Distribution and accumulation of organochlorine contaminants in sediments and biota from iSimangaliso Wetland Park (northern KwaZulu-Natal, South Africa): Ecological risks and implications for conservation

By

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A thesis submitted to the Faculty of Science, University of the Witwatersrand in fulfilment of the requirements for the degree of Doctor of Philosophy

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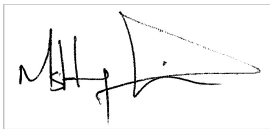
Declaration

The data described in this thesis were collected with approval from the Wits University animal ethics Committee from iSimangaliso Wetland Park, Republic of South Africa, between June 2015 and March 2017. Experimental work was carried out while registered at the School of Chemistry, University of the Witwatersrand, Johannesburg, under the supervision of Doctor Marc S. Humphries.

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of the Witwatersrand, Johannesburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

Archibold Buah-Kwofie
(October 2017)

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.



Dr. Marc S. Humphries
Supervisor
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List of publications and contribution of the authors

This thesis is based on the following papers and report:

I. **The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: Ecological risks and implications for conservation in a biodiversity hotspot**
Archibold Buah-Kwofie, Marc S. Humphries
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Archibold Buah-Kwofie (ABK) conceived of and designed the study with Marc S. Humphries (MSH). ABK and MSH collected the samples. ABK conducted the laboratory experiments. ABK analysed data, and wrote the paper with MSH.

II. **Bioaccumulation and risk assessment of organochlorine pesticides in fish from a global biodiversity hotspot: iSimangaliso Wetland Park, South Africa**
Archibold Buah-Kwofie, Marc S. Humphries, Letitia Pillay
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ABK conceived of and designed the study with MSH. ABK MSH and Letitia Pillay (LP) collected samples. ABK conducted the laboratory experiments. ABK analysed data, and wrote the paper with LP and MSH.

III. **Accumulation of organochlorine pesticides in the fat tissue of wild Nile crocodiles (*Crocodylus niloticus*) from iSimangaliso Wetland Park, South Africa**
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ABK and MSH conceived of and designed the study with input from Xander Combrink (XC) and Jan Myburgh (JM). MSH, XC and JM conducted the field work. ABK conducted the laboratory experiments and analysed the data. ABK and MSH wrote the paper. XC and JM contributed valuable comments.

IV. **A preliminary investigation into the bioaccumulation of organochlorine pesticides in two coral species from the Maputaland reef**

Archibold Buah-Kwofie, Marc Humphries

Sean Porter (SP) conceived of the study. ABK developed the study with MSH, SP and Michael Schleyer (MS). SP and MS collected samples. ABK conducted the laboratory experiments, analysed the data, and wrote the report.

Abstract

iSimangaliso Wetland Park World Heritage site, located in northern KwaZulu-Natal, is one of the most biodiverse conservation regions in southern Africa. It forms part of the Maputaland-Pondoland-Albany biodiversity hotspot and encompasses four Ramsar wetlands of international importance (Lake St Lucia, the Mkhuze wetlands, Lake Sibaya and Kosi Bay). Large breeding populations of numerous species of birds and fish, as well as a host of other endangered species are protected within the Wetland Park. Apart from its ecological importance, resources from the Wetland Park are also used by nearby communities for domestic and agricultural needs. Organochlorine pesticides (OCPs) have been used extensively in the regions surrounding the Wetland Park for agricultural and malaria control purposes, yet few data exist on the local environmental and social impacts of these compounds. This study investigated the distribution and accumulation of OCPs in surface sediments (n = 45), fish tissue (n = 51), crocodile fat (n = 15), and coral tissue (n = 9) from the Wetland Park to assess potential ecological and human health risks.

Substantial OCP concentrations were detected in all samples analysed. Total OCP concentrations in sediment ranged between 120 and 911 ng g^{-1} dw, with ∑HCH (26–283 ng g^{-1} dw) and ∑DDT (34–262 ng g^{-1} dw) the dominant contaminants. These concentrations are among some of the highest detected globally this century, with most far exceeding international soil quality guidelines. Total OCP concentrations in fish tissue samples from *Oreochromis mossambicus* (Mozambique tilapia) and *Clarias gariepinus* (African sharptooth catfish), ranged between 2953 and 8740 ng g^{-1} lw, with all residue concentrations significantly higher than sediment values. Levels in the majority of fish samples exceeded European Commission maximum residue limits and a preliminary risk assessment indicated potential dietary risks associated with exposure to heptachlor, heptachlor epoxide and dieldrin. Nile crocodiles are exposed to OCPs throughout their range within iSimangaliso Wetland Park and contain some of the highest residue concentrations ever recorded in crocodilian fat tissue. 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). The analysis of coral tissues revealed that OCPs also enter the marine environment, with ∑OCP concentrations detected in coral tissues varying between 2300 and 3000 ng g^{-1} ww. ∑DDT (140-460 ng g^{-1} ww), ∑drin (350-900 ng g^{-1} ww), ∑chlor (390-1400 ng g^{-1} ww) were the dominant residues detected.

This study represents the first detailed investigation into the distribution and accumulation of OCPs in iSimangaliso. The concentrations detected in the various environmental components analysed present a number of ecological and human health issues. A full evaluation of the ecological and human health effects associated with OCP contamination are beyond the scope of this thesis, although future assessments would aid in developing appropriate management practices in the region. The continued use of OCPs has potentially serious implications for wildlife conservation in the region and a need for greater understanding of the risks associated with OCP exposure is required. The findings from this study highlight the need for further and detailed investigations into the bioaccumulation and ecotoxicological implications of these OCPs in the aquatic food web, in both freshwater and marine environments. Specifically, further work examining the impact of OCPs on crocodiles and coral reef health is proposed. A more in-depth risk assessment is also recommended to better evaluate the impact of OCP exposure on residents in communities surrounding the Wetland Park.

Dedications

I dedicated this work to my wonderful wife Gloria for the tremendous sacrifices, support, understanding and encouragement during my studies and my lovely children Bradley, Lois and Archibold Jnr for understanding why daddy had to be absent from home for nearly three years.

My parents, thanks for your encouragement, prayers and nurturing. Especially my mother Hagar, for supporting Gloria with the kids in my absence and for single handedly making sure we still had the best of education since the demise of our Dad 19 years ago.

To my siblings Nathaniel and Lawrence and Eugenia, you encouraged and supported me in difficult times and I say thank you and God richly bless you all.

Praise ye the Lord. O give thanks unto the Lord; for he is good: for his mercy endureth forever (Psalm 106:1).

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Table of Content

List of abbreviations and acronyms

$CH_3COONa.3H_2O$	Sodium acetate trihydrate
CRM	Certified reference material
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EC	European Commission
ER-L	Effect range low
ER-M	Effect range medium
EU	European Union
GC	Gas chromatography
GBR	Great barrier reef
HCHs	Hexachlorocyclohexanes
IRS	Indoor residual spraying
IWP	iSimangaliso Wetland Park
KB	Kosi Bay
KNP	Kruger National Park
$MgSO_4$	Magnesium sulfate
MK	Mkhuze wetlands
MS	Mass spectroscopy
NaOAc	Sodium acetate
NOAA	National Oceanic and Atmospheric Administration
OCP	Organochlorine pesticides
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyls
PCNB	Pentachlornitrobenzene
PSA	Primary secondary amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSD	Relative standard deviation
SB	Lake Sibaya

SD	Standard deviation
SL	Lake St Lucia
SPSS	Statistical Package for the Social Sciences
TOF	Time-of-flight
UNEP	United Nations Environmental Program
USEPA	United State Environmental Protection Agency
WHO	World Health Organisation

CHAPTER ONE – General introduction

Organochlorine pesticides (OCPs) have been used extensively for agricultural production and disease vector control since the early 20th century. OCPs are known to persist in the environment, resist different degradation processes, and bioaccumulate through the food chain due to their lipophilicity (UNEP, 2001; Faroon et al., 2002). They may also potentially undergo long-range environmental transport and are known to have adverse effects on their host and non-target organisms (Bouwman et al., 2012; Conis, 2010; Evans and Bouwman, 2000; Makurira et al., 2012). In particular, OCPs and their metabolites have been detected in tissues and organs of animals (Blus et al., 1979; Butler, 1973; Henderson et al., 1971; Henny et al., 1984; Porter and Wiemeyer, 1969; Schmitt et al., 1981), human fatty tissues and breast milk (Bouwman et al., 1989a; Hofvander et al., 1981; Laug et al., 1951; Norén, 1983; Vuori et al., 1977) and blood serum (Bouwman et al., 1989b). Effects of exposure to OCPs in humans are associated with various reproductive, neurological and endocrine disruption (Ferguson et al., 2013; Savitz et al., 1991; Hong et al., 1986; Saxena et al., 1981; Wassermann et al., 1982; Strong et al., 2015). Studies also suggest direct correlation between OCP exposure and the reduction in population of certain species, especially in crocodile (Campbell, 2003; Rauschenberger et al., 2007), different fish species (Kime, 1995; Reijnders, 1986) and some birds including pelicans, cormorants and the bald eagle (Blus et al., 1979; Fry, 1995; Lundholm, 1997). Recognizing the potential adverse impact these compounds have on human health and the environment, the United Nations Environmental Program (UNEP) enacted the Stockholm Convention on Persistent Organic Pollutants (POPs) which was subsequently adopted by Parties in 2001. The aim of the convention is to "eliminate or reduce the production and usage of POPs which include OCPs".

Many industrialized countries banned the use of the first-generation OCPs and other POPs in the early 1970s. In South Africa, OCPs were manufactured and used extensively for agricultural and disease vector control until their eventual ban in 2013, with the exception of DDT which continues to be employed in the malaria endemic regions of the country (Bouwman et al., 2013). In these areas, DDT is typically applied on an annual basis by the

Department of Health as part of an Indoor Residual Spraying (IRS) programme (Department of Health, 2011). The continued use of DDT has proved very effective as reported malaria cases and the budget allocated to malaria control continues to decline (Department of Health; 2011; Bouwman et al., 2006; Sadasivaiah et al., 2007; Tren and Bate, 2004; Craig et al., 2004). Despite these successes, the potential environmental threats posed by OCPs in South Africa have generally been overlooked. Of particular concern is its use in close proximity to ecologically sensitive areas, which serve as breeding grounds for several important species, including nesting turtles, crocodiles, hippos and diverse birds and fish species (iSimangaliso Wetland Park, 2016; Porter et al., 2013). One such area is the iSimangaliso Wetland Park, located in northern KwaZulu-Natal (Fig. 1.1). The Wetland Park encompasses four major Ramsar Sites of international importance; namely Lake St Lucia, the Mkhuze wetlands, Lake Sibaya and Kosi Bay, as well as extensive coral reefs that support abundant marine life.

iSimangaliso Wetland Park (Fig. 1.1) forms part of the Maputaland-Pondoland-Albany biodiversity hotspot and was declared a UNESCO World Heritage site in 1999. With an estimated total area of ~330,000 ha, the park protects a wide range of pristine marine, coastal, wetland, estuarine, and terrestrial environments (UNESCO, 1999). These provide critical habitat to the over 2000 plants species and close to 3000 different species of animals of marine, terrestrial and freshwater origin that breed and reside at iSimangaliso (Porter et al., 2013). There are ongoing efforts by the park management to conserve the over 147 important rare and endangered species, including white and black rhinos, lions, spotted hyenas, cheetahs, leopards, several avian species including saddle-billed storks and pink-backed pelicans, leatherback and loggerhead turtles, and crocodiles within the park (iSimangaliso Wetland Park, 2016; Porter et al., 2013; Zaloumis, 2016)

Apart from its ecological importance, neighbouring communities surrounding the Wetland Park derive resources, including food and water from the lakes for domestic and agricultural activities (Bouwman et al., 2006; Humphries, 2013). According to their 2015/2016 annual report, Wetland Park generated in excess of R1.2 billion which represented ~9 % of the total revenue generated from tourism in South Africa. It also

employs ~7000 people from nearby communities through direct and indirect employment (Zaloumis, 2016).

Despite being formally protected, iSimangaliso is threatened by activities that occur within its catchment areas. This includes the use of pesticides on the numerous agricultural farmlands that surround the park, as well as IRS for malaria vector control. Isolated studies point to the presence of environmentally significant OCP concentrations in the region and high concentrations of DDT have been detected in sediments from Lake Sibaya (Humphries, 2013). Other studies from northern KwaZulu-Natal have shown potential for biomagnification in certain fish and bird species, with OCPs detected in tigerfish from Kruger National Park (Gerber et al., 2016) and Pongolapoort Dam (Wepener and Degger, 2012) and in blood obtained from Pied Kingfishers (Evans and Bouwman, 2000). Eggs collected from other DDT-sprayed areas in South Africa have shown dangerously high levels of DDT, (Bouwman et al., 2013; Davies and Randall, 1989) and DDE has been implicated with eggshell thinning in the African darter (Bouwman et al., 2008) and the African fish eagle (Davies and Randall, 1989).

Human exposure to OCPs, particularly in breastfeeding mothers, has been identified as a major concern in South Africa and other parts of the world where DDT continues to be applied in IRS programs (Bouwman et al., 2012; Mishra and Sharma, 2011; Pirsaheb et al., 2015; Rojas-Squella et al., 2013; Sereda et al., 2009; Tadevosyan et al., 2007). In South Africa, mothers living in the town of Mseleni (near Lake Sibaya) are reported to have some of the highest DDT levels in their breast milk, with concentrations that are almost 100 times higher than the maximum residue limit (Bouwman et al., 2012). Similar concentrations have been reported for work done on breast milk of mothers in the towns of Jozini and Manguzi (Bouwman et al., 2006, 2012) highlighting the potential high risk of exposure to residents in nearby communities.

There is often a delicate connection between humans, wildlife and aquatic ecosystems. Water based-ecosystems in the region are vital for biodiversity conservation, but water also forms a key part of people's everyday life.

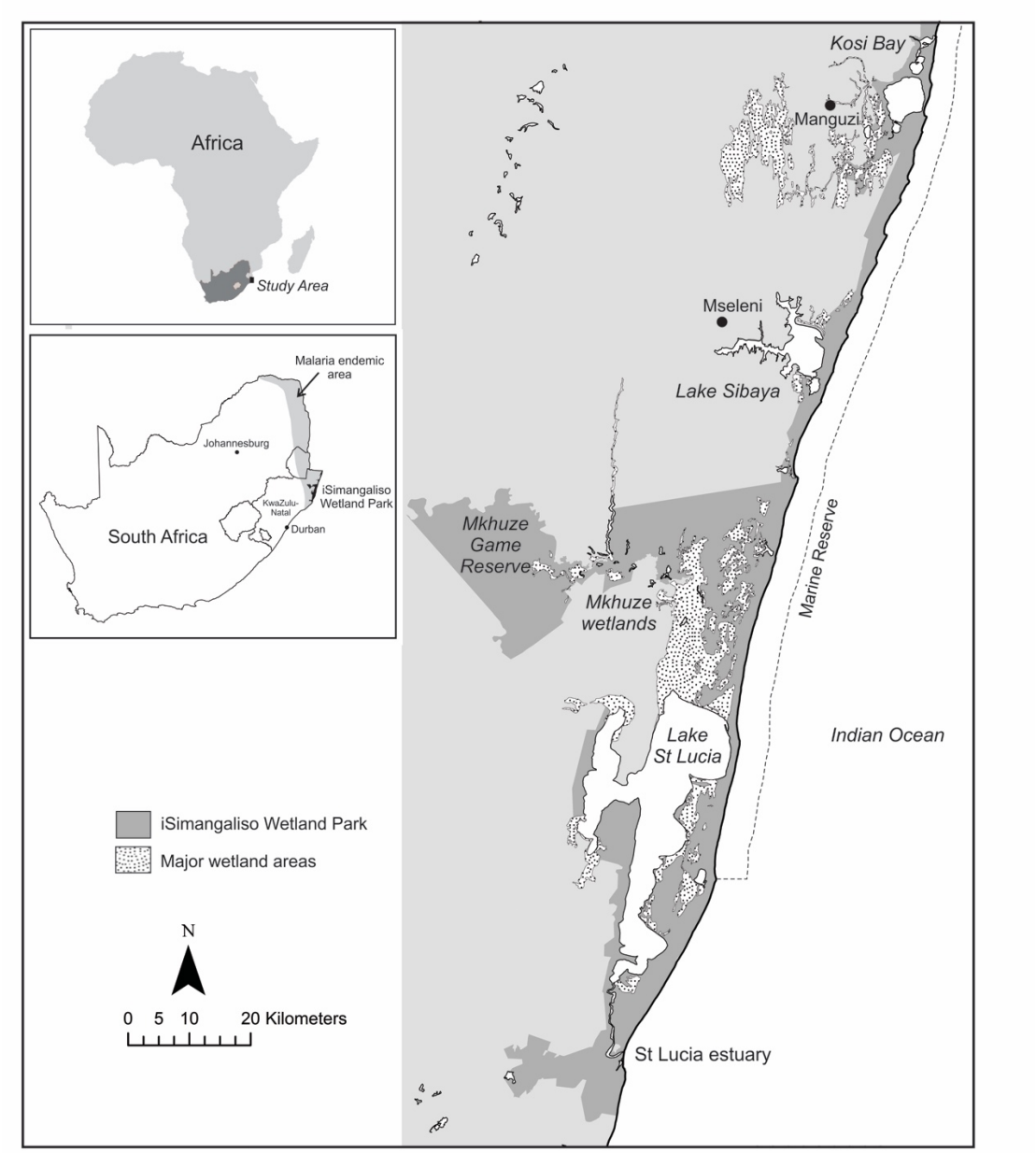

Fig 1.1: Map showing the location of the study area and iSimangaliso Wetland Park (inset), and the four Ramsar systems as well as the marine reserves within the iSimangaliso Wetland Park World Heritage Site where study was undetaken.

Hence, any activity that adversely impacts on the aquatic ecosystem may have direct bearing on human survival. The use of OCPs, and particularly DDT for IRS, is a complex ethical and sustainability issue, and finding a balance between human needs while mitigating the negative environmental effects on aquatic and other wildlife ecosystems is vital. Few data regarding environmental OCP concentrations are reported for the east coast

of South Africa, despite the high ecological value of this region. The continued use of OCPs in the region may have potentially serious implications for both conservation and the local communities who derive resources from the Wetland Park. The implications of the continued use of OCPs, particularly in close proximity to a global biodiversity hotspot, are thus identified as an urgent sustainability and management issue that requires further and more in-depth investigation.

1.2 Aim and structure of the thesis

The aim of this study is to investigate the occurrence and bioaccumulation of organochlorine pesticides in iSimangaliso Wetland Park, and assess their likely impact on both ecosystem and human health. This aim will be addressed in the form of three papers and a preliminary report, which have either been published, or have been submitted for publication. While there may be some minor overlap and repetition between the chapters, the focus of each is distinct. Repetition and any referencing and stylistic inconsistencies have been minimised where possible. References from all chapters are combined in one reference list provided at the end of the thesis. The overall structure of the thesis is summed below:

Chapter 2: *The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: Ecological risks and implications for conservation in a biodiversity hotspot*
This paper examines the distribution of organochlorine pesticides in surface sediments from the main coastal lakes and wetland areas within iSimangaliso Wetland Park and examines their ecotoxicological significance.

Chapter 3: *Bioaccumulation and risk assessment of organochlorine pesticides in fish from a global biodiversity hotspot: iSimangaliso Wetland Park, South Africa*
An investigation into the distribution and accumulation of OCPs in two fish species (*Oreochromis mossambicus* (Mozambique tilapia) and *Clarias gariepinus* (African sharptooth catfish)), commonly found species within the iSimangaliso Wetland Park is presented and the associated human health risk is examined.

Chapter 4: *Accumulation of organochlorine pesticides in the fat tissues of wild Nile crocodiles from iSimangaliso Wetland Park, South Africa*
In this paper, the accumulation of OCPs in the fat tissues of Nile crocodiles, apex predators within iSimangaliso, are examined and their ecotoxicological implications investigated.

Chapter 5: *A preliminary investigation into the bioaccumulation of organochlorine pesticides in two coral species from the Maputaland reef.*
This preliminary study evaluates the susceptibility of two coral species from the Maputaland reef to pesticide contamination and provides a basis for stimulating a full-scale investigation into the likely impact of OCPs on coral health.

Finally, chapter 6 integrates the key findings from all papers and provides directions for future work.

CHAPTER TWO - The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: Ecological risks and implications for conservation in a biodiversity hotspot

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Abstract

The iSimangaliso Wetland Park World Heritage site, located on the east coast of South Africa, spans ~3300 km^2 and constitutes the largest protected estuarine environment for hippopotami, crocodiles and aquatic birds in Africa. Given the ecological importance of this site and continued use of organochlorine pesticides (OCPs) in the region, this study focused on the nature, distribution and potential sources of organochlorine contamination within iSimangaliso Wetland Park. OCPs were widely distributed in surface sediment samples obtained from the four main Ramsar wetland systems within the park (Lake St Lucia, Mkhuze, Lake Sibaya and Kosi Bay). ∑HCH and ∑DDT were the dominant contaminants detected with concentrations in the range of 26.29 – 282.5 ng g^{-1} and 34.49 – 262.4 ng g^{-1}, respectively. ∑DDT concentrations revealed a distinctive gradient, with significantly higher concentrations at Kosi Bay and Lake Sibaya attributed to the application of DDT for malaria control. p,p'-DDE and p,p'-DDD were the dominant isomers detected, but the detection of p,p'-DDT in a number of samples reflects recent inputs of technical DDT. Highest concentrations of HCH, endosulfan and heptachlor were detected in sediments from Mkhuze and reflect the substantial residue load these wetlands receive from agricultural activities within the catchment area. Isomeric compositions indicate that endosulfan and heptachlor residues are derived mainly from historical application, while inputs of HCH, aldrin and endrin could be attributed to more recent usage at several sites. OCP sediment concentrations from iSimangaliso represent the highest yet recorded in South Africa and some of the highest reported globally this century. Sediments found within the lakes and

wetlands of iSimangaliso represent large reservoirs of contaminants that pose ecotoxicological threats to this globally important biodiversity hotspot. Detailed investigation into the bioaccumulation and toxicological risks of OCPs within the wetland park is urgently required.

Keywords:

Organochlorine pesticides; DDT, iSimangaliso Wetland Park, Ecotoxicological Risk

2.1 Introduction

Pesticides have contributed enormously to increases in world agricultural production and improved human health. Despite these achievements, inherent human health and environmental challenges are associated with the use of these compounds because of their toxicity and ability to accumulate in the environment (UNEP, 2001). Of particular concern are organochlorine pesticides (OCPs), which have been linked to a range of physiological disruptions in both humans and wildlife (Conis, 2010; Porter and Wiemeyer, 1969). Despite restrictions on their use, OCP residues continue to be detected in various environmental matrices on a global scale, including water, sediments and aquatic organisms (Fillmann et al., 2002; Iwata et al., 1994; Veljanoska-Sarafiloska et al., 2013). OCPs are toxic, environmentally persistent and able to undergo long-range environmental transport (Dalla Valle et al., 2007; Ritter et al., 1995; Solomon and Schettler, 2000). As a result, their impact on non-target organisms and bioaccumulation potential in the environment are well established (e.g., Barlas, 2002; Davies and Randall, 1989; Gerber et al., 2016). Due to their high hydrophobicity, OCPs tend to adsorb to sedimentary surfaces and often eventually accumulate in estuaries and lakes (Zhou et al., 2006), where they may be transferred through the food chain.

The impact of pesticide residues is of great concern when applied near ecologically sensitive areas. Although banned in many countries, OCPs continued to be used in South Africa until 2013, particularly in the north-eastern parts of the country where DDT is

applied for malaria control. Other OCPs including hexachlorocyclohexanes (HCHs), aldrin, heptachlor, and endosulfan have a long history of usage for agricultural purposes. The continued use of OCPs in South Africa is of particular environmental concern as the north-eastern malaria endemic regions of the country are host to a number of globally important conservation areas, including Kruger National Park and iSimangaliso Wetland Park (Fig. 2.1). The detection of significant OCP levels in different environmental matrices from the region, including human breast milk (Bouwman et al., 2006), surface sediment (Gerber et al., 2015; Sereda and Meinhardt, 2005; Van Dyk et al., 2010), fish tissue (Gerber et al., 2016; Wepener et al., 2012), bird eggs (Bouwman et al., 2008; 2013) and crocodile eggs (Bouwman et al., 2014), presents serious implications for both biodiversity conservation and human health.

The iSimangaliso Wetland Park World Heritage site, located on the east coast of South Africa, spans ~280 km of pristine coastline (Fig. 2.1) and encompasses four major Ramsar wetlands of international importance (Lake St Lucia, Mkhuze wetlands, Lake Sibaya, and Kosi Bay). iSimangaliso forms part of the Maputaland-Pondoland-Albany biodiversity hotspot and includes some of the most important habitats for hippos, crocodiles, and aquatic birds on the continent. The lakes and wetlands also support neighboring communities, many of whom are dependent on the local environment for water, food, and resources. However, these coastal systems drain catchment areas where pesticides are employed for agricultural and mosquito control purposes. Despite the high ecological value of this region, inadequate data are available for iSimangaliso Wetland Park, with previous investigations limited to case studies (e.g., Bouwman et al., 2012; Humphries, 2013). Ecological risk assessment requires knowledge of the nature and spatial distribution of potential chemical stressors, and an investigation into the impact of OCP accumulation within iSimangaliso is urgently needed. Here we present sediment concentration data to examine the nature, distribution and likely source of OCPs within iSimangaliso Wetland Park. The study focuses on sediments from the major coastal lakes and wetlands, as this is where most terrestrially-derived sediments settle and therefore where most persistent chemicals are likely to accumulate. We assess the potential ecotoxicological risk

associated with the continued use of OCPs in the region and provide baseline data for future research on bioaccumulation of these compounds in the study area.

2.2 Materials and Methods

2.2.1 Study site

Sampling focused on the main coastal lakes and wetland areas of iSimangaliso Wetland Park (Fig. 2.1). Lake St Lucia (350 km^2), a shallow estuarine lagoon, is the largest of these. The lake is fed by five major rivers and is characterised by silt and clay-dominated sediment. The Mkhuze River, which drains into the northern end of Lake St Lucia, is the largest contributor to freshwater and sediment supply. The Mkhuze River drains a catchment area of ~6000 km^2 and sustains an extensive wetland area characterised by numerous shallow, clay-filled floodplain lakes (e.g. Nsumo and Muzi). To the north, Lake Sibaya and Kosi Bay occupy deeper (> 20 m) basins that are supplied almost entirely by local groundwater. Lake sediments here are dominated by organic-rich muds.

2.2.2 Sample Collection

During 2015 and 2016, a total of 45 surface sediment samples were collected from iSimangaliso Wetland Park; Lake St Lucia (SL, n = 17), Mkhuze wetlands (MK, n = 4), Lake Sibaya (SB, n = 16) and Kosi Bay (KB, n = 8). Sample sites were selected based on accessibility and to include major drainage lines and river inlets. Sediments (top ~5 cm) were collected using a Van Veen grab sampler, placed in aluminum foil and sealed zip lock bags. The samples were kept on ice in the field and later at below -18^{o}C in the laboratory. Sediments were air-dried at room temperature, crushed and sieved to <42 µm. The sieved samples were then kept in amber bottles and stored below -18^{o}C until analysis.

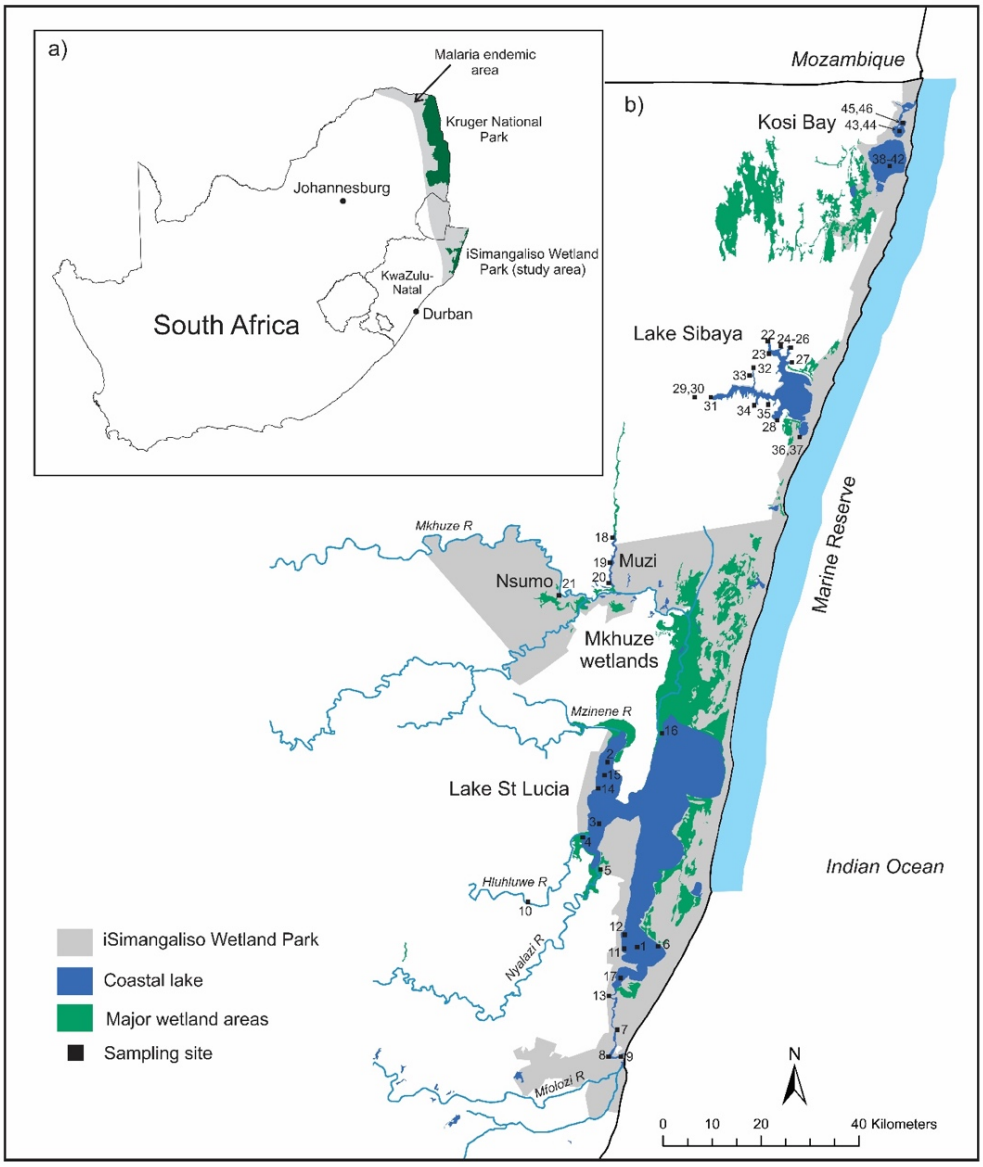

Fig 2. 1. Map showing the location of a) major conservation areas within the malaria endemic region of South Africa, and b) the location of sediment sampling sites (1-46) within iSimangaliso Wetland Park. Sampling focused on the four main Ramsar wetland sites (Lake St Lucia, Mkhuze wetlands, Lake Sibaya and Kosi Bay).

2.2.3 Sample extraction and analysis

The extraction of OCPs from sediment samples was carried out following a modified QuEChERS procedure (Correia-Sá et al., 2012). Briefly, 10 g of sample was weighed into a 50 ml centrifuge tube and spiked with 50 ng g^{-1} pentachloronitrobenzene (PCNB). The samples were allowed to stand for 30 minutes at room temperature and then saturated

with 15 ml of water. After 20 minutes, samples were extracted with 20 ml acetonitrile:acetic acid (99:1 v/v), 8 g anhydrous magnesium sulfate ($MgSO_4$), 1.5 g sodium acetate (NaOAc) and 1.0 g sodium acetate trihydrate ($CH_3COONa.3H_2O$). Samples were shaken vigorously by hand and then vortexed to ensure complete dispersion. The organic extract was separated and transferred into a centrifuge tube containing 1.2 g $MgSO_4$, 0.4 g C18, and 0.4 g primary secondary amine (PSA). This mixture was vortexed and then centrifuged to isolate the clean extract. A 5 ml aliquot of the extract was concentrated to dryness under vacuum at a temperature ≤ 40°C and reconstituted in hexane (1 ml) for final analysis.

2.2.4 Apparatus and Conditions

A total of 17 OCPs were analysed, including dichlorodiphenyltrichloroethane (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 Σdrins), endosulfans (α-, β-endosulfan and endosulfan sulfate; sum expressed as Σendosulfans) and chlor-residues (heptachlor, heptachlor epoxide and methoxychlor; sum expressed as Σchlors).

Two-dimensional gas chromatography–time-of-flight mass spectrometry (GC X GC-TOFMS) 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 with Integra-Guard (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 He as the carrier gas at a constant flow rate of 1.4 ml/min. The GC oven temperature was set to 75 °C (held for 0.5 min), then raised to 280 °C at a rate of 8 °C/min and maintained at this temperature for 2 min. The transfer line and inlet temperatures were set at 260 and 250 °C, respectively. The ion source temperature was 250 °C with a detector voltage of 1850 V. 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 performed against high purity (>98%) reference standards purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Supelco (Bellefonte, PA). Linear regressions obtained from the matrix-matched calibration curves for all pesticides were ≥ 0.99. All solvents used were of HPLC grade and purchased from Sigma-Aldrich (Johannesburg, South Africa).

2.2.5 Quality Assurance and Quality Control

A sediment certified reference material (CRM 804, Sigma-Aldrich) was used to validate the procedure, with all analytes falling within 85-123% of certified values (see Supplementary Table TS 2.1). Blanks and spiked samples were analyzed with each batch, with recoveries for all OCPs ranging between 68 and 115 % (Supplementary Table TS 2.2). The extraction efficiency of the internal standard (PCNB) was 89.3 ± 11.8% (n=3). Reproducibility was typically ≤ 15% for both CRM and spiked recovery studies, with detection limits ranging between 0.42 and 3.1 ng g^{-1} dry weight (dw).

2.2.6 Data treatment

Analysis of variance (ANOVA) using a two-tailed t-test was performed to investigate spatial differences in mean concentrations. All tests were considered significant when $p < 0.05$. Descriptive data analyses were performed using IBM SPSS version 20.

2.3 Results and Discussion

2.3.1 Concentration and spatial distribution of OCPs

The OCPs investigated in this study were widely distributed in surface sediments from iSimangaliso Wetland Park (Table 2.1), with total concentrations (∑OCP) varying between 120 and 911 ng g^{-1} (Fig. 2.2). HCH, DDT, drin, endosulfan and chlor residues were detected in all samples. Among the residues analysed, ∑HCH, ∑DDT and ∑drin were the predominant residues detected, varying from 26.3 to 282.5 ng g^{-1} (average of 95.4 ng g^{-1}), 34.5 to 260 ng g^{-1} (average of 137.7 ng g^{-1}) and 19.4 to 277.5 ng g^{-1} (average of 135.3 ng g^{-1}), respectively.

2.3.1.1 HCH

Highest ∑HCH concentrations were found in sediments from the Mkhuze wetlands where concentrations ranged from 114 to 283 ng g^{-1} (average of 187.1 ng g^{-1}). ∑HCH concentrations in sediments from Lake St Lucia, Lake Sibaya, and Kosi Bay were in the range of 26.3 - 185.8 ng g^{-1} (average of 82.9 ng g^{-1}), 32.4 - 176.0 ng g^{-1} (average of 91.6 ng g^{-1}) and 58.0 - 113.6 ng g^{-1} (average of 83.5 ng g^{-1}), respectively (Fig. 2.2). No significant differences in total mean concentrations between the four systems were observed. α-HCH and β-HCH were the most frequently (98%) detected isomers and were also present in highest concentrations, while γ-HCH was detected less frequently (24% of samples).

2.3.1.2 DDT

Sediments from Lake St Lucia revealed a wide range in ∑DDT concentration, varying between 34 and 158 ng g^{-1}. Highest concentrations were often found near river inlets (Fig. 2.1), with samples from the Mkhuze (120.8 ng g^{-1}), Hluhluwe (135.8 ng g^{-1}) and Mfolozi (119.6 ng g^{-1}) river inlets all showing relatively high ∑DDT concentrations. Samples collected from the Mkhuze wetlands all showed elevated ∑DDT concentrations (averaging

135.9 ± 41.7 ng g^{-1}), with highest concentrations found at Nsumo Pan (193.4 ng g^{-1}). Sediments from Lake Sibaya and Kosi Bay showed the highest ∑DDT concentrations within the study area and were significantly higher than levels at Lake St Lucia (Fig. 2.2). ∑DDT concentrations at Lake Sibaya and Kosi Bay varied from 61.1 to 259.9 ng g^{-1} (average of 162.3 ng g^{-1}) and 182.0 to 209.6 ng g^{-1} (average of 196.5 ng g^{-1}), respectively. p,p'-DDE, and p,p'-DDD were the dominant isomers detected, accounting for on average 45% and 49% of ∑DDT, respectively. Lower concentrations of p,p'-DDT were detected in all samples from Mkhuze and Kosi Bay, but less frequently (56% of samples) at St Lucia and Sibaya.

2.3.1.3 Drin-related compounds

Aldrin (n.d. – 44.02 ng g^{-1}), dieldrin (5.78 – 90.6 ng g^{-1}) and endrin (2.45 – 151.6 ng g^{-1}) were detected in almost all samples analyzed (Table 2.1). ∑drin concentrations found in sediments from Lake St Lucia and Mkhuze were 19.37 – 171.3 ng g^{-1} (average of 93.6 ng g^{-1}) and 90.2 – 163.3 ng g^{-1} (average of 120.7 ng g^{-1}), respectively. Compared to St Lucia, samples from both Lake Sibaya and Kosi Bay contained significantly higher concentrations, averaging 161.5 ± 67.0 ng g^{-1} and 178.9 ± 28.7 ng g^{-1}, respectively.

2.3.1.4 Other organochlorine pesticides

Heptachlor and heptachlor epoxide concentrations ranged between n.d. – 25.8 ng g^{-1} and n.d. – 64.7 ng g^{-1}, and were detected in 61% and 91% of samples, respectively (Table 2.1). Methoxychlor residues were rarely detected and were only present in relatively low concentrations. ∑chlor concentrations from Mkhuze varied between 57.4 and 90.5 ng g^{-1} and were significantly higher than concentrations from Lake St Lucia (35.1 ± 22.5 ng g^{-1}), Lake Sibaya (34.0 ± 14.3 ng g^{-1}) and Kosi Bay (31.6 ± 11.1 ng g^{-1}). α-endosulfan and β-endosulfan residues were detected in most samples. Total endosulfan concentrations at Mkhuze varied between 90.9 and 197.0 ng g^{-1} (average of 141.3 ng g^{-1}) and were significantly higher than those found at St Lucia (73.2 ± 35.5 ng g^{-1}) and Kosi Bay (98.3 ±

23.8 ng g^{-1}). No significant differences in total endosulfan concentration between Lake St Lucia, Lake Sibaya, and Kosi Bay were found.

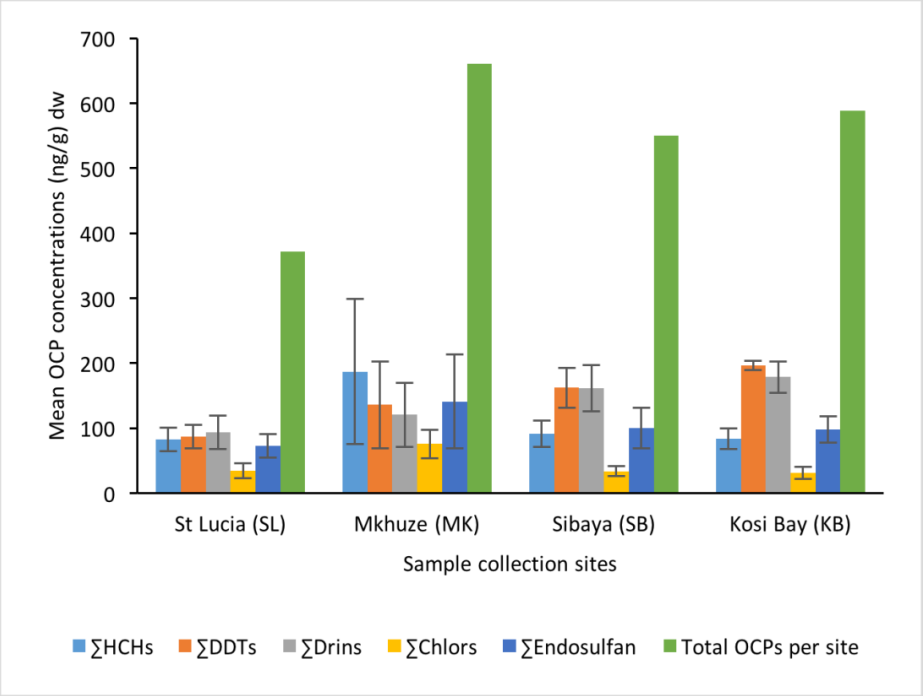

Fig 2. 2**:** Average total concentrations (ng g^{-1} dw) of HCH, DDT, drin, endosulfan and chlor residues for each system within iSimangaliso Wetland Park (n = 45). Error bars show the 95% confidence interval of the mean.

2.3.2 OCP composition and potential sources

Compositional differences in OCP isomer profiles were used to provide insight into potential sources of contamination. DDT was introduced into the region in 1946 (Evans and Bouwman, 2000), and although use for agricultural purposes was banned in 1976, it is still utilized on an annual basis by the Department of Health in malaria control programs. Technical DDT used for malaria control is typically a mixture of p,p'-DDT (72 – 75%) and o,p'-DDT (21%) isomers, with traces of p,p'-DDE and p,p'-DDD (Bouwman et al., 2006). Once in the environment, DDT undergoes degradation to DDE and DDD, which are more stable and persistent than their parent compound. The concentration of DDT relative to its metabolites can, therefore, be used to assess possible pollution sources (Doong et al., 2002).

Table 2.1: Mean concentrations ± SD, (Min – Max) of OCP metabolites in surface sediment samples from iSimangaliso Wetland Park (ng g^{-1} dw). n.d. = not detected.

	Concentration ng g^{-1} dw				
Analytes	Lake St Lucia	Mkhuze	Lake Sibaya	Kosi Bay	% Detection
α-HCH	25.6 ± 13.2 ***(n.d. - 46.0)***	38.6 ± 4.6 ***(34.6 - 44.6)***	40.7 ± 13.3 ***(15.7 - 67.3)***	48.5 ± 8.3 ***(38.2 - 61.8)***	98
β-HCH	27.0 ± 17.7 ***(n.d. - 78.5)***	94.1 ± 70.8 ***(35.9 - 191.0)***	28.4 ± 15.2 ***(4.8 - 64.5)***	28.9 ± 10.4 ***(11.4 - 43.7)***	98
δ-HCH	19.2 ± 8.8 ***(n.d. - 30.6)***	37.4 ± 8.1 ***(27.1 - 46.9)***	18.6 ± 10.9 ***(n.d. - 43.8)***	6.2 ± 6.7 ***(n.d. - 13.9)***	87
γ-HCH	11.0 ± 13.8 ***(n.d. - 38.3)***	17.1 ± 34.2 ***(n.d. - 68.38)***	3.9 ± 14.1 ***(n.d. - 56.3)***	n.d.	24
∑HCHs	**82.9 ± 35.4 (26.3 - 185.8)**	**187.1 ± 70.0 (113.9 - 282.5)**	**91.6 ± 37.9 (32.4 - 176.0)**	**83.5 ± 19.2 (57.9 - 113.6)**	
Aldrin	19.7 ± 11.01 ***(n.d. - 40.3)***	2.9 ± 2.7 ***(n.d. - 5.6)***	28.5 ± 10.8 ***(5.8 - 44.0)***	27.9 ± 8.7 ***(16.7 - 43.4)***	96
Dieldrin	26.4 ± 13.4 ***(5.8 - 54.8)***	58.3 ± 15.6 ***(46.8 - 80.5)***	51.1 ± 21.9 ***(7.8 - 90.6)***	63.9 ± 12.5 ***(46.2 - 85.6)***	100
Endrin	38.9 ± 28.6 ***(2.5 - 102.4)***	43.9 ± 13.1 ***(29.9 - 61.5)***	74.1 ± 42.9 ***(9.7 - 151.6)***	83.6 ± 15.1 ***(58.4 - 110.7)***	100
Endrin ketone	8.7 ± 8.1 ***(n.d. - 26.5)***	15.4 ± 5.7 ***(7.7 - 21.4)***	7.8 ± 8.4 ***(n.d. - 28.9)***	3.5 ± 3.0 ***(n.d. - 7.5)***	72
∑Drins	**93.6 ± 49.3 (19.4 - 171.3)**	**120.7 ± 30.7 (90.2 - 163.3)**	**161.5 ± 67.0 (48.6 - 277.5)**	**178.9 ± 28.7 (139.9 - 227.7)**	
Heptachlor	8.9 ± 8.5 ***(n.d. - 25.4)***	15.5 ± 8.4 ***(5.58 - 25.8)***	4.6 ± 4.6 ***(n.d. - 15.5)***	n.d.	61
Heptachlor epoxide	22.4 ± 12.9 ***(n.d. - 39.9)***	53.9 ± 9.4 ***(42.3 - 64.7)***	27.3 ± 14.9 ***(n.d. - 50.9)***	31.6 ± 11.1 ***(14.8 - 51.7)***	91
Methoxychlor	3.7 ± 7.4 ***(n.d. - 26.9)***	6.4 ± 4.4 ***(n.d. - 9.6)***	2.2 ± 2.9 ***(n.d. - 6.3)***	n.d.	35
∑Chlors	**35.1 ± 22.5 (n.d. - 70.9)**	**75.9 ± 13.9 (57.4 - 90.5)**	**34.0 ± 14.3 (5.6 - 56.3)**	**31.6 ± 11.1 (14.8 - 51.7)**	
p,p'-DDE	40.6 ± 18.0 ***(18.0 - 80.4)***	62.5 ± 14.8 ***(47.5 - 80.9)***	72.2 ± 29.9 ***(14.2 - 122.8)***	86.8 ± 11.2 ***(70.3 - 99.9)***	100
p,p'-DDD	39.3 ± 15.7 ***(16.4 - 70.2)***	54.7 ± 9.3 ***(43.5 - 63.7)***	84.6 ± 26.7 ***(31.4 - 127.2)***	102.3 ± 8.7 ***(88.9 - 112.8)***	100
p,p'-DDT	7.3 ± 10.5 ***(n.d. - 31.5)***	18.8 ± 22.1 ***(6.4 - 51.8)***	5.6 ± 6.3 ***(n.d. - 17.9)***	7.4 ± 2.6 ***(4.5 - 12.2)***	67
∑DDTs	**87.3 ± 35.6 (34.5 - 158.6)**	**135.9 ± 41.7 (104.7 - 193.4)**	**162.3 ± 58.2 (61.1 - 259.9)**	**196.5 ± 8.9 (181.9 - 209.6)**	
α-Endosulfan	32.9 ± 15.4 ***(3.8 - 62.4)***	64.4 ± 14.5 ***(47.9 - 79.1)***	45.8 ± 32.5 ***(6.0 - 112.6)***	56.2 ± 14.6 ***(38.4 - 78.5)***	100
β-Endosulfan	26.9 ± 15.1 ***(n.d. - 52.6)***	43.4 ± 16.6 ***(22.1 - 61.9)***	42.9 ± 22.9 ***(n.d. - 92.6)***	37.5 ± 15.1 ***(15.1 - 54.4)***	96
Endosulfan sulfate	13.4 ± 14.2 ***(n.d. - 43.6)***	33.5 ± 21.0 ***(11.7 - 61.6)***	11.7 ± 10.1 ***(n.d. - 30)***	4.6 ± 2.5 ***(1.5 - 8.3)***	80
∑Endosulfans	**73.2 ± 35.5 (12.2 - 126.5)**	**141.3 ± 45.6 (90.9 - 197)**	**100.4 ± 58.5 (16.4 - 235.1)**	**98.3 ± 23.8 (65.2 - 135.2)**	

In samples from iSimangaliso, DDE and DDD account for ~94 % of the ∑DDT concentration (Fig. 2.3), reflecting long-term degradation and the legacy of DDT use in the region. DDT degrades to DDE under aerobic conditions and to DDD under anaerobic conditions (Hitch and Day, 1992). Sediments from Lake St Lucia and Mkhuze have DDD/DDE ratios that are on average <1, indicating reductive dechlorination of DDT to DDD under aerobic conditions. Lake St Lucia and Mkhuze are shallow water (<1 m) systems, subjected to intensive sediment resuspension that likely favours aerobic degradation processes. In contrast, sediments from the deeper, groundwater-fed coastal lakes of Sibaya and Kosi Bay have DDD/DDE ratios of >1. Limited mixing and restricted water exchange in these environments favour metabolism to DDD under anaerobic redox conditions. Although compositional variations of DDT isomers indicate long-term degradation, the detection of significant concentrations of p,p'-DDT (up to 51.8 ng g^{-1}) within sediments from all systems points to more recent inputs and likely reflects contamination associated with ongoing indoor residual spraying (IRS).

Technical-grade HCH typically contains α-, β-, δ- and γ-isomers with percentages in the range of 50-70%, 5-12%, 6-10% and 10-12%, respectively (Saadati et al., 2012). α-HCH and β-HCH were detected in most sediment samples and on average accounted for ~74% of ∑HCH concentrations. Compared to typical technical compositions, β-HCH was present in much larger quantities relative to ∑HCH (Table 2.1). This can be attributed to the degradation of the α- and γ-isomers to the more stable β-HCH (Walker et al., 1999; Willett et al., 1998). γ-HCH (half-life of 24 days) is easily degraded by microorganisms and photochemically isomerized to α-HCH (Malik et al., 2007). The presence of γ-HCH in samples from Lake St Lucia and Mkhuze suggests recent contamination and is likely related to the fact that use of HCH in South Africa was until recently permitted for agricultural purposes (Bouwman et al., 2013). Ratios of α- to γ-HCH at St Lucia and Mkhuze ranged between 0.5 and 2.9, suggesting that HCH inputs are a mixture of both technical HCH and pure γ-HCH (lindane). γ-HCH was rarely detected in sediments from Lake Sibaya and Kosi Bay.

The abundance of dieldrin relative to aldrin (average ratio ~2:1) reflects the degradation of aldrin to the more stable and persistent dieldrin. This is expected as the drin-related compounds were banned in South Africa for agricultural and public health purposes in the 1980s (Fischer et al., 2011). However, the detection of relatively high aldrin concentrations (up to 44 ng g^{-1}) in samples from all systems suggests more recent inputs. Similarly, the predominance of endrin (half-life of 12 – 20 years) which undergoes photochemical and biodegradation to endrin ketone and endrin aldehyde (Nollet and Gelder, 2000), points to recent contamination.

Technical endosulfan typically consists of α and β isomer in a 7:3 ratio, with the degradation of β-endosulfan being slower than that of the α-isomer (Ghadiri and Rose, 2001; Leonard et al., 2001). Average ratios measured in this study were lower than that of technical endosulfan, reflecting historical contamination. Heptachlor epoxide concentrations were generally higher than those of heptachlor (Table 2.1). This is expected as heptachlor is rapidly metabolized in sediments to the more stable epoxide (WHO, 2004). Heptachlor was not detected at several sampling sites indicating the complete degradation of this compound.

While a quantitative comparison between datasets obtained from different studies is complicated by variances in the time of sampling, nature of the sediment analyzed and analytical methods used, it allows us to place data from iSimangaliso within a regional and global context. The only previous study to examine OCP residues in sediments from iSimangaliso investigated the concentration of DDT and its metabolites in surface sediments from Lake Sibaya (Humphries, 2013). ∑DDT concentrations at Lake Sibaya measured during this study (61.1 - 259.9 ng g^{-1}) were comparable with previously reported levels (0.8 – 123 ng g^{-1}; Table 2.2). Comparison with other regional data reveals that sediment OCP concentrations from iSimangaliso are the highest yet reported for the country. This includes data from other areas in South Africa where DDT continues to be applied for IRS purposes (e.g. Van Dyk et al., 2010; Sereda and Meinhardt, 2005). OCP concentrations from iSimangaliso are also substantially higher than recently reported values from Kruger National Park (Gerber et al., 2015).

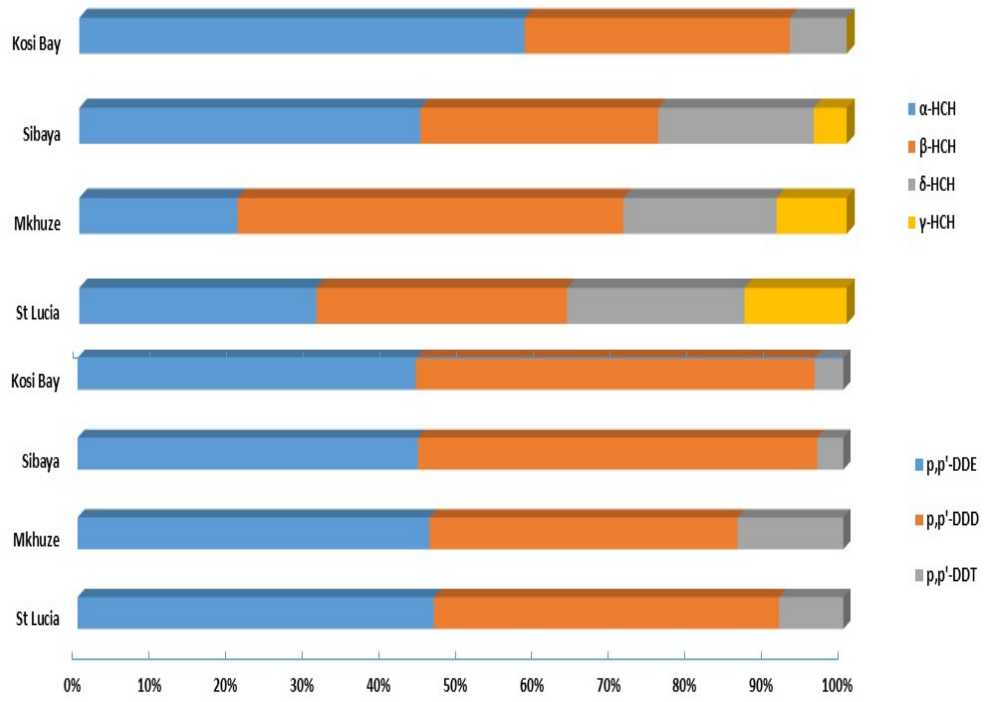

Fig 2. 3**:** Composition of ∑HCH and ∑DDT in surface sediments (n=45) from Lake St Lucia (SL), Mkhuze (MK), Lake Sibaya (SB) and Kosi Bay (KB).

On a global scale, OCP concentrations from iSimangaliso represent some of the highest values reported this century. ∑HCH and ∑DDT levels reported here far exceed the estimated global average ranges of 0.11 – 0.93 and 0.4 – 10 ng g^{-1}, respectively (Camenzuli et al., 2016). OCP concentrations from the study area are relatively high even when compared to polluted industrial regions in parts Asia and Europe (Table 2.2). ∑HCH concentrations were higher than those reported for most other regions in the world, except for sites located near major agricultural activities (e.g., Ogbeide et al., 2016; Table 2.2). ∑DDT concentrations recorded in this study were only exceeded in sediments collected from sites heavily exposed to industrial effluent (e.g., Hong et al., 2006; Wafo et al., 2006). ∑drin, ∑chlor and ∑endosulfan concentrations from the present study were generally higher than those recorded in other regions of the world.

Table 2.2**:** Comparison of organochlorine pesticide concentrations (ng g^{-1} dw) in surface sediments collected from various regions (2000 to present).

Location	Year	∑HCH	∑DDT	∑drin	∑chlor	∑endosulfan	References
iSimangaliso	2015-2016	26.3 - 282.5	34.5 – 259.9	19.4 – 277.5	n.d. – 90.5	12.2 – 235.1	This Study
	Regional Studies						
Lake Sibaya, South Africa	2012		0.8 - 123				Humphries, 2013
KNP, South Africa (river sediment)	2009-2010	0.18 – 1.56	0.25 – 2.98	0.41 – 0.82	0.18 – 2.06		Gerber et al., 2015
Eastern Cape, South Africa (river sediment)	2002	n.d. – 177	n.d.– 110	n.d. – 100	n.d. – 184		Awofolu and Fatoki, 2003
Limpopo, South Africa (soil)	2010		5.7 - 59				Van Dyk et al., 2010
KwaZulu-Natal, South Africa (wetland sediment)	2005		0.001 – 13.74				Sereda and Meinhardt, 2005
	International Studies						
Lake Victoria, Uganda	2011	ND – 5.5	0.01 – 4.0	0.22 – 16	ND – 3.2	0.03 – 9.7	Wasswa et al., 2011
Lake Qarun, Egpyt	2013	0.13 – 62.6	ND – 5.9	ND – 120.1	0.25 – 15.9	n.d. – 18.7	Barakat et al., 2013
Edo State, Nigeria (river sediment)	2016	ND - 5200	ND - 970	-	-		Ogbeide et al., 2016
Lake Prespa, Macedonia	2004-2006	-	1.21 – 4.2	-	-		Veljanoska-Sarafiloska et al., 2013
Marseille, France (marine sediment)	2002	-	2.02 – 254.8	-	-		Wafo et al., 2006
South Carolina, USA (Agric. soils)	2003	<0.1 – 0.5	0.11 – 44.8	-	-		Kannan et al., 2003
São Paulo, Brazil (Soil)	2006	0.05 – 0.9	0.12 – 11.0	-	-		Rissato et al., 2006
Masan Bay, Korea	2002	0.04 – 1.3	0.28 – 89.2	n.d. – 1.03	n.d. – 2.6	n.d. – 5.04	Hong et al., 2003
Coast of Korea (coastal sediment)	1997-2002	n.d. – 5.5	0.01 - 135	n.d. – 2.5	n.d. – 3.3	n.d. – 12.6	Hong et al., 2006
Vietnam (marine sediment)	2008	n.d. – 1.00	0.31 - 274	-	-		Hong et al., 2008
Singapore (coastal sediment)	2003	3.3 – 46.2	2.2 – 11.9	-	-		Wurl and Obbard, 2005
Global average	1993-2012	0.11 – 0.93	0.4 - 10	-	-		Camenzuli et al., 2016

2.3.3 Spatial variations

Results obtained reveal differences in pollutant profiles between the four systems investigated. ∑DDT concentrations reveal a clear south to north gradient, with concentrations at Kosi Bay and Lake Sibaya significantly higher than concentrations at Lake St Lucia. DDT was also the dominant OCP present in sediments from Kosi Bay and Lake Sibaya (Fig. 2.4). Spatial variations in ∑DDT concentrations can be attributed to IRS and reflect the extent to which DDT was, and continues to be, used within respective catchment areas. St Lucia and Mkhuze are considered low-risk malaria areas, while outbreaks of malaria have historically been more common in regions farther north. As a result, DDT has been applied more frequently in communities surrounding Lake Sibaya and Kosi Bay. In contrast, highest concentrations of ∑HCH, ∑chlor, and ∑endosulfan residues were found in sediments from Mkhuze (Fig. 2.4). Historically, application of these compounds has been associated with agricultural activities. The Mkhuze River drains an extensive catchment area (~6000 km^2) and therefore captures a substantial residue load from agricultural activities, which are concentrated primarily in river valleys and floodplains. The wetland areas of Nsumo and Muzi both receive sediment from the Mkhuze River and therefore act as reservoirs for contaminants originating from the catchment. The influence of catchment activities on OCP loads is also evident at Lake St Lucia where highest residue concentrations were generally found in sediments near river inlets. Overall concentrations at Lake St Lucia, however, were lower than those recorded in sediments from Mkhuze. The rivers that discharge into Lake St Lucia drain smaller catchment areas and therefore likely carry lower residue loads. In contrast, Lake Sibaya and Kosi Bay are sustained primarily through groundwater inputs and receive negligible fluvial sediment. The total OCP load that Lake Sibaya and Kosi Bay receive is therefore reduced and associated largely with local small-scale subsistence farming.

To assess the extent of contamination in iSimangaliso, mass inventories were calculated for each system based on average OCP concentrations. The mass inventory (I) for each system was estimated using the following equation

$$I = C_i A_i d\rho$$

where C_i is the average total OCP concentration for the system *i* (ng g^{-1} dw), A_i is the area of the system, d is the thickness of sediment sampled and ρ is the average calculated density of the sediment. A sediment thickness of 5 cm was used and dry bulk density was estimated by water displacement. The total surface burden estimated for Lake St Lucia, Mkhuze, Lake Sibaya and Kosi Bay were 490, 1115, 65 and 40 tons, respectively. While these values are a crude approximation and subject to major uncertainties relating to spatial variations in concentration, they highlight the extent of OCP accumulation in the region. Sediments found within the wetland and lake systems of iSimangaliso represent large sinks for OCPs and may also serve as potential sources of contaminants to the adjacent coastal zone.

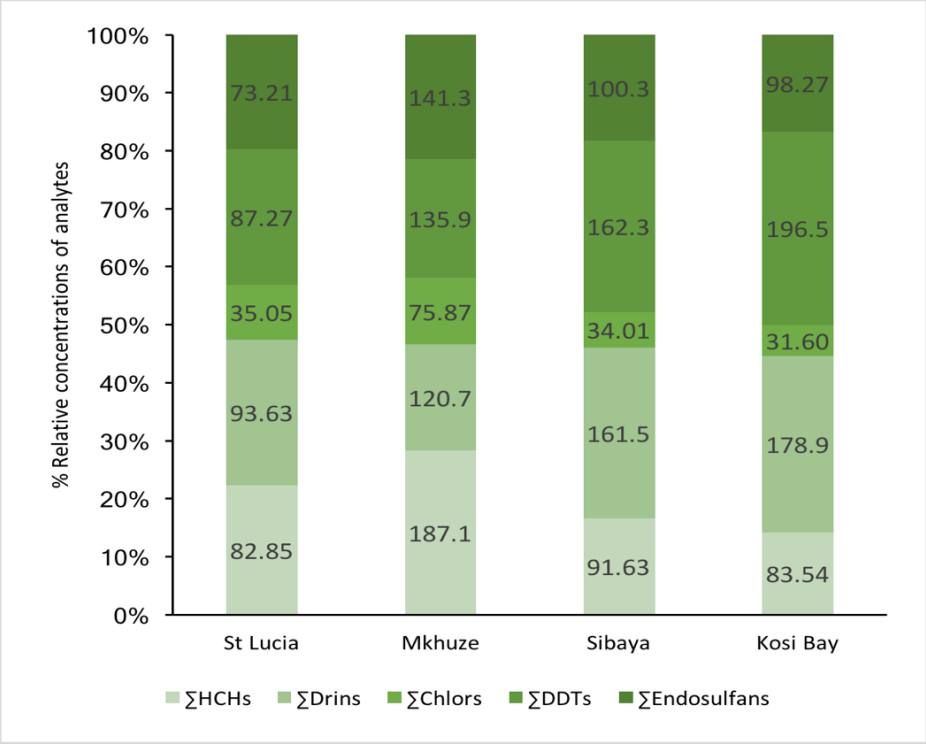

Fig 2. 4: Relative contribution of each class of analyte to total OCP concentration (ng g^{-1}).

2.3.4 Ecotoxicological concerns

Sediments found within the lakes and wetlands of iSimangaliso represent large reservoirs of contaminants that are potentially available for biological uptake. To assess the ecotoxicological significance of OCPs present in sediments from iSimangaliso Wetland Park, concentrations were compared with National Oceanic Atmospheric Administration (NOAA) sediment quality guidelines (Fig. 2.5). All samples analyzed in this study revealed concentrations that greatly exceeded ER-L values for p,p'-DDE, p,p'-DDT and ∑DDT. The concentrations of p,p'-DDE, p,p'-DDT and ∑DDT at most sites were also higher than their respective ER-M guidelines (Fig. 2.5), indicating that these compounds are likely to pose detrimental biological effects. In samples where γ-HCH was detected, concentrations exceeded both ER-L and ER-M guidelines. Similarly, dieldrin and ∑chlor residues exceeded ER-L guideline values, with most samples (93%) also exceeding the ER-M value.

The high concentrations detected in sediments from iSimangaliso have potential to cause adverse biological effects. OCPs such as DDT, dieldrin and HCH have been linked to a number of toxicological effects in various bird species, including brown pelicans in the USA (Blus et al., 1979), great white pelicans, hamerkop, African sacred ibis, and marabou stork in Ethiopia (Yohannes et al., 2017), and white stork nestlings in Spain (de la Casa-Resino et al., 2015). In South Africa, elevated levels of DDE (up to 300 ng g^{-1} wet weight) have been linked to eggshell thinning in African darter populations from regions where DDT continues to be applied as IRS (Bouwman et al., 2013, 2008; Bornman and Bouwman, 2012). The accumulation of high levels of DDT and HCH in tigerfish from Kruger National Park (Gerber et al., 2016) and northern KwaZulu-Natal (Wepener et al., 2012) has also been recorded. This raises concerns regarding the toxicological risk of OCP accumulation to the diverse range of species found within iSimangaliso. The coastal systems of iSimangaliso constitute important nursery areas for estuary-associated fish and invertebrates on the southeast coastline of Africa, and the largest protected estuarine environment for crocodiles and aquatic birds on the continent (Porter et al., 2013). The implications of OCP

accumulation and continued use of DDT in close proximity to ecologically sensitive areas require further investigation and is the focus of ongoing work in the region.

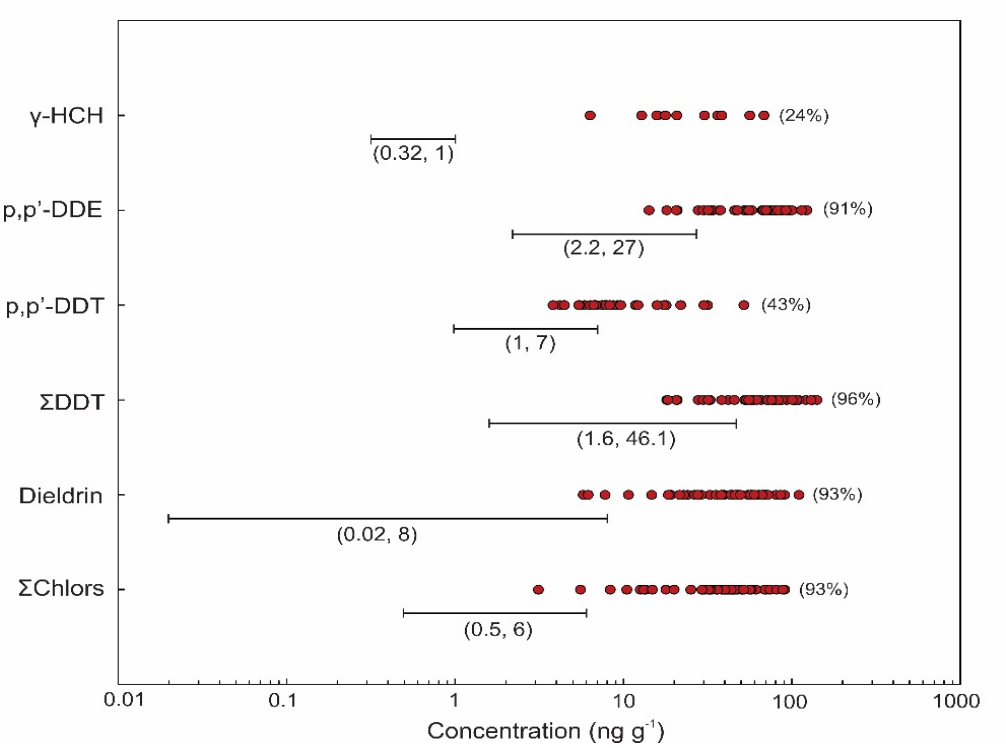

Fig 2. 5**:** Comparison of selected OCP concentrations from iSimangaliso Wetland Park with sediment quality guidelines (ER-L, ER-M). Effect range low (ER-L) and effect range medium (ER-M) values proposed by NOAA (Long et al., 1995). The effects range-low value (ER-L) represents the concentration below which an adverse effect would rarely be observed, while the effect range-median (ER-M) represents the concentration above which adverse effects would frequently occur.

The accumulation of high levels of OCP residues in sediments from iSimangaliso not only poses a threat to aquatic organisms but also to surrounding communities. The ingestion of contaminated food is one of the major pathways through which humans are exposed to OCPs (Gyalpo et al., 2012). Communities living within the catchment areas of iSimangaliso rely on the local environment for food and water. Fish is a major source of protein for most local residents, many of whom also cultivate vegetables and crops within drainage channels and rely on local water for irrigation. Although only a few studies have been conducted, the exposure of humans, particularly breastfeeding mothers, to DDT has been identified as a major concern in areas surrounding iSimangaliso (Sereda et al., 2009; Bouwman et al.,

2012). Considerable levels of DDT (120 mg/kg milk fat) have been reported in the breast milk of mothers living in communities near Lake Sibaya (Bouwman et al., 2012). The chronic exposure of rural communities to elevated levels of OCP residues through dietary intake and the associated health risks requires further investigation.

2.5 Conclusions

This study provides the first investigation into the nature and distribution of OCP contaminants in surface sediments from iSimangaliso Wetland Park. Despite the conservation status of the area and the banning of many of these compounds several decades ago, OCP residues were widely found in sediments throughout the park. Residue concentrations are the highest ever recorded in South Africa and some of the highest reported this century from anywhere in the world. The study indicates that large quantities of OCPs from agricultural and IRS applications have accumulated within wetland and lake sediments. ∑DDT, ∑endosulfan and ∑chlor residues predominantly reflect the historical application of these compounds, while inputs of HCH and drin-residues at several sites can be attributed to recent usage. OCPs banned in South Africa may therefore still be in use in areas surrounding iSimangaliso.

The wetland and coastal systems of iSimangaliso receive and retain large quantities of organic contaminants that are likely to have adverse impacts on both the ecological environment and human health. OCP concentrations exceeded sediment quality guidelines, raising concerns regarding the toxicological risks to a sensitive and ecologically important region of the world. The accumulation of substantial quantities of OCP residues within iSimangaliso indicates the need for further monitoring and this study provides important baseline data for future assessments. Furthermore, a detailed investigation into the bioaccumulation of OCP residues within iSimangaliso Wetland Park and an assessment of the associated toxicological risk to biological ecosystems and human health is urgently required.

Acknowledgements

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CHAPTER THREE - Bioaccumulation and risk assessment of organochlorine pesticides in fish from a global biodiversity hotspot: iSimangaliso Wetland Park, South Africa

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Abstract

Organochlorine pesticides (OCPs) have been used extensively in the eastern regions of South Africa for agricultural and malaria control purposes, yet few data exist on the local environmental and social impacts of these compounds. Such issues have become of increasing concern in the iSimangaliso World Heritage Site, where the continued use of OCPs may pose risks to several sensitive and protected species. This study was designed to examine the bioaccumulation of OCPs in two common fish species, *Oreochromis mossambicus* (Mozambique tilapia) and *Clarias gariepinus* (African sharptooth catfish) from iSimangaliso Wetland Park. These species are targeted by local subsistence fishermen and sustain substantial bird and crocodile populations. Our findings indicate widespread contamination of the aquatic environment, with ΣOCP fish tissue concentrations in the range of 6907-8740 ng g^{-1} lw and 2953-5874 ng g^{-1} lw for *C. gariepinus* and *O. mossambicus*, respectively. HCHs (471-1570 ng g^{-1} lw), DDTs (645-2399 ng g^{-1} lw), drin-related residues (589-1960 ng g^{-1} lw), chlor-related residues (455-2162 ng g^{-1} lw) and endosulfans (457-1495 ng g^{-1} lw) were detected in all tissue samples analysed. Concentrations detected in the majority of samples exceeded European Commission maximum residue limits and a health risk assessment indicated potential dietary risk associated with exposure to heptachlor, heptachlor epoxide and dieldrin. This study represents the first investigation into OCP bioaccumulation in fish species from iSimangaliso Wetland Park. Our findings highlight the need for more detailed investigations into the bioaccumulation and ecotoxicological effect

of these contaminants in the food web and the associated risks to local ecosystems and human health.

Keywords:

Organochlorine pesticides; OCP bioaccumulation; health risk assessment; iSimangaliso Wetland Park

3.1 Introduction

Owing to their widespread use and persistence in the environment, organochlorine pesticides (OCPs) continue to be detected in various environmental compartments, particularly aquatic ecosystems (Barnhoorn et al., 2009; Naigaga et al., 2011; Ogbeide et al., 2016). These compounds are known to bioaccumulate in the tissues of fish, making them useful indicators in assessing ecological health and the potential for contaminants to be transferred to higher trophic levels (e.g., Francour et al., 1994; Saldanha et al., 2010; Gerber et al., 2016). In regions where fish are consumed by people, they may also serve as a barometer in assessing the impact of contaminants on human health (Binelli and Provini, 2003; Bornman et al., 2010).

South Africa has a long history of OCP usage in agriculture and disease-vector control programs (Fischer et al., 2011; Quinn et al., 2011). While the use of many of these compounds has been banned, DDT continues to be applied annually in the north-eastern malaria-endemic region of the country (Fig. 3.1) for indoor residual spraying (IRS) purposes. The continued use of OCPs in this region has attracted recent attention (Barnhoorn et al., 2009; Bouwman et al., 2014; Chapter 2; Gerber et al., 2016; Humphries, 2013; Van Dyk et al., 2010) as it encompasses several globally important areas for biological conservation, including Kruger National Park (KNP) and iSimangaliso Wetland Park. We recently reported highly elevated OCP concentrations present in sediments from the region, which highlighted the need to better understand the potential impacts on the

diverse aquatic organisms that this ecological sensitive region protects (Chapter 2).

The coastal lakes of iSimangaliso are host to an abundant range of fish species and represent some of the most important tropical estuarine systems in the world (Cyrus, 2013). Fish play a key role in the environment, sustaining a considerable biomass that includes substantial breeding bird and crocodile populations. However, few data exist on the specific environmental and social impacts of OCPs within the region, and even less is known about their bioaccumulation and ecotoxicological effect. The ecotoxicological implications are particularly important since fish derived from the wetland park is a primary source of food for many local people. Data on the presence and distribution of OCPs in fish are therefore important from both an ecological and a human health perspective.

This study was initiated to investigate the accumulation of OCPs in two common local fish species; *Oreochromis mossambicus* (Mozambique tilapia) and *Clarias gariepinus* (African sharptooth catfish). These species are widely distributed within the study area, are targeted by local subsistence fishermen, and form an important component in the diet of local bird and crocodile populations. We also assess the potential health risks that the human population living in the region may be exposed to through fish consumption. This study represents the first investigation into OCP bioaccumulation in fish from iSimangaliso Wetland Park and provides key baseline data for assessing exposure in higher trophic levels.

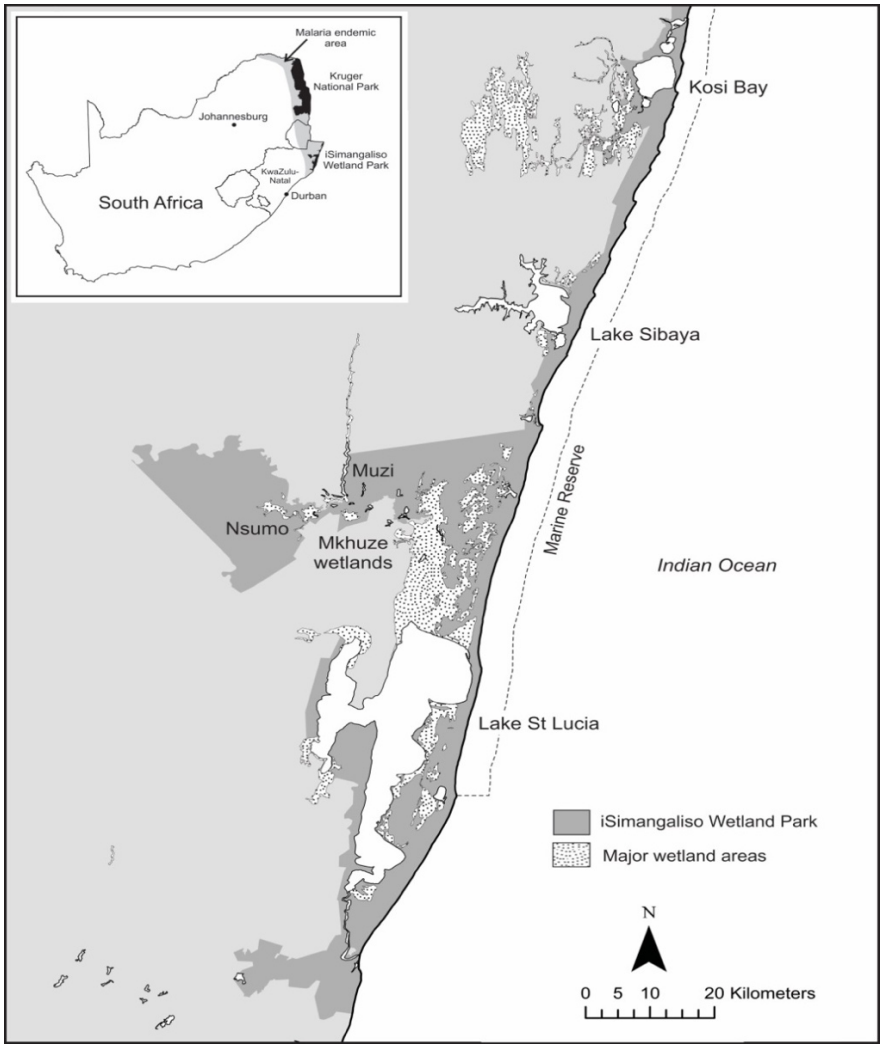

Fig 3. 1**:** Location of iSimangaliso Wetland Park (inset) and the main lake and wetland systems where fish samples were collected from.

3.2 Materials and Methods

3.2.1 Study site

iSimangaliso Wetland Park is situated on the sub-tropical east coast of South Africa (Fig. 3.1) and is characterised by extensive freshwater wetlands and back-barrier coastal lakes. Lake St Lucia (350 km^2) is the largest of these and comprises three shallow, interconnected basins that are sustained primarily through fluvial inputs. The lake drains catchment areas that have all been affected by anthropogenic activities, including agriculture. The system is

connected to the ocean via a long sinuous channel known as the Narrows, although the estuary mouth is prone to prolonged periods of closure. The Mkhuze River, which discharges into the northern end of Lake St Lucia, drains a catchment area of ~6000 km^2 and sustains an extensive wetland area characterised by numerous shallow, freshwater floodplain lakes (e.g., Nsumo and Muzi). To the north, Lake Sibaya and Kosi Bay occupy deeper (>20 m) basins that are supplied almost entirely by local groundwater. Lake Sibaya is an isolated freshwater lake, while Kosi Bay maintains a permanent connection to the ocean via an estuary mouth. Lake Sibaya and Kosi Bay both sustain rapidly growing local communities who live in close proximity to the wetland park and rely heavily on subsistence fishing.

3.2.2 Sample Collection

Four main areas of interest within iSimangaliso Wetland Park were identified; these included Lake St Lucia, the Mkhuze wetland region (Nsumo and Muzi lakes), Lake Sibaya and Kosi Bay (Fig. 3.1). Sampling was undertaken between June 2015 and November 2016 under permit from Ezemvelo KZN Wildlife and the iSimangaliso Wetland Park Authority. Fish were captured over a period of several days using standard angling techniques and scarified by severing the spinal cord. Specimens were weighed and measured for total length. Muscle tissue samples were removed, placed in aluminium foil and stored at -18 ^oC until further analysis. A total of 51 samples were collected and included nine *C. gariepinus* and 42 *O. mossambicus* specimens. All work was performed in compliance with procedures approved by the University of the Witwatersrand Animal Ethics Committee.

Clarias gariepinus and *O. mossambicus* are indigenous species found abundantly within the study area. *Clarias gariepinus* are relatively large, sedentary, bottom-dwelling omnivores, which scavenge and feed on a variety of prey (Skelton, 2001). *Oreochromis mossambicus* is a dominantly detritivorous species (Cyrus, 2013) found in slow flowing rivers and lakes throughout most of southern Africa. Both are relatively hardy species and can survive under varying salinity conditions. Furthermore, these species are an important food source for local communities and form an important component within the ecological food web.

They were therefore identified as good candidates in assessing bioaccumulation and ecotoxicological risk, from both an ecological and human health perspective.

3.2.3 Sample extraction and analysis

Fish tissue samples were freeze-dried and homogenized into a fine powder. OCPs were extracted from accurately weighed (2 g) samples following a modified QuEChERS procedure (Correia-Sá et al., 2012). Samples were rehydrated in 10 ml water and extracted with 10 ml acetonitrile:glacial acetic acid (99:1 v/v). A mixture of 6 g anhydrous magnesium sulfate ($MgSO_4$), 1.5 g sodium acetate (NaOAc) and 1.0 g sodium acetate trihydrate ($CH_3COONa.3H_2O$) was used to aid the partitioning of the organic and aqueous phases. The organic extract was cleaned using a mixture of 1.2 g $MgSO_4$, 0.4 g C-18, 0.4 g primary secondary amine (PSA) and 0.4 g florisil. The resulting mixture was vortexed and then centrifuged to isolate the clean extract. A 4 ml aliquot was concentrated to dryness under vacuum at a temperature ≤ 40 ^oC and reconstituted in hexane (1 ml) for final analysis. Lipid content was estimated gravimetrically after extracting wet subsamples with hexane/acetone (3:1 v/v).

3.2.4 Apparatus and Conditions

Two-dimensional gas chromatography–time-of-flight mass spectrometry (GC X GC-TOF-MS) 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 with Integra-Guard (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coupled to an Rxi-17Sil MS (1.1 m × 0.25 mm i.d. × 0.25 μm thickness) secondary column. A 2 μL sample was injected in splitless mode using ultra high-purity helium as the carrier gas at a constant flow rate of 1.4 mL min^{-1}. The GC oven was set to 55 °C (held for 2 min), then increased to 280 °C at a rate of 5 °C min^{-1} (1 min hold). The transfer line and inlet temperatures were set at 300 and 250 °C, respectively. The scanned mass range was set at 45–550 m/z and a spectral data acquisition rate of 100 spectra s^{-1} was used. Data processing and peak identification was 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 performed using high purity (>98%) reference standards.

Target analytes included hexachlorocyclohexanes (HCHs; α-, β-, γ- and δ-HCH; sum expressed as ΣHCH), drins (aldrin, dieldrin, endrin, endrin aldehyde and endrin ketone; sum expressed as Σdrin), DDTs (p,p'-DDT, p,p'-DDE and p,p'-DDD; sum expressed as ΣDDT), endosulfans (α-, β-endosulfan and endosulfan sulfate; sum expressed as Σendosulfan) and chlors (heptachlor, heptachlor epoxide and methoxychlor; sum expressed as Σchlor). Linear regressions derived from matrix-matched calibration curves for individual pesticides were ≥ 0.99.

3.2.5 Quality assurance

Blank and spiked samples were analysed with each batch of run. Average recoveries for all organochlorine pesticides ranged between 74 and 109%, with all RSDs <15% (Supplementary Table TS 3.1). Quality control (QC) standards were analysed with each batch to assess instrument response and correction factors were applied where appropriate. Detection limits for all analytes ranged between 0.7 and 1.7 ng g^{-1} lipid weight.

3.2.6 Health risk assessment

To assess the potential health risks posed by the consumption of OCP contaminated fish, an estimated daily intake (EDI) was calculated based on average exposure concentrations (WHO/FAO, 1987). This was performed at both the 50^{th} and 95^{th} percentile of the measured concentrations using:

$$EDI = \frac{C \times FR}{BW}$$

where *C* is the average measured concentration of organochlorine pesticides (ng g^{-1} ww), *FR* is the estimated daily fish consumption rate (g d^{-1}) and *BW* is the hypothetical average

body weight (kg) set at 60 kg for adults (WHO, 2012). A daily consumption rate of 150.5 g d^{-1} per person was assumed based on local food consumption studies (Nel and Steyn, 2002).

The risk to humans via exposure to contaminants through a single pathway was assessed using the hazard quotient (HQ) approach (US EPA, 1991):

$$HQ = \frac{EDI}{ADI}$$

where *ADI* is the acceptable (tolerable) daily intake (US EPA, 1991) assumed to be a safe concentration for lifetime exposure. For a preliminary quantitative risk assessment, a HQ ≤ 0.2 is considered to indicate negligible adverse health effects as a result of exposure, while HQ values exceeding this threshold require a further detailed risk assessment or risk management measures to be undertaken (Health Canada, 2004).

3.2.7 Statistical analysis

OCP concentration differences between fish species and between sampling locations were evaluated by analysis of variance (ANOVA) followed by Tukey's posthoc test using Statistica 10. Significance was set at $p < 0.05$. Descriptive statistics were performed using Microsoft Excel, 2016 version.

3.3 Results and discussion

3.3.1 Total residue concentrations

Oreochromis mossambicus specimens sampled in this study were of comparable size with no significant differences in length or weight between individuals. Similarly, *C. gariepinus* samples were obtained from specimens of similar size (892-1450 g), with the exception of one sample from Mkhuze that was significantly larger in mass (3760 g). The lipid content of

muscle tissue samples from *C. gariepinus* varied between 4.9 and 7.1%, and was significantly higher than that measured in *O. mossambicus* samples (2.2-5.0%).

The concentrations of OCPs in muscle tissue samples were normalised to lipid content and are presented in Table 3.1. OCPs were detected in high concentration in all fish tissue samples analysed, indicating widespread contamination of the aquatic environment. Total OCP concentrations ranged from 3563 to 7533 ng g^{-1} for *C. gariepinus* and from 2953 to 5874 ng g^{-1} for *O. mossambicus*. DDT, chlor- and drin-related residues were the predominant compounds detected in both species, although there was considerable variability between samples (Table 3.1; Fig. 3.2). *Clarias gariepinus* generally showed greater variability in ∑DDT, with values ranging between 1034 and 5277 ng g^{-1}. ∑DDT represented on average 28% and 23% of the total OCP burden in tissue samples from *C. gariepinus* and *O. mossambicus,* respectively.

Differences in OCP concentrations and profiles were found between the two species, with highest concentrations generally detected in tissues from *C. gariepinus.* ∑DDT (2148.1 ± 1156.2 ng g^{-1}), ∑drin (1412 ± 540.1 ng g^{-1}), ∑chlor (1661 ± 436.1 ng g^{-1}), and ∑endosulfan (1170 ± 320.4 ng g^{-1}) concentrations were all found to be significantly higher in *C. gariepinus* samples (Fig 3.2). In comparison, tissue samples from *O. mossambicus* exhibited substantially lower OCP levels, with ∑DDT, ∑drin and ∑chlor concentrations of 925 ± 338.5 ng g^{-1}, 969.4 ± 434.1 ng g^{-1} and 1066 ± 507.5 ng g^{-1}, respectively (Fig 3.2).

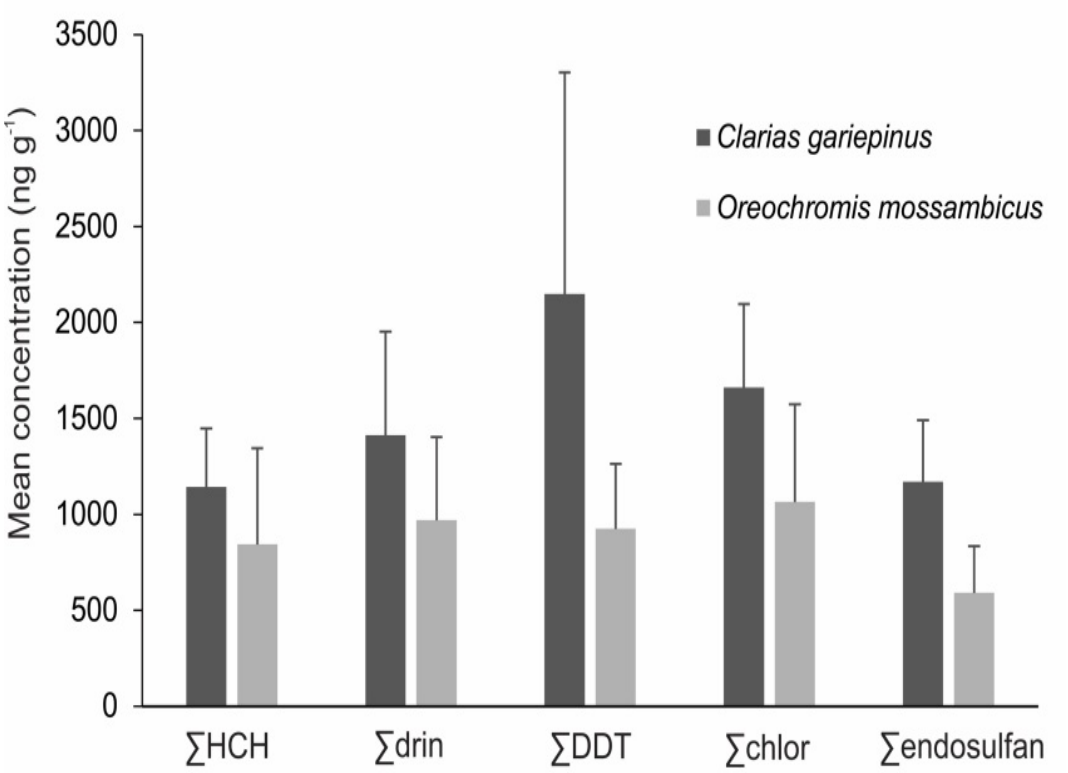

Fig 3. 2**:** Mean OCP concentrations (ng g^{-1} lw) detected in *C. gariepinus* (n = 9) and *O. mossambicus* (n = 42) tissue samples from iSimangaliso Wetland Park. Standard deviation indicated by error bars.

3.3.2 Variation in metabolite concentration profiles

The metabolites, p,p'-DDE and p,p'-DDD, accounted for on average 57% and 73% of ΣDDT concentrations in *O. mossambicus* and *C. gariepinus*, respectively (Fig. 3.3). p,p'-DDT tissue concentrations averaged 612.4 ± 355.1 ng g^{-1} for *C. gariepinus* and were significantly higher than that detected in *O. mossambicus* (429.9 ± 228.8 ng g^{-1}). Highest DDT residue concentrations were detected in a sample from *C. gariepinus*, which contained 1416 ng g^{-1} p,p'-DDT, 2477 ng g^{-1}, p,p'-DDE, and 1384 ng g^{-1} p,p'-DDD. The predominance of p,p'-DDE and p,p'-DDD is similar to trends found in other studies (Covaci et al., 2006; Kaur et al., 2008; Wepener et al., 2012; Yohannes et al., 2013) and is attributed to the breakdown of DDT to more stable metabolite forms. The half-life of p,p'-DDT in fish is approximately eight months, whereas the half-life of p,p'-DDD and p,p'-DDE is seven years (Binelli and Provini, 2003). On this basis, DDT/(DDE+DDD) ratios are often used to identify recent inputs of DDT into the environment. *Oreochromis mossambicus* samples, on average,

contained a larger proportion of undegraded DDT with DDT/(DDE+DDD) ratios varying between 0.17 and 2.7, while *C. gariepinus* exhibited relatively lower values (0.29-0.96). These results suggest that DDT exposure is a mixture of historical as well as more recent inputs of DDT to the environment, likely associated with ongoing malaria control programs in the region.

Large quantities of other OCPs have also been widely used in the region for agricultural purposes. This is reflected in fish tissue residual patterns which generally exhibited prevalence for metabolite compounds. Drin-related metabolites contributed on average 56% and 68% to Σdrin concentrations in *O. mossambicus* and *C. gariepinus,* respectively (Fig. 3.3). Endrin ketone (26-33%) was the dominant metabolite, followed by dieldrin (19%) and endrin aldehyde (12-16%). The use of dieldrin as an insecticide has been banned in South Africa since 1983 (Naidoo et al., 2003) and its presence within fish tissues, therefore, likely reflects the degradation of aldrin (WHO/UNEP, 1989). The concentrations of all drin-related metabolites were significantly higher in *C. gariepinus* samples and ranged between 55 and 786 ng g^{-1} (Table 3.1). The precursors, aldrin and endrin, together contributed on average 43% and 32% in *O. mossambicus* and *C. gariepinus,* respectively. No significant differences in aldrin or endrin concentrations between the two species were observed.

Methoxychlor was the dominant chlor residue detected, accounting on average 44% and 53% of Σchlor concentrations in *O. mossambicus* and *C. gariepinus,* respectively (Fig. 3.3). Methoxychlor concentrations were significantly higher in *C. gariepinus* (884.2 ± 342.3 ng g^{-1}) compared to *O. mossambicus* (467.4 ± 240.0 ng g^{-1}). *Clarias gariepinus* (410.8 ± 95.00 ng g^{-1}) also exhibited significantly higher heptachlor epoxide concentrations, although heptachlor concentrations were similar between species. Methoxychlor was detected in relatively low concentrations within sediments from the region (Chapter 2; Chapter 3) and its prevalence in fish tissue samples indicates the readily bioavailable nature of this compound (Sikka, 1976). Heptachlor is rapidly metabolised to the epoxide form in aquatic systems, which is then readily stored in fatty biological tissues (WHO, 2006). Heptachlor is

still used on limited basis in South Africa (Bouwman, 2003) and the detection of almost equal proportions of heptachlor and its metabolite in fish tissues suggests recent exposure to this pesticide.

The isomers of technical-grade endosulfan (α- and β-) were present in significantly higher concentrations in *C. gariepinus* tissue samples; 394 ± 133.2 ng g^{-1} and 395.5 ± 176.2 ng g^{-1}, respectively. However, their distribution pattern was similar between species, with both isomers occurring in about equal proportions (34%). The measured concentrations of the different isomers from the fish samples gives an indication of how bioavailable the different endosulfan isomers are within iSimangaliso. In *O. mossambicus*, the concentration difference between the two isomers could be accounted for by the persistence and slower degradation of β-endosulfan relative to the α-isomer (Breysse et al., 2015).

HCH compositional profiles exhibited by *C. gariepinus* and *O. mossambicus* were similar, although α- and γ-HCH concentrations were significantly higher in *C. gariepinus,* averaging 295.8 ± 48.2 ng g^{-1} and 270 ± 94.1 ng g^{-1}, respectively. The average contribution of metabolites to ∑HCH concentrations were α-HCH (25-26%), δ-HCH (24%) and γ-HCH (20-24%), respectively (Fig. 3.3). The relatively high contribution of γ-HCH, which was frequently not detected in sediment samples (Chapter 2), can be attributed to its tendency to bioconcentrate readily in aquatic systems (Willet et al., 1998).

Table 3. 1**:** Mean ± standard deviation and range (in parenthesis) of organochlorine pesticide concentrations (ng g^{-1} lipid weight) in *Oreochromis mossambicus* and *Clarias gariepinus* muscle tissue sampled from sites within iSimangaliso Wetland Park (Lake St Lucia, Mkhuze wetlands, Lake Sibaya and Kosi Bay). Concentrations are compared to maximum residue limits (MRLs) set by the European Commission (EC, 2005).

		Mean concentration (ng g^{-1} lw)					
		Oreochromis mossambicus			*Clarias gariepinus*		
Analytes	EC MRL (ng g^{-1} soluble fat)	Lake St Lucia (n = 17)	Lake Sibaya (n = 6)	Kosi Bay (n = 19)	Lake St Lucia (n = 1)	Mkhuze (n = 7)	Kosi Bay (n = 1)
α-HCH	**200**	126.5 ± 84.1 ***(5 - 260)***	132.6 ± 11.7 ***(111 - 144)***	310.3 ± 107 ***(138 - 492)***	247.3	308.8 ± 51.3 ***(239 - 379)***	298.9
β-HCH	**100**	162.5 ± 99.6 ***(28 - 331)***	198.8 ± 22.1 ***(163 - 223)***	369.3 ± 111 ***(218 - 584)***	304.0	277.8 ± 62.6 ***(197 - 383)***	369.6
δ-HCH		108.3 ± 80.4 ***(22 - 268)***	67.4 ± 13.9 ***(51 - 90)***	325.9 ± 104 ***(155 - 505)***	450.4	167.6 ± 57.3 ***(60 - 239)***	489.2
γ-HCH	**10**	83.8 ± 52.8 ***(11 - 210)***	71.9 ± 19.5 ***(44 - 97)***	282.3 ± 115 ***(124 - 468)***	318.0	215.8 ± 56.5 ***(136 - 305)***	412.0
ΣHCH		**481.1 ± 294** ***(140 - 1069)***	**470.7 ± 28.8** ***(431 - 501)***	**1288 ± 337** ***(786 - 1895)***	**1320**	**969.9 ± 216** ***(649 - 1305)***	**1570**
Aldrin		56.1 ± 26.5 ***(21 - 108)***	268.4 ± 39.3 ***(224 - 320)***	124.8 ± 67.1 ***(18 - 248)***	255.0	124.4 ± 108 ***(47 - 358)***	259.0
Dieldrin	**200**	117.3 ± 77.3 ***(34 - 253)***	217.2 ± 68.7 ***(121 - 333)***	223.0 ± 67.2 ***(66 - 331)***	359.0	198.0 ± 131 ***(55 - 460)***	404.0
Endrin	**50**	195.6 ± 125 ***(15 - 390)***	209.5 ± 51 ***(127 - 287)***	433.1 ± 149 ***(175 - 751)***	342.9	262.0 ± 138 ***(39 - 462)***	260.7
Endrin Aldehyde		61.2 ± 46.9 ***(17 - 190)***	76.4 ± 31.7 ***(41 - 123)***	182.5 ± 55.4 ***(85 - 275)***	251.2	208.8 ± 66.5 ***(117 - 290)***	262.0
Endrin ketone		158.6 ± 90.1 ***(42 - 326)***	153.3 ± 30.0 ***(101 - 191)***	360.5 ± 99.0 ***(219 - 530)***	740.1	309.6 ± 110 ***(151 - 447)***	773.7
Σdrins		**588.8 ± 304** ***(180 - 1211)***	**924.8 ± 183** ***(637 - 1180)***	**1324 ± 260** ***(880 - 1979)***	**1949**	**1103 ± 416** ***(443 - 1784)***	**1960**
p,p'-DDE		179.5 ± 98.3 ***(53 - 341)***	241.7 ± 24.4 ***(210 - 264)***	214.3 ± 107 ***(93 - 470)***	687.5	942.1 ± 721 ***(389 - 2477)***	583.5
p,p'-DDD		201.6 ± 111 ***(24 - 381)***	277.4 ± 12.9 ***(261 - 297)***	375.3 ± 103 ***(224 - 605)***	657.5	728.2 ± 458 ***(329 - 1384)***	672.1
p,p'-DDT		516.6 ± 231 ***(195 – 944)***	126.0 ± 21 ***(103 – 161)***	448.2 ± 183 ***(89 – 762)***	384.0	729.1 ± 406 ***(218 - 1416)***	432.0
ΣDDT	**1000**	**897.7 ± 431** ***(309 - 1596)***	**645 ± 41.4** ***(596 - 703)***	**1038 ± 234** ***(615 - 1581)***	**1729**	**2399 ± 1422** ***(1034 - 5277)***	**1687**
Heptachlor		209.2 ± 13 ***(12 - 441)***	139.7 ± 55.6 ***(46 - 211)***	495.0 ± 126 ***(288 - 692)***	536.8	278.7 ± 67.3 ***(165 - 373)***	497.8
Heptachlor Epoxide		175.5 ± 121 ***(41 - 388)***	219.8 ± 30.6 ***(183 - 266)***	369.2 ± 92.3 ***(206 - 502)***	457.0	385.2 ± 114 ***(179 - 508)***	454.0
Methoxychlor		423.0 ± 159 ***(195 - 712)***	95.9 ± 24.9 ***(57 - 121)***	624.5 ± 186 ***(375 - 940)***	1168	748.3 ± 356 ***(162 - 1195)***	1076
Σchlors	**200**	**807.7 ± 394** ***(279 - 1473)***	**455.4 ± 80.1** ***(354 - 560)***	**1489 ± 267** ***(1082 - 2020)***	**2162**	**1412 ± 315** ***(828 - 1796)***	**2028**
α-Endosulfan		177.9 ± 91.9 ***(77 - 331)***	188.3 ± 24.2 ***(161 - 217)***	176.4 ± 117 ***(41 - 408)***	366.5	373.7 ± 160 ***(60 - 516)***	492.8
β-Endosulfan		176.2 ± 99.5 ***(62 - 350)***	80.7 ± 17.5 ***(56 - 106)***	346.0 ± 72.2 ***(242 - 490)***	505.5	307.7 ± 153 ***(94 - 474)***	592.7
Endosulfan sulfate		123.0 ± 97.2 ***(3 - 308)***	187.9 ± 29.1 ***(134 - 210)***	213.3 ± 57.2 ***(99 - 341)***	486.3	341.1 ± 157 ***(100 - 567)***	409.8
Σendosulfans	**50**	**477.1 ± 271** ***(184 - 945)***	**456.9 ± 56.7** ***(351 - 509)***	**735.8 ± 168** ***(512 - 1057)***	**1358**	**1023 ± 311** ***(581 - 1392)***	**1495**
DDT/(DDD + DDE)		**1.5 ± 0.56**	**0.25 ± 0.04**	**0.83 ± 0.36**	**0.29**	**0.52 ± 0.3**	**0.35**
Lipid content (%)		**3.6 ± 0.96** ***(2.2 – 5.0)***			**5.7 ± 0.89** ***(4.9 - 7.1)***		
Specimen mass (g)		**126.1 ± 23.9 (78 – 158)**			**1442 ± 914 (892 – 3760)**		

3.3.3 Bioaccumulation of OCPs

The OCP levels detected in *C. gariepinus* and *O. mossambicus* tissues are significantly higher than previously reported sediment data from iSimangaliso Wetland Park (Chapter 2). All residues detected in the sediment were present in fish muscle tissue samples, often in concentrations that were 5-10 times higher (Fig. 3.4; Table 3.1). However, fish tissue concentrations show high interspecies and intrapopulation variability, which may be related to several factors such as age, metabolism and feeding habits.

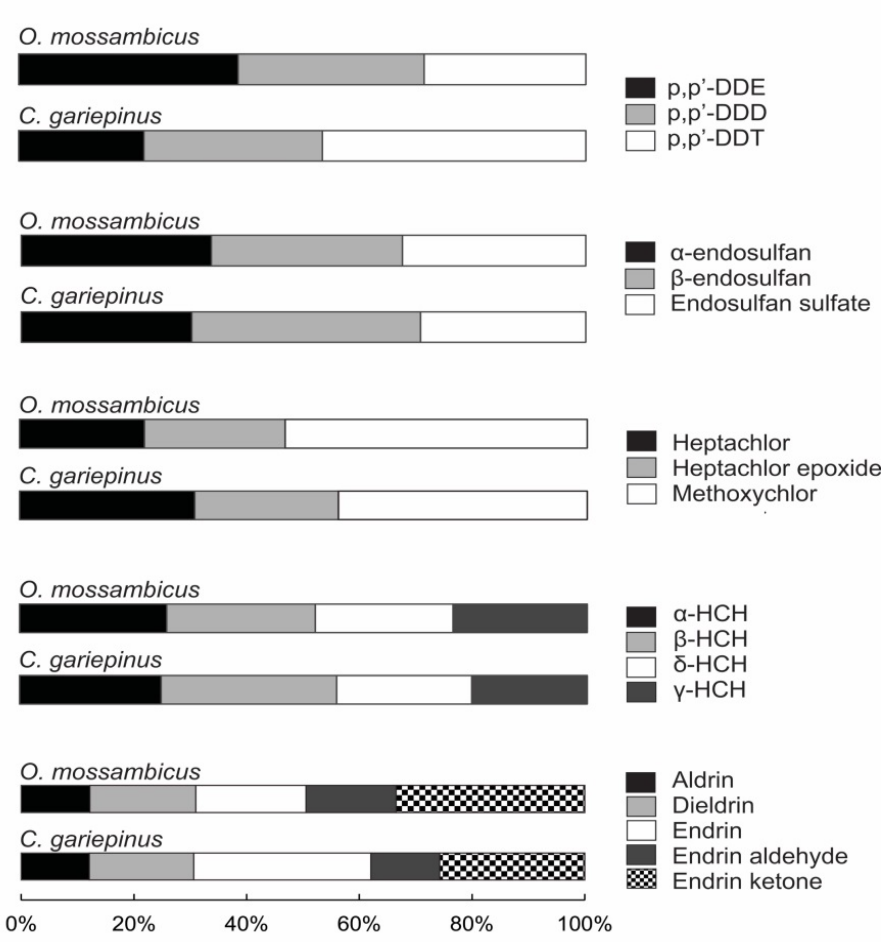

Fig 3. 3**:** The relative contribution of individual residues to ΣDDT, Σchlor, Σdrin, ΣHCH and Σendosulfan concentrations in *C. gariepinus* and *O. mossambicus* muscle tissue.

Highest OCP concentrations were generally detected in tissue samples from *C. gariepinus*, suggesting that this species has a higher potential to accumulate contaminants. *Clarias*

gariepinus scavenges on smaller fish, benthic organisms and decaying matter. This increases their risk of exposure to organic pollutants. As a result, *C. gariepinus* has been shown to accumulate large quantities of pollutants and is often used as an indicator in ecotoxicological studies (Barnhoorn et al., 2010; Bornman et al., 2010; Marchand et al., 2008). In contrast, *O. mossambicus*, which typically feeds on phytoplankton, is likely exposed to lower environmental contaminant concentrations. Similar differences in OCP accumulation between *C. gariepinus* (30-10600 ng g^{-1} lw) and *O. mossambicus* (50-1940 ng g^{-1} lw) have been reported in the Rift Valley lakes of Ethiopia (Yohannes et al., 2013, 2014).

To some extent, variations in fish tissue concentrations may reflect differences in site contamination. While our small sample size precludes a thorough inter-site comparison based on *C. gariepinus*, variations in *O. mossambicus* average tissue concentrations between sampling localities are evident (Fig. 3.4). *Oreochromis mossambicus* samples from Kosi Bay exhibited significantly higher ∑HCH, ∑drin, ∑chlor and ∑endosulfan concentrations compared to those collected from both Lake Sibaya and Lake St Lucia. ∑DDT tissue concentrations were also significantly higher at Kosi Bay compared to Lake Sibaya. Spatial variations in tissue OCP concentrations appear to reflect a local environmental gradient identified by Buah-Kwofie and Humphries (Chapter 2), with sediments from Kosi Bay typically exhibiting highest OCP concentrations.

The fish tissue concentration data reported in this study provide a measure of environmental exposure to OCPs and indicates that higher trophic organisms, including piscivorous birds and crocodiles, are likely to be susceptible to the accumulation of organic contaminants. While no available data currently exists for iSimangaliso, DDT and its metabolites have been linked to eggshell thinning in aquatic birds from other regions of South Africa (Bouwman et al., 2013, 2008). Similarly, OCP residues have been detected in crocodilians from a number of localities worldwide and linked with a range of developmental and reproductive effects (Sherwin et al., 2016; Wu et al., 2014; Charruau and Niño-Torres, 2014; Gonzalez-Jauregui et al., 2012; Stoker et al., 2011; Skaare et al., 1991). Exposure to elevated OCP residue concentrations thus has concerning implications

for the many breeding bird and crocodile populations inhabiting iSimangaliso and warrants further investigation.

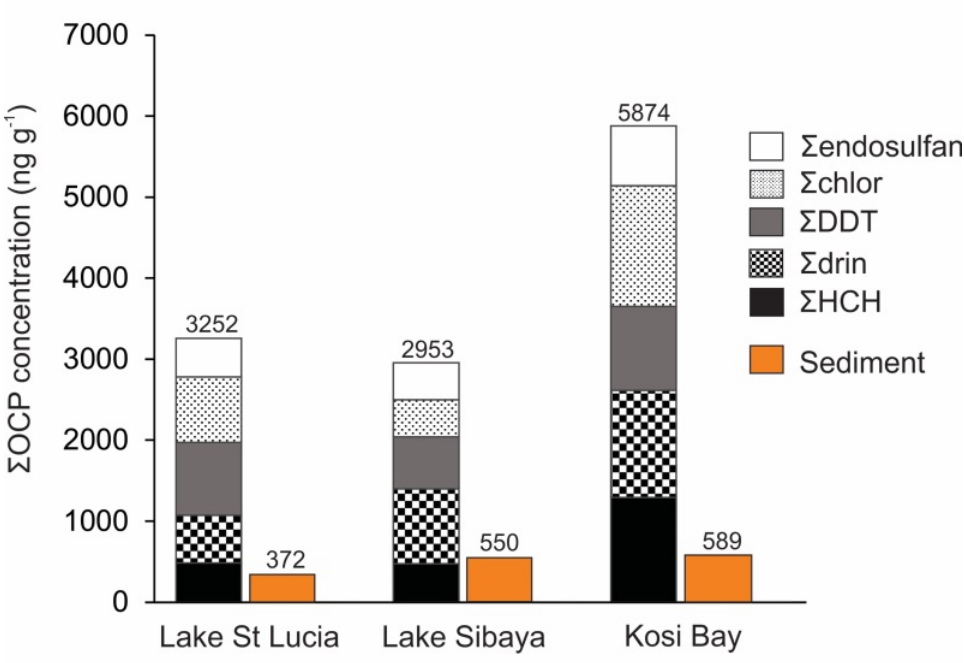

Fig 3. 4**:** Variation in OCP concentrations detected in *O. mossambicus* tissues (ng g^{-1} lw) from sites within iSimangaliso Wetland Park. Tissue concentrations are compared with surface sediment data (ng g^{-1} dw) from Buah-Kwofie and Humphries (Chapter 2).

Table 3. 2: Comparison of organochlorine pesticide concentrations (ng g-1) reported in tissues of Clarias gariepinus and common tilapia species (O. mossambicus and O. noliticus) species from other regions in South Africa and Africa (lw = lipid weight, ww = wet weight, n.d = not detected). For comparative purposes, we report our data in both lw and ww basis.

Location (sampling dates)	Species		γ-HCH	ΣHCH	p,p'-DDT	p,p'-DDE	ΣDDT	Heptachlor	Endrin	Dieldrin	α-Endosulfan	Reference
iSimangaliso, South Africa (2015-2016)	*C. gariepinus*	lw	136-412	649-1594	218-1416	389-2477	1034-5277	165-600	39-462	55-460	60-516	Present study
iSimangaliso, South Africa (2015-2016)	*C. gariepinus*	ww	6.8–20.6	32.4–178.5	10.9-70.8	19.4–123.8	51.7–263.8	8.2-26.9	2.0-23.1	2.7-23	3.0-25.8	Present study
Limpopo, South Africa (2008)	*C. gariepinus*	lw			n.d-6598	1255-63462	1520-81491					(Barnhoorn et al., 2009)
Limpopo, South Africa (2003-2008)	*C. gariepinus*	lw			n.d-18786	n.d-37527	54-67237					(Bornman et al., 2010)
Gauteng/North West Provinces, South Africa (2007)	*C. gariepinus*	ww	n.d-515		n.d-276	n.d-425	n.d-601	n.d-289	n.d-151	n.d-315	n.d-516	(Barnhoorn et al., 2015)
Lake Ziway, Ethiopia (2011-2012)	*C. gariepinus*	lw		4-520			30-10600	10-410				(Yohannes et al., 2014)
iSimangaliso, South Africa (2015-2016)	*O. mossambicus*	lw	11-468	140-1895	88-944	53-470	309-1596	12-692	15-751	34-333	41-408	Present study
iSimangaliso, South Africa (2015-2016)	*O. mossambicus*	ww	0.8-32.8	9.8-132.6	6.2-66.1	3.7-32.9	21.6-111.7	0.8-48.5	1.0-52.6	2.4-23.3	2.8-28.6	Present study
Limpopo, South Africa (2008)	*O. mossambicus*	lw			n.d-7540	n.d-4206	368-14380					(Barnhoorn et al., 2009)
Limpopo, South Africa (5 year period)	*O. mossambicus*	lw	18-48		948-5889	1764-7609	3890-18539			n.d-14	n.d-42	(Barnhoorn et al., 2010)
Lake Victoria Tanzania (1999)	*O. noliticus*	ww					20-30				20-80	(Henry and Kishimba, 2006)
Lake Bosomtwi-Ghana (2004-2005)	*O. mossambicus*	ww	0.7-1.4		3.4-4.7	4.1-7.3	7.5-11.9			0.3-0.6	0.8-2.3	(Darko et al., 2008)
Lake Edward, Uganda (not indicated)	*O. noliticus*	ww					n.d-51					(Ssebugere et al., 2009)
Lake Ziway, Ethiopia (2011-2012)	*O. noliticus*	lw		30-110			50-1940	10-490				(Yohannes et al., 2014)

The OCP concentration data reported here add to a growing body of evidence which highlights the bioaccumulation and environmental impact of OCP use in South Africa. Similar studies in malaria control regions of the country have reported ∑DDT levels in the range of 54-81491 ng g^{-1} lw and 368-18539 ng g^{-1} lw in the tissues of *C. gariepinus* and *O. mossambicus,* respectively (Table 3.2). In comparison, ∑DDT, p,p'-DDT and p,p'-DDE concentrations detected in fish tissue samples from iSimangaliso were substantially lower. Other OCPs, including ∑HCH, heptachlor, α-endosulfan and dieldrin, were generally also detected in lower concentrations when compared to levels found in *C. gariepinus* and *O. mossambicus* from the northern parts of the country (Table 3.2). However, ∑DDT values in this study were substantially higher than levels reported in *C gariepinus* and tilapia species from other African lakes, including regions in Ghana (8-12 ng g^{-1} ww), Tanzania (20-30 ng g^{-1} ww) and Uganda (n.d.-51 ng g^{-1} ww). In Ethiopia, where DDT is still used for malaria control, ∑DDT concentrations detected in *C. gariepinus* were higher than the levels detected at iSimangaliso, although levels present in tilapia were comparable (Yohannes et al., 2014). Little other comparative OCP data exists, although ∑HCH, heptachlor, dieldrin and α-endosulfan concentrations recorded in fish from iSimangaliso were several times higher than those reported for *C. gariepinus* and tilapia from Lake Ziway, Ethiopia (4-520 ng g^{-1} lw for ∑HCH) and Lake Bosomtwi, Ghana (0.8-2.3 ng g^{-1} ww for α-endosulfan).

3.3.4 Human health risk assessment

The bioaccumulation data presented not only reflect the availability of OCPs for biological uptake within the study area, but also provide an indication of the potential concentrations which humans may be exposed to through ingestion. When compared to the European Commission's maximum residue limits (MRLs), the OCP levels detected in *C. gariepinus* samples from iSimangaliso exceeded the guideline values for all analytes with the exception of dieldrin and endrin. For *O. mossambicus,* all samples exceeded the limits for ∑endosulfan, ∑chlor and γ-HCH, while most (52-88%) also exceeded the guideline values for dieldrin and β-HCH.

The estimated daily intake for the targeted OCPs indicated that all the EDI values did not exceed US EPA ADI guideline values at both the 50th and 95th percentile concentrations (Table 3.3). Calculated HQ values revealed that heptachlor, heptachlor epoxide and dieldrin exceeded the threshold value of 0.2 at the 50th and 95th percentile concentrations in both fish species, while aldrin and endrin exceeded the threshold at the 95th percentile. These findings indicate possible health risks associated with the consumption of *C. gariepinus* and *O. mossambicus* from iSimangaliso, although a more detailed risk assessment is required to ascertain the likely impact on human health. Other studies in South Africa have also highlighted potential health risks associated with the consumption of fish contaminated by dieldrin, endrin, and heptachlor (Barnhoorn et al., 2015; Gerber et al., 2016). No immediate health risks were indicated by the HQ values for other analyte concentrations detected. It should be noted, however, that the risk estimates presented here represent a preliminary screening and the increased susceptibility of vulnerable groups (such as children and the elderly) were not taken into account. Other possible limitations to this assessment include potential variations in the quantity of fish consumed by local communities, other possible routes of exposure (inhalation and other contaminated food sources), as well as possible antagonistic and/or synergistic effects. In particular, the contamination of other food sources (e.g., bovine milk and chicken products) in KwaZulu-Natal has previously been highlighted (Barnhoorn et al., 2009; Sereda et al., 2009) and should be factored into any further detailed risk assessment. Additionally, exposure concentrations derived using conventional solvent extraction techniques could lead to an overestimation of the potential health risks, as only a limited proportion of the OCPs present in the fish tissue may be bioaccessible (Wang et al., 2011). To minimize the influence of such factors, any future in-depth risk assessment should take the oral bioaccessibility of the contaminant into account.

Table 3. 3: Estimated daily intake (EDI) (ng kg^{-1} bw d^{-1}) and hazard quotients (HQs) calculated based on average OCP concentrations (ng g^{-1} wet weight) detected in *Oreochromis mossambicus* and *Clarias gariepinus* samples from iSimangaliso Wetland Park, South Africa. EDI are compared with USEPA acceptable daily intake (ADI) values. HQ values in bold exceed the Health Canada guideline

		Oreochromis mossambicus			*Clarias gariepinus*		
Analytes	**ADI (ng kg^{-1} d^{-1})**	**50th (95th) percentile Concentrations**	**50th (95th) percentile EDI**	**50th (95th) percentile Hazard Quotient (10^{-3})**	**50th (95th) percentile Concentrations**	**50th (95th) percentile EDI**	**50th (95th) percentile Hazard Quotient (10^{-3})**
α-HCH	**5000**	12.3 (31.4)	30.9 (78.6)	6.1 (15.7)	14.9 (18.1)	37.4 (45.4)	7.4 (9.0)
β-HCH	**5000**	17.1 (35.5)	42.8 (89.1)	8.6 (17.8)	14.3 (19.2)	35.9 (48.2)	7.2 (9.6)
δ-HCH	**5000**	13.5 (31.5)	33.7 (79.1)	6.8 (15.8)	10.5 (24.5)	26.3 (61.5)	5.3 (12.3)
γ-HCH	**5000**	9.2 (29.2)	23.1 (73.2)	4.6 (14.6)	11.8 (20.6)	29.6 (51.7)	5.9 (10.3)
Aldrin	**100**	6.6 (17.7)	16.4 (44.4)	**165.5 (444)**	7.4 (16.2)	18.6 (40.5)	**185.6 (405)**
Dieldrin	**100**	14.5 (22.6)	36.2 (56.7)	**363.7 (566.9)**	12.2 (21.8)	30.6 (54.6)	**306 (545.6)**
Endrin	**300**	19.1 (41.3)	47.8 (103.6)	**159.6 (345.3)**	14.4 (20.5)	36.1 (51.5)	**180.6 (257.7)**
p,p'-DDE	**10000**	13.1 (25.8)	32.7 (64.7)	3.2 (6.4)	32.7 (87.7)	82 (219.9)	8.2 (22.0)
p,p'-DDD	**10000**	19.6 (34.5)	49 (86.4)	4.9 (8.7)	32.2 (65.2)	80.8 (163.4)	8.1 (16.3)
p,p'-DDT	**10000**	29.6 (59.0)	74.1 (148.0)	7.4 (14.8)	24.3 (57.6)	61 (144.5)	6.1 (14.4)
Heptachlor	**100**	21.9 (44.4)	54.8 (111.5)	**549.3 (1113.7)**	16.8 (28.6)	42.1 (71.6)	**421.4 (716.1)**
Heptachlor Epoxide	**100**	19.1 (34.5)	47.8 (86.5)	**479 (865.3)**	22.8 (24.6)	57.2 (61.6)	**571.9 (615.8)**
α-Endosulfan	**6000**	11.9 (24.7)	29.8 (62.0)	5 (10)	19.4 (25.5)	48.7 (64.0)	8.1 (10.7)
β-Endosulfan	**6000**	18.4 (30.3)	46.2 (76.0)	7.7 (12.7)	20.4 (29.8)	51.2 (74.7)	8.5 (12.5)
Endosulfan sulfate	**6000**	12.5 (20.6)	31.4 (51.6)	5.2 (8.6)	21.7 (27.2)	53.4 (68.2)	8.9 (11.4)

3.4 Conclusions

This study is the first to assess OCP bioaccumulation within the aquatic ecosystems of iSimangaliso Wetland Park. Tissue samples from *C. gariepinus* and *O. mossambicus* reveal that fish bioaccumulate substantial quantities of organochlorine contaminants, reflecting widespread contamination of the aquatic environment. Many of the samples analysed exceeded European Commission maximum residue limits, with potential dietary risk associated with exposure to heptachlor, heptachlor epoxide and dieldrin. While these results highlight the potential health risks associated with the consumption of contaminated fish sourced from iSimangaliso, the exposure of local people to OCP residues could be considerably higher and warrants further investigation. Further work is also

needed to better understand the potential impacts and risks associated with OCP exposure in higher trophic level organisms.

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CHAPTER FOUR – Accumulation of organochlorine pesticides in the 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 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, limited ecotoxicological data exists. 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 residue 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

4.1 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 (Chapter 2), 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 chain 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 moreletti*) (Wu et al., 2000, 2006; Pepper et al 2004), 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, 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 (Marais, 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 exposure from OCPs 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.

4.2 Methods

4.2.1 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 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, 2013, Warner et al., 2016a).

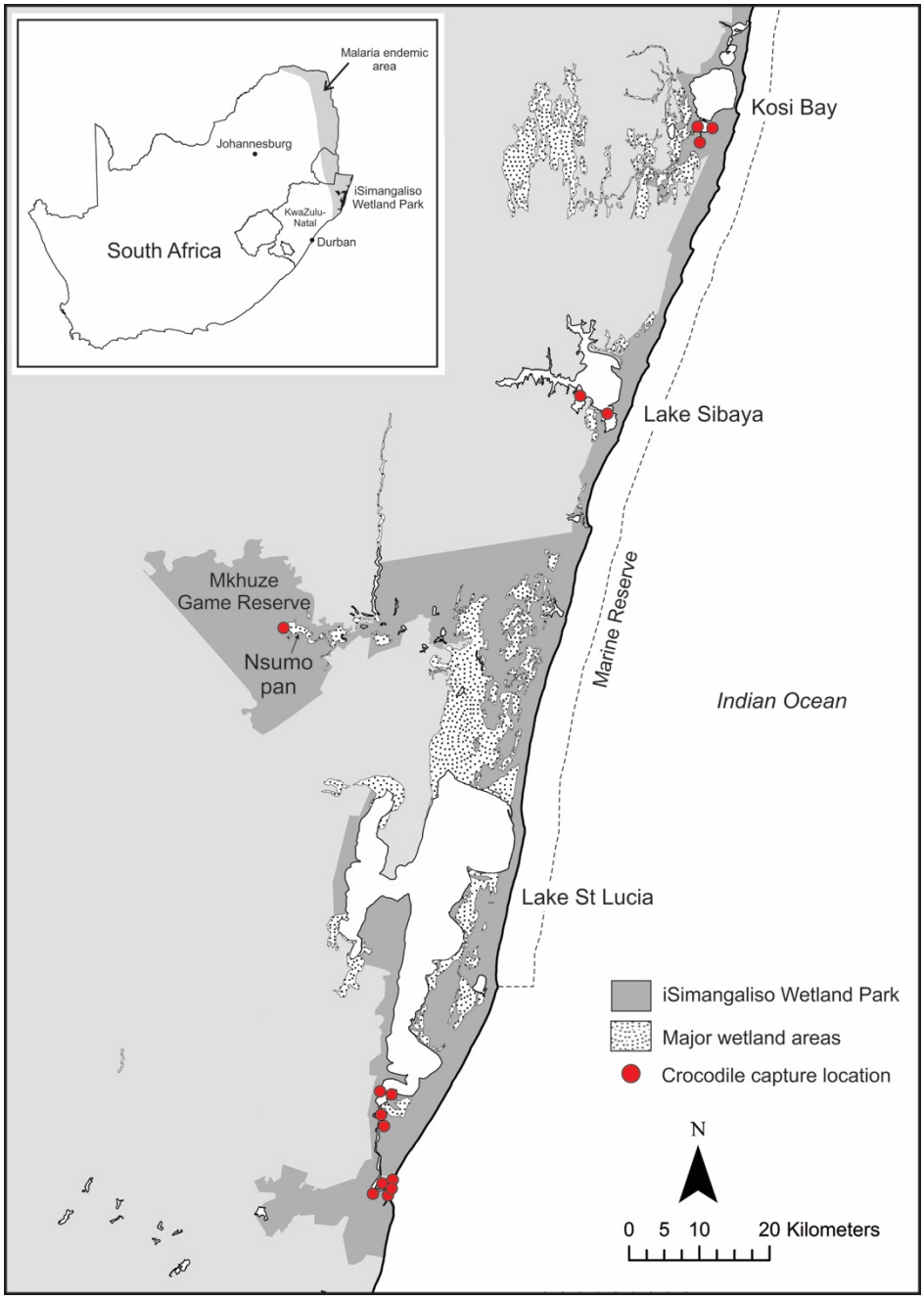

Fig 4.1: Location of iSimangaliso Wetland Park showing crocodile sampling sites.

4.2.2 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, 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).

4.2.3 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.

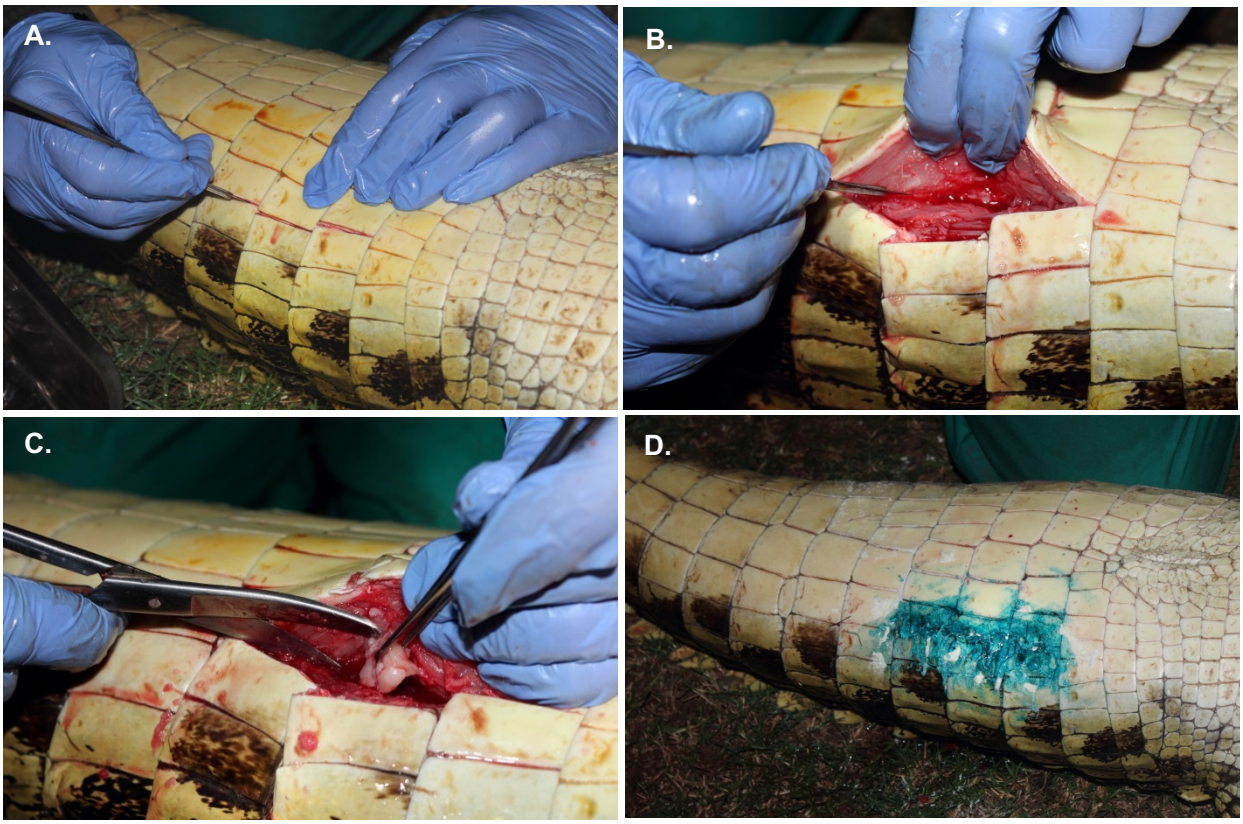

Fig 4.2: Surgical removal of fat samples from the tail area, showing A) the line of incision on the ventro-lateral side of the tail, B) the incision through the *M. ilio-iscchiocaudalis* muscle, C) the removal of the underlying fat sample, and D) the closed wound with applied Wound Gel Powder.

The fat deposit targeted was located between the *Musculus ilio-iscchiocaudalis* and *M. caudofemoralis* muscles of the tail. A careful incision was made through the *M. ilio-iscchiocaudalis*, while *s*terile 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).

4.2.4 Organochlorine pesticide analysis

Fat samples were stored on ice in the field and later frozen at -18 ^{0}C and 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 (Chapter 2). 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 18 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, endrin aldehyde 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 $p < 0.05$.

4.3 Results

4.3.1 Crocodiles sampled

A total of 15 crocodiles were 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.

4.3.2 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. 4.3). ∑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.

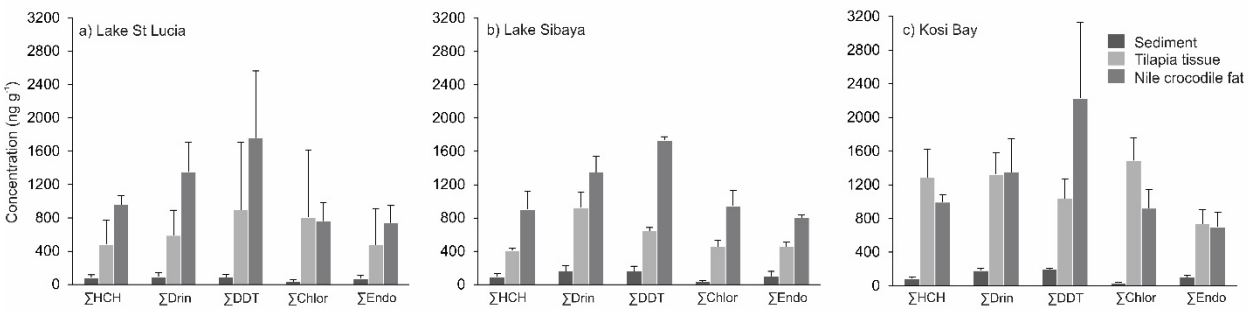

Fig 4.3: Comparison between OCP concentrations detected in surface sediment (ng g^{-1} dw), tilapia tissue (ng g^{-1} lw) and crocodile fat (ng g^{-1} ww) from a) Lake St Lucia, b) Lake Sibaya and c) Kosi Bay. Standard deviation indicated by error bars. Results from Nsumo pan, where only one crocodile was sampled, are not shown.

4.3.3 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 average values 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.

Table 4. 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	160c	3600
4	Lake St Lucia	390	M	120	420	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	420	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 average (± 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**
	Average values 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
	Average values by age														
	Subadults (TL < 2.5 m, n = 6)			190	350	310	300	300	900	370	450	180	260	230	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 (Supplementary Table TS 4.2)

4.4 Discussion

4.4.1 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 (Chapter 2; Chapter 3). 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 (Chapter 2). 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 (Chapter 3). 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 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. 2). 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%). 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, and therefore youngest (1.28 m) 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 containing highest ∑DDT and ∑OCP concentrations measured within the wetland park (Chapter 2).

Crocodile size is generally considered a good predictor of age (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 adult.

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.

4.4.2 Biological significance

Few studies have previously examined OCP 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 values 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, 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 values on the other hand appear to be the highest reported this century for crocodilians (> 15). 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. porous*) 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 (Rauschenberger 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 behaviour 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 (Abdo et al., 2013) and the associated reproductive implications warrant further investigation.

Table 4. 2: Comparison between organochlorine pesticide concentrations detected in the tissues of other crocodilians. Values are presented as means (±SD) based on ng g^{-1} wet weight (ww) unless otherwise stated. Ranges are given in parenthesis. (n.d = not detected; – = not analysed)

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 – 520)	340 (190 – 530)	1070 (210 – 2060)	410 (150 – 670)	330 (160 – 530)	1679 (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 ± 24.2	
	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)	–	–	–	–	–	39.0 (5.64 – 190)	0.47 (0.23 – 22.4)	3.14 (0.13 – 6.40)	48.0 (6.03 – 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 ± 40.0	340.2 ± 81.9	–	254.8 ± 50.5	–

4.5 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 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 and freshwater and estuarine ecosystems in Africa exists. 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 is 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 amount of samples to be collected from live animals and from a consistent location on an animal's body. When compared with concentration data derived from eggs and 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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KZN Wildlife kindly granted us permission to work within iSimangaliso Wetland Park. Any opinion, finding and conclusion or recommendation expressed in this material is that of the authors.

CHAPTER FIVE - A preliminary investigation into the bioaccumulation of organochlorine pesticides in two coral species from the Maputaland reef

Archibold Buah-Kwofie*, Marc Humphries

* This chapter presents data derived from a preliminary set of samples that were collected by Dr Sean Porter and Prof Michael Schleyer from the Oceanographic Research Institute. The results from this work have subsequently been used to motivate for a more comprehensive sampling strategy that will assess variations in OCP accumulation on the Maputaland reef in greater detail.

Abstract

The north-eastern coastline of KwaZulu-Natal is host to one of only two World Heritage site within the southern Indian Ocean and forms part of the iSimangaliso World Heritage Site where the continued use of OCPs may pose substantial risks to several sensitive and protected species. This study examines the bioaccumulation of OCPs within the tissues of two abundant soft coral species, *Sinularia gravis* and *Lobophytum depressum* from iSimangaliso Wetland Park. OCPs were found to have substantially accumulated in the tissues of the coral samples investigated with ∑OCP concentrations in corals tissue in the range of 2300 - 3000 ng g^{-1} ww. ∑HCHs (180-500 ng g^{-1} ww), ∑DDTs (140-460 ng g^{-1} ww), ∑drin (350-900 ng g^{-1} ww), ∑chlor (390-1400 ng g^{-1} ww) and ∑endosulfans (250-670 ng g^{-1} ww) were detected in all samples analysed. This study provides the first preliminary dataset into OCP bioaccumulation in coral reef from the southern Indian Ocean and the Marine protected areas of iSimangaliso World Heritage Site. Our findings highlight the need for more in-depth investigations into the bioaccumulation and ecotoxicological effect of these contaminants in other coral species as well as the spatial distribution of these contaminant with the different coral colonies within iSimangaliso. The potential impact of OCP contaminant on the marine food web and the associated risks to local ecosystems and communities who derive resources from this important ecosystem need further exploring.

5.1 Introduction

Coral reefs are under threat from land-sourced pollutants on a global scale and declines in coral cover have been reported at a number of sites (Nyström et al., 2000; Christin et al., 2004; Markey et al., 2007a). The effects of anthropogenically derived chemical pollutants, which enter reef systems through various pathways, are recognised as one of the main contributing factors for coral reef declines (Burke et al., 2011; Glynn et al., 1989; Morrison et al., 2013; Pandolfi et al., 2003; Ross et al., 2015; Wang et al., 2008). Pesticides have specifically been identified as important non-point source pollutants and residues have been detected in number of coral reef systems (El Nemr et al., 2004; Haynes et al., 2000; Haynes and Johnson, 2000; Morrison et al., 1996). While the effects of pesticides have received increasing attention (e.g. Van Dam et al., 2011; Ross et al., 2015), there is little data to explain the extent to which coral reef ecosystems are affected by chronic exposure. Extremely limited information is available for chemical pollutant concentration from coral reefs worldwide, with most data originating from the coastline of Florida and the Great Barrier Reef (GBR), Australia. In these regions, pesticide runoff and leaching from catchment areas affected by agriculture is the dominant source of pollution to inshore reef communities (Bocquené and Franco, 2005; Dubinsky and Stambler, 1996; Edinger et al., 1998; NOAA, 2011).

Residues of organochlorine pesticides (OCPs) continue to be detected in many areas of the world, despite restrictions on their use. OCPs are considered a serious long-term threat to marine health because of their toxicity, environmental persistence and strong affinity for accumulation in lipid tissues (Araújo et al., 1999; Glynn et al., 1995; Olafson, 1978). Despite this, little is known globally about the chronic effect of OCPs on corals, although suspected adverse effects include bleaching, impaired photosynthesis, partial colony mortality and reduced fecundity (Brodie et al., 2012; Lewis et al., 2012). The effects of long-term exposure to pollution may also combine and interact with other environmental factors, potentially reducing the resilience of corals to global climate change (Hughes et al., 2003;

Negri et al., 2011; Ross et al., 2015; Wooldridge and Done, 2009), although such linkages remain poorly understood.

Coral reef communities found along the Maputaland coastline on the eastern margin of South Africa (Fig. 5.1) constitute the southern limit of their distribution in the Western Indian Ocean region (Schleyer et al., 2000). The Maputaland reefs are relatively pristine, highly biodiverse and dominated by soft corals. Although protected within the iSimangaliso Wetland Park, one of only two marine World Heritage Sites within the Indian Ocean region, the reefs are nevertheless subjected to potential anthropogenic impacts. Long-term observations at monitoring sites indicate a steady decline in soft coral cover of almost 1% per year (Porter and Schleyer, 2016; Schleyer et al., 2008). Due to their marginal geographical location, these communities have not been significantly affected by bleaching (Porter and Schleyer, 2016) and receive negligible direct fluvial input. Factors contributing to apparent long-term declines in soft coral cover therefore remain unknown. However, the reefs are located adjacent to an extensive coastal plain that is host to a number of large coastal lakes and wetlands, where high concentrations of OCPs have been detected within sediments (Humphries, 2013; Chapter 2). The accumulation of pesticide residues within these systems is largely attributed to local agricultural activities and ongoing pest control measures. DDT in particular continues to be used on an annual basis as an indoor residual spray for malaria control (Bouwman et al., 2012; Van Dyk et al., 2010). Sediments found within these systems therefore contain large reservoirs of toxins that may enter coastal waters via groundwater seepage, and the proximity of the Maputaland reefs potentially exposes corals to the long-term impacts from a broad suite of OCPs. To date, the potential for pesticides to impact water quality and the overall health of the Maputaland reefs has not been investigated, and a lack of ecotoxicological data precludes an assessment of the ecological risk posed. This study aims to evaluate the susceptibility of the Maputaland coral reef communities to pesticide contamination, with attention focused on the accumulation of OCPs within the tissues of two species of soft coral (*Lobophytum depressum* and *Sinularia gravis)*. This provides a first step in assessing linkages between terrestrially-derived pesticide residues and coral health for the region.

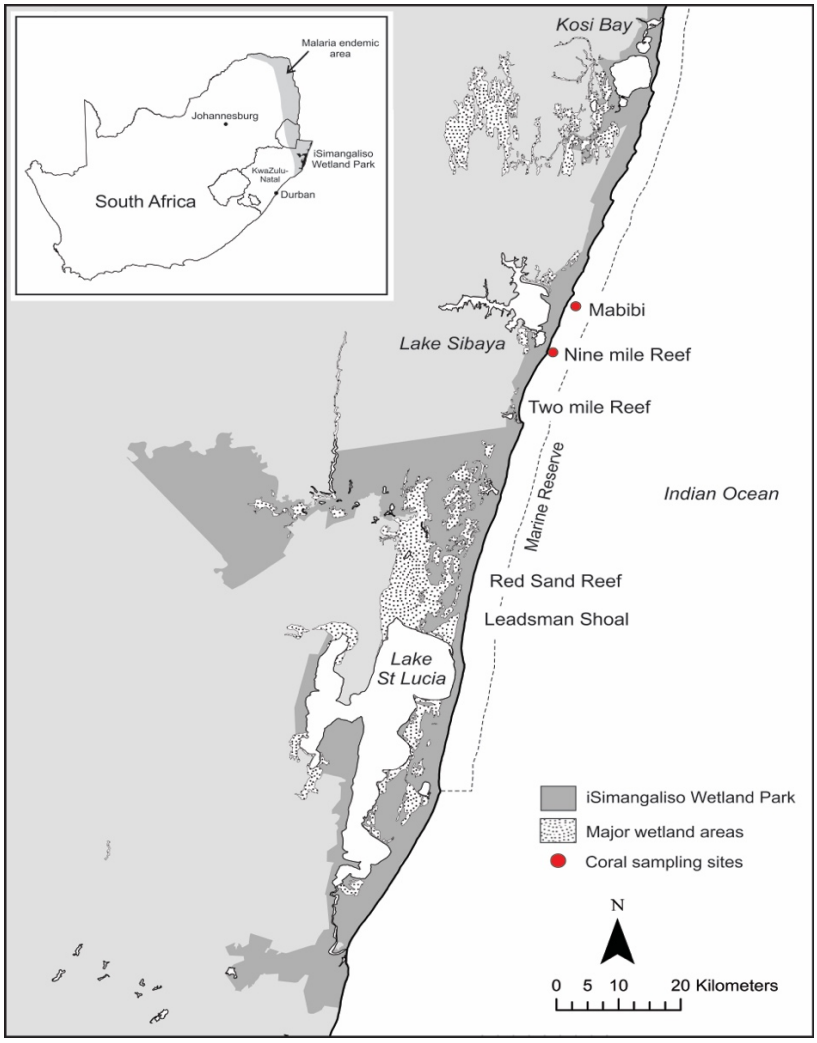

Fig 5. 1: Map showing the location of iSimangaliso Wetland Park with the main lake and wetland systems and the marine protected regions where coral samples were collected from.

5.2 Methods

5.2.1 Sample collection

Sampling was undertaken at two reefs situated along the Maputaland coast; Nine-mile and Mabibi (Fig. 5.1). These reefs were selected for this preliminary investigation as they lie directly offshore of Lake Sibaya, a potentially significant source of OCPs to the coastal zone. Two sessile benthic species were sampled; *Sinularia gravis* and *Lobophytum depressum*. These species were identified as suitable candidates as they are long-lived soft corals and occur abundantly along the Maputaland coastline. Samples of *S. gravis* and *L. depressum*

were collected from Nine-mile, while at Mabibi only *L. depressum* was sampled. In each case, three replicate tissue samples from independent colonies were collected. An apple corer was used to collect a ~20 g sample from the centre of each colony whilst on SCUBA. Samples were immediately placed on ice in the field and stored frozen prior to analysis.

5.2.2 Sample extraction and analysis

The coral samples were initially cryogenically milled with liquid N_2 into a homogenous and fine material. The extraction of OCPs from coral samples was carried out using a modified QuEChERS procedure (Fernandes et al., 2013). In summary, 3 g of homogenized sample was weighed into a 50 ml centrifuge tube and were saturated with 5 ml HPLC water and allowed to stand for ~20 minutes. Samples were then extracted with 6 ml acetonitrile containing 1 v/v% glacial acetic acid, 2.5 g anhydrous magnesium sulfate ($MgSO_4$), 0.8 g sodium acetate (NaOAc) and 0.4 g sodium acetate trihydrate ($CH_3COONa.3H_2O$). They were shaken vigorously by hand and then vortexed to prevent the formation of agglomerates during $MgSO_4$ hydration. Samples were kept frozen for about 3 hours at -18 ^oC to solidify any fat/lipid present. Sample clean-up was achieved by transferring 4 ml of the organic extract into a centrifuge tube containing 0.4 g $MgSO_4$, 0.2 g C18, 0.2 g deactivated florisil and 0.2 g primary secondary amine (PSA). This solution was vortexed and then centrifuged to isolate the clean extract. A 3 ml aliquot was concentrated to dryness under vacuum at a temperature ≤ 40 ^oC and reconstituted in hexane (1 ml) for final analysis.

5.2.3 Apparatus and Conditions

Analysis was performed using an Agilent 7890B gas chromatograph (GC) coupled to a Leco Pegasus 4D GCxGC Time-of-Flight mass spectrometer. Separation was achieved using a Restek Rxi-5Sil MS column with Integra-Guard (30 m × 0.25 mm i.d. × 0.25 µm film thickness) coupled to an Rxi-17Sil MS (1.075 m × 0.25 mm i.d. × 0.25 µm thickness) secondary column. Samples of 2 µl were injected in splitless mode using ultrahigh-purity He helium as the carrier gas at a constant flow rate of 1.2 ml min^{-1}. The GC oven was set to

75 °C (held for 1 min), then raised to 280 °C at a rate of 6 °C min^{-1} and maintained at this temperature for 2 min. The transfer line and inlet temperatures were set at 300 and 250 °C, respectively. The ion source temperature was 250 °C with a detector voltage of 1900 V. The mass range was set at m/z 45–550, with a spectral data acquisition rate of 100 spectra s^{-1}. Data processing and peak identification was performed using the Leco ChromaTOF software and databases. Peaks were identified based on the retention time of target ions (exact masses) and confirmed by two identifier ions.

5.2.4 Target Analytes

Target analytes included hexachlorocyclohexanes (HCHs; α-, β-, γ- and δ-HCH; sum expressed as ΣHCH), drins (aldrin, dieldrin, endrin, endrin aldehyde and endrin ketone; sum expressed as Σdrin), DDTs (p,p'-DDT, p,p'-DDE and p,p'-DDD; sum expressed as ΣDDT), endosulfans (α-, β-endosulfan and endosulfan sulfate; sum expressed as Σendosulfan) and chlors (heptachlor, heptachlor epoxide and methoxychlor; sum expressed as Σchlor).

5.2.5 Quality Assurance and Quality Control

Recovery studies were conducted at three different concentration levels to assess extraction efficiency (supplementary Table TS 5.1). Mean recoveries for all analyte compounds were in the range of 65 –105% (n = 3). Uncertainties for pesticide analytes were typically within < 15%. A standard solution was run every 8–10 samples to monitor instrument variation with accuracy ranging between 83 and 102%. Detection limits ranged between 0.41 and 1.19 ng g^{-1} for all analytes and was computed using International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use guidelines (ICH, 2005). Linear regressions derived from matrix-matched calibration curves for individual pesticides were ≥ 0.99.

5.3 Results

5.3.1 Levels of OCPs in coral tissues

The OCP concentration data is reported on wet weight (ww) basis. The two coral species showed high OCP accumulation with ΣOCP concentration ranging from 2900 to 3100 ng g^{-1} for *S. gravis* and 1700 to 2900 ng g^{-1} for *L. depressum*. The individual OCP classes (ΣHCHs, Σchlors, Σdrins, ΣDDTs and Σendosulfans) were detected in all samples analysed. The three OCP classes detected in highest concentration in both species were Σchlors, Σdrins and Σendosulfans with average concentrations ranging from 750 – 860 ng g^{-1}, 510 – 850 ng g^{-1} and 390 – 600 ng g^{-1}, respectively (Fig. 5.2). In *S. gravis*, Σendosulfan showed the widest range in concentration (140 ng g^{-1}), while Σchlors showed the highest variation in *L. depressum* samples (970 ng g^{-1}). ΣHCHs was among the least varied OCPs detected in both coral species with concentration ranging between 290 and 450 ng g^{-1} for *S. gravis*, and for *L. depressum* 180 and 500 ng g^{-1}. ∑chlors and ∑drins accounted for more than 55 % of the detected ∑OCP concentration, with ∑DDT contributing ≤13 % of the ∑OCP concentration for the two coral species.

In general, OCP concentrations detected in *L. depressum* at Mabibi were higher than at Nine-mile reef. Exceptions to this were ∑HCH and ∑chlors concentrations that were ≥1.5 times greater at Nine-mile (Table 5.1). Notably, ∑DDT concentrations in *L. depressum* on the other hand were 2 times higher at Mabibi compared to Nine-mile (Table 5.1)

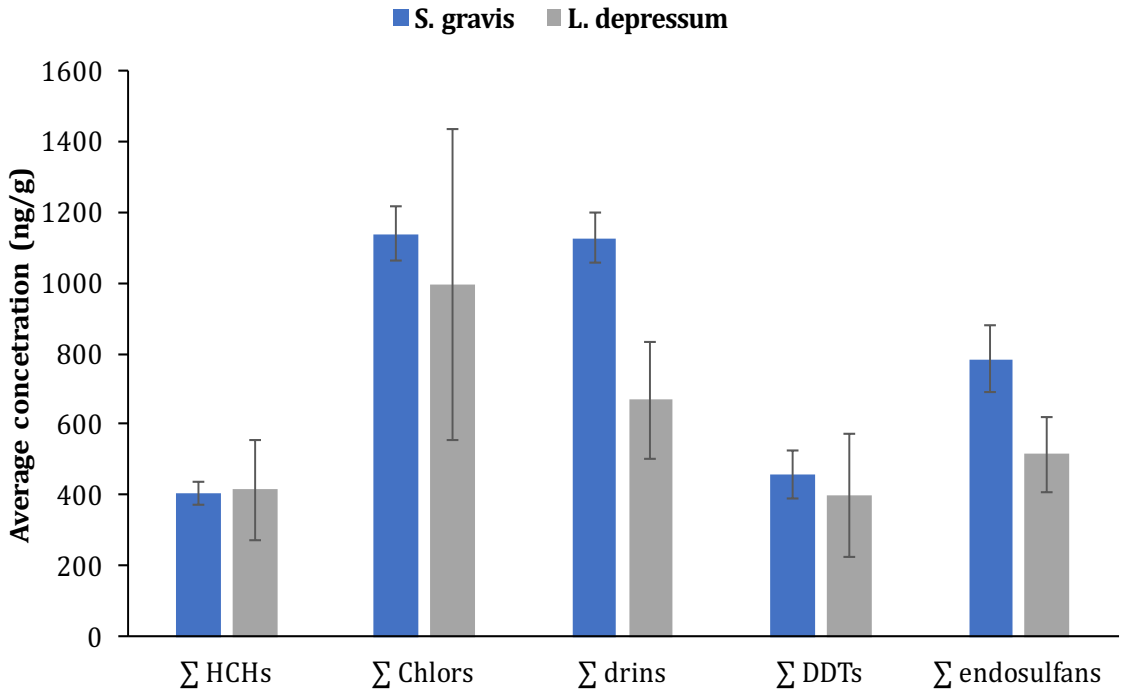

Fig 5. 2**:** OCP concentrations (ng g^{-1} ww) detected in *S. gravis* and *L. depressum* tissues collected from Mabibi and Nine-mile reefs. Standard deviation indicated by error bars

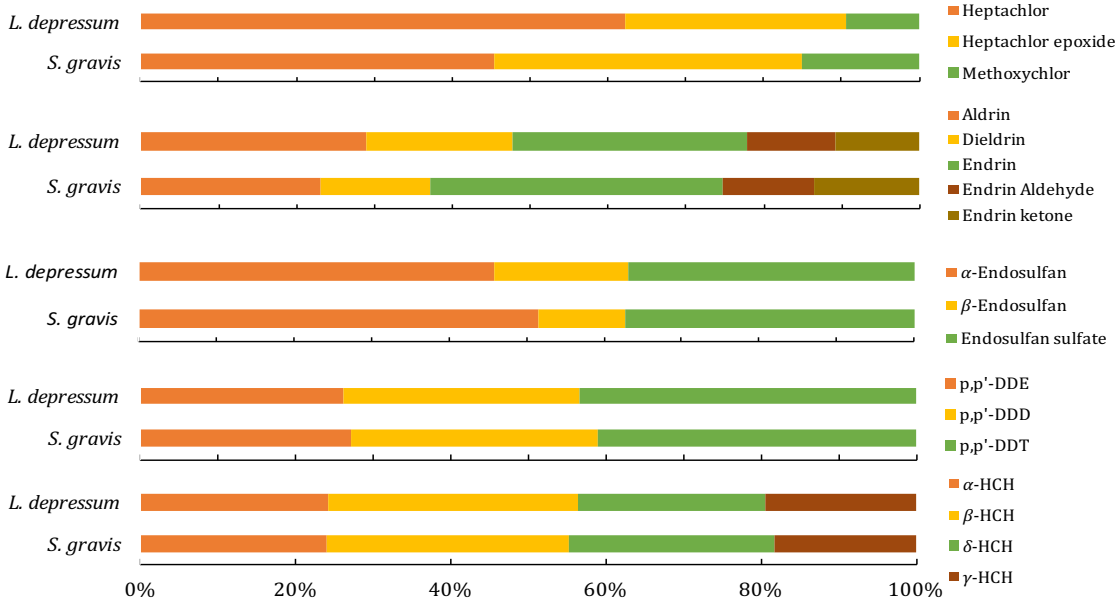

Fig 5. 3**:** OCP metabolite contribution to the concentrations (ng g^{-1} ww) of ΣDDTs, Σchlors, Σdrins, ΣHCHs and Σendosulfans in *S. gravis* and *L. depressum* tissues collected from Mabibi and Nine-mile reefs.

Table 5. 1**:** Mean ± standard deviation and *(range)* of organochlorine pesticides (ng g^{-1} wet weight (ww)) in *Sinularia gravis* and *Lobophytum depressum* tissue sampled from Nine-mile and Mabibi coral reef colonies at iSimangaliso Wetland Park.

	Concentration (ng g^{-1} ww)		
	Nine-Mile (n=3) ***S. gravis***	**Nine-Mile (n=3)** ***L. depressum***	**Mabibi (n=3)** ***L. depressum***
α-HCH	74 ± 9 ***(65-82)***	90 ± 26 ***(66-120)***	63 ± 16 ***(46-77)***
β-HCH	96 ± 10 ***(86-110)***	130 ± 33 ***(97-160)***	72 ± 29 ***(41-98)***
δ-HCH	82 ± 6 ***(77-88)***	94 ± 34 ***(74-134)***	57 ± 11 ***(47-69)***
γ-HCH	56 ± 6 ***(50- 61)***	63 ± 24 ***(48-90)***	60 ± 13 ***(47-73)***
∑- HCHs	*310 ± 2* **(*290-340*)**	**380 ± 110** ***(290-500)***	**250 ± 68** ***(180-320)***
Heptachlor	390 ± 22 ***(370-410)***	610 ± 360 ***(380-1000)***	330 ± 150 ***(180-470)***
Heptachlor epoxide	340 ± 22 ***(320- 360)***	250 ± 83 ***(200-350)***	180 ± 49 ***(140-230)***
Methoxychlor	130 ± 63 ***(61-180)***	60 ± 5 ***(6-120)***	82 ± 29 ***(54-110)***
∑- Chlors	**860 ± 59** ***(820-930)***	**920 ± 390** ***(630-1400)***	**590 ± 210** ***(390-810)***
Aldrin	200 ± 29 ***(170-230)***	150 ± 49 ***(110-200)***	150 ± 14 ***(140-160)***
Dieldrin	120 ± 23 ***(94-140)***	47 ± 8 ***(38-54)***	150 ± 64 ***(94-220)***
Endrin	320 ± 78 ***(250-410)***	190 ± 79 ***(99-240)***	120 ± 50 ***(67-170)***
Endrin Aldehyde	100 ± 37 ***(59-130)***	43 ± 18 ***(22-55)***	72 ± 27 ***(42-93)***
Endrin ketone	120 ± 44 ***(80-170)***	28 ± 4 ***(25-33)***	81 ± 8 ***(72-87)***
∑- drins	**850 ± 55** ***(800- 900)***	**450 ± 96** ***(350-530)***	**560 ± 140** ***(470-730)***
p,p'-DDE	94 ± 13 ***(80-100)***	47 ± 6 ***(42-53)***	110 ± 31 ***(80-140)***
p,p'-DDD	110 ± 10 ***(100-120)***	55 ± 13 ***(40-62)***	130 ± 55 ***(91-190)***
p,p'-DDT	140 ± 55 ***(94-200)***	98 ± 72 ***(26-170)***	160 ± 92 ***(98-270)***
∑- DDTs	**350 ± 52** ***(300-400)***	**200 ± 55** ***(140-250)***	**410 ± 88** ***(300-460)***
α-Endosulfan	310 ± 76 ***(260-400)***	140 ± 96 ***(79-250)***	210 ± 71 ***(130-260)***
β-Endosulfan	70 ± 17 ***(52-86)***	56 ± 9 ***(49-66)***	80 ± 34 ***(53-120)***
Endosulfan sulfate	220 ± 27 ***(190-250)***	150 ± 84 ***(94-250)***	130 ± 50 ***(78-170)***
∑- endosulfans	**600 ± 71** ***(530-670)***	**350 ± 93** ***(250-420)***	**430 ± 57** ***(380-490)***
(DDE+DDD)/DDT	**1.44** ***(0.99- 2.21)***	**1.03** ***(0.48-4.47)***	**1.47** ***(0.69-2.65)***

The OCP metabolite accumulation profiles of the two coral species were similar. On the average, β-HCH, heptachlor, endrin, α-endosulfan and p,p'-DDT were metabolites detected in highest concentrations for the two coral species. In both species, p,p'-DDT accounted for ≥41 % of ∑DDT concentrations, with p,p'-DDE and p,p'-DDD contributing X and Y, respectively. The ratio of the metabolites (DDE + DDD)/DDT was on the average >1.3 for both species. Heptachlor accounted for >45 % of ∑chlors concentrations in both *S. gravis* and *L. depressum*, with average concentrations of 390± 25 ng g^{-1} and 470 ± 290ng g^{-1}, respectively (Fig. 5.3).

Methoxychlor was detected in high concentration in *S. gravis* with an average concentration of 130 ± 63 ng g^{-1} and was approximately twice the average concentration detected in *L. depressum* (Fig. 5.3). Similarly, α-endosulfan concentration in *S. gravis* (average of 310 ± 76 ng g^{-1}) was ~2 times greater than the average concentration detected in *L. depressum* (180± 85 ng g^{-1}). The general order of isomer contribution to ∑endosulfan concentration was similar for both species with α-endosulfan > endosulfan sulfate > β-endosulfan.

The contribution of HCH isomers (α-, β-, δ-, and γ-HCHs) to ΣHCH concentration were also similar between species with each isomer accounting on the average for ~24, 32, 24 and 20%, respectively (Fig. 5.3). β-HCH was the highest contributor to ∑HCH concentration with average concentrations of 96 ± 10 ng g^{-1} and 100 ± 42 ng g^{-1} in *S. gravis* and *L. depressum,* respectively. On the average; endrin, endrin ketone and endrin aldehyde concentrations in *L. depressum* were ~2 times lower than the levels detected in *S. gravis*. On the average, endrin accounted for more than 30% of the ∑drin concentration in both species. Aldrin and dieldrin were the second and third highest contributors to ∑drin concentrations with an average concentration of 200 ± 29 ng g^{-1} and 120 ± 23 ng g^{-1} (*S. gravis*) and 150 ± 32 ng g^{-1} and 95 ± 67 ng g^{-1} (*L. depressum*).

5.4 Discussion

5.4.1 Bioaccumulation of OCPs

Organochlorine pesticides were found to be abundantly present in both coral species investigated from Nine-mile and Mabibi. Accumulation profile as well as the dominant OCP metabolites was similar for the *S. gravis* and *L. depressum* samples investigated (Fig. 5.3). Generally, the contribution of metabolites to ∑OCP concentration for the individual OCP classes were much greater compared to parent compounds. Endrin, Aldrin, Heptachlor and p,p'-DDT in particular showed greater concentration compared to their metabolites (Fig. 5.3). DDT is still applied annually by the Department of Health for malaria vector control in communities surrounding iSimangaliso under exemption from the Stockholm Convention on persistent organic pollutants (UNEP, 2016). In other communities where DDT is used for malaria control, higher levels of DDT metabolites (p,p'-DDE and p,p'-DDD) have been detected relative to p,p'-DDT in sediment and biota (Chapter 2; Wepener et al., 2012; Kaur et al., 2008). This is contrary to the present study where p,p'-DDT remained the dominant metabolite in both coral species, indicating a more recent use of the DDT most likely for IRS application within the catchment of the Wetland Park.

Discharges through run-off and atmospheric deposition from coastal lakes and estuaries have been identified as some of the pathways by which terrestrially derived pesticides contaminate coral colonies within the marine environment from other studies (El Nemr et al., 2004; Imo et al., 2008; Morrison et al., 1996, 2013; van Dam et al., 2011). Coastal lakes and estuaries adjacent to the southern Indian ocean in a recent study have been found to be sinks of substantial quantities of OCPs (Chapter 2) and the likely source of discharge of OCPs to the adjacent coastal zone. Furthermore, OCP metabolites detected in surface sediments and biota were similar to the OCP metabolites detected in coral samples from Maputaland coral colonies. About 72 - 80% of these OCPs and metabolites found in surface sediments and biota from coastal lakes and estuaries at iSimangaliso were metabolites of other OCP classes other than DDT (Chapter 2; Chapter 3). DDTs typically accounted for less

than 13% of all the detected OCPs in the coral samples analyzed in the present study. This high prevalence of other classes of OCP metabolites in present samples and our recent study on sediment and biota underscores the continued and unregulated use of pesticides including banned OCPs in the country and most especially around very sensitive protected habitats.

The two sites (Mabibi and Nine-mile) revealed very little differences in ∑OCPs detected, especially for *L. depressum* (Table 5.1), however their accumulation of the individual classes of OCP metabolites varied. These variations in OCP accumulation could reflect the use patterns of the different classes of OCP within each catchment. Mabibi is situated in close proximity to Lake Sibaya and is predominantly agricultural; DDT especially is used heavily and annually for malaria control programs and thus may account for the high levels detected relative to Nine-mile reef colony which is farther south.

Interspecies variation, revealing higher accumulation of OCPs in *S. gravis* compared to *L. depressum* were also observed (Fig 5.2). These differences in OCP accumulation between the two species may be attributed to differences in their structure and reproduction. *L. depressum* are dimorphic polyps and reach sexual maturity very early in their lifecycle, while *S. gravis* a monomorphic polyp which reaches sexual maturity later in their lifecycle (Achituv and Benayahu, 1990; Helgason, 2015). OCPs are lipophilic and in corals, the lipid rich eggs could be potential reservoirs of these OCPs. The early spawning in *L. depressum* may be a possible mechanism for reducing their OCP burden (Helgason, 2015).

Table 5. 2**:** Comparison of organochlorine pesticide concentrations (ng g^{-1}) reported in different coral species globally (ww = wet weight, dw = dry weight and n.d. = not detected

Location (sampling year)		**Species**	**∑HCH**	**∑DDT**	**∑drin**	**∑chlor**	**∑endosulfan**	**References**
iSimangaliso (2016)	ww	*Sinularia* sp. and *Lobophytum* sp.	180 – 500	140 – 460	350 – 900	390 – 1400	250 – 670	This Study
Great Barrier Reef, Australia (1976-77)	dw	*Acropora* sp. and *Fungi* sp.	0.01 – 13.3	0.1 – 21.7	0.08 – 3.9	0.6 – 7.8		Olafson, 1978
Florida, USA (1985)	ww	*Scleractinian* sp.	n.d. – 854.7	n.d. – 1012.3	n. d. – 2923.5	n.d. – 2711.4	n.d. – 481.5	Glynn et al., 1989
Red Sea Coast, Egypt (1999)	dw	*Acropora* sp.	0.9 – 14.6	1.0 – 11.7	0.5 – 5.5			El Nemr et al., 2004
Hawaii, USA (1998-2001)	dw	*Porites* sp.	0.008 – 0.63	0.08 – 2.7		0.004 – 2.2		Wang et al., 2008
Puerto Rico (2005)	dw	*Porites* sp.	n.d.	n.d. – 0.62				Pait et al., 2009

5.4.2 Comparison with other studies

A search through literature for available data on OCP levels in corals from the southernmost part of the Indian Ocean did not yield any data. This dataset therefore, provides the first insight into the distribution and nature of OCPs in the two corals species analysed from the southern part of the Indian Ocean. Generally, data on levels and distribution of OCPs in corals are limited globally. Table 5.2 provides comparative data for OCP levels in corals from other parts of the world, however, such comparisons are complicated by variances in sampling times, the different species and type of tissues analysed as well as analytical methods used. It, however, allows us to place data from iSimangaliso within a global context. Generally OCP levels from the present study were up to 100 times higher compared to reported concentrations from coral material collected from the Red Sea (El Nemr et al., 2004), Tern Island and Bikini Atoll off the coast of Hawaii (Wang et al., 2008) and the Great Barrier Reef (Olafson, 1978). However, levels from the current study were up to 2-3 times lower than levels determined in corals from the coast of Florida (Glynn et al., 1989) with some contaminants such as endrin as high as 2923 ng g^{-1} ww.

5.4.3 Ecotoxicological importance

The potential adverse effects caused by exposure of corals to pesticides are not well understood. However, a range of adverse impacts affecting metamorphosis, reproduction and fertilization in different life stages of corals (Haynes et al., 2000; Markey et al., 2007; Negri et al., 2005; van Dam et al., 2011) have been suggested. Endosulfan in particular has been linked to the inhibition of electron transport to chloroplast thereby affecting corals photosynthetic activity (van Dam et al., 2011). While corals may recover from short-term exposure to contaminants, reduction in tissue lipid content, bleaching and decreases in photosynthetic activities within coral colonies have been suggested for chronic exposure to anthropogenic chemicals (Lewis et al., 2012, 2009). While it remains difficult to assess the ecotoxicological impacts of the residues detected within coral tissues from Mabibi and

Nine-mile, it is possible that the reported long-term decline in soft coral cover at Nine-mile (Porter and Schleyer., 2016), could be attributed to OCP exposure. However, further and a more in-depth work is necessary to be able to draw such linkages.

5.4.4 Potential human health implications

The iSimangaliso coastline constitutes an important nursery ground for a variety of estuary-associated fish and invertebrates. Fish samples in studies from other coral habitats globally including GBR, Florida Okinawa and French Polynesia have been found to bioaccumulate high levels of pesticides including OCPs (Glynn et al., 1995; Araújo et al., 1999; Imo et al., 2008; Roche et al., 2011). The substantial OCP residues detected in corals from the current study not only poses a threat to aquatic organisms, but the surrounding communities as well. Residents within the catchment areas of iSimangaliso rely heavily on the local environment for food. Many of the residents rely on fish and other marine resources as their main sources of protein. Ingestion of contaminated food has been suggested to constitute one of the major routes through which humans accumulate OCPs (Gyalpo et al., 2012). Therefore, further investigation into the exposure of communities to high levels of OCP residues through their dietary intake and their associated health risks is urgently needed.

5.5 Conclusions

The data presented in this study provides preliminary insight into the bioaccumulation of OCP in coral species from the Maputaland reefs. The coral tissues analysed were found contain substantial quantities of OCPs, indicating a source of contamination to the coastal zone, mostly likely via groundwater seepage. The levels detected are the highest recorded in coral samples globally in almost three decades. Results highlight potential ecotoxicological impacts on coral health as well as for the marine ecosystem in general and requires further and an in-depth investigation. Further work is needed to better understand, contaminant transport mechanisms, spatial variations in accumulation, and

the potential ecotoxicological risks associated with OCP exposure in higher trophic level marine organisms.

Acknowledgements

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CHAPTER SIX – General conclusion and future work

6.1 General conclusions

This study was designed to investigate the occurrence and bioaccumulation of organochlorine pesticides in iSimangaliso Wetland Park, and assess their likely impact on both ecosystem and human health. To address this aim, four key environmental components (sediment, fish tissue, crocodile fat and coral tissue) were sampled and analysed for OCPs.

Substantial concentrations were found in all samples analysed from the Wetland Park. The study identified the lakes and estuaries as important sinks in the environment, with sediment OCP concentrations some of the highest detected in South Africa. All analytes detected in the sediment were found to have accumulated in notably large quantities in the biological tissues analysed. Interestingly, differences in OCP accumulation were observed for organisms occupying different trophic levels in the aquatic food web, an indication of potential threat of biomagnification of OCPs within the aquatic ecosystem. This has far-reaching implications for apex predators such as crocodiles, as well as the numerous species of birds that rely on this habitat for survival and breeding. Furthermore, the Wetland Park provides resources including food including fish which is the main protein source to residents within its catchments. Exposure to OCPs through the ingestion of contaminated food could pose great risk to local residents.

It should be noted that this study on its own does not exhaustively deal with issues of potential bioaccumulation of OCPs, but rather provides the initial data to highlight some of these conservation issues for better management decision. In addition, sampling activities for the study coincided with one of the worst drought periods the country has experienced in recent times (2015 to early 2017), which greatly hampered the collection of other key biological samples, as initially planned. Despite these challenges, the findings provide evidence that OCPs used in the region for agricultural purposes and disease vector control ultimately end up in the wetlands and lakes of iSimangaliso, exposing a variety of

organisms to a potentially dangerous mixture of contaminants. This study provides essential baseline data which should guide both the Wetland Park management as well as government departments to make better-informed management decisions.

6.2 Directions for future research

The findings from this study raise several questions which deserve further investigation. These include:

- The need for an in-depth health risk assessment that incorporates the exposure of local people to OCPs from other food sources. Of particular importance are vegetables being cultivated by local communities in the drainage channels to the lakes and wetlands where high OCP levels have been detected. Such an assessment will provide a better understanding of the risks residents are being exposed to as a result of the continued use of OCPs in the region.

- More detailed investigations are needed to provide a better understanding of the likely impact of OCPs on coral health in the region. This would include expanding on the present work to include additional species and samples from other sites along the Maputaland reef. This will provide a better basis to predict if the decline in soft coral abundance at Nine mile is a result of exposure to terrestrially derived pesticides. There is also the need to explore the effect of multiple stressors and the use of bioindicators to assess potential impacts of contaminants on coral health. Further work is also needed to better understand contaminant transport mechanisms, spatial variations in accumulation, and the potential ecotoxicological risks associated with OCP exposure in higher trophic level marine organisms such as bivalves, different fish species, and nesting turtles.

- OCPs are an emerging new threat to Nile crocodiles within South Africa. The crocodile population in iSimangaliso provides a unique opportunity to study the long-term

consequences (accumulation and possible effects) of these contaminants. An investigation of the different life stages (adults, juveniles and eggs) of both male and female Nile crocodiles will aid in predicting the potential adverse effects of these contaminants on crocodile population within this region.

Environmental sampling such as this in a vast nature reserve such as iSimangaliso is always challenging logistically and often time consuming. With the ever presence of crocodiles and hippopotami in almost all the water systems, the dangers are enormous. However, few studies are as rewarding, both in terms of data collection and personal gratification. It is my hope that findings from this study and the proposed future research will generate new data that will stimulate the old standing debate on OCPs implications on human health and ecology. I am also hopeful it will allow for an improved understanding and lead to better management decisions for both biodiversity conservation as well as human health.

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Appendices

Supplementary Table TS 2.1: The results of certified reference material CRM 804 analysis (n=5) values (ug/kg dry wt.). Data linked to Chapter 2

Analyte	Mean concentration (ug/kg)	%RSD	Certified Value (ug/kg)	% Recovery
γ-BHC	576.74 ± 11.6	2.0	491 ± 43.3	117.5
Aldrin	19.2 ± 1.97	10.28	18 ± 3.01	106.6
Dieldrin	1906.6 ± 34.4	1.8	1860 ± 222	102.5
Endrin	52.9 ± 5.2	9.8	62.2 ± 2.91	85.1
α-Endosulfan	431.7 ± 50.9	11.8	491 ± 44.6	87.9
β-Endosulfan	1013.6 ± 125.1	12.3	1130 ± 138	89.7
p,p'-DDE	1609.7 ± 237.3	14.7	1520 ± 139	105.9
p,p'-DDD	1887.4 ± 107.4	5.7	1530 ± 161	123.4
p,p'-DDT	1018.8 ± 122.8	12.1	1060 ± 93.1	96.1

Supplementary Table TS 2.2: Percentage spiked recoveries for sediment samples (n = 3) and %RSD at three fortified concentrations (ng g^{-1}). LOD = limit of detection. Data linked to Chapter 2

	10 ng g^{-1}		100 ng g^{-1}		500 ng g^{-1}		
Analytes	**Mean**	**RSD**	**Mean**	**RSD**	**Mean**	**RSD**	**LODs**
α-HCH	76.8	3.5	89.3	6.7	88.0	21.8	0.76
β-HCH	90.9	1.9	94.9	11.7	83.0	8.9	1.02
δ-HCH	86	2.8	86.2	5.7	86.9	14.3	1.20
γ-HCH	88.5	3.4	98.6	7.4	85.6	13.9	0.90
Heptachlor	70	1.9	97.5	7.2	86.7	22.5	1.15
Heptachlor epoxide	84.7	2.1	91.7	3.7	95.7	13.2	0.57
Methoxychlor	67.5	2.6	99.1	6.9	112.0	13.5	3.11
Aldrin	88.2	1.5	97.8	9.1	96.4	12.1	0.45
Dieldrin	97.4	2.8	81.6	2.9	78.5	21.1	0.55
Endrin	95.4	1.7	95.3	6.1	80.5	14.2	0.69
Endrin ketone	75.9	2.6	85.5	5.9	88.5	12.8	1.08
p,p'-DDE	114.3	1.3	109.4	5.6	79.4	22.9	0.42
p,p'-DDD	100.6	1.5	102.0	5.9	79.1	18.4	1.77
p,p'-DDT	83.1	1.7	91.3	7.1	92.9	10.8	2.71
α-Endosulfan	63.1	2.4	78.1	7.9	98.4	12.5	0.52
β-Endosulfan	89.1	4.2	86.7	3.9	84.9	9.1	2.18
Endosulfan sulfate	77.8	1.1	107.1	1.8	87.0	12.6	0.59

Supplementary Table TS 2.3: Average concentrations (n = 3) of organochlorine pesticides (ng g^{-1} dw) ± SD in sediment samples collected from iSimangaliso Wetland Park. Data linked to Chapter 2

	Analyte Conc (ng g^{-1})																
Sample ID	α-HCH	β-HCH	δ-HCH	γ-HCH	Aldrin	Dieldrin	Endrin	Endrin ketone	Heptachlor	Heptachlor epoxide	Methoxychlor	p,p-DDE	p,p-DDD	p,p-DDT	a-Endosulfan	b-Endosulfan	Endosulfan sulfate
1	31.76 ± 1.61	27.46 ± 1.17	29.83 ± 1.68	12.86 ± 1.7	19.14 ± 1.85	44.17 ± 2.76	39.5 ± 1.67	7.37 ± 1.27	16.5 ± 1.78	17.73 ± 1.77	4.12 ± 2.72	32.67 ± 0.08	32.72 ± 1.07	n.d.	20.37 ± 2.18	37.59 ± 3.19	16.81 ± 2.68
2	45.95 ± 5.55	21.42 ± 0.1	19.7 ± 0.81	15.87 ± 0.98	33.31 ± 1.31	32.56 ± 0.48	82.66 ± 6.52	22.72 ± 2.15	25.4 ± 0.91	29.05 ± 0.86	16.4 ± 3.11	37.07 ± 5.66	43.24 ± 2.64	21.89 ± 1.05	38.03 ± 2.41	40.41 ± 1.87	27.04 ± 1.64
3	46.03 ± 5.2	32.21 ± 2.33	25.84 ± 3.71	n.d.	40.27 ± 0.63	18.55 ± 2.21	77.94 ± 1.6	26.47 ± 1.79	15.41 ± 0.87	26.66 ± 2.98	26.93 ± 1.9	33.98 ± 3.76	36.55 ± 3.15	7.91 ± 1.7	58.22 ± 5.08	22.4 ± 1.4	12.31 ± 1.42
4	11.49 ± 1.03	24.59 ± 1.86	17.63 ± 1.25	n.d.	13.97 ± 1.36	24.14 ± 2.16	26.13 ± 0.53	6.64 ± 0.34	8.46 ± 0.47	36.17 ± 3.46	n.d.	51.87 ± 3.51	34.93 ± 4.19	4.42 ± 0.18	29.65 ± 1.35	19.41 ± 3.01	15.2 ± 1.09
5	13.21 ± 0.97	23.86 ± 2.32	13.75 ± 0.94	20.73 ± 1.96	n.d.	10.7 ± 0.66	22.01 ± 2.14	n.d.	n.d.	29.26 ± 1.36	n.d.	27.81 ± 2.34	24.77 ± 1.9	n.d.	22.27 ± 2.04	20.37 ± 2.45	n.d.
6	44.69 ± 2.33	78.52 ± 1.39	26.38 ± 3.05	36.23 ± 0.76	26.94 ± 3.92	54.75 ± 2.74	62.14 ± 1.15	n.d.	14.93 ± 1.66	38.5 ± 2.18	7.77 ± 0.49	53.73 ± 5.01	50.09 ± 1.70	5.88 ± 0.51	54.63 ± 4.93	49.06 ± 3.62	22.78 ± 2.40
7	26.24 ± 2.6	39.37 ± 0.88	29.01 ± 1.58	n.d.	36.01 ± 3.51	39.53 ± 3.32	31.92 ± 1.6	11.26 ± 0.99	6.11 ± 0.19	27.18 ± 1.33	n.d.	37.63 ± 2.95	44.95 ± 3.25	7.96 ± 0.35	40.12 ± 3.59	31.37 ± 1.2	30.87 ± 1.82
8	21.77 ± 2.24	46.68 ± 2.69	25.21 ± 2.28	n.d.	14.78 ± 2.26	29.01 ± 3.25	102.35 ± 2.13	12.52 ± 0.99	4.89 ± 0.33	20.29 ± 1.31	n.d.	30.02 ± 1.97	24.72 ± 1.84	8.26 ± 0.95	31.21 ± 1.76	31.01 ± 0.8	25.46 ± 2.19
9	31.54 ± 1.45	31.03 ± 3.5	28.26 ± 1.6	n.d.	26.12 ± 1.02	38.86 ± 3.07	45.16 ± 2.93	11.6 ± 0.28	7.56 ± 0.66	39.93 ± 4.11	n.d.	45.79 ± 3.25	67.04 ± 2.14	6.79 ± 0.64	42.91 ± 2.58	30.08 ± 2.52	28.82 ± 0.42
10	13.97 ± 1.28	n.d.	17.89 ± 1.75	30.25 ± 2.21	17.16 ± 1.02	19.2 ± 1.92	12.6 ± 1.48	16.5 ± 2.06	23.5 ± 1.46	27.04 ± 3.14	n.d.	34.07 ± 2.65	70.22 ± 4	31.53 ± 1.21	36.42 ± 3.11	32.1 ± 1.02	n.d.
11	23.4 ± 1.85	18.78 ± 1.37	11.64 ± 0.57	n.d.	16.66 ± 2.55	35.4 ± 3.58	26.95 ± 3.03	n.d.	n.d.	10.46 ± 0.68	n.d.	32.7 ± 3.05	27.26 ± 2.5	n.d.	23.15 ± 2.5	27.37 ± 2.13	n.d.
12	19.19 ± 0.91	7.1 ± 0.98	n.d.	n.d.	9.36 ± 1.12	5.78 ± 0.47	2.45- ± 0.46	1.78 ± 0.52	3.14 ± 5.99	n.d.	n.d.	18.07 ± 1.79	16.42 ± 0.47	n.d.	29.93 ± 2.38	7.57 ± 0.94	n.d.
13	39.27 ± 1.36	20.55 ± 2.21	7.92 ± 0.23	n.d.	18.5 ± 1.77	18.53 ± 2.37	34.9 ± 2.46	3.02 ± 0.11	n.d.	n.d.	n.d.	57.83 ± 1.21	51.06 ± 2.28	n.d.	24.01 ± 2.72	6.92 ± 0.99	n.d.
14	n.d.	17.14 ± 0.69	13.65 ± 0.59	15.73 ± 0.33	7.76 ± 0.55	6.17 ± 0.59	7.88 ± 0.38	3.47 ± 1.08	n.d.	8.34 ± 0.49	n.d.	20.86 ± 1.09	34.04 ± 2.45	n.d.	3.76 ± 0.47	8.46 ± 0.51	n.d.
15	14.11 ± 1.46	15.05 ± 0.9	30.55 ± 2.38	n.d.	8.32 ± 0.44	22.84 ± 2.57	2.66 ± 1.03	n.d.	n.d.	7.24 ± 1.02	5.35 ± 0.83	20.6 ± 1.22	16.92 ± 0.96	n.d.	16.42 ± 0.91	n.d.	5.42 ± 0.55
16	24.2 ± 2.4	38.51 ± 2.69	12.47 ± 1.69	17.72 ± 0.47	30.59 ± 2.92	21.56 ± 1.73	46.44 ± 0.65	10.77 ± 0.67	17.92 ± 2.14	32.44 ± 1.92	n.d.	80.4 ± 1.22	40.38 ± 2.32	n.d.	62.38 ± 3.83	52.62 ± 3.73	n.d.
17	28.54 ± 0.98	17.47 ± 1.66	15.97 ± 1.29	38.27 ± 0.11	16.51 ± 1.03	26.42 ± 0.25	36.71 ± 3.73	13.63 ± 1.35	8.79 ± 1.56	30.33 ± 1.56	2.1 ± 0.34	75.17 ± 2.28	53.44 ± 4.3	30.02 ± 1.13	25.84 ± 2.08	40.22 ± 3.41	43.55 ± 1.8
18	39.77 ± 3.7	47.11 ± 2.4	27.09 ± 1.55	n.d.	4.86 ± 0.38	47.83 ± 2.3	29.85 ± 2.79	7.67 ± 0.63	5.58 ± 0.98	42.3 ± 1.23	9.56 ± 0.73	54.27 ± 4.41	43.48 ± 3.51	7.73 ± 0.28	47.85 ± 1.42	48.04 ± 1.87	25.79 ± 1.94
19	44.59 ± 3.88	191.03 ± 2.57	46.88 ± 3.07	n.d.	n.d.	80.48 ± 4.2	61.45 ± 3.08	21.36 ± 1.16	25.8 ± 1.81	64.7 ± 3.13	n.d.	67.31 ± 4.49	63.74 ± 5.09	9.21 ± 0.77	79.11 ± 2.27	41.44 ± 5.49	34.98 ± 4.2
20	34.55 ± 1.41	102.15 ± 6.8	37.53 ± 1.32	n.d.	1.49 ± 0.36	57.98 ± 4	41.18 ± 2.83	15.58 ± 1.43	13.61 ± 1.39	51.88 ± 3.72	8.9 ± 0.79	47.48 ± 2.77	50.87 ± 2.95	6.37 ± 1.21	57.12 ± 2.67	22.07 ± 2.47	11.67 ± 0.74
21	35.37 ± 3.59	35.9 ± 2.19	38 ± 0.85	68.38 ± 4.36	5.57 ± 0.49	46.82 ± 1.68	43.41 ± 2.72	17.16 ± 0.14	17.1 ± 0.83	56.81 ± 0.88	7.23 ± 0.63	80.87 ± 1.34	60.69 ± 2.11	51.83 ± 0.13	73.52 ± 5.76	61.91 ± 1.83	61.57 ± 2.61
22	28.27 ± 1.04	47.62 ± 2.43	43.81 ± 3.49	56.34 ± 0.3	44.02 ± 2.12	43.31 ± 3.47	38.53 ± 3.02	9.9 ± 0.92	13.66 ± 1.39	22.92 ± 2.09	5.32 ± 0.96	120.9 ± 2.73	127.2 ± 1.32	n.d.	18.16 ± 1.65	25.66 ± 2.57	27.14 ± 2.02
23	47.84 ± 1.68	34.75 ± 4.25	15.93 ± 0.88	n.d.	11.87 ± 0.33	57.69 ± 2.99	129.15 ± 9.17	n.d.	n.d.	32.31 ± 2.55	n.d.	70.93 ± 3.92	77.56 ± 3.17	n.d.	63.93 ± 4.15	34.47 ± 4.31	n.d.
24	35.86 ± 3.05	13.9 ± 0.86	11.17 ± 0.6	n.d.	30.33 ± 1.34	63.66 ± 1.85	113.46 ± 2.63	7.03 ± 0.66	3.34 ± 0.42	30.47 ± 0.76	5.21 ± 0.73	78.3 ± 4.74	95.88 ± 7.28	8.7 ± 0.48	51.51 ± 3.26	33.58 ± 3.63	2.42 ± 0.43
25	58.33 ± 2.5	40.39 ± 3.03	26.51 ± 1.61	n.d.	38.78 ± 1.16	27.65 ± 0.36	63.93 ± 5.34	n.d.	5.41 ± 0.66	39.91 ± 3.19	n.d.	85.51 ± 4.07	72.04 ± 5.02	7.46 ± 1.48	34.3 ± 2.49	17.9 ± 1.16	15.88 ± 0.85

26	23.69 ± 1.22	6.69 ± 0.28	18.85 ± 0.81	n.d.	21.9 ± 2.5	7.78 ± 0.38	34.13 ± 2.41	n.d.	n.d.	43.2 ± 1.63	n.d.	29.74 ± 1.46	31.35 ± 3.4	n.d.	6.04 ± 0.18	42.1 ± 2.74	2.06 ± 0.23
27	45.57 ± 1.31	27.73 ± 2.43	21.73 ± 2.48	n.d.	24.31 ± 2.07	48.08 ± 1.8	37.79 ± 1.83	14.62 ± 0.92	5.02 ± 0.83	34.99 ± 3.47	6.01 ± 0.19	68.9 ± 3.27	94.45 ± 5.18	7.89 ± 0.11	29.05 ± 1.93	59.91 ± 3.13	9.1 ± 0.66
28	67.33 ± 4.45	64.48 ± 1.51	19.93 ± 1.49	n.d.	26.33 ± 2.79	90.62 ± 5.39	151.62 ± 6.32	8.96 ± 0.98	7.28 ± 1.33	n.d.	6.22 ± 0.54	122.84 ± 1.29	119.18 ± 4.21	17.91 ± 1.17	101.74 ± 1.98	77.98 ± 4.37	28.89 ± 0.93
29	15.72 ± 0.86	4.75 ± 0.6	11.89 ± 0.52	n.d.	5.82 ± 0.18	14.7 ± 0.62	19.77 ± 2.47	8.3 ± 0.69	4.63 ± 0.95	13.24 ± 1.19	n.d.	14.19 ± 0.29	47.68 ± 2.7	4.2 ± 0.83	10.99 ± 1.28	19.44 ± 0.75	10.76 ± 1.51
30	44.66 ± 3.05	30.52 ± 1.82	15.14 ± 1.16	n.d.	27.74 ± 1.74	67.2 ± 5.69	43.52 ± 2.46	n.d.	n.d.	30.55 ± 1.44	n.d.	72.63 ± 5.96	69.47 ± 1.22	n.d.	14.26 ± 0.4	n.d.	2.12 ± 0.18
31	41.28 ± 2.45	27.03 ± 2.95	18.73 ± 1.68	n.d.	33.56 ± 2.98	43.58 ± 1.95	70.17 ± 6.51	28.97 ± 1.73	4.8 ± 0.85	32.49 ± 3.03	6.29 ± 0.57	66.82 ± 5.74	94.25 ± 4.21	17.39 ± 1.01	58.07 ± 1.46	47.1 ± 3.2	12.01 ± 0.19
32	49.28 ± 4.37	25.77 ± 0.36	13.71 ± 1.27	n.d.	36.51 ± 2.89	68.86 ± 1.97	115.6 ± 5.77	20.42 ± 1.73	n.d.	39.91 ± 0.54	n.d.	92.34 ± 6.02	113.1 ± 2.26	9.6 ± 0.37	58.09 ± 3.92	44.54 ± 2.31	9 ± 0.86
33	42.74 ± 2.77	34.57 ± 0.84	10.24 ± 0.28	n.d.	37.68 ± 1.74	70.42 ± 0.89	108.99 ± 4.28	5.29 ± 0.43	3.57 ± 0.32	32.26 ± 1.84	n.d.	78.12 ± 3.24	98.85 ± 6	n.d.	58.8 ± 5.22	53.65 ± 2.09	18.21 ± 1.99
34	52.76 ± 3.59	36.44 ± 1.03	22.29 ± 2.74	n.d.	28.11 ± 2.48	71.43 ± 4.15	112.92 ± 4.65	9.99 ± 0.86	n.d.	50.98 ± 1.47	5.32 ± 0.17	97.96 ± 1.78	98.93 ± 5	11.79 ± 1.04	74.49 ± 4.32	58.13 ± 3.6	12.99 ± 1.12
35	37.62 ± 3.34	19.55 ± 0.66	39.11 ± 1.77	6.34 ± 1.05	43.94 ± 3.37	56.98 ± 4.68	79.36 ± 3.49	10.93 ± 0.76	15.52 ± 0.58	24.51 ± 2.47	n.d.	68.02 ± 4.84	94.86 ± 5.54	3.82 ± 0.65	112.57 ± 5.79	92.55 ± 4.79	30 ± 0.27
36	31.87 ± 1.68	16.15 ± 1.83	n.d.	n.d.	17.05 ± 1.1	48.18 ± 3.42	9.72 ± 0.47	n.d.	5.56 ± 0.88	n.d.	n.d.	31.86 ± 2.59	51.27 ± 4.38	n.d.	7.76 ± 0.4	45.84 ± 3.49	n.d.
37	28.64 ± 0.98	24.15 ± 0.4	8.38 ± 0.66	n.d.	27.62 ± 2.38	38.03 ± 3.13	56.41 ± 1.7	n.d.	4.01 ± 0.43	9.21 ± 0.98	n.d.	55.48 ± 5.02	67.23 ± 4.79	n.d.	32.58 ± 0.74	33.98 ± 2.55	5.83 ± 0.26
38	56.78 ± 4.36	43.74 ± 3.25	n.d.	n.d.	43.39 ± 2.84	67.73 ± 4.26	110.65 ± 7.21	5.9 ± 0.28	n.d.	35.67 ± 2.65	n.d.	88.28 ± 3.31	112.83 ± 4.69	5.49 ± 0.32	59.18 ± 3.28	15.07 ± 0.83	3.22 ± 0.28
39	51.38 ± 1.78	25.05 ± 1.92	n.d.	n.d.	22.89 ± 1.83	63.2 ± 6.01	86.86 ± 4.68	4.67 ± 0.5	n.d.	36.3 ± 2.21	n.d.	82.91 ± 0.95	101.31 ± 4.11	4.46 ± 0.51	61.09 ± 1.48	54.05 ± 1.94	3.72 ± 1.34
40	61.79 ± 1.59	37.78 ± 0.35	13.99 ± 0.23	n.d.	24.34 ± 0.79	85.56 ± 2.49	82.44 ± 4.26	4.76 ± 0.28	n.d.	51.74 ± 3.22	n.d.	93.45 ± 6.54	106.52 ± 4.06	9.64 ± 1.29	72.57 ± 3.5	54.36 ± 5.19	8.25 ± 0.4
41	38.15 ± 3.17	20.32 ± 1.95	n.d.	n.d.	33.49 ± 0.56	46.19 ± 3.81	81.16 ± 7.48	4.75 ± 0.43	n.d.	20.02 ± 2.23	n.d.	70.96 ± 2.35	112.03 ± 6.85	12.18 ± 0.17	45.34 ± 4.84	42.95 ± 1.46	5.15 ± 0.84
42	46.18 ± 3.49	33.05 ± 0.97	10.34 ± 0.48	n.d.	35.63 ± 0.95	72.16 ± 1.22	91.55 ± 8.86	n.d.	n.d.	32.94 ± 3.11	n.d.	96.17 ± 7.29	93.21 ± 5.28	6.85 ± 0.39	55.86 ± 2.98	45.83 ± 1.44	5.78 ± 0.03
43	46.54 ± 2.74	11.42 ± 1.03	n.d.	n.d.	24.33 ± 1.28	49.64 ± 1.26	58.44 ± 1.61	7.56 ± 0.2	n.d.	14.84 ± 1.17	n.d.	99.94 ± 5.44	88.97 ± 8.12	6.66 ± 0.62	39.81 ± 2.03	18.29 ± 1.9	7.08 ± 0.1
44	48.72 ± 2.22	25.08 ± 1.43	12.25 ± 0.32	n.d.	22.7 ± 1.13	66.58 ± 5.69	86.17 ± 7.77	n.d.	n.d.	32.04 ± 1.5	n.d.	92.28 ± 6.26	97.25 ± 7.66	8.31 ± 0.25	78.51 ± 3.7	29.96 ± 1.64	1.46 ± 0.35
45	38.17 ± 1.93	34.6 ± 3.04	13 ± 0.91	n.d.	26.65 ± 2	59.82 ± 3.92	71.69 ± 1.9	n.d.	n.d.	29.25 ± 2.69	n.d.	70.25 ± 5.37	106.3 ± 5.28	5.44 ± 0.21	38.42 ± 2.04	39.43 ± 1.32	1.76 ± 0.04

Supplementary Table TS 3.1: Percentage spiked recoveries for fish samples (n = 3) and %RSD at three fortified concentrations (ng g^{-1}). LOD = limit of detection. Data linked to Chapter 3

	10 ng g^{-1}		50 ng g^{-1}		100 ng g^{-1}		LOD
Analytes	**Mean**	**% RSD**	**Mean**	**% RSD**	**Mean**	**% RSD**	**(ng g^{-1})**
α-HCH	83.5	14.7	92.5	4.9	108.3	12.2	0.79
β-HCH	83.4	9.8	85.6	4.1	91.2	8.0	1.17
δ-HCH	92.7	3.8	87.1	9.9	106.9	10.2	0.80
γ-HCH	89.2	4.7	81.5	7.6	96.1	7.7	1.19
Aldrin	90.5	9.3	85.2	6.9	89.5	5.2	1.01
Dieldrin	88.8	2.5	106.8	4.7	103.2	3.5	1.39
Endrin	85.5	2.4	105.4	3.3	98.3	3.3	1.11
Endrin aldehyde	78.3	9.5	88.9	3.8	94.7	7.7	1.20
Endrin ketone	81.5	6.4	74.3	2.9	84.2	5.4	1.66
p,p'-DDE	83.0	12.4	96.5	9.9	91.9	4.4	0.99
p,p'-DDD	91.5	4.8	97.6	3.4	105.6	3.1	1.23
p,p'-DDT	88.5	10.6	93.0	9.7	95.2	1.7	1.71
Heptachlor	96.2	10.2	92.4	8.9	83.9	5.7	2.05
Heptachlor Epoxide	86.2	14.2	81.9	8.9	89.9	9.7	1.30
Methoxychlor	81.5	13.1	88.3	10.7	84.2	5.4	0.86
α-endosulfan	84.2	9.3	100.9	5.6	95.8	1.5	1.14
β-endosulfan	90.1	5.6	102.1	5.8	113.2	2.1	1.46
Endosulfan sulfate	74.1	6.3	87.7	5.7	84.7	4.7	0.71

Supplementary Table TS 3.2: Mean ± standard deviation and range (in parenthesis) of organochlorine pesticide concentrations (ng g^{-1} wet weight) in *Oreochromis mossambicus* and *Clarias gariepinus* muscle tissue sampled from sites within iSimangaliso Wetland Park (Lake St Lucia, Mkhuze wetlands, Lake Sibaya and Kosi Bay. Data linked to Chapter 3

	Mean concentration (ng g^{-1} ww)					
	Oreochromis mossambicus			*Clarias gariepinus*		
Analytes	Lake St Lucia (n = 17)	Lake Sibaya (n = 6)	Kosi Bay (n = 19)	Lake St Lucia (n = 1)	Mkhuze (n = 7)	Kosi Bay (n = 1)
α-HCH	8.9 ± 5.9 ***(0.3-18)***	9.3 ± 0.8 ***(8-10)***	21.7 ± 7.5 ***(10-34)***	12.4	15.4 ± 2.6 ***(12-19)***	15.0
β-HCH	11.4 ± 7.0 ***(2-23)***	13.9 ± 1.5 ***(11-16)***	25.9 ± 7.8 ***(15-41)***	15.2	13.9 ± 3.1 ***(10-19)***	18.5
δ-HCH	7.6 ± 5.6 ***(2-19)***	4.7 ± 1.0 ***(4-6)***	22.8 ± 7.3 ***(11-35)***	22.6	8.4 ± 2.9 ***(3-12)***	24.5
γ-HCH	5.9 ± 3.7 ***(0.8-15)***	5 ± 1.4 ***(3-7)***	19.8 ± 8.0 ***(9-33)***	15.9	10.8 ± 2.8 ***(7-15)***	20.6
ΣHCH	**33.7 ± 21** ***(10-75)***	**33 ± 2.0** ***(30-35)***	**90.1 ± 24** ***(55-133)***	**66.0**	**48.5 ± 11** ***(32-65)***	**78.5**
Aldrin	3.9 ± 1.9 ***(2-8)***	18.8 ± 2.7 ***(16-22)***	8.7 ± 4.7 ***(1-17)***	12.8	6.2 ± 5.4 ***(2-18)***	13
Dieldrin	8.2 ± 5.4 ***(2-18)***	15.2 ± 4.8 ***(9-23)***	15.6 ± 4.7 ***(5-23)***	18.0	9.9 ± 6.5 ***(3-23)***	20.2
Endrin	13.7 ± 8.7 ***(1-27)***	14.7 ± 3.6 ***(9-20)***	30.3 ± 10.4 ***(12-53)***	17.2	13.1 ± 6.9 ***(2-23)***	13
Endrin Aldehyde	4.3 ± 3.3 ***(1-13)***	5.4 ± 2.2 ***(3-9)***	12.8 ± 3.9 ***(6-19)***	12.6	10.4 ± 3.3 ***(6-15***)	13.2
Endrin ketone	11.1 ± 6.3 ***(3-23***)	10.7 ± 2.1 ***(7-13)***	25.2 ± 6.9 ***(15-37)***	37.0	15.5 ± 5.5 ***(8-22)***	38.7
Σdrins	**41.2 ± 21** ***(13-85)***	**64.7 ± 12.8** ***(45-83)***	**92.7 ± 18** ***(62-139)***	**97.5**	**55.1 ± 21** ***(22-89)***	**98**
p,p'-DDE	12.6 ± 6.9 ***(4-24)***	16.9 ± 1.7 ***(15-19)***	15 ± 7.5 ***(7-33)***	34.4	47.1 ± 36 ***(19-124)***	29.2
p,p'-DDD	14.1 ± 7.8 ***(2-27)***	19.4 ± 0.9 ***(18-21)***	26.3 ± 7.2 ***(16-42)***	32.9	36.4 ± 22.9 ***(16-69)***	33.6
p,p'-DDT	36.2 ± 16.2 ***(14-66)***	8.8 ± 1.5 ***(7-11)***	31.4 ± 13 ***(6-53)***	19.2	36.4 ± 20.3 ***(11-71)***	21.6
ΣDDT	**62.8 ± 30** ***(22-112)***	**45.2 ± 2.9** ***(42-49)***	**72.7 ± 16** ***(43-111)***	**86.5**	**120 ± 71** ***(52-264)***	**84.4**
Heptachlor	14.6 ± 9.3 ***(0.8-31)***	9.8 ± 3.9 ***(3-15)***	34.6 ± 8.8 ***(20-49)***	26.9	13.9 ± 3.4 ***(8-19)***	24.9
Heptachlor Epoxide	12.3 ± 8.5 ***(3-27)***	15.4 ± 2.1 ***(13-19)***	25.8 ± 6.5 ***(14-35)***	22.9	19.3 ± 5.7 ***(9-25)***	22.7
Methoxychlor	29.6 ± 11 ***(14-50)***	6.7 ± 1.7 ***(4-9)***	43.7 ± 13 ***(26-66)***	58.4	37.4 ± 17.8 ***(8-60)***	53.8
Σchlors	**56.5 ± 28** ***(20-103)***	**31.9 ± 5.6** ***(25-39)***	**104.2 ± 19** ***(76-141)***	**108.1**	**70.6 ± 16** ***(41-90)***	**101.4**
α-Endosulfan	12.5 ± 6.4 ***(5-23)***	13.2 ± 1.7 ***(11-15***)	12.4 ± 8.2 ***(3-29)***	18.3	18.7 ± 8.0 ***(3-26)***	24.7
β-Endosulfan	12.3 ± 7.0 ***(4-25)***	5.7 ± 1.2 ***(4-7)***	24.2 ± 5.1 ***(17-34)***	25.3	15.4 ± 7.6 ***(5-24)***	29.6
Endosulfan sulfate	8.6 ± 6.8 ***(0.2-22)***	13.2 ± 2.0 ***(9-15)***	14.9 ± 4.0 ***(7-24)***	24.3	17.1 ± 7.8 ***(5-28)***	20.5
Σendosulfans	**33.4 ± 19** ***(13-66)***	**32 ± 4.0** ***(25-36)***	**51.5 ± 12** ***(36-74)***	**67.9**	**51.1 ± 16** ***(29-70)***	**74.8**

Supplementary Table TS 3.3: Mean ± standard deviation and range (in parenthesis) of organochlorine pesticide concentrations (ng g^{-1} dry weight) in *Oreochromis mossambicus* and *Clarias gariepinus* muscle tissue sampled from sites within iSimangaliso Wetland Park (Lake St Lucia, Mkhuze wetlands, Lake Sibaya and Kosi Bay). Data linked to Chapter 3

	Mean concentration (ng g^{-1} dw)					
	Oreochromis mossambicus			*Clarias gariepinus*		
Analytes	Lake St Lucia (n = 17)	Lake Sibaya (n = 6)	Kosi Bay (n = 19)	Lake St Lucia (n = 1)	Mkhuze (n = 7)	Kosi Bay (n = 1)
α-HCH	55.3 ± 36.8 ***(2-114)***	57.9 ± 5.10 ***(49-63)***	135.8 ± 46.7 ***(60-215)***	68.7	85.8 ± 14.2 ***(66-105)***	83.1
β-HCH	71.1 ± 43.6 ***(12-145)***	87.0 ± 9.60 ***(71-97)***	161.6 ± 48.5 ***(96-256)***	84.5	77.2 ± 17.4 ***(55-106)***	102.7
δ-HCH	47.4 ± 35.2 ***(10-117)***	29.4 ± 6.10 ***(22-39)***	142.6 ± 45.5 ***(68-221)***	125.1	46.5 ± 15.9 ***(17-66)***	135.9
γ-HCH	36.6 ± 23.1 ***(5-92)***	31.6 ± 8.60 ***(20-43)***	123.5 ± 50.1 ***(54-205)***	88.4	59.9 ± 15.7 ***(38-85)***	114.5
ΣHCH	**210.5 ± 129** ***(61-468)***	**205.9 ± 12.8** ***(188-220)***	**563.4 ± 147** ***(344-829)***	**366.6**	**269.4 ± 60.0** ***(180-362)***	**436.0**
Aldrin	24.5 ± 11.6 ***(9-47)***	117.4 ± 17.3 ***(98-140)***	54.6 ± 29.4 ***(8-108)***	70.9	34.5 ± 29.9 ***(13-99)***	72.0
Dieldrin	51.3 ± 33.8 ***(15-111)***	95.0 ± 30.0 ***(53-145)***	97.6 ± 29.4 ***(29-145)***	100.0	55 ± 36.2 ***(15-128)***	112.2
Endrin	85.6 ± 54.5 ***(7-171)***	91.6 ± 22.2 ***(56-125)***	189.5 ± 65.2 ***(77-328)***	95.3	72.8 ± 38.3 ***(11-129)***	72.5
Endrin Aldehyde	26.8 ± 20.5 ***(8-83)***	33.5 ± 13.8 ***(18-54)***	79.9 ± 24.2 ***(37-120)***	69.8	58 ± 18.5 ***(32-80)***	72.9
Endrin ketone	69.4 ± 39.4 ***(18-143)***	67.1 ± 13.1 ***(44-84)***	157.7 ± 43.3 ***(96-232)***	205.6	86 ± 30.4 ***(42-124)***	214.9
Σdrins	**257.6 ± 133** ***(79-530)***	**404.6 ± 80.1** ***(279-516)***	**579.3 ± 114** ***(385-866)***	**541.4**	**306.3 ± 116** ***(123-495)***	**544.4**
p,p'-DDE	78.5 ± 43.0 ***(23-149)***	105.7 ± 10.5 ***(92-115)***	93.8 ± 46.7 ***(41-206)***	191.0	261.7 ± 200 ***(108-688)***	162.1
p,p'-DDD	88.2 ± 48.7 ***(10-167)***	121.4 ± 5.70 ***(114-130)***	164.2 ± 45.2 ***(98-265)***	182.7	202.3 ± 127 ***(91-384)***	186.7
p,p'-DDT	226 ± 101 ***(85-413)***	55.1 ± 9.30 ***(45-71)***	196.1 ± 80.0 ***(39-333)***	106.7	202.5 ± 113 ***(60-393)***	120.0
ΣDDT	**392.7 ± 189** ***(135-698)***	**282.3 ± 18.0** ***(261-308)***	**454.1 ± 103** ***(269-692)***	**480.4**	**666.5 ± 395** ***(287-1466)***	**468.7**
Heptachlor	91.5 ± 58.2 ***(5-193)***	61 ± 24.4 ***(20-92)***	216.6 ± 55.2 ***(126-303)***	149.1	77.4 ± 18.7 ***(46-104)***	138.3
Heptachlor Epoxide	76.8 ± 53.0 ***(18-170)***	96.3 ± 13.3 ***(80-116)***	161.5 ± 40.4 ***(90-220)***	127.0	107 ± 31.5 ***(50-141)***	126.1
Methoxychlor	185 ± 69.5 ***(85-312)***	42.1 ± 10.9 ***(25-53)***	273.2 ± 81.5 ***(164-411)***	324.6	207.8 ± 98.9 ***(45-332)***	298.9
Σchlors	**353.4 ± 172** ***(122-644)***	**199.3 ± 35.1** ***(155-245)***	**651.3 ± 117** ***(473- 884)***	**600.6**	**392.2 ± 87.5** ***(230-499)***	**563.3**
α-Endosulfan	77.8 ± 40.2 ***(34-145)***	82.3 ± 10.6 ***(71-95)***	77.2 ± 51.1 ***(18-179)***	101.8	103.8 ± 44.4 ***(17-143)***	136.9
β-Endosulfan	77.1 ± 43.5 ***(27-153)***	35.4 ± 7.60 ***(25-46)***	151.4 ± 31.6 ***(106-214)***	140.5	85.5 ± 42.5 ***(26-132)***	164.6
Endosulfan sulfate	53.8 ± 42.5 ***(1-135)***	82.1 ± 12.6 ***(59-92)***	93.4 ± 25.0 ***(43-149)***	135.1	94.8 ± 43.4 ***(28-158)***	113.9
Σendosulfans	**208.7 ± 119** ***(81-414)***	**199.8 ± 24.6** ***(154-223)***	**321.9 ± 73.3** ***(224-462)***	**377.3**	**284 ± 86.3** ***(161-387)***	**415.4**

Supplementary Table TS 4.1: Percentage spiked recoveries for Crocodile fat (n = 3) and %RSD at three fortified concentrations (ng g^{-1}). LOD = limit of detection. Data linked to Chapter 4

Analytes	**10 ng g^{-1}**		**50 ng g^{-1}**		**100 ng g^{-1}**		**LOD**
	Mean	%RSD	Mean	%RSD	Meean	%RSD	(ng g^{-1})
α-HCH	80.4	6.9	87.3	5.6	86.1	3.8	0.32
β-HCH	79.0	7.1	93.3	3.3	86.9	5.2	0.36
δ-HCH	84.0	10.0	89.2	4.9	90.9	3.1	0.24
γ-HCH	83.1	7.8	85.7	5.2	90.1	6.4	0.18
Aldrin	81.7	4.9	89.5	3.3	98.3	2.9	0.12
Dieldrin	81.1	3.0	94.0	3.8	94.0	2.4	0.33
Endrin	81.7	6.7	84.7	4.1	89.3	2.1	0.32
Endrin aldehyde	87.2	10.1	91.4	6.5	93.1	7.4	0.29
Endrin ketone	85.4	10.6	92.1	9.6	90.1	5.0	0.18
p,p'-DDE	84.9	7.0	90.4	5.2	108.1	1.7	0.35
p,p'-DDD	80.9	5.9	85.5	5.5	93.6	5.0	0.30
p,p'-DDT	82.1	7.6	85.2	7.4	94.7	2.4	0.35
Heptachlor	82.1	13.1	82.5	3.5	87.9	4.1	0.27
Heptachlor epoxide	79.8	6.9	87.2	5.1	94.6	3.7	0.13
Methoxychlor	82.6	8.4	81.8	7.0	85.5	3.2	0.26
α-Endosulfan	81.3	5.1	91.7	7.8	87.8	2.5	0.20
β-Endosulfan	77.2	8.5	86.2	10.6	83.4	5.7	0.24
Endosulfan sulfate	84.3	10.8	91.2	5.9	86.2	4.4	0.19

Supplementary Table TS 4.2: Organochlorine pesticide concentrations (ng g^{-1} ww) detected in the fat tissue of Nile crocodiles from iSimangaliso Wetland Park, South Africa. Data linked to Chapter 4

	Analyte concentration ng g^{-1} ww														
	Croc 1	Croc 2	Croc 3	Croc 4	Croc 5	Croc 6	Croc 7	Croc 8	Croc 9	Croc 10	Croc 11	Croc 12	Croc 13	Croc 14	Croc 15
α-HCH	275.5	358.9	217.2	300.0	370.8	238.8	278.8	285.6	241.6	206.0	354.5	207.2	271.3	299.2	225.6
β-HCH	210.3	246.4	172.7	291.2	352.4	237.2	283.6	276.0	249.6	240.4	224.7	234.4	159.8	337.2	251.2
δ-HCH	304.3	340.5	214.2	221.9	149.5	174.3	165.7	168.6	228.6	339.0	353.9	98.1	194.9	177.1	271.4
γ-HCH	-	-	235.5	122.0	67.3	288.7	310.0	406.7	345.3	349.3	126.0	204.7	308.3	278.7	204.0
∑HCH	790.1	945.8	839.6	935.1	940.0	939.0	1038.1	1136.9	1065.1	1134.7	1059.1	744.4	934.3	1092.2	952.2
Aldrin	516.8	622.8	339.0	425.2	150.8	338.8	429.6	425.2	364.4	296.0	456.4	279.6	282.8	428.8	316.4
Dieldrin	244.8	508.8	277.4	415.6	158.8	355.6	432.4	433.6	320.8	250.8	400.3	259.6	242.9	415.2	294.0
Endrin	311.4	391.5	189.0	364.4	164.8	406.4	422.8	427.2	380.4	227.6	306.8	286.0	262.7	526.0	409.2
Endrin ketone	160.7	171.5	97.2	94.8	114.4	75.6	122.8	130.4	183.6	144.0	147.4	229.6	84.4	105.6	141.2
Endrin Aldehyde	53.8	162.3	84.3	205.2	74.5	204.5	119.4	222.1	73.6	44.2	172.9	166.1	73.6	268.2	194.2
∑drin	1287.5	1856.9	986.9	1505.2	663.3	1380.9	1527.0	1638.5	1322.8	962.6	1483.8	1220.9	946.4	1743.8	1355.0
p,p'-DDT	209.5	246.9	156.8	366.8	502.0	531.6	426.8	410.0	247.2	342.8	275.7	239.6	319.7	440.8	196.0
p,p'-DDE	635.7	1000.6	213.3	1786.0	938.0	2058.0	794.8	1168.0	455.2	556.0	1099.0	1186.8	1486.8	2004.8	634.8
p,p'-DDD	289.3	299.3	153.5	506.4	599.2	534.8	264.4	502.8	544.4	157.6	329.5	338.0	448.1	670.4	483.6
∑DDT	1134.5	1546.8	523.6	2659.2	2039.2	3124.4	1486.0	2080.8	1246.8	1056.4	1704.2	1764.4	2254.6	3116.0	1314.4
Heptachlor	-	-	452.3	169.0	355.0	855.0	332.0	190.0	411.0	621.0	599.5	288.0	428.1	647.0	388.0
Heptachlor Epoxide	332.8	387.8	208.3	362.0	117.2	284.4	328.8	336.4	286.4	252.4	286.1	225.6	270.6	352.8	270.0
Methoxychlor	155.3	190.2	95.4	112.0	145.5	78.5	233.5	258.9	197.8	164.4	194.2	297.5	133.4	170.9	99.6
∑chlor	488.1	578.0	756.0	643.0	617.7	1217.9	894.3	785.3	895.2	1037.8	1079.8	811.1	832.1	1170.7	757.6
α-endosulfan	348.9	380.1	201.8	362.0	132.4	326.4	366.8	345.2	308.0	161.6	310.2	264.0	179.9	416.8	335.6
β-Endosulfan	267.0	308.5	160.4	442.0	202.4	428.0	228.8	202.8	158.0	130.0	255.9	338.8	173.6	282.4	153.2
Endosulfan sulfate	189.0	222.0	129.7	302.8	115.2	71.2	164.8	178.8	143.6	237.6	216.6	223.6	189.5	188.8	173.6
∑endo	804.9	910.6	491.9	1106.8	450.0	825.6	760.4	726.8	609.6	529.2	782.7	826.4	543.0	888.0	662.4
	Sample biodata														
Location	Lake St Lucia	Lake St Lucia	Lake St Lucia	Lake St Lucia	Lake St Lucia	Lake St Lucia	Lake St Lucia	Lake St Lucia	Lake St Lucia	Nsumo Pan	Lake Sibaya	Lake Sibaya	Kosi Bay	Kosi Bay	Kosi Bay

Capture date	18/06/2016	17/06/2016	18/06/2016	22/03/2017	22/03/2017	23/03/2017	23/03/2017	23/03/2017	24/03/2017	14/03/2017	15/06/2016	19/03/2017	14/06/2016	15/03/2017	17/03/2017
Sex	Male	Female	Male	Male	Male	Male	Male	Female	Male	Female	Male	Male	Male	Female	Male
TL (cm)	360.1	193.2	293	390	217	375	358	262	259	128.4	181	161	320	265	245
SVL (cm)	196.4	125	159	210	114	195	182	135	127	64.5	93.6	84	-	145	135
Neck girth (cm)	-	57	75	113.5	49.5	108	99	70	70	-	39.5	-	-	-	66
Tail girth (cm)	-	51	66.4	92	55.5	95	94.5	65.4	66	-	42.1	-	-	-	63.5
Size classification	Adult	Subadult	Adult	Adult	Subadult	Adult	Adult	Adult	Adult	Subadult	Subadult	Subadult	Adult	Adult	Subadult
GPS location	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	28.295578°	28.344740°	28.386376°	28.278761°	28.262366°	28.384492°	28.384568°	28.257898°	28.384609°	27.660372°	27.357959°	27.398943°	27.033960°	27.026794°	27.034125°
	32.404010°	32.409205°	32.415271°	32.415269°	32.429043°	32.422081°	32.422635°	32.434645°	32.422205°	32.302463°	32.641303°	32.703230°	32.818817°	32.829962°	32.827599°

Supplementary Table TS 5.1: Percentage recoveries for coral samples and RSDs of OCPs spiked at 3 different fortified concentrations (ng g^{-1}). LOD = Limit of detection. Data linked to Chapter 5

	10 ng g^{-1}		**50 ng g^{-1}**		**90 ng g^{-1}**		**LOD**
Analytes	Mean	% RSD	Mean	% RSD	Mean	% RSD	(ng g^{-1})
α-HCH	77.6	5.9	83.5	8.1	80.1	7.3	1.05
β-HCH	80.4	7.3	100.8	3.9	77.9	5.3	1.19
δ-HCH	72.7	17.6	76.6	13.0	93.7	10.5	0.79
γ-HCH	75.6	12.1	85.9	6.4	79.9	6.1	0.59
Aldrin	72.8	14.8	87.6	20.6	106.2	13	0.41
Dieldrin	71.7	8.3	98.0	11.2	92.3	8.5	1.08
Endrin	72.8	12.9	78.9	13.1	80.5	10.1	1.06
Endrin aldehyde	82.6	19.0	77.3	11.8	93.2	4.9	0.97
Endrin ketone	88.0	19.0	90.9	16.7	72.7	8.6	0.60
p,p'-DDE	74.5	11.6	93.4	15.6	104.8	7.8	1.15
p,p'-DDD	79.5	7.0	87.2	13.0	96.6	12.3	0.99
p,p'-DDT	71.0	14.3	79.4	10.1	89.4	6.3	1.17
Heptachlor	65.4	17.5	72.4	6.5	70.4	12.3	0.90
Heptachlor epoxide	74.3	10.9	72.0	12.5	90.3	6.5	0.42
Methoxychlor	77.0	15.6	75.8	20.1	79.7	11.1	0.87
α-Endosulfan	72.2	10.8	91.5	12.0	81.9	9.0	0.66
β-Endosulfan	71.7	16.4	75.8	20.3	80.7	17.0	0.81
Endosulfan sulfate	82.9	13.7	84.0	15.8	74.9	10.6	0.64