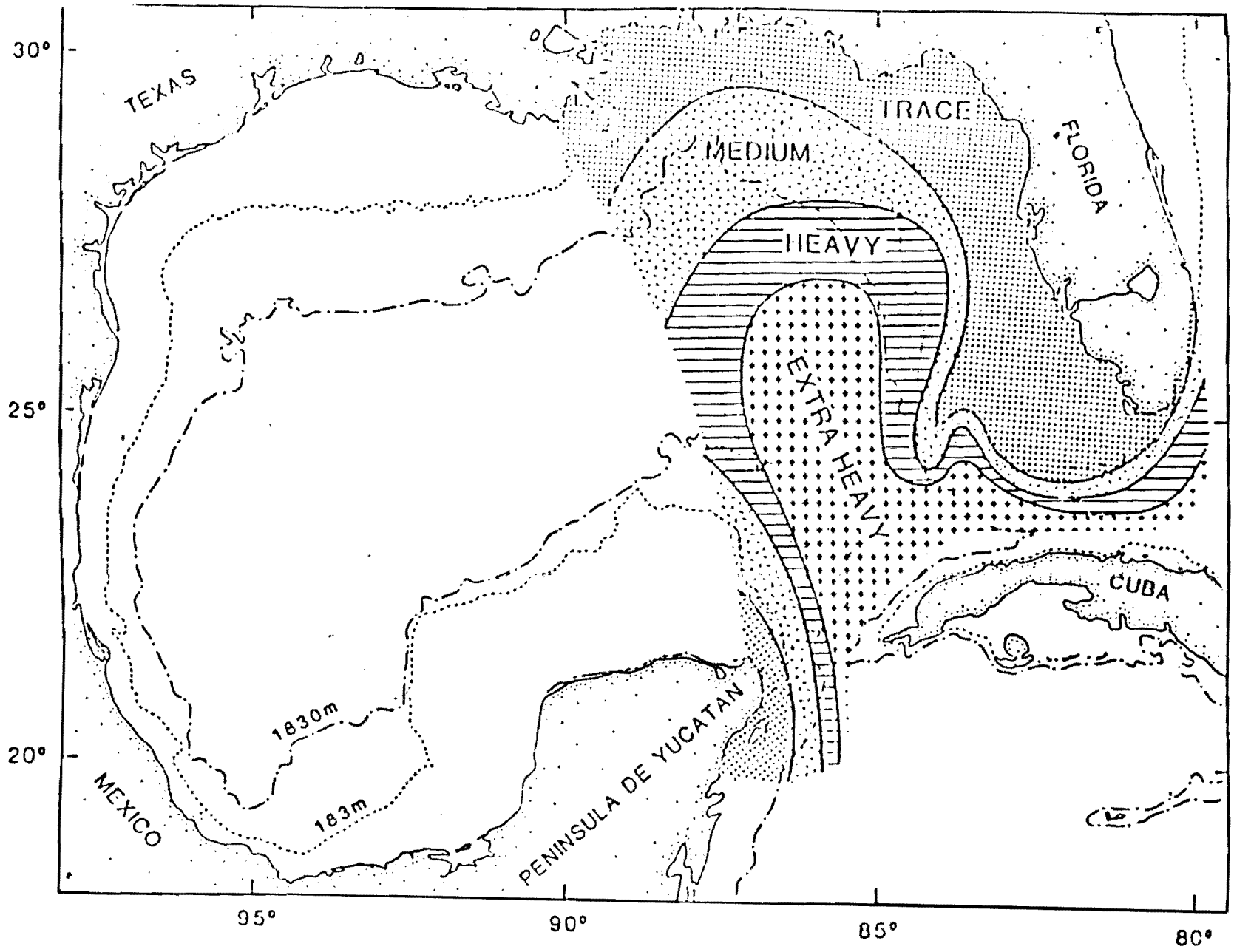

Figure 3-2. Average distribution of pelagic tar in the eastern Gulf of Mexico as determined during the two year study of Van Vleet et al. (1983a, b, c).

investigators in collecting and analyzing pelagic tar in the Gulf would make the proposed alternative straightforward.

b. Methods

Analytical procedures to be used in hydrocarbon analyses have previously been reported in detail (Van Vleet and Reinhardt, 1983; Van Vleet et al., 1983a-d). The general procedures are outlined below.

Extraction and Isolation

Internal standards (androstane and o-terphenyl) are added to the tissues, tarballs, or SLCO used in the laboratory experiments prior to analysis for later quantification of the hydrocarbon fractions. Water samples will be extracted with hexane. Tar and tissue samples will be 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 phase will be transferred to a separatory funnel and partitioned with H_2O. The aqueous-methanol phase will be extracted twice more with hexane and the organic phases combined and evaporated to near dryness. The extract will be diluted to 1 ml with hexane and applied to a 10 cm x 1 cm i.d. glass column containing activated alumina over activated silica get to separate the aliphatic and aromatic hydrocarbon fractions. The aliphatic (f_1) hydrocarbons are eluted with two bed volumes of hexane; after which the aromatic (f_2) hydrocarbons are eluted with two bed volumes of toluene.

Molecular Determinations

The molecular nature of the hydrocarbons will be determined for each fraction by high resolution glass capillary gas-liquid chromatography (GLC) and combined gas chromatography-mass spectrometry (GCMS). Hydrocarbon extracts are evaporated to near dryness and approximately 1 µl of each

fraction is injected into a Hewlett-Packard Model 5880A gas chromatograph equipped with a 30m x 0.2mm i.d. DB-5 fused silica capillary column and flame ionization detector. Column oven temperature is programmed from 90-255°C at 4°C $min.^{-1}$. Chromatograms are recorded and integrated using a Hewlett-Packard Level 4 data data processor equipped with BASIC programming capability. Hydrocarbon fractions will be quantified using the internal standards (f_1-i.s.=androstane, f_2-i.s.=o-terphenyl).

According to Giger and Schaffner (1978), only combined gas chromatography-mass spectrometry (GCMS) can identify each of the aromatic compounds in an oil sample. This is due to the thousands of homologs and isomers that are present. Each aromatic (f_2) fraction collected in this study will be analyzed by GCMS on a Hewlett-Packard Model 5992B computerized GCMS system equipped with a 30m DB-5 fused silica capillary column. Selected aliphatic (f_1) hydrocarbon fractions will also be analyzed by GCMS for structural confirmation.

c. Experimental Design

The intent of the laboratory experiments detailed in the following two subsections is to develop dosing procedures which will insure that the turtles in the exposure studies will be exposed to an oil:water mixture as replicable as possible in concentration and composition throughout the exposure studies. Physical factors alter the behavior of oil when mixed with water and the exposure studies will be carried out under differing ambient environmental conditions over the two year period. The aim of the oil standardization task is to determine the exact concentration and character of petroleum hydrocarbons in the water to which the turtles are exposed.

In addition to these long term effects, in the short-term during each individual exposure study (about 5 days), oil concentration will decline at a

rate depending on temperature, and its composition will alter due to various weathering processes. The proposed laboratory studies will insure that these effects are known in advance and can be corrected where possible or factored into the experimental procedures and interpretation of results.

During the actual exposure studies for determining both behavioral and physiological effects, water and oil samples will be analyzed for each individual experiment to confirm that the dosing procedures are indeed yielding the expected results. Adjustments will be made if necessary. Tar balls will also be analyzed for changes during the course of the experiment. As a minimum, samples will be taken at the beginning and end of each experimental run and, based on the expected rate of weathering, at the midpoint in a run. This will allow clear correlation between the effects on the turtles and the actual oil components to which they are exposed. Such a correlation is essential if a credible data set, useful in management decisions, is to be developed. Tissue samples from the exposed animals will also be analyzed (See Section 4.5).

Effects of Weathering

It is extremely difficult to separate the effects of dissolution and evaporation during weathering. Haegh and Rossemyr (1980) examined the combined effects of dissolution and evaporation on the weathering of oil from two oil spills caused by oil well blowouts: IXTOC-I (Southwestern Gulf of Mexico) and EKOFISK (North Sea). Weathering patterns for these two oils were extremely similar even though the environmental conditions were extremely different. Approximately 65-70% of the oils were lost after two weeks due to combined dissolution and evaporation effects (Figure 3-3). In each case, losses were determined by sequentially examining the residual oil found at the surface. The combined evaporation plus dissolution effects of the SLCO

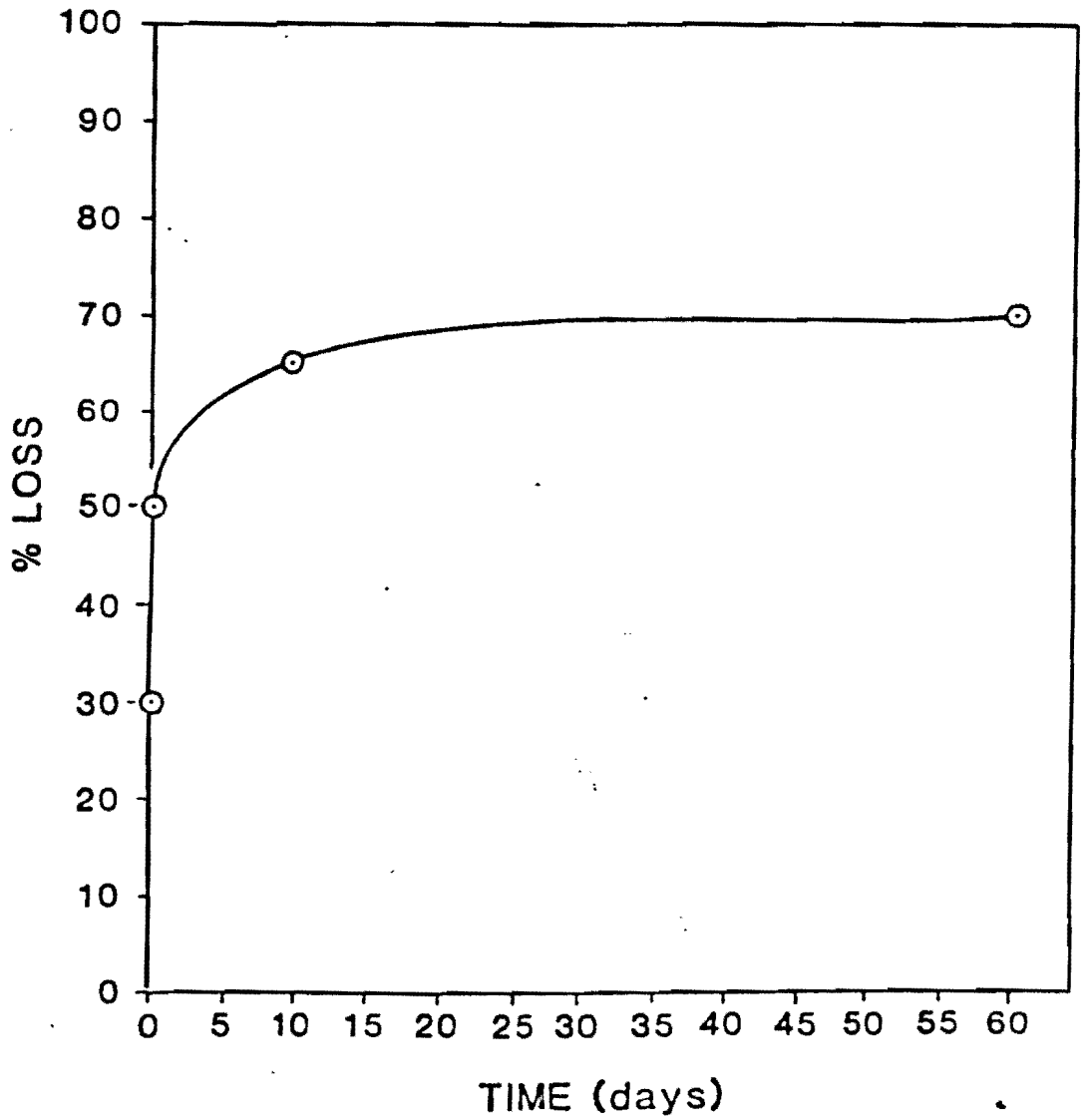

Figure 3-3. Percent loss of hydrocarbons due to evaporation and dissolution as determined by Haegh and Rossemyr (1980) for the IXTOC-I oil well blowout.

used in the present study will be determined by examining the residual oil in each of the previously discussed dosing experiments. Comparison with SLCO analyzed prior to the experiments will reveal these effects. Incorporating the results of all of the above experiments will allow a comparison of the concentration of water accommodated hydrocarbons used in the experiments with concentrations expected for the Gulf of Mexico.

d. Quality Control

Selected tissue, tar, and/or crude oil samples will be sent to independent laboratories for intercalibration of GC and GCMS procedures. GC intercalibrations will be carried out between the University of South Florida, University of Miami, and Mote Marine Laboratory (Sarasota, Florida). GC and GCMS intercalibrations will also be conducted with an independent commercial consulting firm (Interscience, Inc., Tampa, Florida). Previous intercalibration studies between each of the above laboratories have been carried out in conjunction with other research projects. Procedures have been standardized and all previous results have been excellent (see Pierce et al., 1982). Approximately 4 to 6 samples will be distributed to each laboratory for intercalibration during the project.

4.0 EXPOSURE STUDIES (Item 3B)

4.1 General Considerations

a. Source of Oil

As individual batches of SLCO will vary it was determined by the Principal Investigators that it would be necessary to obtain all the oil to be used in the exposure studies at the same time. In this way, the animals would be exposed to the same complex of hydrocarbons throughout the period of the experiments. Preliminary estimates based on the number of experiments planned and the volume of oil required for each experiment indicate that we

will need to stockpile four 55 gallon drums of crude oil. Texaco Inc. has donated four 55 gallon drums of SLCO from its Houma, Louisiana field.

During discussions the Principal Investigators suggested that it would be desirable to use weathered oil rather that fresh oil for the dosing agent. Turtles in the natural environment are much more likely to be exposed to weathered crude oil than fresh crude oil. However, pre-weathering the entire volume of oil required (220 gal) for the exposure experiments would be difficult. The oil standardization experiments indicate that approximately 50% of the oil volume will be lost during weathering. The viscosity did not increase to such a degree that spreading a film over water would be difficult. It was agreed that the SLCO for each exposure experiment (about 20 l) would be weathered for two days over sea water. An air stone will be positioned at the oil-water interface to insure mixing of the oil.

b. Licenses

Appropriate new licenses or extension of existing licenses were requested from Florida Department of Natural Resources. These licenses have been received. Previously existing licenses allowed for the obtaining and holding of marine turtles, therefore the experiments were not delayed by this licensing procedure.

c. Acquisition of Turtles

Initially, the loggerhead turtle (Caretta caretta) will be used in experimental studies. Loggerheads are by far the most abundant species in the geographic study area and are not presently in the precarious survival position of the Kemp's ridley. Loggerheads are known to conduct extensive migrations between natal beaches, developmental areas, and nesting grounds (Carr, 1980; Mendonca and Ehrhart, 1981; Lutcavage, 1981) and thus would be subject to the impacts of development of OCS and Gulf oil resources.

Loggerheads are opportunistic feeders of benthic organisms such as crustaceans and molluscs as well as pelagic prey such as jellyfish (Hendrickson, 1980; Hughes, 1974). These organisms demonstrate the largest enrichment of petroleum components (relative to pelagic species) following pollution exposure (Neff and Anderson, 1981) and could easily pose a source of oil contamination to sea turtles via the food chain. In the second year we plan to use some green (Chelonia mydas) and leatherback (Dermochelys coriacea) turtles.

For the initial experiments approximately twenty (20) sea turtles are currently available at Dr. Lutz's laboratory at RSMAS. Additional animals are being obtained from the following sources:

Texas A & M -	8	10 lb loggerheads
	5	2-3 lb loggerheads
	14	1 lb greens

Clearwater Marine Science Center - 15-20 1/4 lb loggerheads

Arrangements have been made with several of the head start programs for hatchling loggerheads and greens for summer 1984. Approximately 90 hatchlings of each species will be obtained and stocks split between the Shark Institute and Clearwater Marine Science Center (CMSC). The turtles at CMSC will be the back-up stocks. This backup stock is critical as very few loggerheads are held by head start programs beyond the hatchling stage. Thus obtaining replacements for losses during the year due to expected "normal" rates of mortality is difficult if not impossible. If necessary, a given size class of subadults (greater than 20 lb) could be safely and dependably collected in tangle nets set in the Crystal River area (L. Ehrhart, personal communication).

d. Maintenance of turtles

Sea turtles will be held in salt water flow-through facilities at both the RSMAS campus and at Sea World's Shark Institute. Turtles will be

kept under low density conditions either in tanks or in the Shark Institute's lagoonal system. Maximal sea water flow rates will be used to insure rapid turnover of tank water and removal of turtle waste products. Overhead greenhouse shade structures provide a protective, cooling cover for both turtles and workers. Tanks will be emptied, scrubbed and dried at frequent intervals to prevent fouling and planktonic blooms. RSMAS has maintained in good health a research stock of greens and loggerheads ranging from 15-35 turtles. The staff is familiar with all husbandry aspects including identification and successful treatment of health problems. These facilities are complete and ready to receive turtles.

e. Diet and Nutrition

While conducting routine necropsies on captive turtles from several facilities it has been observed at RSMAS that a trend of fatty infiltration and pathology of the liver as well as excessive amounts of body fat develops. This may result from an improper diet of overly fatty food such as mackerel. Since one of the goals of this project is to identify modification of tissues such as the liver that are suspected in metabolism of oil, it is especially important that the diet of turtles held for study is comparable to that of wild turtles. A diet consisting of a balance of invertebrate prey items (e.g. crustaceans, molluscs) and commercially supplied turtle chow will more closely approach a natural diet.

4.2 Behavioral Response to Oil (Item 3BA)

a. Background

Concern over the possible effects of oil pollution on sea turtles was reviewed at the workshop held in Mississippi in 1982 (Adams and Keller, 1982; Witham, 1983). Possible avenues of contamination are ingestion of oil (both directly as in the form of tar balls and as hydrocarbon fractions that

have been accumulated by food items), inhalation of volatile components, and adhesion to the skin/shell of the animal. In the latter case the nostrils could become plugged, the eyes and skin damaged, and some components absorbed through the skin into the circulatory system to possibly affect organ systems and their functions. Witham (1983) expressed particular concern over the ingestion of tar balls by young loggerhead, green, and hawksbill sea turtles. There is a question as to whether these animals will bite/attempt to ingest nearly any object of appropriate size or whether they are attracted to the tar balls by olfactory cues. The tar ball clogs the pharynx and the animal probably dies of starvation/suffocation. Examination of stranded turtles (Item 4) will provide additional information in this area.

The second area of concern is whether or not the sea turtles are able to detect (olfactorily) oil slicks and actively approach or avoid them. The implications of active approach are obvious.

Essentially nothing has been done to study the behavioral response of sea turtles to oil. Kleerekoper and Bennett (1976) have examined the effects of water soluble fractions of Louisiana crude on locomotion in aquatic vertebrates, including green sea turtles. They concluded that these oil fractions did indeed affect sea turtle locomotion.

Studies on sea turtle chemoreception have concluded that these animals "smell" underwater by operating their nostrils and pumping water over the olfactory epithelium by raising and lowering the floor of the oral cavity (Manton, Karr, and Ehrenfeld, 1972a). This raises the strong possibility that sea turtles could be attracted to tar balls and oil slicks by chemical cues.

Sea turtle vision out of the water is apparently poor (Ehrenfeld and Koch, 1967) but presumable they could orient towards objects (i.e. tar balls)

from under water. Given their position in the water, visual cues would be expected to be used at short range whereas olfactory cues can be long range.

Studies of sea turtle feeding and activity patterns suggest they follow a diurnal cycle (Mendonca and Ehrhart, 1982; Ogden et al., 1983). From this, one might expect that any action relative to an oil slick would occur during daylight hours. Turtles could be carried into slicks at night by currents before they could take evasive action. It remains that some sea turtles become contaminated with oil but the circumstances under which this occurs are unknown.

The objectives of this study are twofold:

1) to examine the behavioral response of sea turtles to tar balls;
2) to examine the behavioral response of sea turtles to oil slicks.

b. Methods

Experimental Animals

We will use loggerhead sea turtles obtained through the FDNR for all initial experimentation. Other species used will depend on their availability. We hope to use green sea turtles and Ridley sea turtles. Our intention is to conduct these experiments in a manner that avoids turtle mortality due to experimental protocol. Tar ball experiments will utilize 10 control animals and 10 experimental animals. Experiments will be repeated on the same animals at 3 month intervals (Table 4-1). Sex will not be determined due to financial limitations although blood serum samples collected as a part of routine health examination will be stored frozen should funds become available to cover the cost of radioimmunoassays to determine sex. Experiments will only be conducted on those animals considered to be healthy (by G. Bossart).

Due to a delay in obtaining a stock of SLCO, animals available for the first experiment will be about 9 months old. Hatchlings will not be

available until September, 1984. We will thus be able to test animals from age 3 to age 21 months using the same animals for successive tests. The planned schedule of experiments is shown in Table 4-1. Hatchling turtles, "0" age will not be tested because approximately 10% mortality is expected between 0-3 months. It would, therefore, be difficult to separate the long term effects of oil from natural mortality and maintaining a consistent experimental group would not be possible. It is not feasible, due to cost considerations, to maintain and use totally naive turtles for each age group. Turtles older than 21 months may be obtained from oceanaria or collected in the field. We will attempt to use as many different age classes as can be obtained and remain within the program's (fiscal) limits.

Experimental and control animals will be fasted only for the tar ball experiments, and subsequent tar ball Y-tube experiments if they are necessary. Initial tests with controls will use fed animals. If the strike rate is "low" during the 30 minute test, animals will be fasted for 25 hours prior to testing to see if the strike rate increases. The assumption here is that animals strike at objects in an attempt to find food, and that hungry animals would be more likely to be seeking food than would recently fed animals.

Pre-exposure studies will document diurnal turtle behavior patterns in the 2 m experimental pool, one turtle at a time. The initial expectation is that a diurnal activity pattern (determined respiratory rate and swimming) exists with a peak during daylight hours around mid-day. This, then, would be the time turtles most likely would actively interact with oil slicks and/or tar balls. If, however, there is an unexpected activity peak at night, we would propose conducting both slick and tar ball experiments at night (as well as during the day) to determine if the behavioral responses are different.

	Oil Slick	Tar
Year I		
Start program	10/83	10/83
Control	01/84	01/84
3 month	-----	-----
6 month	-----	-----
9 month	05/84	-----
12 month	09/84	09/84
Year II		
Hatching	08/15 - 09/30	
3 month	10/84	10/84
15 month	11/84	11/84
6 month	01/85	01/85
18 month	03/85	03/85
9 month	04/85	04/85
21 month	06/85	06/85

Table 4-1. Time Schedule Sequence for Experimental Animals

The extent to which learned behavior will affect the experimental results cannot be predicted at this time. Contact with human activity (sounds, handling during health exams and while being placed in the test pool) is unavoidable. Ideally, all animals should be hatched and maintained in an isolated situation (remote feeding, etc.) until testing. This is not possible given the (fiscal) limits of the study. Experiments will be evaluated on a case by case basis to determine if learning occurs. If naive turtles of the appropriate age classes can be readily obtained, some experiments (particularly tar balls) could be easily conducted. For example, animals sequentially tested at 9 and 12 months of age could be compared with 12 month old animals that had no previous testing experience. Again, we can not completely predict what will be available.

The sunlight on the test pool will be reduced by shade cloth to prevent excessive heating. Experiments will be conducted between 0900 and 1600 hours local time unless night experiments are indicated. Observations will be made remotely via the video camera system. It will not be possible to remove all acoustic cues. It still remains that turtle behavior can be altered as a result of human activities. It is not possible to prevent this totally, and the circumstances of the experiments must be considered when an evaluation or interpretation of the results is prepared.

It is our intention to use the same animals for sequential tests in both the tar ball and oil slick studies (Table 4-1). Tar ball studies and slick studies will be conducted at 3 month intervals. In the sequence, tar ball studies will be done first when possible. Each animal will be tested once per day on alternate days over a four day period for the tar ball experiments. Then oil slick experiment will be done one week later. Individual animals will be exposed to oil slick once for 30 min over a four

day period. This leaves a period in excess of 2 months for the animals to recover from any direct contact with oil that may occur. If our (fiscal) limitations permit, we will maintain a separate group of animals (loggerheads) for each type of study. A given turtle will remain in contact with the slick for no longer than 30 minutes. It will then be cleaned (as recommended by G. Bossart). A blood sample will be taken after each exposure series. Blood will also be taken from each turtle prior to the quarterly experimental sessions and more likely at monthly or semi-monthly intervals. Clinical parameters of the blood will be evaluated as another part of the program (G. Bossart). If contamination is minor and/or recovery is rapid, additional tests can be performed on each turtle.

In all cases, the control animals will be the first examined at each quarterly testing session. "Normal" behavior will be established before any experimentation is done. Behavior such as time spent swimming, respiratory rate, location in tank etc., will be used to characterize behavior in the 2 m test pool. One might expect that some sort of acclimation will occur with time but we hesitate to predict this at the present time.

If Y-tube studies are required as a result of the oil slick experiments, it will become necessary to conduct tar ball and oil slick studies at 6 month intervals, alternating with the Y-tube studies.

Experimental Pools

The pool for the tar ball and oil slick experiments will be approximately 2 meters in diameter and 30 cm. deep when small turtles (hatchlings and yearlings) are tested. Adults will require a larger pool if time permits testing the larger sizes. We anticipate that each experimental session will be short enough so that we will not require a continual exchange of water. Pool water temperature will be kept as close to the temperature of

the holding pool as is possible. A video camera will be suspended on a balcony above the pool enabling us to observe (and record) the experiments without the presence of an observer at pool side. This removes the potential for additional visual and acoustic cues that could affect behavior. If necessary, an infrared camera and light source will be installed to observe nocturnal behavior.

Since it is totally suitable and meets all requirements, all behavior experiments will be conducted at the Shark Institute. Also, all behavior experiments will utilize preweathered SLCO as standardized by the Principal Investigator in Item 3AB (E. Van Vleet).

c. Experimental Design

Tar Ball Experiments

These experiments will seek to determine if sea turtles of the several age classes used actively approach and attempt to ingest tar balls. The initial hypothesis to be tested will be that the turtles are not cueing on tar balls but will strike at any object of the appropriate size. They will be given a choice between real and false tar balls. Six tar balls and 6 dummies will be wrapped in plastic mesh and suspended at the surface of the pool via monofilament line attached to an overhead circular frame (Figure 4-1). The frame holding the balls (Figure 4-2) will be lowered into the pool at a time when the turtle is submerged after the turtle has been placed in the pool and a stable behavior pattern reached. The amount of time required to reach stability will be determined using the control animals (10 for each age group). In the control only tar ball substitutes will be used. The experiments will be terminated 30 minutes after the balls have been introduced. Longer exposures may be necessary if strike rate is too low to achieve statistical validity. The data base will be the number of

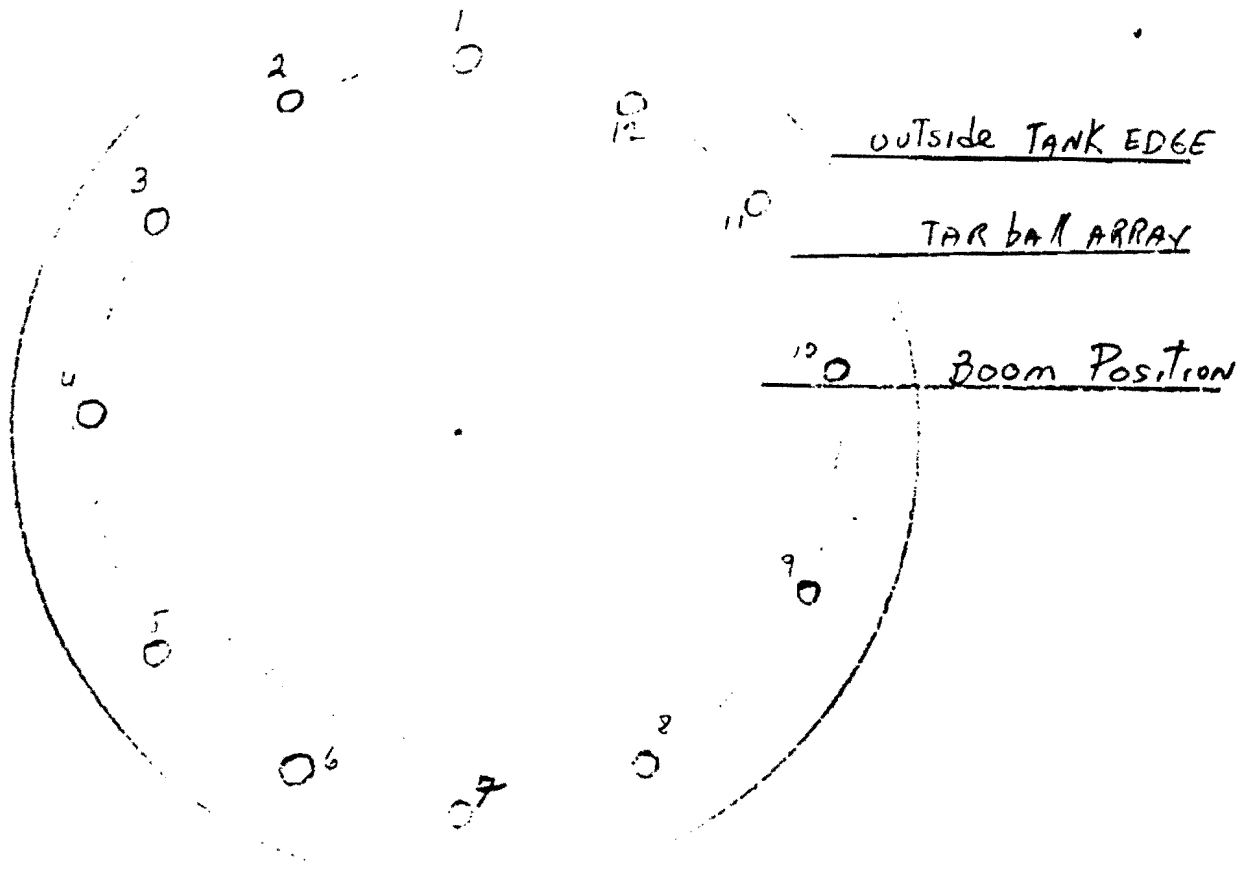

Figure 4-1. Arrangement of tar balls in experimental pool.

Not To Scale; Positions Approximate

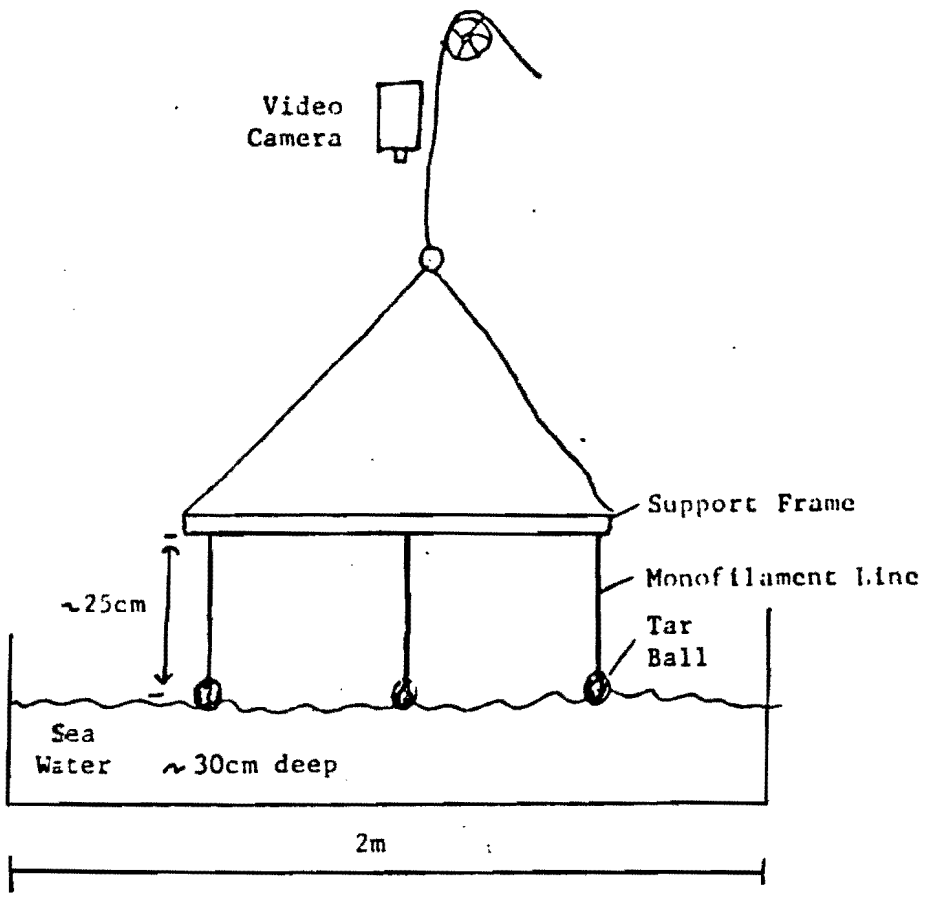

Figure 4-2 Lateral View of Tar Ball Test Apparatus

strikes/unit time/ball. The balls will be evenly spaced over the surface of the pool and will be randomly assigned to the 12 sites for each set of experiment (Figure 4-1). The plastic mesh wrapping presents the same visual stimulus for both real and false tar balls while allowing the soluble fraction of the tar balls to enter the water. The wrapping also protects the turtles from injury from tar ball ingestion. If the turtles are not using any olfactory cues, the initial results should be a statistically identical number of strikes at both types of objects. Each individual turtle will be tested twice once per day on alternate days for a four day period to determine if experience/learning has any effect on the strike pattern. If the strike pattern is non-random (tar balls selected or avoided), additional Y-chamber experiments will be planned to determine if the observed responses are mediated olfactorily. In this experimental situation, a turtle would be placed in a chamber at the base of the Y and be subjected to water flowing from both arms of the Y. One side would be normal water, and the other would contain water that has passed over tar balls only to allow the water soluble components to reach the animal. The animal would approach one of the two arms of the Y depending on whether it was actively approaching or avoiding the tar balls.

Turtles would be tested in both fed and fasted (24 hour) conditions. Depending on the results of these experiments, the same turtles may be used in the oil slick experiment but they will not be fasted.

The random strike hypothesis would be evaluated with the Chi square test for individual animals and the group as a whole. The effect of experience would be evaluated by comparing the strike patterns on successive trials for the same individuals. While more sophisticated statistical methods are available, the Chi-square test is simple and straight forward and will be

used initially. If the results are ambiguous, other tests may be used. These remain unspecified since the test(s) used will depend on the nature of the data base which is dependent on turtle behavior.

Tar ball substitutes will be paraffin or dark wooden beads wrapped in the plastic mesh. Initial ball diameter will be 2 cm. If the initial results are ambiguous, larger balls will be used (0.5 cm. increments) to determine if this factor is real. The experimental protocol would be repeated with 12 balls of the larger size.

The initial assumption is, again, that turtles will strike at any object in their pool (Manton, Karr, and Ehrenfeld, 1972b) and that the response is visually mediated. If there is obvious selection for or avoidance of tar balls, a Y-tube type experiment will be conducted to determine if the response is chemically cued. The planned program will not permit us to distinguish between olfaction and taste because of presently anticipated (financial) limitations.

The Y-tube test apparatus will be a box 1 m. wide and 2 m. long filled with water to a depth of 30 cm. Sea water will flow through the box from two input ports at one end. A 50 cm. partition will separate the two ports. The test animal will be placed in the outflow end of the box with water running from both ports. Input to one port will pass through a chamber containing tar balls (Figure 4-3). Input ports will be selected randomly. Control animals will be tested without the addition of tar ball conditioned water. Water samples will be collected from the outflow of the tar ball chamber and analyzed for hydrocarbon content. A sample will be collected from each test. Test results will be initially evaluated by the Chi-square test. Unspecified additional tests will be used if Chi-square results are inconclusive. If the Y-tube experiments are necessary, the initial hypothesis will be that the

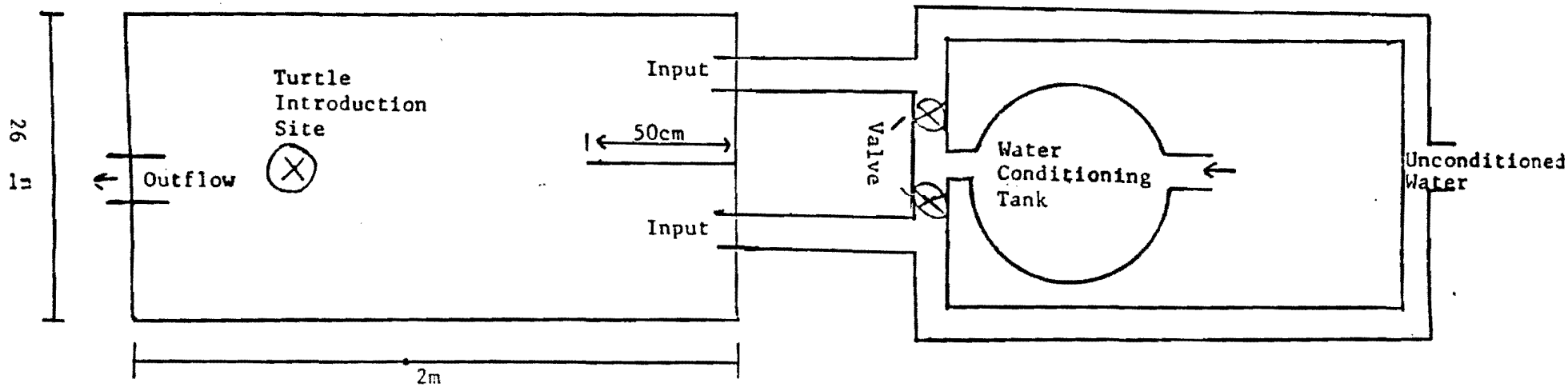

Figure 4-3 Plan View of 'Y-Tube' Test Tank

turtles will select the tar ball conditioned water over unconditioned water. The 10 experimental turtles will be tested in the Y-tube at two week Fig. 2-6 intervals to determine if learning occurs. The length of initial trials will be determined after initial experiments. Tentatively, the experiment will be terminated after 30 minutes if no selection has occurred. Experiments with the control animals will be conducted first to determine how long the animals must be in the chamber before a stable behavior pattern is reached prior to introduction of conditioned water.

Oil Slick Experiments

This set of experiments is designed to determine whether or not sea turtles actively approach or avoid an oil slick. Three groups of 6 turtles will be used for each of the age groups tested. We would hope to be able to use the same turtles as they grow. However, if learning as a result of the initial experiments appears to bias subsequent tests, naive animals of the appropriate age classes will be obtained. (Financial and space limitations preclude holding sufficient numbers of animals in various age classes all at the same time.)

The test pool will be fitted with a circular boom (Figure 4-4) about 1.2 m. in diameter such that the pool is divided into two equal areas of about 2 m. sq. each. The control animals will be in the pool without an oil slick and observed for a standard time period. Animals will be randomly placed either in the center or on the edge of the pool. If the center boom creates a non-random behavioral situation it may be redesigned. Preliminary experiments indicate that the animals swim around the perimeter of the pool. Therefore the oil slick will be placed outside the boom in the annulus. The layer will be 5 mm thick which is a thickness not uncommon under an oil spill. One of the two test groups will be placed (individually) in the

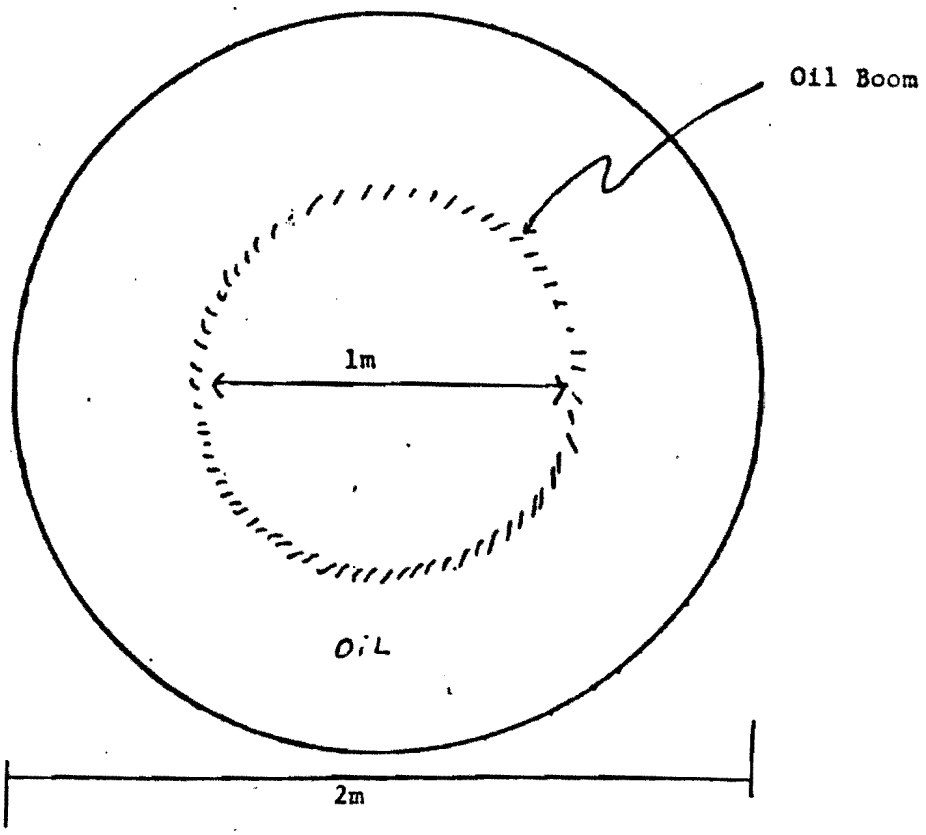

Figure 4-4. Plan View of Oil Slick Test Apparatus
2mm thick oil slick placed in center circle within boom

center with the oil and behavior monitored for a standard time period. The turtle will be removed, cleaned, and allowed to recover before the next test. The third group will be placed in the water along the periphery of the pool. Depending on the initial results, it may be necessary to randomize both the location of the oil and the entry point of the animals. Each animal will be exposed to the oil slick for a total of 30 minutes each 3 month period.

The purpose of the boom is to contain the oil slick, which will be of a uniform thickness of about 5 mm. The boom will be constructed from flexible plastic tubing about 5 cm. in diameter. The boom will be ballasted with water so that approximately 2.5 cm. extends below the surface of the water. If this is not sufficient to contain the slick, additional ballast may be added or a larger diameter tube used.

The initial hypothesis tested will be that the behavior of the turtles will be random with regard to location in the pool and that there will be no difference with regard to point of entry. Chi square tests will be used. As with the tar ball experiments, the results of the initial experiments will suggest additional experiments. If active approach or avoidance appears to occur, Y-tube experiments (as described for tar balls) will be employed to determine if olfactory cues are being used. Learning (approach or avoidance) will be evaluated by examining the behavior of individuals on successive trials. For example, if the turtles placed in the water (rather than in the oil) actively approach and enter the oil, do individuals enter more quickly on successive trials?

In addition to testing the approach/avoidance hypothesis, other aspects of turtle behavior that might be affected by oil will be evaluated. The normal behavior patterns (e.g. respiration, swimming, orientation) of the entire group will be examined on an individual basis before any of the

animals are exposed to oil. The video tape records will enable us to review the pre-test behavior patterns if we encounter unexpected behaviors during the actual tests, thus permitting a better analysis of the results.

In all cases, pre- and post-test water samples will be collected an analyzed for soluble hydrocarbons. Turtle health will be monitored throughout the experiment and the interval between oil exposures adjusted as necessary. Since exposure to oil will be controlled, these animals will be of value to the physiology component of the study.

4.3 Physiological Effects of Exposure to Oil (Item 3BB)

a. Background

Present accounts of oil effects on sea turtles describe and evaluate sources of oiling, yet fail to explain how oil exposure disrupts behavior and physiological functions in these animals. However, it is only by understanding the cause-effect relationship that the full impact of oil exposure on sea turtle populations can be appreciated and realistic mitigating measures formulated.

Studies of toxic substance effects on reptiles (reviewed by Hall, 1980) for the most part, are limited to investigation of pesticide and heavy metal residues. Baseline levels of heavy metals and pesticides in feral sea turtles and their eggs were provided by Hillestad et al. (1974), Schwartz and Flamenbaum (1976), Thompson et al. (1974), Stoneburner et al. (1980), Clark and Krynitsky (1980), and Talbert (1981).

There are very few studies of hydrocarbon effects on sea turtles. Bennet and Kleerekooper examined changes in locomotion caused by oiling (1978). Fritz and McGehee (1981) looked at the effects of oiled sands on incubating Caretta caretta and Lepidochelys kempi eggs. Interestingly, they found that while loggerhead eggs incubated in artificially oiled sands

appeared to suffer reduced survival and altered morphology, L. kempi eggs incubated in oiled sand on their native beach showed no significant differences in embryo survival. These field study results indicate that oil effects on sea turtle eggs may be complex and determined by the interaction of several factors including type of oil, concentration, degree of weathering, and the nest environment itself.

In contrast to the situation for sea turtles, the potential threat of oil pollution has been well recognized with respect to marine mammals (LeBoeuf, 1971; Geraci and Smith, 1976; Kooyman and Costa, 1979; Baker, 1981) and sea birds (Bourne, 1968; Beer, 1970; Clark, 1970; Dieter, 1976; King, 1979; Peakall et al., 1979; 1982; Falandysz and Szefer, 1982) and this awareness has resulted in a considerable amount of research and a solid foundation of knowledge.

Some clues on the possible effects of oil on sea turtles may be obtained from studies of the toxic effects of petroleum fractions on other vertebrates, including marine fishes. These demonstrate wide-ranging physiological alteration caused by acute and chronic exposure to hydrocarbons (e.g. Payne, 1975; Stegeman and Sabo, 1976; Ernst et al., 1977; Solangi and Overstreet, 1982; Weber, 1982). The sensitivity to oil type (crude vs. refined), concentration, degree of weathering, and constituents (aliphatic vs. aromatic) varies tremendously among and within marine vertebrate groups. Several of these factors are likely to affect toxicity of particular oil exposures to sea turtles.

There is evidence that the toxicity of oil is increased by the presence of polycyclic aromatic fractions which are more soluble than alkanes (Neff and Anderson, 1981; Jordon and Payne, 1980). The duration of exposure, life stage, sex, and whether and how the animal metabolizes petroleum also affect

toxicity. In an experimental exposure study of a particular species these factors must be evaluated against potential physiological differences that may occur between sexes and age classes.

Sea turtles are exempt from the obvious damage associated with oiling of structures such as feathers and gills (Solangi and Overstreet, 1982) , and from ingestion of petroleum via preening (Miller et al., 1977). Nonetheless, there are several reasons why sea turtles are exceptionally vulnerable to contamination by petroleum. (1) As air-breathers, sea turtles must come to the surface to breathe, and in an oil spill area would continually come into contact with the oil slick. Surface film oil could be aspirated incidentally via the glottis during ventilation. (2) Unlike marine mammals that actively avoid contaminated areas (as demonstrated during the Santa Barbara spill), there is evidence that in many circumstances sea turtles are transported by passive drift (Carr, 1980). In addition, some species such as the loggerhead appear sometimes to feed on organisms concentrated in surface windrows (Hughes, 1974; Witham, 1980). In both cases turtles might tend to move with any large oil slick. As their behavior appears to be mainly a fixed action pattern, effective avoidance would be minimal and exposure to oil prolonged. (3) Sea turtles, particularly juveniles, have a habit of snapping at, and attempting to swallow any object of an appropriate size. Tarball ingestion has been recorded and is likely to be a serious cause of mortality (Witham, 1978; Frazier, 1980). (4). During the nesting season contaminated beaches could adversely affect reproduction success by deterring egg laying, directly disturbing the developing egg , or changing the composition of the nest sand overlay. In fishes as well as birds, oral ingestion of petroleum hydrocarbons (hereon PHC) are known to disrupt calcium ion transport, as seen in bone deformities of the spinal column (Martin, 1980) and eggshell thinning and disruption of body calcium stores (Miller et al., 1976).

The physiological effects of oil contamination on sea turtles are likely to be widespread and serious. In the pelagic phase for example, the lungs could be particularly vulnerable to volatile or soluble oil fractions. This would be potentially important if turtles occurred in the immediate vicinity of newly released, unweathered crude oil. A severe reduction in their ventilation scope (which, for a reptile is unusually dynamic) caused by pulmonary edema could lead to drowning. However, even minor interference with ventilation could compromise diving capacity, hamper predator avoidance and reduce the time available for feeding and underwater travel.

Both tar balls and soluble products may damage the gastrointestinal tract by disrupting water/ion transport processes. In avian species, lesions, occult blood and enteritis have been observed in the GI tract following ingestion of an oral dose of crude oil. Decreased growth and eventual starvation could result if absorption efficiency were sufficiently reduced in turtles.

Whether sea turtles or reptiles in general accumulate or metabolize PHC has not been demonstrated. In fishes and mammals, mixed function oxidase systems are associated with metabolism of aromatic hydrocarbons localized in the microsomal fractions of the cell (Payne and Penrose, 1975; Malins, 1977; Neff, 1979). Ingestion of petroleum caused hydrocarbon-metabolizing properties of the liver to increase significantly in several animal groups (as reviewed in Lech et al., 1982). The mixed function oxidas system (alternatively, monooxidase system "MO") is utilized in the biotransformation of steroids, drugs, and xenobiotics such as PHC. Increased concentrations of MO enzymes (e.g. cytochrome P-450) have been found as well as altered patterns of lipid metabolism when fish (Stegeman and Sabo, 1976) mammals (Jerina and Daly, 1979) and avian species (Miller et al., 1977; Gorsline and Holmes, 1982) were dosed with petroleum hydrocarbons.

In fishes and sea birds, hydrocarbon residues have been identified in lipophilic tissue such as liver, kidney, gallbladder, and in organs associated with metabolic breakdown (Payne, 1975; Stegeman and Sabo, 1976; Nava, 1980; Whipple et al, 1981). Polycyclic hydrocarbons bind reversibly to lipophilic sites in the cell membrane surface and may disrupt cellular transport functions (Well et al., 1974; Roubal et al., 1977) including osmotic/ionic exchange (Peakall et al., 1977; 1982;) and neurotransmission (DiMichele and Taylor, 1978). In herring gulls and guillemots, ingested crude oil caused disruption of Na^+ K^+ ATPase transport and hypertrophy of nasal salt glands (Peakall et al., 1979; 1982). This was accompanied by a transient increase in plasma sodium. The above factors are indicative of impairment of osmoregulation, which in the sea turtle may be vital for survival.

Elimination of excess Na^+ and K^+ ions in turtles is accomplished via the lacrimal gland in the eye orbit (Schmidt-Nielsen and Fange, 1958; Holmes et al., 1963). This system is likely to be challenged by exposure to oil, since aromatic hydrocarbons have been shown to inhibit enzyme activity and active transport of ions in avian salt glands (Peakall et al., 1979; 1983). As the body fluids of sea turtles are much more dilute than that of the surrounding sea water, any failure of the salt glands would result in a rise in internal ions, which in turn would upset many physiological processes, with likely fatal results.

Significant changes in blood chemistry including elevated serum glucose, protein, and cortisol levels are known to occur in fishes fed petroleum products (Singley and Chavin, 1975; DiMichele and Taylor, 1978). Herring gulls and Atlantic puffins developed severe hemolytic anemia after oral ingestion of Prudhoe Bay crude oil (Leighton et al.,1983). If blood oxygen carrying capacity is reduced by sublethal oil doses, insufficient oxygenation

of red blood cells could lead to tissue hypoxia in active muscle and eventually disrupt aerobic performance. In sea turtles, the consequences of reduced metabolic scope would be far-reaching: growth, migration and reproduction would be compromised.

Based on our literature review, and utilizing what is known about the effects of petroleum on other marine vertebrates, we propose to examine the effects of exposures of sublethal doses of South Louisiana Crude Oil (SLCO) on several basic and critical physiological processes of sea turtles. It is likely that the most immediate effects of oil exposure could be to interfere with swimming, diving, and orientation abilities. These functions can be evaluated in the laboratory by measuring basic metabolic parameters.

b. Methods

Metabolic performance

This study will include measurement of respiration rate, dive length, oxygen consumption, CO_2 production, heart rate, and maximum aerobic and anaerobic scope for activity. Concurrent sampling of blood parameters, including pO_2, pCO_2, pH, lactate, hemoglobin concentration and hematocrit along with plasma constituents as outlined in the following section will allow detection and interpretation of the animal's level of stress.

Recently it has been observed that CO_2 may be lost across the skin of loggerheads subject to stress (Bentley and Lutz, 1983). If this type of nonpulmonary gas exchange functions to maintain a balance of the CO_2/bicarbonate buffer system, the presence of oil on the turtle's integument may compromise this phenomena. Integumentary gas exchange will be examined in oiled as well as unoiled subadult loggerhead sea turtles.

We have measured metabolic parameters in loggerhead and greens using both restrained (Bentley, 1981) and unrestrained turtles (Lutcavage and Lutz,

1983). Confinement and restriction alone may produce striking alterations in ventilation, heart rate and diving patterns. Therefore we will utilize a system that allows remote collection of ventilatory data in unrestrained, free-swimming turtles breathing voluntarily into a funnel fitted with a pneumotach (Figure 4-5). Heart rate may be obtained with our heart rate transmitter or via insulated EKG leads exteriorized through the tank top.

Osmoregulatory functioning

This will involve measurement of blood electrolyte concentration (Na^+, K^+, Mg^+, Cl^-, Ca^+), blood osmotic pressure, functioning of salt gland and Na^+ K^+ - ATPase system. The physiology laboratory at ISMAS previously has measured seasonal blood ionic parameters, lactate, and glucose in loggerheads trawled from Cape Canaveral ship channel (Dunbar-Cooper and Lutz, 1983) which will serve as baseline data for comparison with experimental results.

Digestion and absorption

Qualitative and quantitative analysis of fat, carbohydrate and protein absorption will be determined via analysis outlined in the clinicopathology section. Gut passage time and fermentive bacterial density will be compared for treated vs. controls. Weights of all turtles will be recorded daily as an indication of metabolism.

Liver function

Plasma GOT/GPT will be analyzed. These are useful tests for evaluation of lesions of hepatic parenchymal tissue.

Mixed Oxidose/Mono oxiduse (MO) System

Once basic behavioral and physiological responses to SLCO exposure are thoroughly documented, the uptake and metabolic fate of THC could be studied. We propose to identify those systems in sea turtles that are indicative of MO bioaccumulation and metabolism. These studies have been conducted in

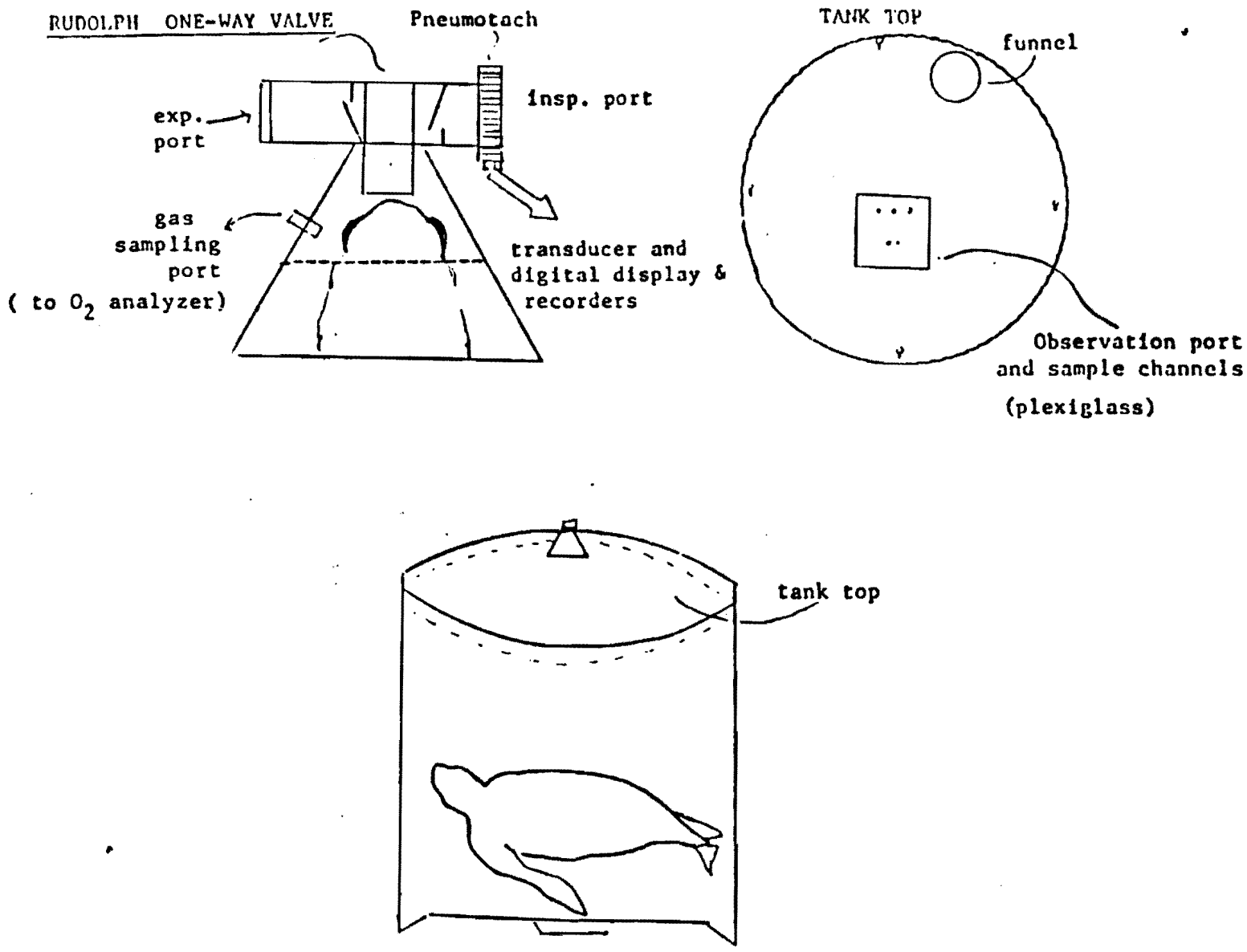

Figure 4-5 Respiration Measurement and Gas Sampling System

mammals, birds, fishes and invertebrates but to our knowledge the effects on the MO system have yet to be identified in reptiles.

Induction of mixed oxidase/monooxidase systems by PHC is known to occur in many animal groups (Lech et al., 1982). Aromatic hydrocarbon hydroxylase (AHH) is induced by crude oil in microsomal liver fractions of these animals. Both from an ecological and comparative physiological viewpoint, it would be of considerable importance to demonstrate whether SLCO induces monooxidase systems in sea turtles.

This study would be initiated on stranded, oiled turtles to identify or isolate the class of MO systems found in turtles. If successful, using a very limited number of experimental animals which could be sacrificed, during Year Two we would determine whether induction of these systems occurs. For this research aspect we would arrange to obtain approximately ten subadult green turtles under 5 kg from the Cayman turtle farm or rear hatching loggerheads specifically for this purpose. These turtles would be fed several levels of dosage of SLCO as described previously. The sex of these turtles will be determined during necropsy. A possible non-lethal approach for this research aspect would be to develop a surgical biopsy technique where appropriate tissues could be extracted from live turtles. The research staff of RSMAS, as well as Dr. Bossart, have considerable experience with both anesthesia and surgical techniques in greens and loggerheads and could potentially develop a biopsy method which would be non-lethal to the animal under study.

These proposed bioaccumulation studies may not meet statistical criteria yet they would be intrinsic to any study that seeks to demonstrate whether and how petroleum hydrocarbons are metabolized in sea turtles. Protocols involved would include first exposure to SLCO then measurement of rates of

MO-catalyzed reactions from liver microsomal fractions. Lethal experiments may prove to be unavoidable; however, these experiments obviously would provide a valuable histopathological and toxicological data base to compare with field-stranded animals. MO extraction and identification techniques according to the method of Omura and Sato (1964) would involve spectrophotometric methods using equipment presently available at our facility. Recent advances in MO research in mammals potentially could yield new techniques to locate and quantify the extent of PHC metabolism in sea turtles.

Information gained in these laboratory studies will be used to formulate scientifically based mitigation procedures and will be crucial for designing turtle rehabilitation programs. These studies also are directly applicable to present conservation programs because the basic physiological functions to be measured determine an animal's overall fitness, and ultimately, its capacity for survival and reproduction.

Data generated in the above studies will be stored and processed on the RSMAS Computer Facility system (RCF). RCF has a DEC PDP-11/44 Computer with 512K3 of memory. This system may be operated independently or as a remote job entry terminal for the University of Miami UNIVAC 1100/81. Support equipment likely to be utilized in our data management include GOULD Electrostatic and CALCOMP plotters, NEC spinwriter, and recent editions of SPSS and SAS statistical analysis software packages.

c. Experimental Design

In sea turtles, the major sources of contamination are likely to be direct contact with oil slicks and ingestion of tar balls, fouled seawater or oiled prey items. Accidental aspiration could occur as the animal breathes at the water's surface. Two major experiments have been designed to determine the effects of these two routes of exposure.

Oil Slick Exposure Experiment

Eight to ten loggerhead turtles (*Caretta caretta*) (10-25 lb) will be used during the physiological effects of oil exposure experiments. This larger size is required to allow sufficient blood volume for all the physiological tests. The control observations will be taken simultaneously with the exposure experiments. Experimental turtles will be those used initially as controls so that each turtle will have a battery of normal physiological data before oil exposure. Additional experimental turtles will also be used. All work will be done at the Rosenstiel School of Marine Science.

Controls

Turtles will be held in cylindrical tanks (65 cm X 75.5 cm) containing approximately 290 l seawater. Turtles will be fasted 24 hrs before initial observations and will continue fasting throughout the observation period.

Oxygen consumption, heart rate, and ventilation rate will be recorded on each turtle for 5 days. Recordings will be taken once daily between 0800 and 1200. Plastic receptacles (modified from syringes) will be secured to cloacae, and excrement will be collected for hydrocarbon analyses. Each turtle will remain in its observation tank throughout the 5 day period.

Three millileters of blood will be drawn daily between 0800 and 1200 from the cervical sinus of each control turtle. Hematocrit, hemoglobin concentration, differential diagnosis, and blood gas analyses will be performed on each whole blood sample. Plasma will be collected from remaining whole blood samples and analyzed immediately for glucose concentrations. Each remaining plasma sample will be divided into two subsamples and frozen. One subsample from each turtle will be assayed for chloride, sodium, and potassium concentrations. One frozen subsample will be

divided and delivered to Dr. Greg Bossart for additional biochemical analyses (serum proteins, metabollic enzymes, hormones). Osmolarity measurements will be taken daily on salt gland secretions.

At the end of the 5 day observation period all turtles will be returned to normal holding facilities and fed. They will be fed normally for 48 hrs prior to oil exposure experiments. This rest period should allow turtles to return to their normal prefasted, prebled physiological statuses.

Exposure

Crude oil will be weathered for 48 hrs prior to experimental exposure. After the turtle is placed in the tank, weathered oil will be poured on top of the tank water to a depth of 5 mm (approximately 715 ml oil for tank diameter of 75.5 cm).

The same turtles used for control baselines will be subjected to oiling. Oxygen consumption, heart rate, and ventilation rate will be recorded as before, as will blood analyses, salt gland secretion osmolarity, and excrement analyses. Turtles will be kept in oiled water for 5 days. If a turtle becomes obviously distressed such that data collection is impaired it will be removed to clean seawater for recovery.

Recovery Phase:

After oil exposure and pending the occurrence of significant physiological changes, turtles will be monitored as before to determine recovery period. Data will be collected on day 11 and 22 after exposure, and at weekly intervals thereafter until normal values are recovered. If after 6 months significant recovery has not occurred data collection will be terminated, and the animal will be considered detrimentally compromised by oil exposure.

Oil Ingestion Experiment

Ingestion studies will be conducted independently of oil slick exposure experiment. We will use SLCO characterized and provided by Dr. Van Vleet. This work will begin during the first quarter of Year Two. With direct ingestion studies, it is reasonable to assume that sublethal effects on sea turtles would parallel those obtained in sea birds. Ecologically and physiologically, some sea turtles are more closely allied with sea birds than other poikilotherms such as fish in that their physiological systems show evidence of endothermy (e.g. bone growth, body heat regulation, metabolic scope). For that reason we believe that design of experimental protocols and SLCO exposure trials should parallel those of sea birds rather than fishes. For example, nonlethal single crude oil doses given to fish may be an order of magnitude greater than effective doses given to sea birds (range 0.1-10 ml/kg). Range of dosage used in our protocols will be adjusted based on the amount a turtle might ingest in the wild and survive as well as loads known to produce sublethal effects in sea birds. At least six subadult loggerhead turtles will be fed a standard dose of SLCO via capsule embedded in squid pieces. Controls will receive a capsule in squid containing an equal amount of gelatin.

4.4 Clinicopathological Effects

a. Background

The objective is to determine selected clinicopathologic parameters in sea turtles involved in the experimental population as well as stranded oil-fouled animals.

The parameters to be measured include selected blood chemistry components to indicate potential specific effects on organ systems:

1. Histopathology, particularly of the renal and gastrointestinal system

2. Blood hemograms to evaluate red blood cell indices, white cell populations and differential counts;
3. Aerobic bacteriologic pharyngeal and fecal cultures to assess any potential pathologic change in bacterial flora between control and experimental/stranded animals. In addition, fecal specimens will be assessed for the presence of occult blood.

The overall goal, therefore, is to determine 'normal' physiological parameters of sea turtles and any changes which may occur due to association with oil. The experimental animal groups utilized for this portion will be utilized concurrently for the other portions of the proposal.

b. Methods

Blood for analytical purposes will be collected aseptically from either the dorsal cervical sinuses, jugular vein, or via cardiac puncture. Since micro methods for analysis will be utilized, only small quantities of blood (less than 3cc) will be required.

Hemograms will be determined from EDTA anticoagulated blood and include red blood cell indices and white blood cell total counts and differentials. The red blood cell indices to be measured include packed cell volume, plasma total solids, hemoglobin, and reticulocyte count.

The following compounds will be measured spectrophotometrically by micromethods with indicator organs in parentheses: serum lipase (pancreatic injury), serum glutamic oxalacetic transaminase and alanine aminotransferase (liver injury), and urea nitrogen and uric acid (kidney injury). Electrolytes including sodium, potassium, and chloride will be determined via flame photometer or by atomic absorption spectra. In addition, glucose, albumin and alkaline phosphatase will be determined spectrophotometerically. Serum electrophoresis will also be run on random individuals in experimental groups to determine alpha, beta, and gamma globulin fraction composition. This will provide data on nutritional and immune status.

Microbiologic studies will be performed utilizing commercially prepared culturettes with transport media. Pharyngeal cultures are obtained simply by nontraumatically opening the mouth with a vaginal spectrum and swabbing the oropharynx. Fecal cultures are obtained from fresh feces or cloacal swabs. The swabs are then returned to the laboratory where they are plated out on commercially prepared agar plates and incubated. Identification of organisms follows recommended incubation periods.

The presence of occult blood in feces, will be determined by a commercially prepared benzidine test. In addition, if any animals should die facilities exist for histopathological evaluation of organs and tissues in order to determine cause of death.

4.5 Tissue Oil Concentrations

a. Background

Tissue samples collected from stranded or beached turtles, as well as possible samples taken from experimentally exposed animals, will be analyzed for hydrocarbons as discussed in Section 3.1,b. Many organisms preferentially accumulate (or depurate) hydrocarbons that have been ingested in an oil. The organisms appear to preferentially depurate alkanes and accumulate aromatic compounds. After initial exposure, a tissue sample may resemble the ingested oil (e.g. SLCO), but after a period of time or after equilibrium has been established, tissue hydrocarbons will not necessarily resemble the original oil. Since aromatic compounds are preferentially retained, these compounds will be analyzed by high resolution gas chromatography-mass spectrometry as discussed below. Only this method can unequivocally identify these compounds. Previous studies at the University of South Florida have analyzed aromatic hydrocarbons in a wide variety of marine organisms (Van Vleet and Reinhardt, 1983; Van Vleet, 1983;

Van Vleet et al., 1983d). Consequently, the organic geochemistry laboratory of the Department of Marine Science (USF) is fully set up to carry out these analyses at the present time.

b. Analytical Methods

The methodology to be use to analyze tissues for hydrocarbon content and molecular composition is detailed in Section 3.1, b.

c. Quality Control

See Section 3.1, c.

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APPENDIX C

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STATISTCS FOR CHRONIC OIL STUDIES
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=0 TYPE=1 ----------					
TAT	% of time at surface	5	26.60	9.07	4.06
BPM	breaths per min	5	0.66	0.21	0.09
WBC	white blood cells x 10e3/cu.mm	5	1.52	0.91	0.41
RBC	red blood cells X 10E6/cu. mm	5	0.28	0.14	0.06
GLU	glucose conc	5	121.74	19.06	8.53
HBG	hemoglobin (g/100ml)	5	6.16	2.36	1.06
PCV	packed cell volume	5	22.10	8.40	3.76
PRO	total protein (g/100ml)	5	3.16	0.31	0.14
BUN	blood urea nitrogen (units/ml)	5	94.92	13.38	5.99
AST		5	225.60	120.07	53.70
ALB	serum albumin(g/100ml)	4	1.35	0.19	0.10
---------- observation day=0 TYPE=2 ----------					
TAT	% of time at surface	6	27.00	36.63	14.96
BPM	breaths per min	6	0.52	0.48	0.19
WBC	white blood cells x 10e3/cu.mm	5	2.16	1.69	0.76
RBC	red blood cells X 10E6/cu. mm	5	0.30	0.17	0.08
GLU	glucose conc	4	137.98	72.25	36.13
HBG	hemoglobin (g/100ml)	5	5.36	2.63	1.18
PCV	packed cell volume	5	19.00	9.83	4.40
PRO	total protein (g/100ml)	5	2.92	0.30	0.14
BUN	blood urea nitrogen (units/ml)	5	94.30	29.28	13.09
AST		5	240.20	92.76	41.49
ALB	serum albumin(g/100ml)	4	1.33	0.18	0.09
---------- observation day=1 TYPE=1 ----------					
TAT	% of time at surface	5	37.60	16.47	7.37
BPM	breaths per min	5	0.76	0.34	0.15
WBC	white blood cells x 10e3/cu.mm	5	2.46	1.39	0.62
RBC	red blood cells X 10E6/cu. mm	5	0.21	0.04	0.02
GLU	glucose conc	5	104.02	26.44	11.83
HBG	hemoglobin (g/100ml)	5	6.66	2.60	1.16
PCV	packed cell volume	5	19.90	6.66	2.98
PRO	total protein (g/100ml)	5	3.04	0.34	0.15
BUN	blood urea nitrogen (units/ml)	3	103.03	17.28	9.98
AST		2	329.00	72.12	51.00
ALB	serum albumin(g/100ml)	1	1.22		

STATISTCS FOR CHRONIC OIL STUDIES
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=1 TYPE=2 ----------					
TAT	% of time at surface	6	23.00	15.90	6.49
BPM	breaths per min	6	0.47	0.22	0.09
WBC	white blood cells x 10e3/cu.mm	5	4.74	3.88	1.73
RBC	red blood cells X 10E6/cu. mm	5	0.22	0.08	0.04
GLU	glucose conc	4	103.88	36.04	18.02
HBG	hemoglobin (g/100ml)	5	5.58	2.73	1.22
PCV	packed cell volume	5	17.10	8.00	3.58
PRO	total protein (g/100ml)	5	2.76	0.31	0.14
BUN	blood urea nitrogen (units/ml)	2	115.25	21.99	15.55
AST		2	329.00	72.12	51.00
ALB	serum albumin(g/100ml)	1	1.51	.	.
---------- observation day=2 TYPE=1 ----------					
TAT	% of time at surface	5	31.00	22.77	10.18
BPM	breaths per min	5	0.58	0.34	0.15
WBC	white blood cells x 10e3/cu.mm	5	3.26	0.66	0.29
RBC	red blood cells X 10E6/cu. mm	5	0.24	0.04	0.02
GLU	glucose conc	5	95.18	11.32	5.06
HBG	hemoglobin (g/100ml)	5	7.22	2.53	1.13
PCV	packed cell volume	5	24.40	10.26	4.59
PRO	total protein (g/100ml)	5	3.14	0.71	0.32
BUN	blood urea nitrogen (units/ml)	2	96.40	25.03	17.70
AST		3	302.33	68.81	39.73
ALB	serum albumin(g/100ml)	3	1.52	0.29	0.17
---------- observation day=2 TYPE=2 ----------					
TAT	% of time at surface	6	42.83	30.27	12.36
BPM	breaths per min	6	0.55	0.34	0.14
WBC	white blood cells x 10e3/cu.mm	5	4.52	3.15	1.41
RBC	red blood cells X 10E6/cu. mm	5	0.18	0.06	0.03
GLU	glucose conc	5	105.36	29.87	13.36
HBG	hemoglobin (g/100ml)	5	5.48	3.47	1.55
PCV	packed cell volume	5	18.50	10.30	4.60
PRO	total protein (g/100ml)	5	2.82	0.23	0.10
BUN	blood urea nitrogen (units/ml)	3	103.43	43.92	25.36
AST		3	304.67	66.16	38.20
ALB	serum albumin(g/100ml)	3	1.32	0.08	0.05

STATISTCS FOR CHRONIC OIL STUDIES
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=3 TYPE=1 ----------					
TAT	% of time at surface	5	18.60	12.50	5.59
BPM	breaths per min	5	0.44	0.21	0.09
WBC	white blood cells x 10e3/cu.mm	5	3.16	2.16	0.97
RBC	red blood cells X 10E6/cu. mm	5	0.27	0.08	0.03
GLU	glucose conc	5	93.98	8.08	3.61
HBG	hemoglobin (g/100ml)	5	7.62	2.70	1.21
PCV	packed cell volume	4	25.75	12.49	6.25
PRO	total protein (g/100ml)	5	3.44	1.28	0.57
BUN	blood urea nitrogen (units/ml)	3	92.43	20.57	11.88
AST		3	346.00	58.89	34.00
ALB	serum albumin(g/100ml)	2	1.26	0.04	0.03
---------- observation day=3 TYPE=2 ----------					
TAT	% of time at surface	6	38.50	34.13	13.93
BPM	breaths per min	6	0.52	0.35	0.14
WBC	white blood cells x 10e3/cu.mm	5	6.02	1.82	0.81
RBC	red blood cells X 10E6/cu. mm	5	0.21	0.08	0.03
GLU	glucose conc	5	79.16	9.80	4.38
HBG	hemoglobin (g/100ml)	4	6.40	4.98	2.49
PCV	packed cell volume	5	19.20	10.82	4.84
PRO	total protein (g/100ml)	5	2.86	0.22	0.10
BUN	blood urea nitrogen (units/ml)	3	89.20	22.20	12.82
AST		3	346.00	58.89	34.00
ALB	serum albumin(g/100ml)	2	1.21	0.04	0.02
---------- observation day=4 TYPE=1 ----------					
TAT	% of time at surface	4	62.75	44.21	22.10
BPM	breaths per min	4	0.78	0.62	0.31
WBC	white blood cells x 10e3/cu.mm	5	2.84	1.53	0.69
RBC	red blood cells X 10E6/cu. mm	5	0.29	0.09	0.04
GLU	glucose conc	5	79.46	6.33	2.83
HBG	hemoglobin (g/100ml)	5	7.80	2.86	1.28
PCV	packed cell volume	5	22.60	9.71	4.34
PRO	total protein (g/100ml)	5	2.80	0.44	0.20
BUN	blood urea nitrogen (units/ml)	5	69.96	28.73	12.85
AST		3	312.00	58.89	34.00
ALB	serum albumin(g/100ml)	2	1.35	0.17	0.12

STATISTCS FOR CHRONIC OIL STUDIES
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=4 TYPE=2 ----------					
TAT	% of time at surface	5	32.60	31.80	14.22
BPM	breaths per min	5	0.58	0.33	0.15
WBC	white blood cells x 10e3/cu.mm	5	4.56	1.66	0.74
RBC	red blood cells X 10E6/cu. mm	5	0.22	0.09	0.04
GLU	glucose conc	5	76.32	18.91	8.46
HBG	hemoglobin (g/100ml)	5	6.94	3.70	1.66
PCV	packed cell volume	5	17.50	8.80	3.94
PRO	total protein (g/100ml)	5	2.72	0.16	0.07
BUN	blood urea nitrogen (units/ml)	5	86.96	21.59	9.66
AST		3	306.67	64.01	36.96
ALB	serum albumin(g/100ml)	2	1.38	0.13	0.09
---------- observation day=7 TYPE=1 ----------					
TAT	% of time at surfsce	5	31.40	29.30	13.10
BPM	breaths per min	5	0.62	0.44	0.20
WBC	white blood cells x 10e3/cu.mm	5	1.64	0.68	0.30
RBC	red blood cells X 10E6/cu. mm	5	0.25	0.04	0.02
GLU	glucose conc	5	102.06	28.72	12.84
HBG	hemoglobin (g/100ml)	5	7.60	2.20	0.98
PCV	packed cell volume	5	23.30	7.68	3.43
PRO	total protein (g/100ml)	5	3.04	0.31	0.14
BUN	blood urea nitrogen (units/ml)	1	115.60	.	.
AST		5	265.60	138.79	62.07
ALB	serum albumin(g/100ml)	5	1.32	0.05	0.02
---------- observation day=7 TYPE=2 ----------					
TAT	% of time at surface	6	33.50	20.23	8.26
BPM	breaths per min	6	0.72	0.40	0.16
WBC	white blood cells x 10e3/cu.mm	5	3.42	1.10	0.49
RBC	red blood cells X 10E6/cu. mm	5	0.19	0.08	0.03
GLU	glucose conc	5	85.72	20.44	9.14
HBG	hemoglobin (g/100ml)	5	5.54	3.33	1.49
PCV	packed cell volume	4	19.12	7.33	3.67
PRO	total protein (g/100ml)	5	2.70	0.30	0.13
BUN	blood urea nitrogen (units/ml)	3	77.07	21.23	12.26
AST		5	277.00	108.58	48.56
ALB	serum albumin(g/100ml)	5	1.27	0.14	0.06

STATISTCS FOR CHRONIC OIL STUDIES
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=11 TYPE=1 ----------					
TAT	% of time at surface	4	33.25	14.64	7.32
BPM	breaths per min	4	0.65	0.24	0.12
WBC	white blood cells x 10e3/cu.mm	5	2.34	2.23	1.00
RBC	red blood cells X 10E6/cu. mm	5	0.25	0.06	0.03
GLU	glucose conc	5	101.88	20.34	9.10
HBG	hemoglobin (g/100ml)	5	6.82	2.43	1.09
PCV	packed cell volume	5	21.00	7.71	3.45
PRO	total protein (g/100ml)	5	3.10	0.37	0.17
BUN	blood urea nitrogen (units/ml)	4	92.65	4.99	2.49
AST		3	231.33	167.41	96.65
ALB	serum albumin(g/100ml)	3	1.30	0.11	0.06
---------- observation day=11 TYPE=2 ----------					
TAT	% of time at surface	5	21.60	12.46	5.57
BPM	breaths per min	5	0.38	0.23	0.10
WBC	white blood cells x 10e3/cu.mm	5	3.98	3.34	1.50
RBC	red blood cells X 10E6/cu. mm	5	0.20	0.08	0.03
GLU	glucose conc	5	95.26	17.82	7.97
HBG	hemoglobin (g/100ml)	5	5.42	3.19	1.42
PCV	packed cell volume	5	17.90	8.56	3.83
PRO	total protein (g/100ml)	5	2.90	0.45	0.20
BUN	blood urea nitrogen (units/ml)	4	54.98	41.28	20.64
AST		4	194.20	157.45	78.73
ALB	serum albumin(g/100ml)	3	93.50	159.78	92.25
---------- observation day=22 TYPE=1 ----------					
TAT	% of time at surface	4	43.00	7.87	3.94
BPM	breaths per min	4	0.93	0.19	0.09
WBC	white blood cells x 10e3/cu.mm	5	1.20	0.57	0.25
RBC	red blood cells X 10E6/cu. mm	5	0.31	0.08	0.04
GLU	glucose conc	5	110.28	11.54	5.16
HBG	hemoglobin (g/100ml)	5	11.18	9.49	4.24
PCV	packed cell volume	4	24.00	10.75	5.37
PRO	total protein (g/100ml)	5	2.96	0.42	0.19
BUN	blood urea nitrogen (units/ml)	3	75.50	22.82	13.17
AST		5	271.80	137.70	61.58
ALB	serum albumin(g/100ml)	4	1.45	0.32	0.16

STATISTCS FOR CHRONIC OIL STUDIES
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
observation day=22 TYPE=2					
TAT	% of time at surface	5	29.80	39.60	17.71
BPM	breaths per min	5	0.40	0.12	0.05
WBC	white blood cells x 10e3/cu.mm	5	1.56	0.81	0.36
RBC	red blood cells X 10E6/cu. mm	5	0.23	0.09	0.04
GLU	glucose conc	5	111.96	23.37	10.45
HBG	hemoglobin (g/100ml)	4	5.23	2.71	1.36
PCV	packed cell volume	5	18.10	8.02	3.59
PRO	total protein (g/100ml)	5	2.78	0.19	0.09
BUN	blood urea nitrogen (units/ml)	2	69.65	24.96	17.65
AST		5	290.40	100.25	44.83
ALB	serum albumin(g/100ml)	4	1.27	0.30	0.15

CHRONIC OIL STUDIES STATISTICS FOR VO2
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=0 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	5	31.56	16.03	7.17
---------- observation day=0 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	5	34.34	19.01	8.50
---------- observation day=1 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	5	29.48	18.12	8.10
---------- observation day=1 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	5	27.16	18.75	8.39
---------- observation day=2 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	5	27.90	20.34	9.10
---------- observation day=2 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	5	30.24	30.14	13.48
---------- observation day=3 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	5	35.88	19.72	8.82
---------- observation day=3 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	4	35.60	32.50	16.25
---------- observation day=4 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	4	22.80	6.73	3.36
---------- observation day=4 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	4	27.53	19.30	9.65
---------- observation day=7 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	5	28.48	16.91	7.56
---------- observation day=7 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	4	36.95	16.67	8.34
---------- observation day=11 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	3	28.07	10.39	6.00

CHRONIC OIL STUDIES STATISTICS FOR VO2
Type 1= Controls , Type 2=Oiled turtles

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
---------- observation day=11 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	3	34.67	13.32	7.69
---------- observation day=22 TYPE=1 ----------					
VO2	oxygen consumption(ml/kg hr)	4	24.25	16.36	8.18
---------- observation day=22 TYPE=2 ----------					
VO2	oxygen consumption(ml/kg hr)	4	25.80	11.32	5.66

Chronic Oil Studies Blood Gases
Type 1= Controls , Type 2= Oiled turtles

OBS	DA	TYPE	PH	SDPH	PCO2	SDPCO2	PO2	SDPO2
1	0	1	7.550	0.068	16.3	8.50	43.4	5.44
2	1	1	7.828	0.151	11.6	4.02	38.8	8.80
3	2	1	7.765	0.161	13.6	5.46	41.0	4.71
4	3	1	7.781	0.218	15.9	6.29	43.9	13.50
5	4	1	7.745	0.050	16.6	2.63	37.1	6.65
6	7	1	7.717	0.065	17.2	2.50	42.2	4.05
7	11	1	7.756	0.132	12.8	4.29	39.4	9.29
8	22	1	7.636	0.127	22.0	5.97	40.2	9.35
9	0	2	7.536	0.107	22.9	5.92	42.7	9.22
10	1	2	7.642	0.176	14.5	7.69	33.9	14.70
11	2	2	7.753	0.135	13.0	4.34	33.7	6.67
12	3	2	7.657	0.113	18.0	3.32	37.4	8.31
13	4	2	7.540	0.219	17.5	5.05	38.9	13.89
14	7	2	7.665	0.147	15.5	2.42	40.9	6.84
15	11	2	7.640	0.117	15.3	3.09	43.8	13.27
16	22	2	7.708	0.117	16.9	7.36	37.9	13.51

DA is day of observation
pH in units pH

SDpH standard deviation

pCO_2 venous pCO_2 (Torr)

$SDpCO_2$ standard deviation pCO_2

pO_2 venous pO_2 (Torr)

$SDpO_2$ standard deviation pO_2

ACUTE OIL STUDIES

VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN
	DAY=0				
VO2	OXYGEN CONSUMPTION as % of day0	4	100.00	0.00	0.00
VT	INSPIRED TIDAL VOLUME (ml/kg)	3	34.37	6.75	3.90
NVP	NON-VENTILATORY PERIOD (min)	3	11.03	8.29	4.78
	DAY=1				
VO2	OXYGEN CONSUMPTION as % of day0	4	267.25	286.78	143.39
VT	INSPIRED TIDAL VOLUME (ml/kg)	3	47.17	42.45	24.51
NVP	NON-VENTILATORY PERIOD (min)	3	19.33	24.66	14.24
	DAY=2				
VO2	OXYGEN CONSUMPTION as % of day0	3	147.70	52.37	30.23
VT	INSPIRED TIDAL VOLUME (ml/kg)	2	52.35	21.28	15.05
NVP	NON-VENTILATORY PERIOD (min)	2	7.55	4.17	2.95
	DAY=5				
VO2	OXYGEN CONSUMPTION as % of day0	2	122.30	85.28	60.30
VT	INSPIRED TIDAL VOLUME (ml/kg)	2	27.85	5.02	3.55
NVP	NON-VENTILATORY PERIOD (min)	2	9.70	3.96	2.80
	DAY=6				
VO2	OXYGEN CONSUMPTION as % of day0	3	158.80	28.75	16.60
VT	INSPIRED TIDAL VOLUME (ml/kg)	3	32.90	4.20	2.43
NVP	NON-VENTILATORY PERIOD (min)	3	10.70	6.53	3.77
	DAY=9				
VO2	OXYGEN CONSUMPTION as % of day0	2	151.45	2.62	1.85
VT	INSPIRED TIDAL VOLUME (ml/kg)	1	31.80	.	.
NVP	NON-VENTILATORY PERIOD (min)	1	5.90	.	.
	DAY=15				
VO2	OXYGEN CONSUMPTION as % of day0	2	115.55	23.55	16.65
VT	INSPIRED TIDAL VOLUME (ml/kg)	2	44.20	14.71	10.40
NVP	NON-VENTILATORY PERIOD (min)	2	6.15	0.49	0.35

Ventilation

Means for all acutes: ventilation
Tidal Volume in ml/kg

Day	0	1	2	5	6	9	15
Means	34.4	47.2	52.4	27.9	32.9	31.8	44.2
Std	5.51	34.66	15.05	3.55	3.43	-	10.40

Day	0	1	2	5	6	9	15
Non ventilatory period			(min)				
Means	11.0	19.3	7.6	9.7	10.7	5.9	6.2
Std.	6.77	20.13	2.95	2.80	5.33	-	0.35

Serum

Glucose (mg) glucose per 100 ml plasma)

Day	0	1	2	5	6	9	15
Mean	153.9	141.9	126.5	101.0	103.6	116.0	130.0
Std	1.33	1.60	14.78	0.00	19.55	0.00	7.00

Glucose percent change from day 0

Day	1	2	5	6	9	15
Mean	-7.8%	-17.7%	-34.8%	-33.1%	-23.7%	-15.3%
Std	1.34%	8.96%	.00%	10.38%	-	4.40%

White Blood Cell (% Change)

WBC as % change from day 0

Day	0	1	2	5	6	9	15
Mean		2.1%	227.5%	355.6%	284.6%	-73.0%	27.0%
Std		26.87%	109.11%	540.40%	373.42%	46.77%	97.62%

CHRONIC EXPOSURE
FECAL SAMPLES
MEAN BACTERIAL COUNTS

SPECIES	DAY 0 MEAN COUNT	N	DAY 4 MEAN COUNT	N	DAY 7 MEAN COUNT	N	DAY 11 MEAN COUNT	N	DAY 22 MEAN COUNT	N	DAY 35 MEAN COUNT	N
1 = Pseudomonas	33.66	6	11.16	6			70.00	1	33.42	7	43.50	2
2 = Salmo-Citro-Esch	10.33	3	32.33	9			60.00	2	25.20	5	8.00	1
3 = Klebsiella	15.00	1	10.00	1					15.00	2		
4 = Proteus-Providencia	23.00	1	8.50	2								
5 = Aero-Vibrio	19.40	5	36.66	3			10.00	1	27.50	4	5.00	1
6 = Micrococcus									60.00	1		
7 = Streptococcus									16.66	3		
8 = Bacillus	100.00	1										
9 = Lactobacillus									95.00	1		
10= Corynebacterium									95.00	1	100.00	1
11= Acinetobacter	2.00	1	5.00	1								
12= Achromobacter												

ACUTE EXPOSURE
OROPHARYNGEAL SAMPLES
MEAN BACTERIAL COUNTS

SPECIES	DAY 0 MEAN COUNT	N	DAY 4 MEAN COUNT	N	DAY 7 MEAN COUNT	N	DAY 11 MEAN COUNT	N	DAY 22 MEAN COUNT	N	DAY 35 MEAN COUNT	N
1 = Pseudomonas	20.28	7	29.16	6	27.50	2			33.83	1	1.00	1
2 = Salmo-Citro-Esch	20.33	3	36.42	7	1.00	1	35.00	1	51.50	2	2.00	1
3 = Klebsiella							25.00	1	15.00	1		
4 = Proteus-Providencia	15.00	2	3.00	1					2.00	1		
5 = Aero-Vibrio	29.80	5	13.00	5	15.00	1			44.25	4	48.50	2
6 = Micrococcus												
7 = Streptococcus			100.00	1			100.00	1	100.00	2		
8 = Bacillus									1.00	1		
9 = Lactobacillus	100.00	1										
10= Corynebacterium			100.00	1					99.00	1		
11= Acinetobacter	18.00	1	2.00	1	30.00	1	40.00	1				
12= Achromobacter												

ACUTE EXPOSURE
FECAL
MEAN BACTERIAL COUNTS

SPECIES	DAY 0 MEAN COUNT	N	DAY 4 MEAN COUNT	N	DAY 7 MEAN COUNT	N	DAY 11 MEAN COUNT	N	DAY 22 MEAN COUNT	N	DAY 35 MEAN COUNT	N
1 = Pseudomonas	15.60	5	12.20	5	5.00	1			25.50	1	15.00	1
2 = Salmo-Citro-Esch	23.75	4	36.00	5	25.00	1	100.00	1	36.37	8	22.50	2
3 = Klebsiella												
4 = Proteus-Providencia					15.00	1			10.00	1		
5 = Aero-Vibrio	36.16	6	36.33	3	55.00	1			24.25	4	20.00	2
6 = Micrococcus	1.00	1	5.00	1					3.00	1		
7 = Streptococcus			55.00	4			100.00	1	75.00	2	100.00	1
8 = Bacillus									2.00	1		
9 = Lactobacillus	99.00	1							95.00	1		
10= Corynebacterium									50.00	1		
11= Acinetobacter	10.00	1	12.50	2								
12= Achromobacter												