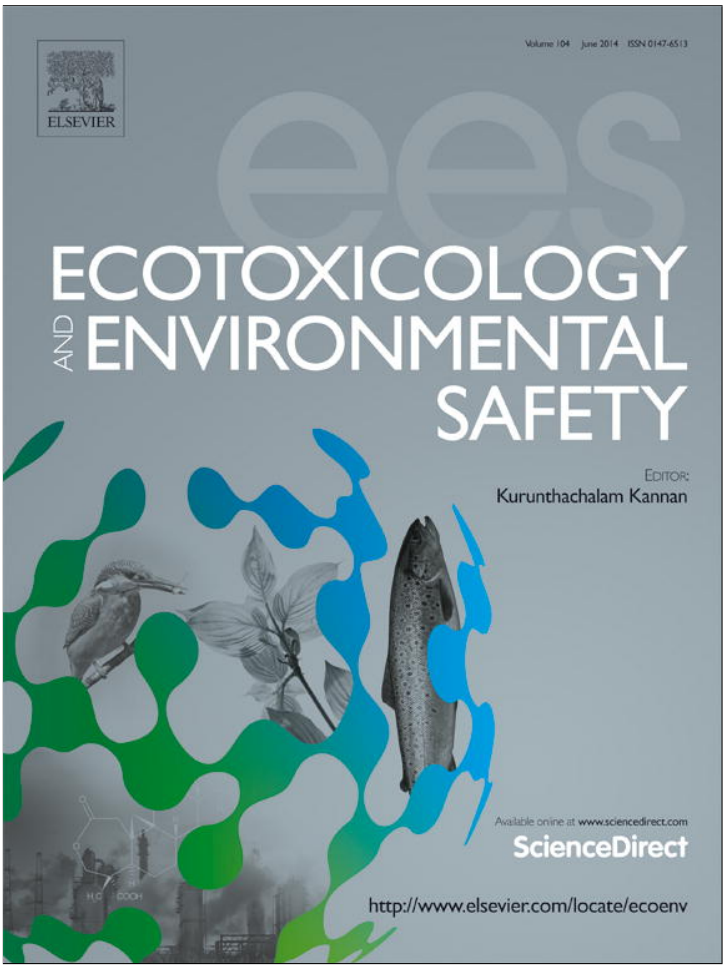

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Chlorinated, brominated, and fluorinated organic pollutants in Nile crocodile eggs from the Kruger National Park, South Africa

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ABSTRACT

Repeated annual episodes of Nile crocodile deaths in two isolated areas of the Kruger National Park prompted the investigation of possible organohalogen pollutant involvement. Crocodile eggs were collected close to one of the mortality sites (Gorge) as well as from a crocodile farm (CF) as reference. ∑DDT was significantly higher in Gorge (450 ng/g wm) than in CF eggs (85 ng/g wet mass). Percentage DDT of ∑DDT was significantly higher in CF (14 per cent) than in Gorge eggs (5 per cent). Mean ∑DDT was almost 70 times higher than mean ∑PCB in Gorge eggs. HCB, β-HCH, mirex, brominated flame retardants (BFRs), and perfluorinated compounds (PFCs) occurred at lower concentrations. We believe that the BFR and PFCs data represent the first published results for any crocodile egg. Thickening of the outer eggshell layer of Gorge eggs was significantly associated with higher concentrations of ∑DDT. Concentrations of ∑DDT and other pollutants were in the same range as eggs from elsewhere, where there were no mortalities. Concentrations of ∑DDT in eggs from healthy Australian crocodiles were of the same orders of magnitude as the current study, making it highly unlikely that the concentrations of pollutants measured in the present study would have caused or substantially contributed towards the mortalities observed. Concerns about reproduction and behaviour remain. As large predators, crocodilians are at the apex of the freshwater aquatic food web. More research is needed to guide measures to manage African freshwater systems so that it will also sustainably accommodate these large, long-lived animals.

1. Introduction

In June 2008, at the onset of the Austral winter, a sudden episode of deaths of large Nile crocodiles *Crocodylus niloticus* was detected in the Olifants Gorge – a steep-walled, rocky river Gorge at the confluence of the Olifants and Letaba rivers in the Kruger National Park (KNP), South Africa. A year later, almost to the week, another bout of deaths occurred. Based on helicopter and night surveys, the estimated number of crocodiles in the Olifants Gorge declined from 780 in 2008 to 505 in 2009. At least 170 carcasses have now been counted (Ferreira and Pienaar, 2011). Downstream of the Olifants Gorge, in Mozambique, is the Massingr Dam that backs up into the gorge (Fig. 1). Smaller mortality episodes occurred yearly from then on, every time at the onset of colder weather. These episodes occurred in a period when the crocodile populations in many other rivers of the KNP were increasing (Ferreira and Pienaar, 2011). Similar mortalities were also detected in the Sabie River in the KNP (also with a dam across the border in Mozambique), and Loskop Dam, upstream of the Olifants Gorge in the Olifants River. The crocodiles of the Flag Boshielo Dam and Phalaborwa Weir, located in the Olifants River between Loskop Dam and Olifants Gorge, were not affected and seemingly healthy.

The deaths of the crocodiles from Olifants Gorge and Sabie River were associated with steatitis, a condition affecting the fat tissue of the crocodile, causing the tissue to harden, becoming inflamed and yellow (Scott et al., 1995; Fytianou et al., 2006). Most of the crocodiles died with large but steatitic fat reserves and empty stomachs. Of the 37 dead crocodiles that were inspected, 83 per cent were males. Steatitis was also detected in fish, terrapins, and herons at different locations on the Olifants River (Myburgh and Botha, 2009; Ashton, 2010; Huchzermeyer et al., 2011). Parasitic and bacterial diseases have been excluded. The exact cause(s) of the steatitis is not yet known, but speculation includes

- microcystins from cyanobacteria in the water and food of the crocodiles (Myburgh and Botha, 2009),

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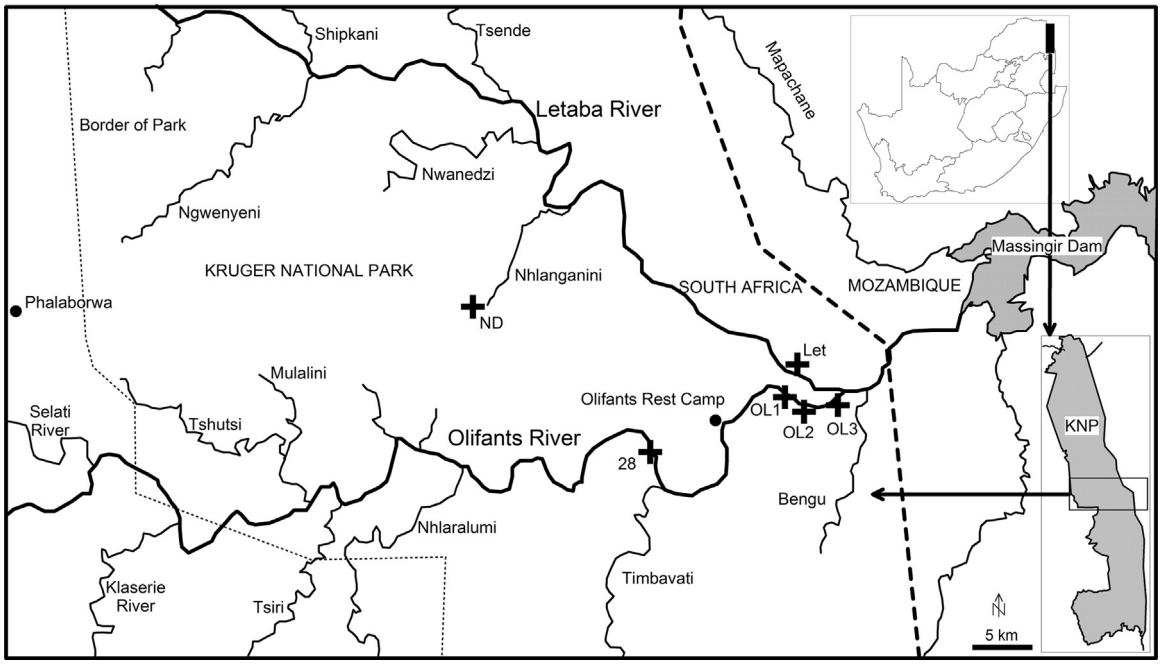

Fig. 1. Locations in the Kruger National Park where Nile crocodile eggs were collected.

- trapping of pollutants settling out of the water due to the effect of the river slowing down in the upper reaches of the Massingr Dam across the border in Mozambique (Osthoff et al., 2010),
- crocodiles feeding on dead and rancid fish caused by anthropogenic ecosystem impacts (Ashton, 2010),
- crocodiles feeding on dead and rancid fish from fishnets (Huchzermeyer et al., 2011),
- broad-scale cascades of environmental deterioration and pollution (Ferreira and Pienaar, 2011),
- crocodiles feeding on local catfish (*Clarias garipienus*) with steatitis with no known cause but pollution is suspected (Huchzermeyer et al., 2011),
- changes in the food web due to changes in the ecosystem, combined with a yet to be found extralimital fish species as vector of the cause (Woodborne et al., 2012),
- high concentrations of aluminium in the body fat of Nile tilapia (*Oreochromus mossambicus*) that interferes with cellular metabolism (including lipid-peroxidation) and affects predators such as crocodiles (Oberholster et al., 2012),
- a seasonal dietary change due to upstream migration of alien silver carp (*Hypophthalmichthys molitrix*) with a fatty acid composition different from local fish (Huchzermeyer, 2012).

Some of the problems when dealing with investigating wildlife mortality episodes in remote areas with an unknown cause(s) are the lack of samples after the event, preceding reference conditions, and a good understanding of the aetiology. Assuming that chemicals were involved, the living crocodiles following such episodes are survivors presumably not as exposed as the dead ones – nevertheless, the concentrations of chemicals in the survivors may contain important information and clues as to the cause, or may exclude potential causes as primary actor. One of the possible contributors to these episodes of steatitis-associated mortalities may be persistent organic pollutants (POPs) that, from previous work (Van Dyk, 1978; Van Dyk et al., 1982; Grobler, 1994; Ferreira and Pienaar, 2011; Roos et al., 2011), are known to be present in the system. Rauschenberger et al. (2004) investigated the ratios between residues in crocodile eggs and maternal body concentrations and found a relationship of close to parity for *p*,*p*-DDE in liver, muscle, bile and adipose tissue. The concentrations in Nile crocodile eggs could therefore be expected to be similar in the maternal body, and therefore informative of involvement or association.

Woodword et al. (2011) reported on the relationships between the deaths of 450 crocodiles in Florida and POPs (especially DDT) but did not mention pansteatitis as one of the symptoms. Although there is no evidence that any POP or similar chemicals can cause pansteatitis, neither has it been entirely excluded as a contributing factor. The concentrations of POPs related to agriculture, malaria control, or industry in crocodile tissue will therefore be informative as for potential involvement of these or associated chemical compounds. High concentrations of PCBs in a single marine turtle with pansteatitis have been found, but the high concentrations could not be linked to the symptoms (Orós et al., 2013).

The likely sources of the POPs are industry, mining, agriculture, electricity generation, and DDT from malaria control. This study would represent the first ever published investigation about POPs in crocodile eggs from the KNP and from South Africa. The aim of this study was to investigate the presence and concentrations of selected chlorinated, brominated, and fluorinated organic pollutants in crocodile eggs from the KNP, and to assess their possible role in the mortalities. For this project, we collected eggs both from the wild populations and from a nearby crocodile farm for comparison.

2. Methods

2.1. Sampling site and egg collection

The crocodile mortalities occurred above and below the confluence of the Letaba and Olifants rivers (Fig. 1). Below the confluence, the river continues as the Olifants River, flowing through a rough and difficult to access Olifants Gorge. In the Olifants Gorge, before the border, the effect of damming by the Massingr Dam is noticeable by heavy sedimentation, shallowing of the river, and the increasing extent of mud banks in an otherwise regularly scoured river. About 170 carcasses have been found, the majority in the Olifants Gorge, and for about 5 km upstream of the confluence in both rivers. Because of the steep slopes and little vegetation, few terrestrial animals are found in the Gorge and the crocodiles here therefore thrive mainly on the plentiful fish.

Egg collection took place between October and December 2009. Field rangers searched for disturbed patches of shore sand by helicopter, marking potential nest

Table 1
Concentrations of halogenated compounds in Nile crocodile eggs (ng/g), based on wet mass and summarised based on lipid mass.

Wet mass (ng/g)	Per cent extracted	LOQ	Crocodile farm (CF), $n=10$						Letaba (Let), $n=6$						Olifants River 1 (OR1), $n=3$					
			Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos
HCB	92	0.010	0.15	0.12	0.073	0.092	0.32	10	0.23	0.22	0.015	0.21	0.26	6	0.22	0.24	0.029	0.19	0.24	3
ß-HCH	96	0.036	1.1	0.31	1.7	0.088	4.3	10	1.3	1.3	0.066	1.3	1.43	6	0.88	0.94	0.10	0.76	0.94	3
Oxychlor	98	0.028	0.71	0.17	1.1	0.036	1.9	3	0.17	0.17	0.012	0.15	0.19	6	0.29	0.33	0.059	0.23	0.33	3
Cis-chlordane	98	0.028	< LOQ					0	< LOQ					0	< LOQ					0
Trans-nonachlor	115	0.024	0.18					1	0.16	0.13	0.024	0.13	0.18	4	0.23	0.19	0.11	0.14	0.35	3
T-Chlor			0.58	0.18	0.90	0.036	1.9	4	0.27	0.31	0.093	0.15	0.37	6	0.52	0.52	0.054	0.47	0.58	3
Mirex	107	0.03	< LOQ					0	0.13	0.10	0.10	0.042	0.32	6	0.095	0.098	0.011	0.083	0.11	3
p,p′-DDE	92	0.024	610	7.6	1300	6.2	3100	10	4000	380	310	3700	4500	6	3500	3500	110	3400	3600	3
p,p′-DDD	103	0.032	75	4.1	150	0.88	380	10	700	700	21	660	720	6	620	630	17	600	630	3
p,p′-DDT	100	0.025	89	1.8	180	0.52	450	10	190	190	9.2	170	190	6	230	230	440	210	240	3
T-DDT			85	1.4	0.49	0.49	420	10	500	510	460	460	520	6	510	540	440	440	560	3
Per cent DDT			13	14	5.1	5.4	19	10	3.9	3.9	0.28	3.4	4.2	6	5.3	5.5	0.41	4.8	5.5	3
PCB-28	107	0.030	0.3	0.4	0.14	0.14	0.45	8	0.14	0.15	0.02	0.10	0.17	6	0.25	0.27	0.051	0.19	0.28	3
PCB-52	130	0.033	0.19	0.18	0.059	0.11	0.28	7	0.23	0.22	0.03	0.19	0.27	6	0.26	0.32	0.12	0.12	0.33	3
PCB-66	137	0.039	0.19	0.17	0.10	0.056	0.36	8	0.14	0.13	0.014	0.12	0.16	6	0.13	0.14	0.038	0.092	0.17	3
PCB-74	93	0.033	0.14	0.073	0.14	0.041	0.36	8	0.10	0.099	0.011	0.082	0.12	6	0.14	0.14	0.028	0.11	0.16	3
PCB-99	105	0.039	0.15	0.16	0.091	0.060	0.25	4	0.17	0.18	0.036	0.126	0.21	6	0.20	0.22	0.067	0.12	0.25	3
PCB-101	104	0.038	0.087	0.072	0.052	0.044	0.14	3	0.32	0.24	0.15	0.19	0.56	6	0.31	0.31	0.20	0.11	0.51	3
PCB-105	102	0.064	0.057	0.034	0.04	0.025	0.12	6	0.093	0.092	0.015	0.073	0.12	6	0.11	0.12	0.017	0.094	0.13	3
PCB-118	95	0.028	0.13	0.087	0.12	0.044	0.34	9	0.21	0.21	0.027	0.17	0.24	6	0.26	0.26	0.0082	0.25	0.27	3
PCB-128	101	0.033	0.083					1	0.09	0.089	0.0095	0.082	0.11	6	0.10	0.10	0.014	0.087	0.12	3
PCB-138	85	0.019	0.35	0.078	0.53	0.045	1.3	8	1.2	1.2	0.074	1.1	1.32	6	1.4	1.5	0.16	1.2	1.5	3
PCB-149	90	0.023	0.16					1	0.29	0.29	0.026	0.26	0.33	6	0.24	0.24	0.043	0.20	0.29	3
PCB-153	95	0.017	0.49	0.18	0.72	0.057	1.9	10	1.5	1.5	0.14	1.32	1.7	6	1.6	1.6	0.18	1.4	1.8	3
PCB-170	91	0.020	0.31	0.43	0.25	0.021	0.48	3	0.27	0.28	0.04	0.21	0.32	6	0.29	0.30	0.020	0.27	0.30	3
PCB-180	89	0.014	0.35	0.089	0.56	0.039	1.4	10	0.86	0.86	0.077	0.77	0.93	6	0.84	0.84	0.045	0.79	0.88	3
PCB-183	83	0.019	0.74					1	0.63	0.63	0.053	0.55	0.72	6	0.46	0.51	0.19	0.26	0.62	3
PCB-187	73	0.016	0.085	0.028	0.13	0.026	0.32	5	0.38	0.38	0.027	0.35	0.42	6	0.40	0.40	0.037	0.36	0.43	3
PCB-194	71	0.017	0.17	0.12	0.16	0.04	0.45	8	0.17	0.16	0.017	0.15	0.19	6	0.13	0.14	0.015	0.12	0.14	3
PCB-206	71	0.018	0.10	0.051	0.13	0.039	0.37	6	0.21	0.21	0.022	0.18	0.23	6	0.19	0.19	0.0046	0.18	0.19	3
T-PCB			2.4	1.1	2.9	0.097	8.8	10	7.0	7.0	0.45	6.5	7.7	6	7.3	7.8	1.1	6.0	7.9	3
BDE-28	98	0.015	< LOQ					0	< LOQ					0	0.015					1
BDE-47	90	0.020	0.069	0.069	0.034	0.038	0.099	3	0.16	0.16	0.015	0.14	0.18	6	0.20	0.21	0.014	0.19	0.21	3
BDE-99	111	0.030	0.089	0.086	0.013	0.077	0.11	4	0.040	0.038	0.0038	0.037	0.044	3	< LOQ					0
BDE-100	115	0.030	0.030					1	0.054	0.051	0.012	0.037	0.069	6	0.057	0.057	0.0080	0.049	0.065	3
BDE-154	102	0.020	0.044	0.039	0.011	0.037	0.064	6	0.050	0.048	0.0059	0.046	0.057	3	0.055	0.045	0.036	0.025	0.094	3
BDE-183	102	0.020	0.080	0.056	0.071	0.023	0.23	10	0.026					1	0.045	0.021	0.042	0.020	0.094	3
T-BDE			0.19	0.11	0.20	0.023	0.55	10	0.28	0.29	0.08	0.18	0.479	6	0.44	0.43	0.037	0.41	0.48	3
PBEB	88	0.010	< LOQ					0	< LOQ					0	0.023	0.023	0.0007	0.022	0.023	2
Lipid mass (ng/g)																				
Per cent lipid			9.58	9.83	1.91	5.1	11.7		10.3	10.3	0.60	9.7	11.4	6	11.8	12.3	1.19	10.4	12.6	3
HCB			1.6	1.2	0.64	1.1	2.9	10	2.2	2.2	0.06	2.1	2.2	6	1.9	1.9	0.074	1.8	1.9	3
T-HCH			10	3.1	15	1.7	39	10	13	13	0.47	12	13	6	7.5	7.5	0.17	7.3	7.6	3
T-Chlor			5.2	1.7	8.0	0.34	17	4	2.7	3.0	0.90	1.5	3.54	6	4.5	4.3	0.92	3.7	5.5	3
T-DDT			780	13	1600	9.3	3900	10	4800	4700	320	4500	5400	6	4400	4400	88	4300	4400	3
Mirex			< LOQ					0	1.3	0.99	1.0	0.36	3.3	6	0.81	0.79	0.041	0.78	0.86	3
T-PCB			2.4	1.1	2.9	0.097	8.8	10	7.0	7.0	0.45	6.5	7.7	6	7.3	7.8	1.1	6.0	7.9	3
T-BDE			1.6	1.2	0.64	1.1	2.9	10	2.2	2.2	0.059	2.1	2.2	6	1.9	1.9	0.074	1.8	1.9	3
PBEB			< LOQ					0	< LOQ					0	0.19	0.19	0.010	0.18	0.19	2
Eggshell thickness (μm)																				
Inner			177	171	37.1	136	234	10	192	198	24	157	221	6	191	190	2.6	189	194	3
Outer			367	360	40	319	443	10	382	381	15	366	406	6	497	497	5	492	502	3
Total			544	526	73	457	648	10	575	569	35	523	627	6	688	691	5.2	682	691	3

Table 1 (*continued*)

Wet mass (ng/g)	Per cent extracted	LOQ	Olifants River (OR2), $n=3$						Olifants River (OR3), $n=3$						Nhlanganini Dam (ND), $n=2$						28
			Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	
HCB	92	0.010	0.19	0.19	0.035	0.16	0.23	3	0.16	0.17	0.03	0.13	0.18	3	0.39	0.39	0.021	0.37	0.40	2	0.21
ß-HCH	96	0.036	0.98	0.86	0.30	0.76	1.32	3	0.70	0.71	0.021	0.68	0.72	3	0.27	0.27	0.0099	0.26	0.27	2	0.33
Oxychlor	98	0.028	0.19	0.19	0.038	0.15	0.23	3	0.13	0.14	0.0061	0.13	0.14	3	0.11	0.112	0.0014	0.11	0.11	2	0.15
Cis-chlordane	98	0.028	< LOQ					0	< LOQ					0	< LOQ					0	0.011
Trans-nonachlor	115	0.024	0.26	0.26	0.13	0.16	0.35	2	0.14	0.14	0.054	0.11	0.18	2	0.045	0.045	0.011	0.037	0.052	2	0.040
T-Chlor			0.36	0.31	0.20	0.19	0.58	3	0.23	0.24	0.098	0.13	0.32	3	0.16	0.16	0.012	0.15	0.17	2	0.20
Mirex	107	0.03	0.085	0.083	0.016	0.071	0.10	3	0.083	0.088	0.013	0.069	0.093	3	0.18	0.18	0.0035	0.18	0.18	2	0.18
p,p′-DDE	92	0.024	3400	3400	43	3400	3500	3	2600	2600	83	2500	2700	3	5000	5000	480	460	5300	2	4400
p,p′-DDD	103	0.032	600	620	40	550	630	3	560	540	51	520	620	3	240	240	7.1	240	250	2	260
p,p′-DDT	100	0.025	210	210	19	190	230	3	170	160	12	160	180	3	81	81	10	74	89	2	130
T-DDT			410	440	300	300	480	3	320	310	280	280	380	3	610	610	580	580	650	2	550
Per cent DDT			5.0	5.1	0.43	4.6	5.5	3	5.0	4.9	0.21	4.9	5.2	3	13	13	2.8	11	15	2	23
PCB-28	107	0.030	0.26	0.27	0.027	0.23	0.28	3	0.19	0.19	0.048	0.15	0.24	3	0.11	0.11	0.050	0.077	0.15	1	0.29
PCB-52	130	0.033	0.19	0.21	0.059	0.12	0.23	3	0.18	0.18	0.052	0.13	0.23	3	0.19	0.19	0.037	0.16	0.22	1	0.1
PCB-66	137	0.039	0.084	0.092	0.024	0.057	0.10	3	0.088	0.085	0.011	0.079	0.1	3	< LOQ					0	0.43
PCB-74	93	0.033	0.087	0.083	0.018	0.071	0.11	3	0.065	0.065	0.006	0.059	0.071	3	< LOQ					0	0.29
PCB-99	105	0.039	0.13	0.12	0.044	0.097	0.18	3	0.13	0.12	0.013	0.12	0.14	3	0.082	0.082	0.016	0.07	0.093	2	0.34
PCB-101	104	0.038	0.17	0.16	0.062	0.11	0.24	3	0.15	0.14	0.018	0.13	0.17	3	0.13	0.13	0.011	0.12	0.14	2	0.34
PCB-105	102	0.064	0.10	0.094	0.039	0.07	0.15	3	0.075	0.069	0.012	0.067	0.089	3	0.037	0.037	0.0050	0.033	0.040	2	0.24
PCB-118	95	0.028	0.23	0.26	0.069	0.15	0.27	3	0.16	0.14	0.044	0.14	0.22	3	0.087	0.087	0.0050	0.083	0.09	2	0.59
PCB-128	101	0.033	0.090	0.087	0.02	0.071	0.11	3	0.064	0.061	0.0089	0.057	0.074	3	0.028	0.028	0.0014	0.027	0.029	2	0.080
PCB-138	85	0.019	1.1	1.2	0.28	0.77	1.3	3	0.86	0.82	0.11	0.77	0.98	3	0.74	0.74	0.045	0.70	0.77	2	2.1
PCB-149	90	0.023	0.24	0.20	0.085	0.17	0.33	3	0.24	0.20	0.085	0.17	0.33	3	0.17	0.17	0.0091	0.16	0.18	2	< LOQ
PCB-153	95	0.017	1.4	1.4	0.42	0.98	1.8	3	1.2	1.1	0.11	1.07	1.3	3	0.67	0.67	0.04	0.64	0.70	2	3.3
PCB-170	91	0.020	0.25	0.27	0.07	0.18	0.31	3	0.20	0.19	0.037	0.17	0.24	3	0.12	0.12	0.0085	0.12	0.13	2	0.85
PCB-180	89	0.014	0.74	0.79	0.20	0.52	0.91	3	0.64	0.61	0.10	0.55	0.75	3	0.40	0.40	0.02	0.39	0.41	2	1.9
PCB-183	83	0.019	0.38	0.41	0.11	0.26	0.46	3	0.21	0.24	0.049	0.16	0.24	3	0.31	0.31	0.035	0.29	0.34	2	0.68
PCB-187	73	0.016	0.34	0.36	0.09	0.24	0.41	3	0.29	0.28	0.040	0.26	0.34	3	0.19	0.19	0.0078	0.19	0.20	2	0.64
PCB-194	71	0.017	0.12	0.12	0.032	0.092	0.16	3	0.12	0.109	0.018	0.11	0.14	3	0.07	0.074	0.0035	0.071	0.076	2	0.5
PCB-206	71	0.018	0.17	0.18	0.05	0.12	0.21	3	0.16	0.15	0.029	0.13	0.19	3	0.11	0.11	0.0078	0.11	0.12	2	0.45
T-PCB			6.1	6.0	1.4	4.6	7.5	3	4.9	4.8	0.60	4.4	5.5	3	3.4	3.4	0.21	3.3	3.6	2	13
BDE-28	98	0.015	< LOQ					0	< LOQ					0	< LOQ					0	< LOQ
BDE-47	90	0.020	0.17	0.18	0.056	0.11	0.22	0	0.13	0.13	0.028	0.10	0.16	3	0.028	0.028	0.0085	0.022	0.03	2	0.22
BDE-99	111	0.030	< LOQ					0	< LOQ					0	< LOQ					0	0.038
BDE-100	115	0.030	0.045					1	0.034	0.034	0.0035	0.03	0.037	3	< LOQ					0	0.075
BDE-154	102	0.020	0.040	0.034	0.012	0.033	0.054	3	0.10					1	< LOQ					0	< LOQ
BDE-183	102	0.020	0.021					1	< LOQ					0	0.485					1	< LOQ
T-BDE			0.26	0.29	0.07	0.18	0.32	3	0.023	0.22	0.039	0.2	0.3	3	0.33	0.33	0.43	0.022	0.63	2	1.1
PBEB	88	0.010	< LOQ					0	< LOQ					0	< LOQ					0	< LOQ
Lipid mass (ng/g)																					
Per cent lipid			9.58	10.4	2.27	7.01	11.3	3	9.78	9.81	1.59	8.18	11.4	3	11.6	11.6	0.070	11.5	11.6	2	11.5
HCB			2.0	2.0	0.23	1.8	2.3	3	1.7	1.6	0.18	1.5	1.9	3	3.3	3.3	0.20	3.2	3.5	2	1.8
T-HCH			10	12	2.7	7.3	12	3	7.3	6.9	1.2	6.3	8.6	3	2.3	2.3	0.095	2.3	2.4	2	2.8
T-Chlor			3.9	4.5	2.0	1.7	5.5	3	2.5	3.0	1.2	1.1	3.3	3	1.4	1.4	0.11	1.3	1.4	2	1.7
T-DDT			4200	4200	17	4200	4300	3	330	3400	130	3200	3400	3	5300	5300	470	5000	5700	2	4700
Mirex			0.90	0.90	0.11	0.79	1.0	3	0.86	0.85	0.041	0.82	0.90	3	1.6	1.6	0.04	1.6	1.60	2	1.5
T-PCB			6.1	6.0	1.4	4.6	7.5	3	4.9	4.8	0.60	4.4	5.5	3	3.4	3.4	0.21	3.3	3.6	2	13
T-BDE			2.0	2.0	0.23	1.8	2.3	3	1.7	1.6	0.18	1.5	1.9	3	3.3	3.3	0.20	3.2	3.5	2	1.8
Eggshell thickness (μm)																					
Inner			182	172	29	160	215	3	205	189	33	182	243	3	203	203	15	192	213	2	179
Outer			428	428	7	421	434	3	412	408	15	399	428	3	453	453	13	444	462	2	424
Total			610	594	29	593	643	3	616	610	32	588	651	3	656	656	2.1	654	657	2	603

sites with a global positioning system. The locations (Fig. 1) were then located by the ground team. Eggs were found by digging in the sand by hand. Six was the maximum number of eggs per nest removed, and signs of digging carefully smoothed away to reduce subsequent chances of predation. One egg (Egg 28) was collected opportunistically in 2008 (after the first mortalities started) upstream of the confluence, and was included as a singleton. For reference, ten eggs were collected from a crocodile farm located south of the KNP. Crocodile eggs were wrapped in foil (pre-cleaned with acetone and hexane), labelled, and frozen.

2.2. Sample preparation and analyses

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 per cent ethanol. The sample preparation, extraction and clean-up procedures were done while protecting the samples from UV to avoid any degradation of light-sensitive brominated compounds. The egg contents were homogenised using ultrasound and then frozen. Samples were shipped to Norway and received frozen. The Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science (NVH) in Oslo is accredited by Norwegian Accreditation for the determination of PCBs and organochlorine pesticides (OCPs) in biological materials according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137). The procedures for extraction, analyses (gas-chromatography and mass spectrometry), and analytical quality control are described in Brevik (1978) and Bouwman et al. (2012). Perfluorinated compounds (PFCs – Table 2) were only determined for pools, using gas-chromatography and mass spectrometry. The compounds detected, their extraction efficiencies and limits of quantification (LOQs) are listed in Tables 1 and 2 as ng/g. We prefer reporting and discussing concentrations based on wet mass (wm) rather than lipid mass (lm), as embryonic metabolism will affect the egg's lipid content (Romanoff, 1932). Wet mass is also used for determining risk. We do however, present summarised lipid-based data for comparisons with others (Table 1).

2.3. Eggshell thickness

After the egg contents were removed, the shells became brittle very quickly and it was impossible to get reliable eggshell thickness measurements physically with a calliper. Instead, three fragments of inner and outer shell each were removed from the same position on the equator of each egg (the two layers separated very easily) and prepared for scanning electron microscopy (SEM). Each inner and outer shell fragment was measured at five different points.

2.4. Data treatment

Descriptive statistics was done using GraphPad Prism 5.05 (GraphPad Software, San Diego California USA www.graphpad.com) using only positive quantifications. The concentration and eggshell thickness data were normally distributed. Multivariate analyses were done, but contributed little more information than presented here. Univariate comparisons of concentrations between clutches were not attempted because of the few eggs per clutch and large variations in measurements. The three clutches from the Olifants River and one from Letaba were, however, combined as Gorge (there were no significant differences (one-way ANOVA) between the OR1-3 and Let for any compound), and compared with the crocodile farm (CF) eggs, using unpaired, two-tailed *t*-tests (Mann Whitney nonparametric test comparing the distributions of two unmatched groups). The two samples of Nhlanganini Dam and one sample representing Egg 28 precluded their meaningful comparisons with other clutches using ANOVA. The data from ND and Egg 28 were included, however, in the graphs for visual comparisons (Fig. 2). We investigated the relationships between compounds and inner-, outer-, and complete eggshell thickness with linear and non-linear regression against pollutant parameters.

3. Results

3.1. Collection

Ethical approval was granted by the North-West University (NWU-00055-07-S3). Twenty-seven eggs were collected from five nests and from a crocodile farm (CF, $n=10$ randomly sampled from hatchery), south of the KNP. Four nests were in the Olifants (OR1 $n=3$, OR2 $n=3$, and OR3 $n=3$) and Letaba (Let $n=6$) rivers upstream of the confluence, and one nest, selected from Nhlanganini Dam (ND $n=2$), a small tributary with a catchment entirely in the KNP (Fig. 1). Therefore, this tributary should be entirely free from any pollutant sources other than airborne and what is brought in by biota. The crocodile farm feeds their animals mainly with chicken sourced from a commercial supplier, and no DDT is used here for any purpose. We have no information for any female for any clutch.

3.2. Concentrations

α- and γ-HCH (hexachlorocyclohexane) could not be quantified. HCB (hexachlorobenzene), β-HCH, oxychlordane, *trans*-nonachlor, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, and almost all PCBs were present in all eggs (Table 1). *Trans*-nonachlor was detected in only one egg from CF. *Cis*-chlordane and BDE-208 were detected in Egg 28 only. Mirex was detected in all wild eggs, but not in any of the CF eggs. The polybrominated diphenyl ethers BDEs −206, −207, −209, PBT (pentabromotoluene), hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDE), pentabromoethylbenzene (PBEB), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), hexabromobenzene (HBB), and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) were determined, but only PBEB could be quantified. BDE-28 was detected in only one egg, from OR3, and BDE-47 was only detected in CF eggs.

The means and distribution statistics of the chemical analyses of the organochlorines and organobromines are presented in Table 1 expressed as ng/g (either as wet mass – wm, or lipid mass – lm), and some compounds are presented graphically, with 95 per cent confidence intervals, in Fig. 2. In Fig. 2A–F, comparisons are shown using the OR1-3 and Let eggs combined as Gorge. Using unpaired, two-way *t*-tests, the following compounds showed significant differences of means between CF and Gorge eggs: HCB ($p=0.0074$), *p,p′*-DDT ($p=0.0336$), *p,p′*-DDE ($p<0.0001$), *p,p′*-DDD ($p<0.0001$), ∑DDT ($p<0.0001$), per cent DDT ($p<0.0001$), and ∑PCBs β-HCH ($p=0.9884$), and ∑BDEs ($p=0.0567$), there were no significant differences between Gorge and CF eggs, and mirex could not be compared, as CF eggs had no detectable concentrations. Although Gorge eggs had higher ∑DDT concentrations than CF eggs, it had a significantly lower mean per cent DDT.

Table 2 provides the concentrations for the PFCs determined, based on wet mass and lipid mass. PFHxS (sodiumperfuoro-1-hexasulfonate), PFDoA (perfluoro-n-dodecanoic acid), and PFTrDA (perfluoro-tri-dodecanoic acid) were not detected in any pool. Perfluoro-1-octane sulphonate (PFOS) and perfluoro-*n*-decanoic acid (PFDA) were detected in all pools. Perfluoro-*n*-octanoic acid

Table 2
Concentrations (ng/g wm) of PFCs in crocodile egg pools. See text for abbreviations.

Compound	Per cent extracted	LOQ	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5
			CF	Let	Gorge	ND	Egg 28
Wet mass							
PFHxS	92	0.45	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PFOS	165	0.45	0.82	12	24	6.6	26
PFOA	115	0.69	< LOQ	< LOQ	< LOQ	0.79	< LOQ
PFNA	126	1.67	< LOQ	< LOQ	< LOQ	2.1	< LOQ
PFDA	88	0.27	0.43	1.4	1.4	0.87	1.3
PFUdA	135	1.35	< LOQ	< LOQ	< LOQ	1.9	< LOQ
PFDoA	104	2.25	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PFTriA	83	2.25	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Total PFC			1.0	14	25	12	27
Lipid mass							
Per cent lipid			9.5	10.6	11.2	12.2	11.5
PFHxS			< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PFOS			8.6	117	212	54	223
PFOA			< LOQ	< LOQ	< LOQ	6.5	< LOQ
PFNA			< LOQ	< LOQ	< LOQ	17	< LOQ
PFDA			4.5	13	13	7.1	11
PFUdA			< LOQ	< LOQ	< LOQ	15	< LOQ
PFDoA			< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
PFTriA			< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Total PFC			13	130	225	100	235

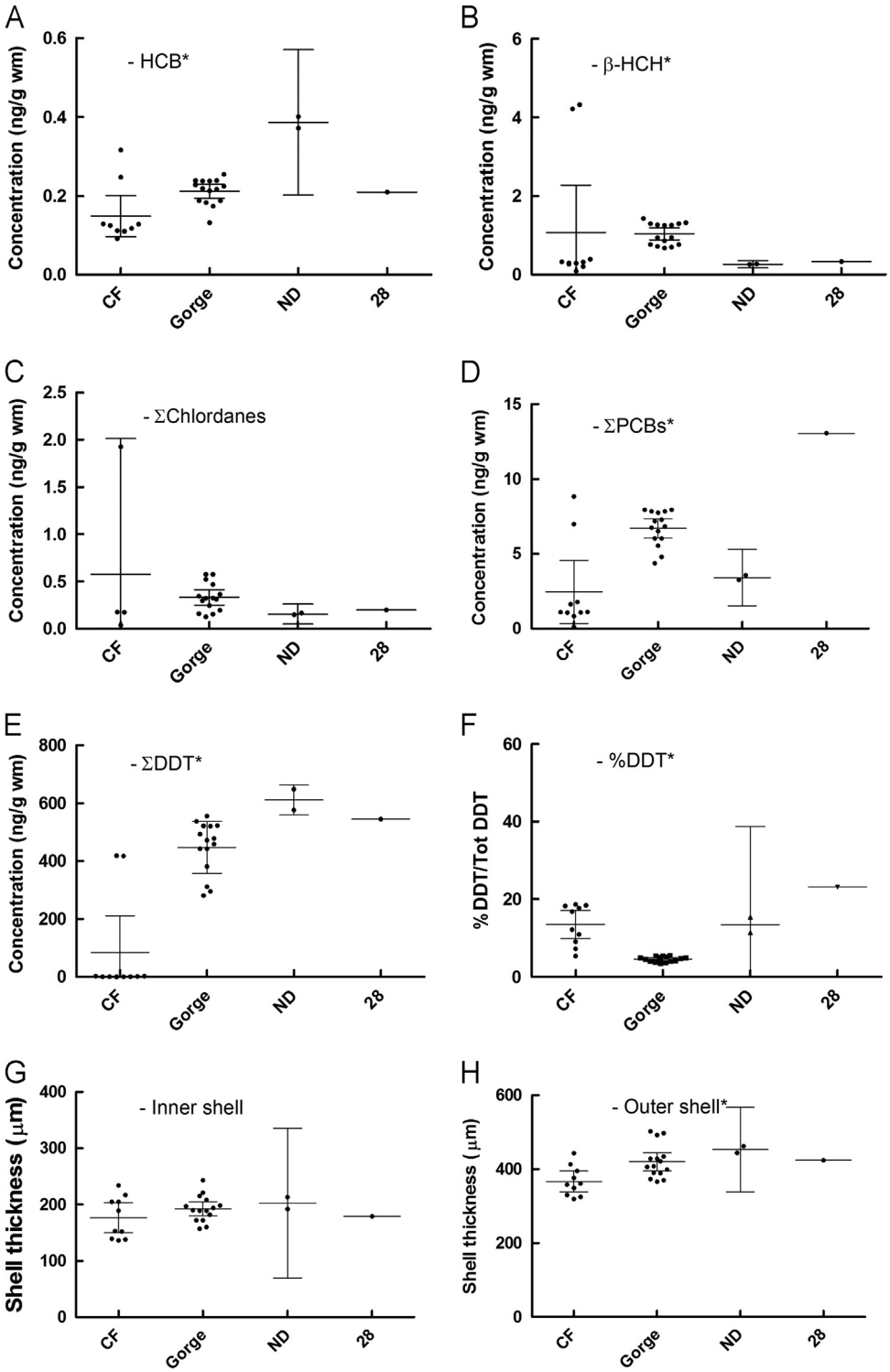

Fig. 2. Graphical represenation of results, indicating means and 95 per cent confidence intervals for Gorge, Crocodile Farm (CF), Nhlanganini, and Egg 28. Compounds indicated with an * means significant difference in means (unpaired, two-tailed, *t*-test, $p < 0.05$) between Gorge and CF egg mean concentrations.

(PFOA) and perfluoro-*n*-nonanoic acid were only detected in Nhlanganini Dam. CF eggs (Pool 1) had the lowest ∑PFC (1 ng/g wm) and Egg 28 (Pool 5) had the highest (27 ng/g).

3.3. *Eggshell thickness*

Alligator eggshells have two major layers: an outer calcified layer consisting of calcite crystals, and an inner mammillary layer, the two layers separated by an organic layer (Wink and Elsey,1994), where the two layers of the crocodile eggshells separated. Fig. 2G and H show the inner and outer eggshell thicknesses of the different groups. There was a significant difference between CF (thinner) and Gorge (thicker) eggs for outer shell thickness (unpaired, two-tailed *t*-test on untransformed data, $p=0.0061$), but not for inner shells (unpaired, two-tailed *t*-test on untransformed data, $p=0.2046$). No meaningful linear or non-linear regressions could be obtained between eggshell and any pollutant parameters. However, it can be seen from Fig. 2E and H that the Gorge eggshells were both

significantly thicker and had significantly higher concentrations of ∑DDT compared with CF.

4. Discussion

4.1. Chlorinated compounds

Almost all chlorinated compounds analysed were detected in crocodile eggs from the KNP (Table 1), confirming that breeding female crocodiles are exposed via water and/or food to these compounds. CF eggs differed significantly from the field collected eggs. Notably, mirex, a fairly ubiquitous pollutant found in bird eggs elsewhere in South Africa (Bouwman et al., 2008, 2013), was below LOQ in CF eggs, but quantifiable in all field-collected eggs (the same laboratory analysed the bird and crocodile eggs). The difference between Gorge and CF eggs was also significant for HCB, ∑PCBs, and ∑DDT; in all cases, the Gorge eggs had the higher concentrations (Fig. 2). Despite the Gorge eggs having ∑DDT concentrations (450 ng/g wm) five times higher than the CF eggs (85 ng/g wm), the CF eggs had a significantly higher mean per cent DDT (13 per cent vs. 4.6 per cent). CF maternal crocodiles were therefore exposed to lower concentrations of DDT in their food, but the ∑DDT seemed to have come from more recent release sources as the proportion of the *p,p′*-DDT to ∑DDT was more than double compared with the Gorge eggs. The chickens used for feeding the CF crocodiles came mainly from commercial broiler production hundreds of kilometres to the west, in part, explaining the differences between CF and Gorge eggs.

Further support for geographical differences comes when Gorge and ND eggs are compared. Although represented by only two eggs (precluding statistical comparisons), ND had visually higher concentrations of HCB and ∑DDT, and lower concentrations of ∑PCBs (Table 1 and Fig. 2) compared with Gorge. The lower concentrations of ∑PCBs suggest that the Nhlanganini tributary of the Olifants River had much less influence from industrial pollution, but the mean ∑BDE concentrations were much the same (Gorge=2.9 ng/g wm; ND=2.8 ng/g wm). Also, the mean per cent DDT in ND eggs was the same as for CF, and much higher than for Gorge eggs (ND=13 per cent vs. CF=13 per cent vs. Gorge=4.6 per cent). The causes of these conflicting patterns may be due to the breeding female crocodile moving between a greater polluted Olifants River (the Olifants River main channel is about 20 km away from the ND nest; Fig. 1), and a presumably less polluted Nhlanganini Stream and Dam (the entire Nhlanganini catchment is within the KNP), combined with differential uptake dynamics for the different classes of compounds. The ages of the females and the number of clutches they have laid can also influence the concentrations of pollutants in crocodiles, but the differences in per cent DDT support a geographic interpretation. As for Wu et al. (2006), we found that there was little inter-nest variation in pollutant concentrations in eggs, indicating that the eggs sampled represented the actual levels in clutches very well. Crocodilians ovulate all eggs simultaneously (Woodword et al., 2011; Lance, 1989), explaining why the pollutant concentrations in eggs from clutches are similar.

The lower mean HCB concentration in the CF eggs indicates that the feed supply chain for battery chickens has lower HCB contamination than the food web in the KNP. HCB has agricultural, combustion, and industrial sources. The higher mean HCB concentration in Gorge and ND eggs suggests combustion as a source, reaching the KNP via river and air transport. The same routes are of course also in play for the other compounds, such as β-HCH and the chlordanes.

Mean ∑DDT in Gorge eggs is almost 70 times higher the corresponding mean ∑PCB (Table 1 and Fig. 2D and E). DDT has been banned for agricultural use in South Africa in the late 1970s but is still used for malaria control (Bouwman, 2003). DDT from malaria control is probably the main source of the residues in crocodile eggs, but secondary legacy sources cannot be ruled out. Malaria has been controlled since the 1950s in the low-lying areas of Mpumalanga and Limpopo provinces (Bouwman, 2003) through which the rivers flow before entering the KNP. The low mean per cent DDT of 4.6 per cent in Gorge eggs might be considered as mitigating against malaria control as the major source as a much higher percentage might be expected. On the other hand, the even higher concentration of ∑DDT and higher per cent DDT in the ND eggs further upstream of the Gorge may suggest a quick breakdown of *p,p′*-DDT (where used for malaria control) as it moves downstream, and this would be reflected in the pollutant parameters of the food items being higher 30 km upstream from the Gorge. In a separate study it was found that Grey Heron eggs (*Ardea cinerea*) collected close to a malaria controlled area had very high concentrations of ∑DDT (mean 13,000 ng/g wm; Table 3), but only 0.97 per cent was *p,p′*-DDT (Bouwman et al., 2013), also suggesting a rather quick breakdown. A wider survey of bird eggs, crocodile eggs, and fish would provide more information.

Most of the Olifants and Letaba rivers flow for only a small part of their lengths through the KNP. Upstream of the KNP, different sections of the river are subject to strong but varying influences of agriculture, mining, electricity generation (Ferreira and Pienaar, 2011), and malaria control. Downstream of the Gorge is the Massingr Dam with agriculture, malaria control, and semi-commercial fisheries. It should be kept in mind that crocodiles are mobile and can cover large distances within and between rivers. Fish, such as the main prey species *C. garipienus*, also migrate upstream (Huchzermeyer, 2012). Therefore, tracking sources of pollutants in highly mobile species that consume highly mobile prey is very difficult, and sources of chemical pollutants in crocodile eggs can originate both upstream and downstream of nesting sites.

It must be noted that maternal transfer is not the only potential source of organochlorine pollutants in crocodile eggs. The eggs may also take up compounds from the environment (Cañas and Anderson, 2002), but the contributions seem to be very small (1.4 per cent for heptachlor, and 0.0 per cent for DDT). These low uptake rates were not presumed to have affected the current concentrations in this study.

4.2. Brominated compounds

To our knowledge, we present here the first data for brominated flame retardants in crocodile eggs. Every egg had at least one PBDE congener at quantifiable concentration. PBEB was only found in two eggs from OR1 (Table 1). The field collected eggs had higher concentrations of ∑BDEs than the CF eggs. The low overall concentrations found in field-collected eggs suggest very little upstream input of BDEs into both rivers, although Egg 28 had a concentration of more than double the means of any other group (Table 1). The dynamics of BDEs under African and tropical conditions however, are not well understood (Polder et al., 2008) and needs more attention before substantive statements can be made.

4.3. Perfluorinated compounds

To our knowledge, we present the first data for PFCs in crocodile eggs (Table 2). Gorge and Nhlanganini pools had the highest ∑PFC concentrations, and CF eggs the lowest. The Hlanganini pool also had detectable concentrations for five PFCs, while the other pools only had two each (PFOS and PFDA). In each pool, PFOS was the dominant compound. The lowest observed adverse effect level (LOAEL) in bird eggs was for injected chicken eggs

Table 3
Comparable results of halogenated compounds in crocodile and alligator eggs (ng/g wm) from other regions.

Species	Location	Sampled	∑DDT	∑PCB	∑HCH	∑Chlordanes	Reference
American alligator	Lake Apopka, USA	1984	3900			250	Heinz et al. (1991)
American alligator	Lake Apopka, USA	1984	640			220	Heinz et al. (1991)
American alligator[a]	Lake Apopka, USA	2001–2002	10,118		2.1	260	Rauschenberger et al. (2004)
American alligator[a]	Lake Apopka, USA	2000–2002	9600			190	Rauschenberger et al. (2007)
American crocodile	Everglades, USA	1972	2000				Hall et al. (1979)
American crocodile	Everglades, USA	1977–1978	13,200	520			Hall et al. (1979)
American crocodile	Belize	1997	33				Wu et al. (2000a)
Morelet's crocodile	Belize	1997	104				Wu et al. (2000a)
Morelet's crocodile	Belize	1998	68				Wu et al. (2000b)
American crocodile	Banco Chinchorro, Mexico	2008	2.0	0.61		0.42	Charruau et al. (2013)
Australian freshwater crocodile	Ord, WA, Australia (mean)	2001	252,000[b]		1	17	Yoshikane et al. (2006)
Morelet's crocodile	Belize	1999	34–326[c]				Wu et al. (2006)
Nile crocodile	Kenya	1980s	666				Skaare et al. (1991)
Nile crocodile	Zimbabwe	1960s	3100				Billings and Phelps (1972)
Nile crocodile	Zimbabwe	1970s	4500			1600	Wessels et al. (1980)
Nile crocodile	Deka Mouth, Zimbabwe	1981	1420		3.0		Phelps et al. (1986)
Nile crocodile	Crocodile farm, Zimbabwe	1981	6650		77		Phelps et al. (1986)
Nile crocodile	Kyle Park, Zimbabwe	1981	2390		76		Phelps et al. (1986)
Nile crocodile	**Gorge, KNP South Africa**	**2009**	**450**	**6.7**	**10**	**0.33**	**This study**
Nile crocodile	**Nhlanganini, KNP, South Africa**	**2009**	**610**	**3.4**	**2.4**	**0.16**	**This study**
Nile crocodile	**Crocodile Farm, South Africa**	**2009**	**85**	**2.4**	**10**	**0.58**	**This study**
Leatherback turtle	French Guiana	2006	1440	6980	410		Guirlet et al. (2010)
Grey heron	Luvuvhu River, South Africa	2009/2010	13,000	33	2.9	98	Bouwman et al. (2013)
House sparrow	Hlengeni, South Africa	2009/2010	16,000	3.7	3.7	1.4	Bouwman et al. (2013)

[a] Concentrations in yolk.
[b] Liver lipid mass, no egg data.
[c] Range of egg clutch means.

(Molina et al., 2006) was 100 ng/g wm. Assuming the same LOAEL for crocodiles, this level is not exceeded in any the crocodile egg pool (Table 2). However, since sensitivities across large taxonomic ranges can be assumed to be also large, a safety factor of 10 would seem acceptable, therefore bringing the concentrations of the current study into range for concern. Also, the eggs analysed here were from female survivors, and it would be prudent to analyse PFCs in tissues of deceased crocodiles.

4.4. Comparisons with other data

We reviewed data from all publications that could be traced with comparable wet mass-based pollutant data in crocodile eggs (Table 3). Surprisingly few have been published on the top freshwater carnivores of the tropics. The ∑DDT concentrations in field-collected South African crocodile eggs were lower than for American crocodile eggs, American alligator eggs, and crocodile eggs from Kenya and Zimbabwe, but higher than for Morelet's crocodile eggs from Belize. For ∑PCBs, and ∑Chlordanes, the South African eggs were also lower than from elsewhere, while for ∑HCH, the concentrations were the same or lower. As mentioned before, the Letaba and Olifants rivers flow through areas where DDT is often used for malaria control. Despite this source of DDT, the concentrations of DDT are surprisingly low. Unpublished data for crocodile tissue from diseased crocodiles in 2008 had a mean of 380 ng/g wm (range 81–550 ng/g wm). This corresponds very well with the Gorge and ND egg means of 450 and 610 ng/g wm, respectively. Rauschenberger et al. (2004) investigated the ratios between residues in crocodile eggs and maternal body concentrations and found a relationship of close to 1 for *p,p*-DDE in liver, muscle, bile and adipose tissue. The concentrations in Nile crocodile eggs could therefore be expected to be similar in the maternal body. In comparison, American Alligators from Lake Apopka had 48,600 ng/g DDE in adipose fat tissue, while similar samples from Lake Griffen, where 450 crocodiles died between 1997 and 2004, had 4600 ng/g DDE. (Woodword et al., 2011).

Yoshikane et al. (2006) reported a mean ∑DDT concentration of 252,000 ng/glm (max 671,000 ng/g lm) in Australian Freshwater Crocodile liver from the Ord River irrigation area, Australia (Table 3). They also reported that the crocodiles seemed healthy with normal blood parameters. Fredericks and Palmer (2008), quoting Yoshikane et al. (2006), recalculated the data to wet mass; 500 ng/g DDE in liver (max 3200 ng/g wm). The highest *p,p′*-DDE concentration measured in eggs of the present study was 5300 ng/g wm, the same order of magnitude recorded in Australia (Table 3).

Recent analyses of aquatic bird eggs from the Luvuvhu River further north (also flowing through malaria controlled areas before entering the KNP) have shown high concentrations of ∑DDT (Table 3); mean 13,000 ng/g wm in Grey Heron (*Ardea cinerea*) eggs (Bouwman et al., 2013). The mean ∑DDT of 450 ng/g wm in the Gorge eggs is two orders of magnitude less. Berg (1995) modelled the dynamics of DDT in Lake Kariba, Zimbabwe, and found that concentrations of DDT in African fish eagle eggs were about twenty times higher than in crocodiles in the same system. If the same relationship holds for the Letaba and Olifants rivers, concentrations in African Fish Eagle (*Haliaeetus vocifer*) eggs would be about 9000 ng/g wm. This exceeds the level associated with impaired reproductive success (4000 ng/g wm) in Brown Pelicans (*Pelecanus occidentalis*) (Blus, 1982). Conversely, at mean concentrations of ∑DDT in fish-eating bird eggs (Grey Heron) from the Luvuvhu of 230,000 ng/g wm, the corresponding concentration in crocodile eggs would be about 11,500 ng/g wm – a cause for concern and a reason to study that system. Helicopter surveys indicated a decreasing crocodile population in the Luvuvhu River, reduced by more than half from a high of about 750 in 1993 to 275 in 2000 (Ferreira and Pienaar, 2011).

4.5. Egg 28

As the only egg from 2008, probably laid soon after the mortalities started, this single sample may shed some light on the contaminant conditions at the time. For all compounds, the concentrations in Egg 28 were about the same as for the Gorge

eggs (Tables 1 and 2 and Fig. 2), except for ∑PCBs, ∑BDEs, and per cent DDT, for which it had the highest for any egg. This may indicate a pollution event in 2008 which was not apparent in 2009, assuming that maternal body burdens in crocodiles respond fairly quickly to environmental changes.

4.6. Eggshell thickness

This present sample of eggs contained a wide range of pollutants at varying concentrations, some of which are known endocrine disruptors. *p,p′*-DDE is known to cause eggshell thinning in birds, while the other isomers of DDT, DDE and DDD do not (Lundholm, 1997). We found four studies that looked at pollutants and changes in eggshell thickness of crocodiles. Hall et al. (1979) measured American Crocodile eggs from Lake Apopka, Florida, and found no relationship between total eggshell thickness (inner and outer layers combined) and DDE (see Table 3 for DDE concentration). Heinz et al. (1991) looked at American Alligator eggs from the same lake and found a non-significant positive association (thicker eggs with higher concentrations). Wink and Elsey (1994) used early embryonic death (EED) as a response factor and found that American and Chinese alligator eggs with EED had an "abnormally" thickened outer shell layer. They did not speculate as to the cause of the thickening or EED. Bryan (2005) found, for reference and polluted lakes, that eggs from the less-polluted lakes had thinner eggs (inner and outer layers combined), ergo, eggs from more polluted lakes had thicker shells. The eggs from the polluted lake (Apopka once again) had, against prediction, significantly thicker shells. We found a significant thicker (by thirteen per cent) outer shell layer in Gorge eggshells (mean 420 μm; 360 ng/g *p,p′*-DDE wm) when compared with the thinner outer shell layer of the CF eggs (mean 367 μm; 67 ng/g *p,p′*-DDE wm), which correspondingly, also had significant less *p,p′*-DDE ($p < 0.0001$). The thickening of the outer shell layer in response to *p,p′*-DDE seems to corroborate the findings of Bryan (2005), and to some extent also Heinz et al. (1991) and Wink and Elsey (1994).

4.7. Relationship to mortalities and possible other effects

The chlorinated pollutants in the crocodile eggs from the KNP, extrapolated on parity to maternal females, are unlikely to have caused or contributed directly to the mortalities observed. The very high DDE concentrations found in Australian crocodiles (Yoshikane et al., 2006) attest to the improbability of DDE being the cause.

Pansteatitis in wildlife has never been associated with POPs and reptile mortalities. Pansteatitis in a single marine turtle is the only exception, but the high concentrations of PCBs could not be aetiologically linked to the symptoms (Orós et al., 2013). Although the eggs for this study came from survivors that presumably were less exposed to the causal agent than the dead ones, the concentrations in crocodiles would have had to be incredibly high to have been the primary cause of the disease which was not the case. It should also be borne in mind that 30 of the 34 crocodiles investigated were males (Ferreira and Pienaar, 2011). Only nineteen of the males were measured and had a mean length of 371 cm, while the mean length of three of the four females was 320 cm (one of which also had a part of the tail missing, so the mean length should be longer; unpublished data). These may be clues that the cause is perhaps gender-related, although it is not known if there would be equal numbers of males and females in a population such as this. Huchzermeyer (2003) implied that reproductive demands slow the somatic growth of females – therefore it is likely that populations of wild crocodiles would be dominated by larger males. From a chemical pollutant perspective, it would seem as if females may have a protective mechanism, possibly depurating the chemicals via egg deposition. It is also possible that the larger females may be senescent as was investigated (but not confirmed) by Woodword et al. (2011), implying that larger females have less opportunity for depuration via egg laying, but for the Nile crocodile, no firm data on age related reproduction seems to be available.

Egg 28 (laid soon after the first mortality episode) had the highest concentrations of ∑PCBs and ∑PFCs (Fig. 2), and assuming co-variance with other pollutants not measured, indicates that gender differences in pollutant concentrations should be investigated, something that cannot be done by investigating eggs. Crocodile samples from both genders and tissue from dead crocodiles need to be analysed.

The sub-lethal effects of halogenated compounds such as the ones studied, however, remain a concern. There is much basis for inferring concern about reproductive performance, based mainly on information from the USA, Argentina, and Mexico (Guillette et al., 1994, 1995; Milnes et al., 2005; Rauschenberger et al., 2007; Stoker et al., 2011; Woodword et al., 2011; Gonzalez-Jauregui et al., 2012). There is also concern about changes in behaviour (Clotfelter et al., 2004; Zala and Penn, 2004; Gonzalez-Jauregui et al., 2012) and gene–pollutant interactions (Moore et al., 2012). We have no knowledge about reproductive effort and hatchling success from crocodiles in the KNP and therefore cannot compare this with pollutant concentrations.

5. Conclusions

We found it unlikely that the pollutants studied in this paper could have caused or contributed substantially to the mortalities as a primary chemical agent. There have to be other causes not yet elucidated, and it may be gender-related. Reproductive, behavioural and genetic concerns remain to be investigated in this and any other crocodile population in Africa. With many increasing threats to their survival in the wild (such as habitat fragmentation and alteration, poaching, human conflict, and pollution), the conservation of the Nile crocodile and its habitat will contribute towards sustainable river systems. As large predators, crocodiles are at the apex of the freshwater aquatic food web, akin to polar bears, orcas, and sharks in the marine environment. Consideration should therefore be given towards additional measures to manage African freshwater systems so that it will also sustainably accommodate these large and long-lived animals. This need is also the case for all regions of the world where crocodiles, alligators, and other crocodilians occur. Water resource management and protective water and sediment quality criteria need to take these long-term users into account; and for this much more knowledge about the Nile crocodile, its ecology, biology, and behaviour are required.

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