Organochlorine pesticide bioaccumulation in wild Nile crocodile (*Crocodylus niloticus*) fat tissues: Environmental influences on changing residue levels and contaminant profiles

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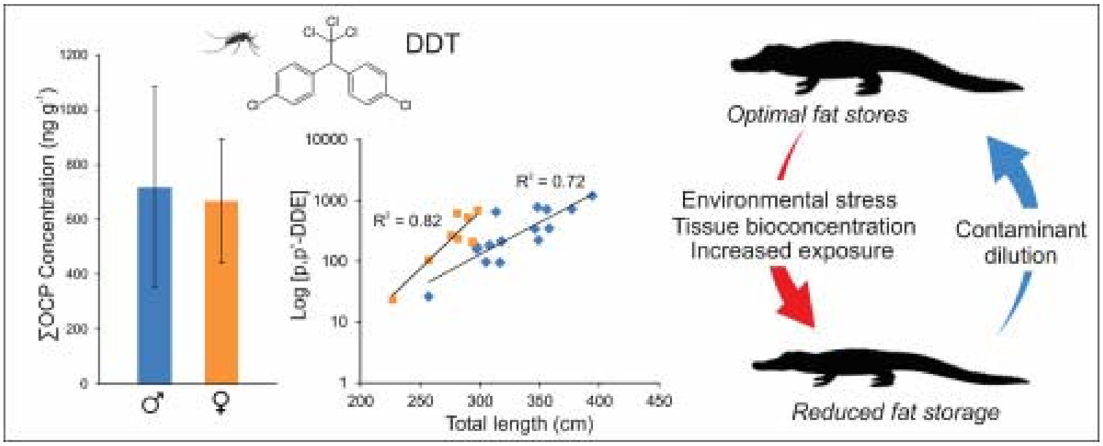
Highlights

- Investigated OCP bioaccumulation patterns in wild and captive Nile crocodiles
- DDT metabolites found to be the principle contaminant in wild animals
- Concentrations between males and females were similar and correlated with body size.
- Significant difference in concentration burdens and profiles between sampling years
- Temporal variations likely linked to environmental stress and dietary exposure

Abstract

Biologically significant concentrations of organochlorine pesticides (OCPs) continue to be reported in wildlife populations and are of particular concern in species that occupy the highest trophic levels. Nile crocodiles (*Crocodylus niloticus*) are important apex predators occurring throughout much of tropical and subtropical sub-Saharan Africa, where they inhabit estuarine and freshwater habitats often impacted by contamination. In this study we examined pesticide residue accumulation in fat tissue from Nile crocodiles at Lake St Lucia, South Africa, where historically large quantities of OCPs have been used for agriculture and disease control. During 2019, we collected tail fat samples from wild (n = 21) and captive (n = 3) individuals to examine the influence of habitat, body size and sex on variations in bioaccumulation. The principal contaminant found was *p*,*p′*-DDE, a major persistent metabolite of DDT, which continues to be used in the region for combating malaria. Tissue *p*,*p′*-DDE concentrations in wild crocodiles (95–1200 ng g^{-1} ww) were significantly ($p < 0.05$) higher compared to captive individuals (23–68 ng g^{-1} ww) and strongly correlated ($R^2 > 0.70$) to body length. Male (n = 14) and female (n = 7) wild crocodiles exhibited similar contaminant body burdens, however, total concentrations were substantially lower than those measured in the same population during 2016/2017. Marked differences in residue levels and profiles appear to reflect changes in food availability and dietary exposure associated with a shift in environmental conditions. These findings suggest that periods of environmental stress may be associated with enhanced toxicological risk in crocodiles. Additional work is needed to better understand contaminant accumulation and elimination mechanisms in crocodiles, and their potential effects on reproductive health.

Graphical abstract



Keywords: Nile crocodile; Organochlorine pesticides; DDT; Ecotoxicology; Bioaccumulation; Biomonitoring

1. Introduction

Even though use of organochlorine pesticides (OCPs) has been banned or severely limited on a worldwide basis, biologically significant concentrations are still observed in many wildlife populations (Tubbs and McDonough, 2017; Dietz et al., 2019; Routti et al., 2019). Due to their persistence in the environment, bioaccumulation potential and long-term toxic effects, OCP exposure is of particular environmental concern to higher-level consumers (Vos et al., 2000; Letcher et al., 2010; Formigaro et al., 2014). Pesticide residues, and specifically DDT metabolites, have long been implicated in reproductive failure in bird populations (Blus, 1995) as well as immunosuppression and reproductive impairment in marine mammals (Delong et al., 1973; De Guise et al., 1995). Nevertheless, DDT is promoted by the World Health Organization for indoor residual spraying purposes and several African countries continue to use the insecticide for malaria vector control (van den Berg et al., 2017). While highly potent and effective in reducing transmission rates in malaria endemic regions, continued use of DDT has potentially serious long-term implications for tropical and sub-tropical biodiversity in Africa.

Although contaminant accumulation and ecotoxicological assessments for birds and aquatic mammals have been frequently reported on (e.g., Hellou et al., 2013), further research is needed to critically evaluate exposure risks in less well-studied wildlife, such as reptiles. Being large-bodied aquatic reptiles, crocodiles are generally considered good ecotoxicological models for monitoring and assessing ecosystem health (Campbell, 2003). Crocodilian populations are especially susceptible to contaminant exposure due to their extreme longevity, high trophic-level position, low metabolism rates and ability to store significant amounts of body fat (Campbell, 2003). They also occur primarily in tropical and sub-tropical regions of the world, where pesticides are often heavily used (de Solla, 2010). As a result, OCPs have been detected in the blood and tissues of wild crocodilian populations from numerous localities, including American alligators (*Alligator mississippiensis*) from Florida (Rauschenberger et al., 2004, Rauschenberger et al., 2007), spectacled caimans (*Caiman crocodilus*, Grant et al., 2013), Morelet's crocodiles (*Crocodylus moreletii*, González-Jáuregui et al., 2012, González-Jáuregui et al., 2018; Sherwin et al., 2016) and

American crocodiles (*Crocodylus acutus*, Rainwater et al., 2007) from central America, as well as in Australian freshwater (*Crocodylus johnsoni*) and saltwater crocodiles (*Crocodylus porosus*, Yoshikane et al., 2006). Apart from potentially having toxicological impacts at the individual level, contaminants may be maternally transferred into eggs where they have been associated with increased rates of embryonic mortality (Rauschenberger et al., 2007), reduced clutch size (Stoker et al., 2011), and altered sex hormone concentrations in juveniles (Guillette Jr. et al., 1996).

In 2018, we reported the presence of high OCP levels in fat tissues from Nile crocodiles (*Crocodylus niloticus*) in South Africa (Buah-Kwofie et al., 2018a). Although ranging throughout sub-Saharan Africa, breeding Nile crocodile populations in South Africa occur exclusively in the sub-tropical eastern region of the country where OCPs have historically been extensively used in agriculture and disease control. DDT continues to be employed in combating malaria in disease risk areas, where it is considered to be a serious threat to local wildlife populations (Gerber et al., 2016; Buah-Kwofie et al., 2018b; Bouwman et al., 2019). This includes Lake St Lucia (Fig. 1), which is host to the most stable nesting Nile crocodile population in the country (Combrink et al., 2013). The St Lucia estuarine system is the oldest formally protected estuary in the world (declared in 1895) and the crocodiles inhabiting the area have benefited from a long research and surveying legacy (Combrink et al., 2013). Crocodiles at Lake St Lucia occur in high densities and are mostly accessible by boat, providing a unique opportunity to further our understanding on OCP accumulation and ecotoxicological risks in crocodilians.

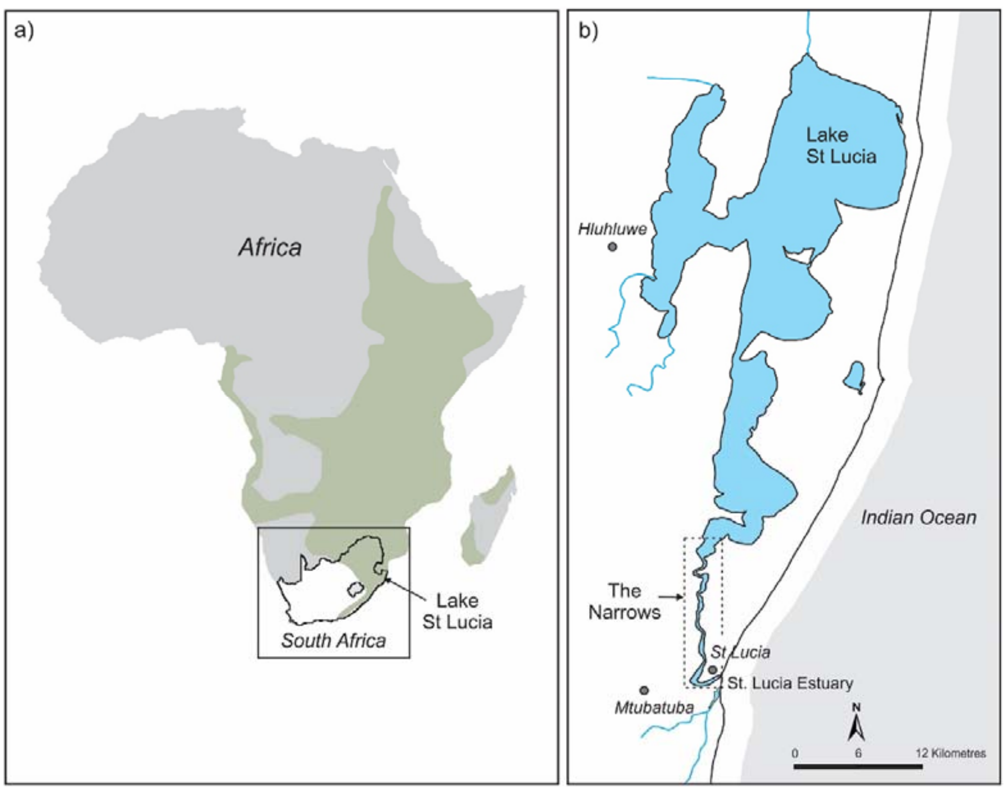


Fig. 1. Buah-Kwofie and Humphries, 2019Maps showing a) the location of the study area and global distribution of Nile crocodiles (redrawn from Grigg and Kirshner, 2015), and b) Lake St Lucia on the east coast of South Africa. All sampling took place within "The Narrows" section of Lake St Lucia.

In this study, we expand on our initial work that showed the surgical removal of fat samples from the tail area to be a reliable, non-lethal sampling strategy for assessing OCP exposure in wild crocodiles (Buah-Kwofie et al., 2018a). The analysis of fat tissue not only provides a good indicator of contaminant accumulation and ecotoxicological risk, but also offers a unique opportunity to examine possible changes in contaminant burdens over time. The objectives of this study are to 1) examine variability in OCP residue levels among reproductive Nile crocodiles and assess the influence of body size and sex on bioaccumulation in fat tissue, 2) compare differences in OCP concentrations of wild and captive crocodiles, 3) examine temporal differences in OCP contaminant profiles and body burdens.

2. Methods

2.1. Study area

Lake St Lucia (28° 00′ 26″ S, 32° 28′51″ E; Fig. 1), located within the UNESCO World Heritage iSimangaliso Wetland Park on the sub-tropical east coast of South Africa, is considered Africa's largest estuarine system (Perissinotto et al., 2013). The system (350 km^2) comprises three shallow, interconnected basins linked to the ocean via a long sinuous channel known as the Narrows. The estuary mouth is prone to prolonged periods of closure and the ecological state of the system is governed largely by seasonal variability in freshwater fluvial inputs. With an average water depth of approximately 1 m, the system is susceptible to unpredictable and often extreme fluctuations in water level and physicochemical conditions (Stretch et al., 2013), which exert strong influence on estuarine biological communities and ecosystem productivity (Cyrus et al., 2010; Nche-Fambo et al., 2015). Approximately 1000 Nile crocodiles are estimated to inhabit Lake St Lucia (Combrink, 2014), with the vast majority typically found within the Narrows. During periods of extended drought, various feeder streams and surrounding freshwater pools and wetland areas become important habitat for crocodiles (Combrink et al., 2013).

2.2. Sample collection

Crocodile captures took place over a two week period during May/June 2019 under permit from the iSimangaliso Wetland Park Authority and Ezemvelo KZN Wildlife (permit number: 639/2019). All wild crocodiles were captured from the Narrows section of Lake St Lucia (Fig. 1), the same area sampled during the previous 2016/2017 study. In addition, three captive individuals were sampled at the St Lucia Crocodile Centre, a provincial conservation managed facility (Ezemvelo KZN Wildlife) established for research, education and conservation purposes.

The months of May and June represent the start of the drier, winter season and would typically correspond with a period of relatively low activity for crocodiles at Lake St Lucia. The majority of wild crocodiles were located by boat at night using spotlights and captured with stainless steel cable snares attached to an aluminium pole. To increase catch efficiency, we also employed baited snare traps (3 mm steel cable with non-relaxing Thompson Snare lock) secured with elastic bands between anchored steel dropper poles in ±70 cm deep water. Trap cables were attached to shoreline mangrove trees. Individuals larger than 2 m in length were targeted as previous experience has shown that these typically yield fat samples of sufficient size for laboratory analysis. Crocodiles were physically restrained and blindfolded immediately following capture. For each individual, we determined the sex through cloacal examination (Brazaitis, 1968) and recorded standard morphometric measurements: total length (TL), snout-vent length (SVL), tail girth (TG) and neck girth (NG). Body condition was assessed using a condition index (CI) calculated based on the ratio between SVL and TG (Leslie, 1997): $\mathrm{CI} = \frac{\mathrm{TG}}{\mathrm{SVL}} * 10$.

Fat samples were surgically removed from the cranial-lateral tail area of crocodiles following a previously described method (Buah-Kwofie et al., 2018a). While this technique is more invasive when compared to other sampling methods (e.g., sampling of scutes), it allows relatively large amounts of sample to be collected from live crocodiles and from a consistent location on the body. Briefly, the area of incision was thoroughly washed using 4% chlorhexidine gluconate soap and disinfected with sterile cotton wool swabs soaked in a chlorhexidine gluconate 0.5% w/v and ethyl alcohol 70% w/v solution. A line block of the area was done using 2% lignocaine HCl (maximum dose 3 mg kg^{-1}) before finally being sterilised with 100 mg mL^{-1} povidone-iodine. A scalpel incision, three scutes in length, was made through the skin between adjacent scutes from a consistent location on the ventro-lateral side of the tail. The fat deposit targeted was located between the *Musculus ilioiscchiocaudalis* and *M. caudofemoralis* muscles of the tail. All visible fat was removed using surgical scissors and placed in a sterile centrifuge tube. Following removal of the fat sample, the incision through the *M. ilio-iscchiocaudalis* layer was sutured using chromic catgut, while the incision through the skins was closed using monofilament nylon. The line of incision was sealed afterwards using a wound gel powder. Crocodiles were injected intramuscularly with a systemic antimicrobial drug (enrofloxacin 10 g 100 mL^{-1}) and then released at their site of capture. All captured animals were permanently marked for future identification by removing a unique series of three caudal scutes using a sterile scalpel (Combrink, 2014). Fat samples were stored frozen at −18 °C prior to analysis.

All field methods and subsequent laboratory work was performed in compliance with procedures approved by the University of the Witwatersrand Animal Ethics Committee (AESC number: 20133201) and South African Department of Environmental Affairs (permit number: 05189).

2.3. OCP residue analysis

Samples were analysed at the University of the Witwatersrand, Johannesburg, following previously established methods (Buah-Kwofie et al., 2018a; Buah-Kwofie and Humphries, 2019). Briefly, OCPs were extracted from cleaned fat samples (2 g) using 8 mL acetonitrile/acetic acid (99:1 v/v) and a mixture of anhydrous magnesium sulfate (4 g), sodium acetate (1.0 g) and sodium acetate trihydrate (0.6 g). Clean-up of the extract was achieved using a combination of $MgSO_4$ (1.0 g), C18 (0.4 g), deactivated florisil (0.4 g) and primary secondary amine (0.4 g), before finally being reduced to dryness under vacuum and reconstituted in hexane for analysis. The recovery efficiency of this method has been shown to range between 77 and 109% (Buah-Kwofie and Humphries, 2019).

OCPs were quantified using two dimensional gas chromatography time-of-flight mass spectrometry (GC X GC-TOFMS). A total of 18 compounds were targeted in this study, which included dichlorodiphenyltrichloroethanes (DDTs; *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD; sum expressed as ΣDDT), hexachlorocyclohexanes (HCHs; α-, β-, γ- and δ-HCH; sum expressed as ΣHCH), drin-residues (aldrin, dieldrin, endrin, endrin aldehyde and endrin ketone; sum expressed as Σdrin), endosulfans (α-, β-endosulfan and endosulfan sulfate; sum expressed as Σendo) and chlor-residues (heptachlor, heptachlor epoxide and methoxychlor;

sum expressed as Σchlor). Analysis was performed on an Agilent 7890 GC coupled to a Leco Pegasus 4D TOF mass spectrometer. Separation was achieved using a Restek Rxi-5Sil MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coupled to a Rxi-17Sil MS (1.075 m × 0.25 mm i.d. × 0.25 μm thickness) secondary column. Samples of 2 μL were injected in a splitless mode using ultrahigh-purity helium as the carrier gas at a constant flow rate of 1.4 mL min^{-1}. Data processing and peak identification were performed using the Leco ChromaTOF software and databases. Peaks were identified based on the retention time of specific ions and confirmed by two identifier ions.

Quantification was achieved using high purity (>98%) reference standards purchased from Sigma Aldrich. Linear regressions derived from matrix-matched calibration curves for all pesticide compounds were ≥ 0.99. Sample extracts were spiked with pentachloronitrobenzene (PCNB) as an internal standard and quality control standards were analysed after every three samples to monitor and correct for variations in instrument response. Sample extracts were analysed in triplicate with variability typically <10%. Detection limits ranged between 0.12 and 0.4 ng g^{-1} wet weight (ww).

Concentration data were tested for normality using a Shapiro-Wilk test and all subsequent statistical tests were performed on untransformed data. Analysis of variance (ANOVA) was used to assess differences in OCP analyte concentrations between difference sexes and between captive and wild crocodile groups. Relationships between body size (TL) and contaminant concentrations were examined using linear regression analysis. Due to previous sampling undertaken in 2016/2017, we had the unique opportunity to examine temporal variations in OCP concentrations. Temporal differences in mean concentrations between sampling periods and in recaptured individuals were examined using an independent *t*-test. All statistical analyses were performed using Statistica 10. Significance was set at $p < 0.05$. All values are expressed as the arithmetic mean ± SD, unless otherwise stated.

3. Results and discussion

3.1. Crocodiles sampled

A total of 22 wild crocodiles from Lake St Lucia were captured. Of these, fat samples of sufficient size (ranging between approximately 3 and 30 g) were obtained from 21 individuals. The sampled population consisted of 14 males (TL: 297–394 cm) and 7 females (TL: 257–298 cm), all of reproductive age. Two of the crocodiles captured (W12 and W17) happened to be individuals that were sampled during our previous 2016/2017 study (Buah-Kwofie et al., 2018a). Calculated body condition indices ranged between 4.0 and 5.3 (4.6 ± 0.35) and were similar to those determined for the population sampled in 2016/2017 (4.8 ± 0.28). The three captive-reared individuals sampled at the St Lucia Crocodile Centre included one male and two females, all of similar size (TL: 246 ± 17 cm). These individuals were all born at the Centre.

3.2. Residue concentrations

The concentrations of major organochlorine residues analysed are summarised in Table 1 (a full set of results provided as supplementary material; Table S1). All target OCP residues were detected in all wild crocodile fat tissue samples, with total OCP concentrations varying between 300 and 1460 ng g^{-1}. The *p,p′*-DDE derivative was the dominant compound detected, which was present in highly variable concentrations (95–1200 ng g^{-1}). Overall, *p,p′*-

DDE accounted for the majority of the total contaminant burden in all individuals, with proportional contributions varying between 20 and 82% (52 ± 18%). Other analytes were detected in significantly ($p < 0.001$) lower levels and exhibited less variability across the sampled population.

Compared to the wild crocodile population, substantially lower OCP concentrations were detected in captive individuals (Fig. 2a). Total OCP burdens averaged 360 ± 72 ng g^{-1}; approximately 50% lower than average levels measured in the wild population and 75% lower than the most contaminated individual sampled. Variation in contaminant patterns between wild and captive subsets was attributable predominantly to *p,p′*-DDE, which was present in significantly lower concentrations (39 ± 25 ng g^{-1}, $p < 0.05$) in captive individuals (Fig. 2b). Although contributing substantially less to overall contaminant body burdens, concentrations of *p,p′*-DDT and *p,p′*-DDD were also significantly ($p < 0.05$) lower in captive individuals compared to the wild population. The other pesticide residues were more evenly distributed and did not vary significantly between captive and wild crocodile groups.

Table 1. Mean (±SD) organochlorine pesticide concentrations (ng g^{-1} ww) measured in fat samples collected from captive and wild Nile crocodiles from Lake St Lucia. All chemical and morphometric data are provided in Table S1.

	Lindane	Aldrin	Dieldrin	Endrin	*p,p′*-DDT	*p,p′*-DDE	*p,p′*-DDD	Heptachlor	Methoxychlor	α + β Endosulfan	∑OCP
Captive (n = 3)	10 ± 5	31 ± 8	27 ± 8	18 ± 6	14 ± 2	52 ± 46	22 ± 6	25 ± 20	9 ± 4	51 ± 12	370 ± 56
Lake St Lucia (n = 21)	15 ± 10	24 ± 8	22 ± 8	14 ± 5	30 ± 13	410 ± 300	48 ± 21	16 ± 5	12 ± 3	37 ± 14	700 ± 320
Lake St Lucia males (n = 14)	18 ± 11	26 ± 8	22 ± 8	14 ± 6	29 ± 15	420 ± 340	50 ± 24	15 ± 5	12 ± 3	37 ± 15	720 ± 370
Lake St Lucia females (n = 7)	11 ± 4	20 ± 6	22 ± 7	15 ± 5	32 ± 7	380 ± 230	44 ± 12	18 ± 5	11 ± 2	37 ± 11	670 ± 330

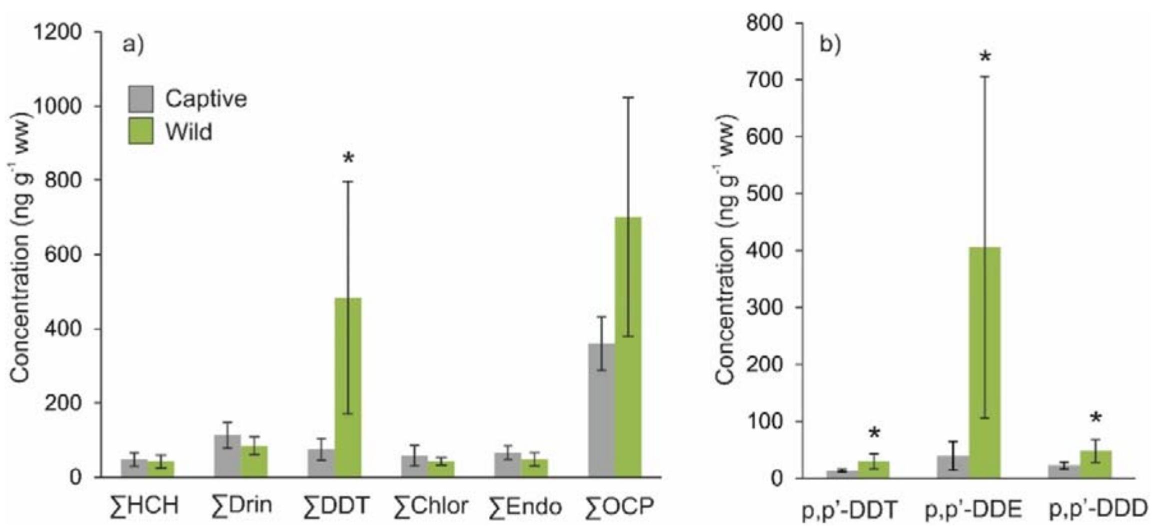


Fig. 2. Comparison between average OCP concentrations measured in wild (n = 21) and captive (n = 3) Nile crocodiles: a) Concentrations by OCP group, and b) Compositional profile for DDT. Significant differences ($p < 0.05$) indicated by asterisk. Error bars represent standard deviation between samples.

Contaminant profiles between male and female crocodiles were similar and we found no significant differences across any of the other analyte groups considered in this study (Fig. 3a). However, males typically exhibited highest *p,p′*-DDE and ∑OCP burdens, with the four most highly contaminated individuals tested all being large male (TL > 3.4 m) crocodiles. Both male ($R^2 = 0.72$, $p < 0.001$) and female ($R^2 = 0.82$, $p < 0.05$) subsets showed significant positive relationships between *p,p′*-DDE tissue concentrations and total body length (Fig. 3b), with larger individuals generally exhibiting higher levels of contamination. A relationship between contaminant concentration and crocodile size was not found for any of the other target analytes.

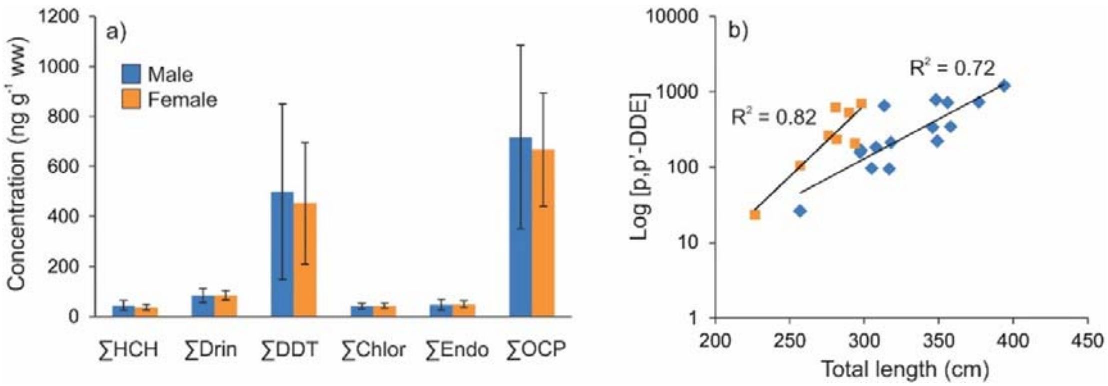


Fig. 3. a) Comparison between average OCP concentrations detected in wild male (n = 14) and female (n = 7) crocodiles by OCP group. b) Relationship between measured *p,p′*-DDE tissue concentrations and total body length in male ($R^2 = 0.72$, $p < 0.00$) and female ($R^2 = 0.82$, $p < 0.05$) crocodiles.

3.3. Bioaccumulation and variability in OCP tissue concentrations

OCPs have a strong affinity for suspended particulate matter and their accumulation within sediments at Lake St Lucia exposes a wide variety of aquatic organisms to elevated levels of contamination. While little is known about the mechanisms of contaminant uptake in reptiles, the ingestion of contaminated food is likely the main route of exposure (Hopkins, 2005). Fish represent the predominant prey of adult crocodiles at Lake St Lucia (Pooley, 1982; Leslie, 1997) and OCP residues have been widely detected in fish species found commonly throughout the St Lucia system (Buah-Kwofie et al., 2018b). The availability of fish and contamination levels associated with this food source are thus considered the most important environmental factors influencing the exposure of crocodiles at Lake St Lucia to OCPs. Despite exhibiting substantially lower concentrations, captive crocodiles at St Lucia are also exposed to multiple OCP residues through various potential dietary pathways, including meat from domestic animals, game meat derived from the culling of wild animals, and fish seized from illegal poachers by management authorities.

Biology may strongly influence the bioaccumulation of lipophilic compounds through differences in food consumption and growth rates, duration of exposure (age of the individual), and reproductive cycles. Several studies have suggested that male crocodilians may be more susceptible to contaminant accumulation as females have the ability to offload a portion of their body burden during egg production and oviposition (e.g., Rauschenberger et al., 2004; Stoker et al., 2011). The maternal transfer of contaminants into eggs is considered an important deposition pathway in some fish (Miller and Amrhein, 1995; Serrano et al., 2008) and bird species (Zheng et al., 2008; Ackerman et al., 2016), where it results in males often having substantially higher contaminant concentrations compared to females. However, we found no significant difference in total OCP concentrations or contaminant profiles exhibited by male and female Nile crocodiles at Lake St Lucia. While there is strong evidence to suggest that chemicals can be maternally transferred to eggs, this appears to have little overall effect on the contaminant body burdens of Nile crocodile females. A significant positive relationship between the predominant compound detected (*p,p′*-DDE) and body size suggests that larger (and therefore older) individuals may be more susceptible to contaminant accumulation in both males and females.

3.4. Changing contaminant profiles: comparison between 2016/2017 and 2019 results

Apart from ∑DDT, wild and captive crocodiles exhibited similar levels of contamination across the other major OCP groups. This was an unexpected result, particularly when compared to previously reported data from Lake St Lucia. Wild crocodile fat collected in 2019 contained significantly ($p < 0.001$) lower OCP concentrations when compared to the population ($n = 9$) sampled in 2016/2017 (Fig. 4a). A distinctive shift in contaminant profiles is also observed (Fig. 4b), with contaminant burdens in 2019 composed predominantly (~70%) of ∑DDT compared to the more evenly distributed OCP composition that characterized the 2016/2017 sample set (∑DDT = ~30%). Extreme variability in OCP concentrations and patterns are highlighted in particular by two crocodiles that were recaptured in 2019 (Fig. 5). Both individuals were large males, which when sampled in 2017 exhibited ∑OCP tissue concentrations that were 5–7 times higher than those measured in 2019. Significant ($p < 0.001$) differences in contaminant profiles were also observed, most notably associated with substantial increases in the proportional contribution of ∑DDT to total contaminant burdens.

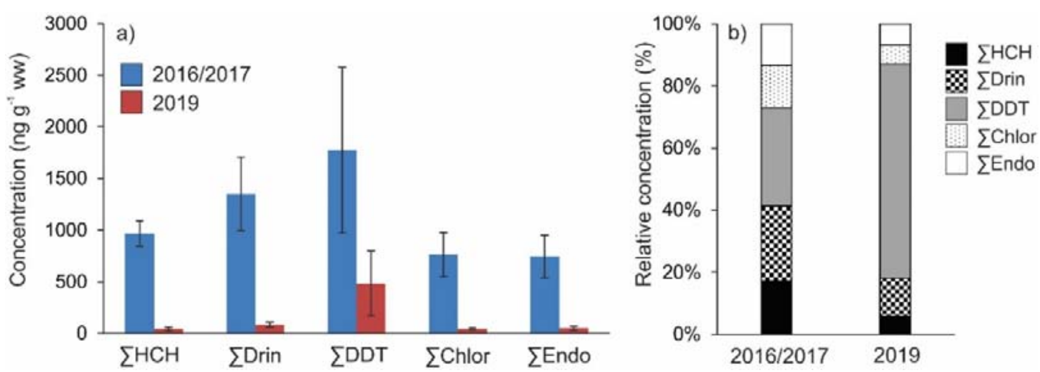


Fig. 4. Comparison between wild crocodiles from Lake St Lucia sampled in 2016/2017 (Buah-Kwofie et al., 2018a) and those sampled in this study. a) Average concentrations by OCP group. b) The relative contribution of individual OCP groups to total body burdens. Concentration differences between 2016/2017 and 2019 datasets are all highly significant ($p < 0.001$).

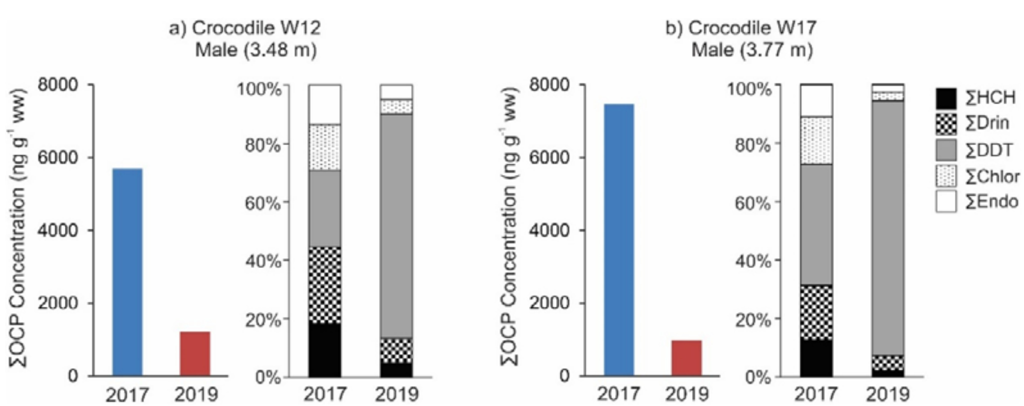


Fig. 5. Comparison between the concentration and distribution of OCPs measured in two recaptured crocodiles (W12 and W17) sampled in March 2017 and June 2019. Differences in total OCP concentration and compositional distribution between years are highly significant ($p < 0.001$) for both individuals.

3.5. OCP assimilation and bioconcentration

Food availability at Lake St Lucia is driven largely by environmental factors such as salinity and water levels, where crocodiles would typically be expected to be in optimal body condition at the end of the warm, wet season (March – April). It is notable that the sampling conducted in June2016/March2017 occurred toward the end of one of the most intense drought events on record (Blamey et al., 2018; Fig. 6). At the peak of the drought, declining water levels had left large portions of Lake St Lucia either sub-aerially exposed or hypersaline. Such conditions are unfavourable for many of the fish species commonly found within the system, typically leading to significant die-offs in biomass (Cyrus et al., 2010) and nutritional stress in crocodiles.

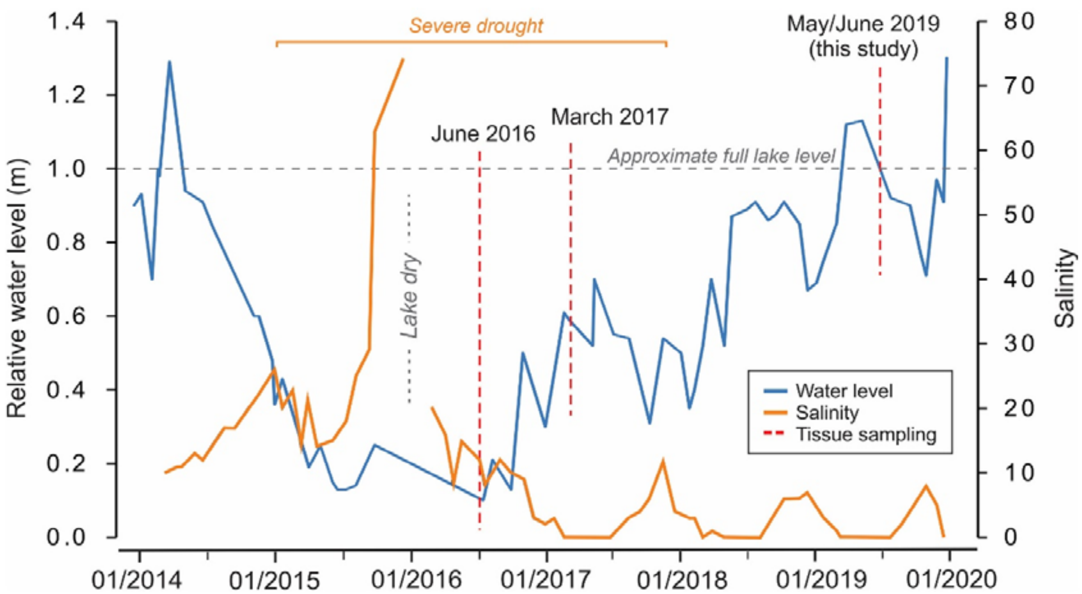


Fig. 6. Water level and salinity measurements recorded at Charter's Creek, Lake St Lucia, January 2014 to December 2019 (Fox and Mfeka, 2019). Crocodile tissue sampling trips undertaken during 2016/2017 (Buah-Kwofie et al., 2018a) and 2019 (this study) are indicated.

Crocodiles are capable of going without food for many months during periods of drought, relying entirely on their own body fat reserves (Grigg and Kirshner, 2015). We observed a significant ($p < 0.001$) difference in the amount of readily available fat for collection from individual crocodiles sampled in 2019 (16.1 ± 7.5 g) compared to 2016/2017 (5.6 ± 3.4 g). This suggests that despite similar body condition estimates, the individuals captured in 2016/2017 were carrying substantially lower tail fat reserves compared to those sampled in this study. In addition, a distinct difference in the appearance of the adipose tissue was observed between sampling periods. Adipose tissue sampled from lean crocodiles in 2016/2017 typically displayed varying degrees of yellow-brown discolouration that contrasted with the white fatty tissue collected in 2019 (Supplementary Fig. 1A). In extreme cases, discolouration was accompanied by a hardening of the adipose tissue (Supplementary Fig. 1B).

We hypothesize that large differences observed in OCP concentrations between crocodiles examined as part of this study and those sampled in 2016/2017 are likely linked to shifts in environmental conditions and its effect on the nutritional status of individuals. Rapid weight loss associated with nutritional stress can result in bioconcentration (sometimes also referred to as bioamplification), whereby chemical residues already present in an animal's tissues become enriched when body lipids are mobilised and used (Daley et al., 2014). Bioconcentration is predicted to have a greater degree of influence in large-bodied animals, which are able to better withstand prolonged starvation events because of their higher ratio of fat reserves to metabolic rate (Daley et al., 2014). For example, bioconcentration has frequently been reported in northern elephant seals and polar bears, which accumulate large amounts of fat to cope with lengthy seasonal fasts (e.g., Polischuk et al., 1995; Debier et al., 2006). During this time, animals may lose up to 90% of their adipose tissue stores. Lipid mobilization during these periods not only releases accumulated contaminants into the bloodstream, but also causes them to concentrate into the remaining adipose tissue (Debier et al., 2006; Louis et al., 2014; Louis et al., 2016; Peterson et al., 2018).

Although bioconcentration models are primarily based on studies of mammals, several lizards have been shown to use abdominal and tail fat stores during seasonal periods of dormancy or extended starvation, resulting in significant loss of fat-body mass (Price, 2017). Crocodiles have two different types of fat stores; somatic fat concentrated primarily under the neck and between the tail muscles, and more readily accessible metabolic fat found in the abdominal fat body (Huchzermeyer, 2003). It is thus plausible that the activation of specific fat deposits may further modify the tissue distribution of contaminants in crocodiles, with compounds released during the early stage of abdominal fat metabolism subsequently being concentrated into somatic fat stores. It is possible that reptiles may be particularly susceptible to such bioconcentration processes as their enzymatic detoxification system is thought to be less developed than in other vertebrates (Hopkins, 2005).

Crocodilians have high food conversion rates and increased access to food following the drought likely served to dilute the contaminant body burden and lower overall tissue concentrations. This was accompanied by a substantial shift in the distribution of OCPs in fat samples. The overwhelming dominance of ∑DDT in crocodiles sampled in 2019 greatly exceeds that which has been measured in sediments and fish from Lake St Lucia (Fig. 7). Given that the majority of the samples collected in 2019 likely represent recently deposited fat tissue, and thus a relatively narrow time window, changes in contaminant profiles suggest that DDT (specifically *p*,*p′*-DDE) may be more efficiently assimilated into the fatty tissues of crocodiles compared to other organochlorines.

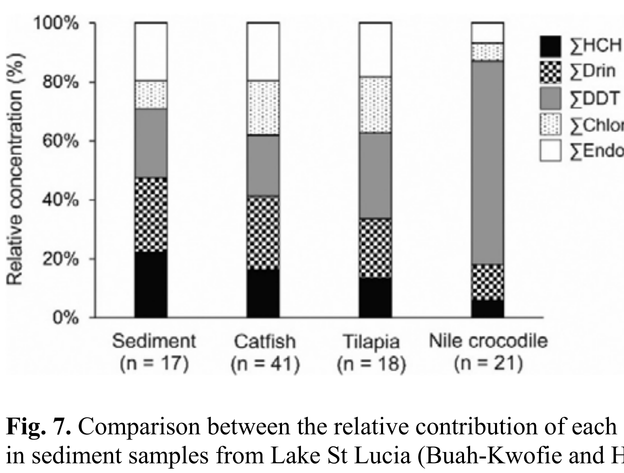


Fig. 7. Comparison between the relative contribution of each analyte group to total OCP concentrations detected in sediment samples from Lake St Lucia (Buah-Kwofie and Humphries, 2017), muscle tissues of two locally abundant fish species sampled in 2018/2019 (African sharptooth catfish, *Clarias gariepinus* and Mozambique tilapia, *Oreochromis mossambicus* (Buah-Kwofie and Humphries, submitted), and Nile crocodile fat tissue (this study)).

Drought conditions and extreme changes in physicochemical state may also potentially expose aquatic organisms to higher OCP concentrations. Ongoing monitoring studies at Lake St Lucia suggest that shifts in environmental conditions result in temporal variations in fish tissue OCP concentrations (Buah-Kwofie and Humphries, submitted). Fish specimens sampled in 2016/2017 during the drought were characterized by substantially higher ∑OCP concentrations (3300 ± 1600 ng g^{-1} lw) compared to those sampled in 2018/2019 (2000 ± 1200 ng g^{-1} lw). These results suggest that crocodiles are likely exposed to higher levels of contaminants during periods of drought, although such variations appear unable to fully account for the marked shifts in OCP concentration and distribution patterns within fat samples. We suggest that a combination of factors linked to food availability (through bioconcentration) and changes in dietary exposure best explains the observed variations in tissue contaminant concentrations.

3.6. Biological implications

Studies examining the extent to which crocodilians are affected by contaminants are limited (Campbell, 2003). Most toxicological risk assessments have focused on DDT metabolite exposure, which has been implicated in having detrimental effects on crocodilian reproduction and development (e.g., Guillette Jr. et al., 2000). Egg production and early life stages appear to be particularly sensitive to the endocrine-altering actions of OCPs. Parental OCP exposure has been reported to contribute to lower clutch viability in American Alligators (Rauschenberger et al., 2007) and smaller clutch sizes in broad-snouted caimans (Stoker et al., 2011), while embryonic pesticide exposure has been associated with reproductive and developmental abnormalities in juveniles. Examinations of the reproductive and endocrine systems of hatchling and juvenile alligators living in pesticide-contaminated lakes have revealed alterations in plasma hormone (estradiol-17β and testosterone) and thyroxine concentrations (Guillette Jr. et al., 1994; Crain et al., 1998), as well as gonadal morphological abnormalities (Guillette Jr. et al., 1996).

The extent to which pesticides may affect embryonic development and endocrine functioning in Nile crocodiles at Lake St Lucia is currently unknown. However, elevated levels of DDT have recently been measured in eggs from Pink-backed Pelican (*Pelecanus rufescens*) at Lake St Lucia, with *p*,*p*′-DDE concentrations strongly associated with eggshell thinning (Bouwman et al., 2019). This raises critical concerns regarding the potential effects of OCP exposure on Nile crocodile reproduction at Lake St Lucia, particularly as maternal transfer in alligators has been found to be strongly correlated with concentrations measured in female tissues (Rauschenberger et al., 2004). The maternal-embryonal transfer of OCPs within the Nile crocodile population at Lake St Lucia remains a major concern and is the subject of ongoing investigation.

4. Conclusions

The results of this study show that Nile crocodiles at Lake St Lucia are exposed to elevated pesticide residue concentrations, especially DDT and its metabolites. We found that male and female crocodiles exhibited similar contaminant profiles, suggesting that contaminant offloading during egg production likely has little lasting effect on the body burden of adult females. The maternal transfer of contaminants may however be of potential biological significance to embryonic development and links between contaminant exposure in female crocodiles and associated reproductive effects remains a particularly distressing gap in knowledge. Comparison between residue levels reported here and those measured in the same population in 2016/2017 following a severe drought suggest that changes in fat-body mass can have a profound influence on tissue contaminant concentrations. Although pesticide toxicokinetics in crocodilians has been little studied, our data suggest that crocodiles may be susceptible to increased toxicological risk during periods of stress, a finding that has potentially important implications for evaluating toxicity in female crocodiles who utilize fat stores during egg production.

Our findings highlight that while crocodilians are widely considered good bioindicators, it is important to take the body condition of an animal into account when assessing tissue bioaccumulation. Selecting what biological tissues to use in assessing contaminant exposure in wildlife is thus crucial. One of the clear advantages of sampling fat tissue, compared with metabolically inert kertinaceous tissues, such as caudal scutes, is the potential insight it offers into temporal changes in contaminant body burdens and toxicity exposure.

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CRediT authorship contribution statement

Marc S. Humphries: Conceptualization, Investigation, Writing - original draft. **Jan G. Myburgh:** Investigation, Writing - review & editing. **Robert Campbell:** Investigation, Writing - review & editing. **Archibold Buah-Kwofie:** Investigation, Writing - review & editing. **Xander Combrink:** Investigation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Research data for this article

Data for: Organochlorine pesticide bioaccumulation in wild Nile crocodile (Crocodylus niloticus) fat tissues and environmental influences on changing residue levels and contaminant profiles
Morphometric and organochlorine residue (ng/g lw) data for Nile crocodiles from Lake St Lucia (https://data.mendeley.com/datasets/mhzxsd6sp2/1)

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