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# Evaluation of the use of dermal scutes and blood samples to determine organochlorine pesticides in *Crocodylus moreletii*: A non-destructive method for monitoring crocodiles and environmental health

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ABSTRACT

This study determines the suitability of using blood plasma and dermal scute samples as a non-destructive technique for monitoring the health of Morelet's crocodiles and the presence of organochlorine pesticides (OCPs) in the environment. We collected samples (blood plasma and caudal scutes) from 30 Morelet's crocodiles (*C. moreletii*); 16 were wild, and 14 were captive. The 30 samples were analyzed for 24 different OCPs and compared in 10 groups based on chemical affinities (isomers and degradation products). Endrin and Chlordane were the most frequent OCP groups detected, found in 63% and 57% of the samples, respectively. We did not find significant differences in OCP concentrations in tissues (plasma and scutes) between wild and captive crocodiles; there were also no significant correlations among concentrations in tissues.

Blood plasma and scutes are good indicators of OCP body burdens in Morelet's crocodiles for monitoring the presence and fluctuation of toxicity in the environment; however, it is not possible to infer the concentrations in one tissue from the concentrations detected in the other tissue.

Selecting which tissues to use for OCP analyses depends on the specific objectives of the researcher. For research, on individual health and local exposure to contaminants, plasma is suitable. For evaluating the presence over time of OCPs in the environment, the caudal dermal scutes are the most appropriate tissue. Overall, it is important to take into account the body condition to avoid biases due to bioamplification effects.

## 1. Introduction

Organochlorine Pesticides (OCPs) such as dichlorodiphenyldichloroethylene (DDT), hexachlorocyclohexane (HCH) and endosulfan, are considered primary pollutants due to their persistence in the environment, their bioaccumulation in the trophic web, and the fact that they are transported long-range with long-term toxic effects (Kelly et al., 2007; Klecka et al., 2009; Newman, 2015). Endocrine disruption is an alteration caused by chemicals on the organism's endocrine system producing adverse developmental, reproductive, neurological, and immune effects. Endocrine disruption caused by OCPs has been reported since the 1960s in many wildlife species (Colborn et al., 1993; Kohler and Triebskorn, 2013; Schug et al., 2016). Some studies indicate that even though the bioaccumulation of some OCPs has decreased as a result of either a ban or severe limitation of their use (e.g., DDT and its degradation products; dieldrin), the decrease among certain populations has been slow, and biologically significant levels are still observed in many wildlife populations (Kohler and Triebskorn, 2013; Schmitt et al., 1990). This is particularly evident in long-lived animals that have experienced the different historic stages of these chemicals, from extensive to controlled to inexistent use (Rowe, 2008).

Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, invasive species, environmental pollution, diseases, unsustainable use or harvest, and global climate change (Gibbons et al., 2000). Some specific characteristics of reptiles, like all ectoderms (Shine, 2013), their high position in trophic food webs (e.g. predators or scavengers), low metabolism rates, and many species are known to store significant amounts of body fat. This allows an accurate diagnosis of the presence of these pollutants in populations and, because reptiles bioaccumulate pollutants, are a good

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indicator of the state of the environment (Selcer, 2006). Several studies reported that OCPs can alter the normal functioning of the endocrine system of reptiles and may cause immunological, behavioral, developmental, and reproductive abnormalities (Burger, 2006; Keller et al., 2004; Willingham, 2006). Therefore, there is a need to identify, validate, and utilize reptile predator species as models in ecotoxicology studies, both in the field and the laboratory, to monitor the presence and effects of OCPs in the environment.

Crocodilians are large-bodied aquatic reptiles that live in tropical regions throughout the world, and many species are threatened or endangered (Huchzermeyer, 2003; Selcer, 2006). Their life history strategy includes late maturity, extreme longevity, and large egg clutches produced over a period of years. Consequently, adverse long-term effects from pesticides in individuals may result in stronger effects at the population level compared to species reaching sexual maturity faster and having a short reproductive life (Wagner et al., 2015). Due to their long life-spans, crocodilians are excellent ecological indicators of environmental contaminants that bioaccumulate, biomagnify, and bioamplify pollutants such as organic contaminants and some heavy metals (Campbell, 2003).

The distribution of Morelet's crocodiles (*Crocodylus moreletii*) is from Tamaulipas to Quintana Roo, in Mexico, to Belize and Northern Guatemala (Escobedo-Galván and González-Salazar, 2011). The reproductive ecology and physiology of Morelet's crocodiles is well documented (Charruau et al., 2013; Escobedo Galván et al., 2009; López-Luna et al., 2015; Merchant et al., 2014; Padilla et al., 2011; Platt et al., 2008, 2006; Sigler, 1991), and the effect of heavy metals and organic pesticides on this species has also been studied (Buenfil-Rojas et al., 2015; Charruau et al., 2013; González-Jáuregui et al., 2012; Rainwater et al., 2007, 2002; Trillanes et al., 2014; Wu et al., 2006).

The most frequently used tissues for monitoring contaminant burdens in wildlife are eggs, liver, adipose tissue or plasma. Other tissues such as muscle, kidney, brain, feathers, and even the whole bodies can be used for OCP concentration analyses. Due to the differences between lipid and protein contents and cellular turnover, measuring contaminants in different tissue types can alter the interpretation of pollutants' body burdens, particularly for long-lived and migratory species (de Solla, 2016; Sherwin et al., 2016).

Most recently, researchers have been using blood and dermal scutes in reptile monitoring studies (Rainwater et al., 2007; Schneider et al., 2015; Sherwin et al., 2016; Tremblay et al., 2017; Trillanes et al., 2014) as a reliable non-lethal sampling technique to monitor the OCP presence in animals and the environment. Blood samples are widely accepted measurements for chemical exposure (Angerer et al., 2007). The presence of chemicals and degradation products in body fluids reflects an individual's actual systemic exposure to a chemical agent from all potential routes of exposure (i.e. inhalation, ingestion, and dermal uptake). Whole blood can be separated into plasma or serum, and packed red blood cells (RBC) by simple centrifugation. Plasma is a preferable way for analyzing OCPs in blood because of its lipid content (Keller et al., 2004). Plasma is obtained from blood samples with an anticoagulant and contains lipoproteins and other proteins which are known to bind and transport OCPs (Norén et al., 1999; Padilla et al., 2011).

Most of the OCPs are lipophilic and get stored in fatty tissues of wildlife. However, fats can be mobilized in the organism of fish, reptiles, or birds, to produce eggs. In mammals, fats are mobilized to produce milk to nurse offspring or as a source of energy during periods of food scarcity or starvation. During these stages, the concentrations of OCPs in circulating media rise, which may have adverse effects on the organism (Bergman et al., 2012; García-Besné et al., 2015; Pelletier et al., 2002). In Morelet's crocodiles, dermis samples have been shown to be useful as a reliable non-lethal sampling and monitoring method to assess OCPs (González-Jáuregui et al., 2012; Rainwater et al., 2007; Sherwin et al., 2016).

The main objective of this study was to determine the suitability of using blood plasma or dermal scute samples as a non-destructive technique for monitoring the health of Morelet's crocodiles as well as monitoring the presence of OCPs in the environment. We wanted: 1) to evaluate the possible correlation between blood plasma and caudal dermal scutes or if there are differences in OCP concentrations among these two types of tissues in crocodiles, and 2) to assess whether OCP bioaccumulation in dermal scutes or blood samples were different between wild and captive conditions. Differences in bioaccumulation could occur because captive crocodiles feed on fish for human consumption and wild crocodiles feed opportunistically on the resources available in the wild.

## 2. Materials and methods

### 2.1. Study sites

Wild crocodiles were randomly captured in two localities in the state of Campeche, Mexico: Los Petenes and Mocu lagoon. The Los Petenes is located on the Gulf of Mexico region in the Yucatan Peninsula. It is a natural protected area, covering 282, 857 ha (from 19°49′00″ to 20°51′35″ N and from −90°45′15″ to −90°20′00″ W). This region is known for its presence of habitat 'hammocks' (locally known as "Petenes"), described as islands of forest surrounded by mangroves and salt marshes with freshwater available year-round from springs (Fig. 1). Mocu lagoon is located in the south of Campeche (18°46'15" N and 90°30'33" W); it is a 400-hectare inland deep lagoon without superficial tributaries. It is surrounded by a well-conserved semi-deciduous forest, and the nearest human settlement is 22 km away (Fig. 1).

Captive crocodiles were randomly sampled at two farm facilities: CETMar No. 2 and Wotoch Aayin. CETMar No 2 (registry number: INE/CITES/DGVS-CR-IN-0519-CAMP/99) is located in the city of Campeche (19°51′50″N, 90°30′33″W) and it is a farm for education and conservation purposes. Wotoch Aayin (Registry number: SEMARNAT-UMA-IN-0054-CAMP) is a commercial crocodile farm located in the rural village of Isla Arena (20°42′11″N, 90°27′08″W) on the north coast of the state of Campeche. Both farms are located within the Los Petenes. The CETMar farm is located on the southern end of Los Petenes and Wotoch Aayin on the northern end.

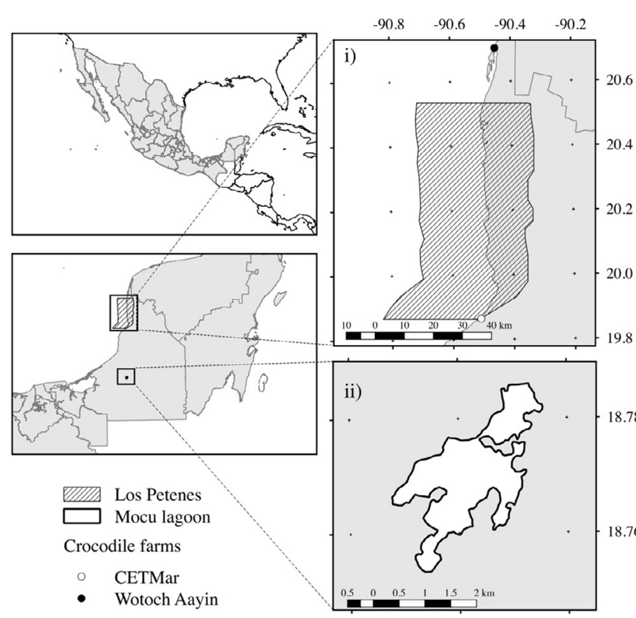


**Fig. 1.** Study areas: wild samples i) Los Petenes, and ii) Mocu lagoon. Wotoch Aayin at the north region of Los Petenes, and CETMar at the south, were the two crocodile farms where tissue samples of captive crocodiles were taken.