Histopathology of the liver and gills of Labeo rosae (rednose Labeo) from Loskop Dam in South Africa

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Abstract

The Loskop Dam is the most polluted impoundment in the Olifants River, because it receives pollutants from the entire mine-dominated upper catchment. However, histopathology of fish inhabiting this polluted dam is scantly explored. The current study aimed to investigate the histopathology of the gills and liver of *Labeo rosae* from Loskop Dam. Alkaline pH was observed throughout the study with most metal concentrations exceeding the water quality guideline for aquatic ecosystems. Regressive changes were the most prominent lesions for both organs with gills showing relatively more pathologies than the liver. Epithelial lifting was 100% prevalent during both seasons in the gills whereas a significant expansion of lipofuscin-laden melanomacrophages (MMCs) showed 100% prevalence in the liver. Gills were significantly different in their prevalence of histopathology between the two seasons, which was not the case for liver. The histopathology recorded in this study shows that the health of *L. rosae* at Loskop Dam was compromised. Given the exacerbating pollution level in the upper Olifants River, these findings serve as a warning to conservation authorities and emphasise the necessity for regular monitoring of fish health at Loskop Dam to assess pollution levels using fish health as a sensitive indicator to altering pollution levels.

Keywords: acid mine drainage, epithelial lifting, fish health, Olifants River, water pollution

Introduction

Freshwater pollution has become a serious threat to aquatic biodiversity globally (Vörösmarty et al. 2010). In South Africa, the Olifants River is one of the most polluted river systems that has experienced episodic mortalities of aquatic biota in its upper and lower catchment (De Villiers and Mkwelo 2009; Bowden et al. 2016). The Loskop Dam (25°26′57.05″ S, 29°19′44.36″ E) is the first main impoundment receiving water from the entire upper Olifants River and it was described as a repository for all pollutants emanating from the upper catchment (Oberholster et al. 2010). The upper Olifants catchment is characterised by extensive mining activities, metallurgic industries, coal-fired power stations and agricultural activities that have consequently affected the water quality of the receiving Loskop Dam (Dabrowski et al. 2013; Oberholster et al. 2017). A high variability of pH, as a result of continuous acid mine drainage, as well as increased concentrations of aluminium and iron in the bottom sediment, water column and aquatic biota has been prominent at Loskop Dam (Oberholster et al. 2010; Oberholster et al. 2012; Lebepe et al. 2016).

Despite elevated pollution levels and occasional mortalities of aquatic biota, macroscopic fish health assessment studies have shown that most fish at Loskop Dam are fairly healthy with internal and external organs exhibiting few to no anomalies (Watson et al. 2012; Huchzermeyer et al. 2017). Macroscopic assessment is highly recommended, because it can provide a simple and rapid indication of how fish cope with stress, however, it could still give misleading results, because it looks at gross changes. Early toxic effects of pollution may be present at a cellular level before gross changes manifest (Camargo and Martinez 2007). Histopathology is known to be a sensitive indicator and provides early warning signs of the effect of pollution on the health of fish (Baiomy 2016). The current study examined the histopathology of the liver and gills of Labeo rosae from the Loskop Dam. It was hypothesised that the histopathology of fish liver and gill tissue at Loskop Dam would be severe, as a result of an elevated pollution level. This study provides the first documentation of the histopathology of fish tissues at Loskop Dam, therefore, these findings could be used as baseline information for future reference.

Material and methods

Water sampling and analysis

Water samples were collected from the inflow, middle and dam wall using acid treated sampling bottles during winter (June) and summer (December) 2011 surveys. The samples were frozen and later sent to the South African National Accreditation System (SANAS) accredited laboratory for chemical analysis. In the laboratory, metals were analysed following Bervoets and Blust (2003) where the water was acidified using nitric acid (HNO_3, 69%) to a pH of 2 and filtered through 0.45µ filter paper. The following metals: aluminium (Al), Arsenic (As), antimony (Sb), barium (Ba), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), selenium (Se), strontium (Sr) and zinc (Zn) were analysed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). These metals are ubiquitous in the Olifants River system and they are important in fish toxicity (Oberholster et al. 2010; Dabrowski et al. 2013; Authman et al. 2015). The pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS) and salinity were measured in situ by using a handheld multi-parameter instrument (YSI 556 Multi Probe System).

Fish sampling and processing

Concurrently with water sampling, 20 fish specimens were sampled as per Marr et al. (2017) using gill nets. The fish were euthanised by severing the spinal cord. Fish and its liver were weighed, and lengths were measured. Fish weight and standard lengths were used to calculate a condition factor (CF) for each fish following the methods of Heath et al. (2004). The liver weight was used to calculate the hepatosomatic index (HSI) as per Nunes et al. (2011) method. The project was approved by the University of Pretoria Animal Use and Care ethical committee (reference number T001-12).

Microscopic assessment

A gill arch and approximately a third of the liver were collected and fixed in 10% neutral buffered formalin. The samples were submitted to the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria for routine histological processing. In the laboratory, samples were dehydrated through a series of ethanol (70%, 80%, 96% and 100%). Xylene was used to clear the samples and make them transparent. The samples were infiltrated through increasing concentrations of Tissue-Tek® III wax in a 60 °C oven. Once thoroughly infiltrated, samples were embedded in wax blocks

(Humason 1962). Each block was sectioned at 4–5 µm using a rotary/sliding microtome (Reichert-Jung 2040). The samples were floated using gelatine and distilled water solution, and then mounted on glass microscope slides and air-dried in a 60 °C oven. Dried samples were prepared for light microscopy analysis using standard technique for Haematoxylin and Eosin (H&E) staining (Bancroft and Gamble (2008). Prepared histological slides were examined using a standard light microscope. Quantitative histopathological assessment was performed using Bernet et al. (1999) protocol. Histopathological lesions were identified and given an importance factor (1–3), which represents the potential for altering the health of the fish. Score values (0–6) were assigned depending on the degree and extent of the changes. Score values and importance factors were used to calculate the organ index (I_{org}). Organ index values were used to classify the severity of the pathology using a classification system based on the scoring scheme developed by Zimmerli et al. (2007).

Data analysis

A Kolmogorov–Smirnov analysis was used to test the normality of the data, whereas a Levene's test was used to check the homogeneity of variance. For histopathology prevalence and organ I_{org}, the data were normally distributed, therefore, an independent sample *t*-test was used to evaluate the differences between the two seasons using R-3.0.2. Because of the small sample size for water, a non-parametric Mann–Whitney *U*-test was used to evaluate the difference between the two seasons. The level of significance was set at 5% ($p < 0.05$).

Results

The conductivity, salinity, and TDS showed a significant difference between the two seasons with higher levels being recorded during summer (Table 1). The DO was significantly higher during winter than summer (Table 1). A neutral to alkaline pH ranging from 7.81 to 9.49 was recorded in winter with 8.07–10.05 being recorded during summer (Table 1). Moreover, SO_4 concentration showed no significant difference between the two seasons. The concentrations of As, Cd, Cu, Pb, Se and Zn were above DWAF (1996) target water quality range (TWQR) during winter and below detection level during summer (Table 1). There is no TWQR set by DWAF (1996) for aquatic ecosystem for Ba, Fe, Mn and Sr, however, substantial concentrations were recorded for these metals during both seasons (Table 1). Metal concentrations have shown no significant difference between the two seasons (Table 1).

Regressive changes were the most prominent lesions for both organs with gills more severely affected than the liver. Lesions in the gills included epithelial lifting, oedema of the secondary lamellae, pillar cell rupture, epithelial hyperplasia, cartilage deformation, chloride cell proliferation, goblet cell hyperplasia and hypertrophy, fusion of primary and secondary lamellae, interstitial oedema, aneurism formation in the primary and secondary lamellae and nuclear degeneration resulting from necrosis (Figure 1; Table 2). Observed lesions exhibited a considerable variation in the degree of injury (Table 2). There was a significant difference ($t = 2.934$, $p < 0.05$) in the prevalence of gill lesions between the two seasons with summer lesions being relatively more severe than those observed in winter. However, the gill index showed no significant difference between the two seasons.

Table 1: Levels of physico-chemical parameters and metals recorded at Loskop Dam during winter and summer 2011. Units are mg l^{-1}, unless specified otherwise. TWQR = Target Water Quality Range. Mann–Whitney *U*-test values and *p*-values are also presented

Physico-chemical parameters	Winter	Summer	TWQR	*U*-value	*p*-value
DO	8.17 ± 2.63	4.71 ± 0.12	na	0.0001	0.04
pH	7.81–9.49	8.07–10.05	6.5–9	8.00	0.12
EC (mS/m)	39.33 ± 0.44	53.20 ± 12.00	na	9.00	0.04
Salinity (‰)	0.19 ± 0.00	0.26 ± 0.06	na	9.00	0.03
TDS	255.66 ± 2.86	345.80 ± 78.00	na	9.00	0.04
Alkalinity as $CaCO_3$	44.00 ± 3.27	42.67 ± 8.22	na	4.50	1.00
SO_4	119.00 ± 6.53	141.67 ± 29.01	na	7.00	0.27
Metals (µg/ℓ)					
Aluminium	0.04 ± 0.03	–	1	–	–
Arsenic	3.67 ± 1.25	–	10	–	–
Antimony	4.67 ± 3.86	–	na	–	–
Barium	53.33 ± 1.89	44.33 ± 6.55	na	1.00	0.10
Cadmium	1.00 ± 0.00	–	0.35	–	–
Copper	2.00 ± 0.00	–	1.2	–	–
Iron	76.67 ± 65.75	82.00 ± 0.00	300	2.00	0.65
Lithium	15.33 ± 1.25	–	na	–	–
Manganese	86.67 ± 33.49	219.00 ± 0.00	180	3.00	0.18
Lead	10.00 ± 2.83	–	1	–	–
Selenium	6.67 ± 4.71	–	2	–	–
Strontium	186.00 ± 6.98	180.67 ± 34.03	na	3.00	0.51
Zinc	2.67 ± 3.09	–	2	–	–

Note: na = not available; (–) = below detection level

Table 2: Histopathology recorded for the gills and liver of Labeo rosae from the Loskop Dam during winter and summer 2011.

Organ	Alterations	Winter (*n* = 20)		Summer (*n* = 20)	
		Importance factor (w)	Prevalence (%)	Importance factor (w)	Prevalence (%)
Gill	Epithelial lifting	2	100	2	100
	Oedema	1	40	1	53
	Haemorrhage	1	47	1	50
	Pillar cell rupture	2	78	2	70
	Epithelial hyperplasia	1	40	1	35
	Fusion of primary lamella	2	46	2	40
	Fusion of secondary lamella	2	48	2	53
	Aneurism	1	35	1	39
	Mucous cells hyperplasia and hypertrophy	1	30	2	50
	Nuclear damage	2	39	2	30
	Necrosis	2	34	2	43
Liver	Hepatocellular hypertrophy	1	48	1	61
	Hydropic degeneration	1	68	1	48
	Melanomacrophages aggregate	1	100	2	100
	Haemorrhage (Sinusoidal congestion)	1	60	1	69
	Lymphocytic infiltration	1	21	1	18
	Fatty vacuolization	1	49	1	56
	Hepatocellular pleomorphism	2	38	1	29
	Nucleus at a peripheral position	2	80	2	86
	Cell rupture	1	42	1	55
	Hyaline droplets	0	0	1	20

Note: Importance factors: 1 = minimal; 2 = moderate; 3 = marked pathological importance

Lipid vacuolisation, sinusoidal congestion, lymphocytic infiltration, steatosis, haemorrhage, peripheral nucleus, hepatocellular pleomorphism and hypertrophy, nuclear degeneration characterised by chromatin clumping resulting from necrosis, hyaline droplet and hydropic swelling, and severe expansion of lipofuscin laden melanomacrophages (MMCs) were observed in the liver during both seasons (Figure 2; Table 2). Hypertrophy, hyaline droplet and hydropic degeneration were prominent during summer (Table 2). The prevalence of the liver lesions was not significantly different between seasons (Table 2) whereas the liver index did differ between seasons ($t = 3.635$, $p < 0.05$). Generally, lesions in the liver were significantly less severe than those observed in the gills ($t = 3.046$, $p < 0.05$). The Zimmerli et al. (2007) classification scheme categorised the liver lesions as moderate ($I_{liver} = 22.00$), with the gill being pronounced ($I_{gills} = 32.80$).

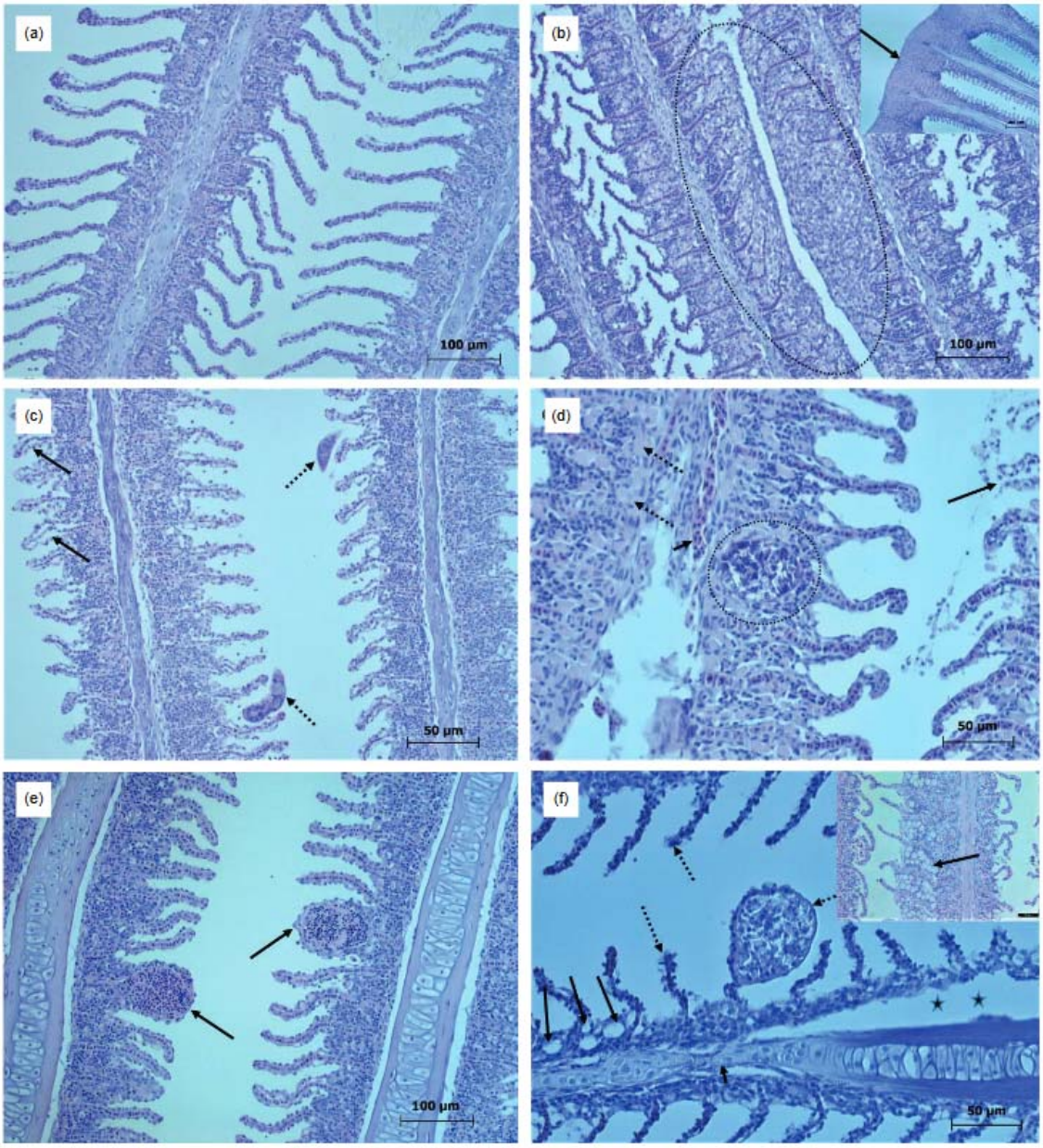

Figure 1: (a) Nearly normal gill; (b) high severity of epithelial hyperplasia that induced complete lamellar fusion (encircled), primary lamella fusion (arrow); (c) epithelial lifting (solid arrows), parasitic infestations (dotted arrows); (d) vascular congestion (short arrow), parasites infestation (solid long arrow), oedema (dotted arrow); (e) aneurism resulted in pillar cells rupture and necrosis (arrows); (f) aneurysm (short dotted arrow), mucous cells hyperplasia and hypertrophy (long solid arrows), oedema (asterisks), regressive change of supporting tissue in a form of cartilage deformity (short solid

arrow

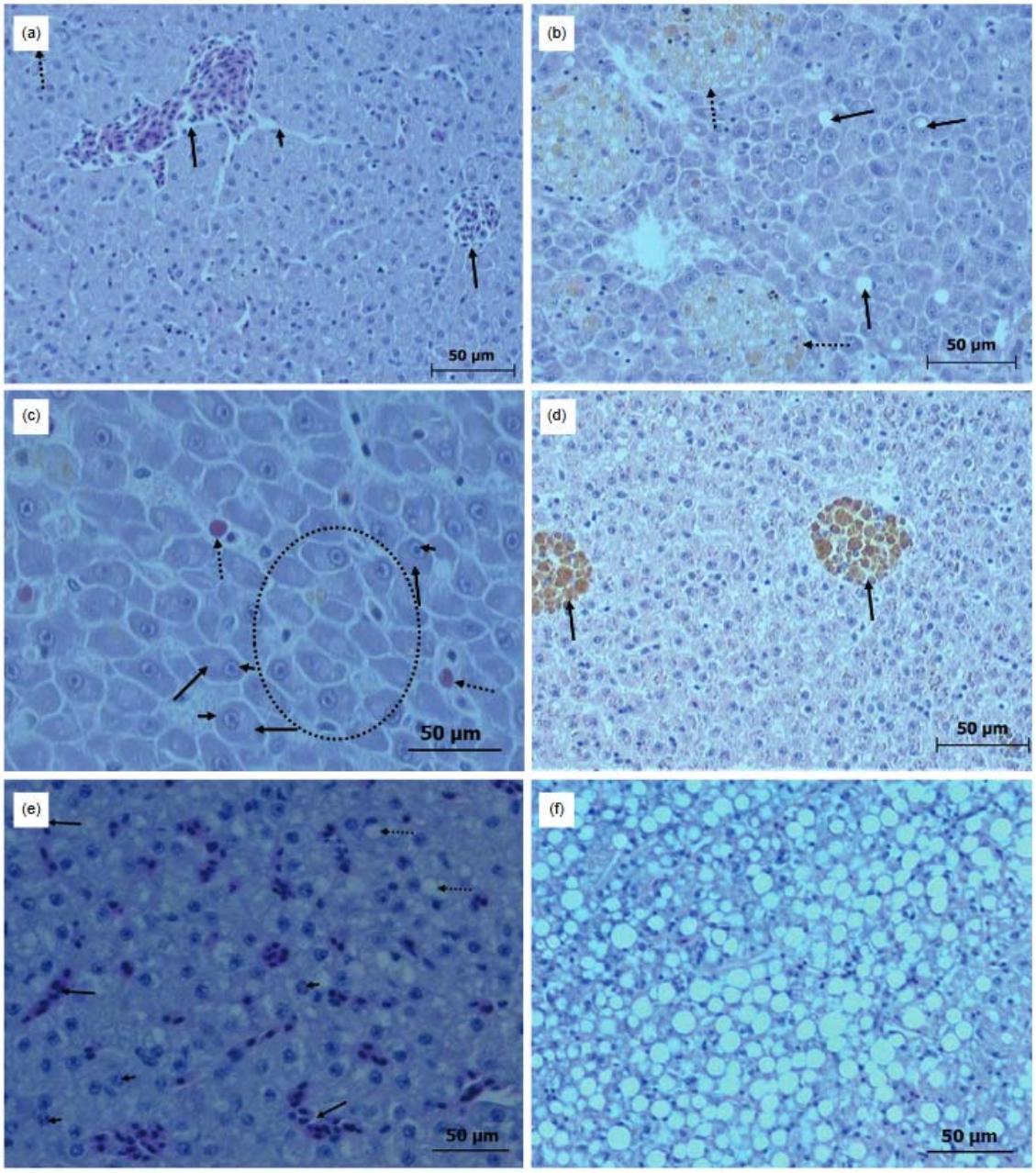

Figure 2: (a) Nearly normal liver showing bile duct (dotted arrows), central vein (solid arrows), sinusoid (short arrow); (b) fatty vacuolisation (solid arrows), melanomacrophages (MMCs) (dotted arrows); (c) hyaline droplets (dotted arrows), hydropic degeneration (long solid arrows), hepatocellular pleomorphism and hypertrophy (encircled); (d) severe accumulation of MMCs (haemosiderin and lipofuscin); (e) sinusoidal congestion (long solid arrows), nuclear degeneration resulting in necrosis (short arrows), fatty vacuolisation (dotted arrows); (f) steatosis

Discussion

The pH was within the DWAF (1996) water quality guidelines throughout the study. There are no TWQR values suggested for aquatic ecosystem for EC, TDS, alkalinity and salinity. However, previous studies reported that the afore-mentioned constituents have shown a significant increase over the past Lebepe, Steyl and Luus-Powell170 two decades (Heath et

al. 2010; Oberholster et al. 2010; Dabrowski et al. 2013). The dam receives contaminants from the entire upper Olifants catchment, which is characterised by mining and metal industries (Oberholster et al. 2012). Sulphate concentration reported in the current study was relatively higher, compared with those reported two decades ago. According to Jooste et al. (2015) an annual median sulphate concentration at Loskop Dam exceeded the 100 mg l^{-1} threshold value for aquatic ecosystems since 2000. Cadmium, Cu, Pb, Se and Zn concentrations were above the DWAF (1996) TWQR during summer. Metal concentration observed in the current study were comparable to those reported in other recent studies (Oberholster et al. 2012; Lebepe et al. 2016).

Gill histopathology reported in the current study were comparable to those reported in different fish species exposed to metals (Abdel-Moneim et al. 2012; Fatima and Usmani 2013) and organic pollution (Schlacher et al. 2007; McHugh et al. 2011). Epithelial lifting, oedema of the secondary lamellae, pillar cell rupture, epithelial hyperplasia, fusion of primary and secondary lamellae, interstitial oedema, and aneurism formation in the primary and secondary lamellae, necrosis and nuclear changes are significant lesions, because they compromise the gill's ability to perform its functions (Roberts 2012). Hypertrophy and hyperplasia of the epithelial and goblet cells reduce the space between lamellae and result in lamellae fusion, which ultimately reduces respiratory surface leading to hypoxia (Authman et al. 2013). Oedema and lifting of the epithelial cells serve as a defence mechanism to chronic exposure, because they increase the distance that waterborne pollutants must diffuse through to reach the bloodstream (Figueiredo-Fernandes et al. 2007; Salamat and Zarie 2012).

The liver has shown a wide spectrum of histopathology with different degrees of alterations. A high accumulation of lipofuscin laden MMCs, lipid vacuolisation, hypertrophy of hepatocytes, nuclear degeneration, hyaline droplets, hydropic swelling, hepatocellular pleomorphism, necrosis and cell rupture were prominent in the liver of L. rosae at both seasons. These lesions were comparable to those reported in the liver of wild fish populations exposed to metals and organic chemicals (Fatima and Usmani 2013; Baiomy 2016; Javed et al. 2017). Liver plays a central role in detoxification, metabolism and excretion of xenobiotic chemicals, hence, a target organ for the contaminants (Marchand et al. 2008). Most histopathological changes in the liver are protective responses to toxicant exposure. Melanomacrophages are involved in disease processes and changes may be brought about by factors such as starvation or chemical exposure (Agius and Roberts 2003; Abdel-Moneim et al. 2012). Hepatocyte hypertrophy and hyperplasia of are adaptive response to pollutant induced stress (Wolf and Wheeler 2018), however, hepatocytes are often swollen, as a result of glycogen or neutral fat accumulation when nutrition is less than ideal, or during cyclical starvation phases (Roberts 2012). Despite high prevalence, histopathology in the gills and liver of *L. rosae* reported in the current study were all reversible with importance factor ranging from 1 to 2 for both seasons.

Histopathology observed in the current study showed clear association with metal concentration, however, similar lesions were observed in fish populations exposed to nutrients (Marchand et al. 2012; van Dyk et al. 2012), pesticides (Yasser and Naser 2011) and organic contaminants (McHugh et al. 2011). Given that the Loskop Dam was reported to be eutrophic (Oberholster and Botha 2011; Dabrowski et al. 2013) with extensive use of pesticides in its vicinity (Bollmohr et al. 2008), it is therefore unlikely that pathologies observed in the liver and gills of L. rosae could solely be explained by metal pollution.

Conclusion

Macroscopic assessment of the health of fish in the middle and lower Olifants River showed no necessity for serious concern over the health of fish, because they appeared well adapted to the water quality (Madanire-Moyo et al. 2012). The current study, nevertheless, reported prominent histopathology in the liver and gills of *L. rosae* that affirm that the health of fish at the Loskop Dam is threatened. Histopathology has shown to be a reliable tool to provide early warning signs of the effects of pollution in fish. Because of the exacerbating water pollution in the upper Olifants River, it is reasonable to deduce that it is unlikely that fish tissue will recover under the current water quality conditions. Therefore, a regular assessment of the histopathology of fish tissues is recommended to monitor deterioration of histopathological changes, using the data obtained from this study as a reference.

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