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GA - Effects of Environmental Contaminants on Loggerhead Sea Turtles (Caretta caretta)

Study Objectives:
To determine the effects contaminants have on the biology of the threatened Loggerhead sea turtle. Recruitment, survivorship as well as contaminant uptake through the shell and resulting anatomical and physiological effects will be measured. To determine if there are contaminant loading differences between sea turtle eggs/hatchlings from northwest Florida and those of the Georgia Coast.

Background:

All seven species of marine turtles are listed as threatened or endangered by the U.S. Fish and Wildlife Service. The southern Atlantic coast of the United States is the largest nesting rookery of one species, the loggerhead (Caretta caretta), but population declines have brought current coastal-usage practices into question. The decrease in nesting females has been attributed to direct human influences such as habitat depletion (National Research Council 1990) and incidental catch of turtles by shrimp trawlers, and indirect influences such as light pollution (Witherington 1992). These sources of human disturbance elicit obvious lethal effects upon sea turtles, but turtle populations may be threatened more by inconspicuous sub-lethal effects such as immune suppression, growth retardation, and reproductive incompetence. These effects can cause rapid population declines by reducing the reproductive output of individual animals.

Insult from environmental contaminants may cause sub-lethal effects in animals. Coastal areas, in particular, are prone to heavy environmental contamination because the areas (1) serve as a "sink" for all contaminants "upstream", (2) are heavily used agricultural regions which contribute pesticide and fertilizer contamination, and (3) have high concentrations of pulp-mill contributing contaminated effluent. Loggerhead sea turtles feed in near-shore and estuarine areas likely to be contaminated and, thus, the turtles are at risk of experiencing damaging sub-lethal effects. Eggs may also be exposed through contaminated sand and soil. The purpose of this study is to determine if loggerhead sea turtles on the Atlantic coast of the United States are adversely affected by environmental contaminants. If so, measures need to be sought to alleviate the adverse effects.

Experimental Overview

The proposed study will assess the affect of environmental contaminants on the number of reproductively viable offspring (and thus future population) produced by adult sea turtles. Due to the threatened status of the loggerhead sea turtle, some experiments will use the yellow-belly slider (Trachymes scripta) as a surrogate species. Current concentrations of contaminants in tissues of loggerhead turtles need to be quantified to determine if bioaccumulation is occurring in the near-shore marine system. Then the reproductive effects of these contaminants may be determined. Eggs will be analyzed for contaminants and monitored for viability. Resultant hatchlings will then be analyzed for (1) hatchling mortality, (2) sex ratio, (3) immune suppression and (4) decreased growth potential. These parameters should adequately assess potential adverse effects resultant from contaminant exposure.

Statistics:

Data collection will consist of physical parameters of the turtles and eggs. These data include but are not limited to shell measurements, approximate volumes of egg clutches as well as atmospheric and stratigraphic data. Analysis of data collected will include descriptive statistics, analysis of variance,

analysis of covariance, analysis of variance of regression, linear regression and Scheffe's test. Data will be transformed prior to analysis using either arcsine or naperian log. All data will be tested for normal distribution and homogeneity of fit. Data will be analyzed using SuperAnova, Statview, (Apple Computer) and SAS 6.04, Excel, Lotus 123 (IBM) computer programs.

Results:

Collection and collation of data and interim analysis will be done as necessary to provide a progress report each year. A comprehensive final report will incorporate all data collected. Data will be accessible at all times and computer backup as well as hard copies will be maintained. Field and laboratory data will be entered into a computer database as soon as possible and not less than once per week.

The results of this study will be formatted for publication in a peer reviewed internationally recognized journal. These would be either Biology of Reproduction, Journal of Reproduction and Fertility, Journal of Ecotoxicology or Journal of Wildlife Management. Additionally, a final report will be presented to the USFWS Regional Office for distribution.. Data from this study will be presented orally at appropriate regional or national meetings such as the Annual Sea Turtle Biologists Conference.

Interpretation:

The results of this study will provide data which presently are not available regarding renourishing activities of beaches. These data will aid in determining the presence of contaminants on tested beaches and their effects on the threatened Loggerhead sea turtle. Additionally, other species may be effected such as the Green sea turtles that use these beaches in Florida for nesting. The benefit of using beaches from a National Wildlife Refuge and those that are renourished or to be renourished will give baseline data for implementation of renourishing projects while not jeopardizing the reproductive efforts of threatened or endangered species.

Roles and Responsibilities:

The environmental contaminants specialist (EC specialist) from Brunswick FWE will be the principle investigator and responsible party to insure the study is completed. Managing, scheduling and report preparation will be the responsibility of the Environmental Contaminants Specialist (EC), Brunswick Field Office, Brunswick, Georgia in cooperation and coordination with the EC, Panama City Field Office, Panama City, Florida, Georgia Department of Natural Resources. Accountability of project funds will be done through the Brunswick Field Office. Field collections will be done using USFWS EC specialists, USFWS personnel from Brunswick FWE, Florida Cooperative Fish and Wildlife Research Unit students, Archie Carr Sea Turtle Center personnel (students), USFWS Savannah Coastal Refuges, USFWS St. Vincent National Wildlife Refuge employees and approved, experienced volunteers.

The EC specialist will be responsible for all data collected, obtaining authorization and permits, coordination of activities, beach patrols, incubation, laboratory analysis, statistical analysis, progress reports, the final report and publication of the data. Personnel from USFWS Brunswick will aid in beach patrols, field and data collections. Refuge personnel will assist in collections, data collection, beach patrols (already being conducted) and providing technical expertise regarding the refuges and turtles nesting there. The students (two have shown great interest and are accomplished sea turtle biologists and laboratory technicians) will be responsible for beach patrols, collections, technical assistance within the laboratory and data collection.

Schedule:

The proposed study will encompass three nesting seasons from fiscal 1994 to the end of fiscal 1996. Samples from stranded turtles will be collected throughout the year. These collections will be made as soon as possible after our notification (within 24 hours) of a stranding. Nesting and egg incubation for each species will begin at the initiation of nesting activity in April and proceed through August 31 or hatching and subsequent release. The laboratory and in-depth analyses will be done from September to March. Additionally, many phases of this project will be overlapping and the necessary time will be devoted to each during the peak collection and data processing periods.

A preliminary (annual) report will be submitted to the Regional Environmental Contaminants Coordinator no later than September 1 of each year. The Final report will be completed and submitted to the appropriate parties (Regional and Washington offices, all participants) by April 1, 1997. Published articles and reports will be made available to interested personnel. Abstracts and reports obtained from these data that are not published in reviewed journals will be distributed to interested parties.

Questions and Experimental Designs

Question 1: Do sea turtle tissues contain environmental contaminants?

Hypothesis: Contaminants are present in quantities capable of causing sublethal effects.

Background Information: Few studies have addressed levels of contaminants in marine turtle tissues and eggs. Thompson et al. (1974) measured levels of PCB's and DDE (the metabolite of DDT) in green turtle eggs from Ascension Island, south Atlantic Ocean. The maximum concentration of DDE observed was 0.009 ppm and the maximum PCB concentration 0.22 ppm (both measured in wet weight). Hillestad et al. (1974) analyzed tissues of loggerhead sea turtles from South Carolina and Georgia and found concentrations of total DDT (DDE+DDD+DDT) from 0.305 to 0.58 ppm. Compared to tissue concentrations of terrestrial mammals, these concentrations are relatively low; however, the implications of such concentrations in migratory marine species are unknown.

Design: Body fat, brain tissue, liver, skeletal muscle and kidney will be obtained from freshly stranded turtles on the Georgia coast. The tissues will be put on ice, frozen, and stored at -20 ^{o}C until analysis. Total DDT, PCB's, dioxin, mercury, and selenium concentrations will be determined. Additionally, blood samples if obtainable will be collected and the plasma assayed for vitellogenin.

Question 2: What is the mode of exposure of eggs to the contaminants?

Hypothesis: Contaminants are bioaccumulated in the female and passed into the egg across the wall of the oviduct.

Background Information: There are two possible ways developing embryos can be exposed to contaminants: (1) the contaminants are incorporated into the albumen or yolk as the egg is assembled or (2) contaminants pass through the shell and into the egg during incubation. The latter possibility would only be expected to occur in highly altered systems such as renourished beaches. Sediments used to renourish beaches are acquired from near-shore areas likely to accumulate contaminants. It is possible during incubation in contaminated sediments, eggs transport contaminants across their permeable shell. Alternatively, the contaminants may already be "packaged" in the egg as a result of the adult female bioaccumulating contaminants and then secreting them into the albumen or yolk.

Experiment A: Immediately after eggs are deposited on a recently renourished beach, one egg will be removed from each of five different nests. The nests will be marked and after the majority of hatchlings emerge from a given nest, the nest will be excavated. Any unhatched eggs will be candled and one unfertilized egg from each of the five nests will be collected and analyzed. The eggs will be analyzed for total DDT, PCB's, dioxin, mercury, and selenium and compared pairwise to determine if contaminants move into the eggs over time.

Experiment B: One hundred yellow-bellied slider (Trachymes scripta) eggs will be purchased at the day of laying from Thane Wibbles (University of Alabama, Birmingham). Twenty will be opened upon arrival and the yolks and albumen separated and stored at -76C. The remaining eggs will be divided equally among four conditions which will be based upon the results of Experiment A. For instance, if

DDT and PCB's are present in significant concentrations, the four conditions will be (1) clean sand, (2) DDT at physiological concentrations (see exp. A), (3) PCB (2',4',6'-trichloro-4-biphenylol) at concentration, and (4) PCB + DDT. Five eggs from each group will be collected at day 10, 20, 40, and 60 of incubation and the albumen, egg fluid, and yolk/embryo will be collected and frozen. All samples will be analyzed under strict QA/QC guidelines to maintain within and between assay parameters. Because these testing procedures are

Question 3: What are the effects of contaminant exposure in loggerhead eggs?

Hypothesis: Contaminants have detrimental effects on sea turtles quantifiable by analyzing (a) mortality, (b) sex ratio, (c) reproductive impairment, (d) immune potential, and (e) growth potential.

Background Information: Studies showing a clear cause-effect relationship between contaminants and turtles are almost non-existent. Crews et al. (in press) showed a "statistical association between contaminant levels in eggs and poor development of these eggs..." for Chelydra serpentina. PCB's and organochlorines were among the contaminants associated with egg mortality.

Many reptiles have temperature-dependent sex determination (TSD). Hot temperatures produce females and cold temperatures produce males in sea turtles and some fresh water turtles (including T. scripta) (Ferguson, 1982). The second trimester of incubation is the critical period (Wibbles et al. 1991), and it has been shown by Crews, et al. (in press) that PCB's act as synthetic estrogens and alter the effects of temperature on sex determination. For instance, an egg incubated at male producing temperature will become female if exposed to PCB's. If PCB's have such effects in the wild, populations would show declines similar to those occurring presently. The effects of estrogen like compounds can be determined with an assay for plasma vitellogenin (yolk protein). If an animal is determined by physical examination to be a male and has vitellogenin in its plasma then it can be determined there has been exposure to estrogens or estrogen like compounds. Furthermore, estrogen/testosterone ratios help establish reproductive injury from exposure to estrogen-like contaminants (Guillette, Gross, Masson unpubl. data).

Contaminants may suppress the immune system. In turtles, immunity is mostly provided by gut associated lymphoid tissue (GALT). Decreases in GALT indicate immune suppression. GALT can be quantified through histological techniques and reduction of GALT is correlative with immune suppression.

Growth of vertebrates is regulated by an intricate hormonal system, but the main regulator is insulin-like growth factor-I (IGF-I) (Sara and Hall 1990). IGF-I acts in endocrine, autocrine, and paracrine fashions to stimulate general transcription and growth of cells and tissues. IGF-I is secreted by the liver into the blood, bound to a binding protein, and escorted to the target cell. Normal levels of IGF-I can be altered by starvation, abnormal insulin levels, and abnormal growth hormone levels. Contaminants may also suppress the IGF-I system. Recent work in our lab indicates that the low clutch viability of alligator and turtle nests associated with contaminated lakes may be due to inhibition of the IGF-I system.

Design: Five hundred eggs will be divided among the four treatment groups identified in Experiment 2B. Eggs will be hatched, and survivorship calculated. Hatchlings will be reared 6 months at which time they will be killed. Blood, gonads, gastro-intestinal tract, spleen, thymus, liver, thyroid, and bone

marrow will be collected. Spleen, thymus, liver, and thyroid will be fixed in Bouin's fixative for histological sectioning. Table 1 outlines experiments to be conducted on turtles from the 4 experimental groups. All methods outlined have been and are currently being used by the principal and co-investigators.

Table 1. Methods of determining potential effects of contaminants.

Effect	**Method of Determination**
mortality	survivorship
sex ratio	Plasma E/T ratio by RIA / Gonad histology
sexual differentiation	Gonad histology, Vitellogenin assay
immune potential	GALT quantification by staining
growth potential	IGF-I concentrations by RIA
contaminant loading	Chemical analysis for Organochlorines, PAHs, Metals Scan

Twenty composite samples (of 20 eggs each, per nest) from Florida populations will be examined, sorted, weighed, and then shipped to the appropriate laboratories for analysis.

Literature Cited

Crews, D., J. Bergeron, J. Bull, D. Flores, A. Tousignant, J. Skipper and T. Wibbles. 1992. Temperature-dependent sex determination: proximate mechanisms and functional outcomes. In press.

Ferguson, M. (1982). Temperature of egg incubation determines sex in Alligator mississippiensis. Nature (London), 296:850-853.

Hillestad, H., R. Reimold, R. Stickney, H. Windom and J. Jenkins. 1974. Pesticides, heavy metals, and radionuclide uptake in loggerhead sea turtles from South Carolina and Georgia. Herpetological Review 5: 75.

National Research Council. 1990. Decline of sea turtles: causes and prevention. National Academy Press. Washington, D.C.

Sara, V. and K. Hall. 1990. Insulin-like growth factors and their binding proteins. Physiological Reviews 70: 591-613.

Thompson, N., P. Rankin and D. Johnston. 1974. Polychlorinated Biphenyls and p,p DDE in green turtle eggs from Ascension Island, South Atlantic Ocean. Bulletin of Environmental Contamination and Toxicology 11: 399-406.

Wibbles, T., J. Bull and D. Crews. 1991. Chronology and morphology of temperature-dependent sex determination. Journal of Experimental Zoology 260: 371-381.

Witherington, B. 1992. Behavioral responses of nesting sea turtles to artificial lighting. Herpetologica 48: 31-39.

Budget

Operational Cost Estimate
Year 1 of 3 (FY 1994)

Supplies (cryo-vials, 5cc syringes, microscope slides & cover slips, transfer pipettes, autoclavable bags for sand)	$ 2000.00
Equipment (liq. Nitrogen container, insulated specimen containers, 2 helmet lights, dissecting tools, incubation trays, hand scales, metric tapes, portable pH meter, ion selective electrodes, thermometers, phase contrast light microscope)	$ 42000.00 **
Miscellaneous (IGF-I mono-clonal antibody, radio-labeled antibody, DDT, PCB's, chemical reagents, T. scripta eggs, office supplies)	$ 2000.00 *
Travel and Personnel (Biological technician, transportation and staging)	$ 20500.00
Non Cost Items Provided (incubators, gamma counter, laboratory, fume hood, surgical supplies)	$ No Cost
TOTAL	$ 67500.00 *

$500.00

1,000 grain size
1,000 per diem
5,000 bio-tech

Analytical Cost Estimate

17,300 - Routine Analysis
4,900 - Dioxin Analysis

Chemical Analysis (DDT, PCB's, Dioxin, Mercury, Selenium)	$ 22,200.00
Testosterone, Estrogens, Progestins	$ 0.00 *
GRAND TOTAL	$ 97700.00

** These costs are for ONE time purchases of necessary field and experimental equipment. The Grand Total is the estimated costs for year 1.

* A significant savings in laboratory analysis has been shown in the budget (> 50% savings) due to the analyses being done by the EC Specialist (Brunswick, FWE) and the biological technician. The RIA, immunocytochemistry, histology and incubation techniques have been and presently are being done by the EC Specialist (Brunswick, FWE) and Biological technician. Furthermore, both these individuals have years of experience in egg biology and sea turtle biology.

Operational Cost Estimate
Years 2,3 of Study Proposal

Operational Costs Year 2	$ 44,500
Operational Costs Year 3	$ 42,500
Three Year Grand Total	$ 184,700

SUMMARY SHEET

STUDY TITLE: Effects of Environmental Contaminants on Loggerhead Sea Turtles (Caretta caretta)

YEAR OF STUDY: 1 of 3

PROJECT CODE:

REGION: 4

REGIONAL PRIORITY: __X__ _____ _____
Refuge Non-Refuge Other

TYPE OF STUDY: ___X___ _____ _____
Background Comprehensive Other

Brief Description: This proposal is to aid in determining if renourishing activities may have an effect on threatened Loggerhead sea turtle nesting populations. The study area includes the Georgia coast involving Blackbeard Island NWR, Wolf Island NWR, Tybee Island and Wassaw NWR. Those areas to be covered in Florida include St. Vincent NWR, Eglin and Tyndall Air Force Bases, Florida State Parks (St. Andrew, St. Joseph, St. George Island). Contaminants are known to be sequestered in vitellogenin of reptilian eggs during folliculogenesis. However, no data are available relating to the uptake of contaminants through the eggshell during incubation. The possibility of using contaminated material to renourish beaches may produce sub-lethal, insidious physiological and anatomical changes (Guillette, Gross, Masson, Percival unpubl. data). These changes introduce sterile young into a population. Unfortunately, these effects may not be noticed for many years until the turtle should reach sexual maturity. Loggerhead sea turtles are long-lived and reproductive maturity is not reached until 12-15 years of age. Therefore, it becomes implicit to identify and alleviate contaminant sources before a population crash or extinction become a reality.
Data from collections of unhatched loggerhead and green sea turtle eggs from nests on St. Vincent NWR, Eglin Air Force Base, and developed and undeveloped beaches in northwest Florida will be compared with those data from Georgia. These comparisons will aid in determining, from analyses, sea turtle species and/or geographical areas having chemical contaminant problems.

PRINCIPAL INVESTIGATORS: Greg R. Masson Ph.D., Michael Brim

FWE FIELD OFFICES: Brunswick, GA; Panama City, FL

REFUGE: Savannah Coastal Refuges, St. Vincent National Wildlife Refuge

FUNDING REQUESTED: 67500.00 + 22,200.00 = 97700.00
Operational Analytical Total

FUNDING SOURCE(s): $ 97,700 $ $
Refuges FWE Other (Specify)