Pansteatitis in polluted Olifants River impoundments: Nutritional perspectives on fish in a eutrophic lake, Lake Loskop, South Africa

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Abstract

This study factorises the occurrence of pansteatitis in Lake Loskop, relative to two other impoundments along the Olifants River. Macroscopic and microscopic pathology, age determination and analysis of stomach content, fatty acids and stable isotopes explain the high prevalence of pansteatitis in *Oreochromis mossambicus* (Peters) and several other species in Lake Loskop. All the dietary indicator comparisons between pansteatitis-affected and healthy fish fail to support a systemic cause. Pansteatitis in Lake Loskop was linked to size and weight of *O. mossambicus*, but not to ontogenic age. Fish in Lake Loskop showed abnormally high omega-3 to omega-6 fatty acid ratios normally only found in marine fish

with no significant difference in degree of assimilation of these fatty acids between pansteatitis-affected and healthy fish. This explains the vulnerability to, but not the occurrence of, pansteatitis. As a cause for the pansteatitis, these results point towards sporadic vitamin E depleting trigger events, known sporadic fish die-off occurrences that provide surviving fish with a rich source of rancid fats on which to scavenge. The mechanism ties pansteatitis to eutrophication and trophic cascade effects, the intrinsic drivers of the disease and suggests an adaptive management strategy that might be applied by relevant conservation authorities.

Keywords

Pansteatitis, eutrophication, omega-3 fatty acids, *Oreochromis mossambicus*, Olifants River, Lake Loskop

Introduction

The disease pansteatitis has been identified as a leading cause of declining natural populations of Nile crocodiles *Crocodylus niloticus* (Laurenti) along the Olifants River in South Africa (Ashton 2010; Ferreira & Pienaar 2011; Lane, Huchzermeyer, Govender, Bengis, Buss, Hofmeyr, Myburgh, Steyl, Pienaar & Kotze 2013). A high prevalence of pansteatitis has also been documented in certain species of fish at several localities where the Olifants River has been dammed to make impoundments (Huchzermeyer, Govender, Pienaar & Deacon 2011; Huchzermeyer 2012; Woodborne, Huchzermeyer, Govender, Pienaar, Hall, Myburgh, Deacon, Venter & Lübcker 2012; Dabrowski, Oberholster, Dabrowski, Le Brasseur & Gieskes 2013; Dabrowski, Hall, Lübcker, Oberholster, Phillips & Woodborne 2014; Bowden, Cantu, Chapman, Somerville, Guillette, Botha, Hoffman, Luus-Powell, Smit, Lebepe, Myburgh, Govender, Tucker, Boggs & Guillette Jr. 2016). The prevalence of the

disease in these lotic and lentic systems is considered to be symptomatic of anthropogenic impacts that range from untreated wastewater effluent and agricultural run-off to heavy industry pollution (particularly mining) and intermittent chemical discharges that affect ecological functioning (de Villiers & Mkwelo 2009; Ashton 2010; Heath, Coleman & Engelbrecht 2010). Despite the significance of the proposed drivers, little evidence has conclusively addressed the aetiology of the disease. The confounding feature is that pansteatitis is not ubiquitous throughout the Olifants River system, and while it has been reported in several locations, these are interspersed with reaches of the river that appear to be unaffected. Many of the studies that have referenced the cause of pansteatitis fail to evince a fully factored set of hypotheses that compare the biotic forcing neither at locations with and without pansteatitis, nor between individuals from the same location that are pansteatitis positive and negative. In addition, few studies have integrated the proposed pollutants that are suggested to cause pansteatitis with the biochemical pathways in the organisms that develop pansteatitis.

The pathogenesis of pansteatitis is closely linked to diets rich in polyunsaturated fats and vitamin E intake. The disease has been described from many species of mammal (Davis & Gorham 1954; Jones, Howard & Gresham 1969; Danse & Verschuren 1978; Juan-Sallés, Prats, Resendes, Domingo, Hilton, Ruiz, Garner, Valls & Marco 2003; Niza, Vilela & Fereira 2003; de Bruijn, Veldhuis Kroeze & Sloet van Oldruitenborgh-Oosterbaan 2006; Fytianou, Koutinas, Saridomichelakis & Koutinas 2006), bird (Nichols, Campbell & Montali 1986; Pollock, Sleeman, Houle & Ramsay 1999; Wong, Mikaelian, Desnoyers & Fitzgerald 1999; Neagari, Arii, Udagawa, Onuma, Odaya, Kawasaki, Tenpaku, Hayama, Harada, Mizukami & Murata 2011), reptile (Wallach & Hoessle 1968; Langham, Zydek & Bennet 1971; Frye & Schelling 1973; Larsen, Buergelt, Cardeilhac & Jacobson 1983; Huchzermeyer 2003; Manawatthana & Kasorndorkbua 2005; Osthoff, Hugo, Bouwman, Buss, Govender, Joubert

& Swarts 2010; Orós, Monagas, Calabuig, Luzardo & Camacho 2013) and fish (Helder 1979; Roberts, Richards & Bullock 1979; Herman & Kircheis 1985; Wada, Hatai & Kubota 1991; Bricknell, Bruno, Bowden & Smith 1996; Guarda, Bertoja, Zoccarato, Tartari & Biolatti 1997; Begg, Bruno & McVicar 2000; Goodwin 2006; Roberts & Agius 2008; Huchzermeyer *et al.* 2011; Huchzermeyer 2012). The aetiology of the disease is thought to be a deficiency in the non-enzymatic role of vitamin E as an anti-oxidant (Danse & Verschuren 1978; White 2000). The aetiopathology appears to depend on two factors; assimilation of large amounts of dietary polyunsaturated fats resulting in a high proportion of these lipids in the adipose tissues of the body, and oxidation of the polyunsaturated fats when tissue antioxidants such as vitamin E are insufficient relative to the amount of stored polyunsaturated lipid (de Bruijn *et al*. 2006). Tissue vitamin E levels may become depleted through two pathways; either through assimilation of overwhelming amounts of highly polyunsaturated lipids that are prone to autoxidation and consequently deplete tissue vitamin E, or through the consumption of already oxidised or rancid polyunsaturated lipids resulting in a diet deficient in vitamin E (Porter 1989; White 2000). In both cases, lipid free-radicals released from lipid hydroperoxides lead to a lipid-damaging chain reaction (Porter 1989). This results in necrosis of adipocytes and the subsequent inflammatory changes in adipose tissues typical of pansteatitis.

Polyunsaturated fats are the most vulnerable and abundant target of free radical attack caused by lipid peroxidation products (Burton 1994). An excessive assimilation of highly polyunsaturated fats by animals thus explains the increased vulnerability to pansteatitis. Peroxidation of polyunsaturated fat results from the oxidative effects of oxygen, but to overcome the dissociation energy of an allylic bond in the polyunsaturated lipid, the reaction needs to be initiated in some way (Burton 1994). As the allylic bonds of polyunsaturated fats are partially activated, only small amounts of a pro-oxidant are required to complete the

reaction. This may be through normal redox cycling in the body of various transition metals, such as iron and copper. Iron, needed for oxygen transportation is abundant in the body and iron-containing complexes, such as heme, are highly soluble and able to reach the vulnerable non-polar mid-zone of biological membranes (Demopoulos 1973). Here, iron acts as pro-oxidant and may initiate the lipid peroxidation chain reaction which under normal circumstances is blocked by the presence of vitamin E.

Lake Loskop, on the upper Olifants River, has a population of Mozambique tilapia, *Oreochromis mossambicus* (Peters), that shows a particularly high prevalence of pansteatitis. Frequent fish die-offs and the decline in the crocodile population since 2005 (Botha, Van Hoven & Guillette 2011) have focussed attention on the impact of pollution on the ecosystem of this lake (Ashton 2010; Oberholster, Myburgh, Ashton, Coetzee & Botha 2011). The lake has a retention time of 12.2 months and is fed by a mean annual run-off of 323.31 Mm^3 (Dabrowski *et al.* 2013). This gathers over a catchment of 12 626 km^2 covering an area intensely mined for coal and known for spillage from largely dysfunctional urban waste water treatment works. Drainage of mine water from abandoned coal mines and phosphorous from excessive sewage discharges impact on the lake. High nutrient levels drive the primary photoautotrophic production of phytoplankton leading to development of substantial algal biomass in the surface waters. With senescence of algae, heterotrophic decomposition at intermediate depth leads to oxygen depletion and oxygen minimum zones which can sporadically accumulate hydrogen sulphide implicated in massive fish kills elsewhere (Schunck, Lavik, Desai, Großkopf, Kalvelage, Löscher, Paulmier, Contreras, Siegel, Holtappels, Rosenstiel, Schilhabel, Graco, Schmitz, Kuypers & LaRoche 2013) and also in Lake Loskop (Dabrowski *et al.* 2013).

Lake Flag Boshielo lies approximately 80 km downstream of Lake Loskop (Figure 1). This oligotrophic impoundment has a thriving population of Nile crocodiles and pansteatitis has

not been documented from fish in this lake. Some 200 km below Lake Flag Boshielo, the Olifants River traverses the 90-km width of the Kruger National Park before entering Lake Massingir, an impoundment just east of the Kruger National Park in Mozambique (Figure 1). Discharges from tailings dams at a copper and phosphate mine at the town of Phalaborwa on the western boundary of the Kruger National Park into the Ga-Selati River, a tributary of the Olifants River, have periodically been blamed for the high phosphate levels measured in the Olifants River downstream of the town. In contrast to Lake Flag Boshielo, Lake Massingir is classed as eutrophic with frequent phytoplankton blooms (Mussagy 2008).

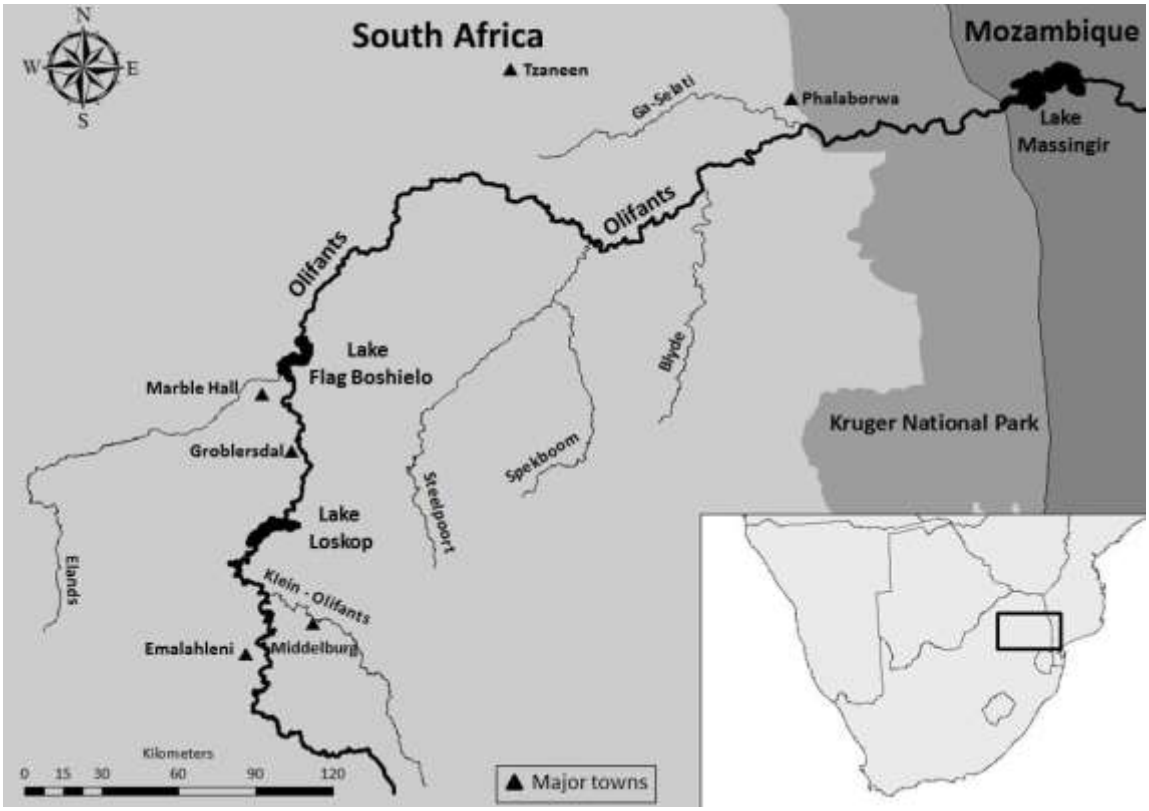


Figure 1 Map of the Olifants River catchment in South Africa showing the respective positions of Lakes Loskop, Flag Boshielo and Massingir. The Olifants River drains through Mozambique into the Indian Ocean.

The Olifants River catchment therefore contains three large impoundments, Lake Loskop and Lake Massingir that are eutrophic, separated by Lake Flag Boshielo which is oligotrophic. Each eutrophic impoundment has a unique combination of biotic and abiotic factors that have

been variously assigned as a cause for pansteatitis, but no synthesis has considered the constraining evidence across the entire catchment. In addition, the methodology that has led to the speculation on the aetiology of pansteatitis at Lake Loskop and the Olifants River Gorge has been inconsistent across sites, further complicating a factorial approach to intersite comparisons.

Raising of the sluice gates of the Massingir dam during 2007 led to the head waters of the lake flooding into a narrow gorge much favoured by crocodiles in the Kruger National Park. This appears to have been the main precipitating factor leading to a massive die-off of crocodiles due to pansteatitis and the subsequent diagnosis of increasing prevalence of pansteatitis in African sharptooth catfish *Clarias gariepinus* (Burchell) at this locality (Ferreira & Pienaar 2011; Huchzermeyer *et al.* 2011; Huchzermeyer 2012). Huchzermeyer, Osthoff, Hugo & Govender (2013) and Woodborne *et al.* (2012) were able to show that a concurrent trophic shift in sharptooth catfish and Nile crocodiles in the Olifants River Gorge was linked to an excessive assimilation of polyunsaturated fats in animals with pansteatitis when compared to healthy individuals from the same locality. This pointed to a change in feeding behaviour possibly linked to sporadic hydrodynamic change within the gorge and to presence of an oil-rich prey species not usually preyed upon by catfish and crocodiles. These authors proposed that in the Olifants River Gorge this may have been the pelagic phytoplanktivorous silver carp, *Hypophthalmichthys molitrix* (Valenciennes). The species is alien to Africa but was accidentally introduced into the Olifants River at Lake Flag Boshielo and is now prolific in Lake Massingir. In the Olifants Gorge, pansteatitis has only been described from crocodiles and sharptooth catfish. Other species, including Mozambique tilapia, appear to be unaffected. Silver carp have also invaded Lake Flag Boshielo, where crocodiles and Mozambique tilapia do not appear to suffer from pansteatitis. Silver carp do

not occur upstream in Lake Loskop where pansteatitis is prevalent in a number of fish species.

Lake Loskop is classified as meso- to eutrophic, and heterogeneous nutrient concentrations result in frequent algal blooms within the transitional zone of the lake (Dabrowski *et al.* 2013). During the summer (October to April) the lake shows a monomictic pattern of stratification with increasing extent of hypoxia in the hypolimnion (Dabrowski *et al.* 2013). A number of stressors have been suggested by Dabrowski *et al.* (2013) to impact on fish in Lake Loskop, including raised ammonia, aluminium, iron and manganese, and periodic exposure to hydrogen sulphide. Apart from plumes of hydrogen sulphide reaching the surface waters, a number of other factors associated with blooms involving *Microcystis aeruginosa* may explain fish die-offs in eutrophic waters. Low dissolved oxygen levels associated with night-time respiration of the bloom biomass as well as the increased biological oxygen demand when the bloom undergoes senescence may drive dissolved oxygen levels below those needed to support respiratory requirements of fish. A number of toxic compounds associated with *M. aeruginosa* may lead to death of fish in particular, if ingested, the hepatotoxin microcystin. In addition, unsaturated lipids released from cyanobacteria when these undergo senescence have been reported to affect gill ion transport by inhibiting ATPase activity resulting in the death of exposed fish (Bury, Codd, Wendelaar Bonga & Flik 1998). How algal blooms might link to pansteatitis has not been previously studied. In Lake Loskop, an increase in phytoplankton blooms involving the cyanobacterium *M. aeruginosa* and dinoflagellate *Ceratium hirundinella* has been observed since 2007 (Dabrowski *et al.* 2013). Frequent fish die-offs have been reported including large male Mozambique tilapia. Amongst such fish, pansteatitis was observed (Dabrowski *et al.* 2013). *Labeo rosae* (Steindachner) occur in the lake in very large numbers. Several large-scale die-offs have been recorded with up to 14 tonnes of dead fish, predominantly of this species, being removed during an event

associated with evidence of hydrogen sulphide release from the water that lasted 30 days in 2007 (Dabrowski *et al.* 2013).

The trophic status of *O. mossambicus* from Lake Loskop was previously assessed through stable light isotope analysis, and it was concluded that pansteatitis prevalence was not linked to piscivory (Dabrowski *et al.* 2014), but this study reported 100% prevalence of pansteatitis, and the only comparative data come from archived samples with unknown pansteatitis prevalence. This appears to conflict with the perspective of Huchzermeyer (2012), Huchzermeyer *et al.* (2013) and Woodborne *et al.* (2012) who argue for a strong role of piscivory in the Olifants River Gorge. Here we explain the apparently anomalous occurrence of pansteatitis in Lake Loskop when compared to occurrence of the disease in the Olifants River Gorge and to provide a perspective on why the disease is not yet known from Lake Flag Boshielo. We reconsider the trophic status of fish from Lake Loskop in the context of their fatty acid composition as well as the presence and absence of pansteatitis. Through age analysis using otoliths we consider the possibility that pansteatitis epidemics result from episodic events that would manifest in cohort-dependent prevalence of the disease.

Materials and Methods

Field sampling and pathology

Live fish were collected from Lake Loskop during January 2016 with the use of baited hooks, lures and a series of gill nets (70 mm, 90 mm, 110 mm stretched mesh) from several sites including the transition zone (25° 28’ 36.64”S; 29° 16’ 13.58”E) where the Olifants River enters the lake and the lacustrine zone along the Mpumalanga Tourism and Parks Agency (MTPA) boat launch site (25° 24’ 59.32”S; 29° 19’ 35.66”E). The focus of the sampling was on *O. mossambicus*, but specimens of other species inadvertently caught were also examined, including *L. rosae* , *Labeobarbus marequensis* (Smith), *Cyprinus carpio* (Linnaeus) and

Clarias gariepinus (Burchell). A total of 33 *O. mossambicus* specimens were sampled to ensure that both fish with and without pansteatitis and of various size and age classes were represented.

Fish were removed from the water live and were stunned by a physical blow to the head. A code was designated to each fish and each specimen was photographed. Length and weight measurements were taken followed by dissection of each fish. The amount of fat deposited in the adipose tissues of each fish was recorded on a scale of zero to five with 0 representing no fat and 5 representing obesity. Pansteatitis severity was scored on clinical appearance of the adipose tissues of the fish, with 0 representing no evidence of pansteatitis, and scores 1 to 5 reflecting increasing severity of pansteatitis, evidenced by the degree of inflammation and displacement of normal fat tissue by golden-brown granulomas. Stomach contents of all fish were examined and composition of food items was recorded.

Otoliths were removed from the skull of all *O. mossambicus* specimens and placed into individual paper envelopes. Otolith sections for age determination were prepared according to the method of Weyl and Booth (2008). From fish that showed signs of pansteatitis, small (10x10x10 mm) pieces of affected adipose tissue were fixed in 10% buffered formalin for histological examination. Histological sections were prepared from formalin-fixed pansteatitis-affected adipose tissue and stained with haematoxylin and eosin following standard histological techniques before being examined by light microscopy to confirm presence of pansteatitis.

From each fish, a minimum of 2 gram mesenteric fat was placed into a plastic Ziplock bag and placed on ice for analysis of fatty acid composition. A sample of muscle tissue measuring approximately 15x 15x 20 mm was collected from each fish and placed into a separate Ziplock bag and kept on ice for analysis of δ^{15}N and δ^{13}C stable isotope ratios.

Fatty acid analysis

Fatty acid composition was determined as described by Osthoff *et al.* (2010) and Huchzermeyer *et al.* (2013), with extraction of total fat from tissue samples following the method of Folch, Lees & Sloane-Stanley (1957). Fatty acid methyl esters (FAME) were transesterified using the method given by Park & Goins (1994).

Stable light isotope analysis

The stable isotope analysis followed the protocol used by Woodborne *et al.* (2012) and Dabrowski *et al.* (2014). Muscle samples were subjected to lipid extraction using a 2:1 mixed solution of chloroform and ethanol (Logan, Jardine, Miller, Bunn, Cunjak & Lucavage 2008) and dried at 70°C. Aliquots of approximately 0.5 mg were weighed into tin capsules and combusted online in a Series 1112 Elemental Analyser. This was interfaced with a Delta V Plus stable light isotope ratio mass spectrometer by a ConFloIV interface (all equipment supplied by Thermo, Bremen). The results for δ^{13}C and δ^{15}N were normalised against a laboratory running standard that was included after every 12 unknown samples. The values are expressed in standard delta notation in which

$$\delta X = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) \times 1000$$

where X = ^{13}C or ^{15}N, and R = ^{13}C/^{12}C or ^{15}N/^{14}N, respectively, and values are reported in per mille units (‰). The values are expressed relative to the standards VPDB for carbon, and air for nitrogen. Reproducability of the standards was 0.08‰ for δ^{15}N and 0.19‰ for δ^{13}C.

Statistical analysis

Mean fatty acid values were compared between pansteatitis-negative and positive fish using a student's t-test with an alpha-error probability of p = 0.05. Logistic regression models were used to estimate the probability of a fish being pansteatitis negative as a function of fish length and body mass. Linear least-squares regression models were used to model pansteatitis severity as a function of fish length and body mass, respectively. Since fish age was not a

significant predictor for either pansteatitis presence or severity, no models are presented with age as the independent variable.

Results

O. mossambicus (n = 33) sampled from Lake Loskop were predominantly large with an average head to tail length of 37.41 cm (range 27–46 cm) and with average mass of 1.27 kg (range 0.4–2.4 kg) (Table 1). The majority of fish had moderate fat reserves with an average fat score of 2.67. Pathology was restricted to the adipose tissues. Multifocal, random, well defined, yellow-brown, firm, nodular to confluent lesions were characteristic evidence of pansteatitis. Presence of pansteatitis was associated with splenic enlargement.

Pansteatitis prevalence in *O. mossambicus* and other by-catch species is reported in Table 1. Lesions typical of pansteatitis were observed in 55% of *O. mossambicus* specimens. In addition to *O. mossambicus*, six *L. rosae* were sampled. Of these, five suffered from pansteatitis with severity scores ranging from 1 to 4. All three specimens of *C. gariepinus* sampled from Lake Loskop suffered from pansteatitis.

Table 1 Pansteatitis prevalence, severity and obesity score and length and mass measurements of *Oreochromis mossambicus* and incidentally caught *Labeo rosae*, *Cyprinus carpio*, *Clarias gariepinus* and *Labeobarbus marequensis* from Lake Loskop during January 2016. Pansteatitis severity was scored on a scale of 1-5, 1 = fat mildly affected by pansteatitis and 5 = fat severely affected by pansteatitis. Obesity was scored on a scale of 0-5, 0 = no fat and 5 = obese.

Species	% with pansteatitis	n	Average pansteatitis score	Average obesity score	Average length (cm)	Average weight (kg)
O. mossambicus	55	33	2.5 (1-5)	2.67 (0-4)	37.4 (27-46)	1.27 (0.4-2.4)
L. rosae	83	6	2.8 (1-4)	2.75 (1-5)	43.75 (41-47)	1.88 (1.5-2.1)
C. carpio	0	2		1 (0-2)	41.5 (36-47)	1.58 (1-2.15)
C. gariepinus	100	3	4 (3-5)	2.5 (0.5-3)	63.4 (76-96)	4.5 (3.3-4.5)
L. marequensis	0	2		4.5 (4-5)	32.5 (29-36)	0.7 (0.45-0.95)

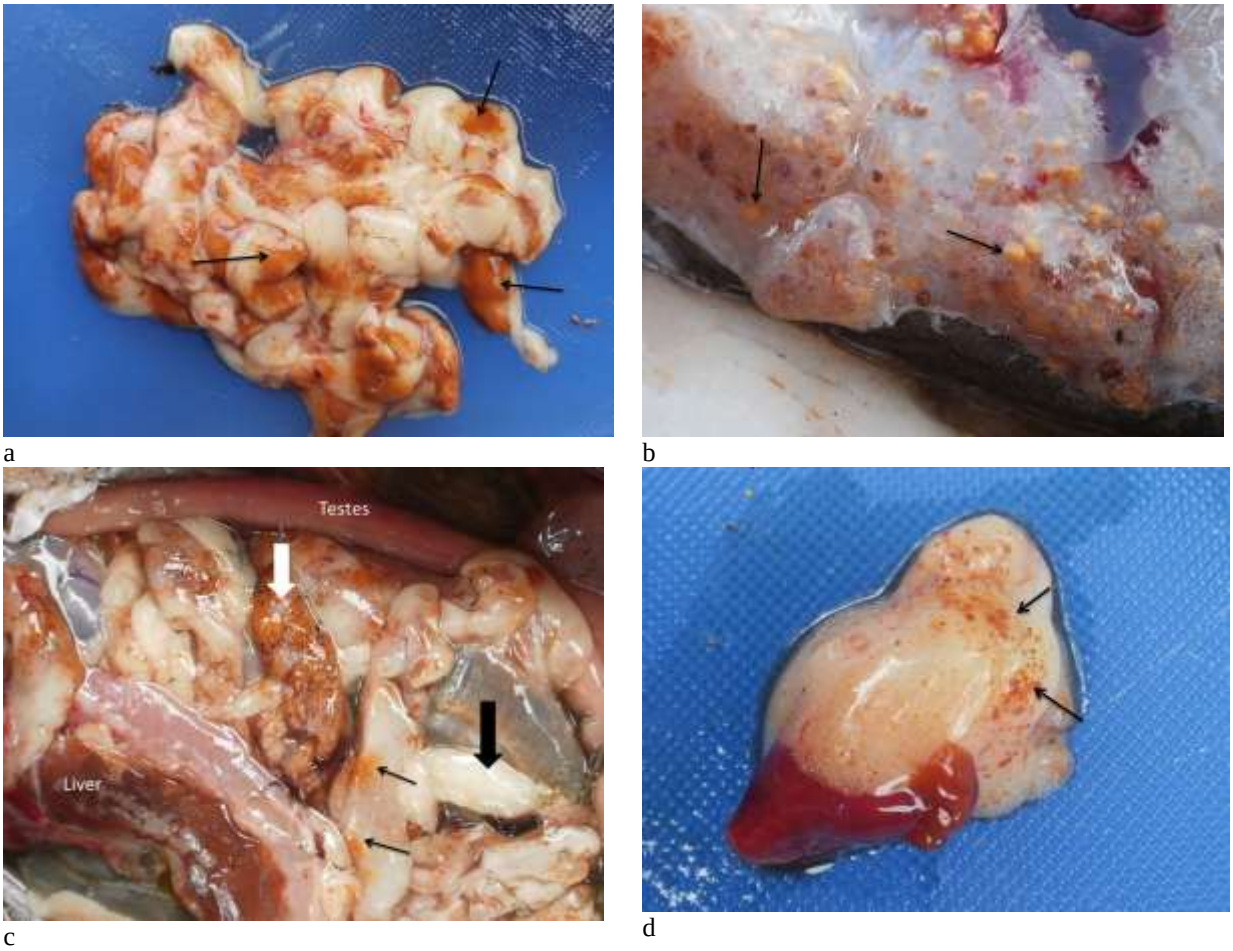


Figure 2 Macroscopic appearance of pansteatitis-affected adipose tissues from *Oreochromis mossambicus* sampled from Lake Loskop, (a) recently affected mesenteric fat showing disseminated foci of even golden-brown discolouration and increased density (arrows) but absence of recognizable granulomas, (b) chronically affected adipose tissue showing focally disseminated small golden to brown granulomas (arrows), (c) presence of normal appearing fat (thick black arrow), acute lesions (thin arrows) and chronic lesions (thick white arrow) in the mesenteric adipose tissues, d. granuloma formation associated with pansteatitis development in the coronary fat (arrows).

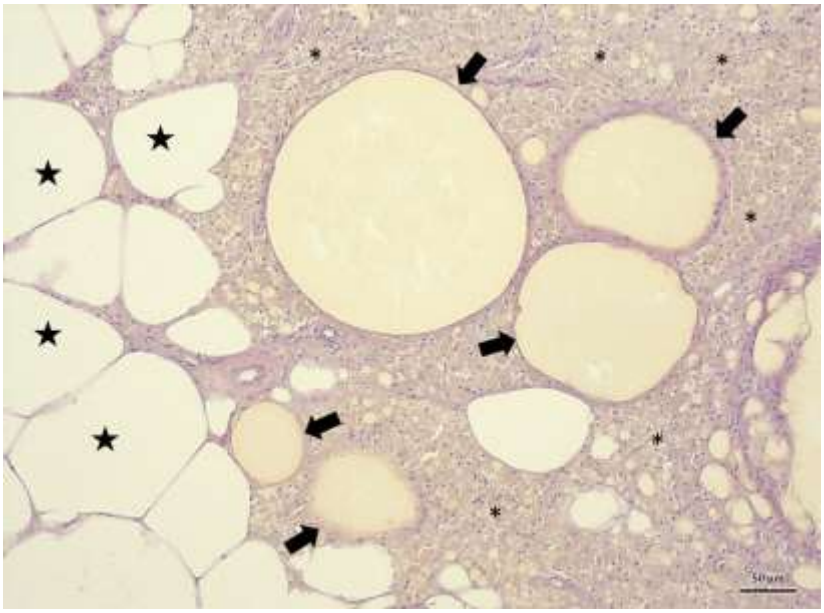
With the exception of one fish, all *O. mossambicus* specimens suffering from pansteatitis had white fat containing either well demarcated, variably diffuse, golden brown areas of increased density (recent inflammatory change) (Fig. 2a) or small variously dense brown to golden granulomas (long standing inflammatory change) disseminated throughout the

adipose tissues (Fig. 2b). Some fish showed a combination of normal fat and lesions associated with both early and advanced pansteatitis (Fig. 2c). In addition to pansteatitis, one fish showed diffuse pale-yellow discolouration of mesenteric fat similar to another fish without pansteatitis. In fish suffering from pansteatitis, fat necrosis and associated granulomatous changes were evident in all adipose tissues including the mesenteric, intermuscular, subcutaneous and coronary fat deposits (Fig. 2d).

Microscopic examination of histological sections of affected adipose tissue confirmed necrosis of adipocytes and associated inflammatory changes typical of pansteatitis (Fig. 3). These were similar to those described with pansteatitis in *C. gariepinus* by Huchzermeyer *et al.* (2011). Variously sized chambers of coalesced extracellular ceroid-type lipopigment derived from necrotic adipocytes and surrounded by an epithelioid sheath and narrow connective tissue capsule were evident throughout the affected adipose tissue (Fig. 3a). Macrophages with eccentric nuclei at the periphery of these chambers appeared to phagocytose small extracellular ceroid-type lipopigment droplets and adjacent areas were often infiltrated by a uniformly dense mass of macrophages interspersed amongst normal appearing adipocytes (Fig. 3b).

The stomachs of two fish contained large numbers of recently ingested fry. One of these fish showed age-related ovarian senescence. Two other specimens had only fish remnants in their stomachs, while algae and fish remnants were detected in the stomachs of three fish. The stomachs of a further three fish were empty. The ingesta of all the remaining fish was made up of phytoplankton (n = 23). Presence of pansteatitis did not appear to affect ovarian development. With the exception of four fish, all, female *O. mossambicus* sampled showed well developed ovaries. Signs of age-related ovarian degeneration were evident in three female fish suffering from pansteatitis, aged 9, 11 and 14 years, and in one fish of 7 years that showed no evidence of pansteatitis.

a



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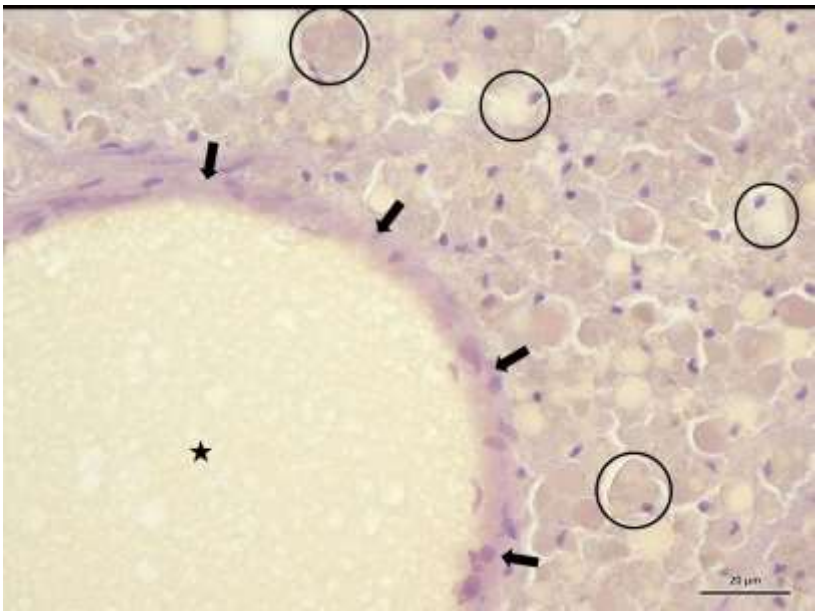
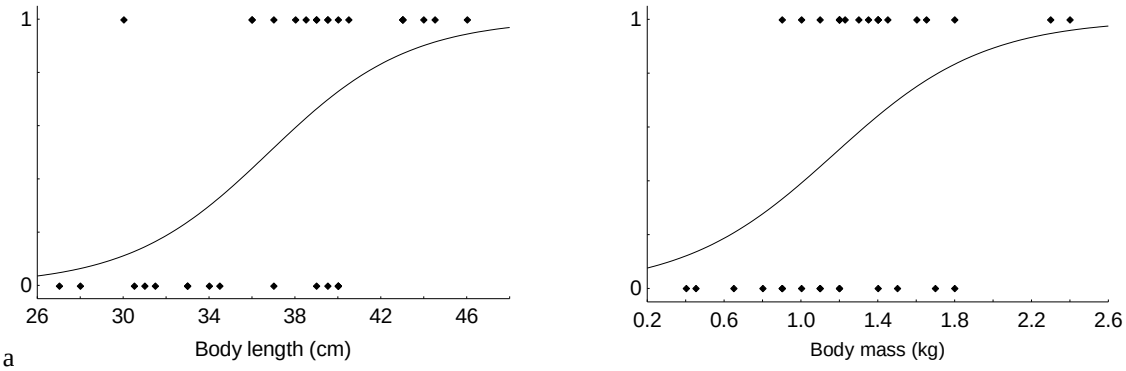


Figure 3 Photomicrographs of pansteatitis affected mesenteric adipose tissue, from *Oreochromis mossambicus* sampled from Lake Loskop, (a) appearance of typical lesions. Note presence of normal appearing adipocytes (stars), extracellular ceroid-type lipopigment containing chambers (arrows) and influx of large numbers of macrophages (asterix) (H&E, X100), (b) coalesced extracellular ceroid-type lipopigment derived from necrotic adipocytes surrounded by an epithelioid sheath and connective tissue capsule (arrows). Macrophages with eccentric nuclei (circles) at the periphery of the chamber phagocytosing small lipopigment droplets (white to yellow) (H&E, X400).



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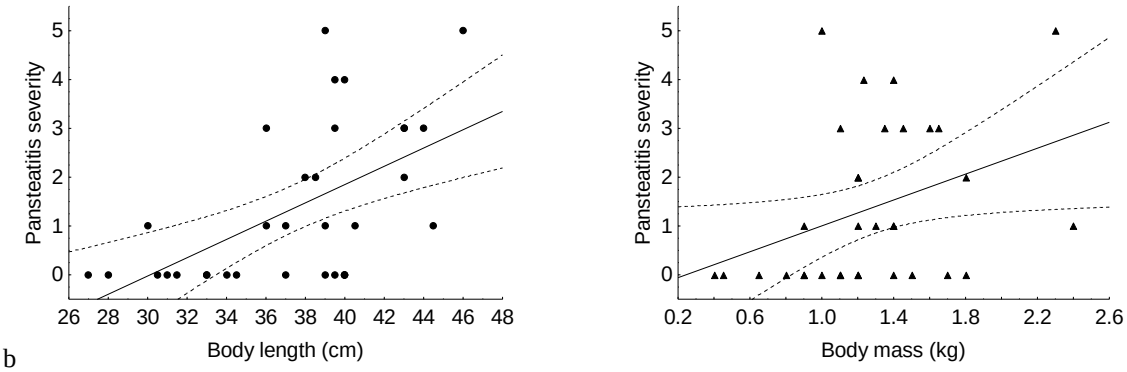

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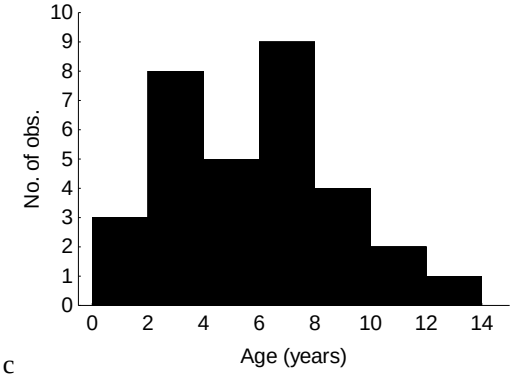

c

Figure 4(a) Presence (1) and absence (0) of pansteatitis in *Oreochromis mossambicus* sampled from Lake Loskop as a function of body length (left) and body mass (right), respectively, estimated using logistic regression modelling. The probability of a fish being pansteatitis-negative can be estimated using its length (L) or mass (W), respectively: $P_L=1/(1+e^{-11.28+0.306*L})$; p = 0.008, $P_W=1/(1+e^{-3.014+2.567*W})$; p = 0.01

Figure 4(b) Pansteatitis severity in *Oreochromis mossambicus* sampled from Lake Loskop as a function of length (left) and body mass (right). Dashed lines represent 95% confidence intervals. The linear models for both length and body mass are Y_L = -5.64 + 0.187 L; p = 0.0006, and Y_W = -0.32 + 1.326 W; p = 0.039, where Y_L and Y_W are pansteatitis severity as a function of length (L) and body mass (W). The dashed lines are 95% confidence intervals.

Figure 4(c) Frequency of *Oreochromis mossambicus*, sampled from Lake Loskop, with pansteatitis by age of fish (years).

There was a significant relationship between presence of pansteatitis and body length and mass (logistic regression) with a higher probability of finding pansteatitis with increasing length and mass (Fig. 4a). Pansteatitis severity also correlated to fish length, and to a lesser extent to body mass (Fig. 4b). There was no relationship between fish age and presence or severity of pansteatitis. Relatively high frequencies for pansteatitis-positive fish in the age categories 2–4 years and 6–8 years, respectively, were noted (Fig. 4c).

The analysis of fatty acids from *O. mossambicus* with and without pansteatitis is presented in Table 2 and that of incidentally caught individuals of several other species is presented in Table 3. A significant difference in the fatty acid composition of healthy and pansteatitis-affected fat was detectable in only four fatty acids; C14:1c9 and C22:5c7,10,13,16,19 (n-3) were increased whereas C16:0 and C20:5c5,8,11,14,17 (n-3) were reduced in fat of individuals with pansteatitis (Table 2). There was no significant difference in the ratio of omega-3 to omega-6 fatty acids (n-3:n-6) between the pansteatitis positive and pansteatitis negative group.

The ratio of n-3:n-6 of 8.2 in the fat of *L. rosae* from Lake Loskop, of which 83% of sampled individuals were suffering from pansteatitis, was twice as high as that of *C. gariepinus* (4.26) and only slightly lower than that of the pansteatitis positive tilapia cohort (11.25) (Tables 2 and 3). The lowest n-3:n-6 ratios of 2.94 and 1.51 were present in the fat of *L. marequensis* and *C. carpio* specimens, respectively (Table 3). Specimens of neither species showed evidence of pansteatitis but numbers sampled were too low to draw conclusions.

When assessing the diet of an organism using stable isotopes, it is important to measure its protein as lipids contain no nitrogen but can affect the carbon isotope values (Post, Layman, Arrington, Takimoto, Quattrochi & Montana 2007). The C:N ratio of the pansteatitis negative *O. mossambicus* was indistinguishable from that of pansteatitis positive individuals (3.88 ± 0.04 versus 3.92 ± 0.08, respectively), and was in the range expected for pure proteins.

Table 2 A comparison of lipid properties of pansteatitis negative and positive *Oreochromis mossambicus* sampled from Lake Loskop during January 2016. The proximate analysis of adipose tissue from the two groups was compared using a t-test at an error level $p<0.05$

		Pansteatitis negative (n=15)	Pansteatitis positive (n=18)	**Prob. Level (p)**
FAME (% of total fatty acids)				
Common name	Abbreviation			
Myristic	C14:0	5.68 ± 0.92	5.96 ± 1.37	0.512
Myristoleic	C14:1c9	0.11 ± 0.03	0.16 ± 0.04	<0.001
Pentadecylic	C15:0	0.11 ± 0.06	0.13 ± 0.04	0.244
Palmitic	C16:0	27.25 ± 2.26	24.44 ± 2.59	0.003
Palmitoleic	C16:1c9	8.38 ± 1.11	8.72 ± 0.73	0.300
Margaric	C17:0	0.27 ± 0.08	0.26 ± 0.14	0.723
Heptadecenoic	C17:1c10	0.23 ± 0.14	0.21 ± 0.08	0.679
Stearic acid	C18:0	2.36 ± 0.37	2.26 ± 0.30	0.437
Elaidic	C18:1t9	0.17 ± 0.13	0.19 ± 0.13	0.753
Oleic	C18:1c9	19.63 ± 2.43	19.57 ± 1.91	0.940
Vaccenic	C18:1c7	3.08 ± 0.42	3.19 ± 0.43	0.484
Nonoadecanoic	C19:0	0.10 ± 0.05	0.10 ± 0.08	0.932
Linoleic	C18:2c9,12 (n-6)	1.82 ± 0.29	1.66 ± 0.39	0.202
Arachidic	C20:0	0.35 ± 0.12	0.42 ± 0.11	0.141
γ-Linolenic	C18:3c6,9,12 (n-6)	0.71 ± 0.23	0.65 ± 0.23	0.472
α-Linolenic	C18:3c9,12,15 (n-3)	2.42 ± 0.56	2.24 ± 0.28	0.242
Heneicosanoic	C21:0	0.02 ± 0.03	0.04 ± 0.07	0.459
Eicosadienoic	C20:2c11,14 (n-6)	0.05 ± 0.05	0.17 ± 0.51	0.380
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.24 ± 0.06	0.28 ± 0.07	0.057
Erucic	C22:1c13	0.31 ± 0.10	0.34 ± 0.13	0.478
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.07 ± 0.04	0.10 ± 0.03	0.098
Arachidonic	C20:4c5,8,11,14 (n-6)	0.34 ± 0.07	0.31 ± 0.04	0.177
Eicosopentaenoic	C20:5c5,8,11,14,17 (n-3)	3.16 ± 0.84	2.67 ± 0.48	0.042
Nervonic	C24:1c15	0.01 ± 0.03	0.03 ± 0.04	0.109
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	7.69 ± 2.36	9.84 ± 2.86	0.027
Docosahexanoic	C22:6c4,7,10,13,16,19 (n-3)	15.43 ± 2.23	16.07 ± 2.43	0.441
Fatty acid ratios				
Saturated Fatty Acids (SFA)		36.15 ± 2.41	33.61 ± 2.50	0.006
Mono Unsaturated Fatty Acids (MUFA)		31.92 ± 2.16	32.41 ± 2.76	0.583
Poly Unsaturated Fatty Acids (PUFA)		31.93 ± 3.69	33.99 ± 4.34	0.157
SFA/PUFA		1.15 ± 0.19	1.01 ± 0.18	0.037
Omega-6 Fatty Acids		2.99 ± 0.58	2.89 ± 0.59	0.618
Omega-3 Fatty Acids		28.94 ± 3.73	31.10 ± 4.48	0.148
Omega-3 / Omega-6 (n-3/n-6)		10.00 ± 2.17	11.25 ± 3.10	0.202

Accordingly, the isotope results can be interpreted in terms of the trophic position of the fish. There was no distinction between the trophic positions of pansteatitis positive and negative individuals (Figure 5). The δ^{13}C values are indistinguishable (-13.4 ± 0.5 ‰ and -13.7 ± 0.6 ‰, respectively), as are the δ^{15}N values (16.3 ± 0.5 ‰ and 16.6 ± 0.5 ‰, respectively). The range of δ^{15}N values (15.4–17.6 ‰) indicates higher levels of piscivory in certain individuals.

Table 3 Fatty acid composition of adipose tissue from *Labeo rosae*, *Clarias gariepinus*, *Labeobarbus marequensis* and *Cyprinus carpio* incidentally caught during sampling from Lake Loskop during January 2016

Species		***L. rosae*** **n=6**			***C. gariepinus*** **n=3**			***L.marequensis*** **n=2**			***C. carpio*** **n=1**
FAME (% of total fatty acids)											
Common name	Abbreviation	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
Myristic	C14:0	4.22	3.32	4.7	1.89	1.48	2.17	4.13	3.68	4.57	2.75
Myristoleic	C14:1c9	0.15	0	0.47	0.12	0	0.26	0.07	0.05	0.09	0.09
Pentadecylic	C15:0	1.89	0.08	10.24	0.33	0.07	0.5	0.08	0.07	0.09	0.84
Palmitic	C16:0	32.16	26.9	37.64	27.04	26.09	27.77	25.03	24.08	25.97	19.91
Palmitoleic	C16:1c9	2.53	0.49	5.18	6.29	5.58	7.67	7.23	6.09	8.36	5.00
Margaric	C17:0	0.73	0.2	2.06	0.89	0.7	1.17	0.34	0.33	0.35	2.00
Heptadecenoic	C17:1c10	0.09	0.03	0.26	0.08	0.04	0.15	0.53	0.33	0.73	0.76
Stearic acid	C18:0	3.78	2.82	5.34	5.16	5	5.28	3.53	3.3	3.76	4.62
Elaidic	C18:1t9	0.08	0	0.5	0.04	0	0.08	0.41	0.27	0.54	0.10
Oleic	C18:1c9	19.49	16.8	21.17	18.34	16.21	22.01	26.53	26.07	26.99	15.04
Vaccenic	C18:1c7	2.86	1.65	4.64	6.35	4.88	7.69	4.18	4.13	4.23	9.19
Nonoadecanoic	C19:0	0.14	0.07	0.22	0.10	0.06	0.17	0.51	0.46	0.55	0.48
Linoleic	C18:2c9,12 (n-6)	2.08	1.43	2.78	3.49	2.75	3.96	5.43	4.5	6.36	7.25
Arachidic	C20:0	0.76	0.51	1.08	0.37	0.34	0.41	0.07	0.05	0.08	0.22
γ-Linolenic	C18:3c6,9,12 (n-6)	0.48	0.27	0.61	0.80	0.56	1.05	0.39	0.36	0.41	0.30
α-Linolenic	C18:3c9,12,15 (n-3)	2.92	2.55	3.3	2.63	2.26	3.32	7.07	7.02	7.11	3.39
Heneicosanoic	C21:0	0.08	0.03	0.14	0.04	0	0.08	0	0	0	0.00
Eicosadienoic	C20:2c11,14 (n-6)	0.25	0	0.65	0.15	0.1	0.22	0.05	0	0.09	1.69
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.11	0.03	0.25	0.31	0.21	0.49	0.11	0	0.22	0.42
Erucic	C22:1c13	0.46	0.23	0.74	0.45	0.34	0.58	0.1	0.08	0.12	1.15
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.11	0.03	0.18	0.31	0.24	0.38	0	0	0	0.19
Arachidonic	C20:4c5,8,11,14 (n-6)	0.95	0.4	2.66	1.51	1.21	1.91	1.16	1.05	1.26	5.64
Eicosopentaenoic	C20:5c5,8,11,14,17 (n-3)	5.97	3.41	9.3	3.05	1.96	4.61	9.18	8.25	10.11	9.73
Nervonic	C24:1c15	0.00	0	0	0.10	0.09	0.11	0	0	0	0.00
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	2.35	1.5	4.36	5.35	3.58	6.96	2.12	2	2.23	2.17

Docosahexanoic	C22:6c4,7,10,13,16,19 (n-3)	15.37	4.79	29.08	14.82	12.74	18.61	1.71	1.22	2.2	7.07
Fatty acid ratios											
Saturated Fatty Acids	SFA	43.75	36.7	50.89	35.81	34.93	37.4	33.67	32.94	34.39	30.82
Mono Unsaturated Fatty Acids	MUFA	25.67	19.5	30.37	31.78	28.03	36.75	39.04	37.99	40.09	31.32
Poly Unsaturated Fatty Acids	PUFA	30.58	18.7	43.78	32.41	28.14	37.04	27.29	26.96	27.62	37.86
SFA/PUFA	SFA/PUFA	1.58	0.84	2.72	1.12	0.94	1.25	1.24	1.22	1.25	0.81
Omega-6 Fatty Acids	n-6	3.86	2.32	6.32	6.25	4.85	7.2	7.02	6.18	7.85	15.08
Omega-3 Fatty Acids	n-3	26.71	12.4	40.38	26.16	23.29	30.35	20.28	19.77	20.79	22.78
Omega- 3 / Omega- 6	n-3/n-6	8.20	1.97	14.72	4.26	3.45	4.8	2.94	2.52	3.36	1.51

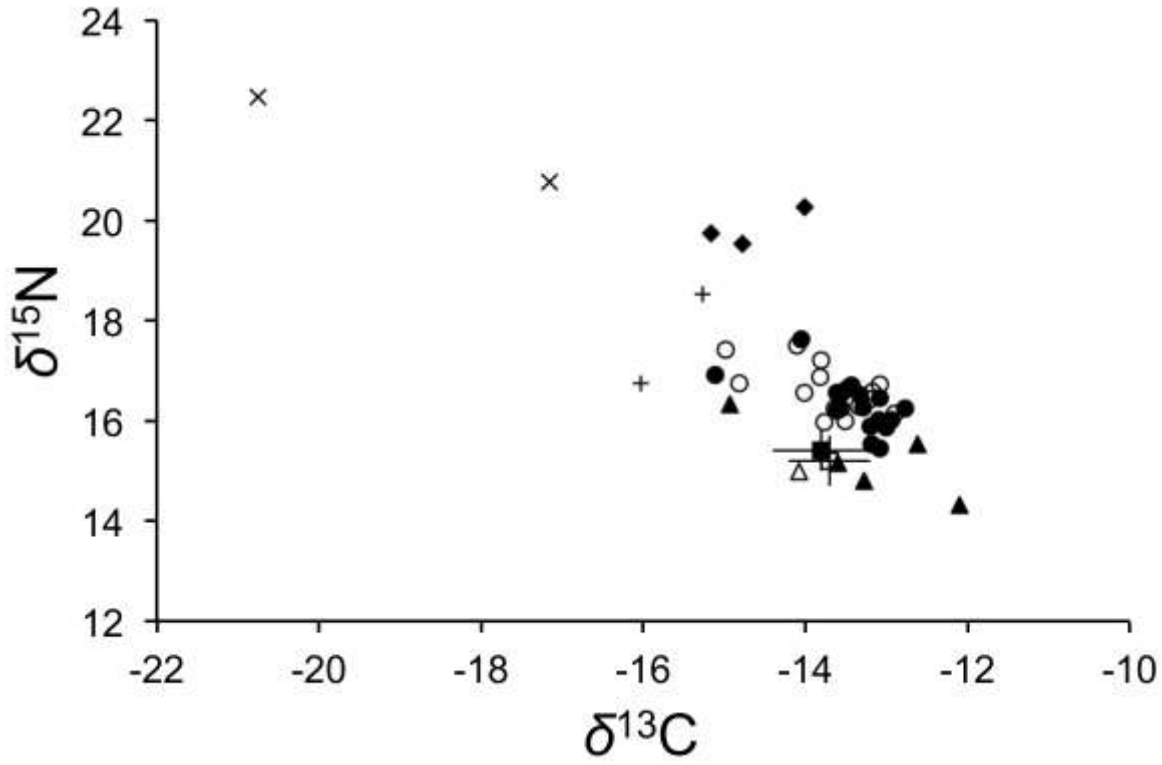

Figure 5 Isotope biplot for fish sampled from Lake Loskop in January 2016. Circles = *Oreochromis mossambicus*, diamonds = *Clarias gariepinus*, triangles = *Labeo rosae*, x = *Labeobarbus marequensis*, + = *Cyprinus carpio*. Open symbols represent pansteatitis negative specimens and filled symbols represent pansteatitis positive specimens. The mean and standard deviation for measurements reported in Dabrowski *et al.* (2014) are also represented (open square = *O. mossambicus* sampled in 2010, filled square = *O. mossambicus* sampled in 2011).

Discussion

The field results confirm that pansteatitis was prevalent in several species of fish in Lake Loskop across a range of trophic levels. However, in contrast to previous reports, 45 % of *O. mossambicus* specimens sampled during this study were healthy. Pansteatitis prevalence in Lake Loskop was higher in larger, heavier individuals of *O. mossambicus* confirming similar findings by Bowden *et al.* (2016). This may indicate a change in feed preference once *O. mossambicus* in Lake Loskop reach a certain size, irrespective of age, as age was not a

significant predictor of either pansteatitis presence or pansteatitis severity within the age range sampled. Similar observations were made by Huchzermeyer *et al.* (2013) in pansteatitis-affected *C. gariepinus* in the Olifants River Gorge. Bowden *et al.* (2016) identified an association between age and pansteatitis in *O. mossambicus* sampled from Lake Loskop during 2014, which may be explained by the presence of younger ages amongst the sampled fish. However, condition factor was not a significant predictor of pansteatitis severity, supporting the findings of Truter, van Wyk, Oberholster, Botha & Luus-Powell (2016) who were unable to link the pansteatitis in Lake Loskop fish to abnormal obesity through hormonal disruption. However, a lack of any relationship between δ^{15}N values (as an indicator of trophic level) and size or age does not support the notion of a shift towards piscivory with age as a systematic adaptive strategy of *O. mossambicus*. That piscivory is not intrinsically driving pansteatitis in Lake Loskop is indicated by the fact that the average δ^{15}N value obtained in the pansteatitis positive population of *O. mossambicus* was marginally lower than the value obtained from the pansteatitis negative population. This is contrary to expectation as higher δ^{15}N values are indicative of higher levels of piscivory. Since both pansteatitis positive and pansteatitis negative populations include individuals that are occupying a piscivorous trophic niche it suggests that piscivory is not an indicator of pansteatitis, apparently confirming the findings of Dabrowski *et al.* (2014). The time for the integration of the δ^{15}N value in muscle tissue takes place over several months, and it may be that episodic intense piscivory events are difficult to discern. The isotopic evidence therefore points towards a systematic change in diet and an increased probability of exposure to concentrated piscivory events with age. Huchzermeyer (2012) showed that pansteatitis severity can be the cumulative effect of repeated oxidative triggers. Presence of normal appearing fat as well as acute and chronic inflammatory change in pansteatitis-affected adipose tissues of *O. mossambicus* from Lake Loskop supports this observation.

In the natural environment, individuals weakened by pansteatitis are expected to fall prey to a variety of aquatic predators. Yet, even fish with a severe degree of pansteatitis, sampled during this study, reached a high age indicating that pansteatitis, at least in *O. mossambicus*, was less debilitating than expected. This is in contrast to findings amongst some farmed fish where pansteatitis has been associated with pathology in other organs and increased morbidity and mortality (Roberts *et al.* 1979; Roberts & Agius 2008)

The ratio of saturated to polyunsaturated fatty acids was slightly, but significantly, lower in the sampled *O. mossambicus* with pansteatitis reflecting a higher assimilation of PUFA in fish that have developed pansteatitis. In catfish from the Olifants River Gorge, the level of C22:6c4,7,10,13,16,19(n-3) in adipose tissues of pansteatitis-affected catfish was significantly higher than that in healthy individuals from the same site (Huchzermeyer *et al.* 2013). A similar differentiation was not evident in *O. mossambicus* from Lake Loskop where both healthy and pansteatitis-affected fish had a similar level of C22:6c4,7,10,13,16,19(n-3) which was much higher than that recorded from the Olifants River Gorge fish.

No significant difference in the ratio of n-3:n-6 was observed when the fat of fish with and without pansteatitis from Lake Loskop was compared (Table 2). This contrasts to findings from the Olifants River Gorge where the ratio of n-3:n-6 was significantly higher in the adipose tissues of both catfish and crocodiles with pansteatitis when compared to that of healthy individuals (Osthoff *et al.* 2010; Huchzermeyer *et al.* 2013). The n-3:n-6 ratio in the fat of *O. mossambicus* from lake Loskop was more than twice that found in *C. gariepinus* suffering from pansteatitis in the Olifants River Gorge (Huchzermeyer *et al.* 2013). This supports an argument for a trophic shift towards an oil-rich prey species in the Olifants River Gorge (Woodborne *et al.* 2012) but not in Lake Loskop.

The spikes in pansteatitis prevalence in the age categories 2–4 years and 6–8 years in fish from Lake Loskop might correspond to major fish die-off events in the lake (2007 and 2012).

Repeated opportunity to feed on dead fish and the scale of such opportunities would influence the number of oxidative event exposures explaining greater severity of pansteatitis in some age categories as well as the presence of normal fat, fat with early pansteatitis and fat with advanced inflammatory changes within the same adipose tissues.

The relative abundance in the diet of the n-6 and n-3 fatty acids that are derived from linoleic and α-linolenic acids, respectively, and that cannot be synthesized by animals, is reflected in the composition of their fat tissues (Hoffman & Prinsloo 1995; Steffens 1997, Huchzermeyer *et al.* 2013). The high n-3:n-6 ratio of polyunsaturated fatty acids (typically between 5 and more than 10) of marine fish oils is a reflection of the fatty acid composition of phytoplankton within marine food webs (Steffens 1997). In fat of freshwater fish, this ratio is generally 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. The n-3:n-6 fatty acid ratio of 10 and higher in adipose tissues of *O. mossambicus* from Lake Loskop exceeds that typically expected from a marine environment. This provides insight into a unique dietary force driving the food web, most likely linked to the intensity of phytoplankton blooms in this impoundment. We suggest that the exceptionally high levels of n-3 polyunsaturated fatty acids assimilated by *O. mossambicus* from Lake Loskop may explain their high vulnerability to pansteatitis.

In the Olifants River Gorge, adipose tissues from catfish and crocodiles with pansteatitis had an n-3:n-6 fatty acid ratio of 2.87 and 2.0 respectively whereas animals without pansteatitis had a ratio of under one (Osthoff *et al.* 2010; Huchzermeyer *et al.* 2013). The findings were mirrored by an increase in δ^{15}N values in muscle of both crocodiles and catfish with pansteatitis, but absent in individuals without pansteatitis suggesting a coincidental trophic shift towards piscivory in these two species (Woodborne *et al.* 2012), neither of which are able to assimilate n-3 fatty acids directly from phytoplankton as neither are filter feeders.

Contrasting to this, fish from Lake Loskop had a significantly higher n-3:n-6 fatty acid ratio, yet showed no significant difference in the n-3:n-6 fatty acid ratio of fish with and without pansteatitis. In a captive farmed population of catfish, Huchzermeyer *et al.* (2013) reported no difference in n-3:n-6 fatty acid ratio between individuals with and without pansteatitis and overall low levels of n-3 fatty acid assimilation when pansteatitis was induced by feeding of a rancid diet not enriched with n-3 fatty acids.

It has been proposed that the yellow discolouration of fat in Mozambique tilapia previously reported in Lake Loskop, but only observed in two fish (one with and one without pansteatitis) during the current study, may be due to bioaccumulation of aluminium and iron via various species of phytoplankton on which these fish feed (Oberholster *et al.* 2011). In a dietary comparison of *O. mossambicus* between Lake Loskop and Lake Flag Boshielo, Dabrowski *et al.* (2014) found the abundance of planktonic food items, dominated by *C. hirundinella* in Lake Loskop, to be the distinguishing feature between the two reservoirs. These authors, using a combination of stomach content and stable isotope analysis over several seasons, maintained that a lack of evidence of piscivory in Lake Loskop points to an aetiology other than the classic documented nutritional cause of pansteatitis. Although the trophic position of *O. mossambicus* in Lake Loskop was found to be higher than in Lake Flag Boshielo, this may be explained by the oligotrophic state of Lake Flag Boshielo forcing *O. mossambicus* to scavenge the benthos of the lake for detritus due to lack of food in the water column. This situation differs substantially from that in Lake Loskop where the cyanobacterium, *M. aerugenosa* and the dinoflagellate *C. hirundinella* provide an ample pelagic source of food for the fish (Dabrowski *et al.* 2014). Based on these findings, Dabrowski *et al.* (2014) conclude that there was no evidence of present or past piscivory to explain the pansteatitis in the fish from Lake Loskop. The current study provides additional evidence, based on stomach content analysis, indicating that piscivory is prevalent amongst

O. mossambicus in Lake Loskop. The presence of fish remains among the stomach contents is associated with isotopic values that are indistinguishable from that reported by Dabrowski *et al.* (2014) suggesting that fish forms a persistent low level component of the diet.

Previous results demonstrated that elevated n-3:n-6 fatty acid ratios were observed in higher trophic levels (sharptooth catfish and crocodiles) in the Olifants River Gorge, but there was no evidence available at that time from the filter feeding guild. This study demonstrates that these high fatty acid ratios are found in the filter feeding guild in Lake Loskop, but this is not intrinsically responsible for pansteatitis as both pansteatitis positive and negative individuals share the high fatty acid ratios, and the same trophic position. Herbivorous fish will take in sufficient vitamin E with their diet to prevent autoxidation of assimilated n-3 fatty acids, but the high levels, to which they assimilate these, increases their susceptibility to pansteatitis. For pansteatitis to develop, tissue vitamin E levels must first be depleted by a trigger event. The presence of demonstrably piscivorous *O. mossambicus* in both pansteatitis positive and negative populations in Lake Loskop confirms that piscivory is not necessarily the fundamental trigger, but contrary to Dabrowski *et al.* (2014) it does occur in *O. mossambicus* in Lake Loskop. We propose that this circumstance is similar to that observed by Huchzermeyer *et al.* (2013) amongst farmed fish, and that pansteatitis among *O. mossambicus* in Lake Loskop is triggered by the intake of rancid fish derived from periodic fish die-offs. The supply of oxidised fats in the diet following a fish die-off event drives tissue vitamin E depletion and the development of pansteatitis. A critical aspect of this proposed aetiology is that stable light isotope values following the intake of oxidised fat from a fish die-off event will be indistinguishable from the persistent background level of piscivory that is not associated with die-off events.

The mechanism proposed here is consistent with the observation in the Oifants River Gorge. Eutrophic conditions and algal blooms are a common feature in Lake Massingir, deriving

almost entirely from the Olifants River Gorge inflow, and these lead to n-3 fatty acid assimilation within the food web as described by Huchzermeyer *et al.* (2013). In the absence of a supply of oxidised fat from fish die-off events, there is no trigger event to deplete tissue vitamin E levels in filter-feeding fish, and it is only the higher trophic levels that are affected by autoxidation of n-3 fatty acids bioaccumulated within the trophic cascade.

In Lake Flag Boshielo where no pansteatitis is reported, the oligotrophic conditions suppress blooms involving the cyanobacterium *M. aeruginosa* and dinoflagellate *C. hirundinella*, and the filter feeding guild that includes *O. mossambicus* and silver carp, have a different diet from their counterparts in Lake Loskop (Dabrowski *et al.* 2014) or the Olifants River Gorge. The n-3 fatty acids are not accumulated through trophic interactions and top predators in the system are not subject to vitamin E stress through this mechanism. In addition, there are no frequent fish die-off events that could trigger direct oxidative stress.

Conclusion

Pansteatitis is an unusual event amongst free-living wild animals. This study provides the first detailed explanation for occurrence of the disease in eutrophic waters. Whereas the causes of pansteatitis in the lower Olifants River have been elucidated (Woodborne *et al.* 2012, Huchzermeyer *et al.* 2013), the causes of the pansteatitis in fish from Lake Loskop have remained unclear. Woodborne *et al.* (2012) and Huchzermeyer *et al.* (2013) identified food web changes associated with eutrophication in the lower Olifants River in the Kruger National Park, South Africa, as the most likely cause for excessive assimilation of certain highly polyunsaturated fatty acids identified in fish and crocodiles suffering from pansteatitis. Close correlation between inversions of the n-3 to n-6 fatty acid ratios characterized both *C.*

gariepinus and crocodiles with pansteatitis when compared to those without pansteatitis from the Olifants River Gorge and other reference populations in the Kruger National Park (Huchzermeyer *et al.* 2013).

The results presented here indicate assimilation of unusually high levels of n-3 fatty acids by filter feeding fish in Lake Loskop. This explains the high vulnerability to pansteatitis in, amongst others, *O. mossambicus*. However, in contrast to the Olifants River Gorge, high assimilation of n-3 fatty acids did not differentiate between pansteatitis positive and negative filter feeding fish in Lake Loskop. Here, the most likely vitamin E-depleting trigger event precipitating pansteatitis in filter feeding and other species is the intake of rancid fats during fish die-off events. The high prevalence of pansteatitis in *O. mossambicus* is symptomatic of the deteriorating trophic conditions in Lake Loskop, and the periodic scavenging opportunities may be linked to an unnaturally high biomass of *L. rosae* thriving on the eutrophic conditions of the lake. In both Lake Loskop and the Olifants River Gorge, the aetiology of pansteatitis is therefore linked to eutrophic conditions affecting the filter feeding guild, with trophic cascade effects either through the higher levels of the food web structure in the case of the Olifants River Gorge, or through scavenging of rancid fats during fish die-off events in Lake Loskop.

Following the mass die-off of large Nile crocodiles in the Olifants River Gorge in the Kruger National Park since 2008 and the decline of the crocodile population in Lake Loskop since 2005, no solution has been found to improve their survival in these waters. Both Lake Loskop and Lake Massingir act as nutrient traps creating unnatural aquatic ecosystems with intense phytoplankton growth sustaining a large biomass of certain species of fish abnormally rich in polyunsaturated fats. Bottlenecks to fish migration (Olifants River Gorge) or fish die-offs (Lake Loskop) increase the risk of pansteatitis developing. Reducing the n-3 rich biomass of such species through strengthened subsistence fisheries and utilisation of fish targeted during

fishing competitions provides an adaptive management response to alleviate the risk of pansteatitis-associated mortality amongst crocodiles at these sites. Lake Massingir, which lies outside of the KNP, supports an artisanal fishery, but the fisher villages do not have the resources to effectively net the pelagic silver carp in the lake. Lake Loskop, on the other hand, falls under the control of MTPA who essentially has a conservation mandate allowing only recreational fishing which has overall little impact on fish biomass in the lake. Further population studies will need to confirm the most abundant species to target. In the case of Lake Loskop this may be *L. rosae*. If confirmed, MTPA Scientific Services may need to manage the numbers of this species directly. This will contribute directly to the survival chances of natural crocodile populations by removing some of the nutrient loading of this impoundment and normalizing the polyunsaturated fat intake from fish on which they prey.

Acknowledgements

The study was funded by the authors personally. Dr P.A. Colly and Sterkspruit Veterinary Clinic in Lydenburg, South Africa are thanked for financial and logistical support. Misters C. and R. Huchzermeyer are thanked for their help with arranging the logistics for the field work and for assisting with the catching and dissection of the fish. Mr N. Huchzermeyer of the Department of Geography, Rhodes University is gratefully acknowledged for preparing the map of the Olifants River catchment. Mr Edward Butler of the Department of Ichthyology and Fisheries Science, Rhodes University is thanked for preparing sections from the otoliths. Dr Grant Hall facilitated the isotopic analysis at the University of Pretoria. The study was approved by the Animal Ethics Committee of the University of Pretoria under project number V072-16.

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