ABSTRACT—We evaluated the concentrations of three heavy metals (cadmium, chromium, lead) in eggs and caudal scutes of nesting female *Crocodylus moreletii* in El Carpintero Lagoon, Tamaulipas, Mexico, in the months of May to August 2013. Samples were processed and analyzed based on the method of flame atomic absorption spectrophotometry at the Centro de Investigación y Tecnología en Saneamiento Ambiental (CITSA). Our results showed the presence of cadmium, chromium, and lead in both caudal scutes and eggs. The three heavy metals showed values in nesting females of 20.3, 5.0, and 28.0 mg kg\(^{-1}\), respectively. We observed significant differences in levels of cadmium and lead among nests but not with levels of chromium. The variations in heavy metal concentrations in eggs suggest that extrinsic and intrinsic factors simultaneously affect crocodiles during the reproductive season. Our levels were high compared with other species of crocodilians and are above thresholds established by Mexican pollution laws.

Resumen—Evaluamos las concentraciones de tres metales pesados (cadmio, cromo y plomo) en huevos y escamas caudales de hembras anidadoras de *Crocodylus moreletii* en la Laguna el Carpintero, Tamaulipas, México, entre los meses de mayo y agosto de 2013. Las muestras fueron procesadas y analizadas en el Centro de Investigación y Tecnología en Saneamiento Ambiental (CITSA), con base en el método de espectrofotometría de absorción atómica de llama. Nuestros resultados mostraron la presencia de los tres metales pesados tanto en las escamas caudales como en los huevos. El cadmio, el cromo y el plomo mostraron valores en hembras anidadoras de 20.3, 5.0 y 28.0 mg kg\(^{-1}\), respectivamente. Se observaron diferencias significativas en los niveles de cadmio y plomo entre los nidos, pero no con los niveles de cromo. Las variaciones en las concentraciones de metales pesados en los huevos sugieren que factores extrínsecos e intrínsecos afectan simultáneamente a los cocodrilos durante la temporada reproductiva. Nuestros niveles fueron altos en comparación con otras especies de crocodilianos y están por encima de los umbrales establecidos por las leyes mexicanas de contaminación.

The Morelet’s crocodile *Crocodylus moreletii* is distributed in the lowlands from Tamaulipas to Yucatan Peninsula, Mexico, including Guatemala and Belize (Platt et al., 2010). This species inhabits several types of waterbodies such as marshes, swamps, ponds, rivers, lakes, lagoons, and man-made habitats (Platt et al., 2010; Sánchez et al., 2011). In relation to its conservation status, population viability analysis across the Morelet’s crocodile range suggested the need to change its conservation status from Appendix I to Appendix II of CITES for Mexico and Belize, which allows the international commercialization of animals coming from breeding farms (Convention on International Trade in Endangered Species, https://www.cites.org/esp/app/appendices.php [20 November 2017]). In addition, this species was removed from the Federal List of Endangered and Threatened Wildlife by the United States Fish and Wildlife Service based on available survey data from Mexico and Belize (United States Fish and Wildlife Service, 2012). Although the United States Fish and Wildlife Service recognizes that water contamination from pesticides and heavy metals is one of the primary threats to Morelet’s crocodile conservation status, the lack of information on the potential effects of contamination on population survival is an impediment to generate appropriate conservation
actions for this species in the future (United States Fish and Wildlife Service, 2012).

Currently, only two studies have been conducted to evaluate heavy metals in Morelet’s crocodiles in southeast Mexico. Trillanes et al. (2014) reported the presence of arsenic, mercury, lead, nickel, cadmium, and chromium in the caudal scutes of captive and wild populations from Yucatan Peninsula. In another study, Buenfil-Rojas et al. (2015) focused on the concentrations of cadmium and mercury in blood plasma and caudal scutes of wild animals from Rio Hondo. However, we are not aware of any study reporting the concentrations of heavy metals in nesting females and their eggs in urban environments. Available information suggests that heavy metals could have negative effects on reproduction, survivorship, and growth in wild populations of crocodilians (Rainwater et al., 2002; Xu et al., 2006). Therefore, a first step is to determine the concentrations of heavy metals in order to know the potential risk for conservation programs based on wild crocodilians. Hence, our study provides useful information about heavy metals in eggs and scutes of Morelet’s crocodile in an urban lagoon at the northern portion of its geographic distribution in Mexico.

The study was conducted in 2013 in El Carpintero Lagoon located in the municipality of Tampico, Tamaulipas (22°12′25"–22°12′01"N, 97°50′11"–97°57′10"W, elev. <5 m, datum WGS84). We located three active nests during April, corresponding to the onset of the nesting season of *C. moreletii* (Platt et al., 2008; López-Luna et al., 2011). Nest sites were separated by at least 0.20 km. We opened the egg chamber carefully to mark each egg using a pencil on the top surface in order to maintain both the original position and orientation (Hutton and Webb, 1992). We manipulated the eggs using latex gloves and plastic trays to avoid contaminating the samples. We determined the egg viability based on the presence of opaque egg bands indicating embryo development was underway (Ferguson, 1985). Upon hatching (from late June to July), 10 eggs from each nest (egg shells, unviable eggs, or eggs with dead embryo) were randomly collected in polypropylene containers and maintained at −10°C. We captured the nesting females during maternal care and Tecnología en Saneamiento Ambiental of the Instituto de Estudios Superiores de Tamaulipas, with a certification code by Mexican Accreditation: AG-0481-055/13. We analyzed for the presence of heavy metals in scutes and eggs in accordance with section 7.0 of the Mexican Official Norm (NOM-117-SSAI-1994; http://www.cofepris.gob.mx/MJ/Paginas/NormasPorTema/Metodos-de-prueba.aspx [20 November 2016]). Briefly, section 7.0 of NOM-117-SSAI-1994 explains the method of open-cup digestion, which consists of having a sample of 10 g fortified with reference material. We placed the samples in a drying oven for 24 h at 100°C, and then placed them in an Erlenmeyer flask of 250 mL with 50 mL of distilled water, 5 mL of nitric acid, and 2.5 mL of sulfuric acid. We heated this mixture to 150°C on a heating grid to the boiling point. We evaluated the concentrations of cadmium, chromium, and lead because they are toxic and persistent in industrial zones. We evaluated these heavy metals because they have been evaluated successfully on eggshells of different crocodilian species, which allows comparisons between the same species and different species (see Table 1). We measured the concentrations of cadmium, chromium, and lead by flame atomic absorption spectrophotometry (model 55B AA, Agilent Technologies, Santa Clara, California). The reference material consisted of a metal solution at 1,000 ppm with a tolerance of ±2 ppm (Merck AccuStandard, New Haven, Connecticut). In this study, the recovery percentage was 92.7 ± 3 for cadmium, 99.0 ± 0 for chromium, and 108.7 ± 3 for lead. We conducted one-way analysis of variance (ANOVA) tests to compare concentrations of cadmium, chromium, and lead among nests. We also used a nonparametric Spearman coefficient to examine the correlation between heavy metals (cadmium-
chromium [Cd-Cr], cadmium-lead [Cd-Pb], and chromium-lead [Cr-Pb]). We performed all statistical analyses using PAST 3.15 (Hammer et al., 2001) and we considered $P < 0.05$ to be significant.

The three nesting females had a total length of 1.97, 2.08 and 2.37 m. Cadmium, chromium, and lead were detected in all females. Cadmium levels measured in nesting females ranged from 4.6 to 20.3 mg kg$^{-1}$, with a mean of 11.2 ± 8.2 mg kg$^{-1}$. Lead levels ranged from 13.8 to 28.0 mg kg$^{-1}$ with a mean of 22.1 ± 7.4, and chromium levels were 5.0 mg kg$^{-1}$ in each nesting female. The mean clutch size in three Morelet’s crocodile nests was 38.7 ± 1.5 eggs (range, 37–40 eggs; Table 2). Heavy metals were detected in all eggshell samples. Cadmium levels differed significantly among nests ($F_{2,27} = 4.5, P = 0.02$; Table 2), with the lowest mean level of 3.4 ± 1.1 mg kg$^{-1}$ at nest 3 and the highest mean level of 8.0 ± 5.4 mg kg$^{-1}$ at nest 1. The mean chromium levels, by nest, were 15.8 ± 11.4 mg kg$^{-1}$ at nest 1, 16.3 ± 13.7 mg kg$^{-1}$ at nest 2, and 10.7 ± 3.8 mg kg$^{-1}$ at nest 3, but this difference was not significant ($F_{2,27} = 0.86, P = 0.43$; Table 2). The mean of lead levels varied among nests ($F_{2,27} = 7.0, P < 0.01$; Table 2), ranging from a nest with low mean levels of 5.3 ± 3.4 mg kg$^{-1}$ to a nest with the highest mean level of 46.5 ± 41.6 mg kg$^{-1}$. The Spearman coefficient showed a positive correlation between cadmium and chromium ($r = 0.9, P < 0.01$), but the correlation was not significant for chromium and lead ($r = 0.3, P = 0.07$) and for cadmium and lead ($r = -0.1, P = 0.66$).

Our results constitute a first step in assessing the population health status of Morelet’s crocodile in an urban ecosystem at its northern distribution. Although Villegas and Reynoso (2013) reported an encounter rate between 8.8 and 27.5 crocodiles per kilometer, only three active nests per year have been recorded around the water body (C.N.C.L., pers. observ.). This limited sample size is insufficient to determine whether there is a relationship between heavy metal levels of nesting females and nests. The high concentrations of heavy metals found in both females and nests suggest a prolonged exposure or an easy metal absorption; however, the potential negative effects (reproductive success, physiological and genetic alterations) of chronic exposure to different heavy metals in urban populations are unclear. Because El Carpintero Lagoon is inside of Tampico City, some anthropogenic contamination sources such as wastewater discharges, combustion of fossil fuels from vehicular traffic, and motor aquatic races may be the main pollution sources. Therefore, future research

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Nest 1</th>
<th>Nest 2</th>
<th>Nest 3</th>
<th>Female 1</th>
<th>Female 2</th>
<th>Female 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>8.034 ± 5.42</td>
<td>4.19 ± 3.13</td>
<td>3.43 ± 1.13</td>
<td>20.30</td>
<td>8.60</td>
<td>4.62</td>
</tr>
<tr>
<td>Cr</td>
<td>15.83 ± 11.45</td>
<td>16.29 ± 13.74</td>
<td>10.73 ± 5.83</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Pb</td>
<td>3.31 ± 3.40</td>
<td>46.55 ± 41.57</td>
<td>25.51 ± 8.87</td>
<td>24.30</td>
<td>13.80</td>
<td>27.98</td>
</tr>
</tbody>
</table>
should attempt to elucidate the impact of heavy metals on crocodilian population dynamics.

The Crocodile Specialist Group of the Species Survival Commission, International Union for Conservation of Nature funded the study, and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad provided partial support. We thank Sandra L. Suastes Acosta for technical support in the laboratory work. The first author is a graduate student at the Programa de Posgrado en Ecología y Manejo de Recursos Naturales, Instituto de Ecología Aplicada of the Universidad Autónoma de Tamaulipas and received a graduate scholarship from the Consejo Nacional de Ciencia y Tecnología (No. 377278). A.H.E.G. thanks Programa para el Desarrollo Profesional Docente para el Tipo Superior, Universidad de Guadalajara (Project ID: 239170) for continuous support. Finally, we are grateful to anonymous reviewers for improving this manuscript.

LITERATURE CITED


United States Fish and Wildlife Service. 2012. Endangered and threatened wildlife and plants; final rule to remove the Morelet’s crocodile from the federal list of endangered and threatened wildlife. Federal Register 77(100):30820–30854.


Submitted 30 June 2017. Accepted 19 June 2018.

Associate Editor was Felipe de Jesus Rodriguez-Romero.