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Bioaccumulation of DDT and other organochlorine pesticides in amphibians from two conservation areas within malaria risk regions of South Africa

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HIGHLIGHTS

- Amphibians from conservation areas in South Africa showed OCP accumulation.
- OCP accumulation differed between species.
- Proximity to sources influenced spatial differences in DDT concentration.

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ABSTRACT

The threat to wildlife from chemical exposure exists regardless of the presence of conservation boundaries. An issue exacerbated by the use of environmentally persistent insecticides for vector control and long-range transport of legacy persistent organic pollutants. In this comparative study between two important conservation regions in South Africa, Kruger National Park (KNP) and Ndumo Game Reserve (NGR), we assessed organochlorine pesticide (OCP) accumulation in several anuran species collected from within the conservation regions. The two conservation regions differ in size and subsequent proximity of collection sites to OCP input sources. Detectable concentrations of OCPs were present in ~ half the frogs analysed from KNP and ~all frogs from NGR and total OCP loads were similar between regions, where measured in the same species. The OCP profiles in KNP frogs were representative of legacy pesticides likely introduced via long-range transport, whereas NGR profiles showed influence of current use of DDT consistent with close proximity to sources. This indicates amphibians can accumulate OCPs within conservation regions and that the exposure of non-target organisms inside conservation regions to current use pesticides has a strong association with proximity to sources. These results serve to inform conservation management decision making with regard to the non-target organism effects of chemical interventions such as vector control pesticide use in and around conservation regions.

1. Introduction

Organochlorine pesticides (OCPs) are a group of chlorinated chemicals historically used as insecticides and are classified as persistent organic pollutants (POPs) based on their extended half-lives in the environment. These pesticides were banned or severely restricted by the Stockholm Convention on Persistent Organic Pollutants of 2001 (Ritter et al., 1995; Bouwman et al., 2011). Effectively use of most OCPs in South Africa were banned by the early 2000s with production ceased between 2004 and 2010. Lindane (γ-HCH), which initially replaced the use of technical grade

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HCH after the POPs ban was put in place, was only banned for agricultural use in South Africa in 2009 (Fisher et al., 2011; DEA, 2019). Dichloridiphenyltrichloroethane (DDT) is an exception, where its agricultural use in South Africa was banned in 1983, but DDT use in malaria vector control is still permitted under strict regulation for indoor residual spraying (IRS; Ritter et al., 1995; Bouwman et al., 2011). Despite having been banned in developing countries for decades, high levels of OCPs are still recorded in various African countries in abiotic matrices, aquatic organisms, and various foodstuff as evident in recent reviews by Thompson et al. (2017a),Gwenzi and Chaukura (2018), and Olisah et al. (2020). It is worth noting that illegal continued use of obsolete OCP stocks in South Africa have been speculated by previous studies (Gerber et al. 2016, 2021), but no definitive evidence has been documented. The constant presence of these pesticides has led to renewed research interest in the levels of vector control pesticides, such as DDT, and other legacy organochlorine pesticides. Fish are frequently included in OCP accumulation assessments to represent the biotic aspect of aquatic ecosystems as well as having human health implications through consumption (Gerber et al., 2016; Verhaert et al., 2017; Pheiffer et al., 2018; Buah-Kwofie et al., 2019; Volschenk et al., 2019). Birds are of concern with regard to OCPs due to the eggshell thinning effect of DDT bioaccumulation (Bouwman et al. 2013, 2019). Domestic chickens kept around homesteads where vector control spraying occurs are also studied due to their close proximity to the spray source and consumption related human health risks (Bouwman et al., 2015; Thompson et al., 2017b). Other taxa are not studied as extensively, and could therefore unknowingly be under as great or greater threat from the effects of OCPs and other pesticides. Reptiles and amphibians are both insufficiently studied taxa in this regard. Reptiles, specifically crocodiles, have recently become the focus of several studies from conservation areas in South Africa that indicated high OCP concentrations in these predators (Buah-Kwofie et al., 2018a; Gerber et al., 2021). Amphibians are specifically understudied in Africa with regard to pesticides (Wolmarans et al., 2020), but OCP accumulation has been confirmed in amphibians from IRS regions in South Africa (Viljoen et al., 2016; Wolmarans et al., 2018).

Since realisation of the global biodiversity crisis and the specific declines in amphibian populations was brought to public attention in the early 1990s (*see* Blaustein et al., 1994), the class Amphibia has continued to show devastating declines. The 2018 Living Planet Report indicates amphibian and reptile populations as the second group most threatened by pollution after birds. Pollution is still considered one of the major threats to amphibian and reptile biodiversity, albeit to a lesser extent than habitat degradation, exploitation, and invasive species (WWF, 2018).

The important role of conservation areas in protection of species from these major threats is undeniable, but whilst physical boundaries and conservation efforts can reduce habitat degradation, exploitation, and invasive species, threats such as chemical pollution can still affect these areas regardless of physical boundaries. The concept of long range atmospheric transport of pesticides and persistent pollutants, with eventual collection and accumulation in aquatic ecosystems is well documented (IUPAC, 1999; Ruggirello et al., 2010; Mackay et al., 2014; Pheiffer et al., 2018). Mast et al. (2012) showed the long range transport of various pesticide groups (including legacy OCPs) into the Yosemite National Park in California, USA, mostly through precipitation in the form of rain and snow. Apart from atmospheric transport majority of OCPs have a high affinity to bind to soils and sediments and organic matter due to high octanol-water portioning coefficients. Pesticides bound to sediments are transported through waterways with those sediment particles and have an extended half-life increasing the travel distance and overall persistence of the pesticide, which is unique to each chemical (*see* Beyer et al., 2000). Pollutants such as OCPs can enter conservation regions through both aquatic and atmospheric pathways and chemical pollution is therefore considered a trans-conservation-boundary threat to animal populations, and specifically amphibians.

The malaria risk region in South Africa occupies the north-eastern part of the country. The distribution of malaria falls within the subtropical climate range and subsequently includes biodiversity hotspots within South Africa. For this reason there is a large overlap in conservation regions in the country that play a critical role in biodiversity conservation whilst also falling within the malaria risk region where DDT and other pesticides are in use for IRS puporses (Buah-Kwofie et al., 2018b). Conservation regions in the country are also often surrounded by rural settlements and agricultural land (Pretorius 2009) where historical OCP input could have occurred. In this study we focussed on two important conservation areas, Kruger National Park (KNP) and Ndumo Game Reserve (NGR), both within the IRS region in South Africa. Both KNP and NGR were surveyed and anurans collected for chemical analysis of OCP residues. The primary aim of this study was to assess whether OCP accumulation occurs in frogs from within conservation regions in South Africa. The secondary aim was to compare OCP accumulation patterns in the same species between the two sampling regions in order to assess possible differences in OCP sources and intensity between the two regions. This study further aimed to assess the differences in OCP patterns between species from the same conservation region in order to assess the role of species specific habits or habitats.

2. Materials and methods

2.1. Study regions

The KNP (Fig. 1) is the largest national park in South Africa and is host to 34 frog species (Vlok et al., 2013; Du Preez and Carruthers 2017). The park is situated in the north-eastern part of South Africa. Mozambique borders the eastern side of the park and Zimbabwe borders the northern side. The park spans over two South African provinces (Limpopo and Mpumalanga) covering 19 485 km^2. During the summer months when malaria risk is highest IRS is actively applied in human settlements surrounding the park as well is housing inside the park, but the use of DDT inside the park is forbidden (SANParks 2006). However, due to the large size of the park, diffuse pollution and long range transport is the main form of organochlorine pesticide input expected at the sample collection sites in this region. The Sabie River and Crocodile River are in close relation to the sampling sites in KNP which increases the possibility of long range aquatic transport of pollutants in the region.

The second conservation area surveyed, NGR (Fig. 1), is much smaller at 102 km^2 and falls within the Phongolo River floodplain on the eastern side of South Africa with Mozambique bordering the northern side of the park. The Phongolo River floodplain is host to a high diversity of birds, fish and specifically anurans in South Africa, with 45 frog species found in the area (Du Preez and Carruthers 2017; Wolmarans et al., 2018). Active IRS occurs inside NGR and in settlements bordering the park (personal communication with NGR staff – Nico Wolmarans). The close proximity of spraying to sampling sites, along with the fact that most wetlands in the area are filled through river overflow or runoff, makes the direct contact or exposure from close-proximity sources (i.e. sprayed homesteads) the expected forms of DDT exposure to amphibians in this region. The Phongolo Catchment is highly utilised for agriculture so legacy OCP exposure via long range aquatic transport is expected as well.

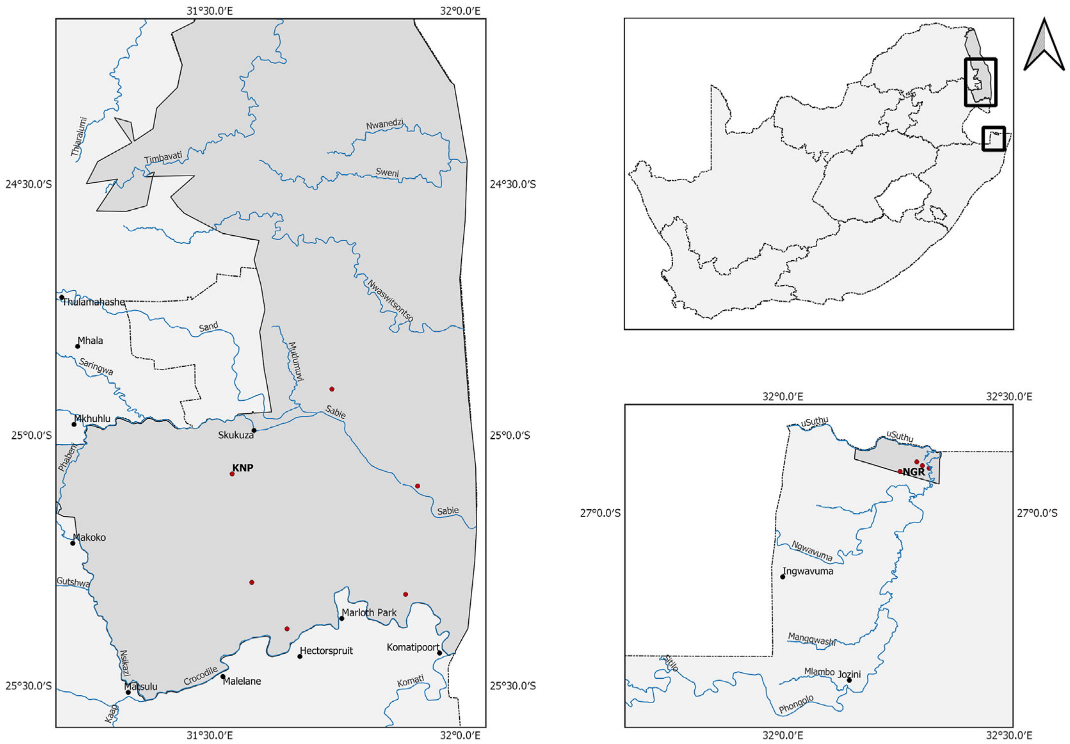

Fig. 1. Survey map showing Kruger National Park (KNP) and Ndumo Game Reserve (NGR) indicating sampling regions (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

There is also long-time practise of livestock dipping (in pesticide) in the land directly bordering the reserve as a form of ectoparasite management, but specific pesticides used could not be confirmed (personal observations and communication with local community members – Nico Wolmarans).

2.2. Selection of species

Amphibian species were selected based on their preferred habitats and behaviour around water that would influence the potential OCP exposure pathways. The classification was based on habitat information from Minter et al. (2004), Du Preez and Carruthers (2017), and personal observations in the field. *Pyxicephalus edulis* (African Bullfrog or Edible Bullfrog), *Hildebrandtia ornata* (Ornate Frog), and *Sclerophrys garmani* (Eastern Olive Toad) are considered semi-terrestrial (ST) as they spend most of their adult lifetime outside water, with only occasional submersion. *Chiromantis xerampelina* (Southern Foam Nest frog) is unique since it is a tree dwelling (TD) frog species that lays its eggs in large foam nests in tree branches overhanging water bodies. These foam nests require a lot of water during production. For this reason, *C. xerampelina* females will soak in water for extended periods during the breeding season in order to hydrate sufficiently. *Ptychadena anchietae* (Common Grass Frog) is considered semi-aquatic (SA) as it stays on river banks and males call from the water edge. If disturbed *P. anchietae* is also more likely to jump towards the water than away from it. *Hyperoluis tuberilinguis* (Tinker Reed Frog) is also considered semi-aquatic as it lives on reeds and has regular contact with water, but males call from outside the water body. Lastly *Xenopus muelleri* (Müller's Clawed Frog) is categorized as fully aquatic (A). Residing in various water bodies and often hiding in the sediment, they have highly permeable skin, which allows for some respiration, extending the time they can spend underwater. Outside of water, they do have the ability to move on wet surfaces and make use of rainy weather to travel. The slightly larger, but also fully aquatic, *X. laevis* has been recorded to travel up to 2.4 km (in six weeks) between water bodies (De Villiers and Measey 2017).

2.3. Sample collection and processing

Frogs were collected using both active and passive sampling methods. Active methods consisted of frogging at night and catching frogs by hand. Passive methods included drift fence pitfall traps for terrestrial species set up on likely migration pathways to and from water bodies. Aquatic species (*Xenopus muelleri*) were collected with bucket traps baited with commercially bought chicken liver. Sampling sites are indicated on Fig. 1. Collection aimed at between 10 and 20 individuals per species sampled evenly between sites based on availability per site, with the exception of *C.xerampelina* in KNP that was collected in higher number for a parallel study. Frogs were therefore treated as one group (per conservation region) representative of the whole region.

Frog samples we collected during November 2011 in KNP (survey 1), November 2012 (survey 2) and April 2013 (survey 3) in NGR. A fourth collection survey took place in May 2016 in NGR for screening of other agricultural pesticides (including other IRS pesticides) in *X. muelleri* only. This region of Southern Africa has a summer rain season spanning from October to March, with October and November considered as onset period and maximal rainfall occurring between December and February (Reason et al., 2005). Surveys conducted in November are therefore considered to be at the start of the rain season, and surveys in April and May at the start of the dry season. The application of IRS occurs in the summer months mostly between November and February. Precise application data for these regions could not be obtained, but based on the combination of the general IRS timeframe and precipitation patterns November surveys are considered to be prior to application and April/May surveys are considered as post application.

Application dates could affect DDT exposure from IRS if direct exposure at sprayed homesteads occur.

Upon collection all frogs were placed in individual plastic containers and for the aquatic and semi-aquatic species water from the sampling site was added. Frogs were then transported to a field station where they were euthanized through double pithing (Amitrano and Tortora, 2012), weighed, and the liver and muscle tissue from the right hind leg were dissected out for separate enzymatic biomarker response analyses not reported in this study (*see* Wolmarans et al., 2018). Chemical euthanasia was not used, to prevent interference with secondary analyses (*see* Wolmarans et al., 2018). The rest of the carcass was wrapped in aluminum foil, labelled, frozen at −20 °C until chemical analysis. The 2016 screening samples did not have livers and muscle samples removed.

2.4. Chemical analysis

Chemical analysis procedures for both KNP and NGR samples (except NGR 2016) were performed following the same method described in Wolmarans et al. (2018) and Yohannes et al. (2017). Briefly, frog carcass (lacking liver and muscle from right hind leg) was homogenized and 5–10 g of sample mixed with anhydrous Na_2SO_4, spiked with a surrogate standard PCB 77 and Soxhlet extracted with 150 mL acetone:hexane (1:3 v/v) mixture. A 20% aliquot of the extract was removed and used to determine lipid content gravimetrically. Excess lipid removal from samples was done through gel permeation chromatography (GPC) with the stationary phase consisting of S-X resin beads (Bio-Rad) and mobile phase of 1:1 hexane:dichloromethane mixture. Final clean-up was done using 6 g 5% deactivated Florisil and eluted with 100 mL hexane:dichloromethane (7:3 v/v) solution. The eluate was then evaporated to near dryness, reconstituted in *n*-decane, and spiked with internal standard 2,4,5,6-tetrachloro-*m*-xylene before instrumental analysis.

Samples were analysed on Shimadzu GC-2014 gas chromatograph coupled with a Ni electron capture detector (GC-ECD). The detector make-up gas flow rate was set at 45 mL min^{-1}. Carrier gas flow rate was set at 1 mL min^{-1} and separation was achieved on an ENV-8MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Splitless injection (1 μl) was used at 250 °C inlet temperature. The oven program was initialised at 100 °C held for 1 min, ramped at 12 °C/min to 180 °C, ramped at 4 °C/min to 240 °C, ramped 10 °C/min to 270 °C and held for 5 min. The detector temperature was set at 320 °C. Five-point calibration curves for a mixture of 22 OCPs (Dr Ehrenstorfer, GmbH) were set up for concentrations ranging between 10 μg/L and 500 μg/L (R^2 ranged between 0.997 and 0.999 for all compounds). The assessed pesticides included the *o,p*- and *p,p*-isomers of DDT, DDD, and DDE (group referred to as DDx); HCB; the α-, β-, δ-, and γ-isomers of hexachlorocyclohexane (HCH; grouping referred to as HCHs); Aldrin, dieldrin, endrin (grouping referred to as Drins); the *cis*- and *trans*-isomers of chlordane and nonachlor, oxychlordane (grouping referred to as CHLs); heptachlor; *cis*- and *trans*-isomers of heptachor epoxide (grouping referred to as HPTs). The PCB# 77 recovery rates were >70% for all samples and concentrations reported were adjusted accordingly. Standard reference material SRM 1947 (Lake Michigan Fish Tissue) analysed using the same method produced recoveries ranging from 75% to 110% with RSD less than 12%. Instrumental limits of quantitation (LOQ) based on 10:1 signal to noise ratio (S/N) were 2.6–4 ng/g for HCHs, 0.53 ng/g for HCB, 0.26–0.4 ng/g for HPTs, 0.3 for CHLs 0.2 to 0.43 for Drins, and 0.13–0.34 ng/g for DDx.

Screening survey samples from NGR (May 2016) were analysed as whole frogs by Primoris analytical laboratories in Belgium using both GC-MS/MS and LC-MS/MS (Primoris Internationally accredited methods: GMSO_01_A, and LMSO_01_A) to screen for the residues of >500 compounds (listed in Supplementary Tables S3 and S4).

2.5. Statistical analysis

All concentration data calculated for this study are reported and discussed in terms of wet mass concentrations (Table 1). Lipid mass concentrations have been provided in Supplementary Table S5 for comparison.

Data followed a non-parametric distribution based on the Shapiro-Wilk normality test. Differences between group (concentration) means were analysed using the Kruskal-Wallis analysis coupled with Dunn's post-hoc test. Significance was set at $p < 0.05$. Isomeric ratios of pesticides were also calculated based on wet mass measurements. Values < LOQ were replaced with 0.5 LOQ for all concentration-based analyses.

For compositional analysis, chemical profiles were transformed into relative composition percentages of total OCPs through the equation $CF_{OCP(x)} = OCP(x)/\text{total OCPs}$ calculated for each sample where $CF_{OCP(x)}$ is the compositional factor of each OCP measured and the sum of CF values for all measured OCP = 1 for each sample. For this specific analysis, OCPs < LOD were set as zero and a datafilter was applied where samples containing values below LOD for all analysed OCPs were excluded from further analysis to prevent skewing OCP composition datasets and only include quantifiable contributing compounds in the compositional analysis. Pearson correlation analysis was performed on CF transformed datasets in order to assess similarities in OCP composition between groups, with significance set at $p < 0.05$ and Pearson's $R > 0.5$. The Kurskal-Wallis analyses and correlation analysis were performed using Graphpad Prism 6.

To supplement correlation analysis, differentiation between OCP compositions based on CF values were assessed in a discriminant function analysis (DFA). Samples were classified into groups pertaining to both survey and species, and OCP accumulation CF values were used as selection variables. Samples where all OCPs analysed were below LOQ were excluded from this analysis. The DFA was performed using IBM SPSS 24.

2.6. Ethical clearance

Frogs from NGR were collected under Ezemvelo-KZN Wildlife permit numbers: OP 5139/2012 & OP 5261/2014. The study was approved by the North-West University AnimCare Ethics Committee (NWU-00095-12-A4). Frogs from KNP were collected under permit number: M-WV08637 and ethics approved by the University of Johannesburg Senate Committee (S02/09).

3. Results

Of the 22 analysed OCPs 12 were detected in samples from both regions. Of the six chemical groupings, no Drins or HCB was detected in any of the samples.

3.1. Spatial differences

No spatial differences were recorded in total OCP concentration between the two species overlapping between conservation areas, *C. xerampelina* and *S. garmani* (Table 1, Fig. 2). Accumulation profiles in all species showed the presence of chlordane in KNP frogs that was below detection limit in NGR frogs and the presence of HCHs in NGR frogs that was not detected in KNP frogs. Exceptions to this include the HCHs not detected from *X. muelleri* (survey four) and

Table 1
The organochlorine pesticide (OCP) concentrations measured in different anuran species, given as mean (and range) of detected measurements (ng/g wet mass) per chemical group. Concentrations were measured in whole frog carcasses from Ndumo Game Reserve (NGR) and Kruger National Park (KNP) across four surveys (SUR1-4). DR% = detection rate expressed as percentage of n. OCP groups: DDTs = total of DDT, DDD, and DDE (all isomers); HCHs = total of all hexachlorocyclohexane isomers; Chls = total Chlordanes; HptChls = total heptachlors. a = in terms of species differences per survey, *C. xerampelina* had significantly higher DDTs and total OCPs ($p < 0.05$) than all other species within SUR3. The categories (Cat) of association with aquatic systems are represented by semi-terrestrial (ST), tree dwelling (TD), semi-aquatic (SA), and aquatic (A).

Site	Survey	Species (*n*)	Cat.	DDTs		HCHs		Chls		HptChls		Total OCPs	
				DR%	Mean (range)	DR%	Mean (range)	DR%	Mean (range)	DR%	Mean (range)	DR%	Mean (range)
KNP	SUR1 (Nov 2011)	*P. edulis* (15)	ST	20	4.45 (0.8–11.6)	0	ND	40	11.09 (1–42.6)	0	ND	40	13.32 (1.2–42.6)
		H. ornata (10)	ST	60	3.98 (1.1–12.1)	0	ND	60	10.13 (1.7–21.3)	0	ND	70	12.1 (2.4–33.5)
		S. garmani (7)	ST	0	ND	0	ND	43	3.94 (1.5–8.6)	0	ND	43	3.94 (1.5–8.6)
		C. xerampelina (29)	TD	31	3.67 (0.9–7.3)	17	3.94 (1–8.7)	3	7.17 (single value)	10	3.35 (0.5–5.9)	38	6.36 (1–12.4)
NGR	SUR2 (Nov 2012)	*C. xerampelina* (4)	TD	100	1.44 (0.5–2.7)	100	0.36 (0.2–0.6)	0	ND	0	ND	100	1.8 (0.9–3.3)
		P. anchietae (6)	SA	33	0.58 (0.4–0.7)	50	0.85 (0.2–2)	0	ND	0	ND	67	1.18 (0.4–3)
		H. tuberilinguis (2)	SA	100	2.16 (2–2.3)	0	ND	0	ND	0	ND	100	2.16 (2–2.3)
		X. muelleri (6)	A	100	3.2 (0.7–6)	67	2.22 (0.5–3.7)	0	ND	0	ND	100	4.68 (0.7–9.58)
	SUR3 (Apr, 2013)	*S. garmani* (11)	ST	91	2.23 (1.3–7.1)	82	0.65 (0.1–1.6)	0	ND	0	ND	100	2.56 (1.3–7.2)
		C. xerampelina (10)	TD	100	^{a} 9.33 (3.2–33.2)	100	0.23 (0.07–0.5)	0	ND	0	ND	100	^{a} 9.5 (3.4–33.3)
		P. anchietae (9)	SA	44	1.39 (0.03–3.3)	100	1.6 (0.4–3.8)	0	ND	0	ND	100	2.25 (0.4–4.4)
		X. muelleri (11)	A	100	1.72 (0.03–9.7)	100	2.15 (0.9–7)	0	ND	36	0.48 (0.1–1.1)	100	4.05 (1–14.1)
	SUR4 (May 2016)	*X. muelleri* (12)	A	83	27.3 (13–51)	0	ND	0	ND	0	ND	83	27.3 (13–51)

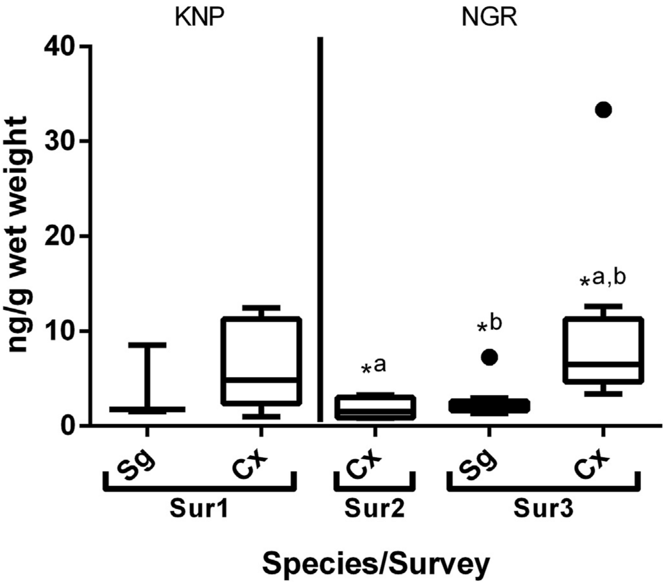

Fig. 2. Box plot (Box = 1st and 3rd quartile with line at the mean, whiskers = 5th and 95th percentile) comparison between total OCPs in Kruger National Park (KNP) and Ndumo Game Reserve (NGR) based on bioaccumulation in two anuran species, *C. xerampelina* (Cx) and *S. garmani* (Sg) collected in November 2011 (KNP), November 2012 (NGR), and April 2013 (NGR). Only species that overlap between sites were included. Shared letters (a, b) represent significant difference ($\alpha < 0.5$) between two groups.

from *H. tuberlinguis* (survey two) in NGR and the presence of HCHs in *C. xerampelina* from KNP during survey one. The detection rate for any of the 22 analysed OCPs for the overlapping species from NGR were 100% for both *C. xerampelina* and *S. garmani*, whereas the same species from KNP showed detection rates <45%. Compositional correlation analysis between overlapping species did not indicate a positive correlation for *C. xerampelina* or *S. garmani* between KNP and NGR, in fact these comparisons showed weak negative correlation (Table 2). In terms of contributing OCPs only *p,p*-DDE and γ-HCH were shared between *C. xerampelina* from the two regions where *S. garmani* had no common OCPs between the two regions (Supplementary Figs. S1 and S2).

3.2. Temporal changes

The DDx (sum of all DDT isomers and metabolites) concentration in *X. muelleri* from NGR showed significant temporal changes between seasons with a statistically significant ($p < 0.0001$) increase toward May 2016 compared to previous April surveys (Fig. 3). Compositional correlations were indicated through moderate to strong positive correlations (All Pearson's $R > 0.55$) between the same species from different surveys within NGR (Table 2).

3.3. Species differences

The frogs from survey one and two showed no statistically significant difference between species (within each survey) for total OCP concentration. In survey three from NGR *C. xerampelina* showed significantly higher total OCP levels than all three other species, *P. acnhietae*, *S. garmani*, and *X. muelleri* (Table 1). Significantly higher DDx concentration in *C. xerampelina* was the main reason for this difference. Heptachlors (consisting of both *cis-* and *trans*-heptachlor epoxide isomers) were detected only in *C. xerampelina* from KNP and *X. muelleri* from survey 3 in NGR (Supplementary Figs. S1 and S2).

Compositional correlation analysis between species within survey one showed no correlation (Pearson's $R < 0.01$) between *C. xerampelina* and all other species from that survey (Table 2). The *C. xerampelina* samples contained α-HCH, *trans*-heptachlor epoxide, and *o,p*-DDE, as major contributing compounds (>10% each) that were below detection limits for the other species (Supplementary Fig. S1). The other three species, *P. edulis*, *S. garmani*, and *H. ornata* (all three in the semi-terrestrial category), had very similar compositions to each other with Pearson's $R \geq 0.83$ between all three species and OCP load consisting only of *trans*-chlordane and *p,p*-DDE. The NGR species' compositional correlations within survey two showed mostly similar OCP compositions between *C. xerampelina* and both *P. anchietae* and *X. muelleri*

Table 2
Correlation table indicating compositional correlations (Pearsons R) for relative contributions of organochlorine pesticides (OCPs) in terms of total OCP accumulation measured in frogs from two conservation areas in South Africa. Survey 1 (SUR1) was conducted in Kruger National Park (KNP). Survey 2, 3, and 4 (SUR2, 3, and 4) were conducted in Ndumo Game Reserve (NGR). SG = *Sclerophrys garmani;* PE = *Pixycephalus edulis*; HO = *Hildebrantia ornata*; CX = *Chiromantis xerampelina*; PA = *Ptychadena anchietae;* HT = *Hyperoluis tuberlinguis*; XM = *Xenopus muelleri*. Grey shading indicates correlations between species within the same survey (indicating compositional similarity between species). Dark blue shading indicates correlations between the same species from NGR over different surveys (indicating temporal compositional similarity). Dark red shading indicates correlations between the same species from different conservation areas (indicating spatial compositional similarity). Significant correlations (Pearson's R values > 0.5 and p < 0.05) are indicated with *.

	SUR1-SG	SUR1-PE	SUR1-HO	SUR1-CX	SUR2-CX	SUR2-PA	SUR2-XM	SUR2-HT	SUR3-XM	SUR3-PA	SUR3-SG	SUR3-CX
SUR1-PE	*0.97											
SUR1-HO	*0.83	*0.95										
SUR1-CX	-0.04	-0.01	0.03									
SUR2-CX	-0.16	-0.001	0.19	-0.11								
SUR2-PA	-0.18	-0.09	0.02	-0.06	*0.54							
SUR2-XM	-0.12	0.14	0.43	0.06	*0.62	0.35						
SUR2-HT	-0.12	-0.07	-0.01	-0.19	0.13	0.45	0.14					
SUR3-XM	-0.15	0.02	0.23	-0.04	*0.77	*0.88	*0.68	0.29				
SUR3-PA	-0.11	-0.1	-0.09	-0.06	0.45	*0.79	0.1	-0.04	*0.72			
SUR3-SG	-0.14	0.1	0.37	0.002	*0.77	*0.69	*0.87	0.42	*0.9	0.36		
SUR3-CX	-0.12	0.09	0.33	-0.02	*0.55	*0.59	*0.74	*0.7	*0.68	0.06	*0.9	
SUR4-XM	-0.08	0.19	0.49	0.12	*0.6	0.33	*0.96	0.18	*0.65	0.01	*0.88	*0.79

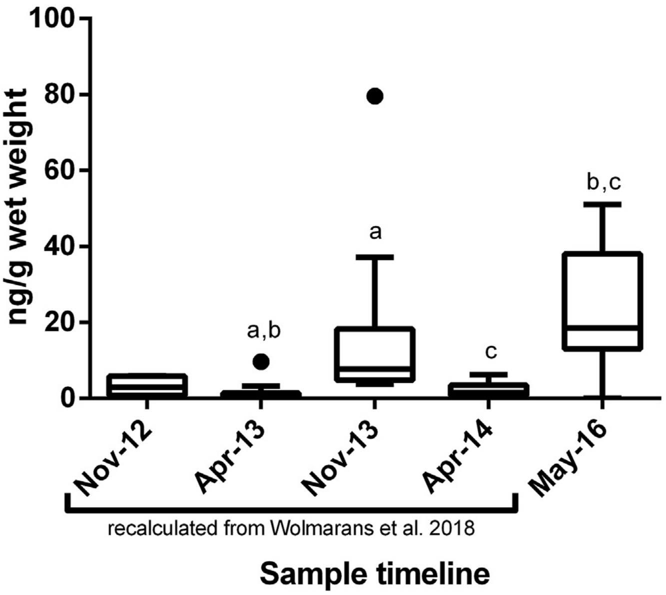

Fig. 3. Box plot (Box = 1st and 3rd quartile with line at the mean, whiskers = 5th and 95th percentile) of the temporal variation in DDT bioaccumulation (ng/g wet mass; detected residues only) in *X. laevis* from NGR. Concentrations for November 2012 to April 2014 were recalculated in terms of wet mass from data in Wolmarans et al. (2018). Shared letters (a–e) represent significant difference (α < 0.05) between two groups. Note: May 2016 samples were analysed as whole animals whereas all other samples were analysed without their livers and muscle from one leg.

(Pearson's R ≥ 0.54; Table 2). These correlations are attributed to the major presence of *p,p*-DDE and γ-HCH in each of these species (Supplementary Fig. S2). *Ptychadena anchietae* did not show significant correlation to *X. muelleri* and *H. tuberlinguis* (Pearson's R > 0.35, p > 0.1). *Hyperolius tuberlinguis* composition showed even lower correlation to that of both *C. xerampelina* and *X. muelleri* (Pearson's R = 0.13 and 0.14 respectively). The survey three species form NGR showed high similarity in OCP composition with *X. muelleri* having a high correlation (Pearson's R > 0.68) with all three other species (*C. xerampelina*, *P. anchietae*, and *S. garmani*; Table 2). Furthermore, *C. xerampelina* showed strong correlation with *S. garmani*, but *P. anchietae* did not show significant correlation to either *C. xerampelina* or *S. garmani*. Correlations between species in this survey were attributed to *p,p*-DDE, *p,p*-DDT, and γ-HCH as major contributing OCPs (Supplementary Fig. S2).

3.4. *Discriminant function analysis*

The DFA results indicated a partial separation of groups (species per survey) based on OCP contributions across the first two functions with *C. xerampelina* from survey one and *H. tuberlinguis* from survey two showing distinct separation from other groups (Supplementary Fig. S3). The first five functions significantly explained variance in the OCP contribution data (α < 0.01 based on Wilks' Lamda). *trans*-Chlordane was the main OCP correlating with the first function. Function two and three did not have strong correlations with singular OCPs. Function four showed strong correlation with *p,p*-DDD and *p,p*-DDE and function five showed strong correlation with p,p-DDT and γ-HCH contributions (Supplementary Table S1). Overall 61.7% of the sample groups (species per survey) were reclassified correctly based on function one and two with 66.8% of variance explained across these two functions. The first five functions cumulatively explained 96% of the variance in the data. *Sclerophrys garmani* from survey one, *H. tuberlinguis* from survey two, *C. xerampelina* from survey three, and *X. muelleri* from survey four were all correctly reclassified for 100% of samples (Supplementary Table S2). This analysis indicated no distinct grouping or separation based on habit/habitat based species classes. Spatial difference was the main factor separated along the first function with the exception of *C. xerampelina* from KNP grouping closer to NGR frogs. Minor species separation is shown along the second function.

3.5. *Isomeric ratios*

Isomeric ratios of importance were calculated that have been shown to serve as indicators of exposure age, type, and distance from source in some scenarios (Table 3). The ratios between *p,p-*/*o,p*-DDTs (DDT + DDD + DDE) were >2 for all species from both locations except for *C. xerampelina* from KNP which had a ratio of 0.8 slightly favouring the *o,p*-isomers of DDT and its metabolites. The parent/daughter (DDT/DDx) compound ratio between DDT itself and metabolites (DDD + DDE) indicated ratios > 1 for *H. tuberlinguis* in survey 2 and *P. anchietae* in survey 3 both from NGR. All other NGR frogs except *X. muelleri* from survey 4 contained parent *p,p*- and *o,p*-DDT, whereas none of the KNP frogs contained any parent DDT. The α-HCH/γ-HCH ratios were only applicable in *C. xerampelina* from KNP, as it was the only species from that region with HCH accumulation, but this species had a ratio of 3.3 where all NGR frogs had α-HCH/γ-HCH ratios <1. The *trans-*/*cis-* CHLs ratios (chlordanes + heptachlors) were >1 in all species containing these

Table 3
Isomeric ratios of importance for OCP accumulation in frogs from Kruger National Park (KNP) and Ndumo Game Reserve (NGR) over four surveys based on mean accumulation per species per survey. SUR1 = Survey 1 (KNP), SUR2 = Survey 2 (NGR), SUR3 = Survey 3 (NGR), SUR4 = Survey 4 (NGR). SG = *Sclerophys garmani*, PE = *Pixycephalus edulis*, HO = *Hildebrantia ornata*, CX = *Chiromantis xerampelina*, PA = *Ptychadena anchietae*, HT = *Hyperolius tuberlinguis*, XM = *Xenopus muelleri*. N/A = not applicable. a = CHLs in this instance includes both chlordanes and heptachlors.

Species	*p,p*-/*o,p*-DDT	*p,p*-/*o,p*-DDD	*p,p*-/*o,p*-DDE	*p,p*-DDT/(DDD + DDE)	*o,p*-DDT/(DDD + DDE)	α-HCH/γ-HCH	*trans*-/*cis*-CHLs[a]
SUR1-SG	N/A	N/A	N/A	N/A	N/A	N/A	79.4
SUR1-PE	N/A	N/A	89.4	0.011	N/A	N/A	222
SUR1-HO	N/A	N/A	137	0.007	N/A	N/A	174
SUR1-CX	N/A	N/A	0.8	0.018	0.015	3.33	16.7
SUR2-CX	0.5	3.3	32.1	0.22	7.94	0.07	N/A
SUR2-PA	5.18	N/A	8.02	0.57	N/A	0.49	5.82
SUR2-HT	50.7	20.4	15.1	1.42	N/A	N/A	N/A
SUR2-XM	0.13	9.44	112	0.008	3.75	0.47	N/A
SUR3-SG	4.11	2.39	54.1	0.42	2.86	0.05	N/A
SUR3-CX	19.15	8.98	203	0.72	4	0.11	N/A
SUR3-PA	4.73	N/A	13.24	0.77	1.16	0.19	N/A
SUR3-XM	2.52	8	177	0.24	8.88	0.01	4.28
SUR4-XM	N/A	N/A	910	0.001	N/A	N/A	N/A

OCPs, but where markedly higher in KNP (KNP $>$ 16 vs. NGR $<$6) frogs due to the dominant presence of *trans*-chlordane.

3.6. Secondary pesticide screening

Out of the 538 analysed pesticides (Supplementary Tables S3 and S4), only DDTs (as *p,p*-DDE) was detected in samples from the May 2016 NGR survey.

4. Discussion

In an effort to convey accurate results within the correct context there are certain limitations to this study that need to be considered when interpreting the data. The first is that only a single survey was conducted in KNP and thus no inferences can be made regarding temporal changes in this region, whereas the multiple surveys from NGR provide a more complete picture of the variation in environmental exposure from that region. The second limitation is that frogs were not sexed during this study. Sexual differences in organohalogen accumulation can occur in amphibians (Kadokami et al., 2002; Viljoen et al., 2016). This is partly due to maternal transfer of lipophilic compounds altering the bioaccumulation in females (Kadokami et al., 2004).

Gaining an understanding of the threat amphibians face with regard to pesticide exposure in conservation regions, and how this exposure relates to location and species, can produce more reliable risk data for future conservation efforts to build upon. In assessing how these concentrations differ between regions, between species, and between amphibians and other taxa the partitioning of these pesticides in the food web and movement through the environment can be better understood.

Organochlorine pesticide accumulation in KNP from the current study are comparable to that of NGR in terms of total OCP concentrations. DDx has previously been measured in *Xenopus laevis* and *X. mueleri* from Limpopo outside the KNP conservation area by Viljoen et al. (2016), but this study analysed frog lipid bodies resulting in values more directly comparable to lipid concentrations rather than whole body wet mass measurements. Lipid content could, however be influenced by physiological species differences. For instance, species making use of brumation and estivation rely on lipid storage for energy during these dormant periods and increase lipid storage during active periods (Fitzpatrick 1976), thus lipid based concentrations may not reflect the most accurate total bioaccumulation of pesticides for comparison between species. It is worth noting that lipid mass conversions of the current study (Supplementary Table S5) resulted in all species containing DDx from KNP and NGR having similar (within the same order of magnitude) DDx concentrations to the levels reported by Viljoen et al. (2016). The frogs from Viljoen et al. (2016) included *p,p*-DDE and *p,p*-DDD, but no *p,p*- or *o,p*-DDT itself, even from sprayed areas. This was also the case for frogs from KNP in this study that only included detectable *p,p*-DDE (and *o,p*-DDE only in *C. xerampelina*), whereas frogs from NGR included *p,p*- and *o,p*-DDT, *p,p*-DDD, and *p,p*-DDE.

Compared to results of the studies by Lambert (2001) on pesticide loads in amphibians from Sub-Saharan Africa measured in sprayed (agricultural) areas during the period when OCPs were still in active use, the current levels are 1000 times lower than that recorded in *P. anchietae* and *Sclerophrys guttaralis* individuals (maximum levels of 1.5 and 3.9 µg/g ww respectively), indicating a significant reduction in amphibian exposure to DDT since its ban for agricultural and unregulated vector control use. Amphibians form a linkage between the aquatic and terrestrial food webs, but are not considered apex predators in either (Kupfer et al., 2006). This unique position also means they can be exposed to pollutants via both aquatic and terrestrial pathways (Todd et al., 2011). Concentrations in frogs from the current study compared to concentrations in the aquatic apex predator, African Tigerfish (*Hydrocynus vittatus*), from NGR (Volschenk et al., 2019) and KNP (Gerber et al., 2016) indicate this complexity in the trophic transfer of OCPs. The mean concentration of DDx in frogs from the current study were in the same order of magnitude as levels measured in *H. vittatus* (mean values: 2–12 ng/g wet mass) for NGR, but one order of magnitude lower than levels in *H. vittatus* (mean values: 12–35 ng/g wet mass) for KNP. Total chlordane was one order of magnitude higher in frogs than in *H. vittatus* (mean values: 0.7–1.5 ng/g wet mass) from KNP. Total chlordane was below detection limit in NGR frogs, but was present at low levels in *H. vittatus* (mean values: 0.01–0.05 ng/g wet mass). Total HCHs were similar between frogs and *H. vitattus* from both KNP (*H. vitattus* mean values: 1.5–2.3 ng/g wet mass) and NGR (*H. vitattus* mean values: 0.01–0.2 ng/g wet mass).

These comparisons place frogs at similar accumulation levels as aquatic top predators from the same regions even though they hold much lower trophic positions. The reason for the poor differentiation in accumulation at different trophic position can be attributed in part to different habitats employed by these animals as Tigerfish occur in the mid to top water column, are active swimmers and do not interact with sediments to the extent amphibians do. A secondary, but likely smaller aspect, is the terrestrial exposure pathways that frogs have and *H. vittatus* does not. Although *H. vittatus* has been shown to consume terrestrial birds in some cases (O'Brien et al., 2014), they are still mainly piscivores (Dalu et al., 2012). The increased chlordane levels in amphibians from KNP (compared to *H. vittatus*) could indicate that chlordane is mostly transferred through the terrestrial habitat (i.e. through terrestrial atmospheric deposits, spray drift, or diet) in this region and not through the aquatic ecosystem. A counter argument to this is the fact that aquatic ecosystems are seen as sinks for OCPs (Arias et al., 2011) and therefore some form or metabolite of chlordane would be expected in the aquatic ecosystem. In this case biotransformation differences between taxa would most likely be responsible for the chlordane concentration differences as frogs and fish do not express the same biotransformation enzymes (*see* Nelson 2009 for CYP450 genomes of different taxa). The accumulation of OCPs in crocodiles from KNP (Gerber et al., 2021) indicated only the presence of nonachlor as a metabolite of chlordane. Crocodiles have higher sediment interaction than *H. vittatus*. This supports the possibility that sediment contact differences would lead to differences in chlordane accumulation patterns between these taxa when coupled with the notion that biotransformation differences between the taxa can cause the differentiation in chlordane accumulation patterns. The Gerber et al. (2021) data do not however exclude the possibility of terrestrial exposure as crocodiles are apex predators in both terrestrial and aquatic ecosystems. The terrestrial exposure pathway possibility for chlordane specifically is somewhat supported by the lower DDx concentrations in frogs compared to *H. vittatus* from KNP. These concentrations follow the expected result for an aquatic exposure route based on trophic differences between the taxa, where DDx in *H. vittatus* is expected (and was measured) at higher concentrations than the partially exposed frogs in comparison (as no fully aquatic frogs were collected at KNP).

An additional factor in comparisons between these different taxa is the fact that for both Tigerfish and crocodiles dietary uptake is the main exposure route to these pollutants based on their status in the food web and behavioural patterns. While exposure pathways in amphibians include both dermal and dietary routes, dermal uptake has been identified as the most important pesticide exposure route for amphibians (Smith et al., 2007; Brühl et al., 2011). This is due to the high permeability of their skin. Terrestrial exposure pathways are likely to include dietary exposure and dermal contact with contaminated soil (or other surfaces such as house walls). Aquatic exposure includes dietary pathways and dermal contact with contaminated water, but in terms of more hydrophobic pollutants such as *p,p*-DDT dermal contact with contaminated sediment is very likely the main exposure pathway.

4.1. Spatial differences

The unique profile of *C. xerampelina* samples from KNP was partly due to being the only dataset from both sites with α-HCH/γ-HCH ratio >1. Technical grade HCH contains majority α-HCH (60–70%) and α-HCH/γ-HCH ratio somewhere between 4 and 7 (Itawa et al., 1993). Under anaerobic conditions HCHs in sediments were shown to follow degradation order of γ-HCH > α-HCH > δ-HCH > β-HCH (Buser and Müller 1995). Long range air transport studies of HCHs in oceanic air suggest that the α-HCH/γ-HCH ratio increases with distance from exposure source with mid oceanic air ratios usually >7. This increase is attributed to photodegradation of γ-HCH to α-HCH (Itawa et al., 1993). Contrary to this, a low ratio (close to zero) can be indicative of “pure” lindane as the source (Itawa et al., 1993). This would suggest that uptake of legacy technical grade HCH from sediments would still contain a majority of α-HCH and thus α-HCH/γ-HCH ratio >1. Diffuse exposure entailing long range aerial transport would further increase the ratio. The *C. xerampelina* from KNP HCH profile shows a slightly lower ratio than expected from technical grade HCH, which could indicate the presence of both technical grade HCH and lindane as combined sources. This is distinct from the NGR samples where all species had ratios <0.5 indicative of legacy lindane exposure (Itawa et al., 1993). Large scale lindane use historically included cattle dip for tick control, being sprayed on trees in forestry for wood borer control (Koerber 1976; Hauzenberger 2004), and large area spraying for tstetse fly control (Grant, 2001). If lindane was in frequent use for tick or tsetse fly control in the NGR region, and remained in use almost a decade after most other OCPs, this could explain the relatively high (compared to DDT that is still in use) levels of γ-HCH measured in this region. The upper catchment of the Phongolo River is also highly utilised for subsistence agriculture and forestry (De Necker et al., 2020), which could also be a source of legacy lindane to the region. The dominant presence of *trans*-chlordane in KNP frogs compared with trace presence of only *trans*-heptachlor epoxide in frogs from NGR is a further indication of different exposure profiles between the regions. While heptachlor itself was also used as insecticide, heptachlor epoxides can be formed through the breakdown of both heptachlor and chlordane parent compounds (Buser and Müller 1993). This suggests that NGR either has a much older chlordane use legacy, or that mainly heptachlor was used in this region. Chlordane use on cotton fields in the Phongolo River region could be a possible historical source. Studies on different fish species have indicated differences in isomeric preferential accumulation of chlordane. Channel catfish (*Ictalurus punctatus*) were shown to accumulate *cis*-chlordane more than *trans*-chlordane and to not accumulate oxychlordane, which usually accumulates as the ultimate metabolite of chlordane in mammals (Murphy and Gooch 1995). On the other hand, Carp (*Cyprinus carpio*) was shown to preferentially accumulate the *trans*-isomer over *cis*-chlordane (Seemamahannop et al., 2005). A study on the degradation of heptachlor through both photo-degradation and mixed function oxidase (MFO) system reactions yielded *cis*-heptachlor epoxide to a larger extent than *trans*-heptachlor epoxide from both degradation methods and the photo-degradation of *trans*-chlordane yielded very low degradation products in comparison to *cis*-chlordane (Buser and Müller 1993). The isomeric ratios of chlordane accumulation in frogs have not yet been assessed in controlled exposure experiments to the best of our knowledge. Data from the current study seem to indicate a non-species specific preference for the accumulation of *trans*-isomers, while also not showing accumulation of oxychlordane as the ultimate chlordane metabolite. However, evidence of oxychlordane accumulation as the major chlordane metabolite has been shown in field samples of two Japanese frog species, *Rana ornativentris* and *Rana japonica* (Kadokami et al., 2004), indicating that species specific differences may exist in this regard.

Both chlordane, lindane, and DDT were produced in the Gauteng Highveld area, approximately 400 km west of KNP with DDT production ending in the 1980s (formulation continued until 2010), and chlordane and lindane production ending around 2001 (Fisher et al., 2011). Industrial air pollution from the Highveld region of South Africa (including Gauteng) has been shown to cause acid rain in KNP (Mphepya et al., 2006), and prevailing winds in this region of South Africa enter the KNP region from Mozambique, and circulate over industrial and agricultural regions of the Highveld back toward KNP (Kruger et al., 2010). Furthermore the rivers flowing through KNP all have catchments closer to Gauteng surrounded by agricultural land where legacy OCP use and production could have attributed to aquatic transport into KNP. The accumulation profiles along with wind patterns and river catchment layout suggest that the chemical profiles of frogs from KNP could largely be due to long range transport (both aerial and aquatic) of legacy OCPs stemming from historical production and use in both the Highveld region of South Africa and southern Mozambique. This hypothesis is supported by the lack of evidence for direct exposure from current IRS around KNP. Only DDE (mostly *p,p*-DDE) was detected in samples from KNP whereas the majority of NGR samples contained both parent *p,p*- and *o,p*-DDT. Results from Bouwman et al. (2019) indicate that parent DDT is unlikely to end up in aquatic systems due to IRS. If IRS is in fact an unlikely source, illegal continued agricultural use of DDT seems a viable scenario that would result in the presence of parent *p,p*-and *o,p*-DDT in aquatic organisms. This is especially likely in cases where *p,p*-DDT concentrations exceed that of *p,p*-DDE, which is considered the more persistent ultimate metabolite. In the current study *p,p*-DDT concentrations only exceeded the sum of *p,p*-metabolite (DDD and DDE) concentrations for *H. tuberlinguis* from NGR during survey 2. As this is not an across-the-board phenomenon for NGR samples the results in no way conclusively indicate illegal continued DDT use in the region. These isomeric ratios do however place DDT input at NGR on a more recent timeline than that of KNP as the presence of only *p,p*-DDE suggests much older input (Ruggirello et al., 2010). Interestingly *o,p*-DDE was only detected in *C. xerampelina* from KNP where it had the largest relative contribution to total OCP concentration (42.5%) in that species. Majority presence of *o,p*-isomers (i.e. higher concentrations than *p,p*-isomers) can in some cases be an indicator of dicofol exposure, which often contains *o,p*-DDT impurities in its commercial formulations, rather than technical grade DDT which contains majority *p,p*-DDT and only around 25% *o,p*-DDT (Qiu et al., 2005; Quinn et al., 2011). Atmospheric long range transport of technical grade DDT would however be expected to lower the *p,p*-/*o,p*-ratio as o,p-DDT is more mobile in air (Van Dyk et al., 2010). Quinn et al. (2011) confirmed that dicofol is a registered insecticide in South Africa mainly for fruit cultivation and garden use, and measured the trace presence ($\leq$0.06 ng/g) of dicofol in both soil and sediment from the Vaal-Orange River system in South Africa. The region surrounding KNP to the south is used for citrus farming (Gerber et al., 2021) and the tree dwelling *C. xerampelina* may possibly have come in contact with dicofol from these farms, but the extent of migration for this species has not yet been documented and dicofol itself was not analysed in this study, thus this remains conjecture and cannot be confirmed. Long range atmospheric transport of technical grade DDT is the more likely exposure scenario for KNP in this regard.

4.2. Species differences

Species categories chosen related in large part to their association with water. Differentiation in OCP concentration at category level that is not seen at species level would therefore substantiate whether OCP exposure routes can be attributed to being mainly through the terrestrial ecosystem or mainly through the aquatic ecosystem.

The unique OCP signature of *C. xerampelina* from KNP along with the significantly higher total OCPs in *C. xerampelina* from the third survey (compared to other species in that survey) indicate that *C. xerampelina* have some tendency to differ in exposure from other frog species. However, these differences are inconsistent between locations. It is possible that the soaking behaviour of *C. xerampelina* females during the mating season (Minter et al., 2004) can explain higher contact with sediment and water and higher exposure in this regard as seen in NGR, but sex data was not recorded and thus this cannot be confirmed. This species also moves toward and away from water sources between summer and winter months, spending winter months hidden beneath bark, in evergreen trees, rock cracks, as well as the rafters of buildings (Minter et al., 2004). This behaviour could bring individuals into closer contact with exposure sources, but does not necessarily explain the vastly different accumulation profile observed in KNP, as closer contact with current use DDT sources would have resulted in parent *p,p*-DDT accumulation. Aquatic systems (water and sediment) tend to contain higher *p,p*-/*o,p* isomer ratios than soil and air (Ricking and Schwarzbauer 2012). If the tree dwelling habits of *C. xerampelina* makes exposure through air contact more likely than in ground dwelling or aquatic species, it could also contribute to the unique *p,p*-/*o,p*-signature observed in that regard. Another species specific observation is that the concentration of DDx in *P. anchietae* was detected at lower rates than other species from NGR. The low detection rate is somewhat contradicted by the fact that *P. anchietae* individuals that did contain DDTs also had high parent/daughter ratios indicating recent exposure to DDT (Ruggirello et al., 2010). The natural behaviour and habitat of this species (Minter et al., 2004) does not provide any sufficient explanation to such high variation in exposure between adult individuals. *Xenopus muelleri*, as aquatic species, had the lowest *p,p*-DDT parent/daughter ratio of all NGR species. Binding to aquatic sediments can lengthen the environmental lifetime of DDTs (Chattopadhyay and Chattopadhyay, 2015). It is possible that this could result in aquatic (sediment dwelling) species being exposed to lower parent/daughter ratios for *p,p*-DDT (than expected from current DDT use) in areas where historic DDT use has occurred. This is mainly because *p,p*-DDE is resistant to biotransformation leading to build-up of DDE in aquatic systems (USEPA, 1979; Chattopadhyay and Chattopadhyay, 2015). The parent daughter ratio in *X. muelleri* from NGR are similar to that in *Synodontis zambezensis* ($\approx$0.42) from NGR analysed by Volschenk et al. (2019). This fish species has similar sediment dwelling and feeding habits to that of *X. muelleri*. The DDT parent/daughter ratios in the aquatic *X. muelleri* and unique aspects to the OCP profile of the tree dwelling *C. xerampelina* were the only species differences strongly related to the habitat and habit based categories of frogs, although the major presence of chlordane in KNP semi-terrestrial frogs could also be attributed to the habitat category, albeit a location specific occurrence, based on the terrestrial exposure theory presented for this region and contrasting accumulation profile in the tree dwelling *C. xerampelina*. The unique separation of *H. tuberlinguis* from other species based on OCP composition in the DFA indicates this species also has unique exposure aspects not encompassed by the current habit and habitat based categories. If extensive species specific behavioural observations are made in future studies a more refined classification system may be obtained, which should have better predictive potential for OCP exposure in amphibians.

4.3. Amphibian conservation implications

As large scale removal of legacy OCPs from the environment is not viable, there is no immediate action that can be taken to reduce exposure to these pollutants. However, it is important that these levels be monitored continuously. The results from this study indicate that amphibians are susceptible to OCP exposure even inside conservation regions. The presence of parent *p,p*-and *o,p*-DDT in frogs from NGR indicates that proximity to input sources could be an important factor in the accumulation of DDT in amphibians as NGR applies IRS inside the reserve, however lack of data on possible illegal use (or other inputs) prevents attributing the results to IRS directly. The results from Viljoen et al. (2016) lacking *p,p*-DDT in spraying areas also support that the *p,p*- and *o,p*-DDT in frogs from NGR may be from sources other than IRS. Amphibian health was not visibly affected in either of the study regions suggesting that the accumulation concentrations do not pose a serious threat to amphibian populations, but sub-lethal effects of these pesticides in amphibians have not been investigated to the extent where field measured residue levels can be related to specific effects or lack thereof (*see* Wolmarans et al., 2020). The screening data from survey 4 indicated amphibians in the NGR region are not under threat from current use pesticides to the extent that detectable tissue accumulation occurs. The presence of parent DDT does however place amphibians from NGR at a higher inherent risk than those from KNP with regard to legacy pesticides. Conservation managers should take into account the trans-boundary contamination of pesticides and especially persistent organic pollutants and their potential effects on wildlife in conservation areas. Monitoring programs for these pollutants in and around conservation regions should ideally be implemented to track seasonal fluctuations and long term patterns in both abiotic and biotic concentrations. Only with sufficient monitoring data will the extent of the hazard held by trans-boundary pollutants in conservation regions be known. There is also an important need for investigation into the sub-lethal effects of these pesticides on amphibians. The unique positioning of amphibians in the food web creates the opportunity to use them as indicators for chemical contamination in the wider ecosystem, but the necessary toxicological data is not yet available.

5. Conclusion

The data presented in this study indicate that frogs in conservation regions of South Africa accumulate detectable levels of OCPs. Spatial differences were marked by higher detection of OCPs in NGR than KNP. Compositional differences were also significant between regions with OCP accumulation in anurans from KNP representing that of legacy DDT and chlordane exposure, while OCPs in anurans from NGR indicated recent exposure to DDT and legacy lindane exposure. This study indicates that the proximity to exposure sources could have an impact on the accumulation of current use DDT in frogs from inside conservation regions, but IRS could not be identified as a definitive source for this accumulation. Other, possibly illegal or accidental, sources need to be thoroughly investigated in the NGR region. In terms of species differences it seems likely that the OCP exposure in different frog species from conservation areas in South Africa is driven by both broader spatial exposure factors as well as species specific differences. These differences are not predictable between species, even with the inclusion of habitat and habit based groupings. Such predictions would require further experimental study and long term exposure monitoring data.

Author credit statement

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.129956.

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