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Ecosystem change and the Olifants River crocodile mass mortality events

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Abstract. Nile crocodile (*Crocodilus niloticus*) mass mortality events in the Olifants River between the Letaba River confluence in South Africa and Lake Massingir in Mozambique have been attributed to pansteatitis: a disease that affects fat depots of the animals. The disease is also found in sharptooth catfish (*Clarias gariepinus*) in the same area, and the cause of the disease is attributed to pollution. Although the Olifants River Valley is polluted, the impact of interventions such as dam construction on biodiversity receives little attention. We show that the onset of the pansteatitis epidemic in crocodiles and sharptooth catfish at the Olifants/Letaba confluence coincided with back-flooding of Lake Massingir that changed the Olifants River from a rock and sand substrate river to a clay substrate lake. Isotopic analysis shows that sharptooth catfish shifted from a predominantly vegetarian to a piscivorous diet that is highly correlated with pansteatitis prevalence, and crocodiles and tiger fish (*Hydrocynus vittatus*) show coincident trophic level increases. The evidence suggests that the ecosystem change altered the structure of the lotic foodweb and that an exotic or extralimital fish has invaded the confluence and is the vector of the pansteatitis epidemic. The invasive fish species is yet to be identified. The pansteatitis epidemic is an unintended ecological consequence of damming this river.

Key words: aquatic biodiversity; *Clarias gariepinus*; *Crocodilus niloticus*; *Hydrocynus vittatus*; lotic foodwebs; pansteatitis; stable isotopes.

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INTRODUCTION

Pansteatitis is an inflammatory reaction accompanying fat cell necrosis that can cause death in a wide range of species (Roberts et al. 1979, Herman and Kircheis 1985, Ladds et al. 1995, Wong et al. 1999, Niza et al. 2003, Goodwin 2006, Roberts and Agius 2008, Neagari et al. 2011). Although the disease is rare in wild animals it has brought the Nile crocodile, *Crocodilus niloticus* Laurenti, population at Lake Loskop in the upper Olifants River Valley, South Africa, to the brink of extinction (Ashton 2010, Botha et al. 2011). Since 2008 crocodile mass mortalities from pansteatitis have also become a seasonally recurring event downstream in an area known

as the Olifants River Gorge between the confluence of the Olifants River with the Letaba River in the Kruger National Park, South Africa, and Lake Massingir, Mozambique (Osthoff et al. 2010, Ferreira and Pienaar 2011). The condition affects fat depots and renders the crocodiles stiff and lethargic and unable to hunt, and death is thought to be through starvation or drowning. Pansteatitis is also diagnosed in sharptooth catfish, *Clarias gariepinus* (Burchell), in the same area of Kruger National Park in which the crocodile mass mortalities occur (Huchzermeyer et al. 2011) and in Lake Loskop it is prevalent in Mozambique tilapia, *Oreochromis mossambicus* (Peters) (Oberholster et al. 2011). Although the cause of the disease is dietary, the co-occurrence of pansteatitis in crocodiles and fish at two different locations of the same river catchment is not related to a potential trophic relationship as the disease is not contagious through ingestion. Pansteatitis may be caused by the consumption of rancid, dead fish (Ladds et al. 1995, Huchzermeyer 2003) but it is the intrinsic fatty composition of the diet (Brooks et al. 1985, Goodwin 2006) rather than pre-existing pansteatitis that affects higher trophic levels.

The pansteatitis epidemic in Lake Loskop followed mass fish die-offs and is attributed indirectly to water pollution from upstream mining, agriculture and human urban waste (Ashton 2010, Oberholster et al. 2011). If the bioaccumulation of pollutants is causing the disease, then it has severe implications for other water users in the catchment. The Olifants River Valley hosts commercial and subsistence agriculture and livestock farmers that irrigate or water directly from the river; it flows through the Kruger National Park where biodiversity is at risk; and there are trans-boundary issues as the river drains the commercial heartland of South Africa into neighbouring Mozambique. Although environmental law in South Africa imposes a "polluter pays" policy in respect of remediation (South African Government Gazette 1998) there is reluctance to impose measures that might affect the coal production and coal-based power generation that support the South African economy. In addition the scientific basis to link specific pollutants with specific industries or land-use patterns in a multi-industry catchment, and the basis to link pollutants with their ecological consequences are poorly developed. As a result it remains unclear if pollution is the underlying cause of the pansteatitis epidemics. Bioaccumulation of pollutants has yet to be demonstrated in the crocodiles or other top predators in the Olifants River system. As Nile crocodiles mature they typically shift from an aquatic foodweb dependence to a terrestrial foodweb (Cott 1961, Wallace and Leslie 2008, Radloff et al. 2012), and so a bioaccumulation mechanism should lead to higher pansteatitis prevalence in juveniles. Affected juveniles may be underrepresented through their vulnerability to predation, but the pansteatitis mortality profiles of crocodiles in the Olifants River Gorge includes large numbers of mature individuals. In addition water pollution is ubiquitous throughout the Olifants River Valley yet substantial reaches remain unaffected by pansteatitis. Lake Flag Boshielo, for example, is located downstream of Lake Loskop and upstream of the Olifants River Gorge and it hosts a crocodile population without apparent pansteatitis symptoms. The dispersed distribution of pansteatitis outbreaks suggests that there may be other factors that are causing the disease.

Since pansteatitis is a dietary disease this research focuses on establishing the structure of the aquatic foodweb in the Olifants River Gorge and to determine if this differs from other systems where no pansteatitis occurs. The objective is to clarify possible exposure pathways for the bioaccumulation of pollutants, or to explore a possible localised ecological trigger that may underpin the pansteatitis epidemic. We use stomach content analysis and stable isotope analysis to determine the dietary niche of healthy and pansteatitis affected populations of sharptooth catfish and crocodiles from Kruger National Park (Fig. 1). The trophic positions of the sharptooth catfish and crocodiles are compared with tiger fish, *Hydrocynus vittatus* Castlenau, because the latter is an obligate piscivore at the top of the fish aquatic foodweb in the Kruger National Park river systems, and also with invertebrate communities lower in the foodweb. The two dietary analysis approaches that are used are complimentary to one another. Carbon and nitrogen isotopes elucidate the trophic relationships between different organisms with $\delta^{13}C$ values reflecting the C_3 or C_4 plant source of

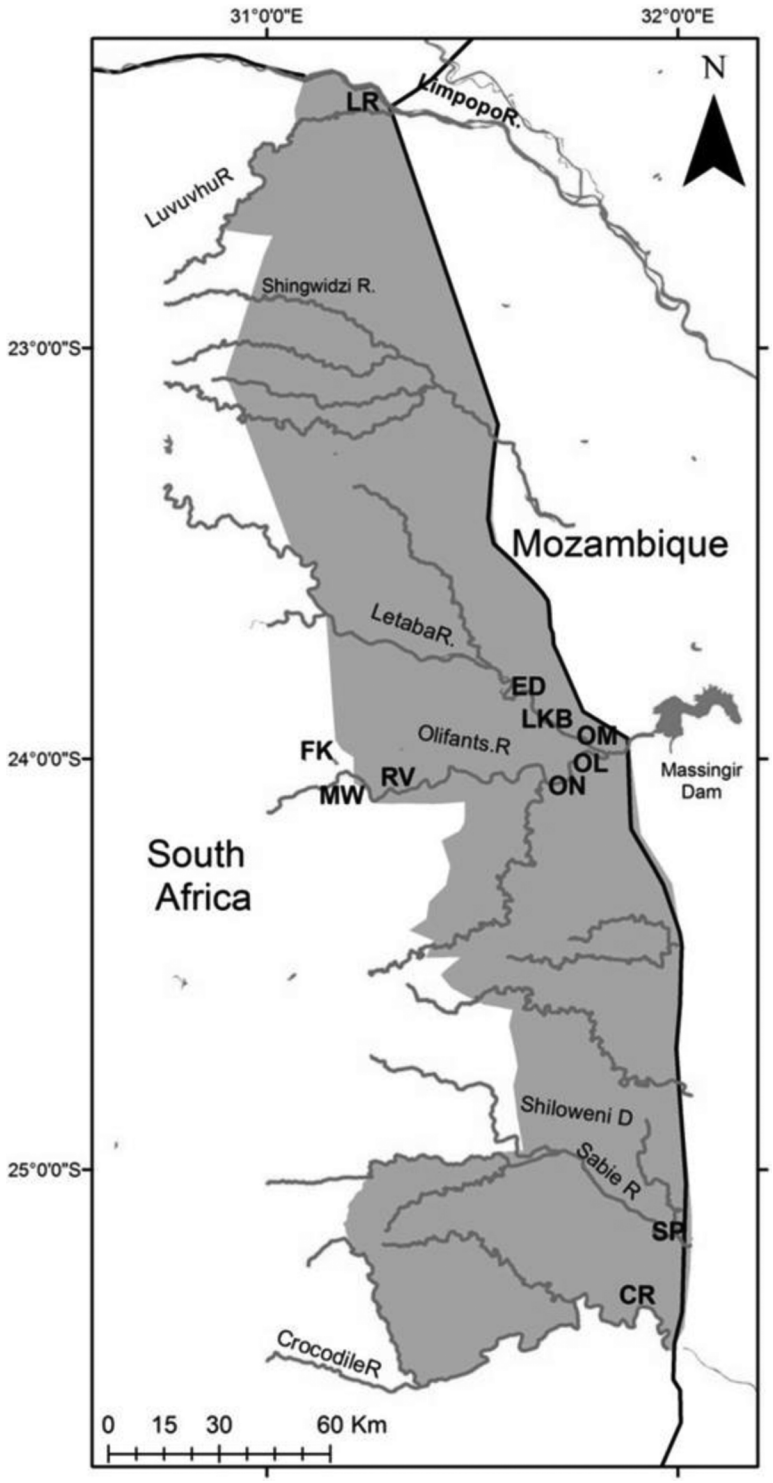

Fig. 1. Location of sampling sites in Kruger National Park. Sampling sites are identified by the site acronyms.

the foodweb with low (<1‰) increases between diet and tissue carbon, while $\delta^{15}N$ values increase by approximately 3–5‰ per trophic level (Minagawa and Wada 1984, Peterson and Fry 1987, Fry 1991, Van der Zanden and Rasmussen 2001). The stomach content of an organism will reflect possibly only the last meal while the carbon and nitrogen isotope values integrate the dietary variance through the turnover time of the tissue, and in Nile crocodile keratin it may be in the order of months (Radloff et al. 2012). The majority of our sample sites were from different river systems and there is no possibility that the organisms might have migrated between the sample sites. However the Letaba River is a tributary of the Olifants River and in this system it is possible that organisms could migrate between the different sampling locations. In order to differentiate the river of origin for fish and crocodiles we analysed sulfur isotope values as this reflects the geology of the different catchments.

Methods

Sampling

Fish samples were collected for histopathological and stomach content analysis in June 2009, August 2009, November 2009, July 2010, January 2011 and June 2011 from rivers and dams in the Kruger National Park (Fig. 1). Sample sites include the confluence of the Letaba and Olifants Rivers (23°59′21.8″ S, 31°49′35.6″ E) and from 1.3km upstream of the confluence in the Letaba River (23°55′57.9″ S, 31°49′02.1″ E) (for sharptooth catfish and tiger fish it can be assumed that these two locations represent a single population) (site OL). Further samples were taken from the Olifants River at Ngotso located 28.5 km upstream of the Letaba confluence (24°03′10.8″ S, 31°43′50.6″ E) (site ON), the headwaters of Lake Massingir near the Mozambique border (23°57′48″ S, 031°52′97″ E) (site OM) and Mamba Weir (24°03′32″ S, 031°14′14″ E) (site MW). Samples were also taken from Reënvoël Dam (23°58′37.2″ S, 031°19′38.4″ E) (site RV) and van Ryssen Dam (24°00′13.6″ S, 31°05′36.9″ E) (site FK) located on tributaries of the Olifants River. The Letaba River was sampled upstream of the confluence at Klipkoppies Bridge (23°56′58″ S, 031°43′89″ E) (site LKB) and from Engelhard Dam (23°50′19″ S, 31°28′28″ E) (site ED) located 17km upstream of the Olifants River confluence. Other river systems that were sampled include the Sabie River in the Sabiepoort (25°10′25.41″ S, 32°2′23.42″ E) (site SP), the Levuvhu River (22°25′51.0″ S, 31°18′04.4″ E) (site LR) and the Crocodile River (25°23′57.1″ S, 31°57′29.9″ E) (site CR). Sharptooth catfish and tiger fish were caught on baited hooks or artificial lures, while other species were sampled using an electrofisher (Samus). The fish that were subject to isotopic analysis comprise a subsample of the June 2011 collection from the OL, LR and CR sites. Invertebrates, diatoms, riparian and aquatic vegetation, sediments and organic detritus were also sampled for isotopic analysis. On 4–7 September 2011 tiger fish samples were collected from the Crocodile River, and both sharptooth catfish and tiger fish were sampled from below the Engelhard Dam wall (considered the same as site ED).

Fish were euthanized in a benzocaine solution. The protocol was approved under the University of Pretoria Animal Care and Use Protocol V013/10 and sanctioned by South African National Parks (SANParks). Crocodile claws were collected from animals that were euthanized as part of the SANParks research into pansteatitis in 2010.

Isotopic analysis

Fish muscle tissues for isotopic analyses were taken from the abdominal area and were degreased in a 2:1 chloroform:ethanol mixture and dried overnight at 70°C. Invertebrates and organic samples were reacted with a 1% HCl solution to remove any trace of carbonates, rinsed to pH neutral in distilled water and dried overnight at 70°C. Analyses were performed on homogenised whole invertebrate samples (Pinnegar and Polunin 1999), and on a time series of samples taken from the dorsal aspect of the crocodile claws extending from the base to the tip of the claw. Claw samples were cleaned by boiling in distilled water during the extraction process but were not further pre-treated. Carbon and nitrogen isotope analyses were undertaken on 0.5–1.0 mg aliquots, while sulphur isotope analyses required 5 mg aliquots. Analyses were done on a Flash EA 1112 Series elemental analyser coupled to a Delta V plus isotope ratio mass spectrometer by a ConFloIV interface (all

equipment supplied by ThermoFisher, Bremen, Germany). Each sample was measured in duplicate with laboratory standards and blanks run after every 12 unknown samples. Precision was <0.1‰ for all analyses.

The degreasing process influences both $\delta^{13}C$ and $\delta^{15}N$ values. The effect on $\delta^{15}N$ values is in the order of 0.25‰ (Post et al. 2007) which is considered negligible in the context of this study, while the effect on $\delta^{13}C$ values is more substantial and a correction based on the C/N ratio (derived from the mass spectrometer measurement) was applied (Post et al. 2007).

Carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel) while sulphur isotopes were referenced against sulphanilamide and NIST bovine liver (Fry et al. 2002). Results are reported using the standard delta notation for stable light isotopes using the equation $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1]/1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. The reference standards against which results are reported are Vienna PeeDee belemnite (VPDB) for $\delta^{13}C$, atmospheric nitrogen (Air) for $\delta^{15}N$ and Vienna Canyon Diablo Troilite (VCDT) for $\delta^{34}S$.

Statistical analysis

The statistical test for significance for the association of pansteatitis with stomach content excluded fish that had empty stomachs. The fish sample was divided into three populations: those that had pansteatitis (P++), those that were sampled at a site at which pansteatitis prevalence was recorded, but did not show any symptoms (P+−), and those sampled at sites from which no pansteatitis was recorded (P−). Stomach contents of fish were classified into three categories: fish, vegetation and mixed. The null hypothesis assumed that the three stomach content categories would be equally sampled in a random selection strategy by the fish populations. The chi-squared test value of 63.9 is significant ($P < 0.0001$) using 4 degrees of freedom and the null hypothesis is rejected implying that there is preferential dietary selection in the three populations.

Results and Discussion

The results of the sulphur isotope analysis of fish from the Olifants River/Letaba River system are plotted in Fig. 2. Distinction is made between sharptooth catfish and tiger fish that are both migratory in river systems versus other fish species that are less likely to migrate (see Appendix A for the species that were sampled). The results for sites ON and ED that are located upstream in the Olifants and Letaba Rivers respectively provide unambiguous sulphur isotope signatures for each river that can be used to trace migratory behavior of fish sampled at the confluence. Migratory-limited fish sampled upstream in the Olifants River had $\delta^{34}S$ values of 4.1 ± 0.7 (mean ± SD, $n = 21$) and those sampled upstream in the Letaba River had $\delta^{34}S$ values of 10.8 ± 0.4‰ (mean ± SD, $n = 13$). Fish sampled at the confluence showed $\delta^{34}S$ that ranged between the baseline values for the two rivers. It is not necessary to characterise the sulphur isotope values for the other sample sites as there is no possibility of migration between these systems, but it is necessary to explore the basis for isotopic foodweb structure comparisons between isolated sites (Post 2002).

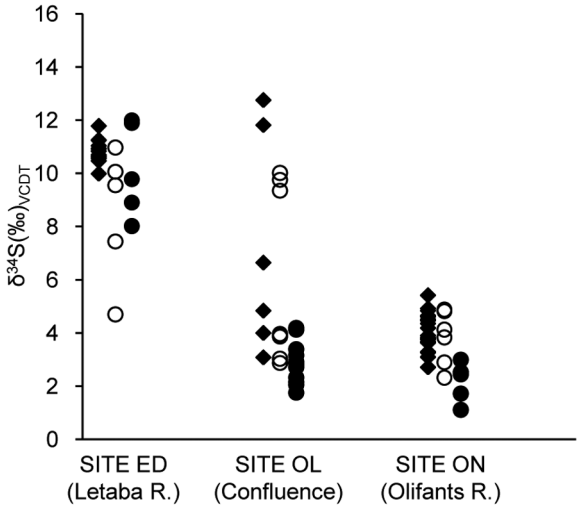

Fig. 2. The $\delta^{34}S$ values for fish characterise the Olifants River source (site ON) and the Letaba River source (site LE). Solid circles represent tiger fish, open circles represent sharptooth catfish and diamonds represent other fish. Fish from the confluence (site OL) show mixed or intermediate $\delta^{34}S$ values.

Isotopic foodweb analysis is based on the predictable diet-to-tissue fractionation of carbon and nitrogen isotopes that leads to isotopic

enrichment in organisms at higher trophic levels. Within a site this allows trophic comparisons to be made between different organisms, but the approach is more complicated if trophic levels are to be compared between sites in different river systems. Local sources of carbon and nitrogen in lotic systems may vary, particularly where rivers flow through different catchment land-uses that may result in different isotopic characteristics at the base of the foodweb (Cabana and Rasmussen 1996, Post 2002). In this study we used the isotopic values for invertebrates as a proxy for the isotopic values for the base of the site-specific foodwebs. We compared species of invertebrates found at all the sampling locations (Appendix B) on the assumption that they will occupy the same trophic level, but only *Gomphidae* (dragonfly larvae) were represented at all sites, and n values at each site were not always sufficient to give confidence to the inter-site comparison. Several other species of invertebrates were represented at most, but not all of the sites. The inter-site isotopic baseline that emerged for selected invertebrate species presented a consistent pattern, and an alternative approach was adopted to overcome the sample bias. Instead of considering individual invertebrate species, the analysis focussed on the averaged isotopic values for all invertebrate species found on each site irrespective of the different trophic levels they represented (Fig. 3). The result that emerged from this approach is very similar to the results observed for individual invertebrate species and the pattern is robust. The average invertebrate values for all sites except the ED site (below the Engelhard Dam on the Letaba River) showed a linear dependence of δ^{15}N on δ^{13}C that is described by Eq. 1.

$$\delta^{15}\mathrm{N} = -0.32\delta^{13}\mathrm{C} + 3.69 \ldots (r^2 = 0.986). \quad (1)$$

The dependence of δ^{15}N on δ^{13}C is related to the variation in the isotopic base of the foodweb between sites, and this equation defines a particular trophic level (in this case the invertebrates). For higher trophic levels the relationship will be defined by Eq. 2.

$$\delta^{15}\mathrm{N} = -0.32\delta^{13}\mathrm{C} + 3.69 + \Delta n \quad (2)$$

where n represents the trophic level of an organism relative to the invertebrates, and Δ represents the diet-to-tissue fractionation factor for nitrogen isotopes. We define the lines in isotope space that are expressed by Eq. 2 as isotrophic lines. We use three isotrophic lines defined by Eqs. 3, 4 and 5.

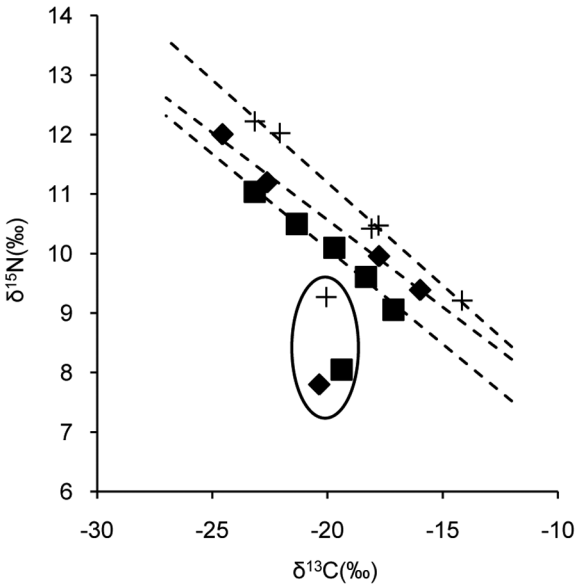

Fig. 3. The isotopic values for aquatic invertebrates are used to define an "isotrophic" comparison between sites from different catchments. *Gomphidae*, represented by crosses, and *Pleurocerida*, represented by diamonds, are represented at most of the sample sites, and display a linear relationship between δ^{13}C and δ^{15}N values. To better represent all invertebrate trophic levels and to provide larger sample size for the comparisons, the isotrophic line regression is based on the average of all invertebrate species from each site, represented by solid squares. The isotopic values for site ED (circled) deviate from the other sites. Regressions through the respective datasets are represented with dotted lines and exclude the ED site.

$$\delta^{15}\mathrm{N} = -0.32\delta^{13}\mathrm{C} + 3.6 \quad (3)$$

$$\delta^{15}\mathrm{N} = -0.32\delta^{13}\mathrm{C} + 6.16 \quad (4)$$

$$\delta^{15}\mathrm{N} = -0.32\delta^{13}\mathrm{C} + 9.16. \quad (5)$$

The first isotrophic line was selected to distinguish fish and invertebrates, the second to represent the population of sharptooth catfish and tiger fish at the confluence of the Olifants and Letaba Rivers, and the third is equally spaced between these endpoints for reference

purposes. Coincidentally the tropic separation in the trophic lines of 3‰ on the nitrogen isotope scale is relatively close to the typical value observed for the trophic level fractionation of $\delta^{15}N$ in a foodweb.

The isotrophic lines apply to all the sites except ED where $\delta^{15}N$ values are systematically offset by a factor of −1.84‰ across all organisms that were analysed. The reason for this excursion is not clear but it may be related to construction work on the Engelhard Dam that involves earthworks and concrete casting that commenced in July 2011 and was still ongoing during foodweb sampling in September. For the remainder of the analysis the isotrophic lines for the ED site are adjusted by −1.84‰.

Trophic results from the Olifants River, the Letaba River, and for reference purposes from the Crocodile and Levuvhu Rivers, are presented in Fig. 4. Emphasis is placed on the relative trophic separation between sharptooth catfish from different river systems and between sharptooth catfish and tiger fish. Stomach contents of tiger fish from the Olifants/Letaba confluence comprised fish remains ($n = 4$) or were empty ($n = 17$) confirming previous observations that this species is an obligate piscivore (Munro 1967, Skelton 1993). The sharptooth catfish is typically a benthic omnivore (Spataru et al. 1987, Bruton 1988, Skelton 1993, Van Weerd 1995) that should occupy a lower trophic level than tiger fish. In the Crocodile River (Fig. 4A) and in the Olifants River upstream from the confluence (Fig. 4B), tiger fish occupy a higher trophic level than sharptooth catfish as expected. In the Letaba River upstream from the confluence a single sharptooth catfish specimen occupies a higher trophic level than tiger fish (Fig. 4C). The sulphur isotope value for this individual was 4.7‰ suggesting that it migrated from the Olifants River system and the carbon and nitrogen isotope signal may not reflect its positioning in the local foodweb. At the Olifants/Letaba confluence (Fig. 4D) sharptooth catfish and tiger fish occupy the same trophic level and the trophic level for both species is higher than any of the other sampled sites. This observation confirms stomach content analysis: of 21 sharptooth catfish sampled at the Olifants/Letaba confluence in June 2011, four had vegetable matter in their stomachs while 11 contained pure fish remnants (Appendix C). One sharptooth catfish specimen with only vegetation in its stomach yielded the lowest trophic level of the isotopic analysis subsample, and its sulphur isotope value of 9.3‰ indicates it was a recent immigrant from the Letaba River.

At the Olifants/Letaba confluence the frequency of pure fish stomach contents in sharptooth catfish varied seasonally between 81.8% in November/January samples (high flow) and 48.7% in June/July samples (low flow). The stomach content analysis only reflects the last food intake of each specimen and cannot be used to infer the overall diet or seasonal variation of each individual fish, but the isotopic analysis aggregates tissue turnover times and reflects the modal diet of each individual. Our combined isotope and stomach content data confirms that there is a high prevalence of piscivory in the Olifants/Letaba sharptooth catfish population. Since the trophic level of both sharptooth catfish and tiger fish at this site is higher than tiger fish from other sites we can make some inference regarding their diet. The trophic similarity between sharptooth catfish and tiger fish seems to indicate predation on a narrow range of fish species at the Olifants/Letaba confluence rather than opportunistic feeding that leads to a broader spread of trophic levels for both species at the comparative sites. The prey species is unlikely to be among the low trophic level fish such as yellowfish, *Labeobarbus marequensis* (Smith), that are extremely abundant. Instead the prey species will have $\delta^{15}N$ values associated with a slightly higher trophic level, or a vegetarian species with a high $\delta^{15}N$ diet. It is noted that diatoms and organic sediment samples from Lake Massingir have elevated $\delta^{15}N$ values relative to all the other sites that were sampled (Appendix D).

This diet specificity appears to underlie the vulnerability of sharptooth catfish to pansteatitis. There is a significant correlation between the frequency of fish stomach contents and the prevalence of pansteatitis: 73.5% of sharptooth catfish with pansteatitis had pure fish stomach contents and 16.3% had pure vegetation in their stomachs compared with 22.2% pure fish stomach contents and 59.3% pure vegetation content from sites without any pansteatitis (Fig. 5, Appendix C). It is not clear if the vulnerability to pansteatitis is a metabolic consequence that is

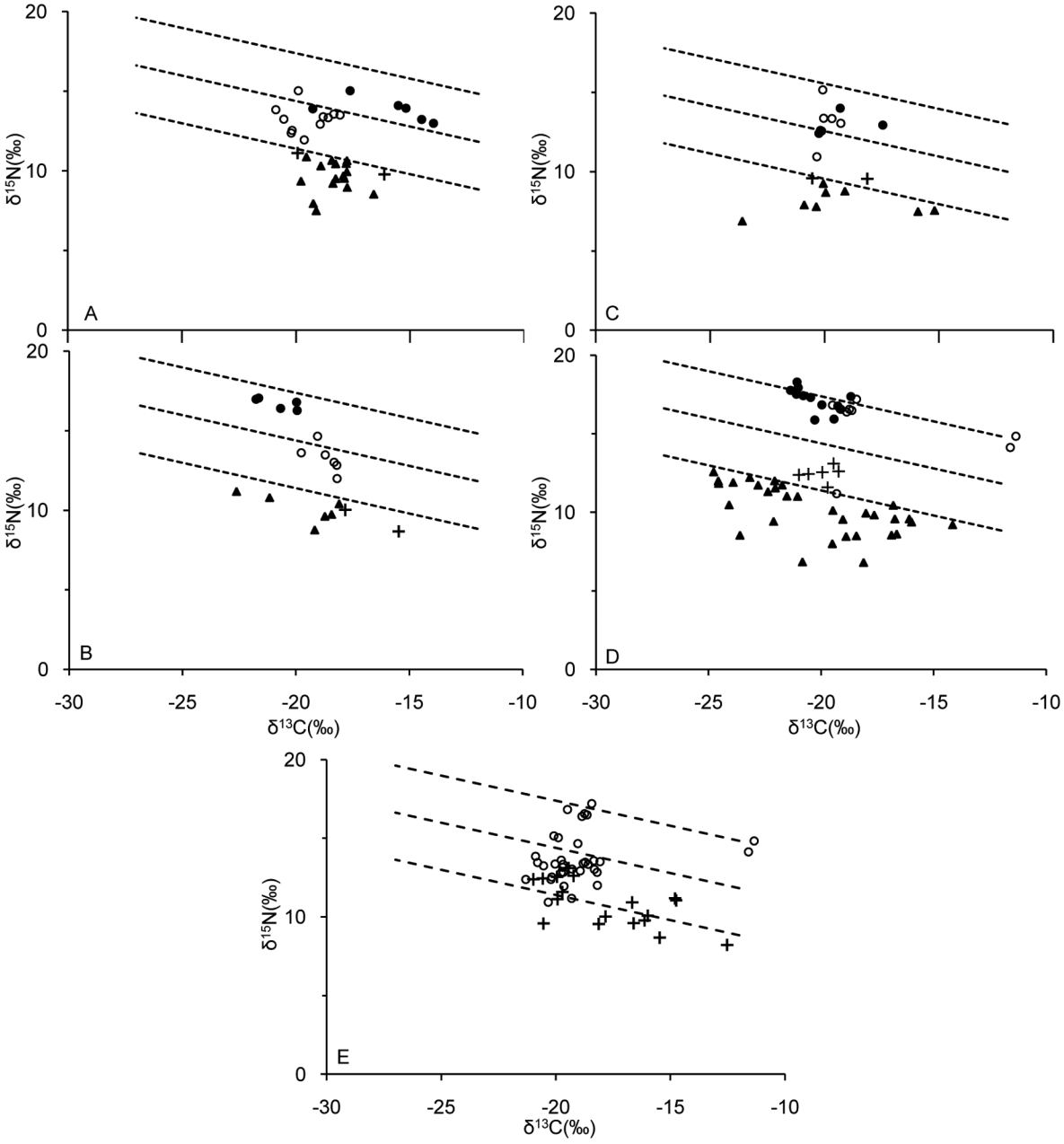

Fig. 4. The relative trophic position of individual sharptooth catfish, tiger fish invertebrate genera and co-occurring crocodiles are represented relative to lines of equal trophic level for sites CR (A), ON (B), ED (C) and OL (D). The relative trophic level of the entire sample of crocodiles ($n = 17$) is compared with all sharptooth catfish samples (E). Sharptooth catfish are represented by open circles, tiger fish by solid circles, invertebrates by solid triangles and crocodiles by crosses. The isotrophic lines for site ED are adjusted by −1.84‰ to compensate for a foodweb base value offset.

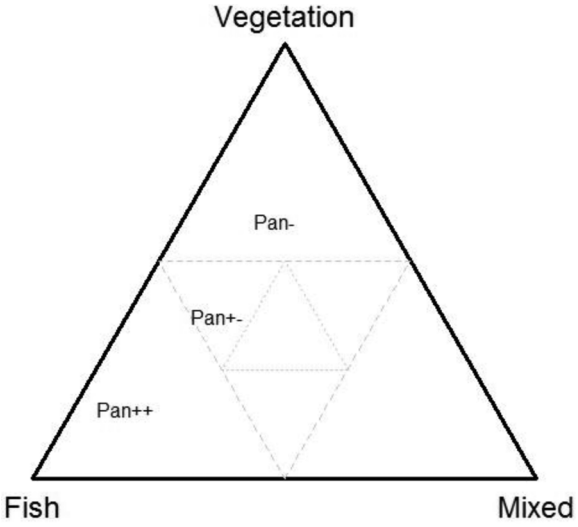

Fig. 5. The population of sharptooth catfish with pansteatitis (Pan++) have stomach contents with a higher proportion of fish than vegetation, or invertebrates and detritus (mixed) when compared with the population of fish from areas that have pansteatitis prevalence, but do not have pansteatitis (Pan+−), and the population from areas without pansteatitis prevalence (Pan−).

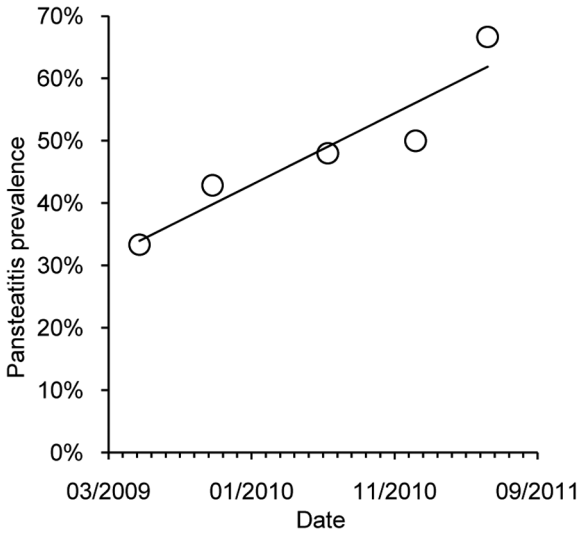

Fig. 6. The trend in the prevalence of pansteatitis in the sharptooth catfish samples through time suggests that the epidemic started in the 2006/2007 austral summer.

unique to sharptooth catfish with a piscivorous diet, as the disease does not occur in lower trophic level fish species or in tiger fish at the Letaba/Olifants confluence. It may be caused by an intrinsic quality of the fish that is ingested as pansteatitis is associated with increased intake of polyunsaturated fatty acids or from a rancid fish fat diet (Brooks et al. 1985, Goodwin 2006). Pansteatitis was, for example, observed in sharptooth catfish fed on rotting trout offal at Lunsklip Fisheries in November 2009 (Huchzermeyer et al., *in press*), but fish die-offs that might be a source of rancid fats are rare at the confluence. A linear regression of pansteatitis prevalence through time suggests that the epidemic started in sharptooth catfish at the beginning of 2007 (Fig. 6).

Interestingly, the role of fish in the crocodile diet is not as high as anticipated. Of the 11 crocodiles for which stomach contents inventories were taken in this study, 6 were empty, 4 contained terrestrial remains, and two contained fish remains. In support of this the isotope values of the crocodiles plot well below sharptooth catfish and most other fish species that were sampled (Fig. 4, Appendix E), implying that terrestrial food sources make up the bulk of their diet. Within the crocodile population that was subject to isotopic analysis, those from the Olifants River Gorge have the highest trophic status and probably consume more fish than in other areas, but it still remains a relatively small part of their overall diet. Time series stable isotope analysis of crocodile claws shows that four of the five animals sampled from the Olifants River Gorge show an unprecedented increase in nitrogen isotope values before their deaths (Fig. 7A–D). This suggests a trophic level increase that may be associated with dietary changes with increased ontogenic age or size (Radloff et al. 2012), but none of the other twelve crocodile claw time series from rivers and dams throughout KNP show this trend. The trophic increase in the Olifants River Gorge crocodiles is similar to that noted in sharptooth catfish and may indicate increased fish intake before the crocodiles died.

A single Olifants River crocodile from the Olifants/Letaba confluence that did not show the gradual nitrogen isotope increase had values that remained within a tight range over most of its growth, but it showed regularly spaced high

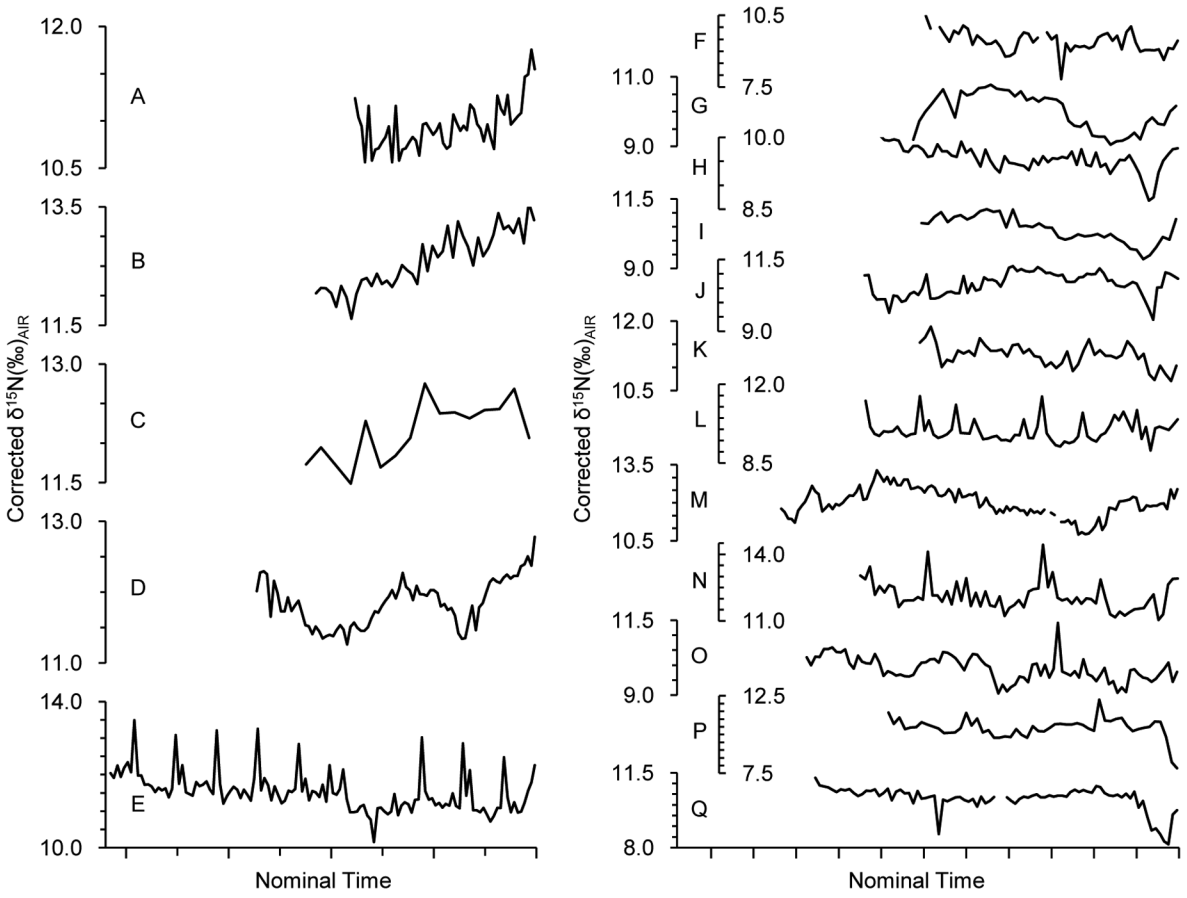

Fig. 7. Most of the crocodiles sampled between the confluence of the Olifants and Letaba Rivers and the Mozambique border show an unprecedented trophic level increase before their death (A–D). A regular spike in one of the crocodile claw time series (E) is thought to be a seasonal signal suggesting that the trophic level increases commenced in the last 3 years. Other crocodiles from Mamba Weir (F, G), the Letaba River (H, I), the Levuvhu River (J, K), the Shingwedzi River (L), Shiloweni Dam (M, N), the Sabie River (O) and the Crocodile River (P, Q) do not show this trend. The $\delta^{15}N$ values have been normalised to the mean $\delta^{13}C$ value of the crocodile claw samples (−17.74‰). The x-axis is a nominal time axis with the value for the tip of each claw plotted on the left, and the base of each claw on the right so that time moves from oldest to youngest in a left to right direction. The crocodiles were sampled in 2010 so the base of each claw represents this date.

nitrogen isotope excursions (Fig. 7E). This pattern is typical of organisms that have a seasonal dietary shift between food sources with distinct isotopic baseline values, for example, in the baleen of migratory whale species that migrate between different food sources in the ocean (Best and Schell 1996, Lee et al. 2005). In some instances the positive nitrogen isotope excursions in the crocodiles are up to 3‰ in magnitude which indicates one trophic level shift. Since the crocodile diet in Kruger National Park appears to be dominated by terrestrial animals the alternative dietary source may be seasonal exploitation of fish which is demonstrated in Fig. 4 to have a higher nitrogen isotope value. We suggest that the regular nitrogen isotope spike is a seasonal marker related to the exploitation of fish breeding migrations during the early rainy season when river flows increase (December to January in Kruger National Park). Determining the precise seasonality of the nitrogen spikes will require further research, but the presumption that it is a seasonal marker provides a means of calibrating the claw growth rate so

that the onset of the trophic shift can be dated. The nitrogen isotope (trophic) increase commenced between 2 and 3 years before the crocodile sample was collected in 2010.

We associate the trophic increase in the sharptooth catfish with the trophic level increase in crocodiles on the basis of geographic and temporal consistencies. The trophic increase in sharptooth catfish and tiger fish without a corresponding shift in invertebrates implies feeding behavior change in the fish. In addition the matching trophic levels of sharptooth catfish and tiger fish populations at the Olifants/Letaba confluence suggest that they are feeding on the same limited range of species. The fish trophic level data requires the presence of a prey fish species that previously did not exist in the region. The same logic does not apply to the trophic increase in crocodiles as this may reflect either increased fish intake or the intake of fish with higher $\delta^{15}N$ values. The latter may be pansteatitis affected sharptooth catfish as the disease renders them vulnerable to predation although eating pansteatitis affected fish will not cause pansteatitis in crocodiles. Controlled feeding experiments in which crocodiles were fed sharptooth catfish from the Olifants/Letaba confluence and pansteatitis affected crocodile tissue have not led to the development of pansteatitis in those crocodiles (J. Myburgh, *personal observations*). Instead it is the presence of fat that is rich in highly polyunsaturated fatty acids in the diet that causes the disease. The simplest explanation for the trophic shift in sharptooth catfish, tiger fish, and crocodiles is a geographically limited, dietary cause brought about by the invasion of a single fish species. The completion of the Lake Massingir sluice gates in 2007 led to back-flooding of the Olifants River Gorge and deposition of large amounts of fine clay where previously there was a rock and sand substrate gorge environment. Rapids that hosted filamentous algae and diverse habitats for fish fauna were lost. We hypothesise that this ecosystem change allowed the invasion of an exotic or extralimital fish species that contains fat rich in highly polyunsaturated fatty acids that causes pansteatitis when eaten either by sharptooth catfish and crocodiles, but apparently not tiger fish. The invasive fish species has yet to be identified, but we hypothesise that the ecosystem change brought about by the back-flooding of Lake Massingir may have allowed silver carp (*Hypophthalmichthys molitrix*, Vallenciennes) that have historically occurred in Lake Massingir to invade the Olifants River gorge. Silver carp were not represented in our fish sample because the sampling methods were inappropriate for this species, and accordingly we cannot place them into the emerging foodweb. The species is not reported in the Sabie River where pansteatitis has been noted in a limited number of crocodiles, and it is abundant in Lake Flag Boshielo where crocodiles do not have pansteatitis. This does not exclude silver carp as the vector for pansteatitis at the Olifants/Letaba confluence as the localised diet of the fish will determine the composition of its fat. Our ongoing research is focussing on the fat composition of the fish in the Olifants River Gorge in order to identify which species are causing the pansteatitis epidemic.

Acknowledgments

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Supplemental Material

Appendix A

Table A1. Isotopic values from fish sampled from rivers in the Kruger National Park, South Africa.

Species	Site	$\delta^{15}N(‰)_{AIR}$	$\delta^{13}C(‰)_{VPDB}$	$\delta^{34}S(‰)_{VCDT}$
Clarias gariepinus	CR	13.5	−18.1	5.5
Clarias gariepinus	CR	15.0	−19.9	6.1
Clarias gariepinus	CR	12.4	−20.2	6.6
Clarias gariepinus	CR	13.8	−20.9	6.5
Clarias gariepinus	CR	13.6	−18.4	5.8
Clarias gariepinus	CR	13.4	−18.8	5.8
Clarias gariepinus	CR	12.5	−20.2	6.6
Clarias gariepinus	CR	11.9	−19.6	5.7
Clarias gariepinus	CR	13.3	−18.6	4.9
Clarias gariepinus	CR	13.2	−20.5	6.2
Clarias gariepinus	CR	12.9	−18.9	6.5
Clarias gariepinus	LR	12.4	−21.3	8.9
Clarias gariepinus	LR	12.8	−19.7	7.2
Clarias gariepinus	LR	13.5	−20.8	7.0
Clarias gariepinus	LR	12.8	−19.8	7.6
Clarias gariepinus	LR	13.2	−19.7	7.5
Clarias gariepinus	OL	11.2	−19.3	9.3
Clarias gariepinus	OL	17.2	−18.4	2.9
Clarias gariepinus	OL	14.8	−11.3	10
Clarias gariepinus	OL	14.1	−11.6	9.8
Clarias gariepinus	OL	16.6	−18.8	4.0
Clarias gariepinus	OL	16.4	−18.9	3.9
Clarias gariepinus	OL	16.8	−19.5	3.0
Clarias gariepinus	OL	16.5	−18.6	3.9
Clarias gariepinus	ED	13.0	−19.3	11.0
Clarias gariepinus	ED	10.9	−20.3	9.6
Clarias gariepinus	ED	13.4	−20.0	7.4
Clarias gariepinus	ED	13.3	−19.7	10.1
Clarias gariepinus	ED	15.1	−20.1	4.7
Clarias gariepinus	ON	13.6	−19.8	4.9
Clarias gariepinus	ON	12.8	−18.2	4.1
Clarias gariepinus	ON	13.5	−18.7	2.9
Clarias gariepinus	ON	12.0	−18.2	4.8
Clarias gariepinus	ON	14.7	−19.0	3.8
Clarias gariepinus	ON	13.0	−18.3	2.3
Hydrocynus vittatus	CR	13.9	−19.3	6.2
Hydrocynus vittatus	CR	13.9	−15.2	3.0
Hydrocynus vittatus	CR	14.1	−15.5	3.4
Hydrocynus vittatus	CR	13.0	−14.0	2.7
Hydrocynus vittatus	CR	15.0	−17.6	5.3
Hydrocynus vittatus	CR	13.2	−14.5	4.5
Hydrocynus vittatus	OL	15.9	−20.3	4.1
Hydrocynus vittatus	OL	17.8	−21.4	3.2
Hydrocynus vittatus	OL	17.9	−21.0	2.2
Hydrocynus vittatus	OL	17.4	−18.7	4.2
Hydrocynus vittatus	OL	16.6	−19.1	2.8
Hydrocynus vittatus	OL	16.6	−19.2	3.4
Hydrocynus vittatus	OL	15.9	−19.4	2.3
Hydrocynus vittatus	OL	17.4	−20.8	2.8
Hydrocynus vittatus	OL	18.3	−21.1	2.8

Table A1. Continued.

Species	Site	$\delta^{15}N(‰)_{AIR}$	$\delta^{13}C(‰)_{VPDB}$	$\delta^{34}S(‰)_{VCDT}$
Hydrocynus vittatus	OL	16.8	−20.0	2.0
Hydrocynus vittatus	OL	17.3	−20.5	2.7
Hydrocynus vittatus	OL	16.8	−19.3	2.9
Hydrocynus vittatus	OL	17.5	−21.1	1.7
Hydrocynus vittatus	ED	12.9	−17.5	9.8
Hydrocynus vittatus	ED	12.6	−20.1	8.9
Hydrocynus vittatus	ED	12.6	−20.2	12.0
Hydrocynus vittatus	ED	14.0	−19.3	8.0
Hydrocynus vittatus	ED	12.4	−20.3	11.9
Hydrocynus vittatus	ON	16.3	−20.0	3.0
Hydrocynus vittatus	ON	16.8	−20.0	2.4
Hydrocynus vittatus	ON	17.0	−21.8	1.1
Hydrocynus vittatus	ON	16.4	−20.7	1.7
Hydrocynus vittatus	ON	17.1	−21.7	2.5
Glossogobius giuris	OL	13.6	−22.0	...
Glossogobius giuris	OL	13.5	−21.5	...
Glossogobius giuris	OL	14.7	−22.4	...
Glossogobius giuris	OL	14.1	−17.6	...
Labeo molybdinus	OL	13.3	−21.2	3.1
Labeo molybdinus	OL	13.0	−24.8	4.0
Labeo molybdinus	OL	12.0	−21.7	7.7
Labeo cylindricus	OL	13.0	−22.0	6.6
Labeobarbus marequensis	OL	13.7	−17.6	12.8
Labeobarbus marequensis	OL	14.0	−16.6	11.8
Labeobarbus marequensis	OL	13.7	−17.7	12.0
Labeobarbus marequensis	ON	13.4	−22.2	2.7
Labeobarbus marequensis	ON	13.0	−21.4	3.1
Labeobarbus marequensis	ON	12.4	−22.9	3.8
Labeobarbus marequensis	ON	13.7	−21.5	3.8
Labeobarbus marequensis	ON	13.9	−21.2	3.7
Labeobarbus marequensis	ON	13.7	−20.1	3.7
Labeobarbus marequensis	ON	13.5	−20.8	3.6
Labeobarbus marequensis	ON	13.5	−20.0	3.9
Labeobarbus marequensis	ON	13.3	−19.7	3.3
Labeobarbus marequensis	ON	13.3	−20.4	4.5
Microlestes acutidens	OL	13.7	−15.7	11.2
Oreochromis mossambicus	OL	10.9	−12.6	8.6
Oreochromis mossambicus	OL	12.9	−15.3	7.2
Oreochromis mossambicus	ED	10.9	−18.3	10.9
Oreochromis mossambicus	ED	10.9	−19.9	10.7
Chiloglanis paratus	OL	16.8	−26.6	4.8
Chiloglanis paratus	OL	14.3	−18.4	11.8
Chiloglanis paratus	OL	14.2	−18.8	12.1
Chiloglanis paratus	OL	14.2	−18.5	14.3
Chiloglanis paratus	ED	12.8	−22.1	10.9
Labeo molybdinus	ED	12.1	−16.3	10.5
Labeo molybdinus	ED	11.3	−19.6	10.6
Labeo molybdinus	ON	12.8	−25.2	4.6
Labeo molybdinus	ON	12.3	−24.7	4.9
Labeo cylindricus	ON	13.6	−26.4	4.8
Labeo cylindricus	ON	12.9	−24.3	4.5
Labeo cylindricus	ON	13.3	−24.2	4.3
Labeo cylindricus	ON	12.3	−25.4	3.3
Labeo cylindricus	ON	12.4	−24.6	4.5
Labeo cylindricus	ON	13.2	−26.4	...
Labeo cylindricus	ON	13.5	−25.2	...
Glossogobius giuris	ED	12.4	−20.1	11.2
Glossogobius giuris	ED	12.3	−22.9	11.8
Glossogobius giuris	ED	11.9	−19.3	10.0
Glossogobius giuris	ED	11.4	−21.5	10.6
Glossogobius giuris	ED	11.5	−19.4	10.8
Glossogobius giuris	ED	12.1	−20.0	11.0
Glossogobius giuris	ED	11.7	−18.6	10.6
Glossogobius giuris	ED	11.6	−19.3	11.3
Labeo spp.	ON	12.1	−24.5	4.6
Labeo spp.	ON	12.1	−24.4	4.6
Labeo spp.	ON	12.1	−23.3	4.2
Shilbe intermedius	ON	12.0	−17.8	5.4

APPENDIX B

Table B1. Isotopic values of aquatic invertebrates sampled from sites OM and OL in the Olifants and Letaba River systems in the Kruger National Park, South Africa.

Species	OM			OL		
	$\delta^{15}N$	$\delta^{13}C$	*n*	$\delta^{15}N$	$\delta^{13}C$	*n*
Atiyidae	...	...	...	10.5 ± 0.2	−16.8 ± 0.5	2
Baetidae	10.1 ± 1.3	−19.5 ± 0.7	7	9.3 ± 1.6	−21.6 ± 3.8	14
Belastomatidae	...	...	...	8.6 ± 0.9	−16.6 ± 0.9	5
Coenagrionidae	11.7 ± 0.4	−21.7 ± 0.6	5	10.2 ± 1.1	−20.1 ± 2.2	7
Corbiculidae	...	...	...	6.8	−18.1	1
Gomphidae	12.0 ± 0.4	−22.1 ± 0.6	2	10.7 ± 1.7	−18.7 ± 4.9	10
Heptagenidae	...	...	...	8.6	−23.6	...
Hydropsychidae	...	...	...	10.3 ± 1.2	−19.89 ± 2.1	7
Leptoceridae	11.0 ± 0.6	−21.5 ± 0.1	2	...	...	...
Libellulidae	...	...	...	9.8 ± 1.1	−22.0 ± 3.2	11
Naucoridae	11.9	−23.9	1	...	...	...
Nepidae	6.8	−20.8	1	10.0	−18.0	1
Notonectidae	11.6 ± 0.3	−22.0 ± 0.9	3	9.6 ± 0.3	−16.1 ± 0.2	4
Physidae	9.8 ± 0.3	−17.6 ± 1.8	3	...	...	...
Pleuroceridae	...	...	...	10.4 ± 1.5	−19.4 ± 5.3	5
Simulidae	11.9	−24.6	1	...	...	...
Tabanidae	...	...	...	12.6 ± 0.2	−24.8 ± 1.4	2
Vellidae	8.0 ± 3.4	−19.5 ± 0.8	2	10.5 ± 0.2	−9.5 ± 3.0	12

Table B2. Isotopic values of aquatic invertebrates sampled from sites ED and ON in the Olifants and Letaba River systems in the Kruger National Park, South Africa.

Species	ED			ON		
	$\delta^{15}N$	$\delta^{13}C$	*n*	$\delta^{15}N$	$\delta^{13}C$(‰)	*n*
Atiyidae	8.7 ± 0.8	−19.9 ± 0.9	15	8.8 ± 1.3	−19.2 ± 2.7	7
Coenagrionidae	8.8 ± 0.5	−19.1 ± 0.8	3	...	...	...
Gomphidae	9.3 ± 0.8	−20.1 ± 1.7	2	10.4 ± 0.5	−18.1 ± 0.6	10
Gyrinidae	...	...	...	10.8 ± 0.4	−21.2 ± 1.2	7
Heptagenidae	7.5 ± 0.8	−15.9 ± 0.5	3	...	...	...
Hydropsychidae	...	...	...	9.6 ± 0.9	−18.7 ± 0.9	2
Libellulidae	...	...	...	9.8 ± 0.3	−18.4 ± 0.5	2
Notonectidae	7.9 ± 1.8	−20.9 ± 4.3	3	...	...	...
Pleuroceridae	7.8 ± 1.3	−20.4 ± 1.6	6	11.2 ± 0.3	−22.6 ± 2.2	8
Sphaeridae	6.9 ± 0.5	−23.6 ± 0.4	8	...	...	...
Potomanautidae	7.6 ± 2.9	−15.2 ± 3.5	2	...	...	...

Table B3. Isotopic values of aquatic invertebrates sampled from site CR in the Crocodile River in the Kruger National Park, South Africa.

Species	CR		
	$\delta^{15}N$	$\delta^{13}C$(‰)	*n*
Atiyidae	10.7 ± 0.8	−18.4 ± 0.9	20
Baetidae	8.0 ± 0.7	−19.2 ± 2.0	24
Coenagrionidae	10.5 ± 0.3	−18.3 ± 0.5	5
Dytiscidae	9.7 ± 0.3	−17.9 ± 0.4	6
Gomphidae	10.5 ± 0.6	−17.8 ± 0.7	13
Gyrinidae	9.5 ± 0.4	−17.9 ± 0.8	4
Heptagenidae	8.6 ± 0.5	−16.6 ± 1.5	8
Hydropsychidae	7.5	−19.1	1
Muscidae	9.4 ± 1.7	−19.8 ± 0.6	3
Nepidae	10.3	−18.9	1

Table B3. Continued.

Species	CR		
	$\delta^{15}N$	$\delta^{13}C$(‰)	*n*
Notonectidae	9.0	−17.7	1
Pleuroceridae	10.0 ± 0.3	−17.8 ± 0.4	7
Sphaeridae	9.2	−18.4	1
Tabanidae	10.9	−19.5	1
Vellidae	9.5 ± 0.5	−18.3 ± 0.8	2
Potomanautidae	10.6	−17.8	1

Appendix C

Table C1. Stomach content analysis for fish sampled in the Kruger National Park, South Africa.

		Steatitis				Healthy			
Site	Date	Fish	Invertebrate and mixed detritus	Vegetation	Empty	Fish	Invertebrate and mixed detritus	Vegetation	Empty
OL	Jun-09	1	0	0	2	2	3	0	1
OL	Aug-09	1	0	0	0	1	0	0	0
OL	Nov-09	5	0	1	3	4	1	0	7
OL	Jul-10	3	1	3	5	2	3	4	4
OL	Jan-11	9	2	0	0	9	2	0	0
OL	Jun-11	8	1	2	3	3	1	2	1
OM	Aug-09	4	0	0	1	3	1	0	3
ON	Jun-09	0	0	0	0	0	3	7	0
LKB	Jun-09	0	0	0	0	2	1	1	0
RV	Nov-09	0	0	1	0	3	2	5	12
RV	Jan-11	0	0	0	0	4	4	3	2
SP	Jul-10	4	0	0	1	5	0	0	1
ED	Jul-10	1	1	0	0	6	1	11	1
MW	Jul-10	0	0	1	0	3	0	13	3
FK	Jan-11	0	0	0	0	9	0	0	1
CR	Jun-11	0	0	0	0	0	2	15	3
LR	Jun-11	0	0	0	0	1	4	9	0

Appendix D

Table D1. Isotopic values from diatoms, organics and riparian vegetation sampled from rivers in the Kruger National Park, South Africa.

	CR		OM		OL		LR		ON	
Species	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Filamentous algae	7.5	−21.3	7.1	−16.9	6.6	−21.6	10.0	−15.8	6.0	−29.0
Diatoms	6.1	−20.7	13.1	−12.9	9.1	−18.3	3.7	−26.4	7.4	−19.6
Sediment	5.6	−18.8	6.8	−21.9	7.2	−19.7	5.3	−21.9	5.7	−19.9
Detritus	4.0	−16.1	...	...	6.4	−14.8	...	...	4.4	−15.7
Vegetation	4.2	−13.3	7.1	−31.6	9.0	−26.2	...	...	9.2	−13.2
Vegetation	...	...	10.3	−12.1	...	...	...	...	9.0	−27.3
Phragmites	8.1	−26.5	6.4	−12.0	14.1	−27.7	7.9	−27.5	8.8	−27.2

APPENDIX E

Table E1. Isotopic values from crocodile claws sampled in the Kruger National Park, South Africa.

Location	$\delta^{15}N$	$\delta^{13}C$	n	$\delta^{34}S$
Olifants River Gorge	12.5 ± 0.4	−19.9 ± 0.3	81	5.1
Olifants River Gorge	12.4 ± 0.6	−20.6 ± 0.1	125	6.8
Olifants River Gorge	12.4 ± 0.3	−21.0 ± 0.7	76	...
Olifants River Gorge	13.1 ± 0.5	−19.4 ± 0.2	44	9.1
Olifants River Gorge	12.6 ± 0.5	−19.2 ± 0.4	15	...
Olifants River Gorge	11.6 ± 0.3	−19.7 ± 0.9	54	...
Olifants River, Mamba Weir	8.7 ± 0.6	−15.5 ± 1.1	53	6.5
Olifants River, Mamba Weir	10.0 ± 0.7	−17.8 ± 0.5	45	6.4
Letaba River, Engelhard Dam	9.6 ± 0.3	−18.1 ± 0.8	63	13.9
Letaba River, Hlanganini Inlet	9.6 ± 0.5	−20.5 ± 0.4	40	13.7
Levuvhu River	10.1 ± 0.3	−16.0 ± 0.8	77	12.0
Levuvhu River	10.9 ± 0.2	−16.7 ± 0.8	48	12.5
Shingwedzi River, Kannidood Dam	9.6 ± 0.6	−16.6 ± 0.8	70	13.3
Shiloweni Dam	11.0 ± 0.4	−14.8 ± 0.9	115	14.2
Shiloweni Dam	11.2 ± 0.6	−14.8 ± 0.6	67	13.6
Sabie River, Lower Sabie Weir	8.2 ± 0.4	−12.5 ± 0.8	88	11.5
Crocodile River	11.1 ± 0.6	−19.9 ± 0.6	53	7.7
Crocodile River	9.8 ± 0.4	−16.1 ± 1.2	84	8.9