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ORGANOCHLORINE PESTICIDES IN CHORIOALLANTOIC MEMBRANES OF MORELET'S CROCODILE EGGS FROM BELIZE

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ABSTRACT: Recent studies examined the utility of the chorioallantoic membrane (CAM) as a nonlethal, noninvasive indicator of environmental contaminant exposure in oviparous wildlife. The CAM is a highly vascularized extraembryonic membrane that functions as a site for respiration, nutrient transport, and waste storage during embryonic development. After hatching, the CAM is usually discarded with the eggshell and can be used for chemical residue analysis. Chorioallantoic membranes have been used successfully to examine contaminant exposure and predict chemical concentrations in multiple species of birds and reptiles. In this study, we examined organochlorine (OC) pesticide concentrations in CAMs from eggs of Morelet's crocodiles (*Crocodylus moreletii*) from northern Belize. Multiple OCs were detected in crocodile CAMs, including aldrin, dieldrin, endrin, dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethane, dichlorodiphenyldichloroethylene (DDE), heptachlor, lindane, and methoxychlor. Number and concentrations of OC compounds in CAMs were variable. The most prevalent contaminant detected was DDE, which occurred in 69% of CAMs, with concentrations ranging from 0.3 parts per billion (ppb) to 17.0 ppb. The OC burdens in crocodile CAMs confirm contamination of eggs and suggest exposure in embryos and maternal females. These results further support the use of CAMs as qualitative indicators of OC exposure in oviparous wildlife. The efficacy of this sampling technique in the field will depend on the logistics and cost associated with CAM collection and the specific life history traits of the wildlife species.

Key words: Belize, chorioallantoic membrane, *Crocodylus moreletii,* eggs, Morelet's crocodile, organochlorine pesticides, reptiles, wildlife.

INTRODUCTION

Examination of environmental contaminant exposure in wildlife often involves killing animals for collection and analysis of various biologic samples (e.g., internal tissues and organs). However, in recent years much emphasis has been placed on the development of nonlethal and noninvasive sampling techniques that provide adequate samples while rendering study animals unharmed (Cobb et al., 2003). For studies involving oviparous wildlife, one such nonlethal sampling technique involves assessment of contaminant exposure in maternal females, eggs, and neonates from contaminant loads in the chorioallantoic membrane (CAM) of hatched eggs. The CAM is a highly vascularized extraembryonic membrane that serves as a site for gas exchange, nutrient transport, and waste storage during embryonic development (Romanoff, 1960; Ferguson, 1985). As such, the CAM receives considerable blood flow from the embryo and is therefore likely to contain the same contaminants to which the embryo is exposed (Bargar et al., 2003). After hatching, the CAM is usually retained in the eggshell and can be easily collected for chemical residue analysis (Bargar et al., 1999). In a laboratory study with white leghorn chickens, Bargar et al. (2003) recently demonstrated that CAMs accurately described polychlorinated biphenyl (PCB) burdens in adults and eggs, and that PCB concentrations in CAMs were significantly related to biologic response (i.e., hepatic cytochrome P450 isozyme activity) in adults and neonates.

The CAM was originally used in ecotoxicological research to assess contaminant levels in birds (Cobb et al., 1994, 1995) and was hypothesized as a potential indi-

cator of chlorinated contaminant exposure in oviparous reptiles. The practicality of use of CAMs as indicators of PCB exposure was evaluated in loggerhead sea turtles (*Caretta carreta*) and American alligators (*Alligator mississippiensis*) (Cobb et al., 1997; Cobb and Wood, 1997), respectively. Detectable levels of PCBs in CAMs were reported in both studies, confirming contaminant accumulation in the membrane and strengthening the case for using CAMs as indicators of exposure in embryos and maternal females. Shortly thereafter, Bargar et al. (1999) found significant correlations between PCB levels in CAMs, fat, and yolk of neonatal alligators, further supporting the use of CAMs to determine organochlorine (OC) burdens in oviparous reptiles.

Morelet's crocodile (*Crocodylus moreletii*) is an endangered, freshwater crocodile found in the Atlantic and Caribbean lowlands of Mexico, Guatemala, and Belize (Ross, 1998; Platt and Thorbjarnarson, 2000). In recent years, multiple environmental contaminants, including OC pesticides, have been detected in nonviable Morelet's crocodile eggs from northern and southern Belize (Wu et al., 2000a, b; Rainwater et al., 2002). The objectives of this study were to examine levels of OC pesticides in CAMs of Morelet's crocodiles from northern Belize and further assess the practicality of CAMs as nonlethal, noninvasive samples for determining OC exposure in oviparous wildlife.

MATERIALS AND METHODS

Morelet's crocodile CAMs were collected in 1998–2000 from Gold Button Lagoon (GBL; 17°55′N, 88°45′W) as part of a study of reproductive ecology and toxicology of this species in northern Belize (Platt, 1996; Platt et al., 2000; Wu et al., 2000a, b; Rainwater et al., 2002). Crocodile nests were located at the onset of the nesting season (late June–early July). Just before hatching (about day 60 of incubation), eggs were removed from the field and hatched in captivity at the Lamanai Field Research Center, Indian Church Village, Orange Walk District, Belize. Upon hatching, CAMs were removed from the discarded eggshells by using sterile forceps, placed individually in chemically cleaned glass jars, and stored at −20 C. The exception was in 1998, when 10 CAMs were left in their respective eggshells and stored at −20 C until shipment to the laboratory. All samples were ultimately shipped to The Institute of Environmental and Human Health at Texas Tech University (Lubbock, Texas, USA) and stored at −20 C until analysis.

Chorioallantoic membranes were analyzed for OC pesticide exposure by using a mixed standard consisting of tetrachloro-meta-xylene (TCMX), heptachlor, γ-benzene hexachloride (γ-BHC; lindane), α-BHC, β-BHC, δ-BHC, endosulfan I, endosulfan II, dieldrin, endrin, *p,p*-dichlorodiphenyldichloroethane (*p,p*-DDD), *p,p*-dichlorodiphenyltrichloroethane (*p,p*-DDT), *p,p*-dichlorodiphenyldichloroethylene (*p,p*-DDE), methoxychlor, aldrin, heptachlor epoxide, γ-chlordane, α-chlordane, endrin aldehyde, endosulfan sulfate, endrin ketone, and decachlorobiphenyl (DCBP) obtained from Ultra Scientific (North Kingstown, Rhode Island, USA). The organic solvents used were either pesticide or gas chromatography–mass spectrometry (GC-MS) grade. Anhydrous sodium sulfate used in the extraction procedure had a purity of 99%.

The entire CAM was extracted after removing (i.e., washed, scraped, and wiped) all residual yolk, albumin, and shell. The CAMs were individually weighed in clean weigh boats, mixed with approximately 10 g of anhydrous sodium sulfate, and dried overnight. Before extraction, all samples were fortified with an internal standard (DCBP and TCMX). Samples were extracted by using a Dionex 200 Accelerated Solvent Extractor (Dionex Inc., Sunnyvale, California, USA) in 33-ml cells. The following parameters were used: preheat=5 min, heat=5 min, static=5 min, flush %=60, purge %=60, cycles=1, pressure=1×10^7 Pa, temperature=100 C, and solvent=100% dichloromethane. Extracts were collected in 60-ml glass vials with Teflon® caps. Each extract was filtered into a clean 125-ml round-bottom flask using filter paper filled with anhydrous sodium sulfate to remove any remaining water. Next, the volume of each filtered extract was reduced to approximately 0.5 ml by using rotary evaporation. Concentrated extracts were transferred into 1-ml volumetric flasks and then filtered by using a 0.45-μm Acrodisc filter (Pall Gelman, Ann Arbor, Michigan, USA) into 2-ml amber GC vials and stored at −20 C until use.

To remove substantial lipid material in CAM extracts, gel permeation chromatography (GPC) was used, following US Environmental Protection Agency (US EPA) Method 3640. A Hewlett-Packard 1100 liquid chromatograph (Hewlett-Packard, Palo Alto, California) equipped

with an ultraviolet detector and a Plgel column (pore size=50 A, Hewlett-Packard) was used to separate and collect the appropriate fraction. A GPC standard consisting of five known compounds (corn oil, phthalate, methoxychlor, perylene, and sulfur) was used to determine the collection interval. Hewlett-Packard ChemStation software was used to control and monitor the chromatography. The fraction collected was after phthalate and through the perylene peak. Fractions were collected into 125-ml round-bottom flasks by using an ISCO Foxy 200 fraction collector (Isco Inc., Lincoln, Nebraska, USA). The collected fractions were then reduced to 0.5 ml in volume by rotary evaporation. The solvent was exchanged into hexane and then evaporated to 0.5 ml. The solvent exchange was repeated and the final volume of extract was 1 ml. Finally, the extracts were filtered and transferred into 2-ml amber GC vials. Each sample was stored at −20 C until GC analysis.

A Hewlett-Packard 6890 gas chromatograph with a ^{63}Ni electron capture detector and a 30-m×0.32-mm column (0.25-μm film thickness) with HP-5 stationary phase (Hewlett-Packard) was used to separate and quantify all OC residues. The gas chromatograph was operated in the splitless mode with helium as the carrier gas (7 ml/min) and argon:methane as the make-up gas (60 ml/min). The temperature program was as follows: initial temperature=80 C; increased to 180 C at 25 C/min; from 180 C to 205 C at 2.5 C/min with 2-min hold; from 205 C to 250 C at 15 C/min with 1-min hold; and from 250 C to 300 C at 20 C/min. Hewlett-Packard ChemStation software was used to control and monitor the chromatography. To quantify OC residues in the CAM samples, a seven-point standard curve consisting of the pesticide mixture described earlier was developed. Detection limits (based on DDE) were 0.33 ng/g for CAM samples.

RESULTS

A total of 122 CAMs from seven clutches of eggs were analyzed for OCs in this study (Table 1). Nine different OC compounds were detected in CAMs, with DDE the most common (69% of the CAMs), ranging in concentration from 0.3 parts per billion (ppb) to 17.0 ppb. Prevalence of the remaining contaminants was as follows: heptachlor (61%), DDT (31%), dieldrin (31%), endrin (25%), methoxychlor (17%), DDD (15%), lindane (13%), and aldrin (10%) (Table 1). Dichlorodiphenyldichloroethylene, heptachlor, and DDT were also the most prevalent OC compounds detected among clutches, occurring in six (86%) of the seven clutches.

Numbers and concentrations of OC compounds detected in CAMs were variable. The number of individual OC compounds in a given CAM ranged from zero to eight, whereas detectable concentrations of individual contaminants in a CAM ranged from 0.3 ppb to 112 ppb. Mean OC concentrations among clutches ranged from 0.3 ppb (DDE) to 34.8 ppb (methoxychlor). Overall, methoxychlor was found at the highest concentrations but occurred in only 21 (17%) of the CAMs. Excluding methoxychlor, variation in mean OC concentrations among clutches was minimal.

To assess the variation in residue data among the complete clutches, we evaluated residue levels for the most frequently detected OC compounds (DDE, heptachlor, DDT, and dieldrin) in CAMs. These results (Table 2) indicate that there is wide variation (large coefficient of variation [CV]) for particular OC compounds in different clutches (CV=0–153%); however, the clutches are reasonably consistent in variation with respect to the OC compounds (for example, clutch 4, CV=50–88%).

DISCUSSION

Results of this study indicate that OC pesticides accumulated in CAMs from Morelet's crocodile eggs in northern Belize, demonstrating OC exposure of embryos and maternal females. Organochlorine concentrations in CAMs were comparable, but less than OC concentrations previously found in Morelet's crocodile eggs from Gold Button Lagoon (Wu et al., 2000a) and other sites in northern and southern Belize (Wu et al., 2000b). Dichlorodiphenyldichloroethylene was the most common OC compound found in CAMs in this study, and also in eggs from Morelet's crocodiles and American crocodiles (*Crocodylus acutus*) from multiple sites in northern, southern, and coastal Be-

TABLE 1. Mean (±SD) concentrations (ng/g) of organochlorine pesticides in chorioallantoic membranes from hatched Morelet's crocodile eggs from Gold Button Lagoon, northern Belize. Values in parentheses are concentration ranges.[a]

		Organochlorine pesticide								
Clutch	Number	Aldrin	Dieldrin	Endrin	DDT	DDD	DDE	Heptachlor	Lindane	Methoxychlor
1998										
Clutch 11	10	ND	ND	ND	ND	ND	ND	ND	ND	64.1[b]
1999										
Clutch 1	4	ND	ND	ND	2.6±0.3 (2.4–2.9)	ND	1.3±0.0	2.7±0.2 (2.6–2.9)	ND	3.9±0.4 (3.6–4.3)
Clutch 8	17	ND	6.0±5.2 (2.5–9.7)	ND	4.7±1.3 (3.3–5.9)	ND	4.4±6.6 (0.5–12.0)	4.1±2.0 (1.5–6.9)	ND	34.8±35.1 (5.1–112)
2000										
Clutch 1	29	ND	1.1±1.2 (0.3–1.5)	0.7±0.5 (0.5–1.5)	6.8±7.9 (0.6–8.1)	1.0±0.24 (0.7–1.2)	0.5±0.3 (0.4–1.0)	0.8±0.5 (0.5–2.0)	ND	17.6±39.0 (0.6–106.0)
Clutch 4	37	0.8±0.1 (0.6–0.9)	7.0±3.5 (0.6–12.1)	6.4±2.0 (3.8–9.6)	5.0±3.2 (1.6–10.8)	1.7±0.8 (0.9–2.4)	1.6±1.4 (0.6–8.1)	1.0±0.7 (0.3–3.2)	2.2±0.6 (1.7–3.0)	ND
Clutch 6	19	2.0±2.2 (0.6–6.9)	10.8±15.4 (0.3–62.0)	12.7±17.5 (0.3–69.0)	9.3±14.2 (0.7–57.0)	6.7±10.0 (0.5–19.0)	2.8±4.1 (0.3–17.0)	1.9±2.5 (0.3–8.6)	2.3±3.5 (0.4–12.0)	ND
Clutch 7	6	ND	0.5±0.2 (0.3–0.7)	0.5±0.04 (0.4–0.5)	1.3±1.1 (0.5–2.1)	ND	0.3±0.1 (0.3–0.4)	0.5±0.2 (0.4–0.6)	ND	ND

[a] DDT = dichlorodiphenyltrichloroethane; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; ND = not detected by the analytical method (limit of detection = 0.33 ppb).

[b] Contaminant detected in only one egg.

TABLE 2. Coefficients of variation (%) for organochlorine residue data[a] from chorioallantoic membranes from hatched Morelet's crocodile eggs from Gold Button Lagoon, northern Belize.

Clutch	DDE	Heptachlor	DDT	Dieldrin
1998				
Clutch 11	NA	NA	NA	NA
1999				
Clutch 1	0	7.4	12	NA
Clutch 8	150	49	28	87
2000				
Clutch 1	60	63	116	109
Clutch 4	88	70	64	50
Clutch 6	146	132	153	143
Clutch 7	33	40	85	40

[a] DDE = dichlorodiphenyldichloroethylene; DDT = dichlorodiphenyltrichloroethane; NA = not applicable.

lize (Wu et al., 2000a, b). Multiple reproductive abnormalities including low clutch viability, altered plasma steroid hormone concentrations, and reduced phallus size have been observed in alligators living in OC-contaminated lakes in Florida, USA (Woodward et al., 1993; Guillette et al., 1994, 1996; Crain et al., 1998). Studies are currently underway to examine similar endpoints in Morelet's crocodiles living in contaminated habitats in Belize (Rainwater, 2003).

The OC contaminants detected in CAMs during this study likely originate from multiple sources. Many OC compounds, particularly DDT, continue to be used in Belize for controlling agricultural pests and disease vectors (Grieco et al., 2000). In addition, chemical spills and poor storage and disposal practices may contribute to environmental contamination. Once released into the environment, these compounds likely enter crocodile habitat in storm-water runoff and by atmospheric deposition. Because of their high environmental persistence, OC compounds may then be bioaccumulated and biomagnified in top-level carnivores (e.g., crocodiles) through trophic transfer. The primary route of OC contamination in eggs is believed to be maternal transfer, whereby contaminants in gravid females are transferred to developing follicles during vitellogenesis (Matter et al., 1998; Russell et al., 1999; Bargar et al., 2001). A secondary route of exposure may be pesticide transfer into eggs from contaminated nest media. Cañas and Anderson (2002) found that eggs of bullsnakes (*Pituophis melanoleucus*) incubated in nest media dosed with OC pesticides accumulated five of the six OC compounds tested. Although this process has not been specifically examined in crocodilian eggs, it is likely to occur given the structure of the eggshell and its gas-exchange capacity (Kern and Ferguson, 1997). Seven of the nine (78%) OC compounds detected in CAMs in this study have been previously found in crocodile nest material from the same lagoon (Wu et al., 2000a).

Most studies evaluating the utility of CAMs as nonlethal and noninvasive indicators of contaminant exposure in oviparous wildlife have compared contaminant burdens in multiple sample tissues from the same organism. Pastor et al. (1996) compared OC concentrations in CAMs from eggs of Audouin's gull (*Larus audouinii*) to their corresponding embryos and yolks. Correspondingly, Cobb et al. (1997) and Cobb and Wood (1997) compared individual CAMs to their corresponding residual yolk in American alligators and loggerhead sea turtles, respectively. In the present study, because of the endangered status of Morelet's crocodile, it was not possible to examine the rela-

tionship between OC compounds in CAMs and other tissues (e.g., embryos and neonatal organs) from corresponding crocodiles, because sacrificing neonates to obtain internal tissues was not an option. In addition, we did not have access to residual yolk contents corresponding to the CAMs collected, although in some instances we did analyze nonviable eggs from the corresponding nest. Thus, although this study qualitatively demonstrates OC contamination in Morelet's crocodile CAMs in Belize, the efficacy of this membrane as a quantitative indicator of OC concentration in developing, neonatal, and maternal crocodiles from the wild remains unknown. Because of the variability in the number and concentrations of OCs in individual CAMs collected in the wild, quantifying OC exposure in an entire clutch or a maternal female from a subsample of CAMs should be avoided, unless one has determined the relationship between OCs in CAMs, maternal females, and offspring through laboratory dosing studies (Bargar et al., 2003).

In some of our previous work with complete clutches of Morelet's crocodile eggs, we used the inherent variation in DDE concentrations within a clutch and a desired confidence interval to design a sampling model (Wu, 2000). The purpose of the model was to estimate the number of eggs in a nest that would need to be sampled in order to predict the average DDE burden within a clutch. We used this same technique on the CAM data by incorporating the average standard deviation (in DDE concentration) and average clutch size of three complete clutches of CAMs. The predicted optimum sample size calculated when using this approach is 15 CAMs and 18 CAMs for the 80% and 90% confidence intervals, respectively. Although useful as a proof of concept, our attempt to determine the optimal number of CAMs was limited by several factors, including small sample sizes and nonuniform DDE contamination. Moreover, the optimal number determined for CAMs in this study was greater than the optimal number of eggs derived by Wu (2000), which suggests greater OC variation in CAMs as compared to eggs.

Although the use of CAMs as indicators of OC contamination in oviparous wildlife shows promise, this technique does have limitations. Most bird studies and all reptile studies that have examined contaminants in CAMs have obtained samples from eggs laid in captivity or collected in the field before hatching. Hatching eggs in captivity assures the recovery of all CAMs, but significant logistics and costs may be involved in collecting and incubating eggs and in caring for and releasing the neonates. In addition, removing eggs from the wild and later releasing captive neonates may disrupt certain aspects of an animal's life history (e.g., growth, behavior, reproduction, and survival) and bias other interesting ecological endpoints. Juvenile farm-raised alligators released into a freshwater marsh in Louisiana exhibited higher mortality rates than wild juveniles (Chabreck et al., 1998). Similarly, survival of captive-raised waterfowl was lower when compared to that of wild birds (Brakhage, 1953; Soutiere, 1989). Neonates hatched in captivity should be released at their respective nest sites as soon as possible after hatching to minimize impacts on survival and other life history parameters.

For the use of CAMs to be nonlethal and noninvasive, they should be collected from nest sites after hatching. However, species-specific life histories may preclude the availability of eggshells after hatching. Many crocodilians, including Morelet's crocodile, consume or dispose of nonhatched eggs and eggshells shortly after hatching occurs (Alvarez del Toro, 1969; Hunt, 1975, 1980, 1987; Pooley, 1977; Deitz and Hines, 1980; Kushlan and Simon, 1981). Opportunistic predators, such as raccoons (*Procyon lotor*) and ants, exacerbate this process, because they are also known to consume nonhatched eggs and eggshells shortly after hatching (Platt, 1996). Conversely, eggshells may be easily

collected from other oviparous wildlife such as colonial nesting birds. Norman (1992) discovered and collected more than 150 freshly hatched eggshells containing CAMs beneath great blue heron (*Ardea herodias*) nests near Puget Sound (Washington, USA). Hence, the efficacy of CAMs as nonlethal and noninvasive indicators of OC contamination in oviparous wildlife will depend on the logistical and financial constraints, as well as the specific life history traits of the study species.

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LITERATURE CITED

ALVAREZ DEL TORO, M. 1969. Breeding the spectacled caiman *Caiman crocodylus* at Tuxtla Gutierrez Zoo. International Zoo Yearbook 9: 35–36.

BARGAR, T. A., C. SILLS-MCMURRY, R. L. DICKERSON, W. E. RHODES, AND G. P. COBB. 1999. Relative distribution of polychlorinated biphenyls among tissues of neonatal American alligators (*Alligator mississippiensis*). Archives of Environmental Contamination and Toxicology 37: 364–368.

———, G. I. SCOTT, AND G. P. COBB. 2001. Maternal transfer of contaminants: a case study of the excretion of three PCB congeners and technical grade endosulfan into eggs by white leghorn chickens (*Gallus domesticus*). Environmental Toxicology and Chemistry 20: 61–67.

———, ———, AND ———. 2003. Chorioallantoic membranes indicate avian exposure and biomarker responses to environmental contaminants: A laboratory study with white leghorn chickens (*Gallus domesticus*). Environmental Science and Technology 37: 256–260.

BRAKHAGE, G. K. 1953. Migration and mortality of ducks hand-reared and wild-trapped at Delta, Manitoba. Journal of Wildlife Management 17: 465–477.

CAÑAS, J. E., AND T. A. ANDERSON. 2002. Organochlorine contaminants in eggs: the influence of contaminated nest material. Chemosphere 47: 585–589.

CHABRECK, R. H., V. L. WRIGHT, AND B. G. ADDISON, JR. 1998. Survival indices for farm-released American alligators in a freshwater marsh. *In* Proceedings of the 14th working meeting of the Crocodile Specialist Group, International Union for the Conservation of Nature and Natural Resources (IUCN)—The World Conservation Union, Gland, Switzerland, and Cambridge, UK, pp. 293–304.

COBB, G. P., AND P. D. WOOD. 1997. PCB concentrations in eggs and chorioallantoic membranes of loggerhead sea turtles (*Caretta caretta*) from the Cape Romain National Wildlife Refuge. Chemosphere 34: 539–549.

———, D. M. NORMAN, AND R. J. KENDALL. 1994. Organochlorine contaminant assessment in great blue herons using traditional and non-lethal monitoring techniques. Environmental Pollution 83: 299–309.

———, ———, M. W. MILLER, AND L. W. BREWER. 1995. Chlorinated contaminants in chorioallantoic membranes from great blue herons at Whidbey Island Naval Air Station. Chemosphere 34: 539–549.

———, P. D. WOOD, AND M. O'QUINN. 1997. Polychlorinated biphenyls in eggs and chorioallantoic membranes of American alligators (*Alligator mississippiensis*) from coastal South Carolina. Environmental Toxicology and Chemistry 16: 1456–1462.

———, T. A. BARGAR, C. B. PEPPER, D. M. NORMAN, P. D. HOULIS, AND T. A. ANDERSON. 2003. Using chorioallantoic membranes for non-lethal assessment of persistent organic pollutant exposure and effect in oviparous wildlife. Ecotoxicology 12: 31–45.

CRAIN, D. A, L. J. GUILLETTE, JR., D. B. PICKFORD, H. F. PERCIVAL, AND A. R. WOODWARD. 1998. Sex-steroid and thyroid hormone concentrations in juvenile alligators (*Alligator mississippiensis*) from contaminated and reference lakes in Florida, USA. Environmental Toxicology and Chemistry 17: 446–452.

DEITZ, D. C., AND T. C. HINES. 1980. Alligator nesting in north-central Florida. Copeia 2: 249–258.

FERGUSON, M. W. J. 1985. Reproductive biology and embryology of the crocodilians. *In* Biology of the reptilia, C. Gans, F. Billet, and P. F. A Maderson (eds.). John Wiley and Sons, New York, New York, pp. 329–491.

GRIECO, J. P., N. L. ACHEE, R. G. ANDRE, AND D. R. ROBERTS. 2000. A comparison study of house entering and exiting behavior of *Anopheles vestitipennis* (Diptera: Culicidae) using experimen-

tal huts sprayed with DDT or deltamethrin in the southern District of Toledo, Belize, C.A. Journal of Vector Ecology 25: 62–73.

GUILLETTE, L. J., JR., T. S GROSS, G. R. MASSON, J. M. MATTER, H. F. PERCIVAL, AND A. R. WOODWARD. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environmental Health Perspectives 108: 680–688.

———, D. B. PICKFORD, D. A. CRAIN, A. A. ROONEY, AND H. F. PERCIVAL. 1996. Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. General and Comparative Endocrinology 101: 32–42.

HUNT, R H. 1975. Maternal behavior in the Morelet's crocodile, *Crocodylus moreletii.* Copeia 4: 763–764.

———. 1980. Propagation of Morelet's crocodile. *In* Reproductive biology and diseases of captive reptiles, J. B. Murphy and J. T. Collins (eds.). Contributions to Herpetology Number 1. Society for the Study of Amphibians and Reptiles, Oxford, Ohio, pp. 161–165.

———. 1987. Nest excavation and neonate transport in wild *Alligator mississippiensis.* Journal of Herpetology 21: 348–350.

KERN, M. D., AND M. W. J. FERGUSON. 1997. Gas permeability of American alligator eggs and its anatomical basis. Physiological Zoology 70: 530–546.

KUSHLAN, J. A., AND J. C. SIMON. 1981. Egg manipulation by the American alligator. Journal of Herpetology 15: 451–454.

MATTER, J. M., D. A. CRAIN, C. SILLS-MCMURRY, D. B. PICKFORD, T. R. RAINWATER, K. D. REYNOLDS, A. A. ROONEY, R. L. DICKERSON, AND L. J. GUILLETTE, JR. 1998. Effects of endocrine disrupting contaminants in reptiles: Alligators. *In* Principles and processes for evaluating endocrine disruption in wildlife, R. J. Kendall, R. L. Dickerson, J. P. Giesy, and W. P. Suk (eds.). SETAC Press, Pensacola, Florida, pp. 267–289.

NORMAN, D. M. 1992. Organochlorine pesticides and polychlorinated biphenyl congeners in great blue herons from the Puget Sound ecosystem. MS Thesis, Western Washington University, Bellingham, Washington, 248 pp.

PASTOR, D., X. RUIZ, L. JOYER, AND J. ALBAIGE. 1996. The use of chorioallantoic membranes as predictors of egg organochlorine burden. Environmental Toxicology and Chemistry 15: 167–171.

PLATT, S. G. 1996. The ecology and status of Morelet's crocodile in Belize. PhD Dissertation, Clemson University, Clemson, South Carolina, 187 pp.

———, AND J. B. THORBJARNARSON. 2000. Population status and conservation of Morelet's crocodile (*Crocodylus moreletii*) in northern Belize. Biological Conservation 96: 21–29.

———, T. R. Rainwater, and S. T. McMurry. 2000. Twinning in Morelet's crocodile (*Crocodylus moreletii*) and a brief review of twinning in crocodilians. Herpetological Natural History 7: 181–185.

POOLEY, A. C. 1977. Nest opening response of the Nile crocodile, *Crocodylus niloticus.* Journal of Zoology (London) 182: 17–26.

RAINWATER, T. R. 2003. Ecotoxicology of Morelet's crocodile in Belize. PhD Dissertation, Texas Tech University, Lubbock, Texas, 156 pp.

———, B. M. ADAIR, S. G. PLATT, T. A. ANDERSON, G. P. COBB, AND S. T. MCMURRY. 2002. Mercury in Morelet's crocodile eggs from northern Belize. Archives of Environmental Contamination and Toxicology 42:319–324.

ROMANOFF, A. L. 1960. The avian embryo. Macmillan Publishing Company, New York, New York, 1305 pp.

ROSS, J. P. 1998. Crocodiles: Status survey and conservation action plan. 2nd Edition. Species Survival Commission–International Union for the Conservation of Nature and Natural Resources (IUCN) Crocodile Specialist Group, Gland, Switzerland, 136 pp.

RUSSELL, R. W., F. A. P. C. GOBAS, AND G. D. HAFFNER. 1999. Maternal transfer and in ovo exposure of organochlorines in oviparous organisms: A model and field verification. Environmental Science and Technology 33: 416–420.

SOUTIERE, E. C. 1989. Survival rates of hand-reared mallards released on two private farms in Maryland. Journal of Wildlife Management 53: 114–118.

WOODWARD, A. R., H. F. PERCIVAL, M. L. JENNINGS, AND C. T. MOORE. 1993. Low clutch viability of American alligators on Lake Apopka. Florida Scientist 56: 52–63.

WU, T. H. 2000. Evaluation of organochlorine residues in Morelet's and American crocodile eggs from Belize. MS Thesis, Texas Tech University, Lubbock, Texas, 120 pp.

———, T. R. RAINWATER, S. G. PLATT, S. T. MCMURRY, AND T. A. ANDERSON. 2000a. Organochlorine contaminants in Morelet's crocodile (*Crocodylus moreletii*) eggs from Belize. Chemosphere 40: 671–678.

———, ———, ———, ———, AND ———. 2000b. DDE in eggs of two crocodile species from Belize. Journal of Agricultural and Food Chemistry 48: 6416–6420.

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