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Metallic elements in Nile Crocodile eggs from the Kruger National Park, South Africa

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A R T I C L E I N F O

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A B S T R A C T

The Nile Crocodile is the largest predator on the African continent. Recent mass mortalities in the Kruger National Park (KNP) raised concerns about possible influence of pollution. We analysed eggs and their eggshells collected from nests inside the KNP and from a crocodile farm for metallic elements. We found that mercury, selenium, and copper occurred at levels of concern. Eggshells had very high concentrations of iron. Apart from toxicological implications associated with elevated concentrations in eggs, we found iron possibly contributing towards thicker eggshells. Thicker shells may act as a barrier to gas and water exchange, as well as possibly increasing the effort required for the hatchling to emerge from tightly packed shells under sand. Pollutants are transported into the KNP via rivers, and possibly via air. Mercury and copper pollution are waste-, industrial- and mining-related; ecotoxicological concern should therefore be extended to all areas where the four African crocodile species occur. Reptiles are under-represented in ecotoxicological literature in general, and especially from Africa. We know of only one previous report on metals and metalloids in crocodile eggs from Africa (Zimbabwe), published 30 years ago. Reduced fitness, endocrine disruption and effects on behaviour are other possible sub-lethal effects associated with metallic elements that may only become apparent decades later in a long-lived species such as the Nile Crocodile. In the face of habitat destruction, pollution, human population increases, and climate change, further research is needed regarding pollutant concentrations and effects in all African reptiles. The rivers that carry water from outside the park sustain its aquatic life, but also transport pollutants into the KNP. Therefore, improved source mitigation remains an important task and responsibility for all involved.

1. Introduction

The Nile Crocodile *Crocodylus nyloticus* is ranked as Least Concern by the International Union for Conservation of Nature (IUCN) (IUCN, 2016). In spite of this, their numbers are declining alarmingly in some areas in South Africa (Botha et al., 2011; Ferreira and Pienaar, 2011; Downs et al., 2015) and Botswana (Bourquin and Leslie, 2011). Reasons advanced for this decline are loss of breeding habitat (Leslie and Spotila, 2001), exploitation (Bourquin and Leslie, 2011), pollution (Botha et al., 2011), and disease (Ferreira and Pienaar, 2011). Adding to the concerns, mass crocodile deaths occurred at the confluence of the Letaba and Olifants rivers situated within the Kruger National Park (KNP; Fig. 1) as well as some deaths in the Sabie River further south in the KNP. These deaths were caused by or associated with pansteatitis, a condition where the fat becomes hardened, inflamed, and yellow (Osthoff et al., 2010). These incidents precipitated much research to elucidate the cause of the deaths and possible associated pollution (Ashton, 2010; Osthoff et al., 2010; Ferreira and Pienaar, 2011; Woodborne et al., 2012; Bouwman et al., 2014; Du Preez et al., 2016; Gerber et al., 2017). Some suspected causes and contributing factors include:

- Microcystins from cyanobacteria (Myburgh and Botha, 2009).
- Pollutants settling out of the water as the river slow down entering the Massingir Dam in Mozambique (Osthoff et al., 2010).
- Crocodiles consuming rancid fish (Ashton, 2010; Huchzermeyer et al., 2011).
- Environmental decline and pollution (Ferreira and Pienaar, 2011).
- Crocodiles feeding on steatitic African Sharp-toothed Catfish *Clarias garipienus* (Huchzermeyer et al., 2011).
- Ecosystem changes combined with extra-limital fish species as vector of the cause (Woodborne et al., 2012).

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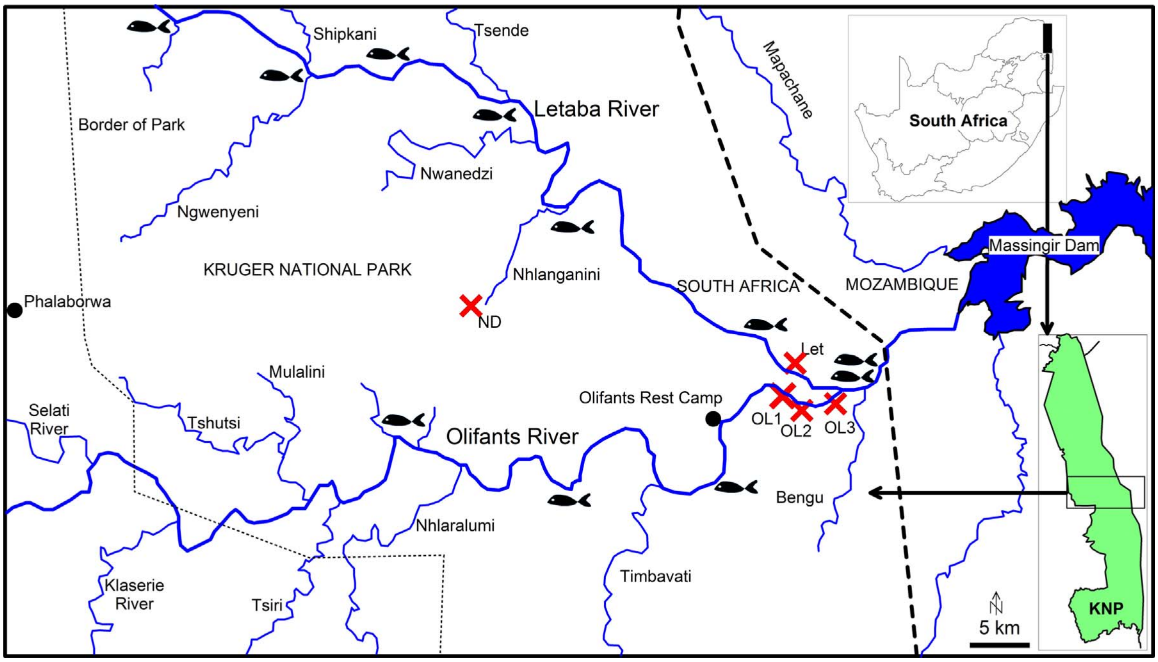

Fig. 1. Map of locations in the Kruger National Park where crocodile eggs were collected, marked with Xs. Sites with fish are recognised national fish sanctuaries. Rivers flow from east to west.

- High concentrations of aluminium in the fat of the Nile tilapia *Oreochromus mossambicus* that may interfere with cellular metabolism such as lipid-peroxidation (Oberholster et al., 2012).
- Seasonal change in diet due to potamodromic migrations of the invasive Silver Carp *Hypophthalmichthys molitrix* that has a fatty acid composition different from indigenous fish (Huchzermeyer, 2012).

Crocodilians are large, amphibious, and apex predators that may be considered bio-indicators because they accumulate a wide range of contaminants over long lifespans (Bouwman et al., 2014; Nifong et al., 2014). In Africa in freshwater ecosystems where they occur, the Nile Crocodile is the apex predator (Nifong et al., 2014). Their trophic levels are similar to sharks in marine ecosystems and polar bears in the Arctic. Total concentrations of contaminants in water and sediments however, do not always reflect their bio-availability (Pheiffer et al., 2014). It is therefore, difficult to assess if the concentrations in water or sediment might cause biological harm (Pheiffer et al., 2014), especially in long-lived animals such as crocodiles. Comparing results with other studies will therefore, assist in the identification of risk associated with the pollutants measured (Cortes-Gomez et al., 2014).

Eggs are often used in bio-monitoring (Klein et al., 2012). Eggs (avian or reptilian) represent contaminant levels within the female as well as the newly developing embryo (Klein et al., 2012; Bouwman et al., 2014; Cortes-Gomez et al., 2014; van der Schyff et al., 2016). Not only the contents, but also the shell may have toxicological implications when considering the distribution of pollutants between shell and contents (Kleinow et al., 1997). Another advantage of using eggs is that no reproducing adult animals have to be captured or killed. Collecting eggs from some species however, are often difficult (and hazardous), especially crocodiles (Bouwman et al., 2014).

According to an assessment of emerging chemical management issues in developing countries that are not covered by international treaties, "heavy metal" pollution is ranked the highest of the 22 issues identified (STAP, 2012). Geology plays an important role in the natural background of metals and metalloids, although anthropogenic activities such as agriculture, mining, and wastes handling may increase bio-available and bio-accessible metals in excess of background (Luoma, 1983). Therefore, elemental concentrations in biota are derived from both natural background and pollution, where pollution has occurred. Some metals like lead, cadmium, arsenic, and mercury have adverse effects on biota, while iron, magnesium, copper, and zinc have important physiological functions (Birch and Taylor, 1999; Hoekstra et al., 2003; Peijnenburg and de Jager, 2003; Grillitsch and Schiesari, 2010). Most metals and metalloids can be toxic at elevated concentrations, some even at relatively low concentrations (Zhou et al., 2008).

We could find only one study reporting metal concentrations in eggs of any of the four species of crocodile occurring in Africa. Phelps et al. (1986) reported on organic micro pollutants and mercury, selenium, cadmium, lead, and zinc in 26 Nile Crocodile eggs from ten sites in Zimbabwe. Organic micro pollutants have been reported in Nile Crocodile eggs from the Kruger National Park, South Africa (Bouwman et al., 2014). Here, we report the findings and interpretation of metallic elements in the same eggs collected in the KNP that were published by Bouwman et al. (2014) for POPs, The collection sites were mainly close to where the crocodile mortalities occurred. We also analysed the corresponding eggshells for the same elements to determine if the shells could serve as a non-lethal bio-monitoring method instead of using egg contents. Using the shells after hatching prevents the destruction of live embryos and may provide important information.

2. Materials and methods

2.1. Description of the Kruger National Park and sampling sites

The Kruger National Park (KNP) has six major river systems. The Letaba and Olifants rivers (Fig. 1) were where the crocodile mortalities occurred. The Letaba River (flowing to the east) confluences with the Olifants River, thereafter running through a gorge known as the Olifants Gorge (Fig. 1), below called the Gorge. The water of the Olifants River is highly mineralized (Du Preez and Steyn, 1992). The Olifants River is a system in which water quality is affected by human activities to a greater degree than from geological background (Gerber et al., 2015a). Metal bio-accumulation studies that were done in the Olifants River on African Sharp-toothed Catfish (*C. gariepinus*) indicated that fish inside the basin of the KNP had lower concentrations of metals than those collected upstream of the KNP (Du Preez et al., 1997). The Letaba