Exposure and Response of Morelet's Crocodile (*Crocodylus moreletii*) Populations to Endocrine Disrupting Compounds in Belize, Central America

Project Scope

Over the last 20 years, evidence of population declines and reproductive impairment in American alligators (*Alligator mississippiensis*) in Florida has increased concern over the effects of endocrine-disrupting contaminants (EDCs) on wildlife and emphasized the importance and utility of reptiles, particularly crocodilians, as a focal species in the field of ecotoxicology. The species examined in this study was Morelet's crocodile (*Crocodylus moreletii*), an endangered, freshwater crocodile found in Mexico, Guatemala, and Belize. During a pilot study in 1995, multiple organochlorine (OC) pesticides considered to be EDCs were found in the eggs of Morelet's crocodiles from three localities in northern Belize. Based on these findings and previous data from Florida showing egg contamination, population declines, and reproductive abnormalities in alligators exposed to many of the same chemicals, this project was initiated to examine various endpoints of contaminant exposure and response in Morelet's crocodiles living on contaminated and reference sites in northern Belize.

The main objectives of this research were to determine:

- OC concentrations in soil, sediment, and nesting material of Morelet's crocodile from contaminated and reference (uncontaminated) sites;
- OC concentrations in nonviable eggs and caudal scute samples;
- Biomarker response of crocodiles to OC exposure at the individual level by monitoring plasma vitellogenin induction, plasma hormone (estradiol-17β, testosterone) concentrations, serum chemistry, and penis size in crocodiles from contaminated and reference sites;
- The response of crocodile populations to OC exposure by monitoring reproductive activity (nesting success), population size structure, sex ratios, and juvenile survival rates on contaminated and reference sites.

To address these objectives, blood, fat, non-viable eggs, caudal scute samples, and population data were collected (non-lethally) from crocodiles on contaminated and reference sites to examine exposure and effects of EDCs at the individual and population levels. To assess effects at the individual level, differences in biochemical endpoints (plasma testosterone, 17β-estradiol, vitellogenin, and serum chemistry) and a morphological endpoint (penis size) between contaminated and reference sites were examined. Crocodile response to EDC exposure at the population level was assessed by examining

Grant Title and Principal Investigator

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EPA STAR Grant #R826310

Key Findings

- Multiple OCs were detected in all environmental and species matrices examined. DDE was found in every egg analyzed, while OC occurrence and concentrations in scutes were more variable.
- It is unclear whether phallus size and plasma T concentrations observed in crocodiles from Gold Button Lagoon and New River Watershed are normal or altered by some stressor (e.g., EDCs).
- There appears to be no population-level effects of EDC exposure on Morelet's crocodiles inhabiting the two study sites.
- Long-term studies are essential for adequately assessing the effects of EDCs on crocodilian populations, as many of the contaminant-induced effects are organizational in nature, occurring during embryonic development, but not appearing until later in life.
- Plasma vitellogenin induction may serve as a reliable biomarker of estrogen exposure in crocodilians.

Project Period: December 1997 to November 2000

differences in endpoints of reproductive success (nesting success, clutch viability) and population structure (density, size structure, sex ratios, juvenile survivability) between contaminated and reference sites.

Relevance to ORD's Multi-Year Research Plan

This project contributes to two important long-term goals of the ORD's MYP: (1) to provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors, and (2) to determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment.

Results of this study provided a better understanding of the widespread organochlorine contamination of Morelet's crocodile habitat in Belize. Multiple organochlorines (most notably DDE) were detected in environmental media and species matrices (e.g., eggs, scutes, blood) at study sites, suggesting contamination of each site and exposure of the crocodiles inhabiting them. Due to the ubiquitous nature of the contamination, an appropriate uncontaminated (reference) site could not be located.

Potential impacts to Morelet's crocodile associated with this contamination were assessed by examining individual- and population-level endpoints. Few significant individual-level effects were noted; no significant population-level effects were observed.

Project Results and Implications

Organochlorine profiles of the "contaminated" site (Gold Button Lagoon) and "reference" site (New River Watershed) were examined by analyzing OC residues in soil, sediment, and crocodile nest material. Multiple OCs, some of which are considered to be EDCs, were found in all three matrices at both sites at similar concentrations; therefore, the New River Watershed did not represent an appropriate uncontaminated reference site. Actual OC exposure in crocodiles was examined through residue analysis of nonviable and unhatched eggs and caudal scute samples. As with the environmental samples, multiple OCs were found in all matrices examined. *p,p´*-Dichlorodiphenylethane (DDE) was found in every egg analyzed; OC occurrence and concentrations in scutes were more variable. Upon finding that both study sites and the crocodiles inhabiting them were contaminated with OCs, and to approximately the same degree, considerable effort was made to locate an uncontaminated crocodile habitat to use as a reference site for comparisons of individual- and population-level endpoints of endocrine disruption. Two additional sites in northern Belize and four additional sites in southern Belize were examined. All crocodile eggs from each locality were shown to contain environmental contaminants, suggesting contamination of each site and the crocodiles inhabiting them. Similar contaminant concentrations were found in American crocodile (*C. acutus*) eggs examined during this study from four sites in the coastal zone of Belize, further illustrating the ubiquitous nature of environmental contamination in the country. After failing to find an uncontaminated reference site, the research focused again on the New River Watershed and the Gold Button Lagoon.

The first individual-level endpoint examined was plasma vitellogenin induction. Vitellogenin is an egg-yolk precursor protein expressed in all oviparous and ovoviviparous vertebrates. Males and juveniles normally have no detectable concentration of vitellogenin in their blood, but can produce it following stimulation by an exogenous estrogen, such as an EDC. Thus, the presence of vitellogenin in the blood of male and immature crocodiles can serve as an indicator of exposure to estrogen-mimicking chemicals. Of 358 males and juvenile females sampled in this study, no vitellogenin induction was observed, suggesting these animals probably were not exposed to estrogenic xenobiotics. Many of the animals sampled, however, later were found to contain OC pesticides in their caudal scutes, confirming they in fact had been exposed to OCs (and EDCs). Previous researchers have stressed that vitellogenin induction is a measure of a biological effect, not merely the presence of a contaminant in the body of an animal. Our results support this notion. The lack of a vitellogenic response, however, should not be interpreted as an indication that no exposure or other contaminant-induced biological response has occurred.

The second individual-level endpoint examined was plasma steroid hormone concentrations. The selection of this endpoint was based on numerous studies reporting altered concentrations of estradiol-

17β (E2) and testosterone (T) in alligators from Lake Apopka and other contaminated lakes in Florida. In the present study, few significant intersite differences in plasma hormone (E2 and T) concentrations were noted. No significant differences in plasma E2 concentrations were detected between sites (see Figure 1). However, large juvenile males and females from the contaminated site (Gold Button Lagoon) exhibited significantly reduced plasma T concentrations compared to large juvenile males and females from the reference site (New River Watershed). This finding was consistent with results from previous studies on alligators in Florida. Reasons for this significant finding are uncertain, given that the contaminated and reference sites exhibited similar contaminant profiles, but may be attributed to physical differences between the sites (water level fluctuations, amount of aquatic vegetation, open versus closed systems). No other significant intersite differences in hormone concentrations were observed.

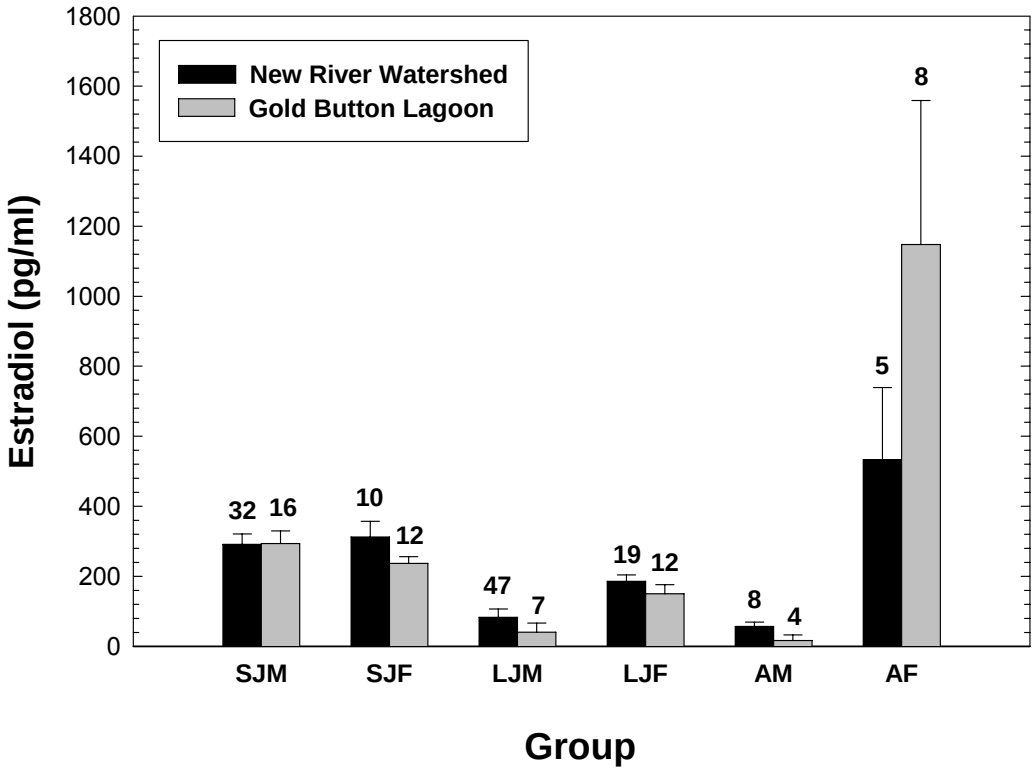

Figure 1. Inter-site comparison of mean (±SE) plasma estradiol-17β (E2) concentrations in Morelet's crocodiles from New River Watershed and Gold Button Lagoon, northern Belize. Numbers above bars indicate the number of animals sampled per site within a group. No significant difference in E2 concentrations within a group was observed. SJM = small juvenile males; SJF = small juvenile females; LJM = large juvenile males; LJF = large juvenile females; AM = adult males; AF = adult females.

The third individual-level endpoint examined was male phallus size. Concurrent with reductions in plasma T concentrations, male alligators from Lake Apopka and other contaminated lakes in Florida have exhibited smaller phallus size compared with animals from a reference lake. Researchers speculate that abnormal hormone concentrations during critical early life stages may affect anatomical structures (e.g., genitalia) dependent on these hormones for proper growth and development. *p,p´*-DDE is one of the primary contaminants of concern at Lake Apopka and has been shown to be anti-androgenic in laboratory animals, inhibiting normal androgen function *in vivo*. This persistent OC has been detected in eggs and blood serum from alligators in Lake Apopka, suggesting its potential role in the reproductive anomalies observed in juvenile males. *p,p´*-DDE also has been detected in Morelet's crocodile eggs and scutes in Belize, confirming EDC exposure in maternal females, neonates, juveniles, and other adults. In the present study, male crocodile phallus size and plasma T concentrations were examined as endpoints of response to *p,p´*-DDE exposure.

No differences in mean phallus size were observed between sites, although mean plasma T concentrations in juveniles from the Gold Button Lagoon were significantly reduced compared with those from the New River Watershed. Juvenile males from both sites exhibited positive relationships between body size and phallus size. Whereas juvenile males from the New River Watershed also exhibited positive relationships between plasma T and body size and plasma T and phallus size, no such relationships were observed for juveniles from the Gold Button Lagoon. For adults, no significant intersite differences were observed in phallus size, plasma T concentrations, or relationships between plasma T and body size or phallus size. Because of the similarity in contaminant profiles between the New River Watershed and the Gold Button Lagoon, it is unclear whether phallus size and plasma T concentrations observed in crocodiles from these two sites are normal or altered by some stressor (e.g., EDCs). Thus, the biological significance of the few site differences observed in this study is difficult to interpret.

The examination of population-level endpoints also was confounded by the fact that both sites exhibited similar contaminant profiles. Intersite differences in population densities (as a function of crocodile encounter rates), population size structure, and sex ratios were observed. We attribute these differences, however, to physical differences between the sites (water level fluctuations, amount of aquatic vegetation, open versus closed systems) and age- and sex-specific wariness and microhabitat use, all of which may have influenced animal catchability and thus interpretation of data on these endpoints. Overall, no population-level effects of EDC exposure on Morelet's crocodiles inhabiting the two study sites were apparent.

More data on the natural pattern of temperature-dependent sex determination in Morelet's crocodile, population size class distribution, sex ratios, and the influence of embryonic exposure to OCs during the temperature-sensitive period are needed to adequately examine the impacts of these chemicals on developing crocodiles and subsequent repercussions at the population level. Data from this study on site-specific size class distribution and sex ratios, however, did not indicate feminization of either population.

Investigators

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For More Information

http://www.tiehh.ttu.edu/research.htm

NCER Project Abstract and Reports:
http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/180/report/0