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

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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), pansteatitis in fish and crocodiles has been shown to be a serious and increasing problem in large man-made lakes fed 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 catfish sampled 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 was also 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, and manuscripts 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 results of the study emphasize the ecological importance and complexity of oxidative stress in a disturbed aquatic environment and 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 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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1 HISTORY AND BACKGROUND OF STUDY

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 study was 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 elucidated previously.

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 these species 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). Catfish specimens 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 catfish were 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 Letaba rivers. 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 specimens collected 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 Sabiepoort was 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. Catfish collected 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 in catfish sampled 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 catfish at 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).

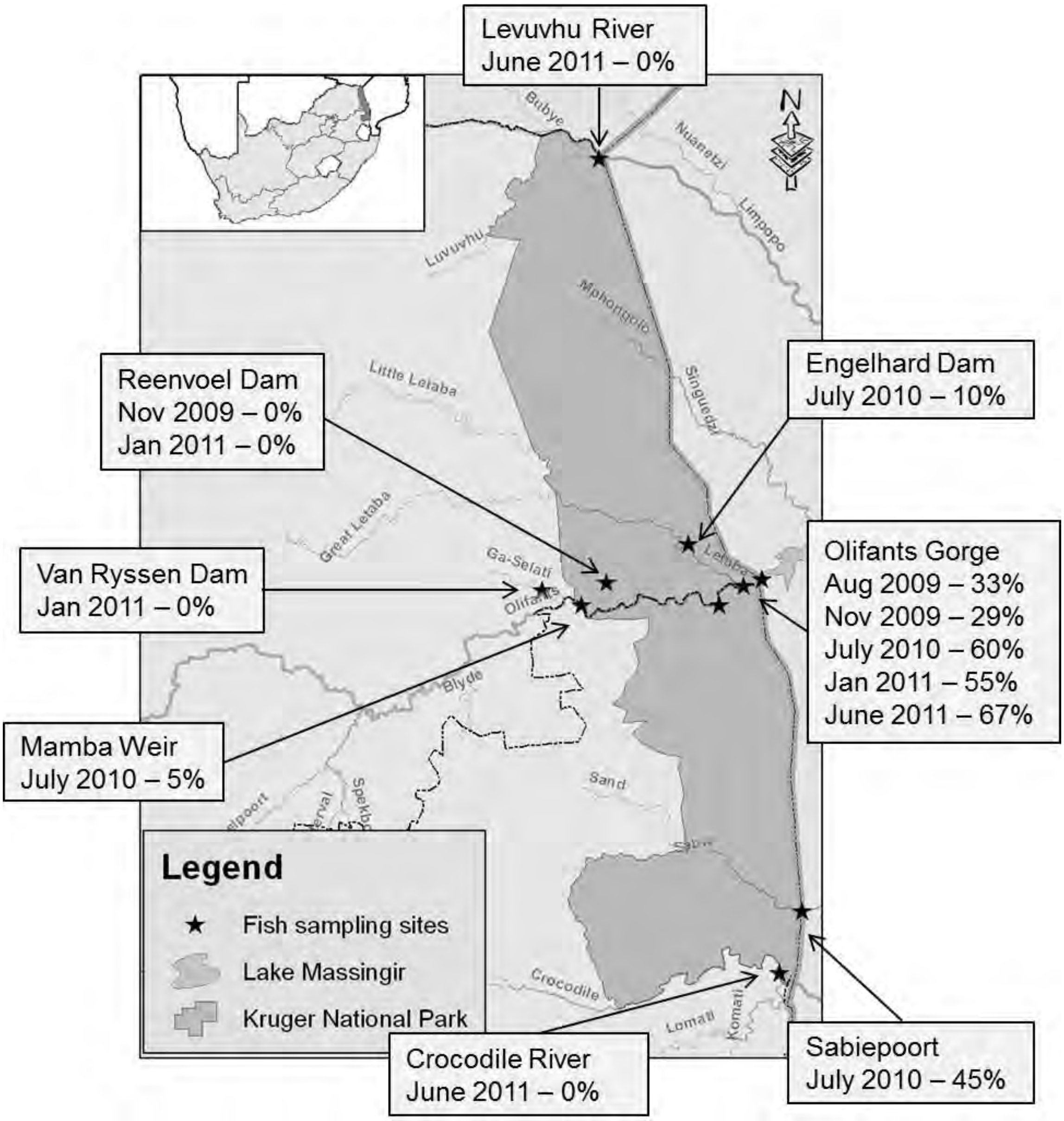

Figure 1: Macroscopic steatitis prevalence as percentage of sampled catfish from various sampling sites in Kruger National Park during the period 2009-2011

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
August 2009	Olifants Gorge	33	9
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

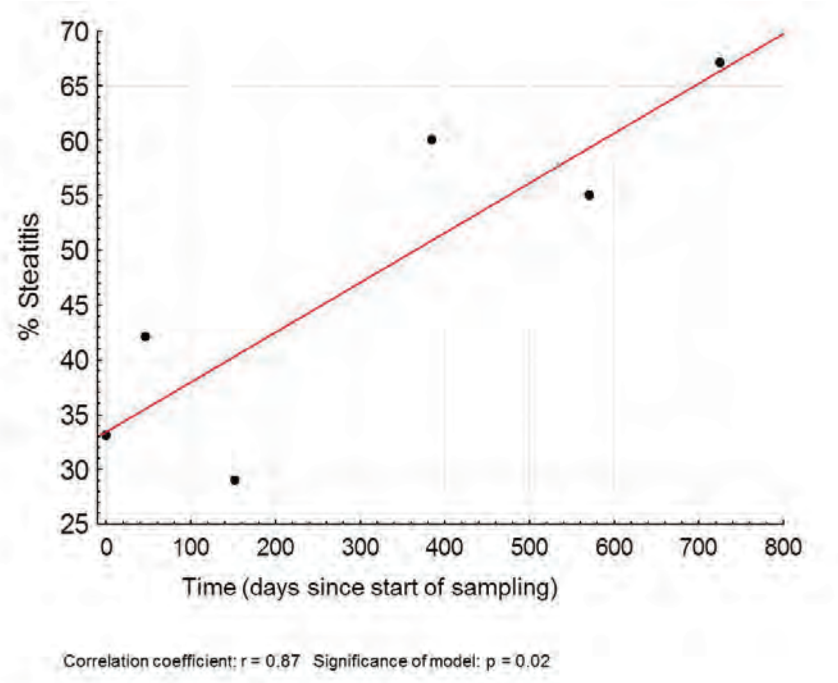

Figure 2: Prevalence of steatitis in catfish sampled from various localities along the Olifants and lower Letaba rivers in the Kruger National Park from 2009 to 2011

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.

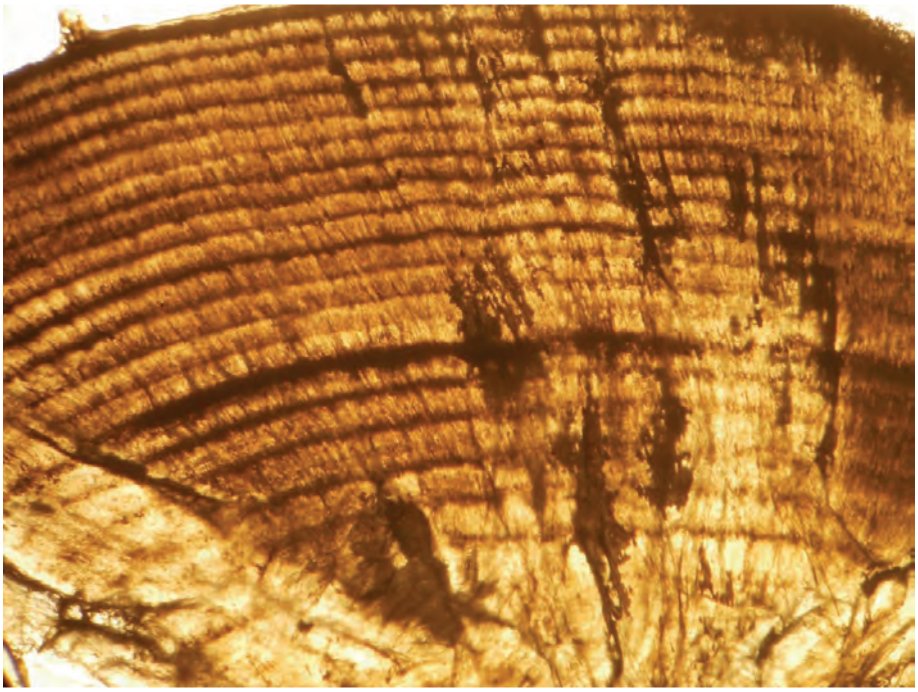

Figure 3A: Growth rings in the otolith of a 19 year old catfish specimen from the Olifants Gorge

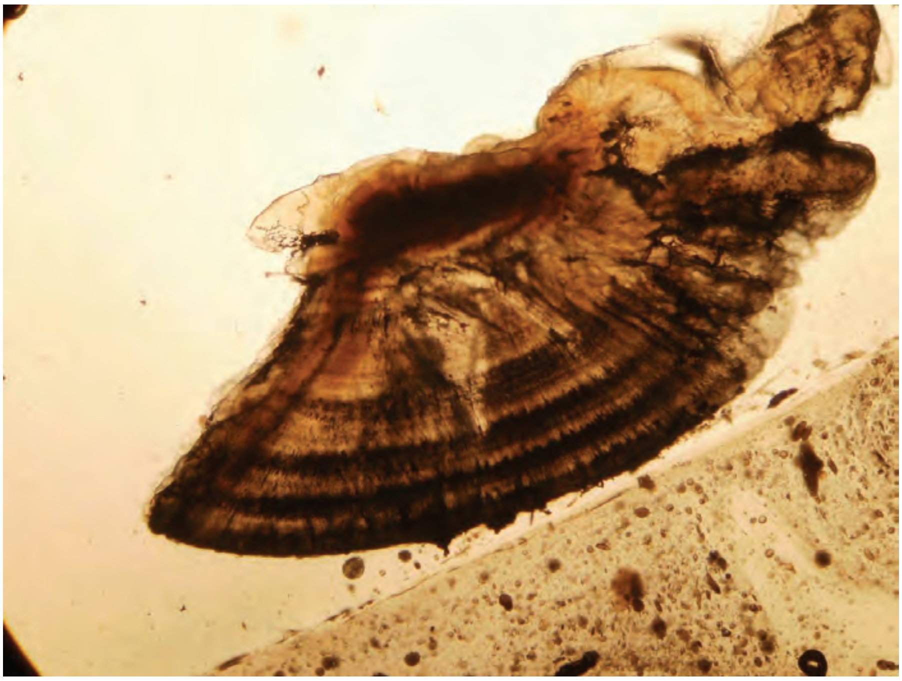

Figure 3B: Growth rings in the otolith of a 4 year old catfish specimen from the Olifants Gorge

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 adhered to 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).

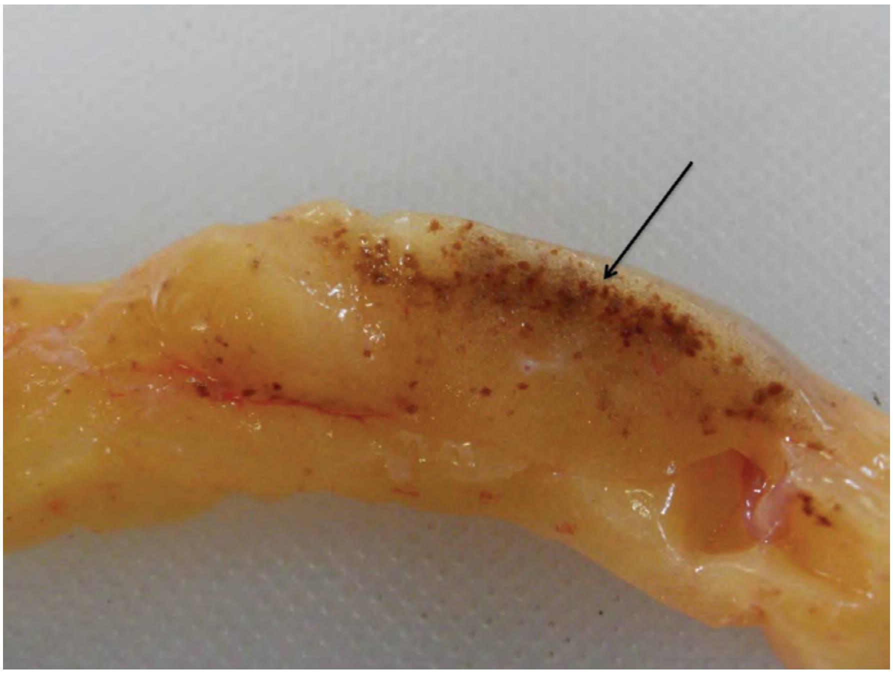

Figure 4A: Early steatitis lesion in catfish sampled from the Olifants Gorge during July 2010. Note the small sharply circumscribed foci of fat cell necrosis and associated ceroid deposition imparting the characteristic brown colour (arrow)

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

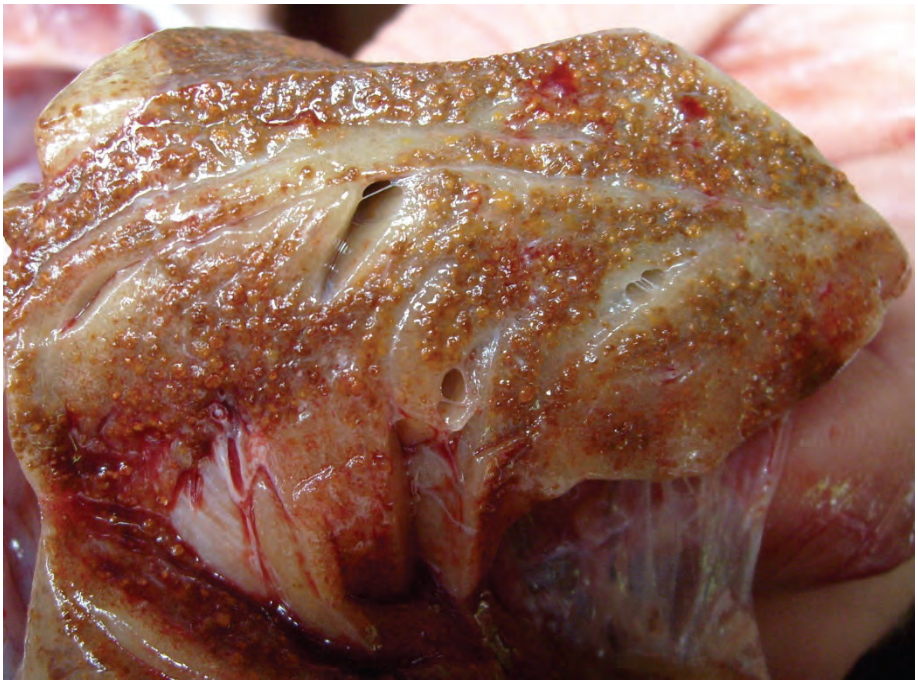

Figure 4C: Cross section of mesenteric adipose tissue from a catfish specimen collected from the Olifants Gorge in November 2009 showing typical severe steatitis. Note brown granuloma formation within the adipose tissue

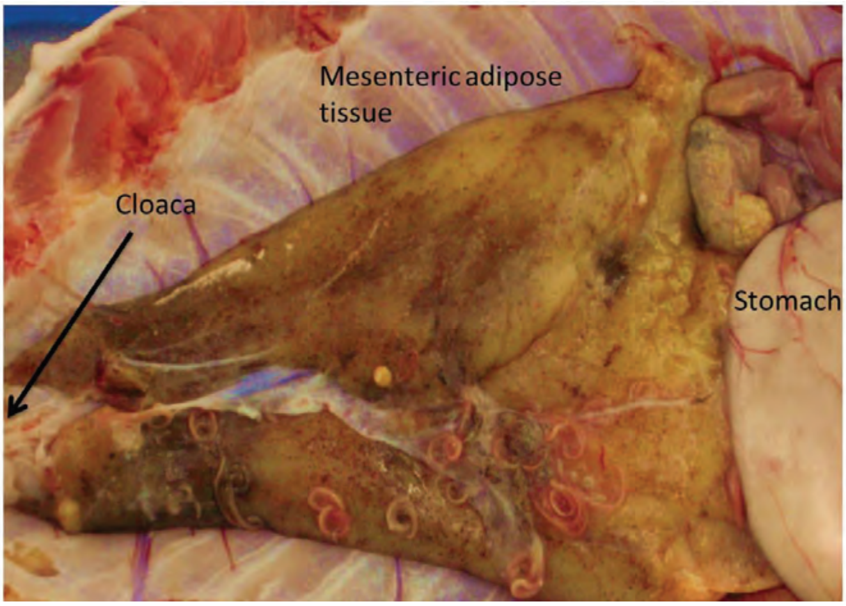

Figure 5: Steatitis of the mesenteric adipose tissues in a catfish sampled from the Olifants Gorge during June 2011. Note that the caudal portion of the mesenteric fat body appears more severely affected

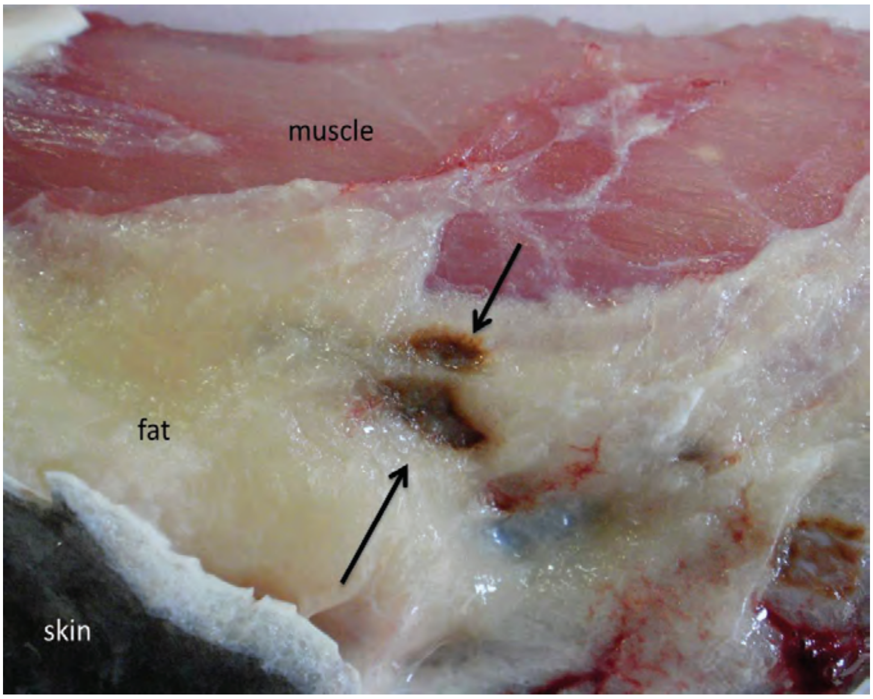

Figure 6A: Focal fat necrosis (arrows) in the pectoral fat of a catfish sampled from the Olifants Gorge during July 2010

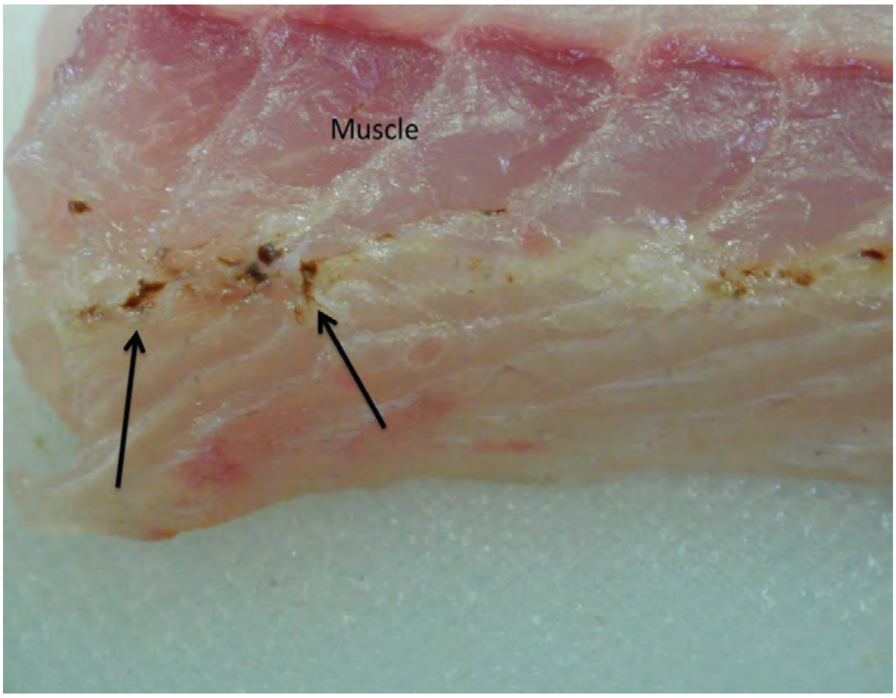

Figure 6B: Focal steatitis (arrows) in the intramuscular fat of a catfish sampled from the Olifants Gorge during July 2010

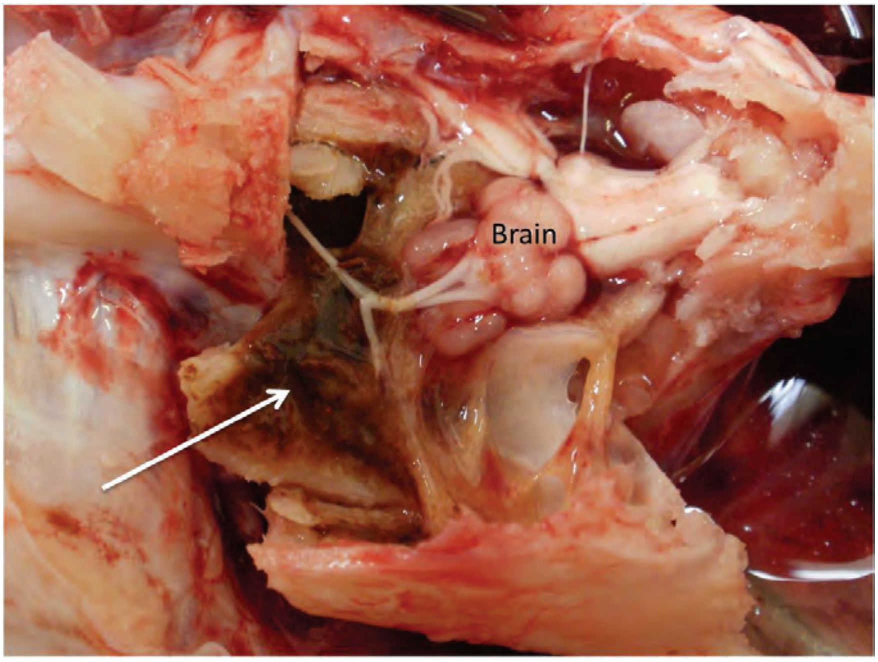

Figure 6C: Generalised steatitis in the fat surrounding the brain of a catfish sampled from the Olifants Gorge during July 2010. Note brown discolouration of fat (arrow) adhering to the opened cranium

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 characterize the 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.

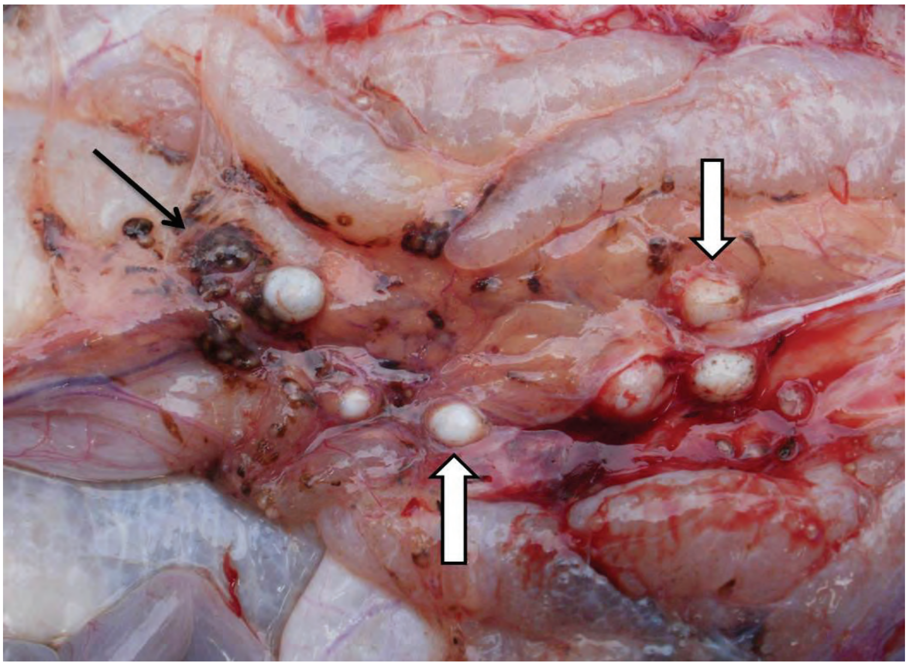

Figure 7: Melanin deposition (black arrow) associated with digenean trematodes cysts (block arrows) in the caudal mesenteric fat between the male accessory sexual glands of a catfish sampled from Reënvoël Dam in KNP

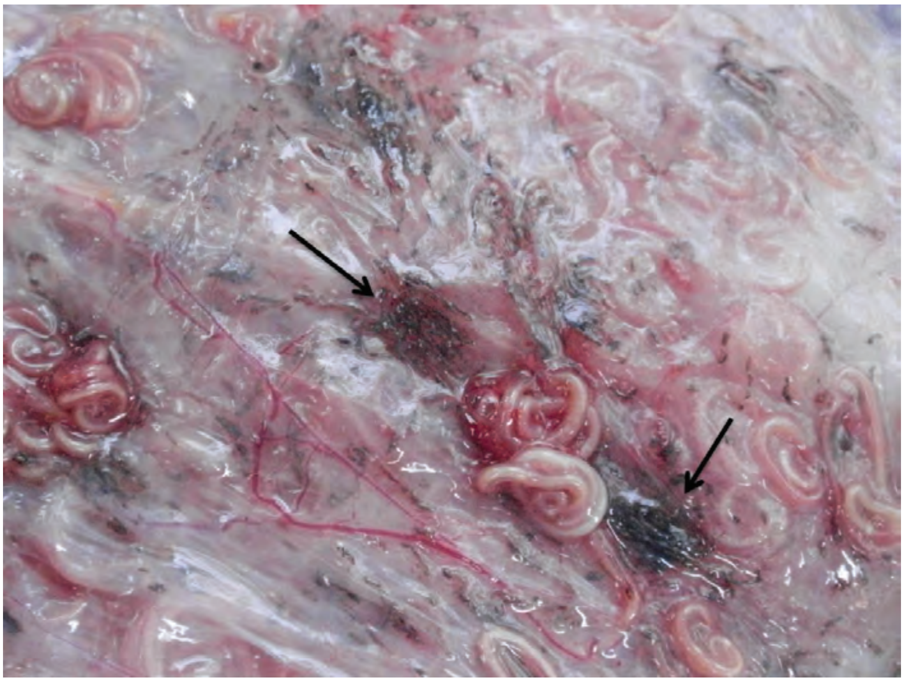

Figure 8: Melanin deposition (arrows) in vicinity of larval nematodes in the mesentery overlying the mesenteric fat in a catfish sampled from van Ryssen Dam at the FOSKOR mine in Phalaborwa

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.

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

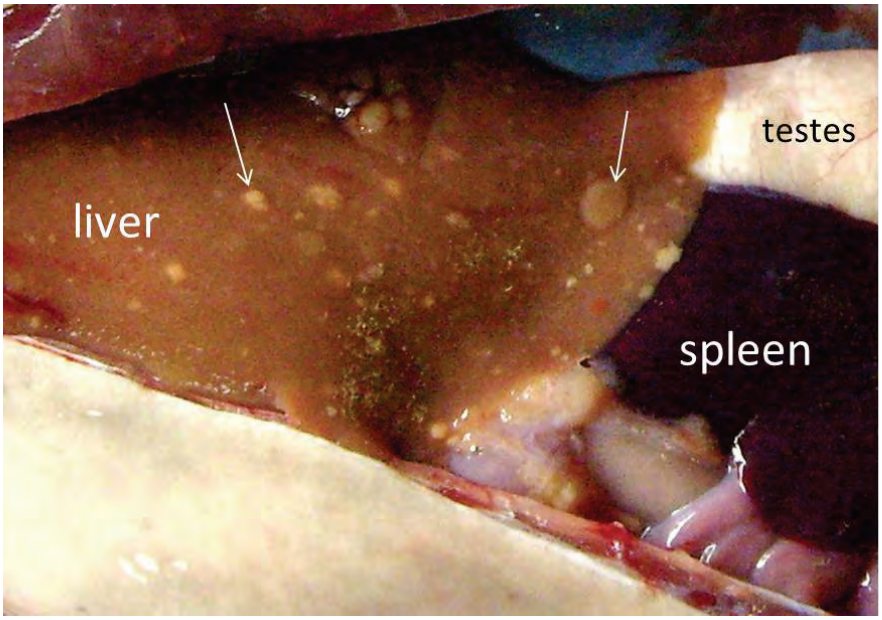

Figure 9B: Focal fat deposits beneith the liver capsule from a catfish with steatitis sampled from Lunsklip Fisheries during November 2009

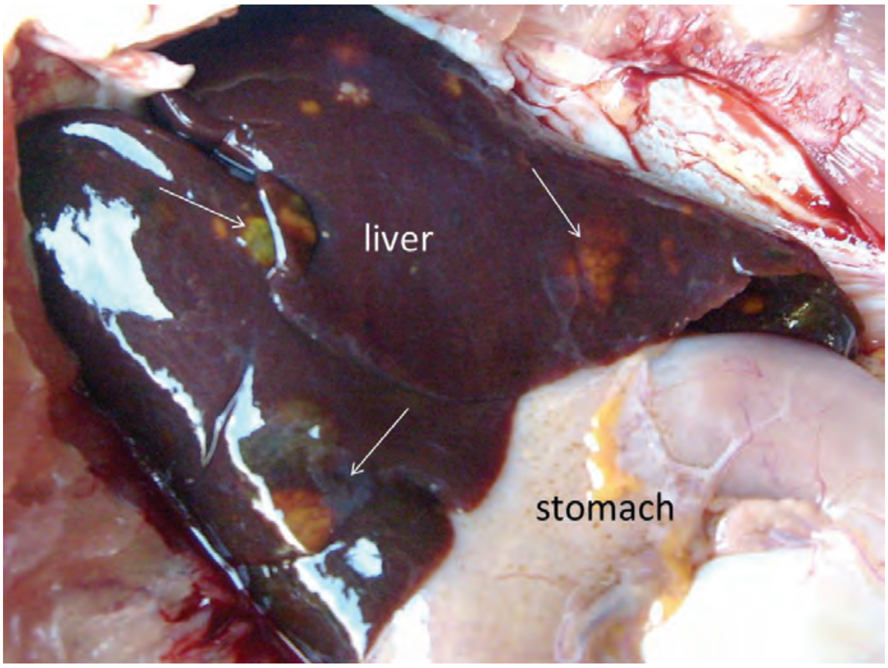

Figure 9C: Liver from a catfish sampled from Reënvoël Dam during November 2009. Arrows show damage associated with parasitic cysts in the liver parenchyma. Note the dark colour and sharp liver borders of an otherwise healthy liver

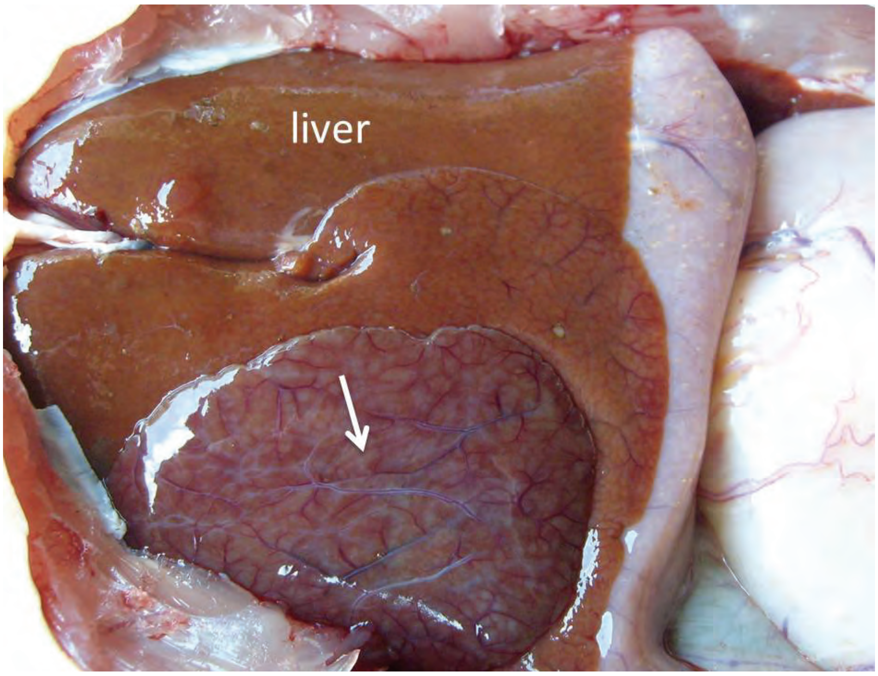

Figure 9D: Outgrowth of regenerating hepatic tissue (arrow), probably associated with parasitism, in a liver from a catfish sampled from Reënvoël Dam during November 2009. Note the normal brown colour of the liver

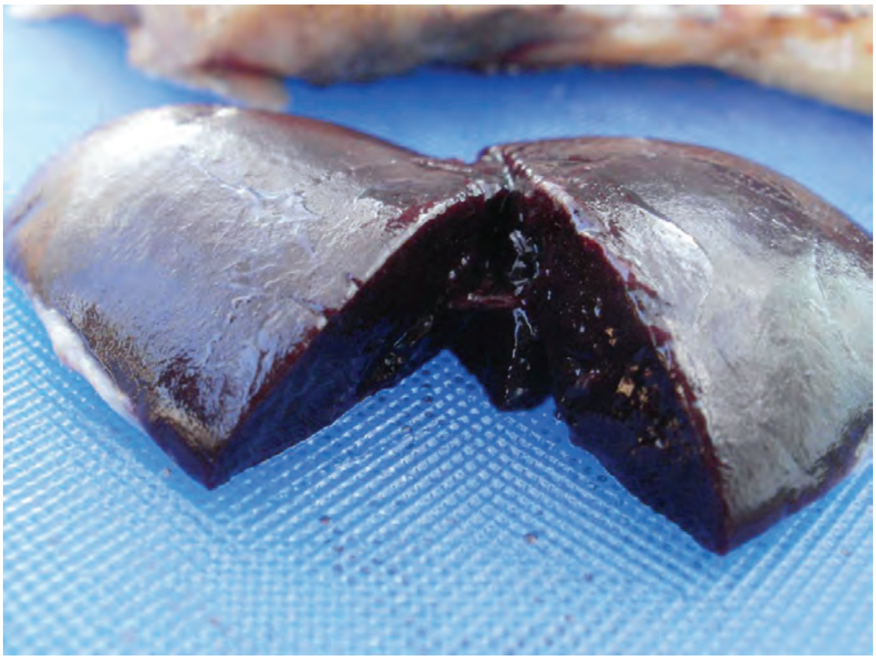

Figure 10A: Typically enlarged spleen of C. *gariepinus*, from the Olifants Gorge, suffering from fat necrosis and steatitis

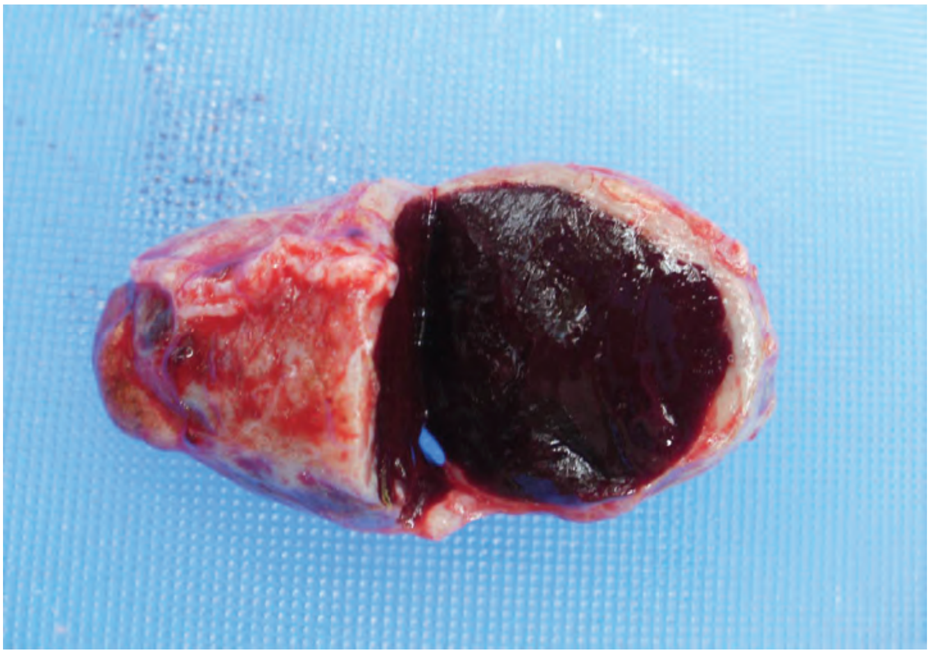

Figure 10B: Spleen of a catfish from Lunsklip Fisheries suffering from severe chronic steatitis showing prominent capsular thickening and splenomegaly. Note the rounded nature of the normally flat spleen

Catfish sampled from Reënvoël Dam showed no steatitis and were used as a negative control population (Table 1). The gills of catfish sampled from Reënvoël Dam during November 2009 appeared 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 2011 had full stomachs, the ingesta consisting of fish 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.

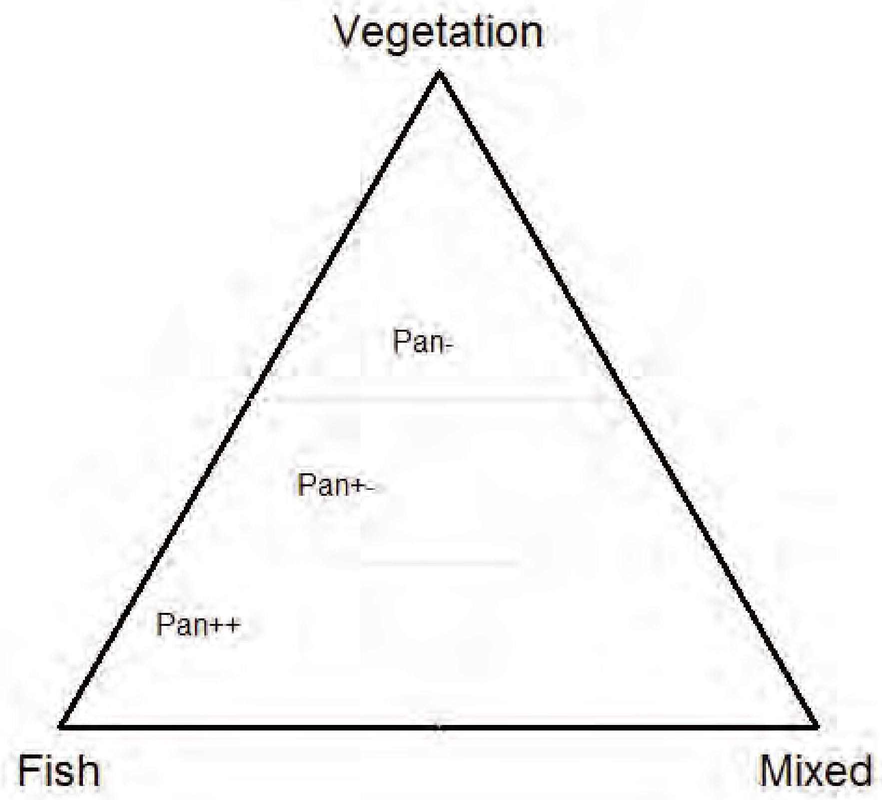

Figure 11: Stomach content analysis of sharptooth catfish showing that the population with pansteatitis (Pan++) at a site where pansteatitis was prevalent had stomach contents with a higher proportion of fish than vegetation, or invertebrates and detritus (mixed) when compared with the population of fish that did 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.

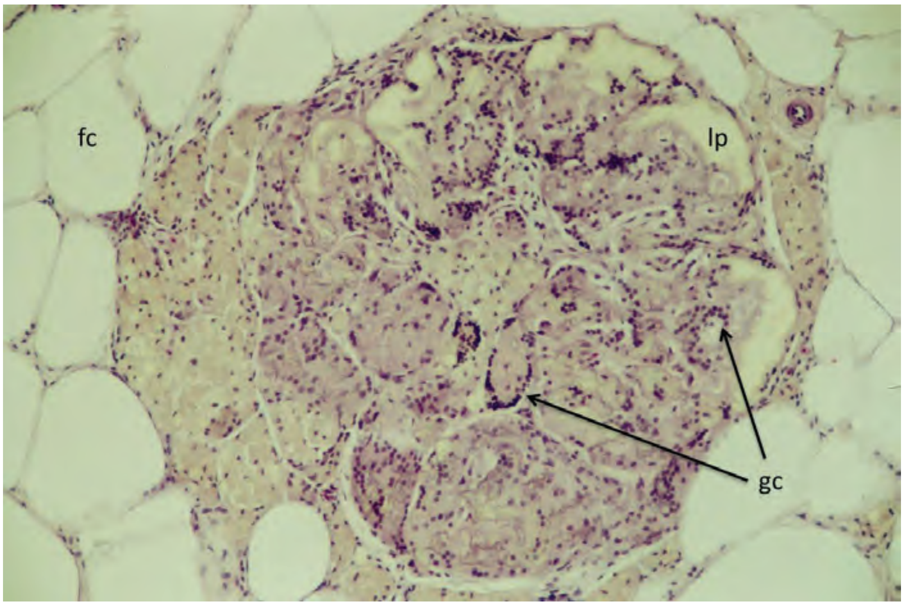

Figure 12A: Giant cell formation (arrows) in a typical steatitis lesion in adipose tissue from a catfish sampled from the Olifants Gorge in July 2009. HE X100

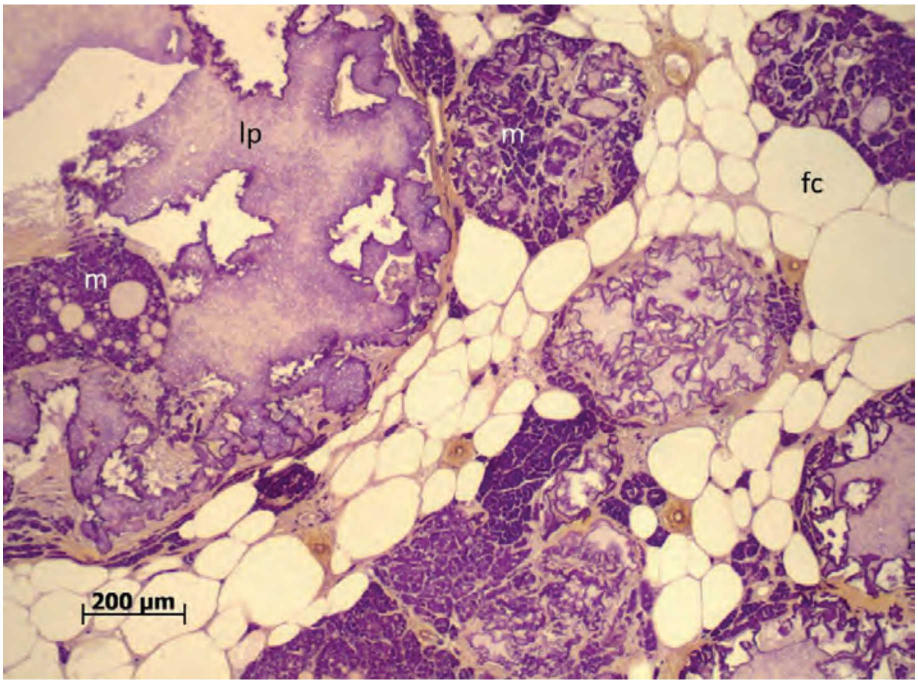

Figure 12B: Positive staining ceroid pigment (purple) within the lipopigment remnants (lp) of ruptured adipocytes and macrophages (m) in mesenteric fat of a catfish sampled from the Olifants Gorge during November 2009. Adipocytes (fc). (GAF)

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.

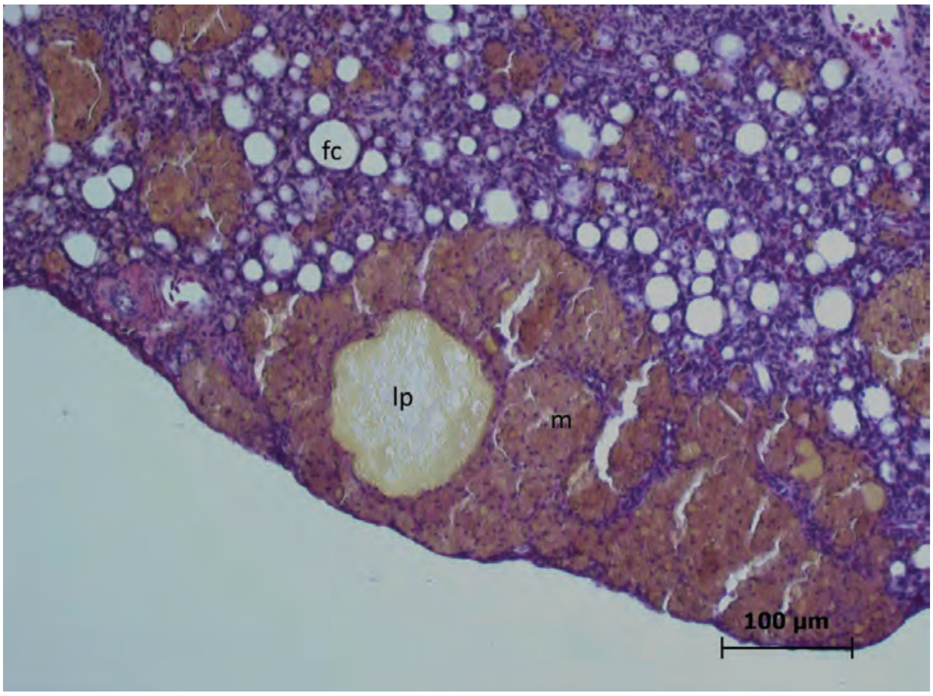

Figure 13: Atrophied mesenteric adipose tissue showing inflammation typical of steatitis in a catfish sampled from the Olifants Gorge during June 2011. Note small size of adipocytes (fc) and aggregates of macrophages (m) containing ceroid, surrounding areas of fat necrosis containing lipopigment (lp).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

In haematoxylin eosin stained sections of affected fat, varyingly sized foci 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 containing intracellular ceroid (Fig. 12B), a pigment 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.

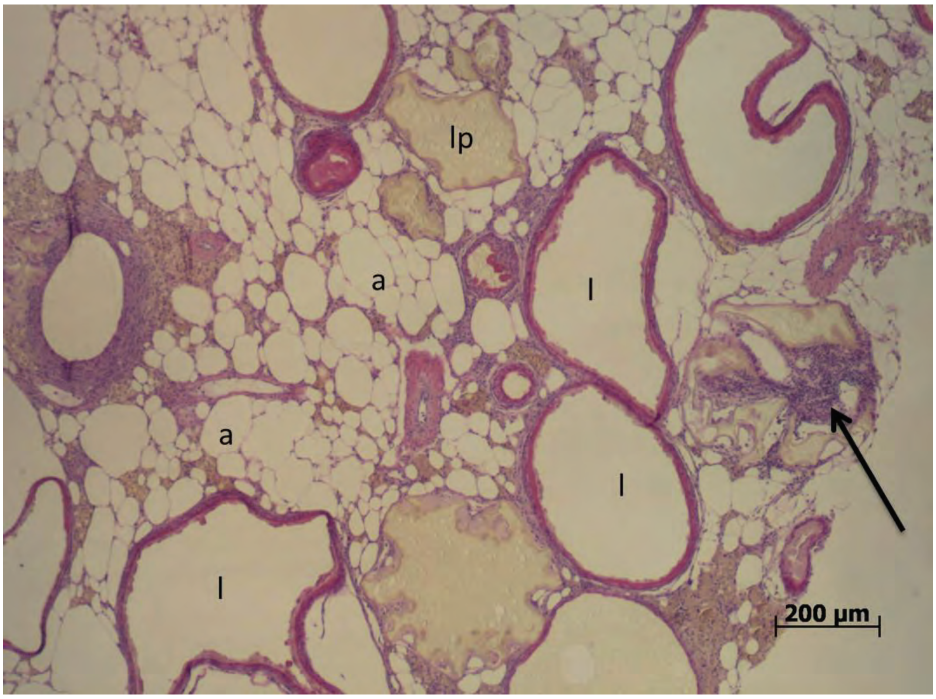

Figure 14: Advanced stage of fat necrosis and steatitis in mesenteric fat of a catfish sampled from the Olifants Gorge during June 2011. Note the apparently empty lacunae (l) where necrotic remnants of oxidised fat (lp) have been lost during processing, surrounded by an epithelioid sheath. Adipocytes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

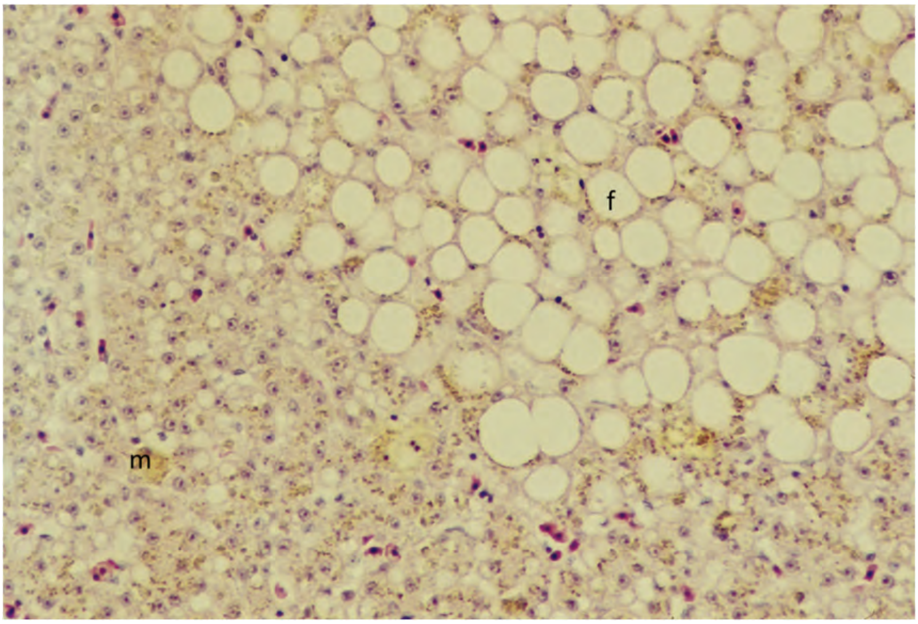

Figure 15A: Liver section of a catfish sampled from the Olifants Gorge during November 2009. Note distinct focus of fat vacuoles (f). (HE X200)

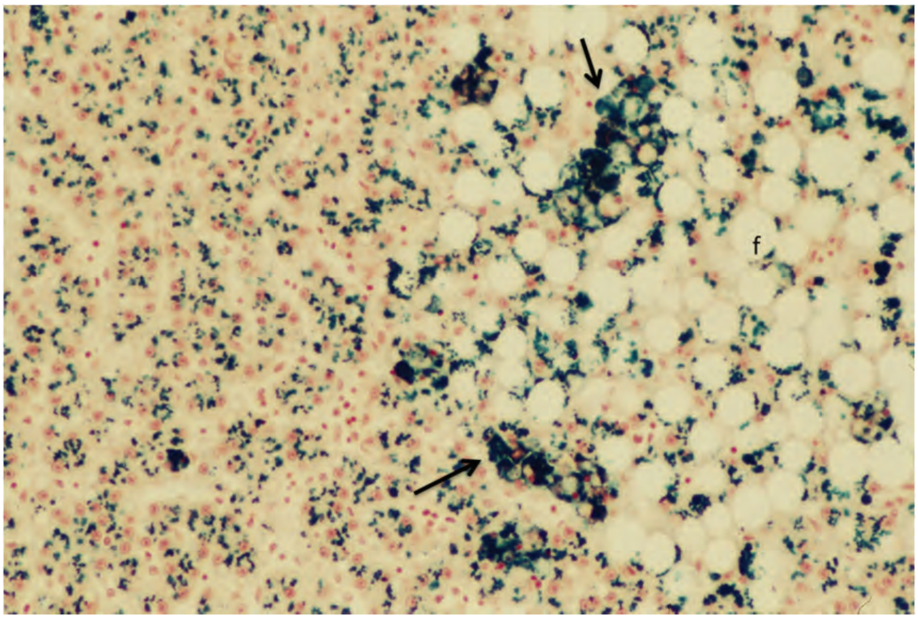

Figure 15B: Liver section of a catfish sampled from the Olifants Gorge during November 2009. Note distinct focus of fat vacuoles (f) and clustering of haemosiderin (arrows) on the perimeter of this focus. (Perl's Prussian blue, X200)

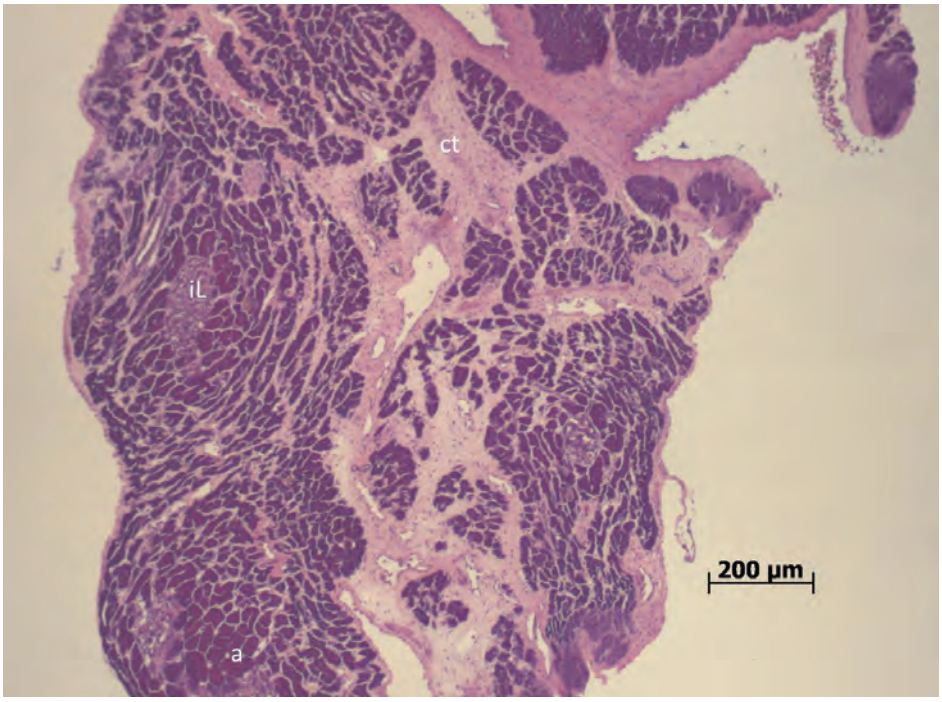

Figure 16: Pancreatic atrophy in a catfish suffering from steatitis, sampled from the Olifants Gorge during June 2011. Note prominence of connective tissue (ct) extending between groups of acinar cells (a). Islet of Langerhans (iL). (HE)

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 of some 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 lined by 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. Only mild hyperplasia of the gill epithelium was evident in some fish from Lunsklip Fisheries; however the absence of digenean gill parasites from these fish was notable.

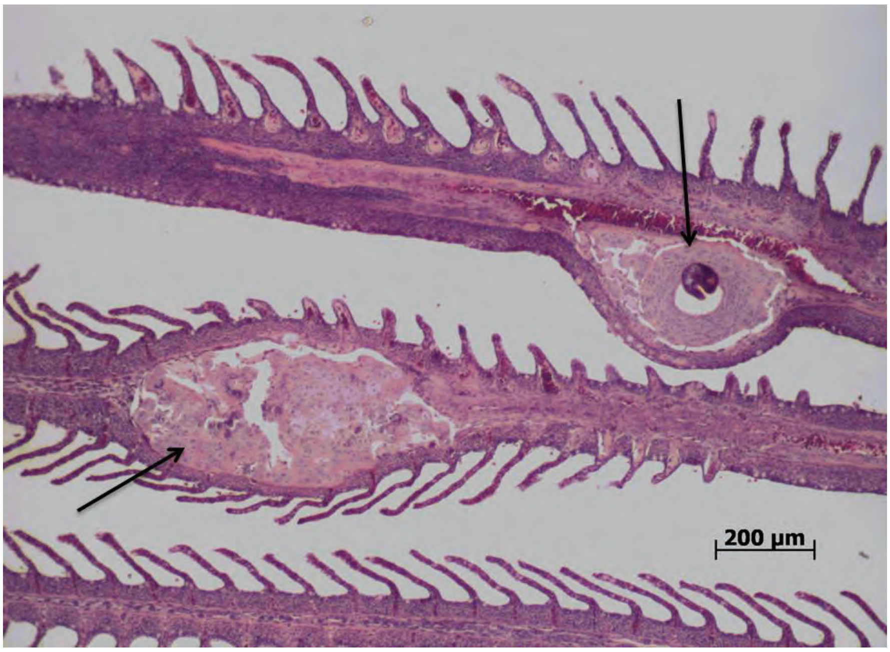

Figure 17: Digenean trematode larva encysted within the cartilage of the primary lamellae of a catfish sampled from the Olifants Gorge during June 2011. Note hyperplastic changes in the cartilage (arrows). (HE)

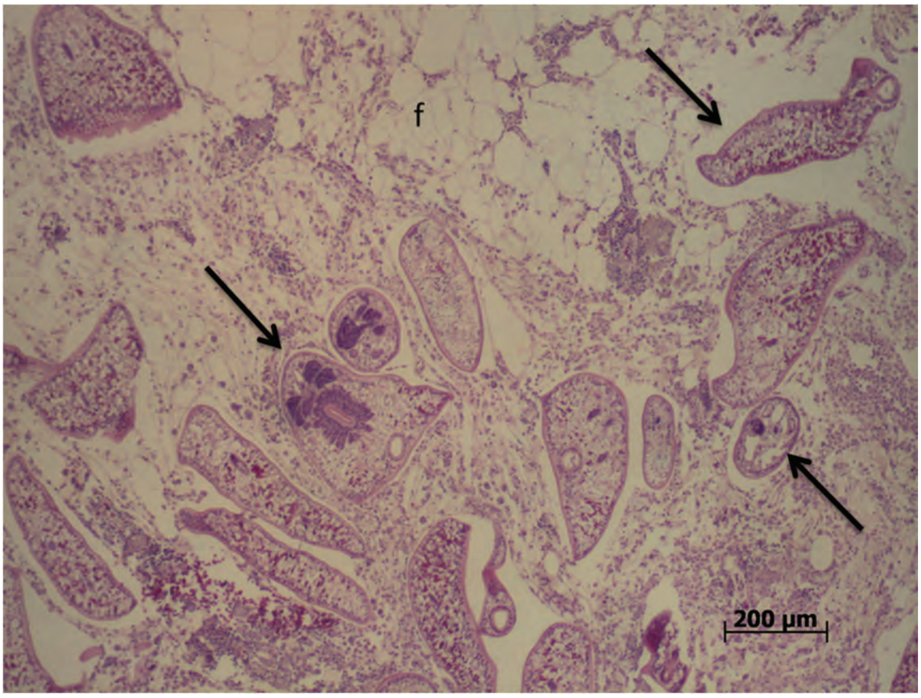

Figure 18: Digenean trematodes of the family Diplostomidae (arrows) within the cerebrospinal space adjacent to fat lining the cranium of a catfish sampled from Engelhard Dam during July 2010. Note the cellular reaction associated with presence of these parasites. (HE)

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 tilapia specimens 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 tilapia specimens 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 2008 indicated 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 catfish normally 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 test showed 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 values of 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.

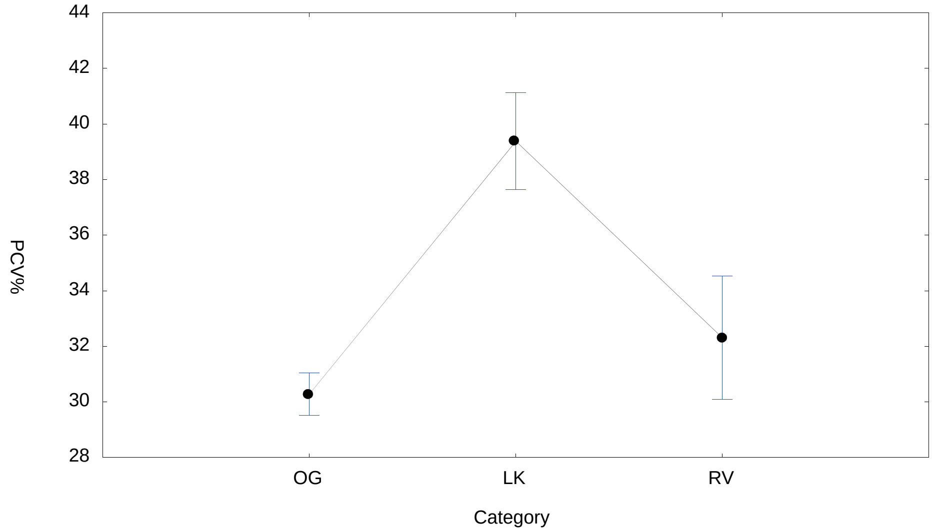

Figure 19: Comparison of mean haematocrit (PCV%) from all catfish sampled from the Olifants Gorge sites (n=111) as compared to mean haematocrit (PCV%) of catfish sampled from Lunsklip Fisheries (n=21) and Reënvoël Dam (n=13). (p=0.00002, vertical bars denote +/- standard errors)

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

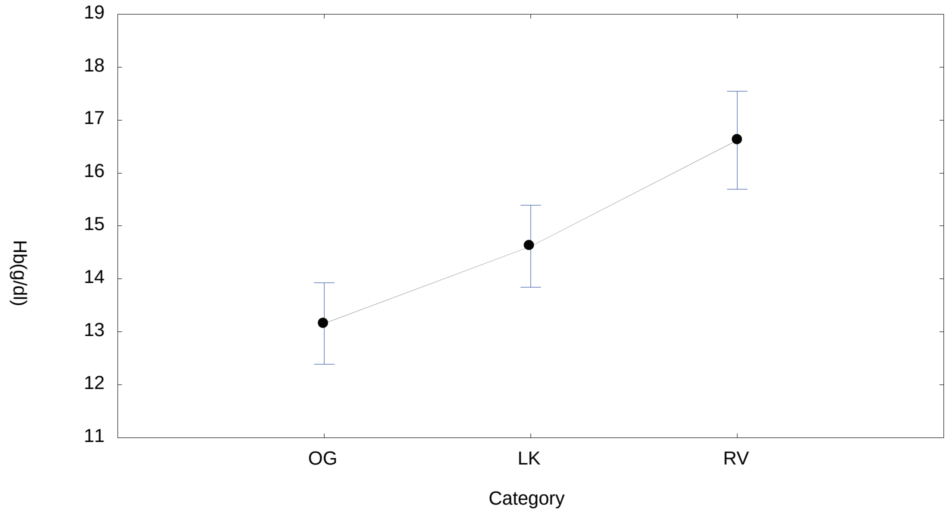

Figure 20: Comparison of mean haemoglobin values (g/dl) of catfish sampled during November 2009. OG=Olifants Gorge (n 20), LK=Lunsklip Fisheries (n 21) and RV=Reënvoël Dam (n 14). (p=0.02136, vertical bars denote +/- standard errors)

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

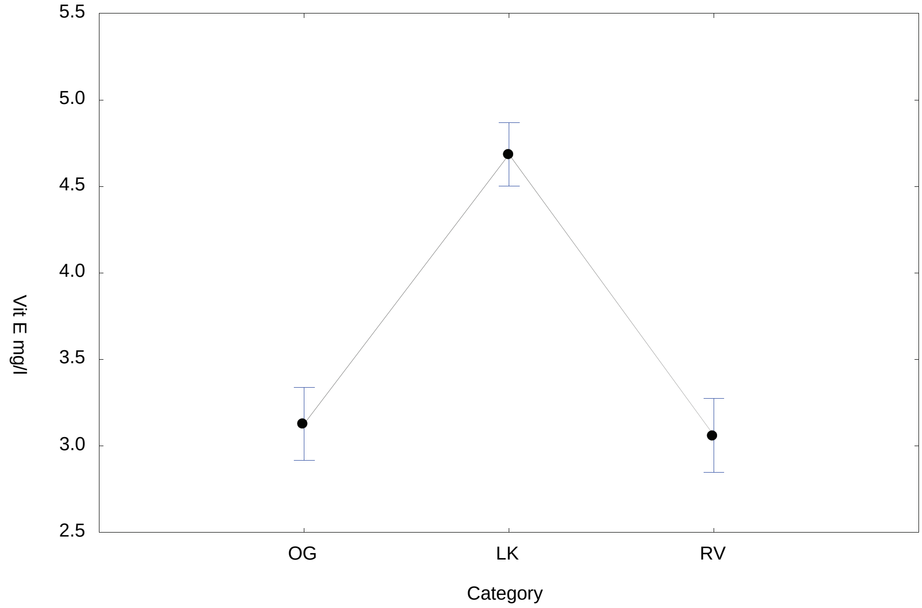

Figure 21: Comparison of mean serum vitamin E values (mg/l) of catfish sampled during November 2009. OG=Olifants Gorge (n 15), LK=Lunsklip Fisheries (n 20) and RV=Reënvoël Dam (n 15). ($p=0.00000$, vertical bars denote +/- standard errors)

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 in erythrocyte 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 date did 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

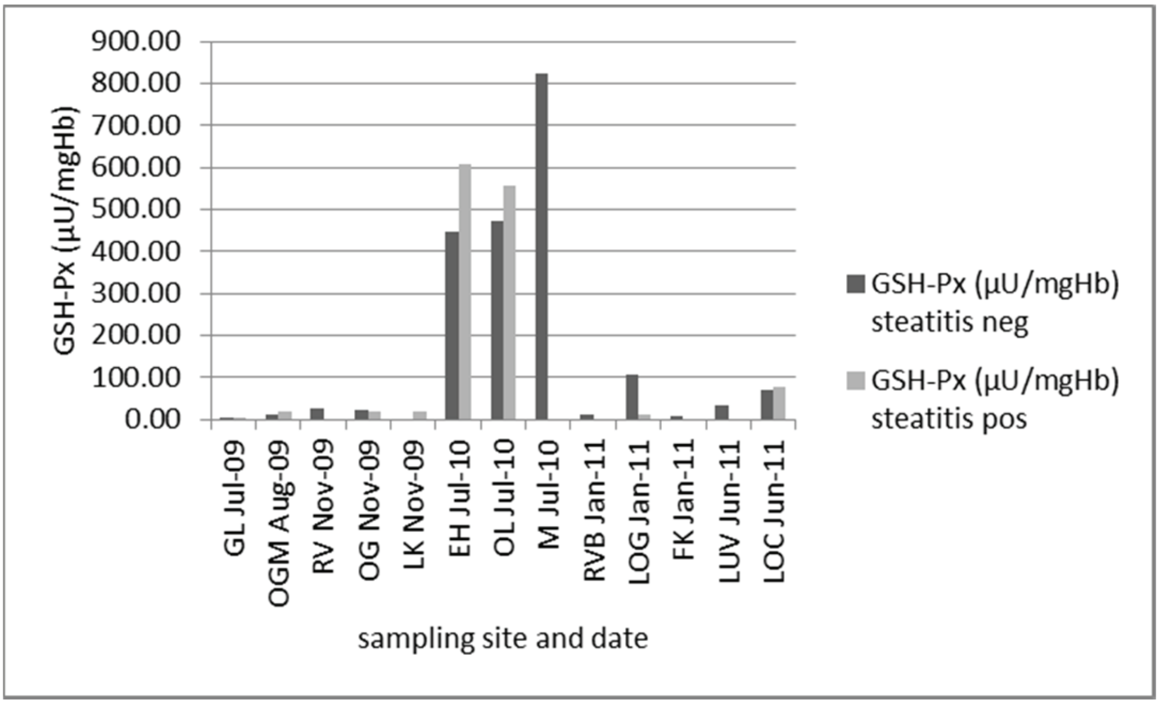

Figure 22A: Average glutathione peroxidase (GSH-Px) values (µU/mgHb) of catfish with and without steatitis sampled from all sampling sites

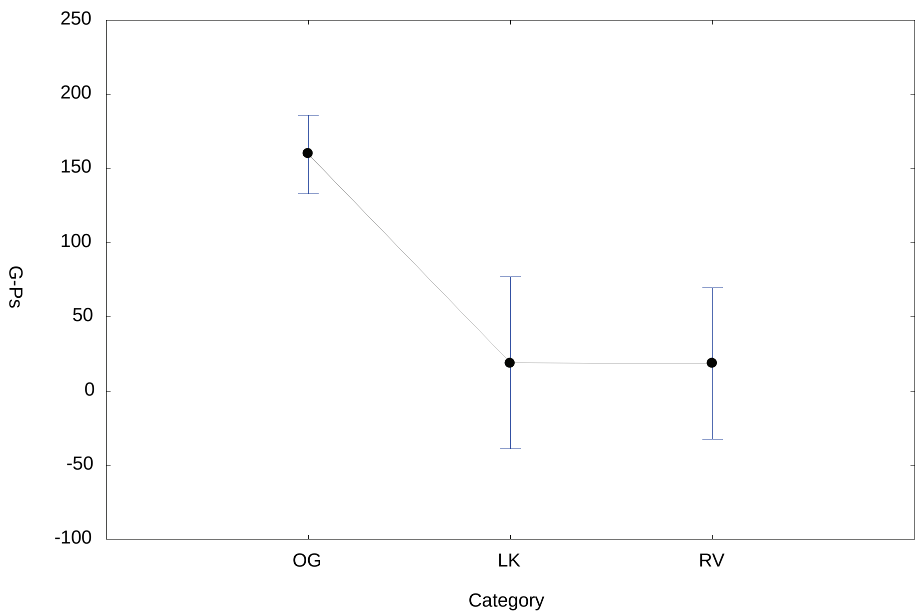

Figure 22B: Comparison of average erythrocyte glutathione peroxidase (G-Ps) values (µU/mgHb) of all catfish sampled from the Olifants Gorge sites (n=101) , Lunsklip Fisheries (n=21) and Reënvoël Dam (n=27). (p=0.01185, vertical bars denote +/- standard errors)

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 a preliminary 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, Letaba and 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 movement of 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 catfish from the Olifants Gorge, fruit of the Sycamore fig trees were the most common constituent of the stomach content of catfish sampled from Mamba Weir.

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, 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 River caused 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 of large 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 of the 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* antioxidants vitamin 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 reduced serum 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 of continuous 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 done before 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 levels when 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 Gorge do 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 catfish from the Olifants Gorge and the Sabiepoort. Fish remnants observed in the stomach content of catfish from 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 only Mozambique 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). The spawning 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 indicated an 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 been a 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 that seasonal 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.

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 in polluted catchments.

The study emphasizes the ecological importance and complexity of oxidative stress in a disturbed aquatic environment and 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 suggesting how 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 fish is 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. Co-workers have 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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