Accumulation of organochlorine pesticides in fat tissue of wild Nile crocodiles (*Crocodylus niloticus*) from iSimangaliso Wetland Park, South Africa

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Abstract

Nile crocodiles (*Crocodylus niloticus*) are important apex predators in many tropical and subtropical aquatic habitats throughout much of sub-Saharan Africa. In South Africa, large crocodile populations inhabit lakes and wetlands that are impacted by organochlorine pesticides (OCPs). Despite the continued use of these compounds and their potential adverse effects on key wildlife populations in southern Africa, limited ecotoxicoloigcal data exist. In this study, we examined the accumulation of OCPs in fat tissues of live, wild Nile crocodiles from iSimangaliso Wetland Park, a region of significant biological importance. All samples (n = 15) contained multiple contaminants in highly elevated concentrations, with total residue burdens varying between 3600 and 8000 ng g^{-1} ww. DDT and its metabolites were the dominant compounds detected in most samples, with ∑DDT concentrations ranging between 520 and 3100 ng g^{-1} ww. Elevated levels of other OCPs were also detected, including lindane (67 – 410 ng g^{-1} ww), aldrin

(150 – 620 ng g^{-1} ww) and heptachlor (170 – 860 ng g^{-1} ww). Our findings show that crocodiles are exposed to OCPs throughout their range within iSimangaliso Wetland Park and contain some of the highest concentrations ever recorded in crocodilian tissue. Results indicate the need for a greater understanding of the impacts of OCP exposure and toxicological responses in crocodiles from iSimangaliso, and in Nile crocodile populations in general. The novel surgical technique described in this study provides an effective method for assessing relationships between contaminant body burdens and their potential reproductive and developmental consequences in crocodilians.

Keywords: Nile crocodile; organochlorine pesticides; DDT; ecotoxicology

1. Introduction

Organochlorine pesticides (OCPs) have been used extensively in South Africa (SA) since the early 1950s in agriculture and for disease-vector control, and DDT continues to be applied in the malaria endemic regions of the country. While the toxic and endocrine-disrupting nature of these compounds is now well established, their continued use in SA is of particular concern as application often occurs in close proximity to key areas of conservation. We recently reported the detection of high concentrations of several OCP residues in sediments from iSimangaliso Wetland Park (Buah-Kwofie and Humphries, 2017), located on the east coast of SA (Fig. 1). The park forms part of the Maputaland-Pondoland-Albany biodiversity hotspot, is a designated World Heritage Site, and is globally recognised as an important protected area for biological conservation (Porter, 2013). Despite the rich biodiversity of the region and widespread environmental occurrence of OCP residues, few studies on contaminant concentrations have been conducted in wildlife here.

OCPs tend to bioaccumulate in the food web because of their environmental persistence and affinity to fatty tissues (Arnot and Gobas, 2006). Owing to their high trophic status and long life span, crocodiles are particularly susceptible to the accumulation of contaminants released into the environment and OCP residues have been detected in crocodilians from multiple localities (e.g., Phelps et al., 1989; Campbell, 2002; Rauschenberger et al., 2004; Yoshikane et al., 2006; Wu et al., 2014). Contaminant studies have focused largely on residues in eggs and include American alligators (*Alligator mississippiensis*) (Heinz et al., 1991; Cobb et al., 1997; Sepúlveda et al., 2004), American crocodiles (*Crocodylus acutus*) (Hall et al., 1979; Wu et al., 2000), Morelet's crocodiles (*Crocodylus moreletii*) (Wu et al., 2000; Pepper et al., 2004; Wu et al., 2006), Nile crocodiles (*Crocodylus niloticus*) (Wessels et al., 1980; Phelps et al., 1986; Skaare et al., 1991; Bouwman et al., 2014) and broad-snouted caimans (*Caiman latirostris*) (Stoker et al., 2011). OCPs have also been detected in the caudal scutes of Morelet's crocodiles (Sherwin et al., 2016) and American crocodiles from Central America (Rainwater et al., 2007; Rainwater et al., 2011). Evidence suggests that OCP residues can be maternally transferred to developing eggs, potentially leading to reduced clutch size, reduced hatchling success and altered plasma steroid hormone concentrations (Guillette et al., 2000; Rauschenberger et al., 2004; Stoker et al., 2011). While such studies highlight the potential developmental and reproductive effects of OCP exposure in crocodilians, assessments based on egg measurements may not necessarily be good indicators of contaminant burdens in tissues of reproductive adults. Furthermore, the analysis of eggs provides no information about the concentrations within adult males. Differences in pesticide residue levels between the sexes of mature individuals of comparable age and condition might be expected. However, the extent to which OCPs accumulated in the fat of female Nile crocodiles can be transferred during vitellogenesis and the influence of this process on total body burdens is unknown.

In this paper, we investigate the accumulation of OCPs in fat tissues collected from wild Nile crocodiles living within iSimangaliso Wetland Park. Nile crocodiles are important apex predators in many tropical and subtropical aquatic habitats throughout much of sub-Saharan Africa. In many environments throughout the continent and particularly in SA, Nile crocodile populations are threatened by habitat destruction, illegal killings, destruction of nesting sites and human disturbance, and as a result, their conservation status is classified as Regionally Vulnerable for SA (Bates et al., 2014). Lake St Lucia, situated within the iSimangaliso Wetland Park, represents the largest Nile crocodile population within a single waterbody in SA and hosts the most southern viable breeding population of the species (Combrink et al., 2013). The park hosts one of only a few remaining viable breeding populations in the country and is also the largest estuarine population in Africa (Combrink et al., 2013). Crocodiles inhabiting this area have been subjected to OCP exposure since the mid-1940s (Quinn et al., 2011) and the potential impact on their physiology and long-term health is thus of concern. This study presents the first attempt to surgically extract fat tissue from live, wild crocodilians for the purposes of chemical analysis and therefore provides a potentially new method in assessing the health threats to populations living within OCP-contaminated habitats.

2. Methods

2.1 Study area

iSimangaliso Wetland Park (325 000 ha) stretches 230 km along the east coast of SA and encompasses a diverse variety of protected habitats, including several major coastal lakes and estuaries, extensive freshwater wetlands, grassland, savannah, coastal forest and coral reef communities (Fig. 1). Crocodiles are present in most waterbodies and wetlands, but the majority are found within four disjunct populations; Lake St Lucia (35 000 ha), the Kosi Bay lakes (3940 ha); Lake Sibaya (7760 ha) and Nsumo pan (380 ha). St Lucia and Kosi Bay are back-barrier coastal lakes that maintain connectivity with the Indian Ocean via an estuary mouth, while Sibaya is a large isolated freshwater lake. Nsumo pan is a shallow, back-filled floodplain lake, situated 25 km west of the ocean within Mkhuze Game Reserve. The size of the crocodile

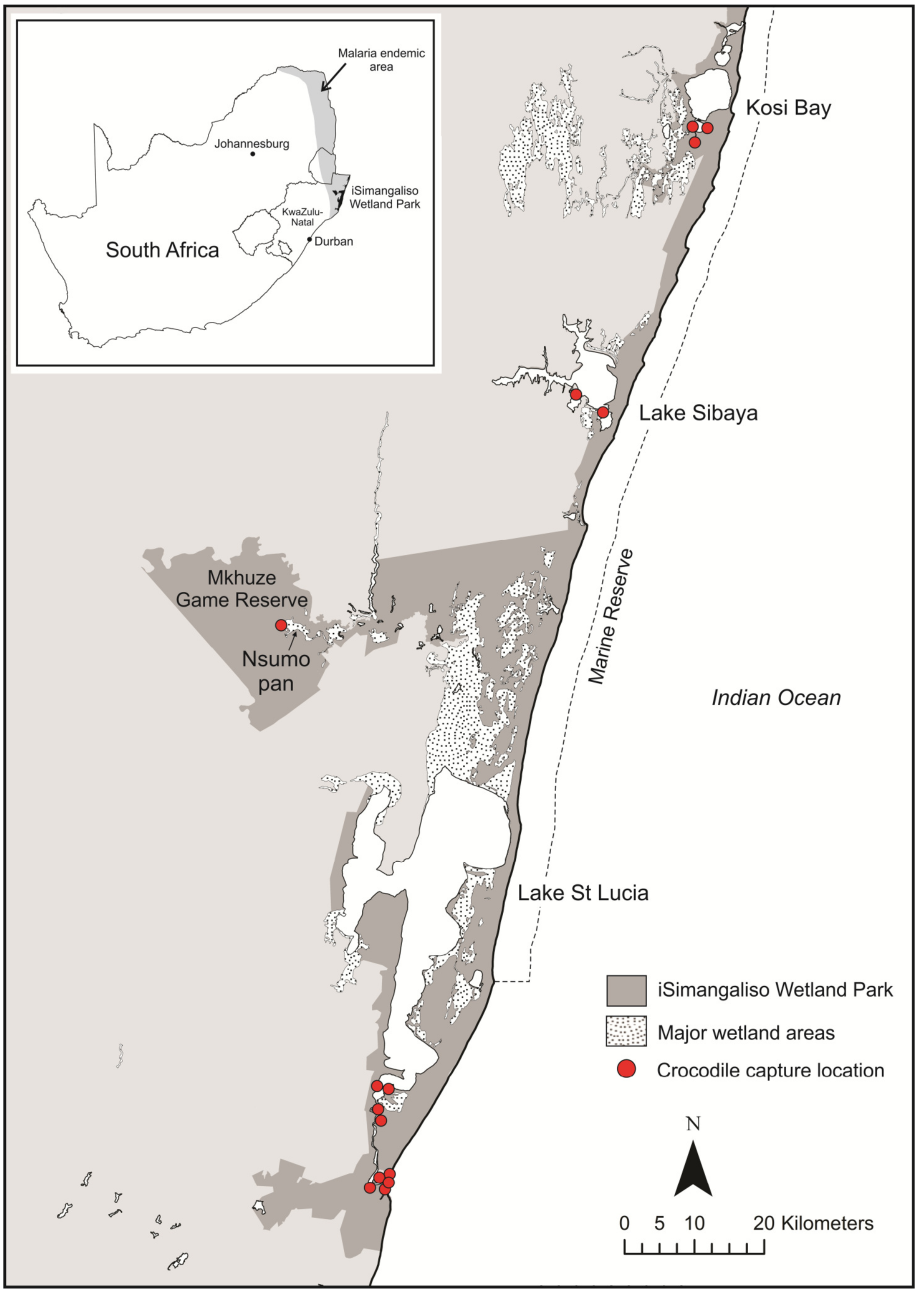

Figure 1. Location of iSimangaliso Wetland Park showing crocodile sampling sites.

population in each of the aforementioned waterbodies seemingly reflects the degree of protection and extent of shared use of the aquatic resource with neighbouring people. Lake St Lucia hosts the largest population with a minimum of 684 adult and sub-adult crocodiles, while Lake Sibaya (26), Kosi Bay (<10) and Nsumo pan (<10) are estimated to contain substantially lower numbers (EKZNW unpublished 2015 aerial survey data). Trend analysis based on aerial count data for the last decade indicates a decline in all four populations (EKZNW unpublished data 2015 aerial survey data) and is attributed largely to direct and incidental anthropogenic pressures, including illegal killings, fish-trap and gillnet mortalities, destructions of nesting sites and eggs, alien plant infestation at nesting sites, boat-collision mortalities, and severe droughts (Kyle, 1999; Leslie and Spotila, 2001; Combrink et al., 2011; Combrink et al., 2013, Warner et al., 2016a).

2.2 Crocodile capture

Crocodile captures took place during 2016 and 2017 under permit from the iSimangaliso Wetland Park Authority and Ezemvelo KZN Wildlife. Crocodiles larger than 2 m in length were targeted as they typically yielded fat samples of sufficient size for laboratory analysis. The capturing of wild crocodiles was handled by an experienced team using standard and approved methods (Manolis and Webb, 2016).

The majority of crocodiles were located at night from a boat with the aid of a spotlight and noosed-captured by securing a self-locking cable snare attached to a 4 m pole around the crocodile’s neck. In some cases, crocodiles were snagged using a small (3/0), weighted barbless treble hook attached to a fishing rod and reel (Cherkiss et al., 2004; Combrink, 2014). Three individuals were captured at the water’s edge during the day. All crocodiles were restrained and blindfolded immediately following capture. In the case of large individuals, the hind legs were tied together to reduce movement. The total length (TL, measured dorsally from the tip of the snout to the tip of the tail) and snout-vent length (SVL, measured from the tip of the snout to the posterior margin of the cloacal vent) of each crocodile was measured using a standard tape measure. The sex of each animal was assessed by cloacal examination (Brazaitis, 1968). Each crocodile was permanently marked for future identification by removing a unique series of three caudal scutes using a sterile scalpel (Combrink, 2014).

2.3 Sample collection

Fat samples were surgically removed from the tails of live Nile crocodiles by making an incision on the ventro-lateral side of the tail, behind the cloaca and hind legs (Fig. 2A). The method of fat extraction from live animals was based on numerous necropsies on crocodile carcasses, especially pansteatitis positive cases (Lane et al., 2013; Myburgh and Botha, 2009). To our knowledge, this study represents the first attempt to surgically extract fat tissue from live, wild crocodilians for the purposes of chemical analysis. The specific area of incision for each crocodile was identified during the pre-surgical inspection of the cranial tail area. The area was thoroughly scrubbed and washed using 4% chlorhexidine gluconate soap (Bioscrub 4%, Dismed Pharmaceuticals (Pty) Ltd, Midrand, South Africa) and a brush. The scrubbed area was disinfected with sterile cotton wool swabs soaked in a chlorhexidine gluconate 0.5% w/v and ethyl alcohol 70% w/v solution (Medicol 0.5%, Acu-Sol, Cape Town, South Africa). This process was repeated until the surgical area was clean. A line block of the area or incision line was done using 2% lignocaine HCl (Lignocaine Injection 2%, Bayer HealthCare, Isando, South Africa). Due to the toxic effects of lignocaine HCl in the Nile crocodile (Jan Myburgh, unpublished data 2017) a maximum dose of 3 mg kg^{-1} was not exceeded. Ample time (20 minutes) was allowed for the local anaesthetic to achieve its maximum analgesic effect before the incision was made. The surgical area was finally sterilised with 100 mg mL^{-1} povidone-iodine spray (Betadine

Antiseptic Solution, Mundipharma (Pty) Ltd, Claremont, South Africa) and wiped with sterile gauze swabs. Surgical drapes were placed over the tail area to maintain a sterile environment during the procedure. A scalpel incision, typically three scutes in length, was made through the skin between adjacent scutes.

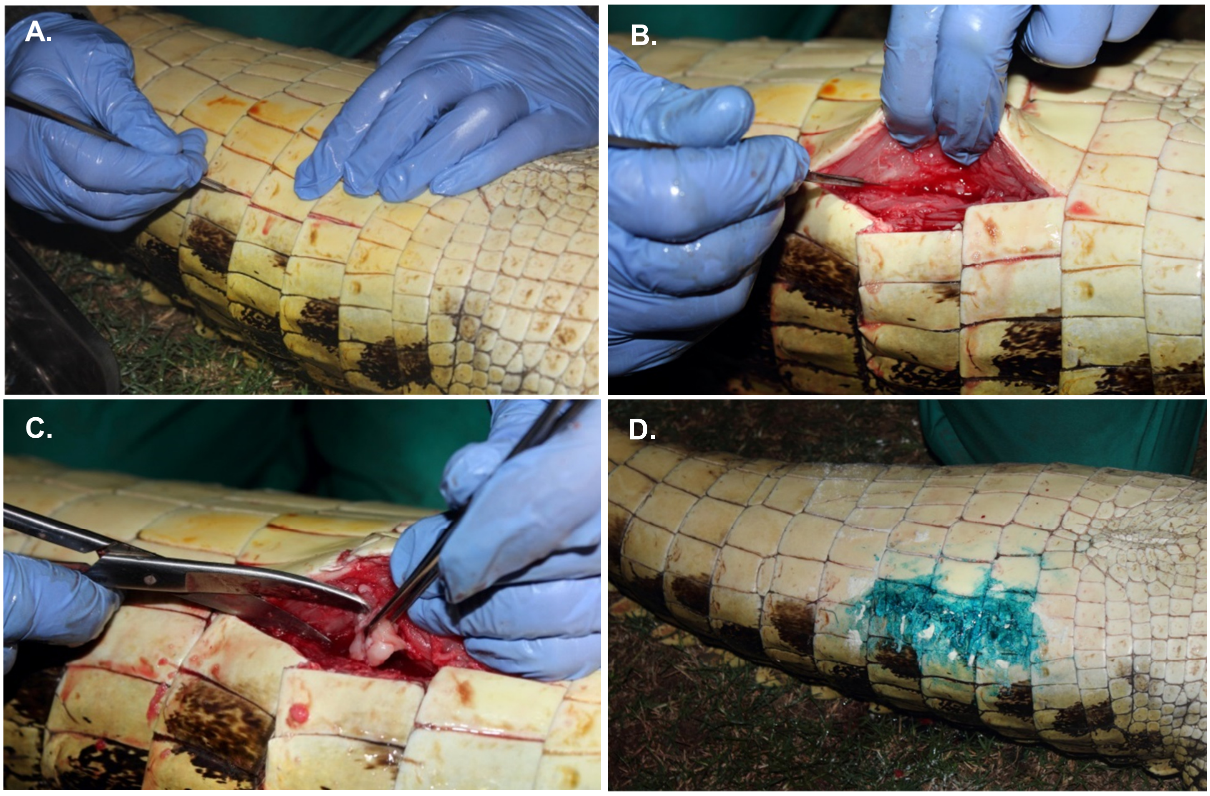

Figure 2. Surgical removal of fat samples from the tail area, showing A) the line of incision on the ventro-lateral side of the tail, B) the incision through the *M. ilio-iscchiocaudalis* muscle, C) the removal of the underlying fat sample, and D) the closed wound with applied Wound Gel Powder. Surgical drape removed for photographic purposes.

The fat deposit targeted was located between the *Musculus ilio-iscchiocaudalis* and *M. caudofemoralis* muscles of the tail. A careful incision was be made through the *M. ilio-iscchiocaudalis*, while sterile swabs were used to remove blood from the surgical wound (Fig. 2B). A sample (2 – 5 g) from the underlying fat layer was removed using blunt-pointed curved scissors (Fig. 2C) and placed in a sterile centrifuge tube. Following removal of the fat sample, the incision through the *M. ilio-iscchiocaudalis* layer was closed using chromic catgut #1 (Cromado Chromic, Ethicon, Johnson & Johnson Medical (Pty) Ltd, Midrand, South Africa) and single interrupted sutures. The incision through the skin was closed using #1 or #0 monofilament nylon (Ethilon, Ethicon, Johnson & Johnson Medical (Pty) Ltd, Midrand, South Africa). The wound (line of incision) was sealed afterwards using a special gel (Wound Gel Powder, Sterkspruit Veterinary Clinic, Lydenburg, South Africa) originally developed for fish surgery and ulcer treatment (Fig. 2D). Crocodiles were injected intramuscularly with a systemic antimicrobial drug (enrofloxacin 10 g 100 mL^{-1}, Baytril 100, Bayer HealthCare, Isando, South Africa) and then released at their site of capture.

All work was performed in compliance with procedures approved by the University of the Witwatersrand Animal Ethics Committee (AESC number: 20133201).

2.4 Organochlorine pesticide analysis

Fat samples were stored on ice in the field and later frozen at -18 ^{o}C before being transported to the University of the Witwatersrand for analysis. They were then washed in deionised water and extracted using a modified QuEChERS method as described in Buah-Kwofie and Humphries (2017). Briefly, OCPs were extracted from 2 g fat samples using 8 ml acetonitrile/acetic acid (99:1 v/v). Anhydrous magnesium sulfate (4 g), sodium acetate (1.0 g) and sodium acetate trihydrate (0.6 g) was used to aid the partitioning of the organic and aqueous phases. Samples were shaken vigorously by hand and then vortexed to prevent the formation of agglomerates. The resulting mixture was frozen at -18 ^{o}C to solidify any lipids and then centrifuged to isolate the organic extract. Clean-up was achieved using a mixture of $MgSO_4$ (1.0 g), C18 (0.4 g), deactivated florisil (0.4 g) and primary secondary amine (0.4 g). A 4 mL aliquot of the clean extract was concentrated to dryness under vacuum and reconstituted in hexane (1 mL) for final analysis.

The analysis of the final extract was achieved by using a two dimensional gas chromatography time-of-flight mass spectrometry (GC X GC-TOFMS). A total of 17 OCPs were analysed, including 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 and endrin ketone; sum expressed as Σdrin), endosulfans (α-, β-endosulfan and endosulfan sulfate; sum expressed as Σendosulfan) 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 Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Supelco (Bellefonte, PA). Linear regressions derived from the matrix-matched calibration curves for all pesticide compounds were ≥ 0.99. Solvents used for the analysis were of HPLC grade and were acquired from Sigma Aldrich. Blank and spiked samples were analysed with each batch, with analyte recoveries ranging between 77 and 109% (Supplementary Table S1). Reproducibility was typically < 10%, with detection limits ranging between 0.12 and 0.4 ng g^{-1} wet weight (ww).

Relationships between residue concentrations and crocodile body size were examined using analysis of variance (ANOVA) followed by Tukey's posthoc test in Statistica 10. Significance was set at $p < 0.05$.

3. Results

3.1 Crocodiles sampled

A total of 15 crocodiles was captured and sampled from Lake St Lucia (n = 9), Lake Sibaya (n = 2), Nsumo pan (n = 1) and Kosi Bay (n = 3). The sampled population consisted of nine adults (≥ 2.5 m) and 6 sub-adults (< 2.5 m), with a male biased female-to-male sex ratio of 0.4:1. The skewed sex ratio was likely due to the small sample size, as a recent study at Lake St Lucia reported a 1:1 sex ratio based on 104 individuals (Warner et al., 2016a). Capture success at Kosi Bay and Lake Sibaya was limited by the low population density of crocodiles present within these systems, while low water levels following a prolonged drought severely hindered capture operations at Nsumo pan.

3.2 Residue concentrations

The concentrations of major organochlorine residues in the 15 fat samples analysed are summarised in Table 1 (full set of results provided as supplementary material; Table S2). OCP residues were detected in all samples analysed, with total residue burdens varying between 3600 and 8000 ng g^{-1}. DDT and its metabolites were the dominant class of OCPs detected in most samples, with ∑DDT concentrations ranging between 520 and 3100 ng g^{-1} (Fig. 3). ∑DDT concentrations were composed largely (~60%) of the metabolite *p,p'*-DDE, with concentrations ranging between 210 and 2060 ng g^{-1} (1070 ± 560 ng g^{-1}). Highest DDT concentrations were measured in fat collected from a 3.75 m male from Lake St Lucia. Although *p,p'*-DDE concentrations varied considerably, concentrations of the parent compound, *p,p'*-DDT, were fairly similar across all samples (330 ± 120 ng g^{-1}).

Elevated levels of other OCPs were also detected, including lindane (67 – 410 ng g^{-1}), aldrin (150 – 620 ng g^{-1}) and heptachlor (170 – 860 ng g^{-1}). The most highly contaminated crocodile was a 2.65 m adult female from Kosi Bay, which contained 2005 ng g^{-1} *p,p'*-DDE, 430 ng g^{-1} aldrin, and 650 ng g^{-1} heptachlor.

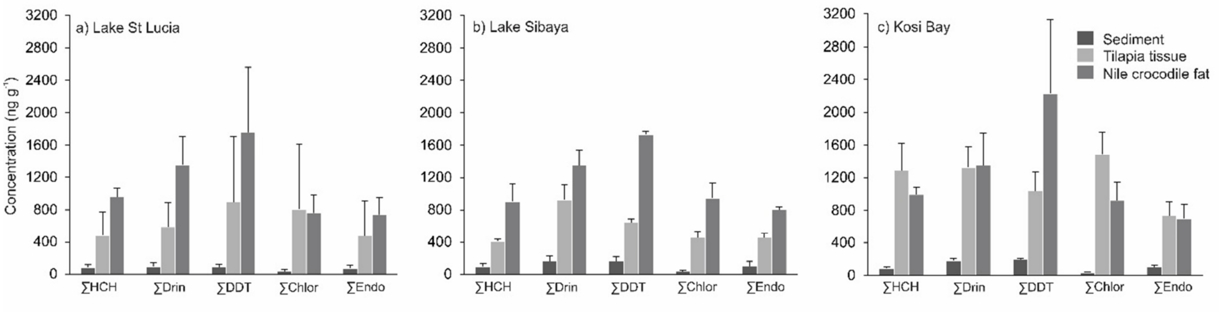

Figure 3. Comparison between OCP concentrations detected in surface sediment (ng g^{-1} dw), tilapia tissue (ng g^{-1} lw) and crocodile fat (ng g^{-1} ww) from a) Lake St Lucia, b) Lake Sibaya and c) Kosi Bay. Standard deviation indicated by error bars. Results from Nsumo pan, where only one crocodile was sampled, are not shown.

3.3 Variability in OCP concentrations

A thorough assessment of inter-site and inter-population variability within our dataset is limited by the small sample size. Nevertheless, calculated mean concentrations at each locality reveal that crocodiles sampled from Kosi Bay had the highest *p,p'*-DDE (1400 ± 690 ng g^{-1}) and total

OCP burdens (Table 1). On average, crocodiles from Lake St Lucia exhibited highest concentrations of aldrin (400 ± 130 ng g^{-1}) and dieldrin (350 ± 110 ng g^{-1}).

A comparison between sub-adult (TL <2.5 m) and adult (TL ≥2.5 m) crocodiles revealed that adults generally had higher OCP burdens (Table 1). In particular, fat samples from adult crocodiles had *p,p'*-DDE concentrations that were on average 30% higher compared to sub-adults. However, we found no significant relationship between crocodile body size (TL) and fat contaminant concentration for any of the residues analysed in this study.

4. Discussion

4.1 Variability in fat tissue OCP concentrations

Results of this study indicate that Nile crocodiles living in iSimangaliso Wetland Park are exposed to, and accumulate, a variety of organochlorine contaminants. This is not an unexpected finding given recently reported OCP residue levels found within sediments and fish from the region (Buah-Kwofie and Humphries, 2017; Buah-Kwofie et al., submitted). The OCPs detected in the fat samples studied here likely originate from agricultural and pest control activities outside the boundaries of the Wetland Park and are introduced via groundwater and fluvial processes. OCPs ultimately accumulate within sediments of the coastal lakes and wetlands, which act as sinks for contaminants in the local environment (Buah-Kwofie and Humphries, 2017). This exposes a variety of fish and invertebrate species to elevated levels of contamination. In particular, elevated levels of OCPs have recently been detected in the tissues of Mozambique tilapia (*Oreochromis mossambicus*) collected from the coastal lakes and estuaries of iSimangaliso (Buah-Kwofie et al., submitted). This species is locally abundant, widely distributed, and forms an important component in the diet of Nile crocodiles from the region. Although adult crocodiles are capable of feeding on large mammals, fish are considered the most important prey items (Pooley, 1982; Leslie, 1997) and the ingestion of contaminated prey is likely the major pathway of exposure for the local crocodile population. The residue concentrations measured in crocodile fat thus likely reflect accumulation over many years as a result of trophic transfer, and in most cases, the concentrations detected in crocodile fat tissues were substantially higher than those found within the muscle tissues (lipid weight) of tilapia (Fig. 3). The potential for biomagnification is particularly evident for DDT and its metabolites, with crocodile fat samples from Lake St Lucia, Lake Sibaya and Kosi Bay all containing *p,p'*-DDE and ∑DDT concentrations significantly higher than that measured in tilapia tissue.

The detection of *p,p'*-DDE as the major residue present in most fat samples is not unexpected as it is the most persistent metabolite of technical DDT, which continues to be used in the region for the control of malaria (Brooke et al., 2013). Technical DDT used for malaria control in the region consists largely of *p,p'*-DDT (75%) and *o,p'*-DDT (21%) (Bouwman et al., 2006). While the accumulation of *p,p'*-DDE reflects the metabolism of DDT, the detection of elevated concentration of *p,p'*-DDT thus suggests recent exposure of crocodiles to the parent compound. The concentration of *p,p'*-DDT measured in the smallest (1.28 m), and therefore youngest crocodile captured, which was within range of some of the largest (>3 m) individuals sampled, supports this observation. The detection of elevated concentrations of lindane, aldrin and methoxychlor, all of which degrade relatively quickly to their respective metabolites, also suggests that crocodile populations within iSimangaliso have been recently exposed to these compounds.

To some extent, variations in OCP concentrations detected in crocodiles from different localities are likely to reflect differences in site contamination. While our dataset (low sample size) precludes a thorough inter-site comparison, fat samples from Kosi Bay crocodiles contained on average highest ∑DDT and ∑OCP concentrations (Table 1). This appears to reflect local environmental gradients, with sediment and fish samples from Kosi Bay also containing highest ∑DDT and ∑OCP concentrations measured within the wetland park (Buah-Kwofie and Humphries, 2017).

Crocodile size is generally considered a good predictor of age (but see Wilkinson et al., 2016) and the age of individuals is relevant in assessing their exposure history. The longevity of crocodiles favours the accumulation of persistent chemicals and some studies have reported significant positive relationships between crocodilian body size and contaminant concentrations (e.g., Yoshikane et al., 2006). In our study, the absence of a significant relationship between crocodile body size and OCP burden may be an artefact of our small sample size. The large (>3.5 m) adult crocodiles sampled are estimated to be >50 years in age and would have lived through several decades of OCP application. A relationship between body size and OCP concentration might therefore be expected, and although highest *p,p'*-DDE burdens were recorded in fat samples collected from adult crocodiles, there appears to be great intra-population variability. Furthermore, the detection of high OCP concentrations in sub-adult crocodiles suggests younger individuals may be just as susceptible to contaminant accumulation. Indeed, Sherwin et al. (2016) found concentrations of the OCP methoxychlor to be two orders of magnitude higher in juvenile Morelet's crocodiles than adults.

A number of studies have suggested that male crocodilians may be more susceptible to contaminant accumulation as females eliminate some of their body burden through egg production and oviposition (e.g., Rauschenberger et al., 2004; Stoker et al., 2011). However, given our small dataset and the biased male to female ratio, this work sheds no light on differences in accumulation between sexes. The analysis of additional samples may allow more meaningful comparisons to be made in the future.

4.2 Biological significance

Few studies have previously examined OC concentrations in bodily tissues of wild crocodilians. We compare our results with available concentration data for fat/muscle tissue obtained from other crocodilians (Table 2). Concentrations of lindane, heptachlor and aldrin detected in Nile crocodiles from iSimangaliso are some of the highest ever reported for crocodilians. These compounds have been rarely detected in crocodilian tissues, with reported concentrations several orders of magnitude lower than levels found in Nile crocodiles from iSimangaliso. Most studies have focused on DDT exposure in crocodilians and have therefore dealt with sites heavily impacted by DDT contamination (e.g., Rauschenberger et al., 2004; Rauschenberger et al., 2007; Yoshikane et al., 2006). Concentrations of *p,p'*-DDE detected in this study are substantially lower than those measured in highly DDT contaminated environments in western Australia (Yoshikane et al., 2006) and Florida (Rauschenberger et al., 2004). The mean concentration of DDT detected in fat tissue in this study was similar to that found in adipose fat of American alligators from Lake Griffin (Florida), a moderately contaminated site (Rauschenberger et al., 2004), but substantially higher than that found in muscle tissues of Chinese alligators (Wu et al., 2014) and caudal scute fat from American crocodiles (Rainwater et al., 2007; 2011; Table 2). *p,p'*-DDT concentrations on the other hand appear to be the highest reported this century for crocodilians (>15 studies). While exposure of crocodilian populations to DDT appears to be declining worldwide, Nile crocodiles from iSimangaliso suffer from chronic exposure as a result of ongoing malaria control operations.

The biological significance of the contaminant concentrations observed in crocodile fat in this study is unknown. Although several studies report body and egg OCP burdens in crocodilians, toxicological data are limited. The available data suggest that, in general, crocodilians can accumulate high concentrations of metals and pesticides, but exhibit a high degree of resistance to the acute toxic effects. For example, Yoshikane et al. (2006) detected high levels of DDE in saltwater (*C. porosus*) and freshwater (*C. johnstoni*) crocodiles in Australia, but reported no obvious effects on individuals of these species. A recent study reported highly elevated blood lead concentrations (960 mg dL^{-1}) in Nile crocodiles from Lake St Lucia (Warner et al., 2016b). However, no clinical effects of lead toxicosis were observed in any of the individuals studied. Nevertheless, crocodilian populations are susceptible to the chronic effects of contaminants on reproduction and long-term health. OCPs accumulated in the fat of females can be transferred to eggs during vitellogenesis (Rauschenburger et al., 2004, 2007; Charruau et al., 2013). Chronic exposure to OCPs is suggested as a cause for significantly decreased hatch rates in American alligators (Rauschenberger et al., 2007) and for reduced clutch size in broad-snouted caimans (Stoker et al., 2011). An association between DDT exposure and abnormal reproductive and endocrine function in juvenile American alligators has also been suggested (Guillette et al., 1994; 1996).

Although the crocodiles sampled in this study appeared healthy, the OCP concentrations reported here suggest they are potentially susceptible to the chronic effects of contaminants. The influence of OCPs on reproduction, egg viability, and hatching success is likely the main concern associated with contaminant accumulation for iSimangaliso's Nile crocodile population. Given the high body burdens reported here, the effect of maternal transfer of contaminants to eggs is also a major cause for concern. While we have no egg-based concentration data from iSimangaliso, the accumulation of OCPs in Nile crocodile eggs has been reported in populations from Kruger National Park (Bouwman et al., 2014) and Kenya (Skaare et al., 1991; Table 2). In Kruger National Park, thickening of the outer eggshell layer was significantly associated with higher concentrations of *p,p'*-DDE. Exposure to a mixture of OCPs increases the possibility for synergistic effects and the associated reproductive implications warrant further investigation.

5. Conclusions

The impact of OCPs on wildlife in southern Africa is of growing concern. The results of this study demonstrate the potential of Nile crocodiles to accumulate substantial quantities of organochlorine contaminants within their fat tissue, with potential long-term reproductive impacts on local populations. While these compounds are not used within the boundaries of iSimangaliso Wetland Park, they may present serious consequences for wildlife in the region and highlight the need to understand the risks associated with OCP exposure, particularly in apex predators such as the Nile crocodile. Further work is needed to better predict the effects of pesticide residues on crocodiles in iSimangaliso, and in Nile crocodile populations in general. In addition to the more obvious threats of habitat destruction, illegal killings and human disturbance, the accumulation of contaminants is emerging as an important threat to Nile crocodile populations in the region. Despite this, few data regarding the possible effects of OCP use on Nile crocodile populations in tropical environments in Africa exist. The iSimangaliso crocodile population provides a unique opportunity to study the long-term consequences of OCPs and thus aid in understanding what risks are posed by contaminants with respect to population survival. Furthermore, the novel surgical technique described in this study was shown to be an effective method for collecting fat tissue from live crocodilians. While this technique is more invasive and requires a longer holding time for the animal when compared to other sampling methods (e.g., scute fat), it allows relatively large amounts of sample to be collected from live animals and from a consistent location on an animal’s body. When compared with concentration data derived from eggs or scute fat, the technique allows for better assessment of the relationships between contaminant body burdens and their potential reproductive and developmental consequences for crocodilians in general.

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Table 1: Organochlorine pesticide concentrations (ng g^{-1} wet wt) measured in fat samples of Nile crocodiles from iSimangaliso Wetland Park

Sample	**Location**	**TL (cm)**	**Sex**	**Lindane**	**Aldrin**	**Dieldrin**	**Endrin**	***p,p'*-DDT**	***p,p'*-DDE**	***p,p'*-DDD**	**Heptachlor**	**Methoxychlor**	**α-Endosulfan**	**β-Endosulfan**	**∑OCP**
1	Lake St Lucia	360	M	–	520	240	310	210	640	290	–	160	350	270	4500
2	Lake St Lucia	193	F	–	620	510	390	250	1000	300	–	190	380	310	5800
3	Lake St Lucia	293	M	240	340	280	190	160	210	150	450	95	200	160	3600
4	Lake St Lucia	390	M	120	430	420	360	370	1800	510	170	110	360	440	6800
5	Lake St Lucia	217	M	67	150	160	160	500	940	600	360	150	130	200	4700
6	Lake St Lucia	375	M	290	340	360	410	530	2060	530	860	79	330	420	7500
7	Lake St Lucia	358	M	310	430	430	420	430	800	260	330	230	370	230	5700
8	Lake St Lucia	262	F	410	430	430	430	410	1200	500	190	260	350	200	6400
9	Lake St Lucia	259	M	350	360	320	380	250	460	540	410	200	310	160	5100
10	Nsumo pan	128	F	350	300	250	230	340	560	160	620	160	160	130	4700
11	Lake Sibaya	181	M	130	460	400	310	280	1100	330	600	190	310	260	6100
12	Lake Sibaya	161	M	205	280	260	290	240	1200	340	290	300	260	340	5400
13	Kosi Bay	320	M	310	280	240	260	320	1500	450	430	130	180	170	5500
14	Kosi Bay	265	F	280	430	420	530	440	2005	670	650	170	420	280	8000
15	Kosi Bay	245	M	200	320	290	410	200	630	480	390	100	340	150	5000
	Residue mean (± SD)			**250 ± 100**	**380 ± 110**	**330 ± 97**	**340 ± 100**	**330 ± 120**	**1070 ± 560**	**410 ± 160**	**440 ± 200**	**170 ± 62**	**300 ± 88**	**250 ± 97**	**5700 ± 1200**
	Mean concentrations by site														
	Lake St Lucia (n = 9)			250	400	350	340	340	1000	410	400	160	310	270	5600
	Lake Sibaya (n = 2)			170	370	330	300	260	1100	330	440	250	290	300	5700
	Kosi Bay (n = 3)			260	340	320	400	320	1400	530	490	140	310	200	6200
	Nsumo pan (n =1)			350	300	250	230	340	560	160	620	160	160	130	4700
	Mean concentrations by age														
	Subadults (TL < 2.5 m, n = 6)			190	350	310	300	300	900	370	450	180	260	233	5300
	Adults (TL > 2.5 m; n = 9)			290	400	350	370	350	1200	440	440	160	320	260	5900

TL = total length
– = not quantified
∑OCP = total organochlorine concentrations calculated based on all analytes presented in the supplementary data (Table S2)

Table 2: Comparison between organochlorine pesticide concentrations detected in the tissues of other crocodilians. Concentrations are presented as means (±SD) based on ng g^{-1} wet weight (ww) unless otherwise stated. Ranges are given in parenthesis.

Species	**Location**	**Tissue analysed**	**Lindane**	**Heptachlor**	**Aldrin**	**Dieldrin**	**Endrin**	***p,p'*-DDE**	***p,p'*-DDD**	***p,p'*-DDT**	**∑DDT**
Nile crocodiles (*Crocodylus niloticus*)											
This study	South Africa, iSimangaliso Wetland Park	Tail fat (n = 15)	250 (67 – 410)	440 (170 – 860)	380 (150 – 620)	330 (160 – 510)	340 (190 – 530)	1070 (210 – 2060)	410 (150 – 670)	330 (160 – 530)	1800 (520 – 3100)
Australian freshwater crocodiles (*Crocodylus johnstoni*)											
Yoshikane et al., 2006	Lower Ord River, Australia	Visceral fat (n = 10)	n.d	n.d	n.d	3.49 (n.d – 29.7)	0.02 (n.d – 0.24)	27747 (1144 – 57403)	74.5 (7.6 – 280)	n.d	27821 (1152 – 55355)
Salt water crocodiles (*Crocodylus porosus*)											
Yoshikane et al., 2006	Lower Ord River, Australia	Visceral fat (n = 10)	0.107 (n.d – 0.79)	n.d	n.d	3.15 (n.d – 11.2)	n.d	3690 (192 – 13708)	n.d	n.d	3690 (192 – 13708)
American alligators (*Alligator mississippiensis*)											
Rauschenberger et al., 2004	Lake Apopka, Florida	Adipose fat (n = 4)	n.d	n.d	n.d	2376 ± 3771	n.d	29840 ± 34366	42.5 ± 67.5	25.9 ± 24	
	Lake Griffin, Florida	Adipose fat (n = 8)	n.d	n.d	n.d	109.3 ± 133.4	n.d	1030 ± 931	10.5 ± 7.7	2.8 ± 1.5	
	Lake Lochloosa, Florida	Adipose fat (n = 3)	n.d	n.d	n.d	13.7 ± 4.7	n.d	297 ± 90.1	1.4 ± 0.1	1.4 ± 0.1	
Chinese alligators, captive (*Alligator sinensis*)											
Wu et al., 2014	Xuancheng, China	Muscle (n = 4)	–	–	–	–	–	39 (5.6 – 190)	0.47 (0.23 – 22)	3.1 (0.13 – 6.4)	48 (6.0 – 220)
American crocodiles (*Crocodylus acutus*)											
Rainwater et al., 2007	Costa Rica	Caudal scute fat (n = 6)	n.d	n.d	n.d	8.0 ± 5.6	229.8 ± 40	340 ± 82	–	255 ± 50.5	–
Rainwater et al., 2011	Costa Rica	Caudal scute fat (n = 7)			n.d – 30		n.d. – 59	n.d. – 118			

n.d = not detected
– = not analysed

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