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Review

Steatitis in wild sharptooth catfish, *Clarias gariepinus* (Burchell), in the Olifants and Lower Letaba Rivers in the Kruger National Park, South Africa

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Abstract

Large numbers of adult Nile crocodiles, *Crocodylus niloticus* (Laurenti), died from pansteatitis during autumn and winter 2008 in the lower Letaba and Olifants River gorge in the Kruger National Park, South Africa. Consequently, the health status of fish from these waters was investigated. The study presents the pathological findings in fish inhabiting these rivers within the boundaries of the Park. Changes typical of steatitis were diagnosed in many of the larger specimens of sharptooth catfish, *Clarias gariepinus* (Burchell), caught within the Olifants River gorge. These fish carried large amounts of mesenteric fat with characteristic small brown granulomata within the adipose tissue. Necrosis and inflammation of the adipose tissues, with characteristic ceroid accumulation within the resultant granulomata and the associated aggregation of ceroid-containing macrophages, were demonstrated histologically and were typical of steatitis. Other changes included mild thickening and pallor of the gill tissues and swollen, orange, fatty livers. Focal hepatic lipidosis was demonstrated histologically, and special stains revealed storage of large amounts of iron in the livers. Blood smears revealed chromatin clumping in erythrocyte nuclei and nuclear and cell membrane irregularities. This is the first record of steatitis in wild-caught *C. gariepinus*.

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Introduction

Unexplained fish and crocodile deaths in the Olifants River, in the north-east of South Africa, have raised concern for a number of years (De Villiers & Mkwelo 2009). Altered hydrodynamics and extensive industrial, mining and agricultural activities in the catchment in Mpumalanga Province have resulted in this river becoming one of the most threatened aquatic ecosystems in South Africa (De Villiers & Mkwelo 2009). The Olifants River flows from west to east, for a distance of some 90 km, through the Kruger National Park (KNP) before being joined by the Letaba River in the Olifants Gorge. The gorge extends for a further 9 km to the Mozambique border where it opens into the Massingir Dam.

The Olifants River gorge and the confluence with the lower Letaba River are home to one of the most dense populations of large Nile crocodiles, *Crocodylus niloticus* (Laurenti), in South Africa. Large numbers of adult crocodiles were found dead during autumn and winter 2008, in both the Olifants and Letaba Rivers in an area stretching from about 10 km upstream of the confluence through the gorge to the Mozambique border. Some 180 dead crocodiles out of a known population of at least 600 were found. Other crocodiles showed impaired movement and an inability to swim. Apparently healthy crocodiles as well as those obviously affected were observed in the same area.

Autopsies performed on some of the crocodiles by KNP veterinarians revealed exceptionally fat carcasses with an abnormal hardening and yellow discolouration of the fat. Histological examination confirmed an inflammation of the fat typical of pansteatitis (E.P. Lane, F.W. Huchzermeyer, D. Govender, R.G. Bengis, E.B. Bus, M. Hofmeyr, J.G. Myburgh, J.C.A. Steyl, D.J. Pienaar & A. Kotze, University of Pretoria, personal communication). A further 24 crocodile carcasses were found during the winter of 2009. The Olifants Gorge consists of a steep sided ravine where the Olifants River cuts through the Lebombo Mountains creating a habitat of sandbanks, deep sand bottomed pools and fast flowing rapids. Since the raising of the Massingir Dam wall in Mozambique in 2008, many of the pools and rapids in the gorge have been flooded and clay-rich sediments carried by the river have been deposited in the gorge where they have inundated the pools.

The Olifants Gorge lies in a remote area where fish mortalities may go unnoticed, but for the first time, in July 2009, a large fish mortality was observed within the gorge. Affected fish were almost exclusively large sharptooth catfish, *Clarias gariepinus* (Burchell), and were found in waters overlying the clay-rich deposits at the point where the gorge widens into the dam. Fish carcasses were observed to be very fat. The fish kill remained localized in space and time, and no mortalities were observed in either the Olifants or Letaba Rivers upstream of the gorge, and fish in Massingir Dam also appeared unaffected.

Pansteatitis is a nutritionally mediated condition characterized by necrosis and inflammation of the adipose tissues. The condition has been described from many species of both warm- and cold-blooded animals, associated with the feeding of rancid or unsaturated fats, often of fish origin, particularly in the absence of sufficient vitamin E (Murai & Andrews 1974; Roberts, Richards & Bullock 1979; Herman & Kircheis 1985; Fytianou, Koutinas, Saridomichelakis & Koutinas 2006; Goodwin 2006; Roberts & Agius 2008). Previously described cases of pansteatitis in crocodiles were associated with consumption of large numbers of dead and rancid fish consumed by farmed crocodiles (Huchzermeyer 2003). Larsen, Buergelt, Cardeilhac & Jacobson (1983) reported steatitis at slaughter in apparently healthy alligators fed an exclusive fish diet. Mass fish mortality, caused by acid mine seepage, was found to be the most likely cause of the deaths of significant numbers of crocodiles and terrapins as a result of pansteatitis in Loskop Dam, higher up on the Olifants River, in 2007 (J.G. Myburgh, J.C.A. Steyl, F.W. Huchzermeyer, M.C. Williams, D.G. Booyse, L.J. Guillette Jr. & J.J. Coetzee, University of Pretoria, personal communication). The 2008 episode of crocodile pansteatitis in the KNP was noteworthy by the absence of observed fish mortality, although circumstantial evidence pointed to illegal fishing activity with gill nets within the gorge as a possible source of dead, rancid fish. This led to extensive sampling and examination of fish from the Olifants and Letaba Rivers within the KNP in an attempt to identify precipitating factors that may have contributed to the development of pansteatitis in the crocodiles.

Fish specimens were collected from the Olifants and Letaba Rivers in the KNP on various occasions as the pansteatitis-related deaths of crocodiles first became apparent in 2008. This project forms part of the multidisciplinary research into the causes of the crocodile mortalities under the auspices of the Consortium for the Restoration of the Olifants Catchment (CROC) initiative.

Materials and methods

Various species of fish, comprising a total of 145 specimens, were collected from five different localities along the Olifants and Letaba Rivers: Olifants Gorge, from the confluence with the Letaba River (S:23°59′32″ E:031°49′57″) to the Mozambique border (S:23°57′48″ E:031°52′97″), Klipkoppies Bridge (S:23°56′58″ E:031°43′89″) above the cascade on the Letaba River and Ludwich's Hut (S:23°58′29″ E:031°47′41″) below the cascade on the Letaba River, Ngotso (S:24°02′96″ E:031°44′24″) on the Olifants River and at Mamba Weir (S:24°03′32″ E:031°14′14″) on the Olifants River near the western boundary of the KNP (Fig. 1). Samplings took place during September 2008 and January, June, July, August and November 2009. Autopsies, blood smears and histological examination were used to determine the degree and variation of pathological changes present in the fish. Initially, a broad range of fish represented in these rivers was examined but sampling was later restricted to *C. gariepinus.*

Baited hook and cast netting were used to catch fish. All fish were anaesthetized for blood collection using a benzocaine hydrochloride bath at approx-

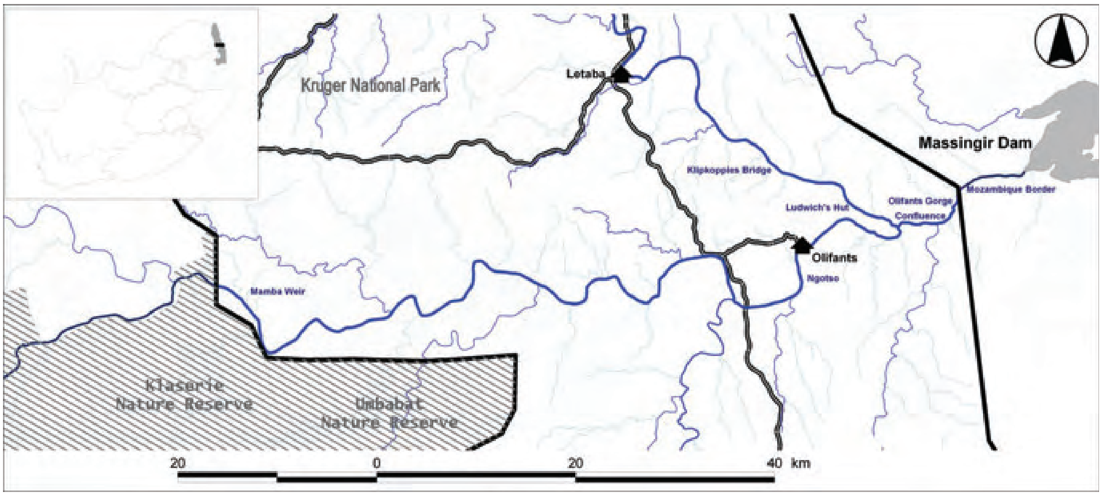

Figure 1 Sampling sites along the Olifants and Letaba rivers in the Kruger National Park, South Africa.

imately 30 ppm strength prior to being killed. Fish were killed through an overdose of benzocaine hydrochloride. Blood samples were drawn from the vessels just ventral to the vertebral column, either from the tail region in the case of anaesthetized fish or through the kidney during post-mortem dissection of the fish. Fresh blood smears were taken from all fish. Gill wet mounts were examined microscopically for gill condition and the presence of parasites. Fish were examined by autopsy for gross pathological changes, and data sheets were compiled. Data included length and weight measurements, body condition, organ descriptions, level of parasitism and stomach and intestinal contents. Samples from a range of organs and tissues were fixed in 10% buffered formalin for histological examination.

Formalin-fixed tissue specimens were processed using standard histological techniques, and 5-μm sections were prepared and stained with haematoxylin and eosin (H&E). Periodic acid-Schiff (PAS), Gomori's aldehyde fuchsin (GAF) and Perl's Prussian blue stain were used to stain selected sections. Blood smears were stained with a CAM's quick stain (Kyro-Quick stain; Kyron Laboratories). Blood smears and histological sections were examined by standard light microscopy.

Results

Characteristic of the *C. gariepinus* specimens collected from the Olifants Gorge and lower Letaba River were the large amount of variably coloured fat in the body cavity and between the muscles of the tail and the distinct white and brown spots observed within the mesenteric fat of some fish. By contrast, other species of fish collected had white fat devoid of lesions. In *C. gariepinus*, fat colour, particularly prominent in the mesenteric fat reserves, varied from almost pure white through shades of cream to yellow. Leaner fish with only small amounts of mesenteric fat invariably had dark yellow to orange fat. Various nodular reactions were observed in the adipose tissues of *C. gariepinus* specimens. Parasitic granulomata were distinguishable from foci of inflammation and granuloma formation associated with non-parasitic causes. Parasitic granulomata in the mesenteric fat of some of the *C. gariepinus* specimens were typically well circumscribed and white in appearance, ranging from 2 to 15 mm in diameter. On incision, these revealed a central encysted parasite and consisted of a well-defined white connective tissue reaction. These parasitic granulomata differed from smaller granulomata of varying shades of brown which when present, were numerous and mostly did not exceed 5 mm in diameter (Fig. 2a). These granulomata were often focally disseminated throughout the entire mesenteric fat, in severe cases imparting a granular grey brown discolouration and in milder cases appearing to cluster along blood vessels. Advanced granulomata presented with a central, < 1 mm in diameter orange area, imparting a granular or rough appearance with a diffuse increase in consistency and in some cases with almost total obliteration of normal fat tissue (Fig. 2b).

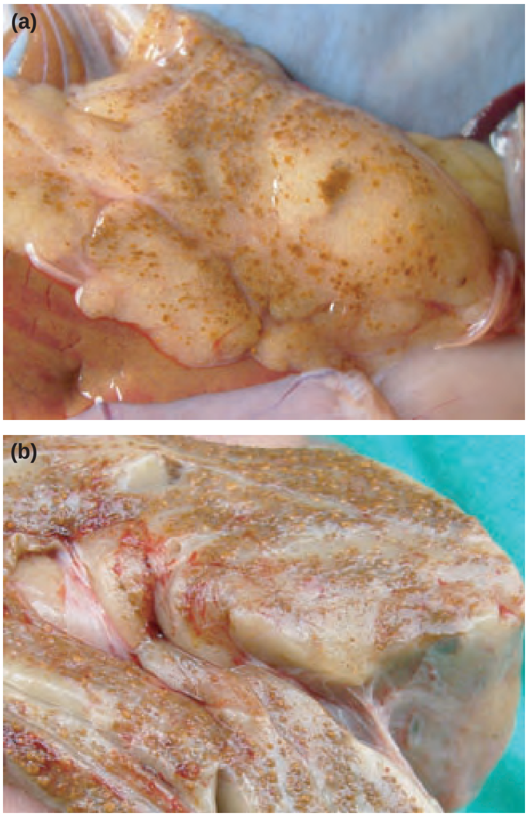

Figure 2 Macroscopic appearance of mesenteric adipose tissue from steatitis affected *Clarias gariepinus* from the Olifants Gorge. (a) Small golden brown granulomata from a mildly affected fish. (b) Advanced lesions from a severely affected fish.

The histological appearance of the non-parasitic granulomata in the adipose tissues was typical of lesions expected with steatitis and consisted of varyingly enlarged as well as ruptured adipocytes surrounded by a dense mass of ceroid-containing macrophages (Fig. 3a), with presence of variable numbers of fibroblasts. These lesions were focally disseminated throughout the affected mesenteric adipose tissue and represented the brown granulomata noted macroscopically. Smaller lesions consisted primarily of ceroid-containing macrophage aggregations within the interstitium of the fat tissues. Presence of ceroid in the macrophages was confirmed by staining with GAF (Fig. 3a, b) and PAS stains. Multinucleate Langhans giant cells surrounding an irregular central necrotic area were evident in most of these granulomata (Fig. 3c). These central areas often consisted of an irregular refractile substance, described by Begg, Bruno & McVicar (2000) as lipopigment (Fig. 3a), evident as yellowish, amorphous material in haematoxylin- and eosin-stained smears. Similar yellow, granular and refractile inclusions of varying size were present in many of the surrounding macrophages of most lesions (Fig. 3d). In some lesions, more compact macrophages were arranged in the form of an epithelioid type sheath surrounding the ruptured fat cells. Advanced cases presented with a clear lacuna surrounded by organized layers of epithelioid cells that in places coalesced and became embedded in fibrous connective tissue. Granulomata were found primarily in the mesenteric fat reserves and could not be demonstrated in the fat cushion behind the pectoral fin or in the epicardial fat, the hypodermal or intermuscular fat. A distinct exudative peritonitis associated with signs of steatitis was observed in a single *C. gariepinus* specimen that was caught in the gorge on the Mozambique border. Parasitic granulomata of varying sizes were common in the mesenteric, hypodermal and intermuscular fat but were not noted in the pectoral fat.

Brown granulomata in the fat were observed in both male and female fish but were absent in specimens under 2 kg body mass and measuring <70 cm in length. The prevalence of macroscopically characteristic lesions of steatitis on various sampling occasions during 2008 and 2009 is illustrated in Table 1. In the week after the July 2009 fish die-off in the gorge, six *C. gariepinus* specimens were caught in the area of the fish kill. All these fish showed presence of brown granulomata in the fat. The same granulomata were again observed in 4 out of 12 specimens caught on the Mozambique border during August 2009.

Livers of *C. gariepinus* from the gorge ranged in colour but appeared distinctly orange, fatty and swollen in appearance compared with fish caught in other sections of the river. A varying degree of fat vacuolation in the livers, often within clearly defined zones, was characteristic of fish collected from the gorge (Fig. 4a). Large amounts of ceroid, golden brown breakdown products of oxidized unsaturated fat as well as iron or haemosiderin were visible within the hepatocytes. In places, well-demarcated foci of fat vacuolation contained distinctly less iron than in surrounding hepatocytes. In other areas, the haemosiderin appeared clumped within the zone of fat vacuolation (Fig. 4b). Adventitious macrophages were numerous in the liver where they contained large deposits of both ceroid and iron, imparting a pronounced golden brown colour in the haematoxylin- and eosin-stained sections with the macrophages staining

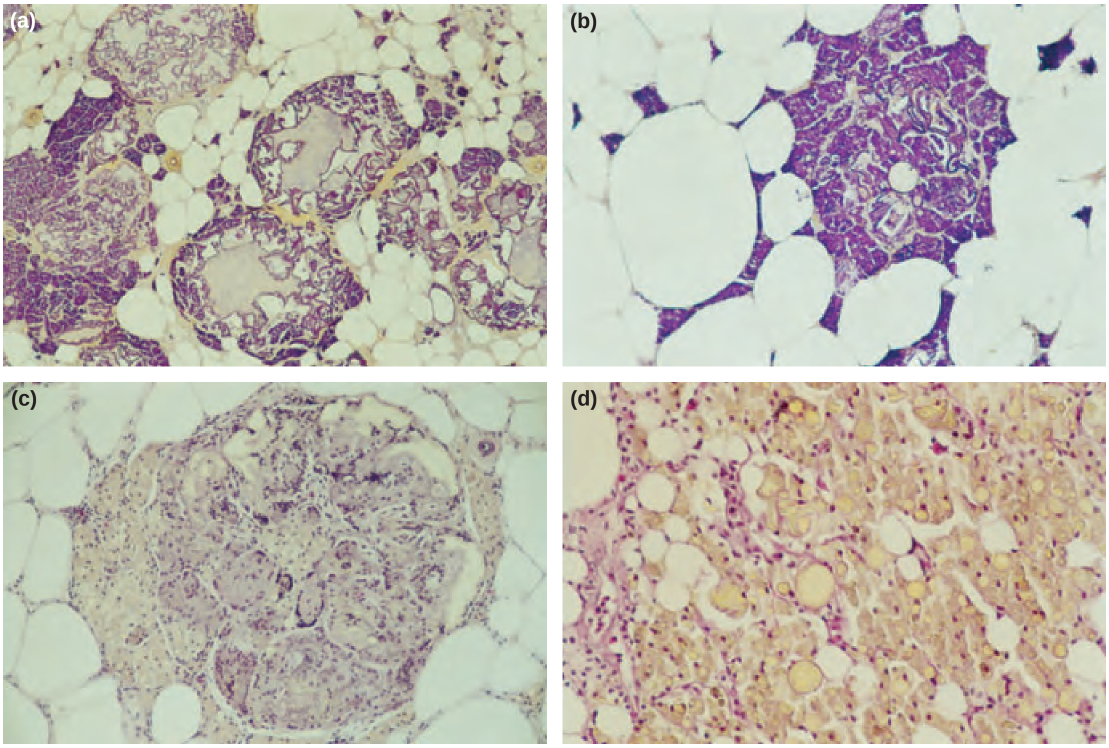

Figure 3 Histological sections of granulomata in the mesenteric fat of *Clarias gariepinus* from the Olifants Gorge. (a) Central area of fat necrosis consisting of lipopigment surrounded by ceroid-containing macrophages and fibroblast reaction (GAF, ×40). (b) Ruptured adipocytes surrounded by ceroid-containing macrophages (GAF, ×100). (c) Multinucleate giant cells surrounding areas of fat cell necrosis and lipopigment (H&E ×100). (d) Xylene-insoluble lipopigment inclusions in macrophages surrounding necrotic fat cells (H&E ×400).

Table 1 Prevalence of macroscopically detectable steatitis lesions in *Clarias gariepinus* from the Olifants and Letaba River sampling sites on various sampling occasions

Date	Site	Fish with steatitis	Total fish sampled
September 2008	Gorge Letaba confluence	1	8
September 2008	Letaba Ludwich's hut	0	4
September 2008	Letaba Klipkoppies bridge	0	1
September 2008	Olifants Mamba weir	0	1
January 2009	Gorge Letaba confluence	0	7
January 2009	Letaba Klipkoppies bridge	0	2
January 2009	Olifants Ngotso	0	9
January 2009	Olifants Mamba weir	0	4
June 2009	Gorge Letaba confluence	3	9
June 2009	Gorge Letaba confluence	3	4
June 2009	Letaba Klipkoppies bridge	0	5
June 2009	Olifants Ngotso	0	10
July 2009	Gorge Letaba confluence	6	6
August 2009	Gorge Letaba confluence	1	2
August 2009	Gorge Mozambique border	4	12
November 2009	Gorge Letaba confluence	6	21

strongly for iron with Perl's Prussian blue stain. Melanomacrophages in the spleen and kidneys of these fish were replete with ceroid, and the splenic macrophages carried large amounts of haemosiderin. Iron was also observed in the macrophages of the kidney, but to a lesser extent than in the spleen and liver. Positive Perl's staining material was observed in some of the renal tubular epithelial cells. Sections of the ovaries of these fish showed numerous adventitious macrophage aggregates containing

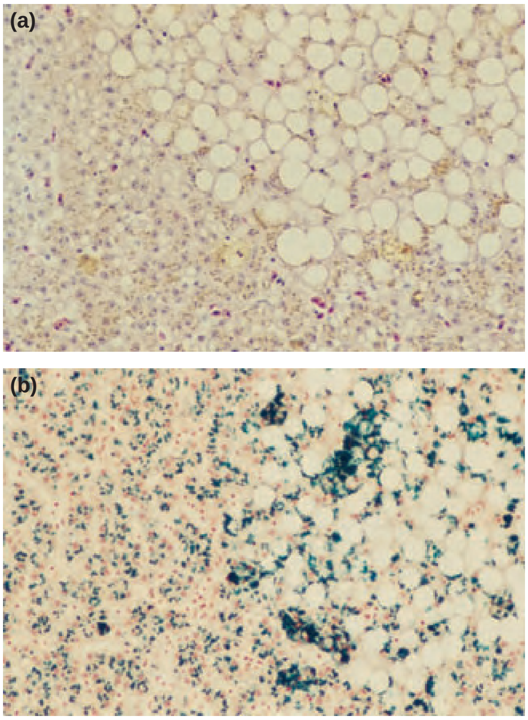

Figure 4 Histological section of liver of *Clarias gariepinus* from the Olifants Gorge. (a) Clearly demarcated zone of lipid vacuoles adjacent to non-vacuolated hepatocytes. Note pigment granules within macrophages and hepatocytes (H&E, ×200). (b) Positive staining reaction for haemosiderin. Note irregular haemosiderin deposition within zone of lipid vacuolation (Perl's Prussian blue, ×200).

large amounts of iron, whereas testicular macrophage centres were devoid of iron. Macrophage aggregations associated with fat cell necrosis in the adipose tissues did not contain iron neither did the macrophages in the hypodermis nor the macrophage centres in the pancreas.

Foci of inflammatory cells were observed in the heart, kidney and liver. In the liver, these were associated with ducts and blood vessels. In the cranial and caudal kidney, they appeared as focally disseminated clusters of dense basophilic cells. Well-encapsulated parasitic granulomata of varying sizes were a common finding in many of the livers. Secondary haemopoietic cell activity was prevalent in the heart and liver of many of the *C. gariepinus* specimens from the gorge. Some of these specimens showed signs of excessive haemolysis and an increase in the phagocytic lining of the ventricle. Pancreatic acinar and islet cells appeared normal in all of the fish, although the prominence of pancreatic tissues, macroscopically, was quite variable.

Gills of many *C. gariepinus* specimens collected from the Olifants Gorge and the lower Letaba River appeared paler than normal and mildly hyperplastic. Some of these gills, particularly in the *C. gariepinus* specimens from the gorge and lower Letaba River collected during January 2009, presented with a two- to threefold increase in the thickness of the epithelium of the secondary lamellae. In many of these specimens, the epithelial hyperplasia increased towards the base of the secondary lamellae imparting a wedge-shaped appearance. These changes were less evident in fish sampled from the same site in November 2009.

Examination of blood smears revealed an abundance of immature erythrocytes in many of the fish collected from the gorge particularly during the January 2009 sampling. Many erythrocytes showed irregular shapes. Nuclear shapes were similarly irregular with a high prevalence of chromatin clumping visible within the nuclei.

Stomach contents from *C. gariepinus* specimens collected in the gorge near the confluence yielded mainly fish remnants, in many cases from fairly large fish. Specimens collected from waters directly over the silt deposits in the gorge on the Mozambique border appeared to have been ingesting only detritus and silt. This area was characterized by a general paucity of other fish species.

A variety of parasites including monogenean trematodes and the cysts of digenetic trematode larvae were found in low to moderate numbers on the gills of many of the *C. gariepinus* specimens. *Argulus japonicus* were found in low numbers on the gills of two *C. gariepinus* specimens. Various parasitic cysts were found in the mesenteric tissues, livers and serosa of the intestines and in the musculature of many of these fish. The peritoneal cavity of many of the *C. gariepinus* specimens yielded low to moderate numbers of nematodes. No difference in the pattern of parasitism between sampling sites could be detected; however, it was noteworthy that no protozoan parasites were observed.

Discussion

Clarias gariepinus was the only fish species found in the Olifants Gorge to show significant lesions in the adipose tissues. Histological examination of the adipose tissues confirmed the presence of steatitis. The presence of ceroid-laden macrophages and characteristic foreign body giant cells surrounding degenerating and ruptured adipocytes as well as the associated granuloma formation are typical findings in all species suffering from pansteatitis, and microscopically, the lesions in *C. gariepinus* resembled those described in other species (Murai & Andrews 1974; Roberts *et al.* 1979; Herman & Kircheis 1985; Begg *et al.* 2000; Goodwin 2006; Roberts & Agius 2008). The macroscopic appearance of the adipose tissues of *C. gariepinus* differed from most other freshwater fish species that have characteristically white fat. A variation in colour from white through various shades of cream and yellow to dark orange appears to be normal in wild *C. gariepinus*. In contrast to pansteatitis in crocodiles and mammalian species, where affected fat tissue takes on a characteristic yellow colour, fat colour alone may be misleading when looking for signs of pansteatitis in *C. gariepinus*. Although fat consistency did change with steatitis in *C. gariepinus*, it did not take on the typically hard consistency noted in crocodiles (Larsen *et al.* 1983; Osthoff, Hugo, Bouwman, Buss, Govender, Joubert & Swarts 2010).

The livers of *C. gariepinus* specimens caught in the gorge were characteristically fatty and showed signs of fatty degeneration of the hepatocytes with accumulation of both ceroid and haemosiderin in hepatocytes and melanomacrophages. Melanomacrophage centres are known to store lipofuscin, a breakdown product of unsaturated fatty acid peroxidation, as well as melanin and haemosiderin (Kennedy-Stoskopf 1993) and represent possible forerunners of the germinal centres in the spleen and lymph nodes of higher animals (Agius 1979). They are a unique feature of the lymphomyeloid tissue of fish (Kennedy-Stoskopf 1993) and play a role in iron storage (Agius 1979). Perl's Prussian blue stain demonstrates the presence of ferric iron. Agius (1979) looked at the pattern of iron storage in the melanomacrophage centres in various organs of 14 different species of healthy and diseased fish. The spleen was the main organ of iron storage by melanomacrophages, whereas melanomacrophage centres in the liver and kidney were found to store insignificant amounts of iron. Whilst certain diseases including pansteatitis resulted in the accumulation of iron in splenic macrophage centres, the same did not happen in hepatic and renal macrophage centres (Agius 1979). In *C. gariepinus* specimens from the gorge, it was noted that the splenic melanomacrophages carried heavy deposits of iron. In the majority of fish, this was also the case in the hepatic melanomacrophage centres and even renal melanomacrophages contained obvious amounts of iron, albeit less than in the liver. Adventitious macrophage aggregations in the ovaries also contained large amounts of iron, whereas macrophage aggregations associated with fat cell necrosis in the adipose tissues and macrophage centres in the pancreas, testes and hypodermis did not contain iron. It is not clear whether the iron deposits represent increased iron storage as haemosiderin because of excessive haemolysis or whether they are indicative of abnormal uptake of iron from the environment. However, Baker, Martin & Davies (1997) have demonstrated heightened oxidative stress in *C. gariepinus* ingesting increased levels of iron under experimental conditions.

The erythrocyte changes observed in blood smears from *C. gariepinus* in the gorge showed the irregularity in shape described by Post (1993) and Murai & Andrews (1974) in cases of vitamin E deficiency in fish. The observed nuclear and cell membrane abnormalities in the blood smears may point towards an increase in apoptosis. Reduced haemopoiesis in the kidney as well as an increase in erythrocyte turnover may explain the secondary haemopoiesis observed in the heart and liver in *C. gariepinus* from the gorge.

Lesions associated with steatitis, other than those observed in the adipose tissues, appear to be variable depending on species involved. Exudative diathesis as described by Murai & Andrews (1974) in channel catfish *Ictalurus punctatus* (Rafinesque), and muscular dystrophy described in channel catfish (Murai & Andrews 1974) and rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Roberts *et al.* 1979) were not a consistent feature of the pansteatitis observed in *C. gariepinus*, although an exudative peritonitis was noted in one fish suffering from steatitis. Fin loss and skin ulceration described in channel catfish in association with steatitis by Goodwin (2006) were not observed in *C. gariepinus* nor was a granulomatous infiltration between the pterygophores as described in wild common dab, *Limanda limanda* (L.), by Begg *et al.* (2000). The swimbladder changes described by Roberts *et al.* (1979) in rainbow trout suffering from pansteatitis could not be demonstrated in *C. gariepinus,* which has a reduced, displaced swimbladder enveloped by a bony capsule formed by the lateral processes of the fourth and fifth vertebrae (Petrick 1975). Roberts & Agius (2008) described lethargy and erratic swimming bursts as presenting signs in farmed northern bluefin tuna, *Thunnus thynnus* (L.), suffering from pansteatitis. No specific pathological changes were observed in the brain tissues of *C. gariepinus* specimens from the Olifants Gorge, but lethargy and frenzied swimming would rapidly attract crocodiles, making it unlikely that such advanced cases would be represented amongst the sampled fish.

There are few references in the literature describing pansteatitis in wild-caught freshwater fish. The condition has been described in various species of farmed fish where it is primarily a nutritional problem involving feeding of unsuitable quantities or types of unsaturated fats (Bricknell, Bruno, Bowden & Smith 1996; Goodwin 2006; Roberts & Agius 2008) and where the condition can be mediated by the presence of adequate vitamin E or addition of ethoxyquin in the diet (Murai & Andrews 1974). Begg *et al.* (2000) reported steatitis in two species of wild marine fish, common dab and long rough dab, *Hippoglossoides platessoides* (Fabricius), with suspicion of a pollution-related aetiology. Bainy, Saito, Carvalho & Junqueira (1996) have linked the effects of oxidative stress from polluted water to changes in antioxidant parameters in Nile tilapia, *Oreochromis niloticus* L. The initiation of lipid peroxidation by cyclic reduction/oxidation has been linked to ingested iron (Minotti & Aust 1992; Elbaraasi, Mézes, Balogh, Horváth & Csengeri 2004) and the iron fraction in expandable clay minerals (Kibanova, Nieto-Camacho & Cervini-Silva 2009). That *C. gariepinus* must have considerable antioxidant protective mechanisms is evidenced by their survival despite extensive lesions. It is noteworthy that lesions appeared to be restricted to the mesenteric fat, possibly indicating differing susceptibility of the various adipose reserves. Similarly, Goodwin (2006) found differences in the susceptibility of various fat tissues in channel catfish suffering from steatitis. In the case of channel catfish, the peritoneal fat reserves appeared to remain intact, whereas lesions were found in the fin bases, the hypodermis and the fat surrounding the brain. This may reflect differences in lipid type stored in the different fat tissues as proposed by Goodwin (2006). Some fat reserves may be more critical to the survival of wild fish, reducing the likelihood of fish with extensive pansteatitis surviving to be caught on hook and line. For the same reason, it is possible that some changes observed in other species of fish under aquaculture conditions would rarely be seen in wild-caught specimens. In the Olifants River gorge, high predator pressure would rapidly remove weakened fish.

Stomach and intestinal contents of the steatitis-affected *C. gariepinus* specimens indicated that appetite was not completely suppressed. This was confirmed by the willingness of the fish to take baited hooks. Similarly, in bluefin tuna suffering from pansteatitis, Roberts & Agius (2008) found that the stomachs and intestines of even moribund fish contained food. *Clarias gariepinus* is an omnivorous benthic scavenger that is also known to actively hunt other fish (Bruton 1996). Significant differences in stomach and intestinal contents were noted between the various sampling sites. Of significance was the presence of clay containing sediment in the intestines of fish caught in waters overlying the clay-rich silt deposits in the gorge as well as in the presence of fish remnants in the stomachs of specimens caught near the Olifants and Letaba confluence, at the entrance to the gorge. This was in contrast to the predominantly vegetable and invertebrate content found in the gastrointestinal tracts of specimens caught at other sampling sites where steatitis did not occur. In the Olifants Gorge, *C. gariepinus* reach a body mass of 9 kg and more. Remnants from often quite large fish were found in the stomach contents of these specimens, confirming that they were either preying on other weakened fish or scavenging off dead fish in the gorge.

The presence of diverse parasites at mild to moderate numbers reflected a relatively healthy host–parasite relationship in the fish. The majority of the parasites noted have a multihost lifecycle, indicating that both the invertebrate intermediate host and the vertebrate final host must be present within the broader ecosystem.

Clarias gariepinus has a broad distribution and is ubiquitous in South African river systems and is hence worthy of investigation where anthropogenic activity has seriously impacted aquatic ecosystems. The variation in pathology that was observed in the fish from the Olifants Gorge appears consistent with oxidative stress. The most significant lesion identified during this fish health assessment was the steatitis present in the adipose tissues of *C. gariepinus* specimens from the Olifants Gorge. Poikilothermic (cold-blooded) animals, such as fish, depend on lipoprotein polyunsaturated fats to maintain membrane fluidity and normal metabolic function especially at colder ambient temperatures (Stewart 1993). They are thus likely to be more sensitive to the effects of lipid autooxidation than warm-blooded animals. This may explain the observed mortalities in both crocodiles and sharptooth catfish during the winter months and the absence of mortality during the summer months.

It is unusual for a benthic scavenger such as *C. gariepinus* to develop steatitis in the wild. This is the first record of steatitis in wild *C. gariepinus* as far as the authors are aware. This species of fish is hardy and well adapted to a wide habitat and food range explaining its extensive distribution throughout Africa. We conclude that it is unlikely that steatitis observed in the sharptooth catfish in the Olifants River is pure coincidence and propose that the finding is significant in relation to the development of pansteatitis in crocodiles occupying the same section of river.

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Prevalence of pansteatitis in African sharptooth catfish, *Clarias gariepinus* (Burchell), in the Kruger National Park, South Africa

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Pansteatitis was confirmed in sharptooth catfish, *Clarias gariepinus* (Burchell), from three main locations within the Kruger National Park (KNP); the Olifants River Gorge, Engelhard Dam on the Letaba River and from the Sabie River in the Sabiepoort. An increasing prevalence of pansteatitis was observed in catfish during repeated samplings from the Olifants Gorge from 2009 to 2011 and co-existence of old and recent lesions indicated on-going incitement of pansteatitis. Only a low prevalence of pansteatitis was observed in catfish sampled from the Olifants River upstream of the Gorge in the KNP and no pansteatitis was observed in catfish sampled from a rain-filled dam not connected to the Olifants River. Common to both the Olifants Gorge and the Sabiepoort is the damming of the rivers in Mozambique to form lakes Massingir and Corumana respectively. Anthropogenic activities resulting in potential pollution of the rivers differ greatly between these two catchments, providing argument against a primary pollution-related aetiology of the pansteatitis found at these two sites. Compared with other sites, analysis of stomach contents of catfish from the Olifants Gorge and the Sabiepoort strongly suggested that consumption of a predominantly fish diet was associated with the development of pansteatitis in these fish. In a farmed population of catfish used as positive control, development of pansteatitis could be ascribed to consumption of rancid fish waste from a trout slaughterhouse. In the Olifants Gorge, alien invasive silver carp, *Hypophthalmychthys molitrix* (Valenciennes), seasonally migrate upstream out of Lake Massingir to spawn. This schooling species is an obligate phytoplankton feeder with consequent high levels of adipose tissue n-3 polyunsaturated fatty acids. In the Olifants Gorge, at least, this may explain seasonal exposure to levels of polyunsaturated fats in the diets of catfish and crocodiles to which these animals are not adapted. The possible roles of diet, membrane lipid composition and metabolic rate of fish, sediment pollution and seasonal drop in environmental temperature in the pathogenesis of pansteatitis in the catfish are discussed. Further studies are needed to verify some of these speculations.

Introduction

Much attention has been focused on the state of the Olifants River in Mpumalanga Province, South Africa. From its origins on the Highveld plateau the river flows eastwards down the escarpment traversing the Kruger National Park (KNP) and Mozambique before discharging into the Indian Ocean. The catchment has been heavily impacted by human activity including mining, coal-fired electricity generation, industrial and urban wastewater discharges, agricultural practices and water impoundments. As a result, the Olifants River is regarded as one of the most threatened aquatic ecosystems in Mpumalanga (Ashton 2010; De Villiers & Mkwelo 2009; Heath, Coleman & Engelbrecht 2010). Since 2003, increasing Nile crocodile, *Crocodylus niloticus* Laurenti, mortalities in Lake Loskop, situated in the upper Olifants catchment, have coincided with periodic mass fish mortalities (Botha, Van Hoven & Guillette 2011). In the KNP, an estimated 180 large crocodiles died in the Olifants River Gorge during the winter of 2008 following the raising of the sluice gates of the Lake Massingir dam wall in Mozambique during 2007 (Ferreira & Pienaar 2011; Huchzermeyer *et al*. 2011). Fewer deaths were recorded in the subsequent two winters. South African National Parks (SANParks) veterinarians established the cause of death as pansteatitis.

Steatitis, the inflammation associated with fat cell necrosis, has been described from many species of warm and cold-blooded animals, including fish. This nutritional disorder is found mainly in farmed and captive animals and rarely in free-living wild animals. Feeding of large amounts of unsaturated fat, particularly if rancid, or diets deficient in vitamin E are known to cause pansteatitis (Goodwin 2006; Herman & Kircheis 1985; Roberts & Agius 2008; Roberts, Richards & Bullock 1979). The condition has been reported in farmed crocodiles fed fish that was no longer fresh (Huchzermeyer 2003; Ladds *et al*. 1995). In Lake Loskop, large-scale fish mortality was observed as a result of acid mine drainage and may explain the development of pansteatitis in the resident crocodiles after the fish die-off (J. Myburgh and co-workers, University of Pretoria, pers. comm., 2009). It has been proposed that bio-accumulation, via algae, of aluminium and iron within body fat was the cause of yellow discolouration of fat of Mozambique tilapia, *Oreochromis mossambicus* (Peters), in Lake Loskop and that this bio-accumulation may have provided a trigger for development of pansteatitis in higher trophic level predators (Oberholster *et al*. 2011). In the Olifants Gorge, significant overt fish mortality has seldom been observed, with the exception of a single localised event affecting almost exclusively large African sharptooth catfish, *Clarias gariepinus* (Burchell), during the winter of 2009 (D. Pienaar & D. Govender, SANParks, Skukuza, pers. comm., 2009). During the 2011 aerial crocodile survey by SANParks, however, three large dead catfish were observed in the Olifants Gorge and in Lake Massingir (D. Pienaar, SANParks, Skukuza, pers. comm., 2011) suggesting that low level mortality was occurring.

There are numerous references in the literature linking lipid peroxidation to pollutants in the aquatic environment (Bainy *et al*. 1996; Baker, Martin & Davies 1997; Kelly *et al*. 1998; Kibanova, Nieto-Camacho & Cervini-Silva 2009), but little has been published linking these effects to pansteatitis, which is regarded as a nutritional disease. Because of a possible link between pansteatitis in catfish and crocodiles, the study of pansteatitis in catfish was initiated as part of the multidisciplinary investigation into the crocodile mortality in the KNP under the auspices of the Consortium for the Restoration of the Olifants Catchment (CROC).

Materials and methods

Sharptooth catfish were collected during the winter and summer months from various localities within and outside the KNP, including two negative reference populations, namely Reënvoël Dam (23°58′37.2″S 31°19′38.4″E) that has its entire catchment within the KNP, and Van Ryssen Dam (24°00′13.6″S 31°05′36.9″E) at the FOSKOR phosphate mine in Phalaborwa just west of the KNP. A farmed population of sharptooth catfish at Lunsklip Fisheries near Lydenburg, Mpumalanga, served as a positive reference population. Subject to catch success, up to 20 fish were collected on each sampling occasion (Table 1). From 2009, fish were sampled repeatedly from the Olifants Gorge. This section of river stretches eastwards from the confluence of the Olifants and Letaba rivers (23°59′21.8″S 31°49′35.6″E), through a 9 km long gorge in the Lebombo Mountains, to where it enters Lake Massingir on the Mozambique border (23°57′48″S 31°52′97″E). Further samplings took place from the Olifants River at Mamba Weir (24°03′32″S 31°14′14″E), where the Olifants River enters the western boundary of the KNP, from Engelhard Dam (23°50′19″S 31°28′28″E) on the Letaba River, and further south in the KNP from the Sabiepoort (25°10′25.41″S 32°02′23.42″E), where the Sabie River enters Lake Corumana on the Mozambique border. In the north of the KNP, fish were sampled from the Levuvhu River (22°25′51.0″S 31°18′04.4″E) and on the southern boundary of the Park from the Crocodile River (25°23′57.1″S 31°57′29.9″E). Fish were caught by baited hook and line and by netting. During the June 2011 sampling in the Olifants Gorge, 21 tiger fish, *Hydrocynus vittatus* Castelnau, were also sampled from the confluence of the Olifants and Letaba rivers.

All fish were anaesthetised using benzocaine hydrochloride (Kyron Laboratories, Johannesburg, South Africa) and subjected to body mass and length measurements, body condition scoring and sexing. Body condition was scored on a scale ranging from 1 (*emaciated*) to 5 (*obese*). Blood was collected from the vessels ventral to the vertebral column in the tail region. Each fish was euthanized with an overdose of benzocaine hydrochloride and a detailed autopsy was performed. Positive pansteatitis cases were identified by presence of grossly observable lesions in the fat. Tissue specimens from organs and abdominal and subcutaneous adipose tissues were collected in 10% buffered formalin from all sampled fish and subjected to standard histological techniques. All tissue sections were stained with haematoxylin and eosin and examined by light microscopy. Selected sections were stained with Gomori’s Aldehyde Fuchsin to demonstrate presence of ceroid within the adipose tissues and in macrophages associated with necrotic lesions in the fat. The stomach contents of each fish were examined and recorded. The sagittal otoliths were removed from all sampled catfish, embedded in resin and sectioned transversely at 0.4 mm thickness by microtome according to the method of Weyl and Booth (2008). Mounted sections were examined under the light microscope and growth zones were counted for age determination.

Ethical considerations

The project was approved by the Animal Use and Care Committee of the University of Pretoria under Protocol VO13/10.

Results

During the period from June 2009 to June 2011, 265 specimens of *C. gariepinus* were examined during 15 sampling episodes from the various localities within and outside of KNP, including the positive and negative control populations (Table 1). Pansteatitis was observed in mesenteric adipose tissues of fish from the Olifants Gorge, Sabiepoort, Engelhard Dam, Mamba Weir and Lunsklip Fisheries and differed little except in degree of severity. Gross lesions consisted of small focally disseminated to coalescing granulomata up to 5 mm in diameter characterised by a brown colour, sometimes with an orange-coloured centre (Figure 1). Affected fat in severe cases had a rubbery consistency. In the adipose tissues, gross lesions of pansteatitis were confirmed histologically by the presence of foci of adipocyte necrosis with extracellular lipopigment surrounded by an intense infiltration of ceroid-containing macrophages. The detailed pathology and histopathology of the organs and the specific lesions associated with fat necrosis in fish from the Olifants Gorge have been published elsewhere (Huchzermeyer *et al.* 2011). Pansteatitis was not found in the 21 tiger fish collected from the Olifants Gorge during June 2011.

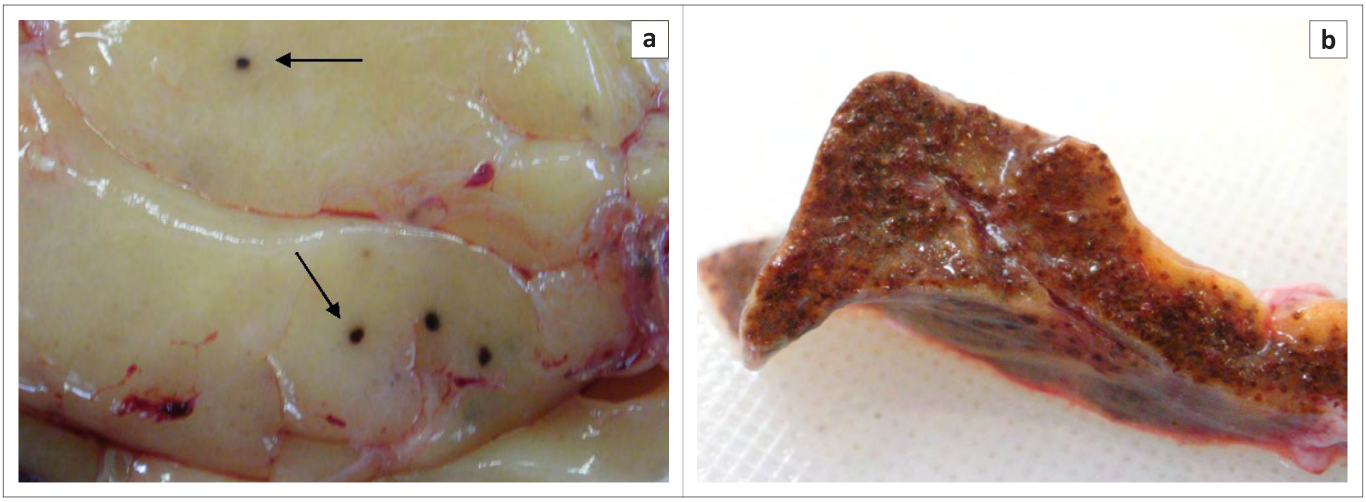


FIGURE 1: (a) Early pansteatitis lesion in mesenteric fat of a sharptooth catfish sampled from the Olifants Gorge during July 2010. Note the sharply circumscribed foci of fat cell necrosis and associated lipopigment deposition imparting the characteristic brown colour (arrow). (b) Advanced pansteatitis of mesenteric fat of a sharptooth catfish sampled from Engelhard Dam during July 2010. Note the diffuse brown granular appearance of the fat and virtual absence of normal-appearing fat.

TABLE 1: Prevalence of gross pansteatitis lesions and fish stomach content, mean body mass, and median body condition and mesenteric fat scores in populations of sharptooth catfish sampled from June 2009 to June 2011.

Site	*n*	Date	% with pansteatitis	% with fish in stomach content	Body mass (grams)		Body condition score (scale 1–5)		Mesenteric fat score (scale 1–5)	
					Mean	Range	Median	Range	Median	Range
G-OL	9	June 2009	33	33	2362	840–4760	3	3–5	2	1–5
G-M	14	Aug. 2009	43	58	3087	1280–5340	3	2–4	2.25	0.5–4
G-OL	21	Nov. 2009	29	43	2679	320–9300	4	3–5	4	2–5
G-OL	25	July 2010	60	20	2730	520–6560	3	2–4	2	1–4
G-OL	22	Jan. 2011	55	82	2570	940–5620	3	2–4	1	0–3
G-OL	21	June 2011	67	57	3220	640–10 000	3	1–4	3	0.5–4
MW	20	July 2010	5	15	1453	580–4600	3	1–4	1	0–3
EHD	21	July 2010	10	33	2175	680–6700	2	1–3.5	1	0–3.5
SP	11	July 2010	45	82	3020	880–7740	3	3–4	2	0–2.5
LKF	21	Nov. 2009	66	38	6893	2820–8680	3	1.5–4	2	0.5–4
RVD	23	Nov. 2009	0	13	4419	2280–8820	3	1–4	2	1–3
RVD	13	Jan. 2011	0	31	1660	540–5860	4	2–4	0.5	0–2
VRD	10	Jan. 2011	0	90	4300	960–6340	3	2–4	3	1–4
LR	14	June 2011	0	7	3174	480–1250	3	1–4	1.5	0.5–4
CR	20	June 2011	0	0	1689	710–4690	3	2–4	0.75	0–2

G-OL, Gorge Olifants Letaba confluence; G-M, Gorge Mozambique border; MW, Mamba Weir; EHD, Engelhard Dam; SP, Sabiepoort; LKF, Lunsklip Fisheries; RVD, Reënvoël Dam; VRD, Van Ryssen Dam; LR, Levuvhu River; CR, Crocodile River.

Mesenteric fat reserves varied from sparse to prominent in catfish represented in samplings from most sites but mesenteric fat stores of most catfish from the Olifants Gorge were larger than those of catfish sampled from other localities in KNP. Catfish from Reënvoël Dam, Engelhard Dam, the Crocodile River and Mamba Weir were leaner. Most fish from Lunsklip Fisheries had prominent mesenteric fat reserves.

Catfish sampled from the Olifants Gorge and from Reënvoël Dam ranged in age from 1 to 19 years. In the Olifants Gorge, pansteatitis was detected in catfish ranging from 3 to 19 years with both sexes equally affected. The main focus of the study was on the Olifants River Gorge, where an increase in prevalence of pansteatitis in sampled catfish was detected since structured sampling began in 2009 (Figure 2). Presence of gross lesions was used to determine prevalence of pansteatitis (Figures 2 and 3). Microscopic examination of histological sections of fat from sampled fish confirmed the macroscopic diagnosis of pansteatitis (Figure 4). Co-existence of coalescing granulomata, scarring of the adipose tissues, and more recent lesions characterised by small foci of brown discolouration of the fat suggested on-going incitement of fat necrosis and attempts at healing in catfish from the Olifants Gorge.

Catfish from Lunsklip Fisheries were fed almost exclusively on untreated waste, rich in polyunsaturated fat, from the trout slaughterhouse on this farm. This waste, consisting largely of fat rich innards, was dumped into the catfish pond where it decomposed until consumed by the fish. These fish showed a high prevalence of pansteatitis as expected under such nutritional conditions, and provided the study with a positive reference population (Table 1). In the Olifants Gorge, analysis of stomach contents showed that catfish fed predominantly on fish as well as insects and small reptiles that had been washed into the river during flood conditions. On the Mozambique border where the Olifants River flows into Lake Massingir and where the sand-bottomed pools and rapids have been inundated with clay deposits, sampled catfish appeared to be feeding off the surface of the clay, and ingesta consisted of algal detritus and clay. In the Sabie River, the Sabiepoort has also been partially inundated with clay sediments as a result of Lake Corumana in Mozambique damming this gorge. A single sampling revealed a similar prevalence of pansteatitis in catfish to that found in the Olifants Gorge (Figure 2). Several of these fish appeared to have been feeding off a dead crocodile with pansteatitis. In the Olifants Gorge both crocodiles and catfish have been observed feeding off the carcasses of dead crocodiles (D. Pienaar, SANParks, Skukuza, pers. comm., 2009). Fish from Mamba Weir were lean and had fed heavily on the fruit of sycamore fig trees, *Ficus sycomorus*, which overhang the river embankment. Prevalence of pansteatitis was significantly lower than that in the Olifants Gorge (Figure 2). Fish sampled from Van Ryssen Dam showed no lesions of pansteatitis (Table 1). They appeared to have fed exclusively on Mozambique tilapia. The stomachs of the majority of fish sampled from the Crocodile River during June 2011 were distended with ingested filamentous algae. Microscopic examination of fluid expressed from stomach contents revealed large numbers of diatoms together with filamentous algae. Fish sampled from the Levuvhu River during June 2011

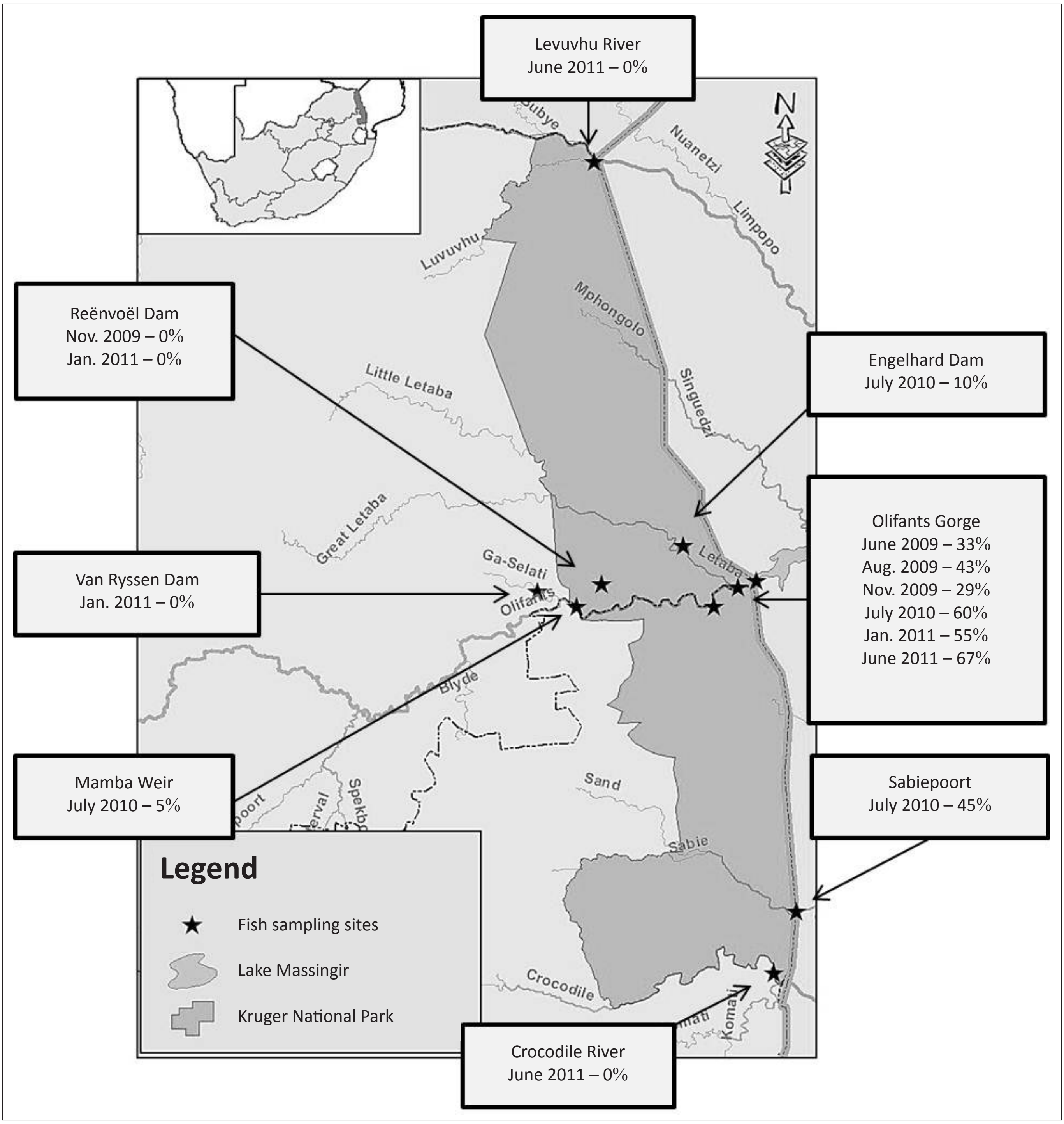


FIGURE 2: Prevalence of pansteatitis in sharptooth catfish sampled from various localities in the Kruger National Park from 2009 to 2011.

had been feeding off algae and sycamore figs. No pansteatitis was detected in fish from the Crocodile and Levuvhu rivers (Table 1). Fish remnants, vegetation, invertebrate and algal detritus were equally represented in stomach contents of fish from Reënvoël Dam. Gross steatitis was not detected (Table 1). Histological examination, however, revealed small numbers of lipopigment-containing macrophages within the mesenteric adipose tissue in one fish (3.6% of sampled fish [*n* = 41]) from Reënvoël Dam. The associated small focus of lipopigment-containing necrotic cell remnants was in the proximity of a large parasitic cyst. Stomach contents of fish from Engelhard Dam included fish remnants, plant and algal detritus. Pansteatitis prevalence was 10% (*n* = 21) (Figure 2).

Discussion

Poikilothermic animals such as fish require inclusion of highly polyunsaturated fatty acids within biological membranes to maintain membrane fluidity necessary for normal metabolism at the relatively colder temperatures at which these animals function (Hulbert 2003). In rainbow trout, *Oncorhynchus mykiss* (Walbaum), it has been shown that the relative proportions of polyunsaturated fatty acids in membranes change with cold acclimation, with particularly the n-3 fatty acids increasing (Hazel 1979). The greater the degree of unsaturation of fatty acids, the more vulnerable they are to oxidative breakdown and intact antioxidant protective mechanisms, particularly presence of adequate vitamin E, are required to prevent *in vivo* autoxidation in bio-membranes (Niki *et al*. 1989). Oxidative stress associated with intense intake of polyunsaturated fat in fresh fish consumed over a short period, or due to ingestion of rancid fish remains, is likely to deplete vitamin E reserves and to result in pansteatitis in catfish as it is known to do in other animals. Vitamin E levels in rainbow trout liver were found to be inversely proportional to dietary level of lipid unsaturation, showing a higher utilisation of vitamin E associated with unsaturated lipid intake, and feeding of such diets may induce apparent vitamin E deficiency symptoms (Watanabe *et al*. 1981). Under conditions of dietary oxidant overload, depletion of vitamin E has also been shown to occur in muscle, liver and plasma of sharptooth catfish (Baker & Davies 1996, 1997). Where vitamin E is insufficient to provide adequate protection against the peroxidation of unsaturated lipids, necrosis and inflammation of the adipose tissues ensues, giving rise to the clinical picture of pansteatitis. Steatitis is the lesion that follows on an oxidative insult to the adipose tissues and the author has demonstrated (unpublished data) that in catfish such lesions can persist for protracted periods of time. From field measurements done by the author (unpublished data) of serum vitamin E values in catfish with pansteatitis in the Olifants Gorge, it would appear that many but not all of these fish had normal serum vitamin E values at the time of sampling, indicating that the oxidative stress exposure was not continuous. Where oxidation of lipids is not currently taking place in the adipose tissues, vitamin E levels may return to normal in animals chronically affected by pansteatitis.

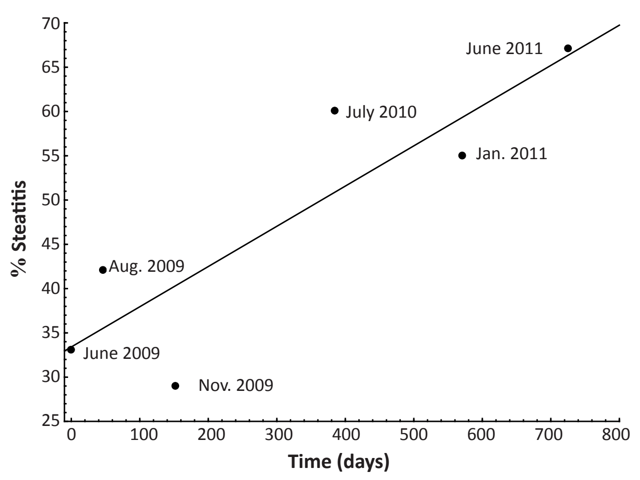
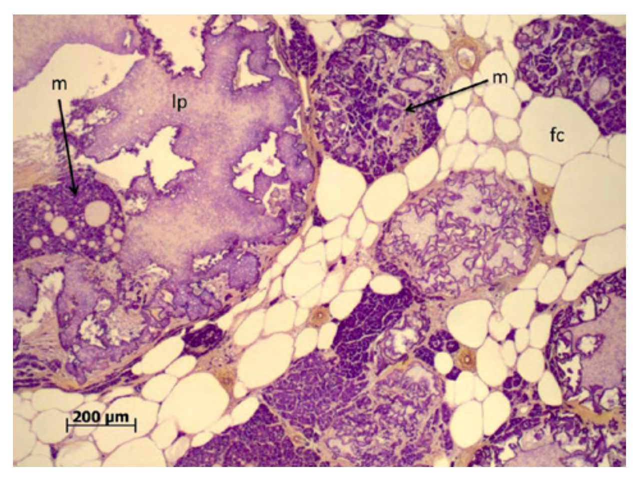


FIGURE 3: Gross pansteatitis prevalence as percentage of sampled sharptooth catfish from the Olifants Gorge during the period June 2009 to June 2011.



Gomori's Aldehyde Fuchsin used to stain ceroid.
lp, lipopigment; m, the intense ceroid containing macrophage reaction; fc, fat cells.

FIGURE 4: Microscopic appearance of granulomata associated with pansteatitis in mesenteric fat of a sharptooth catfish sampled from the Olifants Gorge during November 2009. Note the coalescing fat breakdown product and lipopigment, the intense ceroid containing macrophage reaction and fat cells.

Pansteatitis was confirmed in catfish from three main locations within the KNP: the Olifants Gorge and lower Letaba River at the confluence with the Olifants River, Engelhard Dam on the Letaba River upstream of the Olifants-Letaba confluence, and the Sabiepoort. Pansteatitis in crocodiles was first reported from the Olifants Gorge when large numbers of crocodiles died in this section of the Olifants River in the winter of 2008 (Ferreira & Pienaar 2011). During subsequent samplings from 2009 to 2011 an increasing prevalence of pansteatitis was observed in sharptooth catfish in the Olifants River gorge, yet only a low prevalence was found in catfish inhabiting the Olifants River upstream of the gorge, with none in catfish from a rain-filled dam not connected to the Olifants River. Common to both the Olifants Gorge and the Sabiepoort is the damming of the rivers in Mozambique to form lakes Massingir and Corumana respectively. The inlets of both lakes extend westwards back into the KNP, flooding the respective gorges where these rivers previously traversed the Lebombo Mountains as fast flowing rapids. During 2009, SANParks staff found several dead crocodiles in the Sabie River in the vicinity of the Sabiepoort with pansteatitis also found to be the cause of death (D. Govender, SANParks, Skukuza, South Africa, pers. comm., 2010). A single sampling of catfish from the Sabiepoort, where the Sabie River enters Lake Corumana on the Mozambique border, revealed pansteatitis prevalence similar to that found in the Olifants Gorge, suggesting that the aquatic environment in the two rivers is similar. As in the case of Lake Massingir, Lake Corumana supports a freshwater fishery in Mozambique. Catfish with pansteatitis may have migrated from the Olifants Gorge upstream to Engelhard Dam and this may be one explanation for the presence of pansteatitis-affected fish at this site, but the Sabiepoort has an entirely separate catchment. The anthropogenic activities resulting in potential pollution of the rivers differ greatly between these two catchments, providing argument against primary pollution-related aetiology of pansteatitis at these two sites.

Crocodile mortalities have declined in the Olifants Gorge since the mass die-off of 2008 but co-existence of old and recent lesions in sampled catfish point to an on-going incitement of pansteatitis. Analysis of catfish stomach contents in KNP strongly suggested that consumption of a predominantly fish diet within the Olifants Gorge was associated with the high prevalence of pansteatitis in catfish in this location. Catfish are primarily omnivorous benthic scavengers with wide dietary options but may form schools that actively hunt fish (Skelton 2001) and are known to feed on fish elsewhere (Spataru, Viveen & Gophen 1987). Under natural circumstances, a diet of fish may be well tolerated by sharptooth catfish (Uys 1988), but an intense intake of polyunsaturated fat over a short period is likely to deplete vitamin E reserves and to result in pansteatitis, especially if combined with other oxidative stressors. The Van Ryssen Dam collects treated wastewater from a phosphate mine, and fish from this dam showed no signs of pansteatitis despite a high prevalence of Mozambique tilapia in the stomach contents. The captive population of catfish of Lunsklip Fisheries was fed an excess of fish waste that was observed rotting in the water before being consumed. Under such conditions, pansteatitis could be expected as a result of rancidity of ingested fats leading to oxidative stress and depletion of vitamin E stores.

Tiger fish included in the preliminary samplings of fish from the Olifants Gorge during the winter of 2008, soon after the initial crocodile deaths were discovered, did not have pansteatitis. This finding was repeated during a sampling in June 2011 at a time when prevalence of pansteatitis in catfish from the gorge was high. Tiger fish, being obligate piscivorous predators, possibly evolved more effective antioxidant mechanisms to deal with a higher polyunsaturated fat intake. Being near the top of the aquatic food chain, they would be expected to develop pansteatitis if an inciting agent was bio-accumulated via the fish they kill and eat, or was assimilated from the water. The development of pansteatitis in sharptooth catfish must therefore be linked rather to their benthic scavenging habits or to a change in food source and/or other simultaneous environmental factors to which they are not adequately adapted.

Pansteatitis in free ranging wild animals has rarely been reported except from aquatic birds. Recently an outbreak of steatitis in wild egrets and herons was reported from a reservoir in Japan (Neagari *et al*. 2011). However, steatitis has been reported in wild marine fish, the common dab, *Limanda limanda* (L.), with a suspected pollution-related aetiology (Begg, Bruno & McVicar 2000). Concern about the KNP Olifants River crocodile demise stems from the possible influence of anthropogenic effects on the Olifants River catchment, which covers some 74 500 km^2 and is home to about 8% of South Africa's population (Ashton 2010). Approximately 90% of the country's saleable coal is mined in this catchment and is used to generate 55% of South Africa's electricity, resulting in serious pollution concerns (Coetzee, Du Preez & Van Vuuren 2002; De Villiers & Mkwelo 2009). The area contains numerous dams, including 38 major dams, as well as the country's second largest irrigation scheme (Anon 2001). In addition, large areas of the landscape have been changed by afforestation and agriculture. Huge increases in urban wastewater discharge and on-going high nutrient run-off from agricultural practices raise added concerns of eutrophication (Heath *et al.* 2010). A large phosphate mine is situated near the town of Phalaborwa just east of the KNP near the western entry point of the Olifants River into the KNP. For a number of years prior to 2004, and once in 2008, abnormally high phosphate levels were recorded in the Olifants River within the KNP (J. Venter, SANParks, Skukuza, South Africa, pers. comm., 2012). These were ascribed to the discharge of tailings from the phosphate mine into the Selati River, a tributary of the Olifants River, and to municipal sewerage discharges from the town of Phalaborwa. Such phosphate discharges would have contributed to the inorganic nutrient load trapped in sediments of Lake Massingir. Dissolved phosphate is often the limiting nutrient governing phytoplankton growth in fresh water and phosphates released from sediments will continue to drive the nutrient cycle of the lake. This seasonal stimulus for phytoplankton growth may have contributed to an increasing biomass of fish in the lake.

Contamination of surface waters in the catchment, with accumulation of heavy metals within sediments through adsorption and precipitation processes, has long been recognised as a serious pollution concern, and site specific bio-accumulation of metals has been demonstrated in sharptooth catfish in the upper catchment of the Olifants River (Coetzee *et al.* 2002). Oberholster *et al.* (2011) have suggested that aluminium and iron bio-accumulation by Mozambique tilapia in Lake Loskop may have induced the yellow fat observed from fish at that site. Baker *et al.* (1997) have, however, proposed that sharptooth catfish efficiently regulate iron status and are able to prevent tissue assimilation of dietary iron intake. This is an important adaptation to their benthic habitat, in which they are likely to consume sediment burrowing organisms with inadvertent ingestion of sediment. Large dams in the catchment act as traps for sediments, nitrates, phosphates and heavy metals and are regarded as the epicentre of recent mortalities of fish and crocodiles (Heath *et al.* 2010). Over time, changing water quality may cause sediment-bound contaminants to become bio-available and result in bio-accumulation in fish tissues.

Redox cycling of iron is known to be an initiator of lipid peroxidation (Baker & Davies 1997; Demopoulos 1973; Minotti & Aust 1992; Tappel 1973) and depletion of tissue vitamin E levels by high dietary iron intake may render polyunsaturated fats in the tissues of the fish vulnerable to peroxidation (Baker & Davies 1997). In another study (Huchzermeyer *et al.* 2011), pansteatitis-affected catfish caught during September of 2008 and from January to November of 2009 in the lower Letaba and Olifants Rivers were shown by special histological staining to have accumulated large amounts of iron in the form of haemosiderin in the melanomacrophages of the liver, spleen, ovary and to a lesser extent in the kidney. However, haemosiderin was not detected in the intense macrophage reaction associated with pansteatitis in the adipose tissues. Blood smears of many of these fish showed an abundance of immature erythrocytes as well as irregular erythrocyte shapes as described in cases of vitamin E deficiency in fish (Murai & Andrews 1974; Post 1993; Smith 1979) and increased erythrocyte turnover may have been the source for the increased haemosiderin carried by macrophages. Stomach contents of fish caught in the gorge at the confluence yielded mainly remnants of large fish, whereas specimens caught directly over the silt deposits on the Mozambique border only contained detritus and silt. These findings suggested bio-accumulated iron as an additional oxidative trigger possibly ingested in polluted sediment. As stated before, however, catfish are able to prevent tissue assimilation of dietary iron (Baker *et al*. 1997). This and the absence of haemosiderin in macrophages associated with pansteatitis in the adipose tissues of catfish from the Olifants Gorge thus suggest that the role of iron in the aetiology of pansteatitis in catfish in the Olifants Gorge remains speculative.

As a consequence of raising the dam of Lake Massingir, the heavy silt load of the Olifants River has been deposited in flooded parts of the narrow gorge extending into the KNP. The now inundated sand-bottomed pools between previously fast flowing rapids once formed a favoured habitat for large crocodiles. This altered habitat in the Olifants Gorge may have favoured access to certain fish species not normally consumed in large numbers by crocodiles and catfish. Such species may provide higher levels of polyunsaturated fatty acids than those to which the animals are adapted. An increase in dietary polyunsaturated fat intake has been reported to result in pansteatitis in various animals. Wallach and Hoessle (1968) concluded that a change in diet from smelt (6.7% fat) to mackerel (29.9% fat) was the precipitating cause of pansteatitis in captive American alligators, *Alligator mississippiensis* (Daudin). Goodwin (2006) stressed the dangers of using diets high in fish oils for inappropriate species, and a change from Baltic and Mediterranean clupeids to Moroccan Atlantic pilchards was suspected to have been the cause of pansteatitis in northern bluefin tuna, *Thunnus thynnus* (L.), reported by Roberts and Agius (2008). Similarly pansteatitis could be induced in cats by feeding an oil-rich fish-based diet (Fytianou *et al*. 2006).

Silver carp, *Hypophthalmichthys molitrix* (Valenciennes), an alien invasive schooling species from East Asia (Kolar *et al*. 2005), were introduced into Mozambique from Cuba and also escaped into the Olifants River from South Africa. They are known to occur in Lake Massingir (Skelton 2001) and have been observed on occasion in the Olifants River in large numbers (J. Venter, SANParks, Skukuza, South Africa, pers. comm., 2012). This fish is a specialised plankton feeder that by preference feeds off phytoplankton and is an important consumer of cyanobacterial blooms (Kolar *et al.* 2005), a niche no indigenous South African fish species occupies (P. Skelton, South African Institute of Aquatic Biodiversity, Grahamstown, South Africa, pers. comm., 2012). Such blooms have been observed near the inlet to Lake Massingir (D. Pienaar, SANParks, Skukuza, South Africa, pers. comm., 2009). Phytoplankton naturally contains large quantities of α-linolenic acid and other n-3 polyunsaturated fatty acids, in particular eicosapentaenoic acid C20:5n-3 (EPA) and docosahexaenoic acid C22:6n-3 (DHA) (Steffens 1997). Intake of these fatty acids is reflected in the adipose tissues of silver carp. In one study, these two fatty acids were found to constitute up to 5.28% and 3.41% of body fat triacylglycerols respectively (Buchtová & Ježek 2011). The n-6 and n-3 fatty acids derived from linoleic and α-linolenic acids respectively are essential fatty acids that cannot be synthesised by animals (Steffens 1997). The relative abundance of these fatty acids in the diet of animals is reflected in the composition of their fat tissues (Hoffman & Prinsloo 1995; Steffens 1997). Compared with the fat of farmed crocodiles, a much higher intake of n-3 fatty acids was reflected in the fat of crocodiles in the Olifants Gorge (Osthoff *et al*. 2010). In another study, Huchzermeyer *et al*. (in press) demonstrated that the mesenteric fat of catfish with pansteatitis from the Olifants Gorge showed a similarly high inclusion of n-3 fatty acids, whereas mesenteric fat of healthy catfish from the same site reflected a lower inclusion of n-3 fatty acids.

Seasonal spawning migration of silver carp out of still water bodies into fast flowing rivers occurs over an 8–10 week period once rivers reach their peak summer flows (Kolar *et al.* 2005). During this time, the fish congregate in large numbers and become easy prey for crocodiles and catfish. In the Olifants Gorge this would occur from January to March, a time when fish in the river are difficult to monitor (A. Deacon, SANParks, Skukuza, South Africa, pers. comm., 2012). This may provide an explanation for intense dietary exposure to polyunsaturated fats that could have led to development of obesity and pansteatitis in crocodiles in the Olifants Gorge during the winter of 2008 and to a lesser extent during subsequent winters and to the on-going prevalence of pansteatitis in sharptooth catfish.

The only recorded catfish die-off in the Olifants Gorge was observed during the winter of 2009. The major crocodile die-offs in the Olifants Gorge occurred in 2008 and 2009 respectively and to a lesser extent in subsequent years, after the first really cold weather was experienced around the end of May each year. In many aquatic poikilotherms, especially fish, acclimation to colder water temperatures in winter involves an increase in membrane polyunsaturated fatty acids, in particular DHA (Hazel 1979; Hulbert 2003). Where antioxidant mechanisms have been depleted through oxidative stress, such as that produced by intense dietary polyunsaturated fat intake, compromised bio-membranes in critical tissues might result in impairing adaptation to cold. In an unpublished study the author has demonstrated, however, that catfish severely affected by pansteatitis have survived protracted periods at water temperatures as low as 12 °C with no undue effect. This is well below the lowest water temperature experienced in the Olifants Gorge during winter. Pansteatitis is largely a foreign-body type reaction following on breakdown of fat cells, and presence of the lesion may imply either continuous or preceding oxidative stress. The effect of pansteatitis on cold tolerance may therefore differ depending on the time that has lapsed since the oxidative insult took place.

Conclusion

The deaths of a large number of Nile crocodiles, a keystone species in aquatic conservation, in the Olifants Gorge in the KNP, has raised serious questions about the consequences of anthropogenic activity resulting in altered hydrodynamics and pollution in the catchment of the Olifants River. The objective of this study was to investigate the occurrence of pansteatitis in catfish inhabiting the same waters in the KNP where crocodiles had died of pansteatitis. Catfish were sampled repeatedly over a two-year period from the Olifants Gorge as well as from other sites in and around KNP. In the KNP, pansteatitis in both catfish and crocodiles has been observed in areas where the natural habitat has been drastically altered as a result of damming of rivers, and where the associated deposition of large clay deposits is a potential source of pollution. The increasing prevalence of pansteatitis in catfish in the Olifants Gorge, and the accumulation of lesions over time, points to periodic or seasonal episodes of dietary oxidative stress in these animals. Pansteatitis has also been identified in catfish at other sites in the KNP. The catchment areas feeding these sites differ from that of the Olifants River, providing argument against a primary pollution-related oxidative stress. To date, the only factor common to both the Olifants Gorge and the Sabiepoort, both epicentres of crocodile mortality, and both sites where pansteatitis has been identified in catfish, is the extension of man-made lake inlets into areas favoured by crocodiles. Increasing phosphate levels from anthropogenic activities upstream and in the catchment area of the Olifants River have led to an increase in phytoplankton blooms in Lake Massingir. Whereas this suggests that hydrodynamic change and pollution are the main drivers of this condition in the Olifants Gorge, the presence of large schools of the invasive alien silver carp, benefiting from the nutrient-rich raised water level of Lake Massingir, and known for its high content of n-3 polyunsaturated fatty acids, likely formed much of the seasonal diet of the catfish and crocodiles, either alive or as dead remains, and are thus proposed as the cause of the obesity and a pansteatitis-initiating factor in these animals. The role of bioaccumulation of iron from polluted sediments in initiating lipid autoxidation in catfish is speculative and needs further investigation. The effects on poikilotherm membranes and metabolic rate of a sudden seasonal drop in environmental temperature may have contributed to the die-off of the pansteatitis-affected crocodiles, yet catfish survive cold even when severely affected by pansteatitis. It is not yet clear whether silver carp occur in Lake Corumana and further studies are needed to verify these proposals and answer outstanding questions.

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Competing interests

The author declares that he has no financial or personal relationship(s) which may have inappropriately influenced him in writing this paper.

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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

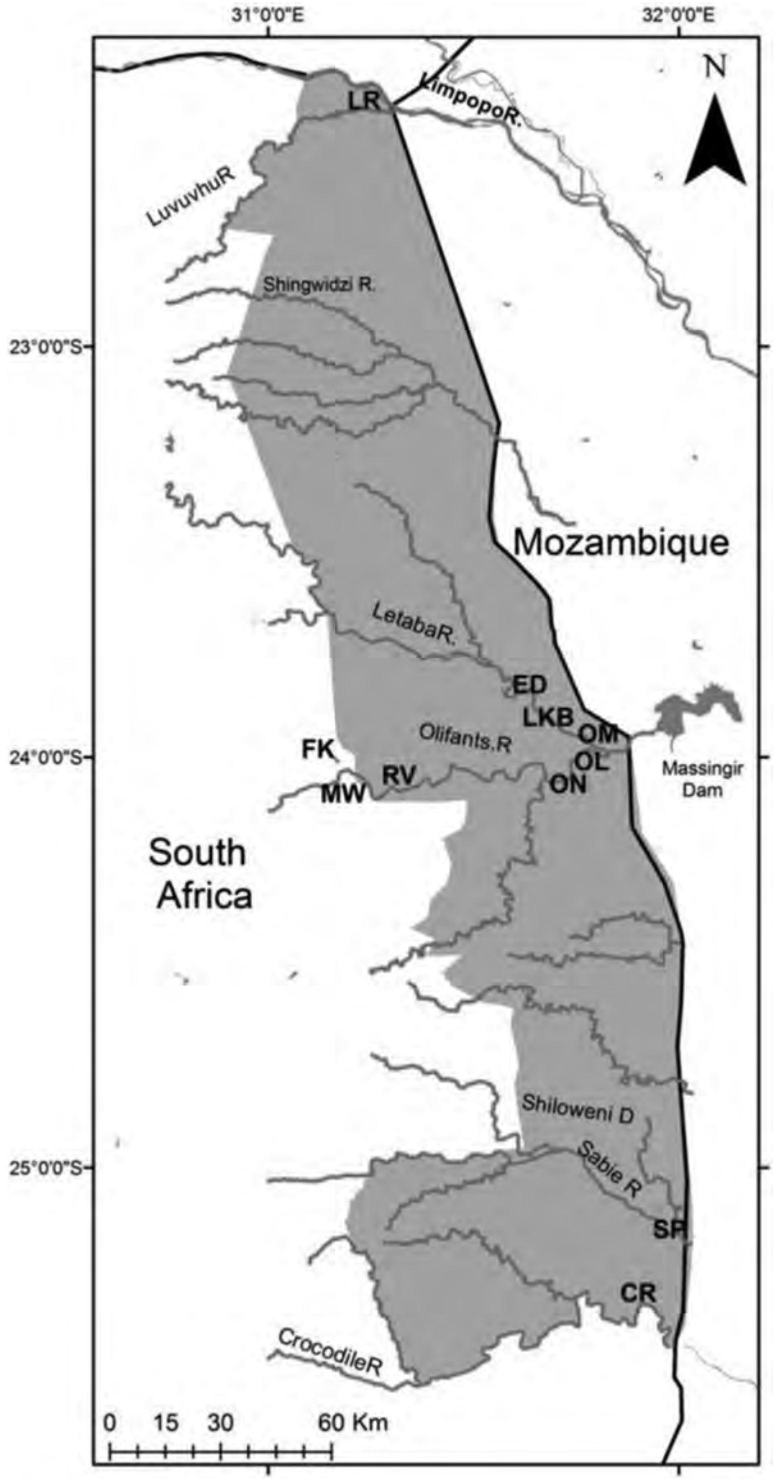

Fig. 1. Location of sampling sites in Kruger National Park. Sampling sites are identified by the site acronyms.

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 δ^{13}C and δ^{15}N values. The effect on δ^{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 δ^{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 $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1]/1000$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. The reference standards against which results are reported are Vienna PeeDee belemnite (VPDB) for δ^{13}C, atmospheric nitrogen (Air) for δ^{15}N and Vienna Canyon Diablo Troilite (VCDT) for δ^{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 δ^{34}S values of 4.1 ± 0.7 (mean ± SD, $n = 21$) and those sampled upstream in the Letaba River had δ^{34}S values of 10.8 ± 0.4‰ (mean ± SD, $n = 13$). Fish sampled at the confluence showed δ^{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).

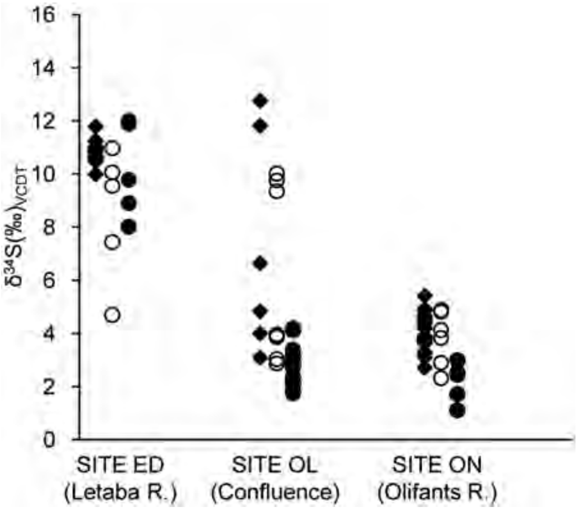

Fig. 2. The δ^{34}S values for fish characterise the Olifants River source (site ON) and the Letaba River source (site LE). Solid circles represent tiger fish, open circles represent sharptooth catfish and diamonds represent other fish. Fish from the confluence (site OL) show mixed or intermediate δ^{34}S 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 $\delta^{15}N$ on $\delta^{13}C$ 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 $\delta^{15}N$ on $\delta^{13}C$ 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

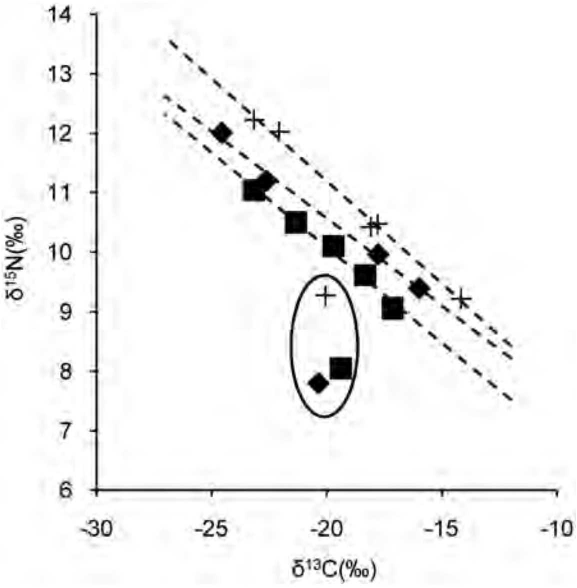

Fig. 3. The isotopic values for aquatic invertebrates are used to define an "isotrophic" comparison between sites from different catchments. *Gomphidae*, represented by crosses, and *Pleurocerida*, represented by diamonds, are represented at most of the sample sites, and display a linear relationship between $\delta^{13}C$ and $\delta^{15}N$ 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.

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.

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

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

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

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

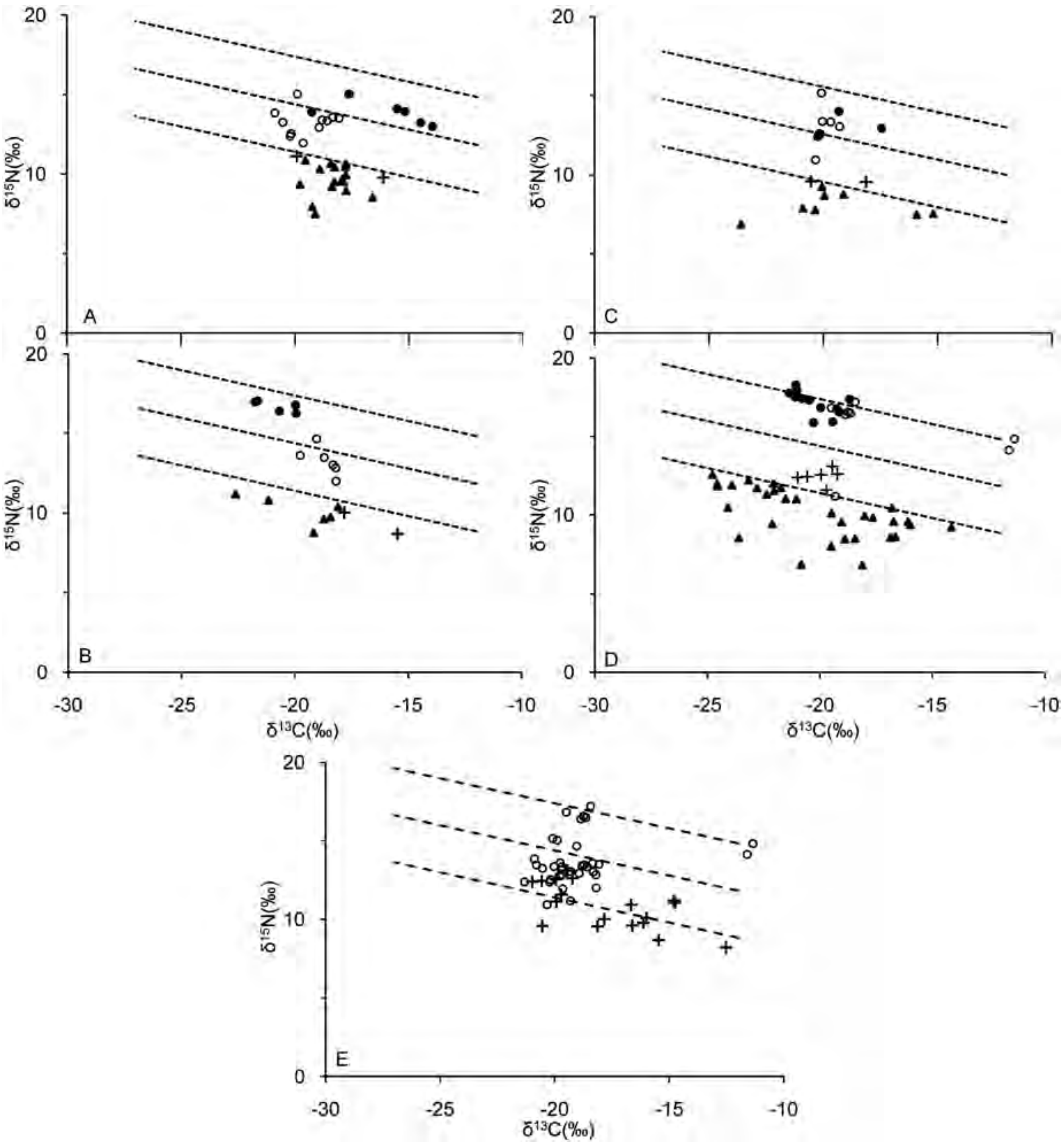

Fig. 4. The relative trophic position of individual sharptooth catfish, tiger fish invertebrate genera and co-occurring crocodiles are represented relative to lines of equal trophic level for sites CR (A), ON (B), ED (C) and OL (D). The relative trophic level of the entire sample of crocodiles ($n = 17$) 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.

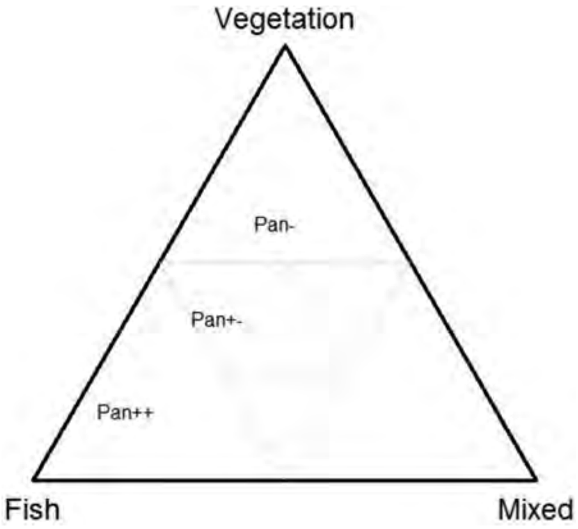

Fig. 5. The population of sharptooth catfish with pansteatitis (Pan++) have stomach contents with a higher proportion of fish than vegetation, or invertebrates and detritus (mixed) when compared with the population of fish from areas that have pansteatitis prevalence, but do not have pansteatitis (Pan+—),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

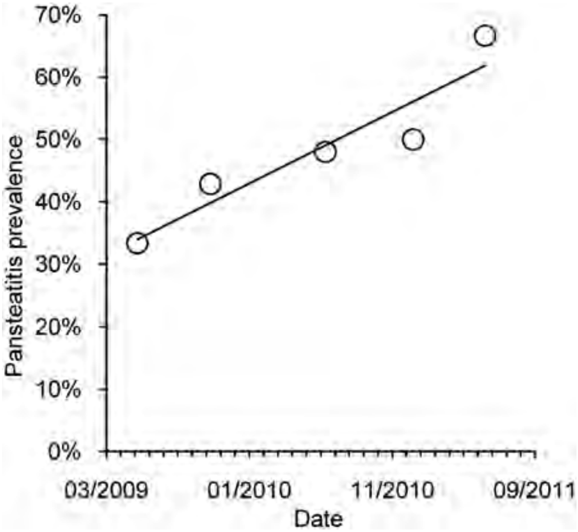

Fig. 6. The trend in the prevalence of pansteatitis in the sharptooth catfish samples through time suggests that the epidemic started in the 2006/2007 austral summer.

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

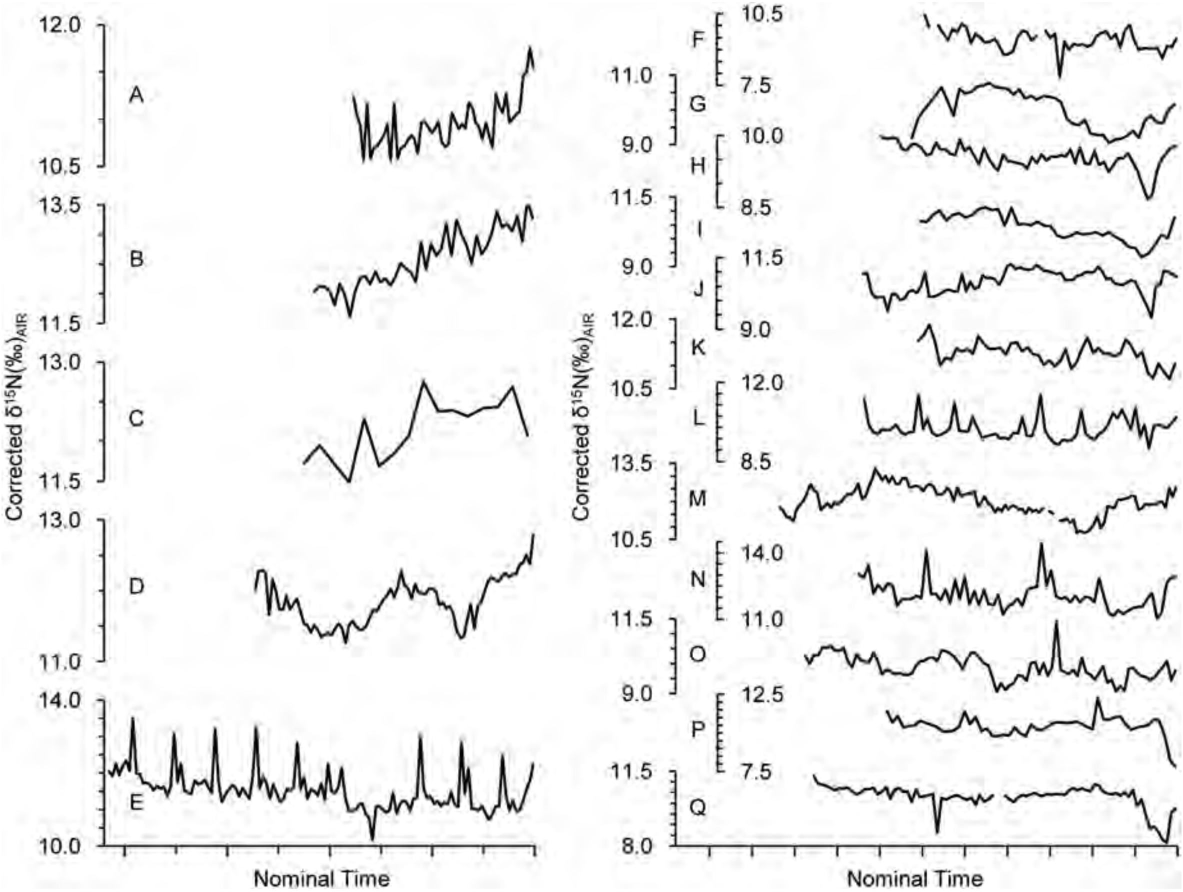

Fig. 7. Most of the crocodiles sampled between the confluence of the Olifants and Letaba Rivers and the Mozambique border show an unprecedented trophic level increase before their death (A–D). A regular spike in one of the crocodile claw time series (E) is thought to be a seasonal signal suggesting that 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 δ^{15}N values have been normalised to the mean δ^{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 δ^{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.

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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	δ^{15}N(‰)$_{\mathrm{AIR}}$	δ^{13}C(‰)$_{\mathrm{VPDB}}$	δ^{34}S(‰)$_{\mathrm{VCDT}}$
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	δ^{15}N(‰)$_{\mathrm{AIR}}$	δ^{13}C(‰)$_{\mathrm{VPDB}}$	δ^{34}S(‰)$_{\mathrm{VCDT}}$
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.

	OM			OL		
Species	δ^{15}N	δ^{13}C	*n*	δ^{15}N	δ^{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.

	ED			ON		
Species	δ^{15}N	δ^{13}C	*n*	δ^{15}N	δ^{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.

	CR		
Species	δ^{15}N	δ^{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.

Site	Date	Steatitis				Healthy			
		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.

Species	CR		OM		OL		LR		ON	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
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	δ^{15}N	δ^{13}C	*n*	δ^{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

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Comparison of the lipid properties of healthy and pansteatitis-affected African sharptooth catfish, *Clarias gariepinus* (Burchell), and the role of diet in pansteatitis outbreaks in the Olifants River in the Kruger National Park, South Africa

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Abstract

Pansteatitis has been identified in wild populations of sharptooth catfish, *Clarias gariepinus* (Burchell), and Nile crocodiles, *Crocodylus niloticus* Laurenti, inhabiting the same waters in the Olifants River gorge in the Kruger National Park, South Africa. Mesenteric and pectoral fat tissue was investigated microscopically and by fatty acid analysis in healthy and pansteatitis-affected catfish from both captive and wild populations. Variation in fatty acid composition between pectoral and mesenteric fat was noted. Composition of mesenteric fat differed between fish from various localities as a result of differences in diet. Pansteatitis in the captive population, resulting from ingestion of high amounts of dietary oxidized fat, reflected higher levels of unsaturated fatty acids within the mesenteric fat. Mesenteric fat of pansteatitis-affected wild catfish was characterized by an increase in moisture content, a decrease in fat content and a decrease in stearic and linoleic acids. The n-3 to n-6 fatty acid ratio of mesenteric fat was higher in pansteatitis-affected wild catfish than in healthy catfish from the same locality, reflecting higher polyunsaturated fat intake by pansteatitis-affected fish. The possible role of alien, invasive, phytoplankton-feeding silver carp, *Hypophthalmichthys molitrix* (Valenciennes), in the aetiology of pansteatitis in both catfish and crocodiles in the Olifants Gorge is discussed.

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Keywords: Clarias gariepinus, lipid properties, pansteatitis, polyunsaturated fat, sharptooth catfish, silver carp.

Introduction

During the winter of 2008, carcasses of more than 170 Nile crocodiles, *Crocodylus niloticus* Laurenti, were found close to the confluence of the Olifants and Letaba Rivers in the Kruger National Park (KNP) in South Africa (Anon 2008; Ferreira & Pienaar 2011). The cause of death was established to be a consequence of severe pansteatitis. Further crocodile mortalities occurred during subsequent winters, but in smaller numbers. Both rivers have their catchments in industrial and agricultural areas, before they enter the Kruger National Park. The Letaba River flows 97 km through the Park, and the Olifants River for some 90 km, where they join (the confluence 23°59′21.8″S 31°49′35.6″E) and flow a further 9 km through the Olifants River gorge before crossing into Mozambique, entering the upper reaches of Lake Massingir. Extensive sampling of fish from the Olifants River gorge has led to the discovery that pansteatitis also affected African sharptooth catfish, *Clarias gariepinus* (Burchell), inhabiting this section of the Olifants River (Huchzermeyer *et al.* 2011). Hydrodynamic change in the Olifants River gorge, brought about by the raising of the sluices of Lake Massingir downstream of the gorge, resulted in the inlet of Lake Massingir extending back into the Olifants Gorge, flooding the previously fast flowing rapids and pools of the gorge. Most of the dead and affected crocodiles were found in both rivers from about 10 km upstream of the confluence, up to the border with Mozambique in the same stretch of river where pansteatitis-affected catfish have been found. It has been speculated that pollutants may be concentrated in the clay deposits trapped by dams along the Olifants River (Heath, Coleman & Engelbrecht 2010) that, when released over time due to local changes in water quality, could be the inciting cause of the crocodile and fish mortalities. High phosphate levels attributed to the discharge of tailings from a phosphate mine and to municipal sewerage spills into the Olifants River near the town of Phalaborwa on the western boundary of KNP prior to 2004 (J. Venter, SANParks, Skukuza, personal communication 2012) have contributed to the nutrient enrichment of Lake Massingir.

Pansteatitis, also called yellow fat disease, has been reported in a variety of animals such as rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Roberts, Richards & Bullock 2006), Sunapee trout, *Salvelinus alpinus oquassa* Girard (Herman & Kircheis 1985), channel catfish, *Ictalurus punctatus* (Rafinesque) (Goodwin 2006), white sturgeon *Acipenser transmontanus* Richardson (Guarda *et al.* 1997), Atlantic halibut *Hippoglossus hippoglossus* L. (Bricknell *et al.* 1996), northern bluefin tuna *Thunnus thynnus* (L.) (Roberts & Agius 2008), red-tailed hawk *Buteo jamaicensis* (Gmelin) (Wong *et al.* 1999), boat billed herons *Cochlearius cochlearius* (L.) (Pollock *et al.* 1999), the domestic cat (Niza, Vilela & Ferreira 2003), wild rabbit (Jones, Howard & Gresham 1969), marmoset, *Callithrix* spp. (Juan-Sallés *et al.* 2003), Amazon River dolphin *Inia geoffrensis* (Blainville) (Bonar & Wagner 2003) and Nile crocodile (Huchzermeyer 2003; Osthoff *et al.* 2010). The occurrence and pathology of pansteatitis in sharptooth catfish in the Olifants River has been described by Huchzermeyer *et al.* (2011). The term yellow fat disease may be misleading in this species as sharptooth catfish naturally show a variable fat colour.

Pansteatitis is defined as a nutritional disorder characterized by necrosis and inflammation of adipose tissue and deposition of a ceroid pigment within macrophages in the associated inflammatory reaction. The disease is linked to the consumption of high levels of unsaturated fatty acids and is exacerbated by intake of oxidized fats and depletion of vitamin E (Scott, Miller & Griffin 1995; Fytianou *et al.* 2006). In many species, the condition can be induced by vitamin E deficiency (Farwer *et al.* 1994). Unsaturated fatty acids in the lipids of cell membranes are vulnerable to oxidation through cyclic reduction–oxidation of oxidants resulting in the formation of lipid hydroperoxides (Bus, Aust & Gibson 1976). Lipid free radicals released from decomposition of lipid hydroperoxides initiate the subsequent lipid peroxidation of cell membranes (Bus *et al.* 1976). A lack of vitamin E may result in an accumulation of reactive peroxides in the tissue, which may lead to pansteatitis (Case, Carey & Hirakawa 1995). Treatment with vitamin E may alleviate the disease (Niza *et al.* 2003), but this is not always the case (Bonar & Wagner 2003; Juan-Sallés *et al.* 2003). The effect of pansteatitis on fatty acid composition has not been described by many researchers. Farwer *et al.* (1994) provided evidence that vitamin E depletion affected the fatty acid composition of liver lipids, and Fytianou *et al.* (2006) showed that the fatty acid composition of adipose tissue is affected by pansteatitis. Osthoff *et al.* (2010) have shown that the lipids of adipose tissue of healthy wild crocodiles differed minimally in the fatty acid composition from diseased ones and that the observed hardness of the fat tissue was not due to changes in fat composition, but rather to high moisture content, the result of physiological changes induced by interstitial inflammation similar to that observed by Niza *et al.* (2003).

Analysis of the fat composition of captive-raised African sharptooth catfish (Hoffman & Prinsloo 1995) and a comparison with the neutral lipids of heart muscle of free-living catfish in Ugandan lakes (Kwetegyeka *et al.* 2011) showed that fatty acid composition of the total fat of captive fish and that of the heart muscle fat of free-living fish was similar. Captive fish were, however, found to contain higher levels of 20:4n3 and lower levels of 18:1 isomers than wild fish (Hoffman & Prinsloo 1995), and the fatty acid composition of the heart muscle fat differed between fish of different localities (Kwetegyeka *et al.* 2011). This comparison could not conclude whether the difference was due to tissue type, locality or diet. However, Steffens (1997) has reported the effect of diet on fatty acid composition. The effect of diet and health conditions on the fat composition of sharptooth catfish has not been reported before. The aim of our study was to determine the impact of pansteatitis on the fat composition of captive and wild African sharptooth catfish and to link this to dietary factors affecting the two populations.

Materials and methods

During November 2009, a minimum of 20 sharptooth catfish were collected from each of three geographically distinct populations: Olifants River Gorge (OG) [23°59′21.8″S 31°49′35.6″E] in the vicinity of the confluence between the Letaba and Olifants rivers in the area regarded as the epicentre of the crocodile mortalities in the KNP; Reenvoël Dam (RV) [23°58′37.2″S 31°19′38.4″E], a rain-filled dam with its entire catchment within KNP; and Lunsklip Fisheries (LK) [25°23′08,9″S 30°15′35″E] on the Lunsklip River near Lydenburg in Mpumalanga Province. Wild fish were caught on baited hook and line. The fish from LK represented remnants of a farmed population of fish and were caught by scoop net.

The LK population of captive catfish was fed an exclusive diet of untreated trout slaughterhouse waste. Since the collapse of the catfish industry many years ago, these fish were no longer used commercially and hence remained in the system reaching a considerable body size. The slaughterhouse waste was made up to a large extent of fat-rich innards of slaughtered trout and was fed in such excess that at any given time a significant amount of waste could be observed uneaten and decomposing in the water.

Fish were killed by an overdose of benzocaine in the holding water. Detailed data sets were collected from all fish and included length and weight measurements, sex determination, macroscopic and histological descriptions of all organs, including adipose tissues. Age determinations were carried out according to the method of Weyl & Booth (2008) using sagittal otolith sections. In addition, samples of the two major adipose tissues, intra-abdominal mesenteric fat and hypodermal fat from the fat cushion behind the pectoral fin, were collected. The sharptooth catfish is unique in that an extension of both the liver and the marrow or haemopoietic part of the anterior kidney extend through the body wall to lie bilaterally under a hypodermal fat cushion just caudal to the pectoral fin. Tissue samples for fat analyses were collected from those fish with adequate fat reserves and were kept on ice until samples could be frozen before being sent to the laboratory. All tissue samples were kept frozen until preparation for fat extraction, which was carried out within 5 days. Tissue samples for histological examination were immediately fixed in 10% buffered formalin before being processed by standard histological techniques. Five-micron-thick tissue sections stained with haematoxylin eosin and Gomori's aldehyde fuchsin were examined under the light microscope. Severity of pathology in the adipose tissues was scored histologically on a scale of 1–4, with 1 representing no steatitis or the presence of only a few lipopigment-containing macrophages in the fat tissue, but without necrosis of adipocytes and 4 showing the greatest degree of necrosis and inflammation.

Extraction of total fat from tissue samples was performed quantitatively according to Folch, Lees & Sloane-Stanley (1957) using chloroform and methanol in a ratio of 2:1. Total extractable fat content was determined gravimetrically and expressed as g fat per 100 g tissue.

Fatty acids were transesterified to form methyl esters (FAME) using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park & Goins 1994). The FAME were quantified using a Varian 430 flame ionization gas chromatograph (Varian), with a fused silica capillary column, Varian CPSIL 88 (100 m length, 0.25 mm ID, 0.2-μm film thickness) (Varian). The column temperature was 40–230 °C (hold 2 min; ramp 4 °C min^{-1}; hold 10 min). The FAME in hexane (1 μL) was injected into the column using a Varian CP-8400 Autosampler (Varian) with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen was used as the carrier gas at 45 psi, and nitrogen was the make-up gas. Chromatograms were recorded using Galaxy, Chromatography, Data System (Varian). Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples with those of standards of all 37 fatty acids obtained from Supelco (Supelco 37 Component Fame Mix 47885-U; Supelco).

Significant differences in means amongst groups were determined using analysis of variance (ANOVA) and multiple comparisons using the Tukey–Kramer test at $\alpha = 0.05$ (Anon 2007).

Results and discussion

The fish sampled from RV showed no macroscopic signs of pansteatitis and, apart from infestation with a variety of parasites natural to this habitat, appeared healthy. Fish from this population ranged in age from 4 to 19 years, with both sexes being represented in the sampling.

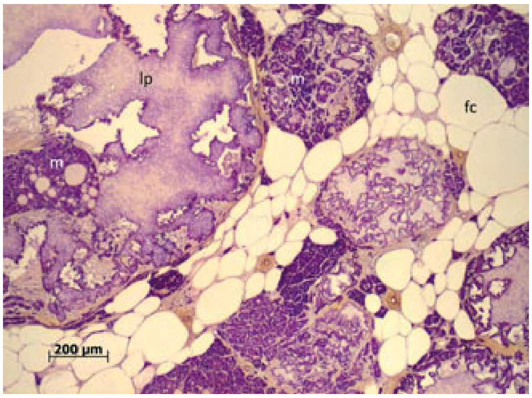
Pansteatitis was found in both male and female fish sampled from the wild population in the OG and from the captive population from LK. In affected fish, lesions were predominantly found in the mesenteric fat reserves. Only a few fish showed lesions in other fat reserves, including the pectoral and hypodermal fat, as well as in fat surrounding the brain. Steatitis was never observed in the pericardial fat. No correlation was observed between age or sex and the presence of pansteatitis in either the wild or captive catfish.

In the OG fish, ratio of adipose tissue to body mass showed little correlation with the presence of pansteatitis, lesions being present in both fat and lean fish. In contrast, easy access to fat-rich food in the captive fish population from LK was reflected in a significantly higher adipose tissue to body mass ratio than in fish from the OG. From the results of the captive fish, it is evident that pansteatitis is not rapidly fatal in these fish, even though the condition may be debilitating. When pansteatitis-affected and healthy fish were kept together in the same tank, clear behavioural differences were observed, the former being slower in finding and taking feed (D. Huchzermeyer, personal observation, 2011).

Comparisons of stomach and intestinal content of fish sampled from the different locations indicated that the sharptooth catfish in OG preyed heavily on other fish, whereas the ingesta of fish from RV reflected a more omnivorous diet consisting of a combination of plants, algae and invertebrates. Stomach content of the LK fish consisted exclusively of decomposing oily fish remnants.

The detailed pathology of pansteatitis in sharptooth catfish from the OG has been described by Huchzermeyer *et al.* (2011). Histological lesions of pansteatitis were similar in the OG and LK fish. Lesions consisted of ruptured adipocytes surrounded by an intense, predominantly macrophage reaction (Figs 1 & 2). Macrophages stained positively for ceroid, a breakdown product of fat, in Gomori's aldehyde fuchsin stained sections (Fig. 1). Coalescing remnants of necrotic fat cells consisted of a refractive yellow staining lipopigment, a fat cell breakdown product, in haematoxylin–eosin-stained preparations (Fig. 2). The same refractive pigment could be observed in macrophages associated with such areas of fat cell necrosis. Fat cell necrosis appeared in distinct foci disseminated through the affected adipose tissue. Such foci were surrounded by macrophages and foreign body giant cells, which in areas coalesced to form epithelioid-like sheaths surrounding lipopigment-containing lacunae. The mononuclear cellular (predominantly macrophage) infiltrate associated with the inflammatory process may have been responsible for the higher moisture content observed in affected fat.

The severest pansteatitis lesions were observed in older, larger fish specimens. In crocodiles, a similar trend was noted with degree of pansteatitis appearing to be related to size and therefore to age (Osthoff *et al.* 2010). On histological examination, the granulomatous inflammation observed surrounding necrotic and ruptured fat cells in pansteatitis-affected catfish was similar to that noted in pansteatitis-affected crocodiles (Osthoff *et al.* 2010).



Colour online, B&W in print

Figure 1 Section of fat from a *Clarias gariepinus* specimen with steatitis, sampled from the Olifants River gorge during November 2009, stained specifically for the presence of ceroid with Gomori's aldehyde fuchsin. Note ceroid-positive staining of macrophages (m) and fat cell breakdown products. Lipopigment (lp), fat cell (fc).

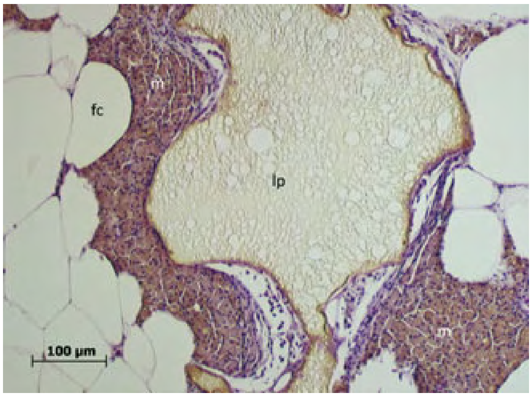


Figure 2 Section of fat from a *Clarias gariepinus* specimen suffering from steatitis sampled from Lunsklip Fisheries during November 2009. Note fat breakdown product with lipopigment (lp) surrounded by macrophages (m). Fat cell (fc). Haematoxylin–eosin.

The fat composition of healthy fish (not affected by pansteatitis) from the three localities, LK, RV and OG is given in Table 1. The mean fatty acid composition of these catfish was in the same order of magnitude as that published by Hoffman & Prinsloo (1995). The monounsaturated acids were found to be higher than the published data, above 36%, mainly due to 16:0, and the levels of polyunsaturated acids were lower, <27% compared with the more than 35% reported. This difference might be ascribed to diet, because the fatty acids of the catfish from the three localities differed significantly (from $P < 0.001$ to $P < 0.05$) amongst each other (Table 1). The greatest differences were observed between the total polyunsaturated fatty acids, mainly due to 18:3c9, 12, 15(n-3), with 1.75% ± 0.68, 1.11% ± 0.28 and 7.78% ± 1.20 for LK, OG and RV respectively, and 20:4c5, 8, 11, 14(n-6), with 0.54% ± 0.02, 1.01% ± 0.18 and 2.72% ± 0.66. Significant differences were also noted in monounsaturated fatty acids, the greatest being in 18:1c9, with respectively 23.71% ± 0.48, 30.62% ± 4.35 and 16.03% ± 2.88. Smaller differences were noted in the content of 18:1c7, 15:0, 17:0 and 18:0. At a lower level of statistical significance, several more differences in fatty acid composition were noted, most obviously in the total unsaturated and polyunsaturated fatty acids. Differences in fatty acid content that might be ascribed to locality, and probably a difference in diet, were similarly reported for heart fat of various fish, including the catfish (Kwetegyeka *et al.* 2011). Of interest is that the moisture content of the fat tissue of fish from OG was lower than that of the other two localities ($P < 0.043$), although the fat content and fat-free dry matter was the same. These results show that the fat of healthy and pansteatitis-affected catfish from the three localities have to be interpreted separately.

The fat composition of normal mesenteric and pectoral fat of captive catfish (LK) is shown in Table 2. Small but statistically significant ($P < 0.001$) differences in content of 14:0, 18:0, 18:1c9, 22:0, 20:5c5, 8, 11, 14, 17(n-3) and 24:1c15 were observed. At a lower statistical significance, $P < 0.05$, more differences in fatty acid composition became apparent. The total saturated fatty acid content of mesenteric fat was higher than that of pectoral fat, 36.37% ± 3.89 vs. 33.95% ± 3.27, whilst the total polyunsaturated content was lower, 26.70% ± 3.80 vs. 30.70% ± 3.14. Mesenteric fat had a higher content of total monounsaturated and lower content of total omega-3 fatty acids, and also a lower content of the very long-chain fatty acids, longer than 18 carbon length, than pectoral fat. The fat content of mesenteric fat tissue was higher than that of pectoral fat tissue, 78.46% ± 6.92 vs. 69.55% ± 4.19 and the water content lower, 16.01% ± 3.21 vs. 21.86% ± 4.44. Similar differences in fatty acid composition of fat of different body locations have been observed in other animals (Sinclair & O'Dea 1987).

In contrast to mesenteric fat, which frequently showed extensive areas of fat necrosis in pansteatitis-affected fish, the pectoral fat only rarely showed foci of fat necrosis. Little difference, as assessed by histological appearance and fat composition of pectoral fat, was observed between the healthy and pansteatitis-affected catfish. Differences observed between mesenteric and pectoral fat might be due to the specific function of the pectoral fat, which is embedded in a connective tissue matrix giving it a spongy feel. This suggests a protective rather than a metabolic function, as the pectoral fat overlies the area behind the pectoral fin where both the liver and anterior kidney extend through a fine canal in the musculature of the body wall to lie subdermally behind the pectoral fin. This is a unique and peculiar feature of sharptooth catfish that is shared with few other fish species. The higher water content of pectoral fat may explain the slightly glassy gross appearance of pectoral fat when compared to mesenteric fat.

Table 1 Analysis of variance (ANOVA) on the effect of locality on chemical composition, fatty acid composition and fatty acid ratios of mesenteric fat of fish with a steatites score of 1

Locality		LK (*n* = 3)	OG (*n* = 7)	RV (*n* = 4)	Significance level
Proximate analysis (%)					
% Fat		78.46 ± 6.92	85.75 ± 3.08	79.55 ± 8.08	$P = 0.129$
% Fat-free dry matter		5.53 ± 4.14	6.92 ± 0.86	8.91 ± 1.30	$P = 0.119$
% Moisture		16.01 ± 3.21^{b}	7.33 ± 2.96^{a}	11.54 ± 6.85^{ab}	$P = 0.043$
FAME (% of total fatty acids)					
Common name	Abbreviation				
Myristic	C14:0	3.41 ± 0.59^{b}	2.37 ± 0.49^{a}	2.05 ± 0.55^{a}	$P = 0.016$
Pentadecylic	C15:0	0.43 ± 0.12^{a}	0.43 ± 0.08^{a}	1.03 ± 0.18^{b}	$P < 0.001$
Palmitic	C16:0	27.05 ± 3.49	29.09 ± 3.90	28.79 ± 1.75	$P = 0.679$
Palmitoleic	C16:1c9	8.17 ± 0.64^{b}	5.89 ± 0.56^{a}	7.53 ± 1.43^{b}	$P = 0.006$
Margaric	C17:0	0.40 ± 0.12^{a}	0.64 ± 0.09^{a}	2.77 ± 0.35^{b}	$P < 0.001$
Heptadecenoic	C17:1c10	0.38 ± 0.10^{b}	0.07 ± 0.02^{a}	0.11 ± 0.01^{a}	$P < 0.001$
Stearic acid	C18:0	4.33 ± 0.48^{a}	6.93 ± 0.72^{b}	6.26 ± 0.26^{b}	$P < 0.001$
Elaidic	C18:1t9	0.31 ± 0.13^{b}	0.03 ± 0.02^{a}	0.15 ± 0.09^{a}	$P < 0.001$
Oleic	C18:1c9	23.71 ± 0.48^{b}	30.62 ± 4.35^{c}	16.03 ± 2.88^{a}	$P < 0.001$
Vaccenic	C18:1c7	4.25 ± 0.27^{b}	3.47 ± 0.11^{a}	6.14 ± 0.71^{c}	$P < 0.001$
Linoleic	C18:2c9,12 (n-6)	13.14 ± 1.80^{b}	8.11 ± 2.96^{a}	6.78 ± 0.90^{a}	$P = 0.012$
Arachidic	C20:0	0.18 ± 0.02^{a}	0.27 ± 0.04^{ab}	0.33 ± 0.08^{b}	$P = 0.009$
γ-Linolenic	C18:3c6,9,12 (n-6)	0.35 ± 0.11^{ab}	0.20 ± 0.08^{a}	0.56 ± 0.23^{b}	$P = 0.006$
α-Linolenic	C18:3c9,12,15 (n-3)	1.75 ± 0.68^{a}	1.11 ± 0.28^{a}	7.78 ± 1.20^{b}	$P < 0.001$
Heneicosanoic	C21:0	0.49 ± 0.11	0.73 ± 0.55	1.14 ± 0.29	$P = 0.167$
Eicosadienoic	C20:2c11,14 (n-6)	0.77 ± 0.03^{b}	0.41 ± 0.13^{a}	0.82 ± 0.15^{b}	$P < 0.001$
Behenic	C22:0	0.08 ± 0.01^{a}	0.11 ± 0.02^{a}	0.29 ± 0.11^{b}	$P < 0.001$
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.47 ± 0.10^{a}	0.44 ± 0.08^{a}	0.76 ± 0.16^{b}	$P = 0.002$
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.05 ± 0.02^{a}	0.14 ± 0.02^{b}	0.90 ± 0.09^{c}	$P < 0.001$
Arachidonic	C20:4c5,8,11,14 (n-6)	0.54 ± 0.02^{a}	1.01 ± 0.18^{a}	2.72 ± 0.66^{b}	$P < 0.001$
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	2.12 ± 1.29^{ab}	0.74 ± 0.25^{a}	2.69 ± 0.95^{b}	$P = 0.004$
Nervonic	C24:1c15	0.11 ± 0.02^{b}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	$P < 0.001$
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.81 ± 0.22	1.77 ± 0.71	1.75 ± 0.21	$P = 0.990$
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.70 ± 1.39	5.41 ± 2.24	2.61 ± 0.63	$P = 0.057$
Fatty acid ratios					
Total Saturated Fatty Acids (SFA)		36.37 ± 3.89	40.57 ± 4.03	42.67 ± 1.03	$P = 0.096$
Total Mono-Unsaturated Fatty Acids (MUFA)		36.93 ± 0.50^{ab}	40.09 ± 3.85^{b}	29.95 ± 4.62^{a}	$P = 0.004$
Total Polyunsaturated Fatty Acids (PUFA)		26.70 ± 3.80^{b}	19.34 ± 0.89^{a}	27.38 ± 4.25^{b}	$P < 0.001$
Total Omega- 6 fatty acids (n-6)		14.85 ± 1.67^{b}	9.88 ± 2.93^{a}	11.79 ± 1.77^{ab}	$P = 0.041$
Total Omega- 3 fatty acids (n-3)		11.86 ± 2.13^{ab}	9.46 ± 2.86^{a}	15.59 ± 2.50^{b}	$P = 0.012$

Means with different superscripts in the same row differ significantly.

Statistical comparison of pansteatitis-affected fish across localities is not possible due to the effects of different diets on fatty acid composition of the fat. Further comparison of fatty tissue in pansteatitis-affected fat will refer only to the mesenteric fat. The comparison of the fat composition of the fish from LK with different scores of pansteatitis is presented in Table 3. No significant differences at $P < 0.001$ were observed between the fats from healthy and pansteatitis-affected fish. At a lower significance level, the saturated fatty acid content of healthy fish at LK, 36.37% ± 3.89 was higher than that of the fish with pansteatitis score 4, 32.49% ± 1.05, the main difference being in 16:0 and 17:0, whereas the total omega-6 content was lower, 14.85% ± 1.67 vs. 17.81% ± 1.42, with 18:2c9, 12 (n-6) being the main component. Although not statistically significant in all aspects, the fatty acid composition of the fish with pansteatitis scores 2 and 3 resembled that of score 4 and may have been a reflection of the uniform prolonged exposure of the fish to oxidized fish waste in the diet. These results are in agreement with Farwer *et al.* (1994) who found that pansteatitis in rats, which was induced by a depletion of vitamin E and a diet high in unsaturated fatty acids, was characterized by lower levels of saturated fatty acids and higher levels of unsaturated fats in the liver and serum. However, when pansteatitis was induced in cats due to a diet high in unsaturated fatty acids, a depletion of vitamin E resulted, and an increase in saturated fatty acids and a decrease in unsaturated fatty acids in the subcutaneous adipose tissue was noted (Fytianou *et al.* 2006). It therefore appears that the changes in tissue fat due to pansteatitis may vary amongst species.

Table 2 Analysis of variance (ANOVA) on the effect of anatomical position (mesenteric vs. pectoral) of fat with a steatitis score of 1 on chemical composition, fatty acid composition and fatty acid ratios of fish from locality LK

Steatitis score		Mesenteric ($n = 3$)	Pectoral ($n = 21$)	Significance level
Proximate analysis (%)				
% Fat		78.46 ± 6.92	69.55 ± 4.19	$P = 0.004$
% Fat-free dry matter		5.53 ± 4.14	8.59 ± 1.57	$P = 0.018$
% Moisture		16.01 ± 3.21	21.86 ± 4.44	$P = 0.040$
FAME (% of total fatty acids)				
Common name	Abbreviation			
Myristic	C14:0	3.41 ± 0.59	5.13 ± 0.71	$P < 0.001$
Pentadecylic	C15:0	0.43 ± 0.12	0.50 ± 0.13	$P = 0.372$
Palmitic	C16:0	27.05 ± 3.49	23.75 ± 2.41	$P = 0.046$
Palmitoleic	C16:1c9	8.17 ± 0.64	9.35 ± 0.56	$P = 0.003$
Margaric	C17:0	0.40 ± 0.12	0.32 ± 0.07	$P = 0.133$
Heptadecenoic	C17:1c10	0.38 ± 0.10	0.53 ± 0.22	$P = 0.255$
Stearic acid	C18:0	4.33 ± 0.48	3.36 ± 0.36	$P < 0.001$
Elaidic	C18:1t9	0.31 ± 0.13	0.39 ± 0.14	$P = 0.362$
Oleic	C18:1c9	23.71 ± 0.48	20.92 ± 0.96	$P < 0.001$
Vaccenic	C18:1c7	4.25 ± 0.27	4.14 ± 0.15	$P = 0.319$
Linoleic	C18:2c9,12 (n-6)	13.14 ± 1.80	15.12 ± 1.44	$P = 0.041$
Arachidic	C20:0	0.18 ± 0.02	0.15 ± 0.02	$P = 0.018$
γ-Linolenic	C18:3c6,9,12 (n-6)	0.35 ± 0.11	0.33 ± 0.10	$P = 0.708$
α-Linolenic	C18:3c9,12,15 (n-3)	1.75 ± 0.68	1.45 ± 0.33	$P = 0.197$
Heneicosanoic	C21:0	0.49 ± 0.11	0.73 ± 0.28	$P = 0.163$
Eicosadienoic	C20:2c11,14 (n-6)	0.77 ± 0.03	0.70 ± 0.12	$P = 0.310$
Behenic	C22:0	0.08 ± 0.01	0.01 ± 0.02	$P < 0.001$
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.47 ± 0.10	0.38 ± 0.06	$P = 0.023$
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.05 ± 0.02	0.06 ± 0.04	$P = 0.535$
Arachidonic	C20:4c5,8,11,14 (n-6)	0.54 ± 0.02	0.66 ± 0.07	$P = 0.011$
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	2.12 ± 1.29	4.02 ± 0.79	$P = 0.001$
Nervonic	C24:1c15	0.11 ± 0.02	0.02 ± 0.03	$P < 0.001$
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.81 ± 0.22	1.64 ± 0.26	$P = 0.284$
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.70 ± 1.39	6.35 ± 1.44	$P = 0.471$
Fatty acid ratios				
Total Saturated Fatty Acids (SFA)		36.37 ± 3.89	33.95 ± 3.27	$P = 0.253$
Total Mono-Unsaturated Fatty Acids (MUFA)		36.93 ± 0.50	35.35 ± 1.04	$P = 0.018$
Total Polyunsaturated Fatty Acids (PUFA)		26.70 ± 3.80	30.70 ± 3.14	$P = 0.056$
Total Omega- 6 fatty acids (n-6)		14.85 ± 1.67	16.86 ± 1.36	$P = 0.028$
Total Omega- 3 fatty acids (n-3)		11.86 ± 2.13	13.83 ± 2.30	$P = 0.175$

Means with different superscripts in the same row differ significantly.

No difference in fat and moisture content was observed between the adipose tissues of healthy and pansteatitis-affected fish from LK. The data differ from results obtained from pansteatitis-affected crocodiles, in which fat tissue was found to have a higher moisture content and contain lower levels of 18:0 and 18:2c9, 12(n-6) than fat tissue of healthy crocodiles (Osthoff *et al.* 2010). High moisture content of pansteatitis-affected fat in crocodiles was thought to be associated with the extensive inflammatory reaction in the adipose tissues accompanying advanced pansteatitis. Although the mesenteric adipose tissue of pansteatitis-affected LK fish took on a rubbery consistency, an equivalent degree of inflammation and hardening of the adipose tissues to that observed in crocodiles was not present. According to Farwer *et al.* (1994), Scott *et al.* (1995) and Fytianou *et al.* (2006) a decrease in unsaturated fatty acids may be due to oxidation, either by ingestion of oxidants or low levels of dietary vitamin E. In the case of the LK fish, the natural antioxidant capability of the catfish may have been overwhelmed by the excessively high unsaturated fat content in the trout slaughterhouse waste on which these fish had been fed over long periods of time.

Due to limited representatives of all pansteatitis scores of fish from OG, the data of scores 1 and 2 were pooled in one score group, whilst 3 and 4 were grouped together, and the fat composition of these are shown in Table 4. Similarly to the finding in crocodiles (Osthoff *et al.* 2010), the moisture content of fat in the higher pansteatitis score fish from OG appeared to be higher than that observed in fat with the lower score, 20.87% ± 14.49 vs. 7.33% ± 2.74.

Table 3 Analysis of variance (ANOVA) on the effect of steatitis score of mesenteric fat on chemical composition, fatty acid composition and fatty acid ratios of fish from locality LK

Steatitis score		1 (*n* = 3)	2 (*n* = 5)	3 (*n* = 3)	4 (*n* = 10)	Significance level
Proximate analysis (%)						
% Fat		78.46 ± 6.92	85.79 ± 2.87	83.70 ± 0.64	81.20 ± 4.80	*P* = 0.142
% Fat-free dry matter		5.53 ± 4.14	4.07 ± 1.09	3.42 ± 1.48	5.03 ± 1.87	*P* = 0.537
% Moisture		16.01 ± 3.21	10.13 ± 3.18	12.88 ± 1.23	13.77 ± 3.71	*P* = 0.122
FAME (% of total fatty acids)						
Common name	Abbreviation					
Myristic	C14:0	3.41 ± 0.59	3.63 ± 0.41	3.26 ± 0.13	3.68 ± 0.53	*P* = 0.546
Pentadecylic	C15:0	0.43 ± 0.12	0.35 ± 0.04	0.31 ± 0.02	0.34 ± 0.05	*P* = 0.077
Palmitic	C16:0	27.05 ± 3.49^{b}	23.75 ± 1.20^{a}	23.66 ± 1.02^{a}	23.57 ± 0.70^{a}	*P* = 0.015
Palmitoleic	C16:1c9	8.17 ± 0.64	8.54 ± 0.38	8.37 ± 0.35	8.65 ± 0.61	*P* = 0.579
Margaric	C17:0	0.40 ± 0.1^{b}	0.31 ± 0.04^{ab}	0.30 ± 0.02^{ab}	0.29 ± 0.02^{a}	*P* = 0.031
Heptadecenoic	C17:1c10	0.38 ± 0.10	0.51 ± 0.06	0.48 ± 0.03	0.47 ± 0.20	*P* = 0.729
Stearic acid	C18:0	4.33 ± 0.48	4.04 ± 0.28	4.17 ± 0.47	3.81 ± 0.51	*P* = 0.340
Elaidic	C18:1t9	0.31 ± 0.13	0.39 ± 0.11	0.31 ± 0.07	0.37 ± 0.15	*P* = 0.750
Oleic	C18:1c9	23.71 ± 0.48	24.28 ± 0.89	24.13 ± 0.69	23.53 ± 1.73	*P* = 0.753
Vaccenic	C18:1c7	4.25 ± 0.27	4.04 ± 0.34	3.99 ± 0.37	3.93 ± 0.21	*P* = 0.406
Linoleic	C18:2c9,12 (n-6)	13.14 ± 1.80^{a}	16.66 ± 0.91^{b}	15.66 ± 0.49^{ab}	16.32 ± 1.44^{b}	*P* = 0.008
Arachidic	C20:0	0.18 ± 0.02	0.15 ± 0.03	0.16 ± 0.04	0.15 ± 0.02	*P* = 0.208
γ-Linolenic	C18:3c6,9,12 (n-6)	0.35 ± 0.11	0.32 ± 0.07	0.30 ± 0.09	0.30 ± 0.04	*P* = 0.650
α-Linolenic	C18:3c9,12,15 (n-3)	1.75 ± 0.68	0.77 ± 0.24	1.14 ± 0.86	1.19 ± 0.59	*P* = 0.183
Heneicosanoic	C21:0	0.49 ± 0.11	0.64 ± 0.10	0.57 ± 0.07	0.59 ± 0.25	*P* = 0.775
Eicosadienoic	C20:2c11,14 (n-6)	0.77 ± 0.03	0.73 ± 0.05	0.75 ± 0.06	0.69 ± 0.06	*P* = 0.087
Behenic	C22:0	0.08 ± 0.01^{b}	0.04 ± 0.02^{a}	0.06 ± 0.01^{ab}	0.05 ± 0.01^{ab}	*P* = 0.022
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.47 ± 0.10	0.40 ± 0.07	0.43 ± 0.06	0.38 ± 0.04	*P* = 0.097
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.05 ± 0.02	0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.02	*P* = 0.224
Arachidonic	C20:4c5,8,11,14 (n-6)	0.54 ± 0.02	0.48 ± 0.06	0.47 ± 0.05	0.48 ± 0.04	*P* = 0.134
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	2.12 ± 1.29	2.70 ± 0.46	2.67 ± 0.16	2.90 ± 0.54	*P* = 0.349
Nervonic	C24:1c15	0.11 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	0.11 ± 0.03	*P* = 0.461
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.81 ± 0.22	1.58 ± 0.19	1.90 ± 0.18	1.71 ± 0.33	*P* = 0.414
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.70 ± 1.39	5.60 ± 0.59	6.81 ± 0.90	6.46 ± 1.42	*P* = 0.424
Fatty acid ratios						
Total Saturated Fatty Acids (SFA)		36.37 ± 3.89^{b}	32.90 ± 1.20^{ab}	32.47 ± 1.60^{ab}	32.49 ± 1.05^{a}	*P* = 0.023
Total Mono-Unsaturated Fatty Acids (MUFA)		36.93 ± 0.50	37.85 ± 0.98	37.39 ± 1.13	37.06 ± 1.37	*P* = 0.628
Total Polyunsaturated Fatty Acids (PUFA)		26.70 ± 3.80	29.26 ± 1.86	30.15 ± 2.34	30.45 ± 1.99	*P* = 0.131
Total Omega- 6 fatty acids (n-6)		14.85 ± 1.67^{a}	18.21 ± 0.86^{b}	17.21 ± 0.51^{ab}	17.81 ± 1.42^{b}	*P* = 0.010
Total Omega- 3 fatty acids (n-3)		11.86 ± 2.13	11.04 ± 1.11	12.94 ± 1.84	12.64 ± 1.65	*P* = 0.299

Means with different superscripts in the same row differ significantly. Steatitis score: 1 = 0–25%; 2 = 25–50%; 3 50–75%; 4 = 75–100%.

The fat content of fish from OG was found to be lower in fish with higher pansteatitis score than in those with lower score, 69.04% ± 18.49 vs. 85.71% ± 2.86, however, only at a significance value of $P = 0.026$. Relative to pansteatitis severity, the greatest differences in fatty acid composition of OG fish were noted amongst the respective levels of 22:5c7, 10, 13, 16, 19(n-3), 22:6c4, 7, 10, 13, 16, 19(n-3) and 18:2c9, 12(n-6). The omega-3 polyunsaturated fatty acids made up 9.28% ± 2.70 of total fatty acids in the lower pansteatitis score group compared with 16.79% ± 5.51 ($P = 0.009$) in the higher pansteatitis score group. The opposite trend was observed for the omega-6 acids, with 10.4% ± 2.75 and 5.85% ± 1.08 ($P = 0.017$) in the low and high pansteatitis groups, respectively. A significant difference between the two groups was also noted in the content of 18:0 with 6.95% ± 0.67 vs. 4.06% ± 0.84, respectively. The lower fat and higher moisture content of the adipose tissue and the lower 18:0 and 18:2c9, 12(n-6) levels in the catfish with the high pansteatitis score is similar to that observed for the Nile crocodiles from the same waters reported by Osthoff *et al.* (2010). The increased firmness of the fat tissue observed in crocodiles (Osthoff *et al.* 2010) and catfish with advanced pansteatitis appears to be unrelated to fatty acid composition and is more likely a reflection of physiological changes associated with interstitial inflammation, as has also been observed in cats with pansteatitis (Niza *et al.* 2003). In catfish, however, the hardening of affected fat tissue was not as severe as that observed in crocodiles.

Table 4 Analysis of variance (ANOVA) on the effect of steatitis score of mesenteric fat on chemical composition, fatty acid composition and fatty acid ratios of fish from locality OG

Steatitis score		1 + 2 (*n* = 8)	3 + 4 (*n* = 4)	Significance level
Proximate analysis (%)				
% Fat		85.71 ± 2.86	69.04 ± 18.49	*P* = 0.026
% Fat-free dry matter		6.96 ± 0.80	10.09 ± 4.23	*P* = 0.060
% Moisture		7.33 ± 2.74	20.87 ± 14.49	*P* = 0.023
FAME (% of total fatty acids)				
Common name	Abbreviation			
Myristic	C14:0	2.44 ± 0.50	2.04 ± 0.88	*P* = 0.338
Pentadecylic	C15:0	0.44 ± 0.08	0.36 ± 0.02	*P* = 0.085
Palmitic	C16:0	28.5 ± 83.89	30.67 ± 2.32	*P* = 0.350
Palmitoleic	C16:1c9	5.87 ± 0.52	7.43 ± 1.55	*P* = 0.024
Margaric	C17:0	0.63 ± 0.09	0.61 ± 0.17	*P* = 0.846
Heptadecenoic	C17:1c10	0.07 ± 0.02	0.15 ± 0.07	*P* = 0.018
Stearic acid	C18:0	6.95 ± 0.67	4.06 ± 0.84	*P* < 0.001
Elaidic	C18:1t9	0.03 ± 0.01	0.06 ± 0.04	*P* = 0.133
Oleic	C18:1c9	31.18 ± 4.32	26.48 ± 4.33	*P* = 0.106
Vaccenic	C18:1c7	3.45 ± 0.12	3.32 ± 1.05	*P* = 0.725
Linoleic	C18:2c9,12 (n-6)	8.29 ± 2.79	4.42 ± 1.05	*P* = 0.025
Arachidic	C20:0	0.26 ± 0.05	0.32 ± 0.09	*P* = 0.137
γ-Linolenic	C18:3c6,9,12 (n-6)	0.19 ± 0.07	0.32 ± 0.07	*P* = 0.013
α-Linolenic	C18:3c9,12,15 (n-3)	1.13 ± 0.27	1.10 ± 0.25	*P* = 0.853
Heneicosanoic	C21:0	0.68 ± 0.52	1.70 ± 0.75	*P* = 0.020
Eicosadienoic	C20:2c11,14 (n-6)	0.42 ± 0.12	0.26 ± 0.07	*P* = 0.037
Behenic	C22:0	0.10 ± 0.03	0.14 ± 0.10	*P* = 0.321
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.44 ± 0.07	0.41 ± 0.16	*P* = 0.616
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.14 ± 0.02	0.23 ± 0.08	*P* = 0.010
Arachidonic	C20:4c5,8,11,14 (n-6)	1.00 ± 0.17	0.62 ± 0.03	*P* = 0.002
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	0.72 ± 0.23	0.79 ± 0.26	*P* = 0.655
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.90 ± 0.76	3.44 ± 1.35	*P* = 0.028
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.09 ± 2.27	11.06 ± 4.47	*P* = 0.010
Fatty acid ratios				
Total Saturated Fatty Acids (SFA)		40.08 ± 3.98	39.92 ± 3.13	*P* = 0.946
Total Mono-Unsaturated Fatty Acids (MUFA)		40.60 ± 3.84	37.44 ± 6.29	*P* = 0.298
Total Polyunsaturated Fatty Acids (PUFA)		19.32 ± 0.83	22.65 ± 5.52	*P* = 0.111
Total Omega- 6 fatty acids (n-6)		10.04 ± 2.75	5.85 ± 1.08	*P* = 0.017
Total Omega- 3 fatty acids (n-3)		9.28 ± 2.70	16.79 ± 5.51	*P* = 0.009

Means with different superscripts in the same row differ significantly. Steatitis score: 1 + 2 = 0–50%; 3 + 4 = 50–100%.

The sharptooth catfish is a benthic opportunistic scavenger that is also known to hunt and prey actively on other fish (Skelton 2001). Food source varied distinctly between sampling sites, and prevalence of fish in the diet correlated with the presence of pansteatitis in free-living catfish from OG. Fish remnants observed in the stomach content of catfish from OG, often in an advanced stage of digestion, frequently consisted of bones and scales of noticeably large unidentified fish. Factors associated with a fish diet appeared to be associated with the development of pansteatitis in catfish in OG, but these must be distinct from a natural healthy fish diet as documented elsewhere in the literature (Spataru, Viveen & Gophen 1987).

An increase in dietary polyunsaturated fat intake has been reported to result in pansteatitis in various animals. A change in diet from smelt (6.7% fat) to mackerel (29.9% fat) was thought to be the precipitating cause of pansteatitis in captive American alligators (Wallach & Hoessle 1968), whereas a change from Baltic and Mediterranean clupeids to Moroccan Atlantic pilchards was suspected to have been the cause of pansteatitis in northern bluefin tuna (Roberts & Agius 2008). Salmonid diets high in fish oils were found to induce steatitis in channel catfish (Goodwin 2006), and in cats, feeding of an oil-rich fish-based diet similarly induced pansteatitis (Fytianou *et al.* 2006). As a consequence of raising the dam wall of Lake Massingir, a habitat change in the OG occurred that may have favoured a change in access to certain species of fish not normally consumed in large numbers by crocodiles and catfish. This may have exposed these animals to levels of polyunsaturated fatty acids in the diet to which they were not adapted. Tiger fish, *Hydrocynus vittatus* Castelnau, sampled from the OG did not to suffer from pansteatitis (D. Huchzermeyer, unpublished data). As obligate piscivores, tiger fish may have developed antioxidant protective mechanisms better enabling them to cope with the consumption of higher levels of dietary polyunsaturated fats than the omnivorous catfish.

The n-6 and n-3 fatty acids derived from linoleic and α-linolenic acids, respectively, are essential fatty acids that cannot be synthesized by animals (Steffens 1997). The relative abundance of these fatty acids in the diet of animals is reflected in the composition of their fat tissues (Hoffman & Prinsloo 1995; Steffens 1997). The fatty acid composition of marine fish oils, and in particular the high n-3 to n-6 ratio of polyunsaturated fatty acids contained in these oils, is a reflection of the fatty acid composition of marine phytoplankton (Steffens 1997). Whereas the ratio of total n-3 to n-6 fatty acids in marine fish oils typically lies between 5 and more than 10 that of freshwater fish is much lower ranging from 1 to 4 (Steffens 1997). In freshwater fish, as in marine fish, these fatty acid ratios are influenced by the composition of the diet. In nutrition trials, the n-3 to n-6 fatty acid ratio in muscle lipid of sharptooth catfish could be manipulated from 0.1 in fish on a sunflower oil diet to 1.8 in fish on a cod liver oil diet (Hoffman & Prinsloo 1995). The fat of captive-farmed crocodiles, receiving a diet of chicken, beef and horse meat, had an n-3 to n-6 fatty acid ratio of 0.08 (Osthoff *et al.* 2010). By contrast, the n-3 to n-6 ratio of fatty acids in the fat of wild crocodiles suffering from pansteatitis from the Olifants and lower Letaba Rivers was found to be 2 (Osthoff *et al.* 2010). This reflected a much higher intake of n-3 fatty acids by crocodiles in the Olifants Gorge. Mean ratios of n-3 to n-6 fatty acids in catfish with mild or no pansteatitis sampled from LK, RV and the OG in November 2009 were 0.8, 1.32 and 0.96, respectively (Table 1). There appeared to be no significant difference in n-3 to n-6 ratio of mesenteric fat between fish from Lunsklip Fisheries with varying degree of severity of pansteatitis. The fish with severe pansteatitis sampled from the OG, however, had an n-3 to n-6 fatty acid ratio of 2.87 compared to 0.92 in fish with only mild or no pansteatitis (Table 4). From these results, it can be inferred that pansteatitis in OG fish was caused by high intake of polyunsaturated fatty acids whereas rancidity rather than high polyunsaturated fatty acid intake was the cause of the pansteatitis observed in catfish from LK. In the light of absence of observed fish mortality in the OG, it would seem unlikely that rancidity associated with intake of dead rotting fish could have been the cause of pansteatitis in the OG catfish and crocodiles.

A significant proportion of the essential fatty acids derived from the diet are stored in the adipose tissues of animals and of these, docosahexaenoic acid 22:6n-3 (DHA) is deposited into the adipose tissues preferentially over eicosapentaenoic acid 20:5n-3 (EPA) (Lin & Connor 1990). Although the polyunsaturated fatty acids are mobilized more rapidly from the adipose tissues than saturated fats, DHA, the most polyunsaturated fatty acid, has been shown to be poorly mobilized (Connor, Lin & Colvis 1996). The higher levels of DHA found in the mesenteric fat of catfish from the Olifants Gorge with pansteatitis (11.06%) compared with mesenteric fat of those without pansteatitis (5.09%) strongly points to a higher intake of DHA in the diet of those fish that developed pansteatitis at this site. A similar differentiation was not observed in the mesenteric fat of catfish with mild and severe pansteatitis from LK, supporting the argument for a different dietary aetiology, most likely associated with rancidity of fats in the slaughter house waste fed to these fish.

The inlet of Lake Massingir, which prior to 2007 lay in Mozambique beyond the OG, now extends into the OG in the KNP within the boundaries of South Africa, flooding the gorge where this river previously traversed the Lebombo Mountains as fast flowing rapids. Phytoplankton blooms have been observed near the inlet to Lake Massingir (D. Pienaar, SANParks, Skukuza, personal communication 2009). Phytoplankton naturally contain large quantities of α-linolenic acid and other n-3 polyunsaturated fatty acids, in particular EPA and DHA (Steffens 1997). It is proposed that by raising the dam of Lake Massingir, the resulting habitat change that occurred in the OG may have seasonally favoured access by crocodiles and catfish to phytoplankton-feeding fish. Of concern in this respect are silver carp, *Hypophthalmichthys molitrix* (Valenciennes), an invasive species outside of East Asia (Kolar *et al.* 2005) that were introduced into Mozambique and have also escaped into the Olifants River from South Africa and are known to occur in Lake Massingir (Skelton 2001). This fish is a specialized plankton feeder that by preference feeds off phytoplankton (Kolar *et al.* 2005) and is known to assimilate n-3 fatty acids (Buchtová & Jezek 2011). It is possible that crocodiles and catfish feed on silver carp when these seasonally migrate from the still waters of the lake into the Olifants River to spawn, and this may provide one explanation for intense intake of excessive polyunsaturated fats by catfish and crocodiles.

Conclusions

The results presented describe and compare the fatty acid composition and pathology found in the adipose tissues of healthy and pansteatitis-affected captive and wild sharptooth catfish. Data indicate possibly differing causes of the pansteatitis observed in the wild and captive fish. A classical nutritional cause, overfeeding of rancid fish waste, adequately explains the pansteatitis found in the captive population of LK fish. Observed fish kills have not been a consistent feature of the Olifants River Gorge, and the results thus strengthen the argument that causes other than consumption of dead fish may be involved in inciting pansteatitis in the OG fish. Similarities in changes in the adipose tissues of catfish and crocodiles inhabiting the same waters were observed. In the OG, the higher n-3 to n-6 fatty acid ratios in the fat of both catfish and crocodiles suffering from pansteatitis, compared with those of healthy catfish and crocodiles, point to an increased intake of polyunsaturated fats as a cause of the observed pansteatitis. The presence of alien invasive, phytoplankton-feeding, silver carp in Lake Massingir and the short seasonal upstream spawning migration of this species through the OG provide one plausible explanation for intense seasonal dietary exposure of catfish and crocodiles to levels of polyunsaturated fats to which they are not adapted. It is proposed that the habitat changes brought about by raising the dam wall of Lake Massingir in 2007 may have improved access of catfish and crocodiles to such fish within the OG, thereby precipitating the pansteatitis outbreaks in these animals.

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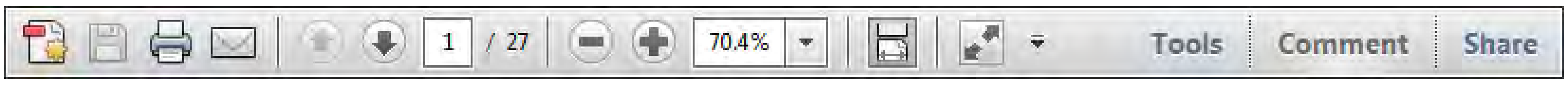

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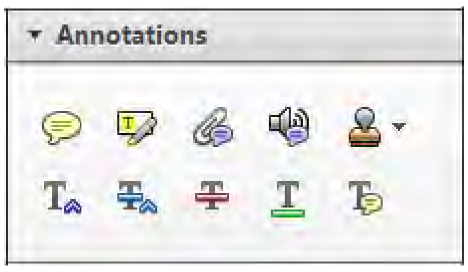

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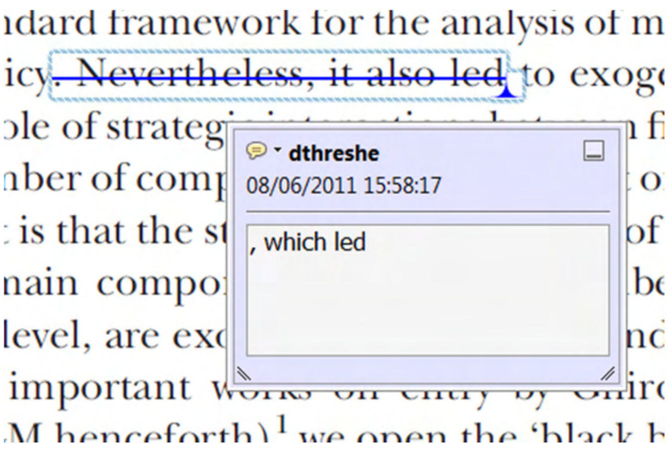

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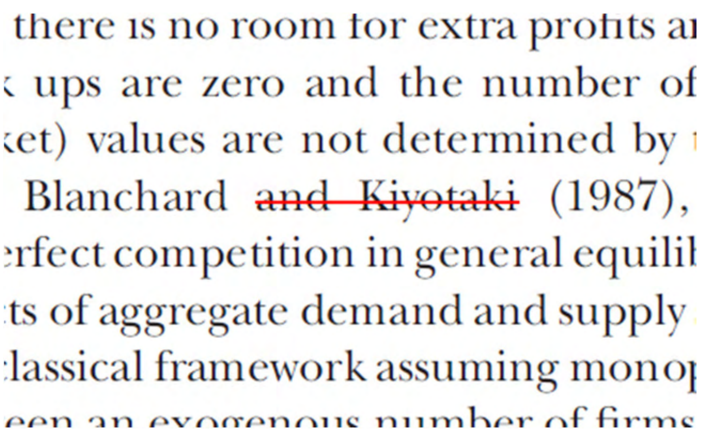

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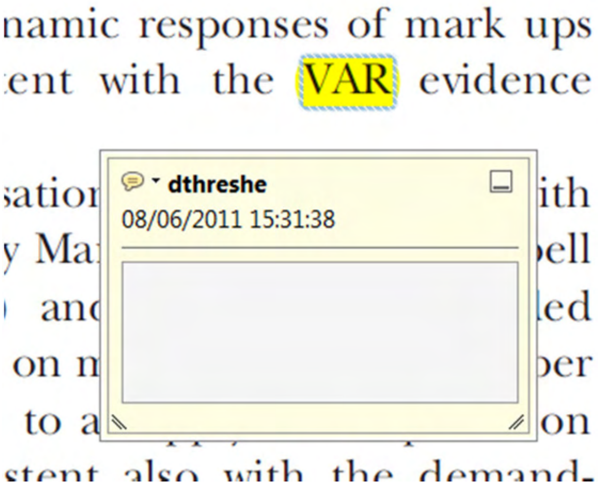

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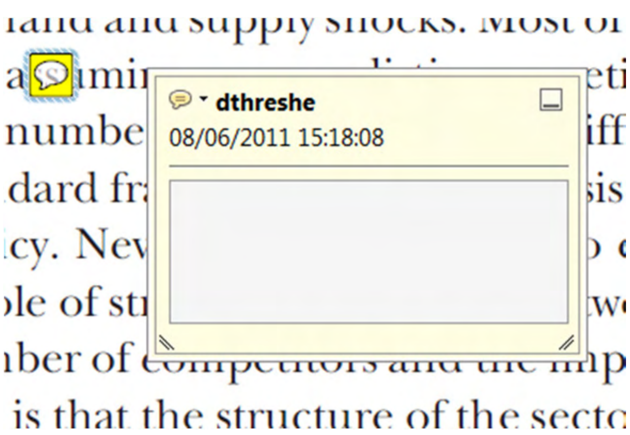

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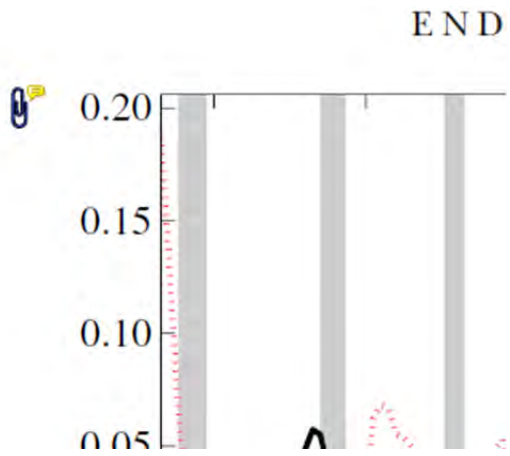

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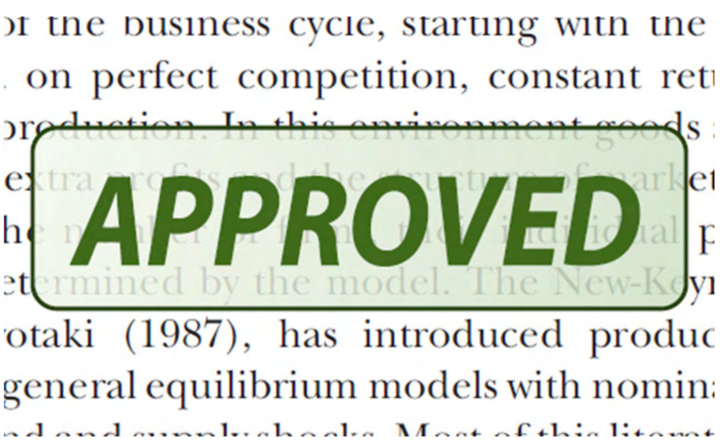

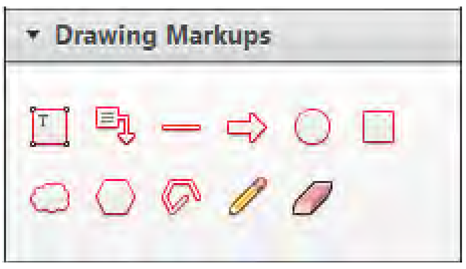

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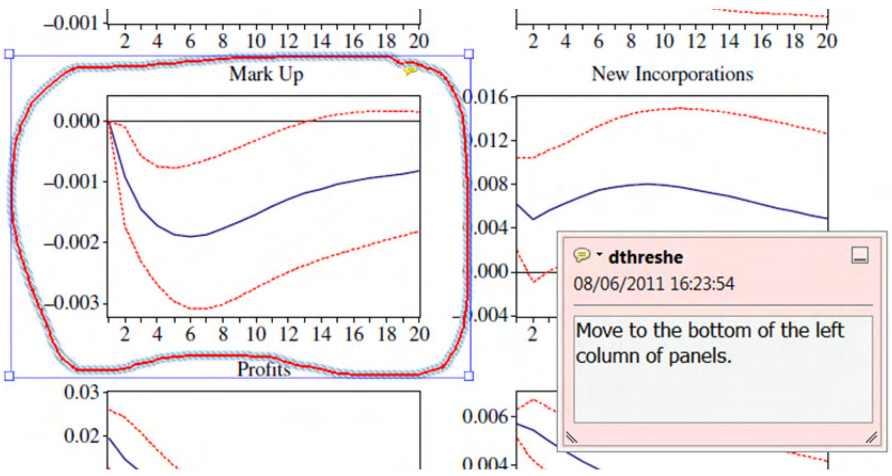

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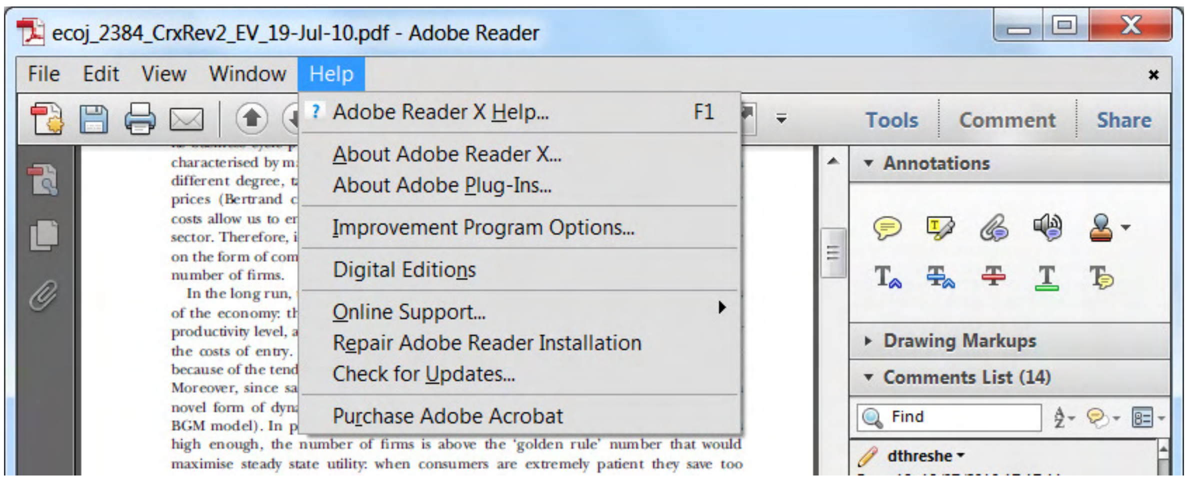

A PRELIMINARY STUDY TO IDENTIFY PATHOLOGY PRESENT IN FISH IN THE LOWER OLIFANTS RIVER FOLLOWING A LARGE CROCODILE MORTALITY EVENT

Report to the
Water Research Commission

by

KDA Huchzermeyer
Department of Paraclinical Sciences
Faculty of Veterinary Science
University of Pretoria

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AUGUST 2012

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EXECUTIVE SUMMARY

Pansteatitis is a nutritional disease that follows on consumption of large amounts of polyunsaturated fats. The reduction in tissue vitamin E levels associated with such a diet is exacerbated where dietary fats have become rancid. In the Kruger National Park (KNP), pansteatitisin fish and crocodiles has been shown to be a serious and increasing problem in large man-made lakesfed by rivers arising in polluted catchments.The objective of this study was to identify the range of pathologies present in fish in the lower Olifants and Letaba rivers within the KNP, to determine the significance of these pathologies in terms of pollution and the development of pansteatitis in crocodiles, to differentiate such pathologies from non-pollution related pathology as would be expected in free-living fish in these rivers and to identify improved sacrificial and non-sacrificial methods of monitoring the fish health in KNP rivers.

During the period June 2009 to June 2011, 285 sharptooth catfish(*Clarias gariepinus*, Burchell) specimens were examined during 17 sampling episodes from various localities within and outside of KNP. Tiger fish were sampled from the Olifants Gorge during June 2011. Fish were subjected to detailed autopsies and subsequent histological examination of the organs.Blood samples were collected from all sampled fish. Detailed data sheets were compiled on all macroscopic findings during field data collection and on subsequent histological and laboratory findings.

Significant pathology in catfish was limited to changes associated with necrosis of fat and the resultant inflammatory reaction in the adipose tissues (pansteatitis) of the fish. Pathology secondary to fat necrosis was also identified in certain other organs. These pathologies were differentiated from pathology associated with parasitosis. Pathology indicative of pollution related aetiology could, however, not be demonstrated.An increasing prevalence of fat necrosis and steatitis was recorded in catfishsampled from the Olifants River gorge in the period 2009 to 2011, and over 60% of catfish collected during the most recent sampling from the Olifants Gorge were affected with steatitis.A decline in amount of fat stored by catfish was noted with repeat samplings from the Olifants Gorge between November 2009 and June 2011. Steatitis wasalso confirmed in catfish collected from the Sabie River in the Sabiepoort at a similar prevalence to that found in the Olifants Gorge. Steatitis was not detected in tiger fish sampled from the Olifants Gorge. Only a low prevalence of steatitis was detected in catfish sampled upstream of the Olifants Gorge within the KNP. No steatitis was observed in catfish sampled from a rain-filled dam unconnected to the Olifants River. Catfish sampled from a dam at a phosphate mine in Phalaborwa showed no sign of steatitis. A high prevalence of nutritionally induced pansteatitis was, however, identified in a farmed population of catfish. This provided the study with valuable comparative pathology. Analysis of stomach content of catfish from the various sampling sites in KNP indicated a higher prevalence of fish in the diet of catfish from the Olifants Gorge and Sabiepoort than in catfish from sites where no steatitis was detected.

With the selected haematological and biochemical parameters it was not possible to differentiate between fish with and without steatitis, reflecting the chronic nature of the condition and possible intermittent exposure to oxidative stress. Whereas haematocrit values proved to be unreliable in detecting presence of oxidative stress in these fish, variation in haemoglobin values between sites pointed to increased erythrocyte turnover,suggestive of oxidative stress in fish from the Olifants Gorge.Determination of serum vitamin E and erythrocyte glutathione peroxidase values, both commercially available tests in South Africa, appeared to indicate that catfish in both the Olifants and lower Letaba rivers within KNP had been exposed to an oxidative stress challenge preceding a sampling during July 2010. These tests require further validation under field conditions before they can be used for non-sacrificial monitoring of catfish in the KNP.

The hypothesis that pansteatitis occurs in sharptooth catfish in the Olifants Gorge has been proven from the results of this project and the findings have been published in the Journal of Fish Diseases.Tissue samples have been stored for toxicological examination from all collected fish. Toxicological analyses fall outside the scope of this contract. Further tissue samples have been collected for co-workers investigating other aspects not covered by this contract. These include analyses of adipose tissue fatty acids and stable isotopes,andmanuscripts have been submitted for publication on these topics.

In the absence of observed fish mortality, periodic exposure to an overabundance of certain fish species in the diet of crocodiles and catfish in the Olifants Gorge may have occurred following the raising of the dam wall of Lake Massingir in 2007. Whereas a contributory role of pollution cannot be ruled out, this study points to the likelihood that the increased abundance of phytoplankton feeding fish species, in particular the alien silver carp (*Hypophthalmichthys molitrix*) in the diet of both catfish and crocodiles in the Olifants and Sabie gorges may have led to the pansteatitis observed in these two species. Silver carp are known to occur in Lake Massingir, but their presence has not been confirmed in Lake Corumana. High phosphate levels measured in the Olifants River within KNP prior to 2004, and trapped in Lake Massingir, would have stimulated phytoplankton growth in this lake. A habitat change in the gorge brought about by raising of the sluices of the lake in 2007, and the consequent extension of the lake into the KNP, may have provided both catfish and crocodiles with an excessive intake of silver carp during the short period during peak summer flow when this species migrates into the Olifants River to spawn.The analysis of heavy metals in the fish tissues collected during the course of this study has not been completed. The possibility of bioaccumulation of iron following the consumption of phytoplankton, (as documented in Lake Loskop by Oberholster et al. (2011)), still needs to be investigated in the fish of Lake Massingir and the Olifants River Gorge.

The results of the study emphasize the ecological importance and complexity of oxidative stress in a disturbed aquatic environmentand the risk associated with the presence of alien invasive fish species within our national parks. The results suggest that pollution-derived nutrient enrichment of rivers can have far reaching effects where man-made hydrodynamic change has altered the aquatic habitat. Such information is important to guide conservation

policy and decisions regarding use of water and the safety of fish consumed from such waters. In KNP, sharptooth catfish and crocodiles appear to show similar sensitivity to pansteatitis within their overlapping habitat. Sharptooth catfish are a suitable monitoring species for the condition and can be used by KNP to monitor pansteatitis in crocodiles. It is recommended that the distribution of alien fish species within rivers traversing the KNP is investigated further and that in dams within KNP and elsewhere in South Africa the effects of hydrodynamic change and nutrient entrapment on the aquatic food chain are monitored with particular reference to the health of top aquatic predators such as crocodiles.The study provides South Africa and its authorities with information to insist that environmental controls ensuring the quality of water in our rivers are implemented and provides SANParkswith information to insist on prevention of pollution and alien fish species entering KNP, thereby ensuring the biodiversity of the KNP rivers and securing the future of the Nile crocodile in the KNP.

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1 HISTORY AND BACKGROUND OF STUDY

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1.1 The Crocodile Mortality Events

The Olifants River Gorge and Lower Letaba River, at the confluence with the Olifants River, in the Kruger National Park (KNP) are home to one of the densest populations of large Nile crocodile (*Crocodylus niloticus*) in South Africa. The raising of the Massingir Dam sluices just inside Mozambique in 2007 has flooded many of the rapids and pools in the gorge. Altered hydrodynamics have resulted in the deposition of clay rich sediments within the aquatic habitat of the area. During the autumn and winter of 2008 and 2009, large numbers of adult crocodiles were found dead in this area, coinciding with flooding of the gorge. Some 180 specimens out of a known population of at least 600 were found dead in 2008 alone. Autopsies performed by KNP veterinarians revealed exceptionally fat carcasses with an abnormal hardening of the fat. Histological examination of tissue specimens by Drs Emily Lane, Johan Steyl and Fritz Huchzermeyer confirmed an inflammation of the fat typical of pansteatitis. On 8 July 2009, for the first time, a large fish mortality event was observed within the Olifants Gorge. Affected fish were almost exclusively large *Clarias gariepinus* specimens and were found in the area overlying the clay rich deposits at the point where the gorge widens into the dam. Fish carcasses were observed to be very fat. The fish kill remained localized in space and time and no mortalities were observed in either the Olifants or Letaba rivers up stream of the gorge, and fish in Lake Massingir appeared unaffected.

1.2 Organisational Response to the Crocodile Mortality

In response to the crocodile deaths in 2008, the Consortium for the Restoration of the Olifants Catchment (CROC) was founded as a multidisciplinary initiative. CROC provides the following preamble: “*Crocodile catastrophe – implications for mankind*

- *It is increasingly clear that the crocodile deaths in the Olifants Basin are symptomatic of a serious and growing environmental problem in which a tipping point has been reached / crossed with dramatic unexpected effects*
- *Such a top predator collapse indicates prolonged and cumulative ecosystem stress caused by human activities in which the implementation of our legislated environmental controls and monitoring response proved inadequate.*
- *There are serious implications for human health and well-being if the situation continues and river health is not restored.”*

As a result, a collaborative team effort was initiated, including KNP researchers, various universities, government departments and private sector consultants, to investigate various aspects that may have played a role leading to the development of pansteatitis in the crocodiles. This studywas based on the assumption that pollution-associated pathology in fish in the Olifants River in the KNP preceded the pansteatitis syndrome that caused the deaths of 180 crocodiles during the winter of 2008 and that certain pathological indicators may be used to monitor the situation along the river.

This report presents the results of the pathology data as collected up until 31 July 2011 for the Consultancy Project K8-948 and discusses the causes of the observed pathology.

2 OBJECTIVE

The objective of this project was to identify the range of pathologies present in fish in the lower Olifants and Letaba rivers within the KNP, to determine the significance of these pathologies in terms of pollution and the development of pansteatitis in crocodiles, to differentiate such pathologies from non-pollution related pathology as would be expected in free-living fish in these rivers and to identify improved sacrificial and non-sacrificial methods of monitoring fish health in KNP rivers.

3 LITERATURE REVIEW

The Olifants River is regarded as one of the most threatened aquatic ecosystems in Mpumalanga Province of South Africa (Ashton, 2010; de Villiers and Mkwelo, 2009; Heath et al., 2010). Since large numbers of Nile crocodiles (*Crocodylus niloticus,* Laurenti) died from pansteatitis in the Olifants Gorge during 2008 (Ferreira and Pienaar, 2011), a link has been sought to the consequences of human activity in the catchment of the Olifants and Letaba Rivers. The Olifants River originates on the Highveld plateau of Mpumalanga then flows eastwards down the escarpment and traverses the Kruger National Park (KNP) where it is joined by the Letaba River at the entrance to the Olifants Gorge. The gorge extends through the Lebombo Mountains for approximately 9 km, exiting into Lake Massingir in Mozambique. From here the Olifants River continues through Mozambique before discharging into the Indian Ocean. The Olifants catchment has been heavily impacted by human activity including; mining, coal fired electricity generation, industrial and urban wastewater discharges, agricultural practices and water impoundments (Heath et al., 2010), whereas the Letaba catchment has been impacted by agriculture and human settlements. Lake Massingir sustains a considerable freshwater fishery to which has been introduced an aggressively invasive planktivorus species, the silver carp (*Hypophthalmichthys molitrix,*Valenciennes) (Skelton, 2001).

Within the upper Olifants catchment lies Lake Loskop. Nile crocodiles mortalities in this lake have coincided with periodic mass die-offs of fish since 2003 (Botha et al., 2011). In the KNP, an estimated 180 large crocodiles died in the Olifants River Gorge during the winter of 2008 following the raising of the sluices of Lake Massingir in Mozambique in 2007 (Ferreira and Pienaar, 2011; Huchzermeyer et al., 2011). However no coincidental fish die-off was observed. The number of deaths has declined during the subsequent winters. Crocodile deaths in the Olifants Gorge continue to be restricted to the winter months and as with the 2008 crocodile mortalities, South African National Parks (SANParks) veterinarians established the cause of death as pansteatitis.

Pansteatitis, resulting from peroxidation of body fat and resultant inflammation of the adipose tissues, has been described from many species of both warm and cold blooded animals. The disease is regarded as a nutritionally mediated condition arising from feeding of diets with low vitamin E content particularly where such diets contain high levels of long chain polyunsaturated fatty acids or rancid fish oils (Wallach and Hoessle, 1968, Smith 1979).

As vitamin E protects against lipid peroxidation, tissue vitamin E levels tend to increase with increase in unsaturated fat intake (Raynard et al., 1991). Where vitamin E intake is insufficient to provide adequate protection against the peroxidation of dietary unsaturated or rancid fats, necrosis and inflammation of the fatty tissues ensues giving rise to the clinical picture of pansteatitis. Fats of fish origin, particularly in the absence of sufficient vitamin E, have most commonly been implicated (Goodwin, 2006; Herman and Kircheis, 1985; Murai and Andrews, 1974; Roberts et al., 1979; Roberts and Agius, 2008, Wallach and Hoessle, 1968).

In crocodiles pansteatitis has been associated with consumption of large numbers of dead and rancid fish following large scale fish mortality (Huchzermeyer, 2003) and with a change in type of fish fed (Wallach and Hoessle, 1968). In Lake Loskop, acid mine seepage was found to be the most likely cause of a mass fish mortality that in 2007 lead to the deaths of significant numbers of crocodiles and terrapins as a result of pansteatitis (Myburgh and co-workers, University of Pretoria, pers. comm. 2009). The 2008 episode of crocodile pansteatitis in the KNP differed in that no mass mortality of fish was observed in the affected region. Circumstantial evidence pointed to illegal fishing activity with gill nets as a possible source of dead fish. A link between pollution-induced *in vivo* lipid autoxidation in fish and the subsequent development of pansteatitis in crocodiles has not been elucidatedpreviously.

The pathology of pansteatitis has been reported in the literature for various species of fish (Herman and Kircheis, 1985; Huchzermeyer et al., 2011; Murai and Andrews, 1974; Roberts et al., 1979; Roberts and Agius, 2008). In fish, pansteatitis has been reported as an incidental finding from apparently healthy slaughter fish (Goodwin, 2006). Similar findings of subclinical pansteatitis found at slaughter have been reported from American alligators (*Alligator mississippiensis*) fed over a period of 14 years on predominantly freshwater fish in the form of whole fish, fish heads, skins and entrails (Larsen et al., 1983). Oxidative deterioration of polyunsaturated lipids, leading to lipid peroxidation through the release of free radicals, initiates a sequence of events leading to molecular damage of subcellular membranes and eventually to cell membrane damage (Tappel, 1973). Fish fats are more susceptible to autoxidation than other polyunsaturated fats due to their high content of long-chain polyunsaturated fatty acids, particularly eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid [22:6(n-3)] (Gonzalez et al., 1992).

Dependency on lipoprotein polyunsaturated fats for normal metabolic function is more pronounced in poikilothermic animals (cold blooded) such as fish and crocodiles than in homothermic (warm blooded) animals. The elongated and desaturated derivatives of linoleic acid (n-6) and α-linoleic acid (n-3) are essential fatty acids that affect the fluidity, flexibility and permeability of membranes (Steffens, 1997). Poikilothermic animals such as fish and crocodiles depend on these dietary polyunsaturated fats to maintain membrane fluidity and normal metabolic function especially at colder ambient temperatures. They are thus more sensitive to the effects of lipid autoxidation than warm-blooded animals (Stoskopf, 1993).

There is evidence in the literature linking pro-oxidant or oxyradical production in aquatic organisms to anthropogenic activity resulting in pollution of the aquatic environment (Bainy et al., 1996, Winston and DiGiulio, 1991). Histopathological tissue changes in fish have been proposed as a sensitive tool for assessing such exposure to pollution in the aquatic environment (Adams et al., 1993; Bernet et al., 1999; Heath et al., 2004, Roberts and Agius, 2003). The useful role of fish in sediment toxicity assessments has been reviewed by Halare et al., 2011, who stress the importance of benthic rather than pelagic fish species in such studies. Histology used to monitor health status of *Hydrocynus vittatus*, *Labeobarbus marequensis*, *Labeo rosae* and *L. cylindricus*, fish representing different trophic levels in the Olifants River in the KNP, showed that thesespecies were in a healthy state (Wagenaar et al., 2012a, Wagenaar et al., 2012b).

It is known that vitamin E plays an important role in nature as an *in vivo* antioxidant preventing the oxidative conversion of polyunsaturated fats into lipid hydroperoxides (Burton, 1994; Bus et al., 1976; Hove 1955;Niza et al., 2003; Stoskopf, 1993). Such lipid peroxides are held responsible for the various changes observed in animals deprived of vitamin E (Hove, 1955). Several exogenous substances have been reported in the literature to promote the peroxidation of fats, including consumption of unsaturated fat (Burton, 1994). These reactions can be mitigated by the presence of adequate dietary vitamin E (Stoskopf, 1993). Under conditions of dietary oxidant overload, depletion of hepatic vitamin E has been shown to occur in sharptooth catfish (Baker et al., 1997).

Basic biological processes such as cellular respiration and the action of certain enzymes lead to production of reactive oxygen species within the body. Imbalances in generation and removal of radical species result in the oxidative stress that has the potential to cause biological injury. Certain xenobiotics capable of redox cycling have the ability to enhance the production of such oxyradicals within cells (Kelly et al., 1998). Xenobiotics capable of redox cycling include quinones, some dyes, bipyridyl herbicides, some transition metals and aromatic nitro compounds (Kelly et al., 1998).The herbicide, paraquot, has been used as an oxidant model causing lipid peroxidation of cell membranes in both humans and animals (Åkerman et al., 2002; Bus et al., 1976; Parvez and Raisuddin, 2005). The cyclical reduction-oxidation of paraquot results in generation of superoxide radicals which dismutate to singlet oxygen. Singlet oxygen reacts with unsaturated lipids in cell membranes to form lipid hydroperoxides. The chain reaction leading to the membrane destructive process of lipid peroxidation results from the spontaneous decomposition of lipid hydroperoxides to lipid free radicals (Bus et al., 1976).The toxic manifestations of other xenobiotics have also been ascribed to oxidative damage. Bachowski et al. (1998) observed a reduction in hepatic and serum α-tocopherol level in mice and rats exposed to dietary dieldrin. It has similarly been proposed that the hepatic lipid peroxidation and DNA damage in rats exposed to the polyhalogenated hydrocarbons, lindane, DDT, chlordane and endrin was a result of oxidative tissue damage that may contribute to the toxic manifestations of these xenobiotics (Hassoun et al., 1993).

Mammalian organisms use three defence mechanisms to counter the destructive process caused by oxidative stress. Super oxide dismutase scavenges toxic superoxide radicals, endogenous anti-oxidants such as vitamin E terminate the free radical chain reaction of lipid peroxidation and glutathione peroxidase enzymatically reduces the unstable lipid hydroperoxides to stable lipid alcohols thus preventing further formation of free radicals (Bus et al., 1976). Vitamin E acts as hydrogen atom donor thereby preventing the reactive lipid peroxyl radicals from abstracting hydrogen atoms from *in vivo* sources such as DNA and proteins (Kelly et al., 1998). These defence mechanisms and related biomarkers have been used in mammalian and fish tissues to measure the effects of pro-oxidant exposure (Awad et al., 1994; Parvez and Raisuddin, 2006) and measurement of thiobarbituric acid reactive substances (TBARS) has been used to determine levels of malondialdehyde, one of the end products of lipid peroxidation, in plasma (Hinchcliff and Piercy, 2000).

Vitamin E is the generic name used to describe the four tocopherols and four tocotrienols that make up this group of lipid soluble substances (Burton, 1994). Of these, α-tocopherol is the most biologically active. For reasons of consistency the term vitamin E will be used with reference to α-tocopherol in the further text of this manuscript. Amongst mammals, cats are known to be particularly sensitive to ingestion of polyunsaturated fats. Fytianou et al. (2006) reported measurable changes in the levels of vitamin E and the enzyme glutathione peroxidase in the blood of kittens exposed to experimental diets rich in rancid fats. In rainbow trout, Bell et al. (1985) demonstrated reduced glutathione peroxidase activity with dietary selenium deficiency whereas serum glutathione peroxidase activity appeared independent of vitamin E intake. Tissue and plasma vitamin E has been shown to increase significantly in response to dietary vitamin E intake in sharptooth catfish but was rapidly utilised in tissues challenged by the oxidative stress caused by ingestion of oxidised dietary fats,leading to appearance of vitamin E deficiency signs and increased free radical tissue damage (Baker and Davies, 1997).

4 MATERIALS AND METHOD

4.1 Fieldwork

The focus of the fieldwork has been on the Sharptooth catfish (*C. gariepinus*, Burchell), as this is a possible major food source for crocodiles along the Olifants River gorge and lower Letaba River. Being an omnivorous benthic scavenger it is likely that this species would be preying on other weakened fish species in the system. Samples were collected and processed over a 2 year period between November 2009 and November 2011. Data from fish sampled prior to this period have been included in the study. Collection of fish samples from the Olifants Gorge has taken place every six months, once during mid to late winter (June-September) when river flow had subsided, most of the rainfall related sediment load had been dropped and water temperatures had reached their winter minimum and once during mid-summer (January to April) when river flow and sediment load were high. A minimum of 20 fish were collected during each of the sampling episodes. Fish were caught by baited hook and line and by netting.A range of fish species, including Mozambique tilapia (*Oreochromis mossambicus,* Peters) were included in samplings done prior to November 2009. During the June 2011 sampling in the Olifants Gorge, tiger fish (*Hydrocynus vittatus*, Castelnau) were also sampled from the confluence of the Olifants and Letaba rivers.

4.2 Description of Study Area

Samplings took place from the confluence of the Olifants and Letaba rivers (23°59'21.8"S 31°49'35.6"E), where the Olifants River enters a 9 km long gorge through the Lebombo Mountains, to where it enters Lake Massingir on the Mozambique border (23°57'48"S 31°52'97"E). Catfishspecimens were also collected from various other localities within and outside the KNP. Subject to catch success, up to 20 fish were collected on each sampling occasion. These included a negative reference population in Reënvoël Dam (23°58'37.2"S 31°19'38.4"E) that has its entire catchment within the KNP, and a wild population in Van Ryssen Dam (24°00'13.6"S 31°05'36.9"E) at the FOSKOR phosphate mine in Phalaborwa just west of the KNP. Upstream of the gorge fish were sampled at Mamba Weir (24°03'32"S 31°14'14"E), where the Olifants River enters the western boundary of the KNP. On the Letaba River fish were sampled from Engelhard Dam (23°50'19"S 31°28'28"E). Further south in the KNP samplings took place from the Sabiepoort (25°10'25.41"S 32°02'23.42"E), where the Sabie River enters Lake Corumana on the Mozambique border and from the Crocodile River (25°23'57.1"S 31°57'29.9"E) on the southern boundary of the Park. In the north of the KNP, fish were sampled from the Levuvhu River (22°25'51.0"S 31°18'04.4"E). Catfish were also sampled from a farmed population at Lunsklip Fisheries (25°23'08,9"S 30°15'35"E) near Lydenburg, Mpumalanga.These fish were fed almost exclusively an excess of trout slaughterhouse waste, rich in polyunsaturated fat. Slaughterhouse waste, consisting largely of fat rich innards, was dumped into the catfish pond where it was left to be consumed by the fish. Trout farmed at Lunsklip fisheries were fed a commercial trout ration which was top-dressed with additional marine fish oil.

4.3 Specimen Collection

All fish were kept alive until they could be examined. Depending on sampling episode catfishwere either processed at the sampling site or transported live in fish transport tanks to various laboratory facilities. All fish sampled prior to November 2009 were examined in a field laboratory set up at the confluence of the Olifants and Letabarivers. During November 2009, catfish sampled from the Olifants Gorge and from Reënvoël Dam were transported live to Lydenburg where the author’s autopsy facility was used. Catfish from Lunsklip Fisheries were examined and processed on site at Lunsklip Fisheries. All catfish specimenscollected during the July 2010 sampling, with the exception of those sampled from the Sabiepoort were transported live to Skukuza where the large autopsy facility was used. A limited field laboratory set up in the Sabiepoortwas used to examine fish from this site. Catfish sampled from the Olifants Gorge during January and June 2011 as well as tiger fish sampled during June 2011 were examined at a field laboratory set up near the confluence of the Letaba and Olifants Rivers. Catfish sampled from Reënvoël Dam during January 2011 were sampled at a field laboratory set up at this site. Catfish from van Ryssen Dam were transported live to the field laboratory at Reënvoël Dam. Catfishcollected from the Levuvhu and Crocodile Rivers during June 2011 were transported live to the field laboratory at the confluence of the Olifants and Letaba rivers. Sampling from the Sabiepoort and from Van Ryssen Dam had not been included in the original protocol and as result of time constraints and limited facilities at the respective sampling sites no blood samples or weights of fat tissues were collected from these fish.

Four examination fish were placed into a water bath containing benzocaine hydrochloride as anaesthetic at approximately 30 ppm. Anaesthetised fish were subjected to weight and length measurements, body condition scoring and blood collection. Detailed data sheets were completed for all gross observations and measurements. Blood was collected through a 20 gauge hypodermic needle into a 5 ml syringe from the large vessels just ventral to the vertebral canal in the tail area caudal to the abdominal cavity or from the large blood vessels running through the kidney. Collected blood was directly transferred to both EDTA and serum tubes. EDTA tubes were gently shaken to avoid clotting and were wrapped immediately in aluminium foil to prevent exposure to sunlight. Samples for serum were centrifuged to separate the blood from the serum after clotting had occurred. Fresh blood smears were made from all fish. Fish were then humanely euthanized through an over-dose of benzocaine hydrochloride. The collected fish were examined by autopsy for gross pathological changes. Samples from a range of suitable organs and tissues were fixed in 10% buffered formalin.

The major part of the visceral fat of sharptooth catfish is stored within the mesenteries forming a discrete body towards the caudal portion of the abdominal cavity. A further discrete body of fat originating from the hypodermal fat layer is situated behind the pectoral fin. This fat cushion overlies an extension of the anterior kidney and liver into the hypodermal space, a feature unique to this species. For the purposes of this manuscript these two discrete fat depots will be referred to as mesenteric and pectoral fat respectively. Samples of liver, mesenteric fat, pectoral fat and eyes were collected on ice for toxicological examination. Pectoral and mesenteric fat were collected for determination of fatty acid composition. Otoliths were collected from all specimens for age determinations.

4.4 Laboratory Work

Tissue specimens fixed in 10% formalin were processed using standard histological technique. Paraffin wax sections were cut at 5µm. All specimens were stained with haematoxylin eosin (HE). Selected specimens were stained in addition with Gomoris aldehyde fuchsin (GAF), periodic acid Schiff's (PAS) and Perl's Prussian blue stain. Sections were prepared from the following organs and tissues of all sampled fish: mesenteric fat, pectoral fat, hypodermal and intramuscular fat, brain fat, liver, spleen, kidney, pancreas, heart, gonad, muscle, skin, gills and brain. Blood smears were fixed and stained with a CAM's quick stain (Kyro-Quick stain, Kyron Laboratories).Histological sections and blood smears were examined by standard light microscopy for presence of pathology. Microtome sections of all otoliths were examined under the light microscope. Growth rings were counted to determine the age of the fish. To determine the haematocrit of the fish, capillary tubes were filled and sealed before centrifugation on a field micro-centrifuge. Packed cell volume (PCV) was expressed as percentage of the height of the red cell column compared to the total column height. All changes were recorded in detail and the relevant information added to the fish data sheets.

Based on the use of blood glutathione peroxidase and vitamin E measurements in studies of the acute effects of steatitis in kittens (Fytianou et al, 2006) and dietary vitamin E and selenium deficiency in rainbow trout (Bell et al., 1985), and the ready availability of these tests from commercial laboratories in South Africa, it was decided to include measurement of these blood parameters in this study in an attempt to identify tests suitable for non-lethal monitoring of steatitis in catfish of the KNP.Blood and serum samples were submitted to IDEXX Laboratories for determination of haemoglobin, erythrocyte glutathione peroxidase and serum vitamin E values.

4.5 Statistical Analysis

Data obtained from the blood chemistry and haematological examinations were grouped into two categories: those collected from fish with steatitis (category 1) and those collected from fish where steatitis could not be demonstrated either macroscopically or histologically (category 2). T-tests were used to compare mean haematocrits (PCV), mean serum vitamin E and mean haemoglobin values between steatitis positive and steatitis negative fish sampled from the Olifants Gorge and from Lunsklip Fisheries. Type 1 error levels below 0.05 (5%) were accepted as significant. A second set of data was arranged to compare means of all data for a particular dependent variable between sampling sites without differentiating whether the samples were obtained from fish with or without steatitis. The data were statistically analysed using analysis of variance followed by the post-hoc Tukey HSD Test(Agresti and Franklin, 2007). The non-parametric Kruskall-Wallis test was used as an additional approach to the data analysis (Agresti and Franklin, 2007).For comparison of the percentage of fish with suppressed serum vitamin E values between sites the chi-squared test was used. All statistical analyses were done using Statistica 10 (Statsoft).

5 RESULTS

5.1 Steatitis Prevalence

The most distinctive pathology observed in catfish from the Olifants Gorge was centred in the adipose tissues of the fish. Presence of macroscopic lesions of fat necrosis and associated inflammation of the adipose tissues was used to determine steatitis prevalence in the KNP rivers(Figure 1). A high prevalence of steatitis was repeatedly identified in catfish sampled from the Olifants Gorge between August 2009 and July 2011. Steatitis prevalence similar to that found in fish from the Olifants Gorge was detected in catfish sampled from the Sabiepoort during a single sampling in July 2010. Lesions in the adipose tissues were identical to those observed in fish from the Olifants Gorge and splenomegaly and pancreatic atrophy were similarly observed. Lower steatitis prevalence was observed in fish sampled from Engelhard Dam. However, the severity of steatitis lesions in one fish from this site was comparable to that of severely affected fish from the Olifants Gorge and Lunsklip Fisheries. In catfish sampled from Mamba Weir a low prevalence of steatitis was noted. Macroscopically no steatitis could be identified in fish sampled from Reënvoël Dam during November 2009 and again during a repeat sampling in January 2011.Similarly, steatitis could not be detected in fish sampled van Ryssen Dam (Figure 1). These fish carried exceptionally heavy burdens of *Contracaecum* spp. larvae in the peritoneal cavity. Steatitis could also not be demonstrated incatfishsampled from the Levuvhu and Crocodile rivers. Fish from both sites carried relatively low parasite burdens. Fish from the Levuvhu River carried more fat in their adipose tissues than fish sampled from the Olifants Gorge during the same period whilst fish from the Crocodile River were notably leaner than fish from the Olifants Gorge (Table 2).

Lesions identical to those found in the adipose tissues of catfish from the Olifants Gorge, the Sabiepoort and Engelhard Dam were observed in a captive population of catfishat Lunsklip Fisheries. The majority of these fish had severe visible changes in the fat associated with pansteatitis (Table 1) and provided the study with an identified positive control for evaluation of gross pathology and histology. Although steatitis was observed macroscopically in 66% of fish sampled from Lunsklip Fisheries, 95% of these fish showed steatitis on histological examination. The majority of these fish had very large mesenteric fat reserves (Table 2, Figure 3).

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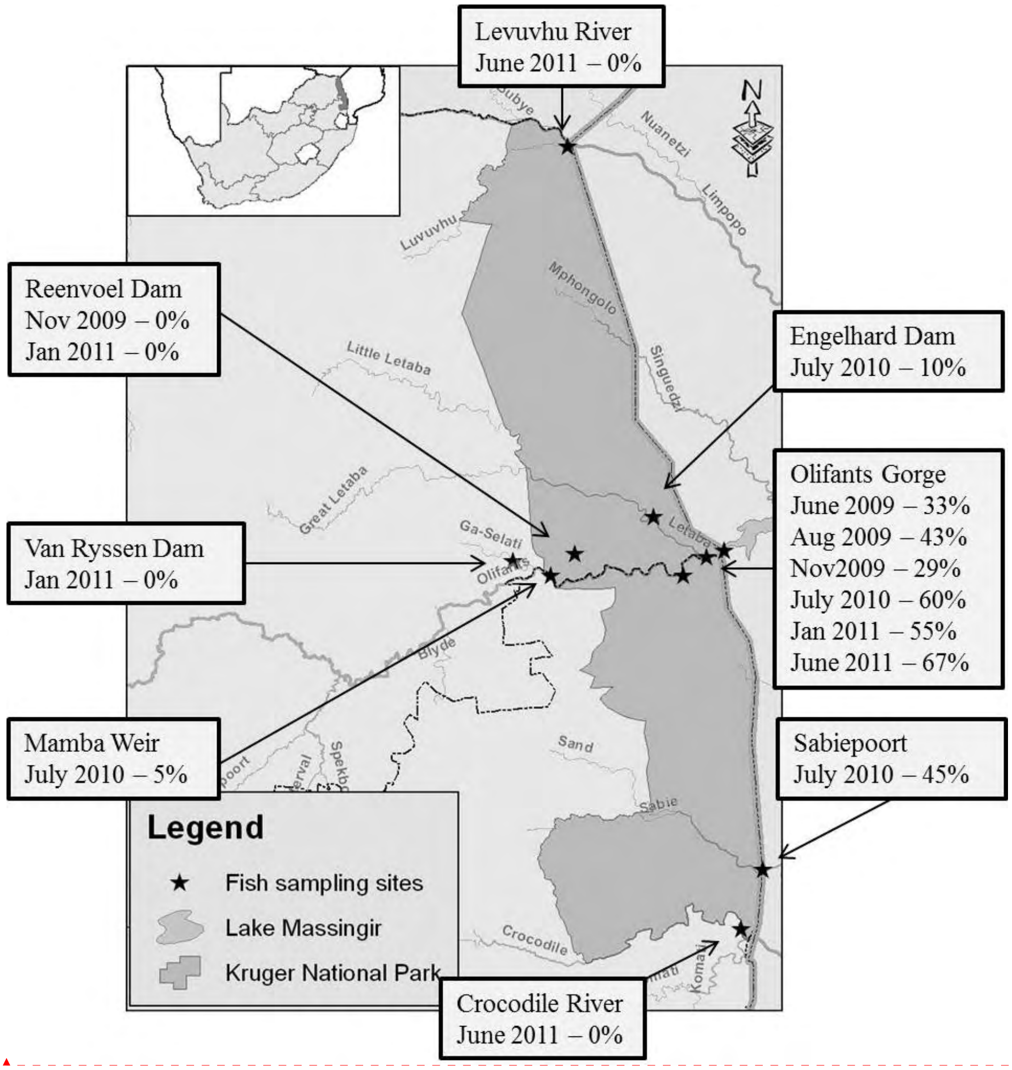
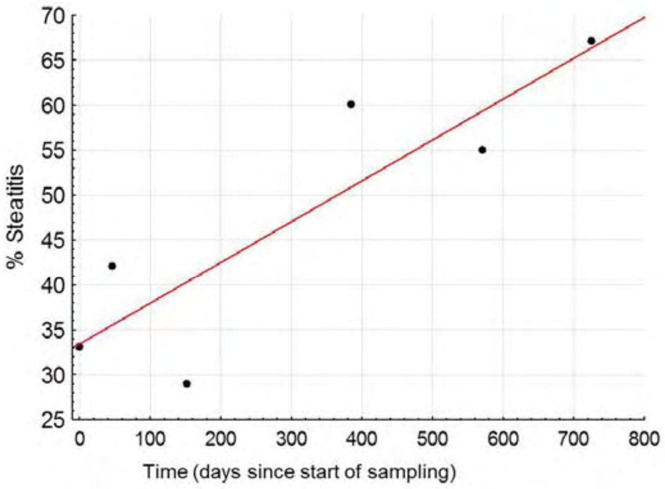


Figure 1: Macroscopic steatitis prevalence as percentage of sampled catfish from various sampling sites in Kruger National Park during the period2009-2011

Table 1: Prevalence of macroscopically detectable steatitis lesions in the Olifants Gorge and other reference populations of catfish

Date	Sampling site	% fish with steatitis	Total fish sampled
June 2009	Olifants Gorge	33	9
August 2009	Olifants Gorge	43	14
November 2009	Olifants Gorge	29	21
November 2009	Reënvoël Dam	0	28
November 2009	Lunsklip Fisheries	66	21
July 2010	Olifants Gorge	60	25
July 2010	Mamba weir	5	20
July 2010	Engelhard Dam	10	21
July 2010	Sabiepoort	45	11
January 2011	Olifants Gorge	55	22
January 2011	Reënvoël Dam	0	13
January 2011	Van Ryssen Dam	0	10
June 2011	Olifants Gorge	67	21
June 2011	Levuvhu River	0	14
June 2011	Crocodile River	0	20



Correlation coefficient: r = 0.87 Significance of model: p = 0.02

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Figure 2: Prevalence of steatitis in catfishsampled from various localities along the Olifants and lower Letaba rivers in the Kruger National Park from 2009 to 2011

In the Olifants River Gorge, where repeated samplings have taken place since 2009, an increase in prevalence of steatitis in sampled catfish was detected (Figure 2).Most catfish from the Olifants Gorge, as well as from the Sabiepoort,were found to store relatively larger amounts of fat compared to catfish sampled from other localities in KNP. However the amount of fat carried was considerably less than that carried by the fish from Lunsklip Fisheries. A decline over time in amount of stored fat in catfish sampled from the Olifants Gorge was noted during repeat samplings between November 2009 and June 2011. Mesenteric fat made up 4.59% of body mass in the most obese specimen from the Olifants Gorge sampled during November 2009, whereas it only constituted 0.88% of body mass in the most obese specimen sampled during June 2011. By contrast, the most obese fish sampled from Lunsklip Fisheries carried more than 12% of body mass as mesenteric fat. Fish sampled from the Olifants Gorge during June 2011 were distinctly leaner than fish sampled during July 2010 and several wasted fish were caught from the Olifants Gorge during the 2011 samplings. One of these fish was extremely emaciated but nevertheless had a reasonable amount of fat stored in the mesenteric adipose tissue. Steatitis was evident in the adipose tissue of this fish. Most catfish sampled from Engelhard Dam, Mamba Weir, Reënvoël Dam and van Ryssen Dam were lean (Table 2).

Table 2: Mesenteric adipose tissue mass relative to body mass of catfish sampled from the Olifants Gorge and other sites on various dates.Olifants Gorge (OG), Engelhard Dam (EH), Mamba Weir (M), Lunsklip Fisheries (LK), Reënvoël Dam (RV), Van Ryssen Dam (FK), Levuvhu River (LUV) and Crocodile River (CR)

Sampling site	Date	Fat % of body mass				
		Mean	Standard Deviation	Sample variance	Range	n
OG	Nov-09	1.17	1.36	1.86	0.08-4.59	20
OG	Jul-10	1.12	1.02	1.05	0-3.68	25
OG	Jan-11	0.4	0.8	0.64	0.02-3.8	22
OG	Jun-11	0.18	0.2	0.04	0.02-0.88	21
EH	Jul-10	0.19	0.4	0.16	0-1.57	21
M	Jul-10	0.16	0.24	0.06	0-0.74	20
LK	Nov-09	4.61	3.45	11.89	1.01-12.07	21
RV	Jul-10	0.32	0.29	0.08	0-0.88	13
FK	Jul-10	0.05	0.08	0.01	0-0.26	10
LUV	Jun-11	0.96	0.78	0.61	0.03-2.2	14
CR	Jun-11	0.14	0.16	0.03	0-0.53	20

Ages of fish sampled from the Olifants Gorge ranged from 1 to 19 years with steatitis being observed in both male and female fish from 3 to 19 years of age (Figure 3). No correlation was observed between age and severity of steatitis.Ages of fish sampled from Reënvoël Dam similarly ranged from 1 to 19 years with both male and female fish represented.

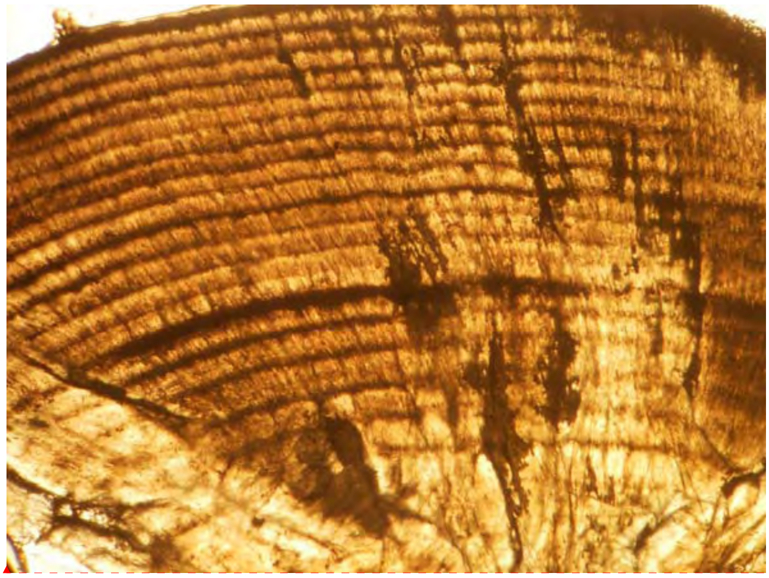

Figure 3A: Growth rings in the otolith of a 19 year old catfish specimen from the Olifants Gorge

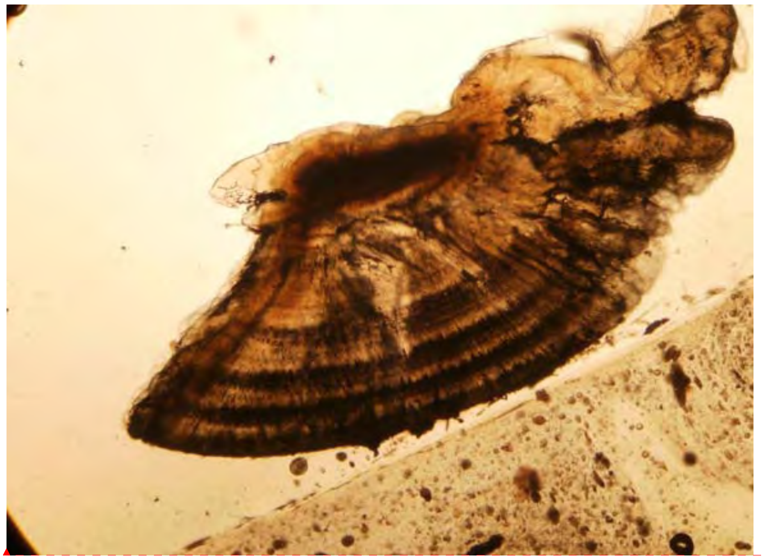

Figure 3B: Growth rings in the otolith of a 4 year old catfishspecimen from the Olifants Gorge

5.2 Macroscopic Pathology

Necrosis and associated steatitis was observed repeatedly in the adipose tissues and, other than severity, there was no distinction between the lesions observed in catfish sampled from the Olifants Gorge, Engelhard Dam, Sabiepoort and the positive reference population at Lunsklip Fisheries. Steatitis lesions presented as distinct white and brown spots (Figure 4A), consisting of small focally disseminated to coalescing granulomata up to 5 mm in diameter. In more advanced cases lesions were characterized by a brown colour, sometimes with an orange coloured centre. Affected mesenteric fat, in severe cases, had a rubbery consistency and brown granulomata were confluent throughout the fat (Figures 4B and 4C).

In catfish with steatitis from both the Olifants Gorge and Lunsklip Fisheries, lesions were mostly restricted to the mesenteric fat tissue. In severely affected fish the caudal section of the mesenteric fat body was often adheredto the hind gut and the caudal section of the gonads (Figure 5). In milder cases granulomata were more densely concentrated on the parietal aspect of the fat body closest to the mesenteric insertion (Figure 4A). Layers of fat with differing severity of steatitis were observed in some fish. Presence of both coalescing granulomata and scarring of the adipose tissues and earlier lesions characterized by focal brown spots in the fat appeared to indicate an on-going incitement of fat necrosis in catfish of the Olifants Gorge. Only occasional fish showed steatitis in the pectoral fat cushion (Figure 6A) and the intramuscular fat (Figure 6B). Steatitis could not be demonstrated in the epicardial fat. In fish with generalised pansteatitis, lesions could however be demonstrated in the brain fat (Figure 6C).

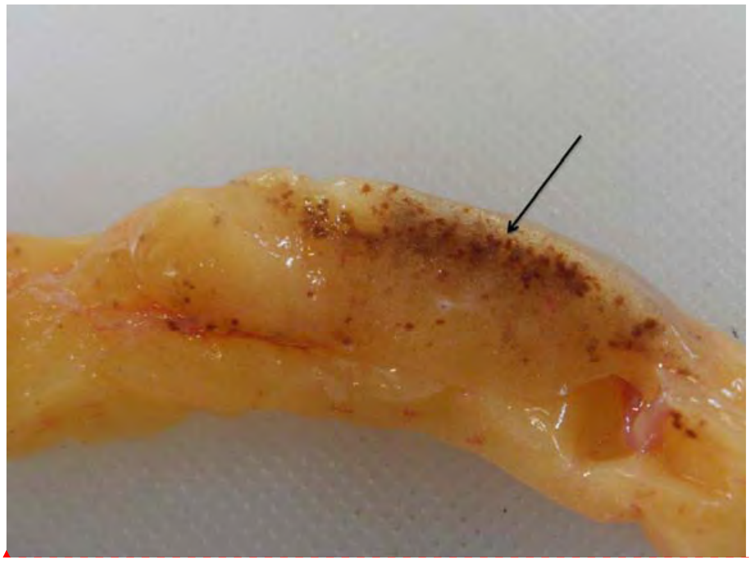

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Figure 4A: Early steatitis lesion in catfish sampled from the Olifants Gorge during July 2010. Note the small sharply circumscribed foci of fat cell necrosis and associated ceroid deposition imparting the characteristic brown colour (arrow)

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Figure 4B: Advanced steatitis of mesenteric fat of a catfish specimen sampled from the Olifants Gorge during November 2009. Note the diffuse brown granular appearance of the fat, the rough surface and virtually total absence of normal appearing fat

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Figure 4C: Cross section of mesenteric adipose tissue from a catfish specimen collected from the Olifants Gorge in November 2009 showing typical severe steatitis. Note brown granuloma formation within the adipose tissue

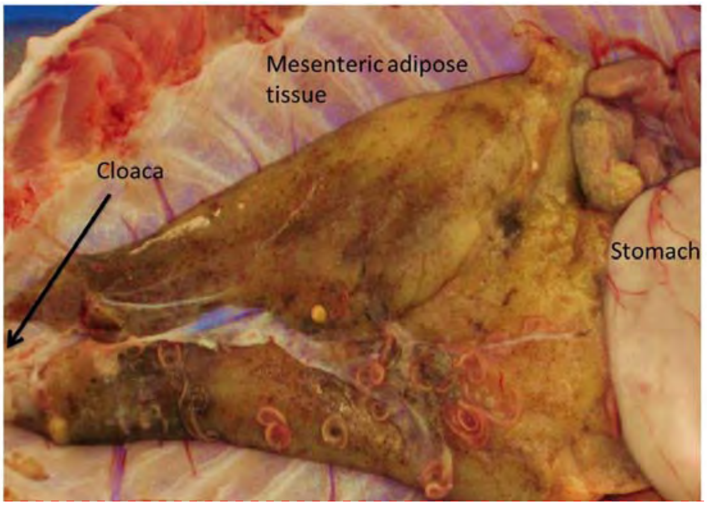

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Figure 5: Steatitis of the mesenteric adipose tissues ina catfish sampled from the Olifants Gorge during June 2011. Note that the caudal portion of the mesenteric fat body appears more severely affected

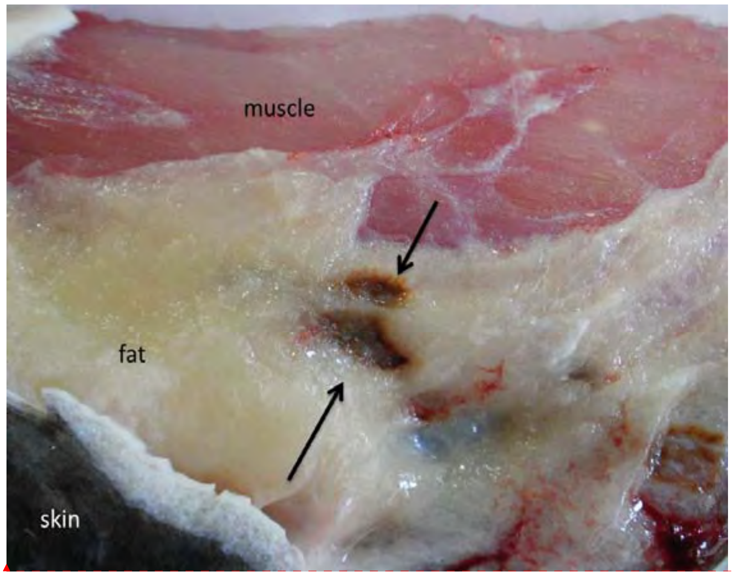

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Figure 6A: Focal fat necrosis (arrows) in the pectoral fat ofa catfish sampled from the Olifants Gorge during July 2010

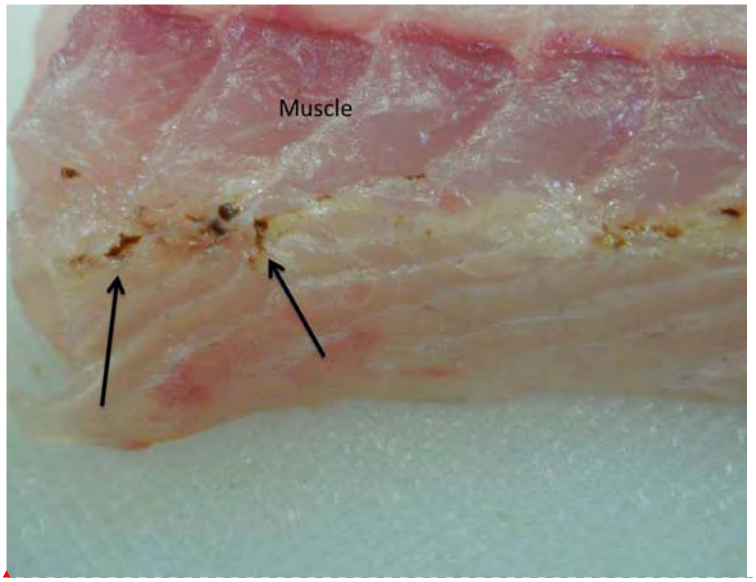

Figure 6B: Focal steatitis (arrows) in the intramuscular fat of a catfish sampled from the Olifants Gorge during July 2010

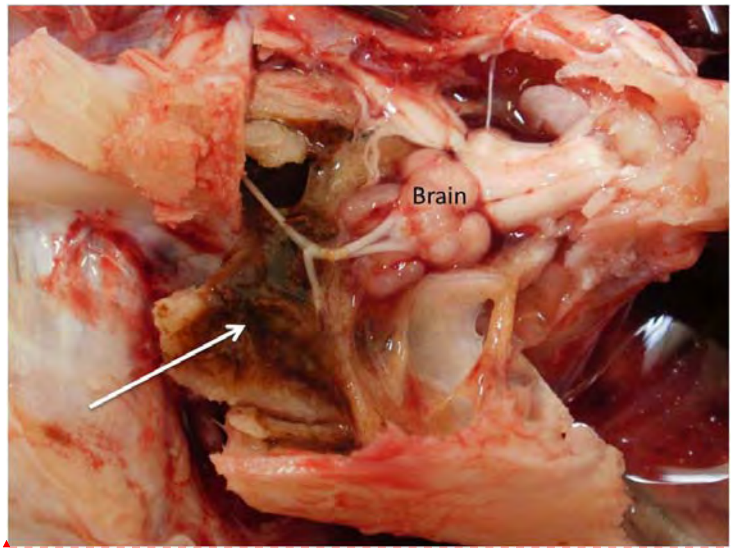

Figure 6C: Generalised steatitis in the fat surrounding the brain of a catfish sampled from the Olifants Gorge during July 2010. Note brown discolouration of fat (arrow) adhering to the opened cranium

Catfish specimens collected from the Olifants Gorge and lower Letaba River during the samplings prior to November 2009 presented with large amounts of variably coloured fat in the body cavity and between the muscles of the tail. The variation in colour, from a cream white to dark yellow, continued to characterizethe mesenteric fat of catfish from all samplings in the Olifants Gorge, despite the reduction in quantity of mesenteric fat noted during subsequent samplings (Table 2). There was no correlation between fat colour and steatitis. Microscopic examination of histological sections of fat from sampled fish confirmed the macroscopic diagnosis of steatitis (Figures 12A and 12B). The detailed pathology and histopathology of the organs and the specific lesions associated with fat necrosis in fish from the Olifants Gorge have been published (Huchzermeyer et al., 2011).

Incidental pathology in adipose tissue associated with presence of parasites was noted in many fish and could be differentiated from changes associated with steatitis in the fat tissues. Cysts of digenean parasites, varying in size from 2 to 15 mm in diameter, were occasionally noted in the mesenteric adipose tissues of fish sampled from the Olifants Gorge. Cysts mostly appeared as well circumscribed, hard, white nodules sometimes focally disseminated throughout the mesenteric fat (Figure 7). On incision these consisted of a dense connective tissue capsule surrounding a central parasitic larva. In some cases an irregular brown discolouration as a result of melanin deposition was noted in the adjoining tissue. Such granulomata were distinct from those caused by fat necrosis. *Contracaecum* spp. larval nematodes were present in variable numbers within the peritoneal cavity of most fish sampled from the Olifants Gorge. Brown melanisation of focal areas of the mesenteries overlying the mesenteric fat was occasionally noted in the presence of severe infestation with *Contracaecum* spp. larvae. This was particularly evident in catfish sampled from van Ryssen Dam (Figure 8). As in the case of larval digenean trematode cysts, such discolouration differed distinctly from the discolouration associated with steatitis. There was no correlation between severity of infestation with nematode larvae or digenean trematodes and steatitis. The brown discolouration of the fat and mesenteries associated with parasites was shown histologically to be caused by melanin. Both lipopigment and ceroid were absent from such lesions.

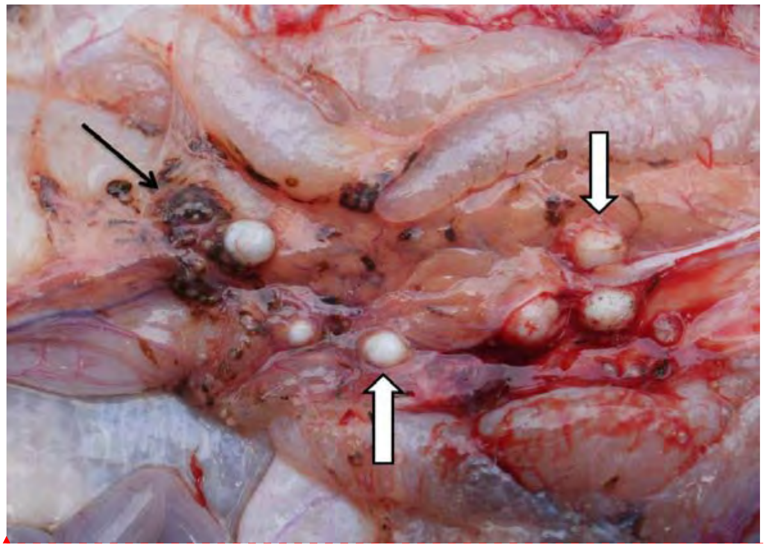

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Figure 7: Melanin deposition (black arrow) associated with digenean trematodes cysts (block arrows) in the caudal mesenteric fat between the male accessory sexual glands of a catfish sampled from Reënvoël Dam in KNP

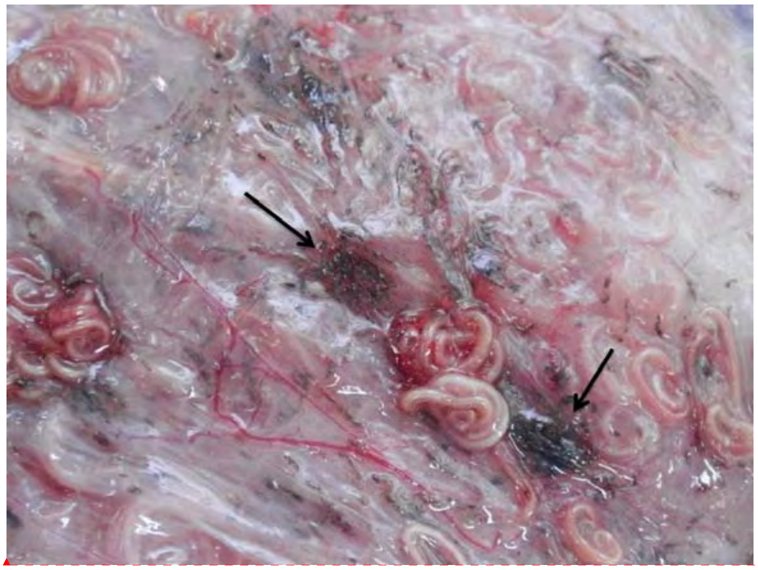

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Figure 8: Melanin deposition (arrows) in vicinity of larval nematodes in the mesentery overlying the mesenteric fat in a catfish sampled from van Ryssen Dam at the FOSKOR mine in Phalaborwa

Livers of fish sampled from the Olifants Gorge varied in colour but appeared more orange, fatty and swollen in appearance than in fish sampled elsewhere (Figure 9A). Livers of fish with good fat reserves often showed small focal deposits of fat visible on the surface of the livers (Figure 9B). Pale zones were sometimes observed in parts of the liver, occasionally extending into the hypodermal lobe of the organ. Most of the pansteatitis-affected fish from the Olifants Gorge and Lunsklip Fisheries showed severely enlarged and rounded spleens with a rough surface (Figure 10A). The healthy spleen of *C. gariepinus* is an oval flat structure with sharp edges and a smooth surface. Atrophy of the pancreas was evident macroscopically in fish suffering from pansteatitis, however the histological picture revealed normal appearing acinar cells and Langerhans islets indicating that the reduction in pancreatic prominence was a result of reduced pancreatic activity in fish with steatitis rather than of specific pathology. During the early samplings in the Olifants Gorge during 2008 and 2009, gills of many catfish appeared paler than normal and mildly hyperplastic. During later samplings this was no longer evident. Furthermore gill pallor appeared to be affected by temperature of the holding water and length of time that fish were kept in the holding tanks. With warmer water temperature and longer holding periods gills appeared paler.

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Figure 9A: Swollen fatty appearing liver typical of a catfish suffering from steatitis, sampled from the Olifants Gorge during June 2011

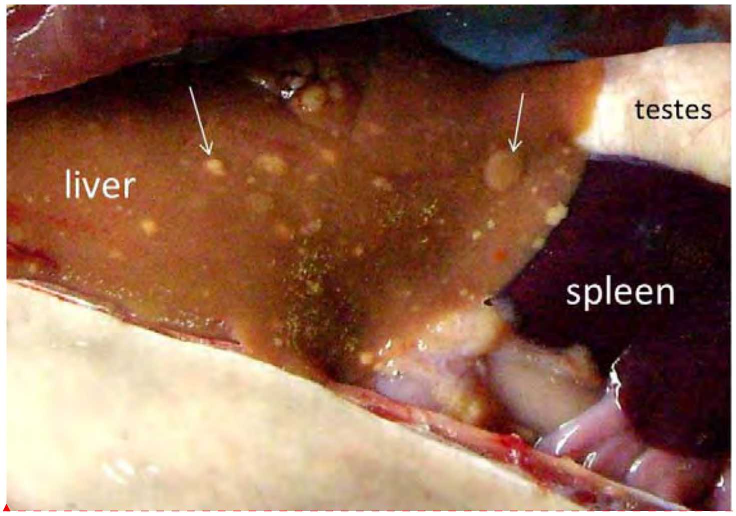

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Figure 9B: Focal fat deposits beneith the liver capsule from a catfish with steatitis sampled from Lunsklip Fisheries during November 2009

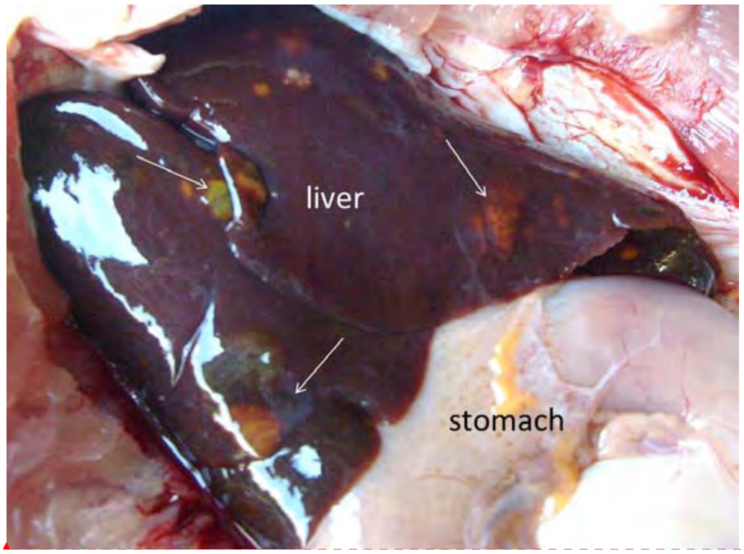

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Figure 9C: Liver from a catfish sampled from Reënvoël Dam during November 2009. Arrows show damage associated with parasitic cysts in the liver parenchyma. Note the dark colour and sharp liver borders of an otherwise healthy liver

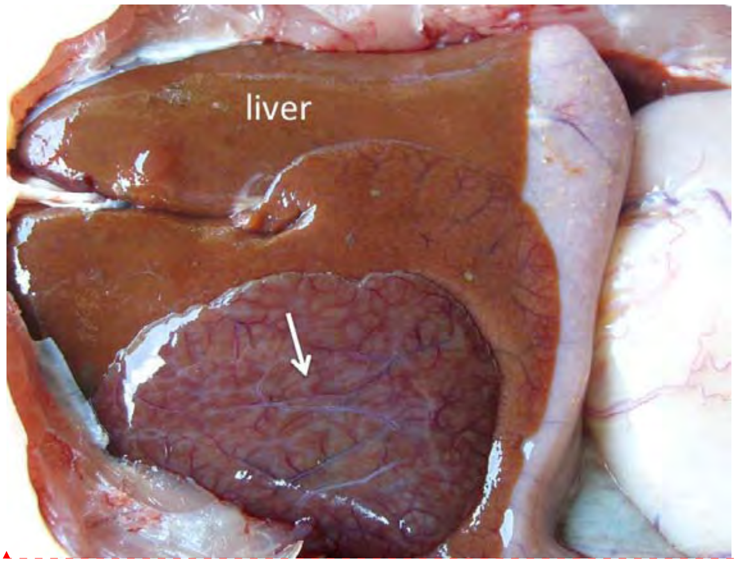

Figure 9D: Outgrowth of regenerating hepatic tissue (arrow), probablyassociated with parasitism, in a liver from a catfish sampled from Reënvoël Dam during November 2009. Note the normal brown colour of the liver

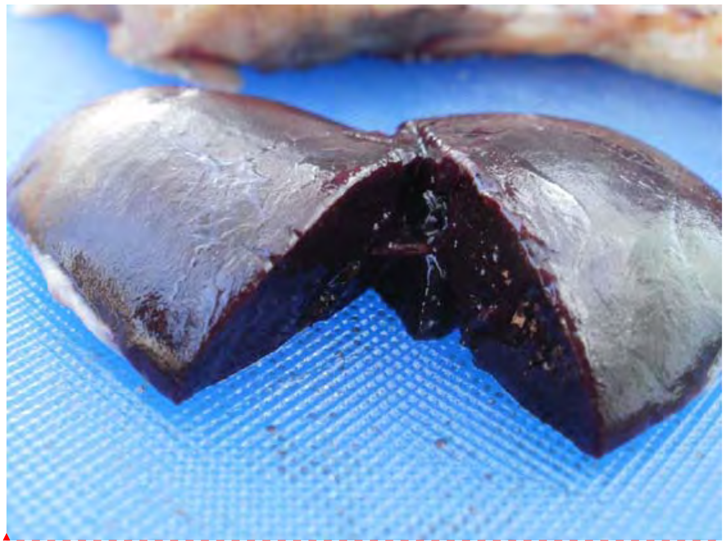

Figure 10A: Typically enlarged spleen of C. *gariepinus*, from the Olifants Gorge, suffering from fat necrosis and steatitis

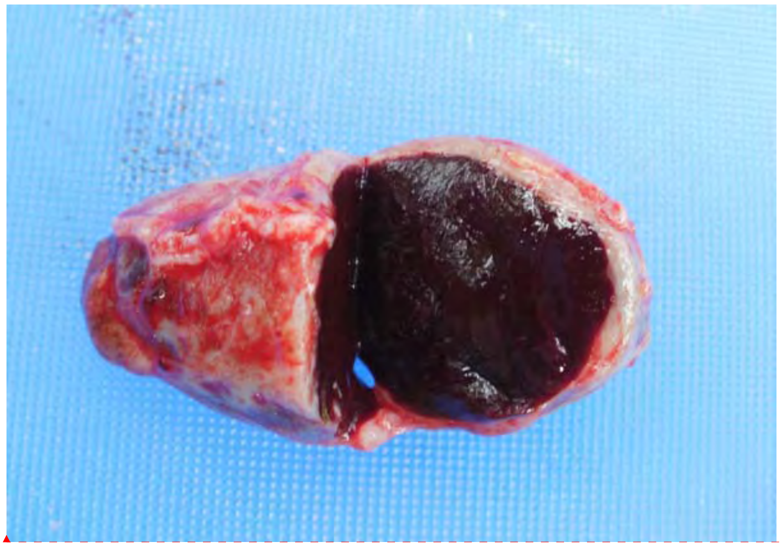

Figure 10B: Spleen of a catfish from Lunsklip Fisheries suffering from severe chronic steatitis showing prominent capsular thickening and splenomegaly. Note the rounded nature of the normally flat spleen

Catfish sampled from Reënvoël Dam showed no steatitis and were used as a negative control population (Table 1). The gillsof catfish sampled from Reënvoël Dam during November 2009appeared normal and in good condition despite heavy parasite burdens.

Livers showed no fatty change but varying pathology was observed, associated with high levels of parasitosis.Extensive changes resulting from heavy parasitosis of the liver (Figure 9C), including outgrowths of regenerating liver tissue, were occasionally observed on the dorsal and ventral surface of some livers (Figure 9D). A further 13 fish were sampled from this site in January 2011 and again no steatitis could be demonstrated despite heavy parasite burdens. *Contracaecum* spp. larval nematodes were present in variable numbers within the peritoneal cavity of most catfish sampled from Reënvoël Dam and parasitic granulomata were frequently present in large numbers in the mesenteric adipose tissue. Brown melanisation of focal areas of the mesenteries overlying the mesenteric fat in the presence of *Contracaecum* spp. larvae and in the vicinity of larval digenean trematode cysts was occasionally noted (Figure 9). Compared to other sampling sites fish from Reënvoël Dam carried the heaviest burdens of digenean trematode cysts in the organs and musculature. Similar deposits of melanin were observed in association with heavy *Contracaecum* spp. larval burdens in catfish from Van Ryssen Dam (Figure 10). Such discolouration differed distinctly from the discolouration associated with steatitis and histologically no steatitis could be demonstrated in these fish.

5.3 Stomach Contents

The sharptooth catfish is an omnivorous benthic scavenger and an active hunter. In the Olifants Gorge there are no trees on the steep riverbanks and catfish stomach contents consisted predominantly of fish. On the Mozambique border where the Olifants River flows into Lake Massingir and where the sand bottomed pools and rapids have been inundated with clay deposits, stomach and intestinal content of sampled catfish consisted of algal detritus and clay. At those sampling sites where pansteatitis was prevalent in catfish, this was repeatedly linked to presence of fish remnants in the stomach contents. Although stomach content only revealed what had been ingested prior to sampling, a relative relationship between diet and presence of pansteatitis appeared to exist as illustrated in the triplot in Figure 11. Fish remnants in stomach content were often in an advanced stage of digestion and consisted of bones and scales from noticeably large fish as well as occasional pectoral spines of *Cynodontis* spp. fish. In a few cases these spines had migrated through the stomach wall and were found lying within the mesenteric cavity with only a mild associated inflammatory reaction. Intestinal content was often considerable and appeared whitish grey and pasty in fish where bones and scales were present in the stomach content. This was distinct from the black brown intestinal content associated with invertebrate and plant stomach content. Almost all catfish sampled in the Olifants Gorge during the peak flow of January 2011had full stomachs, the ingesta consisting offish as well as insects and small reptiles that had been washed into the river during the flood conditions. Despite the murky turbulent water catfish appeared to feed with ease under these conditions. During the winter samplings when the water in the Olifants River is relatively clear far fewer sampled catfish had significant amounts of ingesta in the stomach.

Stomach content of catfish sampled from the Sabiepoort consisted predominantly of fish remnants although stomachs of several of these fish contained recently ingested crocodile fat with visible signs of steatitis still present. The stomachs of catfish sampled from Mamba Weir contained predominantly the fruit of Sycamore fig trees (*Ficus sycomorus*) that overhang the embankment of this stretch of the river.Although more than half of sampled catfish from Reënvoël Dam had empty stomachs, invertebrate and mixed detritus, vegetation and fish were represented in the ingesta of the remaining fish. Recognizable remnants of Mozambique tilapia were found in the stomach content of most catfish sampled from Van Ryssen Dam. The majority of catfish sampled from the Crocodile River during June 2011 had stomachs distended with filamentous algae. Microscopic examination of fluid expressed from the stomach contents revealed that large numbers of diatoms had been ingested together with the filamentous algae. Stomach content of catfish sampled from the Levuvhu River, during June 2011, consisted of algae and sycamore figs.

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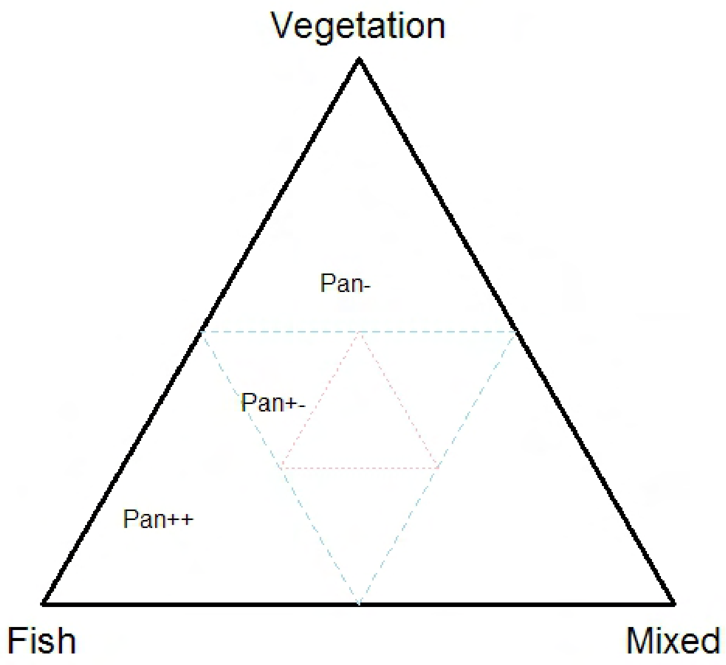

Figure 11: Stomach content analysis of sharptooth catfish showing that the population with pansteatitis (Pan++) at a site where pansteatitis was prevalent had stomach contents with a higher proportion of fish than vegetation, or invertebrates and detritus (mixed) when compared with the population of fish thatdid not have pansteatitis(Pan+-)from areas that had pansteatitis prevalence, and the population from areas without pansteatitis prevalence(Pan-). (Triplot preparation courtesy of S. Woodborne, Centre for Scientific and Industrial Research, Pretoria)

5.4 Histopathology

5.4.1 Histopathology of the adipose tissues

Various granulomatous reactions were observed in the fat tissues of catfish specimens. Parasitic granulomata were distinguishable from foci of inflammation and granuloma formation associated with non-parasitic causes. The histological appearance of non-parasitic granulomata in the adipose tissues was typical of lesions expected with steatitis (Figures 12A and 12B). These lesions were similar in appearance in all fish sampled with macroscopic steatitis, including the fish suffering from nutritional steatitis at Lunsklip Fisheries.

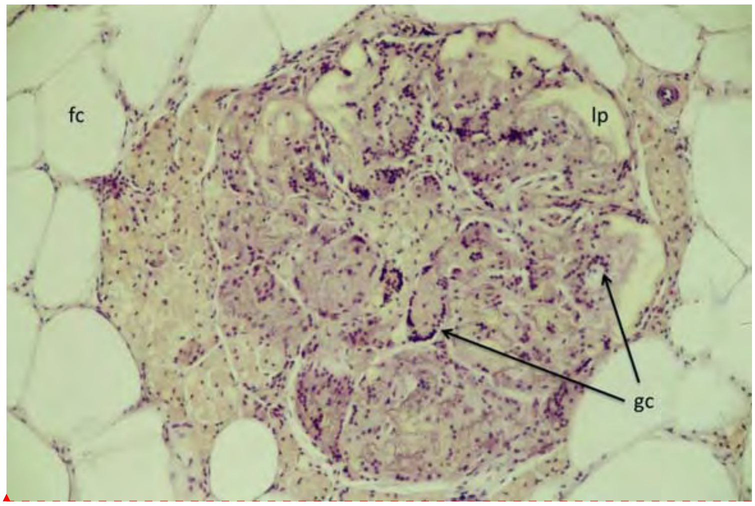

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Figure 12A: Giant cell formation (arrows) in a typical steatitis lesion in adipose tissue from a catfishsampled from the Olifants Gorge in July 2009. HE X100

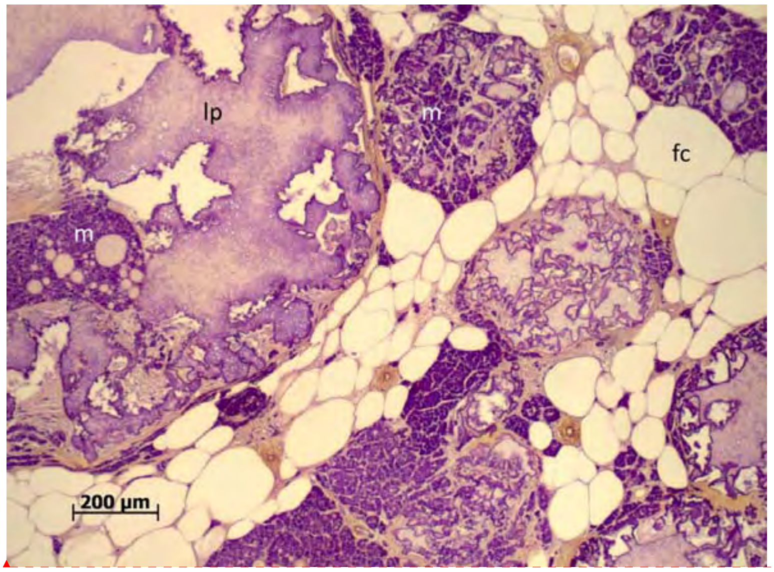

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Figure 12B: Positive staining ceroid pigment (purple) within the lipopigment remnants (lp) of ruptured adipocytes and macrophages (m) in mesenteric fat of a catfish sampled from the Olifants Gorge during November 2009. Adipocytes (fc). (GAF)

Lesions in the adipose tissues were focal and often roughly circular in shape in mild cases and disseminated and coalescing throughout the adipose tissue in severe cases. Surrounding adipocytes often appeared normal although they were reduced in number and displaced by the associated inflammatory reaction in severe cases.

Steatitis was observed in both atrophied adipose tissue (Figure 13) and in adipose tissue where adipocytes were replete with fat. The focal distribution of granulomata in mildly affected fish resulted in lesions sometimes being missed during the sectioning process. Such cases, although positive for steatitis on macroscopic evaluation could not be identified on histological evaluation alone.

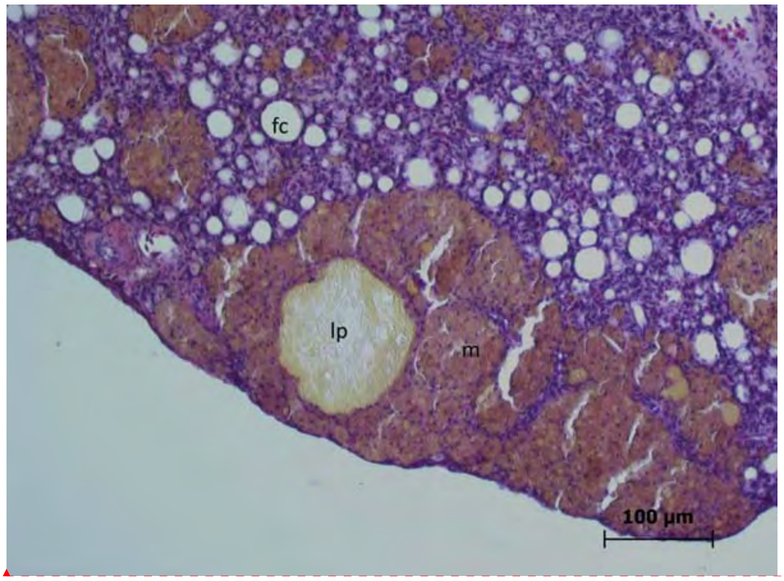

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Figure 13: Atrophied mesenteric adipose tissue showing inflammation typical of steatitis in a catfish sampled from the Olifants Gorge during June 2011. Note small size of adipocytes (fc) and aggregates of macrophages (m) containing ceroid,surrounding areas of fat necrosis containing lipopigment (lp). (HE)

In haematoxylin eosin stained sectionsof affected fat,varyingly sizedfoci consisting of ruptured adipocytes contained a characteristic pigment typical of extracellular ceroid-type (ECC) lipopigment (Elleder, 1991), also called preceroid (Jolly and Dalefield, 1990). This pigment, typical of oxidative damage to fat cells, appeared as yellow, granular and refractive inclusions of varying size. ECC lipopigment was also observed to be phagocytised by the macrophages surrounding steatitis lesions. Necrotic adipocytes and associated cell breakdown debris were surrounded by a dense mass of macrophages containingintracellular ceroid (Fig. 12B),apigment also derived from degeneration and peroxidation of unsaturated lipid (Jolly and Dalefield, 1990; Elleder, M, 1991). Such lesions were associated with presence of variable numbers of fibroblasts and connective tissue deposition. These lesions were focally disseminated throughout the affected mesenteric adipose tissue and represented the brown granulomata noted macroscopically. Lipopigment and ceroid-containing macrophage aggregations within the interstitium of the fat tissues in the absence of adipocyte necrosis were noted in a few fish, and were indicative of mild or early oxidative damage to the fat.

In the absence of frank necrosis in the adipose tissues, the presence of phagocytosed lipopigment within macrophages containing ceroid was used to interpret such lesions as steatitis. Presence of ceroid in the macrophages was confirmed by staining with GAF (Fig. 12B) and PAS stains. Multinucleate Langhans giant cells were invariably associated with the inflammatory response surrounding necrotic areas of fat (Fig. 12A).

In some lesions, more compact macrophages were arranged in the form of an epithelioid type sheath surrounding the ruptured fat cells. Advanced cases presented with a clear or lipopigment containing central lacuna surrounded by organised layers of epithelioid cells that in places coalesced and became embedded in fibrous connective tissue (Figure 14). Clear lacunae were an artefact of sectioning where the central pigmented area of fat breakdown products had been lost during sectioning.

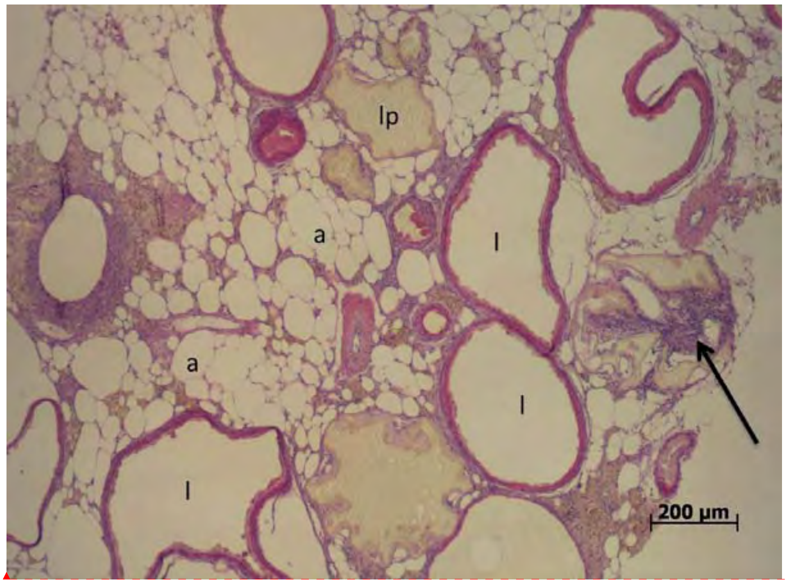

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Figure 14: Advanced stage of fat necrosis and steatitis in mesenteric fat of a catfish sampled from the Olifants Gorge during June 2011. Note the apparently empty lacunae (l) where necrotic remnants of oxidised fat (lp) have been lost during processing, surrounded by an epithelioid sheath. Adipocytes (a), foreign body giant cells (arrow). (HE)

Parasitic granulomata of varying sizes were common in the mesenteric, hypodermal and intramuscular fat but were not observed in the pectoral fat. These granulomata were distinct from granulomata caused by steatitis. Parasitic granulomata showed a greater infiltration of fibroblasts and greater collagen deposition in the capsule than observed with granulomata associated with steatitis. Macrophage clusters on the periphery of parasitic granulomata were less intense and usually in the form of melanomacrophage centres. On haematoxylin eosin stained sections these appeared mildly basophilic in colour with variable amounts of brown melanin pigment. Ceroid- and lipopigment-containing macrophages were not generally associated with parasitic granulomas, and were infrequently observed in the vicinity of parasites in the mesenteric adipose tissues.

5.4.2 Histopathology of other organs

Varying degrees of hepatic lipidosis, often within distinct foci, were observed in the livers of fish suffering from steatitis. Special stains established presence of ceroid, in the hepatocytes of these fish as well as large amounts of haemosiderin (Figures 15A and 15B). Presence of haemosiderin was confirmed by use of Perl’s Prussian blue stain. However, similar changes were observed in livers from some Reënvoël Dam fish in the absence of associated steatitis. Well-encapsulated parasitic granulomata of varying sizes were a common histological finding in many liversectionsof fish from both the Olifants Gorge and Reënvoël Dam but were not observed in livers of catfish from Lunsklip Fisheries. A large focus of hepatocellular disorganization was observed in the liver of one fish from Lunsklip Fisheries suffering from severe steatitis. The affected area showed eosinophilia of hepatocytes and tracts of fibroblasts and associated inflammatory round cells infiltrating the liver parenchyma. The periphery of the focus was demarcated by a zone of melanomacrophage aggregations. The central area of the lesion appeared partitioned by fibrous tracts with islands of hepatocytes undergoing degeneration and necrosis. A clearly demarcated zone of disorganization with enlarged hepatocytes, devoid of pigment, arranged in loose whorls with mild fibroplasia surrounding dilated vascular spaces was observed occasionally in fish from Reënvoël Dam and from the Olifants Gorge. Melanomacrophages in all organs of older fish were replete with melanin. Variable numbers of inflammatory cells associated with ducts and blood vessels were observed in the livers of older fishparticularly. Pancreatic acinar and islet cells appeared normal in all of the fish, although the variable prominence of pancreatic tissues noted macroscopically was reflected in atrophy of the organ, which was most prominent in fish affected by steatitis (Figure 16). No specific pathology was observed in the intestines of sampled fish.

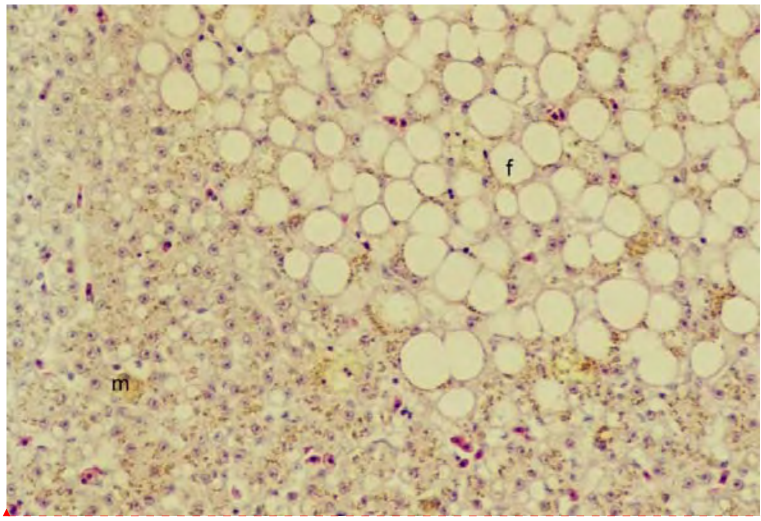

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Figure 15A: Liver section of a catfish sampled from the Olifants Gorge during November 2009. Note distinct focus of fat vacuoles (f). (HE X200)

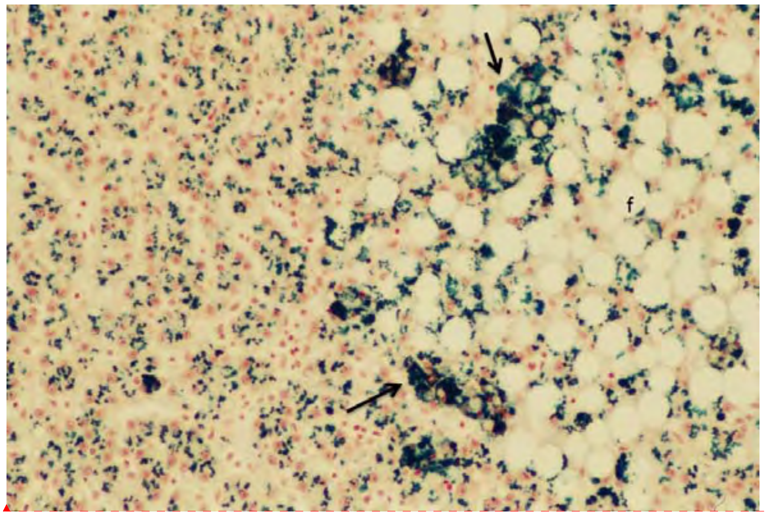

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Figure 15B: Liver section of a catfish sampled from the Olifants Gorge during November 2009. Note distinct focus of fat vacuoles (f) and clustering of haemosiderin (arrows) on the perimeter of this focus. (Perl’s Prussian blue, X200)

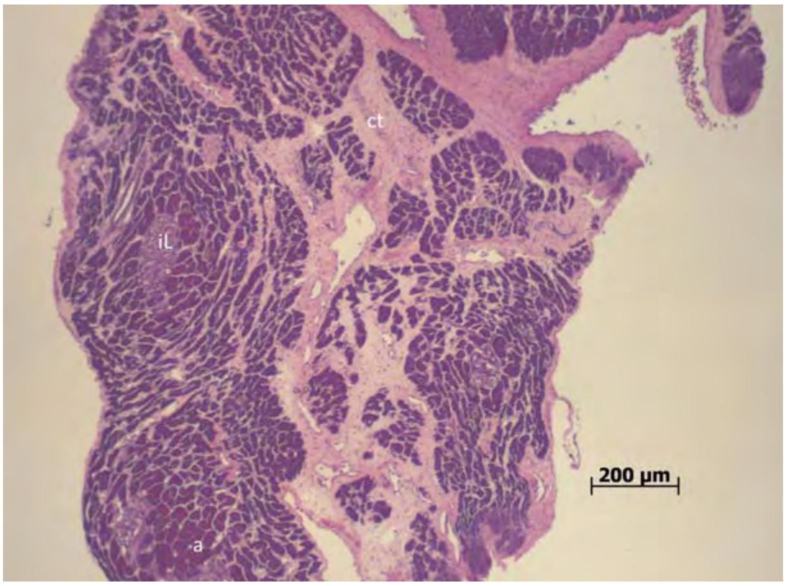

Figure 16: Pancreatic atrophy in a catfish suffering from steatitis, sampled from the Olifants Gorge during June 2011. Note prominence of connective tissue (ct) extending between groups of acinar cells (a). Islet of Langerhans (iL). (HE)

In fish from all sites, variable numbers of focally disseminated clusters of dense basophilic lymphocytes were noted in the cranial and caudal kidney representing variation in the normal lymphocytic tissue within this organ.

Apparent necrotic changes observed in the haemopoietic tissues ofsome catfish specimens collected from the Olifants Gorge during 2008 were not evident in the specimens collected subsequently, but were again noted in fish collected at the time of the fish kill in July 2009. Sampling of these respective fish was done in the absence of the author and autolytic changes had complicated the histological picture. Necrosis of haematopoietic tissues was not observed in fish sampled from any sites between November 2009 and June 2011.

The spleens of fish from various sites were variable in appearance depending on the numbers of erythrocytes held in the splenic sinusoids. Encapsulated necrotic foci, from degenerating parasites within the spleen, were observed in two fish from the Olifants Gorge. Multiple cyst-like mineralized foci were observed in the spleen of one fish that was not suffering from steatitis. Small coccidian type intracellular parasites were observed in macrophages within melanomacrophage centres of both the spleen and kidney of fish from the Olifants River and from Reënvoël Dam. These parasites were not observed in fish from Lunsklip Fisheries. There appeared to be no pathology associated with presence of this parasite.

Multiple focal cyst-like structures that appeared to be of thyroid origin were observed in the hearts of two fish with steatitis from Lunsklip Fisheries. The structures appeared to be linedby an epithelium and were filled with homogenous eosinophilic material. Myocardial lesions were not observed in fish from other sampling sites. No signs of fat necrosis could be detected in epicardial fat cells where these were present on the hearts of sampled fish.

Gills in the catfish specimens collected in the Olifants Gorge during January 2009 presented with a two to three-fold increase in the thickness of the epithelium of the secondary lamellae. In many of these specimens the epithelial hyperplasia increased towards the base of the secondary lamellae imparting a wedge shaped appearance. Such changes were less evident in fish sampled from the Olifants Gorge in November 2009, and during later samplings gills showed minimal signs of hyperplasia. Monogenean trematodes were occasionally visible between the secondary lamellae of the gills. Some fish showed deformity of the cartilage of the primary gill lamellae as a result of infection with a digenean trematode, possibly*Centrocestus formosanus* (Figure 17). These parasites could be observed lying within cysts in the gill cartilage where they appeared to feed off chondrocytes causing considerable damage to the gill cartilage. Infection with this parasite and resultant gill cartilage deformity was also a common finding in fish sampled from Reënvoël Dam. Onlymild hyperplasia of thegill epithelium was evident in some fish from Lunsklip Fisheries;however the absence of digenean gill parasites from these fish was notable.

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Figure 17: Digenean trematode larva encysted within the cartilage of the primary lamellae of a catfishsampled from the Olifants Gorge during June 2011. Note hyperplastic changes in the cartilage (arrows). (HE)

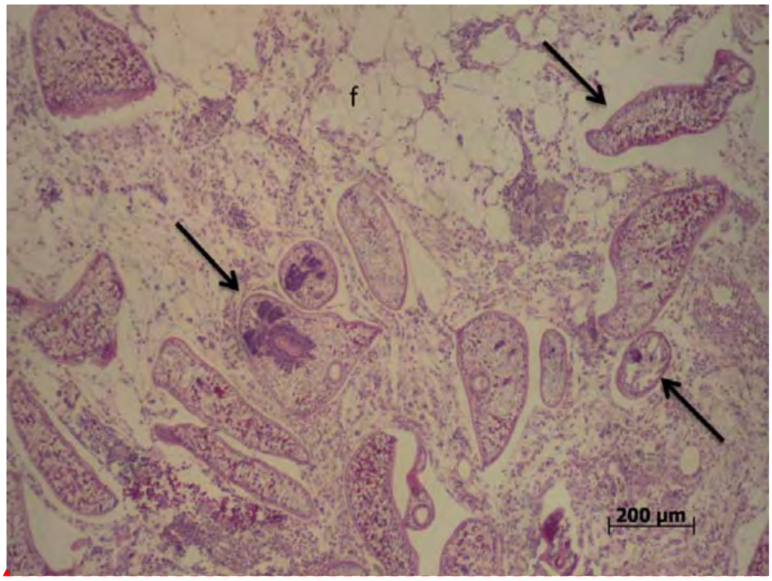

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Figure 18: Digenean trematodes of the family Diplostomidae (arrows) within the cerebrospinal space adjacent to fat lining the cranium of a catfish sampled from Engelhard Dam during July 2010. Note the cellular reaction associated with presence of these parasites. (HE)

No lesions were observed in the brains of fish from any of the sampling sites. At some sampling sites large numbers of digenean trematodes (Fam. Diplostomidae) were observed surrounding the brain and within the brain fat (Figure 18). Histologically an inflammatory reaction with presence of macrophages could be observed in association with these parasites, but the parasites were never observed penetrating the brain tissues. There was no correlation between parasite presence and steatitis in the brain fat.

Maturity of gonads observed in sampled fish depended on age and sampling season. Older female fish showed large numbers of melanomacrophages within the ovaries. In testicular tissue, melanomacrophages were seldom noted. Gonadal development in all cases appeared to be normal and no pathology was noted within the gonads. Development of intersex was not observed in sampled fish.

Muscle atrophy was observed in some fish suffering from steatitis. Other than presence of parasitic cysts, no other pathology was observed in muscle tissue. The fibrous and round cell inflammatory reaction associated with parasites depended on type and stage of parasite but was not correlated with presence of steatitis in the intramuscular fat. No specific pathology was observed in the skin of sampled fish.

5.5 Pathology in Other Species

Mozambique tilapias were difficult to catch in the Olifants Gorge. However a few specimens caught from the Letaba River at the confluence with the Olifants River at the entrance to the gorge appeared thin, despite presence of moderate mesenteric fat reserves. Distinctly demarcated pale areas of discolouration were noted in the livers of some of these fish. These were confirmed by histology to be zones of fat accumulation within hepatocytes. Such zones are not uncommon in farmed tilapias. Gills of Mozambique tilapiaspecimens appeared normal. Mesenteric fat showed no evidence of steatitis. However on histology one fish showed small amounts of lipopigment within macrophages associated with mesenteric adipose tissue. Distinct from catfish, Mozambique tilapiaspecimens showed no ceroid or haemosiderin deposition in the livers. All other organs appeared histologically normal. Only one large specimen of the purple labeo (*Labeo congoro*Peters)was collected in the gorge. The gills of this fish manifested with an unusual severe fusion of the distal ends of the primary lamellae, a change that was not observed in either catfish or Mozambique tilapia. A few purple labeo specimens caught at other sampling sites did not show this same gill fusion.

Steatitis could not be demonstrated in 5 tiger fish collected from the Olifants Gorge during September 2008 or in 21 tiger fish collected from the Olifants Gorge during June 2011. The fish were all in good condition with distinctly white mesenteric fat reserves. Fish sampled during June 2011 ranged in age from 1 to 10 years and both male and female fish were represented. All fish carried low numbers of larval nematodes in the peritoneal cavity however digenean trematode cysts were absent in all but one fish.

5.6 Blood Chemistry and Haematology

5.6.1 Blood smear examinations

Examination of blood smears taken from catfish collected from the Olifants Gorge soon after the mass crocodile mortality in the winter of 2008indicated an increase in numbers of immature erythrocytes in many of the fish as well as erythrocytes with irregular cell shapes and crenated cell membranes. Nuclear shapes were similarly irregular with a high prevalence of chromatin clumping visible within the nuclei in some blood smears. As the changes were not restricted to immature erythrocytes, these may have been an artefact of smear preparation. An increase in polychromatocytes was,however, still evident in blood smears taken during the November 2009 sampling (Table 3). Sharptooth catfishnormally have round nucleated erythrocytes which are distinct from the oval erythrocytes of many other fish species. Compared to mature erythrocytes, polychromatocytes were characterised by a more basophilic cytoplasm and a larger, granular appearing nucleus. Crenation of erythrocyte cell membranes was still present in some blood smears of fish sampled from the Olifants Gorge during November 2009. The finding was more frequent in blood smears taken from catfish at Lunsklip Fisheries at the same time but was rare in blood smears of fish sampled from Reënvoël Dam.

Table 3: Comparison of percentage polychromatocytes in blood smears collected from catfish at three sampling sites during November 2009

Sampling site	% polychromatocytes		
	mean ± SD*	range	n
Lunsklip Fisheries	0.75±0.60	0-2.33	21
Olifants Gorge	3.26±2.73	0.67-12.33	19
Reënvoël Dam	1.95±1.65	0.33-5.33	20

5.6.2 Haematocrit

Average haematocrit values are presented in Table 4. There were no significant differences in haematocrit values between fish with and without steatitis at various sampling sites where steatitis was found to occur. In the Olifants Gorge, mean PCV values of fish ranged between 25 and 41% in apparently healthy fish and between 24 and 37% in fish with steatitis. The mean PCV value for fish sampled from Reënvoël Dam was 32.3% (n=13, standard deviation=6.1). Comparison of mean PCV values from fish sampled from the Olifants Gorge (32.6%) with Lunsklip Fisheries (39.4%) during November 2009 indicated significantly lower haematocrit values in the Olifants Gorge fish ($p<0.05$). Haematocrit values were only available from fish sampled from Reënvoël Dam during January 2011; during the November 2009 sampling of catfish from Reënvoël Dam a field micro-centrifuge was not available.Analysis of variance ($p=0.00002$) followed by post-hoc Tukey’s HSD testshowed significant differences between mean PCV values (39.4%) for fish from Lunsklip Fisheries (positive reference population, n=21) when compared to mean PCV values (30.3%) for all fish sampled from the Olifants Gorge (n=111), and to mean PCV values (32.3%) for fish from Reënvoël Dam (negative reference population, n=13) (Figure 19).

5.6.3 Haemoglobin

Average haemoglobin values for fish sampled from all sites are shown in Table 5 and were noticeably variable. Haemoglobin values (g/dl) did not differ significantly between fish with and without steatitis sampled from the Olifants Gorge, with mean values of 10.1 and 9.7 for fish with and without steatitis, respectively. Analysis of variance showed significant difference ($p<0.05$) in mean haemoglobin valuesof fish sampled during November 2009 from the Olifants Gorge, Lunsklip Fisheries and Reënvoël Dam (post-hoc Tukey’s test) (Figure 20).The average haemoglobin value of fish sampled from Reënvoël Dam during January 2011 was however, much lower than that of fish sampled from the same site during November 2009, with average values being similar to those of catfish sampled from the Levuvhu and Crocodile rivers. The significance of the differences observed in the November results thus remains uncertain.

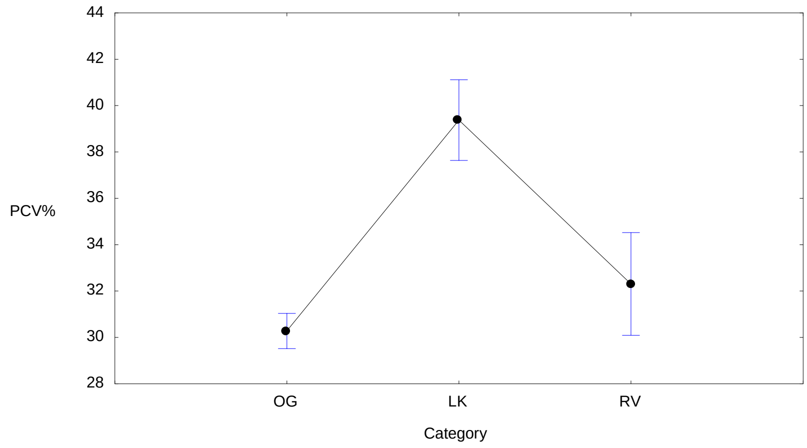

Figure 19: Comparison of mean haematocrit (PCV%) from all catfish sampled from the Olifants Gorge sites (n=111) as compared to mean haematocrit (PCV%) of catfish sampled from Lunsklip Fisheries (n=21) and Reënvoël Dam (n=13). (p=0.00002, vertical bars denote +/- standard errors)

Table 4: Mean haematocrit values (packed cell volume=PCV) of blood collected from *C. gariepinus* from all sites (Olifants Gorge=GL, OGM, OG, OL, LOG, LOC; Lunsklip Fisheries=LK; Reënvoël Dam=RVB; Engelhard Dam=EH; Van Ryssen Dam=FK; Mamba Weir=M; Levuvhu River=LUV; Crocodile River=CR)

Sample site	Date	Packed cell volume (%)		
		mean ± SD*	range	number
GL	Jun-09	26.33 ± 4.97	15-31	9
OGM	Aug-09	39.07 ± 6.75	30-50	14
LK	Nov-09	39.38 ± 7.6	22-50	21
OG	Nov-09	32.55 ± 9.71	10-50	20
EH	Jul-10	29.95 ± 5.96	19-40	19
OL	Jul-10	28.56 ± 6.92	15-38	25
M	Jul-10	27.75 ± 8.34	12-45	20
LOG	Jan-11	30.59 ± 6.04	14-39	22
RVB	Jan-11	32.31 ± 6.06	20-44	13
FK	Jan-11	32.2 ± 4.69	22-38	10
LUV	Jun-11	30.79 ± 5.82	21-40	14
LOC	Jun-11	25.67 ± 7.43	11-40	21

5.6.4 Serum vitamin E

Average serum vitamin E values for catfish sampled from all sites are presented in Table 6. Vitamin E values did not differ significantly between fish with and without steatitis. Although some fish from the Olifants Gorge had very low serum vitamin E values, analysis of variance showed that there was no significant difference in mean serum vitamin E values between fish sampled from the Olifants Gorge and Reënvoël Dam during November 2009 whereas the values in fish sampled from Lunsklip Fisheries were significantly higher at this time (Figure 21).

Table 5: Mean haemoglobin values (g/dl) of blood collected from *C. gariepinus* from all sites (Olifants Gorge=GL, OGM, OG, OL, LOG, LOC; Lunsklip Fisheries=LK; Reënvoël Dam=RV, RVB; Engelhard Dam=EH; Van Ryssen Dam=FK; Mamba Weir=M; Levuvhu River=LUV; Crocodile River=CR)

Sample site	Date	Haemoglobin		
		mean ± SD*	range	number
GL	Jun-09	12.32 ± 1.07	10.7-13.8	9
OGM	Aug-09	11 ± 1.11	8.93-13	14
LK	Nov-09	14.47 ± 3.06	9.53-23	21
OG	Nov-09	13.16 ± 3.84	6.65-21	20
RV	Nov-09	16.62 ± 3.39	10.8-21.3	14
EH	Jul-10	9.39 ± 3.57	3.82-16.9	20
OL	Jul-10	9.66 ± 4.29	3.24-21.3	25
M	Jul-10	8.04 ± 4.52	2.33-17.1	20
LOG	Jan-11	7.69 ± 3.05	0.1-13.7	22
RVB	Jan-11	8.05 ± 2.45	3.5-13.1	13
FK	Jan-11	13.83 ± 6.39	8.15-29.93	10
LUV	Jun-11	9.25 ± 1.68	6.4-12.2	14
LOC	Jun-11	7.9 ± 2.12	4.2-12	16
CR	Jun-11	7.89 ± 1.34	4.2-10.2	20

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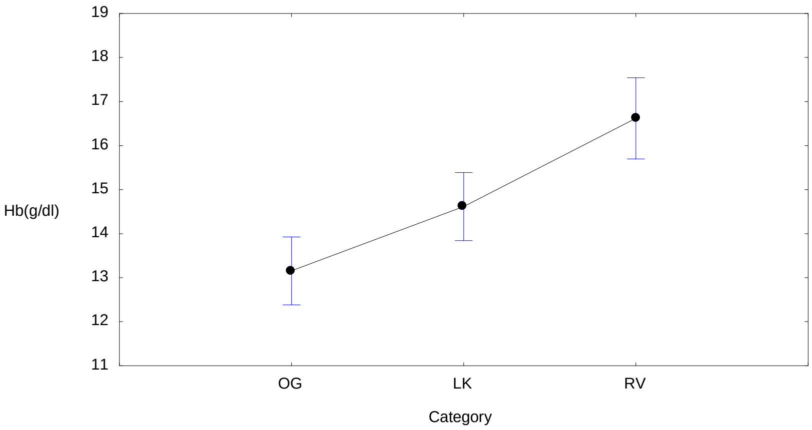

Figure 20: Comparison of mean haemoglobin values (g/dl) of catfish sampled during November 2009.OG=Olifants Gorge (n 20), LK=Lunsklip Fisheries (n 21) and RV=Reënvoël Dam (n 14). (p=0.02136, vertical bars denote +/- standard errors)

Table 6: Average serum vitamin E values of catfish from all sites (Olifants Gorge=OGM, OG, OL; Lunsklip Fisheries=LK; Reënvoël Dam=RV; Engelhard Dam=EH; Mamba Weir=M)

Sample site	Date	Vitamin E (mg/l)		
		mean ± SD*	range	number
OGM	Aug-09	4.67 ± 2.99	1.0-8.9	14
LK	Nov-09	4.6 ± 1.26	2.7-6.7	21
OG	Nov-09	3.13 ± 0.34	2.7-3.9	15
RV	Nov-09	3.06 ± 0.33	2.4-3.7	15
EH	Jul-10	3.33 ± 1.49	1.4-7.8	18
OL	Jul-10	2.75 ± 1.18	1.1-5.4	17
M	Jul-10	2.88 ± 1.4	0.8-5.7	16

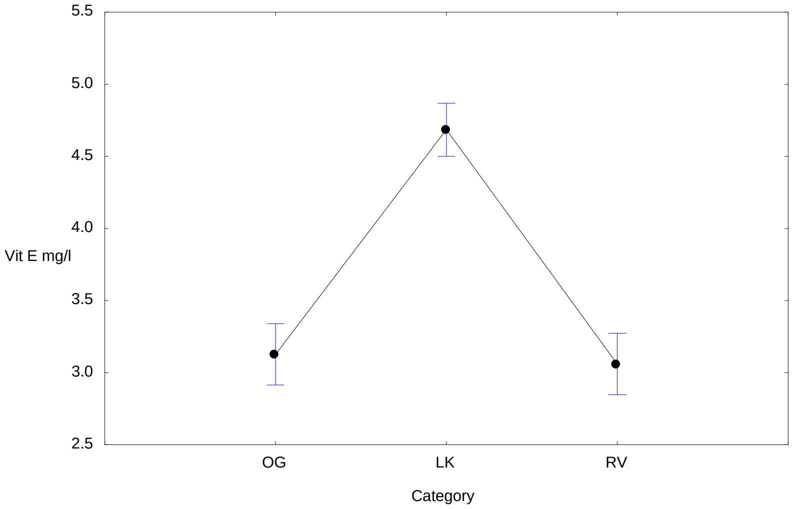

Figure 21: Comparison of mean serum vitamin E values (mg/l) of catfish sampled during November 2009.OG=Olifants Gorge (n 15), LK=Lunsklip Fisheries (n 20) and RV=Reënvoël Dam (n 15).(p=0.00000, vertical bars denote +/- standard errors)

The lower fifth percentile of serum vitamin E values (2.7 mg/l) in healthy fish from Reënvoël Dam was used to identify fish with depressed vitamin E values and subjected to chi-squared analysis ($p<0.05$). Whereas the percentage of fish with depressed serum vitamin E values during the November 2009 sampling from the Olifants Gorge was similar to that of fish sampled from Reënvoël Dam and Lunsklip Fisheries in the same period, significantly higher percentages of fish with depressed serum vitamin E values were sampled from the Olifants Gorge and Mamba Weir during July 2010. Although at p values slightly above 0.05, numbers of fish with low serum vitamin E values sampled from the Olifants Gorge during August 2009 and from Engelhard Dam during July 2010 were indicative of a similar pattern of depression during these sampling episodes(Table 7).

Table 7: Percentage of catfish from the Olifants Gorge and other sites with serum vitamin E levels below the lower fifth percentile of values of healthy fish sampled from Reënvoël Dam. (Olifants Gorge=OGM, OG, OL; Lunsklip Fisheries=LK; Reënvoël Dam=RV; Engelhard Dam=EH; Mamba Weir=M)

Sampling site	Sampling date	% fish with vitamin <2.7 mg/l	n
OGM	Aug-09	36	14
OG	Nov-09	7	15
RV	Nov-09	7	15
LK	Nov-09	10	21
OL	Jul-10	65	17
EH	Jul-10	33	18
M	Jul-10	50	16

5.6.5 Blood glutathione peroxidase

Exceptionally high erythrocyte glutathione peroxidase values were measured in catfish from three sampling sites during July 2010 (Table 8). Fish with steatitis were found at all three of these sites (Figure 22A). A comparison of erythrocyte glutathione peroxidase values measured in blood of catfish from the same sites in the Olifants Gorge on different sampling dates lets these high values appear suspicious.No significant difference inerythrocyte glutathione peroxidase values could be demonstrated in fish sampled during November 2009 from the Olifants Gorge, Lunsklip Fisheries and Reënvoël Dam and there was no significant difference in erythrocyte glutathione peroxidase values in fish with and without steatitis.

After combining data from all sampling sites, including the suspicious data from July 2010,analysis of variance showed that sampling site and datedid have a significant effect on blood glutathione peroxidase values (F=4.57, p=0.0012). According to Tukey’s post-hoc test, there was a significant difference in average values between fish sampled from the Olifants Gorge (159.5) and fish sampled from Reënvoël Dam (18.8). Levene’s test for homogeneity of variances showed that there was significant inequality in variances between treatments and this could not be eliminated after data had been log-transformed. It was therefore decided to use the non-parametric Kruskall-Wallis test as an additional approach to the data analysis. This confirmed the significant effect of sampling site on blood glutathione peroxidase values at p=0.0135 (Figure 22B). The comparison would, however, appear unjustified.

Table 8: Average erythrocyte glutathione peroxidase (GSH-Px) values of catfish from all sites (Olifants Gorge=GL, OGM, OG, OL, LOG, LOC; Lunsklip Fisheries=LK; Reënvoël Dam=RV, RVB; Engelhard Dam=EH; Van Ryssen Dam=FK; Mamba Weir=M; Levuvhu River=LUV; Crocodile River=CR)

Sample site	Date	Glutathione peroxidase (µU/mgHb)		
		mean ± SD*	range	number
GL	Jun-09	2.41 ± 0.22	2.16-2.79	9
OGM	Aug-09	14.13 ± 7.87	6.57-33.72	14
LK	Nov-09	19.13 ± 6.21	11.77-35.93	21
OG	Nov-09	20.3 ± 8.55	10.32-48.08	20
RV	Nov-09	25.41 ± 10.02	4.66-45.34	14
EH	Jul-10	470.24 ± 366.55	76.56-1203.29	20
OL	Jul-10	526.57 ± 464.42	60.87-1664.36	25
M	Jul-10	807.65 ± 712.28	0-2664.48	20
LOG	Jan-11	38.93 ± 119.79	6.29-574.6	22
RVB	Jan-11	11.59 ± 6.14	5.11-30.1	13
FK	Jan-11	7.81 ± 2.14	2.4-9.5	10
LUV	Jun-11	34.64 ± 8.55	24.22-50.7	14
LOC	Jun-11	74.43 ± 118.94	13.68-536.28	20
CR	Jun-11	34.35 ± 12.57	5.8-58.1	20

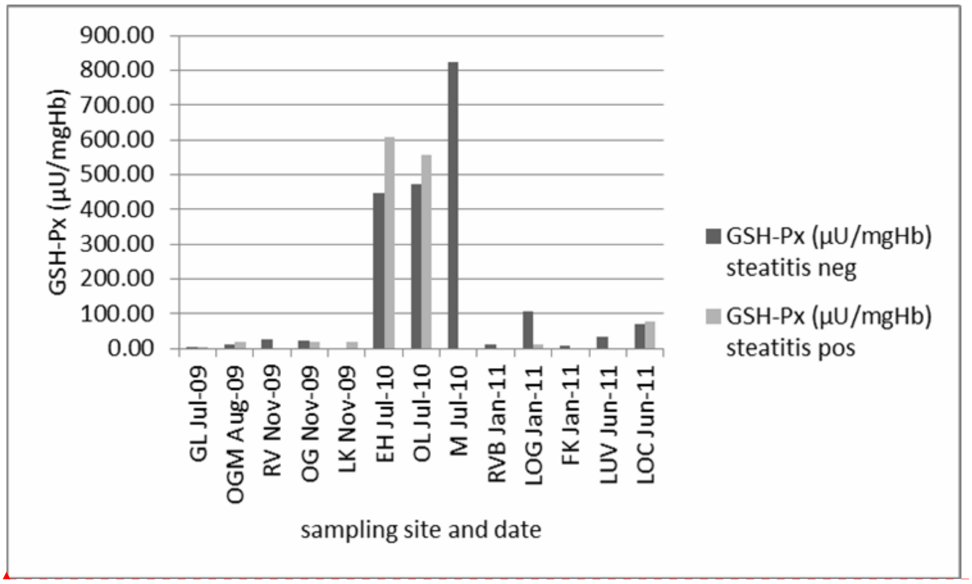

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Figure 22A: Average glutathione peroxidase (GSH-Px) values (µU/mgHb) of catfish with and without steatitis sampled from all sampling sites

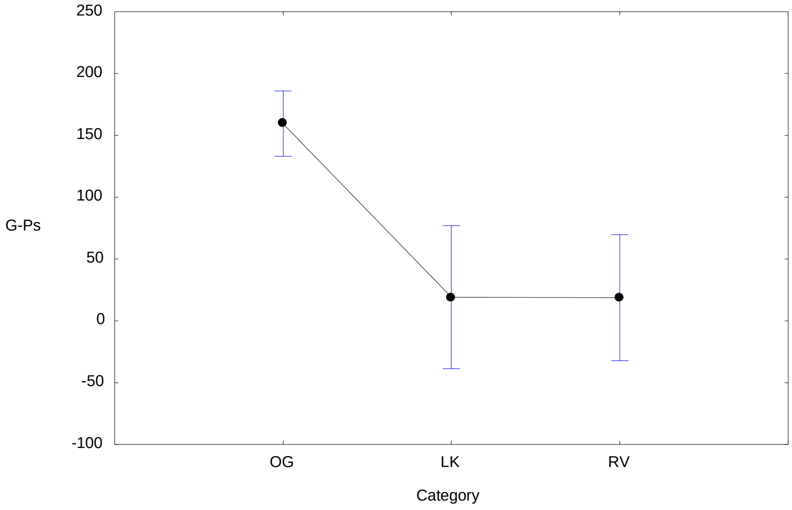

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Figure 22B: Comparison of averageerythrocyte glutathione peroxidase (G-Ps) values (µU/mgHb) of all catfish sampled from the Olifants Gorge sites (n=101) , Lunsklip Fisheries (n=21) and Reënvoël Dam (n=27).(p=0.01185, vertical bars denote +/- standard errors)

6

DISCUSSION

6.1 Prevalence of Steatitis

Steatitis was identified in catfish sampled from the Olifants River at the confluence with the Letaba River where the Olifants River enters the 9 km long gorge that opens into Lake Massingir in Mozambique. This same area has been the epicentre of the recent crocodile mortalities. During repeat samplings, an increasing prevalence of steatitis affecting up to 67% of catfish in this section of river was identified. A lower prevalence of steatitis was found in catfish sampled from Engelhard Dam on the Letaba River a few kilometres upstream of the confluence with the Olifants River and in catfish sampled from Mamba Weir where the Olifants River enters the western boundary of the KNP. In the Sabiepoort of the KNP, where the Sabie River flows through a gorge before entering Lake Corumana in Mozambique, a similar situation was identified with steatitis prevalence similar to that in fish from the Olifants Gorge. Crocodile deaths from pansteatitis have also been observed in the Sabiepoort (D Govender, SANParks, Skukuza, pers. comm. 2010). It is well documented that the Olifants River, draining the eastern side of the Mpumalanga Highveld, has been extensively affected by anthropogenic activity (Ashton, 2010; de Villiers and Mkwelo, 2009; Heath et al., 2010), and this was reflected in apreliminary assessment of metals found in the livers of catfish sampled from the Olifants Gorge (Dixon et el., 2011). There is, however, little commonality between pollution impacts in the respective catchments of the Sabie, Letabaand Olifants rivers. Steatitis could not be detected in fish from the Levuvhu and Crocodile rivers, both of which drain catchment areas subject to divergent anthropogenic impact and neither of which are dammed in or near the KNP. Neither could steatitis be demonstrated in fish from Reënvoël Dam, which is an entirely rain fed water body within KNP and distant from potential pollution sources affecting the Olifants River. It is possible that steatitis in catfish in Engelhard Dam was associated with upstream movementof fish from the Olifants Letaba confluence.

The sediment rich burden of the Olifants River is deposited annually in the Olifants Gorge where the flow of the river has been slowed by damming from Lake Massingir, a situation that arose after the sluices of the Massingir dam wall were raised in 2007 (Ferreira and Pienaar 2011). This brought about a drastic alteration of the aquatic habitat in the Gorge. Mamba Weir differs from the Olifants Gorge in that regular scouring, when the Phalaborwa barrage is opened, removes sediment build-up from the weir. Riparian vegetation along this section of the Olifants River includes Sycamore fig trees. Compared to the predominantly piscivorous diet of catfishfrom the Olifants Gorge, fruit of the Sycamore fig trees were the most common constituent of the stomach content of catfish sampled from Mamba Weir.

Alarge phosphate mine is situated near the town of Phalaborwa just east of the KNP near the western entry point of the Olifants River into the KNP. For a number of years prior to 2004, and once in 2008, abnormally high phosphate levels were recorded in the Olifants River within the KNP (J Venter, SANParks, Skukuza, pers. comm. 2012). These were ascribed to the discharge of tailings from the phosphate mine in Phalaborwa into the Selati River, a tributary of the Olifants River and to municipal sewerage discharges from the town of Phalaborwa.

This discharge was apparently discontinued after 2004 and,except during the winter of 2008, the measurement of phosphate levels in the Olifants River downstream in the KNP has shown acceptable limits (J Venter, SANParks, Skukuza, pers. comm. 2012). Dissolved phosphate is often the limiting nutrient governing phytoplankton growth in fresh water. The high levels of phosphate reaching Lake Massingir may have been a significant stimulus for phytoplankton growth resulting in the blooms observed in 2008 (J Myburgh, Faculty of Veterinary Science, University of Pretoria, pers. comm. 2008).
During periods of flooding the Olifants River carries large loads of silt. From time to time this is exacerbated when the Phalaborwa Barrage, on the Olifants River just west of the KNP, releases water to prevent debris build-up from damaging the sluice gates and to create space to accommodate the increased flow. Downstream, occasional fish kills have resulted from the oxygen depletion in the Olifants Rivercaused by this high silt burden.During such episodes in February 1999 and January 2004 large biomasses of silver carp were identified amongst the dead fish in the Olifants River within KNP, confirming the presence oflarge numbers of this species during these months (J Venter, SANParks, Skukuza, pers. comm. 2012).

The high prevalence of steatitis in captive catfish at Lunsklip Fisheries could be ascribed to the excessive intake of trout slaughterhouse waste that was observed rotting in the catfish pond and hence was likely to contain rancid fats.

6.2 Pathology

Necrosis of the adipose tissue resulting in steatitis was the main pathological change repeatedly observed in sharptooth catfish from the Olifants Gorge and was a consistent indicator of oxidative stress. The fat of *C. gariepinus* is distinct from that of other fish species in that a variation in colour of the mesenteric adipose tissues appears to be normal and fat colour can thus not be used as an indication of lipid peroxidation as in other species.During the study, specific pathology relating to lipid autoxidation and pansteatitis was also observed in a captive population of sharptooth catfish suffering from known nutritionally induced pansteatitis. Observation of these fish indicated that even in severely affected fish, the condition was not rapidly fatal. Results of an unpublished trial done by the author, and not included in this study, confirmed persistence of steatitis in fish from Lunsklip Fisheries after an 11 month period during which these fish were kept in a recirculated facility on a combination of live natural as well as commercial trout food. Despite a 6 month period through the winter during which the fish refused to feed and lost body condition, there was no reduction in the amount of stored mesenteric fat nor in the degree of steatitis in the fat. Similar observations were made in channel catfish (Goodwin, 2006) and in captive alligators (Larsen et al., 1983). Catch methods imposed by the environment in the Olifants Gorge (presence of hippos and crocodiles) have limited most samplings to catching by baited hook and line. The most severely affected fish may not have taken bait and would have been easy prey for crocodiles. This heavily favoured sampling of relatively healthy fish whereas the worst affected fish remained under-represented. Despite this, significant numbers of fish with pansteatitis were caught repeatedly in the Olifants Gorge and on a single occasion in the Sabiepoort.

Compared to control fish,catfish from most sampling sites carried heavy burdens of parasites. Frequent and varied pathology associated with parasites was observed in most of the wild caught fish and varied between sampling sites depending on parasite burdens and prevalence of specific parasites. Despite the associated pathology, presence of parasites appeared to be well tolerated by the fish. Fish from Reënvoël Dam, a population where steatitis could not be demonstrated, showed the heaviest parasite burdens. Focal steatitis with minimal lipopigment formation was observed only infrequently in association with parasites and no correlation could be demonstrated between parasite burden and steatitis.The steatitis described in association with lipoidosis and streptococcosis in cultured silver perch (*Bidyanus bidyanus*) (Deng et al., 2012) was similarly characterised by an absence of ceroid within the necrotic lesions in fat deposits observed in various organs. It is interesting to note the presence of metacercariae of *Centrocestus formosanus* in gills of catfish from KNP, as spread of this zoonotic parasite has been associated with introduction of carp from Asia elsewhere (Velez-Hernandez et al, 1998).

Various degrees of hepatic lipidosis and ceroidosis were observed in fish with severe steatitis.The clustering of haemosiderin around the perimeter of fat accumulation in livers of fish with steatitis is interesting in that redox cycling of iron has been implicated as a cause of iron catalysed lipid peroxidation (Minotti and Aust, 1992), however, such ferric iron compounds may be derived predominantly from haemoglobin catabolism (Mocca et al., 1984) in which case, bound to transferrin or sequestered as haemosiderin, the iron is well tolerated by the liver (Hayes, 2004). Splenomegaly was a consistent finding in fish with steatitis, as was splenic haemosiderosis, indicative of increased haemoglobin catabolism. Reduced feed intake by these fish appeared to result in the observed atrophy of the pancreatic acinar tissues. Muscular dystrophy as described in association with pansteatitis and vitamin E deficiency in other species of fish (Murai and Andrews, 1974, Roberts et al., 1979; Smith et al., 1972) was not observed in fish with pansteatitis from either the Olifants Gorge or Lunsklip Fisheries. This may reflect adequate dietary intake of vitamin E in these fish. Although vitamin E levels are known to deplete with acute pro-oxidant exposure, dietary vitamin E deficiency, rather than lipid peroxidation, has been implicated as the cause of muscular dystrophy observed in various species (Smith et. al., 1972). Although an integral part of the aetiology of pansteatitis, from the results of this study a primary vitamin E deficiency appears unlikely.

6.3 Steatitis and the Crocodile Mortality

During the sampling period from 2009 to 2011 a rising prevalence of steatitis was detected in fish sampled from the Olifants Gorge. However the level of obesity declined in the fish during this period. In the same period there has been a decline in crocodile mortality. The crocodile mortality in the Olifants Gorge began in the winter of 2008 after the sluices of Lake Massingir were raised in 2007 (Ferreira and Pienaar 2011). As the waters of the lake dammed back and flooded a large part of the gorge, the aquatic environment of the gorge was drastically altered with likely changes in the amount of fish available for crocodiles and catfish to feed off. Large crocodiles dying of steatitis in 2008 were found to be extremely fat. By 2009 emaciated crocodiles were also observed in the Olifants Gorge. Recent surveys by SANParks have indicated that younger crocodiles have moved into the gorge (D Pienaar, SANParks, Skukuza, pers. comm. 2012). Some of the surviving large crocodiles have become leaner, and wasted animals have been observed. On autopsy, such animals were found to be suffering from pansteatitis (D. Govender, SANParks, Skukuza,pers. comm. 2011). Similar wasting of catfish, suffering from steatitis, has been observed in the Olifants Gorge. An unpublished trial done by the author, and not included in this study, has confirmed that lesions of nutritionally induced steatitis in the fat of sharptooth catfish remain unchanged over time once the diet has been corrected. These fish are unable to fully access fat reserves damaged by steatitis. If unable to find or catch food, such fish will show wasting of body musculature despite retention of fat in the adipose depots. Danse and Verschuren (1978) have shown that stimulated lipolysis in rats was reduced in adipose tissues affected by steatitis.

As poikilothermic animals do not need energy to maintain homeothermy, starvation leading to death can be protracted over many months during which the starving animal will first utilize available fat reserves. Once fat reserves have become depleted or are no longer accessible, as in the case of pansteatitis, the animal will metabolize amino-acids from the body musculature as a source of energy. Such catabolic processes explain the wasting observed in both crocodiles and catfish chronically affected by pansteatitis in the Olifants Gorge. Poikilothermic animals that are unable to access damaged fat depots need to keep feeding to avoid muscular wasting and starvation. The captive fish at Lunsklip Fisheries were fed throughout the year. At the latitude of the Olifants River, wild crocodiles reduce feeding during the cooler winter months. The accelerated effects of starvation in those individuals affected by pansteatitis may partially explain why crocodile mortalities were restricted to the winter months.

6.4 Haematology and Blood Chemistry

In catfish with steatitis from both the Olifants Gorge and Lunsklip Fisheries the oxidative stress resulting in steatitis appears to have remained specifically limited in location to the adipose tissues as reflected in the pathology. For the selected haematology and blood chemistry parameters no significant differences could be detected between fish with and without steatitis from both the Olifants Gorge and Lunsklip Fisheries and may have been a reflection ofthe chronic nature of the condition in these fish. Significant numbers of catfish with depressed serum vitamin E values correlated to sites with high steatitis prevalence in the Olifants Gorge yet catfish from Lunsklip Fisheries with severe chronic steatitis showed normal serum vitamin E values.

Vitamin E plays an integral role in cell membrane integrity and growth, and in the case of rainbow trout, feeding of diets deficient in vitamin E or containing rancid oxidised fish oils has been held accountable for an increase in the number of polychromatocytes and crenation of immature erythrocytes (Moccia et al., 1984), both changes observed in blood smears from catfish from the Olifants Gorge and Lunsklip Fisheries but not from fish from Reënvoël Dam.

Under similar feeding conditions an increase in polychromatocytes with large rounded granular nuclei has also been reported from channel catfish (Murai and Andrews, 1974). In farmed channel catfish and rainbow trout, microcytic anaemia has been associated with feeding of rancid diets deficient in vitamin E (Smith, 1979; Murai and Andrews, 1974). Where oxidised fish oils were fed to farmed rainbow trout, development of anaemia could be prevented by dietary vitamin E supplementation (Moccia et al., 1984). The fact that fish from Lunsklip Fisheries did not develop anaemia despite developing severe steatitis may be a reflection of the consistently higher serum vitamin E levels found in these fish when compared to those of fish from the Olifants Gorge.The increased haemoglobin catabolism observed in vitamin E deficient rainbow trout, resulting from the degeneration and failure of polychromatocytes to fully mature, has been associated with resultant splenic haemosiderosis (Moccia et al., 1984). Intermittent increased erythrocyte fragility leading to haemolysis and increased erythrocyte turnover at times of oxidative stress challenge may have been responsible for the sometimes high levels of haemosiderin observed histologically in the splenic and hepatic macrophages of catfish from the Olifants Gorge (Huchzermeyer et al., 2011).

Changes in fluid partitioning between blood and lymph are likely to be rapid, variable and pronounced during stressful episodes, such as occur during sampling of wild fish. This is a reflection of the close association of blood and lymph in fish (Branson, 1993). Haematocrits in fish from all sampling sites were very variable, and no significant variation could be demonstrated in haematocrit values between fish with and without steatitis. Comparison of mean haematocrit values however, showed significant variation between fish sampled from the Olifants Gorge and fish sampled from Lunsklip Fisheries and Reënvoël Dam. The mean PCV value from the Lunsklip Fisheries fish was higher than for fish from the Olifants Gorge. This is interesting as the majority of fish from Lunsklip Fisheries were obese and suffering from chronic and protracted steatitis. Fish from Reënvoël Dam showed the lowest mean haematocrit values, but did not show the individual low values found in fish from the Olifants Gorge. Whereas all attempts were made to minimise sampling associated stress, osmotic disruption is impossible to avoid when catching fish. The variability of factors resulting in stress during sampling was likely to have impacted on haematocrit values of the fish limiting the usefulness of this parameter in wild fish.

The haemoglobin values of fish sampled from Lunsklip Fisheries, despite the high prevalence of steatitis, were significantly higher than haemoglobin values in fish from both the Olifants Gorge and Reënvoël Dam. Significantly, the haemoglobin values of fish from the Olifants Gorge were lower than those of fish from Reënvoël Dam, possibly indicating a higher erythrocyte turnover in these fish at the time of sampling, a change consistent with oxidative stress and low vitamin E levels and reflected in the observed haemosiderin deposits within hepatocytes and splenic and hepatic macrophages.

In aquatic systems oxidative stress studies have centred on depletion and induction of various antioxidant defences. In fish the antioxidant protective enzyme glutathione peroxidase shows higher basal activity than the enzymes superoxide dismutase and catalase when compared to other vertebrate systems, making glutathione peroxidase a suitable biomarker of oxidative damage in fish (Kelly et al., 1998). Measurement of blood values of the *in vivo* antioxidantsvitamin E and glutathione peroxidase showed no statistical difference between catfish with and without steatitis from the Olifants Gorge. Apparent differences in erythrocyte glutathione peroxidase values were detected between sampling sites with the highest values detected in fish sampled from the Olifants Gorge during July 2010. Similarly high values were also measured in fish sampled from Mamba Weir and Engelhard Dam at the same time. Different prevalence of steatitis was identified at these three sites. As there were no significant differences in the glutathione peroxidase values of fish with and without steatitis, the significance of these high erythrocyte glutathione peroxidase values should be viewed with suspicion. There was also no significant difference in mean erythrocyte glutathione peroxidase values between fish sampled from Lunsklip Fisheries and Reënvoël Dam, possibly as a result of the higher serum vitamin E values measured in these fish but also a reflection of the chronic nature of the condition in the catfish from Lunsklip Fisheries.

Mean serum vitamin E values in fish from the Olifants Gorge were significantly lower than mean values for fish sampled from Lunsklip Fisheries, but only slightly higher than mean values from fish sampled from Reënvoël Dam. These differences may reflect differences in dietary intake rather than being an expression of oxidative stress. It is interesting to note that a high percentage of individual fish with significantly reducedserum vitamin E values were sampled only from sites in the KNP where steatitis occurred in the fish.

From the pathology observed in catfish from Lunsklip Fisheries it is evident that steatitis observed in these fish was a chronic manifestation of prolonged continuous exposure. The adaptation of antioxidant protective mechanisms to chronic exposure to pro-oxidants, resulting in the chain reaction of lipid peroxidation (Kelly et al., 1998), may explain the similarity in erythrocyte glutathione peroxidase values found in these fish when compared to those of healthy fish from Reënvoël Dam. In the Olifants Gorge, the initiating cause of steatitis may have no longer been present at the time when lesions in the adipose tissues were noted, explaining why only some fish from the Olifants Gorge showed depleted serum vitamin E levels and why these did not necessarily correspond to presence of steatitis in these fish. During this study, only serum vitamin E levels were measured. Kelly et al., (1998) also ascribe a lipid protective role to ascorbic acid which acts through regeneration of tocopherol. Catfish from Lunsklip Fisheries were fed exclusively on trout slaughter house waste that would have reflected the high vitamin E and ascorbic acid inclusion in the commercial diet being fed to the trout. The slightly higher average serum vitamin E values from fish from Lunsklip Fisheries and the absence of fish with depleted serum vitamin E values are a likely reflection ofcontinuous high vitamin E and ascorbic acid intake by these fish, despite the apparent rancidity of the diet which they were fed. It is interesting to note that peroxidation of lipids in the adipose tissues resulting in the observed pansteatitis in these fish took place despite the consistently high serum vitamin E values that were measured.

Serum vitamin E and erythrocyte glutathione peroxidase determinations, shown to be useful in studying the acute manifestations of pansteatitis in cats(Fytianou et al., 2006), appeared to be of limited use as a monitoring tool for oxidative stress exposure of catfish in the lower Olifants and Letaba rivers. The diagnosis of oxidative stress in live fish from the Olifants Gorge is further complicated by the possible intermittent exposure and the resultant protracted chronic nature of the observed lesions. More work needs to be donebefore these or similar tests can be used to monitor the status of fish in these rivers. Determining malondialdehyde levels in the lipid fraction of serum by use of the thiobarbituric acid reactive substances test still needs to be evaluated as a monitoring tool.

6.5 Xenobiotics as Possible Cause of Steatitis

Coetzee et al. (2002) have demonstrated site specific bioaccumulation of metals in sharptooth catfish in the upper catchment of the Olifants River, an area heavily impacted by afforestation, mining, power generation, irrigation and industrial activities. Oberholster et al.(2011) have proposed that aluminium and iron bio-accumulation by fish in Lake Loskop may have induced the yellow fat observed in fish from that site. Baker et al. (1997) have however proposed that African catfish efficiently regulate iron status and are able to prevent tissue assimilation of dietary iron intake. This is an important adaptation to their benthic habitat, in which they are likely to consume sediment burrowing organisms with inadvertent ingestion of sediment. Deposition of sediments in the Olifants Gorge has been of concern regarding release of pollutants at this site and the ingestion of sediment-rich detritus was observed in fish sampled from the Olifants Gorge on the Mozambique border. Hepatic iron levels in fish from the Olifants Gorge were lower than in fish from Lunsklip Fisheries (Dixon et al.,2011), supporting the argument for increased haemoglobin catabolism in pansteatitis affected fish as the cause of these higher iron levelswhen compared to those in fish from Reënvoël Dam.

It is a well-established fact that many xenobiotics exert their harmful effects through oxidative damage to phospholipid structures in various organs and tissues. Exposure to such pollutants would be expected to result in detectable pathology in various organs. In catfish from the Olifants Gorge, significant pathology was restricted to the adipose tissues with the most intense and frequent lesions being present in the mesenteric fat reserves. Further pathology observed in the livers, spleen and pancreas was probably secondary to fat necrosis observed in the adipose tissues. Water-borne pollutants or bio-accumulated xenobiotics moving up the food chain would be expected to exert similar pathology in fish feeding at the same trophic level. Isotopic studies of the lotic food web in the Olifants Gorge have indicated that catfish from this locality had changed their dietary niche to a trophic level similar to that of tiger fish, an obligate piscivore, and that both tiger fish and catfish occupy a higher trophic level in the Olifants Gorge than in other river systems in the KNP (Woodborne et al., 2012), yet tiger fish in the Olifants Gorgedo not develop steatitis. Use of metallothionins, acetylcholinesterase and ethoxyresorufin-O-deethylase, biomarkers respectively of metal, organophosphate and carbamate, and organochlorine exposure, have been proposed for monitoring the state of tiger fish in the KNP (Van Vuuren, Wepener, Smit and Vlok, 2012) and may be found suitable for future monitoring of catfish.

6.6 Dietary Change and Steatitis in the KNP

The sharptooth catfish is a benthic opportunistic scavenger that is also known to actively hunt and prey on other fish (Skelton, 2001). Food source varied distinctly between sampling sites and prevalence of fish in the diet correlated with presence of steatitis in catfishfrom the Olifants Gorge and the Sabiepoort. Fish remnants observed in the stomach content of catfishfrom the Olifants Gorge, often in an advanced stage of digestion, frequently consisted of bones and scales of noticeably large unidentified fish. In the Olifants Gorge both crocodiles and catfish have been observed incidentally feeding off the carcasses of dead crocodiles (D Pienaar, SANParks, Skukuza, pers. comm. 2009) and crocodile fat afflicted with steatitis was found in the stomach contents of some catfish sampled from the Sabiepoort. In contrast, stomach content of catfish from Van Ryssen Dam contained onlyMozambique tilapia. These fish showed no signs of steatitis. Although factors associated with a fish diet appear to be associated with development of steatitis in catfish in the Olifants Gorge, these must be distinct from a natural healthy fish diet as observed in fish from Van Ryssen Dam and documented elsewhere in the literature(Spataru et al., 1987).

As a consequence of raising the sluices of Lake Massingir, the waters of the lake have extended into the KNP causing a habitat change in the Olifants Gorge that may have favoured a change in access to certain species of fish not normally consumed in large numbers by crocodiles and catfish. This could have exposed these animals to levels of polyunsaturated fatty acids in the diet to which they are not adapted. An increase in dietary polyunsaturated fat intake has been reported to result in pansteatitis in various animals. Wallach and Hoessle (1968) concluded that a change in diet from smelt (6.7% fat) to mackerel (29.9% fat) was the precipitating cause of pansteatitis in captive American alligators. Goodwin (2006)stressed the dangers of using diets high in fish oils for inappropriate species,and a change from Baltic and Mediterranean clupeids to Moroccan Atlantic pilchards was suspected to have been the cause of pansteatitis in northern bluefin tuna reported by Roberts and Agius (2008). Similarly pansteatitis could be induced in cats by feeding an oil rich fish-based diet (Fytianou et al., 2006).

The n-6 and n-3 fatty acids derived from linoleic and α-linolenic acids respectively are essential fatty acids that cannot be synthesized by animals (Steffens, 1997). The relative abundance of these fatty acids in the diet of animals is reflected in the composition of their fat tissues (Hoffman and Prinsloo, 1995; Steffens, 1997). The fatty acid composition of marine fish oils and in particular the high n-3 to n-6 ratio of polyunsaturated fatty acids contained in these oils is a reflection of the fatty acid composition of marine phytoplankton (Steffens, 1997). Whereas the ratio of total n-3 to n-6 fatty acids in marine fish oils typically lies between 5 and more than 10, that of freshwater fish is much lower, ranging from 1 to 4 (Steffens, 1997). In freshwater fish, as in marine fish, these fatty acid ratios are influenced by the composition of the diet. In nutrition trials the n-3 to n-6 fatty acid ratio in muscle lipid of sharptooth catfish could be manipulated from 0.1 in fish on a sunflower oil diet to 1.8 in fish on a cod liver oil diet (Hoffman and Prinsloo, 1995). The fat of captive farmed crocodiles,receiving a diet of chicken, beef and horse meat, had an n-3 to n-6 fatty acid ratio of 0.08 (Osthoff et al., 2010). By contrast the n-3 to n-6 ratio of fatty acids in the fat of wild crocodiles suffering from steatitis from the Olifants and lower Letaba Rivers was found to be

2 (Osthoff et al., 2010). Compared to the fat of farmed crocodiles, this reflected a much higher intake of n-3 fatty acids by crocodiles in the Olifants Gorge. Mean ratios of n-3 to n-6 fatty acids in catfish with mild or no steatitis sampled from Lunsklip Fisheries, Reënvoël Dam and the Olifants Gorge in November 2009 were 0.8, 1.32 and 0.96 respectively (Huchzermeyer et al., 2012). There appeared to be no significant difference in n-3 to n-6 ratio between fish from Lunsklip Fisheries with varying degree of severity of steatitis in fish. The fish with severe steatitis sampled from the Olifants Gorge, however, had an n-3 to n-6 fatty acid ratio of 2.87 compared to 0.92 in fish with only mild or no steatitis (Huchzermeyer et al., 2012). From these results it can be inferred that rancidity rather than high polyunsaturated fatty acid intake was the cause of the steatitis observed in catfish from Lunsklip Fisheries. By extension of this argument it would seem unlikely that rancidity associated with intake of dead rotting fish could have been the cause of pansteatitis in the Olifants Gorge catfish and crocodiles.

Steatitis was confirmed in catfish from primarily three locations within KNP; the Olifants Gorge and lower Letaba River at the confluence with the Olifants River, from Engelhard Dam on the Letaba River upstream of the Olifants-Letaba confluence and from the Sabiepoort. Catfish with steatitis may have migrated from the Olifants Gorge upstream to Engelhard Dam and this may be one explanation for the presence of steatitis affected fish at this site, but the Sabiepoort on the Sabie River is in an entirely separate catchment. The anthropogenic activities resulting in potential pollution of the rivers differ greatly between these two catchments providing further argument against primary pollution related aetiology of the pansteatitis incidence at these two sites. Common to both the Olifants Gorge and the Sabiepoort is the damming of the rivers in Mozambique to form lakes Massingir and Corumana respectively. The inlets of both lakes extend back into the KNP flooding the respective gorges where these rivers previously traversed the Lebombo Mountains as fast flowing rapids.

Silver carp (*Hypophthalmichthys molitrix* Valenciennes), an invasive species outside of its home range in East Asia (Kolar et al., 2005), were introduced into Mozambique from Cuba and are known to occur in Lake Massingir(Skelton, 2001). Silver carp are also known to have escaped into the Olifants River in South Africa and may have spread downstream (P Skelton, South African Institute of Aquatic Biodiversity, Grahamstown, pers. comm. 2012). This fish is a specialized plankton feeder that by preference feeds off phytoplankton and is an important consumer of cyanobacterial blooms, with *Microcystis* constituting 20-98% of the food bolus during some seasons (Kolar et al, 2005). Such blooms have been observed near the inlet to Lake Massingir (D Pienaar, SANParks, Skukuza, pers. comm. 2009). Phytoplankton naturally contain large quantities of α-linolenic acid and other n-3 polyunsaturated fatty acids in particular eicosapentaenoic acid C20:5n-3(EPA) and docosahexaenoic acid C22:6n-3(DHA) (Steffens, 1997). Intake of these fatty acids is reflected in the adipose tissues of silver carp, with these two fatty acids, in one study, making up to 5.28 and 3.4% of body fat triacylglycerols respectively (Buchtová and Ježek, 2011). As a result of the high levels of C20 and C22 fatty acids, consumption of the fat of silver carp has been proposed to have health benefits to human consumers equivalent to those of oil-rich marine fish (Buchtová and Ježek, 2011, Steffens, 1997).

A significant proportion of the essential fatty acids derived from the diet are stored in the adipose tissues of animals and of these, DHA is deposited into the adipose tissues preferentially over EPA (Connor et al., 1990). Although the polyunsaturated fatty acids are mobilised more rapidly from the adipose tissues than saturated fats, DHA, the most polyunsaturated fatty acid has been shown to be poorly mobilised(Connor et al., 1996). The higher levels of DHA found in the mesenteric fat of catfish from the Olifants Gorge with steatitis (11.06%) compared to mesenteric fat of those without steatitis (5.09%) strongly points to a higher intake of DHA in the diet of those fish that developed steatitis at this site (Huchzermeyer et al., 2012). A similar differentiation was not observed in the mesenteric fat of catfish with mild and severe pansteatitis from Lunsklip Fisheries, supporting the argument for a different dietary aetiology, most likely associated with rancidity of fats in the slaughter house waste fed to these fish.

Steatitis could not be demonstrated in tiger fish sampled from the Olifants Gorge. Tiger fish, having evolved as obligate piscivores, are likely to have developed anti-oxidant protective mechanisms better enabling them to cope with the consumption of higher levels of dietary polyunsaturated fats than the omnivorous catfish.

Silver carp, a schooling species,seasonally migrate upstream into rivers from the still waters of lakes to spawn (Skelton, 2001). Spawning is associated with an increase in suspended alluvium and a rise in water level of the river and occurs over an 8 to 10 week period (Kolar et al., 2005). Thespawning migration takes place during early to midsummer and in the Olifants Gorge this mass migration may account for intense dietary exposure of crocodiles and catfish to this species and the consequential intake of excessive polyunsaturated fats during a short period each year. This may explain the increase in crocodile mortality during the subsequent autumn and winter as observed in 2008 and to a lesser extent in the following years. In the Olifants Gorge fish surveys are conducted by KNP scientists during the winter months when the river can be safely accessed and the waters of the river become clearer (A Deacon, SANParks, Skukuza, pers. comm. 2012). Movement of silver carp into the Olifants Gorge may thus easily have been over looked. It is proposed that by raising the sluices of Lake Massingir, the resulting habitat change that occurred in the Olifants Gorge may have seasonally favoured access by crocodiles and catfish to large schools of silver carp. The situation in the Sabiepoort is less clear as presence of silver carp in this lake has not been confirmed; however, similarity in habitat to the Olifants Gorge also points to consumption of fish rich in polyunsaturated fats as the cause of steatitis at this site.

7 CONCLUSION AND RECOMMENDATIONS

This study has shown that sharptooth catfish in the Olifants Gorge develop steatitis and that during the study period there was an increasing prevalence of steatitis in these fish. Co-existence of old and recent lesions indicatedan on-going incitement of steatitis. Catfish have been shown to be a suitable monitoring species for pansteatitis in crocodiles as they appear to show similar sensitivity to pansteatitis within their overlapping habitat. Whereas the Nile crocodile is classed as endangered, the sharptooth catfish is an abundant species that in the Olifants Gorge and Sabiepoort is relatively easy to sample.

Several explanations for the cause of pansteatitis in crocodiles and fish in the Olifants Gorge have been proposed. Bio-accumulation of one or more xenobiotics resulting from upstream pollution cannot be ruled out. However, lack of known pollutant related pathology in catfish from the Olifants Gorge and the fact that steatitis was found in catfish in the Sabiepoort, which would have a different pollution profile, makes this aetiology seem unlikely. Consumption of large quantities of dead rotting fish seems unlikely as a cause of the pansteatitis as mass fish mortality has not beena consistent finding at the sites where steatitis was observed and the fatty acid profile of farmed catfish fed rancid fish fats differed from that of catfish suffering from steatitis in the Olifants Gorge. It would also seem unlikely that consumption of catfish suffering from steatitis alone could have precipitated the pansteatitis outbreak in the crocodiles. More convincingly, this study raises the possibility thatseasonal abundance of fish species rich in n-3 polyunsaturated fats in the diet of catfish and crocodiles in the Olifants Gorge may have resulted in development of pansteatitis in these two species and that habitat change brought about by damming of rivers extending into KNP influenced access to such fish. However, it is not clear whether the same factors were responsible for the development of pansteatitis in catfish from the Sabiepoort. The situation at this site warrants further study.

The association between nutrient pollution of the aquatic environment, eutrophication and the influence of phytoplankton on fatty acid composition of fish consuming such phytoplankton needs further study. The role of phosphate discharges into the Olifants River, the impact of dam building and subsequent silt and nutrient entrapment on relative fish species abundance, and particularly the presence of alien silver carp within the KNP need to be investigated.Pansteatitis in wild fish is a unique finding and although work is being done on fish from Lake Loskop (J Myburgh, J Steyl, Faculty of Veterinary Science, University of Pretoria, pers. comm. 2009); further work needs to be done to establish the extent to which steatitis may be present in fish in other artificial lakes inpolluted catchments.

The analysis of heavy metals in the fish tissues collected during the course of this study has not been completed. The possibility of bioaccumulation of iron following the consumption of phytoplankton, (as documented in Lake Loskop by Oberholster et al. (2011)), still needs to be investigated in the fish of Lake Massingir and the Olifants River Gorge.

Comment [a1]: Inseted full stop

The study emphasizes the ecological importance and complexity of oxidative stress in a disturbed aquatic environmentand the risk associated with the presence of alien invasive fish species within our national parks. It is recommended that the distribution of alien fish species within rivers traversing the KNP is investigated and that in dams within KNP and elsewhere in South Africa the long-term effects of hydrodynamic change and nutrient entrapment on the aquatic food chain are monitored with particular reference to the health of top aquatic predators such as crocodiles.

8 BENEFITS

The study provides SANParks with information on the extent of pathology in sharptooth catfish in the Olifants River and other water bodies in KNP and the risks attached to damming of rivers that traverse the Park, particularly where these are nutrient enriched. Such information is important to guide conservation policy and decisions regarding use of water and the safety of fish consumed from such waters. Distinct parallels in the pathology observed in crocodiles and catfish in the Olifants Gorge have been demonstrated. Within KNP the distribution of crocodiles and catfish overlap. Although sharptooth catfish were thought to form a major part of the diet of crocodiles within the river gorges of the Park, this study has provided valuable knowledge suggestinghow the deleterious impacts of hydrodynamic change on the fresh water ecosystems within KNP, brought about by damming of rivers, has allowed crocodiles and catfish to access phytoplankton-feeding fish species in their diet at levels to which they are not adapted.

Sharptooth catfish are an abundant and ubiquitous species in southern African rivers. The results of this study confirm that this fishis a suitable monitoring species in the aquatic environment and can be used by SANParks to monitor fish and indirectly crocodile health. Demonstration of a direct causal relationship between pansteatitis and one or more pollutants could not be demonstrated, but cannot be ruled out. Co-workers have received the entire sample set for toxicological analysis. Should results of such analyses show a causal relationship this will provide SANParks with further information to insist on prevention of pollution entering KNP.

The current study illustrates how the complex interaction of nutrient pollution, construction of dams outside of the borders of South Africa and introduction of alien fish species into such lakes can threaten the biodiversity of the KNP rivers and the future of the Nile crocodile in the KNP. The research was undertaken as part of the CROC initiative at the request of SANParks.The study provides South Africa and its authorities with information to insist that environmental controls ensuring the quality of water in our rivers are implemented and provides SANParks with information to insist on prevention of pollution and alien fish species entering KNP, thereby ensuring the biodiversity of the KNP rivers and securing the future of the Nile crocodile in the KNP.

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