PREDICTING THE EDIBILITY OF FISH IN THE FLAG BOSHIELO SYSTEM

Report to the
WATER RESEARCH COMMISSION

by

JOSEPH R. SARA, SEAN M. MARR, WILMIEN J. LUUS-POWELL, WILLEM J. SMIT and ABRAHAM ADDO-BEDIAKO
University of Limpopo

WRC Report No. 2544/1/21
ISBN 978-0-6392-0262-4

July 2021

WATER
RESEARCH
COMMISSION

Obtainable from
Water Research Commission
Private Bag X03
Gezina, 0031
PRETORIA

orders@wrc.org.za or download from www.wrc.org.za

The publication of this report emanates from a project titled *Investigation of large-scale drivers of seasonal fluctuation on water chemistry and toxicant levels in the Flag Boshielo System* (WRC Project No: K5/2544)

EXECUTIVE SUMMARY

RATIONALE

Despite South Africa being food secure at national level, Statistics South Africa found that almost 21% of South African households experience food insecurity (Statistics South Africa, 2017b). South Africa's National Development Plan aims to eliminate poverty and increase food security in rural areas by 2030. South Africa has more than 4 700 impoundments constructed primarily for domestic, agricultural and industrial water supply. The Department of Agriculture, Forestry and Fisheries have proposed that these water storage and distribution impoundments be developed for inland fisheries and aquaculture in order to improve rural livelihoods and increase food security in South Africa. Fish is a vital source of food for many of the world's people, especially low-income groups, as they are a rich source of protein, micronutrients and essential fatty acids, cheaper than other protein sources, and available from local lakes, rivers and impoundments. A geographic information system model based on the morpho-edaphic index of fisheries potential identified the north-eastern regions of South Africa as having the highest fisheries potential and estimated the total national potential yield for inland fisheries from all South Africa's impoundments to be ~15 000 t/yr (WRC project K5/1954/4); a fraction of South Africa's marine fishery that yields more than 600 000 t/yr. The relatively low production potential of South Africa's inland water bodies precludes the development of industrial or large-scale commercial fisheries on inland waters, however, a combination of recreational, small-scale subsistence and artisanal fisheries for livelihood purposes was considered the optimal model to maximise socio-economic benefit of inland fisheries in South Africa. The Department of Agriculture, Forestry and Fisheries is currently developing an Inland Fisheries Policy.

However, the consumption of fish harvested from contaminated inland waters could result in detrimental long-term health impacts. Flag Boshielo Dam on the main-stem of the Olifants River had been proposed as a suitable location for the development of a fishery and a WRC Project has commenced to determine the fishery potential of this impoundment (WRC project K5/2497/4). WRC project (K5/1929), conducted between 2009 and 2012, identified that the concentrations of selected metals in fish from impoundments in the Olifants River system exceeded safe consumption limits and were potentially detrimental to the health of communities who regularly consumed fish from these impoundments. The Department of Agriculture, Forestry and Fisheries heeded the warnings generated from this work and ensured that the draft Inland Fisheries Policy makes provision to "*establish product quality and safety programmes for freshwater fisheries foods which conform, as far as possible, with relevant local standards and requirements and, as far as possible, with international standards or requirements*." However, understanding of the dynamics of metal accumulation in fish muscle tissue and the environmental processes that drive is insufficient to construct models to allow the fisheries managers to predict when the fish captured from an impoundment would be safe for human consumption. There is, therefore, a need to investigate the seasonal dynamics of metal concentrations in fish muscle tissue and to identify environmental drivers of the observed patterns.

OBJECTIVES

The aim of this project was to evaluate seasonal fluctuations in the metal concentration in fish muscle tissue in Flag Boshielo Dam in order to establish guidelines for the safe consumption of fish from contaminated impoundments. The results of the project are expected to provide information to inform policy for the development of inland fisheries on South Africa's water storage impoundments. The results of this project should directly inform the Inland fisheries Policy, which refers directly to the requirement that the fish products from inland fisheries should be monitored to determine whether they are suitable for human consumption.

In order to achieve the stated aim of the project, the following objectives have been outlined for the project:

- Monitor seasonal fluctuations in selected water quality parameters at Flag Boshielo Dam during drought and flood cycles (if possible, within the duration of the project)
- Monitor seasonal fluctuations in the metal concentration in fish muscle at Flag Boshielo Dam
- Investigate whether environmental and limnological factors can be identified as drivers of the concentration of metals in fish muscle tissue at Flag Boshielo Dam using linear regression and distance-based linear modelling, and
- Use the findings of the study to inform the Inland Fisheries Policy regarding the safe utilisation of fish captured from Flag Boshielo Dam for human consumption.

METHODOLOGY

This project used a combination of analysis of historical data, two-and-a-half years of field data collection, and multivariate regression to evaluate seasonal fluctuations in the metal concentration in fish muscle tissue in Flag Boshielo Dam. Three fish species, *Oreochromis mossambicus*, *Labeo rosae* and *Schilbe intermedius* were chosen for the study due to their abundance in Flag Boshielo Dam and because these species occupy different niches within the system, with the first two species currently targeted by subsistence fishers.

Historical data from the Department of Water and Sanitation's Resource Quality Services for the Olifants and Elands rivers and Flag Boshielo Dam was compiled and analysed to establish whether patterns were evident. The dynamics of river flow and relationships between selected water physico-chemical parameters were explored.

Eleven field surveys of Flag Boshielo Dam were conducted between February 2016 and April 2018. During each survey the water physico-chemical properties were measured and samples of the water, sediment and fish muscle tissue collected for metal content analysis at Waterlab, a SANAS accredited laboratory in Pretoria. A desktop Human Health Risk Assessment, based on US EPA methodology, was conducted for fish muscle tissue from Flag Boshielo Dam over the study period.

Finally, regression analyses were used to determine whether drivers of the metal concentrations in fish muscle tissue could be identified. A suite of environmental and water chemistry parameters was initially selected for the analyses but no clear set of drivers could be identified to predict the metal concentrations in fish muscle tissue. Therefore, a reduced set of ten “limnological drivers” that could be measured in the field and did not require chemical analysis were selected: physico-chemical parameters (water temperature (°C), pH, dissolved oxygen (DO) and electrical conductivity (EC)), lake limnological parameters (depth of thermocline and centre of buoyancy (CB)), river discharge (at the survey time and averaged over the month prior to the survey), dam level (change in level over the month proceeding the survey) and the number of days since the last flood flow of > 20 cumecs. Two approaches were used: Generalised linear models and Distance-based redundancy analysis.

Generalised linear models were developed for a reduced set of eight metals, selected based on having the highest number of non-zero records: Al, Ba, Cu, Mn, Se, Sr, Ti and Zn. Generalised linear models were established for each of the eight metals for each study species. Multi-model inference (Burnham and Anderson, 2002) was used to identify the frequency of the variables in the top models, models that differed by less than 4 AIC points from the best model. Distance-based redundancy analysis (dbRDA) (Anderson et al., 2008) was conducted for the three fish species using a matrix of the 19 metal concentrations in the fish muscle tissue for each fish analysed for the long-term monitoring study as the independent variable and a matrix of the environmental parameters recorded during each field survey matched to the fish data as the explanatory variables. The dbRDA determined the environmental drivers that best describe the patterns in the independent variable matrix. The results were plotted and the importance of the variables was projected over the dbRDA plot. The analyses were conducted for both the environmental and limnological drivers.

RESULTS

The Olifants River system appears to cycle between dry periods of low flow and wet periods with frequent high flow events. The lengths of the wet and dry cycles are variable and are driven through rainfall patterns. The concentration of most water quality parameters increased in the water column of the impoundment over the dry period due to evaporation of water from the impoundment. Floods resulting in river flows of more than 20 cumecs downstream of Flag Boshielo Dam appear to reset the concentrations of ions in the impoundment water column and fully mix the impoundment. Analysis of historical water quality data from Flag Boshielo Dam identified three different groups of water quality parameters:

- parameters that are reset by high flow events and strongly correlated to each other, e.g. electrical conductivity (EC), chlorine (Cl), sodium (Na), fluoride (F), potassium (K) and alkalinity (Alk);
- parameters that are reset by high flow events but not strongly correlated to EC, e.g. calcium (Ca), magnesium (Mg), pH and sulphate (SO_4); and

- parameters present at low concentrations that are not impacted by high flow events or strongly correlated to the other parameters, e.g. ammonium (NH_4), nitrate-nitrite ratio (NO_3:NO_2) and ortho-phosphate (PO_4).

The field studies commenced in a dry cycle of the Olifants River. The impoundment level dropped from about 60% to 20% over 2016 but rainfall events during 2017 maintained the impoundment level between 50% and 60% for 2017. The wet cycle only commenced in March 2018 near the end of the field studies when large rainfall events resulted in the impoundment filling to capacity and spilling, resulting in a flow of more than 20 cumecs in the Olifants River below Flag Boshielo Dam.

Seasonal patterns were found for the water temperature, dissolved oxygen and pH of the water column in the riverine, transition and lacustrine zones of the impoundment. Flag Boshielo Dam fluctuated between mesotrophic and oligotrophic states with ammonium levels generally exceeding the specified Target Water Quality Ranges (TWQR) while the ortho-phosphate levels were below the specified TWQR. There was a chlorophyll-a bloom in the impoundment from 2017 to the end of the study. Sulphate concentrations exceeded the 100 mg/L threshold for aquatic ecosystem health from September 2017 to the end of the study, coinciding with periods of higher inflow into the impoundment. No stratification of temperature, dissolved oxygen or pH was recorded in the riverine zone. Stratification of all three parameters was observed in the warmer months, October to February, for the limnetic sites, while no stratification was evident in the cooler months; indicative of lake turnover in the cooler months.

Of the suite of metals analysed, only aluminium (Al), barium (Ba), boron (B), iron (Fe), strontium (Sr), titanium (Ti) and zinc (Zn) were detected regularly in the water samples. The highest concentration for these metals were recorded in October 2016. These exceed the values recorded for Flag Boshielo Dam in 2010 by Jooste et al. (2013). Sediment samples collected from the impoundment contained higher concentrations of metals and metalloids than the water samples. Aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mg), nickel (Ni), selenium (Se), strontium (Sr), tin (Sn), titanium (Ti), vanadium (V) and zinc (Zn) were detected. Compared to Jooste et al. (2013), sediment concentrations of Sb, B, Ba, Cd, Se, Sn and Zn were lower in this study than in 2010, while Ni concentrations exceed the 2010 values.

Muscle tissue from three fish species were analysed for metals, with the results being used in a US-EPA desk-top Human Health Risk Assessment to determine the risk of long-term consumption of these fish species to human health. For *O. mossambicus*, Al, Ba, B, Cu, Mg and Hg in the muscle tissue were found to be higher during high inflow, while As, Cr, Ni and Sn were higher during periods of low inflow. For *L. rosae*, Al, Ag, As, Cd, Cr, Co, Cu, Mo, Sb, Se, Sn, V and Zn were higher during periods of low inflow. For *S. intermedius*, the levels of Ag, Co and Hg in the muscle tissue were higher during periods of high inflow, while As, Cr, Pb and Ni were higher during periods of low inflow. When comparing these results to the 2010 study from Flag Boshielo Dam, the concentrations of metals in fish muscle tissue were mostly considerably lower than the 2010 values.

The decreased metal concentrations in the fish muscle resulted in the Human Health Risk Assessment finding very few metals exceeding the safe levels for long-term consumption. Hazard Quotients (HQ) greater than 1, indicative of adverse long-term health human impacts, were only found for 19 fish. Using the average concentrations, Co in *O. mossambicus*, As in *L. rosae* and Pb in *S. intermedius* exceeded a HQ of 1. Therefore, the risk of long-term health impacts from consuming fish from Flag Boshielo Dam were lower in the current dry cycle study than in the wet cycle study conducted in 2010.

The generalised linear modelling of eight metals found that the predictor variables that contributed more than 10% to the within group similarity for *O. mossambicus* were pH, DO, EC, thermocline depth, and the time since the last flood, for *L. rosae* pH, thermocline depth, and change in dam level over the last month, with DO contributing more than 5% to the group similarity. For *S. intermedius*, more than 10% to the within group similarity was explained by pH, DO, thermocline depth, and CB. The predictors pH, dissolved oxygen, and thermocline depth were common to the three species, and temperature and electrical conductivity consistently contributed more than 5% similarity for all three species.

The distance-based redundancy analysis found that three variables were shared between the best models for the three species: DO, temperature, and CB. *Oreochromis mossambicus* had eight predictor variables for the metal concentrations in muscle tissue: pH, DO, EC, temperature, thermocline depth, CB, change in dam level over the last month, and the time since the last flood. *Schilbe intermedius* shared all its five predictor variables with *O. mossambicus*: DO, temperature, CB, change in dam level over the last month, and the time since the last flood. *Labeo rosae* shared four of its five predictor variables with *O. mossambicus,* i.e. DO, temperature, thermocline depth and CB, with the fifth predictor variable, river discharge, being unique to *L. rosae*.

KEY FINDINGS

The Olifants River is subjected to wet and dry cycles that influence the concentration of metals in fish muscle tissue. These cycles extend over multiple years and are not related to the annual wet and dry seasons. In the dry cycle, the concentrations of water physico-chemical parameters of the impoundment water column increase gradually. Flood events of greater than 20 cumecs play an important role in resetting the concentration of water physico-chemical parameters of the water column of Flag Boshielo Dam. Stratification of the impoundment was observed during summer, while lake turnover was observed during winter.

Metal concentrations in fish muscle tissue of *O. mossambicus, L. rosae* and *S. intermedius* were lower in the 2016-2018 dry cycle than in the 2010 wet cycle. During the 2016-2018 dry cycle, long-term consumption of these species from Flag Boshielo Dam posed less of a risk to human health than consuming these species from this impoundment in the 2010 wet cycle. Whether this can be extended as a generalisation for the wet and dry cycles requires further research.

The regression analysis to identify drivers of metal concentrations in fish muscle tissue found that all the variables considered were relevant in predicting the concentration of metals in fish muscle. However, three variables (DO, temperature, and CB) were included in the best models for all three species. The best model for *Oreochromis mossambicus* had eight predictor variables, that for *Schilbe intermedius* had five predictor variables, all of which were shared with *O. mossambicus*, and that for

Labeo rosae had five predictor variables, four of which were shared with *O. mossambicus* and three with *S. intermedius.*

The findings of this study will be presented at local conferences and submitted to peer reviewed scientific journals for publication. A community engagement workshop was conducted on the 20th June 2019 involving stakeholders in the Sekhukhune District surrounding Flag Boshielo Dam. The outcome and major findings of this project were presented. The community expressed concern at the findings of the project and inquired about the risks of consuming fish and water from Flag Boshielo Dam, the presence of parasites on the fish, the risks of consuming parasite laden fish, and steps to reduce the health risks of consuming fish from Flag Boshielo Dam. Information about the workshop is provided in Appendix 2.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The wet and dry cycles of the Olifants River are important in driving the biochemical processes in the impoundments of the catchment. The bioaccumulation of metals to fish muscle tissue appears to occur during the wet cycle and elimination of metals from fish muscle appears to take place in the dry cycle. Flood events that result in flows of greater than 20 cumecs in the Olifants River below Flag Boshielo Dam were shown to play an important role in resetting the concentrations of water physico-chemical parameters in the impoundment. During the dry cycle, water physico-chemical parameters appear to concentrate in the impoundment but the metal input appears to decrease.

The environmental drivers that determine the metal concentrations in fish muscle tissue were found to be more complex than expected and our analysis found that all drivers evaluated could be used to describe the observed patterns in fish muscle tissue. Even reducing the number of variables to those that could be measured in the field found that all the variables were relevant. The distance-based redundancy analysis did, however, show that three variables were important for all three species evaluated (DO, temperature, and CB), although the variable sets for the specific species were mostly unique (the variables for *S. intermedius* were a subset of those for *O. mossambicus*). The three species chosen for the study occupy different niches within the impoundment; *S. intermedius* is a pelagic piscivore, *O. mossambicus* is a resident general omnivore, and *L. rosae* is a roaming schooling detritivore. It is therefore not surprising that no common set of environmental variables was found to describe the metal bioaccumulation in the three species.

The results of this project have highlighted a number of areas that require future research. These include:

1. Acid mine drainage is the major driver of the mobilisation of metals in the Olifants River catchment. Research directed towards reducing the generation of acid mine drainage at source and mitigation actions to reduce the impact of acid mine drainage mobilised metals in the ecosystem.
2. The potential threat that pesticides, and other organic compounds pose to the ecological functioning of the Olifants River and its impoundments, including the edibility of fish, needs to be investigated. Several challenges are acknowledged including the exceptionally low concentrations of pesticides

and organic compounds in fresh waters, the presence of multiple breakdown products, the large number of pesticides and organic compounds used in the agricultural, domestic and industrial sectors, and the expense of the analyses.

3. The detection limits for metals in water and fish muscle samples during the laboratory analysis requires attention. The integrity of the data available for statistical analysis is compromised due to the laboratory reporting “below detection limit” or “not detected”. Techniques to concentrate the contents of samples without loss of the chemical content of the sample could be investigated.
4. At present, the only South African guideline for metals in meat are from a 1972 Act and limited to five metals; viz. As, Cd, Pb, Hg and Sn (Department of Health, 2004). These guidelines are in serious need of revision. Where no safe consumption limit is currently available for a specific metal in South Africa, temporary values could be adopted from from international standards.
5. These assumptions of the current risk assessment methodology should be verified through social surveys of the communities consuming fish from Flag Boshielo Dam. Once the average portion size, frequency of fish meals and the adult body mass have been verified, a realistic human health risk assessment can be completed for the fish species evaluated in this study.
6. A medical survey of the communities consuming fish from Flag Boshielo Dam should be undertaken, using data from local clinics and questionnaires, to determine whether the adverse health effects of metals can be identified in the communities. In addition, blood samples of community members who regularly consume fish from the impoundment should be screened to determine whether there are traces of metals. In addition, a nutritional assessment using questionnaires should be conducted to determine the importance of fish in the diet of the communities surrounding Flag Boshielo Dam.
7. The size and participation in the existing fishery at Flag Boshielo Dam need to be determined, including current catch rates for the species exploited. Prioritisation of the existing fishery should be evident when the possible expansion of the fishery is considered.
8. A good understanding of the dynamics of the fish populations designated for exploitation is required for sustainable and effective management of these populations. Understanding of the recruitment to the populations, the cues for spawning (or spawning migrations) and the impact of the water level in the impoundment on recruitment are required when establishing exploitation rates. The dynamics of recruitment and population size during wet and dry cycles identified in the current study is required.
9. Further studies are required to elucidate the relationships between environmental or limnological drivers and the dynamics of metal concentrations in fish muscle tissue. It would be ideal if the dynamics of metal concentrations in fish muscle can be modelled to provide the Department of Agriculture, Forestry and Fisheries a toll to predict when the fish from the impoundment are safe for long-term human consumption. These models can then be added to models of fish population and projected exploitation in a Building Block of DRIFT type model to help the Department of Agriculture, Forestry and Fisheries, the Department of Health and other trade organisations to regulate the harvesting effort in Flag Boshielo Dam such that the fish are safe for human consumption.

10. The conservation of rare and endangered species should also be carefully managed and included in the fishery management guidelines from the establishment of the fishery. The presence of crocodiles at Flag Boshielo Dam should be managed such that crocodiles do not become a conflict species between the conservation and fishery users of the impoundment.
11. The Department of Water and Sanitation's water quality monitoring program should be expanded to include metals, especially in catchments where metal pollution has historically been present. This would provide early warning signals to warn fisheries managers of potential metal bioaccumulation in fish and provide researchers with tools to evaluate long-term trends in metal pollution in these catchments. Not all metals need to be included in such a monitoring programme, just the metals that have been identified in risk assessment for their potential risk to human health and more abundant metals that could be used as controls or indicators of disturbances.
12. This project has demonstrated that developing a model to predict when the fish from an impoundment are safe for human consumption is a far from trivial exercise. Therefore, in order to implement the food security monitoring from the Inland Fisheries Policy, the DAFF will require direct sampling of the fish captured from the impoundments where inland fisheries have been developed. The sample collection and analytical procedures can follow those used in this project.
13. The DAFF has proposed Flag Boshielo Dam as a potential site for the establishment of an inland fishery and aquaculture ventures. However, based on the available evidence, we believe that Flag Boshielo Dam is not suitable for the establishment of inland fisheries or aquaculture ventures due to the health risks posed by long-term consumption of metal contaminated fish.
14. The current utilisation of fish from the impoundment by subsistence fishers poses a complex problem that requires a solution. Subsistence fishers currently capture fish from the impoundment for personal consumption and as an income source. Fish captured from the impoundment are sold locally and possibly in Johannesburg and Pretoria. The current informal subsistence fishery is largely unquantified, but could provide an important source of income and protein for the local rural community. Daily, and weekly, consumption of fish from the impoundment should be strongly discouraged as this could pose a significant long-term health risk to the consumers. However, discouraging regular consumption of fish from the impoundment could counteract the benefit of utilising fish as a protein supplement in protein deficient communities. The communities should, therefore, be informed of the long-term health risks resulting from regular consumption of fish from the impoundment and regular monitoring of the metal concentrations in the fish muscle tissue should take place to generate advisories on fish consumption to reduce the risks to long-term human health. Advisories could include recommendations on fish consumption frequency based on specified portion sizes.

ACKNOWLEDGEMENTS

The authors would like to thank the Water Research Commission (WRC) for funding this research project. Members of the Steering Committee of our WRC project No. K5/2544 are hereby gratefully acknowledged for their assistance and the constructive discussions and inputs during the duration of the project:

REFERENCE GROUP

Mr B Madikizela	Water Research Commission (WRC: Chairperson)
Ms P Dubula	Department of Water and Sanitation (DWS)
Mr M Elijah	Department of Water Affairs and Environment (RQS)
Prof. R Greenfield	University of Johannesburg (UJ)
Ms B Makhafola	Department of Health (DoH)
Prof. R Pieters	North West University (NWU)
Mr T Nepfumbada	Department of Agriculture Forestry and Fisheries (DAFF)
Mr DR Mogakabe	Department of Water and Sanitation (DWS)
Ms G Kruger	(Project Administrator)

PROJECT TEAM MEMBERS

Prof WJ Luus-Powell	University of Limpopo (UL: project leader)
Prof A Addo-Bediako	University of Limpopo (UL: deputy project leader)
Dr SM Marr	South African Institute of Aquatic Biodiversity (SAIAB)
Dr JR Sara	University of Limpopo
Mr WJ Smit	University of Limpopo
Dr KD Bal	Independent

Our gratitude is extended to our University of Limpopo colleagues: Mr KD Kunutu and Mr HA Hlungwani for their valuable assistance during the field surveys. Our postgraduate students: Ms BM Phala and Mr SS Motimele (MSc students) for all their hard work during the course of this project.

We would also like to thank the DST-NRF SARChI (Ecosystem Health) and the Belgium VLIR-IUC (Vlaamse Interuniversitaire Raad – University Development Cooperation) Funding Programme, for additional funding.

TABLE OF CONTENTS PAGE

LIST OF FIGURES

LIST OF TABLES

Note on the reorganization of the government departments in South Africa.

During the compilation of this report several government departments were reorganized. Of significance to this report, the Fisheries Department was moved from the Department of Agriculture, Forestry and Fisheries into the Department of Environment, Forestry and Fisheries. Similarly, the Department of Water and Sanitation, formerly in the Department of Water Affairs and the Department of Water Affairs and Forestry, was moved to the Department of Human Settlements, Water and Sanitation.

For this report, please note that the names of the government departments as of the beginning of 2019 were used, i.e. the Department of Water and Sanitation and the Department of Agriculture, Forestry and Fisheries

LIST OF ABBREVIATIONS

AEV	Acute Effect Value
ANOVA	Analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
CB	Centre of buoyancy
CCME	Canadian Council of Ministers of the Environment
CEV	Chronic Effect Value
DAFF	Department of Agriculture, Forestry and Fisheries
DHHS	Department of Health and Human Services
DO	Dissolved oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWAS	Department of Water and Sanitation
EC	Electrical Conductivity
EDC	Endocrine Disruptive Chemicals
FBD	Flag Boshielo Dam
IARC	International Agency for Research on Cancer
ICP-OES	Inductively coupled plasma-optical emission spectrometry
KNP	Kruger National Park
PB	Phalaborwa Barrage
PCBs	Polychlorinated biphenyls
POPs	Persistent Organic Pollutants
SAWQG	South African Water Quality Guidelines
SQG’s	Sediment Quality Guidelines
TWQR	Target Water Quality Range
UL	University of Limpopo
US-EPA	US Environmental Protection Agency
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

South Africa's National Development Plan, Vision 2030, aims to eliminate poverty and reduce inequality in South Africa by 2030 (National Planning Commission, 2013). The proportion of the population living in poverty was estimated to be 56% (30,4 million) in 2015 (Statistics South Africa, 2017a). One of the consequences of poverty is inadequate or severely inadequate access to food – food insecurity. Despite South Africa being food secure at national level, almost 21% of South African households experience food insecurity (Statistics South Africa, 2017b). Food insecurity varies by province (Limpopo (94%) and Gauteng (84%) had the highest food security while North West (64%) and Northern Cape (67%) had the lowest), population group of household head, and household size (larger households are more likely to have food insecurity) (Statistics South Africa, 2017b).

In addition to poverty, environmental stressors and conflict are strong drivers of food insecurity in southern Africa (Misselhorn, 2005). In particular, global climate change represents a major challenge to the future livelihood and food security of social groups currently vulnerable to inadequate access to food (Bohle et al., 1994). Furthermore, global climate change poses a significant threat to water security in many areas. Water is essential for human survival and changes to water supply and quality can have devastating implications to rural populations dependent on lakes, rivers and wetlands for water, irrigation and fish to supplement income and dietary protein needs.

South Africa is a semi-arid country with an average annual rainfall less than 450 mm, approximately half the global average (Nomquphu et al., 2007; Nare et al., 2011). South Africa is regarded as the 30th most water stressed country globally with an annual freshwater availability of less than 1700 m^3 per capita (Nare et al., 2011; Aphane and Vermeulen, 2015). In addition, the geographical distribution of rainfall is highly variable with the northern and eastern regions receiving more rain than the southern and western regions (Cessford and Burke, 2005). South African rivers have strongly seasonal flows with considerable year-to-year variability in runoff (Davies et al., 1993). In order to compensate for the seasonality in water availability, the South African government has constructed numerous water storage impoundments and inter-basin transfer schemes to store water during the dry periods and to move water to areas where the demand for water exceeds the natural supply from run-off.

Africa is more vulnerable to the deleterious impacts of climate change than other continents with climate model scenarios predicting higher air temperatures and greater rainfall variability over the continent through the next century (Fischer et al., 2005; De Wit and Stankiewicz, 2006; Boko et al., 2007). Coupled with ever increasing domestic, industrial, agricultural and mining demand for water, the impact of climate change on freshwater ecosystems and communities reliant on the ecosystem services they provide is expected to become dire in future (Whitehead et al., 2009). In addition to long-term climate change, there are short-term wet and dry cycles that drive localised incidences of droughts and floods. The drivers for these short-term cycles are poorly understood but climate change predictions concur that as global temperatures rise, the severity of the short-term cycles are likely to become more pronounced leading to prolonged droughts and more intense flooding events.

Water scarcity is exacerbated by the pollution of surface and groundwater sources. In recent years, attention has focused on the impact of metal contaminants in South African freshwater systems and the health risks to communities that consume fish from contaminated water bodies. The Olifants River (Limpopo River System), in particular, has been the focus of a number of studies highlighting the potential impact to human health of consuming fish from impoundments, e.g. Flag Boshielo Dam (Addo-Bediako et al., 2014a, 2014b; Jooste et al., 2013, 2014, 2015; Lebepe et al., 2016; Marr et al., 2015, 2017; Sara et al., 2017a, 2018), as a result of the acid mine drainage mobilisation of metals from the basement geology of this mineral rich catchment (Netshitungulwana and Yibas, 2012). The potential health consequences of communities consuming untreated water or other aquatic resources, e.g. fish, from contaminated water bodies is an important consideration in the management of water resources and the promotion of inland fisheries and freshwater aquaculture in water storage impoundments. It is, therefore, important to understand the dynamics of the contaminant concentrations, in say the edible portions of fish, in order to optimise the utilisation of aquatic resources while reducing the potential human health impacts of consuming food and water that may lead to deleterious health consequences.

One of the proposals forwarded by the Department of Agriculture, Forestry and Fisheries (DAFF) to improve food security in South Africa is the use of water supply impoundments for the development of inland fisheries and aquaculture ventures (DAFF, 2019a). When food security is based on the exploitation of living resources, management of these living resources to meet long-term species conservation goals need to be incorporated in the setting of the exploitation levels. Freshwater living resources are particularly complex to manage and uncontrolled over-exploitation of fisheries has resulted in the collapse of seemingly endless resources; e.g. the collapse of the river fisheries on the Zambezi River (Tweddle et al., 2015). When the impoundments contain rare and/or threatened species, the protection and conservation of these species should be incorporated in the management plans for proposed fisheries before their inception such that these species do not become an area of conflict when the fishery has been established. Furthermore, it is important to establish the acceptable levels of exploitation that can be sustained by the proposed fishery through scientifically rigorous studies under different water level scenarios and restrictions should be placed on gears such that only specific species are targeted by the fishery.

South Africa's National Development Plan (Vision 2030) (National Planning Commission, 2013) is closely aligned to UNDP's 17 Sustainable Development Goals (SDGs) for 2030 (UNDP, 2015). The proposal to utilise the water storage network of impoundments to establish inland fisheries could be measured in eight of the 17 SDGs. The overarching goal of food security and the creation of opportunities to earn an income are encapsulated in SDGs 1 and 2, No Poverty and Zero Hunger, respectively, and to a lesser extent SDG 8 Decent Work and Economic Growth if the income opportunities are fully realised. Because the proposal relates to the use of the water supply network for the development of inland fisheries, SDG 6 of Clean Water and Sanitation is incorporated. In addition, because of the concerns about the long-term health impacts of consuming fish from contaminated impoundments, SDG 3 Good Health and Well-Being becomes an important consideration, in addition to the considerations of the impact of contaminated water sources on SDG 6. Utilisation of living resources requires that these living resources be managed sustainably and SDG 14 Life in Water, although more orientated towards marine living resources, could also be invoked for freshwater living resources exploited for inland fisheries. Similarly, the health of rivers and other inland water bodies is directly related to state of the catchment and the land-use activities taking place within the catchment, relating to SDG 15 Life on Land. Finally, the future of both life in water and life on land is dependent on the impacts of global climate change. The SDG 13 Climate Action relates more to activities to mitigate and reduce global climate change, but the impacts of climate change could detrimentally impact proposed inland fisheries and the need to address and mitigate against global climate change is imperative for the successful implementation of the proposed development of inland fisheries in water storage and distribution networks of South Africa.

Sustainable Development Goals relevant to the development of inland fisheries in South Africa include:

- SDG 1 – No Poverty – end extreme poverty in all forms by 2030
- SDG 2 – Zero Hunger – end hunger, achieve food security and improved nutrition and promote sustainable agriculture
- SDG 3 – Good Health and Well-Being – ensure healthy lives and promote well-being for all at all ages
- SDG 6 – Clean Water and Sanitation – ensure availability and sustainable management of water and sanitation for all
- SDG 8 – Decent Work and Economic Growth – promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
- SDG 13 – Climate Action – take urgent action to combat climate change and its impacts
- SDG 14 – Life Below Water – conserve and sustainably use the oceans, seas and marine resources for sustainable development [should include freshwater ecosystems]
- SDG 15 – Life on Land – sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss [the state of the water in the streams and rivers of the catchment is directly related to the state of the land surface of that catchment and the land-use within the catchment]

INLAND FISHERIES

Hara and Backeberg (2014) estimated that South Africa has over 4 700 storage dams constructed primarily for domestic, irrigation and industrial water supply, of which about 700 of which are owned and controlled by Government. Given widespread rural unemployment, poverty and undernourishment, Hara and Backeberg (2014) suggested that the development of inland fisheries on public dams and natural water bodies has potential for improving rural livelihoods and food security, including the potential for the inclusion of communities in other value chains linked to economic activities around public dams. However, Britz (2015) identified that the governance of South Africa's inland fishery resources needed a guiding policy, supporting legislation and government capacity based on the social, economic and environmental objectives. The reader is directed to McCafferty et al. (2012), Hara and Backeberg (2014), Britz (2015) and Britz et al. (2015) for a comprehensive history of inland fisheries in South Africa, the current status on inland fisheries, and the issues pertaining to the establishment of sustainable inland fisheries in the country.

1.1.1 Inland Fisheries Potential

A geographic information system model based on the morpho-edaphic index of fisheries potential (Ryder et al., 1974; Ryder, 1982) was constructed to identify regions of South Africa with high fisheries potential based on the relationships between climate, geography and fish yield (Britz et al., 2015). Britz et al. (2015) estimated the total national potential inland fisheries yield from all of South Africa's impoundments to be approximately 15 000 tonnes/yr. This is comparatively small in comparison to South Africa's marine fishery that yields more than 600 000 tonnes/yr. Britz et al. (2015) concluded that the relatively low production potential of South African inland water bodies precluded the development of industrial or large-scale commercial fisheries on inland waters, recommending that recreational and small-scale subsistence and artisanal fishing for livelihood purposes were the optimal inland fishery utilisation towards maximal socio-economic benefit.

Due to climatic constraints, most impoundments in the country were shown to have relatively low productivity in terms of fish yields. As South Africa currently lacks an inland fisheries sector, Britz et al. (2015) recommended that fisheries development initiatives be prioritised in areas where success is most likely. Limpopo and northern KwaZulu-Natal provinces were identified as regions likely to have the most productive fisheries with Flag Boshielo Dam being considered to be the most suitable location for the establishment of a small-scale fishery in Limpopo Province.

1.1.2 Inland Fisheries Policy

The lack of an inland fisheries policy, both at the provincial and national level, was identified by Weyl et al. (2007) as a major bottleneck for the sustainable development of inland fisheries resources. Recognising the need for an Inland Fisheries Policy, The Department of Agriculture, Forestry and Fisheries drafted a policy document (DAFF, 2018) and circulated it for public comment in June-July 2018. Subsequently, a second draft policy document has been compiled (DAFF, 2019b) and circulated for public comment in June-July 2019.

The purpose of the Inland Fishery Policy is to guide the sustainable development of inland fisheries; including legislative reform and harmonization, the definition of access rights, criteria for ensuring sustainable harvest levels, government organisational structure and capacity, cooperative governance and co-management arrangements and the empowerment of rural communities to participate equitably in sustainable resource use (DAFF, 2019b). The policy is grounded in the principles of inclusivity, inland fisheries being an economic sub-sector, equitable access to freshwater aquatic resources, transformation, sustainable development, follows an ecosystem approach to fisheries, embraces the precautionary principle approach, encourages the value chain approach and a developmental approach, and supports good governance. Focus areas for the implementation of the Inland Fisheries Policy include a legal and regulatory framework; access rights and authorisations; resource sustainability; maximising economic and social benefits; corporate governance and co-management; research development and monitoring, inland fisheries development support; transformation and broadening of participation; capacity building; monitoring, evaluation and enforcement; and **food safety monitoring**. Until national legislation is promulgated, inland fisheries will continue to be governed in terms of the cooperative governance provisions of the NEMA and the provincial environmental acts and ordinances (DAFF, 2019b).

1.1.3 Inland Fisheries Case Study: Flag Boshielo Dam

Building on the findings of Britz et al. (2015), the WRC solicited a project to determine the fisheries potential of Flag Boshielo Dam (WRC Project K5/2497/4: TOWARDS ENHANCING CONTRIBUTIONS OF INLAND FISHERIES TO RURAL LIVELIHOODS). Assessment methods such as the yield-per-recruit (YPR) and spawner biomass-per-recruit (SBR) analysis were used to identify and develop a sustainable management strategy based on maximising yield for high value target species, i.e. *Labeo rosae* and *Oreochromis mossambicus,* while minimising the risk to vulnerable conservation priority species. Even though at the time of writing the results of the study have not been formally published, a summary of the preliminary findings is discussed in Section 1.3.2 below.

FLAG BOSHIELO DAM

Flag Boshielo Dam, formally Arabie Dam, is one of four major impoundments on the main-stem of the Olifants River, Limpopo River system; the third largest river system in southern Africa after the Zambezi and Orange River systems. The Olifants River, catchment area 54 570 km^2 (Ballance et al., 2001), drains Gauteng, Mpumalanga and Limpopo provinces (Figure 1.1) and is one of the most polluted river systems in South Africa (van Vuren et al., 1999). The upper Olifants sub-catchment area is dominated by coal and ferro-chrome/ferro-manganese mines, coal-fired power stations, and chrome and steel smelters, which are concentrated mainly in the Olifants and Klein Olifants River catchments near the cities of Witbank and Middelburg, upstream of the Witbank and Middelburg dams respectively.

Intensive and subsistence agriculture and uncontrolled release of treated and raw sewage in the upper Olifants catchment, in conjunction with urban development, mining and industrial activities in the Witbank-Middelburg area, significantly impact the water quality of the upper Olifants River (Claassen, 2005; Heath et al., 2010; Ashton and Dabrowski, 2011), resulting in contamination by acidification, metals, industrial and agricultural chemicals, organic pollutants and domestic waste (Ashton, 2010; Ashton and Dabrowski, 2011). Increased delivery of nutrients (nitrogen and phosphorus) from agricultural runoff and effluent from domestic sources and sewage results in the eutrophication of freshwater systems (Codd, 2000; De Villiers and Thiart, 2007). Agricultural pesticides, including organophosphates and carbonates, are regularly applied (aerial application) to crops in the Groblersdal and Marble Hall areas with little or no regulation of pesticide application taking place in the area (Bollmohr et al., 2008). There is a growing concern of long-term impact of water pollution on the aquatic ecosystem and the health risks to rural communities, especially those still reliant on untreated water and aquatic resources from the Olifants River and its impoundments (Oberholster et al., 2010; Oberholster et al., 2012).

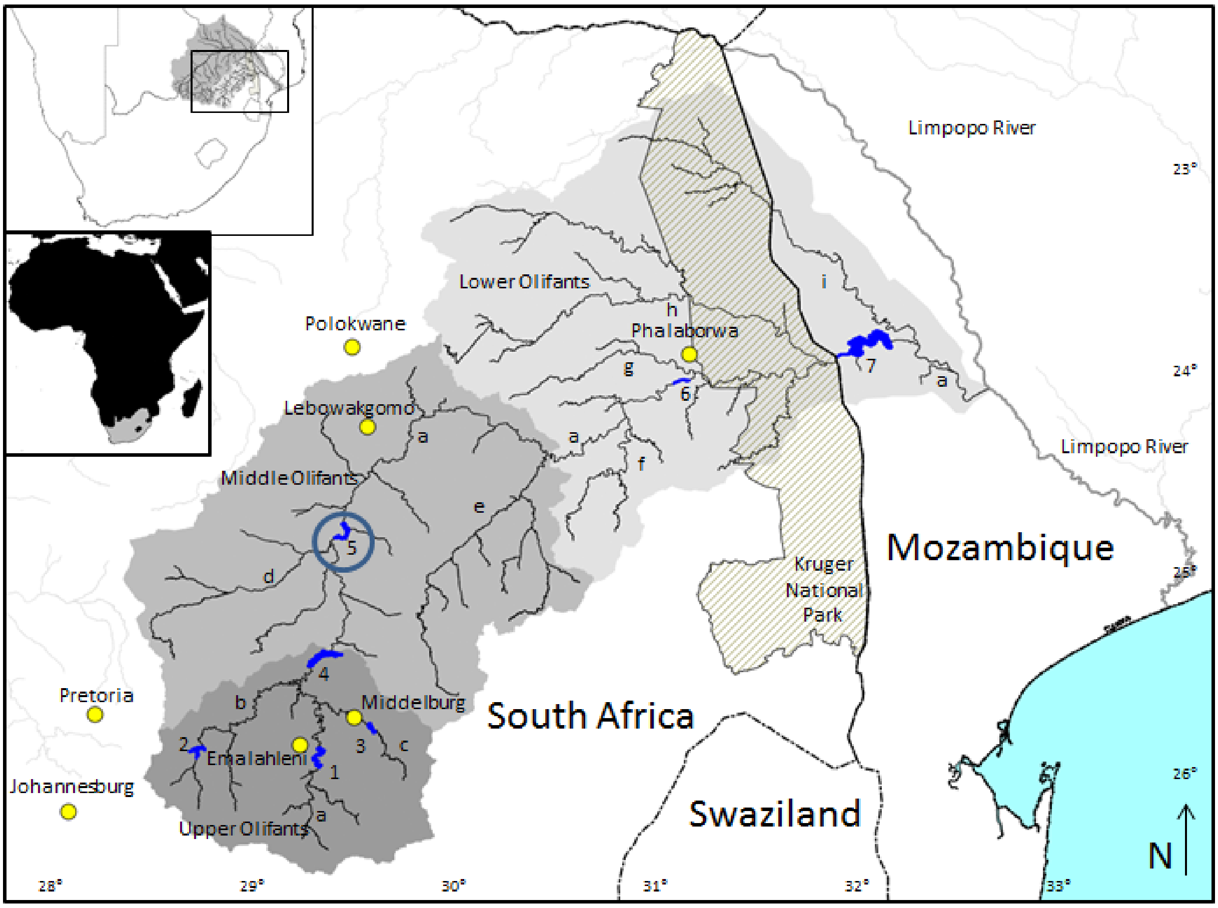

Figure 1.1: The Olifants River basin with the different sub-catchments shaded in grey, showing the location of major towns, impoundments and tributaries. Major impoundments are depicted by numbers: 1) Witbank Dam, 2) Bronkhorstspruit Dam, 3) Middelburg Dam, 4) Loskop Dam, 5) Flag Boshielo Dam, 6) Phalaborwa Barrage and 7) Massingir Dam. The Olifants River and its tributaries are depicted by letters: a) Olifants mainstem, b) Wilge, c) Klein Olifants, d) Elands, e) Steelpoort, f) Blyde, g) Ga-Selati, h) Letaba and i) Shingwedzi. The study site, Flag Boshielo Dam, is circled.

Flag Boshielo Dam is located downstream of the confluence of the Olifants and Elands rivers (Figure 1.2). With a mean depth of 8.15 m, the catchment area of the dam covers 4 213 km^2 (Dabrowski et al., 2014). Flag Boshielo Dam provides water to neighbouring municipal and rural districts for domestic, agriculture and mining purposes (De Villiers and Mkwelo, 2009). To increase water capacity, the dam wall was raised by five meters in 2007/8 increasing the storage capacity from 100 to 188 million m^3 and the yield from 56 to 72 million m^3 per annum (DAFF, 2005). A water treatment plant at the dam ensures a water supply of 8.0 ML per day and raw water consumption of 2920 ML per annum. Lepelle Northern Water Board estimated the consumption to be 2 515 ML per annum (Mahlatji, 2014).

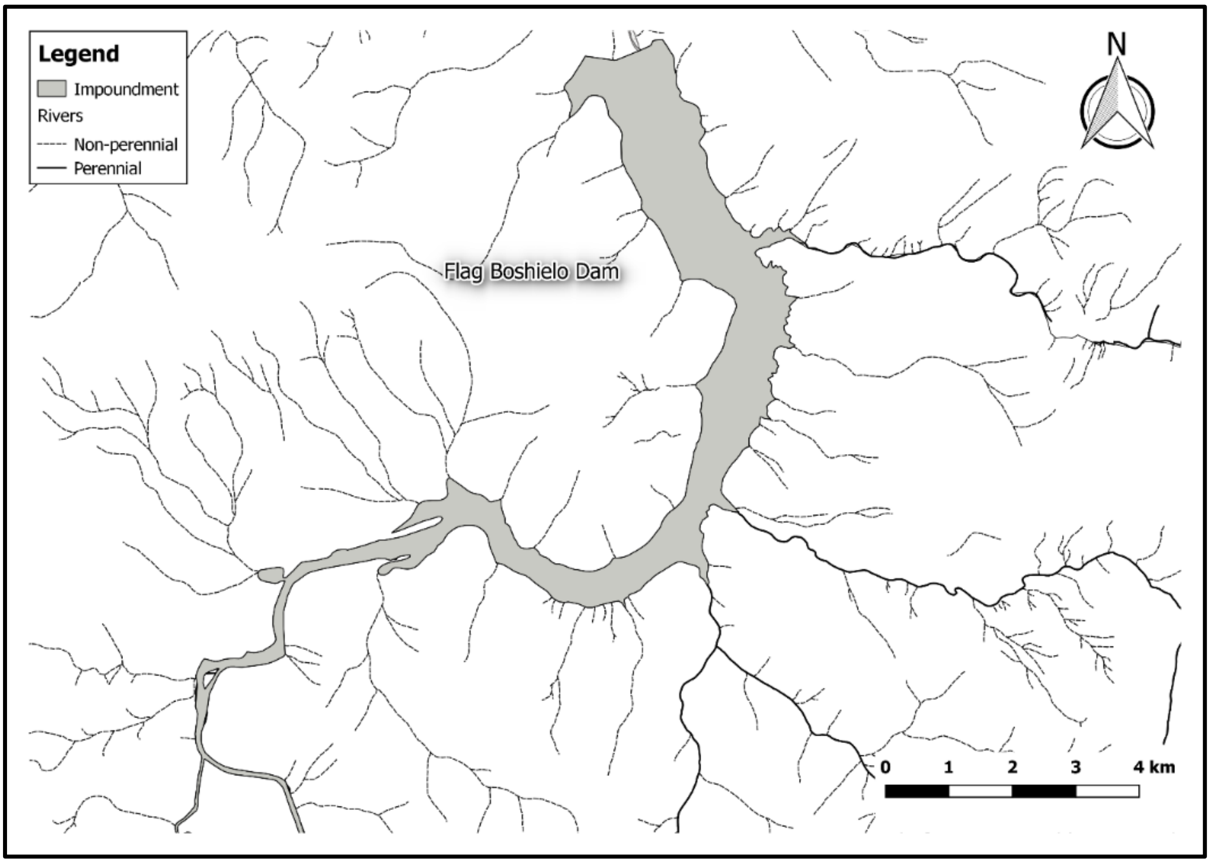

Figure 1.2: A map of Flag Boshielo Dam and neighbouring perennial and non-perennial rivers that flow into the system. Location: Dam wall: 24°46' 51.46"S: 029°25' 32.57"E.

The Schuinsdraai Nature Reserve, Limpopo Province, forms most of the western border of the dam. The reservoir is surrendered by typical savannah vegetation (Bushveld Basin Ecoregion), while the many partly submerged dead trees provide roosts for piscivorous birds like darters, herons, cormorants and fish eagles. The dam hosts a considerable, but declining, population of Nile crocodiles (Botha, 2010; Botha et al., 2011). Land use activities surrounding Flag Boshielo Dam can be categorized into four groups: (1) forest and woodland; (2) agricultural including commercial and semi-commercial; (3) urban including residential; (4) other land use, including degraded forest and woodland (Figure 1.3).

1.1.4 Fish community structure of Flag Boshielo Dam

The north-eastern area of South Africa (east flowing Limpopo, Phongolo and Incomati river systems) have a relatively larger fish fauna than the rest of the country due to historical connections to the tropical Zambezian Lowveld Ecoregion (Abell et al., 2008). This area contains a number of fish species that are considered to have fisheries potential, species that are generally larger and have a high market value, e.g. large cichlids of the genus *Oreochromis* (Tweddle et al., 2015). Flag Boshielo Dam, the second largest impoundment in Limpopo Province, has become an important recreational fishing venue, hosting regular angling competitions targeting species such as the indigenous Mozambique tilapia *Oreochromis mossambicus* and alien sport fishes including largemouth bass *Micropterus salmoides*, common carp *Cyprinus carpio* and silver carp *Hypophthalmichthys molitrix*. The latter species has become a major draw-card for competitive and recreational anglers after it "accidently" escaped from an aquaculture facility at Tompi Seleka College of Agriculture in the early 1990s (Brits, 2006). Since its introduction, silver carp have invaded the Olifants River downstream of Flag Boshielo Dam and their distribution range now extends to the lower reaches of the Olifants and Limpopo rivers into Mozambique.

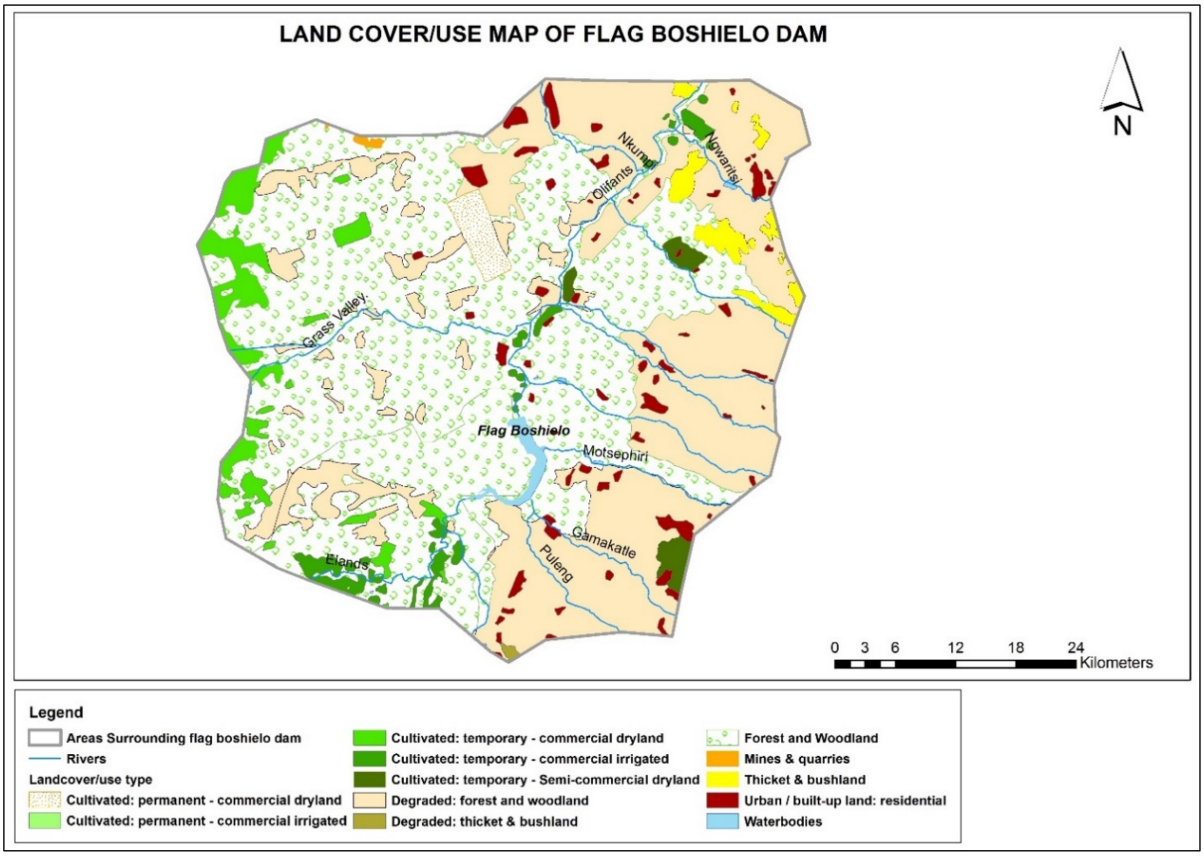

Figure 1.3: A map of land use activities associated with the Flag Boshielo System.

Brits (2006) conducted a comprehensive assessment of the fish assemblage of Flag Boshielo Dam using a suite of gears summarising the abundance and composition of the fish community (Table 1.1). The most abundant species of fisheries potential were *O. mossambicus*, *L. rosae*, *Coptodon rendalli* and *Schilbe intermedius,* comprising approximately 16, 11, 6 and 4% of the catch, respectively. Surveys conducted using gillnets in 2013 by Sara et al. (2017b) revealed that *L. rosae, O. mossambicus, Labeobarbus marequensis* and *S. intermedius* were the most abundant fish species constituting about 37, 16, 10 and 10% of the total catch, respectively.

Table 1.1: The relative abundance (% composition by weight; *n* = 52 kg) of fish species captured in Flag Boshielo Dam by Brits (2006) during October 1998 to December 2001.

Species	Common name	%
Enteromius paludinosus (Peters, 1852)*	Straightfin Barb	> 0.1
Enteromius rappax (Guimarães, 1884)*	Papermouth	0.6
Enteromius trimaculatus (Peters, 1852)*	Threespot Barb	3.1
Enteromius unitaeniatus Günther, 1866*	Slender Barb	13.1
Clarias gariepinus (Burchell, 1822)	Sharptooth Catfish	0.2
Coptodon rendalli (Boulenger, 1896)***	Redbreast Tilapia	5.6
Ctenopharyngodon idella (Valenciennes, 1844)**	Grass Carp	> 0.1
Cyprinus carpio Linnaeus, 1758**	Common Carp	0.1
Hypophthalmicthys molitrix (Valenciennes, 1844)**	Silver Carp	0.5
Labeo cylindricus Peters, 1852	Redeye Labeo	> 0.1
Labeo molybdinus Du Plessis, 1963	Leaden Labeo	0.2
Labeo rosae Steindachner, 1894	Rednose Labeo	11.4
Labeobarbus marequensis (A. Smith, 1841)	Largescale Yellowfish	2.3
Labeobarbus polylepis (Boulenger, 1907)	Smallscale Yellowfish	> 0.1
Marcusenius pongolensis (Folwer, 1934)†	Bulldog	0.1
Engraulicypris brevianalis (Boulenger, 1908)‡	River Sardine	25.0
Micralestes acutidens (Peters, 1852)	Silver robber	16.6
Micropterus salmoides (Lacepède, 1802)**	Largemouth Bass	> 0.1
Oreochromis mossambicus (Peters, 1852)	Mozambique Tilapia	15.9
Pseudocrenilabrus philander (Weber, 1897)	Southern Mouthbrooder	0.2
Schilbe intermedius Rüppell, 1832	Butter Catfish	3.6
Synodontis zambezensis Peters, 1852	Brown Squeaker	0.1
Tilapia sparrmanii Smith, 1840	Banded Tilapia	1.0

* indicates the genus previously known as *Barbus sensu* Skelton (2016) where ** indicates alien species, *** the genus previously known as *Tilapia sensu* Dunz and Schliewen (2013), ‡ the genus previously known as *Mesobola sensu* Riddin et al. (2016) and † the species previously known as *Marcusenius pongolensis (*Fowler, 1934) *sensu* Maake et al. (2014).

1.1.5 Inland fisheries case study: Flag Boshielo Dam

In addition to being ecologically and socio-economically important, Flag Boshielo Dam currently supports a subsistence fishery that is important to rural communities surrounding the impoundment (Dabrowski and De Klerk, 2013; Tapela et al., 2015). In the Water Research Commission study introduced in Section 1.2.3 above, nine fish surveys, using a standardized fleet of gillnets, were conducted from April 2016 to April 2017 at Flag Boshielo Dam to determine the fisheries potential of the impoundment, identify species of fisheries potential, and make recommendations regarding gear and effort restrictions to ensure sustainable utilisation of the resource. A total of 1376 fish (11 species from six families) were recorded (Table 1.2). Native species (*L. roase*, *O. mossambicus*, *Synodontis zambezensis* and *S. intermedius*) dominated the catches in terms of abundance and biomass (kg/100 m net/hr^{-1}). Cumulatively, the remaining species contributed 18% of the total catch. Based on their abundance, the degree by which they are targeted by fishers, the differences between their mode and feeding behaviour and the niche occupied by each species within the Flag Boshielo system, *O. mossambicus*, *L. rosae* and *S. intermedius* were selected for this current study.

Table 1.2: The relative abundance (% composition by number) of fish species captured from Flag Boshielo Dam during surveys conducted from April 2016 to April 2017 using gill nets. *Indicates alien species.

Species	Count	(%)
*Cyprinus carpio**	6	0.44
Clarias gariepinus	9	0.65
Coptodon rendalli	4	0.29
Enterobius rappax	9	0.65
Labeobarbus marequensis	6	0.44
Labeo rosae	808	**58.72**
Marcusenius pongolensis	12	0.87
*Micropterus salmoides**	6	0.44
Oreochromis mossambicus	291	**21.15**
Schilbe intermedius	15	1.09
Synodontis zambezensis	170	**12.35**
Crocodile damage	40	2.91
Total	**1376**	**100**

1.1.6 Fish edibility studies

People who regularly consume fish from contaminated water bodies are at risk of genotoxic, carcinogenic and non-carcinogenic health impairment from long-term exposure to toxic contaminants (du Preez et al., 2003). Addo-Bediako et al. (2014a), using a desk-top human health risk assessment assuming a weekly meal comprising a 150 g fish muscle, established the concentrations of lead (Pb), antimony (Sb) and chromium (Cr) in the muscle tissue of *O. mossambicus* from Flag Boshielo Dam exceeded the acceptable levels for safe consumption (Table 1.3). Similarly, Jooste et al. (2014) determined that Pb, Cr and Sb exceeded acceptable safe consumption levels for *L. rosae* and Addo-Bediako et al. (2014b) found that Pb, Cr and Sb exceeded acceptable levels for the safe consumption of *S. intermedius* (Table 1.3). Based on historical data, it appeared that the metal concentration in the muscle tissue of Mozambique tilapia was increasing particularly with for aluminium (Al), Cr, copper (Cu) and iron (Fe) (Addo-Bediako et al., 2014a). These disturbing results were based on samples collected in 2009-2010 (Jooste et al., 2013). Subsequent samples of *L. rosae* from surveys in 2012 revealed a different picture with only Pb and Sb being found to exceed safe consumption limits in only 50% of the fish analysed (Lebepe et al., 2016). Subsequent studies revealed that many of the metal concentrations in fish muscles were decreasing (Luus-Powell, unpublished data) highlighting the need to investigate the drivers of the metal concentrations in fish muscle in order to establish guidelines for the safe consumption of fish from contaminated impoundments. An explanation on the source, introduction and fate of metals in aquatic environments and their impact and accumulation in fish is described in **Appendix A**.

Table 1.3: The hazard quotients (HQs) for *Oreochromis mossambicus*, *Labeo rosae* and *Schilbe intermedius* from Flag Boshielo Dam calculated by Jooste et al. (2013) based on the average metal content in the muscle tissue assuming one fish meal (150 g) is consumed once a week. MC = metal concentration ($mg.kg^{-1}.fw$); ADD = average daily dose ($mg.kg^{-1}$), R_fD = reference dose ($mg.\ kg^{-1}$). HQ > 1 indicate a health risk to humans.

Metal	*Oreochromis mossambicus*				*Labeo rosae*				*Schilbe intermedius*			
	MC	ADD	R_fD	HQ	MC	ADD	R_fD	HQ	MC	ADD	R_fD	HQ
Al	15.00	4.58	1000.00	0.00	15.60	4.78	1000.00	0.00	13.80	4.23	1000.00	0.00
Sb	5.00	1.52	0.40	3.79	3.90	1.21	0.40	3.02	0.10	0.05	0.40	0.11
As	0.20	0.06	0.30	0.19	0.20	0.08	0.30	0.19	0.90	0.28	0.30	0.95
Ba	5.80	1.77	200.00	0.01	6.70	2.05	200.00	0.01	81.10	24.82	200.00	0.12
B	24.90	7.62	200.00	0.04	29.90	9.16	200.00	0.05	65.00	19.89	200.00	0.10
Cd	0.00	0.00	3.00	0.00	0.10	0.02	0.50	0.01	0.00	0.00	0.05	0.00
Cr	9.20	2.82	3.00	0.94	11.20	3.44	3.00	1.15	3.80	1.15	3.00	0.38
Co	0.50	0.15	0.40	0.38	0.70	0.20	0.40	0.51	0.10	0.02	0.40	0.06
Cu	2.60	0.81	40.00	0.02	1.40	0.44	40.00	0.01	1.10	0.33	40.00	0.01
Fe	161.70	49.49	700.00	0.07	266.90	81.70	700.00	0.12	28.40	8.70	700.00	0.01
Pb	1.00	0.31	0.06	5.15	0.80	0.25	0.06	4.18	0.80	0.24	0.06	4.06
Mn	1.30	0.40	140.00	0.00	2.40	0.74	140.00	0.01	1.10	0.34	140.00	0.00
Ni	0.50	0.15	20.00	0.01	0.60	0.19	20.00	0.01	0.20	0.07	20.00	0.00
Se	0.40	0.13	5.00	0.03	3.30	1.02	5.00	0.20	0.60	0.19	5.00	0.04
Ag	0.00	0.00	5.00	0.00	3.30	1.02	5.00	0.00	0.40	0.13	5.00	0.03
Sr	0.40	0.12	600.00	0.00	3.10	0.95	600.00	0.00	1.90	0.57	600.00	0.00
Sn	1.10	0.32	600.00	0.00	0.90	0.26	600.00	0.00	0.40	0.19	600.00	0.00
V	7.70	2.37	5.00	0.47	8.00	2.45	5.00	0.49	0.10	0.02	5.00	0.00
Zn	6.40	1.97	300.00	0.01	12.60	3.84	300.00	0.01	52.80	16.15	300.00	0.05

Where fw = fresh muscle weight and Al (Aluminium), Sb (Antimony), As (Arsenic), Ba (Barium), B (Boron), Cd (Cadmium), Cr (Chromium), Co (Cobalt), Cu (Copper), Fe (Iron), Pb (Lead), Mn (Manganese), Ni (Nickel), Se (Selenium), Ag (Silver), Sr (Strontium), Sn (Tin), V (Vanadium), Zn (Zinc). ADD calculated according to Heath *et al.* (2004b). Risk assessments evaluating non-carcinogenic toxic effects of contaminants use reference doses (RfDs) as thresholds above which adverse health impacts could be expected with RfD levels published by the US-EPA being used (US-EPA, 2012). HQ calculated as ADD ÷ RfD.

AIM AND OBJECTIVES

The aim of this project is to evaluate seasonal fluctuations in the metal concentration in fish muscle tissue in Flag Boshielo Dam in order to establish guidelines for the safe consumption of fish from contaminated impoundments. The results of the project are expected to provide information to inform policy for the development of inland fisheries on South Africa's water storage impoundments. The results of this project should directly inform the Inland fisheries Policy, which refers directly to the requirement that the fish products from inland fisheries should be monitored to determine whether they are suitable for human consumption.

In order to achieve the stated aim of the project, the following objectives have been outlined for the project:

- Monitor seasonal fluctuations in selected water quality parameters at Flag Boshielo Dam during drought and flood cycles (if possible, within the duration of the project),
- Monitor seasonal fluctuations in the metal concentration in fish muscle at Flag Boshielo Dam,
- Investigate whether environmental and limnological factors can be identified as drivers of the concentration of metals in fish muscle tissue at Flag Boshielo Dam using linear regression and distance based linear modelling, and
- Use the findings of the study to inform the Inland Fisheries Policy regarding the safe utilisation of fish captured from Flag Boshielo Dam for human consumption.

STRUCTURE OF THIS REPORT

This report is presented in five chapters. Each chapter addresses specific components of the scope of work outlined for this project. Here, an outline of each chapter is presented to provide the reader a quick overview of the content of the respective chapters and their contribution to the report.

***Chapter 1*: Introduction and Literature Review**

This chapter provides the context for the study including the aim and objectives of this work. A literature review is presented to provide the necessary background for each of the subjects addressed in the report. Flag Boshielo Dam, the study locality, is introduced and a review of recent studies presented.

***Chapter 2*: Historical Data**

This chapter provides an overview of the historical water quality and fluctuations in dam levels and flow at Flag Boshielo Dam prior to the collection of water, sediment and fish samples for this study.

***Chapter 3*: Long-term Monitoring**

This chapter presents the long-term monitoring data for Flag Boshielo Dam including the limnology, water, sediment, and fish muscle tissue of three fish species. In addition, a Human Health Risk Assessment was conducted to establish whether consumption of these fish poses a threat to human health.

***Chapter 4*: Drivers of metal concentrations in fish tissue**

This chapter evaluates environmental and limnology drivers of the metal concentration in the muscle tissue of the three fish species from Flag Boshielo Dam using generalised linear models and distance-based redundancy analysis.

***Chapter 5*: Conclusion and recommendations**

This chapter discusses the results of the study and provides guidelines for the Inland Fishery Policy regarding the safe utilisation of fish from Flag Boshielo Dam for human consumption and provides suggestions for future work based on the findings of the project.

CHAPTER 2: HISTORICAL DATA

INTRODUCTION

The Olifants River (Limpopo River System) plays a key role in supplying water that sustains the rapid socio-economic development of the Limpopo Province. For the past few decades, the Olifants River has been experiencing rapid development in its upper catchment. The upper reaches of the Olifants River catchment are characterised by large-scale coal mining, coal-fired power generation, intensive irrigation agriculture, a variety of industrial practices, including ferro-chrome and ferro-manganese smelters, as well as several town and urban centres (Driescher, 2008; Ashton, 2010) and the development of small and large informal settlements. Land use activities in the middle sub-catchment include widespread irrigation agriculture and several platinum, chrome and vanadium mines, ferro-chrome refineries, and a number of urban and rural settlements. In turn, the lower sub-catchment is populated by large game conservancies, several mines and a major copper and phosphate operation near the town of Phalaborwa prior to where the Olifants River flows into the Kruger National Park (KNP) (Ashton, 2010; Heath et al., 2010).

Over the years the Olifants River has been systemically impaired by mining, industrial, agricultural and domestic pollution, including acid mine drainage (AMD) (Ashton and Dabrowski, 2011). The influx of toxins has resulted in the Olifants River being one of the most polluted river systems in South Africa (Heath et al., 2010; Ashton and Dabrowski 2011; Huchzermeyer et al., 2011; Jooste et al., 2015; Marr et al., 2015; Azeez et al., 2017; Oberholster et al., 2017). Intensive and subsistence agriculture practices in all the sub-catchment areas, in conjunction with mining and industrial activities in the Witbank-Middelburg areas have impacted the water quality of the Olifants River, particularly in the upper Olifants catchment. Agricultural, mining and industrial effluents contain complex mixtures of chemicals, many of which may have deleterious effects on aquatic systems. In addition, acid mine drainage from abandoned mines and smouldering mine dumps in the upper catchment is resulting in the acidification of streams and the mobilisation of metals (McCarthy, 2011; Netshitungulwana and Yibas 2012). Further, the uncontrolled release of treated and raw sewage in the upper reaches has been identified as a major concern (Huchzermeyer et al., 2017; Oberholster et al., 2017). As a result, there is increased concern over the potential long-term impact of water pollution on the health of rural communities of the Olifants River catchment, especially those still reliant on untreated water from the river.

HISTORICAL FLOW DATA

Historical water flow data for the Olifants River since the construction of Flag Boshielo Dam was compiled from the Department of Water and Sanitation’s Resource Quality Services web site. Data for the Elands River (B3H020 and B3H021) and the Olifants River above (B3H001 and B3H025) and immediately below Flag Boshielo Dam (B5H004) were downloaded and analysed for patterns in, or correlations between, the river flow and water quality parameters available from this source. Data from some gauging stations were not complete and data from multiple stations were required to complete the data set.

From these data, a clear pattern emerges in the flow of the Olifants and Elands rivers. The system appears to cycle between dry periods of low flow and wet periods with frequent high flow events (Figure 2.1). The lengths of the wet and dry cycles are variable and are driven through rainfall patterns. As a result of global climate change, De Wit and Stankiewicz (2006) predict a reduction in rainfall of more than 10% over the Olifants River catchment by the end of the 21st Century. A 10% decrease in precipitation could result in a greater than 50% decrease in the runoff of the system (De Wit and Stankiewicz, 2006). Climate change predictions forecast greater stochasticity in rainfall events where the annual precipitation will be received in less frequent rainfall events of greater intensity. For a catchment already experiencing wet and dry cycles, this could mean more frequent and longer durations for the dry cycles and more intense flooding events in the wet cycles.

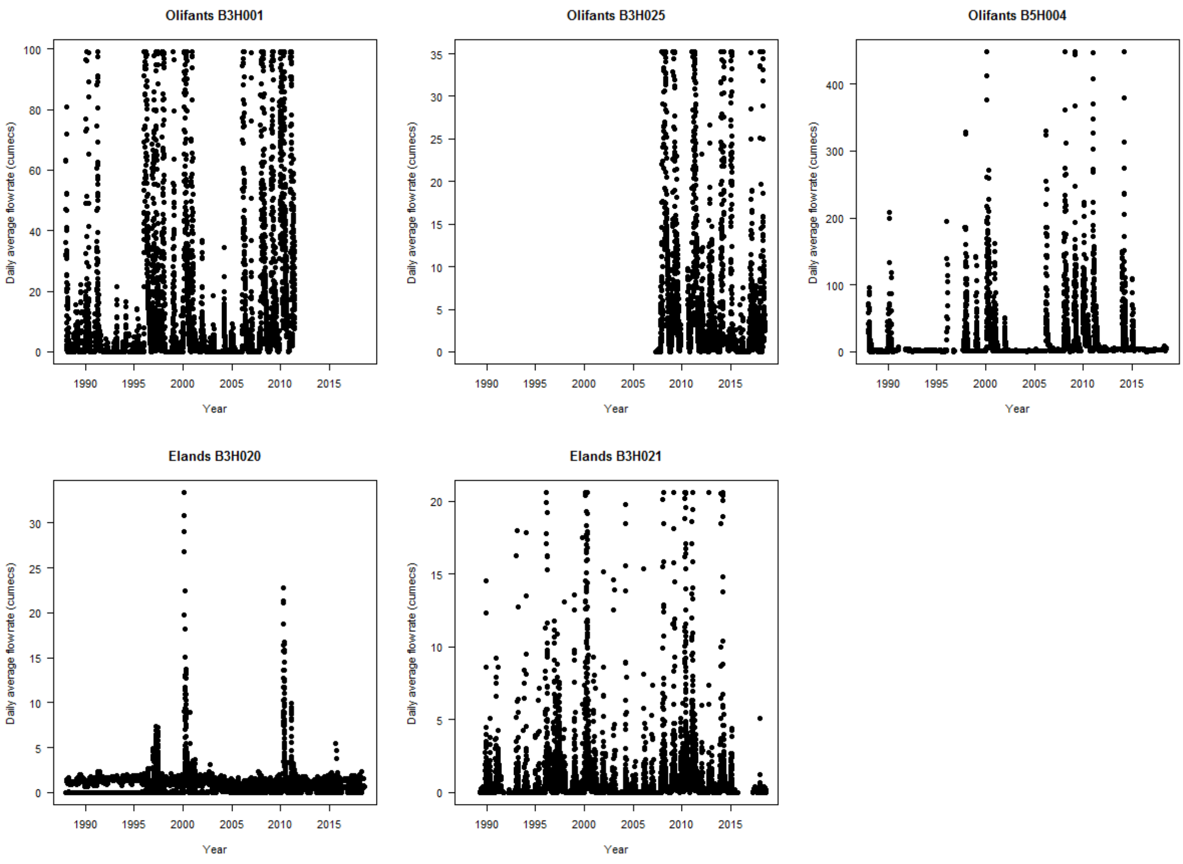

Figure 2.1: Time series plots for water flow in the Olifants and Elands rivers upstream of Flag Boshielo Dam and the Olifants River downstream of the dam wall.

HISTORICAL WATER DATA

Historical water quality data was acquired from the Department of Water and Sanitation's Resource Quality Services web site. Data for Flag Boshielo Dam wall (B5R002), the Elands River (B3H021) and the Olifants River above (B3H001) and below (B5H004) Flag Boshielo Dam was downloaded and analysed to determine whether there were patterns or correlations between various water quality parameters.

Analysis of water quality data from Flag Boshielo Dam identified three different groups; parameters that are strongly related to high flow events and correlated with each other, e.g. electrical conductivity (EC), chlorine (Cl), sodium (Na), fluoride (F), potassium (K) and alkalinity (Alk); parameters that are impacted by high flow events but not correlated to EC, e.g. calcium (Ca), Magnesium (Mg), pH and sulphate (SO_4); and parameters present at low concentrations but not impacted by high flow events or correlated to the other parameters, e.g. nutrients such as ammonium (NH_4), nitrate-nitrite ratio (NO_3:NO_2) and ortho-phosphate (PO_4).

By plotting total dissolved solutes of Cl, Na, F, K and Alk (a measure of the carbonate/bicarbonate ions) with EC, results showed strong correlations towards each other (Figure 2.2). Moreover, the correlated water parameters show distinct high flow related patterns, where spikes in parameter concentrations can be observed when plotted as a time series (Figure 2.3). The time series data suggests that multiple year droughts are experienced, punctuated by large floods. The decrease in concentration of all of these parameters at the beginning of 2006 (Figure 2.3) can be attributed to the raising of the dam wall by 5m and the large flood that occurred in late January 2006 (Dabrowski et al., 2014). A three-year drought preceded the raising of the dam wall demonstrating that drought conditions can increase the concentration of salts in an impoundment.

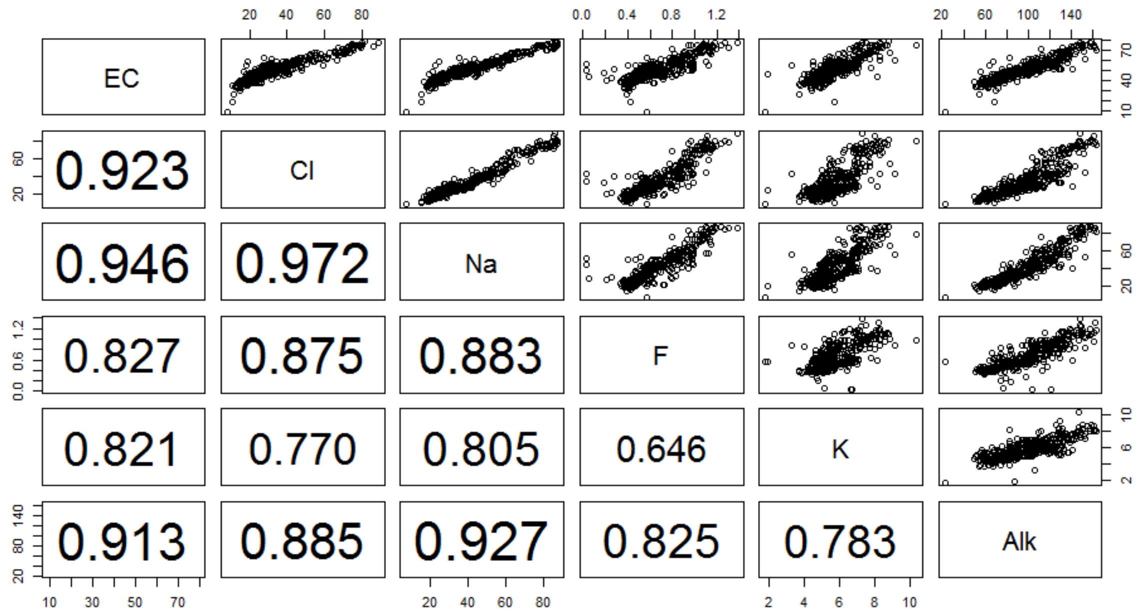

Figure 2.2: Pearson correlation between electrical conductivity (EC), chloride (Cl), sodium (Na), fluoride (F), potassium (K) and alkalinity (Alk) in the water of Flag Boshielo Dam.

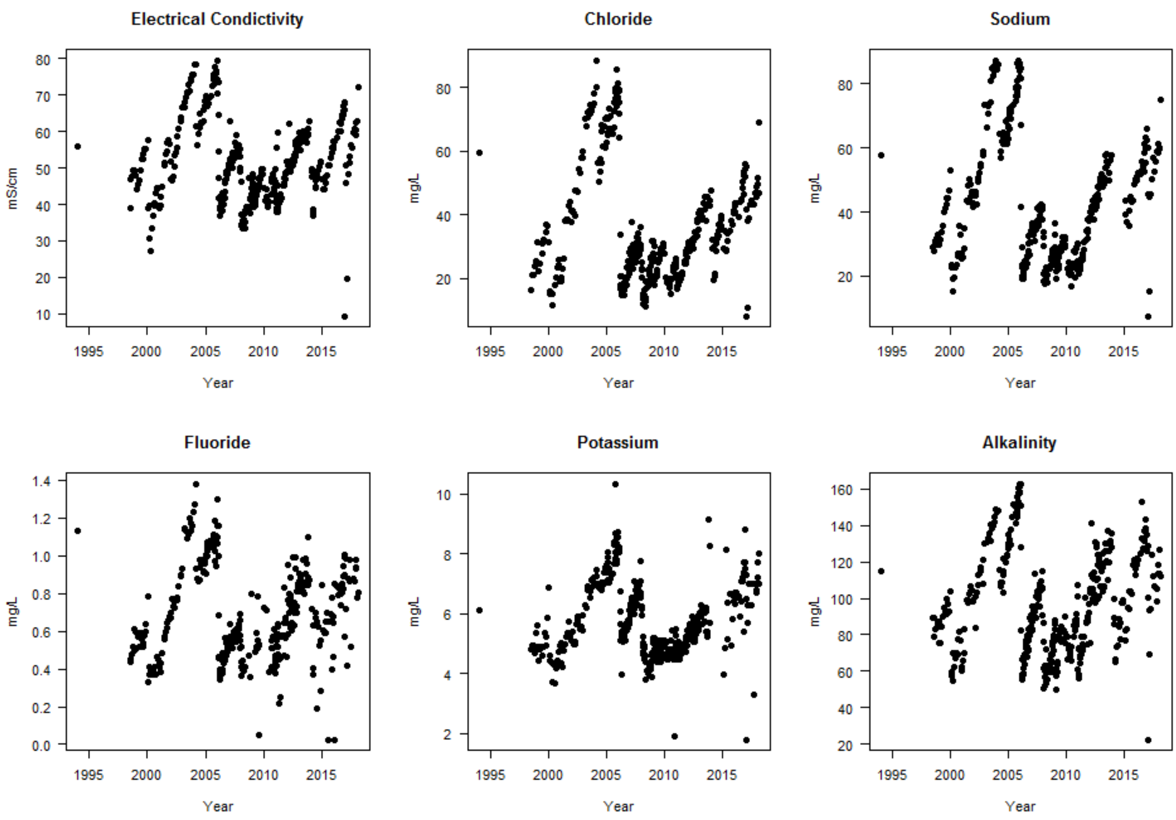

Figure 2.3: Time series plot for electrical conductivity (EC), chloride (Cl), sodium (Na), fluoride (F), potassium (K) and alkalinity (Alk) in the water of Flag Boshielo Dam.

For the second group of water parameters, i.e. Ca, Mg, pH and SO_4, there is a weaker correlation with EC (Figure 2.4) and the impact of high flow events is less pronounced (Figure 2.5).

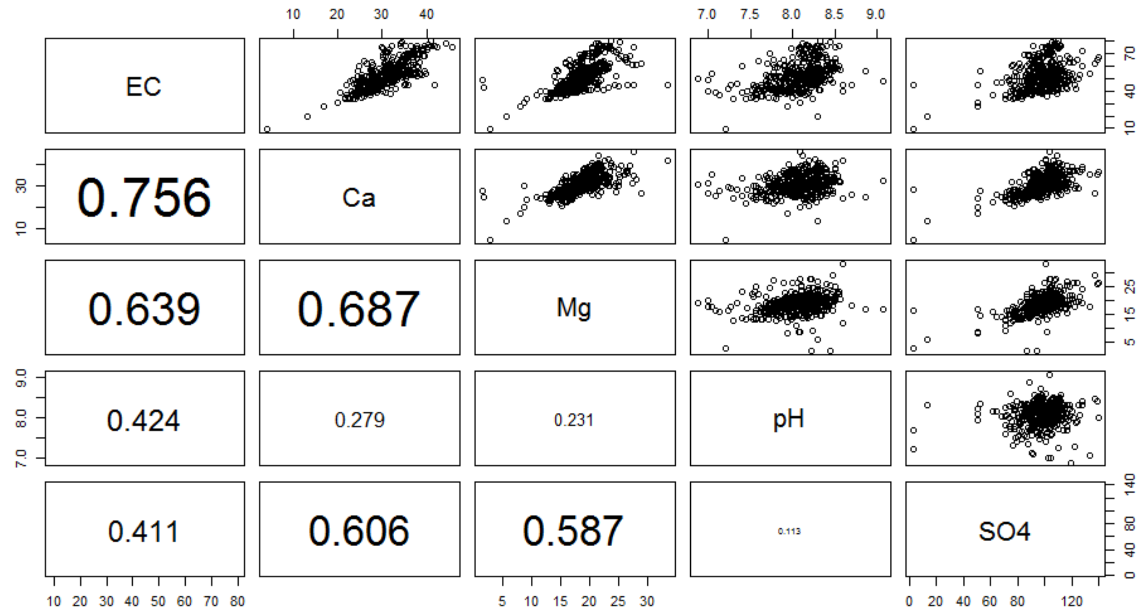

Figure 2.4: Pearson correlation between electrical conductivity (EC), calcium (Ca), magnesium (Mg), pH and sulphate (SO_4) in the water of Flag Boshielo Dam.

The impact of flow events on the second group of water parameters are more constrained, or buffered, than the first group and appear to have upper and lower limits to their concentrations. The SO_4 concentration does not appear to be increasing as suggested by Jooste et al. (2015) but it should be noted that these authors based their statements on the trajectory of the annual median value based on the data evaluated here.

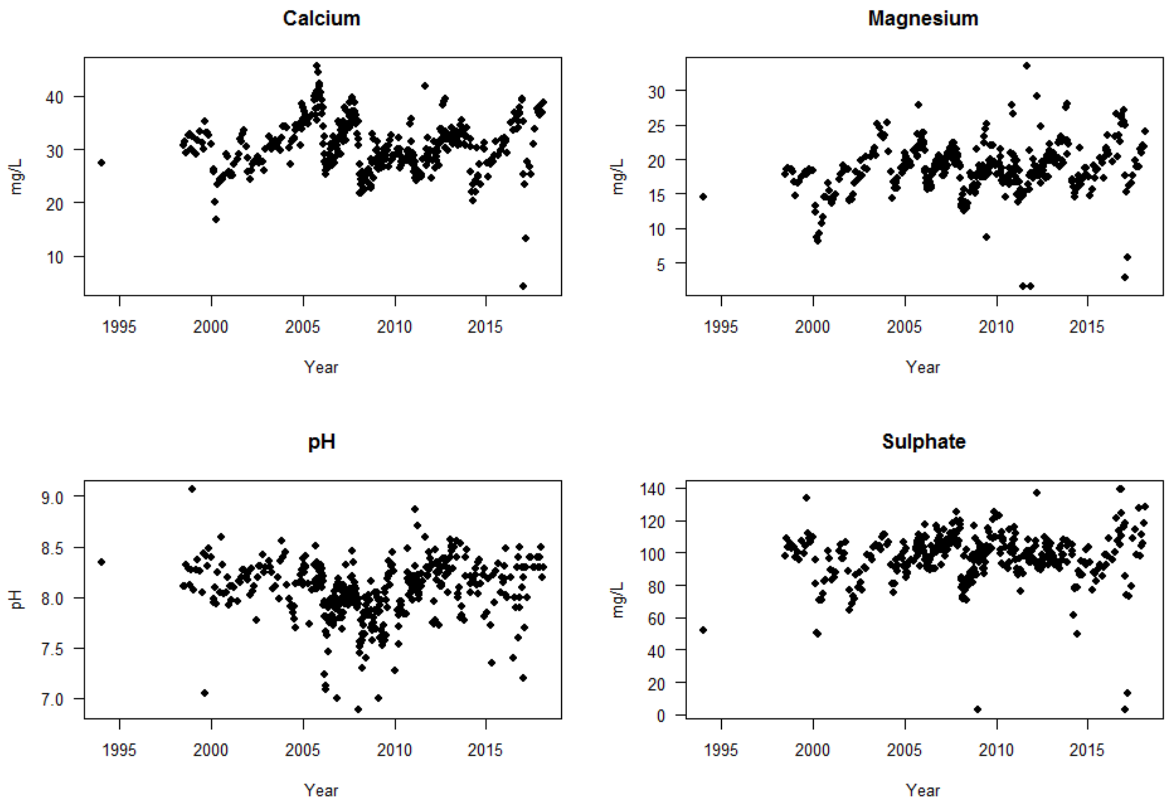

Figure 2.5: Time series plots for calcium, magnesium, pH and sulphate in the water of Flag Boshielo Dam.

The analysis of the Olifants River site below the Dam Wall unsurprisingly reflected the patterns shown by the water parameters of the impoundment. The samples from the riverine sites on the Olifants and Elands rivers above the lake showed similar patterns with almost all water parameters being highly correlated with each other, including pH. The exception being the third group of parameters, i.e. nutrients; PO_4, NH_4 and NO_3:NO_2 and potassium K. These data still displayed the wet-dry cycle pattern observed for the dam, but these are less clearly defined for the sites upstream of the dam (Figures 2.6 and 2.7). For all water parameters presented in Figures 2.6 and 2.7, a gradient exists: the concentration of the specific parameters is highest in the Olifants River upstream of the lake and lowest in the Olifants River downstream of the impoundment. The lake appears to have a self-cleansing impact on the water parameters, probably resulting in the water parameters being bound in the sediment or biota of the impoundment. It can be inferred that a similar process may be occurring with the metals.

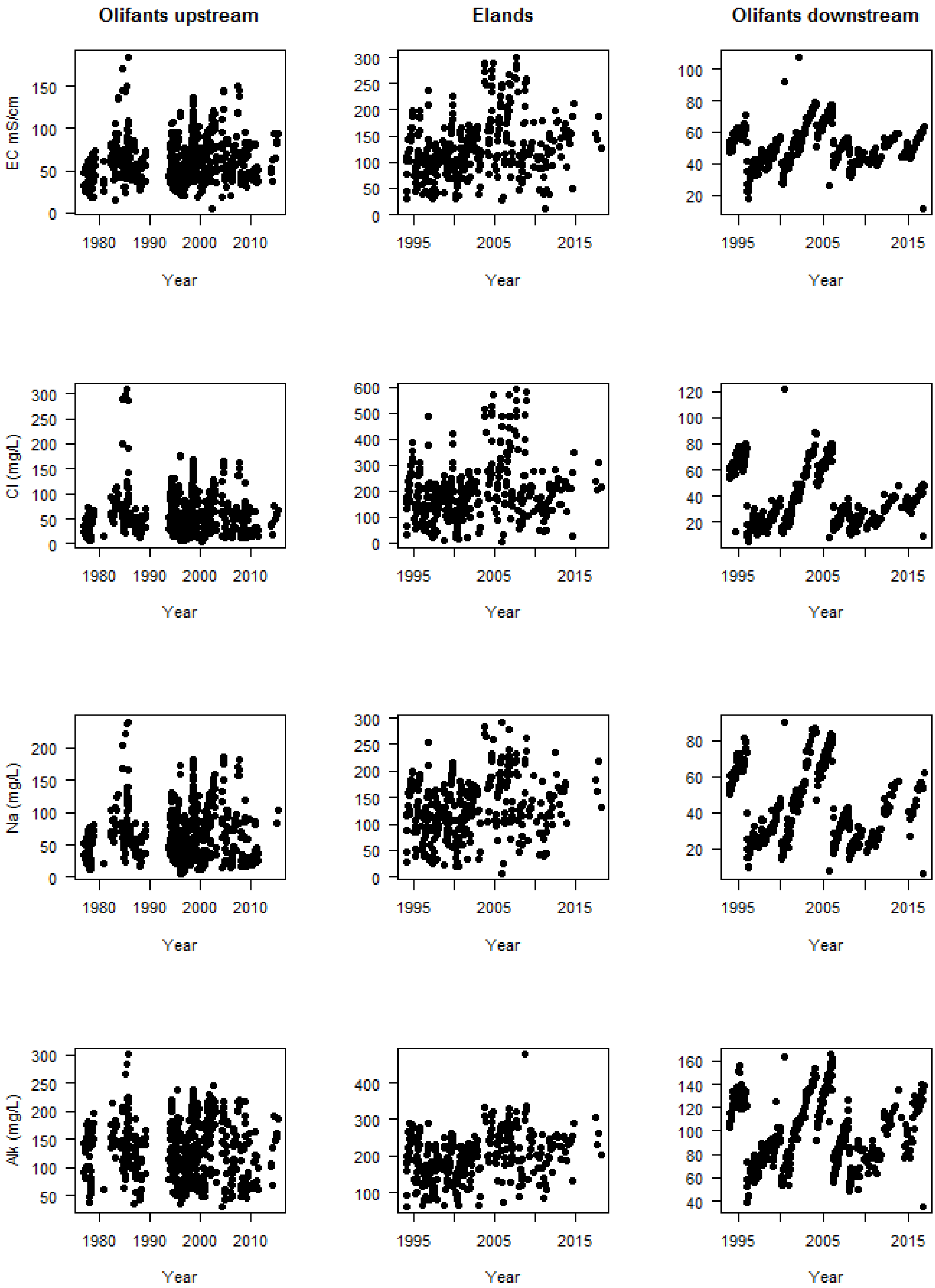

Figure 2.6: Time series plots for electrical conductivity (EC), chloride (Cl), sodium (Na) and alkalinity (Alk) in the water of the Olifants and Elands rivers upstream of Flag Boshielo Dam and the Olifants River downstream of the dam wall.

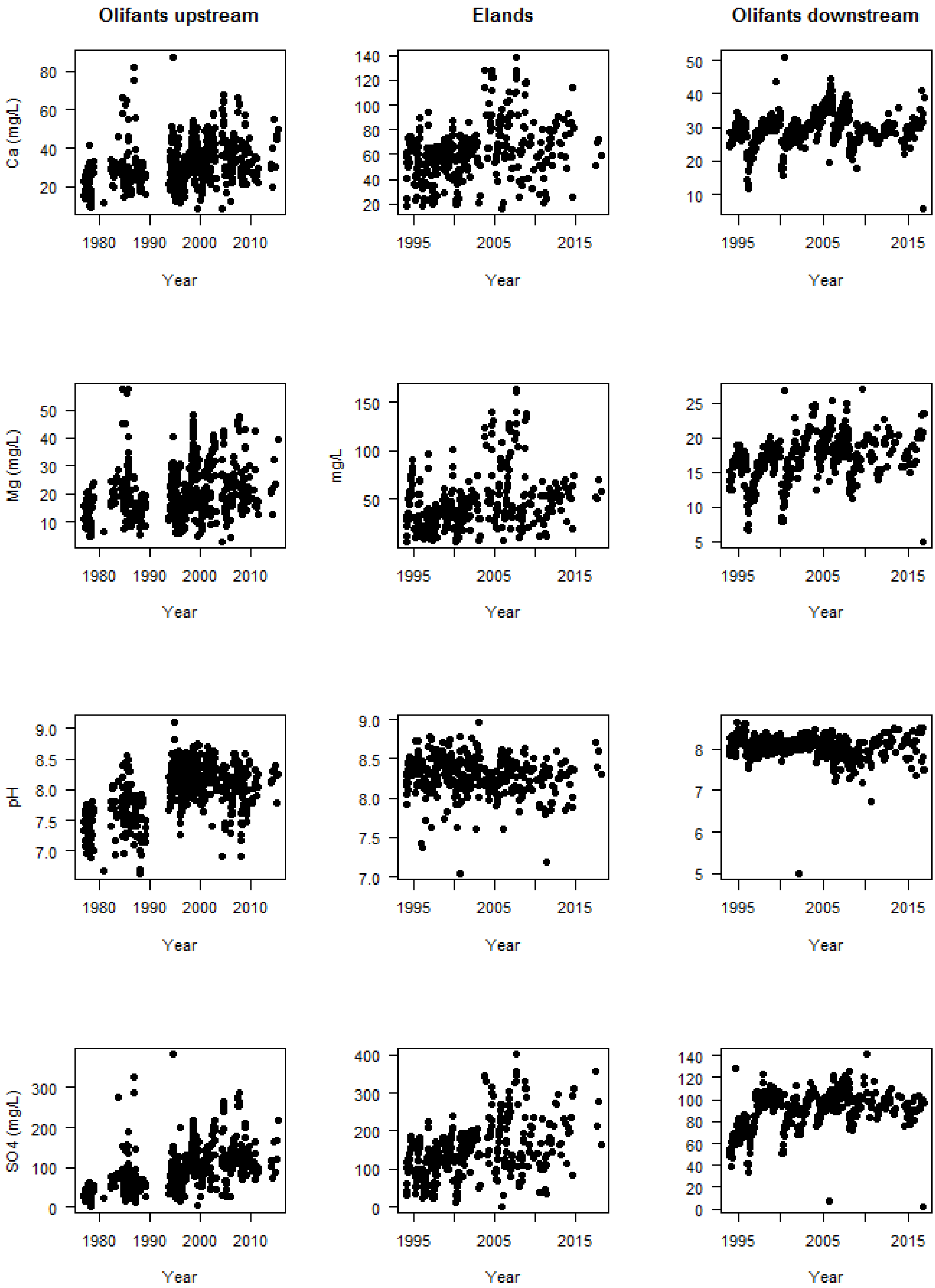

Figure 2.7: Time series plots for calcium (Ca), magnesium (Mg), pH and sulphate (SO_4) in the water of the Olifants and Elands rivers upstream of Flag Boshielo Dam and the Olifants River downstream of the dam wall.

In order to evaluate whether the wet-dry cycle patterns in the water quality parameters were truly driven by high flow events that putatively reset the concentration of ions in the impoundment, the flow pattern was overlaid on the data for the EC for the site B5H004, which is just below the dam wall (Figure 2.8). It becomes apparent that the high flow events all coincide with a reset of the conductivity (Figure 2.8). Flood events are, therefore, important at reducing the concentration of the salts in the waters of Flag Boshielo Dam with a gradual increase in salt concentrations occurring over periods of lower flows and water draw-down during dry periods and protracted droughts.

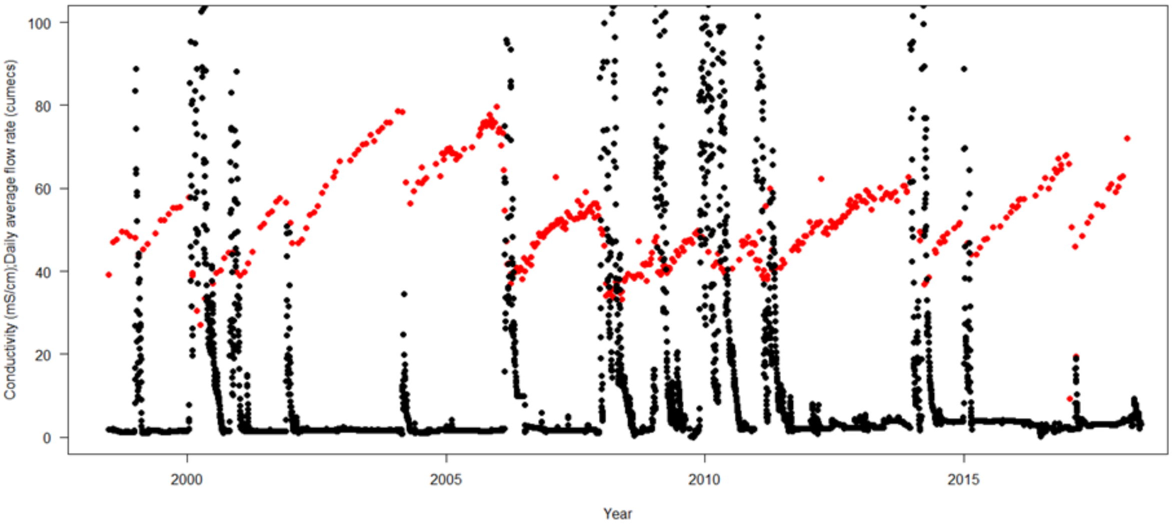

Figure 2.8: Time series plot of the electrical conductivity (EC) and water flow in the Olifants River downstream of Flag Boshielo Dam with EC signified by black dots while the flow in cumecs is signified by the red dots.

WET AND DRY CYCLES OF FLAG BOSHIELO DAM

The Olifants River appears to go through wet cycles characterised by frequent high flow events that lead to Flag Boshielo Dam reaching full capacity for extended periods. These are followed by dry cycles when inflow into the system is low for prolonged periods resulting in water draw-down and low levels in the impoundment. This raises the question as to how wet and dry cycles impact the concentrations of metals in the environment and in the muscle tissue of fish? From physical chemistry theory, a number of predictions can be made.

Environmental chemical reactions are governed by temperature, pH, redox and time. At ambient temperatures, which are low in chemistry terms, the rates of reactions are reduced and consequently take considerable time to reach equilibrium. At low flow, the residence time of the water in the river system increases and it is possible for the chemical reactions to progress further towards equilibrium. In contrast, at high flows the residence time is reduced and chemical reactions do not progress as far towards equilibrium in the same spatial scale. In addition, during the low flow dry cycles, the water passes more slowly through biological filters in the catchment, such as reed beds, and the lack of scouring flows results in the reed beds extending further into the river channel thereby increasing the biological uptake of metals to aquatic vegetation. During the low flow dry cycle, the extent of the reactions would progress further towards equilibrium and the biological uptake of metal is higher than in the high flow wet cycle and, therefore, concentrations of metals in the water column would be reduced in comparison to those of the high flow wet cycle.

The impoundment has a buffering, or smoothing, effect on the concentrations of metals and other chemical concentrations in the water column. This can be explained by the residence time of the water in the impoundment. During the low flow events, the residence time of the water in the impoundment is increased, as is evaporation from the impoundment surface. The increased evaporation results in an increase in the concentration of chemicals in the water column. The concentration is reset when a high flow event occurs. Depending on the magnitude of the high flow event, and the degree of mixing that takes place as a result of the increased river discharge, the concentrations of the chemicals in the impoundment's water column are reduced to those of the incoming river water. This explains the pattern of the EC, and other chemical concentrations, increasing during periods of low flow only to be reset by periods of high flows (Figure 2.8). From the flow data, it appears that flooding events greater than 20 cumecs flow are required to reset concentration of chemicals in in the water column of Flag Boshielo Dam.

Since floods are shown here to be a major driver resetting the chemical concentrations of the water column, it can be inferred that high flows also have the potential to increase the concentrations of metals in the water column of the impoundment making the time and duration since the last flood flow an important factor to consider with regard to the concentration of metals in fish muscle tissue. Furthermore, since the wet and dry cycles are determined by rainfall patterns with river discharge highly correlated to rainfall, rainfall was not considered further as an environmental driver of the metal concentration in fish muscle tissues.

Finally, historical land-use change was initially proposed as a potential environmental driver of the metal concentrations in fish muscle tissue. However, when the available land-use data was reviewed, we found that it was not available on a suitable temporal resolution such that could be incorporated in the statistical procedures used in this study.

.

CHAPTER 3: LONG-TERM MONITORING

INTRODUCTION

Studies conducted by Addo-Bediako et al. (2014a; 2014b), Jooste et al. (2014; 2015), Lebepe et al. (2016), Marr et al. (2017) and Sara et al. (2017a; 2018) on the metal content in muscle tissue of various fish species from Flag Boshielo Dam revealed that the consumption of these fish was not advisable due to unacceptably high levels of certain metal that would pose a health risk to humans if consumed over an extended period. These studies, conducted when the storage capacity of the dam was in excess of 80%, raised the question whether it is prudent to initiate and develop a fishery in this impoundment. In this chapter, we evaluate whether the metal concentrations in the muscle tissue of the three selected fish species (Mozambique tilapia *O. mossambicus*; the rednose labeo *L. rosae* and silver catfish *S. intermedius*), and the associated health risks of long-term consumption to humans, fluctuates temporally as the impoundment's storage capacity changes. This aim was achieved by completing the following objectives:

- Monitor the storage capacity of Flag Boshielo Dam to establish the extent by which water levels fluctuate over time;
- to determine the nutrient and physico-chemistry properties of water and the metal concentrations in water and sediment collected during surveys conducted at Flag Boshielo Dam over the study period;
- to establish the concentration of selected metals in the muscle tissue of the three fish species collected when conducting fish surveys at Flag Boshielo Dam over the study period, and
- to conduct a Human Health Risk Assessment so as to ascertain whether the consumption of fish collected from the Flag Boshielo Dam over the study period posed a health risk to humans.

METHODOLOGY

For logistical reasons, Flag Boshielo Dam was sampled randomly from February 2016 and May 2018 whereby 11 fish surveys were conducted during this period (see Figure 3.1). During each survey the water physico-chemical properties were measured and the metal content in water, sediment and fish established.

3.1.1 Changes in recorded water levels

Water level data for Flag Boshielo Dam was acquired from the Department of Water and Sanitation web site (https://www.dwa.gov.za/hydrology/weekly/percentile.aspx?station). At the commencement of this study, water levels in Flag Boshielo Dam were below 50% of full capacity (Figure 3.1) decreasing to approximately 18% in November and early December 2016. Low rainfall in the catchment resulted in that neither the Olifants nor the Elands rivers contributed towards water flow into the system. In contrast during November 2016, Loskop Dam was at 60.4% capacity while water levels in the Rust de Winter and Rhenosterkop dams, upstream on the Elands River, were at 66.3% and 27.5% respectively. During

December 2016 and January 2017, rainfall over the catchment raised water levels in Flag Boshielo Dam to 43.1%. High rainfall over September-December 2017 and January-February 2018 resulted in the reservoir reaching full capacity in March 2018. The extent by which water levels have fluctuated in Flag Boshielo Dam on a month to month basis over the study period is depicted by the solid black line in Figure 3.1.

3.1.2 Physico-chemical parameters

During each survey, water temperature (°C), pH, dissolved oxygen concentration (mg/L), electrical conductivity (EC; µS/cm), total dissolved solids (TDS; ppm) and salinity (‰) were measured *in situ* at each site using a handheld multi-parameter probe (YSI Model 554 Data logger with a 4 m or 20 m multiprobe). Measurements within the littoral zone were taken immediately below the surface and at 1 m intervals to a depth of 2 m. Measurements were recorded between 6:00 and 10:00 am. Sites representative of the riverine (Site 1, 2 and 3); transition (Sites 4 and 5) and lacustrine (Site 7 and 8) regions were selected (Figure 3.2).

Water parameters measurements were recorded thrice at each of these sites and the mean and standard error reported. By comparison water readings in the limnetic zone of the impoundment, at Site 6 (M) and Site 8 (L18), were taken at 1 m intervals using the same multi-probe meter to a depth of 20 m. At these sites the top and bottom depth of metalimnion, thermocline depth and centre of buoyancy was calculated using the rLakeAnalyzer package (Winslow et al., 2018) in R statistical software (R Development Core Team, 2018). Similarly, the centre buoyancy of water was determined using the c.b () function from rLakeAnalyzer package and the R statistical software. A Shapiro-Wilk test was performed to determine whether the water data was normally distributed. The non-parametric Kruskal-Wallace test was performed to determine for significant differences between surveys. During periods of low or no inflow, sampling at the confluence of the Olifants and Elands Rivers (Site 1) had to be terminated. Similarly, further drawdown and receding water levels resulted in that sampling at sites 2 and 3 had to be terminated. The sites surveyed are depicted in Figure 3.2. Recordings of water parameters were compared with the Minimum Allowable Values (MAV) and Target Water Quality Range (TWQR) specified by DWAF (1996a).

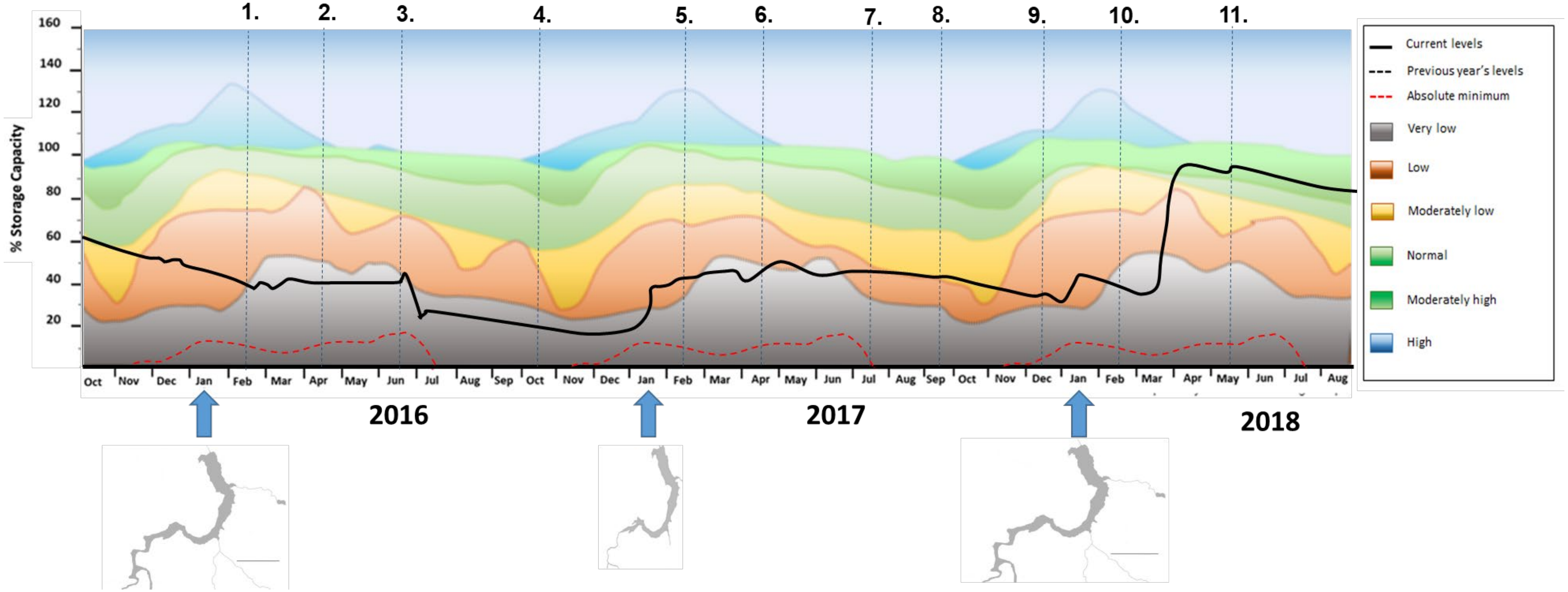

Figure 3.1: The sampling events conducted at, and changes in storage capacity (%) of Flag Boshielo Dam between October 2015 and August 2018. Blue arrows indicate the start of each year while the month and number of surveys undertaken are indicated by numbers 1 to 11. The black solid line signifies the change in the storage capacity (%) of Flag Boshielo Dam over time.

(Source:https://www.dwa.gov.za/hydrology/weekly/percentile.aspx?station = B5R002; Accessed September 2016, August 2017 and January 2018).

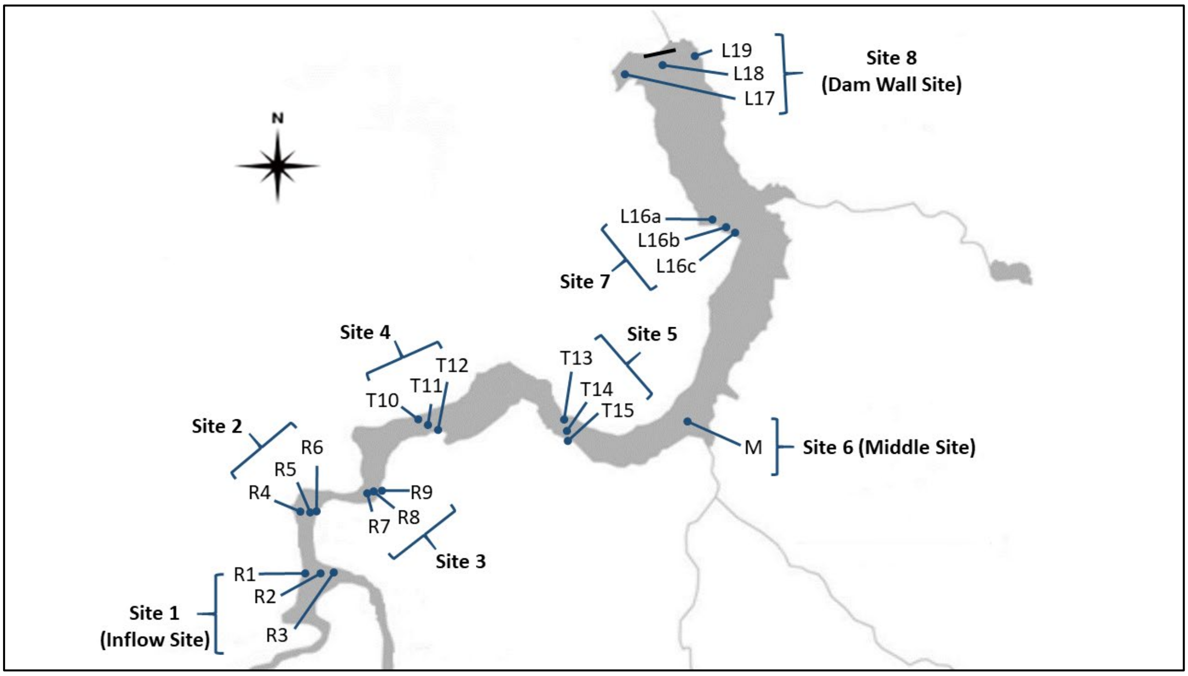

Figure 3.2: Sites surveyed in Flag Boshielo Dam with sites 1, 2 and 3 located within the riverine zone (R), sites 4, 5 and 6 within the transition zone (T) and sites 7 and 8 in the lacustrine zone (L).

3.1.3 Water and sediment

Water and sediment samples analysed for metal content were collected seasonally (i.e. quarterly) at site 1 (R1), site 5 (T14) and site 8 (L18); see Figure 3.2. These sites were chosen to provide a profile from the inlet to the dam wall along the thalweg of the impoundment. Subsurface water samples were collected at a depth of 30 cm in 1000 mL polyethylene bottles (acid pre-treated) and stored frozen at -5°C prior to the analyses of nutrients and a suite of metals, metalloids and anions at Waterlab, a SANAS accredited laboratory (ISO/IEC 17025:2005) in Pretoria. The water samples were analysed in batches with blanks using inductively coupled plasma-optical emission spectrophotometry (ICP-OES; Perkin Elmer, Optima 2100 DV). Analytical accuracy was determined using certified standards (De Bruyn Spectroscopic Solutions 500MUL20-50 STD2) and recoveries were within 10% of certified values. Instances when nutrient data were not provided by the laboratory, the desired and relevant information was sourced from the Department of Water and Forestry website (www.DWAF.gov.za/IWQS/WMS/Data/000key.Asp). From the website, datum from the month proceeding, during and following, the surveys were extracted and an average established.

Sediment samples were collected using a Friedlinger mud grab. Three to five sediment samples from each of the three sites were taken, combined and transferred to a 500 mL acid pre-treated polyethylene sampling bottle. These samples were frozen for storage at -5°C and sent to Waterlab in Pretoria for the analyses. At the laboratory sediment samples were dried, digested according to the methods of Bervoets and Blust (2003) and analysed for metals and metalloids using ICP-OES and reported as mg/kg dry weight. The ICP-OES spectrophotometer was set to scan sequentially for all metals and metalloids rather than for a limited set of elements. The spectral lines were examined to ensure that there was no interference between elements. All samples were subjected to the same QC/QA as the water samples. Recoveries were within 10% of the certified values.

The mean and standard deviation of the respective water chemistry, water and sediment metal concentrations were calculated. A two-way ANOVA was performed to determine whether the water chemistry and/or water column metal concentration varied seasonally using the aov() function in the R statistical package (R Development Core Team, 2018).

3.1.4 Fish and muscle tissue collection

During each survey a fleet of four composite gill nets comprising 5 m panels having stretch mesh sizes of 44, 60, 70, 100 and 144 mm were placed randomly at the various sampling sites indicated in Figure 3.2. Gill nets were generally placed approximately two hours before sunset and left to soak for four to six hours. Specimens captured were identified in accordance with identification keys in Skelton (2001). The Mozambique tilapia *O. mossambicus,* rednose labeo *L. rosae*, and silver catfish *S. intermedius* were selected and the live mass (g) and the standard/total length (mm) determined using a weighing balance and measuring board, respectively. During each survey the objective was to collect a maximum of ten specimens per species but this was not always possible. *Oreochromis mossambicus* specimens were collected throughout whereas *L. rosae*, and *S. intermedius* were collected from June 2017 onwards due to logistical and practical reasons. Specimens collected were sacrificed and a skinless sample of the muscle tissue (± 5 g) extricated and individually placed into a labelled self-sealing plastic bag and frozen at -20°C on site. The samples were stored in a -80°C freezer at the Department of Biodiversity, University of Limpopo, prior to the analyses of metals at Waterlab in Pretoria.

In the laboratory, muscle tissue samples were freeze dried, weighed and digested in nitric acid following Bervoets and Blust (2003). The analytical procedure used to establish fish muscle tissue metal content followed the same basic procedure applied for water and sediment. Results were expressed in mg/kg dry weight.

For the Human Health Risk Assessment (Section 3.2.5), the mean and standard deviation of the metal concentration in the muscle tissue were calculated for each fish species. These data were also used in determining the environmental drivers of the metal concentrations (Chapter 4). Box plots were generated using the R statistical software package (R Development Core Team, 2018) to help visualise the data and identify outliers. A Shapiro-Wilk test was performed to determine whether the metal data was normally distributed. The test returned a significant result for all metals in all three fish species. The non-parametric Kruskal-Wallace test was performed to determine whether there were significant differences in metal content in the muscles tissue of each species existed between surveys. The Dunn-Test was performed *post hoc* to determine the pair-wise comparisons between the respective surveys that were contributing to the significant result in the Kruskal-Wallace test. The Kendall tau-B correlation coefficient was calculated for each species and survey to determine whether there was any correlation between the respective metals in the muscle tissue using the corr() function in the R statistical software (R Development Core Team, 2018).

To evaluate whether there were differences in the multivariate metal concentrations of the fish species between surveys and within fish species, a resemblance matrix was constructed using Euclidian distances and non-metric multi-dimensional scaling (NMDS) to visualise the differences using PRIMER-E 6.1.5 (Clarke and Warwick, 2001; Clarke and Gorley, 2006). The metal concentrations in the fish muscle were transformed using a 4th root transformation to reduce the confounding impact of metal species which were present at high concentrations and to minimise the dispersion within the groups such that the PERMANOVA routine could be performed.

A distance-based test for homogeneity of multivariate dispersion was performed to test whether there was a statistically significant difference in multivariate dispersion for the water chemistry between surveys using the PERMDISP and PERMANOVA routines in PRIMER-E 6.1.5 (Anderson, 2001; Anderson et al., 2008). The PERMDISP routine was used to determine whether the multivariate dispersion about the group centroid differed between surveys for each species, while the PERMANOVA routine was used to determine whether the position of the group centroids differed between surveys for each species (Anderson, 2001; Anderson et al., 2008).

A SIMPER analysis was performed to determine the main factors contributing to the differences between surveys and between the fish species.

3.1.5 Human Health Risk Assessment

A human health risk assessment was carried out according to the methodology outlined by the Environmental Protection Agency of the United States (US-EPA, 2000) and the World Health Organization (WHO, 2003) as summarised for use in South Africa (du Preez et al., 2003; Heath et al., 2004a). The risk of chronic non-cancer health effects from oral exposures is calculated using the Average Daily Dose (ADD) received during the period of exposure, expressed in mg/kg body weight per day, as follows:

$$\mathrm{ADD} = \frac{(\text{average metal concentration in fish muscle}) \times (\text{mass of portion})}{(\text{adult body weight}) \times (\text{no. of days between fish meals})} \quad \ldots (1)$$

where the average fresh weight of metal concentrations in muscle tissue is in mg/kg, the mass of meal portion, adult body mass in kg and the number (no.) of days between fish meals (US-EPA, 2000). In order to calculate the ADD, a number of assumptions were required to characterize the population at risk. These assumptions were that a 150 g portion of fish muscle was consumed once a week by a 70 kg adult over a 30-year period; the latter assumption was not used in the calculation but formed the basis of the risk assessment).

A Hazard Quotient (HQ) for each metal was calculated comparing the expected exposure of the populations to the Reference doses (RfD); a threshold above which adverse health impacts could be expected. For oral exposures, the HQ is calculated as follows:

$$\mathrm{HQ} = \frac{\mathrm{ADD}}{\mathrm{RfD}} \quad \ldots (2)$$

Hazard Quotients less than 1 are considered to be safe for a lifetime exposure. The average metal concentration in the muscle tissues for the respective fish species were used in the health risk assessment. Reference Dose levels developed by the US-EPA were used for the evaluation of the Hazard Quotients (US-EPA, 2012). No RfD for lead (Pb) was available from the US-EPA because the EPA researchers believed it inappropriate to develop RfDs for Pb due to the wide range of health impacts, concluding that some haematological and neurological health impacts of Pb occurred at such low levels that these may be without threshold (US-EPA, 2004). For our analysis, we used the value used by Ashraf et al. (2012) who referenced the US-EPA as their source.

The use of averages for the risk assessment could be misleading because of the presence of outliers in the sample data set. To reduce the risk of misrepresenting the risk assessment results, the HQ values were calculated for the individual fish and the results summarised using box and whisker plots.

RESULTS

To investigate whether low water levels induced by drought conditions compounded metal accumulation in water, sediment and the muscle tissue of fish, eleven surveys were conducted between February 2016 and April 2018. Dam water levels in Flag Boshielo Dam during October of 2016 were recorded to be below 20%, rising to around 60% capacity in April 2017 and 100% in April 2018 (See Figure 3.1). The extreme fluctuation of water levels during this study presented the opportunity to collect samples under a variety of environmental conditions. Muscle tissue from 131 *O. mossambicus*, 40 *L. rosae* and 41 *S. intermedius* were analysed for metals.

3.1.6 Water quality in the littoral zone

At sites sampled within the littoral zone, water temperatures (°C) revealed the expected low winter and high summer readings indicative of the colder and warmer periods, respectively (Figure 3.3). There was a highly significant difference in both temperature and dissolved oxygen (DO) between the months surveyed ($p < 0.0001$). Saturation levels of DO recorded were within the Minimum Allowable Values (MAV) and Target Water Quality Range (TWQR) specified by DWAF (1996a) to be essential for aquatic organisms. Dissolved oxygen concentrations (mg/L) showed no distinct trend with DO levels in June 2016 recorded to be the highest. Throughout the study mostly alkaline conditions (pH 6.5-11.7) persisted with higher pH values recorded near the dam wall (Figure 3.3). Average temperature readings in Flag Boshielo Dam were erratic between low and high inflow periods, while DO and pH values were recorded to be higher and above the specified TWQR during periods of low inflow.

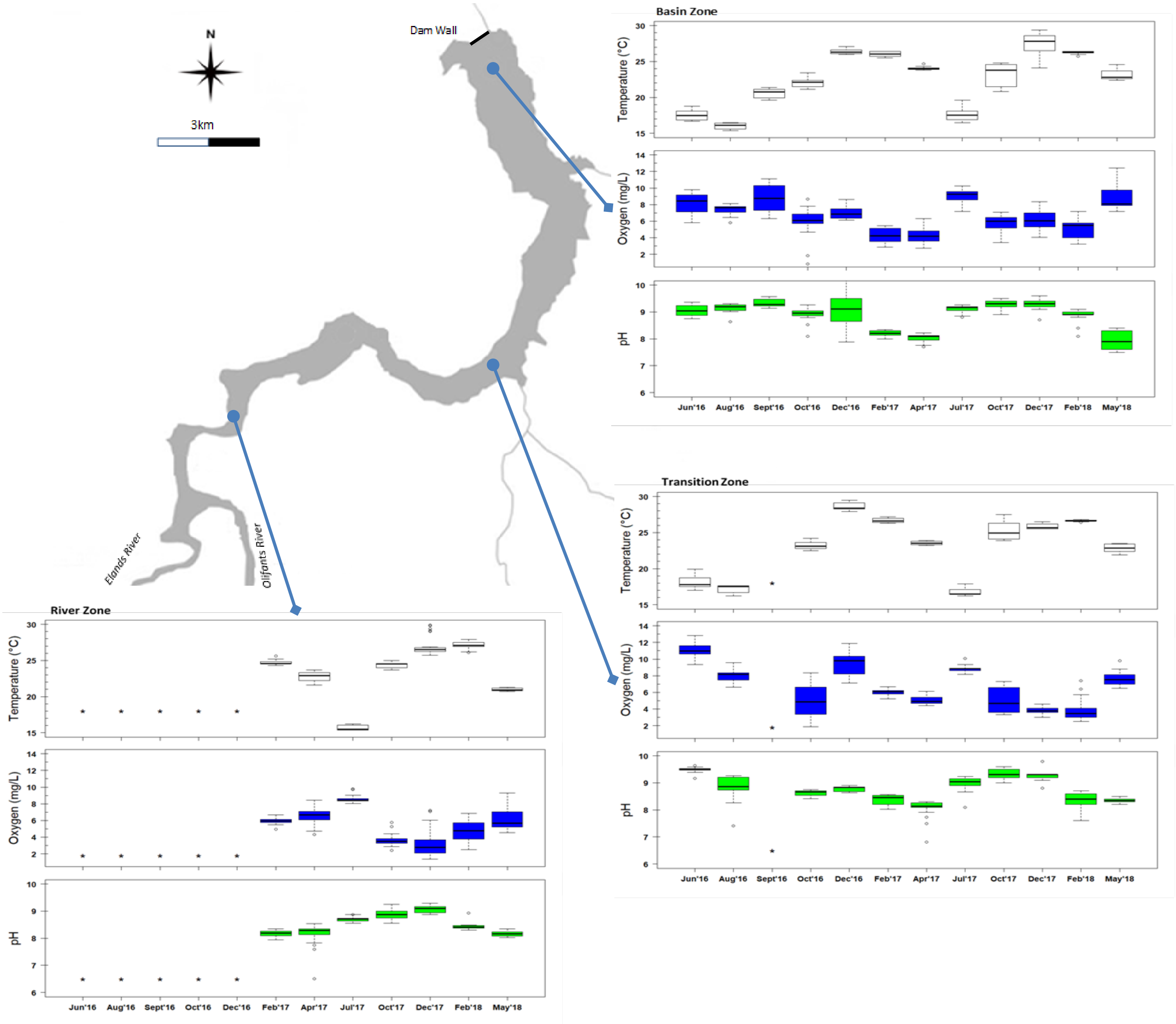

Figure 3.3: Boxplots of temperature (°C), dissolved oxygen (mg/L) and pH recorded in Flag Boshielo Dam during June, August, September, October, December 2016 and February, April, July, October, December 2017 and February, May 2018 at each of the littoral zones sampled. Months void of data are indicated with an (*) due to water levels being too low to access by boat.

Variation of average total dissolved solids (TDS) and electrical conductivity (EC) values were highly significant ($p < 0.001$) between surveys. Higher EC values were recorded during the period of low inflow with lower values recorded when dam levels were higher. Similarly, salinity levels increased slightly when water levels in the impoundment were low (Table 3.1).

The mean and standard deviation reported for EC was 607.4 ± 111.1 µS/cm and 429.8 ± 59.2 ppm for TDS. Higher levels of EC and TDS were reported in December 2016, December 2017, February 2018 and May 2018. Levels that were attributed to higher rainfall and water inflow. Surveys conducted in September, October and December 2016 could not be conducted adjacent Site 4 and upstream of this site due to the drawdown and receding water levels. Elevated concentrations of EC and TDS during periods of high inflow, which indicate high levels of electrolytes and ions in solution, can also be attributed to Loskop Dam being a major contributor of dissolved and organic compounds into Flag Boshielo Dam (Dabrowski and De Klerk, 2013) and the possible influx of metals and salts. Similarly, silt, nutrients, metal and salts from Elands River are expected to flow into Flag Boshielo Dam during periods of high inflow. Salinity showed no significant seasonal variation ($p > 0.05$) and did not affect changes in water density in Flag Boshielo Dam.

Although not measured in this study, elevated TDS readings during summer can also be due to the occurrence of phytoplankton blooms which can account for lower DO levels being recorded. Large phytoplankton blooms can deplete DO at night (Osman and Kloas, 2010) and given that most water measurements were taken within a couple of hours after sunrise this may account for lower levels of DO being reported during warmer months.

Chlorophyll-*a* and phosphorous levels (PO_4^{3-}), were recorded by the Department of Water Affairs and Forestry (Figure 3.4). Compared to four years prior, high chlorophyll-*a* concentrations reported during 2017 and 2018 indicated that Flag Boshielo Dam cycles between oligo-, meso- and eutrophic states. Not all waste water treatment plants in the catchment above Flag Boshielo Dam function optimally and high inputs of nutrients are received by this impoundment (Dabrowski et al., 2014). Elevated concentrations of ammonium (NH_4^+), nitrate (NO_3^-) and ortho-phosphate (PO_4^{-3}) in surface waters are commonly associated with the influx of sewage that leads to eutrophication and alkaline conditions (Oberholster et al., 2017). The introduction of organic matter from sewage leads to a high biological demand for oxygen (BOD) from the accumulation of metabolic products whereas eutrophic and alkaline conditions lead to the production algae (Codd, 2000; Osman and Kloas, 2010). By comparison, low nutrient concentrations can be attributed to high primary productivity whereby large algal blooms deplete aqueous levels of NH_4^+, NO_3^- and PO_4^{-3} (De Villiers and Thiart, 2007). An alternative source for nutrients in Flag Boshielo Dam is the intermitted release of fertilisers from the intensive agriculture upstream of the impoundment with nutrient containing agricultural runoff entering the impoundment during rainfall events (De Klerk et al., 2012).

Concentrations of NH_4^+, NO_3^{-2} and PO_4^{-3} varied significantly between surveys ($p < 0.05$). Ammonium levels recorded during August 2016, October 2016 and February 2017 exceeded the TWQR guidelines, an indication that eutrophic conditions existed within Flag Boshielo Dam at the time (Table 3.1). In contrast PO_4 levels were generally low throughout the study with the highest concentrations recorded during the June 2016 survey (Table 3.1).

Table 3.1: The mean ± the standard error (SE) of physico-chemical parameters measured at Flag Boshielo Dam during monthly surveys. Values in grey indicate where the Target Water Quality Range (TWQR) specified by DWAF (1996a) have been exceeded. Months highlighted in yellow indicate higher in flow and higher water levels when compared to the month previous (See Figure 3.1).

Parameter	Surveys												TWQR
	June '16		August '16		September '16		October '16		December '16		February '17		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Temperature (°C)	17.62	0.13	16.12	0.14	20.92	0.11	21.88	0.22	26.53	0.23	24.71	0.05	-
DO (mg/L)	**9.45**	0.39	7.64	0.13	**10.00**	0.31	5.38	0.55	6.74	0.20	5.28	0.00	6.0-9.0
pH	8.83-9.49		8.83-9.35		**9.14-9.57**		8.53-9.04		8.23-11.38		8.03-9.11		6.0-9.0
EC (µS/cm)	528.74	8.28	588.11	36.48	572.56	1.54	612.75	6.78	712.42	13.25	434.30	0.00	-
TDS (mg/L)	404.81	8.74	466.17	27.28	403.00	0.00	422.50	3.39	450.05	6.54	275.60	0.00	-
Salinity (ppt)	0.30	0.00	0.35	0.02	0.30	0.00	0.32	0.00	0.34	0.01	0.20	0.00	-
Ammonium (mg/L)	0.05	0.00	**0.31**	0.14	0.18	0.00	**0.91**	0.86	0.05	0.00	**1.10**	0.48	0.2*
Nitrate (mg/L)	**0.76**	0.71	**0.92**	0.53	0.05	0.00	**1.45**	0.62	**0.45**	0.00	**0.33**	0.25	0.2
Ortho-phosphates (mg/L)	0.09	0.00	**0.23**	0.19	0.01	0.00	0.03	0.02	0.04	0.00	0.01	0.00	0.1*
Sulphate (mg/L)	98.40	0.00	50.20	47.50	99.00	0.00	49.30	48.00	2.30	0.00	2.30	0.00	100**
Chloride (mg/L)	44.70	3.10	25.75	16.25	48.70	0.00	28.75	19.05	8.40	0.00	-	-	600
Fluoride (mg/L)	0.91	0.01	0.43	0.27	0.44	0.00	0.47	0.22	0.62	0.00	-	-	0.75

Key: SE = standard error, † Ortho-phosphate expressed as phosphorus; TWQR = target water quality range for South African water quality guidelines for aquatic ecosystems (DWAF 1996a) * = World Health Organisation Guidelines (1984), ** = Canadian Guidelines (2012).

Table 3.1 (cont): The mean ± standard errors (SE) of physico-chemical parameters measured at Flag Boshielo Dam during monthly surveys. Values in grey indicate where the TWQR guidelines have been exceeded. Months highlighted in yellow indicate higher in flow and higher water levels when compared to the month previous (See Figure 3.1).

Parameter	Surveys												TWQR
	April '17		July '17		September '17		December '17		February '18		May '18		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Temperature (°C)	22.80	0.12	15.70	0.06	24.37	0.07	27.11	0.28	27.20	0.09	20.99	0.04	-
DO (mg/L)	6.51	0.81	8.59	0.09	3.61	0.14	3.38	0.34	4.77	0.27	6.15	0.24	6.0-9.0
pH	6.5-8.5		8.6-8.9		8.6-9.3		8.9-9.3		8.3-8.9		8.0-8.3		6.0-9.0
EC (µS/cm)	368.79	23.55	448.47	3.22	557.41	47.53	954.26	19.00	921.72	2.12	733.48	0.47	-
TDS (mg/L)	314.37	23.66	439.59	2.09	603.06	15.44	580.52	11.11	457.86	1.41	377.00	0.00	-
Salinity (ppt)	0.25	0.03	0.33	0.00	0.36	0.01	0.43	0.01	0.34	0.00	0.28	0.00	-
Ammonium (mg/L)	0.12	0.11	0.05	0.00	0.07	0.03	0.11	0.07	0.05	0.00	0.05	0.00	0.2*
Nitrate (mg/L)	0.28	0.03	0.34	0.40	-	-	-	-	0.10	0.01	0.10	0.00	0.2
Ortho-phosphates (mg/L)†	0.02	0.01	0.02	0.01	0.03	0.03	0.01	0.01	0.02	0.01	0.01	0.01	0.1*
Sulphate (mg/L)	55.20	36.58	107.17	8.01	**112.70**	20.93	**102.80**	8.88	**123.55**	4.99	**140.90**	6.32	100**
Chloride (mg/L)	-	-	-	-	-	-	-	-	-	-	-	-	600
Fluoride (mg/L)	-	-	-	-	-	-	-	-	-	-	-	-	0.75

Key: SE = standard error, † Ortho-phosphate expressed as phosphorus; TWQR = target water quality range for South African water quality guidelines for aquatic ecosystems (DWAF 1996a) * = World Health Organisation Guidelines (1984), ** = Canadian Guidelines (2012).

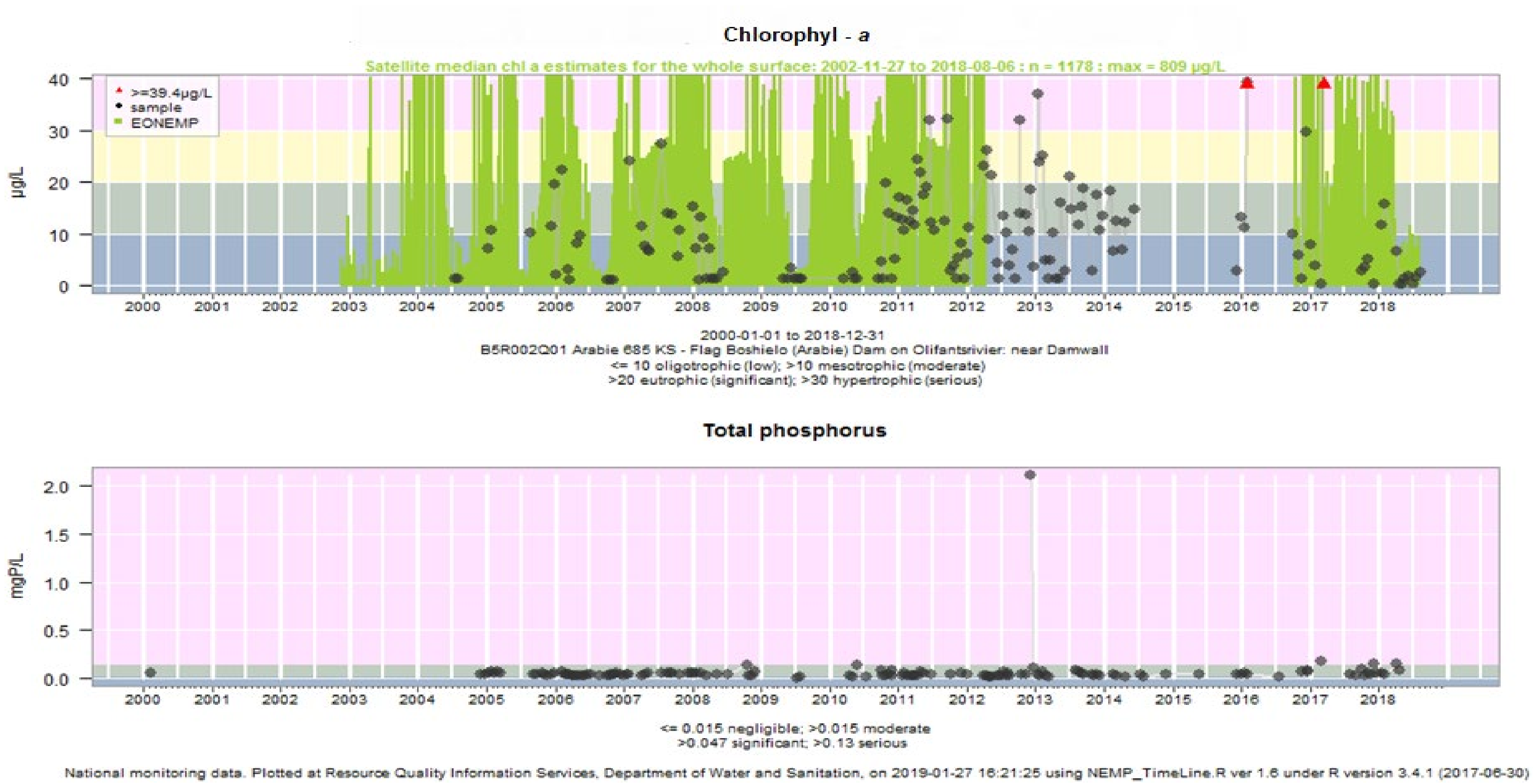

Figure 3.4: The Chlorophyl-*a* and total phosphorous readings recorded by the Department of Water and Forestry at Flag Boshielo Dam for the period from 2000 to 2018. (Source: https://www.dwaf.gov.za/iwqs/wms/data/000key.asp; Accessed January 2018).

De Villiers and Mkwelo (2009) note that the annual median of SO_4 concentrations at Flag Boshielo Dam is approaching the 100 mg/L threshold for aquatic ecosystem health. In this study SO_4^2 concentrations > 100 mg/L were recorded in the September 2017 and December 2017 and February 2018 and May 2018 surveys, when inflow into the system continued to increase (see Figure 3.1 and Table 3.1).

Concentrations of chlorine (Cl^-) and fluoride (F^-) did not vary significantly between surveys ($p > 0.05$) and fell within the permissible TWQR throughout the sampling period. Day and King (1995) reported the waters of the Olifants River System to be dominated by Ca, Mg and to a lesser extent, Na and bicarbonate anions. Geological strata are known to contribute to the increase in cations and anions through groundwater discharge. For example, limestone form efficient aquifers that introduce minerals such as Mg, while metamorphic or igneous rocks contribute to Na, K, SO_4^2 and Cl^- concentrations (De Klerk et al., 2012). High concentrations of these ions can also be attributed to natural processes such as leaching caused by the weathering of riverbed substrate. Alternatively, a decrease of these ions in the water column can be attributed to their uptake and retention by macrophytes and/or because they settle on the bottom substrate during periods of low inflow only to be re-suspended when water flow increases (Potasznik and Szymczyk, 2015).

3.1.7 Water quality in the limnetic zone

Overall the littoral zone had higher oxygen levels than limnetic sites M and L18. Water pH at these sites became more acidic with an increase in depth (Figure 3.5 and Figure 3.6). Survey profiles of temperature for these sites revealed little stratification during the colder periods. In contrast October and December 2017 surveys revealed that the temperature in upper stratum or epilimnion to be uniform to a depth of approximately 8 m. No stratification of temperature, oxygen and pH occurred in the riverine zone. As expected, limnetic waters had lower oxygen levels than the littoral zone with conditions becoming anoxic at depths > 11 m.

Seasonal fluctuations in water temperature exhibited thermal stratification and water mixing during winter. Thermal stratification occurs when increasing air temperatures exceed those of the water (Manasrah et al., 2006). Conversely, a decrease in ambient temperature can lead to a loss of buoyancy and cause the density of the surface waters to pass a critical threshold, after which vertical convection occurs and becomes dominant during winter (Manasrah, 2002). There existed an inverse relationship between temperature and DO whereby higher DO concentrations were recorded under colder conditions and *vice versa*. Higher DO concentrations recorded during the colder months can also be attributed to the presence of lower water levels and a high turnover of the water column from wind action over shallower and deeper waters. Conversely, lower DO concentrations is the result of higher water temperatures, a decrease in phytoplankton photosynthesis activity (Bartram and Balance, 1996) and a high BOD that emanates from a high influx of nutrients and organic matter into the system.

At the middle site (Site 6) the depth of the metalimnion varied between surveys and was recorded to be at shallower depth (Figure 3.7) than in the basin adjacent the dam wall (Figure.3.8). The middle or transitional zone between the well mixed epilimnion and the colder hypolimnion layers indicates a stratified lake. This layer contains the thermocline, but is loosely defined depending on the shape of the temperature profile (Horne and Goldman, 1994). The metalimnion was found to be absent during the August 2016 and between March and June 2017 surveys. A phenomenon attributed to the mixing and turnover of the water column.

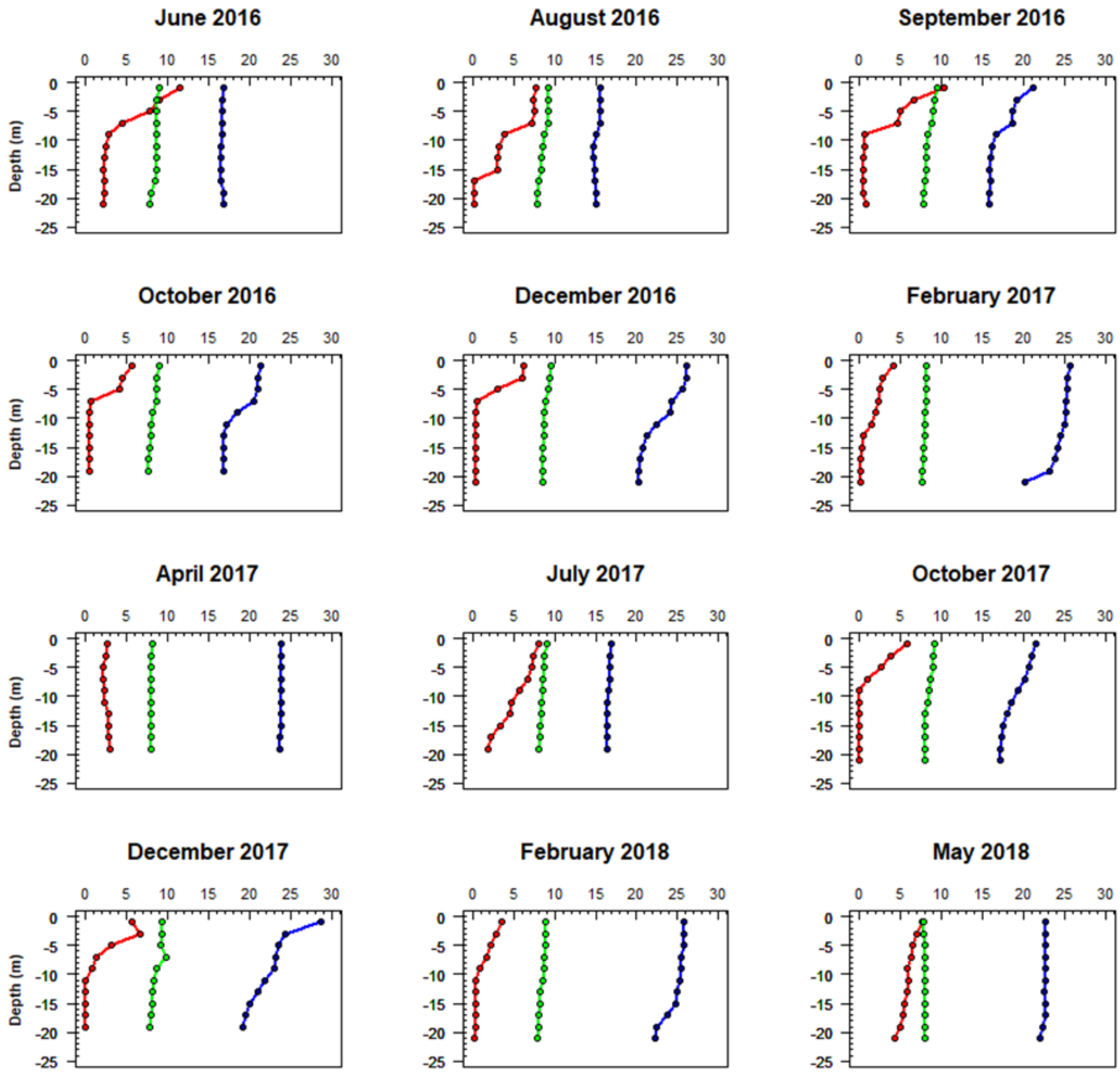

Figure 3.5: The temperature (°C), dissolved oxygen (mg/L) and pH profile recorded at limnetic site L18 in Flag Boshielo Dam during June, August, September, October, December 2016 and February, April, July, October, December 2017 and February, May 2018 for the littoral zones sampled.

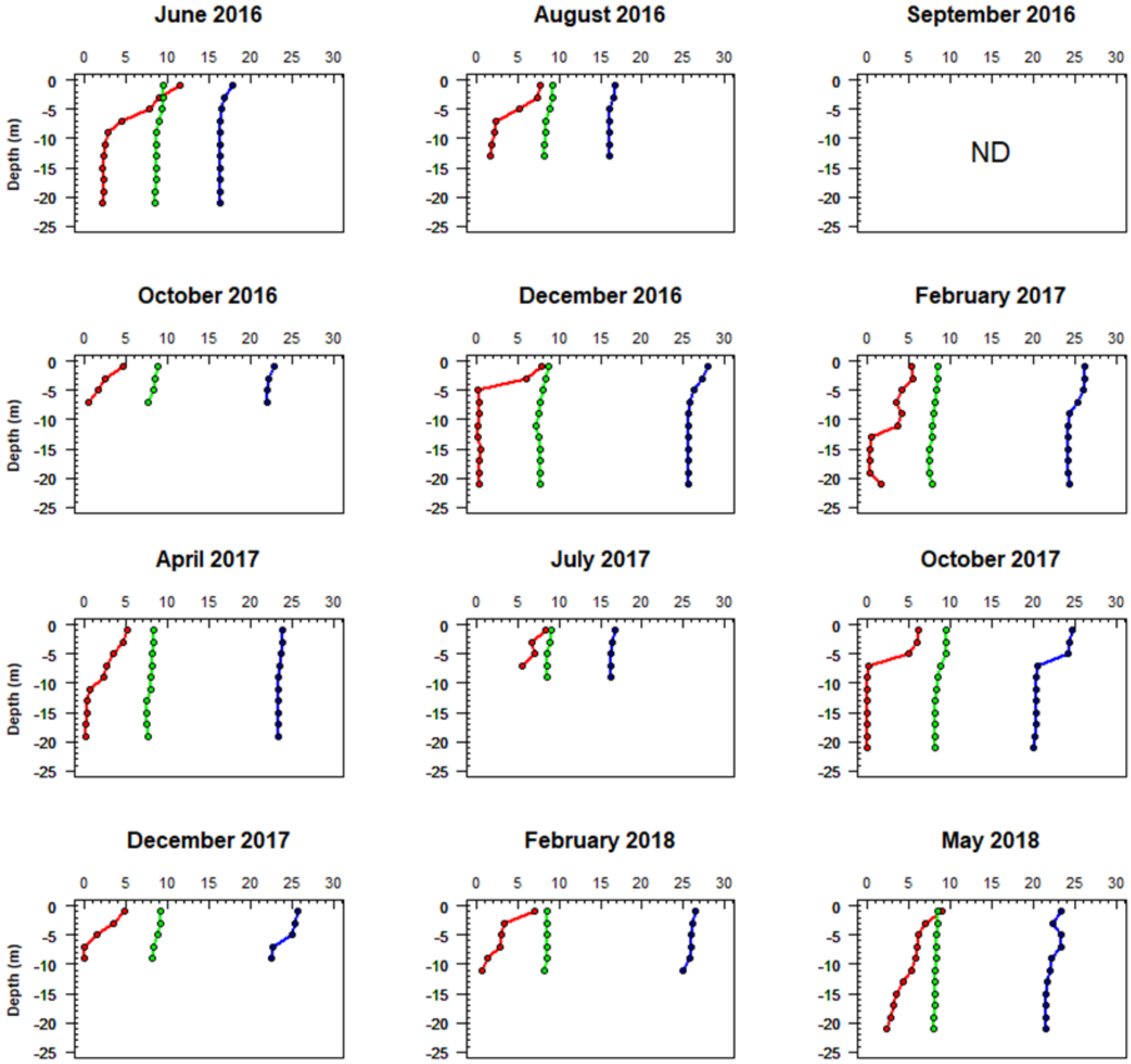

Figure 3.6: The temperature (°C), dissolved oxygen (mg/L) and pH profiles recorded at limnetic site M in Flag Boshielo Dam during June, August, September, October, December 2016 and February, April, July, October, December 2017 and February, May 2018 for the littoral zones sampled. ND indicates no data recorded for September due to theft of team's equipment.

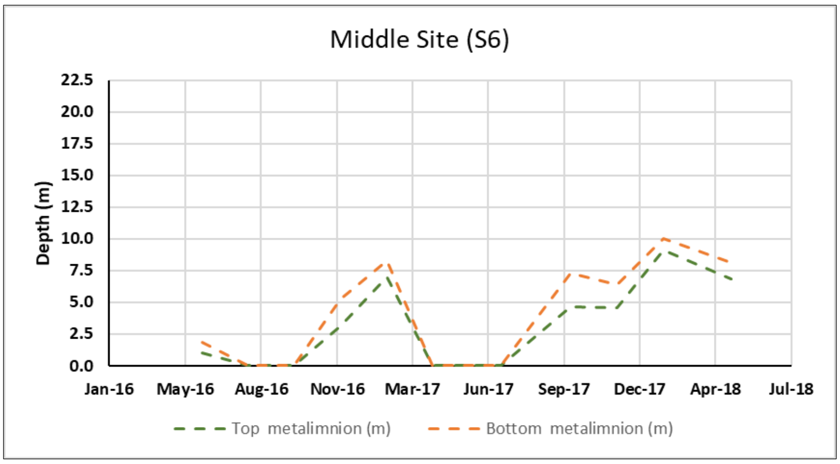

Figure 3.7: The top and bottom depth of the metalimnion calculated at the middle site (Site 6) during surveys conducted at Flag Boshielo Dam from June 2016 to May 2018.

In the basin the metalimnion zone was absent during the August 2016 and March 2017 survey with the metalimnion recorded at greater depths in the basin than at the middle site (Site 6). The depth of the metalimnion was recorder to be more expansive during the October 2016 and November 2017 surveys.

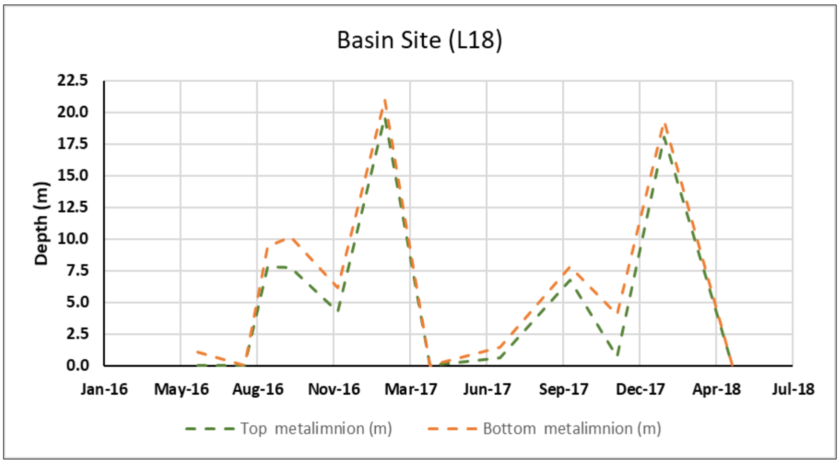

Figure 3.8: The top and bottom depth of the metalimnion calculated at the lancustrine site (Site L18) during surveys conducted at Flag Boshielo Dam from June 2016 to May 2018.

The absence of the metalimnion in May 2016 and May/June 2017 is indicative of a strong water turnover. During this period the weather conditions cool air and water temperatures that result in the water density between the epilimnion and hyperlimnion to increase. Similarly, the centre of buoyancy and thermocline depth was calculated to be shallower at the middle site (Figure 3.9) than in the basin (Figure 3.10). The thermocline extended to deeper depths during the December 2017 and February 2018 surveys when ambient temperatures were higher.

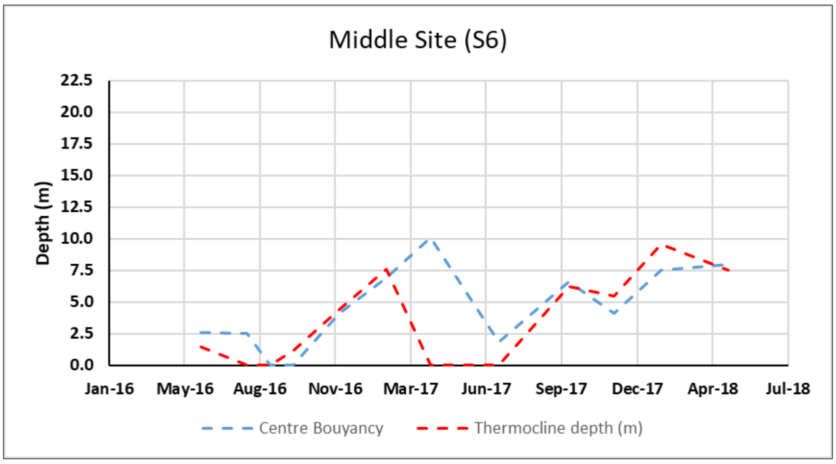

Figure 3.9: The centre of buoyancy and thermocline depth calculated at the middle site (Site 6) during surveys conducted at Flag Boshielo Dam from June 2016 to May 2018.

With the gradual increase in water temperatures the centre of buoyancy was observed to increase. An indication that algae and certain metals in solution will be more buoyant and more available to the ichythiofauna in Flag Boshielo Dam during this period.

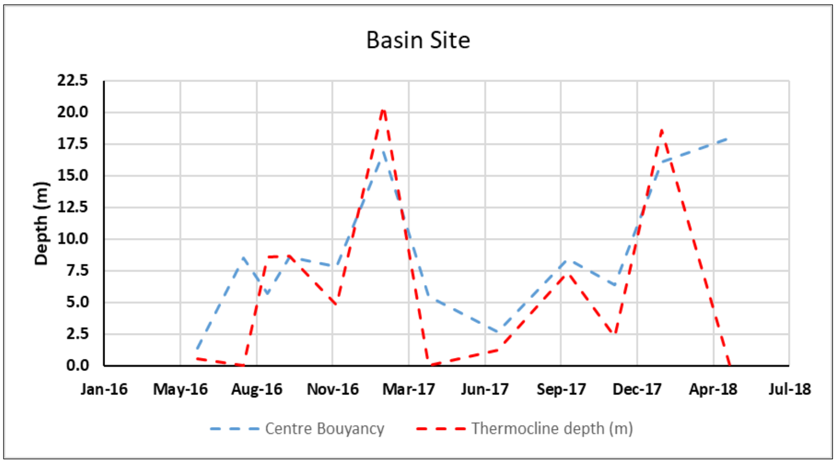

Figure 3.10: The centre of buoyancy and thermocline depth calculated at the lancustrine site (Site L18) during surveys conducted at Flag Boshielo Dam from June 2016 to May 2018.

3.1.8 Water metal data

Metals can enter surface waters through natural processes such as the weathering of igneous and metamorphic rocks and volcanic activity (Dallas and Day, 2004). The main anthropogenic sources of metals are domestic sewage, industrial effluents, oil and chemical spills, agricultural runoff, mining and metallurgical activities (Dallas and Day, 2004). According to Chapman (1996), more than 50% of total metals present in water are usually adsorbed by suspended particles. Thus, the undetectable concentrations of some metals can be as a result of adsorption and accumulation of metals by suspended solids. There are currently no ideal thresholds available in terms of the TWQR for barium (Ba), boron (B), calcium (Ca), sodium (Na), phosphorous (P), silicon (Si), strontium (Sr) and titanium (Ti) (Phala, 2019).

Of the suites of metals analysed in the water samples collected, aluminium (Al), barium (Ba), boron (B), iron (Fe), strontium (Sr), titanium (Ti) and zinc (Zn) were detected. The highest readings of these metals were recorded in October 2016, exceeding levels recorded in 2013 by Jooste et al. (2013). However, a clear trend between water metal concentrations and a change in dam levels was not evident (Figure 3.11).

Aluminium (Al) is one of the most common elements in the earth's crust that is inversely correlated to pH (Crafford and Avenant-Oldewage, 2010). Solubility of Al may be mobilised to aquatic environment under acidic (pH < 6) or alkaline (pH > 8) conditions (Mmualefe and Torto, 2011). This element occurs primarily as alumina-silicate that are too insoluble to participate in geochemical reactions. Toxicity of Al in fish occurs through respiration where it interferes with the ionic and osmotic balance, leading to hypoxia due to the clogging of interlamellar spaces in fish gills (Dallas and Day, 2004; Sara et al., 2014). Given that the TWQR for Al to be 0.01 mg/L (DWAF, 1996a) it was expected that higher levels of Al would be detected in Flag Boshielo Dam due to mining and industrial practices in the upper catchment of this impoundment. Except for surveys conducted in October 2016, March 2017 and September 2017, aqueous concentrations of Al were below detection levels. Low Al concentrations was inferred to be due to this metal having deposited into sediments. Elevated levels of Al in surface waters can also be linked to and coal combustion (Svobodova et al., 1993). According to Oberholster et al. (2012) and Dabrowski et al. (2014), benthic filamentous algae have the ability to accumulate high concentrations of metals, Al in specific. However, Al concentrations reported to be below detection in samples collected during February and May 2018, which is a period of high inflow whereby aqueous metal concentrations become diluted. This phenomenon has been reported to occur elsewhere (Kotze et al., 1999).

Barium (Ba) is a metal that occurs in combination with other elements such as sulphur, oxygen and carbon (US-EPA, 2008). This metal can react with almost all non-metallic elements, forming substances that are toxic to organisms at both high and low concentrations (Lenntech, 2009). Naturally, Ba originates primarily from natural sources and is present as a trace element in both igneous and sedimentary rocks (WHO, 2003). A possible explanation of Ba concentrations detected here can be that this metal occurs through natural processes and because Ba is extensively used in mining, coal and oil combustion (Phala, 2019). Anthropogenic practices occurring in the upper and middle reaches of the Olifants River catchment can possibly explain Ba levels recorded in Flag Boshielo Dam.

Boron (B) is a natural component of freshwaters arising from weathering of parent rocks, soil leaching and volcanic action (Chapman, 1996). Anthropogenic sources of B in surface waters include industrial practices, municipal wastewaters, coal-fired electric power generation plants and AMD (Eisler, 1990), and can possibly explain B concentrations detected in Flag Boshielo Dam. In addition, agricultural run-offs can contain B, particularly in regions where it is used in pesticides to improve crop yields (Hasenmueller and Criss, 2013). Elevated levels of B recorded in Flag Boshielo Dam can possibly be attributed to effluents from agricultural runoff from citrus orchards along the Elands River.

Iron (Fe) is the fourth most abundant element that constitute 5% of the earth's crust and is naturally released into surface waters from the weathering of sulphide ores (FeS_2) and igneous, sedimentary and metamorphic rocks (DWAF, 1996a; 1996b). Under alkaline or neutral conditions, dissolved Fe decreases while under acidic conditions Fe is highly detectable (Akcil and Koldas, 2006). Factors that may contribute to fluctuations of Fe levels detected in samples collected here. Anthropogenic sources of Fe also include mining, sewage effluent, landfill leachates and some industrial effluents (DWAF, 1996a). The TWQR for Fe is 0.2 mg/L (DWAF, 1996a) and was exceed in samples taken in October 2016 and April 2017. Although pH values were recorded to be alkaline during these surveys, elevated Fe concentrations recorded at these times may be due to direct inputs from Loskop Dam, the Elands River or from atmospheric deposits containing this metal.

Strontium (Sr) is a non-radioactive element naturally occurring in rocks, soil, dust, coal and oil (Irwin et al., 1997). This element forms about 0.034% of all igneous rock in the form of the sulphate mineral; celestite ($SrSO_4$) and the carbonate; strontianite ($SrCO_3$). Strontium compounds are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, and medicines (Irwin et al., 1997). High concentrations of Sr at Flag Bosheilo Dam can be attributed to this metal occurring naturally within the catchment and main stem of this system.

Titanium (Ti) is one of the most abundant chemical elements in the earth's crust present in rocks, soils and bottom sediments of water bodies (Linnik and Zhezherya, 2015). Among the most common minerals containing Ti is titanium dioxide (TiO_2) which is a compound widely used in the manufacturing of pigments, production of nanomaterials and as nanotubes in wastewater treatment (Linnik and Zhezherya, 2015). Elevated levels of Ti in Flag Boshielo Dam can be explained by the underlying rock formation surrounding this impoundment.

Zinc (Zn) is an essential micro-nutrient for all organisms. Natural processes such as the weathering rocks yields this metal (DWAF, 1996a). Anthropogenic sources of Zn include industrial wastes, pharmaceuticals, fertilizers and insecticides. Zinc concentrations detected at Flag Boshielo Dam were above the TWQR of 0.002 mg/L. Levels that can be attributed to natural processes and anthropogenic factors in the catchment of the impoundment.

During dry periods and low inflow, the rise in temperature, high evaporation and drawdown causes metals to settle and become concentrated (Davies et al., 2006) resulting in the residual build-up of metals in sediments. Consequently, diffusion of contaminants takes place naturally when the concentration in the overlying water is less than that of the underlying water (De Klerk et al., 2012). The influx and increase of metal concentrations recorded during high inflow can be attributed to a concentration gradient created by rainfall whereby metals are diffused out of the sediment and into the overlying water column (Kotze et al., 1999) or because of the scouring effect high flow rates have on the river substrate whereby metals trapped in the sediment become re-suspended in the water column. In turn, seasonal changes in metal concentrations is influenced by various physico-chemical parameters such as total hardness, organic matter, water temperature, DO, pH and increased rainfall (De Klerk et al., 2012) whereby a combination of these may explain for the elevated concentrations of B, Ba, Sr, Ti and Zn reported during a period of high inflow.

3.1.9 Metal sediment data

Sediment samples collected in the riverine, transition and lacustrine zones revealed a far greater number of metals and metalloids than those in water samples (Table 3.2). Aluminium (Al), antimony (Sb), arsenic (As), Ba, B, cadmium (Cd), chromium (Cr), cobolt (Co), copper (Cu), lead (Pb), manganese (Mg), nickel (Ni), selenium (Se), strontium (Sr), tin (Sn), Ti, vanadium (V) and Zn were among metals and metalloids detected. Compared to the study by Jooste et al. (2013) sediment concentrations of Sb, B, Ba, Cd, Se, Sn and Zn were recorded to be lower in this study (Figure 3.12). In contrast average Ni levels recorded in the February and April 2016 far exceed that recorded by Jooste et al. (2013) for the same metal. Except for Sb, B, Cd, Ni and Se trends indicate a general increase in sediment concentrations during periods of high-water inflow (Figure 3.12).

During periods of low rainfall and drought, large areas of previously submerged sediment can be exposed to the atmosphere and become desiccated, whereby nutrients dynamics, microbial communities and the oxidative state of elements are affected (Osman and Kloas, 2010; Dabrowski et al., 2017). The rewetting of sediments can result in acidification and eutrophication (Dabrowski and De Klerk, 2013; Dabrowski et al., 2017). Therefore, low accumulation of metals during high inflow period suggest that the concentration of metals in the bottom substrate was brought about by changes in water flow.

Table 3.2: The average concentrations of metals detected in sediment collected from Flag Bosheilo Dam in this study.

Sediment Metal mg/kg dry mass (dm)	Mean	± SE	Sediment quality guidelines (CCME 2012)*
Aluminium (Al)	47404.41	5021.77	No guidelines
Antimony (Sb)	0.14	0.05	No guidelines
Arsenic (As)	8.27	1.21	5.9 mg/kg dm
Barium (Ba)	333.12	28.95	No guidelines
Boron (B)	8.15	3.28	No guidelines
Cadmium (Cd)	0.01	0.01	0.6 mg/kg dm
Chromium (Cr)	128.95	15.04	37.3 mg/kg dm
Cobalt (Co)	16.90	2.25	No guidelines
Copper (Cu)	31.55	4.75	35.7 mg/kg dm
Iron (Fe)	50630.75	2778.94	No guidelines
Lead (Pb)	26.39	2.18	35.0 mg/kg dm
Manganese (Mn)	1356.89	133.39	No guidelines
Nickel (Ni)	136.77	64.23	No guidelines
Selenium (Se)	0.36	0.28	No guidelines
Silver (Ag)	0.00	0.00	No guidelines
Strontium (Sr)	36.34	3.91	No guidelines
Tin (Sn)	2.60	0.41	No guidelines
Titanium (Ti)	5696.87	490.24	No guidelines
Vanadium (V)	74.57	10.05	No guidelines
Zinc (Zn)	102.94	21.55	123 mg/kg dm

*CCME (2012) Canadian Council of Ministers of the Environment: Sediment Quality Guidelines – aquatic life

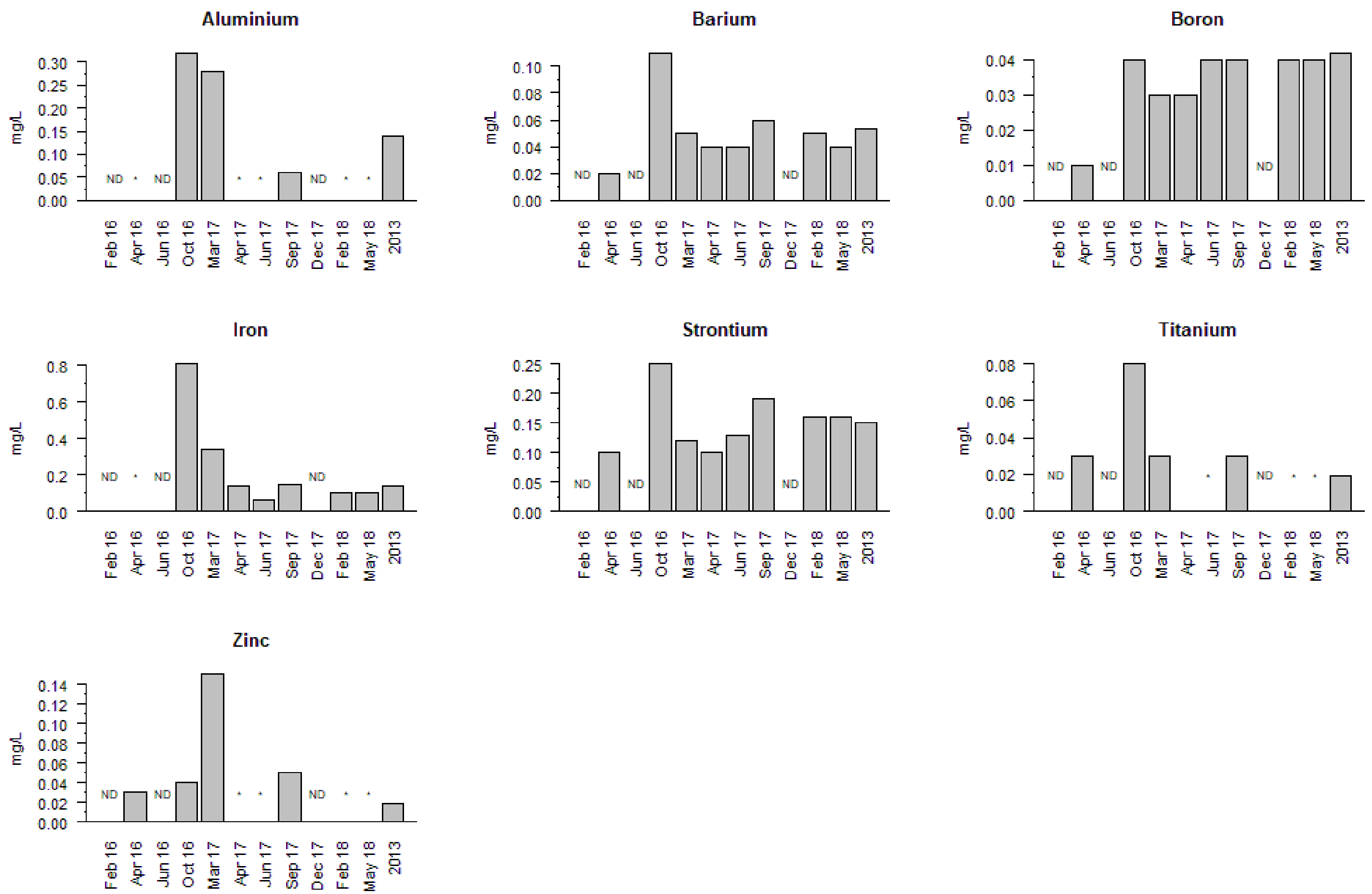

Figure 3.11: Comparison of the average metal concentrations (mg/L) in water samples collected from Flag Boshielo Dam during monthly surveys of the current study to those published by Jooste et al. (2013).

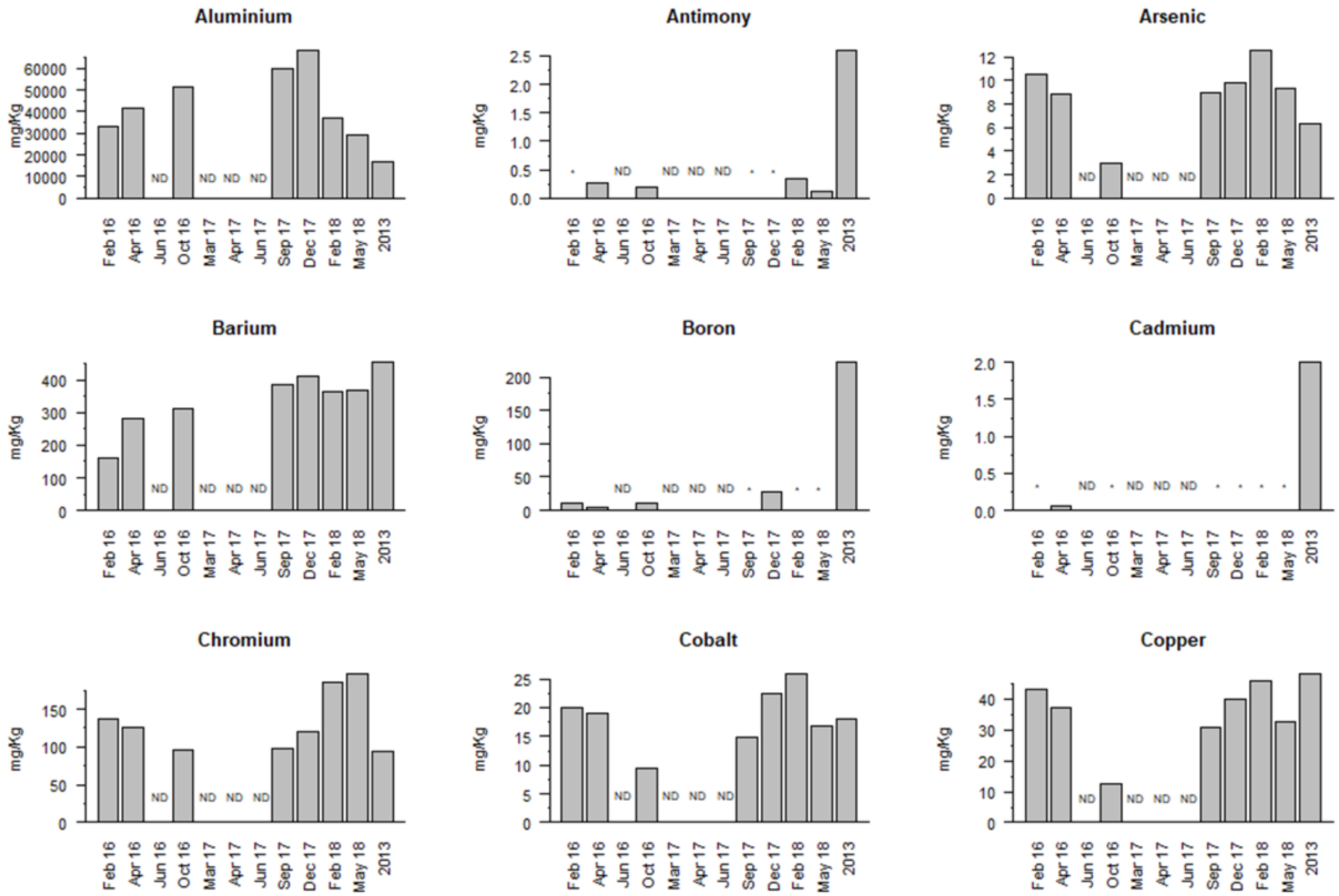

Figure 3.12: Comparison of the average metal concentrations (mg/kg) in sediment samples collected from Flag Boshielo Dam in the surveys of the current study to those published by Jooste et al. (2013).

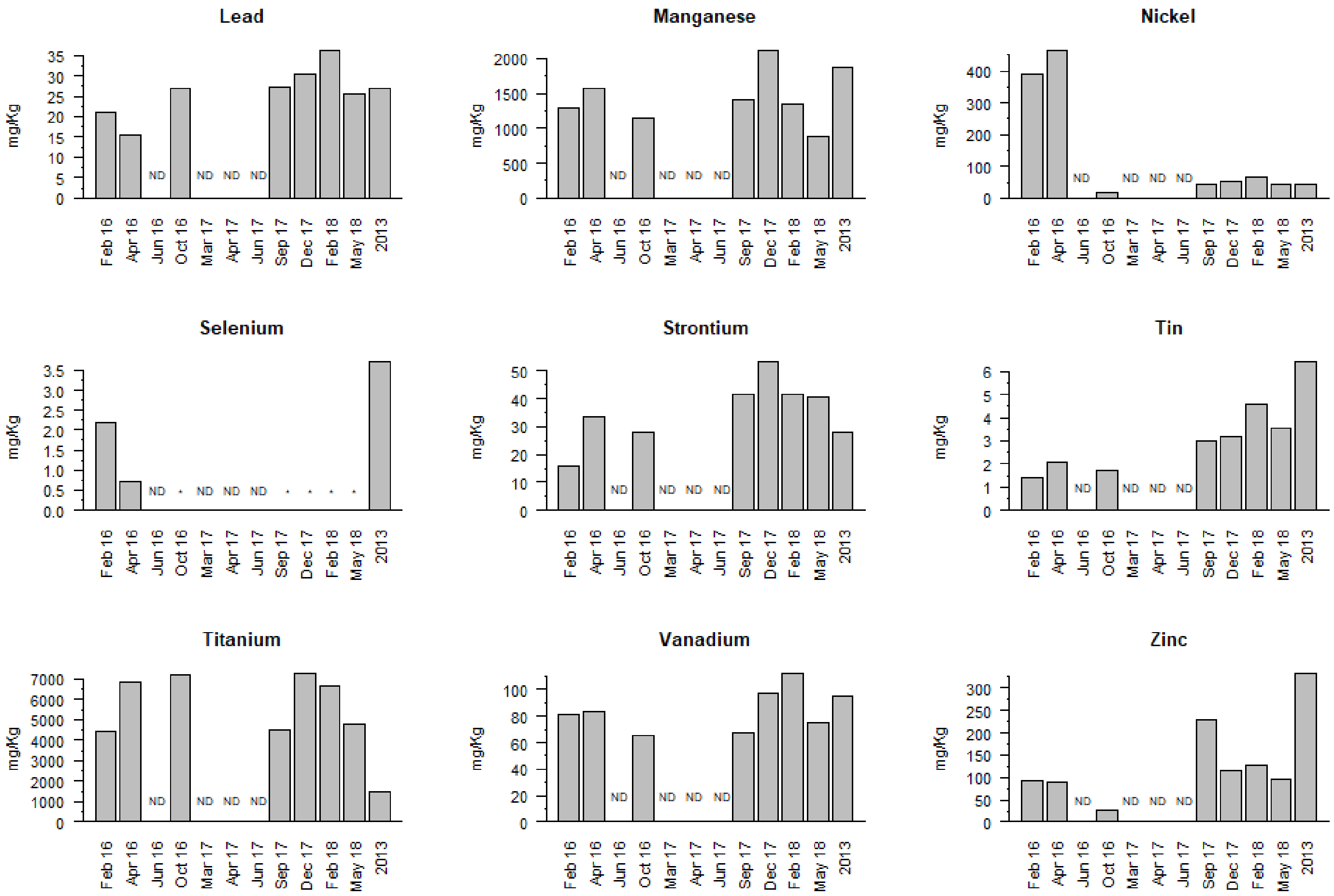

Figure 3.12 (cont): Comparison of the average metal concentrations (mg/kg) in sediment samples collected from Flag Boshielo Dam in the surveys of the current study to those published by Jooste et al. (2013).

3.1.10 Metal concentrations in fish muscle

A number of metals were consistently below the detection limit of the analytical equipment and were not included in the subsequent analyses. These included Au, Be, Bi, Cs, Dy, Er, Eu, Gd, Ge, Hf, In, Ir, Li, Lu, Nb, Os, Pd, Rh, Ru, Sn, Ta, Tn, Te, Th, Tl, Tm, Yb and Zr for all three fish species.

Oreochromis mossambicus

The metal concentrations on fish muscle tissue for Mozambique tilapia *O. mossambicus* are summarised in box and whisker plots (Figure 3.13). A general pattern of the April and June 2017 and February and May 2019 surveys was that metal concentrations were significantly different than those reported for surveys prior to April 2017, e.g. Cd, Co, Cu, Pb, Mn, Mo, Ni, Se, Ag, Ti, V and Zn. For some of these metals, the four surveys were also significantly different to the September and December 2017 surveys, e.g. Pb, Mn, Mo, Ag and Zn.

The correlation coefficients between the metals in the muscle tissues were calculated using Kendall's tau-B (Table 3.3). Four metals, Cd, Co, Se and V, exhibited correlation coefficients > 0.6 with each other and a suite of metals: Ag, Cu, Mn, and Ni. In addition, correlation coefficients > 0.6 were found for the following metal pairs: As-Co, As-Zn, Cd-Pb, Cd-Mo, and Cu-Mn.

The average metal concentrations for each survey were then compared to the average metal concentrations reported previously for this species in Flag Boshielo Dam by Jooste et al. (2013); see Figure 3.14. The results reported by Jooste et al. (2013) were considerably higher than the results reported in this survey for Sb, Ba, B, Cr, Pb, Sn, and V, while the remainder of the metals reported on were similar or lower in the 2013 results.

The NMDS plot of the metal concentrations in muscle tissues shows a clear separation between the respective surveys (Figure 3.15). The PERDISP result for multivariate dispersion of the metal concentrations in muscle tissues of *O. mossambicus* across the surveys was significant ($p = 0.0001$) and the pair-wise *post hoc* comparison between surveys found a significant difference in multivariate dispersion between surveys prior to the June 2017 survey and from that survey onwards. The PERMANOVA routine returned a significant result ($p < 0.001$) and the pair-wise *post hoc* comparison between surveys was significant for all comparisons. The NMDS plot confirms that the data from each survey was positioned at a unique point in multivariate space.

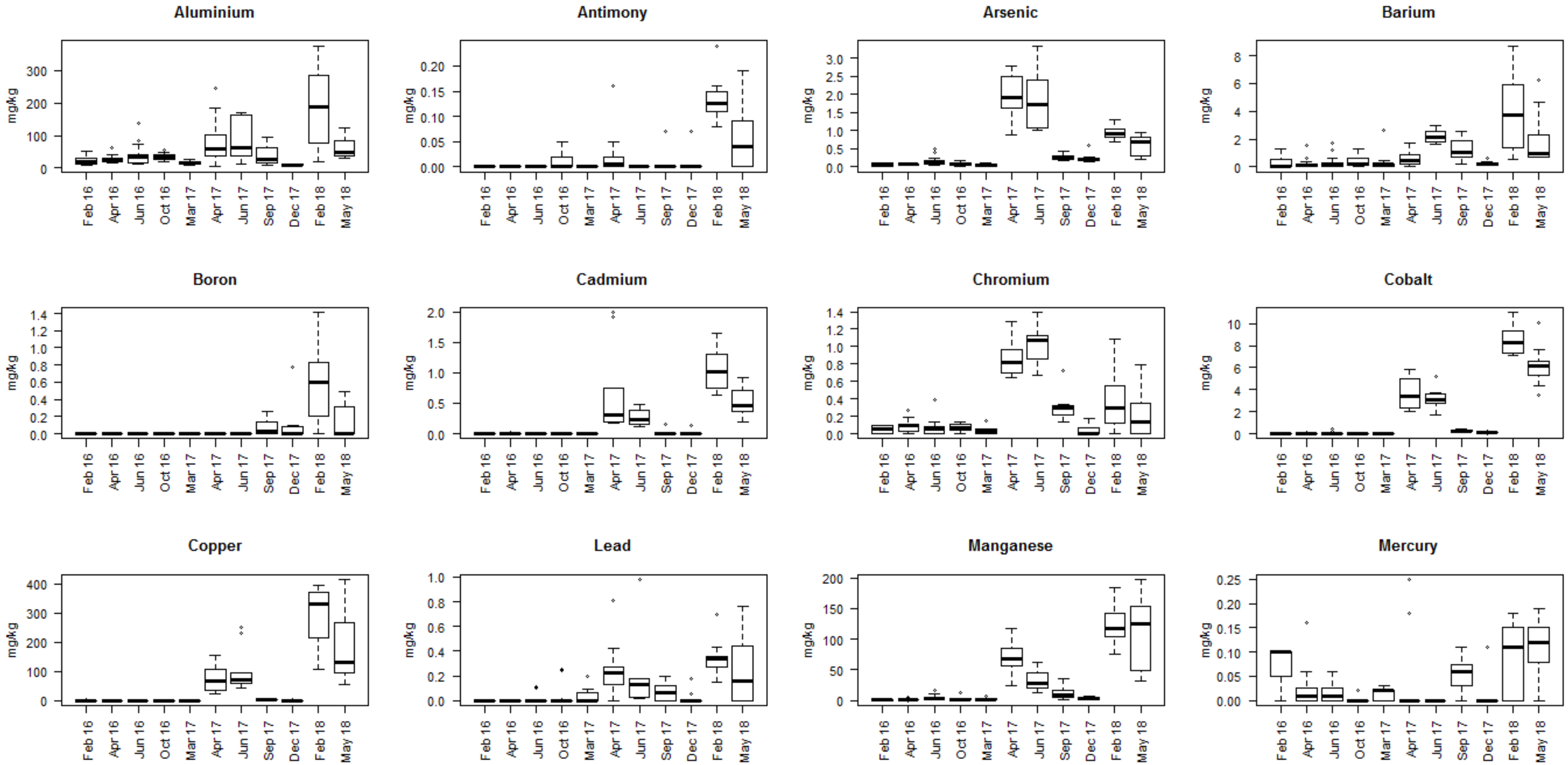

Figure 3.13: Box and whisker plots of metal concentrations (mg/kg dry weight), in the muscle tissue of *Oreochromis mossambicus* from Flag Boshielo Dam.

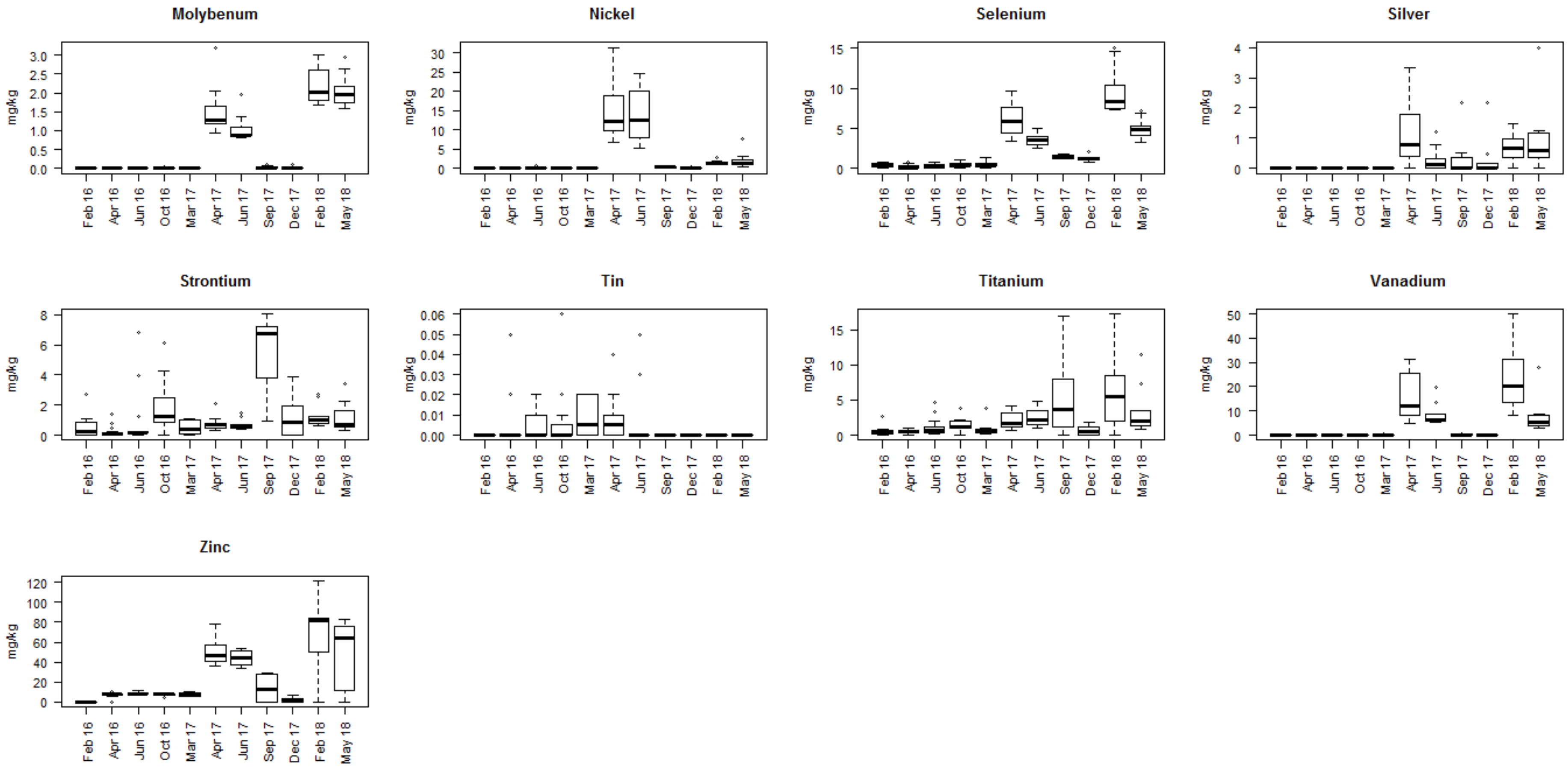

Figure 3.13 (cont): Box and whisker plots of metal concentrations (mg/kg dry weight), in the muscle tissue of *Oreochromis mossambicus* from Flag Boshielo Dam.

Table 3.3: The Kendall tau-B correlation coefficient between the metal concentrations in the muscle tissue of *Oreochromis mossambicus* collected from Flag Boshielo Dam. Correlation coefficients greater than ± 0.6 are highlighted.

	Ag	**Al**	**As**	**B**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Mn**	**Mo**	**Ni**	**Pb**	**Sb**	**Se**	**Sn**	**Sr**	**Ti**	**V**	**Zn**
Ag																					
Al	0.27																				
As	0.54	0.32																			
B	0.40	0.12	0.27																		
Ba	0.38	0.41	0.40	0.28																	
Cd	0.65	0.42	0.56	0.36	0.45																
Co	0.62	0.32	0.64	0.39	0.48	0.74															
Cr	0.35	0.40	0.52	0.08	0.39	0.43	0.39														
Cu	0.57	0.35	0.55	0.36	0.47	0.61	0.67	0.38													
Hg	0.10	0.05	-0.02	0.24	0.08	0.18	0.15	-0.03	0.08												
Mn	0.54	0.37	0.57	0.32	0.56	0.61	0.70	0.35	0.57	0.09											
Mo	0.51	0.37	0.45	0.23	0.34	0.66	0.55	0.25	0.49	0.13	0.55										
Ni	0.55	0.31	0.72	0.22	0.40	0.60	0.65	0.51	0.56	0.07	0.59	0.45									
Pb	0.58	0.42	0.49	0.32	0.46	0.65	0.56	0.45	0.50	0.18	0.55	0.53	0.52								
Sb	0.46	0.33	0.32	0.45	0.32	0.54	0.40	0.27	0.34	0.24	0.37	0.44	0.25	0.53							
Se	0.60	0.29	0.56	0.37	0.45	0.64	0.64	0.31	0.65	0.09	0.62	0.57	0.52	0.56	0.40						
Sn	-0.04	0.00	-0.01	-0.20	-0.09	-0.08	-0.09	0.03	-0.09	-0.16	-0.08	0.03	-0.05	-0.02	-0.14	-0.08					
Sr	0.20	0.18	0.18	0.22	0.45	0.14	0.20	0.19	0.19	-0.03	0.33	0.06	0.15	0.21	0.19	0.22	-0.05				
Ti	0.32	0.55	0.39	0.17	0.59	0.38	0.36	0.44	0.34	0.00	0.50	0.29	0.35	0.42	0.29	0.36	-0.01	0.46			
V	0.61	0.37	0.63	0.39	0.55	0.70	0.70	0.46	0.60	0.11	0.66	0.50	0.61	0.58	0.43	0.65	-0.10	0.27	0.46		
Zn	0.37	0.44	0.38	0.13	0.42	0.52	0.47	0.32	0.36	0.03	0.51	0.52	0.46	0.45	0.24	0.40	0.05	0.19	0.41	0.49	

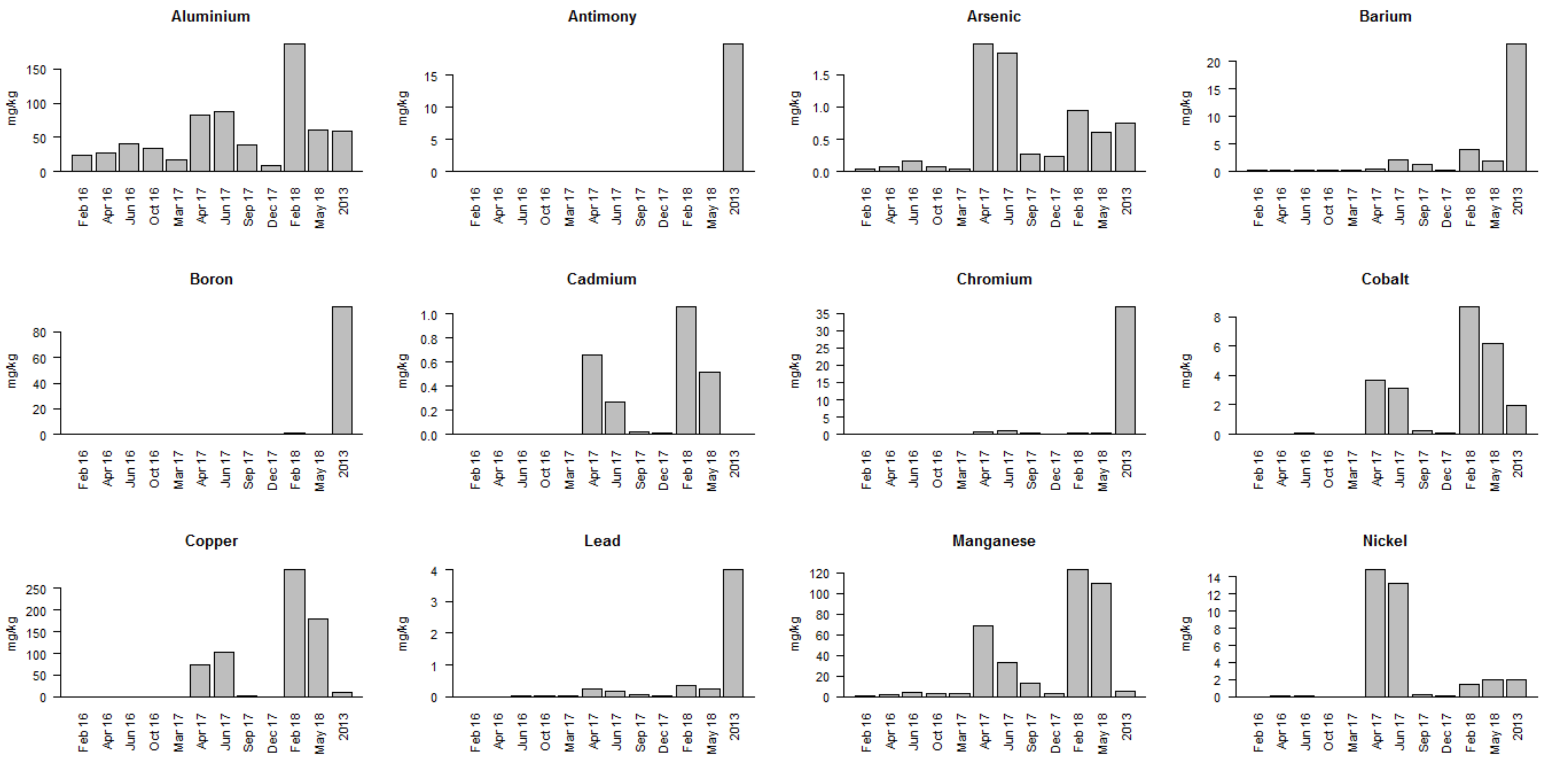

Figure 3.14: Comparison of the average metal concentrations (mg/kg dry weight) in the muscle tissue of *Oreochromis mossambicus* from Flag Boshielo Dam collected in the surveys of the current study to those published by Jooste et al. (2013).

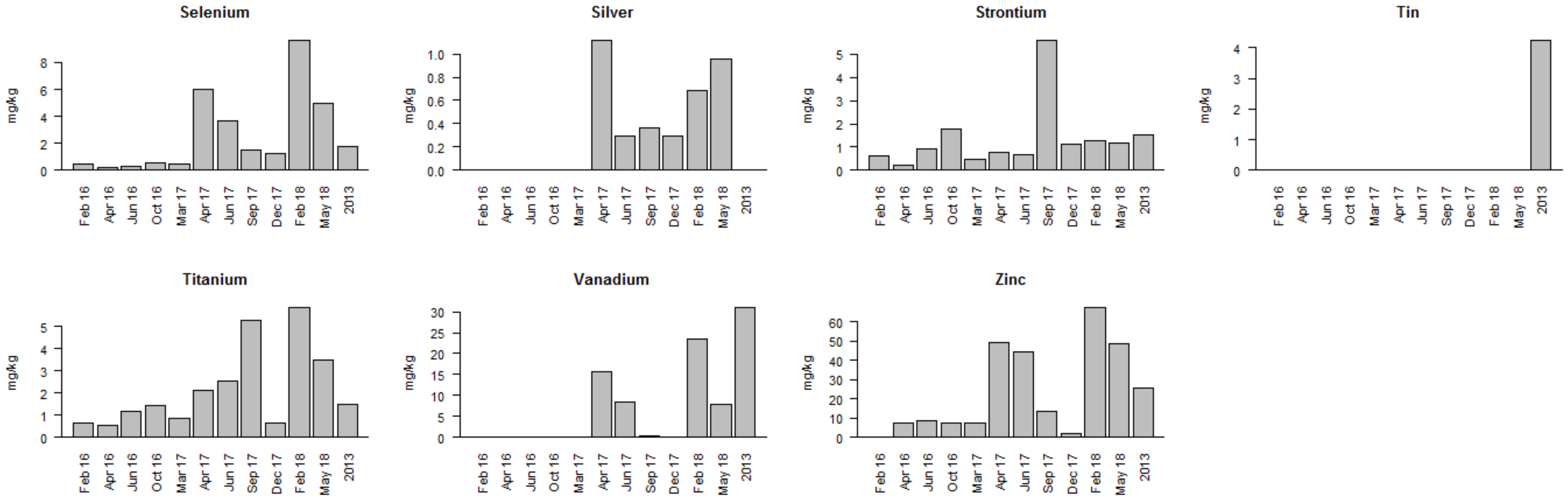

Figure 3.14 (cont): Comparison of the average metal concentrations (mg/kg dry weight) in the muscle tissue of *Oreochromis mossambicus* from Flag Boshielo Dam collected in the surveys of the current study to those published by Jooste et al. (2013).

Two major groups are evident from the NMDS plot; the one to the right containing surveys from February 2016 (Feb 16) to March 2017 (Mar 17), September 2017 (Sep 17), and December 2017 (Dec 17) while the group to the left contains April (Apr 17) and June 2017 (Jun 2017), and February 2018 (Feb 18) and May 2018 (May 2018). Within each of the major groups the position of the respective surveys appears to be unique, based on the outcomes of the PERMANOVA analysis. For the right group, Feb 16 is separate from the overlapping groups of the April 2016 (Apr 16) to March 2017 (Mar 17) surveys. The September and December 2017 surveys are loosely associated with the right-hand group. The left-hand group is a loose association of four surveys with little overlap between the sample spaces.

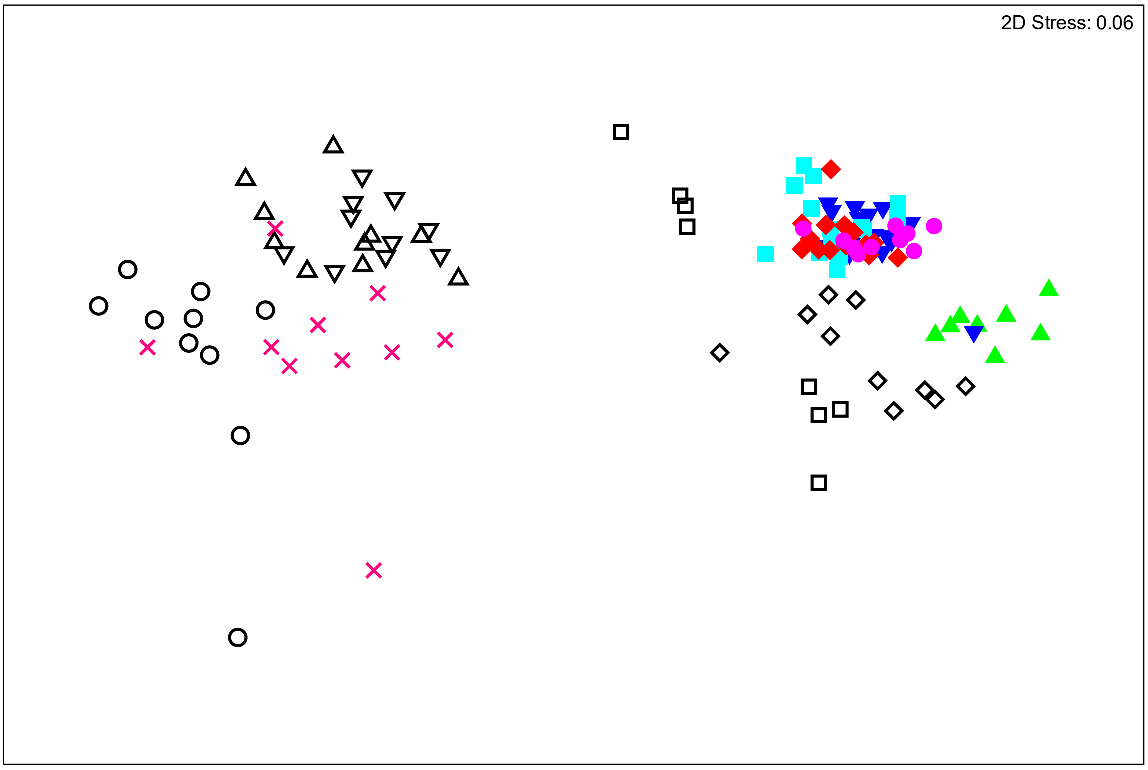

Figure 3.15: Non-metric multi-dimensional scaling plot for the metal concentration in the muscle tissue of *Oreochromis mossambicus* from the Flag Boshielo Dam based on Euclidian distance and a 4th root transform of the original data. The data points for the field trips are represented by unique symbols: Feb 16 (▲), Apr 16 (▼), Jun 16 (■), Oct 16 (♦), Mar 17 (●), Apr 17 (Δ), Jun 17 (∇), Sep 17 (□) Dec 17 (◇), Feb 18 (O), and May 18 (X).

The SIMPER analysis showed that the metals in *O. mossambicus* muscle tissue contributing to the similarity within the respective groups varied across surveys (Table 3.4). Zinc was a major contributing metal for five surveys (April 2016, September 2017, December 2017, February 2018 and May 2018) while Al and Cu contributed most to the within group similarity for the April and July 2017 surveys and Ba and Sr contributed most to the similarities within the February 2016 and March 2017 surveys.

The SIMPER results for the dissimilarity in the metals recorded in *O. mossambicus* muscle tissue between surveys also provides some interesting patterns (Table 3.5). Zinc is the most important distinguishing metal between the February 2016 survey and all other surveys because the samples from this survey did not appear to contain any Zn. In contrast, Zn is a major distinguishing metal between the September and December 2017 surveys and all other surveys because of the lower concentrations of Zn found in these samples in relation to the April and July 2017 and February and May 2018 surveys. Copper, Mn and Zn contributed most to the dissimilarity between the February and May 2018 surveys and all other surveys while Cu, Mn, Ni and V contributed the most to the dissimilarity between April 2017 surveys and those proceeding it, while Cu, Ni and V contributed the most to the dissimilarity between July 2017 surveys and those proceeding it. Prior to the April 2017 survey, and excluding the February 2016 survey, dissimilarities between the April 16 survey and the others was due to Se and Sr, dissimilarities between the June 16 survey and the others was due to Sr, and dissimilarities between the October 2016 survey and the others was due to Ba, Al and Sr.

Table 3.4: SIMPER Analysis results for the metals contributing more than 10% to the similarity within the field surveys for *Oreochromis mossambicus* from Flag Boshielo Dam.

Survey	Metals contributing to similarity within groups
February 2016	Sr 20%; Ba 18%; Mn 16%; Ti 11%
April 2016	Zn 20%; Se 14%; Sr 11%
June 2016	Al 17%; Sr 13%; Se 13%
October 2016	Sr 17%; V 10%
March 2017	Ba 22%; Sr 12%; Se 11%
April 2017	Al 34%; Cu 14%
July 2017	Al 33%; Cu 21%; Ag 14%
September 2017	Zn 49%, Ti 13%
December 2017	Zn 25%; Sr 15%; Ti 14%; Ag 10%
February 2018	Zn 34%; Al 22%; Ti 12%
May 2018	Zn 34%; Cu 12%

Table 3.5: SIMPER Analysis results for the metals contributing more than 10% to the dissimilarity between the field surveys for *Oreochromis mossambicus* in Flag Boshielo Dam.

Survey	Feb '16	April '16	June '16	Oct '16	March '17	April '17	July '17	Sep '17	Dec '17	Feb '18
April '16	Zn 50%									
June '16	Zn 45%; Mn 11%	Sr 13%; Se 12%; Al 11%								
October '16	Zn 47%	Sr 22%; Se 14%	Sr 16%; Ni 10%							
March '17	Zn 52%	Se 15%; Ba 13%; Sr 11%	Al 15%; Ba 13%; Ni 10%; Sr 10%	Sr 16%; Ba 14%; Al 10%						
April '17	Zn 21%; Mn 14%; Cu 14%; Ni 11%; V 11%	Cu 20%; Ni 14%; Mn 13%; V 13%	Cu 23%; V 14%; Mn 11%; Ni 11%	Cu 21%; Ni 15%; V 13%; Mn 13%	Cu 21%; V 15%; Ni 14%; Mn 11%					
July '17	Zn 22%; Cu 18%; Ni 12%	Cu 27%; Ni 14%; V 11%	Cu 31%; Ni 12%; V 12%	Cu 29%; Ni 17%; V 11%	Cu 28%; Ni 15%; V 12%	Al 25%; Cu 14%; Mn 13%; Ag 13%				
September '17	Zn 25%; Mn 14%; Sr 11%	Zn 22%; Sr 15%	Zn 25%; Sr 12%	Zn 26%	Zn 21%; Sr 10%	Zn 21%; Cu 20%	Cu 28%; Zn 22%			
December '17	Zn 16%; Mn 11%	Zn 29%; Se 11%	Zn 29%; Al 13%	Zn 29%; Al 11%	Zn 30%	Zn 19%; Cu 17%; V 11%; Mn 10%	Cu 24%; Zn 20%; Ni 10%	Zn 28%; Ti 14%; Sr 12%		
February '18	Cu 24%; Zn 16%; Mn 15%	Cu 31%; Mn 14%; V 11%	Cu 35%; Mn 12%; V 11%	Cu 33%; Mn 13%; V 11%	Cu 32%; Mn 12%; V 12%	Cu 23%; Al 18%, Zn 12%	Cu 17%; Al 14%; Mn 13%	Cu 35%; Zn 16%	Cu 29%; Zn 14%; Mn 11%; Al 11%	
May '18	Cu 23%; Mn 18%; Zn 18%	Cu 32%; Mn 17%	Cu 36%; Mn 15%	Cu 34%; Mn 17%; Co 10%	Cu 33%; Mn 15%	Cu 16%; Zn 16%; Ni 13%	Mn 16%; Zn 15%; Cu 12%; Ni 11%	Cu 34%; Zn 19%; Mn 12%	Cu 30%; Zn 17%; Mn 14%	Zn 25%; Al 18%; Cu 11%

Labeo rosae

The metal concentrations on fish muscle tissue for rednose labeo *L. rosae* are summarised in box and whisker plots (Figure 3.16). A general pattern of the March and April 2017 field trips revealed significantly different metal concentrations than the field surveys conducted after April 2017, e.g. Sb, Cd, Co, Cu, Mn, Mo and Sr and to a lesser extent As, Cr, Cu, Pb, Se, Ag, V and Zn.

The correlation coefficients between the metals in the muscle tissues were calculated using Kendall's tau-B (Table 3.6). The metals, As, Cd, Cr, Co, Mo, Ni, Pb, Sb, Se and V, exhibited correlation coefficients > 0.6 with each other. In addition, correlation coefficients > 0.6 were found for the following metal pairs: Ag-Cd, Ag-Sb, and Al-R, and Zn with Co, Cu, and Mo.

The average metal concentrations for each survey were then compared to the average metal concentrations reported previously for this species in Flag Boshielo Dam by Jooste et al. (2013); see Figure 3.17. The results reported by Jooste et al. (2013) were considerably higher than the results reported in this survey for Sb, Ba, B, Cr, Co, Pb, Sn, and V, while the remainder of the metals reported on were similar or lower in the 2013 results.

The NMDS plot of the metal concentrations in muscle tissues shows a clear separation between the respective surveys (Figure 3.18). The PERDISP result for multivariate dispersion of the metal concentrations in muscle tissues of *L. rosae* across the surveys was significant ($p = 0.0001$) and the pair-wise *post hoc* comparison between surveys found a significant difference in multivariate dispersion between the May 2018 survey and the 2017 surveys. The PERMANOVA routine returned a significant result ($p < 0.001$) and the pair-wise *post hoc* comparison between surveys was significant for all comparisons. The NMDS plot confirms that the data from each survey was positioned at a unique point in multivariate space (Figure 3.18).

The SIMPER r analysis showed that the metals in *L. rosae* muscle tissue contributing to the similarity within the respective groups were unique for each survey (Table 3.7). The SIMPER results for the dissimilarity in the metals recorded in *L. rosae* muscle tissue between surveys also provides some interesting patterns (Table 3.8). Copper and Ni contributed the most to the dissimilarities between the March and April 2017 surveys and all other surveys. Zinc contributed most to the dissimilarity between the December 2017 survey and all other surveys while Al contributed the most to the dissimilarity between the February 2018 survey and those after September 2017, and the September 2017 surveys and those following it.

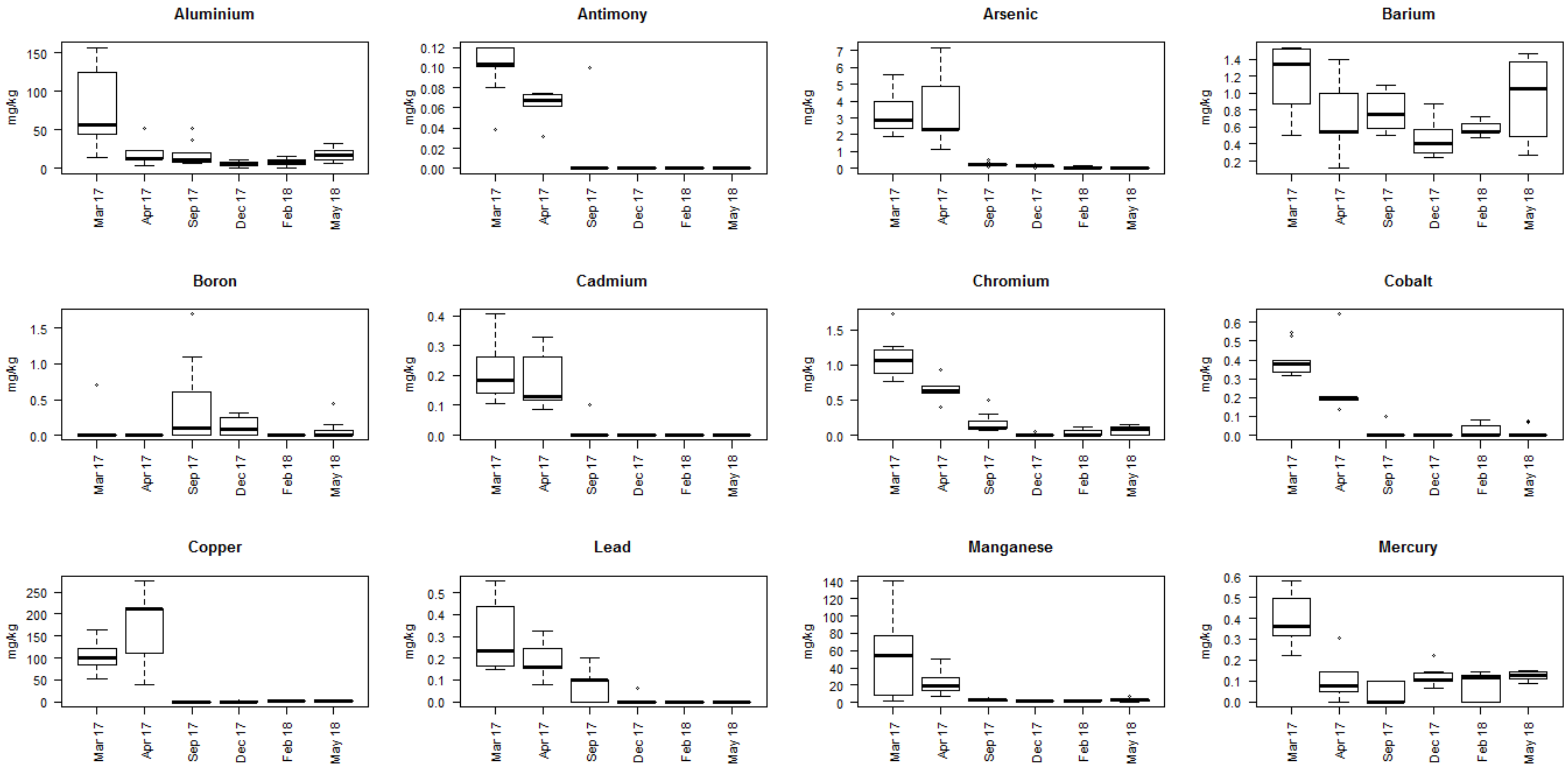

Figure 3.16: Box and whisker plots of metal concentrations (mg/kg dry weight), in the muscle tissue of *Labeo rosae* from Flag Boshielo Dam.

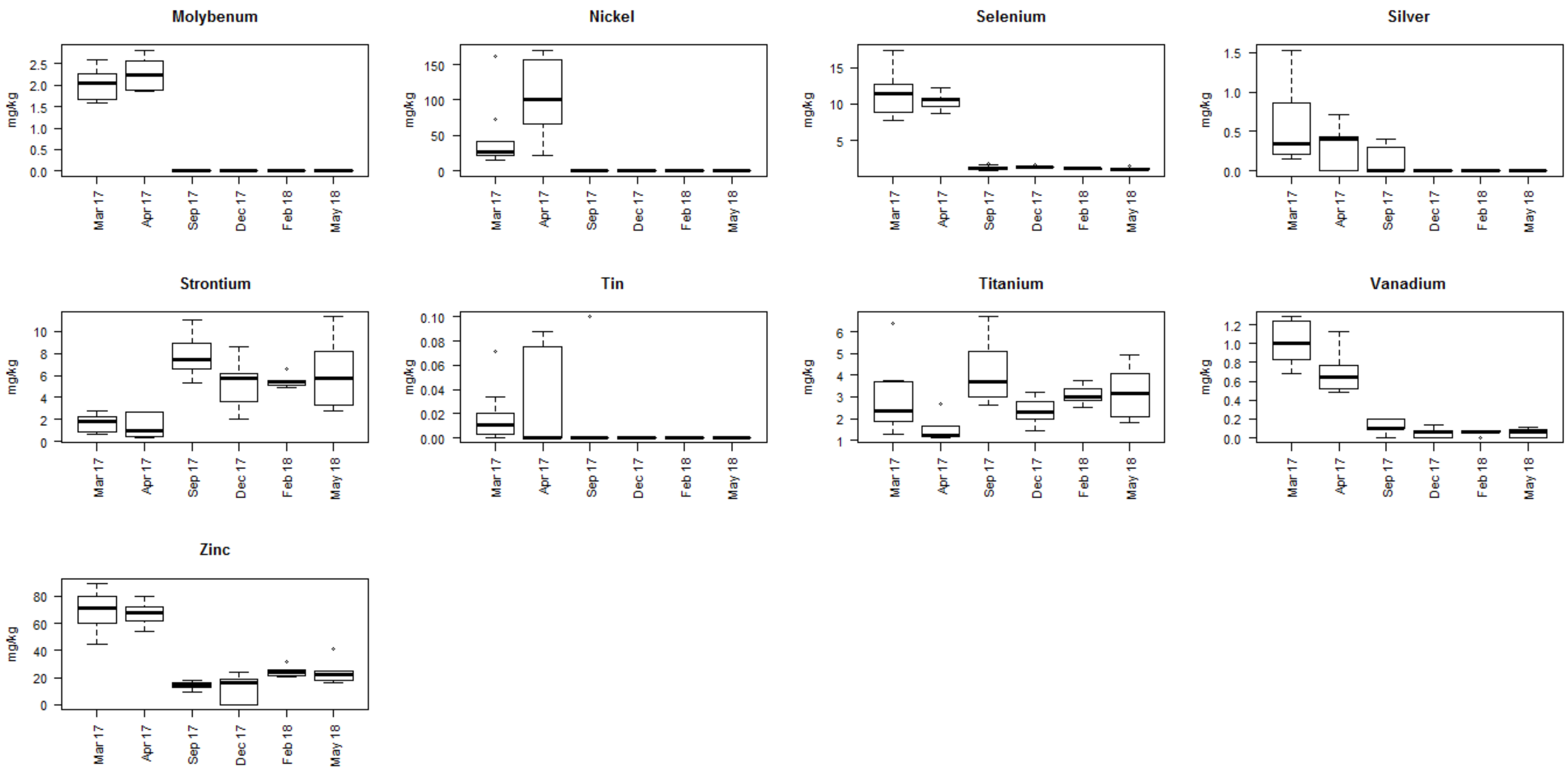

Figure 3.16 (cont): Box and whisker plots of metal concentrations (mg/kg dry weight), in the muscle tissue of *Labeo rosae* from Flag Boshielo Dam.

Table 3.6: The Kendall tau-B correlation coefficient between the metal concentrations in the muscle tissue of *Labeo rosae* collected from Flag Boshielo Dam. Correlation coefficients greater than ± 0.6 are highlighted.

	Ag	**Al**	**As**	**B**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Mn**	**Mo**	**Ni**	**Pb**	**Sb**	**Se**	**Sn**	**Sr**	**Ti**	**V**	**Zn**
Ag																					
Al	0.40																				
As	0.63	0.26																			
B	-0.06	-0.15	-0.09																		
Ba	0.16	0.39	0.11	-0.08																	
Cd	0.77	0.45	0.70	-0.16	0.21																
Co	0.59	0.43	0.58	-0.18	0.32	0.76															
Cr	0.58	0.64	0.57	-0.15	0.35	0.69	0.61														
Cu	0.42	0.33	0.27	-0.24	0.22	0.57	0.57	0.40													
Hg	0.29	0.30	0.10	-0.19	0.16	0.37	0.31	0.22	0.41												
Mn	0.41	0.50	0.35	-0.18	0.36	0.51	0.55	0.53	0.47	0.27											
Mo	0.55	0.40	0.60	-0.29	0.27	0.74	0.74	0.60	0.58	0.39	0.51										
Ni	0.54	0.38	0.66	-0.21	0.20	0.62	0.66	0.60	0.38	0.09	0.41	0.69									
Pb	0.53	0.49	0.64	-0.13	0.33	0.70	0.71	0.69	0.36	0.27	0.56	0.71	0.65								
Sb	0.66	0.46	0.68	-0.19	0.30	0.82	0.76	0.68	0.54	0.43	0.53	0.76	0.64	0.74							
Se	0.50	0.22	0.62	-0.07	0.07	0.67	0.58	0.40	0.39	0.28	0.29	0.61	0.49	0.57	0.63						
Sn	0.48	0.44	0.44	-0.33	0.26	0.50	0.47	0.51	0.36	0.16	0.43	0.52	0.44	0.54	0.49	0.32					
Sr	-0.38	-0.16	-0.42	0.25	0.13	-0.51	-0.47	-0.28	-0.44	-0.31	-0.11	-0.59	-0.44	-0.31	-0.51	-0.44	-0.29				
Ti	-0.10	0.22	-0.22	0.01	0.28	-0.21	-0.17	0.06	-0.18	-0.12	0.14	-0.29	-0.17	-0.06	-0.22	-0.29	0.03	0.56			
V	0.54	0.42	0.60	-0.14	0.38	0.65	0.68	0.63	0.37	0.28	0.57	0.62	0.55	0.76	0.66	0.44	0.46	-0.12	0.06		
Zn	0.37	0.41	0.27	-0.28	0.31	0.54	0.60	0.43	0.66	0.40	0.45	0.62	0.49	0.43	0.57	0.36	0.35	-0.45	-0.13	0.41	

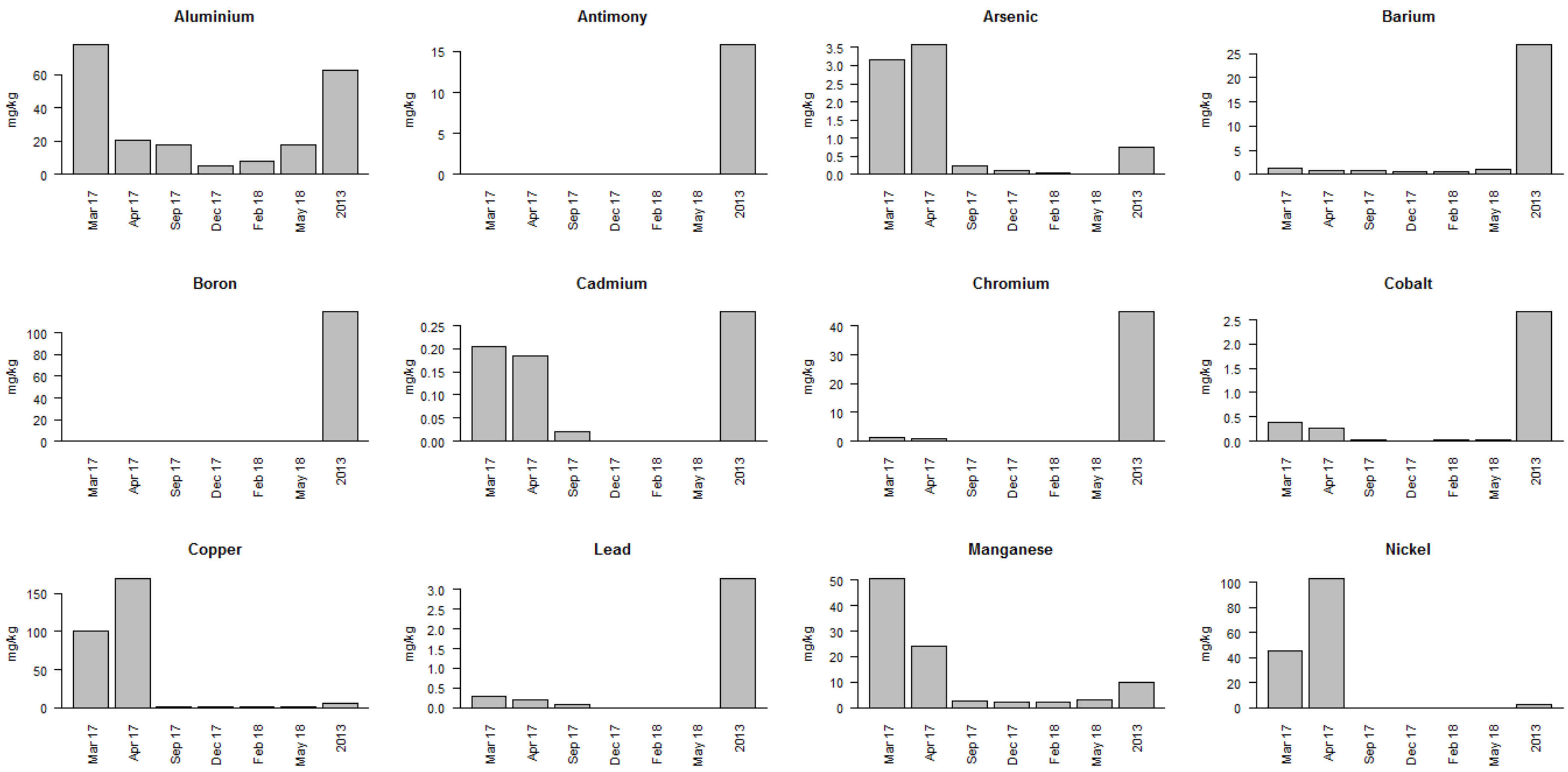

Figure 3.17: Comparison of the average metal concentrations (mg/kg dry weight) in the muscle tissue of *Labeo rosae* from Flag Boshielo Dam collected in the surveys of the current study to those published by Jooste et al. (2013).

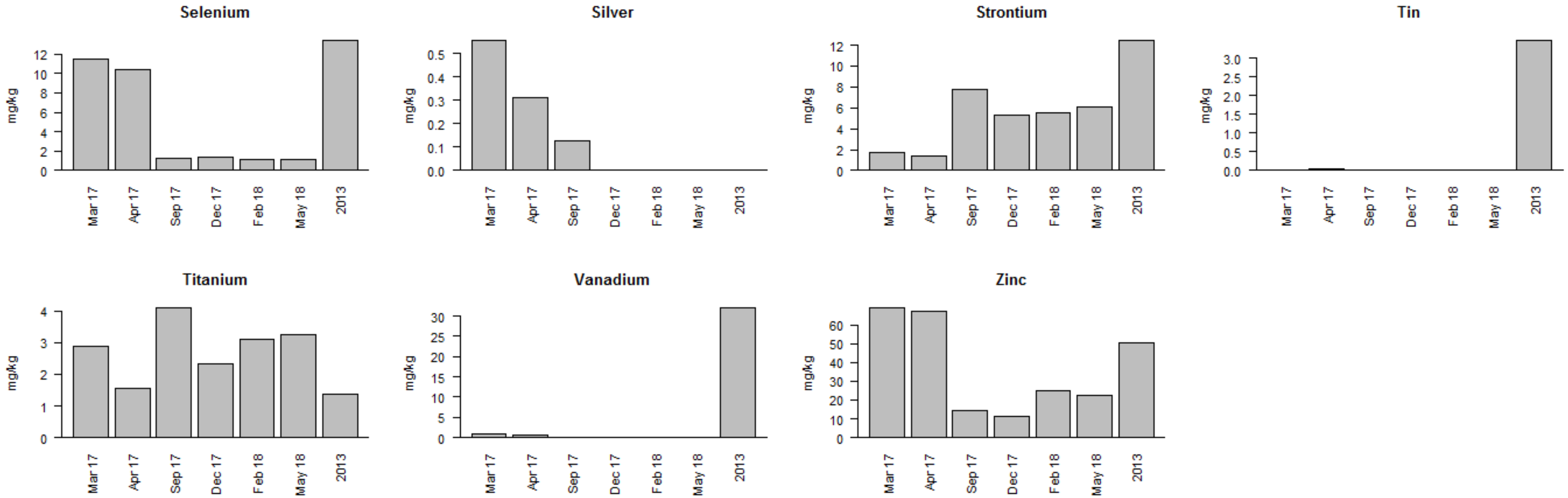

Figure 3.17 (cont): Comparison of the average metal concentrations (mg/kg dry weight) in the muscle tissue of *Labeo rosae* from Flag Boshielo Dam collected in the surveys of the current study to those published by Jooste et al. (2013).

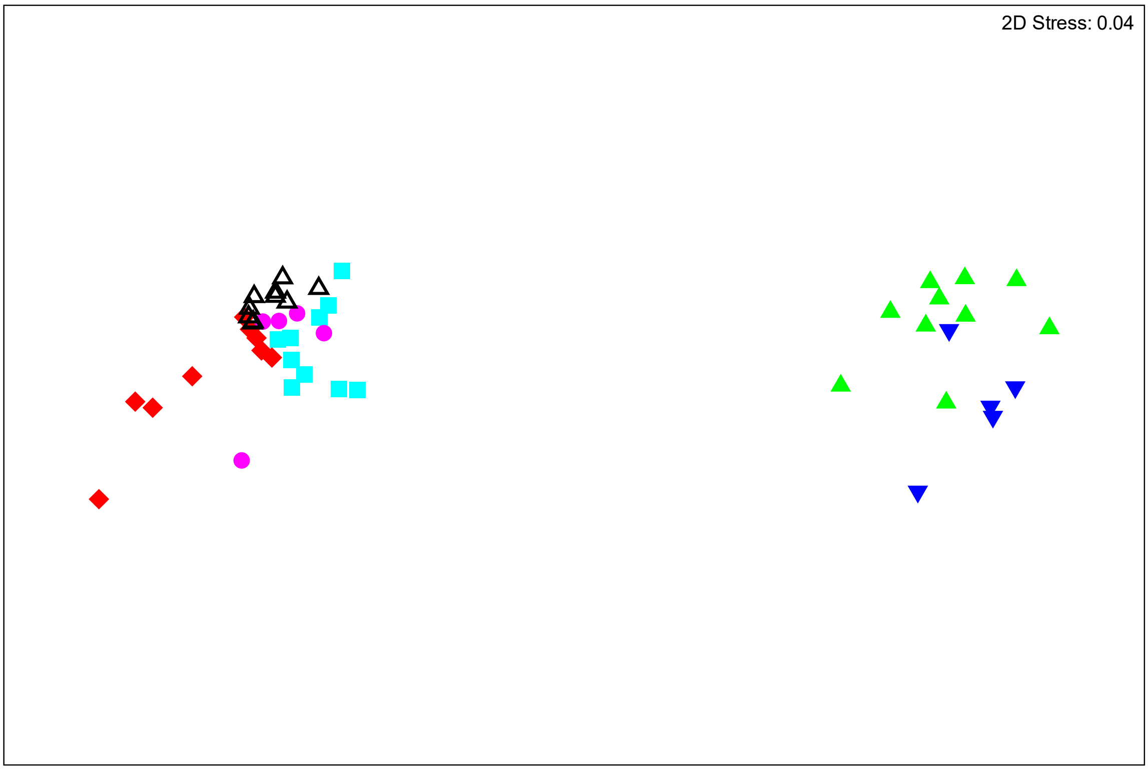

Figure 3.18: Non-metric multi-dimensional scaling plot for the metal concentration in the muscle tissue of *Labeo rosae* from the Flag Boshielo Dam based on Euclidian distance and a 4th root transform of the original data. The data points for each field trip are represented by unique symbols: Mar 17 (▲), Apr 17 (▼), Sep 17 (■) Dec 17 (♦), Feb 18 (●), and May 18 (∆).

Table 3.7: SIMPER Analysis results for the metals contributing more than 10% to the similarity within the field surveys for *Labeo rosae* in Flag Boshielo Dam.

Survey	Metal contributing to similarity within groups
March 2017	Mn 41%; Al 20%; Ni 15%
April 2017	Cu 22%; Ni 20%; Al 14%; Ag 12%
September 2017	B 22%; Ag 15%; Al 13%
December 2017	Zn 59%; Al 17%
February 2018	Al 59%
May 2018	B 19%; V 14%; Cr 13%; Al 11%

Table 3.8: SIMPER Analysis results for the metals contributing more than 10% to the dissimilarity between the field surveys results for *Labeo rosae* in Flag Boshielo Dam.

Survey	Mar '17	Apr '17	Sep '17	Dec '17	Feb '18
Apr '17	Al 27%; Ni 20%; Mn 17%; Cu 12%				
Sep '17	Cu 37%; Ni 21%	Cu 33%; Ni 31%			
Dec '17	Ni 22%; Cu 19%; Zn 13%; Al 11%	Ni 33%; Cu 25%; Zn 12%	Zn 30%; Al 18%		
Feb '18	Cu 21%; Ni 18%; Al 14%	Ni 30%; Cu 28%	Al 30%; B 14%	Zn 46%; Al 20%	
May '18	Ni 27%; Cu 21%	Ni 37%; Cu 26%	As 17%; B 13%	Zn 46%; Al 21%	Al 43%; Ni 11%

Schilbe intermedius

The metal concentrations on fish muscle tissue for silver catfish *S. intermedius* are summarised in box and whisker plots (Figure 3.19). The Kruskal-Wallis test returned a significant result for all tests with the exception of Sb (p = 0.441). A general pattern of the March and July 2017 field surveys revealed significantly different metal concentrations than the field surveys after April 2017, e.g. for As, Cr and Ni. In addition, the September and December 2017 surveys revealed concentrations of Ba, B, Co, Cu, Pb, Mn, Ti, V and Zn to be significantly different when compared to levels recorded during the other surveys.

The correlation coefficients between the metals in the muscle tissues were calculated using Kendall's tau-B (Table 3.9). Correlation coefficients > 0.6 were found for V with Co, Cu, Mo and Pb, Mo with Co, Cu and Se, and for Cd-Co, As-Ni, and Ag-Sb.

The average metal concentrations for each survey were then compared to the average metal concentrations reported previously for this species in Flag Boshielo Dam by Jooste et al. (2013); see Figure 3.20. The results reported by Jooste et al. (2013) were considerably higher than the results reported in this survey for Al, Sb, Ba, B, Cr, Co, Pb, Mn, Sr, Sn, V and Zn, while the remainder of the metals reported on were similar or lower in the 2013 results.

The NMDS plot of the metal concentrations in muscle tissues shows a clear separation between the respective surveys (Figure 3.21). The PERDISP result for multivariate dispersion of the metal concentrations in muscle tissues of *S. intermedius* across the surveys was significant (p = 0.0001) and the pair-wise *post hoc* comparison between surveys found a significant difference in multivariate

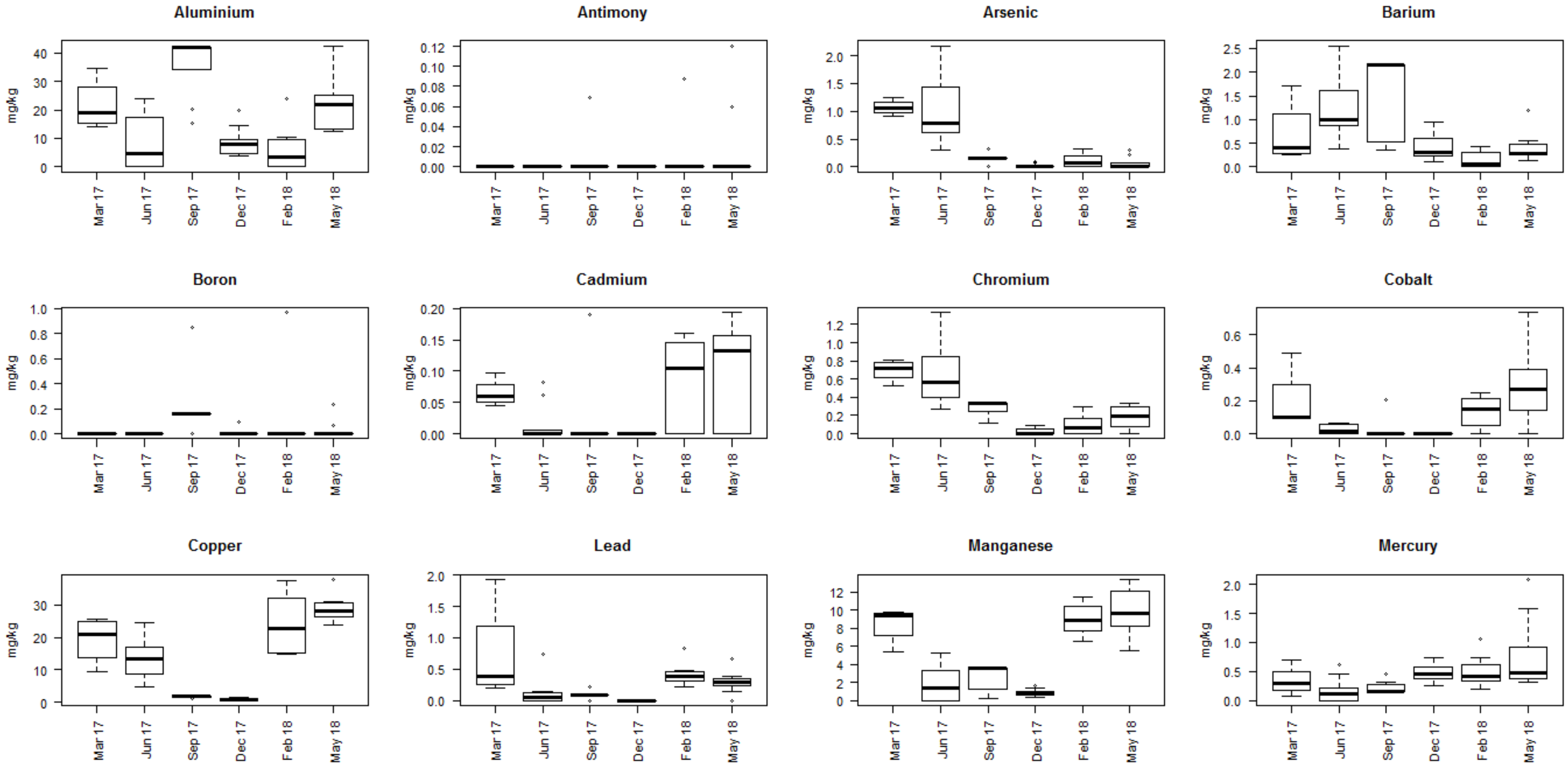

Figure 3.19: Box and whisker plots of metal concentrations (mg/kg dry weight), in the muscle tissue of *Schilbe intermedius* from Flag Boshielo Dam.

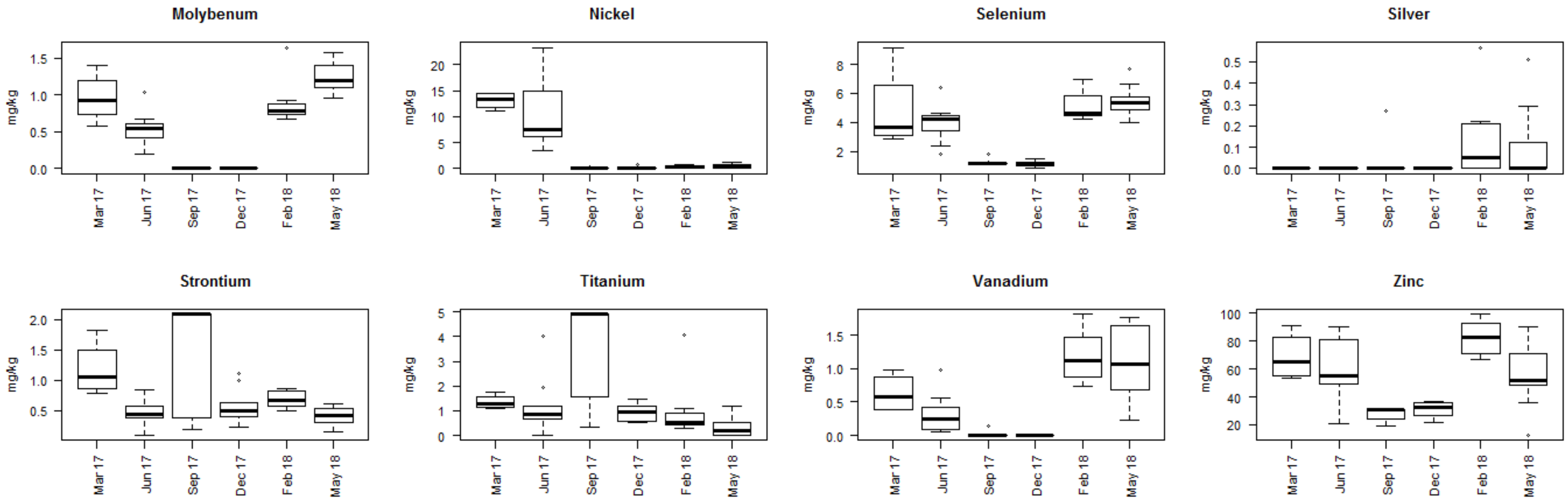

Figure 3.19 (cont): Box and whisker plots of metal concentrations (mg/kg dry weight), in the muscle tissue of *Schilbe intermedius* from Flag Boshielo Dam.

Table 3.9: The Kendall tau-B correlation coefficient between the metal concentrations in the muscle tissue of *Schilbe intermedius* collected from Flag Boshielo Dam. Correlation coefficients greater than ± 0.6 are highlighted.

	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	Ti	V	Zn
Ag																					
Al	0.09																				
As	0.07	0.05																			
B	0.26	0.32	-0.03																		
Ba	-0.21	0.25	0.27	0.22																	
Cd	0.52	-0.05	0.15	0.05	-0.22																
Co	0.51	0.02	0.19	-0.03	-0.17	0.63															
Cr	-0.21	0.16	0.57	0.06	0.47	0.00	0.09														
Cu	0.26	0.02	0.20	-0.14	-0.13	0.33	0.52	0.16													
Hg	0.27	-0.17	-0.29	-0.06	-0.30	0.38	0.26	-0.33	0.03												
Mn	0.23	0.14	-0.05	0.02	-0.22	0.31	0.46	-0.01	0.49	0.24											
Mo	0.27	-0.04	0.14	-0.23	-0.21	0.42	0.61	0.08	0.76	0.16	0.49										
Ni	-0.05	-0.01	0.66	-0.11	0.21	0.19	0.25	0.59	0.35	-0.21	0.10	0.31									
Pb	0.28	-0.01	0.16	0.08	-0.06	0.47	0.53	0.15	0.53	0.20	0.51	0.51	0.29								
Sb	0.60	0.12	0.05	0.38	-0.09	0.46	0.39	-0.06	0.11	0.32	0.13	0.16	0.04	0.26							
Se	0.32	-0.09	0.17	-0.12	-0.11	0.37	0.61	0.14	0.67	0.21	0.45	0.71	0.29	0.57	0.23						
Sn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA					
Sr	0.05	0.23	0.14	0.34	0.22	-0.02	-0.05	0.08	0.00	-0.11	0.16	-0.03	0.03	0.11	-0.04	-0.02	NA				
Ti	-0.21	0.34	0.17	0.16	0.36	-0.26	-0.32	0.12	-0.25	-0.29	-0.15	-0.32	0.00	-0.16	-0.24	-0.32	NA	0.37			
V	0.35	-0.18	0.13	-0.18	-0.24	0.47	0.62	0.06	0.69	0.25	0.43	0.72	0.28	0.62	0.24	0.69	NA	-0.06	-0.37		
Zn	0.27	-0.16	0.25	-0.18	-0.08	0.39	0.42	0.15	0.44	0.14	0.27	0.49	0.26	0.50	0.14	0.50	NA	0.12	-0.12	0.54	

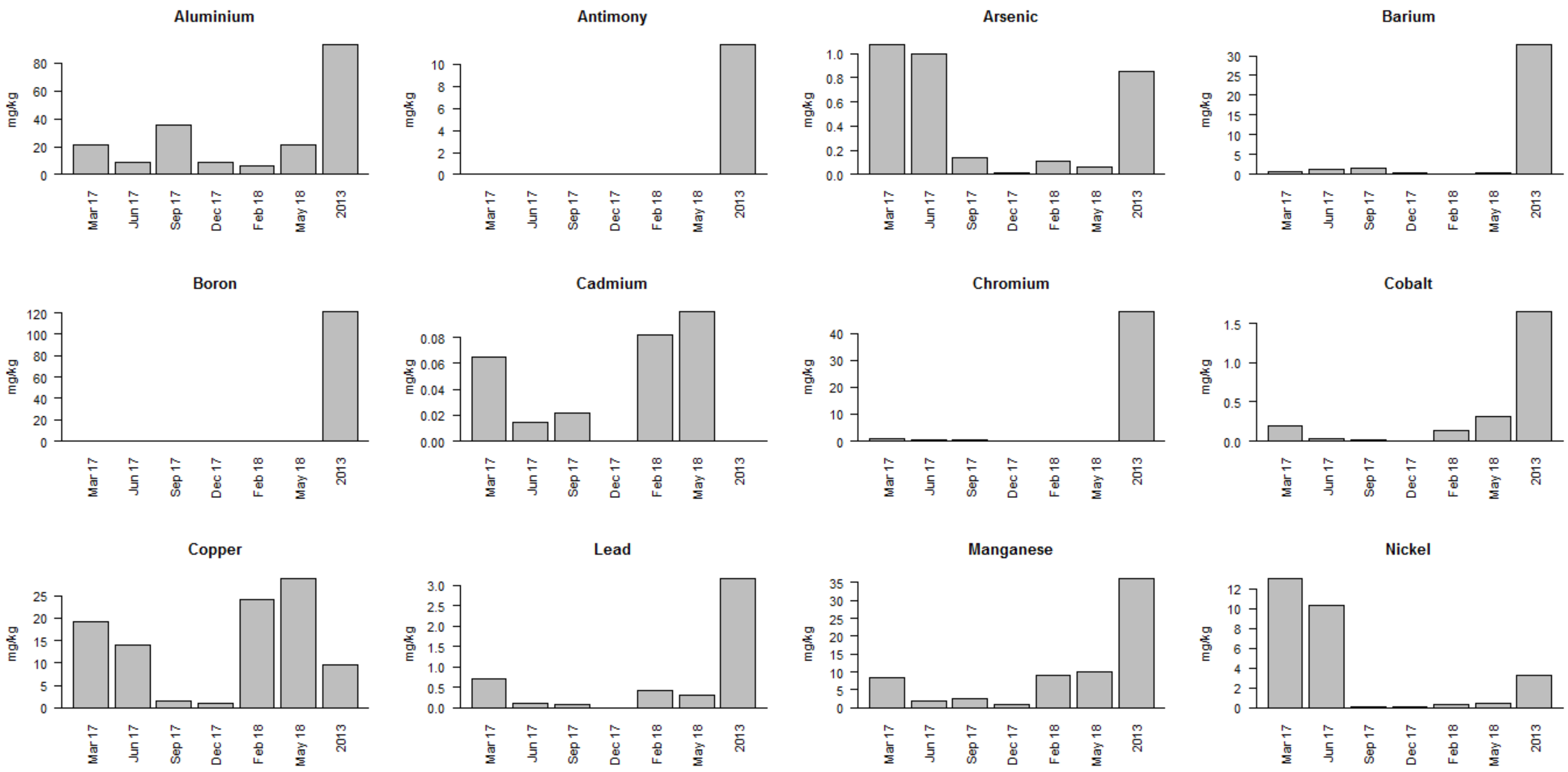

Figure 3.20: Comparison of the average metal concentrations (mg/kg dry weight) in the muscle tissue of *Schilbe intermedius* from Flag Boshielo Dam collected in the surveys of the current study to those published by Jooste et al. (2013).

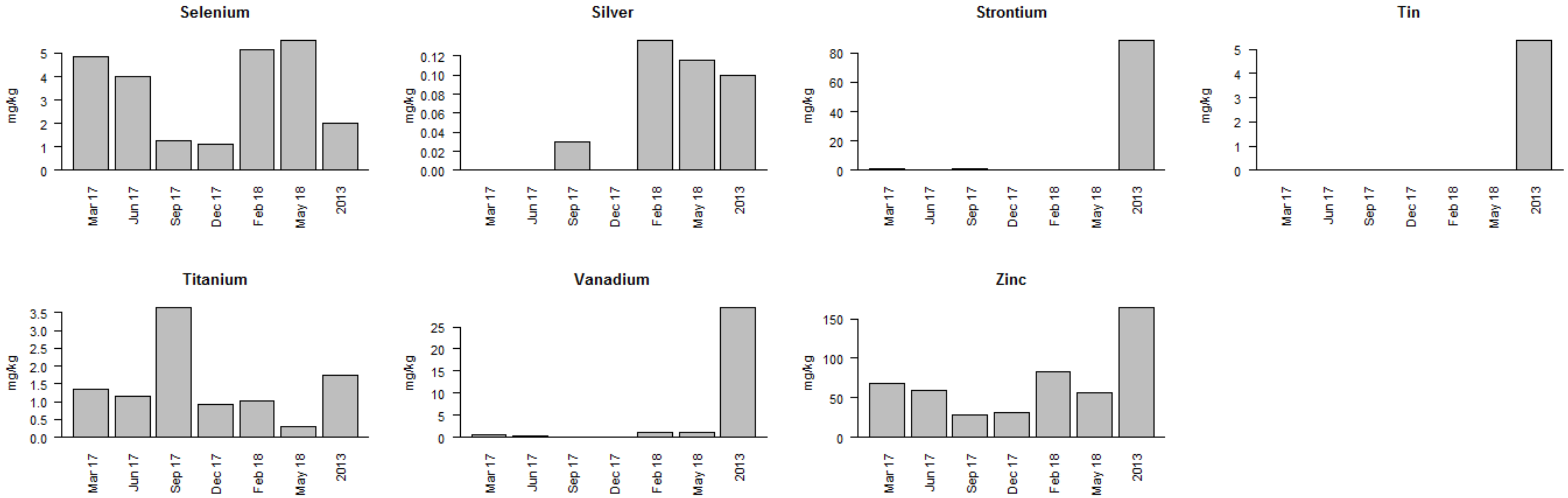

Figure 3.20 (cont): Comparison of the average metal concentrations (mg/kg dry weight) in the muscle tissue of *Schilbe intermedius* from Flag Boshielo Dam collected in the surveys of the current study to those published by Jooste et al. (2013).

dispersion between the May 2018 survey and all other surveys with the exception of June 2017, February 2018 survey and all other surveys with the exception of April 2017, and the April 2017 survey and all other surveys with the exception of February 2018. The PERMANOVA routine returned a significant result ($p < 0.001$) and the pair-wise *post hoc* comparison between surveys was significant for all comparisons. The NMDS plot confirms that the data from each survey was positioned at a unique point in multivariate space.

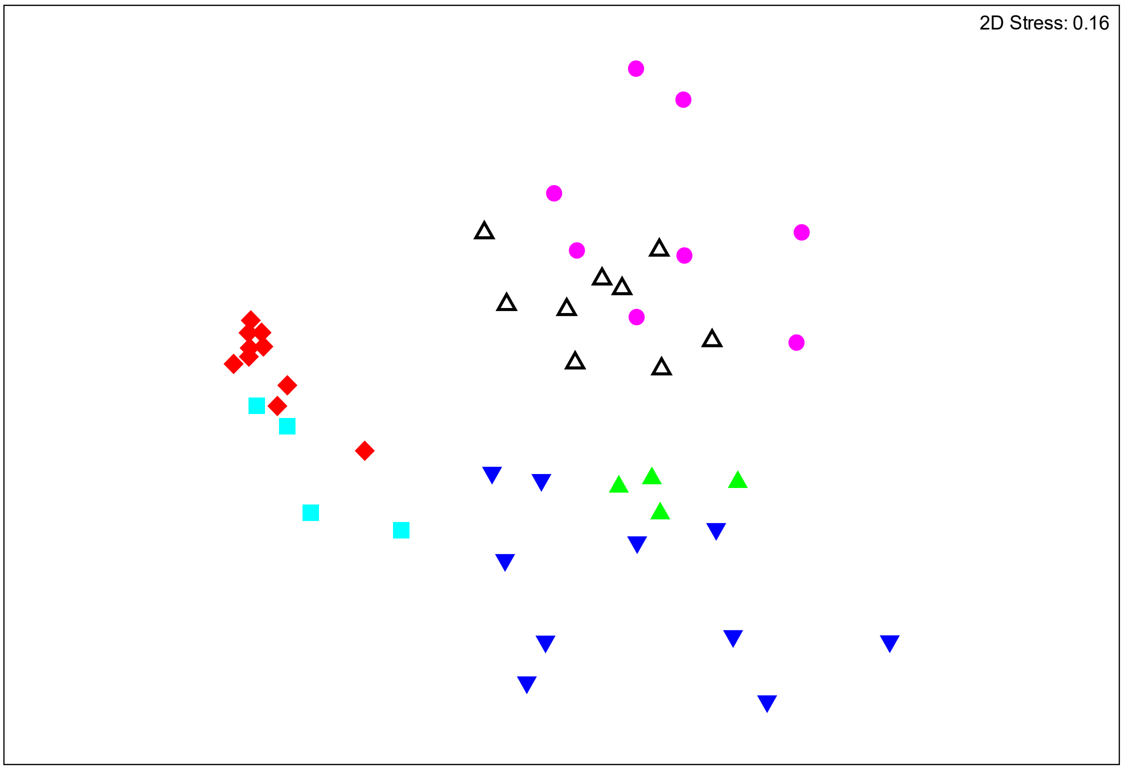

Figure 3.21: Non-metric multi-dimensional scaling plot for the metal concentration in the muscle tissue of *S. intermedius* from the Flag Boshielo Dam based on Euclidian distance and a 4th root transform of the original data. The data points for field trip are represented by unique symbols: Mar 17 (▲), Jun 17 (▼), Sep 17 (■) Dec 17 (♦), Feb 18 (●), and May 18 (Δ).

The SIMPER r analysis showed that the metals in *S. intermedius* muscle tissue contributing to the similarity within the respective groups were unique for each survey, although the July 2017 and February 2018 survey results were both dominated by Al (Table 3.10). The SIMPER results for the dissimilarity in the metals recorded in *S. intermedius* muscle tissue between surveys also provides some interesting patterns (Table 3.11). Aluminium contributed the most to the dissimilarities between the July 2017 and February 2018 surveys and all other surveys. Nickel contributed most to the dissimilarity between the December 2017 survey and the proceeding surveys while Cu contributed the most to the dissimilarity between the December 2017 survey and subsequent surveys. Nickel also contributed most to the dissimilarity between the September 17 survey and the proceeding surveys while Cu, Mo and V contributed the most to the dissimilarity between the May 2018 survey and the December 2017 and February 2018 surveys.

Table 3.10: SIMPER Analysis results for the metals contributing more than 10% to the similarity within the field surveys for *Schilbe intermedius* in Flag Boshielo Dam.

Survey	Metal contributing to similarity within groups
March 2017	Cu 15%; Pb 14%; Al 13%; Ba 11%; Se 11%
July 2017	Al 41%; Mn 22%
September 2017	B 12%; As 10%
December 2017	Ni 23%; Cr 18%; As 14%; Al 13%
February 2018	Al 45%
May 2018	Ti 16%; Ag 11%; Zn 10%

Table 3.11: SIMPER Analysis results for the metals contributing more than 10% to the dissimilarity between the field surveys results for *Schilbe intermedius* in Flag Boshielo Dam.

Survey	Mar '17	Jul '17	Sep '17	Dec '17	Feb '18
Jul '17	Al 34%; Mn 29%				
Sep '17	Ni 25%; Mo 13%; Cu 12%	Al 25%; Ni 18%			
Dec '17	Ni 31%; Cu 11%	Ni 31%; Al 11%; Cu 10%	Al 20%; Cr 12%; B 11%; Ni 10%		
Feb '18	Al 34%; Ni 30%	Al 21%; Ni 21%; Mn 16%	Al 30%; Cu 11%	Cu 17%; Al 16%; V 11%; Mo 10%	
May '18	Ni 35%; As 17%; Ti 13%	Al 20%; Mn 19%; Ni 16%	Cu 18%; Mo 14%; Ti 13%; V 11%	Cu 22%; Mo 13%; V 11%	Al 45%

3.1.11 Fluctuations of water levels in Flag Boshielo Dam

The data collected during this study needs to be considered in the context of the fluctuations that took place in Flag Boshielo Dam over the duration of the study; see Figure 3.22. The river discharge showed the typical low values of the dry cycle of the Olifants River at the beginning of the study. The level of dam was about 50% when the study commenced but this dropped dramatically to about 20% in the latter half of 2016 when a tree limb became lodged in the outlet of the dam. The outlet of the dam could only be closed after the tree limb had been removed. The dramatically low levels of Flag Boshielo Dam are illustrated graphically in the Google Earth images of the dam in January 2015 and December 2016 are viewed side-by-side (Figure 3.23).

Good rains at the end of 2016 resulted in the level of the dam rising to 50% by February 2017 after which a high flow event was released to the Olifants River below the dam. The rapid increase in dam level resulted in a sharp decrease in the conductivity of the water in the impoundment, a reset in ionic concentrations similar to those caused by flood events with river discharges exceeding 20 cumecs; see Figure 2.8. This dilution of the waters of the impoundment revealed a second mechanism through which the water chemistry of the impoundment can be reset other than by flood events revealed in the analysis of the historical data. Good rains in the autumn of 2018 resulted in a flood event that raised the impoundment's capacity whereby the dam was spilling in April 2018.

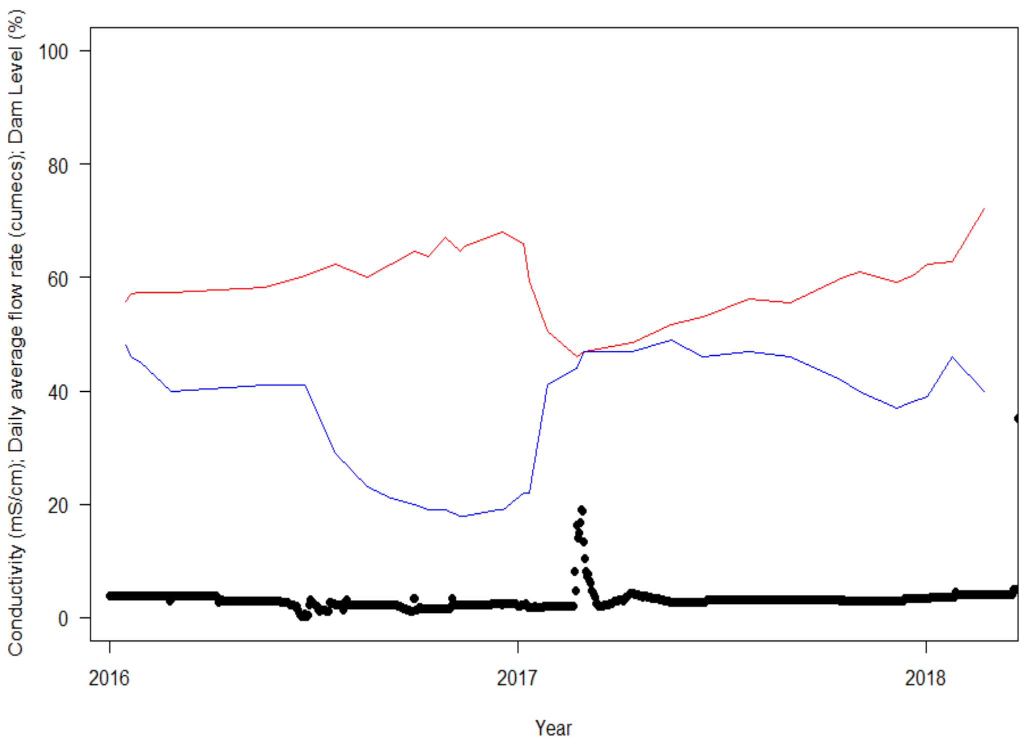

Figure 3.22: Fluctuations in the Olifants River discharge (black), dam level (blue) and electrical conductivity (red) in Flag Boshielo Dam during the study.

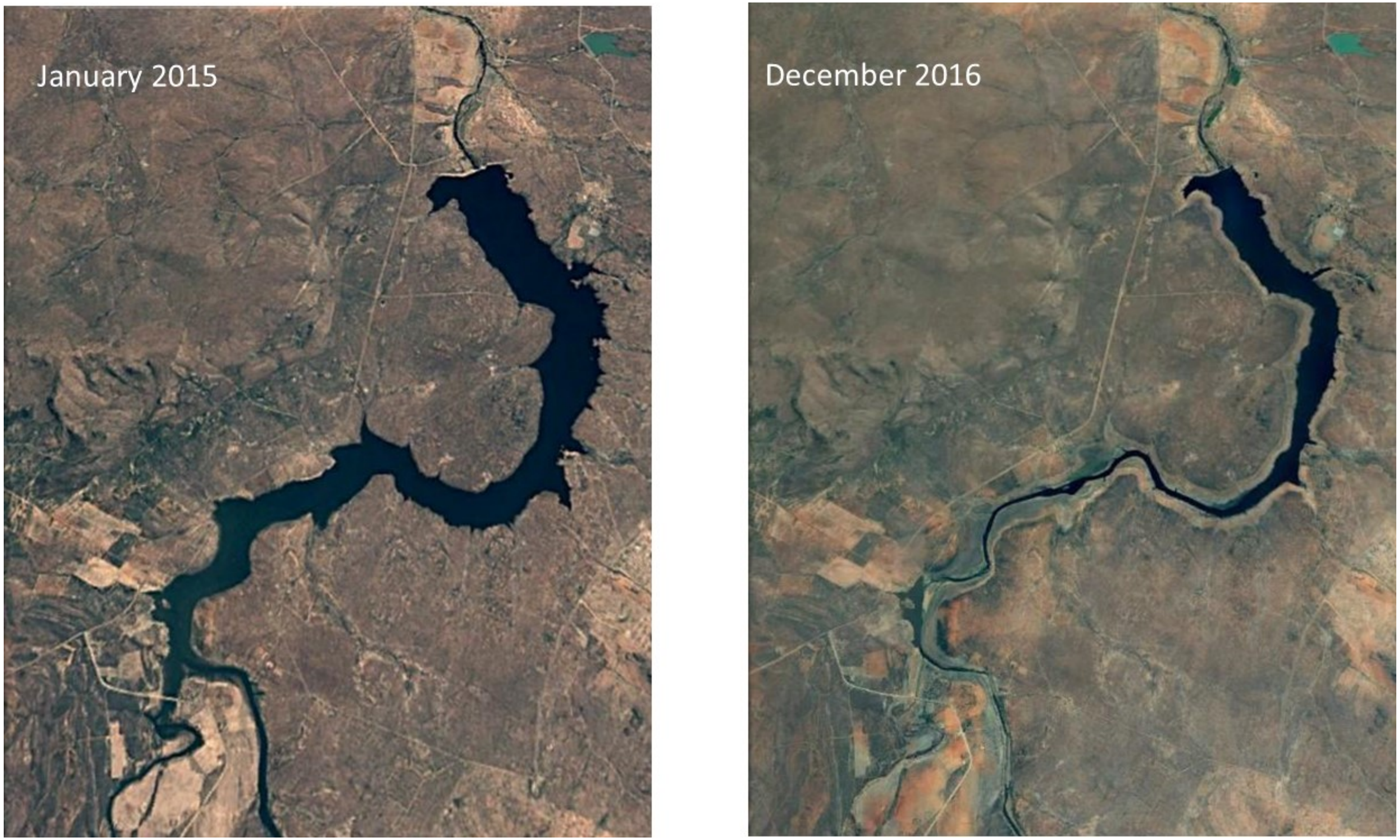

Figure 3.23: Change in the level of Flag Boshielo Dam from near capacity in January 2015 to 20% in December 2016; images from Google Earth.

3.1.12 Human health risks and edibility of fish

Concentrations and uptake of metals in fish is subject to environmental and species-specific biological factors as well as chemical and physical states of metals (Coetzee et al., 2002). Metal bioaccumulation in fish can be influenced by seasonality due to fluctuations in exposure routes, i.e. diet as well as aqueous concentrations and bioavailability (Luoma and Rainbow, 2005).

Based on the metal content in the muscle tissue of *O. mossambicus*, cobalt (Co) was the only metal to have a Hazard Quotient (HQ) greater than one (Figure 3.24) for fish collected during the February 2018 survey (Table 3.12). Overall the metals in the muscle tissue of *O. mossambicus* did not accumulate to levels that posed a health risk to humans should this species have been consumed over the period surveyed. By comparison, the average HQ previously calculated for *O. mossambicus* from Flag Boshielo Dam by Jooste et al. (2013) for Cr, Co, Pb, Sb and V was 9, 4, 50, 40 and 5 respectively (see Figure 3.25).

With regard to Co, exposure of the general population to this metal occurs through inhalation of ambient air and ingestion of food and drinking water. In general, intake from food sources is much greater than from drinking water and inhalation. Cobalt intake from food sources has been estimated to be 5.0-40.0 µg/day. Adequate chronic studies of the oral toxicity of Co or Co compounds in humans and animals are not presently available. The most sensitive endpoint following oral exposure to Co in humans appears to be an increase in erythrocyte numbers (polycythaemia) (ATSDR, 2004a). This effect has been observed in both normal subjects and in patients who were anaemic as a result of being anephric. However, treatment of pregnant women with Co did not prevent the reduction in haematocrit and haemoglobin levels often found during pregnancy (ATSDR, 2004a). Exposure of humans to beer containing Co used as a foam stabilizer resulted in severe effects on the cardiovascular system, including cardiomyopathy and death, as well as gastrointestinal effects (nausea, vomiting) and hepatic necrosis. However, the subjects in these studies were alcoholics, and it is not known what effect excessive alcohol consumption may have played in the development of the observed effects (ATSDR, 2004a).

Available studies of the carcinogenic effects of Co in occupationally-exposed humans have reported mixed results, with both positive and negative results recorded (IARC, 2012). Oral data on the carcinogenic effects of Co and Co compounds are not available. IRIS does not report a cancer classification for Co or Co compounds. The International Agency for Research on Cancer (IARC) has classified Co and Co compounds as possibly carcinogenic to humans (IARC, 2012).

In *L. rosae* a HQ > 1 was calculated for As in individual specimens collected during the March and April 2017 surveys (see Figure 3.26 and Table 3.13). However, the average HQ for As did not exceed one. In general, metals detected in this study in the muscle tissue of *L. rosae* did not accumulate to levels that posed a health risk to humans consuming this fish. In contrast the study by Jooste et al. (2013) reported average HQ values of 2, 12, 5, 40, 30, 2 and 5 for As, Cr, Co, Pb, Sb, Se and V respectively for the same species (see Figure 3.27).

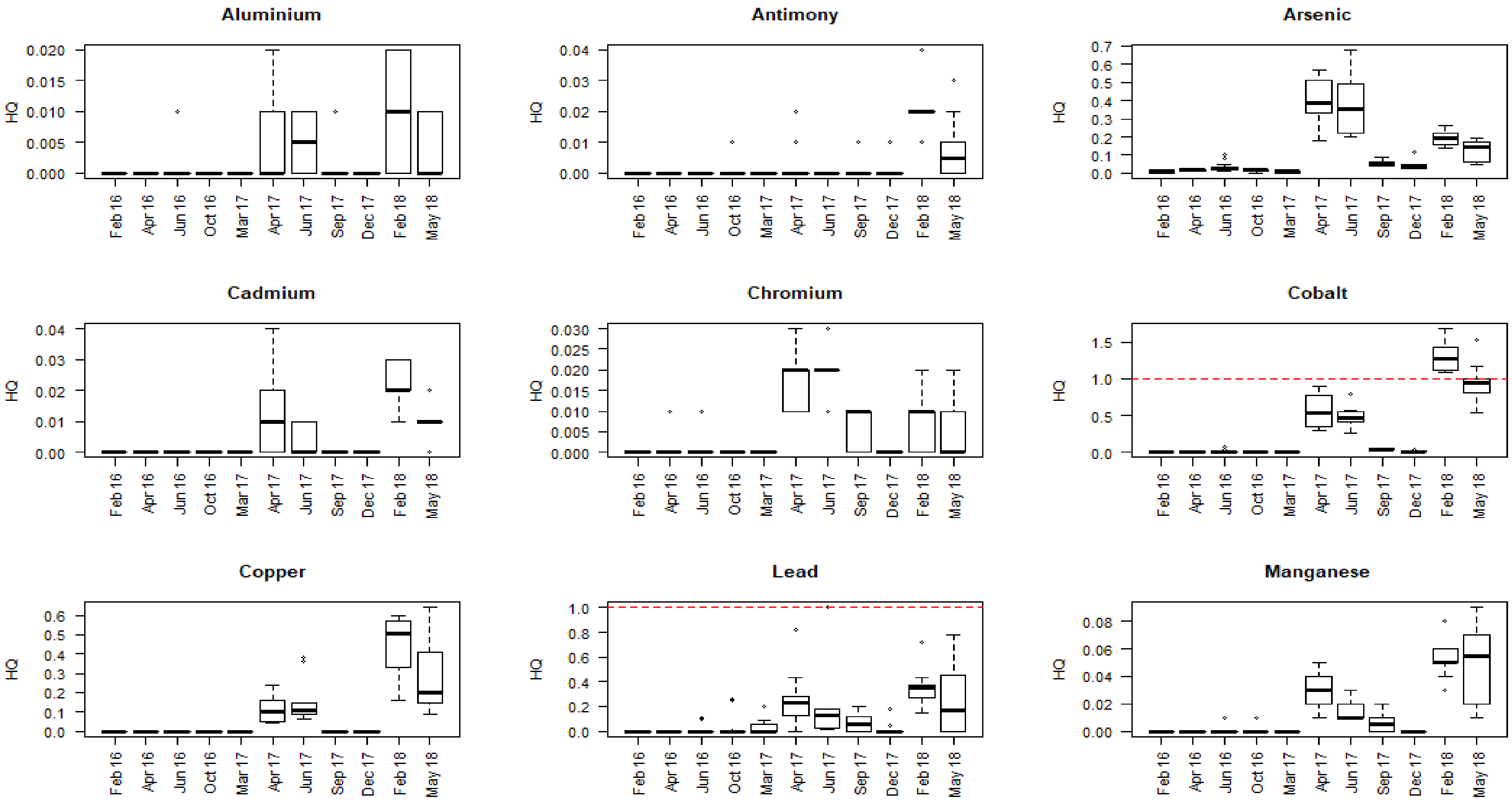

Figure 3.24: Box plots of the Hazard Quotient (HQ) calculated for metal and metalloids in *Oreochromis mossambicus* sampled from Flag Boshielo Dam during surveys conducted between from February 2016 to May 2018. The broken line (red) indicates an HQ of 1 above which long-term health impairment is possible.

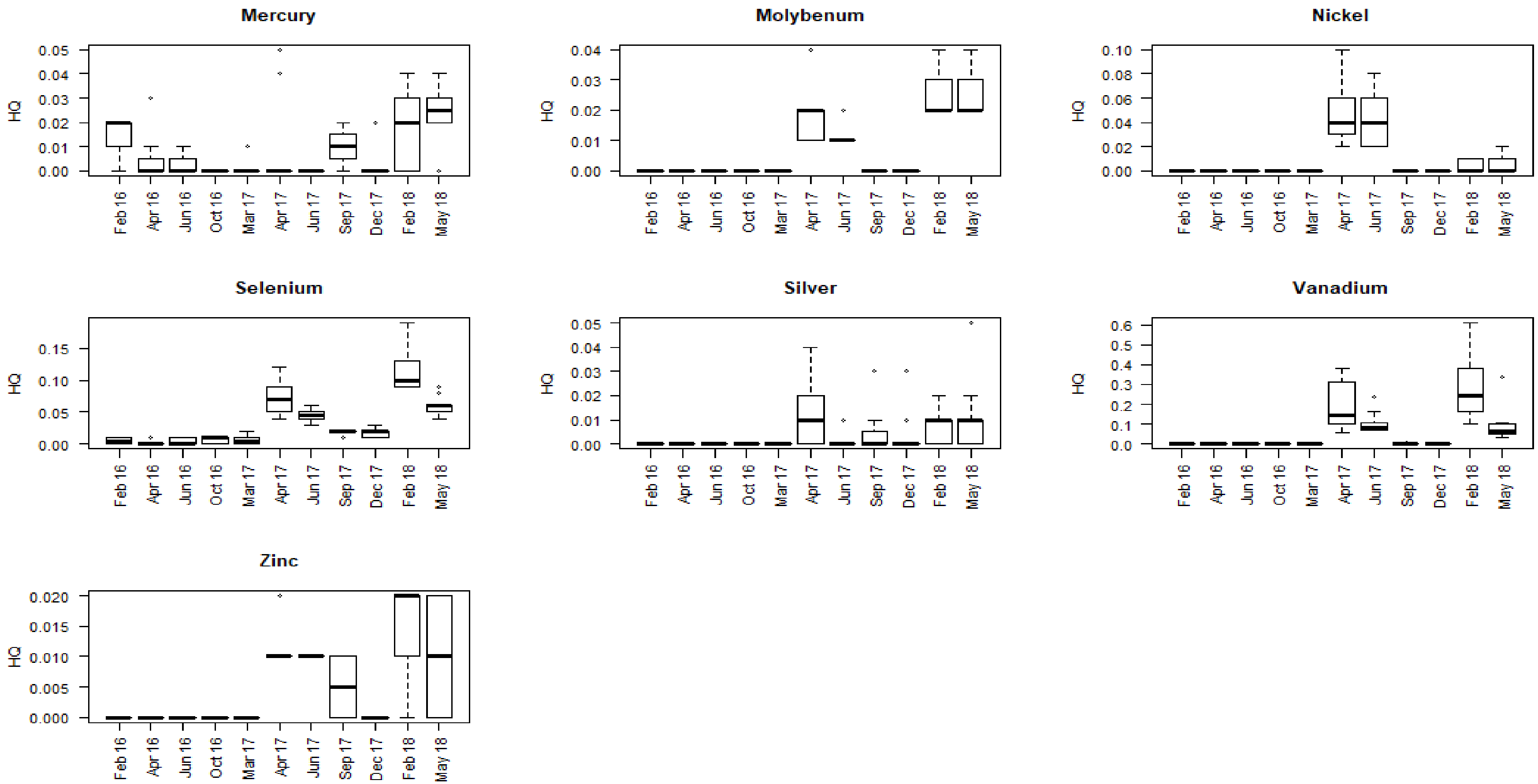

Figure 3.24 (cont): Box plots of the Hazard Quotient (HQ) calculated for metal and metalloids in *Oreochromis mossambicus* sampled from Flag Boshielo Dam during surveys conducted between from February 2016 to May 2018. The broken line indicates an HQ of 1 above which long-term health impairment is possible.

Table 3.12: The Hazard Quotients (HQ), based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Oreochromis mossambicus* sampled during surveys conducted at Flag Boshielo Dam from February 2016 to May 2018. Cells highlighted in red indicate where the HQ exceeds 1.

Survey 1	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	4.66	0.01	0.00	0.06	0.00	0.00	0.01	0.07	0.01	0.13	0.00	0.00	0.00	0.00	0.09	0.00	0.12	0.00	0.00
Average Daily Dose (µg/kg)	0.00	1.43	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.04	0.00	0.00	0.00	0.00	0.03	0.00	0.04	0.00	0.00
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 2	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	5.60	0.01	0.00	0.05	0.00	0.00	0.02	0.07	0.00	0.36	0.00	0.01	0.00	0.00	0.04	0.00	0.05	0.00	1.48
Average Daily Dose (µg/kg)	0.00	1.71	0.00	0.00	0.01	0.00	0.00	0.01	0.02	0.00	0.11	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.45
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 3	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	8.06	0.03	0.00	0.06	0.00	0.01	0.01	0.06	0.00	0.92	0.00	0.03	0.00	0.00	0.06	0.00	0.18	0.01	1.66
Average Daily Dose (µg/kg)	0.00	2.47	0.01	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.28	0.00	0.01	0.00	0.00	0.02	0.00	0.06	0.00	0.51
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 4	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	6.89	0.02	0.00	0.08	0.00	0.00	0.01	0.07	0.00	0.56	0.00	0.00	0.01	0.00	0.10	0.00	0.36	0.01	1.48
Average Daily Dose (µg/kg)	0.00	2.11	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.17	0.00	0.00	0.00	0.00	0.03	0.00	0.11	0.00	0.45
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 3.12 (cont): The Hazard Quotients (HQ), based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Oreochromis mossambicus* sampled during surveys conducted at Flag Boshielo Dam from February 2016 to May 2018. Cells highlighted in red indicate where the HQ exceeds 1.

Survey 5	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	3.41	0.01	0.00	0.08	0.00	0.00	0.01	0.04	0.00	0.51	0.00	0.00	0.01	0.00	0.10	0.00	0.10	0.01	1.52
Average Daily Dose (µg/kg)	0.00	1.04	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.16	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.47
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 6	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.22	16.48	0.39	0.00	0.12	0.13	0.73	0.17	14.94	0.01	13.79	0.31	2.98	0.05	0.00	1.21	0.00	0.16	3.13	9.82
Average Daily Dose (µg/kg)	0.07	5.05	0.12	0.00	0.04	0.04	0.22	0.05	4.57	0.00	4.22	0.09	0.91	0.02	0.00	0.37	0.00	0.05	0.96	3.01
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.4**	**0.0**	**0.0**	**0.0**	**0.6**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.1**	**0.0**	**0.0**	**0.2**	**0.0**

Survey 7	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.06	17.52	0.37	0.00	0.44	0.05	0.63	0.21	20.44	0.00	6.62	0.21	2.65	0.04	0.00	0.74	0.00	0.14	1.67	8.86
Average Daily Dose (µg/kg)	0.02	5.36	0.11	0.00	0.14	0.02	0.19	0.06	6.26	0.00	2.03	0.06	0.81	0.01	0.00	0.23	0.00	0.04	0.51	2.71
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.4**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**

Survey 8	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.07	7.81	0.05	0.01	0.25	0.00	0.05	0.06	0.26	0.01	2.52	0.00	0.06	0.01	0.00	0.29	0.00	1.11	0.05	2.72
Average Daily Dose (µg/kg)	0.02	2.39	0.02	0.00	0.08	0.00	0.02	0.02	0.08	0.00	0.77	0.00	0.02	0.00	0.00	0.09	0.00	0.34	0.02	0.83
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 3.12 (cont): The Hazard Quotients (HQ), based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Oreochromis mossambicus* sampled during surveys conducted at Flag Boshielo Dam from February 2016 to May 2018. Cells highlighted in red indicate where the HQ exceeds 1.

Survey 9	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.06	1.69	0.05	0.02	0.05	0.00	0.02	0.01	0.12	0.00	0.70	0.00	0.03	0.00	0.00	0.26	0.00	0.22	0.02	0.32
Average Daily Dose (µg/kg)	0.02	0.52	0.01	0.01	0.01	0.00	0.01	0.00	0.04	0.00	0.21	0.00	0.01	0.00	0.00	0.08	0.00	0.07	0.01	0.10
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 10	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.14	37.05	0.19	0.13	0.81	0.21	1.73	0.07	58.79	0.02	24.64	0.43	0.29	0.07	0.03	1.92	0.00	0.26	4.68	13.49
Average Daily Dose (µg/kg)	0.04	11.34	0.06	0.04	0.25	0.06	0.53	0.02	18.00	0.01	7.54	0.13	0.09	0.02	0.01	0.59	0.00	0.08	1.43	4.13
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**1.3**	**0.0**	**0.4**	**0.0**	**0.1**	**0.0**	**0.0**	**0.4**	**0.0**	**0.1**	**0.0**	**0.0**	**0.3**	**0.0**

Survey 11	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.19	12.25	0.12	0.03	0.39	0.10	1.23	0.05	35.76	0.02	22.00	0.41	0.41	0.05	0.01	1.00	0.00	0.24	1.57	9.71
Average Daily Dose (µg/kg)	0.06	3.75	0.04	0.01	0.12	0.03	0.38	0.01	10.95	0.01	6.74	0.13	0.12	0.02	0.00	0.30	0.00	0.07	0.48	2.97
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**3.0**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.4**	**5.0**	**600.0**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.9**	**0.0**	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.1**	**0.0**	**0.0**	**0.1**	**0.0**

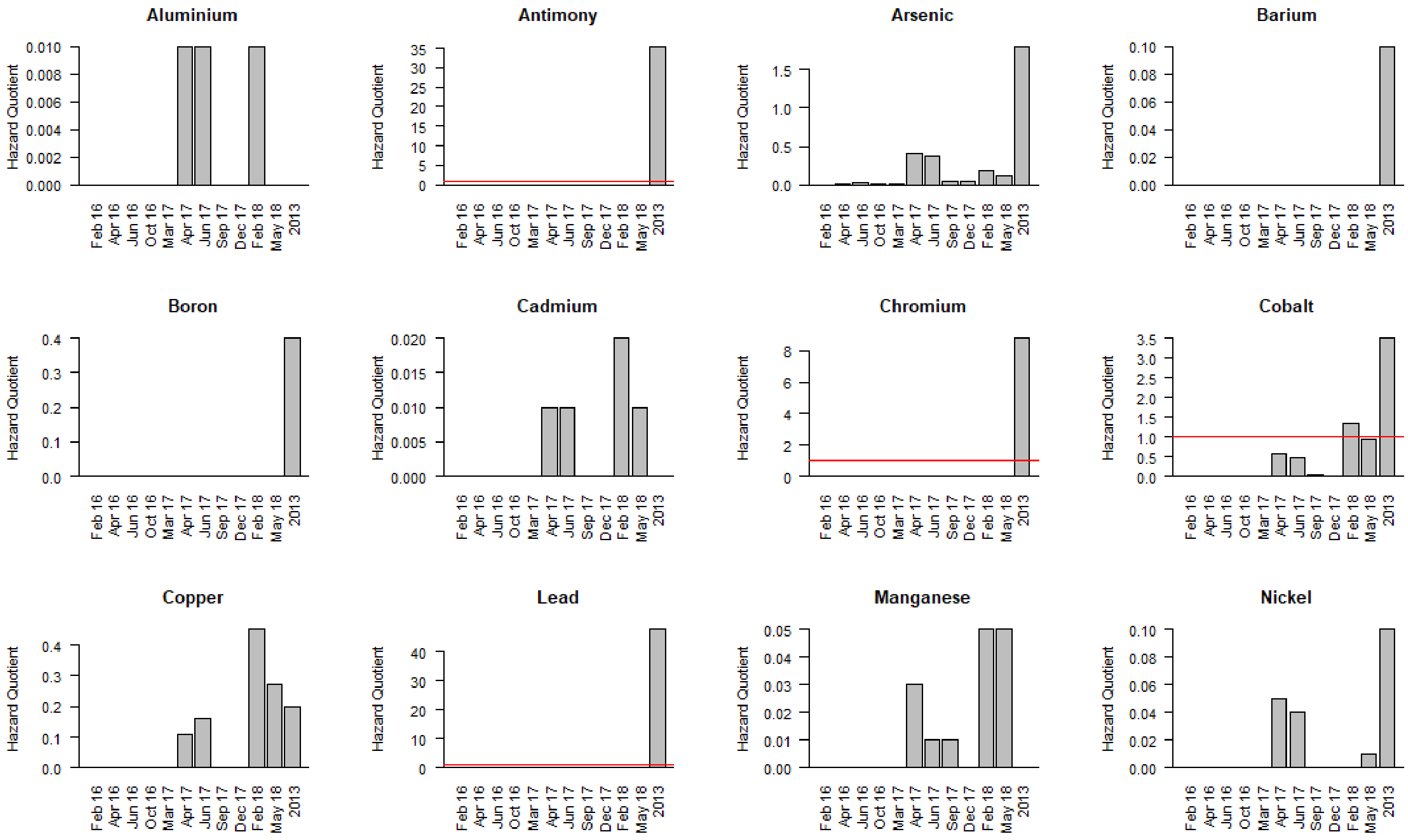

Figure 3.25: Bar graphs of average Hazard Quotient (HQ) values calculated for metal and metalloids in *Oreochromis mossambicus* sampled from Flag Boshielo Dam during the current study in comparison to those published by Jooste et al. (2013). The red line indicates an HQ of 1 above which long-term health impairment is possible.

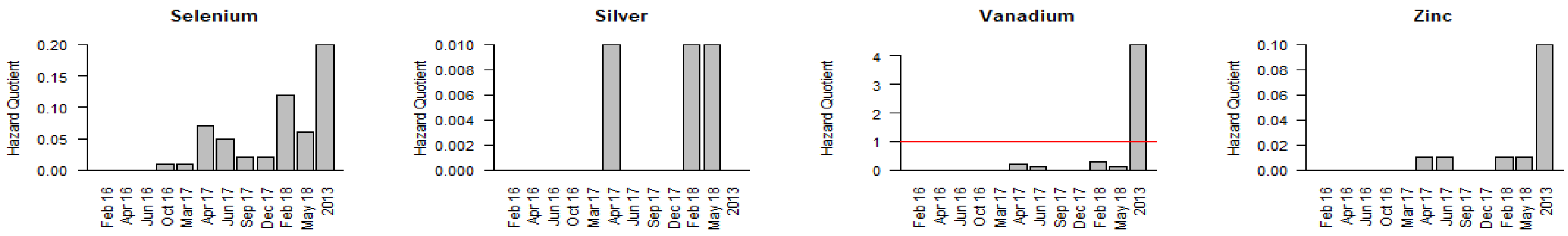

Figure 3.25 (cont): Bar graphs of average Hazard Quotient (HQ) values calculated for metal and metalloids in *Oreochromis mossambicus* sampled from Flag Boshielo Dam during the current study in comparison to those published by Jooste et al. (2013). The red line indicates an HQ of 1 above which long-term health impairment is possible.

Several studies have shown that inorganic As can increase the risk of lung cancer, skin cancer, bladder cancer, liver cancer, kidney cancer, and prostate cancer (ATSDR, 2007a) resulting in the World Health Organization (WHO), the Department of Health and Human Services (DHHS), and the US-EPA declaring inorganic As a human carcinogen. Oral exposure has resulted in a pattern of skin changes that include the formation of warts or corns on the palms and soles along with areas of darkened skin on the face, neck, and back (ATSDR, 2007a). Blackfoot disease, a disease characterized by a progressive loss of circulation in the hands and feet, leading ultimately to necrosis and gangrene, is associated with As exposure (ATSDR, 2007a). Other effects noted from chronic oral exposure include peripheral neuropathy, cardiovascular disorders, gastrointestinal disorders, haematological disorders, and liver and kidney disorders (ATSDR, 2007a). Studies in animals show that large doses of As that cause illness in pregnant females, can also cause low birth weight, foetal malformations, and even foetal death (ATSDR, 2007a). Ingesting very high levels of As can result in death whereas exposure to lower levels can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart rhythm, damage to blood vessels, and a sensation of "pins and needles" in hands and feet (ATSDR, 2007a).

For *S. intermedius* a HQ > 1 was calculated for Pb in individual specimens collected in the March 2017 survey (see Figure 3.28 and Table 3.14). However, the average HQ calculated for Pb did not exceed one. Similar to *L. rosae* the metal content in the muscle tissue of *S. intermedius* did not accumulate to levels that were a health risk to humans consuming this fish. By comparison, Jooste et al. (2013) calculated an average HQ of 3, 10, 17, 80, 120 and 4 for Cr, Co, Pb, Sb and V respectively (see Figure 3.29).

The toxic effects of Pb have been known for centuries, but the discovery in the past few decades that levels of exposure resulting in relatively low levels of Pb in blood (e.g. < 20 μg/dL) are associated with adverse effects in the developmental phase of an organism is a matter of great concern. The most sensitive targets for Pb toxicity are the developing nervous system, the haematological and cardiovascular systems, and the kidney. However, due to the multi-modes of action of Pb in biological systems, Pb could potentially affect any system or organs in the body (ATSDR, 2007b).

Population studies suggest that there is a significant association between bone-Pb levels and small elevations in blood pressure. Lead also affects kidney functions with glomerular filtration rates the function affected by low Pb. Lead has long been known to alter the haematological system (inhibiting the activities of several enzymes involved in heme biosynthesis), anaemia, premature skeletal maturation in children (8-10 years) that might predispose them to osteoporosis in later life, increased occurrence of dental cavities in children and periodontal bone loss, circulating levels of thyroid hormones, serum levels of reproductive hormones, immune parameters, and the development of encephalopathy in children, a general term that describes various diseases that affect brain function. Neurological symptoms develop following prolonged exposure and include dullness, irritability, poor attention span, epigastric pain, constipation, vomiting, convulsions, coma, and death. Lead poisoning in children can leave residual cognitive deficits that can be still detected in adulthood (ATSDR, 2007b).

Lead has produced primarily renal tumours in rodents by a mechanism not yet known. Some non-genotoxic mechanisms that have been proposed for lead-induced cancer include inhibition of DNA synthesis and repair, alterations in cell-to-cell communication, and oxidative damage. The DHHS, US-EPA and IARC have determined that Pb and Pb compounds are probable human carcinogens (IARC, 2012).

Carcinogenic Risks

In this study levels of Co, As and Pb were reported in some fish at concentrations that are expected to cause both toxic effects (with hazard quotients ranging from close to double to twenty times the safe dose) with carcinogenic risks of between 1 and 4 people in 1,000 having the chance of developing cancer from As concentrations measured in the fish studied here. According to the US-EPA, carcinogenic risks are considered to be unacceptable if in excess of 1 in 10,000. The carcinogenic effect of Pb requires further evaluation. However, compared to Jooste et al. (2013) the HQ calculated in this study were considered negligible and indicates that there was possibly a higher influx of toxins into the system due to higher water flow and water levels during the Jooste et al. (2013) study.

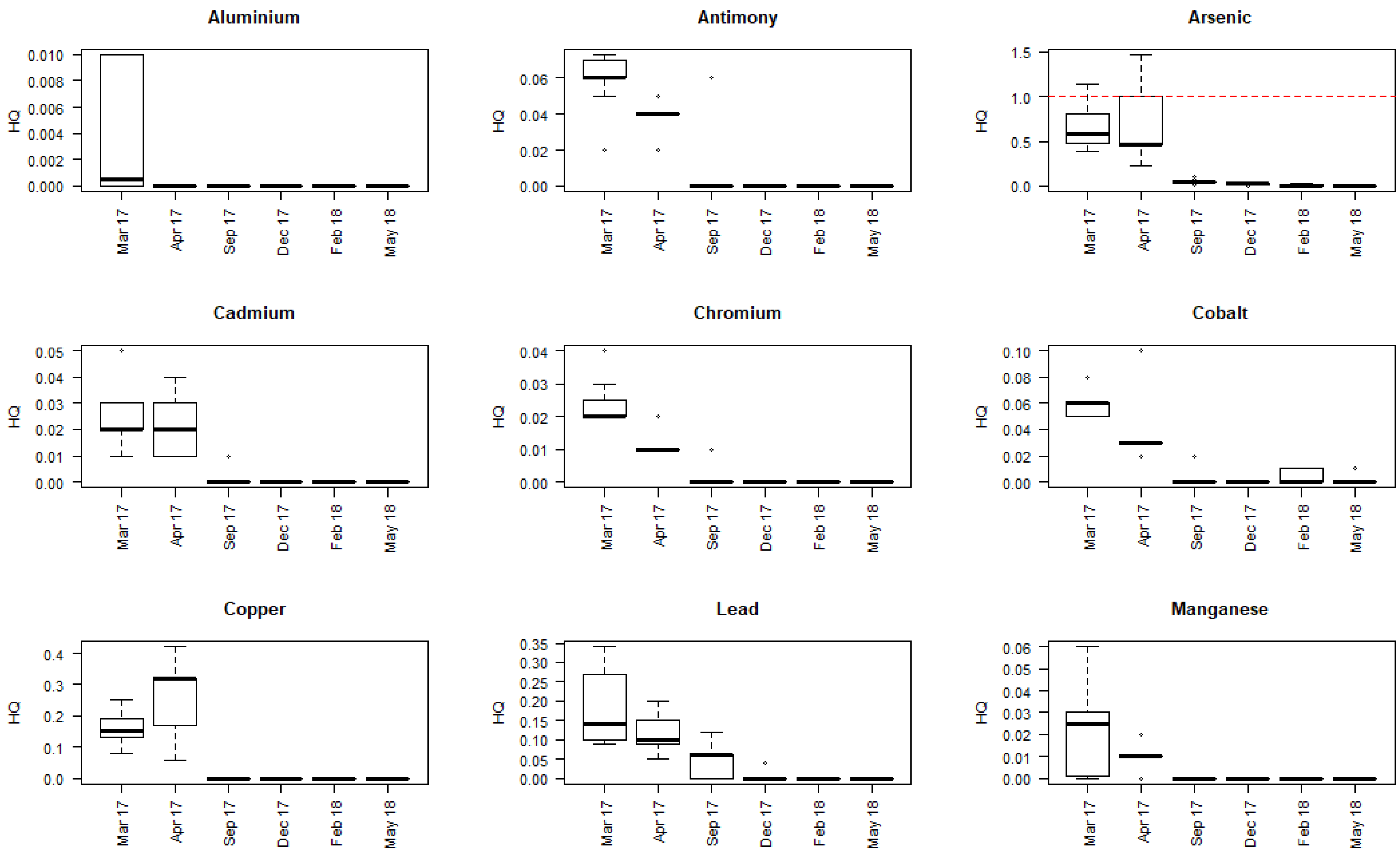

Figure 3.26: Box plots of the Hazard Quotient (HQ) calculated for metal and metalloids in *Labeo rosae* sampled from Flag Boshielo Dam during surveys conducted between from February 2016 to May 2018. The broken line (red) indicates an HQ of 1 above which long-term health impairment is possible.

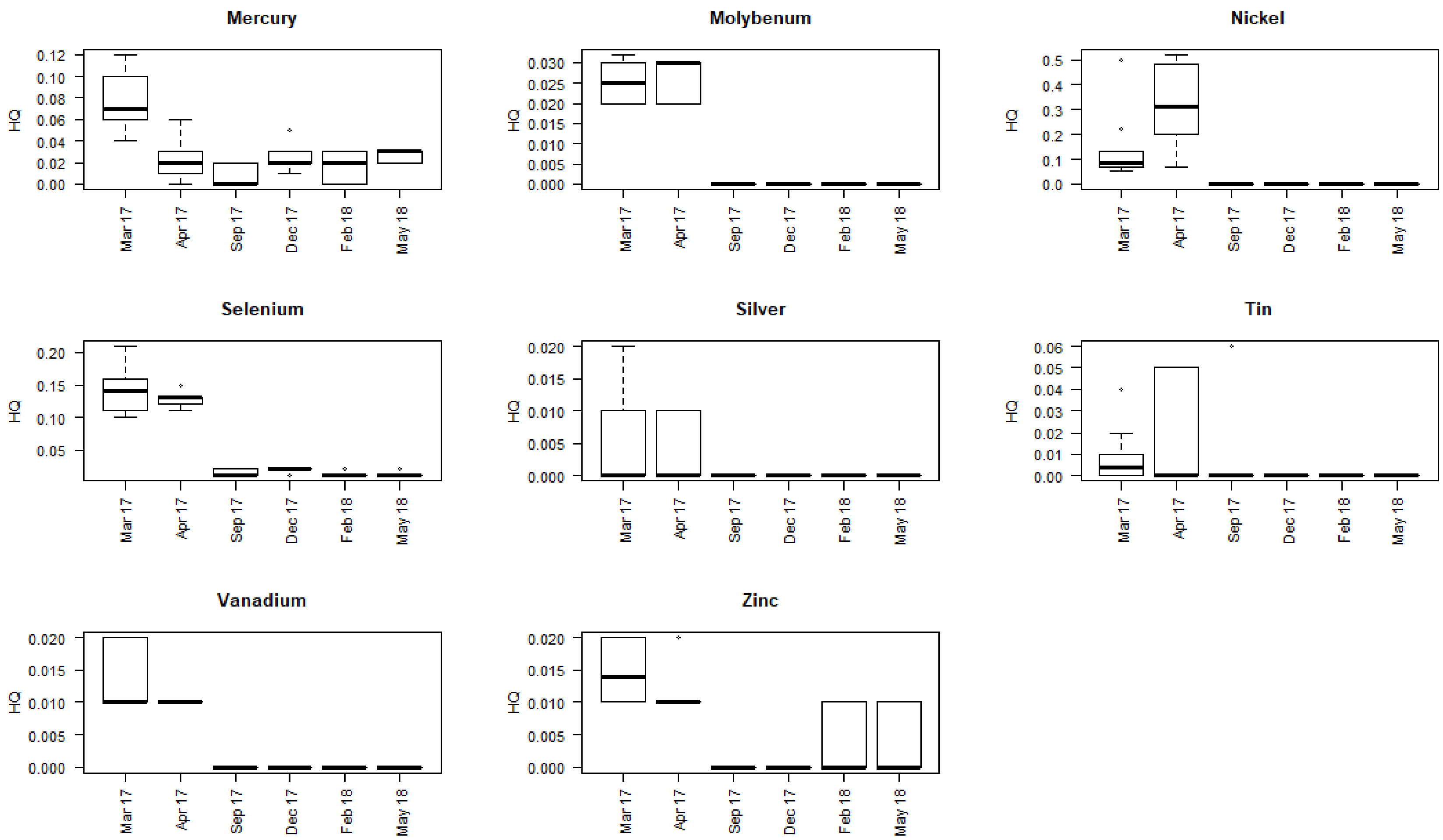

Figure 3.26 (cont): Box plots of the Hazard Quotient (HQ) calculated for metal and metalloids in *Labeo rosae* sampled from Flag Boshielo Dam during surveys conducted between from February 2016 to May 2018. The broken line indicates an HQ of 1 above which long-term health impairment is possible.

Table 3.13: The Hazard Quotients, based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Labeo rosae* sampled during surveys conducted at Flag Boshielo Dam from March 2017 to May 2018.

Survey 1	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.11	15.56	0.63	0.01	0.24	0.04	0.08	0.22	20.14	0.08	10.13	0.40	9.07	0.06	0.02	2.29	0.00	0.34	0.20	13.82
Average Daily Dose (µg/kg)	0.03	4.76	0.19	0.00	0.07	0.01	0.02	0.07	6.16	0.02	3.10	0.12	2.78	0.02	0.01	0.70	0.00	0.10	0.06	4.23
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.6**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.2**	**0.1**	**0.0**	**0.0**	**0.1**	**0.2**	**0.1**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 2	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.06	4.19	0.71	0.00	0.14	0.04	0.05	0.13	33.96	0.02	4.79	0.45	20.63	0.04	0.01	2.08	0.01	0.29	0.14	13.43
Average Daily Dose (µg/kg)	0.02	1.28	0.22	0.00	0.04	0.01	0.02	0.04	10.40	0.01	1.47	0.14	6.32	0.01	0.00	0.64	0.00	0.09	0.04	4.11
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.0**	**0.0**	**0.3**	**0.1**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 3	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.03	3.57	0.05	0.08	0.16	0.00	0.00	0.04	0.13	0.01	0.55	0.00	0.02	0.01	0.00	0.25	0.00	1.55	0.02	2.82
Average Daily Dose (µg/kg)	0.01	1.09	0.01	0.03	0.05	0.00	0.00	0.01	0.04	0.00	0.17	0.00	0.01	0.00	0.00	0.08	0.00	0.48	0.01	0.86
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 4	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	1.01	0.02	0.02	0.10	0.00	0.00	0.00	0.15	0.02	0.39	0.00	0.00	0.00	0.00	0.28	0.00	1.05	0.01	2.28
Average Daily Dose (µg/kg)	0.00	0.31	0.01	0.01	0.03	0.00	0.00	0.00	0.05	0.01	0.12	0.00	0.00	0.00	0.00	0.08	0.00	0.32	0.00	0.70
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 3.13 (cont): The Hazard Quotients, based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Labeo rosae* sampled during surveys conducted at Flag Boshielo Dam from March 2017 to May 2018.

Survey 5	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	1.58	0.01	0.00	0.12	0.00	0.01	0.01	0.24	0.02	0.45	0.00	0.02	0.00	0.00	0.23	0.00	1.10	0.01	4.94
Average Daily Dose (µg/kg)	0.00	0.48	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.00	0.14	0.00	0.01	0.00	0.00	0.07	0.00	0.34	0.00	1.51
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 6	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	3.50	0.00	0.01	0.19	0.00	0.00	0.01	0.21	0.02	0.63	0.00	0.00	0.00	0.00	0.22	0.00	1.22	0.01	4.51
Average Daily Dose (µg/kg)	0.00	1.07	0.00	0.00	0.06	0.00	0.00	0.00	0.06	0.01	0.19	0.00	0.00	0.00	0.00	0.07	0.00	0.37	0.00	1.38
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

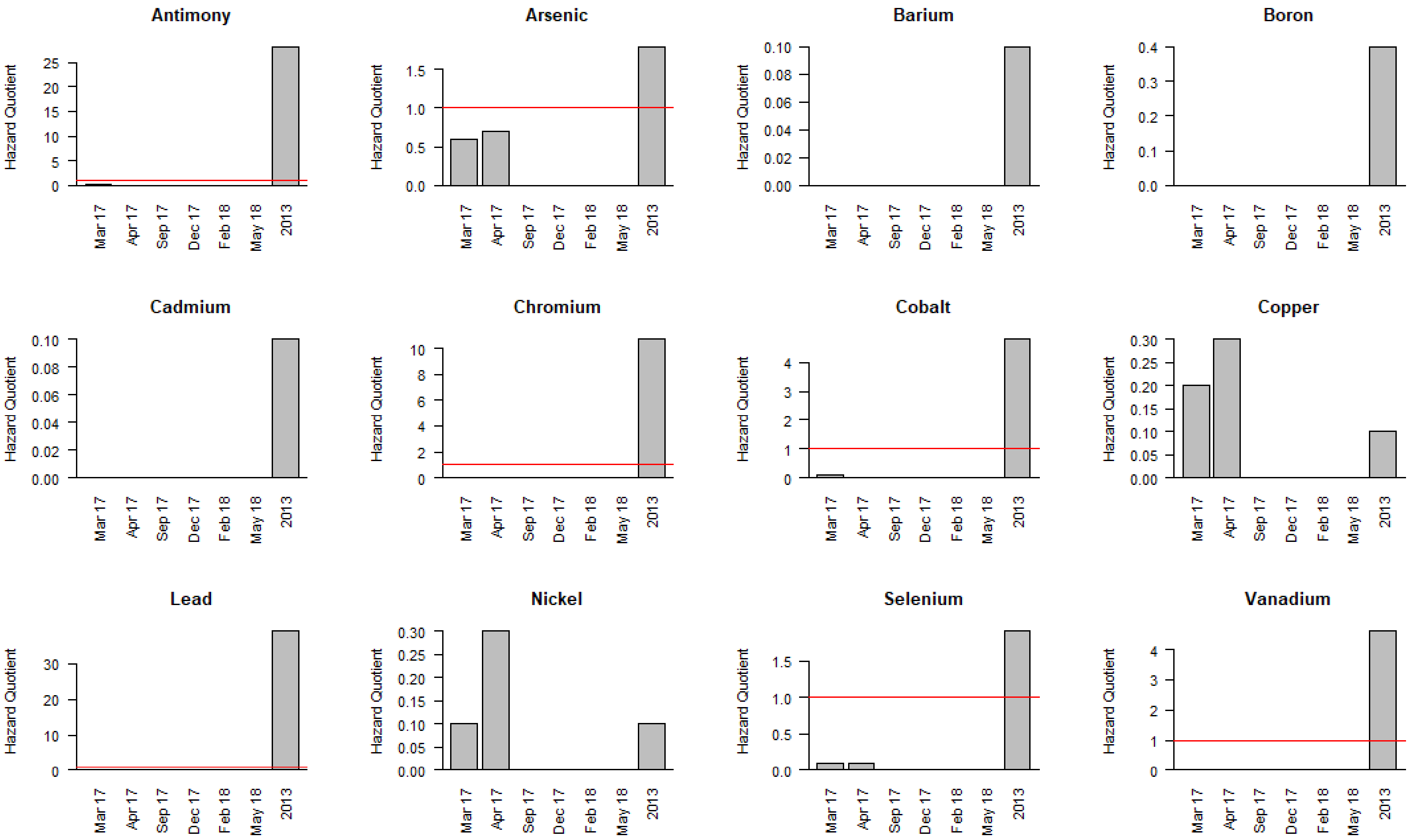

Figure 3.27: Bar graphs of average Hazard Quotient (HQ) values calculated for metal and metalloids in *Labeo rosae* sampled from Flag Boshielo Dam during the current study in comparison to those published by Jooste et al. (2013). The solid red line indicates an HQ of 1 above which long-term health impairment is possible.

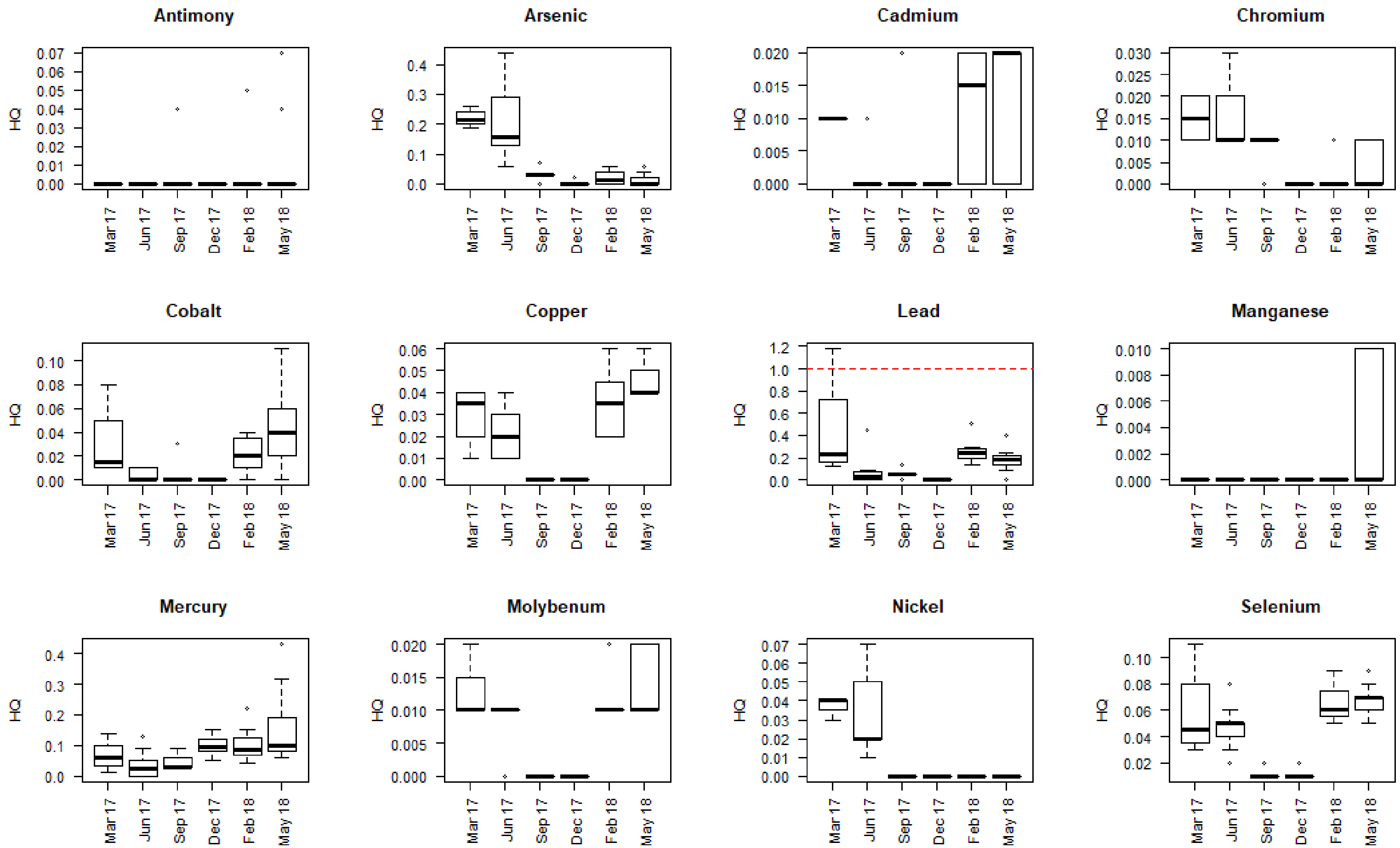

Figure 3.28: Box plots of the Hazard Quotient (HQ) calculated for metal and metalloids in *Schilbe intermedius* sampled from Flag Boshielo Dam during surveys conducted between from February 2016 to May 2018. The broken line (red) indicates an HQ of 1 above which long-term health impairment is possible.

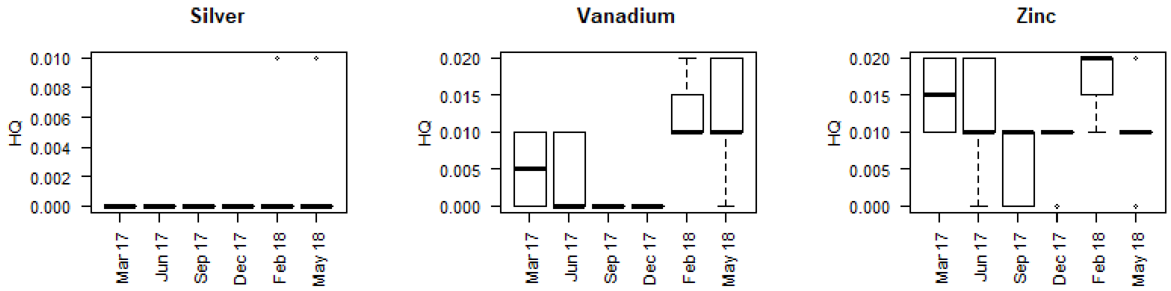

Figure 3.28 (cont): Box plots of the Hazard Quotient (HQ) calculated for metal and metalloids in *Schilbe intermedius* sampled from Flag Boshielo Dam during surveys conducted between from February 2016 to May 2018. The broken line indicates an HQ of 1 above which long-term health impairment is possible.

Table 3.14: The Hazard Quotients, based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Schilbe intermedius* sampled during surveys conducted at Flag Boshielo Dam from March 2017 to May 2018.

Survey 1	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	4.33	0.21	0.00	0.14	0.01	0.04	0.14	3.86	0.07	1.70	0.19	2.61	0.14	0.00	0.97	0.00	0.24	0.13	13.73
Average Daily Dose (µg/kg)	0.00	1.33	0.07	0.00	0.04	0.00	0.01	0.04	1.18	0.02	0.52	0.06	0.80	0.04	0.00	0.30	0.00	0.07	0.04	4.20
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.4**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 2	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	1.81	0.20	0.00	0.25	0.00	0.00	0.13	2.81	0.03	0.35	0.11	2.05	0.02	0.00	0.80	0.00	0.09	0.06	11.73
Average Daily Dose (µg/kg)	0.00	0.55	0.06	0.00	0.08	0.00	0.00	0.04	0.86	0.01	0.11	0.03	0.63	0.01	0.00	0.24	0.00	0.03	0.02	3.59
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 3	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.01	7.12	0.03	0.04	0.32	0.00	0.00	0.06	0.32	0.04	0.53	0.00	0.03	0.02	0.00	0.25	0.00	0.30	0.00	5.63
Average Daily Dose (µg/kg)	0.00	2.18	0.01	0.01	0.10	0.00	0.00	0.02	0.10	0.01	0.16	0.00	0.01	0.00	0.00	0.08	0.00	0.09	0.00	1.72
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 4	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.00	1.76	0.00	0.00	0.08	0.00	0.00	0.00	0.18	0.09	0.17	0.00	0.02	0.00	0.00	0.23	0.00	0.11	0.00	6.27
Average Daily Dose (µg/kg)	0.00	0.54	0.00	0.00	0.02	0.00	0.00	0.00	0.05	0.03	0.05	0.00	0.00	0.00	0.00	0.07	0.00	0.03	0.00	1.92
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 3.14 (cont): The Hazard Quotients, based on one fish meal eaten on a weekly basis, calculated for metals found in the muscle tissue of *Schilbe intermedius* sampled during surveys conducted at Flag Boshielo Dam from March 2017 to May 2018.

Survey 5	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.03	1.25	0.02	0.02	0.03	0.02	0.03	0.02	4.81	0.10	1.80	0.18	0.06	0.08	0.00	1.03	0.00	0.14	0.24	16.47
Average Daily Dose (µg/kg)	0.01	0.38	0.01	0.01	0.01	0.01	0.01	0.01	1.47	0.03	0.55	0.05	0.02	0.03	0.00	0.31	0.00	0.04	0.07	5.04
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**

Survey 6	Ag	Al	As	B	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
Metal concentration (mg/kg)	0.02	4.28	0.01	0.01	0.08	0.02	0.06	0.04	5.77	0.16	2.01	0.25	0.09	0.06	0.00	1.10	0.00	0.08	0.21	11.20
Average Daily Dose (µg/kg)	0.01	1.31	0.00	0.00	0.02	0.01	0.02	0.01	1.77	0.05	0.61	0.08	0.03	0.02	0.00	0.34	0.00	0.03	0.06	3.43
Reference Dose (µg/kg)	**5.0**	**1000.0**	**0.3**	**200.0**	**200.0**	**0.5**	**0.4**	**3.0**	**40.0**	**0.3**	**140.0**	**5.0**	**20.0**	**0.1**	**0.1**	**5.0**	**0.1**	**600.0**	**5.0**	**300.0**
Hazard Quotient	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**

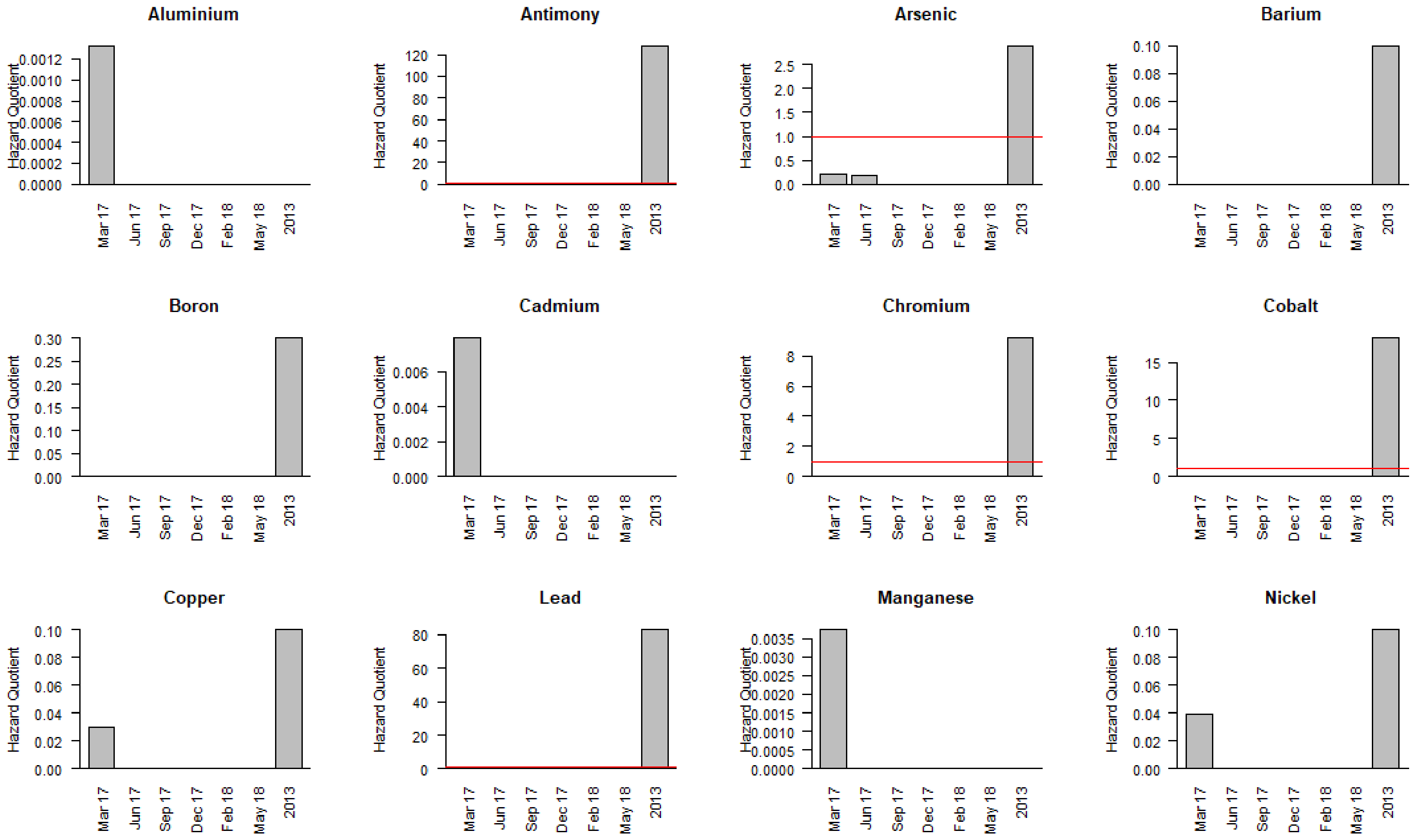

Figure 3.29: Bar graphs of average Hazard Quotient (HQ) values calculated for metal and metalloids in *Schilbe intermedius* sampled from Flag Boshielo Dam during the current study in comparison to those published by Jooste et al. (2013). The solid red line indicates an HQ of 1 above which long-term health impairment is possible.

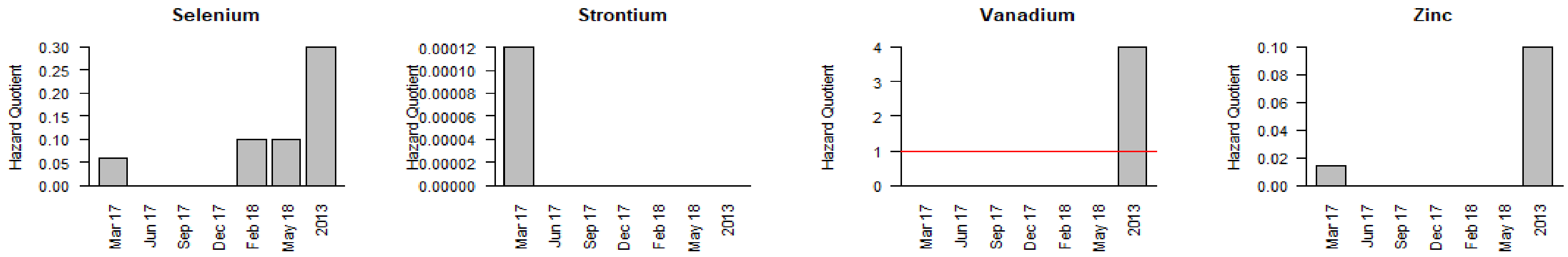

Figure 3.29 (cont): Bar graphs of average Hazard Quotient (HQ) values calculated for metal and metalloids in *Schilbe intermedius* sampled from Flag Boshielo Dam during the current study in comparison to those published by Jooste et al. (2013). The solid red line indicates an HQ of 1 above which long-term health impairment is possible.

DISCUSSION

Acid mine drainage, prevalent in the upper catchment mobilises metals from the sediment and bedrock in the Olifants River (Ashton and Dabrowski, 2011). This phenomenon is assumed to be the main driver for the increase of metal concentrations reported in fish muscle tissues from this system (Jooste et al., 2014). Metal concentrations detected in sediments were higher than those reported in water and in fish muscle tissue. Addo-Bediako et al. (2014a) and Jooste et al. (2014), found no association between metals detected in water and sediment, and those detected in fish muscle tissues. The lack of association can be because fish bio-accumulate metals from their surroundings over time as opposed aqueous metal concentrations that fluctuate depending on physical parameters such as flow rate, water volume, and the source and nature of the pollution including chemical properties such as water pH and water hardness (Goswami et al., 2016).

Although metals were not measured in other organs such as gonads, gills and liver, low metal concentrations detected in the muscle tissues of fish from Flag Boshielo Dam can possibly be attributed to more metals accumulating in these organs. Studies by Canli and Atli (2003) have shown that various fish accumulate metals at different rates and at different concentrations and that different metals can accumulate differently within the same species. In fish, gender differences in metal accumulation seem to occur only in gonads and during certain stages of their reproductive cycles (e.g. spawning). For example, zinc is deposited in gonads during gonadal development and later disposed of when spawning (Seymore et al., 1996). The gills are a good reflection of metal concentrations in the water, while liver concentrations represent the storage site for metals (Arain et al., 2008). Metals absorbed through the gills or across the intestinal wall are circulated and distributed throughout the body to be utilised for essential life functions or be rendered neutral by binding to the protein, metallothionein (Olsson et al., 1998). Therefore, it is imperative that future studies consider investigating metal accumulation in various organs of fish.

Temporal variation of metal levels in fish is usually ascribed to the variation in environmental conditions such as rainfall and the extent by which pollutants occur within a system (Kotze et al., 1999). Lower levels of metals detected in muscle tissues of *L. rosae* sampled during high inflow was consistent with most trends often described in the literature whereby metals in solution tend to be diluted during periods of high rainfall and inflow (Seymore et al., 1996). Alternatively, metals levels in *O. mossambicus* and *S. intermedius* were overall higher during periods of high inflow. Besides the direct uptake of aqueous metals, *O. mossambicus* feeds on filamentous green algae which can results in the intake and accumulation of high metal concentrations (Froese and Pauly, 2010; Oberholster et al., 2012). The pH in the stomach of *O. mossambicus* decreases significantly from 6 to as low as 2.9 after ingestion has commenced (Kotze et al., 1999) resulting in metal contaminants becoming more bioavailable during the digestion and assimilation process (Kotze et al., 1999). In turn the feeding preference and behaviour of *S. intermedius* may make this species more susceptible to metal uptake. The biomagnification of metals in Flag Boshielo Dam requires investigation to determine whether the uptake of metals and their subsequent accumulation and release from food sources within trophic levels occurs and to what extent.

Results appear to indicate that drought conditions reduced the concentrations of metals in fish muscle tissue to levels sufficient for safe consumption. In this study a HQ > 1 for Co was calculated for specimens of *O. mossambicus* sampled during a period of high inflow whereas a HQ > 1 was establish for As in specimens of *L. rosae* during period of high inflow while the same was true for Pb in *S. intermedius*. The differences in the metals detected in the different species can possibly be related to the trophic level these species occupy. Results would, therefore, indicate that a mixed diet of fish is safe for humans when consuming a meal portion of 150 g fillet per week. However, one needs to stress caution since organs such as gonads, gills and liver were not analysed for their metal content and consequently the retention and accumulation of metals in these organs in fish from Flag Boshielo Dam are currently unknown. This especially if consumed by subsistence fisher folk who may include these organs in a stew or feed portions thereof to infants. A survey on the local and surrounding populace from poor households is required to establish how fish are prepared and to what extent are they consumed.

Anecdotal information collected during the field surveys revealed that large portions of the rural communities are consuming at least one fish meal per week. Increasing population density and rural poverty will result in a greater reliance on fish for protein. At the level of weekly consumption of fish from Flag Boshielo Dam, there is a risk of health impairment because Co and to a lesser extent As, exceed the recommended HQ of 1 in *O. mossambicus* and *L. rosae* respectively. Fish species that are commonly targeted by local fisher folk.

The HQ's calculated in this assessment are based on the assumptions of one fish meal (approximately 150 g portion) eaten weekly by a 70 kg adult. The health risk posed to children of the community would be considerably higher than those reported here because children would be expected to weigh less than adults. The US-EPA recommended that a weight of 14.5 kg be used for 0-1 age children and 30 kg for 1-10 age children (US-EPA, 2000), making the risk to infants almost five times that of an adult. With many rural communities caring for the infants of siblings or children working or residing in the urban centres, findings in this study reveal an increased potential of exposure of infants in rural areas to the debilitating impacts of toxic metals such as Co, As and Pb.

CHAPTER 4: DRIVERS OF METAL CONCENTRATIONS IN SELECTED FISH SPECIES

INTRODUCTION

In order to establish a management strategy for the development of an exploitation fishery on South Africa's inland water bodies, it is important to understand the drivers of not only the exploitable portion of the fishery resource, but also those that may limit the utilisation of the resource. Previous studies have shown that the fish from Flag Boshielo Dam contain certain metals whose long-term consumption could be detrimental to the people consuming these fish. It is therefore important to understand which drivers determine the levels of metals in the muscle tissue of fish and whether these drivers can be mitigated against.

For this work, two approaches were explored to evaluate the relationship between the concentration of selected metals in the fish muscle tissue and selected environmental drivers using 1) generalised linear models (glm) and 2) Distance based linear modelling (DISTLM) using distance-based redundancy analysis (Anderson et al., 2008). Distance based linear modelling is a routine for analysing and modelling the relationship between a multivariate data cloud, as described by a resemblance matrix, and one or more predictor variables. The routine allows predictor variables to be fit individually or together in specified sets. P-values for testing the null hypothesis of no relationship (either for individual variables alone or conditional on some variables) are obtained using appropriate permutation methods. The DISTLM partitions the variation in a data cloud by a resemblance matrix according to a regression (or multiple regression) model.

METHODS

4.1.1 Fish muscle analysis

Skinless samples of fish muscle tissue were sent to Waterlab, a SANAS accredited laboratory (ISO/IEC 17025:2005) in Pretoria, and analysed for a suite of metals, metalloids and anions (see Chapter 3 for details). The results were expressed in mg/kg dry weight. The following metals were selected for the DISTLM analyses: aluminium (Al), arsenic (As), antimony (Sb), barium (Ba), boron (B), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), tin (Sn), titanium (Ti), vanadium (V) and zinc (Zn). A reduced set of eight metals were selected for the generalised linear modelling (glm) based on the number of non-zero metal concentration in the fish muscle. The metals with the highest number of non-zero values were selected: Al, Ba, Cu, Mn, Se, Sr, Ti and Zn.

4.1.2 Environmental drivers

Initially, we envisaged using data collected during the field surveys, augmented with selected large scale data (e.g. rainfall, land-use change, river discharge and dam levels), but most of the monitoring data collected during the study was not usable due to missing samples (sediment samples could not be collected for several field trips following the theft of the mud grab) or concentrations of variables below the detection limit of the laboratory analysis (e.g. metals in the water samples). For both statistical approaches, a complete set of variables was required. Therefore, physico-chemical parameters (water temperature (°C), pH, dissolved oxygen concentration (DO; mg/L and % saturation), electrical conductivity (EC; µS/cm) and lake limnological parameters (depth of thermocline and centre of buoyancy) collected during the field surveys were augmented with lake water chemical analyses (potassium (K), magnesium (Mg), ammonium (NH_4), nitrate and nitrite, phosphate (PO_4), silicon (Si), and sulphate concentrations (SO_4)), river discharge (within the survey time and averaged over the month prior to the survey), and dam levels (level at the time of the survey, change in level over the month proceeding the survey, change in level since the previous survey), extracted from the Department of Water and Sanitation's Resource Quality Services web site. The historical data (presented in Chapter 2) showed that there was a reset in the water chemistry of Flag Boshielo Dam after a flow exceeding 20 cumecs. For the purposes of this study, a flow exceeding 20 cumecs was considered a "flood", and the number of days since the last flood was calculated from the daily averaged flow data. Data for Flag Boshielo Dam (B5R002) were used for the water chemistry and from the weir on the Olifants River below Flag Boshielo Dam (B5H004) were used for the flow. Because multiple fish were collected during each field survey, the environmental data for each field survey was replicated for each fish collected during that survey.

4.1.3 Limnological drivers

Following the analysis of the environmental drivers, a reduced set of more limnological drivers were selected for a different perspective on the drivers of metal concentrations in fish muscle tissue. The limnological drivers were selected on the basis that they did not require chemical analysis such that decisions on utilisation of the fish resources from Flag Boshielo Dam could be made on the basis from data measured in the field. A set of ten drivers were selected from the environmental variables used for the initial assessment of environmental drivers. These were physico-chemical parameters (water temperature (°C), pH, DO% and EC), lake limnological parameters (depth of thermocline and centre of buoyancy), river discharge (within the survey time and averaged over the month prior to the survey), dam levels (change in level over the month proceeding the survey) and the number of days since the last flood flow of 20 cumecs.

4.1.4 Modelling metal concentrations in fish muscle tissue

The environmental variables were first evaluated to determine whether there was co-linearity within the variable set. Pearson correlation coefficients were calculated for the pairwise comparisons between each of the environmental predictor variables using the corr function in the core stats package of R 3.5.0 statistical software (R Development Team, 2018). Pairwise comparisons with correlation coefficients greater than 0.9 were highlighted and one of the variables was selected for elimination from the variable set. The two dissolved oxygen variables (mg/L) had a correlation coefficient > 0.9 and two dam level variables (level at the time of the survey and change in level since the previous survey) had a correlation coefficient > 0.9 with the river discharge averaged over the month prior to the survey. One dissolved oxygen variable (mg/L) and the two dam level variables were excluded from the environmental variables leaving 17 variables in the environmental data.

Separate generalised linear models were established for each of the eight selected metals for each of the three fish species. A generalised linear model was established for each metal using the glm function in the core stats package of the R statistical software (R Development Team, 2018). Because the objective of the analyses was to determine drivers of the metal concentration in fish muscle, multi model inference (Burnham and Anderson, 2002) was used to compile a list of all possible models list and calculate the Akaike Information Criteria (AIC) for each of these models using the dredge function in the R package MuMIn (Barton, 2018). The top models, defined as models with a difference in AIC of less the 4 to that of the top model, were extracted using the get.models function in MuMIn. The importance of the respective variables in the top models was then evaluated using the importance function in MuMIn. This function returns the proportion of the top models that contain the respective environmental variables.

To evaluate patterns in the frequency of the respective environmental predictors for each metal for the three fish species, a resemblance matrix was constructed using Euclidian distances and a non-metric multi-dimensional scaling (NMDS) performed to visualise the differences using PRIMER-E 6.1.5 (Clarke and Warwick, 2001). No transformation of the variables was performed because all the variables were on the same scale. A combined NMDS was prepared for all three species to examine whether the environmental drivers differed between species. A distance-based test for homogeneity of multivariate dispersion was performed to test whether there was a statistically significant difference in multivariate dispersion for using the PERMDISP and PERMANOVA routines (Anderson et al., 2008) in PRIMER-E 6.1.5 (Clarke and Warwick, 2001). The PERMDISP routine was used to determine whether the multivariate dispersion about the group centroid differed between the impoundments/species, while the PERMANOVA routine was used to determine whether the position of the group centroids in multivariate space and/or multivariate dispersion about the group centroid differed between the impoundments/species (Anderson et al., 2008). A significant result from the PERMDISP test reduces the interpretability of significant result from a PERMANOVA tests, because it is not possible to determine whether a significant result in PERMANOVA is due to multivariate dispersion, difference in the position of the group centroids, or both (Anderson et al., 2008). A SIMPER analysis was performed to determine the main factors contributing to the differences in environmental drivers of metal concentrations between the fish species using PRIMER-E 6.1.5.

Distance based linear modelling, using distance-based redundancy analysis (dbRDA) (Anderson et al., 2008), was conducted for the three fish species using the PERMANOVA extension of the PRIMER-E 6.1.5 statistical software. Two datasets were entered into the software for each fish species: 1) a matrix of the 19 metal concentrations in the fish muscle tissue for each fish analysed for the long-term monitoring study, and 2) a matrix of the environmental parameters recorded during each field survey that were matched to the fish data collected. The metal matrices were 4^{th} root transformed and a resemblance matrix constructed using Euclidean distance. The distance-based redundancy analysis determined the environmental drivers that best describe the patterns in the resemblance matrix. The dbRDA results were plotted and the importance of the variables overlain on the dbRDA plot. The aforementioned analyses were repeated for the limnological drivers.

RESULTS

The three fish species used in the study were expected to respond to different environmental drivers with the piscivorous pelagic *S. intermedius* expected to respond to changes in the lake water column while the detritivorous benthic *L. rosae* is adapted to faster flowing waters but is found in the littoral zone of the lake. Finally, the omnivorous *O. mossambicus* is also benthic and associated with the slower waters in the littoral zone. *Labeo rosae* is an active forager and is expected to experience a wider range of environments than the more territorial *O. mossambicus*.

4.1.5 Generalised linear model of environmental drivers

The eight metals used in the regression analyses included two alkali earth metals (Ba and Sr), one metal from the Boron Group (Al), one non-metal from the Oxygen Group (Se) and four transition metals from the 3-d row (Ti, Mn, Cu and Zn). With the variation in the metals selected, it was expected that metals from the same group, e.g. the Alkali Earths Ba and Sr, would have a similar set of drivers while vastly different elements, e.g. Se or Al and the Alkali Earths, would have different profiles of environmental drivers.

Oreochromis mossambicus

All the environmental variables selected for the glm analyses were included as predictors for each of the eight metals evaluated (Figure 4.1). The importance of the environmental variables as drivers varied for the respective metals with some metals having an even distribution of importance across the predictor variables, e.g. Cu and Mn, while other metals having biases towards specific predictor variables, e.g. Al, Ba and Ti, and to a lesser extent Zn. The two Alkali Earth metals, Ba and Sr, did not have similar biases in predictors as was expected.

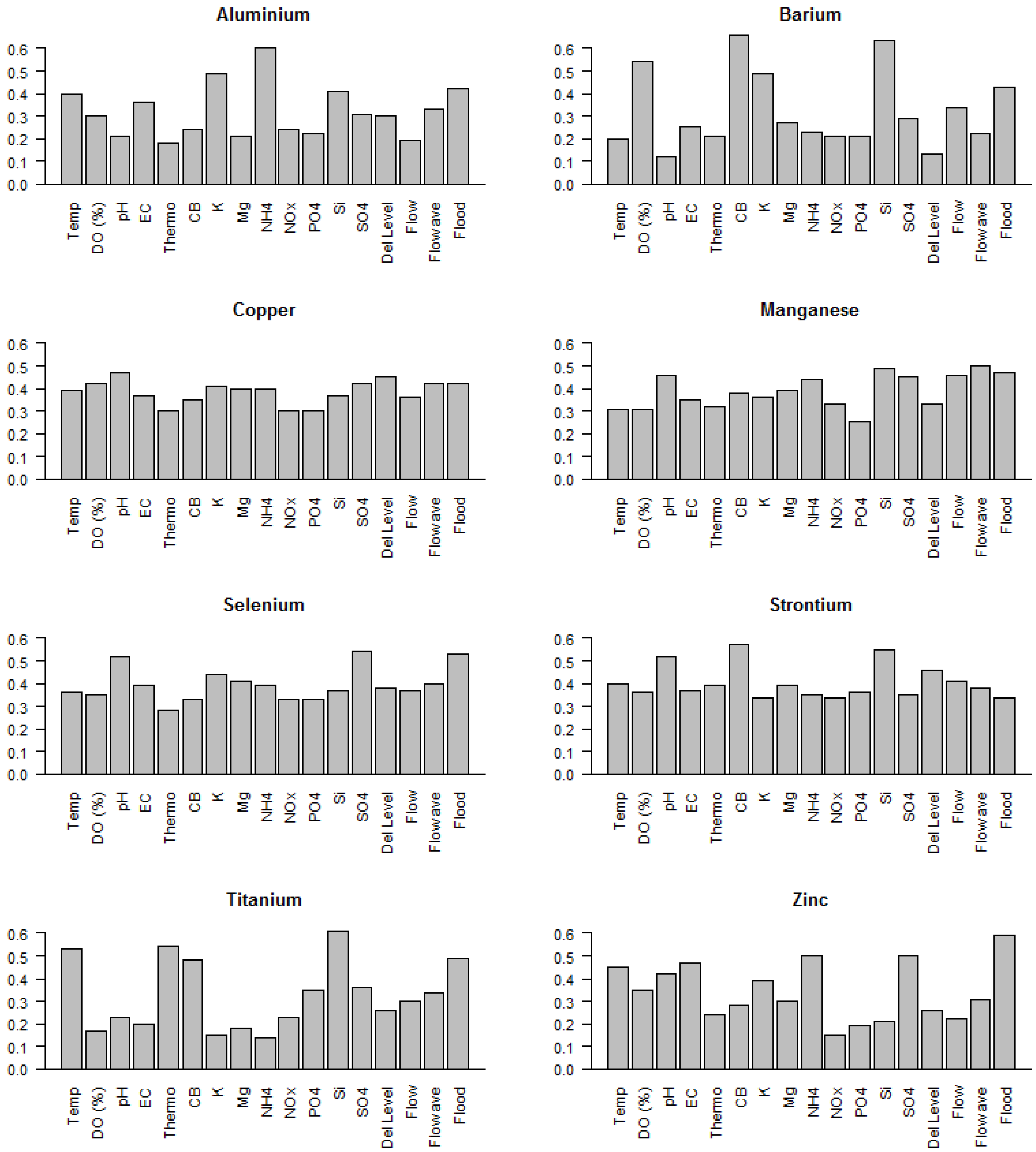

Figure 4.1: Bar plots of the proportion of the top models that contain the respective environmental drivers for the concentration of eight metals in *Oreochromis mossambicus* muscle tissue. Where Temp = temperature, DO = dissolved oxygen (%), EC = electrical conductivity, Thermo = thermocline depth, CB = centre of buoyancy, K = potassium, Mg = magnesium, NH4 = ammonium, NOx = nitrate:nitrite ratio, PO4 = phosphate, Si = silicon, SO4 = sulphate, Del Level = change in dam level over the last month, Flow = river daily average discharge, Flow ave = average river discharge over the last month, Flood = the number of days since the last flood (a discharge exceeding ≥ 20 cumecs).

Evaluating the predictor variables for the respective metals for the species, the NMDS plot shows a rough grouping of the metals whose predictor variable profile does not show any major biases in predictor variables (Cu, Mn, Se, Sr) separated from three distinct groups of metals whose predictors show biases in predictor variables: 1) Zn and Al, 2) Ti, and 3) Ba (Figure 4.2). Zinc and Al show a bias towards ammonia as a predictor while Ba and Ti show a bias towards silicon as a predictor, but for the latter group there is minimal overlap between the other main predictors.

The SIMPER analysis showed that the environmental factors contributing more than 10% to the similarity within the *O. mossambicus* group included pH (12%), centre of buoyancy (11%), ammonia (11%) and silicon (10%), with thermocline depth, change in dam level over the last month, K and % dissolved oxygen saturation all contributing more than 5% to the group similarity.

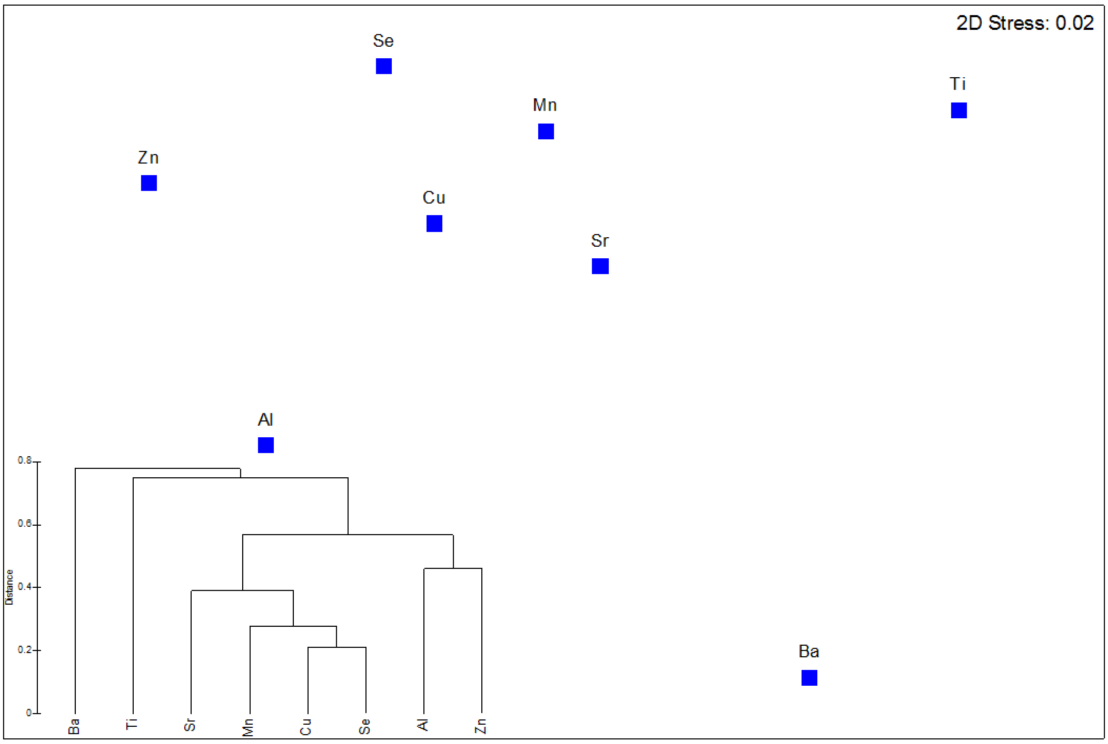

Figure 4.2: Non-Metric multidimensional scaling plot of the environmental drivers for the eight metals in *Oreochromis mossambicus* muscle tissue. The Cluster analysis dendrogram is inset on the lower right of the plot.

Labeo rosae

All the environmental variables selected for the glm analyses were included as predictors for each of the eight metals evaluated (Figure 4.3). The importance of the environmental variables as drivers varied for the respective metals with some metals having an even distribution of importance across the predictor variables, e.g. Cu, Mn and Zn, while other metals having biases towards specific predictor variables, e.g. pH for Sr and Ti, phosphate for Se, magnesium for Al, and change in dam level for Ba. As for *O. mossambicus*, the two Alkali Earth metals, Ba and Sr, did not have similar biases in predictors as was expected.

Evaluating the predictor variables for the respective metals for the species, the NMDS plot shows a rough grouping of the metals whose predictor variable profile does not show any major biases in predictor variables (Cu, Zn, Se) separated from three distinct groups of metals whose predictors show biases in predictor variables: 1) Mn and Al, 2) Ti and Sr, and 3) Ba (Figure 4.4). Manganese and Al have a bias towards magnesium as a predictor while Sr and Ti show a bias towards pH as a predictor.

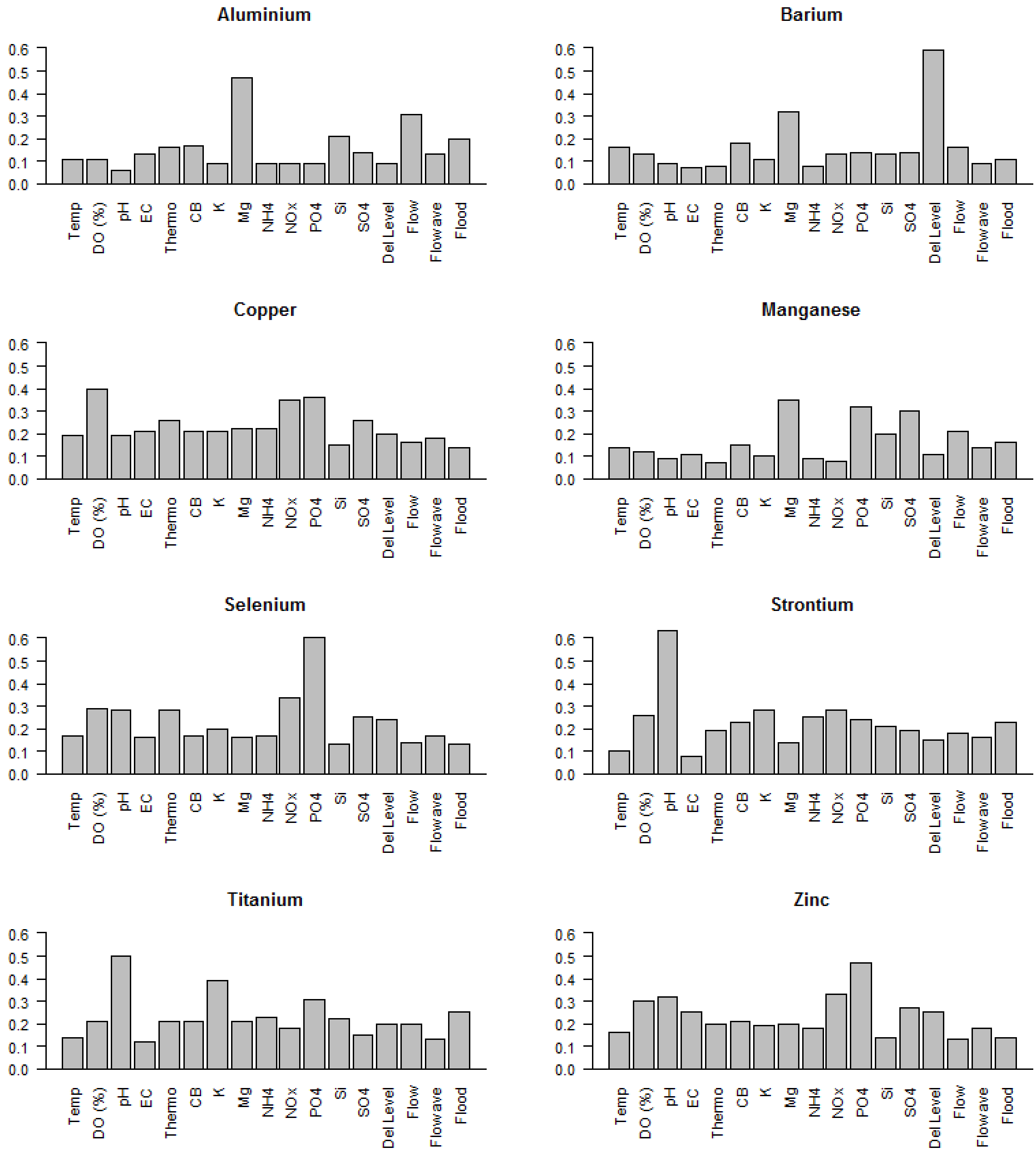

Figure 4.3: Bar plots of the proportion of the top models that contain the respective environmental drivers for the concentration of eight metals in *Labeo rosae* muscle tissue. Where Temp = temperature, DO = dissolved oxygen (%), EC = electrical conductivity, Thermo = thermocline depth, CB = centre of buoyancy, K = potassium, Mg = magnesium, NH4 = ammonium, NOx = nitrate:nitrite ratio, PO4 = phosphate, Si = silicon, SO4 = sulphate, Del Level = change in dam level over the last month, Flow = river daily average discharge, Flow ave = average river discharge over the last month, Flood = the number of days since the last flood (a discharge exceeding ≥ 20 cumecs).

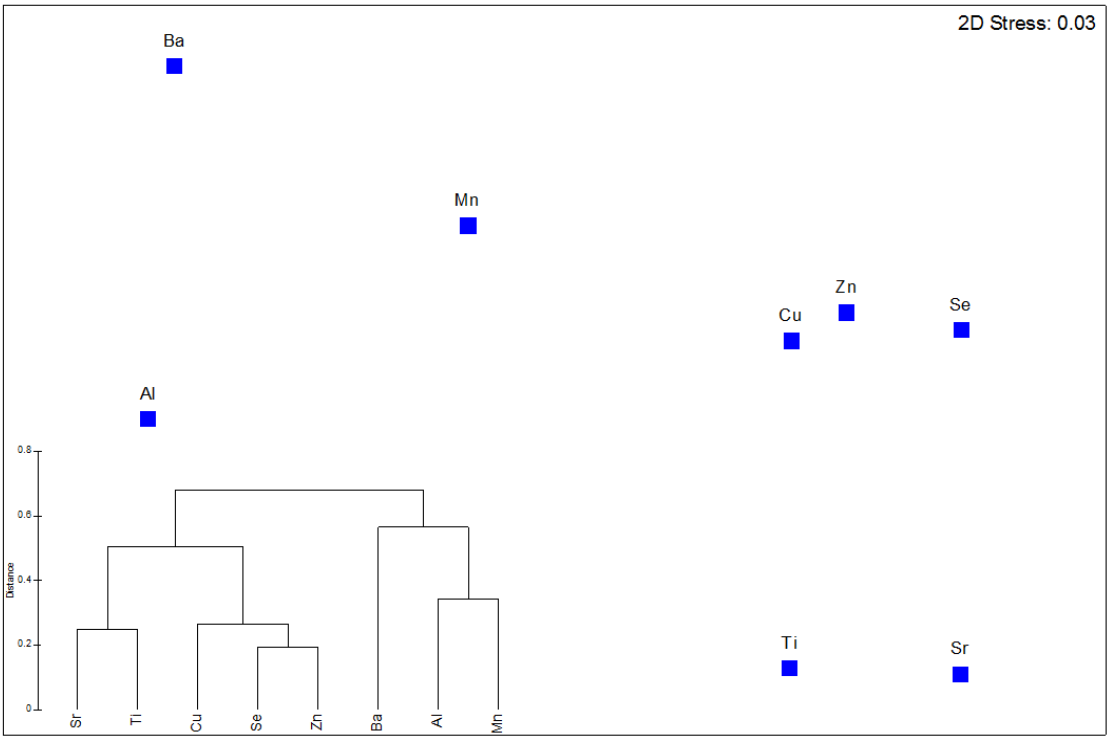

Figure 4.4: Non-Metric multidimensional scaling plot of the environmental drivers for the eight metals in *Labeo rosae* muscle tissue. The Cluster analysis dendrogram is inset on the lower right of the plot.

The SIMPER analysis showed that the environmental factors contributing more than 10% to the similarity within the *L. rosae* group included pH (25%), PO_4 (16%) and change in dam level over the last month, NH_4 (14%), with nitrate-nitrite, Mg, % DO and K all contributing more than 5% to the group similarity.

Schilbe intermedius

All the environmental variables selected for the glm analyses were included as predictors for each of the eight metals evaluated (Figure 4.5). The importance of the environmental variables as drivers varied for the respective metals with some metals having an even distribution of importance across the predictor variables, e.g. Cu and Zn. In contrast to the other two species, the two Alkali Earth metals, Ba and Sr, had similar biases in predictors and were grouped with Ti and Al.

Evaluating the predictor variables for the respective metals for the species, the NMDS plot shows a rough grouping of the metals whose predictor variable profile does not show any major biases in predictor variables (Cu and Zn) separated from three distinct groups of metals whose predictors show biases in predictor variables: 1) Ba, Sr, Ti and Al, 2) Mn, and 3) Se (Figure 4.6). Manganese shows a bias towards centre of buoyancy as a predictor while Se shows a biases towards the nitrate-nitrite ratio and river discharge as predictors.

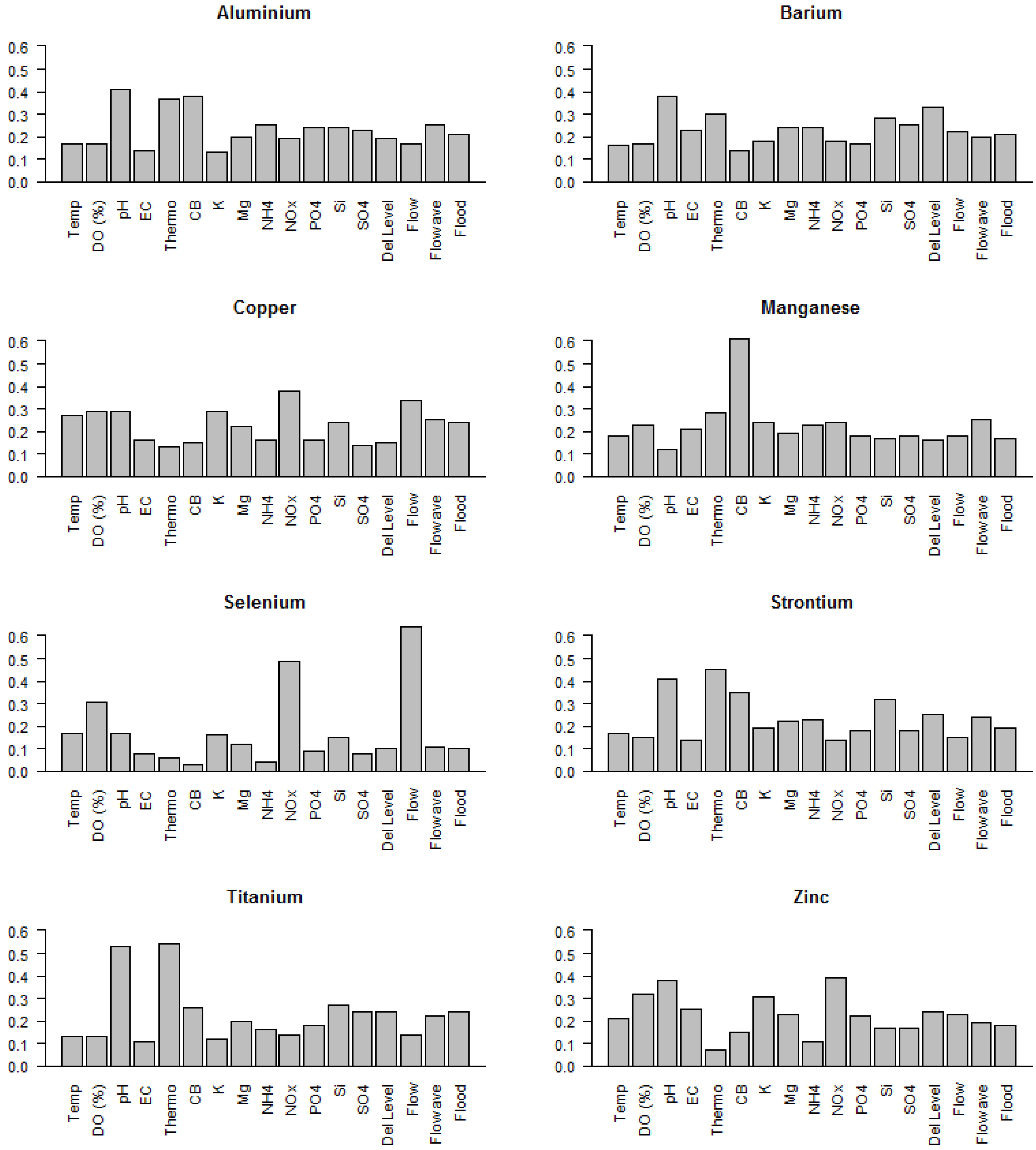

Figure 4.5: Bar plots of the proportion of the top models that contain the respective environmental drivers for the concentration of eight metals in *Schilbe intermedius* muscle tissue. Where Temp = temperature, DO = dissolved oxygen (%), EC = electrical conductivity, Thermo = thermocline depth, CB = centre of buoyancy, K = potassium, Mg = magnesium, NH4 = ammonium, NOx = nitrate:nitrite ratio, PO4 = phosphate, Si = silicon, SO4 = sulphate, Del Level = change in dam level over the last month, Flow = river daily average discharge, Flow ave = average river discharge over the last month, Flood = the number of days since the last flood (a discharge exceeding ≥ 20 cumecs).

The SIMPER analysis showed that the environmental factors contributing more than 10% to the similarity within the *S. intermedius* group included the centre of buoyancy (20%), thermocline depth (18%), average discharge (16%), pH (11%) and the nitrate-nitrite ratio (10%). These five variables contributed more than 65% to the group similarity.

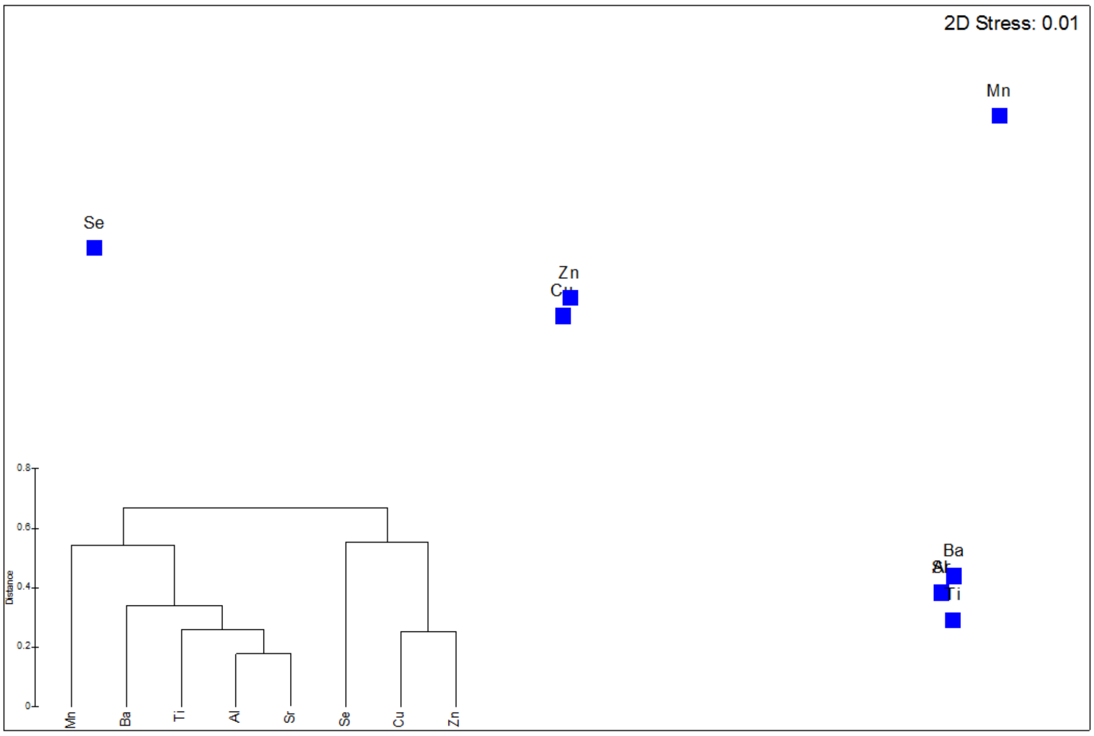

Figure 4.6: Non-Metric multidimensional scaling plot of the environmental drivers for the eight metals in *Schilbe intermedius* muscle tissue. The Cluster analysis dendrogram is inset on the lower right of the plot.

When the metals in muscle tissue for the three species are plotted on the same NMDS plot, an interesting pattern emerges. A degree of overlap is evident for *Labeo rosae* and *S. intermedius* while *O. mossambicus* appears to be separate from the other two species (Figure 4.7). The multivariate dispersion of the groups for the respective species was not significantly different (PERMDISP F = 0.1469, df1 = 2, df2 = 21; P(perm) = 0.902) indicating that the deviations from the centroids for the respective groups were not different. The PERMANOVA test returned a significant result (Pseudo F = 7.633, df = 2, P(perm) < 0.001) and a pairwise PERMANOVA was performed to determine which groups contributed towards the significant result for the overall test. The pairwise test returned a significant result for pairwise comparisons including *O. mossambicus* (both P(perm) < 0.001) but not for the *L. rosae-S. intermedius* pairwise comparison (P(perm) = 0.204). This result indicates that there is a significant difference in the position of the centroid of the *O. mossambicus* metals to the positions of the centroids of the other two species. There is no significant differences in the position of the centroids of the *L. rosae* and *S. intermedius* metals, indicating that the environmental drivers determining the metal concentrations in the muscle tissue for *S. intermedius* and *L. rosae* are more similar than those determining the metal concentrations in the muscle of *O. mossambicus*. It is worth noting that although the drivers of metal concentrations for *S. intermedius* and *L. rosae* may be similar, the position of the individual metals for the respective species in multivariate space are not necessarily the same.

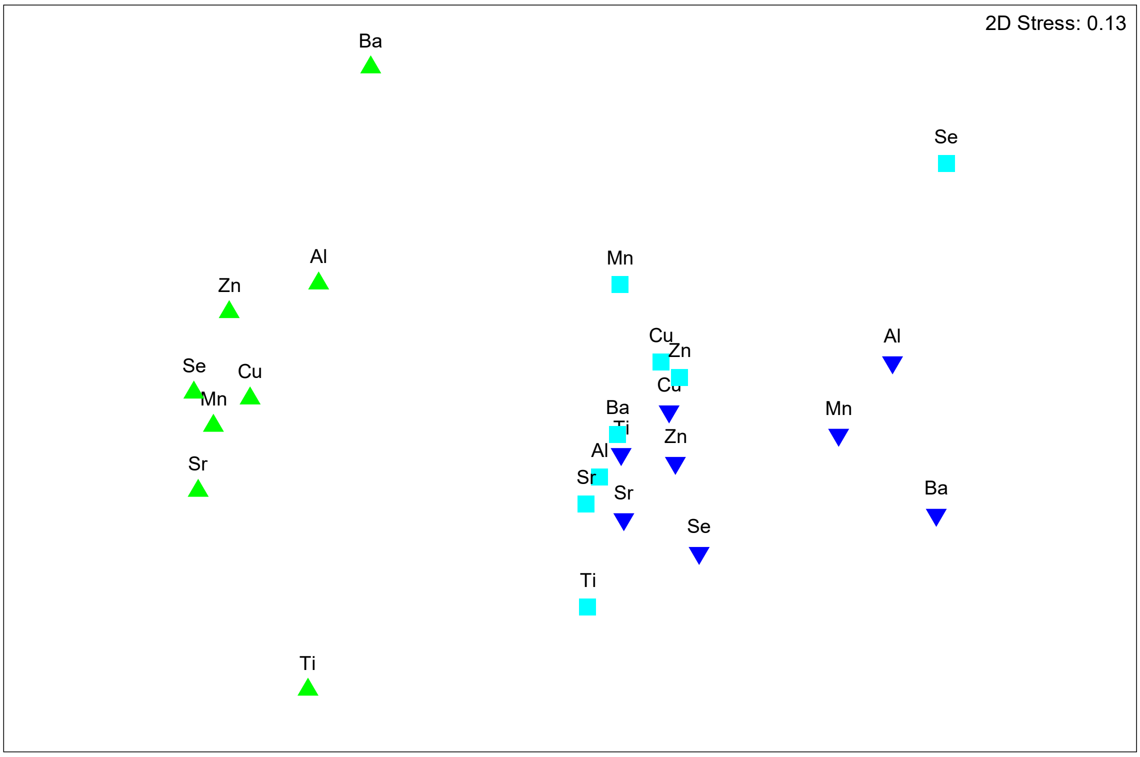

Figure 4.7: Non-Metric multidimensional scaling plot of the environmental drivers for the eight metals in *Oreochromis mossambicus* (green icons), *Labeo rosae* (blue icons) and *Schilbe intermedius* (cyan icons) muscle tissue.

The PERMANOVA result is supported by the average Euclidean distance within and between the groups for the respective species. Within group distance is in a similar range for each of the three species (*O. mossambicus* – 0.602; *L. rosae* – 0.559 and *S. intermedius* – 0.546) although the *O. mossambicus* within group distance is a little higher than the other two species. The between group distance between *L. rosae* and *S. intermedius* is within the range of the within group distances (0.585) but those between *O. mossambicus* and the other two species are considerable higher than the within group distances (*O. mossambicus* – *L. rosae* = 0.928, *O. mossambicus* – *S. intermedius* = 0.853).

The SIMPER analysis showed that the environmental factors contributing more than 10% to the dissimilarity between the *O. mossambicus* and *L. rosae* metals included Si (11%) and the number of days since the last flood (11%), with NH_4, pH, CB, temperature, K, average river discharge over the last month, EC and SO_4 concentration all contributing more than 5% to the dissimilarity between the groups.

The SIMPER analysis showed that the environmental factor contributing more than 10% to the dissimilarity between the *O. mossambicus* and *S. intermedius* metals was the number of days since the last flood (11%), with Si, CB, NH_4, SO_4 concentration, temperature, K, EC, thermocline depth and pH all contributing more than 5% to the dissimilarity between the groups.

The SIMPER analysis showed that the environmental factors contributing more than 10% to the dissimilarity between the *L. rosae* and *S. intermedius* metals included pH (16%), PO_4

concentration (13%) and thermocline depth (12%), with CB, river discharge, nitrate-nitrite ratio and the change in the dam level over the last month all contributing more than 5% to the dissimilarity between the groups.

4.1.6 Distance-based linear modelling of environmental drivers

The distance-based redundancy analysis presents a different picture of the drivers for the metal concentrations in the muscle tissue of the respective species because the entire suite of metals reported on in the long-term monitoring are included in the analysis.

Oreochromis mossambicus

The dbRDA found that 88% of the fitted variation, and 70% of the total variation in metal concentrations in muscle tissue could be explained by the first axis (pH (0.470), nitrate:nitrite (0.446), EC (0.374), SO_4 (-0.358), NH_4 (-0.342), DO (-0.341), K (0.272) and thermocline depth (-0.071); Figure 4.8). The best model explained almost 80% of the variation in the dataset (with an AIC of 58.16, R^2 = 0.788 comprising 8 variables). All of the best predictor variables for the metal concentrations in *O. mossambicus* muscle tissue were physico-chemical variables with the exception of the thermocline depth. This indicates that the more resident *O. mossambicus* is effected more by physico-chemical variables than larger scale limnological variables.

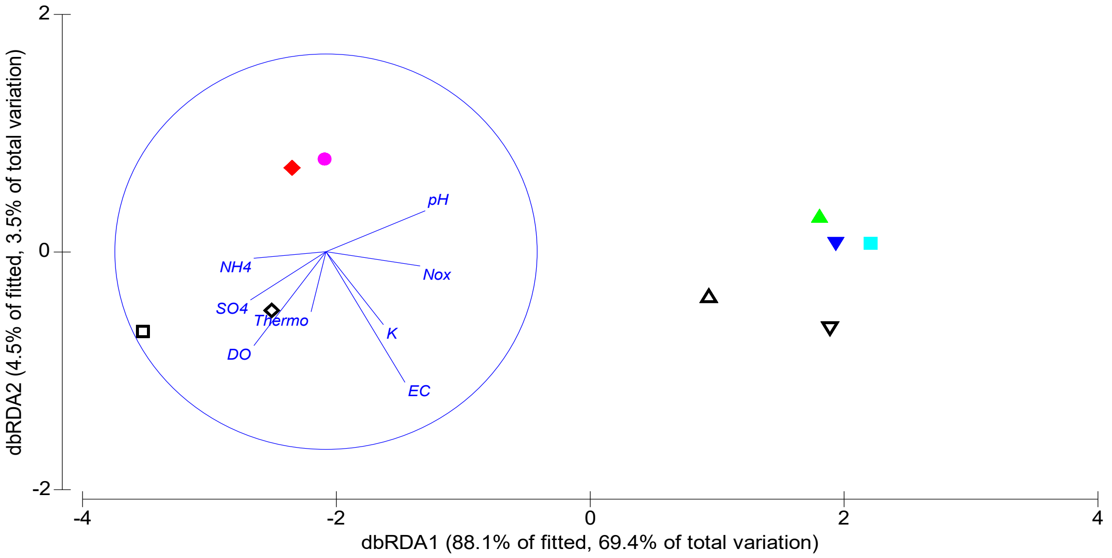

Figure 4.8: Distance based redundancy analysis plot of the environmental drivers of metal concentrations in *Oreochromis mossambicus* muscle tissue. The data points for the field trips are represented by unique symbols: Jun 16 (▲), Oct 16 (▼), Mar 17 (■), Apr 17 (♦), Jun 17 (●), Sep 17 (Δ) Dec 17 (∇), Feb 18 (□), and May 18 (◇). Where NH4 = ammonium, SO4 = sulphate, DO = dissolved oxygen (%), Thermo = thermocline depth, EC = electrical conductivity, K = potassium, Nox = nitrate:nitrate ratio.

Labeo rosae

The dbRDA found that 92% of the fitted variation, and 74% of the total variation in metal concentrations in muscle tissue could be explained by the first axis (number of days since last flood (-0.857), Mg (-0.449), PO_4 (0.254) and the average river discharge (0.017); Figure 4.9). The best model explained almost 81% of the variation in the dataset (with an AIC of 15.26, R^2 = 0.806 comprising 4 variables). The most prominent predictor for the metal concentrations in *L. rosae* muscle tissue was the time since the last flood. This indicates that the frequency of flood events is a major driver for the metal concentrations in the muscle tissue of the benthic detrivorous *L. rosae*.

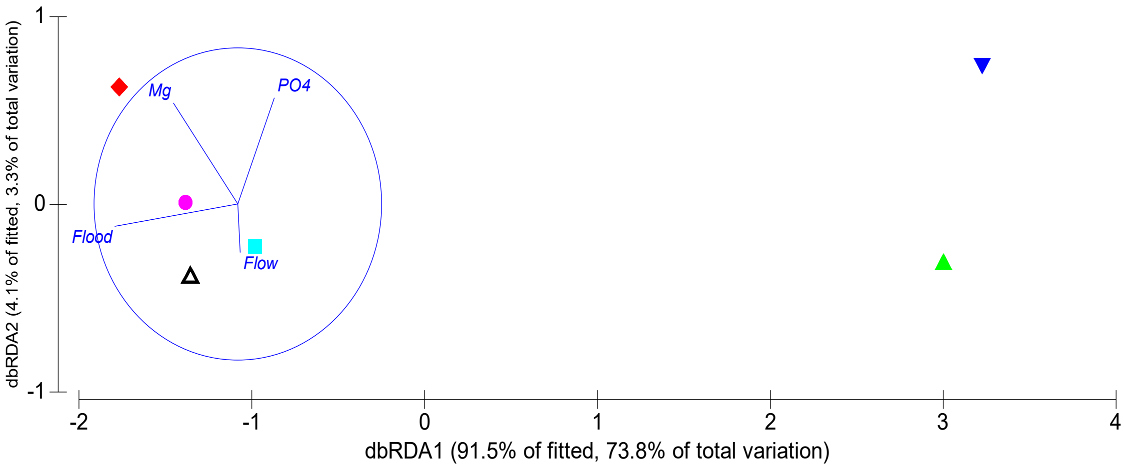

Figure 4.9: Distance based redundancy analysis plot of the environmental drivers of metal concentrations in *Labeo rosae* muscle tissue. The data points for each field trip are represented by unique symbols: Mar 17 (▲), Apr 17 (▼), Sep 17 (■) Dec 17 (♦), Feb 18 (●), and May 18 (∆). Where Mg = Magnesium, Flood = number of days since last flood, Flow = river daily average discharge and PO4 = phosphate.

Schilbe intermedius

The dbRDA found that 55% of the fitted variation, and 36% of the total variation in metal concentrations in muscle tissue could be explained by the first axis (EC (0.844), pH (0.507), DO (-0.127), number of days since last flood (-0.115) and nitrate:nitrite (0.020); Figure 4.10) while the second axis (number of days since last flood (-0.885), pH (0.332), EC (-0.308), nitrate:nitrite (-0.084) and DO (0.066)) explained 25% of the fitted variation, and 17% of the total variation. The best model explained almost 67% of the variation in the dataset (with an AIC of 17.20, R^2 = 0.666 with 5 variables). The most prominent predictor for the metal concentrations in *S. intermedius* muscle tissue was the time since the last flood. This indicates that the frequency of the flood events is a major driver for the metal concentrations in the muscle tissue of the pelagic piscivorous *S. intermedius*.

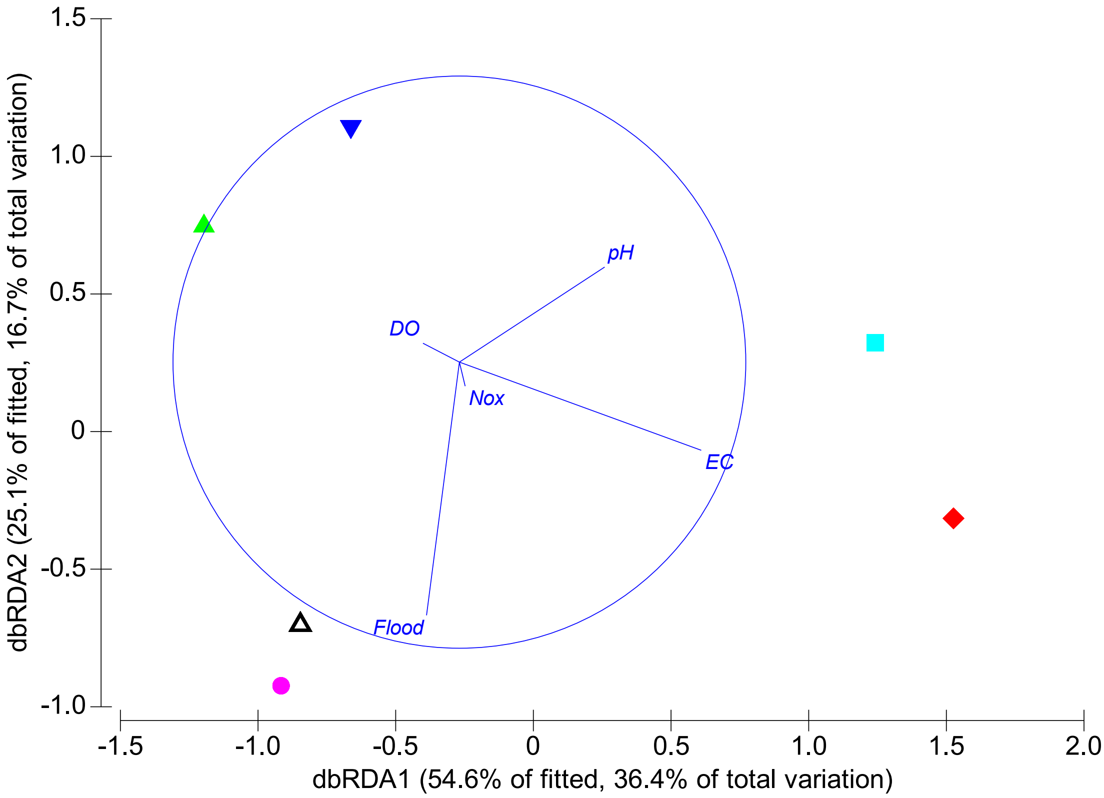

Figure 4.10: Distance based redundancy analysis plot of the environmental drivers of metal concentrations in *Schilbe intermedius* muscle tissue. The data points for field trip are represented by unique symbols: Mar 17 (▲), Jun 17 (▼), Sep 17 (■) Dec 17 (♦), Feb 18 (●), and May 18 (∆). Where DO = dissolved oxygen (%), Flood = number of days since last flood, Nox = nitrate:nitrite ratio, EC = electrical conductivity.

4.1.7 Generalised linear model of limnological drivers

The glm and DISTLM analyses were repeated with a reduced set of ten "limnological" variables that can be measured in the field (i.e. temperature, DO (%), pH EC, thermocline depth, centre of buoyancy (CB), change in dam level over the last month, river daily average discharge, daily average river discharge over the last month and the number of days since the last flood (a discharge exceeding 20 cumecs).

Oreochromis mossambicus

All the environmental variables selected for the glm analyses were included as predictors for each of the eight metals evaluated (Figure 4.11). The importance of the environmental variables as drivers varied for the respective metals with some metals having an even distribution of importance across the predictor variables, e.g. Cu, Mn and Se, while other metals having biases towards specific combinations predictor variables, e.g. Ba and Ti.

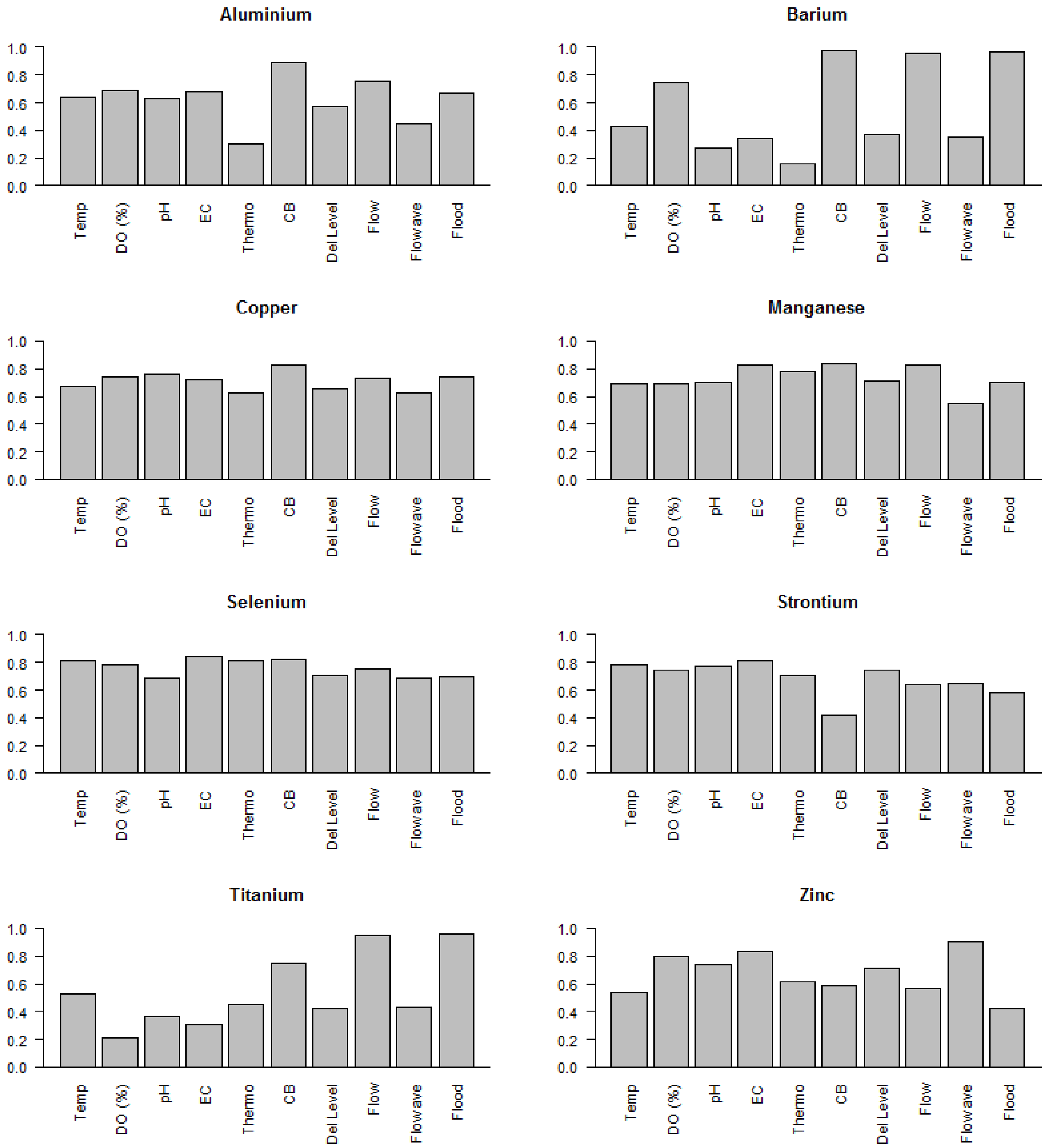

Figure 4.11: Bar plots of the proportion of the top models that contain the respective limnological drivers for the concentration of eight metals in *Oreochromis mossambicus* muscle tissue. Where Temp = temperature, DO = dissolved oxygen (%), pH, EC = electrical conductivity, Thermo = thermocline depth, CB = centre of buoyancy, Del Level = change in dam level over the last month, Flow = river daily average discharge, Flow ave = average river discharge over the last month, Flood = the number of days since the last flood (a discharge exceeding ≥ 20 cumecs).

Evaluating the predictor variables for the respective metals for the species, the NMDS plot shows a rough grouping of the metals whose predictor variable profile does not show any major biases in predictor variables (Cu, Mn, Se) separated from the other metals (Figure 4.12). Barium and Ti show a bias towards centre of buoyancy, river discharge and the time since the last flood as predictors but there is minimal overlap for the other main predictors.

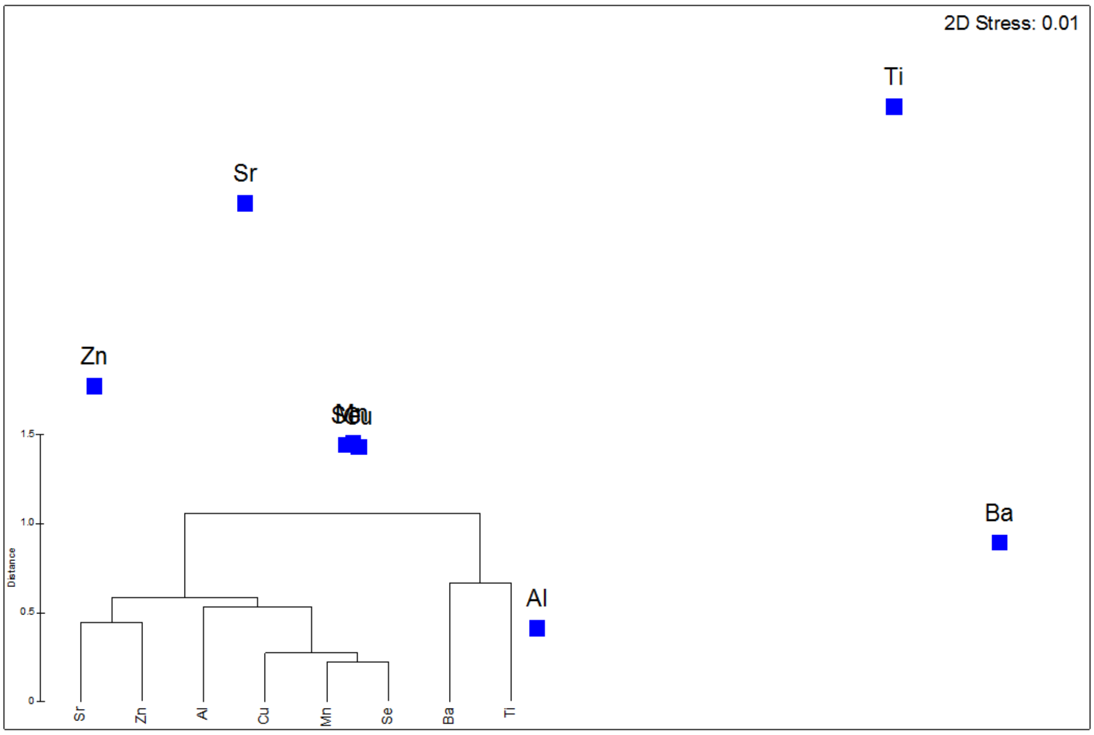

Figure 4.12: Non-Metric multidimensional scaling plot of the limnological drivers for the eight metals in *Oreochromis mossambicus* muscle tissue. The Cluster analysis dendrogram is inset on the lower right of the plot.

The SIMPER analysis showed that the limnological factors contributing more than 10% to the similarity within the *O. mossambicus* group included thermocline depth (16%), EC (15%), pH (11%), DO (11%) and the number of days since the last flood (10%), with CB, average river discharge over the last month, the change in the dam level over the last month, river discharge and temperature all contributing more than 5% to the group similarity.

Labeo rosae

All the limnological variables selected for the glm analyses were included as predictors for each of the eight metals evaluated (Figure 4.13). The importance of the environmental variables as drivers varied for the respective metals with some metals having an even distribution of importance across the predictor variables, e.g. Cu and Se, while other metals having biases towards specific predictor variables, e.g. pH for Sr, Ti and Zn, time since last flood for Mn and change in dam level for Ba.

Evaluating the predictor variables for the respective metals for the species, the NMDS plot shows a rough grouping of the metals whose predictor variable profile does not show any major biases in predictor variables (Cu and Se) separated from three distinct groups of metals whose predictors show biases in predictor variables: 1) Mn and Al, 2) Mn and Sr, and 3) outliers of Ba and Ti (Figure 4.14).

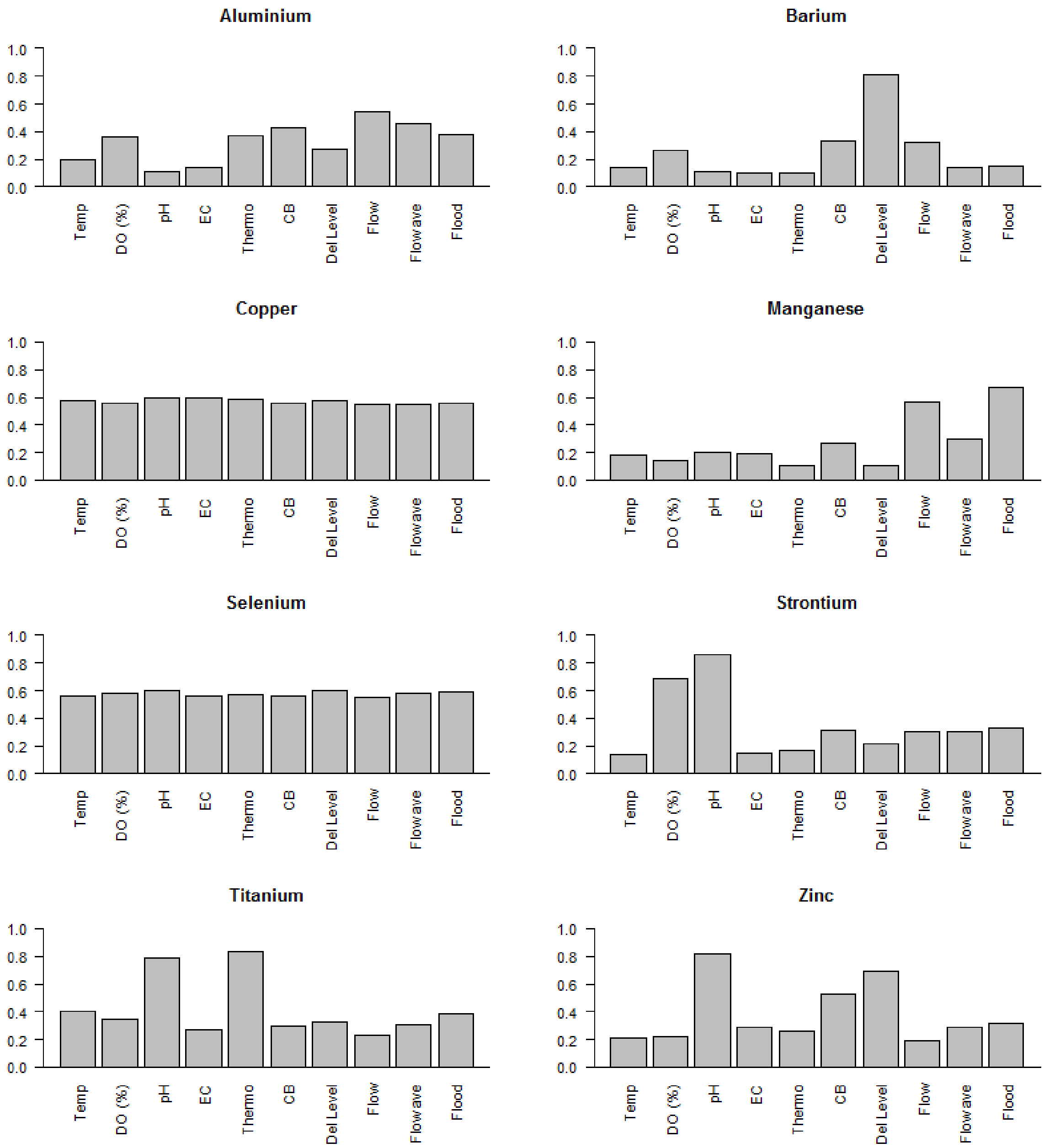

Figure 4.13: Bar plots of the proportion of the top models that contain the respective limnological drivers for the concentration of eight metals in *Labeo rosae* muscle tissue. Where Temp = temperature, DO = dissolved oxygen (%), pH, EC = electrical conductivity, Thermo = thermocline depth, CB = centre of buoyancy, Del Level = change in dam level over the last month, Flow = river daily average discharge, Flow ave = average river discharge over the last month, Flood = the number of days since the last flood (a discharge exceeding ≥ 20 cumecs).

The SIMPER analysis showed that the limnological factors contributing more than 10% to the similarity within the *L. rosae* group included pH (24%), thermocline depth (16%) and the change in the dam level over the last month (14%) with DO, EC, temperature, the number of days since the last flood and river discharge all contributing more than 5% to the group similarity.

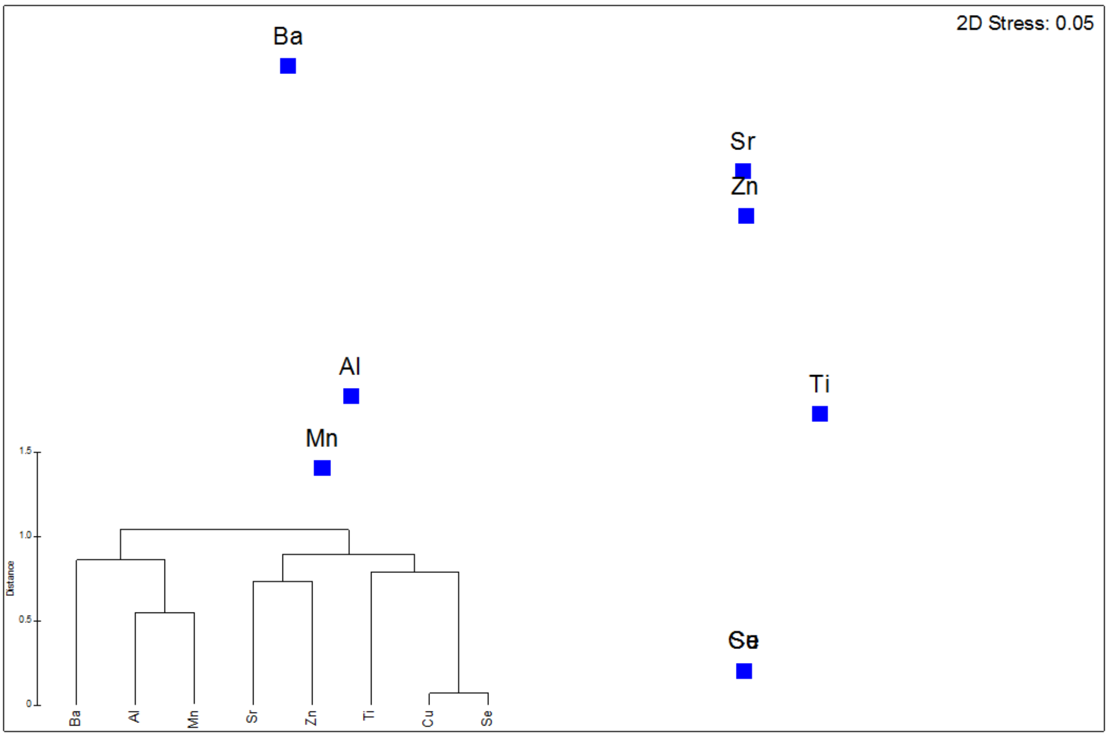

Figure 4.14: Non-Metric multidimensional scaling plot of the limnological drivers for the eight metals in *Labeo rosae* muscle tissue. The Cluster analysis dendrogram is inset on the lower right of the plot.

Schilbe intermedius

All the limnological variables selected for the glm analyses were included as predictors for each of the eight metals evaluated (Figure 4.15). The importance of the limnological variables as drivers varied for the respective metals with some metals having an even distribution of importance across the predictor variables, e.g. Cu and Zn.

Evaluating the predictor variables for the respective metals for the species, the NMDS plot and cluster analysis shows a rough grouping of the metals into two distinct groups 1) Sr, Ti and Al with Ba as an outlier and 2) Cu and Zn with Se and Mn as outliers (Figure 4.16). Manganese shows a bias towards centre of buoyancy as a predictor, while Al, Sr and Ti are biased towards pH and Se showing a bias toward river discharge as a predictor, while Cu and Zn have similar profiles of predictor variables.

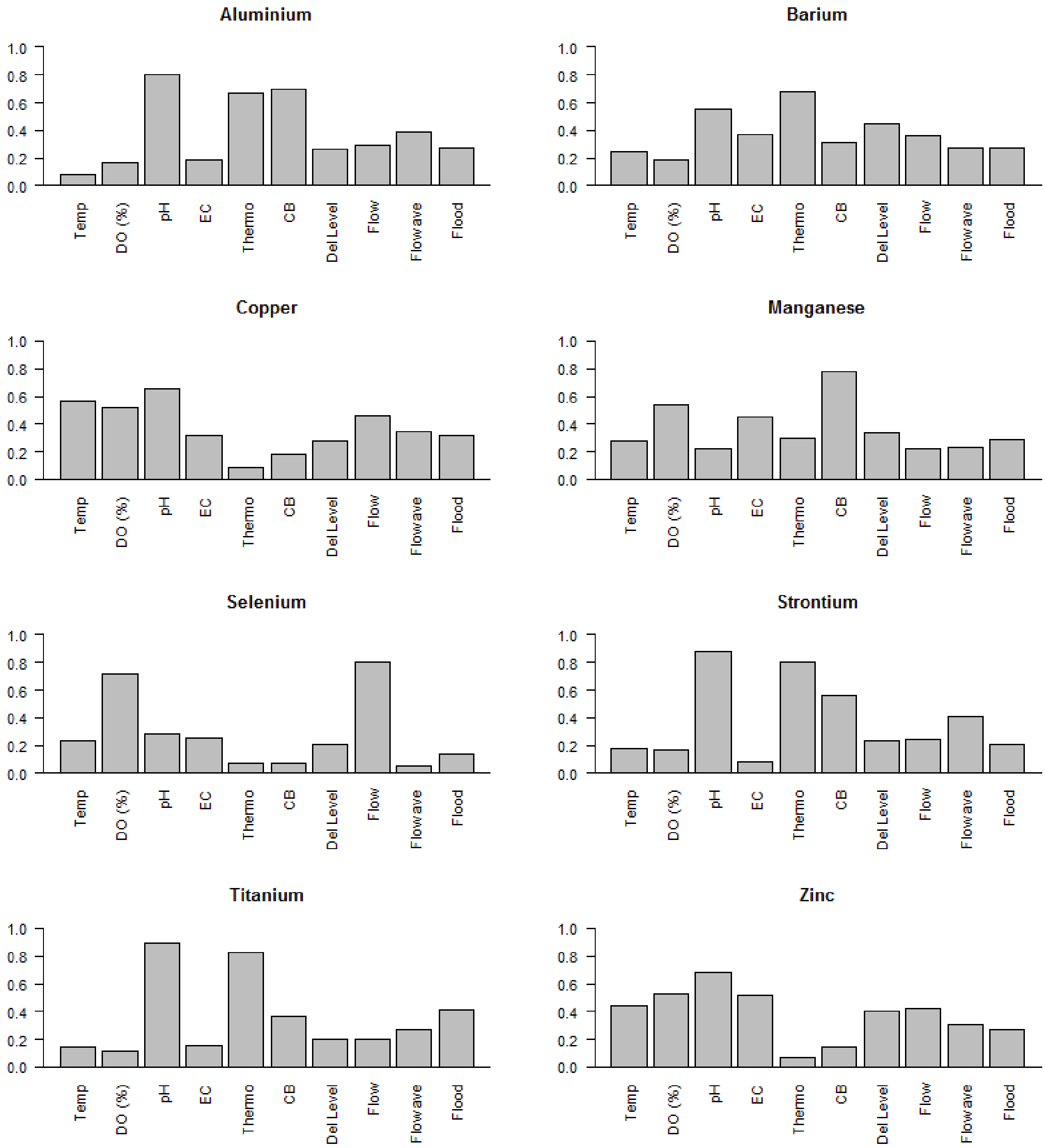

Figure 4.15: Bar plots of the proportion of the top models that contain the respective limnological drivers for the concentration of eight metals in *Schilbe intermedius* muscle tissue. Where Temp = temperature, DO = dissolved oxygen (%), pH, EC = electrical conductivity, Thermo = thermocline depth, CB = centre of buoyancy, Del Level = change in dam level over the last month, Flow = river daily average discharge, Flow ave = average river discharge over the last month, Flood = the number of days since the last flood (a discharge exceeding ≥ 20 cumecs).

The SIMPER analysis showed that the limnological factors contributing more than 10% to the similarity within the *S. intermedius* group included thermocline depth (27%), centre of buoyancy (17%), pH (16%) and DO (13%), with river discharge, temperature and EC all contributing more than 5% to the group similarity.

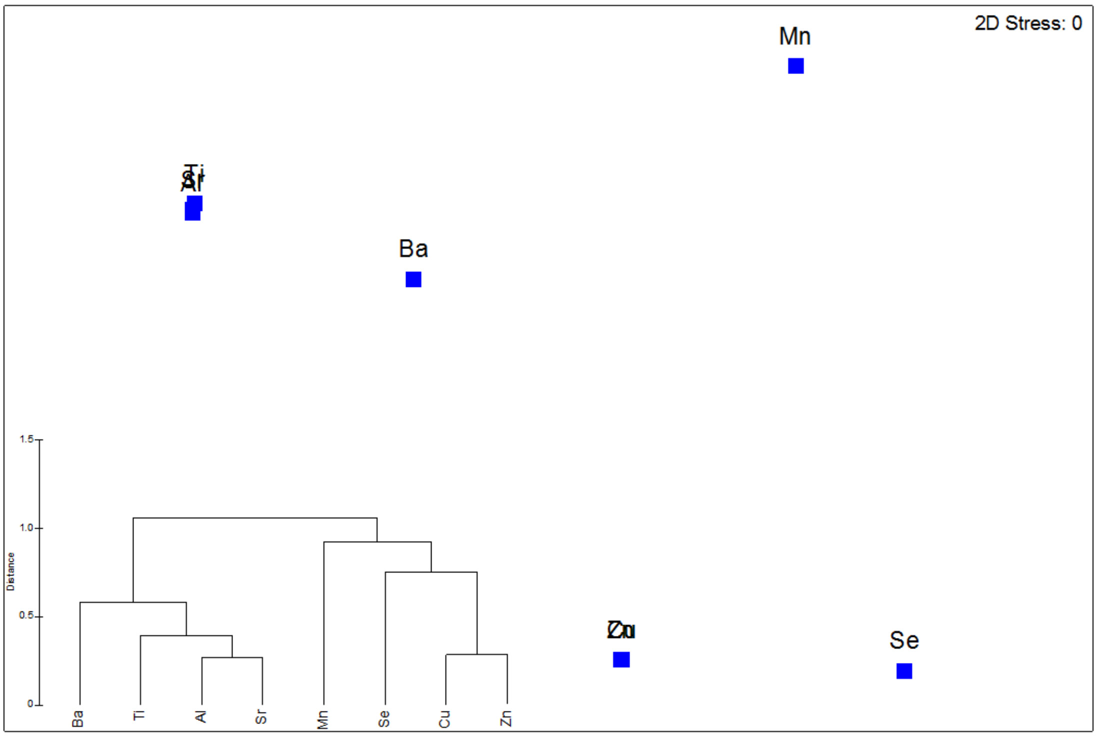

Figure 4.16: Non-Metric multidimensional scaling plot of the limnological drivers for the eight metals in *Schilbe intermedius* muscle tissue. The Cluster analysis dendrogram is inset on the lower right of the plot.

When the metals in muscle tissue for the three species are plotted on the same NMDS plot, an interesting pattern emerges. A degree of overlap is evident for *L. rosae* and *S. intermedius* while *O. mossambicus* appears to be separate from the other two species (Figure 4.17). The multivariate dispersion of the groups for the respective species was not significantly different (PERMDISP F = 1.341, df1 = 2, df2 = 21; P(perm) = 0.380) indicating that the deviations from the centroids for the respective groups were not different. The PERMANOVA test returned a significant result (Pseudo F = 6.506, df = 2, P(perm) < 0.001) and a pairwise PERMANOVA was performed to determine which groups contributed towards the significant result for the overall test. The pairwise test returned a significant result for pairwise comparisons including *O. mossambicus* (both P(perm) < 0.001) but not for the *L. rosae*-*S. intermedius* pairwise comparison (P(perm) = 0.684). This result indicates that there is a significant difference in the position of the centroid of the *O. mossambicus* metals to the positions of the centroids of the other two species. There is no significant difference in the position of the centroids of the *L. rosae* and *S. intermedius* metals, indicating that the environmental drivers determining the metal concentrations in the muscle tissue for *S. intermedius* and *Labeo rosae* are more similar than those determining the metal concentrations in the muscle of *O. mossambicus*. Of significance is that although the drivers of metal concentrations for *S. intermedius* and *L. rosae* may be similar, the position of the individual metals for the respective species in multivariate space are not necessarily the same.

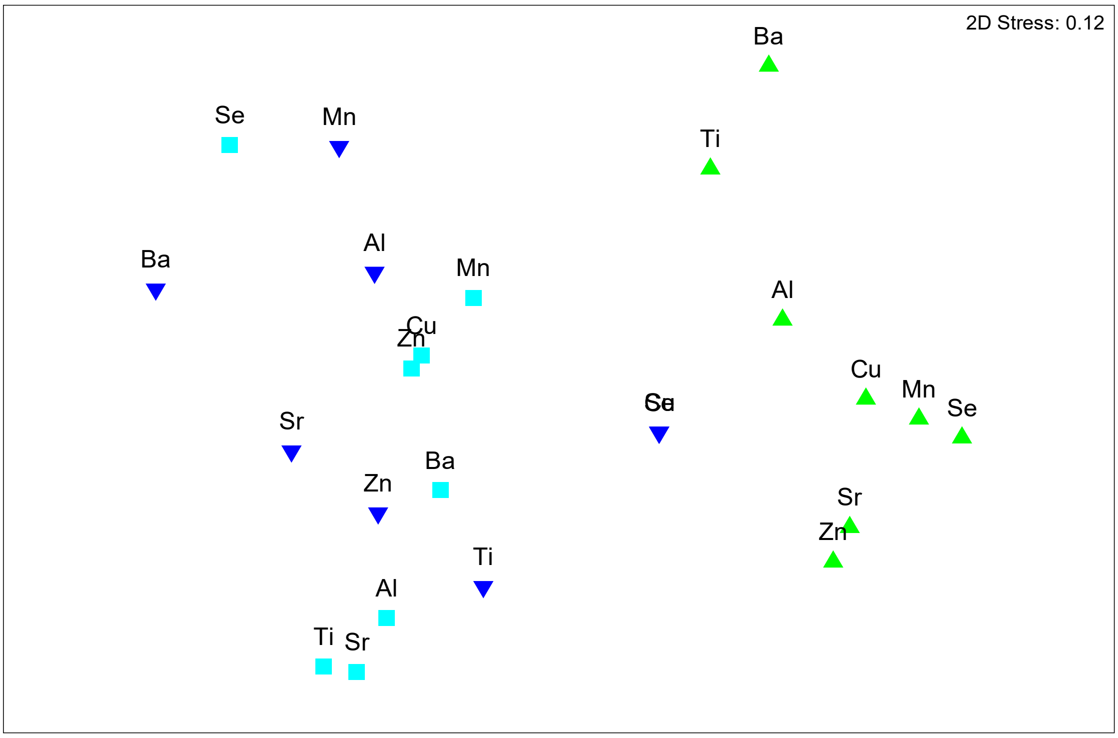

Figure 4.17: Non-Metric multidimensional scaling plot of the environmental drivers for the eight metals in *Oreochromis mossambicus* (green icons), *Labeo rosae* (blue icons) and *Schilbe intermedius* (cyan icons) muscle tissue.

The PERMANOVA result is supported by the average Euclidean distance within and between the groups for the respective species. Within group distance is in a similar range for each of the three species (*O. mossambicus* – 0.746; *L. rosae* – 0.913 and *S. intermedius* – 0.866) although the *O. mossambicus* within group distance is a little higher than the other two species. The between group distance between *L. rosae* and *S. intermedius* is within the range of the within group distances (0.889) but those between *O. mossambicus* and the other two species are considerable higher than the within group distances (*O. mossambicus* – *L. rosae* = 1.160, *O. mossambicus* – *S. intermedius* = 1.304).

The SIMPER analysis showed that the environmental factors contributing more than 10% to the dissimilarity between the *O. mossambicus* and *L. rosae* metals included EC (15%), river discharge (12%), CB (11%) and temperature (11%), with thermocline depth, DO, the number of days since the last flood pH and the change in the dam level over the last month all contributing more than 5% to the dissimilarity between the groups.

The SIMPER analysis showed that the environmental factor contributing more than 10% to the dissimilarity between the *O. mossambicus* and *S. intermedius* metals included the number of days since the last flood (13%), CB (13%), river discharge (12%) and EC (12%), with DO, temperature, thermocline depth, average river discharge over the last month and the change in the dam level over the last month all contributing more than 5% to the dissimilarity between the groups.

The SIMPER analysis showed that the environmental factors contributing more than 10% to the dissimilarity between the *L. rosae* and *S. intermedius* metals included thermocline depth (20%), pH (19%) and the change in the dam level over the last month (11%), with DO (%), CB, river discharge, temperature, the number of days since the last flood and EC all contributing more than 5% to the dissimilarity between the groups.

4.1.8 Distance based linear modelling of limnological drivers

Oreochromis mossambicus

The dbRDA found that 88% of the fitted variation, and 69% of the total variation in metal concentrations in muscle tissue could be explained by the first axis (change in dam level over the last month (0.665), CB (-0.436), DO (-0.328), EC (0.297), pH (-0.273), number of days since last flood (0.236), thermocline depth (0.193), and temperature (0.069); Figure 4.18). The best model explained almost 80% of the variation in the dataset (with an AIC of 58.16, R^2 = 0.788 comprising 8 variables). At least seven variables were required for the ΔAIC to be less than 4 from the top model's AIC. The best seven variable model also explained almost 80% of the variation in the dataset.

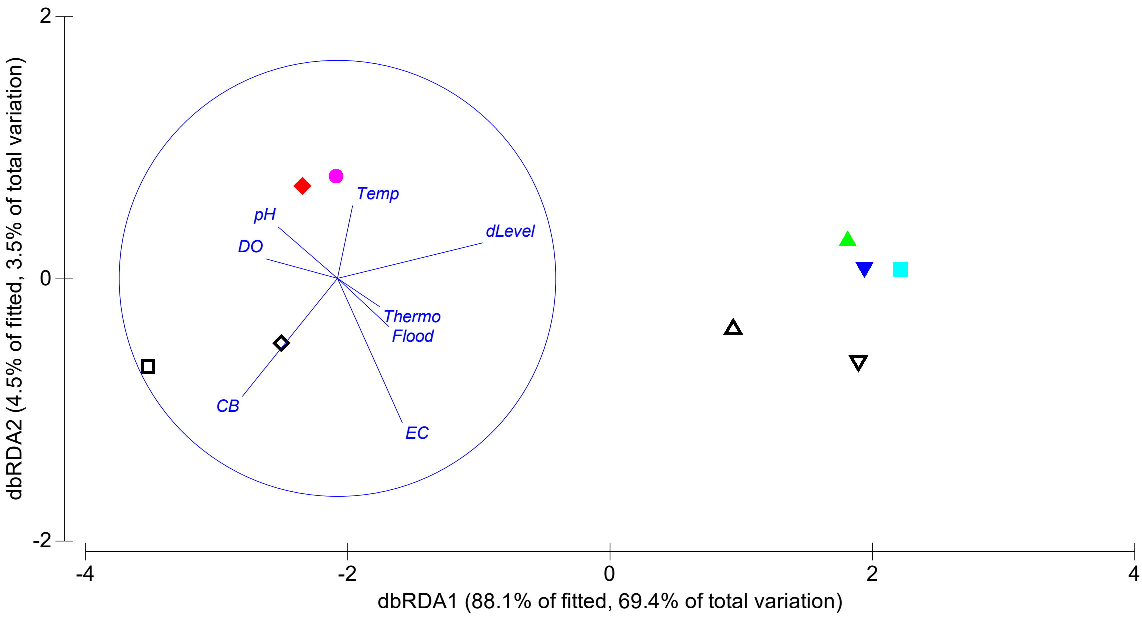

Figure 4.18: Distance based redundancy analysis plot of the limnological drivers of metal concentrations in *Oreochromis mossambicus* muscle tissue. The data points for the field trips are represented by unique symbols: Jun 16 (▲), Oct 16 (▼), Mar 17 (■), Apr 17 (♦), Jun 17 (●), Sep 17 (Δ) Dec 17 (∇), Feb 18 (□), and May 18 (◇). Where Temp = temperature, DO = dissolved oxygen (%), CB = centre of buoyancy, EC = electrical conductivity, Flood = number of days since last flood, Thermo = thermocline depth, and dLevel = change in dam level over the last month.

The magnitude of the coefficients for the respective predictors on the first RDA axis show that environmental physico-chemical parameters are important in determining the distance within the response matrix; pH (-31.121), temperature (-10.375), DO (-5.085), CB (-1.718), change in dam level over the last month (1.328), thermocline depth (1.270), EC (0.45793), and number of days since last flood (-0.064).

Labeo rosae

The dbRDA found that 91% of the fitted variation, and 74% of the total variation in metal concentrations in muscle tissue could be explained by the first axis (DO (-0.835), thermocline depth (0.381), river discharge (0.329), temperature (0.207) and CB (0.077); Figure 4.19). The best model explained almost 81% of the variation in the dataset (with an AIC of 15.71, R^2 = 0.812 comprising 5 variables). At least three variables were required for the ΔAIC to be less than 4 from the top model's AIC. The best three variable model also explained 78% of the variation in the dataset. The most prominent predictor for the metal concentrations in *Labeo rosae* muscle tissue was the dissolved oxygen saturation.

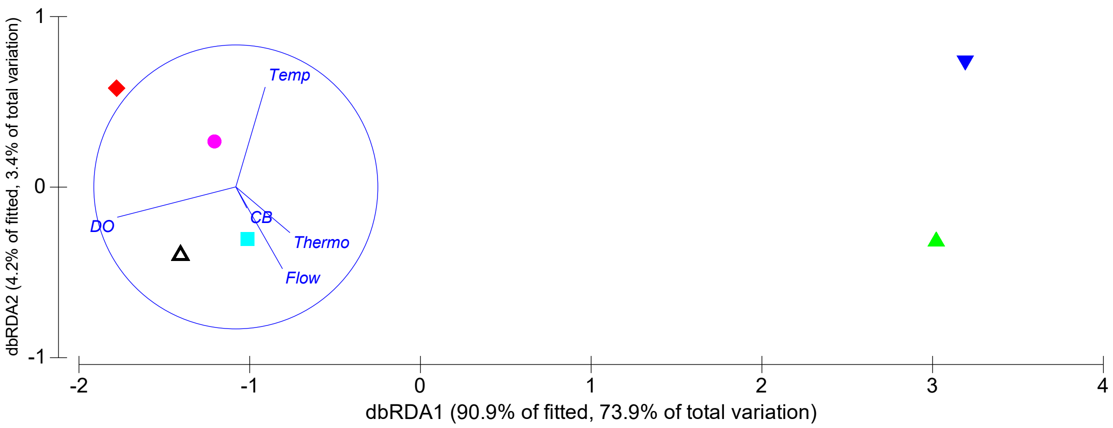

Figure 4.19: Distance based redundancy analysis plot of the limnological drivers of metal concentrations in *Labeo rosae* muscle tissue. The data points for each field trip are represented by unique symbols: Mar 17 (▲), Apr 17 (▼), Sep 17 (■) Dec 17 (♦), Feb 18 (●), and May 18 (Δ). Where Temp = temperature, DO = dissolved oxygen (%), Flow = river discharge, CB = centre of buoyancy andThermo = thermocline depth.

The magnitude of the coefficients for the respective predictors on the first RDA axis show that environmental physico-chemical and limnological parameters are important in determining the distance within the response matrix; DO (-124.25), river discharge (-77.562), CB (73.65), temperature (24.289), and thermocline depth (-8.884).

Schilbe intermedius

The dbRDA found that 55% of the fitted variation, and 36% of the total variation in metal concentrations in muscle tissue could be explained by the first axis (temperature (0.717), CB (-0.655), DO (-0.229), change in dam level over the last month (0.066) and number of days since last flood (-0.024); Figure 4.20) while the second axis (number of days since last flood (-0.898), temperature (-0.259), CB (-0.258), change in dam level over the last month (0.227) and DO (0.090)) explained 25% of the fitted variation, and 17% of the total variation. The best model explained almost 67% of the variation in the dataset (with an AIC of 17.20, R^2 = 0.666 consisting of 5 variables). At least four variables were required for the ∆AIC to be less than 4 from the top model's AIC. The best four variable model also explained 65% of the variation in the dataset. The most prominent predictor for the metal concentrations in *S. intermedius* muscle tissue was the time since the last flood. This indicates that the frequency of the flood events is a major driver for the metal concentrations in the muscle tissue of the pelagic piscivorous *S. intermedius*.

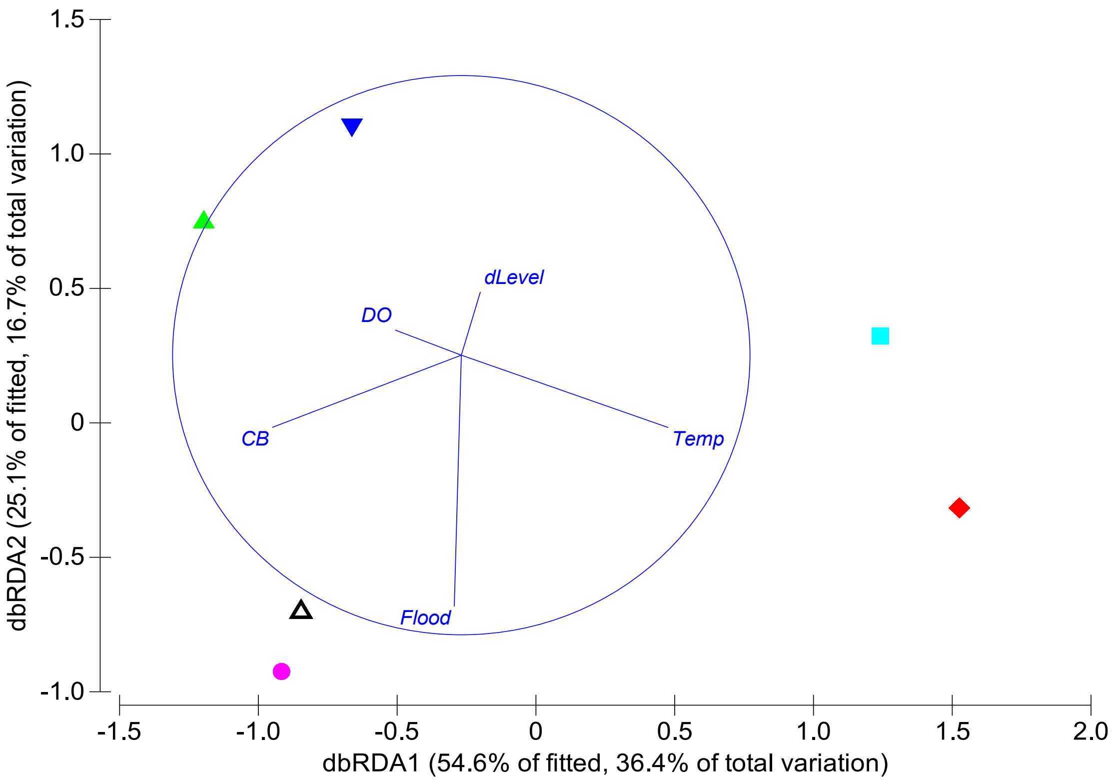

Figure 4.20: Distance based redundancy analysis plot of the limnological drivers of metal concentrations in *Schilbe intermedius* muscle tissue. The data points for field trip are represented by unique symbols: Mar 17 (▲), Jun 17 (▼), Sep 17 (■) Dec 17 (♦), Feb 18 (●), and May 18 (∆). Where dLevel = change in dam level over the last month, DO = dissolved oxygen (%), CB = centre of buoyancy, Flood = number of days since last flood and Temp = temperature.

The magnitude of the coefficients for the respective predictors on the first two RDA axes show that environmental physico-chemical and limnological parameters are important in determining the distance within the response matrix; first RDA axis DO (377.18), temperature (248.95), change in dam level over the last month (166.69), CB (-39.337), and number of days since last flood (5.296); second RDA axis (DO (25.296), temperature (16.611), change in dam level over the last month (11.189), CB (-2.661), and number of days since last flood (0.350).

DISCUSSION

When comparing the AIC values for the best distance based redundancy analysis models generated from the Environmental and Limnological predictor datasets, it is evident that the AIC values for the best models from the respective predictors are within 4 AIC points of each other, and can therefore be considered as equivalent models (Burnham and Anderson, 2002) for each of the three species. Since the limnological predictors are measured in the field, or at least can be calculated in the field, and do not require chemical analyses these are preferred as a predictor set and will form the basis for the ensuing discussion.

4.1.9 Regression analysis for eight metals

Based on the generalised linear modelling regression analysis for eight metals, the predictor variables that contributed more than 10% to the within group similarity for *O. mossambicus* were pH, DO, EC, thermocline depth, and the time since the last flood, for *L. rosae* pH, thermocline depth, and change in dam level over the last month, with DO contributing more than 5% to the group similarity. For *S. intermedius*, more than 10% to the within group similarity was explained by pH, DO, thermocline depth, and the CB. The predictors pH, dissolved oxygen, and thermocline depth were common to the three species. In addition, temperature and electrical conductivity consistently contributed more than 5% similarity for all three species.

The contributions or more than 10% to the dissimilarity between *O. mossambicus* and the other two species were centred on EC, CB and river discharge, with each species having a unique additional predictor; temperature for *L. rosae* and the time since the last flood for *S. intermedius*. More than 10% to the dissimilarity between *L. rosae* and *S. intermedius* was found to be attributed to pH, thermocline depth, and the change in dam level over the last month.

4.1.10 Distance-based redundancy analysis

Based on the distance-based redundancy analysis, three variables were shared between the best models of the three species: dissolved oxygen, temperature, and centre of buoyancy. *Oreochromis mossambicus* had the largest set of eight predictor variables for the metal concentrations in muscle tissue: pH, DO, EC, temperature, thermocline depth, CB, change in dam level over the last month, and the time since the last flood. Models for *O. mossambicus* with seven variables produced equivalent models to the best model and it may be possible to reduce the number of variables required for this species.

Schilbe intermedius shared all five its best predictor variables with *O. mossambicus*: dissolved oxygen, temperature, CB, change in dam level over the last month, and the time since the last flood. Models for *S. intermedius* with four variables produced equivalent models to the best model and it may be possible to reduce the number of variables required for this species. *Labeo rosae* shared four of its five best predictor variables with *O. mossambicus,* i.e. DO, temperature, thermocline depth, CB, and the time since the last flood. River discharge was the fifth variable for *L. rosae* and was unique to this species as a predictor variable. Models for *L. rosae* with three variables produced equivalent models to the best model and it may be possible to reduce the number of variables required for this species to pH, centre of buoyancy, and change in dam level over the last month.

4.1.11 Comparing approaches

The two approaches provided slightly different perspectives of the drivers of the metal concentrations in the muscle tissue of the three fish species. Comparing the predictors that contributed more than 5% to the similarities for the metals in muscle tissue for the three species to the best predictors from the distance-based redundancy analysis, the same eight predictors were identified for *O. mossambicus* by both approaches. For *L. rosae*, four of the five predictors from the redundancy analysis contributed more than 5% to the similarity within the group from the regression analysis; centre of buoyancy did not contribute more than 5% to the similarity for *L. rosae*. For *S. intermedius*, only three of the five predictors from the redundancy analysis contributed more than 5% to the similarity within the group from the regression analysis; change in dam level over the last month and the time since the last flood did not contribute more than 5% to the similarity for *S. intermedius*.

Overall, because the redundancy analysis evaluates a full set of metal concentration simultaneously, while the regression analysis explores eight metals individually using generalised linear models, the redundancy analysis provides a more holistic view of the response of the metals in fish muscle tissue to the predictor variables. So, while the two approaches are complementary and provide some confirmation of important predictors of the concentration in fish muscle, the results of the redundancy analysis are considered the more robust, a basis for management recommendations.

4.1.12 Comparing species' responses

The response variable in the redundancy analysis is a distance matrix based on the concentration of the respective metals in the fish muscle tissue. A lower concentration of the respective metals would result in an overall lower distanced of the response variable and therefore predictors that have negative coefficients would indicate that an increase in the value of the variable would reduce the distance within the response variable. However, predictor variables shared between the three species' best models do not result in the change within the response variable, e.g. temperature increases the distance within the response variable for *O. mossambicus* while it decreases the response variable for *L. rosae* and *S. intermedius*; centre of buoyancy (CB) increases the distance within the response variable for *L. rosae* while it decreases the response variable for *O. mossambicus* and *S. intermedius*; while dissolved oxygen (DO) increases the distance within the response variable for *O. mossambicus* and *L. rosae* while it decreases the response variable for *S. intermedius.* Increased thermocline depth decreased the response variable for *L. rosae* but increased it for *O. mossambicus* while an increase in the change in dam level over the last month increased the response variable for both *O. mossambicus* and *S. intermedius.* An increase in the time since the last flood increased the response variable for *S. intermedius* but decreased it for *O. mossambicus*.

For *O. mossambicus*, pH, temperature and DO were the predictor variables with the largest coefficients (in absolute value) and all were negatively related to the response variable. This again emphasises the importance for the physico-chemical parameters in the more resident *O. mossambicus*. The other two species school more and are more mobile, using more of the environment and are therefore exposed to a greater variation in environmental conditions.

For *L. rosae*, DO, river discharge and CB were the predictor variables with the largest coefficients, the first two being negatively related to the response variable while the latter was positively related to the response variable. The finding that an increase in river discharge decreases the response variable is counter intuitive because it contradicts the hypothesis that increased river discharge increases the metal input into the reservoir. However, increased river discharge could result in lower metal concentrations in the detritus, the major food of *L. rosae*. This would need to be confirmed through additional research. Alternatively, the impact of river discharge may be specific to the low flow conditions that dominated the period of the study.

For *S. intermedius*, DO, temperature, change in dam level over the last month were the predictor variables with the largest coefficients and all were positively related to the response variable. An increase in dam level is indicative that the river discharge has increased in the last month and it could be inferred that this indicates an increase in the metal input into the impoundment over the last month. An increase in dam level also resulted in a positive response for *O. mossambicus* possibly confirming the hypothesis that the environmental concentrations of metals increases with the dam level. Interestingly, a drop in the dam level, indicative of reduced river discharge entering the impoundment over the month prior to the sample. This is different to the river discharge variable which was the average daily value at the time of the survey. It is expected that time required for the metals to be taken up by the fish and one would expect a delay in the response variable in relation to instantaneous higher flow conditions.

It should be remembered that correlation does not reflect causality. Therefore, the mechanisms of the predictor variable influencing the response variable need to be explored further. This work could only infer relationships based on large scale processes. Further exploration of the mechanisms is required before a detailed picture of the factors influencing the metal concentration in fish muscle are better understood. Such studies should explore longer term data sets that include both the wet and dry cycles of the Olifants River System.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The aim of this project was to evaluate seasonal fluctuations in the metal concentration in fish muscle tissue in Flag Boshielo Dam in order to establish guidelines for the safe consumption of fish from contaminated impoundments. The results of the project were expected to provide information to inform inland fisheries policy for the development of inland fisheries on South Africa's water storage impoundments, specifically to the requirement that the fish products from inland fisheries should be monitored to determine whether they are suitable for long-term human consumption.

To achieve this aim, the following objectives were accomplished through the project:

- Monitoring the seasonal fluctuations in selected water quality parameters at Flag Boshielo Dam through drought and flood cycles,
- Monitoring the seasonal fluctuations in the metal concentration in fish muscle at Flag Boshielo Dam,
- Investigating whether environmental and/or limnological factors can be identified as drivers of the concentration of metals in fish muscle tissue at Flag Boshielo Dam using linear regression and distance-based linear modelling, and
- Using the findings of the study to inform the Inland Fisheries Policy regarding the safe utilisation of fish captured from Flag Boshielo Dam for long-term human consumption.

SUMMARY OF FINDINGS

A brief literature review of the issues relating to food security showed that poverty, environmental stressors and conflict are strong drivers of food insecurity in southern Africa (Misselhorn 2005). Despite South Africa being food secure at national level, almost 21% of South African households have food insecurity (Statistics South Africa 2017b). A number of factors contribute to the environmental stressors that impact food security including global climate change (Bohle et al., 1994), water security including competition with increasing domestic, industrial, agricultural and mining demands for water, the contamination of inland water bodies, and sustainable management of utilised living resources including the conservation of rare and endangered species and ecosystems.

The Department of Agriculture, Forestry and Fisheries have proposed the utilisation of the water storage impoundments, established to ensure water security and the supply of safe water for domestic, industrial and agricultural utilisation, for the establishment of inland fisheries and aquaculture facilities (DAFF, 2019a). At the time of writing (June 2019), the second draft of the Inland Fisheries Policy was being circulated for public comment. Under the heading "Food Safety Monitoring", the second draft of the Inland Fisheries Policy (DAFF, 2019b) states:

"The DAFF will, in consultation with the departments responsible for health and trade, establish product quality and safety programmes for freshwater fisheries foods which conform, as far as possible, with

relevant local standards and requirements and, as far as possible, with international standards or requirements."

This project investigates a very specific aspect of food security; ensuring that fish captured from contaminated water are suitable for human consumption. The results of the project should inform the Inland Fisheries Policy. The study took place at Flag Boshielo Dam on the main-stem of the Olifants River, Limpopo River System, because this impoundment has been proposed to be suitable for the development of aquaculture and inland fisheries (Britz et al., 2015) while also having been identified as an impoundment where selected fish species were found to contain metal concentrations in their muscle tissue that are considered unsafe for long-term human consumption (Addo-Bediako et al., 2014a; b; Jooste et al., 2014; 2015; Marr et al., 2015; Lebepe et al., 2016; Sara et al., 2017; Sara et al., 2018).

5.1.1 Historical data from Flag Boshielo Dam

The Olifants River system appears to cycle between dry periods of low flow and wet periods with frequent high flow events. The lengths of the wet and dry cycles are variable and are driven through rainfall patterns. De Wit and Stankiewicz (2006) predict a reduction in rainfall of more than 10% over the Olifants River catchment by the end of the 21st Century. A 10% decrease in precipitation could result in a greater than 50% decrease in the runoff of the system (De Wit and Stankiewicz, 2006). Climate change predictions forecast greater stochasticity in rainfall events where the annual precipitation will be received in less frequent rainfall events of greater intensity. For a catchment already experiencing wet and dry cycles, this could mean more frequent and longer durations for the dry cycles and more intense flooding events in the wet cycles.

Analysis of historical water quality data from Flag Boshielo Dam identified three different groups of water quality parameters; parameters that are reset by high flow events and strongly correlated to each other, e.g. electrical conductivity (EC), chlorine (Cl), sodium (Na), fluoride (F), potassium (K) and alkalinity (Alk); parameters that are reset by high flow events but not strongly correlated to EC, e.g. calcium (Ca), magnesium (Mg), pH and sulphate (SO_4); and parameters present at low concentrations that are not impacted by high flow events or strongly correlated to the other parameters, e.g. ammonium (NH_4), nitrate-nitrite ratio (NO_3:NO_2) and ortho-phosphate (PO_4).

The concentration of most water quality parameters increased in the water column of the impoundment over the dry period due to evaporation of water from the impoundment. Floods resulting in river flows of more than 20 cumecs downstream of Flag Boshielo Dam appear to reset the concentrations of ions in the impoundment water column and fully mix the impoundment. This pattern of concentration followed by a reset is in contrast to the assertion of Jooste et al. (2015) who suggested that the median annual concentration of the water entering Flag Boshielo Dam is increasing at a rate of 1.32 mg/L/year.

5.1.2 Monitoring of Flag Boshielo Dam

The study mostly took place in a dry cycle of the Olifants River. The impoundment level dropped from about 60% to 20% over 2016. A rainfall event at the beginning of 2017 resulted in an increase in the impoundment level to 50% with the impoundment level remaining between 50 and 60% for 2017. The wet cycle only commenced in March 2018 near the end of the study when a large rainfall event resulted in the impoundment filling to capacity and spilling, resulting in a flow of more than 20 cumecs in the Olifants River below Flag Boshielo Dam.

Seasonal patterns were found for the water temperature, dissolved oxygen and pH of the water column in the riverine, transition and lacustrine zones of the impoundment. Data from DWS indicated that there was a chlorophyll-a bloom in the impoundment from 2017 to the end of the study. The reason for this bloom is difficult to ascertain because there was no evidence of an elevation in the ortho-phosphate levels in the water column. Sulphate concentrations exceeded the 100 mg/L threshold for aquatic ecosystem health from September 2017 to the end of the study, coinciding with periods of higher inflow into the impoundment.

No stratification of temperature, dissolved oxygen or pH was recorded in the riverine zone. Stratification in all three parameters was observed in the warmer months, October to February, for the limnetic sites, while no stratification was evident in the cooler months. The absence of the metalimnion in the cooler months is indicative of a strong water turnover.

Of the suites of metals analysed for the samples of water collected from Flag Boshielo Dam, only aluminium (Al), barium (Ba), boron (B), iron (Fe), strontium (Sr), titanium (Ti) and zinc (Zn) were detected. The highest values of the concentration of these metals were recorded in October 2016. These exceed the values recorded for Flag Boshielo Dam in 2010 by Jooste et al. (2013). However, there was no clear trend between water metal concentrations in the water and the level of the impoundment.

Sediment samples collected in the riverine, transition and lacustrine zones of the impoundment contained higher concentrations of metals and metalloids than the water samples. Aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mg), nickel (Ni), selenium (Se), strontium (Sr), tin (Sn), titanium (Ti), vanadium (V) and zinc (Zn) were detected. Compared to Jooste et al. (2013), sediment concentrations of Sb, B, Ba, Cd, Se, Sn and Zn were lower in this study than in 2010 while Ni concentrations exceed those recorded by Jooste et al. (2013). With the exception of Sb, B, Cd, Ni and Se, a general increase in sediment concentrations of metals was noted during periods of high-water inflow into the impoundment.

Muscle tissue from three species of fish identified as having fisheries potential were analysed for metals and the results of these analyses used in a US EPA developed desk-top study to determine the risk to human health of long-term consumption of these fish species. For *O. mossambicus* Al, Ba, B, Cu, Mg and Hg in the muscle tissue of were found to be higher during high inflow while As, Cr, Ni and Sn were higher during periods of low inflow. For *L. rosae* Al, Ag, As, Cd, Cr, Co, Cu, Mo, Sb, Se, Sn, V and Zn were higher during periods of low inflow. For *S. intermedius* the levels of Ag, Co and Hg in the muscle tissue were higher during periods of high inflow while As, Cr, Pb and Ni were higher during periods of low inflow. When comparing these results to the 2010 study from Flag Boshielo Dam reported by Jooste et al. (2013), the concentrations of metals in fish muscle tissue were considerably lower than the 2010 values, with some exceptions. This was a surprising outcome because the expectations were that the drought conditions prevailing for most of the study would result in increased concentrations of metals in the fish muscle tissues.

With the decreased metal concentrations in the fish muscle, the Human Health Risk Assessment found that very few metals exceeded the levels safe for long-term consumption. Hazard Quotients (HQ) greater than 1, indicative of adverse long-term health effects to humans, were only found for Co in *O. mossambicus*, As in *L. rosae* and Pb in *S. intermedius*.

5.1.3 Water chemistry in wet and dry cycles

Environmental chemical reactions are governed by temperature, pH, redox and time. At ambient temperatures, which are low in chemistry terms, the rates of reactions are reduced and consequently a considerable time taken to reach equilibrium. At low flow, the residence time of the water in the river system increases and it is possible that chemical reactions to progress further towards equilibrium before reaching Flag Boshielo Dam. In contrast, at high flows the residence time is reduced and chemical reactions may not progress as far towards equilibrium over the same spatial scale. In addition, during the low flow dry cycles, the water passes more slowly through biological filters in the catchment, such as reed beds, and the lack of scouring flows results in the reed beds extending further into the river channel thereby increasing the biological uptake of metals to aquatic vegetation. Therefore, during the low flow of the dry cycle, lower water column concentrations of metals are expected to be entering Flag Boshielo Dam while, during the high flow wet cycle, higher water column concentrations of metals are expected to be entering Flag Boshielo Dam.

During the dry cycle, most of physico-chemical parameters in the impoundment water column are concentrated. These concentrations are reset during the wet cycle by flood events that result in greater than 20 cumecs flows in the Olifants River downstream of Flag Boshielo Dam. During the wet cycle, frequent flood events maintain the impoundment at close to capacity. During the dry cycle, fluctuations in the impoundment level occur, even down to 20% of capacity. The impoundment is managed to conserve water during these low-level periods and no water is released downstream to the Olifants River.

5.1.4 Environmental drivers of fish muscle metal concentrations

In order to identify potential drivers of the metal concentrations in fish muscle tissue in Flag Boshielo Dam, multi-variate regression analyses where used to determine which drivers contributed most towards explaining the patterns of metal concentrations observed in the fish muscle tissue of the three species selected for this study. A suite of environmental and water chemistry parameters was selected for the analyses but the results of the analyses did not identify a clear set of drivers that could be used to develop model to predict metal concentrations in fish muscle tissue. Therefore, a reduced set of "limnological drivers" that could be measured in the field and did not require chemical analyses were selected: physico-chemical parameters (water temperature (°C), pH, DO% and EC), lake limnological parameters (depth of thermocline and centre of buoyancy), river discharge (at the survey time and averaged over the month prior to the survey), dam level (change in level over the month proceeding the survey) and the number of days since the last flood flow of > 20 cumecs. Two approaches were used: Generalised linear models and distance-based redundancy analysis.

Generalised linear models were developed for a reduced set of eight metals (selected based on having the highest number of non-zero metal concentration in the fish muscle: Al, Ba, Cu, Mn, Se, Sr, Ti and Zn). Generalised linear models were established for each of the eight metals for the three study species. Multi-model inference (Burnham and Anderson, 2002) was used to identify the frequency of the variables in the top models, models that differed by less than 4 AIC points from the best model, selected considering all possible models generated from the set of explanatory environmental variables. The generalised linear modelling analysis for the eight metals found that the predictor variables that contributed more than 10% to the within group similarity for *O. mossambicus* were pH, DO, EC, thermocline depth, and the time since the last flood, for *L. rosae* pH, thermocline depth, and change in dam level over the last month, with DO contributing more than 5% to the group similarity. For *S. intermedius*, more than 10% to the within group similarity was explained by pH, DO, thermocline depth, and the CB. The predictors pH, dissolved oxygen, and thermocline depth were common to the three species, and temperature and electrical conductivity consistently contributed more than 5% similarity for all three species.

Distance-based redundancy analysis (dbRDA) (Anderson et al., 2008), was conducted for the three fish species. The independent variable was a matrix of the 19 metal concentrations in the fish muscle tissue for each fish analysed and the explanatory variables were a matrix of the environmental parameters recorded during each field survey that were matched to the fish data collected. The distance-based redundancy analysis determined the environmental drivers that best describe the patterns in the independent variable matrix. The dbRDA results were plotted as an RDA plot with the importance of the variables overlain on the RDA plot. The analyses were repeated for the limnological drivers. The dbRDA found that three variables were shared between the best models of the three species: dissolved oxygen, temperature, and centre of buoyancy (CB). *Oreochromis mossambicus* had eight predictor variables for the metal concentrations in muscle tissue: pH, DO, EC, temperature, thermocline depth, CB, change in dam level over the last month, and the time since the last flood. *Schilbe intermedius* shared all its five predictor variables with *O. mossambicus*: dissolved oxygen, temperature, CB, change in dam level over the last month, and the time since the last flood. *Labeo rosae* shared four of its five predictor variables with *O. mossambicus,* i.e. DO, temperature, thermocline depth and CB, with the fifth predictor variable, river discharge, being unique to *L. rosae*.

CONCLUSIONS

Conclusions drawn from the study are discussed below.

5.1.5 Wet and dry cycles of the Olifants River

The wet and dry cycles identified for the Olifants River are important in driving the biochemical processes in the impoundments of the catchment. The bioaccumulation of metals to fish muscle tissue appears to occur during the wet cycle and elimination of metals from fish muscle appears to take place in the dry cycle. Flood events that result in flows of greater than 20 cumecs in the Olifants River below Flag Boshielo Dam were shown to play an important role in resetting the concentrations of water physico-chemical parameters in the impoundment. During the dry cycle, water physico-chemical parameters appear to concentrate in the impoundment due to evaporation. There is currently a lack of understanding of the drivers and dynamics of the wet and dry cycles. These are most likely driven by meteorological process and global climate change is most likely to change the dynamics of these meteorological processes.

5.1.6 Drivers of metal bioaccumulation

The environmental drivers that determine the metal concentrations in fish muscle tissue were found to be more complex than expected and our analyses found that all drivers evaluated could be used to describe the observed patterns in metal concentrations in fish muscle tissue. Even reducing the number of variables to variables that could be measured in the field found that all the variables were relevant. The distance-based redundancy analysis did, however, show that three variables were important for all three species evaluated, although the variable sets for the specific species were mostly unique (the variables for *S. intermedius* were a subset of those for *O. mossambicus*). The three species chosen for the study occupied different niches within the impoundment; *S. intermedius* is a pelagic piscivore, *O. mossambicus* is a resident general omnivore, and *L. rosae* is a roaming schooling detritivore. It is therefore not surprising that no common set of environmental variables was found to describe the metal bioaccumulation in the three species.

5.1.7 Human Health Risk Assessment

The desktop risk assessment found that the fish of Flag Boshielo Dam analysed in the current study posed a lower risk to human health than those analysed in previous studies of this impoundment. There were still fish that had metal concentrations that exceeded the safe limits for human consumption for certain metal, but these were the exception rather than the rule.

RECOMMENDATIONS

Fish are a vital source of food for many of the world's people, especially low-income groups (Sayer and Cassman, 2013), as they are a rich source of protein, micronutrients and essential fatty acids (Beveridge et al., 2013), cheaper than other protein sources, and available from local lakes, rivers and impoundments. However, the consumption of fish could result in detrimental health impacts when harvested from contaminated inland waters. In this section we present recommendations derived from the experience gained from completing this project and from the results of the study.

5.1.8 Combat the problem at source

Acid mine drainage seeping from abandoned mines and smouldering mine dumps in the upper Olifants River is resulting in the acidification of streams and the mobilization of metals from the sediment

(McCarthy, 2011; Netshitungulwana and Yibas, 2012). In order to minimise the metal contamination of the biota of the Olifants River mainstem and impoundments, addressing the major driver of the metal contamination source would logically be the place to start. However, although many engineering approaches and devices have been trialled to address acid mine drainage at source, no economically feasible options have been identified or implemented. Acid mine drainage is a global environmental problem, greater than metal contamination of fish in the impoundments of the Olifants River. South Africa has numerous mines that are generating acid mine drainage and the Olifants River is not the only catchment where acid mine drainage poses serious environmental risks. Research directed towards reducing the generation of acid mine drainage at source and mitigation actions to reduce the impact of acid mine drainage mobilised metals in the ecosystem are recommended.

5.1.9 More than metals

The contamination of fish muscle tissue by metals and the associated risks to human health have dominated the literature regarding the consumption of fish from the impoundments of the Olifants River. However, numerous agricultural fertilisers and pesticides are used in the Olifants River catchment and elsewhere in South Africa (Ansara-Ross et al., 2012). The potential threat that these pose to the ecological functioning of the Olifants River and its impoundments, including the potential impact to the edibility of fish, needs to be investigated. Several challenges are acknowledged when investigating pesticides in fresh waters including the exceptionally low concentrations of pesticides in fresh waters, the presence of multiple breakdown products generated by pesticides, the large number of pesticides and organic compounds used in the agricultural, domestic and industrial sectors and the expense of the analyses of samples.

5.1.10 Laboratory detection limits

The detection limits for metals in water and fish muscle samples during the laboratory analysis requires attention. The integrity of the data available for statistical analysis is compromised due to the laboratory reporting “below detection limit” or “not detected”. These are obvious limitations of the analysis technique used for the study. Laboratory analyses of metals in fresh waters usually fail to detect most metals due to the self-cleansing nature of water, where metals are precipitated and only present in low concentrations. For pesticides, the low concentrations of the pesticides, and the numerous breakdown products, provide a similar challenge. Lowering the detection limit of the analysis will obviously increase the cost of the analyses. Techniques to concentrate the contents of samples without loss of the chemical content of the sample could be investigated.

5.1.11 Metal concentration guidelines

At present, the only South African guideline for metals in meat are limited to five metals; viz. As, Cd, Pb, Hg and Sn (Department of Health, 2004). These guidelines are based on a 1972 Act and are in serious need of revision. The FAO recommended concentration limits (Nauen, 1983) are frequently cited as the basis for assessments of edibility of food stuffs and presents a summary of the concentration limits for contaminants applicable in various countries. However, other guidelines are available, notably from Australia and New Zealand (FSANZ, 2012) and the European Union

(Commission of the European Communities, 2006), but these only contain guidelines for selected metals (Table 5.1). A comparison between the South African recommended safe limits for metals/metalloids in fish for human consumption are presented in Table 5.1, which has been augmented with international values from the FAO (Nauen, 1983), the European Union (Commission of the European Communities, 2006) and Australia and New Zealand (FSANZ, 2012).

Table 5.1: The Comparison of safe limits for metals/metalloids in fish for human consumption for South Africa (Department of Health, 2004) and selected other countries.

	South Africa	International	References
Al			
Sb		Australia 1mg kg^{-1}	FSANZ (2012)
As	3 mg kg^{-1}	Australia 1 mg kg^{-1}	Nauen (1983)
Ba			
B			
Cd	1 mg kg^{-1}	European Union 0.05 mg kg^{-1}	Commission of the European Communities (2006)
Cr		USA (value for shellfish) 12 mg kg^{-1}	Food and Drug Administration (2009)
Co			
Cu		Australia 10 mg kg^{-1}	Nauen (1983)
Fe			
Pb	0.5 mg kg^{-1}	European Union 0.3 mg kg^{-1}	Commission of the European Communities (2006)
Mn			
Hg	0.5 mg kg^{-1}	European Union 0.5 mg kg^{-1} [1]Australia 0.5 mg kg^{-1}	Commission of the European Communities (2006); FSANZ (2012)
Ni		USA (value for shellfish) 40 mg kg^{-1}	Food and Drug Administration (2009)
Se		Australia 1 mg kg^{-1}	Nauen (1983)
Ag			
Sr			
Sn*	50 mg kg^{-1}	Australia 50 mg kg^{-1}	Nauen (1983)
V			
Zn		Australia 150 mg kg^{-1}	Nauen (1983)

* Note: The South African and Australian levels for tin apply to uncanned meat and meat products (Nauen, 1983; Department of Health, 2004)

The US EPA based desktop risk assessment used in this report uses a reference dose to determine whether the food portion exceeds the levels for safe consumption (US-EPA, 2000). The reference doses used in this study were sources from the Integrated Risk Information System database managed by the US EPA (US-EPA, 2013). From the reference doses, it is easy to back calculate the acceptable levels for metal concentration in meat or fish muscle using the assumptions upon which the desktop risk assessments are based. Where no safe consumption limit is currently available for a specific metal in

South Africa, a temporary value could be calculated from the US EPA reference dose values and revised when such values are generated by the Department of Health.

5.1.12 Community Risk Assessment

The current risk assessment is based on a desktop study based on assumptions of the average portion size, frequency of fish meals and the adult body mass as recommended for South Africa by Heath et al. (2004b). These assumptions should be verified through social science surveys of the communities consuming fish from Flag Boshielo Dam using questionnaires. Once the values of the average portion size, frequency of fish meals and the adult body mass have been verified, a human health risk assessment should be completed for the fish species evaluated in this study.

In addition, a medical survey of the communities consuming fish from Flag Boshielo Dam should be undertaken, using data from local clinics and questionnaires, to determine whether the adverse health effects of metals can be identified in the communities. In addition, blood samples of community members who regularly consume fish from the impoundment should be screened to determine whether there are traces of metals. Finally, a nutritional assessment using questionnaires should be conducted to determine the importance of fish in the diet of the communities surrounding Flag Boshielo Dam.

5.1.13 Continued monitoring of fish muscle metal content

The results of the current study showed that there were distinct wet and dry cycles in the Olifants River catchment. Previous studies conducted during the wet cycle reported alarming levels of certain metals in the muscle tissue of fish from Flag Boshielo Dam. The current study conducted mostly during a dry cycle did not find the same levels of metals in fish muscle tissue. Greater understanding of the drivers of the metal concentrations in fish muscle tissue is required in order to fully inform the management of inland fisheries in Flag Boshielo Dam. Further studies are required to elucidate the relationships between environmental or limnological drivers and the dynamics of metal concentrations in fish muscle tissue. It would be ideal if the dynamics of metal concentrations in in fish muscle can be modelled to provide DAFF a tool to predict when the fish from the impoundment are safe for long-term human consumption.

5.1.14 New modelling approaches

Rivers around the world are being degraded as ecosystems under increasing demands for fresh water and changing weather patterns (Vörösmarty et al., 2010; Tonkin et al., 2019). In a recent paper in Nature, Tonkin et al. (2019) argue that ongoing human development and climate change mean that conventional management techniques that aim to restore ecosystems to their original state are no longer feasible. The authors call for a different approach because models based on past correlations are poor at predicting how species might respond to unprecedented changes in future. Tonkin et al. (2019) recommend that rivers be managed adaptively to enhancing their resilience, maintain water supplies and limit the risk of devastating population crashes; e.g. mass fish mortalities. The use of models is put forward to project how key species, life stages and ecosystems might respond to environmental changes, requiring a move beyond simply monitoring the state of ecosystems to the modelling of the biological mechanisms underpinning the survival of the aquatic biota within these ecosystems. Four steps are recommended by Tonkin et al. (2019) for the establishment of these models: data collection should focus on mechanisms; key processes should be described; management of bottlenecks should be concentrated upon; and uncertainties should be explicitly evident.

South Africa has a rich history of developing models for predicting the impacts of changes in flows on aquatic biota and ecosystems: e.g. DRIFT (King et al., 2004) and the Building Block Methodology (King et al., 2008). While these models rely heavily on expert opinion, the recommendations by Tonkin et al. (2019) encourage researchers to upgrade the expert opinion to laboratory and field-based models that have been verified using scientific methods. The work presented in this report represents an attempt to identify variables that could be incorporated in models to describe the metal concentration in fish muscle tissue. While the results of this study have identified a suite of suitable variables, the study was not able to reduce the suite of variables. Further research is required to continue the work initiated in this project to develop models with the capacity to predict the concentrations of metals in the muscle tissue of fishes in Flag Boshielo Dam. These models can then be added to models of fish population and projected exploitation in a Building Block of DRIFT type model to help the Department of Agriculture, Forestry and Fisheries (DAFF), the Department of Health and other trade organisations to regulate the harvesting effort in Flag Boshielo Dam such that the fish are safe for human consumption.

5.1.15 Expanding the Department of Water and Sanitation (DWS) monitoring

The DWS runs a water quality monitoring program that samples a suite of water quality parameters at monitoring sites across South Africa. The current monitoring program includes chemical analyses of nutrients, alkali and alkali earth metals, and major anions important in describing freshwater ecosystems. Metals are currently not reported in the monitoring program. Expanding the monitoring to include metals, especially in catchments where metal pollution has historically been present, would provide early warning signals to warm fisheries managers of potential bioaccumulation of metals into fish muscle tissue. Such databases would also provide researchers with tools to evaluate long-term trends in metal pollution in these catchments. Not all metals need to be included in such a monitoring programme, just the metals that have been identified in risk assessment for their potential risk to human health and more abundant metals that could be used as controls or indicators of disturbances.

5.1.16 Inland Fisheries Management

Inland fisheries should be managed sustainably. A good understanding of the dynamics of the fish populations designated for exploitation is required for sustainable and effective management of these populations. Understanding of the recruitment to the populations, the cues for spawning (or spawning migrations) and the impact of the water level in the impoundment on recruitment are required when establishing exploitation rates. The dynamics of recruitment and population size during wet and dry cycles identified in the current study is required, e.g. *Clarias gariepinus* and *Labeo rosae* spawn over flooded grasses and without flooded grasses available, it is unlikely that either of these species would recruit that year. During the dry cycles, exploitation rates may need to be adjusted to compensate for the lower levels of the impoundment which may concentrate the fish populations and increase catch per unit effort in comparison to during the higher impoundment levels of the wet cycle. In addition, the conservation of rare and endangered species should also be carefully managed and included in the fishery management guidelines from the establishment of the fishery. The presence of crocodiles at Flag Boshielo Dam should be managed such that crocodiles do not become a conflict species between the conservation and fishery users of the impoundment.

The size and participation in the existing fishery at Flag Boshielo Dam need to be determined, including current catch rates for the species exploited. Prioritisation of the existing fishery should be evident when the possible expansion of the fishery is considered.

5.1.17 Inland Fisheries Policy

The DAFF should be applauded for including food safety monitoring in the draft Inland Fisheries Policy. This inclusion has been a direct result of the work of researchers at the University of Limpopo's Biodiversity Department who have highlighted the potential risk to human health posed by consuming contaminated fish from impoundments in the Olifants River catchment, in particular Flag Boshielo Dam. Having committed to including food safety monitoring in the inland fisheries policy, the DAFF needs to develop methodologies to implement food safety monitoring. While it would be advantageous to have a model to predict when the fish from an impoundment are safe for human consumption, this project has demonstrated that this is a far from trivial exercise. Further projects developed from the findings of this project will be required before the dynamics of metal bioaccumulations in fish muscle tissue is beginning to be conceptually understood.

Therefore, in order to implement the food security monitoring, the DAFF will require direct sampling of the fish captured from the impoundments where inland fisheries are developed. The sample collection and analytical procedures can follow those used in this and similar projects conducted by the University of Limpopo. However, clear guidelines for the limits to metal concentrations need to be developed before the food safety monitoring program commences. In particular, these guidelines are required for metals and metalloids already highlighted in recent studies of the edibility of fish from the impoundments of the Olifants River for which no guidelines are currently available for South Africa; e.g. antimony, cobalt, chromium and vanadium.

5.1.18 Fishing at Flag Boshielo Dam

The DAFF has proposed Flag Boshielo Dam as a potential site for the establishment of an inland fishery and aquaculture ventures. However, the results of the current study, and other studies referenced in this report, have shown that there is a risk to human health of metal toxicity associated with the long-term consumption of fish from this impoundment. These studies have also shown that the risk to human health varies in time and appears to increase in the wet cycle of the Olifants River system and decrease during the dry cycle. Understanding of this dynamic is embryonic and requires further research. However, based on the available evidence, we believe that Flag Boshielo Dam in not suitable for the establishment of inland fisheries or aquaculture ventures due to the health risks posed by long-term consumption of metal contaminated fish.

The current utilisation of fish from the impoundment by subsistence fishers, however, poses a complex problem that requires a solution. Subsistence fishers currently capture fish from the impoundment for personal consumption and as an income source. Fish captured from the impoundment are sold locally, with anecdotal information suggesting that fish are sold as far away as Johannesburg and Pretoria. The current informal subsistence fishery is largely unquantified, but could provide an important source of income and protein for the local rural community. Daily consumption of fish from the impoundment should be strongly discouraged as this would pose a significant long-term health risk to the consumers. Even weekly consumption of fish should be discouraged. However, discouraging regular consumption of fish from the impoundment would counteract the benefit of utilising fish as a protein supplement in protein deficient communities. The communities should be informed of the long-term health risks resulting from regular consumption of fish from the impoundment and regular monitoring of the metal concentrations in the fish muscle tissue should take place to generate advisories on fish consumption to reduce the risks to long-term human health. Advisories could include recommendations on fish consumption frequency based on specified portion sizes.

REFERENCES

ABELL R, THIEME ML, REVENGA C, BRYER M, KOTTELAT M, BOGUTSKAYA N, COAD B, MANDRAK N, BALDERAS SC, BUSSING W and STIASSNY ML (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience* 58(5):.403-414.

ADAMS WJ, CONRAD B, ETHER G, BRIX KV, PAQUIM PR and DI TORO D (2000) The challenges of hazard identification and classification of insoluble metals and metal substances for the aquatic environment. *Human and Ecological Risk Assessment* 6: 1019-1038.

ADDO-BEDIAKO A, MARR SM, JOOSTE A and LUUS-POWELL WJ (2014a) Are metals in the muscle tissue of Mozambique tilapia a threat to human health? A case study of two impoundments in the Olifants River, Limpopo province, South Africa. *International Journal of Limnology* 50: 201-210.

ADDO-BEDIAKO A, MARR SM, JOOSTE A and LUUS-POWELL WJ (2014b) Human health risk assessment for silver catfish *Schilbe intermedius* Ruppell, 1832, from two impoundments in the Olifants River, Limpopo, South Africa. *Water SA* 40: 607-614.

AKCIL A and KOLDAS S (2006) Acid mine drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production* 14: 1139-1145.

ANSARA-ROSS TM, WEPENER V, VAN DEN BRINK PJ and ROSS MJ (2012) Pesticides in South African fresh waters. *African Journal of Aquatic Science* 37: 1-16.

ANDERSON MJ (2001) A new method for non-parametric multivariate analysis of variance. *Australia Ecology* 26: 32-46.

ANDERSON MJ, GORLEY RN and CLARKE KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods, PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK.

APHANE V and VERMEULEN PD (2015) Acid mine drainage and its potential impact on the water resources in the Waterberg coalfield, South Africa. *South African Journal of Geology* 118(1): 55-70.

ARAIN MB, KAZI TG, JAMALI MK, JALBANI N, AFRIDI HI and SHAH A (2008) Total dissolved and bioavailable elements in water and sediment samples and their accumulation in *Oreochromis mossambicus* of polluted Manchar Lake. *Chemosphere* 70: 1845-1856.

ASHRAF MA, MAAH MJ and YUSOFF I (2012) Bioaccumulation of heavy metals in fish species collected from former tin mining catchment. *International Journal of Environmental Research* 6: 209-218.

ASHTON PJ (2010) The demise of the Nile crocodile (*Crocodylus niloticus*) as a keystone species for aquatic ecosystem conservation in South Africa: The case of the Olifants River. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 489-493.

ASHTON PJ and DABROWSKI JM (2011) *An overview of water quality and the causes of poor water quality in the Olifants River Catchment*, WRC Project No. K8/887. Water Research Commission, Pretoria.

ATSDR (2004a) Toxicological profile for cobalt, Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/index.asp, Atlanta, GA.

ATSDR (2004b) Toxicological profile for strontium, Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/index.asp, Atlanta, GA.

ATSDR (2007a) Toxicological profile for arsenic, Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/index.asp, Atlanta, GA.

ATSDR (2007b) Toxicological profile for lead, Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/index.asp, Atlanta, GA.

AZEEZ OI, MYBURGH JG, MEINTJES RA, OOSTHUIZEN MC and CHAMUNORWA JP (2017) Histomorphology, ultrastructure and fatty acid composition of the adipose tissue in pansteatitis, the potentials in understanding the underlying mechanism and diagnosis of pansteatitis in the Nile crocodile. *Lipids in health and disease* 16(1):1-47.

BALLANCE A, HILL L, ROUX D, SILBERBAUER M and STRYDOM WF (2001) *Crocodile, Sabie-Sand and Olifants River Systems. River Health Programme (RHP)*, WRC Report No.TT 147/01. Water Research Commission, Pretoria, South Africa.

BARTON K (2018) MuMIn: Multi-model inference. R Package version 1.42.1. http://R-Forge.R-project.org/projects/mumin/.

BARTRAM J, BALLANCE R and WORLD HEALTH ORGANIZATION (1996) Water quality monitoring: a practical guide to the design and implementation of freshwater quality studies and monitoring programs. 348 pp.

BEVERIDGE MCM, THILSTED SH, PHILLIPS MJ, METIAN M, TROELL M and HALL SJ (2013) Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *Journal of Fish Biology* 83: 1067-1084.

BERVOETS L and BLUST R (2003) Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. *Environmental Pollution* 26: 9-19.

BERVOETS L, BLUST R and VERHEYEN R (2001) Accumulation of metals in the tissues of three spined stickelback (*Gasterosteus aculeatus*) from natural fresh waters. *Ecotoxicology and Environmental Safety* 48: 117-127.

BEVERIDGE MCM, THILSTED SH, PHILLIPS MJ, METIAN M, TROELL M and HALL SJ (2013) Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *Journal of Fish Biology* 83: 1067-1084.

BOHLE HG, DOWNING TE and WATTS MJ (1994) Climate change and social vulnerability: toward a sociology and geography of food insecurity. *Global Environmental Change* 4: 37-48.

BOKO M, NIANG I, NYONG A, VOGEL C, GITHEKO A, MEDANY M, OSMAN-ELASHA B, TABO R and YANDA P (2007) Africa Climate Change 2007: Impacts, adaptation and vulnerability. in PARRY ML, CANZIANI OF, PALUTIKOF JP, VAN DER LINDEN PJ and HANSON CE (eds), *Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change,* Cambridge University Press, Cambridge UK, pp. 433-467.

BOLLMOHR S, THWALA M, JOOSTE S and HAVEMANN A (2008) *An assessment of agricultural pesticides in the Upper Olifants River Catchment*, Report No. N/0000/REQ0801. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, South Africa.

BOTHA PJ (2010) The distribution, conservation status and blood biochemistry of Nile crocodiles in the Olifants River System, Mpumalanga, South Africa, PhD thesis, University of Pretoria, Pretoria.

BOTHA H, VAN HOVEN W and GUILETTE LJ (2011) The decline of the Nile crocodile population in Loskop Dam, Olifants River, South Africa. *Water SA* 37: 103-108.

BRITS DL (2006) Distribution and status of the silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) in the Flag Boshielo Dam (Arabie Dam) and its influence on the ecology of the Dam. Unpublished PhD thesis, University of Limpopo, Sovenga, South Africa. pp 253.

BRITZ P (2015) The history of South African inland fisheries policy with governance recommendations for the democratic era. *Water SA* 41: 624-632.

BRITZ PJ, HARA MM, WEYL OLF, TAPELA BN and ROUHANI QA (2015) *Scoping study on the development and sustainable utilisation of inland fisheries in South Africa. Volume 1: Research Report*. WRC Report No. TT 615/1/14, Water Research Commission, Pretoria. 272pp.

BURNHAM KP and ANDERSON DR (2002) Model Selection and Multimodel Inference – A Practical Information-Theoretic Approach. Second edition. Springer, New York. 488 pp.

CANLI M and ATLI G (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental pollution* 121: 129-136.

CESSFORD F and BURKE J (2005) National State of the Environment Project. Inland Water. Background Research Paper Produced for the South Africa Environment Outlook Report: Inland Water. Department of Environmental Affairs and Tourism, Pretoria, South Africa.

CHAPMAN PM (1996) Hazard identification, hazard classification and risk assessment for metals and metal compounds in the aquatic environment. *International Council on Metals and the Environment*. Ottawa, Canada.

CHAPMAN PM and WANG F (2000) Issues in ecological risk assessment of inorganic metals and metalloids. *Human and Ecological Risk Assessment* 6: 965-988.

CHEN CY, STEMBERGER RS, KLAUE B, BLUM JD, PICKHARDT PC and FOLT CL (2000) Accumulation of heavy metals in food web components across a gradient of lakes. *Limnology and Oceanography* 45: 1525-1536.

CLAASSEN M (2005) *Olifants River water resources development project; environmental impact assessment (12/12/20/553) – Water quality assessment.*, Department of Water Affairs and Forestry (DWAF) and CSIR Environmentek Report no.: P WMA 04/B50/00/3104.

CLARKE KR and GORLEY RN (2006) PRIMER v6: User Manual/Tutorial, PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK.

CLARKE KR and WARWICK RM (2001) Change in marine communities: An approach to statistical analysis and interpretation, 2nd edition, PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK.

CODD GA (2000) Cyanobacterial toxins, the perfection of water quality, and the prioritisation of eutrophication control. *Ecological engineering* 16: 51-60.

COETZEE L, DU PREEZ HH and VAN VUREN JHJ (2002) Metal concentrations in *Clarias gariepinus* and *Labeo umbratus* from the Olifants and Klein Olifants River, Mpumalanga, South Africa: zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. *Water SA* 28: 433-448.

COMMISSION OF THE EUROPEAN COMMUNITIES (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, Brussels, 19 December 2006.

COUTURE P and PYLE G (2011) Field studies on metal accumulation and effects in fish. in WOOD CM, FARRELL AP and COLIN JB (eds), *Fish Physiology*, Academic Press, pp 417-473.

CRAFFORD D and AVENANT-OLDEWAGE A (2010) Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (sharptooth catfish) from the Vaal River system – strontium, aluminium, lead and nickel. *Water SA* 36: 621-640.

DABROWSKI, JM and DE KLERK, LP (2013) An assessment of the impact of different land use activities on water quality in the upper Olifants River catchment. *Water SA* 39(2): 231-244.

DABROWSKI J, OBERHOLSTER PJ and DABROWSKI JM (2014) Water quality of Flag Boshielo Dam, Olifants River, South Africa: Historical trends and the impact of drought. *Water SA* 40: 345-357.

DABROWSKI J, BALDWIN DS, DABROWSKI JM, HILL L and SHADUNG J (2017) Impact of temporary desiccation on the mobility of nutrients and metals from sediments of Loskop Reservoir, Olifants River. *Water SA* 43(1): 7-16.

DAFF (2018) *National Inland Fisheries Policy Framework for South Africa: 1st Draft*. Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa.

DAFF (2019a) *Developing Inland Fishing key to Food Security and Marine Conservation*. Available at: https://www.daff.gov.za/daffweb3/Home/aid/161 (accessed 20/06 /2019).

DAFF (2019b) *National Freshwater (Inland) Wild Capture Fisheries Policy for South Africa: 2nd Draft*. Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa.

DALLAS HF and DAY JA (2004) *The effect of water quality variables on aquatic ecosystems: A review*, WRC Report No. TT224/04. Water Research Commission, Pretoria, South Africa.

DALLINGER R, PROSI F, SEGNER H and BACK H (1987) Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia* 73: 91-98.

DAVIES BR and DAY JA (1998) *Vanishing waters*, University of Cape Town Press, South Africa.

DAVIES BR, O'KEEFFE JH and SNADDON CD (1993) *A synthesis of ecological functioning, conservation and management of South African river ecosystems*, Water Research Commission Report TT62/93. Water Research Commission, Pretoria, South Africa.

DAVIES OA, ALLISON ME and UYI HS (2006) Bioaccumulation of heavy metals in water, sediment and periwinkle (*Tympanotonus fuscatus* var *radula*) from the Elchi Creek, Niger Delta. *African Journal of Biotechnology* 5(10): 968-973.

DAY JA and KING JM (1995) Geographical patterns, and their origins, in the dominance of major ions in South African rivers. *South African Journal of Science* 91: 299-306.

DE KLERK AR, DE KLERK LP, CHAMIER J and WEPENER V (2012) Seasonal variations of water and sediment quality parameters in endorheic reed pans on the Mpumalanga Highveld. *Water SA* 38(5): 663-672.

DEPARTMENT OF HEALTH (2004) Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). Regulation R.500 30 April 2004. *Regulation relating to maximum levels for metals in foodstuffs*. Government Gazette No. 26279, 30 April 2004, Pretoria, South Africa.

DWAF (1996a) *South African Water Quality Guidelines, Volume 1: Domestic Use* (second edition), Department of Water Affairs and Forestry, Pretoria. South Africa.

DWAF (1996b) *South African Water Quality Guidelines, Volume 3: Industrial Use* (second edition), Department of Water Affairs and Forestry, Pretoria. South Africa.

DWAF (2005) *Olifants River Water Resources Development Project. Environmental Impact Assessment*, Final Environmental Impact Report No 90. Department of Water Affairs and Forestry, Pretoria, South Africa.

DE VILLIERS S and THIART C (2007) The nutrient status of South African rivers: Concentrations, trends and fluxes from the 1970s to 2005. *South African Journal of Science* 103: 343-349.

DE VILLIERS S and MKWELO ST (2009) Has the monitoring failed the Olifants River, Mpumalanga? *Water SA* 35: 671-676.

DE WIT M and STANKIEWICZ J (2006) Changes in surface water supply across Africa with predicted climate change. *Science* 311: 1917-1921.

DI TORO DM, KAVVADAS CD, MATHEW R, PAQUIN PR and WINFIELD RP (2001) *The persistence and availability of metals in aquatic environments*. International Council on Metals and the Environment, Ottawa, Canada.

DRIESCHER AC (2008) A water quality study of Loskop Dam and the upper catchment of the Olifants River. Unpublished M.Sc Dissertation, University of the Free State, Bloemfontein, South Africa. 150 pp.

DUNZ AR and SCHLIEWEN UK (2013) Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "Tilapia". Molecular Phylogenetics and Evolution 68(1): 64-80.

DU PREEZ HH, HEATH RGM, SANDHAM LA and GENTHE B (2003) Methodology for the assessment of human health risks associated with the consumption of chemical contaminated freshwater fish in South Africa. *Water SA* 29: 69-90.

EISLER R (1990) Boron Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. *U.S. Fish Wildlife Service*, Biological Report 85: 20 pp.

FISCHER G, SHAH M, TUBIELLO FN and VAN VELHUIZEN H (2005) Socio-economic and climate change impacts on agriculture. *Philosophical Transactions of the Royal Society B* 360: 2067-2083.

FOOD AND DRUG ADMINISTRATION (2009) *Guide for the control of molluscan shellfish. 2007 Revision*, National Shellfish Sanitation Program, Interstate Shellfish Sanitation Conference and US Food and Drug Administration. U.S. Department of Health and Human Services, Public Health Service, Office of Seafood (HFS-416), 200 C Street, SW, Washington, D.C. 20204.

FROESE R and PAULY D (2010) FishBase 2010: concepts, design and data sources. In, *International Center for Living Aquatic Resource Management*, Manila. www.fishbase.org. 344 pp.

FSANZ (2012) *Australia New Zealand Food Standards Code – Standard 1.4.1 – Contaminants and Natural Toxicants: F2011C00542*, Food Standards Australia New Zealand.

GOSWAMI P, KAUSHIK U, DAMOR S, SHARMA P and SHARMA N (2016) Effects of cadmium chloride on biochemical profile and enzyme activity in Tilapia mossambica. *International Journal of Pharma Research and Health Sciences* 4(6): 1462-1465.

HARA MM and BACKEBERG GR (2014) An institutional approach for developing South African inland freshwater fisheries for improved food security and rural livelihoods. *Water SA* 40: 277-286.

HASENMUELLER EA and CRISS RE (2013) Multiple sources of boron in urban surface waters and ground waters. *Science of the Total Environment* 447: 235-247.

HEATH AG (1995) *Water pollution and fish physiology. 2nd Edition*, CRC Press. Taylor and Francis Group, Boca Raton, Florida, USA.

HEATH RGM, DU PREEZ HH, GENTHE B and AVENANT-OLDEWAGE A (2004a) *Freshwater fish and human health. Overview guide*, WRC Report No.TT213/04. Water Research Commission, Pretoria, South Africa.

HEATH RGM, DU PREEZ HH, GENTHE B and AVENANT-OLDEWAGE A (2004b) *Freshwater fish and human health. Reference guide*, WRC Report No.TT212/04. Water Research Commission, Pretoria, South Africa.

HEATH RGM and CLAASSEN MC (1999) *An overview of the pesticide and metal levels present in populations of the larger indigenous fish species of selected South African Rivers*, WRC Report No. 428/1/99. Water Research Commission, Pretoria, South Africa.

HEATH R, COLEMAN T and ENGELBRECHT J (2010) *Water quality overview and literature review of the ecology of the Olifants River*, WRC Report No.TT452/10. Water Research Commission, Pretoria, South Africa.

HOBBS P, OELOFSE SH, and RASCHER J (2008) Management of environmental impacts from coal mining in the upper Olifants River catchment as a function of age and scale. *International Journal of Water Resources Development* 24(3): 417-431.

HUCHZERMEYER KDA, GOVENDER D, PEINAAR DJ and DEACON AR (2011) Steatitis in wild sharptooth catfish, *Clarias gariepinus* (Burchell), in the Olifants and Lower Letaba Rivers in the Kruger National Park, South Africa. *Journal of Fish Diseases* 34: 489-498.

HUCHZERMEYER KDA, WOODBORNE S, OSTHOFF G, HUGO A, HOFFMAN AC, KAISER H, STEYL JCA and MYBURGH JG (2017) Pansteatitis in polluted Olifants River impoundments: nutritional perspective on fish in a eutrophic lake, Lake Loskop, South Africa. *Journal of Fish Diseases* 40(11): 1665-1680.

IARC (2012) Agents classified by the IARC Monographs. Volumes 1-105. In, *International Agency for Research on Cancer*. IARC, Lyon, France.

IRWIN R J, VAN MOUWERIK M, STEVENS L, SEESE MD and BASHAM W (1997) Environmental Contaminants Encyclopaedia: Vanadium entry. *National Park Service, Water Resources Division*. Fort Collins, Colorado. 30pp.

JEZIERSKA B and WITESKA M (2006) The metal uptake and accumulation in fish living in polluted waters. In: Twardowska I, Allen HE, Häggblom MM, Stefaniak S (eds) *Soil and Water Pollution Monitoring, Protection and Remediation*. Springer, Krakow, Polanda, pp 107-114

JOOSTE A, LUUS-POWELL WJ and ADDO-BEDIAKO A (2013) *The impact of water and sediment quality on the health of fish and the diversity of fish parasites in two impoundments of the Olifants River, Limpopo province.* WRC Project No. K5/1929. Water Research Commission, Pretoria. 189pp.

JOOSTE A, MARR SM, ADDO-BEDIAKO A and LUUS-POWELL WJ (2014) "Metal bioaccumulation in the fish of the Olifants River, Limpopo province, South Africa, and the associated human health risk: A case study of rednose labeo *Labeo rosae* from two impoundments." *African Journal of Aquatic Science* 39: 271-277.

JOOSTE A, MARR SM, ADDO-BEDIAKO A and LUUS-POWELL WJ (2015) Sharptooth catfish shows its metal: a case study of metal contamination at two impoundments in the Olifants River, Limpopo river system, South Africa. *Ecotoxicology and Environmental Safety* 112: 96-104.

KALAY M and CANLI M. 2000. Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissues of a freshwater fish *Tilapia zilli*. *Turkish Journal of Zoology* 24: 429-436.

KING JM, BROWN CA, PAXTON BR and FEBRUARY RJ (2004) *Development of DRIFT, a scenario-based methodology for environmental flow assessments*. WRC Report No: 1159/1/04, Water Research Commission, Pretoria. 159 pp.

KING JM, THARME RE and DE VILLIERS MS (2008) *Environmental flow assessments for rivers: manual for the Building Block Methodology*. WRC Report No: TT 354/08, Water Research Commission, Pretoria. 340 pp.

KOTZE P, DU PREEZ HH and VAN VUREN JHJ (1999) Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepiunus*, from the Olifants River, Mpumalanga, South Africa. *Water SA* 25(1): 99-110.

LEBEPE J, MARR SM and LUUS-POWELL WJ (2016) Metal contamination and human health risk associated with the consumption of *Labeo rosae* from the Olifants River system. *African Journal of Aquatic Science* 41: 161-170.

LENNTECH. 2009. Elements of the Periodic Table. Online at http//.www.lenntech.com accessed on October 2018.

LINNIK PN and ZHEZHERYA VA (2015) Titanium in natural surface waters: the content and coexisting forms. *Russian Journal of General Chemistry* 85(13): 2908-2920.

LUOMA SN and RAINBOW PS (2005) Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environmental Science & Technology* 39: 1921-1931.

MAAKE PA, GON O and SWARTZ ER (2014). "Descriptions of three new species of *Marcusenius* Gill, 1862 (Teleostei: Mormyridae) from South Africa and Mozambique. *Zootaxa* 3780(3): 455-480.

MARR SM, JOOSTE A, ADDO-BEDIAKO A and LUUS-POWELL WJ (2015) Are catfish from metal polluted impoundments in the Olifants River, South Africa, safe for human consumption? *Inland Waters* 5: 215-223.

MARR SM, LEBEPE J and LUUS-POWELL WJ (2017) Bioaccumulation of selected metals in the gill, liver and muscle tissue of rednose labeo Labeo rosae from two impoundments on the Olifants River, Limpopo river system, *South Africa. African Journal of Aquatic Science* 42: 123-130.

MAHLATJI ML (2014) The impact of water and sediment quality on the health of *Clarias gariepinus* (Burchell 1822) and *Labeo rosae* (Steindachner, 1894) at the Phalaborwa Barrage, Olifants River, Limpopo Province. Unpublished PhD thesis, University of Limpopo, Sovenga, South Africa. pp 110.

MANASRAH R (2002) The general circulation and water masses characteristics in the Gulf of Aqaba and northern Red Sea. *Meereswissenschaftliche Berichte* (Marine Science Report) 50: 120 pp.

MANASRAH R, RAHEED M and BADRAN MI (2006) Relationships between water temperature, nutrients and dissolved oxygen in the northern Gulf of Aqaba, Red Sea. *Oceanologia* 48(2): 237-257.

MCCAFFERTY JR, ELLENDER BR, WEYL OLF and BRITZ PJ (2012) The use of water resources for inland fisheries in South Africa: review. *Water SA* 38: 327-343.

MCCARTHY TS (2011) The impact of acid mine drainage in South Africa. *South African Journal of Science* 107: 1-7.

MISSELHORN AA. (2005) What drives food insecurity in southern Africa? A meta-analysis of household economy studies. *Global Environmental Change* 15: 33-43.

MMUALEFE LC and TORTO N (2011) Water quality in the Okavango Delta. *Water SA* 37(3): 411-418.

NARE L, ODIYO JO, FRANCIS J and POTGIETER N (2011) Framework for effective community participation in water quality management in Luvhuvhu catchment of South Africa. *Physics and Chemistry of the Earth* 36: 1063-1070.

NATIONAL PLANNING COMMISSION (2013) *Our future – make it work. National Development Plan 2030. Executive Summary*. Department of the Presidency, Republic of South Africa, Pretoria. 70 pp.

NETSHITUNGULWANA R and YIBAS B (2012) Stream sediment geochemistry of the Olifants catchment, South Africa: Implication for acid mine drainage. In: McCullough CD, Lund MA, Wyse L (eds) *Proceedings of the International Mine Water Association Symposium. 29 September-4 October 2012*, International Mine Water Association, Bunbury, Australia, pp 257-264.

NOMQUPHU W, BRAUNE E and MITCHELL S (2007) The changing water resources monitoring environment in South Africa. *South African Journal of Science* 103: 306-310.

OBERHOLSTER PJ, MYBURGH JG, ASHTON PJ and BOTHA A-M (2010) Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. *Ecotoxicology and Environmental Safety* 73: 326-335.

OBERHOLSTER PJ, MYBURGH JG, ASHTON PJ, COETZEE JJ and BOTHA A-M (2012) Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. *Ecotoxicology and Environmental Safety* 75: 134-141.

OBERHOLSTER PJ, BOTHA A-M, HILL L and STRYDOM WF (2017) River catchment responses to anthropogenic acidification in relationship with sewage effluent: an ecotoxicology screening application. *Chemosphere* 189: 407-417.

OSMAN AGM and KLOAS W (2010) Water quality and heavy metal monitoring in water, sediments, and tissues of the African catfish *Clarias gariepinus* (Burchell, 1822) from the river Nile, Egypt. *Journal of Environmental Protection* 1: 389-400.

OLSSON PE, KLING P and HOGSTRAND C (1998) Mechanisms of heavy metal accumulation and toxicity in fish. In: *Metal metabolism in aquatic environments*. Springer, Boston, MA, USA. pp. 321-350.

PHALA BM (2019) Establishing biological and environmental drivers that influence the health assessment index as a biomonitoring tool. Unpublished M.Sc Dissertation, University of Limpopo, Sovenga, South Africa.

PARAMETRIX INC. (1995) Persistence, bioaccumulation and toxicity of metals and metal compounds. *International Council on Metals and the Environment*. Ottawa, Canada.

POTASZNIK A and SZYMCZYK S (2015) Magnesium and calcium concentrations in the surface water and bottom deposits of a river-lake system. *Journal of Elementology* 20(3): 677-692.

R DEVELOPMENT CORE TEAM (2018) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

RIDDIN MA, BILLS IR and VILLET MH (2016) Phylogeographic, morphometric and taxonomic re-evaluation of the river sardine, *Mesobola brevianalis* (Boulenger, 1908) (Teleostei, Cyprinidae, Chedrini). *ZooKeys* 641: 121.

RYDER RA (1982) The morphoedaphic index – use, abuse, and fundamental concepts. *Transactions of the American Fisheries Society* 111: 154-164.

RYDER RA, KERR SR, LOFTUS KH and REGIER HA (1974) The morphoedaphic index, a fish yield estimator—review and evaluation. *Journal of the Fisheries Board of Canada* 31: 663-688.

SARA JR, SMIT WJ, ERASMUS LJC, RAMALEPE TP, MOGASHOA ME, RAPHAHLELO ME, THERON J and LUUS-POWELL WJ (2014) Ecological status of Hout River Dam, Limpopo province, South Africa, using fish condition and health assessment index protocols: a preliminary investigation. *African Journal of Aquatic Science* 39(1): 35-43.

SARA JR, MARR SM, SMIT WJ, ERASMUS LJC and LUUS-POWELL WJ (2017a) Human health risks of metals and metalloids in muscle tissue of *Synodontis zambezensis* Peters, 1852 from Flag Boshielo Dam, South Africa. *African Journal of Aquatic Science* 42: 287-291.

SARA JR, WEYL OLF, MARR SM, SMIT WJ, FOUCHE PSO and LUUS-POWELL WJ (2017b) Gill net catch composition and catch per unit effort in Flag Boshielo Dam, in Limpopo Province, South Africa. *Water SA* 43: 463-469.

SARA JR, MARR SM, CHABALALA NM, SMIT WJ, ERASMUS LJC, LUUS-POWELL WJ (2018) Human health risks of metalloids and metals in muscle tissue of silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) from Lake Flag Boshielo, South Africa. *African Journal of Aquatic Science* 43(4): 405-411.

SAYER J and CASSMAN KG (2013) Agricultural innovation to protect the environment. *Proceedings of the National Academy of Sciences* 110: 8345-8348.

SEYMORE T, DU PREEZ HH and VAN VUREN JHJ (1996) Concentration of zinc in *Barbus marequensis* from the lower Olifants River, Mpumalanga, South Africa. *Hydrobiologia* 332: 141-150.

SKELTON PH (2001) A complete guide to the freshwater fishes of Southern Africa, Struik Publishers, Cape Town, South Africa.

SKELTON PH (2016) Name changes and additions to the southern African freshwater fish fauna. *African Journal of Aquatic Science* 41(3): 345-351.

STATISTICS SOUTH AFRICA (2017a) *Poverty Trends in South Africa: An examination of absolute poverty between 2006 and 2015*. Report No. 03-10-06. Statistics South Africa, Pretoria. 141pp.

STATISTICS SOUTH AFRICA (2017b) *Towards measuring food security in South Africa: An examination of hunger and food inadequacy*. Report No. 03-00-14. Statistics South Africa, Pretoria. 36pp.

STRYDOM C, ROBINSON C, PRETORIOUS E, WHITCUTT JM, MARX J and BORNMAN MS (2006) The effect of selected metals on the central metabolic pathways in biology. *Water SA* 32(4): 543-554.

SVOBODOVÁ Z (1993) Water quality and fish health. EIFAC Technical Paper (54). Food and Agriculture Organisation. Rome. 66 pp.

TAPELA BN, BRITZ BJ, ROUHANI QA (2015) *Scoping study on the development and sustainable utilisation of inland fisheries in South Africa. Vol 2: Case studies of small-scale inland fisheries.* WRC Report No. TT615/2/14. Water Research Commission, Pretoria pp 156.

TWEDDLE D, COWX IG, PEEL RA and WEYL OLF (2015) Challenges in fisheries management in the Zambezi, one of the great rivers of Africa. *Fisheries Management and Ecology* 22: 99-111.

UNDP (2015) *Sustainable Development Goals*. United Nations Development Programme. 24 pp.

US-EPA (2000) Guidance for assessing chemical contaminant data for use in fish advisories. Volume 2: Risk assessment and fish consumption limits, Third Edition, EPA 823-B-00-008. *Office of Health and Environmental Assessment*. Washington, DC.USA.

US-EPA (2004) Integrated Risk Information System (IRIS): Lead and compounds (inorganic) (CASRN 7439-92-1). http://www.epa.gov/iris/subst/0277.htm#oralrfd, *United States Environmental Protection Agency, Environmental Criteria and Assessment Office*. Cincinnati, OH. USA.

US-EPA (2008) Health effects support document for Boron. Health & Ecological Criteria Division. Washington DC 20460. www.epa.gov/safewater/ccl/pdf/boron.pdf accessed November 2018.

US-EPA (2012) Regional Screening Table: Composite Table May 2012. in, *United States Environmental Protection Agency*. Mid-Atlantic Risk Assessment.USA.

VAN VUREN JHJ, DU PREEZ HH, WEPENER V, ADENDORFF A, BARNHOORN IEJ, COETZEE L, KOTZÈ P and NUSSEY G (1999) *Lethal and sublethal effects of metals on the physiology of fish: An experimental approach with monitoring support*, WRC Report No. 608/1/99. Water Research Commission, Pretoria, South Africa.

WARREN LA and HAACK EA (2001) Biogeochemical controls on metal behaviour in freshwater environments. *Earth-Science Reviews* 54: 261-320.

WEYL OLF, POTTS WM, ROUHANI Q and BRITZ PJ (2007) The need for an inland fisheries policy in South Africa: A case study of the North West Province. *Water SA* 33: 497-504.

WHITEHEAD PG, WILBY RL, BATTARBEE RW, KERNAN M and WADE AJ (2009) A review of the potential impacts of climate change on surface water quality. *Hydrological Sciences Journal* 54: 101-123.

WHO (2003) WORLD HEALTH ORGANISATION State of the art report: Health risks in drinking-water. Background document for development of WHO guidelines for drinking-water quality, aquifer recharged using reclaimed water, water, sanitation and health, protection and human environment. SDE/WSH/03.08, World Health Organization, Geneva. WHO/SDE/WSH/03.04/76. 21 pp.

WINSLOW L, READ J, WOOLWAY R, BRENTRUP J, LEACH T, ZWART J, ALBERS S and COLLINGE D (2018). rLakeAnalyzer: Lake Physics Tools. R package version 1.11.4. https://CRAN.R-project.org/package=rLakeAnalyzer.

APPENDIX 1: METALS IN FRESHWATER ECOSYSTEMS

INTRODUCTION

Due to underlying geology and geochemical process, metals and metalloids (hereafter referred to as metals) occur naturally in ground and surface waters and play an essential role for the normal function and development of aquatic organisms (Dallas and Day, 2004). However, natural levels of metals are augmented when released into inland waters from industrial and mining sources with further mobilizing occurring due to acid mine drainage (Strydom et al., 2006; Hobbs et al., 2008). In a geological context, trace elements refer to all elements except the eight abundant rock-forming elements namely oxygen, silicon, aluminium, iron, calcium, sodium, potassium and magnesium. Trace metals are metals occurring at 1000 ppm or less in the earth's crust and are usually found in small (trace) amounts in water (Davies and Day, 1998). Metals can also be divided into those which occur naturally in trace amounts in most waters, most of which are essential micronutrients for plants (and animals) but may become toxic to the aquatic biota at elevated levels (e.g. cobalt (Co), copper (Cu), manganese (Mg), and zinc (Zn)); and those which usually do not occur in measurable amounts in natural waters, are potentially toxic even at low concentrations, but have been widely distributed as a result of human activities (including cadmium (Cd), lead (Pb), mercury (Hg)) (Davies and Day, 1998; Dallas and Day, 2004). The US EPA considers only beryllium (Be) and Hg as hazardous, and barium (Ba), Cd, Pd, nickel (Ni), tin (Sn), vanadium (V), and Zn as potentially hazardous (Davies and Day, 1998; Dallas and Day, 2004), see Table A1.1.

Table A1.1: Metals classified according to their toxicity and availability in natural aquatic systems (Dallas and Day, 2004).

Non-critical	Toxic but insoluble or very rare	Very toxic and relatively accessible
Na*, K*, Mg*, Al*, Li, Ca*, Fe*, Rb, Sr	Ti*, Hf, Zr, W, Nb, Ta, Re, Ga, La, Os, Rh, Ir, Ba	*Be*, Co, Ni, Cu, Zn, Sn, As, Se, Te, Pd, Ag, Cd, Pt, Au, Hg, Tl, Pb, Sb, Bi

Italics: atomic mass < 40.078 (i.e. not a heavy metal)
* abundant in the earth's crust (i.e. not defined as trace metal)

Metals usually exert their biological effects (either as essential micronutrients or as toxins) forming stable co-ordinate bonds in proteins where they function as catalysts in Redox reactions (e.g. iron (Fe), Cu, molybdenum (Mo)) or form part of the active centre of enzymes (e.g. Co, Zn) (Dallas and Day, 2004). The metals known to be essential in one or more biological systems are Mn (in enzymes), Fe (most important in haeme-containing pigments), Co (in vitamin B12), Cu (in enzymes involved in Redox reactions), Zn (in enzymes) and Mo (proteins involving electron transfer and photosynthesis) (Parametrix Inc., 1995; Dallas and Day, 2004). The particularly toxic metals tend to form more stable bonds with the SH group than with anions such as CO_3^{2-}, HCO_3^-, OH^- and Cl^- and thus affect proteins by combining with their thiol groups and thus altering their functioning (Dallas and Day, 2004). The utilisation of essential metals requires that a concentration of metabolically available metal must reach a minimal concentration in an organism (Parametrix Inc., 1995).

Metals do not degrade in the environment but are transformed from one form to another. Some forms of metals are inert while others are biologically available to organisms. Understanding the impact of metals on aquatic life requires knowledge of the form of the metal in the environment and the routes of exposure of aquatic organism. Figure A1.1 below provides a conceptual representation of an aquatic system: the water column and the underlying sediment. A metal may be present in the water column or sediment in one of the following three forms:

- Dissolved inorganic metal, including both the ionic form and the inorganic complexes of the metal
- Dissolved organically complexed metal and,
- Particulate metal including the absorbed and precipitated forms of the metals and mineral phases that may be present.

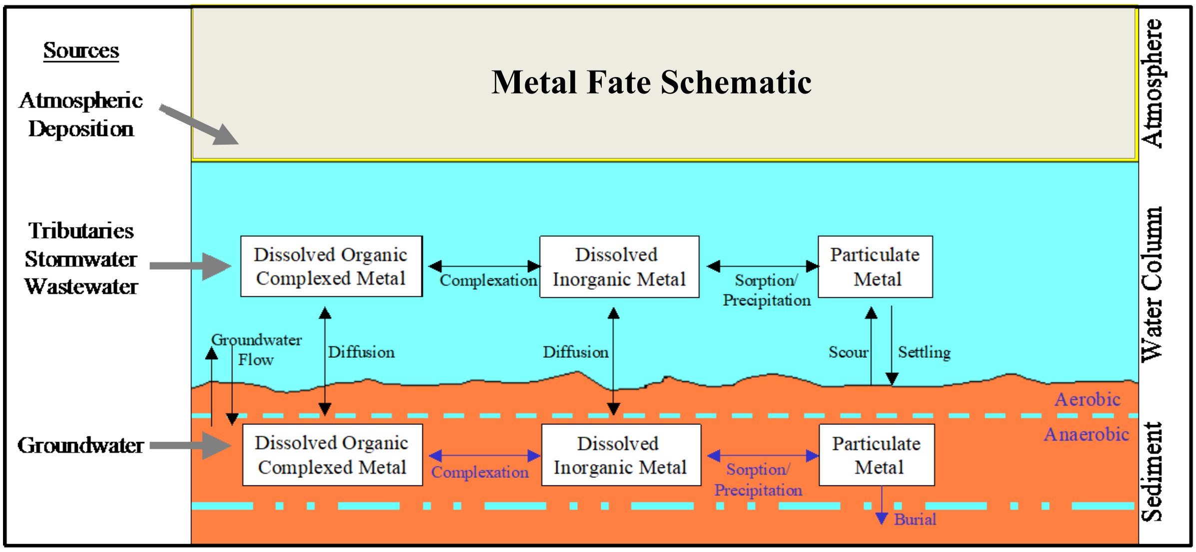

Figure A1.1: Fate of metals in the aquatic environment (di Toro et al., 2001).

In aquatic systems the water column and sediment layer are in direct contact. This leads to an exchange of constituents, including metals, between these media. The distribution, form, and chemical speciation, of the metal in the sediment will differ considerably from that of the water column. The Redox potential in the sediment determines the stable valence state of the metals present in the sediment. Distribution of the metal between the water column and sediment in a river is dependent on the hydraulics (movement of water, i.e. fluid transport, and movement of particulate matter, i.e. sediment transport) and chemistry (speciation of the metal and its sorption to particulate matter in both the water column and the sediment) of the system (Adams et al., 2000; Chapman and Wang, 2000; di Toro et al., 2001).

Re-suspension or the scouring of particulate material from sediments may occur through extreme flow or wind mixing events. Short term increases in total metal concentration in the water column may result following such events. The pH, Redox potential, solids sorption partition coefficient, water hardness, salinity/total dissolved solids, dissolved oxygen concentration, and the presence of sulphides all have an impact on the complexation reactions taking place in the water column and the sediment (di Toro et al., 2001). Differences in speciation of the metals result in dramatic differences in the metal availability of surface waters and sediments.

Concentration of metals in sediments usually exceeds those of overlying water column by three to five orders of magnitude. With such high concentrations, the bioavailability of even minute fractions if re-suspended in solution, assumes considerable importance. The composition of sediments is so complex and variable that it is often difficult to assess the bioavailability of sediment bound metals. In the water column and sediments, the most important factors in determining the bioavailability of metals are pH, Redox potential, hardness, alkalinity, and the composition and concentration of ions, particulate matter and organic carbon (Parametrix Inc., 1995).

Bioaccumulation of metals in fish

Freshwater fish take up pollutants from their environment through their diet (phytoplankton, periphyton, macrophytes, aquatic invertebrates and detritus), from the sediment while foraging or ingestion of sediment with food, and directly from the water column through their skin, gills and intestines (Dallinger et al., 1987; Bervoets et al., 2001; Couture and Pyle, 2011). Environmental factors affecting the uptake of metals into fish tissues include exposure time, exposure concentration, the presence of metal complexing agents (e.g. EDTA), temperature, acidity, hardness, and salinity (Kalay and Canli, 2000; Jezierska and Witeska, 2006). Metal accumulation rates and thresholds in the respective tissues differ due to the diet, mode of feeding or habitat use of fish species (Jezierska and Witeska, 2006). Biological factors responsible for determining the response of a fish to metals include its life history stage, size, age, sex, body condition, levels of starvation, metabolic activity, tolerance levels and acclimation ability, all of which may also affect the susceptibility of the fish to other pollutants (Kalay and Canli, 2000; Dallas and Day, 2004; Jezierska and Witeska, 2006).

Tissues such as liver, spleen, kidney and gills are metabolically highly active in fish and may accumulate metals faster, and to a higher degree, than other tissues, e.g. muscle (Kalay and Canli, 2000, Jezierska and Witeska, 2006). Specific tissues display varying affinity for metals, e.g. liver accumulate copper and the gonads zinc (Jezierska and Witeska, 2006). In contrast to other tissues, the liver produces metal binding proteins, e.g. metallothionein, that bind and detoxify metals allowing higher metal concentrations to accumulate than in other tissues (Kalay and Canli, 2000, Jezierska and Witeska, 2006). For this reason, it is generally expected that the highest concentration of metals occurs in the liver, followed by kidneys, gills and muscle (Jezierska and Witeska, 2006). Fish can eliminate excessive metals from their tissues (Kalay and Canli, 2000, Jezierska and Witeska, 2006). The elimination of metals is influenced by temperature, interactions with other metals, metabolic activity and the specific tissue (Kalay and Canli, 2000). The elimination routes include bile, urine, gills and mucus (skin and intestine), however, the rate of metal absorption is faster than elimination implying that metal accumulation in tissues is reversible but once metals have been accumulated in tissues, it is difficult for the fish to eliminate them, particularly non-essential metals (Kalay and Canli, 2000). Metals are also moved within the body of fish from gills to liver for detoxification, then gradually released to muscle tissue or excreted (Kalay and Canli, 2000).

Several biotic and abiotic factors affect the toxicity of metals to aquatic organisms including physiological condition, tolerance, growth and reproduction, species variation, inter- and intra-specific variation in life history stages, adaptive capabilities and behavioural responses of the organisms whereas the abiotic factors include the presence of metals or pollutants, nature of dissolved organic matter, pH, water temperature, alkalinity and water hardness, dissolved oxygen and interactions between all these factors in the ecosystem (Heath, 1995).

Freshwater organisms absorb contaminants from the surrounding environment, i.e. sediment, water and food (Chen et al., 2000; Warren and Haack, 2001) and incorporate them into aquatic food webs, concentrating them up the food chain (Adams et al., 2000). As a consequence, the bio-magnification of metals pose a toxicity risk to top level predators, e.g. piscivorous fish and humans. This is a major concern as fish are a vital dietary source of protein for low income groups and many people in rural communities neighbouring inland waters (Beveridge et al., 2013; Sayer and Cassman, 2013).

APPENDIX 2: REPORT ON A WORKSHOP/INFORMATION SESSION TO ALL STAKEHOLDERS IN THE SEKHUKHUNE DISTRICT SURROUNDING FLAG BOSHIELO DAM

Compiled by: Prof. Wilmien Luus-Powell

Researchers from the University of Limpopo's Biodiversity Department joined the Limpopo Provincial Government during their 'Sekhukhune District Environment Day' on 20 June 2019 to inform the community of the findings of the WRC Project K5/2544. The aim of the workshop was to inform the Olifants River/Sekhukhune District community about ecosystem health including metal pollution, water quality and fish health in Flag Boshielo Dam and the human health risk posed by long-term consumption of fish from Flag Boshielo Dam.

Background

This workshop emanated from our Water Research Commission (WRC) project '**Predicting the edibility of fish in the Flag Boshielo System**'.

The risk assessment used to calculate the Hazard Quotient (HQ) assumes the consumption of a single fish meal of 150 g muscle tissue once a week by a 70 kg adult whereby a HQ > 1 indicates an unacceptably high risk. For fish collected during periods when flow rates and water levels in the dam were recorded to be low (i.e. < 80%) a HQ < 1 was calculated for all three fish species sampled. By contrast, when water levels in the dam exceeded 80% capacity the HQ for cobalt (Co) was calculated to be greater than one for O. mossambicus and for arsenic (As) in L. rosae.

The WRC indicated that we should present the findings of our recently completed WRC report at a stakeholder workshop. Consequently, we have joined the Sekhukhune District Environment Day organized by Limpopo Provincial Government to inform the stakeholders, including local community members, about fish health and parasites as well as human health risk upon consumption of the fish from Flag Boshielo Dam.

Workshop programme

Date: 20 June 2019 Time: 10:00-15:00 Venue: Matlala-Dichoeung Traditional Hall, Ephraim Mogale Local Municipality

Programme Director: Councillor TL Mabaso

FORMAL AGENDA FOR SEKHUKHUNE DISTRICT WORLD ENVIRONMENT DAY

2019 Theme: "Air pollution"

Venue: Matlala-Dichoueng Traditional Hall — Date: 20 June 2019

Programme Director: Cllr Mabaso T L

LIMPOPO PROVINCIAL GOVERNMENT REPUBLIC OF SOUTH AFRICA DEPARTMENT OF ECONOMIC DEVELOPMENT, ENVIRONMENT & TOURISM

Time	Programme	Facilitator
10:00	Welcome	Moshate wa Matlala-Dichoueng
10:05	Opening Remarks	P.D
10:10	Acknowledgment of delegates	P.D
10:15	Purpose of the day	Tsheola M.P
10:25	Item by learners	
Presentations		
	1. Environmental Governance	Mr. Leshabane Patrick
	2. Environmental Compliance Challenges	Ms. Takalo Tshegofatso
	3. Waste Management/Collection	Mr. Phuti Mabotha
	4. Water Research Commission-Flag Boshielo Dam Research Findings by University of Limpopo.	Prof. Wilmien Luus-Powel
Motivation Talk		
	1. Thotse tsa Lerotse	Matentshi M/ Riba S
	2. Dept. of Health	Elandskraal Clinic
	3. Dept. of Social Development	Mr. Makokga T.A
	Questions and Clarifications	P.D
	Vote of Thanks	E.M Local Municipality
	Wayfoward	Ms. Marokana M.A
	Announcements	Ms. Masemola P
	Closure	P.D

An interpreter translated the presentations to ensure that all community members understood the content of the presentations. This was much appreciated.

The following departments and forums were represented at the workshop: Department of Environmental Affairs (DEA), Department of Economic Development, Environment and Tourism Limpopo (LEDET), Mpumalanga Tourism and Parks Agency (MTPA), members of the ward committee, Ephraim Mogale Local Municipality, members from Regae Primary School, community members from Dichoueng, Pioneers, Matlala, PSI, MKM. More than 100 people attended the workshop.

Members from the local community and other stakeholders at the workshop responded well to the presentations given by Prof Luus-Powell and Prof Addo-Bediako. Some of the questions raised were 'Is it safe to eat fish contaminated with metals from the Dam? Are all the fish contaminated? How 'large' is 150 g fish? Will it be better to remove the guts of fish before preparation? What can be done to reduce the metal levels? Which is the best way to prepare fish to ensure parasites are eliminated? Is it safe to drink water from the river?

We discussed the concerns raised with all stakeholders by informing them that some fish species have higher levels of metals (depending on their trophic level), and it is not safe to eat fish from this Dam on a regular basis (especially during high flow seasons). We couldn't confirm whether the method of fish preparation will influence metal levels as it did not form part of our study. We highlighted that if fish is well cooked (doesn't matter whether baked in oil or boil in water), it should be safe to eat concerning parasite infections. To reduce metal levels, the pollution upstream should be mitigated. We also discussed the reasons why it is not safe to drink water directly from the dam or river (e.g. coliform contamination) and encouraged them to boil the water first it should be safe.

In conclusion, the workshop was a great success rendering the various informal discussions we had with the participants during and after the workshop as well as the interest shown and questions raised by community members and other stakeholders

Acknowledgements

We would like to thank the WRC for funding and guiding this project and workshop.

Our thanks are extended to:

Members of the Steering Committee for assistance with arranging the workshop;

University of Limpopo for supporting our research;

UL Research Development and Administration for their financial management and support of the WRC contract;

DST-NRF SARChI Chair (Ecosystem Health) and VLIR-UOS for additional funding;

All the team members and the postgraduate students of the WRC project.


LIMPOPO
www.ledet.gov.za | +27 15 293 8300


LIMPOPO


ENVIRONMENTAL AFFAIRS
LIMPOPO