Histopathological changes in two potential indicator fish species from a hyper-eutrophic freshwater ecosystem in South Africa: a baseline study

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Summary

Histopathological changes were identified in selected target organs from two freshwater fish species, *Clarias gariepinus* and *Oreochromis mossambicus*, inhabiting a hyper-eutrophic freshwater aquatic system. The approach was to use a histology-based fish health assessment protocol which included a semi-quantitative histopathological assessment of six target organs (gills, liver, ovaries, testes, kidney, and heart). Results of water quality analysis showed selected variables to be above the recommended levels including pH, ammonia, nitrogen, chloride, and phosphorus. A number of histopathological alterations were identified in the target organs of both species, with the highest number observed in the liver. Species differences were identified in terms of the severity of the alterations within all the organs, with most alterations being regressive in nature. The results of this study provide valuable baseline data for two indicator species inhabiting a hyper-eutrophic system that could be useful for future bio-monitoring studies.

Keywords: Roodeplaat, fish histology, bio-monitoring, *C. gariepinus*, *O. mossambicus*.

Introduction

Roodeplaat Dam (RD) is located within the Roodeplaat Nature Reserve (RNR), 22km north-east of the city Pretoria, South Africa (Figure 1). The dam is an important freshwater resource as it augments the supply of water to the northern areas of the city through the Montana, Wonderboom and Magaliesberg reservoirs. The Roodeplaat water treatment plant was commissioned in 2006, and is also used as a direct water supply to the Doornpoort area. The reserve itself was proclaimed in 1977 and is now a well known destination for bird watching, game viewing, and a range of water sports including freshwater angling (Roodeplaat Reserve 2008).

Although the dam is situated within a reserve, it has no bearing on its ecological status. It should by no means suggest that it supports a pristine ecosystem. The RD has a history of water quality problems (Steyn and Toerien 1976; Jones and Lee 1984; Versfeld and van Veelen 2007), and a fish kill was reported in 2004 (Hohls and van Ginkel, 2004). Presently it is considered as a hyper-eutrophic system (van Ginkel et al. 2001), and receives discharge from two sewage treatment plants, industry, housing, and agriculture, upstream of the reserve. Fifty percent of its capacity comes from the return flows of the secondary treated and chlorinated domestic wastewater

effluent from the Baviaanspoort and Zeekoegat wastewater treatment works (Versfeld and van Veelen 2007). The two wastewater treatment works are also two primary point sources of orthophosphate contributing to the orthophosphate load within the dam (Hohls et al. 1998), particularly from domestic and industrial soaps and/or detergents (DWA 2010).

Due to its importance as a water resource and recreational facility, particularly for fresh water angling, there is continued concern about RD and its eutrophication-related water quality characteristics. It has been listed fourth in the top ten dams in South Africa requiring priority eutrophication management (van Ginkel et al. 2001), and in 2010 was still considered eutrophic as reported by the Minister of Water and Environmental affairs (DWA 2010). The degradation of water quality through eutrophication restricts its uses and can render a whole water body not only useless, but hazardous to both aquatic life and to the people who rely on the water resource base itself (Codd 2000; Croke 2002a, b; South African Research and Documentation Center (SARDC), 2009).

With the above considered, it is evident that an environmental monitoring program is essential for potential and necessary long term mitigation. In 2007 the Gauteng Provincial Department of Agriculture, Conservation and Environment (GDACE) compiled an ecological management plan for the Roodeplaat Dam Nature Reserve (GDACE, 2007). One of the key objectives found within

the report was to conserve aquatic ecosystems in the reserve (GDACE, 2007). However, for effective monitoring to take place, baseline data needs to be obtained, including data related to the current health status of fish inhabiting the system and possible differences between species occupying different trophic levels.

The aim of this study was to carry out a histology-based fish health assessment for two freshwater fish species, *Clarias gariepinus* and *Oreochromis mossambicus*, inhabiting the RD in order to establish baseline data for future environmental monitoring studies. The objectives included: 1) analysis of physical water quality parameters and chemical constituents within water and sediment samples; 2) a standard fish necropsy to identify any external or internal macroscopic abnormalities; 3) a semi-quantitative histopathological assessment of six selected target organs (gills, liver, ovaries, testes, kidney, and heart) to identify and compare organ specific histopathological alterations; and 4) to identify possible species-specific differences.

Materials and Methods

Water analysis

Single measurements of physical water quality parameters were recorded at three sites (Figure 1) in the RD, in November 2007, between 11:00am and 13:00pm [Site 1: Inflow to RD (Leewfontein estate); Site 2: Fish sampling site (Hengelaarsvriend); Site 3: Most northern part of the dam (Dam

wall)]. Water temperature (°C), pH, total dissolved solids (TDS µg L^{-1}), oxygen concentration (mg L^{-1} and %) and conductivity (µS) were measured. The pH was measured with a pH Scan 2 pH meter (Eutech Instruments) and the remaining physical parameters with a Cyberscan CON 300, Conductivity/TDS/°C meter RS 232 (Eutech Instruments).

Water samples were collected at each site for chemical analysis of selected metals and potential endocrine disrupting chemicals (EDCs). The samples were collected in 14 mL Falcon tubes, acidified with 1mL of 65 % nitric acid (Merck), and stored at 4°C until analysis. Water samples were analysed for metal concentrations using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). The metal concentrations in the water were compared to the acceptable levels of the Target Water Quality Range (TWQS) stipulated by the South African water quality guidelines (DWAF 1996).

For EDC analysis, glass bottles (2.5 L, pre-rinsed in 100% methanol, chromatography grade) were filled with sample water. The neck and opening of the bottle was covered with aluminum foil and the lid replaced. These samples were stored at 4°C until analysis. Glass honey jars (250 mL, pre-rinsed with ethanol) were used to collect sediment. Analytical chemistry was carried out by an ISO 17025 accredited laboratory to determine organochlorines (OCs), polychlorinated biphenyls (PCBs), and alkylphenol (AP) levels. Organochlorines, PCBs and AP were extracted from water

samples using C18 Solid phase extraction (SPE) (Cacho et al. 1995). Gas chromatography coupled to a mass spectrometer detector operated in negative chemical ionization mode (GC-MS), was used for the OCs and PCBs. A high performance liquid chromatograph coupled to a fluorescence detector (HPLC-FLD) was used for the APs. Organochlorines, PCBs and AP were extracted from sediment samples using a combination of liquid extraction with methanol and hexane (Naudé et al. 1998).

Fish collection

Clarias gariepinus and *O. mossambicus* were selected as indicator species as they are indigenous to South Africa and important for their angling and commercial value. They represent different trophic levels as *O. mossambicus* feed on algae and larger individuals also feed on insects and other invertebrates while *C. gariepinus* is an omnivorous species and prey on fish including *O. mossambicus.* Sampling was carried out during a high flow summer season and gill nets were used to acquire the sample size (*C. gariepinus* n = 20; *O. mossambicus* n = 18).

Necropsy, blood parameters and biometrical indices

Fish were weighed and measured for the calculation of a condition factor (CF) (Carlander 1969). Blood was drawn from live specimens using cold vacutainers, containing heparin and EDTA for *O. mossambicus* and *C. gariepinus* samples respectively, and kept at 4°C. Selected parameters

including haematocrit (Hct), leukocrit (Lct) and total proteins (TP) were measured. All fish were sacrificed by severing the spinal cord anterior to the dorsal fin. Each fish was examined macroscopically to identify any external and/or internal abnormalities, parasites or injuries. The whole liver was removed and weighed for calculation of the HSI by the formula liver weight / body weight x 100.

Tissue processing

Gill samples were the first to be removed to prevent possible postmortem changes. The innermost left gill arch of each fish was removed and divided into 10 mm pieces for fixation. After surgical opening of the ventral abdominal wall, selected abdominal organs (liver, gonads, and kidney) were removed. Finally, the heart was removed through a ventral incision in the pericardial cavity. Each respective organ was sampled immediately for histopathology after being removed. A 5 mm piece of liver tissue was sampled centrally, around the main portal vein. A 5 mm mid-section of the gonads and a section of the posterior kidney were sampled. The hearts of fish from both species were fixed as whole organs. All tissue samples, with the exception of gonadal tissue, were placed immediately into 10% neutrally buffered formalin (NBF) for 48 hours. Gonadal tissue was fixed in Bouin's solution for 24 hours. Samples were washed in tap water and dehydrated through a graded series of ethanol concentrations (30% - 50% - 70%) and stored in 70% ethanol until further laboratory processing. Tissue samples were then sent to the

Onderstepoort Veterinary Institute, University of Pretoria, South Africa, for final histological processing and preparation for light microscopy analysis using standard techniques for Hematoxylin and Eosin staining.

Histopathological assessment

A semi-quantitative assessment protocol was used to quantify histopathological alterations observed in the sections of each organ. A qualitative histopathological assessment was done jointly by two assessors using a multi-headed Olympus light microscope. Tissue sections were scanned on 40, 200, and 400x magnification. The results were semi-quantitatively assessed using part of a scoring system (van Dyk et al. 2009), modified from the protocol by Bernet et al. (1999). In brief, the tissue samples were assessed by identifying histopathological alterations in terms of six reaction patterns including (1) circulatory disturbances; (2) regressive changes; (3) progressive changes; (4) inflammatory responses; (5) neoplasia and (6) intersex (applicable only to the gonads). If identified, the alteration was given an importance factor which represents the potential of the alteration to affect fish health: 1 (alteration is reversible); 2 (alteration is reversible if the stressor is neutralized); 3 (alteration is irreversible). A score value, representing the occurrence of the alteration throughout the tissue was also assigned: 0 (absent), 2 (mild), 4 (moderate), and 6 (severe). The score value and the importance factor for each alteration were multiplied and these results for all the alterations identified in a single organ were then summed to

give an organ index per fish. Thus, six organ indices were calculated: Gill Index; Heart Index; Liver Index; Testes Index; Ovary Index; Kidney Index. These organ indices were calculated for each specimen. A mean of each organ index was calculated for each sample group (*C. gariepinus* and *O. mossambicus*) and was used to compare the same organs between species. The sum of the six organ indices per fish yielded a total Fish Index value. This index indicates the combined histological response of the sampled organs for the individual fish. A mean Fish Index was calculated for the total sample group per species.

Statistical analysis

Descriptive statistics are presented in the form of means, standard deviations, medians and percentage prevalence. The HSI, CF and semi-quantitative histopathological results were compared between species using the non-parametric Mann-Whitney *U*-test. Statistical differences were considered to be significant at $p < 0.05$. Data was checked for normality using the Kolmogorov-Smirnov and the Shapiro-Wilk tests.

Results

Water and sediment analysis

The water quality results (Table 1) showed high oxygen concentrations. Dissolved oxygen concentrations of 80 – 120% of saturation are considered to constitute the TWQR for aquatic

ecosystems (DWAF 1996). Elevated pH levels, above the normal range of 6 – 8, were recorded at all three sites. Table 2 presents the results on detected levels of micronutrients, selected metals, metalloids and non-metals in water samples. The micronutrient and non-metal variables that proved to be of greatest concern were ammonia as N (5.8 mg L^{-1}), Kjeldahl (organic) nitrogen (42 mg L^{-1}), chloride as Cl (48 mg L^{-1}), and phosphorus (0.737 mg L^{-1}). All other micronutrient variables were considered to be within a normal range. The results of the organic chemical analysis of water and sediment showed undetectable levels, reporting < 10ng L^{-1} for all hormones, < 1.0 µg L^{-1} for all phthalates, and < 0.50 mg L^{-1} for all OCs.

Necropsy

The results of the external macroscopic examination revealed no abnormalities for any of the fish. There were however notable internal abnormalities, particularly concerning the liver of *C. gariepinus*, where 65% of the group had livers with a discoloured, fatty and nodular appearance. The only internal abnormalities in *O. mossambicus* were deformation of the testes seen in two specimens and a kidney with a soft, fragmented structure in one specimen.

Blood parameters and biometric indices

The results of the biometric indices (mean values) are shown in Table 3. All variables were within normal ranges (Adams et al. 1993; van Dyk, 2006), except for the mean Hct of *C. gariepinus.* Sixty five percent of the group had values above the normal range (30 – 45 %).

Histological assessment

The percentage prevalence of histopathological alterations identified in the target organs of both species is presented in Table 4. The highest number of histopathological alterations were observed in the liver (Figure 2, a, b & c). These included primarily regressive changes in the form of structural alterations i.e. no clear hepatic plates or cords, mainly as a result of multiple focal areas of cellular alterations (FCA) leading to a loss of uniform hepatocyte structure. The FCAs were mostly associated with steatosis. Furthermore, cytoplasmic and nuclear alterations (hypertrophic and pyknotic nuclei) of hepatocytes, an increase in the size of melanomacrophage centers (MMCs), and focal areas of necrosis were identified.

A number of alterations were identified in the gills (Figure 2, d & e) including telangiecstasia or aneurysms and epithelial lifting, rupture of pillar cells, and hyperplasia of epithelial and mucous cells. Alterations identified in the kidney included dilation of glomerular capillaries and cytoplasmic

alterations of the tubular epithelium in the form of granular degeneration. MMCs were also identified in the interstitial tissue.

Testicular oocytes were identified in 44% of *O. mossambicus* males (Figure 2, f), however not in *C. gariepinus*. The testicular oocytes identified were all developing in nature and were not found to be prolific throughout the seminiferous tissue. Spermatogenesis did not seem to be affected by this condition as all spermatogenic stages were present, including large numbers of spermatozoa. The only alteration identified in the ovaries was increased MMCs in *C. gariepinus.* Focal inflammation and vacuolation of the myocardium was also identified in the ventricles of 25% of *C. gariepinus* specimens.

The results of the semi-quantitative histopathological assessment showed that *C. gariepinus* had higher mean values, though not all significantly so, than *O. mossambicus* for all organ indices and the Fish Index (Table 5), except the Testes Index where *O. mossambicus* had a significantly higher value (Table 6), due to the occurrence of testicular oocytes (intersex).

Discussion and conclusion

The Roodeplaat Dam is regarded as a hyper-eutrophic freshwater aquatic ecosystem (van Ginkel et al. 2001; DWA 2010). Evidence of the eutrophic status was observed during fish collection including increased biomass, algal blooms, reduced water clarity, reduction in the perceived

aesthetic value of the water body as well as through selected water quality parameters. The variables that proved to be of greatest concern were the elevated pH, ammonia, nitrogen, chloride and phosphorous levels. The eutrophic conditions were most likely due to the return flow from the two wastewater treatment works, as well as residential, agricultural, and industrial contributions (DWA 2010).

Previous studies have found that fish inhabiting hyper-eutrophic systems can be negatively affected (Smith et al. 1999; Nijboer and Verdonschot 2004). In the current study biometrical indices, selected blood parameters as well as histopathological alterations of selected target organs were assessed as selected health parameters. Of these, haematocrit levels above the normal range were found in 60% and 44% of *C. gariepinus* and *O. mossambicus* respectively. It has been shown, that nocturnal hypoxia results from the respiration and photosynthesis cycle of algal populations (Kirby-Smith and Costlow 1989; Paerl 1995; Paerl et al. 1995). This could result in increased haematocrit levels in fish as was shown in a study on juvenile flounder *Paralichthys lethostigma* by Taylor and Miller (2001). It is therefore possible that the haematocrit levels of fish inhabiting the RD could be affected by such conditions. However, the current study did not investigate diurnal fluctuations in dissolved oxygen concentrations in water of the RD and the related Hct measurements in fish. Thus, the cause of the increased Hct levels in some of the sampled fish can only be speculated at this stage.

Histopathological alterations were identified in all the selected target organs of both species. However, there was a clear difference in the percentage prevalence of the same alterations comparing different organs. A smaller number of similar alterations were identified in the gills of both species. The kidneys, hearts and gonads were the least affected in terms of the number of alterations identified. The types of responses in these organs were however different between the two species. Most of the alterations in all the target organs were regressive in nature, with progressive changes and circulatory responses being mostly associated with the gills. A higher prevalence of inflammatory responses was identified in the livers compared to the other target organs.

The liver was the most severely affected target organ in terms of both macroscopic and microscopic abnormalities. The number of histopathological alterations was the highest in *C. gariepinus*, resulting in a significantly higher mean Liver Index compared to *O. mossambicus*. Histopathological alterations in *C. gariepinus* livers (with the most severe macroscopic abnormalities) included loss of hepatic architecture, FCAs associated with severe megalocytosis (hypertrophy of hepatocytes and nuclei), inflammation, steatosis and necrosis. Similar alterations have been observed in fish exposed to microcystins (Gupta and Guha 2006). Microcystin is a highly potent hepatotoxin produced by *Microcystis aeruginosa*, frequently associated with

cyanobacterial algal blooms in hyper-eutrophic systems. Atencio et al. (2008) found pathological lesions in the liver of different tilapia species including cord-disarray, megalocytosis, necrosis, and microvesicular steatosis following intraperitoneal exposure to microcystins. It is therefore likely that fish in the RD could be exposed to the hepatotoxin, micrcocystin, resulting in the liver histopathological alterations identified.

A possible explanation for the more severe hepatic response identified in catfish in the current study, might be because this species occupies a different trophic level within the aquatic ecosystem, as a complete omnivorous species and predator, compared to tilapia feeding mostly on algae and small invertebrates (Skelton 2001). As a result, catfish are more likely to be exposed to higher concentrations of potential contaminants through biomagnification. Previous studies has shown that the liver can be a useful biomarker of toxicant exposure (Schlacher et al., 2007; Lang et al., 2006; Nero et al., 2006; Feist et al., 2004; Au, 2004; Stentiford et al., 2003; Wester et al., 2002; Schmidt-Posthaus et al., 2001; Schmidt et al., 1999; Swee et al., 1996; Braunbeck and Völkl, 1993; Hinton et al., 1992). The results therefore provide valuable liver baseline data for future monitoring programmes in the RD.

No macroscopic abnormalities were identified in the gills. Both species were affected similarly in terms of the type of histopathological alterations identified. No significant difference in the Gill

Index was found between the two species. The progressive changes identified in *C. gariepinus* were more prevalent than in *O. mossambicus,* however, the opposite was true for the selected regressive and circulatory alterations identified. It should be noted, that the regressive change, namely rupture of pillar cells, is related to the formation of telangiecstasia. This circulatory response is regarded as an acute response to different stressors including possible damage from gill nets or rough handling. It should therefore be considered carefully as a toxicopathic lesion. Alternatively, the progressive changes, including mucous cell and epithelial hyperplasia, could be considered as toxicopathic lesions following chronic exposure to a causative agent (Nero et al. 2005; Lease et al. 2003; Benli et al. 2008; Miron et al. 2008; Spencer et al. 2008). The same type of gill degenerative changes identified in the current study was described by Gupta and Guha (2006) in Bloch *Heteropneustes fossilis* exposed to microcystin through intraperitoneal injection. No parasites were present on the gills. Thus, parasitic infestation was not a likely cause of the progressive changes identified.

Some other factors that may cause gill alterations include elevated pH. This may result from particular industrial effluents and eutrophication (DWAF 1996; Dallas and Day 2004), and may act as a stressor on fish by increasing the O_2 diffusion distance in the gills (Lease et al. 2003) i.e. hyperplasia identified in the gills of both species. In addition, under alkaline conditions, the toxicity of ammonium increases and may cause a stress response in fish. Wastewater treatment plant

discharges, degradation of nitrogen-containing organic matter, fertilizer runoff, and industrial sources, aquatic ecosystems can have ammonia concentrations high enough to negatively impact fish (Benli et al. 2008; Miron et al. 2008; Spencer et al. 2008; Capkin et al. 2009). DWAF (1996) recommends a TWQR concentration of ≤ 7µg N L^{-1} for un-ionised ammonia in aquatic ecosystems. The level of ammonia as N measured in water samples in the current study far exceeded the recommended TWQR. It is therefore likely that the gill histopathological alterations identified in the current study could be as a result of the elevated ammonia concentrations.

The target water quality range (TWQR) for chlorine according to DWAF (1996) is 0.2 µg L^{-1}. The concentration found in the water of RD far exceeds this limit. Free forms of chlorine occur in aquatic ecosystems as a result of chlorination of drinking water, the textile industry, sewage treatment, and swimming pools. Chlorine itself does not bioaccumulate, but chlorinated organic substances may bio-concentrate in aquatic organisms (DWAF, 1996). Various studies on fish have shown the relationship between exposure to chlorine and histopathological alterations to gills (hyperplasia and lamellar fusion, swelling of epithelial cells, hyperplastic lesions, proliferation of mucous cells, clubbed lamellae) (Bass et al. 1977; Black & McCarthy, 1990). These types of alterations would be expected to result in increased diffusion distances of the gill membrane and decreased functional gill surface area. Histopathological alterations, as mentioned above, were identified in this study, and could be as a result of exposure to high chlorine concentrations; again

likely from the return flow from the wastewater treatment works, and possibly run off from a number of swimming pools in many town house complexes nearby.

The histopathological response of the kidneys in the two species was notably different in terms of the type of histopathological alterations identified. Of concern in terms of pathological importance, was the occurrence of necrosis in *O. mossambicus*, and the cytoplasmic granular degeneration, nuclear alterations and the dilation of glomerular capillaries in catfish. Of all the alterations identified in the kidneys, only the circulatory changes were found to be significantly different comparing species. Ammonia has been shown to have a negative effect on the kidneys of fish. However, the histopathological alterations in the kidneys identified in this study did not correlate with these results, which included hyperemia and glomerulonephritis in Nile tilapia *Oreochromis niloticus* (Benli et al. 2008), hydropic degeneration in cutthroat trout *Oncorhynchus clarkia* (Thurston et al. 1978) and glomeruli congestion in rainbow trout *Oncorhynchus mykiss* (Larmoyeux and Piper 1973). It has also been documented that low concentrations of microcystins cause kidney damage in european carp *Cyprinus carpio* (Fischer and Dietrich 2000) and in different tilapia species (Atencio et al. 2008) and can accumulate in the kidneys of Nile tilpia *Oreochromis niloticus* (Mohamed et al. 2003). The same type of kidney degenerative changes identified in the current study was described by Gupta and Guha (2006) in Bloch *Heteropneustes fossilis* exposed to microcystin through intraperitoneal injection. The differences

in terms of the renal response between species may, as with the liver, be due to the difference in exposure to potential contaminants as the feeding habits of the species differ.

An inflammatory response and vacuolation of the ventricular myocardium was only identified in *C. gariepinus*. This resulted in a significantly higher Heart Index for catfish compared to tilapia. The cardiac response was mild and focal in nature and no clinical signs were apparent to suggest that such lesions affected the fish negatively. Further research is recommended to investigate the possible association between toxicant exposure and cardiac inflammation.

An interesting finding identified in *O. mossambicus* males was the presence of testicular oocytes (intersex). This condition in fish has been associated with exposure to potential endocrine disrupting chemicas (EDCs) (Kime et al. 1999; Cheek et al. 2001; Zaroogian et al. 2001; Jobling and Tyler, 2003; Barnhoorn et al. 2004, 2010; Marchand et al. 2008; Pieterse et al. 2010). These include organochlorines, polychlorinated biphenols and phthalates (Kime et al. 1999; Menditto and Turrio-Baldassarri 1999). EDCs are known to mimic the function of natural hormones in different ways, causing estrogenic activity. There are increasing incidences of intersex and skewed sex ratios (favouring the females) among various organisms, especially fish (Kime et al. 1999; Viganò et al. 2001). This was not the case in the current study. Although the results of the water and sediment analysis did not show detectable levels of EDCs, testicular oocytes may be

indicative of prior exposure during fetal development (Damstra et al. 2004), or exposure to low concentrations on a chronic level. The testes of affected *O. mossambicus* males seemed to be in a functional state as all stages of spermatogenesis were present, including spermatozoa. The fact that testicular oocytes were only identified in *O. mossambicus* males in the current study suggests possible species specific sensitivity. A study by Barnhoorn et al. (2010) also showed the presence of testicular oocytes in *O. mossambicus* while this condition was absent in *C. gariepinus*. Further research in terms of the exact causative agent and biological processes involved in the formation of testicular oocytes is necessary.

In terms of the overall organ response, the semi-quantitative histopathological assessment showed that the liver was the most affected target organ in the hyper-eutrophic RD. Considering the water quality results did not show elevated levels of metals or potential EDCs, it is more likely that microcystin (a hepatotoxin) could be the cause of the identified liver lesions. It would, in future, be beneficial to conduct bioaccumulation studies on liver tissue of fish from the RD to identify a possible association between microcystin levels and the liver histopathology.

Melanomacrophage centers were a common occurrence in the kidneys of both species as well as the livers and gonads of catfish. It has been suggested that MMCs are a normal characteristic in fish tissue (Leknes 2004) but an increase in the number or size of these structures can be as a

result of a number of factors, including toxicant exposure (Agius and Roberts, 2003) and possibly age. A limitation to this study was the fact that the age of fish was not determined and should in future be included as part of the standard fish health assessment protocol. This will enable a better understanding of potential age related histopathological alterations. Previous studies in our laboratory (Unpublished results) showed a 100% prevalence of MMCs in *C. gariepinus* from a natural, unpolluted system. It is likely that MMCs are a normal occurrence in *C. gariepinus*. It is clear that inconsistencies exists relating water quality to an increase in MMCs. Therefore, further research is necessary.

In conclusion, the histopathological assessment showed a number of alterations in the target organs of both fish species from the polluted RD. Species differences were identified in terms of the severity of the alterations within all the organs, as well as in terms of the type of alterations identified in the gonads and the kidneys. An important consideration in evaluating histopathological alterations in wild fish is that they are exposed to a mixture of various pollutants at low concentrations on a chronic level and that these alterations cannot be regarded as toxicant specific. Histopathological responses, similar to those identified in fish from the RD, have been associated with microcystin exposure in previous studies. It is therefore likely that fish in the RD, known for its active algal blooms, are possibly exposed to this biotoxin, resulting in the histopathological changes identified. The fact that microcystin is known to bioaccumulate in the

liver (Mohamed et al. 2003), might explain the more severe abnormalities identified in this organ compared to the other target organs. Although exact causative agent/s cannot be pinpointed at this stage, the results of this study provides valuable baseline data for the two indicator fish species, *C. gariepinus* and *O. mossambicus* for future biomonitoring initiatives in the RD.

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Table 1: Water quality parameters measured at three sites at the Roodeplaat Dam (RD) in November 2007

Parameter	Site 1	Site 2	Site 3	Mean
pH	10.97	9.81	10.20	10.33
Temperature (°C)	27.40	24.40	23.90	25.23
TDS ppm (µg L^{-1})	253	232	616	367
Conductivity µS cm^{-1}	500	460	1200	740
Oxygen (%)	358	222	216	265
Oxygen (mg L^{-1})	27.36	17.58	17.36	20.77

Table 2: Detected levels of micronutrients, selected metals, metalloids, and non-metals in the water from Roodeplaat Dam (RD). * indicates algae.

Variable (mg L^{-1})	RD
Total Hardness as $CaCO_3$	172
Total Alkalinity as $CaCO_3$	116
Ammonia as N	5.8
Nitrate as N	0.2
Nitrite as N	< 0.1
Kjeldahl Nitrogen	42
Chloride as Cl	48
Sulphate as SO_4	44
Faecal Coliform Bacteria / 100mL	0*
Fluoride as F	0.2
Boron	0.087
Barium	0.060
Calcium	31
Iron	0.290
Magnesium	16
Potassium	9.9
Phosphorous	0.737
Silicon	4.2
Sodium	41
Strontium	0.107
Sulfur	25.1

Vanadium	0.010

Table 3: Mean (± standard deviation) blood parameters and biometric indices of *C. gariepinus* and *O. mossambicus* from Roodeplaat Dam (RD)

	RD	RD
Variable	*C. gariepinus*	*O. mossambicus*
Hct (%)	46.25 ± 9.78	43.49 ± 9.53
Lct (%)	0.46 ± 1.60	1.78 ± 1.89
TP (mg dL^{-1})	52.15 ± 8.35	51.61 ± 10.33
HSI (%)	1.69 ± 0.61	1.37 ± 0.24
CF	0.75 ± 0.07	1.68 ± 0.12

Table 4: Percentage prevalence of histopathological alterations identified in target organs of *C. gariepinus* and *O. mossambicus*. L – liver, G – gill, O – ovary, T – testis, K – kidney, H – heart.

	C. gariepinus						*O. mossambicus*					
Alteration	L	G	O	T	K	H	L	G	O	T	K	H
Circulatory Disturbances												
Telangiecstasia	na	**55**	na	na	na	na	na	**66**	na	na	na	na
Epithelial lifting	na	**55**	na	na	na	na	na	**83**	na	na	na	na
Congestion	0	0	0	0	0	0	**44**	0	0	0	0	0
Glomerular capillary dilation	na	na	na	na	**45**	na	na	na	na	na	0	na
Regressive Changes												
Rupture of pillar cells	na	**55**	na	na	Na	na	na	**66**	na	na	na	na
Cord disarray	**30**	na	na	na	Na	na	**16**	na	na	na	na	na
Intracellular deposits	**35**	0	0	0	0	0	**50**	0	0	0	0	0
Steatosis	**40**	0	0	0	0	0	**22**	0	0	0	0	0
Vacuolation (non-steatosis)	**60**	0	0	0	0	**25**	**27**	0	0	0	**27**	0
Structural alterations	0	0	0	0	0	0	0	0	0	0	**27**	0
MMCs	**95**	0	**50**	**25**	**95**	0	**33**	0	0	0	**88**	0
Nuclear alterations	**95**	0	0	0	**55**	0	**83**	0	0	0	**27**	0

Granular degeneration	0	0	0	0	**40**	0	0	0	0	0	0	0
Hyaline droplet degeneration	0	0	0	0	0	0	0	0	0	0	**66**	0
Necrosis	**20**	0	0	0	0	0	0	0	0	0	**27**	0
Progressive changes												
Hyperplasia (gill epithelium)	na	**100**	na	na	na	na	na	**50**	na	na	na	na
Hyperplasia (mucous cells)	na	**30**	na	na	na	na	na	**11**	na	na	na	na
Hypertrophy	**60**	0	0	0	0	0	0	0	0	0	0	0
Inflammation												
Infiltration	**55**	0	0	0	0	**35**	**66**	0	0	0	0	0
Intersex												
Testicular oocytes	na	na	na	0	na	na	na	na	na	**44**	na	na

Table 5: Mean (± standard deviation) organ indices and mean Fish Index of the semi-quantitative histopathological assessment of *C. gariepinus* and *O. mossambicus* from Roodeplaat Dam (RD)

	RD	RD
Mean Index	*C. gariepinus*	*O. mossambicus*
Gill Index	12.20 ± 4.10	10.89 ± 3.95
Heart Index	4.20 ± 4.15	0.00 ± 0.00
Liver Index	20.80 ± 8.59	12.89 ± 5.83
Testes Index	2.20 ± 2.90	13.00 ± 6.50
Ovary Index	4.40 ± 3.75	1.80 ± 1.99
Kidney Index	8.90 ± 2.79	8.56 ± 7.51
Mean Fish Index	49.40 ± 10.44	39.11 ± 12.06

Table 6: Significant differences between mean indices of *C. gariepinus* (*C. gariep*) and *O. mossambicus* (*O. moss*) from Roodeplaat Dam (RD) shown by the Mann-Whitney *U* Test (p = < 0.05).

Variable	Species	Mean	ST DEV	Median	*U*	*z*	*p*
Heart Index	*C. gariep*	4.20	4.15	4	72.000	-3.836	0.000
	O. moss	0.00	0.00	0			
Liver Index	*C. gariep*	20.80	8.59	18	75.500	-3.072	0.002
	O. moss	12.89	5.83	12			
Testes Index	*C. gariep*	2.20	2.90	1	3.000	-3.349	0.001
	O. moss	13.00	6.50	12			
Fish Index	*C. gariep*	49.40	10.44	47	91.000	-2.606	0.009
	O. moss	39.11	12.06	35			

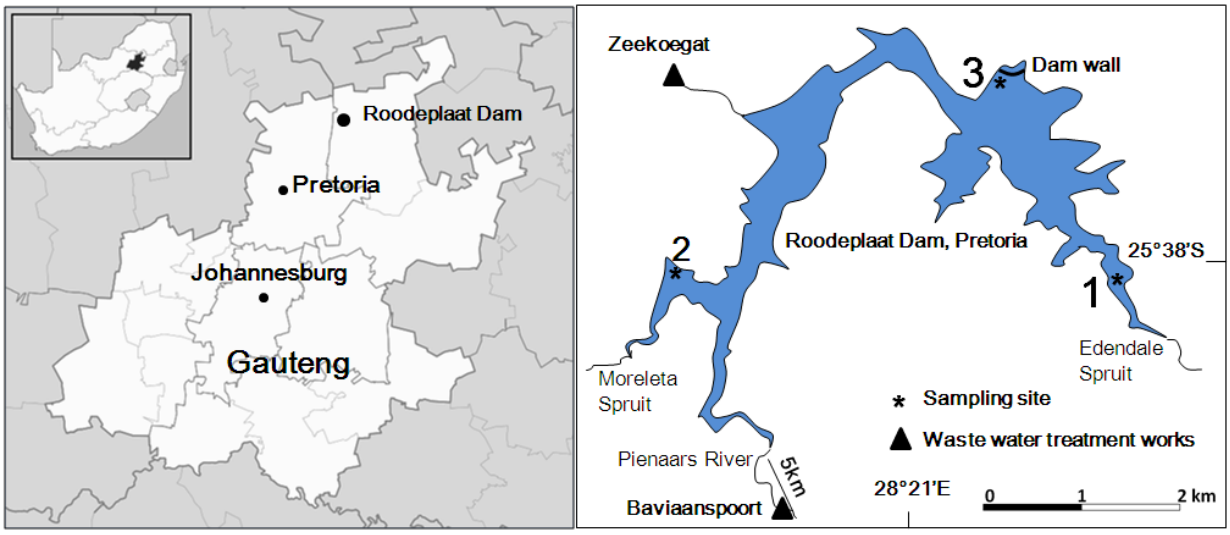

Fig. 1: Location of RD within South Africa and position of water quality sampling sites and waste water treatment works in relation to the dam.

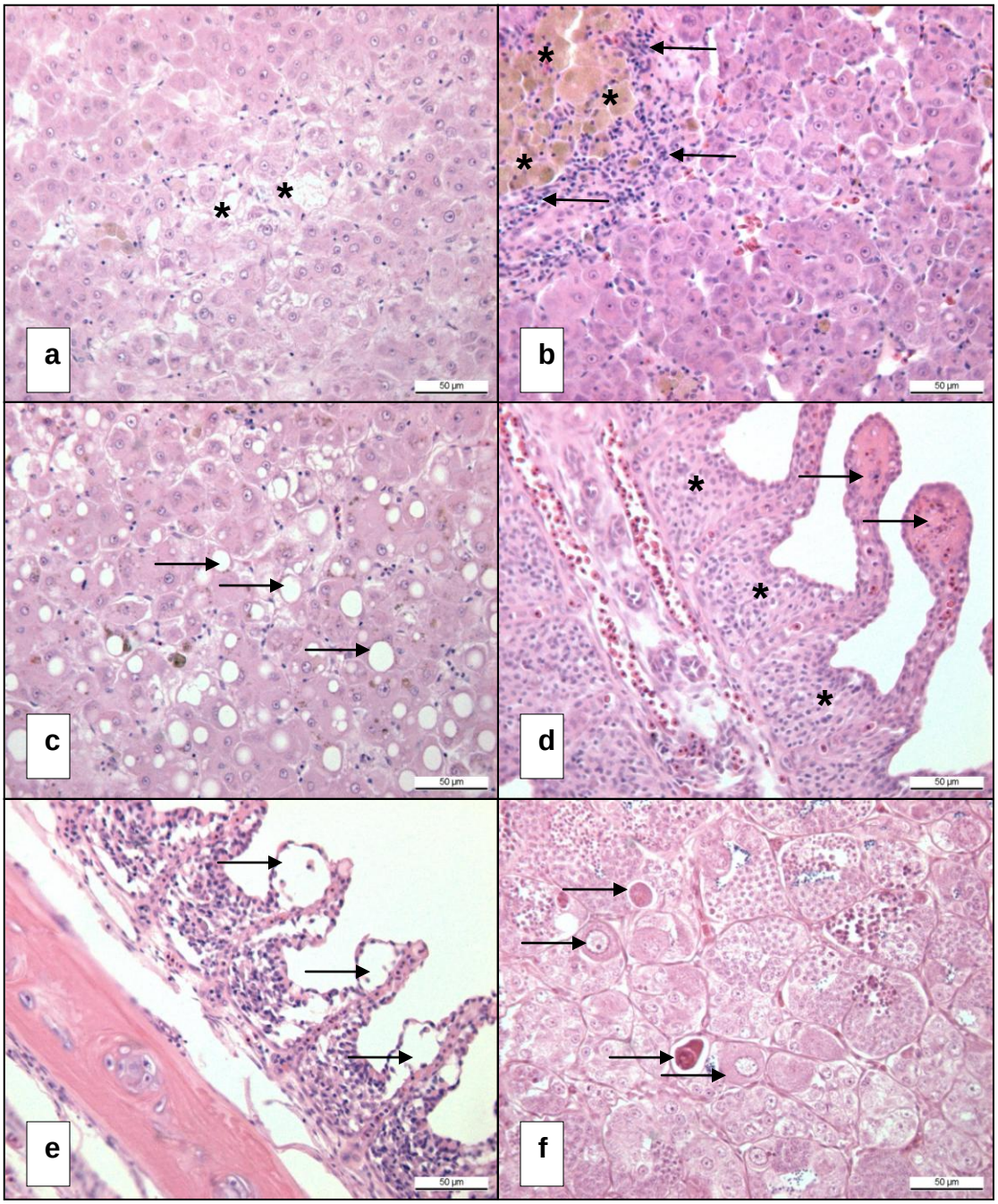

Fig. 2**:** Examples of histopathological alterations identified in the livers (a, b, c; H & E) and gills of *C. gariepinus* (d, e; H & E), and the testes of *O. mossambicus* (f; H & E). a: focal necrosis (asterisk) of hepatocytes and nuclear alterations (hypertrophy and pyknosis); b: MMCs (asterisk) and an infiltration of inflammatory cells (arrows); c: macrovesicular steatosis (arrows) within hepatocytes; d: hyperplasia of epithelial tissue (asterisk) and telangiecstasia of secondary lamellae (arrows); e: epithelial lifting of secondary lamellae (arrows); f: developing oocytes within testis tissue (arrows).