Pol. J. Environ. Stud. Vol. 32, No. 5 (2023), 4739-4752
DOI: *10.15244/pjoes/168136* ONLINE PUBLICATION DATE: 2023-08-02

Original Research

Biochemical and Histopathologic Biomarkers of Pollution in the uMgeni River System in KwaZulu-Natal, South Africa

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Received: 27 March 2023
Accepted: 12 June 2023

Abstract

The uMgeni River is one of the most polluted freshwater ecosystems in KwaZulu-Natal. The study aims to assess the response of *Clarias gariepinus* to water contamination at the Nagle and Inanda dams using selected biomarkers. Samples were collected and preserved as per the analyses to be carried out. Generally, Nagle Dam showed good quality water compared to Inanda Dam. The Inanda Dam population showed a relatively higher prevalence of alterations in the gills and liver than that from Nagle Dam. The degree of alterations showed some variability within each population, however, there was no significant difference ($p>0.05$) between the two populations. Organ indices of 20 denoting moderate alterations were observed for both populations. Although the mean AChE activity and VtG induction showed no significant difference between the two populations ($p>0.05$); the lowest AChE activity and the highest VtG level, were observed in the Inanda Dam populations. Both histopathologic and biochemical biomarkers show signs of severely compromised fish health in the Inanda Dam compared to those from Nagle Dam. It is thus, evident that the tributaries feeding the uMgeni River downstream of Nagle Dam are resulting in the increase of pollution level in the Inanda Dam.

Keywords: *Clarias garipienus*, fish health, histopathology, vitellogenin, acetylcholinesterase, Inanda Dam, Nagle Dam

Introduction

Freshwater constitute less than 1% of the water on Earth, yet they support many life forms, enhance economic development, and provide goods and services to people [1]. However, the global evolution of industries, mining, and urbanization, improved agricultural practices, and the growing human population results in the contamination of freshwater bodies [2, 3]. Once polluted, restoring the freshwater ecosystem to its pristine state may be challenging, and its function cannot be substituted [4]. Contamination of aquatic ecosystems results in mortalities and even local extinction of biodiversity [5]. Therefore, there is a need to keep a closer eye on the health of aquatic biota, particularly those inhabiting potentially polluted water bodies. Kroon [6] reported that biomarkers might be

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used as early warning signs of pollution exposure before gross changes are observed at the tissue or organ level.

Numerous biomarkers have successfully been employed to discriminate pollution effects in water bodies exhibiting different water quality [7, 8]. However, contaminants may target particular tissues depending on their functions and location in an organism [9]. Gills are in direct contact with the external water environment, and the liver is a primary site for detoxification; hence, they are among the target tissues in fish [7, 8]. Although most tissues have the ability to metabolize contaminants, their capacity can be overwhelmed by elevated concentrations and result in biochemical and/or structural damage [10]. Tissue damage due to exposure to contamination has been observed in various rivers in South Africa [11, 12] and beyond [13, 14].

The uMgeni River, is one of the largest river systems in KwaZulu-Natal, South Africa. The river is home to about 48 fish species, both native and alien. About 3.5 million people in the eThewini and uMsunduzi metropolitan areas depend on this river for their livelihoods [15]. However, the pollution level has shown to increase over the past few decades due to the discharge of treated and partially treated effluents from wastewater works, agricultural run-off, and industrial activities in the catchment [16, 17]. Moreover, the river has recently experienced a toxic chemical spillage which resulted in fish mortalities [18]. Despite signs of water quality deterioration in this river system, no study has explored how the inhabitant fish are responding to pollutant exposure. According to Hamilton [19], fish tend to respond physiologically under pollutant exposure. Therefore, the present study aims to use biochemical and histopathologic biomarkers to assess the health status of *Clarias gariepinus* from the Inanda and Nagle dams in the uMgeni River system. Given that Inanda Dam receives polluted water from polluted uMsunduzi, Umngcweni, and Sikelekehleni rivers, it was hypothesized that its inhabitant fish would exhibit poorer health status relative to those from Nagle DamMaterials and methods

Study Area

The uMgeni River (29º48’36’’S, 31º02’08’’) is one of the largest freshwater ecosystems in KwaZulu-Natal, South Africa. The river is 220 km long with four impoundments and it drains a catchment area of 4000 km^2. The catchment receives an average annual rainfall of 600-1500 mm per year [16]. Nagle Dam is located in the middle catchment of the uMgeni River, upstream of the uMgeni-uMsunduzi rivers confluence. In contrast, Inanda Dam is situated downstream of the uMgeni-uMsunduzi rivers confluence. The uMsunduzi River is the most polluted tributary of the uMgeni River as it drains a highly industrialised catchment [20]. Other tributaries are uMngcweni and Sikelekehleni rivers which also joins the uMgeni River before in feeds the Inanda Dam (Fig. 1).

Water and Sediment Sampling, and Analysis

Water and sediment samples were collected at the Inanda and Nagle dams at the inflow, middle, and dam wall during the low flow (Winter) and high flow (Summer) seasons in 2020 and 2021. The physical parameters such as temperature, salinity, dissolved oxygen levels (DO), pH, and electrical conductivity were measured *in situ* (HANNA multiparameter instrument Model: HI98494). Water and sediment samples were collected using bottles pre-treated with hydrochloric acid and kept in a cooler box with ice. Samples were transported to the lab, and sediment was kept at -4ºC, whereas water was kept in the fridge prior to chemical analysis. Approximately 150 ml water sample was transferred to a 250 ml beaker. The sample was filtered a 0.45 µm SIMPLEPURE filter, and 50 ml was transferred into the 250 mL volumetric flask. Thereafter, 1 ml of 32% hydrochloric acid (HCl) was added to the sample and diluted to 250 ml with deionised water. Thereafter, 15 ml of sample was transferred to the pre-cleaned vials, and aluminium (Al), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) concentrations were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES). Metal concentrations were measured in triplicate water samples and the results were expressed in mg/l. The nutrient analysis i.e. nitrite, nitrate, ammonia, and phosphate was carried out at the uMgeni water laboratory using an Ion Chromatography System (ICS-5000 Single Pump) with a Suppressor and Conductivity Detector.

Sediment was digested following Gaudino [21] protocol where aqua regia, 3 parts 32% hydrochloric acid, and 1 part 36% nitric acid (3-HCl: 1-HNO_3) was modified by adding hydrogen peroxide 30% (H_2O_2) to enhance the destruction of organic matter. The metal analysis was carried out using ICP-OES. For all digestions, the acid used was of ultra-pure analytical grade. Metal concentrations were measured in triplicate digested sediment samples, and results were expressed in mg/kg.

Fish Sampling and Processing

Fish were collected from Nagle (n = 18, 11 males and 7 females) and Inanda (n = 28, 16 males and 12 females) dams during each sampling using gill nets (25 m long and 2 m deep) and an electro-shocker. Sampled fish were euthanized by severing the spinal cord at the back of the head. Fish, liver, and gonads were weighed, and fish lengths were measured. A piece of liver and middle gill arch were fixed in 10% neutral buffered formalin for histopathologic analysis. Brain tissue for AChE analysis and male liver for VtG analysis were cut out and kept in dry ice and later stored at -80ºC. The protocol was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ref: AREC/019/018).

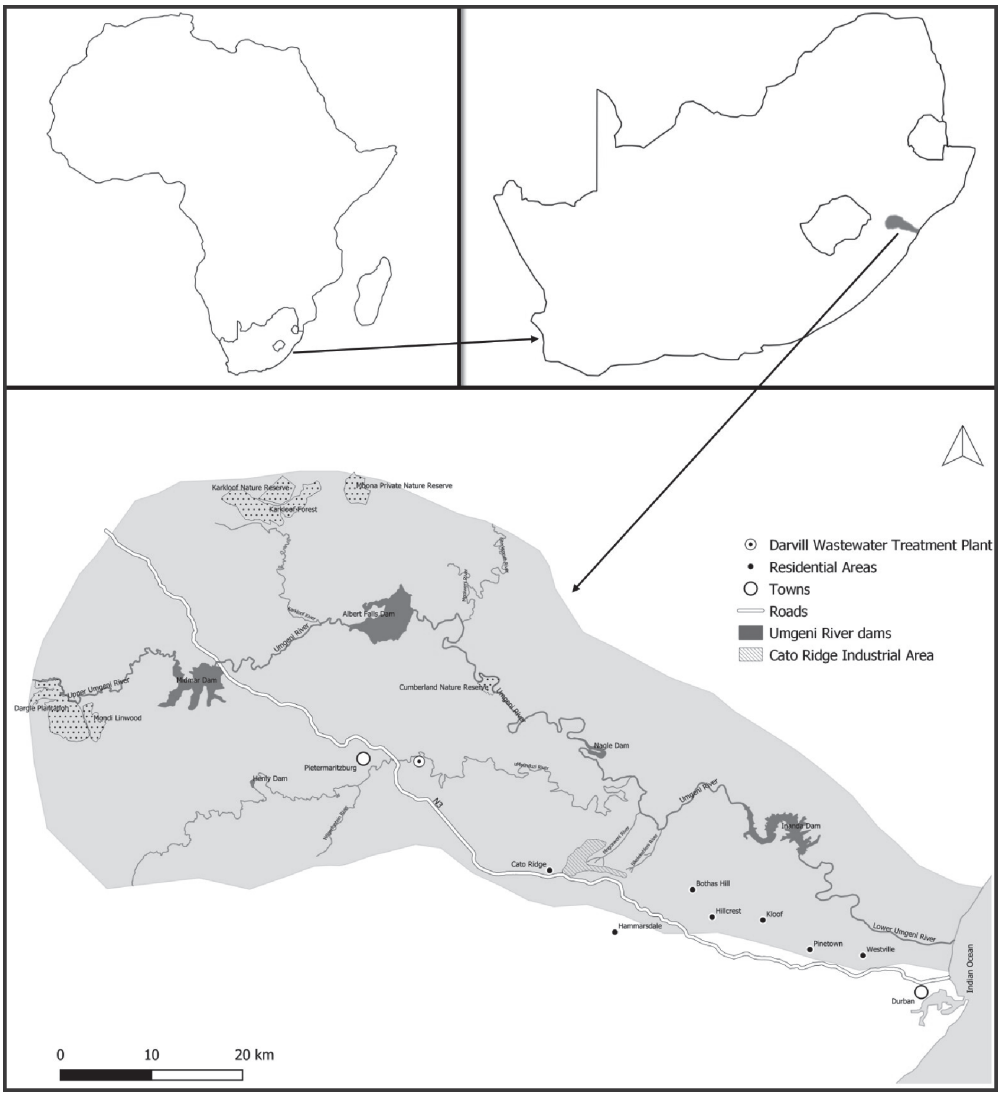

Fig. 1. The locality map of uMgeni River system showing the four impoundments attached to its course, land uses, tributaries, and neighbouring communities along the catchment.

The fish weight (g) and total length (mm) were used to calculate Fulton's condition factor (K) using Equation (1). Fish-weight relationships were determined following Ak [22], where W is a weight (g), L is the total length (mm), and a is an intercept generated from the regression analysis of fish length and weight (Equation (2)). The fish growth classified as negative allometric (b<3), isometric (b=0) and positive allometric (b>3). Organ indices were calculated using the following Equation (3).

$$K = \frac{W\,(g) * 100}{L(mm)^3} \quad (1)$$

$$W = aL^b \quad (2)$$

$$\text{Organosomatic index} = \frac{\text{organ weight (g)}}{\text{fish weight (g)}} * 100 \quad (3)$$

Tissue Processing and Histopathologic Assessment

The processing of tissue was carried out following the method described in Lebepe [23]. Tissues were dehydrated through a series of ethanol (70%, 80%, 96% and 100%). To make the sample clear and transparent, xylene was used. The samples were infiltrated through increasing concentrations of Tissue-Tek® III wax in a 60ºC oven and them embedded in wax blocks [24]. A rotary/sliding microtome (Reichert-Jung 2040) was used to section the block at 4-5 µm. The samples were floated using gelatine and distilled water solution, and then mounted on glass microscope slides and air-dried in a 60ºC oven. The slide was stained using standard technique for Haematoxylin and Eosin (H&E) staining [25]. The histopathological assessment was done using a compound microscope to identify alterations in the target organs.

The prevalence percentage was calculated by counting the number of specimens that showed the alteration, divided by the total number of specimens examined. The organ alterations were quantitatively assessed following Agamy [26] protocol which was adapted from Bernet [27]. Histopathological alterations were categorized into four reaction patterns which are i) circulatory disturbances, ii) regressive, iii) progressive and iv) inflammation. Each reaction pattern shows different histological characteristics and is very specific in the areas in which they affect. Thereafter, each alteration was identified and given an important score (how the lesion affects the organ function and the fish's ability to survive) (1-6). The score values were assigned based on the extent of the lesion (1-3). Furthermore, the important scores and score values determined during the assessment were used in calculating the organ index (I_{org}) as per Bernet [27] using the equation below.

$$I_{org} = \sum_{rp} \sum_{alt} (a_{\text{org rp alt}} \text{ x } w_{\text{org rp alt}})$$

Where rp is the reaction pattern, alt is the alterations. The Iorg values were used to categorize the severity of the alteration following the classification system developed by Zimmerli [28]:

a) Histological index <10: normal tissue; no pathological changes.
b) Histological index 11–20: slight modifications are present.
c) Histological index 21–30: moderate modifications.
d) Histological index 31–40: pronounced modifications.
e) Histological index >40: severe alterations.

The scoring values was also used to categorize the reaction patterns.

Acetylcholinesterase Assay

Acetylcholinesterase activity in fish brain tissue was measured in duplicate samples, with an ELISA kit (ELabScience, catalogue number: E-BC-K174-M) that is based on the Ellman [29] method and later modified by Villescas [30]. Approximately 100 mg of brain tissue was homogenized in a 0.9 mL of 0.1 mol/L lysis buffer, pH of 7.5, and centrifuged at 10000 rmp for 10 minutes. Thereafter, 20 µl of sample, 170 µl of reagent 3 working solution, and 10 µl of reagent 4 working solution were added to each well (reagents 3&4 as per the assay kit). It was then mixed in a multiplate reader for 5 seconds, and changes in absorbance were measured at 412 nm within 5 minutes. Protein concentrations were assayed using the BCA protein assay kit (ThermoScientific, catalogue number: 23225), and AChE activity was expressed in U/mg protein, where 1 unit is the amount of AChE enzyme needed to catalyze the production of 1 nmol TNB per minute. The kit used has a published intra-assay coefficient of variance (CV) of 4.7%, an inter-assay CV of 9.3% and a recovery rate is 104%

Vitellogenin Assay

Vitellogenin induction in male liver tissue was measured in duplicate samples, with an ELISA kit (Bioassay Technology Laboratory, catalogue number: E0020Fi). Approximately 100 mg of liver tissue was homogenized in a 0.9 mL of 0.1 mol/L phosphate buffer (PBS), pH of 7.4, and centrifuged at 3000-4000 rmp for 20 minutes. All reagents, standards, and samples were prepared before the use in the assay. Thereafter, 50 µl of the prepared standards, 50 µl of controls (the samples without the anti-VTG antibody), and 40 µl of pre-washed samples (tissue samples were washed twice with PBS to remove excess blood prior to the homogenization process) were added to the allocated wells, and 10 µl of anti-VtG antibody was added to sample wells only. Note: The anti-VTG was not added to the standards because they already had the biotinylated antibody. Thereafter, 50 µl of streptavidin-HRP was added to the sample and standard wells. The wells were covered and incubated at 37ºC for 1 hour. After incubation, the contents of the wells were washed five times with the 350 µl wash buffer for 1 minute for each wash. The plate was then blotted into absorbent paper towels. Thereafter, 50 µl of substrate A and substrate B were added to each well consecutively, allowed to incubate for 10 minutes at 37ºC in the dark. To terminate the reaction, 50 µl of stop reaction was added to each well. The color reaction was measured at 450nm within 10 minutes in a multiplate reader. VtG concentration was calculated with the standard curve equation and was expressed in µg/ml. The kit used has a published intra-assay CV of <8%, and an inter-assay CV of <10%

Statistical Analysis

R-commander software was used for data analysis [31]. Normality assumption was tested using the Shapiro-Wilk test, and equality of variances was tested using Levene's test. Based on the assumptions' tests, an independent sample t-test or Mann-Whitney test was used to test the differences in water and sediment parameters between Nagle and Inanda dams. Moreover, an independent sample t-test or Mann-Whitney test was also used to test the differences in condition factor, hepatosomatic index, gonad-somatic index, organ index, AChE activity, and VtG induction between Nagle and Inanda dam populations. A simple linear regression test was used to determine the fish weight-length relationships and population growth between *C. gariepinus* from Nagle and Inanda dams. The relationship between fish size and AChE activity, and fish size and VtG induction was evaluated using correlation analysis. Statistical significance was set at $p<0.05$.

Results

Water and Sediments Quality

Water quality results are presented in Table 1. Most physical variables showed no significant difference between the two dams ($p>0.05$) with only electrical conductivity, showing a significantly higher level at Inanda Dam ($p<0.05$) (Table 1). No significant difference was observed for ammonia concentration between the two dams ($p>0.05$). In contrast, nitrite, nitrate, sulphate, and phosphate showed significantly higher concentrations at Inanda Dam than at Nagle Dam ($p<0.05$) (Table 1). Metal concentrations in the water column showed no significant difference between the two dams ($p>0.05$) with copper and Mn being below detection level at both dams (Table 1). In contrast, considerable concentrations were reported in the bottom sediment at both dams. Significantly higher concentrations were observed for Al, Cu, Fe, and Zn in sediment at Inanda Dam than at Nagle Dam ($p<0.05$) (Table 1).

Fish Weight-Length Relationships, Condition Factor, and Organ Indices

The weight-length relationships of fish from Nagle Dam were as follows; $W = 0.066L^{2.568}$ (females), $W = 0.00967L^{3.03}$ (males), with $W = 0.021L^{2.926}$ (females) and $W = 0.274L^{2.262}$ (males) being observed for Inanda Dam populations. The Nagle Dam females exhibited negative allometric growth (b<3), with males showing positive allometric growth (b>3). Both male and female fish at Inanda dam exhibited a negative allometric pattern (b<3). The condition factor showed a greater variability for the Inanda Dam population compared to Nagle Dam (Fig. 2). Moreover, the condition factor showed a significant difference between the two populations ($p<0.05$), with the Inanda Dam population

Table 1. Mean levels of water and sediment quality parameters recorded at Nagle and Inanda dams during low and high flow surveys in 2020 and 2021. The table also presents water quality guidelines stipulated by DWAF [80] and CCME (2012, and CCME (2012) sediment quality guideline.

Water column			
Parameters	Nagle Dam	Inanda Dam	Guidelines
Temperature (ºC)	24.09±2.24	25.16±3.74	n/a
pH	7.95±0.46	8.33±0.12	6.5-9.0
Dissolved oxygen (%)	90.2±12.72	152.35±111.67*	80-120
Dissolved oxygen (mg/L)	7.46±1.31	12.99±10.48	6-9
Conductivity (µS/cm)	117±17.12	299±37.97*	n/a
Ammonia (mg/L)	0.23±0.21	0.13±0.05*	0.007 (DWAF 1996)
Nitrite (mg/L)	<0.10	0.62±0.65*	0.06 (CCME 2012)
Nitrate (mg/L)	0.23±0.12	1.08±0.96*	13 (CCME 2012)
Sulphate (mg/L)	3.92±0.75	19.83±2.58*	n/a
Phosphate (mg/L)	15.97±1.88	47.85±56.39*	n/a
Aluminium (mg/L)	0.30±0.49	0.26±0.31	n/a
Copper (mg/L)	<0.010	<0.010	n/a
Iron (mg/L)	0.53±0.77	0.38±0.49	n/a
Manganese (mg/L)	0.04±0.02	0.05±0.02	0.18
Zinc (mg/L)	<0.025	<0.025	n/a
Sediment			
Aluminium (mg Al/kg)	127±87.48	200±77.76*	n/a
Copper (mg Al/kg)	0.34±0.14	0.52±0.15*	35.7
Iron (mg Al/kg)	295. 35±190.08	441.67±162.44*	n/a
Manganese (mg Al/kg)	7.99±6.23	9.12±2.61	n/a
Zinc (mg Al/kg)	0.71±0.51	1.87±1.97*	n/a

Key: n/a = not available; * denote values that showed significant differences between the two dams

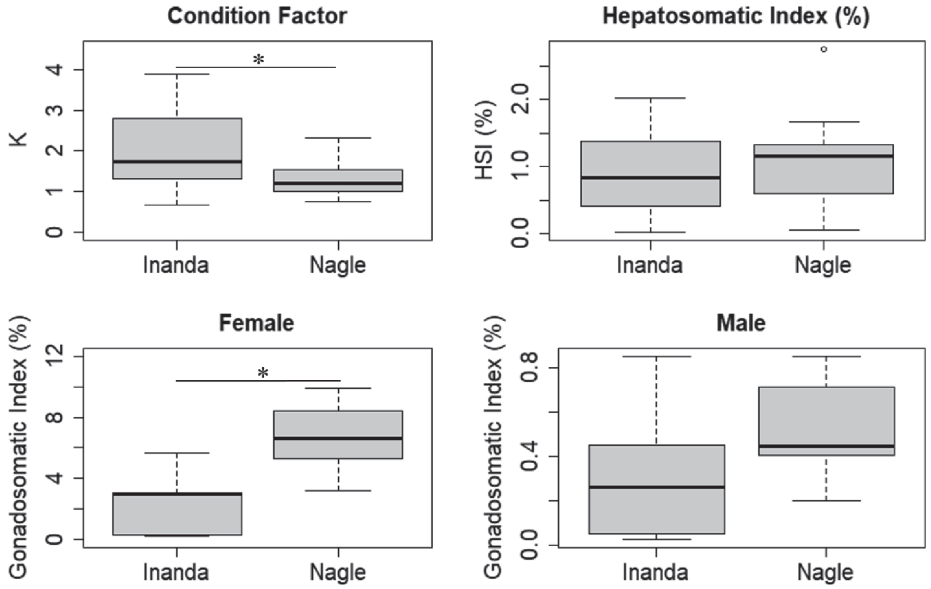

Fig. 2 Box and whisker plots presenting condition factor, and hepatosomatic and gonadosomatic indices observed for *Clarias gariepinus* at Nagle and Inanda dams during low and high flow surveys in 2020 and 2021.

exhibiting a higher value. The hepatosomatic index of *C. gariepinus* showed no significant difference between the two populations (Fig. 2); however, Inanda Dam population exhibited a greater variability compared to Nagle Dam (Fig. 2). Gonadosomatic index showed no significant difference ($p>0.05$) for males, whereas a significant difference ($p<0.05$) was observed for females between the two populations (Fig. 2).

Histopathology

The gill and liver histopathology are presented in Table 2 and Figs 3 and 4. For the gill, epithelial lifting, oedema, hyperplasia, and leukocyte infiltration were the most prevalent pathologies at Inanda Dam, whereas lamella fusion, leukocyte infiltration, and epithelial hyperplasia were common at Nagle Dam (Table 2). Nuclear degeneration, pillar cell rupture, and necrosis were among the regressive changes reported in both dams, with a higher prevalence being observed for the Nagle Dam population (Table 2). Other regressive alterations which were common at both dams included a fusion of lamella and epithelial lamella. The Inanda Dam population has shown a higher prevalence of alterations with a higher degree of severity compared to Nagle Dam. This was also supported by the gill index, which was 16±6.57 for the Nagle Dam population and 19.85±5.97 for that from Inanda Dam. The gills indices were less than 20 for both Nagle and Inanda dam populations which classify the histological alterations as slight for both populations. The degree of severity for gill alterations has shown a great variability within each population; however, there was no significant difference between the two populations ($p>0.05$).

The liver histopathology showed a higher prevalence for Inanda Dam population compared to Nagle Dam (Table 2). The liver of *C. gariepinus* exhibited congestion of sinusoids, melanomacrophages (MMCs), hepatocellular and nuclear pleomorphism, nuclear degeneration, hydropic degeneration, hypertrophied hepatocytes, hepatocytes hyperplasia, and leukocyte infiltrations for both populations, whereas steatosis was only observed at Inanda Dam (Table 2). There was no significant difference for the liver index between the two populations ($p>0.05$).

Acetylcholinesterase

The AChE activity were not significantly different between Nagle and Inanda dam populations ($p>0.05$) (Fig. 5). Approximately 16% of the Inanda Dam population exhibited a considerably low AChE activity ranging from 246 to 375.62 U/mg protein. Moreover, 26% of the Nagle Dam population has shown substantially low AChE activity ranging from 217.17 to 421.88 U/mg protein. A weak negative relationship between AChE activity and fish body weight ($r = -0.264$, $p>0.05$) was observed for the Nagle Dam population, whereas the Inanda Dam population showed no relationship ($r = -0.016$, $p>0.05$).

Vitellogenin in Male Fish

There was a higher variability of VtG levels observed in male *Clarias gariepinus* for Inanda Dam populations than that from Nagle Dam (Fig. 5). However, no significant difference was observed for the overall VtG levels between the two populations ($p>0.05$) (Fig. 5). A strong positive relationship between fish weight and

Table 2. Prevalence percentage of gill and liver histopathology observed for *Clarias gariepinus* at Nagle and Inanda dams during 2020 and 2021 surveys.

Gills			
Reaction pattern	Alterations	Nagle Dam	Inanda Dam
Circulatory	Haemorrhages	30	55
	Aneurysm	35	60
	Intracellular oedema	33	65
Regressive	Complete fusion of lamella	10	46
	Partial fusion of lamella	67	62
	Epithelial lifting	45	80
	Shortening of secondary lamella	17	8
	Rupture of pillar cells	50	70
	Nuclear degeneration	10	30
	Necrosis	10	25
Progressive	Epithelial hyperplasia	83	70
	Mucus cell hyperplasia	30	55
	Chloride cell hyperplasia	10	30
	Mucus cell hypertrophy	20	55
Inflammatory	Leukocyte infiltration	83	77
Liver			
Circulatory	Haemorrhages	40	70
	Congestion of sinusoids	88	90
Regressive	Melanomacrophages centres	100	70
	Fatty vacuolization	11	30
	Steatosis	0	40
	Nuclear degeneration	20	45
	Hepatocellular pleomorphism	40	60
	Hydropic degeneration	30	60
	Necrosis	20	50
Progressive	Hepatocyte hyperplasia	34	44
	Hepatocyte hypertrophy	40	60
Inflammatory	Leukocyte infiltration	56	40

VtG level was observed for the Nagle Dam population ($r = 0.759, p<0.05$), and no association was observed for the Inanda Dam population ($r = -0.149, p>0.05$).

Discussion

Water Quality

Physical variables were not different between the two dams, except conductivity which was higher at Inanda Dam. Electrical conductivity is influenced by total dissolved solids and primary drivers include wastewater effluents and agricultural runoff [32]. Inanda Dam receives water from Darvill Wastewater Works through uMsunduzi River, which could explain the higher conductivity. The nitrite, phosphate and sulphate were substantially high at Inanda Dam compared to Nagle Dam. The nutrient levels exhibited metrophic condition at Nagle Dam and eutrophic at Inanda Dam. Nitrogen, phosphate and sulphate in freshwaters are mostly associated with wastewater effluents, agricultural runoff, and industrial and mining activities [33]. The concentrations observed at Inanda

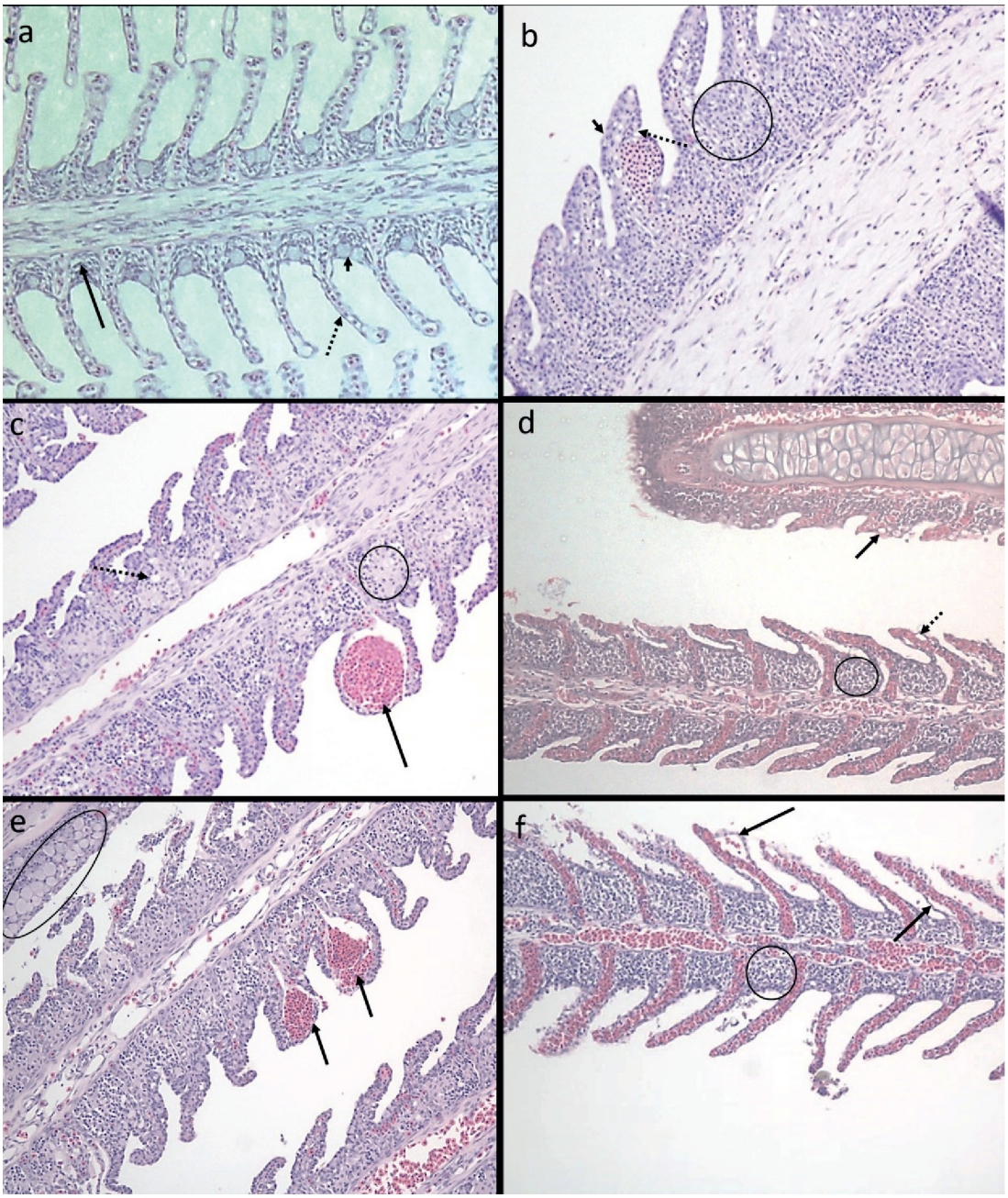

Fig. 3. Histopathology of the gill observed for the *Clarias gariepinus* from the Nagle and Inanda dams during 2020 and 2021 surveys. a) Normal primary lamella (arrow), normal secondary lamella (dotted arrow), mucus cell (short arrow), b) Haemorrhage/aneurism (dotted arrow), epithelial hyperplasia (short arrow and encircled), c) oedema (encircled), mucus cells hyperplasia (dotted arrow), aneurism resulting in pillar cell rupture and epithelial lifting (arrows), d) chloride cell hyperplasia (arrow), oedema and epithelial lifting (dotted arrow), epithelial hyperplasia (encircled); e) Mucus cells hyperplasia (encircled), and aneurism, epithelial lifting and pillar cell rupture (arrows); f) Infiltration of leukocytes, epithelial lifting (arrow), hyperplasia (encircled). Haematoxylin and Eosin staining (H&E).

Dam are comparable to those reported in other water bodies receiving treated, partially treated and untreated wastewaters [34].

Metal concentrations in the water column followed a descending order, Fe>Al>Mn with Zn and copper being below detection level. Coinciding with the aforementioned trend, a descending order, Fe>Al>Mn>Zn>Cu was observed in sediment. The metal levels were not significantly different between the two dams in the water column; however, sediment exhibited a substantially higher metal concentration in Inanda Dam than Nagle Dam. Metals may occur naturally due to rock weathering, soil erosion, volcanic activities or the dissolution of the water-soluble salts; however, anthropogenic sources such as industrial activities, mining, and wastewater effluents may result in extreme concentrations [34, 35]. Therefore, the presence of metallurgic industries in the uMsunduzi River catchment could explain the increased metal concentration in the Inanda Dam. This trend is comparable to those observed in other related studies

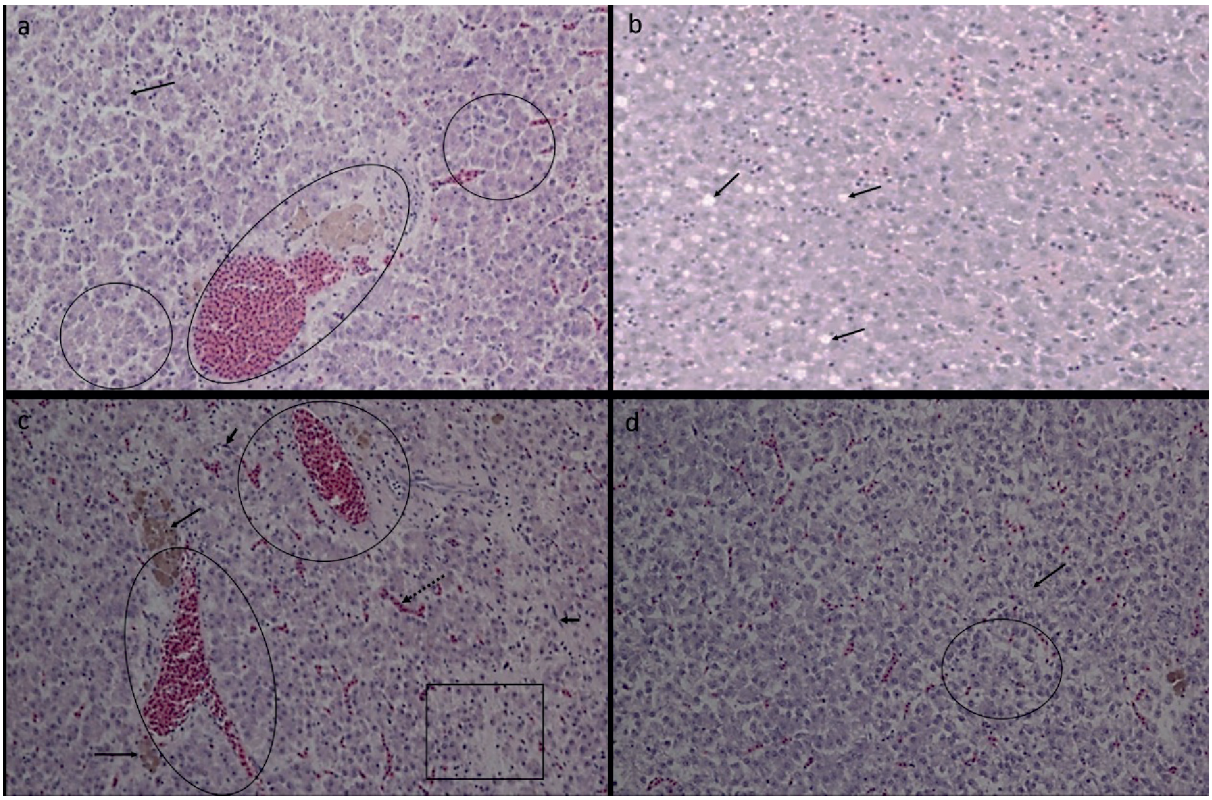

Fig. 4. Histopathology of the liver observed for the *Clarias gariepinus* from the Nagle and Inanda dams during 2020 and 2021 surveys. a) Hypertrophied hepatocyte (arrow), hepatocellular pleomorphism accompanied by nuclear degeneration and necrosis (circle), hepatic portal triad (oval); b) Scattered steatosis (arrow), leukocyte infiltration; c) Central vein (oval), Melanomacrophages (arrow), congestion of the sinusoids (dotted arrow), hepatocellular and nuclear pleomorphism with necrosis (square), Kupffer cells (short arrows); d) Hydropic degeneration (arrow), hepatocellular pleomorphism and hypertrophied hepatocytes. Haematoxylin and Eosin staining (H&E).

[36, 37]. Upon entering aquatic ecosystems, metals settle to the bottom sediment and may be resuspended into the water column due to changes in physico-chemical properties such as water pH, salinity, the presence of other metals etc. [38, 39]. Therefore, the Inanda Dam water is at risk of experiencing high metal concentration without input from external sources.

Overall Fish Health and Histopathologic Biomarker

The length-weight relationship, condition factor and hepatosomatic index have successfully been used in fishery assessment studies to explore the general well-being of fish and their growth [40, 41]. In the present study, both populations exhibited negative allometric growth whereas the condition factor was within the normal range for Nagle Dam population and some beyond the normal range for Inanda Dam population. These results are comparable to those reported by Kumolu-Johnson and Ndimele [42] and contradict with those reported by Verma and Prakash [43] for *Mystus vittanus* for polluted waters. Moreover, Anene [44] recorded a condition factor ranging from 4.3-5.27 in four Cichlids species at a polluted polluted lake. Although food availability, nutrition, parasites infestation and diseases have shown to influence condition factor [45, 46], the water pollution effect in the Inanda Dam population may not be dismissed.

Another index that has shown reliability in discriminating two populations from dissimilar water quality is the hepatosomatic index [41, 47]. According to Traven [48], an increased hepatosomatic index may be an indication of an enhanced detoxification process in response to exposure to increased level of contaminants. In contrast, Liebel [49] emphasized that exposure to increased pollutants may result in low hepatosomatic index and possibly decreased lipid storage. Morever, hepatosomatic index can be influenced by physiological development, age, sex, seasonal cycles, food availability, presence of different contaminant mixtures and parasites infestation [48, 50]. In the present study, the hepatosomatic index was not conclusive in discriminating the health of the two populations.

Histopathogy has proven to provide an insight and early warning signal of pollutants exposure in fish [51]. The fish gill plays an important role in osmoregulation, acid-base balance, respiration, and excretion and are in direct contact with the external environment making them susceptible to environmental contaminants [10]. In the present study, alterations such as epithelial lifting, hyperplasia of the secondary lamella, intracellular

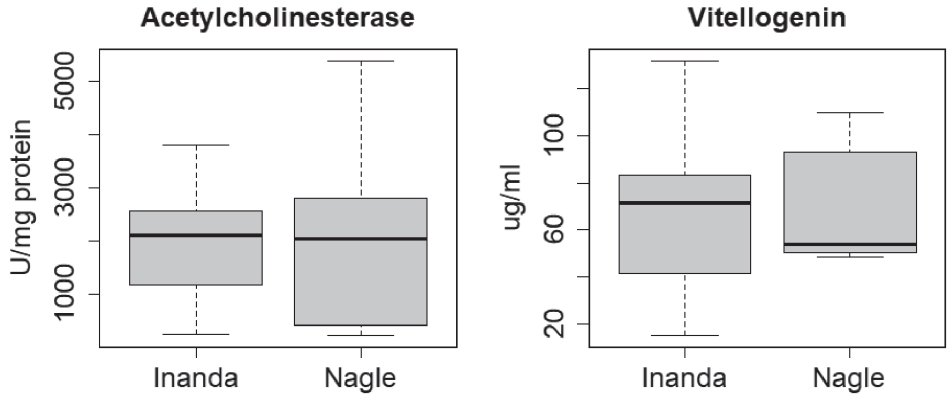

Fig. 5. Box and whisker plots presenting the acetylcholinesterase activity in the brain and vitellogenin concentrations in *Clarias gariepinus* sampled at Nagle and Inanda dams during low and high flow surveys in 2020 and 2021.

oedema, and leukocyte infiltration were prevalent in *C. gariepinus* gills at both dams, with most pathologies showing a relatively higher prevalence and degree of severity in the Inanda Dam population. These findings coincide with those reported in other related studies [52, 53]. Alterations such as epithelial lifting, hyperplasia and intracellular oedema could be an indication of a defense mechanism from contaminants by creating an increased distance between the external environment and blood, they also resulting in impairing oxygen uptake [23, 54]. Epithelial lifting may also be a result of fluid infiltration between epithelial and basement membrane [53].

A partial fusion of secondary lamella was moderately prevalent at both dams, with a complete fusion being observed only in fish from Inanda dam. Complete and partial fusion of the secondary lamella may reduce free gas exchange and thus, affecting general fish health [55]. Other gill alterations that were more prominent in the Inanda Dam population include aneurysm, hemorrhages and oedema. Aneurysm and hemorrhages are mostly accompanied by a pillar cell system collapse as a result releasing large quantity of blood into the lamella [56]. Corroborating with these findings, Dane and Şişman [55] and Ikisa [54] recorded a higher degree of alterations for aneurysm, hemorrhages and oedema in fish from polluted water bodies compared to those from control site. Most gill alterations observed in the present study showed a great variation on the degree of severity with Inanda Dam population showing a relatively higher severity compared to Nagle dam population. However, the gill index classifies abnormalities as slight histological alteration at both dams, hence, they have not influenced the functioning of the organ. A gill index of <20 was observed for *Prochilodus lineatus* exposed to atrazine [57]. Moreover, McHugh [58] recorded a gill index of <10 for *Hydrocynus vittatus* from dichlorodiphenyltrichloroethane (DDT) polluted Pongolapoort Dam.

The liver is a primary organ for pollutants metabolism and detoxification; hence, susceptible to toxicants [59]. In the present study, alterations such as MMCs, congestion of sinusoids, hepatocellular and nuclear pleomorphism, hepatocytes hypertrophy, necrosis, hydropic degeneration and leukocyte infiltration were prevalent in *C. gariepinus* liver from both dams. These results are comparable to those observed in other related studies [23, 60]. Hyperplasia, which was more prevalent for Inanda Dam population, is commonly regarded as a compensatory action to interstitial cell death [61]. Hyperplasia and hypertrophy are associated with the hepatocellular pleomorphism which was observed at both dams with Inanda Dam population exhibiting the higher prevalence and the degree of alteration. Melanomacrophages in fish liver may be an indication of a recycle of endogenous material from damaged cells [60]; however, an increased concentration may be attributed to pollutants exposure [62].

There was an association between water quality and the prevalence of histopathologic alteration in the liver. Some alterations were common for both populations with a difference on the degree of alterations. The total liver indices from both populations were found to be <20 which indicate slight histopathological alterations. Corroborating these findings, McHugh [12] recorded a liver index of <10 for *H. vittatus* from Pongolapoort Dam polluted with DDT whereas Nero [63] recorded a liver index of <20 for *Perca flavescens* exposed to naphthenic acid toxicity. Inanda Dam receives partially treated and treated wastewater from the wastewater work and metals from metallurgic industries through uMsunduzi River. Although the liver index was not successful in discriminating the two populations, most anomalies showed higher prevalence for Inanda Dam population.

Acetylcholinesterase

Acetylcholinesterase is a primary target for organophosphates and carbamates and has been extensively used as an indicator of environmental exposure in fish. Major sources of pesticides into water bodies include wastewater effluents and run-off from agricultural activities [64]. Despite Inanda

Dam receiving additional contaminants from polluted uMsunduzi, uMngcweni and Sikelekehleni rivers, the mean activity of AChE has shown no difference between Inanda and Nagle Dam populations. However, AChE activity showed great variability within each population with the lowest levels being recorded for the Inanda Dam population.

Üner [65] reported a significant decrease in AChE activity in the brain of *Oreochromis niloticus* exposed to diazinon and Mdegela [66] recorded a 50% reduction AChE brain activity in *C. gariepinus* exposed to pesticides. Sabullah [67] highlighted that AChE inhibition due to heavy metals may have resulted from the attraction of metal ions to negatively charged amino acid side chains containing carboxyl groups thus cause changes in the active site. Pan [68] recorded a decreased AChE activity in *Danio rerio* exposed to Cd whereas Sabullah [67] recorded more than 70% activity loss in *Periophthalmodon schlosseri* exposed to cadmium, chromium, lead, copper and zinc. Although AChE activities showed no difference between the two dams, the pollution effect at Inanda Dam may not be dismissed because this population is the one that showed lowest activity.

Acetylcholinesterase activity in the fish brain prevents the unceasing nerve firing, which is important in the functioning of the neuromuscular and sensory systems [67]. The AChE inhibition occurs because of phosphorylation of the serine residue in the enzyme's active site resulting in a depolarized post-synaptic membrane and synaptic transmission failure [64]. Intrinsic factors such as fish size and age may also have an influence on the AChE activity [69]. Bigger fish generally have a larger surface area to volume ratio (SA:V) and may assimilate and detoxify contaminants at a faster rate [69]. However, fish populations from Inanda Dam exhibited no relationship between fish size and AChE activity whereas Nagle Dam population showed a weak relationship. These results coincide with Rodríguez-Fuentes [70] that recorded an inverse relationship between fish size and AChE activity in *Gambusia yucatana* in a polluted Peninsula River in Mexico, and Fajardo and Ocampo [71] in *Glossogobius giurus* in a polluted lagoon lake, Philippines.

Vitellogenin

Vitellogenin is an egg yolk precursor protein that is produced in the liver of egg-laying female organisms; however, exposure to xenoestrogen showed to induce it in male and immature fish [72]. Vitellogenin induction in juvenile and male fish has long been established as a reliable biomarker for oestrogenic effects in aquatic environments [73, 74]. In the present study, males showed considerable levels of VtG for both Nagle and Inanda dam populations; however, higher induction and variability were observed in individuals from Inanda Dam. This trend is comparable to those observed in the other related studies conducted in the field and in laboratories [75, 76]. Yang [77] recorded a significant increase in VtG concentrations in male zebrafish, *Danio rerio* exposed to high concentrations of bisphenol A whereas Paschoalini [78] observed VtG induction in males exposed to heavy metals due to their ability to interfere in the action of oestrogenic hormones. Moreover, vitellogenin becomes a Zn-binding protein in some fish species and result in induction in male fish [79].

It is evident that vitellogenin may be induced by various pollutants and the isolated agricultural and mild industrial activities in Howick in the upper uMgeni River catchment [16], may be some of the explanations for the vitellogenin induction in Nagle Dam. Despite mild pollution from the upper catchment, the Inanda Dam receives water from a severely contaminated uMsunduzi River which drains a highly industrialised catchment with poorly performing wastewater treatment plants [16, 20], which could be the explanation for the observed variability in the Inanda Dam population.

Conclusion

The cumulative effects of different land-use occurring in the vicinity of the uMgeni River have resulted in the increase of metal concentration in sediment at Inanda Dam and should be viewed with concern. The condition factor was successful in discriminating the two populations whereas the hepatosomatic index was not conclusive. The histopathology-based assessment has proven to provide an insight and early warning signal of pollutants exposure, with the gill severe cumulative effect of water-borne pollutants. The populations have shown no significant difference for AChE inhibition and VtG induction, however, it is evident that there are signs of neurotoxic and estrogenic effect in *C. gariepinus* populations from both dams given the observed variability on the AChE and VtG levels. Generally, both histopathologic and biochemical biomarkers show signs of severely compromised fish health in the Inanda Dam compared to those from Nagle Dam. It is thus, evident that the tributaries feeding the uMgeni River downstream of Nagle Dam are resulting in the increase of pollution level in the Inanda Dam. *Clarias gariepinus* is known for its resistance to pollution effect, therefore, it is likely that other species may be severely affected. Further exploration of pollution effect on fish health is recommended on other native species which are known to be pollution sensitive. Moreover, a multi-biomarker approach is recommended to get more insight on the effects of contaminants on fish species in the uMgeni River system.

Acknowledgment

This work is based on the research supported wholly by the National Research Foundation of South Africa

(Grant Numbers: 121848). The authors would like to thank the University of KwaZulu-Natal, School of Life Sciences and postgraduate students for technical support.

Conflict of Interest

The authors declare no conflict of interest.

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