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An assessment of the influence of multiple stressors on the Vaal River, South Africa

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ABSTRACT

The Vaal River is situated in the mining and industrial heartland of South Africa. It is regarded as a "work horse" river in South Africa and as a consequence it receives treated waste water from the largest metropolitan area in South Africa. It is only with the more frequent occurance of fish kills in the Vaal Barrage area during the past few years that public attention has been drawn towards the decreasing water quality and subsequent deterioration in the aquatic health of the Vaal River system. The aim of this study was to apply a multi-metric approach to assessing the risk of the multiple stressors to fish populations of the Vaal River system. A relative risk assessment approach was applied to divide the Vaal River Barrage into four risk regions. Field sampling was undertaken to validate the predicted risks in each region. The sampling included abiotic (i.e. water and sediment quality) and biotic (fish components) assessment. General water quality parameters (pH, conductivity, dissolved oxygen) together with nutrient, bacteriological and metal concentrations were measured in the four regions. Sediment quality was determined through physical (particle size distribution) and chemical (metal and organic pollutant) analyses. The fish assessment was undertaken at different levels of biological organisation ranging from biomarkers at subcellular levels (cytochrome P450-EROD, metallothionein, acetycholine esterase, antioxidant enzymes, cellular energy), tissue (histopathology), whole organism (fish health index), population and community level. These biological responses were related to environmental exposure through bioaccumulation analyses of metals and organic pollutants in fish tissues. Multivariate statistical analyses were applied to integrate the environmental exposure and effects. The results indicated that those regions that were predicted to be at greatest risk to exposure of multiple stressors did indeed display the greatest disturbance in fish community structures. This was related to decreased fish health as demonstrated by increased oxidative stress due to exposure to metals such as copper and nickel as well as organic pollutants such as PCBs, HCHs and bromated flame retardants. This study clearly demonstrates the importance of the inclusion of higher tier assessment endpoints to elucidate the effects of multiple stressors in aquatic ecosystems. The study further allowed for the identification of specific effect endpoints that need to be included in future monitoring programmes such as viral immunoassays.

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1. Introduction

The Vaal River has been described as one of Africa's work horse rivers (Braune, 1986). The Vaal River ecosystem is not only of immense value to Gauteng it is also pivotal in providing water to surrounding water management areas and as a result the demands on the Vaal River system are envisaged to increase in the future (DWAF, 2004). Of the 1300 km-long Vaal River, the Vaal Barrage region is regarded as the hardest-working region river in South Africa (Tempelhoff, 2009). The 63 km from the Vaal Dam to the Barrage constitutes less than 5% of the total catchment but 10 million people reside in this catchment and the run-off water of three large metropolitan cities, some 13,600 wet industries and a number of gold mines flow into the Vaal River between the wall of the Vaal Dam and the Barrage (McCarthy et al., 2007; Tempelhoff, 2009).

Due to continued degradation and pollution of the main tributaries such as the Klip (McCarthy and Venter, 2006; McCarthy et al., 2007), Blesbokspruit (Roychoudhury and Starke, 2006) and Rietspruit (Steynberg et al., 1995; Lukhele, 2002), the water quality of the Vaal Barrage is seriously impacted (CDE-BLSA, 2010). The deteriorating water quality of the Vaal Barrage resulted in an increasing number of mass fish mortalities; particularly yellow fish, occurring in the region of the Barrage (Tempelhoff, 2009). Although degraded water quality conditions continue to pose the greatest threat to fish health in this system (de Villiers, 2007), additional impacts such as habitat alteration, flow regime modifications, barriers for migration, disturbance to wildlife and or the impact of non-endemic alien or introduced fishes may be affecting the fish communities in the Vaal River.

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The use of fish and the attributes of fishes have long been applied to assess the integrity or ecological state of and/or identify impacts affecting freshwater aquatic ecosystems (Barbour et al., 1999). Initially fish community attributes were used to assess ecological integrity of stressed ecosystems (Karr, 1981). The notion of using fish endpoints at different levels of biological organisation ranging from subcellular to ecosystem is now firmly entrenched in ecological risk assessment (Adams, 2001; van der Oost et al., 2003). Many studies have used a combination of endpoints to link exposures with effects e.g. at lower levels of biological organisation studies using subcellular biomarkers with metal bioaccumulation (Knapen et al., 2009; Wepener et al., 2005) and subcellular biomarkers and condition indices with metal and organic bioaccumulation (Linde-Arias et al., 2008; Bervoets et al., 2009). Linking of higher tier organisation with pollutant exposure has been achieved using histopathology with metals and organic pollutants (Poleksic et al., 2009; van Dyk et al., 2009a), fish health with water quality changes (Crafford and Avenant-Oldewage, 2009), biological traits and water quality (Benejam et al., 2010) and fish communities and ecological traits associated with water quality changes (Martinho et al., 2008; O'Brien et al., 2009). In order to integrate the biological exposure results with biological effects, multivariate statistical techniques are commonly applied (Wepener et al., 2005, 2008). This causal relationship between the exposure and effect provides the basis for the application of these techniques within an ecological risk assessment framework (van der Oost et al., 2003; Wepener, 2008).

The aim of this study was therefore to determine the risk that fish are exposed to in selected regions of the Vaal Barrage using a suite of biomarkers representing the spectrum of biological organisation as proposed by Wepener (2008). The purpose of this paper was not to compare and expand on spatial differences in bioaccumulation and biomarker responses but rather to apply multivariate procedures to determine whether a relationship could be detected between water quality, sediment quality, pollutant exposure (bioaccumulation) and the concomitant responses of fish.

2. Materials and methods

2.1. Description of the study area

The study area is presented in Fig. 1 and includes the Vaal River and its associated riparian ecosystem from, and including the lower portion of the Vaal Dam to below the Vaal Barrage. Five important tributaries of the Vaal River enter the study area within this reach and are considered as individual sources of stressors contributing to the risks within the study area. Four risk regions (A–D) were selected as sub-areas in this study based on the location of barriers and taking into consideration the sources of stressors that may affect the study area.

2.1.1. Risk region A

Risk region A includes the reach of the Vaal River below the Vaal Barrage. In this section the Vaal Barrage is an important barrier that acts as a geographical barrier affecting upstream movement of aquatic organisms. This lotic reach of the Vaal River is dominated by fast flowing riffle and rapid habitats that are separated by large pools, glides and backwater areas. Sources of pollutants within this reach include informal and limited formal settlements, some agriculture activities and recreation activities.

2.1.2. Risk region B

Risk region B includes the Vaal Barrage and Loch Vaal upstream to above the confluence of the Vaal River and Taaibosspruit. Within this risk region the Vaal Barrage, that acts as a dam wall, has created a large lentic ecosystem consisting of slow moving deep pool areas. Four tributaries of the Vaal River including the Klein Rietspruit and Rietspruit, Leeuspruit and Taaibosspruit enter the Vaal River in this reach and contain numerous types of water quality related stressors. In addition, the urban areas of Vanderbijlpark and Sasolburg occur within this risk region. These urban areas contain heavy and light industries, waste water treatment works, informal and formal/urban settlements, recreational activities and golf courses. Through the tributaries entering this risk region this area is further exposed to water quality related impacts from heavy industries and mining operations in the catchment.

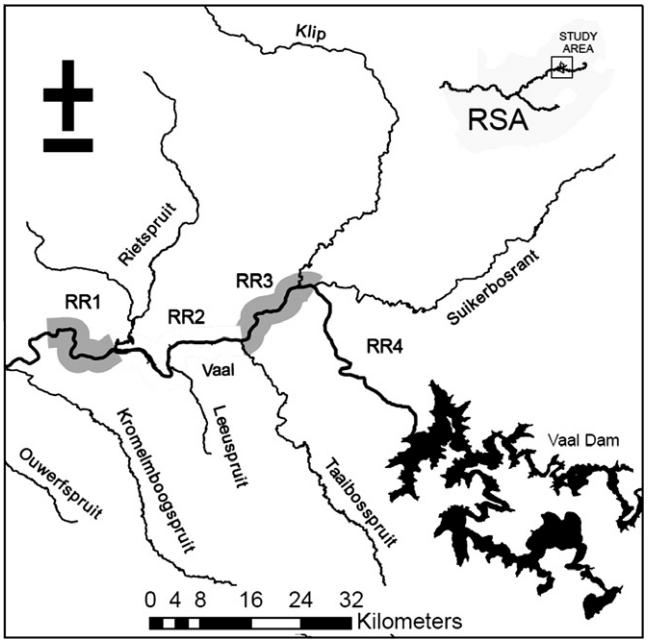

Fig. 1. Diagram of the study area presenting the four risk regions selected for assessment in this study. Tributaries of the Vaal River that were considered to be delivering stressors into the study area are indicated as arrows.

2.1.3. Risk region C

Risk region C includes the Vaal River from the Taaibosspruit confluence to above the confluence with the Klip and Suikerbosrant Rivers. These are sources of water quality related impacts arising from mine, heavy industry and waste water treatment works. A small portion of the urban areas of the town of Vereeniging extends into this risk region resulting in local sources including industries, waste water treatment works, storm water runoff, informal and formal/urban settlements and recreational activities. This reach contains lotic and lentic habitat types but is dominated by slow flowing glides and pool areas.

2.1.4. Risk region D

Risk region D extends from above the confluence of the Vaal and the Suikerbosrant Rivers to and including a portion of the Vaal Dam. The Vaal Dam wall is an important geographical barrier within this risk region that restricts the movement of aquatic organisms. In addition the Vaal Dam causes flows to be regulated affecting the timing, durations and volumes of flows. Important sources of stressors include a water abstraction point for the water utility, Rand Water, some informal and formal settlements and agricultural activities. Diverse habitat types occur within this reach, i.e. rapids and riffle habitats with pools and glides.

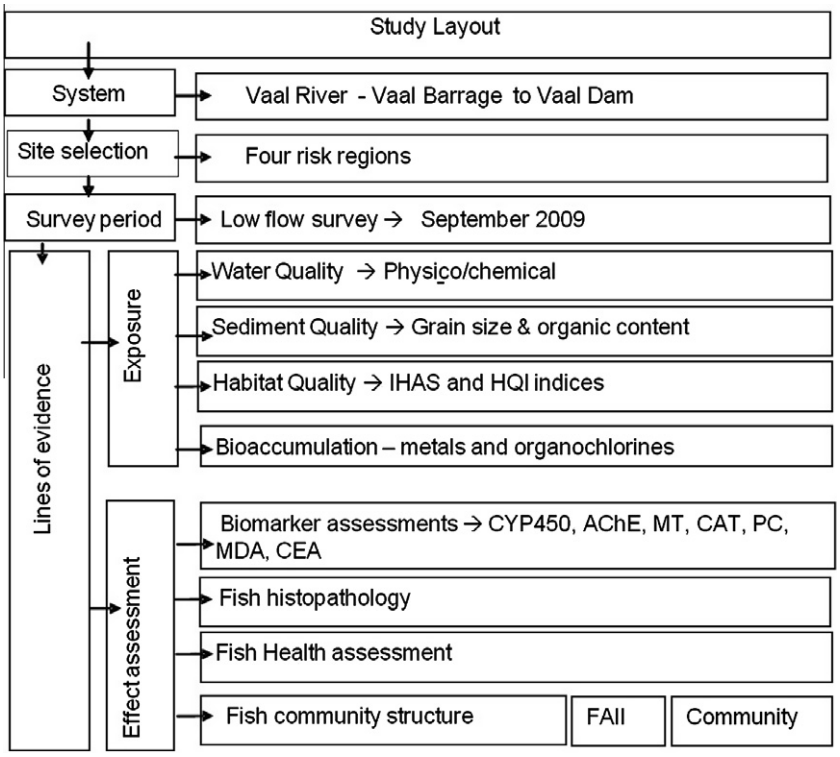

Fig. 2. Study design and lines of evidence used in the low flow survey.

2.2. Study design

To determine the risks posed to fish associated with each of the four risk regions, a number of abiotic and biotic endpoints or lines of evidence (LoEs) were selected for this study (Fig. 2). The LoE related to exposure assessment were: water and sediment quality and bioaccumulation as indication of fish exposure to metals and organic pollutants in the four risk regions. The effects assessment LoEs were based on subcellular biomarker responses, histopathological alterations, whole fish health assessment and fish community assessment.

2.3. Exposure assessment lines of evidence

2.3.1. Water quality

Water samples were collected in acid-washed polyethylene bottles from each of the study sites. The polyethylene bottles were rinsed with water from the site before a sample was taken and after collection, these samples were stored at −4 °C until further analysis. Along with the collection of the samples, *in situ* measurements of the following variables were taken using a WTW 340i Multi meter: oxygen saturation (%), dissolved oxygen concentration (mg/l), temperature (°C), pH and electrical conductivity (μS/cm). The frozen samples were transported to the laboratory where they were allowed to defrost. Additional chemical variables were analysed at an independent accredited laboratory. The variables analysed were: chemical oxygen demand (COD), redox potential, total alkalinity, ammonium, chloride, nitrates, sulphates, total phosphates, fluorides and phenols.

2.3.2. Sediment quality

All the techniques applied in this study are based on the standard protocols of the United States Environmental Protection Agency (USEPA, 2001). Surface sediment samples were collected at each site during each survey using a Petit Ponar grab. Sediment was collected in polyethylene jars and was frozen to prevent the loss of organic material through the digestion by invertebrate fauna or organic decomposition. In the laboratory the sediment samples were allowed to thaw before determining the moisture and organic carbon content according to methods described by Wepener and Vermeulen (2005). The percentage particle size distribution was determined by sieving dried sediment in an Endecott EFL 2000/1 sieve system with sieves ranging from >4000 μm to 53 μm. Most of the substrate consists largely of clay (very fine sand and mud) and the content of the final two sieves were thus combined. The following grain size categories were applied in this study (Wepener and Vermeulen, 2005): gravel (>4000 μm), very course sand (4000–2000 μm), course sand (2000–500 μm), medium sand (500–212 μm), fine sand (212–53 μm) and mud (<53 μm).

2.3.3. Metal and organic bioaccumulation

Specimens of the Orange River mudfish, *Labeo capensis*, were collected from the four risk regions (RR) in the Vaal River (RRA $n = 20$) (RRB $n = 6$) (RRC $n = 14$) and (RRD $n = 20$) using gill nets. Following capture fish were transported to a field laboratory and kept in aerated portable ponds pending analysis. For bioaccumulation studies the fish were sacrificed by severing the spinal cord and liver and muscle tissue were excised, transferred to pre-cleaned polyethylene tubes and frozen at −20 °C pending metal analyses. Muscle samples were also collected for organic analyses and placed in aluminium wrap prior to freezing at −20 °C.

2.3.3.1. Metal analyses. Tissue samples were thawed and dried at 60 °C for 48 h and were digested using HNO_3/H_2O_2 and microwave digestion (Blust et al., 1988). The digested samples were then diluted in 1% HNO_3 (AR) with Mili-Q water and metals were determined using a Thermo inductive coupled optical emission spectrophotometer (ICP-OES) and an inductive coupled plasma mass spectrophotometer (ICP-MS). All samples were analysed for

Table 1
Mean recoveries for metals from Standard Reference Material (SRM).

Element	BCR 278 mussel tissue (μg/g)	Recovery (μg/g)
Cd	0.35 ± 0.01	0.36 ± 0.02
Cr	0.78 ± 0.06	0.76 ± 0.04
Cu	9.45 ± 0.13	9.58 ± 0.12
Mn	7.69 ± 0.23	7.65 ± 0.13
Pb	2.00 ± 0.04	2.0 ± 0.10
Zn	83.1 ± 1.7	79 ± 0.2

cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn).

Yttrium was used as an internal standard to correct for interference from high-dissolved solids in the different matrices. Concentrations were expressed on a dry weight basis. Quality control of metal measurements in sediment and mussel tissue was verified by including process blanks and certified reference material (CRM 278, mussel tissue Community Bureau of Reference, Geel, Belgium). All recoveries were within 10% of the certified value (Table 1).

2.3.3.2. Organic analyses. Analyses were undertaken in pooled muscle samples with five replicates from each risk region.

Sample preparation – fish tissue: Dried fish muscle (typically between 1 and 2 g) was accurately weighted into an extraction thimble and spiked with internal standards (20 ng CB 143, 4 ng ε-HCH and 4 ng BDE 77). Samples were extracted for 2 h by hot Soxhlet with 100 ml mixture of acetone/hexane (1/3, *v*/*v*). The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% *w*/*w*), from which pollutants were eluted with 20 ml hexane and 15 ml DCM. The eluate was evaporated to dryness and redissolved in 100 μl iso-octane (Voorspoels et al. 2003; Covaci et al. 2008). Minor adaptations were required as PCBs, HCB and DDTs were analysed by GC-EI/MS, while PBDEs, HCHs and CHLs were analysed by GC-ECNI/MS.

GC analysis: The determination of PBDEs, HCHs and CHLs was performed with an Agilent 6890GC-5973MS equipped with a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column and operated in electron capture negative ionisation (ECNI) mode. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. The electron multiplier voltage was set at 2200 V. The MS was operated in SIM mode (*m*/*z* 79 and 81 were monitored for the entire run, and two specific ions for OCPs). Dwell times were set to 40 ms. Helium was used as carrier gas at constant flow (1.0 mL/min), while methane as moderating gas. One microliter of the extract was injected in cold pulsed splitless (initial injector temperature at 90 °C, stay 0.03 min, then heated at 700 °C/min to 300 °C, pressure pulse 25 psi, pulse time 1.50 min, splitless time 1.50 min). The temperature of the DB-5 column was programmed from 90 °C, kept for 1.5 min, then increased with 15 °C/min to 300 °C, kept for 15 min.

For the analysis of PCBs, HCB and DDTs, extracts were injected into a GC/MS operated in electron ionisation (EI) mode and equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The mass spectrometer was used in SIM mode with the two most intense ions (typically from the molecular cluster) acquired for each homologue group or isomer. One μL of the extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C at 700 °C/min, pressure pulse 25 psi, pulse time 1.50 min, splitless time 1.50 min). Helium was used as carrier gas at constant flow (1 mL/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, holding for 20 min.

Quality Assurance and Quality Control: was performed through the analysis of procedural blanks, and a Standard Reference Material (SRM 1945, OCPs, PCBs, and PBDEs in whale blubber). For the replicate and SRM 1945, the relative standard deviations (RSDs) were <10% for most analytes. Additionally, the method performance was assessed through successful participation to inter laboratory studies organised by the National Institute for Standards and Technology (NIST) (OCPs, PCBs and PBDEs in biological tissues). Procedural blanks were consistent (RSDs < 20%) and therefore the mean value of each analyte in the procedural blanks was used for subtraction. Method quantification limits (LOQs) for individual OCPs, PCB and PBDE congeners were based on procedural blanks (3× SD) and the amount of sample taken for analysis.

2.4. Effects assessment lines of evidence

2.4.1. Biomarkers

Liver samples were collected and immediately frozen in liquid nitrogen in vials containing Henrikson's stabilising buffer (Wepener et al., 2005). In the laboratory all samples were transferred to a −80 °C freezer pending analysis. Samples were homogenised using the appropriate volume and buffer for each biomarker. All biomarker activities are expressed per mg of protein in the sample. Protein content was determined according to the method of Bradford (1976).

Biomarkers of exposure were used during this study. Acetylcholine esterase (AChE) was measured using the protocol of Ellman et al. (1961), while the ethoxyresorufin-o-deethylase (EROD) was analysed using the direct flourometric method of Burke and Mayer (1974). Metallothionein (MT levels were determined according to a modified procedure of Viarengo et al. (1997).

Biomarkers of effect involved the determination of the influence of reactive oxygen species through the measurement of enzymatic and non-enzymatic indicators of antioxidant activity, as well as the cellular energy budgets in liver tissue of *L. capensis*. The following antioxidant biomarkers were analysed during this study: catalase (CAT) using the method of Cohen et al. (1970), malondialdehyde (MDA) that measures the lipid peroxidation through the formation of thiobarbituric acid reactive substances (Ohkawa et al., 1979) and protein carbonyl (PC) levels using the method described by Parvez and Raisuddin (2005). Cellular energy allocation (CEA) is a biomarker of available cellular reserves and was adapted from the technique described by De Coen and Janssen (1997) with some minor changes. Muscle tissue samples were homogenised on ice in 1 ml of ice-cold distilled water. The energy reserves (E_a) determined were carbohydrate, lipid, and protein content in muscle samples. Carbohydrate content was determined using a glucose test kit (GOD-PAP1 448 668, Roche) and glucose standard (C-FAS 759 350; Roche), total lipids were extracted following the method of Bligh and Dyer (1959) and protein content was determined using Bradford's reagent (Bradford, 1976). The energy consumption (E_c) or cellular respiration rate was determined by measuring the electron transport system activity (De Coen and Janssen, 1997). The different energy reserves (E_a) were transformed into energetic equivalents using the enthalpy of combustion values used by De Coen and Janssen (1997). And the total energy budget was calculated using the equation:

$$\mathrm{CEA} = E_a - E_c,$$

where $E_a = E_{\mathrm{carbohydrate}} + E_{\mathrm{lipid}} + E_{\mathrm{protein}}$ and $E_c = E_{\mathrm{ETS}}$.

2.4.2. Histopathology

Concurrent with the tissue sampling for bioaccumulation analyses, tissue samples of the selected target organs were sampled for

histopathology and processed using standard techniques. The samples were prepared for light microscopy analysis using Haematoxylin and Eosin staining. A qualitative histological analysis was done to identify any histological alterations. These results were subsequently quantified using a histological quantification index protocol (van Dyk et al., 2009b). Each organ was assessed in terms of five reaction patterns including circulatory disturbances, regressive changes, progressive changes, inflammatory responses, and neoplasms. For each organ, an organ index was calculated. This index is indicative of the histological response in the respective tissue type. The sum of the four organ indices (Liver Index (I^L), Gill Index (I^G), Kidney Index (I^K) and Testis Index (I^T) or Ovary Index (I^O)) per fish yielded a Fish Index (I^{FISH}) value. This index indicates the combined histological response of the sampled organs per fish. Due to the different sample sizes per sample group, mean index values were calculated and are thus presented for comparative purposes.

2.4.3. Fish health assessment index

The methodology described by Heath et al. (2003) was followed. The fishes were transferred into a small pool supplied with water from the river through a centrifugal pump. Fish were killed with a cut through the spinal cord and an autopsy was performed in a field laboratory. Each fish was investigated in accordance with the methodology prescribed in the manual for the HAI (Heath et al., 2003). Gills and intestines were removed from the fish, placed in saline and investigated with the aid of a dissection microscope. Blood was drawn from the dorsal aorta into heparinised blood vials. Samples were placed on ice prior to analyses in the laboratory. Blood values were obtained through analyses with a Sysmex microcell counter CC120 and plasma concentration with an EL_X 800 BIO-TEK INSTRUMENTS automated plate reader. The condition factor was calculated for each fish according to the formula (weight $\times$ 10^5/length^3) of Carlander (1969).

2.4.4. Fish community assessment

The diversity and abundances of fish were determined by using the following range of sampling techniques:

- *Netting techniques*: Gill nets with 25 m segments consisting of 30, 47, 58, 90, 120 and 150 mm mesh sizes as well as fyke nets of two sizes (small net with two 700 mm opening traps separated by a 10 m wing and a large net with a 1200 mm opening to a single trap containing three 25 m guiding wings) where used to sample deep pools and deep areas of the Vaal River and the Vaal Dam and Vaal Barrage.
- *Electrofishing*: A standard SAMUS battery pack or generator operated electrofisher was used in the shallow wade-able areas of the Vaal River.
- *Angling*: Angling techniques were used in selected area to target large predatory yellowfishes and bass as well as carp.

The fish diversity and abundance data were recorded in the field and voucher photographs and if deemed necessary specimens where collected to later be sent to the South African Institute for Aquatic Biodiversity.

2.4.4.1. Habitat assessment. In accordance with the Fish Response Assessment Index (FRAI) methodology (Kleynhans, 2005) the habitat assessment for this portion of the study refers to an evaluation of fish habitat potential at a site in terms of the diversity of velocity-depth classes present and the presence of various cover types within each of these velocity–depth classes. This provides a framework within which the presence, absence and frequency of occurrence of species can be interpreted. The habitat assessment includes a general consideration of impacts that may influence the condition or integrity of fish habitat at a site (Kleynhans, 2005).

2.4.4.2. Fish Response Assessment Index (FRAI). In order to establish the present ecological state of the riverine or lotic sites assessed in this study, the ecological category of the fish community was determined by applying the FRAI methodology (Fish Response Assessment Index) using desktop and field survey data (Kleynhans, 2005; Kleynhans et al., 2007).

2.5. Statistical analyses

The graphical presentations were performed using the GraphPad Prism software and data are reported as mean + SE (standard error of the mean). The variations in each assessment endpoint were tested by one-way analysis of variance (ANOVA), considering sites as variables. Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively. When the ANOVA revealed significant differences, post-hoc multiple comparisons between sites were made using the appropriate Scheffé (parametric) or Dunnette-T3 (non-parametric) test to determine which values differed significantly. The significance of results was ascertained at $p < 0.05$ (Zar, 1996).

In this study Principle Component Analysis (PCA) (Canoco for Windows Version 4.53) was used to assess the spatial patterns associated with water and sediment quality, bioaccumulation in fish tissue, biomarker responses and fish community structures (Ter Braak and Smilauer, 2004). The PCA is based on a linear response model relating species and environmental variables (van den Brink et al., 2003). Results of the ordination are a map of the samples being analysed on a two dimensional basis, where the placements of the samples reflect the dissimilarities or similarities between the samples; in this case the sampling sites. To determine which factors were responsible for the structure or groupings obtained in the PCA a redundancy analysis (RDA) assessment was carried out. An RDA is a derivative of a PCA with one additional feature which allows for the selection of the driving variables which are intended to be overlaid onto the PCA. The values entered into the RDA analysis are not the original data but the best-fit values estimated from a multiple linear regression between each variable in turn and a second matrix of complementary biological or environmental data. The RDA plots are interpreted through 2-dimentional bi-plots that present the similarities or dissimilarities between the samples analysed (Shaw, 2003).

3. Results

3.1. Water and sediment quality

The water and sediment quality results collected from the four risk regions are presented in Tables 2 and 3. The data were subjected to PCA ordination and Fig. 3 presents an integrated spatial ordination of the environmental variables. The biplot explains 95.7% of the variance in the data on the two axes. There are distinct spatial differences in the water and the sediment quality during the survey with RRB and RRC displaying more similar quality characteristics than the other two regions. These two risk regions were characterised by increased nutrient and bacterial levels with concomitant finer substrate composition with very high organic carbon content. Risk region D was characterised by increased phenol concentrations and chemical oxygen demand while the sediment grain size was dominated by gravel and course sand. The water and sediment quality of RRD was distinctly different to the other three regions and generally had lower levels of nutrients and salts with substrate consisting of fine sand.

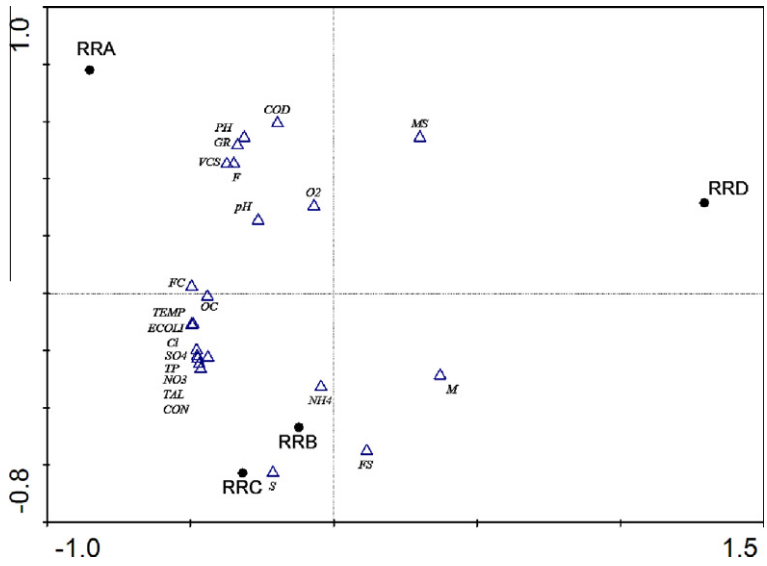

Fig. 3. PCA biplot of the water and sediment quality variables measured at the four risk regions. The ordination explains 95.7% of the variance in the data with 62.7% and 32% on the first and second axes respectively. Refer to Tables 2 and 3 for an explanation of the abbreviations.

Table 2
Water and sediment quality variables analysed at the four risk regions in the Vaal Barrage. The codes for each variable are used in the ordination diagrams.

	Code	RRA	RRB	RRC	RRD
Water quality variable					
Ammonium (mg/l)	NH4	0.1	0.2	0.1	0.1
Chemical oxygen demand (mg/l)	COD	143	80	95	100
Chloride (mg/l)	Cl	69	65	76	5
Dissolved oxygen (mg/l)	O2	9.83	10.56	5.72	8.03
Electrical conductivity (μS/cm)	CON	755	711	787	178
E. coli (cfu/100 ml)	ECOLI	19.7	8.6	18.5	1
Faecal coliforms (cfu/100 ml)	FC	5794	2613	4106	1046
Fluoride (mg/l)	F	0.5	0.3	0.2	0.2
Nitrate (mg/l)	NO3	2.5	2.5	2.8	0.2
pH	pH	9.14	9.09	7.88	8.08
Phenols (mg/l)	PH	0.1	0.01	0.01	0.01
Sulphate (mg/l)	SO4	149	142	141	31
Temperature (C)	TEMP	19	18.1	18.3	14.5
Total alkalinity (mg/l)	TAL	132	128	148	68
Total phosphate (mg/l)	TP	0.9	1.1	0.8	0.2
Sediment quality variable					
Organic carbon	OC	17.65	6.645	18.22	3.92
Gravel (%)	GR	33	0.51	0.22	0.07
Very course sand (%)	VCS	9.87	0.93	1.7	0.72
Course sand (%)	MS	19.49	5.3	5.99	46.17
Medium sand (%)	FS	30.65	66.45	45.08	42.36
Fine sand (%)	S	6.17	24.71	41.1	4.05
Mud (%)	M	0.54	1.51	5.43	6.51

Table 3
Mean ± standard error concentrations of metals in muscle (μg/g dry mass) and organic pollutants in muscle (ng/g lipid mass) tissue of *Labeo capensis* sampled in the four risk regions in the Vaal Barrage. Within rows concentrations with common superscript represent significant differences ($p < 0.05$) between risk regions.

	RRA	RRB	RRC	RRD
Cd	0.005 ± 0.001	0.004 ± 0.001	0.006 ± 0.002	0.004 ± 0.002
Cr	0.261 ± 0.03^{ab}	0.142 ± 0.01^{ab}	0.215 ± 0.017^{a}	0.209 ± 0.029^{b}
Cu	4.47 ± 0.99^{ab}	1.84 ± 0.28^{a}	1.78 ± 0.15^{b}	14.3 ± 0.17^{ab}
Ni	0.428 ± 0.065^{a}	0.517 ± 0.134^{ab}	0.423 ± 0.035^{b}	0.187 ± 0.032^{ab}
Pb	0.067 ± 0.027^{a}	0.025 ± 0.013^{ab}	0.063 ± 0.019^{b}	0.045 ± 0.035^{c}
Zn	16.31 ± 1.29^{a}	13.1 ± 0.98^{abc}	18.46 ± 1.46^{b}	14.67 ± 0.98^{c}
ΣPCBs	911 ± 181^{ab}	641 ± 193.6^{a}	371.8 ± 113.2^{ab}	583.3 ± 89.2^{b}
HCBs	8.5 ± 2.2^{abc}	135.4 ± 89^{a}	257 ± 204.6^{b}	588.3 ± 442.6^{c}
ΣDDTs	261.8 ± 67.3^{a}	227.7 ± 56.2^{b}	160.5 ± 47.1^{c}	468.1 ± 147.4^{abc}
OxC	2.5 ± 0.9	1.1 ± 0.5	<0.5	2.7 ± 0.7
TN	23.6 ± 7.1^{a}	15 ± 5.1^{b}	5.8 ± 1.8^{ab}	2.7 ± 0.7^{ab}
CN	9.8 ± 2.8^{a}	7.7 ± 2.8^{b}	2.6 ± 0.6^{abc}	9.8 ± 1.9^{c}
ΣHCHs	80.8 ± 9.8^{a}	70.1 ± 34.9^{b}	10.2 ± 0.9^{ab}	156 ± 29.6^{ab}
ΣPBDEs	43.4 ± 15.2^{a}	15.3 ± 5.2^{a}	5.9 ± 1^{a}	27.4 ± 11.3^{a}

PCB – polychlorinated biphenyls; HCB – hexachlorobenzenes, OxC – oxychlordane, TN – trans-nonachlor, CN – cis nonachlor, HCH – hexachlorocyclohexanes, and PBDE – polybrominated diphenylethers.

3.2. Metal and organic exposure in L. capensis

Metal and organic bioaccumulation in tissue of *L. capensis* was used as a surrogate for environmental exposure to these contaminants in the four risk regions (Table 3). Significant spatial differences were observed for all toxicants (Table 3) with three distinct bioaccumulation patterns evident (Fig. 4). Risk region C was characterised by a predominance of metal bioaccumulation, whilst RRAs A and D showed greater organochlorine bioaccumulation patterns. Risk region B displayed a mixture of organic and metal bioaccumulation. Risk region A displayed higher concentrations of PCBs, flame retardants (PBDEs) and nonachlor's, whereas RRD the DDTs, HCHs and HCBs predominated.

3.3. Biological effects in L. capensis

The biomarkers of exposure and effect are presented in Fig. 5A–C and D–G respectively. The AChE activities were significantly lower at RRA and B ($p < 0.05$) when compared to the other regions. The EROD activity was significantly ($p < 0.05$) higher at RRA while the MT levels were significantly higher at RRB and C (Fig. 5C). There were less significant spatial differences when comparing the biomarkers of effect with only the MDA (Fig. 5E) and PC levels (Fig. 5F) increasing significantly at RRA.

The histological assessment showed normal hepatic structure of most livers sampled. The cytoplasm of hepatocytes was mostly granular or clumped in appearance (Table 4). Melano-macrophage centers were a common feature in specimens from all sites (Fig. 6A). Steatosis (fatty change) was present in the majority of specimens from all sites (Fig. 6B). Intercellular deposits and nuclear alterations were observed in a number of specimens from all sites. The histological analyses also confirmed parasitic infections in specimens from all sites (Fig. 6C) and inflammatory responses

Table 4
Histological alterations and parasitic infections identified in the livers, gills and kidneys of *L. capensis* from the four risk regions. Results are presented as the percentage prevalence (%).

	RRA	RRB	RRC	RRD
Liver				
Structural alterations	15	0	0	0
Melano macrophages aggregates	100	83	100	100
Inflammatory response	15	0	0	10
Steatosis	50	67	64	65
Intra-cellular deposits	25	33	0	15
Nuclear alterations	0	0	64	5
Parasitic infections*	40	50	21	35
Gills				
Telangiectasia	15	50	43	25
Epithelial lifting	25	33	14	35
Pillar cell rupture	35	50	79	65
Epithelial hyperplasia	95	100	93	100
Vacuolated epithelial cells	25	0	0	15
Clubbing of secondary lamellae	75	67	79	50
Structural alterations	5	50	0	5
Parasitic infections*	100	100	100	100
Kidney				
Melano-macrophages aggregates	100	83	100	100
Hyaline droplet degeneration	90	83	93	90
Intra-cellular deposits	65	17	50	35
Parasitic infections*	70*	50*	43*	80*

* Parasitic infections per organ were assessed as 'present' or 'not present'. The number of infections per organ per fish was not quantified and is rather included as part of the HAI.

only observed in specimens from RRA and D (Fig. 6D and E). Epithelial hyperplasia, clubbing (Fig. 7A) of secondary gill lamellae, and telangiectasia (Fig. 7B) with the associated rupture of pillar cells were the most marked alterations identified in the gills of fish from all sites (Table 4). In some cases, the proliferation of the epithelial cells resulted in the fusion of the primary gill lamellae at the distal regions (Fig. 7C). Epithelial lifting was also identified in fish from all sites (Fig. 7D). The histological analyses also confirmed parasitic infections in specimens from all sites. The histological assessment of the kidneys showed a number of histological alterations in fish from all sites (Table 4). The most prominent of these were hyaline droplet degeneration (Fig. 8A) and Melano-macrophage centers (Fig. 8C). The histological analyses also confirmed parasitic infections in the kidneys of specimens from all sites (Fig. 8B). No histological alterations or abnormalities were identified in the gonads of the sampled fish. The histological analyses confirmed a sex ratio of 65% male and 35% female (RR A), 100% female (RR B), 7% male and 93% female (RR C), 55% male and 45% female (RR D). The gonads of all males and females were in the mature stages of gametogenesis.

The quantitative histological results (histological index values), HAI and CI values are presented in Table 5. The histological indices for the same organ varied slightly between the different risk regions and all organ indices fell within the range of 0–20. The highest liver, kidney and overall fish histopathology index values and thus by implication the most histological damage, were recorded at RRA. The lowest index values were found at RRC and D even though the highest gill index value was recorded in the latter risk region. The CI values were very similar in all four regions. The highest HAI scores were recorded at RRC while the healthiest fish (i.e. lowest HAI values) were sampled at RRA.

3.4. Fish community structure

Fourteen species were collected from the four risk regions (Table 6) and of all of the species expected to occur within the study area only two indigenous barbs namely the Chubbyhead barb (*Barbus anoplus*) and the Goldie barb (*B. pallidus*) were not collected. Four species are alien species that have the potential to impact on the indigenous fish community structures in the form of competition for food or predation (such as the Largemouth bass – *Micropterus salmoides*) or alter habitats indirectly affecting fish communities (such as the Common carp – *Cyprinus carpio*). Two of the species have conservation value namely the Vaal River rock catfish (*Austroglanis sclateri*) that belongs to the Bagridae family of which all species are protected in South Africa and the Largemouth yellowfish, a threatened and protected species. The FRAI assessment results indicate that the status of the fish community decreases from a B-class in RRD to a largely modified D-class in RRC and B. The ecological state recovers to a B/C (between a largely natural to moderately modified condition). A redundancy analysis plot (RDA) was constructed where the environmental conditions were superimposed on the diversity and abundance data. The ordination describes 90.3% of the variation in the data (Fig. 9). The hab-

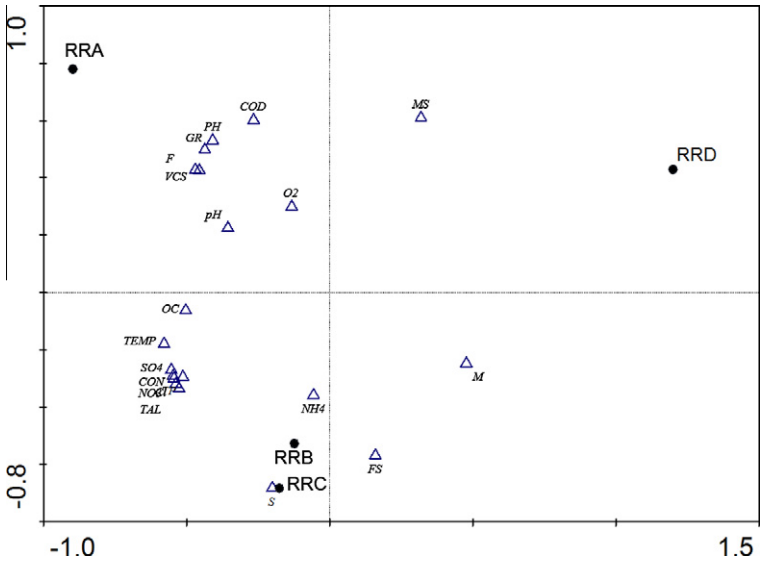

Fig. 4. PCA biplot of the metal and organic pollutant bioaccumulation in muscle tissue of *Labeo capensis* from the four risk regions. The ordination explains 94.8% of the variance in the data with 87.5% and 7.3% on the first and second axes respectively.

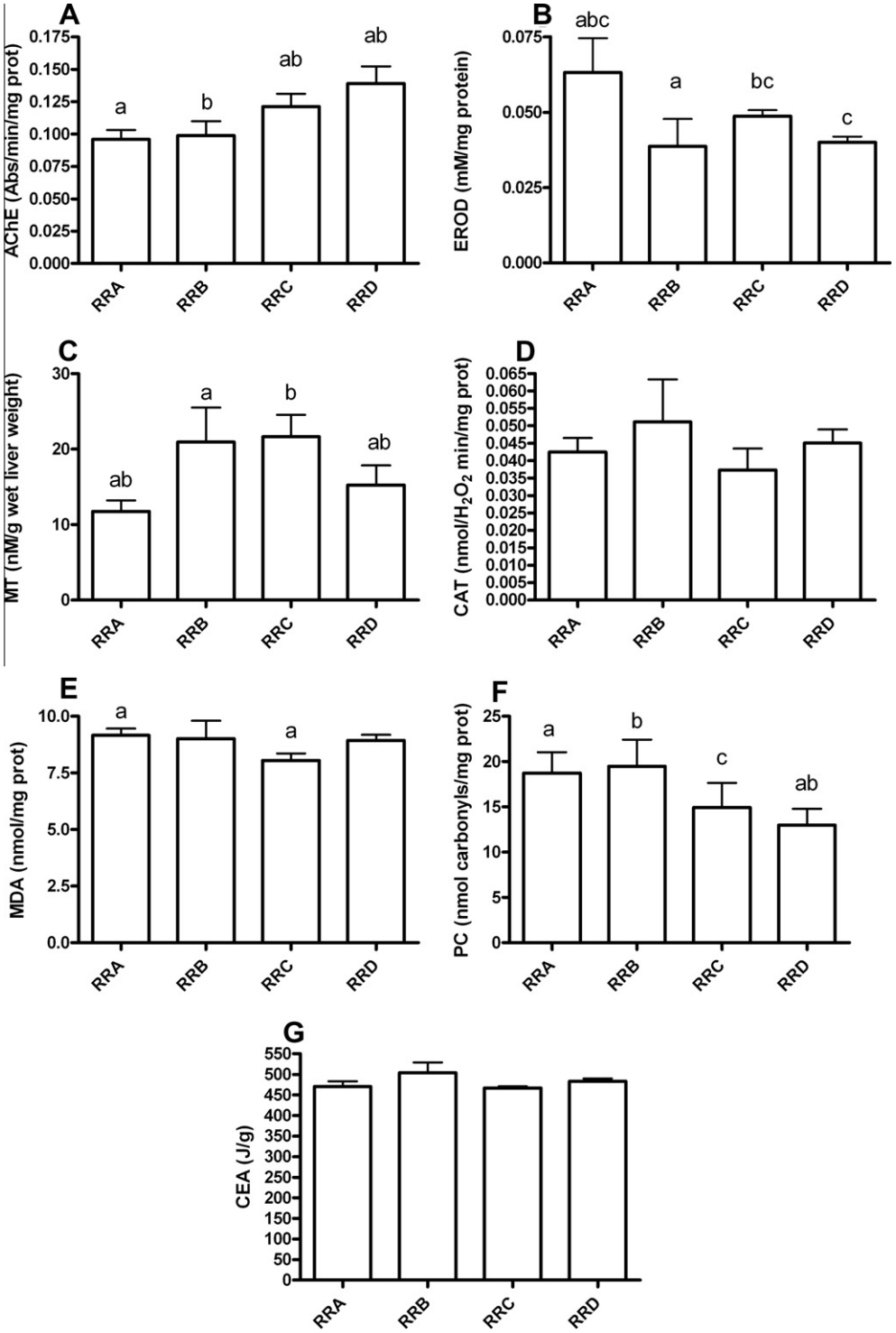

Fig. 5. Mean + standard error of biomarkers of exposure (A–C) and effect (D–G) in liver tissue of *Labeo capensis* from the four risk regions in the study area. Bars with common superscript are statistically significant different ($p < 0.05$).

itat type (based on depth and flow) described 100% of the groupings.

4. Discussion

In this study a multi-metric approach recommended by Wepener (2008) was applied to evaluate the risk that different fish attributes are exposed to in the Vaal Barrage region. The study area was divided into four risk regions and risks posed by identified stressors to three fish attribute endpoints, i.e. health of individual fish, the health of an indicator population and the integrity of the fish community structure was studied. These endpoints were selected since the specific problem that led to the initiation of this study was the fish kills that occurred within the Vaal Barrage region during the last 2 years.

The Vaal Barrage catchment receives extensive volumes of water from point source discharges. These point source discharges

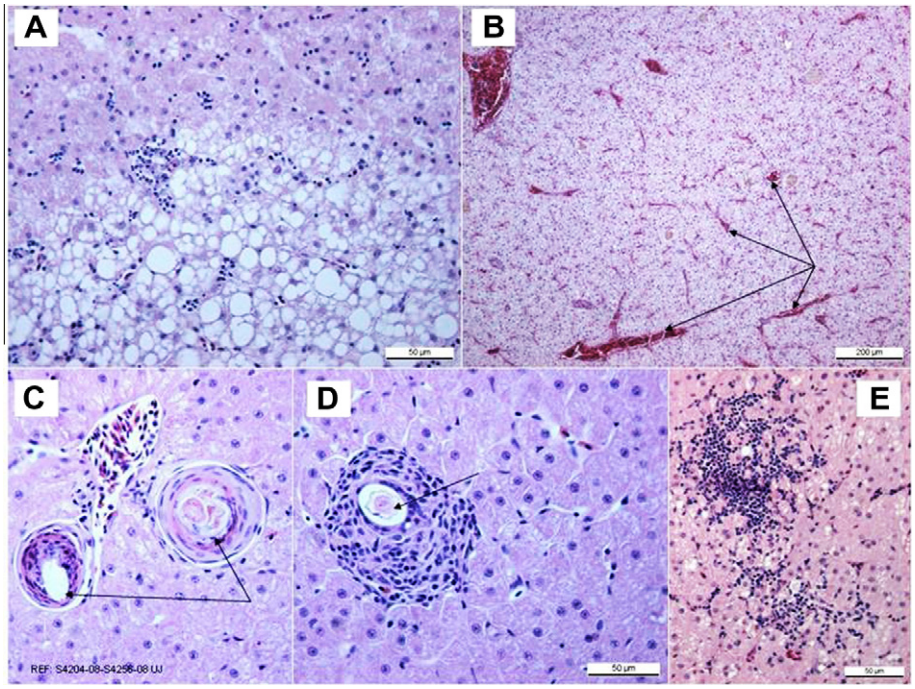

Fig. 6. Histological alterations identified in the liver (H&E). A: Steatosis within the hepatocytes (×40). B: Congestion (arrow) of the blood sinusoids (×10). C: Parasitic infection (arrow) (×40). D: Parasitic infection (arrow) with a surrounding inflammatory response (×40). E: Leukocyte infiltration within the liver tissue (×40).

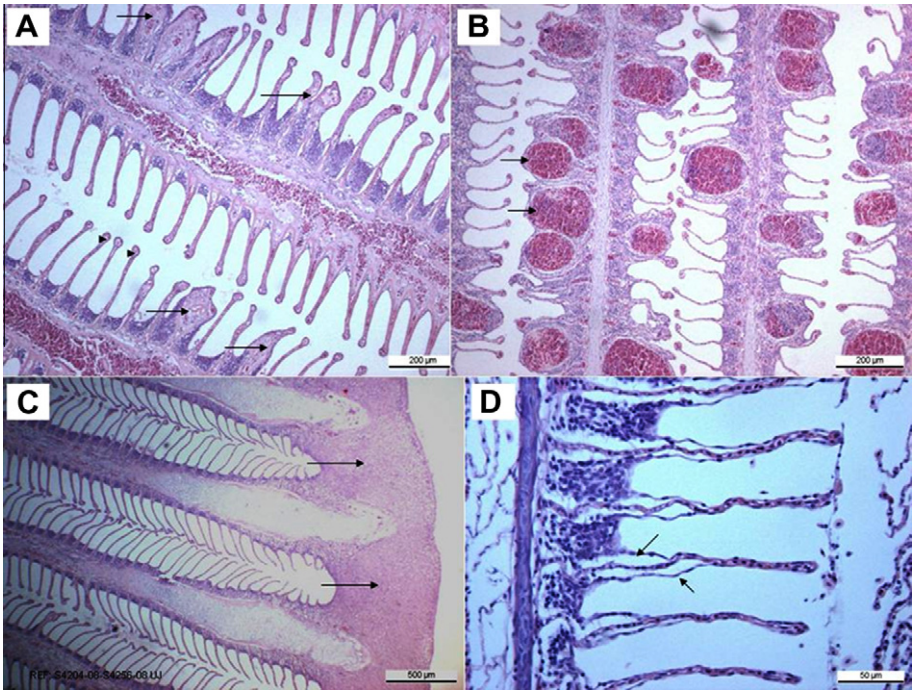

Fig. 7. Histological alterations identified in the gills (H&E). A: Structural alterations (arrow) and clubbing (short arrow) of the secondary gill lamellae (×10). B: Telangiectasia (arrow) of the secondary gill lamellae (×10). C: Fusion of the primary gill lamellae at the distal regions (arrow) (×10). D: Epithelial lifting (arrow) (×40).

include the major wastewater treatment works run by Johannesburg Water, East Rand Water and Metsi-a-Lekoa, as well as the discharges from gold mines. The major salt loads from the mines are discharged from Petrex (formerly Grootvlei) and ERPM mines (Schoeman and Steyn, 2001). All of the aforementioned enter the Vaal River in RRB. There are also industrial effluent discharges, the largest being from SAPPI Enstra (RRC), Sasol Sasolburg (RRA) and the stormwater runoff from Mittal Steel Vanderbijlpark (RRB). The discharge volumes from the wastewater treatment plants will grow with time as the water requirements grow and the level of services are improved with the expansion of water borne sewerage systems in the urban areas (DWAF, 2008). It is particularly the Rietspruit and Klein Rietspruit Rivers that receive sewage from extensive formal and informal settlements (Venter et al., 1997) and deposits it into the Vaal Barrage (RRB). This was the major source of the organic-rich, silt sediment as well as the

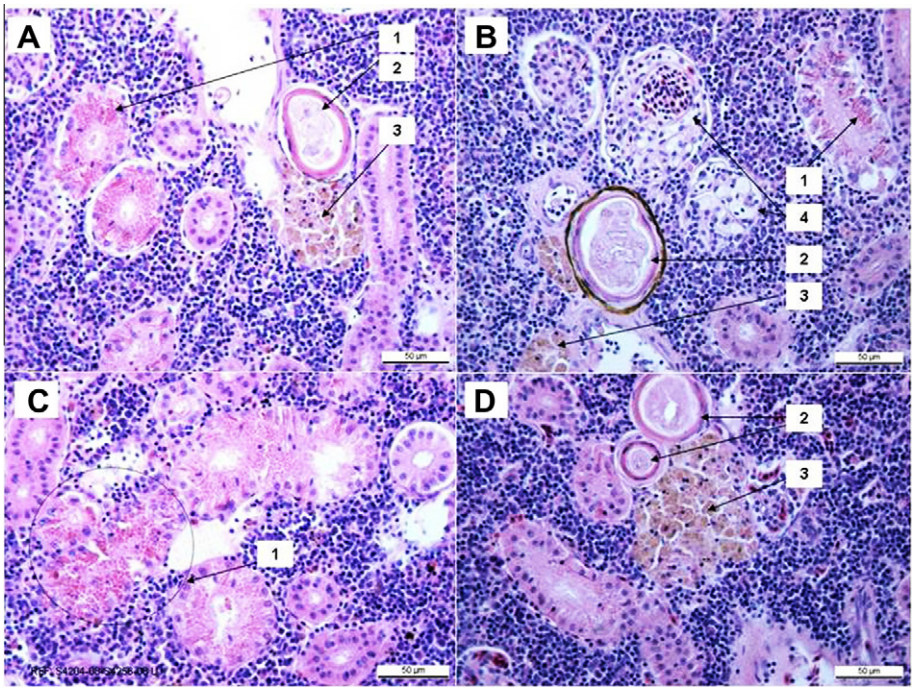

Fig. 8. Histological alterations identified in the kidney (H&E). A: Hyaline droplet degeneration. B: Parasitic infection. C: Melano macrophage aggregates. D: Enlarged glomeruli with dilated capillaries (×40).

Table 5
Mean histological Index values calculated for each target organ. Ranges are indicated in parenthesis. I^L = Liver Index, I^G = Gill Index, I^K = Kidney Index, I^{FISH} = Fish Index. Health Assessment Index (HAI: mean and range) and Condition Index (CI: mean ± standard error) values for *Labeo capensis* from each site are also presented.

	RRA	RRB	RRC	RRD
I^L	7.4 (2–18)	5.6 (0–8)	5.4 (2–12)	4.7 (2–10)
I^G	11.8 (4–20)	14.6 (0–28)	10.4 (4–16)	12.9 (6–22)
I^K	8.5 (4–14)	5.3 (0–12)	7.8 (2–12)	6.1 (2–10)
I^{FISH}	27.7	25.5	23.6	23.7
HAI	6 (0–30)	23.3 (0–60)	45.7 (0–80)	24.5 (0–70)
CI	1.07 ± 0.12	1.2 ± 0.2	1.08 ± 0.24	0.87 ± 0.1

low dissolved oxygen and high microbial counts that were found within this risk region (RRB).

Bioaccumulation of metals and organic compounds in the muscle tissue of fish was used as an indication of contaminant-specific bioavailability and therefore possible causative agent(s) of toxicity (Chapman, 1997; Rainbow, 2007). The higher metal bioaccumulation in RRC and D can be attributed to the increased metal exposure in these areas as a result of large scale mining operations and contamination of the tributaries flowing into the Vaal River in these regions (Venter et al., 1997; Roychoudhury and Starke, 2006; McCarthy et al., 2007). The higher bioaccumulation of PCBs and PBDEs in RRA can be attributed to the influence of large scale industries in the Sasolburg and Vanderbijlpark regions. Nieuwoudt et al. (2009) found that high levels of these substances in the soils and sediments were the result of a combination of point source releases into the Vaal River and associated tributaries as well as diffuse aerial deposition. The higher levels of organochlorine pesticides recorded in the Vaal Dam region – RRD (i.e. DDTs, HCHs and HCBs) are related to the large scale commercial farming activities in the upper catchment of the Vaal River (Quinn et al., 2009).

To relate the environmental exposure to biological responses, a suite of biomarkers representing different levels of biological organisation was applied (Wepener et al., 2005, 2008). The interpretation of the biomarker responses are summarised in Table 7. The

Table 6
Fish diversity and abundances sampled at the four risk regions in the Vaal Barrage. The codes are used in the RDA triplot.

Scientific name	Common name	Code	RRA	RRB	RRC	RRD
Austroglanis sclateri	Vaal rock catfish	ASCL	0	0	0	3
Barbus paludinosus	Straightfin barb	BPAL	12	0	0	0
Barbus trimaculatus	Threespot barb	BTRI	7	0	0	0
Clarias gariepinus	African sharptooth catfish	CGAR	2	34	1	2
Ctenopharyngodon idella	Grass carp	CIDE	1	0	0	1
Cyprinus carpio	Common carp	CCAR	5	11	11	1
Gambusia affinis	Mosquito fish	GAFF	0	0	0	3
Labeo capensis	Orange River labeo	LCAP	39	0	0	44
Labeo umbratus	Moggel	LUMB	0	0	0	1
Labeobarbus aeneus	Smallmouth yellowfish	LAEN	55	0	0	16
Labeobarbus kimberleyensis	Largemouth yellowfish	LKIM	7	0	0	0
Micropterus salmoides	Largemouth bass	MSAL	0	7	0	4
Pseudocrenilabrus philander	Southern mouthbrooder	PPHI	2	0	0	9
Tilapia sparrmanii	Banded tilapia	TSPA	38	0	0	19
Fish response assessment index (FRAI) scores			B/C	D	D	B

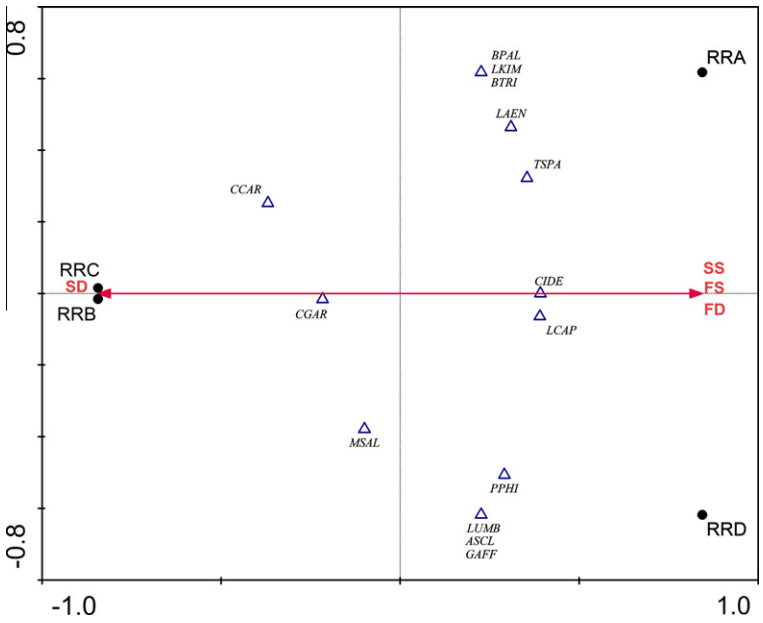

Fig. 9. RDA triplot of the fish community structures in the four risk regions with the depth and flow classes superimposed. The ordination explains 90.3% of the variance in the data with 71.3% and 19% on the first and second axes respectively. Depth and flow classes are represented by FD = fast-deep, FS = fast-shallow, SD = slow-deep and SS = slow-shallow.

Table 7
Summary of the diagnostic nature of the biomarker responses and their interpretation (adapted from van der Oost et al., 2003).

Biomarker	Increase/ decrease	Exposure or effect interpretation
Acetylcholine esterase (AChE)	↓	Inhibition due to organophosphate and carbamate pesticide exposure
Cytochrome P450 activity (EROD)	↑	Stimulation in the presence of organochlorine compounds
Metallothionein (MT)	↑	Stimulation in response to metal exposure
Catalase (CAT)	↑	Produced in response to reactive oxygen species (ROS) formation
Malondialdehyde (MDA)	↑	Indicative of liver peroxidation due to ROS
Protein carbonyl (PC)	↑	Damage to proteins due to ROS
Cellular energy allocation (CEA)	↓ and ↑	Decrease due to stress compensation requiring additional energy sources. Increases associated with additional energy sources.

interpretive value of the increasing or decreasing nature of the particular biomarker was taken from the extensive review on fish biomarkers by van der Oost et al. (2003). The metal exposure that was shown by the bioaccumulation in RRB and C are supported by the MT induction found in the same regions. Similarly the induction of EROD activity in RRA in response to organochlorine exposure is supported by the bioaccumulation results. The only biochemical effects that were significant were the increases in MDA and PC levels in RRA and B that are indicative of lipid peroxidation and protein damage due to pollutant exposure at these sites. The CEA biomarker provides an indication of metabolic energy requirement changes in response to stress caused by e.g. exposure to pollutants (Smolders et al., 2004). In this study no changes in CEA values between the different risk regions were recorded.

In order to identify biological responses at higher levels of biological organisation, histopathological alterations were studied. The results found in this study were indicative of normal tissue structure with slight to moderate histological alterations (van Dyk et al., 2009b). In terms of the total organ indices, little variation between the different sample groups was found and fish from the risk regions seemed to be in a similar condition regarding histological characteristics. All of the organs assessed appeared to be in a functional state in terms of tissue structure and the alterations identified are generally considered reversible should the stressor (biological or chemical) be removed or neutralised (van Dyk et al., 2009a). However, the presence of selected alterations identified (e.g. kidney tumours and fusion of the primary lamellae) could lead to adverse health effects should any additional stressors occur. The general health of the fish as reflected in the HAI was markedly weaker than the values previously recorded in the Vaal Dam and Vaal Barrage (Crafford and Avenant-Oldewage, 2009). During these earlier surveys no anomalies were recorded for *L. umbratus* for skin, haematocrit and plasma protein values. During this study these parameters are consistently high (abnormal). Based on the HAI index values it can be concluded that the water quality has deteriorated during the intervening period. Notwithstanding the decreased health, the general condition as reflected in the CI was not different between the risk regions. Condition indices are excellent indicators of whole organism health (Smolders et al., 2004). Stressed organisms generally have decreased condition due to depletion of energy reserves in an attempt to physiologically compensate for damage caused by the stressor. The unchanged CI also support the unchanged energy expenditure (CEA biomarkers) found during this study.

The fish community structures of the study area were assessed to determine whether the water quality, increased exposure to pollutants and ensuing health effects has had any effects on the community structure. The state of the fish communities within the Vaal

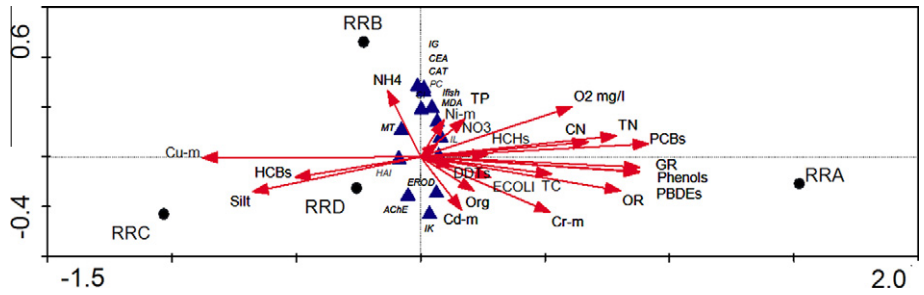

Fig. 10. RDA triplot of the biomarker responses in the four risk regions with the environmental (water and sediment quality and bioaccumulation) variables superimposed. The ordination explains 94.8% of the variance in the data with 87.1% and 7.7% on the first and second axes respectively. Refer to Table 6 for an explanation of the abbreviations.

River reduced noticeably from a largely natural state upstream of the confluence between the Vaal and Suikerbosrant/Klip rivers (RRD) to a largely modified state below the Vaal Barrage (RRA). The extremely low abundance and diversities of fishes obtained within the Vaal Barrage (RRB) suggest that the state of the fish communities in the Barrage are lower than the "D" largely modified state obtained downstream of the Barrage. The situation may be as a result of the combined effects of unfavourable habitat (as reflected in the predominance of the slow–deep habitat) and altered water quality causing indigenous fish to avoid the Barrage. They may only occupy these areas if population pressure forces them into these areas. Some of the very tolerant indigenous species such as the Sharptooth catfish, Vaal River mudfish and the alien Common carp and Largemouth bass dominate the fish community assemblages within the Vaal River. To these tolerant hardy species the current state of the Vaal River within the Vaal Barrage may be marginal to favourable for species that are able to take advantage of the modified habitat (such as the Largemouth bass). The multivariate statistical assessment of changes in the fish community structures, complement the findings of the FRAI assessment. It is evident that a noticeable change in the fish community structures exists within the study area and that this change is in the form of an increase in dominance of the hardy exotic species such as the Sharptooth catfish within the Vaal Barrage. Potential drivers of this change in community structure include changes in the water quality of the Vaal River below the confluence between the Vaal and Blesbokspruit/Klip rivers and or due to habitat alterations. The consequences of these drivers has been the complete removal of indigenous species that are sensitive to modified water quality and those species that have a high preference for specialised riverine habitats or substrates. Below the Vaal barrage the riverine habitats are re-established but the water quality impacts remain high. Below the Vaal Barrage the diversity, abundance and overall ecological state of the fish communities increase suggesting that the Vaal River itself has a high assimilation potential whereby the water quality impacts are reduced to allow for the recovery of the fish communities.

To integrate the responses of the different fish attributes to environmental parameters a RDA was completed where the risk regions were ordinated based on the subcellular and whole fish attributes. The water and sediment quality as well as the bioaccumulation results (viz. indicators of environmental pollutant exposure) were superimposed on the ordinations to provide an indication of the driving variables responsible for the specific site ordination (Fig. 10). The risk regions where the impaired fish health (RRC) and histopathological lesions (RRB) were predominant were related to decreased oxygen levels and increased nutrient levels. The biomarkers of exposure (i.e. EROD and MT) were related to the risk regions where organochlorines (RRA and RRD) and metals (RRC) were highest. Thus those biomarkers that indicated potentially lethal responses (i.e. fish health and histopathological lesions) were related more to low oxygen levels than pollutant exposure.

Even though we were able to demonstrate clear relationships between exposures to specific pollutants and biological responses at cellular and whole fish level, none of these attributes were deemed to be sufficiently negative to induce mortalities in any of the risk regions. This is further emphasised by probably the most ecologically-relevant data, i.e. the fish community structures, where the fish communities in RRA were very similar to the structures within the risk region that was at risk the least (RRD) from the identified pollution sources.

Just prior to the survey conducted for this study mass fish mortalities occurred in RRB and A. Water quality logger data in the period preceding the fish kill showed that the dissolved oxygen levels were very low. This was attributed to increased algal growth in response to increased nutrient loads. Detailed histopathology assessments in the only species affected during that event, i.e. carp showed limited damage with the exception of the gill tissue, which was severely affected by hyperplasia. The HAI assessment (HAI score of 126) found that externally the fish had lesions and high parasite loadings while the gills displayed massive damage. The internal organs displayed signs that would be consistent with oxygen and water regulation impairment, which could be linked to the gill damage. The symptoms of Spring Viremia of Carp Virus (SVCV), i.e. gill damage, internal oedema, haemorrhaging and inflammation were observed in all the fish sampled (Monette et al., 2006). Since the water was not inherently toxic (i.e. based on the acute toxicity tests using the standard fish and daphnid tests, carried out on water samples) it was concluded that the fish kills were the result of chronic stress conditions that required only a single trigger (e.g. sustained lower oxygen concentrations) for pathogens to increase. Thus opportunistic infections due to secondary suppression of the immune system, which was caused by a combination of environmental (i.e. low dissolved oxygen or increased temperatures) and physiological (i.e. spawning) factors most likely resulted in the mass mortalities of the affected species (e.g. carp). These conclusions could not empirically be substantiated but it was noticeable that the fish kill episodes would target specific species and did not result in mass mortalities of all species in the study area.

5. Conclusion

Notwithstanding the evidence of sustained pollutant exposure and stress conditions experienced by the test species, the fish community assessment did indicate that the fish structures in RRA and D were in a good condition. This clearly demonstrates the high degree of resilience of the fish communities to the

stressors within this region. It therefore becomes a political and socio-economical decision as to what degree of environmental stress, with its ensuing consequences will be allowed within the Vaal Barrage. The suite of biomarkers that were used during this risk assessment was not able to identify the causative agents for the periodic mass fish mortalities. However, based on the hypothesis that was formulated future studies should address the identification of the presence of co-occuring viruses (e.g. SVCV), using appropriate histopathological and/or genetic marker techniques.

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