USE OF STABLE ISOTOPE ANALYSIS TO DESCRIBE AQUATIC FOODWEBS IN THE KRUGER NATIONAL PARK

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EXECUTIVE SUMMARY

This project set out to determine (a) the value of using Stable Isotope Analysis (SIA) as a forensic tool to identify foodchain links between primary producers and apex predators (crocodiles) in the Olifants and Sabie River systems in the Kruger National Park and (b) to attempt to "top-down" link the crocodiles to a potential cause of the pansteatitis-related mortalities of crocodiles.

The project was constrained by being chronological de-linked from the crocodile death events by a considerable extent. Accordingly, the conditions prevailing in either of the two river systems at the time of the incident could not be sampled. In particular, no cyanobacterial blooms were present in the Olifants Gorge and the SIA results were dependent on a single crocodile sample, but were bolstered by fish samples collected both during and prior to this project.

At the time of this survey the phytoplankton of Massingir Dam and the lower Olifants River was dominated by the K-strategist dinoflagellate, *Ceratium hirundinella*. This organism is an alternate dominant in mild eutrophic conditions, typically alternate to a species such as *Microcystis*. Although *Microcystis* was present in Massingir Dam, so was *Cylindrospermopsis raciborskii*, a genus that can outcompete other cyanobacterial species by virtue of being able to suppress their ability to take up phosphorus.

The results indicate that while the crocodile diet is centered in the fish typical of Massingir Dam and the lower Olifants Gorge, no links to phytoplankton via fish-zooplankton could be demonstrated. This is further unlikely given the general inedibility and low food value that most colonial cyanobacteria offer to zooplankton. The results suggest that the relevant fish species are feeding via an invertivore linkage, either in the benthos or the littoral of Massingir Dam.

The presence of the toxin-producing *C. raciborskii* in Massingir Dam is significant in that this organism has been linked to large reptile (alligator) mortalities, as well as other wildlife, for many years. The rapid appearance (globally) of this cyanobacterium is a relatively new and poorly-understood phenomenon. Its possible role in the KNP crocodile mortalities cannot be underestimated and cannot be linked as no samples were collected at the time, nor was a comprehensive assessment made of the conditions prevailing in Massingir Dam. The presence of *C. raciborskii* has major implications for the future use of water from this dam – either local or downstream.

It is considered likely that the cause and effect pathway to the crocodile deaths was centered in Massingir Dam and its limnological ageing process following filling, rather than a riverine-based cause. Again, however, the absence of primary data

coincident with the event, as well as more time-based (seasonal) data, preclude the further development of this line of thinking at the present time.

The project has shown that SIA-based forensic interpretation of foodweb characteristics can be deployed rapidly and cheaply in response to an event such as the crocodile mortalities.

The data assembled in this investigation are likely to be of mutual, complementary value to other surveys being conducted around the issue of crocodile deaths in the Kruger National Park.

ACKNOWLEDGEMENTS

This report acknowledges the input, help and support of the following:

- Dr Danny Govender for her kind guidance and support;
- SANPARKS and KNP for permission to undertake research in the KNP, and for the provision of GIS data;
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Water Research Commission Consultancy K8/890/2

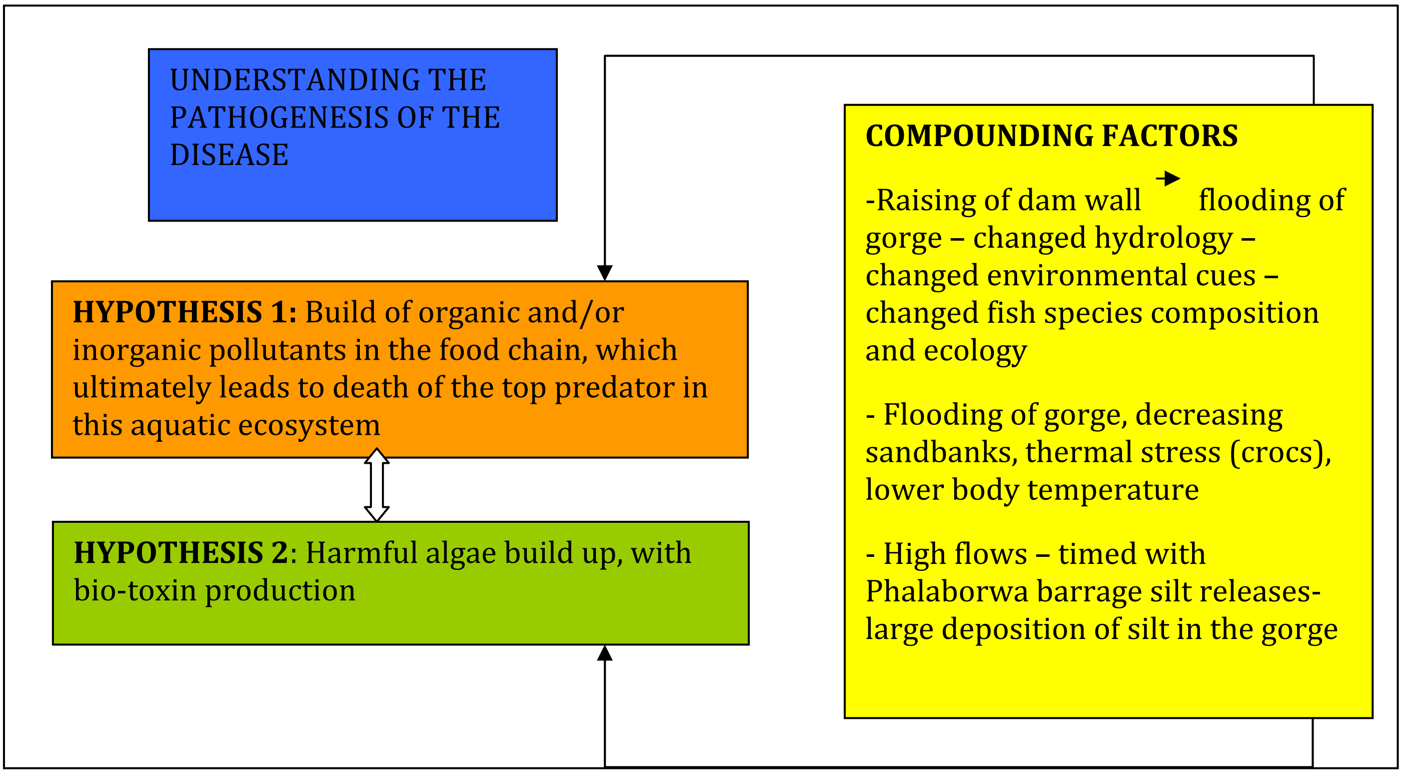
Final Report (October 2010)

WR Harding & RC Hart, DH Environmental Consulting

BACKGROUND

Following the scientific speculation surrounding possible reasons the spate of crocodile deaths that occurred in the Olifants Gorge of the Kruger National Park (KNP) during 2008, the first author proposed that the use of Stable Isotope Analysis (SIA) could provide a means to define the aquatic foodwebs and biota feeding patterns – the "who eats whom and where" approach.

During 2008 SANPARKS became aware of crocodile mortalities in the Olifants River and in which all the affected animals suffered from pansteatitis. A total of 170 dead animals were recorded in 2008 and a further 28 in 2009. A further three animals also died in the Sabie River during 2009 (Dr D Govender, SANPARKs, pers. comm.). These incidents led to SANPARKs formulating provisional hypotheses (see **Box**):



Investigation of both hypotheses (1 and 2, **Box**) centers on foodchain analysis and confirmation of foodchain links. To undertake such an investigation using 'classical' means (gut contents) is/was neither feasible nor practical in this case. Accordingly, the use of an SIA-based approach was proposed.

Principally, the use of SIA was intended to identify links between catfish and crocodiles, additionally to examine possible involvement of cyanobacteria. The use of SIA provides a powerful 'natural tracer' tool for examining trophic relationships in terrestrial and aquatic foodwebs – highlighting feeding relationships between the biota within a particular foodweb. There exists, generally, a stable relationship between the carbon (^{13}C) and nitrogen (^{15}N) isotopic compositions (signatures), with carbon defining food source (and by implication the associated feeding habitat) (= 'eats what (and where)') and nitrogen the trophic level – with the latter increasing by approximately 3-5 ppt between trophic levels (= 'eats what'). Carbon thus indicates the source of the energy, while nitrogen indicates the length of the foodchain and the individual trophic positions.

The technique, when applied at appropriate frequencies of sampling, also helps to define migration patterns and/or omnivorous feeding patterns by higher level predators. Using SIA to track feeding of pelagic biota in riparian marshes, for example, can be compared to its use for tracking feeding habits of migratory herbivores in the terrestrial environment.

An additional advantage of SIA is that it allows definition of the trophic transfer of pollutants (e.g. nutrients originating from wastewater) in aquatic systems. Sources of nutrients such as wastewater have higher ^{15}N signatures – which are then reflected in the foodweb as quite distinct from the autochotonous food sources. Similarly, SIA applications have been used to track toxic trace metals (e.g. mercury) through aquatic foodwebs. These types of forensic application require, however, a reasonably sound understanding of the unaffected underlying foodweb.

In order to apply this approach in the KNP, the Water Research Commission (WRC) funded a consultancy to undertake exploratory sampling and analysis. It should be noted that this work was undertaken more than a year after the first deaths were noted, i.e. the sampling undertaken here was by no means coincident with or representative of conditions pertaining at that time. The allocated budget also significantly constrained the amount of time that could be devoted to fieldwork, this further complicated by the remoteness of the sampling locations and that sampling had to be undertaken both in South Africa and Mozambique.

This project formed one of a suite of investigations initiated to delve into the cause of the reported crocodile mortalities. Of significance is the fact that Massingir Dam was filled to capacity for the first time only in 2007 – following the installation of sluice gates on the dam wall. This resulted not only in the loss of basking sites (sandbanks) for the crocodiles, but also inundated a massive area of previously-farmed land. The limnological response of the dam to this sudden change in its hydro-morphology was not monitored but is likely to have brought about a massive change in the chemical and biophysical characteristics of the impoundment.

METHODS

Sampling

The initiation of the use of SIA in the KNP occurred some considerable time after the crocodile death incidents, i.e. the collection of samples for SIA analysis was not temporally coincident with the events. For this project (WRC #890), provision was made for two sampling trips, an initial screening of various locations and habitats, followed by a second, focused sampling informed by the results of the initial foray.

The sampling trips took place during late August 2009 (24-26 August 2010) and again during April 2010 (14-21 April 2010). A longitudinal transect of sampling sites, starting at Mamba Weir on the Olifants River near Phalaborwa, and ending at the Massingir Dam wall, were visited. Additionally, other sites linked to crocodile mortalities on the Sabie River and Couramana Dam (Mozambique) were variously visited on each of the sampling trips (see **Figure 1a & b**).

Sampling focused on water chemistry, sediments, aquatic macrophytes, algae (phytoplankton and epiphytes) and zooplankton. Fish, apart from limited samples purchased from artisanal fishermen, were obtained from material provided by KNP (these were caught during June 2009), and crocodile samples (3), provided by KNP (collection dates unknown). Only one of these crocodile samples (from the Olifants Gorge) was representative of an animal that had died as a result of pansteatitis.

Sampling sites

A total of eleven sites were visited, seven on the Olifants system and four on the Sabie (see **Figure 1a & b**).

Sample collection

Sampling was undertaken using a boat in the Gorge and from the shoreline at all other sites. Comprehensive sampling at Sunset Dam was precluded by the presence of tourists. Depth profiles were recorded for all sites deeper than 2 m, with temperature profile data being collected at Couramana Dam.

Water quality

Water samples were collected either as grab samples (shallow water) or by means of a Van Dorn closing-bottle in deeper waters (> 2 m). Measurements of water temperature, electrical conductivity, dissolved oxygen and pH were made in the field using a Hach HQd multi-probe data-logging instrument. Analyses for nitrate-nitrogen and orthophosphate-phosphorus were undertaken immediately after sampling using Hach field colorimeters.

Sediments

Sediment samples were collected using a Birge-Ekman grab or by scooping where littoral samples were the only collecting option. Samples were dried in air at 60°C prior to homogenization and analysis. The sediment samples were retained for possible future use by other related projects.

Macrophytes

Macrophyte and/or macroalgal samples were collected as cut stems (reeds) or canopy (pondweed, macroalgae). The samples were cleaned and washed of extraneous material prior to drying and analysis.

Phyto- and zooplankton

Phytoplankton and zooplankton samples were collected by means of vertical or horizontal hauls using standard plankton nets of, respectively, 20µm and 80µm mesh. Samples were screened and examined on the day of sampling, with sub-samples preserved using Lugol's iodine (phytoplankton), ethanol (diatoms) and ethanol or formalin (zooplankton) for later examination.

Quantitative data on abundance and composition were obtained from measured vertical net haul samples, but at various sites, sampling was confined to the shoreline 'casts' of the net, yielding only qualitative information from horizontal net tows. Routine procedures were used to determine quantitative data.

Diatoms

Diatom samples were collected off rocks or plant surfaces and preserved using standard procedure (Taylor et al. 2007). Diatoms were used as a proxy for the phytobenthos.

Invertebrates

Aquatic invertebrates were collected using a 1000 µm sweep/scoop net and preserved fresh for SIA and in ethanol.

Fish

Samples of fish tissue, provided by KNP, comprised three batches caught at Ngotso (8 specimens), in the Olifants Gorge at the Letaba confluence (9 specimens) and at Klipkoppies (Letaba) Bridge (4 specimens). The specimens from Ngotso and Klipkoppies were all sharptooth catfish (*Clarias gariepinus*), while the Gorge specimens comprised catfish and Mozambique tilapia (*Oreochromis mossambicus*). All of these fish were caught during August 2009).

Fish purchased from local fishermen in the Olifants River Gorge at the head of Massingir Dam during August 2009 comprised *Synodontis zambezensis* ('squeaker', 1 specimen), tilapia (*O. mossambicus*, 1 specimen) and *Labeo ruddi* (1 specimen).

No fish samples were available for Sunset Dam. This waterbody has been observed to contain a population of cichlids (RC Hart, pers. comm.) and probably also contains sharptooth catfish.

During April 2010, fresh single specimen samples of catfish (*Clarias gariepinus*), Mozambique bream (*Oreochromis mossambicus*), Tiger fish (*Hydrocynus vittatus*), imberi (*Alestes imberi*), rock catlet (*Chiloglanis* sp.), butter barbel (*Schilbe intermedius*) and squeaker (*Synodontis zambezensis*) were purchased from local fishermen at various points around the lake.

Samples of dorsal muscle were excised from each specimen and retained frozen prior to drying.

Crocodiles

Three samples of crocodile tissue, one from the Olifants Gorge and two from Sunset Dam, were provided by KNP. As noted above, only the Olifants Gorge specimen (a single sample) was directly associated with the pansteatitis mortalities. As such the availability of tissue from crocodile specimens was severely limited.

Stable isotope analysis

All of the samples were retained frozen prior to air-drying (60°C) and submission for SIA analysis. Samples were weighed into tin cups to an accuracy of 1 microgram on a Sartorius micro-balance. The cups were then squashed to enclose the sample.

The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany).

The in-house standards used were:

- Choc – a commercial chocolate/egg mixture;
- Sucrose – "Australian National University (ANU)" sucrose;
- Valine – DL Valine purchased from Sigma;
- MG – Merck Gel – a proteinaceous gel produced by Merck;
- Seal – a seal bone, crushed, demineralized and dissolved in acid, and then reconstituted in gel form;
- Lentil – dried lentils;
- Nastd – Dried nasturtium leaves;
- NH_4Cl – As purchased from a chemical supplier.

All the in-house standards were calibrated against IAEA (International Atomic Energy Agency) standards. Nitrogen is expressed in terms of its value relative to atmospheric nitrogen, while carbon is expressed in terms of its value relative to Pee-Dee Belemnite.

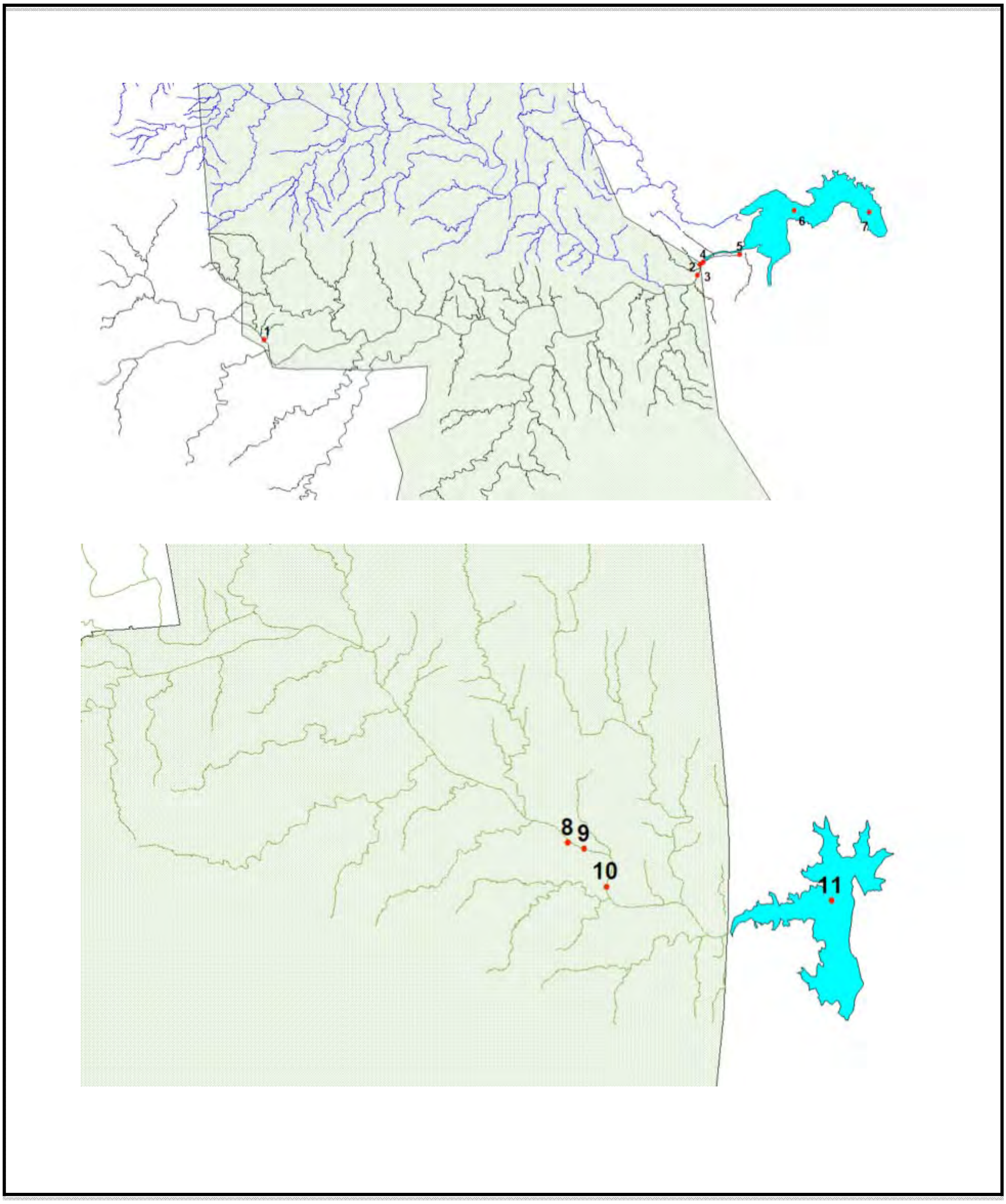


Figure 1: Top (a) Positions of sampling sites on the Olifants River and Massingir Dam; Bottom (b) sampling sites on the Sabie River, Sunset Dam and Couramana Dam. Kruger National Park area shaded.

RESULTS

Water quality

The water quality data for this project are based on one-off samplings and, accordingly, the inferring of ecological inferences would be presumptuous. However, certain key attributes are noted here, with reference to **Table 1**, as follows:

- Although Massingir showed no signs of thermal stratification to a depth of 15 meters, a very slight oxycline was detected. This reflects the mixing power of light winds on a very large (surface area) dam. Similar deep mixing is evident for the temperature profile data collected for Couramana Dam;
- Nutrient levels in Massingir (April 2010) were relatively low, with phosphorus concentrations at the oligo-mesotrophic boundary. No measureable inorganic nitrogen was detected, resulting in ostensibly high N:P ratios that would tend to favour diazotrophic (nitrogen fixing) cyanobacteria such as *Cylindrospermopsis;*
- Nutrient availability in the gorge during August 2009 was relatively high, with N:P ratios less than 20. This suggests that flow rates were of an order high enough to offset any algal development, including that of cyanobacteria. This is confirmed by the very low biomass of phytoplankton and zooplankton captured in the net hauls.

Stable Isotope Analysis

Stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) are now widely used by ecologists to explore trophic interactions (who eats what) in aquatic and terrestrial ecosystems. Relative amounts of C^{13} to C^{12}, denoted as $\delta^{13}C/^{12}C$ ‰ (parts per mille) vary according to food identity, and can be used as a signature of particular food types in a food web. Likewise, the ratio of N^{15} to N^{14} ($\delta^{15}N/^{14}N$ ‰) indicates the hierarchical progression of food within a food web. While N^{15} enrichment between trophic levels can vary considerably (from -1 to 6 ‰), an average $\delta^{15}N/^{14}N$ enrichment of 3.4 ‰ per trophic level is widely assumed (Karasov & del Rio, 2007).

TABLE 1: OLIFANTS AND SABIE RIVER SIA SURVEYS (AUGUST 2009 AND APRIL 2010): SUMMARIZED WATER QUALITY DATA

SITE #	River	Date	Site Detail	Latitude	Longitude	Distance (km)	Depth (m)	Secchi depth (m)	Temp (°C)	EC (mS/m)	DO (mg/l)	Oxysat (%)	NOx-N (ug/l)	PO_4-P as P (ug/l)
1	Olifants	Aug-09	Mamba Weir	24.066694	31.242433	0	0	-	18.6	44	9.6	102	0.0	0.19
2	Olifants	Aug-09	Upstream border	23.972417	31.877158	90	0	0.6	20.0	43	8.0	88	0.7	0.02
							1		20.0	43	8.4	93	-	-
							2		19.9	43	8.3	93	-	-
							3		19.9	43	8.3	93	-	-
							4		19.9	43	8.3	93	-	-
3	Olifants	Aug-09	SA/Mozambique border	23.956989	31.88085	91.8	0	0.6	20.7	43	8.0	89	0.9	0.08
							1		20.7	43	7.9	87	-	-
							2		20.6	43	7.9	87	-	-
							3		20.5	43	7.5	83	-	-
							5		20.5	43	7.9	87	-	-
							7		20.1	43	7.3	79	-	-
4	Olifants	Aug-09	Downstream border	23.953461	31.885	92.4	0	0.65	21.0	43	8.0	88	0.9	0.09
							1		20.8	43	7.6	88	-	-
							2		20.4	43	7.6	88	-	-
5	Olifants	Aug-09	Outlet of Olifants Gorge into Massingir	23.941836	31.93895	98.4	0	0.6	21.4	38	6.9	79	2.1	0.11
							2		21.2	39	7.2	80	-	-
							4		21.0	40	5.8	64	-	-
							6		20.9	40	5.2	58	-	-
6	Olifants	Apr-10	Massingir Dam (West)	23.877669	32.018781	109.4	0	0.8	28.0	39	7.6	99	0.0	0.05
							5		27.3	39	7.0	89	0.0	0.07
							10		27.5	39	6.3	81	0.0	0.02
							15		27.9	38	6.2	79	0.0	0.03
							20		27.5	38	5.8	75	0.0	0.03
7	Olifants	Apr-10	Massingir Dam (East)	23.880325	32.128189	123.4	0	1.2	27.3	36	8.7	111	0.3	0.01
							1		27.4	36	8.6	110	-	-
							2		27.4	36	8.2	105	-	-
							3		27.4	36	7.9	101	-	-
							4		27.3	36	7.1	90	-	-
							5		27.3	36	6.3	80	0.5	0.02
							6		27.2	36	6.3	80	-	-
							7		27.2	36	6.3	80	-	-
							8		27.0	36	6.2	78	-	-
							10		27.0	36	5.9	75	0.0	0.03
							15		26.3	36	5.8	73	0.8	0.01
8	Sabie	Aug 09/April 2010	Sunset Dam	25.116353	31.912144	0	0	-	21.5'/25.9	41'/33	2.6	29	0.0	0.81
9	Sabie	Aug-09	Sabie River High Level Bridge	25.121022	31.924639	1.4	0	-	21.5	12	6.7	79	0.0	0.07
10	Sabie	Aug-09	Sabie River Weir	25.149247	31.94125	6	0	-	20.7	12	8.2	91	-	-
11	Sabie	Apr-10	Couramana Dam	25.159819	32.109381	27	0	0.35	26.4	13	-	-	-	-
							1		26.4	-	-	-	-	-
							2		26.4	-	-	-	-	-
							3		26.4	-	-	-	-	-
							4		26.4	-	-	-	-	-
							5		26.4	-	-	-	-	-
							6		26.4	-	-	-	-	-
							7		26.2	-	-	-	-	-
							8		26.0	-	-	-	-	-

The stable isotope signatures obtained for different functional groups at different sites and dates on the Olifants River from Mamba Weir downstream, virtually to the Massingir Dam wall, during this study are illustrated in **Figure 2**. These consolidated data illustrate considerable coherence in the ^{13}C signatures of a range of fish species and a single crocodile that succumbed to pansteatitis in the Olifants River gorge in the Kruger National Park, strongly suggesting this crocodile's reliance on fish as its food source (in contrast to signatures obtained for crocodiles from Sunset Dam – see below). The Olifants River fish values span a range of some two trophic levels, with the crocodile 'centrally' located within this range.

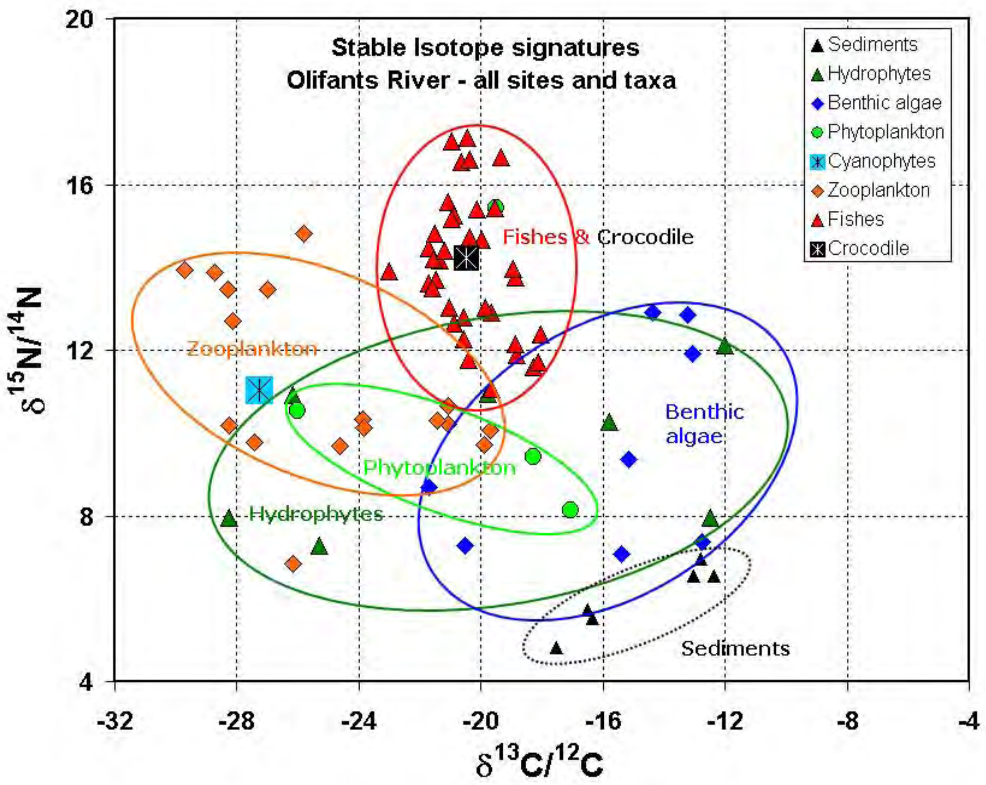


Figure 2. Stable isotope signatures of various functional food-web components at sites along the Olifants River from Mambo Weir to Massingir Dam, incorporating samples collected in August 2009 and April 2010. Envelopes enclose the vast majority of values determined for particular functional groupings.

Functional groupings other than fish show much greater variation in their ^{13}C signatures, and commonly span as wide a range of trophic levels as evident among the fishes. The few phytoplankton values (phytoplankton biomass was extremely low at most sites) range greatly in C^{13} values (nearly 10 ‰), and the horizontal alignment with their putative zooplankton consumers is weak. Here it should be noted, with reference to **Table 2**, that the phytoplankton present were generally unsuited as prey for zooplankton. Furthermore, phytoplankton values are insufficiently ^{15}N-depleted relative to zooplankton to indicate firm trophic couplings. Many of the zooplankton C^{13} values are also greatly depleted relative to prospective fish consumers. However, some values do align appropriately to suggest trophic couplings and consumption by fish. Benthic algae (substrate-attached diatoms and filamentous green algae) show relatively high C^{13} signatures, while emergent and submerged hydrophytes span a particularly wide range of C^{13} values. A single invertebrate sample, comprised of notonectid organisms sampled from Site 4 in the Olifants Gorge, suggested a possible stronger role of an invertivore linkage (phytoplankton, invertebrates, fish) in the fishes foodchain.

Figure 3 identifies the constituent taxonomic components within the consolidated fish-crocodiles SI signature envelope. Although some N^{15} values of catfish (*Clarias gariepinus*) and Mozambique bream (*Oreochromis mossambicus*) exceed those for the crocodile, others lie appropriately to reflect a crocodile food source. Piscivorous taxa among the fish assemblage include tiger fish (*Hydrocynus vittatus*) showing slight ^{15}N enrichment relative to the crocodile, although other invertivorous fishes – the imberi (*Alestes imberi*) and rock catlet (*Chiloglanis* sp.) as well as more generalist feeders such as butter barbel (*Schilbe intermedius*), squeaker (*Synodontis zambezensis*), and some *C. gariepinus* individuals are also more ^{15}N-enriched than the crocodile.

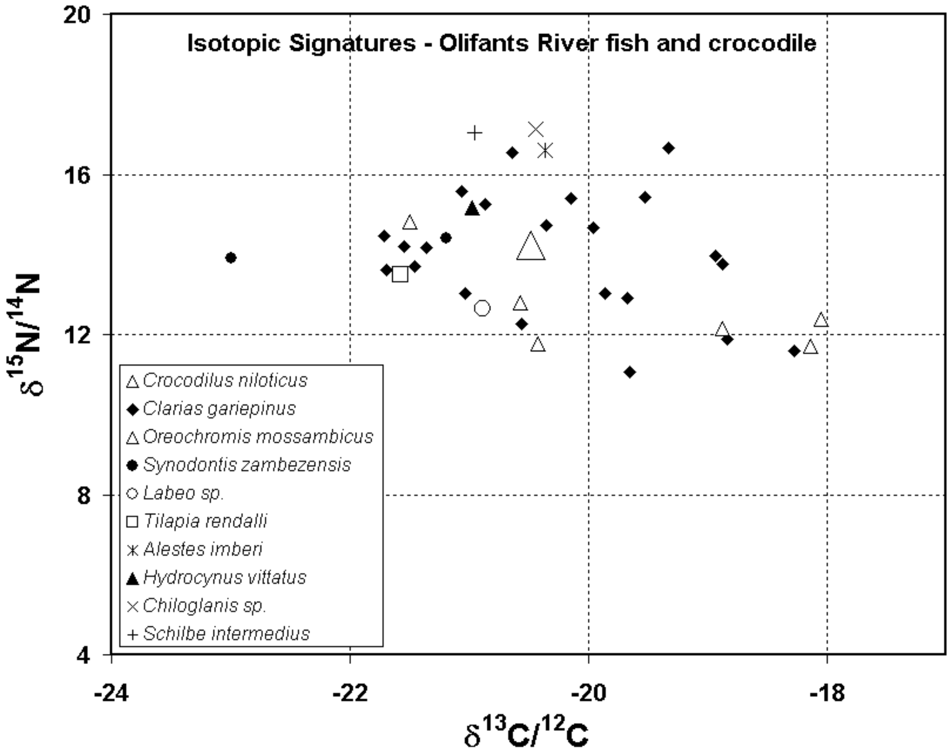


Figure 3. Species-specific fish signatures in the Olifants River system in relation to the crocodile value (enlarged triangular symbol). Note expanded scale used for $^{13}C/^{12}C$ abscissa.

The positive slope of sediment values reflects accords with progressive distances downstream, although the values for Mamba Weir at the beginning of the 123 km downriver transect are as high as the terminal values – both representing lentic (impounded) habitats.

Stable isotope values were obtained for three size fractions of zooplankton, hereafter identified as Large (> 500 µm), Medium (500-200 µm) and Small (200-80 µm). (Note that these consistent size classes should not be considered as comparable to the same arbitrary abbreviations employed in Table 2). Considerable and incongruous incoherence exists among and between the isotope signatures of planktonic autotrophs (algae and cyanobacteria) and heterotroph (zooplankton) assemblages (**Figure 4**). C^{13} values vary widely, and the expected trophic ascendancy from planktonic autotrophs to heterotrophs is not consistently reflected in their respective N^{15} signatures. Where Small and Medium zooplankton size fraction samples were N^{15}-enriched relative to phytoplankton, corresponding C^{13}-depletion was apparent in relation to the former putative food. One highly N^{15}-enriched phytoplankton sample is considered an incongruous outlier, and is excluded from the group envelope.

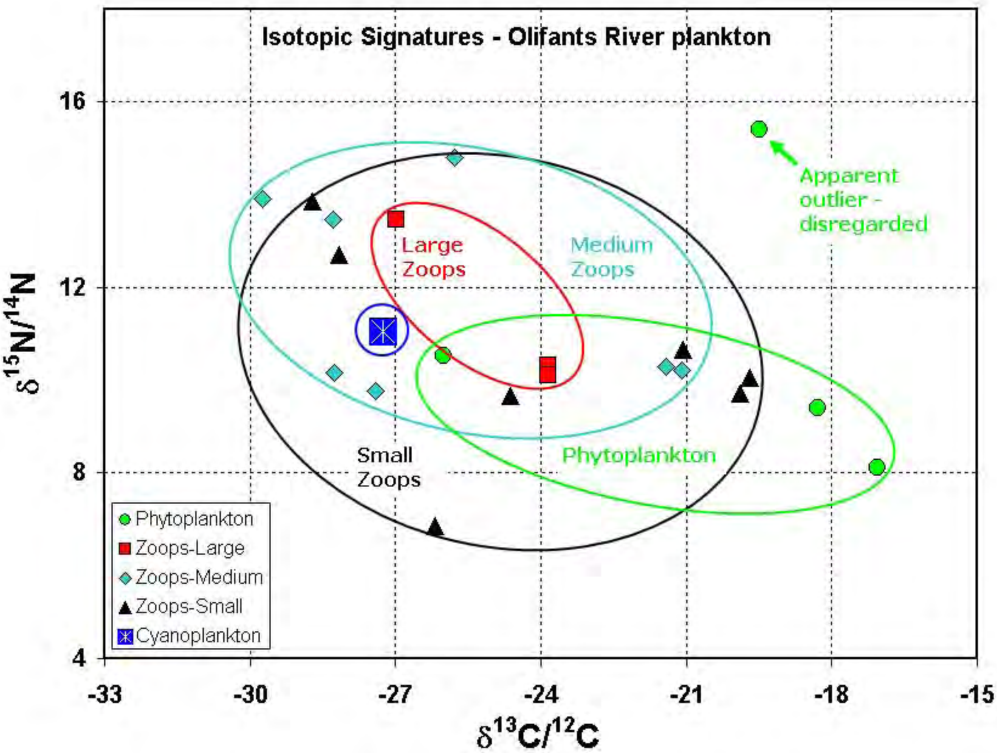


Figure 4. Expanded plots of C^{13} and N^{15} signatures among planktonic components in the Olifants River system.

No pansteatitis-induced crocodile fatalities comparable to those observed in the Olifants River system were evident in the Sabie River system, which accordingly provides an effective experimental 'control' environment. **Figure 5** shows the corresponding stable isotope signatures determined in the latter system, from Sunset Dam (at Lower Sabie rest camp) to the Couramana Dam in Mozambique.

Some anomalous signatures are also apparent in the Sabie River too, but for the purposes of this report, the important finding is reflected in the considerably enriched C^{13} signatures of crocodiles culled in Sunset Dam relative to the two fish data for Couramana Dam. This plausibly reflects the reliance of crocodiles in this Sunset Dam on terrestrial vertebrates rather than on aquatic food sources as revealed in the Olifants System, although in the absence of fish signatures for Sunset Dam, this remains speculative conjecture. Phytoplankton and zooplankton C^{13} signatures are notably better aligned horizontally in **Figure 5**.

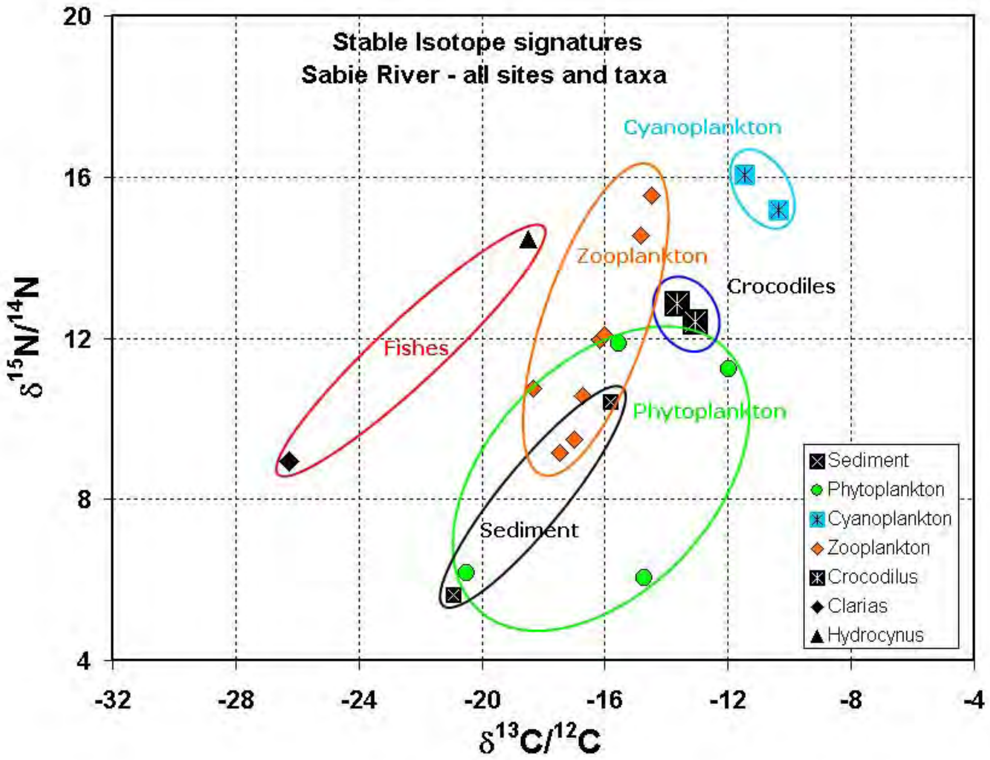


Figure 5. Stable isotope signatures in the Sabie River system. Other details as per legend to **Figure 1**.

Phytoplankton composition and abundance

Phytoplankton were generally depauperate in all samples except those collected from Sunset and Massingir Dams. Dominant phytoplankton, where present, are summarized per sampling site in **Table 2**:

TABLE 2: Summarized phytoplankton assemblages (sampling sites as per Figure 1 and **Table 1**).

Site	Date	Dominant assemblage
1	Aug 2009	*Spirogyra*
3	Aug 2009	*Gyrosigma*
4	Aug 2009	*Gyrosigma*
5	Aug 2009	*Ceratium hirundinella*
6 7	April 2010	*Ceratium hirundinella, Aulacoseira granulata, Cylindrospermopsis raciborskii, Microcystis aeruginosa, Coelastrum sp., Oocystis sp., Merismopedia sp., Staurastrum sp.*
8	Aug 2009 April 2010	*Microcystis aeruginosa, Closterium sp., Tetraedron sp., Oocystis sp., Trachelomonas sp., Oscillatoria sp., Cryptomonas.*
11	April 2010	*Ceratium hirundinella, Anabaena sp.*

The presence of *Cylindrospermopsis* in Massingir Dam is possibly significant. As yet unexplained associations between *Ceratium hirundinella* and *Cylindrospermopsis raciborskii* have been widely reported. Both species are K-strategists and have a wide range of environmental drivers and tolerances and may reflect early onset of climate change responses in poorly-enriched, particularly riverine, ecosystems. Additionally, *Cylindrospermopsis* has been linked to wildlife mortalities worldwide, including temporal associations with moribund alligators (Ross, 2000). Its presence in Massingir Dam could suggest an impoundment-related linkage to the crocodile mortalities, as opposed to a riverine connection (see below).

At the time of the reported crocodile deaths, dense blooms of cyanobacteria were recorded in the Olifants Gorge. No cyanobacteria were present in the gorge during August 2009 and, regrettably, no samples of algae were collected and preserved at the time of the crocodile deaths.

Zooplankton composition and abundance.

As expected, zooplankton was only prominent in the major standing water environments sampled – Massingir, Couramana and Sunset Dams. Far fewer occurred in the two weir habitats sampled (Mamba and Lower Sabie), while very little zooplankton was evident in flowing waters of the Olifants River gorge and its headwater inflows into Massingir Dam. On account of small sample size, data from the Lower Sabie River itself are not considered further.

Quantitative estimates of zooplankton abundance were obtained from measured vertical net hauls in Massingir and Couramana Dams. In these samples, zooplankton assemblages were of 'typical' composition and diversity, with many taxa present in high densities (**Table 3**). The crustacean zooplankton consisted predominantly of small-bodied cladocerans (*Bosmina*, *Ceriodaphnia*, *Diaphanosoma*), the calanoid copepods (*Tropodiaptomus*) and a generic mixture of cyclopoid copepods was numerically significant. The virtual absence of large-bodied cladocerans like *Daphnia* is plausibly attributable to the presence of a strong population of dinoflagellates (*Ceratium*), and/or reasonably high mineral turbidity (suspended sediments) – both of which are inimical to *Daphnia*'s feeding ability. Depredation by visual zooplanktivores (various fishes) is also a possible contributor to their effective absence, although stable isotope signatures provide little support for this prospect (see above).

TABLE 3: Zooplankton assemblages recorded in Massingir and Couramana Dams. Densities (number/m^3) of crustacean macrozooplankton (explanation in text) at sites and dates as in **Table 1**.

	Massingir			Couramana
Date:Sites #	Apr: 2	Apr: 1	Aug: 1	Apr:
Bosmina longirostris	21,606	15,348	21,479	15,910
Ceriodaphnia reticualta	16,846	15,161	6,894	33,411
Daphnia lumholtzi	0	0	265	0
Diaphanosoma excisum	2,514	7,861	8,751	15,115
Tropodiaptomus spectabilis	3,957	10,911	7,160	11,535
Cyclopoid copepods	18,156	29,387	3,978	41,764

Zooplankton was also well developed in Sunset Dam, where no quantitative estimates of abundance could be obtained, as sampling was necessarily restricted to unmeasured net-cast hauls from the shoreline. Samples obtained using this method revealed the predominance of a small-bodied cladoceran – *Ceriodaphnia*, and strong populations of cyclopoid copepods. Rotifers – especially *Keratella* and very large individuals of the predatory *Asplanchna*, were also prominent. Rather surprisingly, however, *Diaphanosoma* and *Bosmina* were effectively absent. Calanoid copepods were also absent.

Considering the crustacean macrozooplankton alone (i.e. disregarding rotifers and copepod naupliar larvae), in order to focus on elements of potential importance in food webs plausibly leading directly to fish and thus crocodiles, the broad composition of zooplankton taxa (in percentage terms) collected at each of the sites on the two sampling dates is summarized in **Table 4**. Seasonal changes in community structure appear slight, but this evaluation is confounded by spatial variation between actual sampling sites. In Massingir for example, *Diaphanosoma* appeared less abundant in April than in the previous August, but a gradient of increasing abundance upstream is evident within and between dates (compare values in successive columns which are arrayed progressively 'upstream' in this dam).

TABLE 4: Percentage numerical contribution of crustacean macrozooplankton at the various sites sampled in July 2009 and April 2010. Within each major group – cladocerans and copepods – taxa are arrayed according to general size, denoted by L, M and S (Large, Medium and Small, respectively). Note however that this is a subjective 'relative' size classing, unlike the 'absolute' size fractions subsequently considered in terms of stable isotope signatures. Shading identifies sites where total abundance in collected samples taken was too low for composition to be determined reliably.

	Olifants/Massingir						Couramana	Sunset Dam		Mamba Weir	
	Dam wall	Dam centre	Dam upper	Lower gorge	Mid gorge	Gorge inflow	Mid/upper Dam	Shoreline edge		Inshore edge	Mid stream
	Site 2 April	Site 1 April	Site 1 Aug	Site 2 Aug	Site 3 Aug	Site 4 Aug	Apr	Aug	Apr	Aug	Aug
Cladocera											
Daphnia (L)	0	0	0.5	0	0	0	0	0	0	0	0
Simocephalus (L)	0	0	0	0	0	0	0	0	0	74.1	48.8
Diaphanosoma (L/M)	4.0	10.0	18.0	92.3	50.0	23.0	12.8	0	0	0	0
Ceriodaphnia (M/S)	26.7	19.3	14.2	0.5	0	0	28.4	81.7	35.9	0	0
Bosmina (S)	34.3	19.5	44.3	1.8	0	94.7	13.5	0	0	0	0
Copepoda											
Tropodiaptomus (L/M)	6.3	13.9	14.8	0.5	0	0.4	9.8	0	0	0	0
Cyclopoida (M/S)	28.8	37.4	8.2	5.0	50	4.9	35.5	18.3	64.1	25.9	51.2

SUMMARY & CONCLUSIONS

This project set out to determine (a) the value of using Stable Isotope Analysis (SIA) as a forensic tool to identify foodchain links between primary producers and apex predators (crocodiles) in the Olifants and Sabie River systems in the Kruger National Park and (b) to attempt to "top-down" link the crocodiles to a potential cause of the pansteatitis-related mortalities of crocodiles.

The project was constrained by being chronological de-linked from the crocodile death events by a considerable time margin. Accordingly, the conditions prevailing in either of the two river systems at the time of the incident could not be sampled. In particular, no cyanobacterial blooms were present in the Olifants Gorge and the SIA results were dependent on a single crocodile sample, but were bolstered by fish samples collected both during and prior to this project.

At the time of this survey the phytoplankton of Massingir Dam and the lower Olifants River was dominated by the K-strategist dinoflagellate, *Ceratium hirundinella*. This organism is an alternate dominant in mild eutrophic conditions, typically alternate to a species such as *Microcystis*. Although *Microcystis* was present in Massingir Dam, so was *Cylindrospermopsis raciborskii*, a genus that can outcompete other cyanobacterial species by virtue of being able to suppress their ability to take up phosphorus.

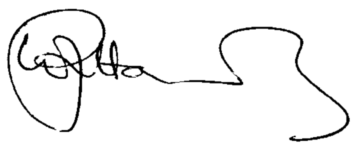
The results indicate that while the crocodile diet is centered in the fish typical of Massingir Dam and the lower Olifants Gorge, no links to phytoplankton via fish-zooplankton could be demonstrated. This is further unlikely given the general inedibility and low food value that most colonial cyanobacteria offer to zooplankton. The results suggest that the relevant fish species are feeding via an invertivore linkage, either in the benthos or the littoral of Massingir Dam.

The presence of the toxin-producing *C. raciborskii* in Massingir Dam is significant in that this organism has been linked to large reptile (alligator) mortalities, as well as other wildlife, for many years. The rapid appearance (globally) of this cyanobacterium is a relatively new and poorly-understood phenomenon. Its possible role in the KNP crocodile mortalities cannot be underestimated and cannot be linked as no samples were collected at the time, nor was a comprehensive assessment made of the conditions prevailing in Massingir Dam. The presence of *C. raciborskii* has major implications for the future use of water from this dam – either local or downstream.

It is considered likely that the cause and effect pathway to the crocodile deaths was centered in Massingir Dam and its limnological ageing process following filling, rather than a riverine-based cause. Again, however, the absence of primary data coincident with the event, as well as more time-based (seasonal) data, preclude the further development of this line of thinking at the present time.

The project has shown that SIA-based forensic interpretation of foodweb characteristics can be deployed rapidly and cheaply in response to an event such as the crocodile mortalities.

The data assembled in this investigation are likely to be of mutual, complementary value to other surveys being conducted around the issue of crocodile deaths in the Kruger National Park.



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