Stable isotopes of wild Nile crocodiles as a trace for pollution in the upper Olifants River, South Africa

Albert H. Myburgh; **Hannes Botha**; **Xander Combrink**[3]; **Jan Myburgh**; **Louis J. Guillette Jr**; **Grant Hall**[6]; **Chris T. Chimimba** & **Stephan Woodborne**

Introduction

In the autumn and winter of 2008, more than 180 Nile crocodiles died in the Olifants River Gorge in the Kruger National Park (KNP) (Ferreira & Pienaar 2011). Evidence indicated that the deaths were caused by a disease called pansteatitis, a dietary disease also reported in the Mozambique tilapia (*Oreochromis mossambicus*), crocodiles and several other species in Lake Loskop (Oberholster *et al.* 2012; Huchzermeyer *et al.* 2017) higher in the Olifants River catchment. Studies on the ecology of crocodiles suggests a broad range of feeding behaviors that include both terrestrial and aquatic food sources, with an ontogenic shifts towards larger food items (Pooley & Gans 1976; Hutton 1987; Wallace & Leslie 2008). A key question in the mass mortality was whether a specific dietary item was the cause of the disease. Stable isotopes evidence of the aquatic food web in the Olifants River Gorge suggested that fish were unlikely to be the dominant diet of crocodiles, but rather a seasonal component in an otherwise terrestrial diet (Woodborne *et al.* 2012).

In order to test the fish-diet dependence of crocodiles in the Olifants River we make use of stable isotope analyses of fish carried out at different impoundments along the Middle Olifants River. Fish in Lake Loskop have mean nitrogen (δ^{15}N) isotopic values of 16.8 ± 1.6 ‰ (Huchzermeyer *et al.* 2017) associated with high levels of pollutants, and downstream in Lake Flag Boshielo the δ^{15}N values for fish average 13.9 ± 1.1 ‰ (Lübcker *et al.* 2016), representing a decreased pollutant load. This region of the river is also home to a substantial population of crocodiles. The intervening reach of the river will show an isotopic gradient in the fish population that can be used to test the level of fish dependence and pollutant integration in the diet of crocodiles. We hypothesis that a predominantly fish diet will be supported by a shift in the isotope values in the crocodiles that matches that of the local fish communities. Stable isotope ratios of nitrogen were used to compare crocodile and fish populations along this length of the Olifants River.

Methods

Study region

The study area extended from the inflow region of Lake Loskop through to Lake Flag Boshielo in the Middle Olifants River catchment, Mpumalanga and Limpopo Provinces, South Africa. The inter-lake lotic system comprises approximately 100km of river reach over a 65 km distance. Lake Loskop is hypertrophic due to an influx of pollutants from the upper regions of the catchment. The shallower reaches of the river downstream of Lake Loskop are moribund with *Phragmites sp.* reed beds, and water quality undergoes drastic improvements downstream toward Lake Flag Boshielo. Several key indicators (nitrogen, phosphorous,

heavy metals) decline substantially to often untraceable levels (Dabrowksi *et al.* 2014a). River flow is almost entirely controlled by water releases from Lake Loskop.

Sampling

Samples representing the entire food web including primary productivity (aquatic macrophytes), invertebrates, and fish as well as crocodiles were collected at five locations between Lake Loskop and Lake Flag Boshielo.

Sampling took place during January (summer) and September (winter/spring) 2018, which normally should represent high and low flow conditions during summer and winter, but water releases from Lake Loskop were similar in both sampling seasons. Fish were captured using a combination of electrofishing (Samus model 452) and baited rod and reel. Small fin clips were removed for isotopic analysis and the fish were returned into the river.

Crocodiles were captured using cage traps baited with bovine lungs obtained from a local butcher. Smaller individuals (<1.5m total length (TL) measured dorsally from the tip of the snout to the tip of the tail) were hand-captured from a boat at night. The crocodiles were blindfolded and the jaws, hind and fore legs were restrained. The TL (total length) and SVL (Snout vent length, measured from the tip of the snout to the posterior margin of the cloacal vent) of each crocodile was measured using a standard tape measure. The sex of juveniles, sub-adults and adults were assessed by cloacal examination (Brazaitis 1968). Crocodiles were marked for future identification using a passive integrated transponder (PIT) tag, web tags and by permanently removing a unique series of three caudal scutes using a sterile scalpel. All scute samples were frozen on site. Scute samples and the claws of three post-mortem crocodiles from Lake Loskop were analyzed.

Fieldwork was conducted under collecting permit numbers MPB 5605 from Mpumalanga Tourism and Parks Agency, AUCC 012/16 & 001-CPM402-00002 from the Limpopo Department of Economic Development, Environment and Tourism. Sampling protocols were approved by the University of Pretoria animal ethics committee (ethics clearance number ec009-17)

Laboratory analysis

Samples were analyzed using a Flash EA 1112 Series elemental analyzer coupled to a Delta V plus isotope ratio mass spectrometer by a ConFlo IV interface (all supplied by Thermo Fisher, Bremen, Germany). We only report on the δ^{15}N values of the fish and crocodiles in this analysis although δ^{13}C were obtained as were the C/N ratios

Results

The results of the isotopic analysis of the crocodile scutes was plotted with the fish δ^{15}N values as a function of distance from Lake Loskop head-waters . An increase in δ^{15}N values below the sluices of Loskop Dam was attributed to denitrification. The fish in the Olifants River Gorge are subject to additional pollution sources and showed a slightly elevated δ^{15}N value (15.2 ± 2.0 ‰, $n=39$) relative to Lake Flag Boshielo.

The δ^{15}N values of the crocodiles varied within a much tighter range along the Olifants River than that of the fish samples.

Although the nitrogen isotopic values of the crocodiles vary within a limited range, part of the variability might be attributed to dietary shifts with ontogenic age. The size range of crocodiles that were sampled in this study is wide (TL=32cm to TL=470cm).

Conclusions

Clear differences in the δ^{15}N values of fish along the Middle Olifants River is indicative of a very localized structure to the food web. The differences in δ^{15}N between the fish along the river is a function of the nutrient gradient that is driven by pollution in Lake Loskop, and is mitigated by denitrification and biological incorporation of pollutant derived nutrients in *Phragmites sp.* beds in the reach to Lake Flag Boshielo. The spatial patterning in the δ^{15}N values of the fish assemblages provides a local baseline of available fish for crocodile consumption as a possible pathway for the integration of pollutants. If it is assumed that the crocodiles are eating fish, and that they have a constant diet to tissue discrimination factor, then the nitrogen isotope values for the crocodiles should track the changes in the resident fish populations along the reach of the river. This was clearly not the case, as the crocodile δ^{15}N values tend to be relatively invariable relative to the fish

There are three scenarios that might explain the isotopic relationship between the crocodiles and the local fish populations. The first scenario is the possibility that the fish are migratory, but this was rejected on the basis of several weirs and the patently different nitrogen isotope values for fish at each sampling location. The second scenario is that the crocodile population is highly mobile and that their diet is not necessarily assimilated at the location at which they were sampled. We rejected this scenario because it would imply that all the crocodiles have a single dietary source that is downstream from Lake Loskop. This would have to be Lake Flag Boshielo, and it is untenable that the crocodile population migrate up to 90 km in order to feed on fish in Lake Flag Boshielo when there are abundant fish in the intermediate river reach. The third scenario is that we reject the hypothesis that the crocodiles are largely dependent on fish for their diet.

The notion that an unprotected population of Nile crocodiles is not dependent on fish for their diet reconciles with the data of Woodborne *et al.* (2012) who suggested that episodic trophic shifts apparent in isotopic time series from crocodile claws represented occasional fish over-exploitation in the Olifants River Gorge. If the crocodiles are not eating fish then it suggests that their diet comprises elements of the terrestrial niche, most likely in the riparian zone. The use of nitrogen isotopes in dietary analysis allows us to conclude that crocodiles are not dependent on fish for their diet and subsequently are less exposed to pollutants from the upper region of the Olifants river as they rely on the riparian zones for the bulk of their diet. Unfortunately, this has additional implications for human-wildlife conflict as the riparian zones of this section of river are further developed for agriculture, housing and recreation.