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Oxidative stress biomarkers in the African sharptooth catfish, *Clarias gariepinus*, associated with infections by adult digeneans and water quality

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ABSTRACT

Parasites and environmental features could synergistically act as stressors to the health of their hosts. The objectives of this study were to evaluate the effect of: (i) water quality, host sex, size and body condition on adult digenean parasite infections; (ii) digenean infections and host sex and size on the oxidative stress biomarkers and body condition of hosts; and (iii) water quality on the oxidative stress biomarkers and body condition in *Clarias gariepinus*. Water quality variables were measured and *C. gariepinus* were collected each month for a year for examination of two intestinal digeneans, *Masenia nkomatiensis* and *Glossidium pedatum*, and determination of body condition and measurement of biomarkers in the host. The results indicated that the intensity of *M. nkomatiensis* was positively correlated with electrical conductivity and total dissolved solids. Prevalence of *G. pedatum* was negatively correlated with electrical conductivity, salinity and total dissolved solids. High summer water temperature was strongly associated with high digenean infections. There was no host body condition, sex or size bias for any of the parasite infection variables. Differences in the biomarker levels and body condition between uninfected fish and those infected with *M. nkomatiensis* or *G. pedatum* were insignificant indicating a low effect of the digenean parasites on oxidative stress biomarkers and body condition in the fish. However, total protein levels were positively associated with host size, and lipid peroxidation was negatively related to host body condition; total protein levels were also positively correlated with temperature and negatively correlated with dissolved oxygen. Host body condition was only negatively correlated with dissolved oxygen. Overall the trends observed in the data showed that the parasites have a negligible effect on oxidative stress in host fish and the trends observed for all variables (water quality, stress biomarkers, body condition and parasite infections) showed a strong seasonal pattern.

1. Introduction

The quality of the freshwater environment has a profound effect on the health status of fish (Abdel-Gaber et al., 2016). Changes in environmental parameters (eg. dissolved oxygen, pH, carbon dioxide, temperature, salinity) and parasite infections elicit an immune response in the host (Dezfuli et al., 2008; Cabillon and Lazado, 2019). Producing an immune response involves the release of reactive oxygen species (ROS), but excess intracellular concentrations of ROS are harmful to the physiological function of the host (Mougeot et al., 2010). Therefore, if excess ROS is not reduced in tissues of the host, this could influence the host condition negatively or weaken the protective function of the immune system leaving the host more susceptible to diseases and parasites (Nabi et al., 2017).

Vertebrates have evolved remarkable antioxidant defence systems to cope with the damaging effects of high ROS through antioxidant enzymes, which decrease intracellular ROS concentrations (Birnie-Gauvin et al., 2017). Most antioxidant systems are found particularly in organs with high metabolic activity such as the liver, kidney and brain (Stanca et al., 2013). The superoxide dismutase-catalase system is the first line of enzymatic defence to remove $H_2O_2^-$ and O_2^- radicals and has been widely used to evaluate the health status of fish (Ighodaro and Akinloye, 2018).

Studies have reported oxidative stress biomarkers in fish in relation to helminth infections and environmental conditions, and have further shown that oxidative stress associated with helminth parasites and altered environments is highly variable (Lushchak and Bagnyukova,

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2006; Nabi et al., 2017). For instance, low levels of superoxide dismutase (SOD), lipid peroxidation (LPX) and total protein (TP) have been associated with infection by acanthocephalans (Radovanović et al., 2010; Nabi et al., 2017); TP and catalase (CAT) have been found to be lower, and SOD and LPX higher in hosts infected with digeneans (Belló et al., 2000; Stumbo et al., 2012). Superoxide dismutase and CAT were higher in fish co-infected with nematode and myxosporean parasites (Skuratovskaya et al., 2013); SOD and CAT were high in fish hosts infected with nematodes (Skuratovskaya et al., 2018). Higher levels of CAT and low TP have been recorded in fish infected with cestodes (Dautremepuits et al., 2002).

Oxidative stress biomarkers have also been related to environmental parameters and showed variable trends such as increased LPX associated with high temperature (Lushchak and Bagnyukova, 2006); increased SOD associated with low dissolved oxygen concentration (Víg and Nemcsók, 1989) and low SOD levels associated with high salinity (Wang et al., 2016). Body condition is another quantitative indicator of the welfare of fish associated with nutritional conditions and is, therefore, an important tool to study the relationship between host health and intestinal parasites (Guidelli et al., 2011).

Clarias gariepinus (Burchell, 1822) is of great importance for livelihoods and income generation in many parts of Africa. Parasites have the potential to drastically impact aquaculture of this fish species (Walakira et al., 2014) and therefore, the health of this fish is of major concern. Many endoparasitic helminths have been reported to infect *C. gariepinus* (see Kuchta et al., 2018) suggesting that the fish plays an important role in life cycles of many metazoan parasites, particularly helminths, in freshwater ecosystems (Madanire-Moyo et al., 2010). It hosts the highest richness of adult helminth species in Africa (Khalil and Polling, 1997) and therefore, it is a suitable model to study the effect of parasites on immune and oxidative stress responses.

Although *C. gariepinus* is an important source of nourishment to people living in many parts of Africa, from an aquaculture perspective, it is noteworthy that the health of this fish exposed to parasites has been poorly studied. Additionally the effect of water quality on the health of *C. gariepinus* has been less explored because it is considered to be highly adaptable and hardy toward adverse environmental conditions. At present, the paucity of research in fish diseases/health in Africa is not seen as a factor that will negatively impact aquaculture development and as such is not a target research area (Ajala and Fawole, 2014).

Two digenean species parasitize the intestine of *C. gariepinus*; namely, *Masenia nkomatiensis* Dumbo, Dos Santos & Avenant-Oldewage (2019) (Cephalogonimidae) and *Glossidium pedatum* Looss, 1899 (*incertae sedis*). These species have been recorded only in Africa, infecting the intestines of clariid fishes (Dumbo et al., 2019a, b). The life cycle of both parasite species is unknown, however, in general, the life cycle of digeneans include various stages namely, miracidia, sporocysts, rediae, cercariae and metacercariae that develop in multiple hosts such as molluscs (first obligate intermediate host), crustaceans and vertebrates (Poulin and Cribb, 2002). Dumbo and Avenant-Oldewage (2019) reported immune and pathological changes in the intestine of *C. gariepinus* caused by adults of *M. nkomatiensis*, yet a similar study is not available for *G. pedatum*. To the best of our knowledge, no studies have dealt with the effect of both, digenean parasites and physico-chemical water quality parameters on selected physiological biomarkers in this fish.

In this study, we examined the relationships between water quality, host attributes and digenean infections, and assessed whether water quality and digenean infections affect the health of *C. gariepinus* from the Incomati River in Mozambique. More specifically, we tested the (1) correlation of host size and water quality with intensity and prevalence of *M. nkomatiensis* and *G. pedatum*; (2) difference in digenean infection between male and female hosts; (3) differences in concentrations of biomarkers and body condition among uninfected fish, *M. nkomatiensis*-infected fish and *G. pedatum*-infected fish; (4) correlation of water quality, host size and body condition with concentrations of biomarkers in the host; and (5) difference in concentrations of biomarkers between male and female fish.

2. Material and methods

2.1. Study site

The Incomati River is a major component of the Incomati Basin, located in an international drainage basin situated in the Kingdom of eSwatini (previously Swaziland), in the Republic of South Africa and the Republic of Mozambique (Vaz and Pereira, 2000). Monthly sampling of fish and measurement of water quality parameters were carried out in the Incomati River, in the Manhiça municipality, Mozambique (25°25′50.4″S, 32°48′45.1″E) (Fig. 1) between October 2016 and September 2017. The depth of the river varied from 80 cm in dry season–winter (April–September) to 200 cm in rainy season– summer (October–March). The river was not accessible in May and June due to the intense cover by water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach, 1883. Fish diversity at the study site includes *Opsaridium zambezense* (Peters, 1852); *Oreochromis mossambicus* (Peters, 1852); *Synodontis zambezensis* Peters, 1852; *Tilapia rendalli* (Boulenger, 1897); *Protopterus annectens brieni* Poll, 1961; *Serranochromis meridianus* Jubb, 1967 and *C. gariepinus*. The latter species was the object of the present study.

2.2. Water quality parameters in the study site

In situ water quality parameters and water samples were measured and collected 20 cm below the surface in the midstream of the river during the sampling survey. Monthly quadruplicate *in situ* measurements of temperature, pH, electrical conductivity (EC) and dissolved oxygen (DO) were done with a Multi-Probe meter pH PCE-PHD1 (PCE Ibérica S.L, Spain). For salinity, turbidity (TB) and total dissolved solids (TDS), monthly quadruplicate water samples were collected in labelled, tightly-sealed plastic bottles and cooled to ~2–4 °C (Śliwka-Kaszyńska et al., 2003) for approximately 2 h until the measurements were done in the laboratory at room temperature, with a Multi-Probe meter OAKTON PCD650 (EUTECH Instruments, Singapore) and microprocessor turbidity meter HI593703 (HANNA Instruments, Hungary).

2.3. Ethical standards, fish collection and identification of parasites

Sampling was carried out according to the specifications of a permit issued by the Ministry of Sea, Inland Waters and Fisheries of Mozambique, number 1148/590/GM-MIMAIP/SIC/2016 of June 16, 2016. All procedures and manipulations of the animal were approved by the Ethics Committee of the Faculty of Science, University of Johannesburg, nr 2016-11-28/Dumbo to comply with the ethical standard guidelines for manipulation and use of laboratory animals in South Africa. In the present study, 154 fish (40 ± 8.19 (20–60) cm total length) were collected using 50 × 1 m gill nets (5.08 cm and 10.16 cm stretched mesh size). Fish were euthanized by severing the spinal cord with a single cut; this was followed by measurement of fish (weight and total length) and determining the host sex. Thereafter, fish were dissected and the intestine and liver excised. Thirty-four liver samples from infected hosts and 37 from uninfected hosts were obtained soon after euthanasia and immediately frozen by immersion in liquid nitrogen for oxidative stress biomarker analysis. The uninfected fish used as control were free of any digeneans; free of any external morphological changes; internal organs with no apparent aberrations and especially the liver with no distinct localized or general discolouration, nodules or cysts.

The intestines were removed and examined for digenean parasites using a stereomicroscope. Parasites were flattened in a drop of alcohol-formalin-acetic acid (AFA) between a glass slide and coverslip and then preserved in 70% ethanol. Parasites were stained with acetocarmine, differentiated with 70% ethanol–0.5% hydrochloric acid and

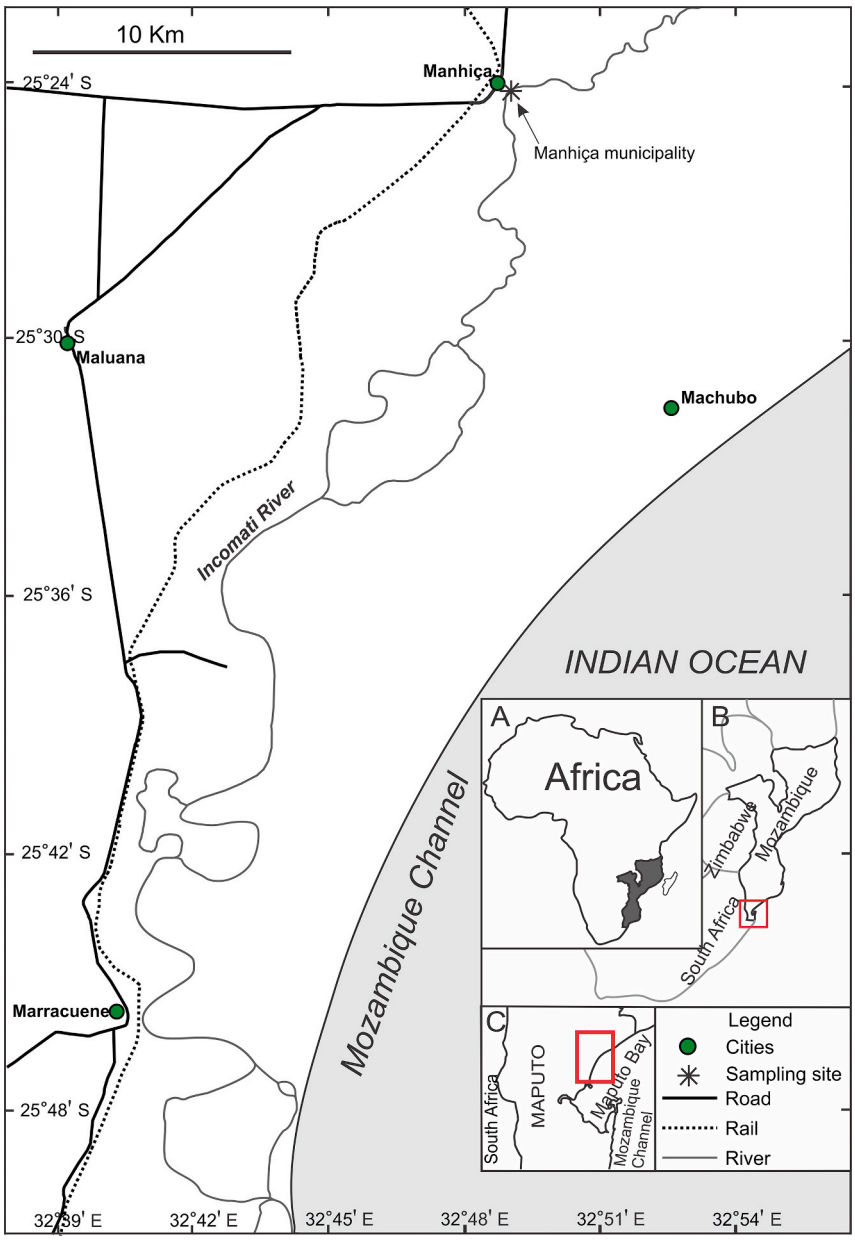

Fig. 1. Various maps of the Incomati River showing the position of the sampling site. A– Mozambique shaded on the African continent; B– shows position of Maputo Province in Mozambique; C– indicates the position of the Incomati River and the sampling site.

dehydrated through an ascending ethanol series, cleared in beachwood creosote, mounted in Canada balsam and viewed with a microscope (Thatcher, 2006). Taxonomic identification was based on recent descriptions and re-descriptions of these parasites by Dumbo et al. (2019a, b).

2.4. Body condition (KN) of the host

The total length (*Lt*) and total weight (*Wt*) of *C. gariepinus* were used to calculate the body condition (KN) of the fish by adjusting to the relationship curve,

$$Wt = a \times Lt^{b}$$

The values of the regression coefficients "a" and "b" were estimated and later used to estimate the theoretically expected values for weight "We". The body condition was set as the ratio between measured (observed) weight and expected weight for a given length (Le Cren, 1951).

$$KN = Wt/We$$

2.5. Preparation of tissue homogenate for antioxidant biomarkers

Three subsamples of individual liver tissue were weighed (100–150 mg) and homogenized with a CAT X120 homogenizer (Ingenieurbüro CAT M. Zipperer GmbH Staufen, Germany). One part, was homogenized in 0.05 M phosphate-buffered saline (PBS; pH 7.0) (1:4 w/v) and centrifuged for 10 min at 10 000×*g* (4 °C) for TP and LPX analysis; the second part was homogenized in 0.05 M PBS (1:10 w/v) and centrifuged for 10 min at 7500×*g* (4 °C) for estimation of GSH and SOD activities; and the third part was homogenized in 0.05 M PBS (1:3 w/v) and centrifuged for 10 min at 10 000×*g* (4 °C) for CAT activity. The homogenate of each sample was collected and stored in −80 °C until further analysis.

For each biomarker, the sample was read in triplicate in the KC junior

software in ELx800 universal Microplate reader (Bio-Tek Instruments, Inc, USA) for the TP content, LPX, and CAT while Gene 5™ 1.05 in FLx800 Microplate reader (Bio-Tek Instruments, Inc, USA) for GSH and SOD levels. Total protein in the samples was measured at 595 nm following the Bradford method, where proteins are bound to Coomassie Brilliant Blue G250 (Bradford, 1976). Lipid peroxidation was measured as described by Ohkawa et al. (1979). The total reaction volume of 1.25 mL contained tissue homogenate, 8.1% (w/v) sodium dodecyl sulphate, 20% (v/v) acetate buffer (pH 3.5) and 0.8% (w/v) aqueous solution of thiobarbituric acid (Merck KGaA, Darmstadt, Germany). The reaction medium was heated at 95 °C for 30 min, the red pigment produced was extracted with n-butanol–pyridine mixture and the absorbance of the product (Malondialdehyde) was measured at 532 nm and expressed as nmol.mg^{-1} protein.

Reduced glutathione activity was assayed according to Cohn and Lyle (1966). Final concentrations in a volume of 250 µL of 0.05 M potassium phosphate buffer (pH 7.0), 0.1% (w/v) O-pthalaldehyde and the sample diluted (1:10) with Milli-Q water. Specific activity was read at 420 nm and expressed as ug.mg^{-1} protein. Superoxide dismutase activity was determined following the inhibition of pyrogallol autoxidation as described by Del Maestro and McDonald (1989). The total volume of the reaction medium (250 µL) contained the sample, 50 mM diethylenetriamine pentacetic acid/Tris base and pyrogallol. The reaction was incubated in darkness while the pyrogallol was added and the reaction read at an absorbance of 405 nm. The SOD activity was expressed as ng SOD.mg^{-1} protein. Catalase activity was assayed following the method of Cohen et al. (1970), which is based on the measurement of residual H_2O_2 during the reaction with $KMnO_4$. Enzyme activity was read at an absorbance of 492 nm for 30–60 s and was expressed as µmol H_2O_2.min^{-1}.

2.6. Statistical analyses

The infection indices (prevalence, mean intensity (MI) and mean abundance (MA)) were calculated according to Bush et al. (1997). Normality of the data was tested using the Shapiro-Wilk (W) test. Monthly differences in physico-chemical water quality variables and the intensity of the infections were tested using one way ANOVA and when the difference was significant pairwise difference comparison was conducted using Tukey's HSD; whereas monthly body condition and biomarkers levels were tested using Kruskal-Wallis test due to non-normal distribution of the variables. The Mann-Whitney U test was used to test seasonal differences between water quality parameters and differences in biomarkers and parasite infections between male and female fish. Differences in oxidative stress biomarkers between uninfected fish, *M. nkomatiensis*-infected fish and *G. pedatum*-infected fish were also tested using ANOVA while for body condition, the distribution of the variable was non-normal and therefore the non-parametric test, Kruskal-Wallis test was used. A Spearman's correlation (R) was used to test the significance of the relationships between each individual water quality variables, host body condition and size and intensity and prevalence of *M. nkomatiensis* and *G. pedatum*; water quality variables, host size and body condition with stress biomarkers. The Principal Component Analysis (PCA) was used to explore the association between parasitism, physico-chemical variables and concentration of stress biomarkers. Statistical analyses were performed using STATISTICA (StatSoft, Inc. USA) and Excel (version 2013), except the Spearman's correlation that was analysed in SPSS V. 26 (Statistical Package for the Social Sciences, SPP Inc.) and graphically presented with GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA, USA). Significance difference was set at $p \leq 0.05$.

3. Results

3.1. Water quality variables

Monthly data for physico-chemical water quality variables in the Incomati River (Fig. 2) varied significantly across the study period: pH (ANOVA $F_{11, 36} = 2.8$, $p < 0.001$); EC (ANOVA $F_{11, 36} = 7.5$, $p < 0.001$); temperature (ANOVA $F_{11, 36} = 4.6$, $p < 0.001$); DO (ANOVA $F_{11, 36} = 4.6$, $p < 0.001$); salinity (ANOVA $F_{11, 36} = 7.6$, $p < 0.001$); TB (ANOVA $F_{11, 36} = 6.4$,$p < 0.001$) and TDS (ANOVA $F_{11, 36} = 6.8$, $p < 0.001$). Based on significant differences, water quality parameters could be grouped into seasons based on rainfall patterns (ie. dry and rainy seasons). The temperature (Mann-Whitney U = 4.0, $p = 0.025$) was higher in rainy season (summer) than the dry season (winter). The highest temperature was observed in January (summer) and the lowest in September (winter) (Fig. 2). The remaining water parameters showed insignificant seasonal difference over the period of sampling, DO (Mann-Whitney U = 11, $p =$

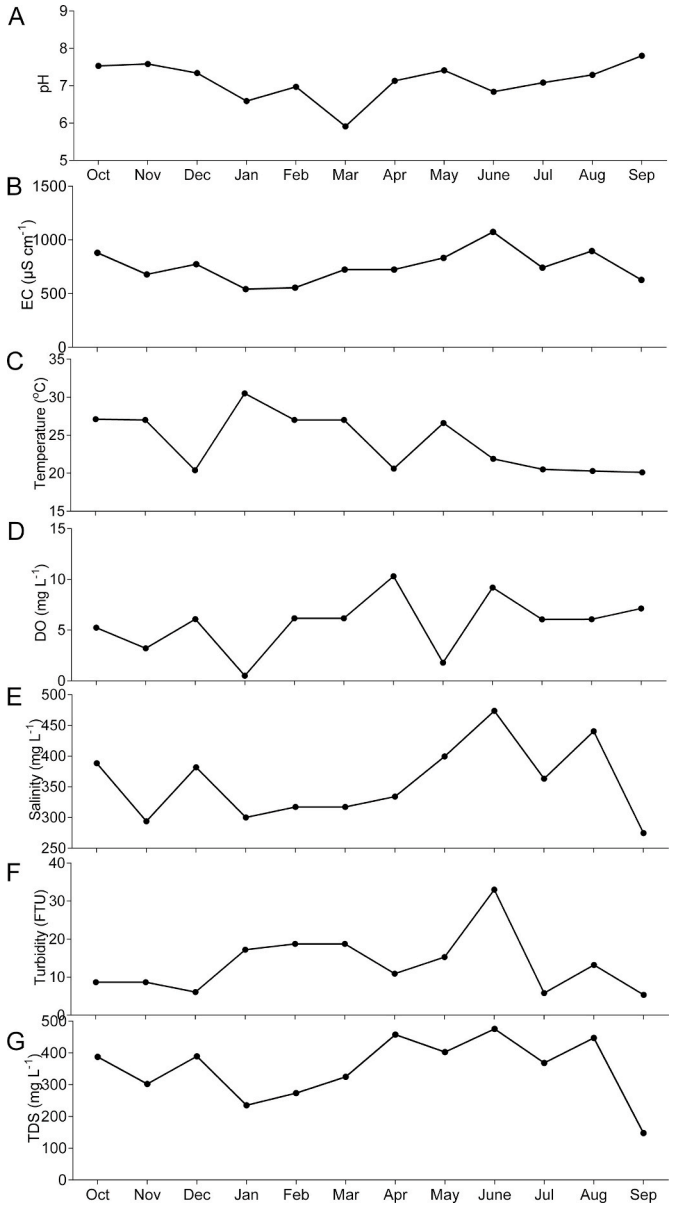

Fig. 2. Monthly variation of physico-chemical parameters during the fish collection period, October 2016–September 2017. A– pH; B– Electrical conductivity; C– Temperature; D– Dissolved oxygen; E– Salinity; F– Turbidity; G– Total dissolved solids.

0.262); TDS (Mann-Whitney test U = 8, $p = 0.109$); pH (Mann-Whitney test U = 15, $p = 0.631$); salinity (Mann-Whitney test U = 10, $p = 0.2$); turbidity (Mann-Whitney test U = 15, $p = 0.63$); and EC (Mann-Whitney test U = 9.5, $p = 0.173$).

3.2. Infection biology of *M. nkomatiensis* and *G. pedatum*

A total of 1024 digeneans were collected and identified as being adults of *M. nkomatiensis* (731) and *G. pedatum* (293) specimens. Statistically, monthly intensity for *M. nkomatiensis* (ANOVA $F_{9, 141} = 8.33$, $p < 0.001$) and *G. pedatum* (ANOVA $F_{9, 143} = 9.72$, $p < 0.001$) showed significant difference with highest mean intensity, for both parasites, in February (rainy season) (Fig. 3). In four specimens (three in dry and one in rainy seasons), hosts were found to be co-infected by both digeneans and this did not show any seasonal pattern due to low number of co-infected hosts. The effect of host attributes on the intensity of parasites showed that host size and body condition did not significantly correlate with intensity of *G. pedatum* and *M. nkomatiensis* ($p > 0.05$) (Table 1). Additionally, pairwise test showed insignificant differences in digenean intensity between male and female hosts (*G. pedatum*, Mann-Whitney U = 2837, $p = 0.084$; *M. nkomatiensis*, Mann-Whitney U = 2674, $p = 0.536$).

3.3. Antioxidant biomarkers and body condition

All biomarkers showed insignificant monthly differences (Fig. 4) during the study period (Kruskal-Wallis; $p > 0.05$). The uninfected fish, *M. nkomatiensis*-infected fish and *G. pedatum*-infected fish showed insignificant differences in TP (ANOVA $F_{2, 73} = 1.99$, $p > 0.05$), CAT (ANOVA $F_{2, 72} = 1.01$, $p > 0.05$), GSH (ANOVA $F_{2, 73} = 0.013$, $p > 0.05$), LPX (ANOVA $F_{2, 73} = 1.14$, $p > 0.05$), SOD (ANOVA $F_{2, 48} = 0.906$, $p > 0.00.5$) and KN (Kruskal-Wallis $H_{2, 70} = 0.231$, $p > 0.05$). An increased level of GSH and CAT in all three groups and only for LPX in *Masenia*-infected fish was observed from March to April. Total protein showed the opposite trend where levels decreased from March to April in *G. pedatum* and *M. nkomatiensis* infected fish but showed a slight increase in uninfected fish.

3.4. Analysis of interaction between parasitic infection and water variables

The lowest DO (0.5 mg.L^{-1}) in January and the highest DO (10 mg.L^{-1}) in April, corresponded with a decreased MI and MA of the parasites. From January to March, increased levels of DO, EC, TDS, salinity and decreased pH and temperature were related to increased prevalence of *M. nkomatiensis* (Figs. 2 and 3A) and decreased prevalence of *G. pedatum* (Fig. 3B). In February–April, the MI and MA of both parasites and prevalence of *G. pedatum* decreased and no clear pattern was observed in water quality variables for this period. A decreased EC, temperature, DO, TDS, salinity and TB, from August to September corresponded to the increase in prevalence, MI and MA of *G. pedatum*; whereas no change was observed in infection indices of *M. nkomatiensis* (Figs. 2 and 3). Spearman's correlation (Table 1) showed that only the intensity of *M. nkomatiensis* correlated positively and significantly with EC and TDS. Negative and significant correlations were observed between prevalence of *G. pedatum* with EC, salinity and TDS. For intensity of *G. pedatum*, correlations between EC, TDS and temperature were negative but not significant.

3.5. Analysis of interaction between water parameters and parasitic infection with biomarkers

The SOD was low in fish collected when the TDS, DO, EC and salinity were elevated in December, and the MI and MA for both parasites were low. Fish exposed to DO below to 5 mg.L^{-1} and elevated temperature (30.5 °C) (in January) showed a low MI and MA for both parasite species and an increasing level of SOD. Notwithstanding higher MI and MA for both parasites in February, not much variation in biomarker responses was observed. In March, when the pH was low (pH = 5.91) decreased levels of TP, CAT and LPX were detected and this coincided with a decrease of MI and MA for both parasites from February to March. An increased level in CAT, GSH and LPX, in April, match with increased pH up to 7, but the MI and MA for both parasites continued to decrease.

Overall, to improve understanding of the effect of water quality variables on parasite intensity and biomarkers, data were subjected to ordination by PCA and compared using Spearman's correlation. In Fig. 5

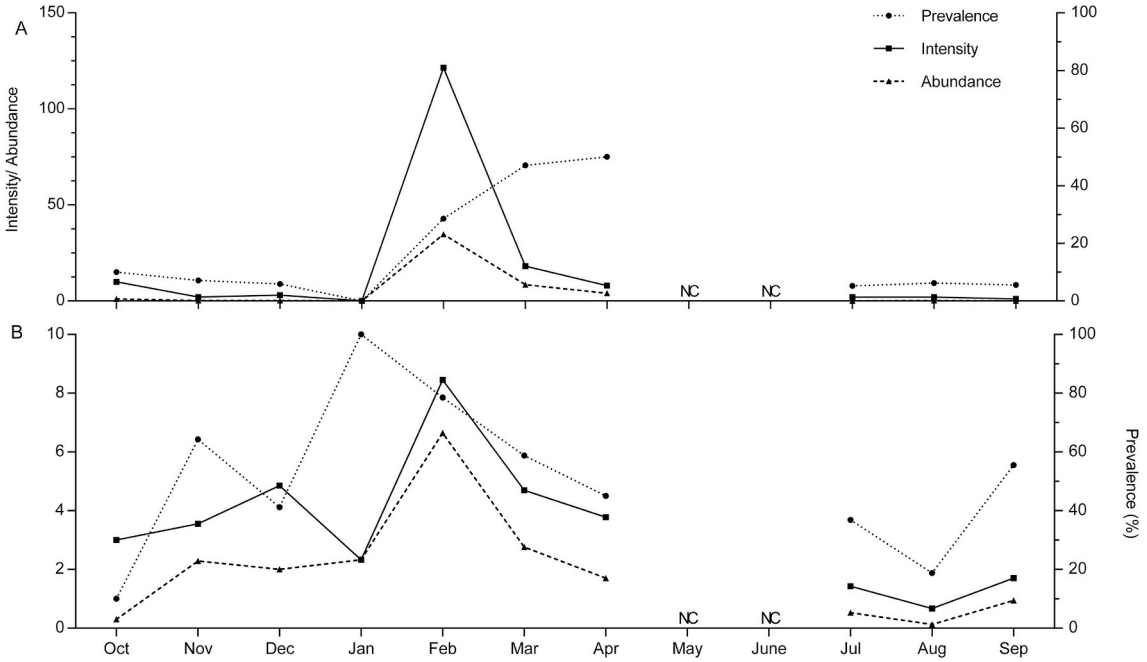

Fig. 3. Monthly variation of prevalence (%), mean intensity and mean abundance. A– *Masenia nkomatiensis*; B– *Glossidium pedatum*. NC – no collection was performed.

Table 1
Spearman's correlation (R) for physico-chemical water quality, host attributes and biomarkers with *Masenia nkomatiensis* and *Glossidium pedatum* infections in *Clarias gariepinus* from the Incomati River. Dashes indicate not applicable; bold text significant correlations between variables.

Variable	Intensity *M. nkomatiensis*	Prevalence *M. nkomatiensis*	Intensity *G. pedatum*	Prevalence *G. pedatum*	Total protein	Catalase	Gluthatione reduced	Lipid peroxidation	Superoxide dismutase	Host body condition
pH	0.198	0.159	0.148	0.33	−0.181	0.061	−0.028	−0.144	−0.049	0.159
Electrical conductivity	**0.282**[a]	0.611	−0.138	**−0.67**[a]	−0.157	−0.053	−0.037	−0.127	−0.132	0.059
Temperature	−0.21	−0.296	−0.103	−0.167	**0.347**[a]	−0.014	0.069	0.227	−0.038	−0.052
Dissolved oxygen	0.143	0.336	0.105	0.083	**−0.288**[a]	0.201	0.071	−0.176	0.117	**−0.246**[a]
Salinity	0.197	0.367	−0.15	**−0.694**[a]	−0.101	0.018	0.04	−0.029	0.008	0.03
Turbidity	−0.049	0.089	−0.096	−0.322	0.213	−0.027	0.174	0.21	0.071	−0.115
Total dissolved solids	**0.242**[a]	0.463	−0.147	**−0.646**[a]	−0.12	−0.049	0.017	−0.069	−0.069	0.034
Intensity *M. nkomatiensis*	–	–	−0.025	–	<0.001	0.025	0.022	0.06	0.236	−0.068
Prevalence *M. nkomatiensis*	–	–	–	−0.469	−0.445	−0.091	0.226	−0.098	−0.128	0.213
Intensity *G. pedatum*	−0.025	–	–	–	−0.029	0.081	−0.149	−0.075	0.103	0.047
Prevalence *G. pedatum*	–	−0.469	–	–	−0.317	0.037	−0.152	−0.146	0.244	0.061
Host sex	−0.15	–	−0.014	–	–	–	–	–	–	–
Host size	−0.07	–	0.024	–	**0.289**[a]	−0.016	0.153	0.097	−0.055	0.022
Host body condition	–	–	–	–	−0.06	0.121	−0.109	**−0.304**[a]	−0.024	–

[a] Correlation significant at $p \leq 0.05$.

the PCA biplot explained 45.2% (Factor 1 = 29.24%; Factor 2 = 14.56%) of the variation between biomarkers, infected fish and water quality variables. Based on Eigen values obtained from the comparison, highest loadings were found for EC (Eigen value = 0.426) and temperature (Eigen value = – 0.438). All correlations between biomarkers and parasite infections were insignificant. Correlations between biomarkers and water quality were mixed and in the majority of comparisons were not significantly correlated. Total protein was an exception which showed significant positive correlation with temperature, while significant negative correlation was observed with dissolved oxygen. Body condition was also negatively correlated with temperature (Table 1). Correlations among host attributes (body condition and size) and oxidative stress biomarkers were mostly insignificant. Only total protein levels were positively correlated to host size, and only lipid peroxidation negatively correlated to host body condition (Table 1). Additionally, pairwise comparisons of stress biomarkers between male and female showed insignificant differences [TP (Mann-Whitney test U = 517, p = 0.141); CAT (Mann-Whitney test U = 556, p = 0.303); GSH (Mann-Whitney test U = 546, p = 0.253); LPX (Mann-Whitney test U = 550, p = 0.272); SOD (Mann-Whitney test U = 505, p = 0.108) and KN (Mann-Whitney test U = 531, p = 0.189)].

4. Discussion

In the present study, water quality parameters fluctuated throughout the year corroborating the findings by Chilundo et al. (2008), that variability in water discharge during seasonal periods characterised by high and low rainfall is the main cause of fluctuation in temperature, pH, DO, TDS and EC. As such, significant correlations between intensity of *M. nkomatiensis* and prevalence of *G. pedatum* infecting *C. gariepinus* with EC, TDS and salinity suggest a possible impact of seasonal changes of these water quality variables on the life cycle and transmission of both digeneans (Ojwala et al., 2018). These water quality parameters are however, interdependent; EC is an estimator of the amount of total dissolved salts or total dissolved ions in water (Peterson, 2000). According to Sreenivasan (1976) and Bhatnagar and Devi (2013), waters with high conductivity are more productive and hence likely to harbour more invertebrates (intermediate hosts) and enabling parasites to thrive.

Larsen and Mouritsen (2014), Brunner and Eizaguirre (2016) and Gehman et al. (2018) have indicated that temperature is negatively correlated with DO with the former being the main driving variable for seasonal changes in parasite populations. In the present study, there was a recorded increase in temperature in January (southern hemisphere summer), which coincided with a decreased DO and the opposite trend was observed in April as temperature decreased.

In summer time when the temperature was higher (27–30.5 °C) (Fig. 2), prevalence and intensity of digenean infections were high (Fig. 3). This corroborates with Upadhyay et al. (2015) who recorded a significant positive correlation between the intensity of *Cephalogonimus yamunii* Upadhyay, Jaiswal, Malhotra & Malhotra, 2013 (Digenea) in the gut of *Heteropneustes fossilis* (Bloch, 1794) with water temperature. Elevated temperatures typically increase the metabolic rate of ectothermic organisms– an increase in parasite development rate– due to a decrease in the embryonation period. Similarly increasing temperature (in summer) will propagate the population of snails (Barber et al., 2016). As digeneans use snails as first obligate intermediate host (most limiting factor for digenean dispersal) (Paperna and Dzikowski, 2006), a seasonal cycle of snail density associated with a reduced parasite embryonation period may be a plausible reason for the increased parasite infections in summer detected in host fish from the present study. More snails mean more prey would be available for the definitive host (*C. gariepinus*) and if metacercariae intensities are high in snails then the intensity in the definitive host would be greater. If the life cycles of *M. nkomatiensis* and *G. pedatum* involves metacercarial stages in the snails, then infection of fish hosts would be through ingestion of infected snails where the metacercariae encyst. Studies on life stages of

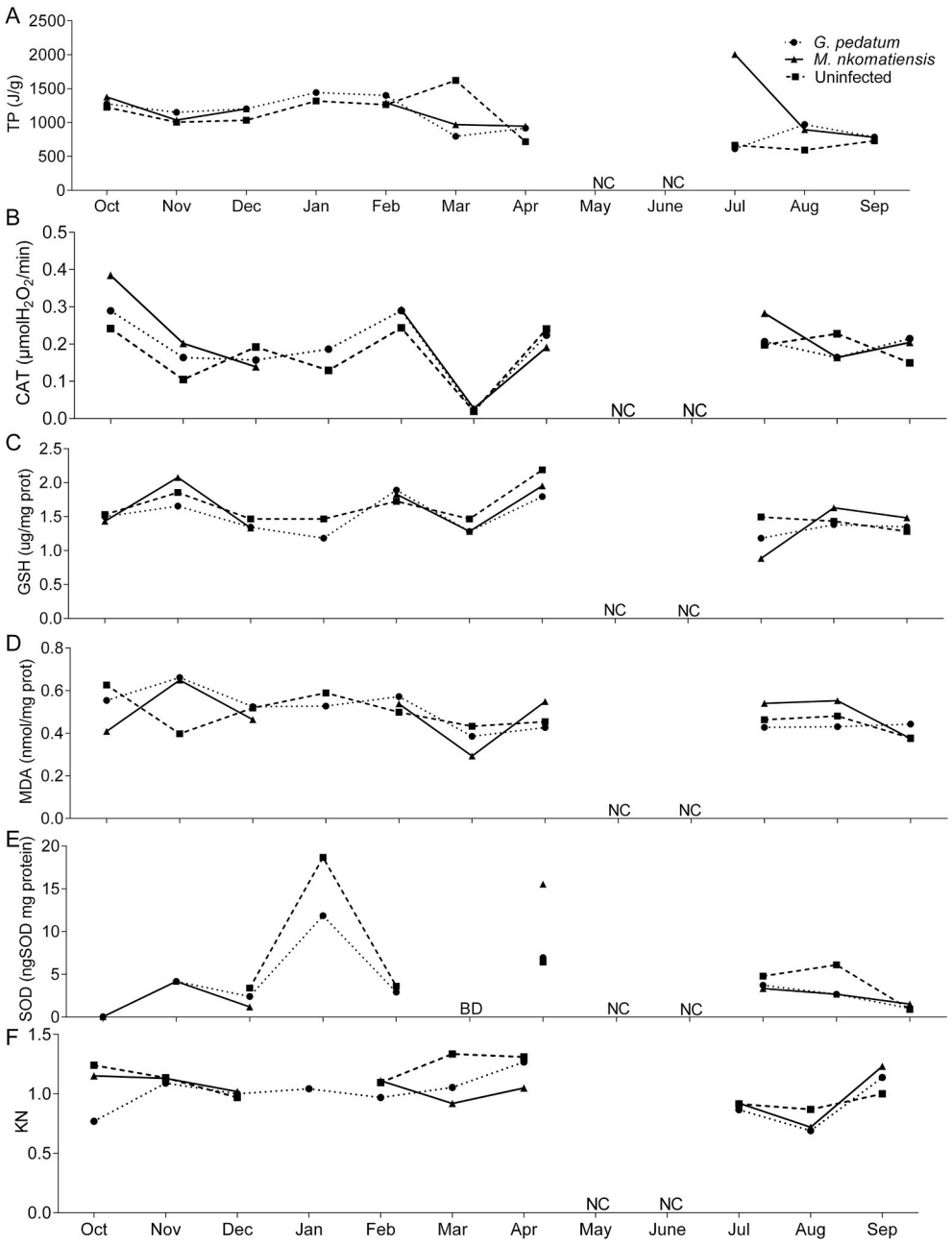

Fig. 4. Monthly variation of stress biomarkers measured in the liver of *Clarias gariepinus* and body condition. A– Total protein (TP); B– Catalase (CAT); C– Glutathione reduced (GSH); D– Lipid peroxidation (LPX); E– Superoxide dismutase (SOD); F– body condition (KN). NC – no collection was performed; BD - below detection.

trematodes, such as miracidia, cercariae and metacercariae, have shown that an increasing temperature may act as a trigger for initiating parasite egg hatchability, maturation of developmental stages of trematodes and infectivity (Morley and Lewis, 2015, 2017), and this should increase the parasites success to encounter their hosts.

The insignificant relationship between parasite intensity and host sex is likely due to the similarity in habitat and feeding preferences, and resulting behaviour between male and female *C. gariepinus*. Biu and Akorede (2013) and Kawe et al. (2016) recorded sex bias in cestodes and nematodes in *C. gariepinus*. Though, there are contrasting reports in this regard as some authors have observed higher infection intensity for nematodes and cestodes in male *C. gariepinus* as compared to females (Akinsanya and Otubanjo, 2006; Olofintoye, 2006; Aliyu and Solomon, 2012) and others indicate a greater susceptibility of female catfish for digeneans, nematodes and acanthocephalans (Emre, 2000; Uzodinma et al., 2016). Regarding host size, the finding of the present study contradict those of Mwita (2014) and Kawe et al. (2016). Inconsistencies of the effect of host size on infection indices may be attributed to factors related to host species (intermediates and definitive hosts), parasite species, infective stage and water quality conditions (Gautam et al., 2018).

Studies of relationships between infection and body condition have shown contrasting results. For instance, Lagrue and Poulin (2015) obtained a positive correlation between digenean parasite load and body condition in *Gobiomorphus cotidianus* McDowall, 1975. Conversely, Özer et al. (2016) reported an opposite pattern in *Mugil cephalus* Linnaeus, 1758 suffering from infections by monogeneans, digeneans and acanthocephalans; whereas in the present study, the correlation between body condition and digenean infections/intensities was insignificant. Inconclusive data on this matter is likely due to direct interactions

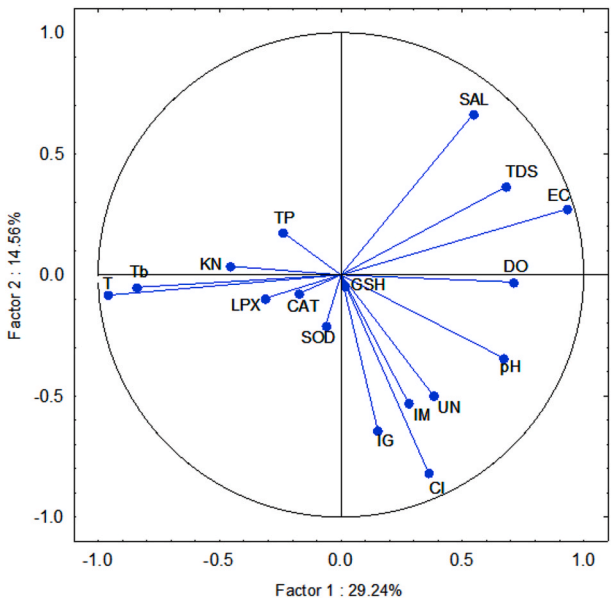

Fig. 5. Principal Component Analysis (PCA) of physico-chemical variables, biomarkers and parasitism in *Clarias gariepinus* collected in the Incomati River in Mozambique. Two principal components (PC1 and PC2) explained 45.45% of the total variation between water variables, biomarkers and occurrence of parasites. The EC, TDS and salinity (SAL) are associated with Component 1 while LPX, CAT, SOD, turbidity (TB) and temperature (T) are negatively associated with these variables. CI = co-infection; IM = *M. nkomatiensis* intensity; IG = *G. pedatum* intensity, UN = uninfected.

between hosts and parasites, as well as, external factors affecting both host condition and parasite infection independently. Such complex interactions are therefore the product of multiple mechanisms operating simultaneously in different directions (Beldomenico and Begon, 2010).

Insignificant difference in the levels of biomarkers between the uninfected fish and the *M. nkomatiensis*-infected fish and *G. pedatum*-infected fish, suggests that the biomarkers in the liver assessed here were poorly associated with infections. The PCA biplot also indicated that regardless of whether fish were uninfected, infected by single parasite species or co-infected, biomarker concentrations were not associated with infections. This corroborates with findings of Belló et al. (2000) and Nabi et al. (2017) for CAT, SOD, TP and LPX for digenean-infected fish and uninfected fish. The absence of effect from parasite infections on oxidative stress biomarker levels and body condition is also supported by two incidents in the present study: (i) unchanged levels of biomarkers despite increased intensity for both parasites in February and (ii) increased levels of biomarkers when parasite intensity decreased from March to April. This finding corroborates with Chambers et al. (2001) who argued that even though high intensity of digenetic trematodes may be present in the fish, they cause negligible and no overt change due to their small size, limited mobility (do not create permanent feeding scars) and do not feed deeply on host tissue.

Expression of oxidative stress biomarkers in host liver and muscle were mostly associated with host attributes and water quality. The positive correlation between host size and total protein is in line with Kurhaluk (2019) who demonstrated that a significant increase in lipid peroxidation lead to the release of free radicals which reacted with proteins and resulted in increased levels of total proteins in liver tissue during aging in *Salmo trutta* m. *trutta* Linnaeus, 1758. A negative correlation between LPX levels in *C. gariepinus* liver samples and body condition suggest that fish with better body condition have lower levels of lipid peroxidation in the liver.

Environmental temperature is considered an important factor in the biology of fishes and the rate of biochemical and physiological functions (Singh et al., 2013). Changes in water temperature, and consequently the DO levels can cause stress in wild fish and result in a misbalance between ROS production and elimination (Lushchak, 2011). For instance, exposure of organisms to hypoxia or hyperoxia can lead to increased generation of ROS (Lushchak, 2011). In the present study, a negative association between DO concentration and TP level corroborates with Lushchak et al. (2001), who observed an increase of TP levels under hypoxic conditions (0.04–3.91 mg O_2 L^{-1}) in the liver and brain of *Carassius auratus* (Linnaeus, 1758). The mechanisms of hypoxia-induced oxidative stress have not been established yet, however, two hypotheses are suggested to explain it; reduction of the carriers in electron-transport chains and the oxidation of the xanthine reductase/xanthine oxidase system (Lushchak, 2011). Elevated temperature stimulates metabolic processes and often induce oxidative stress and antioxidant response in ectothermic organisms (Bagnyukova et al., 2007). On the other hand temperature decrease weakens ROS elimination systems, and/or enhances ROS production, but the mechanisms involved are not well known (Lushchak, 2011). The positive association between TP and temperature indicates that the increase of TP level is a physiological response to compensate for increased temperature, therefore contributing to neutralize heat-induced oxidative stress. This reasoning is supported by Madeira et al. (2013) who claimed that exposure to high temperatures might alter the oxidative stress biomarkers.

5. Conclusions

The water parameters measured showed seasonal fluctuations. Intensity of *M. nkomatiensis* and prevalence of *G. pedatum* were related to EC and TDS, and salinity compared to the latter species. This finding supports the importance of water quality parameters as drivers of seasonal variation in digenean infections. The host body condition, size and sex were not related to the mean intensity of either parasite. However, high intensity of the two digeneans studied here may be linked to the effect of season (summer) on the density of intermediate hosts. Increased temperature during summer could lead to an increase in the density of infected snails and therefore an increase in possible prey items for *C. gariepinus*. The effect of the digenean infections, host sex and size on oxidative stress biomarkers and body condition of *C. gariepinus* is negligible. However, the correlations between dissolved oxygen concentration, temperature and total protein and body condition, body condition and lipid peroxidation were significant; this may serve as a physiological mechanism against increases in free radical levels, therefore, preventing cellular damage.

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Author contribution

JCD Conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft preparation, funding acquisition.

BMG Methodology, data curation, writing – reviewing and editing, visualization.

AAO Conceptualization, formal analysis, resources, writing – reviewing and editing, visualization, supervision, project administration, funding acquisition, final approval of the version to be submitted.

Declaration of competing interest

None.

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