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Halogenated pollutants in terrestrial and aquatic bird eggs: Converging patterns of pollutant profiles, and impacts and risks from high levels

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ABSTRACT

We investigated the presence, levels, relationships, and risks of HCHs, DDTs, chlordanes, mirex, PCBs, and brominated flame retardants (BFRs) in terrestrial and aquatic bird eggs from an area in South Africa where DDT is used for malaria control. We found one of the highest ΣDDT levels reported this century; 13 000 ng/g wm (wet mass) in Grey Heron eggs which exceeds critical levels for reproductive success (3000 ng/g wm) calculated for Brown Pelicans, with a no-effect level estimated at 500 ng/g wm. Even higher ΣDDT levels at 16 000 ng/g wm were found in House Sparrow eggs (possibly the highest ever recorded for sparrows), with a maximum of 24 400 ng/g wm. Significant eggshell thinning in Cattle Egrets (33% between thickest and thinnest) was associated with increased levels of *p,p′*-DDT and *p,p′*-DDE. There were indications of unknown use of DDT and lindane. Relative to DDT, PCBs and BFRs levels were quite low. Ordinated data showed that different terrestrial pollutant profiles converged to a homogenised aquatic profile. Converging profiles, high levels of DDT in heron and sparrow eggs, and thinning eggs shells, indicate risk and impacts at release, in the aquatic environment, and in between. If characteristic life-strategies of birds in warm areas (e.g. longer-lived and fewer eggs per clutch) increases the risk compared with similar birds living in colder regions when both experience the same environmental pollutant levels, then malaria control using DDT probably has more significant impacts on biota than previously realised. Therefore, risk assessment and modelling without hard data may miss crucial impacts and risks, as the chemical use patterns and ecologies in Africa and elsewhere may differ from the conditions and assumptions of existing risk assessment and modelling parameters. Consideration of other findings associated with DDT from the same area (intersex in fish and urogental birth defects in baby boys), together with the findings of this study (high levels of DDT in bird eggs, eggshell thinning in the Cattle Egrets, and the apparent absence of breeding piscivore birds in the sprayed area) are strongly suggestive of negative impacts from DDT spraying for Malaria control. Our data presents strong arguments for an expedited process of replacing DDT with sustainable methods.

1. Introduction

Many reports about pollutants in birds concentrate on a single species (e.g. Elliott et al., 2012; Gentes et al., 2012; Gomez-Ramirez et al., 2012), compare related species (e.g. Hoffman et al., 1998; Morales et al., 2012), or consider the relationships of a specific compound between different species (e.g. Ramirez et al., 2011; Rudel et al., 2011). Only a few studies such as Gao et al. (2009) considered and compared different pollutants in disparate terrestrial and aquatic bird eggs from the same region. We collected and analysed aquatic and terrestrial bird eggs from a region in the Limpopo Province of South Africa with a mosaic of uses and releases of chlorinated and brominated compounds. Part of this region is controlled for malaria by indoor residual spraying (IRS) using DDT and other insecticides (Bouwman et al., 2012b). Additionally, chlorinated pesticides could occur in the region due to legacy as well as current use and through industrial chemicals occurring in imported products. The patterns and risks of DDT in breast milk from the region is reasonably well understood (Bornman et al., 2010; Bouwman et al., 2012b), there is good understanding of DDT in and around treated homesteads (Bornman et al., 2012; Van Dyk et al., 2010), and there is some understanding of the impacts of DDT in the aquatic environment (Barnhoorn et al., 2009, 2010).

The aim of this study was to investigate the presence, levels, relationships, impacts, and risks of HCHs, DDTs, chlordanes, mirex, PCBs, and brominated flame retardants (BFRs) in bird eggs in a complex rural source and release scenario, where it is assumed

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that the primary contaminant would be DDT from malaria control. It is predicted that, at least for DDT, the levels in eggs will increase from west to east, as this is the direction of increased DDT use, and thereby also the risk. With such knowledge, recommendations about protective interventions can be motivated.

2. Methods

2.1. Sampling sites and species

Fig. 1 shows the sample region for this study, located in the Limpopo Province of South Africa. Thohoyandou (the capital of the Province) is the largest city in this area with about 500 000 inhabitants. Towards the north is Zimbabwe, and towards the east is the northern part of the Kruger National Park (KNP). Many smaller villages and settlements are located here, interspersed with subsistence and small-scale agriculture. All rivers drain east to east towards the Indian Ocean. No large industries occur in the area, with the exception of commercial agriculture (fruit and forestry) that could contribute to legacy use of organochlorine pesticides. Electricity is readily available via substations in many of the villages, but transformers are mainly of recent origin as electrification of rural areas only became a priority after 1994. However, many households still cook over open fires. Small-scale motor vehicle repair and artisanal brick making does occur. Malaria is controlled with DDT and other insecticides using IRS from Thohoyandou eastwards, up to the KNP border, protecting about 1.84 million people. Very little if any malaria control has ever been done towards the west because of little or no malaria. In the 2009/10 malaria transmission season (when the eggs were collected), about 52 000 kg of 75% water wettable DDT powder, and 11 600 kg of α-cypermethrin was applied as IRS in South Africa. IRS insecticides are applied indoors on walls and outdoors under rafters, at 2 g/m^2. More on malaria control in this region can be gleaned from Bornman et al. (2010) and Bouwman et al. (2012b).

2.2. Egg collection and preparation

The project was approved by the ethics committee of the North-West University (NWU) (NWU-00055-07-S3). Permits for collection of bird eggs were obtained from the Department of Economic Development, Environment and Tourism of the Limpopo Provincial Government.

A local bird guide and interpreters were employed to assist in locating known and potential breeding colonies, and to communicate with the local communities. Sampling of heron and egret eggs was done by scaling stable trees using rock climbing techniques with a double-belay system. A team of two experienced rock climbers (including IMV) was used. Reachable nests were inspected and sampled. Where possible and safe, and depending on the number of eggs in a nest, either one or two eggs were collected, leaving the rest behind.

The abundant sparrow nest within the roofs of thatched houses drew our attention. We collected all the eggs (normally 4–6) from selected nests. Southern Masked Weavers eggs were also collected at Nandoni Dam from nests overhanging the water. All eggs were wrapped in pre-cleaned foil and marked. The eggs were then frozen. It must be noted that the nests in roof thatch are in very close proximity to the sprayed inner surfaces—only centimetres away. Since eggs were collected from the outside, no additional contamination of the eggs via handling is foreseen. Care was also taken not to contaminate the egg contents with the eggshells that may have extraneous DDT residues on the outside when removing the egg contents.

All equipment used for sample preparation was rigorously pre-cleaned using soap and water, rinsed three times with double distilled water, and washed three times with 96% ethanol. Eggs were measured and carefully broken open in the absence of direct light to protect light-sensitive compounds. The sparrow eggs from each nest were pooled as the small volumes of the eggs precluded individual analysis. The contents were homogenised using an ultrasonic homogeniser such that as little foam as possible was formed. Samples were shipped frozen and received so in Norway. The analyses were done at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science (NVH) in Oslo—it is an accredited analytical laboratory (NS-EN ISO/IEC 17025 (TEST 137)). The sample preparation, extraction and clean-up procedures were done while protecting the samples from UV-light to avoid the degradation of brominated compounds.

2.3. Extraction and analyses

Procedures and quality control for extraction and analyses (Brevik, 1978) are described and referenced in Bouwman et al. (2012a). Because we suspected very low levels of brominated compounds, only pooled samples from each species and site were analysed. We prefer reporting and discussion concentrations based on wet mass (wm) rather than lipid mass (lm), as embryonic metabolism affects lipid content of the egg (Romanoff, 1932). Wet mass is also the basis for determining risk. However, we do present summarised lipid-based data (Table 1), and when comparing with other studies (Table 2). Mean relative recoveries were 110% for organochlorines, and 99% for BFRs. The data were not corrected for recoveries. Detection limits are provided in Table 1, and calculated as 3 × noise level, except BDE-183, -207, -208 and -209 that was mean of blank value+2 × standard deviation due to problems with blanks. Compounds below quantification limits in all eggs were α-HCH (at LOD=0.006 ng/g wm), PBEB, (pentabromoethylbenzene), DPTE (2,3-dibromopropyl-2,4,6-tribromophenyl ether), and HBB (hexabromobenzene) (all at LOD=0.01 ng/g wm). BDE-206 and HBCD were analysed for, but because of

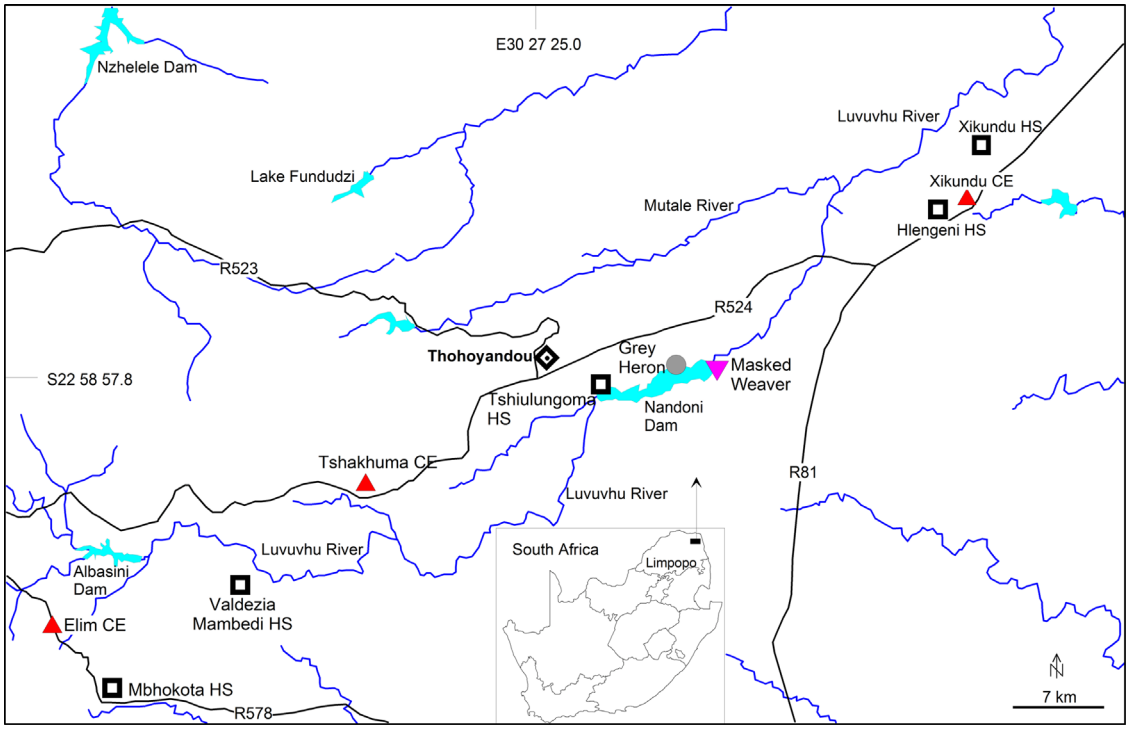

Fig. 1. Map of the region. The river flows west to east. HS—House Sparrow; CE—Cattle Egret.

chromatographic interferences from other compounds, we chose not to use the results.

2.4. Eggshell thickness

Eggshell thickness was measured for Cattle Egret eggs as there was good representation across the west-to-east transect (Fig. 1). The shells were gently washed to remove the membrane, and allowed to dry for at least three weeks. Three locations of fragments of the apex of the each shell were measured three times with an electronic digital calliper and the mean used for further calculations. House Sparrow eggshells were too thin to measure reliably.

2.5. Data treatment

Descriptive data analyses were done using GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com). All concentration data on the graphs were log-transformed, using only positive quantifications. Univariate comparisons were not attempted between species because of few eggs or pools per species. Instead, we concentrated on investigating the relationships between species and sites with multivariate analysis using MjM Software PC-ORD version 6.07 (www.pcord.com). Nonmetric Multidimensional Scaling (NMS) was chosen as the best model as it avoids the assumption of linear relationships among variables by using ranked distances to linearise the relationships between measured distances in ordination space. NMS deals much better with zero (equivalent to data points less than LOD) compared with other ordination methods (McCune and Grace, 2002). The distance measure used was Sørensen. Pollutant data were relativised for each egg to investigate pollutant profiles rather than absolute concentrations. Random starting configurations were used with 250 runs of real data. Monte Carlo tests were done with 250 runs of randomised data. Convex hulls for each species were drawn.

3. Results

3.1. Collection

Information gathered on the bird species occurring in the sampling area indicated that the following breeding species could be expected: Pied Kingfisher *Ceryle rudis*, White-breasted Cormorant *Phalacrocorax carbo*, Reed Cormorant *Phalacrocorax africanus*, African Darter *Anhinga melangogaster*, Grey Heron* *Ardea cinerea*, Blackheaded Heron *Ardea melanocephala*, Cattle Egret* *Bubulcus ibis*, Hadeda Ibis *Bostrychia hagedash*, Glossy Ibis *Plegadis falcinellus*, Sacred Ibis *Threskiornis aethiopicus*, Redknobbed Coot *Fulica cristata*, Moorhen *Gallinula chloropus*, Crowned Plover *Vanellus coronatus*, Blacksmith Plover *Vanellus armatus*, Greyheaded Gull *Larus cirrocephalus*, House Sparrow* *Passer domesticus*, Cape Sparrow *Passer melanurus*, Greyheaded sparrow *Passer griseus*, and Southern Masked-Weaver* *Ploceus velatus*. Of these species the Pied Kingfisher was of particular interest as its occurrence in the area was well documented. However, after an extensive search only nests for species indicated by an * could be located. No active breeding Pied Kingfishers could be found although old and newly excavated nests and adult birds were seen. The focus for egg collection was then directed towards other species. Although we would expect breeding colonies of herons and egrets, only a few were located. Even more worrisome was that only two heron breeding colony could be found—aquatic feeding Grey Herons (*A. cinerea*, very similar to the Great Blue Heron *Ardea herodias* of the Americas) at Elim and Nandoni Dam. The heron nests at Elim were unreachable as they were located high on thin branches. The Nandoni Dam colony was located between the DDT-sprayed and non-DDT sprayed areas (Fig. 1). This information becomes important later on. Three Cattle Egret colonies (terrestrial feeding) were found; at Elim, Tshakhuma, and a dam close to Xikundu (Fig. 1). Granivorous Southern Masked Weavers were sampled at Nandoni Dam. House Sparrow (granivorous but also scavenged food items) eggs were obtained from five different villages along a west–east transect (Fig. 1). Mbhokota and Valdezia Mambedi were outside the DDT sprayed area.

3.2. Compounds (based on Table 1 and Fig. 2)

Table 1 present the summarised analytical results. Figs. 2 and 3 represent the log-transformed data illustrating means, medians, 5–95% confidence intervals, and minima and maxima. Please note that the discussion will include consideration of the west to east transect is followed for Cattle Egrets and House Sparrows, so that geographic trends can be observed (see also Fig. 2).

Mean HCB levels were generally low; the Grey Heron eggs, and the sparrow eggs from Mbakota and Hlengeni, were higher than the others. The highest level was 1.5 ng/g wm in two sparrow egg pools—one from each town (data not shown). There was no clear west–east pattern for the Cattle Egret eggs.

Mean ΣHCH was highest in a pooled sparrow egg sample from Valdezia Mambedi; one pooled sample (Lim63) measured 620 ng/g wm (equivalent to 28 000 ng/g lm), while the other two pools were 2.5 and 0.61 ng/g wm, giving a mean ΣHCH of 210 ng/g wm from that town. The very high level in Lim63 was checked more than once, and consisted almost exclusively of γ-HCH (620 ng/g wm). The mean β-HCH and γ-HCH levels for all egg samples excluding Lim63 were 0.78 and 0.64 ng/g wm, respectively (Lim63 is not included in the vector for HCH in Fig. 3). For all other compounds analysed, Lim63 had levels no different from the other samples. The next highest mean ΣHCH levels were also in sparrow eggs; 4.0 and 3.7 ng/g wm from Hlangeni and Mbakota, respectively. Excluding Lim63, there was a clear decrease in HCH levels from west to east. The aquatic feeding Grey Heron had a mean of 2.9 ng/g wm, while the terrestrially feeding Cattle Egrets had less. There was no clear west–east pattern for the Cattle Egret eggs.

The Cattle Egret eggs from Xikundu Dam had the highest levels of ΣChlordanes (mean and maximum 42 and 200 ng/g wm, respectively), consisting mainly of *trans*-nonachlor. One egg had a maximum of 200 ng/g wm, equivalent to 2900 ng/g lm. The next highest mean ΣChlordanes was the Grey Heron at 9.8 ng/g wm. The House Sparrows had mean levels equivalent to or less than the Cattle Egret eggs. The Southern Masked Weaver eggs from Nandoni Dam had the lowest levels (0.35 ng/g wm). There was no clear west–east pattern for the Cattle Egret eggs.

Mirex had low levels overall, between 0.12 and 0.50 ng/g wm. There was no clear west–east pattern for the Cattle Egret or sparrow eggs.

Sparrow eggs from Xikundu and Hlangeni, the furthest east, had the highest ΣDDT for sparrows, and the mean ΣDDT for Hlengeni was the highest of any species and locality, at 16 000 ng/g wm, equivalent to 210 000 ng/g lm. One pooled sample from Hlengeni had the highest wet mass levels for sparrows of any single sample at 24 000 ng/g wm, equivalent to 310 000 ng/g on a lipid basis, less than the highest for Grey Heron. Curiously, sparrow eggs from Mbakota, furthest west and outside the sprayed area, had the next highest mean ΣDDT of 5600 ng/g wm. The three pooled sparrow egg samples from Valdezia Mambedi (which had the highest ΣHCH concentration) had a mean ΣDDT of 70 ng/g. Except for Mbakota, there seem to be an increase in ΣDDT levels in sparrow eggs from west to east. The three Southern Masked Weaver egg pools had a mean of 1400 ng/g wm, greater than the Cattle Egrets. Cattle Egret eggs showed a clear increase in means and medians from west to east.

ΣDDT was 2nd highest in Grey Heron eggs (mean and maximum 13 000 and 24 000 ng/g wm, respectively). This equates to 230 000 ng/g and 380 000 ng/g lm. Cattle Egret eggs had much lower levels, but there was a clear increase from west to east (means of 19, 92 and 290 ng/g wm for the three sites, respectively). The one-way ANOVA for log-transformed ΣDDT for the Cattle Egrets was significant ($p=0.0081$), and the Bonferroni multiple comparison post-test showed a significant difference ($p<0.05$) between eggs from Elim and Xikundu.

Table 1
Detection limits, concentrations of halogenated compounds, and eggshell thickness in wild bird eggs from the Limpopo Province. Detailed data are presented based on wet mass, and summarised data based on lipid mass.

Wet mass ng/g	Detection limit	Grey Heron						Cattle egret					
		Nandoni Dam ($n=6$)						Elim ($n=4$)					
		Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos
HCB	**0.005**	0.58	0.57	0.088	0.47	0.70	6	0.5	0.46	0.24	0.3	0.78	4
β-HCH	0.025	2.8	1.1	2.6	0.79	6.0	6	0.28	0.21	0.18	0.16	0.53	4
g-HCH	0.012	0.05	0.053	0.01	0.038	0.063	6	0.24	0.16	0.28	0.027	0.62	4
T-HCH		2.9	21	40	17	98	6	0.53	6.26	9.0	3.0	22.8	4
Oxychlordane	0.021	1.6	1.7	0.72	0.83	2.3	6	1.5	0.77	1.7	0.34	4.0	4
cis-Chlordane	0.022	0.082	0.08	0.011	0.07	0.1	6	0.085	0.08	0.044	0.04	0.14	4
trans-Nonachlor	0.022	5.2	2.9	8.0	0.1	19	6	1.2	0.91	1.4	0.09	2.7	3
T-Chlor		9.8	9.1	8.0	3.2	23	6	3	1.6	3.4	0.66	8.0	4
Mirex	0.010	0.38	0.32	0.29	0.29	0.59	6	0.35	0.25	0.14	0.14	0.74	4
p,p′-DDE	0.020	13000	7700	8600	5800	23000	6	18	18	11	6.0	29	4
o,p′-DDD	0.038	2.8	1.3	2.3	0.91	5.3	6	0.046	0.043	0.014	0.034	0.064	4
p,p′-DDD	0.029	170	100	120	71	320	6	0.24	0.16	0.21	0.1	0.55	4
o,p′-DDT	0.038	5.3	4.1	2.2	3.1	8.2	6	0.27	0.27	0.19	0.09	0.46	4
p,p′-DDT	0.018	100	99	32	72	150	6	0.96	1.0	0.35	0.5	1.4	4
T-DDT		13000	8000	8800	5900	24000	6	19	20	11	7.2	31	4
%DDT		0.97	1.2	0.35	0.58	1.2	6	6.5	4.5	4.8	3.2	14	4
PCB-28	0.005	0.50	0.46	0.13	0.36	0.68	6	0.08					1
PCB-52	0.039	0.32	0.36	0.15	0.07	0.45	6	0.30	0.30	0.045	0.24	0.35	4
PCB-74	0.070	0.78	0.70	0.41	0.41	1.4	6	< LOD					0
PCB-66	0.061	0.91	0.62	0.56	0.42	1.7	6	0.09					1
PCB-101	0.074	0.29	0.29	0.01	0.28	0.3	2	0.083	0.05	0.067	0.040	0.16	3
PCB-105	0.041	0.54	0.49	0.18	0.37	0.81	6	0.1	0.075	0.068	0.050	0.2	4
PCB-99	0.071	0.35	0.24	0.33	0.08	0.72	3	0.057	0.05	0.021	0.040	0.08	3
PCB-118	0.061	1.6	1.6	0.20	1.4	1.9	6	0.25	0.13	0.31	0.040	0.71	4
PCB-128	0.027	0.75	0.91	0.30	0.4	1.1	6	0.25	0.25	0.060	0.18	0.31	4
PCB-138	0.046	9.9	8.0	4.1	6.5	15	6	0.53	0.24	0.68	0.10	1.5	4
PCB-149	0.054	0.30	0.27	0.06	0.25	0.38	6	0.35	0.34	0.097	0.24	0.47	4
PCB-153	0.066	7.3	7.1	0.75	6.5	8.2	6	2.6	1.6	2.6	0.86	6.5	4
PCB-156	0.036	0.44	0.44	0.083	0.33	0.55	6	0.082	0.068	0.050	0.04	0.15	4
PCB-157	0.048	0.13	0.17	0.061	0.06	0.18	6	< LOD					0
PCB-170	0.031	1.8	1.3	1.3	1.1	4.2	6	0.21	0.13	0.22	0.04	0.53	4
PCB-180	0.037	3.7	3.7	0.43	3.1	4.3	6	1.5	1.1	1.0	0.8	3.1	4
PCB-183	0.038	0.66	0.64	0.081	0.59	0.78	6	0.22	0.074	0.32	0.027	0.70	4
PCB-187	0.033	1.2	0.95	0.53	0.81	2.0	6	0.29	0.17	0.27	0.12	0.69	4
PCB-194	0.033	0.67	0.69	0.091	0.57	0.79	6	0.24	0.16	0.26	0.04	0.62	4
PCB-206	0.033	0.93	0.67	0.54	0.58	1.9	6	0.35	0.17	0.39	0.14	0.93	4
T-PCB		33	30	6.1	27	42	6	7.8	5.4	6.3	3.2	17	4
BDE-28	0.01	0.01					P	< LOD					P
BDE-47	0.01	0.99					P	< LOD					P
BDE-100	0.02	0.43					P	< LOD					P
BDE-99	0.02	0.21					P	< LOD					P
BDE-154	0.01	0.46					P	< LOD					P
BDE-153	0.02	0.46					P	0.03					P
BDE-183	0.05	0.08					P	< LOD					P
BDE-208	0.14	< LOD					P	< LOD					P
BDE-207	0.16	< LOD					P	< LOD					P
BDE-209	0.22	< LOD					P	< LOD					P
T-BDE		3.1					P	0.03					P
PBT	0.01	0.03					P	< LOD					P
PTBPE	0.03	< LOD					P	< LOD					P

Table 1 (*continued*)

Wet mass ng/g	Detection limit	Grey Heron						Cattle egret					
		Nandoni Dam ($n=6$)						Elim ($n=4$)					
		Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos
Lipid mass ng/g		**Mean**	**Median**	**SD**	**Min**	**Max**	**Pos**	**Mean**	**Median**	**SD**	**Min**	**Max**	**Pos**
HCB		11	12	2.5	8.0	13	6	8.8	7.6	4.7	4.7	15	4
T-HCH		49	40	17	17	98	6	9.6	9.0	3.0	3.0	23	4
T-Chlor		190	150	170	68	480	6	56	25	70	12	160	4
T-DDT		230 000	150 000	130 000	130 000	380 000	6	340	290	210	150	620	4
Mirex		7.2	6.6	3.1	4.8	12	6	5.7	4.5	3.8	3.0	11	4
T-PCB		610	620	51	550	680	6	140	82	130	66	340	4
T-BDE		61					P	0.55					
PBT		0.57						< LOD					
PTBPE		< LOD						< LOD					
Eggshell thickness (mm)								0.19	0.20	0.02	0.16	0.20	4.00
		Cattle egret											
		Tshakhuma ($n=6$)						**Xikundu Dam ($n=5$)**					
		Mean	**Median**	**SD**	**Min**	**Max**	**Pos**	**Mean**	**Median**	**SD**	**Min**	**Max**	**Pos**
HCB		0.47	0.50	0.10	0.3	0.61	6	0.36	0.39	0.12	0.19	0.48	5
β-HCH		0.30	0.21	0.20	0.12	0.66	6	0.59	0.33	0.52	0.29	1.5	5
g-HCH		0.027	0.028	0.0056	0.020	0.032	6	0.04	0.039	0.01	0.023	0.056	4
T-HCH		0.32	5.7	3.0	1.8	8.7	6	0.63	6.3	7.8	4.4	23	5
Oxychlordane		1.6	1.15	1.4	0.33	4.2	6	7.3	1.8	14	0.11	32	5
cis-Chlordane		0.18	0.19	0.12	0.04	0.37	6	2.2	0.05	4.9	0.04	11	5
trans-Nonachlor		0.98	0.84	0.82	0.03	2.4	6	40	0.91	79	0.03	160	4
T-Chlor		3.1	2.6	2.2	0.84	7.3	6	42	3.4	89	0.48	200	5
Mirex		0.29	0.31	0.21	0.21	0.34	6	0.50	0.64	0.18	0.18	0.75	5
p,p′-DDE		81	27	130	18	350	6	270	200	270	50	720	5
o,p′-DDD		0.042	0.040	0.0073	0.035	0.052	4	0.044	0.039	0.018	0.029	0.070	4
p,p′-DDD		0.88	0.25	1.6	0.080	4.2	6	4.3	1.2	7.2	0.35	17	5
o,p′-DDT		0.36	0.38	0.25	0.080	0.66	6	5.5	0.2	12	0.052	27	5
p,p′-DDT		9.20	1.8	19	0.43	48	6	8.9	6.1	7.1	1.3	20	5
T-DDT		92	30	150	20	400	6	290	210	270	51	730	5
%DDT		6	7	3.9	1.7	12	6	4.3	2.9	3.4	0.84	9.4	5
PCB-28		0.05					1	0.06					1
PCB-52		0.34	0.35	0.078	0.23	0.42	6	0.30	0.33	0.090	0.2	0.4	5
PCB-74		0.06					1	0.13	0.13	0.039	0.08	0.17	4
PCB-66		0.055	0.055	0.0058	0.050	0.06	4	0.11	0.07	0.093	0.05	0.22	3
PCB-101		0.13	0.13	0.048	0.080	0.18	5	0.09	0.09	0.071	0.04	0.14	2
PCB-105		0.068	0.065	0.023	0.040	0.11	6	0.22	0.085	0.30	0.05	0.67	4
PCB-99		0.075	0.075	0.019	0.050	0.1	6	0.45	0.055	0.80	0.04	1.6	4
PCB-118		0.136	0.17	0.067	0.050	0.2	5	0.12	0.12	0.052	0.05	0.17	4
PCB-128		0.23	0.23	0.028	0.18	0.26	6	0.24	0.25	0.021	0.22	0.26	4
PCB-138		0.30	0.3	0.10	0.16	0.45	6	0.39	0.31	0.31	0.1	0.83	4
PCB-149		0.24	0.22	0.043	0.21	0.31	6	0.19	0.23	0.076	0.08	0.25	5
PCB-153		1.6	1.70	0.61	0.95	2.7	6	1.4	1.3	0.48	0.94	2.2	5
PCB-156		0.059	0.067	0.015	0.042	0.069	3	< LOD					0
PCB-157		< LOD					0	< LOD					0
PCB-170		0.133	0.12	0.085	0.050	0.29	6	0.13	0.09	0.094	0.04	0.28	5
PCB-180		1.203	1.2	0.34	0.79	1.8	6	1.2	1.2	0.34	0.99	1.8	5
PCB-183		0.077	0.080	0.013	0.06	0.093	6	0.089	0.075	0.036	0.061	0.13	3

PCB-187	0.17	0.18	0.025	0.14	0.21	6	0.18	0.16	0.051	0.15	0.27	5
PCB-194	0.153	0.13	0.10	0.040	0.31	6	0.27	0.19	0.21	0.11	0.5	3
PCB-206	0.198	0.18	0.096	0.10	0.32	6	0.17	0.17	0.079	0.07	0.26	4
T-PCB	5.433	5.6	1.5	3.5	7.8	6	5.6	4.6	2.1	3.8	8.2	5
BDE-28	NA						< LOD					
BDE-47	NA						< LOD					
BDE-100	NA						0.02					
BDE-99	NA						< LOD					
BDE-154	NA						0.01					
BDE-153	NA						0.10					
BDE-183	NA						0.09					
BDE-208	NA						< LOD					
BDE-207	NA						< LOD					
BDE-209	NA						0.51					
T-BDE	NA						0.7					
PBT	NA						< LOD					
PTBPE	NA						< LOD					
Lipid mass ng/g	**Mean**	**Median**	**SD**	**Min**	**Max**	**Pos**	**Mean**	**Median**	**SD**	**Min**	**Max**	**Pos**
HCB	7.6	7.2	1.2	6.3	9.5	5	5.7	6.0	1.3	3.6	6.9	5
T-HCH	5.4	3.0	1.8	1.8	8.7	5	10	7.8	4.4	4.4	23	5
T-Chlor	52	34	39	15	120	5	620	43	1300	9.1	2900	5
T-DDT	1600	420	2700	240	7100	5	4300	2600	3700	970	11 000	5
Mirex	5.0	4.0	2.4	3.0	9.5	5	8.0	9.3	4.2	3.4	13	5
T-PCB	87	84	14	75	110	5	89	84	26	58	120	5
T-BDE	NA						15					
PBT	NA						< LOD					
PTBPE	NA						< LOD					
Eggshell thickness (mm)	0.205	0.21	0.010	0.19	0.22	6	0.228	0.23	0.01	0.22	0.24	5

	House sparrow					
	Mbk (3)	**VMa (3)**	**Tsg (3)**	**Hlg (2)**	**Xik (2)**	**Nnd (3)**
	Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
HCB	0.77	0.16	0.31	0.9	0.31	0.49
β-HCH	0.65	0.23	0.22	0.82	0.53	0.49
g-HCH	3	210	0.26	3.2	0.16	0.34
T-HCH	3.7	210	0.49	4.0	0.69	0.83
Oxychlordane	0.24	0.12	0.049	0.78	0.15	0.08
cis-Chlordane	0.093	< LOD	< LOD	0.1	0.04	0.037
trans-Nonachlor	0.33	< LOD	< LOD	1.14	< LOD	0.32
T-Chlor	1.4	0.25	0.087	3.5	1.1	0.35
Mirex	0.08	0.07	0.28	0.35	0.12	0.17
p,p′-DDE	3300	69	1300	7400	6800	1300
o,p′-DDD	0.74	< LOD	< LOD	2.1	0.47	< LOD
p,p′-DDD	290	0.17	4.6	820	350	57
o,p′-DDT	45	0.24	0.93	200	8.1	0.34
p,p′-DDT	2000	0.83	61	4700	540	8.6
T-DDT	5600	70	1300	16000	8300	1400
%DDT	31	1.2	2.2	46	13	1.8
PCB-28	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCB-52	0.43	0.31	0.34	0.42	0.46	0.29
PCB-74	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCB-66	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCB-101	0.17	0.13	0.65	0.27	< LOD	0.080
PCB-105	0.04	0.04	0.18	0.090	< LOD	0.047
PCB-99	< LOD	< LOD	1.07	0.41	< LOD	< LOD
PCB-118	0.07	0.135	0.095	0.090	< LOD	0.06
PCB-128	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

Table 1 (*continued*)

	House sparrow					
	Mbk (3)	**VMa (3)**	**Tsg (3)**	**Hlg (2)**	**Xik (2)**	**Nnd (3)**
	Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
PCB-138	0.92	0.4	0.44	0.86	1.4	0.66
PCB-149	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCB-153	0.74	0.98	1.3	0.68	0.71	1.1
PCB-156	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCB-157	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCB-170	0.065	0.22	0.12	0.06	0.075	0.073
PCB-180	0.83	0.95	1.3	0.83	0.82	0.86
PCB-183	0.11	0.23	< LOD	0.13	< LOD	0.072
PCB-187	0.16	0.18	0.19	0.25	0.52	0.18
PCB-194	0.065	0.15	< LOD	0.065	< LOD	0.07
PCB-206	0.14	0.08	0.17	0.11	< LOD	0.17
T-PCB	3.7	3.3	4.7	4.2	4.2	3.7
BDE-28	< LOD	NA	NA	NA	< LOD	NA
BDE-47	0.03	NA	NA	NA	0.04	NA
BDE-100	0.06	NA	NA	NA	0.04	NA
BDE-99	0.13	NA	NA	NA	0.10	NA
BDE-154	0.03	NA	NA	NA	0.03	NA
BDE-153	0.24	NA	NA	NA	0.18	NA
BDE-183	0.56	NA	NA	NA	0.44	NA
BDE-208	0.18	NA	NA	NA	< LOD	NA
BDE-207	0.35	NA	NA	NA	0.19	NA
BDE-209	1.02	NA	NA	NA	0.48	NA
T-BDE	2.6	NA	NA	NA	1.5	NA
PBT	0.02	NA	NA	NA	0.02	NA
PTBPE	< LOD	NA	NA	NA	0.05	NA
Lipid mass ng/g	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
HCB	21	8.5	9	16	6.3	11
T-HCH	68	9500	7.9	51	12	17
T-Chlor	26	7.4	1.6	47	23	7.3
T-DDT	110 000	1500	19 000	210 000	140 000	29 000
Mirex	1.4	2.2	9.8	7.4	3.8	3.9
T-PCB	64	73	87	57	74	76
T-BDE	41	NA	NA	NA	2.5	NA
PBT	0.32	NA	NA	NA	0.32	NA
PTBPE	< LOD	NA	NA	NA	0.8	NA
Eggshell thickness (mm)						

Table 2
Reports with comparable data based on lipid mass.

ng/g	Location	Sampled	HCB	ΣHCH	ΣChlord	Mirex	ΣPCB	ΣDDT	ΣBDE	Reference
Great Blue Heron	Canada	1994	NA[a]	NA	NA	NA	3 454	1 616	NA	Thomas and Anthony (1999)
Great Blue Heron	Canada	1997/82	184	NA	80	48	6 650	3 031	NA	Harris et al. (2003)
Great Blue Heron	Canada	1997	480 000	NA	800		148 000	52 000	NA	Laporte (1982)
Great Blue Heron	Canada	1998	700 000	NA	400	NA	280 000	359 000	NA	Laporte (1982)
Grey Heron	Namibia	1970s	1200?	NA	NA	NA	NA	9 200	NA	Van Dyk et al. (1982)
Grey Heron	**Nandoni Dam**	**2009**	**11**	**49**	**190**	**7.2**	**610**	**230 000**	**61**	**This study**
Night Heron	Hong Kong PRC	2006	241	NA	158	84	880	2 500	NA	Wang et al. (2011)
Great Egret	Hong Kong PRC	2006	40	NA	348	107	2 000	16 000	NA	Wang et al. (2011)
Cattle Egret	China	2000–02	NA	NA	NA	NA	NA	NA	200	Lam et al. (2007)
Cattle Egret	India	2007	NA	3855	NA	NA	NA	984	NA	Malik et al. (2011)
Cattle Egret	Spain	1970s	NA	NA	NA	NA	NA	1 930	NA	Ruiz et al. (1983)
Cattle Egret	RSA	2004/5	9	12	11	4	46	330	2	Polder et al. (2008)
Cattle Egret	RSA	2004/5	18	12	7	19	120	430	4	Polder et al. (2008)
Cattle Egret	**Elim**	**2009/9**	**8.8**	**9.6**	**56**	**5.7**	**140**	**340**	**0.55**	**This study**
Cattle Egret	**Tshakhuma Dam**	**2009/10**	**7.2**	**3.0**	**34**	**4.0**	**84**	**420**	**< LOD[b]**	**This study**
Cattle Egret	**Xikundu Dam**	**2009/10**	**5.7**	**10**	**620**	**8.0**	**89**	**4 300**	**15**	**This study**
Double Crested Cormorant	USA	2008	NA	NA	NA	NA	NA	NA	5 500	Klosterhaus et al. (2012)
Imperial Shag	Falklands	2008	204	2.3	NA	NA	140	106	1.92	van den Steen et al. (2008)
European Shag	Norway	2003–04	165	54	903	NA	4 600	1 500	90	Herzke et al. (2009)
Great Cormorant	Netherlands	1988/9	NA	NA	NA	NA	466 000	132 000	NA	Dirksen et al. (1995)
African Darter	RSA	2004/5	69	1 700	150	33	5 100	4 400	17	Polder et al. (2008)
Reed Cormorant	RSA	2004/5	41	82	56	34	2 700	7 000	14	Polder et al. (2008)
Lapwing	Belgium	2006	50	7.3	205	NA	4 360	97	109	Dauwe et al. (2009)
White Stork	Spain	1998–04	NA	NA	NA	NA	NA	NA	9.1	Munoz-Arnanz et al. (2011)
African Sacred Ibis	RSA	2004/5	17	40	230	5	1 000	1 200	230	Polder et al. (2008)
Sooty Tern	Mauritius	2009	3.6	2.4	0.48	8.7	20	17	0.07	Bouwman et al., 2012a,b
American Robin	USA	1993–95	NA	NA	NA	NA	NA	225 000[c]	NA	Harris et al. (2000)
Blue Tit	Spain	2006	NA	NA	NA	NA	2 000	NA	110	van den Steen et al. (2008)
Great Tit	Belgium	2006	19	4.4	9.2	NA	2 760	535	49	Dauwe et al. (2009)
House Sparrow	India	2001–06	NA	4 066	NA	NA	NA	14 176	NA	Dhananjayan et al. (2011)
House Sparrow	**Mbhokhota**	**2009/10**	**21**	**68**	**26**	**1.4**	**64**	**110 000**	**0.32**	**This study**
House Sparrow	**Valdezia Mabedi**	**2009/10**	**9.0**	**25[d]**	**7.4**	**2.2**	**73**	**1 500**	**< LOD**	**This study**
Lim63	**Valdezia Mabedi**	**2009/10**	**7.3**	**28 000**	**16**	**2.2**	**86**	**1 600**	**< LOD**	**This study**
House Sparrow	**Tshiulungoma**	**2009/10**	**9.0**	**7.9**	**1.6**	**10**	**87**	**19 000**	**< LOD**	**This study**
House Sparrow	**Hlengeni**	**2009/10**	**16**	**51**	**47**	**7.4**	**57**	**210 000**	**< LOD**	**This study**
House Sparrow	**Xikundu**	**2009/10**	**6.0**	**12**	**23**	**3.8**	**74**	**140 000**	**2.5**	**This study**
Southern Masked Weaver	**Nandoni Dam**	**2009/10**	**11**	**17**	**7.3**	**3.9**	**76**	**29 000**	**< LOD**	**This study**

Possibly misnomer for HCH.

[a] Not analysed.
[b] Detection limit.
[c] Wet mass.
[d] Without Lim36.

p,p′-DDE made up the bulk of ΣDDT. Because of this, the distribution almost mirrors that of ΣDDT. The sharp increase from west to east in both Cattle Egret eggs and sparrow eggs (except for Mbhokota) is clear. The one-way ANOVA for log-transformed *p,p′*-DDE for the Cattle Egrets was significant ($p=0.0057$), and the Bonferroni multiple comparison post test showed a significant difference ($p<0.05$) between eggs from Elim and Xikundu (very much the same as for ΣDDT).

o,p′-DDT, which makes up about 22% of the applied DDT (Bouwman et al., 2006), was very low throughout (0.27–45 ng/g wm), except in sparrow eggs from Hlengeni at 200 ng/g. There seem to be an increase from west to east for sparrow eggs, again, except for Mbhokota. For Cattle Egrets eggs, there was a slight increase in mean *o,p′*-DDT from west to east, but not when considering medians. Mean *p,p′*-DDD, the breakdown product of *p,p′*-DDT, although quite low throughout, was highest in Grey Heron eggs at 2.8 ng/g wm.

Mean %DDT (% of *p,p′*-DDT in ΣDDT) was highest in the sparrow eggs from Hlengeni (46%, range 24–50%), followed by Mbhokota (31%) and Xikundu (13%). The mean %DDT was low in Tshiulungoma (2.2%) and Valdezia Mambedi (1.2%). Cattle egrets had intermediate mean %DDT (4.3–7%), and a slight decreasing trend from west to east.

ΣPCB was low throughout, except in Grey Heron eggs at a mean of 33 ng/g wm (610 ng/g lm). There were almost no differences in mean and median ΣPCB between the rest of the bird eggs and localities.

PBDEs could not be quantified in some samples, notably in three of the domestic breeding sparrow localities. The highest mean ΣBDE level was for Grey Heron eggs at 3.1 ng/g wm, equivalent to 61 ng/g lm. There was not enough data to look at west to east trends.

3.3. Multivariate analyses

Fig. 3 shows the results of the NMS joint plot. Two dimensions were derived. The final stress was 6.15 reached after 103 iterations. Final stress is interpreted as follows: < 5 excellent, 5–10 good, 10–20 general picture good, but not in detail, > 20 not good (McCune and Grace, 2002). Final instability was 0.0000, therefore very stable. The Monte Carlo tests were significant for Axis 1 and 2 ($p<0.05$). Axis 1 explained 77% of the variation, and Axis 2, 21%, for a cumulative explanation of 98%, and adjusted cumulative for lack of orthogonality to 100%. The two terrestrial species (Cattle Egrets and House Sparrows) occupied large but mostly separate ordination spaces. There was a small overlap to the left, indicating some eggs with comparable pollutant profiles. The two aquatic species (Southern Masked Weaver and Grey Heron) occupied comparatively much

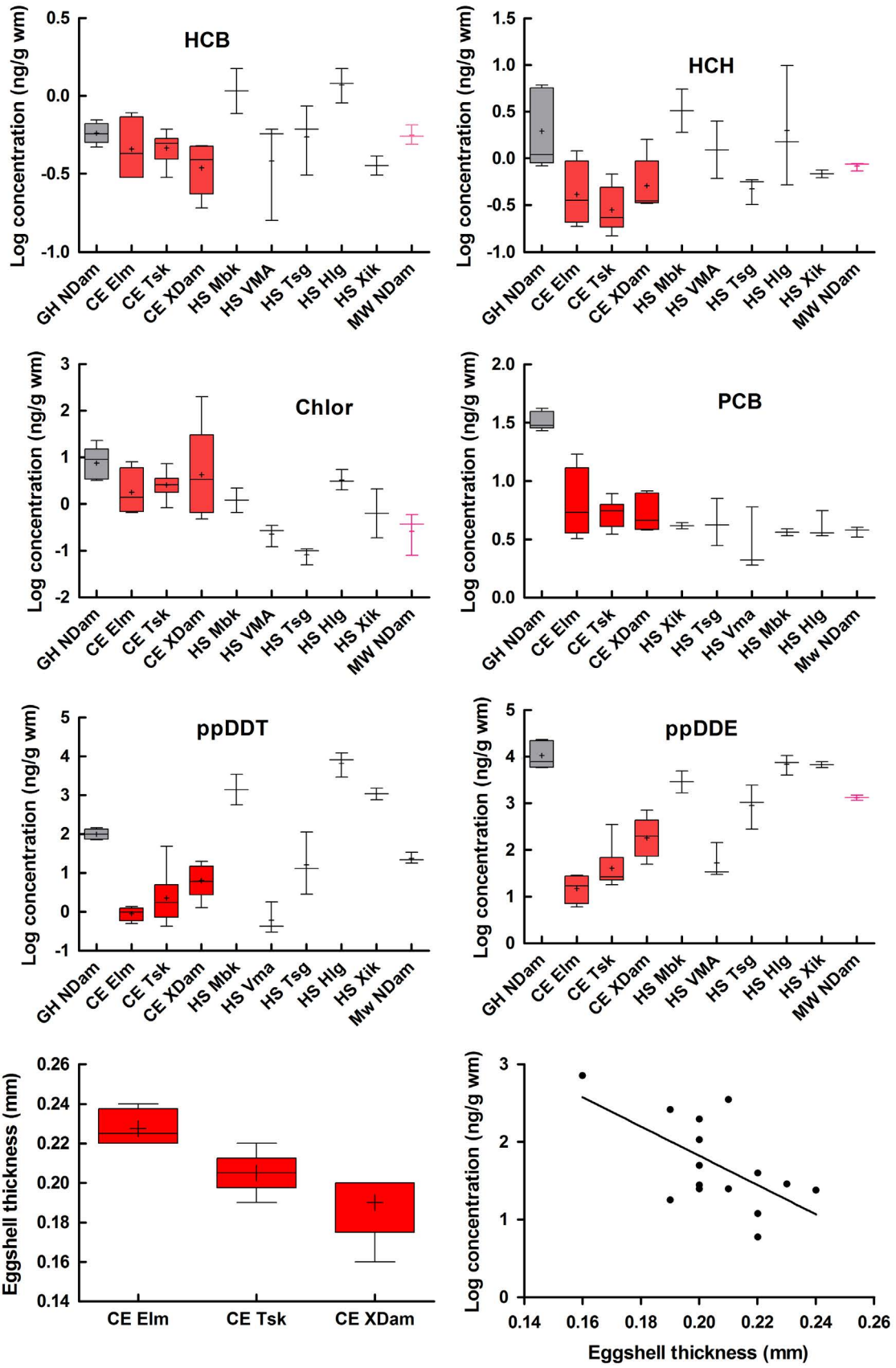

Fig. 2. Box and whisker plots (horizontal lines are medians, 5 and 95% confidence intervals, and minima and maxima). Log concentrations (wet mass) per site of (A) HCB, (B) ΣHCH, (C) Σchlordanes, (D) PCBs, (E) *p,p′*-DDT, (F) *p,p′*-DDE. (G) Eggshell thickness of Cattle Egrets per site, and (H) linear regression of eggshell thickness against log *p,p′*-DDE.

smaller ordination spaces which were located close together, and both were closely located in the same region where the Cattle Egret and House Sparrows pollutant profiles overlapped. In this region therefore, the pollutant profiles of four different species with different life histories showed convergence.

3.4. Eggshell thickness

Table 1 presents the eggshell thickness data for the Cattle Egret samples, since they had a well-spaced west-to-east transect (Fig. 1). Fig. 2G shows the metrics for the three different localities. There was a significant decrease in thickness from west to east (Kruskall–Wallis one-way ANOVA; $p=0.009$). The Dunn's multiple comparisons test showed a significant difference between Elim and Xikundu Dam. The linear regression of transformed *p,p′*-DDE against thickness of individual eggshells (Fig. 2H) was also significantly different from zero at $p=0.0152$. The runs test showed no significant deviation from linearity ($p=0.9487$). This effect was almost the same for *p,p′*-DDT with the regression line significantly different from zero at $p=0.0159$. Investigation of the other compounds, including the PCBs, showed no influence on eggshell thickness. The difference between thickest and thinnest shells was 33%.

4. Discussion

4.1. Collection

We initially concentrated on trying to collect Pied Kingfisher eggs because we have Pied Kingfisher data from northern KwaZulu-Natal (Evans and Bouwman, 2000), another region where DDT is sprayed for malaria control. We could not get any eggs back in 1993, but from Pied Kingfisher blood levels we calculated means of ΣDDT of 1390 ng/g wm and 2910 ng/g wm for eggs from different regions, with a maximum of 4190 ng/g wm. These levels were judged to be close to or exceeding the levels associated with impaired reproductive success (4000 ng/g wm) in Brown Pelicans (*Pelecanus occidentalis*) (Blus, 1982) and that the population might be at risk. The absence of breeding colonies of water birds east of Thohoyandou (Cattle Egrets are colonially breeding terrestrial feeders) needs to be kept in mind when considering further discussion. Although the Grey Herons colony at Nandoni Dam is located on the edge where DDT is started to be sprayed, the birds would feed in a large radius from there, including the spayed areas to the east where they would encounter higher levels of DDT in their prey as DDT levels in fish is increasing downstream (Barnhoorn et al., 2009). Laporte (1982) found that the colony that had the highest levels of ΣDDT in their

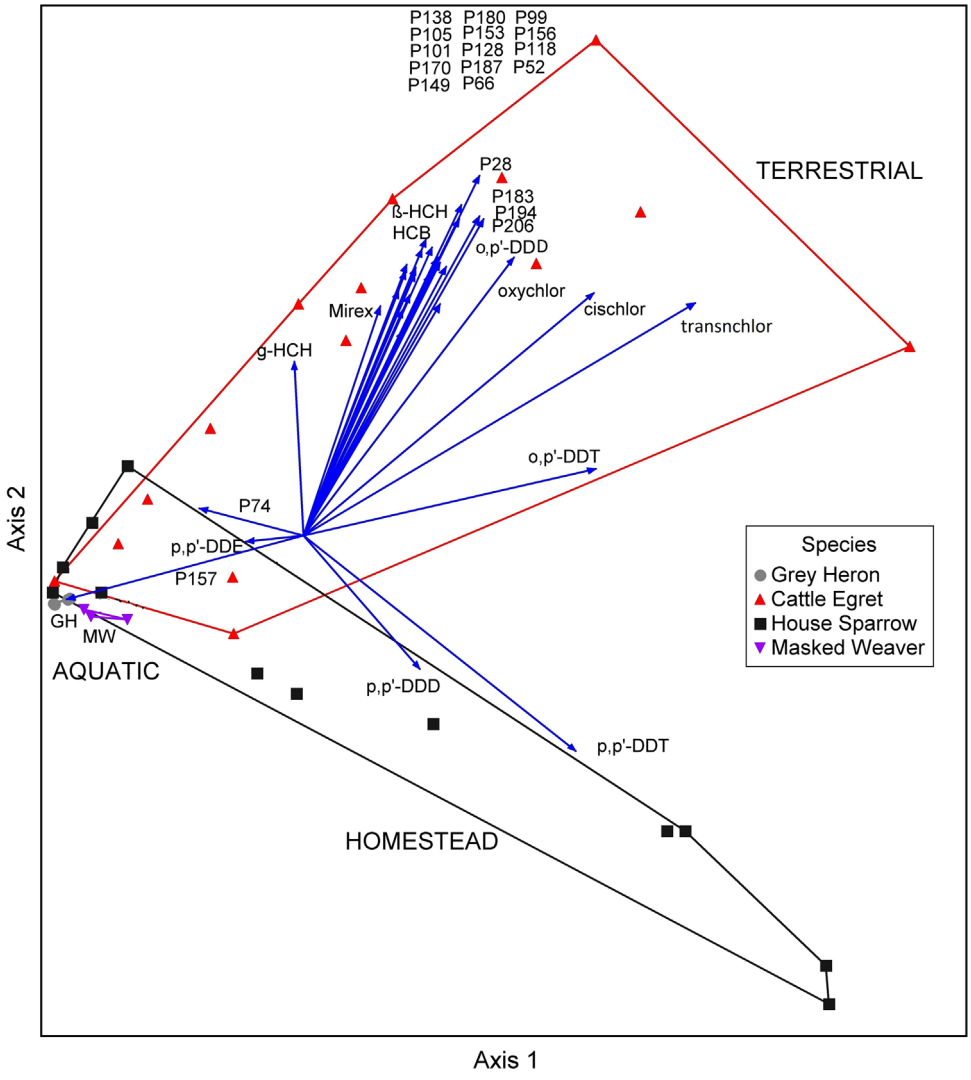

Fig. 3. NMS plot of all the data. The concentration data are relativised. The distance between the points (samples) is approximately proportional to the dissimilarities between them. The length of the vectors shows the strength of association in the direction of higher proportion. The convex hulls delineate the pollutant profile of each species.

eggs of a community of Great Blue Heron colonies in Canada (359 000 ng/g lm—compare with levels in Table 2), was later deserted, while all the others with lower levels remained. However, other compounds such as HCB and PCBs were also high and may have contributed to the desertion.

4.2. HCB

HCB has multiple sources such as use as from pesticide or combustion. Edvidence of its reported use in locust control can be seen in Grey Heron eggs from Namibia (1200 ng/g wm; Table 2), although there is likely confusion with HCH and the later is more likely. Low levels of HCB is therefore attributed to ambient background due to historic use or unintentional formation during combustion. The House Sparrow eggs from villages (where there is biomass combustion for cooking and heating) were generally the same or higher than for the egret and heron eggs feeding further away, also suggesting that HCB was probably generated by domestic combustion. Compared to other regions, HCB levels in eggs were low (Table 2).

4.3. HCH

All registrations of HCH, including γ-HCH or lindane, have only recently been withdrawn for all uses in SA (personal communication, SA Government). Lindane used to be an active ingredient in a dust formulation frequently used for home garden and indoor insect control in Limpopo (personal observations). However, the very low levels in House Sparrow eggs (with the exception of sample Lim63; 621 ng/g wm, equivalent to 28 000 ng/g lm) suggest that little residue remains associated with the homesteads. The high temperatures in the region probably played a role. Slightly lower mean levels in Cattle Egret eggs suggest legacy from agricultural application. The higher level in Blue Heron eggs suggests transfer to the aquatic environment. There was no west–east pattern, indicating that HCH in bird eggs were not associated with any geographic factor. The very high level for ΣHCH in Lim63 (one of three pooled sparrow egg samples from Valdezia Mambedi) was most likely due to recent domestic use of old stock, as the levels for the other pools and pesticides were all comparable (Table 2). As far as we are aware, this represents the highest HCH level yet reported for any sparrow egg sample. The high level in Lim63 is a third of the highest mean level recently measured in SA eggs, at 1700 ng/g wm; Table 2) in African Darter, collected in 2004/5 in a commercial agricultural region 500 km to the south-west (Bouwman et al., 2008).

4.4. Chlordane and mirex

ΣChlordanes increased slightly from west to east in Cattle Egrets from Xikundu Dam. We have no explanation for this pattern. The eggs of the aquatic feeding Grey Heron had the next highest mean. ΣChlordanes in Grey Heron eggs were lower than in Shag eggs from Norway and most samples from other regions and countries (Table 2). There was no geographic pattern to be seen in the House Sparrow egg concentrations of ΣChlordanes.

The levels for mirex were uniformly low with no pattern. This is in line with other findings from South Africa (Table 2), and less than in eggs from elsewhere. Mirex was never registered for use as an insecticide in South Africa. Bouwman et al. (2008) discussed the levels and possible sources of mirex in South Africa.

4.5. PCBs

PCBs had relatively low levels in almost all samples from Limpopo when compared with results from elsewhere, including South Africa (Table 2). The Grey Heron eggs had the highest ΣPCB (610 ng/g wm), with the other samples from Limpopo at between 64 and 140 ng/g lm mean levels (Table 1). PB153 had very much the same pattern. There was an almost equal distribution between lower and higher chlorinated congeners (Table 1). There are no industrial sources in the vicinity of the north-west region of Limpopo, and most of the electrification has occurred post 1994 and is therefore unlikely to contain PCBs. The low levels of ΣPCBs in House Sparrows suggest that very little PCBs are imported into the region via products. The only remaining sources would be biomass combustion and vegetation fires. The African Darter, African Sacred Ibis and Reed Cormorant eggs samples from South Africa (Table 2) had much higher levels of PCBs, most likely as a result of industry and electrical equipment. The Blue Tit eggs from Spain, and the Great Tit eggs from Belgium (slightly smaller in size than the sparrow), had ΣPCB levels two orders of magnitude higher than the sparrows from Limpopo (Table 2). This region is therefore particularly clean of PCBs, suggesting that causes of effects would largely be ascribed to other chlorinated compounds, particularly the DDTs (see next section).

4.6. DDT

Because of the ongoing spraying of DDT in Limpopo Province since 1945 (Bornman et al., 2012), and its historic use in agriculture terminated in the late 1970's (Bouwman, 2003), the presence of DDTs was not unexpected. Unexpected were the very high levels found in some birds (Tables 1 and 2). This study reports one of the highest ΣDDT levels in wild bird eggs this century from anywhere in the world (in the classic expression as 380 ppm lm as maximum in Grey Heron). No other study is known where Grey Heron was sampled in South Africa. From Namibia, a calculated level of 9200 ng/g lw in Grey Heron eggs is geographically the closest known historic datum for this species (Van Dyk et al., 1982). The mean ΣDDT in Grey Heron eggs of this study at 13 000 ng/g wm (equivalent to 13 mg/kg wm) exceeds the "critical level for reproductive success" (3 mg/kg wm) calculated for Brown Pelicans in field studies in the USA (USDoI, 1998), with the non-effect level estimated at 0.5 mg/kg wm (Cooper, 1991). This assessment was based on the findings of Blus (1982) who estimated that there was substantial impaired reproductive success at 3 mg/kg wm, and failure at 4 mg/kg wm for the Brown Pelican. DDE concentrations of 2.8 mg/kg wm have been associated with reproductive impairment of piscivore bird populations, while 1 mg/kg wm has been linked to reduced heron survival (Connell et al., 2003). It is therefore likely that Grey Herons and other piscivore birds in Limpopo Province are at risk of effects of DDT. The Limpopo Grey Heron eggs were collected at Nandoni Dam, straddling the divide between DDT-sprayed and non-DDT-sprayed areas (Fig. 1), and the birds would in all likely hood also forage downstream (east) into areas with DDT use. It would be speculative to imply that the notable lack of breeding colonies of aquatic birds in the DDT-sprayed area would be due to DDT, but the suggestions are there, and it would be very interesting to look at similar DDT-sprayed areas elsewhere in South Africa and Africa.

No other biota in this Province comes close to the levels found in Grey Heron eggs, except House Sparrow eggs from two DDT-sprayed villages. The mean ΣDDT levels in House Sparrow eggs (Table 1) are possibly the highest ever recorded for any sparrow worldwide (16 000 ng/g wm, equivalent to 210 000 ng/g lm). One of the two clutch-samples from Hlengeni had the highest mean ΣDDT levels for sparrows of any single sample at 24 400 ng/g wm (equivalent to 310 000 ng/g lm), higher than the highest level for Grey Heron. The sparrows do reproduce at these levels, seemingly unaffected, although we did not monitor this. Mitchell (1946) sprayed DDT (at 5 lb/acre) directly on nests with eggs and found no mortality, effects on hatching or hatchlings, and no nest abandonment. ΣDDT in American Robin eggs (225 000 ng/g wm),

at levels that have caused mortalities elsewhere, did not show obvious signs of reproductive failure (Harris et al., 2000). The authors suggested that the lack of toxic effect might have to do with the 'aged' nature of the DDT in the orchard soils. Chicken tissues of free roaming chickens from DDT-sprayed homesteads had very high levels (Van Dyk et al., 2010), as did their eggs (unpublished data; highest was 48 000 ng/g wm). In our case, with the sparrows and chickens exposed to annually applied DDT for generations since 1945, they may have developed tolerance to DDT, although there is no such suggestion in literature.

Uptake of DDT by House Sparrows is probably related to the very close proximity of application to the nest, centimetres away from where the DDT is deposited on the thatch within which they make their nests. However, uptake from feeding on the ground in the vicinity of the treated house with known high levels of DDT in soils (van Dyk et al., 2010) may also play a role. The almost equivalent DDT level in chicken eggs (unpublished data) is strongly indicative of the terrestrial route of uptake. The finding that House Sparrow eggs from a non DDT-sprayed village (Mbhokota) had higher levels than eggs from the same species from DDT-sprayed villages needs further investigation. It perhaps points towards an unknown recent use of DDT.

The levels of DDT in Cattle Egret eggs, although much lower than in the Grey Heron (Table 1), are also relatively high, higher than in any other known record of this species we could trace (Table 2). The route of uptake is not clear as these birds feed terrestrially on insects and small animals near cattle and game, but may pick up DDT from sometimes venturing near sprayed homes possibly predating on insects killed or affected by DDT or other pesticides. The ΣDDT levels in eggs from Elim and Tshakhuma (outside the sprayed area) indicate that they do roam to some extent, and that the roaming distance for the Xikundu colony falls mainly inside the sprayed area. Cattle Egrets forage up to 15 km from their breeding colonies (Butler and Kok, 2011).

Considering the west–east transect of eggs collected at Cattle Egret colonies away from (Elim), near (Tshakhuma) and within the DDT-sprayed area (Xikundu), it is clear that with a significant increase in *p,p*′-DDE and *p,p*′-DDT (Fig. 2E and F) there was also a significant thinning of the eggshells (Sections 3.3 and 3.4 and Fig. 2G). The regression (Fig. 2H) was also significant (Section 3.4) with a 33% difference between thinnest and thickest shells. Ruiz et al. (1983) found for Cattle Egrets in the Ebro Delta in Spain, egg breakage at mean levels of *p,p*′-DDE at 1930 ng/g wm. In a review of literature, Lundholm (1997) concludes that in field situations a thinning of below 10% in eggshell thickness is not associated with egg breakage and population decline. In another review, Blus and Henny (1997) found that a mean reduction in eggshell thickness of $>18\%$ was associated with population declines. Cooke (1973) associated 13 000 ng/g wm with 20% thinning in Great Blue Heron eggs, suggestive of impaired reproduction. It is obvious that there is good cause for concern about the reproductive performance of the Cattle Egrets in the study area, and probably also in other DDT-spayed areas in Africa.

The three clutches of granivorous Southern Masked Weaver eggs breeding at Nandoni Dam, had remarkable uniform levels of ΣDDT (1300, 1300, and 1600 ng/g wm). This indicates relatively uniform exposure acquiring DDT at levels higher than the Cattle Egrets feeding on insects and other small animals. Why the Southern Masked Weaver feeding at a lower trophic level than the Cattle Egrets had higher levels is unknown and would bear further ecological and/or metabolic investigations. It has been pointed out that the passerines (including weavers and sparrows) are less well studied and might also be at risk in polluted areas (Douthwaite, 1995).

4.7. BFRs

Tables 1 and 2 shows the low levels of BFRs in all egg samples, but with higher levels in the Grey Heron eggs, followed by Cattle Egrets and House Sparrows. Compared with data from elsewhere, including South Africa (Polder et al., 2008), the levels were low. The low levels indicate that very little BFRs reach this area probably because there are no large industries. These levels should be monitored as it might increase with development, increased welfare (with more possessions), and possible dumping. It also means that any effects seen are unlikely to be caused by BFRs.

4.8. Observations and impacts

DDT used in malaria control clearly reaches the environment, but also human inhabitants. Previously, the highest ever DDT level recorded in any biological matrix from the Limpopo Province was 1700 000 ng/g wm (25 000 000 ng/kg lm) in a human breast milk sample collected to the east of Nandoni Dam (Bouwman et al., 2012b). Both the Provisional Tolerable Daily Intake for DDT by infants and the Maximum Residue Limit for milk were significantly exceeded.

In birds and other animals, endocrine disrupting chemicals (EDCs), including DDE and PCBs, have been linked to effects on gonadal steroid hormones (Beard et al., 2000) and P450 enzymes, altered thyroid hormone function and neuro-endocrine systems, as well as activation of the stress response (Dawson, 2000; Fry, 1995; Langer et al., 1998). *p,p*′-DDE has been associated with delayed sexual maturation and impaired mating behaviour (Ottinger et al., 2005), thinning of eggshells, developmental effects and embryo mortality (Lundholm, 1997; Zimmermann et al., 1997). Pollutants can also increase oxidative stress and may affect the survival and genetic variation in wild bird populations (Eeva et al., 2006a, 2006b). PCBs and DDT seemingly can also cause fluctuating asymmetry in bird wings (Jenssen et al., 2010), reduce hatchability, induce wasting syndrome, cause skeletal abnormalities, and impair differentiation of reproductive and neurological systems (Fry, 1995; Weiss, 2011). Organic pollutants (such as mirex, chlordane, HCHs, HCB, DDT, PCBs, PCDDs, PCDFs, PBDEs, and HCB) have been associated with a variety of behavioural (Bustnes et al., 2001; Fry, 1995), and developmental and toxic effects in birds (Allen and Thompson, 1996; Fry, 1995) that may impact negatively on wild bird populations.

Whether the levels and eggshell thinning we measured actually impacted on the birds through any mode of action (Giesy et al., 2003), we cannot say. However, endocrine disruption has been detected in the same region we collected the eggs. We have some understanding of DDT in fish from the Luvuvhu River. Mean ΣDDT significantly increased in fish from Nandoni Dam (0.40 mg/kg lm) to Xikundu (8.1 mg/kg lm) 35 km downstream, probably as a result of cumulative DDT loading into the river through runoff (Barnhoorn et al., 2009). The different DDT isomers and congeners also have different endocrine disrupting effects, and signs of endocrine disruption were found in the Mozambique Tilapia. Intersex in male fish was present throughout the Luvuvhu system (Barnhoorn et al., 2010). In addition, the high levels in humans from the DDT-sprayed areas of the Limpopo Province, evident from the high levels in breast milk, has been associated with urogenital birth defects in baby boys, which was significantly higher in boys from mothers living in sprayed homes, than reference mothers (Bornman et al., 2010). The endocrine disrupting signs from fish and humans, very high levels of DDT in bird eggs, eggshell thinning in the Cattle Egrets, and the apparent absence of breeding piscivore birds in the sprayed area (also compare with the desertion of the Great Blue Heron colony in Canada (Laporte, 1982) in Table 2 with the highest ΣDDT at levels higher than found in this study), are strongly suggestive of negative impact on colonially breeding fish-eating birds and terrestrially feeding Cattle Egrets.

4.9. Convergence of pollutant profiles

DDT applied indoors for malaria control finds its way outdoors contaminating the environment and biota. Fig. 3 illustrates this well, and shows all the data integrated through NMS. It is apparent that the homestead-associated House Sparrow has a pollutant profile different form the terrestrially feeding Cattle Egret. Both pollutant profiles intersect to the left, in very close ordination space proximity with the two aquatic-associated species, the Grey Heron and Southern Masked Weaver. The sparrows and egrets occupy much larger profile ranges in ordination space, probably because they are exposed to a wider variety of pollutant profiles through food and environment, explaining why some of their eggs have profiles similar to the aquatic birds. The weaver and heron eggs on the other hand, have much more compact profiles in ordination space, indicating a homogenising effect of the water environment.

It is also clear that the *p,p′*-DDT, applied to the homesteads remains strongly associated with House Sparrows, despite also occurring in all other eggs. The vector for *o,p′*-DDT is intermediate between terrestrial and aquatic, probably reflecting its lower vapour pressure and therefore more likely to escape from the application sites. The *o,p′*-DDT vector (associated with the Cattle Egrets) is almost opposite the *p,p′*-DDE vector that points towards the aquatic birds meaning that where *o,p′*-DDT increases in the terrestrial environment, *p,p′*-DDE increases towards the aquatic environment. All the agriculturally used pesticides (chlordanes, HCB, and HCHs), mirex, and all the PCBs (except CB157) were associated with the Cattle Egrets. CB157 was only detectable in Grey Heron eggs (Table 1).

The convergence of the pollutant profiles of four different species characteristic of the aquatic-associated species is indicative of a homogenizing effect whereby different terrestrial sources of chemicals homogenises into a profile associated strongly with the aquatic environment. Very high levels of DDT were found in House Sparrow eggs (where DDT was applied) and again in Grey Heron eggs (far away from application) where DDT eventually accumulates in aquatic associated food webs. In between (represented by Cattle Egrets) the levels were comparatively lower. It can therefore be deduced that different terrestrial sources of chemicals converge in aquatic-associated systems, indicating that risk from high levels occurs at source, in the associated terrestrial environment, and again where accumulation occurs.

5. Conclusions

DDT applied indoors for malaria control finds its way outdoors contaminating the environment and birds. We have previously argued that some characteristic life-strategies of birds in warm areas (e.g. longer-lived and fewer eggs per clutch) would actually increase the risk to birds compared with similar birds living in colder regions when exposed to the same environmental pollutant levels (Bouwman et al., 2008). If this is the case, then malaria control using DDT probably has more significant impacts on biota than previously realised. Many of the malaria controlled regions of Southern and East Africa also host migratory bird species from Europe and Asia, and consideration should be given to prevent further contamination of the terrestrial and aquatic environments.

The ordination (Fig. 4) presents a striking example of how different profiles of terrestrial sources of pollutants converge into a homogenised aquatic profile in the same area. Although the picture represents relativised and not absolute data, combined with the results in Table 1 and Figs. 2 and 3, motivates for the protection of the aquatic system. However, the eggshell thinning of Cattle Egrets and the high levels in sparrow eggs also motivates for protection closer to the source—the use of DDT in IRS.

The findings of this study indicates that risk assessment and modelling without hard data may miss crucial impacts and risks, as the use patterns and ecosystems in Africa and elsewhere may differ in unexpected ways from the conditions and assumptions of general risk assessment and modelling parameters. Following from this, study designs based on classic assumptions may also miss critical nodes of impacts, supporting explorative study designs, albeit with more samples than the current study.

Combined with the high levels found in breast milk (Bouwman et al., 2012b), IRS with DDT associated with birth defects in baby boys (Bornman et al., 2012), intersex in fish from the Luvuvhu River, high levels of DDT in heron and sparrow eggs, eggshell thinning in Cattle Egrets significantly associated with DDT, and the apparent absence of fish-eating birds in the sprayed area, this paper presents strong arguments for an expedited process of replacing IRS using DDT with other sustainable methods.

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