KNP CROC DEATHS – Groundbreaking study narrows suspect list

It has been four years since this magazine first reported on efforts to discover the exact cause of alarming, large-scale deaths of Nile crocodiles in the Kruger National Park (KNP). Results of a groundbreaking project, partly funded by the Water Research Commission, provide astonishing insight into the probable trigger leading to these mortalities. Article by Lani van Vuuren.

Aquatic ecosystems

In 2008, the world watched in horror as a third of the estimated crocodile population of the densely populated Olifants River gorge in the KNP perished. While deaths of crocodiles, fish and terrapins had been recorded in the Olifants River system (particularly around Loskop Dam) since at least 2003, the large-scale demise of a keystone species spelled disaster for an aquatic ecosystem long since affected by upstream pollution and land use activities.

Post mortem studies revealed the cause of death to be pansteatitis – a nutritional disease usually found in captive animals as a result of being fed on a diet comprising large amounts of unsaturated or rancid fat. The Olifants River crocodiles were among the first recorded cases in the world of wild crocodilians succumbing to the disease.

While fingers pointed to various anthropogenic impacts on the Olifants River catchment, including mining and agricultural activities as well as the cumulative effect of numerous large dams in the system, what triggered the large-scale crocodile mortalities remained a mystery. The research community responded by establishing the Consortium for the Restoration of the Olifants Catchment (CROC), and a series of multidisciplinary research projects followed. (For more on CROC, read 'Experts Unite to Save Abused River from Extinction', the Water Wheel January/February 2009).

Independent fish pathologist, Dr David Huchzermeyer, initiated one of these studies following speculations regarding the possible impact of pollution and toxic bioaccumulation on the health of the KNP crocodiles. "I made the suggestion that if the crocodile deaths were caused by pollution we should look for related pathology in the fish on which the crocodiles were likely to be feeding," he tells *the Water Wheel*.

PANSTEATITIS EXPLAINED

onsidered a nutritional disease, pansteatitis develops when susceptible animals consume an overwhelming amount of polyunsaturated fats, typically fatty or oily fish or fats that have become rancid. In the case of the Olifants Gorge, both crocodiles and sharptooth catfish are known to hunt and eat fish. However, diets containing excessive amounts of certain highly polyunsaturated fats are problematic for these animals. Polyunsaturated fats are unstable and easily oxidise. Physiologically they exert a level of oxidative stress that is capable of overwhelming the natural anti-oxidant defence mechanisms of the animal, in particular tissue vitamin E. The oxidative breakdown of fats initiates a chain reaction that eventually consumes the vitamin E in the adipose tissues and leads to the death of fat cells. Dead fat cells release the breakdown products of fat oxidation and these cause the acute and chronic inflammation that lead to clinical pansteatitis. Pansteatitis causes fat to harden reducing mobility and affecting the ability to swim. This is particularly noticeable in crocodiles, and in severe cases leads to drowning or starvation of affected animals.

PREDATORY FISH ALSO AFFECTED

Dr Huchzermeyer's theory that the health of crocodiles and fish species in the Olifants River system was linked was confirmed following a rare, large-scale fish mortality event in the Olifants Gorge in the winter of 2009. Most of the dead fish turned out to be African sharptooth catfish (*Clarias gariepinus*), an opportunistic omnivorous species known to predate on other fish. The fish were mostly found in water overlying the clay-rich deposits at the point where the gorge widens into the recently raised Massingir Dam, of Mozambique. Fish carcasses were observed to be extremely fat, but a definitive diagnosis could not be made due to advanced putrefaction.

Catfish were subsequently sampled regularly over a two-year period from the Olifants Gorge as well as from other sites in and around the KNP. The project brought together researchers from South African National Parks, the universities of Pretoria, North West and the Free State, the CSIR, South African Police Service Forensics Laboratory, and IDEXX Laboratory. Much of the financing was borne by the research institutions and the researchers themselves.

The investigation led to the discovery that the catfish in the Olifants Gorge were also affected by pansteatitis – the first reported case of its kind in the world. Since sharptooth catfish are easier to

Cross sections of pansteatitis-affected fat from a sharptooth catfish (**top**) and a crocodile (**bottom**).







sample than crocodiles, it then provided the project team the opportunity to study the disease in greater detail.

ONCE OFF OR CONTINUOUS?

key question that needed to be answered was whether the disease was a once-off occurrence or episodic in nature. "My experimental work with captive catfish demonstrated that, in the case of catfish, pansteatitis was not rapidly fatal. Affected fish could survive protracted periods and lesions were likely to accumulate over time," reports Dr Huchzermeyer. "This pointed to periodic or seasonal episodes of dietary oxidative stress in these animals." This explains why crocodile fatalities have occurred in the years following 2008, albeit in much lesser numbers.

Furthermore, pansteatitis has also been identified in catfish at two other sites in the KNP. The catchment areas feeding these sites differ from that of the Olifants River, providing argument against a primary polluted-related oxidative stress.

FISHY CULPRIT

A nalyses of the fatty acid composition of crocodiles and catfish with and without pansteatitis by Garry Osthoff and Arno Hugo at the University of the Free State found that the ratio of omega 3 to omega 6 fatty acids was similar in catfish and crocodiles with pansteatitis but differed from that of healthy catfish and crocodiles. Pansteatitis-affected animals had particularly high levels of docosahexaenoic acid (DHA) in their fat.

"This polyunsaturated fatty acid is an essential nutrient that cannot be synthesised by catfish and crocodiles," notes Dr Huchzermeyer. "It is poorly mobilised from adipose tissues and thus reflects what the catfish and crocodiles have been feeding on." Phytoplankton is rich in omega 3 fatty acids, including DHA. Crocodiles and catfish would need to feed on phytoplankton feeding fish species in the Olifants Gorge to assimilate these fatty acids. In a related study, farmed catfish that developed pansteatitis from consuming rotting fish waste did not assimilate the characteristic fatty

Above: Deep water damming back into the Olifants Gorge from the raised Lake Massingir, in Mozambique.

Below: University of Cape Town student, Richard Huchzermeyer, with a large sharptooth catfish from the Olifants Gorge. acids observed in the catfish from Crocodile Gorge.

As part of the project, Stephan Woodborne from the CSIR examined stable isotopes in tissue samples from crocodiles and catfish while investigating the food web in the Olifants Gorge (for more on his research see, 'Searching for clues inside the claws', *the Water Wheel* July/August, 2011)

Abnormally high nitrogen isotope values in the crocodiles and catfish from the Olifants Gorge pointed to a trophic level increase in these animals when compared to animals sampled from sites where pansteatitis did not occur. This, together with Dr Huchzermeyer's work which indicated that catfish from the Olifants Gorge had changed to a predominantly fish diet, raised the question as to whether the catfish and crocodiles were feeding on a species of fish not normally consumed by these animals.

"We still needed to explain an intense dietary intake of polyunsaturated fats," notes Dr Huchzermeyer. "This led me to look for a migratory species that was an obligate phytoplankton feeder and would migrate





"Part of the explanation is still speculative and further research is needed to confirm these speculations." into the Olifants Gorge seasonally in large enough numbers to provide the catfish and crocodiles with an overwhelming intake of omega 3 fatty acids."

Silver carp (*Hypophthalmichthys molitrix*), an invasive alien species originating from East Asia, are known to occur in Lake Massingir. Though speculative, this provides an explanation for high polyunsaturated fat intakes by crocodiles and catfish. Silver carp are pelagic phytoplankton feeding fish that migrate into fast flowing waters of the Olifants River to spawn. In order to do so, they have to swim through the Olifants Gorge where it is suspected that they become an easy prey for crocodiles and catfish.

Silver carp are known to congregate in large numbers in deep water. The previously shallow, fast-flowing rapids of the gorge have now become a deep water system during the summer as a result of the enlarged Massingir Dam, providing an ideal environment for these invasive alien fish.

High phosphate levels measured in the Olifants River within the KNP prior to 2004 have contributed to the **Top left:** Dr David Huchzermeyer dissecting a fish on site in the Olifants Gorge.

Top Right: Dr David Huchzermeyer and his team at the field laboratory on the banks of the Letaba River.

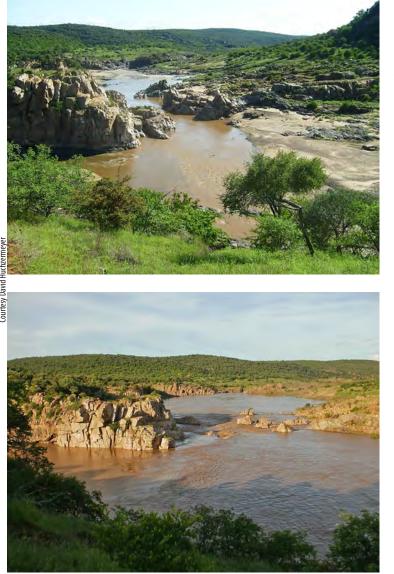
Above left: Johann Müller of IDEXXSA Laboratory and independent fish pathologist, Dr David Huchzermeyer with a pansteatitis mortality in the Kruger National Park

Above right: Johann Müller working at the field laboratory in the Olifants Gorge.

Aquatic ecosystems

Top right: The Olifants/ Letaba river confluence during normal summer flow showing the few remaining shallow rapids.

Bottom right: The same confluence in full flood.



eutrophication of Lake Massingir. This, in turn, has provided a rich dietary source of phytoplankton on which the silver carp and possibly other fish species have thrived. "To date this provides the only link between water pollution and the pansteatitis deaths of crocodiles in the Olifants Gorge, however, work on toxin bio-accumulation has not yet been completed," Dr Huchzermeyer notes.

He believes that the story leading to the large-scale crocodile mortality in 2008 unfolded as follows: Large man-made lakes (in this case Massingir) act as traps for nutrient pollutants, particularly phosphates. Once trapped in the lake they drive phytoplankton growth leading to eutrophication. The raising of Massingir Dam extended the dam waters into the Olifants gorge altering the habitat of the gorge. This has possibly improved the ease with which crocodiles and catfish hunt and feed on silver carp.

Silver carp thrive on phytoplankton blooms and the fat in their adipose tissues reflects a high omega 3 to omega 6 fatty acid ratio assimilated from phytoplankton. The high intake of omega 3 fatty acids by crocodiles and catfish, associated with consumption of large numbers of such fish, results in an oxidative stress that overwhelms the antioxidant protective mechanisms, particularly tissue vitamin E, of the crocodiles and catfish. As the fats in the adipose tissues of the animals begin to oxidise, fat cells die, leading to inflammation of the adipose tissues and pansteatitis.

Part of the explanation is still speculative and further research is needed to confirm these speculations. While crocodile mortalities have declined since 2008 it is not known how many crocodiles are still suffering from subclinical pansteatitis. The prevalence of pansteatitis in catfish probably reflects the similar dietary exposure to a polyunsaturated fat-rich diet.

Dr Huchzermeyer notes that this could vary from year to year depending on the intensity of phytoplankton blooms in Lake Massingir and on the degree of fat deposition in silver carp and other plankton-feeding fish. "Silver carp in Lake Flag Boshielo located upstream of the Olifants Gorge, for example, have been observed to be thin and crocodiles in this lake remain healthy. We also do not know to what extent consumption of rotting fish from fish die-offs is contributing to pansteatitis, particularly at sites where the presence of silver carp has not been confirmed."

LESSONS FOR THE FUTURE

Dr Huchzermeyer believes that this case study has many lessons for the way we manage and conserve our natural environment. "As this case has shown, human interference in aquatic systems can have far-reaching consequences, particularly for top predators. Several anthropogenic factors have come together which individually may not have caused the deaths of the crocodiles. Ultimately it was the combination of these factors that led to the tragedy."